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Experimental study on the performance of microwave assisted Hydrogen peroxide (H_2O_2) pretreatment of rice straw

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Abstract: Biofuels are essentials as they can provide impending substitute for fossil fuels. Rice straw has gained much attention from researchers because of its usability as a potential feedstock for bioethanol production. Pre-treatments are crucial for enzymatic hydrolysis of rice straw. In this study, combination of microwave and hydrogen peroxide (H_2O_2) is employed for the delignification of rice straw. The Response surface methodology is used to optimize the pretreatment conditions with respect to H_2O_2 concentration, microwave power and irradiation time. Under optimum conditions, maximum reducing sugar obtained through microwave assisted H_2O_2 is 1,453.64 μg/mL. The chemical and morphological analysis ascertained that the surface of the samples treated with microwave assisted H_2O_2 was more ruptured and has a significantly high crystalline index (63.64%) than untreated rice straw sample (52.2%). Microwave assisted H_2O_2 pre-treatmentdisrupted the silicon waxy structure and broken down allether linkages between lignin and carbohydrates and thus, efficiently remove lignin. The present study proves that microwave assisted H_2O_2 is an effective pretreatment technique for the conversion of rice straw into bioethanol production by enhancing enzymatic saccharification.

Keywords: pre-treatment, enzymatic hydrolysis, rice straw, biofuel, microwave, hydrogen peroxide

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1 Introduction

Continued use of fossil fuel resources causes depletion of resources and global warming. The promising sources of energy which can solvethe crisis of energy scarcity andclimate change are “biofuels”. Biofuels are of several types such as bioethanol, biodiesel, bio-hydrogen etc. Ethanol has gained much attention as an alternative fuel or oxygenate additive for fossil fuels. Production of bioethanol not only reduces the energy dependency on fossil fuels but also prevents environmental pollution. Ligno-cellulosicfeedstocks are abundant, cheap and renewable and therefore, can be used for ethanol production through microbial intervention (Zhao et al., 2007). However, there effective utilization requires separation of polymeric components i.e. cellulose, hemicellulose and lignin (Sun and Cheng, 2002). Cereal straws are available in large quantities that can provide potential substrate as a starting constituent for biofuel production (Sun et al., 2010). Rice straw is one of the most abundant ligno-cellulosic crops that can be converted into fermentable sugars, ethanol and other useful products (Jeya et al., 2009; Zhu et al., 2005a; Lo et al., 2010). It is one of the major crops of India and constitutes 23% straw of its total weight (Gadde et al., 2009). Major components of rice straw are: cellulose, hemicellulose, lignin, silicon and ash content. Among all the components, cellulose is present in larger quantity which can be hydrolyzed into glucose by cellulase enzyme. Among different methods of hydrolysis of ligno-cellulose, enzymatic hydrolysis is the

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most common method because of its mild reaction conditions, lack of corrosion and positive environmental effects (Gong et al., 2010). The presence of lignin and complex structure of polysaccharides have made the rice straw hydrolysis very complicated. Thus, pretreatments are necessary for increasing the digestibility of polysaccharides and removal of lignin (Jorgensen et al., 2007). It reduces cellulosecrystallinityand increases the porosity of the materials (Karimi et al., 2006). Various pretreatment methods including steam explosion, liquid hot water, dilute acid, flow through acid pretreatment, lime pretreatment, wet oxidation, ammonia fiber/freeze explosion, milling and grinding, microwave energy and high energy radiation etc. have been developed for this purpose (Liu et al., 2005 and Fan et al.,1982).

Microwave heats the target object directly by applying an electromagnetic field to dielectric molecules as compared to conduction/convection heating which is based on intra-molecular heat transfer (Newnham et al., 1991). Many researchers explored microwave pretreatment technology as a potential method for the pretreatment of various ligno-cellulosic materials (Eskicioglu et al., 2007(a,)(b); Taherzadeh and Karimi, 2008; Alvira et al., 2010; Shi et al., 2011; Jackowiak et al., 2011) as well as to damage the recalcitrant lignin (Hu and Wen, 2008). It can degrade lignin and hemicelluloses as well as increase enzymatic susceptibility (Zhu et al., 2005b). Several researchers have been reported that enzymatic hydrolysis (Azuma et al., 1984; Ooshima et al., 1984; Kitchaiya et al., 2003) of rice straw can significantly be increased by microwave pretreatment. A pretreatment process can be improved by the combination of microwave irradiation and chemical pretreatment technique.

In the current study, microwave assisted hydrogen peroxide (H_2O_2) pretreatment is employed for enzymatic hydrolysis of rice straw. Statistical analysis is performed using Response Surface methodology (RSM). It is a collection of mathematical and statistical techniques for empirical model building. By careful design of experiments, the objective is to optimize a response (output variable) which is influenced by several independent variables (input variables). For optimization, the user required to supply minimum and maximum values for each factor (Ferreira et al., 2007). Since, for rice straw peroxide pretreatment along with microwave treatment is not yet explored. In this work,a systematic study of microwave assisted H_2O_2 was carried out by optimizing the pretreatment conditions. For optimization, a Box- Behnkenfactorial design (BBD) was selected. The design would help in investigating the effects of power (W), treatment time (min) and concentration of chemicals (%) on reducing sugar yield. Further, the morphological characteristics of rice straw are analyzed through scanning electron microscope (SEM) and biomass crystallinity through X-ray diffraction (XRD). The goal of this study is to optimize an efficient, microwave pretreatment technology for the hydrolysis of rice straw which in turn enhances ethanol production.

2 Material and methods

2.1 Raw materials and microwave-peroxide pretreatment

In the present research work Indian straw from rice variety “PUSA SUGANDH” has been used. The samples of rice straw were locally harvested at Indian Agriculture Research Institute. Firstly, rice straw has been cut into pieces of size 1-2 cm. Now, the prepared samples of rice straw are cleaned thoroughly using tap water until the washings became clean and colourless. Before any pretreatment, samples have been air dried. The chemical composition of rice straw is given in Table 1. Microwave pretreatment is one of efficient way and a modified type domestic microwave oven is used in the present study. The microwave power is varied between 70 W to 700 W respectively. About 5 g of rice straw was suspended in 30 mL of H_2O_2 concentration ranged from 0.1% to 2% (v/v) and left for overnight as per RSM fitted design. It was then radiated at in the range of 70-700 W for 1-5 min in microwave. All the pretreatment

Table 1 Chemical composition of Rice straw variety (PusaSugandh) (Manjunath et al., 2008)

S. No	Characteristics of rice straw	Values/%
1	Cellulose	39.04 ± 0.50
2	Hemi-cellulose	21.64 ± 0.50
3	Lignin	16.2 ± 0.30
4	Ash	18 ± 1.1

conditions i.e. power 70-700 W, concentration of chemicals 0.1 to 2% (v/v) and treatment time 1 to 5 min is designed by Response Surface Model (RSM), Design Expert software Version 7.

2.2 Enzymatic saccharification of pretreated rice straw

Saccharification or hydrolysis of the wet pretreated paddy straw samples is carried out using E-CELAN, endo-1, 4-β-glucanase from *Aspergillusniger* supplemented with EBGLUC (endo-β-glucosidase), β-glucosidase from *Aspergillusniger*(Megazyme International and Genecor) (Saritha et al., 2012). All other chemicals employed in this study are of reagent grade. Enzyme saccharification is carried out in 50 mL screw capped bottles, which consisted of 1.0 g microwave treated rice straw, 10 units of E-CELAN and 5 units of EBGLUC. The final volume of reaction mixture has been made using 10 mL of citrate buffer (pH 4.8). Bottles are kept at 50ºC and 150 r min^{-1} in a constant temperature shaker water bath. Samples has been collected from reaction mixture at different time intervals and analyzed for sugar by DNSA method (Miller, 1959). All the experiments have been performed in triplicate and the average values are reported.

2.3 Morphological characterization through scanning electron microscope (SEM)

In this study, the morphology of rice straw is examined through scanning electron microscope (ZEISS, Evoma-10). Firstly, samples are dried in a vacuum dryer oven at 45ºC for 24 h and then gradually dehydrated using acetone-water mixtures. Same process is being repeated with 50%-100% acetone. The samples have been mounted on aluminium stubs and coated with gold and platinum mixtures prior to imaging under SEM.

2.4 X-ray diffraction (XRD) of the pretreated raw materials

Crystallinity of untreated and pretreated rice straw samples has been determined using X-ray diffraction (PW 1710, copper Kα radiation). Rice straw treated with water-microwave served as a control. Crystallinity index is calculated by using following Equation (1) (Segal et al., 1959);

$$CrI = I002 - IamI002 \times 100 \quad (1)$$

where, $I002$ is intensity for the crystalline part of the biomass (i.e. cellulose) and Iam is intensity for the amorphous part of the biomass (i.e. cellulose, hemi-cellulose and lignin). In this research work, intensity of crystalline portion was at 2θ = 22.4 and intensity for amorphous portion was at 2θ = 10.1.

For the estimation of comprising crystalline area in plant ($d002$) Equation (2) is used to calculate crystalline size of (002) plane based on Scherrer equation (Gumuskaya and Usta, 2002).

$$d002 = 0.9\lambda\beta \mathrm{Cos}\theta \quad (2)$$

where, λ is wavelength of X-ray tube (λ =1.5406 Å); β is FWHM (full width at half maximum) of (002) peak; θ is diffraction angle of (002) plane.

2.5 Removal and recovery of lignin

The extent of lignin removal is mainly determined on the basis of lignin fragments and monomers present in the alkali extract according to the NREL LAP-004. The absorbance is measured at 205 nm through spectrophotometer (Ehrman, 1996). Through acidification, value added acid-precipitable polymeric lignins are recuperated from the extracts (Pometto and Crawford, 1986). In the next step extract is acidified to pH 1-2 with concentrated sulphuric acid. Centrifugation process took 30 min at 13,000 rpm. The precipitates are washed with distilled water and dried at 60ºC till the constant weight has been achieved.

2.6 Experimental designs and data analysis

Design Expert software Ver. 7 naming Box- Behnken factorial design (BBD) is used with three factors and three levels, including three replicated at centre point to evaluate the effect of concentration of chemicals (A), power (B) and treatment time (C) on hydrolysis of rice straw (Y) obtained from the pretreatment experiments. The range of variables for $H_2O_2$is power 70-700 W, concentration of chemicals 0.1% to 2% (v/v) and treatment time 1 min to 5 min (Table 2 and Table 3). The design matrix with 17 experimental runs in one block with five replicates. The Box- Behnken factorial design (BBD) matrix is shown in Table 3. A polynomial quadratic equation was fitted to evaluate the effect of each independent variable to the response:

$$Y = \beta o + \beta 1A + \beta 2B + \beta 3C + \beta 11A2 + \beta 22B2 + \beta 33C2 + \beta 12AB + \beta 13AC + \beta 23BC$$

where, *Y* is the predicted response; βo is a constant; $\beta 1$, $\beta 2$, $\beta 3$ are the linear coefficients; $\beta 12$, $\beta 22$, $\beta 13$ are the cross-coefficients; $\beta 11$, $\beta 22$, $\beta 33$ are the quadratic coefficients. The response surfaces of the variables inside the experimental domain were analyzed using Design Expert. Subsequently, five additional confirmation experiments were conducted to verify the validity of the statistical experimental strategies.

Table 2 Independent variables tested in the Box-Behnken design

Independent variables	Code	Actual factor level at coded factor levels		
		- 1	0	+ 1
H_2O_2 Concentration/%	A	0.1	1.05	2
Power/W	B	70	385	700
Time/min	C	1	3	5

Table 3 Box-Behnken design matrix

S. No.	H_2O_2 Concentration /%	Power /W	Time /min	Reducing sugar (Y)/μg mL^{-1}	
				Predicted	Measured
1	0.10	70	3	1438.12	1440.63
2	2.00	70	3	1436.38	1453.64
3	0.10	700	3	1440.38	1445.56
4	2.00	700	3	1446.73	1449.43
5	0.10	385	1	1435.87	1433.14
6	2.00	385	1	1400.24	1403.01
7	0.10	385	5	1369.52	1377.34
8	2.00	385	5	1362.35	1371.01
9	1.05	70	1	1369.75	1372.77
10	1.05	700	1	1338.29	1344.48
11	1.05	70	5	1357.63	1353.78
12	1.05	700	5	1348.33	1347.45
13	1.05	385	3	1351.22	1354.48
14	1.05	385	3	1341.52	1346.66
15	1.05	385	3	1344.22	1345.99
16	1.05	385	3	1354.11	1352.02
17	1.05	385	3	1347.28	1349.45

3 Results and discussion

3.1 Response surface methodology (RSM) results

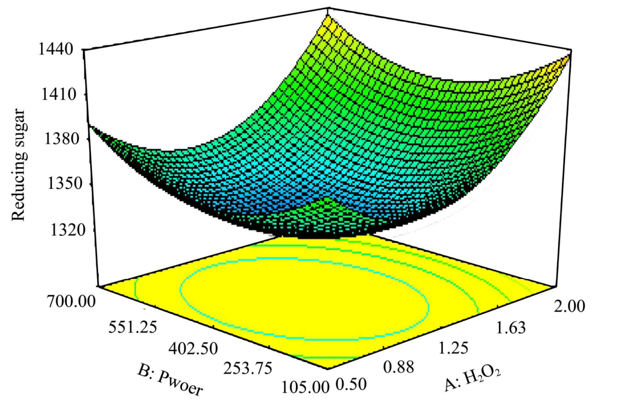
For optimization of microwave effect and other factors on saccharification of rice straw, experiments based on BBD model are employed. Design expert software is used for data analysis, analysis of variance (ANOVA), regression coefficients and regression equations. ANOVA model represents that model is significant for H_2O_2 at Fisher's F-test value 8.54 (Table 4). The coefficient of variation (R^2) for H_2O_2 has been found 0.92. The model appears statistically sound as the lack of fit test used for testing of model shows p value of 0.2893 and it is not significant. The most significant parameter for H_2O_2 is quadratic termsH_2O_2 concentration (A^2) and power (B^2). Analysis of residuals showed no abnormality. The 3D response surface plots for different conditions are shown in Figure 1. To depict the interactive effect of independent variables on responses, one variable should be kept constant while the other two variables were varied at different ranges. The interaction between different factors has been shown through the shape of response surfaces.

3.2 Optimum conditions

Same Design expert software is used for deciding optimum conditions (Table 5). The reducing sugar obtained through H_2O_2-microwave pretreatment under optimum condition is 1,453.64 μg mL^{-1}. The reducing sugar concentration in the saccharified rice straw under H_2O_2-microwave pretreatment was increased 2005% (Table 6) as compared to raw straw.

Table 4 ANOVA of the quadratic model $H_2O_2$and its influential factors

Source	Sum of squares	Degree of freedom	Mean square	F- value	P-value	
Microwave and H_2O_2 treatment						
Model	38409.55	9	4267.73	8.54	0.0049	Significant
Quadratic terms H_2O_2 concentration (A^2)	28075.65	1	28075.65	56.20	0.0001	
Power (B^2)	5203.49	1	5203.49	10.42	0.0145	
Residual	3496.80	7	499.54			
Lack of fit	2001.10	3	667.03	1.78	0.2893	Not Significant



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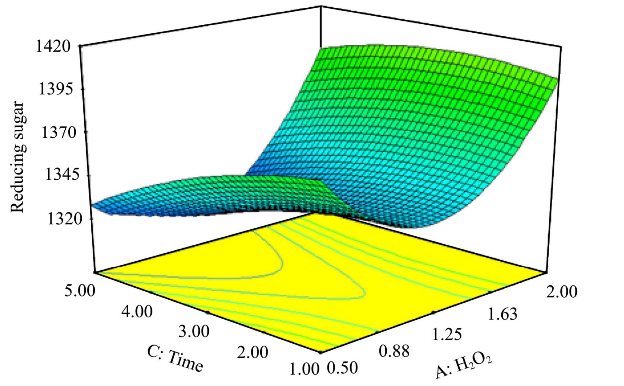

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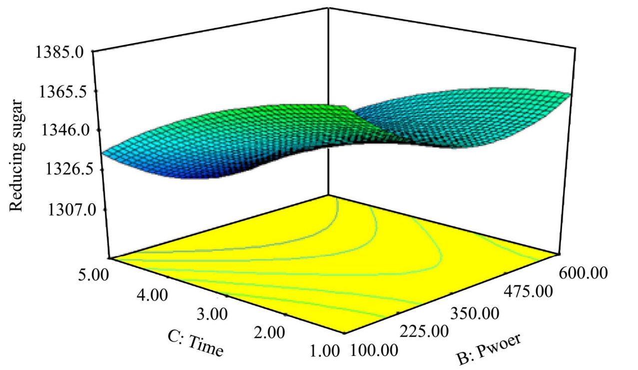

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Figure 1 Three dimensional response surface graphs between (a) power (microwave) and H_2O_2 concentration (b) time and H_2O_2 concentration and (c) time and power show the effect on reducing sugar

Table 5 Optimum conditions for delignification of rice straw

Pretreatment methods	Chemicals concentration/%	Power/W	Time/min
H_2O_2-microwave pretreatment	2	100	3

Table 6 Predicted and experimental reducing sugar obtained under optimum conditions

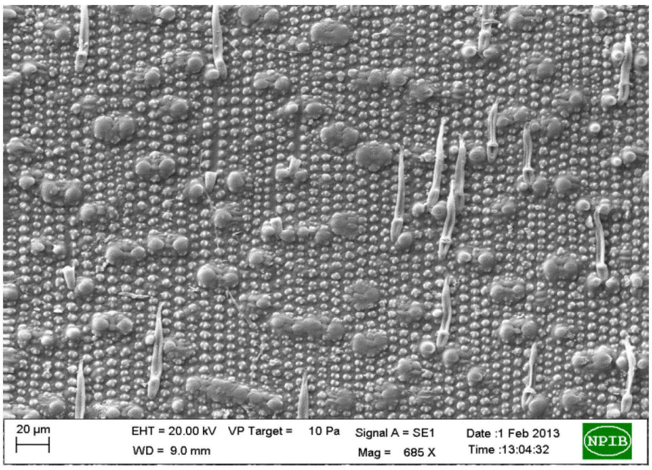
Pretreatment in combination with microwave	Control [a] /μg mL^{-1}	Predicted /μg mL^{-1}	Measured[b] /μg mL^{-1}	Increasing rate /%
H_2O_2	69	1436	1453 ± 14.3	2005

Note: [a] Rice straw used in control was untreated.

[b]Mean ± standard deviation of five replicates.

3.3 Scanning electron microscope (SEM) analysis

The morphological changes occurred due to the pretreatment could be analyzed with the help of scanning electron microscope (SEM) (Namasivayam and Kavitha, 2006). SEM images of the untreated sample showed that there is less number of cracks and the surfaces of the samples are densely packed as compared to the microwave assisted H_2O_2 pretreated sample (Figure 2). The silicon waxy structure, lignin-hemicellulose complex of rice straw is broken down significantly. Previous studies have also shown that the surface of the samples treated with microwave assisted organic acid become loose and irregular (Gong et al., 2010). Also, microwave assisted $FeCl_3$ damage the cell wall structure and altered the fibrillar structure of rice straw (Lu and Zhou, 2011). It proves that microwave pretreatment has effectively improved the straw digestibility by removing silica content (Rezanka and Sigler, 2008).



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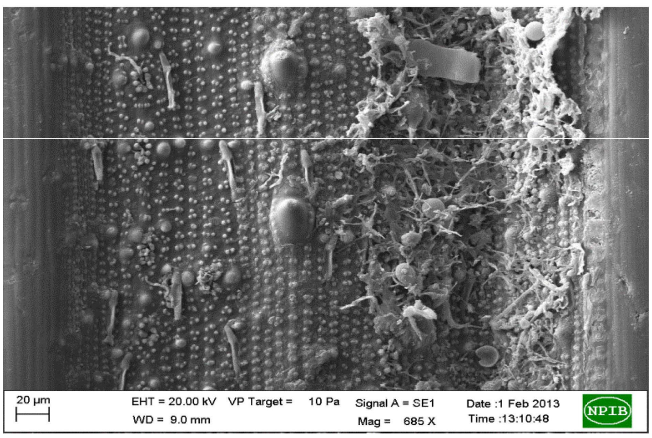

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Figure 2 SEM images of (a) untreated sample (b) sample pretreated with microwave assisted Hydrogen peroxide (H_2O_2)

3.4 Effect on chemical composition of rice straw

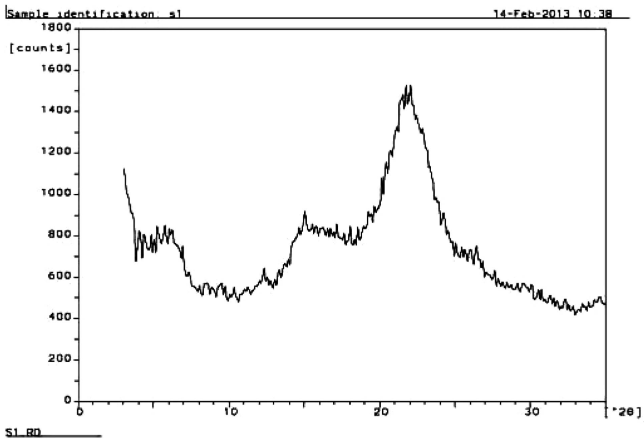
Chemical components of rice straw changed after pretreatment with microwave assisted H_2O_2 (Table 7). There is an increase in percentage of cellulose contents in treated rice straw samples with comparison to untreated. However, other components e.g. lignin, hemi-cellulose and ash have been reduced significantly. This indicates that the pretreatment method is capable of removing lignin and other components. It damaged the cell wall by disrupting the lignin structure. It led to increase in the surface area and thereby better enzymatic accessibility. All these conditions are greatly beneficial for enzymatic hydrolysis.

Table 7 Chemicalcomposition of rice straw after pretreatment

S.No	Characteristics of rice straw	H_2O_2-microwave pretreatment/%
1	Cellulose	45.3 ± 1.5
2	Hemi-cellulose	15.25± 1.9
3	Lignin	3.2 ± 0.4
4	Ash	13.2 ± 0.1

3.5 X-ray Diffraction (XRD) analysis

Crystallinity index is the percentage of crystalline material in the biomass (Segal et al., 1959). It is a major factor that affected enzymatic hydrolysis (Kim and Lee, 2005; O' Dwyer et al., 2007). XRD analysis (Figure 3) showed that the crystallinity index of rice straw treated with microwave assisted H_2O_2 is high as compared to the untreated and blank sample. For untreated and blank (without addition of any chemicals) sample, it is 52.2% and 49.07% respectively as listed in Table 8. By disrupting inter and intra chain hydrogen bonding of cellulose fibrils pre-treatments can change the cellulose structure (Mosier et al., 2005). In biomass, Hemi-cellulose and lignin are amorphous in nature, while cellulose is crystalline (Jeoh et al., 2007). The results demonstrated that the removal of amorphous part of the rice straw i.e. lignin, hemi-cellulose was more in sample treated with microwave assisted H_2O_2 than untreated and blank. This increase in value showed that the pretreatment has potentially removed the amorphous components i.e. lignin, hemi-cellulose and increased the crystalline component i.e. cellulose in the rice straw. It is being found that the cellulose content has been increased but, only in small amount, whereas imperfect microcrystalline cellulose has been hydrolyzed and large perfect cellulose was left. Previous research has also suggested that the crystallinity index of rice straw could enhance by hot acid treatment (Yu et al., 2009). Several studies showed increase in crystalline index value after biomass pretreatment (Chang and Holtzapple, 2000; Kim and Holtzapple, 2006; Bak et al., 2009; Liu et al., 2009).



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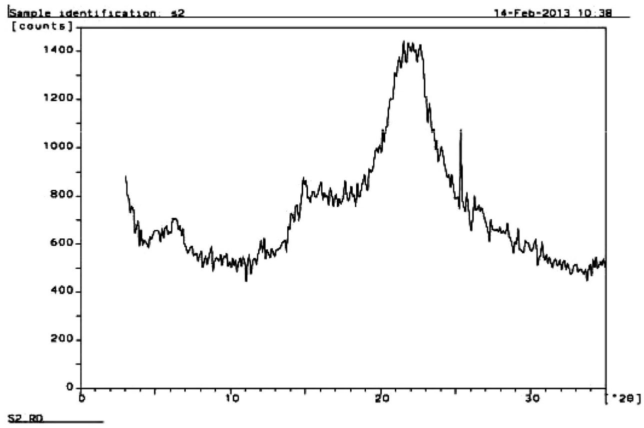

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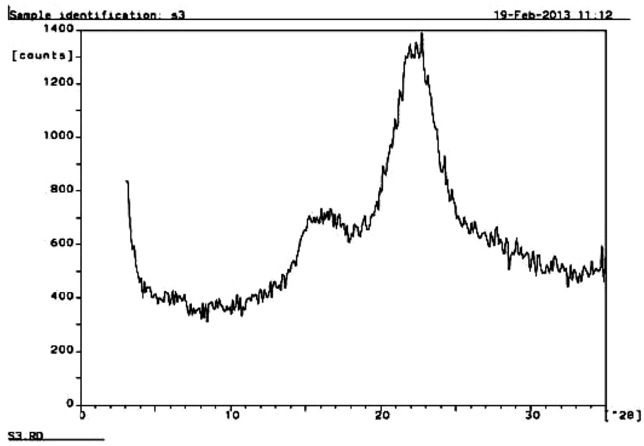

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Figure 3 X- ray diffraction pattern of (a) untreated sample (b) blank (c) microwave assisted H_2O_2 pretreated sample

Table 8 Crystallinity index of rice straw samples

S. No.	Treatments	Crystalline Index (CrI)/%
1	Untreated rice straw	52.2
2	Blank (without addition of any chemicals)	49.07
3	H_2O_2- microwave pretreatment	63.64

Scanning electron microscope (SEM) analysis, changes on chemical composition of rice straw and X-ray Diffraction (XRD) analysis used in the present study proved that microwave assisted hydrogen peroxide pretreatment method had the potential of exposing cellulose and increasing cellulose contents. The study also proves that huge availability of rice straw in Indian livestock has tremendous potential for ethanol conversion using microwave-chemical pretreatment methods and technology is working well for them in Indian conditions and varieties of rice straw.

4 Conclusions

The current research work substantiatesthat microwave is an efficient heating method for the pretreatment of rice straw. Microwave assisted H_2O_2 enhances the saccharification of rice straw by removing lignin and hemicelluloses in large quantity which in turn increases enzymatic accessibility. The optimal conditions have been deducted by using Box- Behnken design (BBD). Maximum reducing sugar was obtained through microwave assisted H_2O_2 pretreatment (1,453.64 μg mL^{-1}) using 2% H_2O_2 (v/v) at 100 W for 3 min. Analysis of chemical composition of rice straw, the images obtained through scanning electron microscope (SEM) and X-ray diffraction (XRD) analysis revealed the eradication of lignin and hemi-cellulose, although lignin has not been recovered significantly. SEM images certified that the surface is more ruptured and damaged in microwave assisted H_2O_2 pretreatment than blank sample. Moreover, crystallinity index for rice straw samples treated with microwave assisted H_2O_2 is significantly high 63.64 % in comparison to untreated sample 52.2%. The removal of lignin and hemi-cellulose enhances the enzymatic hydrolysis with microwave treatment and thus, the enzymatic saccharification of rice straw can be assisted with microwave efficiently.

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