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RESEARCH ARTICLE

Delignification of corncob using ultrasonic pretreatment

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ABSTRACT

An ultrasonic pretreatment was evaluated for the delignification of the corncob biomass. The ultra-sonication process was carried in an ultrasonic water bath for 1 h, 33 kHz, and 100 watts. Three different pretreatment combinations were studied: corncob with distilled water, corncob with buffer, and corncob with buffer and enzyme (*Trametes versicolor*), and assessed for lignin reduction, hemicellulose reduction, and cellulose increase. The results indicated that sonication of corncob biomass with the enzyme yielded 44.19%, 6.74%, and 15.39% of lignin and hemicellulose reduction and cellulose increase, respectively. In FTIR, a reduction in peak intensity for lignin at wavenumbers 1697.05 cm^{-1}, 1534.1 cm^{-1} and 1328.71 cm^{-1} was noticed. Thus, ultrasonic pretreatment can be effectively used for the delignification of corncob biomass.

Keywords: *Corncob; Pretreatment; Laccase; Ultrasonication; Delignification*

INTRODUCTION

Lignocellulosic biomass (LCB) is considered as the future of sustainable energy sources, which consists of cellulose, hemicellulose (fermentable sugars), and lignin (phenyl propanoid units). Though, the use of LCB is restricted by its low digestibility, which is mainly attributed to the high crystallinity nature of cellulose and the lignin covering (Himmel *et al.*, 2007). The process of lignocellulosic conversion into glucose is via hydrolysis, for that the lignin bound to xylan and glucomannan (Lawoko *et al.,* 2006) is recognized to be a recalcitrant compound. Therefore, pretreatment is necessary to delignify and facilitate the disruption of the lignocellulosic moiety. Pretreatment alters the cellulose structure and making it more accessible to the enzyme that converts carbohydrate polymer into fermentable sugar (Bak *et al.,* 2009; Gabhane *et al.,* 2014).

Many strategies have been recommended for the efficient pretreatment, including lime, steam explosion, dilute acid, liquid hot water, and ammonia fiber explosion (Mood *et al.,* 2013). However, conventional acid pretreatment at high temperature (160 °C) repeatedly causes excessive hemicellulose degradation, resulting in furfural formation that strongly inhibits fermentative microbes (Klinke *et al.,* 2004). Therefore, the pretreatment conditions are requisite to be mild, ideally room temperature and atmospheric pressure, but sufficiently effective. Ultrasonication can be a promising alternative to conventional hydrolysis techniques (Wong *et al.*, 2009). The ability of ultrasonication in degrading polymeric sequences has been well documented, particularly in synthetic materials dissolved in various solvents (Gronroos *et al.*, 2004) and in extracting lignin and hemicellulose from lignocellulosic materials (Sun and Tomkinson, 2002; Gabhane *et al.*, 2014).

Applications of ultrasonication are diverse in various fields, such as sono-assisted lignocellulosic pretreatment (Iskalieva *et al.*, 2012; Bussemaker and Zhang, 2013), extraction of natural products (Shirsath *et al.*, 2012), sonochemistry of carbohydrate compounds (Kardos and Luche, 2001), catalytic esterification and transesterification of lipids (Veljkovi *et al.*, 2012; Badday *et al.*, 2012; Gole and Gogate, 2012), food processing (Bhaskaracharya *et al.*, 2009), pretreatment and fermentation of organic wastes (e.g., bio-sludge) to gaseous products (e.g., H_2, CH_4) (Pilli *et al.*, 2011; Yin *et al.*, 2004) and biochemical engineering/ biotechnologies (Gogate and Kabadi, 2009; Rokhina *et al.*, 2009; Kwiatkowska *et al.*, 2011) like biological wastewater treatment and bioremediation. Therefore, the objective of the present study is to study the effect of ultra-sonication on the deliginification of corncob biomass.

MATERIAL AND METHODS

2.1 Biomass preparation

The biomass was reduced in size by shredding,

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*Corresponding author's e-mail: rameshd@tnau.ac.in milling, and then sieving at 212 microns using ASTM No. 70 sieves. The sieved corncob was dried in a hot air oven at 45 °C until constant weight.

2.2 Compositional analysis of biomass

For the compositional analysis of pretreated biomass, the biomass sample was filtered using Whatman No.1 filter paper to separate biomass and filtrate. In order to neutralize (pH 7.0), the pretreated biomass was washed twice with distilled water, and the drying of samples was done in a hot air oven at 45 °C. National Renewable Energy Laboratory (NREL) procedure was adopted for biomass compositional analysis of both raw and pretreated biomass samples (Sluiter *et al*., 2008).

The percentage of reduction in lignin and hemicellulose and the increase in the cellulose of corncob samples after pretreatment can be calculated with the help of the following equations.

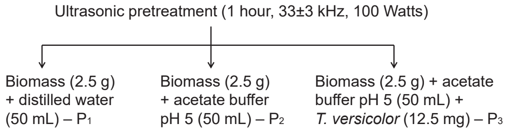
$$Percentage\ of\ lignin\ reduction = \frac{Lignin\ in\ raw\ biomass - lignin\ in\ pretreated\ biomass}{Lignin\ in\ raw\ biomass} \times 100$$

$$Percentage\ of\ hemicellulose\ reduction = \frac{Hemicellulose\ in\ raw\ biomass - hemicellulose\ in\ pretreated\ biomass}{Hemicellulose\ in\ raw\ biomass} \times 100$$

$$Percentage\ of\ cellulose\ increase = \frac{cellulose\ in\ pretreated\ biomass - cellulose\ in\ raw\ biomass}{Cellulose\ in\ pretreated\ biomass} \times 100$$

2.3 Ultrasonic pretreatment of corncob biomass

The dried extractive free sample (2.5 g) was taken in a Duran bottle in 50 mL of distilled water and kept in an ultrasonic water bath for 1 h at 33±3 Hz (100 watts); afterward, the sample was filtered and dried at 45 °C in a hot air oven.



2.4 FTIR analysis

The FTIR spectra of the test samples were obtained via FTIR (FTIR-6800 JASCO, Japan) for selected wavenumber (4000 to 400 cm^{-1}) with a spectral resolution of 4 cm^{-1} and 64 scans per sample.

RESULTS AND DISCUSSION

3.1 Ultrasonic pretreatment of corncob biomass

All the experimental trials were conducted thrice to confirm the reproducibility of the data. The cellulose, hemicellulose, and lignin content of raw corncob were found to be 36, 27.38, and 17.60%, respectively. The comparisons of different pretreatment combinations are presented in Fig. 1. The corncob biomass subjected to ultrasonication with acetate buffer and enzyme *T. versicolor* (P_3) resulted in an increase in cellulose content of 44.35%, decrease in lignin and hemicellulose content of 10.84 and 25.8 compared to other pretreatment combinations (P_1 and P_2). The percentage of lignin reduction (44.19%), hemicellulose reduction (6.74%), and cellulose increase (15.39%) was observed in P_3 (corncob biomass sonicated with enzyme) (Table 1).

Delignification of corncob is due to the collapse of the cavities and shock waves generated during the pretreatment. Ultrasonic waves produce cavities (microbubbles) in a solution, and several of the micro-bubbles collapse during compression of the wave, leading to a locally generated extreme state with a temperature higher than 5000 K and pressure of around 50 MPa commonly called a hot spot (Bernstein and Zakin, 1996; Thompson and Doraiswamy, 1999).

Table 1. Comparison between untreated and treated corncob and its per cent change

Treatments		Untreated	Treated	% Change
Corncob+ distilled water +ultrasonication	Lignin reduction	15.63	12.75	22.58
	Hemicellulose reduction	27.54	26.32	4.64
	Cellulose increase	38.44	42.54	10.67
Corncob+buffer + ultrasonication	Lignin reduction	15.63	11.31	38.18
	Hemicellulose reduction	27.54	26.49	3.96
	Cellulose increase	38.44	43.31	12.68
Corncob+buffer+ Enzyme+ ultrasonication	Lignin reduction	15.63	10.84	44.19
	Hemicellulose reduction	27.54	25.80	6.74
	Cellulose increase	38.44	44.35	15.39

This local high energy disrupts the hydrogen bonds in the microfibrils of cellulose, resulting in bundles disassociation and biomass swelling. Furthermore, asymmetric bubble collapse near a solid surface induces a microjet that hits the solid surface at high speed (>100 m/s) (Suslick, 1990). A microjet generated at the biomass surface would greatly impact the biomass and pit the lignocellulose (Bussemaker *et al*., 2013).

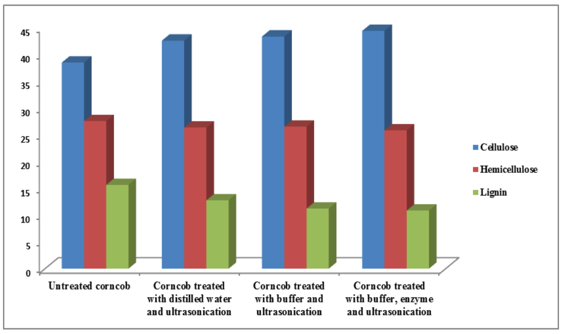


Figure 1. Comparison of different combinations of pretreatment

Subhedar *et al*., 2018 reported that in ultrasound-assisted approach, the extent of delignification for groundnut shells, coconut coir, and pistachio shells were 71.1, 89.5, and 78.9%, respectively under the optimum conditions of alkali concentration of 1 N, biomass loading of 0.5% (w/v), sonication power of 100 W, a duty cycle of 80% and pretreatment time of 70 min. Patil *et al*., (2019) reported that an 35.08% delignification of sawdust was observed at an alkali concentration of 1.5 N in ultrasound-assisted delignification. The mechanism of ultrasonic intensification is even different for different reactions, different enzymes, and operations (O'Donnell *et al*., 2010). The high-intensity shearing stress may influence it through the vigorous bubble implosion or it depended on the concentration of chemical radicals such as ·OH and ·H generated by ultrasonic cavitation (Riesz and Kondo, 1992).

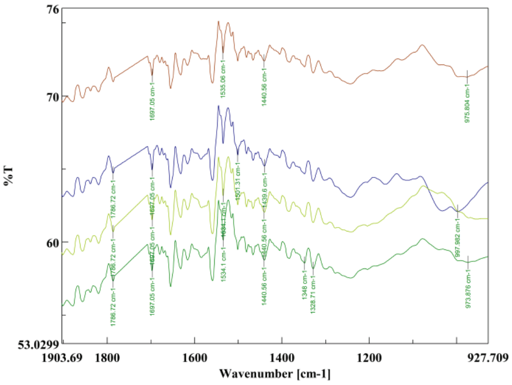


Figure 2. FTIR spectra of different combinations of ultrasonic pretreatment of corncob

FTIR analysis of pretreated biomass

Raw and pretreated corncob samples were analyzed using Fourier Transform Infra-Red (FTIR), and FTIR transmittance spectrum for each treatment is given in Fig. 2. From the results, it was evident that raw corncob showed clear peaks for cellulose, hemicelluloses, and lignin at the corresponding wavenumbers. Due to biomass pretreatment, reduction in hemicelluloses peak intensity is attributed to solubilization of hemicelluloses; wave number 1786.72 cm^{-1} with a functional group of free ester was also greatly reduced. Similarly, a reduction in peak intensity for lignin at wavenumbers 1697.05 cm^{-1}, 1534.1 cm^{-1}, and 1328.71 cm^{-1}, which represents a functional group of C=O stretching (unconjugated), aromatic ring vibration, and C-O of syringyl group, respectively, was noticed. The cellulose, hemicellulose, and lignin corresponds to a wavenumber of 1440.56 cm^{-1} and 1348 cm^{-1}, which represents to a functional group of O-H in-plane bending and C-H vibration, O-H in-plane bending, respectively. The wavenumber 973.87 cm^{-1} and 997.98 cm^{-1} represents cellulose with a functional group of C-O valence vibration.

CONCLUSION

The present work demonstrated an effective approach for the successful utilization of the corncob, which is otherwise discarded as redundant agricultural waste. Raw corncob pretreated with ultrasound-assisted with enzyme showed better delignification of 44.19% at 33 kHz, sonication power of 100 watts, and pretreatment time of 60 min. Overall, the work demonstrated the use of ultrasound in the processing of sustainable biomass.

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