



Microwave Assisted Chemical Pretreatment Method for Bio-ethanol Production from Rice Straw

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Continuous depletion in fossil fuel reserves and their contribution towards greenhouse gas emissions compelled the scientist to explore renewable sources of energy. Abundance of rice straw and its poor utilization is one major research question addressed through the present research work. The microwave assisted chemical treatment for Indian rice straw for bio-ethanol production has not been investigated so far and present study has provided insight in to the area of research. In the present research work, feasibility of microwave assisted alkali, acid and peroxide pretreatment has been investigated for rice straw. Mainly three chemicals NaOH, H₂SO₄ and H₂O₂ have been used. It has been found that the combination of microwave pretreatment with H₂O₂, H₂SO₄ and NaOH enhances the saccharification of rice straw, respectively by removing lignin and hemicelluloses in large quantity. Maximum reducing sugar is found through H₂O₂-microwave pretreatment (1453.64 µg/mL). SEM images also confirmed that the surface of the samples treated with microwave assisted H₂O₂ were more ruptured than H₂SO₄ and NaOH. It becomes quite evident from experimental analysis that the enzymatic saccharification of rice straw can be assisted with microwave-chemical pretreatment.

Keywords: Enzymatic digestibility, Rice straw, Microwave, Chemical pretreatment.

INTRODUCTION

In recent decades, due to population explosion and industrialization, demand of energy has increased manifolds. The consumption of global petroleum is increased from 57×10^6 barrels/day in 1973 to 82×10^6 barrels/day by 2004 and further increase of 50 % is predicted by 2025 [1]. Fossil fuel based energy resources are the main greenhouse gas emitters and the increase in amount of CO₂ in the atmosphere leads to the global warming which in turn causes climate change. Due to fossil fuels burning, the atmospheric CO₂ levels rises from about 275 to 380 ppm [1]. Increasing concerns for climate change and greenhouse gas (GHG) emissions made scientific community inclined towards sustainable and renewable energy research. Ethanol from renewable resources has been of great interest as an alternative fuel or oxygenate additive for fossil fuels. Worldwide production capacity of ethanol in 2005 and 2006 were about 45 and 49 billion litres, respectively and the total projected demand in 2015 is over 115 billion litres [2]. Ligno-cellulosic materials are abundance, cheap and renewable. They may be used as a substrate for ethanol production

through microbial intervention [3]. Among all the lingo-cellulosic biomass residues, cereal straws are the most abundant resource which can serve as a potential feedstock for the production of bio-fuel [4]. India is an agriculture based country producing large quantity of crop residues and wastes that can be utilized for ethanol production [5]. Rice is one of the major crops of India and contains 23 % straw of its total weight. Most of the time rice straw is either left in the field or burnt before the next crop which leads to the air pollution. In India, the open-field burning of rice straw contributes up to 0.05 % of total greenhouse gas emissions [6]. This makes rice straw as a viable and potential resource for bioethanol production.

Rice straw mainly consists of cellulose, hemi-cellulose, lignin, silica and ash. Among all the components, cellulose is present in larger quantity which can be hydrolysed into glucose by cellulose enzyme. Among different methods of hydrolysis of ligno-cellulose, enzymatic hydrolysis is the most common used method because of its mild reaction conditions, lack of corrosion and positive environmental effects [7]. This hydrolysis can be affected by porosity (accessible surface area) of ligno-cellulosic biomass, cellulose fibre crystallinity, lignin and

hemicellulose content. Conversion of rice straw to fermentable sugar is a complicated process due to the presence of complex structure of lignin and hemicelluloses with cellulose [8,9]. The production of ethanol from rice straw requires four major steps *viz.*, pretreatment, hydrolysis, fermentation and distillation [2]. A pretreatment process is necessary in order to remove lignin and hemicellulose to reduce cellulose crystallinity and to increase the porosity of the materials [10]. Different types of pretreatment methods such as steam explosion, liquid hot water, acid pretreatment, lime, wet oxidation and ammonia fibre/freeze explosion [11] are used for this purpose. Also, some quite frequently used physical pretreatment processes are milling and grinding, microwave energy, steam explosion, wet oxidation and high energy radiation *etc.* [12].

Microwave irradiation has high heating efficiency and can be operated easily. It can degrade lignin and hemicelluloses as well as increases enzymatic susceptibility [13]. Enzymatic hydrolysis [14-16] of rice straw can significantly be increased by microwave pretreatment. 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 [17]. The technology of microwave pretreatment has been explored by many researchers as a potential method for pretreatment of various ligno-cellulosic materials [18-24] as well as to damage the recalcitrant lignin [25]. Microwave pretreatment can effectively enhance the sugar yield [26]. Microwave treatment can enhance pretreatment process if it is followed by induction of chemicals into ligno-cellulosic materials. Microwave-alkali pretreatment is an effective process for the improvement of enzymatic hydrolysis and has been used for many other ligno-cellulosic materials. However, the research on using microwave-chemical pretreatment method for rice straw has not been done extensively. Microwave-chemical pretreatment is beneficial because of many reasons *e.g.*, (i) the microwave irradiation could enhance the lignin degradation and (ii) presence of aqueous NaOH increases soapnification of inter-molecular ester bonds cross-linking hemi-cellulose and lignin [27].

In present research work, microwave treatment is combined with different concentration of alkali *i.e.* sodium hydroxide, sulphuric acid and hydrogen peroxide for enzymatic hydrolysis. Response surface methodology (RSM) is used for statistical analysis. Response surface methodology 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). During the optimization procedure, the user is required to supply minimum and maximum values for each factor [28]. Since acid and alkali pretreatment is not yet explored along with microwave treatment. In this work for systematic study of effectiveness of microwave with combination of other chemicals on the hydrolysis of rice straw, a Box-Behnken design was selected for the optimization of pretreatment conditions. The design would help in investigating the effects of power (W), treatment time (min) and concentration of chemicals (%) on 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 overall goal of this study is to optimize an efficient, microwave pretreatment technology for the hydrolysis of rice straw for ethanol production.

EXPERIMENTAL

Raw materials and microwave-alkali/acid/peroxide pretreatment: In present work, rice variety “PUSA SUGANDH” has been used and the samples of rice straw were locally harvested at Indian Agriculture Research Institute (Table-1). 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 every pretreatment, sample has been air dried. The chemical composition of rice straw is given in Table-1. For the microwave pretreatment modified type domestic microwave oven is used. Detailed schematic of the procedure adopted for chemical pretreatment is given in Fig. 1.

TABLE-1
CHEMICAL COMPOSITION OF RICE STRAW
VARIETY (PUSA SUGANDH) [Ref. 29]

Characteristics of rice straw	Values (%)
Cellulose	39.04 ± 0.5
Hemi-cellulose	21.64 ± 0.50
Lignin	16.2 ± 0.3
Ash	18 ± 1.1

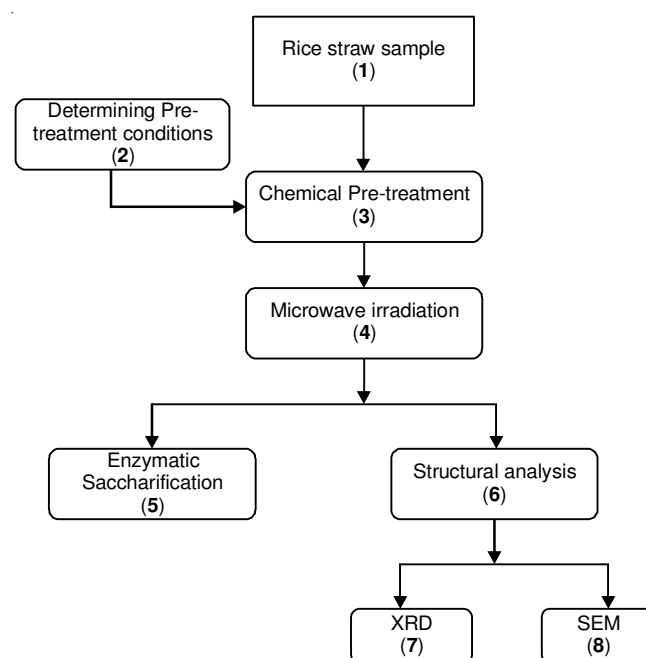


Fig. 1. Procedural framework for the microwave-chemical pretreatment of rice straw; (1) Variety pusa sugandh: Composition: Cellulose 39.04 ± 0.50 %; Hemicellulose 21.64 ± 0.50 %; Lignin 16.2 ± 0.30 %; Ash 18 ± 1.1 %; (2) Pretreatment conditions (power, conc. and treatment time) designed by response surface model (RSM), Design Expert V7; (3) Three chemicals NaOH, H₂SO₄, H₂O₂; Sample soaked overnight in 30 mL of chemical (conc. 0.1-2 %); (4) Microwave irradiation in the range of 70-700 W for 1-5 min; (5) Enzymatic saccharification, 10 units of E-CLEAN + 5 units of EBUGLUC + Volume make up to 10 mL using citrate buffer (pH 4.8), saccharification bottles at 50 °C and 150 rpm in a constant temperature shaker water bath. Analysis of reducing sugar by DNSA method; (6) Two major techniques used to analyze the change in structure of the rice straw; (7) X-ray diffraction (XRD) analysis; (8) Scanning electron microscope (SEM) analysis

The microwave power is varied between 70 to 700 W, respectively. Three microwave power set point of 70, 385 and 700 W are used for 1-5 min duration for pretreatment of samples. About 5 g of rice straw was suspended in 30 mL of NaOH, H₂SO₄, H₂O₂ (concentration 0.1 to 2 %) and left for overnight as per RSM fitted design. The pretreatment conditions are designed by response surface model using Design Expert 7 and further same programme is used for data analysis.

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 *Aspergillus niger* supplemented with EBGLUC (endo- β -glucosidase), β -glucosidase from *Aspergillus niger* (Megazyme International and Genecor) [30]. All other chemicals employed in this study are of reagent grade. Enzymatic saccharification occurred in a 50 mL screw capped bottles which consisted of 1 g microwave treated rice straw, 10 units of E-CELAN and 5 units of EBGLUC. By using 10 mL of citrate buffer (pH 4.8), the final volume of reaction mixture has been made up. Bottles are kept at 50 °C and 150 rpm 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 [31]. All the experiments have been performed in triplicate and the average values are reported.

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 45 °C 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.

X-ray diffraction of pretreated raw materials: Crystallinity of untreated and pretreated rice straw samples has been determined using X-ray diffraction (PW 1710, copper K α radiation). Rice straw treated with water-microwave served as a control. Crystallinity index is calculated by using following formula [32];

$$C_r I = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad (1)$$

where; I_{002} is intensity for the crystalline part of the biomass (*i.e.* cellulose) and I_{am} 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 $2\theta = 22.4$ and intensity for amorphous portion was at $2\theta = 10.1$.

For the estimation of comprising crystalline area in plant (d002) eqn. 2 is used to calculate crystalline size of (002) plane based on Scherrer equation [33].

$$d002 = \frac{0.9\lambda}{\beta \cos \theta} \quad (2)$$

where λ is wavelength of X-ray tube ($\lambda = 1.5406 \text{ \AA}$), β is FWHM (full width at half maximum) of (002) peak, θ is diffraction angle of (002) plane.

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 [34]. Through acidification, value added acid-precipitable polymeric lignins are recuperated from the extracts [35]. In the next step extract is acidified to pH 1-2 with concentrated sulphuric acid. Centrifugation process took 0.5 h at 13000 rpm. The precipitates are washed with distilled water and dried at 60 °C till the constant weight has been achieved.

Experimental designs and data analysis: Indoor test facilities are used for the experimental study of various chemical pretreatment of rice straw.

Box-Behnken factorial design (BBD) is used in Design Expert 7 with three factors namely concentration of chemicals (A), power (B) and treatment time (C) in order to replicate their effect on hydrolysis of rice straw (Y). The range of variables for NaOH, H₂SO₄ and H₂O₂ are given in Table-2. The design matrix with 17 experimental runs in one block with five replicates. A polynomial quadratic equation was fitted to evaluate the effect of each independent variable to the response:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC$$

where, Y is the predicted response; β_0 is a constant; $\beta_1, \beta_2, \beta_3$, are the linear coefficients; $\beta_{12}, \beta_{23}, \beta_{13}$ 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
RANGE OF VARIABLES FOR NaOH, H₂SO₄ AND H₂O₂

Name of chemical substance	Concentration of chemicals (%)	Power (watt)	Time (min)
Sodium hydroxide	0.1-2	70-700	1-5
Sulfuric acid	0.1-2	70-700	1-5
Hydrogen peroxide	0.1-2	70-700	1-5

RESULTS AND DISCUSSION

Response surface methodology (RSM): For optimization of microwave effect and other factors on saccharification of rice straw, experiments based on Box-Behnken design (BBD) model are employed. Data analysis is carried out by analysis of variance (ANOVA), regression coefficients and regression equations using Design Expert 7. ANOVA model represents that the model is significant for NaOH, H₂SO₄ and H₂O₂ (Table-3). The Fisher's F-test value 13.23 for NaOH, 13.78 for H₂SO₄ and 8.54 for H₂O₂ implies that the model is significant. The coefficient of determination (R^2) for NaOH, H₂SO₄ and H₂O₂ has been found 0.94, 0.98 and 0.92, respectively. The model appears statistically sound as the lack of fit test used for testing of model shows p value of 0.0784, 0.7061 and 0.2893 for NaOH, H₂SO₄ and H₂O₂, respectively and is not significant. The most significant parameter for NaOH is treatment time (C), quadratic terms NaOH concentration (A^2), power (B^2) and time (C^2), for H₂SO₄ is treatment time (C), power (B), quadratic terms H₂SO₄ concentration (A^2), power (B^2) and interaction term (AC) H₂SO₄ concentration (A) and time (C) and for H₂O₂ quadratic terms H₂O₂ concentration (A^2), power (B^2). Analysis of residuals showed no abnormality. The 3D response surfaces

TABLE-3
ANOVA OF THE QUADRATIC MODEL NaOH, H₂SO₄ AND H₂O₂ AND THEIR INFLUENTIAL FACTORS, RESPECTIVELY

Source	Sum of squares	Degree of freedom	Mean square	F-value	P-value	
Microwave and NaOH treatment						
Model	50337.85	9	5593.09	13.23	0.0013	Significant
Time (C)	2804.82	1	2804.82	6.64	0.0367	
NaOH concentration (A ²) Quadratic terms	31134.89	1	31134.89	73.66	0.0001	
Power (B ²) Quadratic terms	10504.96	1	10504.96	24.85	0.0016	
Time (C ²) Quadratic terms	3411.50	1	3411.50	8.07	0.0250	
Residual	2958.84	7	422.69			
Lack of fit	2330.33	3	776.78	4.94	0.0784	Not Significant
Microwave and H ₂ SO ₄ treatment						
Model	19299.36	9	2144.37	137.87	0.0001	Significant
Time (C)	1151.89	1	1151.89	74.06	0.0001	
Power (B)	741.27	1	741.27	47.66	0.0002	
Quadratic terms H ₂ SO ₄ concentration (A ²)	9281.90	1	9281.90	596.77	0.0001	
Power (B ²)	6680.06	1	6680.06	429.49	0.0001	
Interaction term (AC)	522.40	1	522.40	33.59	0.0007	
Residual	108.88	7	15.55			Not Significant
Lack of fit	29.40	3	9.80	0.49	0.7061	
Microwave and H ₂ O ₂ treatment						
Model	38409.55	9	4267.73	8.54	0.0049	Significant
Quadratic terms H ₂ O 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

for NaOH, H₂SO₄ and H₂O₂ are respectively shown in Fig. 2. 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.

Optimum conditions: Same Design expert software is used for deciding optimum conditions (Table-4). Maximum reducing sugar is obtained through H₂O₂-microwave pretreatment (1453.64 µg/mL) with comparison to H₂SO₄-microwave pretreatment (1376.99 µg/mL) and NaOH-microwave pretreatment (1334.79 µg/mL) at optimum conditions. The reducing sugar concentration in the saccharified rice straw under different pretreatment methods *i.e.* NaOH-microwave, H₂SO₄-

microwave and H₂O₂-microwave are increased significantly by 17, 18 and 21 number of times than actual values in untreated samples of rice straw (Table-5). The experimental results about sugar concentration shows that microwave assisted chemical treatment have significant impact on the reducing sugar value in sampled rice straw.

TABLE-4
OPTIMUM CONDITIONS FOR DIFFERENT PRETREATMENT METHODS FOR DELIGNIFICATION OF RICE STRAW

Pretreatment methods	Chemicals conc. (%)	Power (watt)	Time (min)
NaOH-microwave pretreatment	2	110	3.16
H ₂ SO ₄ -microwave pretreatment	2	115	3.32
H ₂ O ₂ -microwave pretreatment	2	100	3.00

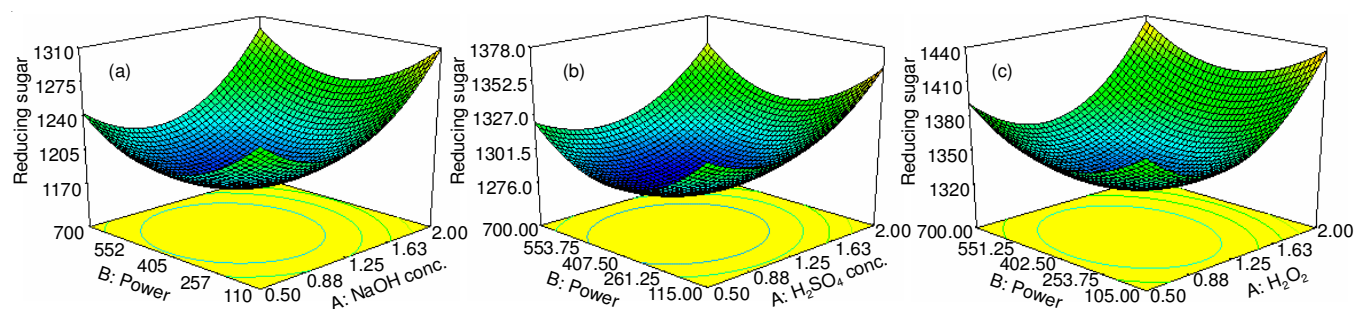


Fig. 2. Response surface for the effect on reducing sugar at constant time using power (microwave) (a) NaOH (b) H₂SO₄ (c) H₂O₂

TABLE-5
PREDICTED AND EXPERIMENTAL REDUCING SUGAR OBTAINED UNDER OPTIMUM CONDITIONS

Pretreatment in combination with microwave	Control (µg/mL)	Predicted (µg/mL)	Measured (µg/mL)	Number of times (approx.)
NaOH	72	1324	1334 ± 15.2	17
H ₂ SO ₄	73	1351	1376 ± 20.3	18
H ₂ O ₂	69	1436	1453 ± 14.3	21

Scanning electron microscope analysis: The morphological changes arise in rice straw due to pretreatment can be analyzed with the help of scanning electron microscope (SEM) [36]. Pretreatment solubilize the hemicellulose, modify the lignin content and enhances the cellulose content, thereby increasing the sugar content [37]. SEM images showed that the surfaces of the samples are densely packed and there is less number of cracks in the untreated sample as compared to microwave-chemical pretreated sample (Fig. 3). The surface of H_2O_2 -microwave treated sample has been found more ruptured and changed more drastically than that of H_2SO_4 -microwave and NaOH -microwave. The silicon waxy structure, lignin-hemicellulose complex of rice straw is broken down extensively. Several research studies have also confirmed that the microwave assisted pretreatment methods are capable of breaking the surface bonds, damage the cell wall structure and altered the fibrillar structure of rice straw [6,38,39]. Microwave pretreatment has effectively improved the straw digestibility by removing silica content [40].

Effect on chemical composition of rice straw: Chemical composition of rice straw has been altered after pretreatment with microwave assisted treatment containing NaOH , H_2SO_4 and H_2O_2 (Table-6). There is increase in percentage of cellulose contents in treated rice straw samples with comparison to un-

TABLE-6
CHEMICAL COMPOSITION OF RICE
STRAW AFTER PRETREATMENT

Characteristics of rice straw	NaOH -microwave pretreatment	H_2SO_4 -microwave pretreatment	H_2O_2 -microwave pretreatment
Cellulose	40.5 ± 2.2	42.6 ± 2.4	45.3 ± 1.5
Hemi-cellulose	48.2 ± 1.9	49.2 ± 1.5	51.2 ± 1.9
Lignin	4.1 ± 0.2	3.8 ± 0.5	3.2 ± 0.4
Ash	13.9 ± 0.1	13.5 ± 0.2	13.2 ± 0.1

treated rice straw samples. However, other components *e.g.* lignin, hemicellulose and ash has been reduced significantly. This indicates that the pretreatment method is capable of removing lignin and other components as well. 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.

X-ray diffraction analysis: Crystallinity index is the percentage of crystalline material in the biomass [32]. It is a major factor that affected enzymatic hydrolysis [41,42]. XRD analysis (Fig. 4) shows that the crystallinity index of rice straw treated with microwave-assisted H_2O_2 , H_2SO_4 and NaOH is higher as compared to the untreated and blank sample [Blank sample refers to sample of rice straw with water only; no

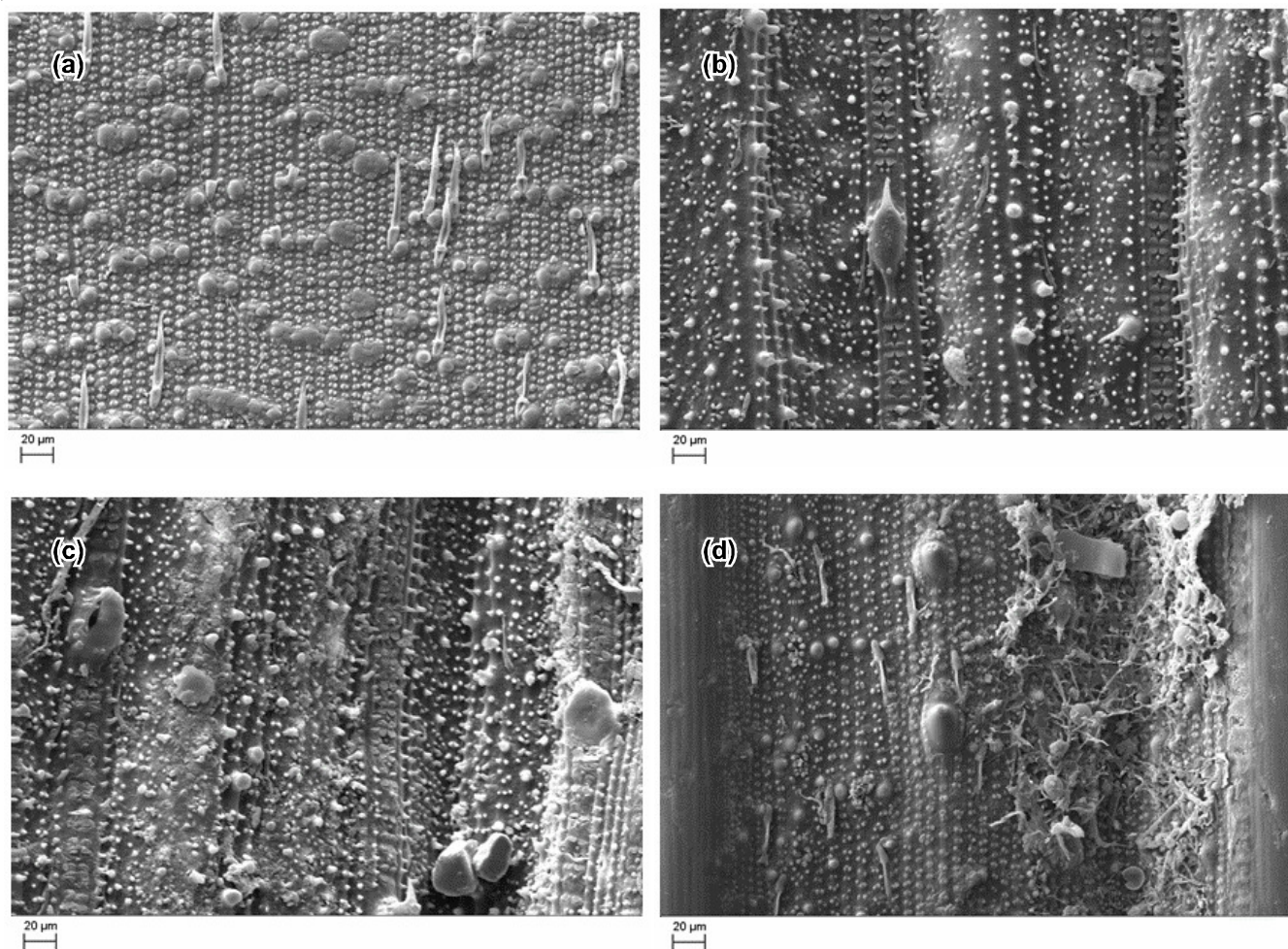


Fig. 3. SEM images of (a) untreated sample (b) sample pretreated with microwave assisted sodium hydroxide (NaOH) (c) sample pretreated with microwave assisted sulphuric acid (H_2SO_4) (d) sample pretreated with microwave assisted hydrogen peroxide (H_2O_2)

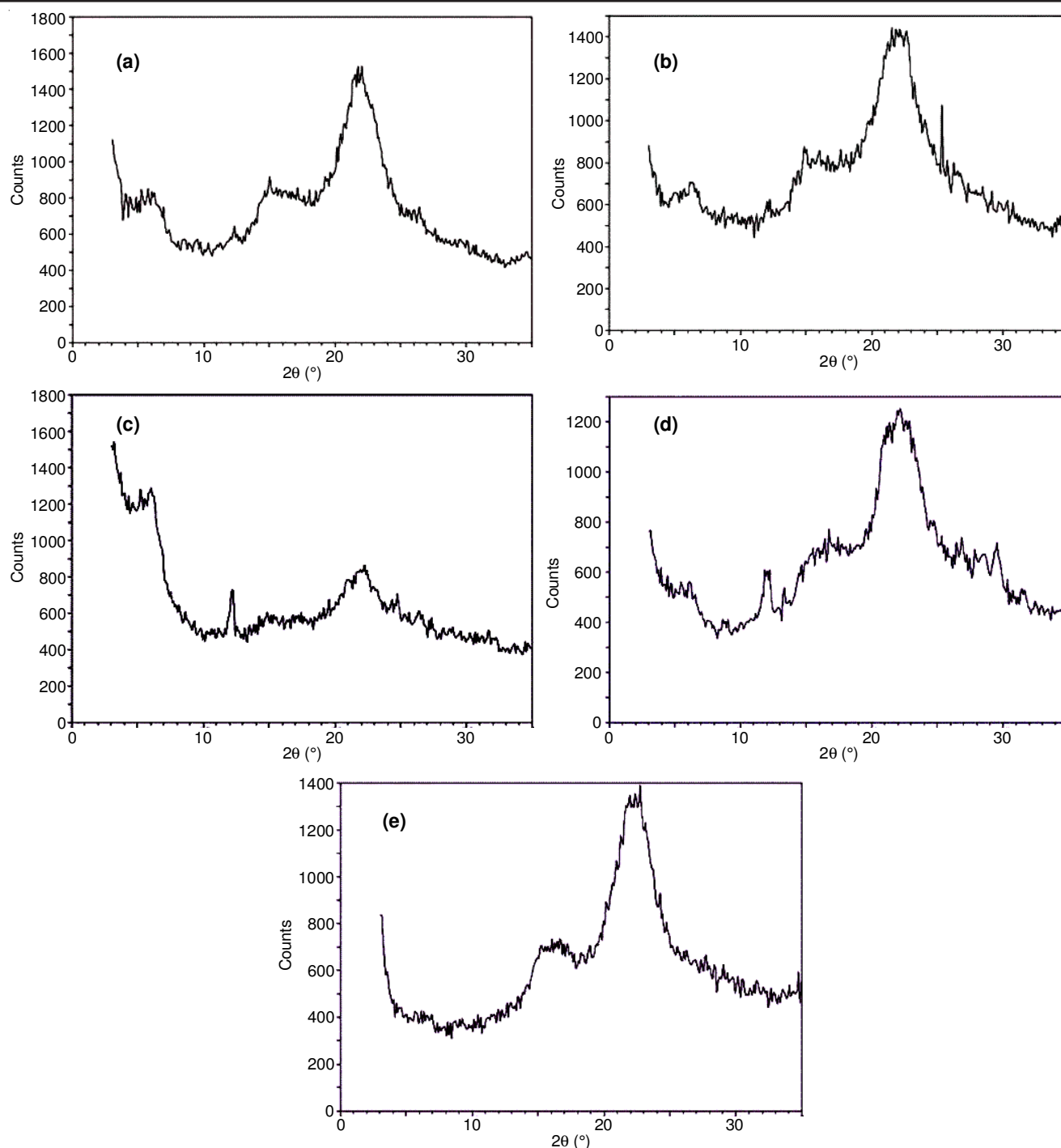


Fig. 4. X-ray diffraction pattern of (a) untreated sample (b) blank (c) NaOH-microwave treated sample (d) H_2SO_4 - microwave treated sample (e) H_2O_2 -microwave treated sample

chemicals are used for blank samples. However for untreated rice straw samples, dry rice straw without water and chemical is used]. However, crystallinity index of rice straw samples treated with microwave assisted H_2O_2 is highest. For untreated and blank sample, it is 52.2 and 49.07 %, respectively as listed in Table-7. By disrupting inter and intra chain hydrogen bonding of cellulose fibrils pre-treatments can change the cellulose structure [43]. In biomass, hemicellulose and lignin are amorphous in nature while cellulose is crystalline [44]. The results demonstrated that removal of amorphous parts of the rice straw *i.e.* lignin, hemicellulose was more in sample treated with

microwave-assisted NaOH, H_2SO_4 and H_2O_2 than untreated and blank. This increase in value showed that pretreatment has potentially removed the amorphous components of rice straw *i.e.* lignin, hemicellulose and increased the crystalline component *i.e.* cellulose in the rice straw, but only 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 can be enhanced by hot acid treatment [45]. Several research studies reported an increase in crystalline index value after biomass pretreatment [46-51].

TABLE-7
CRYSTALLINITY INDEX OF RICE STRAW TREATED WITH DIFFERENT CHEMICALS

Treatments	β (°)	Crystalline index (CrI) %	Microcrystalline size (nm)
Untreated rice straw	2.51	52.2	11.8
Control	2.75	49.07	11.22
NaOH and microwave pretreated	2.01	54.55	15.11
H ₂ SO ₄ and microwave pretreated	2.02	61.36	15.32
H ₂ O ₂ and microwave pretreated	2.31	63.64	14.30

SEM and XRD analysis used in the present study proved that microwave assisted hydrogen peroxide, sulphuric acid as well as sodium hydroxide pretreatment methods had the potential of exposing cellulose and increasing cellulose contents. However, maximum reducing sugar has been found in microwave-H₂O₂ pretreatment. The study also proves that huge availability of rice straw in Indian livestock have tremendous potential for ethanol conversion using microwave-chemical pretreatment methods and technology is working well for them in Indian conditions and verities of rice straw.

Conclusion

Present study suggests that the microwave assisted chemical pretreatment can be an efficient and feasible pretreatment solution for bio ethanol production from Indian rice straw. The combination of microwave pretreatment with H₂O₂, H₂SO₄ and NaOH increases the saccharification of rice straw by removing lignin and hemicelluloses in large quantity and also increases its accessibility to enzymes. Maximum reducing sugar was obtained through H₂O₂-microwave pretreatment (1453.64 µg/mL) compared to H₂SO₄-microwave pretreatment (1376.99 µg/mL) and NaOH-microwave pretreatment (1334.79 µg/mL) under optimum conditions. Analysis of chemical composition of rice straw treated with microwave assisted H₂O₂, H₂SO₄ and NaOH showed the removal of lignin and hemicellulose, although lignin has not been recovered significantly. SEM images confirms that the surface of the samples treated with microwave assisted H₂O₂ are more ruptured than H₂SO₄ and NaOH. Also, maximum crystallinity index has been found for rice straw samples treated with microwave assisted H₂O₂. The removal of lignin and hemicellulose enhanced the enzymatic accessibility with microwave treatment and proves that the enzymatic saccharification of rice straw can be assisted with microwave efficiently.

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REFERENCES

- A.J. Ragauskas, C.K. Williams, B.H. Davison, G. Britovsek, J. Cairney, C.A. Eckert, W.J. Frederick, J.P. Hallett, D.J. Leak, C.L. Liotta, J.R. Mielenz, R. Murphy, R. Templer and T. Tschaplinski, *Science*, **311**, 484 (2006); <https://doi.org/10.1126/science.1114736>.
- S.A. Ravoo, K. Prateepa, T. Supassri and S. Chittibabu, *Int. J. Med. Biosci.*, **1**, 13 (2012).
- X.B. Zhao, L. Wang and D. Liu, *J. Chem. Technol. Biotechnol.*, **82**, 1115 (2007); <https://doi.org/10.1002/jctb.1775>.
- R.C. Sun, *Cereal Straw as a Resource for Sustainable Biomaterials and Biofuels*. Elsevier Publications, Amsterdam, The Netherlands (2010).
- R. Singh, A. Shukla, S. Tiwari and M. Srivastava, *Renew. Sustain. Energy Rev.*, **32**, 713 (2014); <https://doi.org/10.1016/j.rser.2014.01.051>.
- B. Gadde, C. Menke and R. Wassmann, *Biomass Bioenergy*, **33**, 1532 (2009); <https://doi.org/10.1016/j.biombioe.2009.07.018>.
- G. Gong, D. Liu and Y. Huang, *Biosyst. Eng.*, **107**, 67 (2010); <https://doi.org/10.1016/j.biosystemseng.2010.05.012>.
- Y. Sun and J. Cheng, *Bioresour. Technol.*, **83**, 1 (2002); [https://doi.org/10.1016/S0960-8524\(01\)00212-7](https://doi.org/10.1016/S0960-8524(01)00212-7).
- S.D. Zhu, Ph.D. Thesis, Pretreatment by Microwave/Alkali of Rice Straw and Its Saccharification and Fermentation Ethanol Production, Huazhong Agriculture University, Wuhan, China (2005).
- K. Karimi, G. Emtiazi and M.J. Taherzadeh, *Enzyme Microb. Technol.*, **40**, 138 (2006); <https://doi.org/10.1016/j.enzmictec.2005.10.046>.
- C.G. Liu and C.E. Wyman, *Bioresour. Technol.*, **96**, 1978 (2005); <https://doi.org/10.1016/j.biortech.2005.01.012>.
- L.T. Fan, Y.H. Lee and M.M. Gharpuray, *Adv. Biochem. Eng.*, **23**, 157 (1982).
- S. Zhu, Y. Wu, Z. Yu, J. Liao and Y. Zhang, *Process Biochem.*, **40**, 3082 (2005); <https://doi.org/10.1016/j.procbio.2005.03.016>.
- J. Azuma, F. Tanaka and T. Koshijima, *J. Ferment. Technol.*, **63**, 377 (1984).
- H. Ooshima, K. Aso, Y. Harano and T. Yamamoto, *Biotechnol. Lett.*, **6**, 289 (1984); <https://doi.org/10.1007/BF00129056>.
- P. Intanakul, M. Krairiksh and P. Kitchaiya, *J. Wood Chem. Technol.*, **23**, 217 (2003); <https://doi.org/10.1081/WCT-120021926>.
- R.E. Newnham, S.J. Jang, M. Xu and F. Jones, in eds.: D.E. Clark, F.D. Gac and W.H. Sutton, *Fundamental Interaction Mechanisms Between Microwaves and Matter*, In: *Ceramic Transactions, Microwaves: Theory and Application in Materials Processing*, America Ceramic Society, Westerville, Ohio, vol. 21 (1991).
- C. Eskicioglu, K.J. Kennedy and R.L. Droste, *Water Environ. Res.*, **79**, 2304 (2007); <https://doi.org/10.2175/106143007X184069>.
- C. Eskicioglu, N. TerZian, K.J. Kennedy, R.L. Droste and M. Hamoda, *Water Res.*, **41**, 2457 (2007); <https://doi.org/10.1016/j.watres.2007.03.008>.
- M.J. Taherzadeh and K. Karimi, *Int. J. Mol. Sci.*, **9**, 1621 (2008); <https://doi.org/10.3390/ijms9091621>.
- P. Alvira, E. Tomas-Pejo, M. Ballesteros and M.J. Negro, *Bioresour. Technol.*, **101**, 4851 (2010); <https://doi.org/10.1016/j.biortech.2009.11.093>.
- J. Shi, Y. Pu, B. Yang, A. Ragauskas and C.E. Wyman, *Bioresour. Technol.*, **102**, 5952 (2011); <https://doi.org/10.1016/j.biortech.2011.03.027>.
- D. Jackowiak, J.C. Frigon, T. Ribeiro, A. Pauss and G. Guiot, *Bioresour. Technol.*, **102**, 3535 (2011); <https://doi.org/10.1016/j.biortech.2010.11.069>.
- J. Gabhane, S.P.M. Prince William, A.N. Vaiya, K. Mahapatra and T. Chakrabarti, *Biomass Bioenergy*, **35**, 96 (2011); <https://doi.org/10.1016/j.biombioe.2010.08.026>.
- Z. Hu and Z. Wen, *Biochem. Eng. J.*, **38**, 369 (2008); <https://doi.org/10.1016/j.bej.2007.08.001>.
- R. Choudhary, A.L. Umagiliyage, Y. Liang, T. Siddaramu, J. Haddock and G. Markevicius, *Biomass Bioenergy*, **39**, 218 (2012); <https://doi.org/10.1016/j.biombioe.2012.01.006>.
- S. Zhu, Y. Wu, Z. Yu, X. Zhang, H. Li and M. Gao, *Bioresour. Technol.*, **97**, 1964 (2006); <https://doi.org/10.1016/j.biortech.2005.08.008>.

28. S.L.C. Ferreira, R.E. Bruns, E.G.P. da Silva, W.N.L. dos Santos, C.M. Quintella, J.M. David, J.B. de Andrade, M.C. Breikreitz, I.C.S.F. Jardim and B.B. Neto, *J. Chromatogr. A*, **1158**, 2 (2007); <https://doi.org/10.1016/j.chroma.2007.03.051>.
29. B.L. Manjunath, H.R. Prabhu Desai, S. Talaulikar and V.S. Korikanthimath, *Indian J. Agric. Sci.*, **8**, 78 (2008).
30. M. Saritha, A. Arora and L. Nain, *Bioresour. Technol.*, **104**, 459 (2012); <https://doi.org/10.1016/j.biortech.2011.10.043>.
31. G.L. Miller, *Anal. Chem.*, **31**, 426 (1959); <https://doi.org/10.1021/ac60147a030>.
32. L. Segal, J. Creely, A. Martin and C. Conrad, *Text. Res. J.*, **29**, 786 (1959); <https://doi.org/10.1177/004051755902901003>.
33. E. Gumuskaya and M. Usta, *Turk. J. Agric. For.*, **26**, 247 (2002).
34. T. Ehrman, Determination of Acid-soluble Lignin in Biomass, NREL Chemical Analysis and Testing Task Laboratory Analytical Procedure-004, pp 1-7 (1996).
35. A.L. Pometto III and D.L. Crawford, *Appl. Environ. Microbiol.*, **51**, 171 (1986).
36. C. Namasivayam and D.I.R. Kavitha, *Microchem. J.*, **82**, 43 (2006); <https://doi.org/10.1016/j.microc.2005.07.002>.
37. J. Xu, H. Chen, Z. Kádár, A.B. Thomsen, J.E. Schmidt and H. Peng, *Biomass Bioenergy*, **35**, 3859 (2011); <https://doi.org/10.1016/j.biombioe.2011.04.054>.
38. J. Lu and P. Zhou, *Bioresour. Technol.*, **102**, 6966 (2011); <https://doi.org/10.1016/j.biortech.2011.04.044>.
39. H. Ma, W.-W. Liu, X. Chen, Y.-J. Wu and Z.-L. Yu, *Bioresour. Technol.*, **100**, 1279 (2009); <https://doi.org/10.1016/j.biortech.2008.08.045>.
40. T. Rezanka and K. Sigler, *Phytochemistry*, **69**, 585 (2008); <https://doi.org/10.1016/j.phytochem.2007.09.018>.
41. T.H. Kim and Y.Y. Lee, *Bioresour. Technol.*, **96**, 2007 (2005); <https://doi.org/10.1016/j.biortech.2005.01.015>.
42. J.P. O' Dwyer, L. Zhu, C.B. Granda and M.T. Holtzapple, *Bioresour. Technol.*, **98**, 2969 (2007); <https://doi.org/10.1016/j.biortech.2006.10.014>.
43. N. Mosier, C.E. Wyman, B.E. Dale, R.T. Elander, Y.Y. Lee, M. Holtzapple and M.R. Ladisch, *Bioresour. Technol.*, **96**, 673 (2005); <https://doi.org/10.1016/j.biortech.2004.06.025>.
44. T. Jeoh, C.I. Ishizawa, M.F. Davis, M.E. Himmel, W.S. Adney and D.K. Johnson, *Biotechnol. Bioeng.*, **98**, 112 (2007); <https://doi.org/10.1002/bit.21408>.
45. C.T. Yu, W.H. Chen, L.C. Men and W.S. Hwang, *Ind. Crops Prod.*, **29**, 308 (2009); <https://doi.org/10.1016/j.indcrop.2008.06.005>.
46. V.S. Chang and M.T. Holtzapple, *Appl. Biochem. Biotechnol.*, **84-86**, 5 (2000); <https://doi.org/10.1385/ABAB:84-86:1-9:5>.
47. S. Kim and M.T. Holtzapple, *Bioresour. Technol.*, **97**, 583 (2006); <https://doi.org/10.1016/j.biortech.2005.03.040>.
48. J.S. Bak, J.K. Ko, Y.H. Han, B.C. Lee, I.-G. Choi and K.H. Kim, *Bioresour. Technol.*, **100**, 1285 (2009); <https://doi.org/10.1016/j.biortech.2008.09.010>.
49. L. Liu, J.S. Sun, M. Li, S.H. Wang, H.S. Pei and J.S. Zhang, *Bioresour. Technol.*, **100**, 5853 (2009); <https://doi.org/10.1016/j.biortech.2009.06.040>.
50. D. Vamvuka, *Int. J. Energy Res.*, **35**, 835 (2011); <https://doi.org/10.1002/er.1804>.
51. W.H. Chen, Y.J. Tu and H.K. Sheen, *Int. J. Energy Res.*, **34**, 265 (2010); <https://doi.org/10.1002/er.1566>.