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ORIGINAL PAPER

Grain sorghum is a viable feedstock for ethanol production

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Abstract Sorghum is a major cereal crop in the USA. However, sorghum has been underutilized as a renewable feedstock for bioenergy. The goal of this research was to improve the bioconversion efficiency for biofuels and bio-based products from processed sorghum. The main focus was to understand the relationship among "genetics–structure–function–conversion" and the key factors impacting ethanol production, as well as to develop an energy life cycle analysis model (ELCAM) to quantify and prioritize the saving potential from factors identified in this research. Genetic lines with extremely high and low ethanol fermentation efficiency and some specific attributes that may be manipulated to improve the bioconversion rate of sorghum were identified. In general, ethanol yield increased as starch content increased. However, no linear relationship between starch content and fermentation efficiency was found. Key factors affecting the ethanol fermentation efficiency of sorghum include protein digestibility, level of extractable proteins, protein and starch interaction, mash viscosity, amount of phenolic compounds, ratio of amylose to amylopectin, and formation of amylose-lipid complexes in the mash. A platform ELCAM with a base case showed a positive net energy value (NEV) = 25,500 Btu/gal EtOH. ELCAM cases were used to identify factors that most impact sorghum use. For example, a yield increase of 40 bu/ac resulted in NEV increasing from 7 million to 12 million Btu/ac. An 8% increase in starch provided an incremental 1.2 million Btu/ac.

Keywords Grain composition · Sorghum · Bioconversion · Bioprocess · Ethanol · Fermentation efficiency · Energy life-cycle analysis

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Introduction

The US demand for ethanol has increased sharply in recent years. A major driver for this demand has been the value of ethanol as an oxygenate, replacing methyl tert-butyl ether (MTBE) in gasoline. The domestic oxygenate market volume, based on an E10 blend (10% ethanol:90% gasoline blendstock), is projected to be 14–15 B gallons of ethanol. US ethanol production has been increasing with 4.8 B gallons in 2006 and a projected production volume of ~6.2B gallons in 2007, and ethanol imports have been ~600 to 700 K gallons/year [1]. Clearly, the potential volume for ethanol based on oxygenate use alone, irrespective of additional use as an alternative fuel, remains much higher than current production plus import volume. Consequently, there

has been relatively rapid growth in new construction of ethanol facilities, especially across the US Corn Belt region. In some local areas within the Corn Belt, the concentration of ethanol production facilities is reaching near saturation, relative to the volume of corn grain being available (over and beyond other uses such as feed) within the collection vicinity. Opportunities for continued expansion of ethanol production still exist in several areas around the Corn Belt and in other agricultural regions. One area in particular has high potential for increased contribution—that being the sorghum production region of the Central Plains. Currently, feedstock for commercial ethanol production is ~95% corn grain and ~4% sorghum grain [1]. Researchers and ethanol producers have shown that grain sorghum is a reasonable feedstock (technically acceptable, fits the infrastructure, and can be economically viable) for ethanol and could make a larger contribution to the nation's fuel ethanol requirements [2–4].

In approximate terms, ethanol yield from sorghum grain is comparable to that from corn grain. However, in the past, factors impacting ethanol yield were less well studied for sorghum than for corn. Little research has been conducted on performance of sorghum varieties in ethanol fermentation. Zhan et al. studied the effect of genotype and location on ethanol and lactic acid production of a limited number of sorghum genotypes [5]. Several researchers have investigated the digestibility of sorghum starch [6–8] and sorghum protein [7, 9–11] as related to its use in feed or food. Others have investigated the isolation of sorghum starch [12–16] and its properties [17–19]. The economic viability of an ethanol production facility depends on several factors, including ethanol yield, efficiency of conversion, and quality of the "distiller's grain" (grain residue and yeast mass remaining after the fermentation process). After several years of research on ethanol production from grain sorghum, our group has discovered some important factors that significantly impact the performance of grain sorghum for ethanol production. These factors have been quantified via use of our energy life cycle analysis model (ELCAM) for grain sorghum, which allows a comparative determination of the improvement in relation to energy use and profitability. It is expected that both sorghum growers and ethanol plant managers will benefit from subsequent application of our basic findings on factors impacting ethanol yield, conversion process, quality of distiller's grain, and relative energy conversion that may be achieved.

Materials and methods

Materials

Seventy sorghum genotypes and elite hybrids with a broad range of chemical compositions and physical properties were used for this study. The sorghum samples (~100 g) were cleaned by removing the debris and other contaminants, and milled through a 0.5-mm screen in a Udy cyclone mill (Udy Corp., Fort Collins, CO, USA) and used for chemical analysis. Samples for ethanol fermentation were milled in a Magic Mill III Plus Grain Mill (Magic Mill Products & Appliances, Monsey, NY, USA) set at level III.

Ethanol fermentation

Ground samples containing 30.00 g dry mass were mixed with 100 mL of preheated (~60 to 70 °C) enzyme solution containing 0.1 g of KH_2PO_4 and 20 μL of Liquozyme SC DS in a clean 250-mL Erlenmeyer flask to form an evenly suspended slurry. The flasks were kept at 70 °C in a rotary water-bath shaker operating at ~180 rpm. The temperature of the water bath was raised from 70 to 90 °C in 35–40 min, kept at 90 °C for a few minutes, and then lowered to 85 °C; liquefaction continued for 60 min. Materials sticking on the inner surface of the flasks were pushed back into the mashes with a spatula. The spatula and inner surface of the flasks were rinsed with 3–5 mL of distilled water. After cooling to room temperature (~25 to 30 °C), pH of the mashes was adjusted to around 4.2 with 2 N HCl.

Before inoculation, the dry yeast was activated by adding 1.0 g of active dry yeast into 19 mL of preculture broth (containing 20 g of glucose, 5.0 g of peptone, 3.0 g of yeast extracts, 1.0 g of KH_2PO_4, and 0.5 g of $MgSO_4 \bullet 7H_2O$ per liter) and incubated at 38 °C for 25–30 min in an incubator operating at 200 rpm. The activated yeast culture had a cell concentration of 1×10^9 cells/mL.

The simultaneous saccharification and fermentation (SSF) process started with the addition of 1.0 mL of activated yeast culture, 100 μL of Spirizyme Fuel (750 AGU/g, ~1.15 g/mL), and 0.30 g of yeast extract into mashes in each flask. Fermentation was conducted at 30 °C for 72 h in an incubator shaker operating at 150 rpm. The conversion efficiency was calculated from the theoretical yield of 56.72 g of ethanol produced from 100 g of dry starch (assuming 1 g of starch could be hydrolyzed into 1.11 g glucose and each gram of glucose could generate 0.511 g of ethanol).

Viscosity measurement

A 10-min liquefaction test was carried out using a Rapid Visco Analyzer (model RVA-4, Newport Scientific, Warriewood, Australia) as described by Wu et al. [2]. Ground sorghum samples (8.00 g, 14% moisture content) were dispersed in 21.0 mL of distilled water in aluminum RVA cups to give mashes with ~24% solid contents. The solids were dispersed by stirring at high speed (960 rpm) for 10 s before the measurement phase. Viscosity properties of the

tested samples were recorded for 10 min at 95 °C, with a constant stirring speed of 160 rpm.

Differential scanning calorimetry analysis

Differential scanning calorimetry (DSC) analysis of meal and starch samples of sorghum and corn were performed with a Pyris 1 differential scanning calorimeter (Perkin Elmer Corp., Norwalk, CT, USA) equipped with the Pyris 1 Data Analysis System for Windows. Samples (~10 mg) were weighed in a stainless steel pan on a CAHN 21 automatic electrobalance (Cerritos, CA, USA) and were mixed with distilled water (~35 μL) to give mixtures with 75–80% moisture content. The sealed samples were kept at 4 °C overnight before being tested. A sealed, empty stainless steel pan was used as reference. All samples were held at 20 °C for 1 min and heated from 20 to 160 °C at 10 °C/min.

Confocal laser scanning microscopy (CFLSM) images

Proteins in original sorghum or mash residue samples were labeled with fluorescein isothiocyanate (FITC) and examined by confocal laser scanning microscopy. Samples of 100 mg of sorghum flour (milled through 0.25-mm screen with a Udy cyclone mill) or 200 mg of mash residues (separated by centrifuge before inoculation of yeast) were mixed with 1 mL of 0.05% FITC solution (in 0.5 mM NaOH) and incubated in the dark for 1 h at room temperature. After being centrifuged at 10,000*g* for 4 min, the pellet was spread on a glass slide and allowed to dry at room temperature in dark. Protein microstructure was visualized using a laser-scanning confocal microscope (Zeiss LSM 5 PASCAL, Carl Zeiss MicroImaging, Inc., Thornwood, NY, USA). Prior to imaging, one drop of oil was added to the sample. A cover slip was placed on it, and another drop of oil was added on top of the cover slip to achieve higher resolution [20]. Sorghum protein fluorescence was analyzed using 488-nm excitation through a 505–530 band-pass barrier filter for detection of FITC. Optical sections of samples were collected with a z-step of 0.9 μm throughout the sample thickness. 3-D images comprised more than 25 laser-generated optical planes in z-sectioning. Only the single optical plane in the middle of the z-series overlaid from transmitted and fluorescence images, is presented.

Analytical methods

Nitrogen contents were analyzed by combustion [21] using a nitrogen determinator (FP-528, Leco Corp., St. Joseph, MI, USA). Nitrogen values were multiplied by 6.25 to convert to protein values. Total starch contents were determined using Megazyme total starch kits [22]. In vitro protein digestibility tests were modified from the method of Mertz et al. [23] as follows: 200 mg of sorghum samples were suspended in 35 mL of pepsin solution (1.5 g of enzyme/L of 0.1 M potassium phosphate buffer, pH 2.0) and incubated with vigorous shaking at 37 °C. Pepsin digestion was stopped at 2 h with the addition of 2 mL of 2 M NaOH. After centrifuging at 4,000 × *g* for 15 min, the supernatant was discarded, and the residue was washed in 10 mL of 0.1 M phosphate buffer (pH 2.0) and centrifuged as before. After the second washing and centrifugation steps, the residue was frozen and then lyophilized. The freeze-dried residue was then weighed and analyzed for nitrogen content. The pepsin used was porcine pepsin 1:10,000 (Sigma P-7000; activity 924 units per mg of protein). Ethanol concentration in the finished beer was determined by HPLC after distillation as described by Wu et al. [3].

Results and discussion

Effects of sorghum variety and type of starch

The ethanol production process basically converts starch from grain sorghum into ethanol. Therefore, the higher-starch-content grains might be expected to result in higher ethanol yield. Starch content in the sorghum genotypes that we studied ranged between 64 and 74% of grain dry weight. On average, this starch differential should result in up to a 15% calculated difference in ethanol volume per unit of grain used. However, our research also showed that not all starches in the different sorghum varieties contribute equally for ethanol production. An analysis of sorghum varieties with similar starch percentage amounts demonstrated that variations in ethanol yields could be as large as 7.4% (Fig. 1).

In general, waxy and heterowaxy sorghum varieties have higher ethanol yields than other non-waxy trait varieties, at the same starch level. These differences can be explained by the adverse effects of higher amylose content in regular

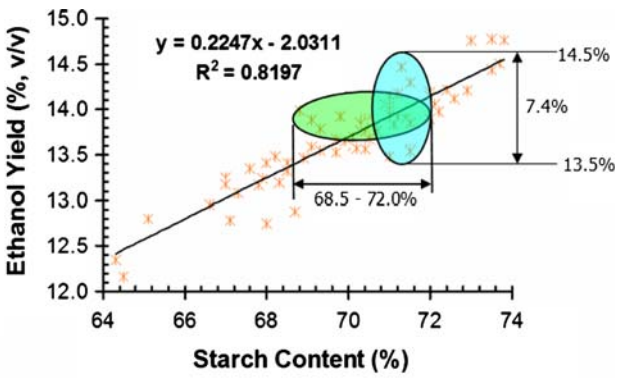

Fig. 1 Relationship between ethanol yield and starch content of sorghum

variety during gelatinization. Poor gelatinization subsequently restricts hydrolytic enzyme access to the starch molecules, resulting in poorer conversion to glucose sugar. The DSC thermogram of normal sorghum with 25% amylose content before enzymatic hydrolysis (or cooking process) showed a prominent endothermic peak at 90–105 °C (Fig. 2), which was assigned to an amylose–lipid complex [24]. After enzymatic hydrolysis, the area representing amylose–lipid complexes increased and the peak occurred at 105–120 °C. This result indicates that some starch reacted with lipids to form new polymers. The starch in the amylose–lipid complex cannot be converted into fermentable sugar; therefore, the formation of amylose–lipid complexes reduced the starch-conversion rate and final ethanol yield. This result further confirmed that low amylose grains are preferred for ethanol fermentation.

Protein quality and starch–protein interaction

In general, ethanol yields decreased as protein content increased, due to an inverse relationship between starch and protein content in a unit mass of grain. However, at the same protein level, ethanol fermentation efficiency varied as much as 8%; this level was higher than typical experimental variations, indicating that factors in addition to protein content were also affecting the starch-conversion rate (Fig. 3). Nine sorghum genotypes (hybrids or breeding lines) covering a broad range of ethanol fermentation efficiencies were selected and used to study the effect of protein quality on ethanol-fermentation efficiency. The results showed a strong linear relationship between protein digestibility and fermentation efficiency ($R^2 = 0.91$) (Fig. 4). Conversion efficiency increased as protein digestibility increased.

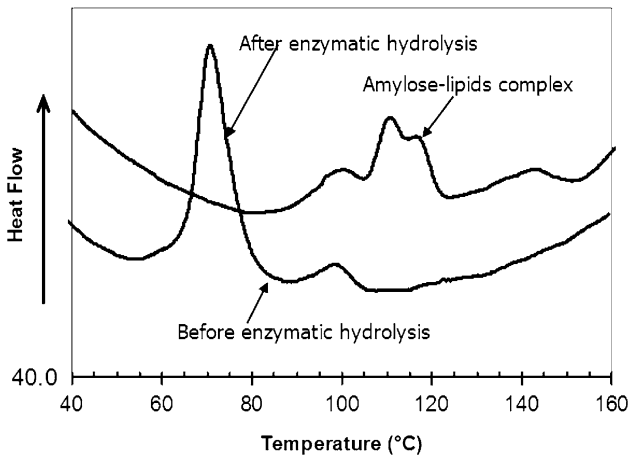

Fig. 2 DSC thermograms of sorghum grain before and after enzymatic hydrolysis

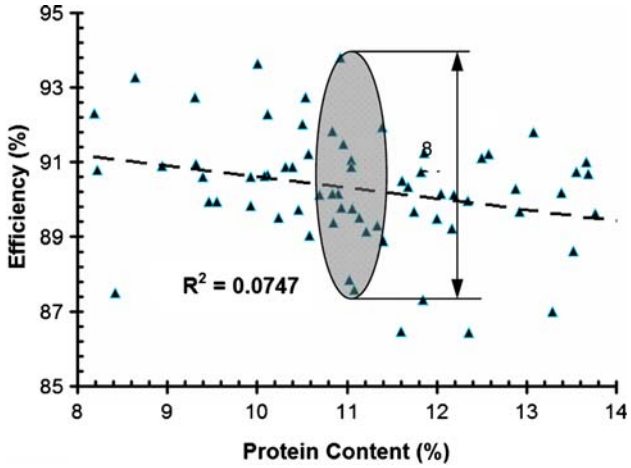

Fig. 3 Relationship between ethanol fermentation efficiency and protein content

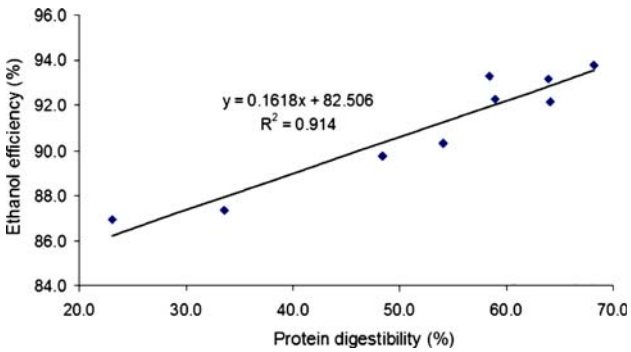

Fig. 4 Relationship between ethanol fermentation efficiency and protein digestibility

It is possible that sorghum samples with high protein digestibility provided more free-amino acid for yeast growth during fermentation. However, it is more probable that the starch–protein interaction had a major effect on conversion efficiency and fermentation yield. Small starch granules may be embedded in the protein matrix during grain development and remained ungelitanized during cooking, and some starch may remain embedded in the protein matrix due to protein denaturation during cooking (Fig. 5). It appears that the embedded starch granules are inaccessible to hydrolytic enzyme degradation and, consequently, are not converted into glucose for yeast fermentation. In addition, the relatively low digestibility of the sorghum protein matrix will contribute to this effect. Our mechanistic hypothesis is that cross-linking of sorghum proteins during cooking, and the formation of a web-like protein matrix, restricts the accessibility of hydrolyzing enzymes to the starches within the protein matrix (Fig. 5). The research results showed that sorghums with the highest degree of protein cross-linking had the lowest fermentation efficiency under all conditions.

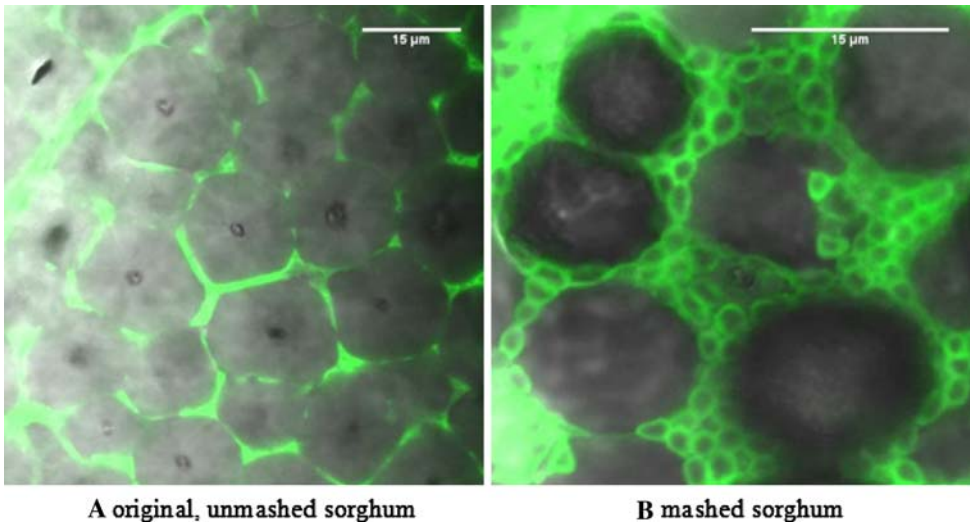

Fig. 5 Confocal laser scanning micrographs (single optical planes) of a typical sorghum sample before (**a**) and after (**b**) mashing, with the protein matrix (*green areas*) stained with FITC. Mashed residue shows highly extended, strong web-like protein microstructure

Tannin and mash viscosity

Tannins have been previously recognized as having adverse effects on starch digestion because of their ability to interact with proteins (including hydrolytic enzymes). Therefore, the starch hydrolysis process of tannin sorghum is usually slower than that for waxy and normal sorghums. At any given point in time during liquefaction, the final mash viscosity of tannin sorghum is higher than that for waxy and normal sorghums. Since ethanol fermentation efficiency increased as mash viscosity decreased (Fig. 6), the more viscous mash is expected to have negative consequences for the commercial conversion process. Viscous mashes not only lead to incomplete hydrolysis of starch but also increase the required process energy due to more sluggish flow and mixing properties, and generally lower the efficiency of heat exchange. The average ethanol conversion efficiency ranged from 86 to 93.8% for the 70 sorghum varieties evaluated. The difference in efficiencies among the sorghum color was not significant ($P < 0.05$), except for the brown tannin-containing lines. Results with these genetic lines confirmed that high-tannin varieties are not a good choice for ethanol production.

Particle size of ground sorghum meal

Particle size of the ground sorghum meal also plays an important role in the starch-to-ethanol conversion process. Fermentation efficiencies of the finely ground samples were approximately 5% higher than the coarsely ground samples. This effect may have been a consequence of the difference in gelatinization temperature and accessibility of starch to the hydrolyzing enzymes. Gelatinization temperatures of larger particles were 5–10 °C higher than those for smaller

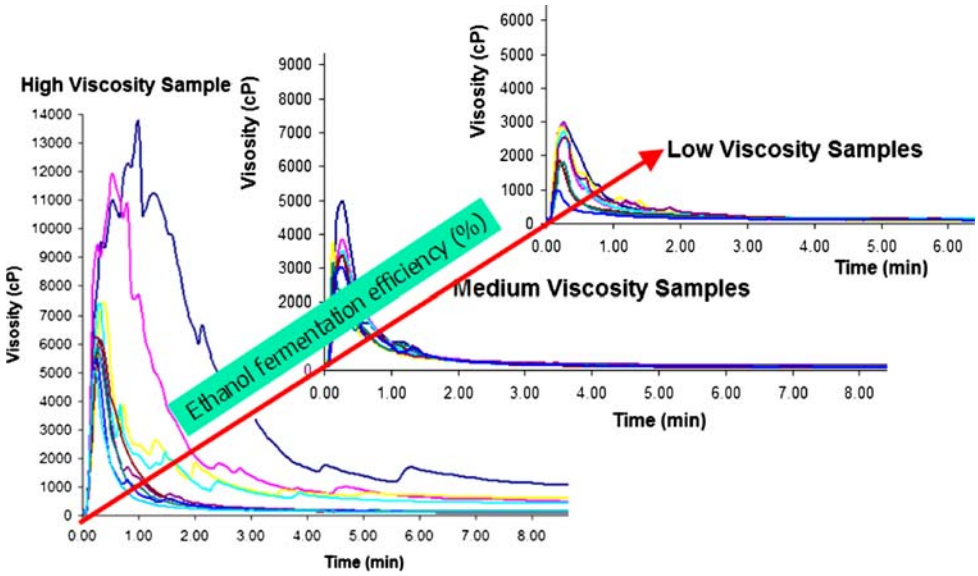

Fig. 6 Effect of mash viscosity on ethanol fermentation efficiency

particles by DSC, which likely influenced the completeness of starch hydrolysis resulting in lower fermentation efficiency for the larger particles. Several other researchers have reported similar results: for example, ethanol yield from finely ground (0.5-mm sieve) corn meal was 2.2% (v/v) higher than that from coarsely ground (5-mm sieve) meal, representing a ~20% gain [25]. Therefore, ethanol plants should grind the feedstock grain as fine as possible, balanced by grinding costs and avoidance of downstream process issues.

Quality of distiller's grain (DG)

Sale of DG accounts for 15–20% of the annual revenue of an ordinary dry-grind ethanol plant and returns the "unused" portion of the processed grain back into the feed system. The quality of DG will directly impact its feeding value and final revenue to the ethanol plant. Most of the DG from dry-grind ethanol plants is used as animal feed for ruminants such as dairy and beef cattle. A small amount (typically less than 15%) may be added to monogastric (hogs, poultry) diets, with the limitation being due to the presence of difficult-to-digest lignocellulosic material. In practice, it is also becoming more common to feed DG wet rather than dry, which saves energy costs in drying but necessitates a local market within truck-delivery range. Another important compositional factor impacting DG quality is protein content: since grain starch is largely converted into ethanol and the protein remains, final protein content in the DG is about triple that in the starting grain. As we discussed above, grain starch content is typically inversely related to grain protein content, thus the high starch varieties provide higher ethanol yield but typically give lower protein DG. Therefore, ethanol plants should closely monitor protein contents of their feedstock and final DG to guarantee it will meet minimum requirements for particular customers.

A secondary impacting factor is that of mycotoxins, which arise from fungal infection of the grain. The FAO has estimated that ~25% of the world's grain supply is contaminated by various kinds of mycotoxins. These toxins can negatively impact the performance of the fermentation yeast in the ethanol conversion process and can also become concentrated in the DG (up to threefold) feed material. The FDA has set limits for some of the common mycotoxins such as aflatoxins, vomitoxin, fumonisins, and zearalenone in animal feeds. Incidence and contamination level of mycotoxin-producing fungi are generally lower in sorghum than in corn. While all DG production must be monitored for any "local" contamination by mycotoxins, in general, DG from grain sorghum is less likely to have mycotoxin problems than DG from corn.

Energy life cycle analysis model

An ELCAM was developed to provide a reference platform for the total energies involved in grain sorghum production and processing to ethanol and DG (Fig. 7). While this model is basically a full-life cycle energy-accounting system, several aspects on the use (and misuse) of such models should be placed into context:

- All conversion processes appear to "lose" energy, e.g., the conversion ratio for gasoline production from oil is generally taken as ~0.8. Of course, energy is neither created nor destroyed and is usually dissipated as heat or an increase in entropy.
- In the case of ethanol from corn or sorghum, the typical ratio is between 1.0 and 1.3 with the actual number being heavily assumption dependent. The conversion ratio is only above 1.0 because crop systems capture solar radiation as an additional external input of energy. The solar radiation energy component is inherent, but not specified, in typical life cycle analyses.
- Energy, as used by humans, has many different values that are not directly proportional to the physical measurement of energy content. For example, electricity costs more than coal for the exact same amount of British thermal units (Btus). Therefore, there is a "social value" beyond the physical energy content, which includes factors such as convenience, safety, security, and availability. A reasonable case can be made that the absolute physical Btus of ethanol, or the conversion process, are irrelevant since ethanol provides a "social value" beyond that of unit energy alone. For example, ethanol is an excellent oxygenate that can be added to gasoline to provide accelerant power plus cleaner combustion of the blended gasoline, and all in a convenient liquid form.

Notwithstanding the above points, the ELCAM was used as an internal reference platform to evaluate the experimental variables. The results highlight some areas in production and process improvements where energy capture could be improved in systems similar to our particular set of events. From the input modules (Fig. 7), the ELCAM was used to generate a series of results for each set of input variables. A typical output set of energies is shown in Fig. 8—this was then used as an internal platform case against which we evaluated production and process variables.

The difference between energy inputs and energy returned in the products is termed the "net energy value" (NEV). If NEV is positive, then there is an apparent gain in energy. The fact that the NEV is positive for the sorghum case (positive 25,500 Btu/gal ethanol for this particular case) is because captured solar radiation energy is in the products but is not accounted for as a direct input.

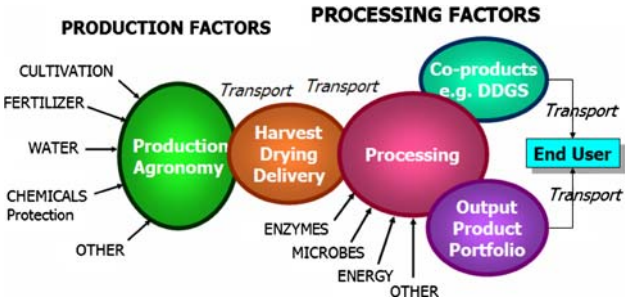

Fig. 7 Major factors and the related sub-routines in the ELCAM for grain sorghum

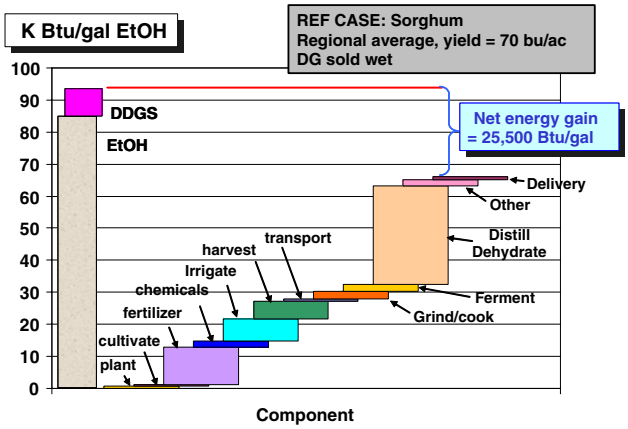

Fig. 8 Energy balance for ethanol production from grain sorghum. These particular values are from one ELCAM run that we call the base reference case

An important aspect that must be considered is whether we want to improve the efficiency of ethanol production per unit volume of product, per unit weight of feedstock, or per unit area of land resource used. These factors clearly do not necessarily result in the same issue and will require different solutions; yet very seldom are any of these factors explicitly explained in the results of energy models and are never referenced in the selective headline proclamations concerning biofuels. Figure 9 shows the $NEV_{refcase}$ for two different units of output and the impact of yield (weight of feedstock per unit land) on the projected results.

Clearly, grain yield is a large determinant of the NEV obtained. However, these results are for a given case of assumptions. The probability of obtaining an overall average yield of 150 bu/acre declines in the absence of irrigation, and irrigation is an energy intensive input (Fig. 8). We have run the model with and without irrigation energy inputs, but for simplicity, the results are shown for only the 150 bu/acre situation. For example, in Fig. 9, the circles represent the NEV for the respective units if the land had to be irrigated to achieve that level of yield.

The ELCAM was used to evaluate several potential factors for improvement, especially the experimental variables

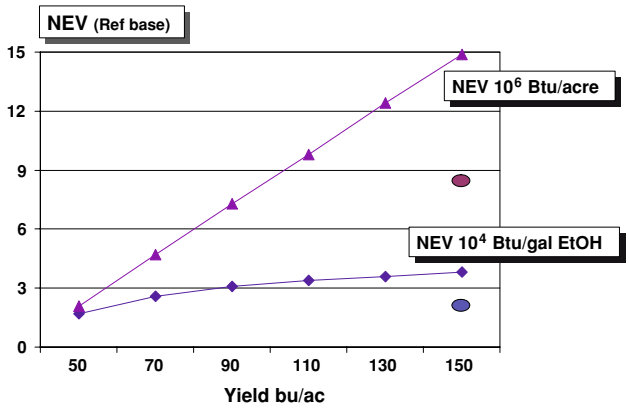

Fig. 9 NEV values for different levels of grain yield per acre, either on a per gal ethanol basis or per unit land. Note that the power of the Btu units has been adjusted to show both on the same chart. The *circles* represent the NEV for the respective units if the land had to be irrigated to achieve that particular level of yield

studied in the research project. For example, grain starch composition varied across different germplasm lines (and locations), and the impact of increasing starch level within our system was very positive (Table 1). The results indicate that increasing starch level, even at the same grain yield level, has a large effect: Table 1 shows that an increase in starch of 17.6% (across the range of numbers shown) translates into a 40% increase in NEV per unit area. The "multiplier" effect arises from a combination of factors, such as transport energy being the same, irrespective of grain starch level.

The empirical research and model simulations carried out in this project have clearly shown that grain sorghum is a viable feedstock for ethanol, and that improvements in ethanol yield and efficiency of conversion can be achieved via yield per unit land using genotypes that store higher levels of starch, altering starch types in the grain, manipulating the protein matrix in the grain to allow easier access for hydrolytic enzymes, avoiding the higher-tannin-content phenotypes, and by physical grinding of the grain at the ethanol plant. Sorghum is more tolerant to water–stress than corn and is often grown in the drier regions (generally to the west of the Corn Belt). We propose that such areas

Table 1 The NEV on a unit product basis and unit land used basis for differing levels of grain starch content

Starch content (% db)	Energy recovery	
	10^4 Btu/gal EtOH	10^6 Btu/acre
68	2.38	4.2
72	2.56	4.7
76	2.71	5.3
80	2.85	5.9

could be utilized more for the production of ethanol from grain sorghum. Existing sorghum is a viable feedstock with a known cropping system, germplasm programs, and agronomic and cultural knowledge. With a relatively small amount of research focus, the existing grain sorghum germplasm could be further enhanced in several ways, including those demonstrated in our research and reported here. Thus, sorghum grain can be provided that would make a significant additional contribution to the ethanol market, and this could be achieved in the near term and with a high return on R&D funds.

Conclusion

Results from this research will allow more focused development and improvement of processing methods based on knowledge of how particular grain properties impact bioconversion to commercial products. With confirmation of key impact factors found in our research, it is envisaged that utilization of processed sorghum for industrial uses could be increased. Sequencing of the sorghum genome is underway, which will provide a stronger genetic foundation to assist in the functional applications of the results reported here. Application of research findings to bioprocessing of sorghum grain could benefit both grain producers and the bio-industry via the following areas: (1) approaches and capabilities to further improve the efficiency of sorghum processing; (2) improvement in sorghum conversion yield to industrial products, thereby improving sorghum economics; (3) information to assist in development of new and improved sorghum hybrids; and (4) enhancement of economic rural development through expanded sorghum production, especially across the many drier sorghum-growing states.

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