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# Sorghum as a Multifunctional Crop for the Production of Fuel Ethanol: Current Status and Future Trends

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## 1. Introduction

Nowadays, there is a growing interest for alternative energy sources because of the reduction of fossil fuel production. Ethanol used as automotive fuel has increased at least six times in the current century. According to the Renewable Fuels Association, in 2010 the USA bio-refineries generated 13 billion gallons of fuel ethanol and the year before worldwide production reached 19 billion. This noteworthy increment is in its majority based on maize and sugar cane as raw materials (Berg, 2004; Renewable Fuels Association, 2010). The use of these feedstocks has triggered concerns related to food security especially today when the world population has reached 7 billion people.

The relatively sudden rise in food prices during 2008, 2010 and 2011 has been attributed mainly to the use of maize for bioethanol even when other factors like droughts or changes in global consumption patterns have also played a major role (World Food Program, 2008). Food price projections indicate that this situation will worsen, breaking the downward trend registered in food prices in the last thirty years (The Economist, 2007).

Even if there was not a food-fuel controversy especially due to the current conversion of millions of tons of maize for bioethanol, the use of only this crop cannot support the ambitious objectives of renewable fuel legislation in countries like the United States of America, where a target of 36 billion gallons of liquid biofuels have been established for 2022. In order to meet this requirement all the 333 million tons of maize yearly produced by USA should be channelled to biorefineries. This production represents 2 and 16 times the maize harvested in countries like China and Mexico respectively, which in turn are two of the five top world producers.

Environmental factors have been also pushing for the quest of new crops dedicated exclusively for liquid automotive fuel in order to reduce the use of prime farming land, irrigation water and other resources. A dedicated energy crop ideally must meet several requirements such as: high biomass yield and growth rate, perennial, with reduced input necessities, fully adapted to the geographic regions where will be planted, easy to manipulate via genetic improvement, non-invasive, tolerant to stress and with a good carbon sequestration rate among others (Jessup, 2009). At the present time, energy crops are

mainly represented by perennial grasses as switchgrass (*Panicum virgatum* L.), energy cane (*Saccharum* spp), sweet and forage sorghum (*Sorghum bicolor*), miscanthus (*Miscanthus* spp.) as well as other short-rotation forest resources (willow –*Salix* spp- and poplar –*Populus* spp) (Jessup, 2009; McCutchen et al., 2008).

The development of new and improved enzymes, bioprocesses and feedstocks could lead to cost reduction from an estimated of 0.69 cents to below 0.51 cents/L that nowadays is the benchmark established for starchy raw materials (Kim & Day, 2011). Besides the development of dedicated crops for energy, one of the best approaches for cost reduction and optimal use of resources is the use of flexible facilities allowing the integration of different streams of same or different feedstocks. Flexibility, balance, diversification and regionalization are indeed keywords in the development of solutions to meet future world energy demands.

In tropical, subtropical, and arid regions from the United States, Mexico, China, India, Southern Africa, and other developing countries, where agronomic harsh conditions prevail, one of the most promising crops for fuel is sorghum (*Sorghum bicolor* (L.) Moench) (Reddy et al., 2005; Zhang et al., 2010). This is a high efficient photosynthetic crop that reached a worldwide production of 56 million tons of grain in 2009 (FAOSTAT, 2011), just behind maize, wheat, rice and barley. Almost 30% of this production is harvested in North America where sorghum is mainly used for feed. Sorghum is a C4 plant, highly resistant to biotic and abiotic factors as insects, drought, salinity, and soil alkalinity. Furthermore, this crop has one of the best rates of carbon assimilation (50 g/m<sup>2</sup>/day) which in turn allows a fast growth and a better rate of net CO<sub>2</sub> use (Prasad et al., 2007). Sorghum requires one third of the water with respect to sugar cane and 80 to 90% compared to maize (Almodares & Hadi, 2009; Wu et al., 2010b). Thus, sorghum is considered as one of the most drought resistant crops. Furthermore, sorghum requires approximately one third of the fertilizer required by sugar cane (Kim & Day, 2011) and its growth cycle is between 3 to 5 months allowing two or three crops per year instead of one commonly obtained with sugarcane. Besides environmental advantages, sorghum is one of the more acquiescent plants to genetic modification because is highly variable in terms of genetic resources and germplasm. This facilitates plant breeding and development of new cultivars adapted to different regions around the globe (Zhang et al., 2010).

Sorghum can be classified in four broad groups: grain, sweet, forage and high biomass. All belong basically to the same species and virtually there are no biological or taxonomic differences (Wang et al., 2009). Grain sorghum is used mainly as food, feed and for starch production. In the United States only a small percentage of fuel ethanol (around 2-3%) is obtained from grain sorghum (Renewable Fuels Association, 2010; Turhollow et al., 2010; Zhao et al., 2008), but in 2009 about 30% of the U.S. grain sorghum crop was used for ethanol production (Blake, 2010).

On the other hand, forage sorghum is characterized as a high biomass crop. This capacity has been boosted by intensive research programs worldwide, focused in the design of new varieties tailored for ethanol production (Rooney et al., 2007). The main product obtained from sweet sorghums is the fermentable sugar rich juice that is produced and accumulated in the stalks in a similar fashion as sugar cane. The extracted sweet juice is mainly composed of sucrose, glucose, and fructose, and thus can be directly fermented into ethanol with efficiencies of more than 90% (Wu et al., 2010b). According to Almodares & Hadi (2009) sorghum yields a better energy output/input ratio compared to other feedstocks such as sugar cane, sugar beet, maize and wheat. Altogether with the

juice, the residue or bagasse can be also converted to ethanol or used for other traditional applications.

In summary, sorghum is a crop well adapted to adverse climatic conditions which at this time is one of the growing concerns in agronomic projections. This is mainly due to the change of rain patterns and climate, greenhouse effect and the steadily rise of world temperature. Given all these advantages of sorghum as a potential source of biofuels, the objective of this chapter is to explore its potential, as an integrated crop for fuel production in terms of yield and technologies available for processing. The chapter especially focuses on optimum technologies to produce bioethanol from sweet sorghums, starchy grains and biomass from dedicated crops.

## 2. Botanical features and agronomic characteristics

Sorghum is a member of *Poaceae* family, a high-efficient photosynthetic crop, well adapted to tropical and arid climates. As a result, sorghum is extremely efficient in the use of water, carbon dioxide, nutrients and solar light (Kundiyana, 1996; Serna-Saldívar, 2010). This crop is considered one of the most drought resistant, making it one of the most successful in semi-desert regions from Africa and Asia (Woods, 2000). This resistance is due mainly to its photosynthetic C4 metabolism that allows sorghum to accumulate CO<sub>2</sub> during the night, to lower the photorespiration rate in presence of light, to reduce the loss of water across the stoma and the waste of carbon (Keeley & Rundel, 2003).

The leaves of sorghum and maize are similar but in the case of sorghum they are covered by a waxy coat that protects the plant from prolonged droughts. The sorghum grain is grouped in panicles and the plant height ranges from 120 to 400 cm depending on type of cultivar and growing conditions. An advantage of sorghum compared to maize is that it has a comparatively lower seed requirement because only 10 to 15 kg/ha are used compared with 40 kg/ha required by other cereals (Kundiyana, 1996). In some regions is possible to produce multiple crops per year, either from seed (replanting) or from ratoon (Saballos, 2008; Turhollow et al., 2010).

## 3. Chemical composition

### 3.1 Juice from sweet sorghum

The mature stems of sweet sorghum contain about 73% moisture and the solids are divided in structural and non-structural carbohydrates. Approximately 13% are non-structural carbohydrates composed of sucrose, glucose and fructose, in variable amounts according to cultivar, harvesting season, maturity stage, among other agronomic factors (Mamma et al., 1996; Phowchinda et al., 1997). Anglani (1998) suggests a classification of sweet sorghums based on proportion of soluble sugars in the juice. The first group with a high content of sucrose (sugary type) and the second with more monosaccharides (glucose and fructose) compared to other soluble carbohydrates (syrup type). Smith et al. (1987) in their evaluation of six sweet sorghum varieties throughout four years in nine different locations did not find significant differences in sugar content or composition. The typical composition indicates that around 70% was sucrose and the rest glucose and fructose in equal parts. In stem dry basis, Woods (2000) reported fermentable sugars content between 41 to 44% in Keller and Wray varieties with 80 and 63% represented by sucrose and the rest by glucose and fructose. A fiber variety analyzed by the same author (H173) reached only 20% fermentable sugars based on the dry stem weight; sucrose, glucose and fructose were found in equivalent

amounts (around 7% for each sugar). Compared to sugar cane, the main difference is that the sucrose content in cane is significantly higher compared to glucose and fructose (90, 4 and 6% respectively) and the total content sugar is 49% of the dry stem weight. In general terms, composition of simple sugars in sweet sorghum juice is 53-85, 9-33 and 6-21% for sucrose, glucose and fructose, respectively (Gnansounou et al., 2005; Mamma et al., 1996; Phowchinda et al., 1997; Prasad et al., 2007).

Beyond the proportion of soluble sugars in sweet sorghum plants, the yield of total sugars per harvested area is a better guide in the analysis for fuel production. Woods (2000) reported for sweet sorghum cultivars (Keller, Wray and H173) an average of 7, 10 and 4 ton of fermentable sugars/ha respectively, significantly lower compared to the 17 ton/ha for sugarcane indicated by the same author. The varieties studied by Davila-Gomez et al. (2011) yielded an average of 1.85 to 2.03 ton of sugar/ha, whereas Smith et al. (1987) in a extensive study performed in several locations of continental United States and Hawaii, obtained from 4.5 to 10.6 ton/ha. In other varieties evaluated in China, the best yields reached 18 ton/ha (Zhang et al., 2010).

Sugars in sweet sorghum are very sensitive to microbial contamination especially after crushing stalks for juice production. In data reported by Davila-Gomez et al. (2011), the percentage of sugars, as °Brix before fermentation, was lower (11 to 24% lower) than the obtained immediately after harvest in summer time, when temperatures easily reached 32°C in Northeast Mexico. The microbial contamination was the most obvious explanation of this phenomenon. Besides, the sucrose proportion in the fermented juices was lower in relation to glucose and fructose (0 to 10% of total). This can be related to invertase activity of contaminating wild yeasts that hydrolyzed sucrose into glucose and fructose. These monomers are quickly metabolized by means of facilitated diffusion into the yeast cell. Wu et al. (2010b), working with cultivars with 16 to 18% of fermentable sugars, found that as much as 20% of substrate can be lost in 3 days at 25°C. This loss corresponds to approximately 700 L ethanol/ha when a yield of 50 ton of sorghum stems/ha is considered. Daeschel et al. (1981) reported that juices can be preserved during 14 days at 4°C without detectable changes or deterioration (sour odor and foaming). These authors also reported that the dominant spoilage microorganisms were *Leuconostoc mesenteroides* and *Lactobacillus plantarum* at 25 and 32°C, respectively and recommended to process the juice within five hours after extraction.

### 3.2 Sorghum grain

Sorghum grain is a naked caryopsis composed of three major anatomical parts: pericarp, germ, and endosperm. The pericarp is composed of epicarp, mesocarp and endocarp (cross and tube cells). Among cereals, sorghum is the only one that can contain significant amounts of starch granules in the mesocarp cells. The starch-devoid germ is rich in fat, soluble sugars and proteins (albumins and globulins) whereas the endosperm is divided into the single layered aleurone and the starchy endosperm cells positioned in the corneous and floury or chalky regions of the endosperm. The endosperm constitutes the largest fraction of the kernel and where almost all the starch is contained. Similar to maize, sorghum contains 60 to 70% of starch. The endosperm texture and hardness are highly related to the performance of the grain during several stages of ethanol production. In general terms, composition of sorghum is similar to maize with a few small but significant differences mainly in protein and fat concentrations. Sorghum for instance, has an average 1% less fat and 1.5 to 2.0% more crude protein compared to maize. Both

sorghum and maize have more than 50% of this protein as prolamins named kafirins and zeins, respectively. In sorghum, approximately half of the prolamins are bound. In contrast, approximately 70% of the maize prolamins are free or alcohol-soluble. There are some sorghum varieties that contain significant amounts of condensed tannins in the testa. These sorghums are classed as type III and have a lower nutritional value compared to other sorghums and maize. This is due to the presence of tannins that bind proteins and inactivate enzymes. As a result, high tannin sorghums may have reduced ethanol yields (Serna-Saldivar, 2010).

One of the most noteworthy differences between sorghum and maize is its starch granule-protein matrix interaction that negatively affects the susceptibility of both proteins and starch to enzyme hydrolyses. These structural differences affect protein digestibility and the speed of dextrans and glucose production during liquefaction and saccharification and thereafter the efficiency of yeast fermentation. Kafirins, despite the high sequence homology with zeins, tend to be less digestible especially after wet-cooking indicating the change in conformational structure attributed to formation of disulphide bonds. This is due to its high hydrophobicity which also makes possible the formation of additional protein aggregates that enhance the formation of more covalent bonds compared to zeins (Wong et al., 2009). Prolamins in the kernel are concentrated in protein bodies arranged among starch granules. The protein body composition in maize and sorghum is also similar, with *alpha* kafirin in the inner core surrounded by *beta* and *gamma* kafirins. The difference with maize is that during wet thermal processes the external part of protein body seems to form a net that makes difficult to access the *alpha* portion that is in turn more digestible than the *beta* and *gamma* counterparts. This phenomenon affects starch digestibility because in sorghum is 15 to 25% less digestible compared to maize. Taylor & Belton (2002) indicate that in sorghum, a complex rather than a simple obstruction mechanism between kafirins and starch is more likely to occur. This is the main reason why sorghum has lower susceptibility to hydrolysis and fermentation and yields less fuel ethanol compared to maize. Besides the starch-protein relationship, some other factors such as mash viscosity, amount of phenolic compounds, ratio of amylose to amylopectin and formation of amylose-lipid complex in the mash, limit the rate of enzymatic hydrolysis and fermentation efficiency during bioethanol production. For instance, starch in amylose-lipid complex cannot be converted into fermentable sugars, reducing conversion rate and final ethanol yield (Wang et al., 2008).

### 3.3 Sorghum bagasse and straw

As stated in section 3.1, besides water-soluble sugars (sucrose, glucose and fructose), sorghum is composed by structural cell wall carbohydrates primarily cellulose and hemicellulose, which in turn can be hydrolyzed and used as substrate for ethanol production (Sipos et al., 2009).

Sorghum bagasse is the residual fraction obtained after juice extraction from sweet sorghum whereas sorghum straw is the remaining material usually left on the field after threshing. The composition and proportion of fibrous-structural fractions in sorghum is widely reported and varies according to intrinsic and extrinsic factors such as cultivar type, maturity and climatic conditions. An average of 15% of the total weight corresponds to the fibrous portion within a range from 12 to 17% (Woods, 2000).

In sweet sorghum bagasse, average content of cellulose, hemicelluloses and lignin is 34-44%, 27-25%, and 18-20% respectively (Ballesteros et al., 2003; Kim & Day, 2011; Sipos et al., 2009).

Table 1 summarizes chemical composition of sweet sorghum bagasse and straw compared to energy-dedicated sugar cane, maize, wheat and rice counterparts.

Feedstock	Fiber(%)	Cellulose(%)	Hemicellulose (%)	Lignin (%)	Ash (%)
Sweet sorghum	13.0	44.6	27.1	20.7	0.4
Sweet sorghum <sup>2</sup>	-	25.0	22.0	4.0	-
Sweet sorghum bagasse <sup>3</sup>	-	41.3	24.6	14.0	3.7
Sorghum straw	-	32.4	27.0	7.0	0.7
Sugar cane	13.5	41.6	25.1	20.3	4.8
Energy cane	26.7	43.3	23.8	21.7	0.8
Corn stover	-	40.0	28.0	21.0	7.0
Wheat straw	-	38.0	32.0	19.0	8.0
Rice straw	-	36.0	28.0	14.0	20.0

<sup>1</sup> Modified from Kim & Day (2011) and Reddy & Yang (2005). All data expressed in dry weight basis. Percentage of fiber is based in 100% of original material and cellulose, hemicellulose, lignin and ash are percentages of the total fiber; <sup>2</sup>Wray variety (Woods, 2000); <sup>3</sup>Data yet not published from sweet sorghum bagasse harvested in Central Mexico and manually pressed for juice extraction.

Table 1. Fiber composition of different ethanol feedstock <sup>1</sup>

#### 4. Ethanol fuel from sweet sorghum juice

Sweet sorghum juice can be used for syrup, molasses, sugar and ethanol production with average fermentation efficiencies from 85 to 90% (Almodares & Hadi, 2009; Prasad et al., 2007; Wang et al., 2009; Wu et al., 2010b). The sweet sorghum juice is not commonly used for crystallized sugar production because of the presence of significant amounts of inverted sugars (glucose and fructose) that makes difficult crystallization in large-scale processes. However, the sweet sorghum juice, rich in fermentable sugars, has an excellent potential for yeast fermentation (Turhollow et al., 2010; Woods, 2000).

The sweet sorghum juice is obtained through a mechanical operation with a roller mill composed by a set of cylinders, similar to the ones employed by the sugar cane mills. Water is added during the last stage of the crushing process with the aim to augment the solubilization of residual sugars associated to the bagasse. The sweet sorghum juice yields around 50% in relation to the initial weight of the stems (Wu et al., 2010b). However, these authors describe an extraction process by pressing, which results in lower yields compared to roller mills. Furthermore, pressing is a batch process which is difficult to optimize for industrial conditions.

Approximately 90% of fermentable sugars from sorghum stalks can be obtained after conventional roller-milling, yielding an extraction ratio of 0.7 in relation to the initial plant weight (Almodares & Hadi, 2009). Gnansounou et al. (2005) reported extraction ratios ranging from 0.59 to 0.65 for the sweet sorghum cultivars Kelley, Wray, Río and Tianza. On the other hand, Kundiyana (1996) observed that extraction percentages varied between 47 to 58%, close to values observed by our research group in central Mexico (unpublished data).

After extraction, the sweet sorghum juice is fermented, distilled and the ethanol finally dehydrated (Fig. 1). This is the simplest way to produce fuel ethanol because the grain and

fiber processes require the hydrolysis of starch and fiber components into fermentable sugars. These steps are considered expensive, take time and expend energy and other additional resources (i.e. enzymes, chemical reagents, etc.) (Fig. 2 and 3). Despite these benefits, some challenges must be solved in order to efficiently convert the sweet sorghum crop into fuel ethanol. The main setbacks are the relatively higher rate of sugar degradation at ambient temperature and the low nitrogen content for yeast growth (Mei et al., 2009; Wu et al., 2010b). Thus, the logistics of just in time harvesting and the storage of the feedstock in facilities that retard decomposition and degradation of fermentable carbohydrates should be considered and stressed. In relation to nitrogen availability, this disadvantage can be overcome with the supplementation of urea, ammonia or yeast extract in order to avoid sluggish fermentation.

Besides sugar and nitrogen content, fermentation performance of sweet sorghum juice can also be affected with processing parameters and bioreactor configuration. Nuanpeng et al. (2011) observed in a repeated-batch study that very high gravity (VHG) fermentation is a good alternative to produce high ethanol concentrations from sweet sorghum juice when an adequate level of yeast cell concentration, nitrogen, and agitation are used. On the other hand, Laopaiboon et al. (2007) reported better results in fed-batch fermentation compared to batch configuration, in terms of ethanol concentration and product yield but not in productivity (measured as grams of ethanol generated/L/hr). These findings indicate the need to optimize parameters as feeding and withdrawn rate in order to optimize yields.

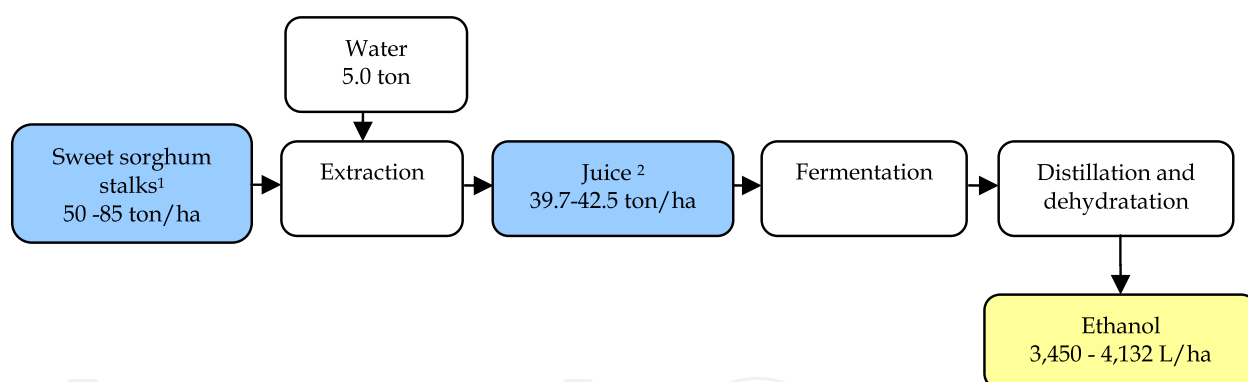


Fig. 1. Flowchart for ethanol production from sweet sorghum juice; <sup>1</sup>Water 73%, sugars (sucrose, glucose and fructose) 13.0%; <sup>2</sup>Water 84%, sugars (sucrose, glucose and fructose) 14.2%. Data from: Almodares & Hadi (2009) and Gnansounou et al. (2005).

The microorganism used, as indicated in the next sections, is also a factor that is worthwhile exploring. In the case of sweet sorghum juice, fermentation with different yeast strains has been evaluated and productivity varies significantly, but most of the strains showed an efficiency of more than 90% (Wu et al., 2010b). Liu et al. (2008) reported the use of immobilized yeast in a fluidized bed reactor that shortened process time and increased conversion efficiency. These results can be optimized when parameters as temperature, agitation rate, particles stuffing rate and pH are modified. Liu & Shen (2008) found that fermentation with immobilized yeast at 37°C, 200 rpm, 25% particles stuffing rate and pH of 5.0 in shaking flasks and 5 L bioreactor corresponds to the optimal conditions derived from an orthogonal experimental design.



## 5. Ethanol fuel from sorghum grain

### 5.1 Conventional dry grind

The five basic steps in the conventional dry-grind ethanol process are milling, liquefaction, saccharification, fermentation and ethanol distillation/dehydration (Fig. 2). Mashing goes throughout the entire process beginning with mixing the grain meal with water (and possibly backset stillage) to obtain a mash ready for fermentation. Mashing is a wet-cooking process to turn the gelatinized starch into fermentable sugars first with the use of thermostable *alpha*-amylase and then with amyloglucosidase (Zhao et al., 2008; Solomon et al., 2007; Wu et al., 2007). Starch is the substrate for grain fuel ethanol. Unlike maize, the starch content of sorghum is not the best indicator of ethanol yield obtained by the dry-grind process because this carbohydrate greatly differs in availability or susceptibility to amylases.

The comparatively higher protein content of sorghum compared to maize should be advantageous because the protein is partially degraded into free amino nitrogen compounds during biocatalysis. These compounds are a source of nitrogen for yeast nutrition. However, the relatively lower protein digestibility and nature of the endosperm proteins associated to sorghum counteracts its higher protein concentration. As a result, sorghum mashes almost always contain less free amino nitrogen compared to maize mashes. The use of proteases during or after liquefaction is a good alternative to increase free amino nitrogen in sorghum mashes (Perez-Carrillo & Serna-Saldivar, 2007). Protein digestibility in wet-cooked sorghum is relatively lower compared to other cereals, mainly because of the cross-linking of prolamins. This phenomenon reduces the availability of nitrogenous compound in sorghum mashes needed to support yeast metabolism during fermentation.

Yeast cannot use proteins as source of nitrogen, instead it utilizes amino acids and short peptides (di or tri), indicating the importance of protein fragmentation altogether with starch hydrolysis in mashing. Beyond yeast nutritional quandary, there are also issues related to starch digestibility that affects the performance of amylolytic enzymes during liquefaction and saccharification. This trend is also related to proteins because of the interaction between protein and starch that in sorghum reduces the susceptibility of this polysaccharide in both native and gelatinized conditions. Sorghum starch has higher gelatinization temperature compared to maize and more prolamins containing bodies within the endosperm, differences that can restrict gelatinization of starch granules (Zhao et al., 2008).

It has been reported that ethanol yields from sorghum decreases as protein content increases; however, at the same protein level, ethanol fermentation efficiency can vary as much as 8%. The difference is higher than typical experimental variations which indicate that additional factors to protein affects starch-conversion rate. In a work reported by Wang et al. (2008), nine sorghum genotypes were selected and used to study the effect of protein availability on efficiency of ethanol fermentation. The results showed a strong positive linear relationship between protein digestibility and fermentation efficiency, indicating the influence, and at the same time, the usefulness of this sorghum grain features as predictor of ethanol yield (Rooney et al., 2007; Wang et al., 2008; Wu et al., 2007; Wu et al., 2010a).

In Fig. 2 a typical process of dry-grind ethanol production is depicted. An average yield of 390 L of ethanol from 1 ton of sorghum can be obtained, but yields as high as 400 L/ton with fermentation efficiencies of more than 90% has been achieved and reported (Chuck-Hernandez et al., 2009; Pérez-Carrillo & Serna-Saldivar, 2007). The Dried Distillers Grains with Solubles (DDGS) obtained in these processes contribute to the economics of biorefineries. The wet distillers grains can be dried to 12% moisture with the aim to produce a shelf-stable byproduct.

Its nutritional composition (39 and 49% of protein and carbohydrates respectively) makes it an excellent option for livestock feed, especially for ruminants.

## 5.2 Use of biotechnology to improve ethanol yields

### 5.2.1 Genetic modified sorghum

Nowadays, advances in transformation and genetic modification in plants make the development of special sorghum cultivars one of the best tactics to overcome the various known factors that reduce ethanol yields. Previous research works have concluded that fermentation efficiencies and ethanol yields are influenced by genotype and chemical composition (Wu et al., 2007, 2008; Zhao et al., 2008). These investigations have determined important traits that enhance or reduce yields. Starch, protein and tannins are the principal components related to ethanol production from sorghum grain and these characteristic can be associated to genotype and also, in the case of starch and protein, to environmental factors as sowing season and location (Wu et al., 2008). Starch composition, specifically the amylose:amylopectin ratio, is related to fermentation efficiency. Raw materials with less amylose are more efficiently converted into ethanol (Wu et al., 2006). The improvement is related to digestibility of starch, reported as higher in waxy types (Rooney & Pflugfelder, 1986). Wu et al. (2006) also attributed the increased efficiency to the lower content of amylose-lipid complexes in mashes.

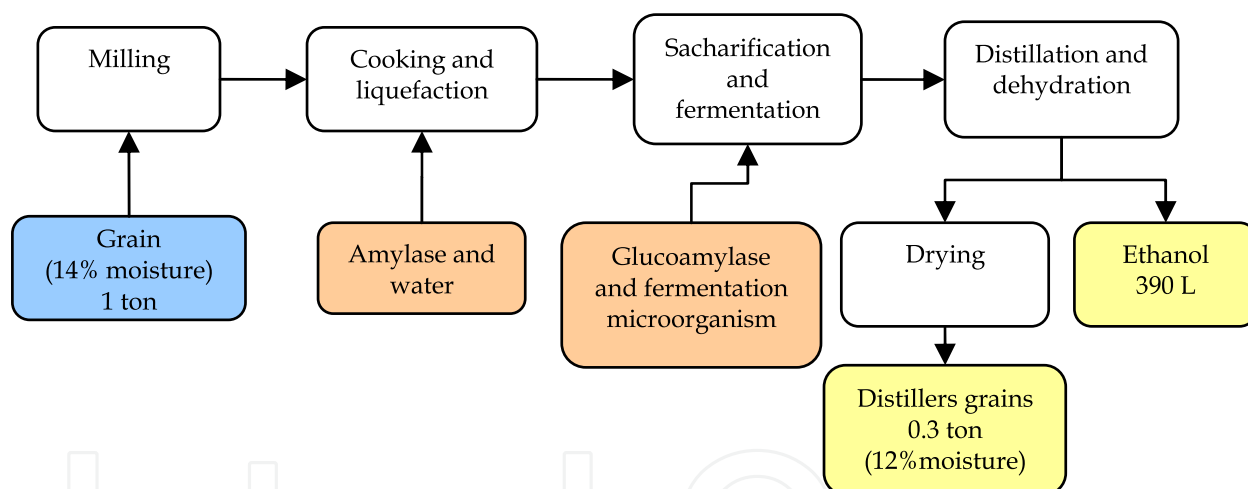


Fig. 2. Flowchart for ethanol production from sorghum grain. Data from: Serna-Saldívar (2010).

The DSC thermograms of starches from waxy sorghum and waxy maize are essentially the same: both display a single, smooth endothermic peak, with approximately the same onset, peak, and ending temperatures in the range of 60-80°C. However, in normal sorghum a second peak appears around 85 to 105°C corresponding to an amylose-lipid complex that reduces the availability of starch. Waxy starches are thereby easily gelatinized and hydrolyzed, giving high conversion efficiencies (Wu et al., 2007). Thus, the waxy characteristics improved the susceptibility of the endosperm matrix for low-energy gelatinization, enzymatic hydrolysis and total ethanol production (Wu et al., 2010a).

In the case of proteins, Wu et al. (2010a) indicate that high-lysine, high-protein-digestibility (HD) sorghum lines have been developed. These genotypes have several potential advantages for their use as feedstocks in biorefineries. First, the starch granules swells and

pastes more easily at lower temperatures; second, the proteins have improved feed value with higher bioavailability even for monogastrics. Interestingly, these high-lysine genotypes can contain 60% more of this essential amino acid compared to regular counterparts and similar content compared to quality protein maize (QPM) genotypes (Wu et al., 2010a). The enhanced protein digestibility of these lines is attributed to an improved kafirin digestibility as a result of the unique, abnormal and highly invaginated protein bodies. Segregated progeny with HD population lack the kafirin protein body matrix that surround starch granules and restrict swelling and pasting.

While modification in starch and protein digestibility affects ethanol production, one of the most important traits in starch conversion is total starch harvested per area. The primary goal of sorghum breeding programs has been and continues to be the development of high-yielding, drought-tolerant and pest-resistant hybrids. This effort will continue and additional gains in yield can be expected which will result in higher ethanol production from each hectare dedicated to sorghum (Rooney et al., 2007).

### 5.2.2 Exogenous enzymes

As explained before, protein digestibility is related to ethanol production and this digestibility in turn is related to the tendency of sorghum proteins to form web-like structures during mashing which reduces the possibility of enzymes to access starch. Protein solubility should decrease with the increase of protein cross-linking; thus, this parameter can be used as a quality indicator in sorghum biorefineries (Zhao et al., 2008).

The utilization of proteases before conventional starch liquefaction can be used as an alternative method to improve rate of starch hydrolysis and yield hydrolyzates with high FAN concentration (Perez-Carrillo & Serna-Saldivar, 2007).

Perez-Carrillo et al. (2008) proposed the use of protease before starch gelatinization and liquefaction of both decorticated and whole sorghum meals. The use of decortication to remove the sorghum outer layers and the exogenous protease had a positive synergic effect in terms of ethanol yield and energy savings because mashes required about half of the fermentation time compared to conventionally processed sorghum. Decorticated meals with more starch were more susceptible to *alpha*-amylase during liquefaction and produced more ethanol during fermentation (Alvarez et al., 2010). This technology produced similar ethanol yields compared to soft yellow dent maize and 44% more ethanol compared to the whole sorghum control treatment. The other advantage of mechanical decortication is that the bran, separated beforehand, is shelf-stable and can be directly channeled for production of animal feeds and consequently the yield of wet distilled grains from decorticated sorghum is significantly lower compared to the obtained after processing whole sorghum meals. Thus, if dried distilled grains are produced, the biorefinery plant will spend less energy when processing decorticated sorghum.

### 5.2.3 Germination and sprouting

Germinated or sprouted regular and high-tannin sorghums have improved ethanol yields compared to the unmalted kernels. Yan et al. (2009, 2010) reported a reduction in fermentation time and reported higher yields when sprouted sorghum was processed. The improved yield and efficiency is attributed to the action of intrinsic enzymes in starch, proteins and cell walls. Thus, the use of purposely malted or field sprouted sorghums can be advantageous for fuel ethanol biorefineries. Nevertheless, the industries should consider that malting requires important inputs in terms of water, labor, energy for drying and logistics.

#### 5.2.4 Very High Gravity (VHG) fermentation

Very High Gravity (VHG) mashes are used for fuel ethanol production at industrial scale. Among the benefits include an increased productivity, a reduced capital cost, a higher ethanol concentration in the fermented mash (from 7-10% to 15-18% v/v or more), and a decrease in water requirements. The most concentrated ethanol in fermented mashes also reduces distillation requirements, being an important issue because after feedstock, energy is the biggest production input, representing 30% of total ethanol cost (Pradeep et al., 2010; Wang et al., 2007). This economic consideration indicates the importance of the substrate concentration at the beginning of the process. The use of mashes with higher sugar concentration influences the decision of which fermentation microorganism will be selected and used.

Yeast osmotolerance is determined by genetics and by the carbohydrate level present in mashes, fermentation temperature, osmotic pressure/water activity and substrate concentration. Osmotolerant yeast fermenting in batch conditions can produce and tolerate levels of 16 to 17% (v/v) alcohol (Casey & Ingledew, 1986). According to the same authors, higher alcohol beers can be produced if oxygenation and nitrogen sources are supplemented to worts. Pradeep et al. (2010) reported a maximum ethanol concentration of 15.6% (v/v) converted with about 86.6% efficiency when finger millet mashes were fermented with *Saccharomyces bayanus*. Fermentation temperature is also an important factor affecting productivity, and generally speaking, at higher temperatures the time required to finish fermentation is decreased. Jones & Ingledew (1994) reported an increment in fermentation efficiency when dissolved solids concentration increased from 14 to 36.5 g/100 mL and also observed that the use of urea accelerated the rate of reaction and decreased time required to complete fermentation.

Working with VHG sweet sorghum juice rather than with ground sorghum grain, Wu et al. (2010b) reported an increase in glycerol (0.3 to 0.6%) and residual sugars (0.2 to 5.1%) when sugar in juices increased from 20 to 30%. A reduction in fermentation efficiency (93 to 72%) was also observed after 72 hours fermentation. Authors recommend the use of juices with no more than 20% soluble sugars in order to obtain the highest efficiency.

In general terms, yeasts can exhibit osmotic inhibition starting at 15% sugar, and this inhibition is higher in glucose followed by other carbohydrates such as sucrose and maltose. Sumari et al. (2010) stated that very few types of yeasts were known to tolerate sugar concentration above 40% and normally at this concentration their growth is sluggish. For this reason, the screening for osmotolerance and the development of new strains is necessary for industrial purposes. Sumari et al. (2010), using a molecular genetic approach, characterized a set of yeasts isolated from African brews and wines. One strain was able to ferment a medium with sucrose concentration of 1000 g/L. The phylogenetic analysis with rDNA clustered this microorganism away from the typical osmotolerant yeast. This indicates the opportunity to explore and look for new strains in nature.

Besides yeast, other microorganisms, as bacteria, are especially designed for ethanol fermentation. *Escherichia coli* is the typical modified microorganism for ethanol production because of the wide spectrum of metabolized carbohydrates, its well-known genetic makeup and the easiness of manipulation. *Zymomonas mobilis*, a rod shaped, gram negative, non-spore forming bacteria is naturally ethanologenic and compared to yeast, has higher rates of glucose uptake. *Z. mobilis* has also a higher ethanol production, increased yield and tolerance, making it a good option to use in VHG fermentation. Kesava et al. (1995), working with *Z. mobilis*, reported 95% conversion rates after 35 hours fermentation and ethanol yields of approximately 70 g/L when fermenting mashes containing 150 g glucose/L. The

bacterium was able to ferment mashes containing 200 g glucose/L in a step-fed system. Perez-Carrillo et al. (2011) observed that *Z. mobilis* had lower nitrogen requirements compared to *S. cerevisiae* when fermenting mashes adjusted to 20° Plato. This bacterium has potential and possible advantages for commercial use in biorefineries.

### 5.3 Physico-mechanical technologies to improve ethanol yield

Several approaches to increase ethanol yield from sorghum involve physical or mechanical treatments, v.gr: reduction of particle size, decortication or steam flaking. The aim of these treatments is to reduce physical barriers to hydrolytic enzymes in order to yield more fermentable sugars in shorter reaction times.

#### 5.3.1 Particle size

Particle size of ground sorghum meals also plays an important role in the starch-to-ethanol conversion process. Wang et al. (2008) observed that fermentation efficiencies of finely ground samples were approximately 5% higher compared to coarsely ground counterparts. This effect is a consequence of differences in gelatinization temperature and accessibility of starch to hydrolyzing enzymes. Wang et al. (2008) reported that gelatinization temperatures of larger or coarser particles are 5-10°C higher compared to finer particles.

The conversion of meals with smaller particles enhanced digestibility due to an improvement in the relative surface-contact area. Mahasukhonthachat et al. (2010) indicate that starch digestion proceeded by diffusion mechanisms is based on an inverse square dependence of rate coefficient on average particle size.

#### 5.3.2 Decortication

According to Rooney & Serna-Saldivar (2000) pericarp, testa, aleurone and mainly peripheral endosperm are grain tissues directly related to the lower nutrient digestibility of sorghum. These layers can be removed through decortication or pearling, an abrasive process used on a regular basis for production of refined flours or grits (Serna-Saldivar, 2010). Commercial mills are typically batch type and are equipped with a set of abrasive disks or carborundum stones to mechanically remove from 10 to 30% of the grain weight. The resulting mixture of bran and decorticated sorghum is separated via air aspiration or sifting (Serna-Saldivar, 2010). The classified pearled grain is then conventionally milled into a meal or flour. This technology requires little capital investment or alteration of existing facilities (Wang et al., 1999). The mechanical removal of the sorghum outer layers increases starch concentration and decreases fiber, fat and phenolics. The ground decorticated sorghum kernels are more susceptible to thermoresistant *alpha*-amylase hydrolysis (Perez-Carrillo & Serna-Saldivar, 2007). Furthermore, the removal of the sorghum outer layers allows greater starch loading and results in improved ethanol yields.

#### 5.3.3 Steam-flaking

Other proposed alternative to process sorghum before dry-milling is steam-flaking. This technology, widely used in feedlots, disrupts the endosperm structure with the injection of live steam in a period of 15 to 30 min, followed by flaking through grooved rolls. Before flaking, moisture of sorghum is increased to at least 21% and a conditioning or surfactant agent as lecithin is added in order to obtain whole flakes and reduce processing losses (Serna-Saldivar, 2010). After drying and cooling, sorghum flakes can be milled using

traditional processes. The pregelatinized starch associated to the ground and steamed flaked sorghum had higher susceptibility during liquefaction and produced more ethanol during fermentation. Compared to the whole sorghum counterpart the steam-flaked sorghum yielded approximately 40% more ethanol (Chuck-Hernandez et al., 2009). Currently, the cost of steam flaking one ton of sorghum is approximately \$7.5 US dollars.

#### **5.3.4 Supercritical Fluid Extrusion (SCFX)**

Extrusion has been widely used for the processing of cereal grains because this thermoplastic technology is continuous and saves unit operations and energy. In extrusion, the materials are subjected to heating, mixing, and shearing, resulting in physical and chemical changes during its passage through the extruder. The major advantages of extrusion include: improvement of starch digestibility and reduction of its molecular weight, production of free sugars and dextrans, changes in the native structure of both starch granules and proteins and reduced viscosity of fermentation broths. Therefore, extrusion could be an effective process to improve the bioconversion rate of sorghum starch (Zhan et al., 2006).

An innovative processing technology patented by researchers of Cornell University combines extrusion process and supercritical-fluid technology. The main difference between supercritical-fluid (SCFX) and conventional extrusion is the injection of supercritical carbon dioxide, which replaces water as blowing agent for expansion. The injection of supercritical-fluid carbon dioxide breaks the intimate bonds between starch granules and protein matrix and results in the improvement of starch availability (Zhan et al., 2006). These researchers suggested that SCFX produces molecular degradation of starch during extrusion of sorghum. This process also increased about 8% the protein digestibility, the measurable starch content, the free sugar concentration and gelatinized starch and other parameters that increased ethanol yield (+5%) and boosted fermentation efficiency compared to the non-extruded counterpart. The SCFX cooking also affected the crude fiber, chemical fraction that after microscope examination showed disruption and fissures. These authors describe the sorghum extrudates with "porous structure". Thus, this thermoplastic procedure was indeed effective as pretreatment to improve bioconversion of sorghum into ethanol.

## **6. Ethanol from sorghum bagasse and straw**

### **6.1 Raw material conditioning**

After extraction of juice or grain harvesting, the lignocellulosic residue is chopped, milled, and dried at 50-60 °C to reduce the moisture content to about 10 to 15% (Herrera et al., 2003; Sipos et al., 2009). There are many options to reduce particle size; the most commonly used are hammer or rotary mills. Grinding can be used on both dry and wet materials, and the cost is one of the lowest compared with others methods used for milling biomass. The grinder reduces the particle size to a fine powder by mechanical shearing and this operation can also be made with rotating and stationary abrasive stones (Mizuno et al., 2009).

### **6.2 Fiber extraction**

One of the most significant problems in ethanol production from lignocellulose is production cost (Mizuno et al., 2009) because the fiber conversion requires of high energy investments in order to obtain high concentrations of fermentable sugars from the insoluble polymers (Kurian et al., 2010; Mamma et al., 1996). A pre-hydrolysis step releases both the

hemicellulosic and cellulosic fractions of the fiber (Herrera et al., 2003). The main processes related to the pretreatment of sorghum biomass for ethanol production are the acid and/or enzyme-catalyzed hydrolyses (Mamma et al., 1996; Sipos et al., 2009). Generally, the acid hydrolysis precedes the enzymatic in order to optimize production of C6 and C5 fermentable sugars (Sipos et al., 2009).

### 6.3 Pretreatments used for sorghum bagasse

The extraction of structural carbohydrates from bagasse cell walls is highly related to the effectiveness of pretreatments. Nowadays there are many proposed treatments for cellulose and hemicellulose extraction, but only few have been commercially implemented. In the following sections some of the proposed technologies for sorghum biomass are discussed.

#### 6.3.1 Steam explosion

The ground sorghum bagasse is rehydrated with steam at atmospheric pressure and impregnated with low amounts (up to 3% w/w) of sulfur dioxide (SO<sub>2</sub>) in plastic bags for 20-30 minutes in order to improve enzymatic saccharification (Sipos et al., 2009; Stenberg et al., 1998; Öhgren et al., 2005). The impregnated bagasse is introduced into a reactor and the temperature is maintained by injection of saturated steam, varying in a range of 170-210°C (Sipos et al., 2009; Stenberg et al., 1998; Öhgren et al., 2005). After 2 to 10 minutes, the blow-down valve is opened and the hydrolyzate is released into a cyclone (Stenberg et al., 1998). Sipos et al. (2009) achieved an extraction of 89% to 92% of cellulose with steam explosion, up to 18 g glucose, 23 g xylose and 5.5 g arabinose/L hydrolyzate. Ballesteros et al. (2003) used steam explosion pretreatment without sulfur dioxide and obtained around 50% of solids recovery and only 20% solubilization of the cellulose. Hemicellulose sugars were extensively solubilized because the raw material had originally 25% xylose and after the treatment only 2% remained on the fibrous residue.

#### 6.3.2 Dilute acid hydrolysis

Acid hydrolysis, the most common fiber pretreatment method (Ban et al., 2008), generates significant amounts of sugars from hemicellulose. Besides it is a process relatively cheap (Gnansounou et al., 2005). Sulfuric, hydrochloric, hydrofluoric or acetic acids have been tested as catalysts (Herrera et al., 2003). The process consists on the addition of diluted aqueous acid solution (0.1 to 10 % w/v) to the ground raw material and hydrolyzing in an autoclave. A solid residue, rich in cellulose and lignin, is formed after acid hydrolysis and subsequently treated with enzymes in order to increase the amounts of fermentable sugars (Tellez-Luis et al., 2002). Kurian et al. (2010) achieved extract with 92 g/L of total sugars from sweet sorghum bagasse treated with sulfuric acid at a concentration of 5 g/kg and treated at 140°C for 30 minutes. Ban et al. (2008) treated the same raw material at a solid-liquid mass ratio of 10% with 80 g phosphoric acid/L at 120°C for 80 minutes. These authors reported 302 g reducing sugars/kg with this pretreatment.

#### 6.3.3 Alkali pretreatment

Unlike other pretreatments, the use of strong alkali delignifies biomass by disrupting the ester bonds of cross-linked lignin and xylans, resulting in cellulose and hemicellulose enriched fraction. Alkali pretreatment processes generally utilize lower temperatures, pressures and residence times compared to other technologies (McIntosh & Vancov, 2010).

The main compounds used as pretreatment agents in alkali processes are: sodium hydroxide, ammonia and lime, because of their comparatively lower cost and the possibility of chemical and water recycling (McIntosh & Vancov, 2010). Usually two temperature conditions are used for hydrolysis: mild (60°C) or high (121°C).

#### 6.4 Enzymatic extraction

There are several enzymes generally used to convert cellulose and hemicellulose into soluble sugars. They are a mixture of pectinases, cellulases and hemicellulases (Lin et al., 2011; Reddy & Yang, 2005). Cellulose can be hydrolyzed by the synergistic action of endo-acting enzymes known as endoglucanases, and exo-acting enzymes, known as exoglucanases (Lin et al., 2011). Today it is common to employ enzyme complexes consisting of seven or more degrading enzymes that act synergistically. The enzyme mixture is added before or after chemical or mechanical treatments (Reddy & Yang, 2005). Enzymes appear to be the best prospects for continued improvements because can reduce production costs (Gnansounou et al., 2005).

Sipos et al. (2009) observed that the separation of the solid and the liquid phases after chemical pretreatment is beneficial to the whole process because the xylose-rich liquid fraction can be fermented into ethanol through the pentose pathway or as substrate for microbial cellulase production or transformed into other various valuable products. On the other hand, the solid fraction can be further hydrolyzed and fermented into ethanol. The use of alkali treatment before enzyme hydrolysis generated 540 g glucose/kg raw material, equivalent to a 90% conversion of available cellulose to monomeric sugars. On the other hand, 235 g xylose/kg was released after pretreatment of sorghum straw (McIntosh & Vancov, 2010). These hydrolysates were obtained with an enzyme complex containing endoglucanase, exoglucanase, xylanase, beta-glucosidase and cellulase.

#### 6.5 Hydrolysis by-products or fermentation inhibitors

The fiber chemical hydrolysis process can produce a large number of sugar degradation products which are known to inhibit bacteria and yeast and thus the conversion of fermentable sugars into bioethanol (Ban et al., 2008). The most important inhibitors are furfural, 5-hydroxymethylfurfural and acetic acid. After the acid hydrolysis, it is necessary to adjust the pH with alkalis in order to obtain the adequate conditions for the subsequent step of fermentation. Lime or calcium hydroxide is commonly added to increase the pH to 9-10. This alkali treatment precipitates inhibitors in the form of insoluble salts and therefore acts as detoxifying treatment (Kurian et al., 2010).

#### 6.6 Fermentation

Hydrolyzates obtained from sorghum fiber are solutions rich in both hexoses and pentoses (Kurian et al., 2010). Production of ethanol from these mashes is possible only with the use of osmotolerant and pentose fermenting yeast or bacterial strains (Table 2).

Ballesteros et al. (2003) obtained 16.2 g ethanol/L when hydrolyzates obtained from sweet sorghum bagasse were fermented with *Kluyveromyces marxianus*. On the other hand, Kurian et al. (2010) working with *Pichia stipitis* obtained 38.7 g ethanol/L with a theoretical conversion of 82.5%. In Fig. 3, a flowchart of ethanol production from sorghum bagasse is depicted. A yield of 158 L ethanol/ton biomass (wet basis) can be obtained after a sulfuric acid hydrolysis. The process yielded 110 kg of lignin and other non-fermentable materials. Almodares & Hadi (2009) and Gnansounou et al. (2005) reported that the cellulase used in Simultaneous



Microorganism	Characteristics
<i>Clostridium acetobutlicum</i>	Useful in fermentation of xylose to acetone and butanol; bioethanol produced in low yield
<i>Clostridium thermocellum</i>	Capable of converting cellulose directly to ethanol and acetic acid. Bioethanol concentrations are generally less than 5 g/l. Cellulase is strong inhibition encountered by cellobiose accumulation
<i>Escherichia coli</i>	Native strains ferment xylose to a mixture of bioethanol, succinic, and acetic acids but lack ethanol tolerance; genetically engineered strains predominantly produce bioethanol
<i>Klebsiella oxytoca</i>	Native strains rapidly ferment xylose and cellobiose; engineered to ferment cellulose and produce bioethanol predominantly
<i>Klebsiella planticola</i> ATCC 33531	Carried gene from <i>Zymomonas mobilis</i> encoding pyruvate decarboxylase. Conjugated strain tolerated up to 4% ethanol
<i>Lactobacillus pentoaceticus</i>	Consumes xylose and arabinose. Slowly uses glucose and cellobiose. Acetic acid is produced along with lactic in 1:1 ratio
<i>Lactobacillus casei</i>	Ferments lactose, particularly useful for bioconversion of whey
<i>Lactobacillus xylosus</i>	Uses cellobiose if nutrients are supplied: uses glucose, D-xylose and L-arabinose
<i>Lactobacillus pentosus</i>	Homolactic fermentation. Some strains produce lactic acid from sulfite waste liquors
<i>Lactobacillus plantarum</i>	Consumes cellobiose more rapidly than glucose, xylose, or arabinose. Appears to depolymerize pectins; produces lactic acid from agricultural residues
<i>Pachysolen tannophilus</i> <i>Saccharomyces cerevisiae</i> ATCC 24 860	Co-culture of <i>S. cerevisiae</i> and strains resulted in the best ethanol yield
<i>Pichia stipits</i> NRRL Y-7124, Y-11 544, Y-11 545	NRRL strain Y-7124 utilized over 95% xylose based on 150 g/L initial concentration. Produced 52 g/L of ethanol with a yield of 0.39 g ethanol per g xylose
<i>Pichia stipits</i> NRLL Y-7124 (floculating strain)	Maximum cell concentration of 50 g/L. Ethanol production rate of 10.7 g/L.h with more than 80% xylose conversion. Ethanol and xylitol yield of 0.4 and 0.03 g/ g xylose
<i>Saccharomyces cerevisiae</i> CBS 1200 <i>Candida shehatae</i> ATCC 24 860	Co-culture of two yeast strains utilized both glucose and xylose. Yields of 100 and 27% on glucose and xylose, respectively

<sup>1</sup> With data from: Balat et al. (2008) and Lee (1997).

Table 2. Native and engineered microorganisms capable of fermenting xylose to bioethanol<sup>1</sup>

Saccharification and Fermentation (SSF) can be added directly or from material previously deviated from pretreatment and inoculated along with *Trichoderma reesei* or other fungi such as *Neurospora crassa* and *Fusarium oxysporum*. These microorganisms were capable of directly fermenting cellulose (Mamma et al., 1996). *F. oxysporum* was used in a SSF along with *S. cerevisiae*, yielding 5.2 to 8.4 g ethanol per 100 g of fresh sorghum. The efficiency was calculated based on soluble sugars and not in total polysaccharides (Mamma et al., 1996).

## 7. Estimated ethanol yields

Fig. 1 to 3 summarizes and compares average ethanol yields from sorghum grain, sweet juice and biomass. Ethanol yields vary according to variety, geography, soil fertility and temperature.

Sweet sorghums usually yield from 50 up to 120 tons of stalks after the first cut. This feedstock contains 73% moisture, 13% soluble sugars, 5.3% cellulose, 3.7% hemicelluloses and 2.7% lignin. The stalks yield up to 70% sweet juice and 15.33 ton/ha of spent bagasse (Almodares & Hadi, 2009; Prasad et al., 2007).

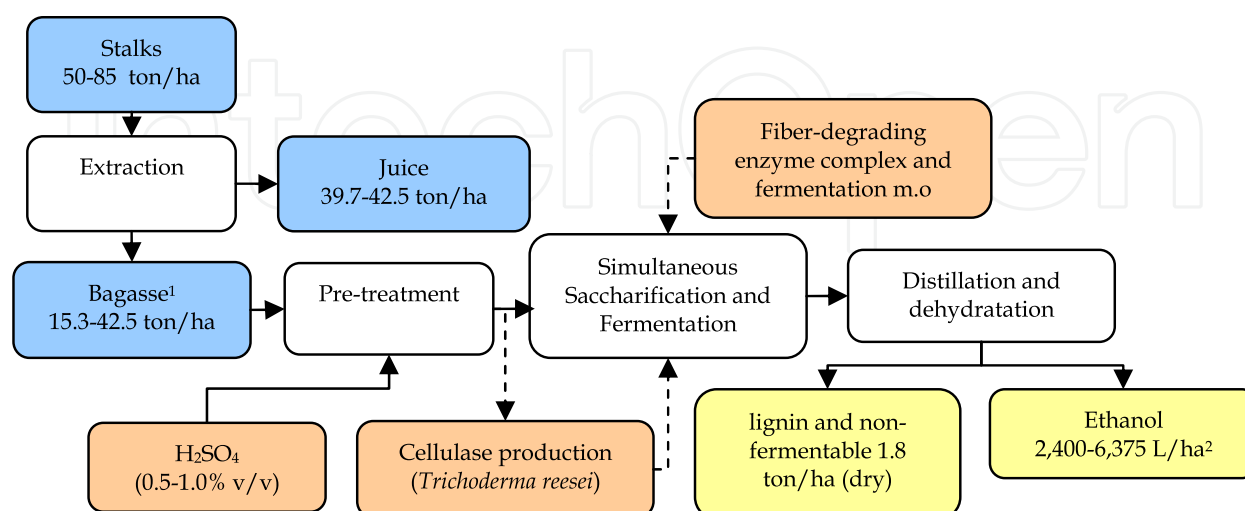


Fig. 3. Flowchart for ethanol production from sweet sorghum bagasse. <sup>1</sup> Average composition of sweet sorghum bagasse: Water 54%, simple fermentable sugars 5.4%; Cellulose 17%; Hemicellulose 12%; Lignin 11.7% <sup>2</sup> Practical yield from fermentation with *I. orientalis*: 3,865 L/ha. From: Almodares & Hadi (2009); Gnansounou et al. (2005).

Water added during extraction is considered part of the sweet juice yield (Fig. 1) and the sweet juice commonly contains around 14% soluble sugars. This substrate allows the production of 3,450 L ethanol/ha with a fermentation efficiency of 95%, similar to the result reported Kim & Day (2011) (3,296 L/ha). These last researchers did not consider losses that negatively affect fermentation efficiencies. Almodares & Hadi (2009), on the other hand, reported a yield of 3,000 L ethanol/ha directly when processing juice extracted from varieties that yielded from 39 to 128 ton stalks/ha. Although Wu et al. (2010b) did not report ethanol yields per hectare, the calculated ethanol production from the amount of total fermentable sugars extracted from a high yielding M81E cultivar planted at two different locations and bioconverted with a 95% of fermentation efficiency was in the range of 4,750 to 5,220 L/ha. These potential ethanol yields are equivalent to the bioconversion of 12 to 13 tons of maize kernels.

Experimental data obtained from sweet sorghums cultivated in Central Mexico indicated that these materials are capable of yielding 6.38 tons of sugar/ha/cut. Consequently, when are adequately bioconverted have the potential of producing 4,132 L ethanol (unpublished data). Regarding to the lignocellulosic fraction, if 15.33 ton of bagasse/ha is obtained containing 29% cellulose and hemicellulose and 5.4% of remaining unextracted soluble sugars, up to 2,400 L of ethanol can be obtained (Fig. 3). This yield represents almost half of the 4,058 L/ha described by Kim & Day (2011) as theoretical ethanol.

In central Mexico, 42.5 ton of bagasse/ha with 50% fermentable sugars are commonly obtained. This biomass is capable of yielding 6,375 L ethanol with perfect conversion efficiency. However, experimental data where the acid-treated biomass was fermented with *Issatchenkia orientalis* indicated only 60% fermentation efficiency (3,865 L/ha) (unpublished

data). These results indicate that there are still many areas for potential improvements especially when processing spent biomass.

Almodares & Hadi (2009) reported that a yield up to 2 ton of grain/ha can be expected from sweet sorghum. If this material is milled, hydrolyzed and fermented, a final ethanol yield of 780 L can be expected. Nevertheless, the sweet sorghum grain during optimum harvesting is not fully matured and generally collected along the vegetative parts of the plant. Thus, the immature sweet sorghum kernels are usually processed with the bagasse and not fermented using grain technologies.

The biomass production per cultivated surface (Fig. 3) is the key and most important factor that affects ethanol yields indicating the importance of both plant breeding for the generation of new improved cultivars and the agronomic conditions mainly affected by soil fertility and water availability. The new biomass cultivars should adapt to marginal lands in order to minimize competition with basic grain production. The potential to obtain ethanol yields of 6630, 7000 and 10000 L/ha (with 95% of extraction and fermentation efficiency) can be achieved because yields of 50 to 120 tons of biomass/ha are reported. Comparatively Kim & Day (2011) indicated that the theoretical yield of maize kernels can be as high as 5,100 L/ha and up to 8,625 L/ha when the whole plant is bioconverted into ethanol (grain + corn stover).

One of the most important factors to be addressed during yield calculation is indeed the energy required for ethanol production. Biomass and starch require more energy for hydrolysis compared to sweet sorghum juice. The technologies for starchy kernels and sweet juice are matured but the conversion and estimation of energy balances when processing lignocellulosic material will be critically important for the evaluation of economic advisability.

## 8. Future trends

One of the most promising research priorities in agricultural production is the genetic improvement of crops with high economic relevance. In the case of sorghum for fuels there are important advances in the development of biomass, sweet and high yielding grain varieties and hybrids, but is yet one of the most important and critical research topics. The new cultivars should be adapted to marginal lands and also they must be resistant to pests, other phytopathogens and stable facing water stress.

The creation of new varieties for ethanol production is not an easy task because the relevant traits, such as plant height, total soluble solids, juice production and lignin : cellulose : hemicellulose ratio are "non additive" (Reddy et al., 2005). On the other hand and according to Turhollow et al. (2010), the genetic mapping combined with its relatively fast hybridation and field tests, can facilitate the design and development of dedicated bioenergy cultivars.

It is also of utmost importance to develop machinery to harvest sweet and biomass sorghums because the use of existing sugarcane equipments reduce yields and efficiencies. Furthermore, it is also imperative to development new agronomical and technological packages that include "just in time" harvesting.

The use of biomass sorghum represents one of the most relevant topics in research even when there are not economic and energy efficient technologies. However, there have been important advances in terms of fiber degradation to yield extracts rich in C5 and C6 fermentable sugars. The development of new and more environmental-friendly pretreatments that include the use of fiber degrading enzymes and hot water and new strains of yeast and bacteria are critical points for the economics of biomass transformation.

The new microorganisms must be designed or genetically engineered to be more efficient in terms of enhanced capacity to fully ferment C5 and C6 sugars at high temperatures (Canizo, 2009). The development of new strains of *Saccharomyces cerevisiae* designed for pentose utilization, with high tolerance to inhibitors, and with a better genomic stability has not been yet fully addressed despite the recent advances in genetic engineering. Unfortunately, there are only few industrial and commercial strains in the market.

Process wise, biorefineries should focus on designing new bioreactors, flow-patterns, new cocktails of enzymes to optimize hydrolysis, the utilization of immobilized microorganisms and the development of new distillation and ethanol dehydration technologies that favors the total energy balance.

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## **Bioethanol**

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Recent studies have shown strong evidence of human activity impact on the climate of the planet. Higher temperatures and intensification of extreme weather events such as hurricanes are among the consequences. This scenario opens up several possibilities for what is now called "green" or low carbon economy. We are talking about creating new businesses and industries geared to develop products and services with low consumption of natural resources and reduced greenhouse gases emission. Within this category of business, biofuels is a highlight and the central theme of this book. The first section presents some research results for first generation ethanol production from starch and sugar raw materials. Chapters in the second section present results on some efforts around the world to develop an efficient technology for producing second-generation ethanol from different types of lignocellulosic materials. While these production technologies are being developed, different uses for ethanol could also be studied. The chapter in the third section points to the use of hydrogen in fuel cells, where this hydrogen could be produced from ethanol.

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