Chapter 4

Technological Advancements in 1G Ethanol Production and Recovery of By-Products Based on the Biorefinery Concept

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4.1 INTRODUCTION

The constant and growing interest in cleaner, more secure, and lower cost energy systems has increased the attention on biofuels production worldwide. Ethanol, one of the main sustainable biofuels, has been used for more than 50 years. The use of bioethanol reduces the levels of carbon monoxide and carbon dioxide emissions relative to fossil fuel, and has been classified by the US Environmental Agency as an advanced biofuel, once it can reduced greenhouse gas emissions up to 61% when compared to gasoline (UNICA, 2011).

A wide range of renewable feedstocks can be used for ethanol production, including: (1) fermentable sugars (sugar cane, sugar beets, sweet sorghum); (2) starches and fructosans (corn, potatoes, rice, wheat, agave); and (3) cellulosics (stover, grasses, corn cobs, wood, sugarcane bagasse) (Amorim et al., 2009). Despite the potential of various feedstocks, due to regional availability and technological challenges, 87% of the 101,380,178 m^3 of ethanol produced worldwide in 2015 was produced from corn and sugarcane (based on Fig. 4.1 data). These feedstocks are employed by Brazil and the United States, which produced 75% of the ethanol in the world (Balat et al., 2008; OECD-FAO Agricultural Outlook, n.d.).

Despite the biggest production of ethanol being based on starch derived from corn in the United States, ethanol produced from sugarcane presents

Advances in Sugarcane Biorefinery. DOI: https://doi.org/10.1016/B978-0-12-804534-3.00004-5

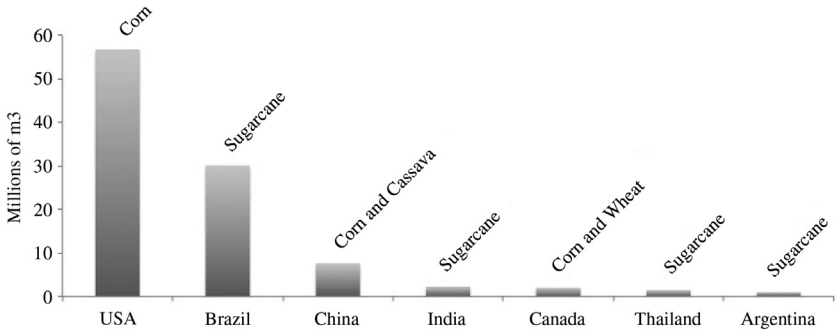

FIGURE 4.1 Ethanol production in 2015: main producing countries and feedstocks employed. *OECD Stats. Data viewed on 06.03.16.*

higher net energy and greenhouse gases balances (Cerqueira Leite et al., 2009). Sugarcane presents very high biomass productivity, amounting to 80–120 ton/ha/year, with an industrial ethanol production of 8000 L/ha, compared to 3000 L/ha from corn (McLaren, 2009). Thus, the sugarcane crop has drawn global interest as a raw material for the production of energy. Indeed, sugarcane is used as feedstock in many different countries (Fig. 4.1).

In Brazil, sugarcane is the first source of renewable energy, and it accounts for 18% of the national energy matrix (Empresa de Pesquisa Energética-EPE, 2015). The production of sugarcane in the country increased 8.6 times in the last 40 years, reaching 642,118,352 tons of sugarcane in 2015–16 (Fig. 4.2) (MMA, 2016). The increased production of sugarcane resulted from the expansion of cultivated areas to the south and middle west regions, as well as by the increment in the productivity of the crop.

The success of sugarcane in Brazil may be explained by the possibility that many mills produce both ethanol and sugar (Basso and Rosa, 2010). This flexibility allowed industrial development, even when ethanol or sugar prices where low in national and export markets. Indeed, sugar production increased considerably from 1994 to 2004, while ethanol production remained at similar levels by that time. Brazil has been a world leader in sugar exportation for several years, with a production of 38,069,510 tons in 2010–11 (Brasil, 2016). However, its manufacture slightly decreased to 32,745,169 tons in the last 3 years, due to the increased profitability of ethanol (UNICA, n.d.; USDA, 2015) (Fig. 4.2).

Ethanol production in Brazil increased considerably with the Pró-álcool Program in 1975, going from a production of 59,499 m^3 in 1974–75 to 12,765,910 m^3 in 1994–95 (Brasil, 2012, 2016) (Fig. 4.2). A second period of high increments in ethanol production took place between 2004 and 2011, due to a big increase in the Brazilian fleet of flex fuel cars (running with ethanol and/or gasoline). This growth was directly influenced by the fluctuation of

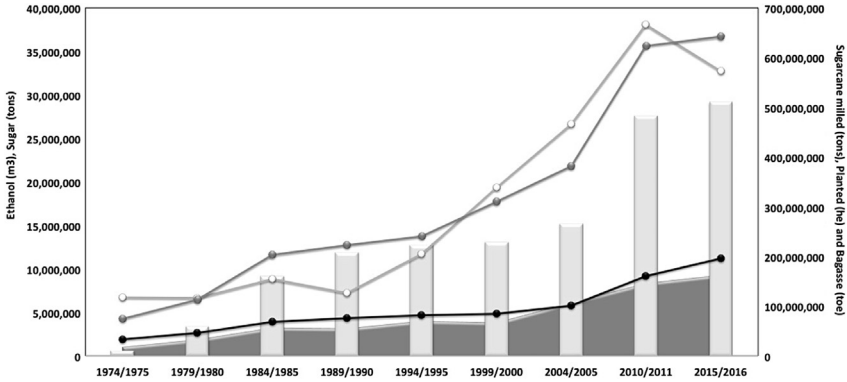

FIGURE 4.2 Brazil's ethanol, sugar and sugar cane production along the years. *Black* circle: sugarcane production area; *Gray* circle: sugarcane milled; *White* circle: sugar production; Bars: total ethanol production; Area: sugarcane bagasse production.

petroleum prices, tax incentives, and subsidized credit lines given by the government to the sector. In the last 5 years, ethanol production stabilized (Fig. 4.2), due mainly to a drought that affected sugarcane crop productivity and the absence of political incentives for ethanol production (CONAB, 2015).

Brazil has used sugarcane as feedstock for large-scale bioethanol production for more than 30 years in a biorefinery model. The biorefinery concept embraces a wide range of technologies to process and convert biomass or biomass-derived components to energy, chemicals, and other materials. Its purpose is analogous to a petroleum refinery, which produces multiple fuels and products (Dias et al., 2011; Kamm and Kamm, 2004) and has the potential to settle the growing demand for energy, fuels, chemicals, and materials worldwide. In the process, sucrose from sugarcane juice is converted to ethanol and sugar, and the sugarcane bagasse is burnt to generate steam and power (Mariano et al., 2013). In addition, yeast and vinasse can be used as animal feed and fertilizers, respectively.

4.2 TECHNIQUES IN ETHANOL PRODUCTION FROM SUGARCANE

The ethanol production process improved considerably over the years. Initially, production of ethanol was established to process molasses from the sugar industry (annexed distilleries), but with the increasing importance of ethanol in the 1980s, many mills began to run as autonomous ethanol plants (i.e., autonomous distilleries, which only produce ethanol). The main steps in the ethanol production process, which are similar in both type of distilleries, are summarized in Fig. 4.3, and briefly described below.

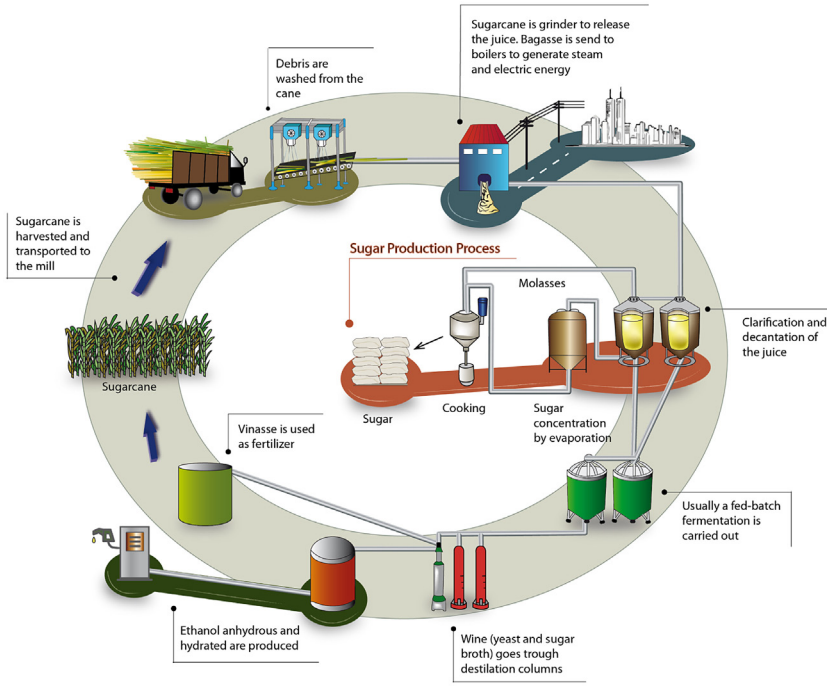

FIGURE 4.3 Ethanol production process from sugarcane and coproducts generated.

4.2.1 Crop Production, Harvesting and Transportation

The first step to produce ethanol is to obtain sugarcane fields with high productivity. Sugarcane is a semiperennial plant that requires crop rotation with other crop types to maintain the stability of the soil, which is usually employed every 5 or 8 years. Between cycles, leguminous plants are planted with the purpose of recycling nutrients and adding nitrogen by biological fixation in the soil. Sugarcane is usually harvested every 2 years from the same canebrake. Most of the harvesting is done mechanically nowadays, due to investments from the sector and legislation that prohibits straw burn. After harvesting, the cane is cut into smaller pieces and transported by trucks to the mills as soon as possible (not more than 8 hours), to avoid degradation of the cane (CGEE, 2008).

4.2.2 Cleaning, Grinder and Juice Extraction

Once sugarcane arrives in the mill, it passes through a cleaning process to wash impurities and other residues. The cleaning can be wet or dry; dry cleaning is more ecologically friendly as it does not waste water, and does not lead to sucrose loss. After that, sugarcane is ground to crush the fiber

and to increase the density of the material from 175 to 450 kg/m^3 (CGEE, 2008; Pacheco et al., 2013).

The juice extraction process is made by a crusher (some mills use a diffusion process) to release sucrose. The milling process extracts the juice using 4−7 mill suits with 3−4 pressure rollers each, while hot water (around 70°C) is passed through the bagasse to improve extraction yield. The remaining bagasse, with a humidity of 50%−52%, is sent to boilers to generate high-pressure steam. The steam turbine converts thermic energy to mechanical or electrical energy (Pacheco et al., 2013).

4.2.3 Sugar Production and Molasses Generation

In annexed distilleries, the amount of juice that is going to be directed to produce sugar, usually, depends on the market demand. Due to quality reasons, only the sugarcane juice extracted in the first mill suit is used for sugar production. The juice obtained in following mill suits, which accounts for at least 30% of the total reduction sugar, is directed to ethanol production. After extraction, the juice is sieved and clarified by decanting of impurities to prevent the undesired sugar inversion. Clarification uses chemical compounds such as sulfate (sulfuric gas) and liming, followed by heat and decanting (Pacheco et al., 2013). The clarification process generates a sludge that is used as a fertilizer in the sugarcane crops (CGEE, 2008).

During clarification, the juice is heated to 105°C to lower microbial contamination, and to facilitate the coagulation of colloids and emulsification of grease and wax. At sugar processing, high temperatures are used to concentrate the juice through evaporation and to crystallize sucrose. After sugar production, the residual sugar solution is called molasses (or honey), which contains high amounts of glucose (5%−20%) and sucrose (45%−60%) (CGEE, 2008). In annexed sugarcane distilleries, molasses can be mixed with sugarcane juice (originating must) to produce ethanol. This is advantageous, because the juice has some nutritional deficiencies, whereas molasses has inhibitory compounds for yeast fermentation. In some plants, only one or the other is used as a substrate to produce ethanol (Basso et al., 2015).

4.2.4 Fermentative Processes

As discussed above, sugarcane goes through cleaning, extraction, and physical and chemical treatments (Fig. 4.3). After that, sugars are fermented to ethanol by yeasts. Fermentation starts by mixing sugarcane juice or must (molasses and sugarcane juice), which contains 18%−22% (w/w) total reducing sugars, to a yeast cell suspension. Different types of fermentation processes were developed over time, such as: batch process, fed-batch process, and continuous process. Fed-batch process is commonly used in 70%−75%

of the ethanol distilleries, the feeding time normally lasts for 4–6 hours, and fermentation is finished within 6–10 hours.

In the batch process, the fermentation vat is loaded with a carbon source (must or sugarcane juice) prior the addition of yeast (*Saccharomyces cerevisiae*). The yeast suspension (with 30% of yeast cell, on a wet basis) represents 25%–30% of the total volume of fermentation, which is performed in tanks of 300–3000 m^3. This method is not used in industrial ethanol plants, since it can lead to low productivity of ethanol due to the presence of contaminants once the process takes place under aerobic conditions (Basso and Rosa, 2010; Cheng et al., 2009), being used only on a laboratory scale, in small distilleries, or in yeast propagation.

In the fed-batch process, yeast is added to the fermentation vat and the juice is added continuously during the fermentation process until the maximum volume of the vat is reached (Cheng et al., 2009). This process is performed in serial fermentation vats, where the must is added with constant feed flow rate or intermittently. After the vat reaches its maximum volume, the fermentation continues until the total reduction of the sugar is complete, and the product is collected followed by a cleaning and sterilization process of the fermenter for the next batch. This fermentation method has some advantages, such as maintenance of the maximum concentration of viable cells, prolongation of cell lifetime, and less inhibition of yeast by the high substrate concentration (Zabed et al., 2014). The fed-batch system is widely used in Brazilian industry, being employed in approximately 75% of the mills, due to a higher ethanol yield at the end of fermentation and being less subject to contamination (Basso et al., 2015).

The continuous fermentation process is characterized as a system that can operate for long periods at steady state. The fermentation vat works with a constant high volume and flows feed of must, while the juice is withdrawing at the same flow rate of the inlet flow. Continuous fermentation is a process that requires greater knowledge of the microorganism's behavior in the environment in which it operates. Operating factors such as pH, temperature, substrate concentration, ethanol, and biomass influence the system productivity, requiring greater control of the process. The biggest disadvantage is that the continuous fermentations are more susceptible to bacterial contamination for long exposure times (Cysewski and Wilke, 1978).

When fermentation ceases, the resulting broth (called wine) has about 6%–12% (v/v) of alcohol. Yeast cells are separated from wine by centrifugation, resulting in a concentrated yeast cell suspension (the yeast "cream") with 60%–70% (wet weight basis/volume) of cells (Basso et al., 2015). As the yeast suspension is recycled, the yeast cream is diluted with water and treated with sulfuric acid for 2 hours to reduce contamination (Basso et al., 2015). Remaining wine is sent to a distillation vat, and ethanol is recovered in a hydrated form (96° GL), producing stillage or vinasse as a by-product. This by-product is usually sent to cane fields to be used as fertilizer (Fig. 4.3). The hydrated ethanol can be stored, or sent to a dehydration vat where cyclohexane

is added to produce anhydrous ethanol. Another way to produce anhydrous ethanol is through molecular sieves that consume less energy (CGEE, 2008).

4.3 NEW TECHNOLOGIES INVOLVED IN THE PRODUCTION OF ETHANOL 1G

In the first 20 years of industrial ethanol production in Brazil (1975–94) there was a very significant improvement in several variables of the process, since the process was maturing. In the 20 subsequent years, the most significant improvement was in the alcohol content present in the wine, and consequent lower vinasse production (Table 4.1). Among others factors, this was possible due to the characterization, selection, and development of more tolerant yeast strains, with higher ethanol yields and implantation capability in the distilleries. Thus, in next section we discuss the main topics on yeast and sugarcane research that allowed this improvement.

4.3.1 Yeast Research

4.3.1.1 Selection and Genetic Improvement of Yeasts

In Brazil, *S. cerevisiae*, including industrial and wild yeast strains, is the most common microorganism used in industrial bioethanol production from sugarcane juice and molasses (Marques et al., 2016).

TABLE 4.1 Technological Evolution of Sugarcane Ethanol Production Process

Indicators	Initial Phase of Pro-Alcohol Program (1975)	1994	2013
Fermentation time (h)	24	6	6–8
Alcohol content (°GL)	6.5	10	Up to 16[a]
Fermentation efficiency (%)	80	91	92
Distillation yield (%)	98	99	99.5
Ethanol yield (l hydrated bioethanol/t cane)	66	86	87
Bagasse surplus (%)	Up to 8	Up to 78	Up to 78
Vinasse produced/bioethanol (L/L)	13	–	10–15/1

[a]*Value obtained using perfect fermentation conditions and tolerant strains.*
Dedini S.A. Indústrias de Base (2005, 2012, 2013); Abarca (2005).

Investigations of the yeast population from distilleries by karyotyping analyses have shown that starter cultures (brewing or baker's yeast) could not survive over the crop season (Basso et al., 2008; Lopes et al., 2015). As the ethanol production is carried out without complete asepsis, contaminant microorganisms are able to enter in the process and replace the initial cultures. Indeed, studying yeast population dynamics in industrial alcoholic fermentations, Basso and coworkers (2008) observed that baker's yeast was replaced by wild strains in a period of 20–60 days, despite 250 days of recycling during the season. In an extensive study, covering a period of 12 years (1993–2005) and approximately 70 distilleries, approximately 340 contaminant strains were isolated and identified. Most of them showed undesirable traits like excessive foam formation, flocculation, and incomplete fermentation, regardless of their dominance and persistence, and only 14 strains presented desirable fermentation features and performances. Of them, PE-2, CAT-1, and BG-1 were the best performing strains, with higher implantation capability (Basso et al., 2008). Due to their interesting traits, PE-2 and CAT-1 have been used as selected starter cultures in more than 200 Brazilian distilleries that account for 60% of the entire national ethanol production (Basso et al., 2015). Application of selected strains such as PE-2 represents a yield gain of ~3%, compared with baker's strain. In a distillery, this difference generates an impact in ethanol production of approximately 2,000,000 L of ethanol per crop season (Basso et al., 2008).

Following dynamic populations of yeast in their own distilleries has allowed the selection of new strains, with improved capabilities and more adapted to their microenvironment (Lopes et al., 2015). Using a process-driven selection strategy, FT858 (2007) and FERMEL (2014) yeast strains were recently selected. The first one shows high dominance and persistence rates, tolerance to aluminum, pH changes, and high alcohol conversion, while the former strain was selected for molasses-based musts, molasses, or sugarcane juice fermentation, demonstrating robustness, higher tolerance to acid treatment, and alcohol content and high fermentation speed ("Fermentec," 2016).

Currently, on the Brazilian market for fuel ethanol, few yeast strains are being produced on a large scale for commercialization. In addition to the above-mentioned yeast strains (CAT-1, PE-2, BG-1, FT-858, FERMEL), there are other strains also widely used in the distilleries, such as VR-1, SA1, and JP1. All of them can be used alone, or even in combination, to start the fermentation processes (Della-Bianca et al., 2013; "Fermentec," 2016; "LNF," n.d.).

4.3.1.2 Genetics Behind Yeast Robustness and Dominance

Understanding the mechanisms of stress tolerance and dominance of natural isolates is a prerequisite to exploit desirable features for directed evolution and genetic engineering of yeasts in first-generation bioethanol production.

Whole-genome sequencing and other genetic approaches revealed important traits of bioethanol yeasts, such as CAT-1 and PE-2 strains, and a haploid derivative of the YJS329 (Argueso et al., 2009; Babrzadeh et al., 2012; Stambuk et al., 2009; Zheng et al., 2012). The copy number amplification of genes related to the metabolism of vitamins B1 (thiamine) and B6 (pyridoxine) was observed in five industrial strains (BG-1, CAT-1, PE-2, SA-1, and VR-1) compared to baker's yeast and S288C laboratory strain (Stambuk et al., 2009). Besides the role of these cofactors in the amino acid metabolism and other biochemical pathways, they are also required for sugar catabolism and oxidative stress tolerance, which could explain the competitive advantage and positive effect in the predominance of these strains in a fermentation industry that uses high sugar concentration broth (Argueso et al., 2009; Babrzadeh et al., 2012; Stambuk et al., 2009).

4.3.1.3 Increasing Ethanol Yields by Reducing By-Product Formation

Glycerol is one of the major by-products obtained during bioethanol industrial fermentation. Approximately 4−8% of the substrate is converted to this compound (Gombert and van Maris, 2015). In this way, a reduction in the quantity of glycerol produced during fermentation could positively affect the ethanol yield. Adjusting feeding rates in fed-batch processes, or selecting low glycerol-producing strains, are strategies to increase the ethanol production (Basso et al., 2015).

Recently, an approach for reducing glycerol formation has been described. Studying yeast natural biodiversity, Hubmann et al. (2013a,b) applied pooled-segregant whole genome sequence analysis to identified major and minor Quantitative trait loci (QTLs) related to low glycerol and high ethanol yields in yeast fermentation. Multiple alleles of regulatory and structural genes of glycerol metabolism were identified as causative of a low glycerol formation phenotype in CBS6412 strain. Introduction of these mutants in an industrial-derived strain reduced the glycerol/ethanol ratio, without compromising the osmotic stress tolerance or ethanol productivity.

Another strategy employed to minimize the glycerol formation was the direct modification of central reactions associated with the formation of the by-product, as deletion or regulation of genes encoding glycerol-3-phosphate dehydrogenase GPD1 and/or GPD2 enzymes. Despite decreased glycerol production in the case of a single deletion, or even eliminating it when the double deletion is present in the strain, these modifications are also implicated in negative effects on growth, fermentation, and osmotolerance (Björkqvist et al., 1997; Gombert and van Maris, 2015). Fine-tuned reduction in GPD activity by promoter engineering showed improved ethanol yields, but also led to loss of productivity and stress tolerance (Pagliardini et al., 2013). In an attempt to solve this problem, Guadalupe-Medina et al. (2014)

applied evolutionary engineering to obtain an evolved osmotolerant *gpd1Δ gpd2Δ* acetate-reducing *S. cerevisiae* strain, which already carried an alternative redox sink, coupling NADH reoxidation to the reduction of acetate to ethanol. Although further improvements are required for industrial implementation, the strain showed 11% higher ethanol yields, and at least 10-fold lower glycerol production compared to the reference strain. The mentioned advances were not yet implemented in Brazilian industries; however, they show the potential of engineered yeast strains to increase the efficiency in bioethanol production.

4.3.1.4 Tolerance to Ethanol, High Temperatures and High Osmotic Pressure

The implementation of Very High Gravity (VHG) fermentation, in which the initial sugar concentration can reach 250−400 g/L, represents a challenge to increase the efficiency of first generation fuel ethanol. Using this technology, the ethanol content in the fermentation broth will increase, then, less energy will be necessary for ethanol distillation, and less waste will be produced. VHG affects directly the overall production cost, causing a reduction of it, and contributing to environmental sustainability (Basso et al., 2015).

The polygenetic nature and complexity of ethanol tolerance make the rational traditional methods not the most adequate for strain improvement, thus nontargeted approaches, such as evolutionary engineering, mutagenesis, and genome shuffling, have shown more successful results (Snoek et al., 2015; Steensels et al., 2014; Swinnen et al., 2012). Swinnen et al. (2012) mapped QTLs involved in tolerance to high ethanol levels (up to 18% ethanol) in the Brazilian bioethanol strain VR-1. Strategies like that can provide genes for strain improvement through genetic engineering. The use of genome shuffling has been applied to generate strains with improved phenotypes, as performed by Tao et al. (2012) and Zheng et al. (2014). In their work, the strains obtained showed higher stress tolerance and enhanced ethanol titers under VHG conditions. Using large-scale robot-assisted genome shuffling, Snoek et al. (2015) obtained hybrids with increased ethanol accumulation and tolerance compared to the commercial yeast Ethanol Red. The hybrids are derived from eight strains initially selected among 318 *Saccharomyces* isolated in different niches, such as wine, ale, and biofuel production industry.

4.3.2 Improvement of Sugarcane

Genetic improvements in sugarcane have caused a great impact on the ethanol production in Brazil. Currently, more than 600 commercial varieties of sugarcane are available, derived from traditional genetic improvements developed mainly by Ridesa and CTC (Center of Sugarcane Technology). New cultivars

have been developed searching for desirable traits like high productivity, increased sugar content, adaptation to weather and soil conditions, tolerance to drought, or resistance to diseases and pests (Matsuoka et al., 2009).

Breeding programs have shown a good potential and effectiveness for sugarcane improvement. Thanks to them, sugarcane productivity increased 50% in the last 40 years (Matsuoka et al., 2009). However, biotechnology and genetic approaches have come to accelerate this process, introducing new genes into the sugarcane genome or developing genetic tools or methods to identify quickly clones with advantageous characters (CGEE, 2008).

The complete genome sequence of sugarcane is not available yet, due to the high polyploidy and complexity of the *Saccharum* genome. Considering this aspect, different strategies are being used to reveal some genetic information. de Setta et al. (2014) constructed and analyzed a set of 317 BAC (Bacterial Artificial Chromosome) sequences produced from the R570 sugarcane cultivar and, more recently, another BAC collection was generated from a different variety, SP80-3280 (Okura et al., 2016). Both analyses provide relevant information about the structure and sequence composition of the sugarcane genome. Furthermore, thousands of sugarcane protein-coding genes and several noncoding genes were sequenced and annotated, allowing, e.g., access to important biological information about metabolic pathways, promoters, and intergenic regions. The SUCEST, a consortium of Brazilian researchers, produced the largest collection of sugarcane ESTs (expressed sequence tags). The database contains 237,954 sugarcane ESTs from cDNA libraries constructed from different organs and tissues sampled at different developmental stages or conditions (Hotta et al., 2010; Vettore, 2003). This collection provides important tools for the identification of genes involved in growth development, sugar accumulation, and response to biotic and abiotic stresses, and for the development of molecular markers associated with interesting traits in the cultivars (Matsuoka et al., 2009).

Several groups have been working on identification of molecular markers and genes that can be used in molecular-assisted breeding to develop improved cultivars in less time and with lower costs (Garcia et al., 2006; Marconi et al., 2011; Margarido et al., 2015; Pastina et al., 2012). Using simple sequence repeats or microsatellite markers from the SUCEST database, Marconi et al. (2011) could detect high levels of polymorphism across 18 sugarcane genotypes tested. Some of them, e.g., those related to bacterial defense responses or carbohydrate metabolism, can be used efficiently for genetic mapping studies of segregating populations. In another approach, Pastina et al. (2012) performed a QTL detection based on mixed models for multienvironment data to identify QTLs for cane yield, sugar yield, fiber percentage, and sucrose content, taking into account two locations and three consecutive harvest years.

On the other hand, genetic engineering has been employed in many laboratories to generate new varieties of sugarcane, and field trials have been

conducted to evaluate the incorporated features (da Cunha et al., 2015). In Brazil, CTC was the pioneer in the creation of transgenic varieties in 1994, and since then, other studies with transgenic varieties have been conducted (CTC, 2016). Research groups are focused on the development of genetically modified sugarcane to increase sugar content (Wu and Birch, 2010), and biotic and abiotic resistance (da Cunha et al., 2015; Reis et al., 2014). As well, alternative approaches to use sugarcane expressing heterologous protein to produce value adding products, such as the biopolymers polyhydroxyalkanoate and polyhydroxybutyrate (PHAs and PHBs), or introducing changes to facilitate second-generation ethanol production or increase biomass production (Ferreira et al., 2016; McQualter et al., 2014; Somleva et al., 2013). However, there is still much to be understood and developed about transgenic sugarcane. Sugarcane transformation is not trivial and shows low efficiency. Transgene inactivation (gene silencing and regulation), genetic instability, somaclonal variation, and the long time required for regeneration and regulatory approval of the construction for commercial release (Hotta et al., 2010) are examples of some limitations in the area (Arruda, 2012).

4.3.3 Coproducts in the Bioethanol Industry

For the immediate future, the combination of emerging biorefineries with other industries is a potential solution to mitigate the threat of climate change, and also provide a means to support the demand for energy, fuels, chemicals, and materials (Ragauskas et al., 2006). The new challenge is to develop technologies for more valuable coproducts, and better utilization of crop residues in biorefineries. In this context, much has been done and considered regarding the development of the sugarcane industry into biorefineries. Here, we describe the main products of the sugarcane industry, and the potentially new products in addition to ethanol and sugar.

4.3.3.1 Sucrose

Sucrose is a common, naturally occurring disaccharide formed by glucose−fructose found in many plants. In sugarcane, sucrose contents can reach up to 26%. Traditionally, sucrose is used for sugar and ethanol production in Brazil (Basso and Rosa, 2010). However, developments in biorefinery research opened up the possibility of using sucrose for the production of high value added chemicals (Amyris, n.d.; "SolazymesIndustrial," n.d.). In this sense, sucrose could be directed, for instance, to production of oils and ingredients through genetically modified algae and microorganisms. Even with new uses, sucrose availability is still not limiting sugar and ethanol production; however, with the increment in production and release of new products in the market it could be affected. It is expected that ethanol

production could be compensated by the use of cane biomass (bagasse and straw) in a coupled second-generation process.

4.3.3.2 Power Generation

Sugarcane bagasse, one of the main by-products of sugarcane processing (Fig. 4.3), is used as fuel in cogeneration systems, which provide steam and electric energy to supply the bioethanol production process. The bagasse represents 25% of the sugarcane weight (Rezende et al., 2011). According to Dias et al. (2010), conventional plants can be equipped with boilers for the production of 22 bar steam. The steam produced in the boilers is used to produce electricity in steam turbines and thermal energy for the process, besides being used in mechanical drivers in the sugarcane preparation and juice extraction systems. However, for the past few years, growing interest in the production of electricity in ethanol production plants has been observed, which may improve revenues and the competitiveness of sugarcane ethanol (Dias et al., 2010). In this way, many sugar factories are presently producing considerable amounts of electricity for export to the utility grid, while at the same time meeting on-site energy needs by installing modern condensing−extraction steam turbines for cogeneration (Dias et al., 2010).

In addition to bagasse, sugarcane is composed of leaves and tops, which nowadays are left in the field. For the near term it is expected that part of this can be recovered from the field, to enable further power generation throughout the year.

In all cases where excess steam is produced, it is condensed in steam turbines, increasing the amount of electricity produced. Considering these commercial steam cycle cogeneration systems, the mills' electricity surplus could leap from the current 0−10 kWh/t cane level (pure cogeneration at 22 bar/300°C) to more than 140 kWh/t cane (CEST, 90 bar/520°C, using bagasse), and with competitive costs for the current electricity market. Indeed, in 2010, the capacity installed to produce electric energy from bagasse in Brazil was about 6011.6 megawatts (568,148.76 kW/h of energy), which corresponded to 5% of the countries energetic matrix (Filho, 2011). In 2015, 10,793 MW was produced from biomass energy, equivalent to 7.7% of the total power generation energy in Brazil (CCEE, 2015). New technologies and strategies to increase the capacity of the boilers and power turbines continue to be developed, with the aim of using bagasse in a more profitable way (NovaCana, n.d.).

4.3.3.3 Vinasse

Vinasse is a main by-product of the sugar−ethanol industry. It is usually an acidic (pH 3.5−5) dark brown slurry, with a high organic content (COD: 50−150 g/L), and an unpleasant odor to humans. According to Wilkie et al. (2000), the production of ethanol from sugar crops, starch crops, and/or

cellulosic material, generates on average, 10−15 L of vinasse for each liter of ethanol produced, depending on the distillery equipment (Cortez et al., 1992). Vinasse was considered highly toxic to animals, plants, microbes, and microflora from freshwater. In addition, it disturbed marine animals that came to the coast to reproduce, and had a high pollution potential, approximately 100 times more than household sewage, due to the high organic matter content, causing a depletion of oxygen and high levels of biochemical demand for oxygen (BOD) (Kannan and Upreti, 2008). However, vinasse use as fertilizer became usual in sugarcane refineries since the beginning of the 1980s. When applied in nature to the soil, in controlled and small quantities to avoid the damaging effect, sugarcane vinasse help fertilize the sugarcane crop, lowering the costs of chemical fertilizers (Laime et al., 2011).

An alternative that has increasingly been used for vinasse valoration in the ethanol industry is its anaerobic biodigestion. This process consists of the biodegradation of the organic load of vinasse to produce biogas. The anaerobic process occurs in two phases: acidogenic and methanogenic, with the action of facultative and obligate anaerobic bacteria. Due to its high methane content, biogas is mainly used to produce energy. In the sugar−ethanol industry, biogas can be used to: operate gas turbines combined to an electric generator; substitute part of the fuels used in the agroindustry during harvesting time; or use in boilers to generate vapors and to mill sugarcane (Szymanski et al., 2010).

Initiatives towards the use of vinasse for microalgae mixotrophic cultivation are also being evaluated. Recently, researchers from Embrapa have reported the selection of native wild-type *Chlorophyta* strains capable of producing up to 440 mg/L/day of biomass using crude vinasse as the sole growth media. Among them is a starch-rich *Chlamydomonas* sp. strain that is a priority within Embrapa's research program, given its potential use for ethanol (carbohydrate fraction) and electricity (residual biomass) production (Brasil, 2016).

4.3.3.4 Dry Yeast

Generally, fermentation starts by adding must to a yeast cell suspension. As there is always yeast growth during the fermentation step, an excess of yeast is generated during alcohol production. Thus, around 20% of yeast is intended for drying, while the remaining 80% is reused in the process of fermentation. The yeast cream is sent to a drying chamber (Spray Dryer), where it comes into direct contact with the hot air. The dried and powdered form can be packed and marketed as a supplement for animal feed ("Jalles Machado", 2016).

The dry yeast is used as a nutritional supplement in feed formulations due to its high protein content, high concentration of vitamin B complex, and excellent balance of amino acids. In addition, it also improves the flavor

of the feed, and has prophylactic effects (acts as a natural antibiotic). It is indicated for use in feeds for poultry, pigs, cattle, goats, fish, shrimps, horses, dogs, cats, and others, with indexes of specific inclusions recommended for each case (discretion of the vet/additions in feeds are usually from 0.1% to 1% in relation to its weight). In the stage of the preparation of this yeast, there is an increase in protein content and partial reduction of minerals, acidity, and organic substances that interfere with the color, smell, and taste of dry yeast ("Jalles Machado," 2016).

4.3.3.5 Bioplastic

Production of chemicals from renewable sources is essential to mitigate detrimental effects of a petroleum-based economy on the environment. In this sense, there is increasing interest in the production of bioplastics. Bioplastic is a plastic that is made partly or wholly from polymers derived from renewable sources, such as sugarcane, potato starch, or cellulose, straw, and cotton. There are three main types of bioplastics in commercial scale production: (1) plastics derived from fossil carbon source but biodegradable; (2) plastics derived from polymers converted from biomass and biodegradable; and (3) plastics derived from polymers converted from biomass but not biodegradable (Pei et al., 2011).

Living organisms, such as plants, fungi, or bacteria, produce bioplastics, derived from bio-based polymers. Some microorganisms are capable of converting biomass into biopolymers employing a set of catalytic enzymes. The bioplastics available in the market are made from polymers such as starch-based, polyhydroxyalkanoates (PHAs), polylactic acid (PLA), and others. Polymers for bioplastics are usually produced in biological fermentation processes using sugar, starch, oil, or lignocellulosic biomass-derived carbohydrates. For instance, xylose, which is one of the most abundant sugars in nature, can be converted to lactic acid and acetic acid by an anaerobic fermentation. These can subsequently be used as feasible feedstocks for PHA production (Pei et al., 2011).

4.3.3.6 CO_2

Carbon dioxide is produced in significant amounts during sugarcane juice fermentation and bagasse burning. Ethanol and CO_2 are produced equimolarly in the fermentation step, thus carbon dioxide (CO_2) from ethanol production facilities increases proportionally as more ethanol is produced. For instance, 90 kg of glucose becomes about 46 kg of ethanol and 44 kg of CO_2.

Carbon dioxide released from the ethanol fermentation process can be captured, stored, and utilized for beverages, oil recovery, refrigerant, fertilizer, and food processing (Hunt et al., 2010). For the recovery of CO_2, several combined processes are used, such as washing, liquefaction, compression, and refrigeration (Farla et al., 1995). One common method used for

the capture and purification of CO_2 is cryogenic distillation, which is a physical–chemical process that separates noncondensable contaminants from the gas of interest. The cryogenic distillation to obtain pure CO_2 is advantageous when the feed stream originating from the fermentation step is relatively pure (not less than 90%, v/v) (MAPA, 2012). Such a highly-concentrated source of CO_2 is a potential candidate for capture and utilization by the CO_2 industry (Xu et al., 2010).

In food industries, the carbonation process intends to improve organoleptic properties, such as taste and texture, and increase the shelf-life of soft drinks, water, and beer. Additionally, the gas is used in refrigeration systems, especially in the transport and storage of frozen foods, in the form of dry ice. The extraction and processing of natural materials with supercritical CO_2 can be used as raw materials for pharmaceutical, cosmetic, and food industries (Filho et al., 2013). Other opportunities for CO_2 uses are growing in the industrial sector. Novel chemistry is evaluating opportunities in polymer processing, renewable methanol, and the production of formic acid, all using CO_2 as a feedstock.

In the advanced biofuels industry, algal CO_2 fixation is an excellent means of utilizing carbon dioxide in renewable energy. Other energy twists include using the commodity in wind energy projects as a cushion gas, enhancing the recovery of natural gas in coal bed seams, and in situ uranium leaching ("Ethanol Producer Magazine", n.d.).

4.3.3.7 Other Chemicals

In addition to traditional coproducts that can be generated during bioethanol production as described above, other innovative products with high benefits and environmentally friendly appeal can be coupled to the sugarcane agroindustry sector. Many kinds of biofuels and chemicals are created from chemical building blocks of sugars or alcohols by fermentation pathways or chemical–physical transformations.

Dehydration of bioethanol can produce ethylene, the largest petroleum-based chemical commodity used in the production of many other compounds and plastics (IEA, 2009). Other products, such as polypropylene, ethylene, and polyvinyl chloride, which are applied in industries using plastic resins, solvents, and textile fibers, can also be produced from bioethanol. Acetaldehyde, which can be produced through ethanol dehydrogenation, is an important chemical intermediate for the production of compounds such as acetic acid, pentaerythritol, acetic esters, butadiene, and polybutadiene. Moreover, several other products, such as paints, adhesives, agrochemicals, and fertilizers, can be produced from alcohol, showing bioethanol as an alternative for petrochemical products (BNDES and CCGE, 2008). As an example, the agrochemical company Oxiteno has implemented production routes for ethylene and green alcohols, such as isoamyl alcohol and isobutyl

alcohol derived from sugarcane (http://www.oxiteno.com/). Fusel oil is a by-product obtained during bioethanol production, which is composed of a mixture of higher alcohols, such as isoamyl alcohol, isobutyl alcohol, and propanol, where the first one is the major component. Considering the annual production of bioethanol in Brazil, and that 2.5 L of fusel oil is produced per 1000 L of ethanol (Ferreira et al., 2013), it is estimated that approximately 80,000,000 L of fusel oil can be produced per year in the country. However, normally the distilleries sell the fusel oil to chemical industries, or burn it to supply energy to the mill. In order to implement the production of high value added compounds in distilleries, and recover the waste generated in the process, Ferreira et al. (2013) have proposed an integrated process system to increase ethanol recovery and to purify isoamyl alcohol from fusel oil. This new proposed process can be integrated independently, or coupled to the existing plant distilleries (Meirelles et al., 2016). In another approach, fusel oil was used in the generation of organic carbonates via capture and fixation of carbon dioxide (CO_2), also a coproduct generated in the distillery (Pereira et al., 2015). Organic carbonates can be used as fuel additives, solvents, and reagents in the chemical industry and medical chemistry.

Braskem is one of the largest producers of green plastics in the world. It started polyethylene production derived from sugarcane ethanol on a commercial scale in Triunfo—Brazil, in 2010, and nowadays its annual production is estimated at 200,000 m^3. The company is also investing in the improvement of process technologies, quality of products, and in the production of other products from renewable raw materials, such as butadiene (Braskem, n.d.).

In another innovative project, Amyris and Total launched in 2010 ongoing collaborative research to develop a renewable biodiesel and an aviation jet fuel made from a hydrocarbon, farnesene. In the process, genetically-modified yeasts ferment sugars present in the sugarcane syrup to produce farnesene instead of ethanol. This compound is chemically hydrogenated and converted to farnesane, which is used in diesel fuel. The first production plant was built in São Paulo, Brazil. In 2014, the jet fuel began to be commercialized, after industry acceptance and regulatory approval (Amyris, n.d.).

4.4 CONCLUSIONS

The sugarcane industry has been in development for decades in Brazil, contributing to one of the most renewable energy matrixes in the world. Sugarcane alone contributes to 13%−18% of the Brazilian energy matrix, and allows the country be one of the main ethanol and sugar producers worldwide. Nowadays, technologies for ethanol production are well-established, and more than 300 mills work throughout the year producing the fuel, as well sugar, electric energy, and other by-products. However, the increasing demand for more sustainable production processes for energy and chemicals

in a biorefinery context brought new opportunities and challenges for the sector. Current developments include improvements in biomass, yeast performance, and process technologies.

On the biomass side, major advancements in ethanol production from sugarcane might come from the generation of GMO cultivars and exploitation of energy cane. In the first case, genetic improvement programs of sugarcane to increase drought tolerance and resistance to pests are expected to yield cultivars with increased productivity. Whereas the energy cane planted area is growing in the country. Energy cane accumulates less sucrose than sugarcane, however, its higher fiber and productivity will allow increased ethanol and energy production.

The ethanol fermentation process has yields above 90%, however, yeast performance may be improved in a variety of ways. Reduction of by-product formation, increased robustness, and better tolerance to high ethanol contents are important traits being considered in the genetic improvement of yeast. High ethanol tolerance allows VHG fermentation, which, in turn, will increase process efficiency by reducing the energy required for distillation, and less waste generation.

Other minor improvements in the different steps of the production process, such as milling and steam generation, are expected to be included in the bioethanol industry. Adaptation of the operational units in the mills are mainly expected due to the new characteristics of the biomass, which has a higher fiber content. All taken together, these factors will improve ethanol yield and productivity in Brazilian sugarcane biorefineries.

REFERENCES

Amorim, H., Basso, L., Lopes, M., 2009. Sugar cane juice and molasses, beet molasses and sweet sorghum: composition and usage. The Alcohol Text Book. Nottingham University Press, Nottingham, p. 541.

Amyris, n.d. Amyris [WWW Document]. http://amyris.com.

Argueso, J.L., Carazzolle, M.F., Mieczkowski, P.A., Duarte, F.M., Netto, O.V.C., Missawa, S.K., et al., 2009. Genome structure of a *Saccharomyces cerevisiae* strain widely used in bioethanol production. Genome Res. 19, 2258–2270. Available from: http://dx.doi.org/10.1101/gr.091777.109.

Arruda, P., 2012. Genetically modified sugarcane for bioenergy generation. Curr. Opin. Biotechnol. 23, 315–322. Available from: http://dx.doi.org/10.1016/j.copbio.2011.10.012.

Babrzadeh, F., Jalili, R., Wang, C., Shokralla, S., Pierce, S., Robinson-Mosher, A., et al., 2012. Whole-genome sequencing of the efficient industrial fuel-ethanol fermentative *Saccharomyces cerevisiae* strain CAT-1. Mol. Genet. Genomics 287, 485–494. Available from: http://dx.doi.org/10.1007/s00438-012-0695-7.

Balat, M., Balat, H., Cahide, O.Z., 2008. Progress in bioethanol processing. Program Energy Combust. 34, 551–573.

Basso, L.C., Rosa, C.A., 2010. Sugar cane for potable and fuel ethanol, in: Institute of Brewing & Distilling (Ed.), Distilled Spirits: New Horizons; Energy, Environmental and

Enlightenment; [proceedings of the Third Worldwide Distilled Spirits Conference; Edingburgh in September 2008; Organised by the Scottisch Section of the Institute of Brewing & Destilling]. Univ. Press, Nottingham.

Basso, L.C., de Amorim, H.V., de Oliveira, A.J., Lopes, M.L., 2008. Yeast selection for fuel ethanol production in Brazil. FEMS Yeast Res. 8, 1155–1163. Available from: http://dx.doi.org/10.1111/j.1567-1364.2008.00428.x.

Basso, L.C., Basso, T.O., Rocha, S.N., 2015. Ethanol production in Brazil: the industrial process and its impact on yeast fermentation. Biofuel Production-Recent Developments and Prospects. Intechopen.

Björkqvist, S., Ansell, R., Adler, L., Lidén, G., 1997. Physiological response to anaerobicity of glycerol-3-phosphate dehydrogenase mutants of *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. 63, 128–132.

BNDES e CGEE, 2008. Bioetanol de cana-de-açúcar: energia para o desenvolvimento sustentável. www.bioetanoldecana.org.

Brasil, 2012. Anuário Estatístico da Agroenergia.

Brasil, 2016. Brazilian Sugarcane, Sugar and Ethanol Production.

Braskem, n.d. Braskem [WWW Document]. http://www.braskem.com.br.

CCEE, 2015. Geração das usinas à biomassa cresce 15% no primeiro semestre de 2015.

Cerqueira Leite, R.C., de, Verde Leal, M.R.L., Barbosa Cortez, L.A., Griffin, W.M., Gaya Scandiffio, M.I., 2009. Can Brazil replace 5% of the 2025 gasoline world demand with ethanol? Energy 34, 655–661. Available from: http://dx.doi.org/10.1016/j.energy.2008.11.001.

CGEE, 2008. Bioetanol de Cana-de-açucar. Energia para o desenvolvimento sustentável.

Cheng, N.G., Hasan, M., Kumoro, A.C., Ling, C.F., Tham, M., 2009. Production of Ethanol by Fed-Batch Fermentation. Sci. Technol. 17, 399–408.

CONAB, 2015. Acompanhamento da Safra Brasileira - Cana-de-Açucar. V.2 - SAFRA 2015/2016 - N. 03 Terceiro levantamento.

Cortez, L., Magalhães, P., Happi, J., 1992. Principais subprodutos da agroindústria canavieira e sua valorização. Rev. Bras. Energ. 2, 111–146.

CTC, 2016. Sugar Technology Center.

Cysewski, G.R., Wilke, C.R., 1978. Process design and economic studies of alternative fermentation methods for the production of ethanol. Biotechnol. Bioeng. 20, 1421–1444. Available from: http://dx.doi.org/10.1002/bit.260200908.

da Cunha, B.A.D.B., Martins, P.K., Kobayashi, A.K., Molinari, H.B.C., 2015. Biotecnologia aplicada ao sistema de produção da cana-de-açúcar.In: Sistema de produção mecanizada da cana-de-açúcar integrada à produção de energia e alimentos.

Della-Bianca, B.E., Basso, T.O., Stambuk, B.U., Basso, L.C., Gombert, A.K., 2013. What do we know about the yeast strains from the Brazilian fuel ethanol industry? Appl. Microbiol. Biotechnol. 97, 979–991. Available from: http://dx.doi.org/10.1007/s00253-012-4631-x.

de Setta, N., Monteiro-Vitorello, C., Metcalfe, C., Cruz, G.M., Del Bem, L., Vicentini, R., et al., 2014. Building the sugarcane genome for biotechnology and identifying evolutionary trends. BMC Genomics 15, 540. Available from: http://dx.doi.org/10.1186/1471-2164-15-540.

Dias, M.O.S., Cunha, M.P., Jesus, C.D.F., Scandiffio, M.I.G., Rossell, C.E.V., Filho, R.M., et al., 2010. Simulation of ethanol production from sugarcane in Brazil: economic study of an autonomous distillery. Computer Aided Chemical Engineering. Elsevier, pp. 733–738.

Dias, M.O.S., Modesto, M., Ensinas, A.V., Nebra, S.A., Filho, R.M., Rossell, C.E.V., 2011. Improving bioethanol production from sugarcane: evaluation of distillation, thermal integration and cogeneration systems. Energy 36, 3691–3703. Available from: http://dx.doi.org/10.1016/j.energy.2010.09.024.

Farla, J.C.M., Hendriks, C.A., Blok, K., 1995. Carbon dioxide recovery from industrial processes. Clim. Change 29, 439–461. Available from: http://dx.doi.org/10.1007/BF01092428.

Empresa de Pesquisa Energética-EPE, 2015. Brazilian Energy Balance.

Ethanol Producer Magazine, n.d.

Fermentec, 2016. Fermentec [WWW Document]. http://www.fermentec.com.br/.

Ferreira, M.C., Meirelles, A.J.A., Batista, E.A.C., 2013. Study of the fusel oil distillation process. Ind. Eng. Chem. Res. 52, 2336–2351. Available from: http://dx.doi.org/10.1021/ie300665z.

Ferreira, S.S., Hotta, C.T., Poelking, V.G., de, C., Leite, D.C.C., Buckeridge, M.S., et al., 2016. Co-expression network analysis reveals transcription factors associated to cell wall biosynthesis in sugarcane. Plant Mol. Biol. 91, 15–35. Available from: http://dx.doi.org/10.1007/s11103-016-0434-2.

Filho, Â.B., 2011. A Geração Termoelétrica com a Queima do Bagaço de Cana-de-Açúcar no Brasil.

Filho, R.B., de, A., Danielski, L., de Carvalho, F.R., Stragevitch, L., 2013. Recovery of carbon dioxide from sugarcane fermentation broth in the ethanol industry. Food Bioprod. Process. 91, 287–291. Available from: http://dx.doi.org/10.1016/j.fbp.2012.09.009.

Garcia, T.A., Santiago, M.F., Ulhoa, C.J., 2006. Properties of laccases produced by Pycnoporus sanguineus induced by 2,5-xylidine. Biotechnol. Lett. 28, 633–636. Available from: http://dx.doi.org/10.1007/s10529-006-0026-3.

Gombert, A.K., van Maris, A.J., 2015. Improving conversion yield of fermentable sugars into fuel ethanol in 1st generation yeast-based production processes. Curr. Opin. Biotechnol. 33, 81–86. Available from: http://dx.doi.org/10.1016/j.copbio.2014.12.012.

Guadalupe-Medina, V., Metz, B., Oud, B., van Der Graaf, C.M., Mans, R., Pronk, J.T., et al., 2014. Evolutionary engineering of a glycerol-3-phosphate dehydrogenase-negative, acetate-reducing *Saccharomyces cerevisiae* strain enables anaerobic growth at high glucose concentrations: laboratory evolution of osmotolerant Gpd $^{-}$ yeast. Microb. Biotechnol. 7, 44–53. Available from: http://dx.doi.org/10.1111/1751-7915.12080.

Hotta, C.T., Lembke, C.G., Domingues, D.S., Ochoa, E.A., Cruz, G.M.Q., Melotto-Passarin, D. M., et al., 2010. The biotechnology roadmap for sugarcane improvement. Trop. Plant Biol. 3, 75–87. Available from: http://dx.doi.org/10.1007/s12042-010-9050-5.

Hubmann, G., Foulquié-Moreno, M.R., Nevoigt, E., Duitama, J., Meurens, N., Pais, T.M., et al., 2013a. Quantitative trait analysis of yeast biodiversity yields novel gene tools for metabolic engineering. Metab. Eng. 17, 68–81. Available from: http://dx.doi.org/10.1016/j.ymben.2013.02.006.

Hubmann, G., Mathé, L., Foulquié-Moreno, M.R., Duitama, J., Nevoigt, E., Thevelein, J.M., 2013b. Identification of multiple interacting alleles conferring low glycerol and high ethanol yield in *Saccharomyces cerevisiae* ethanolic fermentation. Biotechnol. Biofuels 6, 87. Available from: http://dx.doi.org/10.1186/1754-6834-6-87.

Hunt, A.J., Sin, E.H.K., Marriott, R., Clark, J.H., 2010. Generation, capture, and utilization of industrial carbon dioxide. ChemSusChem 3, 306–322. Available from: http://dx.doi.org/10.1002/cssc.200900169.

Kamm, B., Kamm, M., 2004. Principles of biorefineries. Appl. Microbiol. Biotechnol. 64, 137–145. Available from: http://dx.doi.org/10.1007/s00253-003-1537-7.

IEA, 2009. World Energy Outlook. Int. Energy Agency.

Jalles Machado [WWW Document], 2016. www.jallesmachado.com/2016.

Kannan, A., Upreti, R.K., 2008. Influence of distillery effluent on germination and growth of mung bean (Vigna radiata) seeds. J. Hazard. Mater. 153, 609–615. Available from: http://dx.doi.org/10.1016/j.jhazmat.2007.09.004.

Laime, E.M.O., Fernandes, P.D., Oliveira, D.C.S., Freire, E.A., 2011. Possibilidades tecnológicas para a destinação da vinhaça: uma revisão. Rev. Trópica – Ciênc. Agrár. E Macromol. 25, 31–36.

Lopes, M.L., Paulillo, S.C., de, L., Cherubin, R.A., Godoy, A., de Amorim Neto, H.B., et al., 2015. Linhagens de Leveduras Personalizadas para Produção de Etanol: Seleção Dirigida pelo Processo, first ed FERMENTEC, Piracicaba.

LNF [WWW Document], n.d. Lat. Am. LTDA. http://www.lnf.com.br/.

MAPA, 2012. Brazil's Ministry of Agriculture, Livestock and Supply.

Marconi, T.G., Costa, E.A., Miranda, H.R., Mancini, M.C., Cardoso-Silva, C.B., Oliveira, K. M., et al., 2011. Functional markers for gene mapping and genetic diversity studies in sugarcane. BMC Res. Notes 4, 264. Available from: http://dx.doi.org/10.1186/1756-0500-4-264.

Margarido, G.R.A., Pastina, M.M., Souza, A.P., Garcia, A.A.F., 2015. Multi-trait multi-environment quantitative trait loci mapping for a sugarcane commercial cross provides insights on the inheritance of important traits. Mol. Breed. 35. Available from: http://dx.doi.org/10.1007/s11032-015-0366-6.

Mariano, A.P., Dias, M.O.S., Junqueira, T.L., Cunha, M.P., Bonomi, A., Filho, R.M., 2013. Butanol production in a first-generation Brazilian sugarcane biorefinery: technical aspects and economics of greenfield projects. Bioresour. Technol. 135, 316–323. Available from: http://dx.doi.org/10.1016/j.biortech.2012.09.109.

Marques, W.L., Raghavendran, V., Stambuk, B.U., Gombert, A.K., 2016. Sucrose and *Saccharomyces cerevisiae*: a relationship most sweet. FEMS Yeast Res. 16, fov107. Available from: http://dx.doi.org/10.1093/femsyr/fov107.

Matsuoka, S., Ferro, J., Arruda, P., 2009. The Brazilian experience of sugarcane ethanol industry. Vitro Cell. Dev. Biol. – Plant 45, 372–381. Available from: http://dx.doi.org/10.1007/s11627-009-9220-z.

McLaren, J., 2009. Sugarcane as a feedstock for biofuels.

McQualter, R.B., Somleva, M.N., Gebbie, L.K., Li, X., Petrasovits, L.A., Snell, K.D., et al., 2014. Factors affecting polyhydroxybutyrate accumulation in mesophyll cells of sugarcane and switchgrass. BMC Biotechnol. 14, 83. Available from: http://dx.doi.org/10.1186/1472-6750-14-83.

Meirelles, A.J. de A., De Oliveira, M.J., Batista, E.A.C., 2016. Sistema integrado para aumento da recuperação de etanol e coprodução de álcool isoamílico, processo integrado para aumento da recuperação de etanol e coprodução de álcool isoamílico e, produtos assim obtidos. WO2016054706 A1.

MMA, 2016. Moagem de Cana-de-Açucar.

NovaCana, n.d. NovaCana [WWW Document]. http://www.novacana.com/.

OECD-FAO Agricultural Outlook (No. 1563-0447), n.d. Organisation for Economic Co-operation and Development, Paris.

Okura, V.K., Souza, R.S.C., de, de Siqueira Tada, S.F., Arruda, P., 2016. BAC-Pool Sequencing and Assembly of 19 Mb of the Complex Sugarcane Genome. Front. Plant Sci. 7. Available from: http://dx.doi.org/10.3389/fpls.2016.00342.

Pacheco, T.F., Machado, C.M.M., Golçalves, S.B., 2013. Produção de Etanol. Microrganismos Na Produção de Biocombustíveis Líquido. Embrapa.

Pagliardini, J., Hubmann, G., Alfenore, S., Nevoigt, E., Bideaux, C., Guillouet, S.E., 2013. The metabolic costs of improving ethanol yield by reducing glycerol formation capacity under anaerobic conditions in *Saccharomyces cerevisiae*. Microb. Cell Factories 12, 29. Available from: http://dx.doi.org/10.1186/1475-2859-12-29.

Pastina, M.M., Malosetti, M., Gazaffi, R., Mollinari, M., Margarido, G.R.A., Oliveira, K.M., et al., 2012. A mixed model QTL analysis for sugarcane multiple-harvest-location trial data. Theor. Appl. Genet. 124, 835–849. Available from: http://dx.doi.org/10.1007/s00122-011-1748-8.

Pei, L., Schmidt, M., Wei, W., 2011. Conversion of biomass into bioplastics and their potential environmental impacts. In: Elnashar, M. (Ed.), Biotechnology of Biopolymers. InTech.

Pereira, F.S., Pereira, L.J., Crédito, D.F.A., Girão, L.H.V., Idehara, A.H.S., González, E.R.P., 2015. Cycling of waste fusel alcohols from sugar cane industries using supercritical carbon dioxide. RSC Adv 5, 81515–81522. Available from: http://dx.doi.org/10.1039/C5RA16346C.

Ragauskas, A.J., Williams, C.K., Davison, B.H., Britovsek, G., Cairney, J., Eckert, C.A., et al., 2006. The path forward for biofuels and biomaterials. Science 311, 484–489.

Reis, R.R., Andrade Dias Brito da Cunha, B., Martins, P.K., Martins, M.T.B., Alekcevetch, J.C., Chalfun-Júnior, A., et al., 2014. Induced over-expression of AtDREB2A CA improves drought tolerance in sugarcane. Plant Sci. 221–222, 59–68. Available from: http://dx.doi.org/10.1016/j.plantsci.2014.02.003.

Rezende, C., de Lima, M., Maziero, P., deAzevedo, E., Garcia, W., Polikarpov, I., 2011. Chemical and morphological characterization of sugarcane bagasse submitted to a delignification process for enhanced enzymatic digestibility. Biotechnol. Biofuels 4, 54. Available from: http://dx.doi.org/10.1186/1754-6834-4-54.

Snoek, T., Picca Nicolino, M., Van den Bremt, S., Mertens, S., Saels, V., Verplaetse, A., et al., 2015. Large-scale robot-assisted genome shuffling yields industrial *Saccharomyces cerevisiae* yeasts with increased ethanol tolerance. Biotechnol. Biofuels 8, 32. Available from: http://dx.doi.org/10.1186/s13068-015-0216-0.

SolazymesIndustrial [WWW Document], n.d. Solazymes. http://solazymeindustrials.com

Somleva, M.N., Peoples, O.P., Snell, K.D., 2013. PHA bioplastics, biochemicals, and energy from crops. Plant Biotechnol. J. 11, 233–252. Available from: http://dx.doi.org/10.1111/pbi.12039.

Stambuk, B.U., Dunn, B., Alves, S.L., Duval, E.H., Sherlock, G., 2009. Industrial fuel ethanol yeasts contain adaptive copy number changes in genes involved in vitamin B1 and B6 biosynthesis. Genome Res. 19, 2271–2278. Available from: http://dx.doi.org/10.1101/gr.094276.109.

Steensels, J., Snoek, T., Meersman, E., Nicolino, M.P., Voordeckers, K., Verstrepen, K.J., 2014. Improving industrial yeast strains: exploiting natural and artificial diversity. FEMS Microbiol. Rev. 38, 947–995. Available from: http://dx.doi.org/10.1111/1574-6976.12073.

Swinnen, S., Schaerlaekens, K., Pais, T., Claesen, J., Hubmann, G., Yang, Y., et al., 2012. Identification of novel causative genes determining the complex trait of high ethanol tolerance in yeast using pooled-segregant whole-genome sequence analysis. Genome Res. 22, 975–984. Available from: http://dx.doi.org/10.1101/gr.131698.111.

Szymanski, M.S.E., Balbinot, R., Schirmer, W.N., 2010. Biodigestão anaeróbia da vinhaça: aproveitamento energético do biogás e obtenção de créditos de carbono – estudo de caso. Semina Ciênc. Agrár. 31, 901. Available from: http://dx.doi.org/10.5433/1679-0359.2010v31n4p901.

Tao, X., Zheng, D., Liu, T., Wang, P., Zhao, W., Zhu, M., et al., 2012. A novel strategy to construct yeast *Saccharomyces cerevisiae* strains for very high gravity fermentation. PLoS One 7, e31235. Available from: http://dx.doi.org/10.1371/journal.pone.0031235.

UNICA, 2011. União da Agroindústria Canavieira de São Paulo [WWW Document]. www.unica.com.br.

UNICA, n.d. Dados e cotações – Estatísticas.

USDA, 2015. Sugar: World Markets and Trade.

Vettore, A.L., 2003. Analysis and Functional Annotation of an Expressed Sequence Tag Collection for Tropical Crop Sugarcane. Genome Res. 13, 2725–2735. Available from: http://dx.doi.org/10.1101/gr.1532103.

Wilkie, A.C., Riedesel, K.J., Owens, J.M., 2000. Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks. Biomass Bioenergy 19, 63–102. Available from: http://dx.doi.org/10.1016/S0961-9534(00)00017-9.

Wu, L., Birch, R.G., 2010. Physiological basis for enhanced sucrose accumulation in an engineered sugarcane cell line. Funct. Plant Biol. 37, 1161. Available from: http://dx.doi.org/10.1071/FP10055.

Xu, Y., Isom, L., Hanna, M.A., 2010. Adding value to carbon dioxide from ethanol fermentations. Bioresour. Technol. 101, 3311–3319. Available from: http://dx.doi.org/10.1016/j.biortech.2010.01.006.

Zabed, H., Faruq, G., Sahu, J.N., Azirun, M.S., Hashim, R., Nasrulhaq Boyce, A., 2014. Bioethanol Production from Fermentable Sugar Juice. Sci. World J. 2014, 1–11. Available from: http://dx.doi.org/10.1155/2014/957102.

Zheng, D.-Q., Wang, P.-M., Chen, J., Zhang, K., Liu, T.-Z., Wu, X.-C., et al., 2012. Genome sequencing and genetic breeding of a bioethanol *Saccharomyces cerevisiae* strain YJS329. BMC Genomics 13, 479. Available from: http://dx.doi.org/10.1186/1471-2164-13-479.

Zheng, D.-Q., Chen, J., Zhang, K., Gao, K.-H., Li, O., Wang, P.-M., et al., 2014. Genomic structural variations contribute to trait improvement during whole-genome shuffling of yeast. Appl. Microbiol. Biotechnol. 98, 3059–3070. Available from: http://dx.doi.org/10.1007/s00253-013-5423-7.