Sustainability of the Sugar and Sugar-Ethanol Industries

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Foreword

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Preface

Sucrose sugar is primarily produced industrially from sugarcane (*Saccharum officinarum*) and sugar beet (*Beta vulgaris*). In 2008/09, 157 million tonnes of sugar (raw value) were produced world-wide, and the sugar industry is a significant component of the economy in many countries. Surplus sugar on the world market, competition from high-intensity sweeteners, fluctuating prices, demand for production of high quality raw sugars, government policies, water- and energy-intensive factories and refineries, and in particular the current worldwide impetus to produce alternatives to petroleum-based fuels, are putting pressure on the sugar industry to diversify and add value for sustainability. Sustainability in this book is defined as the balancing of economic, environmental, and societal performance of the sugar and sugar−ethanol industries for generations to come.

All biomass from sugarcane and sugar beet plants, including leaves and tops, are being intensely investigated for utilization, including cogeneration of heat and bioelectricity in some countries. Thus, the sugar industry is increasingly being regarded as a biomass-based industry that not only manufactures food, but also fuel ethanol and other value-added products. It is expected in the next few years that sugar will be the "new oil" because sugar is superior feedstock for the production of platform chemicals for a wide range of industrial products. Sugar crops, including sweet sorghum (*Sorghum bicolor*) fit well into the emerging concept of a renewable carbohydrate feedstock because of their availability, and because they are amongst the plants giving the highest yields of carbohydrates per hectare. For sugarcane, sweet sorghum, and sugar beet, their significant potential as food and fuel ethanol (bioenergy) crops is currently driving rapid expansion of production areas throughout the world.

Another world-wide trend in the sugarcane industry is the manufacture of higher quality VHP (very high pol) and VLC (very low color) raw sugar for supply to new refineries. The quality of the sugarcane supplied to the factory directly impacts the manufacture of VHP/VLC raw sugar. Strategies to manufacture VHP/VLC sugar include the supply of higher quality sugarcane to the factory, as well as process manipulations.

All these dramatic developments were the primary reasons to produce this book, and the associated American Chemical Society (ACS), Division of Carbohydrate Chemistry and ACS Thematic Programming One-Day Symposium, *Sustainability of the Sugar and Sugar−Ethanol Industries* that was organized by Dr. Gillian Eggleston. This symposium was held on March 22, 2010 in San Francisco at the 239th National ACS Meeting.

The objective of this book is to provide an overall increasing awareness, understanding, and implementation of the recent great advances in the measurement

of sustainability in the sugar and sugar−ethanol industries, sustainable changes in processing and production of different sugar crops for multiple end-products including biofuels, and sustainable value-added products. Multiple industries around the world are purposely highlighted so that various viewpoints are exchanged.

The chapters are arranged so as to provide the reader with an understanding of the sustainability issues facing the sugar and sugar−ethanol industries. The first introductory section gives an overall perspective to background issues in both industries, as well as recent sustainable developments in the production of sugar and biofuels, especially bioethanol from different sugar crops. The second section discusses the measurement and evaluation of sustainability with case examples. The third section highlights successful sustainable efforts in sugar and sugar−ethanol industries around the world, emphasizing both production and processing practices plus new innovations. The fourth section includes the sustainable production of fuel ethanol from multiple sugar crops. Production of cellulosic ethanol from sugar industry cellulosic materials such as sugarcane bagasse and sugar beet pulp are also included. The fifth section discusses sustainable improvements in the quality supply of sugar feedstocks. This includes new approaches to raw sugar quality improvement as a route to sustaining a reliable feedstock and the control of sugarcane and sugar beet deterioration which can still be a major technological constraint in processing. In the sixth and final section, value-added products that could underpin future sustainable developments are highlighted. With contributing authors from industry, government, and academia, worldwide, the text should provide the readers with an up-to-date review of this important and rapidly developing field.

The distinguished reviewers who made this book possible by their thorough and professional reviews are acknowledged: Sarah Lingle, John R. Vercellotti, Mary An Godshall, Barbara Muir, Harold Birkett, Dennis Walthew, Jurgen Bruhns, Isabel Lima, J. Mitchell McGrath, Geoff Parkin, and Wolfe Braude.

I deeply appreciate the Symposium sponsors without whose donations the Symposium and book could not have been realized: ACS Sustainability Theme Committee, in particular Laura Pence, ACS Division of Carbohydrate Chemistry, the American Sugar Cane League, and V-Labs, INC. I also thank Dr. Sharon Shoemaker of UC Davis for helping to organize a most successful Symposium dinner at the North Beach Restaurant in San Francisco.

Many thanks to Tim Marney of ACS publications who worked hard with me to get this book finished and edited in a timely manner. Thanks also go to Dr. John Vercellotti for useful comments on the book. Finally, I thank all the authors for their valuable contributions to this book, and I sincerely hope that the readers will enjoy it.

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Dedication

This book is dedicated to:

Duane Legendre, Mill Manager, Lafourche Sugars LLC, Thibodaux, Louisiana – for being with me from the start on the journey in the factories, and his support.

Adrian Monge, Fabrication Consultant, Cora Texas Manufacturing Co., White Castle, Louisiana – for his mentorship in factory processing.

Eldwin St. Cyr, Physical Science Technician, USDA-ARS-SRRC, New Orleans, Louisiana –for excellent technical assistance <u>always</u>.

Future Sustainability of the Sugar and Sugar−Ethanol Industries

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Like many other food and chemical industries, the sugar and sugar−ethanol industries are facing important sustainability issues. The relatively low and fluctuating profit for sugar, the world-wide impetus to produce alternatives to petroleum-based fuels and reduce green house gases, and water- and energy-intensive factories and refineries are putting pressure on the industries to diversify for sustainability. In sugar manufacture, there is a world-wide trend to produce very high purity (VHP) and very low color (VLC) raw sugars for vertical integration from the field to white sugar. All biomass from the sugarcane and sugar beet plants including tops and leaves, are being intensely investigated for utilization, including cogeneration of both heat and bioelectricity in some countries. Sugar, in a few years, is expected to be the "new oil" as sugar is a superior feedstock for the production of platform chemicals for the manufacture of a range of end-products, e.g., bioplastics, industrial solvents, and chemicals. Sugarcane, sugar beet, and sweet sorghum fit well into the concept of a renewable carbohydrate feedstock for fuel ethanol production because of their availability, and they are amongst the plants giving the highest yields of carbohydrates per hectare. Green sustainability criteria are now in place in the European Union for the EU biofuels sector that have to be met to count against national biofuel targets. Processes to convert high-fiber, energy sugarcanes and sugar beets as well as traditional cellulosic

by-products into fuel ethanol have been developed but are not yet commercialized.

Introduction

Sucrose (α-D-glucopyranosyl-(1→2)-β-D-fructofuranose) is ubiquitously known as common table sugar, and crystalline sucrose is primarily produced industrially from sugarcane (*Saccharum officinarum*) and sugar beet (*Beta vulgaris*) (Figure 1).

Like many other food and chemical industries, the sugar industry and sugar−ethanol industries are currently facing tough sustainability issues. Sustainability in this chapter is defined as the balancing of the three, interdependent pillars of the environment (ecology), society, and the economy (Figure 2). For some industries the core principles for sustainable manufacture are renew, reuse, and and recycle, which are applied to every production step and business practice (*1*).
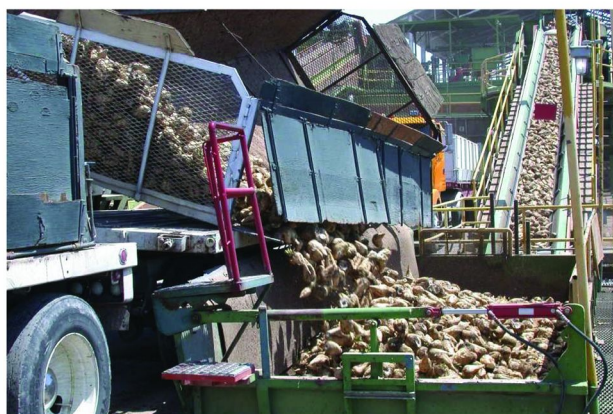
Figure 1. Sugarcane harvested into billets (top) and sugar beets being delivered for processing (bottom).
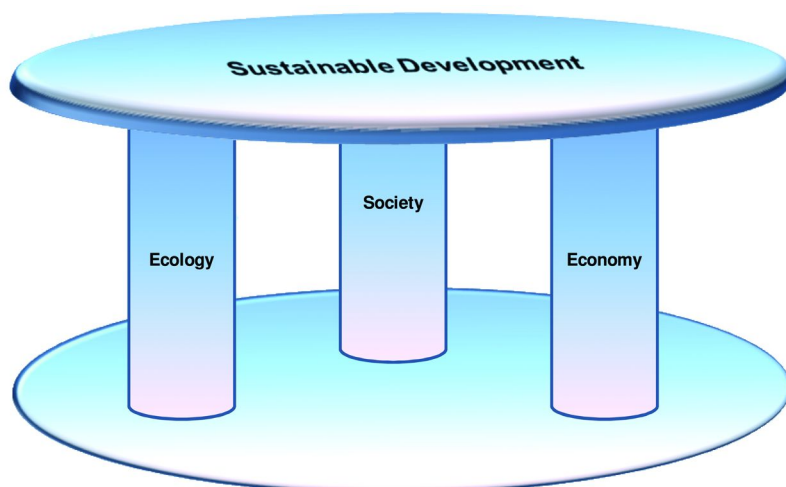
Figure 2. Sustainability focuses on the triple bottom line: the integration of (i) ecological integrity, (ii) social responsibility and (iii) economic viability.

Table I. Unsustainable Versus Sustainable Mindsets and Practices in the Current Sugar Industry. (Adapted from (3))

Key dimension	Unsustainable	Sustainable
Society/Policy Goals	Economic growth	Growth in well-being
Approach to Nature	Control over nature	Work with nature
Predominant Work Mode	"Big is Better"	"Smart is Better"
Focus on Business Activities	Goods	Services, needs
Energy Sources	Fossil fuels	Renewable energy (including biofuels)
Predominant Chemistry	Energy intensive	Low energy
Waste Production	High waste	No waste
Typical Materials	Iron, steel and cement	Bio-based materials

The twentieth century saw enormous growth in chemicals manufacturing which fed the parallel growth in the developed world. However, the growth came at a cost. Inefficient processes leading to unacceptable levels of pollution, hazardous operations resulting in a number of well-publicized disasters, inadequate product testing causing often irrational public concerns over product safety, have all led to an exponential growth in chemicals legislation (2). Chemical industries, including the sugar and emerging sugar−ethanol industries, now need to achieve environmentally acceptable and economically viable manufacturing in a tough legislative framework while meeting the high demand of a growing

population. Sustainable production of sugar, ethanol and other bioproducts from sugar crops, will only be realized through a reassessment of the entire chemical product life-cycle from resources, to manufacturing and production, through to product use and ultimate fate (*2*).

To achieve sustainable sugar and sugar−ethanol industries several critical changes are required both in mindset and practice that are listed in Table I.

This chapter describes current trends and needs in the sugar and sugar−ethanol industries that are expected to strongly contribute to their sustainability.

Industrial Sugar Production: Background

Commercially available sucrose has very high purity (>99.9%) making it one of the purest organic substances produced on an industrial scale. To obtain such a pure product from both sugarcane and sugar beet, rather complex isolation and purification process units are followed. Industrial sucrose production is essentially a series of separations of non-sucrose compounds (usually termed non-sugars or impurities) from sucrose, and the chemistry of the sequential process units is designed for maximum removal of non-sugars with minimum destruction of sucrose (Figure 3). Sugarcane is grown in tropical and sub-tropical areas of the world and processing often occurs in two stages. Firstly, the juice is extracted from sugarcane (sucrose yields range between 10-15% weight of sugarcane) and converted to raw sugar (~97-99% pure sucrose; golden yellow/brown crystals) at factories. Secondly, after raw sugar has been transported to a refinery, it is refined using very similar unit processes used in raw sugar manufacture, to the familiar white, refined sugar (>99.9% sucrose). In some tropical areas of the world, particularly Asia, plantation white, mill white, or direct white sugar (>99% sucrose with a higher color than white, refined sugar) is produced directly from sugarcane (*4*).
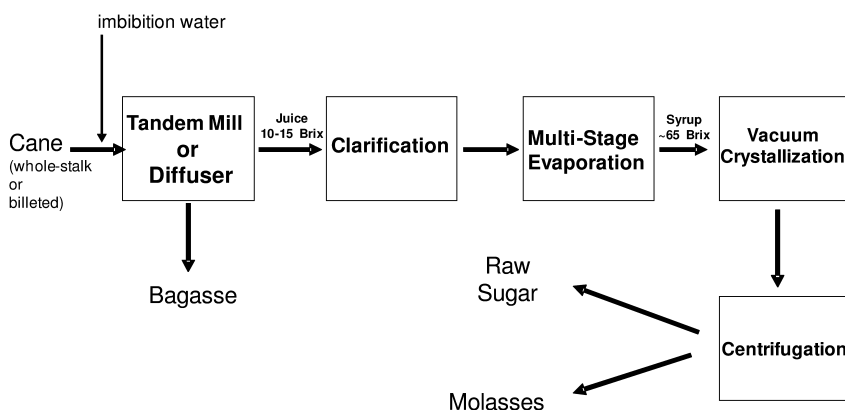

Figure 3. Basic scheme of the raw sugar manufacturing process in a sugarcane factory (4). Brix is % dissolved solids.

In comparison to sugarcane, sugar beets are grown in more temperate areas and are processed directly into white sugar (>99.9% sucrose) at nearby factories. Production of refined sugar from sugar beets has some similarities to refined cane sugar production. However, disimilarites exist as sugar beet is a tuberous root and sugarcane a grass. Sugar beets are harvested defoliated and delivered to the factory, with excess sugar beets stored in long-term storage piles on factory or remote grounds. Sugar beets are introduced to the factory, washed, and sliced into "V" shaped cossettes. Cossettes are added to a diffuser and sucrose (~98%) and impurities are extracted with hot water. Diffusion juice contains ~12% sucrose and 2% soluble impurities on sugar beet weight, and is heated to ~85 °C before it is purified with a double-carbonatation clarification process. The resulting clarified "thin" juice is then concentrated from ~14 to ~60-65 Brix ("thick" juice) across multiple-effect evaporators, then triple-crystallized and centrifuged to produce white, refined sugar (>99.7% purity). In some sugar beet factories additional purification steps are employed, such as color removal with ion-exchange resins or carbon adsorbants. Additionally, sucrose is also recovered from beet molasses with chromatography, a process that is much easier in sugar beet than sugarcane processing. For more detailed information on the industrial production of sucrose from sugarcane and sugar beet, the reader is referred to other comprehensive texts (*4–8*).

The major by-products of industrial sucrose production are cane bagasse, beet pulp, and molasses. Minor by-products include fly ash, filter cake, lime and calcium carbonate residues. By volume, bagasse is the most important by-product and is the primary source of fuel for the generation of steam and bioelectricity to run sugarcane factories. Beet pulp is a source of animal feed as wet pulp, pressed pulp silage, and dried pulp, with or without added molasses. Molasses is presently the most valuable by-product of sugar manufacture and exists in a range of grades: edible molasses, cane and beet molasses, and refinery molasses. It is used as an animal feed additive, in the industrial production of rum and other beverage alcohols, bakers' yeast, citric acid, and other fermentation processes.

VHP (Very High Pol) and VLC (Very Low Color) Sugar Production–A Sustainable Trend

There is presently a trend in the U.S. and worldwide to manufacture VHP and VLC raw sugars for supply to refineries, i.e., a trend of vertical integration from the field to the white sugar output. Furthermore, a concomitant trend exists to build refineries of the VHP/VLC cane raw sugar close to the consumption areas to satisfy the needs of the food industry. This trend to integrate factory and refinery operations began in Australia in the mid 1990's (*9*) to reduce the overall cost structure and enhance product quality. There is also a growing demand for exports of VHP and VVHP (very, very high pol) raw sugars, particularly from Brazil, mainly for overseas markets. In the U.S., many factories have combined into the Louisiana Sugar Cane Products, Inc. (LSCPI) and are investing, with Imperial Sugar and Cargill companies, in a new sugar refinery in Gramercy, LA, which is expected to be operational in mid-to-late 2011 (*10*). Some refineries

also want lower ash concentrations in the VHP/VLC sugar because (a) some of the refined sugar will be manufactured into liquid sugar, which requires low ash, and (b) lower ash is needed for short, medium, and long term refinery strategies (Chapman, LSCPI, personal communication). The request for the supply of such higher quality raw sugars is expected to create additional efficiencies at the new refineries, particularly at the early, energy-intensive affination stage. The higher quality raw sugars will also allow factory processors to gain premiums from the new refineries. Furthermore, manufacture of higher quality raw sugars at the factory where the energy source is the sustainaible, renewable by-product bagasse (Figure 3), will save fossil energy utilization by the refiners.

One of the main keys to manufacturing VHP/VLC sugar is the removal of color. While color removal at the refinery is mostly perfected (*11*), the processes are capital- and equipment-intensive, which further justifies the refiners request that more of the color removal work be undertaken at the factory. Current color removing strategies at the factory can be separated into three categories: (i) improved unit process operations and designs, (ii) chemical processes, and (iii) physical processes (*11*, *12*). However, all three categories are typically expensive. Moreover, great variations in the color of the raw sugars produced still exist because of the large variations in the quality of the cane supply (*12*, *13*). Muir and Eggleston (*12*) recently suggested that even a small reduction, e.g., <10% in total trash levels processed at the factory, could be more efficient and cost-effective than other factory color removal processes and have the additional advantages of improving sugar yields and ash contents.

Large-Scale Cogeneration of Bioelectricity from Sugarcane Bagasse

Most sugarcane factories cogenerate steam and bioelectricity from bagasse to run the factory and, in the early years of the sugar industry they were viewed as the original cogenerators of the world (*14*). Nowadays, some countries' sugar industries, e.g., Mauritius, Brazil, India, and the Philippines, also operate large-scale cogeneration and sell the surplus electricity to the local or national grid, and there is great potential for many other countries to follow. Furthermore, cogeneration contributes to sustainability as the negative environmental impact of Green House Gases (GHGs) from traditional thermal power stations are reduced (*15*). A case example is the Mauritius sugar industry. In a typical year, 19-21% of the electricity in Mauritius is generated from bagasse. Because of the seasonal character of sugarcane, the contribution of bagasse to the Mauritian grid fluctuates seasonally. Thus, to ensure year-round supply of electricity, the plants co-fire with coal (*16*).

One of the main technological improvements leading to higher efficiency cogeneration of bioelectricity from bagasse has been the use of new high-pressure boilers, i.e., up to 82-92 bars (producing superheated steam at 525 °C). Efficiency gains leading to a surplus of electricity generation for export to the grid have also been accomplished through the retro-fitting of turbo-alternators with high steam pressure/temperature (*14*), the optimization of other process parameters,

including steam consumption, increasing fiber content of cane through genetic manipulation, lower moisture content of bagasse, and reducing the consumption of electricity in the factory tandem mill and power plant (*15*). The development of year-round bagasse cogeneration in Mauritius was promoted by providing incentives for the cogenerator and a number of policies and policy instruments drivers: (i) reform of the Mauritian sugar industry, (ii) planning and regulatory paths, (iii) financial and tax incentives, (iv) power purchase agreements including the pricing of electricity, (v) research and development, (vi) equity participation to broaden ownership of the industry, and (vii) carbon dioxide emission reductions (*16*). This will be discussed in much further detail in Chapter 4 of this book (*17*).

Sugarcane trash biomass, e.g., leaves and tops, from the fields allows even more scope for cogeneration (*14*); a 2007 study in Brazil (*18*) showed utilization of trash with bagasse doubled the MWh production of energy compared to when just bagasse alone was utilized. However, the sugar industry's world-first attempt in Australia to send the entire green cane crop, i.e., with all the trash, to the factory to fuel its electricity cogeneration plants, was halted in November 2009 (*19*) because less than acceptable sugar recoveries occurred in factories. This was "extremely disappointing" to growers who had spent millions of dollars modifying equipment to no gain and now have debts (*19*). A cleaning plant may be necessary to remove the trash at the factory so it is not processed (*19*). This is discussed more fully in the next section.

A New Reverence for Sugarcane Trash (Leaves and Tops)

Although sugarcane trash continues to be under-utilized in numerous countries there is a growing reality that it represents a rich source of biomass for production of a multitude of biomaterials, including bioelectricity as described in the above section, cellulosic ethanol, and biochar. Moreover, separation of trash from stalks before processing would dramatically improve the efficiency of processing and the quantity and quality of raw and VHP sugar produced (*12*). Sugarcane trash includes green and brown dried leaves plus growing point region [apical internodes] or top. Compared to bagasse, sugarcane trash contains approximately the same or slightly less lignin and is, therefore, as easily degraded. Singh *et al.* (*20*) recently reported the effect of biological treatments on sugarcane trash for its conversion to fermentable sugars.

The use of sugarcane trash as a biomass source for bioelectricity, cellulosic ethanol, and biochar production is dependent on the amount of dry mass available. Typical percent tissue weights on a dry mass basis for U.S. and South African commercial sugarcane varieties grown mid-season are listed in Table II. It can be seen (Table II) that varietal variation occurs, and U.S. sugarcane had ~34% total trash compared to 41% for the South African variety (Table II). Thus, over one third of the total dry mass from sugarcane is from trash. In the case of the U.S. varieties the green leaves deliver the most dry mass of all the trash tissues (*21*), whereas for the South African variety that was ~23 months age compared to 12 months age for U.S. cane varieties (Table II) the growing point region delivered

the most dry mass. The amount of available dry mass from trash will also fluctuate across the season.

Often sugarcane trash is burned in the field or left as a cover in the field after harvesting to contribute as an organic soil fertilizer or delivered to the factory where it detrimentally affects upstream and downstream processing (*21*). Leaving excess trash on the field can reduce subsequent ratoon (new crop) yields (*22*). Although some trash should be utilized as a soil fertilizer there is still plenty available for use as biomass. Furthermore, the world-wide shift away from the harvesting of burnt to unburnt (green) sugarcane for environmental reasons means even more trash is becoming available to collect in the field or at the factory.

For trash to work as a biomass source, research into economical ways to collect and transport excess trash in the field is needed, preferably after solar drying in the field to create greater dry mass (*24*). Trash that is harvested and delivered with the stalks at the factory could also be separated there; trash separation technologies at the factory are available (*25*), including dry cleaning before the sugarcane is shredded. However, questions still remain on how efficiently trash separation technologies perform while *not* removing valuable sucrose from the stalks (*22*, *26*). Furthermore, the excessively large piles of trash that could be created at the factory will have to be utilized quickly (*22*). Trash shredders can reduce trash to bagasse-like consistency (*25*).

Table II. Average % Tissue Weights on a Dry Weight Basis (Potential Biomass) of Field Sugarcane Varieties in the U.S. and South Africa (Mid-season). (From Eggleston *et al.* (*22*) and Muir *et al.* (*23*))

	% Tissue on a Dry Weight Basis[ab]			
Tissue	U.S. (Louisiana)			South Africa (Midlands)
	HoCP 96-540[c]	L 99-226[c]	L 99-233[c]	N12[c]
Stalk	63.7	64.0	71.0	58.9
Growing Point Region	4.8	4.5	4.8	22.4
Green Leaves	17.2	20.7	16.2	13.6
Brown Leaves	14.3	10.7	8.1	5.2
Total Trash: GPR + GL + BL	35.3	35.9	29.1	41.1

[a] N=4   [b] % tissue on a dry weight basis was calculated as wet weight x (100-% moisture content)/Total plant dry weight x 100   [c] The Louisiana season is 3-months from Oct to Dec (winter). The South Africa season is 8-months from April to Dec; sampling occurred in June (winter). U.S. sugarcane was ~12 months age whereas N12 was ~23 months.

Value Added Products from Sucrose

Value-added products from sucrose that meet existing needs can increase the demand, value, and consumption of sucrose, and improve the competitiveness of the sugar industry in a world increasingly turning to agriculturally-derived chemicals from renewable feedstocks because of the surging costs and detrimental climate impacts of petroleum and gas feedstocks. However, only a small percentage of the sugar produced in the world is used in non-food applications, with ~1.7% at present in the U.S. (*27*). This is unfortunate as much research effort and funds have been expanded on the identification and development of value-added products from sucrose. Part of the reason for such little impact of this research is that the scientists inventing the products have not fully considered the market, and do not have the business acumen to sell such products to industry (*4*). More involvement by industry, particularly at the conception phase, would help to gain more impact (*4*).

Sucrose is a likely source for many value-added products because of its chemical and enzymatic reactivity. The basis for the reactivity of sucrose is the eight hydroxyl groups present on the molecule. Generally, the three primary hydroxyls have greater reactivity but they often prove a hindrance as they are difficult to react exclusively (*28*). The synthesis of an enormous number of sucrose derivatives is possible; substitution with just one group type could theoretically give two hundred and fifty five different compounds! Moreover, the alcohol group can be derivatized to form esters, ethers, and substitution derivatives (*28*). Sucrose can be readily degraded by acids, oxidizing agents, alkalis, and catalytic hydrogen to compounds of lower molecular weight. Sucrose is also an exceptional molecule for enzymatic synthesis reactions (*27*, *29*). Sucrose can act as a donor molecule for enzymatic transfer reactions to form oligosaccharides and polysaccharides. Products formed from chemical and enzymatic reactions will be discussed in chapter 15 of this book including the manufacture of bioplastics and biofibers (*30*).

Further Optimization of Sugar Processing

Like for other chemical industries, there is always room for improvement in sugar processing. The large topic is beyond the scope of this chapter but two critical areas needing improvement – (a) measurement of deterioration at the factory and (b) optimized application of enzymes - are briefly described in the next two sub-sections.

Improved Control of Sugarcane and Sugar Beet Deterioration

Better control and processing of sugarcane and sugar beet deterioration will contribute to the sustainability of the sugar industry. The delivery of consignments of deteriorated sugarcane or sugar beet to factories in many countries still often detrimentally affect multiple process units, reduce valuable sucrose and ethanol yields, and even lead to a factory shut-down. Until the last few years, there was no validated, reliable, rapid, easy, and inexpensive method to measure deterioration

at the factory. This has meant that factory personnel have not been able to screen individual consignments of sugarcane or sugar beet and, thus, they do not know which consignments will detrimentally affect processing and are unable to reject unsuitable consignments. Furthermore, grower payment formulas incorporating a deterioration quality parameter may serve as a deterrent against the delivery of overly deteriorated sugarcane, improve processing, and encourage better sugarcane management as prevention is always better than the cure.

The major (but not sole) contributor to sugarcane and sugar beet deterioration in the U.S. and many other countries, particularly where warm and humid conditions prevail, is infection by hetero-fermentative *Leuconostoc mesenteroides* lactic acid bacteria (*31–33*). Previously, the sugar industry has considered dextran, a viscous glucopolysaccharide, as the major deterioration product of a *L. mesenteroides* infection. Current methods to determine dextran, however, are either too time consuming and complicated, not specific enough, too expensive, too imprecise, or too difficult in the interpretation of results (*33*). Moreover, none of these dextran methods can be used in a payment system for growers. In recent years it has emerged that mannitol, a sugar alcohol, is also a major degradation product of *L. mesenteroides* sugarcane deterioration, sugar beet deterioration, and even some bacterial contamination of fuel ethanol produced from sugarcane (*33*). Mannitol is also produced by other hetero-fermentative *Lactobacillus* bacteria, although *L. mesenteroides* is the greatest producer (*34*). An enzymatic factory method that is rapid, simple, accurate, and inexpensive is now available to measure mannitol in juices (*33*). Greater than ~500 ppm/Brix of mannitol in sugarcane juice predicts downstream processing problems, but this threshold value may vary from region to region (*33*). The increasing awareness of how mannitol detrimentally effects processing is fully discussed in Chapter 13 of this book (*35*).

Optimized Industrial Enzyme Applications

In the sugar industry, α-amylase is frequently used to hydrolyze starch into lower MW (molecular weight) dextrins and maltooligosaccharides in sugarcane factories, and dextranase is used to hydrolyze dextran into lower MW dextrans and isomaltooligosaccharides when sugarcane or sugar beet deterioration has occurred. Unfortunately, large enzyme manufacturing companies only regard the sugar industry as a small enzyme market. As a consequence, there has been limited or no research and development by such companies to tailor the properties of commercial α-amylases and dextranases to the harsh sugar processing conditions, and none is expected in the near future. Thus, both α-amylases and dextranases used in the sugar industry were developed for larger markets, e.g., α-amylases for the detergent industry, which has caused their sub-optimum and mis-applications in sugar factories (*36*, *37*). For this reason, since 2005 factory optimization studies for both α-amylase and dextranase in sugar processing were conducted (*38–40*). These have included providing methods to measure the activities of these commercial enzymes at the factory. Results from these studies have already positively impacted the industry, and further optimization may be achieved by installing serpentine pipes to increase mixing of the enzyme

and substrate and reduce the need for more retention time in the factory, as well as applying promising low level, uniform ultrasound technology (*41*). More long-term solutions to overcome the non-tailored processing properties of α-amylases and dextranases in the sugar industry could be protein engineering of the enzymes. Protein engineering techniques include site-directed mutagenesis and random mutagenesis (directed evolution) (*36*).

Sugar−Ethanol Industries

Continued reliance on fossil fuel energy resources is unsustainable, owing to both depleting world reserves and the GHG emissions associated with their use, as well as national security. Consequently, there are currently vigorous research initiatives aimed at developing renewable and potentially carbon neutral, solid, liquid and gaseous biofuels as alternative energy resources. Sugar crops, mainly sugarcane, sugar beet, and sweet sorghum (*Sorghum bicolor*), fit well into the emerging concept of renewable carbohydrate feedstocks for alternative fuels because of their availability, that they are amongst the plants giving the highest yields of carbohydrates per hectare, have high sugar content and are remunerative for growers.

First Generation Biothanol from Sugar Crops

As of January 2010, approximately 50% of the world's fuel ethanol production (mostly first generation) was from sugar crops utilizing conventional fermentation, with the remaining 50% from starchy grain crops (Table III). Sugar crops have the advantage over grain crops because they can be grown in a much larger area of the world (*42*) and are directly fermentable. Although in the U.S., the dominant feedstock for ethanol production is corn (*Zea mays*) grain, most other ethanol producing countries use sugarcane and sugar beet as their primary feedstocks (Table III). Both Brazil and India have large-scale sugarcane based fuel ethanol production (Table III). European fuel ethanol production has continued to increase strongly in the last three years because the new EU Sugar Regime has driven major restructuring of the European sugar industry (*43*). The new EU Regime rules allow non-sugar quota beet to be produced for industrial use - mostly ethanol production. Developments are also taking place at European sugar−ethanol plants to recover carbon dioxide produced by the plant to produce a liquified CO_2 value-added product, as well as make use of *all* the materials delivered to and generated at the plant (*43*).

Since 2006 there has been a near doubling of European Union (EU) production (*44*, *45*), mostly because of robust growth in France and Germany, and more than twenty sugar beet ethanol plants now exist in Europe. The EU Renewable Energy Directive (RED) has recently put forth sustainability criteria for the EU biofuels sector that have to be met to count against national biofuel targets (*46*). The sustainability criteria have three elements (*46*):

- Obligatory minimum Green House Gas (GHG) savings

- Restrictions on land use for growing biofuel crops
- Social standards which have to be met

The most tangible criteria is the GHG saving of at least 35% which a given biofuel has to achieve to comply with the RED, which will rise to 50% in 2017 for existing plants. Ethanol from sugar beet and even more so from sugarcane exceed this 35% threshold by a large margin (*46*).

Currently, the U.S.A. is a net importer of energy and there is a goal to be completely independent and sustainable in energy production.

In recent years, there has been a dramatic increase in interest of sweet sorghum for large-scale conventional bioethanol manufacture (*47*, *48*) especially when integrated with sugarcane (*49*). One sugarcane-sweet sorghum industrial plant is currently under construction Florida. Highlands Envirofuels company is constructing a plant (20 million gal) northwest of Lake Okeechobee, Florida, after receiving a U.S. $7 million Florida State grant in 2008 (*50*). As sugarcane factories sit idle for many months of the year, processing of sweet sorghum to syrup in sugarcane factories before or after the harvest would allow for the greater use of capital equipment. Converting sugarcane and sweet sorghum juice to syrup that can be stored is one way of making the feedstock available year-round.

The use of first generation bioethanol in the transport sector has shown rapid global growth in recent years. It is projected that the growth in its production and consumption will continue (*51*) but its contribution toward meeting the overall energy demands in the transport sector will remain limited because of (i) competition with food and fiber production for the use of arable land, particularly in vulnerable regions of the world, (ii) regionally constrained market structures, (iii) lack of well managed agricultural practices in emerging economies, (iv) high water and fertilizer requirements, and (v) a need for conservation of biodiversity. However, some countries have the natural resources to grow large amounts of first generation biofuel crops without jeopardizing food production (*52*). For example, less than 7% of current Brazilian agricultural land is needed to expand sugarcane derived ethanol for the displacement of a further 5% of projected gasoline use by 2025 (*52*).

Second Generation Bioethanol from Sugar Biomass Crops

Second generation, advanced biofuels including bioethanol and biobutanol, derived from wastes, residues, and non-food cellulosic and lignocellulosic feedstocks address some of the problems associated with first generation biofuels, e.g., the strain on world food markets, contribution to water shortages and destruction of the world's forests (*52*). Second generation ethanol production from sugar biomass crops offers advantages over first generation ethanol because (i) lignocellulosics and cellulosics are abundant and less expensive than agricultural food feedstocks, (ii) they have a lot of potential growth, and (iii) can be grown in marginal lands that often require less fertilizer and water inputs (*42*).

The different technological steps required for the sustainable production of second generation bioethanol from sugar biomass crops are illustrated in Figure 4 as well as the need for an integrated research approach. However, the processing

technology for conversion in the most part has not reached commercial scales. Currently, the production of second generation biofuels are still in the research and development or demonstration phases (*42*). Furthermore, commercialization of second generation bioethanol will depend mostly on economic factors such as values for agricultural feedstocks that have been estimated to range between 50-80% of the total ethanol's cost (*53*), government tax incentives for ethanol production, and mandatory ethanol/gas blends (*54*).

Table III. World fuel ethanol production by country in 2009. (From: F. O. Lichts World Sugar Statistics (*45*))

Country	Million cubic meters per year	Percent of world production
United States (*corn*)	**41,072**	**46.8**
Brazil (*sugarcane juice and molasses*)	**27,165**	**31.0**
China (*corn and wheat*)	**4,450**	**5.1**
India (*sugarcane molasses*)	**1,725**	**2.0**
France (*sugar beets*)	**1,850**	**2.1**
Germany (*sugar beets*)	**1,040**	**1.2**
World Total:	**87,703**	**100.0**
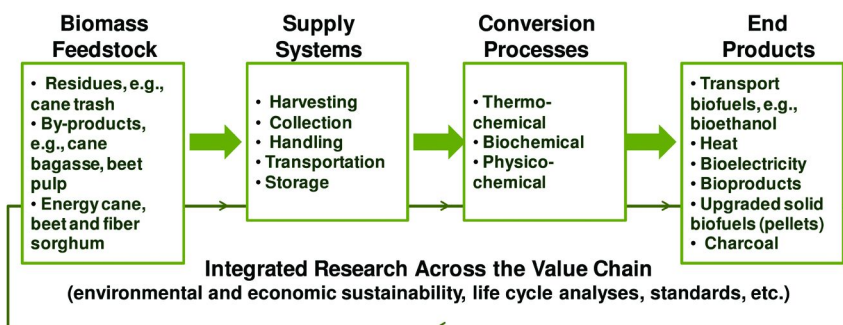
Figure 4. Sustainable biomass-based technologies for the second generation, sugar−ethanol and related industries. To achieve success, different fields of research must be integrated. Adapted from EUBIA (55).

Energy Sugar Crops for Emerging Second Generation, Cellulosic-Ethanol Industries

For truly sustainable sugar and sugar−ethanol industries, research is needed to find the most profitable, productive and responsible ways to manage the natural-resource base so that production of sugar crops can be more sustainable. New genetic lines of crops are being developed that yield well under various stress conditions and have advantageous processing characteristics (*56*). Other improved agricultural practices can also reduce dependence on pretroleum-based agricultural chemicals. Moreover, it is the close relationship among the available amount of light, water, and nitrogen inputs and the amount of plant mass that they can produce – not human demand – that will determine how much biofuel the world can produce (*57*). Conversely, as crop residues of sugarcane and sugar beet are being proposed for ethanol production and other biofuels, a delicate balance has to be struck between how much is removed for energy and how much is left on the ground to protect soil from erosion, maintain soil organisms, and store carbon in the soil.

High-fiber "energy" or "biomass" crops, sugarcane, sugar beet and fiber sorghum can be converted to second generation cellulosic fuel ethanol as well as energy and bioelectricity. Companies and government agencies in several countries are currently sponsoring research into the development of energy canes and sugar beets. Processes to convert energy canes and beets into fuel ethanol are under intense investigation (*29*, *58*). The challenge is to develop energy crops with a suite of desirable physical and chemical traits while increasing biomass yields by a factor of two or more (*59*). Only little work has been accomplished on the breeding and cultivation of sugarcane and sugar beet for increased biomass yields. Thus, the time is ripe for intensive breeding of energy cane and beet varieties.

Energy Canes

In sugarcane, more rapid genetic gain can occur for total biomass yield than for sugar yield because growth does not have to be intentionally restricted during the life cycle of the crop and a wider array of germplasm of potential value is available to the breeder once stringent standards for sucrose and fiber levels are relaxed. A few energy cane varieties have already been developed and released (*60*) for the Louisiana, U.S., sugar industry in a cooperative effort between the USDA's Agricultural Research Service's (ARS) Sugarcane Research Unit, the Louisiana State University's (LSU) Agricultural Center, and the American Sugar Cane League of the U.S.A. Inc. During the 13-year selection process for varietal development, the sugar yield potentials of candidate varieties are compared to commercial standards. Often varieties are discarded because their fiber levels exceed 16%, a level which raw sugar manufacturers consider unacceptable for processing (*48*). Some of these discarded varieties continue to be used as parents in the breeding programs conducted by ARS and LSU because of their positive attributes. Three of the high fiber sugarcane varieties (L 79-1002, HoCP 91-552, and Ho 00-961) were released for commercial planting in 2007 (*60*) produce dry

biomass yields in excess of 25 tonnes/ha. As marginal land to grow energy canes in Louisiana are mostly north and, therefore, colder during the winter, a major emphasis of the breeding program is to breed for cold tolerance.

Energy Beets

Higher biomass yields for energy beets are also possible using fodder beet germplasm as a parent in hybrids with sugar beet (*61*, *62*). Biomass yield potential is dependent upon interception of solar radiation which gives beets grown in areas with long growing seasons a decided advantage. Winter beets in the U.S. have a longer growing season and, therefore, a much higher yield potential (*42*).

In the current economic situation, most U.S. growers want beets that can be grown for either sucrose or ethanol, ensuring flexibility. Sugar beet pulp and molasses are also potentially excellent feedstocks for ethanol (*42*). It makes economic sense to co-locate ethanol plants or at least enzymatic digestion facilities next to sugar beet factories where the pulp is produced. As with all potential feedstocks, economics will determine the feasibility of developing the sugar beet crop as an ethanol feedstock (*63*).

Future Platform Chemicals from Sugar Industry Biomass

Novozymes CEO Steen Riisgaard recently said "in a few years sugar [crops] will be the new oil" as sugar is a superb feedstock for the production of platform chemicals for the manufacture of a range of end-products, e.g., bioplastics, industrial solvents, and chemicals (*3*). Efforts in "green chemistry" have been ramped up to transform crop biomass, e.g., from sugar crops, into the basic chemical ingredients that go into many everyday products (*64*). One of the major bottlenecks to using cellulosic biomass has been the depolymerization step. Low, moderate (~500 °C) and high temperatures (gasification temperatures) are being studed to convert biomass, but it is still too early to say which ones will be the most useful (*64*). Although there is no current, effective one-step method for converting raw lignocellulose to finished products, progress is being made. The firm KiOR (Pasadena, TX) recently demonstrated a one-step procedure for transforming cellulose into 5-hydroxymethylfurfural (HMF), which is a versatile biomass "platform" chemical for the production of solvents, fuels, and monomers for polymer production (*64*). Furthermore, increasing investments in the sugar−ethanol industry could facilitate the contruction of the physical infrastructure, and associated technologies that could also be used for the production of bioproducts (*3*). Biotechnology processes are particularly suited for the transformation of natural feedstock from sugar crops into the necessary sugars and building blocks of secondary bioproducts, and bioethanol itself can also be used as a platform chemical (*3*).

However, at the moment there are few budding entrepreneurs in the sugar industry taking advantage of the advances in process conversion technologies driving the biobased products sector (*3*).

Overall Future Outlook

In many areas of the world, particularly in Europe, there is currently a rapid diversification of the sugar and sugar−ethanol industries into "sugar processing industries" that are deeply involved in the maximization of sugarcane and sugar beet biomass (*65*), and more areas are expected to diversify for sustainability in the future. Furthermore, it is expected that "sugar" and "sugar−ethanol" companies, just like many other chemical companies, will be more and more eager to become greener (*66*) as they realize that they can reduce pollution and increase profits simultaneously (*67*). Companies will want to be able to select greener starting materials and use cleaner chemical processes to make environmentally preferred products (*66*).

Sustainability of the sugar and sugar ethanol industries should be viewed as a continuous improvement journey (*1*). Behavior change and education will be linchpins in effective sustainability programs. Traditionally, chemical/food process development has focused on economic criteria, but additional criteria for sustainability have become and will continue to be increasingly important and integrated into decision making processes (*68*). Assessment tools, standards, and enhanced metrics to measure "green, greener, or greenest" are being developed to achieve this tool in the U.S. (*66*). Ecological or environmental sustainability, one of the three pillars of sustainability (Figure 2) can be examined using Life Cycle Assessment (LCA) (*66*). This can be applied to new processes for converting sugar biomass. Furthermore, for sustainability of the sugar and sugar−ethanol industries, there will be a need for new analytical methods and standards in ethanol manufacture and for areas of grower payment with new biomass crops.

Acknowledgments

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Chapter 2

Measuring and Monitoring Sustainability in the Sugar and Sugar−Ethanol Industry

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Methods for assessing and monitoring sustainability are the focus of a number of new initiatives worldwide. The production of bioethanol and the prospect of its importation into the EU have lead to various initiatives to ensure that only biofuels which are produced in a sustainable way are acceptable. In general many companies and organizations are becoming aware of the need for sustainable production. Methods of measuring and monitoring sustainability are in various stages of development. The process of developing measurements and incorporation into accepted standards is illustrated by the development of the Better Sugarcane Initiative standards, to be applied specifically to the cane sugar industry for sugar and ethanol production. The major issues and problems surrounding measurement standards are highlighted. Progress in the sugar industry is briefly discussed.

Introduction

There is increasingly wide acceptance of the fact that all agricultural and industrial enterprises need to operate in a manner in which not just the economic but also the social and environmental factors are promoted. At the same time energy and water use, production efficiency, elimination of wastage, a range of social and labor issues and the effect on global climate change are all being more carefully monitored.

There is a growing corporate move to address sustainable development. Companies are beginning to appreciate that there are sound business reasons to adopt more sustainable production and processing practices. Further evidence

of the importance of sustainability is contained in sustainability reports being prepared by more and more companies, including 8 out of the 10 chemical companies in the Fortune Global 500 list (*1*). Managing social and environmental risks is important for growers, processors, traders and food companies due to regulatory pressures as well as shareholder and consumer expectations. Increasingly environmental and social performance is affecting access to markets and to capital as well.

The pressure for a system to certify that sustainable practices are being adhered to has come largely from the market place. A number of large industrial consumers of sugar want to be able to certify that sugar and other ingredients in their products are produced by means of sustainable practices. This initiative has been given additional momentum with biofuels, where for instance the import of biofuels into Europe requires that these fuels are produced following sustainable practices. The discussion on sugarcane ethanol has largely centered on conditions in Brazil (*2*, *3*). Several initiatives are being developed in Europe and the United States relating to certification for sustainable production of biofuels. A multi-stakeholder initiative, the Roundtable on Sustainable Biofuels is well advanced in developing guidelines for sustainable biofuel production.

However it is not only the consumers that are the driver for measuring sustainability. Society at large realizes the responsibility it has to the greater welfare of the planet. Many people and organizations see sustainable development and the urgent need to move to a low carbon economy as the most significant issue facing society today.

The sugar industries have made significant progress over the last decade, particularly in improving their efficiency of production and their environmental performance. Although some progress has been made, in the sugar industry, there is still considerable room for improvement. There is now a need for the sugar industry to be able to show that it undertakes its activities in a sustainable way; this requires a system which can be applied to sugar production to measure its alignment with sustainable practices.

Sustainability

There are various ways in which sustainability can be defined. A generally accepted definition would be along the lines of sustainable development providing for human needs without compromising the ability of future generations to meet their needs. Savage (*4*) elaborated on the difficulties associated with a precise definition of sustainability e.g. how are needs defined, and what are appropriate standards, now and in the future. The ACS defines sustainability as the balancing of economic, environmental and societal performance of industry for generations to come. The American Institute of Chemical Engineers definition is "the path of continuous improvement, wherein the products and services required by society are delivered with progressively less impacts upon the earth" (*1*). They have devised a Sustainability Index for organizations, composed of seven critical elements:

- strategic commitment to sustainability
- safety performance
- environmental performance
- social responsibility
- product stewardship
- value-chain management
- innovation

The impact of industry on sustainability is sometimes summarized in the "triple bottom line", covering the three components of environmental responsibility, economic return (wealth creation), and social development. Many companies now recognize and monitor these three parallel strands, using their assessment to guide their product, process and personnel development and to secure their position in the rapidly changing climate of environmental legislation and stakeholder concerns.

Development of Sustainability Measures/Standards

Lifecycle assessment is often used as a framework for comparing two or more options. This considers all of the environmental impacts associated with every step of every process involved in manufacturing, using and disposing of a product. The results of these exercises often lead to conclusions that are counter-intuitive. They are also very sensitive to assumptions about the system boundaries and the functional unit.

Lifecycle analysis is often used in establishing a carbon footprint, i.e. greenhouse gas emissions associated with a product or activity. It can also be applied to the use of resources such as water and raw materials.

Various standard systems for reporting on sustainability issues have been devised.   Of particular interest are the Sustainability Reporting Guidelines published by the Global Reporting Initiative (*5*). They require some standard disclosures relating to an organization's profile, strategy, management approach, governance and engagement with stakeholders. In addition they require reporting on a set of performance indicators, split into the three categories of economic, environmental and social issues. Within each category some core indicators must be reported on, which are generally expected to be applicable and material for most organizations, and some additional indicators which should be reported on as appropriate.

Various studies on the net energy value of ethanol from corn have been compared by Farrell and co-workers at UC Berkeley (*6*). Their EBAMM (ERG Biofuels Analysis Meta-Model) spreadsheets are available on the internet. A number of other carbon calculators are available on the internet, mostly designed for the production of biofuels, which also take into account the distribution and use of the biofuels. The Renewable Fuels Agency in the UK provides an on-line calculator, which has been used by sugar companies in the UK, as does the GREET (Greenhouse Gases, Regulated Emissions and Energy Use in Transport)

model produced by the Argonne National Laboratory in the US (*3*). This list is not exhaustive and various other calculators are available from specialist consultants.

Environmental and social concerns have been the main reason for the requests for the inclusion of sustainability criteria in the international trade of biofuels. The most important issues seem to be the GHG (greenhouse gas) emissions savings, sustainable agricultural practices, protecting biodiversity and ecosystem services, and labor practices. The economic sustainability is sometimes overlooked, but is equally important. Improving business and technical efficiencies inevitably also benefits the people and the environment, and needs to be an integral part of any sustainability exercise.

Various organizations have been active in developing sustainability standards for the production of biofuels, from all feedstocks including ethanol from cane. The Better Sugarcane Initiative (BSI) has set about developing standards to evaluate sustainability specifically for the sugar industry, whether its products are sugar, ethanol, power export or any other by-products. The process used in developing measurement standards is most important if widespread acceptability and credibility is to be achieved.

All countries have their own sets of regulations and laws governing environmental and social issues. Internationally recognized standards may be seen as a prescription by one country or customs union of the standards that a supplying country must meet as a condition for access to their markets. In some respects it levels the playing fields amongst producers, e.g. developed nations presently consider that they have to meet harsher environmental and labor standards than some of the developing world's standards. Others may question whether linking such standards to trade is motivated by altruism or protectionism.

It is for this reason that any certification system must be developed in an entirely transparent way, involving a multi-stakeholder process. Only then can it be claimed that the system of certification is not open to abuse.

It is also necessary at the outset to decide on how the standards are to be implemented. Options include a benchmark for self-assessment, trade guidelines, rules for procurement, a reporting obligation (as for instance in implementation of the UK Renewable Transport Fuels Obligation), or a certification scheme with third party certification, which may be either a business-to-business standard or a consumer label.

The Processes Involved

The first step is the establishment of Principles, which are universal statements about sustainability and define the objectives. From the Principles, flow the Criteria and Indicators. Criteria are the conditions to be met in order to adhere to a Principle. Indicators are measurable states that indicate whether or not associated criteria are being met. This is shown schematically in Figure 1.

The process of developing standards and indicators must be entirely transparent and inclusive. This is vital if the standards developed are to have international credibility. In this respect it is necessary to engage widely with the
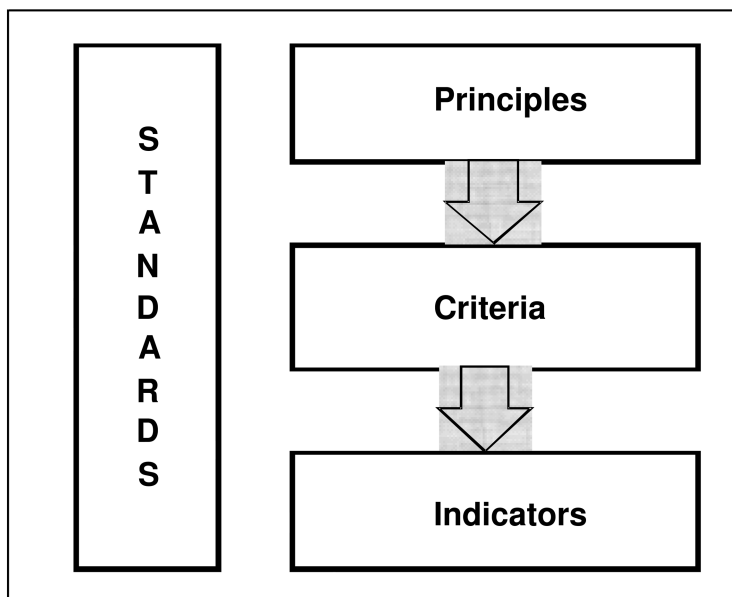
Figure 1. Nomenclature used in standards.

stakeholders in all spheres of operation and to encourage participation through comments, suggestions and input of any kind.

The International Social and Environmental Accreditation and Labeling (ISEAL) Alliance has developed a Code of Good Practice for Setting Social and Environmental Standards to evaluate and strengthen voluntary standards, and to demonstrate their credibility on the basis of how they are developed (www.isealalliance.org). Adhering to procedures that constitute good practices for setting standards ensures that the application of the standard results in measurable progress towards social and environmental objectives, without creating unnecessary hurdles to international trade.

Following the ISEAL code, the following steps *inter alia* are envisaged:

- Documented procedures for the process under which the standard is developed shall form the basis of the activities of BSI. These procedures are developed with the involvement of a balance of interested parties.
- Allowance will be made for a complaints resolution mechanism for the impartial handling of any procedural complaints. All interested parties must have access to this complaints resolution mechanism.
- A public review phase in the development of the standard is necessary, and shall include at least two rounds of comment submissions by interested parties. The first round shall include a period of at least 60 days for the submission of comments, and the second period at least 30 days.
- All comments must be recorded and a synopsis of how they have been dealt with must be available to the public.

- Final international standards will be placed in the public domain. ISEAL dictate that, with the exception of reasonable administrative costs, they must be made freely available in electronic format.
- Standards will be reviewed on a periodic basis for continued relevance and effectiveness in meeting their objectives and periodically revised as necessary. A review process must occur at least every five years.

The process of establishing standards and indicators is iterative, as shown schematically in Figure 2.

ISEAL suggest that in order for standards to be mutually consistent and free from contradiction for the largest number of user communities, standard-setting organizations should pursue harmonization of standards and/or technical equivalence agreement between standards. Generation of multiple standards can lead to confusion and constitute a reason or excuse to postpone commitment to better standards. In the case of sugar enterprises, this requires that the standards should as far as possible be consistent with the schemes proposed for biofuels.

It is also important to ensure that participation reflects a balance of interests in the subject matter and in the geographic scope. Thus an international standard requires input from stakeholders in all significant sugar producing areas. This is necessary in spite of the fact that the efficiency and speed at which decisions can be made may be negatively affected by the diversity of stakeholder engagement in the decision making.

Stakeholders and Their Roles

Stakeholders are individuals or groups with an interest in the initiative succeeding in its objectives, as well as those external to the organization e.g. communities.   They include producers, traders, retailers, consumers, trade unions, social NGOs (Non-Governmental Organizations), environmental NGOs, indigenous groups, government, researchers and academics and certification bodies.   All stakeholders need to be encouraged to participate in agreeing measurement standards, and they need to see that their contribution is able to influence the final outcomes. It is inevitable that conflicting views exist among different stakeholders, and the standard setting organization has to be able to explain how it balanced these in reaching the final standards.

In the early stages, some producers seem to be unwilling to commit the time and resources when they are uncertain of the eventual outcome.

Governments have thus far been little involved in these voluntary standard setting initiatives, with the exception of the initiatives on sustainable biofuels. However the existence of viable standards could assist governments in making better choices on policy options.

In general the NGOs are most supportive of standard setting and have an important role to play. They bring different insights to the process, often helping to raise the bar when standards are being established. However a minority of NGOs seem to want to undermine standard setting procedures. This is unfortunate, since
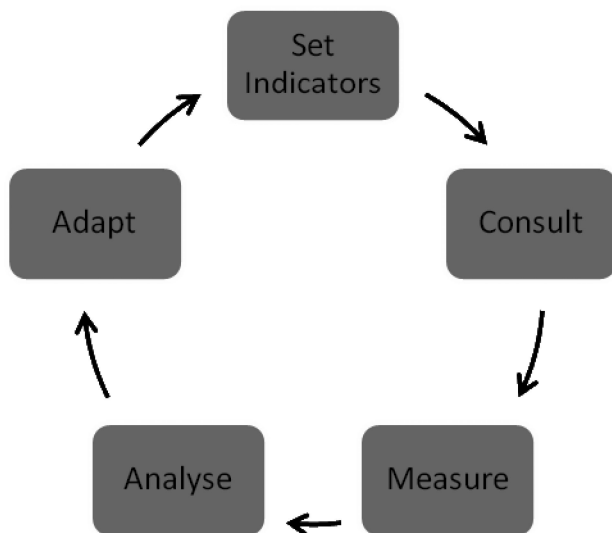
Figure 2. Process of establishing standard indicators.

NGOs can substantially assist business and consumers in providing guidance in the furthering of sustainable practices.

The WWF were particularly responsible for initiating, promoting and sponsoring of the establishment of sustainability standards for sugarcane.

Better Sugarcane Initiative

The Better Sugarcane Initiative (BSI) is a collaboration of sugar retailers, investors, traders, producers and NGOs who are committed to sustainable sugar production by establishing principles and criteria that can be applied in the sugarcane growing regions of the world. With the initial guidance and support of the WWF, the BSI has embarked on an exercise to promote measureable standards in the key environmental and social impacts of sugarcane production and primary processing while recognizing the need for economic viability. The BSI is funded by members, among whom are consumer companies (e.g. Coca Cola, Cadbury Schweppes), commodity traders (e.g. ED & F Man, Cargill), NGOs (e.g. WWF, Solidaridad), producers (e.g. Cosan, EID Parry), producer associations (e.g. UNICA, ASSOCANA) and oil companies (e.g. Shell, BP). The BSI web site explains its activities in more detail (www.bettersugarcane.org).

The BSI aims to reduce the impact of cane sugar production on the environment in measurable ways, while also contributing to social and economic benefits for sugar growers and all others concerned with the sugar supply chain. The goal of the BSI is to reduce farm and other sugar processing impacts, while increasing sugar's competitiveness in markets that are becoming increasingly competitive.

The Principles and Criteria for BSI have been drawn up, discussed, modified and finally accepted by the BSI members. The Principles accepted are:

- Obey the Law
- Respect human rights and labor standards
- Manage input, production and processing efficiencies to enhance sustainability
- Actively manage biodiversity and ecosystem services
- Commit to continuous improvement in key areas of the business

The sugar industry is well-placed as an agro-industrial business. Sugarcane is a particularly efficient crop in terms of its photosynthetic capacity to produce biomass, it contains a fibrous structure which provides a renewable fuel resource, and processing of the cane does not involve the use of any toxic or hazardous products or waste streams. Sugarcane produces more biomass dry matter per hectare than any other crop species. It can, therefore, have a strong positive influence on the environment and so has a great future in providing food and/or energy in a sustainable way.

The ISEAL Alliance comments as follows on standards: "A good standard is equally applicable anywhere within its geographic scope and focuses on achieving outcomes rather than prescribing methods for reaching these outcomes". It is for this reason that the BSI has attempted to set indicators which measure outcomes, the impacts of their activities, rather than recording the existence of good practices. It is hoped that the values of the indicators will be universally applicable, with a minimum of regional variation required by local circumstances.

It is important to differentiate between the Standards and Best Management Practices (BMPs). BMPs are a means to an end and not an end in itself. BMPs have been drawn up in many parts of the sugarcane world, which are valuable and useful, but they do not identify the impact on the environment of the activity considered. They will also be different in different cane growing areas. In addition, today's BMPs are likely to be superseded by tomorrow's better ones. It has been suggested that the term BMP should therefore refer to Better Management Practices (7). ISO 14001 standards are also available to guide sustainable practices, but focus on organizational processes and not products or impacts.

Best management practices have been drawn up for use in a number of sugar producing countries, including Australia and South Africa. These are important initiatives and have an important part to play in ensuring sustainability. Their adoption is likely to result in sustainable sugar production in the countries where they have been developed.

BSI has chosen to use in its standards measurable indicators. Great importance is attached to devising metrics, numbers that can be put to each of the indicators. It is assumed that credibility comes with metrics; without metrics, certification programs can become subjective rather than science-based. However choosing the appropriate metrics is not simple. The metrics employed may vary radically in the degree to which they capture the full character of an individual effect. Some effects are intrinsically more readily quantifiable than others (e.g. particulate emissions vs. aesthetic landscape effects). This is most difficult in the area of social issues.

BSI established expert groups with relevant expertise to identify standards that can be measured. Three Technical Working Groups (TWGs) covered the three areas of (1) social and labor issues, (2) processing/factory issues and (3) agronomic practices. The membership of the TWGs covered most of the important sugarcane producing areas and the task of putting together the standards and indicators required has been completed.

BSI has been incorporated as a not-for-profit company in the UK, and has drawn up procedures for good governance. In addition, Articles of Association have been established, which allow for open membership, subject to approval by a Supervisory Board.

Major Sustainability Issues

One of the key challenges of sustainable development is that it demands new and innovative choices and ways of thinking. The boundary of the sugar producing organization should encompass both growing and processing activities, but must also make allowances for the production of energy and biofuels, and in the longer term, effective use of sugarcane biomass.

The major issues which standards and certification systems do not address are the indirect land use effects, namely the displacement of agriculture into other areas and macro-effects such as rising food prices. Indirect land use change continues to be an area of concern, and will be for some time because of the difficulty in measuring its effects. The major product from sugarcane is still a food product, sugar. Expansion in Brazil to produce increased quantities of ethanol from sugarcane has at the same time resulted in increased quantities of sugar. Thus the food security issue is somewhat different in the case of sugarcane. Klenk and Kunz (8) have shown that in the case of ethanol production from sugar beet and wheat, the co-products replace other feedstuffs which would have required additional land, and so actually free up land for other crop production.

Biodiversity and High Conservation Value areas are also among the main concerns of many stakeholders. Some disagreement on what constitutes such areas and how they should be measured still exists. These are natural habitats where conservation or biodiversity values are considered to be of outstanding significance or critical importance. In addition, some standards require that crops must not be obtained from land with a high carbon stock, including wetlands, continuous forest, highly diverse grasslands and peat lands. This generally excludes what has historically been in use as croplands, and applies to land changed to cropland after a cut-off date.

The aspect of sustainability standards which perhaps attracts the most attention is the greenhouse gas (GHG) emissions. This is derived together with estimates of energy used. In this respect both direct effects and indirect effects need to be taken into account. The latter include the energy required for the production of chemicals, fertilizers and other materials used, emissions from land use change, and the additional energy necessary for the manufacture and construction of farm, transport and industrial equipment and buildings. Direct land use change has to be taken into account, but indirect land change is generally

excluded, largely because the effects of these are difficult to estimate and subject to too much uncertainty.

The results of GHG emission calculations are subject to some uncertainty, depending on how co-products are handled, how emissions from fertilizers are handled and on which items are included in indirect effect accounting. In the case of sugarcane, burning of the cane before harvesting has a significant effect on emissions and also has to be accounted for. The results of these calculations show clearly that the best way to reduce the overall emissions is to cogenerate and export power (*9*). The use of bagasse in sugarcane factories, or other biomass fuel in the case of sugar beet and cane sugar refining, also has a substantial beneficial effect on the emissions.

A concern expressed by producers is that a need to meet standards will impose reporting and measurement demands which soak up manpower, time and money. For there to be buy-in by sugar producers, there must be some benefits in adopting standards. These are likely to include:

- A means of self-assessment and performance improvement demonstration.
- A means of benchmarking against others.
- Some credits as a premium for producing sugar sustainably.
- Alternatively a way of by-passing trade barriers.
- For industries already meeting the conditions, a leveling of the playing fields in terms of meeting environmental and labor related issues.
- Management of risk and liability
- Enhancement of brand image and reputation

In the long run it is expected that conforming to such standards will save money, as inputs such as energy and raw material are used more efficiently, losses and wastage are minimized and manpower is used more productively. It is certainly one of the objectives of BSI to achieve a system of standards which result in benefits to producers which outweigh any costs.

BSI Indicators

Some guidance in the metric indicators to be used was obtained from the Institution of Chemical Engineers sustainability metrics (*10*) and the Global Reporting Initiative (*5*). Some indicators have numerical values, often in the form of ratios. Ratio indicators can be chosen to provide a measure of impact independent of the scale of operation, or to weigh cost against benefit, and in general facilitate comparison between different operations. Others are just yes/no responses e.g. compliance with ILO labor requirements, compliance with local and international laws, clear title to land.

The requirements for selecting sustainability metrics are:

1. Clear definition of what is to be assessed, and why.
2. Available data – quantifiable empirical data, not qualitative judgments.

3. Coverage – inclusion of key aspects.
4. Avoidance of duplication and needless complexity.
5. Materiality – impacts requiring active management.
6. Use of composite metrics where appropriate
7. Ability to be audited by a third party

The goal is to achieve inclusion with a minimum number of criteria and indicators. The current BSI standards include 21 criteria and a relatively small but focused number of 49 indicators.

An advantage of the use of metrics is that they can be used as a means of assessing ongoing improvement, by monitoring how the values of the metrics change over time. It also facilitates comparisons and benchmarking with other producers. Setting baseline values represents an on-going challenge. It is not intended to be an "elitist" initiative intended to discriminate against certain industries. The standards should not be "best achievable" but true reflections of what experts define as a minimum acceptable level that can realistically be achieved by responsible operators. Baseline values will be set following further experience with application of the standards in the sugarcane industries in a number of different regions of the world.

The BSI standards have been posted on a specially designed web site to elicit comments (www.bettersugarcane.com) for two periods of public consultation, as ISEAL guidelines require. Behind the standards is a document which is necessary to explain the terms, and specific methods used to gather, analyze and present the data, particularly for the use of auditors. The standard is intended to be an auditable document according to ISO 65 and not only a reporting framework

The Sustainability Reporting Guidelines proposed by the Global Reporting Initiative (*5*) suggest the adoption in the first instance of Core Indicators, which are the most important and material for most organizations. Additional Indicators are proposed for later inclusion and adoption. An approach based on "major" and "minor" indicators was considered by BSI, but not adopted in the first instance. It is possible however that additional indicators will appear in time, as global conditions and expectations change.

Implementation of Standards

Once the standards are approved for use, the issue of conformity assessment needs to be addressed. At the lowest level, a company can undertake its own assessment against the standards, in order to assess compliance and if necessary to identify areas for change. In most cases it is assumed that third party certification will be necessary, particularly if a certification scheme is instituted which bestows additional value on the certified product. This requires verification by an assessor or inspector, certification as a result of the assessment, and accreditation based on the demonstrated competence of the certification body. It is anticipated that the course of independent third party audit adopted by most other roundtables will be followed by BSI as well.

Several initiatives are being developed in Europe and the United States related to certification, traceability and definition of standards for sustainable production of biofuels. For example, the European Commission has launched its Biofuels Directive establishing a legal basis for blending biofuels and fossil fuels. The BSI standards are a feedstock specific set of standards, which it is hoped will find acceptance as a recognized sustainability standard where ethanol from sugarcane is traded.

Branding or labeling can be used to generate income, which it is hoped could cover the cost of accreditation, the on-going costs of the standard setting body, and still return money to producers, to provide incentives for them to cover the cost of improved performance. This route also requires the setting up of a system of traceability or chain of custody standards and registering and protecting the certification mark. A further requirement would be procedures in place that guarantee audit and certification quality.

Progress in Monitoring Sustainability in the Sugar Industry

In the sugarcane industry, Brazil has been the most active in embracing and reporting sustainability performance. This is largely due to the need to meet sustainable standards in producing biofuels for export to first world countries. In the absence of agreed standards for sugarcane, a number of factories are reporting their results based on the Sustainability Reporting Guidelines proposed by the Global Reporting Initiative (*5*).

Some companies are now using the Fairtrade label on their products, assuring that production has followed generally acceptable practices. Otherwise the major activity has been in assessing the GHG emissions (or carbon footprint) associated with sugar. Tate & Lyle report a figure for cane sugar of 0.5 g CO_2eq / g sugar, taking into account refining, packing and transport, and recycling and disposing of packaging waste (*11*). The growing and milling activities only are responsible for 0.2 g CO_2eq / g sugar. The figure estimated by Tate & Lyle for beet sugar in the same study is almost 1 g CO_2eq / g sugar.

British Sugar used the procedure of PAS 2050 (*12*) to arrive at a figure of 0.6 g CO_2eq / g sugar (*13*). However this is the B2B figure, as provided to the industrial user. Use of cogeneration in the manufacture of ethanol from wheat or sugar beet particularly in combination with a gas-fired turbine can significantly improve energy and emission improvements relative to gasoline (*14*). This is put to good use in British Sugar's operations. Tate & Lyle report that the carbon footprint of sugar produced at Thames refinery will reduce by 25 % when new biomass boilers are commissioned.

Florida Crystals market "carbon-free" sugar, achieved through the cogeneration and sale of electric power. Their power generation facility can produce 80 MW from 103 bar steam, using the factory bagasse as well as 900 000 tonnes of wood waste/year diverted from landfills as the fuel source. Nordic Sugar have reported an emission of 0.675 g CO_2eq/g sugar.

More work has been done on emissions from the production of ethanol than from sugar. For instance the EU RED (Renewable Energy Directive) default

values for field to wheel ethanol from sugarcane are 24 g CO_2eq/MJ and 40 g CO_2eq/MJ for ethanol from sugar beet. The difference reflects to a large extent the use of sugarcane bagasse as fuel for the boilers.

Conclusions

A means of measuring and monitoring sustainable production of sugar is being driven by a number of factors, including legislative requirements, investor expectations, consumer / market advantage and reputation and brand image.

The sugar industry has an obligation to run its activities in a sustainable way. This is an obligation to society as well as to its consumers and clients. The BSI aims to involve the sugarcane industry in setting reasonable standards for sustainable operation, leading to realistic, practical and achievable standards. This should assist in the management of the triple bottom line components of environmental responsibility, economic return and social development.

It is intended that sustainability standards such as those being developed by BSI will find international acceptance. It is anticipated that most significant sugar producers already produce sugar in a way that will meet most of the standards. Metric standards provide a useful means of assessing progress and improvements. It is already evident that awareness of sustainability issues is influencing business decisions, to the benefit of the environment and sustainable production into the future.

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Chapter 3

Major Challenges and Changes in the European Sugar Sector

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Over the last five years a number of changes have taken place within the European Sugar Sector, mostly driven by the reform of the European Sugar Regime. This Regime had been in place since 1968 and was designed to "maintain employment and standards of living for EU growers of sugar beet" by making the continent self sufficient in sugar production. This book chapter highlights the changes that have taken place to the EU Sugar Regime and how the Sugar Industry within Europe has altered to meet the new requirements. Sugar beet growers and processors are examining alternative strategies, resulting in new R&D initiatives, to ensure the stability and continuation of the industry in the future. These have included biofuel production, greater power generation involving Combined Heat and Power (CHP) plants, alternative fuel sources, product diversification, and refining of imported cane raw sugars. These initiatives will illustrate what a European sugar producer could be producing and using in the near future.

Introduction

The European Sugar Regime was established in 1968 where it regulated the sugar sector of the original six members of the European Economic Community (EEC). As part of the Common Agricultural Policy (within Europe) it was designed to make the continent self-sufficient in food production from the 1980s onwards (*1*). To meet this goal it was important to ensure that the growers received enough money for their goods to make it profitable and maintain employment and standards of living. In 1973, the EEC expanded to nine member states with

the addition of Denmark, Ireland, and the UK. This change also brought with it a history of raw cane sugar refining with the UK's longstanding agreement to import sugar from African, Caribbean, and Pacific (ACP) countries (*2*). This volume of raw sugar was taken into account in setting the UK quota.

The Sugar Regime introduced a minimum price that had to be paid for sugar beet and also a minimum price that the sugar producer would receive for the sugar. Additionally import tariffs were set which limited the amount of imported sugar into the EEC and any producer selling surplus sugar onto the world market received a refund to compensate for the difference between the world market and EEC price for sugar. To make this all work each country received a production quota which was aimed at ensuring national and group needs were met.

The Sugar Regime was seen as a stable, self-financing system where the levies paid by the producers compensated for the export refunds, hence it survived without much change for many years. During that time other parts of the Common Agricultural Policy were reformed to address areas of overproduction. Sugar users however were not happy with the arrangement and complained that the difference between the world market price for sugar and that paid by them within Europe was too great. Sugar producing nations outside of Europe were also concerned that the Sugar Regime restricted their ability to sell their sugar into the European market. Despite these pressures the Sugar Regime remained in place largely unchanged for many years until pressure from within the EU for reform, coupled with pressure from the World Trade Organization (WTO), convinced the European Commission to reform it.

The reform of the European Sugar Regime has driven a lot of changes within the EU sugar industry including major restructuring. Many of the changes and initiatives driven by the reform had been ongoing for some years, e.g., improved process efficiency, but the reforms prompted acceleration of these and developments in other areas, such as product diversification, which will be discussed in this book chapter.

Reform of the European Sugar Regime

At the Doha meeting of the WTO in 2001 the EU agreed to limits on export and this started the process of reform for the Sugar Regime. Additionally, in a bid to improve aid to developing countries, the Everything-But-Arms agreement came into being where the 46 Least Developed Countries (LDCs) were given free access to EU markets, i.e., tariffs were eliminated for almost all imports, for all their goods except for arms (*1*). Many of these LDC countries are sugar producing nations and so this presented a new opportunity to them. The full effects of this are still to be seen as it only fully came into force from 1 October 2009.

In 2003, the WTO ruled that the EU needed to reduce it's import tariffs. This resulted in the phasing out of the export of some 5.1 million tonnes of sugar. In 2005, the EU agreed on a reform package of the Sugar Regime which started in the 2006/2007 sugar beet campaign. Rather than just reduce national quotas across the whole of the EU, and also in an attempt to maintain the more efficient production areas in Europe, a set of changes were implemented. These Sugar Regime changes

included: (i) 36% cut on the Sugar Reference Price spread over 4 years, (ii) 40% cut in the price of sugar beet raw material, (iii) quotas cut by 24%, (iv) exports cut from ~6 million tonnes of sugar to 1 million tonnes, and (v) substantial increases in imports. These changes have meant that the EU has changed from being the second largest sugar exporter in the world to being the second largest importer in just 3 years from 2006/2007. These dramatic changes are illustrated in Figure 1 and listed in Table I.

From Table I it can be seen that the stock of sugar in Europe has reduced by 70% over the last 4 years, and in the same period the quotas received by the member states have reduced from a total of 17.4 to 13.3 million tonnes. Imports have increased by 85% and are likely to increase further (Table I). Consumption is expected to be relatively stable, growing slightly if anything, and most importantly the amount of export has reduced dramatically by approximately 86% (Table I).

An estimated 140,000 growers (or 45% of the total) have stopped growing sugar beet since 2004 and the area sown has been reduced by more than 30%. One hundred and forty seven factories have closed since 2000 with subsequent employee redundancies. Five member states of the EU have closed their industries and a further six have cut back production by over 40%. This has left eighteen sugar producing countries in Europe with 70% of the sugar produced in seven member states. Importantly 20-25% of the sugar consumed in the EU is imported.
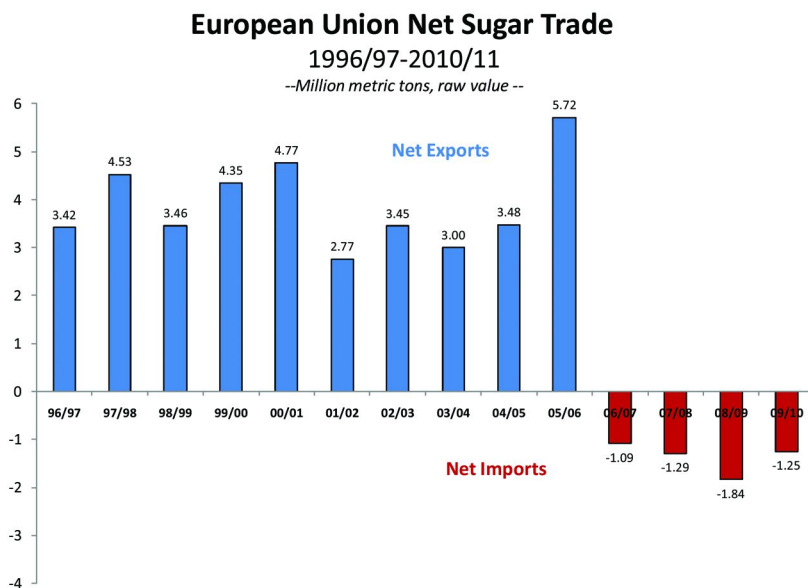

Figure 1. European Union net sugar trade from 1996 to 2011. Source: USDA (4). 2010/2011 data is projected. (see color insert)

Table I. European Union Beet Sugar Market (All Figures in Million Tonnes).

	Pre-Reform (2005/06, EU 25)	*Reform* (2009/10, EU 27)*	*Post-Reform (2014/15, EU 27)*
Beginning stocks (1 July)	6.2	1.8 (-70%)	n.a.
Production quota	17.4*	13.3* (-24%)	13-14#
Imports	2.1	3.9 (+85%)	4.5-5.5
Consumption	16	16.6	17-18
Exports	8.1	1.1 (-86%)	1

* These figures are limited to quota sugar and do not include out-of-quote sugar or other uses of sugar beet not covered by the CMO Regulation nor raw cane sugar refining. Exports and imports are for sugar as such (quota and out-of-quota sugar). Several sources including figures from Sugar Management Committees of 07.10.08 and 02.26.09 and presentations by DG Agri at CIBE and CEFS Congresses in June 2009.   # European Commission (DG Agriculture) Forecasts made in July 2009.
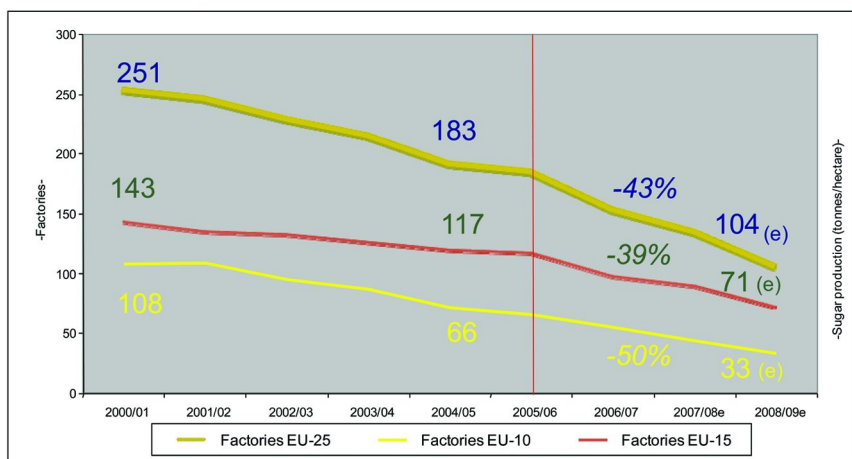
Figure 2. EU sugar industry rationalization. Factory closures have also led to a focus of production in those factories which are more efficient in extracting sugar. Both agronomic and factory improvements, therefore, are in the same direction. Source: CEFS Statistics (3). 2008/2009 values are shown as estimates (e) (see color insert)

Sustainability of the European Sugar Sector in the Face of Reform

Factory Rationalization

To meet the challenges posed by the changes to the EU Sugar Regime the European sugar producing industry has been through a period of rationalization, both in terms of the number of operating companies and also in the number of manufacturing sites. In 2000 there were 251 sugar factories in the 25 member states of the EU and that number was expected to be down to 104 in 2009. Over the same time frame the sugar yield in the field has increased from 7.9 tonnes per hectare to an estimate of 11 tonnes. The chart in Figure 2 illustrates the change in factory numbers throughout the EU member states.

European sugar companies have achieved reducing the number of factories by closing smaller factories and thus increased the average size so that economies of scale can be realized, and the crop processed more efficiently and economically in those that remain. For instance an automated control system, which the factory needs to control its process, costs the same to install, maintain, and operate regardless of whether the factory can slice 4000 or 20000 tonnes of sugar beet per day. Figure 3 shows the volume of sugar beet those factories have processed during the same time period.
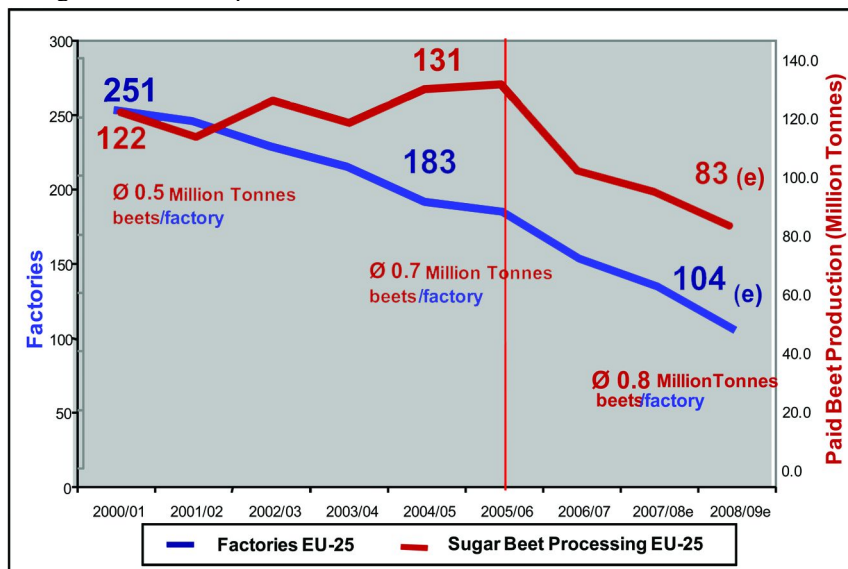

Figure 3. Weight of sugar beet processed by individual European beet sugar factories pre and post EU Sugar Regime changes. This figure is a mere average of the EU that confirms a known trend but is not necessarily representative of the actual average or median size of a beet factory in the EU. Actual figures will vary strongly from factory to factory. Average distance from farm to factory is 40 km in the EU. Source: CEFS statisticsand CEFS/CIBE Sustainability Leaflet (3). (see color insert)

From Figure 3 it can also be seen that the amount of beet processed per factory has changed from approximately 500,000 tonnes to 800,000 tonnes per campaign year with factories in continental Europe increasing their operational period from around 80 to 100 days (on average). Prolonging the campaign decreased the capital costs per kg of sugar produced. Wissington Sugar Factory in the UK (owned by British Sugar plc) finished the 2009/10 campaign after slicing over 3 million tonnes of sugar beet and producing 530 thousand tonnes of white sugar equivalent (note some of this sugar is stored as an intermediate syrup for refining later in the same year).

Agronomic Improvements

With the reduction in the price paid for sugar beet caused by the change in the EU Sugar Regime, it is vital that the yield per hectare increases and the cost of production also decreases for the grower. Figure 4 shows how the yield per hectare in France has consistently increased since 1977. The downward spike in 2001 was due to the anomalous weather during the growing season. Figure 4 also shows how the use of nitrogen fertilizer has been reduced over the same time period from approximately 150 kg per hectare to less than 80. These advancements are both welcome and necessary for the EU growers to continue to maintain sugar beet production.
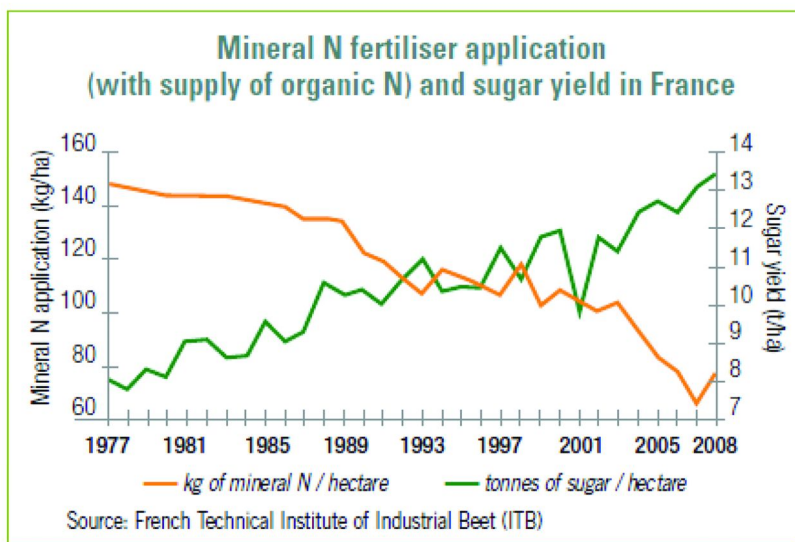

Figure 4. Changes in mineral nitrogen fertilizer application and sugar yields from sugar beet production in France from 1977 to 2008. (see color insert)

At the same time the seed breeders have been developing new sugar beet varieties with ever increasing sucrose content, lower non-sucrose composition, and better disease resistance. Beet quality is a key driver for process efficiency and improved yields within a sugar beet factory. Higher beet quality can result in lower use of processing aids to produce the same quality white sugar. As the sugar beet yield increases the grower requires less area of land to produce the same amount of sugar and, therefore, has the option of producing other crops or finding alternative outlets for the excess sugar beet grown.

One major difference between the sugar beet grown in Europe compared to the U.S. is the use of Genetically Modified (GM) sugar beet (*5*). Whilst this is fairly commonplace now in the U.S. there has not been the same level of acceptance by the consumers in Europe for Genetically Modified crops. Some field trials have taken place but, so far, no sugar in Europe has been produced from GM sugar beet.

Reduction in the Use of Energy

Sugar production, like other similar "first vegetable transformation industries" (e.g., oil and starch), requires a lot of energy to process sugar beet and sugarcane through to white, refined sugar. This is also one of the major differences between the production of sugar from sugar beet and sugarcane. Sugarcane factories burn the renewable bagasse (cane fibre residue after diffusing or tandem milling juice from the plant stalk) in their boilers to produce the steam for heat, power, and electricity requirements in the factory. In Europe, sugar beet factories tend to use either non-renewable coal, oil, or gas in their boilers as the fuel source, with the bagasse equivalent (fibrous beet pulp) instead being sold as a high value animal feed. Steam from the boiler is put through a turbo alternator to produce the electricity for the site with the exhaust steam from the turbine providing the heat for the process.

Combined Heat and Power (CHP) plants have been in place for many years in EU sugar beet factories, but more recently innovations such as inclusion of a gas turbine into these plants have developed (e.g., at British Sugar's Wissington factory and Nordzucker's Klein Wanzleben factory). This uprates the electrical output significantly and allows for supply of significant levels of electricity to the host countries national power system. These Combined Heat and Power (CHP) plants are approximately twice as efficient as a gas-fired power station solely supplying electricity due to the use of the steam generated by the plant to provide heat. Thus, the overall efficiency approaches 85%.

The addition of larger CHP plants to EU sugar beet factories has also brought further opportunities for product diversification. The waste heat and carbon dioxide produced by the boiler can be used to provide excellent conditions for the growth of horticultural crops that are discussed in detail in the Product Diversification section of this book chapter.
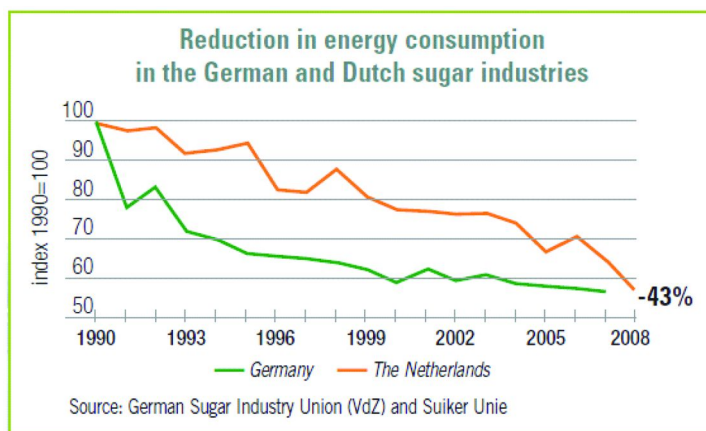
Figure 5. Reduction in energy consumption in the German and Dutch sugar industries since 1990. (see color insert)

Of course the efficiency of the manufacturing process is continually under review in the key areas of energy, yield, and cost. Price and sustainability have been the key drivers for more efficient energy utilization. In Figure 5 the reduction of energy consumed in German and Dutch factories since 1990 is illustrated, with a 43% reduction achieved. This has been achieved through investment in energy saving projects such as multiple-effect evaporator stations (up to 7 effect evaporators) and better re-use of waste heat.

Improved Process Efficiencies

Increasing the yield of sugar produced from sugar beet is very important in driving profitability of sugar factories and investment in new technology, such as chromatographic systems for separating sucrose and non-sugars, can help recover the sucrose normally lost to molasses in a conventional factory. Longer processing seasons and, therefore, better utilization of factory assets has been a long-term aim of European beet sugar factories. In British Sugar plc in the UK this has been achieved by building storage tanks to store an intermediate but stable sugar syrup. These storage tanks have enabled factory throughputs to be increased by uprating beet end capacity alone. Factories in British Sugar now produce sugar for 44 weeks out of the year compared to less than 20 a few years ago. Of course the use of processing aids is also under review from both a cost, final product specification and environmental impact position. Reduction in use is achieved through improvements in process control, new formulations and increased understanding of route cause issues.

Product Diversification

The aim of European sugar beet factories, especially after the change in the Sugar Regime, is to try and utilize all of the material delivered to site which also reduces waste going to landfill and helps improve the overall profitability

of the site. Figure 6 illustrates many of the possible, diversified products that can be produced from the delivery of sugar beet to the the sugar factory. All of this is on the back of the core role of the factory (sugar production) and the site infrastructure needed for this to produce materials for agriculture, construction, electricity, animal feed, energy etc. Beet sugar factories can now be truly thought of as "Biorefineries" given the range of products made at them. Figure 6 also illustrates how new biorefineries based on sugar beet as the major feedstock are a model of sustainability.

At British Sugars' factories the soil delivered with the beet is washed off and then allowed to settle in large lagoons. The soil is then removed from these lagoons and blended to produce a range of products for application in areas such as golf courses, racecourses, and building sites. Any stones delivered with the sugar beet are also separated and go to use in construction projects such as road building. Sugar factories use limestone in the juice clarification or purification unit process, and calcium carbonate is precipitated as a by-product. This by-product is removed by press filters, which produces a friable material with approximately 70% solids content, that can be easily handled and used as a soil pH adjuster. Its particle size and neutralizing value makes it ideal for this purpose and it can also be used in other areas such as blending with soil for mushroom compost.

As well as the product sucrose, or table sugar, sugar beet also contains a range of other valuable compounds that can be extracted (*6*). One such compound is betaine which is extracted from beet molasses using industrial chromatography, whilst at the same time recovering more sucrose that would normally be lost to molasses. This, therefore, has the benefit of increasing sugar yield and producing a further product with a number of diverse uses such as fish food. Another compound is lysine from the fermentation of a number of factory beet products, e.g., raw juice, syrup, and molasses. The obtained lysine can be added to dry pulp to enrich its nutritional value (*6*).

As previously stated the addition of larger CHP plants to sugar factories has brought further opportunities for product diversification. For example, the waste heat and carbon dioxide is being used to provide excellent conditions for horticulture at British Sugar's Wissington Sugar Factory, UK. At Wissington, 11 hectares (approx 27 acres) of glasshouses (see Figure 7) have been built which produces approximately 8000 tonnes of tomatoes per year. This is the biggest glasshouse in the UK, and Wissington is now the largest producer of classic salad tomatoes in the UK. The tomatoes are all grown hydroponically utilizing recycled rainwater supplied from the factory site, and carbon dioxide is delivered to the plants through a plastic pipe situated beneath each row. The glasshouse also acts as an excellent carbon sink taking in approximately 500 tonnes of carbon dioxide each day and, therefore, reducing the overall carbon emissions from the site. The bottom half of Figure 7 shows the glasshouse position in relation to the main factory site. In the bottom right hand portion of the photograph (Figure 7) can be seen the CHP plant and the pipe leading from this to the glasshouse for the heat supply. Calculations have shown that there is sufficient heat and carbon dioxide to expand the capacity of the glasshouse by approximately 70%.
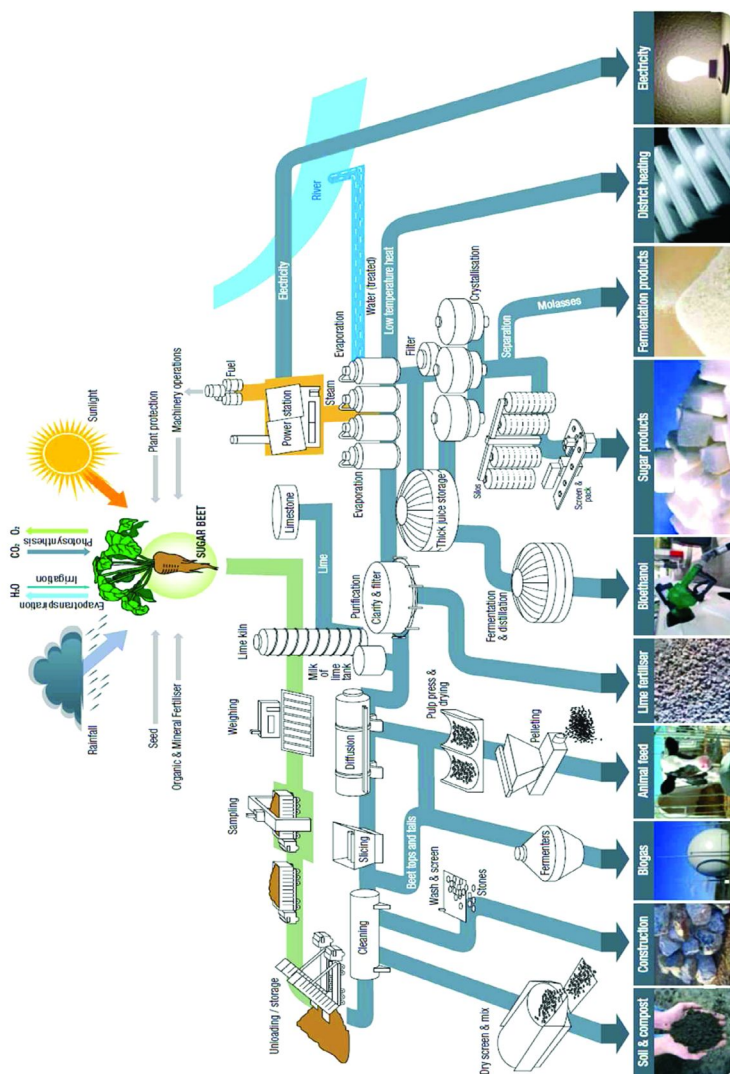
Figure 6. Diversification of sugar beet plants/refineries in Europe. Source: CEFS & CIBE Sustainability Leaflet (3). (see color insert)

Biofuel Production from Sugar Beet in Europe

Changes to the EU Sugar Regime has driven major restructuring of the European sugar industry; in particular European fuel ethanol production from sugar beet has increased strongly in the last three years. Since 2006 there has been a near doubling of European Union production (*7*, *8*), mostly because of robust growth in France and Germany, and more than twenty sugar beet ethanol plants now exist in Europe. The EU Sugar Regime Rules allow non-quota sugar

In Sustainability of the Sugar and SugarEthanol Industries; Eggleston, G.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

beet to be produced for industrial use and one such outlet is to use sugar beet to produce bioethanol. This allows the grower to plant sufficient sugar beet to ensure his sugar quota target is met and any surplus can then go to ethanol production. It is very difficult for the grower to produce just the amount needed to fulfil quota given the variable growing conditions in any year and, clearly, he wouldn't wish to produce less. Production of bioethanol, therefore, gives both the grower and sugar producer the flexibility required to grow sufficient sugar beet for sugar production and to manage the excess.

The current sugar beet ethanol plants in the EU produce ethanol for potable use as well as inclusion within fuel. Developments are also taking place to capture the carbon dioxide produced by such plants to produce a liquified CO_2 product; again showing the desire of the sugar factories to minimize waste and create a diverse product range. Other industrial applications include feedstock for other fermentation industries such as yeast and citric acid manufacture.

Figure 7. The glasshouses of tomatoes at British Sugar plc Wissington Sugar Factory. Top: Rows of tomatoes grown in the green house. Bottom: Aerial view of 11 hectares of glasshouse at Wissington. (see color insert)

Figure 8 illustrates the current position of sugar beet growing areas, beet sugar factories, and beet ethanol plants as of 2010.
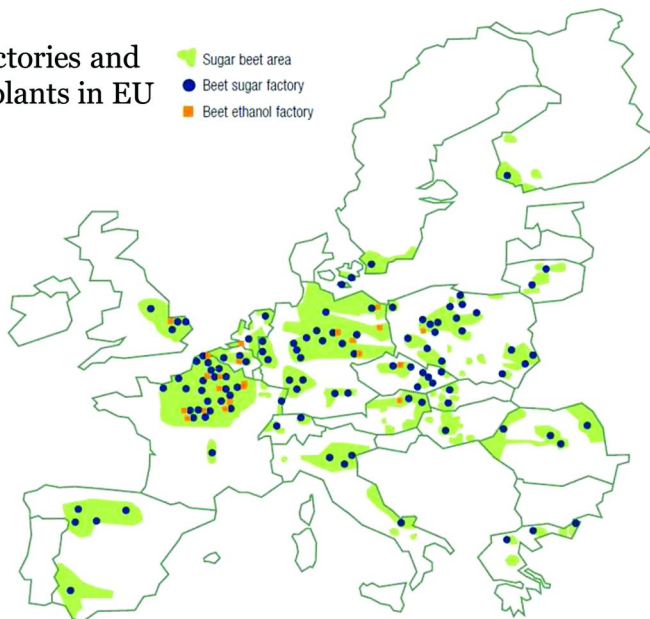

Figure 8. A map showing the current positions of beet growing areas, beet sugar factories, and beet ethanol plants in the EU as of 2010. The greatest number of ethanol plants are in France and then Germany (3). (see color insert)

As seen in Figure 8, the greatest number of ethanol plants are in France with a further five in Germany. Currently in the UK there is only one beet ethanol plant attached to the sugar factory at Wissington in Norfolk.

Wissington Sugar Factory and beet ethanol plant of British Sugar plc is used as an example to illustrate the dramatic diversification of the sugar beet industry that is occurring in Europe at the present time. An aerial photograph of the Wissington Sugar Factory/refinery is illustrated in Figure 9.

The ethanol plant at Wissington became operational in 2007 and is, currently, capable of producing 70 million litres of ethanol per year. All of the produced ethanol is used in fuel. The ethanol plant is visible in the bottom left hand corner of Figure 9.

Increased Refining of Cane Raw Sugars in Europe

Reforms of the EU Sugar Regime have also prompted the growth of raw cane refining in the EU with companies looking to obtain sugar from the LDCs for refining – this can be sold in excess of the national quota rather than as part of it. Raw cane sugar has been refined in Europe for many years, particularly in the UK, France, Finland, and Portugal, at longstanding refineries such as Silvertown in London. The EBA initiative has increased the volume of raw cane sugar imports

into Europe with new refineries being built such as Guadalete on the southern coast of Spain.

Additionally a number of beet sugar producers are looking at the possibility of co-refining raw cane sugar in beet sugar factories. Here the quality of the raw cane sugar will be important as the color profile and elimination in the affination process will be affected and potentially, therefore, the final product quality. Such Co-Refineries will generally require VHP (very high pol) and Very Low Color (VLC) raw sugars with lower impurities. These high quality raw sugars are discussed in more detail in Chapter 1 of this book (*9*).

Current Research and Development

The rising cost of energy and concern over the continuity of gas supply in Europe has led to increased levels of R&D aimed at reducing energy use and also moving towards more use of renewable sources of energy. A major initiative is that of anaerobic digestion of biomass, such as pressed pulp and beet tails to produce biogas for burning in the factory boilers. Sugar beet is ideally suited to biogas production and is a cost effective substrate for this process (*10*).

Figure 9. Wissington Sugar Factory and ethanol complex in Norfolk, UK. This is currently the largest beet biorefinery in Europe and is owned and operated by British Sugar, plc. The storage tanks allow the site to produce sugar for longer periods of time in comparison to other beet sugar factories. Addtionally there is the CHP plant, the glasshouse, chromatographic separation, and ethanol production. (see color insert)

In Sustainability of the Sugar and SugarEthanol Industries; Eggleston, G.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

Sugar beet pulp has the potential to be used as the fuel in hot gas generators which are used by some companies for animal feed drying. Using the material as the fuel reduces the amount of animal feed that can be produced but it does mean that the animal feed made is done so in a more sustainable manner. Energy beets with higher fiber and biomass yields would be a better source of raw material for this product (*11*).

Projects have also been undertaken to examine the potential of isolating further products from the sugar beet such as amino acids (*6*). Sustainable environmental considerations are always part of the process and the project's impact on the environment is a major consideration for sugar producers in Europe.

The longer beet campaigns have necessitated longer periods of beet storage, and research is ongoing to optimize this.

Acknowledgments

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Chapter 4

The Sugarcane Crop for the Sustainable Production of Sugar and Other Cane Derived Products in Mauritius

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Sugarcane was introduced in Mauritius four centuries ago. Through its considerable resistance and resilience to very adverse climatic conditions like drought and, in particular, intense tropical cyclones, it has proved beyond doubt its capacity to be sustainably cultivated on a long-term commercial basis and to play a multifunctional role. The sugar industry in Mauritius has constantly been faced with challenges and it has always stood up to convert these challenges into opportunities to ensure sustainable productions of sugarcane and derived products derived. Actions taken were mainly in the way of reforms to address technical, financial, socio-economic, and environmental viability of the industry. Appropriate legislations (bearing in mind the specificities of Mauritius as a small island developing state) were put in order to facilitate the sustainability.

The History of Sugar in Mauritius

Mauritius is a tropical island in the Indian Ocean located at 20° south of the equator. It has a total area of 1,860 km² and has a population of around 1.2 million people. Sugarcane was introduced in Mauritius as far back as 1639 by the Dutch (*1*). Over the period of their occupation of the island (1598-1710), only around 4 hectares (ha) of land was under cane cultivation, essentially for the production of potable alcohol (rhum). The plantation at maturity became infested with rats and was, therefore, not expanded. During the subsequent French occupation of

the island (1710-1810), cane cultivation increased slowly and by 1810 two sugar factories produced 3,000 tonnes of sugar from a 3,600 ha crop.

With the colonization of the island by the British in 1810, more impetus was given to agriculture and sugar occupied a prominent position. By the year 1825, the area under cane cultivation increased to 11,000 ha, the number of factories reached 110, and 10,800 t of sugar was produced.

After the abolition of slavery in 1835, labor shortages were addressed through the recruitment of indentured labor from India. This, coupled with the introduction of new cane varieties and the use of fertilizers, resulted in a significant expansion of the sugarcane industry. Infrastructure for cane transport and a new railway system linking the various parts of the island were established. By 1862, cane grown on 72,000 ha was processed in 259 factories producing 150,000 t of sugar. From that time, the industry faced a number of unfavorable events, including pests and diseases in the cane, a severe cyclone in 1892, malaria tragically affecting the population with a death toll of 10%, a fall in world market sugar prices, the introduction of customs duties on British imports, and a sharp increase in wages during the First World War that led to a deterioration in the socio-economic situation in Mauritius culminating in social unrests and riots on sugar estates.

By 1938, although the area under cane had declined to 60,000 ha and the number of operating factories to 37, sugar production reached 320,000 tonnes, thanks to the cultivation of pest and disease resistant cane and a marked increase in cane yields.

After the Second World War, the British Government in need of sugar supplies from its Dominions and Colonies promoted sugar production through the assurance of a long-term market and remunerative prices. A Commonwealth Sugar Agreement (CSA) was negotiated and signed in 1951. The quota for Mauritius in the CSA amounted to 386,000 tonnes, allowing the industry to adopt a longer term focus. Cane production was expanded together with the modernization of the factories, adoption of better cultural practices, and overhaul of its financial and administrative centers. In 1973, the area under cane cultivation reached 87,000 ha and sugar production 720,000 t with 21 operational factories.

Mauritius obtained independence from Britain in 1968. When Britain joined the European Economic Community (EEC) in 1975, the guarantees provided in the CSA formed part of the conditions for Britain to enter the EEC. As a result, a Sugar Protocol was negotiated with all concerned parties assuring this remunerative market in the long-term. This protocol laid the foundation that enabled the subsequent socio-economic development of Mauritius and its sugar industry.

The Sugar Protocol provided for around 1.3 million tonnes of sugar to be supplied from the African, Caribbean, and Pacific group of states (the ACP) and India. Mauritius managed to secure a significant share of this quota at 0.5 million tonnes of sugar. Moreover, in 1995 Mauritius was able to secure an additional 85,000 t of sugar under a special preferential sugar agreement (*2*) to meet a sugar refining deficit in the European Union (EU), and the price offered was equivalent to 80% of the Sugar Protocol price.

However, preferential market agreements and other barriers to trade received increasing criticism with the opening of global markets. In 2001, tariffs were eliminated on almost all imports from 48 of the Least Developed Countries (LDC) under the Everything-But-Arms Regulations of the EU. The exceptions, beside armaments, were sugar, rice, and bananas and these were to be fully liberalized by 2009. In 2003, three sugar producing countries, Australia, Brazil, and Thailand, challenged the legality of the sugar regime claiming that the regulations were not compatible with the rule of the World Trade Organization (WTO). The WTO ruled that the EU needed to reduce its import tariffs (*3*). This resulted in the phasing out of the export of some 5.1 million tonnes of sugar. In 2005, the EU agreed on a reform package of the sugar regime from 2006/2007 with a price reduction of 36% spread over 4 years. This reform had significant implications on the world sugar market and, in particular, the EU former colonies that had been deriving beneficial treatment under the preferential trade agreement. The ACP guaranteed sugar price fell from £ sterling 524 to £ 335 in 2008. The EU offered some adjustment assistance to the affected countries, which included Mauritius.

Specific Reforms in the Mauritian Sugar Industry

There exists in Mauritius a special sugar regime inherited from the colonial past, whereby the sugar industry and the relationship between factory processors and growers have always been regulated by the authorities. The government has thus always ensured the benefits emanating from the Sugar Protocol trickled down to all stakeholders – the processors, the growers, the workers, and the population at large. Any single partner could thus not claim ownership of the benefits accruing to the industry and each partner has always played a key role in the sustainable development of the industry.

Prior to 1980, efforts were concentrated on improving cane production and optimizing installed capacity of cane factory facilities to cope with the progressively increasing amount of cane being produced. Up to that period, the industry was practically the sole meaningful industrial and agricultural activity and also the sole earner of foreign exchange. Sugar exports represented more than 40% of the economy and contributed 13% of total revenue to the Government in the form of export duty. At that stage other sectors, such as tourism, were introduced to the economy. Focused plans and programs were, therefore, put in place to keep the Mauritian sugar industry sustainable.

The Sugar Sector Action Plan (1985-1990)

The aim of the Sugar Sector Action Plan (*4*) was to provide a clear definition of the policies and programs in the Mauritian sugar sector to secure the future of the sugar industry. It was acknowledged that the sugar industry was already a mature industry and its growth potential was limited by market factors and the availability of arable land. Prospects for increased production, therefore, depended on:

- productivity improvement in respect of land, machinery, and employment

- utilization of sugarcane by-products, in particular bagasse for production of electricity and cane tops for livestock production
- agricultural diversification and
- a new spirit of cooperation and trust between all parties in the industry.

The potential for increased productivity of small-scale growers was identified. It was noted that cane production from growers cultivating less than 2 ha was 80% of that achieved by the corporate and large growers. Special services were, therefore, offered through extension works and the creation of farmers' service centers, establishment of demonstration plots on small farmers' land, an accelerated de-rocking program and appropriate credit schemes, supervision, and technical assistance.

In consultation with the Ministry of Energy and the Central Electricity Board, a project aimed at energy export from bagasse was initiated. The study covered the following areas:

- rate of development and supplies of bagasse
- optimum location and phasing of two power stations
- electricity
- national demand projections
- policy of the utility on use of coal
- technical factors of plant design and specifications
- capital and operating costs coupled with a financial analysis
- ownership and responsibility for project and ongoing management

Factory areas were redefined considering issues like factory capacity, both individually and regionally, cane availability predictions, geographical factors, and cane transport. The objectives of this exercise were to minimize transport costs and ensure maximum use of factory capacity. The Plan also made provision for establishing Regional Milling (factory) companies to ensure the long-term factory development in each region.

To facilitate the implementation of the above measures, legislative changes were needed. These revolved mainly around exemption from payment of taxes on property transfer, restructuring payment of export duty payable by the corporate and large growers, conduct of studies to establish cost of production of cane by growers and estates, and finally consideration of widening of share ownership of milling (factory) companies and bagasse electricity generation companies. Most of the above issues were the subject of a detailed sugar industry efficiency study in 1985 with the support of the World Bank and in consultation with all stakeholders, resulting in the creation of the Mauritius Sugar Authority (1984) and the Sugar Industry Efficiency (SIE) Act (1988).

The SIE Act provided measures to improve sugarcane processing and growing to enable the sugar industry to continue functioning on an efficient basis and to be competitive on an international market. The Act made provision for an efficient and viable sugar industry, preservation of agricultural land, promotion of agricultural diversification and diversification within sugar, ensuring that all commitments under the Sugar Protocol are met, and finally ensuring fairness,

equity and transparency within the sugar industry. Incentives were given to the processers to improve factory performance and to invest in modern equipment, bagasse savings, factory energy production, and the reduction of environmental pollution.

Bagasse Energy Development Program (1991)

Mauritius, as a small island, has no reserves of oil, gas, or coal and it depends heavily on imported fossil fuel resources to meet its demand in energy. However, the island has so far identified and exploited two renewable sources of energy, namely hydro-energy and sugarcane bagasse. Whereas the potential of hydro resources were fully realized by 1985 with a peak capacity of 100 GWh, that from sugarcane bagasse has been constantly increasing over the years.

Bagasse has a calorific value of around 8,000 KJ/kg and is burnt to meet the energy (steam and electricity) requirements for processing of cane into sugar and its by-products. The process in which electricity and heat (in the form of steam) are generated simultaneously in a single power plant (steam boiler coupled with turbo alternator) is known as cogeneration. In a sugarcane factory with a well-balanced energy system, the energy potentially available in the bagasse is in excess of that required for recovery of sugar from cane, and this excess can potentially be exported in the form of electricity to the grid.

Interest in the use of bagasse for electricity generation started in Mauritius in 1957, when one sugar factory exported 0.28 GWh of electricity to the public grid in an intermittent form, that was not modulated to the needs of the utility. The price was low but attractive enough to encourage other factories to export such electricity. By 1980, 14 out of the 21 factories were exporting a total of 27 GWh. Rapid improvements as well as the establishment of a Continuous Power Purchase Agreement (PPA) with the local electricity utility increased the contribution to total electricity generation from the sugar industry to 116 GWh in 1986 of which 73 GWh was from bagasse and the remainder from coal imported from South Africa.

In the light of these successes and experiences and prompted by the oil price hikes during the Gulf War, the Mauritian Government, with support from the World Bank, established a Bagasse Energy Development Program in 1991 (*5*) to devise a strategy to maximize electricity export from bagasse. A World Bank loan (US$ 15 million) and a Global Environment Facility grant of US$ 3.3 million were secured to achieve the following objectives:

- displacement of investments based on fuel oil by the electricity utility
- reducing reliance on imported fuel
- enabling modernization of the sugar industry and improving its viability
- saving on foreign exchange linked to fossil fuel imports
- contribution to the mitigation of greenhouse gas emissions.

In addition, energy production is linked to centralization. The approach brings about economies of scale given that the centralized factory receives together with the cane its fiber. Hence more bagasse is available for electricity generation and

export to the grid. In such cases, the total investments in power plants can represent up to 50% of the total investment in a sugar factory with its matching energy generation facility.

The most significant development in bagasse energy has been the investments in two new power plants – the first one with 2 x 35 MW installed capacity started in 2000 and the second one with 2 x 41.5 MW capacity commissioned in 2007. These plants are located next to sugar factories which supply all their bagasse and condensed water in exchange for process steam and electricity from the power plant. The steam pressure and temperature are 82 bars and 525 °C and the plants are exporting around 125 to 135 kWh/ tonne of cane to the grid depending on the fibre content of the cane. The plants burn the totality of the bagasse from the sugar factory, and coal as complementary fuel when bagasse is not available, in particular during the off-crop season.

Table I gives the evolution of electricity export from the sugar industry located power plants with bagasse and complementary coal as feedstocks, as well as that from all sources in Mauritius (*6*).

Five new power plants each with 42 MW installed capacity and 82 bar operating pressure have been identified for investment in the cane sub-clusters, making a total electricity generation of 125 KWh/tonne cane achievable for the country.

Table I. Evolution of bagasse electricity production in Mauritius[*]

Period	Sugar Industry (GWh)			Island	Bagasse %	
	Bagasse	Coal	Total	Total GWh	Island Total	KWh/tc
1971-1975	24	0	24	208	18.4	4
1976-1980	26	0	26	355	9.0	4
1981-1985	43	0	43	363	15.0	7
1986-1990	72	34	106	549	16.7	12
1991-1995	85	43	128	805	14.8	15
1996-2000	194	62	256	1365	22.7	33
2001-2005	318	407	725	1923	16.5	55
2006-2010	366	999	1365	2241	16.3	81

[*] Figures given are annual peaks over 5-year periods.

The Sugar Investment Trust (1994)

In 1994, the Sugar Investment Trust was established, enabling small-scale growers and workers to become shareholders in milling (factory) companies up to 20%. This measure coincided with the total abolition of payment of export duty by the factory processors.

Centralization of Sugarcane Milling (Factory) Activities (1997)

The decision on factory closure falls under the authority of the Minister responsible for Agriculture, and the Government has constantly been under pressure from processors to close down sugar factories. In the early eighties, Government denied a request for closure of 2 of the 21 factories. The processors filed a case in the Supreme Court against the Minister, requesting re-examination of the application. Approval was only granted in 1984.

The issue of centralization became topical again in 1994 against the background of major reforms in agricultural policies across the world in the context of the General Agreement on Tariffs and Trade (GATT) and the optimal use of bagasse for energy.

The Mauritian government devised a Blue Print on Centralization of Sugar Activities in 1997 (*7*). The objectives were the possible reduction in foreign income from sugar as well as reduction of production costs through economies of scale and factory modernizations (both short and long-term investments). It also included environmental considerations and socio-economic issues related to the vulnerable partners, namely the workers and the small-scale growers. As per the Blue Print, the Centralization process would enable:

i.   the full use of existing spare capacity thereby reducing fixed costs
ii.   investments in modern, efficient, and larger sized equipment which would be geared towards energy savings and energy generation. The new equipment will be coupled with pollution abatement technology.
iii. an improvement in health and safety conditions through the installation of "workers friendly equipment".

Provisions of the Blue Print included tax exemptions applicable to cash and land compensations associated with voluntary termination of employment contracts. These tax exemptions were in relation to land conversion, land transfer, morcellement (reduction into smaller pieces), and capital gain, and registration duties.

Moreover, the workers were entitled to a cash compensation and a land compensation depending on their salary and their length of service. The land would be provided with the necessary infrastructures such as roads, drains, electricity, and water supply. Provisions had also been made by the factory processors for social amenities like a volleyball or football pitch, maintenance of roads and refuse collections, as well as payments for legal and procedural costs involved in the transfer of the plots of land to the workers.

Table II. Evolution of Number of Manpower in the Mauritian Sugar Industry[*]

Period	Factory workers	Field workers[**]		Total (Factory +Field)
		Men	Women	
1971-1975	6,157	36,404	14,592	57,153
1976-1980	8,868	38,690	15,364	62,922
1981-1985	8,306	na	na	58,316
1986-1990	6,937	33,634	12,166	52,737
1991-1995	7,062	29,125	10,147	46,334
1996-2000	6,266	23,523	8,083	37,872
2001-2005	4,532	na	na	18,335
2006-2010	3,636	na	na	13,511

[*] Figures given are annual peaks over 5-year periods; na - breakdown not available. [**] Sugar estates and planters growing cane on more than 10 ha of land.

In the package for growers, the processors were required to maintain an operational weighbridge, cane testing facilities, and cane unloading devices at the closed factory site, to transport at his cost the cane from this site to the centralized factory, to make available at the closed factory filter mud accruing to the grower, to maintain the same daily cane supply quota, to provide the grower with cane sets, to construct a store for fertilizer and other inputs and, finally, to credit 3 million Mauritian Rupees (MUR) per year over 5 years, that is a total of MUR 15 million to a Growers Fund to be used to enhance the productivity of growers.

This Blue Print thus facilitated the centralization of cane processing activities so much that it has enabled closure of a number of small factories in favor of ones with higher cane crushing capacity, thus, benefiting from economies of scale, reduction in cost of processing and, more importantly, adoption of modern and more efficient processing technology while meeting its social obligations towards the most vulnerable partners – the workers and the small growers. Table II indicates the change in manpower in the industry from 1971 to current date (*8, 9*).

Further amendments to the SIE Act in 1999 were made to enable processing and related companies to convert around 506.5 ha of agricultural land into residential land provided that they undertake to sell 25% of the land to the Government and to plough back 60% of the proceeds of which half would go into sugar production or diversification within sugar in Mauritius (as per an approved list of schemes/projects/equipment) and the remainder to any other economic activity in Mauritius. This piece of legislation enabled a land owner and any other person jointly and severally engaged in cane growing, processing, power generation or non sugar activity as well as a person involved in factory closure, to recoup 100% of the cost incurred from the proceeds of land conversion. In addition, these amendments solved the problem of having separate legal processing and growing activities and enabled the processers and the factory growers to derive full benefits from the incentives provided in the Act.

Sugar Sector Strategic Plan (2001-2005)

In 2000, further difficulties were being faced by the sugar industry. Such difficulties related to the erosion of preferential access for sugar in our traditional export markets and the challenges resulting from trade liberalization. The long-term viability of the industry thus depended on its ability to reduce the cost of production and ensure a selling price for Mauritian sugar that would enable it to compete with the least developed country supplier. Further reforms were thus imperative and a 5-year Sugar Strategic Plan Strategic Plan – SSSP (2001-2005) was prepared in consultation with stakeholders and approved by Government. This Plan (*10*) was meant to create the proper environment to enable the industry to rethink its operations, ensure its efficiency and viability, and win the competitiveness battle.

The objectives of this reform were as follows:

- Ensure that export market commitments are fulfilled. Around 620,000 t of sugar production was to be achieved. Promote special sugars in other markets in addition to the EU. Producers of special sugars and packing plants should opt for Hazard Analysis and Critical Control Point (HACCP) norms.
- Reduce the number of sugar factories from 14 to ideally 7 or 8. Concurrently bring down sugar losses at harvest and in cane processing to a strict minimum. A low cost centralization program was the preferred option.
- To maximize electricity generation from bagasse as a renewal resource. Power plants operating at the commercially proven technology adopting 82 bars pressure were the preferred option and such plants would be located next to sugar factories and use coal as complementary fuel.
- Ensure that the maximum extent of land under cane is prepared, de-rocked and provided with irrigation. The ultimate objective is to adopt complete mechanization over 60,000 ha of which 32,000 ha would be provided with irrigation facilities by the year 2010.
- Create the enabling environment for dynamic and efficient field operations. A task force was set up to address funding of de-rocking/ irrigation and mechanization project in the small/medium grower sector.
- Effect a manpower rightsizing involving a reduction in the labor force through a socially acceptable Voluntary Retirement Scheme (VRS).
- Rationalize the Global Cess and make a more productive use thereof.
- Ensure a more efficient and judicious use of land and water resources. Water consumption in cane sugar factories should be brought down to 0.6 m^3/tonne cane.
- Further democratize the industry, in particular, through the sale of agricultural land. A sale of up to 5% of shares to planters in existing power plants and the forthcoming ones was proposed.
- Develop R & D so as to fully tap the benefits of the forthcoming quantum leaps in respect of biotechnology, biotics, and cane biomass. As an

immediate step, production of 'rhum agricole" had to be initiated. In addition, a co-product development program had to be worked out.

The Sugar Industry Efficiency Act of 1988 was completely overhauled and a new SIE Act 2001 came into force. A new mission was defined to ensure that a viable sugar industry is passed on to future generations who would then avail themselves of the multifaceted opportunities offered by a strategic crop, the cane plant. The Act provided for the enabling framework for democratization of ownership of land, as well as for cane processing activity, investment in modernization of cane growing and processing and in power generation facilities manpower rightsizing, recoupment of cost through land conversion and sale, and finally, exemption of fiscal duties and taxes applicable to land and capital gains.

The Multi Annual Adaptation Strategy Action Plan (2006-2015)

In 2005, the reform of the Common Agricultural Policy of the EU had a profound and adverse impact on the Mauritian sugar industry. The impact was felt in three major areas:

- a reduction in price by 36%,
- elimination of the intervention/guaranteed price mechanism and
- the reduction of production in the EU by some 6 million tonnes of sugar.

One major consequence of the Reform was that the EU will no longer export sugar to the world market becoming a net importer of essentially ACP and LDC sugar. The EU sugar producers have been compensated by around 64% of the loss incurred and the ACP producers have been entitled to support in the form of accompanying measures with conditions related to economic and social performance indicators attached.

Under the above new environment coupled with

i. developments which have taken place under the Regional Economic Partnership Agreement,
i. phasing out of the Sugar Protocol as from 1 October 2009,
ii. limit of 3.5 million tonnes of sugar imposed on imports from all ACP states (non-LDC and LDC) and
iii. unlimited access on quantities of sugar as from October 2015,

Mauritius prepared, in consultation with all stakeholders, a Multi Annual Adaptation Strategy (MAAS) for its sugar sector together with an Action Plan spanning over the period 2006 to 2015. In the Action Plan (*11*), sugarcane in Mauritius is recognized as much more than a cash crop. It has a multifunctional role and the country has no other alternative but to continue cultivating it. This role encompasses the economic, energy, social, and environmental issues. Cane cultivation has been recognized as essential for Mauritius to meet its commitments for sugar in its various markets, to generate electricity from an

annually renewable resource (bagasse from cane), to produce ethanol from cane molasses, and to provide a green landscape for the tourism industry. More importantly, it constitutes a major source of foreign earnings which, *inter alia*, is required for its food procurement. The overall objectives of the Plan have been to ensure, upon implementation of a number of measures and projects, the long term viability and sustainability of the industry while at the same time fulfilling its multifunctional role in the country.

Cost reduction in processing activities is to be achieved through centralization as per the Blue Print on centralization approved by Government in 1997. The main project relates to the reduction of the number of sugar factories from 11 to 4 and with around 1200 workers leaving the factory sector. This will enable the establishment of a sugarcane cluster, made up of 4 sub-clusters operating around the 4 factories. The factories will operate in a flexible manner to produce a mix of direct consumption sugars (white refined and speciality) and co-products, mainly electricity from bagasse and ethanol from molasses. The targeted amount of sugar to be produced would be 520,000 tonnes of which 80% would be in the form of refined white sugar and the rest as speciality sugars. Cane production to meet this target is set around 5 million tonnes annually.

The other project that would enable cost reduction is the adoption of mechanization of field operations and cultural practices which will be facilitated after proper land de-rocking and preparation. This will, in addition, bring improvement in cane and sugar yields. This project will be extended to the small/medium growers (cultivating up to 25 ha) sector after plots of land owned by such growers have been regrouped into bigger units. These regrouped plots will also benefit from irrigation wherever the need arises. The targeted area for regrouping is around 12 000 ha and a yield increase by 20% and a cost decrease by 20% are achievable.

Besides sugar, each factory in the 4 sub-clusters will generate electricity for sale to the grid. In this context, investments in new power plants and enhancement in the capacity of existing ones will be undertaken around each sub-cluster so much so that the electricity export from the sugar factory located power plants will increase to around 1700 GWh (600 GWh from bagasse and 1100 GWh from complementary coal).

Ethanol from molasses and, depending on market conditions of sugar, even from cane juice has been projected in the Plan. Around 30 million litres of ethanol are potentially obtainable from the 120,000 t of molasses currently being exported out of the 150,000 t being produced annually. This amount of ethanol can conveniently substitute up to 20% in a blend with gasoline.

Sugarcane is also being cultivated in so-called difficult areas located on mountain slopes and very rocky soils. Yields of cane are low and cost of cultivation is high given that such areas cannot be mechanized. But if such areas are not used to cultivate cane, they will suffer from very adverse environmental as well as social consequences. Provisions have been made in the Plan to maintain cane production on a substantial part of the difficult areas through an income support to the growers in the areas.

The Plan makes provision for a greater role on research and technological development given that the industry will move from a traditional single commodity

– sugar - to an agro-industrial complex producing a portfolio of products derived from an annually renewable cane biomass resource.

A Sustainable Industry

The first and foremost revenue generating activity of the industry has always been to produce sugar as the main product and to concurrently develop and tap additional revenue through peripheral activities like:

i.   production of agricultural crop from land under cane through use of cane interline or rotational land,
ii. specialty sugars in an attempt to diversify its portfolio of products from sugar cane,
iii. cogenerated electricity from bagasse for export to the public grid to benefit from an additional revenue stream,
iv. ethanol from molasses with an initial emphasis on potable alcohol for subsequent extension to fuel ethanol, and finally
v.   agricultural rum to benefit from the advantage of an alcoholic beverage originating from a tropical island, comparable to similar value added products produced in the Caribbean region.

However, before discussing statistics related to the above, it is relevant to highlight the role of the sugar industry in the Mauritian economy over time.

The Sugar Industry in the Mauritian Economy

The sugar industry is a major net foreign exchange earner and it is an accepted fact that 80% of the earnings are generated locally and 20% represents imports of inputs like equipment and machinery, fertilizer, and other agrochemicals. The contribution of the industry in the GDP has always been significant, but has been decreasing in relative terms due to development of other sectors in the economy such as manufacturing (in the form of an Export Processing Zone or EPZ), hotel and tourism, business and finance, information and communication technologies, and construction.

Table III gives a summary of the statistics related to sugar cane and derived products as well as food crops since 1971 (*12*, *13*). It should be highlighted that sugarcane is an agricultural crop and the amount of cane harvested annually is very dependent on the prevailing climatic conditions especially in a tropical island. Events like cyclones, droughts, excessive rainfall, and fire affect cane and sugar production. The figures have therefore been split into sub-periods of 5 years and the peak production for each item within each 5 year period has been given.

Cane Production

Cane yields have constantly been on the increase due to development and adoption of pest and disease free cane varieties bred locally. Yields of up to 80 t/ha

have consistently been obtained over the past decades. Cane production peaked at around 6.6 million tonnes in 1982 but it has been between 5.0 to 5.5 million tonnes regularly. However, there has been constant pressure on land under cane cultivation for conversion into other uses so as to meet the demand for residential, industrial, urbanization, and tourism development. Such demand has brought about a constant decrease in the area under cane cultivation but there has always been pressure on the sugar industry to maintain its cane production to set targets through improvement in cane varieties and adoption of good crop husbandry.

In an attempt to bring down the cost of production and increase cane yields, the industry has been investing in de-rocking (both coarse and fine) and land preparation to facilitate mechanized cultural practices, in particular, cane harvesting. The evolution of tonnage of cane harvested or loaded mechanically is given in Table IV. It can be seen that the amount of cane loaded mechanically after it had been cut manually had been constantly on the increase and reached almost 3 million tonnes in 1995. On the other side, mechanically harvested (chopped cane) had been initiated in 1979 and was stopped in 1986. It was restarted in 1992 and the amount has now reached almost 2 million tonnes. 90% of the cane is harvested green.

In addition, the industry corporate sector and a number of large growers have been adopting irrigation systems which, over the years, are more and more efficient both in terms of water usage and cost of operation. The evolution of area under various irrigation systems (*12*, *14*) is given in Table V. The investment in irrigation had been prompted by the uncertainties in the long term price of sugar, labor shortages, and cost of water. The new systems – drip, centre pivot, drag line fits with those criteria.

Table III. Production of Cane and Related Products Over Time and Food Crop[*]

Period	*Land* *x10³ (ha)*	*Cane* *million (t)*	*Sugar* *000(t)*	*Molasses* *x10³ (t)*	*Ethanol* *million (L)*	*Food crop* *x10³ (t)*
1971-1975	81	6.2	720	185	1.8	47
1976-1980	80	6.3	690	200	3.3	41
1981-1985	80	6.6	690	190	3.8	49
1986-1990	78	6.0	710	170	3.5	66
1991-1995	75	5.8	650	170	5.1	80
1996-2000	74	5.8	630	170	6.9	101
2001-2005	73	5.8	650	175	6.5	130
2006-2010	71	4.8	510	140	8.5	105[**]

[*] Figures given are annual peaks over 5-year periods.   [**] Provisional for period.

Table IV. Cane Harvest and Loading – Evolution of Mechanization in Mauritius*

Period	Cane Harvested (million t)	
(peak year)	*Mechanical loading*	*Mechanical harvesting*
1971-1975	0	0
1976-1980	2.2	0
1981-1985	2.0	0
1986-1990	2.4	0
1991-1995	3.0	0.2
1996-2000	na	0.6
2001-2005	na	1.4
2006-2010	na	1.8

* Figures given are annual peaks over 5-year periods; na – not available.

Table V. Evolution of Irrigation Systems Implemented in Mauritius

Period	Agricultural land (ha)					
	Irrigation				Drip	Total
	Surface	*Overhead*	*Dragline*	*Centre Pivot*		
1971-1975	7479	5543	0	0	0	13,022
1976-1980	5579	9406	0	0	64	15,049
1981-1985	5942	8285	0	0	175	14,402
1986-1990	na*	na	0	0	0	na
1991-1995	na	na	0	1214	na	na
1996-2000	2774	5971	2519	3491	1462	16,217
2001-2005	313	4286	3554	5061	1255	14,469
2006-2010	310	4364	3330	5501	1163	14,668

* na – not available.

To boost cane production and facilitate mechanization and irrigation, the small/medium grower sector has also been investing in land preparation/de-rocking/irrigation systems with the support of Government. This support is being intensified with funds being made available to this sector under the EU Accompanying Measures as per the MAAS Action Plan (2006-2015).

This category of producers (around 28 000 in number) grows cane on areas varying from 0.1 to 25 ha and owns a total acreage of around 20 000 ha. Significant acreage of such land is marginal to cane cultivation and even more for agriculture.

However the cane supply from this source is important for the cane processing plants and the sub-clusters would not be economically viable without supply of such cane on a sustainable manner. This consideration has in no small measure facilitated an agreement on the enhanced equity participation of these producers in sugar factories, power plants, refineries, and projected distilleries. This equity participation seals the commonality of interests between the various categories of stakeholders.

Given that the small/medium growers operate in small plots, their production methods are inefficient. This group of producers has the following characteristics:

i.   Their land is neither prepared for mechanization (either partial or total) nor is it ready to benefit from economies of scale.
ii. Cane varieties cultivated are, in many cases, not appropriate and, on account of re-plantation costs, the planters go for a long crop cycle (beyond the 7-8 ratoons recommended) and cane yields are thus poor.
iii. Cane is not always harvested at optimal maturity and, even when harvested, is not processed promptly. These factors result in loss of sucrose.
iv. Cutting, loading, and transport are costly due to diseconomies of scale and these items are associated with hassles. Growers are induced to abandon their cane lands.

To further improve cane production and reduce the cost of production, new farming techniques are being adopted. Such techniques include farm planning using GPS which enable adoption of control traffic in fields for all mechanized cultural and harvest operations. Moreover, a cropping system is being promoted which includes a leguminous crop, between two cane crop cycles, being ploughed back to enable an improvement in soil organic matter, and a saving in nitrogenous fertilizer.

Diversification Within Sugar – Food Crop Production

In the past, the arable land of Mauritius was mainly under sugarcane cultivation with some tea in the uplands, as well as tobacco and vegetables. However, due to the fact that land is a very scarce resource on the small island, it had become essential that maximum use is made of such land. Over time, Mauritius became one of the leaders in using cane interline (land under plant cane before the field closes in) to grow short cycle cash crops like potato, maize, and groundnuts as well as rotational land between two cane cycles for other vegetables. The island is self-sufficient in its vegetable requirements. Moreover, incentives were also provided for creation of permanent gardens, production of mushroom, vanilla, spices and medicinal plants, storage of fruits and vegetables, aquaculture, and finally the establishment of orchards.

Resolute efforts were made by the corporate sector supported by Government in the form of incentives and enactments which enabled production of food crops and fruits both for local consumption and export. As a result, production increased

over the years (Table III) and this was achieved without any substantial reduction in sugar output. It should be noted that the production was around 45,000 tonnes in the 1980s and has reached almost 130,000 tonnes in 2001 (*13*) to meet demand of the local population and the booming tourism sector.

Ethanol

Ethanol can potentially be produced from any sugar containing products and molasses and cane juice from sugar cane are potential substrates available from the sugarcane industry. Under current sugar market circumstances, the cane juice to ethanol option is not financially viable. However, molasses can be economically converted to ethanol with oil prices at around US$ 60/barrel (*11*). 30 million litres of ethanol can potentially be obtained from 120,000 t of molasses currently being produced on an annually renewable basis in Mauritius. This amount of ethanol corresponds to a 20/80 blend with gasoline, the annual consumption of which is around 120 million litres. The bulk of the molasses is exported *as is* and part of it is converted into ethanol. This ethanol is used in the production of potable alcohol and a number of alcohol derived products like vinegar and perfumes. Recently, one distillery was commissioned using molasses as feedstock and had been exporting its ethanol but it ceased operation.

Rhum Agricole

The SIE Act 2001 provided for the enabling legislation to produce Rhum Agricole from cane and derived products. Rhum Agricole is produced from cane juice instead of cane byproducts such as molasses, and has been produced on small artisanal scale along similar lines to that produced in the Caribbean region, e.g., Jamaica. This new activity, started in 2002, has been on constant increase since then, and an equivalent of 450 tonnes of sugar was used in 2009 to produce Rhum Agricole.

Other Diversification Activities

Using the sugar industry as an economic base, the country has been able to diversify its activities in other sectors after its independence in 1968. For example, an Export Processing Zone was set up with original emphasis on the textile industry. Tourism is another sector where the sugar industry has a strong foothold. The closed sugar factories have been converted into industrial estates. The Mauritian sugar industry has also undertaken investment in the African continent, e.g., Mozambique, Ivory Coast, Reunion, and Tanzania. All these activities were possible given that Mauritius was able to sustain a healthy and efficient sugar industry.

Environmental Issues

On a small tropical island devoid of any natural resources and heavily dependent on a tourist industry, protection of the environment is imperative. Maintaining an agricultural crop in a sustainable manner widely upholds the environment and protects the coral reef barrier that protects the lagoon, marine life, and white sandy beaches. Moreover, the lands have a relatively thin layer of top soil. The absence of sugarcane or a large scale abandonment of cane would bring about irreversible damage to the whole ecosystem with far reaching implications to the environment, the fishing sector, the tourism industry, as well as the economy at large.

Sugarcane, which has been cultivated for over three and a half centuries, has enabled the island to have a stable and homogeneous soil stratum within which equilibrium has been reached. Sugarcane provides a permanent cover on the land throughout the year. It prevents soil erosion, maintains soil moisture, and improves soil organic matter content. No soil erosion implies absence of sedimentation and/or eutrophication problems in downstream reservoirs and, more importantly, the lagoons. Moreover, the sugarcane crop requires relatively low doses of agrochemicals given that the commercial varieties adopted are resistant to pests and diseases. In addition, these varieties are wind resistant and have a strong root system which binds the soil.

From an environment life cycle perspective, the sugarcane industry is associated with significant benefits. All the co-products are utilized in an environmentally friendly manner. For example, bagasse and potentially other cane derived fibrous fractions are used in electricity generation. Filter cake and bagasse furnace ash are sent to the field as fertilizer, molasses is used in livestock feeds and/or converted into biofuel. Vinasse, the effluent from ethanol distilleries is used *as is* or, after concentration into a concentrated molasses stillage (CMS), as an organic fertilizer.

Furthermore, sugarcane is, amongst all cultivated plants on a commercial scale, the most efficient sequestrator of carbon dioxide which is fixed in the cane biomass through photosynthesis. More than 120 tonnes of biomass are obtained per hectare of land under cane cultivated on an annually renewable basis and, in the process 18 tonnes of carbon (or an equivalent of 66 t of carbon dioxide) are fixed. Around 12,000 kWh of electricity and 750 litres of ethanol are recovered in power plants/distilleries from 120 t biomass per hectare of land under cane cultivation. Furthermore, sugarcane is associated with aesthetic benefits in that it provides a green cover attractive for the tourism industry.

In the process of recovery of sugar from cane, factories consume large amounts of water and if such water is not properly managed, it will impact the quality of the receiving water bodies and its fauna and flora or biodiversity. In Mauritius, this amount had been varying from 0.5 to even 14 m³/tonne cane depending on whether such factories are located in areas with plentiful availability of water (downstream of super humid region) or low rainfall or dry areas (Northern plain and the West of the island). The source of such water is normally rivers flowing in the neighborhood of the factories. After use, such water is discharged, normally after some form of treatment into the receiving

In Sustainability of the Sugar and SugarEthanol Industries; Eggleston, G.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

water bodies – like the river or lagoons. Now, most factories send such water to irrigate cane fields, while ensuring that it meets legal norms applicable to same.

Studies have established that all factory effluents are loaded with organic substances, have low dissolved oxygen, high BOD-5, high temperature, and contain oil and grease. The untreated effluents have a marked depression on biodiversity at the outfall points compared to that in the upstream of the river. Such water can recover only at a certain distance from the outfall ranging between 1.2 to even 3.5 km.

Over the years, as from 1984, the number of factories in operation has decreased from 21 to 7 currently. The implication from a biodiversity perspective is that all the negative impacts of effluents on the receiving water bodies due to the outfalls from the closed factories into rivers and lagoons have been eliminated. Moreover the remaining factories in operation have invested in water conservation, recycling, and re-use measures which have reduced their water intake per tonne of cane processed. Centralization is to continue as per the MAAS Action Plan with only 4 factories in operation by 2015. Hence, impact of effluents on biodiversity will decrease significantly to enable sustained cane/sugar/energy production from cane biomass.

A Strategic Environmental Assessment (SEA) was conducted in 2007 (*15*) with the objective of describing, identifying, and assessing the likely significant challenges and effects of implementing the strategic action plans with regard to the environmental impact of the sugar cane industry restructuring. All issues associated with the reform of the sugar sector starting from sugarcane farming to ethanol production were addressed. The Environmental Assessment was undertaken through a thorough consultation with 63 experts and involved in-country research and policy makers. The process included site visits, statistical data consultation and analysis, as well as comparative reviews of in-country research results with international literature. The study concluded that the overall implications are likely to have positive environmental effects. However, a number of environmental concerns associated with some aspects have been identified. The potential risks associated with those concerns can be managed if adequate precautionary measures are taken during implementation. Upon adoption of the mitigation and enhancement measures recommended in the SEA, any adverse impacts on the environment can be offset and even exceeded by the environment.

Another major advantage from an environmental perspective is that the both air and water qualities have improved. The centralized factories are also associated with power plants – normally equipped with state-of-the-art pollution abatement technologies in the form of electrostatic precipitators. Such equipment brings down the particulate matter content in the stack emission to a very low level, well below the permissible limits. Such power plants have also adopted closed circuit systems for cooling purposes. Only make-up water is used as boiler feed water and for condenser cooling purposes. Power plants have invested in costly demineralization plants as well.

Investment in the power plants coupled with centralization has implied that the number of discharges be it for air emission or water effluents have decreased. The negative impacts arising out of particulate matter, dissolved oxygen, BOD,

temperature, suspended solids, and oil and grease on the receiving water bodies like the rivers and marine sites/lagoons, have been eliminated.

In terms of carbon emissions, the 450 GWh of equivalent energy generated from bagasse (both for internal use and export) has avoided the use of around 300,000 tonnes of coal annually. The coal ash avoided would be equivalent to around 85,000 tonnes/year. Moreover, a cane biomass yield of 120 t/ha is equivalent to a carbon sequestration of 18 t carbon or 66 t of carbon dioxide. This has a beneficial impact on greenhouse gas emissions.

Conclusions

Mauritius has, over time, been able to produce cane and sugar on a sustainable basis through plans, policies, and measures which have incorporated technological advancements, both in cane growing and processing. It has also tapped as many opportunities as possible to increase its portfolio of products from cane (specialty and white refined sugars, electricity, and ethanol) and enhance its revenue from the sugarcane industry. All through the process, the socio-economic and environmental issues have been given due consideration. In terms of local value addition, sugarcane relies only on 15% of imports compared to 80% in the other economic activities like manufacturing and tourism.

The sugar industry is thus highly integrated in the Mauritian economy and society and to a greater degree than any other economic activity. It has contributed to the diversification of the economy into sectors like food crop for local consumption, the Export Processing Zone (EPZ), manufacturing and tourism which benefited from the capital and technical expertise of the industry. Such expertize relates to management and skilled workers who are able to dispense and transfer their know-how in the emerging sectors.

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Chapter 5

The Success and Sustainability of the Brazilian Sugarcane−Fuel Ethanol Industry

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Currently, Brazil has 410 sugar and ethanol plants that crush about 660 million tons of cane per crop, producing about 28.5 billion liters of ethanol and 38.7 million tons of sugar. New sugarcane varieties launched in the last two years are less demanding in water, have high sugar concentration, and are more adaptable to mechanical harvesting. Regarding the sustainability of ethanol production from sugarcane, it is essential to consider the use of the land, reduction of greenhouse gases (GHG), bioelectricity production from bagasse, energy balance of ethanol produced from sugarcane and reduction of vinasse. No other technology available to date has been able to transform the sun's energy and to reduce carbon emissions as efficiently and economically as the production of ethanol from sugarcane and its use as biofuel. This amazing combination of the sun's energy, fixation of CO_2 by sugarcane, and the transformation of sugars into a high quality, clean, liquid fuel has made the ethanol industry in Brazil a success as well as an example of sustainability.

Introduction

Brazilian sugarcane production dates back 400 years. At the beginning, sugar was the main product, and later the distillate "cachaça" and then ethanol fuel in 1920-30. Ethanol production until 1975 was marginal totaling 300 to 600 million

liters, used chiefly for industry and neutral alcohol for beverages. In this same year the Brazilian government launched the ethanol program due chiefly to the high international oil prices in 1973.

Because of the international oil crises and dependence on petroleum imports, it was necessary to choose alternative energy sources that could replace oil derivatives. In 1975, the Alcohol National Program, named "ProÁlcool" was instituted by the Brazilian government (*1*). Initially the program was based on anhydrous alcohol production to be mixed with gasoline. However, after the new oil crisis in 1979, beyond the mixture to the gasoline, it was initiated the manufacture of automobiles that used only ethanol. The success of ethanol cars changed the automotive industry. The concern about pollution came in the 1980s to replace the lead in gasoline with ethanol, chiefly in the city of Sao Paulo. The brain defects caused by lead contamination in the air decreased significantly after substitution of lead with ethanol in gasoline.

In the middle of 1980s the production of cars running with hydrated ethanol reached 98% of all vehicles produced in Brazil, increasing the consumption of this fuel and reducing oil imports. At this time, due to decrease of oil prices, and the lack of subsides for ethanol, the ethanol plants in Brazil started producing sugar as well. To overcome the low prices in the market and its costs of production, without government subsides, the sugar and alcohol industry improved their fermentation processes. New technologies were developed and transferred to industries that allowed them to survive different crises that occurred in the last 20 years (*2*).

Today, another step has been reached regarding the sustainability of sugarcane production with the cogeneration of electricity program by the sugar and ethanol industries. Production of fuel ethanol from sugarcane in Brazil has many advantages in comparison with other biofuels produced from different raw materials such as corn, sugar beet, and sorghum in other countries. Despite low investments in research it has been essential to keep production costs at low levels and to improve sustainability of ethanol production. In addition, the development of new technologies and diversification of products such as bioelectricity, biodegradable plastics, yeast as feed, and other co-products open new opportunities of expansion.

The Use of the Land: Fuel versus Food

Unlike the European Union (and other parts of the world) dilemma of fuel versus food, so far Brazil has no problem regarding this matter. Figures 1 and 2 show an increase in sugarcane, corn, and soybean production in Brazil over the same time period. It is important to emphasize that sugarcane has not occupied or limited the expansion of other important crops such as corn and soy bean that uses 36.3 million hectares in 2009, 13.1 for corn and 23.2 for soy (*3*, *4*).

Regarding the use of land, another important initiative from the Brazilian government was to carry out an agroclimatic zoning for sugarcane and several crops in the whole country, to conserve watersheds, forests, and aquatic reserves (*5*). Most of the sugarcane expansion areas are degraded pastures that do not support more than one cow per hectare. These areas of degraded pastures exceed
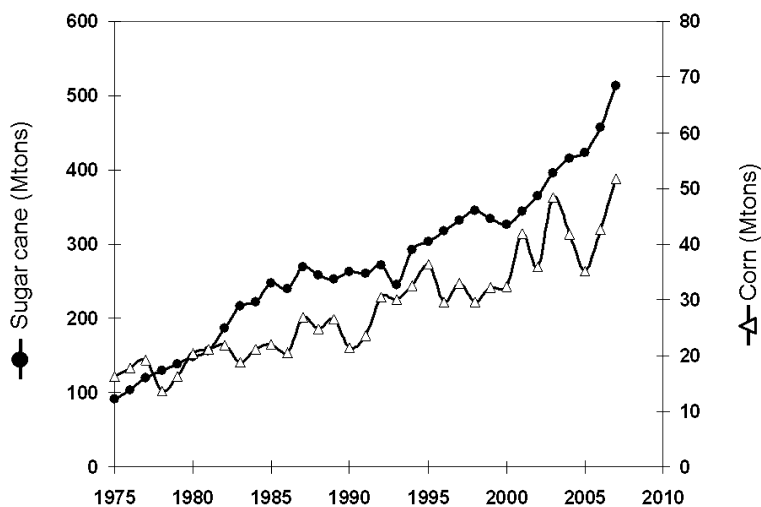
Figure 1. Sugarcane and corn production in Brazil

100 million hectares and are located very far from the Amazon wet tropical forest where sugarcane does not grow well (*6*).

Concerning deforested areas, there is no evidence that it has been used for the expansion of sugarcane fields. In Brazilian states (Sao Paulo, Minas, Parana and Goias) where the growth of sugarcane areas was 1.2 million hectares there was also a simultaneous growth of forested areas (3.6 million hectares). Moreover, some states where sugarcane was not cultivated have increased their deforested areas. This means that sugarcane expansion in relation to land use has been insignificant to deforestation as well as to food crops such as corn and soy bean (*7*). Moreover, it is important to emphasize that 45% of all sugarcane produced in Brazil is destined for sugar production for internal consumption as well as to export to several countries worldwide. The remaining sugarcane (55%) is crushed and processed for fuel ethanol production.

In 2009 just 0.87% of all land in Brazil was used to produce sugarcane. Half of this sugarcane area was destined for ethanol fuel production while corn and soybean occupied 4.23% of Brazilian lands (*3*). In 2009, each hectare cultivated with corn and soybean produced in average 4.2 and 2.9 tons of grains, respectively, while the same area produced 81.6 tons of sugarcane, 10.1 tons of sugars, 6,218 liters of ethanol, and 9.8 tons of bagasse (*4*). In addition, sugar production processes generate molasses, a by-product very rich in sucrose, glucose, and fructose. Molasses has been used by the distilleries for ethanol production where it is mixed with low quality sugarcane juice to be fermented by yeast cells. After the fermentation step the yeast cells are recycled in order to be used in the next fermentation step, while the wine is distilled to produce ethanol and vinasse (*8*).

Vinasse is the resulting liquid after removal of ethanol by distillation. It is rich in minerals, chiefly potassium but also in calcium, magnesium, nitrogen and phosphorous. Vinasse is used to fertilize the sugar cane fields, recycle nutrients, improving water use and physical conditions of the soil. It was demonstrated that soil pH drops after application of vinasse but it increases gradually after some days
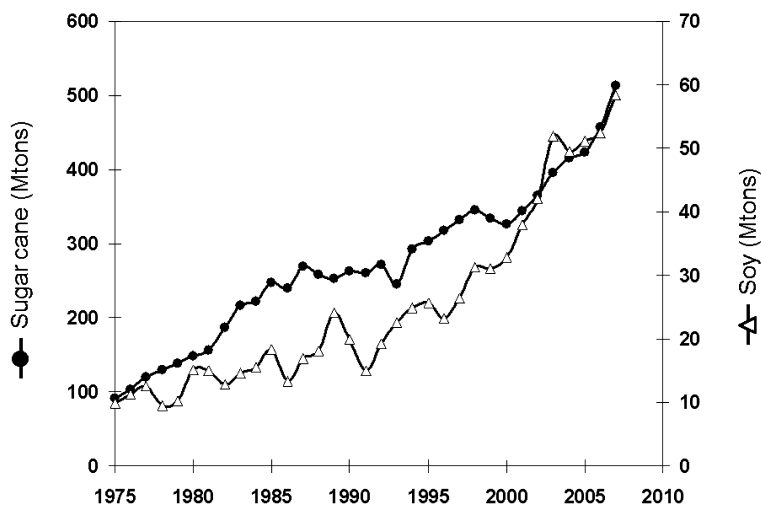
Figure 2. Sugarcane and soy production in Brazil

because of microbial activity (*9*). Activity of soil microorganisms is stimulated by the organic compounds and nutrients from vinasse. However, application in the field requires careful procedures to avoid contamination of table waters.

Minerals from filter cake and fly ash are also incorporated into the soil. Some nutrients such as nitrogen and sulfur can be lost to the atmosphere during the burning of sugarcane in the field for manual harvesting. The replacement of manual harvesting by mechanical harvesting preserves a layer of vegetative sugarcane trash (leaves and tops) on the soil, increasing the recycling of nutrients (*10*). Moreover, some sugarcane varieties can fix atmospheric nitrogen in association with *Glucoacetobacter diazotrophicus*. In addition, other species of diazotrophic bacteria associated to sugarcane have been isolated since the pioneer work carried out by Dobheiner 50 years ago (*11*) that opened new possibilities for biological fixation of nitrogen. Regarding the need for nitrogen fertilizers from the petroleum industry, sugarcane is less exigent than other crops. The low rates of nitrogen application in Brazilian sugarcane fields have allowed sugarcane to have a favorable energy balance in comparison with other crops (*12*). Just carbon, oxygen and hydrogen are removed in large quantities through sugar and ethanol production.

Ethanol is a liquid fuel that contains 93% of the energy found in sugar. While the sugar might be poetically called "the crystallized energy from the sun" formed through photosynthesis, water, and CO_2 by sugarcane, the ethanol represents the sugar energy converted to liquid fuel by a living process carried out by yeast cells. A great biodiversity of yeast strains have been observed in industrial processes and enormous efforts have been undertaken to select new yeast strains with improved fermentative abilities (*13*). Recently, the genome of two Brazilian yeast strains (CAT1 and PE2) selected by Fermentec in the 90s were sequenced and characterized by molecular techniques (*14*, *15*).

Nowadays, the gasoline sold in Brazil contains 25% anhydrous ethanol. This percentage is controlled by the Brazilian government and might vary according

to offers for and prices of anhydrous ethanol in the market. Besides the addition to gasoline, in the last years there were an expansion of the use of ethanol by a growing fleet of light vehicles running with flex fuel engines (*16*). In January 2009, ethanol surpassed gasoline as fuel for cars in Brazil. Today more than 95% of all cars sold in the Brazilian market are flex fuel. This means that they can run with gasoline, which contains anhydrous ethanol, or any blend with ethanol. Besides of the fleet of light cars moved to ethanol, most of the planes used for aerial spraying crops also use ethanol as fuel, reducing sulfur and carbon monoxide emissions.

Reduction of GHG Emissions

Nowadays, sustainability of biofuel production has been one of the main issues focused in current discussions over the world. Because of its prompt availability to displace fossil fuels, ethanol production from different feedstocks has gained attention due to the need to mitigate GHG emissions worldwide. Of course other reasons also include oil prices and diversification of the energy matrix.

The balance of GHG emissions from Brazilian bioethanol production has been considered the best among all biofuels currently produced (*7*). Considering a full life-cycle analysis of ethanol production and consumption, the reduction of GHG emissions reached 90% compared to gasoline. This result is much better than avoided emissions for ethanol produced from corn (30%) and sugar beet (45%).

According to studies carried out by Macedo *et al.* (*17*) along of all life-cycle of ethanol production from sugarcane production and processing, the most significant GHG emissions were attributed to soil emissions, followed by agricultural operations and transport, burning of the sugarcane in the fields before manual harvesting, use of fertilizers, and ethanol distribution. However, current technologies available to sugar and ethanol industries such as mechanical harvesting and surplus of electricity from bagasse and sugarcane trash have contributed to reduce the emissions of GHG in relation to total energy produced by sugar and alcohol industries.

With the prohibition of burning the cane for harvest, many people have to be trained to cope with the evolution of fully mechanized harvesting and direct planting. In the state of Sao Paulo, more than 60% of the cane is mechanized and harvested green. On the other hand, re-composition of the forest reserves in crop areas, including riparian forest, is mandatory for sustainability programs, not only for the reduction of GHG but also to preserve water reserves, biodiversity, soil conservation, and others.

Variation in the land use, due to massive deforestation, would be an additional source of GHG emissions. However, the expansion of sugarcane fields in Brazil is not taking place on forests but on degraded pastures that do not involve deforestation. For this reason the impact of GHG emissions due to change of land is very small (*18*).

On the other hand, the global warming caused mainly by industrialized countries and emerging economies based on use of fossil fuels (petroleum and coal) has changed the CO_2 concentration in the atmosphere as well as the global

climate. These changes in the world climate have also induced the change of crop practices in several countries and the use of lands. This means that while we have focused the discussion on the very small impact of bioethanol on GHG emissions due to use of land, the largest change has been caused by fossil fuels. Thousands of tons of CO_2 have changed the climate in several places in the world. These changes have affected small communities, forests, rivers, as well as the use of land for agriculture. For this reason, ethanol also represents an excellent alternative to coal and oil for dependent countries. Emerging economies such as China, India, and Africa may improve the bioethanol production from sugarcane reducing environmental impacts caused by coal, oil and its derivates.

In summarizing the sugarcane industry, Figure 3 shows that from 1 ton of sugarcane one third (145 kg) is sugar, one third (280 kg) is bagasse with 50% moisture, and one third (140 kg) is trash (tops and leaves of the sugar cane that are burned off before harvesting or left on the field to decompose in Brazil). The sugar can be crystallized and sold as sugar, and the bagasse can be burned to produce steam to run the factory and transformed in electrical energy. Bagasse can also be used to make paper and other products. Today, the trash can be burned to produce steam and/or electricity, or sold to feed boilers (orange juice concentration factory, soybean extraction oil, etc.). In the future, it could also be used to produce ethanol.

From Bagasse to Bioelectricity

The energy matrix of the World vs Brazil is remarkable different. While the world uses 88% non-renewable energy and 12% renewable, Brazil uses 45% renewable and 55% nonrenewable (*18*). In the region where sugarcane is planted it does not rain in the harvest season and there is a shortage of electricity due to the low level of water of the hydroelectric power plants. Just at that time, the plants produce electricity from the bagasse, and in the future, from sugarcane trash also.
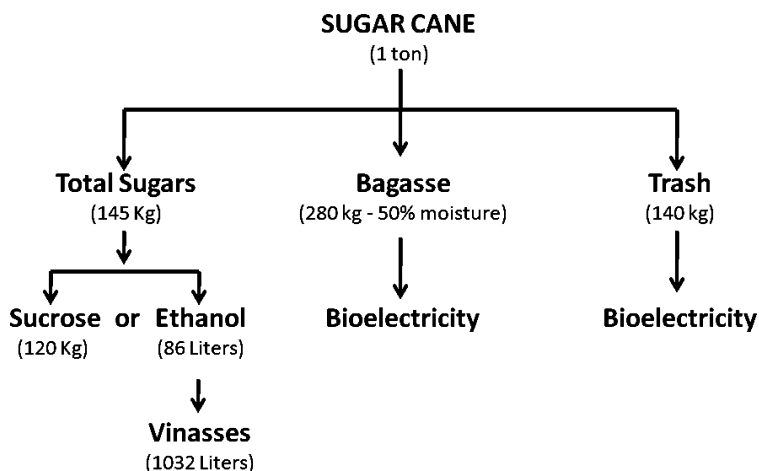
Figure 3. Sugarcane potential to produce energy

The first Brazilian sugar factory started to produce surplus electricity in the second half of 1980s. Considering the sugarcane production in 2009, about 4.6 TWh of surplus electricity could be produced and commercialized. However, for the same amount of sugarcane produced, but using both sugarcane bagasse and trash as fuels, the potential could be 5-7 times higher than what has been produced.

In 2009, the electricity produced from sugarcane bagasse contributed 3% of the total electricity production in Brazil. It has been recognized by the sugar factories in Brazil that diversification of end-products as sugar, ethanol, bioelectricity, dry yeast, and other products is essential to enhance the industry's competitiviness as well as to survive market oscillations and economc crises. In addition, there are new opportunities to transform factories into biorefineries. However, it is necessary to invest in research, transfer of new technologies, and training of technical staff.

Presently, the energy balance between corn and sugarcane are both positive, however, the output in sugarcane is 7.2 times higher than corn. The energy in cane production and transport shall increase in terms of MJ/ton, because of the increase in mechanization, not only in harvesting, but also in planting. The total fossil energy input should increase from 233.8 to 262.0 MJ/ton in 15 years. However, the total renewable output will increase from 2,185 to 3,032 MJ/ton in the same time (*9*). Considering the production of bioethanol, bioelectricity, bagasse, and trash, the energy balance will increase from 9.3 to 11.6 in 2020 (*9*). The highest positive balance for sugarcane can be explained due to use of bagasse as a source of heat and bioelectricity (biomass) in the production of ethanol, including crushing, evaporation, and distillation (*19*).

The development of new technologies and improvement of industrial processes have reduced the consumption of energy, making the distilleries more efficient. In the industry, to improve efficiency in energy, boilers passed from 21 bar to 86 and 92 bar saving 2.6 times more steam per KWh (from 12.5 to 4.7 Kg steam/KWh) than older systems. Ethanol produced from Brazilian sugarcane is the biofuel with the best energy balance. It means that a positive ratio between renewable products and the energy input as fossil fuel. For Brazilian sugarcane ethanol this balance is 9.3 much more than ethanol from corn (1.3) and wheat from Europe (2.0) (*18*).

This energy balance represents the ratio between renewable energy output (ethanol + electricity + bagasse as fuel) to the fossil energy input in different stages of the supply chain. The advantage of sugarcane can be explained due to the fact that bagasse (a fibrous material) can be used as fuel at the factories, producing steam and electricity. One third of all sugarcane produced in 2009 by Brazilian factories (600 million tons) was bagasse with 50% moisture. All these crop and industrial characteristics make the ethanol production from sugarcane a success in sustainability. Based on Brazilian experience, other countries where sugarcane grows well may adopt similar programs for bioethanol, bioelectricity, and sugar production with low impact on the environment.

Solutions to Vinasse

In the ethanol plant there is a co-product, vinasse, which is rich in potassium and some organic matter. All the plants uses this vinasse, mixed with water, to wash the cane and fertilize the cane field, and with this procedure the cane and sugar yield increases significantly. In this way, potassium and other minerals return to cropped soils. Brazil imports more than 85% of the potassium it needs, and this process brings a tremendous economic gain.

However, for each liter of ethanol produced, 12 liters of vinasse are generated. This happens because the alcoholic content in the wine at the end of fermentation reaches 8-9% in the Brazilian distilleries. This means that for 26 billion liters of ethanol produced in 2009 another 312 billion liter of vinasse were generated. Nowadays the government keeps track of the levels of potassium, nitrate, and others elements in the soil, to avoid table water contamination.

An interesting idea arose six years ago to reduce vinasse volumes with savings in energy and transport costs. Fermentec Ltda developed a process which increases the ethanol concentration in the fermented mash up to 16% with yeast recycling, and with this process the vinasse is reduced by half or 6 liters per liter of ethanol (Table I). The vinasse volume is reduced while ethanol increases because this fermentation process works with higher sugar concentrations in the wort (aka as must) than traditional fermentations used by Brazilian distilleries. Consequently ethanol concentrations reach 16% (v/v) while traditional processes work with 8-9% (v/v) of ethanol. While sugar concentrations in the wort and ethanol contents in the wine increase, the volume of vinasses is reduced because less water is used in the fermentation. However, because Brazilian distilleries re-use yeast cells, several industrial parameters were modified to maintain yeast cells with high viability during successive recycles with high ethanol concentrations in the wine. Without these improvements would be impossible to increase the ethanol content in the wine and maintain the yeast cells with high viability.

Table I. Reduction of vinasse volume with higher ethanol % in the wine

Ethanol % in the wine (v/v)	Volume of vinasse / liter of ethanol	Concentration factor[a]
5.0	20	1.00
7.5	13	1.53
10	10	2.00
14	7.0	2.86
16	6.0	3.33
18	5.5	3.63

[a] Obtained in relation to vinasse volume produced by fermentations with 5% of ethanol in the wine.

In Sustainability of the Sugar and SugarEthanol Industries; Eggleston, G.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

This process will decrease the energy for distillation by 0.56 to 0.80 Kg steam/ liter of ethanol, and bring an economy of two U.S. dollars per ton of crushed sugar cane for vinasse distribution in the field.

Overall Conclusion

In summary, it has been demonstrated that the development and transference of new technologies to factories need to be aligned to economical and sustainability proposals. The success of first and second ethanol generations of biofuels production depends upon investment in research. In this way, we will build the sustainable knowledge for biofuels production.

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Chapter 6

The South African and Southern African Regions – Part I: Sugarcane Production

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South Africa is the main producer of sugarcane on the African continent and cultivates around 20 million tonnes of sugarcane on an annual basis from just under 400,000 ha of land. It is one of the world's leading cost competitive producers of high quality sugar. As an exporter, it is the world's sixth largest net-exporter of raw sugar and makes an important contribution to employment, particularly in rural areas, sustainable development, and the national and regional economy. In line with global changes, the sugar industries of southern Africa have adopted a longer-term focus when it comes to sugarcane research, in terms of the development of new varieties suitable to each region and industry needs, crop management, and the transfer of information to growers through extension services. Other issues of importance to sustainability include responding to changes in climatic conditions, support services for small-scale and first-generation growers, and the effect of HIV/AIDS on the industry.

Introduction

The developing countries of the African region are particularly well positioned to contribute significantly to some unique demands in the wake of a global recession. The need for renewable energy resources has focused attention on those areas where abundant or underused land is available and where the climate is conducive to potential 'energy' crops. These crops (including sugarcane) could allow for the cost-effective production of bioenergy, biofuels, and chemical intermediates in regions where unemployment is extensive, thus fulfilling economic, social, and environmental needs simultaneously. To this end, issues such as hunger and poverty, the HIV/AIDS pandemic, corruption, governance and poor service delivery, and lack of infrastructure and proliferation of totalitarian leaders or cultures will most certainly have to be addressed.

The Republic of South Africa

South Africa is by far the largest sugar producing country in Africa. The South African sugarcane industry comprises roughly 390,000 ha (*1*) and is situated in three of the country's nine provinces – KwaZulu-Natal, the Eastern Cape, and Mpumalanga (Figure 1).

The industry is well established in terms of infrastructure and supply chains. It is strictly governed by The Sugar Act of 1978 and associated Sugar Industry Agreement (SIA 2000) which has survived the political realignment of 1994 only because it was recognized that the conditions on the international sugar market are severely distorted and could lead to the demise of the industry if no protection was retained. Despite a recent decision by the South African Government that the provisions of the Sugar Act will be revised, sugar will continue to be treated as a special case due to the large contribution of sugar to the GDP and exports of the country. Direct employment in the industry is approximately 79,000 with an estimated 1 million people relying on the sugar industry for their livelihood.

The SA Sugar Act provides the industry with three pillars of support, namely: a) protection against low world sugar prices, b) provisions for the establishment of equitable export obligations for processers (aka millers) and growers alike, and c) maintenance of a managed, equitable special arrangement for sugar trading within the Southern African Development Community (SADC) region as Annex VII of the SADC Trade Protocol (www.sadcpf.org).

A substantial obstacle in this strict regulatory environment between growers and processers is the issue of revenue sharing if, for example, sugarcane biomass is to be used for cogeneration. This will need to be addressed and resolved quickly and efficiently to facilitate and encourage investment, value-addition and diversification which are crucial for sustainability in this industry (*2*).

A total of 2.3 million tonnes of sugar and 115 million liters of ethanol are produced per annum. Approximately 37% of the sugar is exported, rendering the industry particularly vulnerable to volatile world prices.

Most of the sugarcane (76%) is rainfed, with the balance being irrigated. While rainfed cane usually results in much reduced yields (longer growing seasons), irrigation is expensive and power-intensive with limitations on its
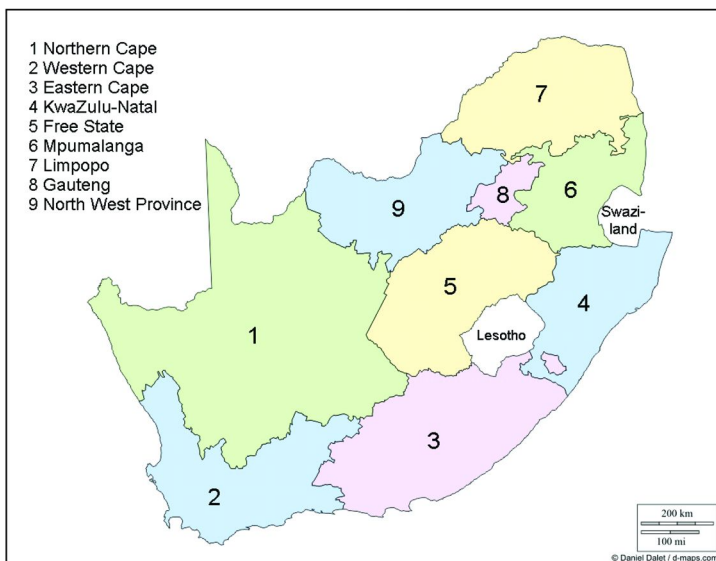
1 Northern Cape
2 Western Cape
3 Eastern Cape
4 KwaZulu-Natal
5 Free State
6 Mpumalanga
7 Limpopo
8 Gauteng
9 North West Province

Figure 1. The nine provinces of the Republic of South Africa (d-maps.com)

application. Sugarcane requires roughly 850 mm mean annual precipitation, with an optimum of 1,300 mm, while daily mean temperatures should range between 22 and 32 °C (*3*). The annual rainfall for South Africa during the 2008/09 season (June-May) was only 964 mm (*1*).

Because of the relatively low rainfall, poor topography throughout most of the sugarcane cultivated land, a variety of soil types, and high incidence of pests and diseases, the country is not ideally suited to sugarcane. Despite a highly optimized industry, South Africa still obtains the lowest yields of cane per ha (refer to Table I) compared to neighboring countries. It is, therefore, not surprising that the South African owned sugarcane companies are rapidly expanding operations and investment in other African countries, although this is to a large degree also influenced by the export opportunities to the European Union (EU) enjoyed by these countries, with South Africa being the only developing country in the 79 member African, Caribbean, and Pacific (ACP) group that has been denied access to the EU market.

The Southern African Development Community (SADC)

SADC was formed in 1992, essentially creating a regional free trade area under instruction of the SADC Trade Protocol. The SADC region currently extends over 3,300 km (2,050 miles) from Cape Town in South Africa to Kinshasa in the Democratic Republic of Congo (DRC) and consists of: Angola, Botswana, the DRC, Lesotho, Madagascar, Malawi, Mauritius, Moçambique, Namibia, Seychelles, South Africa, Swaziland, Tanzania, Zambia and Zimbabwe (Figure 2).

The SADC vision is one of a common future, within a regional community that will ensure economic well-being, improvement of the standards of living and

1 DR Congo
2 Tanzania
3 Seychelles
4 Malawi
5 Zambia
6 Angola
7 Namibia
8 Botswana
9 Zimbabwe
10 Mocambique
11 Madagascar
12 Mauritius
13 Swaziland
14 Lesotho
15 South Africa

1000 km
600 mi
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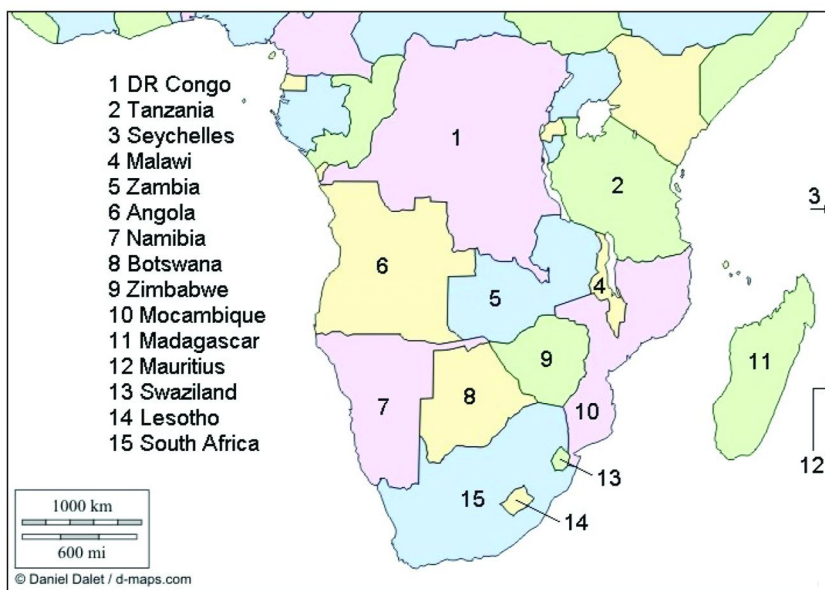
Figure 2. The Southern African Development Community (d-maps.com)

quality of life, freedom and social justice, and peace and security for the peoples of southern Africa. A free trade area was officially launched in August 2008 (achievement of 85% free trade within the SADC region).

A total of 661,000 ha is currently under sugarcane cultivation (*1*). Rainfall, climate and soil conditions favor cultivation in the countries closer to the equator and a slow migration of sugarcane northwards is predicted. Cane production and rainfall figures for the 2008/09 season are given in Table I.

While Mauritius is part of the SADC region, their technological development has been notably different mainly due to their dependence on coal importation and large share in preferential sugar trade agreements with the European Union, and significant investment from Britain. Mauritius started with sugar production more than a century before the rest of the SADC and is better associated with the developed world sugar industries. The Mauritian sugar industry is, therefore, discussed separately in Chapter 4 of this book (*4*).

Due to the distorted condition of the international sugar market, a special annex (Annex VII) to the Trade Protocol was created to regulate sugar trade within the SADC region. The objective of this annex is the full liberalization of trade in sugar by 2012, dependent on the progress of reforms to the international sugar market. To this end, a Technical Committee on Sugar was established, consisting of government and private sector members, to develop a regional strategy for the optimal development of the SADC sugar industries. Until now, the sugar sector has been shown to be the most highly integrated agricultural sector in the SADC region and provides an example of successful private sector involvement in regional structures and processes (*5*).

Table I. Cane production and rainfall figures in the SADC sugarcane producing countries (2008/9 harvesting/processing season) (*1*)

Country	Area under cane (1,000 ha)	Yield (tonne/ha)	Total cane (mln tonnes)	Rainfall (mm pa)
Malawi	19	114	2.3	1,225
Mauritius	66	73	4.5	2,190
Moçambique	38	68	2.1	610*
South Africa	389	67	19.3	940
Swaziland	52	98	4.9	530
Tanzania	43	77	2.7	1,020
Zambia	11	124	1.3	925
Zimbabwe	43	69	2.6	n/a
Total	661	690	40	8,070

* Average of 404 mm in the south and 817 mm in the north of Moçambique.

Climate Change

Atmospheric concentrations of greenhouse gases are on the increase and there is strong evidence that such changes are affecting the earth's climate (*6*). The sustainability of the SADC sugarcane industries, where rainfall and temperature play a dominant role, will depend upon the industry's ability to monitor the changes, and adapt accordingly. Active programs are, therefore, in place in an attempt to predict the effects of climate change, breed location-specific sugarcane varieties, and improve agronomic and crop protection strategies.

Rainfall

Despite the difficulties in climate change predictions, experts believe that rainfall in KwaZulu-Natal and Mpumalanga is likely to increase slightly in the intermediate future (*7*). Increased rainfall bodes well for improved sugarcane yields, but the rainfall may be more erratic and perhaps more extreme (*8*). Soil conservation structures (e.g., waterways, contours) will need to be improved, wetlands and watercourses maintained, and in the irrigated areas, rainwater harvesting optimized.

Temperature

The mean global temperature has displayed an upward trend since the early 1900s, with an even steeper rate of increase since the 1960s (*7*). A mean annual increase in South Africa's daily temperatures will affect a number of agronomic factors, such as evaporation, soil moisture, yields and growing season, amongst others. It is believed that South African temperatures may increase by 1.5 to 2.5 °C during the next 30 years, with even greater increases possible by 2080

(*7*). Increased temperature could affect sugarcane production in the country in a number of ways:

- Increased temperature in the cooler inland areas could enable an expansion in the area suitable for sugarcane production. This in turn has implications on the location of sugarcane factories.
- Less frequent frost events and higher average temperatures in the cooler midlands of KwaZulu-Natal could allow expansion of sugarcane into previously frost-prone areas, as well as enabling a shorter growing season for cane in this traditionally long-season (18-24 month) region.
- The incidence and distribution of sugarcane pests and diseases are likely to be affected by future climate change in South Africa.

The eldana stalk borer (*Eldana saccharina* Walker), for example, is one of South Africa's most serious sugarcane pests (*9*). Eldana moths require optimal temperatures after sunset to ensure mating success. In South Africa's sugarcane industry, Mpumalanga and north-eastern KZN display these conditions for the greatest number of days per annum (*10*). As climate change drives temperature increases, the area with temperatures favourable to eldana mating will increase (*7*), potentially allowing this pest to spread through more of the industry.

A similar effect could occur with the spotted sugarcane stalk borer, chilo (*Chilo sacchariphagus*). Although this pest has not yet been found in South Africa's sugarcane industry, it has recently been identified in sugarcane fields in Mozambique (*11*) – one of South Africa's northern neighbors. Mpumalanga and north-eastern KZN currently display temperatures conducive to chilo mating and survival success (*12*), and further temperature increases could allow future spread of this pest into other areas of the industry.

The brown rust fungus (*Puccinia melanocephala*) is currently one of South Africa's most challenging sugarcane diseases. High temperatures and humidity levels, as well as prolonged periods during which the sugarcane leaf is wet, are most conducive to rust infection (*13*). Mpumalanga and north-eastern KZN are currently the areas most favourable for rust infection (*14*). Increased temperatures could favor the spread of rust to other areas of the sugarcane industry, but greater duration and intensity of rainfall events are detrimental to rust infection rate (*15*), potentially limiting such a spread in the future.

Research Direction

The South African Sugar Association Experiment Station (SASEX) was established in 1925 as the research arm of the South African Sugar Association (SASA). One of the principal functions of the station was the introduction and testing of sugarcane varieties suitable for South African conditions – one of the driest sugarcane producing areas in the world. In 2004, SASEX changed its name to the South African Sugarcane Research Institute (SASRI) in line with its evolved longer term research focus. SASRI is the cornerstone of sustainable

sugarcane agriculture in the entire region, as underpinned by the Institute's vast support services and the four multidisciplinary research programs:

1. Variety Improvement
2. Crop Protection
3. Crop Production & Management
4. Systems Design & Optimisation

Stage	Number of Varieties	Year Midlands	Irrigated
Crossing	(1,500 crosses)	0	0
Nursery	250,000 seedlings (potential varieties)	1	1
Single stools	175,000	1	1
Single lines	20,000	3	2
Observation Plots	2,000	6	4
1° Variety Trials	330	8	6
2° Variety Trials	100	10	8
Propagation	25	13	10
Bulking	5	14	11
Release	2-3	15	12

Figure 3. Flow chart of the variety selection program at SASRI. Crops in the KZN Midlands have a longer growing period – 18-24 months – making the selection process longer. Crops in the irrigated areas grow for 12 months. Source: Varieties, Breeding and Selection course note. Senior Certificate Course in Sugarcane Agriculture (SASA), 2010.

The **Variety Improvement Program** encompasses activities that facilitate the development and release of varieties with sucrose, yield, pest and disease, agronomic and processing characteristics that are desirable to both processers and growers. The first South African-selected variety, NCo376, was released in 1955, resulting in a substantial increase in sugar yield and number of ratoon crops (*16*).

Sugarcane breeding (i.e., seed production) started in 1944 (*17*). Today, modern biotechnological methods are used to enhance parental selection, deliver novel, desirable traits, develop systems for the rapid bulking and distribution of high-quality seedcane and investigate the biological basis of sucrose accumulation in sugarcane, with a view to enhancing the process.

Figure 3 describes typical activities in the variety selection program. Initial crosses are made between suitable 'parent' varieties each year. Seeds from these crosses are assigned to the research stations in five major agroclimatic regions for field testing. At each stage of the selection process, the number of varieties is reduced, with the selected varieties being planted in larger plots in which their performance is more reliably evaluated. This process takes 11 to 15 years. A Variety Release Committee (VRC), consisting of plant breeders, pathologists, entomologists, extension specialists and senior managers consider one or two of the resulting potential varieties for bulking, where they are inspected for trueness to type and freedom from serious diseases (*17*). A number of 'N' varieties have been released to date; the most recent, N51, in 2009.

Continued variety trials are conducted throughout the industry to assess performance of the commercial varieties under different climatic and management conditions (*18*). The wide range of climate and soil conditions makes it important to breed a "basket" of varieties to cover the different conditions. The importance of long-term forward planning and strategies to ensure that varieties are bred that will meet the industry's needs in 10 to 15 years' time (*17*) is recognized, thus ensuring the sustainability of the industry with suitable, profitable sugarcane varieties.

The key objective of the **Crop Protection Program (CPP)** is to minimize the effects of pests, diseases, and weeds on crop production. The CPP includes research projects run by entomologists, pathologists, weed scientists, biotechnologists, and nematologists who work in multi-disciplinary project teams to develop novel methods of protecting the sugarcane crop. The Program has a large number of projects and procedures in place. Better management practices (BMPs) have been developed, whereby growers are encouraged to follow guidelines to reduce pest and disease impact. These BMPs address management strategies such as appropriate planting procedures (using disease-free seedcane), field hygiene, chemical pest and weed control, harvesting procedures that limit disease spread, and habitat management. In conjunction with this program, a disease identification service is provided which supports Local Pest and Disease and Variety Control Committees, whose function is to monitor pest and disease levels in the various areas and control the use of released varieties.

Two of the research programs target crop management and systems design – Crop Performance and Management (CPM) (*19*, *20*), and Systems Design and Optimization (SDO) (*21*). The main objective of the CPM program is to enable sustainable and increasingly profitable use of resources to produce and deliver

quality sugarcane to the factory. It achieves this by designing situation-specific best management practices for variety, soil and water use, harvest and transport systems, and chemical and nutrient use. The CPM program includes research projects run by agronomists, modelers, soil scientists and bioresource engineers who work on multi-disciplinary project teams to develop and refine the sustainable use of resources to produce sugarcane. Researchers in the **SDO** program include soil scientists, agronomists, and bioresource engineers to investigate and develop innovative systems to optimize crop production through modeling, technology design and a farming systems approach.

Finally, the SASRI Extension Service provides the valuable link between researchers and growers, enabling research results and advice to be disseminated to growers in an effective way. Extension specialists are located in every sugarcane growing region and cater to both large and small scale growers.

The Environment

The South African sugar industry actively promotes sound and sustainable environmental practices in line with national legislation and international requirements.   This is achieved through national and local environmental structures and has an extensive set of guidelines for environmental protection and benchmarking of individual farms.   These cover field practices such as soil conservation, cane extraction and management practices (land preparation, pest and disease control, harvesting, fire protection etc.); conservation of water resources, with regard to water courses and wetlands, irrigation and drainage; air pollution; soil management; traffic regulation and cane spillage; and the management of employee village sites, farm pollution and chemical storage. Natural resource and cultural asset management is also covered, including on-farm public recreational facilities (*22*).

The industry is further involved in sustainable resource management through a Memorandum of Understanding with the Worldwide Fund for Nature - South Africa, and is also involved in a SADC-wide initiative aimed at establishing a guide on environmental best management practices for the regional sugar industries.

Small-Scale Sugarcane Growers

In South Africa, as in many other sugarcane producing areas, individual ownership of land title by small-scale farmers is not common, and land users are mostly farming on untitled traditional authority land that may have a 'permission to occupy' (PTO) attached. Growers may only have access to limited areas for both cultivation and grazing, fearing the loss of use of land to another deemed more worthy if it is not used for production.

Since 1994, the South African Government has embarked on Black Economic Empowerment land reform and restitution programs. The land reform program has resulted in a vast increase in the number of titled land transfers between willing buyers and sellers of freehold sugarcane land, to meet the target of 30 %

black ownership of freehold sugarcane land by 2014. There are now indications, however, that this target date may be set back as there is a lack of available funds within Government to pay the sellers and to settle and support the new black farmers. The sugar sector is the most advanced agricultural sector in terms of land reform, with around 19% of freehold land under sugarcane already transferred; far in advance of the total agricultural sector figure of 7.5%.

The current distribution between small-scale individual growers and large-scale commercial growers is shown in Table II. For various reasons, the numbers of both large- and small-scale growers have been on the decline for the last number of years.

Support services, both formal and informal, specifically aimed at small-scale growers and growers new to farming, exist in all production areas. These institutional arrangements require strengthening to have improved leverage and capacities in purchasing inputs as well as managing the production of a quality product. The growers are often at the mercy of community leadership or contractors in defining the timing and operations of their businesses and the decisions taken are not always in the best interests of the individual growers. Due to the scale, growers are unable to justify the machinery required to plant and harvest a bulky crop such as sugarcane, so the use of contractors is unavoidable (*23*).

There remains an unreasonable expectation by growers as to what the land is able to deliver in terms of financial reward, and this expectation is perpetuating a system of less and less investment into successive sugarcane ratoon crops, allowing a downward spiral of yields from year to year. If a grower was able to generate an additional income from production of biomass, there may be an additional incentive to re-invest, also generating a certain degree of security of cane supply to the 14 sugar factories in South Africa.

Recognized as one of the greatest socio-economic challenge facing the South African sugar industry is the ability to transfer appropriate information and technology to communal first generation growers, who may be absentee, old or part-time farmers and may lack the capacity, will and resources to apply recommended practices or inputs. There is, therefore, a concerted effort to encourage the growers to pool their land resource to increase the viability of larger production units. These co-operative farming units are then professionally managed with the growers receiving a land rental and annual dividend proportionate to their area contribution, and being able to sell their labor into the farming unit.

The Effect of HIV/AIDS

Of the 33.4 million people living with HIV globally, 22.4 million are living in Sub-Saharan Africa; South Africa has the largest number of HIV infections in the world (5.7 million) (*24*) with the highest incidence in the KwaZulu-Natal rural areas.

Manual labor in the South African sugar industry is favored and sometimes unavoidable due to the steep slopes, the high cost of mechanical harvesters and the perceived sucrose losses associated with mechanization. The sugar producing

areas are found predominately in rural areas where the incident of HIV/AIDS is the highest.

Figure 4 depicts the projected loss in labor due to HIV/AIDS from 1985 to 2020 (25 years) in nine of the SADC region countries (*25*). These figures put the impact of HIV/AIDS on the regional workforce in the years to come in stark perspective. An anonymous HIV survey involving the voluntary testing of 1,500 South African farm employees revealed that 30% of the workers surveyed were infected with HIV with a significantly higher prominence in females.

Small Scale Growers

HIV/AIDS has no doubt partly contributed to the declining numbers of small-scale growers who deliverd cane to the factories, from 23,577 in 2004/5 to 16,280 in the 2008/9 season (*1*).

The specific impact on small-scale growers in rural areas is summarized as follows (*25*):

- Decrease in the area cultivated
- Decrease in weeding
- Increase in fallow land returning to bush
- Growers are moving to less labor-intensive crops and animal production
- Missed planting seasons

Commercial Growers

There are few figures to show the impact of HIV/AIDS on commercial farming in South Africa. There is general consensus in the literature that the most immediate effects are as follows:

- Increased absenteeism amongst the labor force.
- Loss of workers.
- Loss of valuable agricultural skills.

Case studies show that more workers are often required to do the same work, as they are not strong enough to work to full capacity. In some cases whole villages of seasonal workers have disappeared.

In addition to the shortage of skilled, unskilled labor and the costs of replacing and training staff, farmers are experiencing huge additional costs that include:

- Transport to clinics, and time off for visits
- Support of workers' families: each worker infected has dependants and is usually supporting a number of orphans and extended family members
- Funeral costs
- Payment for anti-retrovirals (ARVs) and other medical bills, since clinics are generally under resourced
- Time for assistance with government processes such as obtaining of grants

Table II. Distribution of small- and large-scale growers in South Africa (*1*)

	Large-scale	*Small-scale*
Total land holding (ha)	330,000	70,000
Number of registered growers	1,626	16,280
Average land holding (ha) per grower	200	4
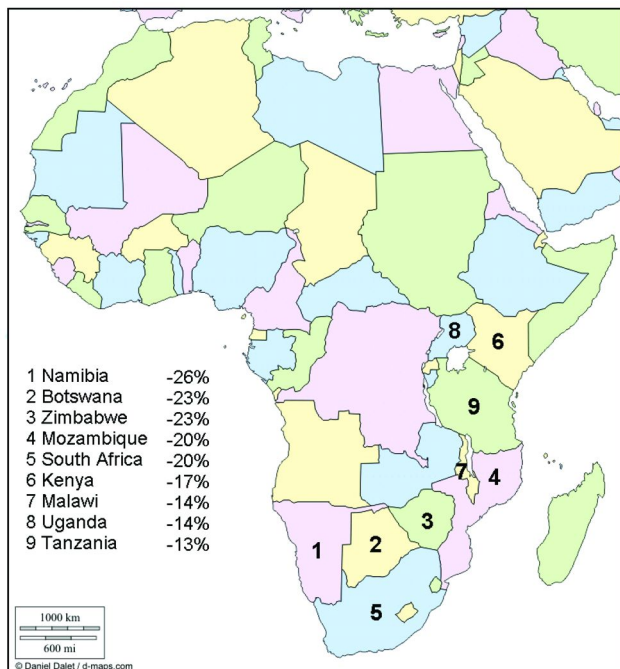
Figure 4. Projected loss in agricultural labor force through HIV/AIDS in the nine hardest-hit African countries (1985-2020) (22)

Response from the Sugar Industry

The sugar industry through SASA supports and contributes to initiatives that will ensure the sustainability of both small-scale and commercial growers (www.sugarindustrydev.co.za). The sugar industry, in comparative context, has been a leader in corporate social investment initiatives. Every single sugar factory village has an active HIV/AIDS program and functional clinic, and both growers and processer efforts appear to be in advance of what is happening elsewhere in agriculture.

The SASA health program's main objective is to increase communities' capacities to coordinate and respond to health challenges in a sustainable manner. Its other objective is to invest in the prevention, protection and improvement of the people's overall physical and mental well-being. SASA's support therefore focuses on:

- Community awareness programs
- Income generation programs
- Home based care programs
- Food parcels programs
- Support programs for child headed households
- AIDS orphans

Sustainable Farming

The long-term sustainability of the South African sugarcane industry will no doubt depend on sound management of economic, social and environmental factors. Sugarcane growers now have access to a management system which takes all of these factors into account, and plots their progress towards attaining better management practices. In 2004, the Worldwide Fund for Nature (WWF), in conjunction with its South African-based Mondi Wetlands Project, formed a partnership with the Noodsberg Cane Growers in the KwaZulu-Natal Midlands. Workshops were held with all major stakeholders including government policy makers, environmental non-governmental organizations, and sugarcane growers. The result of this process was the establishment of the Sustainable Sugarcane Farm Management System (SuSFarMS), designed to encourage sustainable sugarcane production through the implementation of Best Management Practices (BMPs). The SuSFarMS system is a tool which takes into account relevant local and international legislation and BMPs developed by the sugar industry. A list of criteria, against which cane growers are scored, is used as an audit by the growers themselves or extension specialists, and the status of the farm determined (*26*).

The BMPs chosen needed to be practical, workable, sustainable, and suitable for use as an extension tool. The main framework of SuSFarMS is underpinned by three main principles of sustainability: economic (economically viable sugarcane production maintained or enhanced); social (rights of employees and the community upheld); and environmental (natural assets conserved, and ecosystem services maintained). All BMPs chosen needed to address some or all of these principles. The audit check sheet allows the grower's current performance level to be determined against the BMPs listed, areas of strengths or weaknesses to be identified, and a corrective action plan to be developed (*26*). Growers can conduct a self-audit using the SuSFarMS check sheet in order to prepare for the second-party audit, which can then be conducted by extension specialists. A field visit is included to gain an overall impression of the farm.

Growers exposed to the system thus far have commented on how the system has improved their management by highlighting areas of weakness, and shown willingness to exchange management information with fellow growers and extension specialists. Numerous grower groups throughout the South African sugar industry have expressed interest in adopting the system (*26*). It is hoped that, with the spread of the SuSFarMS auditing system, South African growers will identify areas of strength and weakness, which will ultimately equip them to farm – environmentally, economically and socially sustainably – into the future.

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Chapter 7

The South African and Southern African Regions – Part II: Sugarcane Processing

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The optimization of sugar recovery from sugarcane has always been the main focus of the southern African sugar industries. Except for Mauritius which was forced to invest in the cogeneration of electricity, the abundance of coal and resulting availability of electricity has shaped the industry into a supplier of mainly crystal sugar and minor producer of by-products such as bioethanol, animal feed, and paper products. Legislative changes in the European Union policies that impact directly on sugar trade agreements and the recent drive towards renewable energy and chemical intermediates have forced these industries to realign their strategic direction to ensure sustainability both in the short- and long-terms. With vast arable land suitable to sugarcane production available in the region, ethanol production, cogeneration, biomass gasification, and a host of other by-products have moved to the forefront of research and development efforts. Availability of research funds, both public and private, will ensure the survival of the industries.

Introduction

The ability to control and utilize earth's natural resources to the betterment of an individual or community is unique to the human species on this planet. In the last century alone, phenomenal technological advances were made to aid in these activities. Recently, changes in world mentality have springboarded the responsibility that is vital to ownership of such powerful abilities, and industrial efforts worldwide are increasingly geared towards responsible practices to sustain human efforts for future generations.

In South Africa, the recent political realignment has heralded a strong emphasis and commitment from the new government to the principles of Sustainable Development, already underpinned by vast efforts, resources and funding that have been poured into all sectors of the country and region. International trade reforms, the global recession, and the new focus to produce alternatives to petroleum-based fuels and chemicals, are putting pressure on sugar industries worldwide to consider adding value to their core business and sustainability issues. This chapter considers the southern African sugarcane processing activities with specific emphasis on South Africa, as the biggest sugar producer on the continent. Current production, planned expansions and diversification into cogeneration, ethanol and other value-added products are discussed.

Sugar in the Southern African Development Community (SADC)

With the exception of South Africa, all of the SADC sugar producing countries have, until 2009, enjoyed preferential sugar export prices through agreements with the European Union (EU). These agreements were built on the Commonwealth Sugar Agreement (CSA) established by Britain after World War II in 1951 with their former colonies to a) ensure development and independence of sugar industries in the Commonwealth countries (excluding Australia) and b) secure raw sugar imports for Britain. Due to political pressures, South Africa withdrew from the Commonwealth in 1961 and was, therefore, not included in subsequent agreements. When Britain joined the European Economic Community (EEC) in 1974, the CSA evolved into the African, Caribbean and Pacific (ACP)/EEC Sugar Protocol and in 2000 the ACP/European Union (EU) Sugar Protocol. A Special Preferential Sugar (SPS) Agreement was introduced when Portugal joined the EU in 1995 (www.acpsugar.org). Table I lists sugar prices in different markets, including the USA Tarrif Rate Quota for developing countries, compared to the world sugar price in February 2000 (*1*).

When South Africa negotiated its own Free Trade Agreement with the EU in the late-1990's, the Trade and Development Cooperation Agreement (TDCA), sugar was intentionally not included by the South African government as its inclusion would have meant that the quotas allocated to other SADC member states would have had to have been reduced to accommodate the South African ambitions.

As part of reforms to the EU's Common Agricultural Programme, the European Commission embarked on reforms to the EU's sugar regime. This

Table I. Sugar prices obtained in different markets - February 2000 (*1*)

Market	Price per ton (US$)	Index (World=100)
ACP/EU – Sugar Protocol	530.76	425.6
Special Preferential Sugar	448.67	358.9
USA – Tariff Rate Quota	354.39	283.5
World	125.00	100.0

involved gradual liberalization of the sugar market, including reductions in EU sugar production and also the domestic reference price paid for sugar imports. Fixed sugar quotas for ACP members were replaced by import thresholds for various ACP regions, under the system of Economic Partnership Agreements. As part of these reforms, in 2009 the Everything but Arms (EBA) initiative saw that all duties and levies be removed for all imports from 46 least developed ACP countries, to be phased out over a number of years, forcing many of the industries into major reforms. The removal of guaranteed quotas and reduction in the reference price likewise affected developing country ACP sugar producers. Mauritius was particularly affected, having enjoyed a major share of the sugar export quotas under the former agreements (refer to Chapter 4 of this book (*2*)).

SADC countries currently produce 4.8 million tonnes of sugar annually (*3*) and are among the lower-cost producers of high quality sugar in the world. The long processing season (33-37 weeks) enables better use of capital equipment than in most other sugar producing areas.

Of the sugar produced (raw, very high pol VHP, and refined) 43% is exported (*3*), which constituted 4.6% of the world sugar exports in 2007 (*4*). The main producer in the region is South Africa with 47% of the total sugar produced by 14 of the 40 factories in the region. However, sugarcane cultivation in the rest of SADC is expected to increase due to higher yields and better conditions for sugarcane cultivation compared to the South African conditions. There is thus a strong drive for South African and other sugar companies to invest in the rest of SADC. Availability of arable land and labor, and concerted effort towards the economical and social upliftment of the developing countries is stimulating investment further, with sugarcane being one of the main sources of biomass under consideration. Sugar activities in some of the relevant SADC countries for the 2008/09 crushing season are shown in Table II (*3*).

It is interesting to note from Table II that countries like Mauritius can export all of their locally produced sugar into the EU market at preferential rates and then import sugar for local consumption at much lower world market prices.

Green Cane Harvesting

Sugarcane non-stalk residues (such as leaves) have a detrimental effect on the factory during processing, and the best and most economical way to get rid of these, both for the grower and the processer in the southern African region, is through burning of the cane prior to harvest. Coinciding with the Australian industry's

move towards total green cane handling (i.e., without burning), by the 1950s a sub-committee of the South African Sugar Technologists' Association declared that cane burning was outlawed and would thus be phased out over a three-year period (*5*). That was more than six decades ago and yet 92% of the cane in South Africa, and most of the cane in the rest of southern Africa, is still burnt prior to harvest. The exception is Mauritius that has been practicing green cane harvesting for the last number of decades, mainly due to the emerging tourism industry and for electricity generation to avoid coal importation from South Africa.

Amongst the main reasons why green cane harvesting in Africa is not very attractive, are:

- most of the cane in the region is harvested manually; manual cutting of green cane is 30% less productive (cutting and bundling) and, therefore, more costly than burnt cane (*6*),
- wildlife residing in the cane (snakes, spiders, scorpions, and small mammals) are not only life threatening to cane cutters when not properly contained but some may also provide a free meal for the family back home if trapped during a controlled burn, and
- environmental pressures are only effective around the up-market urban and tourism areas which constitute a relatively small percentage of sugarcane cultivated land on the continent.

Nonetheless, there is some evidence that alternative grower incentive schemes and education efforts are convincing growers and cutters to reconsider (Figure 1).

There has recently been renewed effort towards utilization of the potential value of biomass (such as sugarcane leaves) for energy and bioproducts (*7*). In South Africa the national energy crisis of 2007 and beyond left most major businesses and households powerless and in complete darkness. This was because of scheduled blackouts by the parastatal electricity supplier in an effort to manage inadequate power supply. Major resources have since been made available for renewable energy sources, as is discussed elsewhere in this chapter.

As the local leader in this field of study, trials at Swaziland's Ubombo factory to investigate cleaning of cane in-field, followed by drying the crop residue, baling, transport, and grinding at the factory for use as fuel for electricity generation has been ongoing for the last 10 years. Sadly the outcomes show overwhelmingly that such a system is simply not economical, mostly because of high transport costs (road freight).

In 1939 the South African Sugarcane Experiment Station (now The South African Sugarcane Research Institute SASRI) had the foresight to start a long-term trial at a dryland site to investigate the effects of burning compared to green cane harvesting (trashing) on agronomy under varying conditions and scenarios (*8*). Now in its 70th year, this experiment, called the BT1 trial, is the longest running sugarcane experiment in the world and has culminated in a wealth of knowledge that includes the effects of trash blanketing or tops left in the field on organic matter retention, fertilizer treatment, pest management, water retention, and ultimately yield improvements in the main sugarcane varieties used in the southern African sugar industry (*9*). This information was recently assimilated into a model, called

Table II. Total sugar* production, consumption and trade for the sugar producing SADC countries (2008/09 crushing season) (*3*)

Country	Production (1,000 tonnes)	Consumption (1,000 tonnes)	Imports (1,000 tonnes)	Exports (1,000 tonnes)
Malawi	300	200	-	70
Mauritius	450	40	40	450
Moçambique	250	170	-	130
South Africa	2,260	1,430	140	840
Swaziland	630	320	-	300
Tanzania	280	350	80	-
Zambia	260	130	-	130
Zimbabwe	390	160	-	140
Total	**4,820**	**2,800**	**260**	**2,060**

* Total sugar includes raw and VHP sugar as well as refined sugar

the Economics of Trashing (ET) Decision Support Program (DSP), developed over a period of 8 years (*10*). The program aimed to assist any particular grower in the decision on whether to trash or burn; and if they trashed, how much trash to leave behind if they wanted to collect the trash and sent it to the factory. The model was recently extended to assess costs of trash collection (including agronomic costs) and value of trash as a coal replacement, thereby assisting growers and processors in deciding the price to which coal must rise to justify trash collection/ buying, under various agricultural conditions (*7*). Interestnigly, the outcome for each and every grower, be it a small- or large-scale operation, differed and the model has, therefore, been instrumental in the optimizations of individual farms and community farming efforts alike.

From the factory perspective, an assortment of trials - from laboratory to pilot plant to short and long-term large scale factory endeavors - have been conducted over approximately the last 50 years. The more substantial of these include the industry-wide investigation of options to purposefully increase the volume of bagasse produced by altering harvesting techniques and consequently factory operations. This study concluded that the concept was simply not economically viable for either the growers or the factory. A number of trials followed to elucidate some of the findings and to extend the concept to factories operating cane diffusers (*11–13*)

Throughout southern Africa there is a continuous focus on improving harvesting and transport logistics. This includes recognition of the potential need to haul trashy cane and to develop trash-based by-products while protecting the sugar manufacturing process from adverse effects such as decreased extraction, increased colour and poor juice clarification. A particular characteristic of the South African sugarcane industry is the incredibly expensive transport systems, and vast over-capitalization, compared to mainly centralized transport operations elsewhere in the SADC region.

Figure 1. A cane cutter in the field in South Africa

Cogeneration

Production of electricity (through cogeneration) by sugarcane factories in excess of what is needed by the factory and related operations is still limited in the region to isolated cases, such as Mauritius where ~16% of the island's electricity demand is generated from bagasse. Being a volcanic island, Mauritius's lack of natural resources such as coal, which is abundant on the rest of the continent, helped to advance its cogeneration operations.

Historically, the electricity price in South African has been low due to a surplus of installed generating capacity and an abundance of cheap coal. However, this is rapidly changing. Profitable export markets for coal have become available, leading to an increase in the price of this fuel in the local market. In addition, due to the rapid economic growth in the country, there is a shortage of generating capacity that started to show severe effects in 2007, in the form of scheduled electricity blackouts. To fund new capacity, price increases have been severe, and further increases of 24% per annum over the next three years will be implemented. This sharp increase in price will make electricity sales by sugar factories far more profitable than they have been in the past. Although this driver may appear to be specific to the South African electricity market, it will have a broader impact since South Africa is a substantial exporter of electrical power to neighboring countries and thereby affects the price and availability of electricity within the entire region.

The South African government has undertaken to promote renewable energy technologies. The use of a range of measures to integrate renewable energy into the mainstream energy economy is proposed, with market incentives being utilized to promote these technologies. It appears likely that incentives will be offered to renewable energy projects by South African companies for implementation

anywhere within the SADC region. A renewable energy contribution of 10,000 GWh to the total energy consumption by the year 2013 has been targeted (*14*), with the production of electricity from sugar factory bagasse being specifically highlighted.

Cogeneration and Sustainable Development

The generation of renewable electrical power from sugarcane biomass yields positive benefits in all three spheres (i.e., economic, societal and environmental spheres) of sustainability.

In terms of social benefits, the provision of electricity to all areas of the country is important to free, particularly women, from the burden of collecting wood for fuel. One of the highest causes of infant mortality is from acute respiratory illness associated with the inhalation of wood smoke (*14*) and this can also be alleviated by the provision of electricity to all. Renewable electricity can assist with the electrification programme by expanding generating capacity within South Africa. The cultivation of biomass for renewable energy generation (e.g., sugar cane growing) also provides for income generation in rural areas. Sugar factories, in general, provide important centres of economic activity in rural communities. Ensuring the sustainability of sugar processing and growing through cogeneration, therefore, provides social benefits in these areas.

The generation of renewable energy can lead to substantial environmental benefits both locally and globally, and can reduce the dependence of the region on fossil fuels with their associated greenhouse gas emissions. Electrification will reduce the dependence on wood as a fuel, which will reduce air pollution and the environmental degradation caused by unsustainable harvesting practices. Renewable electricity is inherently more sustainable than current electricity generation technologies in the region, which are based primarily on coal as a fuel.

Cogeneration in South African Sugar Factories

Cogeneration is still only being undertaken on a limited scale in South African sugar factories. At current price levels (which averages around 17 to 18 SA cents (2-3 US cents) per kWh, Dec 2009) the export of electrical power is only economically viable when it is carried out using back-pressure turbo-alternators, by means of the high pressure steam, with the low pressure exhaust steam being used in sugar processing. Back-pressure turbo-alternators typically exhaust steam at a pressure of 200 kPa(a), which is useful for heating purposes in a sugar factory. Only a portion of the total energy value of the high pressure steam is, therefore, used for electrical power production. The marginal cost of electricity generation under these conditions is in the order of 14 SA cents per kWh, based on the cost of the fuel alone.

The marginal (fuel only) cost of cogeneration using condensing turbo-alternators (in the order of 65 to 70 SA cents per kWh under current conditions) is too high to be profitable at this present time. Condensing turbo-alternators typically exhaust steam at a very low pressure of 15 kPa(a), which is of no use for heating purposes in sugar production. The entire heating

In Sustainability of the Sugar and SugarEthanol Industries; Eggleston, G.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

value of the high pressure steam is therefore used in power production, which increases the associated costs. The same applies to generation using back-pressure turbo-alternators when making use of steam that is not essential for processing (i.e., wastefully increasing the steam consumption of the factory to allow for more electricity generation does not generally make economic sense). Consequently, electricity export is currently carried out only by those factories with a surplus of installed back-pressure turbo-alternator capacity and a steam demand high enough to allow for marginal generation at a reasonably low price. However, unique conditions may exist at select factories to justify higher levels of export.

Although government incentives for renewable electricity production will increase the amount of cogeneration carried out by the sugar industry, the impact is unlikely to be substantial under current conditions. The proposed incentive payment for solid biomass-based generation is R 1.18 per kWh (*15*). While this tariff will cover the marginal operating cost of generation using condensing turbo-alternators, it is completely inadequate to cover the capital cost of the boiler upgrades, turbo-alternator capacity and factory energy efficiency modifications that would be required to carry out cogeneration on a sizeable scale. A further problem with the current incentive proposed by government is that bagasse-based electricity has been excluded from receiving the additional tariff, on the incorrect assumption that bagasse is a zero-value waste fuel. These barriers need to be overcome before large-scale cogeneration by the South African sugar industry can become a reality.

Ethanol from Molasses

The main focus of the South African sugar industry has been the optimization of sucrose recovery from cane to sugar. The current seasonal average extraction (14 factories) is 97.6% (sucrose based) which is much higher than most other sugarcane processing countries. Crystal sugar has been regarded as the only product of the process, with molasses being a by-product of limited value. This molasses has been sold at a fixed price regardless of the actual quality (the product must only conform to a minimum Brix level), although the price is adjusted annually.

Current bioethanol production is shown in Table III. However, a range of new ventures for bioethanol and cogeneration are currently being developed and deployed. Prior to the world recession (2009) much international funds were earmarked for such development projects.

The production of ethanol from sugarcane in South Africa has been limited to the fermentation of molasses and its use, since the 1960s, has been restricted to potable and industrial applications. No fuel ethanol is produced and none of the existing factories have an annexed ethanol plant. In southern Africa the Simunye factory in Swaziland has a full-scale ethanol plant (32 ML capacity), as does the Triangle sugar factory in Zimbabwe (25 ML; Figure 2). A small plant is also attached to the Hippo Valley factory in Zimbabwe.

Table III. SADC bioethanol production (molasses fermentation) – 2010

Country	Bioethanol (ML)
Mauritius	26
Malawi	30
South Africa	115
Swaziland	30
Zimbabwe	35

Ethanol as a Fuel in South Africa

Prior to 1954, all fuel consumed in South African was imported in a refined form. After the Second World War the demand for fuel products increased to such an extent that the establishment of a viable refining industry became possible.

For political and strategic reasons it was decided to embark on a synthetic fuel program through the parastatal company then known as *Suid-Afrikaanse Steenkool en Olie* or SASOL (translated: South African Coal and Oil). The SASOL I plant was established in 1954 to convert coal into synthetic fuel. In 1964, due to the uncertainties of the international crude oil supply situation and the oil embargo imposed on South Africa, the Strategic Fuel Fund Association (SFF) was established, whose brief was the acquisition of crude oil and the administration of the strategic crude oil stockpile.

The synthetic fuel industry was expanded with the commissioning of SASOL II in 1982 and SASOL III in 1983. Now known as Sasol Limited, the company is currently also South Africa's biggest producer of ethanol, which has frustrated alternative ethanol sources such as molasses. In 1987, a new parastatal producer of fuel, called Mossgas, was established to convert natural gas found off the eastern coastline to synthetic fuels. About one-third of South Africa's fuel demand is currently met by the synthetic fuels industry, and tariff protection is afforded to the producers of these fuels, although these tariffs are being progressively lowered.

Approximately 15% of South Africa's primary energy consumption is met by imported crude oil. Taking synthetic fuel production into consideration, liquid fuels meet approximately 28% of South Africa's final energy needs. As a result of the historical development of the liquid fuels industry and economic and political influences, the industry is characterized by a unique regulatory framework and a significant degree of government involvement. It is, however, recognized by the South African government that the liquid fuels industry would probably function better in an environment of minimum governmental intervention and regulation. Government has therefore agreed to take a step back in principle to create such an environment (*16*).

In addition, there is growing recognition of the need to employ renewable energy sources, of which biofuels are an important component. It is also recognized that the two most common biofuels are ethanol and biodiesel (*17*). This change in policy opens the door to the use of ethanol as a component of the fuel mix in South Africa. The government admits, however, that the biggest

Figure 2. The Triangle ethanol distillery in Zimbabwe

challenge will be to provide sufficient incentive for renewable energy based industries to develop, grow and be sustainable in the long run (*17*).

Currently, there is no legislation allowing for mandatory blending of ethanol in South Africa. Moçambique allows for a 5% ethanol blend (*18*), Malawi a 10% blend, and Zimbabwe a 10% (still to be ratified) blend. The proposed blending ratio for South Africa is E8 (8% ethanol blend). However, the use of ethanol as a fuel substitute will obviously only be viable if the cost of producing the ethanol is lower than the price obtained for the product.

The petrol price in South Africa is regulated and a large component of the price is fuel tax (20%). The government is aware of this, and the December 2007 Biofuels Strategy (*19*) proposed a 100% fuel tax levy exemption.

The government's biofuels strategy expects the costs of the biofuels to be ring-fenced, and remunerated separately, as the biofuels will be blended at the wholesale level. The cost to the motorist will be equivalent to US$65 per barrel (bbl) crude oil based refined products and therefore will present a benefit, although this will be limited if the price of crude oil stays above $65/bbl. The average oil price for October 2009 was $75/bbl.

Initially, it seemed that the government was hoping that an ethanol based biofuels program would become a strong driver of economic growth, investment, and especially employment. However, the economic incentive was not adequate to permit development of new areas under cane and new processing and distilling processing plants. This in turn, raised concerns of food security when it became clear that existing maize farmers would divert a portion of their crops to satisfy the biofuels market. As a result government support for a strong liquid biofuel component has waned.

Value Added products

Value addition to sugarcane products in southern Africa is currently limited to the following:

- bagasse used as a fuel for steam generation in sugar factory boilers
- bagasse used for its particulate fibre properties to make paper
- bagasse used as a source of xylose to make furfural and related products
- bagasse and molasses as an animal feed or supplement
- molasses as a substrate for alcohol production by fermentation

In South Africa relatively small production facilities exist that produce the following by-products from either bagasse or molasses (Figure 3):

- furfural (20,000 tonnes per annum)
- flavor compounds (150 tonnes per annum)
- ethanol (Merebank 50, Glendale 5 and NCP 60 ML per annum)
- paper – board and tissue (bagasse usage: Sappi Stanger 60,000, Mondi Felixton 155,000 tonnes bagasse per annum
- animal feed (2 facilities)

While the isolation and refining of sugarcane wax on a small-scale has been investigated extensively and achieved, lack of appropriate markets has stymied these endeavors.

Figure 3. The Sezela furfural plant on the south coast of KwaZulu-Natal in South Africa

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Within SADC, the SADC Secretariat commissioned a study in 2005 which analysed the production potential for seven energy crops in member states - oil palm, sweet sorghum, sugarcane, sunflower seed, soybeans, jatropha, and cassava. The findings were that sugarcane topped the list because (*20*):

- it is already being widely grown in the region and its production can easily be expanded wherever there is irrigation and water,
- its impact on employment is high,
- considering that ethanol is produced from a by-product of sugar, molasses, there is a double benefit in terms of income,
- ethanol is already widely used as a petrol blend and the processing technology is known and available, and
- its direct benefit on foreign exchange savings are easy to calculate, depending on the blending rate adopted.

Drivers for Change

The industry is on the verge of entering a significant downstream/co-product production phase. Firstly, there is a strong global drive to move the manufacturing base from one which was largely founded on oil to a sustainable platform based on renewable resources. Secondly, the sugar industry has had to come to terms with the changes in the industry that have driven it towards larger economies of scale, rationalisation and the quest to recover more value from every stick of cane crushed.

Two driving forces are recognised, namely economic necessity and technological ability. Economic necessity requires adding money to the bottom line without the luxury of a long lead time. This sees sugar factories turning to cogeneration and distilleries since the technologies are familiar and can be purchased off-the-shelf. On the other hand, technological ability is focussing on the more fundamental possibilities such as turning C6 and C5 building blocks from plant material into commodity as well as fine-chemical products. This confronts the industry with a more long-term vision and starkly unfamiliar territory.

The secret to growing the sector will be to find the synergies between the two driving forces and these are unlikely to be the same for each sugar growing region.

Research Direction

South African sugarcane research is undertaken by two institutes, the South African Sugarcane Research Institute (SASRI, previously the South African Sugar Association Experiment Station – SASEX), and the Sugar Milling Research Institute (SMRI). SASRI was founded in 1925 and is funded by growers and processers through the South African Sugar Association (SASA). Research at SASRI is clustered within four multidisciplinary programmes, namely Variety Improvement, Crop Protection, Crop Production & Management, and Systems Design & Optimization. An Extension Service provides the essential link between

researchers and sugarcane growers. SASRI also offers a range of services including soil analysis, fertilizer advice, disease diagnoses and education courses.

The SMRI was founded in 1949 and is funded by processers only through the South African Sugar Millers' Association Limited. Research at the SMRI is focused on two thrust areas, namely a) reduction of production costs by up to 40% and b) development of new value added products. In both of these thrusts, innovative approaches and new technologies are at the forefront of the research. In addition, the SMRI provides a range of support services and plays a vital role in weekly benchmarking of the industry factories through its industry database. Most of the factories in the SADC region contribute to and benefit from this database that now spans over more than 50 years.

The research institutes realized that, to sustain the South African sugar industry into the future, there was a need for the industry to diversify towards a biorefinery concept. In 2009, the concept of a Strategic Sugarcane Research Platform was developed between the consortium members of SMRI, SASRI and the University of KwaZulu-Natal (UKZN), to attract substantial funding for sugarcane research from the Government's Department of Science and Technology. The aim of the Sugarcane Platform is the creation of sugarcane research and development leadership and innovation to enhance economic empowerment and human capital development in KwaZulu-Natal, in South Africa and in Africa. This will be done through developing varieties, technologies and production systems aimed at extending the existing industry lifecycle as well as producing competitive, high value products from sugarcane for a wide range of market sectors to create new revenue streams for the industry.

Much emphasis is being put on energy implication of all parts of the sugarcane value chain from biomass production systems to biomass processing systems and finally to the end products, which may include a variety of bioenergy products. To this end, the SMRI has recently joined the International Sugarcane Biomass Utilization Consortium (ISBUC) to strengthen collaboration with international researchers in the field in an effort to determine potential processes for energy and cost reduction to direct the research strategies of the SMRI and the Platform.

Conclusions

Concerted efforts and substantial investment within the SADC region are evident and geared toward the sustainable development of the sugarcane production industry. As such the sugar sector is being heralded as one of the most highly integrated agricultural sectors in SADC and one of the success stories of private sector involvement in regional governance. A regional Sugar Strategy was approved by the region's Trade Ministers in 2008, which is in the process of being implemented. The South African sugar industry is cohesive, with the South African Sugar Association playing a co-ordinating and managing role in terms of issues affecting the industry as a whole. Since South African companies are major operators in other African countries the strengths of the South African industry are readily transferred to these countries.

With the emergence of sugarcane as the biomass source of choice, due to its high energy value, the vast potential of cultivation and processing of this commodity in Africa cannot be overemphasised. The optimization of biomass recovery, cogeneration of electricity, and diversification into a range of by-products is being actively considered and pursued. The possibilities for employment, technological development, and diversification of domestic economies are significant. Internationally, the agricultural sector within developing countries is being seen as a potential driver of development once again. Appropriate involvement and support from the various governments will be absolutely essential. However, success will ultimately depend on the timely management of obstacles in each region as well as those particular to Africa. As such, economic, social, and environmental integrity can only be obtained if all driving forces can be suitably aligned towards a common vision.

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Chapter 8

Pretreatment Technologies for the Conversion of Lignocellulosic Materials to Bioethanol

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The concept of energy crops, a renewable source of energy, has been around for decades. It was not until the discovery of fossil fuels, a non-renewable source of energy, in 1859 that agricultural and forestry crops and their residues stopped being the primary source of energy. Since then, fossil fuels have become the major source of energy generation and transportation fuels supplying 85% of the United States total energy demand. According to the U.S. National Renewable Energy Laboratory ethanol produced from energy crops could displace as much as 25% of gasoline currently consumed in the United States. Ethanol produced from energy crops mitigates many of the limiting factors associated with corn−ethanol or sugarcane−ethanol production such as competition with the food supply, availability of feedstocks, and transport costs. Nevertheless, the use of energy crops for ethanol production is still in the developmental stage. Available processing technologies suffer from relatively low sugar yields, severe reaction conditions, large capital investment, high processing costs, and great investment risks.

Introduction

Energy consumption of non-renewable fuels has increased steadily over the last century as the world population has grown and more countries have become industrialized. The United States, China, and Japan account for more than 40% of the total world consumption resulting in large volumes of crude

oil being traded and redistributed on a competitive basis (*1*). In the USA, the demand for transportation fuels is currently around 140B gal/year for gasoline, and 48B gal/year for diesel. Increasing energy security concerns, fluctuating oil prices, problems with CO_2 emissions, and concerns over possible future supply constraints have strengthened the interest in alternative non-petroleum based sources of energy. Biomass is a suitable and renewable energy resource that can be used for the generation of biobased transportation fuels such as ethanol.

A study supported by both the U.S. Department of Energy (DOE) and the U.S. Department of Agriculture (USDA) has indicated that the USA has sufficient land resources to sustain production of over 1 billion dry tons of biomass annually, enough to replace at least 30% of the nation's current consumption of liquid transportation fuels (*2*, *3*). This supply of biomass could represent a seven-fold increase over the 190 million dry tons of biomass per year being used for the production of bioenergy and bioproducts, of which only 18 million are used for the production of biofuels, primarily corn-grain ethanol (*2*, *4*, *5*). Corn ethanol or first generation liquid fuel has been used in oxygenated fuels since the 1980's at concentrations of up to 10% ethanol by volume or recently in blends as E85 (85% ethanol and 15% gasoline by volume). The use of ethanol blended fuels for transportation not only minimizes greenhouse gas emission and petroleum use but it is a safer alternative to methyl tertiary butyl ether, a toxic chemical used in gasoline to provide cleaner combustion (*6*, *7*). The sustainable and economic production of first generation fuels has, however, come under close scrutiny in the last decade attributed in most part to the competition for limited land and water used for food and fiber production (*8*). Demand for transportation fuels is expected to increase and the use of non-food biomass as a source for the development of second generation biofuels represents a reasonable approach.

Lignocellulosic biomass such as agricultural residues, forest products, and dedicated crops are considered potential sources for second generation bioethanol. Ethanol from lignocellulosic biomass can be produced mainly by two different conversion routes, biochemical or thermochemical. The thermochemical route involves the use of pyrolysis, liquefaction, and gasification technologies to produce synthesis gas (CO, H_2) from which a wide range of fuels can be derived. The biochemical route employs enzymes and microorganisms to convert cellulose and hemicellulose fractions to sugars prior to their fermentation into ethanol. At present, there is currently no clear commercial or technical advantage between the biochemical and thermochemical pathways, and both technologies remain unproven at full commercial scale (*9*).

The conversion of lignocellulosic biomass to ethanol is more complicated than that of first generation fuels from corn starch or sugarcane juice, and this has limited its commercialization. Published production costs for second generation fuels are in the range of $0.60 to $1.30/L (*9*). The wide cost range is attributed to the varying assumptions made for feedstock supply cost, pretreatment costs, performance efficiencies, and availability of both the feedstock supply and conversion technologies. Ethanol yields from the bioconversion of agricultural residues range between 29 to 70 gal/t dry biomass, whereas ethanol yields from forest residues range between 33 to 79 gal/t dry biomass (*10*, *11*). Furthermore,

lignocellulose at a theoretical rate of conversion of 85 gal ethanol/ton could provide biofuels for approximately 65% of the transport fuel market (*1*).

Sugarcane bagasse, the fibrous residue obtained from extracting the juice from sugarcane during the sugar production process, has great potential as substrate for second generation fuels as it is found in large quantities in the USA (Louisiana, Florida, and Texas) and in tropical countries. Sugarcane bagasse can also be generated by first generation fuel industries in countries like Brazil, India, and China where sugarcane juice is the substrate for ethanol conversion. Approximately, 5.4×10^8 dry tons of sugarcane bagasse are processed annually worldwide (*12*). Most sugar factories and distillery plants generate their own energy by burning close to 50% of bagasse residue. The remaining material is either stored in a stockpile or burned, hence the growing interest in developing technologies for its conversion not only to ethanol but other petroleum-based chemicals as well (e.g., polymers, resins). Approximately, 90% of the dry weight of lignocellulosic biomass is in the form of cellulose, hemicellulose and lignin. For the biochemical conversion of sugarcane bagasse or any other lignocellulosic material to ethanol, cellulose and hemicellulose must be broken down into their corresponding monomeric sugars (fermentable sugars) prior to their conversion into ethanol by microbial fermentation. The overall process can be summarized into six main steps: pretreatment, enzyme hydrolysis, detoxification, fermentation, ethanol recovery, and effluent treatment (Figure 1). Pretreatment of biomass is an extremely important step in the conversion of lignocellulosic biomass to fuels and adds approximately 30% to the total processing cost.

This book chapter provides an overview of the chemical and physical characteristics of lignocellulosic biomass and on the processing of sugarcane bagasse to ethanol by the biochemical route with emphasis on new developments of pretreatment technologies. Product separation, purification, and effluent treatment processes are not discussed in this review as these are already established technologies.

Lignocellulose Components

Lignocellulosic biomass is any plant material produced by photosynthesis. Potential lignocellulosic biomass for bioethanol production include crop residues (sugarcane bagasse, corn stover, corn fibers, rice straw, wheat straw, barley straw, coconut husks, sorghum bagasse), hardwood (black locust, poplar, eucalyptus), softwood (pine, spruce), herbaceous biomass (switchgrass, Bermuda grass), cellulose wastes (newsprint, recycled paper sludge) and municipal solid wastes (*13*, *15*). Lignocellulosic biomass is composed mostly of carbohydrate polymers (cellulose and hemicellulose) and lignin with compositions varying amongst plant materials (Table I). Proteins, ash and oils make up for the remaining fraction (*16*). The cellulose and hemicellulose portions make up almost two thirds of the total dry mass. Only the carbohydrates are hydrolyzed to simple sugars and eventually fermented to ethanol.
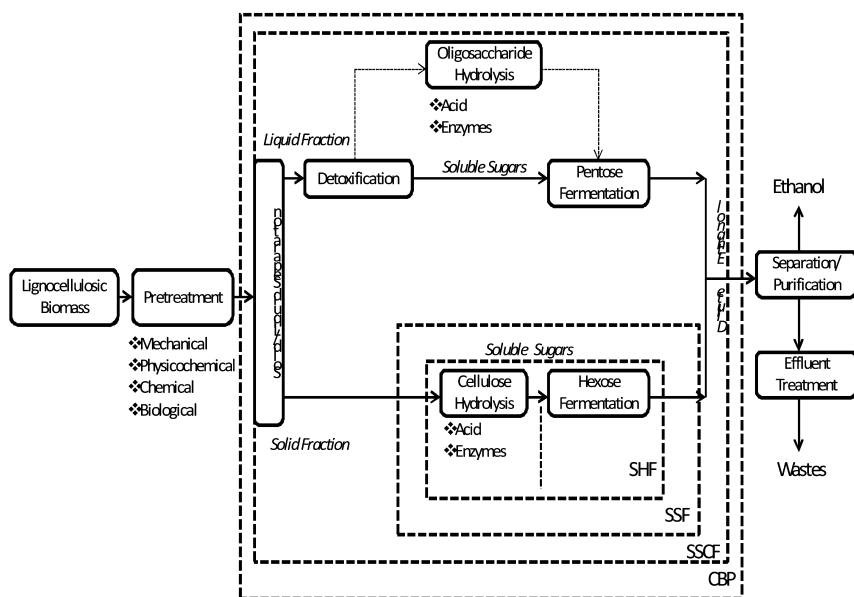
*Figure 1. Flow sheet for ethanol production from lignocellulosic biomass. SHF:
Separate Hydrolysis and Fermentation; SSF: Simultaneous Saccharification and
Fermentation; SSCF: Simultaneous Saccharification and Co-Fermentation;
CBP: Consolidated Bioprocessing, where enzyme production, hydrolysis and
fermentation of all sugars are performed in one step (13, 14).*

Cellulose

Cellulose is a linear macromolecular chain of D-glucose units linked by β-1,4-
glucosidic bonds with organized (crystalline) and not-well organized (amorphous)
structures (*17*). The orientation and linkages of these structures make cellulose
water insoluble and highly resistant to chemical and biological degradation (*18*).

Hemicellulose

Hemicellulose is located between the lignin and the collection of cellulose
fibers underneath. Unlike cellulose, hemicellulose is heterogeneous and is
composed of polymers like pentoses (xylose, arabinose), hexoses (glucose,
mannose, and galactose) and/or uranic acids (glucuronic, methylgalacturonic,
galacturonic) (*14*). The dominant component in agricultural wastes and hardwood
is xylan. Xylan is a complex polysaccharide with backbone chains of 1, 4-linked
β-D-xylopyranose units (*19*). Softwood hemicelluloses consist mostly of
galactoglucomannans (*20–22*). The xylan layer with its covalent linkage to lignin
and its non-covalent interaction with cellulose may play a role in protecting the
plant against enzymatic degradation (*23*).

Table I. Chemical composition of lignocellulosic biomass (percent dry basis)
(*4, 18, 36–42*)

Feedstock	Cellulose (glucan)	Hemicellulose (xylan)	Lignin
Softwoods			
Douglas fir	50.0	2.4-3.4	28.3
Pine	44.6	5.3-8.8	27.7
Spruce	45	6.6	27.9
Hardwoods			
Black locust	41.6	17.7	26.7
Hybrid poplar	44.7	18.6	26.4
Eucalyptus	49.5	13.1	27.7
Populus tristis	40-49.9	13-17.4	18.1-20
Crop Residues			
Corn cobs	45.0	35.0	15.0
Corn fiber	14.3	16.8-35.0	8.4
Corn stover	36.8-39	14.8-25.0	15.1-23.1
Cotton gin trash	20.0	4.6	17.6
Grasses (sugarcane, sorghum bagasse)	25-50	25-50	10-30
Rice straw	35-41	25-14.8	9.9-12.0
Rice hults	36.1	14.0	19.4
Wheat straw	30-38	21-50	20-23.4
Herbaceous Materials			
Bermuda grass	25.0	35.7	6.4
Switchgrass	31-32	20.4-25.2	14.5-18.1
Cellulose Wastes			
Newsprint	40-64.4	4.6-40	18.3-21
Paper	85-99	0.0	0-15

Lignin

Lignin is a highly branched heteropolymer present in plant cell walls (*24*). It provides the plant with structural support, impermeability, and resistance against microbial degradation and oxidative stress. The structure of lignin is very complex, disordered, and random. It consists mainly of ether linked phenylpropane units (*p*-coumaryl, coniferyl, and sinapyl alcohol) that add elasticity to the cellulose and hemicellulose matrices (*17, 22*). It is widely accepted that lignin acts as the "glue" that binds cellulose and hemicellulose, giving both rigidity and resistance to the lignocellulosic structure. Cross-linking of lignin and cell wall polysaccharides by ferulic acid and *p*-coumarate bridges may be one of the most essential obstacles in lignocellulosics degradation (*25*). The presence of ferulate ethers affect cell wall degradation more than *p*-coumarate ethers which are linked only to lignin and do not act as bridges to polysaccharides

(*26*). Warm-season grasses such as sugarcane bagasse have high levels of phenolic compounds (ferulate and *p*-coumarate ethers) which can be used for the development of value-added products but also increase its recalcitrance to hydrolysis (*27*).

Lignocellulose Recalcitrance

The polysaccharides (hemicellulose and cellulose) present in native lignocellulosic biomass are not readily available for bioconversion to ethanol. The close association and complexity of the carbohydrates-lignin complex make this a challenging task. Degree of polymerization (number of glucose units per cellulose chain) and cellulose crystallinity are important factors in determining the hydrolysis rates of lignocellulosic biomass (*28*). The precisely arranged glucose residues and linkages in and between cellulose sheets make crystalline cellulose hydrophobic and resistant to acid hydrolysis (*29*). It is the hydrogen bonding network in crystalline cellulose that makes it more resistant to enzyme hydrolysis than amorphous cellulose (*30*, *31*). Nevertheless, these parameters alone do not explain the recalcitrance of lignocellulosic biomass (*32*). Lignin and hemicellulose content and the heterogeneous character of biomass particles have also been recognized as limiting factors. Both lignin and hemicellulose act as barriers by preventing the access of cellullase enzymes to cellulose thus reducing the efficiency of hydrolysis. Hemicelluloses are thought to coat the cellulose fibrils resulting in a reduced accessibility of the cellulose structure. The removal of hemicellulose leads to an increase in the pore size of the biomass material thus favoring cellulose hydrolysis (*33*). The degree of acetylation in the hemicellulose structure and the linkages between acetyl groups and lignin further enhance biomass recalcitrance thus preventing the breakdown of polysaccharides (*34*). Lignin also appears to reduce enzyme hydrolysis by acting as an attractant to cellulase enzymes resulting in non-productive binding (*35*).

Pretreatment Technologies

Pretreatment of lignocellulosic biomass is required to make cellulose accessible to enzymes for their conversion into fermentable sugars and further bioconversion to ethanol. The purpose of a pretreatment is to reduce cellulose crystallinity, remove lignin and/or hemicellulose, and to increase biomass porosity. An effective pretreatment favors the release of sugars or enhances their breakdown by enzyme hydrolysis, minimizes the loss of sugars and the formation of by-products that are inhibitory to hydrolysis and fermentation processes, reduces energy demands and is cost-effective. Numerous pretreatment methods have been suggested in the past decades and are generally categorized as mechanical (e.g., milling, grinding), physico-chemical (e.g., autohydrolysis, liquid hot water, steam, supercritical fluids), chemical (e.g., alkali, acid, organic solvents, oxidizing agents) and biological (e.g., fungi) processes or combinations of these approaches. A summary of pretreatment methods and their effect on the physical and chemical composition or structure of lignocellulosic biomass is presented in

Table II. Pretreatment affects the cost of most other process operations, including size reduction prior to pretreatment and enzymatic hydrolysis post pretreatment. It can also influence downstream costs by determining fermentation toxicity, enzymatic hydrolysis rates, enzyme loadings, and other process variables (*43*). Advantages and disadvantages of pretreatment technologies are summarized in Table III.

Mechanical Pretreatment

The objective of the mechanical pretreatment is to breakdown the lignocellulosic biomass into small particles. Chopping, grinding or milling (e.g., ball milling, hammer milling, colloid milling) reduces cellulose crystallinity, increases the degree of polymerization and also the specific surface area of the lignocellulosic biomass thus increasing the total hydrolysis yield by 5-25% (*44*). Minimum effect on the hydrolysis yield of biomass has been reported on particle sizes below 40 mesh (0.0165 in) (*34*). Mechanical pretreatment if often used in combination with other technologies. The high energy requirement of this pretreatment is a major drawback and it is almost unlikely to be economically viable at a commercial scale.

Physico-Chemical Pretreatment

Autohydrolysis

Autohydrolysis or steam explosion refers to a pretreatment method in which lignocellulosic biomass is heated to less than 240 °C by high-pressure steam lasting a few seconds to several minutes followed by an explosive decompression (*42*). This pretreatment takes place when hydrogen ions are generated by the auto-ionization of water or from acetic acids favoring the solubilization of hemicellulose. Acetic acid is produced from the acetyl groups in hemicellulose. Acetic acids and other weak acids (e.g., formic and levulinic) released during this pretreatment may further catalyze hydrolysis and sugar degradation. Laser et al. (*45*) reported hemicellulose removal as a function of time and temperature. An increase in hemicellulose solubilization (15 to 89%) was observed in sugarcane bagasse pretreated for 2 to 10 min at 200 °C, respectively. Similar observations were made at 220 °C with an increase in hemicellulose solubilization of 88 to 99%. Cellulose was mostly preserved in the solid fraction with a 4% average removal. The rapid thermal expansion used in this technology opens up the biomass structure but cellulose digestibility is only weakly correlated with this effect (*46*). Some lignin is removed by this pretreatment but it is redistributed on the fiber surfaces as result of the melting and depolymerization/repolymerization reactions (*47*). A major drawback of steam explosion pretreatment is the partial degradation of hemicellulose to sugar monomers (xylose). Boussarsar et al. (*48*) reported a 49% xylose recovery in sugarcane bagasse pretreated at 170 °C for 2 h. The addition of impregnating agents such as sulfuric acid (H_2SO_4) and sulfur dioxide (SO_2) have been reported as means to improve hemicellulose digestibility, lower the optimal pretreatment conditions and allow for a partial hydrolysis

of cellulose (*49*, *50*). Martin et *al.* (*51*) observed higher xylose yields in 1% (w/w) SO_2 impregnated bagasse (82 g/g dry biomass) and in non-impregnated bagasse (61 g/g dry biomass) than in 1% (w/w) H_2SO_4 impregnated bagasse (36 g/g dry biomass) treated at 205 °C for 10 min. The lower xylose yields in H_2SO_4 impregnated bagasse were attributed to the severity of the pretreatment resulting in a higher degree of degradation of the released fermentable sugars into furfurals and 5-hydromethyl furfural (HMF). In contrast, only negligible amounts of glucose were released after sulfur dioxide and without any impregnation (10 g/g dry biomass) as compared to 230 g/g dry biomass seen in H_2SO_4 impregnated bagasse. Rudolf et *al.* (*52*) reported 87% xylose recovery with a 2% (w/w) SO_2 impregnation of sugarcane bagasse at room temperature for 1 h followed by 1 min steam pretreatment at 190 °C. Hemicellulose degradation can be minimized by separating the biomass from the condensate during pretreatment, by maintaining the pH between 5 and 7 or by applying a two step steam pretreatment (*53*, *54*). At first, pretreatment is performed at lower temperatures to dissolve the hemicellulose and the cellulose fraction is subjected to a second pretreatment at temperatures higher than 210 °C.

Steam is an effective method to heat lignocellulosic biomass to a targeted temperature without excessive dilution of resulting sugars. A disadvantage is the generation of toxic compounds. Detoxification methods are needed for the removal of these compounds to reduce their inhibitory effect on enzymes and yeasts during hydrolysis and fermentation, respectively. However, owing to the additional cost, detoxification should be avoided whenever possible.

Liquid Hot Water

In this process (a.k.a. hydrothermolysis, un-catalyzed solvolysis, steam/aqueous fractionation, or aquasolv) water is used under pressure and at elevated temperatures to remain in the liquid state (*57*, *58*). This pretreatment takes place at temperatures in the range of 140 to 300 °C with a 15 min residence time, which results in the removal of 4 to 22% cellulose, 35 to 60% lignin and all of the hemicellulose. Liquid hot water cleaves the acetyl and uronic acid groups in hemicellulose generating acetic and other organic acids. The release of these acids helps catalyze the formation and removal of oligosaccharides. However, depending on the pretreatment conditions, polysaccharides can be further hydrolyzed to monomeric sugars and be partially degraded to furfurals and HMF (*59*). A neutralization step is not required with this technology since water is the catalyst. Three reactor configurations are used with liquid hot water processes, co-current, counter current, and flow-through. In the co-current configuration, biomass slurry is heated to the desired temperature by passing though heat exchangers for a fixed residence time before being cooled. Countercurrent pretreatment is designed to move the biomass slurry and water in opposite directions in a jacketed reactor. Flow-through designs pass hot water over a stationary bed of lignocellulosic biomass. Size reduction of the lignocellulosic biomass prior to pretreatment is not needed since the particles break apart when heated in water (*53*).

Table II. Effect of various pretreatment methods on the structure of lignocellulosic biomass (*18*, *42*, *55*)

Pretreatment	Increase accessibility surface area	Decrystallization cellulose	Solubilization hemicellulose	Removal lignin	Generation of toxic compounds	Alteration lignin structure
Mechanical	+	+				
Liquid hot water	+	-	+	+/-	-	+/-
Acid	+	+	+	-	+	+
Alkaline (lime)	+	+/-	-	+/-	-	+
oxidative	+	-	+/-	+/-	-	+
Organosolv	+	-	+/-	+/-	+/-	+
Organosolv + acid	+	+/-	+	+	+	+
Lime	+	ND	+/-	+	+/-	+
Thermal + acid	+	ND	+	+/-	+	+
Thermal + lime	+		-	+/-	-	+
Thermal + oxidative	+	ND	-	+/-	-	+
Thermal + alkaline + oxidative	+	ND	-	+/-	-	+
Autohydrolysis	+	-	+	+/-	+	+
Ammonia Fiber Expansion	+	+	-	+	-	+
ARP	+	+	+/-	+	+/-	+
CO_2 explosion	+		+			
Microbiological	+	ND	+	+		+

Table III. Summary of the advantages and disadvantages of various processes used for the pretreatment of lignocellulosic biomass (*31, 56*)

Pretreatment Process	Advantages	Disadvantages
Mechanical comminution	Reduces cellulose crystallinity	High power and energy consumption
Steam explosion	Causes lignin degradation and hemicellulose solubilization	Incomplete disruption of the lignin-carbohydrate complex
	Cost effective	Partial hemicellulose degradation
		Generation of inhibitory compounds
Liquid hot water	Causes lignin degradation and hemicellulose solubilization	Partial hemicellulose degradation
	Most cellulose is preserved	Generation of inhibitory compounds
	Neutralization step is not needed	Detoxification is needed
Supercritical fluids	Increases accessible surface area; cost effective	High pressure requirements
	Do not imply generation of toxic compounds	
Alkaline hydrolysis	Increases accessible surface area	Irrecovarable salts are formed and incorporated into biomass
	Removes lignin and hemicellulose	Requires long residence times
AFEX	Increases accessible surface area	No efficient in high-lignin content biomass
	Removes lignin and hemicellulose to an extend	Cost of ammonia
	No inhibitors produced for downstream	
Acid hydrolysis	High glucose yields	High cost
	Ambient temperatures	Acids need to be recovered
		Equipment corrosion problems
		Formation of inhibitory compounds
		Neutralization step is needed
Oxidation	Efficient removal of lignin	High cost of catalysts
	Minimizes energy demand (exothermic)	Incomplete hemicellulose hydrolysis
	Low formation of inhibitors	Uncontrollable reaction of reactive oxygen species
Organosolv	Hydrolyzes lignin and hemicellulose	High cost
	Recovery of relatively pure ligin as byproduct	Catalysts need to be drained and recycled
		Safety, high solvent volatility
Biological	Low energy requirements	Low rate of hydrolysis
	Degrades lignin and hemicellulose	

Supercritical Fluids

This refers to a fluid that is in a gaseous form but it is compressed at temperatures above its critical point to a liquid like density but below the pressure required to condensate it into a solid (*60*). Carbon dioxide (CO_2), water and propane are the most studied supercritical fluids. The high pressure facilitates the faster penetration of CO_2 molecules into the lignocellulosic structure. Under these conditions, carbon dioxide forms carbonic acid when in water, resulting in the hydrolysis of cellulose and hemicellulose fractions (*61*). The hydrolyzed monomers can be further converted to furfurals, HMF, and other toxic by-products under increasing temperatures and reaction times. Zheng et *al.* (*62*) found that CO_2 explosion at 35 °C and 73 Bars was more cost effective than ammonia explosion for the pretreatment of recycled paper mix, sugarcane bagasse, and pulping waste. The explosive release of the carbon dioxide pressure enhances substrate hydrolysis by disrupting the cellulosic structure. Glucose yields for treated sugarcane bagasse were increased by 50 to 70%. The use of supercritical

fluids as pretreatment is beneficial but more research is needed before these fluids are implemented at a larger scale.

Chemical Pretreatment

Alkaline

Alkaline pretreatment processes can be divided into two major groups depending on the catalyst used, metal based (calcium, sodium, potassium) and ammonia based catalysts. Unlike acid processes, alkaline pretreatments are highly effective in removing lignin exhibiting minor cellulose and slightly higher hemicellulose solubilization (*34*, *38*). By this process, lignocellulose biomass can be fractionated into soluble lignin, hemicellulose and solid residue (mostly cellulose). Nevertheless, the effectiveness of this process depends on the lignin content of the biomass.

Metal-Based Alkaline Pretreatment

Sodium, potassium, and calcium hydroxides remove a small percentage of acetyl groups from hemicelluloses in addition to lignin (*63*). Removal of lignin and hemicelluloses increases effectiveness by reducing non-productive adsorption sites for enzymes (*64*). Sodium hydroxide causes swelling of the biomass which increases the internal surface of cellulose and decreases the degree of polymerization and crystallinity (*65*). Peng et *al.* (*66*) reported a 74.9% removal of the original hemicellulose after sequential extractions of de-waxed sugarcane bagasse with water, 1% (w/v) and 3% (w/v) sodium hydroxide solutions at 50 °C for 3 h. Sodium hydroxide solutions significantly cleaved the ether bonds between lignin and hemicellulose resulting in their dissolution.

Calcium hydroxide, also known as lime, has been widely studied. The process of lime pretreatment of sugarcane bagasse has been performed at various temperatures, reaction times and concentrations (*67–69*). Reaction times can be shortened by increasing temperature conditions. According to Puri and Pearce (*70*), pretreatment of sugarcane bagasse for 5 min at 200 °C under 3.45 MPa gas pressure (steam and nitrogen) resulted in 70% digestibility. Rabelo et *al.* (*68*) reported that sieving sugarcane bagasse before lime pretreatment was necessary to improve the enzymatic digestibility of the material. Optimal conditions for maximum glucose yield (203 mg/g dry bagasse) were pretreatment of screened bagasse (0.248 to 1.397 mm) with 0.15 g of lime/g dry biomass at 87 °C for 66 h. According to Chang et *al.* (*63*), lime loading has a critical value of approximately 0.1 g/g dry biomass. Below this critical value, biomass digestibility decreases significantly. Use of lime beyond the amount required for maintaining a saturated lime solution is unnecessary due to its poor solubility in water (*71*). Playne (*67*) evaluated the digestibility of sugarcane bagasse soaked at various lime concentrations (0.12 to 0.3 g/g bagasse) for 8 days at ambient conditions. Cellulose digestibility improved from 20% before pretreatment to 72% after pretreatment with lime loading of 0.3 g/g bagasse. Ibrahim and Pearce

(*72*) reported that soaking of sugarcane bagasse with 0.1 g lime/g dry biomass had a slightly higher digestibility (43%) than spraying (41%) at 55 °C for 24 h. Combinations of lime with other alkali such as ammonia, sodium hydroxide, and sodium carbonate have also been evaluated to enhance biomass digestibility (*67*, *73*). Addition of oxygen to the lime mixture improves delignification by 77.5%, especially in highly lignified materials such as poplar (*34*, *74*). Pretreatment with lime has lower cost and less safety requirements than sodium hydroxide and potassium hydroxide pretreatments (*18*). Lime pretreatment is required to employ either biomass washing or pH adjustment with acids prior to hydrolysis with enzymes because it is performed at much higher pH ranges (pH 11.0 and 12.0) than the optimum pH of enzymes (pH 4.5 to 5.5) (*63*). Lime can be recovered as calcium carbonate by neutralization with carbon dioxide at pH 9.5 (*63*). Lime can be subsequently regenerated using established kiln technology (*75*). Lime neutralization with acetic acid resulted in a 3 to 18% inhibition of cellulase activity due to the formation of calcium acetate (*63*). Scaling takes place in calcium-containing liquors particularly on heated surfaces, and this disadvantage has to be considered in any operations involving calcium hydroxide (*67*).

Ammonia-Based Alkaline Treatment

Ammonium hydroxide (NH_4OH) or liquid ammonia enhances the surface area of cellulose, disrupts crystalline structures, removes lignin and can be easily recovered due to its high volatility (*76*).

Ammonia fiber/freeze expansion (AFEX) process is very similar to steam explosion. AFEX is usually conducted at temperatures of 60 to 110 °C for 5 to 30 min with a liquid ammonia dosage of 1 to 2 Kg/Kg dry biomass, followed by an explosive pressure release (*39*). During pretreatment, some lignin and hemicellulose are removed while decrystallizing the cellulose. The structure of the material is changed resulting in increased water holding capacity and higher digestibility (*41*). Over 90% hydrolysis of cellulose and hemicellulose has been reported after pretreatment of low lignin (15%) containing biomass (*77*). Low-lignin containing biomass such as sugarcane bagasse is well suited for AFEX; however, this technology works moderately on hardwoods, and not at all on softwoods (*78*). The cost of ammonia and ammonia recovery drives the cost of the AFEX pretreatment (*79*).

Ammonia recycle percolation (ARP) involves the passing of an aqueous ammonia solution of 5 to 15% (w/w) through a flow-through column reactor packed with biomass at elevated temperatures (150 to 170 °C) with a fluid velocity of 1 cm/min and a residence time of 14 min. This process enables the un-reacted ammonia to be separated and recycled (*18*, *80*). Under these conditions, aqueous ammonia reacts with lignin and causes its depolymerization and cleavage of lignin-carbohydrate linkages. ARP solubilizes lignin and hemicellulose, whereas cellulose remains intact (*81*). This technology does not produce inhibitors for the downstream biological processes, so a water wash is not necessary (*82*). A degree of delignification ranging from 60 to 85% with negligible cellulose solubilization (<10%) has been reported in agricultural residues post treatment with 2.5 to 20%

(w/w) ammonia solution at 170 °C for 1 h (*83*). The main economical constraints of ARP are the cost of ammonia recovery, energy consumption, safety and the need for detoxification processes due to the high solubilization of lignin.

Another type of process utilizing ammonia is soaking aqueous ammonia (SAA) in which lower processing temperatures are used (30 to 75°C). SAA is one of the few pretreatment methods in which both hemicellulose and cellulose remain in the solid fraction. Reports on AFEX, ARP, and SAA have indicated delignification values of 53 to 85% under various ammonia concentrations (1–15 parts ammonia per part biomass), reactor pressures (atmospheric to 334 psi), processing temperatures (20 to 210 °C) and reaction times (1–60 days) (*80, 84–86*).

Salvi et al. (*87*) reported the removal of 44% lignin after the pretreatment of an agricultural residue with a 28% (v/v) ammonia solution with a loading of 0.5 g/g dry biomass at 160°C for 1 h. More than 90% of the cellulose remained in the solid fraction. Kim et al. (*88*) evaluated the effect of ammonia (0.03 to 0.3% w/w, ammonium hydroxide) on sugarcane bagasse stored at 30 °C at atmospheric pressure for 40 days without agitation. Maximum lignin removal (46%) was observed with biomass stored for 40 days with a 0.3% ammonia solution. Approximately, 100% cellulose and 73% hemicellulose were retained in the solid fraction.

Acid Pretreatment

The objective of acid pretreatments is to solubilize the hemicellulose fraction and to enhance cellulose digestibility in the residual solids. This type of pretreatment can be performed with concentrated or diluted acids and it has been tried on a wide range of feedstocks ranging from hardwoods to grasses to agricultural residues. Although the use of concentrated acids enables the hydrolysis of hemicellulose and cellulose, its use for ethanol production is less favored because of the high operational and maintenance costs due to the formation of inhibiting compounds, and equipment corrosion problems (*89*). Sulfuric acid (H_2SO_4) is the most studied acid. Others include hydrochloric acid (HCl), nitric acid, phosphoric acid, peracetic acid and organic acids such as fumaric and maleic. Teixeira et al. (*90, 91*) employed a silo type system in which sugarcane bagasse was placed in plastic bags along with concentrated peracetic acid solutions. A 93% cellulose conversion rate was obtained with either 21% or 60% (w/w) acid concentrations for 120 or 24 h, respectively.

The hydrolysis of lignocellulosic biomass with diluted acids have been evaluated at concentrations ranging from 1 to 10% (w/w) (*13, 39*) and at wide temperature ranges, higher than 160 °C for low solids loadings (5 to 10%, weight of substrate/weight of reaction mixture) (*92, 93*), and lower than 160 °C for high solids loadings (10-40%) (*94, 95*). Diluted acid pretreatment favors the solubilization of hemicellulose, xylan in particular, but it also breaks down the solubilized hemicellulose to fermentable sugars. Depending on the operational conditions, decomposition products from pentoses (furfural) and hexoses (HMF) sugars, generation of acetic acids from the acetyl groups linked to hemicellulose,

and aromatic lignin degradation compounds can be found in the liquid phase of the hydrolysates. Nevertheless, diluted acid pretreatments generate lower degradation products than concentrated acid pretreatments (*56*). Yu and Stahl (*96*) recovered 30.7 g/L of total reducing sugars and 18.9 g/L of xylose after pretreating sugarcane bagasse with 0.75% (w/w) H_2SO_4 for 120 min at 115 °C. The hydrolysate, however, was inhibitory to *Ralstonia eutropha*, an organism used in the biosynthesis of polyhydroxyalkanoates. Aguilar et *al.* (*97*) reported a higher xylose concentration (21.6 g/L) at 2% (w/w) H_2SO_4 for 24 min at 122 °C. Only 3 g glucose/L and 0.5 g furfural/L were detected in the hydrolysate. According to Gamez et *al.* (*98*), 21 g/L fermentable sugars and less than 4 g/L inhibitors were obtained with 6% (w/w) H_2SO_4 at 100 °C for 300 min. Pattra et *al.* (*99*) evaluated the hydrolysis of sugarcane bagasse at various concentrations of sulfuric acid (0.25 to 7% w/w) and reaction times (15-240 min) at 121 °C. Optimal conditions were reported at 0.5% (w/w) H_2SO_4 for 60 min with concentrations of 11 g/L glucose, 11.3 g/L xylose, 2.2 g/L arabinose, 2.5 g/L acetic acid, and 0.1 g/L furfural. Glucose losses were only observed between 1 to 5% (w/w) H_2SO_4. A pilot scale (350 L) study conducted by Rodrigues et *al.* (*100*), reported xylose (19.2 g/L) as the major hydrolysate product after pretreating sugarcane bagasse with dilute H_2SO_4 at 121 °C for 10 min. Coumarilic acid (0.15 g/L) was the highest lignin degradation product followed by ferulic acid and galllic acid.

Studies on the use of hydrochloric acid for the pretreatment of lignocellulosic biomass has also resulted in favorable hydrolysis yields, however, environmental impact and corrosive properties limits its application. Hernández-Salas et *al.* (*101*) reported 35 and 37% reducing sugars for 1.2% (v/v) hydrochloric acid-treated depithed sugarcane bagasse and pith, respectively. The interest in the use of phosphoric acid for the deconstruction of lignocellulose biomass is because after neutralization of the hydrolysate with sodium hydroxide, sodium phosphate is formed which can be used as a nutrient by microorganisms (*98*). Gamez et *al.* (*102*) reported concentrations of 17.6 g/L xylose, 2.6 g/L arabinose, 3.0 g/L glucose, 1.2 g/L furfural, and 4 g/L acetic acid in the hydrolyzate of 4% (w/w) phosphoric acid-pretreated sugarcane bagasse at 122 °C for 300 min. Geddes et *al.* (*103*) soaked sugarcane bagasse in 1% (w/w) phosphoric acid or 1% (w/w) H_2SO_4 for 4 h at 22 °C followed by an additional 1 h at 145 °C. Both treatments generated similar total sugars yields of 246 g and 257 g/kg dry bagasse, respectively. The main difference between both acid pretreatments was found in their sugar degradation products. The amount of degradation products (furfural) with phosphoric acid were one third the levels observed with H_2SO_4. Levulinic acid and formic acid were absent in phosphoric acid hydrolysates but abundant along with acetic acid in sulfuric acid hydrolysates. Rodriguez-Chong et *al.* (*104*) observed optimal pretreatment conditions for nitric acid-treated sugarcane bagasse at 6% (w/w) for 9.3 min at 122 °C resulting in the release of 18.6 g/L xylose, 2.0 g/L arabinose, 2.87 g/L glucose, 0.9 g/L acetic acid, and 1.3 g/L furfural.

Acid removal and/or neutralization are required for both concentrated and diluted acid approaches before the sugars proceed to fermentation, generating large amounts of waste as salts. Acid removal and disposal of neutralization salts (e.g., gypsum) results in added cost.

Oxidative Pretreatment

An oxidative pretreatment employs the addition of an oxidizing agent such as hydrogen peroxide (H_2O_2), air or oxygen (wet oxidation). The objective is to remove lignin and hemicellulose with minimal sugar degradation and toxic compound formation. In many cases the oxidant used is non-selective and losses in cellulose and hemicellulose can occur (*44*).

Hydrogen peroxide (H_2O_2) does not leave residues in biomass because it degrades into O_2 and H_2O (*105*). Under alkaline conditions, hydrogen peroxide decomposes into hydroxyl and perhydroxyl radicals, solubilizing the lignin fraction and proteins (*106, 107*)). Sugarcane bagasse treated with 2% alkaline H_2O_2 solution (pH 11.5) at 30 °C for 8 h removed approximately 50% lignin and most of the hemicellulose resulting in 95% cellulose conversion (*108*). An increase in the processing temperature from 30 to 60 °C promoted delignification with minimal decomposition of the cellulose fraction. Krishna et *al.* (*109*) reported a 92% cellulose conversion in the alkaline oxidative treatment of sugarcane leaves. According to Gould (*110*), a good delignification can be obtained at hydrogen peroxide concentrations of at least 1%, at pH 10-11.5, and with a 0.25 weight ratio between hydrogen peroxide and biomass. Sugarcane bagasse mixed with 0.15 g H_2O_2/g dry biomass in an airtight polythene bag and incubated for 21 days resulted in 25% hemicellulose loss, 10% cellulose loss with 83% enhanced cellulosic digestibility (*111*). Although alkaline peroxide pretreatment is known to be an effective method, it has a cost disadvantage. Cheng et *al.* (*112*) evaluated a five stage recycle process to minimize water and chemical consumption during a sodium hydroxide NaOH/H_2O_2 pretreatment of sugarcane bagasse. It was concluded that a two cycle of alkaline peroxide pretreatment was reasonable for the delignification and enzymatic hydrolysis of sugarcane bagasse with a 26 and 40% consumption savings for NaOH and water, respectively. Bas et *al.* (*113*) recommended the supplementation of alkaline hydrogen peroxide pretreated sugarcane bagasse with an appropriate protein source if to be used as animal feed. Amjed et *al.* (*114*) reported that alkaline hydrogen peroxide (0.25 g/g dry biomass) pretreated bagasse at pH 11.5 and at ambient temperatures for 24 h did not affect the composition of core lignin (polymeric), but it rather changed the composition of noncore lignin (phenolic acids). These observations are inconsistent with those reported by Kerley et *al.* (*115*) in which both core and noncore lignin fractions were affected by alkaline hydrogen peroxide in wheat straw. Significant amounts of esterified ferulic acid, esterified *p*-coumaric acid, or the ether-linked form of either phenolic acid were removed in treated sugarcane bagasse and wheat straw. Esterified ferulic acid has been reported to limit hemicellulose digestion more than esterified *p*-coumaric acid (*116*). Most *p*-coumaric acid is esterified to core lignin rather than to hemicellulose (*117*). According to Kondo et *al.* (*118*), alkaline hydrogen peroxide removes ferulic acid molecules that may cross-link lignin with hemicellulose via both an ester bond through their carboxyl groups to arabinoxylan and an ether bond to core lignin through their phenolic hydroxyl groups. Hydrogen peroxide combinations with sodium hypochlorite have also been investigated (*119*).

Oxygen or air can also be used as catalysts at temperatures above 120 °C and under pressure (10 to 12 bar O_2) during a process known as wet oxidation (*120*). Martin et *al.* (*121*) reported sugarcane bagasse with 70% remaining cellulose and a 75% cellulose digestibility post pretreatment. Lignin derived products (phenolic acids) are formed but are further degraded to carboxylic acids. The addition of sodium carbonate has been shown to decrease the formation of toxic compounds by keeping the pH in the neutral to alkaline range (*56*). Additionally, Klinke et *al.* (*122*) demonstrated that combinations of alkali and wet oxidation reduce the formation of fermentation inhibitors, furaldehydes and phenol aldehydes. The hydrolysis of hemicellulose to sugar polymers rather than monomers and the high cost of catalysts are major drawbacks of this technology.

Organosolv Process

Solvents evaluated include those with low boiling points (methanol and ethanol), high boiling points (ethylene glycol, glycerol), ethers, ketones and phenols (*123*). Methanol and ethanol are mostly favored. For most organosolv solvents, pretreatment is conducted at temperatures below 180 °C with the addition of acids as catalysts (sulfuric, phosphoric, hydrochloric, formic, oxalic, acetylsalicylic, salicylic) to dissolve the hemicellulose fraction and increase xylose yields (*39*). Acid addition can be avoided by conducting the pretreatment (auto hydrolysis) at temperatures higher than 185 °C. Preferred conditions depend on the nature of the biomass but are generally conducted at heating temperatures of 180 to 195 °C, reaction times of 30 to 90 min, ethanol concentrations of 35 to 70% (w/w), and liquor to solid ratio in the range of 4:1 to 10:1 (w/w). The organic acids (e.g., acetic acid) released during pretreatment act as catalysts for the breakdown of the lignin-carbohydrate complex (*124*). Organosolv pretreatment yields three separate fractions: dry lignin, an aqueous hemicellulose stream, and a pure cellulose fraction. After delignification, the pretreated solid is washed with methanol or ethanol before water washing. The organic fraction is drained from the reactor, evaporated and condensed, and the solvent is recycled to the reactor. Water is added to the liquid fraction to precipitate the dissolved lignin. An organosolv alternative is its combination with supercritical carbon dioxide. This process combines the use of pressurized (liquid) carbon dioxide (50%) and a lower amount of organic solvent (50% alcohol/water mixture). Lignin removal is facilitated by the release of pressure after pulping. Pasquini et *al.* (*125*) evaluated the delignification effect of a mixture of ethanol and water (50 to 100% ethanol) in the presence of carbon dioxide at high pressures (14.7 to 23.2 MPa) and temperatures (142 to 198°C) for 30 to 120 min in depithed sugarcane bagasse. The highest delignification yield (88.4%) was obtained with 75% ethanol at 160 °C for 60 min at 16 MPa. Similar results were observed in sugarcane bagasse after exposing it to 90% (v/v) formic acid for 80 min at atmospheric pressure (*126*). Formic acid disrupts the lignin and dissolves the hemicellulose followed by solvation of the fragments into soluble components, oligosaccharides, monosaccharides and acetic acids (*127*). Some pentose sugars are further hydrolyzed to furfurals thus lowering sugar recovery. Lignin is

hydrolyzed into lower molecular weight molecules. The serious corrosion problems associated with formic acid limits its use at the industrial level.

Advantages of organosolv pretreatment are solvent recovery by distillation and the recovery relatively pure lignin as a by-product (*43*). However, organic solvents are expensive and their recovery increases energy consumption. Another disadvantage of organic solvents is that they are not environmentally friendly or "green" and, therefore, not sustainable. Due to the high volatility of the solvents, this pretreatment must be performed under extremely tight and efficient conditions. Additionally, pretreated solids need to be washed with organic solvents previous to water washing to avoid lignin precipitation. Therefore, this pretreatment is too expensive to be used for biomass pretreatment at present.

Biological Pretreatment

In biological pretreatment processes, microorganisms such as white, brown and soft-rot fungi are used in the degradation of lignocellulosic biomass. Brown-rots attack cellulose, whereas white and soft-rots attack both cellulose and lignin. White rot fungi degrade lignin through the action of peroxidases and laccases. The white-rot fungus *Phanerochaete chrysosporium* produces peroxidases during secondary metabolism in response to the absence of carbon or nitrogen (*128*). The C/N ratio is higher in fungi (30:1) than in bacteria to (10:1), hence fungi are more capable of degrading lignocellulosic material as their dependency of nitrogen is relatively lower (*129*). Singh et *al.* (*130*) evaluated the effect of eight biological agents including fungi and bacteria on sugarcane bagasse at varying C/N ratios. The maximum drop in C/N ratio was observed with *Aspergillus terreus* (61%), followed by *Cellulomonas uda* (52%) and *Trichoderma reesei* and *Zymomonas mobilis* (49%). A 35% bioconversion of biomass to reducing sugars was observed in wheat straw with *Pleurotus ostreatus* after a five-week incubation period (*131*). Akin et *al.* (*27*) reported hydrolysis rates in Bermuda grass of 29-32% and 63-77% by using *Ceriporiopsis subvermispora* and *Cyathus stercoreus* for 6 weeks, respectively.

Aerobic fungi are thought to be the most effective lignin degrading organisms. The major families of lignolytic enzymes in fungi are lignin peroxidases, Mn-dependent peroxidases, versatile peroxidases and laccases (*132*). A common attack of lignocelluloses by these organisms is a simultaneous decay of polysaccharides and lignin resulting in the total degradation of lignocelluloses (*26*). However, patterns and degrees of delignification vary among species and strains. Lignolytic enzymes produced by white-rot fungi are effective degraders. *Phanerochaete chrysosporium, Pleurotus pulmonaris, Pleurotus sapidus*, *Phlebia radiate*, *Phlebia tremellasa* are among wood-rotting fungi with highest lignolytic activities (*133, 134*). Li et *al.* (*135*) reported more than 50% lignin degradation in sugarcane bagasse by the marine fungus *Phlebia* sp. MG-60. Only 10% of the cellulose was lost. *P. chrysosporium*'s genome contains ten lignin peroxidases, five Mn-dependent peroxidases, and a number of other related genes (*136*). Instability of lignin degrading enzymes presents a major difficulty in using these organisms for enzyme production. *Phanerochaete sordida* YK-624 is

a hyper lignin-degrading basidiomycete possessing greater lignin selectivity than either *P. chrysosporium* or *Trametes versicolor*. Extensive research on basidiomycetous fungi has been conducted to isolate lignolytic enzymes (*137*). Two lignin-degrading products, Depol 740 L™ and TP 692 L™, are available commercially. Depol 740 L™ reportedly removes free phenolic acids and fermentable sugars from plant material. TP 692 L™ is a complex mixture of cellulases, hemicellulases and ferulic acid esterases (*138*). A 60% weight loss was observed in corn fibers (size < 1mm) when using extremely high concentrations of 692 L™ at an enzyme to biomass ratio of 2 g: 0.5 g.

In general, microbial processes offer advantages such as no chemicals requirement, low energy input, low capital cost and mild environmental conditions. However, the main drawback is the slow rate of hydrolysis as compared to other existing technologies.

Detoxification of Hydrolyzates

Fermentable sugars and a wide range of unwanted degradation products which are toxic to enzymes and fermenting organisms are formed or released during pretreatment. The existence of these toxic compounds is most likely favored at high temperatures and/or in the presence of acids. Some common inhibitors have been identified including furfural, hydroxymethylfurfural (HMF), acetic acid, levulinic acid, and formic acid derived from sugars degradation and phenolics such as 4-hydroxybenzoic acid, vanillin, and catechol from lignin degradation. Furfural and HMF are considered to be the most potent and representative inhibitors of yeast growth and fermentation. These compounds break down DNA, inhibit protein and RNA synthesis, and damage the microbial cell wall (*139–141*). Weak acids have a growth inhibiting effect on microorganisms due to the inflow of un-dissociated acids into the cell membrane resulting in the drop of intracellular pH (*142*). Growth of *S. cerevisiae* at pH as low as 2.5 in the absence of acetic acid has been observed in model fermentations (*59*). However, the minimum growth pH increased to 4.5 in the presence of acetic acid (10g/L). Low pH fermentations are favored in industrial settings as a means to controlling bacterial infection. Lignin degradation products, in particular low molecular weight phenolic compounds, affect the ability of cell membranes to act as selective barriers and enzyme matrices (*143*).

Detoxification procedures are often applied to remove these undesired products and facilitate fermentation. However, these additional steps not only add cost and complexity to the process but result in the generation of extra waste products (*144*, *145*). Removal of toxic compounds begins by the separation of the pretreated slurry or hydrolysate into solid and liquid fractions. Water is sometimes used to wash away water soluble compounds from the pressed solid fraction. Detoxification technologies include neutralization (*145*), overliming (*144*), enzyme detoxification with laccase (*146*), activated charcoal (*147*), electrodialysis (*148*), and ion exchange (*149*). Chandel et al. (*146*) evaluated the effect of various detoxification technologies in sugarcane bagasse hydrolysates pretreated with 2.5% (v/v) HCl at 140 °C for 30 min. The hydrolysate contained

30 g/L reducing sugars and various fermentation inhibitors such as furans (1.89 g/L), phenolics (2.75 g/L), and acetic acids (5.45 g/L). Among the evaluated detoxification methods, ion exchange was the most efficient in removing furans (63%), total phenolics (76%), and acetic acids (85%) resulting in 94.5% ethanol conversion. Laccase treatment reduced total phenolics by 77.5%. However, no effect on the removal of acetic acid was observed with either lacasse or overliming. Neutralization resulted in the lowest ethanol conversion (43%).

Enzymatic Hydrolysis

The term hydrolysis refers to the cleavage of carbohydrate chains with acids (diluted or concentrated) or enzymes before their fermentation into alcohols. Acid hydrolysis, in particular diluted, is fast and easy to perform but drawbacks include non-selectivity and byproduct formation. During hydrolysis with diluted acid, temperatures of 200 to 240 °C at 1% acid (H_2SO_4 or HCl) concentrations are employed to degrade the crystalline cellulose (*150*). However, the further degradation of glucose into HMF and xylose into furfural and other undesirable products is unavoidable.

Enzyme hydrolysis of cellulose is carried out using microbial cellulytic enzymes. Prior to enzymatic hydrolysis, however, the lignocellulose structure must be made available to enzymes by pretreating the material mechanically, thermally, chemically, biologically or with combined processes. The type of feedstock and choice of pretreatment dictates whether the linkages that hold together the lignin-carbohydrate complex are or are not accessible to enzymatic attack (*151*). Unlike acid hydrolysis, no degradation products are formed but the process is much slower. Enzymatic hydrolysis is considered the most promising approach for obtaining high sugar yields which are critical to the economic success of lignocellulosic ethanol (*152*). The key is in achieving high sugar yields (>85% theoretical) at high substrate loadings (>10% w/v) over short residence times (< 4 days).

Cellulytic enzymes are produced by fungi (e.g., *Trichoderma, Aspergillus, Schizophyllum,* and *Phanerochaete)*, bacteria (e.g., *Clostridium, Bacteroides, Cellulomonas, Bacillus, Erwinia, and Acetovibrio)* and protozoans (*153*). Plants and animals also produced cellulases. In animals, cellulose hydrolysis takes place in the stomachs of ruminants. Enzymatic hydrolysis of cellulose involves three steps: adsorption of cellulase enzymes onto the surface of cellulose, biodegradation of cellulose to fermentable sugars and desorption of cellulase enzymes from the surface of cellulose (*39*). The cellulose enzyme system is a mixture of endo-1, 4-β-glucanase, exo-1, 4-β-glucanase, and β-glucosidase (*154*). Endoglucanase acts randomly on the regions of low crystallinity on the cellulosic fiber, whereas exoglucanase removes cellobiose from the non-reducing ends of cellulose chains. Glucosidase hydrolyzes the cellobiose into two glucose molecules. Cellulase enzyme loadings in hydrolysis depend on the substrate type and solids concentration.

The activity of cellulases has been shown to decrease during hydrolysis due partially to the irreversibly adsorption of cellulase on cellulose (*155*). The

use of non-ionic surfactants (e.g. Tween 80™, Tween 20™, polyoxyethylene glycol) during enzyme hydrolysis reverses this effect by modifying the surface properties of cellulose (*156–158*). Intermediate and end products of hydrolysis, glucose and cellobiose, also inhibit cellulase activity. This can be avoided by adding extra enzymes (β-glucosidases) during the reaction, by ultrafiltration or by simultaneously hydrolyzing and fermenting the reducing sugars. Additionally, lignin acts as a competitive adsorbent for cellulases (*51*).

Cellulases can be recovered from the liquid supernatant or the solid residues. Enzyme recycling can effectively increase the rate and yield of the hydrolysis and lower the enzyme cost ((*159*). Ramos et *al.* (*160*) was able to recycle a mixture of commercial enzymes, Celluclast and Novozyme, for five consecutive steps with an elapsed time of 48 h between each recycling step.

Hemicellulose enzymatic-degradation requires endo-β-1,4-xylanase, β-xylosidase, and several other accessory enzymes such as α-L-arabinofuranosidase, α-glucuronidase, acetylxylan esterase, ferulic acid esterase, and p-coumaric acid esterase (*21*, *161*). Accessory enzymes require a partial degradation of hemicellulose prior to cleaving the side chains. Enzyme degradation products include mostly xylose and some mannose, galactose, glucose, and acetic acid. Organisms such as *Penicillium capsulatum* and *Talaromyces emersonii* possess complete xylan degrading enzyme systems (*162*). For effective hydrolysis of xylan, a proper mix of endoxylanase with accessory enzymes is essential (*40*). Beukes et *al.* (*69*) compared the synergistic degradation of untreated and lime pretreated sugarcane bagasse (0.4 g calcium hydroxide/g dry bagasse at 70 °C for 36 h) using recombinant hemicellulases expressed in *Escherichia coli* BL21 (DE3). The highest amount of reducing sugars was 91.834 umol/min for untreated bagasse obtained with an enzyme combination of 37.5% ArfA and 62.5% ManA, with a 1.87 degree of synergy. The hydrolysis of lime pretreated bagasse with the enzyme combination of 37.5% ArfA, 25% ManA and 37.5% XynA resulted in the release of 593.65 mol reducing sugars/min, with a degree of synergy of 2.14.

Cellulases currently used in the industry are both slow and unstable (*163*). The hydrolysis process in cellulosic ethanol production remains expensive compared to that of corn−ethanol despite the funding efforts from the U.S. government and the private sector to reduce the costs. Known cellulases and hemicellulases from nature typically will not function at temperatures higher than about 50 °C. Therefore, improvements are still needed on enzymes required for the industrial degradation of lignocellulose.

Fermentation of Lignocellulosic Hydrolysates

The six-carbon sugars or hexoses (glucose, galactose, and mannose) are readily fermented to ethanol by many microorganisms, but the five-carbon sugars or pentoses (xylose and arabinose) are fermented to ethanol by few naturally occurring strains at relatively low yields. *Saccharomyces cerevisiae* (a.k.a. Baker's yeast), the best know alcohol-fermenting organism, can ferment only hexose sugars to ethanol. Ethanol production from cellulose can be either sequentially performed (separate hydrolysis and fermentation, SHF) or performed

in a single reaction vessel (simultaneous saccharification and fermentation, SSF). A promising alternative is the inclusion of the pentose fermentation in the SSF, a process called simultaneous saccharification and co-fermentation (SSCF). A schematic representation of the various saccharification and fermentation configurations is depicted in Figure 1. Furthermore, for lignocellulosic ethanol to be economical, fermentation of both hexose and pentose sugars must result in high yields. A way to overcome this obstacle is through genetic engineering of bacteria and yeast. Targeted organisms include *Zymomonas mobilis*, *Escherichia coli* and *Saccharomyces cerevisiae*.

Separate hydrolysis and fermentation (SHF) is the classic configuration employed for fermenting biomass hydrolysates. SHF involves a sequential process where enzyme hydrolysis and microbial fermentation steps are carried out separately thus allowing each step to be performed at its optimal operating conditions (e.g., pH, temperature). Regarding substrate concentration, solid loads of 10 % (w/w) are defined as the most adequate considering arising mixing difficulties and accumulation of inhibitors in the medium (*150*).

Simultaneous saccharification and fermentation (SSF) consolidates hydrolysis of cellulose with fermentation of the released sugars. Higher ethanol yields and less energy consumption are achieved than SHF by reducing the accumulation of hydrolysis products, cellobiose and glucose, which are inhibitory to cellulolytic enzymes. Major drawbacks include incompatible hydrolysis (45 to 50 °C) and fermentation (30 °C) temperatures, ethanol tolerance of microorganisms, and enzyme inhibition by ethanol. Reports by Wu and Lee (*164*) indicated that a 9%, 36%, and 64% cellulose activity was lost during SSF at ethanol concentrations of 9 g/L, 35 g/L, and 60 g/L at 38 °C, respectively. A study conducted with 10% sugarcane bagasse pretreated with two recycles of $NaOH/H_2O_2$ combination resulted in 25 g/L ethanol with a yield of 0.2 g/g using *Kluyveromyces maxianus* DW08 as the ethanol fermenting yeast (*112*). Ethanol has a theoretical yield of 0.51 g per g glucose. Ballesteros et al. (*165*) reported ethanol concentrations of 38 g/L after 78 h fermentation by *Kluyveromyces marxianus* at 42°C using Solka Floc 2000 as the substrate. The bacterium *Zymomonas mobilis* has an ethanol potential that is comparable if not higher than *S. cerevisiae,* and it has been the subject of numerous investigations (*166*). Dos Santos et al. (*167*) evaluated numerous conditions for SSF using *Z. mobilis* CP4 in sugarcane bagasse pretreated with 1% (v/v) H_2SO_4 followed by alkaline delignification with 4% (v/v) NaOH at 121 °C for 30 min. The maximum ethanol concentration (60 g/L) was obtained at 30 °C with 76 g/L of SSF initial glucose concentration, a solid: liquid ratio of 3:10, an enzymatic load of 25 FPU/g glucan, and a cell concentration of 4 g/L.

Configurations involving the separate fermentation of glucose and xylose have been evaluated. Xylose fermenting yeasts like *Pichia stipitis* and *Candida shehatae* can assimilate hexoses but their ethanol production rate from glucose is at least five times less than that observed for *S. cerevisiae* (*168*). Additionally, these organisms have low tolerance to inhibitory compounds in un-detoxified lignocellulose hydrolysates. Canilha et al. (*169*) compared the fermentation yields of *P. stipitis* DSM 3651 using hydrolysates derived from sugarcane bagasse pretreated with 2% (w/v) H_2SO_4 for 30 min at 150 °C. Fermentations with *P. stipitis* DSM 3651 using the non-detoxified hydrolyzate resulted in 4.9 g/L ethanol

in 120 h, with a yield of 0.20 g/g. Detoxification by pH alteration and active charcoal adsorption resulted in 6.1 g/L ethanol in 48 h, with a yield of 0.30 g/g. The highest ethanol concentration (7.5 g/L) and yield (0.30 g/g) was observed with ion-exchange detoxification. *Saccharomyces cerevisiae* TMB3400, a xylose-fermenting recombinant strain, and *P. stipitis* CBS6054, a naturally xylose fermenting strain, were compared in SSF of non-detoxified hydrolyzate from steam pretreated sugarcane bagasse (190 °C for 5 min) previously impregnated with 2% (w/w moisture) SO_4 for 1 h at room temperature (*52*). The highest ethanol yield (0.35 g/g) and concentration (26.7 g/L) were obtained with *S. cerevisiae* TMB3400. Fermentations with *P. stipitis* under aerated conditions resulted in an ethanol yield of 0.28 g/g and concentration of 19.5 g/L. Lower results (0.05 g/g and 4.6 g/L, respectively) were observed in fermentations with no aeration. Aeration is costly and may even be technically difficult to scale up for lignocellulosic ethanol production.

Unlike SSF, a separation procedure for dividing the mixture of cellulose and hemicellulose into individual streams is not required in the SSCF process. However, drawbacks of SSCF include the high by-product formation in the form of CO_2 and xylitol, poor enzyme stability, and incompatible processing pH and temperature (*170*). The U.S. National Renewable Energy Laboratory has used SSCF in a model process for the production of fuel ethanol from aspen wood chips (*171*). In this design, a 92% conversion of glucose to ethanol and a xylose to ethanol conversion of 85% by a recombinant strain of *Z. mobilis* was proposed. As in the case of SSF, the development of microbial strains able to growth at elevated temperatures may improve techno-economical indicators of SSCF process significantly (*172*).

Consolidated bioprocessing (CBP) is the logical culmination of reaction–reaction integration for the transformation of lignocellulosic biomass into ethanol (*172*). In CBP, ethanol and all required enzymes are produced by a microbial community, in a single reactor (*173*). Xylose-fermenting thermophilic bacteria such as *Clostridium thermocellum* and *Clostridium thermohydrosulfuricum* are prospective organisms to be co-cultured with cellulose hydrolyzing bacteria to directly convert pretreated biomass into ethanol. In addition to converting pentoses and aminoacids into ethanol, *Clostridia* can grow on a wide variety of non-treated waste material. The main drawback is their low tolerance to ethanol having reached concentrations of less than 30 g/L. As of today, no microorganisms or compatible combinations of microorganisms are available that exhibit the whole combination of features required for the development of CBP (*173*). The success of this approach relies heavily on genetic and metabolic engineering for the development of CBP enabling microorganisms for the industrial production of fuel ethanol.

At the end of fermentation, ethanol is recovered from the fermentation broth by existing technologies such as distillation or by a combination of distillation and adsorption methods. Typically, residual lignin, unhydrolyzed cellulose and hemicellulose, enzymes and microorganisms accumulate at the bottom of the distillation column. Lignin is extracted from the solid fraction and may be used for heat and power generation.

Conclusions

Bioethanol production from lignocellulosic biomass represents an alternative source of fuel and could contribute to the sustainable development of the sugar and sugar−ethanol industries by reducing the use of non-renewable resources. Morphological complexity and crystallinity of the lignocellulosic biomass remains as one of the major hurdles in the bioconversion process. Although numerous pretreatment technologies are available for the decomposition of lignocellulosic biomass, one technology that is efficient for a particular type of biomass might not work for another substrate due to compositional differences. Pretreatment of biomass is an extremely important step in the conversion of lignocellulosic biomass to fuels and chemicals and the chosen pretreatment technology will dictate the susceptibility of the substrate to hydrolysis and fermentation of the released sugars. Moreover, the ability of microorganisms to utilize all released sugars is vital to the economic success of lignocellulosic ethanol. Metabolic engineering and systems biology approaches offer new insights for the development of robust microbial strains capable of growth and fermentation in the presence of inhibitors released during the deconstruction process. These new technologies are expected to play a major role in process integration for the successful commercialization of second generation biofuels.

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Chapter 9

Sustainable Production of Energycane for Bio-Energy in the Southeastern United States

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The U.S. Energy Independence and Security Act of 2007 mandates that at least 36 billion gallons of biofuels are to be used by 2022 and that 16 billion gallons be derived from cellulosic sources. Sugarcane as a biofuels feedstock has tremendous potential for production of bioethanol and second-generation biofuels; growers could be paid for not just cane stalks but also for leafy material that is usually not harvested and left on the field. Sugarcane grown solely for the production of energy is referred to as energycane; energycane fiber can range from 14-30%, sucrose 4-15%, and leafy material 2-20%. Energycanes appear to have greater biomass yield potential, ratoon vigor, flood tolerance, and cold tolerance than commercial sugarcane varieties. Energycane may be grown for more ratoon crops, on marginal land, in cooler environments, and with fewer inputs than sugarcane. Economic sustainability will depend on biofuel yields from the conversion of feedstocks such as sugarcane and energycane via multiple techniques such as combustion and cellulosic conversion. Currently, there is a very broad range for fuel yields and conversion costs are also unknown. Once an economically viable and commercial conversion process is established, then the level of resources required to produce a sustainable feedstock from energycane can become more specific.

Introduction

First generation ethanol production, through conventional fermentation of sugar or starch has centered on corn (*Zea mays*) in the U.S. Corn-based ethanol accounts for approximately 97% of the total ethanol produced in the United States. Production of ethanol in Brazil has focused mainly on juice and molasses from sugarcane (*Saccharum spp*) as a primary feedstock; this successful industry demonstrates the technical feasibility of sugar-to-ethanol production. Although technically feasible, juice and molasses-based ethanol from sugarcane is not economically feasible in the U.S. because the production cost would be twice that of corn into ethanol (*1*, *2*). Currently, Louisiana growers are paid US$ 18-32/tonne of cane stalks; economic research shows positive returns on investment for processors if sugarcane stalks can be bought from growers at US$ 15/tonne (*3*). Thus, first generation production of ethanol from sugarcane in the US is not an economically feasible option at this time. However, the Energy Independence and Security Act of 2007 mandates that at least 36 billion gallons of biofuels are to be used by 2022 and in particular 16 billion gallons be derived from cellulosic sources. When one considers this new demand for cellulosic ethanol, then sugarcane as a biofuels feedstock has tremendous potential for production of bioethanol and second-generation biofuels (*1*); growers could be paid for not just cane stalks but also for the leafy material that is usually not harvested. Sugarcane grown solely for the production of energy is commonly referred to as energycane (Figure 1) (*2*). For energycane, fiber can range from 14-30%, sucrose 4-15%, and leafy material 2-20%. For energycane to be sustainable, it must economically produce high and consistent yields (*4*). This chapter will discuss the sustainability of energycane production in the southeastern region of the USA.

During the past decade, bioenergy supplied 47% of total renewable energy, and 4% of the total energy produced in the U.S. (*5*). Once established, perennial energy crops that are well adapted to the climatic and soil conditions of a region do not require annual re-seeding (*6*). They also require lower energy inputs of fertilizer and pesticide than annual crops (*7*), have a high production of biomass, and can often be grown on marginal cropland (*8–10*). Other indirect benefits include enhanced carbon sequestration, greenhouse gas mitigation, and the potential to increase soil stabilization on slopes (*11*, *12*). Grasses, such as sugarcane, can be an energy source by conversion into liquid fuels such as ethanol; sole combustion or companion combustion with fossil fuels to produce heat, steam, or electricity; and through gasification (*13*). The southeastern region of the U.S. has favorable climates for extended plant growth and high yields, with cellulosic biomass as the most attractive renewable energy source; estimated net primary production for this area is 1200 g C/m^2/year (*14*). Temperate and sub-tropical regions can support grass crops that have the efficient C$_4$ metabolic system that returns 4-5 units of energy for each unit used making carbon (C). Of the tropical grasses, members of the *Saccharum* genera are some of the most efficient in converting solar energy to biomass. Crop management practices may change if sugarcane is grown for sugar and/or biofuels and will be influenced by climatic regions where the crop is grown. For sugarcane to be sustainable as a source of food and fuel, the longevity of the crop must be balanced with actual

yearly crop yield. Unlike most other row crops, sugarcane is a perennial with the ability to produce several ratoon crops. Therefore, management choices may not only affect the crop during the current season, but practices may also impact future crops for several additional growing seasons.

Energycane Germplasm

Sugarcane is primarily propagated for sucrose production. Fiber content is inversely related to the level of juice extraction for sugar production and tandem milling efficiency, thus there has been limited efforts for high-fiber sugarcane varietal development (*15*). Sugarcane varieties typically have 13-15% sucrose, 15% or less fiber, and about 70% water (*16*). Sugarcane breeding programs have traditionally discarded clones with excess of 13.5% fiber due to poor suitability for sugar production due to decreased tandem milling efficiency. Many related species and genera, though, have been incorporated into variety development in an effort to broaden the genetic base of the crop (*17*). Early generation F_1 progeny from crosses involving elite sugarcane varieties with a male parent from these related species and genera have exhibited high levels of hybrid vigor, which is hypothesized to potentially impart cold tolerance; greater ratooning ability; enhanced levels of tolerance to moisture extremes, insects, and diseases; and more efficient nutrient utilization. However, these early generation clones had extremely low sugar content, so backcrossing with elite sugarcane varieties/clones with high levels of sugar was required to increase sugar yields to a profitable level. Backcrossing of wild-type germplasm to elite sugarcane parents resulted in a marked reduction of many important biomass yield components, including fiber content, soluble solids concentration (Brix), and stalk population (*16*). If sugar production is not the goal but instead high dry biomass (DB) yields, then these early generation hybrids are ideal candidates for cellulosic biomass production (*4*). Many of these hybrids can produce over 30 Mg/ha DB annually over four fall harvests, with about 20 Mg/ha being fiber and 10 Mg/ha being Brix (*18*).

The DB yields of interspecific and intergeneric hybrids are assumed to surpass that of conventional sugarcane varieties, but there have been few statistical comparisons (*16*). Several hybrids with wild-type germplasm had twice the fiber content of traditional sugarcane varieties, thus they were evaluated as a potential biomass crop in the early 1970s when high oil prices encouraged finding alternative fuel supplies for the co-generation of electricity. The primary objective of initial germplasm development involved identifying crops with high biomass-producing potential (*19*). Sugarcane has a tremendous amount of genetic diversity. One of the first clones to be identified for biomass production was the energycane 'L 79-1002', which is described as a cold-tolerant genotype (*20*). The cross for 'L 79-1002', an F_1 hybrid, was made in 1974 using the sugarcane variety 'CP 52-68' as the female parent and 'Tainan', a *Saccharum spontaneum* clone, as the male parent. Original testing was done from 1976-1983 in yield trials conducted in the traditional sugarcane growing area of south Louisiana and in the colder, non-traditional sugarcane growing regions of north Louisiana. Yield testing resumed from 2002 through 2005 as interest in biofuels research resurged.

Figure 1. HoCP 96-540 (sugarcane) on left; L 79-1002 (energycane) on right.

Experiments in the northern area indicated a broader range of adaptability than sugarcane varieties grown for the production of sugar (*21*).

Early studies reported average cane yield (on a wet-weight basis) of 'L 79-1002' was 170 Mg/ha compared to the traditional sugarcane 'CP 65-357' (*22*) yields of 50.4 Mg/ha. This energycane also produced an additional 41.7 Mg/ha of leafy material, while the sugarcane produced 7.5 Mg/ha (*20*). Both of these plant parts have potential as feedstocks for cellulosic fuel production (Figure 2). In another study investigating the comparison of yields of energycane and sugarcane at multiple non-sugarcane growing areas in Florida and Alabama, 'L 79-1002' averaged 18 Mg/ha more DB than 'CP 72-1210' (*23*), a sugarcane variety commonly grown in south Florida (*24*). Other studies have compared energycane and sugarcane to other tall bunchgrasses including elephantgrass (*Pennisetum purpureum* Schum.) and to forage and sweet sorghums (*Sorghum bicolor* L. Moench). Yield rankings varied with geographical location, but energycane and elephantgrass consistently had higher yield than annual grasses and sweet and forage sorghums in sub-tropical and temperate zones. Another study evaluated the energy potential of energycane relative to canarygrass and pearl millet. The energycane 'L 79-1002' produced the highest dry weight biomass of 49 Mg/ha (*25*). To estimate gross energy yields, bomb calorimetric procedures were conducted on several bunchgrasses. 'L 79-1002' energy equivalent yields were 141 barrels of crude oil/ha/year (*24*).

Yield Attributes

Early biomass studies were initiated to identify plant attributes responsible for high DB yields of C4 elephant grass and energycane. Daily DB accumulation rates were 0.23 Mg/ha/d, which is within the normal range for C_4 plants, but surprisingly was lower than reports of 0.29 Mg/ha/d for irrigated, well fertilized grain sorghum (*26*). Moreover, tropical maize has been reported to have DB rates of 0.33 Mg/ha/day (*27*). However, this period of DB accumulation ranged from 140-196 days for energycane in comparison to 47 and 50 days for sorghum and tropical maize. Thus, the duration of the grand growth period of energycane was responsible for the high yielding ability of L 79-1002 rather than a higher daily rate of DB accumulation. Total solar accumulation was highly influential on DB yields of 'L 79-1002'. Its radiation use efficiency was 1.24 g DB produced above ground per MJ of total solar radiation and 1.30 g per MJ intercepted total solar radiation. The energy concentration for annual DB and solar energy recovery was 18.0 kJ/g and 2.24%, which was similar to other bunchgrasses evaluated (*24*). Solar energy recovery is the percentage of energy from total solar radiation available that is stored in chemical-bound energy within a plant. Solar energy recoveries for energycane, sorghum, and tropical maize have independently been reported as 2.24, 2.23, and 2.85%, respectively (*24*, *28*). Thus, energycanes are not the most efficient converters of solar energy into chemical energy, but their extended growth phase allows them to capture more solar energy over an entire growth season relative to other C_4 grasses. This extended growth phase of energycane is attributed to tillers remaining functional and the continuous activity of apical meristems throughout most of the year in subtropical areas. This allows energycane to maintain light interception and radiation use efficiency over extended periods relative to other C_4 plants such as sorghum and tropical maize. Moreover, continued stem elongation, increased internode density, and continued canopy development of tillers allows for storage of solar energy as DB in an extended spatial area (*24*). Selection against flowering could further increase duration of the vegetative growth period.

Non-Stalk Feedstock

A majority of sugarcane in Louisiana is harvested green, whereby the chopper harvester separates some of the leafy material from the actual stalks. This post-harvest residue left of the field is typically removed from the field surface by burning because retention causes yield loss. With burning much of the C-H-O-N is lost but most of the minerals are returned to the soil. With complete-cane harvest, all of the minerals in the leafy material would be removed from the field. Removal of aboveground biomass for cellulosic feedstock harvesting operations may affect sustainability by negatively impacting soil health. Parameters used to define soil health include the ability of a soil to retain and release nutrients and water, provide an acceptable medium for root growth, and resist degradation or erosion (*29*). Bare or sparsely covered soil is highly susceptible to wind and water erosion. The amount of residue needed for erosion protection varies with management practice. Limited soil sustainability work has been conducted

Figure 2. Sugarcane billets (left) and post-harvest residue (right).

specifically on energycane, so we must consider research involving other crops. Graham et al. (*30*) constrained corn (*Zea mays*) stover removal limits to the tolerable soil erosion loss (T-values) as defined in the revised universal soil loss equation (RUSLE). Based on this constraint, universal adoption of no-tillage allowed for greater residue removal than mulch till or current tillage practices with U.S. estimates of 80, 60, and 50 million dry Mg/year, respectively (*30*). However, others have indicated that significant erosion can occur even if removal rates are constrained to T-values (*31*). Wilhelm et al. (*32*) stated that the corn stover needed to maintain soil organic C (primary component of soil organic matter) was greater than that needed to control soil erosion. Thus, carbon neutrality should be considered when determining optimal residue removal rates.

Crop residue provides the primary input for soil organic matter in agricultural systems. Soil organic matter benefits include increased soil structure (enhanced aeration and infiltration), increased water holding capacity, erosion control, higher biological activity, ion exchange, and nutrient release. Mineralization of soil organic matter releases plant nutrients, mainly N, P, and S. Soil organic matter also contains a cation exchange capacity as high as 200 $cmol_c$/kg soil at neutral pH, which can bind and retain the macronutrients (Ca, Mg) and micronutrients (Cu, Zn) required for crop growth. Varvell et al. (*33*) reported that 51% removal of corn stover resulted in significant reductions of corn grain and corn stover yield over a six year period. Similarly, Wilhelm et al. (*34*) reported a 0.1 Mg/ha grain yield reduction for each Mg/ha of stover removed. Karlen et al. (*35*) reported a loss of 4.5 and 0.28 Mg of soil C and N per hectare after 10 years of corn stover removal as compared to non-removal. However, yield reductions were variable. Lemke et al. (*36*) modeled soil C reductions in 30 years continuous wheat (*Triticum aestivum*) production in Canada. They found removal of 50% and 95% of wheat residue would marginally and significantly reduce soil C mass, respectively. Less work has been accomplished in sugarcane and none in energycane. However, current sugarcane cultural practices associated with residue management offers a starting point for research.

Green harvesting of sugarcane deposits 6 to 24 Mg blanket of post-harvest residue/ha (*37*), which consists of brown and green leafy material and some fragments of cane stalk. Full retention of this residue negatively impacts ratoon yields in temperate areas due to several factors, including cooler, wetter spring growth conditions, leached autotoxic chemicals, and reduced weed management options (*37*, *38*). This contrasts to the effects of residue in the drier tropical regions, where irrigation is required and where full retention resulted in higher cane yields as compared to preharvest burning in 8 of 13 years evaluated, and the remaining years were not different (*39*). Current options in Louisiana for the handling of the post-harvest residue blanket include the controlled burning of the residue or the brushing of the residue from the row top containing the planted line of sugarcane into the wheel furrow (Figure 3). For cultivar 'LCP 85-384' (*40*), postharvest controlled burning at predormancy or complete dormancy during the winter months increased cane and sugar yields compared to mechanical removal or full retention (*37*). However, controlled burning of sugarcane fields is facing legal and air-quality obstacles, especially in those areas near urban centers.

Soil Health

The percentage of the residue to be returned to the field at harvest can be selected during harvest using existing chopper harvester technology. With the chopper harvesters currently utilized to harvest the majority of the sugarcane grown in the USA, harvested stalks are chopped into 15 to 20 cm long pieces (billets) and large extractor fans are used to separate some of the leaf trash from stalk pieces based on plant material density. The settings on the extractor fan can be adjusted to remove all or just a portion of the leafy material. Consequently, the amount of postharvest residue can be adjusted depending on the harvester's extractor fan speed settings (*41*). However, similar to corn and wheat systems above, removal of excess nutrients will negatively impact soil organic matter (SOM), as cane residue C along with root C represent the primary inputs for SOM. Blair et al. (*42*) observed an increase in total soil C (0-1 cm depth) for green-cane harvest as compared to burning of leaves prior to harvest in Australia. Total C values were similar for deeper depths (1-10 cm, 10-25 cm, or 1-25 cm). In Brazil, soil C was found to increase between harvest and 6 and 12 months in 0-1 cm depth for green-cane harvest (*42*). But again, the lower depths were similar for green-cane harvest or burning. After plant-cane and two ratoon crops, total soil C (0-10 cm depth) was greater in burnt green-cane as compared to a no removal control (full retention). However, there was a trend for higher labile C where the residue was not burned. Additionally, wet aggregate stability was greater in the non-burned plots (*43*). This preliminary evidence may indicate that soil C sequestration mechanisms (e.g., macroaggregate formation) are in early stages (*44*). Many of these studies are conducted in tropical areas where organic matter decomposition rates can be double that of temperate regimes (*45*). Therefore, this data may not completely extrapolate to all potential energycane growing areas, indicating a need for long-term research.

Figure 3. Postharvest residue mechanically removed from the row top.

One possibility to mitigate nutrient removal effects on soil health is by using by-products from sugarcane processing (e.g., filter press mud, fly ash), fermentation (e.g., vinasse), or pyrolysis (e.g., biochar). A positive interaction between fertilizer and factory filter mud was observed for cane and sucrose yields in Florida (*46*). Factory mud, when applied with fertilizer, increased plant cane and sucrose yields as compared to the control (no mud or fertilizer) or fertilizer alone. Similarly, Prasad (*47*) reported consistent increases in plant-cane and ratoon-cane yield when filter press mud was applied to sugarcane land. Using 2009 USDA fertilizer economic data, the value range of 6 Mg sugarcane biomass at N, P, and K levels of 0.7, 0.07, and 0.7%, respectively, would be about USD 120/ha. However, date of harvest needs to be considered due to nutrient translocation within the plant. Other considerations include the cultural practices now used in sugarcane production and how these may evolve over time. Reduced tillage, furrow dikes, precision leveling, precision agriculture, cover-crops, short-season rotational crops, and development of new implements may all play a role in sustainability of energycane as a cellulosic feedstock.

Crop Establishment and Ratooning

The geographic boundaries of sugarcane in the southeastern U.S. have always been limited to areas where cane can survive harsh winters. Sugarcane in Louisiana is propagated from vegetative plantings in late summer and early fall as either whole-stalks with 4 to 16 nodal buds or as stalk pieces (billets) with 2 to 4 buds (*48*). Winter survival is a problem in Louisiana due to saturated soils that encourage stalk rotting organisms and re-ccurring freezing temperatures and is the reason for the higher planting rate relative to tropical areas (*49*). Furthermore, stalk rot is a major concern in sub-tropical and temperate areas and becomes

more severe when seed-cane is exposed to environmental stress (*50*, *51*). Viator et al. (*48*) indicated that planting in August, averaged across variety and planting method, results in the highest plant-cane yields and that this increase in yield can carry over into the first-ratoon crop. Research on elephantgrass and energycane in Florida demonstrated better establishment if grasses are planted during the winter season and allowed to grow the entire warm season prior to the next winter instead of spring planting (*25*). Therefore, planting in early fall may even further increase levels of establishment compared to winter plantings; this crop should be allowed to emerge and develop a below ground stool with above-ground tillers before a killing frost. New shoots can then emerge from this well-developed stool. Preliminary data from studies located in Louisiana on plantings of energycane and sugarcane in August, September, and October demonstrate that yields of both types of canes are higher when planted earlier (Viator, unpublished data).

One must consider not just winter survival of newly planted sugarcane, but also over wintering of ratoon crops. Multiple ratoon crops are necessary for sustainable energycane production because planting cost is relatively high compared to annual, seed-propagated crops such as corn. Louisiana's temperate climate, which is somewhat representative of the southeastern U.S., is unique compared to most other sugarcane producing areas. In tropical climates, the sugarcane plant does not undergo a dormant growth stage due to low soil temperatures; it re-emerges immediately after being harvested and continues to grow (except for short periods of stress such as drought) until the subsequent harvest. In a temperate climate, the crop does reemerge after harvest but is killed due to frost events (*52*). The crop remains dormant until soil temperatures exceed 18 °C (*53*). As soils warm beyond this threshold, primary tillers emerge and then secondary tillers (post-dormancy) (*37*). Without multiple ratoon crops, economic sustainability is not possible due to the high planting costs of sugarcane. Cold tolerant bunchgrasses like elephantgrass and energycane should over winter in USDA Plant Hardiness Map Zones 8-11 if it can be established well before the winter (*24*). Burner et al. (*54*) conducted some initial work on cold tolerance of sugarcane. Ratoon cold tolerance was defined as the ability of the plant to produce viable shoots in the spring of the year after transplanting. *Saccharum* sp. (SAC) by *S. spontaneum* (SPT) crosses were compared to *Saccharum* sp. by *Miscanthus* sp. (MIS) crosses. SAC/MIS hybrids exhibited ratoon cold tolerance in west-central Arkansas (35°05′N, 93°59′W), unlike SAC/SPT hybrids (*54*). On the other hand, there were only four sources of SPT germplasm represented, thus there was an extremely limited sampling of SPT (*55*).

Harvest

Another challenge facing yield stability of energycane is harvest timing. An extensive study was conducted to determine the best harvest time to maximize DB yields using energycane 'US 72-1153'. Plant cane (initial harvest) was harvested when stalks reached 1.2, 2.5, and 3.7 m, (stalks measured to the leaf collar of the uppermost fully expanded leaf); October (4.9 m height); and December (4.9 m height). Early harvests at stalk heights of 1.2 and 2.5 resulted in significantly lower

yields, 19 and 36 Mg/ha, compared to the yields of all other harvest dates, which ranged from 52 to 65 Mg/ha (*56*). Harvest date may affect biomass quality such as moisture content and relative amounts of lignin, cellulose, and hemicellulose. Besides harvest dates affecting the current crop yields, harvest date can also have carryover effects into the subsequent ratoon crops. Viator et al. (*57*) conducted research with four Louisiana sugarcane varieties ('LCP 85-384', 'Ho 95-988', 'HoCP 96-540', and' L 97-128' (*40*, *57–59*) in which plant-cane was harvested on 1 October (early) and 1 December (late-season). Averaged across all four varieties, the October harvest of plant-cane reduced sucrose yields (p = 0.01) of the first-ratoon (7.7 t/ha) compared to the late-season harvest date (10.1 t/ha.). A second experiment was conducted to determine the effects of two consecutive years of early harvest (plant-cane and first-ratoon) on yields of the second-ratoon. Averaged across all varieties, the October harvest of both plant cane and first ratoon reduced sugar yields (p = 0.01) of the subsequent second-ratoon (5.5 t/ha) compared to the December harvest (10.0 t/ha). It is currently suggested that Louisiana growers not harvest cane early in consecutive seasons because yields are reduced below the point of a positive return on input costs (*60*).

Research with ratooning with various harvest dates utilizing energycane showed similar results. When repeatedly harvested at an immature stage of 1.2 m or at full maturity, the energycane 'US 72-1153' produced a 4-year average yield of 10 and 48 Mg/ha/yr dry biomass, respectively. This resulted in decreased DB of 89% (1.2 m harvest) and 53% (mature harvest) between years 1 and 4 of the four-year crop cycle (*61*). Similar results were obtained with 'L 79-1002' (*56*). Fresh-weight and dry-matter yields decreased linearly across crops (plant-cane through the fourth-ratoon crop) for sugarcane varieties, but there was no consistent trend for the early-generation hybrids which could be utilized as energycane (*16*). Energycanes, unlike sugarcanes, produced consistent yields of fresh-weight and dry-matter biomass/ha for 5 years with no evidence of decreasing yield (*16*). Deren et al. (*8*) stated that interspecific and intergeneric sugarcane hybrids manifest attributes of parental sugarcane and wild relatives which results in good ratooning ability that contributes to increased stand duration. They also reported slow stand establishment in plant-cane but prolonged ratooning with wild-type germplasm. Future work needs to be conducted on maximal stand longevity. It is not known how many ratoon crops could be harvested from a single planting of energycane, but germplasm has been grown for over ten ratoons in nurseries containing many of the same clones at Houma, LA (A. Hale, unpublished data). Conversely, sugarcane is normally productive for only two to three ratoon crops in Louisiana, as demonstrated by the linear decrease in biomass yields across crops (*16*). Moreover, continually harvesting energycane at the early stage of 1.2 m stalk height consistently produced the lowest DB yields (10 Mg/ha) compared with the other harvest dates where yields ranged from 30 to 52 Mg/ha. In contrast, ratoon yields were not different for the October and December harvest dates. These studies suggested that harvest management is an important factor for energycane biomass yield and ratoon-crop sustainability (*56*).

Sustainability

Continued DB production will require soil nutrient replenishment because high yield cannot be sustained over the long-term without adequate inputs. Based on a set of nine sustainability indicators (resource use efficiency, soil quality, net energy production and greenhouse gas emissions, and disregarding socio-economic or biodiversity aspects and land use change), biofuel produced from oil palm (South East Asia), sugarcane (Brazil), and sweet sorghum (China) appeared to be the most sustainable (*62*). Thick-stemmed perennials require high inputs, but in the appropriate locations the high yields can offset the high inputs required. Herbaceous perennial energy crops such as energycane had lower N-P-K and herbicide application rates relative to corn and some short-rotation woody crops. Energycane also had relatively lower input costs in terms of both dollars invested per unit dry biomass and energy output relative to switchgrass and woody crops with near equivalent input costs to that of sorghum (*63*).

Other research on methane production demonstrated that there were no significant differences in terms of methane per unit of energycane DB for 0, 168, 336 kg N/ha (*2*). DB yields of over 40 Mg/ha/year were obtained with adequate rainfall and high fertilization rates of 168, 18, and 70 kg/ha of N, P, and K with energycane (*24*). Mislevy et al. (*61*) reported DB yield increased 9.6 Mg/ha for plots with 336 kg N/ha compared to plots receiving 168 kg N/ha. These yield differences were somewhat reflected in the nitrogen content in above ground biomass, which was 5 and 4 g/kg on a DB basis for the 336 and 168 kg N/ha rates, respectively. Additional nitrogen may be partitioned and stored in underground biomass, which could explain why the higher nitrogen rates resulted in lower yield reductions than the lower nitrogen rates when one compares the plant-cane crop to the third-ratoon crop. Moreover, the lower rate of N resulted in twice the amount of stand loss when one compares stalk counts in plant cane to stalks counted in the third ratoon. Mislevy et al. (*61*) also noted that immature harvesting can result in complete stand failure within 2 years.

Feedstock Quality

Optimal production of energy from biomass requires the identification, production, harvest, transport, and storage of high yielding perennial grasses such as energycane (*61*). One of the reasons that sugar based biofuels from sugarcane is not feasible in the U.S. is that ethanol cannot be produced year-round. Ethanol can only be produced immediately after sugarcane is harvested because it will soon begin to deteriorate (*3*). The deterioration could be mitigated by the use of high-fiber energycanes with very low amounts of juice. To achieve economies of scale, large quantities of biomass will need to be converted into biofuels over an extended time period. One option is ensiling, which is storage of wet plant matter in a silo or some other type of storage like plastic bags. Work where energycane was harvested with a silage harvester chopping it into 2-3 cm pieces after which it was ensiled in plastic silo bags resulted in a 90% DB recovery (*25*). Ensilage also produced similar methane yields relative to yields produced from fresh energycane (*2*). Another option to consider is harvest timing. Delayed harvest

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could improve the quality of feedstock for cellulosic conversion by reducing tissue water concentration, and this would reduce yield of leaves, lignin, ash, and cellulose (*54*). However non-stalk material reduction would represent losses of about 33% of total biomass and loss of considerable energy because lignin has fuel value equivalent to coal (*54*).

Biomass partitioning work has revealed important information in regards to crop growth and maturity. Stem and dead leaf plant components increased quadratically as plant height increased, and as the crop matured the amount of green leaves decreased from 70% to 17% as the plant increased in height from 0.6 to 4.3 m. This resulted in decreased crude protein concentrations of 51% for green leaves and 81% for stems, respectively, as plant height increased (*61*).

Conclusions

To conclude, energycane has tremendous potential as a renewable biomass source for energy production in the southeastern U.S. Energycanes appear to have greater biomass yield potential, ratoon vigor, flood tolerance, and cold tolerance than commercial sugarcane varieties. Energycane may be grown for more ratoon crops, on marginal land, in cooler environments, and with fewer inputs than sugarcane. Economic sustainability will depend on biofuel yields from the conversion of feedstocks such as sugarcane and energycane via multiple techniques such as combustion and cellulosic conversion. Currently there is a very broad range for fuel yields and conversion costs are also unknown (*12*). Once an economically viable and commercial conversion process is established, then the level of resources required to produce a sustainable feedstock from energycane can become more specific.

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Sugar Beet (*Beta vulgaris* L) as a Biofuel Feedstock in the United States

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Sugar beet is a biennial plant, which produces an enlarged root and hypocotyl in the first year, in which it stores sucrose to provide energy to flower in the next season. Technically, conversion of sugar to ethanol is a simple process requiring only yeast fermentation. A 2006 USDA study calculated the yield of ethanol from the sucrose in a sugar beet was 103.5 L per tonne of root (wet weight). Life cycle analysis (LCA) indicates that bioethanol from sugar beet reduces green house gases as well or better than maize. Both nitrogen and water use efficiency may be superior to maize on average. However, sugar beet with an area of 465,000 ha in 2009, compared with about 32 million ha of maize, likely will not displace maize as the primary feedstock for bioethanol in the U.S. More likely, co-products like pulp and molasses will find use as bioenergy feedstocks, probably for high value specialty fuels or as feedstocks for a whole generation of petroleum plastic substitutes.

Introduction

Sugar beet (*Beta vulgaris*, L) is a biennial plant. In the first year, it produces an enlarged root and hypocotyl, in which it stores sucrose that provides energy used to flower in the next season. Sugar beet typically is cultivated in the northern temperate zones, between 30° and 60° (*1*), where it is primarily a spring

planted crop. There also are areas of cultivation in the southern temperate zones, including Chile, Venezuela, and Uruguay (*2*). It also can be cultivated as a winter crop, "winter beet", (planted in the autumn and harvested the next summer) in Mediterranean regions and some arid tropical and sub-tropical areas, if irrigation water is available or rainfall is sufficient.

Although domestication of beet as a leafy vegetable and root crop took place in prehistoric times, sugar beet is a relatively new crop plant (*3*). The European beet sugar industry was able to develop once the technology to measure sucrose concentration in solution was discovered, and the spread of this industry was accelerated by increased demand for beet sugar caused by the British blockade of continental Europe in the early 19th century. Starting in France and Germany, the beet sugar industry spread throughout Europe, to North and South America, Asia, and North Africa (*2*).

About 35% of global sugar production and 50-55% of the domestic (U.S.) sugar production comes from sugar beet, equating to about 8.4 million metric tons (*4*). Some sugar beet currently is used for fuel ethanol production and, in Europe over the past three years, this has increased sharply because of restructuring of the European Sugar Regime (*5*). Production of sugar beet in 2009 in the U.S. was 26.7 million tonnes on 465.6 thousand hectares at a value of approximately $1.3 billion (*6*). Sugar beet was grown in 12 states and processed in 22 sugar beet factories.

Sugar beets are refined directly into white sugar at processing plants (see (*7*) for details of this process). Sucrose content in sugar beet ranges from 16-20% (wet weight). The major co-products from sugar beet processing are molasses, consisting of soluble impurities including some sucrose, which remains after sucrose extraction from the juice; and pulp, which consists of root material from which the sucrose has been extracted (*8*). Both are used as animal feed.

Technically, conversion of sugar to ethanol is a simple process requiring only yeast fermentation, whereas producing ethanol from maize, e.g., requires enzymes to convert starch to sugars (*9*).

The Sugar Beet Plant as a Biofuel Feedstock

Sugar beet is planted as early as possible in temperate areas because there is a direct correlation between the amount of solar radiation intercepted by sugar beet leaves and the sucrose stored in the root (*10*, *11*). However the sugar beet seedling is sensitive to cold and will not survive prolonged exposure to air temperatures below -2.5 °C (*12*).

The amount of sucrose extracted per area is dependent on three factors, the weight of the beets harvested, the percentage sucrose in those beets, and the amount of the sucrose that is extractable. Even though the beet root may contain up to 20% sucrose by fresh weight, the average percent extracted is less. Cations such as Na^+ and K^+ and small amino nitrogen compounds (e.g., glycine, betaine, and glutamine) interfere with the extraction and re-crystallization of sucrose (*13*). The average percent sucrose recovered from the U.S. crop from 2000-2009 was 15.3% (*6*). The portion of juice that is left over once all of the extractable sucrose

has been removed is the molasses, which represents about 4% of the weight of one tonne of sugar beet and has a sucrose percentage of about 50% (*14*).

Pulp or marc remains after the sucrose and molasses have been extracted from the crop. The pulp represents the 22-28% of the dry mass of the sugar beet root that is not solubilized during the sugar beet extraction process (*15*). The weight of beet tops ranges from 4.6 to 7.5 tonnes per hectare (*15*), and beet tops have feed value, but are usually left in the field at harvest.

In a 2006 USDA study, it was estimated that the yield of ethanol from the sucrose in an average sugar beet crop was 103.5 L per tonne of root (wet weight) (*14*). This calculation was based on a refined sucrose recovery of 15.5% (of wet weight), and a yield of 20 kg of sucrose from a tonne of beet molasses (*14*). The authors based their calculations on a theoretical (stoichiometric) yield of 680 liters per metric tonne of sucrose and then assumed an obtainable yield of 86.6% (*14*).

In the 2006 USDA study, only sucrose or molasses was examined as a potential biofuel feedstock. The pulp contains 80 to 94% fermentable components (pentosans, pectins, and cellulose) and only 12 to 16% lignin, crude protein and mineral substances (*16*). Therefore, much of the pulp could provide additional biofuel feedstock if the sugars were released from the biomass. Atlantic Biomass Conversions (Frederick, MD) has reported that it is theoretically possible to solubulize 50-60% of the available sugars with an enzyme digestion method (*17*). The co-product of this process is a protein pellet of about 35% crude protein, which has value as animal feed (*17*). If pulp could be solubilized to fermentable sugar, it would provide an alternative feedstock source from sugar beet that was not considered into the 103.5 L/tonne calculation of Shapouri *et al.* (*14*).

The beet root dry weight is about 24% of the root yield fresh weight (*15*). The pulp of the sugar beet root is about 25% dry weight of the sugar beet root, (sucrose averaging about 75% of the dry root weight), therefore one tonne of sugar beet (fresh weight) yields about 6.0% pulp (fresh weight) (*15*). If 40% of a tonne of pulp (dry weight) could be converted to fermentable sugars (sucrose equivalent), the pulp would yield approximately 235 liters of ethanol (using the predicted yield of Shapouri *et al.* (*14*). One tonne (dry weight) of pulp is produced for every 17 tonnes (fresh weight) of beets harvested. The total ethanol yield per tonne of sugar beet than could equal 117 liters (assuming 40% conversion of the pulp) rather than the 103.5 liters estimated in the USDA study (*14*). The enzymatic digestion of the pulp would add a cost to the ethanol production, however, very little additional energy costs. Alternatively, von Felde (*18*) has estimated that a larger amount of energy is extractable from beets using anaerobic digestion methods for whole beets to produce bio-methane, compared to ethanol. This is a potential energy resource that should be studied in more depth.

Potential U.S. Sugar Beet Yields and Acreage

Sugar beet sucrose yield (all three components) or energy yield depends on a number of environmental and cultural factors. These include whether the crop is irrigated or rain fed, length of growing season, latitude (determining day length), disease pressure, soil type and fertility, and presence or absence of other

abiotic stresses (drought, temperature, CO_2 levels, etc.) (*19*). Assuming no other significantly limiting factor, the sucrose concentration of the harvested root is proportional to the amount of solar radiation intercepted by a full canopy (*11*). Sugar beet is well adapted to a wide range of soil types and is able to thrive in soils with a pH above 6.5. In the United States sugar beet has been cultivated in soil types ranging from peat soils (San Joaquin Delta, CA) to rich loam soils of the Midwest and in low organic matter, slightly saline, mineral desert soils with a pH greater than 8.0. In arid to semi-arid sub-tropical areas, with sufficient irrigation, sugar beet will survive temperatures upwards of 40 °C. However, in humid tropical and sub-tropical areas, disease can limit production at high temperature.

Within the U.S., the four main growing regions have very different root yields per hectare (Figure 1) as well as total regional production based on total regional area cultivated (Figure 2). The per hectare yield differences are due to different agronomic practices and growing conditions. The highest yields are in the Far West, which consists primarily of Idaho, with smaller acreages in Oregon, Washington, and California. The only growing area left in California is in the Imperial Valley, where sugar beet is grown as a winter crop, i.e., planted in the fall and harvested early the next summer. The crop is irrigated in this area and the growing season is long (from mid-September until mid-July). The Far West region was about 17% of the 2009 growing area (*6*).
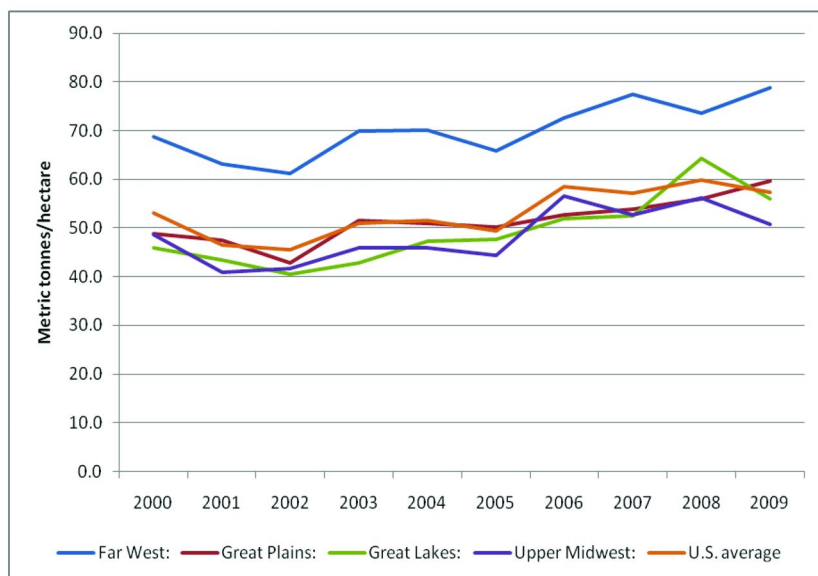
Figure 1. Yield per hectare in the four U.S. growing regions over the last ten years. Although there are year to year fluctuations, the general trend is increasing yield.
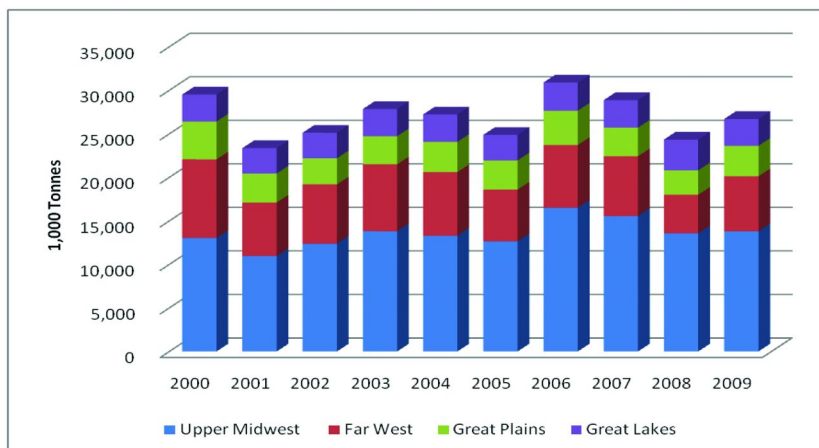
*Figure 2. This graph indicates total production in metric tonnes in the United
States over the last ten years by growing area. There is a trend toward higher
yields even though the area cultivated is below historical highs.*

The largest growing area in the U.S. is the Upper Midwest consisting of
Minnesota and North Dakota. It is in the northernmost part of the continental
U.S. and, therefore, has long summer days. The Great Lakes region consists of
beets cultivated in Michigan (and Ontario, Canada). However the crop grown in
both regions is not irrigated and has a short growing season, therefore. average
yields are the lowest in these growing areas. Nonetheless, with about 58% of the
2009 growing area, the Upper Midwest's total production leads the U.S. (Figure
2). The Great Lakes area's production (12.6% of the U.S.) is similar to production
in the Great Plains (Montana, Wyoming, Colorado, and Nebraska (11.8 % of the
U.S.) (*6*). The yields of the two growing areas are similar despite the fact that the
Great Plains' crop is grown with irrigation.

Sugar beet production in the United States is determined by domestic
marketing allotments allowing the Cooperatives producing sugar to market an
amount of sugar based on historical production in their growing area (*20*). For this
reason, sugar beets are planted only if the grower has a contract for processing.
Current sugar prices are high and projected to stay that way throughout 2010
(*21*) and, therefore, there is little interest in diverting refined sucrose into biofuel
processing. However, sugar beet cultivation has moved into the Upper Midwest
over the past 20 years due, at least in part, to the lower cost of production in this
region. Therefore many former growing areas have less area cultivated for sugar
beet than their historical highs.

If sugar beet were grown exclusively as an energy beet, many of the areas
where it has been grown in the past would be the logical first places to look to
for increased production. A recent study for the Washington State Department
of Agriculture (*22*) looked at the feasibility of ethanol production from a sugar
beet feedstock. In the past, sugar beet has been produced on 37,000 hectares in
Washington State (*6*), however, only about 650 hectares were grown in 2008 and
none in 2009. Nonetheless, Washington State has had high yields, comparable

to California, which has had the highest in U.S. (Table I) (*6*). The report concludes that three factors would have to converge to increase the likelihood of successfully producing ethanol from sugar beet in Washington State. They are: the simultaneous (i) increase of the price of oil, (ii) increase of the cost of corn (maize), and (iii) the decrease of the price of refined sugar (*22*). This would increase the economic competitiveness of sugar beet as an ethanol feedstock nationwide.

Table I. Area Harvested and Average Yield for the Last Three Years by U.S. State within Growing Region

	Hectares Harvested			*Yield Mg/ha*		
	2009	*2008*	*2007*	*2009*	*2008*	*2007*
Great Lakes:						
Michigan	55039	55039	60300	56.0	64.3	52.4
Upper Midwest:						
Minnesota	184139	161475	194661	51.5	55.3	53.3
North Dakota	87415	79726	99961	49.3	58.0	51.7
Great Plains:						
Colorado	14165	11574	11817	62.1	59.4	58.7
Montana	13315	12424	19021	65.4	60.0	55.3
Nebraska	21247	15095	17928	54.9	50.6	52.6
Wyoming	10118	10967	12222	58.2	54.9	48.8
Far West:						
California	9956	10279	15824	89.6	88.9	79.5
Idaho	65966	46945	67585	76.8	69.9	77.1
Oregon	4249	2388	4452	82.3	74.0	71.5
Washington	---	648	809	---	93.8	94.1

Winter Beets

Another area, in which the production of biofuels from sugar beet is being considered, is California (Figure 3). Storing the harvested sugar beet roots is one of the largest obstacles to using only sugar beet as a biofuel feedstock, because they degrade in quality much more quickly than does grain in storage. The Sacramento and San Joaquin Valleys of California are climates in which beets can be grown as both spring and fall planted crops, and harvested daily for 6 to 7 months. If anaerobic digestion were the primary conversion technology, additional beets might be ensiled to allow additional months of operation.

When sugar beet is treated as a fall planted crop in some areas of the world (sub-tropical and tropical, plus arid), including the Imperial Valley of California, it is planted late summer and harvested the following late spring and summer (210 to 300 days from planting). The advantage to growing winter beet is that yields can be much higher due to longer growing season (nine months instead of six or seven). In Mediterranean climates, Fall-planted beets have better water use efficiency than spring planted beets due to greater water use efficiency during periods with cooler temperatures and more frequent rainfall throughout the winter. Disease pressure also may be reduced. Disadvantages to growing winter beet include breeding for extreme tolerance to bolting because the cooler winter temperatures may approach the temperature needed for vernalization and flowering. Although the disease pressure may be reduced, there often is a different spectrum of disease and insect problems than seen in spring planted sugar beet, and winter beet hybrids must contain a different suite of resistances to these pests and diseases. Finally the logistics of harvest are more complicated because roots cannot be stored for more than a few days before processing (23, 24). Many of the specific practices are reviewed in Cooke and Scott (25) and Draycott (19). Irrigation of winter beet has been reviewed (26, 27).

Figure 3. Harvesting over-wintered beets in Brawley, California, in June, 2008.

**Table II. Imperial Valley of California Harvest Results from 1998 – 2007
(Personal Communication, Ben Goodwin)**

Crop Year	Hectares Harvested	Tonnes/ha	% Sucrose	Kg Refined sucrose/ha
2007	9620.9	85.2	17.3	14739.2
2006	9616.5	82.9	16.8	13932.8
2005	9471.6	86.4	16.7	14441.3
2004	10439.2	96.3	16.5	15921.9
2003	10565.5	96.7	16.2	15633.0
2002	10367.2	95.0	16.7	15904.0
2001	10634.7	93.3	15.5	14416.6
2000	12750.5	86.5	16.3	14050.4
1999	12902.6	90.3	17.0	15374.2
1998	13822.9	80.9	17.2	13932.8
Average	11019.2	89.3	16.6	14834.6

Yields in California averaged 86.0 tonne/ha during the years of 2007 through 2009 (Table I). In the Imperial Valley, where only fall-planted beets are grown, that average over 10 years was 89 tonnes/ha (Table II, Ben Goodwin, personal communication). However, there is an approximate doubling of yield between the fields harvested in April (60 t/ha) and early August (120 t/ha) because the beet crop continues to accumulate dry matter until harvest (*28*). For this reason, the winter beet yield potential is much greater in irrigated Mediterranean and semi-arid to arid conditions with modern agronomic practices than in regions with more temperate or continental climates. For example, the highest known commercial yield (142.4 tonnes/ha) was observed in 2004 in the Imperial Valley of California from a 33 ha field (80 acres), harvested in July, which produced an average 23.5 tonnes/ha gross sugar (*28*). This is a tremendous potential ethanol yield per hectare.

The theoretical ethanol yield from crops with such high yields is very large. For 2007 average yields in the Imperial Valley, approximately 9,400 L of ethanol can be produced per ha on average (1000 gal/ac). This is more than double average ethanol yields from United States maize in 2009 (4660 L/ha), average estimated sugarbeet ethanol yields (5,100 L/ha), or average sugarcane ethanol yields in Brazil of 6,800 L/ha) (*14*, *29*, *30*).

Life Cycle Analysis (LCA)

Life cycle analysis is a methodology that attempts to evaluate the net green house gas (GHG) effects generated from the extraction of the raw materials to the end of their use during the production of a product or service. There are international standards that provide the framework, guidelines, principles,

requirements, etc. for conducting LCA studies (ISO 14040:2006 and 14044:2006) (*31*). LCAs are used by a number of governmental agencies to make decisions to promote or mandate biofuels (*32*). LCA calculates the direct effects of biofuel production and use from feedstock production and assembly to transformation and ultimate use in vehicles. Based on the analysis of direct effects, most LCAs indicated that first generation biofuels result in GHG savings compared to petroleum based gasoline or diesel. There is debate, however, that calculating only direct effects misses other important consequences from crop use for biofuels, resulting from market-mediated pressures to convert new lands to agriculture to substitute for land diverted from traditional food or feed production (*33*, *34*). This issue is in dispute and discussion is beyond the scope of this review. First generation biofuels (bioethanol from maize or sugarcane) and biodiesel from fats, oils, and greases (FOG) (principally soybean oil) have been subjected to a number of LCAs (*31*, *35*). Some second generation biofuels like switchgrass also have received attention. Since LCA methods and assumptions differ, they are not easily compared with each other. A recent and thorough assessment estimates that direct green house gas emissions from sugar beet produced in Europe on average are 40 g CO /MJ of fuel energy. This compares in the same analysis 70 for ethanol made from wheat, 43 for maize and 24 for Brazilian sugarcane (*36*). Based on this analysis and excluding any calculations for indirect GHG emissions, sugar beet would qualify as an advanced biofuel under US EPA's classification system (*32*).

Because the commercial production of biofuel (ethanol) from sugar beet occurs in Europe (*37*), most LCAs for sugar beet have been done for central European conditions (*31*, *38*, *39*). In these evaluations, GHG reduction from sugar beet is comparable or better than that of maize or sugarcane (see Table 5.1, p 85 in Menichetti and Otto (*31*) comparing maize, sugarcane, wheat and sugar beet). However, both sugar beet and maize production in central Europe is different from production in the United States. Maize yields in the United States are typically higher and sugar beet growing areas in the western U.S. are irrigated. Sugar beet production in western, irrigated regions like California and Washington have both higher yields and additional, regionally variable energy costs associated with irrigation that are not accounted in most European estimates. The need to qualify LCA analysis under different environmental conditions is noted in the literature (*35*, *40*).

Nitrous oxide (N_2O) is a more potent GHG than CO_2. It is released in small amounts from soils and is related to fertilizer, manure or cover crop use in farming (*41*), but since it is 300 times more effective at atmospheric warming than CO_2, its loss is important. In a broad-scale analysis, Smeets et al. (*41*) concluded that sugar beet and sugarcane reduced N_2O emissions more than maize, with resulting greater GHG savings (*41*). Sugar beet production was based on estimates from the EU25 nations or East Europe, and the authors emphasized that 'optimized management' for cultivation of the crop had a significant effect on N_2O generation, especially optimization of nitrogen fertilization (*41*). Increased fertilizer use efficiency, resulting in greater biomass yields at the same or reduced levels of fertilizer use has been reported for sugar beets in Europe and California (*42*, *43*). Increasing resource use efficiency, where it occurs, is a positive basis for the use of crops and crop residues for biofuels, while static or declining resource

use efficiency would make it unwise to use sugar beets or any other crop for bioenergy purposes (*43*).

Another resource requirement that has been evaluated is the water needed (or water footprint) for bioenergy crop production. Gerbens-Leenes *et al.* (*44*), in a country-scale study, found sugar beet and potato, followed by sugarcane to be more efficient than maize and sorghum as sources for biofuels in most regions of the world. In most respects, bioethanol from sugar beet compares favorably with maize in most environments.

Breeding an Energy Beet for Production in the United States?

In the United States over the next few years, economic conditions imply that sugar beet will be grown as a sugar crop. However, if sugar beet is eventually used solely as a biofuel feedstock, depending on the conversion technology used, biomass yield may become a more important breeding goal than sucrose yield (*18*). Previous research has shown that higher biomass yields are obtainable using fodder beet germplasm as a parent in hybrids with sugar beet (*45*, *46*). In an older study, Geng *et al.* (*47*) compared fodder beet, sugar beet, sweet sorghum, and maize for potential ethanol yields and reported that fodder beet resulted in the largest ethanol yields of the group under equivalently well-managed conditions. This research should be repeated with modern sugar beet and fodder beet germplasm. Because the potential yield of biomass is correlated with interception of solar radiation (*48*), winter beets, typically with a longer growing season than spring-planted beets, have a much higher yield potential. This is one reason that sugar beet is being investigated throughout the semi-arid tropics as a potential bioenergy feedstock (*49*) as well as in temperate regions of Asia (*50*, *51*).

Even though LCA may indicate that sugar beet is a better feedstock than maize, because area of sugar beet cultivation in the United States in 2009 was about 465 thousand hectares (*6*) and the area of maize cultivation about 32 million hectares (*52*), sugar beet cannot displace maize as a feedstock for bioethanol. Beets also are more costly to produce than maize in the United States and result in larger estimated per unit costs of ethanol, while sugar also remains a more valuable commodity than ethanol (*14*).

Sucrose is a source for many value-added feedstock chemicals or sucrose derivatives, but currently only about 2% of sucrose worldwide is used for such purposes (*7*). In addition to sucrose, sugar beet roots contain about one-third each of cellulose, hemicelluloses, and pectin, with very little lignin (*8*). Each compound is used or can be used as the source of several important industrial feedstock chemicals, and use for this purpose has significant potential for growth (*53*). For example, Fishman and co-workers (*54*, *55*) recently reported on the use of sugarbeet pulp as a source of carboxyl methyl cellulose and polysaccharides with industrial uses. As modern economies reduce or transform their use of oil, it is possible that sugar beets will become a feedstock for a range of chemicals and new biomass-derived specialty materials.

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<center>Chapter 11</center>

Opportunities and Challenges of Sweet Sorghum as a Feedstock for Biofuel

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Sorghum (Sorghum bicolor L. Moench) is a grass crop with thick stalks adapted to warm climates. Sweet sorghum has a juicy, sweet stalk. The juice can be pressed from the stalks, and the sugars either directly fermented or evaporated to make syrup, which can be fermented after storage. The plant residue remaining can be burned to run the factory or cogenerate electricity, or used as feedstock for cellulosic ethanol. It can also be used as animal feed. Sweet sorghum has wide environmental adaptation, rapid growth, high productivity, relative tolerance to marginal growing conditions, and high concentrations of the easily fermentable sugars sucrose, glucose, and fructose. The sugars in sweet sorghum start to deteriorate once the stalk is harvested. Leaves and leaf sheaths are difficult to remove from the stalk. They add microorganisms, organic acids, and starch to the juice. Microorganisms deteriorate the sugars, organic acids react with the sugars when the juice is heated, and starch thickens or gels when the syrup cools after boiling. Ideas for addressing these challenges are discussed.

Introduction

Sorghum (Figure 1) is a widely adapted grass crop with thick stalks native to Africa (*1*). Sorghums are grown for grain, forage, fiber, and sugar or syrup. Sorghum with sweet, juicy stalks is known as sweet sorghum or sorgo. Sweet sorghum is used to make edible syrup. It can also be used to make crystalline

sugar, although this is technically difficult and not economical. The sugar in the juice is also directly fermentable for ethanol production.

Sorghum utilizes the highly efficient C_4 photosynthetic pathway. It has a fibrous root system that makes it very efficient in the utilization of soil nutrients (*2*). It also has advantages over sugarcane (*Saccharum* spp. hybrids) as a sugar-producing biofuel crop because it requires much less water for economic production and is tolerant of drought. Maturity of the grain occurs 60 to 300+ days after planting, depending on variety (*3*). In warm climates it will regrow (ratoon) after harvest to produce a second crop.

Interest in sweet sorghum as a feedstock for biofuel began at least in the 1970s, during the first "energy crisis." In recent years, a rising demand for energy, coupled with concern about possible climate change has once again sparked interest. In 2005, the U.S. Departments of Energy and Agriculture published a vision of replacing 30% of the U.S. energy needs with biomass-based energy by the year 2030 (*4*). Because sweet sorghum stores high concentrations of easily fermentable sugar and is also more widely adapted than either sugarcane or sugar beets, it is seen as a viable feedstock for ethanol production from sugar (*5–9*). The plant material left over after the juice is removed (bagasse) could be used for cellulosic ethanol production once the techniques for this have been worked out and commercialized (*10*). If the seed head can be separated from the stalk before or during harvest, the starch in the seeds can be converted to ethanol similarly to corn-based ethanol, or used for food or animal feed. This review will focus on the use of the sugars from sweet sorghum as a feedstock for ethanol production.

Characteristics of Sweet Sorghum

The sweet sorghum crop is planted as seeds when the soil is at least 15 °C (*2*), although 21 °C is better as noted by Kresovich (*11*). The above-ground parts of the plant are leaves and leaf sheaths which are attached to the stalk at the nodes. The nodes are separated by internodes which undergo expansive growth through cell elongation. The stalk forms the bulk of the sugar-storing tissue in sweet sorghum. Most of the nodes will also form vegetative buds. Buds at the base of the plant will sometimes grow to form their own stalks, named tillers. The number of tillers formed is determined by variety and by environment. Buds in the middle or top of the plant generally remain quiescent unless the apical meristem is damaged, at which time they can form branches. In sorghum, the sheath tightly surrounds the stalk, and most of the leaves stay green until the grain is mature. This has implications for harvesting and processing, which will be described below. There are several phases of development of the plant. These have been classified by several agronomists such as Vanderlip (*12*). The phases critical to sweet sorghum production are: a) germination and stand establishment; b) vegetative growth; and c) reproductive development. The total biomass harvested is determined by germination, stand establishment, and vegetative growth, while the soluble sugar accumulated in the stalk is somewhat dependent on reproductive development, since large concentrations of sugar do not accumulate until after the inflorescence forms (*13*, *14*). Many varieties are photoperiod sensitive; that is, they require a
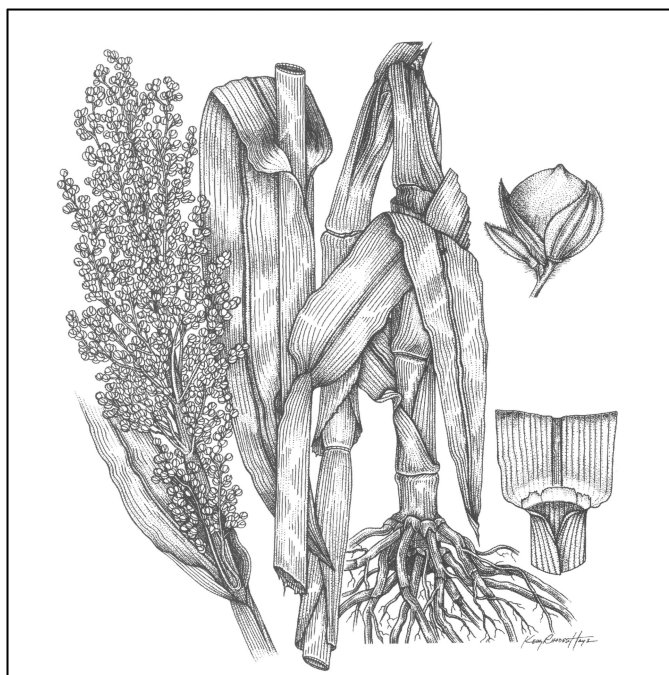
Figure 1. Illustration a of sorghum plant. (Reproduced with permission from reference (15). Copyright 2004, American Society of Agronomy)

specific length of night to initiate flowering (*15*). While grain sorghums have been selected to be short to reduce lodging, sweet sorghums are generally taller, ranging from 2 to 4 m in height (Figure 2).

Sweet sorghum stalks are solid (Figure 3). Like sugarcane, the sugars sucrose, glucose, and fructose accumulate in the parenchyma cells of the stalk. The amount of sugar stored is influenced by variety, environment, and the growth stage of the crop at harvest. Stalk, sugar, and theoretical ethanol yield from several studies are shown in Table I. Dry stalk and sugar yield vary considerably due to differences in growing conditions. The length of the growing season is a major factor limiting biomass and sugar yield. Sweet sorghum requires at least 120 frost-free days to produce a good crop (*16*). Biomass yield may be increased by moderate applications of nitrogen (*17*, *18*), but sugar yield may not be (*17*, *19*). Moderate water stress does not appear to have a big impact on biomass or sugar yield in sweet sorghum (*20*, *21*). Sugar content of the stalk juice increases after floral initiation, and reaches a maximum approximately when the developing grain reaches the soft or hard dough stage of development (*22*, *23*).

Figure 2. Sweet sorghum at flowering stage. Photo courtesy of Arisbel Ambrossi, Acichan.com, Uruguay. Used with permission.

Much of what is known about sweet sorghum juice comes from research to optimize the use of sweet sorghum as a feedstock for making crystalline sugar in sugarcane factories. In the continental United States, sugarcane factories stand idle for 6 to 9 months of the year. For many years, it was thought that incorporating sweet sorghum as a feedstock would expand the use of the capital equipment of existing factories by three or more months. However, as a feedstock for crystallizing sucrose, sweet sorghum was lacking in many respects compared to sugarcane (Table II). Sweet sorghum has less sucrose and more glucose and fructose than sugarcane. It is also higher in starch and aconitic acid. Careful clarification or the use of α-amylase (*25*) removes much of the starch. However, the oligosaccharides produced by α-amylase reduce the efficiency of sucrose crystallization. The higher aconitic acid concentration in sweet sorghum juice also causes problems with sucrose crystallization. Ventre (*26*) demonstrated that much of the aconitic acid could be removed by the addition of calcium chloride and lime to the syrup. The combination of hydrolyzing the starch and removing much of the aconitic acid allowed the crystallization of sucrose from sweet sorghum juice (*27*), but the increased cost made the process uneconomical, so the idea of sweet sorghum as a feedstock for sucrose was dropped in the U.S in the early 1980s.

Harvesting

Sweet sorghum can be harvested by hand, with a corn binder, which cuts the stalks at the base and bundles them, with a forage harvester, with a sugarcane whole-stalk "soldier" harvester, with a sugarcane combine or billet harvester, or with a dedicated sweet sorghum harvester. If it is possible, it is recommended that the seed head be removed up to 2 weeks before harvest to maximize the sugars in

*Figure 3. Sweet sorghum stalks. Photo courtesy of Arisbel Ambrossi,
Acichan.com, Uruguay. Used with permission.*

**Table I. Stalk Yield, Fermentable Sugar (Sucrose, Glucose, and Fructose)
Yield, and Theoretical Ethanol Yield from Sweet Sorghum**

Location	Dry Stalk Yield	Sugar Yield	Theoretical Ethanol Yield	Source
	Mg/ha		L/ha	
Tucson, AZ	25	4.2	2726	(*21*)
Nebraska	7.5- 4.8	1.4 – 6.2	967 – 4131	(*24*)
Logan, UT	7.9 – 19.4	2.5 – 16.5	1330 – 8784	(*16*)
Aiea, HI	17.0 – 33.0	13.7 – 20.9	7293 – 11127	(*16*)
Fort Collins, CO	18.0 – 18.3	5.7-6.1	3793 – 4059	(*19*)
Ames, IA	13.1 – 13.8	5.1 – 6.9	3394 – 4591	(*19*)

the stalk (*33*). Harvesting by hand or with a corn binder is very labor-intensive,
and useful only for small planting areas.

A forage harvester chops stalks, leaves, and seed heads (if they have not
been removed) into small pieces of 15-20 mm long (*34*). This has the advantage
of increasing the bulk density of the harvested crop compared to whole-stalk or
billet harvesters, thus increasing the amount that can be loaded onto each truck
and increasing the distance that the sorghum can be economically transported.
However, the leaves cannot be separated from the stalks. In addition, small pieces
deteriorate very quickly (*34*, *35*) and cannot be stored at ambient temperature for
more than a few hours. Webster et al. (*34*) showed that juice Brix declined from 10
to 8 Brix by 19 hours after harvest with a forage harvester, while Eiland et al. (*35*)

Table II. Juice Properties of Sweet Sorghum and Sugarcane. Titratable Acidity is mL 0.1 N NaOH Required to Adjust 10 mL Juice to pH 8.3; Other Compounds are in % Soluble Solids

Character	Sweet Sorghum (source)	Sugarcane (source)
Juice °Brix	10.5 – 20.7 Brix (16)	16 - 20 Brix[1]
Sucrose	69 – 74 % (18)	70 – 88 % (28)
Reducing sugars	5 – 19% (29)	4 – 8 % (28)
Starch	0.4 – 5.3% (29)	0.001 – 0.050% (28)
pH	4.9 – 5.5 (29)	5.2 – 5.4 (28)
Titratable acidity	3.6 –4.8 (26)	2.0 – 3.2 (30)
Organic acids	NA[2]	1.5 – 5.5% (28)
Aconitic acid	3.6 – 4.8% (26)	1.0 – 2.1% (31)
Protein	0.9 – 1.3% (32)	0.5 – 0.6% (31)

[1] Unpublished data.   [2] NA, not available.

noted a decline from 15.4 to 12.8 Brix in 1 day. Eckoff et al. (36) demonstrated that 0.5% SO_2 controlled microbial deterioration of sugar in chopped sweet sorghum.

Sugarcane soldier harvesters cut the stalks and lay them to the side; they must then be transferred to a truck or trailer for transport in a separate operation. If undamaged, whole sorghum stalks can be stored for up to a week without significant loss of sugar (35).

A sugarcane billet harvester cuts the stalks into pieces. A limitation is that the sugarcane harvester can cut only one row at a time. Webster et al. (34) reported that sweet sorghum billets cut with a cane harvester were about 200 to 250 cm long. The sugarcane harvester has fans that can be set to blow off some of the leaves, although Webster et al. (34) noted that the sorghum billets weighed less than sugarcane billets and the fan speed had to be turned down to decrease the loss of stalk pieces.

At least two major equipment companies, Case New Holland and John Deere, are developing sweet sorghum harvesters based on the sugarcane harvester, but able to cut more than one row at a time. They also differ from the sugarcane harvester in where they cut the stalks. Sugarcane harvesters cut the stalks as close to the soil as possible, because sugar in the lower part of the stalks is very high whereas in sweet sorghum the lowest internodes do not contain much sugar. Therefore, the sweet sorghum harvester cuts the stalk higher than a sugarcane harvester.

In addition, there have been several designs of harvesters that include a single-pass 3-roller mill to extract the juice in the field (37, 38). This method extracts only about 40-60% of the juice, and leaves the remainder in the field with the bagasse. It would probably not be suitable for large-scale industrial ethanol production, but would allow individual growers to produce ethanol on-farm for their own use.

Processing

Small Scale Syrup Production

Sweet sorghum juice is processed to edible syrup in many countries around the world. In the U.S, sorghum syrup was an important sweetener until white sugar became easily available and inexpensive. Today, production of sorghum syrup in the U.S. persists mainly in the southern states. In 1975, the Agricultural Census reported that 972 ha of sorghum were grown for syrup in the U.S. (*39*). Syrup production in the U.S. is now so minor that production statistics are no longer kept by the U.S. Department of Agriculture. Farmers generally grow from 4 to 40 ha of sweet sorghum to make syrup for personal use or for sale (M.J. Bitzer, personal communication). At this scale, sorghum is cut by hand or using a corn binder or forage harvester (*40*). If possible, the leaves and seed heads are removed from the stalks (*33*). Leaving harvested whole-stalk sorghum several days before milling can improve syrup quality, possibly by allowing the plant's own amylase enzymes to hydrolyze some of the starch (Bitzer, personal communication). The juice is expressed from stripped whole stalks or chopped stalks by passing the material through a 3-roller mill. Small 3-roller mills express from 40 to 60% of the juice from the stalks. The juice is filtered to remove large pieces of trash, then allowed to settle for 2 or 3 hours (*33*, *40*). Amylase enzyme may be added to help hydrolyze the starch (*41*). Clear juice is then drawn off and transferred to an evaporator. There are many styles of on-farm evaporator. One of the more common types is a continuous evaporator where juice enters one end and finished syrup leaves the other end (*40*, *41*). During evaporation, the juice is skimmed as it heats to remove the coagulated proteins and pigments that rise to the top. The final syrup is bottled or canned for storage or sale.

Large Scale Processing

On a much larger scale, sweet sorghum grown near existing sugarcane factories can be processed to juice or syrup using that equipment (*8*). Methods for processing sweet sorghum this way are not completely worked out, but the previous experience of the U.S. sugar industry with making sugar from sweet sorghum gives researchers and the industry a starting point in developing methods. Key considerations for the processing of sweet sorghum are: (i) more fiber and green leaves in sweet sorghum compared to sugarcane; (ii) high glucose and fructose content; (iii) more starch; and (iv) more aconitic acid (Table II).

There are two methods of extracting sugarcane juice from the cane: tandem roller mills, and diffusers. Tandem roller mills are five or six 3-roll mills joined in series (Figure 4) which press the juice from the cane. Diffusers use hot water or juice to extract sugars from shredded cane without milling (*42*). Excess moisture is pressed from the bagasse at the end of the process. Diffusers are uncommon in U.S. sugarcane factories. There are few reports on extracting juice from sweet sorghum using sugarcane mills. Polack and Day (*43*) noted that sweet sorghum tended to choke the mill tandems more than sugarcane. Webster et al. (*34*) showed that sweet sorghum was 24 to 28% fiber, depending on how much of the leaves were included with the stalks. In comparison, the sugarcane in their study was 16%

fiber. The high fiber of sweet sorghum decreases sugar extraction, partly because of inefficiencies in milling the higher fiber material, and partly because the excess fiber tends to absorb juice and sugar. Each of these studies was done using tandem roller mills

In making crystallized sugar from sweet sorghum, the larger concentration of starch in sweet sorghum, up to 5.3% (Table II), mandated that juice be heated to a lower temperature than sugarcane juice initially (*25*) to avoid gelatinization of starch granules. Smith et al. (*25*) demonstrated that 95% of the starch in sweet sorghum juice could be removed during clarification by adding lime to pH 7.7 – 7.9, a flocculating agent, and heating to 55-60 °C. However, glucose and fructose degrade in alkaline conditions (*44*), reducing fermentable sugars in the syrup. Starch could also be hydrolyzed by adding heat-stable α-amylases during juice clarification (*45*). These are commercially available. Maltose and other oligosaccharides that are formed by the action of α-amylase on starch are fermentable sugars, but it is unclear if the cost of the enzyme would be offset by amount of fermentable carbohydrate produced. Extraction of sugar from sweet sorghum using diffusion might result in less starch being expressed. Rein (*42*) and Koster (*46*) mentioned that there was less starch in sugarcane juice following diffusion as compared to milling.

The second major obstacle to crystallizing sucrose from sweet sorghum was the aconitic acid concentration. Ventre (*29*) discovered that aconitate crystals formed during the later phases of sucrose crystallization from sorghum syrup, and that these aconitate crystals interfered with the centrifugation of the sucrose crystals. Ventre and others (*26, 47*) later developed a method for removing excess aconitic acid from sweet sorghum juice. Sorghum syrup used as a feedstock for ethanol may not require the reduction of aconitic acid. It is not known if this or other compounds are inhibitory to fermentation. Polack and Day (*43*) noted the presence of a fermentation inhibitor in sweet sorghum juice, although they did not provide evidence for inhibition. They did show that different yeast strains had different fermentation productivity. Further research in this area is necessary.

Other Processing Ideas

There is a lot of interest by farmers and researchers in alternate methods of producing ethanol from sweet sorghum. These systems are being developed in part because transporting harvested sweet sorghum long distances increases the cost and decreases the energy yield ratio of the ethanol produced (*48, 49*). Systems are being developed to harvest and extract juice from sweet sorghum in one operation (*37*). This could be combined with on-farm fermentation. The fermented "beer" could be distilled on-farm for local use or transported to a regional distillation facility such as the maize-based ethanol plants now scattered across the U.S. Midwest. Alternately, regional processing and fermenting facilities could be set up to produce the initial ethanol product, which could be transported to existing maize-based ethanol plants for distillation.

Figure 4. Tandem roller mills. Photo courtesy of Fulton Iron Works. Used with permission.

Fermentation

A major challenge in fermenting sweet sorghum juice is keeping it from fermenting before one is ready. Healthy, undamaged stalks protect the sugars stored inside. However, once the crop is harvested, bacteria and yeasts can make their way to the stored sugars through cut ends and damaged areas on the stalks. Daeschel et al. (*50*) noted that fresh sweet sorghum juice contained about 10^8 microorganisms per mL, and that these were predominantly *Leuconostoc mesenteroides*, although other bacteria and yeasts were also present. Without treatment or refrigeration, juice began to spoil after about 5 hours. The number and type of microorganisms present on the harvested stalks and juice will vary with the environment, the presence or absence of insects and diseases on the sorghum, and possibly the amount of leaves included when the sorghum is milled.

A complete discussion of industrial fermentation is beyond the scope of this chapter. There are, however, a few things to keep in mind. Fermenting the sugars in sweet sorghum can be as simple as adding yeast to the juice (*38*). Delaying yeast inoculation of raw juice will decrease the amount of ethanol produced, presumably because of the growth of other microorganisms (*37*). Rein et al. (*51*) got greater fermentation efficiency if the juice was heated to 60 °C before inoculating with yeast, although they couldn't explain poor conversion efficiency in unheated juice. Ratnavathi et al. (*52*) showed significant variation in fermentation efficiency between genotypes, even though the sugar concentrations in the juices were the same. They attributed some of the difference to acid invertase activity in the juice. Acid invertase would break the sucrose into glucose and fructose, making the sugar more available to the yeast. Day and Sarkar (*53*) noted that sweet sorghum juice generally did not ferment as well as sugarcane juice, and that alcohol yield did not appear to always relate to the sugar content of the juice. They attributed the difference to unknown inhibitors in the

sorghum juice. However, they also pointed out that different yeast strains had different productivities. Yeast strains are highly variable and adaptable. Brazil's experience in fermenting sugarcane juice and molasses have shown that wild yeast strains will sometimes overgrow the applied yeast, but a few of these strains have been selected to have superior fermentation performance (*54*).

Conclusions

Sweet sorghum is a very attractive biofuel crop. Given good growing conditions, it produces large amounts of readily fermentable soluble sugars. With staggered planting of varieties of different maturities, sweet sorghum can be available for several months every year. It can be processed by existing sugarcane factories in a way that does not interfere with the production of sugar from sugarcane, but it can also be grown and processed in areas that cannot grow sugarcane because of environmental constraints.

Research to maximize biomass and sugar yields from sweet sorghum, and processing and fermenting techniques is ongoing in many areas of the world. The potential for sweet sorghum as a crop can also be seen in the willingness of major equipment companies to develop dedicated sweet sorghum harvesters. It is clear that sweet sorghum can contribute significantly to the 30% biobased energy production envisioned for the U.S. by 2030.

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Chapter 12

Approaches to Raw Sugar Quality Improvement as a Route to Sustaining a Reliable Supply of Purified Industrial Sugar Feedstocks

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Energy costs in the sugar industry are outstripping costs of manufacture, particularly in refineries. This, as well as increasing transportation costs and the need to meet manufacturers' tight specifications, has increased the demand for a sustainable supply of purified, raw sugar. Agricultural commodity delivery of purified, raw sugar as an adequately refined raw material for manufacturing value-added products demands consistently high quality to to be competitive. To achieve very low colorant and high pol values in purified, raw sugars, components in raw juice inhibiting the crystallization of sugar must be identified. Micro- and nanoparticulate materials can foul sensitive surface properties of adsorbents such as powdered activated carbons (PACs) or resins. Improved approaches to clarification, such as combined centrifugation, microfiltration or nanofiltration of sugar juices or syrups permit more efficient decolorizing with solid adsorbents. Lower quality sugars can thus be upgraded to permit isolation of product while sustaining energy utilization.

Introduction

"Sustain" is derived in the English language from the Latin word *sustinere*, the *sus-* prefix (from the preposition *sub*) meaning "from below" and the verb *tenere*, "to hold" (*1*). John Ikerd, Professor Emeritus of Agricultural Economics, University of Missouri, Columbia, often used this equation to account for distributive confluence of sustainability (*2*):

$$I = P \times A \times T$$

where I = Environmental impact, P = Population, A = Affluence, T = Technology.

At the early part of the last century a typical quarter section, 160 acre family farm in northern Illinois survived because certain energy and investment inputs could be balanced. Although this report is about purified raw sugar production from sugarcane, calculations about corn yield and total investment input one hundred years ago still hold. It then took about 85 gallons (322 liters) of gasoline to produce a harvested acre of corn. This takes a lot of inputs into account such as the cost of plowing or harvesting machinery, drying, fertilizer application, and tilling. Even at a high yield of 200 bushels of corn per acre, at US$4.00 per bushel today that is a sale price of $800 per acre. With gasoline today at about $2.50 per gallon, the fuel alone costs $212.50 or one-fourth the sale price of the corn. Furthermore, this does not account for the hybrid seed costs, pesticide use, special fertilizers, tax structure, labor per hour, depreciation or maintenance of equipment, and fluctuations in the grain markets (*3*, *4*). A similar calculation with respect to sugarcane from field to final raw sugar is as complex.

Economic Significance of Raw Sugar for Sustaining the Future of the Sugar Industry

Current world demand for high quality sucrose-based sweeteners is very great. Raw sugar, as recorded on the current and extensive U.S.D.A.-E.R.S. survey tables, is very heterogeneous with respect to quality (*5*). With the rising costs of energy, raw sugar manufacturing costs reflect a tightening market demand for raw sugar. World raw sugar prices in January and February, 2010, were 28.94 and 27.29 US cents/lb and March 16, 19.33 cents/lb. In the commercial sweetener industries, the use of high fructose corn syrup (HFCS) use has been steadily falling according to the U.S.D.A.-E.R.S. as well as the Corn Refiners Association, due to consumer concerns and preferences. Values of HFCS reduction have ranged between 13-17% less since 2001 (*5*). Sucrose based sweeteners, including purified, raw sugars are meeting consumer preference demands and have contributed considerably to the world-wide increase of raw sugar remelting.

There is no such thing as a "reagent grade raw sugar." Although demand for purified, raw sugar is increasing, energy costs for a sustainable level of this product outstrips cost of manufacturing in many areas of the world. Agricultural commodity delivery of sugar as an adequately refined raw material for manufacturing value-added products demands that the highest quality yields of purified, raw sugar be realized to be competitive. Lower quality sugars can

thus be upgraded to permit isolation of acceptable products while sustaining more favorable energy utilization. Since such large amounts of energy and labor are already expended on the manufacture of raw sugars, salvaging useful final products for use in high end sweetener applications makes a lot of sense. Marginal raw sugars for remelting processes have not only high International Commission for Uniform Methods of Sugar Analysis (ICUMSA) specified color (IU) values but also considerable turbidity values because of the presence of both sediment and colloidal particulate (*6*, *7*). Various strategies must be employed to achieve high pol, low ICUMSA color (less than 50 IU), with low invert sugar, turbidity, dextran or polysaccharides, 5-hydroxymethylfurfural, ash, especially iron salts, little olfactory off-flavors that could prejudice a high end product's flavor quality, and absence of pesticide residues, mycotoxins, polluting chemicals, and polyphenolic color compounds (*8–10*).

The objective of this work was to compare the quality of raw sugar samples from various countries where they are utilized by carbonated beverage and other high end manufacturers after they have been remelted, clarified, and decolorized with powdered activated carbon (PAC) and finally polished with nanofiltration. In countries where final refining of raw sugar into white, refined sugar is not economically feasible, practical utilization of purified, raw sugars, that are borderline for higher end use, can be made at cost savings.

Effective Powdered Activated Carbons (PACs) for Decolorization of Raw Sugars

Critical to this work has been the identification of PACs that can survive applied loads of raw sugars containing large amounts of impurities, *i.e.*, with turbidity in ≥ 600 ICUMSA Unit (IU), which can deactivate the carbon by fouling. We have examined hundreds of raw sugars from world-wide markets that represent many types and ranges of impurities. The following examples make the point that further utilization of such purified, raw sugars can increase energy savings from the expensive refining of raw sugar into white sugar and, thus, contribute to a more sustainable sugar production.

There are two key issues involved in the ability of PAC to treat high color raw sugars: the higher PAC dose rates required to achieve a final color < 50 IU, for example, and the corresponding filtration issues which relate to the loading capacity of the typical filters in industrial use today (*11*, *12*). Carbochem® CA-50 powdered activated carbon (source: wood, chemically activated) was used in these studies as a wide range sugar decolorizer due to its high decolorizing efficiency and particle size distribution which minimize the filtration limitations. The particle sizes range from 1 - 45 μm diameter which eliminates the fines fraction defined as particles <1 μm (Malvern Analysis, (*13*)). The fines fraction improves decolorizing performance due to improved kinetics and surface area but presents filtration problems due to the need for finer size filter-aid and higher dose rates which impact the loading capacity. The ability to treat high color raw sugars requires PAC like Carbochem® CA-50 that can provide high decolorizing capability without the fines fraction. There is little nanoparticulate dust in

CA-50. Pore size engineering of these carbons permits adsorbents of identical compositions with tailored pore size and surface area. Each PAC particle consists of both micropores (<20 Å or 2 nm), which involve inclusion of colorant or nanoparticulate, as well as macropores (>500 Å or 50 nm), both of which make up very large areas of adsorptive capacity (*12*). Activated carbons are non-polar and possess affinity for organic compounds. The general mechanism of adsorption is through intermolecular attraction with carbon atoms similar to partitioning of soluble solutes between solvents of differing polarities (*14*). The adsorption forces involved are short-range London dispersion forces from induced molecular charges or van der Waals attractions of delocalized aromatic unsaturation. Steam activation at high temperature creates porosity in the carbonaceous material through the water gas reaction with the removal of carbon as CO. Performance is determined by surface area and correct pore structure for molecules to be removed.

The Lewis acid, zinc chloride, was widely used to activate carbons sourced from wood, but has been discontinued due to concerns regarding zinc ion as a potentially toxic heavy metal. Zinc ion has been replaced with phosphoric acid as the most common method of chemical activation today. Adsorption capacity is related to pore structure and surface area and maximized at equilibrium with solutes. Adsorption capacity is also related to temperature, pH, concentration, and contact time of the colorant in solution with the powdered activated carbon (*11*). Adsorption is not a selective process and is related to parameters such as molecular size and solubility. Particle size only affects the rate of adsorption and not adsorption capacity. Surface area of granular activated carbon (GAC) is only approximately 10 m^2/g less than PAC, as most of the surface area is determined by the internal surface area and not the external surface area. Surface area of a typical powdered activated carbon is approximately 1000 m^2/g (1 kg = 1,000,000 m^2 adsorption surface area).

There is no universal PAC for every application and it is, therefore, necessary to match the carbon properties with the application requirements (*14*). Physical properties such as apparent density reflect porosity and volume activity. Adsorption of molecular iodine is used to determine iodine number as a measurement of surface area and micropore content. The other characterization of PAC for macropore content is by the molasses number or degree of caramel decolorization. The robustness of the PAC particles is measured as hardness, which is a resistance to attrition with formation of fines that block filtration. This is mainly applicable to the use of granular activated carbon or GAC. Minimizing water soluble ash in PAC is also important to avoid extractables as contaminants in final decolorized products.

Chemistry of Raw Sugar Colorant as a Challenge to Decolorizing Processes

With these background remarks about PAC properties the nature of the colorants in raw sugar will be considered. The carbon removes non-enzymatic and enzymatic-based colorants formed during isolation of sugar (*6*, *7*, *11*).

The primary colorants, are the result of caramelization and Maillard reactions to produce melanoidins. Plant pigments can also be carried forward with the cane juice. Both types of colorants must be removed from sugar syrups to achieve higher quality products Caramelization occurs from the dehydration of sugars and limited polymerization of the reactive species generated at elevated temperatures. The Maillard reaction is initiated by the condensation of amino acids and reducing sugars which are transformed to produce polymeric melanoidins. 5-Hydroxymethylfurfural (HMF) is produced from the dehydration and 1,2-enone rearrangement of fructose. Fructose derived via the enediol of glucose also is driven to form 5-hydroxymethylfurfural. Water soluble HMF as a low melting solid participates in polymerizations similar to the Maillard reaction between amino acids and glucose/fructose. These colorants are typically yellowish-orange to light brown absorbing in the ultraviolet-visible range of 350-500 nm (*15*). Natural pigments in the sugarcane, such as polyphenolics, anthocyanins, chlorophyll degradation products, and fatty polymers formed from cross linking of lipids, are colorants or color precursors, and are mainly responsible for the color of raw and affinated sugars (*6*). Formation of melanins, produced by the polymerization of quinones with amino group molecules, is related to enzymatic oxidations of phenolic aromatic molecules (*16*). Such colorants are termed enzymatic browning products. The reactant quinones are produced by catalytic action of polyphenol oxidase (PPO) through the aromatic ring oxidation of phenolic compounds. Melanins are dark, high molecular weight, insoluble polymers resulting from amino groups reacting with quinone carbonyls in Schiff base linkages. Enzymatic browning can be controlled by temperature, use of reducing agents such as bisulfite or ascorbic acid, and pH (*17*).

Sediments and Hydrocolloids in Raw Sugars That Contribute to Turbidity

Another factor greatly influencing the upgrading of raw sugars by PAC to industry-acceptable products is colloidally suspended micro- and nanoparticulate. The high turbidity in raw sugar samples is composed of many types of particles. Such particles coat the surface of the PACs and reduce their effectiveness. The composition of these particles varies from country to country, region to region, and from harvest area to handling and processing at the factories. Extractables from crushed cane plants include the following turbidity forming particles: pulverized bagacillo, waxy lipid micelles, starch granules, microbial bodies, and mineral materials from clays in the abundant soil delivered with sugarcane stalks and trash (leaves and tops). Such turbidity forming particles extracted into the processing stream often escape conventional clarification techniques (*6*, *16*, *18*, *19*).

Parameters Necessary To Optimize the Upgrading of Raw Sugar Feedstocks by PAC

To achieve purification of raw sugar with PAC a number of factors must be taken into consideration. Optimization of the traditional hot melt process for

raw sugar upgrade requires that test protocols include objectives with these fixed parameters: (i) quality of sugar feedstock, (ii) treatment temperature, (iii) contact time of PAC with sugar and colorant, (iv) physical properties of the PAC such as resistance to turbidity fouling and colorant adsorption activity, (v) PAC dose rate of application, (vi) particle size distribution of the PAC, (vii) filtration media for final clarification and (viii) pH of reaction medium throughout to stabilize the sugar and avoid spectrophotometric indicator effects (*7*, *15*, *16*). A key component in the success of this concept is the filtration process, as this is the limiting factor in terms of the higher PAC dose required to treat high color sugar (*18*, *19*). The extra loading on the filter will not be possible with most industrial filters in use today. The design of the filtration process is very important for matching the particle size characteristics of the filter and/or filter-aid with those of the PAC and the impurity levels and types contained in the sugar. In the current work many commercial PACs were compared for efficiency with respect to the above parameters and filtering properties. Most commercial PAC grades contain a high amount of fines (fraction <1 μm), which requires a finer filter-aid and greater filter loading. Such PACs cannot be used to treat high color raw sugars (*11*, *12*). To resist fouling of the carbon surface or filter blockage, as well as to permit good decolorizing ability without high fines, Carbochem Inc., U.S.A., has developed a proprietary grade of PAC named Carbochem® CA-50 for raw sugar remelting processes. In this study Carbochem® CA-50 was compared to competing commercial PACs following the experimental protocol described in Table I.

Table I. Test Conditions for Initial Screenings of Raw Sugars Devised According the ICUMSA methods (*20*)

Parameter	Test Conditions
Syrup Brix	60 Brix (wt/wt in water)
Temperature	80°C
Activated carbon dose rate	0.1 to 1% PAC wt/wt % sugar -depending on quality
Contact time	60 min with agitation
Filtration for ICUMSA color	0.45 μm Nylon filter covered with two1.6 μm spun glass prefilters
pH determined before and after treatment	If pH > 6 but < 8 no pH adjustment done.
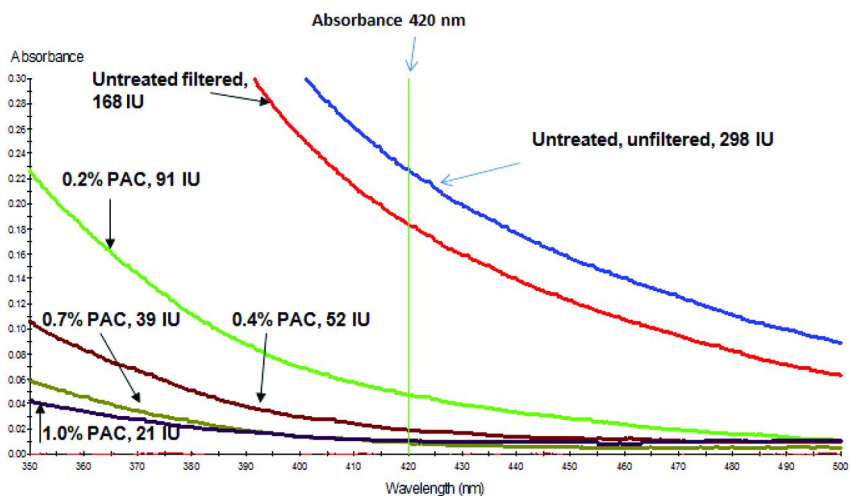
Figure 1. Carbochem CA-50R PAC at 0.2, 0.4, 0.7 and 1% wt/wt solids. Color removal, 60 Brix Mexican raw sugar, 168 IU, 80 °C for 1 hour (20). (see color insert)
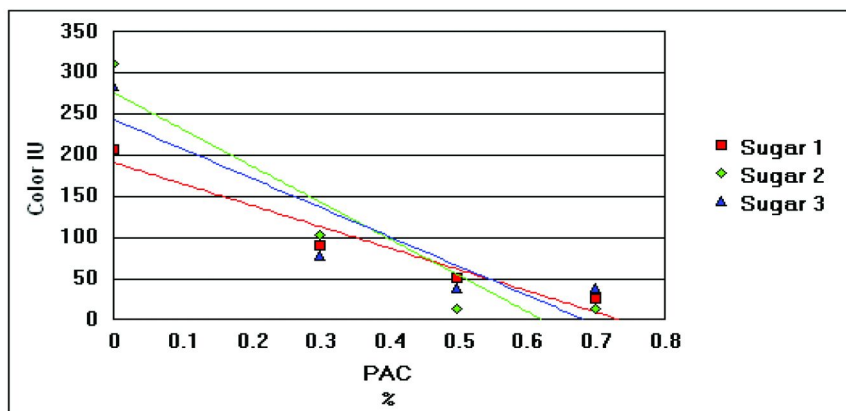
Figure 2. Example of linear dose responses of PAC on three raw sugars during decolorizing. Plot of ICUMSA units color vs. percent of PAC (20). Data plotted are listed below in Table II. (see color insert)

Typical dose response measurements for decolorizing a raw sugar were examined for a number of samples. Figure 1 illustrates the changes in ultraviolet-visible absorbances that result from treating a raw sugar with PAC using the test conditions in Table I. The treatment levels of PAC are reflective of a linear trend in the response activity (Figure 1).

Each of the raw sugars is unique in its collective impurities. Consequently each raw sugar lot must be tested, responses generated for the PAC, and correlated with a linear regression of the PAC dose. The regression suggests the dose

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concentration of the PAC for treatment. Figure 2 represents linear response plots
for three raw sugars at various treatment levels.

**Table II. Linear Regression Analysis Data of PAC Dose Responses for Three
Typical Raw Sugars (20)**

PAC	Sugar Color IU, 60° Brix		
%	1	2	3
0	207	311	285
0.3	91	104	78
0.5	52	13	39
0.7	26	13	39

**Table III. Compositional Ranges of International Sugars Representing
the Heterogeneity Often Encountered in Remelting Processes Analyzed
According to ICUMSA Protocols (20)**

Analyses	Concentration Range	Concentration Range after Reduction by PAC
Sediment	3477-5542 ppm	NA
Polysaccharide	3986-27204 ppm	3693-14645 ppm
Polyphenolics	35-74 ppm	11-44 ppm
Total invert	0.025-0.131%	0.014-0.034%
Iron	0.30-3.10 ppm	0.33-1.33 ppm
Ash	0.05-0.10 ppm	0.03-0.08 ppm
Sulfur Dioxide	None detected	(D/L = 4ppm)
Dextran	86-247 ppm	NA

Cross Sectional Proximate Analyses of International Raw Sugars

Heterogeneity of the raw sugars in this study is illustrated in Table III. The
analyses were undertaken using ICUMSA (20) methods and reflect typical ranges
of colorant found in these products. More than one hundred international raw
sugars from twelve countries were examined in these trials. The samples reported
here were from India, the Philippines, Brazil, Mexico, and the U.S. Data from
representative samples are presented here to give a cross section of developing
effective raw sugar upgrading conditions. ICUMSA colors and responses of
several of the raw sugars from Table III to PAC decolorizing are compared in
Table IV.

Table IV. Decolorizing Responses of Several International Raw Sugars with Compositions in Table III Indicating Variations in Response to 0.3% CA-50 PAC Treatment. ICUMSA Color Determination (*20*)

	Unfiltered color IU	Turbidity IU	Treated filtered color IU	Color reduction %
Mexico	388	168	13	94
Mexico	375	194	26	86
Mexico	582	323	39	85
India	595	349	39	84
India	1099	621	103	78
Brazil	582	336	39	84
Brazil	944	582	52	86
pH range: 5.8-6.0 Remained unchanged after PAC treatment				

Effectiveness of Micro- and Nano-Filtration of Particulate on Decolorization

Raw sugar turbidity can have negative effects on a PAC's ability to decolorize sugars. The effect of the PAC on removing turbid particulate in the raw sugars was also studied. A study of sequential particle size membrane pre-filtration on color removal from raw sugars has been previously demonstrated in this laboratory as well as with other industrial researchers (*17–19*, *21*, *22*). The comparison of pretreated syrups was undertaken using 60 Brix unfiltered syrups, and 8, 1.6, and 0.45 µm pore size membrane filtered syrups. The syrups treated represented large micron size and nanoparticulate populations of sediment removed by membranes. The following photographs (Figures 34567) depict the colloidal light transmissions of these respective syrups. The relative effects of the membrane clarification of the raw syrups on the effectiveness of the PAC used for decolorizing are also presented.

In Figure 3, a 60 Brix syrup of raw sugar is shown as well as three types of microfiltration removing progressively smaller particles. The photographs illustrate the contrast changes, from one membrane size to the next, in the Tyndall effects of light scattering on the microscopic suspended particles. Some of the diffusion of light in the unfiltered syrup could also be from the presence of more macroscopic particles in the raw sugar such as bagacillo. Progressing through the descending filter pore sizes, better clarification occurred after treatment with a 0.45 µm Nylon filter with no colloidal suspension light scattering apparent.

Unfiltered 60°Brix Mexican raw sugar

8µm pore size cellulose nitrate

1.6µm pore size spun glass fiber

0.45µm pore size Nylon membrane
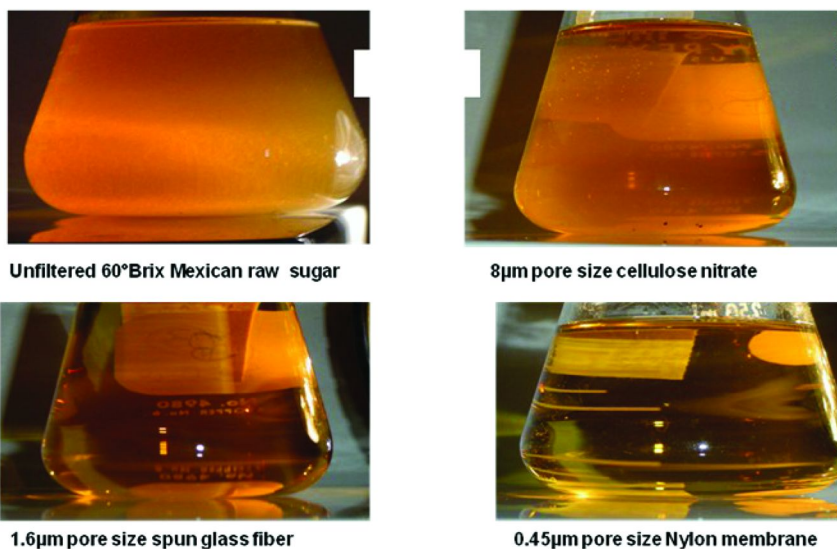
Figure 3. 60 Brix syrup of raw sugar with three types of microfiltration removing progressively smaller particles. Unfiltered, 594 IU, 8 µm filtered, 439 IU, 1.6 µm filtered, 426 IU, 0.45 µm filtered, 349 IU (20). (see color insert)

Not prefiltered 1

Untreated   0.1% CA-50   0.2% CA-50   0.3% CA-50
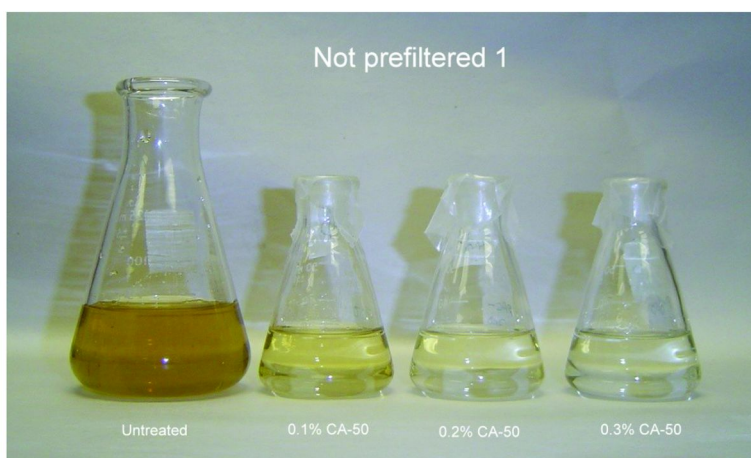
Figure 4. Untreated, unfiltered 60 Brix syrup decolorized with increasing doses of activated carbon with visible decrease in color at the highest concentration of PAC on the right. Untreated,unfiltered, 594 IU, treated 0.1% PAC, 193 IU, 0.2% PAC, 103 IU, 0.3% PAC, 64 IU (20) . (see color insert)
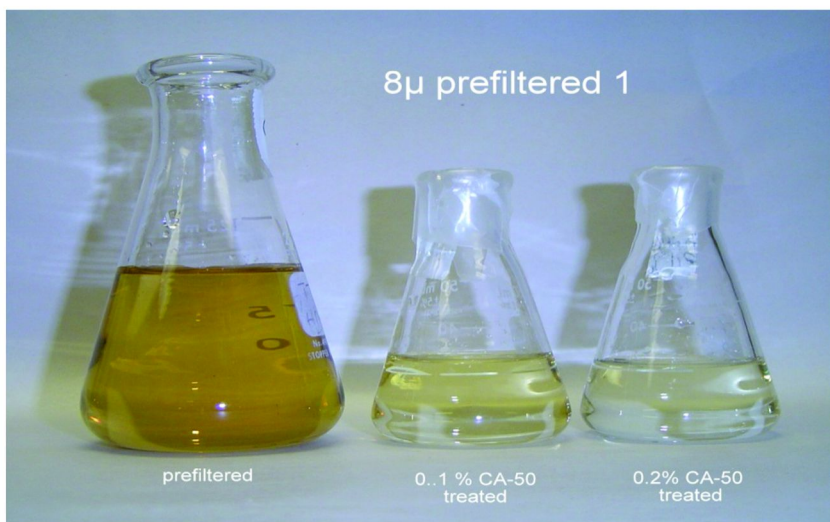
Figure 5. Effect of 8 μm prefiltration on the ease of PAC decolorizing of filtered syrup. 8 μm prefiltered, untreated with PAC, 439 IU, 0.1% PAC, 193 IU, 0.2% PAC, 103 I U (20). (see color insert)
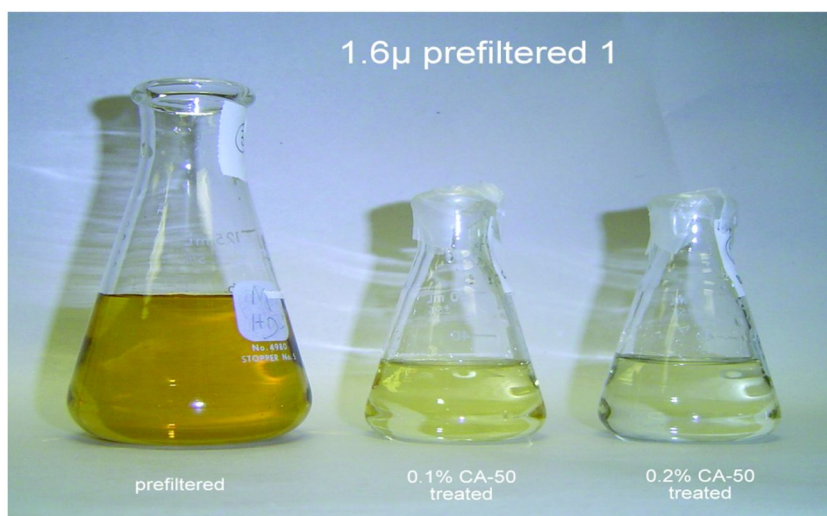
Figure 6. Filtration of the 60 Brix with 1.6 μm spun glass filter to remove further microparticulate and permit adequate decolorizing of syrup at 0.2% PAC. 1.6 μm prefiltered, untreated with PAC,426 IU, 1.6 μm prefiltered, 0.1% PAC, 181, 0.2% PAC, 51 IU (20). (see color insert)
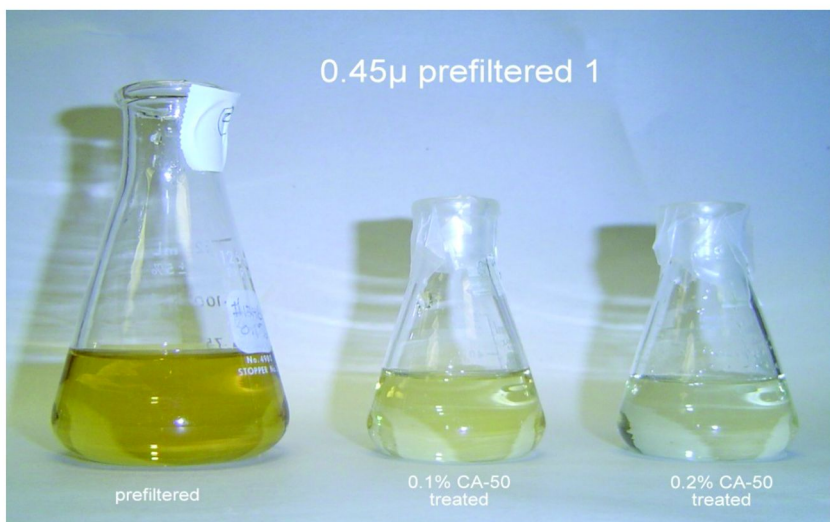
Figure 7. Removing the micro- and much of the nanoparticulate with the 0.45 μm membrane permits very efficient decolorizing of the 60 Brix syrup with the activated carbon to low ICUMSA values. 0.45 μm prefiltered, 349 IU, 0.1% PAC, 37 IU, 0.2% PAC, 18 IU (20). (see color insert)
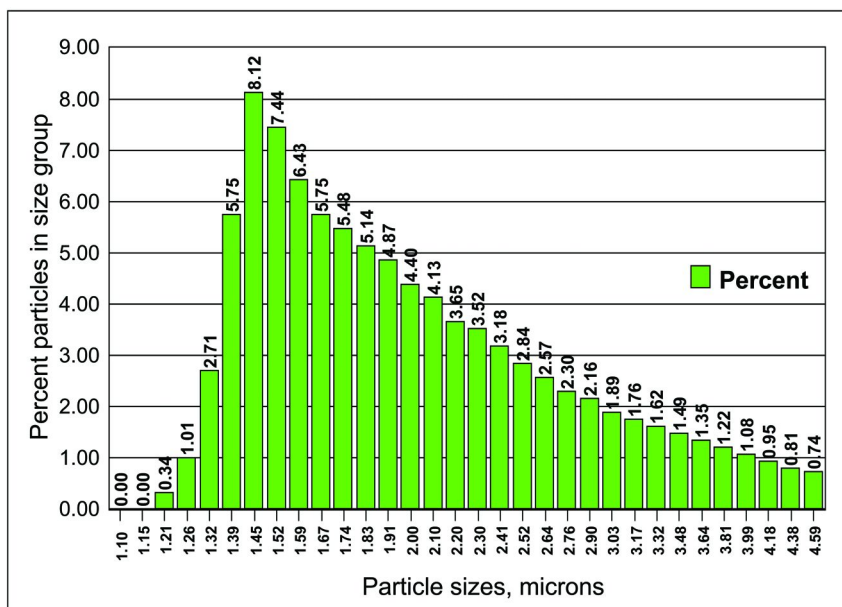
Figure 8. Multiple angle light scattering particle size distribution of typical international sugar sediment particulates in the 1.2-6.0 μm range. 25% between 1.2 -1.5 μm; 69.5%, 1.6-6.0 μm s; 5.5%, 6.1-110 μm. (see color insert)

When treated with PAC the unfiltered sample lost its color with an increasing order of CA-50R PAC dose, 0.3%>0.2%>0.1% wt/wt (Figure 4).

In Figure 5, the 8 µm pore size prefiltration step, which removes larger particles from the unfiltered raw sugar, permitted the PAC to remove more colorant than from the unfiltered raw sugar syrup shown in Figure 4. With this 8 µm filtration step the same color reduction was achieved with 0.2% PAC as was done with 0.3% PAC alone in Figure 4.

The 1.6 µm spun glass filter permitted even more effective removal of the colorant with PAC as more microparticulate was removed by the filter than with the 8 µm filter (Figure 6).

The PAC was most effective after the 60 Brix syrup was prefiltered with the tightest membrane (Figure 7). The 0.45 µm Nylon membrane removed not only all of the microparticulate but also a large share of nanoparticles. These nano-range colloidal suspensions no longer enter into the pores of carbon particles and mask over large areas of decolorizing surface; thus, the colorant was most efficiently adsorbed.

Laser Light Scattering Particulate Analyses of Raw Sugars

Ten representative international raw sugars and associated sediments, were analyzed for particulate distribution by multiple angle laser light scattering analysis. Light scattering particle size distribution of sediment and particulate in raw sugars was undertaken to further compare the compositions of the raw sugars studied. The broad range of particle sizes did not permit the inclusion of all the percentages for the range of particle sizes. Figure 8 displays a cross-section of one of the typical Mexican raw sugars in this study. Each sugar from the various factories is different having its own specific profile. The range of particle sizes from nanoparticulate to higher micrometer diameters is relevant to the mechanisms of fouling of the activated carbon pores and adsorbent surfaces. There are strong arguments for pretreatment of raw sugars before application of further decolorizing processes. This pretreatment could remove nanoparticulate which fouls PAC as well as clogs necessary filter media for clarification and decolorizing. The sample in Figure 8 is diagnostic of the particle interferences to PAC treatment during processing.

Future Developments in the Upgrading of Raw Sugars for Remelting

Practical application of the knowledge gained in this laboratory study to the industrial scale involves many of the same factors. The carbon used in the present studies, Carbochem® CA-50 PAC, has been very successfully used world-wide without need of prior industrial micro- or nanofiltration (*23*). Industrial reports of recent factory trials using Carbochem® CA-50 PAC on raw sugar samples within reasonable turbidities and colors, 600 IU or less (*23*), suggest that multiple ton quantities of raw sugar can be remelted to very pure, high quality product. Previous reports of processes for refining raw cane sugar in

a remelting operation (*21*) have depended on initial clarification with tangential microfiltration and resin demineralization followed by PAC treatment. In the 1990's trials were held (*17–19*, *22*) to evaluate on-line disk stack centrifugation as an advanced clarification process to eliminate micro- and nanoparticulate. Not only were greater yields of crystallization found, but the raw sugars contained much less microparticulate or colloidally stable polymers. More recently the Brazilian firm, *Mecat Filtracoes Industrias* (Golas, Brazil) has developed very efficient continuous centrifugal clarification of raw sugar syrups (MECAT Turbo [centrifugal] Filters™) (*24*). This new technology used in both Brazilian sugar and food processing industries has resulted in highly reliable centrifugal nano- or microfiltration. Such equipment is capable of handling heavy loads of sediments which prevent easy decolorizing in raw sugar. The MECAT Turbo Filter™ does not remove colorant, but as a prefilter removes all of the turbid sediment (*24*). Consequently, this pretreatment permits more effective PAC treatment as demonstrated above in the membrane prefiltrations before PAC treatment.

Conclusions

Agricultural commodity delivery of sugar as an adequately refined material for manufacturing value-added products demands high yields of purified, raw sugar, if it is to be realistically competitive. Demand for purified, raw sugar is increasing because of expensive energy costs for refined white sugar production. Micro- and nanoparticulate can foul sensitive surface properties of adsorbents such as PACs or resins. Components in raw juice inhibiting the decolorizing of sugar must be removed to achieve very low colorant and high pol values. Development of improved powdered activated carbons with particle size limits, as well as optimal porosity, greatly increases resistance to fouling from turbid particles in raw sugars. At the same time elimination of excess particle fines enhances filterability of treated syrups. Commercial PACs such as Carbochem® CA-50 can achieve these goals and effectively removes a wide spectrum of colorant. Improved clarification processes currently being developed, such as centrifugation/ microfiltration or nanofiltration of sugar juices or syrups, will permit more efficient decolorizing with such an adsorbent that optimizes decolorizing performance and filtration properties.

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Chapter 13

Enzymatic Analysis of Mannitol as a *Leuconostoc mesenteroides* Deterioration Marker in Sugarcane and Sugar Beet Factories

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Sugarcane and sugar beet deterioration can still be a major technologocial constraint in processing, and better control of deterioration will contribute to the sustainability of the industries. The major (but not sole) contributor to deterioration in the U.S. and many other countries, particularly where warm and humid conditions prevail, is infection by hetero-fermentative *Leuconostoc mesenteroides* lactic acid bacteria. In recent years it has emerged that mannitol is a major product of *L. mesenteroides* deterioration of both sugarcane and sugar beet and a sensitive marker that can predict processing problems. An enzymatic factory method that is rapid, simple, accurate, and inexpensive is now available to measure mannitol in consignment juices and molasses. Cost of juice preparation was improved considerably by using Celite™ filter-aid and glass filters rather than PVDF microfilters. Precision and accuracy of the enzymatic method to measure low mannitol concentrations in sugar products were improved by spiking the buffer with mannitol and then calculating the final mannitol concentration by difference, although this was much better for diluted molasses than juice. Fructose up to 18% on a dissolved solids (Brix) basis was unequivocally shown *not* to interfere in the enzymatic determination of mannitol in both juices and molasses by comparing with gas and ion chromatography results. The increasing awareness of how

mannitol detrimentally affects processing, e.g., crystallization, is discussed.

Introduction

The delivery of consignments of deteriorated sugarcane or sugar beet to factories can detrimentally affect multiple process units, and even lead to a factory shut-down. Until the last few years, there was no reliable, rapid, simple, and inexpensive method to measure deterioration at the factory. This has meant that factory personnel have not been able to (a) screen individual consignments of sugarcane or sugar beet and, thus, they do not know which consignments will detrimentally affect processing and are unable to reject unsuitable consignments, and (b) rapidly detect deterioration inside the factory. Furthermore, with respect to sugarcane there is currently a world-wide emphasis of delivery of higher quality sugarcane to the factory (*1*). Consequently, grower payment formulas incorporating a deterioration quality parameter may serve as a deterrent against the delivery of severely deteriorated sugarcane, improve processing, and encourage better sugarcane management.

The major (but not sole) contributor to sugarcane deterioration in the U.S. and many other countries, particularly where warm and humid conditions prevail, is infection by hetero-fermentative *Leuconostoc mesenteroides* lactic acid bacteria (*2*, *3*). Similarly, sugar beets also suffer from *L. mesenteroides* infections under similar conditions as well as other bacterial infections (*4*). Factors affecting infection are ambient temperature and humidity, level of rainfall and mud, billet or root damage, delays between harvesting and subsequent processing, and factory hygiene.

Previously, the sugar industry has considered dextran (α-(1→6)-α-D-glucan), a viscous glucopolysaccharide, as the major deterioration product of a *L. mesenteroides* infection (Figure 1). Although ethanol has also been considered an indictor of deteriorated cane in South Africa (*5*), it was shown by Eggleston and Legendre (*6*) to be only weakly correlated with dextran and *Leuconostoc* cane deterioration under humid U.S. conditions, and is most likely more prevalent under dry South African conditions because of yeast induced deterioration. High concentrations of dextran (>1000 ppm/Brix measured with the Haze method; (*7*, *8*) can reduce evaporation and crystallization rates, and the factory can be penalized by refiners for excessive dextran in the raw sugar. Unfortunately, present factory methods to determine dextran are either too time consuming and complicated (ASI enzymatic method; (*9*)), not specific enough (haze method; (*7*)), too expensive (antibody method; (*10*)), too imprecise (antibody method), or too difficult in the interpretation of results (haze method). Moreover, none of these dextran methods can be used in a grower payment system. In recent years it has emerged that mannitol, a sugar alcohol, is also a major product of *L. mesenteroides* deterioration of sugarcane (*6*, *11–13*) and sugar beet (*14*, *15*). Mannitol is even known to be a product from the bacterial contamination of fuel ethanol produced from sugarcane products by hetero-fermentative *Lactobacilli*

including *L. mesenteroides* (*16*). In contrast, mannitol is not produced by homo-fermentative bacteria (*17*).

In 2002, Eggleston (*11*) first reported that mannitol was a major deterioration product in sugarcane, using ion chromatography with integrated pulsed amperometric detection (IC-IPAD aka HPAEC). In this laboratory study (*11*), an excellent correlation ($R^2=0.98$) was shown to exist between mannitol and dextran (measured by the ASI-II enzymatic method). Later, in a field study, Eggleston et al (*12*) showed a strong correlation ($R^2=0.85$) between mannitol and dextran (ASI-II enzymatic method) in juices from sugarcane varieties that had been freeze deteriorated. The slightly lower correlation for the field study was most likely because of greater variation in biological (field) samples. Therefore, mannitol, is at the least as good as if not better than, dextran at predicting sugarcane deterioration problems caused by *L. mesenteroides*.

Up until circa 2005, mannitol continued to be measured in sugar products from both sugarcane and sugar beet mainly using IC chromatography techniques (see Figure 2). However, chromatography techniques are too sophisticated for use at the factory, very expensive, and a high level of expertize is required by the operator.
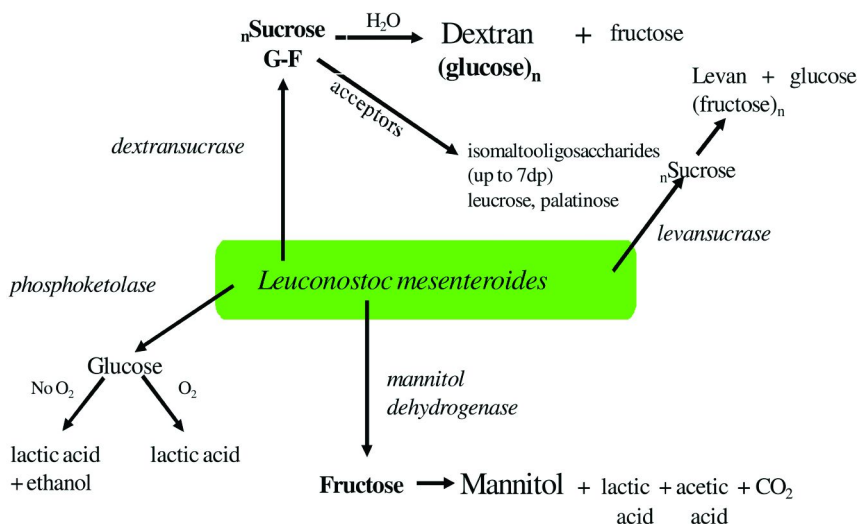
Figure 1. Major metabolites of hetero-fermentative Leuconostoc mesenteroides bacteria of interest to the sugarcane and sugar beet industries (8). Levan polysaccharide is much more predominant in sugar beet deterioration.
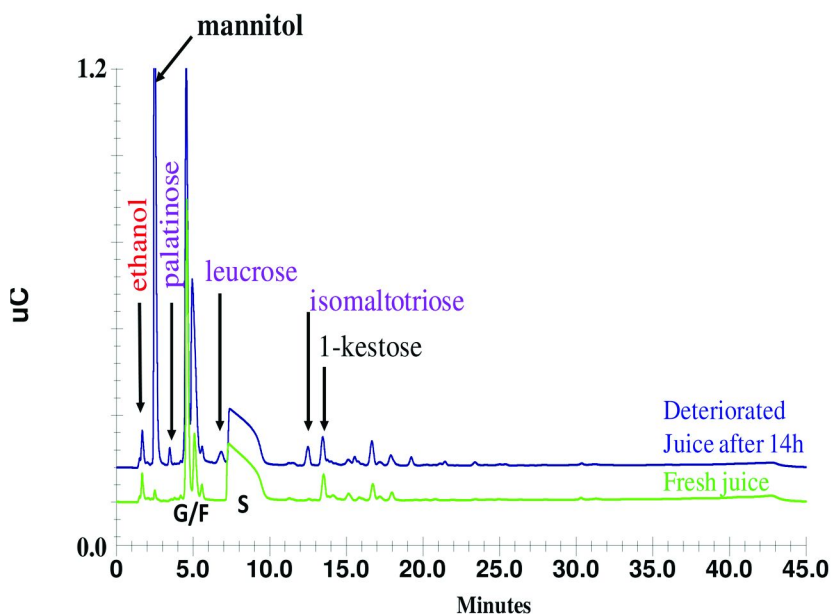
Figure 2. Change in IC-IPAD chromatograms after sugarcane juice is deteriorated. Palatinose, leucrose and isomaltotriose are oligosaccharide markers of severe dextran formation.

$$\text{Mannitol} + \text{NAD}^+ \xrightleftharpoons[]{\substack{\textit{Mannitol}\\ \textit{Dehydrogenase}}} \text{Fructose} + \text{NADH} + \text{H}^+$$
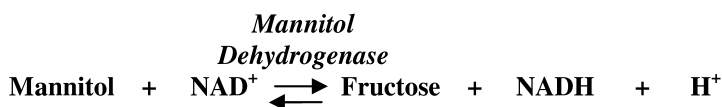
Figure 3. Enzymatic reaction of mannitol dehydrogenase

As a consequence, Eggleston and Harper (*13*) developed an enzymatic method to measure mannitol in sugarcane juices at the factory, which can also be applied to sugar beet juices (see later sections of this chapter). The method utilizes mannitol dehydrogenase (MDH) to convert mannitol to fructose in the presence of co-enzyme NAD$^+$. The NADH formed can be easily measured spectrophotometrically at 340 nm (Figure 3).

At the initial development of the method (*13*), problems were found relating to the stability of the MDH enzyme. Adding 30% glycerol to buffer in the stock and diluted MDH solutions, was found to be optimum for stabilization of the enzyme. The freeze dried MDH is stable in a -20 °C freezer for up to 6 months. The MDH stock and diluted solutions can be stored in conventional -20 °C freezers, but -40 °C freezers are preferable.

Sugarcane juice is a complex matrix and contains numerous particles, including bagacillo fibers, soil, and starch granules. Such particles in undiluted and unfiltered cane juice can interfere with the enzymatic mannitol determination (*13*). Therefore, cane juice must be diluted then filtererd for this method (*13*). The relationship between the mannitol concentration and the absorbance at 340

nm after 5 min was found to be only approximately linear up to 1000 ppm, and a quadratic fit is often better (*13*). The method is rapid (~7 min at room temperature [25 °C] and within 4 min if a 40 °C waterbath is used to incubate the juice), accurate, highly specific for mannitol, and was not affected by the presence of sucrose, dextran, and up to 3% (on a Brix basis) of glucose or fructose (*13*). Precision was acceptable although it was less reliable at low mannitol concentrations in juice (*13*). The method can be easily performed using existing equipment at the factory. The current cost of reagents per analysis of mannitol in a sugarcane load at the factory is only ~60 U.S. cents (U.S.$0.60), with the largest cost being NAD at 45 cents per analysis (*13*). Kits, for example, by Biosentec™ and Megazyme™, are now also available to measure mannitol in juices, but they cost over US$4 per analysis. Another option, would be to use immobilized enzyme technology, e.g., from Biosentec™, but after the initial purchase of the instrument the cost per analysis would be >U.S.$1 (*8*).

Eggleston (*8*) reported that a strong polynomial relationship (R^2=0.912) existed between mannitol and haze dextran (α-(1→6)-α-D-glucan) in 188 sugarcane press and crusher juices obtained across a 3-month processing season at a Louisiana sugarcane factory. Although mannitol concentrations were lower than haze dextran - a non-specific measure of dextran - mannitol was, generally, higher than dextran when dextran was measured by a specific method, i.e., ASI-II enzymatic or antibody dextran methods. This highlights (i) the usefulness and sensitivity of mannitol to predict sugarcane deterioration from *L. mesenteroides* and other hetero-fermentative *Lactobacillus* bacteria, and (ii) the underestimation by sugar industry personnel of the relatively large amounts of mannitol present in deteriorated sugarcane or sugar beet that can affect processing. Greater than ~500 ppm/Brix of mannitol in sugarcane juice predicted downstream processing problems (*8*). However, this threshold value may vary from region to region and by what is acceptable to individual factory personnel. This mannitol value can only be considered an approximate predictor of dextran because mannitol is produced by various *Lactobacillus* species and strains, although *Leuconostoc mesenteroides* is the greatest *Lactobacillus* producer of mannitol (*16*).

This chapter reviews the analysis of mannitol as a chemical marker of *L. mesenteroides* infections at sugarcane and sugar beet factories, and reports further research to optimize the enzymatic method (*8*) for measurement of low mannitol concentrations in upstream juices and downstream factory molasses.

Experimental

Chemicals, Enzymes and Juice Samples

Mannitol dehydrogenase (EC 1.1.1.67) was purchased as a freeze-dried powder (8.45 U/mg dry weight) from Biocatalyst Ltd, Wales. All chemicals used were analytical grade. Sugarcane molasses were obtained from a South Africa factory courtesy of Dr. Barbara Muir of the SMRI, Durban, South Africa and from four Louisiana sugarcane factories (Cora Texas, St. Mary, Raceland, and Alma). Molasses were stored at 4 °C. Sugarcane juices were obtained by pressing sugarcane through a roller mill and courtesy of Dr. Ryan Viator,

USDA-ARS-SL, Houma, Louisana. Some of the sugarcane had been exposed to freezing temperature -3.3 °C for 5 h followed by warm weather that caused considerable deterioration. All juices (120 mL) were stored with 5 drops of biocide (Bussan 881™, Buckman Labs.), in a -60 °C freezer until analyzed.

Buffers

Phosphate Buffer

Phosphate buffer (25 mM; pH 6.0) with 30% glycerol was prepared by adding potassium dihydrogen phosphate (0.34 g) and glycerol (30 g) into a 100 mL volumetric flask, then dissolved in deionized water (50 mL) and adjusted to pH 6.0 with NaOH (1 M). Dithiothreitol (15.4 mg) was added and the final volume made to 100 mL with de-ionized water.

Glycine Buffer Not Spiked with Mannitol

To prepare glycine buffer (100 mM; pH 10.5), glycine (7.51 g) was dissolved in deionized water (800 mL) and adjusted to pH 10.5 with NaOH (1 M), then made up to 1 L with de-ionized water.

Glycine Buffer Spiked with Mannitol

To prepare glycine buffer with 30 ppm mannitol (100mM; pH 10.5), glycine (7.51 g) and mannitol (0.03 g) were dissolved in deionized water (800 mL) then adjusted to pH 10.5 with NaOH (1 M), then made up to 1 L with de-ionized water. For other spiking levels with mannitol the amount of mannitol dissolved was changed accordingly.

NAD Solution

NAD (0.22 g) was dissolved in 10 mL of deionized water; NAD solution has to be prepared daily.

Preparation of Enzyme

A stock solution of enzyme was first prepared by dissolving 0.01 g of the freeze-dried MDH in 1 mL of ice cold phosphate + 30% glycerol buffer. For the assays, a further dilution was made by pipetting 100 μL of stock into a 10 mL volumetric flask and making to the final volume with phosphate + 30% glycerol buffer (10,000-fold dilution). Both the stock solution and diluted enzyme solutions were stored in a -20 °C freezer. The stock solution can be stored for ~1 month, and the diluted enzyme for 1-2 weeks. During analysis, the diluted enzyme was kept on ice at all times.

Molasses Preparation

Molasses were first diluted to ~14 Brix using de-ionized water then treated the same as for juices described below.

Juice Preparation

To measure mannitol by the enzymatic method in cane juice, the juice was first filtered by using either (i) PVDF (polyvinyldene fluoride) filters or (ii) using celite ™ filter-aid and a glass filter, as described below:

Filtering with PVDF Filters

Juice was first diluted 1:1 (i.e., 2-fold) in glycine buffer and then filtered through 0.45 μm pore-size PVDF filter (Millex^{R}HV, Millipore Corp., Ireland) a 0.22 μm pore-size PVDF filter (Millex^{R}GV). For difficult to filter samples, celite can be first added to the juice before filtering through the PVDF filters or the juice can first be filtered through Whatman™ 91 filter paper (185 mm; 10 μm).

Filtering with Celite™ Filter-Aid and a Glass Filter

Celite™ diatomaceous earth from Celite Corpn., Lompoc, CA (0.5 g) was added to cane juice (10 mL) in a syringe body (30 mL), mixed well, then filtered through a glass filter (25 mm diameter; Pall Corp., MI), with discard of the first 2 mL of filtrate. The filtrate (5 mL) was then diluted with glycine buffer (5 mL).

Factory Mannitol Enzymatic Method

A standard curve and equation were first generated using four mannitol standards (1, 10, 100, and 500 ppm) diluted in de-ionized water. A new standard curve must be generated for each batch of enzyme. To two test-tubes glycine buffer (1.4 mL), diluted and filtered juice (0.2 mL), and NAD (0.2 mL) were added (Table I). For the blank, 0.2 mL deionized water was added and the mixture was vortex stirred then immediately added to a 1 cm quartz cuvette and placed in a Shimadzu UV-1201™ spectrophotometer (Table I). For the test sample, 0.2 mL of 10,000-fold diluted MDH was added then immediately stirred on a vortex mixer then the timer started immediately. The solution was then added to a separate 1 cm cuvette, and the Δ absorbance measured at 340 nm after 0 and 5 min. The final absorbance was the Δ sample absorbance - blank absorbance. Calculations were based on the equation of the standard curve and dilution factors (*8*). For deteriorated juices containing high concentrations of mannitol which cause the mannitol absorbance to be higher than the upper limit of the standard curve, further dilutions of 1:3 (4-fold) or 1:7 (8-fold) in glycine buffer are required.

Table I. Mixing of Sample and Blank Reagents in Test-Tubes for the Enzymatic Mannitol Method (*8*)

Sample Test-Tube	*Blank Test-Tube*
1.4 mL glycine buffer	1.4 mL glycine buffer
0.2 mL diluted and filtered juice	0.2 mL diluted and filtered juice
0.2 mL NAD	0.2 mL NAD
0.2 mL MDH enzyme	0.2 mL deionized water

Mannitol Determined by IC-IPAD

See Eggleston (*11*) for method. Dilutions varied, depending on the juice, from 1 g/50 ml to 1 g/500 ml.

Gas Chromatography (GC) Analysis

See Eggleston et al (*18*) for the GC method.

Statistics

Statistical differences between two samples was conducted using *t*-test assuming equal variances using Microsoft Excel™ version 2007 with SP-2.

Results and Discussion

Improved Cost of Juice Preparation for the Enzymatic Mannitol Method

The use of 0.45 then 0.22 μm pore-size PVDF filters to filter diluted cane juice in the enzymatic mannitol method (*13*) are relatively expensive, i.e., ~U.S.\$1 each, and factory personnel complained about their cost. In comparison, filtering of juice with Celite™ filter-aid and a glass filter is less expensive and the required syringe and filter holder can be re-used after washing. Factories often have such equipment and filter-aid available in their laboratories for other methods (*7*). We, therefore, compared the use of 0.45 and 0.22 μm pore-size PVDF filters to Celite™ with a glass filter (see Experimental Section) on four sugarcane juices and results are illustrated in Figure 4.

As seen from Figure 4, there was an excellent relationship between the two preparation treatments for sugarcane juices. No significant (P<.05) differences existed between either treatment for three of the juices analyzed. There was only a significant (P<.05) difference for the juice with the lowest mannitol concentration and the coefficient of variation (CV) was an uacceptable 9.53% for the PVDF filtered juice compared to an acceptable 3.12% with Celite™ filtered juice Furthermore, the CV for all the juices with PVDF treatment varied considerably more than with the Celite™ treatment, indicating that the Celite™ treatment was better.
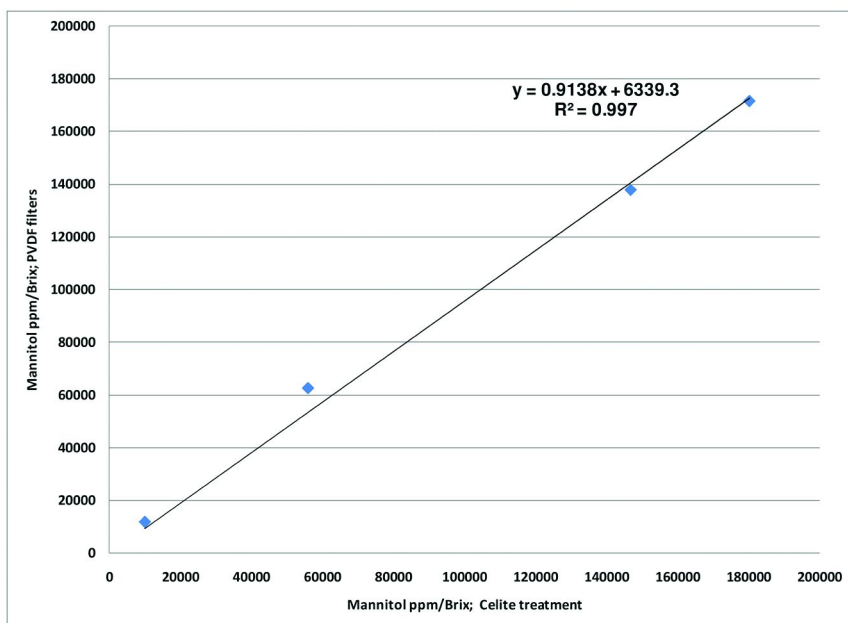
Figure 4. The difference between filtering molasses juice with PVDF (0.45 and 0.22 μm pore-size) syringe filters to using Celite™ with a glass filter. N=3.
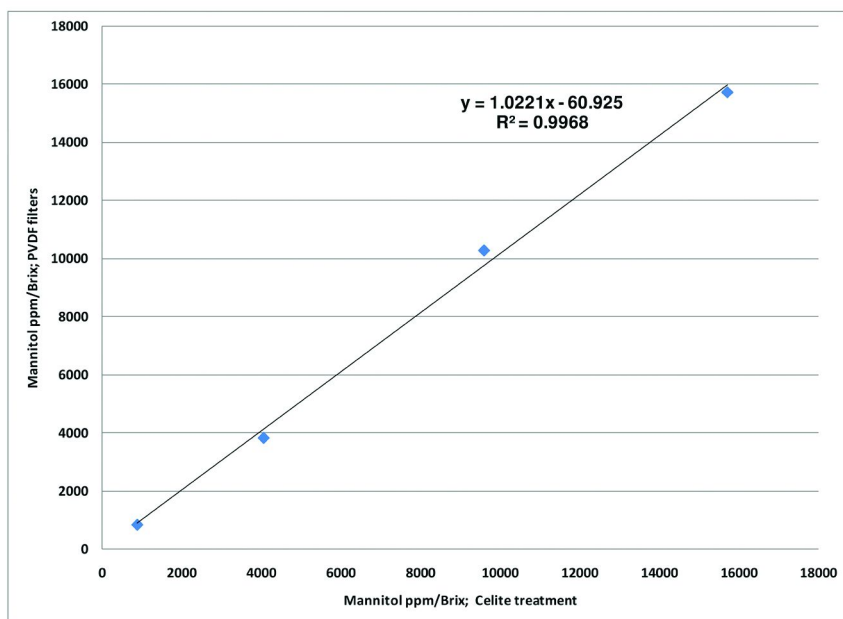
Figure 5. The difference between filtering molasses juice with PVDF (0.45 and 0.22 μm pore-size) syringe filters to using Celite™ with a glass filter. N=3.

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Further comparison of both treatments for four molasses juices are illustrated in Figure 5. There was also an excellent relationship between the two preparation treatments for molasses juices (Figure 5). No significant (P<.05) differences existed between either treatments for each of the four molasses juices analyzed.

As a consequence of these results, all further optimization of the enzymatic method for factory sugar products was undertaken using the less expensive Celite™ filter-aid and glass filters to filter the product juice.

Improved Precision of Enzymatic Mannitol Method at Low Concentrations in Sugarcane Molasses

Eggleston and Harper (*13*) reported that precision of the enzymatic mannitol method was worse when low concentrations of mannitol occurred in sugarcane juices. Eggleston et al (*19*) further reported that the use of the mannitol enzymatic method (*13*) in twenty raw sugars "often indicated there was no mannitol in the raw sugars, yet ion chromatography results indicated small amounts were present." Thus, not only was precision worse when low mannitol concentrations occurred but the enzymatic method (*13*) also under-estimated mannitol in downstream factory products. We decided to improve the enzymatic method for low concentrations of mannitol in the downstream end-product molasses. Initially, six molasses from the U.S. or South Africa were studied as they were known to represent a range of mannitol concentrations. GC analyses of common sugars and mannitol in these six molasses are listed in Table II.

Glucose and fructose concentrations were much lower in the South African molasses as sugarcane is hand-cut and, therefore, less trash and reducing sugars are present. St. Mary A and B molasses from the U.S. had markely higher GC mannitol concentrations since they were processed from deteriorated cane that had caused difficulties in the factory boiling house (*20*).

Both the effect of fructose concentration on mannitol meansurements as well as the relationship between GC and enzymatic mannitol results are illustrated in Figure 6. As mannitol and other sugars are physically separated from each other in chromatography, this technique is considered highly accurate. There was no significant relationship between fructose and mannitol (measured by both GC and enzymatic methods) which confirms that, even at very high fructose concentrations fructose does not significantly (P<.05) interfere with the enzymatic mannitol method (Figure 6). Eggleston and Harper (*13*) previously reported that the presence of 3% fructose on a Brix basis in sugarcane juices did not significantly affect the enzymatic mannitol assay.

Although mannitol measured by the enzymatic method was higher than by the GC method in the molasses that contained the highest concentration of mannitol (St. Mary B molasses) (Figure 6), this was not significant at the 5% probability level. Significant (P<.05) differences in the measurement of mannitol by the two methods only occurred when the concentration of mannitol was very low, i.e., <1000 ppm/Brix measured with the enzymatic mannitol method in the South African molasses (Figure 6).

**Table II. GC Analysis Results for Four Sugarcane Molasses Samples
Obtained from U.S. and South African Factories; N=3**

Sample	GC Analyses			
	Average Sucrose % on Brix basis	Average Glucose % on Brix basis	Average Fructose % on Brix basis	Average Mannitol ppm/Brix
Cora Texas U.S., C molasses	39.2	8.7	10.5	4111.1
South Africa molasses	71.9	3.3	2.4	3028.2
St. Mary, U.S. A molasses	67.1	5.9	5.7	9799.1
St. Mary U.S., B molasses	54.5	7.4	7.8	13038.5
Raceland U.S., C molasses	36.4	4.8	10.3	3761.5
Alma U.S., B Molasses	58.6	4.9	7.1	2163.3
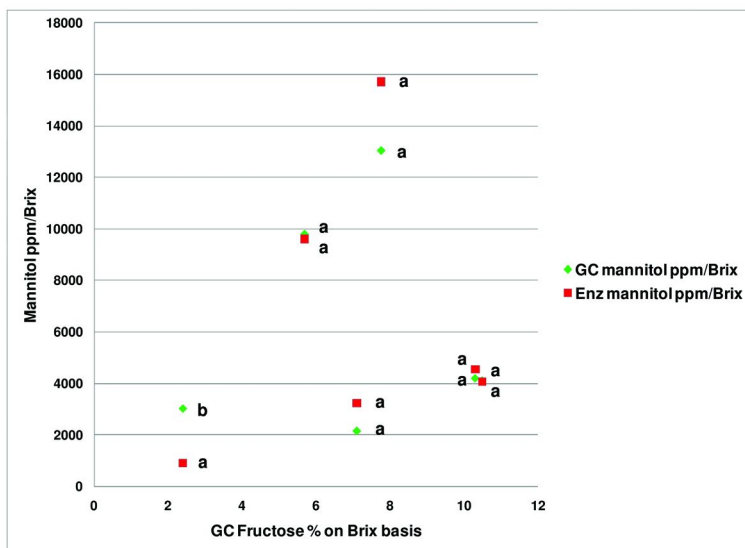
*Figure 6. The effect of fructose on the mannitol measurement in four molasses
samples, and the difference between the measurement of mannitol by GC or
enzymatic methods. The same lower case letters represent no statistical difference
(P<.05) between the two different mannitol methods for each sample only.*

Table III. Effect of Different Levels of Spiking the Buffer with Mannitol on the Precision and Accuracy of the Eggleston (*8*) Enzymatic Method to Measure Mannitol in Sugarcane Molasses Expressed as the Coefficient of Variation (CV)

Sample	GC Method			Enzymatic Method					
				No spiking			With spiking		
	N	Average ppm/Brix	CV %	N	Average ppm/Brix	CV %	N	Average ppm/Brix	CV %
							50 ppm spiking		
South African-Molasses	3	3028.2a*	29.6	3	895.8b	16.9	3	6390.8c	8.2
							40 ppm spiking		
South African-Molasses	3	3028.2b	29.6	3	895.8c	16.9	3	4798.2a	7.5
							30 ppm spiking		
South African-Molasses	3	3028.2a	29.6	3	895.8b	16.9	3	3203.4a	6.0
							10 ppm spiking		
South African-Molasses	3	3028.2a	29.6	3	895.8c	16.9	3	1355.4b	10.4
St. Mary B Molasses	3	13038.5a	1.3	3	15710.9a	12.1	3	26284.3b	4.9

* The same lower case letters represent no statistical difference (P<.05) among the three different methods for each juice and one spiking level only.

The enzymatic reaction in the presence of MDH (Figure 3) is a reversible reaction and a low mannitol concentration hinders the drive of the reaction to the right, i.e., to production of fructose and NADH. Therefore, to drive the reaction to the right in the South African molasses sample which contained only a low concentration of mannitol and, concomitantly improve enzymatic analysis of mannitol, we spiked the glycine buffer with mannitol and then calculated the final mannitol concentration by difference. Results are listed in Table III. For comparison purposes only, we also spiked the St. Mary B molasses that contained a very high concentration of mannitol, i.e. >13,000 ppm/Brix. As expected, for the St. Mary B molasses spiking did not improve the enzymatic assay and just caused it to become further over-estimated (Table III).

Spiking the South African molasses juice (with a low mannitol concentration) with a starting level of 50 ppm in the buffer significantly (P<.05) over-estimated mannitol but did improve precision as the CV decreased (Table III). Spiking with

40 ppm still significantly (P<.05) over-estimated mannitol but to a lesser extent than at 50 ppm spiking, and slightly improved precision (Table III). However, at 30 ppm spiking there was no significant difference between the mannitol measured by the enzymatic and GC methods and the CV was an acceptable 6%. Furthermore, these results were reproducible. We tried to further decrease the spiking level, but as can be seen in Table III, at 10 ppm spiking there was still a significant (P<.05) under-estimation of mannitol compared to the GC method and precision was worse.

Further 30 ppm spiking of two more U.S. molasses from Raceland and Alma sugarcane factories, which both contained <4550 ppm/Brix mannitol measured with the unspiked enzymatic method, only overestimated mannitol compared to GC (results not shown). Thus spiking is only valid for molasses with very low concentrations of mannitol (less than ~1000 ppm/Brix measured first with unspiked buffer).

Improved Precision and Accuracy of the Enzymatic Mannitol Method in Sugarcane Juices Containing Low Concentrations of Mannitol

We also tried to improve the precision and accuracy of measuring mannitol by the enzymatic method in nine sugarcane juices. The juices were pressed directly from commercial sugarcane and sugarcane hybrids in Louisiana and represented a large range of mannitol concentrations (Table IV). For some of the juices the mannitol concentrations were extremely high because the sugarcane had been purposely allowed to deteriorate after a freeze. Table IV also lists the fructose concentrations in the juices that also varied markedly from 4.6 to 18.5% on a Brix basis.

Statistical differences between the measurement of mannitol in the juice by both the IC or enzymatic methods (8) are also shown in Table IV. The GC method used to measure mannitol in the molasses samples was found not to be sensitive enough to detect the very low concentrations of mannitol in some of the juices so IC was used as a comparison instead. Similar for the molasses results (compare Table III and Figure 6), significant (P<.05) differences in the measurement of mannitol by the two different methods, generally, only occurred at low concentrations of mannitol (Table IV). However, in some cases no significant diffences occurred, i.e., juices V9002 and V153 in Table IV. When significant differences did occur the enzymatic method tended to under-estimate mannitol and precision was low (Table IV) although precision by the IC method was also low. In contrast, in juices with higher concentrations of mannitol there were generally no significant differences between results from the two mannitol methods and the precision was acceptable (Table IV).

Table IV. Fructose and Mannitol Analysis Results for Nine Different Sugarcane Juices from the U.S.; N=3

Juice Sample	GC Analysis	IC Method		Enzymatic Method	
	Average Fructose % on Brix basis	Average Mannitol ppm/Brix	CV %	Average Mannitol ppm/Brix	CV %
V9002	4.6	238.8a*	11.7	174.2a	16.7
V114	11.8	1203.4a	13.1	116.1b	42.7
V149	11.0	1676.2a	18.7	298.0b	43.9
V153	18.5	157.8a	2.6	151.6a	25.5
V147	11.4	15216.4a	4.4	12226.0b	2.8
V7	5.0	64250.8a	6.3	61068.1a	0.6
V1003	7.7	179394.1a	4.6	171773.0a	2.0
V540	10.3	nd	nd	296039.0	1.0
V1004	7.3	230007.1a	3.7	246475.4a	7.1

* The same lower case letters represent no statistical difference (P<.05) between the IC and enzymatic methods to measure mannitol in each juice only.

Figure 7 illustrates the effect of fructose concentration on the measurement of mannitol by IC and enzymatic methods. Similar for molasses (Figure 6) there was no significant relationship between fructose and mannitol which unequivocally confirms that fructose levels do *not* significantly interfere with the enzymatic mannitol method. Furthermore, low mannitol concentrations sometimes occurred when fructose concentrations were very high and vice versa (Figure 7).

As 30 ppm spiking of the glycine buffer had improved the accuracy and precision of the enzymatic measurement of mannitol in molasses (Table III), we tried this level of spiking in juices (Table V). However, 30 ppm spiking significantly (P<.05) increased the concentration of mannitol measured in two juices (Table V) and and precision was not consistently improved either. Similar over-estimated results were obtained for the same two juices at 15 ppm spiking level. Even at the 2 ppm spiking level mannitol measured in juices were still significantly (P<.05) over-estimated, although precision was improved (Table V). In contrast, however, at the 1 ppm spiking level there was no significant difference between the measurement of mannitol in juice using either the spiked or unspiked enzymatic method; furthermore, there was also no significant diffence with the IC results (Table V). Although precision improved at the 1 ppm spiking level (Table V), at extremely low intial concentrations of mannitol, i.e., <~365 ppm/Brix, precision was less than an acceptable 5% level - but this also applied to IC results (Table V).
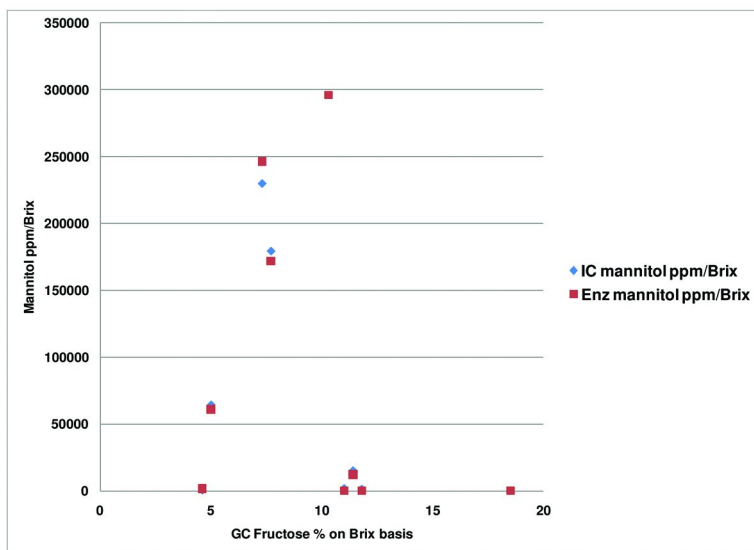
Figure 7. The effect of fructose on the mannitol measurement in nine sugarcane juices, as well as the difference between the measurement of mannitol by IC or enzymatic methods.

Overall, spiking buffer was not as effective at improving the accuracy and precision of the enzymatic mannitol method at measuring low concentrations of mannitol in juices as for molasses juice. This suggests that one or more enzyme inhibitors are present and active in juices compared to molasses. Inhibitors could include proteinaceous inhibitors that are denatured downstream in the factory at elevated temperatures, which cause them not to be present in molasses and raw sugar. Inhibitors in the juice may also be complexing with calcium downstream. Another explanation is that in the juice there is the possibility of competing activities. Another compound may be utilizing the NADH produced by MDH. Investigations are continuing on the pre-treatment of juices to remove inhibiting factors. However, it must be noted that, although precision was less at low concentrations of mannitol, these concentrations are below the threshold values that cause processing problems in the factory (*8*).

Use of the Enzymatic Mannitol Method in Sugar Beet Factories

Application of the enzymatic mannitol method (*8*) to sugar beet juices from U.S. and French factories has been successful (Table VI), although just like for sugarcane juices, the precision decreases at low concentrations of mannnitol.

Table V. Effect of Different Levels of Spiking the Glycine Buffer with Mannitol on the Precision and Accuracy of the Eggleston (*8*) Enzymatic Method to Measure Mannitol in Sugarcane Juices

Sample	IC method			Enzymatic Method					
				No spiking			With spiking		
	N	Average ppm/Brix	CV %	N	Average ppm/Brix	CV %	N	Average ppm/Brix	CV %
								30 ppm spiking	
Juice 1	3	315.8a*	24.1	3	253.5a	54.4	3	2735.7b	11.8
Juice 2	4	73694.3a	1.4	3	99598.4b	2.4	3	209134.0c	6.9
								15 ppm spiking	
Juice 1	3	315.8a	24.1	3	253.5a	54.4	3	2106.5b	1.6
Juice 2	4	73694.3a	1.4	3	99598.4b	2.4	3	148087.5b	3.0
								2 ppm spiking	
Juice 1	3	315.8a	24.1	3	262.0a	16.8	3	593.1b	5.8
Juice 2	4	73694.3a	1.4	3	84620.5b	4.9	3	101704.5c	1.4
Juice 3	3	721.7a	9.2	3	761.8a	39.5	3	1447.3b	26.6
								1 ppm spiking	
Juice 3	3	721.7a*	9.2	3	766.2a	13.0	5	786.8a	1.1
Juice 4	3	365.0a	7.0	6	405.8a	38.0	6	332.9a	20.7

* The same lower case letters represent no statistical difference ($P<.05$) among the three different methods for each juice and one spiking level only.

Table VI. Precision of the Eggleston (*8*) Enzymatic Method to Measure Mannitol in Sugarbeet Juices Expressed as the Coefficient of Variation (CV)

Sample	Source	N	Average Mannitol ppm/Brix	CV %
Sugar beet juice A	U.S.	11	1539.7	3.7
Sugar beet juice B	U.S.	10	137.6	9.9
Sugar beet diffusion juice	France	10	200.8	6.7

Huet (*21*) also tested, adapted, and improved the initial enzymatic method (*13*) for determining mannitol in beet sugar processing products. The absorbance of raw juices in sample cells increased sometimes before the enzyme was added. Adding a 3 min stabilization time step or treating with Carrez solution (or other clarification agents) before the enzyme addition overcame this problem (*21*). The accuracy and precision was acceptable (precision was less at low mannitol concentrations) and the method has been validated as an Official ICUMSA (International Commission for Uniform Methods in Sugar Analysis) method (*22*). Mannitol correlated strongly with acetic (R^2=0.97) and lactic acids (R^2=0.97), and was 5-6 fold and 3-4 fold higher than acetic and D-lactic acids, respectively (*22*). Furthermore, mannitol correlated moderately well with dextran (R^2=0.74) (*22*).

In beet factory extraction plants, the greatest amounts of mannitol were always found in diffuser and juice/cossette heat exchanger areas (*21*). At a Pfeifer & Langen sugar beet factory in Germany the enzymatic mannitol method is being used to monitor for dextran formation within raw juice heaters to know when to treat regularly with sodium hydroxide, i.e., when the mannitol became greater than a limit value of 50-60 ppm (*22*). At the Raffinerie Tirlemontose Co. beet factory in Belgium the enzymatic mannitol method is used to monitor raw juice stations. At greater than 160 ppm mannitol steam disinfections of juice/cossette heat exchanges occur which reduced filterabilitiy problems (*22*). Many other sugar beet factories in Europe are similary using mannitol to predict and control processing problems (*22*). As with sugarcane, mannitol determinations are necessary within each factory to define critical warning thresholds which may vary from factory to factory and with size of the plant.

The mannitol method has been investigated at Amalgamated™ Twin Falls beet factory in Idaho, U.S., to detect *Leuconostoc* infections during beet diffuser processing. Initial results are shown in Table VII. Mannitol concentrations were low in most products and because no spiking of buffer was used, they are most likely underestimations. Nevertheless, there was a higher mannitol concentration in the diffuser mid-tower juice on 21 Dec., 2009 (Table VII) that indicated an infection occurred there or that the beet that entered the diffuser was from a deteriorated load and action needed to be taken in the factory.

How Mannitol Affects Industrial Processing

As the awareness of mannitol in the sugar industry is growing, so is the knowledge on how it affects processing. Mannitol ($C_6H_{14}O_6$; mol wt. 182.2) has a low positive optical rotation $[\alpha]_D$ at 20 °C is +23° and is hygroscopic. Early in 1975, Bliss reported that mannitol affects processing by reducing sugar recovery (*23*).

**Table VII. Mannitol Concentrations Measured by the Eggleston (*8*)
Enzymatic Method in Sugar Beet Juice and Press Water Products from
Amalgamated Twin Falls Sugar Beet Factory in Idaho, U.S.**

Date	Twin Falls Factory Product	Brix	Mannitol ppm/Brix
4/11/2009	Raw juice	14.0	839.3
	Diffuser juice; 6:00 pm	14.2	67.9
	Diffuser mid-tower juice; 6:00 pm	3.2	66.3
	Press water from pulp exiting the diffuser; 6:00 pm	2.1	242.3
	Re-circulated juice in diffuser*; 6:00 pm	13.9	96.5
	Re-circulated juice in diffuser*; 8:00 pm	13.0	351.7
	Re-circulated juice in diffuser*; 10:00 pm	13.6	87.5
12/21/2009	Diffuser juice; 12:00 am	14.0	124.2
	Diffuser mid-tower juice; 12:00 am	4.6	4255.2
	Press water from pulp exiting the diffuser; 12:00 am	2.2	39.9
	Re-circulated juice in diffuser*; 12:00 am	13.7	28.9
	Re-circulated juice in diffuser*; 2:00 am	14.4	93.6
	Re-circulated juice in diffuser*; 4:00 am	14.0	112.5

* From pump 50D.

Mannitol, unlike sucrose and reducing sugars, does not degrade under industrial conditions found in sugar factories (*12*). Thus, when mannitol is delivered to the factory in either deteriorated sugarcane or sugar beet it can travel through the factory and very large amounts of mannitol have been found in factory syrups, massecuites, and molasses (*20*). Furthermore, mannitol has been linked to the hard-to-boil (HTB) massecuites phenomenon in Louisiana, U.S., whereby the thermal transfer properties of massecuites formed after processing severely deteriorated sugarcane are markedly reduced (*20*). Greater than 24,000 ppm/Brix mannitol was found in one HTB massecuites (*20*). Overall, as mannitol increased there was a concomitant resistance to heat transfer. However, as Eggleston et al. (*20*) reported that an intermolecular (gel) network was a major factor contributing to higher viscosities in HTB, mannitol is only a contributing not major factor. Large amounts of mannitol have also been found in betaine (125858 ppm/Brix) obtained from the chromatographic separation from feed molasses (19623 ppm/Brix) in a a U.S. sugar beet factory that had highly deteriorated sugar beet from freezes.
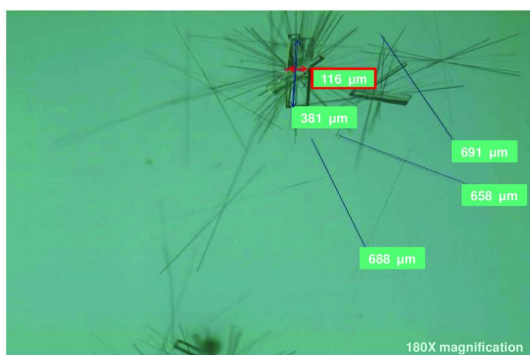
Figure 8. Formation of needle-like crystals of mannitol in a 33 Brix pure mannitol solution . Over time star-like formations occur.
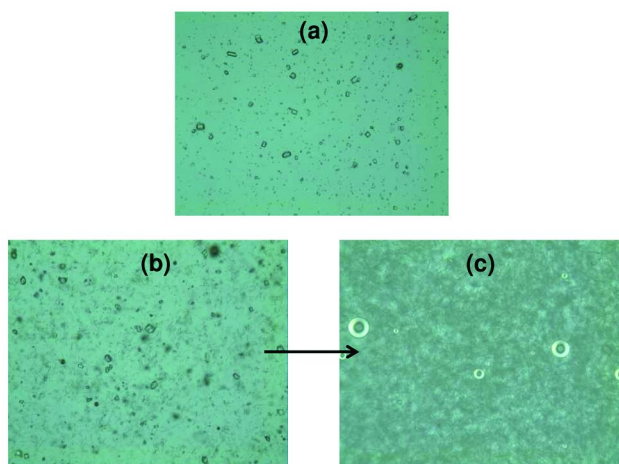
Figure 9. Digital micrographs of seed sucrose crystals added to 71 Brix solutions of (a) pure sucrose after 45 min, (b) 90% sucrose and 10% mannitol after 30 min, and (c) 90% sucrose and 10% mannitol after 40 min. Mannitol needle crystals are predominant in (c).

Steinmetz et al. (*14*) reported that mannitol correlated the best with filterability of first carbonation slurries after processing freeze deteriorated sugar beets. Mannitol has also been shown to predict sucrose losses and dextran related problems such as viscosity and, to a lesser extent, filterability problems in sugarcane (*12*).

Mannitol is much less soluble than sucrose at all temperatures (*24*). For example, at 60 °C sucrose solubility is 283 g/100cm^3 and mannitol is only 56 g/100 cm^3. Mannitol forms needle-like crystals as illustrated in Figure 8 and star-like conglomerations of mannitol crystals are frequently observed at high concentrations of mannitol (Figure 8).

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At ≥10% mannitol levels in sucrose solutions (71 Brix), mannitol crystals become prevalent (Figure 9). The presence of calcium increases the solubility of mannnitol in such solutions as well as the solution viscosity (results not shown). This may be because of the formation of calcium-mannitol adducts (*25*), and the authors are continuing investigations on the effect of mannitol on sucrose crystallization under raw sugar manufacturing conditions.

Summary

In recent years it has emerged that mannitol, a sugar alcohol, is a major product of *Leuconostoc mesenteroides* deterioration of both sugarcane and sugar beet and a sensitive marker that can predict processing problems. An enzymatic factory method (*8*) is now available to measure mannitol in juices and molasses, and warrants further investigation for use in downstream factory products including raw sugars.

Cost of juice preparation was improved considerably by using Celite™ filter-aid and glass filters rather than PVDF microfilters.

Precision and accuracy of the enzymatic method to measure low mannitol concentrations in sugar products were improved by spiking the buffer with mannitol and then calculating the final mannitol concentration by difference, although this was much better for diluted molasses than juice. Investigations are continuing on improving the method for sugarcane juice containing low concentrations of mannitol so that it can be used in grower payment systems. However, although precision is less at low concentrations of mannitol in cane juices, these concentrations are below the threshold values that cause processing problems.

Fructose up to 18% on a Brix basis was unequivocally shown not to interfere in the enzymatic determination of mannitol in cane juices and molasses.

Mannitol is stable under industrial processing conditions and the authors are continuing on the detrimental effects of mannitol on industrial crystallization.

Overall, the enzymatic measurement of mannitol as a marker of hetero-fermentative *Leuconostoc* deterioration of sugarcane and sugar beet is only a tool for management. Change will only occur after a management decision.

Acknowledgments

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Chapter 14

Sustainability of Low Starch Concentrations in Sugarcane through Short-Term Optimized Amylase and Long-Term Breeding Strategies

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Starch negatively affects the quantity and quality of raw sugar produced. Starch reduces crystallization and centrifugation rates, occludes into sucrose crystals, and impedes refinery decolorization processes. The problem of starch in sugarcane juice has been exacerbated by the widespread adoption of green cane harvesting and also, perhaps by the necessity to incorporate useful traits from wild *Saccharum* germplasm into cultivated sugarcane. Use of α-amylase to hydrolyze starch during processing should be viewed as a short-term solution as the enzyme is relatively expensive and not always efficient. Availability of sugarcane varieties low in starch content should present a more sustainable, long-term solution. This chapter highlights problems caused by starch during the processing of sugarcane, as well as presents data suggesting that it would be possible to deploy sugarcane varieties low in starch content.

Introduction

Starch is an impurity in sugarcane juice that can impede the extraction of sugar during processing as well as affect the quantity and quality of raw and refined sugars (*1*, *2*). Starch can reduce crystallization and centrifugation rates, occlude into the sucrose crystals, increase the production of molasses, reduce filterability and affination of raw sugars, and impede refinery decolorization processes. These processing problems are currently being mitigated in the factory by using α-amylase to hydrolyze starch, which is a short-term solution because the enzyme is relatively expensive and not always efficient. The problem of starch in sugarcane juice has been exacerbated by the widespread adoption of green cane harvesting and also perhaps by the necessity to use wild *Saccharum* germplasm for incorporation of useful traits into cultivated sugarcane.

Availability of sugarcane varieties low in starch content would be a more preventative, economical, and efficient solution. Therefore, research focused on breeding for low starch in sugarcane could provide the envisaged long-term solution. Sugarcane breeding programs consider several traits during selection. Adding an additional selection trait such as starch would encumber the selection process and may result in the reduction of selection gains. A good strategy would involve using parents with low starch during crossing and selecting for yield and quality from the progeny that are expected to produce low starch. In this chapter, processing issues related to starch in sugarcane juice are reviewed. This is followed by a review of data from multiple, recent studies that were designed to survey relative starch content in a wide collection of varieties and wild *Saccharum* species used in sugarcane breeding programs. Finally, we propose a more sustainable, longer-term strategy to lower starch content in sugarcane varieties, without putting a burden on breeding programs with the introduction of a new selection trait.

Starch in the Sugarcane Plant

Starch (α-1→4-glucan) is a sugarcane juice impurity that adversely affects factory and refinery processes and subsequently the quantity and quality of sugar products (*1*, *2*). Unfortunately, the delivery of sugarcane starch to U.S. and other countries' factories has risen markedly in recent times because of the increased adoption of green (unburnt) cane harvesting (*3*) and the introduction of newer varieties with higher contents of starch (*4*).

Starch exists as semi-crystalline granules (1 to 10 μm) in sugarcane tissue and extracted juice. These granules are smaller than those from corn (5 to 25 μm) and potato (15 to 100 μm) (*5*). Sugarcane starch granules contain two glucose polysaccharides: ~19% amylose and 81% amylopectin (*6*). Amylose is linear with the glucose molecules α-D-(1→4) linked (Figure 1). Amylopectin, in addition to the α-D-(1→4) linked glucose found in amylose, has α-D-(1→6) linked branch points (Figure 1). Amylose forms a blue color in the presence of iodine (*5*), while amylopectin forms a red-violet color.

Starch is produced in the sugarcane plant as a storage polysaccharide (carbohydrate reserve) and utilized during periods of rapid growth, e.g., during
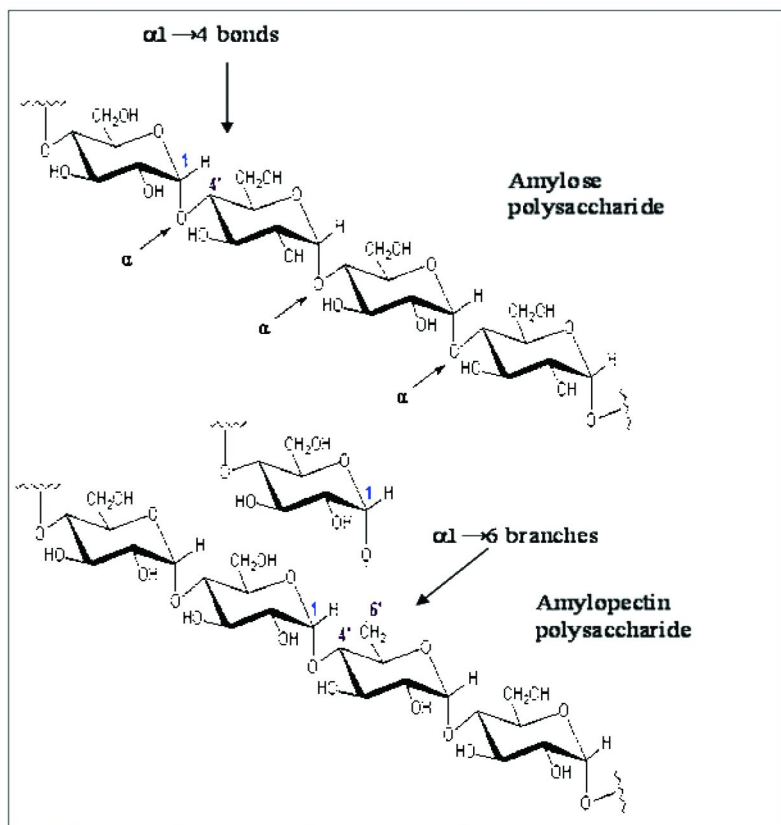
Figure 1. Chemical structure of amylose and amylopectin starch polysaccharides (12).

the sprouting of roots and buds, seedling germination, and emergence (*5*, *7*). Starch granules are present in stalks, leaves (both green and brown; (*8*)), and roots of the sugarcane plant (*9*), but are most abundant in the green leaves and growing point region (Table I). There is strong varietal effect on starch content in the total juice (*8*, *10*) and the distribution of starch among different plant tissues (*11*) (Table I). Starch decreases with sugarcane maturity.

In sugarcane stalks, starch granules are deposited mainly at the nodes and disappear during rapid growth. Growing conditions such as soil type, nutrients, agronomic practices, water supply, and temperature have been reported to affect the levels of starch found in sugarcane stalks (*5*). Although starch levels in stalks are relatively low compared with other tissues (*11*), when calculated on a % tissue wet weight basis, it is observed that stalks actually deliver a considerable amount

of starch to the factory, just because of their much higher weight and volume (*11*). Therefore, starch delivery to the factory by stalks should *not* be underestimated.

Sugar Processing Problems Associated with Starch

In recent years, there have been warnings by some U.S. raw sugar refineries that they may impose a penalty on high-starch levels in raw sugar if starch control is not improved (*1*, *2*). Processing costs increase, not only in terms of additional processing aids, but also from increased viscosity of massecuites, reduction of crystallization and centrifugation rates, occlusion of starch into the sucrose crystal, increased molasses production (*13*), reduced filterability and affination of raw sugars, and impediment of refinery decolorization processes (*1*, *2*). Mud filtration is particularly impeded when a carbonatation refinery processes raw sugar containing >250 ppm/Brix starch. For these reasons, U.S. factories are being encouraged to deliver raw sugar containing <250 ppm/Brix starch with, however, a level <200 ppm/Brix being preferred for carbonatation refineries. In comparison, the South Africa industry has imposed a penalty on raw sugar containing starch >130 ppm/Brix (P. Schorn, Tongaat-Hulett Sugar Ltd., personal communication). In the U.S., however, there is, not yet, a current penalty on high-starch concentrations in raw sugar. Instead, an informal policy of encouragement and cooperation exists between the carbonatation refinery and factory, which has worked to the satisfaction of the refinery staff in the last 3 to 4 years (F. Goodrow, Domino Sugar, personal communication). Cooperation includes the application of α-amylase in the factory to hydrolyze starch (*1*, *2*, *4*). However, not all U.S. raw sugar factories apply α-amylase and some just apply it intermittently.

Starch at the Factory

Starch granules are extracted into the sugarcane juice during tandem milling or diffusion upstream at the factory. The behavior of starch granules upon hydration and heating in the factory influences their effects on downstream processing. In cold juice, starch is not soluble. If hot maceration or imbibition water is added at the factory, the granules are washed out of the shredded plant tissue into the juice, and heat causes the granules to swell, become partially soluble and gelatinized (*14*). Solubilization and gelatinization of the granules are completed at elevated temperatures during clarification and evaporation (Figure 2).

During clarification and evaporation, the starch granules are heated further, swell progressively, and finally rupture to release amylose and amylopectin into the solution, which becomes amorphous and viscous (Figure 2). Linear amylose molecules are capable of forming helices and can readily associate in water by hydrogen bonding and increase viscosity. Upon cooling, they can re-associate to form a gel network, whereas branched amylopectin cannot. This explains why the amylose fraction is responsible for the deleterious effect of starch in the factory (*14*). This amylose molecular association phenomenon is known as retrogradation, and influences the distribution of starch in factory and refinery products. Due to

Table I. The distribution of starch in different tissues of the sugarcane plant from two Louisiana commercial varieties (*8*)

Sugarcane Tissue	Mean starch concentration (ppm/Brix)			
	LCP 85-384		HoCP 96-540	
	24 Oct, 2005	18 Nov, 2005	24 Oct, 2005	18 Nov, 2005
Green Leaves	1244	1246	1139	1372
Growing Point Region	2263	1780	2528	1523
Middle Stalk	502	495	1831	282
Lower Stalk	458	336	973	485
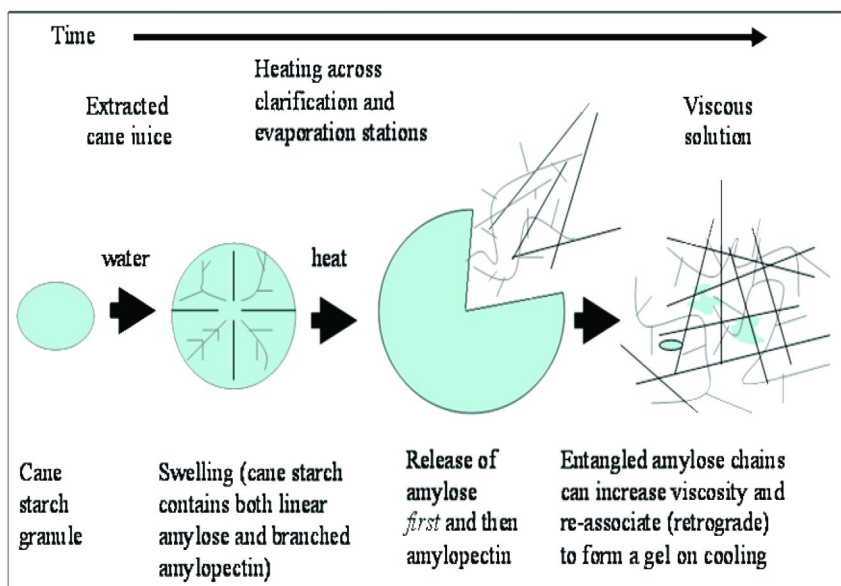
Figure 2. Starch solubilization and gelatinization across the sugarcane factory (2).

the starch physical transformation and concentration effects taking place across the factory, the viscosity of products in the boiling house can increase (*13*).

Factory Processes That Contribute to the Removal of Starch

In some countries such as Australia, natural amylases (diastases) have been utilized to hydrolyze starch in juice. In Louisiana, there is only one factory with a large enough incubation tank to allow the time for the natural amylases to work. Filtrate from the clarifier can be recycled into the incubation tank to reduce sugarcane juice acidity, enabling the natural amylases to better hydrolyze starch (*15*). However, starch hydrolysis is more efficient when starch granules have been partially solubilized and gelatinized by hot maceration or imbibition

water. Furthermore, there is an unfortunate possibility that some unwanted acid degradation of sucrose may occur in the tank because of the retention time. Moreover, often the naturally occurring amylases occur at concentrations that are too low to hydrolyze all the starch. Eggleston *et al.* (*15*) reported ~10 to 20% degradation of starch by natural amylases in a 12 min retention time incubation tank.

If intermediate or hot temperature lime clarification is operated in the factory, some starch will be removed by precipitation, just from pre-heating the juice before liming and clarification (*15*), but this will not occur with cold lime clarification. However, the combined heating, incubation, or clarification of juice usually does not reduce starch to a concentration that substantively alleviates process problems in the boiling house. Therefore, subsequent treatment of the evaporator syrup with α-amylase is regularly used in some factories to hydrolyze the remaining starch.

α-Amylase Properties and Factory Application

α-Amylase is usually added to the penultimate (next-to-the-last) or last evaporator body because starch is in a completely solubilized and gelatinized form that is much more conducive to α-amylase hydrolysis. The pH, Brix, temperature, and retention time are also more conducive to α-amylase action, but are still not fully optimal (*1, 2, 4*).

α-Amylases (endo-1→4-α-D-glucan glucohydrolases; EC 3.2.1.1) are endo-hydrolases that, in the presence of water, randomly cleave 1→4-α-D-glucosidic linkages between adjacent glucose molecules in the amylose chain of the solubilized/gelatinized starch. The viscous solution is progressively "thinned" into lower molecular weight (MW) dextrins and finally maltodextrins (oligosaccharides) of smaller chains (often in the 2 to 7 dp range) (Figure 3).

α-Amylases are classified according to their action and properties (*16*) and derived from several bacteria, yeasts, and fungi. Bacterial α-amylases, particularly from *Bacillus sp.*, are generally preferred for commercial production and widely used in numerous industries because they have the most diverse biochemical properties and are generally recognized as safe. Most commercial α-amylases used by the U.S. sugar industry to control starch have intermediate temperature stability (up to 85 °C with an optimum ~70 °C) and are produced from *Bacillus subtilis*. They are calcium-dependent α-amylases, but this is not a concern for sugar industry applications because lime is added during the clarification process and, therefore, free calcium concentrations are adequate. Unfortunately, some factories in the U.S. and worldwide have applied bio-engineered high temperature (HT) and stable (up to 115 °C) α-amylases from *Bacillus licheniformis* and *B. stearothermophilus*, which were developed for much larger markets than the sugar industry. They are not specifically tailored to sugar industry conditions (*1, 2*): for example, they are less active in high Brix syrups. HT α-amylases are *too temperature-stable* for the sugar industry and may not denature or inactivate after application, resulting in carry-over activity in raw sugar and molasses. α-Amylase activity in the raw sugar can even carry through subsequent refinery processes and eventually reside in refined sugar, molasses, and food products.
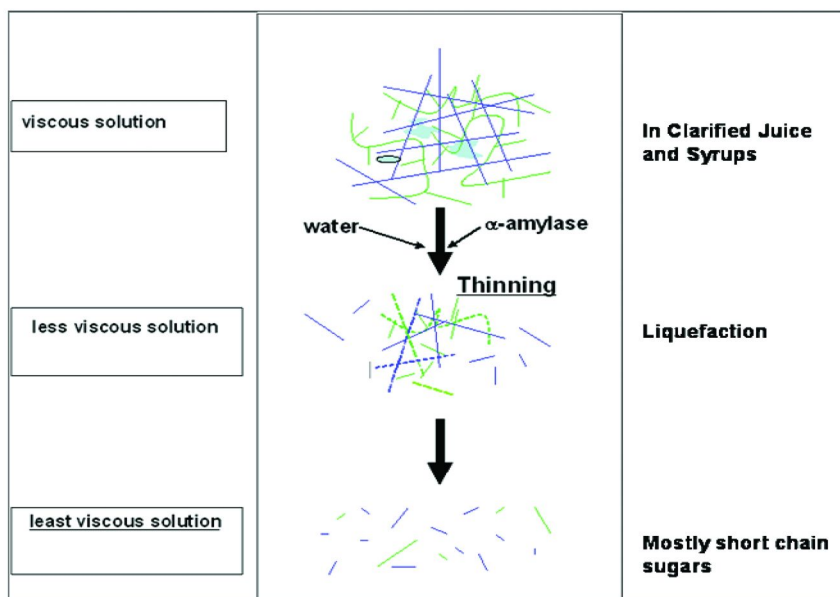
Figure 3. Action of α-amylase on a gelatinized, viscous solution of starch at the sugarcane factory (2).

Two U.S. refineries sold final molasses that contained residual α-amylase activity to barbeque sauce manufacturers, which caused barbeque sauce to detrimentally "liquefy" (*1*, *2*). To avoid this, high-volume customers of refineries have requested that α-amylases *not* be applied at the refinery. Concomitantly, refineries in Louisiana have requested factories not to apply HT-stable α-amylases.

Another added complication in the application of α-amylases in sugarcane factories is the existence of a wide variation in the activities and activity per unit cost of *B. subtilis* α-amylases (*1*, *2*). This is compounded by there being no uniform or standard method to measure the α-amylase activity in the sugar industry or the existence of a regulatory body to issue or regulate standard activity methods and units for the commercial enzyme. The efficiency of α-amylase action to hydrolyze starch in syrups is related to the activity of the α-amylase used (*17*). Application of relatively high-activity α-amylase, as a working solution diluted 3-fold in water at the factory with the penultimate evaporator body, can improve contact between the starch and α-amylase and improve hydrolysis and is cost-effective (*2*, *4*). Eggleston *et al.* (*2*, *4*) recently also observed that it is more difficult to hydrolyze starch with α-amylase at the factory when starch levels are low (~1000 ppm/Brix). This is because of lower contact between the starch (substrate) and α-amylase (enzyme). This problem may be mitigated by increasing the dose of working solution of high- activity α-amylase added to the penultimate evaporator body (*2*, *4*).

Method of Determining Starch Content in Sugarcane Juice

Stalk Sampling and Juice Extraction

The procedures described here were used to determine relative starch content among a set of sugarcane genotypes. At crop maturity, five stalks were randomly collected from each genotype in a field plot or pot culture. The stalks were cut at the base using a cane knife. The cane was topped at the growing point region but the leaves were not intentionally removed before the stalks were bundled together and labeled. The bundles were then either shredded and the juice extracted by a hydraulic press or roller mill. Juice Brix and other quality variables were immediately measured. Juice (12 mL) was pipetted into test-tubes (15 mL) and heated on a dry block heater for 10 min at 90 °C, to denature the natural amylase enzyme in the juice and stop any further starch degradation The juice was cooled on ice and stored in a -80 °C freezer until starch analysis.

Starch Analysis Protocol

Starch in juice was analyzed using the Sugar Processing Research Institute (SPRI) method (*18*) used in some factories as modified by Eggleston *et al.* (*1*). The principles, reagents and procedures used for the analysis are briefly described below.

Principles

Starch is more easily measured when it has been gelatinized (solubilized). Therefore, to ensure the granules in juice were fully gelatinized sufficient boiling time is required. The gelatinized starch is then reacted with iodine to form a blue/purple starch-iodine complex. The absorbance of the reacted starch is then read at 600 nm on a spectrophotometer. The µg of starch in the sugar product is then determined from a starch calibration curve.

Procedure for Starch Analysis in Sugarcane Juice

Sugarcane juice (3 mL) was transferred to three test tubes (labeled A, B, and C) and covered with aluminum foil. The test tubes were placed in a boiling water bath for 10 min. After boiling, the juice was allowed to cool on ice. The following chemicals were pipetted into each of the sample test tubes in the following order: 2N acetic acid (1.2 mL), 10 % KI (0.25 mL), and KIO_3 (2.5 mL). A blank tube was prepared where the juice (3 mL) was replaced with distilled water. The contents of the test-tubes were inverted three times and centrifuged to settle the solid material. The absorbance of the supernatant was measured at 600 nm on a spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan). The final absorbance was calculated as the sample absorbance minus the blank absorbance. The starch content estimated in µg was determined directly from the calibration curve.

Starch Content Among Wild and Cultivated Germplasm

In recent years, there has been renewed interest in wild *Saccharum* species germplasm across sugarcane breeding programs. Modern sugarcane varieties were derived from inter-specific hybridization particularly between two major *Saccharum* species, namely *S. officinarum* and *S. spontaneum*, in the early 1900s (*19*). *S. officinarum* is believed to be the primary source of genes for sucrose accumulation whereas *S. spontaneum* contributed genes for general adaptability (*20*). Unfortunately, the *S. spontaneum* genome probably contributed unfavorable attributes, for example juice quality, that were not fully removed during the nobilization process. Very few *Saccharum* species were used in the breeding of modern commercial sugarcane varieties (*21*), thereby resulting in a narrow genetic base. This has led to a plateau effect with respect to the improvement of certain traits such as sucrose content (*22*). Recently, there has been renewed effort to widen the genetic base of cultivated sugarcane by using wild *Saccharum* species in germplasm introgression programs.

Efforts to broaden the genetic base, while introducing novel genes into cultivated sugarcane varieties, have continued to place priority on *S. spontaneum*. In Louisiana, resistance to mosaic virus was successfully transferred to BC_4 progenies in cultivar x *S. spontaneum* crosses culminating in the release of LCP85-384 (*23*). Despite this success, only a limited number of new *S. spontaneum* clones are represented in the genetic background of Louisiana varieties. For example, all current commercially recommended varieties in Louisiana, LCP85-384, HoCP85-845, L97-128, and HoCP96-540 share the same single *S. spontaneum clone*, US56-15-8, in their pedigree. Although the issue of genetic diversity is being addressed, incentives that could encourage more diverse use of the *S. spontaneum* germplasm available in the collection are warranted.

In Louisiana, starch content is not currently considered when deciding which *S. spontaneum* clones to use for germplasm enhancement, whereas F_1 (commercial x *S. spontaneum*) clones are severely penalized for low total recoverable sugar. Starch content in the *S. spontaneum* parent may inadvertently influence total recoverable sugar in the F_1 and subsequent generations and may be responsible for the slow progress in improving sucrose content during germplasm enhancement. Knowledge about the starch content of *S. spontaneum* might be of interest to breeders seeking to use this germplasm. Clones with low starch content, when used as parents, may minimize the unfavorable juice quality of *S. spontaneum*. Characterization of clones in the germplasm collection could serve this purpose.

Variation Among Wild *Saccharum* Species for Starch Content

Among the relatives of cultivated sugarcane, starch was originally thought to occur only in *Saccharum* species other than *S. officinarum* (*24*). However appreciable levels of starch have since been reported in *Erianthus, S. barberi, S. sinense* (*25*). Recent studies have shown that the little or no starch recorded in *S. officinarum* was because of the less sensitive analytical methods used (*17*). In our studies, starch was analyzed using the SPRI method (*17*) that was modified by Eggleston *et al.* (*1*). This method detected very low (134 ppm/Brix) levels

of starch in sugarcane juice. Simple correlation coefficients among pairs of sub-samples were highly significant (r>0.90; *P*<0.001) indicating that the starch analysis method was reliable and can be used for screening large populations (*26*).

Variation among *Saccharum* species for starch content has been assessed in several experiments using germplasm originating from three USDA-ARS centers: Houma (Louisiana), Canal Point (Florida), and the Miami (Florida) world collection of sugarcane germplasm (Table II). A clear picture emerged from these studies with regards to the relative differences in starch content among the *Saccharum* species. Significant differences (*P*=0.01) in starch content were found between species and among clones within species (data not shown), suggesting a wide variation in starch accumulation. Starch content was generally lowest for *S. officinarum*, followed by *S. robustum, S. barberi*, and *S. sinense*, whereas *S. spontaneum* had the highest mean starch content (Table II).

From these results, the *Saccharum* and allied species can be grouped into three categories based on their starch content: high starch (*S. bengalense, Erianthus* and *S. spontaneum*), medium starch (*S. barberi, S. sinense* and *S. robustum*) and low starch (*S. officinarum* and *Miscanthus*) species. Generally, the cultivated *Saccharum* species produced less starch than their wild relatives, supporting observations in Figure 4, which suggests that accumulating lower levels of starch is advantageous for sucrose production in the *Saccharum* species. These results agree with Dutt and Narasimhan (*25*), who tested starch accumulation in 215 wild species and cultivars of sugarcane and found that *S. officinarum* and *S. robustum* (cultivated species) had, at most, traces of starch, whereas *S. spontaneum, S. barberi*, and *S. sinense* accumulated much greater amounts of starch.

In a study aimed at identifying *S. spontaneum* clones with low starch content, 52 *S. spontaneum* and one *S. officinarum* (control) clones were evaluated for starch (*26*). The *S. officinarum* control produced significantly ($P \leq 0.01$) less starch than the *S. spontaneum* clones (Table III). Starch content among the *S. spontaneum* clones varied widely and non-discretely, from 869 to 7805 ppm/Brix, with a few *S. spontaneum* clones producing less starch than the *S. officinarum* control (Figure 5).

These results demonstrated the potential of selecting sugarcane germplasm with low starch for use in germplasm enhancement as a method to minimize the negative effect of starch during introgression. However, the extent to which starch content in these germplasm influences starch among the progeny, and presumably total recoverable sugar (TRS) during introgression, remains to be elucidated. TRS generally decreases with increasing starch levels (Figure 4). Also, when using *S. spontaneum* germplasm for introgression, backcrossing to a high-sucrose, low-starch parent (in this case a variety) indirectly reduces the overall starch levels among the resulting progeny, from a high selection pressure put on sucrose levels (Table IV). Overall, the results highlight the importance of screening for low-starch content among sugarcane germplasm and further suggest that using germplasm low in starch could lead to lower starch content and most likely higher TRS among the progeny. Most importantly, fewer resources would be expended to achieve

Table II. Starch (mean, standard deviation, and % content of *Saccharum* and related species compared to the starch content value for *S. officinarum*) within the experiment. The genotypes evaluated were sampled from sugarcane germplasm collections located at the USDA-ARS in Houma, Louisiana and Canal Point, Florida and from the world collection of sugarcane germplasm at Miami, Florida

Source	Saccharum Species	No. of clones	Starch (ppm/Brix)		
			Mean	Std Dev	% S. officinarum
Houma	S. barberi	13	1913	243	131
	S. bengalense	1	2581	53	176
	Erianthus	1	2454	12	168
	Miscanthus	1	1537	332	105
	S. officinarum	9	1464	270	100
	S. robustum	11	1748	423	119
	S. sinense	8	1929	843	132
	S. spontaneum	5	2349	846	160
Houma	S. hybrids	6	789	225	94
	S. officinarum	4	841	125	100
	S. robustum	3	2064	583	245
	S. spontaneum	50	2231	999	265
Canal Point	S. barberi	7	477	290	381
	S. hybrids	14	320	244	256
	S. officinarum	1	125	9	100
	S. sinense	36	380	407	304
	S. spontaneum	4	737	340	590
Miami	S. barberi	4	572	301	96
	S. edule	1	1019	28	172
	S. officinarum	15	593	275	100
	S. robustum	9	661	323	111
	S. sinense	6	620	219	104

low levels of starch in varieties since fewer generations of backcrossing would be required.

Variation Among Varieties for Starch Content

High levels of starch in sugarcane varieties have presented major difficulties during juice processing. Noteworthy examples were reported in South Africa (Natal Uba and NCo310) and Australia (CP29-116 and NCo310) (*27*). The only active research in addressing the sugarcane starch problem has been at the factory despite the acknowledgement by several authors (*7*, *9*, *10*, *28*, *29*) that variety effects were important in determining starch content. This is because problems associated with starch in sugarcane juice have been viewed by most breeders as one that can be alleviated more economically by adding α-amylase during processing. This view has stemmed from the need to minimize the number of selection criteria in order to maximize genetic gain (*30*). While adding α-amylase
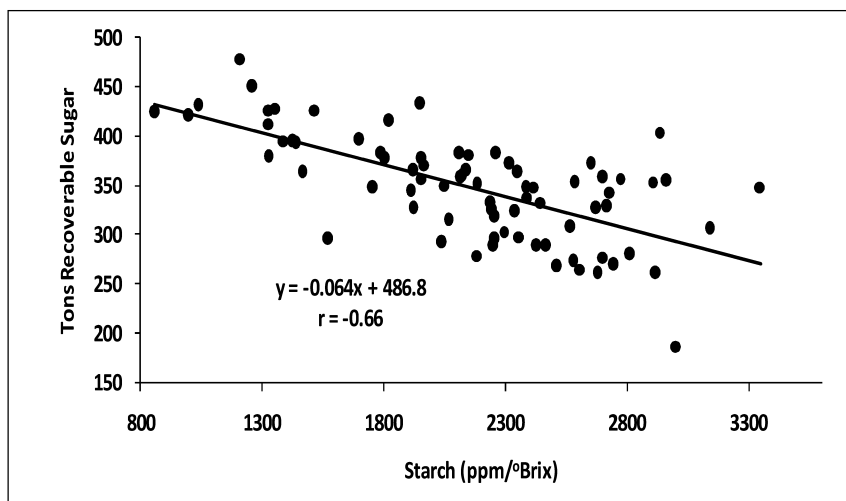
Figure 4. Tons of recoverable sugar plotted against starch content (26).

Table III. Starch content (ppm/Brix) among *S. spontaneum* clones compared to *S. officinarum* control

Species	Number of clones	Starch (ppm/Brix)	Standard Deviation	% of S. officinarum
S. officinarum		2144	86	100
S. spontaneum	52	3756	1504	175

has proved to be an economical control method in countries such as Australia, it may not be feasible or entirely efficient and economical in other countries. In Thailand, Muangmontri *et al.* (*10*) recently surveyed starch content among sugarcane varieties and recommended that low starch varieties should be used to alleviate problems associated with starch. In Louisiana, only one factory has a large enough incubation tank to allow the natural amylases to work (*1*). Also, in sub-tropical environments like Louisiana, the 9-month growing period imposed by freezing temperatures has meant that more immature sugarcane is processed than in tropical environments. Starch levels are generally higher in immature cane.

Genetic and Environmental Effects on Starch Accumulation

Starch accumulation appears to be under genetic control, although it is also highly affected by the environment. Depending on the precision of the experiment and on the type of genetic material being evaluated, broad-sense heritability estimated (degree of genetic determination) in our studies have ranged from 75 to 80% (Table V). This range supports the feasibility of selecting clones low in starch, either from among varieties or inter-specific hybrids.
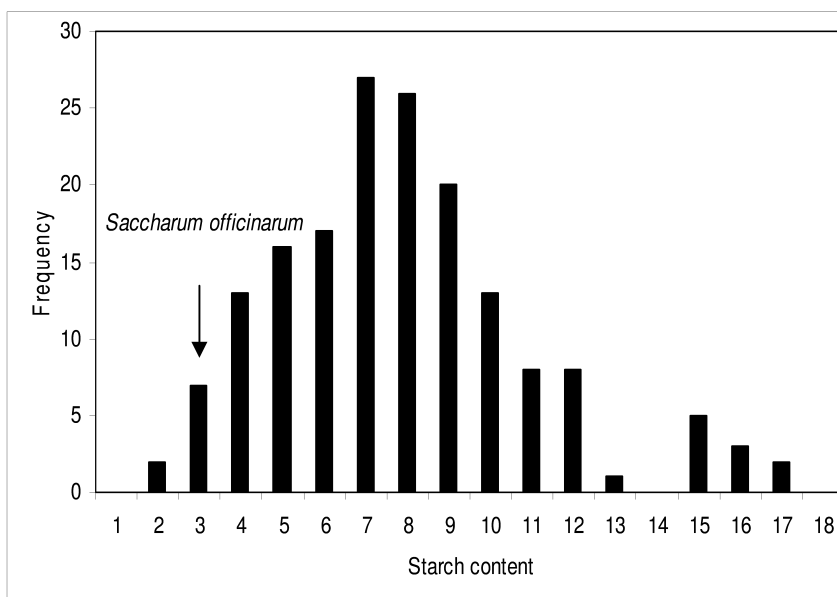
*Figure 5. Frequency distribution of starch content (ppm/Brix) among 52
Saccharum spontaneum clones compared to an S. officinarum control. The
corresponding values for starch content were divided into 18 classes of
500ppm/Brix (26).*

**Table IV. Mean starch content (ppm/Brix) among varieties, F_1 and BC_1
clones (*26*)**

Entry	Number of clones	Starch (ppm/Brix)	Standard error
Varieties	6	1264	75
BC1	29	1944	38
F1	41	2436	34

Clones selected for their relative differences in starch content are also
expected to maintain the difference over time, providing further support of
genetic control for this trait. Studies reported by Godshall *et al.* (*28*) showed
that differences in starch among varieties were more consistent than seasonal
differences. Recent studies have corroborated this result (Figures 6, 7 and 8;
Table V). Generally, correlation coefficients of $r > 0.70$ were found between
replications, locations or crop years in these studies. In one study, 76 clones
including 6 varieties and 70 unselected clones of F_1 and BC_1 origin, derived
from crosses between varieties and *S. spontaneum* (see Tables IV and V), were
evaluated in 3 replicates over 2 crop years. The starch content for each clone was
averaged over replicates and crop years and the lowest and highest 10% of clones
were plotted for each replicate within a crop year (Figure 6). Starch content varied
across replicates within crop years, but amid all these environmental variation,

Table V. Variance components and broad sense heritability estimates for starch content in different sugarcane populations

Population parameters	Population		
	SESpop	Larta	Advanced clones
Population and trial description	120 clones derived from a *S. officinarum x S. spontaneum* cross evaluated across three replications at one location.	70 clones of F_1 and BC_1 origin derived from crosses between cultivars and *S. spontaneum* evaluated across three replications over two years at a single location.	19 varieties evaluated across two replications at three locations with each location harvested on a different date
σ_g^2	7754	127786	9422.26
σ_{yv}^2	N.A.	31372	N.A.
σ_{lv}^2	N.A.		8441.73
σ_e^2	7629.72	85805	225.23
Formula	$\sigma_g^2/(\sigma_g^2+\sigma_{e/r}^2)$	$\sigma_g^2/(\sigma_g^2+\sigma_{yv/y}^2+\sigma_{e/ry}^2)$	$\sigma_g^2/(\sigma_g^2+\sigma_{lv/l}^2+\sigma_{e/rl}^2)$
Heritability	75.3	80.9	76.8

clones in the lowest 10% group produced consistently less starch than those in the highest 10%.

Environmental Temperature Effects on Sugarcane Starch

Temperature is an environmental factor most likely to influence starch accumulation in sugarcane. In a separate study, the mean starch value from a population of 300 clones derived from selfing the variety LCP 85-384 (*23*) was averaged across two replications and ranked from lowest to highest. The mean 10% of clones with the lowest and highest starch content were plotted for each replication (Figure 7). Replication 1 was sampled on November 13, 2006 (before a freeze) and replication 2 was sampled on December 27, 2006, after 5 days of freezing temperature (Table VI). The clones accumulated significantly ($P<0.0001$) more starch before than after the freeze (Replication 1) (Table VI). In comparison with the high group, the low-starch clones accumulated lower levels of starch before and after the freeze and showed very little decrease in starch content after the freeze. In contrast, the high-starch clones showed higher levels of starch before the freeze, and after the freeze experienced a larger decrease in starch levels (Figure 7).

In another freeze study, 19 advanced clones from a commercial breeding program were grown in two replicates across three locations (Tables V and VI). Starch content for each of the 19 clones was averaged across replications and locations and the mean of the lowest and highest 5 clones was plotted against locations (Figure 8). The low starch clones consistently accumulated relatively
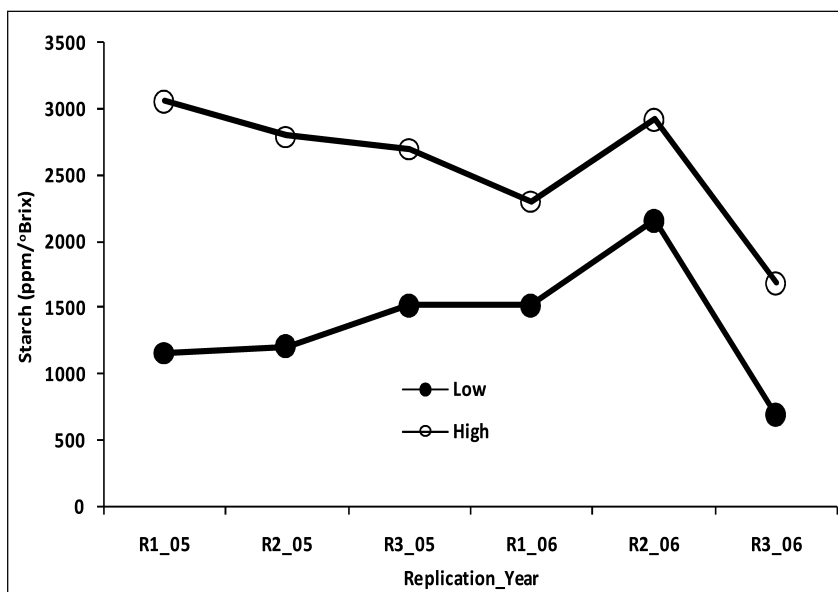
Figure 6. Mean starch content of the highest and lowest 10% of clones in replications 1, 2, 3 for crops sampled in 2005 and 2006. The mean starch content for each of the 76 clones was derived by averaging starch content over three replications and two crop years.
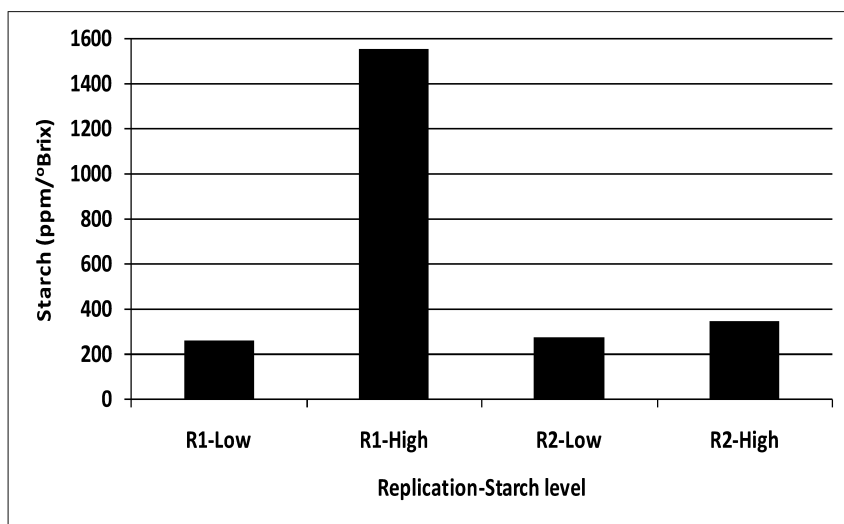
Figure 7. Starch content of the high- (10%) and low- (10%) starch clones sampled before a freeze in replication 1 (R1) and after the freeze in Replication 2 (R2) at Houma, Louisiana. The mean starch content for each of the 300 clones was derived by averaging starch content over the two replications.

lower levels of starch across the locations. The locations/harvest dates with lower mean freeze temperatures were associated with lower starch content compared to locations/ harvest dates with higher mean freeze temperatures (Table VI). Although in each of the two aforementioned experiments, replications or locations were confounded with sampling dates, when the results from both experiments are considered together, they suggest that low-starch clones were relatively more stable and less susceptible to environmental fluctuations than high-starch clones. High-starch clones would accumulate high levels of starch when conditions are favorable for starch accumulation and the starch levels could decrease sharply when conditions change, particularly after experiencing freezing temperatures. Thus, from a breeding standpoint, varieties developed or selected for low levels of starch are likely to produce relatively low and stable starch content over a wide range of conditions.

The decrease in starch content after a freeze appeared to be proportional to the severity of the freeze (Table VI and Figure 8). A similar dynamic where stress causes starch levels to drop has been reported in other crops. In alfalfa, starch levels in the roots of plants of a cloned genotype were drastically reduced following defoliation compared to their non-defoliated counterparts (*31*). In the green algae *Chlorella vulgaris*, analysis of products formed in cells during photosynthesis in air containing 3000 ppm $^{14}CO_2$ at various temperatures, revealed that the level of ^{14}C-starch was maximum around 20–24 °C and decreased with further rise in temperature until 40 °C, while ^{14}C-sucrose greatly increased at temperatures above ~28 °C (*32*). Elevating the temperature from 20 to 38 °C during photosynthetic $^{14}CO_2$ fixation resulted in a remarkable decrease in ^{14}C in starch and a concomitant increase in ^{14}C in sucrose and this conversion of starch to sucrose when shifting the temperature from 20 to 38 °C proceeded even in the dark.

Artificial Ripener Effects on Starch

In Louisiana, sugarcane is typically harvested and processed from late September to the beginning of January (Autumn to Winter). Sugarcane is immature in early season and sucrose levels are usually low and, generally, increase as the season progresses. As a consequence, chemical ripeners, such as glyphosate, are used on approximately 75% of the total area planted to sugarcane for increasing sucrose content in the stalk and, consequently, yield of sugar per ton of cane and per acre (*8*). The effect of glyphosate ripener (Polado™) on starch accumulation was investigated in four varieties namely, L 97-128, LCP 85-384, HoCP 96-540 and HoCP 95-988. On average, starch content decreased by 17% in Polado™ treated plots compared with untreated plots (Table VII). There were significant differences in starch among the varieties in response to ripener treatment. Some varieties such as LCP 85-384 and HoCP95-988 appeared not to respond, whereas other varieties (L 97-128 and HoCP 96-540) were highly responsive to ripener treatment. Variety L 97-128 produced the greatest reduction in starch (37%) after treatment with Polado™, while variety LC85-384 showed little change in starch content after ripener treatment. Similar results on

Table VI. Date of sampling, mean starch content on the date of sampling, number of frozen days before sampling, and mean temperature of frozen days for two experiments sampled in November and December in Louisiana

Source of Data	Date of Sampling	Mean Starch ppm/Brix	Number of Frozen Days Before Sampling	Mean Temperature of Frozen Days (°C)
Selfed progeny of LCP85-384 planted to 2 replicates and 1 location (n = 300)				
Replication 1	Nov. 13, 2006	748	0	-
Replication 2	Dec. 27, 2006	299	5	-2.7
Advanced clones from the commercial breeding program planted to 2 replicates and three locations (n = 19)				
Location NN	Dec. 5, 2006	356	7	-2.9
Location NL	Dec. 6, 2006	215	8	-3.2
Location NA	Dec. 14, 2006	106	11	-3.9
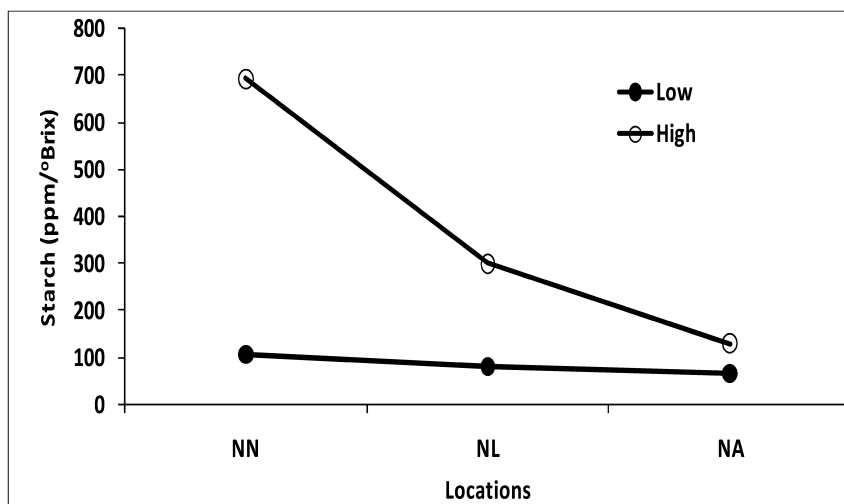
Figure 8. Starch content of the high- and low-starch clones sampled from three locations NN, NL and N. The mean starch content for each of 19 clones in the study was derived by averaging starch content over the two replications and three locations.

differential response of varieties to ripener treatment were reported by Eggleston et al. (*8*), who evaluated glyphosate effects on LCP 85-384 and HoCP 96-540.

Therefore, the dynamics between starch and sucrose in sugarcane following temperature (freeze, heat) and non-temperature (application of chemical ripeners) related stresses may follow a similar pattern and warrants further investigation.

The results may have implications in the way starch is managed during processing when harvesting is preceded by exposure of the crop to stress.

Molecular Markers for Starch Content in Sugarcane

Molecular markers provide a potentially quick and efficient method of screening genotypes for use as parents or selecting seedlings harboring a gene (trait) of interest during breeding and selection. Studies have been undertaken to identify potential markers associated with starch in sugarcane. Data for starch content and SSR markers was collected from 51 *S. spontaneum* clones grown in a replicated pot trial at the USDA-ARS, Sugarcane Research Laboratory, Houma, Louisiana. The mixed procedure of SAS was used to determine markers that were significantly associated with starch. The analysis accounted for structure in the population, which was mostly due to country of origin of the clones. Out of a total of 357 markers, 39 were significantly (P<0.05) associated with starch. Of the 39 markers, 18 were positively associated with starch content and 21 were negatively associated (unpublished data). On average, the presence of markers that were positively associated with starch resulted in an increase in starch content by 39%, while the absence of markers negatively associated with starch resulted in an increase in starch content by 57%. It would be prudent to select using markers positively and negatively associated with starch content, although it appears much more progress can be made by selecting markers negatively associated with the trait when breeding with clones from this population.

In another molecular marker study, starch data collected from 227 individuals over two harvesting seasons in a replicated field trial of progeny derived from selfing the variety LCP 85-384 was used to identify QTAs (Quantitative Trait Alleles) associated with starch content. The population was genotyped using AFLP, TRAP and SRAP markers. A total of 10 putative QTAs were associated with the starch trait. The QTAs explained 5.4 to 32.3% of the phenotypic variation with positive additive effects for nine of the ten QTAs.

A third study was undertaken using a bi-parental mapping population (SESpop; see Table V) of a *S. spontaneum* x *S. officinarum* (SES-147B x LA Striped) cross that was genotyped using AFLP, TRAP and SRAP markers (*33*). A total of 100 progeny were evaluated in a replicated field trial across two harvesting seasons. Out of the 38 significant QTAs derived from LA striped, 21 were negatively associated and 17 were positively associated with starch content. From the 8 significant QTAs derived from SES-147B, two were negatively associated while 6 markers were positively associated with starch. The result indicated that *S. officinarum* (known to produce low starch and high sucrose content) and *S. spontaneum* (known to produce high starch and low sucrose) can both contribute positively as well as negatively towards starch accumulation in cultivated sugarcane, highlighting the important role that molecular markers can play during selection and introgression.

Conclusions and Future Outlook

Starch is a sugarcane impurity that adversely affects the quantity and quality of sugar products during processing. Starch has increased in sugarcane delivered to factories in recent years because of increased production of combine and green (unburnt) harvested sugarcane and the increasing, but necessary, use of wild germplasm to improve cultivated sugarcane. The behavior of starch granules on hydration and heating influences directly its effects on sugar processing. α-Amylase used to hydrolyze starch in the factory is expensive and not always efficient. Deploying low-starch varieties would be a more long-term, sustainable solution.

Motivated by the need to minimize the number of selection criteria in sugarcane breeding programs, problems associated with starch in sugarcane juice have traditionally been alleviated through the application of α-amylase during processing. This may have worked in some countries, but it is not universally feasible or entirely efficient and economical in subtropical areas, where immature sugarcane is harvested before the onset of freezing temperatures. Furthermore, the widespread adoption of billeted and green (unburnt) sugarcane harvesting methods has exacerbated the problem because starch is higher in green leaves and tops.

It may be possible to bio-engineer an intermediate-temperature α-amylase to be more active in high Brix syrups. However, bio-engineering of enzymes is extremely expensive, and such an investment by a large enzyme company is not foreseen as the sugar market is viewed as being too small. Moreover, water is a necessary reactant in the enzymatic hydrolysis reaction and would, therefore, limit any progress of bio-engineering the enzyme to be high Brix tolerant. Alternative methods such as selection and crossing among varieties low in starch content most likely will alleviate the problem and provide a more economical and long- term solution without adding an extra trait at the selection stages.

Starch content is known to be high among some *Saccharum* germplasm (e.g., *Saccharum spontaneum*) frequently used to improve cultivated sugarcane in Louisiana. However, little is known about how the starch content may affect selection progress during introgression because in the past no effort was made to screen for starch content in the germplasm or their progeny. Screening of germplasm for starch content and subsequent selection of low-starch clones for introgression may now offer an opportunity to lower starch concentration during introgression. Moderate to high broad-sense heritability estimates for starch content indicate the potential to select for low starch genotypes among varieties or introgression lines.

Starch analysis of the *Saccharum* species collection showed significant variation among the species and clones within the species. The magnitude of the variation indicated strong genetic control. These factors offer the opportunity of using "among species" and "within species" variation to develop populations and parents for developing low-starch parents. Crossing low-starch parents produces progenies with low starch while crossing high- starch parents produces progenies with high starch. Therefore, introgression with selected low-starch parents can result in progeny with low starch for variety development. Use of germplasm

Table VII. Glyphosate ripener effect (treated vs untreated) on starch accumulation in four sugarcane varieties

Variety	Starch ppm/Brix			
	Untreated	Treated	Treated % Untreated	Mean
L97-128	372	234	63	303a*
LCP85-384	384	386	100	385ab
HoCP96-540	770	577	75	673c
HoCP95-988	535	522	98	528bc
Mean	515	430	83	

* The same lower case letter represents no statistical difference at the 5% probability level

with low starch should result in fewer backcrossing cycles to reduce starch and increase sucrose content in clones.

Low-starch clones consistently produced lower and more stable starch across replications, years and locations compared to high-starch clones. A reduction in starch content was associated with the severity of freeze temperatures before sampling. Low starch clones produced low and more stable starch levels as temperatures fluctuated. Generally starch content in sugarcane decreased in response to treatment with chemical ripeners, but a wide range of responses was recorded for individual varieties ranging from non-responsive to very responsive. Knowledge of varietal response to stimuli such as temperature and ripener treatment could be useful in managing harvest scheduling during the season where high starch varieties are harvested later in the season.

To avoid increasing selection traits for breeding programs, future research to lower starch in varieties should focus on selecting parents with low starch in the introgression and commercial crossing programs. Molecular markers associated with the starch trait could be used to expedite this process through marker assisted selection. Low-starch clones are stable and consistently produce low starch, which warrants further investigation into the potential of scheduling harvesting of varieties based on their starch content. It is likely to be more beneficial to harvest low-starch varieties early and high-starch varieties later, when their starch content would have declined due to maturity and decreasing temperatures. This approach may have the overall effect of lowering the amount of starch delivered to the factory and can potentially lower the costs associated with the recurrent use of α-amylase in the factory. Lowering the amount of starch delivered to the factory should at least lower the concentration or quantity of α-amylase required for processing the cane.

Acknowledgments

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Chapter 15

Value-Added Products for a Sustainable Sugar Industry

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Sugar production, from both beet and cane, is energy and water-intensive. In today's social and political environment, industries strive to be environmentally sustainable and "green," while maintaining profitability. The sugar industry has three avenues for achieving these goals: (i) Improve the over-all efficiency of the process by reducing power and water usage; (ii) expand its markets with a range of innovative edible products; and (iii) enter into the 21st century's bio-based economy by developing products to replace petrochemical-derived products. The industry has done well with the first two of these, but has found barriers to exploiting the latter possibility. Nevertheless, many possibilities exist for utilizing the co-products and waste-products of sugar manufacture, as well as the sugar itself, to produce new and useful products and chemicals that reduce the world's dependence on petrochemical feedstocks.

Introduction

Sustainability can be defined in many ways. One of the most succinct definitions is a recent one from Wikipedia (*1*), "Sustainability is the capacity to endure." In many contexts, sustainability requires the replacement of nonrenewable petrochemical resources with renewables. At the 2005 World Summit it was noted that sustainability requires the reconciliation of environmental, social, and economic demands - the "three pillars" of sustainability (*2*). Sustainability issues include recycling (no waste production), water use, protection of the environment, land and air, and societal issues, such as fair wages for workers. Sustainability is care for the earth and its inhabitants, but it must also

include sound business practices, which allow the company to produce a profit and continue in business.

The Biorefinery Concept

Much has been written, and continues to be written, about the biorefinery concept. In the biorefinery scenario, feedstock, which can be any source of suitable biomass, enters the factory, whereupon conversion processes take place, which can include extraction, fermentation, hydrolysis, chemical conversion, etc., to produce a variety of products such as food, feed, fiber, power and useful chemicals.

The sugarcane factory is a prototypical biorefinery, in which sugarcane is brought into the factory and a variety of products, including raw sugar, molasses, ethanol, bagasse and electricity are produced. Some factories may also produce a variety of other products, such as citric acid or confectionery (Colombia) or cogeneration of power as is done in factories in India and Florida; but for the most part, the products mentioned above are the main output of a conventional sugar factory operation. Thus, there is a great deal of potential for the sugar factory to expand as a biorefinery, given the recent advances in sucrochemistry and other areas.

Raw Materials for the Biorefinery

The concept of utilizing the products from a sugarcane factory for value addition is not new. There were several early proponents of the industrial utilization of the by-products of the cane sugar industry (*3–5*). A comprehensive review was published in 1997 by Rao (*6*).

Sugar production from sugarcane proceeds in basically two stages. In the first stage, sugarcane is harvested from fields and brought to a nearby factory, where it is processed into raw sugar, the main commercial product. If there is an attached distillery, ethanol will be the other major commercial product. The second stage of cane sugar production is refining the raw sugar into white (refined) sugar. In the most common situation, raw sugar is transported long distances, often overseas, to refineries that are close to urban areas.

Considering a sugar factory with an attached distillery as the biorefinery unit, the main "raw" materials for further processing into sustainable value-added products are bagasse, fly ash, vinasse, molasses, filter cake, and sugar/sucrose.

Bagasse

Bagasse is the fibrous residue remaining after the cane stalk has been milled to remove the sugar-laden juice. The amount of fiber in commercial canes ranges from 12 to 14% of the stalk weight. Cane varieties that have been bred to produce a high amount of fiber, called energy canes, contain up to 20 to 30% fiber and are in consideration for cellulosic ethanol production.

Figure 1. Bagasse paper production in a sugarcane mill in China

The most important current use of bagasse is to produce electricity for the factory. Firing the bagasse in boilers to produce power allows the factories to be self-sustaining. Excess electricity can be sold to the grid.

Bagasse has been made into a variety of value-added fibrous and paper products, including paper, newsprint, fiber board (*7*), particle board, briquettes, and erosion control mats. Its potential use in nonwoven fabrics is felt to be underrated (*8*). Mixed with molasses, it is a valuable animal food. In China, there is a program to produce high quality paper from bagasse (*9*). Figure 1 shows paper production in a mill in Nanning, China.

A recently reported new use for bagasse is the production of biodegradable packages ("bagasse boxes") in Thailand that are being used as an alternative to plastic packaging. The biodegradable packaging market is predicted to grow by about 20% a year (*10*). A search of the Internet showed a lively market in bagasse paper plates, takeaway boxes, and lunch boxes that are waterproof and heat resistant, as well as biodegradable.

Fly Ash

Bagasse fly ash is the residue remaining after bagasse has been burned in the boiler. It is considered a waste product with only a few uses, such as a replacement for bagacillo to improve mud filtration and as an amendment for potting soil. Figure 2 shows a pile of fly ash outside a Louisiana sugar factory.

Recent research has shown that it has potential as an adsorbent to trap organic and inorganic materials, including pesticides, dyes and heavy metals (*11–14*). Work at the Sugar Processing Research Institute, Inc., showed that it effectively removed textile dye waste, benzalkonium chloride (used as a standard for quaternary amines) and heavy metals, in particular chromium, mercury and

lead, with less adsorption of arsenic and cadmium. The removal of heavy metals is shown in Figure 3 (*13*).

A novel use for fly ash was recently reported by Balakrishan *et al.* (*15*), in which ceramic membranes were produced from fly ash and tested for the clarification of sugarcane juice. The membranes were reported to remove up to 88% of the turbidity and 35% of the color in cane juice. The authors did not test the removal of other components, such as heavy metals and pesticides, but that may be another promising use for this product

Filter Cake

The precipitated impurities contained in the cane juice, after removal by filtration, forms a fibrous filter cake, also called filter mud, which is high in phosphate, calcium, and magnesium, making it a good source of fertilizer to add back to the cane field. The volume of filter cake produced is about 3-4% of the weight of the cane (*3*).

Filter mud also contains a high concentration (5-14%) of crude wax, fat and plant sterols, which has been of interest for many years because of its high quality (*16*) and businesses have, from time to time, been created to extract and market cane wax. At one time, a Cuban company was selling a mixture of cane waxes and sterols as a dietary supplement. Work by the Sugar Processing Research Institute identified the presence of 2.9% hemicellulose in filter cake, along with a fraction enriched in p-hydroxycinnamic acid, ferulic acid, palmitic, linoleic, oleic and stearic acids, and a number of waxy alcohols (hexacosanol, ocatacosanol, C29-OH, C31-OH, and C32-OH) and plant sterols (stigmasterol, beta-sitosterol, and campesterol) (*17*).

Figure 2. Pile of bagasse fly ash outside of a Louisiana sugar factory.

Heavy Metal Removal by 5.0 g Bagasse Fly Ash
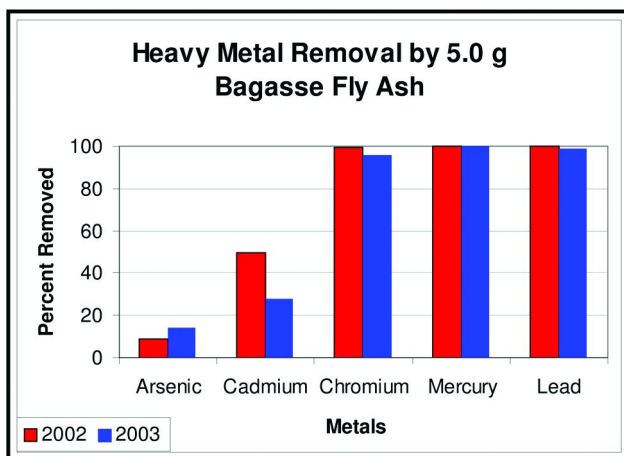
Figure 3. Removal of 100 ppm each of heavy metals by bagasse fly ash (13)

Vinasse

The liquid remaining after the distillation of bio-ethanol is known as vinasse or distillery slops. Ten liters of vinasse is produced per liter of ethanol, which represents a huge disposal challenge. The vinasse, about 12% solids, is high in ash and organic matter with a high COD and BOD (*18*). A recent article reviewed the composition, uses and disposal of vinasse (*19*). Table I shows the composition of a sample of vinasse. Although some disposal is accomplished by spraying it on fields, as a fertilizer, overuse can result in damage to soils, and controls for its disposal are becoming much more stringent. Given the huge volumes of vinasse that are produced at a distillery, some thought could be given to recovering some of the valuable compounds in it.

Vinasse can be used as a composted bio-fertilizer (*20*), a fuel source (either converted to biogas by anaerobic digestion or burned in concentrated form), in plant disease control for apple scab (*21*), and as the sole carbon source for fodder yeast production (*22*). Many volatile flavor compounds and other organic compounds have been identified in vinasse, shown in Table II (*19*). This list, as with Table I, shows the potential for further exploitation of vinasse as a source of valuable compounds.

Vinasse can be partially converted to methane by anaerobic digestion (*23*). It can also be concentrated, mixed with bagasse and burned in boilers to produce electricity. Systems that utilize all the vinasse in this manner are known as zero liquid discharge systems and are highly desirable (*23*).

An important advance in effluent control from the distillery is the development of yeast with a higher alcohol tolerance. The volume of vinasse can be significantly reduced when the amount of alcohol tolerated by yeast during fermentation is

increased. If yeast can tolerate up to 18% alcohol, from the usual 10%, the amount of vinasse produced is cut nearly in half (*24*).

The Carbohydrate Economy – Platform Chemicals

In 1998, Yalpani stated that "Carbohydrate technology is the sleeping giant of the next century" (*25*). It is now the "next century" and technologies for transforming carbohydrates into useful chemicals to replace petroleum are developing rapidly.

In 2004, the U.S. Department of Energy (DOE) produced a report on potential candidate chemicals from sugars that should be emphasized for further development (*26*). These chemical candidates were referred to as "platform chemicals," small molecules that serve as building blocks for useful chemicals, polymers and products, and which are "economic drivers for the biorefinery." The platform chemicals in the DOE list that are derived from carbohydrates included the following (*26*):

Succinic acid
    Fumaric acid
    Malic acid
    2, 5-Furan dicarboxylic acid
    3-Hydroxy propionic acid
    Glucaric acid
    Itaconic acid
    Levulinic acid
    3-Hydroxybutyrolactone
    Glycerol
    Sorbitol
    Xylitol
    Arabinitol

Also important as platform chemicals in the developing carbohydrate economy. but not on the DOE's original list, are other sugar-derived molecules, including lactic acid, ethylene, ethylene glycol, 5-hydroxymethylfurfural (HMF), methyl ethyl ketone, 1,3-propanediol and 1,4-butanediol.

All of these chemicals can be made from sucrose by one or another synthetic route. In a recent article on the production of bio-based bulk chemicals, the authors concluded that "Bio-based bulk chemicals from industrial biotechnology offer clear savings in non-renewable energy use and green house gas emissions with current technology compared to conventional petrochemical production." The authors further stated, "Of all feedstocks, sugar cane is to be favored over lignocellulosics, which in turn is preferable to corn starch as a source of fermentable sugar to maximize savings" (*27*).

Table I. The Major Organic Compounds in a Colombian Vinasse. (Ash = 12.4% Of Dry Matter) (*19*)

Compound	% of dry matter
Polysaccharides	5.21
Colorant polymers >12,000 DA	2.31
Glycerol	4.17
Sorbitol	2.15
Myoinositol	0.56
Trehalose	0.47
Sucrose	0.32
Fructose + Glucose	2.00
Aconitic acid	2.71
Citric acid	1.24
Lactic acid	1.97
Quinic acid	1.09
Malic acid	0.35
2,4-Dihydroxy-pentanedioic acid	1.09
Butanediol	0.32

Commenting on a report from the World Wildlife Federation (*28*), the CEO of Novozymes, Steen Riisgaard, stated that "sugar is the new oil" and that he expected biotechnological transformation of cellulosic feedstock to give rise to a "sugar economy" that will replace our oil-dependent economy.

Availability of Sucrose

Before discussing the use of sucrose as a source of chemicals to replace petroleum, the ethical issue of food versus fuel must be addressed. It is expected that the main use of sugar (sucrose) will always be as a food source. However, sugar crops can be cultivated in most of the world – sugarcane in tropical and semi-tropical climates and sugar beets typically in temperate and colder climates, and excess stocks of sucrose over food demand could be considerable.

World sugar production for the 2009/10 marketing year was estimated at 153.3 million tons. Excess world sugar stocks are in the range of 25-26 million tons. This figure fluctuates about 5 million tons per year (*29*). Additionally, with sugar reform and other controls, the United States and Europe have excess capacity for sugarcane and/or sugar beet agriculture which cannot be used to produce sugar for food. Parkin reported that the beet area in the EU has been reduced by 500,000 ha (*30*). Brazil is also reported to have the capacity to grow much more sugarcane (*24*).

Table II. Compounds Found in Trace Quantities in Colombian Vinasse (*19*)

Acetic acid	Ethyl succinate	4-Methylcyclohexanol
Acetone	Formic acid	2-Methyl furfural
Alanine	Fumaric acid	2-Methylfuran
Aspartic acid	2-Furancarboxylic acid	Palmitic acid
Benzaldehyde	Furfuryl alcohol	2-Phenyl-ethanol
Benzoic acid	Glyceric acid	Phenylethyl alcohol
Butanoic acid, butyl ester	Glycolic acid	Phenyl lactic acid
2,3-Dihydrobenzofuran	1-Hydroxyacetone	Propylene glycol
2,3-Dihydro-3,5-di-OH-6-methyl-4H-pyran-4-one	p-Hydroxybenzoic acid	Pyroglutamic acid
2,5-Dimethylfuran	3-Hydroxy-2-butanone	Pyrrolyl ethanone
2,4-Dimethyl-4-OH-3-(2H)- furanone	p-Hydroxycinnamic acid	Resorcinol
2,6-Dimethoxyphenol	2-OH-furancarboxylic acid	Stearic acid
Dimethylsulfide	2-Hydroxyhexanoic acid	Succinic acid
Ethanol	Itaconic acid	Syringic acid
Ethyl palmitate	Methoxyphenylethanone	3,4,5-Trimethylpyrazole
2-Methylbutanal & 3-Methylbutanol	Vanillic acid	Xylitol

Sucrochemistry

Sucrochemistry is the branch of chemistry in which the sucrose molecule is chemically manipulated to make it into other chemical compounds. The sucrose molecule can be transformed into high value compounds by fermentation, enzymatic transformation, and chemical transformation. In fermentation, living cells are grown in vats under optimum conditions to make a product, which is then extracted from the medium. Enzymatic transformation is a subset of fermentation, in which the active enzyme has been extracted from the organism, purified, and put to use in ways that the lifespan activity of the enzyme can be extended, such as by immobilization onto an inert column. Enzymatic transformation allows the production of targeted products of high purity. Living cells are not involved in enzymatic transformations, making for a cleaner, more efficient and better controlled process.

A new process has recently been reported for converting sucrose into chemicals using aqueous catalytic technology, known variously as liquid phase reforming (*31*), catalytic reforming (*32*), aqueous phase reforming or BioForming® (patented process of Virent Technology) (*33*). The process applies catalytic petroleum processing technologies to convert biomass into a variety of liquid hydrocarbon fuels. Any biomass source of soluble sugars can be used,

but Virent Technologies is currently using processed cane sugar for pilot scale operations (*34*).

Many thousands of chemicals and chemical intermediates have been reported synthesized from sucrose. Some of these compounds have potential usefulness and others are mainly of research interest. Some have been successfully commercialized and some have future potential.

Throughout the second half of the Twentieth Century, interest in sucrose as a raw material for production of industrial chemicals waxed and waned, depending on the prevailing economic conditions and the difficulties and expense of using sucrose as an industrial raw material in practical terms (*35*, *36*). Interest picked up again in the 1990s and continues to increase because of the availability of better reaction pathways, the continuing rise in the cost of petrochemical resources as well as the dwindling supply, and a desire to produce chemicals in an environmentally benign manner from renewable biomass resources, as well as to achieve energy independence.

Feedstocks for the use of sucrose as a substrate for new products may include cane juice, molasses, raw sugar and refined sugar, as well as a variety of intermediate sucrose syrups. The required purity and form of the sugar needed would determine which feedstock/substrate to use in any given process.

Sucrose is a versatile compound that can be, and has been, transformed into many derivatives and products, including fine chemicals, pharmaceuticals, polymers, building and structural materials, fermentation substrate for chemical production, and fuel. However, there are limitations and constraints to using sucrose as an industrial raw material, which include:

(i)   The high reactivity of the sucrose molecule – its many hydroxyl functional groups make reactions difficult to control. There can be too many products from one reaction.

(ii) Competition from other low-cost agricultural biomass, such as corn or glucose

(iii) Availability of lower-cost alternatives from petrochemical feedstocks. Therefore, one wishes to find products for which sucrose is the preferred or only substrate or for which sucrose can compete successfully with other agricultural or petrochemical processes. There are also breakpoints in the price of petroleum products which can be favorable to using agricultural feedstocks instead of petroleum feedstocks

(iv) Sucrose hydroxyls are less reactive than water, so it is relatively difficult to make sucrose derivatives in aqueous medium, the preferred reaction medium. This limitation can be overcome to some extent by using catalytic conditions, such as an acid or alkaline environment, high temperature, high pressure and/or metal catalysts.

(v) The relative insolubility of sucrose in organic solvents limits reactions to only a few solvents. However, the development of sophisticated catalysts has helped to overcome much of this problem (*33*).

(vi) Yields are generally lower than theoretical because the oxygen-rich environment of the sucrose molecules tends to produce carbon dioxide

and water, with a subsequent loss in yield, often only to 50% of theoretical yield.

Sucrose Esters

One of the largest classes of currently produced sucrose compounds is sucrose esters, of which there are many. Sucrose esters have a wide range of food, cosmetic, pharmaceutical, and industrial applications because of their low toxicity, biocompatibility, and biodegradability. Sucrose esters have a range of water solubilities, from fully water soluble to fully oil soluble, giving them a lot of flexibility for different applications, from surfactants, emulsifiers, stabilizers, texturizers, detergents, paint additives, etc. Some important sucrose esters are mentioned here.

Sucrose Acetate Isobutyrate (SAIB)

The highest volume commercial sucrose ester is SAIB, used both in food and industry. More than 100,000 tons are produced annually, with a value ranging from about US \$4.50/lb for the industrial grade to US \$7-8.00/lb for food grade. Among its many uses are as a clouding and stabilizing agent in beverages, in automotive paints, nail polish and hair spray.

Sucrose Octaacetate

This is a multi-use ester used both as a food additive and as a pesticide. It is produced in good yield by reacting sucrose with acetic anhydride and sodium acetate. All of the sucrose hydroxyl groups are esterified with an acetate group, giving the molecule an extremely bitter taste, so it can be used as a bitter additive in foods and as a denaturant for alcohol. Other uses include adhesive for laminating glass, glossing agent for paper, and plasticizer. It was approved in 1999 by the U.S. Environmental Protection Agency (EPA) to control mites and soft-bodied insects on food and non-food crops, in media for growing mushrooms to protect from gnats, and to control Varroa mites on adult honey bees; it also discourages mice.

Sucrose Benzoate

This is a stable, odorless, glassy solid or white powder with stability to ultraviolet light, compatible with a broad range of resins, plasticizers and solvents. It is used as a denaturant in the paint, ink, resin, plastics and printing industry and is an important ingredient in nail polish. It may be used to particular advantage in UV coatings and inks. It imparts film hardness, gloss, and depth of gloss to coatings.

Sucrose Cocoate

This is a natural soap derived from sucrose and coconut oil which is used as a facial cleansing agent and emollient. Sucrose cocoate provides moisturizing properties to liquid soaps and is a favored ingredient in the cosmetic industry, having no known toxicity. It enhances the foaming characteristics of liquid soaps.

Olestra

This is a mixed sucrose polyester which functions as a liquid fat substitute, a product of Procter & Gamble, which can be used as a frying oil substitute with zero calories. It is a mix of octa, hepta, and hexa esters.

Sefose

This is a new family of mixed sucrose polyesters from Proctor & Gamble, made from sucrose and vegetable oil, used industrially for paints and lubricants. Sefose performs the same function as resins and solvents in paint but doesn't release volatile organic compounds (VOC). They create a tough glossy finish that is resistant to scratching (*37*). The architecture of the molecules can be tailored for reactivity and physical properties by manipulating the fatty acid chain length, the degree of esterification, the level of unsaturation, and adding functional groups (*38*). Many potential uses are envisioned.

Current Scene: Bulk Chemicals and Bio-Plastics

The shift away from petroleum and toward renewable feedstocks is accelerating tremendously. The world market for biobased chemicals, including bioplastics and platform chemicals (excluding biofuels) was $1.6 billion in 2008 (*39*). This is expected to rise to $5 billion by 2015. These products are mostly fermentation products, using highly optimized and rugged organisms that can feed on glucose, sucrose and other carbohydrates. Current products include ethylene, propylene, 1,3-propanediol, polyhdroxyalkanoate polymers, polylactide polymers, 1,4-butanediol, methyl ethyl ketone, and succinic acid (*39*). Below are some examples that use sucrose to make useful chemicals that replace petroleum feedstocks.

Isosorbide Resins

An excellent example of replacing a problematic chemical is the development of a family of isosorbide-based epoxy resins that have the potential to replace bisphenol A in products such as the linings of food cans. Although the patent emphasizes corn and glucose, a number of renewable sugars, including sucrose, can be used in the technology (*40*).

1, 4-Butanediol (BDO)

Approximately 1.3 million tons of the platform chemical 1,4-butanediol (BDO) is manufactured annually from petroleum sources for use in the production of solvents, fine chemicals, polymers, fibers and polybutylene terephthalate plastics. A process has recently been developed using engineered E. coli to produce BDO by fermentation using sucrose as the primary feedstock (*41*).

Ethylene and Propylene

Braskem, a Brazilian petrochemical producer, has partnered with Novozymes, using sugarcane as the raw ingredient to produce polyethylene and polypropylene from ethylene and propylene, respectively, using ethanol as the intermediate. These two polymers represent the first and second most widely used plastics.

Ethylene Glycol

Ethylene glycol is a key component of polyethylene terephthalate (PET) used to make beverage bottles. Recently, the Coca Cola Company announced that it is using sugarcane and molasses sourced from Brazil as feedstock to produce ethylene glycol so that 30% of their new bottle (the PlantBottle™) is bio-based (*42*).

Table III. Polyhydroxybutyrate (PHB) accumulation in microorganisms, percent of dry cell weight (*43*)

Alcaligenes eutrophus	96
Azospirillum sp.	75
Azotobacter sp.	73
Baggiatoa sp.	57
Leptothrix sp.	67
Methylocystis sp.	70
Pseudomonas sp.	67
Rhizobium sp.	57
Rhodobacter sp.	80

Polyhydroxybutyrate (PHB)

An area that generated considerable excitement a decade ago was the production of natural biodegradable plastics (polyesters) by microorganisms. The discovery of other ways to produce bio-sourced plastics (above) appears to have lessened the interest in bacterial polyhydroxyalkanoates for the time being. Various bacterial species produce biodegradable plastics as storage polymers within their cells. From 50-90% of the microorganism's body weight can be bioplastic (Table III) (*43*). Sucrose is a preferred carbohydrate source. The gram-negative bacterium *Alcaligenes eutrophus* is the favored production organism, with intracellular accumulation of polyhydroxy butyrate (PHB) over 90% of the cell dry matter being reported. PHB is probably the most common type of polyhydroxyalkanoate, but many other polymers of this class are produced by a variety of organisms.

Poly Lactic Acid, Polylactide (PLA)

Polylactic acid or polylactide (PLA) is a biodegradable, thermoplastic, aliphatic polyester derived from renewable resources, such as corn starch or sugarcane. It has become of commercial interest in recent years because of its biodegradability. PLA is produced by a combination of fermentation and chemical synthesis, beginning with the bacterial fermentation of a carbohydrate source to produce lactic acid, which is then catalytically dimerized to the cyclic lactide monomer. Polymerization proceeds by a ring opening reaction using stannous octoate catalyst.

Depending on processing conditions, a wide range of molecular weights can be achieved. PLA has a range of applications, including biomedical applications, such as sutures, stents, dialysis media and drug delivery devices. It also makes strong fibers and moldable products. Cargill Company is marketing its PLA biopolymer under the trade name Ingeo™.

Conclusion

The sugarcane factory produces a variety of raw materials with potential for added value products that can replace those made from non-renewable resources, and which, in turn, contribute to sustainability of the environment. Bagasse can serve as a cellulosic feedstock for ethanol, chemicals, paper, structural materials and power. Fly ash has potential in purification of contaminated streams. Vinasse can be a source of fertilizer, and chemicals as well as biogas from anaerobic digestion or power from burning it in concentrated form. The recent development of alcohol tolerant yeast can potentially reduce the amount of vinasse produced. The various sources of sucrose from raw cane juice to crystalline sugar to molasses can provide feedstock for transformation into many bulk/platform chemicals by fermentation, aqueous phase reforming and other chemical conversions. Further processing of these leads to bio-based plastics, fibers and building materials. Even such well-known derivatives as sucrose esters are being chemically manipulated to form new products that can replace petroleum based products.

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Liquid Sugars Produced in Sugar Refineries: Advantages of Large Central Units Serving the Sustainable and Competitive Needs of the Food Industry

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In the present world economics, the prices of sugar are significantly influenced by large cane sugar producers/exporters like Brazil, and sustainable domestic sugar production in other cane producing countries. In comparison, cereals whose prices have been very volatile since 2008, are now considered a more questionable raw material for the production of liquid sugars such as high fructose syrups. The production of liquid sugars (sucrose or medium invert) at the Cane Sugar Refinery is now gaining new recognition for several reasons: (i) the raw material is available in large quantities at competitive prices, (ii) because of the proximity of the Sugar Refinery to the Food Industry there is the possibility to build large-scale efficient central units for liquid sugars, and (iii) energy usage can be saved by avoiding the costs of crystallization, when supplying directly a liquid product ready to use. This book chapter describes various production systems, starting from different types of raw material and using a combination of purification technologies, to produce high quality liquid sugars suitable for the market.

Introduction

Industrial utilization and market recognition of liquid sugars has significantly developed over the last decade (*1*) as a result of several factors:

- good control of specific quality parameters related to liquid sugars and their utilization as food and beverage manufacturing ingredients
- development of liquid sugar applications in emerging countries
- development of large-scale production and associated logistics

The development of the large-scale production of liquid sugar is a renewed opportunity for optimizing the technology and process integration within existing sugar refineries. Significant optimization of global production costs is possible by better integrating the production of liquid sugar in the refinery process.

Three main production systems are compared in this book chapter:

1. Production of crystalline, refined sugar at the refinery, with shipping and dissolving of the liquid sugar at the end user's facility
2. Production of liquid sugar at the refinery, using crystalline refined sugar as raw material
3. Direct production of liquid sugar, concurrently with crystalline refined sugar, from decolorized melt liquor

Production of Crystalline Refined Sugar at the Refinery, with Shipping to and Dissolving of the Liquid Sugar at the End User's Facility

This production system is illustrated in Figure 1. According to this system, no liquid sugar is produced at the sugar refinery. Standard granulated refined sugar is delivered to the end user's facility. There, it must be dissolved with high quality water to prepare a 50-68 Brix liquid sugar solution which can be used as a food and beverage manufacturing ingredient.

While this system (Figure 1) is very simple for the sugar refinery, it requires significant additional costs and operating requirements at the end user's facility:

- Superior quality water must be available to dissolve the refined sugar at the end user's site. This often requires specific local investment in a water demineralization process by using ion-exchange or reverse osmosis processes.
- The sugar dissolving process is completed at high temperature, typically consuming steam (0.06 – 0.1 ton of steam/ton of sugar depending on final Brix)
- Liquid sugar must, generally, be cooled after dissolving to the required temperature, to be used as a processing ingredient
- If various qualities of liquid sugars are required to be produced at the end user's facility, then a polishing process might be required at a relatively small production scale for the reduction of color or conductivity ash

(soluble ash). Such polishing systems are typically a lot more expensive in operation in US$/ton, compared to large-scale processing plants.

- If color or conductivity ash reduction is required at the end user's facility, it will produce solid or liquid waste with related disposal issues. Bottler's plants and many food processing plants are not designed for handling and disposing of these types of effluents.
- Storage of granulated sugar at the end user's facility requires specific investment in silos, hoppers, and handling equipment (e.g., belt conveyors, sugar elevators, etc…)
- Additional manpower is necessary for operating the manufacturing of liquid sugar prior to using it as a food and beverage ingredient. It is sometimes difficult to find local manpower with the required qualifications or technical expertize for operating such liquid sugar manufacturing facilities.
- Local cost for liquid sugar manufacturing inputs, such as steam and high quality water, can be expensive, compared to the cost of these utilities in large-scale production plants. Such extra costs have been estimated and are listed in Table I, according to usual minimum and maximum costs for such liquid sugar production, and based on the experience of Novasep Process™ with many liquid sugar projects world-wide.
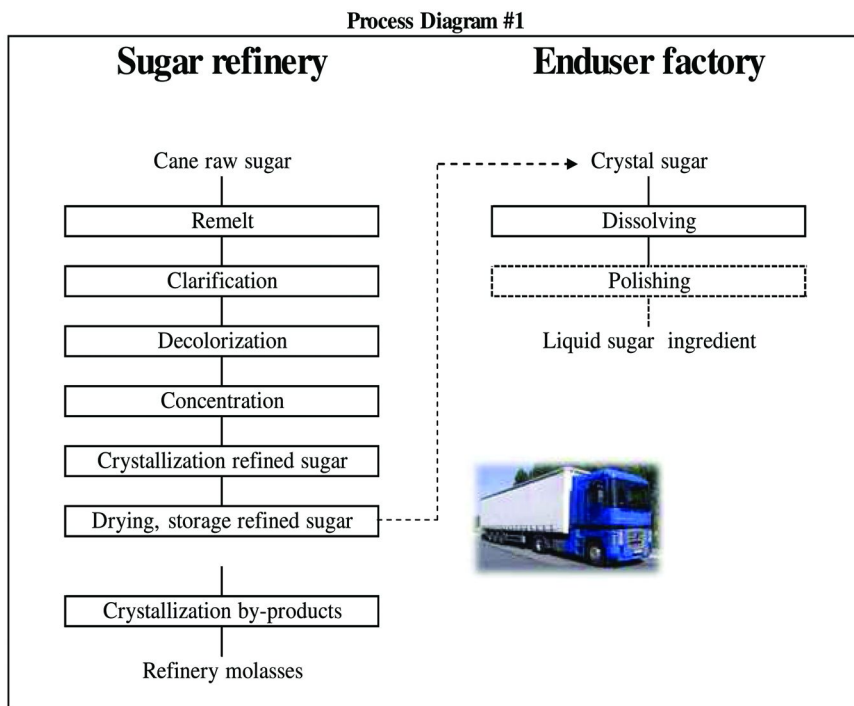
Figure 1. Process diagram of a system for the production of crystalline, refined sugar at the refinery, with shipping to and dissolving of the liquid sugar at the end user's facility.

Table I. Operation Costs for Production of Crystalline Refined Sugar at the Refinery, with Shipping to and Dissolving of the Liquid Sugar at the End User's Facility (Figure 1)

Process Imput	Consumption per tonne of sugar	Range of cost/tonne or kg	Cost per tonne of sugar*
Steam	0.06 – 0.1 t/t TS	15 – 30 US$/t	0.9 – 3.0 US$/t TS
High quality water	0.5 – 1.0 m³/t TS	1 - 3 US$/m³	0.55 – 3.0 US$/t TS
Manpower	0.03 h/t TS	25 – 50 US$/h	0.75 – 1.5 US$/t TS
Total			2.2 – 7.5 US$/t TS

* Results of second column multiplied by third column.

All these operating requirements at the end user's facility have a significant cost which also depends on the Brix for dissolution and can be estimated according to Table I.

As can be seen in Table I, the operation costs can be very variable according to local conditions at the end-user's facility. These costs must be added to the base cost of purchasing crystalline refined sugar from the refiner.

According to this production system (Figure 1) with shipping of crystalline, refined sugar, a sugar refinery can supply the needs of very remote markets as long as the cost of delivery remains competitive at the end-user's facility. There is no real advantage for the local market around the location of the refinery. The end-users can easily switch from one supplier of crystalline sugar to another depending on the total cost of delivery.

Production of Liquid Sugar at the Refinery, Using Crystalline Refined Sugar as the Raw Material

This production system is illustrated in Figure 2. According to this system the liquid sugar is produced at the refinery by dissolving crystalline refined sugar in water as shown in Figure 1. However, the liquid sugar manufacturing plant is installed in the refinery and can use steam, water, and manpower from the main refinery process. This by itself can provide significant optimization of the production cost for liquid sugar, along with the following additional potential benefits:

- Liquid sugar can be manufactured using by-products from the refinery's sugar house such as lumps or fines from the screening of crystals. This can also reduce the amount of sugar recycled in the refinery process.
- If installing a color/conductivity ash polishing process in the liquid sugar plant, it is possible to produce various qualities of liquid sugars for specific markets, and keep a single specification of crystalline refined sugar. In some existing refineries producing liquid sugar from crystalline

refined sugar, the color of all the fine liquor prior to decolorization is reduced to 90-120 ICU (International Commission for Uniform Methods in Sugar Analysis ICUMSA color units), only for producing specific quality of liquid sugar after dissolution with water in the liquid sugar plant. This results in an additional production cost for all the production of crystalline refined sugar. There is no such need if the liquid sugar can be polished in the liquid sugar plant, for meeting the requirement of a specific end user.

- Effluents from color-reduction or conductivity ash removal processes installed in the liquid sugar plant are minimal compared to the larger volume of effluents produced by the sugar refinery, and can be handled more easily.
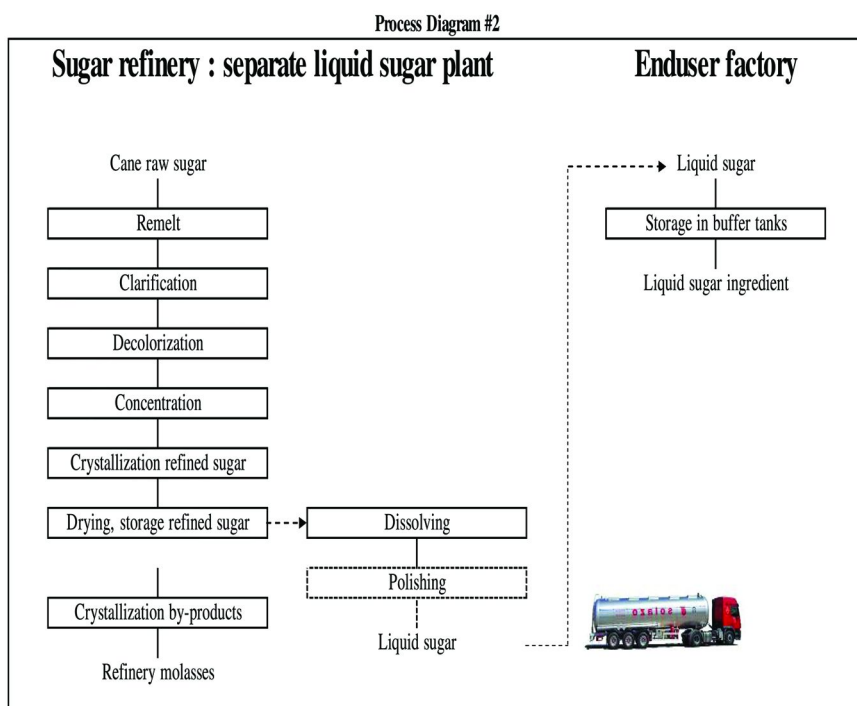
Figure 2. Process Diagram of a System for the Production of Liquid Sugar at the Refinery, Using Crystalline Refined Sugar as the Raw Material

**Table II. Estimated Processing Costs for the Liquid Sugar Production
System illustrated in Figure 2**

Process Imput	Consumption per tonne of sugar	Range of cost/tonne or kg	Cost per tonne of sugar*
Steam	0.06 t/t TS	10 – 20 US$/t	0.6 – 1.2 US$/t TS
High quality water	0.5 m³/t TS	1 – 2 US$/m3	0.5 – 1.0 US$/t TS
Manpower	0.015 h/t TS	25 – 50 US$/h	0.4 – 0.75 US$/t TS
Total			1.5 – 3.0 US$/t TS

* Results of second column multiplied by third column.

Comparing combined operations in the sugar refinery and at the end user's facility, this system clearly provides significant cost benefits. However the refinery must invest in additional production capacities:

a. install a modern liquid sugar dissolver unit that is specific to the liquid plant
b. store liquid sugars in specific tanks, equipped with air and temperature control
c. plan for ensuring liquid sugars deliveries, even at times when the main refinery process is down for maintenance operations
d. implement and maintain a specific quality system for controlling the various qualities of liquid sugar produced at the refinery, including testing their quality and microbiology.

Some of the required capital investment and additional operating costs can normally be shared with the end users, who will mostly benefit from the reduction in the overall production cost of liquid sugars. Liquid sugar is typically produced at the refinery with 67 Brix, with the estimated basic processing costs listed in Table II, excluding any additional cost for color or conductivity-reduction (again a minimum-maximum costs range has been considered, not reflecting any specific condition at a given refinery site).

In practice, liquid sugar can only be delivered at the end user's facility with competitive price, within a limited area and local end users around the refinery. Delivery areas of 100-300 miles around the location of the refinery are often considered as a potential local market. Once established, this local market is more likely to remain a good and loyal customer, as long as the refinery can meet these specific requirements:

•   on-time delivery of liquid sugars with sometimes short notice
•   systematic control and good conformity with agreed quality specifications, especially microbiology stability
•   good flexibility in the production/shipping capacity: most of the end-users for industrial liquid sugars have a highly seasonal activity which is difficult to predict and can see very large variations.

All these requirements are sometime difficult to meet and manage when liquid sugar is produced from crystalline refined sugar. Therefore, for the largest production capacity and more cost-effective operation, refineries can consider a complete integration of the production of liquid sugar in their main process line, concurrently with crystalline sugar, which is discussed below.

Direct Production of Liquid Sugar, Concurrently with Crystalline Refined Sugar, from Decolorized Melt Liquor

This production system is illustrated in Figure 3. Liquid sugar can be produced directly without crystallization, in liquid form starting from decolorized fine liquor as the raw material (*2*).

Typical specifications for fine liquor as the raw material area (*3*) as follows:

- Concentration 65%TS (total solids)
- Color 100-150 ICU
- Ash content 0.10-0.15% on TS
- Invert content 0.1-0.2% on TS

Such characteristics are quite close to liquid sugar specifications:

- Concentration 67%TS
- Color 20-50 ICU
- Ash content 0.015-0.1% on TS

By removing 50-75% of color and conductivity ash from decolorized fine liquor, it is possible to meet most specifications of liquid sugars. The best purification technology for this final purification stage includes the following operations:

- Decolorization and de-ashing by ion-exchange mixed beds
- Final color/taste/odor polishing using activated carbon, either in granular carbon columns or with powdered activated carbon
- Pasteurization or sterile filtration, or a combination of both for most demanding microbial specifications
- Final concentration to optimum Brix for storage and delivery

Some of the polishing steps in this system (Figure 3) are often already present in the liquid plant using crystalline refined sugar as raw material. Mostly ion-exchange mixed-beds columns must be installed for primary decolorization and de-ashing of decolorized liquor. No additional filtration system is required, as decolorized fine liquor is already exiting from the main ion-exchange or granular carbon decolorization system and contains no suspended solids. Mixed-beds columns are filled with cationic and anionic ion-exchange resins. The two resin beds are intimately mixed during production, providing both the decolorization and de-ashing requirement at the same time and in the same column. Normally

a single-pass process is used from good quality decolorized liquor. When decolorized liquor has a higher color or ash content, a double-pass process can be used with little increase of the operating cost.

Mixed-bed ion-exchange technology provides several benefits during a single processing step:

- Color removal from 100-150 to 25-50 ICU, depending on the final specification of liquid sugar
- Conductivity ash reduction from 0.10-0.15% to 0.015-0.05% on TS
- Efficient pH control of the low-conductivity liquid sugar, without any added chemical. This is obtained by adjusting the relative volume of the cationic and anionic resin beds in the mixed-beds column.
- Processing of fine liquor up to 65 Brix

To avoid sucrose inversion during the mixed-beds purification process, the operating temperature must be adjusted much lower than for the decolorization columns. It is possible to limit any increase in invert content to 0.1% maximum on TS. However this process is dependant upon the initial content of invert sugar in the fine liquor and, in some cases, may not be able to produce less than 0.5% invert on TS. This point has to be specifically checked with all potential end-users.

After processing a certain volume of fine liquor, depending on its color and ash conductivity levels, the resins must be regenerated. This is completed after separation of the cationic and the anionic resin beds inside the column, according to their density: ~1.2 for the cationic resin bed, and ~1.05 for the anionic resin bed. An intermediate distributor is installed at the interface between the two resin beds, and allows for separate regeneration of the cationic resin by hydrochloric acid (HCl), and of the anionic resin by sodium hydroxide NaOH (or potassium hydroxide KOH if preferred by the end-user). After the final rinse with low-conductivity water, the two resin beds are re-mixed in the column and ready for a new production cycle.

Considering the high sucrose purity already reached in the fine liquor, only small amounts of ash and color needs to be removed. This makes ion-exchange technology more efficient compared to crystallization for the production of liquid sugar. The polishing step is useful for the final removal of specific impurities which may not be efficiently removed by ion-exchange, such as aromatic and organic compounds.

A final treatment with granular carbon or powdered carbon, at low dosage, is very efficient and cost-effective for ensuring required taste and odor specifications, or removing HMF (Hydroxy-Methyl-Furfural) in the case of invert syrup.

A number of existing industrial plants have provided evidence that this process can completely replace crystallization and meet all end-user's specifications. Estimated basic operating costs for the direct production of liquid sugar (Figure 3) are listed in Table III.
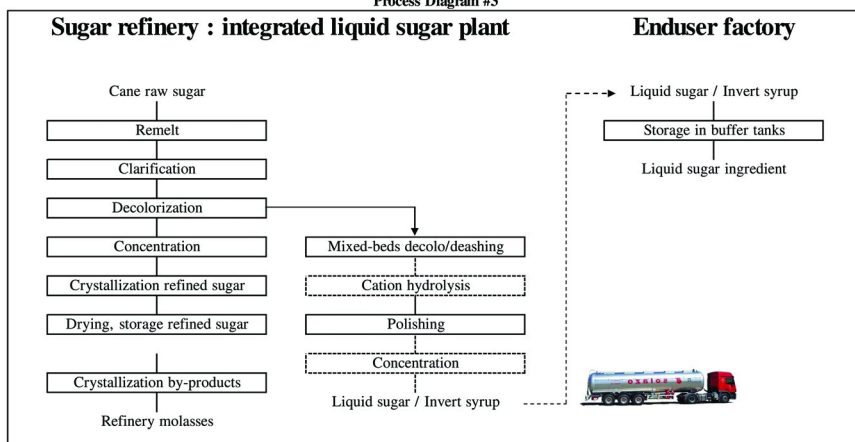
Figure 3. Production System for the Direct Production of Liquid Sugar Concurrently with Crystalline Refined Sugar, from Decolorized Melt Liquor

Table III. Operating costs for direct production of liquid sugar at 67 Brix (based on Novasep Process technology, and possible variable local costs of process imputs)

Process Imput	Consumption per tonne of sugar	Range of cost/tonne or kg	Cost per tonne of sugar*
Steam	0.05 t/t TS	10 – 20 US$/t	0.5 – 1 US$/t TS
NaOH 100%	4.8 Kg/t TS	400 - 800 US$/t	2 – 3,8 US$/t TS
HCl 100%	2.4 Kg/t TS	150 - 300 US$/h	0.4 – 0,7 US$/t TS
Water	0.6 m³/t TS	1 – 3 US$/m³	0.6 – 1,8 US$/t TS
Effluent	0.5 m³/t TS	0.5 – 2 US$/m³	0.3 – 1 US$/t TS
IX resin	0.045 L/t TS	7.5 US$/L	0.3 US$/t TS
PAC	0.5 Kg/t TS	3 US$/Kg	1.5 US$/t TS
Total			5.6 – 10 US$/t TS

* Results of second column multiplied by third column.

Comparison of the Three Systems for Producing Liquid Sugar

To finally compare the combined operation cost of the three production systems outlined in Figures 1, 2, and 3, it is necessary to include the specific cost for crystallization, drying, handling and storage of crystalline refined sugar (*4*). This cost has been estimated and is listed in Table IV, specifically for the cost of steam used for crystallization which can be in single-effect (0.9 t steam/t sugar) or double-effect (0.6 t steam/t sugar) and are listed in Table IV. Additional costs have been considered including electricity, drying, storage, and handling (all costs

are not reflecting the situation of any specific sugar refinery, but rather variable costs range for all refineries).

Table V lists the comparison for the overall combined production cost of liquid sugar at the refinery and at the end-user's facility, for the three production systems.

This preliminary comparison (Table V), although based on cost range analysis and not specific costs of a single project case study, shows that direct production of liquid sugar (System 3 outlined in Figure 3) is a more cost-effective production system compared to crystallization, when properly integrated in the main refinery process. It will also provide greater flexibility to the sugar refinery for maximizing the production of crystalline refined sugar, or liquid sugar, depending on the season (5).

If the liquid sugar production stream is sized accordingly, it could be possible to divert a large part of the production of the refinery toward liquid sugar during peak demands of the end-users, when market can absorb huge amounts of liquid sugar very quickly. The greater the direct production of liquid sugar, the most effective production cost for the refinery. This process can also be installed in an existing refinery to increase significantly its overall throughput without any modification to the sugar house and crystalline sugar storage and handling. Only the front end of the refinery needs to be expanded from raw sugar melt up to decolorized fine liquor. Fine liquor is then directed in parallel to the sugar house and to the liquid sugar process. Furthermore, there is no additional production of refinery molasses.

Table IV. Comparative Cost of Steam, Electricity, Drying, Storage, Bagging, and Manpower for the Three Production Systems Outlined in Figures 1, 2, and 3

Process Imput	System 1	System 2	System 3
Steam	0.6 – 0.9 t/t TS	10 – 20 US$/t	6 – 18 US$/t TS
Electricity			
Drying			
Storage			15 US$/t TS
Bagging			
Manpower			
Total			21 - 33 US$/t TS

Table V. Overall Comparison of Costs for the Three Production Systems Outlined in Figures 1, 2, and 3

	System 1	*System 2*	*System 3*
Refinery cost range			
Crystallization	21 – 33 US$/t	21 – 33 US$/t	
Dissolution		1.5 – 3.0 US$/t	
Direct production			5.6 – 10.0 US$/t
Typical shipping cost	20 US$/t	27 US$/t	27 US$/t
End-user cost range			
Dissolution	2.2 – 7.5 US$/t		
Total production cost range	43 – 60 US$/t	50 – 63 US$/t	33 – 37 US$/t
Median production cost	52 US$/t	56 US$/t	35 US$/t
	100%	110%	70%

Production of Medium Invert Syrup

An interesting variation of the direct liquid sugar process depicted in Figure 3, is the production of medium invert syrup in very competitive conditions. By installing one additional ion-exchange column, loaded with a specific catalytic, cation resin in acid form, it is possible to control the inversion of sucrose in the syrup at a desired level. Control parameters include Brix, temperature, and specific the flow in the column. There is no need to add a chemical in the invert syrup, unlike previous technology using acid hydrolysis. Moreover, no further purification is required after inversion, except eventually HMF removal if a high degree of inversion has been requested. Most typical Medium Invert Syrups are Medium Invert 50, Medium Invert 66 and Invert Syrup 90.

Typical specifications for Medium Invert 66 Syrup:
Concentration: 73 Brix
Sucrose: 34% on TS
Glucose + Fructose: 66% on TS
Color: 25 – 50 ICU
Ash content: 0.05 – 0.10% on TS
Sugars concentration: 1000 g/L

A higher concentration can be used for storage and delivery, which provides significant benefits both for the refinery and end-users:

a. reduced volume for storage and transport (73 Brix instead of 67 Brix)
b. better microbial stability (less water activity in the syrup) and longer shelf-life

c. easy formulation for European end users : 1 litre = 1 Kg of total sugars
d. better stability of the sugars profile : liquid sugar is usually inverted in
    acid beverages in a few days to the same final sugars profile as Medium
    Invert. This inversion is causing a volume retraction and potential
    difficulties for proper filling when using liquid sucrose.
    When using Medium Invert 66 Syrup, the filling process is much better
    controlled and stable over time.

Despite these significant benefits compared to the production of crystalline
refined sugar and liquid sucrose, Medium Invert 66 Syrup is not well developed
except in a few specific countries. It could be an excellent sweetener syrup for
future large-scale and most cost-effective production in sugar refineries.

Example of a Modern Liquid Sugar Production Facility

A good example of a modern production facility is the new liquid sugar
production facility designed by Novasep Process™ for Cevital Spa in their large
sugar refinery at Bejaia, Algeria. This new facility was commissioned in 2008
for a nominal production capacity of 600 tonnes dry solids per day, and the
production of liquid sugar or medium invert syrup directly from fine liquor after
ion-exchange decolorization. The specifications for their fine liquor and medium
invert syrup are listed in Table VI.

From 140 m³/h decolorized fine liquor in the refinery, 30 m³/h are diverted to
direct production of liquid sugar through mixed-beds, powdered carbon polishing,
sterile filtration, and concentration. One large hydrolysis column can convert all
the production to Medium Invert Syrup 66 when desired. Medium Invert 66 Syrup
was added to conventional liquid sugar, for higher concentration at 73%TS and
improved microbiological stability during storage and transport.

Table VI. Specifications of fine liquor and medium invert syrup

	Decolorized fine liquor	*Medium Invert Syrup*
Concentration	62 %TS mini	73%TS +/- 0.5%
Sugars conc.		1000 g/L
Color	80 – 100 ICU	25 ICU
Ash content	0.10 – 0.15% on TS	0.05% on TS
Invert	0.10 – 0.20% on TS	66% on TS
Microbiology Mesophiles Yeast Mold		200/10 g max 10/10 g max 10/10 g max
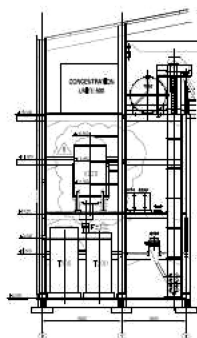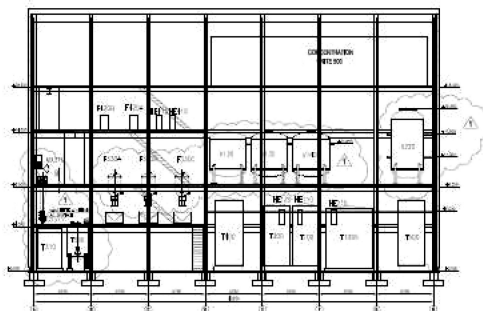
Figure 4. New liquid sugar production facility designed by Novasep Process™
for Cevital Spa in their large sugar refinery at Bejaia, Algeria.

     With a potential production capacity of 150,000 – 200,000 tons per year in equivalent dry solids, this new line is a good example of large-scale and cost-effective production of liquid sugar for the local market around the refinery.

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Chapter 17

The Role of Sugar Beet Pulp Polysaccharides in the Sustainability of the Sugar Beet Industry

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Sugar beet pulp was sequentially extracted with a microwave heating source under pressure to produce pectin, alkaline soluble polysaccharides, and cellulose which was converted into carboxymethyl-cellulose. The solution physical-chemical properties of these polysaccharides were compared to those obtained using other extraction methods. The molar mass, radius of gyration, and intrinsic viscosity quality of these sugar beet polysaccharides was very high compared to values reported in previous literature. A sugar beet biorefinery is discussed that could produce valuable polysaccharide co-products in addition to providing feedstocks for biofuel fermentation in conjunction with sucrose production. These methods and new co-products would improve the sustainability of sugar beet processing by reducing energy costs, replacing petroleum-based products, and decreasing chemical input to produce a common food gum.

Introduction

Production of sugar from sugar beets is an energy and water intensive process with several heating, drying, and washing steps (Figure 1) (see (*1*)). With sucrose, ethanol, betaine, uridine, sugar beet pulp and molasses as the main products of the sugar beet industry, additional co-products would be beneficial to improve the process economics and sustainability. Process modifications with less energy, water, greenhouse gas emissions, carbon footprint, agricultural input (fertilizer, herbicide, and tillage) or transportation costs are needed. Additional bioactive,

biobased, biofuel, and green products from the existing sugar beet process would also improve sustainability.

Agricultural production of sugar beets has become more sustainable with the advent of glyphosate-tolerant (Roundup Ready) sugar beets in the U.S. In addition to easier weed control, farmers have been able to reduce tillage savings and fuel and fertilizer, and they observed less soil erosion when glyphosate-tolerant sugar beets were planted (*2*). Glyphosate-tolerant sugar beets have been planted in the U.S. since the spring of 2008 and now represent 95% of this biennial plant grown in this country (*2*). While genetically modified crops cannot be grown in Europe, the European Union allows sugar as well as food and feed products made from glyphosate-tolerant sugar beets to be imported for human and animal consumption. Therefore, additional co-products of U.S. sugar beet processing will improve the economics and sustainability of sugar beet processing globally. A global sugar beet processing biorefinery would be possible where sugar beet pulp is utilized for various fractions depending on the scale of production and local market conditions.

1. Sugar beet green tops (currently left in the field). 2. Beet slices (cossettes). 3. Cossettes with steam application. 4. Wet pulp after sucrose extraction (~10% solids). 5. Pressed pulp (~25% solids). 6. Dehydrated pellets (~90% solids). 7. Sucrose and/or molasses.
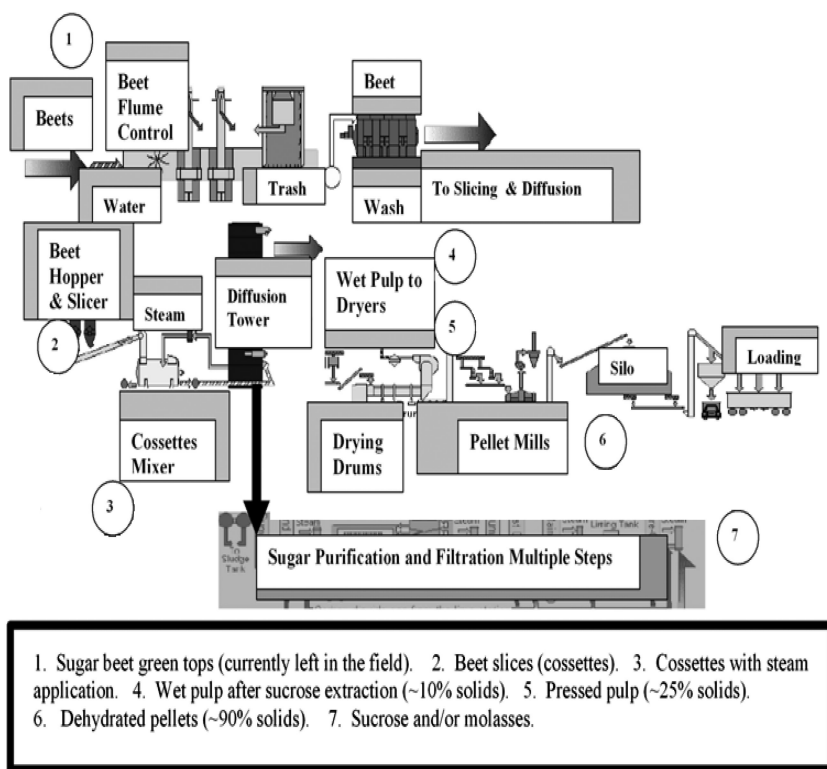
Figure 1. Sugar beet processing diagram.
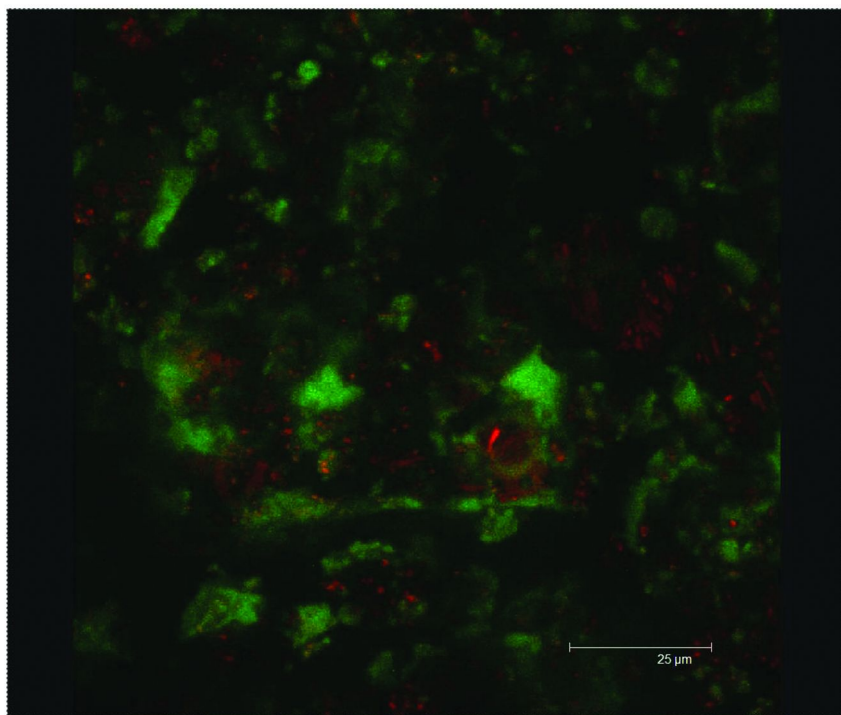
Figure 2. Confocal microscopy of 50:50 (w/w) sugar beet pulp and PLA. The confocal reflection (633 nm) for PLA is red and confocal fluorescence (excitation, 488; emission, 500-530 nm) for sugar beet pulp is green. (see color insert)

In Europe, ethanol biofuel from surplus sugar beet production is the chief co-product of sugar beet processing. Biofuel production from sugar beets is more economically viable in Europe due to incentives and sugar production limitations. There are also ten times more sugar beets produced in Europe compared to the U.S. This chapter will focus on the use of plant polysaccharides in sugar beet pulp as bioactive food ingredients and biobased products. These high value co-products would not compete with the food supply of sugar but would utilize some of the sugar beet pulp used for animal feed.

Sugar beet pulp is produced in 10^7 ton quantities annually and consists of roughly equal parts of cellulose, pectin, and pectin-associated arabinan and galactan (*3–6*). All of these polysaccharides are potentially valuable co-products of sugar beet processing with only limited current commercial production of sugar beet pectin. In collaboration with Joy Peterson (University of Georgia), we demonstrated that fermentation of partially pectin-extracted sugar beet pulp was possible (*7*). Additionally, we produced bioplastic composites from sugar beet pulp and polylactic acid (PLA) (*8–10*) (Figure 2). It is now possible to produce these composites with 95% sugar beet pulp (data not shown). Therefore, in a sugar beet biorefinery, a variety of valuable carbohydrates and carbohydrate-based materials can be produced in addition to biofuels. These

products would make sugar beet processing more sustainable particularly if they replace a petroleum-based products.

Sugar Beet Pulp Polysaccharides

Sugar beet pulp was systematically fractionated into pectin, alkaline soluble polysaccharide, and cellulose (Figure 3) (*11–13*). Microwave assisted extraction (MAE) of sugar beet pulp under hot acidic conditions and 30 psi pressure followed by precipitation with isopropyl alcohol (IPA) was used to extract sugar beet pectin. An alkaline hydrogen peroxide MAE under pressure of the remaining residue produced ASP I and ASP II. The final residue represented sugar beet cellulose, which was converted to carboxymethyl cellulose (CMC) using chloroacetic acid (Figure 3).

Pectin

Sugar beet pectin is unlike commercial pectins, extracted primarily from citrus, in that it does not gel which is due to a higher degree of acetylation, neutral sugar content, and degree of feruloylation (*14–16*). Rhamnogalacturonan II-boron diester complexes which cross-link this pectin sub-component were first reported in sugar beet pectin (*17*). Sugar beet pectin has been widely investigated as an oil in water beverage emulsifier (*12*, *18–20*). The high acetyl and protein content of sugar beet pectin contribute to its emulsion stabilization properties (*18*, *19*). We identified extensin as a protein associated with sugar beet pectin (*21*). Sugar beet pectin has also reported uses in cholesterol absorption from food, stabilization of acidified yogurt beverages, and as a water soluble pectin fiber (*6*).

With MAE, we produced an acid-extracted sugar beet pectin with 532,000 to 1.2 million Da molar mass, 35 to 51 nm radii of gyration and 3.00 to 4.30 dL/g intrinsic viscosity (*11*). These pectin solution physical chemical properties were generally higher quality than a typical commercial sugar beet pectin sample but similar high values were previously reported in the literature when fresh sugar beets were extracted (*16*, *22*).

Alkaline Soluble Polysaccharides

Alkaline soluble polysaccharides (ASP) consist of arabinans, galactans and arabinogalactans that form the neutral sugar side chains of pectin as well as rhamnogalacturnonan (*15*, *26*). ASP has potential as bioactive food ingredients. Arabinose-rich pectic oligosaccharides from citrus peel were reported to have prebiotic (*23*) and food pathogen anti-adhesive properties (*24*). While sugar beet arabinan is an intricately branched polysaccharide (*25*), these prebiotic pectic oligosaccharides are likely to be present in sugar beet ASP. Sugar beet ASP also functions as an oil in water emulsifier (*12*).
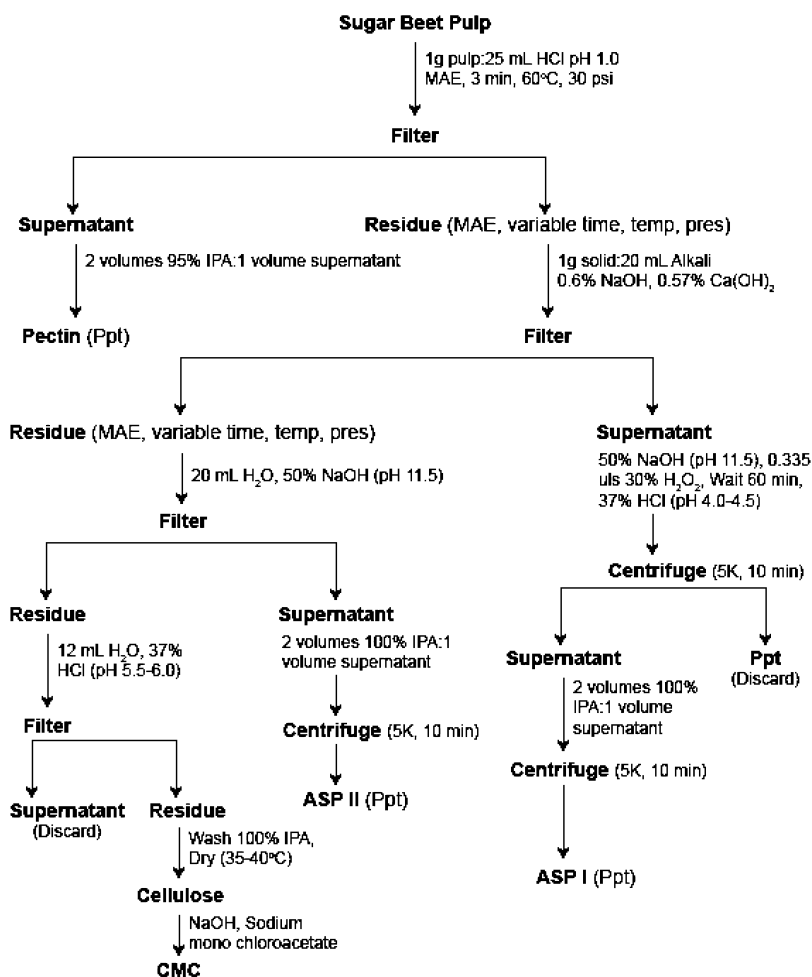
Figure 3. Fractionation of sugar beet pulp using microwave assisted extraction (MAE) into pectin, alkaline soluble polysaccharides (ASP) and cellulose which was converted to carboxymethyl-cellulose (CMC).

The sugar beet ASP produced by alkaline hydrogen peroxide MAE had 83,000 to 324,000 Da molar mass, 10 and 16 nm radii of gyration, and 0.31 and 0.33 dL/g intrinsic viscosities for ASP I and ASP II, respectively (*12*). While these molar mass values are similar to those reported for sugar beet ASP extracted with 2% NaOH at 45°C (*26*), the monosaccharide compositions of the ASPs produced in the different laboratories are not consistent. Our ASP has the monosaccharide composition of the arabinose and ferulic acid rich pectic polysaccharides extracted from sugar beet pulp under alkaline conditions or by autoclave hydrolysis at pH 5.2 (*3*) and the autoclave 2 sample (*27*). The molar mass, radius of gyration and intrinsic viscosity values reported for the autoclave 2, pool II fraction (*27*) agree exactly with what we observed for sugar beet ASP. While MAE is similar

to an autoclave as a means of extraction under carefully controlled heat and pressure conditions, we did not observe the high molar mass (1.3 million Da), radius of gyration (36 nm) and intrinsic viscosity (0.97 dL/g) values reported for the autoclave 2 pool I fraction (*27*) in our ASP fractions. Minor amounts of glucuronic acid observed in both our sugar beet pectin and ASP suggest that a glucuronoxylan is present similar to the (4-O-methyl-D-glucurono)-D-xylan (GAX) extracted from sugar beet pulp previously (*28*).

Cellulose

Alkaline extraction (2% NaOH) of sugar beet pulp and homogenization of the cellulosic residue produced some very interesting properties (*4*). The cellulose microfibrils produced a creamy suspension in water that didn't flocculate or sediment. The suspension was a birefringent liquid crystalline phase of loosely arranged microfibrils that produced electron and powder x-ray diffraction patterns confirming cellulose I (*4*). However, once the sugar beet cellulosic fraction was treated with 10% or higher concentration of alkali or 100°C trifluoroacetic acid (TFA), then the microfibrillar structure was lost and a gel-like membranous aggregation of particle strings was observed, a cellulose II powder x-ray diffraction pattern was observed (10% NaOH treatment) and the sample flocculated (*4*). One of the polysaccharides released from the cellulose microfibrils by the TFA treatment was GAX (*28*). Therefore, pectic, alkaline soluble and hemicellulosic polysaccharides were essential for sugar beet cellulose microfibrils to maintain their structure, physical-chemical and crystallographic properties. Once removed, the cellulose microfibrils broke down into nanofibrils in water. Sugar beet cellulose is a parenchyma cellulose, meaning that the cellulose is like that in primary cell walls present in the parenchyma growing tissue of the plant and not like the thickened secondary cell walls found in wood (*29*). Cellulose whiskers or nanofibrils (210 nm long) were described in an acid-treated sugar beet pulp cellulosic fraction (*30*) that was very similar to the flocculated sugar beet cellulose produced by Dinand et al. (*4*). These cellulose whiskers were used to reinforce nanocomposite materials (*30, 31*).

We converted sugar beet cellulose into carboxymethyl-cellulose (CMC) (*31*). The CMC molar mass ranged from 96,000 to 220,000 Da, the intrinsic viscosity ranged from 1.9 to 4.1 dL/g, and the degree of substitution ranged from 0.59 to 1.38 (*13*). When carboxymethylation of sugar beet cellulose was optimized for solvent medium, alkali concentration, sodium chloroacetate amount, temperature and time of reaction, a CMC with a 0.667 degree of substitution was obtained (*32*). A sugar beet CMC opens markets in adhesives, paper products, battery manufacture, drug delivery, dairy substitutes, fat replacement, antibiotic stabilizer and laxatives for sugar beet processing co-products. We observed that the sugar beet cellulose fraction retained some galacturonic and glucuronic acids (data not shown), which could provide unique properties in these application areas. Therefore, it is likely that less sodium monochloroacetate was necessary to produce sugar beet CMC compared to CMC prepared from cotton linters or wood pulp. This would make sugar beet CMC a greener more sustainable product. This suggests that the value

of sugar beet cellulose may be much greater than that as a feedstock for biofuel production.

Conclusions

Valuable polysaccharides including pectin, alkaline soluble polysaccharides, and cellulose are present in sugar beet pulp that can be utilized for bioactive food ingredient and biobased product applications. Microwave assisted extraction of sugar beet pulp in a biorefinery could fully capture the carbohydrate potential of this biomass. Functional food ingredients and biobased materials could be produced that would replace petroleum-based products using much shorter heating times compared to other extraction methods. The sustainability of the sugar beet industry would improve by the addition of valuable co-products from a renewable crop, reduction of energy costs, replacement of petroleum-based products and less chemical input to produce a common food gum.

Acknowledgments

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Subject Index

VLC. *See* Very low color