

Use of cultivars of low cost, agroindustrial and urban waste in the production of cellulosic ethanol in Brazil: A proposal to utilization of microdistillery



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ABSTRACT

Ethanol production from sucrose from sugarcane allowed Brazil to become a world reference in the production of biofuels. Presenting the 2013/2014 crop harvest of 653,519 thousand tons of sugarcane, thus producing 37,713 thousand tons of sugar and 27,543 thousand m³ of ethanol and generating 91,493 thousand tons of bagasse. However, as referenced since 1970, Brazil could exploit sugarcane waste surpluses in order to use bagasse and straw for ethanol production, but the data presented in the last decade emphasized in a significant direction and on increasing the use of these wastes in the generation of bioelectricity. Currently there are 486 sugar-energetic power plants with the productive capacity of 12.056 MW to the power network (autoconsumption of sugar-energetic power plants corresponds to 50% of the energy produced), representing 8.4% of the Brazilian energy matrix. Therefore, this review reports that the offer of bagasse and straw for ethanol production in industrial scale will be insufficient, thus, arising the need to find possible lignocellulosic materials with the potential to be used for ethanol production, thus allowing the supplemental absence of straw and bagasse sugarcane that could be available according to the locality, both in the rural area as well as in the urban area. Furthermore, the review reports the application of sugarcane wastes in the production of bioethanol, the difficulties encountered in the implementation of cellulosic ethanol power plants based only on the use of bagasse and straw of sugarcane, the possibility to use alternatives of lignocellulosic materials with potential to be applied in Brazil, besides the production of cellulosic ethanol, the production of co-products and by-products using microdistillery, based on the biorefinery context in an efficient manner.

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Contents

1. Contemporary situation of Brazilian sugar-energetic power plants	1288
2. Microdistillery	1289
2.1. Implantation of microdistillery	1289
2.2. Implantation of microdistillery in the context of biorefinery	1290
2.3. Production of co-products and by-products of the productive process of cellulosic ethanol	1291
2.4. Enzymes	1292
3. Sources of biomass	1292
3.1. Terrestrial photosynthetic biomass	1292
3.2. Coconut bagasse	1293
3.3. Cactus	1294
3.4. Halophyte	1294
3.5. Urban solid waste of plant origin	1294
4. Biotechnological advances strategic	1295
4.1. Alcoholic fermentation of mixed sugars	1295
4.2. Consolidated bioprocessing (CBP)	1296

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4.3. Others advances.....	1296
5. Disadvantages, difficulties, challenges and perspectives in the production of cellulosic ethanol.....	1297
6. Final considerations.....	1299
Acknowledgments.....	1299
References.....	1299

1. Contemporary situation of Brazilian sugar-energetic power plants

Brazilian sugar-energetic power plants focused only on the production of ethanol and sugar, however, recently attention turned back also to the production of bioelectricity, alcohol chemistry and commercialization of carbon credits [1–4]. That way, it made possible the increase in the offer of products generated by sugar-energetic power plants [2,5]. Furthermore, stands out the global production of cellulosic ethanol as an alternative to ethanol produced based on food sources [5–10], as evidenced by Sastri and Lee [10] (Fig. 1). In Brazil, the production of cellulosic ethanol is being projected, at first, from waste sugarcane [1,5,7,11–13], estimating that the first Brazilian power plants of this type will be in operation in 2014 [14].

The harnessing of the Brazilian energetic potential referring to the straw and bagasse of sugarcane in a short and medium term is directed for electric power production [1,4,5,15–19], considered economically viable in Brazil [1,20], that configures itself like a potential competitor in the destination of straw and bagasse of sugarcane for ethanol production, especially if adopted specific policies such as the energy auctions related to bioelectricity generation.

According to Dias et al. [5], Castro et al. [21] and Menon and Rao [22] the cellulosic ethanol can be competed with the production of bioelectricity, but initially it requires the use integral waste of sugarcane, lower cost of enzymes involved in the process and industrial scale production. However, despite expectations, great uncertainty remains about the performance of cellulosic ethanol production in industrial scale [22], like that the implantation of the power plants in industrial scale is hampered by the absence of public–private funding, derived from the world economic crisis [23,24]. Therefore, this biotechnological route has yet to prove soundness, efficiency and competitiveness.

In 2010, there existed 432 sugar-energetic power plants in activity in the Brazil, whereas 129 were exporting energy to the power network, adding up to 1002 MW [4,25]. In 2012, there were 348 sugar-energetic power plants exporting energy to the power network [26] and currently there are 486 sugar-energetic power plants with the productive capacity of 12,056 MW, representing 8.4% of the Brazilian energy matrix. Presenting the crop 2013/2014

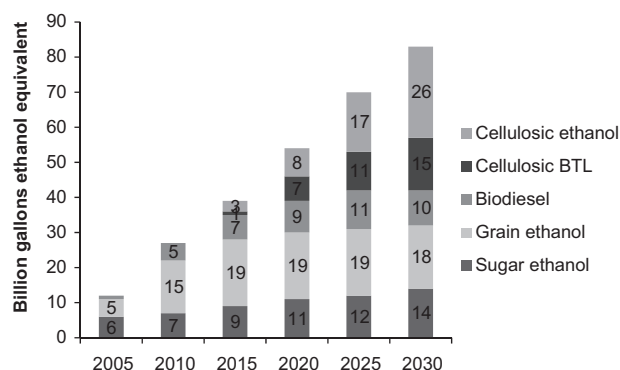


Fig. 1. Total biofuels production by type in the reference scenario. .
Source: [10]

harvest of 653,519 thousand tons of sugarcane, thus producing 37,713 thousand tons of sugar and 27,543 thousand m³ of ethanol (being 12,223 m³ of anhydrous ethanol and 1532 thousand m³ of hydrous ethanol). 91,493 thousand tons of bagasse was generated [26]. In this trend, it has been verified the use of straw and bagasse from sugarcane for the production of electricity [19]. According to Sousa and Macedo [4] bioelectricity presents a potential to supply about 20% of the Brazilian electricity demand by the end of this decade. It is estimated that the production and distribution of bioelectricity in the Brazilian electricity network, through 2021, can be compared to three hydroelectric power plants of Belo Monte, as shown in Fig. 2 [25].

In this context, the Brazilian production of ethanol from straw and bagasse of sugarcane can also be penalized by the adaptive structures of sugar-energetic power plants that use such wastes for the production of electricity, because the Brazilian environmental law places some restrictions on the installations of new sugar-energetic power plants. For example, the State of Mato Grosso do Sul limits the installation of a new sugar-energetic power plant to a minimum radial distance of 25 km between the power plants and limits at 20 km, the distance between the urban area and the power plant. It means that in a few years it will be occupy a majority of permissible areas for implantation of these sugar-energetic power plants, and in the current situation it is designed for the waste of sugarcane for the production of electricity. This destination of waste from sugarcane for the production of electricity in Brazil was also motivated by the delay in implantation of the power plants producing ethanol from these lignocellulosic materials, besides the increase in public–private incentives for the production of bioelectricity [19], therefore reducing the offer of waste of sugarcane for ethanol production. All of these events were also favored by the appellants “energy

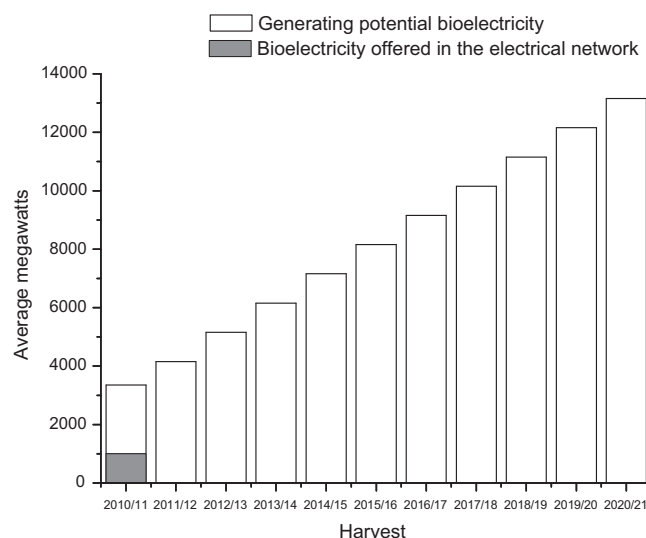


Fig. 2. Market potential of bioelectricity to electrical network – Brazil (2010–2021).
Source: [25]

blackouts” and by the low levels of hydrological reservoirs of the Brazilian hydroelectric power plants.

According to Dias et al. [27] and Pellegrini and Junior Oliveira [28] the sugar-energetic power plants need installation of low pressure boilers and use the energy generated internally, or in accordance with the market, putting investments in high pressure boilers which allows export surplus energy to the efficient power network. In this sense, in Brazil the power plants are gradually replacing low pressure boilers for high pressure boilers and new power plants already have high pressure boilers, focusing the interest of these power plants in exporting the surplus energy produced to the power network [3,19,28].

Bioelectricity linked to the production of ethanol and sugar has exceptional conditions to represent strategic role in the expansion of the national power system, as an important energy source to supplement the offer of energy generated by the hydroelectricity, besides providing low cost production, transmission and distribution (electric energy generated nearest the consumer centers), environmental benefits (source of renewable energy and reducing emissions of greenhouse gases) and socioeconomic benefits (employment generation, guarantee of the supply of energy, reductions in electricity transmission losses and decentralization of electric energy offer). All these factors strengthen the competitiveness of the national economy [4]; furthermore, bioelectricity allows the generation of energy during the months with lower pluviometrics levels [4,19], making possible the decrease in the cost of production of sugar, ethanol and cellulosic ethanol, when bioelectricity generation is present in the productive process [28,29], providing fixed values and financial contracts exceeding 15 years [19]. Therefore this situation stimulates the choice of public–private policies favorable to the generation of bioelectricity as a possible energetic solution, to the energetic market in a country in full socioeconomic expansion, with growing need for electric power availability.

According to Dias et al. [5] the results obtained by simulation demonstrated the possibility of improving the internal return rate of conventional autonomous distilleries when using integrally sugarcane and surplus of electric power passed along to the network. The data obtained by Dias et al. [5] have evidenced the best results in scenarios where electricity production is maximized while, that for cellulosic ethanol, the best internal return rates were obtained from the use of sugarcane and efficient enzymatic hydrolysis integrally.

Yet, according to Dias et al. [30] the evaluative simulation of some productive scenarios, considering different levels of integration between the power plants for ethanol production and cellulosic ethanol as from the sugarcane shows significant differences (Table 1). Integrated production of ethanol and cellulosic ethanol with the current technology of enzymatic hydrolysis (scenario 2) possesses the largest investment between the scenarios studied; it shows the internal return rate greater than just

autonomous power plant for cellulosic ethanol (scenario 5) and it shows the highest production costs. Use of enzymatic hydrolysis advanced technologies in the integrated process improves the ethanol production (scenarios 3 and 4), but only takes place together with the fermentation of pentoses (scenario 4), being the return rate greater than the autonomous ethanol power plant (scenario 1). Autonomous cellulosic ethanol power plant (scenario 5) has the lowest return rate, due to the large initial investment, similar to the (1a) scenario, besides the low ethanol production. If the solvent used in alkaline delignification is recovered, this configuration provides the best environmental indicators [30]. All of the tested scenarios allow exportation of the energy surplus in the form of bioelectricity to the electrical network.

These facts corroborate for shortage in the offer of straw and bagasse of sugarcane as the main lignocellulosic materials for ethanol production in Brazil. Considering this, the production of Brazilian cellulosic ethanol shall be performed not only from the use of straw and bagasse from sugarcane, but also from various sources of lignocellulosic materials. In this case, it could be produced not only by large-scale power plants, but also by power plants of medium and small-scale, such as microdistilleries. In this sense, this review evidences the difficulties encountered in Brazil to produce ethanol from straw and bagasse from sugarcane in industrial scale; it demonstrates some lignocellulosic materials with high potential as a raw material for the production of enzymes, cellulosic ethanol, co-products and by-products in the context of the biorefinery.

2. Microdistillery

2.1. Implantation of microdistillery

Brazil is currently the largest exporter and second largest world producer of ethanol [31], but it has an internal shortage of ethanol, especially during off-season harvest of sugarcane, which provides a decrease in the amount of ethanol present in gasoline and increases the difficulty in acquiring hydrous ethanol, being that these obstacles cause an increase in the value of ethanol. In a country exporter of ethanol, these factors provide disrepute to the market. That way, in order to avoid such facts, it emerges as an alternative, the possibility of producing cellulosic ethanol in microdistilleries, with the implementation this project in large individual estates, agroindustrial cooperatives, urban and family agriculture.

Ethanol production in microdistillery by means of simple structure and economic accessibility, allows the daily production up to 5000 l of ethanol, whereas the for the installation of these microdistilleries it would not need great homogeneous area for its operation, thus allowing its installation in different places and the use of many raw materials, besides allowing the production in places difficult to access, as well as the possibility of consumption in situ [32–34]. These factors related to the size of the installation, location of raw material, infrastructure for production and distribution of the product are interrelated and interdependent, generating substantial impacts about the cost of cellulosic ethanol production [29,34–38]. That way, installation in a site that minimizes the transportation costs of raw materials and the product represents an economy from 15% to 25% of the total production cost of the cellulosic ethanol [37].

Ethanol production in microdistillery still allows a considerable increase in the human development index, as from the improvement in quality of life in rural areas [32]. With income generation (discouraging rural exodus) and absence of social problems related to large-scale production of ethanol [33], it also allows to increase the amount of food produced through the use of the by-products

Table 1
Characteristics of the evaluated scenarios.
Source: [30].

Parameter	Scenario					
	1	1a	2	3	4	5
Ethanol production	X	X	X	X	X	
Cellulosic ethanol production			X	X	X	X
Sell of surplus electricity	X	X	X	X	X	X
Sell of surplus bagasse		X				
Current technology for cellulosic ethanol			X			
2015 Technology for cellulosic ethanol				X	X	X
Pentoses biodigestion			X	X		
Pentoses fermentation					X	X

of microdistillery [39], being an integrated system for food production, energy and environmental services as a promising alternative for rural properties, especially if the aim is related to the energy and environmental performance, compared to the large-scale production of ethanol [33]. This productive concept becomes stronger, in addition to energetic questions, socioenvironmental parameters [33] and the potentialities to neutralize the generated carbon [40]. Thus allowing the production of various co-products and by-products in the context of biorefinery in an efficient manner [33], without necessarily competition with the food production [41].

According to Pereira and Ortega [42] the ethanol production carried out on a large-scale shows renewability of only 30%, besides presenting worries about environmental impacts and high consumption of natural resources. A study carried out by Cavalett et al. [43], corroborates the results obtained by Pereira and Ortega [42], which shows renewability of 64% in ethanol production in microdistillery and makes it more sustainable than ethanol production on a large-scale (renewability of 23%). According to Ortega et al. [44] by using systemic analysis, it becomes possible to analyze the disappearance of economy of scale, while the integrated ecological agricultural system in microdistillery can display optimum economic and environmental performance.

Apart from that, the processing may be advantageous in microdistillery concerned to a large-scale production [22,34]. Ethanol production on a large-scale will reduce the arable land for the cultivation of food [8,42,45], therefore resulting in necessity of careful approach in the large-scale production [41]. Furthermore, the demand for use of lignocelluloses material for bioenergy production may potentiate the pressure on productive systems generating negative impacts on biodiversity [46,47], the expansion of monocultures and chemical products applications [47]. Besides biodiversity, the main negative impacts of biofuels production on a large-scale are the threats to forests; it will increase food prices and competition for the water resources [48].

Successful experiences in the production of ethanol in Brazilian microdistillery as from the sucrose of sugarcane are presented by Moreno and Ortiz [49], such as cooperatives agroindustrial COOP-ERCANA, CRERAL and COOPERBIO. COOPERCANA produces about 2% of the fuel consumed in Rio Grande do Sul and about 2500 ha of cultivation of sugarcane propagated in over 300 properties is distributed in the municipalities of Porto Xavier, Roque Gonzales and Lucena Porto, with production of 9 million liters of ethanol per year [49]. COOPERBIO has a network of 64 municipalities in Rio Grande do Sul and it has developed a program for the production of food and biofuels based on agroecological principles, directed to small family farmers, with daily production capacity of 600 l of ethanol [50].

In comparative terms, in the Nordic countries that are in search of meeting the demand for ethanol, they also bet on the regional production of cellulosic ethanol in microdistillery [34]. For example, biorefinery Borregaard (Sarpsborg, Norway) constitutes one of the most advanced biorefineries in operation, with an experience of over 40 years of operation currently produced as from lignocellulosic material, such as: lignosulphonates, oxylignin sulphonates, vanillin, cellobiose octaacetate, microfibrillar cellulose, speciality cellulose, bioethanol and protein [51]. Kadam et al. [52] reported a biorefinery with the capacity to convert corn stover into ethanol, soluble cellulose and lignin for the production of resin. The biorefinery based on sweet sorghum stem has produced after 54 h, approximately 140 g/L of ethanol, with ethanol yield of 0.49 g/g [53] and sweet sorghum bagasse pretreated with acetic acid was hydrolyzed about 85% of hemicellulose and using as raw material (55 g/L of sugar) has produced 19.21 g/L of total solvent (9.34 g of butanol, 2.5 g of ethanol and 7.36 g of acetone) by *Clostridium acetobutylicum* and the residual

bagasse pretreated was extruded with poly (lactic acid) for the production of bioplastics [53]. According to Mao et al. [54] and Fornell et al. [55] there is technical viability and it is economic in the production of ethanol from Kraft mill. This productive process is also attractive for production in microdistillery [54].

Starting at public–private incentives it is possible to imagine the implantation of thousands of microdistilleries in Brazilian territory, thus providing an increase in the offer of energy, in addition to the incorporation of social gains, environmental and economic to the population. It is emphasized that, from the development of this model, Brazil may contribute to the development of other countries. As an example, African continent presents characteristics similar to Brazil and bioenergy production may permit an increase of food security (increase in food production), investment in technology and infrastructure, recovery of agricultural areas and human resources training [56], configuring itself a region with high productive capacity still unexploited energetically.

2.2. Implantation of microdistillery in the context of biorefinery

The possibility of implantation of thousands of microdistilleries for the production of cellulosic ethanol based on the context the biorefinery will provide a biotechnological gain to Brazil. Biorefinery term was created in 1990 by NREL [57] and according to NREL [58] the biorefinery is an industrial installation that integrates conversion processes and equipment for the production of fuels, power and other chemical products from biomass [35,41,57,59–61].

Biorefinery consists of three main models: the biorefinery based on lignocellulosic raw material (LCF), “whole-crop biorefinery” and green biorefinery [62,63]. In accordance with Uihlein and Schebek [63] the LCF biorefinery is considered the most promising. Recently, it appeared that the biorefinery model based on photosynthetic aquatic biomass [64], beyond the biorefinery model is associated with the urban solid waste of plant origin. Biorefineries are classified into three phases, being the biorefinery of phase 1, limited use of a raw material for production of one product. Biorefinery of phase 2, it uses a raw material and converts it into various products. Biorefinery of phase 3, the most advanced, which aims at the use of various conversion processes in the production of multiple products from various raw materials [65].

Biorefineries can perform interlinked activities that enable the creation of industrial networks, which generally provide social benefits, environmental and economic [41,66], by combining the technologies needed for the processing of biological raw materials, of the intermediate products and finals [62], carried out by thermochemical processes, chemists, biochemicals, physicists or a combination of these processes [41,65], thus forming an industrial symbiosis [66].

Furthermore, biorefineries use resources in more sustainable ways, without producing waste and other environmental pollutants [60,67]. Being of great importance under the vision of a sustainable economy based on biological resources [22,61,63], it allows sustainable economic growth as from insurance resources for industrial production [62–64]. According to Rødsrud et al. [51] the biorefinery operation based on vegetable biomass consists of a challenge, both of the processing viewpoint, and from the market. Highlighting the fact that green industrialization cannot always be clean and sustainable [41].

In this context, the biorefinery can improve their sustainability through diversification of products from biomass. Being the diversity of raw materials and processing technologies the precursors of various industrial combinations, in order to meet different needs, geographic location, economies of scale and national priorities [22,41,64]. Thus, a group of specific technologies

shall be developed in order to convert each fraction more efficiently and generating products of higher added value [22,35,60]. Similarly, the production of multiple products in a certain location, but at different time, may permit the continuous operation of biorefinery [53].

2.3. Production of co-products and by-products of the productive process of cellulosic ethanol

The prospects are optimistic for the production of cellulosic ethanol and its derivatives (co-products and by-products generated and used efficiently) in Europe [68], Malaysia [69], Korea [70], China [71,72], India [73,74], Turkey [75], Canada [76], Ghana [77], Serbia [78], Australia [79], Colombia [80] and Brazil [11].

Some lignocellulosic materials enable the extraction of protein before the pretreatment stage; the protein can be used for protein enrichment in food industry and formulation of nitrogen compounds [81–83].

Based on cellulosic ethanol production, the degradation of lignocellulosic materials promoted by the pretreatment and chemical hydrolysis or enzymatic may result in the release of monomeric sugars, furan derivatives, weak acids, phenolic compounds, lignin and other [41,60,84–86], generating even, by-products originating from industrial process such as carbon dioxide, vinasse and vegetable biomass waste, by-products that enable the production of synthesis gas or biogas, electricity and heat [41,84].

In the conventional power plants for ethanol production, the residual lignin is considered as a low aggregate value and generally burned to provide energy and heat [71]. However, on the concept of biorefinery this lignin must be efficiently integrated into the productive process [87]. According to Ghatak et al. [41] the use of lignin in the non-energetic form can significantly improve the economic and environmental sustainability of biorefineries. In this scenario, the lignin can be converted to synthesis gas [71,88], or degraded into smaller fractions for the production of polyurethane foam, phenolic resins and epoxy, as sources of phenol and ethylene [89–92], adsorbents and carbon precursors [89,93], polymer formulations [94,95] and raw material for numerous aromatic substances of low molecular weight [89,96]. Besides the possibility of producing additive for the use in renewable biofuels from the ozonolysis of lignin [97].

Degradations of cellulose and hemicellulose during the pretreatment could generate compounds that may promote inhibition effects during the stages of enzymatic hydrolysis and fermentation, such as furan derivatives, hydroxymethylfurfural (HMF), product of dehydration of hexoses and furfural, and product of dehydration of pentose. Degradation of HMF releases the levulinic acid and formic acid. Formic acid can also be released by the degradation of furfural. Hydrolysis of acetyl groups linked to sugars generates acetic acid. Phenolic compounds and aldehydes are formed from the degradation of lignin and carbohydrates. Acetic acid, formic acid and levulinic acid are weak acids most common in the lignocellulosic hydrolysates [85,86]. These compounds show the possibility of harnessing, for example, furfural can be hydrolyzed into maleic acid or resin form with addition of urea or phenol [98], HMF can be cleaved in formic acid and levulinic acid, this later can be used as raw material for production of polyesters [99], acetic acid can be used as chemical reagent or in the form of vinegar, besides providing a starting material for the synthesis of various polymers such as vinyl acetate and acetic anhydride, which are traditionally produced as from raw materials at petroleum base [100,101].

Production of ethanol from lignocellulosic materials increases the supply of ethanol and enables the generation of many co-products, such as ethanol vapor in hydrogen production to obtain fuel cells [102,103], ethylene production, ethylene glycol, acetaldehyde, acetate, ethyl acetate, glycols, acrylates, ethyl chloride, butane, propylene and butadiene [99] and the production of ethane resulting from the

dehydration of ethanol, precursor of wide range of products such as polyethylene, polypropylene and polyvinyl chloride (PVC). In Brazil, the production of these co-products from ethanol is due the expanding ethanol production in the country [104]. It can be cited, for example, the use of ethanol in replacement of methanol during the production of biodiesel [105], as well as conversion of ethanol into ethylene for the production of bioplastics, scenarios that contribute to the increasing demand for ethanol. It is emphasized that ethanol used during the stage of transesterification of biodiesel can be obtained from the lignocellulosic residues resulting from the oil extraction during the biodiesel production and directed to the production of cellulosic ethanol [106]; currently, 80% of the biodiesel produced in Brazil uses soybeans as raw material [107]. According to Visser et al. [106] the ethanol demand varies with the lignocellulosic material used, in the case of cottonseed, 470% of ethanol used during the stage of transesterification of biodiesel production can be supplied from the production of ethanol from the waste of cottonseed.

The microbial biomass produced during alcoholic fermentation can be used as a source of protein (single cell protein) and can be incorporated into the feeding [108–110]. Microbial biomass still contains nucleic acids, carbohydrates, compounds of the cell wall, lipids, minerals and vitamins [109].

During the industrial process exists the generation of waste liquids with high organic load and inorganic, for example, vinasse, which has a high efficiency as a fertilizer. According to Silva et al. [111], Bekatorou et al. [112] and Selim et al. [113] is also potentially feasible the use of vinasse in the production of single cell protein by *Saccharomyces cerevisiae*, providing a less toxic effluent [112]. Furthermore, many organic wastes can be used in the microbial protein production, for example, the culture medium containing papaya [114] and the culture medium containing soybean hull [115], made it possible to increase the concentration of protein in *S. cerevisiae* at 20% and 25%, respectively. That way, it strengthens the idea to use several raw materials for the production of protein (single cell protein) in microdistillery.

Fermentation stage produces a flow of high purity CO₂ [116] and stored CO₂, derived from the fermentation it does not need the later treatment [106]. The costs of installation, operation and maintenance for the capture and storage of CO₂ as from the fermentation stage are generally low [106]. This CO₂ generated during the production of ethanol can be used in the production of microbial biomass [71,117] and of synthesis gas, enabling the increase in offer, together with the gasification of residual vegetable biomass of the fermentation process, this latter is rich in CO and H₂, which can be used in the synthesis in any hydrocarbon [41].

This posterior capture and storage of carbon from the residual vegetable biomass of the fermentative process enables real reduction of the global concentration of CO₂ in the atmosphere [116], combined with the capture of carbon emissions from point sources, as one of several necessary strategies for mitigating the greenhouse gases in the atmosphere [118]. It has also become possible the use of non-gasified CO₂ generated in the fermentative process for the production of microalgae, which helps in the cycling of CO₂ and permits the use of this biomass, mainly in the form of lipids for biodiesel production and the use of residual microbial biomass in the production of cellulosic ethanol, acetone and butanol [119].

In relation to the synthesis gas, its fermentation offers a path for the sustainable synthesis of fuel and chemical products with many advantages over the synthesis gas conversion by catalyst generating, mainly, ethanol, hydrogen, acetic acid, butyric acid, butanol, methane, single cell protein and biopolymer [67,120–123]. Such fermentation of the synthesis gas presents a highly biocatalytic conversion efficiency in various biochemical compounds and biofuels [122,124]. For example, butanol is used as raw material for the production of butyl acetate and butyl acrylate, which can be used as fuel additives for improving the gasoline

Table 2
CMCase-producing, xylanase-producing and β -glucosidase-producing microorganism.

Enzymes	Microorganisms	Substrates	Production U/mL	Reference
CMCase	<i>Trichoderma harzianum</i>	Wheat bran	1.64	[131]
	<i>Chaetomium erraticum</i>	Wheat bran	0.04	[132]
	<i>Termitomyces clypeatus</i>	Mustard stalk and straw	2.95	[133]
	<i>Lysinibacillus</i> sp.	Wheat bran	0.43	[134]
	<i>Neosartorya spinosa</i>	Wheat bran	0.11	[134]
Xylanase	<i>Streptomyces flavogriseus</i>	Wheat bran	1.59	[135]
	<i>Streptomyces</i> sp. C-248	Wheat bran	4.48	[135]
	<i>Thermoascus aurantiacus</i>	Corn bran	130	[136]
	<i>Streptomyces</i> sp. C-254	Wheat bran	6.44	[135]
	<i>Aspergillus sydowii</i>	Wheat bran	1.10	[137]
	<i>Termitomyces clypeatus</i>	Mustard stalk and straw	14.12	[133]
	<i>Lysinibacillus</i> sp.	Wheat bran	5.40	[134]
	<i>Neosartorya spinosa</i>	Wheat bran	2.10	[134]
	<i>Chaetomium globosum</i>	Delignified palm fibers	9.80	[138]
	<i>Termitomyces clypeatus</i>	Mustard stalk and straw	2.30	[133]
β -glucosidase	<i>Aspergillus niger</i>	Wheat bran	2.84	[74]
	<i>Trichoderma reesei</i>	Wheat bran	0.22	[74]
	<i>Thermoascus aurantiacus</i>	Wheat bran	7.0	[139]

octane index [125]. According to Mohammadi et al. [122] and Munasinghe and Khanal [67], several microorganisms are capable of fermenting synthesis gas in biofuels, such as *Rhodospirillum rubrum*, *Rhodobacter sphaeroides*, *Methanobacterium thermoautotrophicum*, *Methanosarcina barkeri*, *Clostridium thermoaceticum*, *Rhodopseudomonas gelatinosa*, *Bacillus smithii* ERIH2, *Rhodopseudomonas palustris* P4 and *Acetobacterium kivui*. Experiments carried out by Mohammadi et al. [126] aiming the production of bioproducts starting from the synthesis gas, containing 55% of CO, 20% of H₂, 10% of CO₂ and 15% of Ar by *Clostridium ljungdahlii* in continuous stirred bioreactor, at 37 °C and agitation of 500 rpm, resulted in 2.34 g/L of dry weight, 96% conversion of CO, concentrations of 6.50 g/L of ethanol and 5.43 g/L of acetate.

Microbial modification, in order to use the greenhouse gases, mainly CO₂, as a substrate for biofuel production becomes reality [127]. Li et al. [76] have modified *Ralstonia eutropha* for use of CO₂ in the production of isobutanol and 3-methyl-1-butanol, resulting in 140 mg/L of the branched chain alcohols. Lu et al. [128] have modified *R. eutropha*, resulting in 10 mg/L of isobutanol from fructose; the experiment carried out with nitrogen limitation resulted in the production of 170 mg/L of isobutanol. Residual vegetable biomass of the productive process of cellulosic ethanol also makes possible the production of biogas, produced by the digestion of this organic material [129].

2.4. Enzymes

Steps during enzyme production as well as enzymatic hydrolysis need some attention when the production of cellulosic ethanol in microdistillery is intended, because these steps may make ethanol production unfeasible economically, in particular on this scale. Therefore, there is an option of using commercial enzymes or enzymes produced locally with regional substrate. The possibility of local production (in situ) of the enzymes starting from by-products and agroindustrial wastes becomes a promising alternative [130], mainly for the production of enzymes in semi-solid bioprocess (Table 2). Production of these enzymes from agroindustrial and urban wastes provides sustainability and profitability for the productive chain, in addition to reducing the carbon emissions into the atmosphere and the possibility of using lignocellulosic materials of low cost [130].

Production of enzyme in situ allows the use of crude enzymatic extract, without the need to concentrate and purify it. For instance, the production of β -glucosidase by *Lichthemia ramosa* using wheat bran, with production of 15 U/mL of β -glucosidase [140] and the possibility

of in situ production and application of non-concentrated form was carried out by Gonçalves et al. [141], as from the synergistic mixture of the hemicellulolytic and cellulolytic enzymes produced by *Trichoderma reesei* and *L. ramosa* in the hydrolysis of mature coconut shell pretreated with hydrogen peroxide and sodium hydroxide, resulted in 82.3% conversion of reducing sugars and 83.9% of glucose, after 96 h of hydrolysis.

According to Castro and Castro [130] Brazil is one the biggest producers of biofuels of the world, having in the biodiversity and environmental characteristics for possibility of making it a great generator of biotechnological products, such as enzymes for bioenergy industry. The results obtained by Castro and Castro [130] indicate the possibility of the Brazilian production of concentrated enzymatic from agroindustrial wastes based on amylases, cellulases, xylanases and lipases estimated at 3.1×10^7 , 3.2×10^7 , 3.1×10^8 e 2.9×10^9 t, respectively.

Besides the search for improving production stages to obtain efficient enzymes and enzymatic hydrolysis, it also becomes important to perform recovery of the enzymes after the fermentation process [142]. Eckard et al. [143] carried out enzymatic recycling in simultaneous saccharification and fermentation (SSF) and separate hydrolysis and fermentation (SHF) of corn stover evaluating the use of novel enzyme stabilizers of casein, Tween 20 and polymeric micelles of polyethylene glycol (PEG)-casein and PEG-Tween20. With the addition of these compounds significant recoveries of enzymatic activity were obtained. According to Rodrigues et al. [144] the recovery of cellulases can be carried out by alkaline washing of the lignin and cellulose residual. Using this simple alkaline washing the enzyme recovery, showed more than 60% of enzymatic activity in synthetic substrate (4-methylumbelliferyl- β -D-cellobioside), therefore, this technique consists of a promising strategy for recycling enzymatic, that can allow a simple implement on an industrial scale, in addition to being effective and economical [144], providing a reduction in production costs of cellulosic ethanol [145].

3. Sources of biomass

3.1. Terrestrial photosynthetic biomass

Terrestrial biomass production consists of approximately 100 billion tons of dry organic matter [64,146]. Part of this biomass is used (1.25%) and the remainder is recycled by the biotic system. Therefore, part of this biomass can be used as a raw material for the production of many products [64].

Table 3
Brazilian harvest in 2011.
Source: [159,161].

Vegetable	Production (t)	Estimation of vegetable biomass left on the field (t) ^a
Beans (grain)	3,550,107	1,171,535
Upland cotton (seed)	5,059,618	1,669,674
Groundnut (in shell)	275,460	90,902
Rice (paddy)	13,456,369	4,440,602
Oats (grain)	340,995	112,528
Potato	3,943,146	1,301,238
Onion	1,402,758	462,910
Corn (grain)	56,099,662	18,512,888
Soybeans (grain)	74,829,383	24,693,696
Sorghum (grain)	1,941,267	640,618
Wheat (grain)	5,646,166	1,863,235
Triticale (grain)	147,078	48,536
Garlic	8962	2957
Cactus	60,000	
Sunflower (grain)	78,690	25,968
Fruit	Production (t)	Estimate of bagasse (t) ^b
Coconut	1,899,355	949,678
Cashew nut	229,319	114,660
Cocoa	248,165	124,083

^a 33% of vegetable.

^b 50% of fruit.

Biofuel production generally uses substrates derived from food crops (e.g. corn in the United States), which have afforded polemic between the destination of this vegetable biomass for food or biofuel production [7,9,41,147–149]. Therefore, there is a great research effort for obtaining sustainable sources of biomass for biofuels production, mainly ethanol. Due to the risk of food insecurity, this question should be carefully issued, for example, the Chinese government has laws that discourage use of feed raw material for ethanol production [72].

As an alternative to food sources arises the use of agroindustrial and urban wastes [7,150–153] and plants cultivated in inhospitable areas [154–157]. This scenario enables aggregate value to these biomasses, allowing increased production of fuel without the need for expansion in the use of agricultural lands, still providing subsidies to meet the targets established in the diplomatic accords such as the Montreal Protocol in 1987, Kyoto in 1997 and Copenhagen in 2009.

The advancement in the use of lignocellulosic materials increases the concern to maintain the natural forests, mainly in tropical countries [158]. Since the conservation of forests consists in a precondition for the sustainable production of cellulosic ethanol [158] Popp et al. [158] results obtained by simulation indicate the need to increase the yield of energy crops and conservation of forests. Therefore, the demand for cellulosic bioenergy will put additional pressure about the agricultural productive system and it will provide increase in the level of CO₂ [158]. Everything reinforces the importance of the use of agroindustrial and urban wastes, beyond the cultivated plants for the production of ethanol and enzymes (Tables 2 and 3). The Brazilian harvest in 2011, shown in Table 3, evidences the potential of lignocellulosic material left on the field, based on methods to calculate existing biomass [159]. Some of these lignocellulosic materials exhibit little information in the literature, as for example, the lignocellulosic materials discussed below.

3.2. Coconut bagasse

Coconut trees are ideal for cultivation in a humid tropical climate, they thrive in poor soils, sandy and tolerate short period exposure to saline water and are distributed in more than 200 countries [160]. According to FAO [160] the world production of coconut in 2009 was about 55 million tons, with highlighted production for the Philippines (36%), Indonesia (28%) and India (20%). Brazil is the fourth largest world producer of coconut, with production of about 3 million tons [161]. In South America, Brazil is responsible for more than 80% of production, being the North-east Region responsible for 82.28% of the total planted area and 69.25% of the total value of the coconut produced in 2009 [161].

The search for adequate food as an alternative to improve the health generates numerous food products produced at coconut base, such as coconut water fresh and powder, coconut milk, grated coconut and coconut oil. Due to this advancement, the productive coconut chain does not possess the correct destination of their agroindustrial and urban wastes. In seaside towns, mainly in areas with tourist vocation, green coconut shells account for up to 80% of total volume of solid wastes collected on the waterfront and due to its high concentration of lignin, hemicellulose and cellulose have slow decomposition, being 85% of the weight of the fruit made up of lignocellulosic material [12]. The discard of the green coconut shells in garbage dumps, sanitary landfills, patios processing industries and common areas of public use and result in environmental pollution [162].

Bagasse from mature coconut when processed can result in a long fiber, generally employed in the automotive industry [163], but generates waste material. It is estimated that for every kilogram of produced fiber there are generated about 2 kg of powder and short fibers [164]. Composition of green coconut fiber has lignin (43.14%), cellulose (45.93%) and ash (3.60%), diameter of natural maximum of 495 µm and minimum of 69 µm [165]. Coconut husk has lignin (29.79%), cellulose (39.31%), hemicellulose (16.15%) extractives (2.48%) and ash (3.19%) [166].

Gonçalves et al. [167] reported that composition of the mature coconut fibre has lignin (26.69%), cellulose (31.60%), hemicellulose (26.31%), extractives (5.44%) and ash (3.31%). Green coconut shell has lignin (26.88%), cellulose (32.88%), hemicellulose (26.50%), extractives (3.27%) and ash (4.34%). Mature coconut shell has lignin (33.15%), cellulose (30.47%) hemicellulose (25.42%), extractives (2.71%) and ash (4.84%). Coconuts were pretreated by sequential alkaline hydrogen peroxide–sodium hydroxide process, resulting in composition of mature coconut fibre which contains lignin (8.92%), cellulose (51.80%), hemicellulose (25.81%), extractable (0.05%) and ash (2.98%). Green coconut shell has lignin (7.89%), cellulose (54.14%), hemicellulose (28.36%), extractable (0.26%) and ash (1.07%). Mature coconut shell has lignin (10.22%), cellulose (53.88%) hemicellulose (23.02%), extractable (0.53%) and ash (3.80%) [167].

These results were further confirmed by the corresponding glucose conversion yields in the enzymatic hydrolysis of 70.20%, 76.21% and 74.50% for green coconut shell, mature coconut fibre and mature coconut shell, respectively. Subsequently, the comparison between Simultaneous saccharification and fermentation (SSF) and semi-simultaneous saccharification and fermentation (SSSF) using *S. cerevisiae*, *Pichia stipitis*, *Zymomonas mobilis* and pretreated mature coconut fibre was done, being shown that a short presaccharification step at 50 °C for 8 h in the SSSF had a positive effect on the overall ethanol yield, with an increase of 79.27–84.64% to 85.04–89.15% [167].

These lignocellulosic materials have conditions to be employed as a substrate for the production of bioproducts, for example, ethanol. Coconut husk pretreated with sodium hydroxide was used in the process of simultaneous saccharification and fermentation by *S. cerevisiae*, resulting in a yield of ethanol of 0.4 g/g [166].

Table 4
Composition of halophytic biomass.
Source: [173].

<i>Aeluropus lagopoides</i>	26.6	29.3	7.6
<i>Aerva javanica</i>	15.6	13.3	6.3
<i>Arthrocnemum indicum</i>	11.3	13.0	7.0
<i>Calotropis procera</i>	12.3	11.0	5.0
<i>Cenchrus ciliaris</i>	22.6	23.2	7.0
<i>Chloris barbata</i>	25.3	23.0	8.3
<i>Desmostachya bipinnata</i>	26.6	24.7	6.6
<i>Dichanthium annulatum</i>	19.0	24.3	7.0
<i>Eleusine indica</i>	22.0	29.6	7.0
<i>Halopyrum mucronatum</i>	37.0	28.6	5.0
<i>Ipomea pescaprae</i>	12.6	17.0	5.3
<i>Lasiurus scindicus</i>	24.6	29.6	6.0
<i>Panicum turgidum</i>	28.0	27.9	6.0
<i>Paspalum paspaloides</i>	20.3	32.0	2.3
<i>Phragmites karka</i>	26.0	29.0	10.3
<i>Salsola imbricata</i>	9.0	18.3	2.6
<i>Salvadora persica</i>	22.0	13.3	7.0
<i>Sporobolus ioclados</i>	15.3	30.6	2.0
<i>Suaeda fruticosa</i>	8.6	21.0	4.6
<i>Suaeda monoica</i>	10.6	11.3	2.3
<i>Tamarix indica</i>	12.2	24.6	3.3
<i>Typha domingensis</i>	26.3	38.6	4.6
<i>Urochondra setulosa</i>	25.3	25.0	6.3

Some countries in Asia and Oceania utilize coconut oil as biofuel in diesel engines [168,169], usually mixed with the kerosene and diesel; however, to certain conditions it fully replaces the diesel [168], it is used in the transport sector [168–170] and electric power generation [168] as well as in the transport sector, mainly in the Thailand and Philippines [169]. Some Pacific Island Country such as Fiji Islands, Kiribati, Marshall Islands, Papua New Guinea, Samoa, Solomon Islands, Tuvalu and Vanuatu also use coconut oil as an energetic strategy to decrease fuel imports, besides taking advantage of the abundance of coconut in the region [169]. This increase in the use of biofuels in Pacific island countries also contributes to the reduction of greenhouse gases, employment creation and strengthening of the economy [170]. According to Tan et al. [171] the use of coconut oil reduces the CO₂ emissions from 80.8% to 109.3%, when compared to the diesel. The production of coconut oil is carried out on a small industrial scale situated in the rural properties [168–170], therefore, the integration of the productive system of coconut oil coupled to microdistillery for production of cellulosic ethanol and it becomes a promising alternative.

3.3. Cactus

Cactus is a plant adequate for bioenergy production and is commonly cultivated in arid environments [154]. These characteristics allow its cultivation in areas with low rainfall levels, mainly located on the African continent and countries such as Mexico, Chile, Brazil, Australia, China, India and United States. That is, it can be cultivated in inappropriate areas for cultivation of plants intended for conventional foods [155]. In Brazil, production of cactus was 60 thousand tons in 2009, concentrated in the North-east Region and destined integrally for animal feed [161]. Gonçalves et al. [167] reported that cactus was pretreated by sequential alkaline hydrogen peroxide–sodium hydroxide process, with initial composition of cactus which has lignin (20.90%), cellulose (38.33%) hemicellulose (22.19%), extractable (5.82%) and ash (6.64%). Resulting in the composition of pretreated cactus was lignin (9.45%), cellulose (54.91%) hemicellulose (17.65%), extractable (0.48%) and ash (8.77%). Resulted in the enzymatic hydrolysis in 96 h was 68.44%.

A study of ethanol production using cactus was carried out by Retamal et al. [172], in cladodes of cactus pretreated with

perchloric acid, fermented by *S. cerevisiae* and resulted in 9 L of ethanol from 100 kg cladodes.

3.4. Halophyte

Halophyte consists of a category of native plants typical from saline soils, capable of growing in environments with elevated concentration of salt and irrigated with saline water or marine water, without any serious negative effect on growth [156]. These are commonly found in semi-deserts saline or alkaline, saline, steppes and seacoasts [157]. This is considered one of the most productive areas in terms of bioenergetic potential [157], besides no use of the appropriate area for cultivation of biomass intended for food [155]. The Brazil presents no reported use of halophytes in bioprocesses, being an energetic alternative unexploited. However, some countries already use it, as referenced below.

According to Abideen et al. [173] the species such as *Halopyrum mucronatum*, *Desmostachya bipinnata*, *Phragmites karka*, *Typha domingensis* and *Panicum turgidum* found in the coastal region of Pakistan possess high potential as a source of biomass for ethanol production. These perennial grasses are tolerant to salt, possess high growth rates and present the composition between 26% and 37% of cellulose, between 24% and 38% of hemicellulose and less than 10% of lignin (Table 4), in addition to extractives between 1.00% and 2.51%, crude protein between 3.20% and 8.27% and crude fiber between 30.89% and 58.53% [173]. Therefore, the cultivation of halophytes becomes an excellent alternative of biomass that can compete favorably with other conventional sources for biofuels production. Highlighting that they are not inserted into the human food chain and have lower production cost [173] with a yield of *Tamarix* sp. 17–20 t/hm², *Panicum* sp. 9.4–25 t/hm² and *Suaeda* sp. 1.3–2.4 t/hm². Considering that 43% of the terrestrial climate are arid or semi-arid and 98% of the global water offering is seawater [174], 800 million hectares are affected by the water salinity [175] and of the 230 million hectares of irrigated lands, 45 million have become saline [176]; the cultivation of halophyte can be an excellent alternative as a raw material for the production of cellulosic ethanol.

Díaz et al. [177] carried out the cultivations of *Salicornia bigelovii*, *Atriplex lentiformis*, *Distichlis spicata*, *Spartina gracilis*, *Allenrolfea occidentalis* and *Bassia hyssopifolia* irrigated with saline water drainage and these species were seen as a good alternative of raw material for bioenergy production, in addition to cultures with high potential for the use of water of saline drainage.

The global search in generating energetic source based on the opportunity to offer a large amount of energy combined with quality energetic becomes an obstacle in the contemporary society. As an example, China has energetic bottleneck limiting the socioenvironmental development. According to Xian-Zhao et al. [157] it becomes impossible to extensively cultivate vegetable biomass energetic in arable land in China, because this country needs these lands to guarantee food safety of its citizens. Therefore, the cultivation of halophytes in coastal saline land arises as an important source of biomass to produce bioenergy [157].

3.5. Urban solid waste of plant origin

Management and the destination of inadequate urban solid waste cause socioenvironmental impacts, such as soil degradation, damage of water bodies and fountains, intensifying floods, besides contributing to air pollution and the proliferation of vectors of sanitary importance [178]. The adequate destination of urban solid waste for the production of bioproducts minimizes such problems, because according to Hussin et al. [153] the urban solid waste contains significant amounts of sugars from plant origin. Utilization of these wastes relieves sanitary landfills, but the increase in

the generation of these urban municipal solid wastes is motivated by the socioeconomic development of citizens, whose consumption increases day after day. Therefore, it increases the offering of substrate for the production of ethanol, mainly using urban solid waste from plant origin [7,153].

Brazil generates 597 million tons per year of organic waste [12]. According to CETESB [179] it is estimated that in a city with a population above 500 thousand inhabitants, an individual daily production of solid waste consists of 0.7 kg, part of this amount may be destined as a substrate for production of by-product. Approximately 60% of urban wastes are formed by organic compounds [180]. Annual pruning of trees arising from the maintenance of the distribution networks of electrical energy carried out in the municipalities generate huge amount of organic matter, also being a sustainable alternative to its disposal as a substrate for ethanol production. For example, in Singapore, this material is the most common in municipal solid waste; in 2008 it was generated over 229,300 t, which showed the following average compositions: 34.5% of cellulose, 28.6% of hemicellulose and 36.0% of lignin [181]. The harnessing system of urban solid waste plant origin in Brazil is deficient, not taking an advantage of this potential energetic, which commonly accumulates in sanitary landfills, being one of the alternatives to its use in the production of cellulosic ethanol.

Shi et al. [150] have analyzed 173 countries and concluded that 82.9 billion gallons of cellulosic ethanol can be produced from waste papers, thus allowing the replacement of 5.36% global consumption of gasoline and significant decrease of greenhouse gases emissions. Champagne and Li [151] carried out enzymatic hydrolysis in lignocellulosic materials contained in urban wastewater, waste separated by primary residue decomposition and anaerobically digested biosolids, which have undergone pretreatment acid and alkaline. Best results were obtained from the primary residues hydrolyzed at 40 °C and an enzymatic load of 800 U/g, for 24 h, with 54.2% of the material pretreated and converted into reducing sugars [151]. These biosolids and waste sludge contain large amounts of lignocellulosic materials, polysaccharides and proteins; the conversion of these residues into aggregated value products can consist of an attractive alternative [151].

Yang et al. [182] have estimated that the total weight (wet basis) of urban and rural domestic waste in China are about 500 million tons each year. From the recycling of urban waste, 2.19 million tons of ethanol would be produced from toilet paper.

According Jensen et al. [152] the use of enzymes during liquefaction of organic material of plant origin, contained in urban solid waste showed significant results, thus allowing efficient recovery of the sugars contained in these wastes. In Canada it was estimated the possibility of production of 22.6 million tons of sugar based on the amount produced from livestock manures, municipal biosolids and sludge [183–185]. The use of urban solid waste contributes to the mitigation of emissions of greenhouse gases, recycling of nutrients, reduces the air potential contaminant, water and soil, broadens the diversity of source materials emerging for industrial bioproducts and reduces the pressure on non-renewable resources, such as petroleum [151].

According to Li et al. [76] 1 t urban solid waste has 45 kg of metal, 65 kg of glass, 90 kg of plastic, 600 kg of lignocellulosic materials and 200 kg of other elements. Lignocellulosic materials have the following composition: 65% of cellulose and hemicellulose, 10% of lignin and 20% of inorganic compounds. Enzymatic hydrolysis of this material resulted in a conversion of 53% of reducing sugars. These results indicate that 152 L of ethanol can be obtained from 1 t of urban solid waste [76].

Schmitt et al. [186] have analyzed the production of ethanol from three residues from lignocellulosic materials: urban solid

waste, paper waste and organic waste garden pretreated by dilute sulfuric acid. The results obtained during the enzymatic hydrolysis showed high yields of conversion in the three residues. Fermentations of hydrolysates were carried out by *Rhodotorula mucilaginosa* and have resulted in elevated yields of ethanol, about 100% of theoretical ethanol [186].

Kemppainen et al. [187] have used fiber and sludge waste recovered from the paper productive process; these lignocellulosic materials showed conversion of 75% of reducing sugar and fermentative yield of 84%. In this scenario, 1 t of dry lignocellulosic materials can produce 170 kg of ethanol, 310 kg of biogas, 360 kg of residual sludge and 170 kg of CO₂ [187].

4. Biotechnological advances strategic

4.1. Alcoholic fermentation of mixed sugars

Hydrolysates of terrestrial lignocellulosic materials can present mixed sugars, for example: glucose, cellobiose, xylose, arabinose and galactose. Therefore, the development of a microorganism capable of fermenting simultaneously different sugars present in these hydrolysates becomes a fundamental aspect in the implantation of conversion process profitable for the production of biofuels [190]. In this scenario, it gains importance for the use of Synthetic Biology, Genetic, Metabolic and Evolutive Engineering for the development of efficient and robust microorganisms to be used in the process of simultaneous saccharification and fermentation (SSF), semi-simultaneous saccharification and fermentation (SSSF) and consolidated bioprocessing (CBP) [191–194]; prospection also consists of an alternative for these industrially viable microorganisms [195,196]. Such microorganisms are expected to increase the efficiency of fermentation of hydrolysates containing hexoses and pentoses; it should be resistant to high concentrations of sugars, the presence of inhibitory compounds generated during the pretreatment and fermentation, as well as it must tolerate the ethanol itself, besides to ferment the hydrolyzate without purification and endure high concentration of solid [191]. Although numerous approaches to overcoming the glucose repression are mentioned in the literature, none of them allows efficient co-fermentation of glucose and other sugars, with conversions similar to fermentation of glucose [190].

Microbial fermentation of cellobiose and xylose will contribute to the production of cellulosic ethanol commercially viable [72,190], however, without microbial modification it becomes difficult to obtain this ideal scenario. An exception consists of the *Flammulina velutipes*, which has the capacity to convert glucose, xylose and cellobiose into ethanol [197,198]. As reported by Ha et al. [199] the *S. cerevisiae* may not use cellobiose directly, but can be modified to ferment cellobiose through the simultaneous introduction of cellodextrin (CDT-1) transporter and intracellular β -glucosidase gene (GH1-1) of *Neurospora crassa*. These authors reported that modified strain of *S. cerevisiae* expresses a putative transporter gene of hexose HXT2.4 of *Scheffersomyces stipitis*. Other modifications were carried out in this recombinant strain of *S. cerevisiae* and resulted in the mutant HXT2.4 (A291D), which resulted in the consumption of 75 g/L of cellobiose and production of 32 g/L of ethanol in 36 h, with a ethanol yield of 0.43 g/g [199]. Guo et al. [200] have modified *S. cerevisiae* with the insertion of the gene BGL1 of *Saccharomycopsis fibuligera*, resulting in consumption of 5.2 g/L of cellobiose and production of 2.3 g/L of ethanol, in 48 h. Ethanol production by recombinant yeast pYBGA1, cultivated in 100 g/L of cellobiose, in 96 h, resulted in a conversion rate of 85% ethanol [201].

4.2. Consolidated bioprocessing (CBP)

CBP consists of the simultaneous realization of three main biological processes: enzymes production (cellulases and hemicellulases), hydrolysis of cellulose and hemicellulose into monomeric sugars, fermentation of hexose and pentose [22,202,203]. Therefore, CBP has an important advantage in the industrial process, with the elimination of isolated stage of enzyme production [202–204]. In this scenario, engineering of cellular surface plays a key role, where it is possible to hydrolyze the lignocellulosic materials, providing the production of several cellulolytic and hemicellulolytic enzymes in the cellular surface microbial [204], beyond the elimination of stage of purification and enzymatic concentration [202,203]. Furthermore, the possibility to obtain higher conversion rates provides a volume reduction in the reactor, consequently, decreasing the capital investment [22]. Moreover, CBP consists of one of the principal routes to reduce the cost and increase the efficiency of cellulosic ethanol production, when compared with the other productive processes [22,203–205].

However, one of the main bottlenecks of the CBP consists of the difference in optimum temperature between enzymatic hydrolysis and fermentation; in this case, the application of thermotolerant strains in CBP will allow to win this bottleneck, making possible the realization of enzymatic hydrolysis and fermentation at elevated temperature [204]. Another bottleneck of CBP is related to the use of pentose, mainly of xylose, by showing lower conversion into ethanol and longer duration of fermentation [206]; finding solutions to these questions it will lead to higher profitability in the production of ethanol from lignocellulosic materials [191,206].

There are two main avenues for the production of strains to CBP. The category CBP I, aims to modify a microorganism producer of cellulases and hemicellulases, making it also a producer of ethanol, while the category CBP II, aims to modify a producer of ethanol, making it also a producer of cellulases and hemicellulases [22,205]. The researchers, in its majority, address the CBP category II [204,207,208].

Fujita et al. [209] carried out the ethanol production from cellulose pretreated with phosphoric acid, without the addition of enzymes using recombinant strain *S. cerevisiae* expressing genes of *T. reesei* (EGII e CBHII) and *A. aculeatus* BGL1 on the cell surface. This strategy resulted in ethanol yield of 0.45 g/g. A diploid strain of recombinant yeast for the production of cellulolytic enzymes produced 7.5 g/L of ethanol from 100 g/L of rice straw hydrothermally pretreated, without addition of exogenous enzyme [210]. Wang et al. [211] carried out ethanol production by *S. cerevisiae* recombinant for expression of β -glucosidase in corn cob pretreated, resulting in 77.7% of theoretical ethanol, with ethanol production of 33.1 g/L.

Some filamentous fungi produce a great repertoire of enzymes for the degradation of lignocellulosic materials assimilating the sugars present in lignocellulosic materials and converting them into ethanol, that way, these filamentous fungus naturally possess all of the metabolic pathways for conversion of lignocellulosic materials into ethanol [205]. Concentration of ethanol produced by filamentous fungus is surprisingly high for microorganisms traditionally considered non-fermentative, however, are still low for industrial production [212], formations show by-products and low rates of fermentation [203]. The research using filamentous fungus has been stimulated by attributes that make them attractive for industrial ethanol production [212,213]. Limited capacity of yeast and bacteria in enzymes production in quantity and quality to degrade lignocellulosic materials, can make filamentous fungus a promising alternative as a candidate for CBP [203], such as *Monilia*, *Fusarium*, *Rhizopus*, *Paecilomyces*, *Aspergillus*, *Mucor*, *Neocallimastix*, *Neurospora*, *Trametes* and *Trichoderma*

[212,214–222], mainly the *Aspergillus* and *Trichoderma*, because they are the filamentous fungi which are most studied and have their enzymes commercially available, inclusive, the latter has its genome sequenced.

Skory et al. [212] carried out the isolation of 19 strains of *Aspergillus* and 10 strains of *Rhizopus* that showed the ability to ferment sugars into ethanol (glucose, xylose, arabinose, cellulose, oat-spelt xylan, corn fiber and corn germ pressing). Of these microorganisms tested, three strains of *Rhizopus* have produced more than 31 g/L ethanol under anaerobic stress, in 72 h of cultivation [212]. Stevenson and Weimer [217] have cultivated *Trichoderma* sp. in culture medium, the base of glucose and resulted in 5.0 g/L of ethanol.

SSF using *Fusarium oxysporum* to convert cellulose resulted in ethanol yield of 0.35 g/g [214]. According to Ruiz et al. [223] the *F. oxysporum* possesses capacity to convert glucose and xylose into ethanol, with an ethanol yield of 0.38 and 0.25 g/g, respectively. The use of a mixture of glucose (50%) and xylose (50%) resulted in sequential consumption of the two substrates with an ethanol yield of 0.28 g/g. Furthermore, *F. oxysporum* has a capacity to produce ethanol from lignocellulosic material. Maehara et al. [198] carried out fermentation with *Flammulina velutipes* in the cellulose of sugarcane bagasse, with an ethanol yield of 0.05 g/g (10% theoretical ethanol) without addition of enzymes. However, when 9.0 mg/g of commercial cellulase was added in the cultivation, an ethanol yield of 0.36 g/g (69.6% theoretical ethanol) was obtained.

Genetic modification of the filamentous fungus becomes reality and provides a better adequacy for CBP. As an example, modification of the hexose transporter (Hxt) of *F. oxysporum* has allowed high affinity of the glucose transport and an increasing of 33% in the ethanol production [224].

4.3. Others advances

The possibility of performing the biological process with two or more microorganisms provides higher harnessing of lignocellulosic materials in bioproducts. As an example, the production of hydrogen and ethanol by the mixed microflora where seeds pretreated by heat, in a concentration of 80 g/L, at 50 °C and pH 6 can be considered, which resulted in the productivity and hydrogen yield of 7.9 mmol H₂/L/d and 0.40 mmol H₂/g-COD, respectively, productivity and ethanol yield of 0.22 g EtOH/L/d and 3050 mg COD/L, respectively. Bioenergy yield was 41 J/g, obtained from the 21% and 79% of hydrogen and ethanol, respectively [191].

Use of metagenomics technology also arises as a powerful tool for prospection for new enzymes and insertion of special characteristics for the production of biofuels in industrial scale [45].

Another tendency consists of the genetic manipulation to facilitate the degradation of lignocellulosic material. According to Wang et al. [225] the reduction of the amount of lignin present in the transgenic lineages is important in the use of this biomass in the production of cellulosic ethanol. Reduction of lignin in 10% did not alter the growth rate and biomass yield of transgenic of *Populus tomentosa*. Results obtained by Wang et al. [211], indicate that the lignin modification may facilitate the enzymatic hydrolysis stage. In this sense, there was renewed interest in the energy cane as raw material for cellulosic ethanol production, being an important alternative for production of biomass modified, which instead accumulates high levels of total soluble sugars; energy cane consists of a vegetable with high fiber content and elevated biomass productivity, these desired characteristics are obtained by genetic modification and retrocrossing [226].

The possibility of performing the fermentative process under low temperature has been reported by Tsuji et al. [227] who carried out the SSF with *Mrakia blollopis* in cultivation condition at

10 °C. SSF converted paper filter (5% w/v), *Japanese cedar* and eucalyptus (10% w/v) in 12.2 g/L, 12.5 g/L and 7.2 g/L of ethanol, respectively. On the addition of 1% (v/v) of tween, 80 resulted in higher ethanol concentration.

5. Disadvantages, difficulties, challenges and perspectives in the production of cellulosic ethanol

The ethanol production from waste from sugarcane mitigates the use of food sources [5–10], not causing an increase in food prices, besides to providing increased availability of ethanol. Furthermore, increase in the quality of life of Brazilian was influenced by proportionate contributions by the sugar-energetic power plants (since the implementation of the Proalcool Project in 1975), in this way, ethanol produced from residues of the sugarcane can provide the same way. The GranBio power plant installed in São Miguel dos Campos, Alagoas (using bagasse and straw) and Raizen in Piracicaba, São Paulo (using bagasse) shows optimistic projections, evidencing economically viable production of cellulosic ethanol [228]. Thus, GranBio projected the value of cellulosic ethanol in 20%, less than the value of ethanol currently produced in the sugar-energetic power plants and producing the world's cleanest fuel at commercial scale [228–230]. The increase in the availability of ethanol will be easily consumed by national and international markets by contributing to sustainable development and will strengthen this consumption from 2016 by the UN Climate Change Conference (COP-16, Paris), that signals to mitigating emissions of greenhouse gases from the understanding between the major world leaders, especially of the USA (Barack Obama) and China (Xi Jinping) [231].

In this sense, the use of wastes from sugarcane for ethanol production allows addition value to these biomasses, without the need of expansion in the use of agricultural land for biomass production, besides ethanol production also enables employment generation, sugar-energetic power plants operate all months of the year, energy self-sufficiency of the region, increasing vegetal productivity using the treated vinasse [81,232], mitigating the effects of fossil fuels on human health (reduction in the emission of greenhouse gases and fine particles emitted in the air, reducing the quantity of fertilizers and pesticides dumped into rivers and lakes) [40,233]. Beyond the money with the sale of straw, producers of sugarcane see other advantages to partially remove the material, such as a reduction in the level of infestation by insects and lower risk of fires in the area [228].

The Raizen had financial aid from the BNDES of US\$ 85 million and the Granbio had financial aid from the BNDES of US\$ 150 million and the BNB financed machinery and equipment. In this sense, the two sugar-energetic power plants of commercial scale in Brazil had public-private financing and federal and state tax incentives for their functioning [228,234,235].

The sugar-energetic power plants sector in Brazil had some incentives, such as the Commerzbank, Inter-American Development Bank and Banco Pine S.A. closed US\$ 115 million syndicated A/B loan to expand access to financing for environmentally sustainable projects. According to the BNDES, the sugar-energetic power plants had a demand of US\$ 1.7 billion, being US\$ 1 billion have already been approved [234,235]. Credits of BNDES for the renewal of sugarcane plantations in 2013/2014 were US\$ 2 billion [234,235]. Thus, BNDES released US\$ 3 billion to the sugar and ethanol sector in 2013. Between the lines of credit for working capital and marketing, the National Program of Support to the Middle Rural Producer (Pronamp) reached a total loan of US\$ 2.1 billion in 2013. The agribusiness portfolio of the Banco do Brasil reached US\$ 50 billion, being a Family Farming Program (Pronaf) had a credit of US\$ 10 billion in 2014 [236]. The Banco do Brasil

also has a significant participation in the Pronamp with US\$ 5 billion and the low carbon agriculture program with US\$ 1 billion [228,234,235].

The available credit line for the implementation and adaptation of microdistilleries in Brazil is the Pronaf (US\$ 10 billion in 2014). A great technological incentive conducted by the Brazilian Agricultural Research Corporation (Embrapa) was of the microdistilleries installations in the Piauí in 2014. Indicating the possibility of future installations of microdistilleries for the production of cellulosic ethanol [235].

However, not all sugar-energetic power plants have such financing and public incentives. In addition to the sugar-energetic power plants, also needs aid the entire production chain of cellulosic ethanol. But, according to Peng [233] the absence of financial aid is not exclusive to Brazil and mentions the example of China, the Government policies have had a significant impact, discouraging or encouraging development of bioenergy in the country. Furthermore, there are still numerous difficulties for implementations of microdistilleries, being necessary the support of the government policy with financing lines (BNDES and Pronamp), incentives (minimum price guarantee of cellulosic ethanol, increasing in the proportion of ethanol into gasoline, gasoline price agreement next to the world price and use of ethanol 80% (wt)) and tax exemptions (subsidies and commercialization in situ) [228,234–236].

The first company to reach commercial scale production of cellulosic ethanol was Beta Renewables in Crescentino (cost of € 150 million), Italy. Its patented Proesa process technology subjects the biomass to high temperature and pressure, enabling the necessary separation of the cellulose and hemicellulose from the lignin, followed by subsequent enzymatic treatment releasing simple sugars which are fermented by yeast into ethanol. The lignin and biogas derived from the processes are recovered and used as fuel in the boiler, generating heat and power. Using waste from wheat and rice, besides *Arundo donax* (planted area: 150,000 m² (37 acres)) with biomass total used 200,000 t/year, production of 50 million liters of ethanol/year, electricity production of 13 MW and 100% of water recycling. The success of Crescentino plant, which became operational in 2012 resulted in the construction of several cellulosic plants. The plants that are presently under construction include Alpha Project and CanEnergy in the USA, Granbio in Brazil and more 3 in Italy (Sulcis, Termini Imerese and Puglia), using energy grasses, wheat straw and corn stover as feedstocks. In early 2014, the technology was also licensed in China (Fuyang Bioproject) [228,230,234–237].

The GranBio started producing cellulosic ethanol at its Bioflex 1 unit in São Miguel dos Campos, Alagoas, Brazil, in 2014. The new facility is the first commercial-scale cellulosic ethanol production unit to be brought online in the Southern Hemisphere. Using Beta Renewable's Proesa pretreatment technology, along with yeast from DSM and enzymes from Novozymes will generate up to 21.66 million gal/y of bioethanol with a carbon intensity rating of 7.55 g/megajoule of CO₂. Construction of the project, which was completed in 20 months, was made possible by GranBio's US\$ 190 million investment. GranBio, along with Carlos Lyra Group's Caete unit, also invested US\$ 75 million in developing a cogeneration system adjacent to the plant to produce steam and electricity [229]. BNDES approved US\$ 150 million loan for the construction of Bioflex 1 and BNB financed the machinery and equipment from Biochemtex (subsidiary of Mossi and Ghisolfi), that enabled the operation of Bioflex 1 [228].

The steps in the Bioflex 1 for obtaining sugars by Proesa technology (licensed by Beta Renewables) are steam pretreatment (licensed by Beta Renewables); enzymatic hydrolysis (licensed by Novozymes); and fermentation using an engineered strain to convert pentose and hexose sugars into ethanol (licensed by the

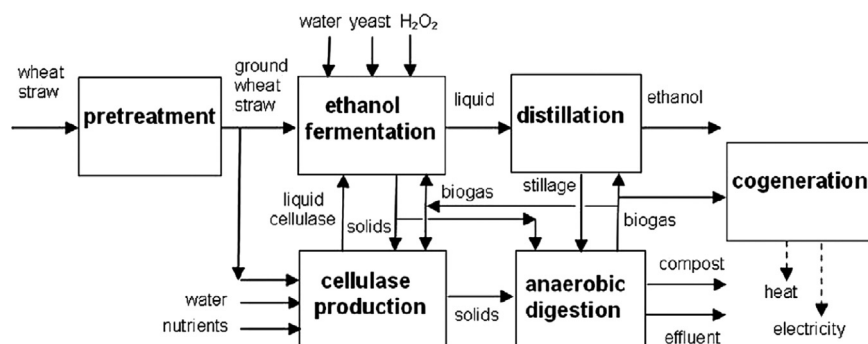


Fig. 3. Flowcharts for cellulosic ethanol production.

Source: [241]

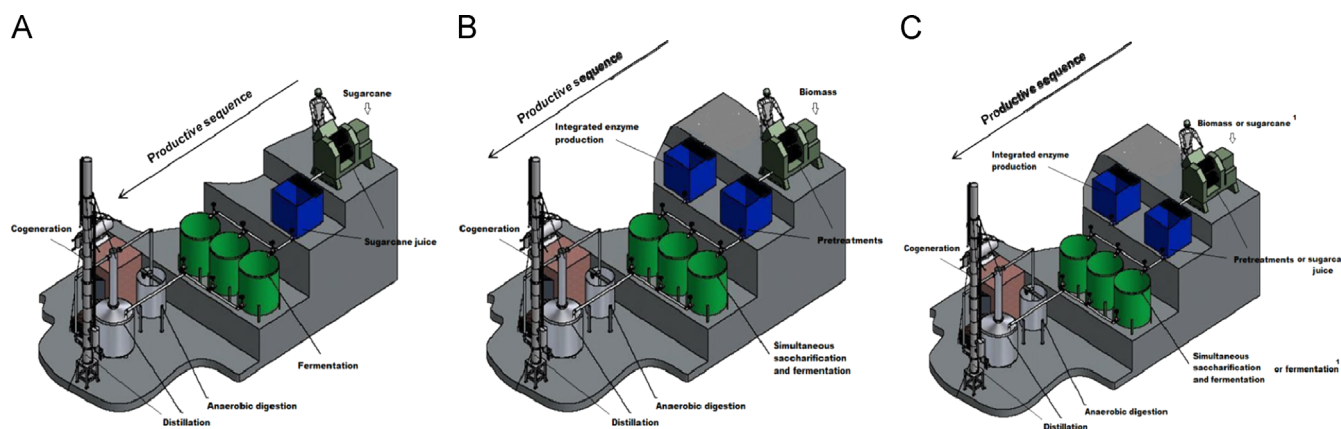


Fig. 4. Flowcharts of ethanol production. (A) Ethanol production from sugarcane juice; (B) ethanol production from sugarcane wastes; (C) ethanol production from sugarcane and/or sugarcane wastes. Source: Adapted from [240].

Note: 1 denotes possibility of producing cellulosic ethanol and ethanol in the same production system.

DSM). The lignin removed by the process can be used for energy generation (licensed by Carlos Lyra). The Bioflex 1 began operating in September 2014 and up to mid-November 2014 had produced more than 600,000 l of cellulosic ethanol from straw. With the annual flow of harvest 2014/15 will reach a volume close to 350,000 t of biomass. The cellulosic ethanol GranBio (Bioflex 1) will be competitive with ethanol in early 2015 and expectation of GranBio is that in the coming months the cost of cellulosic ethanol production it will be 20% lower than the ethanol [228]. Over the next ten years, GranBio intend to install another ten sugar-energetic power plants for the production of cellulosic ethanol in the country, in partnership with sugar-energetic power plants (which carry out planting sugarcane) to provide the straw and bagasse. These sugar-energetic power plants will be invested around US\$ 2 billion. The second sugar-energetic power plant is expected to go into operation as early as 2016. By 2020, the GranBio expected produce 1 billion liters of cellulosic ethanol.

Besides the waste from sugarcane the GranBio will use energy cane in 2015. This energy cane was developed by genetically crossing commercial hybrids with ancestral types of sugarcane. The result is a cane that is more robust, with higher fiber content and productive potential (3 times more productive than the sugarcane) [228].

The production of co-products and by-products from productive process of cellulosic ethanol are presented in Section 2.3. In this context, there is a need for successful utilization of the co-products and by-products; the GranBio uses the residual lignin of the productive process for the generation of heat and bioelectricity, and the excess the energy is exported to the power grid. In addition, the vinasse is subjected to the evaporation process and used in the fertigation. Moreover, in August of 2013, GranBio

sealed a partnership with the multinational Rhodia to produce chemicals from renewable sources. In Brazil, build the world's first plant for bio n-butanol, a key chemical compound in producing paints and solvents [228].

Another initiative in Brazil to produce cellulosic ethanol on a commercial scale has been implemented by Raizen (São Paulo, Brazil) integrated Costa Pinto sugar-energetic power plants. The company will use Iogen Energy's technology (Ottawa, Canada), which is similar to that of PROESA's. The main difference between them is that the latter uses simultaneous saccharification and fermentation, while Iogen's technology has separated saccharification and fermentation. The plant is located in Piracicaba (São Paulo) with investment of US\$ 100 million (being US\$ 85 million financed by BNDES) and may produce up to 40 million liters of cellulosic ethanol a year [238]. Raizen, which is the world's largest sugar and ethanol producer, plans to build nine mills producing cellulosic. Expectations consist of the production of the cellulosic ethanol at the same cost of ethanol, in three years (US\$ 0.4 l). Raizen until the moment has made 200,000 l and are selling cellulosic ethanol in Brazil [236]. According to Raizen, the marketing on a large scale in Brazil will open the door to the global deployment of the technology [236].

The microdistillery is in a reduced technology projection and simplified of a commercial large-scale aimed at the lower cost of investment and operational facilities. Indicated to meet the energetic needs of farms, cooperatives, urban and isolated communities. According to Ho et al. [239] the cellulosic ethanol technologies are relatively mature, with a few commercial scale units, around 100 plants at pilot and demonstration scale worldwide. The microdistilleries established in Brazil produce only ethanol and usually has the same productive configuration

(Fig. 4A). Thus, we have two ways of introducing the cellulosic ethanol production in small-scale in Brazil. The first consists of installing microdistilleries to produce only cellulosic ethanol with integrated enzyme production (production concept of GranBio) (Fig. 4B). The second consists of adapting the existing microdistilleries to produce ethanol and cellulosic ethanol (Fig. 4C) and adding the integrated enzyme production and pretreatment step (production concept of Raizen).

The small-scale process for converting wheat straw into ethanol was modeled by Lever [241] and evaluates the energy performance to produce 8000 l of cellulosic ethanol in two batches per week. The process shows six interconnected unit operations: pretreatment (grinding), cellulase production (on-site production of crude unprocessed liquid cellulase produced via solid-state fermentation under conditions of 30 °C for 8–12 days), alcoholic fermentation (simultaneous saccharification and fermentation carried out at 37 °C for 60 h), anaerobic digestion of wastes, distillation (produced ethanol at the azeotropic concentration of 96% (wt)) and cogeneration (power cogeneration) (Fig. 3). The modeled process resulted in high-energy yield ratios, reductions between 80% and 90% of the energy required to produce and transport the cellulase compared to commercial preparations, and a net surplus of on-site heat and electricity. Thus, Lever [241] demonstrates the productive viability of ethanol in small-scale from wheat straw.

In this sense, the proximity of the composition of wheat straw is cellulose (35.4%); hemicellulose (21.6%); lignin (22.6%); extractives (1.2%) and ash (4.4%) [242]. With the bagasse from sugarcane: cellulose (37.74%); hemicellulose (27.33%); lignin (20.57%); extractives (4.07%); ash (6.53%) and protein (1.13%) [243]. Straw from sugarcane: cellulose (33.77%); hemicellulose (27.38%); lignin (21.28%); extractives (7.02%); ash (6.23%) and protein (3.72%) [243]. As reported earlier, the mature coconut fibre has cellulose (31.60%), hemicellulose (26.31%), lignin (26.69%), extractives (5.44%) and ash (3.31%) [167]. Green coconut shell has cellulose (32.88%), hemicellulose (26.50%), lignin (26.88%), extractives (3.27%) and ash (4.34%) [167]. Mature coconut shell has cellulose (30.47%) hemicellulose (25.42%), lignin (33.15%), extractives (2.71%) and ash (4.84%) [167]. The proximity in the compositions of these lignocellulosic materials allows to emphasize the ethanol production feasibility in small-scale using these raw materials.

Furthermore, Macrelli et al. [244] reported that results simulations of ethanol production from sugarcane bagasse and leaves in Brazil are already competitive with ethanol production from starch in Europe. Furthermore, cellulosic ethanol could be produced at a lower cost if subsidies were conceived and reduced the cost of enzymes.

The lignocellulosic materials mentioned above may present some difficulties in the ethanol productive process, such as the coconut has high rigidity and volume, hindering the reduction of granulometry and storage; the cactus has high amounts of thorns (ash) and high viscosity in the hydrolyzed the halophytic has a high salt concentration present in the biomass. In addition, the sugarcane plantation represents 1% of agricultural lands of Brazil and strengthening of the sugar-energy sector can use larger amount of land. In this sense, GranBio has as a possibility to plant energy cane, and it can be planted in degraded pastureland and is potentially enormous, because Brazil has 32 million hectares of degraded pastureland that can be used for energy cane [228]. However, it can bring a socioenvironmental unbalance.

According to Gupta and Verma [245] and Lever [241] cellulosic ethanol production has four obstacles, being the pretreatment, enzymatic hydrolysis, fermentation and distillation for an efficient technology. These difficulties are common to cellulosic ethanol production in large-scale and small-scale (microdistillery). However, the production of cellulosic ethanol in small-scale is more sensitive to market fluctuations [241].

Therefore, some difficulties encountered are: lack of labor-qualified and high salary; added value to the co-products and by-products; low capacity of yeast to live in a substrate with high alcohol concentration and low thermoresistance; very high cost of enzymes production and access to production technologies; different residual biomass will enable different inhibitors and inhibitor concentrations in the productive process; the pretreatment in industrial scale (GranBio and Raizen) steam explosion, but small-scale generates high cost for implementation and use of equipment; reduced availability of credit and financial incentives; production of cellulosic ethanol process has long-term (5 days) in relation to the production of ethanol (8–12 h); lower temperature in the distiller; efficient enzymatic hydrolysis and fermentation under high solids loading; adequate use of pentose; maintaining the sterility of the production process; avoiding regional deforestation; implementation of minimum price of ethanol (cost of the barrel of oil in December/2014 was US\$ 55.91, discouraging the production of cellulosic ethanol); operating all months of the year; area to the stock of biomass and security to prevent fires; ANP certify the microdistillery for the sale of ethanol to the consumer and release the sale of ethanol 80% (wt); and avoiding freight payment of wastes and cellulosic ethanol [228,232,239,241,245,246].

6. Final considerations

Brazil is currently the second largest world producer of ethanol, however, when evaluating only the productive chain of cellulosic ethanol, the Brazilian influence in the international market becomes insignificant. The prospect of using straw and sugar cane bagasse produced by the sugar-energetic power plants can be frustrating, because most of these lignocellulosic materials are destined for the production of electricity.

Therefore, there is a need for searching alternative and renewable sources for ethanol production, as the use of vegetables grown in inhospitable places, agroindustrial and urban wastes. Besides the possibility of producing cellulosic ethanol in large-scale power plants, it highlights the opportunity of implantation of power plants of medium and small size, especially microdistilleries, with a productive capacity of up to 5000 l of ethanol daily.

These microdistilleries would be distributed throughout the national territory and would use various lignocellulosic materials for the production of many products, co-products and by-products, operating based on the concept of the biorefinery. These strategy leads to gain social, environmental and economic for the population. Therefore, it is believed that Brazil shows significant potential for the production of bioproducts, as biocatalysts and biofuels from renewable materials.

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