# Perspectives on Bioenergy and Biotechnology in Brazil

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#### **Abstract**

Brazil is one of the world's largest producers of alcohol from biomass at low cost and is responsible for more than 1 million direct jobs. In 1973, the Brazilian Program of Alcohol (Proalcool) stimulated the creation of a bioethanol industry that has led to large economic, social, and scientific improvements. In the year 1984, 94.5% of Brazil's cars used bioethanol as fuel. In 2003/2004, 350.3 million of sugarcane produced 24.2 million t of sugar and 14.4 billion L of ethanol for an average 4.3 million cars using ethanol. Since its inception, cumulative investment in Proalcool totals US\$11 billion, and Brazil has saved US\$27 billion in oil imports. The ethanol production industry from sugarcane generates 152 times more jobs than would have been the case if the same amount of fuel was produced from petroleum, and the use of ethanol as a fuel is advantageous for environmental reasons. In 2003, one of the biggest Brazilian ethanol industries started consuming 50% of the residual sugarcane bagasse to produce electrical energy (60 MW), a new alternative use of bioenergy for the Brazilian market. Other technologies for commercial uses of bagasse are in development, such as in the production of natural fibers, sweeteners (glucose and xylitol), single-cell proteins, lactic acid, microbial enzymes, and many other products based on fermentations (submerged and semisolid). Furthermore, studies aimed at the increase in the biosynthesis of sucrose and, consequently, ethanol productivity are being conducted to understand the genetics of sugarcane. Although, at present, there remain technical obstacles to the economic use of some ethanol industry residues, several research projects have been carried out

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and useful data generated. Efficient utilization of ethanol industry residues has created new opportunities for new value-added products, especially in Brazil, where they are produced in high quantities.

Index Entries: Ethanol; bioenergy; sugarcane; agroindustry; sugar powder.

#### Introduction

Sugarcane is one of the oldest crops under cultivation and has played a central role in Brazil's culture and economy since the beginning of the colonial period. The cultivation of sugarcane generates financial resources for the country, mainly through the production of sugar powder, anhydrous ethanol (a gasoline additive), and hydrated ethanol (carburent, destined for biofuels). Sugarcane also has several alternative applications as a source of energy, because electrical energy can be produced through the burning of bagasse (after extraction of the cane juice), and in the production of biodegradable plastic, such as polyhydroxybutyrate (PHB). Furthermore, all produced industrial residues (bagasse, stillage, and so on) can be used for the production of energy (ethanol and biogas) as well as component sugars (glucose, xylose, xylitol) (1,2).

#### Sugarcane

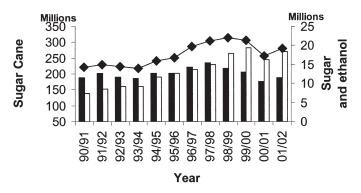
Brazil is the main sugarcane producer worldwide, followed by India and Australia. During the 2003–2004 growing season in Brazil, approx 350.3 million t of sugarcane was cultivated, of which 298 million t (or 85%) came from the Center-South Brazilian region (Fig. 1). For the 2003–2004 crop, 11.05% more sugarcane was processed than for the 2002–2003 crop (1). About 55% of Brazilian sugarcane production is used for the production of alcohol (ethanol) and 45% for powdered sugar. At present, about 4.5 million ha of Brazilian territory is devoted to the cultivation of sugarcane in two main regions: Center-South and Northeast. Because the climate varies significantly in these regions, it is possible to plan two harvesting periods: from May to November in the Center-South region, and from December to June in the Northeast region. Once planted, sugarcane takes from 12 to 18 mo to be harvested and processed for the first time, and its cultivation can provide up to five harvests before it has to be replanted (Fig. 2).

# **Ethanol and Sugar Powder**

Ethanol production from the 2003–2004 crop reached 14.4 billion L in the whole country, with the Center-South region responsible for 12.9 billion L of this total, and production increased 16.72% compared to the previous crop (2002–2003) and surpassed the greatest crop (1997–1998) registered in Brazil. In São Paulo, the production reached 8.74 billion L, 14.71% higher

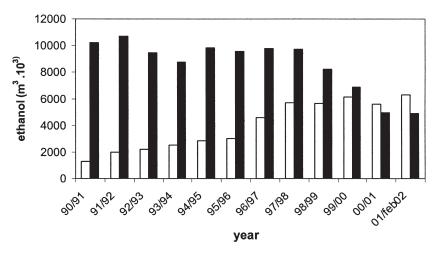


Fig. 1. Map of Brazil and its regions.



**Fig. 2.** Production of  $(\spadesuit)$  sugarcane,  $(\Box)$  sugar powder, and  $(\blacksquare)$  ethanol production as function of crop (year).

than the amount produced in the prior period. One hectare of land produces about 81 to 82 t of sugarcane, and about 7000 L of ethanol. The Brazilian production of powdered sugar in 2003 reached 24.2 million t (Figs. 2 and 3). The Center-South region was responsible for 20.35 million t, and 10.1 million t was exported (3).



**Fig. 3.** Production of ( $\blacksquare$ ) hydrated and ( $\square$ ) anhydrous ethanol in Brazil as function of crop (year).

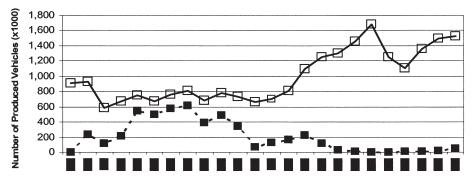
### **Productivity**

In 2003–2004, crop production of sugarcane, sugar powder, and ethanol surpassed expectations. The highest overall production was in the Center-South region, influencing significantly the increase in the Brazilian agricultural productivity. The factors that favored productivity were the use of new varieties of cane (genetically modified), the favorable climatic conditions, more appropriate harvesting time, and improvement in transportation and grinding. The 2003–2004 crop showed a satisfactory sugarcane production average, reaching about 149.62 total reducing sugars/t of cane, resulting in a 1.93% increase compared to the crop registered in 2002–2003.

The average cost of sugarcane production in Brazil was US\$180/t of sugar or US\$0.20/L of ethanol, whereas in Australia, the cost was higher than US\$335/t of sugar (2). In Europe, a subsidized ton of sugar powder reached US\$710 (beet root sugar) (2).

# **Energy Production of Sugarcane Bagasse**

In 2003, sugarcane bagasse, a byproduct of the sugar powder and ethanol factory, stocked electrical energy similar to that of the Brazilian nuclear power plant, considering only the production of the São Paulo State. The production of sugarcane in Brazil has the potential to produce 12,000 MW of the electrical power in excess of process energy requirements, representing a significant fraction of the total national installed capacity of 70,000 MW. The sugarcane industries can supply from 2000 to 3000 MW of electrical energy to the market in the period of July to November, when hydroelectric reservoirs, responsible for 95% of the energy produced in the country, are at their lowest levels. In 2003, a generator unit of electrical energy, based on sugarcane bagasse, started working



**Fig. 4.** Production of ( $\blacksquare$ ) ethanol and ( $\square$ ) ethanol + gasoline + diesel as function of time (year).

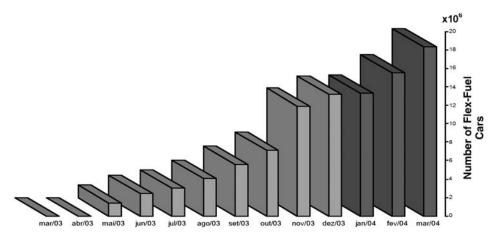
(Santa Elisa industry, Sertãozinho, SP) with an installed capacity of 60 MW. The same industry planned to consume half of the energy produced and sell the other part to the federal government.

#### Brazilian Production of Ethanol

Anhydrous ethanol (ethanol concentration higher than 99.58% [v/v] and specific mass of 791.5 kg/m³) has been used in Brazil as an additive in gasoline. In 1973, in search of energy autonomy, the Brazilian government developed the National Alcohol Program (PROALCOOL) for the production of this fuel, and the development of the first car fueled by hydrated ethanol (ethanol concentration of 95.56  $\pm$  0.43% [v/v] and specific mass of 809.3  $\pm$  1.7 kg/m³) in the world. PROALCOOL was stimulated by fiscal incentives, and the sugarcane industries started producing ethanol in parallel with sugar powder. Brazil developed important technology for ethanol production and created a renewable source of fuel, produced independent of the world petroleum market.

In Brazil, 1% of the total cultivated area, or about 4.5 million ha, is presently devoted to the production of sugarcane. This area is equivalent to 19% of the territory of the United Kingdom and 8% of France. Each ton of sugarcane has the energy potential equivalent to that generated by 1.2 barrels of petroleum.

Every year, Brazil produces about 10.4 billion L of ethanol, and about 62% is provided by the state of São Paulo. Approximately 3 million vehicles are fueled with hydrated ethanol and consume 4.9 billion L/yr (Figs. 3 and 4). Anhydrous ethanol (a production of 5.5 billion L/yr) is utilized in a 22–25% blend with gasoline. Installed ethanol production capacity is 16 billion L, equivalent to 84 million petroleum barrels/yr (3). The production of ethanol reduces petroleum importation, saving Brazil billions of dollars. In the last 22 yr, an economy of US\$1.8 billion/yr was registered, with a significant substitution of gasoline by ethanol, equivalent to 200,000 barrels of gasoline/d (3). As a consequence, the number of jobs also increased.



**Fig. 5.** Number of flex-fuel cars produced in Brazil as function of time (month/year).

#### **Influence of Ethanol on Environmental Conditions**

Ethanol fuel is a renewable and clean product and has contributed to the reduction in the greenhouse effect and substantially diminished air pollution, minimizing impacts on public health. In Brazil, the addition of ethanol to gasoline has a main objective to minimize the emission of pollutants by vehicles, such as carbon monoxide, sulfur oxides, and organic toxic compositions (benzene and lead compositions).

When fuel ethanol production started, ethanol was mixed with gasoline in the form of anhydrous ethanol. After PROALCOOL was formed, motors using only hydrated ethanol started being used. Today, the mixture of anhydrous ethanol (22–25%) with gasoline is consolidated, tested, and approved on a large scale by federal law.

However, since 1995, the number of cars fueled by ethanol decreased (Fig. 4). The three most important factors that contributed to this reduction were a drop in international prices of petroleum, incentives for exportation of sugar powder, and a collapse of hydrated ethanol production in some Brazilian regions. The drop in demand for hydrated ethanol was compensated by a greater use of anhydrous ethanol, which accompanied the growth of fleet vehicles in Brazil. At present, 60% of the consumption of ethanol fuel in a country is anhydrous, after mixing with gasoline in a proportion of 20–25%. This combination is unique in the world and has decreased Brazilian dependence on imported petroleum. To increase the demand of ethanol cars, the government recently reduced taxes on cars that utilize 100% ethanol. To avoid a lack of ethanol supply, the government has guaranteed stocks of the product. However, the development of flex-fuel cars that can use gasoline or ethanol as fuel has significantly impacted the consumption of ethanol (Fig. 5).

In the more than 30 yr of the (PROALCOOL) program, Brazil has developed new technology for motors, transport logistics, and efficient distribution of ethanol and now has a network of more than 25,000 filling stations with hydrated ethanol pumps to supply about 3 million vehicles, or 20% of the national fleet. Furthermore, the industry has solved all technological difficulties associated with vehicles.

The energy originating from 1 L of ethanol corresponds to 20.5 MJ, and from 1 L of gasoline, 30.5 MJ. The average distance traveled per liter of fuel varies between 9.8 (ethanol) and 13.5 km (gasoline). However, because ethanol is cheaper than gasoline, the ethanol-fueled car travel further in relation to the gasoline-fueled car (4). In flex-fuel cars, ethanol consumption is 30% higher than that of gasoline, so its price should be up to 75% that of gasoline. Today, ethanol is more advantageous for the consumer than gasoline, because its price is about 65% that of gasoline.

#### **Stillage**

In the alcohol industry, sugar (mainly sucrose), present in sugarcane juice, is converted into ethanol during the alcoholic fermentation process, and afterward the broth (fermented medium) is forwarded to distillation. During distillation, ethanol is separated, and all the other components (water, potassium, calcium, iron, phosphorus, and organic compounds) are transformed into residue. This residue can be utilized as fertilizer. One liter of alcohol generates 12–15 L of stillage.

At the beginning of the PROALCOOL program, stillage was discharged directly into riverbeds and caused very serious environmental problems owing to its biologic oxygen demand. However, the high content of nutrients and water in stillage makes it potentially useful for fertilization and irrigation of the soil. This has been a very important solution for some of the dry regions of Brazil. The use of stillage as a fertilizer increased sugarcane productivity, because the physical structure of the soil (mainly porosity) improved the water absorption capacity. Therefore, agrotoxics and mineral supplements were saved.

Stillage can be converted by anaerobic digestion into biogas and biofertilizer because it presents less acidic pH, lower biochemical oxygen demand, and a phytosanitary effect with an increase in plant resistance. In addition, biogas can be used directly in industrial boilers as another source of energy.

# Urban Pollution in the City of São Paulo: Benefits of Ethanol

The city of São Paulo has 5.2 million cars and 11,000 buses. Air quality is poor, leading to respiratory and health problems. Air pollution consists of gases and solid particles emitted by the exhaust pipes of vehicles. To reduce air pollution, one option could be to diminish the quantity of automobiles in urban areas. A more viable solution for the short to medium

Table 1 Consumption of Electrical Energy per Inhabitant in Different Countries

Country	Electrical energy (kwh/inhabitant)
Norway	25,000
Canada	16,000
United States	12,500
Australia	9500
Belgium	7300
United Arab Emirates	6800
France	6600
Hong Kong	5200
Ireland	5000
Russia	4800
Spain	4300
Belgium	7300
South Africa	4000
Portugal	3600
Hungary	3300
Poland	3200
Kazakhstan	3150
Lebanon	3000
Venezuela	2850
Croatia	2800
Libya	2680
Malaysia	2600
Uruguay	2400
Jamaica	2350
French Guiana	2300
Romania	2200
Argentina	2100
Brazil	2000

term is the total replacement of gasoline with ethanol as fuel for automobiles, buses, and trucks.

Gas from the exhaust pipe of an ethanol motor contains water vapor, carbon monoxide, and oxides of nitrogen, as well as unburned hydrocarbons such as acetaldehyde. The gas emitted by gasoline and diesel oil motors also contains oxides of sulfur, solid microparticles of carbon, and aromatic hydrocarbons. Some studies conducted at the University of São Paulo showed that an increase in the number of ethanol cars decreased the number of deaths of the elderly by lung infections (4).

### **Production of Electrical Energy from Sugarcane Bagasse**

The Brazilian market for electrical energy has experienced rapid and sometimes turbulent growth, owing to the steady growth in demand and the shortage of infrastructure for power utilization, and to distribution.

The relatively low per-capita power consumption (Table 1) provides opportunities for power production from new sources such as sugarcane residues.

Sugarcane bagasse represents a quantity of energy equivalent to the nuclear power plant located in the State of Rio de Janeiro. The Brazilian energy matrix, which is the sum of all types of energy, is composed mainly of petroleum (fuel) and water (hydroelectrics). The hydroelectric reservoirs pass through a period of low water level, from April to October, corresponding exactly to the sugarcane production period in the Center-South region. This situation gives sugarcane bagasse a strategic position in the logistic structure of the Brazilian energy matrix (5).

#### **Steam Generation**

Bagasse is a very important raw material in the industrial sugarcane unit. It is the fuel used in the productive process. It contains a low sugar concentration ( $\leq$ 4% [w/w]) and humidity (about 50% [w/w]) and generates enough steam to drive heavy machines in the manufacture of sugar powder and ethanol. Part of the steam is currently used to produce electrical energy for the industry, and for the government.

### **Projects Among Industries and Academics**

In the next 10 yr, the generation of electrical energy from sugarcane bagasse could, be equivalent to 25% of that produced by the Itaipu Hydroelectric Company (located in Brazil, Paraguay, and Argentina), which is responsible for providing electricity to 20% of the Brazilian population. Today, Brazil has 308 sugar powder and ethanol facilities, each processing an average of 1 million of cane/yr. One ton of sugarcane produces about 140 kg of bagasse (~50% w<sub>water</sub>/w<sub>bagasse</sub>), of which 90% is used in the production of energy (thermal and electric). The same ton of sugarcane is associated with 140 kg of straw, which, at present, is burned or abandoned in the field. The energy potential represented by these residues is impressive. In the next 10 yr, the aforementioned 308 facilities would be able to generate 3 to 4 GW of energy, sufficient for the annual consumption of 8.5 million people. The challenge is to supply the industries with appropriate technology for the energy production. The São Francisco industry, located in Sertãozinho City, State of São Paulo, has a 12-yr contract with the Companhia Paulista de Força e Luz (Paulista Company of Power and Light/CPFL) to provide 12 MW every month, sufficient to illuminate a city of 300,000 inhabitants for 1 mo. The production of energy corresponds to 5% of the company profit (5).

# Alternative Products Proceeding from Reutilization of Sugarcane Bagasse

In Brazil, various technologies have been developed for producing commercial products from bagasse, such as natural fiber for use as sanitation

tubes, reservoirs, swimming pools, piping, and water and sewage treatment stations. Sugarcane bagasse also represents an inexpensive source of raw material, which can be converted into several chemical feedstocks using biotechnology. Sugarcane bagasse is composed of three major components: cellulose, hemicellulose, and lignin. Cellulose and hemicellulose (>60% of sugercane's dry matter) are converted by hydrolysis into glucose and xylose, respectively. The glucose can be used directly as a sweetener, or it can be converted into ethanol and many other products based on fermentations. Xylose can be manufactured into xylitol, ethanol, microbial enzymes, and other industrial chemicals. Although, at present, there remain several technical obstacles to the economic use of the hemicellulose component of bagasse, several research projects, basic and applied, have been carried out and many useful data generated. These projects include the production by submerged and semisolid fermentation of xylose, single-cell protein, enzymes, and lactic acid. From these observations it can be concluded that bagasse not only is a fuel but also has a very high potential for producing different products with high commercial values.

## Production of Biodegradable Plastic in Brazil

A project dedicated to the production of biodegradable plastic (PHB and its copolymer polyhydroxybutyrate/valerate [PHB-HV]) using sugarcane juice as a substrate started in 1992 through a partnership among Copersucar (Technology Center Copersucar [CTC]), the industry Usina da Pedra, the Institute of Technological Research (IPT) of the State of São Paulo, and the Institute of Biomedical Sciences of the University of São Paulo, with financial support from PADCT/FINEP. In this project, IPT studied the fermentation parameters and CTC developed the technology for extraction and purification of the plastic, and, in 1995, a pilot plant was installed in the Usina da Pedra industry. The production capacity was about 1.5–2.0 t/mo. In the year 2000, with the creation of the firm PHB Industrial S/A, the pilot plant was remodeled and started operating with a capacity of 60 t/yr. After the conclusion of an economically viable production process, in 2004, a commercial plant was installed with a capacity of 2000 t/yr.

The biodegradable plastic is basically composed of carbon, oxygen, and hydrogen and can derivate a copolymer (PHB-HV). The most important property of this plastic is its production from a renewable raw material, such as sugarcane. This plastic is a biocompatible product, and its production is carried out by means of fermentation (by Alcaligenes sp.) of sugar from sugarcane that initially is converted into a mixture of glucose and fructose by an enzymatic process. In the process, a superior alcohol is also utilized as a solvent to extract the biopolymer. The effluents are basically water and organic matter produced by the bacteria and can be employed as an organic fertilizer. At present, the main applications for

PHB are in the technology of extrusion and thermoforming; in the substitution of olefinic polymers; in the packaging of cosmetics, food, and medical solutions, and in agriculture.

# Biotechnology as a Tool to Increase Sucrose Content in Sugarcane

Molecular biology and genetic engineering are the most recent tools that plant breeders are using to cope with the growing demands from the sugarcane industry. To discover the genes from sugarcane, a network of 22 Brazilian laboratories participated in the Sugarcane Expressed Sequence Tag Project (SUCEST) launched in September 1998. The project was supported by CTC, a private company, and the State of São Paulo Research Foundation (Fundação de Amparo à Pesguisa do Estado de São Paulo. [FAPESP]). The strategy was based on expressed sequence tags, which are DNA sequences derived from the coding region of a gene, i.e., a region that contains information on the product of a gene (sucrose synthase, xylanase, and so on). This avoids the sequencing of noncoding DNA regions, which have no function attributed so far and may represent >50% of the genome. The sequencing, completed in 2001, produced 291,689 DNA sequences, which were grouped, forming a set of 33,620 unique genes representing about 90% of all sugarcane genes (6). Another 15 laboratories joined the sequencing group to organize the knowledge derived from this large number of genes (7). A series of 37 articles was published in a special 2001 issue of Genetics and Molecular Biology describing the genes that sugarcane uses in signal transduction, pathogen response, photoreceptors, cell cycle, and aluminum tolerance, among many others aspects.

The real test for the biotechnological use of a gene is the performance of transgenic plants in which the expression of the gene is changed. In Brazil, two laboratories have produced transgenic sugarcane resistant to the herbicide ammonium gluphosinate (8) and a second cultivar expressing a proteinase inhibitor from soybean, aimed, thus far without success, at insect resistance (9). A partnership between SUCEST and Cropdesign, a company in Belgium, was established in 2001, and 1000 genes were selected to be transferred to rice, a model plant. The first transgenic plants with interesting characteristics were obtained in 2003, and the genes producing useful traits will be transferred to sugarcane by Brazilian laboratories in cooperation with local industries (10). Two other private companies, Allelyx and Canavialis, started in 2003 and are developing work on genomics, biotechnology, and classic breeding. It is expected that in 2 or 3 yr they will present the first results.

To discover genes involved in sugar production, a functional genome approach is under way by Brazilian laboratories and two local private companies, Copersucar and Center de Álcool Lucélia, and FAPESP. The strategy is to discover genes that are expressed in association with high levels of

sugar production in leaves and stalks of selected sugarcane varieties. This will be done using DNA array technologies, which allows quantification of the expression levels of thousands of genes in parallel. Preliminary results using about 3000 genes have shown that more than a hundred genes are more active in tissues with high sucrose content (unpublished results). Among them are genes encoding sugar transporters and enzymes known to be involved in sucrose metabolism. Interestingly, most of the genes that were discovered in these analyses have not previously been related to sugar metabolism. Transgenic plants expressing high levels of these proteins will be evaluated this year at the Sugarcane Technology Center, a research center supported by 80 sugarcane mills. The objective is to produce about 20 new transgenic sugarcane varieties in the next 3 yr.

In addition to sugar production, several other biotechnological applications of genes are under way: insect resistance, phosphate absorption, interaction with beneficial microorganisms, and water deficit stress. In fact, Nogueira et al. (7) identified several genes that are activated by sugarcane under low-temperature stress. Transgenic plants containing one of the genes encoding a transcriptional factor have been obtained and their evaluation will be finished soon.

#### Acknowledgments

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