Renewable and Sustainable Energy Reviews 44 (2015) 888–903

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

journal homepage: www.elsevier.com/locate/rser

Anaerobic digestion of vinasse from sugarcane ethanol production in Brazil: Challenges and perspectives

Bruna S. Moraes [a,*], Marcelo Zaiat [b], Antonio Bonomi [a]

[a] Brazilian Bioethanol Science and Technology Laboratory (CTBE), Brazilian Center for Research in Energy and Materials (CNPEM), Rua Giuseppe Máximo Scolfaro, 10.000, Polo II de Alta Tecnologia, PO Box 6170, Campinas 13083-970, SP, Brazil

[b] Biological Processes Laboratory (LPB), Center for Research, Development and Innovation in Environmental Engineering (CPDI-EA), São Carlos Engineering School (EESC), University of São Paulo (USP), Av. João Dagnone, 1100, Santa Angelina, São Carlos 13563-120, SP, Brazil

ARTICLE INFO

Article history:
Received 30 April 2014
Received in revised form
1 December 2014
Accepted 4 January 2015
Available online 29 January 2015

Keywords:
Sugarcane ethanol production
Vinasse
Pentoses liquor
Anaerobic digestion
Biogas
Sustainable biorefinery

ABSTRACT

The replacement of fossil fuels by biofuels has been extremely important worldwide to stimulate the growth of economies based on the sustainability through the use of renewable resources. Anaerobic digestion for biogas production is recognized as a clean technology that allies the suitability of wastes with energy generation, fulfilling the requirements for a sustainable alternative to provide the optimization of the biofuels production. This alternative is especially interesting for the sugarcane ethanol production in Brazil, in which the generation of vinasse, the main liquid waste, is very expressive. Nevertheless, the use of vinasse for anaerobic digestion has been finding some challenges to its establishment in the Brazilian sugarcane biorefineries. This paper reviews the actual context of anaerobic digestion within the sugarcane ethanol production in Brazil, presenting the main obstacles for its full application and the directions to promote it as well. Alternatives for biogas use are also presented and compared, highlighting the environmental and energy advantages of applying anaerobic digestion in the sugarcane biorefineries. This scenario is envisaged as a suitable way to achieve the future biorefineries model, based on the use and recovery of renewable resources with economic, social, and environmental benefits.

Contents

* Corresponding author.
E-mail address: bruna.moraes@bioetanol.org.br (B.S. Moraes).

http://dx.doi.org/10.1016/j.rser.2015.01.023
1364-0321/

1. Background

The growing need to expand the use of renewable energy sources in a sustainable manner has boosted the production of biofuels worldwide. Within this scenario, Brazil stands out due to its use of ethanol from sugarcane; the first-generation production process is already established on a large scale in the country, whereas the second-generation process is still in a developing stage. From an environmental perspective, the replacement of fossil fuels by ethanol would reduce greenhouse gas emissions. However, this biofuel production process generates large volumes of wastewater, especially vinasse (also termed stillage), which may constitute a serious environmental problem depending on its final destination. Another biorefinery liquid stream with considerable impact is the pentoses liquor obtained during the pretreatment of bagasse from the sugarcane used in the production of ethanol from lignocellulosic material (second-generation ethanol). Although several studies have examined the production of ethanol and other products from pentoses, this process stream should be given an appropriate destination to avoid environmental damage; however, these alternatives are not technologically feasible on a full scale. The same statement can be made for vinasse from second-generation ethanol production, which must also be treated and properly disposed.

Brazilian sugarcane ethanol production began to develop in the 1970s as a result of the oil crisis, which boosted the search for alternative fuels [1–3]. Since then, Brazil has been implementing biofuels policies to not only reduce the country's dependence on fossil fuels but also benefit from the many environmental, economic, and social advantages associated with the sustainable production and use of biofuels [4]. Nevertheless, although the disposition, treatment, and reuse of vinasse in the sugar and ethanol sectors has improved over the past 30 years, the current policies and regulations that provide guidelines are still inefficient and outdated. Prior to the 1970s, the discharge of vinasse in watercourses was identified as a serious environmental problem, increasing the pollution load of rivers and streams near the sugarcane plant area. In 1967, the Federal Government issued Decree-Law no. 303 prohibiting this action. In 1978, Ordinance no. 323 [5] was enacted for the same purpose, aiming to protect the ecological balance and environment in response to the increasing amount of distilleries promoted by the Brazilian Alcohol Program (Proálcool), which was created in 1975 to increase the production of alcohol for fuel purposes [2]. Thus, alternatives for vinasse disposal were sought; application in soil as a fertilizer for sugarcane crops (fertirrigation) was the most common practice until now. However, the criteria for vinasse application in soil were regulated only recently, by a statewide technical norm decreed in São Paulo State [6]. This regulation only forecast the impacts caused by vinasse on soil, water, and groundwater, prescribing vinasse application according to its potassium content but neglecting organic matter content and atmospheric impacts due to air emissions. Additionally, in some sugarcane processing plants, vinasse application in soil is carried out in a rather indiscriminate manner, intensifying the environmental impacts associated with this action, e.g., soil salinization [7], leaching of metals and sulphate [8–10], and groundwater contamination [11–14]. The release of malodours and attraction of insects are also commonly associated to this practice.

The situation is more complex in the case of the other liquid streams (second-generation vinasse and pentoses liquor), for which there are no environmental regulations thus far. This lack of regulation is understandable because the second-generation ethanol production process is still in a research phase; however, the destination of the wastewater generated in this process must be planned. Additionally, the composition of these liquid streams prevents them from being used as fertilizers in sugarcane crops because their nutrient content (nitrogen, phosphorous, and potassium) is very low, unlike the vinasse from first-generation ethanol production. Thus, apart from its associated environmental impacts, fertirrigation is not suitable in this case, and an alternative disposal method of such liquid wastes must be pursued.

In this context, and considering the current available technologies for wastewater treatment, anaerobic digestion stands out as an interesting alternative to be applied to the liquid wastes of sugarcane biorefineries. Anaerobic digestion can reap environmental and energy advantages. From an environmental perspective, such technology reduces the organic matter content of those wastes while maintaining the inorganic nutrient content, which is particularly important in the case of vinasse generated in first-generation ethanol production. In this manner, the biodigested vinasse can still be used as fertilizer in the sugarcane crop while having a lower pollutant load. In the case of pentoses liquor and vinasse from second-generation ethanol production, a decrease of their pollutant load would facilitate their final disposal. Regarding energy aspects, the biogas generated from the anaerobic process would be an attractive alternative energy source due to the high heat of combustion of the methane present in the biogas. Although these advantages of applying the anaerobic digestion process to the liquid wastes of sugarcane biorefineries are well known, several challenges and obstacles remain for its full implementation, mainly associated to the insufficient understanding of the bioprocess applied to that specific wastes, the lack of appropriate legislation on fertirrigation practice as well as the non appreciation of biogas as an alternative fuel in Brazil. Thus, to overcome those barriers, the combined efforts from the government, scientific community and environmental agencies are indispensable.

This paper presents an overview of the actual stage of development of sugarcane ethanol production in Brazil as well as the main trends in this field, with a focus on the destination of the principal liquid waste, vinasse. This paper also identifies the barriers for the full application of anaerobic digestion in the treatment and use of effluents from sugarcane biorefineries and provides potential directions for overcoming these challenges. Based on the indicated paths, future prospects are outlined to highlight the importance and advantages of the application of this biotechnology in the biorefinery context.

2. Sugarcane biorefinery concept: Current status and trends

2.1. First-generation ethanol production

In Brazil, the technology applied to first-generation ethanol production from sugarcane is already consolidated, considering its experience of almost four decades in the development and production of this biofuel. Nevertheless, there are still many opportunities for investment in research, development, and innovation to enhance the production of first-generation ethanol from sugarcane, increasing the financial return and productivity of the overall conversion process [3,4,15,16].

First-generation ethanol is produced from sugarcane juice or molasses (or a mixture thereof) depending on the processing plant: in autonomous distilleries, ethanol is produced from sugarcane juice, whereas in annexed plants, a fraction of the sugarcane juice is diverted for sugar production, and the remaining fraction along with the molasses is used for ethanol production. According to CONAB [17], 63.5% and 30.5% of the sugarcane processing units in Brazil are annexed and autonomous plants, respectively, and the remaining units produce only sugar. The prevalence of annexed plants in the country is related to its flexibility to produce more ethanol or more sugar depending on the market demands, which

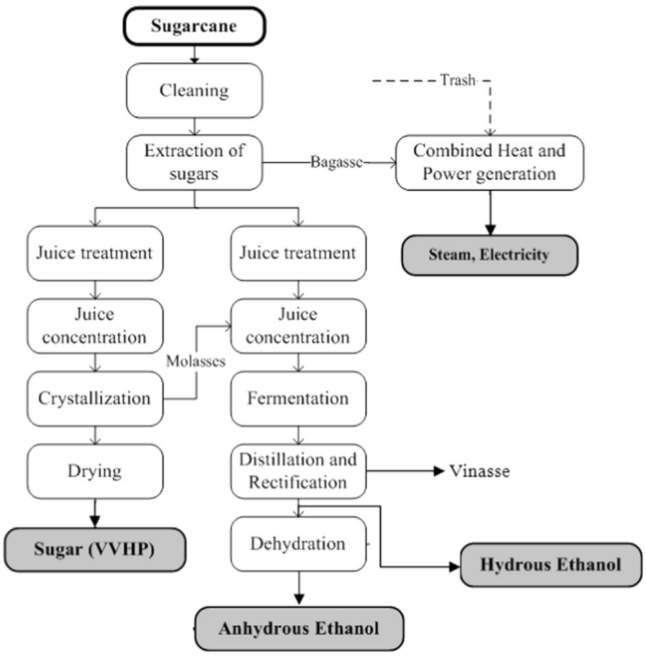

Fig. 1. Block flow diagram of the production of sugar, first-generation ethanol, and electricity from sugarcane (Modified from Bonomi et al. [18]).

is part of the reason for the success of bioethanol production in Brazil. However, the range of operation of an installed plant is somewhat limited to the existing design restrictions and available facilities [18].

In terms of energy consumption, the sugarcane processing facilities (either annexed or autonomous) are self-sufficient through the use of sugarcane bagasse as a source of energy. Bagasse is burned in combined heat and power (CHP) systems to produce all of the thermal and electric energy required for the production process as well as the sugarcane straw when it is recovered from the field. The energy source is usually used for the processes of evaporation and ethanol distillation, which require steam at low pressure (2.5 bar). During the off season periods, however, the energy generated in CHP system is usually exported to the grid, since the operation of the mills is stopped. The steam consumption generally occurs by using back pressure turbines (inlet at 65 bar and 490 °C), coupled to generators to produce electric energy. The exhaust steam can be blown off by using condensing turbines, where the exhaust steam pressure is sub-atmospheric. This creates greater enthalpy difference between the high pressure and exhaust steam condition, resulting more electrical energy per steam mass unit [19]. The CHP system efficiency depends on the technology and the energy integration degree of each sugarcane mill.

A scheme of the sugar, the first-generation ethanol, and electricity production process from sugarcane is illustrated in Fig. 1. In an autonomous distillery, the unit operations related to sugar production is not included in the sugarcane mill.

2.2. Second-generation ethanol production

The search to increase the ethanol productivity per sugarcane planted area has facilitated the development of second-generation ethanol production in Brazil. Through this technology, ethanol is produced from the lignocellulosic material of sugarcane (bagasse and straw). Thus, all of the sugarcane biomass could be used industrially when the first- and second-generation ethanol production processes are integrated, optimizing the ethanol production chain.

Table 1
Composition of hemicellulose hydrolyzed in monosaccharides [14].

Saccharide	g/100 g of hemicellulose of sugarcane bagasse
D-Xylose	20.5–25.6
L-Arabinose	2.3–6.3
D-Mannose	0.5–0.6
D-Galactose	1.6

The current development stage of second-generation ethanol production in Brazil is still in the research phase, with some promising studies for developing large-scale bioethanol production in this area [4,20,21]. However, the production cost of ethanol from lignocellulose is still overly high, which is the major reason why ethanol from this feedstock has not yet made its breakthrough [22]. In addition, a number of bottlenecks in the investigation of an efficient conversion process of the lignocellulosic material present in the bagasse and straw to ethanol prevent their reproduction on an industrial scale. For this conversion, the lignocellulosic material must be subjected to some pretreatment and hydrolysis to break the polysaccharides of this material into fermentable sugars.

Cellulose and hemicellulose are among the polysaccharides present in the sugarcane bagasse and straw of greatest interest for the production of byproducts [23]. A liquid fraction, the hemicellulosic hydrolyzate, is extracted after bagasse pretreatment. This hydrolyzate is composed of pentoses (monosaccharides that contain five carbons in their structure), with xylose as the main constituent (Table 1). The solid material resulting from the pretreatment of bagasse (cellulignin), composed mainly of cellulose, may be hydrolyzed to obtain fermentable hexoses. The hexoses may in turn be converted into second-generation ethanol through a fermentative process. Thus, the production of bioethanol from sugarcane bagasse requires suitable pretreatment (to facilitate the hydrolysis of lignocellulosic material), hydrolysis, and fermentation [24,25].

Efforts have been made in recent years to develop efficient technologies for second-generation ethanol production, especially in the pretreatment phase. Although pretreatment is one of the most expensive and least technologically mature steps in the process for converting biomass to fermentable sugars [26], this step also has great potential for improving efficiency and lowering costs through research and development [20,27,28]. The pretreatments can be classified as physical, chemical, physical–chemical, and biological, as well as combinations thereof. Within these main groups, different techniques can be applied, as shown in Table 2 [29–37]. According to Rabelo et al. [28], chemical pretreatments have received more attention due to their high efficiency and improved lignocellulose digestibility compared to other treatments. Currently, the most widely used methods include alkaline pretreatment, steam explosion, wet oxidation, and acid hydrolysis [25,33]. Although such pretreatments are the best current alternatives, improvements are still required in these technologies to prevent the formation of metabolic inhibitory products (such as furfural and 5-hydroxymetil furfural) and improve their economic competitiveness through the use of inexpensive chemicals and simple equipment and procedures [38–40]. In this scenario, some promising research has been currently performed in the pretreatment technology field, aiming to obtain higher efficiency process allied to satisfactory sustainability indicators, i.e., integrating economic and environmental criteria to the technical stages of process design [36,41,42]. Furthermore, the scale-up of the technologies also has been strongly prognosticated, being assessed the integration of such processes in pilot or demo scale. A report from the International Energy Agency Bioenergy Task 39 group [43],

Table 2
Summary of pretreatment processes for lignocellulosic biomass.

Methods	Pretreatment process	Advantages	Disadvantages	References
Physical	Mechanical comminution	Reduces cellulose crystallinity, increases superficial area and pore size; no inhibitors production	Power consumption typically higher than inherent biomass energy; time-consuming, expensive; does not remove lignin	[29–33]
	Pyrolysis	Produces gas and liquid products	High temperature; ash production	[30–32]
Physical–chemical	Steam explosion	Causes hemicellulose degradation and lignin transformation; cost-effective; complete sugar recovery; lower environmental impact; less hazardous process chemicals and conditions; feasibility at industrial scale development	Destruction of a portion of the xylan fraction; incomplete disruption of the lignin-carbohydrate matrix; generation of inhibitory compounds to microorganisms; need to wash the hydrolysate	[29–35]
	CO_2 explosion	Increases accessible surface area; cost-effective; does not cause formation of inhibitory compounds	Does not modify lignin or hemicelluloses; very high pressure requirements	[31,32,34]
	Ammonia fiber explosion (AFEX)	Increases accessible surface area, removes lignin and hemicellulose to an extent; does not produce inhibitors for downstream processes	Not efficient for biomass with high lignin content; high cost for ammonia recovery	[31,32,34]
Chemical	Acid hydrolysis	Hydrolyzes hemicellulose to xylose and other sugars; alters lignin structure; increase accessible surface area	High cost; equipment corrosion; formation of toxic substances	[31,33,34,36]
	Alkaline hydrolysis	Removes hemicelluloses and lignin; increases accessible surface area	Long residence times required; irrecoverable salts formed and incorporated into biomass	[31,33,35,36]
	Ozonolysis	Reduces lignin content; does not produce toxic residues; usually performed at room temperature and normal pressure	Large amount of ozone required; expensive	[31,34]
	Organosolvent process	Hydrolyzes lignin and hemicelluloses; recovery of relatively pure lignin as a by-product	Solvents must be drained from the reactor, evaporated, condensed, and recycled; high cost	[31,34,35,37]
	Oxidative delignification	Almost total solubilization of hemicellulose; degrades lignin	losses of hemicellulose and cellulose can occur; risk of formation of inhibitors	[32,33]
	Wet oxidation	Solubilization of major part of hemicelluloses; degrades lignin; phenolic compounds are further degraded to carboxylic acids	Formation of inhibitors; cost of oxygen and catalyst	[32–35]
Biological	Brown, white, and soft-rot fungi	Degrades lignin and hemicelluloses; low energy requirements; no chemical requirements; environmentally friendly approach; low cost	Rate of hydrolysis is very low; limit application at industrial scale	[31,32,34,35]
Combined	Alkaline treatment associated with steam explosion; milling followed by acid or alkaline treatment	Degrades lignin and hemicelluloses, increases superficial area and porous size	Provides lower digestibility when compared with simple treatments	[29]

in which is outlined the progress on more than 100 advanced biofuel projects under development worldwide, contains many of these scaled-up plants.

After the pretreatment step, pretreated lignocellulosic materials are subjected to a hydrolysis process to obtain fermentable sugars. Hydrolysis can be carried out as acid hydrolysis (diluted or concentrated acids) or enzymatic hydrolysis. Sulfuric acid, hydrochloric acid, and phosphoric acid are typically used in hydrolysis with concentrated acids at low temperatures ($<100\,^{\circ}C$) [44]. However, the use of concentrated acids requires reactors that are highly resistant to corrosion, which increases the cost of the process. In addition, the effluent generated must be recovered to avoid environmental impacts due to the presence of acids. In contrast, dilute acid hydrolysis was found to significantly improve the cellulose hydrolysis, but the drawbacks associated with this technique are the requirements of high temperature and neutral pH [25,39]. Additionally, considerable amounts of sugar and soluble lignin are degraded due to the high temperatures, which cause inhibition during the fermentation process [32].

Enzymatic hydrolysis has yielded better results for the subsequent fermentation because no degradation components of glucose are formed, although the process is slower [22,32]. The cellulases break down cellulose to cellobiose, which is subsequently cleaved to glucose by β-glucosidase [28]. The advantages of enzymatic hydrolysis when compared with acid hydrolysis are a higher yield of fermentable sugars due to the milder conditions, which causes less formation of byproducts, and the use of biodegradable inputs (enzymes), which make the process more environmentally friendly [28,45].

The conversion of lignocellulosic materials involving the hydrolysis of cellulose to glucose and the alcoholic fermentation of the resulting sugar can be performed in a single step or in two sequential phases. The advantage of the latter process is that each step can be carried out at its optimum conditions, ensuring more flexibility for the control of operational conditions. However, process integration reduces the capital cost [46] and the inhibition caused by the final product that occurs in the two-phase process because the presence of fermentative microorganisms and cellulolytic enzymes reduces the accumulation of sugars in the fermenter [44].

Focusing on the concept of sugarcane biorefinery, second-generation ethanol production should be integrated with the first-generation process to maximize the production of ethanol while reducing waste. A block-flow diagram of the second-generation ethanol production process, integrated with the first-generation process, is provided in Fig. 2. This representation does not completely fulfill the biorefinery concept because not all possibilities of exploitation and production of byproducts are considered.

2.3. *Liquid streams: Residues or raw materials?*

The main liquid stream from the first-generation ethanol production process consists of vinasse, characterized as an effluent of high pollutant potential, containing high levels of organic compounds and nutrients (mainly potassium but also nitrogen and phosphorous). It is derived from the ethanol distillation step (Fig. 1, Section 2.1), leaving the columns at a temperature in the range of 85–90 °C. The presence of melanoidins and the high

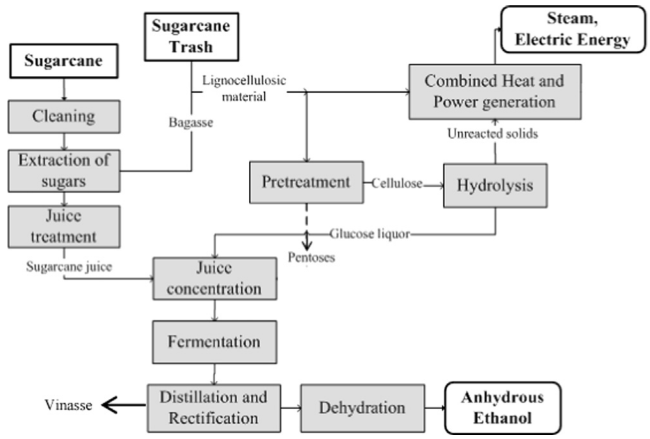

Fig. 2. Block-flow diagram of the integrated 1st- and 2nd-generation ethanol production process from sugarcane (Modified from Bonomi et al. [18]).

organic acid content provide the dark-brownish color and low pH, respectively, of this liquid waste [47]. In Brazil, sugarcane processing plants generally generate from 10 to 15 L of vinasse per liter of produced ethanol [18,48,49]. Considering that the total ethanol production in the 2009–2010 harvest season was approximately 25,750 m^3 [50], the volume of vinasse generated nationwide is considerable.

For decades, the main destination of vinasse in Brazil has been its application in sugarcane crops as fertilizer, a practice known as fertirrigation. Nevertheless, Brazilian environmental legislation only recently established criteria and procedures for vinasse application on soil [6]. Such legislation can be considered superficial because vinasse application (vinasse per area) is prescribed only according to its potassium content. Its high organic matter content and the potential environmental impacts associated with it are not considered. Such impacts are mainly related to greenhouse gas emissions [51,52], soil salinization [7], leaching of metals and sulfate [8–10], and groundwater contamination [11–14], as well as the release of unpleasant odors and the possible attraction of insects. Therefore, the lack of regulation of vinasse chemical composition for soil application allows fertirrigation with *in natura* vinasse to act as a source of potential environmental impacts.

The intensity of such impacts may differ as a function of the variable vinasse composition. According to Sheehan and Greenfield [53], vinasse characteristics are dependent on the raw material and thus on the agricultural practices influencing plant composition. In the case of sugarcane vinasse, its composition also varies according to the fermentation feedstock, namely, sugarcane juice and/or molasses. In the autonomous plants, which produce only ethanol, vinasse originates from sugarcane juice. In the annexed plants, which produce both sugar and ethanol, vinasse derives from both molasses and juice. Regardless of its origin, the main organic compounds present in sugarcane vinasse reported in the literature consist of organic acids (mainly lactate and acetate), as well as alcohols (mainly glycerol and ethanol) and a minor amount of carbohydrates [14,54–57]. Table 3 compiles studies in which vinasse characterization was performed, presenting the main parameters that define its composition according to the fermentation feedstock [47,48,58–62].

Vinasse from molasses generally presents higher values of chemical oxygen demand (COD) and biochemical oxygen demand (BOD) than vinasse from sugarcane juice, as was also observed by Wilkie et al. [61]. According to the authors, the concentration of sugars in molasses, through the crystallization and evaporation of juice, increases the content of non-fermentable organics that remain in the vinasse after fermentation, thus increasing the COD

Table 3
Compilation of the main parameters for vinasse characterization from different feedstocks, collected from the literature.

Feedstock	Parameters													
	COD ($g L^{-1}$)	BOD ($g L^{-1}$)	N ($g L^{-1}$)	P ($g L^{-1}$)	K ($g L^{-1}$)	SO_4^{2-}–S ($g L^{-1}$)	Ca ($g L^{-1}$)	Mg ($g L^{-1}$)	TS[a] ($g L^{-1}$)	VS[b] ($g L^{-1}$)	Phenols ($mg L^{-1}$)	Reduced sugars ($g L^{-1}$)	pH	Reference
Sugarcane juice	15–33	6–17	0.2–0.7	0.004–0.1	1.0–1.7	0.2–0.3	0.1–1.1	0.1–0.3	24	20	n.d.	7.9	3.7–4.6	[58]
	30	n.d.	0.52	0.25	1.4	0.46	0.11	0.11	n.d.	1.8[c]	n.d.	n.d.	n.d.	[59]
	31.3	17.1	0.41	0.11	1.5	n.d.	n.d.	n.d.	21.1	15.6	n.d.	n.d.	3.9	[60]
	23.4	n.d.	0.28	0.04	0.6	0.44	0.32	0.17	n.d.	n.d.	n.d.	n.d.	3.7	[62]
	30.4 ± 8.2 [d](6)	16.7 ± 3.4 (5)	0.63 ± 0.32 (6)	0.13 ± 0.11 (6)	2.0 ± 1.2 (5)	1.4 ± 1.4 (5)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.0 ± 0.5 (7)	[61]
Sugarcane molasses	65	25	0.5–1.6	0.04–0.13	3.1–6.5	2.2	0.3–3.6	0.3–0.9	82	60	n.d.	9.5	4.2–5.0	[58]
	52.0	n.d.	0.77	0.04	2.5	1.2	1.75	0.62	n.d.	n.d.	n.d.	n.d.	4.4	[62]
	84.9 ± 30.6 (22)	39.0 ± 10.8 (19)	1.23 ± 0.63 (20)	0.19 ± 0.35 (17)	5.1 ± 3.1 (12)	3.5 ± 2.5 (16)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.5 ± 0.4 (7)	[61]
Mixed	42	11.3	0.07	0.2	2.3	1.3	0.46	0.29	158	n.d.	1.1	1.0	3.9	[47]
	32.6	n.d.	0.46	0.05	1.2	0.89	0.84	0.32	n.d.	n.d.	n.d.	n.d.	4.1	[62]
	45	20	0.5–0.7	0.004–0.1	2.8–3.8	1.3	0.9–3.3	0.3–0.4	53	13	n.d.	8.3	4.4–4.6	[58]
	31.5	n.d.	0.37	0.024	1.3	0.15	n.d.	n.d.	n.d.	3.7[c]	n.d.	n.d.	3.9	[48]

[a] Total solids (TS).
[b] Volatile solids (VS).
[c] Volatile Suspended Soilds (VSS).
[d] (*n*) is the number of literature values used.
: n.d.=not determined.

and COD/BOD ratio. Higher potassium concentrations are also observed in vinasse from molasses, as well as calcium, magnesium, and phosphorous, due to the addition of such nutrients during the sugar processing phase, e.g., the application of magnesia in clarification, the use of calcium in carbonation and liming, and the use of phosphoric acid for phosphating. The presence of sulfate in vinasse from both sugarcane juice and molasses are mainly due to the addition of sulfuric acid to yeast cell suspensions for bacterial control in alcoholic fermentation. Regardless of the origin of sugarcane vinasse, the organic matter content and potassium concentration stand out from among the other elements, followed by sulfate, nitrogen, phosphorus, calcium, and magnesium.

Vinasse composition also varies throughout the harvesting season, mainly due to the raw material (e.g., milling different varieties of sugarcane, with different maturation indexes, and grown in different soils with different levels of fertility) and to the industrial process (e.g., variations in the operation of the fermentation and distillation steps). Thus, vinasse is considered a complex wastewater within the same production process, varying throughout the operation time.

There is a lack of information in the literature on the composition of the liquid streams generated in second-generation ethanol production, mainly due to the current stage of development of the process for second-generation bioethanol production; the investigation for an efficient technological process is still underway. As reported in Section 2.2, different technologies can be applied to the pretreatment of sugarcane bagasse, and thus, the composition of the pentoses liquor generated during the process is not precisely defined. Table 4 compiles the range of values of the main components present in the pentoses liquor according to the pretreatment applied to the sugarcane bagasse [44,63,64]. Depending on the pretreatment, the hydrolyzed cellulose can contain glucose, cellobiose, hydroxymethylfurfural, sucrose, and galactose, whereas the solubilized hemicellulose includes xylose, arabinose, furfural, and acetic acid. The total lignin content encompasses both soluble and insoluble parts. A more detailed characterization of pentoses liquor for different pretreatments is presented in Table 5, illustrating that pentoses liquor is primarily composed of hemicelluloses, with xylose as the main reduced sugar [63–67].

Regarding the vinasse generated in the production of bioethanol from sugarcane bagasse, the only available information was found in an international patent application [65]. The composition of such vinasse is reported in Table 6. Such vinasse presents a higher organic matter content than the vinasse from first-generation ethanol production. However, the BOD/COD ratio is comparable for both types of vinasse (0.4–0.5). In contrast, the content of nutrients and minerals, especially potassium, is considerably lower for second-generation vinasse. Thus, the application of this liquid stream in the soil cannot be justified for its fertilizer characteristics, as occurs with vinasse from first-generation ethanol production. In addition, the same environmental impacts caused by the

Table 4
Chemical composition of pentoses liquor based on solubilized compounds according to the pretreatment applied to sugarcane bagasse.

Pretreatment	Main components (%)			Reference
	Solubilized cellulose	Solubilized hemicellulose	Lignin	
$Ca(OH)_2$	4.1–2.9	31.5–21.0	41.7–41.9	[44]
H_2O_2	2.0–n.d.	32.2–22.3	35.7–46.2	[44]
Steam explosion (180 °C)	30	67.1	16.8	[63]
Steam explosion (180 °C)	11.8 ± 3.7	82.7 ± 4.3	7.9 ± 9.1	[64]

Table 6
Characterization of vinasse obtained in 2nd-generation ethanol production from sugarcane [65].

Parameter	Values
pH	4.0–4.9
COD (g L^{-1})	75.8–109.7
BOD (g L^{-1})	31.5–87.7
N (g L^{-1})	0.205–0.462
P (g P_2O_5 L^{-1})	0.1005
K (g K_2O L^{-1})	0.040–0.088
SO_4^{2-} (g S L^{-1})	0.0146–0.122
Ca (g CaO L^{-1})	0.008–0.012
Mg (g MgO L^{-1})	0.016–0.024
TS (g L^{-1})	0.467–5.805
VS (g L^{-1})	0.454–5.715
Carbon (g C L^{-1})	22.7–33.2
Reduced substances (g L^{-1})	9.166
Phenols (mg L^{-1})	0.4–12.4

Table 5
Chemical composition of pentoses liquor according to the pretreatment applied to sugarcane.

Pretreatment	Component	Concentration	Reference
Acid hydrolysis	Total reduced sugars	42.9 g L^{-1}	[63,64]
	Xylose	34–45 g L^{-1}	
	Glucose	3–8 g L^{-1}	
	5-Hydroxymethylfurfural	0–99 ppm	
	Acetic acid	8–26 g L^{-1}	
Steam explosion (12 bar steam, 190 °C, 15 min)	Total reduced sugars	142 g L^{-1}	[65]
	Xylose	84.6%	
	Glucose	4.5%	
	Sucrose	10.9%	
Hydrothermal (170 °C, 250 min)	Xylose	78%	[66]
	Glucose	10%	
	Arabinose	9%	
	Galactose	3%	
Hydrothermal (190 °C, 10 min, direct steam injection)	Xylose	23.88 g L^{-1}	[67]
	Glucose	4.01 g L^{-1}	
	Arabinose	1.39 g L^{-1}	
	Formic acid	0.63 g L^{-1}	
	Acetic acid	5.79 g L^{-1}	
	5-Hydroxymethylfurfural	0.19 g L^{-1}	
	Furfural	0.32 g L^{-1}	

presence of organic matter content are expected, which further hinders fertirrigation with second-generation vinasse.

Aiming to optimize the energy potential and sustainability of bioethanol production, the above liquid streams should not be considered as residues of the process. Accordingly, the valorization of such streams shall be sought so that they become raw materials for other processes. This concept is now inherent in the field of biological treatment of wastewater, in which the scientific and technological advances made in recent years have driven the creation of new lines of research aimed at not only the environmental suitability of the wastes generated but also the recovery of energy and products from these wastes. Through this approach, the wastewater is considered raw material for a biotechnological process that can generate energy and products with high added value while also fulfilling the primary function of controlling environmental pollution.

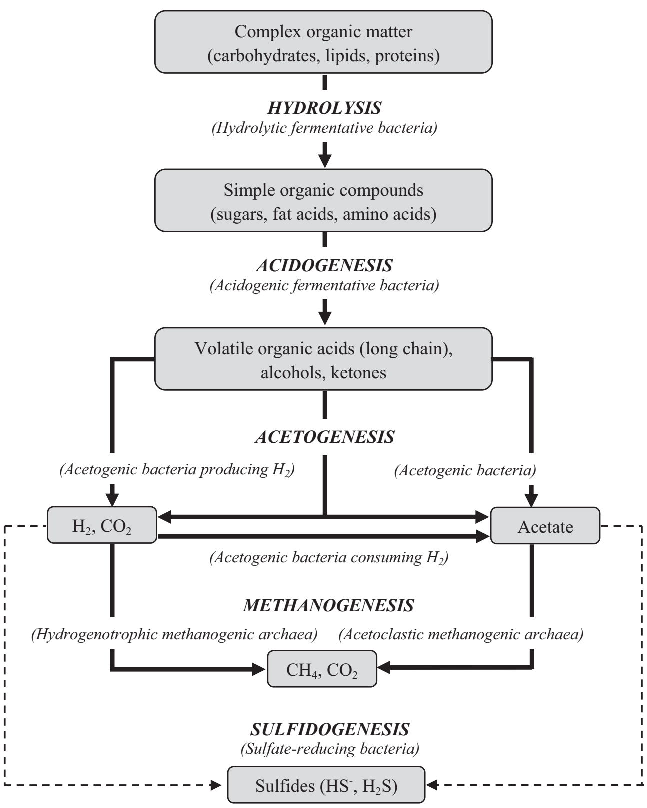

Fig. 3. Scheme of the anaerobic digestion of complex organic matter, depicting the steps and microbial populations involved.

3. Anaerobic digestion

3.1. Fundamentals of the bioprocess

Anaerobic digestion consists of a set of complex and sequential metabolic processes that occur in the absence of molecular oxygen and depend on the activity of at least three distinct groups of microorganisms to promote the stable and self-regulating fermentation of organic matter, resulting mainly in methane and carbon dioxide gases [68–71]. These groups of microorganisms include acidogenic (or fermentative) bacteria, acetogenic (or syntrophic) bacteria, and methanogenic archaea [72–74]. In the presence of sulfate, sulfite, or thiosulfate, there is also activity from sulfate-reducing bacteria (SBR), responsible for the reduction of oxidized sulfur compounds to sulfide dissolved in the effluent ($HS^-/S^{-2}/H_2S$) and to hydrogen sulfide (H_2S) in the biogas [70]. Fig. 3 illustrates the scheme of the anaerobic digestion of complex organic matter and identifies the respective groups of microorganisms involved in each step.

Fermentative microorganisms are the first to act in this complex process for substrate degradation and are those who obtain the greatest energetic benefit. Most acidogenic bacteria convert the hydrolysis products of the complex organic matter in volatile organic acids (mainly acetic, propionic, and butyric), alcohols (mainly ethanol), ketones (mainly acetone), carbon dioxide, and hydrogen. Such biological reactions are thermodynamically favorable (Table 7), and thus, acidogenic microorganisms present the lowest minimal generation time and highest growth rates [75–77]. Therefore, acidogenesis will only limit the process if the substrate to be degraded is not readily hydrolyzed. In contrast, acetogenic reactions are thermodynamically unfavorable in standard conditions; however, they occur naturally in anaerobic reactors due to the interaction between methanogenic and acetogenic microorganisms. To overcome thermodynamic limitations, the products of acetogenesis must be maintained in low levels, which occur through the activity of hydrogenotrophic methanogenic microorganisms. Because methanogenesis strongly depends on the availability of acetate (70% of the methane formed is from acetate), hydrogen produced in acetogenesis must be continually removed to ensure that the production of acetate is not interrupted or drastically decreased [69,78]. The thermodynamic relationship between the partial pressure of hydrogen and acetogenic respiration associated with anaerobic digestion illustrates that such biological reactions occur favorably under low hydrogen partial pressure [68,78]. The coexistence of microorganisms that produce and consume hydrogen is possible under this condition (approximately 10^{-6}–10^{-4} atm), as the Gibbs free energy is negative for both processes.

It is important to provide favorable environmental conditions for the microbial populations inside the anaerobic reactors to ensure that the autoregulatory process occurs in a stable manner [72,79]. Environmental factors that influence anaerobic digestion

Table 7
Energy comparison of some common reactions in anaerobic degradation [75–77].

Step	Reaction	ΔG° (kJ/reaction)
Acidogenesis	$C_6H_{12}O_6+2H_2O \rightarrow 2CH_3COO^-+2CO_2+2H^++4H_2$	−206
	$C_6H_{12}O_6+2H_2 \rightarrow 2CH_3CH_2COO^-+2H_2O+2H^+$	−358
	$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COO^-+2CO_2+H^++2H_2$	−255
Acetogenesis	$CH_3CH_2COO^-+3H_2O \rightarrow CH_3COO^-+HCO_3^-+H^++3H_2$	+76.1
	$CH_3CH_2COO^-+2HCO_3^- \rightarrow CH_3COO^-+H^++3HCOO^-$	+72.2
	$CH_3CH_2CH_2COO^-+2H_2O \rightarrow 2CH_3COO^-+H^++2H_2$	+48.1
Methanogenesis	$CH_3COO^-+H_2O \rightarrow CH_4+HCO_3^-+2H_2$	−31.0
	$H_2+1/4HCO_3^-+1/4H^+ \rightarrow 1/4CH_4+3/4H_2O$	−33.9
	$HCOO^-+1/4H_2O+1/4H^+ \rightarrow 1/4CH_4+3/4HCO_3^-$	−32.6

mainly involve temperature, pH, alkalinity, adequate macronutrients (N, P, SO_4^{-2}) and micronutrients (trace metals), adequate metabolic time, and a carbon source (for synthesis and energy), which affect the chemical and biochemical reaction rates.

Fair and Moore [80] were the first to demonstrate that the highest reaction rates of sludge digestion occur in two optimal temperature ranges, termed mesophilic (approximately 30 °C) and thermophilic (approximately 50 °C). This behavior is due to the existence of a variety of microbial species present in the medium: each species is expected to answer differently to temperature variations, and thus, the community can achieve maximum biological activity in distinct optimal temperatures [78]. A lack of temperature control can affect the balance of production and consumption of intermediary products (mainly volatile organic acids), the microbial growth (especially for the methanogenic archaea), and the hydrolysis rate of proteins, lipids, and particulates, decreasing the global efficiency of the process [68,78].

It is also important to provide sufficient alkalinity to the system to maintain the pH in the optimal range (6.5–8.2) because the anaerobic digestion process generates intermediary organic acids. Alkalinity is generated during the anaerobic digestion process: the main sources in wastewater are proteins, which release ammonia and salts of weak organic acids when hydrolyzed [78]. However, alkalizing agents can be added to the influent to increase the buffering of the medium. The monitoring of alkalinity in anaerobic reactors is more efficient than the monitoring of pH, mainly because alkalinity is expressed on a linear scale, whereas the pH scale is logarithmic. Thus, a small decrease in pH implies a large consumption of alkalinity, resulting in a considerable loss in the buffering capacity.

Apart from fundamental theory, a working knowledge of the basic principles of anaerobic biotechnology, including potential operational problems, is necessary for the successful application of the technology to industrial use. Techniques for evaluating performance, knowledge of the factors that determine effluent quality and temperature sensitivity, and protective procedures that optimize the acclimation to toxicants inherent in the feedstock must all be mastered before practical application of the process is possible [68,81]. Regarding anaerobic treatment of sugarcane biorefinery liquid streams, the acclimation of biomass to the wastewater is perhaps the most crucial factor. Although this application has been known since the 1970s, it was not encouraged until recently due to a number of reasons, which will be discussed in the next section.

3.2. Integration of anaerobic digestion in a sugarcane biorefinery: Background

The recovery of energy and production of multiple products are principles inherent to the concept of a biorefinery, which has been developed recently. The biorefinery integrates the biological production process for the production of fuels, bioenergy, and chemical by-products from biomass, analogous to an oil refinery [82]. According to Luo et al. [83], the generation of multiple products is a benefit to the biorefinery, maximizing the value of by-products from the biological process. Therefore, anaerobic digestion is suitable for a sugarcane biorefinery because in addition to the environmental suitability of wastewaters, it also allows energy to be generated through the use of biogas and the generation of other by-products from that biological process.

The processing of industrial effluents by anaerobic digestion has been the subject of several studies and industrial applications focused primarily on the reduction of the organic load content and potential energy use of biogas [64,79]. The simple design and low capital/operational costs have made such technology a ubiquitous starting point for the treatment of wastewaters with a high organic load [84]. In the case of sugarcane vinasse, business processes were developed in the late nineteenth century but found no interest from the sugarcane sector due to the low economic viability of electricity generation from biogas [58,60,85]. This technological possibility is now being reconsidered due to the need to reduce the organic load of the effluent to the soil while maintaining the nutrient and mineral content, as well as to the interest in optimizing the energy balance of sugarcane biorefineries [82–84].

Regardless of this renewed interest, the use of energy from biogas is still not widespread in Brazil. The only vinasse treatment plant mentioned in the literature [48] that produces biogas is located in the São Martinho mill, located in the state of São Paulo. The reactor consists of a 5000 m^3 up-flow anaerobic sludge blanket (UASB) operated in thermophilic conditions; this reactor was constructed in the 1990s. The biogas produced is applied to dry the yeast used in the fermentation step. However, the treatment efficiency is typically modest because the main concern in this case is only the production of adequate biogas for yeast drying. Therefore, the optimization of the anaerobic treatment has not been pursued, and the main barriers to the anaerobic biodigestion of vinasse are not being researched in that mill. Even less information is available for the pentoses liquor from sugarcane bagasse and straw: no reports were found in the available literature regarding the anaerobic digestion of such streams, even at the bench-scale. Thus, the use of biogas from the anaerobic digestion of pentoses liquor in biorefineries remains a possibility. According to Salomon and Lora [86], the major obstacles to the use of biogas in Brazil are (i) the high investment costs, (ii) insufficient funding and little research in the area of anaerobic digestion, (iii) a lack of a national biogas program, specific financing, and government incentives, (iv) the difficulties faced by small biogas plants in selling their carbon credits, (v) a lack of information and funding for farmers, (vi) the need to define biodigestion technology for each case separately, and (vii) a lack of studies in the specialized literature for the selection and assessment of economic viability.

Energy recovery through the anaerobic digestion of liquid streams from a biorefinery provides incentives for second-generation ethanol production. Currently, sugarcane bagasse from the first-generation ethanol production process is burned for energy cogeneration in the mills. In this sense, energy from biogas produced in the anaerobic digestion of waste could partially or even completely replace the energy obtained from burning bagasse, releasing this material for second-generation ethanol production. Junqueira et al. [87] performed economic and environmental assessments of this scenario and concluded that the anaerobic digestion of vinasse and pentoses liquor in a biorefinery with integrated first- and second-generation ethanol production would allow 100% of the lignocellulosic material to be used for second-generation ethanol production. Given this scenario, the productivity in liters of ethanol per hectare-year of planted cane could be increased because an integrated first- and second-generation process would allow for the complete utilization of plant biomass for ethanol production.

Aside from energy generation, other by-products from anaerobic digestion could be recovered, such as sulfur; specifically, generated sulfide can be recovered as elemental sulfur in microaerated reactors [88,89] combined with the prior anaerobic digester. Thus, sulfur could be a new value-added byproduct of the ethanol production chain, and its commercialization may generate a new revenue source for the processing plants. Biodigested vinasse could also be a byproduct in a biorefinery: it could be used to formulate a fertilizer and be applied in the sugarcane crop, reducing or even eliminating the environmental impacts associated with the organic matter present in vinasse. Other byproducts from anaerobic digestion can also be considered, such as the generation of intermediary compounds of such bioprocess,

Table 8
Experimental data obtained in anaerobic digestion of vinasse in mesophilic and thermophilic conditions considering the production of ethanol from sugarcane.

Temp. (°C)	Configuration (Useful volume—L)	Vinasse origin	$COD_{influent}$[a] ($g\ L^{-1}$)	HRT[b] (d)	$OLR_{máx}$[c] ($g\ L^{-1}\ d^{-1}$)	ε_{COD}[d] (%)	η_{CH4}[e] ($NL\ g^{-1}$)	Reference
Mesophilic (32–37 °C)	AFBR with activated[f] carbon (0.75)	Cane molasses	33.0	0.3	10.0	76.0	0.032	[94]
	AFBR with zeolite (1.0)	Cane molasses	66.0	0.6	10.0	80.0	0.023	[94]
	UASB[g] (2.3)	Cane molasses	69.0	3.2	21.5	58.0	0.26	[95]
	UASB (11,000)	Cane molasses + juice	15.2	0.83	18.3	76.0	0.47	[58]
	UASB (11)	Cane juice	31.3	4.9	10.5	88.5	0.22	[60]
	Upflow anaerobic filter (21)	Cane juice	21.5	6.0	3.4	89.0	0.34	[96]
	Upflow floc digester (10)	Cane juice	30.0	1.2	25.4	70.0	0.29	[59]
Thermophilic (53–55 °C)	UASB (140)	Cane molasses	10.0	0.42	28.0	67.0	0.29	[97]
	UASB (70,000)	Cane molasses + juice	31.5	0.45	26.5	72.0	0.42	[48]
	HAIB[h] (2)		10	1.1	9.1	70.0	n.a.	[98]
	2-CSTR[i] (8.6)	Cane molasses	130	5.6	20.0	65.0	0.40	[99]

n.a. = not available.
[a] Influent chemical oxygen demand.
[b] Hydraulic retention time.
[c] Maximum organic loading rate.
[d] COD removal efficiency.
[e] Methane yield coefficient in $NL\ CH_4\ g\ COD_{removed}^{-1}$ (theoretical value = $0.35\ NL\ g^{-1}$).
[f] Anaerobic fluidized bed reactor.
[g] Upflow anaerobic sludge blanket.
[h] Anaerobic horizontal-flow immobilized biomass.
[i] Continuous stirred-tank reactor.

e.g., acetate, propionate, and butyrate, which could become value-added byproducts in a biorefinery. However, these suppositions are still only possibilities in sugarcane biorefineries because even the anaerobic digestion process is not yet consolidated in existing processing plants.

3.2.1. *Current stage of the research*

Some research on the anaerobic digestion of vinasse has been performed, considering different reactor configurations, operational conditions, and raw materials [89–95]. However, limited information is available when considering only vinasse from sugarcane processing. Table 8 depicts the most significant works regarding vinasse treatment in anaerobic reactors and their experimental results considering only the use of sugarcane as feedstock for ethanol production [48,58–60,94–99]. Most of the studies date from the mid-1980s and early 1990s, given that it is difficult to find recent data in the literature about the operation of anaerobic reactors treating sugarcane vinasse. The few recent experimental reports have focused on the evaluation of pretreatments for the removal of phenols or color [47,100] or on the assessment of the potential methane or hydrogen production [101,102]. Moreover, all of these studies were performed in bench-scale batch reactors (flasks), and they were not intended to provide conclusions about sugarcane vinasse anaerobic treatment (e.g., reactor configurations, and operational conditions).

Table 8 illustrates that the removal efficiency of organic matter in mesophilic and thermophilic conditions has comparable values in terms of the maximum organic loading rate (OLR_{max}) applied. However, specifically for UASB reactors, the highest OLR_{max} values were obtained for thermophilic treatment, as was also inferred by Bitton [103]. In addition, such treatment may be economically more advantageous because vinasse leaves the distillation process at a temperature of approximately 90 °C and, thus, cooling to 55 °C may occur naturally [61]. In contrast, in mesophilic conditions, forced cooling to temperatures lower than 40 °C is required. Although higher organic loads were applied in thermophilic conditions, higher COD removal efficiencies were observed in mesophilic reactors. Such efficiencies were obtained with a higher hydraulic retention time (HRT), which could make such an anaerobic digestion project spatially and economically unfeasible for industrial applications.

Regardless of the treatment conditions, another important topic lies in the vinasse composition in terms of organic matter (COD), as discussed in Section 2.3. The COD values in Table 8 for vinasse generated from cane juice and/or cane molasses differ considerably among the aforementioned scientific works, even when the same vinasse origin is considered. Similarly, methane yield data are also variable. Several factors can affect such parameters, such as vinasse composition, operational and environmental conditions, reactor configurations, and the microbial community established in the reactor. Among these factors, vinasse composition is perhaps the most crucial, considering the stage of research on sugarcane vinasse anaerobic digestion. Some authors have reported that the presence of recalcitrant compounds can be toxic or inhibitory for microorganisms, commonly phenols, melanoidins, and a variety of sugar decomposition products [99,100,104–106]. Additionally, some methane yield values higher than the theoretical value are indicative of cellular death, which may cause an overestimation of the methane production per unit of COD removed. However, other studies have asserted that acclimation is the key factor for the anaerobic treatment of wastewater containing toxic compounds [107–110], which may make this technology applicable to sugarcane vinasse. Thus, are such recalcitrant compounds truly (or solely) responsible for hampering the anaerobic treatment of sugarcane vinasse? The literature has provided only few reports of the feasible presence of another group of compounds that is more toxic to the anaerobic microbial consortium, namely, antibiotics. In Brazil, the application of antibiotics during alcoholic fermentation is common when bacterial contamination over the yeast activity is detected. Such application is typically made qualitatively. Thus, antibiotics are likely to be over- or under applied due to the lack of information and formal instruction in sugarcane processing plants [111]. If no thermolabile antibiotics are used, they will persist after the distillation process and remain in the vinasse. According to Oliva-Neto and Yokoya [112], penicillin, virginiamicin, and Kamoran HJ are the antibiotics typically applied in industrial fuel alcoholic fermentation in Brazil, as also reported for bioethanol fermentation from other feedstocks [111,113,114]. Additionally, other biocides can also be added for bacterial control, such as carbamates, quaternary ammonium compounds, and halogenated phenols [112], all of which are also potential inhibitors for the subsequent anaerobic digestion process.

Some research has reported the inhibition of acetogenesis and methanogenesis – steps in which the most sensitive microorganisms of anaerobic digestion act – for different wastewaters due to different types of antibiotics [115–118]. However, no studies have investigated sugarcane vinasse in particular. The presence of antibiotics may be noted in the few scientific works that characterize the composition of such liquid waste, but their specification and determination has not been reported thus far. The application of antibiotics during ethanol production is not constant. Thus, their presence in the vinasse is variable, which may hamper a standard characterization of such compounds in this effluent. Additionally, such discontinuity also impairs a possible adaptation of the anaerobic microbial population to these toxic compounds. Given this context, further studies on the characterization of antibiotics in sugarcane vinasse and their effect on anaerobic digestion are required.

The current stage of research on sugarcane vinasse anaerobic digestion is unsatisfactory. The few scientific studies are often inconclusive, even conflicting. The state of research on pentoses liquor is even less satisfactory; there is no information available in the literature about the operation of anaerobic reactors treating such liquid streams, except for the attempts of its use for the production of ethanol [29,119–121] or other products in a biorefinery [122,123]. The only information on pentoses liquor biodigestion consists of computational simulations [16,21,124,125] based on parameters obtained in bench-scale biomethane potential tests [126]. In these tests, pentoses liquor from two specific bagasse pretreatments was used and displayed potential for methane production from anaerobic digestion. A maximum CH_4 yield of 0.18 NL per gram of COD added was obtained for pentoses liquor from lime pretreatment, containing an initial COD of approximately $10\ g\ L^{-1}$. However, the authors highlighted that the highest global methane production per kilogram of bagasse was obtained with alkaline hydrogen peroxide (AHP) pretreatment ($72.1\ NL\ kg^{-1}$).

Considering that second-generation ethanol production is still in the development stage, available research on anaerobic digestion of second-generation sugarcane vinasse is either nonexistent or still in progress. Data in the literature are related to the anaerobic treatment of vinasse from other cellulosic feedstocks than sugarcane bagasse (e.g., eucalyptus, hardwoods, or pinus), as compiled earlier by Wilkie et al. [61]; new scientific studies in this area are currently scarce [127]. Wilkie et al. [61] highlights that such types of vinasse might contain high levels of metals and uncommon inhibitors after the pretreatment of cellulosic feedstocks, which may hamper the anaerobic biodigestion process. Nevertheless, a lack of further information hinders confident predictions.

3.2.2. *Challenges for full application*

Although anaerobic digestion appears to be a suitable and promising technology for the concept of a sugarcane biorefinery, there are several obstacles that hamper its implementation on a full industrial scale. The barriers can be categorized into four main reasons:

1) Current feasibility of disposing of vinasse *in natura* in sugarcane cultivation (fertirrigation);
2) Predominance of empirical approaches in the fundamental studies of anaerobic digestion of vinasse;
3) Unsatisfactory results obtained in the few full-scale anaerobic reactor plants;
4) Lack of valorization of biogas as an alternative energy source.

Fertirrigation in sugarcane crops has been the most traditional destination given to vinasse since the late 1970s. Despite its traditionalism, it is unclear whether one can safely assert that this action does not result in environmental impacts. However, few research groups in that period searched for alternative pathways, which served to reinforce that application [85,128–130]. Considering anaerobic digestion as an alternative pathway, one of the obstacles is the lack of environmental and economic stimulus for investment in large-scale plants for vinasse treatment. Such a lack of stimuli is partially related to the existence of only a few studies on anaerobic digestion of vinasse thus far: the limited knowledge of the fundamental aspects of this process hampers the formation of a solid scientific base to provide efficient and optimized treatment on a large scale. In addition, the environmental benefits of this application have not yet been clearly quantified and disclosed.

The poor understanding of the fundamental aspects of vinasse anaerobic digestion is a result of the scarce research in this area. The few current bench-scale studies have mainly been performed using a "black-box" approach, neglecting the kinetic aspects of cellular growth, substrate utilization, and product formation, as well as mass transfer phenomena and the hydrodynamics of actual reactors. According to [131], such an empirical approach only allows an interpolation of the results, whereas an experimental-mechanistic approach (based on the fundamental elucidation of the processes involved) allows for both interpolation and extrapolation. Thus, the application of experimental-mechanistic approaches is not restricted to the experimental conditions of the "black-box" approach. López and Borzacconi [95] conducted one of the only studies found in which such a "black-box" investigation was performed in the field of vinasse biodigestion; the investigation was performed by modeling an expanded granular sludge bed (EGSB) anaerobic reactor using a simple model with two steps (acidogenesis and methanogenesis), two populations, two substrates, and completely mixed conditions. In addition to this scarcity of research, the variability of vinasse composition when comparing different experiments available in the literature provides inconsistent or even conflicting results, as demonstrated in Section 3.2.1.

The lack of fundamental data governing the global conversion of the bioprocess has led to inefficient reactor design since this technology was first considered in the late nineteenth century. The failure to comprehend the complexity of anaerobic digestion process was the main factor leading to mistakes in the past. The application of unsuitable configurations of reactors and the lack of fundamental engineering information related to the operation of anaerobic reactors applied to vinasse treatment discouraged the establishment of full-scale reactors in the plants. This situation is reflected in the current near-absence of anaerobic digestion plants in sugarcane biorefineries. Therefore, technology and science should join together in the quest to find a common objective of developing efficient, safe, and reliable anaerobic reactors, always searching for conclusive answers and consolidated technologies. An approach focusing on experimental support for models based on fundamental phenomena may allow for the discovery of the "limits" of anaerobic treatment, as well as the best engineering conditions for performance optimization, eliminating or reducing the risk of attributing the "burden of failure" of an anaerobic treatment system to the organisms or to chance [132].

The low appreciation of biogas as an alternative energy source is also a factor that discourages the application of anaerobic digestion of vinasse. According to Salomon and Lora [133], the lack of a national biogas program, specific funding, and incentives from the government in conjunction with the difficulties faced by small biogas plants in commercializing their carbon credits and the high associated investment costs are among the main obstacles to the use of biogas in Brazil. In the case of electric energy from biogas, for example, its commercialization is hampered by the current relatively low price paid for new energy sources through public auctions for renewable energy in Brazil compared to traditional energy sources. According to Cruz et al. [11], with a lower profit margin than other energy sources, such as wind, biomass, bagasse, and sugarcane straw, projects using biogas from biodigesters for power generation, although economically feasible, have remained absent from recent energy auctions. Therefore, additional government incentives for renewable energy could be directed to add value to this type of energy. Another option to stimulate the trade market of energy from biogas would be the adoption of vinasse biodigestion in partnership with electric utilities, as currently occurs with sugarcane bagasse cogeneration in the state of São Paulo [11].

Other biogas applications were demonstrated by Moraes et al. [134], who assessed the potential of using energy from biogas derived from the anaerobic digestion of first-generation vinasse in cogeneration systems, for electricity generation, and as diesel replacement. The best scenario for a sugarcane biorefinery, considering energy, environmental, and economic aspects, was biogas applied as a replacement for diesel. However, the authors question the current stage of development in the area of biogas engines, highlighting that further research is needed to implement such an application for biogas.

Therefore, the first step toward changing some consolidated but obsolete principles related to sugarcane vinasse from first-generation ethanol production is increasing the efforts from the scientific community to further investigate the field of anaerobic digestion of vinasse and its potential biogas applications. It is important that this investigation occurs in conjunction with practical applications so that it contributes to technological development. In this manner, further fundamental engineering knowledge will enable the demonstration of potential benefits, which may boost the full application of this process, as well as some incentives from the government and environmental agencies. This scenario must also be extended to the other liquid streams from first- and second-generation sugarcane biorefineries to structure a more sustainable bioethanol sector in Brazil in the near future.

3.2.3. *Short-term approach*

Considering the current status of research and applications of anaerobic digestion of the liquid streams from the sugarcane ethanol production process in Brazil, the immediate actions that can be taken are related to the fundamental knowledge of such processes. Thus, further research on the fundamental aspects governing biological conversions should be pursued to investigate the kinetic characterization and optimization of anaerobic digestion as applied to vinasses and pentoses liquor. An experimental-mechanistic approach should be taken considering mass transfer fluxes, the intrinsic kinetics involved in organic matter conversion and microbial growth, and the hydrodynamic behavior of the reactor, as well as a detailed investigation into the microbiology and biochemistry of the conversion processes [132]. From this starting point, it would be possible to project the design and progressive scale-up of the process more successfully based on previous rational scientific research. The empirical design criteria used now would be secondary, as the validated rational models would be the main inputs for safer anaerobic system projects. It would be possible to develop a detailed model of the anaerobic digestion process as applied to the liquid streams of sugarcane biorefineries. Such information would be useful for feed tool simulations that allow for the rapid verification and/or validation of models from the literature and could serve as a tool for application in industrial units.

The development and implementation of technology solutions that are more appropriate and optimized, with a stronger theoretical base, are essential for maintaining the low production costs of ethanol from sugarcane and for leveraging the sustainability of enterprises in the area. In this sense, the Technological Assessment Program (PAT) of the Brazilian Bioethanol Science and Technology Laboratory (CTBE) is developing a tool that allows for the measurement of the stage of development and its success, as well as the improvement of new technologies applied to the production of ethanol and other products derived from sugarcane. This tool, the Virtual Sugarcane Biorefinery (BVC), consists of a custom computing platform, built using techniques from mathematical modeling and computer simulations of processes that should be used to assess the impacts of technologies when applied to the production chain of the sugar and ethanol industry [18]. The use of computer simulations of processes should enable the evaluation of various scenarios and technological routes in less time and with lower costs when compared to laboratory experiments or pilot plant operation; this approach represents a useful tool for preliminary studies of new processes and for the optimization of operating conditions.

Thus, tool simulation should also be used as a short-term action in the area of anaerobic digestion of the liquid streams of sugarcane biorefineries. This has already been carried out by the above cited institution (CTBE); however, in these preliminary simulations, anaerobic digestion has been considered as a "black-box" due to the lack of deeper information and knowledge about this bioprocess. Data about kinetic aspects and reactor hydrodynamics should be included in the simulations to provide more accurate results. This discussion highlights the need for fundamental research in this area as a short-term measure.

4. Biogas from vinasse in the context of biorefineries

Successful experiences in biogas production and its energy conversion have been reported, especially in the European Union (EU). Landfills, sewage sludge, decentralized agricultural plants, municipal solid waste, methanization plants, co-digestion, and multi-product plants are the most common sources used. The main forms of biogas recovery in these countries are electricity and heat production through cogeneration, which have increased in recent years. In 2011, the gross electricity production from biogas reached 35.9 TW h, 18.4% higher than the previous year, while biogas heat sales to factories or heating networks increased by 52.2% in the same period [135]. The main applications for biogas heat are on site in the biogas plants, for drying sludge, heating buildings, and maintaining optimum digester temperatures. In this scenario, Germany leads the primary production of biogas, with more than 50% of the total production, which is a reflection of the favorable support schemes related to government incentives. Sweden is a leader in using biogas as biofuel for transport: approximately 13% of the total fuel consumption for transport was provided from biogas in 2011 [135]. A third use for biogas that is dawning in the EU countries is its injection into the natural gas grids as biomethane (purified biogas), which appears to be the trend for the next few years.

The European Union case can be taken as a successful reference for biogas use within biorefineries in Brazil, considering all potential biogas applications. In this manner, the energy balance of a biorefinery can be improved considerably through biogas production and its energy conversion. According to Moraes et al. [134], for a single ethanol processing plant that processes 2.0 million tons of sugarcane per season, the potential power generated from biogas production would be approximately 18 MW per season, considering a CH_4 content of 60% (v/v) in the biogas and a low heat value of 21,500 kJ N m^{-3}. In national terms, this potential would reach approximately 3500 MW per season if all of the vinasse generated in Brazil were biodigested. This value corresponds to approximately 1% of the entire Brazilian internal energy supply from renewable sources, which was 120.2 Mtep in 2012 [136], including hydraulic energy and electricity, sugarcane biomass, firewood and charcoal, and other renewables. Within the latter source (in which biogas is classified), the energy from biogas from only vinasse in Brazil accounts for approximately 13% of the renewable category, totaling 11.8 Mtep for the same year.

Based on the same work cited above [134], when biogas generated from vinasse biodigestion in a single sugarcane biorefinery is applied for electricity generation in stationary engines (38% efficiency) and cogeneration in industrial boilers (30% efficiency), the energy generated would reach approximately 27,000 MW h (6.9 MW) and 22,000 MW h (5.4 MW), respectively, per season. These values are comparable with a successful case of anaerobic digestion of vinasse from the cognac production of an industrial plant located in Bordeaux, France. This plant, Revico, receives vinasse from different cognac producers from a specific region of Bordeaux. During the season (April to August), between 500,000 and 600,000 m^3 of vinasse (with 22 kg COD m^{-3} on average) is received and converted into biogas, energy, and byproducts. Annually, 20,000 MW h is produced from biogas from the anaerobic digesters and is applied for both steam generation and cogeneration (45% efficiency for heat generation and 33% efficiency for electricity generation). The heat produced, approximately 8500 MW h per year, is used in situ in the industrial plant, whereas the electricity is sold to the grid (approximately 3300 MW h per year).

Considering the Brazilian scenario, Salomon and Lora [86] reported that the potential electricity generation from the anaerobic digestion of all vinasse generated would be approximately 820 or 880 MW if biogas was used in microturbines (27% efficiency) or engine generators (29% efficiency), respectively. Similarly, Moraes et al. [134] presented electricity generation values of approximately 1300 and 1000 MW when biogas is burned in stationary engines (38% efficiency) and industrial boilers (30% efficiency), respectively. These values correspond to between 15% and 19% of all electricity produced from biogas in EU countries in 2011 (derived from different sources, as previously reported), which highlights the great potential of energy generation from vinasse in Brazil. Considering the Brazilian electric matrix, this energy range corresponds to 14–17% of the electric generation from biomass, estimated as 40.3 TW h in 2012, according to the National Energy Balance [136].

Salomon et al. [133] also assessed different alternatives for biogas use in a sugarcane biorefinery. For electricity generation, approximately 5.4 and 5.8 MW would be generated if biogas were used in reciprocating combustion engines (30% efficiency) and gas microturbines (32% efficiency), respectively. The use of biogas for yeast drying was also an interesting and economically attractive alternative: approximately 55% of the internal rate of return would be achieved with dried yeast sales, a market that has been growing mainly for animal feed. However, the biogas generated in the reactor for this scenario would be sufficient to dry an amount of yeast 13 times greater than the amount produced in a conventional sugarcane mill in Brazil. Rocha et al. [137] performed a case study of a Brazilian sugarcane processing plant (2.0 million tons of sugarcane per season) considering the anaerobic digestion of vinasse and biogas applied to electricity generation as one of the alternatives: approximately 4.5 MW or 20,000 MW h would be produced considering a power generator system with a capacity of 17 kW h per m^3 of treated vinasse.

De Souza et al. [138] assessed the potential for biogas use in urban buses in Brazil. According to the authors, the biogas produced from the biodigestion of vinasse from the biggest sugarcane processing plant in Brazil (São Martinho) could supply a bus fleet of nearly 800 vehicles, considering buses with a 400 km range and a daily biogas consumption of approximately 290 N m^{-3} per bus. In broader terms, the biogas that could be produced from all of the vinasse generated in Brazil, estimated as approximately 4 billion Nm^3 of biogas per season, would be sufficient to replace 50% of the Brazilian urban bus fleet. Similarly, Moraes et al. [134] assessed the use of biogas as an alternative fuel in a sugarcane biorefinery, replacing the diesel demand in agricultural operations and the transport of sugarcane, inputs, and vinasse. Considering the trucks used to transport sugarcane from the field to the processing plant, the biogas produced for a single sugarcane plant would be sufficient to supply fleets consisting of up to 249 *rodotrens* or 307 *treminhões*. In national terms, the values would be equivalent to approximately 48,000 *rodotrens* and 59,000 *treminhões* (given the energy equivalence of 1 N m^{-3} of biogas to 0.55 L of diesel).

In the case of cogeneration, biogas can still replace part of the bagasse used for steam generation. As an energetic comparison, 298.5 N m^{-3} of biogas (70% CH_4 v/v) produced from vinasse is equivalent to the burning of 1 t of bagasse (50% moisture). The leftover bagasse could be used for second-generation ethanol production, increasing the ethanol productivity of the sugarcane biorefinery. According to Moraes et al. [134], the anaerobic digestion of vinasse from sugarcane biorefineries with a capacity of 2 million tons of sugarcane per season would produce approximately 14.5×10^6 m^3 of biogas (60% CH_4 v/v; 55 °C), which would release nearly 12% of the bagasse from burning.

Apart from the energetic gains, economic and environmental indicators reinforce the advantages of using biogas in the context of a biorefinery (Table 9) [87,134,139]. The data from economic and environmental assessments are scarce, as this concept is still being introduced in the Brazilian bioethanol sector. Regardless, the first results illustrate that the use of biogas in biorefineries could increase its profitability, consistently reducing the production costs, and reduce some environmental impacts as well.

5. Future prospects

The concept of biorefineries is increasingly spreading among the scientific community [4,82,83,140,141]. According to Corrêa do Lago et al. [4], the biorefinery concept embraces a wide range of technologies able to separate biomass or biomass-derived resources into their building blocks (i.e., lignin, cellulose, and hemicellulose), which can be converted into energy and material products (e.g., ethanol, sugar, and others). According to the authors, a future biorefinery model should be designed to produce renewable substitutes for the oil and chemical industry with economic, social, and environmental benefits, as presented in Fig. 4. In this sense, anaerobic digestion can be integrated in this concept, although such an approach has not yet been realized effectively. This integration is expected to occur over the long term, with full-scale industrial anaerobic reactors in operation in sugarcane biorefineries in Brazil. In the short and medium term, it is envisaged that the fundamentals of anaerobic digestion as applied to the liquid streams of sugarcane mills will be better clarified and understood upon the development bench-scale reactors that operate stably and

Table 9
Summary of the main economic and environmental results of the production and use of biogas from vinasse within the sugarcane biorefinery concept.

Biogas use	IRR per year (%)	Anhydrous ethanol cost (US$ L^{-1})	Avoided GHG emissions (t CO_{2eq} $year^{-1}$)	Reference
Base scenario[a]	11.9	0.379	0	[134][b]
Electricity	12.2	0.365	19,100	
Cogeneration	11.8	0.377	20,100	
Diesel replacement	12.3	0.365	25,000	
Base scenario[a]	11.6	0.367	0	[139][b]
Electricity	12.0	0.345	19,800	
Cogeneration	12.2	0.345	21,100	
Diesel replacement	12.7	0.336	28,250	
Biomethane sale	12.6	0.341	14,700	
Base scenario[a]	16.8	–	–	[87][c]
Cogeneration	18.2	–	–	

[a] Base sugarcane mill with no biogas production.
[b] Based on first-generation annexed biorefineries processing 2 mi TC per season.
[c] Based on first- and second-generation annexed biorefineries processing 2 mi TC per season.

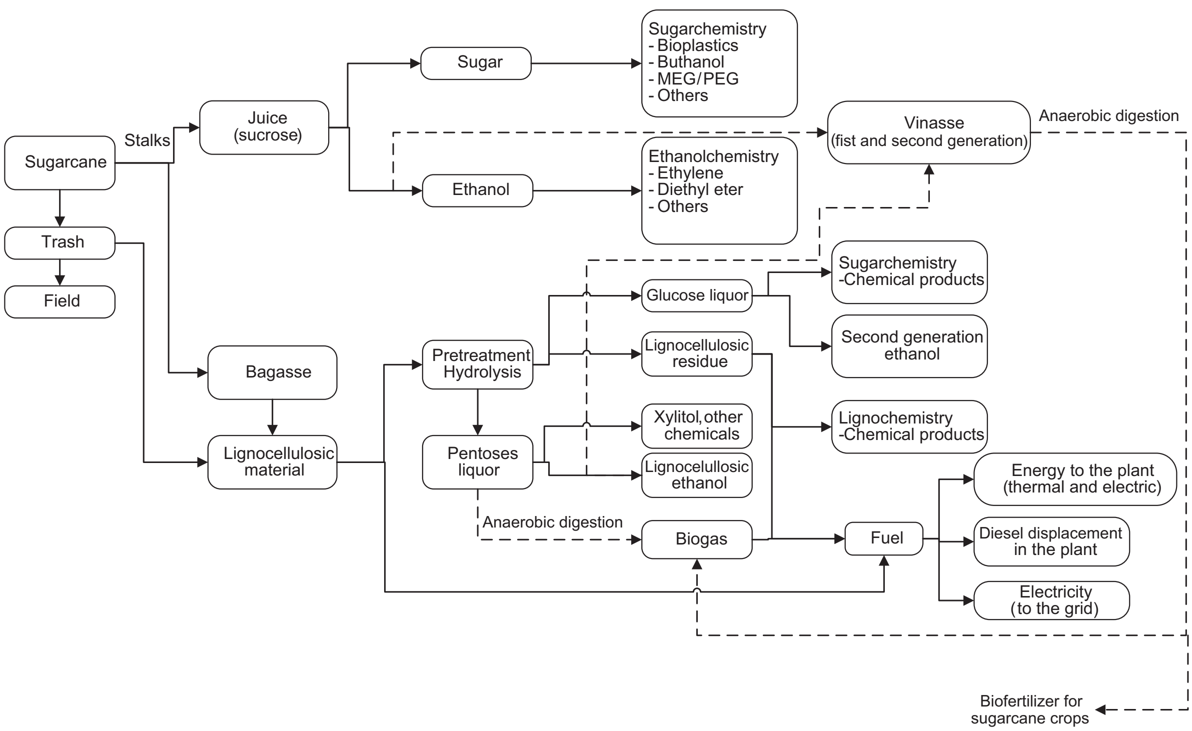

Fig. 4. Simplified process scheme of a conceptual future sugarcane biorefinery, including anaerobic digestion of the liquid streams, indicated by the dotted lines (Modified from Corrêa do Lago et al. [4]. Copyright 2012, with permission of Elsevier).

efficiently. The bottlenecks that impair the satisfactory production of biogas and organic matter removal from the anaerobic reactors must be overcome in such a period.

Further technological assessments using simulation tools are also expected in the short-term considering the environmental, energy, economic, and social aspects of sugarcane biorefineries with different scenarios, including an anaerobic digestion process. These scenarios could cover different considerations, e.g., different reactor configurations, operational conditions, and biogas applications, as well as integrated first- and second-generation or stand-alone biorefineries. Thus, simulations of the anaerobic digestion of vinasses and pentoses liquor as part of the ethanol production chain will work as a tool for preliminary studies of new processes and the optimization of operational conditions, aimed toward future industrial application.

6. Concluding remarks

For ethanol production to be described as a sustainable process, considerations about the treatment and use of the liquid streams from the production process are essential. The anaerobic treatment process is a sustainable option for the treatment and utilization of organic waste due to its low power consumption, the possibility to produce energy and by-products, and the potential to lower emission factors for greenhouse gases, given the suitable treatment of vinasse. This notion fits into the concept of a sugarcane biorefinery, integrating ethanol production, energy recovery, and the generation of value-added by-products. Set in this context, the anaerobic digestion of vinasse (from first- and second-generation ethanol production) and pentoses liquor presents itself as an interesting alternative for the utilization of the residue in conjunction with the energy recovery from biogas. However, some obstacles and bottlenecks must be overcome to achieve full industrial application in the future. For this to occur, combined incentives from the government and environmental agencies, and the support of the scientific community are required, without which the practical and widespread application of such a process into the ethanol production chain may become hampered or even unfeasible.

Acknowledgements

This work was supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) process no. 2012/00414-7.

References

[1] Moreira JR. Sugarcane for energy—recent results and progress in Brazil. Energy Sustainable Dev 2000;4:43–54.
[2] Coelho ST, Goldemberg J, Lucon O, Guardabassi P. Brazilian sugarcane ethanol: lessons learned. Energy Sustainable Dev 2006;10:26–39.
[3] Amorim H, Lopes M, de Castro Oliveira J, Buckeridge M, Goldman G. Scientific challenges of bioethanol production in Brazil. Appl Microbiol Biotechnol 2011;91:1267–75.
[4] Corrêa do Lago A, Bonomi A, Cavalett O, Pereira da Cunha M, Pinheiro Lima MA. Sugarcane as a carbon source: the Brazilian case. Biomass Bioenergy 2012;46:5–12.
[5] Provides for control of the release of vinasse in water collection Brazil; Nov 29 1978. [In Portuguese].
[6] Environmental Sanitation Technology Company (Companhia de Tecnologia de Saneamento Ambiental). Vinasse—criteria and procedures for application to agricultural soil. Technical norm P4231. São Paulo: CETESB; 2006. [in Portuguese].
[7] Madejón E, López R, Murillo JM, Cabrera F. Agricultural use of three (sugar-beet) vinasse composts: effect on crops and chemical properties of a Cambisol soil in the Guadalquivir river valley (SW Spain). Agric Ecosyst Environ 2001;84:55–65.
[8] Madrid L, Díaz-Barrientos E. Release of metals from homogeneous soil columns by wastewater from an agricultural industry. Environ Pollut 1998;101:43–8.
[9] Alves ME, Lavorenti A. Sulfate adsorption and its relationships with properties of representative soils of the São Paulo State, Brazil. Geoderma 2004;118:89–99.
[10] Ribeiro BT, Lima JMd, Guilherme LRG, Julião LGF. Lead sorption and leaching from an Inceptisol sample amended with sugarcane vinasse. Sci Agric 2010;67:441–7.
[11] Cruz R, Righetto A, Nogueira M. Experimental investigation of soil and ground water impacts caused by vinasse disposal. Water Sci Technol 1991;24:77–85.
[12] Lyra MR, Rolim MM, Silva JA. Toposequence of soils fertigated with stillage: contribution towards the quality of ground water table. Rev Bras Eng AgrícAmbient 2003;7:525–32.
[13] da Silva MA, Griebeler NP, Borges LC. Use of stillage and its impact on soil properties and groundwater. Rev Bras Eng Agríc Ambient 2007;11:108–14.
[14] España-Gamboa E, Mijangos-Cortes J, Barahona-Perez L, Dominguez-Maldonado J, Hernández-Zarate G, Alzate-Gaviria L. Vinasses: characterization and treatments. Waste Manage Res 2011;29:1235–50.
[15] Goldemberg J, Guardabassi P. The potential for first-generation ethanol production from sugarcane. Biofuels, Bioprod Biorefin 2010;4:17–24.
[16] Dias MOS, Junqueira TL, Jesus CDF, Rossell CEV, Maciel Filho R, Bonomi A. Improving second generation ethanol production through optimization of first generation production process from sugarcane. Energy 2012;43:246–52.
[17] National Supply Company (Companhia Nacional de Abastecimento). Profile of sugar and ethanol sector in Brazil—Edtion for 2009–2010 harvesting season. Brasília: CONAB; 2012. 62p. [in Portuguese]. 〈http://www.conab.gov.br/conabweb/download/safra/perfil.pdf〉 [cited 25.06.13].
[18] Bonomi A, Dias MOS, Junqueira TL, Cardoso TF, Cavalett O, Franco HCJ, et al. The virtual sugarcane biorefinery (VSB)—2011 report [Internet]. Campinas: Brazilian Bioethanol Science and Technology Laboratory (CTBE), Technological Assessment Program (PAT); 2012. 128 p. [cited 25.06.13].〈https://dl.dropbox.com/u/129726003/Informa%C3%A7%C3%B5es%20Institucionais/Virtual%20Sugarcane%20Biorefinery%20Report%202011.pdf〉.
[19] Rein P, Attard R. Cane sugar engineering: Bartens 2007.
[20] Soccol CR, Vandenberghe LPdS, Medeiros ABP, Karp SG, Buckeridge M, Ramos LP, et al. Bioethanol from lignocelluloses: status and perspectives in Brazil. Bioresour Technol 2010;101:4820–5.
[21] Dias MOS, Junqueira TL, Rossell CEV, Maciel Filho R, Bonomi A. Evaluation of process configurations for second generation integrated with first generation bioethanol production from sugarcane. Fuel Process Technol 2013;109:84–9.
[22] Cardona CA, Quintero JA, Paz IC. Production of bioethanol from sugarcane bagasse: status and perspectives. Bioresour Technol 2010;101:4754–66.
[23] Gírio FM, Fonseca C, Carvalheiro F, Duarte LC, Marques S, Bogel-Łukasik R. Hemicelluloses for fuel ethanol: a review. Bioresour Technol 2010;101:4775–800.
[24] Dermibas A. Bioethanol from cellulosic materials: a renewable motor fuel from biomass. Energy Sources 2005;27:327–37.
[25] Velmurugan R, Muthukumar K. Utilization of sugarcane bagasse for bioethanol production: sono-assisted acid hydrolysis approach. Bioresour Technol 2011;102:7119–23.
[26] Mosier N, Wyman C, Dale B, Elander R, Lee Y, Holtzapple M, et al. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol 2005;96:673–86.
[27] Wyman CE, Dale BE, Elander RT, Holtzapple M, Ladisch MR, Lee Y. Coordinated development of leading biomass pretreatment technologies. Bioresour Technol 2005;96:1959–66.
[28] Rabelo SC, Maciel Filho R, Costa AC. A comparison between lime and alkaline hydrogen peroxide pretreatments of sugarcane bagasse for ethanol production. Appl Biochem Biotechnol 2008;148:45–58.
[29] Balat M. Production of bioethanol from lignocellulosic materials via the biochemical pathway: a review. Energy Convers Manage 2011;52:858–75.
[30] Zheng Y, Pan Z, Zhang R. Overview of biomass pretreatment for cellulosic ethanol production. Int J Agric Biol Eng 2009;2:51–68.
[31] Kumar P, Barrett DM, Delwiche MJ, Stroeve P. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. Ind Eng Chem Res 2009;48:3713–29.
[32] Sánchez ÓJ, Cardona CA. Trends in biotechnological production of fuel ethanol from different feedstocks. Bioresour Technol 2008;99:5270–95.
[33] Hendriks ATWM, Zeeman G. Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour Technol 2009;100:10–8.
[34] Alvira P, Tomás-Pejó E, Ballesteros M, Negro MJ. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. Bioresour Technol 2010;101:4851–61.
[35] Agbor VB, Cicek N, Sparling R, Berlin A, Levin DB. Biomass pretreatment: fundamentals toward application. Biotechnol Adv 2011;29:675–85.
[36] Galbe M, Zacchi G. Pretreatment of lignocellulosic materials for efficient bioethanol production. In: Olsson L, editor. Biofuels. Springer Berlin Heidelberg; 2007. p. 41–65.
[37] Zhao X, Cheng K, Liu D. Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. Appl Microbiol Biotechnol 2009;82:815–27.
[38] Laser M, Schulman D, Allen SG, Lichwa J, Antal Jr MJ, Lynd LR. A comparison of liquid hot water and steam pretreatments of sugar cane bagasse for bioconversion to ethanol. Bioresour Technol 2002;81:33–44.
[39] Sun Y, Cheng J. Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour Technol 2002;83:1–11.
[40] Barakat A, Monlau F, Steyer J-P, Carrere H. Effect of lignin-derived and furan compounds found in lignocellulosic hydrolysates on biomethane production. Bioresour Technol 2012;104:90–9.
[41] Chiaramonti D, Prussi M, Ferrero S, Oriani L, Ottonello P, Torre P, et al. Review of pretreatment processes for lignocellulosic ethanol production, and development of an innovative method. Biomass Bioenergy 2012;46:25–35.
[42] Scott F, Quintero J, Morales M, Conejeros R, Cardona C, Aroca G. Process design and sustainability in the production of bioethanol from lignocellulosic materials. Electron J Biotechnol 2013;16:13.
[43] Bacovsky D, Dallos M, Wöergetter M. Status of 2nd generation biofuels demonstration facilities in June 2010. A report to IEA Bioenergy Task 39: commercializing 1st and 2nd generation liquid biofuels from biomass. Report T39-P1b; 2010. 126p. 〈http://task39.org/publications/〉 [cited 04.11.2014].
[44] Rabelo SC. Evaluation and optimization of pretreatments and enzymatic hydrolysis of the sugarcane bagasse for second generation ethanol production. (PhD thesis). Department of Chemical Process, School of Chemical Engineering, University of Campinas; 2010 ([in Portuguese]).
[45] Menon V, Rao M. Trends in bioconversion of lignocellulose: biofuels, platform chemicals & biorefinery concept. Prog Energy Combust Sci 2012;38:522–50.
[46] Hahn-Hägerdal B, Galbe M, Gorwa-Grauslund MF, Lidén G, Zacchi G. Bio-ethanol—the fuel of tomorrow from the residues of today. Trends Biotechnol 2006;24:549–56.
[47] Romanholo Ferreira LF, Aguiar MM, Messias TG, Pompeu GB, Queijeiro Lopez AM, Silva DP, et al. Evaluation of sugar-cane vinasse treated with Pleurotus sajor-caju utilizing aquatic organisms as toxicological indicators. Ecotoxicol Environ Saf 2011;74:132–7.
[48] Souza M, Fuzaro G, Polegato A. Thermophilic anaerobic digestion of vinasse in pilot plant UASB reactor. Water Sci Technol 1992;25:213–22.
[49] Cavalett O, Junqueira T, Dias MS, Jesus CF, Mantelatto P, Cunha M, et al. Environmental and economic assessment of sugarcane first generation biorefineries in Brazil. Clean Technol Environ Policy 2012;14:399–410.
[50] National Supply Company (Companhia Nacional de Abastecimento). The thermoelectric generation by burning sugarcane bagasse in Brazil—performance analysis of 2009–2010 harvesting season. Brasília: CONAB; 2011. 160p. [in Portuguese]. 〈http://www.conab.gov.br/OlalaCMS/uploads/arquivos/11_05_05_15_45_40_geracao_termo_baixa_res.pdf〉 [cited 26.06.13].
[51] de Oliveira BG, Carvalho JLN, Cerri CEP, Cerri CC, Feigl BJ. Soil greenhouse gas fluxes from vinasse application in Brazilian sugarcane areas. Geoderma 2013;200–201:77–84.
[52] Carmo JBd, Filoso S, Zotelli LC, de Sousa Neto ER, Pitombo LM, Duarte-Neto PJ, et al. Infield greenhouse gas emissions from sugarcane soils in Brazil: effects from synthetic and organic fertilizer application and crop trash accumulation. GCB Bioenergy 2013;5:267–80.

[53] Sheehan GJ, Greenfield PF. Utilisation, treatment and disposal of distillery wastewater. Water Res 1980;14:257–77.
[54] Dowd MK, Johansen SL, Cantarella L, Reilly PJ. Low molecular weight organic composition of ethanol stillage from sugarcane molasses, citrus waste, and sweet whey. J Agric Food Chem 1994;42:283–8.
[55] Benke MB, Mermut AR, Chatson B. Carbon-13 CP/MAS NMR and DR-FTIR spectroscopic studies of sugarcane distillery waste. Can J Soil Sci 1998;78:227–36.
[56] Decloux M, Bories A. Stillage treatment in the French alcohol fermentation industry. Int Sugar J 2002;104:509–17.
[57] Parnaudeau V, Condom N, Oliver R, Cazevieille P, Recous S. Vinasse organic matter quality and mineralization potential, as influenced by raw material, fermentation and concentration processes. Bioresour Technol 2008;99:1553–62.
[58] Costa F, Rocha BBM, Viana C, Toledo A. Utilization of vinasse effluents from an anaerobic reactor. Water Sci Technol 1986;18:135–41.
[59] Cail RG, Barford JP. A comparison of an upflow floc (tower) digester and UASB system treating cane juice stilage. Agric Wastes 1985;14:291–9.
[60] Craveiro AM, Soares HM, Schmidell W. Technical aspects and cost estimations for anaerobic systems treating vinasse and brewery/soft drink wastewaters. Water Sci Technol 1986;18:123–34.
[61] Wilkie AC, Riedesel KJ, Owens JM. Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks. Biomass Bioenergy 2000;19:63–102.
[62] Luz PHC, Vitti GC. Manejo e uso de fertilizantes para cana-de-açúcar. In: Marques MO, et al., editors. Tecnologias na agricultura canavieira. Jaboticabal: FCAV; 2008. p. 141–67.
[63] Oliveira FMV, Pinheiro IO, Souto-Maior AM, Martin C, Gonçalves AR, Rocha GJM. Industrial-scale steam explosion pretreatment of sugarcane straw for enzymatic hydrolysis of cellulose for production of second generation ethanol and value-added products. Bioresour Technol 2013;130:168–73.
[64] Rocha GJM, Gonçalves AR, Oliveira BR, Olivares EG, Rossell CEV. Steam explosion pretreatment reproduction and alkaline delignification reactions performed on a pilot scale with sugarcane bagasse for bioethanol production. Ind Crops Prod 2012;35:274–9.
[65] Cammarota MC, CAMPORESE SEF, DA CGA, De CAL, Groposo SCJ, Machado DCA, et al. Method for producing energy-rich gases from lignocellulosic material streams. Google patents 2012.
[66] Santa ALMM, Pereira N, Da Conceicai Gomes A, Vasques MP. Process for the fermentative production of ethanol from solid lignocellulosic material comprising a step of treating a solid lignocellulosic material with alkaline solution in order to remove the lignin. Google patents 2008.
[67] Mariano AP, Dias MOS, Junqueira TL, Cunha MP, Bonomi A, Filho RM. Utilization of pentoses from sugarcane biomass: techno-economics of biogas vs. butanol production. Bioresour Technol 2013;142:390–9.
[68] Speece RE. Anaerobic Biotechnology for Industrial Wastewaters. Archae Press; 1996.
[69] Leitão RC, van Haandel AC, Zeeman G, Lettinga G. The effects of operational and environmental variations on anaerobic wastewater treatment systems: a review. Bioresour Technol 2006;97:1105–18.
[70] O'Flaherty V, Collins G, Mahony T. The microbiology and biochemistry of anaerobic bioreactors with relevance to domestic sewage treatment. Rev Environ Sci Biotechnol 2006;5:39–55.
[71] Madsen M, Holm-Nielsen JB, Esbensen KH. Monitoring of anaerobic digestion processes: a review perspective. Renewable Sustainable Energy Rev 2011;15:3141–55.
[72] Mosey F. New developments in the anaerobic treatment of industrial wastes. Water Pollut Control 1982;81:540–52.
[73] Guiot S, Pauss A, Costerton J. A structured model of the anaerobic granule consortium. Water Sci Technol 1992;25:1–10.
[74] Wirth R, Kovacs E, Maroti G, Bagi Z, Rakhely G, Kovacs K. Characterization of a biogas-producing microbial community by short-read next generation DNA sequencing. Biotechnol Biofuels 2012;5:41.
[75] Decker K, Jungermann K, Thauer R. Energy production in anaerobic organisms. Angew Chem Int Ed Engl 1970;9:138–58.
[76] Thauer RK, Jungermann K, Decker K. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol Rev 1977;41:100.
[77] McCarty P, Mosey F. Modelling of anaerobic digestion processes (a discussion of concepts). Water Sci Technol 1991;24:17–33.
[78] Foresti E. Fundamentals of anaerobic digestion process. In: Proceedings of III Taller y Seminario Latinoamericano de Tratamiento Anaeróbio de Aguas Residuales. Motevideo, Uruguay; 1994. [in Portuguese].
[79] Rajeshwari KV, Balakrishnan M, Kansal A, Lata K, Kishore VVN. State-of-the-art of anaerobic digestion technology for industrial wastewater treatment. Renewable Sustainable Energy Rev 2000;4:135–56.
[80] Fair GM, Moore EW. Time and rate of sludge digestion, and their variation with temperature. Sewage Works J 1934:3–13.
[81] Merlin Christy P, Gopinath LR, Divya D. A review on anaerobic decomposition and enhancement of biogas production through enzymes and microorganisms. Renewable Sustainable Energy Rev 2014;34:167–73.
[82] Cherubini F. The biorefinery concept: using biomass instead of oil for producing energy and chemicals. Energy Convers Manage 2010;51:1412–21.
[83] Luo G, Talebnia F, Karakashev D, Xie L, Zhou Q, Angelidaki I. Enhanced bioenergy recovery from rapeseed plant in a biorefinery concept. Bioresour Technol 2011;102:1433–9.
[84] Ryan D, Gadd A, Kavanagh J, Barton GW. Integrated biorefinery wastewater design. Chem Eng Res Des 2009;87:1261–8.
[85] Walter A. Potential of electricity production from sugarcane residues. Luis Augusto Barbosa Cortez (Org.). Sugarcane bioethanol: R&D for productivity and sustainability. 1st ed. São Paulo, SP, Brasil: Editora Blucher; 2010. p. 577–82.
[86] Salomon KR, Silva Lora EE. Estimate of the electric energy generating potential for different sources of biogas in Brazil. Biomass Bioenergy 2009;33:1101–7.
[87] Junqueira TL, Dias MOS, Cavalett O, Cunha MP, Jesus CDF, Mantelatto PE, et al. Maximizing feedstock availability for second generation in integrated first and second generation ethanol production from sugarcane. In: Poster presentation on 34th symposium on biotechnology for fuels and chemicals. New Orleans, USA; 2012.
[88] Diaz I, Lopes A, Pérez S, Fdz-Polanco M. Determination of the optimal rate for the microaerobic treatment of several H2S concentrations in biogas from sludge digesters. Water Sci Technol 2011;64:233.
[89] Vlissidis A, Zouboulis AI. Thermophilic anaerobic digestion of alcohol distillery wastewaters. Bioresour Technol 1993;43:131–40.
[90] Bories A, Raynal J, Bazile F. Anaerobic digestion of high-strength distillery wastewater (cane molasses stillage) in a fixed-film reactor. Biol Wastes 1988;23:251–67.
[91] Boopathy R, Tilche A. Anaerobic digestion of high strength molasses wastewater using hybrid anaerobic baffled reactor. Water Res 1991;25:785–90.
[92] Driessen W, Tielbaard M, Vereijken T. Experience on anaerobic treatment of distillery effluent with the UASB process. Water Sci Technol 1994;30:193–201.
[93] Pant D, Adholeya A. Biological approaches for treatment of distillery wastewater: a review. Bioresour Technol 2007;98:2321–34.
[94] Wang W, Xie L, Chen J, Luo G, Zhou Q. Biohydrogen and methane production by co-digestion of cassava stillage and excess sludge under thermophilic condition. Bioresour Technol 2011;102:3833–9.
[95] Lopez I, Borzacconi L. Modelling of an EGSB treating sugarcane vinasse using first-order variable kinetics. Water Sci Technol 2011;64:2080–8.
[96] Fernndez N, Fdz-Polanco F, Montalvo S, Toledano D. Use of activated carbon and natural zeolite as support materials, in an anaerobic fluidised bed reactor, for vinasse treatment. Water Sci Technol 2001;44:1–6.
[97] Espinosa A, Rosas L, Ilangovan K, Noyola A. Effect of trace metals on the anaerobic degradation of volatile fatty acids in molasses stillage. Water Sci Technol 1995;32:121–9.
[98] Russo C, Sant' Anna Jr GL, de Carvalho Pereira SE. An anaerobic filter applied to the treatment of distillery wastewaters. Agric Wastes 1985;14:301–13.
[99] Harada H, Uemura S, Chen A-C, Jayadevan J. Anaerobic treatment of a recalcitrant distillery wastewater by a thermophilic UASB reactor. Bioresour Technol 1996;55:215–21.
[100] Siles JA, García-García I, Martín A, Martín MA. Integrated ozonation and biomethanization treatments of vinasse derived from ethanol manufacturing. J Hazard Mater 2011;188:247–53.
[101] Fernandes BS, Peixoto G, Albrecht FR, Saavedra del Aguila NK, Zaiat M. Potential to produce biohydrogen from various wastewaters. Energy Sustainable Dev 2010;14:143–8.
[102] Peixoto G, Pantoja-Filho J, Agnelli J, Barboza M, Zaiat M. Hydrogen and methane production, energy recovery, and organic matter removal from effluents in a two-stage fermentative process. Appl Biochem Biotechnol 2012;168:651–71.
[103] Bitton G. Wastewater microbiology. Wiley; 2011.
[104] Kumar V, Wati L, FitzGibbon F, Nigam P, Banat IM, Singh D, et al. Bioremediation and decolorization of anaerobically digested distillery spent wash. Biotechnol Lett 1997;19:311–4.
[105] Pandey RA, Malhotra S, Tankhiwale A, Pande S, Pathe PP, Kaul SN. Treatment of biologically treated distillery effluent—a case study. Int J Environ Stud 2003;60:263–75.
[106] Martín Santos MA, Bonilla Venceslada JL, Martín Martín A, García García I. Estimating the selectivity of ozone in the removal of polyphenols from vinasse. J Chem Technol Biotechnol 2005;80:433–8.
[107] Goodwin JAS, Stuart JB. Anaerobic digestion of malt whisky distillery pot ale using upflow anaerobic sludge blanket reactors. Bioresour Technol 1994;49:75–81.
[108] Lettinga G. Anaerobic digestion and wastewater treatment systems. Antonie van Leeuwenhoek 1995;67:3–28.
[109] Wolmarans B, De Villiers GH. Start-up of a UASB effluent treatment plant on distillery wastewater. Water SA 2004;28:63–8.
[110] Satyawali Y, Balakrishnan M. Wastewater treatment in molasses-based alcohol distilleries for COD and color removal: a review. J Environ Manage 2008;86:481–97.
[111] Muthaiyan A, Limayem A, Ricke SC. Antimicrobial strategies for limiting bacterial contaminants in fuel bioethanol fermentations. Prog Energy Combust Sci 2011;37:351–70.
[112] Oliva-Neto Pd, Yokoya F. Susceptibility of Saccharomyces cerevisiae and lactic acid bacteria from the alcohol industry to several antimicrobial compounds. Braz J Microbiol 2001;32:10–4.
[113] Hynes SH, Kjarsgaard DM, Thomas KC, Ingledew WM. Use of virginiamycin to control the growth of lactic acid bacteria during alcohol fermentation. J Ind Microbiol Biotechnol 1997;18:284–91.
[114] Islam M, Toledo R, Hamdy MK. Stability of virginiamycin and penicillin during alcohol fermentation. Biomass Bioenergy 1999;17:369–76.
[115] Gartiser S, Urich E, Alexy R, Kümmerer K. Anaerobic inhibition and biodegradation of antibiotics in ISO test schemes. Chemosphere 2007;66:1839–48.

[116] Fountoulakis MS, Stamatelatou K, Lyberatos G. The effect of pharmaceuticals on the kinetics of methanogenesis and acetogenesis. Bioresour Technol 2008;99:7083–90.

[117] Álvarez J, Otero L, Lema J, Omil F. The effect and fate of antibiotics during the anaerobic digestion of pig manure. Bioresour Technol 2010;101:8581–6.

[118] Ding C, He J. Effect of antibiotics in the environment on microbial populations. Appl Microbiol Biotechnol 2010;87:925–41.

[119] Ceccato-Antonini SR, Martini C, Heluany MH, Buzolin AL, Tauk-Tornisielo SM, Rosa-Magri MM. Isolation and screening of pentose-fermenting yeasts for the bioethanol production. J Biotechnol 2010;150:531–2.

[120] Balat M, Balat H, Öz C. Progress in bioethanol processing. Prog Energy Combust Sci 2008;34:551–73.

[121] Ge J, Liu G, Yang X, Sun H, Ling H, Ping W. Optimization of xylose fermentation for ethanol production by Candida shehatae HDYXHT-01. Chin J Biotechnol 2011;27:404–11.

[122] Cheng K-K, Zhang J-A, Ling H-Z, Ping W-X, Huang W, Ge J-P, et al. Optimization of pH and acetic acid concentration for bioconversion of hemicellulose from corncobs to xylitol by Candida tropicalis. Biochem Eng J 2009;43:203–7.

[123] Wei D, Liu X, Yang S-T. Butyric acid production from sugarcane bagasse hydrolysate by *Clostridium tyrobutyricum* immobilized in a fibrous-bed bioreactor. Bioresour Technol 2013;129:553–60.

[124] Dias MOS, Junqueira TL, Cavalett O, Cunha MP, Jesus CDF, Rossell CEV, et al. Integrated versus stand-alone second generation ethanol production from sugarcane bagasse and trash. Bioresour Technol 2012;103:152–61.

[125] Dias MOS, Cunha MP, Jesus CDF, Rocha GJM, Pradella JGC, Rossell CEV, et al. Second generation ethanol in Brazil: can it compete with electricity production? Bioresour Technol 2011;102:8964–71.

[126] Rabelo SC, Carrere H, Maciel Filho R, Costa AC. Production of bioethanol, methane and heat from sugarcane bagasse in a biorefinery concept. Bioresour Technol 2011;102:7887–95.

[127] Taherzadeh MJ, Karimi K. Acid-based hydrolysis processes for ethanol from lignocellulosic materials: a review. BioResources 2007;2:472–99.

[128] Freire WJ, Cortez LAB. Sugarcane vinasse—agricultural engeneering series. Guaiba: Agropecuária; 2000. p. 203 ([in Portuguese]).

[129] Camhi JD. Treatment of stillage, byproduct from alcohol distilation. Brasil Açucareiro 1979;94:18–23.

[130] Glória NA. Agricultural utilization of vinasse. Brasil Açucareiro 1975;86:11–7.

[131] Biological wastewater treatment: principles, modelling and design. IWA Pub.; 2008.

[132] Rodrigues JAD, Ratusznei SM, Zaiat M, Foresti E. New configurations and operation strategies of anaerobic biofilm bioreactors applied to wastewater treatment. Focus Biotechnol Res 2006:1–58.

[133] Salomon KR, Lora EES, Rocha MH, del Olmo O. Cost calculations for biogas from vinasse biodigestion and its energy utilization. Coop Sugar 2011;42:23–31.

[134] Moraes BS, Junqueira TL, Pavanello LG, Cavalett O, Mantelatto PE, Bonomi A, et al. Anaerobic digestion of vinasse from sugarcane biorefineries in Brazil from energy, environmental, and economic perspectives: profit or expense? Appl Energy 2014;113:825–35.

[135] Eurobserv'ER, The state of renewable energies in Europe. 12th Eurobserv'ER report; 2012. 241p. ⟨http://www.energies-renouvelables.org/observ-er/stat_baro/barobilan/barobilan12.pdf⟩ [cited 20.11.2013].

[136] National Energy Balance (Balanço Energético Nacional). 2012 base year: summary report. Rio de Janeiro: EPE; 2013. 55p. ⟨https://ben.epe.gov.br/downloads/S%C3%ADntese%20do%20Relat%C3%B3rio%20Final_2013_Web.pdf⟩ [cited 20.11.2013].

[137] Rocha M, Lora E, Venturini O, Escobar J, Santos J, Moura A. Use of the life cycle assessment (LCA) for comparison of the environmental performance of four alternatives for the treatment and disposal of bioethanol stillage. Proc Int Soc Sugar Cane Technol 2010.

[138] de Souza SNM, Borsoi A, Santos RF, Secco D, Frigo EP, da Silva MJ. Production potential of biogas in sugar and ethanol plants for use in urban buses in Brazil. J Food Agric Environ 2012;10:908–10.

[139] Moraes BS, Junqueira TL, Chagas MF, Morais ER, Mantelatto PE, Cavalett O, et al. Assessment of biogas and biofertilizer production from anaerobic digestion of vinasse integrated to a sugarcane biorefinery. In: Proceedings of fifth international conference on engineering for waste and biomass valorisation. Rio de Janeiro, Brazil; 2014.

[140] Taylor G. Biofuels and the biorefinery concept. Energy Policy 2008;36:4406–9.

[141] Fatih Demirbas M. Biorefineries for biofuel upgrading: a critical review. Appl Energy 2009;86(Suppl 1):S151–61.