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Enhancement of bio-ethanol production potential of wheat straw by reducing furfural and 5-hydroxymethylfurfural (HMF)

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ABSTRACT

The present study intended to enhance the bioethanol production potential of wheat straw by reducing furfural and 5-hydroxymethylfurfural. The combination of 180 °C and 2% H_2SO_4 was optimized for pretreatment of wheat straw, which resulted significantly higher total soluble sugar. The maximum amount of furfural and HMF were observed when wheat straw pretreated at 180 to 220 °C, using 4% (v/v) dilute sulfuric acid. Amendment of pretreated acid hydrolysate using activated charcoal (5%, w/v) reduced up to 84.01% furfural and up to 76.42% HMF concentration in filtrate. The maximum ethanol yield of 5.29% (v/v) was obtained from charcoal amended acid hydrolysate, equivalent to 87.9% theoretical yield. Ethanol yield coefficient (Y_{ps}) was found to be 0.44 g ethanol g^{-1} sugar utilized. These results indicate that activated charcoal treated acid hydrolysate will be effective among the available technologies and could make lignocellulosic biomass-based ethanol production process economically viable by maximizing ethanol yield.

1. Introduction

The increased concern for energy security and severe consequence of fossil fuels on public health and environment, primarily global warming, climate change and air pollution, have been putting pressure on science and society to find new sustainable energy alternatives (Hasunuma and Kondo, 2012). Lignocellulosic biomass has been studied comprehensively and projected as a carbon-neutral source of liquid biofuels and other useful chemicals and eco-friendly polymeric materials (Saha et al., 2005; Ragauskas et al., 2006; Prasad et al., 2012). Currently, ethanol has been promoted as an alternative transportation fuel, because of its antiknocking properties which help to increase octane ratings and improve fuel efficiency (Prasad et al., 2014). Also, the use of biomass-based transportation biofuels can help in reducing CO_2 build up by recycling CO_2 that is liberated when ethanol is combusted as fuel (Hasunuma and Kondo, 2012).

Agriculture is the most prominent sector of India, and produces a huge volume of agri-residues, in the form of stalks and stubble (stems), leaves, and seed pods during crop harvesting seasons (Sharma and Dikshit, 2016). These residues are being used as animal feed and cooking fuels in rural areas. However, a considerable amount of the agri-residues is unutilized and left on farms. The proper disposal of such a large volume of crop residues is a significant challenge (Prasad et al., 2012; Kumar et al., 2016). Wheat is the world's most important and most widely grown cereal crop, cultivated in over 115 nations under a wide range of agro-climatic conditions (Ortiz et al., 2007; Cardoen et al., 2015). Therefore, the wheat straw would serve as potentially attractive raw material for ethanol production in future. The rigid crystalline structure of cellulose and complex structure of hemicellulose and lignin with cellulose in wheat straw make the pretreatment more critical before its enzymatic saccharification (Paulov et al., 2015; Kumar et al., 2016).

Wheat straw has been reported as a low-cost and most sustainable raw material for the large-scale production of ethanol. However, production of ethanol from lignocellulosic biomass is facing many barriers and technical challenges (Rathore et al., 2016). One barrier to the ethanol production of from cellulosic biomass is that the sugars necessary for fermentation are trapped inside the lignocellulose. In order to produce fermentable sugars from cellulosic biomass, a pretreatment process is used. The presence of inhibitory by-products in lignocellulosic hydrolysates that are released during the pretreatment process remains a significant challenge. These products especially furfural and HMF adversely affect the growth of ethanol fermenting yeast and hamper the ethanol productivity (Carolina et al., 2011; Ali et al.,

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2012). Therefore, the quantitative estimation of furfural and HMF is crucial for the optimization of the pretreatment process to reduce their formation. Many approaches have been proposed to remove or degrade furfural and HMF from lignocellulose hydrolysates, such as ion exchange, over-liming, enzymatic conversions and also some limited work on adsorption using activated charcoal (Mussatto and Roberto, 2004; Chandel et al., 2007; Canilha et al., 2008; Ali et al., 2012).

High ethanol yielding ability of ethanologenic yeasts and bacterial strains is one of the most important factors that affect the efficiency of ethanol production on an industrial scale. However, many of the ethanologenic yeasts strains are susceptible to inhibitory compounds originated from acid pretreatment, particularly to the presence of furfural and HMF (Díaz et al., 2009; Carolina et al., 2011; Ali et al., 2012). These compounds adversely affect the efficiency of ethanologenic yeasts by hampering enzymatic and biological activities (Hsu et al., 2010), thus reducing the overall efficiency for bioconversion of lignocellulosic biomass to ethanol. Thus, the present investigation was undertaken to determine the optimal conditions for microwave-assisted acid pretreatment and its impact on total soluble sugar recovery, quantification of furfural, and HMF production during pretreatment and its removal with activated charcoal, and subsequently impact on ethanol yield from wheat straw.

2. Material and methods

2.1. Feedstock characterization

The feedstock used was wheat straw of variety HD 2932, collected from the fields of IARI, Pusa, New Delhi by random selection in triplicate. The collected wheat straw was air dried, cleaned, cut into smaller pieces and then powdered using a laboratory grinder so that it passes through 20–40 mesh sieves. The wheat straw compositional analysis was carried out using standard protocols. Total solids were estimated as per the method described by Sluiter et al. (2008). The estimation of nitrogen and potassium was done using Kjeldahl, and flame photometry, respectively according to the procedure described by Piper (1950). The determination of phosphorus was accomplished using spectrophotometer according to Jackson (1973). The straw fibers were estimated as cellulose, hemicellulose, and lignin by following 2022-AN 380 application note using Foss Tecator Fibertec Analyzer (Goering and Van Soest, 1975).

2.2. Pretreatment conditions

Microwave assisted acid pretreatment was conducted using completely randomized design (CRD) with three replications to optimize the condition of temperature and concentration of dilute sulfuric acid in combination to achieve maximum sugar recovery from wheat straw. A WD700 (MG-5062T) type domestic microwave convection oven (LG) was used in this study. For pretreatment, 100 mg of oven dry, powdered wheat straw was taken in 50 ml glass tubes, and 10 ml of 1%, 2%, 3% and 4% (v/v) diluted sulfuric acid (H_2SO_4) was added, keeping a solid loading ratio of 1:10 (solid: liquid). Different treatments were given at all combinations of 4 sets of temperatures (100 °C, 140 °C, 180 °C, and 220 °C) with a residence time of 10 min. The control consisted of separate sets of wheat straw submerged in distilled water, keeping a solid loading ratio of 1:10 (solid: liquid) and treated with microwave at various temperatures as mentioned above.

2.3. Analysis of pretreated wheat straw

Total soluble sugars or fermentable sugars of the filtrate were estimated by the Anthrone reagent as described by Thimmiah (1999). The liquors filtered through 0.45 µm pore size filter membrane were collected and analyzed for furfural and HMF by high-performance liquid chromatography (HPLC) using PDA (Photo Diode Array) detector (Servin et al., 2005). At least three parallel samples size were used in all analytical measurements, and data are reported as the mean (n = 3). HPLC grade chemicals were used for the preparation of standard solutions and further analysis. Furfural GR Reag by Merck, KGaA and HMF extra pure for analysis were purchased from SRL (Sisco Research Laboratory). Methanol and deionized water for HPLC, Li Chrosolv R were purchased from Merck.

2.3.1. Preparation of standard solution and the calibration curve for furfural and HMF

The optimum detection wavelength of 275 nm is chosen, at which furfural and 5-HMF gave relatively good absorptions. A stock solution containing the pure grade 5-HMF and furfural was prepared independently and diluted to a series of appropriate concentrations level for the making of calibration curves. Standard solutions of 5, 10 and 15 parts per million (ppm), were prepared in 100% methanol for HPLC analysis. Then the standards were run simultaneously in Shimadzu HPLC (photodiode array detector) with methanol (HPLC grade) as the mobile phase. As a result, a gradient elution of A (water) and B (methanol) (20% in pump A and 80% in pump B (v/v)) at 275 nm and 40 °C exhibited the desired separation within 20 min run time. On the basis of the area obtained, standard calibration curves were prepared. The regression equation revealed a very good linear relationship ($r2 = 0.998 \pm 0.001$ for furfural; $r2 = 0.978 \pm 0.0012$ for HMF) within the test ranges.

2.3.2. Sample preparation and analysis

The diluted acid pretreated slurry was filtered through a Whatman grade No. 1 filter paper to separate residues (solid) and liquid. The filtrate fraction was collected and centrifuged at 0 °C at 5000 rpm for 10 min and stored at 4 °C in the refrigerator for HPLC analysis. The liquid fraction samples were degassed and filtered through syringe filter into auto-sampling vials and loaded into the Shimadzu HPLC for determination of furfural and HMF. Methanol was used as the mobile phase. In the binary mode, deionized water was pumped at a flow rate of 0.2 ml/min in pump A and methanol at 0.80 ml/min in pump B in C18 (liquid chromatographic) column (Temperature 40 °C). The samples were injected using a fixed injection loop volume of 20 µ and run time was 10 min. After separation, chromatograms were compared with the calibration curves according to the retention times of furfural and HMF for quantification of the amounts present in the samples.

2.4. Substrate for saccharification

The pretreated wheat straw of variety HD 2933 was taken as the substrate for enzymatic saccharification. The microwave assisted acid pretreated wheat straw was neutralized and thoroughly washed, before the saccharification. The wet material after pretreatment was directly used for the study, as drying is reported to cause irreversible pore collapse in the microstructure of biomass and thus decrease the enzymatic release of sugars (Brown and Torget, 1996). Besides, soluble sugars, the hydrolysate contain many intermediate inhibitors compounds in varying quantities such as water-soluble lignins (WSL), furfurals and acetic acid are known to be inhibitory to both yeast and bacterial strains (Nigam, 2001; Ali et al., 2012).

Enzymatic saccharification of pretreated wheat straw was carried out according to the protocol described by NREL LAP-009 (Brown and Torget, 1996). The samples obtained from the pretreatment, after washing and neutralization, were used in the study of enzymatic saccharification. Untreated wheat straw was used as the control. Pretreated samples equal to the equivalent of 0.1 g of cellulose was taken in 250 ml glass Erlenmeyer flask, and to each conical flask, 5 ml sodium citrate ($Na_3C_6H_5O_7$) buffer (0.05 M, pH 4.8), cellulase enzyme to equal around 60 FPU g^{-1} cellulose, β -glucosidase to equal 64 pNPGU g^{-1} cellulose were added. 20 mg/ml sodium azide (NaN_3) was added as an antibiotic agent to prevent the growth of organisms. Then, the volume

was adjusted to exactly 100 ml of distilled water. The flasks were plugged with cotton and incubation was done at 50 °C and 68 rpm in a BOD incubator-cum-shaker for 96 h. Samples were taken every 24 h of the hydrolysis and subjected to total sugar analysis by anthrone method (Thimmiah, 1999). The saccharified slurry was filtered through a Whatman Grade-1 filter paper to separate residues and filtrate. The filtrate was transferred into a sterilized 100 ml volumetric flask and stored at −20 °C till fermentation study.

2.5. Amendment of the substrate using activated charcoal

The presence of inhibitors like furfural and HMF in lignocellulosic hydrolysate is an industrial problem. Efficient removal of these inhibitors from pretreated lignocellulosic hydrolysate is necessary to increase the ethanol yield. Activated charcoal has the potential to remove these problematic chemicals. Hence, in this study, activated charcoal was used for the removal of furfural and HMF from the pretreated acid hydrolysate (hydrolysate obtained after pretreatment with acid). Activated charcoal, 5% (w/v) was added in hydrolysates and then stirred at room temperature for 30 min. Subsequently, the activated charcoal was separated from the hydrolysates by filtration. The filtered liquid fraction was collected and centrifuged at 0 °C at 5000 rpm for 10 min and stored at 4 °C in the refrigerator for HPLC analysis. The quantification of furfural and HMF in the amended substrate was done as described in Section 2.3.2.

2.6. Fermentation and estimation of bio-ethanol

The *Pichia stipitis* NCIM 3498 procured from NCIM (National Collection of Industrial Microorganisms), National Chemical Laboratory, Pune was used for ethanol fermentation. The yeast strain was maintained on malt extract, glucose, yeast extracts; peptone (MYGP) agar slants and sub-cultured periodically. Batch fermentation experiment was carried out in two sets of wheat straw hydrolysate (detoxified and non-detoxified) to compare the effect of inhibitors on ethanol yield. Detoxification was done using activated charcoal as mentioned earlier. Each set of batch fermentation experiment was carried out in triplicates for each sample, using 250 ml flasks containing 100 ml detoxified and non-detoxified wheat straw hydrolysate and inoculated with 10% inoculum of *Pichia stipitis* and incubated at 30 °C for 72 h. After completion of fermentation, 10 ml of samples in triplicate were withdrawn for analyzing ethanol using HPLC as mentioned below.

2.7. Estimation of ethanol by HPLC

A stock solution containing the HPLC grade ethanol was prepared separately and diluted with deionized water (HPLC grade) to a series of appropriate concentrations for calibration curves. Standards of 5, 10, 15 and 20% were prepared. Then standards were run simultaneously in Refractive Index Detector (RID-10A) in Shimadzu HPLC with 0.001 M sulfuric acid (H_2SO_4) as a mobile phase in isocratic mode. Runtime was 10 min. On the basis of the area of chromatograms, the standard calibration curve was prepared. The curve showed excellent linearity, and the regression coefficient was found to be in the range of 0.987 ± 0.0013.

The liquid fraction was collected and centrifuged at 0 °C at 5000 rpm for 10 min. After centrifugation, the supernatant was collected in a clean microcentrifuge tube, and immediately stored at 4 °C in the refrigerator until ethanol estimation. The pretreated liquid samples were degassed and filtered through 0.2 µm syringe filter into autosampling vials and loaded into the Shimadzu HPLC. 0.001 M H_2SO_4 was used as mobile phase. In the isocratic mode, 0.001 M H_2SO_4 was pumped at a flow rate of 1.0 ml/min in pump 'A' in C18 (liquid chromatographic) column (Column temp. 55–65 °C). The standard and all samples injection volume were kept at 20 µl and run time was 15 min. Refractive Index Detector (RID-10A) was used for estimation of ethanol in pretreated samples. After separation, chromatograms were compared with the calibration curves, and ethanol was estimated. Percentage of conversion efficiency or yield efficiency (Ey) was calculated as per following formula (Prasad et al., 2009).

$$Ey = Yps^{*}100/0.51$$

where, Yps is ethanol yield expressed as a gram of ethanol per gram of sugar utilized (g g–1), and 0.51 is the highest theoretical ethanol yield of glucose used (Prasad et al., 2009).

2.8. Statistical analysis

Statistical analysis of observed experimental data set was carried out using state-of-the-art Statistical Analysis System (SAS) for Windows version 6.11. The results of variability across three replicates were pooled and expressed as mean (n = 3), and ± standard deviations. The least significant difference (LSD) at 5% probability levels ($P = 0.05$) were figured by multiplying the standard error of the difference between any two treatment means (SED values with tabulated t values. The difference between two treatment means was considered as significant when the value exceeded that of LSD (Panse and Sukhatme, 1985).

3. Results and Discussion

3.1. Characterization of wheat varieties

The results of wheat straw characterization before pretreatment and saccharification showed that wheat straw is rich in carbohydrate polymers (cellulose, hemicellulose), total solids, nitrogen (N), phosphorus (P) and potassium (K). Total solids value was observed 94.89%; N content was estimated 0.34%, while P and K contents were 0.015 and 1.67%, respectively (Table 1). The fiber content in the wheat straw of variety HD 2932 was analyzed as cellulose, hemicellulose, and lignin. The fibrous constituents of wheat straw especially cellulose, hemicellulose and lignin contents were 46.63, 27.47 and 15.20%, respectively. Similar results on cellulose, hemicellulose, and lignin content in the wheat straw have also been reported by Yasin et al. (2010).

3.2. Total sugar recovery after acid pretreatment

The sugar recovery from wheat straw hydrolysates obtained after microwave-assisted acid pretreatment was in the range of 11.03 to 19.32%. The sugar recovery obtained was significantly higher at 180 °C (19.32%), followed by 140 °C (17.78%) with 2% sulfuric acid and it was minimum at 220 °C with 4% acid treatment (Table 2). The combination of 180 °C and 2% sulfuric acid concentration was found the optimal condition for pretreatment of wheat straw, where significantly higher total soluble sugar recovery was obtained. These results were in agreement with that of Yang and Wyman (2008) and Yoswathana et al. (2010). Yemis and Mazza (2012) also demonstrated that the microwave-assisted reaction temperature (140–200 °C) was very effective for the sugar recovery from wheat straw with diluted acid catalysis.

Table 1
Chemical characterization of wheat straw (var. HD 2932).

Constituents	(%, oven dry weight basis)
Cellulose	46.63 ± 4.16
Hemicellulose	27.47 ± 1.96
Lignin	15.20 ± 1.61
Nitrogen	0.34 ± 0.028
Phosphorus	0.015 ± 0.004
Potassium	1.67 ± 0.14
Total solids	94.89 ± 1.95

Results are expressed as the mean (n = 3) ± standard deviations.

Table 2
Total soluble sugars from pretreated wheat straw.

Dilute acid conc. temperatures	% total soluble sugars				
	1%	2%	3%	4%	Control
100 °C	14.81[b]	15.24[b]	14.51[b]	13.62[c]	4.10[c]
140 °C	16.57[b]	17.78[a]	15.58 [ab]	15.54[b]	4.77b[c]
180 °C	16.93[a]	19.32[a]	15.78[a]	14.48[ab]	6.40[a]
220 °C	13.51[b]	13.66[b]	12.63[a]	11.03[c]	5.24[b]
LSD (p = 0.05)	1.29	1.66	1.09	1.21	0.64

Mean with the same letter (s) along same column are not significantly different (p = 0.05).

Jaisamut et al. (2016) reported that the pretreatment condition especially temperature strongly affect the solid phase composition of wheat straw, as well as yields of monomeric sugars in enzymatic hydrolysis and subsequent ethanol fermentation. The levels of intermediatory inhibitors produced during pretreatment and decomposition of hemicellulose into simple sugars are also extremely affected by increasing pretreatment temperature. The increment in sugar content may be due to the cleaving of the β-glycosidic linkages of hemicellulose and liberation of non-numeric sugars. However, the combination of higher acid concentration and high temperature for pretreatment had a detrimental effect on sugar recovery from wheat straw (Table 2). The detrimental effect on sugar recovery may be due to high temperatures caused more sugar degradation, aiding the formation of inhibitors (Yang and Wyman, 2008).

3.3. Effect of acid pretreatment on furfural and HMF production

The furfural production from wheat straw by microwave assisted sulfuric acid pretreatment at different temperatures ranged from 1.54 to 39.21 ppm (Fig. 1). It was significantly higher at 220 °C (39.21 ppm) followed by 180 °C (36.32 ppm) with 4% sulfuric acid assisted microwave pretreatment. This may be because of pretreatment at higher acid concentration and temperature conditions, which usually produce these inhibitory compounds due to degradation of sugar (Saha et al., 2005). Ambalkar and Talib (2012) also reported that increasing sulfuric acid concentration, temperature and pressure level, and CO_2 flow rate would increase furfural yield. Similar trends were also observed for HMF and obtained in the range of 2.41 to 30.10 ppm (Fig. 2). HMF production was also significantly higher at 220 °C (30.10 ppm) followed by 180 °C (28.11 ppm) with 4% sulfuric acid assisted microwave pretreatment. In control at 100 °C, furfural and HMF production was negligible. Guarnieri et al. (2017) have also reported that furfural and HMF are usually formed during high-temperature pretreatment of lignocellulosic biomass. That could be due to autohydrolysis by liberated acetic acid in biomass, which can lead to the dehydration of hexose and pentose sugars into the HMF and furfural, respectively.

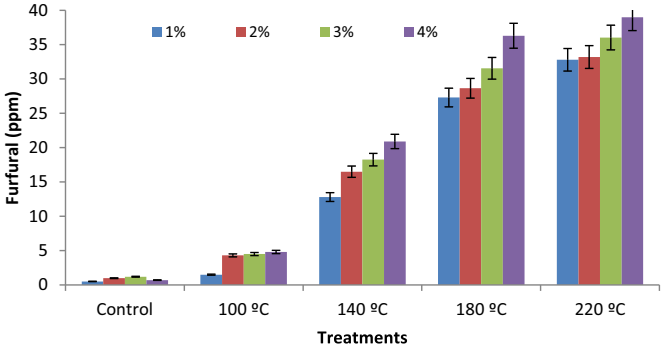

Fig. 1. Furfural production during pretreatment of wheat straw at varying temperature and H_2SO_4 concentration.

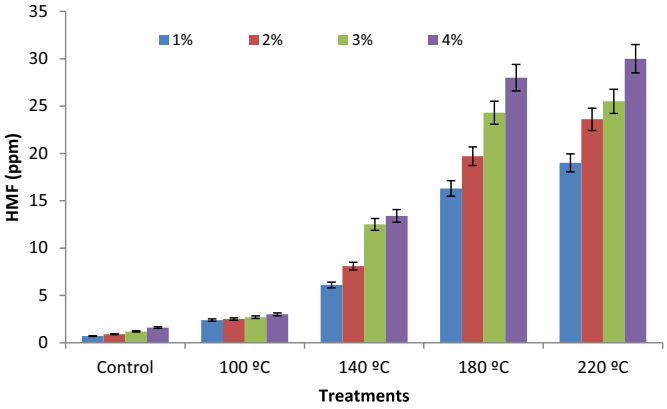

Fig. 2. HMF production during pretreatment of wheat straw at varying temperature and H_2SO_4 concentration.

3.4. Effect of activated charcoal on pretreated hydrolysate

Application of activated charcoal reduced furfural in the range of 72.32 to 84.01% and HMF in the range of 28.53 to 76.42% in the microwave assisted acid pretreated wheat straw hydrolysate (Tables 3 and 4). This reduction may be attributed due to the high capacity of activated charcoal to absorb furfural, HMF and other inhibitory compounds without affecting sugar levels in hydrolysate (Mussatto and Roberto, 2004; Chandel et al., 2007; Canilha et al., 2008;). Seo et al. (2009) observed that activated carbon treatment followed by hydrolysate preparation using 20 g of corn hull and 4% (v/v) H_2SO_4 lowering the phenolic compounds from 2015.2 to 153.3 mg/l, which shows 92.3% reduction in total phenolic compounds. Ali et al. (2012) also used activated carbon to minimize the level of inhibitors (by-products) and found the significant reduction in furfural, HMF, acetic acid, ethyl vanillin, and syringaldehyde.

3.5. Total sugar recovery after saccharification of pretreated wheat straw

In this study, for refining previous experimental finding, wheat straw was further pretreated at 180 °C with 1–4% sulfuric acid. Then the pretreated samples were washed and neutralized. These washed and neutralized pretreated samples were taken for subsequent use as the substrate for enzymatic saccharification according to the protocol described by NREL LAP-009 (Brown and Torget, 1996). Untreated wheat straw was used as a control. The soluble sugars obtained after enzymatic saccharification was significantly higher from the pretreated wheat straw at 2% (23.8%), followed by at 3% (19.32%) and it was minimum in control (Table 5). The result observed for total sugar recovery after acid pretreatment and subsequent enzymatic saccharification of wheat straw is shown in Table 5 and Fig. 3. Total sugar recovery was found significantly higher (43.12%), after saccharification in sample pretreated at 180 °C with 2% sulfuric acid (Table 5 and Fig. 3). Minimum sugar recovery was obtained in control (11.8%). The results exhibited in the investigation are in accordance with those reported by Sun et al. (2005). Delgenes et al. (1996) applied concentrated H_2SO_4 (72%, w/v) for the pretreatment of wheat straw at 30 °C for 30 min and obtained 11.1 g monomeric sugars in total from 18.8 g dry feed stalks accounting for 59% of theoretical yield. The enhancement in efficacy of saccharification was observed after pretreatment of wheat straw samples at 180 °C with 2% H_2SO_4 (diluted acid) resulted in significantly higher sugar recovery (43.12%), due to pretreatment ability to increase the removal of lignin from hemicelluloses and, consequently, decreased the degree of cellulose chain polymerization and improved enzymatic digestibility (Pu et al., 2013; Jaisamut et al., 2016). Jaisamut et al. (2016) estimated 311 kg glucose from one ton of dry wheat straw pretreated at 180 °C, and 155 kg of ethanol in the

Table 3
Effect of charcoal amendment on the furfural in the hydrolysate of pretreated wheat straw at 180 °C.

Particulars	Hot water	Dilute sulfuric acid conc.			
	Control	1%	2%	3%	4%
Furfural without charcoal amendment (ppm)	1.22 ± 0.12	27.30 ± 1.00	28.65 ± 1.34	31.56 ± 1.17	36.51 ± 0.79
Furfural after charcoal amendment (ppm)	0.34 ± 0.11	5.81 ± 1.06	5.12 ± 1.02	4.98 ± 0.79	5.83 ± 0.81
% Reduction in furfural	72.32 ± 1.48	78.71 ± 1.39	82.10 ± 1.08	84.22 ± 0.95	84.01 ± 1.40

Results are expressed as the mean (n = 3) ± standard deviations.

Table 4
Effect of charcoal amendment on the HMF in the hydrolysate of pretreated wheat straw at 180 °C.

Particulars	Hot water	Dilute sulfuric acid conc.			
	Control	1%	2%	3%	4%
HMF without charcoal amendment (ppm)	0.78 ± 0.04	16.33 ± 0.72	19.70 ± 1.20	24.32 ± 1.09	28.11 ± 0.86
HMF after charcoal amendment (ppm)	0.56 ± 0.10	4.11 ± 0.86	5.31 ± 0.72	5.86 ± 1.07	6.63 ± 1.28
% Reduction in HMF	28.53 ± 1.40	74.81 ± 0.82	73.04 ± 0.87	75.91 ± 0.98	76.42 ± 1.68

Results are expressed as the mean (n = 3) ± standard deviations.

Table 5
Total sugar recovery from pretreatment at 180 °C with varying concentration of H_2SO_4 and enzymatic saccharification of wheat straw.

Treatments	Total soluble sugars (%)		
	Pretreatment	Saccharification	Total recovery of sugars from pretreatment and saccharification
Hot water (Control)	5.40^{c}	6.40^{d}	11.80^{e}
1% H_2SO_4	14.93^{b}	16.42^{b}	31.35^{c}
2% H_2SO_4	19.32^{a}	23.80^{a}	43.12^{a}
3% H_2SO_4	15.78^{b}	19.32^{b}	35.10^{b}
4% H_2SO_4	14.48^{b}	12.90^{c}	27.38^{d}
LSD (p = 0.05)	1.95	2.97	3.25

Mean with the same letter (s) along the same column are not significantly different (p = 0.05).

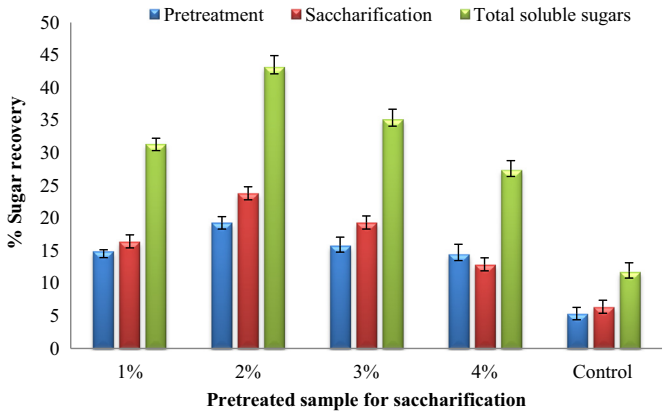

Fig. 3. Total sugar recovery from pretreatment at 180 °C with varying concentration of H_2SO_4 and enzymatic saccharification of wheat straw.

subsequent fermentation.

3.6. Effect of the activated charcoal amendment in acid hydrolysate on bio-ethanol production

A critical factor preventing industrial fermentation of dilute acid hydrolyzate is the inability of the fermentative yeast strains to resist intermediate inhibitory compounds produced during the pretreatment process, and usually, a detoxification step is required to increase fermentability of sugar to ethanol (Chandel et al., 2007; Panagiotou and Olsson, 2007; Huang et al., 2009). The yeast strain *Pichia stipitis* NCIM 3498 was used for ethanol production from detoxified with activated charcoal and non-detoxified microwave assisted acid pretreated wheat straw hydrolysate. The results showed positive effects on fermentation and higher ethanol yield (Table 6) by *Pichia stipitis* NCIM 3498 from detoxified wheat straw hydrolysate (5.29%v/v) as compared to non-detoxified hydrolysates (4.97%v/v). The maximum ethanol yield obtained from detoxified hydrolysate was 5.29% (v/v), which is equivalent to 87.9% of the theoretical yield. Suh et al. (2003) and Huang et al. (2009) also reported similar findings of the effect of these inhibitory compounds on ethanol fermentation by *Pichia stipitis* for fermentation efficiency as high as 84.7 to 90.7% per unit substrate consumption. The positive effects of activated charcoal on the removal of inhibitors have also been reported by many other researchers (Mussatto and Roberto, 2004; Canilha et al., 2008).

Ethanol yield from non-detoxified and detoxified microwave assisted acid pretreated wheat straw hydrolysate was 4.97 and 5.29% (v/v), respectively. However, the non-detoxified wheat straw hydrolysates had comparatively lower ethanol yield than the detoxified samples (Table 6). This detrimental effect on ethanol yield may be due to inhibitors, and amount of furfural and HMF present in non-detoxified wheat straw hydrolysates (Díaz et al., 2009; Carolina et al., 2011). The ethanol yield coefficient (Yps) was found to be 0.44 g ethanol g-1 sugar utilized. Nigam (2001) also reported ethanol yield, i.e., 0.41 ± 0.01 g/

Table 6
Ethanol yield from charcoal amended and without amended acid hydrolysate obtained after pre-treatment at 180°C with 2% H_2SO_4 concentration and enzymatic saccharification.

Treatments	Ethanol yield (%v/v)	
	Detoxified (Amended with activated charcoal)	Non-detoxified (without amendment of activated charcoal)
Hot water (Control)	1.66^{c}	1.54^{c}
1% H_2SO_4	3.62^{b}	3.41^{ab}
2% H_2SO_4	5.29^{a}	4.97^{a}
3% H_2SO_4	4.18^{ab}	3.94^{ab}
4% H_2SO_4	3.18^{b}	2.89^{bc}
LSD (p = 0.05)	1.46	1.72

Mean with the same letter (s) along the same column are not significantly different (p = 0.05).

g equivalent to 80.4 ± 0.55% theoretical yield from the wheat straw hydrolysate by *P. stipitis* NRRL Y-7124. Koti et al. (2016) also observed higher ethanol concentration from wheat straw dilute acid pretreated hydrolysate, i.e., 9.61 ± 0.39 g/l equivalent to the yield 0.33 ± 0.008 g/g and fermentation efficiency of 64.53 ± 0.248% by mutating the strain of *Pichia stipitis*.

Some other studies were also carried out by using wheat straw hydrolyzates as a substrate for ethanol production by using ethanologenic yeasts strains *Kluyveromyces marxianus* (Tomas-Pejo et al., 2009) and recombinant strains of *S. cerevisiae* (Panagiotou and Olsson, 2007). These studies were also indicated the efficient utilization of fermentable sugar and a higher ethanol yield coefficient. In this study, ethanol yield obtained at optimized pretreatment condition with detoxified wheat straw hydrolysate equivalent to 87.9% of the theoretical yield could be economically viable ethanol production process by utilizing the wheat straw. This study will also help in reducing the environmental pollution by avoiding the unhealthy biomass disposal systems.

As India is suffering from severe air pollution problem due to biomass burning and lack of efficient management of excess wheat straw and other crop residues on the farm (Sharma and Dikshit, 2016; Kumar et al., 2016). In the Indian context, the investigation will help to manage the crop residues not only on the farm but also reduce the biomass burning and remediate air pollution problems. The efficient pretreatment of biomass to maximize fermentable sugar production, removal of inhibitory compounds from hydrolysates and use of native and recombinant microbial strains is the key to higher ethanol productivity. The application of this novel technology will provide enhancement of bio-ethanol production potential of wheat straw and also create the sustainable development of the ethanol production process, which can utilize lignocellulosic biomass efficiently.

4. Conclusions

This study examined and point out the fact that wheat-straw is a potential feedstock for ethanol production. The combination of temperature and acid pretreatment conditions were optimized as 180 °C with 2% sulfuric acid and subsequently saccharified wheat straw hydrolysate for maximum sugar recovery. With the increase in pretreatment temperature and sulfuric acid concentration, production of furfural and HMF also increases. The amendment of pretreated hydrolysate with activated charcoal significantly reduces the amount of furfural and HMF production, resulted in the maximum ethanol yield 5.29% (v/v), equivalent to 87.9% of the theoretical yield. The ethanol yield obtained in this study could be sufficient to produce ethanol at a breakeven price, which will make the process economically viable.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biteb.2018.09.007.

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