Research Article

Microwave Assisted Alkali Pretreatment of Rice Straw for Enhancing Enzymatic Digestibility

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Received 8 August 2013; Accepted 6 January 2014; Published 25 March 2014

Academic Editor: S. Venkata Mohan

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Rapid industrialization, increasing energy demand, and climate change are the conditions that forced the researchers to develop a clean, efficient, renewable, and sustainable source of energy which has a potential to replace fossil fuels. Ethanol is one of the attractive and suitable renewable energy resources. In present study, effectiveness of microwave pretreatment in combination with sodium hydroxide (NaOH) for increasing enzymatic hydrolysis of rice straw has been investigated and under optimum conditions obtained a maximum reducing sugar (1334.79 $\mu\text{g/mL}$) through microwave assisted NaOH pretreatment. Chemical composition analysis and scanning electron microscope (SEM) images showed that the removal of lignin, hemicellulose, and silicon content is more in microwave assisted NaOH pretreatment than the blank sample. X-ray diffraction (XRD) analysis revealed that the crystallinity index of rice straw treated with microwave assisted alkali (54.55%) is significantly high as compared to the blank (49.07%). Hence, the present study proves that microwave assisted alkali pretreatment can effectively enhance enzymatic digestibility of rice straw and it is feasible to convert rice straw for bioethanol production.

1. Introduction

In recent times, due to increase in concerns for climate change and greenhouse gas emissions, research work has been inclined towards the development of sustainable and renewable energy resources. The atmospheric CO_2 levels increased from ~275 to ~380 ppm [1]. From renewable resources, ethanol has been of great interest as an alternative fuel or oxygenate additive for fossil fuels. In 2005 and 2006, worldwide production capacity of ethanol was about 45 and 49 billion litres, respectively, and the total projected demand in 2015 is over 115 billion litres [2]. Lignocellulosic materials are abundant, cheap, and renewable and may be used as a substrate for ethanol production through microbial intervention [3]. Cereal straws are the most abundant resource which can serve as a potential feedstock for the production of biofuel [4]. Rice contains 23% straw of its total weight. Usually, farmers burnt the rice straw left on the field in order to clear the field for the next crop. In India, open-field burning of rice straw contributes up to 0.05% of total GHG emissions [5].

Use of rice straw for bioethanol production not only provides a renewable fuel but also prevents climate change.

Rice straw is mainly composed of cellulose, hemicellulose, lignin, silica, and ash contents. Conversion of rice straw to fermentable sugar is a very complicated process due to the presence of complex structure of lignin and hemicelluloses with cellulose [6–8]. A pretreatment process is required as it can remove cellulose and hemicellulose, reduce cellulose crystallinity, and increase the porosity of the materials [9]. Vast varieties of pretreatment methods are available such as steam explosion, liquid hot water, dilute acid, flow through acid pretreatment, lime, wet oxidation and ammonia fiber/freeze explosion, milling and grinding, microwave energy, wet oxidation, and high energy radiation [10, 11].

Microwave irradiation has high heating efficiency, and thus it is able to degrade lignin and hemicelluloses as well as increase in enzymatic susceptibility [12]. Microwave heats the target object directly by applying an electromagnetic field to dielectric molecules as compared to conduction/convection heating which is based on intramolecular heat transfer [13].

It can significantly increase the enzymatic hydrolysis [14–16] of rice straw. Several researchers have used microwave pretreatment as a potential method for pretreatment of various lignocellulosic materials [17–22] and to damage the recalcitrant lignin [23]. Microwave-chemical pretreatment can be proved as a beneficial method for hydrolysis of rice straw; however, extensive research work on using microwave-chemical pretreatment method for rice straw has not been done effectively.

In present research work, microwave treatment is combined with different concentration of alkali, that is, sodium hydroxide (NaOH) for enzymatic hydrolysis. Response surface methodology (RSM) is used for statistical analysis. Response surface methodology (RSM) 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 [24]. For Indian rice straw variety, alkali pretreatment is not yet explored along with microwave treatment. In this work, for systematic study of effectiveness of microwave assisted NaOH, a Box-Behnken design was selected for the optimization of pretreatment conditions. Further, the morphological characteristics of rice straw are analyzed through scanning electron microscope (SEM) and biomass crystallinity is analyzed through X-ray diffraction (XRD). The aim of this study is to optimize an efficient microwave pretreatment technology for the hydrolysis of rice straw for ethanol production.

2. Material and Methods

2.1. Raw Materials and Microwave Alkali 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 colorless. Before any pretreatment, samples have been air dried. The chemical composition of rice straw is given in Table 1. Microwave pretreatment is one of the efficient ways and modified type domestic microwave oven is used in the present study. The microwave power varied between 70 and 700 W, respectively. About 5 g of rice straw was suspended in 30 mL of NaOH concentration ranged from 0.1 to 2% and left for overnight as per RSM fitted design. It was then radiated in the range of 70–700 W for 1–5 min in microwave. All the pretreatment conditions, that is, power 70–700 W, concentration of chemicals 0.1 to 2%, and treatment time 1 to 5 min, are 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 *Aspergillus niger* supplemented with EBGLUC (endo- β -glucosidase), and β -glucosidase from

TABLE 1: Chemical composition of rice straw variety (Pusa Sugandh) [45].

S. number	Characteristics of rice straw	Values (%)
1	Cellulose	39.04 $\pm$ 0.50
2	Hemicellulose	21.64 $\pm$ 0.50
3	Lignin	16.2 $\pm$ 0.30
4	Ash	18 $\pm$ 1.1

Aspergillus niger (Megazyme International and Genencor) [25]. 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 50°C and 150 rpm in a constant temperature shaker water bath. Samples have been collected from reaction mixture at different time intervals and analyzed for sugar by DNSA method [26]. 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 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.

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 K α radiation). Rice straw treated with water-microwave served as a control. Crystallinity index is calculated by using the following formulae [27]:

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

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, hemicellulose, 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 (d_{002}) (2) is used to calculate crystalline size of (002) plane based on Scherrer equation [28]:

$$d_{002} = \frac{0.9\lambda}{\beta \cos \theta}, \quad (2)$$

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

TABLE 2: ANOVA of the quadratic model microwave and NaOH pretreatment and its influential factors.

Source	Sum of squares	Degree of freedom	Mean square	F value	P value	
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

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 [29]. Through acidification, value added acid-precipitable polymeric lignins are recuperated from the extracts [30]. In the next step extract is acidified to pH 1-2 with concentrated sulphuric acid. Centrifugation process took 30 minutes at 13000 rpm. The precipitates are washed with distilled water and dried at 60°C till the constant weight has been achieved.

2.6. Experimental Designs and Data Analysis. Design Expert software Version 7 named 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 NaOH is power 70–700 W, concentration of chemicals 0.1% to 2%, and treatment time 1 to 5 min. The design matrix with 17 experimental designs runs in one block with five replicates. A polynomial quadratic equation was fitted to evaluate the effect of each independent variable to the response:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC, \quad (3)$$

where Y is the predicted response; β_0 is a constant; β_1 , β_2 , and β_3 are the linear coefficients; β_{12} , β_{23} , and β_{13} are the cross-coefficients; β_{11} , β_{22} , and β_{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.

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

FIGURE 1: Response surface for the effect on reducing sugar using power (microwave) and NaOH concentration at constant time.

regression equations. ANOVA model represents that model is significant for NaOH at Fisher's F -test value 13.23 (Table 2). The coefficient of variation (R^2) for NaOH has been found as 0.94. The model appears statistically sound as the lack of fit test used for testing of model shows P value of 0.0784 and it is not significant. The most significant parameter for NaOH is treatment time (C) and quadratic terms NaOH concentration (A^2), power (B^2), and time (C^2). Analysis of residuals showed no abnormality. The 3D response surfaces for NaOH treatment 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 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 3). The reducing sugar obtained through NaOH-microwave pretreatment under optimum condition is 1334.79 $\mu\text{g/mL}$ (Table 4). The reducing sugar concentration in the saccharified rice straw under NaOH-microwave pretreatment was increased 1752% (Table 3) as compared to raw straw.

3.3. Scanning Electron Microscope (SEM) Analysis. The morphological changes that occurred due to pretreatment could be analyzed with the help of scanning electron microscope

TABLE 3: Optimum conditions for delignification of rice straw.

Pretreatment methods	Chemicals concentration (%)	Power (Watt)	Time (min)
NaOH-microwave pretreatment	2	110	3.16

TABLE 4: Predicted and experimental reducing sugar obtained under optimum conditions.

Pretreatment in combination with microwave	Control ^a ($\mu\text{g/mL}$)	Predicted ($\mu\text{g/mL}$)	Measured ^b ($\mu\text{g/mL}$)	Increasing rate (%)
NaOH	72	1324	1334 $\pm$ 15.2	1752

^aRice straw used in control was untreated.^bMean $\pm$ standard deviation of five replicates.

(SEM) [31]. 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 NaOH-microwave pretreated (Figure 2). The silicon waxy structure is ruptured and lignin-hemicellulosic complex of NaOH-microwave pretreated rice straw is broken down drastically. Previous studies have also shown that the surface of the samples treated with microwave assisted organic acid became loose and irregular [6]. Also, microwave assisted FeCl_3 damaged the cell wall structure and altered the fibrillar structure of rice straw [32]. It proves that microwave pretreatment has effectively improved the straw digestibility by removing silica content [33].

3.4. Effect on Chemical Composition of Rice Straw. Chemical components of rice straw changed after pretreatment with microwave assisted treatment containing NaOH (Table 5). There is increase in percentage of cellulose contents in treated rice straw samples with comparison to untreated. However other components, for example, lignin, hemicellulose, 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.

3.5. X-Ray Diffraction (XRD) Analysis. Crystallinity index is the percentage of crystalline material in the biomass [28]. It is a major factor that affected enzymatic hydrolysis [34, 35]. XRD analysis (Figure 3) shows that the crystallinity index of rice straw treated with microwave assisted NaOH 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 6. By disrupting inter- and intrachain hydrogen bonding of cellulose fibrils pretreatments can change the cellulose structure [36]. In biomass, hemicellulose and lignin are amorphous in nature while cellulose is crystalline [37]. The results demonstrated that removal of amorphous parts of the rice straw, that is, lignin and hemicellulose, was more in sample treated with microwave assisted NaOH than untreated and blank. So, microwave-assisted NaOH pretreated rice straw had lower lignin and hemicellulose, but higher cellulose. It is being found that cellulose content has been increased but only in

TABLE 5: Chemical composition of rice straw after pretreatment.

S. number	Characteristics of rice straw	NaOH-microwave pretreatment (%)
1	Cellulose	40.5 $\pm$ 2.2
2	Hemicellulose	14.2 $\pm$ 1.9
3	Lignin	4.1 $\pm$ 0.2
4	Ash	13.9 $\pm$ 0.1

TABLE 6: Crystallinity index of rice straw samples.

S. number	Treatments	Crystalline index (CrI) %
1	Untreated rice straw	52.2
2	Blank (without addition of any chemicals)	49.07
3	NaOH-microwave pretreatment	54.55

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 [38]. Several studies showed increase in crystalline index value after biomass pretreatment [39–43].

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 sodium hydroxide pretreatment method had the potential of exposing cellulose and increasing cellulose contents. Similar results were obtained from previous research studies. They also found it beneficial as the microwave irradiation could enhance the lignin degradation and presence of aqueous NaOH increases saponification of intermolecular ester bonds cross-linking hemicellulose and lignin [44].

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.

FIGURE 2: SEM images of (a) untreated sample and (b) sample pretreated with microwave assisted sodium hydroxide (NaOH).

FIGURE 3: X-ray diffraction pattern of (a) untreated sample, (b) blank, and (c) NaOH-microwave treated sample.

4. Conclusions

The present research work proves that microwave is an efficient heating method for the pretreatment of rice straw. The combination of microwave pretreatment with NaOH increases the saccharification of rice straw by removing lignin and hemicelluloses in large quantity and increases its accessibility to enzymes, respectively. The optimal conditions have been deduced by using Box-Behnken design (BBD). Maximum reducing sugar was obtained through microwave

assisted NaOH pretreatment ($1334.79 \mu\text{g/mL}$) under optimum conditions as compared to the blank. Analysis of chemical composition of rice straw, the images obtained through scanning electron microscope (SEM), and X-ray diffraction (XRD) analysis showed the removal of lignin and hemicellulose, although lignin has not been recovered significantly. SEM images showed that the surface rupture is more in microwave assisted NaOH pretreatment than blank sample. Crystallinity index for rice straw samples treated with microwave assisted NaOH is significantly high 54.55% in

comparison to untreated sample 52.2%. The removal of lignin and hemicellulose increased the enzyme accessibility with microwave treatment and thus the enzymatic saccharification of rice straw can be assisted with microwave efficiently.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgment

The authors are grateful to Science and Engineering Research Board, Department of Science and Technology, Government of India, for providing funding during the course of the study.

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