Enzymatic Conversion of Sugarcane Lignocellulosic Biomass as a Platform for the Production of Ethanol, Enzymes and Nanocellulose

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Received May 18, 2017; Accepted July 26, 2017

ABSTRACT: The conversion of sugarcane lignocellulosic biomass into fuels, chemicals and high-value materials using the biochemical pathway is considered the most sustainable alternative for the implementation of future biorefineries. Actually, the first large-scale cellulosic ethanol plants that have started operating worldwide apply the enzymatic hydrolysis process to convert biomass into simple sugars that are fermented to ethanol by yeasts. However, several technological challenges still need to be addressed in order to obtain commercially competitive products. This review describes current challenges and perspectives regarding the enzymatic hydrolysis step for processing sugarcane lignocellulosic biomass within the biorefinery. Recent developments in terms of process configuration strategies and opportunities for the implementation of a sugarcane biorefinery, in which the production of ethanol is integrated into the production of high-value products such as enzymes and nanocellulose, are discussed in view of the demands of the current bioeconomy.

KEYWORDS: Enzymatic hydrolysis, bioethanol, sugarcane, nanocellulose, biorefinery

1 INTRODUCTION

The biorefinery concept has been identified as one of the most promising routes to build the new industries of the future, as it allows the use of renewable lignocellulosic biomass for the production of biofuels, chemicals and novel materials [1–4]. Therefore, the application of the biorefinery concept to the sugarcane industry is a potential strategy to diversify and expand the markets of this important agro-industrial sector. This approach could have a significant impact on the economy of countries like Brazil, which is the world's largest producer of sugarcane and together with the United States leads the global production of bioethanol [5].

In the sugarcane industry, large amounts of lignocellulosic residues (bagasse and straw) are generated during the production of ethanol and sugar. In this process, every ton of sugarcane processed generates about 140 kg of bagasse and 140 kg of trash on a dry basis (db) [6]. The ethanol produced using lignocellulosic biomass as feedstock, also called second generation (2G) ethanol, has been considered as being the biofuel with the greatest potential to replace fossil fuels, especially in terms of sustainability [7]. Even though the current 2G technology is less economically feasible than that of conventional first generation (1G) ethanol [8, 9], the first commercial-scale plants for the production of 2G ethanol have already begun operating worldwide [10].

One of the key technological challenges still holding back the industrial production of 2G ethanol is related to the conversion of lignocellulosic biomass into simple sugar molecules, which will then be fermented into ethanol by yeasts. Among the possible alternatives, the biochemical pathway using enzymes has been considered the most sustainable approach for the implementation of this process. Among the advantages of using enzymatic over chemical catalysis include the possibility of using milder operating conditions. Due to the high specificity of such biocatalysts, there is no formation of side products, which in turn leads to a high conversion efficiency. Thus, the enzymatic conversion of the polysaccharides present in sugarcane lignocellulosic biomass will certainly be a key technology in future biorefineries, since the biochemical pathway

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DOI: 10.7569/JRM.2017.6341578

using biocatalysts is highly advantageous both technically and environmentally, being compatible with the demands of the bioeconomy [4, 11–13].

However, due to the complexity and recalcitrance of lignocellulose, high enzyme loadings are needed in the conversion process and the cost of these enzymes significantly affects the economic feasibility of 2G ethanol production [10, 14, 15]. There is therefore a need to develop enzymatic cocktails with improved performance in lignocellulose hydrolysis. In order to address this important issue, several studies have focused on increasing the efficiency in the production of cellulolytic enzymes by selection of microorganisms capable of secreting a high and diversified amount of enzymes [16–20] as well as by optimizing the composition of the cellulolytic cocktail [21–23]. Studies addressing bioprocess engineering strategies to improve cellulolytic enzymes production by manipulation of process variables, bioreactor type and cultivation methods have also been reported [20, 24–29]. The production of enzymes on-site, within the sugarcane mills, is also being considered as a potential strategy that could be used to reduce costs [10, 15, 27, 30–33]. Furthermore, it has been suggested that use of the enzymes secreted from microorganisms grown on the same lignocellulosic material that will be converted into ethanol could be a possible means of better modulating the enzyme complex [20, 30, 34]. Thus, the use of sugarcane lignocellulosic biomass as carbon source and inducer for microbial production of the cellulolytic enzymes required in the saccharification step could be a potential strategy for the sugarcane industrial sector.

Another strategy to overcome the technical and economic challenges that hinder the commercial large-scale production of 2G ethanol is to integrate the production of high-value products such as nanocellulose. Recent reports concerning the integration of 2G ethanol and nanocellulose production processes have demonstrated the potential of this approach for different lignocellulosic feedstocks [35–44]. The production of nanocellulose from the solids residues of the enzymatic hydrolysis of sugarcane bagasse has been recently demonstrated as a potential strategy for the application of the biorefinery concept to the sugarcane industry as well [35]. Nananocellulosic materials present excellent mechanical properties, good biocompatibility, tailorable surface chemistry, and interesting optical properties [45]. Due to these distinguished properties, nanocellulose-based materials have attracted interest for applications in food packaging [46,47], biomedicine [48], as mechanical reinforcement of matrices [49], among several other applications [45,47,50-55]. Therefore, the implementation of the biorefinery concept in the sugarcane sector, in which the production of ethanol is integrated into the production of higher value products such as enzymes and nanocellulose, could contribute to the expansion of the sugarcane industry into new and diversified markets, generating additional income and providing an important strategy to cope with market and economic fluctuations.

Despite the variety of platforms available for biomass conversion, the selection of the most appropriate technology and the best target products for the successful implementation of the biorefinery concept in the sugarcane industry can represent a great challenge from the techno-economic and environmental sustainability standpoints. This review describes current challenges and perspectives regarding enzymatic hydrolysis as a platform for processing sugarcane lignocellulosic biomass within the biorefinery concept. Recent developments in terms of process configuration strategies for the conversion of lignocellulosic sugarcane biomass into biofuels and high-value materials, such as nanocellulose, as well as the production of enzymes on-site using sugarcane biomass as feedstock are discussed.

2 PRETREATMENT OF LIGNOCELLULOSIC SUGARCANE BIOMASS

The plant cell wall is composed of a mixture of polysaccharides, proteins, phenolic compounds and mineral salts. Polysaccharides represent about 90% of the dry mass of the plant cell wall and consist of cellulose, which comprises 20 to 40%, hemicelluloses (15–25%) and pectins (~ 30%). In addition to the polysaccharides, the plant cell wall is also impregnated with lignin, an aromatic polymer that provides rigidity to the plant [56, 57]. An interlaboratory comparison study on the characterization of sugarcane bagasse revealed that the composition of this biomass includes 42.3% glucan, 22.3% xylan, 21.3% total lignin, 6.7% total extractives, and 1.5% whole ash [58]. As for sugarcane straw, the overall average cellulose, hemicellulose, lignin, and ash contents determined from four different varieties were 41.1 ± 0.9, 36.2 ± 0.9, 11.4 ± 0.4, and 2.2 ± 0.5%, respectively [59]. Moreover, a recent physical-chemical-morphological characterization study of the whole sugarcane lignocellulosic biomass also revealed the presence of spectral and morphological differences among sugarcane bagasse, straw and tops [60].

In order to effectively process this complex lignocellulosic structure through biochemical conversion, a pretreatment step is usually required for increasing the accessibility of enzymes to the polysaccharides [61–64]. Different pretreatment technologies employing either physical or chemical methods have been

investigated and the fundamentals, advantages and disadvantages of each pretreatment method have been previously reported [61, 62, 65].

Developments on sugarcane bagasse pretreatment using steam explosion [66–68], hydrothermal [69], ionic liquids [70–72], dilute acid [73, 74], lime [75], organosolv [76], and chemi-thermomechanical processing [77] have been reported. Each pretreatment technique, however, can result in the release of distinct amounts of chemical compounds, such as lignin-derived phenolic compounds, furan and organic acids, which can inhibit and/or deactivate enzymes as well as impair the ethanolic fermentation by yeasts [64]. Moreover, the remaining exposed lignin adsorbs enzymes nonproductively and reduces the amount of enzymes available to hydrolyze cellulose [78–80]. Therefore, a compromise should be reached when defining the choice of pretreatment technique as well as the severity of each process condition.

3 INHIBITORY PRODUCTS GENERATED DURING PRETREATMENT OF LIGNOCELLULOSIC BIOMASS

Economic constraints to implement future biorefineries raise the need for process options that reduce the number of unit operations and increase the concentration of products, such as working at high-solids loading (above 15% w/w). It has been widely reported that processing of biomass at high-solids loading will be required in order to implement future large industrial-scale processes as this will lead to a higher sugar concentration, improved ethanol productivity, and reduced capital costs due to lower energy inputs [81–83]. For instance, running pretreatment at high-solids loading can reduce the heating, cooling and mixing costs [83]. However, the negative effect of the inhibitors generated during pretreatment can be even more pronounced when processing biomass at high-solids loading [84]. Consequently, besides the technical challenges for working at a high-solids loading in the pretreatment reactors, the subsequent hydrolysis and fermentation reactions are also highly affected [83].

The higher solids content results in a reduced enzymatic hydrolysis yield, due to limitations caused by factors such as poorer mass transfer [82, 83, 85, 86], end-products inhibition [87, 88], nonproductive enzyme adsorption into lignin [78, 82], and mixing difficulties caused by the high initial viscosity [89]. On top of that, there is a need to minimize the enzyme loading applied in the hydrolysis step, because the high cost of the enzymatic cocktail required for biomass saccharification has a significant impact on the economics of the overall process [10, 14]. Therefore, the implementation of large industrial-scale processes involving sugarcane lignocellulosic materials requires overcoming technical limitations related to working with high-solids loading and the consequent increased levels of inhibitors in the hydrolysis and fermentation reactions.

Different strategies have been proposed to minimize the effects of inhibitors generated during pretreatment and to improve biomass conversion efficiency [64, 80, 84]. A potential strategy to mitigate the issues related to unproductive adsorption of enzymes onto lignin is the addition of lignin-blocking agents to the hydrolysis reaction medium. Although the use of an additive may increase the cost of the cellulosic ethanol production process, there are clear benefits in terms of improving the saccharification reaction [78–80, 90–93]. Reduction of unproductive binding to lignin enables more effective use of the added enzymes and, most importantly, can help to decrease the enzyme loading required [90].

Several studies using additives, such as surfactants (Tween 20 or 80), polyethylene glycol (PEG), and bovine serum albumin (BSA), showed increased yield and rate of enzymatic hydrolysis for different lignocellulosic materials [78, 79, 81, 92–97]. However, there is a clear need to find more cost-effective additives for use in large industrial-scale processes. Alternative lower-cost additives that could be used for the purpose of lignin blocking that have been suggested include soybean and whey protein as well as polypeptides that have an affinity for lignin [91]. Among these possible options, soybean protein stands out as a promising cost-effective candidate, since it is one of the cheapest proteins available on the market [14].

A recent study showed that the addition of soybean protein to the enzymatic hydrolysis of pretreated sugarcane bagasse led to an approximately 2-fold increase in hydrolysis for both *Aspergillus niger* and *Trichoderma reesei* enzymatic cocktails [80]. Moreover, the findings indicated that the responses of *A. niger* enzymes to the presence of soybean protein were significantly affected by the cultivation method used to produce them, with the strongest responses in the case of solid-state fermentation (SSF). The *T. reesei* enzymes were significantly favored by the addition of soybean protein, independent of the cultivation method used to produce them. The difference between the proteins secreted by each fungal strain, as well as the cultivation methods used to produce the enzymatic cocktails, may have greatly contributed to the differential enzymatic hydrolysis effects obtained in the presence of the soybean protein additive [80]. Therefore, the production of enzymes using different fungal strains and cultivation methods is a potential strategy to obtain enzymatic cocktails with different characteristics

towards lignin adsorption. Moreover, understanding the mechanisms involved and reducing the loss of enzymes due to unproductive adsorption onto lignin are critical to improve the efficiency of bioconversion of lignocellulosic materials into fuel, chemical, and other high-value products.

4 ENZYMES INVOLVED IN THE DECONSTRUCTION OF SUGARCANE LIGNOCELLULOSIC BIOMASS

Multiple enzymes are required for the complete hydrolysis of lignocellulosic materials, including cellulases, hemicellulases, pectinases, ligninases and other accessory enzymes [98, 99]. The cellulolytic enzymes comprise a set of glycoside hydrolases whose action involves hydrolysis of the β-1,4-glycosidic bonds of cellulose [98]. The most widely accepted mechanism of action of cellulases involves three classes of enzymes: endoglucanases, exoglucanases, and β-glucosidases. Endoglucanases hydrolyze accessible intramolecular β-1,4-glycosidic bonds of the cellulose chains randomly, producing new chain ends; exoglucanases progressively cleave cellulose chains at the ends to release soluble cellobiose or glucose; and β-glucosidases hydrolyze cellobiose to glucose [100, 101].

A hierarchical model proposed for the enzymatic hydrolysis of sugarcane biomass includes feruloyl esterases as important to break ferulic bridges among hemicellulose and facilitate the action of celullases [102]. Other important enzymes required for depolymerization of hemicellulose are the endo-1,4-β-xylanase (xylanase) enzymes, which cleave the β-1,4-glycosidic linkage between xylose residues in the backbone of xylans [103]. Supplementation with feruloyl esterases and xylanase enzymes produced on-site improved the hydrolysis of sugarcane bagasse by up to 36% [22].

The role of oxidative enzymes, such as lytic polysaccharide monooxygenases (LPMO) and other accessory proteins, in increasing the degradation of cellulose suggests that the action of the classical hydrolytic cellulases is also facilitated by the lytic action of the polysaccharide monooxygenases [99]. Addition of LPMO activity to a *T. reesei* enzyme formulation resulted in a 2-fold reduction of the total protein loading required to hydrolyze biomass [104]. The lignin content has been reported to affect these oxidative enzymes, as the highest activity of LPMO was observed for pretreated biomasses that contained the highest level of lignin [105]. Recently, a notable improvement in the action of LPMOs has been reported by combining it with pigments and reducing agents that when exposed to light resulted in a 100-fold increase in catalytic activity and also broadened LPMO substrate specificity to include both cellulose and hemicellulose [106].

This enzymatic complex containing cellulases and accessory enzymes is produced by a wide variety of microorganisms (bacteria and fungi). However, the aerobic fungi are especially known for their high growth and protein secretion rates [101, 107, 108]. Several filamentous fungi have been used for the industrial production of cellulolytic cocktails. Among them, *Trichoderma* and *Aspergillus* strains are considered the workhorses, presenting good fermentation characteristics, such as high protein secretion rates and the ability to produce a wide range of extracellular enzymes [109]. Nevertheless, enzyme-prospecting research continues to identify opportunities to enhance the activity of enzyme preparations by supplementation with enzymatic diversity from other microbes [17, 21, 22].

In view of the enzyme cost contribution in the overall economics of 2G ethanol, the production of enzymes on-site, using lignocellulosic residues as feedstocks, can significantly reduce the cost and provide a promising alternative for large-scale industrial process [10, 15, 110]. Recent studies have shown a significant reduction in the cost of the enzyme when produced on-site due to its simplified purification and logistics, as well as the potential use of low-cost carbon source from lignocellulosic material [10, 15, 110]. Therefore, the use of on-site enzyme production within the sugarcane ethanol mills is a potential approach to reduce the costs associated with enzymes, thus contributing to the feasibility of applying the biochemical route in the sugarcane biorefinery.

5 ON-SITE PRODUCTION OF ENZYMES USING SUGARCANE BAGASSE AS FEEDSTOCK

Different process configurations, using either commercial enzymes or locally produced enzymatic preparations, have been described for the hydrolysis of sugarcane bagasse [21, 29, 111–115]. The use of on-site enzyme production within the sugarcane ethanol mills is a potential approach to reduce the costs associated with enzymes, as described in several reports [10, 15, 27, 29–33, 114, 115]. Besides, this strategy allows the production of an enzymatic cocktail specially tailored to degrade sugarcane biomass, since the enzymes secreted from microorganisms grown on the same lignocellulosic material that will be converted to ethanol have been reported as a possible means of better modulating the enzyme complex [29, 30, 34, 115].

A recent study compared the production cost of cellulase using three approaches (off-site, on-site using glucose as carbon source and integrated using

cellulosic biomass as carbon source) and concluded that the integrated method has the lowest cost [10]. Moreover, the cellulase produced on-site in such an integrated approach was found to be better or equal to the commercial enzymatic cocktail for cellulose conversion into sugars [10]. These findings are corroborated by another recent comparative cost study where the authors concluded that production of enzymes on-site should be evaluated at industrial scale to yield an economically sound enzyme supply for cellulosic ethanol production [15]. However, a deeper cost evaluation specifically addressing cellulase enzymes is difficult due to a variation of data related to the cost of enzymes reported in terms of dollars per gallon of cellulosic ethanol, which will depend on other factors as well [14]. For instance, some reports show that the cost of cellulase can range from $0.1 to $0.4/gal of ethanol, supporting the idea that current technology is economically feasible, but when based on saccharification and fermentation yields the values can reach up to $1.47/gal ethanol [14]. In terms of the actual purchase price of cellulase in the industrial enzyme market and the conventional ethanol yield, the enzyme cost could be up to $2.71/gal ethanol, accounting for 48% of the minimum ethanol selling price [15]. Such results certainly support further bioprocess development studies addressing the economic issues for the production of enzymes integrated into the sugarcane biorefinery.

The microbial cultivation processes for enzyme production can be conducted in a solid medium, called solid-state fermentation (SSF), or in liquid medium, called submerged fermentation (SFm). Although most of the advances related to the microbial production of cellulases have been developed for SFm, the growth of filamentous fungi, the main producers of cellulolytic enzymes, occurs naturally under conditions similar to SSF [109]. Both processes have advantages as well as limitations, which should be considered according to the desired product and the selected microorganism [109,116-118]. A potential advantage of the SSF is that it enables the use of agro-industrial residues, such as sugarcane bagasse, as carbon source and inducer for microbial enzyme production [27, 29, 109, 114, 119–121].

Alternatively, the use of the entire SSF medium, containing the enzymes, mycelia, and residual solid substrate for the saccharification of sugarcane biomass has been described as a potential process configuration to reduce enzyme costs, as well as to avoid generation of effluent streams [122–125]. A major advantage of this configuration is that it enables the use of a single reactor system, avoiding any need for the additional extraction and separation steps required in traditional SSF used for enzyme production (Figure 1). A comparison of the conversion of steam-exploded sugarcane bagasse using the *A. niger* and *T. reesei* enzymes from either the extracts (EE) or the whole fermentation media (WM) resulted in similar yields for EE and WM in terms of both glucose and total reducing sugar, giving a clear indication that the SSF enzyme extraction step could be eliminated [122–124]. Such results open new opportunities for developing a process

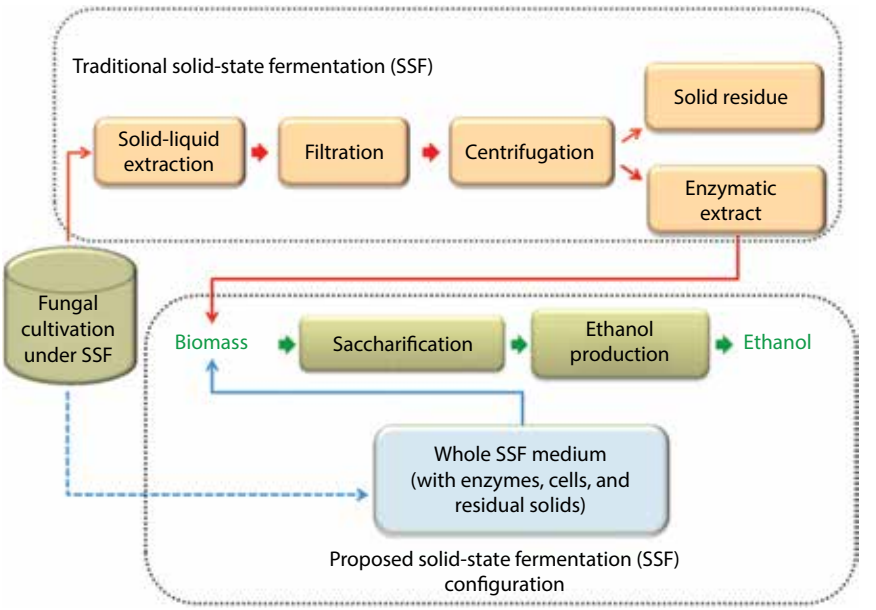

Figure 1 Schematic illustration of the on-site enzyme production under SSF and use of the enzymes from the whole medium of fungal cultivation in the saccharification and fermentation process to obtain ethanol. Conceptually, this process could be carried out in a single reactor system, avoiding the need for additional separation steps [123].

configuration using a sugarcane biomass as feedstock for enzyme production under SSF, and to use it again during the saccharification step, thus eliminating the enzyme extraction/filtration steps.

Recently, a combination of the SSF and SFm cultivation techniques, defined as sequential fermentation (SeqF), has been effectively applied for the production of cellulolytic enzymes using sugarcane bagasse as carbon source and inducer [20, 28, 114, 115, 126, 127]. The sequential fermentation is characterized by a preculture preparation with initial stage of fungal growth under solid state, followed by a transition to submerged state. The SeqF presented significant results in relation to the conventional submerged process of cellulase production, both in agitated flasks [20, 27, 114, 115, 127] and in conventional stirred-tank bioreactors as well as in air-lift type bioreactors [28, 126]. Endoglucanase productivity was 3-fold higher in SeqF compared to conventional FSm, suggesting the potential of the technique as a promising alternative for the production of cellulolytic enzymes by *A. niger* [126]. The SeqF methodology was also validated for strains of the genus *Trichoderma*, resulting in an enzymatic profile with greater activities of xylanase, endoglucanase, β-glucosidase, avicellase and FPase [127]. As a follow-up study, the secretome of the *T. reesei* and *A. niger* strains cultured in FSm and Fseq was evaluated [20]. The proteomic analysis of the *A. niger* strain showed that the SeqF presented a higher number of proteins identified and higher enzymatic activities as well. In addition, the higher enzymatic activities and/or a better balance of the secretome composition from fungal cultivation under SeqF lead to a 3-fold increase in the saccharification of sugarcane bagasse pretreated by steam explosion [20]. Overall, these findings suggest that the integration of the enzyme production process using sugarcane biomass as feedstock is of potential interest for implementation in future sugarcane biorefineries.

6 INTEGRATED PRODUCTION OF NANOCELLULOSE AND 2G ETHANOL

The conversion of biomass into simple sugars via enzymatic hydrolysis usually results in high amounts of a residual solid fraction, due to the high recalcitrance of lignocellulosic materials [59, 60, 83, 128]. Such residues from the enzymatic hydrolysis step contain highly crystalline cellulose, because the enzymes degrade the amorphous part of the cellulose at a much faster rate than the crystalline fraction [129]. Thus, a potential use of this solid residue is for the production of nanocellulose, which is a high-value material. Nanocellulosic materials can be obtained in the form of cellulose nanofibers (CNFs) or cellulose nanocrystals (CNCs), depending on the extraction procedure used [130]. CNFs are micrometer-long entangled fibrils which form a web-like network structure while the CNC presents a rod-like shape, also referred to as nanocrystalline cellulose and cellulose nanowhiskers [45]. Besides the differences in terms of morphology, CNF and CNC will vary in terms of their applications as well [47, 48, 52, 54, 55, 131, 132].

Nanocellulose is considered the most attractive renewable material for advanced applications due to its excellent mechanical properties, good biocompatibility, tailorable surface chemistry, and interesting optical properties [45]. Due to these distinguished properties, nanocellulose-based materials have attracted interest for applications in food packaging [46, 47], biomedicine [48], as mechanical reinforcement of matrices [49], enzyme immobilization [133], membrane filtration [134], among many other applications [45, 47, 50–55]. Actually, the first large-scale plants for the production of nanocellulose have already begun operating worldwide [135, 136], making these nanomaterials usable in commercial applications of high-value products. Different sources may be used to obtain nanocellulose such as curaua fibers [137, 138], sisal [139], cotton [140–142], cassava bagasse [49], and sugarcane bagasse [143–146], among others.

The CNFs are obtained from natural sources mostly using mechanical processes, which include high-pressure homogenization, grinding and refining treatments, while the rod-like CNCs can be isolated using an acid hydrolysis process [130]. The conventional methods used to obtain CNC require high concentrations of strong acids such as sulfuric acid or hydrochloric acid, which react rapidly with amorphous cellulose, with interruption of the reaction before the hydrolysis of crystalline cellulose [139, 147]. Currently, both the mechanical treatment and acid hydrolysis are the most studied methods to obtain CNF and CNC, respectively. However, from the environmental point of view such procedures have some drawbacks, as the acid hydrolysis needs to consume a large quantity of acid while the mechanical treatment consumes large amounts of energy [149]. In contrast, the use of enzymatic hydrolysis to produce nanocellulose is highly advantageous from the environmental point of view and results in materials with distinct properties [44, 144, 148, 149].

Most of the studies addressing the production of CNC using the enzymatic route have employed additional steps such as mechanical or ultrasound treatment in combination with the enzymes [144, 148–150]. However, the use of enzymatic reactions to hydrolyze the biomass to obtain nanocellulose usually results in

solid materials with characteristics of cellulose nanofibers [40]. Recently, the feasibility of integration of cellulosic ethanol production with the manufacture of CNC using only enzymatic hydrolysis was demonstrated by using eucalyptus cellulose pulp as feedstock and employing a new strategy with temperature reduction [44]. The CNC obtained using only enzymatic hydrolysis reaction showed a crystallinity index of 83%, length of 260 nm, diameter of 15 nm, aspect ratio (L/D) of 15, and initial temperature of degradation of 325 °C, which makes this material suitable for many applications. Moreover, the sugars released from eucalyptus pulp were efficiently fermented into ethanol, showing the viability for this integrated process [44].

Recent research addressing the idea of integration of nanocellulose and 2G ethanol production has demonstrated the potential of this technological route for different lignocellulosic material feedstocks (Table 1). Among these sources, the feasibility of using the residual solids remaining after the enzymatic hydrolysis of sugarcane bagasse for the production of CNC was demonstrated by Camargo *et al.* [35]. Sugarcane bagasse subjected to steam explosion (SEB) and liquid hot water pretreatment (LHWB) was hydrolyzed using different loadings of a commercial cellulase cocktail and the solid residues after the enzymatic hydrolysis step were used to obtain CNC by a chemical treatment (Figure 2). The CNC produced from sugarcane bagasse residues presented morphology, dimensions, physical-chemical characteristics, thermal stability, and crystallinity within the ranges reported for this type of material (Table 2). Most important, the enzyme loading or the type of hydrothermal pretreatment employed showed no significant effects on the CNC obtained, indicating that these variables could be flexibly adjusted according to specific interests [35]. Furthermore, the potential of using sugarcane bagasse to obtain nanocellulose, either as CNF or CNC, has been previously demonstrated by using different approaches [144–146].

From an economic standpoint, a detailed cost analysis showing the positive impact of including the production

Table 1 The literature reports addressing the integration of nanocellulose and 2G ethanol production using different lignocellulosic materials as feedstocks.

Feedstock	Procedure	Nanocellulose type	Reference
Residue from wood bioethanol pilot plant	Chemical extraction and bleaching followed by ultrasonication, high-pressure homogenization and chemical acid hydrolysis	Cellulose nanowhiskers	[37]
Wet bleached Kraft eucalyptus pulp	Enzymatic hydrolysis followed by mechanical homogenization	Nanofibrillated cellulose	[40]
Microcrystalline cellulose; bioresidue from wood ethanol plant (BR)	Acid hydrolysis (MCC); bleaching and homogenization (BR)	Cellulose nanowhiskers	[38]
Cotton cellulose (filter paper)	Acid hydrolysis	Cellulose nanocrystals	[36]
Citrus processing waste from orange	Enzymatic hydrolysis followed by bleaching and sonification	Nanofibrillated cellulose	[42]
Hardwood and softwood pulp	Enzymatic hydrolysis followed by sonification	Nanofibrillated cellulose	[43]
Unbarked wood chips of spruce	Chemical extraction and bleaching followed by high-pressure homogenizer or chemical acid hydrolysis	Cellulose nanocrystals	[39]
Spruce bark	Chemical extraction and bleaching followed by chemical acid hydrolysis and sonication	Cellulose fibers and cellulose nanocrystals	[41]
Pure cellulose, Eucalyptus holocellulose, unbleached Kraft pulp, and sugarcane bagasse	Wet disk milling followed by enzymatic hydrolysis	Cellulose nanocrystals	[150]
Sugarcane bagasse	Enzymatic hydrolysis followed by bleaching and chemical acid hydrolysis	Cellulose nanocrystals	[35]
Kraft eucalyptus cellulose pulp	Enzymatic hydrolysis	Cellulose nanocrystals	[44]

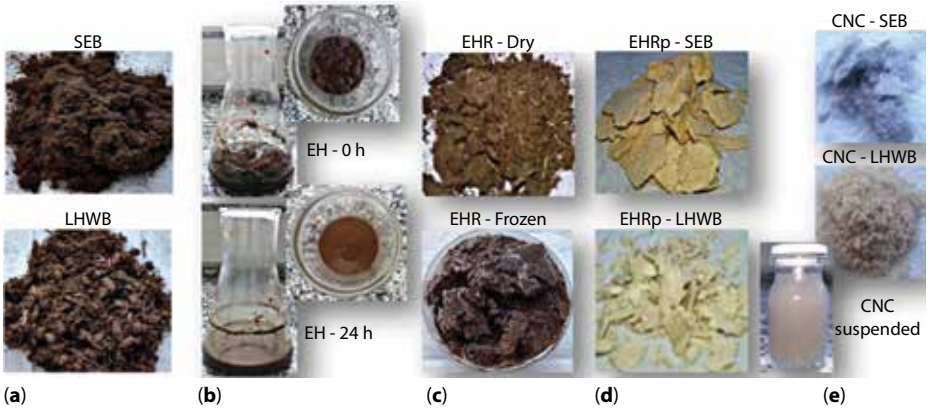

Figure 2 Schematic illustration of the samples used to obtain CNC from sugarcane bagasse. (**a**) Sugarcane bagasse pretreated by steam explosion (SEB) and liquid hot water (LHWB); (**b**) Suspensions obtained before and after the enzymatic hydrolysis step (EH); (**c**) Solid residue after the enzymatic hydrolysis (EHR); (**d**) Solid residue after the enzymatic hydrolysis and purification step (EHR_p); (**e**) Dry CNC from SEB and LHWB and CNC suspension in water [35].

Table 2 Properties of cellulose nanocrystals (CNCs) obtained from sugarcane bagasse pretreated by steam explosion (SEB) and liquid hot water (LHWB) after enzymatic hydrolysis, purification and acid hydrolysis using different enzyme loadings [35].

			SEB			*LHWB*		
			CI (%)	**T_{onset} (°C)**	**L/D**	**CI (%)**	**T_{onset} (°C)**	**L/D**
Sugarcane bagasse			**68.0**	**296.0**	**–**	**71.9**	**298.1**	**–**
Enzymatic hydrolysis 	**Enzyme loading (mg/g)**	07	60.3	280.2	–	64.6	290.2	–
		12	53.5	276.7	–	63.5	300.6	–
		22	52.0	285.3	--	63.3	295.0	–
Purification 		07	85.2	–	--	83.4	–	–
		12	83.7	–	–	82.0	–	–
		22	82.0	–	–	81.4	–	–
Acid hydrolysis 		07	81.7	259.9	11	81.4	262.0	11
		12	77.7	252.2	11	81.6	246.7	13
		22	78.4	249.7	15	77.9	238.5	15

CI (%) – crystallinity index; T_{onset} (°C) – temperature; L/D – length to diameter ratio.

of CNC in an integrated biorefinery with ethanol has been demonstrated for crop residues such as wheat straw [151]. Considering engineering and economic parameters for a 50 million gallon per year ethanol process and 1,050 tons of CNC per year, the production cost of nanocellulose was estimated to be $1.25 per kg as compared to the production cost of ethanol of $0.41 per liter. Such economic analysis indicated that production of CNC would be an enhancement to the economic performance of a wheat straw to ethanol mill, thus contributing to the profitability of the biorefinery [151].

Overall, these previous studies demonstrate the feasibility of producing nanocellulose as a valuable co-product from the 2G ethanol process using sugarcane bagasse as feedstock. The co-production of nanocellulose in addition to ethanol in such an integrated sugarcane biorefinery can contribute to a higher return on investment than with the production of biofuels alone.

7 CONCLUDING REMARKS

The enzymatic conversion of the polysaccharides present in sugarcane lignocellulosic biomass will certainly be a key technology for the implementation of future biorefineries from sugarcane and for the development of this important agro-industrial sector. A discussion related to current technological challenges in the enzymatic hydrolysis step and developments in terms of process configuration strategies for the conversion of sugarcane biomass has been presented. The integrated production of ethanol, enzymes and nanocellulose is suggested as a possible strategy for the implementation of future sugarcane biorefineries.

Further studies focusing on process scale-up and on the techno-economic and environmental evaluation of the overall sugarcane biorefinery should be addressed. For that, process models should be studied in order to determine economic feasibility and process efficiency. Moreover, the effects of lignocellulose-derived inhibitors on both enzymes and fermentative microorganisms should be carefully evaluated in such process models as they represent key impediments to cost-effective conversion of biomass to ethanol and other bioproducts. Therefore, future 2G ethanol production facilities and sugarcane biorefineries should consider the incorporation of a detoxification step as well as the use of additives and more efficient biocatalysts to overcome the presence of inhibitors generated during the pretreatment step. Considering that each feedstock requires specific processing conditions, optimized process configurations for an efficient use of the whole sugarcane lignocellulosic biomass into fuels and high-value products will be needed for the successful implementation of future sugarcane biorefineries.

ACKNOWLEDGMENTS

The author would like to thank Embrapa, CNPq (Process 401182/2014-2), SISNANO/MCTIC, Capes, and FAPESP (Processes 2014/19000-3 and 2016/10636-8) (all from Brazil) for financial support, and the students and technicians from Embrapa Instrumentation for their contribution.

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