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# Sugarcane Bagasse Pretreatment Methods for Ethanol Production

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## Abstract

Lignocellulosic biomass such as sugarcane bagasse (SCB) is a renewable and abundant source for ethanol production. Sugarcane bagasse is composed of cellulose, hemicellulose, lignin, extractives, and several inorganic materials. Pretreatment methods of SCB are necessary for the successful conversion of SCB to ethanol. Each pretreatment process has a specific effect on the cellulose, hemicellulose, and lignin fraction. The conversion of SCB to ethanol typically consists of four main steps: pretreatment, enzymatic hydrolysis, fermentation, and distillation. Hence, different pretreatment methods should be chosen according to the process design for the following hydrolysis, fermentation, and distillation steps. There are many types of pretreatments such as physical, chemical, physico-chemical, and biological pretreatments. This chapter reviews the chemical and physico-chemical pretreatment methods of SCB which are often used by many researchers for ethanol production. Different chemical and physico-chemical pretreatment methods of SCB are introduced and discussed based on relevance to the sugar yield, lignin removal, and cellulose content after pretreatment.

**Keywords:** sugarcane bagasse, pretreatment, ethanol

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## 1. Introduction

According to the latest report produced by the United Nations Food and Agricultural Organization, there are 10 largest sugarcane producing countries in the world in 2018. The 10 countries are Brazil, India, China, Thailand, Pakistan, Mexico, Colombia, Indonesia, Philippines, and United States. About 540 million metric tons per year of sugarcane bagasse are produced globally [1]. **Table 1** presents sugarcane bagasse production annually for several

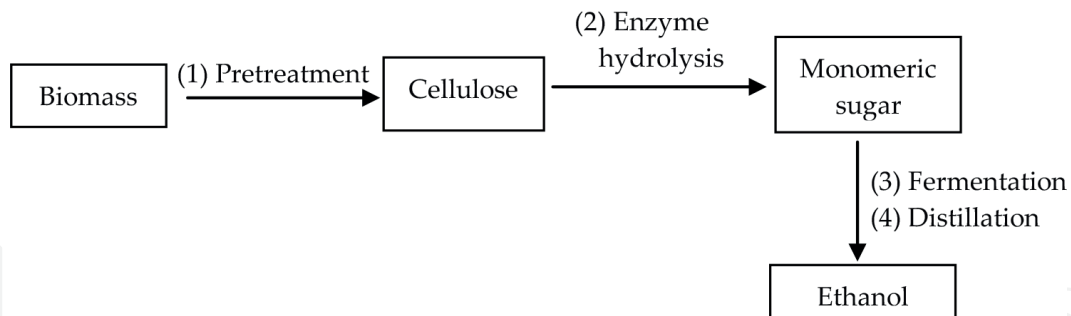
countries. Sugarcane bagasse is the solid residue obtained after extraction of the juice from sugar cane (*Saccharum officinarum*) and can be a potential substrate for ethanol production since it has high sugar content and is a renewable, cheap, and readily available feedstock.

Sugarcane bagasse is mainly composed of cellulose (33–36%), hemicellulose (28–30%), and lignin (17–24%). Cellulose is the most abundant polysaccharide polymer which comprised of a linear chain of  $\beta(1 \rightarrow 4)$  linked D-glucose units that generates crystalline regions and consequently increases resistance to the hydrolytic process. Hemicellulose is the second most abundant polysaccharide after cellulose and is a short and highly branched polymers which comprised of pentose (xylose and arabinose) and hexose (mannose, glucose, and galactose) sugars. It possesses a heteropolysaccharide composition that varies according to the source. Sugarcane bagasse hemicellulose is composed of heteroxylans, with a predominance of xylose. Hence, it can be chemically hydrolyzed more easily than cellulose. Lignins are complex phenylpropanoid polymers formed by the polymerization of aromatic alcohols. The combination of the cellulose-hemicellulose-lignin matrix is conferring resistance to enzymatic and chemical degradation [10, 11]. Bagasse could represent the main lignocellulosic biomass in many tropical countries since it is available at the sugar factory without additional cost and contains high sugar and low lignin content [12].

Production of bioethanol from SCB has a major advantage, like its less carbon intensive, than fossil fuel which reduces air pollution [13]. The bioethanol produced from lignocellulosic materials is named as second-generation (2G) ethanol or cellulosic ethanol, while the first generation ethanol is produced from sucrose (juice extracted from sugarcane, sugarbeet, or sweet sorghum) or starch (typically extracted from grains) [14]. The second-generation ethanol production from lignocellulosic biomass has been considered to be the biofuel with the greatest potential to replace oil-based fuels ([15, 16], and it can be produced from various lignocellulosic biomasses such as wood, agricultural, or forest residues. Typically, bioethanol can be produced in a four-step process, that is, pretreatment, enzymatic hydrolysis, fermentation, and distillation (**Figure 1**), where hydrolysis and fermentation may be combined. Currently, bioethanol is produced mostly in U.S and Brazil (**Table 2**) [17].

Country	Sugarcane bagasse production (million metric ton/year)	References
Brazil	181	[2]
India	101.3	[3]
China	80	[4]
Thailand	20	[5]
Mexico	15	[6]
Colombia	7	[7]
Philippines	5.1	[8]
United States	3.5	[9]

**Table 1.** Sugarcane bagasse production annually for several countries.



**Figure 1.** A four-step process for ethanol production from biomass.

Country	Bioethanol production (million gallon)
United State	15,250
Brazil	7295
European Union	1377
China	835
Rest of World	490
Canada	436
Thailand	322
Argentina	264
India	225

**Table 2.** Bioethanol production by country, million gallons, 2017 [17].

## 2. Pretreatment

The main objective of the pretreatments is to break down the lignin structure and disrupt the crystalline structure of cellulose for enhancing enzymes accessibility to the cellulose during the hydrolysis step [18]. These pretreatments may be biological, chemical, and physical processes that are used individually, combined, and/or sequentially [19, 20]. The natural structure of lignocellulosic material is extremely recalcitrant to enzymatic hydrolysis. Therefore, the pretreatment step is required for efficient enzymatic hydrolysis of cellulose by removal of lignin and hemicellulose, reduction of cellulose crystallinity and increase the porosity of the biomass [21]. Each pretreatment has a different effect on the cellulose, hemicellulose, and lignin fraction.

It is necessary to choose suitable pretreatment methods for SCB since different lignocellulosic materials have different physico-chemical characteristics [22]. An efficient pretreatment should (1) improve the formation of fermentable sugars, (2) avoid the loss or degradation of carbohydrates, (3) avoid the formation of inhibitory by-products, and (4) be cost-effective [23]. According to Puligundla et al. [24], an ideal pretreatment should be economically efficient,

low energy consumption, and producing less or no residues. High digestibility of cellulose and versatility of feedstock are also important in the pretreatment process. In addition, other factors such as low sugar decomposition, low water or high solids, and low chemical consumption during the process should be considered. Besides that, the pretreatment should be performed at low operational risk and safe.

## 2.1. Chemical pretreatment

### 2.1.1. Dilute acid pretreatment

There are two types of acid pretreatments either using concentrated acid or diluted acids. Concentrated acid hydrolysis can be performed at a low temperature (30–60°C) using acid with the concentration around 40–80%. High sugar yield can be obtained using this method, however, requires large volumes of acid which are toxic and corrosive. Thus, corrosion resistant reactors are needed if concentrated acid is employed. Furthermore, the acid concentration must be recovered after hydrolysis to make the process economically feasible [10]. The development of effective acid recovery technologies has made this process renewed its interest [25]. On the other hand, dilute acid hydrolysis is the most widely used and has been considered to be one of the treatment methods with greater potential for wide-scale application. This process can be performed using diluted acids in the range of 0.5–6% and high temperatures from 120–170°C, with variable treatment times from minutes up to an hour.

Dilute acid pretreatment has received numerous research interests, and it has been successfully developed for pretreatment of lignocellulosic biomass. Dilute acid pretreatments are normally used to degrade the hemicellulosic fraction and increase the biomass porosity, improving the enzymatic hydrolysis of cellulose. The dilute acid pretreatment is important to weaken the glycosidic bond in the hemicellulose and lignin-hemicellulose bond and the lignin bond. This will lead to the dissolution of the sugar in the hemicellulose and also increase the porosity of the plant cell wall for effective enzyme digestibility [26]. Acid pretreatment is a very commonly used technology for biomass to ethanol conversion due to its low cost and the fact that the used acids are easily available. However, acid pretreatments can cause side effects such as the formation of furan and short chain aliphatic acid derivatives, which are considered strong inhibitors in microbial fermentation [27, 28].

Several different acids used in pretreatments of SCB, including dilute sulfuric acid [29–35], dilute hydrochloric acid [36], dilute phosphoric acid [32, 37], and dilute nitric acid [38], have been reported. High hydrolysis yields have been obtained when lignocellulosic biomass was pretreated with dilute sulfuric acid compared with hydrochloric, phosphoric, and nitric acid [22]. Sulfuric ( $\text{H}_2\text{SO}_4$ ) and phosphoric ( $\text{H}_3\text{PO}_4$ ) acids are widely used for acid pretreatment since they are relatively inexpensive and efficient in hydrolyzing lignocellulose.  $\text{H}_3\text{PO}_4$  also gives less negative impact on the environment compared to  $\text{H}_2\text{SO}_4$ , meanwhile hydrochloric (HCl) acid had better penetration to biomass and more volatile and easier to recover than  $\text{H}_2\text{SO}_4$  [39]; similarly, nitric acid ( $\text{HNO}_3$ ) possesses good cellulose to sugar conversion rates [40]. However, both acids are expensive compared to  $\text{H}_2\text{SO}_4$ . Sulfuric acid is the most commonly used acid in the pretreatment of SCB [41, 42]. **Table 2** shows the yield of sugar at different types of acid pretreatment of SCB.

According to **Table 3**, the acid concentration used in the range of 0.5–6.0%, temperature 120–170°C and time is around 10 to 300 min. Dilute acid at moderate temperature effectively removes most of the hemicelluloses and recovers as dissolved sugars.

### 2.1.2. Alkali pretreatment

Beside acid pretreatment, alkaline pretreatment is also one of the chemical pretreatment technologies receiving numerous attention for SCB pretreatment. It employs various bases, including sodium hydroxide (NaOH) [43–53], calcium hydroxide (Ca(OH)<sub>2</sub>) [54, 55], potassium hydroxide (KOH) [56], aqueous ammonia (NH<sub>3</sub>) [57], ammonia hydroxide (NH<sub>4</sub>OH) in combination with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) [58], NaOH in combination with Ca(OH)<sub>2</sub> (lime) [59], and NaOH in combination with H<sub>2</sub>O<sub>2</sub> [60]. Alkaline pretreatment is basically a delignification process. It disrupts the cell wall of SCB by (1) dissolving hemicelluloses, lignin, and silica, (2) hydrolyzing uronic and acetic esters, and (3) swelling cellulose under mild conditions. This process results in two fractions, a liquid (hemicellulose oligomers and lignin) and a solid fraction (cellulose). **Table 4** depicts the composition of lignin in SCB and pretreated SCB with NaOH. It shows that the lignin content decreased when SCB was pretreated with NaOH for all different pretreatment conditions.

The physical structure and chemical composition of the substrate as well as the treatment conditions are important factors for the effectiveness of alkaline pretreatment. In general, alkaline pretreatment is more effective on hardwood, herbaceous crops, and agricultural residues with a low lignin content than on softwood with a high lignin content [61]. Although hydroxides are not expensive, the drawback of this process is that it consumes a lot of water for washing the sodium (or calcium) salts that incorporate into the biomass so that the treatment of a large amount of

Type of acid	Pretreatment conditions	Yield of sugar		References
		mg/g	g/L	
Sulfuric acid	1.5% H <sub>2</sub> SO <sub>4</sub> , 170°C, 15 min	350		[29]
	0.5% H <sub>2</sub> SO <sub>4</sub> , 120 °C, 120 min	452.27		[30]
	2.0% H <sub>2</sub> SO <sub>4</sub> , 155°C, 10 min		22.74	[31]
	0.5% H <sub>2</sub> SO <sub>4</sub> , 130°C, 15 min	414.9		[32]
	1.25% H <sub>2</sub> SO <sub>4</sub> , 121°C, 2 h		59.1	[33]
	0.5% H <sub>2</sub> SO <sub>4</sub> , 121°C, 60 min		24.5	[34]
	2.5% H <sub>2</sub> SO <sub>4</sub> , 140°C, 30 min		30.29	[35]
Hydrochloric acid	1.2% HCl, 121°C, 4 h	37.21		[36]
Phosphoric acid	3.5% H <sub>3</sub> PO <sub>4</sub> , 130°C, 180min	404.5		[32]
	4% H <sub>3</sub> PO <sub>4</sub> , 122°C, 300 min		23.2	[37]
Nitric acid	6% HNO <sub>3</sub> , 122°C, 9.3 min		23.51	[38]

**Table 3.** Yield of sugar at different types of acid pretreatment of SCB.



Lignin (% w/w)		Pretreatment conditions	References
SCB	Pretreated SCB		
21.5	10.6	1.0% NaOH, 120°C, 10 min	[43]
27.9	9.2	0.9% NaOH, 80°C, 2 h	[44]
25.4	7.8	2% NaOH, 121°C, 30 min	[45]
18.0	1.8	15% NaOH, 175°C, 15 min	[46]
17.8	4.3	4% NaOH, 121°C, 30 min	[47]
25.0	9.0	2.5% NaOH, 126°C, 45 min	[48]
30.1	18.5	1.0% NaOH, 120°C, 60 min	[49]
23.4	5.2	5% NaOH, 121°C, 60 min	[50]
25*	6	1% NaOH, 100°C, 30 min	[51]
34.3*	5.7	1% NaOH, 100°C, 1 h	[52]
22.0	9.5	2.0% NaOH, 120°C, 40 min	[53]

\*Lignin content of SCB pretreated by steam explosion.

**Table 4.** Composition of lignin in SCB and pretreated SCB.

salts becomes a challenging issue for alkaline pretreatment. In addition, some enzyme inhibitors can be generated during lignin depolymerization [62]. In comparison with other pretreatment technologies, alkali pretreatment usually uses lower temperatures and pressures, even ambient conditions. Pretreatment time, however, is recorded in terms of hours such as 24 hours or days that are much longer than other pretreatment processes [63].

Alkaline pretreatments differ from acid pretreatments so that they are more efficient in lignin removal, substantially increasing cellulose digestibility, even after removing only part of the lignin. The hydrolysis of ester linkages between hemicellulose residues and lignin promotes an increase of porosity in the biomass, and as a result, cellulose and hemicellulose become more accessible to enzyme action [10, 64]. As this pretreatment results in a large fraction of both cellulose and hemicellulose to remain intact, it has the potential for hydrolysis of a much larger fraction of the pretreated biomass, releasing glucose from cellulose and additional pentose sugars from hemicellulose. In addition, this occurs in an environment free of strong acids and fermentation inhibitors. Under these conditions, the degradation of sugars is minimal [65]. Sodium hydroxide shows the greatest lignin degradation when compared to other alkalis, such as sodium carbonate, ammonium hydroxide, calcium hydroxide, and hydrogen peroxide.

Lime (calcium hydroxide) pretreatment is another attractive alkali pretreatment technology due to the low formation of fermentation inhibitors, which increases pH and provides a low-cost alternative for lignin solubilization where the process is removing approximately 33% of lignin and 100% of acetyl groups. Even though the action of lime is slower than other pretreatments, lime is much cheaper than other alkalis and has low toxicity to the environment and safe handling [66]. The effectiveness of lime pretreatment in improving sugarcane bagasse susceptibility to enzymatic hydrolysis was studied by Rabelo et al. [54]. The result showed that lime pretreatment improved the enzymatic digestibility of SCB.

### 2.1.3. *Organosolv pretreatments*

The organosolv process is a delignification process, with varying simultaneous hemicellulose solubilization. The organosolv process uses organic or aqueous organic solvent mixtures with or without an acid or alkali catalysts to extract lignin from lignocellulosic biomass. Numerous organic solvent mixtures including methanol, ethanol, acetone, ethylene glycol, triethylene glycol, and tetrahydrofurfuryl alcohol have been used. The advantages of ethanol as a solvent are that it is produced in many biorefineries. It is easily replenished and recycled as a solvent for the pretreatment process. Ethanol is also inexpensive and less toxic to humans compared to other solvents such as methanol [67].

The ethanol organosolv process is among the chemical pretreatment being studied for the conversion of SCB to ethanol. In this pretreatment, high degrees of delignification can be achieved for SCB following ethanol organosolv pretreatment using formic acid as a catalyst. The degree of delignification increased with increasing pretreatment temperature. The maximum degree of delignification of sugarcane bagasse reached 80% at 210°C [68]. Mesa et al. [69] reported that the combination of a dilute-acid pretreatment followed by the organosolv pretreatment with NaOH at a temperature of 195°C for 60 min using 30% (v/v) was an efficient technique for SCB fractionation for the subsequent use on the enzymatic hydrolysis process, since yielded a residual solid material containing 67.3% (w/w) glucose. Novo et al. [70] showed that one of the best pretreatment conditions for lignin removal from SCB by the organosolv method could be achieved at 190°C and 150 min.

Beside ethanol, glycerol is an excellent solvent for organosolv pretreatment [71]. Glycerol, a high-boiling-point organic solvent derived from the oleochemical industry as a by-product has become very attractive. Martín et al. [72] studied the effect of glycerol pretreatment on the main components of SCB. The result shows that the glycerol acted more selectively on lignin than on xylan where cellulose was almost completely recovered in the pretreated solids, accounting for 72% (g/g) of the pretreated substrate. Meanwhile, Novo et al. [70] reported that the glycerol pretreatment attained good cellulose preservation (>91%) and 80% lignin removal. However, Zhang et al. [73] found that >96% of the cellulose was recovered, whereas the lignin and hemicellulose removal were almost 60 and 80%, respectively, when SCB was treated with an acid-catalyzed glycerol organosolv pretreatment.

## 2.2. **Physico-chemical pretreatment**

### 2.2.1. *Steam explosion pretreatment*

Steam explosion is one of the most efficient methods to deconstruct the plant cell wall macromolecular organization [19, 74]. This process occurs both chemically and physically by revealing the lignocellulosic materials to high temperatures ranging from 160 to 260°C for reaction times varying from 2 to 30 min in the saturated steam either in the absence or presence of an exogenous acid or basic catalyst. The steam is able to expand the cell wall of the polysaccharide fiber and destroys cell structure into small pieces and breaks down the lignin network. This process would increase the accessibility of the enzyme to cellulose by exposing internal cellulose surface, which acetyl groups of hemicellulose can be hydrolyzed to acetic acid [75, 76]. The physical forces cause partial hemicellulose solubilization and lignin reorganization. The



major variables that affect steam explosion pretreatment efficacy include biomass origin, particle size, temperature, residence time, and moisture content [77, 78].

When pretreatment is performed in the presence of an acid catalyst such as sulfuric ( $\text{H}_2\text{SO}_4$ ) or phosphoric ( $\text{H}_3\text{PO}_4$ ) acids, the need for time and temperature decreases substantially depending on the strength of the acid and its actual concentration in relation to the dry mass of the biomass. In addition, this process can remove hemicelluloses almost completely, whereas lignin is modified to a deeper extend, thus making the cellulosic materials more susceptible to enzymatic or acid hydrolysis [27, 74, 79]. There are several advantages of steam explosion pretreatment which includes lower environmental impact, cost-effectiveness, greater energy efficiency, and less or no chemical usage [22]. Also, to obtain the same particle size of the substrate, steam explosion method requires a 70% lower energy consumption compared to the conventional mechanical process [10]. The main drawbacks of steam explosion pretreatment are the partial degradation of hemicelluloses and the formation of toxic components that could affect the enzymatic hydrolysis and fermentation process [76].

### 2.2.2. Liquid hot water

According to Sánchez et al. [80], liquid hot water (LHW) pretreatment is performed at various temperatures from 160 to 240°C in the liquid state with water instead of steam. The LHW process primarily maximizes the solubilization of hemicellulose, partial removal of lignin, and making cellulose more accessible to the enzyme. In addition, the formation of the undesirable side products in liquid fraction can be reduced due to solubilized hemicellulose mostly appears in oligomers forms [18]. The LHW pretreatment cleaves hemicellulose linkages and liberates various acids during the process. These acids help to hydrolyze hemicellulose to monomeric sugars, which can be subsequently degraded to aldehydes (i.e., furfural from five carbon sugars and HMF from six carbon sugars). LHW has a great potential to be chosen as a pretreatment step in the biorefinery process as it can be considered as a green technology [81].

During high temperature pretreatment processes, water molecules penetrate the biomass cell wall and hydrate cellulose, with the partial removal of hemicellulose and minor amount of lignin [82]. The advantage of using the neutral method compared to the dilute-acid and alkaline catalyzed pretreatments is to avoid the chemical use in excess, because pH close to neutral does not cause corrosion from occurring, and the formation of excess furans during sugar degradation reactions can be eluded. [83]. However, sugar release yields from LHW pretreated biomass are lower than diluted acid pretreated biomass, otherwise higher pretreatment temperature and longer residence time are required for comparable performance [84]. The LHW has a few advantages compared to other pretreatment methods such as no additional catalysts or chemicals, operates at relatively moderate temperature, high hemicelluloses recovery, low levels of inhibitory by-products and cost-effective [85].

**Table 5** presents the comparison between the cellulose content before and after pretreatment of LHW and steam explosion. The temperature range used in LHW is around 170–200°C, whereas in steam explosion the temperature is in the range of 180–195°C. Compared to the untreated SCB, cellulose content increased in pretreated SCB for both LHW and steam explosion pretreatments. The LHW pretreatment of SCB led to an excellent preservation of glucan (cellulose) fraction [88]. Meanwhile, steam explosion with and aid of  $\text{H}_2\text{SO}_4$  acid during

Physico-chemical pretreatment	Pretreatment conditions	Cellulose content of SCB (%)		Reference
		Before pretreatment	After pretreatment	
Liquid hot water	Temp. 200°C, time 10 min, LSR 4	39.5	41.7	[86]
	Temp. 200°C, time 30 min, LSR 10	37.53	53.02	[87]
	Temp. 180°C, time 20 min, LSR 9	43.43	66.53	[88]
	Temp. 170°C, time 60 min, LSR 3	42.6	48.5	[89]
Steam explosion	Temp. 180°C, time 5 min, LSR 20	42.8	49.1	[90]
	Temp. 190°C, time 10 min, LSR 10, impregnated with 4%(v/v) H <sub>2</sub> SO <sub>4</sub>	50.7	61.4	[91]
	Temp. 195°C, time 7.5 min	36.9	62.8	[92]
	Temp. 190°C, time 15 min	43.1	57.5	[93]

LSR: liquid solid ratio.

**Table 5.** Cellulose content of SCB before and after pretreatment by LWH and steam explosion.

pretreatment also increases the cellulose content in the pretreated SCB [91]. The increment of cellulose in pretreated SCB is related to the lignin removal during the pretreatment process either in LHW or steam explosion.

### 2.3. Biological pretreatment

Biological pretreatment of lignocellulosic biomass is considered as an efficient, ecofriendly, and cheap alternative [94]. The biological pretreatment of lignocellulosic biomass is usually performed using cellulolytic and hemicellulolytic microorganisms. The commonly used microorganisms are filamentous fungi which are ubiquitous and can be isolated from the soil, living plants or lignocellulosic waste materials [95]. White-rot fungi have been reported as the most effective microorganisms for the pretreatment of most of the lignocellulosic materials [96]. These microorganisms degrade lignin through the action of lignin-degrading enzymes such as peroxidases and laccases [97]. Brown-rot fungi mainly attack cellulose, while white and soft rot fungi attack both cellulose and lignin [10]. **Table 6** shows the type of fungal species commonly used in biological pretreatment. The biological pretreatment appears to be a promising technique and has very apparent advantages, including low-capital cost, low energy requirement, no chemical requirement, and mild environmental conditions. However, the main disadvantages are the long incubation time, low efficiency, considerable loss of carbohydrate requirement of careful control of growth conditions, and space restrain its applications [98].

Jiraprasertwong et al. [99] investigated the effect of different microbial strains on biological pretreatment of SCB for enzymatic hydrolysis. The results showed that the pretreatment with the white-rot fungus gave the highest glucose concentration around two-fold higher when compared with the others. Hernández et al. [100] reported that SCB pretreated with *Pycnoporus sanguineus* promotes better lignin decay, glucose release, and hydrolysis yields. Studies by Khuong et al. [101] have shown that the initial moisture content of the bagasse was found to affect biological delignification by MG-60, and the 75% moisture content was

Type of fungus	Fungal species
White rot	<i>Phanerochaete chrysosporium</i>
	<i>Pleurotus ostreatus</i>
	<i>Cyathus stercoreus</i>
	<i>Penicillium</i> sp.
Brown rot	<i>Aspergillus niger</i>
	<i>Fomitopsis palustris</i>
	<i>Gloeophyllum trabeum</i>
Soft rot	<i>Trichoderma reesei</i>

**Table 6.** Type of fungal species commonly used in biological pretreatment.

suitable for selective lignin degradation and subsequent ethanol production when white-rot fungus *Phlebia* sp. MG-60 was applied to sugarcane bagasse.

3. Conclusions

There are several pretreatment methods available for SCB; however, the final choice for the selection of pretreatment methods depends upon the effective delignification or hemicellulose removal, low sugar loss, time savings, being economic, and causing less environmental pollution. Each pretreatment method has its own advantages and disadvantages. Instead of performing the chemical pretreatment alone, it is good to combine the pretreatment with other physico-chemical pretreatment such as steam explosion in order to improve the sugar yield and increase the lignin removal from SCB. The combination of pretreatment is a promising method to improve enzymatic hydrolysis and ethanol production from SCB.

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## References

- [1] Zhao Y, Chen M, Zhao Z, Yu S. The antibiotic activity and mechanisms of sugarcane (*Saccharum officinarum* L.) bagasse extract against food-borne pathogens. *Food Chemistry*. 2015;**185**:112-118
- [2] Santos VEN, Ely RN, Szklo AS, Magrini A. Chemicals, electricity and fuels from 28 bio-refineries processing Brazil's sugarcane bagasse: Production recipes and 29 minimum selling prices. *Renewable and Sustainable Energy Reviews*. 2016;**53**:1443-1458
- [3] Kumari S, Das D. Improvement of gaseous energy recovery from sugarcane bagasse by dark fermentation followed by biomethanation process. *Bioresource Technology*. 2015;**194**:354-363
- [4] Li Z, Ge Y. Antioxidant activities of lignin extracted from sugarcane bagasse via different chemical procedures. *International Journal of Biological Macromolecules*. 2012;**51**:1116-1120
- [5] Sukyai P, Anongjanya P, Bunyahwuthakul N, Kongsin K, Harnkarnsujarit N, Sukatta U, et al. Effect of cellulose nanocrystals from sugarcane bagasse on whey protein isolate-based films. *Food Research International*. 2018;**107**:528-535
- [6] Barrera I, Amezcua-Allieri MA, Estupinan L, Martinez T, Aburto J. Technical and economical evaluation of bioethanol production from lignocellulosic residues in Mexico: Case of sugarcane and blue agave bagasses. *Chemical Engineering Research and Design*. 2016;**107**:91-101
- [7] Bezerra TL, Ragauskas AJ. A review of sugarcane bagasse for second-generation bioethanol and biopower production. *Biofuels, Bioproducts and Biorefining*. 2016;**10**(5):634-647
- [8] Conag AT, Villahermosa JER, Cabatingan LK, Go W. Energy densification of sugarcane bagasse through torrefaction under minimized oxidative atmosphere. *Journal of Environmental Chemical Engineering*. 2017;**5**(6):5411-5419
- [9] Webber CL, White PM Jr, Landrum DS, Spaunhorst DJ, Wayment DG. Sugarcane field residue and bagasse Allelopathic impact on vegetable seed germination. *Journal of Agricultural Science*. 2017;**9**(11):10-16
- [10] Sun Y, Cheng J. Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresource Technology*. 2002;**83**(1):1-11
- [11] Ververis C, Georghiou K, Danielidis D, Hatzinikolaou DG, Santas P, Santas R, et al. Cellulose, hemicelluloses, lignin and ash content of some organic materials and their suitability for use as paper pulp supplements. *Bioresource Technology*. 2007;**98**(2):296-301
- [12] Martin C, Galbe M, Nilvebrant NO, Jonsson LJ. Comparison of the fermentability of enzymatic hydrolysates of sugarcane bagasse pretreated by steam explosion using different impregnating agents. *Applied Biochemistry and Biotechnology*. 2002;**98**:699-716
- [13] Canilha L, Chandel AK, Milessi TSDS, Antunes FAF, da Costa Freitas WL, Felipe MGA, et al. Bioconversion of sugarcane biomass into ethanol: An overview about composition,

- pretreatment methods, detoxification of hydrolysates, enzymatic saccharification, and ethanol fermentation. *Journal of Biomedicine and Biotechnology*. 2012;**2012**:1-15
- [14] Brienzo M, Fikizolo S, Benjamin Y, Tyhoda L, Görgens J. Influence of pretreatment severity on structural changes, lignin content and enzymatic hydrolysis of sugarcane bagasse samples. *Renewable Energy*. 2017;**104**:271-280
- [15] Macrelli S, Mogensen J, Zacchi G. Techno-economic evaluation of 2nd generation bioethanol production from sugar cane bagasse and leaves integrated with the sugar-based ethanol process. *Biotechnology for Biofuels*. 2012;**5**:22-39
- [16] Seabra JEA, Tao L, Chum HL, Macedo IC. A techno-economic evaluation of the effects of centralized cellulosic ethanol and co-products refinery options with sugarcane mill clustering. *Biomass and Bioenergy*. 2010;**34**(10):65-78
- [17] RFA. Ethanol industry outlook 2017. In: Building Partnerships and Growing Markets. Washington, DC: Renewable Fuels Association; 2017
- [18] Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, et al. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology*. 2005;**96**:673-686
- [19] Silveira MHL, Morais ARC, Da Costa Lopes AM, Oleksyszzen DN, Bogel-Lukasik R, Andreus J, et al. Current pretreatment technologies for the development of cellulosic ethanol & biorefineries. *ChemSusChem*. 2015;**8**(20):3366-3390
- [20] Galbe M, Zacchi G. Pretreatment: The key to efficient utilization of lignocellulosic materials. *Biomass & Bioenergy*. 2012;**46**:70-78
- [21] Benjamin YH, Cheng H, Görgens JF. Optimization of dilute sulfuric acid pretreatment to maximize combined sugar yield from sugarcane bagasse for ethanol production. *Applied Biochemistry and Biotechnology*. 2014;**172**:610-630
- [22] Alvira P, Tomas-Pejo E, Ballesteros M, Negro MJ. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresource Technology*. 2010;**101**:4851-4861
- [23] Martín C, Klinke HB, Thomsen AB. Wet oxidation as a pretreatment method for enhancing the enzymatic convertibility of sugarcane bagasse. *Enzyme and Microbial Technology*. 2007;**40**:426-432
- [24] Puligundla P, Oh S-E, Mok C. Microwave-assisted pretreatment technologies for the conversion of lignocellulosic biomass to sugars and ethanol: A review. *Carbon Letters*. 2016;**17**(1):1-10
- [25] Moe ST, Janga KK, Hertzberg T, Hägg MB, Øyaas K, Dyrset N. Saccharification of lignocellulosic biomass for biofuel and biorefinery applications—A renaissance for the concentrated acid hydrolysis. *Energy Procedia*. 2012;**20**:50-58
- [26] Jiang L, Fang Z, Li X, Luo J, Fan S. Combination of dilute acid and ionic liquid pretreatments of sugarcane bagasse for glucose by enzymatic hydrolysis. *Process Biochemistry*. 2013;**48**:1942-1946



- [27] Hendriks ATWM, Zeeman G. Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology*. 2009;**100**:10-18
- [28] Kumar R, Mago G, Balan V, Wyman CE. Physical and chemical characterizations of corn Stover and poplar solids resulting from leading pretreatment technologies. *Bioresource Technology*. 2009;**100**:3948-3962
- [29] Soares KC, Mendes S, Benachour M, Abreu CAM. Evaluation of the effects of operational parameters in the pretreatment of sugarcane bagasse with diluted sulfuric acid using analysis of variance. *Journal Chemical Engineering Communications*. 2017;**204**(12):1369-1390
- [30] Timung R, Deshavath NN, Goud VV, Dasu VV. Effect of subsequent dilute acid and enzymatic hydrolysis on reducing sugar production from sugarcane bagasse and spent citronella biomass. *Journal of Energy*. 2016;**2016**:1-12
- [31] Dussan KJ, Silva DDV, Moraes EJC, Arruda PV, Felipe MGA. Dilute-acid hydrolysis of cellulose to glucose from sugarcane bagasse. *Chemical Engineering Transactions*. 2014;**38**:433-438
- [32] Gomez MRS, Andrade RR, Santander CG, Costa AC, Rubens MF. Pretreatment of sugarcane bagasse with phosphoric and sulfuric diluted acid for fermentable sugars production by enzymatic hydrolysis. *Chemical Engineering Transactions*. 2010;**20**:321-326
- [33] Cheng KK, Cai BY, Zhang JA, Ling HZ, Zhou YJ, Ge JP, et al. Sugarcane bagasse hemicellulose hydrolysate for ethanol production by acid recovery process. *Biochemical Engineering Journal*. 2008;**38**(1):105-109
- [34] Pattra S, Sangyoka S, Boonmee M, Reungsang A. Bio-hydrogen production from the fermentation of sugarcane bagasse hydrolysate by *Clostridium butyricum*. *International Journal of Hydrogen Energy*. 2008;**33**:5256-5265
- [35] Chandel AK, Kapoor RK, Singh A, Kuhad RC. Detoxification of sugarcane bagasse hydrolysate improves ethanol production by *Candida shehatae* NCIM 3501. *Bioresource Technology*. 2007;**98**:1947-1950
- [36] Hernández-Salas JM, Villa-Ramírez MS, Veloz-Rendón JS, Rivera-Hernández KN, González-César RA, Plascencia-Espinosa MA, et al. Comparative hydrolysis and fermentation of sugarcane and agave bagasse. *Bioresource Technology*. 2009;**100**(3):1238-1245
- [37] Gámez S, González-Cabriaes JJ, Ramírez JA, Garrote G, Vázquez M. Study of the hydrolysis of sugar cane bagasse using phosphoric acid. *Journal of Food Engineering*. 2006;**74**:78-88
- [38] Rodríguez-Chong A, Ramírez JA, Garrote G, Vázquez M. Hydrolysis of sugarcane bagasse using nitric acid: A kinetic assessment. *Journal of Food Engineering*. 2004;**61**:143-152
- [39] Demirbas A. Products from lignocellulosic materials via degradation processes. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*. 2008;**30**(1):27-37
- [40] Tutt M, Kikas T, Olt J. Influence of different pretreatment methods on bioethanol production from wheat straw. *Agronomy Research Biosystem Engineering*. 2012;**1**:269-276



- [41] Canilha L, Santos VTO, Rocha GJM, Silva JBA, Giulietti M, Silva SS, et al. A study on the pretreatment of a sugarcane bagasse sample with dilute sulfuric acid. *Journal of Industrial Microbiology & Biotechnology*. 2011;**38**:1467-1475
- [42] Zhao X, Zhou Y, Liu D. Kinetic model for glycan hydrolysis and formation of monosaccharides during dilute acid hydrolysis of sugarcane bagasse. *Bioresource Technology*. 2012;**105**(1):160-168
- [43] Yu N, Tan L, Sun Z-Y, Nishimura H, Takei S, Tang Y-Q, et al. Bioethanol from sugarcane bagasse: Focused on optimum of lignin content and reduction of enzyme addition. *Waste Management*. 2018;**76**:404-413
- [44] Ye G, Zeng D, Zhang S, Fan M, Zhang H, Xie J. Ethanol production from mixtures of sugarcane bagasse and *Dioscorea composita* extracted residue with high solid loading. *Bioresource Technology*. 2018;**257**:23-29
- [45] Wunna K, Nakasaki K, Auresenia JL, Abella LC, Gaspillo PD. Effect of alkali pretreatment on removal of lignin from sugarcane bagasse. *Chemical Engineering Transactions*. 2017;**56**:1831-1836
- [46] de Carvalho DM, de Queiroz JH, Colodette JL. Assessment of alkaline pretreatment for the production of bioethanol from eucalyptus, sugarcane bagasse and sugarcane straw. *Industrial Crops and Products*. 2016;**94**:932-941
- [47] Guilherme AA, Dantas PVF, Santos ES, Fernandes FAN, Macedo GR. Evaluation of composition, characterization and enzymatic hydrolysis of pretreated sugar cane bagasse. *Brazilian Journal of Chemical Engineering*. 2015;**32**(01):23-33
- [48] Nadeem M, Aftab MU, Irfan M, Mushtaq M, Qadir A, Syed Q. Production of ethanol from alkali-pretreated sugarcane bagasse under the influence of different process parameters. *Frontiers in Life Science*. 2015;**8**(4):358-362
- [49] Maitan-Alfenas GP, Visser EM, Alfenas RF, Nogueira BRG, de Campos GG, Milagres AF, et al. The influence of pretreatment methods on saccharification of sugarcane bagasse by an enzyme extract from *Chrysoporthe cubensis* and commercial cocktails: A comparative study. *Bioresource Technology*. 2015;**192**:670-676
- [50] Khuong LD, Kondo R, Leon RD, Anh TK, Shimizu K, Kamei I. Bioethanol production from alkaline-pretreated sugarcane bagasse by consolidated bioprocessing using *Phlebia* sp. MG-60. *International Biodeterioration & Biodegradation*. 2014;**88**:62-68
- [51] Soares ML, Gouveia ER. Influence of the alkaline delignification on the simultaneous saccharification and fermentation (SSF) of sugar cane bagasse. *Bioresource Technology*. 2013;**147**(1):645-648
- [52] Wanderley MCA, Martín C, Rocha GJM, Gouveia ER. Increase in ethanol production from sugarcane bagasse based on combined pretreatments and fed-batch enzymatic hydrolysis. *Bioresource Technology*. 2013;**128**:448-453

- [53] Rezende CA, de Lima MA, Maziero P, de Azevedo ER, Garcia W, Polikarpov I. Chemical and morphological characterization of sugarcane bagasse submitted to a delignification process for enhanced enzymatic digestibility. *Biotechnology for Biofuels*. 2011;**11**:4-54
- [54] Rabelo SC, Filho RMF, Costa AC. Lime pretreatment of sugarcane bagasse for bioethanol production. *Applied Biochemistry and Biotechnology*. 2009;**153**:139-150
- [55] Grimaldi MP, Marques MP, Lalluce C, Cilli EM, Sponchiado SRP. Evaluation of lime and hydrothermal pretreatments for efficient enzymatic hydrolysis of raw sugarcane bagasse. *Biotechnology for Biofuels*. 2015;**8**(205):1-14
- [56] Paixão SM, Ladeirab SA, Silvaa TP, Areza BF, Roseiroa JC, Martinsb MLL, et al. Sugarcane bagasse delignification with potassium hydroxide for enhanced enzymatic hydrolysis. *RSC Advances*. 2016;**6**:1042-1052
- [57] Hedayatkah A, Motamedi H, Varzi HN, Ghezelbash G, Bahnamiry MA, Karimi K. Improvement of hydrolysis and fermentation of sugarcane bagasse by soaking in aqueous ammonia and methanolic ammonia. *Bioscience, Biotechnology, and Biochemistry*. 2013;**77**(7):1379-1383
- [58] Zhu Z-S, Zhu M-J, Xu W-X, Liang L. Production of bioethanol from sugarcane bagasse using  $\text{NH}_4\text{OH-H}_2\text{O}_2$ . Pretreatment and simultaneous saccharification and co-fermentation. *Biotechnology and Bioprocess Engineering*. 2012;**17**:316-325
- [59] Ahmadi F, Zamiri MJ, Khorvash M, Ziaee E, Polikarpov I. Pre-treatment of sugarcane bagasse with a combination of sodium hydroxide and lime for improving the ruminal degradability: Optimization of process parameters using response surface methodology. *Journal of Applied Animal Research*. 2016;**44**(1):287-296
- [60] Ayeni AO, Adeeyo OA, Ayoola A. Effective gravimetric characterization for lignocellulosic biomass: Comparison of  $\text{NaOH-H}_2\text{O}_2$  and  $\text{Ca(OH)}_2\text{-H}_2\text{O}_2$  oxidation pretreated sugarcane bagasse. *International Journal of Scientific Engineering and Technology*. 2015;**4**(1):5-9
- [61] Chen Y, Stevens MA, Zhu Y, Holmes J, Xu H. Understanding of alkaline pretreatment parameters for corn stover enzymatic saccharification. *Biotechnology for Biofuels*. 2013;**6**(8):1-10
- [62] Chaturvedi V, Verma P. An overview of key pretreatment processes employed for bio-conversion of lignocellulosic biomass into biofuels and value added products. *3 Biotech*. 2013;**3**:415-431
- [63] Sabiha-Hanim S, Norazlina I, Noraishah A, Nor Suhaila MH. Reducing sugar production from oil palm fronds and rice straw by acid hydrolysis. In: 2012 IEEE Colloquium on Humanities, Science & Engineering Research (CHUSER 2012); 3-4 December; Kota Kinabalu. 2012. pp. 642-645
- [64] Cardona CA, Quintero JA, Paz IC. Production of bioethanol from sugarcane bagasse: Status and perspectives. *Bioresource Technology*. 2010;**101**:4754-4766

- [65] Visser EM, Falkoski DL, de Almeida MN, Maitan-Alfenas GP, Guimarães VM. Production and application of an enzyme blend from *Chrysosporthe cubensis* and *Penicillium pinophilum* with potential for hydrolysis of sugarcane bagasse. *Bioresource Technology*. 2013; **144**:587-594
- [66] Wyman CE, Dale BE, Elander RT, Holtzapple M, Ladisch MR, Lee YY. Coordinated development of leading biomass pretreatment technologies. *Bioresource Technology*. 2005; **96**:1959-1966
- [67] Zhao X, Chang K, Liu D. Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. *Applied Microbiology and Biotechnology*. 2009; **82**:815-827
- [68] Agnihotri S, Johnsen IA, Bøe MS, Øyaas K, Moe S. Ethanol organosolv pretreatment of softwood (*Picea abies*) and sugarcane bagasse for biofuel and biorefinery applications. *Wood Science and Technology*. 2015; **49**:881-896
- [69] Mesa L, González E, Cara C, González M, Castro E, Mussatto SI. The effect of organosolv pretreatment variables on enzymatic hydrolysis of sugarcane bagasse. *Chemical Engineering Journal*. 2011; **168**:1157-1162
- [70] Novo LP, Gurgel LVA, Marabezi K, da Silva Curvelo AA. Delignification of sugarcane bagasse using glycerol–water mixtures to produce pulps for saccharification. *Bioresource Technology*. 2011; **102**:10040-10046
- [71] Sun FF, Zhao X, Hong J, Tang Y, Wang L, Sun H, et al. Industrially relevant hydrolyzability and fermentability of sugarcane bagasse improved effectively by glycerol organosolv pretreatment. *Biotechnology for Biofuels*. 2016; **9**(59):1-13
- [72] Martín C, Puls J, Saake B, Schreiber A. Effect of glycerol pretreatment on component recovery and enzymatic hydrolysis of sugarcane bagasse. *Cellulose Chemistry and Technology*. 2011; **45**:487-494
- [73] Zhang Z, O'Hara IM, Doherty WO. Pretreatment of sugarcane bagasse by acidified aqueous polyol solutions. *Cellulose*. 2013; **20**:3179-3190
- [74] Ramos LP, Silva L, Ballem AC, Pitarelo AP, Chiarello LM, Silveira MHL. Enzymatic hydrolysis of steam-exploded sugarcane bagasse using high total solids and low enzyme loadings. *Bioresource Technology*. 2015; **175**:195
- [75] Sassner P, Mårtensson CG, Galbe M, Zacchi G. Steam pretreatment of H<sub>2</sub>SO<sub>4</sub>-impregnated *Salix* for the production of bioethanol. *Bioresource Technology*. 2008; **99**:137-145
- [76] Oliva JM, Sáez F, Ballesteros I, González A, Negro MJ, Manzanares P, et al. Effect of lignocellulosic degradation compounds from steam explosion pretreatment on ethanol fermentation by thermotolerant yeast *Kluyveromyces marxianus*. *Applied Microbiology and Biotechnology*. 2003; **105**:141-154
- [77] Rabemanolontsoa H, Saka S. Various pretreatments of lignocellulosics. *Bioresource Technology*. 2016; **199**:83-91
- [78] Jedvert K, Saltberg A, Lindström ME, Theliander H. Mild steam explosion and chemical pre-treatment of Norway spruce. *BioResources*. 2012; **7**:2051-2074

- [79] Chiaramont 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 and Bioenergy*. 2012;**46**:25
- [80] Sánchez ÓJ, Cardona CA. Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresource Technology*. 2008;**99**:5270-5295
- [81] Gullón P, Romaní A, Vila C, Garrote G, Parajó JC, editors. Potential of hydrothermal treatments in lignocellulose biorefineries. *Biofuels, Bioproducts and Biorefining*. 2012;**6**:219-232
- [82] Jönsson LJ, Martín C. Pretreatment of lignocellulose: Formation of inhibitory byproducts and strategies for minimizing their effects. *Bioresource Technology*. 2018;**199**:103-112
- [83] Geddes CC, Peterson JJ, Roslander C, Zacchi G, Mullinnix MT, Shanmugam KT, et al. Optimizing the saccharification of sugar cane bagasse using dilute phosphoric acid followed by fungal cellulases. *Bioresource Technology*. 2010;**101**:1851-1857
- [84] Yang B, Tao L, Wyman CE. Strengths, challenges, and opportunities for hydrothermal pretreatment in lignocellulosic biorefineries. *Biofuels, Bioproducts and Biorefining*. 2017;**12**:125-138
- [85] Gírio FM, Fonseca C, Carvalheiro F, Duarte LC, Marques S, Bogel-Lukasik R. Hemicelluloses for fuel ethanol: A review. *Bioresource Technology*. 2010;**101**:4775-4800
- [86] Wang Z, Dien BS, Rausch KD, Tumbleson ME, Singh V. Fermentation of undetoxified sugarcane bagasse hydrolyzates using a two stage hydrothermal and mechanical refining pretreatment. *Bioresource Technology*. 2018;**261**:313-321
- [87] Michelin M, Ximenes E, de Lourdes Teixeira de Moraes Polizeli M, Ladisch MR. Effect of phenolic compounds from pretreated sugarcane bagasse on cellulolytic and hemicellulolytic activities. *Bioresource Technology*. 2016;**199**:275-278
- [88] Gurgel LVA, Pimenta MTB, Curvelo AAS. Ethanol–water organosolv delignification of liquid hot water (LHW) pretreated sugarcane bagasse enhanced by high–pressure carbon dioxide (HP–CO<sub>2</sub>). *Industrial Crops and Products*. 2016;**94**:942-950
- [89] Vallejos ME, Zambon MD, Area MC, Curvelo AAS. Low liquid–solid ratio (LSR) hot water pretreatment of sugarcane bagasse. *Green Chemistry*. 2012;**14**:1982-1989
- [90] Silva TAL, Zamora HDZ, Varão LHR, Prado NS, Baffi MA, Pasquini D. Effect of steam explosion pretreatment catalysed by organic acid and alkali on chemical and structural properties and enzymatic hydrolysis of sugarcane bagasse. *Waste and Biomass Valorization*. 2017;**9**(11):2191-2201
- [91] You Y, Zhou Z, Zhou P, Bu L, Jiang J, Jang W. Comparison of pretreatment methods for production of ethanol from sugarcane bagasse. *BioResource*. 2016;**11**(1):2297-2307
- [92] Neves PV, Pitarelo AP, Ramos LP. Production of cellulosic ethanol from sugarcane bagasse by steam explosion: Effect of extractives content, acid catalysis and different fermentation technologies. *Bioresource Technology*. 2016;**208**:184-194

- [93] Rocha GJM, Gonçalves AR, Oliveira BR, Olivares EG, Rossella CEV. Steam explosion pretreatment reproduction and alkaline delignification reactions performed on a pilot scale with sugarcane bagasse for bioethanol production. *Industrial Crops and Products*. 2012;**35**(1):274-279
- [94] Wan C, Li Y. Fungal pretreatment of lignocellulosic biomass. *Biotechnology Advances*. 2012;**30**:1447-1457
- [95] Vats S, Maurya DP, Shaimoon M, Agarwal A, Negi S. Development of a microbial consortium for the production of blend enzymes for the hydrolysis of agricultural waste into sugars. *Journal of Scientific and Industrial Research*. 2013;**72**:585-590
- [96] Kumar R, Wyman CE. Effects of cellulase and xylanase enzymes on the deconstruction of solids from pretreatment of poplar by leading technologies. *Biotechnology Progress*. 2009;**25**:302-314
- [97] Kumar P, Barrett DM, Delwiche MJ, Stroeve P. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Industrial and Engineering Chemistry Research*. 2009;**48**(8):3713-3729
- [98] Zheng Y, Pan Z, Zhang RN. Overview of biomass pretreatment for cellulosic production. *International Journal of Agricultural and Biological Engineering*. 2009;**2**:51-68
- [99] Jiraprasertwong A, Gulari E, Chavadej S. Effect of different microbial strains on biological pretreatment of sugarcane bagasse for enzymatic hydrolysis. *World Academy of Science, Engineering and Technology International Journal of Bioengineering and Life Sciences*. 2014;**8**(9):968-972
- [100] Hernández CA, Ziarelli F, Gaimé I, Silva AMFD, García G, García-Pérez JA, et al. Chemical and biological pretreatments on sugarcane bagasse to enhance its enzymatic hydrolysis. *ChemistrySelect*. 2017;**2**(15):4213-4218
- [101] Khuong LD, Kondo R, Leon RD, Anh TK, Meguro S, Shimizu K, et al. Effect of chemical factors on integrated fungal fermentation of sugarcane bagasse for ethanol production by a white-rot fungus, *Phlebia* sp. MG-60. *Bioresource Technology*. 2016;**167**:33-40