Ultrasonics - Sonochemistry 58 (2019) 104656

Contents lists available at ScienceDirect

Ultrasonics - Sonochemistry

journal homepage: www.elsevier.com/locate/ultson

Intensification of delignification of sawdust and subsequent enzymatic hydrolysis using ultrasound

Rucha S. Patil, Saurabh M. Joshi, Parag R. Gogate*

Department of Chemical Engineering, Institute of Chemical Technology, Matunga, Mumbai 400 019, India

ARTICLE INFO

Keywords:
Lignocellulosic materials
Delignification
Ultrasound
Enzymatic hydrolysis
Biofuels

ABSTRACT

The current work investigates intensification of delignification of sawdust and subsequent enzymatic hydrolysis to produce reducing sugars with the use of ultrasound. Alkaline hydrolysis of sawdust was initially performed to remove lignin which hampers the rate of enzymatic hydrolysis. Effect of different parameters in the case of ultrasound (US) assisted and conventional processes such as alkali concentration (0.5–2.5 N), substrate loading (0.2–1.0% w/v) and temperature (40–80 °C) have been investigated. Optimized parameters obtained for US assisted process showed better trends as compared to conventional process with about 1.25 times higher yields and significant reduction in time by about 4 h. The process parameters for US assisted and conventional enzymatic hydrolysis to produce reducing sugars were also optimized by varying substrate loading (0.5–10% w/v), reaction temperature (30–70 °C) along with variation in US power (10–80 W) and US duty cycle (30–90%). US assisted enzymatic hydrolysis performed at 4% w/v substrate loading along with 50 W US power and 50% duty cycle at 50 °C resulted in 7.46 mg/mL of reducing sugars yield within 1 h while conventional stirring with 6% w/v substrate loading and 50 °C resulted in approximately same yield of reducing sugars within 3 h. The requirement of lower time for similar yields or in other words higher yield in same time clearly highlights the process intensification benefits due to the use of ultrasound. Overall it can be concluded from the study that US assisted processes resulted in efficient delignification along with higher yield of reducing sugars in lower treatment time as compared to conventional process.

1. Introduction

The environmental and economic concerns along with rise in population have resulted in increased use of renewable sources to replace conventional fossil fuels. Utilization of waste lignocellulosic biomass to produce biofuels is one of the potential alternatives to curb pollution and reduce dependency on crude oil. Sawdust is a byproduct of the timber and wood industry abundantly available and can be used in the production of bio-ethanol and other useful products. Production of bioethanol from sawdust consists of delignification, hydrolysis and fermentation steps; these processes are reported to be time consuming and tedious. Delignification with acid, or steam explosion treatments is widely reported but disadvantages like expensive reactor setups and higher reaction time make them infeasible. Alkaline delignification is reported to be the most efficient process in lignin removal among the conventional approaches with requirement of lower reaction temperature as compared to other processes such as acid based treatment [1,2]. Similarly in the case of hydrolysis, the processes like steam explosion, acid treatment (dilute and concentrated), ammonia fiber explosion etc. are observed to be expensive also giving lesser yields. Enzymatic hydrolysis is a process with lesser utility costs and can be performed at lower reaction temperatures but require higher processing time [2,3]. Overall newer processes need to be developed requiring lesser quantum of chemicals along with economic feasibility. Cavitation is one such upcoming technology with a potential to overcome disadvantages associated with conventional processes also possibly giving higher yield of products.

Cavitation is a phenomenon of cavity generation followed by growth and implosion at a specific point resulting in physical and chemical effects in the medium. Physical effects produced are microstreaming and intense turbulence while chemical effects are the production of hydroxyl radicals. Cavitation is mostly generated by ultrasonic or hydrodynamic cavitation reactors at multiple locations in a reactor. Ultrasonic reactors are reported to produce intense cavitation as compared to hydrodynamic cavitation reactors and come with different configurations like US horn, US flow cell and US bath depending upon the processing volumes [4–6]. US assisted alkaline delignification can result in intensification attributed to the physical effects and

* Corresponding author.
E-mail address: pr.gogate@ictmumbai.edu.in (P.R. Gogate).

https://doi.org/10.1016/j.ultsonch.2019.104656
Received 11 April 2019; Received in revised form 28 May 2019; Accepted 21 June 2019
Available online 22 June 2019

enhanced mass transfer resulting in increased lignin removal with lesser requirement of chemicals and time [7]. US assisted enzymatic hydrolysis can also result in higher rate of reducing sugars production with significant reduction of reaction time [2]. Cavitation is reported to modify enzyme structure leading to increased surface contact between substrate enzyme complexes along with increased mass transfer rates for the substrates towards the enzymes. There are various studies reported on US assisted delignification and enzymatic hydrolysis on different biomasses like sugarcane bagasse, rice straw, wheat straw, waste newspaper etc. [8–13] but very few studies are reported on combined delignification and enzymatic hydrolysis for specific biomass quantifying the intensification obtained due to the use of ultrasound. Also studies reporting the use of saw dust as sustainable feed stock are not seen in the open literature to the best of our knowledge confirming the importance of the presented work. The current work focuses on US assisted alkaline delignification of sawdust followed by US assisted enzymatic hydrolysis of pretreated sawdust to produce higher quantity of reducing sugars. The main objective of the work is understand the effect of different operating parameters so as to arrive at optimum conditions giving maximum process intensification benefits.

2. Materials and methods

2.1. Materials

Sawdust was locally obtained from a carpentry shop in Mumbai. Standard lignin sample, sodium hydroxide pellets, glacial acetic acid, perchloric acid, carboxy methyl cellulose (CMC), dinitrosalicylic acid, phenol crystals, sodium potassium tartrate, coomassie brilliant blue G250, bovine serum albumin and glucose were obtained from Hi-Media Laboratories, Mumbai. Acetyl bromide and hydroxylamine hydrochloride were obtained from Thomas Baker, Mumbai. Citric acid (anhydrous), tri-Sodium citrate (monohydrous), hydrochloric acid, sodium sulphite and phosphoric acid were obtained from S.D. Fine Chemicals, Mumbai. Cellulase enzyme was procured from Advanced Biotechnologies, Mumbai.

2.2. Compositional analysis of the sawdust

Compositional analysis of sawdust was performed using thermo gravimetric analyzer (TGA). TGA analysis records the loss in weight of samples over a programmed temperature range and gives details about the components present based on the specific rates of loss of weight. PerkinElmer STA 6000 instrument with Pyris software was used for TGA analysis. To get the TGA curve for the sawdust and to establish the composition, the temperature was gradually increased from 30 °C to 600 °C at a constant heating rate of 10 °C per min. Sample required for analysis was 13 mg and it is expected that, after the removal of moisture, hemicelluloses present in the lignocellulosic material decompose first followed by cellulose and lignin. The degradation temperature range for each component was observed; hemicellulose showed degradation in the temperature range of 200–300 °C, cellulose in the range of 300–370 °C and finally lignin in the range of 370–600 °C. The obtained TGA curve for the sawdust is depicted in Fig. 1. Based on the TGA curve analysis, the amount of different components was established as 38.19% cellulose, 11.2% hemicelluloses, 16.01% lignin and 8.67% moisture.

2.3. Experimental methodology

2.3.1. Preprocessing of feed stock

The sawdust was screened through the sieves of 0.5 mm particle size mesh. The particle size less than 0.5 mm was selected for further study with an aim of offering higher surface area for the delignification. Using still lower particle sizes can offer difficulty in separation and hence the grinding was not continued below this optimum mesh size [7,11]. Sieved sawdust was water washed and then dried in the muffle furnace for 2 h at 105 °C. The dried sawdust was further used in the experiments.

2.3.2. Delignification pretreatment

Delignification pretreatment was performed in three neck jacketed reactor with 250 mL capacity. Temperature of reaction mixture was maintained by circulating water through cooling jacket. Sodium hydroxide solution (100 mL) was transferred to the reactor as per the required concentration followed by addition of sawdust. Immediately after the addition of sawdust, a sample was withdrawn and agitation was started using mechanical stirrer operating at a speed of 300 rpm (conventional approach). To study the effect of ultrasound on delignification, a probe sonicator obtained from M/s Dakshin, Mumbai with fixed frequency of 20 kHz and tip diameter of 20 mm was introduced in place of mechanical stirrer and submerged 10 mm into the reaction mixture. Experimental setup used in the work for the ultrasound assisted approach is shown in Fig. 2. Sonication parameters i.e. supplied electric power of 100 W (the actual calorimetric power dissipation would be around 12 W for a observed energy efficiency of 12%) and duty cycle of 70% were kept constant based on optimum conditions reported in the literature [7]. Agitation was provided using magnetic stirrer. During the experiments, samples were withdrawn at regular intervals. The clear supernatant was used for determination of the lignin concentration. Parameter optimization study was performed to obtain optimum alkali concentration and substrate loading.

2.3.3. Enzymatic hydrolysis of pretreated sawdust

The enzymatic hydrolysis of pretreated sawdust was performed using cellulase enzyme having CMCase activity of 34.84 CMCU and specific activity of 394.35 IU/mg. Hydrolysis study was performed in a 100 mL reactor. An overhead glass stirrer was provided for mechanical agitation in the case of conventional approach. The temperature of the reaction mixture was controlled with help of water bath. Initially, the pre-treated sawdust was taken at required loading in the reactor with subsequent addition of citrate buffer with pH maintained at 4.8 and desired amount of cellulase enzyme. To study the effect of US on enzymatic hydrolysis of sawdust, another probe sonicator procured from M/s Dakshin, Mumbai operating at fixed frequency of 20 kHz was introduced vertically into the reaction mixture instead of the mechanical stirrer. The schematic representation of the assembly for the ultrasound assisted approach is shown in Fig. 3. US horn had a tip diameter of 11 mm and was submerged 10 mm into the reaction mixture. Sonication was performed at different US power (over the range of 10–90 W) and duty cycle (range of 30–90%) respectively to understand the effect of the operating parameters. Effect of other process parameters like substrate loading (0.5–10% w/v) and temperature (30–70 °C) on the sugar yield was also studied. For the analysis of the progress of reaction, samples were withdrawn at regular intervals. All the withdrawn samples were centrifuged at 10,000 rpm for 10 min. The supernatant obtained after centrifugation was used subsequently for the analysis of reducing sugars.

2.4. Analysis

2.4.1. Determination of lignin concentration

The lignin concentration in supernatant solution after treatment was analyzed using UV spectrophotometer. Acetyl bromide in glacial acetic acid (25%) was added to withdrawn sample (0.5 mL) in test tubes. Sample tubes, after proper tight capping, were kept in water bath at 70 °C for 30 min. Subsequently the samples were cooled and 2.5 mL of glacial acetic acid, 1.5 mL of 0.3 M NaOH and 0.5 M hydroxylamine hydrochloride solution were added to all the tubes. The final volume was made to 10 mL based on addition of glacial acetic acid. Absorbance was measured at 280 nm and then the lignin concentration was calculated from the standard lignin curve [14].

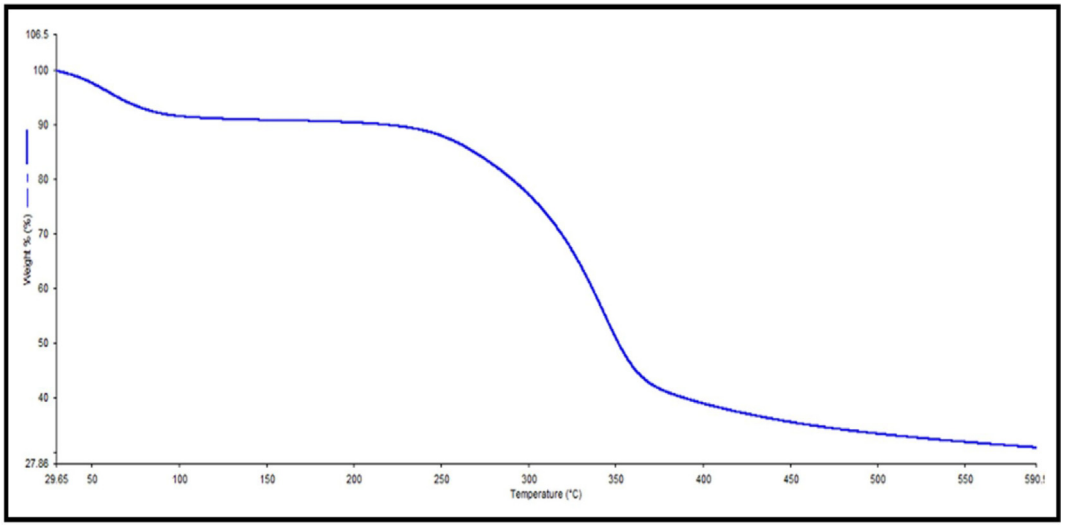

Fig. 1. TGA curve of untreated sawdust.

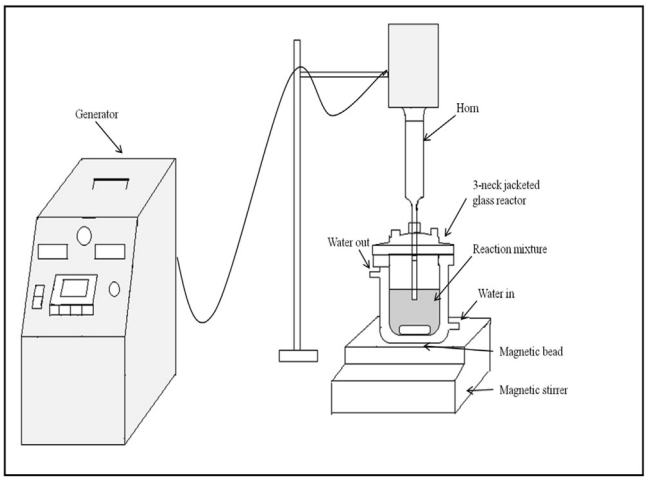

Fig. 2. Experimental setup for US assisted alkaline delignification.

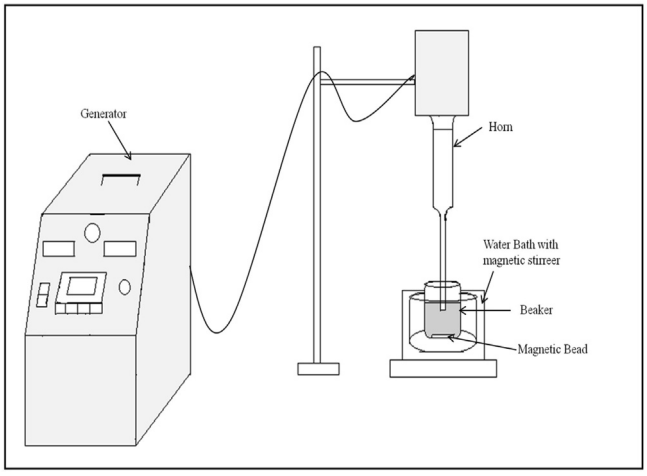

Fig. 3. Experimental setup for US assisted enzymatic hydrolysis of pretreated sawdust.

2.4.2. Determination of the reducing sugar

Concentration of reducing sugars was estimated using dinitrosalicylic acid (DNS) assay method [15]. A standard DNSA protocol has been followed to determine the residual glucose content. DNSA reagent containing 1% 3,5-Dinitrosalicylic Acid, 0.2% Crystalline Phenol, 0.05% Sodium Sulphite and 1% Sodium Hydroxide was freshly prepared in laboratory. To measure the amount of reducing sugar obtained after enzymatic hydrolysis, 200 µL of clear supernatant was transferred to test tube and solution diluted five times to make up to 1 mL volume. 1 mL of DNS reagent was added with the sample kept in a tightly capped test tube. The reaction mixture was heated at 90 °C for 30 min when the red-brown colour was obtained. 1 mL of a 20% potassium sodium tartrate (Rochelle salt) solution was added to stabilize the colour of the reaction mixture. 9 mL of distilled water was then added to the test tube and absorbance was measured at 540 nm against reagent blank to calculate the amount of reducing sugar. The standard calibration curve of absorbance v/s glucose concentration in mg/mL was established using analysis based on known concentrations.

3. Results and discussion

3.1. Pretreatment of sawdust

Sawdust was subjected to a pretreatment to remove lignin with conventional as well as US assisted approach. The effects of important process parameters on the alkaline delignification were investigated and following sections presents the obtained results with discussion on the governing mechanisms for intensification.

3.1.1. Effect of alkali concentration on extent of delignification in conventional approach

The effect of alkali concentration on the extent of delignification was studied by varying concentration of sodium hydroxide in range of 0.5–2.5 N keeping constant substrate loading at 0.5% w/v, treatment time as 8 h, temperature of 50 °C and stirring speed as 300 rpm. The obtained results for the effect of alkali concentration on the extent of delignification are depicted in Fig. 4a and it can be observed from the results that the extent of delignification increased with an increase in alkali concentration. The extent of delignification was reported to increase with alkali concentration confirming that higher alkalinity is favourable. Initially, the extent of delignification increased at higher rate with a change in alkali concentration from 0.5 to 1.5 N resulting in increased lignin amount in the supernatant from 7.5 to 14.5 mg. Subsequently, when alkali concentration was increased to 2.5 N, the lignin amount increased only marginally to 15.1 mg. Considering these results, concentration of 1.5 N was considered as optimum and used in further treatment. The obtained values of the lignin removal with different alkali concentrations have been given in the Table 1. Aryl ether

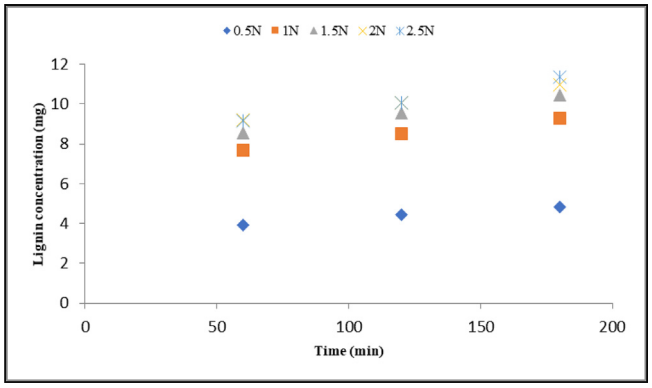

Fig. 4a. Effect of alkali concentration on extent of sawdust delignification using conventional approach.

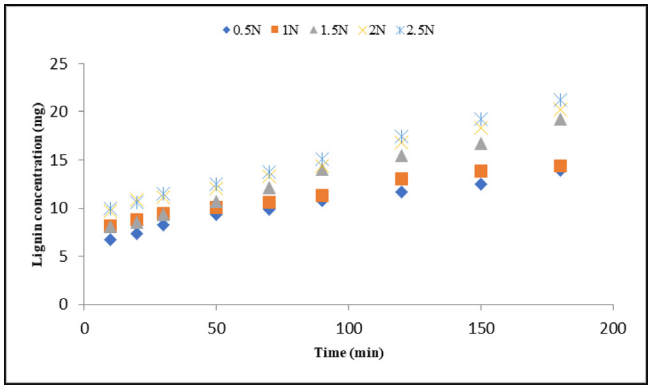

Fig. 4b. Effect of alkali concentration on extent of sawdust delignification using US assisted approach.

linkages are reported to be 50–60% of the total lignin linkages and are broken by alkali treatment. The increased alkali concentration catalyses the cleavage of ether linkages at a faster rate along with release of free sodium phenolates in the liquid [7]. As the alkali concentration increases, the hydroxyl ion concentration also increases which further helps in removal of lignin at a faster rate. At 1.5 N alkali concentration around 26.5% delignification has been observed. Study performed by Chang et al. [16] reported 77.5% lignin removal from wooden chips with oxidative lime pretreatment at 0.1 g calcium hydroxide ($CaOH_2$) loading at 150 °C for 6 h. Another study on delignification of wheat straw reported that treatment using 6% NaOH loading for 24 h at 25 °C resulted in about 85% increased reducing sugars production attributed to significant lignin removal [17]. Study performed by Subhedar et al. [7] on delignification of sustainable biomass as waste news paper also established that an increase in alkali concentration leads to increase in delignification. Compared to the reported extents of delignification, lower delignification obtained in current study can be attributed to the compositional difference of raw materials also highlighting the importance of detailed study for different substrates.

3.1.2. Effect of alkali concentration on extent of delignification using US assisted approach

The effect of alkali concentration on the delignification of sawdust was also investigated in the case of the US assisted approach over the range of 0.5 to 2.5 N. US parameters as 100 W power and 70% duty cycle were kept constant based on reported literature [7] and other parameters like substrate loading (0.5% w/v), treatment time (180 min), and temperature (50 °C) were also kept constant for this set of investigations. The obtained results for the effect of alkali concentration on US asssisted delignification are shown in Fig. 4b and it can be observed that as the alkali concentration increased from 0.5 to 1.5 N, the lignin concentration in the supernatant (this represents the lignin being removed from the sawdust and hence indication of the extent of delignification) also increased from 13.89 to 19.26 mg. Subsequently, when the alkali concentration was further increased to 2.5 N, the lignin concentration in the supernatant only marginally increased to 21.25 mg. The comparison of lignin removal for the US assisted and conventional approach has been given in Table 1 (expressed both as amount of lignin and the extent of delignification in terms of percentage of the original quantity) and it can be observed that, in both the processes there is only marginal increase in the delignification when alkali concentration was increased beyond 1.5 N. The observed delignification was 35.08% for alkali concentration as 1.5 N in the case of US assisted approach whereas the extent of delignification was 26.4% in the case of conventional approach. Time required by US assisted approach for 35.08% delignification was 3 h while conventional approach required 8 h to achieve the best delignification in conventional approach, clearly highlighting the benefits of using ultrasound. A study performed on delignification of waste newspaper also reported 1 N to be optimal alkali concentration with 80% delignification [7] with similar favorable results for ultrasound. Another study on delignification of different raw materials also reported 1 N alkali concentration to be optimum but giving around 70% delignification [11], again confirming the dependency of extent of delignification on the substrate. It is important to understand that the optimum alkali loading is also different in the reported cases again highlighting the importance of the presented work dealing with the specific sustainable biomass. The higher degree of intensification obtained due to the use of US is attributed to the increased mass transfer rate due to the cavitation effects. Studies performed on delignification have reported lesser requirement of alkali loading in US assisted processes as compared to conventional process [7], though not observed in the present work. Typically, high intensity turbulence helps to open up the matrix of lignocellulosic materials resulting in higher penetration of alkali giving increased delignification along with lesser reaction time and requirement of chemicals. It is important to note that the observed effects would indeed be dependent on the specific biomass in question.

3.1.3. Effect of sawdust loading on delignification using the conventional approach

The sawdust loading is another important parameter deciding the extent of delignification. The effect of sawdust loading was studied over the range of 0.2 to 1.0% w/v, keeping other parameters constant at 1.5 N as the alkali concentration, treatment time as 7 h, temperature at

Table 1
Comparison of the effect of alkali concentration on the final lignin concentration and extent of delignification using US assisted and conventional approach.

Alkali concentration (N)	US assisted delignification		Conventional method for delignification	
	Lignin concentration (mg)	Extent of Delignification (%)	Lignin concentration (mg)	Extent of Delignification (%)
0.5	13.88	25.28	7.53	13.71
1	14.40	26.23	12.51	22.79
1.5	19.26	35.08	14.54	26.48
2	20.23	36.84	15.08	27.48
2.5	21.25	38.7	15.18	27.65

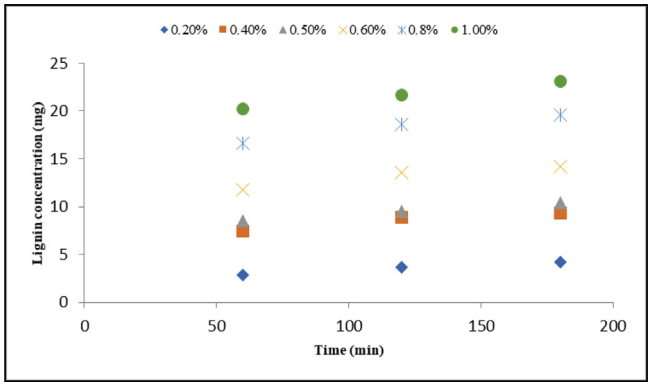

Fig. 5a. Effect of substrate loading on extent of sawdust delignification using conventional approach.

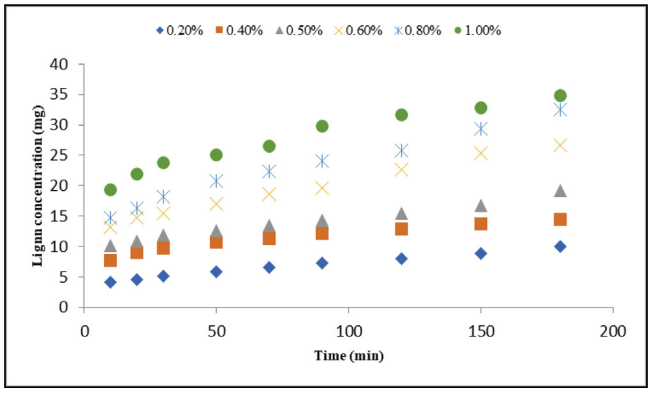

Fig. 5b. Effect of substrate loading on extent of sawdust delignification using US assisted approach.

Table 2
Comparison of the effect of substrate loading on the final lignin concentration and extent of delignification using US assisted and conventional approach.

Substrate loading (%w/v)	US assisted delignification		Conventional method of delignification	
	Lignin concentration (mg)	Extent of Delignification (%)	Lignin concentration (mg)	Extent of Delignification (%)
0.2	9.99	45.48	6.42	29.23
0.4	14.46	32.93	12.93	29.4
0.5	19.26	35.08	14.54	26.47
0.6	26.67	40.48	16.96	25.73
0.8	32.56	37.06	26.0	29.6
1.0	34.89	31.77	31.33	28.53

50 °C and stirring speed as 300 rpm. The obtained results for the effect of substrate loading have been shown in Fig. 5a. The amount of lignin removed increased from 6.4 to 26 mg when the substrate loading increased from 0.2 to 0.8% w/v but a further increase in the loading to 1% w/v resulted in only marginal increase to 31.3 mg with 28.5% as the extent of delignification. The obtained data for the variation in the extent of lignin removal (both in terms of amount of lignin and the percentage delignification) with substrate loading is given in Table 2. The data shows that 0.8% w/v substrate loading gave best results with higher extent of delignification as 29.6%. Considering these results, 0.8% w/v loading was established as the optimum loading. Another study also reported 0.8% w/v as the optimum with 40% delignification using conventional stirring based approach [7]. Using too high substrate loading may affect the delignification adversely as the ratio of available hydroxyl ions to the substrate may get affected adversely and also mixing plays a major role in the processing. Thus it is important to select an optimum substrate loading for the effective processing.

3.1.4. Effect of sawdust loading on extent of delignification in the case of US assisted approach

The effect of substrate loading on the extent of delignification was also investigated in the case of US assisted approach over the sawdust loading range of 0.2 to 1% w/v and other parameters were maintained constant at 1.5 N as the alkali concentration, treatment time of 3 h and temperature as 50 °C along with US power and US duty cycle at 100 W and 70% respectively. The obtained results for the effect of sawdust loading are depicted in Fig. 5b and it can be observed from the figure that the lignin concentration in the supernatant increased from 9.9 to 26.7 mg when the substrate loading was increased from 0.2 to 0.6% w/v. Further increase in the sawdust loading upto 1% w/v resulted in higher lignin quantity in the supernatant of 34.8 mg. The data obtained for the variation in the extent of lignin removal (again both in terms of amount of lignin and percentage delignification) with substrate loading in the case of US assisted treatment is also given in Table 2. It was observed that substrate loading of 0.6% w/v with US assisted approach was able to remove 26 mg of lignin while with conventional approach at 0.8% w/v substrate loading, similar lignin removal was obtained. At 1% w/v substrate loading, the extent of delignification was reported to decrease (31%), though the total lignin in the supernatant is higher and this can be attributed to the fact that above certain point the substrate loading dampens the effects of US resulting in lesser delignification. Higher extent of presence of solids leads to attenuation effects reducing the amount of energy transferred into the solution finally giving lower degree of cavitational effects. Comparing the results for the conventional and ultrasound assisted processing, it was observed that US assisted approach was able to give the same lignin yield with lesser substrate loading or in other words higher extent of delignification. US assisted approach also required lesser reaction time of 3 h as compared to 7 h required by conventional process. Similar trend of higher extent of delignification in lower duration was also reported by Subhedar et al. in delignification studies performed on waste newspaper [7]. Another study on delignification of napier grass using US reported optimum substrate loading of 5% w/v which was found to be very high as compared to optimum established in the present work [10]. Substrate loading is mainly dependent upon the nature of feedstock to be processed as few materials like waste newspaper and sawdust form a thick slurry and limit the substrate loading to lower ratios. The presented discussion clearly highlights the importance of establishing the optimum loading for different processed feed stocks.

3.1.5. Effect of temperature on the extent of delignification using the conventional approach

The effect of temperature on the extent of delignification was studied over the range of 40–80 °C keeping other parameters constant at 0.8% w/v as the substrate loading, 1.5 N as the alkali concentration, treatment time of 7 h and stirring speed as 300 rpm. The obtained results are shown in Table 3 and Fig. 6. The amount of lignin in the supernatant was observed to increase from 21.4 to 46.6 mg with an increase in temperature from 40 to 80 °C. The increase in the extent of lignin removed from sawdust and hence appearing in the supernatant with an increase in temperature can be attributed to enhanced reactivity due to favored kinetics. Experiments were not performed above 80 °C as it was observed that significant heating resulted in loss of

Table 3
Effect of temperature on extent of sawdust delignification using conventional approach.

Temperature (°C)	40	50	60	80
Lignin concentration (mg)	21.47	26.0	31.74	46.66
Extent of Delignification (%)	24.44%	29.6%	36.12%	53.11%

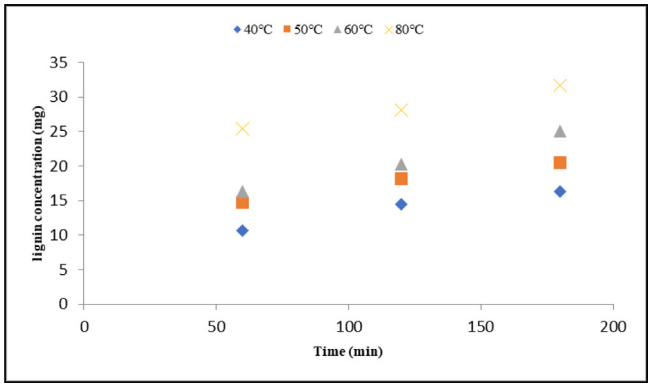

Fig. 6. Effect of temperature on extent of sawdust delignification using conventional approach.

reaction mixture. Use of significantly higher temperatures may also cause degradation of polysaccharides especially cellulose which may further affect the yield of reducing sugars in hydrolysis step [7]. Extent of delignification was observed to increase from 24.4 to 53.1% with an increase in temperature from 40 to 80 °C. Considering these results, 80 °C was considered as the best operating temperature and used in further studies related to delignification. Use of higher temperatures results in intensified removal of lignin providing increased access to cellulose and hemicellulose in the subsequent enzymatic hydrolysis. Delignification is reported to be effective at higher temperatures and 80 °C was also observed to be optimum in earlier reported US assisted delignification studies [7,10,11] matching the observed trends for the conventional processing in the current work. Considering this analysis, the temperature used for the ultrasound assisted processing was also 80 °C and detailed investigation was not performed for US assisted approach.

3.2. Enzymatic hydrolysis of sawdust

The delignified sawdust was further subjected to enzymatic hydrolysis using cellulase enzyme. The yield of reducing sugar was quantified under different sets of operating parameters to obtain the set of optimum conditions. The enzymatic reactions are comparatively slow yielding higher processing costs. Process intensification studies based on ultrasound assisted approach were hence performed to possibly minimize the processing costs.

3.2.1. Effect of temperature on enzymatic hydrolysis of sawdust using the conventional approach

The effect of temperature on the enzymatic hydrolysis was investigated for the conventional approach over the range of 30–70 °C keeping constant substrate loading as 2% w/v, enzyme loading as 0.5% w/v, reaction time of 4.5 h and speed of stirring as 300 rpm. The obtained results are shown in the Fig. 7a and it can be observed from the figure that the reducing sugar concentration increased substantially with an increase in temperature till an optimum. Quantitatively, as the temperature increased from 30 to 50 °C, the concentration of reducing sugar increased from 1.94 to 2.24 (mg/mL) and further increase in the temperature to 70 °C led to a decrease in the reducing sugar yield to 1.63 (mg/mL). Increase in temperature increases the rate of reaction but in the case of enzymatic reactions, higher temperature may deactivate the enzyme giving lower reducing sugar yield as enzymes are proteins in nature and they denature at high temperatures. The values of reducing sugar yield obtained at different conditions are given in Table 4. Study reported on enzymatic hydrolysis of sugarcane bagasse also reported 50 °C to be the optimum temperature yielding maximum amount of reducing sugars [18]. Another study on enzymatic hydrolysis also reported 50 °C as the optimum temperature [19]. Considering the

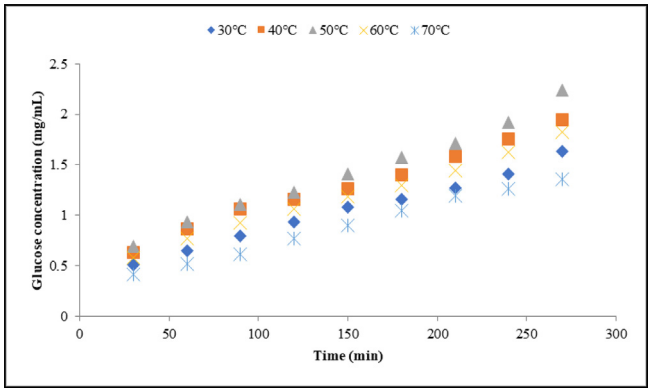

Fig. 7a. Effect of temperature on enzymatic hydrolysis of pretreated sawdust using conventional approach.

Table 4
Comparison of the effect of temperature on final glucose concentration and yields in the enzymatic hydrolysis of pretreated sawdust using US assisted and conventional approach.

Temperature (°C)	US assisted hydrolysis		Conventional method hydrolysis	
	Glucose concentration (mg/mL)	% Glucose yield (g/g)	Glucose concentration (mg/mL)	% Glucose yield (g/g)
30	2.71	13.55	1.63	8.17
40	3.21	16.05	1.94	9.72
50	4.08	20.40	2.24	11.19
60	3.00	15.03	1.83	9.13
70	2.06	10.30	1.36	6.78

results obtained in current study, 50 °C was set as the optimum reaction temperature for subsequent studies.

3.2.2. Effect of temperature on enzymatic hydrolysis of the sawdust using the US assisted approach

The effect of temperature on the US assisted enzymatic hydrolysis was also investigated over the range of 30–70 °C. The US parameters were kept constant at 50 W power and 50% duty cycle along with other constant process parameters as substrate loading as 2% w/v, enzyme loading as 0.5 w/v and reaction time as 1 h. The obtained results are depicted in Fig. 7b and it can be observed from the figure that the sugar concentration increased from 2.1 to 3.8 mg/mL initially with an increase in temperature from 30 to 50 °C. Further increase in temperature to 70 °C led to a significant decrease in reducing sugar production (actual value as 2.06 mg/mL). Based on the results obtained in Table 4,

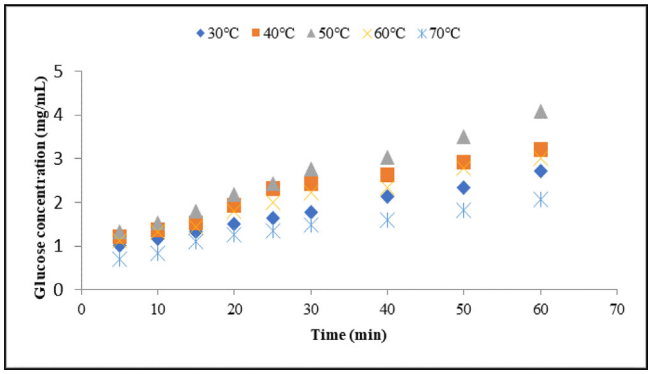

Fig. 7b. Effect of temperature on US assisted enzymatic hydrolysis of pretreated sawdust.

50 °C was set as the optimum reaction temperature. The observed optimum can be attributed to the fact that cavitational intensity decreases at higher temperatures due to increase in vapor pressure of the system, which gives too many nuclei and cavitation events leading to cushioned collapse [20].

US assisted hydrolysis was observed to produce 1.6 times higher reducing sugars as compared to the conventional approach with significant reduction in reaction time from 5 h to 1 h. Study performed by Subhedar et al. [8,11] on US assisted enzymatic hydrolysis of waste newspaper and other lignocellulosic materials also reported 50 °C as optimum reaction temperature with increased yield of reducing sugars and requirement of lesser reaction time for the ultrasound assisted approach. Another study reported on US assisted enzymatic hydrolysis of corn cobs also reported 50 °C as the optimum reaction temperature though with different degree of intensification for the sugar yields [21]. The observed intensification due to the use of ultrasound is attributed to the physical effects of cavitation induced by passage of ultrasound.

3.2.3. Effect of US power on enzymatic hydrolysis of sawdust

US power is an important factor deciding the cavitational activity and hence the degree of intensification in the case of US assisted enzymatic reactions. The reaction was performed for 70 min keeping duty cycle constant as 50% along with substrate loading as 2% w/v, enzyme loading as 0.5% w/v and temperature as 50 °C. The power dissipation was varied from 10 to 80 W with the obtained results for the progress of reaction depicted in Fig. 8. Initially, it can be seen that with an increase in power from 10 to 50 W reducing sugar production is also found to increase from 1.85 to 2.45 mg/mL. Further increase in power to 80 W, resulted in sudden drop in reducing sugar production to 1.82 mg/mL. The decrease in reducing sugars production can be attributed to the fact that higher US power dissipation result in inactivation of enzymes (as demonstrated in our earlier work of Subhedar and Gogate [22]) and also too many cavities at higher power gives a cushioning effect inhibiting the implosion of cavities. The percent yield of reducing sugars at different power dissipations has been given in Table 5 and it can be observed that the glucose yield increased from 18.51 to 24.97% with an increase in power from 10 to 50 W and with further increase in power to 80 W, the yield reduced to 18.18%. Considering the obtained results in the present work, 50 W power was considered as the optimum power dissipation. Other studies on US assisted enzymatic hydrolysis have also reported existence of the optimum US power though with different actual value as 60 W [8,11]. Another study reported 7.2 W/mL as the optimum power density in the case of US assisted enzymatic hydrolysis [9]. Two other studies reported on enzymatic hydrolysis using US bath reported 100 W and 132 W as the optimum US power [10,18]. It can be thus established that depending upon the processing volumes and reactor configurations, the optimum value of the US power dissipation tend to change.

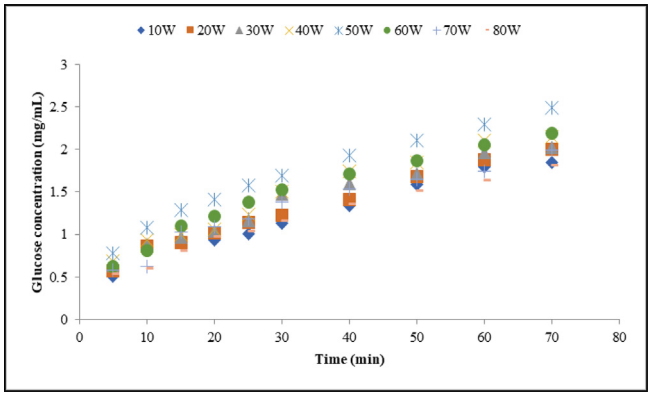

Fig. 8. Effect of US power on US assisted enzymatic hydrolysis of pretreated sawdust.

3.2.4. Effect of duty cycle on US assisted enzymatic hydrolysis of sawdust

The duty cycle is also one of the important factors deciding the progress of enzymatic hydrolysis and the overall economics of operation. The effect of duty cycle was investigated by changing the ON and OFF time of the US operation. The hydrolysis reaction was performed for 60 min at constant US power of 50 W, 2% w/v as the substrate loading and 0.5% w/v as the enzyme loading. The effect of duty cycle was studied over the range of 30–90% (3 s to 9 s on time in cycle of 10 s). The presented data in the Fig. 9 establishes that the reducing sugar yield increased from 3.53 to 4.21 mg/mL with an increase in the duty cycle from 30 to 70% and marginal increase in reducing sugar was observed from 4.21 to 4.24 mg/mL on further increase in duty cycle from 70 to 90%. The obtained data for the percentage yield is given in Table 6. The glucose yield was reported to increase from 17.65 to 21.05% with an increase in duty cycle from 30 to 70%, beyond which marginal effects were observed. Another study performed on US assisted enzymatic hydrolysis have reported similar results with 70% duty cycle as optimum resulting in 25 g/L as the reducing sugars concentration [8]. An increase in the duty cycle initially increases the extent of cavitational effects favorably but beyond the optimum it has a negative effect on the enzyme stability and also results in cushioning similar to the effect of power dissipation. Thus using optimum duty cycle is a major factor in obtaining proper progress of the enzymatic hydrolysis. Using too high duty cycle will also cause problems like increase in reaction temperature and inefficient energy dissipation as well as erosion of transducers and thus should be avoided. Another study on US assisted enzymatic hydrolysis reported 50% duty cycle to be the optimum [10]. Considering the observed results in the present work, 70% duty cycle was set as optimum for US assisted enzymatic hydrolysis process. It is important to note that exact optimum duty cycle depends on the specific system and need to be established using laboratory studies as per the methodology presented in current work.

3.2.5. Effect of substrate loading on enzymatic hydrolysis of sawdust using the conventional approach

The effect of substrate loading on enzymatic hydrolysis was studied in the case of conventional approach with variation of substrate loading in the range of 0.5 to 10% w/v keeping other treatment parameters constant at 50 °C as the temperature, 0.5% w/v as the enzyme loading, 4.5 h as reaction time and stirring speed as 300 rpm. The obtained results are shown in Fig. 10a and it can be observed from figure that with an increase in substrate loading from 0.5 to 6% w/v reducing sugar concentration increased from 1.14 to 6.5 mg/mL. Further increase in substrate loading to 10% w/v, resulted in marginal increase to 7 mg/mL as the reducing sugar concentration. The observed trends can be attributed to the fact that with lower substrate loadings the enzyme active sites become easily accessible while at the higher substrate loadings beyond optimum, enzymes get saturated quickly and rate of reaction becomes constant irrespective of substrate loading. Considering the obtained results for reducing sugar yield, 6% w/v substrate loading was decided as the optimum. Another study performed on enzymatic hydrolysis reported 5% w/v substrate loading to be optimum with 11.5 g/L yield of reducing sugars [8]. Study performed on enzymatic hydrolysis of sugarcane bagasse reported 2% w/v as the substrate loading with the actual production of sugars as 0.26 g sugar/g of dried biomass [18]. It is important to see that the observed optimum loading and yield of sugars is different meaning that it is important to perform a detailed study for the specific substrate in question.

3.2.6. Effect of substrate loading on enzymatic hydrolysis of sawdust in the case of US assisted approach

The effect of substrate loading on the progress of US assisted enzymatic hydrolysis approach was also investigated over the range of 0.5–10% w/v as the loading keeping constant conditions of temperature (50 °C), enzyme loading (0.5% w/v) and reaction time (1 h) along with US power and duty cycle as 50 W and 50% respectively. The results

Table 5
Effect of power dissipation on the final sugar yields in US assisted enzymatic hydrolysis of pretreated sawdust.

US power (W)	10	20	30	40	50	60	70	80
Glucose concentration (mg/mL)	1.85	1.99	2.02	2.15	2.5	2.18	1.99	1.82
% Glucose yield (g/g)	18.51	19.99	20.22	21.58	24.97	21.80	19.89	18.18

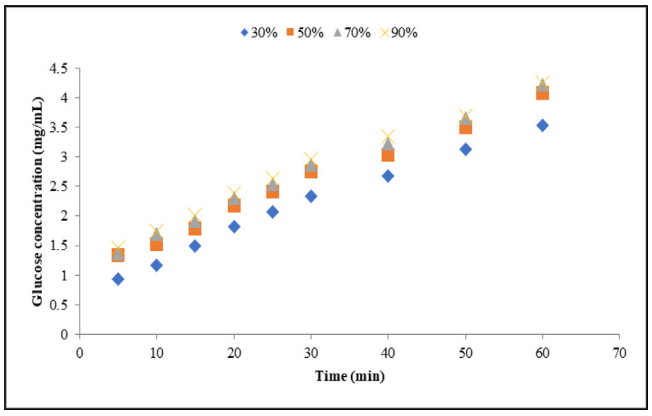

Fig. 9. Effect of US duty cycle on US assisted enzymatic hydrolysis of pretreated sawdust.

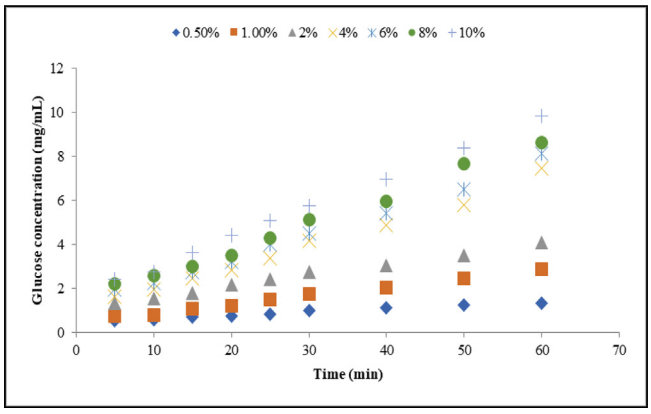

Fig. 10b. Effect of substrate loading on enzymatic hydrolysis of sawdust using US assisted approach.

Table 6
Effect of duty cycle on the final sugar yields in US assisted enzymatic hydrolysis of pretreated sawdust.

US duty cycle (%)	30	50	70	90
Glucose concentration (mg/mL)	3.53	4.08	4.21	4.24
% Glucose yield (g/g)	17.65	20.40	21.05	21.20

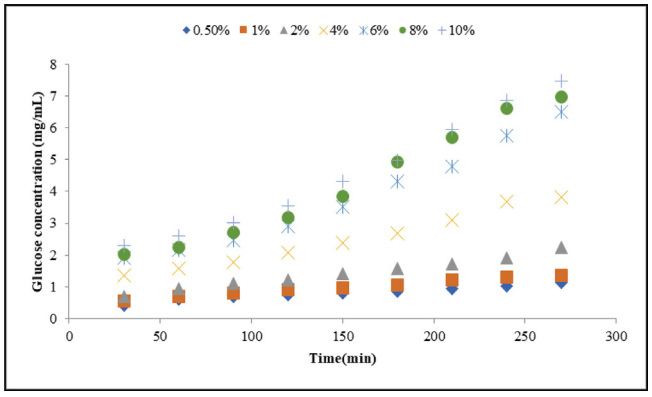

Fig. 10a. Effect of substrate loading on enzymatic hydrolysis of pretreated sawdust using conventional approach.

obtained for substrate loading variation are depicted in Fig. 10b and it can be observed from the reported data that production of reducing sugars increased from 1.35 to 7.46 mg/mL with an initial increase in substrate loading from 0.5 to 4% w/v. Further increase in substrate loading from 4 to 10% w/v resulted in a comparatively marginal increase in the sugar yield from 7.46 to 9.8 mg/mL. Based on the results obtained, 4% w/v substrate loading was considered as the optimum with 7.46 mg/mL of reducing sugars production as there was no significant increase in yield above 4% w/v at the maintained constant enzyme loading of 0.5% w/v. Comparison of reducing sugars production indicate that the production of reducing sugars from US assisted approach was twice that of conventional process till 4% w/v substrate loading at the maintained constant enzyme loading of 0.5% w/v. US induced cavitation reduces the diffusion barrier and accelerates contact between substrate and enzyme yielding increased rate of hydrolysis [2]. The combination of turbulence along with thermal energy dissipation also increases the reaction rate constants resulting in higher glucose yield. Study performed by Rodriguez et al. [21] dealt with US assisted enzymatic hydrolysis of corn cobs and it was reported 1:10 (w/v) substrate loading resulted in higher amount of biogas production. Another study performed on US assisted enzymatic hydrolysis reported 3% w/v to be the optimum substrate loading giving 27.6 g/L reducing sugars from waste newspaper [8]. In the current study, 4% w/v substrate loading was reported to be optimum with 7.46 mg/mL reducing sugar concentration. Overall, it was demonstrated that US assisted enzymatic hydrolysis approach resulted in intensified processing with significantly higher production of reducing sugars with lesser requirement of time and chemicals as compared to conventional process.

4. Conclusions

In the present work, delignification of sawdust as pretreatment followed by enzymatic hydrolysis has been investigated providing detailed understanding into effects of the important process parameters and intensification benefits due to the application of US. Studies revealed that alkali concentration of 1.5 N was the optimum alkali concentration beyond which only marginal increase in delignification was obtained in the case of both conventional and US assisted approach. The optimum loading of biomass established for conventional and US assisted delignification was 0.8% w/v and for enzymatic hydrolysis, the optimum substrate loading for the conventional approach was 6% w/v whereas for US assisted hydrolysis, it was 4% w/v giving almost the same yield. In the case of alkaline pretreatment, higher temperatures were observed to give enhanced effects and hence the best operating temperature established was 80 °C. For enzymatic hydrolysis, 50 °C was observed as the optimum temperature for both the approaches. It was established that higher US power dissipation and duty cycle can result in deactivation of enzyme and hence it is necessary to use optimum power/duty cycle for efficient processing. In the present study for enzymatic hydrolysis, 50 W as the US power dissipation and 70% US duty cycle were observed as the optimum. Overall, it was established that the US assisted delignification and enzymatic hydrolysis resulted in about 1.5 times higher lignin removal and 1.6 times higher yield of reducing sugars respectively as compared to conventional treatment approach.

Significant reduction in reaction time was also reported in the case of US assisted processes with delignification requiring 3 h and enzymatic hydrolysis requiring 1 h to give the same yield as that of conventional process taking 6–7 h. Overall the work clearly demonstrated the process intensification benefits due to the use of ultrasound in processing of sustainable biomass.

Acknowledgement

RP would like to acknowledge the funding of Department of Biotechnology, New Delhi for fellowship during the course of M. Tech. (Bioprocess Technology).

References

[1] R.A. Silverstein, Y. Chen, R.R. Sharma-Shivappa, M.D. Boyette, J. Osborne, A comparison of chemical pretreatment methods for improving saccharification of cotton stalks, Bioresour. Technol. 98 (2007) 3000–3011.
[2] P.B. Subhedar, P.R. Gogate, Intensification of enzymatic hydrolysis of lignocellulose using ultrasound for efficient bioethanol production: a review, Ind. Eng. Chem. Res. 52 (2013) 11816–11828.
[3] Y. Sun, J. Cheng, Hydrolysis of lignocellulosic materials for ethanol production: a review, Bioresour. Technol. 83 (2002) 1–11.
[4] P.R. Gogate, A.B. Pandit, Engineering design method for cavitational reactors: I. Sonochemical reactors, AIChE J. 46 (2000) 372–379.
[5] P.R. Gogate, A.B. Pandit, Engineering design methods for cavitation reactors II: hydrodynamic cavitation, AIChE J. 46 (2000) 1641–1649.
[6] A. Iskalieva, B.M. Yimmou, P.R. Gogate, M. Horvath, P.G. Horvath, L. Csoka, Cavitation assisted delignification of wheat straw: a review, Ultrason. Sonochem. 19 (2012) 984–993.
[7] P.B. Subhedar, P.R. Gogate, Alkaline and ultrasound assisted alkaline pretreatment for intensification of delignification process from sustainable raw-material, Ultrason. Sonochem. 21 (2014) 216–225.
[8] P.B. Subhedar, N.R. Babu, P.R. Gogate, Intensification of enzymatic hydrolysis of waste newspaper using ultrasound for fermentable sugar production, Ultrason. Sonochem. 22 (2015) 326–332.
[9] D. Wang, X. Ma, L. Yan, T. Chantapakul, W. Wang, T. Ding, X. Ye, D. Liu, Ultrasound assisted enzymatic hydrolysis of starch catalyzed by glucoamylase: investigation on starch properties and degradation kinetics, Carbohydr. Polym. 175 (2017) 47–54.
[10] K.N. Nagula, A.B. Pandit, Process intensification of delignification and enzymatic hydrolysis of delignified cellulosic biomass using various process intensification techniques including cavitation, Bioresour. Technol. 213 (2015) 162–168.
[11] P.B. Subhedar, P. Ray, P.R. Gogate, Intensification of delignification and subsequent hydrolysis for the fermentable sugar production from lignocellulosic biomass using ultrasonic irradiation, Ultrason. Sonochem. 40 (2018) 140–150.
[12] R. Velmurugan, K. Muthukumar, Ultrasound-assisted alkaline pretreatment of sugarcane bagasse for fermentable sugar production: optimization through response surface methodology, Bioresour. Technol. 112 (2012) 293–299.
[13] H. Wu, X. Dai, S.L. Zhou, Y.Y. Gan, Z.Y. Xiong, Y.H. Qin, J. Ma, L. Yang, Z.K. Wu, T.L. Wang, W.G. Wang, C.W. Wang, Ultrasound-assisted alkaline pretreatment for enhancing the enzymatic hydrolysis of rice straw by using the heat energy dissipated from ultrasonication, Bioresour. Technol. 241 (2017) 70–74.
[14] R.S. Fukushima, R.D. Hatfield, Extraction and isolation of lignin for utilization as a standard to determine lignin concentration using the acetyl bromide spectrophotometric method, J. Agric. Food Chem. 49 (2001) 3133–3139.
[15] G. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chem. 31 (1959) 426–428.
[16] V.S. Chang, M. Nagwani, C.-H. Kim, M.T. Holtzapple, Oxidative lime pretreatment of high-lignin biomass: poplar wood and newspaper, Appl. Biochem. Biotechnol. 94 (2003) 1–28.
[17] M. Zheng, X. Li, L. Li, X. Yang, Y. He, Enhancing anaerobic biogasification of corn stover through wet state NaOH pretreatment, Bioresour. Technol. 100 (2009) 5140–5145.
[18] F.C. Lunelli, P. Sfalcin, M. Souza, E. Zimmermann, V. Dal Prá, E.L. Foletto, S.L. Jahn, R.C. Kuhn, M.A. Mazutti, Ultrasound-assisted enzymatic hydrolysis of sugarcane bagasse for the production of fermentable sugars, Biosyst. Eng. 124 (2014) 24–28.
[19] C. Álvarez, F.M. Reyes-Sosa, B. Díez, Enzymatic hydrolysis of biomass from wood, Microb. Biotechnol. 9 (2016) 149–156.
[20] P.R. Gogate, A.B. Pandit, Theoretical and experimental sonochemistry involving inorganic systems, in: M. Ashokkumar, Pankaj (Eds.), Theor. Exp. Sonochemistry Involv. Inorg. Syst., 2011, pp. 69–106.
[21] N. Pérez-Rodríguez, D. García-Bernet, J.M. Domínguez, Effects of enzymatic hydrolysis and ultrasounds pretreatments on corn cob and vine trimming shoots for biogas production, Bioresour. Technol. 221 (2016) 130–138.
[22] P.B. Subhedar, P.R. Gogate, Enhancing the activity of cellulase enzyme using ultrasonic irradiations, J. Mol. Cat. B Enz. 101 (2014) 108–114.