

# Effect of alkaline peroxide pretreatment on fibre composition of various lignocellulosic residues

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## Abstract

**Purpose:** The aim of this work is to determine the effect produced by alkaline hydrogen peroxide (AHP) pretreatment on the composition of several lignocellulosic residues. These residues represent an abundant carbon-neutral renewable energy source. However, the major challenge in their hydrolysis to obtain fermentable sugars for several bio-products production is the cell wall recalcitrance, which must be reduced by an adequate pretreatment of the residue.

**Method:** Rice husk, exhausted sugar beet cossettes, wheat straw and sunflower stem were used as the raw materials in this work. AHP pretreatment was performed under the same conditions for all the residues. Alkaline hydrogen peroxide at 4% (w/v) and pH = 11.5 at a solid/liquid ratio of 1:20 (w/v) was added to each solid and the mixtures were incubated at 30°C for 24h. After that, the pre-treated solids were filtered, washed and dried in an oven at 40°C for 24h. The composition of the solids, before and after the pretreatment was analysed.

**Results:** Fibre composition varies between the tested wastes, showing rice husk the highest lignin content. After the pretreatment, a high percentage of lignin was lost (55- 85%), while cellulose content remained almost constant for all the solids. However, hemicellulose content losses differed between the different residues.

**Conclusion:** AHP pretreatment is a simple and efficient method for solubilisation of lignin, although hemicellulose is also solubilized. The main advantage of this pretreatment is that cellulose content is not affected resulting in a substantial concentration. Thus, as a consequence of AHP pretreatment the total fermentable sugars content of the residues increases between 10% and 25%.

## Introduction

Currently, the environmental issues associated to the production of petroleum derived products has induced the interest on the production of fuels, plastic, and other products from renewable resources. Among them, lignocellulosic biomass produced as a result of agriculture and forestry industries has turned out to be interesting, giving its potential for the production of chemical and fuels in a sustainable way [1]. Moreover, this raw material presents an abundant carbon-neutral renewable source and does not compete with primary food production. Rice husk (RH), wheat straw (WS), sunflower stalk (SS), and exhausted sugar beet cossettes (ESBC) are some samples of lignocellulosic biomass. In particular, RH, WS and SS are the residues left after the seed collection process, while ESBC is obtained after sugar extraction by diffusion in the sugar production process from sugar beet. After the sugar extraction stage, the solid residue is pressed, dehydrated, and conformed as pellets, being normally used as animal feed [2].

Lignocellulosic biomass, composed of cellulose, hemicellulose and lignin, can be a low-cost and abundant feedstock which can be transformed in different value-added products by biotechnological pathways [3]. The most common process consists of an enzymatic hydrolysis to produce fermentable sugars which are converted to the desired product in a subsequent fermentation. The structural characteristics of lignocellulosic materials form strong native recalcitrant structures which decrease enzyme accessibility. For this purpose, a pretreatment stage is required [4]. In general, the aim of the pretreatment step is to break down the recalcitrant structure of lignocellulose to make cellulose and hemicellulose more accessible to the enzymes [5]. Given that lignin acts as a physical barrier in the enzymatic degradation process, it hinders the cellulolytic enzyme attack. Consequently, the rate and extent of the enzymatic hydrolysis of agricultural residues is inversely related to the lignin content, with

maximum degradation occurring only when 50% or more of the lignin is removed from the original biomass [6].

With the aim of improving enzyme hydrolysis yields and remove or reduce the lignin content, numerous pretreatments, such as biological, physical (mechanical comminution, extrusion), chemical (alkali, acid, ozonolysis, etc.) or physicochemical (steam explosion, liquid hot water, ultrasound, etc.) have been studied [7]. Some advantages of the pretreatment includes higher hydrolysis yields, avoiding the degradation of sugars (hexoses and pentoses), and the production of fermentation inhibitors [5, 8]. The effect on the lignocellulosic biomass depends on the pretreatment used [9]. So, for example, physical pretreatment breaks down the biomass size and crystallinity by milling or grading, while biological pretreatment involves the use of microorganisms to degrade lignin and hemicellulose, but leaving the cellulose intact. As regards chemical pretreatment, alkali, acid, steam or supercritical fluids, etc could be used.

Alkaline hydrogen peroxide (AHP) pretreatment is an oxidative process which has shown effective results on RH [3, 10, 11], WS [12, 13], sugarcane bagasse [14], softwood [15], corn stover [16], among others. Furthermore, it has been proved that AHP pretreatment could significantly improve biomass digestibility as it does not only selectively remove lignin, but also it deconstructs the cell walls [17–19]. Hydrogen peroxide produces the oxidizing radicals necessary to degrade lignin by means of the dissociation reaction produced at alkaline pH [20].  $\text{H}_2\text{O}_2$  dissociates into hydrogen and the hydroperoxyl anion ( $\text{HOO}^-$ ). The anion reacts with remaining peroxide to form highly-reactive hydroxyl radicals which attack the lignin structure [20].

The main advantage of AHP pretreatment of lignocellulosic biomass is the utilization of chemicals at low concentration and temperature. Moreover, the reactors at industrial scale do not need to work at high pressure and temperature, reducing the process cost [21]. Moreover, it is an attractive, low cost and environmentally friendly pretreatment method as the only final products obtained from the decomposition of hydrogen peroxide are water and oxygen [22]. However, it shows some disadvantages, such as, the production of inhibitory compounds, like phenolic compound and volatile fatty acids, which can affect the subsequent fermentation stage. So, these inhibitors have been proved to affect the fermentation for ethanol and lactic acid production [22, 23].

The aim of this study is to evaluate the effect of AHP pretreatment on the composition of several lignocellulosic residues, specifically RH, WS, SS and ESBC. For this study, pretreatment parameters, such as, temperature, time, pH or hydrogen peroxide concentration were fixed based on previous studies carried out by the research group [3, 6, 24]

## **Materials and methods**

### **Raw materials**

Rice husks (RH) were provided by the Spanish company Herba Rice Mills (Seville, Spain). Wheat straw (WS) and sunflower stalks were provided by IFAPA Center Rancho de la Merced (Jerez de la Frontera, Spain). Exhausted sugar beet cossettes (ESBC) were provided by the British company AB-Sugar situated in Jerez de la Frontera (Cadiz, Spain). Samples were collected and stored at 4 °C until use. ESBC were conformed in dried pellets by the company, which show 6mm diameter and variable length (10–40 mm).

### **Solid conditioning**

RH were milled and sieved to obtain different particle size; 0-0.5 mm, 0.5-1 mm, 1-1.5 mm and 1.5-2 mm. SS, WS and ESBC were milled and sieved to obtain a unique particle size between 0.5-1mm. All the solids were stored at room temperature.

### **Chemical pretreatment**

AHP pretreatment was performed by mixing each solid fraction with hydrogen peroxide at 4 % (w/v) in a solid/liquid ratio of 1:20 (w/v) and the pH was adjusted at 11.5 with NaOH. The erlenmeyer flasks, containing this mixture, were covered with aluminium foil and incubated at 30 °C during 24 h. After the pretreatment, the slurry was filtered through a Whatman No. 1 filter paper to separate solid and liquid fractions. Afterwards, the solid was washed with distilled water to remove undesired chemicals

until a neutral pH was reached. Finally, the solid was dried in an oven at 40°C during 24h. Before and after the pretreatment, the solid was weighted in order to know the amount of lost solid during the pretreatment. The conditions used for AHP pretreatment were based on our previous research [3, 6, 10]

## Fibre analysis

Fibre compositional analysis was carried out before and after the pretreatment of each fraction of rice husk. This analysis was developed in Fibertec™ 8000 and FT 121 Fibertec. The former was used to determine fats and ADL method, while the second one was used to NDF method and ADF method. Fibre compositional analysis consists on the determination of acid detergent fibre (ADF) and acid detergent lignin (ADL) according to EN ISO 13906:2008 and the determination of amylase treated neutral detergent fibre (NDF) according to AOAC 2002:04/ISO 16472:2006.

All the samples were milled to obtain a particle size smaller than 1 mm. Then, they were dried in an oven during 2 h at a temperature of 105 °C in order to remove the humidity. Then, they were kept at room temperature in a desiccator for 30 min.

Sample fractions, compliant substances and denomination used in this paper are shown in Table1.

Code	Sample fraction	Compliant substances	Denomination
A	removed with acetone	Fats, oils, wax	fats
B+C	removed with neutral detergent	Proteins, enzymes, pectins, soluble salts, etc.	Salts and no cellulosic
B	no calcined removed	soluble salts	Salts
C	calcined removed	rest of removable material no saline	no cellulosic
D	removed with acid detergent	Hemicellulose, etc.	Hemicellulose
E	removed with concentrated acid	Cellulose, soluble lignin	Cellulose
D+E	removed with acid	hemicellulose, cellulose and soluble lignin	Cellulose and Hemicellulose
F	calcined not removed	insoluble lignin, etc.	Lignin
G	not calcined and not removed	insoluble salts, minerals, etc.	Minerals
B+G	total no calcined	Total salts	Salts and minerals

Table 1. Description of analysed fractions in fibre composition analyses

## Results and discussion

### Influence on the solid particle size

Feedstock particle size is an important aspect to consider in the economy of a bio-process where a pretreatment is needed. Normally, smaller particle sizes improve the yields of the subsequent hydrolysis step because there is an increase of the specific surface area [25]. However, as milling process consumes a significant amount of energy, a pretreatment which no requires a previous particle size reduction should be more profitable for the global economy of the process [14].

AHP pre-treatment was applied on milled rice husk and four different ranges of size were assayed (0-0.5, 0.5-1, 1-1.5 and 1.5-2 mm). The solids composition (fats, salts, no cellulosic, hemicellulose, cellulose, lignin and minerals percentages) before and after AHP pretreatment were measured and the results obtained are shown in Figures 1 and 2.

As it can be observed in Figure 1, milling of rice husk does not affect significantly its composition, although salts and no cellulosic fractions percentages differ between the two sizes analysed (0-0.5 mm and 0.5-1 mm). In the same way, no significant differences were observed in the fibre composition of rice husk after AHP pretreatment (Figure 2) when different particle sizes were assayed. The small differences that were registered between the fractions can be caused by the low accuracy of the method followed for the fibre composition analysis.

Many papers report that a small particle size improves the hydrolysis yield [14]. For example, Khullar *et al.* analysed the effect of different particle sizes on the hydrolysis yield of Miscanthus solid particles, previously pre-treated by several methods. They concluded that the highest yields were obtained

with smaller particle sizes [26]. However, not only the particle size has to be considered on the hydrolysis yield of a solid but also other factors, such as the enzyme source and loading, the presence of auxiliary enzymes in the hydrolytic preparation employed, the solid loading, the reaction time, etc [25]. This means that the comparison of hydrolysis efficiencies should be made with caution.

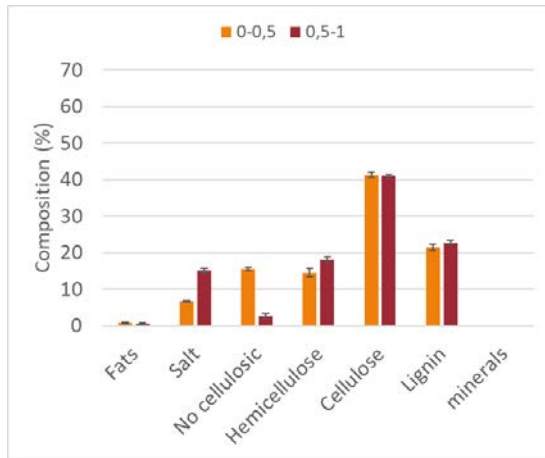


Figure 1. Rice husk composition before AHP pretreatment at the different particle sizes assayed.

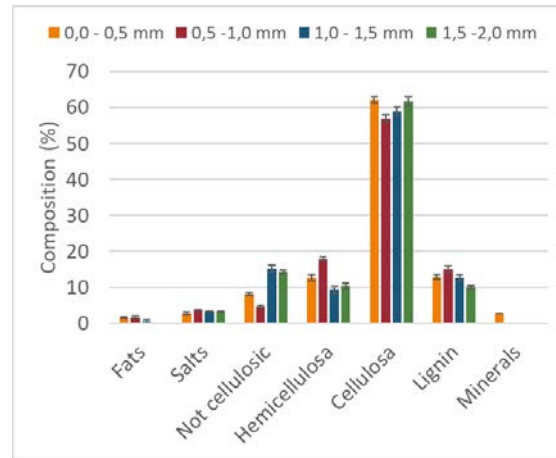


Figure 2. Rice husk composition after AHP pretreatment at the different particle sizes assayed

Additionally, the loss in weight produced in each fraction after AHP peroxide pre-treatment, expressed as total-solid losses percentage (%TSL), was also measured and results obtained are shown in Table 2. As it can be observed, the average percentage of total-solid losses was around 25%, independently of the particle sizes range. Consequently, solid losses produced by AHP pretreatment do not depend on the size of the rice husk particles.

Particle size (mm)	%TSL
0.0 – 0.5	25.73 ± 0.21
0.5 – 1.0	24.97 ± 1.14
1.0 – 1.5	25.37 ± 0.76
1.5 – 2.0	25.05 ± 0.71
<b>Average</b>	<b>25.31 ± 0.38</b>

Table 2. Percentage of total-solid losses (%TSL) on rice husk fractions after the AHP pretreatment,

As the pre-treatment was not affected by the particle size of the solid, a particle size range of 0.5-1 mm was used for the following experiments. This range was selected because the filtration step needed to remove the filtrate from the pretreated solid is quicker, as particles smaller than 0.5 mm settle first and hinder the filtration step. Moreover, fibre compositional analyses must be applied on particles smaller than 1 mm.

### AHP pretreatment effect on different agro-industrial residues

The effect of the alkaline peroxide pretreatment on the fibre composition of different agro-industrial residues was studied. Rice husks (RH), wheat straw (WS), sunflower stalk (SS) and exhausted sugar beet cossettes (ESBC) fibre composition was analysed before and after the pretreatment. As it can be observed, RH, WS, SS and ESB showed quite different initial fibre compositions (Figure 3) and therefore, the effect of AHP pretreatment was expected to be also different.

As hydrolysis technologies usually target the production of fuels or chemicals from the cellulose and hemicellulose in the biomass, the initial amount of both polysaccharides in a residue is an important factor to be considered. As it can be seen in Figure 3, SS showed the highest cellulose and hemicellulose content ( $56.08 \pm 1.22\%$  of cellulose and  $19.57 \pm 0.96\%$  of hemicellulose) followed by WH ( $39.15 \pm 0.98\%$  and  $29.66 \pm 1.12\%$ ) and RC ( $41.08 \pm 0.64\%$  and  $16.31 \pm 1.02\%$ ); while ESBC showed the lowest content ( $20.02 \pm 3.94\%$  and  $17.4 \pm 0.84\%$ ). As it is well-known, lignin content is another crucial factor to be considered in the selection of a lignocellulosic residue to be used as raw material to obtain the desired bio-product. So, the higher the lignin content, the more difficult the hydrolysis process will be. Lignin can

hinder the enzymatic hydrolysis by acting as a physical barrier, restricting the accessibility of cellulose to enzymes, but also by non-productive binding of cellulolytic enzymes [27]. RH showed the highest insoluble lignin content ( $22.07 \pm 0.65\%$ ), meanwhile this content was  $8.87 \pm 0.83\%$  and  $4.85 \pm 0.59\%$  in SS and WS, respectively. Insoluble lignin content in ESBC was negligible.

As residues with high potentially hydrolysable polysaccharides and low lignin content are the preferred ones for enzymatic hydrolysis processes, SS and WS would be the most adequate. RC shows high values of both cellulose and hemicellulose content but also a significant fraction of lignin. On the contrary, ESBC shows the lowest cellulose and hemicellulose content, but lignin fraction is negligible.

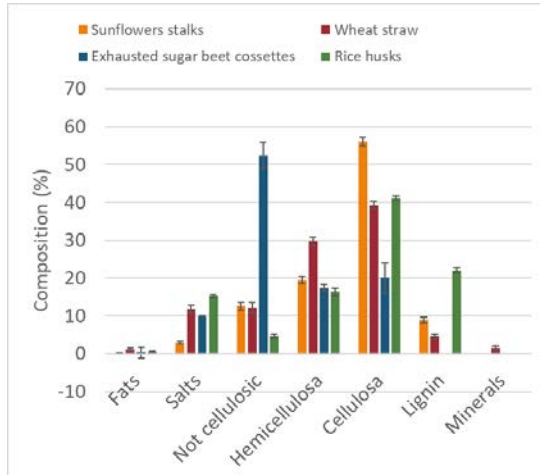


Figure 3. Fibre composition analysis of sunflowers stalks, wheat straw, exhausted sugar beet cossettes, and rice husks before the AHP pretreatment

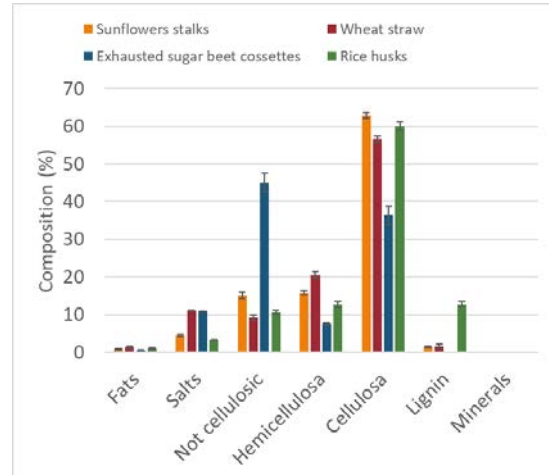


Figure 4. Fibre composition analysis of sunflowers stalks, wheat straw, exhausted sugar beet cossettes, and rice husks after the AHP pretreatment

The fibre composition analysis of the AHP pretreated solids is represented in Figure 4. After the pretreatment (figure 4), it can be observed in all the residues a reduction in practically all the components analysed except in cellulose, which significantly increased. Lignin was almost eliminated on SS and WS, obtaining values of  $1.48 \pm 0.61\%$  and  $1.41 \pm 0.41\%$ , respectively. In the case of rice husk, which is the residue with the highest initial lignin content among the residues tested, this content was reduced from  $22.07 \pm 0.65\%$  to  $12.68 \pm 0.69\%$ . Moreover, in spite of hemicellulose content was reduced after AHP pretreatment of all the residues, the total amount of cellulose and hemicellulose increased for all of them.

The effect of AHP pretreatment on the main residues components (hemicellulose, cellulose and lignin) is summarized in Figure 5. As it can be seen, lignin was reduced in all the residues. However, part of hemicellulose was also solubilized. This effect is expected with AHP, given that oxidizing radicals produced with hydrogen peroxide at alkaline pH, are able to degrade lignin and also solubilize part of hemicellulose [12, 22]. As it was mentioned before, cellulose content increased in all cases. Given that for the hydrolysis technologies hemicellulose and cellulose represent the most important polymers to be converted into fermentable sugars, the total amount of both polysaccharides is an important factor to be considered. For all residues tested, the sum of cellulose and hemicellulose content increased after AHP.

The effectiveness of the reactions produced during AHP pretreatment depends on pH, temperature, hydrogen peroxide concentration and time reaction. Thus, the efficiency of AHP pretreatment might have been improved if these variables had been optimized to each residue [28]. The conditions are optimized for rice husk on previous researches of our group [10]. Despite this fact, lignin content was almost eliminated in SS and WS with the conditions tested

Conditions used in this paper are similar to other published in literature. So, Chen et al., employed 4 %  $H_2O_2$  at room temperature (pH 13.1) in order to pretreat WS, however, a previous pretreatment with steam explosion was applied before AHP. On the other hand, Yuan et al used less hydrogen peroxide concentration (0.4% w/v), but higher temperatures ( $50^\circ C$ ) and a pre-extraction with sodium hydroxide before AHP pretreatment were needed in this case [12, 29]. On both articles, the

addition of  $H_2O_2$  improved the delignification of WS, observing an increase on cellulose content and a decrease on hemicellulose and lignin content.

Saha et al [11] also used this method to pretreat RH, however, higher hydrogen peroxide concentration (7.5% v/v) and temperature (35°C) were needed in this case compared with the conditions tested in the present work.

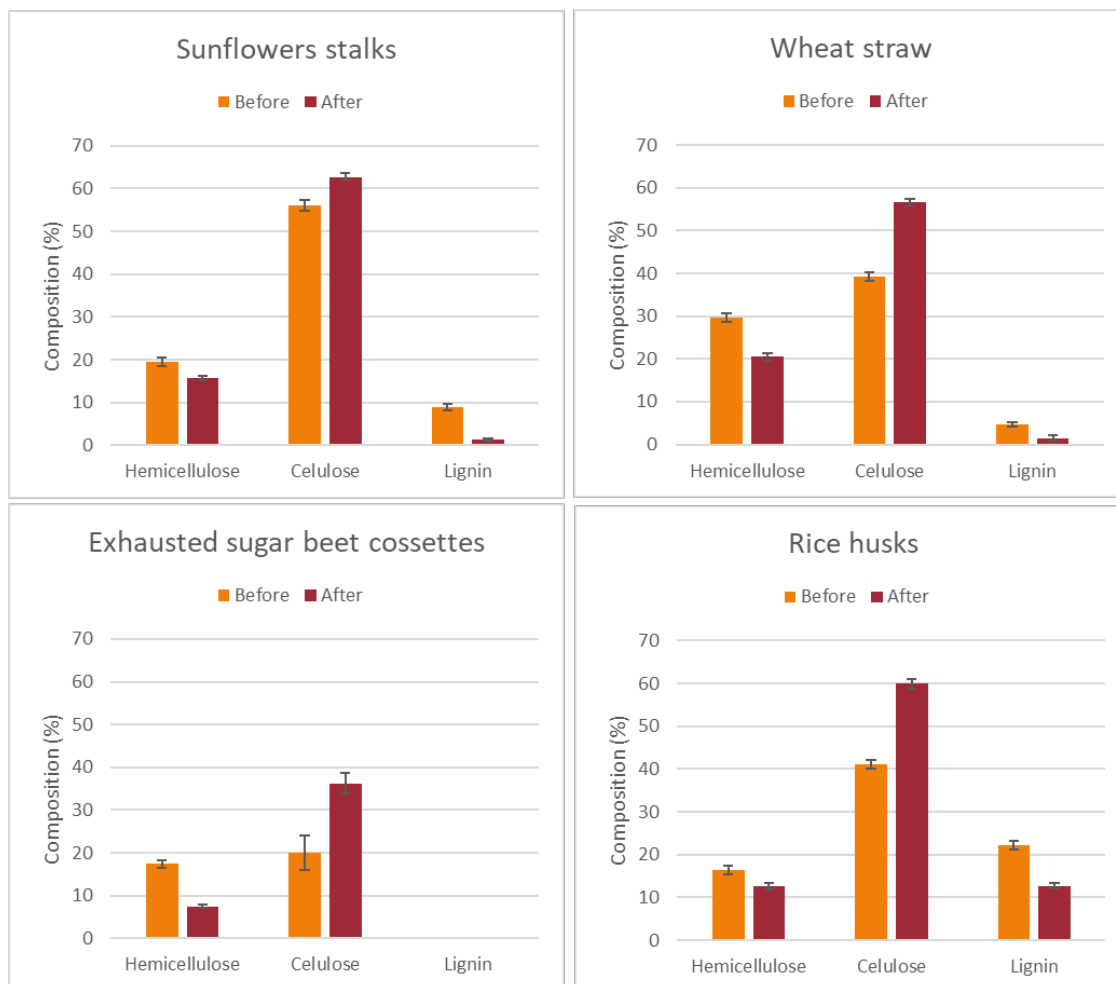


Figure 5. Composition of main components before and after AHP pretreatment

## Conclusion

Lignocellulosic biomass is a potential raw material for the production of added-value products through fermentation of monomeric sugars. Nevertheless, a previous pretreatment stage is needed to make the polymers more accessible to the enzymes in the hydrolyses step producing the fermentable sugars. Alkaline hydrogen peroxide has turned out to be an efficient pretreatment method to remove a significant amount of lignin in sunflowers stalks, wheat straw and rice husk, being cellulose and hemicellulose more accessible to the enzymes in the hydrolysis step. In spite of hemicellulose was partially solubilized in the four residues tested, cellulose fraction was not affected achieving its concentration. As a result of AHP, higher concentrations of hydrolysable polymers (hemicellulose plus cellulose) were attained with all the studied residues, which will provide higher hydrolysis yields.

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## References

1. Isikgor, F.H., Becer, C.R.: Lignocellulosic biomass: A sustainable platform for the production of bio-based chemicals and polymers. *Polym. Chem.* 6, 4497–4559 (2015)
2. Díaz, A.B., Marzo, C., Caro, I., de Ory, I., Blandino, A.: Valorization of exhausted sugar beet cossettes by successive hydrolysis and two fermentations for the production of bio-products. *Bioresour. Technol.* 225, 225–233 (2017)
3. Diaz, A., Le Toullec, J., Blandino, A., De Ory, I., Caro, I.: Pretreatment of rice hulls with alkaline peroxide to enhance enzyme hydrolysis for ethanol production. *Chem. Eng. Trans.* 32, 949–954 (2013)
4. Sun, S., Sun, S., Cao, X., Sun, R.: The role of pretreatment in improving the enzymatic hydrolysis of lignocellulosic materials. *Bioresour. Technol.* 199, 49–58 (2016)
5. Xu, Z., Huang, F.: Pretreatment methods for bioethanol production. *Appl. Biochem. Biotechnol.* 174, 43–62 (2014)
6. Díaz, A.B., Blandino, A., Belleli, C., Caro, I.: An effective process for pretreating rice husk to enhance enzyme hydrolysis. *Ind. Eng. Chem. Res.* 53, 10870–10875 (2014)
7. Alvira, P., Tomás-Pejó, E., Ballesteros, M., Negro, M.J.: Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresour. Technol.* 101, 4851–4861 (2010)
8. Brodeur, G., Yau, E., Badal, K., Collier, J., Ramachandran, K.B., Ramakrishnan, S.: Chemical and Physicochemical Pretreatment of Lignocellulosic Biomass: A Review. *Enzyme Res.* 2011, 1–17 (2011)
9. Chiamonti, D., Prussi, M., Ferrero, S., Oriani, L., Ottonello, P., Torre, P., Cherchi, F.: Review of pretreatment processes for lignocellulosic ethanol production, and development of an innovative method. *Biomass and Bioenergy.* 46, 25–35 (2012)
10. Cabrera, E., Muñoz, M.J., Martín, R., Caro, I., Curbelo, C., Díaz, A.B.: Alkaline and alkaline peroxide pretreatments at mild temperature to enhance enzymatic hydrolysis of rice hulls and straw. *Bioresour. Technol.* 167, 1–7 (2014)
11. Saha, B.C., Cotta, M.A.: Enzymatic saccharification and fermentation of alkaline peroxide pretreated rice hulls to ethanol. *Enzyme Microb. Technol.* 41, 528–532 (2007)
12. Chen, H., Han, Y., Xu, J.: Simultaneous saccharification and fermentation of steam exploded wheat straw pretreated with alkaline peroxide. *Process Biochem.* 43, 1462–1466 (2008)
13. Saha, B.C., Cotta, M.A.: Ethanol Production from Alkaline Peroxide Pretreated Enzymatically Saccharified Wheat Straw. *Biotechnol. Prog.* 22, 449–453 (2006)
14. Rabelo, S.C., Andrade, R.R., Filho, R.M., Costa, A.C.: Alkaline hydrogen peroxide pretreatment, enzymatic hydrolysis and fermentation of sugarcane bagasse to ethanol. *Fuel.* 136, 349–357 (2014)
15. Alvarez-Vasco, C., Zhang, X.: Alkaline hydrogen peroxide pretreatment of softwood: Hemicellulose degradation pathways. *Bioresour. Technol.* (2013)
16. Mittal, A., Katahira, R., Donohoe, B.S., Black, B.A., Pattathil, S., Stringer, J.M., Beckham, G.T.: Alkaline Peroxide Delignification of Corn Stover. *ACS Sustain. Chem. Eng.* 5, 6310–6321 (2017)
17. Yuan, Z., Wen, Y., Kapu, N.S.: Ethanol production from bamboo using mild alkaline pre-extraction followed by alkaline hydrogen peroxide pretreatment. *Bioresour. Technol.* 247, 242–249 (2018)

18. Banerjee, G., Car, S., Scott-Craig, J.S., Hodge, D.B., Walton, J.D.: Alkaline peroxide pretreatment of corn stover: effects of biomass, peroxide, and enzyme loading and composition on yields of glucose and xylose. *Biotechnol. Biofuels.* 4, 16 (2011)
19. Bhalla, A., Bansal, N., Stoklosa, R.J., Fountain, M., Ralph, J., Hodge, D.B., Hegg, E.L.: Effective alkaline metal-catalyzed oxidative delignification of hybrid poplar. *Biotechnol. Biofuels.* 9, 34 (2016)
20. Fernando, T.N., Aruggoda, A.G.B., Ariadurai, S.A., Disanayaka, C.K., Kulathunge, S.: Evaluation of Alkaline Peroxide Pretreatment for Extraction of Cellulose from Selected Plant Biomasses. *J. Eng. Technol. Open Univ. Sri Lanka.* 3, (2015)
21. Zhu, M.Q., Wen, J.L., Wang, Z.W., Su, Y.Q., Wei, Q., Sun, R.C.: Structural changes in lignin during integrated process of steam explosion followed by alkaline hydrogen peroxide of *Eucommia ulmoides* Oliver and its effect on enzymatic hydrolysis. *Appl. Energy.* 158, 233–242 (2015)
22. Michalska, K., Ledakowicz, S.: Alkaline peroxide pretreatment for an effective biomass degradation. In: *Biomass fractionation technologies for a lignocellulosic feedstock based biorefinery.* pp. 483–498 (2016)
23. Cubas-cano, E., González-Fernández, C., Ballesteros, M., Tomás-Pejó, E.: Biotechnological advances in lactic acid production by lactic acid bacteria: lignocellulose as novel substrate. *Biofuels, Bioprod. Biorefining.* 12, 290–303 (2018)
24. Cabrera, E., Muñoz, M.J., Martín, R., Caro, I., Curbelo, C., Díaz, A.B.: Comparison of industrially viable pretreatments to enhance soybean straw biodegradability. *Bioresour. Technol.* 194, 1–6 (2015)
25. Dutra, E.D., Santos, F.A., Alencar, B.R.A., Reis, A.L.S., de Souza, R. de F.R., Aquino, K.A. da S., Morais, M.A., Menezes, R.S.C.: Alkaline hydrogen peroxide pretreatment of lignocellulosic biomass: status and perspectives. *Biomass Convers. Biorefinery.* 8, 225–234 (2018)
26. Khullar, E., Dien, B.S., Rausch, K.D., Tumbleson, M.E., Singh, V.: Effect of particle size on enzymatic hydrolysis of pretreated miscanthus. *Ind. Crops Prod.* 44, 11–17 (2013)
27. Arslan, B., Egerton, K., Zhang, X., Abu-Lail, N.I.: Effects of the Surface Morphology and Conformations of Lignocellulosic Biomass Biopolymers on Their Nanoscale Interactions with Hydrophobic Self-Assembled Monolayers. *Langmuir.* 33, 6857–6868 (2017)
28. Michalsk, K., Ledakowicz, S.: Alkaline hydrogen peroxide pretreatment of energy crops for biogas production. *Chem. Pap.* 68, 913–922 (2014)
29. Yuan, Z., Wen, Y., Li, G.: Production of bioethanol and value added compounds from wheat straw through combined alkaline/alkaline-peroxide pretreatment. *Bioresour. Technol.* 259, 228–236 (2018)