BioEnergy Research (2021) 14:761–773
https://doi.org/10.1007/s12155-020-10169-w

Cellulosic Ethanol from Sugarcane Straw: a Discussion Based on Industrial Experience in the Northeast of Brazil

Pollyanna Ximenes Oliveira Bezerra[1,2] · Carlos Eduardo De Farias Silva[2] · João Inácio Soletti[2] · Sandra Helena Vieira de Carvalho[2]

Received: 10 February 2020 / Accepted: 20 July 2020 / Published online: 24 September 2020

Abstract

Second-generation technology (2G) is currently the industrial route that is being developed with the potential to meet the demand for biofuels and bioproducts. The technology studied in the present work is based on the non-food processing of sugarcane straw, which is unlike the bagasse processing route (a lignocellulosic residue) that is commonly used in boilers for steam and electricity generation. In the last years, cellulosic ethanol plants worldwide include the utilisation of corn stover, sugarcane bagasse, wood waste and plant residues. Therefore, a communication based on industrial aspects using sugarcane straw is important in order to expand the knowledge regarding this promising technology. The main objective of this work included listing the results obtained in a cellulosic-ethanol production industry using sugarcane straw, located in the Northeast region of Brazil and taking into account the efficiency of pre-treatment, enzymatic hydrolysis and fermentation processes and comparing these results with data found in the literature regarding studies with similar efficiency. It was possible to observe that the production of ethanol from sugarcane straw at an industrial scale achieved a productivity of 200 l of ethanol per tonne of biomass, exhibiting 90, 70 and 85% of pre-treatment, hydrolysis and fermentation efficiency, respectively.

Keywords Biofuels · Bioproducts · Second generation · 2G ethanol

Introduction

Second-generation (2G) technology is currently the industrial route developed with the potential to meet the demand for renewable energy and bioproducts. This technology is based on non-food biomasses for sugar and lignin extraction [1, 2]. The worldwide production of ethanol has almost doubled in the past decade, with a production of over a 100 million cubic metres a year, being a sign of the great dependency on this source of energy at a global scale. The drawback of expanding this production is related to the limitation regarding the use of soils, as well as the competition with other raw materials used in the food industry (sucrose and starch, for instance), the changes in oil prices and the difficulties in scaling-up the promising second generation route [1, 3].

✉ Pollyanna Ximenes Oliveira Bezerra
pso.mcz@gmail.com

[1] Instituto Federal de Educação, Ciência e Tecnologia de Pernambuco, Recife, Pernambuco, Brazil

[2] Centro de Tecnologia, Universidade Federal de Alagoas, Maceió, Alagoas, Brazil

It is also important to point out that the production of bioethanol based on this type of biomass can be divided into four different generations: the first generation includes biofuels produced from saccharide and starch feedstock (such as sugarcane, sugar beet and corn); the second generation regards biofuels produced from lignocellulosic biomass (such as sugarcane straw); the third from the use of micro/macroalgae; and the fourth generation includes the production of biofuels from genetically modified cyanobacteria, through the photofermentation process. However, the last two still do not have an industrial application [3, 4]. It is also worth noting that the approach of this current article addresses the concept of second-generation biofuels.

Industrial plants that seek to validate this type of technology (lignocellulosic ethanol) include the use of corn stover, wood waste, plant residues and sugarcane bagasse [1, 5]. Difficulties regarding the efficiency of the pre-treatment, enzymatic saccharification and fermentation processes, combined with the appropriate use of pentoses from hemicellulose, prevent the industrial efficiency of this technology [6]. In Brazil, there are currently three 2G ethanol production plants using lignocellulosic residue from sugarcane crops (bagasse and sugarcane straw). Two of these industrial plants

produce ethanol at an industrial scale (with one being located in the Northeast region) and one at a pilot scale. This paper will discuss the efficiency of a second-generation ethanol industrial plant in the Northeast of Brazil under an industrial point of view.

Second-Generation Technology—Theoretical Background

The main difference between first- and second-generation technologies is that sugars, such as saccharified and starchy biomass, are not directly accessible in the 2G route, being obtained from cellulose and hemicellulose sources [1, 5]. The process of making these sugars accessible consists of biomass pre-treatment, followed by hydrolysis, transforming polymer chains into monomers. This step is fundamental to take advantage of the full potential of the lignocellulosic biomass, which includes the use of isolated fractions in a biorefinery context, namely the potential of sugar fermentation for ethanol production.

Figure 1 shows a schematic diagram of the industrial plant studied in this article for the production of 2G ethanol and lignocellulosic residue from sugarcane straw.

In this process, biomass bales are bilaterally supplied from the conveyor, which, after being weighed, are debaled and their strings removed by an automatic string cutter system. The biomass is subsequently transported to a trommel screen for removing any impurities and then milled. Subsequently, the cleaned and milled biomass is fed into the pre-treatment system through the biomass conveyor.

In the pre-treatment process, the biomass is transported through screw conveyors until the low-pressure reactor, where the auto-hydrolysis (LHW—liquid hot water) process takes place. The *t-piece* is the part of the system which acts as a biomass seal, being responsible for feeding the reactor, as this component separates the equipment that operate under atmospheric pressure (*screw conveyor*, *feeder stuffing screw*, *biomass feeder*) from the downstream equipment (*roto steamer*), which operates under the pressure of the reactor. When reaching the residence time, the boiled biomass is depressurised through the blow line and loaded into the cyclone, with the pre-treatment process being then complete.

The pre-treated biomass is then transported to the viscosity reduction tank and to the hydrolysis tank. In the enzymatic hydrolysis area, the following parameters are controlled: enzyme dosage, temperature, residence time and pH. Following this step, the monomers (xylose and glucose) are transported to the fermentation tanks. One of the particularities of this process is the need to carry out yeast batch propagation for every fermentation batch, as yeast cannot be reused due to the excess of solids.

The fermented must is then distilled in the distillation columns and dried in the molecular sieves, obtaining anhydrous 2G ethanol. The lignocellulosic residue is then separated from the vinasse through filtration (*lignin filters*), being then sent to the boilers, thereby increasing the energy efficiency of the plant.

In the subsequent section, each step (pre-treatment, hydrolysis and fermentation) will be discussed in detail, taking into account a widely used industrial process and pointing out the respective objectives and specific peculiarities of each process.

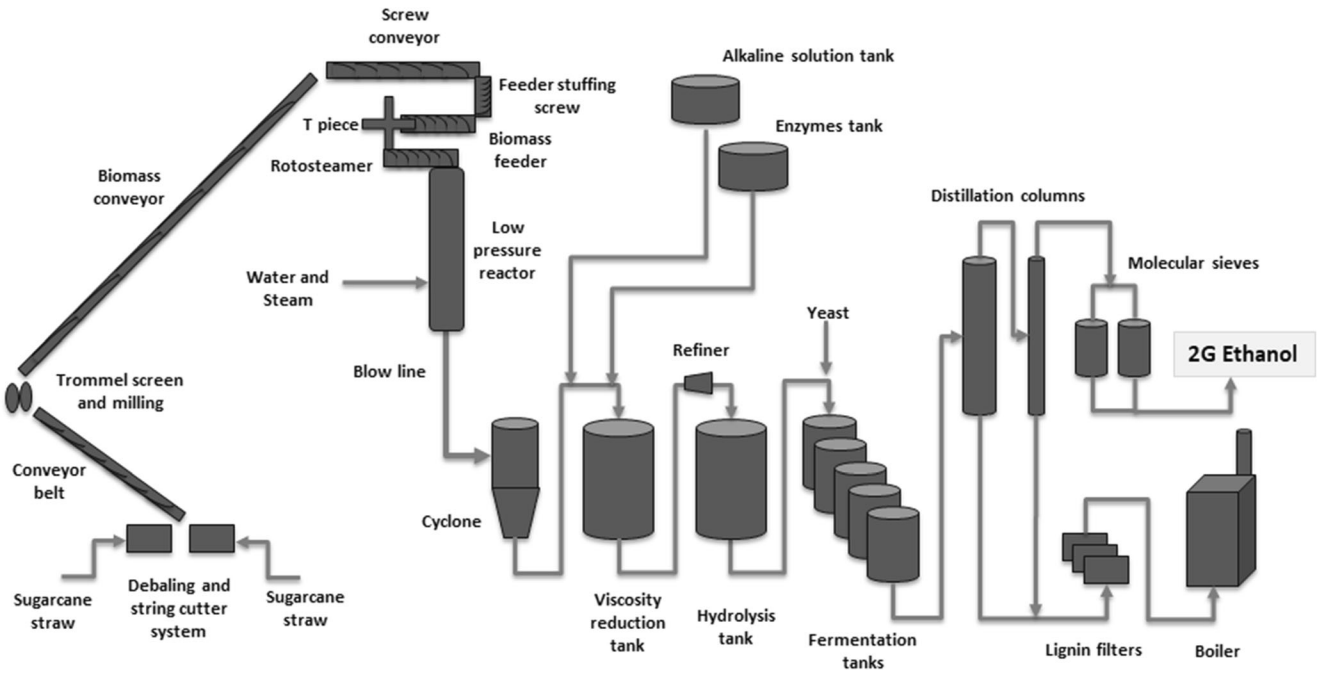

Fig. 1 Schematic diagram of the production process of 2G ethanol and lignocellulosic residue

Pre-treatment: Auto-hydrolysis (Liquid Hot Water)

Pre-treatment is a hydrothermal process in which the lignocellulosic material is mixed with hot water in controlled temperature and pressure conditions. Under these conditions, hydronium ions are generated in situ as a result of water ionisation, releasing acid compounds such as acetic acid from hemicellulose. In turn, these acid compounds help autocatalyse hemicellulose hydrolysis, resulting in greater accessibility to cellulose [7]. Both saccharification and the amount of degradation products generated in the process are influenced by the severity parameter (severity factor) of the process [8, 9], defined through an empiric equation (Eq. 1) as a function of residence time and reactor temperature. Such parameters are related to the length of the reaction from an initial kinetic model, such as the Arrhenius equation [8–10].

$$\log(R_o) = \log\left(t\, e^{\frac{|T-100|}{14.75}}\right) \quad (1)$$

where T = temperature (°C), t = time (min) and R_o = severity factor.

Slightly moderate temperatures (181 to 196 °C) are recommended to be used in order to avoid excessive cellulose depolymerisation after the extraction of xylo-oligomers, xylose, furfural and by-products [8]. In turn, more severe auto-hydrolysis pre-treatment conditions facilitate the removal of hemicellulose, despite reducing enzyme efficiency in the conversion of cellulose into glucose. Auto-hydrolysis pre-treatment with a severity factor between 3.6 and 4.2 results in high solubilisation levels of hemicellulose and in a sharp improvement in cellulose enzymatic hydrolysis [7]. This technology is attractive under an economic and environmental point view, as no acid or base needs to be added and due to a relatively high xylose recovery (from 88 to 98%) [11].

Enzymatic Hydrolysis

Following the pre-treatment of the lignocellulosic biomass, a large sugar fraction remains in forms of polysaccharides and oligosaccharides. These polymers must be broken into monomeric sugars to enable the conversion into ethanol. Therefore, several approaches can be applied for breaking cellulose, including enzymatic hydrolysis, which employs a mixture of proteins, commonly called enzymatic cocktails, to break glycosidic bonds, as well as acid hydrolysis, which uses strong concentrated acid such as H_2SO_4.

Enzymatic hydrolysis has been the most common approach used in industrial applications, due to the following aspects: milder operating conditions (temperature in the range between 50 and 60 °C and pH from 4.5 to 5.5), higher yields, and lower consumption of steam and water. Moreover, enzymatic hydrolysis reduces corrosion issues in the equipment, which are typically associated to acid hydrolysis [12]. Several factors can interfere with enzymatic hydrolysis, namely: the type of pre-treatment which the biomass was submitted to; thermostability of enzymes; concentration and adsorption of enzymes in the substrate, such as lignin residue; substrate concentration, hydrolysis residence time; pH in the medium; as well as temperature and stirring rate [12, 13].

Enzyme cocktails are used for hydrolysis purposes in different industries, among them the textile, detergent, food, paper and cellulose industries. However, the production of biofuels such as ethanol and butanol, as well as of other biomass-derived biochemicals, can transform this industry into the largest enzyme market in the world. In addition, the production of enzymatic cocktails at a competitive cost is an important issue and a requirement to ensure the economic feasibility of the production of second-generation ethanol [13].

Nowadays, the commercial preparation of enzymes available for the hydrolysis of lignocellulosic biomass is produced via the fermentation of genetically modified strains of *Trichoderma reesei*. This filamentous fungus is widely known as being an efficient producer of cellulase and hemicellulase, which jointly act in the degradation of lignocellulosic material [11]. Three companies represent more than 75% of the market that consolidates the global production of enzymes for biofuel: Novozymes (Denmark), Genencor-Dupont (United States) and DSM (Netherlands). Consequently, most scientific studies use the enzymes from these companies in the hydrolysis process, especially the following enzyme cocktails: Celluclast, Novozym, Cellic and Accellerase [14].

Although the production of enzymes as a cocktail is already available to be used in the conversion of lignocellulosic biomass at an industrial scale (such as Cellic CTec3 and Accellerase), their costs still need to be optimised in order to ensure a more competitive production of second-generation ethanol. New enzymatic extracts obtained from wild-type strains collected in different environments continued to be studied, with the main objective of reducing the costs of the process through the development of independent technologies as an alternative to new biorefineries [14]. However, enzymatic hydrolysis at an industrial scale still has many challenges that need to be overcome in terms of efficiency, costs, dosage, residence time and process configuration. Therefore, residence times, high concentrations of solids and enzyme dosages must be improved in order to reach greater hydrolysis yields.

Residence time is considered an important factor to reduce capital and operating costs, as lower residence times require less volume in tanks and less peripheral equipment (pumps and heat exchangers) in the process. Therefore, the hydrolysis process must be carried out at the lowest residence time possible. It is worth pointing out that hydrolysis currently has a total duration of approximately 72 h at an industrial scale [12].

The initial total solid content in hydrolysis is related to the sugar concentration at the end of the hydrolysis process. Thus, the greater the amount of total solids in the beginning of hydrolysis, the greater the sugar concentration in the hydrolysate. High sugar concentrations typically enable to obtain greater ethanol fractions during fermentation, reducing the volume of tanks and resulting in a more profitable ethanol distillation. Nevertheless, it is necessary to assess the end-product inhibition effect, which is when the sugar produced during the hydrolysis step is inhibitory to enzymes. Thus, the greater the solid content, the greater the sugar concentration released and, consequently, the greater the end-product inhibition effect. Accordingly, sugar concentrations higher than 100 g/L represent a strong enzymatic-inhibitory effect [12–14].

Therefore, hydrolysis should be carried out with 20 to 25% *w*/*w* of total solids in order to reach between 10 and 12% *w*/*w* of fermentable sugars (considering at least 70% hydrolysis yield) and obtain an ethanol concentration in fermentation greater than 4% *w*/*w* (considering approximately an 88 to 92% fermentation yield) [12]. Enzyme costs represent approximately 20% of the total cost of cellulosic ethanol production [11]. The optimisation of enzyme dosage without increasing hydrolysis time nor reducing hydrolysis yields is an important issue to be considered to ensure the feasibility of enzymatic hydrolysis at an industrial scale.

Looking at the optimisation of enzyme dosage to achieve an economically relevant conversion of biomass to sugars, Novozymes published results of studies that show the best correlation of enzyme dose (using Cellic CTec3, a multi-enzyme cocktail) for a required conversion (based on all available glucose and xylose sources).

For example, considering enzyme hydrolysis at 50 °C and pH 5.0 of an unwashed sample of dilute acid-pretreated corn stover (18% total solid loading), if a range of 60 to 80% conversion is required, the recommended dosage for this feedstock under the hydrolysis conditions mentioned above would be about 1.5 to 3% *w*/*w* (g/100 g). Based on those Novozymes studies, the optimal performance of that multi-enzyme cocktail occurs at a temperature range of 50 to 55 °C and pH 4.75 to 5.0 [15, 16].

Fermentation

In the alcoholic fermentation process, microorganisms transform sugars, producing ethanol and CO_2 [13]. Several processing conditions can affect fermentation, resulting in an efficiency decrease. Among the factors that can hinder fermentation efficiency, the following can be pointed out: physical factors, such as temperature and osmotic pressure; chemical factors, such as pH, oxygen content, as well as nutrient and inhibitory concentrations; and biological factors, such as yeast species and concentrations, as well as the contamination of the fermentation media by other microorganisms [12, 13]. Lignocellulosic hydrolysates are rich in sugars that are easily fermented, such as glucose, mannose and galactose (although the last two be metabolised in lower rates when compared with the glucose–catabolic repression when glucose is present), as well as sugars that require the use of genetically modified yeast to be converted into ethanol, such as xylose and arabinose (pentoses).

The pre-treatment of lignocellulosic biomass can produce degradation products with an inhibitory effect in the fermentation process. These inhibitors have toxic effects on yeast, reducing ethanol yields and productivity. The level of toxicity partly depends on certain fermentation variables, including cell physiological conditions, dissolved oxygen concentration and pH in the medium. In addition, yeast can, up to a certain point, be resistant to inhibitors or can be progressively adapted to their presence. Nevertheless, the initial approach consists of avoiding the formation of inhibitors as much as possible, by controlling pre-treatment conditions [17].

The main types of inhibitors commonly present in liquors are furfural, 5-hydroxymethylfurfural (HMF), acetic acid, phenols, levulinic acid and formic acid. The inhibitory effect of these compounds is greater when they are jointly present, due to their synergic effect. The relative toxicity of the various inhibitors for ethanol fermentation can be outlined as follows: phenolic compounds >0.1 g/L, furfural >0.25 g/L, HMF ≥ 0.25 g/L and acetic acid ≥ 3 g/L [17].

Within the context of second-generation fermentation, yeast propagation is necessary to obtain sufficient cell mass to start the fermentation process. In the Brazilian market, this step is not part of the usual process of first-generation ethanol production from sugarcane juice (saccharose), as fermentation is carried out from the recovery of cells through centrifugation during the entire harvesting period. Therefore, the cell mass produced during fermentation is used as inoculum for the next fermentation. However, for second-generation ethanol, yeast recovery is more difficult, given the high concentration of insoluble solids, such as lignin, insoluble sugar and ashes. Even if yeast reuse was possible, its use would not be recommended, as genetically modified yeast can be replaced by wild-type strains and other contaminants. Thus, yeast propagation is an important step before each fermentation batch for the production of second-generation ethanol.

Saccharomyces cerevisiae yeast has the ability of growing under either anaerobic or respiratory metabolism, depending on culture conditions. For the production of ethanol, anaerobic conditions are preferred. However, for yeast propagation, a complete respiratory metabolism is essential to reach high cell yields. Under total respiratory metabolism, yields of approximately 0.5 g of cell mass per gram of glucose can be reached (even if observing the Pasteur, Crabtree and reverse-Pasteur effects) [12].

Yeast propagation is a process that depends on several factors, such as sugar concentration, aeration, culture medium composition, yeast strain and propagation reactor configuration. In order to promote respiratory metabolism in *Saccharomyces cerevisiae,* high aeration rates are necessary (approximately 1 to 2% vvm), as well as sugar concentrations below 5 g/L and a specific bioreactor configuration (i.e. airlift) [12, 18].

Maintaining a sugar concentration below 5 g/L during propagation is important as yeast usually consumes glucose for ethanol fermentation in excess of sugar, even in sufficient aeration levels. This anaerobic ethanol fermentation is known as the Crabtree effect (or catabolite repression), being one of the primary reasons for low cell yields in the presence of aeration [19, 20]. This low sugar concentration can be obtained by the use of fed-batch or continuous yeast operations in several tanks.

The substrate and nutrients are other important factors to be considered. For the production of industrial yeast, sugarcane molasse is the most commonly used substrate for yeast propagation. Its composition not only contains sugars, but also phosphates, salts and the vitamins required for cell growth. By using sugarcane molasse as substrate, only one nitrogen source, such as urea or ammonia, is usually supplemented.

In the current Brazilian scenario, the availability of sugarcane molasse is not an issue, as a result of the stability faced by the sucrose sector. Nevertheless, in cases of unavailability of molasse, the following can be used: VHP sugar, urea, monopotassium phosphate, vitamins (such as biotin and thiamine) and minerals (Mg, Mn, Fe, among others) are added to supplement the substrate used, providing the growth conditions necessary for yeast development [12, 20].

A robust yeast strain and a well-designed process can ensure large amounts of yeast over a short period of time, with low production costs. Large amounts of yeast are preferable during the fermentation stage, as a high yeast mass as inoculum can reduce fermentation time and consequently increase ethanol productivity.

Fermentation is considered efficient when high yields are reached, producing great concentrations of ethanol. For first-generation fermentation, these performance parameters are well known. However, for second-generation fermentation, the scenario still needs to be optimised. The main challenges to be overcome include robustness of genetically modified yeast, xylose consumption rates, tolerance to inhibitors, costs and equipment configuration.

Due to the close relation between hydrolysis and fermentation, the configuration of both processes are usually designed simultaneously. Some studies have discussed the processes of separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), hybrid hydrolysis and fermentation (HHF) and consolidated bioprocessing (CBP). SHF and HHF currently stand out given that the technologies developed for industrial enzymatic hydrolysis and fermentation (enzyme cocktails and commercial yeast) are related to these concepts [12, 14].

SHF involves hydrolysis and fermentation in separate tanks, enabling to adjust each process to their optimal conditions (hydrolysis: 50 to 55 °C; fermentation: 30 to 35 °C). However, the drawbacks of this process include enzyme inhibition via subproducts, as well as the high costs (associated to the need of additional tanks and high energy consumption). On the other hand, HHF involves hydrolysis in a separate tank until glucose release decreases. The reaction is then transferred to another tank, with the temperature being reduced for fermentation, during which hydrolysis continues at low rates concomitant with ethanol production. Therefore, this process can optimise capital and operational costs when compared to SHF, as the volume of tanks and residence times would be lower. Nonetheless, the success of this process depends on fine tuning hydrolysis to fermentation transfer, as well as on enzyme features and on hydrolysis at high total solids concentration [11].

SHF and HHF are currently used at a commercial scale in Brazil. The logen technology, employed by Raízen (São Paulo), is a SHF process, while the *PROESA* technology, used by GranBio – BioFlex1 (Alagoas), is a process similar to the HHF concept [12].

In addition to the considerations pointed out above, during the hydrolysis process with 20 to 25% of total solids, the hydrolysate must contain approximately 5 to 10% *w*/*w* of insoluble solids, which are primarily composed of lignin, insoluble sugars and ashes. These solids (mainly lignin) are important for the processes that direct them to the boiler, for steam and energy generation. Nevertheless, the stage at which the solids are removed is another factor that determines the configuration of the process [11].

The separation of solids can be carried out upstream or downstream of fermentation. Removing the solids before fermentation can enable an easier integration with first-generation distillation (if only sugars containing six carbons—C6—are used) through a mixture of first- and second-generation streams (at a beer well or at a fermentation tank itself), thus providing flexibility to the process. This configuration can reduce the investment by using equipment already installed at first-generation mills, which reduces distillation costs for low ethanol concentrations (4% *w*/*w*), as well as allowing yeast recovery. However, solid separation before fermentation can lead to significant losses of sugar, as sugars would be dragged with solids during separation, as well as restricting the process for the use of SHF [12].

Another alternative is solid separation after distillation, which avoids sugar losses and allows the use of SHF or HHF approaches. Nevertheless, it involves special equipment design (such as fermentation tanks, heat exchangers, pumps, distillation equipment and a cleaning system) for working

with high solids contents. For instance, Iogen technology uses solid separation upstream of fermentation, while PROESA technology removes the solids after distillation.

First- and second-generation processes can also be integrated through other operational configurations. Lignocellulosic residues from the first-generation process (bagasse and straw) can feed the second-generation process. In the cogeneration unit, residues from the second-generation process (mainly lignin), together with bagasse and straw, supply steam and electricity for both processes, with the possibility of the surplus being sold to local electric energy distribution network. Brazil currently has a great competitive advantage for the installation of new integrated units, as mills already installed provide raw materials and facilities for such deployment [12].

Materials and Methods

All the data analysed in this study was generated in an industrial plant of cellulosic ethanol production, taking into account 4 months of operation. In order to reach the objectives foreseen in this work, the following samples of the process were necessary:

a Sugarcane straw *in natura*, collected from the reception area of raw material;
b Pre-treated material, collected after the straw was submitted to auto-hydrolysis;
c Hydrolysate, collected after reaching enzymatic hydrolysis residence time;
d Fermented sample collected after 12, 24, 36 and 40 h of fermentation.

Preparation of Straw *In Natura* and Pre-treated Straw Samples

This procedure was carried out in accordance with norm ASTM E1757-01 (Standard Practice for Preparation of Biomass for Compositional Analysis) [21], being applied to separate soluble and insoluble compounds from straw *in natura* samples and after pre-treatment in order to determine the chemical composition of the respective samples.

The samples were washed, centrifuged, separated from the washing liquid and dried in an oven at 105 °C. The respective washing liquid (liquid fraction) was collected after centrifugation and then filtered. After filtration of the liquid fraction, monomeric soluble sugar and solid contents were determined, with soluble oligomer content being determined after acid hydrolysis of the liquid. In turn, lignin and ash contents were determined in the dry fraction and, after acid hydrolysis of the solid, insoluble oligomer/polymer content was verified.

Preparation of Enzymatic Hydrolysis and Fermentation Samples

This procedure was carried out in accordance with norm ASTM E1757-01 (Standard Practice for Preparation of Biomass for Compositional Analysis) [21] and was applied to separate the solid from the liquid fraction of the samples collected, in order to determine the chemical composition of the hydrolysed and fermented samples.

For the samples of the solids in suspension, a test tube and a centrifuge were used aimed at separating the liquid and the solid fractions. After centrifugation, soluble monomeric sugar content and solid content were determined in the liquid fraction, with soluble oligomer content being determined after acid hydrolysis of the liquid.

The solid fraction separated after centrifugation was then washed and centrifuged once again. The separated liquid was discarded, and the solid fraction was dried in an oven at 105 °C until constant mass was reached. Insoluble oligomer and polymer contents were then determined after acid hydrolysis through the dried solid fraction.

Acid Hydrolysis for the Solid and Liquid Sample

This procedure was carried out in accordance with norms: ASTM E1757-01 (Standard Practice for Preparation of Biomass for Compositional Analysis) and NREL/TP-510-42618 (Determination of Structural Carbohydrates and Lignin in Biomass) [21, 22]. Acid hydrolysis of the solid sample was carried out in order to convert oligomers/polymers into monomeric sugars by adding a 72% sulphuric acid solution. The liquid fraction produced during acid hydrolysis contains the monomers originated from the initial oligomers and polymers. These sugars were then quantified through liquid chromatography (HPLC: Dionex Ultimate 3000; column: Biorad Aminex 87P).

Determination of Moisture Content

Moisture content was determined through direct drying in an oven at 105 ± 2 °C. This method is based on the mass loss in an oven until the sample reaches a constant mass. Moisture content was then determined according to Equation 2.

$$\text{Moisture}\ (\%) = \frac{100 \times (W_i - W_f)}{(W_i - W_c)} \quad (2)$$

where W_i = crucible mass + biomass before drying (g); W_f = crucible mass + biomass after drying (g) and W_c = crucible mass (g).

Determination of Solid Content

This procedure was carried out in accordance with norms: ASTM E1756-01 (Standard Test Method for Determination of Total Solids in Biomass) and NREL/TP-510-42621 (Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples) [23, 24]. The total solid content was determined by drying the homogenised liquid at 105 °C until complete water removal. The soluble solid content was determined by drying a sample of filtered liquid (0.45 μm) at 105 °C until reaching constant mass. The solid content of a sample is then used to convert the analytical results obtained on a dry basis.

Determination of Process Efficiency

The analytical procedures for characterising the samples previously described enabled to determine the efficiency of the pre-treatment and fermentation processes. The efficiencies of these processes can be calculated according to Equations 3, 4 and 5.

$$\text{Pre-treatment efficiency } (\%) = \frac{\text{Monomeric sugars of pre-treated sample}}{\text{Monomeric sugars (clean raw material)}} \times 100 \quad (3)$$

$$\text{Hydrolysis efficiency } (\%) = \frac{\text{Soluble monomers after hydrolysis}}{\text{Monomeric sugars (pre-treated sample)}} \times 100 \quad (4)$$

$$\text{Fermentation efficiency } (\%) = \frac{(\text{final ethanol} - \text{initial ethanol})}{\text{Fermentable sugars at the beginning of fermentation}.0.511^{*}} \times 100 \quad (5)$$

*the factor of 0.511 corresponds to the conversion of fermentable sugars into ethanol, considering Gay-Lussac stoichiometry.

Results and Discussion

In this section, the following aspects will be presented and discussed: (1) the results obtained from the characterisation of sugarcane straw and (2) a comparison with data from the literature regarding the results of the efficiencies of the processes of pre-treatment, enzymatic hydrolysis and fermentation in a cellulosic ethanol production plant using sugarcane straw.

All the data presented refer to the results achieved in practice, at an industrial scale, taking into account an industrial plant for cellulosic ethanol and lignocellulosic production from sugarcane straw, with the data collected representing 4 months of operation. It is important to point out that the results of this study were presented in such a way so as to protect the technological confidentiality of the company, although valuable information that could aid the understanding of the large-scale operation be provided.

Sugarcane Straw Characterisation

Moisture content is a parameter necessary to determine the biomass composition on a dry basis. Figure 2 shows the results obtained for sugarcane straw in the 4 months during which the industrial was monitored.

The composition of the sugarcane straw studied was similar to that found in the literature [25, 26], consisting of approximately 35% of cellulose, 24% of hemicellulose and 20% of lignin (dry basis). Considering this composition of sugarcane straw *in natura* and after the necessary conversions to a monomeric base, it is possible to observe that this biomass presents, on average, 60% of sugars available for 2G transformation processes.

Pre-treatment

The pre-treatment configuration analysed in this work was based on the technological concept of auto-hydrolysis or liquid hot water (LHW) in a single boiling stage. This technology is a physicochemical process and consists of boiling the biomass using only water and steam, operating under optimal temperature, pressure and residence time conditions. The objective of the process was to solubilise hemicellulose, making

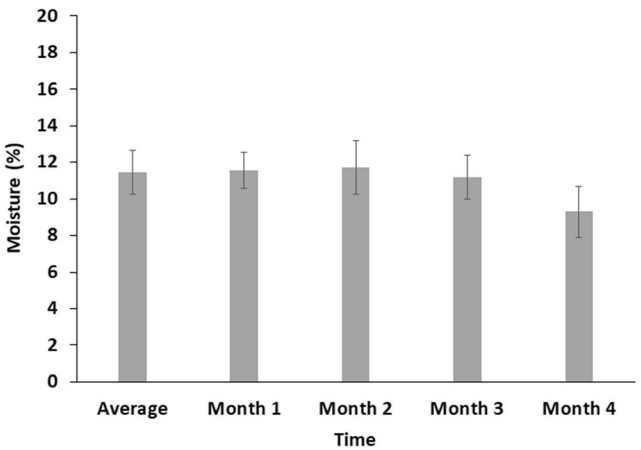

Fig. 2 Moisture content of sugarcane straw *in natura*

cellulose more accessible and avoiding the formation of degradation products and/or inhibitors.

The biomass was provided to the industry in study in the form of bales. The strings present in the bales were removed in the beginning of the process, in order to avoid problems in the machines and equipment (especially in the rotating equipment). In addition, the biomass was baled, sieved and cut, ensuring under optimal feeding conditions for the pre-treatment process.

The main control parameters of the pre-treatment process are operating temperature, residence time and liquid/solid ratio in the reactor. Additionally, the two rapid response parameters in the pre-treatment process area include pH, which provides an indication of the severity of the reaction regarding sugar solubilisation, and solid percentage, which provides a rapid response regarding the amount of water being fed to the reactor [12]. Table 1 presents the control parameters adopted, as well as the average pre-treatment efficiency (efficiency in terms of the non-generation of inhibitors and sub-products) presented in the literature and compatible with the LHW process studied during the period analysed.

Enzymatic Hydrolysis

The enzymatic hydrolysis process addressed in this article consists of the concept of hybrid hydrolysis and fermentation (HHF), which involves hydrolysis in a tank until glucose release decreases. The reaction is then transferred to another tank and the temperature reduced for fermentation, in which hydrolysis continues at low temperatures simultaneously with ethanol production.

The efficiency of the hydrolysis process depends on several factors, such as residence time, pH, temperature, enzyme dosage, percentage of total solids and accessibility of the pre-treated material. The cost of enzymatic hydrolysis is low when compared to acid or basic hydrolysis, as enzymatic hydrolysis is usually carried out in milder temperature (50 to 60 °C) and pH conditions (4.5 to 5.5), besides not having any corrosion issues [12, 28].

In the process presented and studied in the present work, pH was efficiently adjusted through the addition of a weak soluble base, with temperature being controlled by the heat exchangers. Enzyme dosage is one of the primary factors which have an impact on the efficiency of enzymatic hydrolysis. The higher the enzyme dosage, the greater the efficiency of the hydrolysis process. However, in order to determine the dosage to be used, the economic balance between efficiency and enzyme costs must be taken into account.

Figure 3 presents the efficiency profile of enzymatic hydrolysis as a response to the main parameters of the process. In phase A, residence time and enzyme dosage remained constant, with T in hours and E in % (g of enzyme/100 g of total solids), respectively. In phase B, residence time was increased by 30% for an enzyme dosage equal to 93% in relation to the initial dosage, with the average yield maintained. For phase C, residence time was 1.4 times higher than the initial time, with an enzyme dosage equal to 92% in relation to the initial dosage. During this period, the average yield increased 3% in relation to the initial figure. Finally, in phase D, residence time remained constant with an enzyme dosage equal to 97% in relation to the initial dosage. The average yield in D was 11% higher than the average initial yield.

Aimed at reaching economic feasibility of industrial enzymatic hydrolysis process, besides the appropriate adjustment of the main parameters of the process (such as temperature and enzyme dosage as mentioned by [15, 16] and residence time as mentioned by [12]), hydrolysis yield must be of at least 70% [12], with this result being reached in the industry for the production of 2G ethanol from sugarcane straw through LHW treatment.

Table 1 Pre-treatment: control parameters, response variables and efficiency reached

Parameter	Results	Source
Residence time	~ 30 min	[12]
Severity Factor	3.6 ~ 4.2	[13]
Reactor temperature	170 ~ 200 °C	[8, 27]
Efficiency	> 90%	[11, 27]

Fermentation

After biomass pre-treatment and enzymatic hydrolysis, the hydrolysed material was submitted to fermentation. In the context of second-generation fermentation, yeast recovery is more difficult given the high concentrations of insoluble solids, such as lignin, insoluble sugar and ashes. Therefore, yeast propagation is an important step before each batch fermentation for cellulosic ethanol production [12].

The objective of propagation is to multiply yeast cell mass, which usually starts with low cell masses in order to reach final satisfactory values. In order to reach this final yeast mass, appropriate culture and nutrient conditions need to be provided. After propagation, yeast is submitted to fermentation, with the success of the process depending on the quality and quantity of yeast produced, among other factors. For the period analysed, yeast mass was optimised in order to be multiplied 200-fold. Throughout the operation period, yeast propagation was stabilised when reaching the final yeast mass.

An efficient fermentation process depends on a combination of several factors, namely the quality of yeast propagated, conditions of the process (pH, temperature and nutrients), substrate quality (hydrolysate), operational stability and human factors (compliance with the procedures adopted).

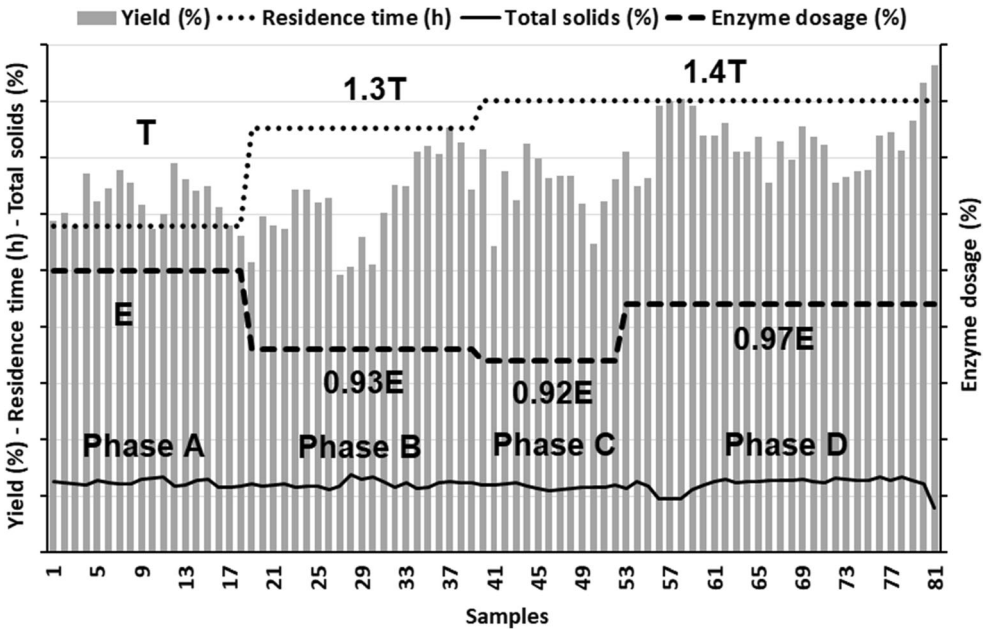

Fig. 3 Enzymatic hydrolysis efficiency after varying the process parameters. E refers to enzyme dosage, expressed in % (g of enzymes/100 g of total solids); T is residence time in hours (the scale used was omitted in order to preserve technological confidentiality). Eighty-one independent fermentation processes were analysed

As it is the last area before downstream, fermentation strongly depends on the quality of the previous process areas, such as enzymatic hydrolysis and mainly pre-treatment. Despite correctly following all conditions established for the process and using a robust-type yeast, 2G fermentation yield can be low if, during pre-treatment, high inhibitor concentrations are generated, or if the biological limit of yeast is reached.

Figure 4 shows the consumption profile of sugars and cellulosic ethanol production during the period analysed in this study. It is possible to observe that glucose is totally consumed in the first 24 h of fermentation. Due to the catabolic repression phenomenon, xylose consumption is slower, becoming faster as glucose concentration in the medium is reduced. Catabolic repression is common in ethanol fermentation processes with multiple substrates (monosaccharides) in which glucose metabolisation is favoured [29–31].

During the period analysed, fermentation contamination due to bacteria and/or wild-type yeast was not a recurring problem and, when any contamination events were observed, the due cause was identified. The main causes of contamination included long cooling times of the hydrolysed material, which favours the proliferation of contaminant microorganisms, and when asepsis procedures were not correctly followed.

No microbiological analyses were carried out to monitor contamination by bacteria or wild-type yeast in fermentation due to the difficulties in performing this type of analysis in samples with high total solid content, which could indicate a false negative or positive concentration, depending on the agglomeration state of microorganisms in the solid, as well as due to the extraction re-suspension process for subsequent incubation in solid medium. This is a drawback of microbial control in fermentation processes carried out with solids in suspension.

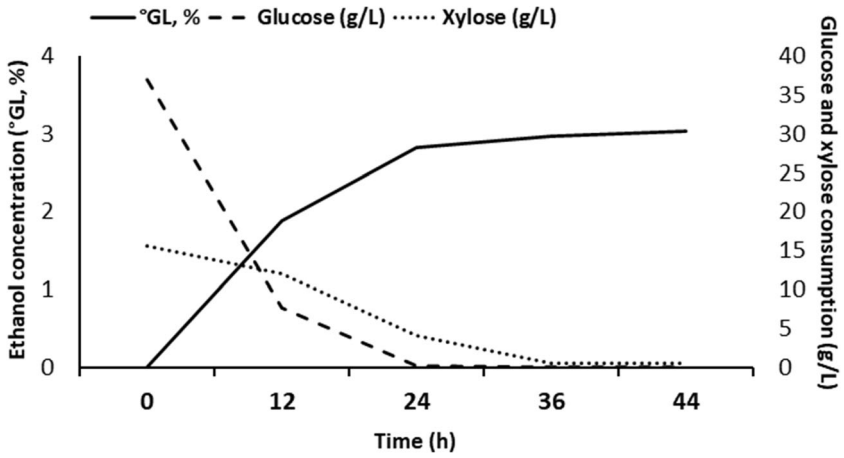

Fig. 4 Consumption profile of sugars and cellulosic ethanol production in second-generation fermentation (scale was omitted in order to preserve technological confidentiality)

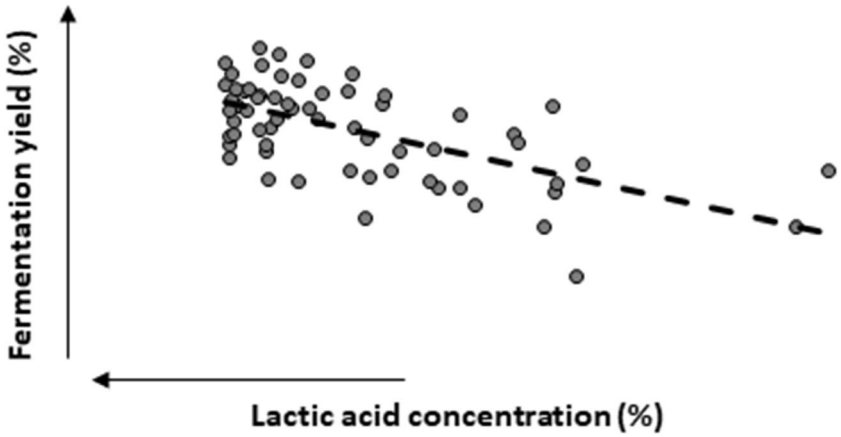

Fig. 5 Correlation between cellulosic ethanol fermentation yield and lactic acid concentration at the end of the fermentation process (scale was omitted in order to preserve technological confidentiality)

Nevertheless, indirect monitoring was carried out using pH and mainly lactic acid produced during fermentation. Lactic acid can be used as a strong indicator of bacterial contamination in ethanolic fermentation processes [32, 33]. Figure 5, drawn from the results of ten different fermentations, conducted in the industrial plant analysed in this work, illustrates an inversely proportional correlation between fermentation yield and lactic acid concentration at the end of the fermentation process.

During the period analysed, the pre-treatment and hydrolysis steps were well-controlled, ensuring that the high quality of the hydrolysed material for fermentation. The literature [34, 35] contemplates yields higher than 85% for second-generation fermentation, a compatible figure when compared to the process studied at an industrial scale.

Taking into account the results presented per process area and the results widely available in the literature regarding the process, it is possible to calculate the global efficiency of cellulosic ethanol production at an industrial scale, as shown in Fig. 6. Therefore, considering auto-hydrolysis as a pre-treatment technology and the concept of enzymatic hydrolysis and hybrid fermentation, it is possible to reach a production of approximately 200 l of ethanol per tonne of processed sugarcane straw.

Regarding productivity, it can be observed by Table 2 that the productivity exhibited by the lignocellulosic sugarcane biomass, especially when adding straw and bagasse, could at least double the production of ethanol per hectare in countries dependant on this technology, such as Brazil. This information is important, as sugarcane has specific climate and soil requirements, which prevents this culture from being cultivated in many regions around the world. Moreover, third-generation (3G) bioethanol (micro and macroalgae), despite exhibiting promising numbers, does not yet have a developed technology and it is not clear whether the results obtained in the laboratory can be replicated at an industrial scale. Brazil has developed ethanol from corn both in sugarcane off-season periods and at mills specialised in this technology, thus increasing the Brazilian autonomy in terms of the production of this biofuel.

Furthermore, the most diverse innovate processes for bioethanol production from unusual feedstock, such as mango bark residue, waste hamburger and cattle manure, are being

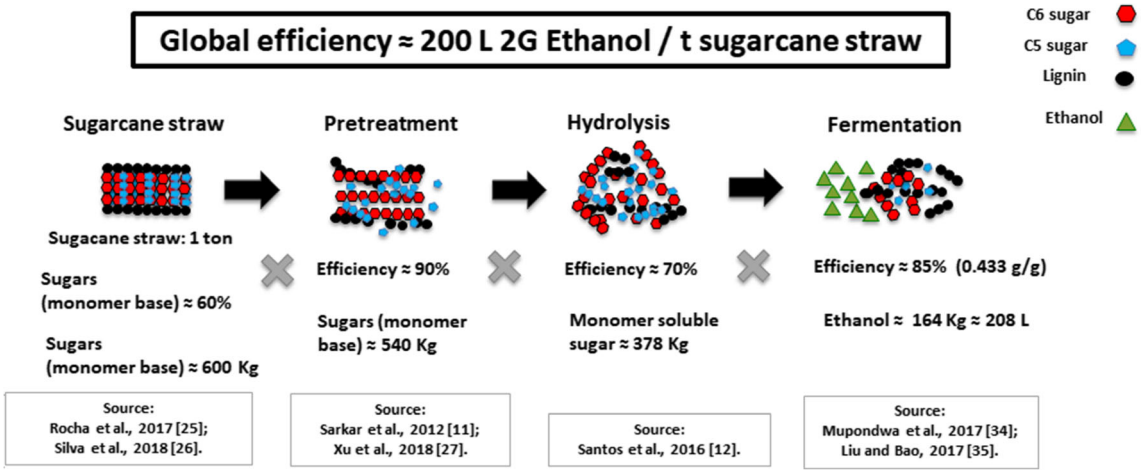

Fig. 6 Efficiency per process area and global efficiency of the process of cellulosic ethanol production

Table 2 Comparison between plant and microalgae productivity for biofuel production

Raw material	Carbohydrate content ($\%_{dry\ biomass}$)	Production ($L_{bioethanol}\ Ton_{biomass}^{-1}$)	Land use (m^2 year $L_{bioethanol}^{-1}$)	Productivity ($L_{bioethanol}\ ha^{-1}\ year^{-1}$)
Corn	-	460	2.5	3450–4600
Sugar beet	-	100	1.3	5000–10000
Sugarcane	-	90	1.2	5400–10800
Lignocellulosic Biomass[a]	50–70	~400	1.0	~10,000
Microalgae[b]	45–60	-	-	11,000–31,000

[a] Lignocellulosic sugarcane biomass, considering sugarcane straw and bagasse

[b] Productivities extrapolated in continuous and laboratory scale flat-panel photobioreactors using *Chlorella vulgaris* and *Scenedesmus obliquus* and considering hydrolysis and fermentation yields of 90%. Adapted from Silva and Bertucco, 2019 [36]

developed to increase the autonomy for biofuel production routes. The combination of alternative feedstock with hybrid systems processes and biomass genetic modification presents good perspectives to improve bioethanol production, enhancing its feasibility by: increasing the amount of cellulose in the biomass, reducing severe pre-treatment methods, reducing enzyme costs, improving energy recovery and reducing waste generation [37–42].

The costs of bioethanol production from sugarcane (0.16–0.22 US$/L) are lower than those from corn (0.25–0.40 US$/L), sugar beet (0.43–0.73 US$/L) and lignocellulosic materials (0.43–0.93 US$/L) [5]. However, certain aspects also need to be considered for sugarcane residues, such as the simultaneous production of saccharose juice and lignocellulosic biomass in the same area [4], as well as the productivity obtained by recovered lignin, either for combustion [43] or for compounds and materials of high added value [44], which are not often considered in the financial balance of the process.

Conclusions, Challenges and Future Perspectives Towards Feasibility

The production of cellulosic ethanol at an industrial scale from sugarcane straw exhibited (global and process) efficiencies compatible with several studies regarding second-generation routes. Considering the main results of the three processes (pre-treatment, enzymatic hydrolysis and fermentation), it is possible to produce approximately 200 l of cellulosic ethanol per tonne of biomass processed. However, production costs associated to second-generation production still represent a great challenge to the sector. Government incentives, such as those offered in the United States through the RFS (Renewable Fuel Standard) program, make the cellulosic ethanol sector more profitable for exportation purposes. Brazil is also putting forward other alternatives, such as *RenovaBio*, to add value to the internal market of biofuels. Nonetheless, despite all the benefits outlined, validating the industrial process is still considered a drawback, especially in economic terms, when compared with sugarcane used in first-generation production.

The main drawbacks of the steps pointed out in this article include purity of the material fed to the pre-treatment reactors, as well as its recovery and efficient use of the pentose (C5) fraction of hemicellulose, formation of inhibitors in the pre-treatment process, price and enzyme stability, co-fermentation of hexoses and pentoses (C6 and C5), as well as the recovery and efficient use of the solid fraction (primarily formed by lignin).

So, with the industrial scale validation of global and process efficiencies as presented in this study, for future perspectives, investment on different initiatives can be pointed aiming to solve the main bottlenecks of this process, from a feasibility point of view, such as

- The development and use of Cana Vertix® as a potential and commercial feedstock, which is a kind of energy cane that is genetically modified to become more productive for biofuel and biochemical manufacturing, as well as for renewable energy generation;

- The development and use of own commercial yeast, called Celere-2L, capable of converting hexoses and pentoses present in sugarcane straw and bagasse into ethanol in a single fermenting process;

- The more add-value use of lignocellulosic waste generated from 2G ethanol, as a low-cost raw material for the biochar and bio-oil production. Nowadays, this waste is used in boilers for steam and electricity generation.

Acknowledgements The authors would like to thank GranBio, the Federal University of Alagoas (UFAL) and the Laboratory of Separation Systems and Process Optimisation (LASSOP/UFAL). C.E.F.S thanks CNPq for the financial support, Processes No 407274/2018-9 and 440070/2019-8.

References

1. Jin E, Mendis GP, Suttherland JW (2019) Integrated sustainability assessment for a bioenergy system: a system dynamics model of

switchgrass for cellulosic ethanol production in the U.S. Midwest. J Clean Prod 234:503–520
2. Liu C, Xiao Y, Xia X, Zhao X, Peng L, Srinophakun P, Bai F (2019) Cellulosic ethanol production: progress, challenges and strategies for solutions. Biotechnol Adv 37(3):491–504
3. Abud AKS, Silva CEF (2019) Bioethanol in Brazil: status, challenges and perspectives to improve the production. Bioethanol Production from Food Crops 115:417–443
4. Silva CEF, Barbera E, Bertucco (2019) Biorefinery as a promising approach to promote ethanol industry from microalgae and cyanobacteria. Bioethanol Production from Food Crops 90:343–359
5. Gupta A, Verna JP (2015) Sustainable bio-ethanol production from agro-residues: a review. Renew Sust Energ Rev 41:550–567
6. Silva CEF, Bertucco A (2016) Bioethanol from microalgae and cyanobacteria: a review and technological outlook. Process Biochem 51:1833–1842
7. Michelin M, Teixeira JA (2016) Liquid hot water pretreatment of multi feedstocks and enzymatic hydrolysis of solids obtained thereof. Bioresour Technol 216:862–869
8. Overend RP, Chornet E (1987) Fractionation of lignocellulosics by steam-aqueous pretreatments. Philos Trans R Soc 321:523–536
9. Chen S, Mowery RA, Chambliss CK, Peter G, Walsum PVL (2007) Pseudo reaction kinetics of organic degradation products in dilute-acid-catalyzed corn stover pretreatment hydrolisates. Biotechnol Bioeng 98(6):1135–1145
10. Silva CEF, Bertucco A (2018) Severity factor as an efficient control parameter to predict biomass solubilization and saccharification during acidic hydrolysis of microalgal biomass. Bioenergy Res 11:491–504
11. Sarkar N, Ghosh SK, Bannerjee S, Aikat K (2012) Bioethanol production from agricultural wastes: an overview. Renew Energy 37:19–27
12. Santos LV, Grassi MCB, Gallardo JCM, Pirolla RAS, Calderón LL, Netto OVC, Parreiras LS, Camargo ELO, Drezza AL, Missawa SK, Teixeira GS, Lunardi I, Bressiani J, Pereira GAG (2016) Second-generation ethanol: the need is becoming a reality. Ind Biotechnol 12:40–57
13. Silva RO, Pinheiro IR, Ferreira INT (2018) Estudo do reaproveitamento dos resíduos sólidos da indústria cacaueira. Avanços Científicos e Tecnológicos em Bioprocessos 1:65–69
14. Perrone OM, Rossi JS, Moretti MMS, Nunes CCC, Bordignon SE, Gomes E, Silva R, Boscolo M (2016) Influence of ozonolysis time during sugarcane pretreatment: effects on the fiber and enzymatic saccharification. Bioresour Technol 224:733–737
15. Teter SA. 2012. DECREASE final technical report: development of a commercial ready enzyme application system for ethanol. United States: N. p., 2012. Web. doi:https://doi.org/10.2172/1039767 https://www.osti.gov/servlets/purl/1039767
16. Teter SA, Sutton KB, Emme B. 2014. Enzymatic processes and enzyme development in biorefining. Advances in Biorefineries: Biomass and Waste Supply Chain Exploitation 199–233. Doi: https://doi.org/10.1533/9780857097385.1.199
17. Harmsen PFH, Huijgen W, Bermudez L, Bakker R (2010) Literature review of physical and chemical pretreatment processes for lignocellulosic biomass. Food & Biobased Research 1184:1–54
18. Hanotu J, Kong D, Zimmerman WB (2016) Intensification of yeast production with microbubbles. Food Bioprod Process 100:424–431
19. Imura M, Iwakiri R, Bamba T, Fukusaki E (2018) Metabolomics approach to reduce the Crabtree effect in continuous culture of Saccharomyces cerevisiae. J Biosci Bioeng 126:183–188
20. POET RESEARCH. NARENDRANATH, V.; LEWIS, S. M. Propagating microorganisms & related methods & systems. Estados Unidos, patente n. US 2018 / 0171285 A1, 2015 Jun 17, 2018 Jun 21
21. ASTM International. ASTM E1757–01 (2015) Standard practice for preparation of biomass for compositional analysis. West Conshohocken, PA; ASTM International, 2015. doi: https://doi.org/10.1520/E1757-01R15
22. SLUITER, A. et. al. Determination of structural carbohydrates and lignin in biomass. National Renewable Energy Laboratory – NREL, 2012
23. ASTM International. ASTM E1756–08 (2015) Standard test method for determination of total solids in biomass. West Conshohocken, PA; ASTM International, 2015. doi: https://doi.org/10.1520/E1756-08R15
24. SLUITER, A. et. al. Determination of total solids in biomass and total dissolved solids in liquid process samples. National Renewable Energy Laboratory – NREL, 2008
25. Rocha MSRS, Pratto B, Almeida RMRG, Cruz AJG (2017) Otimização de parâmetros operacionais do pré-tratamento da palha de cana-de-açúcar. Scientia Plena 13:1–16
26. Silva JS, Malta VRS, Rocha MSRS, Almeida RMRG, Gomes MA, Vicente CD, Barbosa KL (2018) Hidrólise enzimática, fermentação e produção de biocombustíveis através da coroa de *Ananas comosus*. Química Nova 41:1127–1131
27. Xu Y, Li J, Zhang M, Wang D (2018) Modified simultaneous saccharification and fermentation to enhance bioethanol titers and yields. Fuel 215:647–654
28. Sun Y, Cheng J (2002) Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour Technol 83:1–11
29. Taguchi M, Tohma T, Itaya T, Fuji M (1997) Ethanol production from a mixture of glucose and xylose by co-culture of *Pichia stipitis* and a respiratory-deficient mutant of *Saccharomyces cerevisiae*. J Ferment Bioeng 83(4):364–370
30. Fu N, Peris P (2008) Co-fermentation of a mixture of glucose and xylose to ethanol by *Zymomonas mobilis* and *Pachysolen tannophilus*. World J Microbiol Biotechnol 24(7):1091–1097
31. Vilela LF, Araujo VPG, Paredes RS, Bom EPS, Torres FAG, Neves BC, Eleutherio CA (2015) Enhanced xylose fermentation and ethanol production by engineered *Saccharomyces cerevisiae* strains. AMB Express 5(16):1–7
32. Bischoff KM, Liu S, Leathers TD, Worthington RE, Rich JO (2009) Modeling bacterial contamination of fuel ethanol fermentation. Biotechnol Bioeng 103(1):117–122
33. Becker M, Ivey ML, Phister TG (2011) Microbial contamination of fuel fermentations. Lett Appl Microbiol 53:387–394
34. Mupondwa E, Li X, Tabil LG, Sokhansanj S (2017) Status of Canada's lignocellulosic ethanol: part II: hydrolysis and fermentation technologies. Renew Sust Energ Rev 79:1535–1555
35. Liu G, Bao J (2017) Maximizing cellulosic ethanol potentials by minimizing wastewater generation and energy consumption: competing with corn ethanol. Bioresour Technol 245:18–26
36. Silva CEF, Bertucco A (2019) Bioethanol from microalgal biomass: a promising approach in biorefinery. Braz Arch Biol Technol 62(e19160816):1–14
37. Carrillo-Nieves D, Ruiz HA, Aguilar CN, Ilyina A, Parra-Saldivar R, Torres JA, Martínez Hernández JL (2017) Process alternatives for bioethanol production from mango stem bark residues. Bioresour Technol 239:430–436
38. Han W, Liu Y, Xu X, Huang J, He H, Chen L, Qiu S, Tang J, Hou P (2020) Bioethanol production from waste hamburger by enzymatic hydrolysis and fermentation. J Clean Prod 264:121658
39. Rocha-Meneses L, Ferreira JA, Mushtaq M, Karimi S, Orupõld K, Kikas T (2020) Genetic modification of cereal plants: a strategy to enhance bioethanol yields from agricultural waste. Ind Crop Prod 150:112408
40. Sambusiti C, Monlau F, Barakat A (2016) Bioethanol fermentation as alternative valorization route of agricultural digestate according to a biorefinery approach. Bioresour Technol 212:289–295

41. Vancov T, Schneider RCS, Palmer J, McIntosh S, Stuetz R (2015) Potential use of feedlot cattle manure for bioethanol production. Bioresour Technol 183:120–128
42. Werlang, E. B., J. Julich, M. V. G. Muller, F. de Farias Neves, E. Sierra-Ibarra, A. Martinez and R. C. S. Schneider (2020). Bioethanol from hydrolyzed Spirulina (Arthrospira platensis) biomass using ethanologenic bacteria. Bioresources and Bioprocessing 7(1)
43. Welker CM, Balasubramanian VK, Petti C, Rai KM, DeBolt S, Mendu V (2015) Engineering plant biomass lignin content and composition for biofuels and bioproducts. Energies 8:7654–7676
44. Vishtal A, Kraslowski A (2011) Challenges in industrial applications of technical lignins. Bioresources 6(3):3547–3568