Chapter 5

# Conversion of High Biomass/Bagasse from Sorghum and Bermuda Grass into Second-Generation Bioethanol

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## Abstract

Sorghum (Sorghum bicolor) and Bermuda (Cynodon dactylon) grass are examples of annual and perennial forage crops produced throughout the globe. These crops should be harvested at the peak of biomass production when the levels of lignin are relatively low. The high biomass sorghum, sweet sorghum bagasse (2 cuts or crops year$^{-1}$) or Bermuda grass capable of yielding up to 50, 60 and 27 tons of dry forage ha$^{-1}$ year$^{-1}$ rich in cellulose and hemicellulose can be efficiently transformed into bioethanol using second-generation technologies consisting of milling, pretreatment (chemical and/or enzymatic) and fermentation with microorganisms capable of transforming C5/C6 sugars to obtain ethanol. An alternative process contemplates the extrusion aimed toward the physical disruption of cell walls minimizing the use of considerable amounts of water and chemicals commonly used during pretreatment. Extruded feedstocks treated with fiber-degrading enzyme cocktails had conversion efficiencies between 60 and 78% of the hemicellulose and cellulose similar to the ones achieved after acid/enzyme hydrolyses. The chief advantages of this continuous process are that hydrolysates are practically free of enzymes and yeast inhibitors. These feedstocks can produce up to 310 L anhydrous bioethanol dry t$^{-1}$ and have a great potential for widespread use.

**Keywords:** high biomass sorghum, sorghum bagasse, Bermuda grass, second-generation bioethanol

## 1. Introduction

The family of true grasses, botanically known as *Gramineae* or *Poaceae*, is a large group of genus and species commercially grown and distributed practically in all places around the globe. These monocotyledonous flowering plants are the most common sources of food for mankind and forages for domestic animals. The value of these plants is that they produce kernels rich in starch and protein that constitute the main staple for most cultures around the globe. Furthermore, these grasses provide most of the fiber consumed by ruminant domestic animals that provide mankind with milk and meat. The family contains about 10,000 domesticated and wild species, which represent the fifth largest plant family. Grasslands make up one-fifth of the vegetation cover of the globe and are considered as one of the chief sources of fibrous rich feedstocks. Annual and perennial grasses are classed into three broad categories: bunch-type, stoloniferous and rhizomatous. The success of the various types of grasses is mainly attributed to their morphological and physiological diversity. According to the physiological activity and more specifically to the photosynthetic pathways for carbon fixation, grasses are divided into C3 or C4 plants. The C4 plants have a photosynthetic pathway that particularly adapts them to hot climates and atmospheres low in $CO_2$ [1]. The commercial cereal grains are annual whereas most of the grasses that provide forages perennial. The chemical composition of these forages is affected by genotype, maturity and soil fertility. However, independently of the source, these feedstocks are highly attractive for biorefineries because of their abundance, relative low cost and quality of the fiber that it can be successfully converted into second-generation bioethanol, lactic acid, or other high value chemicals.

According to the Renewable Fuels Association, the world biorefineries generated more than 100 billion L of bioethanol in 2016. The USA is the major producer with approximately 58% of the present production (57.7 billion L) followed by Brazil with 27% of the total production. The contemporary fuel ethanol production is based chiefly on maize and sugarcane [2] and the use of these feedstocks triggers concerns related to food security especially as world population surpasses 7600 million people [3]. More biofuels production is particularly expected in the USA where the Energy Independence Act mandates the manufacturing of 136 billion L of bioethanol and biodiesel by year 2022. In order to meet these expectations, biorefineries will convert lignocellulosic biomass to energy using forages and dedicated energy crops such as high biomass sorghum, switchgrass and Miscanthus, which could be planted in marginal zones or alternatively serve to protect ecosystems especially from soil erosion.

The C4 sorghum (*Sorghum bicolor* L. Moench) plant has been identified as one of the best potential bioenergy crops. This member of the Gramineae family poses considerable potential as a dedicated lignocellulosic crop because of its broad genetic diversity, which provides plant breeders the opportunity to develop high biomass types adapted to different environments under dryland or irrigated conditions or sweet sorghums varieties, which can be effectively converted into first- and second-generation bioethanol [4]. The annual or

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short perennial high biomass sorghums can grow to a height of 6 m tall, depending on the genotype and growing conditions. This capacity has been boosted by intensive plant breeding programs focused in the design of new genotypes that can be effectively converted into second-generation ethanol [5, 6]. Sorghum is resistant to both abiotic and biotic stress factors such as drought, soil salinity and alkalinity and insects. Besides, this cultivar possesses one of the best rates of carbon incorporation (50 g $m^{-2}$ $d^{-1}$), which promotes its fast growth and enhanced rate of $CO_2$ utilization [7]. The sorghum growth cycle regularly lasts 3–5 months, and therefore, it can be produced twice or even three times throughout the year instead of only one crop obtained with sugarcane. Bermuda grass (*Cynodon dactylon*) is also a C4 perennial season forage crop, mainly used in the United States and northern of Mexico for ruminant feed and soil remediation from animal wastes. This grass has a short growth period and is usually cut monthly during the spring and summer seasons. The grass is highly susceptible to cold temperatures so it passes the winter inactive. The optimum growth conditions are 24–37°C maintaining a well biomass yield under water-stress conditions [8]. Together with these characteristics, the Bermuda grass is composed of a high amount of holocellulose (>50%) and low amounts of lignin (up to 20%) [9].

The aim of this review is to describe conversion technologies of sweet sorghum bagasse, high biomass sorghum and perennial grasses like Bermuda into second-generation fuel bioethanol.

## 2. Ethanol production from high biomass or sorghum bagasse and Bermuda grass

Among annual cultivars, the high biomass and sweet sorghums offer the most efficient and fastest means of producing large quantities of usable biomass [1, 3, 6, 10], whereas the perennial Bermuda grass is a good example of a forage crop which can be effectively converted into second-generation bioethanol using currently available technologies. High yielding sweet sorghums planted in good soils and with adequate agronomic practices are able to produce 3500 L of anhydrous ethanol $ha^{-1}$ from sweet juice, 3000 from the spent bagasse and 500 L from the starchy kernels (a total of about 7000 L) after 4 months of sowing, whereas high biomass dedicated crops up to 5600 L of second-generation ethanol $ha^{-1}$.

Amosson et al. [6] estimated dry yields of high biomass sorghum planted on dryland and irrigated areas in 8 and 18.5 t $ha^{-1}$. Likewise, Habyarrimana et al. [11] evaluated the biomass yield and drought resistance in field conditions of Italian sorghum genotypes. This study which evaluated 75 lines and two hybrids concluded that tropical sorghum landraces yielded total aboveground dry biomasses of 33–51 t $ha^{-1}$ under irrigation and 20–29 t $ha^{-1}$ under rain-fed conditions. Dryland and irrigated high biomass sorghum planted in the high plains of Texas was capable of yielding from 2400 to 5600 L ethanol $ha^{-1}$, respectively, assuming that one ton of dry biomass yields approximately 300 L of second-generation ethanol. The estimated cost of producing 1 ton of dry weight on the dryland and irrigated lands was estimated at 76.6 and 82 US dollars, respectively [6].

The short rotational sweet sorghums yield high sugar in their stalks, whereas the high biomass sorghums developed for second-generation bioethanol are mainly composed of fibrous chemical compounds. In terms of conversion into ethanol, the extracted sweet juice can be easily and highly efficiently converted into bioethanol leaving the spent bagasse as other potential feedstock for second-generation ethanol manufacture. Vencor Green [12] reported that the cost of ethanol production from sweet sorghum juice was 20% lower than either sugarcane or corn. According to Serna Saldivar et al. [1], the mature stems of sweet sorghum contain about 73% moisture and 27% solids which are manly comprised of structural and non-structural carbohydrates. Approximately 13% of the solids are non-structural carbohydrates composed of the disaccharide sucrose and monosaccharides glucose and fructose, in variable amounts according to cultivar, maturity stage and harvesting season [13, 14]. The sweet sorghum cultivars are classified based on their juice sugar composition into sugar and syrup types. The first is rich in sucrose while the second in glucose and fructose. According to the same authors, Wray, Keller and H173 sweet sorghum cultivars harvested post-anthesis yielded an average of 10, 7 and 4 t $ha^{-1}$ of fermentable sugars. Other studies [15, 16] reported sugar yields varying from 4.5 to 18 t $ha^{-1}$. These sugars are considerably highly fermentable and able to yield approximately 46% ethanol after 48 h fermentation. Therefore, the conceivable production of anhydrous ethanol fluctuates from 1000 to 8000 L.

On the other hand, an established perennial Bermuda grass field is capable of producing from 6 to 27 t $ha^{-1}$ $year^{-1}$ of dry forage [8], which can be converted into 1200–5400 L fuel ethanol. The large yield variability is due to water availability, nitrogen fertilization, soil fertility and number of monthly cuts during the year. Generally, the Bermuda grass is cut monthly except during the cold season of the year.

## 2.1. Second-generation ethanol from high biomass or sorghum bagasse and Bermuda grass

### *2.1.1. Fiber composition*

The high biomass forage or sorghum bagasse leftover after juice extraction of sweet cultivars is a fiber-rich feedstock with some variation in composition according to intrinsic and extrinsic factors such as genotype, maturity or degree of lignification and climatic conditions.

Nagaiah et al. [17] determined the structural composition of six high biomass sorghums differing in plant height (3.9–6.2 m) and yield (53.6–90.5 t of fresh stalks). The cultivars contained from 27 to 52% cellulose, 17 to 23% hemicellulose and 6.2 to 8.1% lignin (**Table 1**). According to Woods [18], from 12 to 17% (average 15%), 15% of the total sweet sorghum plant weight is constituted by the fibrous portion. Typically, the sweet sorghum harvested at optimum time yields half juice and half bagasse after milling or crushing the stalks. The spent bagasse with 52% moisture contains 5.4% residual fermentable sugar, 17% cellulose, 11.9% hemicellulose and 8.5% lignin [19, 20]. Heredia-Olea et al. [20], evaluating the fiber and structural carbohydrate composition of sweet sorghum bagasse, concluded that the main structural carbohydrates were cellulose derived β-glucans and xylans and arabinans related to hemicellulose. The quantity of lignin of 13.5% (dwb) was similar to that assayed by Gnansounou et al. [19] and

| Crop | Composition (%) | | |
|---|---|---|---|
| | Cellulose | Hemicellulose | Lignin |
| High biomass sorghum[2] | 27–52 | 17–23 | 6.2–8.1 |
| Sweet sorghum bagasse[3] | 31–34 | 18–25 | 10–18 |
| Bermuda grass[4,5] | 31–35 | 26–30 | 14–23 |

[1]Composition and yields are expressed on dry matter basis.
[2]Nagaiah et al. [17].
[3]Stenberg et al. [27], Heredia-Olea et al. [20].
[4]Canizo et al. [22].
[5]Lee et al. [9].

**Table 1.** Chemical composition of high biomass sorghum, sweet sorghum bagasse and Bermuda grass[1].

slightly higher compared to the value (11.1% dwb) reported by Prasad et al. [7]. Importantly, lignin is directly related to plant maturity and thus inversely correlated to the proneness of the other fiber components to hydrolysis and ethanol production.

According to Lee et al. [21], the Bermuda grass contains 30.4, 22.6, 4.9 and 23.2% of glucans, xylans, arabinans and lignin, respectively. On the other hand, Canizo [22] determined a similar composition of the potentially fermentable sugars but a slightly lower lignin content (**Table 1**).

## 2.2. Second-generation ethanol production

### *2.2.1. Pretreatments and sugars yields*

Production of lignocellulosic anhydrous ethanol consists of five sequential steps: milling of the feedstock, chemical or physical pretreatment, enzyme catalysis or saccharification, fermentation of resulting C6 and C5 sugars and ethanol distillation-dehydration [4]. Cellulose and hemicellulose upon hydrolysis yield the C6 and C5 fermentable sugars, respectively. The effectiveness of the process is strongly affected by the availability of cellulose and hemicellulose which must be separated from lignin, which hinders rate of hydrolysis and therefore ethanol yields.

The lignocellulosic residue is usually milled in hammer or rotary mills with the objectives of reducing the particle size and disrupt cell walls so fiber components are more prone to subsequent chemical, enzymatic and fermentation treatments. Then, the ground feedstock is hydrolyzed using chemicals such as acid, alkalis and ammonia and/or a set of fiber-degrading enzymes. Generally, the chemical or physical hydrolysis precedes the enzymatic in order to further release C6 and C5 sugars [4].

Extrusion cooking and steam explosion are two alternative types of physical pretreatments that are used to expose cellulose and hemicellulose associated with lignified cell walls. The first has been effectively used to disrupt fiber components especially hemicellulose minimizing the use of water [23, 24]. Heredia-Olea [25] effectively employed a thermoplastic twin extruder to modify the fiber structure of ground sorghum bagasse. Results indicated that that thermoplastic extrusion additionally reduced the particle size of the ground feedstock

and exposed cellulose which was more susceptible to fiber-degrading enzymes. The extruded feedstock treated with the kit of fiber-degrading enzymes had conversion efficiencies of 77.5 and 60 of cellulose and hemicellulose, respectively. These conversion rates are comparable to rates attained when acid and enzyme hydrolyses. The main benefits of the continuous extrusion process are that hydrolysates are virtually free of yeast inhibitors such as acetic acid, furfural and hydroxymethyl furfural [1, 24].

Steam explosion consists of placing the feedstock in a pressurized reactor where steam is injected. The reactor's operation temperature is in the range of 170–210°C [26, 27]. After a 2–10 min holding cycle, the blown down valve suddenly opens and the resulting pressure variation disrupts the fiber matrix [27]. Sipos et al. [26] achieved an extraction of 89–92% of cellulose associated with sweet sorghum after the use of this technology. The same authors documented an impregnation process for ground sorghum bagasse with up to 3% w/w $SO_2$ in plastic bags for up to 30 min. The $SO_2$ impregnated bagasse prior to steam pretreatment improved the subsequent saccharification step [27]. The ammonia fiber explosion process known as AFEX is a novel pretreatment that disrupts the internal fiber structure and molecular characteristics of the raw material without the production of liquid. Lee et al. studied the effectiveness in terms of fermentable sugars generation of autohydrolysis or AFEX applied to coastal Bermuda grass [9]. The AFEX process conducted at 100°C for 30 min yielded 94.8% sugars of the theoretical possible value, whereas autohydrolysis at 170°C for 1 h yielded just 55%. The study clearly demonstrated that the proposed AFEX pretreatment enhanced significantly the enzymatic accessibility of the Bermuda grass.

The most employed chemical pretreatment employed for second-generation ethanol production is acid hydrolysis because it is relatively cheap, releases significant quantities of sugars and improves the susceptibility of disrupted fiber components to the next stage of the process consisting of treating the biomass with fiber-degrading enzymes [28]. The major advantage and disadvantage of acid hydrolysis is that it enhances cell wall delignification and generates relevant quantities of known yeast inhibitors. Sulfuric, hydrochloric, hydrofluoric or acetic acids have been used. The process involves adding diluted acid solution (0.1–10% mass fraction) to the milled feedstock followed by pressure-cooking in a reactor. The major control points of acid hydrolysis are the strength of the acid solution, the pressure and temperature and the processing time.

Several investigators have researched the effectiveness of acid hydrolysis of sweet sorghum bagasse. Kurian et al. [29] pressure-cooked sorghum bagasse with sulfuric acid at a concentration of 5 g $kg^{-1}$ for half an hour and obtained an extract with 92 g $L^{-1}$ of total sugars, whereas Ban et al. [28] treated the same raw material at a solid-liquid ratio of 10% with 80 g phosphoric acid $L^{-1}$ at 120°C for 80 min. These authors reported 302 g $kg^{-1}$ bagasse of reducing sugars after applying this specific acid treatment. Recently, Heredia-Olea et al. [20] researched through surface response methodologies two different acid pretreatments (sulfuric or hydrochloric acid) and one blend of these acids on ground sweet sorghum bagasse harvested post-anthesis. Resulting acid hydrolysates were treated with calcium hydroxide in order to detoxify hydrolysates. The sweet sorghum bagasse free of the sweet juice contained 41.2% cellulose and 24.5% hemicellulose. The response variables were production of C5 and C6 sugars and the three major inhibitors of yeast (acetic acid, 5-hydroxymethylfurfural, and furfural). Results

indicated that the different acid pretreatments produced similar quantities of fermentable carbohydrates. These pretreatments liberated from 56 to 57% of the total sugars present in the sorghum bagasse (390–415 mg sugar $g^{-1}$ bagasse) and from 44 to 61 mg total inhibitors $g^{-1}$ (**Table 2**). Among the three pretreatments, the HCl treatment was the best alternative due to its relatively lower hydrolysis time (less energy expenditure) and satisfactory yield of C5 and C6 fermentable sugars.

On the other hand, the use of strong alkalies breaks ester bonds of cross-linked lignin and xylans producing an enriched cellulose and hemicellulose fraction. The preferred alkalis are sodium hydroxide, ammonia and calcium hydroxide or lime. The alkaline pretreatments are regularly conducted at relatively lower temperatures, pressures and times compared to other technologies. The main withdraw of chemical treatments is the production of known yeast inhibitors divided into three categories: organic acids (i.e. acetic, formic and levulinic), furans (furfural and 5 hydroxymethylfurfural) and phenolics such as *p*-hydroxybenzoic acid released from lignin moieties [28]. The concentration of these hydrosoluble compounds is known to upset cell physiology and viability and thus the efficacy of ethanologenic microorganisms. According to Amartey and Jeffries [30], the removal of these inhibitors before fermentation can reduce approximately 25% the production cost.

Wang and Cheng investigated the efficiency of lime or calcium hydroxide pretreatment in order to enhance reducing sugar recovery in coastal Bermuda grass [31]. These authors studied the effects of temperature (21–121°C) and lime loadings (0.02–0.2 g/g of dry biomass) followed by biocatalysis with cellulases and cellobiases. The best pretreatment combinations removed approximately 20% of the original total lignin content, which was over twice more than that from the untreated counterpart. The best total fermentable sugar yield for the lime pretreated Bermuda grass (100°C, 15 min, and 0.1 g lime $g^{-1}$ dry biomass) was 78% of the theoretical maximum. Moreover, this specific pretreatment converted 87%, and 68% of the

| Compound (%) | Sweet sorghum bagasse[4] | | | Bermuda grass[5] | |
|---|---|---|---|---|---|
| | HCl hydrolysis | HCl hydrolysis + enzymatic hydrolysis | Extruded + enzymatic hydrolysis | $H_2SO_4$ hydrolysis | $H_2SO_4$ hydrolysis + enzymatic hydrolysis |
| Glucose | 52.2 | 82.2 | 66.5 | 20.9 | 90.5 |
| Xylose | 53.3 | 75.7 | 94.1 | 66.6 | 84.6 |
| Arabinose | 42.6 | 50.2 | 28.7 | 90.0 | 90.0 |
| Total sugar yield[3] | 55.6 | 80.6 | 67.5 | 40.9 | 89.2 |

[1]The compounds are percentage of sweet sorghum bagasse or Bermuda grass.

[2]Results are in dry matter basis.

[3]Sugars yield = total sugars (mg/g)/[1.11× glucans (mg/g) + 1.14 (xylans (mg/g) + arabinans (mg/g))].

[4]Heredia et al. [25].

[5]Canizo et al. [22].

**Table 2.** Sugars and second generation ethanol generated from sorghum bagasse or Bermuda grass after different pretreatments[1,2].

glucan and xylan into glucose and xylose, respectively. A microwave-assisted alkali pretreatment of coastal Bermuda grass followed by enzyme hydrolysis proved to be an effective technology to improve fermentable sugars. Pretreatments were performed by immersing the feedstock in different dilute alkalis and microwaving the resulting slurries at 250 W for 5–20 min [32]. Sodium hydroxide was the most effective alkali for microwaving of the coastal Bermuda grass. Approximately 87% glucose and 59% xylose yields were achieved after the hydrolysate previously treated with 2% NaOH and microwave treated for 10 min was treated with the fiber-degrading enzymes. For pretreated Bermuda grass, the hypothetical yields of ethanol based on glucose or glucose and xylose were 147 and 208 L $ton^{-1}$, respectively.

Heredia-Olea et al. [20] assayed the quantities of furfural, hydroxymethyl furfural and acetic acid produced after diverse acid hydrolyses. These compounds were generated in higher extents in hydrolysates obtained with higher acid concentrations and processed for longer periods of time. The principal inhibitor was acetic acid which was freed from hemicellulose covalently bound by acetic moieties to lignin [33, 34]. The different sorts of acids broke these linkages generating this acid inhibitor. Detoxification strategies are commercially used to lower inhibitors. The most usual approaches are the use of calcium hydroxide (lime) or activated carbon which entraps phenolic compounds from lignin, ion-exchange resins and enzymes such as laccase [4]. Heredia-Olea et al. [20] successfully detoxified with lime different sorts of acid hydrolysates obtained from sorghum bagasse. Results indicated that the lime treatment removed 19% of the acetic acid and 38% of the hydroxymethylfurfural.

### 2.3. Enzymatic hydrolysis and fermentation

The enzyme hydrolysis of fiber is one of the fundamental steps for second-generation alcohol production. Normally, this biocatalysis is performed in chemically treated biomass or alternatively and less frequently with ground untreated fiber. This last process is more recommended for feedstocks low in lignin and has the advantages of saving energy, processing time. Besides the sole utilization of enzymes without the chemical pretreatment is less harmful for the environment. There are several enzymes normally utilized to convert cellulose and hemicellulose into fermentable carbohydrates. They consist of blends of endo and exocellulases, cellobiase, hemicellulases, pectinases, xylanases, B-glucosidase and others [4]. Cellulose is more effectively hydrolyzed by the synergistic activity of endo and exo-acting enzymes (exoglucanases). Nowadays, it is common practice to employ enzyme kits consisting of seven or more cell wall degrading enzymes which act synergistically. Heredia-Olea et al. [25] investigated the efficacy of two concentrations of a fiber-degrading enzyme kit supplemented directly to ground sorghum bagasse and concluded that the higher concentration produced about 20% more fermentable sugars. The main soluble carbohydrates generated during the enzymatic treatment were glucose and xylose. Moreover, the thermoextruded feedstock treated with the fiber enzyme complex was effective when applied in a SSF system. This combination yielded hydrolysates with high amounts of fermentable sugars employing less energy and processing time.

Production of ethanol from physically, chemically and/or enzymatically treated hydrolysates is feasible with the utilization of osmotolerant and C5 (pentose) yeast or bacterial strains. The recent advances in biotechnology has generated numerous genetically modified or engineered yeast and bacteria proficient of fermenting hydrolyzates containing significant amounts of

C5 and C6 sugars. The most important and applicable are strains of *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, *Pichia stipitis*, *Klebsiella oxytoca*, *Klebsiella planticola*, *Candida shehatae*, *Flammulina velutipes* and *Issatchenkia orientalis.* These microorganisms have been innoculated alone or in blends at temperatures around 37°C and pH varying from 5.2 to 6 [1, 32].

Heredia-Olea et al. [20] fermented detoxified sweet sorghum bagasse worts obtained after acid and/or enzyme treatments with a genetically-engineered *S. cerevisiae* or *I. orientalis* which were modified so they were able to metabolize C5 and C6 sugars. *S. cerevisiae* and *I. orientalis* were able to produce 18.4 and 20.9 g ethanol·100 $g^{-1}$ dry sweet sorghum bagasse, respectively, and were capable of fermenting 64.4 and 73.3% of the fermentable sugars. Remarkably, most of the bioethanol was produced during the first day of fermentation. Likewise, Ballesteros et al. [35] obtained 16.2 g ethanol $L^{-1}$(10% solids) from sweet sorghum bagasse hydrolyzates fermented with *K. marxianus*, whereas Kurian et al. [29] working with *P. stipitis* produced 38.7 g ethanol $L^{-1}$ with a theoretical conversion of 82.5%.

Although similar pretreatments have been also applied to Bermuda grass (alkaline, hot water, ozonolysis, steam explosion), the diluted acid hydrolysis had better effectiveness in terms of releasing monomeric sugars, specially xylose [23]. Canizo et al. [22] concluded that $H_2SO_4$ hydrolysis (121°C, 75 min, 1.25% acid concentration, and 12.5 solid to liquid fraction) released 20.9% glucose, 66.6% xylose and 90% arabinose. Likewise, Ballesteros et al [35] used a hydrolysis scheme with more acid concentration (1.5% w/w) and time (90 min) achieving 66 and 52% xylose and glucose yield, respectively. Wang and Cheng studied the efficiency of lime pretreatment and enzyme hydrolysis in terms of reducing sugar production and bioethanol yield of costal Bermuda grass [31]. The highest total reducing sugar production of 449.8 mg/g of raw biomass was attained using the pretreatment conditions of 0.1 g of lime/g of dry biomass, 100°C, and 15 min. Fermentation tests of the hydrolysates indicated that more than 99% glucose was converted by the yeast with ethanol yields of approximately 95% of the theoretical maximum. Assuming that the annual production of coastal Bermuda Grass was 14.8 tons/ha, on the basis of the theoretical ethanol yield from raw Bermuda grass of 167 L/dry ton of biomass for only glucose fermentation or 273 L/dry ton of biomass for co-fermentation of glucose and xylose, could be 2056–2755 L/ha. Redding et al. [36] employed higher temperatures (140°C for 30 min) achieving hydrolysis rates of 83% of total xylose and 28.3% glucose. A significant improvement in fermentable carbohydrate yield from Bermuda grass is documented after using a fiber-degrading enzyme complex [23]. Likewise, Canizo et al. [22] hydrolyzed Bermuda grass solids after separating the acid pretreated hydrolysate using 27 FPU celullase $g^{-1}$ cellulose, 0.6% β-glycosidase and 0.5% xylanase for 168 h at 50°C, pH 4.8 and 150 rpm and concluded that the dual technology released 90.1% of glucose from cellulose and 18% xylose from hemicellulose. Thus, the dual treatment yielded 89.2% of the total sugars from the Bermuda grass cell walls (**Table 2**). Sun and Cheng [37] used 25 FPU $g^{-1}$ cellulase plus 75 IU $g^{-1}$ cellobiase to increase approximately 20% the yield of glucose. Likewise, Redding et al. [36] used 40 FPU $g^{-1}$ cellulase plus 70 IU $g^{-1}$ cellobiase in order to obtain 95% of the theoretical fermentable sugar yield.

With the estimated ethanol yield obtained by Canizo et al. [22] is feasible to attain a theoretical amount of 180 mg of ethanol $g^{-1}$ of dried Bermuda grass. This means a theoretical ethanol yield between 1.08 and 4.86 t $ha^{-1}$ $year^{-1}$ (**Figure 1**).

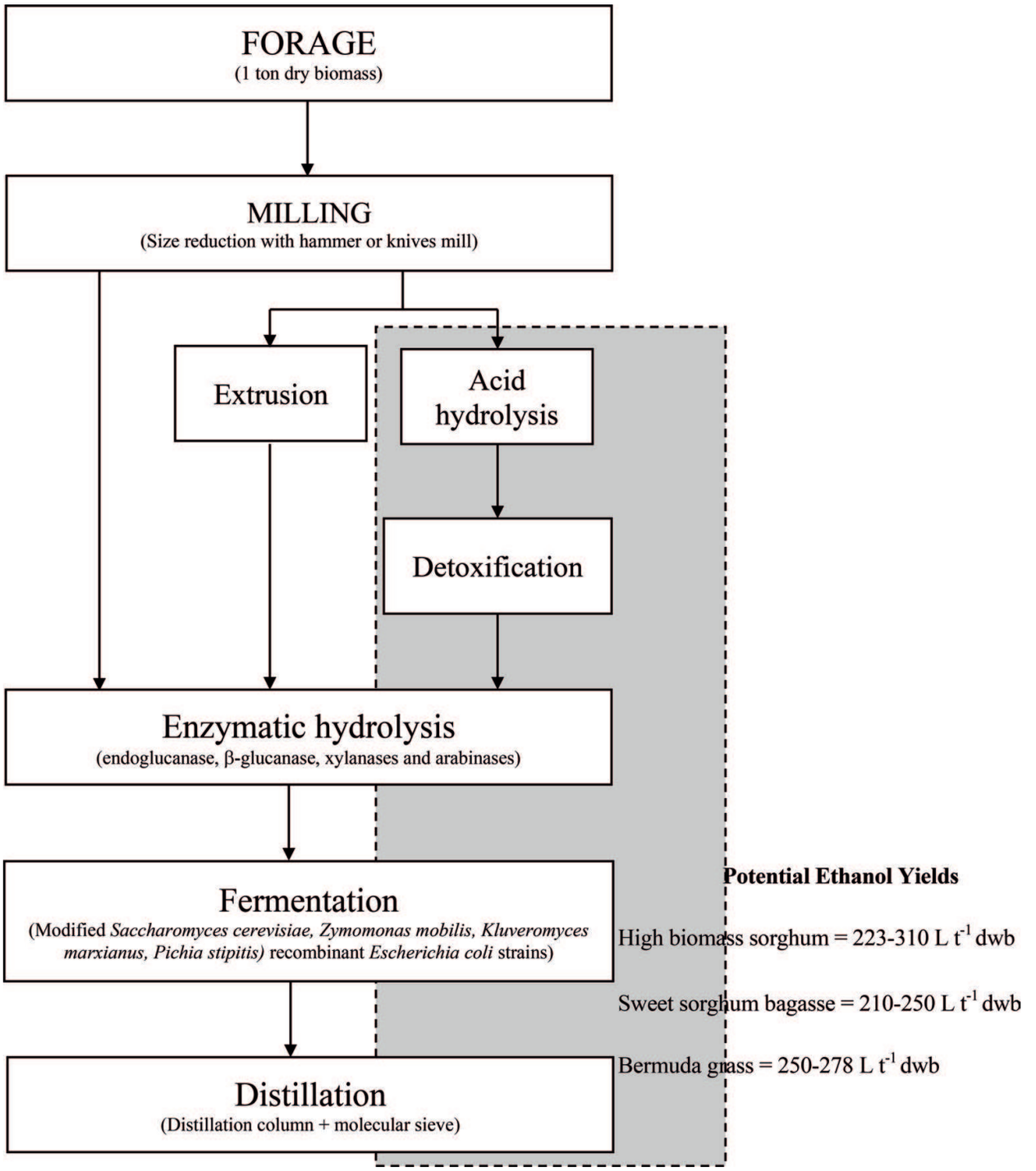


**Figure 1.** General schemes for production of second-generation ethanol from high biomass sorghum, sweet sorghum bagasse or Bermuda grass.

## 3. Conclusions

Both biomass sorghum and Bermuda grass adapted to tropical, subtropical and temperate agriculture regions of the world can be effectively converted into second-generation bioethanol due to the composition of the fiber rich in cellulose and hemicellulose and low in lignin.

Sweet and biomass dedicated sorghums can yield up to 60 tons of dry forage $ha^{-1}$ year $^{-1}$, whereas the perennial Bermuda grass up to 20 tons of dry forage $ha^{-1}$ year.

These lignocellulosic feedstocks can be converted into bioethanol using the conventional process of milling and pretreatment (chemical and/or enzymatic) with the aim of producing both C5 and C6 sugars in preparation for fermentation with yeast or genetically modified microorganisms capable of fermenting these sugars. After fermentation, the fermented broth is distilled in order to obtain high concentrated ethanol, which is further dehydrated in order to get anhydrous alcohol. An alternative process contemplates the use of thermoplastic extrusion aimed toward the physical disruption of the cell walls of the biomass minimizing the use of considerable amounts of water and chemicals commonly used during pretreatment. These feedstocks can yield up to 310 L anhydrous ethanol dry $t^{-1}$ and have a great potential for general use.

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