ISSN: 0971-1260

Vol-22-Issue-17-September-2019

Bioprospecting cost-effective delignification of lignocellulosic biomass for enhanced biofuel production

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Abstract

The utilization of lignocellulosic biomass as a raw material represents a potential candidate for biofuel production, thereby, preventing energy crisis. However, the technology for the production of biofuels suffers from certain technological barriers which do not make the overall process to be economically feasible. Current research focuses on key steps of biofuel production from lignocellulosics, viz. efficient pretreatment process, high yielding synergistic enzymatic cocktails, co-fermenting microorganisms and process integration. The present study aims to investigate several pretreatment processes, i.e. physical (irradiation, microwave pretreatment); chemical (acidic- sulphuric acid, hydrochloric acid, nitric acid; alkaline- sodium hydroxide, potassium hydroxide, calcium hydroxide); physicochemical (steam explosion, acid assisted steam explosion, alkali assisted steam explosion, acid assisted microwave pretreatment, alkali assisted microwave pretreatment) with varied time and temperature for both wheat bran and rice husk. The pretreatments were qualitatively assessed for delignification by phloroglucinol-HCL staining where the effective treatments were screened. Further FTIR analysis was carried out which depicted 100 % delignification with 2 % sodium hydroxide assisted microwave pretreatment at 400^0 C at

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230 V, 1200 W for 2 min, and sulphuric acid (2 %) assisted steam explosion pretreatment at 121^0C, 15 psi for 120 min, for rice husk and wheat bran, respectively. Completely delignified lignocellulosic biomass plays a pivotal role for cost effective saccharification and biofuel production.

Keywords: Pretreatment; lignocellulosic biomass; biofuel production; lignin degradation; bioenergy

Introduction

During the last few decades, defies of global oil crisis and climate change have led to overexploitation of fossil fuels and many public health problems. So, alternative source of fuel should be sustainable, renewable, efficient, cost-effective and safe [1]. Recent research is more focused on the use of lignocellulosic biomass (LCB) for the prevention of competition between the crops for food and fuel [2,3]. However, the technology for the biochemical conversion of second generation raw material viz. agricultural waste, municipal solid waste, forestry residues etc. suffers from few setbacks including recalcitrance nature of lignin, costly delignification strategies, lack of cheap enzyme cocktails for saccharification and lack of efficient co-fermenting ethanologens etc. Lignin is associated with cellulosic and hemicellulosic components of paddy straw via covalent bonds, forming lignin-carbohydrate complexes (LCCs) containing ferulic bridges attached via ether and ester bonds. Lignin removal is an essential step for increasing the accessible sites for enzymes and aid in effective enzymatic saccharification [4,5].

Among the various steps of biochemical conversion of LCB to biofuels, pretreatment is the major step which affect the overall cost of the process [6]. The cost of pretreatment is attributed to type of reactor used, chemicals, solvents, gases, detoxification techniques for the removal of inhibitory compounds etc. [7]. Hence, advancement of efficient pretreatment technologies along with standardization of different parameters is indispensible for minimal loading of the

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ISSN: 0971-1260

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chemicals and maximal loading of biomass, thereby, boosting the enzymatic hydrolysis and making the process economically viable [8].

Several physical, chemical or physicochemical strategies have been reported in the literature for pretreatment for different LCBs [9,10]. However, the method of pretreatment should be specific to the type of LCB used. Physical pretreatment mainly destroys the cell structure and decreases the crystallinity of cellulose, whereas chemical pretreatment aims to alter the structure of lignin and sometimes partial hydrolysis of hemicellulose as well. On the other hand, physicochemical pretreatment, increases the porosity of the biomass and avoid the formation of fermentation inhibitors [11,12]. Hence, advancement of efficient pretreatment technologies along with standardization of different parameters is indispensible for minimal loading of the chemicals and maximal loading of biomass, thereby, boosting the enzymatic hydrolysis and making the process economically viable [8].

De-lignification of LCBs poses a significant role in the saccharification of LCB, thereby, efficient conversion of overall process [13]. Although several studies have been reported in literature for biochemical conversion of different LCBs for biofuel production, for eg., sugarcane bagasse [14] corn cobs [15] cotton stalks, wheat straw [16] paddy straw [17]; bamboo [18], however, to the best of our knowledge, evaluation of pretreatment method with 100 % delignification for rice husk and wheat bran has not been reported earlier. The present study was carried out with the objective of development of a bioprocess for complete delignification of rice husk and wheat bran. In addition to this, this comparison would aid in the better understanding of the interaction of different pretreatment parameters with the biomass used.

Materials and Methods

Preparation of raw material

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ISSN: 0971-1260
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The samples of rice husk (RH) and wheat bran (WB) were obtained from Sonu Atta Chakki, Deepnagar, Jalandhar (31.3260° N, 75.5762° E). Both the samples were washed thrice with water, air-dried for 4 days, and finely grounded.

Physical Pretreatment

Irradiation pretreatment

Samples of both rice husk and wheat bran were irradiated with Helium Neon Laser for three hours without prior soaking. Irradiation was also done for the samples soaked with solid : liquid ratio of 1:10 using water and 2 % sodium hydroxide for three hours. All the samples were dried in hot air oven at 100^0C for 6 hours after irradiation.

Microwave pretreatment

Microwave pretreatment of both rice husk and wheat bran was done at 230 volt, 1200 W by varying the temperature at 150, 200, 300 and 400^0C. All the samples were dried in hot air oven at 100^0C for 6 hours after microwave treatment.

Chemical pretreatment

Acid pretreatment

Acid pretreatment of both rice husk and wheat bran was done by soaking the samples in different acid solutions, viz. 2 % sulphuric acid, 2 % hydrochloric acid and 2 % nitric acid, for 3 days with solid: liquid ratio of 1:10. After acidic treatment, the hydrolysate was drained and all the samples were dried in hot air oven at 100^0C for 6 hours.

Alkali pretreatment

Alkaline pretreatment of both rice husk and wheat bran was done by soaking the samples in different alkaline solutions, viz. 2 % sodium hydroxide, 2 % potassium hydroxide and 2 %

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ISSN: 0971-1260

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calcium hydroxide, for 3 days with solid: liquid ratio of 1:10. After alkaline treatment, the hydrolysate was drained and all the samples were dried in hot air oven at 100^0C for 6 hours.

Physicochemical pretreatment

Steam explosion

Steam explosion of both rice husk and wheat bran was done at 121^0C, 15 psi by varying the time for 30, 60, 90 and 120 min. All the samples were dried in hot air oven at 100^0C for 6 hours after steam explosion.

Acid assisted steam explosion

Acid assisted steam explosion of both rice husk and wheat bran was done in different acid solutions, viz. 2 % sulphuric acid, 2 % hydrochloric acid and 2 % nitric acid, with solid: liquid ratio of 1:10 with prior overnight soaking, at 121^0C, 15 psi by varying the time for 30, 60, 90 and 120 min. All the samples were dried in hot air oven at 100^0C for 6 hours after acid assisted steam explosion.

Alkali assisted steam explosion

Alkali assisted steam explosion of both rice husk and wheat bran was done in different alkaline solutions, viz. 2 % sodium hydroxide, 2 % potassium hydroxide and 2 % calcium hydroxidewith solid: liquid ratio of 1:10 with prior overnight soaking, at 121^0C, 15 psi by varying the time for 30, 60, 90 and 120 min. All the samples were dried in hot air oven at 100^0C for 6 hours after acid assisted steam explosion.

Acid assisted microwave pretreatment

Acid assisted microwave pretreatment of both rice husk and wheat bran was done in different acid solutions, viz. 2 % sulphuric acid, 2 % hydrochloric acid and 2 % nitric acid, with solid: liquid ratio of 1:10 by varying the temperature at 100, 200, 300 and 400 ^{o}C time for 2 min. All

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ISSN: 0971-1260
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the samples were dried in hot air oven at 100^{0}C for 6 hours after acid assisted microwave treatment.

Alkali assisted microwave pretreatment

Alkali assisted microwave pretreatment of both rice husk and wheat bran was done in different alkaline solutions, viz. 2 % sodium hydroxide, 2 % potassium hydroxide and 2 % calcium hydroxide, with solid: liquid ratio of 1:10 by varying the temperature at 100, 200, 300 and 400^{0}C time for 2 min. All the samples were dried in hot air oven at 100^{0}C for 6 hours after alkali assisted microwave treatment.

Phloroglucinol-HCl staining

The dried samples were taken and their phloroglucinol-HCL staining was performed. On the basis of colour intensity for delignification, the samples with very light to no colour were screened for further FTIR analysis.

FTIR analysis

FTIR spectroscopy was performed for precise prediction of the chemical composition of both rice husk and wheat bran (Shimadzu, FTIR-8400S) and suitable changes before and after pretreatment. Dried mixed powder of the sample and potassium bromide (KBr), in the ratio of 1:10was used for scanning from 4000 and 400 cm^{-1} with a resolution of 4 cm^{-1} [19, 20]. The reproducible results of all the samples were confirmed with three consecutive samples. Necessary corrections for frequency variations and penetration depth were applied by using Shimadzu IR solution 1.5 software supplied with the equipment.

Results and Discussion

Screening of pretreated samples for delignification

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ISSN: 0971-1260

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Screening of pretreated samples for enhanced delignification was done by staining the pretreated samples with phloroglucinol-HCl dye as it specifically stains lignin, where the cinnamaldehyde group of lignin reacts with the dye to give red-violet color. The amount of lignin present was reflected by the intensity of the colour retained by the sample, thus, qualitatively, the samples with very light or no colour were screened for our further study. Figs. 1 and 2 showed the untreated samples of rice husk and wheat bran for reference of colour intensity. Physical and chemical pretreatments were found to have intensely stained samples, whereas physicochemically pretreated samples had no or very less colour. Table 1 showed the screened samples, both for rice husk and wheat bran, on the basis of colour intensity. The screened samples were subjected to FTIR analysis to verify delignification.

Table 1: Screened samples after phloroglucinol-HCl staining

S.No.	**Pretreatment**
Rice Husk	
1.	Steam exploded for 30 min
2.	Sulphuric acid assisted steam exploded for 90 min
3.	Hydrochloric acid assisted steam exploded for 30 min
4.	Nitric acid assisted steam exploded for 30 min
5.	Nitric acid assisted steam exploded for 90 min
6.	Nitric acid assisted steam exploded for 120 min
7.	Sodium hydroxide assisted steam exploded for 120 min
8.	Potassium hydroxide assisted steam exploded for 30 min
9.	Potassium hydroxide assisted steam exploded for 90 min
10.	Potassium hydroxide assisted steam exploded for 120 min
11.	Sulphuric acid assisted microwave pretreatment at 400 ^{o}C
12.	Hydrochloric acid assisted microwave pretreatment at 200 ^{o}C
13.	Nitric acid assisted microwave pretreatment at 200 ^{o}C

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ISSN: 0971-1260

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14. Sodium hydroxide assisted microwave pretreatment at 200 ^oC
15. Sodium hydroxide assisted microwave pretreatment at 300 ^oC
16. Sodium hydroxide assisted microwave pretreatment at 400 ^oC
17. Potassium hydroxide assisted microwave pretreatment at 400 ^oC

Wheat bran

1. Sulphuric acid assisted steam exploded for 120 min
2. Sodium hydroxide assisted steam exploded for 90 min
3. Potassium hydroxide assisted steam exploded for 60 min
4. Sulphuric acid assisted microwave pretreatment at 400 ^oC
5. Sodium hydroxide assisted microwave pretreatment at 300 ^oC
6. Potassium hydroxide assisted microwave pretreatment at 200 ^oC

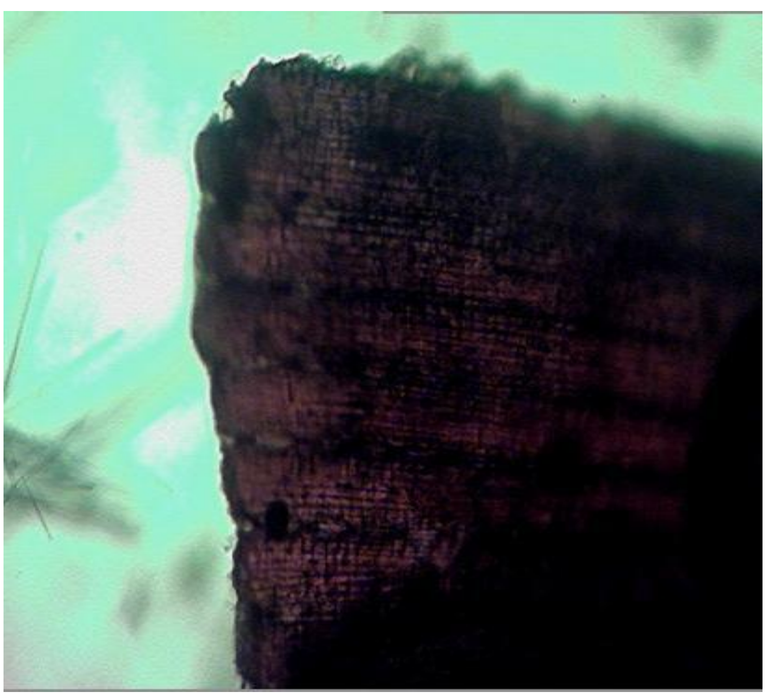

Fig. 1 Untreated sample of rice husk stained with phloroglucinol-HCl staining

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ISSN: 0971-1260
Vol-22-Issue-17-September-2019

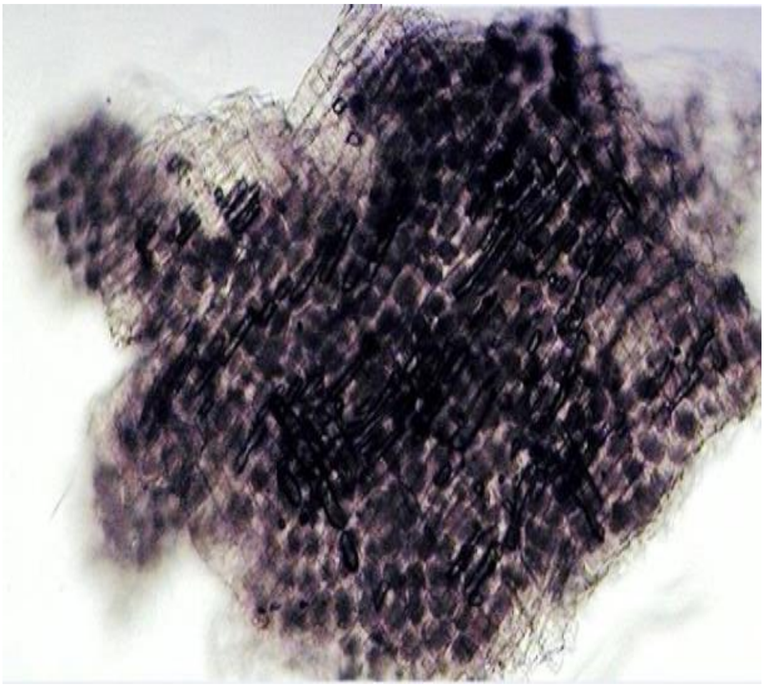

Fig. 2: Untreated sample of wheat bran stained with phloroglucinol-HCl staining

FTIR analysis

Cellulose, hemicellulose and lignin peaks are generally found at the wavenumbers of 1316-1368, 1346-1384, 1425-1462 cm^{-1}, respectively. Figs. 3 and 4 show the FTIR analysis of raw and pretreated samples of rice husk and wheat bran, respectively, with complete delignification. FTIR analysis of all the screened samples showed reduction or absence of the peaks specific for the functional groups of lignin. The bands at 1512 and 1464 cm1 relate to aromatic ring stretching to authorize the aromatic ring structure of lignin molecules [18].

In case of rice husk maximum delignification was achieved by 2 % NaOH assisted microwave pretreatment at 400^0C, 230 V and 1200 W. Other treatments that proved to be effective for removal of the lignin functional groups include 2 % NaOH assisted steam explosion

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ISSN: 0971-1260

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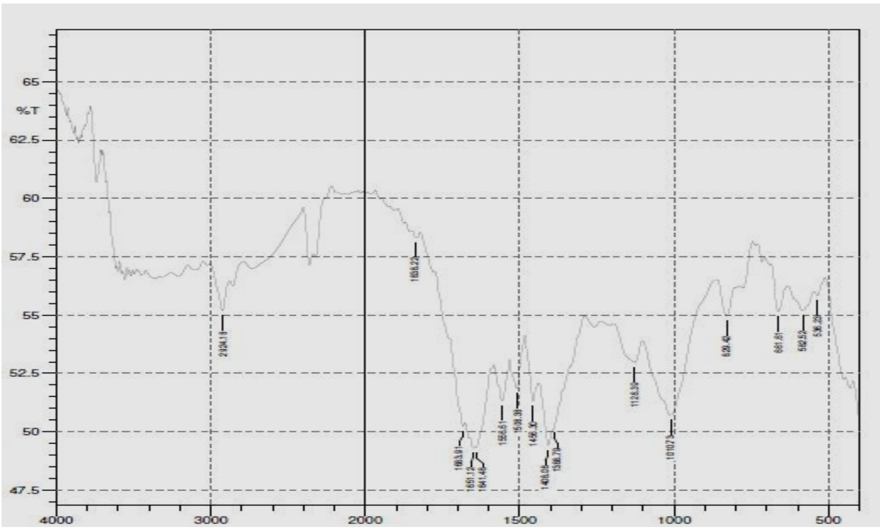
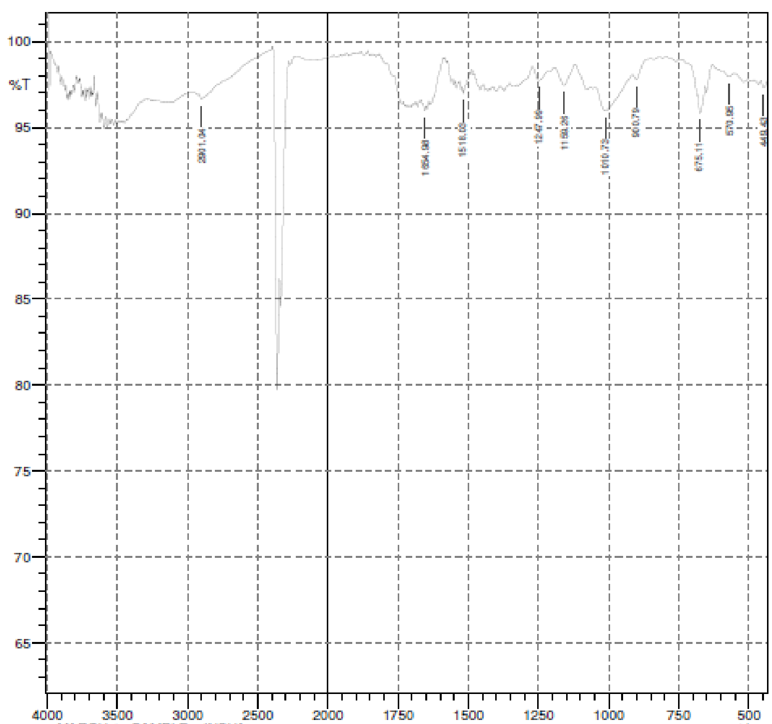
for 120 min, 2 % KOH assisted microwave at 400^0C, 2 % H_2SO_4 assisted SE for 90 and 120 min, and 2 % NaOH assisted microwave at 300^0C.



(A)



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ISSN: 0971-1260

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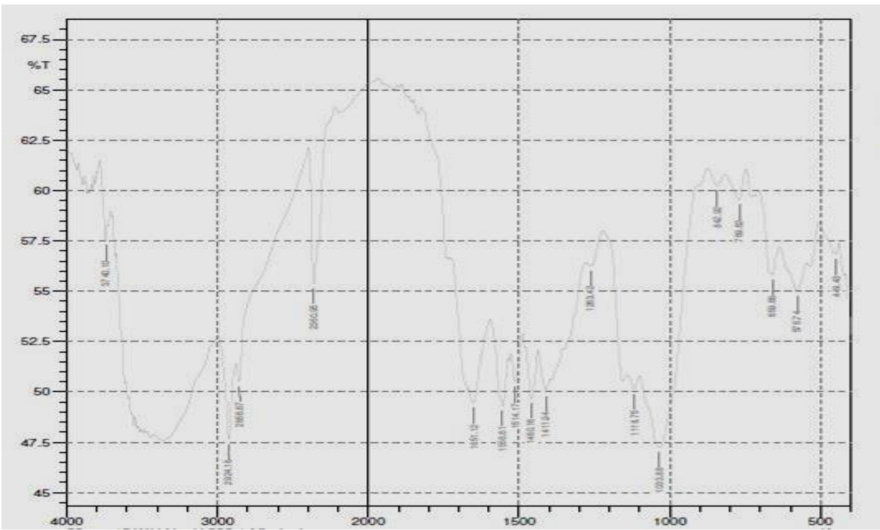
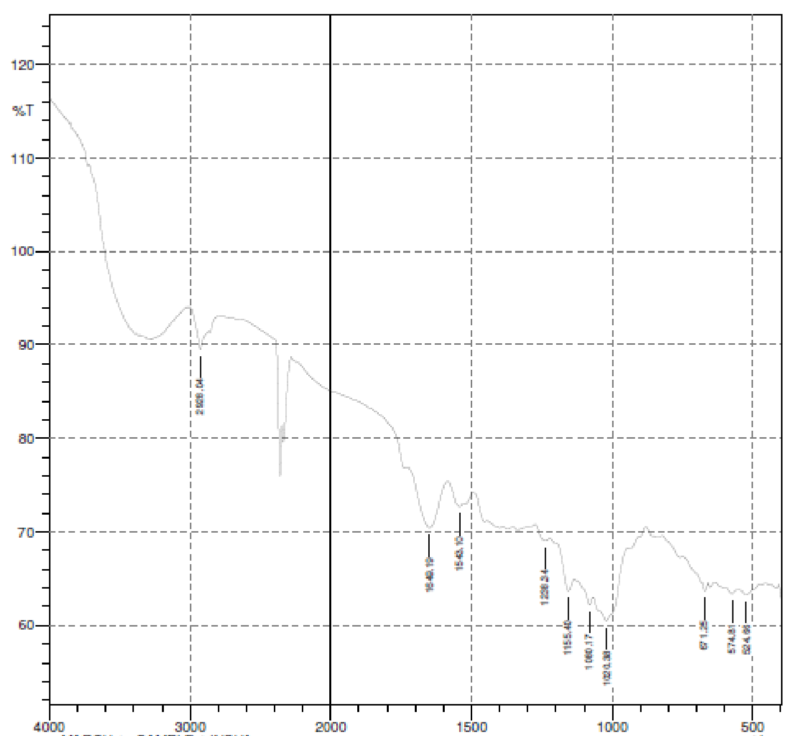
(B)

Fig. 3: FTIR analysis of rice husk (A) Raw (B) 2 % NaOH microwave pretreated at 400 ºC 230 V 1200W



(A)



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(B)

Fig. 4 : FTIR analysis of wheat bran(A) Raw (B) 2 % H_2SO_4 steam exploded at 121 ºC 15 psi

On the other hand, in case of wheat bran maximum delignification was achieved by 2 % H_2SO_4 assisted SE for 120 min at 1210C and 15 psi. Other effective pretreatment for effective delignification was H_2SO_4 assisted microwave at 400^0C.

Conclusions

The study highlights the impact of physicochemical pretreatment on rice husk and wheat bran on delignification for increasing the accessibility of the enzymes for saccharification and easy bioconversion to biofuels. However, further work is required to optimize different pretreatment and saccharification conditions so that the process may be integrated in a single reactor to make the process economically feasible.

Acknowledgement

Authors are thankful to authorities of Lovely Professional University for providing support to conduct this study.

Compliance with ethical guidelines

Competing interests

The authors declare that they have no competing interests.

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