

Springer Proceedings in Energy

Sachin Kumar  
Samir Kumar Khanal  
Y.K. Yadav *Editors*

# Proceedings of the First International Conference on Recent Advances in Bioenergy Research

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Y.K. Yadav  
Editors

# Proceedings of the First International Conference on Recent Advances in Bioenergy Research

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# Preface

The increasing prices of crude oil, the depleting nonrenewable energy resources, and the growing concern for climate change have led to a significant interest in renewable energy, including bioenergy. There is a need to shift the current energy spectrum to green energy by creating advanced technologies to utilize renewable resources (such as agro-forestry biomass) for bioenergy production to keep pace with the growing demand for energy. The research in bioenergy is receiving unprecedented attention from all over the world due to the potential of biofuels as a replacement for fossil fuels.

The First International Conference on “Recent Advances in Bio-energy Research” (ICRABR-2015) organized by the Sardar Swaran Singh National Institute of Bio-Energy as an attempt to bring together researchers to form a meaningful conversation on directions of bioenergy. This proceedings volume comprises the top 26 papers contributed by authors who participated and presented their findings at ICRABR 2015. These chapters are peer-reviewed by experts in the field.

We hope that the contents of this volume will help researchers, students, and policy makers involved in this research field.

Kapurthala, India  
March 2015

Sachin Kumar  
Samir Kumar Khanal  
Y.K. Yadav

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## About the Editors



**Dr. Sachin Kumar** is Deputy Director in the Biochemical Conversion Division at the Sardar Swaran Singh National Institute of Bio-Energy, Kapurthala, India. Dr. Sachin Kumar obtained his Ph.D. in Chemical Engineering from Indian Institute of Technology Roorkee, India in 2011. He has more than 11 years of research experience in biochemical conversion of biomass to biofuels. His areas of research interest include biofuels including bioethanol, biogas, biobutanol, and biohydrogen, algal biomass, bioprocess engineering, enzyme technology, and metabolic engineering.



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**Prof. Dr. Y.K. Yadav** received his Ph.D. in Energy Studies from Indian Institute of Technology, Delhi and at present he is the Director General, Sardar Swaran Singh National Institute of Bio-Energy (Formerly Sardar Swaran Singh National Institute of Renewable Energy), Kapurthala, India. Professor Yadav has been actively engaged in research, teaching, consultancy, administration, and management in the field of renewable energy for more than two decades. He developed and taught renewable energy courses to undergraduate and postgraduate students at CCS Haryana Agricultural University, Hisar, India and established a Renewable Energy Laboratory there. He also contributed in the establishment the Department of Energy at Tezpur University, Assam, India.

## About the Conference

The First International Conference on “Recent Advances in Bio-energy Research” ICRABR-2015 was organized by Sardar Swaran Singh National Institute of Bio-Energy, Kapurthala, India during March 14–17, 2015. The conference was inaugurated by Shri Upendra Tripathy, Secretary, MNRE. Dr. B.S. Dhillon, Vice-Chancellor, Punjab Agricultural University, Ludhiana, Dr. N.P. Singh, Adviser, MNRE, Prof. B.S. Pathak, Former Director, SPRERI, Vallabh Vidhyanagar, and Shri Balour Singh, Director, PEDDA, Chandigarh, and Prof. Y.K. Yadav, Director General, SSS-NIBE were also present during the inaugural function. The prominent personalities among delegates from overseas were Dr. Lalini Reddy, Cape Peninsula University of Technology, South Africa, Dr. Hanna Tashyreva, Zabolotny Institute of Microbiology and Virology of National Academy of Sciences of Ukraine, and Dr. Baba Shehu Ibn Abubakar, University of Maiduguri, Nigeria.

Eminent speakers including Prof. B.S. Pathak, Dr. Lalini Reddy, Dr. Abubakar, Dr. Hanna Tashyreva, Prof. A.K. Jain, Dr. D.K. Adhikari, Mr. Vasudeo Joshi, Dr. A.S. Mathur, Dr. R.C. Ray, Dr. D.K. Sahoo, Prof. I.M. Mishra, and Mr. Parikshit Dhingra delivered the plenary speeches. Two plenary speeches were conducted through video conferencing by Prof. Lee R. Lynd, Thayer School of Engineering, Dartmouth College, USA and Prof. Ram B. Gupta, School of Engineering, Virginia Commonwealth University, USA.

Fifteen technical sessions were conducted in three parallel slots each day with different themes such as Biomass & Energy Management; Thermo-chemical Conversion; Biochemical Conversion; Chemical Conversion; Electrochemical Processes; and Integrated/Waste to Energy. About ten invited speakers delivered their research findings and review papers. More than 30 participants presented their work through oral presentation, whereas 38 participants presented their work through posters. About 218 abstracts were published in the souvenir under the different sections including plenary speakers, invited speakers, biochemical conversion, chemical conversion, biomass and energy management, thermochemical conversion, electrochemical processes, waste to energy, and integrated systems.



On the last day of event, one session was conducted for the formation of a “Bio-Energy Alliance.” During the session, the representatives of different organizations including academic and research institutions, universities, industry, and international groups were present and agreed on fully supporting the Bio-Energy Alliance.

**Part I**  
**Biochemical Conversion**

# Comparison Between Separate Hydrolysis and Fermentation and Simultaneous Saccharification and Fermentation Using Dilute Acid Pretreated Lignocellulosic Biomass

Madhuri Narra, Jisha P. James and Velmurugan Balasubramanian

**Abstract** In the present study, two different processes, separate hydrolysis and fermentation (SHF), and simultaneous saccharification and fermentation (SSF) were compared. Three different lignocellulosic biomass viz. rice straw (RS), wheat straw (WS), and sugarcane bagasse (SB) were pretreated with dilute acid at two different concentrations (2 and 4 % H<sub>2</sub>SO<sub>4</sub> w/v) and at two different time intervals, i.e., 30 and 60 min. RS, WS, and SB with 4 % H<sub>2</sub>SO<sub>4</sub> at 121 °C for 30 min yielded maximum reducing sugars (110, 90, and 95 g l<sup>-1</sup>). Delignification of the solid residues were carried out with 0.5 % NaOH, at 121 °C for 30 min. In-house cellulase produced by *Aspergillus terreus* was used for separate hydrolysis studies at 10 % solid loading and 9 FPU g<sup>-1</sup> substrate enzyme loading for 0–48 h at 42 °C. Maximum yield of reducing sugars from RS, WS, and SB were 266, 242, and 254 mg g<sup>-1</sup> substrate, respectively. Acid and enzymatic hydrolysates from RS, WS, and SB produced 5.1, 4.9, 5.2 g l<sup>-1</sup>, and 14.0, 13.9, 12.9 g l<sup>-1</sup> of ethanol with *Pichia stipitis* and *Saccharomyces cerevisiae* in 24 and 36 h, respectively. Whereas SSF at 10 % solid loading and 9 FPU g<sup>-1</sup> substrate enzyme loading for different time intervals 0–72 h at 42 °C was carried out using in-house thermotolerant yeast strain *Kluyveromyces* sp. RS, WS, and SB yielded maximum ethanol of 23.23, 18.29, and 17.91 g l<sup>-1</sup>, respectively. Ethanol yield was enhanced by addition of Tween 80 1 % (v/v) by 8.39, 9.26, and 8.14 % in RS, WS and SB, respectively.

**Keywords** Lignocellulosic biomass · Delignification · Thermotolerant yeast strain · SHF · SSF · Ethanol

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## 1 Introduction

Bioethanol can be produced from any lignocellulosic biomass such as RS, WS, and SB as they are readily available renewable resources of carbohydrates for biological conversion to fuels and chemicals (Borbala et al. 2013). One of the most abundant lignocellulosic biomass in the world is RS. About 731 million tones of RS is produced annually which are distributed in Africa (20.9 million tones), Asia (667.6 million tones), Europe (3.9 million tones), America (37.2 million tones), and Oceania (1.7 million tones). Around 205 billion liters bioethanol per year can be potentially produced from this quantity of RS, which is the largest amount from a single biomass (Faveri et al. 2004). Sugarcane industries also generate huge amount of bagasse annually and some of this residue is currently used for energy cogeneration in sugar mills while the surplus being stockpiled.

Lignocellulosic biomass primarily consists of cellulose, hemicellulose, and lignin and its composition varies with different feed materials used. They are very complex materials; hence a single pretreatment method cannot be applied to all lignocellulosic biomass. Various pretreatment methods have been developed including chemical, physical, physico-chemical, and biological for lignocellulosic biomass and commonly used methods are steam, dilute acid, alkaline, and oxidative pretreatment methods. At current scenario, an up-to-date-technology like dilute acid pretreatment is used for pretreating any lignocellulosic biomass. In acid hydrolysis, removal of hemicellulosic content with small fraction of lignin takes place and the remaining part of lignin remains fixed to the cellulosic content (Kaya et al. 2000). Delignification of lignocellulosic solid biomass is essential to achieve maximum cellulose hydrolysis due to the greater affinity of cellulase components,  $\beta$ -glucosidase, and endoglucanase towards lignin than to the carbohydrates, resulting in lower saccharification efficiency during enzymatic hydrolysis. Agricultural residues such as RS and herbaceous crops are very effectively pretreated using alkali agents. (Chen et al. 2007). Advantages of alkali pretreatment over other pretreatment technologies include lower temperature, pressure, and time requirement. Sodium hydroxide (alkali) has been widely studied for many years to increase the ease of access of cellulases towards cellulose and hemicelluloses by disrupting the lignin structure of the lignocellulosic biomass.

During enzymatic hydrolysis, addition of cellulases to pretreated material containing holocellulosic (cellulose and hemicelluloses) material converts into monomeric sugars and subsequent addition of yeast ferment these sugars to ethanol. SHF is a two step process where enzymatic hydrolysis is followed by fermentation. However, in SSF using single reactor both the process steps can be carried out simultaneously. SSF is more advantageous compared to SHF as it reduces the cost of reactors by performing saccharification and fermentation in single vessel and better ethanol yields by reducing the product inhibition exerted by saccharification products (Scordia et al. 2013).

The need of the hour is to develop a suitable technology for bioethanol production as a partial replacement of gasoline from lignocellulosic biomass as most of

these materials are found in surplus and burnt in the open fields, thereby creating environmental pollution. In order to have an economically viable ethanol plant, the primary focus should be on low cost pretreatments, novel enzymes with higher activities, innovations on fermentation technologies for complete sugar utilization, bioreactors design as well as strains for SSF to provide higher ethanol productivity. Hence, it is essential to develop an indigenous technology with low capital cost and operational expenditure.

In this study, two different processes, SHF and SSF were compared with respect to production of ethanol from dilute acid pretreated lignocellulosic biomass. Both SHF and SSF were carried out at 10 % solid loading and 9 FPU g<sup>-1</sup> substrate enzyme loading at 42 °C by the in-house cellulase produced by *Aspergillus terreus* for different time intervals 0–48 h and using thermotolerant in-house yeast strain *Kluyveromyces* sp. for different time intervals 0–72 h, respectively. Comparative performance of both the processes was reported in this paper.

## 2 Materials and Methods

### 2.1 Lignocellulosic Biomass, Media and Chemicals

Raw materials such as RS and WS were collected from local farmers, Anand, Gujarat, India. SB was procured from a local sugar factory. After collection, the raw materials were exposed to physical pretreatment, i.e., passing through 5 mm mesh in hammer mill prior to chemical pretreatment (Finex, India). The physically pretreated lignocellulosic biomass were washed thoroughly with tap water, air dried, and stored at room temperature in air tight containers. All the chemicals, reagents, and media of analytical grade were purchased from local vendors.

### 2.2 Acid Pretreatment

One fifty grams of pre-sized RS, WS, and SB were mixed with 750, 1500, and 3000 ml of 2 and 4 % H<sub>2</sub>SO<sub>4</sub>. Substrate to acid ratio (w/v) maintained were 1:5, 1:10, and 1:20, respectively. Pretreatment of RS, WS, and SB were conducted at temperature 121 °C for time period 30 and 60 min as described earlier (Narra et al. 2015). Double layered muslin cloth was used to filter the acid hydrolysate. The acid hydrolysate was detoxified with calcium hydroxide as described by Kuhad (2010) and analyzed for sugars, phenolics, and furans. The leftover solid residues after acid pretreatment were thoroughly washed with continuous flow of water till neutral pH and dried under sunlight. The dried acid pretreated solid residues were further subjected to delignification using 0.5 % NaOH at 121 °C for 30 min of 1:20 substrate to alkali ratio. The cellulosic material separated from lignin portion were

filtered using double layered muslin cloth and washed thoroughly with tap water till neutral pH and sun dried. These dried solid residues were further used for SHF and SSF studies or stored at 4 °C in air tight bags.

### 2.3 *Microorganisms and Culture Conditions*

Standard cultures for SHF were viz. *Saccharomyces cerevisiae* 3570 and *Pichia stipitis* NCIM 3499 were procured from National Chemical Laboratory (NCL), Pune, India whereas, newly isolated thermotolerant yeast strain isolated from fruit waste was used for SSF. Partial sequencing of purified strain was carried out at National Collection for Industrial Microorganisms (NCIM), Pune, India. The hexose fermenting yeast strains were maintained on agar slants containing ( $\text{g}^{-1}$  l): glucose, 30.0; yeast extract, 3.0; peptone, 5.0; agar, 20.0 at pH  $6.0 \pm 0.2$ , and *Pichia stipitis* was maintained on agar slants containing ( $\text{g}^{-1}$  l) xylose, 20.0; yeast extract, 4.0; peptone, 5.0;  $\text{KH}_2\text{PO}_4$ , 1.5;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5; agar, 20.0 at pH  $5.0 \pm 0.2$  and temperature 30 °C, respectively. The cultures were stored at 4 °C.

### 2.4 *Yeast Inoculum Preparation*

The *Saccharomyces cerevisiae* inoculum was grown for 12 h at  $30 \pm 2$  °C in a culture medium containing ( $\text{g}^{-1}$  l); glucose, 30.0; yeast extract, 3.0; peptone, 5.0;  $(\text{NH}_4)_2\text{HPO}_4$ , 0.25 at pH  $6.0 \pm 0.2$  (Chen et al. 2007). *Kluyveromyces* inoculum was prepared at  $42 \pm 2$  °C for 12 h. After incubation, the flask contents were aseptically collected, centrifuged, and used for SHF and SSF studies. An optical density of 0.6–0.8 at 620 nm was used for cell culture. Inoculum of *Pichia stipitis* was prepared as described by Nigam (2001) using ( $\text{g}^{-1}$  l); xylose, 50.0; yeast extract, 3.0; malt extract, 3.0; peptone, 5.0 at pH  $\pm 0.2$ , and temperature 30 °C. A 12 h seed culture of *Pichia stipitis* with 1 % v/v were inoculated in separate flasks.

### 2.5 *SHF of Cellulosic Residue*

#### 2.5.1 *Cellulase Preparation*

Crude cellulase used for separate hydrolysis and SSF was indigenously produced by *Aspergillus terreus* under solid state fermentation as described earlier (Narra et al. 2012). RS was used as a substrate for cellulase production and the crude enzyme contained FP activity,  $\beta$ -glucosidase, and endoglucanase, of  $0.98 \pm 0.13$ ,  $5.2 \pm 0.30$ , and  $14.2 \pm 0.40$  U  $\text{ml}^{-1}$ , respectively.

### 2.5.2 Enzymatic Hydrolysis of Delignified Cellulosic Residue

Enzymatic hydrolysis was carried out with cellulosic solid residues of RS, WS, and SB at 42 °C in 50 ml capacity oak ridge wide mouth bottles at 16 rpm for 4–40 h with an enzyme load of 9 FPU g<sup>-1</sup> substrate. Total volume of the system was 20 ml (0.05 M citrate buffer, pH 4.8). Other conditions were kept constant as described earlier (Narra et al. 2012). At regular intervals, the supernatant samples were analyzed for total reducing sugars by DNSA method (Miller 1959) after centrifugation at 10,000 g for 15 min. Saccharification efficiency was calculated as mentioned previously (Narra et al. 2012).

### 2.5.3 Ethanol Fermentation

The enzymatic and acid hydrolysate were fermented with *Saccharomyces cerevisiae* and *Pichia stipitis*, respectively, in a 100 ml stoppered flask at 30 ± 2 °C for 40 h. Nutrients containing NH<sub>4</sub>Cl, 0.5, KH<sub>2</sub>PO<sub>4</sub>, 0.15, yeast extract, 3.0 were added to the acid hydrolyse (20 ml) containing (20.0 g l<sup>-1</sup>) sugars and the pH was adjusted to 5.5 ± 0.2. While the cellulosic hydrolysate having 40.0 g l<sup>-1</sup> sugar was supplemented with yeast extract 3.0 g l<sup>-1</sup> and (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, 0.25 g l<sup>-1</sup>. The flasks were inoculated with 10 % (v/v) inoculum and incubated at 30 °C for 60 h at 150 rpm.

## 2.6 SSF

SSF was performed with cellulosic solid residues of RS, WS, and SB in 50 ml capacity oak ridge wide mouth bottles at 42 °C. The total volume of the system maintained was 20 ml (0.05 M citrate buffer, pH 4.8). Crude cellulases were used to hydrolyze the cellulosic substrates at 10 % solid loading and 9 FPU g<sup>-1</sup> substrate enzyme loads. After 6 h of hydrolysis at 42 °C, an in-house yeast strain *Kluyveromyces* sp. was added under sterile conditions for better conversion of cellulosic material. The experimental flasks placed in a rotating assembly and were rotated at 16 rpm for 60 h as described earlier (Narra et al. 2015).

## 2.7 Analytical Methods

Endoglucanase activity was assayed using 2 % carboxymethyl cellulose (CMC, Sigma Chemical Co.) in 0.05 M sodium acetate buffer, pH 4.8 as substrate. The release of reducing sugars in 20 min at 50 °C was determined by DNSA (Miller, 1959). β-glucosidase assay was carried out using *p*-nitrophenyl—β-D-glucopyranoside (PNPG, Sigma Chemical Co.) as substrate at 50 °C for 30 min. The reaction was terminated by addition of 4 ml NaOH-glycine buffer (0.2 M, pH 10.6).

FP activity was measured as described earlier (Narra et al. 2012). Lignin, cellulose, and hemicellulose contents of the untreated and pretreated RS, WS, and SB were analyzed according to Goering and Vansoest (1975).

The samples from SHF and SSF were withdrawn at regular intervals, centrifuged at  $10,000 \times g$  for 15 min, and the supernatant was analyzed for residual sugars by DNSA method as described earlier. Ethanol was estimated using high performance liquid chromatography (Shimadzu, Japan) equipped with a refractive index detector (RID) and packed with an Aminex-HPX-87 column (Biorad, Hercules, USA, CA) with dimension of  $300 \text{ mm} \times 7.8 \text{ mm}$ . Samples were eluted using  $5 \text{ mM H}_2\text{SO}_4$  with the flow rate of  $0.6 \text{ ml min}^{-1}$ . Column temperature was maintained at  $65 \text{ }^\circ\text{C}$ . The saccharification efficiency and the theoretical yield of ethanol were calculated as described by Narra et al. (2012, 2015).

### 3 Results and Discussion

#### 3.1 Biomass Composition Analysis

RS, WS, and SB contained cellulose content of  $41.02 \pm 1.45 \%$ ,  $38.50 \pm 1.07 \%$ ,  $39.00 \pm 1.83 \%$ ; hemicellulose content of  $28.47 \pm 1.91 \%$ ,  $27.00 \pm 1.36 \%$ ,  $25.00 \pm 1.44 \%$ ; lignin content of  $9.20 \pm 1.12 \%$ ,  $12.82 \pm 1.27 \%$ ,  $14.21 \pm 1.62 \%$  and moisture content of  $7.04 \pm 1.21 \%$ ,  $6.93 \pm 1.17 \%$ ,  $8.14 \pm 1.15 \%$ , respectively. After pretreatment, the cellulose, hemicellulose, and lignin contents of RS, WS, and SB were ( $80.00 \pm 1.58 \%$ ;  $67.00 \pm 1.60 \%$ ;  $70.00 \pm 1.32 \%$ ); ( $3.00 \pm 1.91 \%$ ;  $6.54 \pm 1.42 \%$ ;  $4.36 \pm 1.44 \%$ ); ( $2.01 \pm 1.16 \%$ ;  $4.98 \pm 1.97 \%$ ;  $5.13 \pm 1.48 \%$ ), respectively (Narra et al. 2015).

#### 3.2 Dilute Acid Hydrolysis of Lignocellulosic Biomass

RS yielded maximum amount of reducing sugars ( $110 \text{ g l}^{-1}$ ) followed by WS and SB ( $90$  and  $95 \text{ g l}^{-1}$ ) when treated at optimum conditions, i.e.,  $4 \%$   $\text{H}_2\text{SO}_4$  at  $121 \text{ }^\circ\text{C}$  for 30 min. The substrate to acid ratio maintained was 1:5 (w/v). As the treatment time increased from 30 min to 60 min, there was no substantial difference in the reducing sugar yield ( $102$ ,  $85$ , and  $91 \text{ g l}^{-1}$ ). The acid hydrolysate obtained at optimum conditions of hydrolysis contained major amount of xylose ( $90.23 \pm 2.34 \text{ g l}^{-1}$ ), glucose ( $5.01 \pm 1.29 \text{ g l}^{-1}$ ), arabinose ( $7.48 \pm 2.69 \text{ g l}^{-1}$ ), phenolics, furfural, and HMF. The major degradation products of pentose, hexose sugars, and lignin are furfural, HMF, and phenolics, respectively (Kuhad et al. 2010). These toxins have the ability to decrease the activities of several yeast enzymes, e.g., alcohol dehydrogenase, aldehyde dehydrogenase, and pyruvate dehydrogenase during fermentation (Modig et al. 2002). In order to reduce the inhibitor concentrations in acid hydrolysates, sequential addition of overliming and



activated charcoal was used. Overliming followed by activated charcoal treatment resulted in reduction of furfural (96.31, 95.15, and 96.20 %), HMF (93.36, 92.18, and 92.19 %), and phenolic (93.36, 92.18, and 92.19 %) in RS, WS, and SB, respectively. As the acid hydrolysate was rich in xylose it was used to produce ethanol using pentose utilizing yeast strain *Pichia stipitis* NCIM 3499.

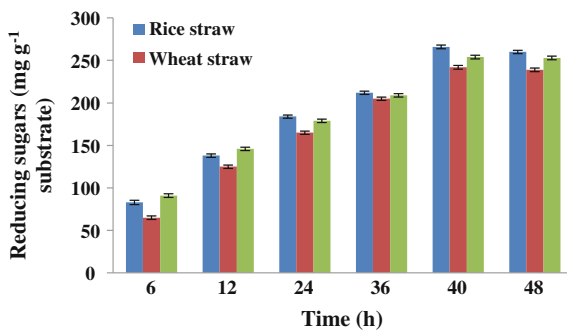
### 3.3 *Delignification of Lignocellulosic Biomass*

Delignification of acid pretreated solid biomass was carried out with 0.5 % NaOH at 121 °C for 30 min to maximize reducing sugar yield. The cellulose content in the pretreated biomass increased substantially with simultaneous reduction in lignin content after alkaline pretreatment (Narra et al. 2015). The cellulose content was found to be increased by 95.02, 74.02, and 79.48 %, while the lignin removals were 78.16, 61.15, and 63.90 % in RS, WS, and SB, respectively. It was also observed that during the treatment most of the pentosan was solubilized. The increase in cellulose content during pretreatment might be due to removal of lignin which might have increased the enzyme effectiveness by eliminating nonproductive adsorption sites and increasing access to cellulose and hemicelluloses (Lu et al. 2002). Kumar et al. (2009) reported that solubilization of other components in the aqueous alkali solution is also responsible for increase in the cellulose content. These findings were consistent with earlier published data on effects of delignification of different lignocellulosic biomass RS, WS, and rapeseed straw by alkaline pretreatment (Narra et al. 2012; Nopparat et al. 2013).

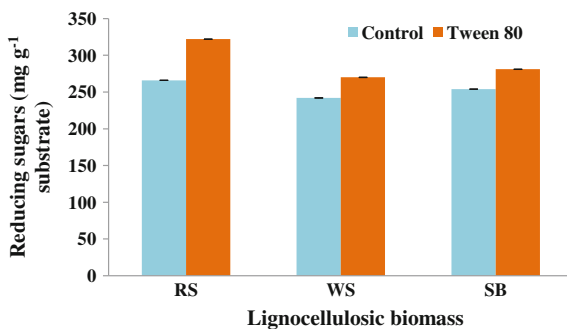
### 3.4 *Enzymatic Saccharification of Delignified Cellulosic Material*

When delignified cellulosic substrates were subjected to enzymatic saccharification, an increase in reducing sugar concentration was observed till 40 h time period and thereafter it remained almost constant. Maximum yield of saccharification from RS, WS, and SB was 266, 242, and 254 mg g<sup>-1</sup> substrate, respectively (Fig. 1). Tween 80 1 % (v/v) addition also found to increase the saccharification yield by 12.1, 11.4, and 10.6 %, respectively (Fig. 2). According to Kaar and Holtzapple (1998), all through the enzymatic saccharification process, thermal deactivation of enzymes was prevented by the addition of Tween. This outcome may be due to the surface activity of Tween which resulted in shorter enzyme contact with air-liquid interface. The decrease in surface tension of the solution not only permits the saccharifying exoglucanase more active sites to cellulose, but it also prevents the nonproductive part of the exoglucanase to the lignin surface which yielded in increased sugar level (Hematinejad et al. 2002).

**Fig. 1** Enzymatic hydrolysis of RS, WS, and SB at different time intervals. Temperature 42 °C, substrate load 10 % (w/v), enzyme load 9 FPU g<sup>-1</sup> substrate, pH 4.8, rpm 16



**Fig. 2** Influence of Tween 80 on enzymatic hydrolysis of RS, WS, and SB. Substrate load 10 % (w/v), enzyme load 9 FPU g<sup>-1</sup> substrate, pH 4.8, time 48 h, rpm 16, Tween 80 1 % (w/v)



### 3.5 Fermentation of Hemicellulosic and Enzymatic Hydrolysate

Maximum amount of ethanol production from RS, WS, and SB was obtained up to 5.1, 4.9, 5.2 g l<sup>-1</sup>, respectively. Table 1 shows the profile for ethanol fermentation by *Pichia stipitis* 3499 NCIM. It was observed that there was increase in ethanol

**Table 1** Fermentation profile of hemicellulosic hydrolysate of lignocellulosic biomass using *Pichia stipitis*

Time (h)	Ethanol (g l <sup>-1</sup> )			Sugar (g l <sup>-1</sup> )			Ethanol yield (g g <sup>-1</sup> )		
	RS	WS	SB	RS	WS	SB	RS	WS	SB
0	0.04	0.05	0.05	20.00	19.80	20.00	0.00	0.00	0.00
6	1.62	2.25	1.97	19.12	18.45	19.26	0.08	0.11	0.09
12	3.11	3.78	3.95	12.58	13.54	12.95	0.16	0.19	0.19
24	5.10	4.90	5.20	6.21	6.92	6.09	0.26	0.25	0.26
36	3.27	4.17	4.19	5.98	5.21	5.04	0.16	0.21	0.21
48	2.61	3.09	3.35	5.26	4.97	4.70	0.13	0.16	0.17
60	2.21	2.85	3.01	4.18	4.26	4.13	0.11	0.14	0.15

**Table 2** Fermentation profile of enzymatic hydrolysate of lignocellulosic biomass using *Saccharomyces cerevisiae*

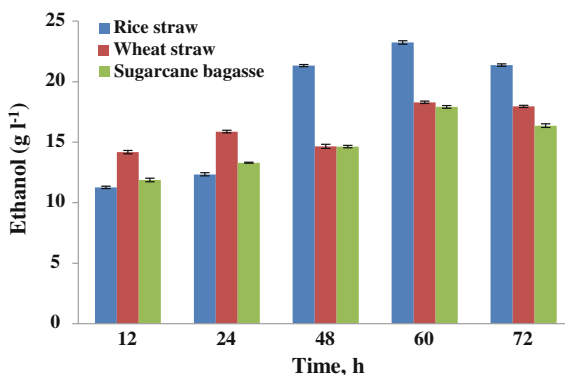
Time (h)	Ethanol (g l <sup>-1</sup> )			Sugar (g l <sup>-1</sup> )			Ethanol yield (g g <sup>-1</sup> )		
	RS	WS	SB	RS	WS	SB	RS	WS	SB
0	0.08	0.07	0.08	40.00	40.00	40.00	0.00	0.00	0.00
6	10.18	6.95	6.29	11.89	13.28	14.09	0.25	0.17	0.16
12	12.84	9.01	7.58	10.37	12.04	12.54	0.32	0.22	0.19
24	13.53	11.26	9.26	8.43	9.30	9.01	0.34	0.28	0.23
36	14.00	13.90	12.90	7.27	6.95	8.15	0.35	0.35	0.32
48	13.84	11.78	11.27	5.31	5.29	6.26	0.34	0.29	0.28
60	12.63	11.05	10.98	4.98	4.42	5.01	0.32	0.32	0.27

and increase in the growth of fermenting yeast but the consumption of reducing sugars was relatively poor. The incomplete utilization of reducing sugars by fermenting yeast may be due to some kind of inhibitors present in the acid hydrolysate which might have not been removed during the detoxification process. Similar observation was also made by Kuhad et al. (2010) that the yeast could not tolerate the higher amount of sugar concentration. The ethanol yield obtained from cellulosic hydrolysate (RS, WS, and SB) using *Saccharomyces cerevisiae* was 0.35, 0.35, and 0.32 g g<sup>-1</sup>, respectively (Table 2).

### 3.6 SSF

Maximum ethanol yield achieved at 60 h with 10 % solid load from RS, WS, and SB (23.23, 18.29, and 17.91 g l<sup>-1</sup>) which was equivalent to 51.29, 48.22, and 45.19 % of maximum theoretical yield (Fig. 3). The earlier reports have shown that increase in substrate load beyond certain extent could have caused decrease in hydrolysis rate due to the product inhibition. The extent of inhibition usually

**Fig. 3** SSF of RS, WS, and SB at different time intervals. Temperature 42 °C, substrate load 10 % (w/v), enzyme load 9 FPU g<sup>-1</sup> substrate, pH 4.8, rpm 16

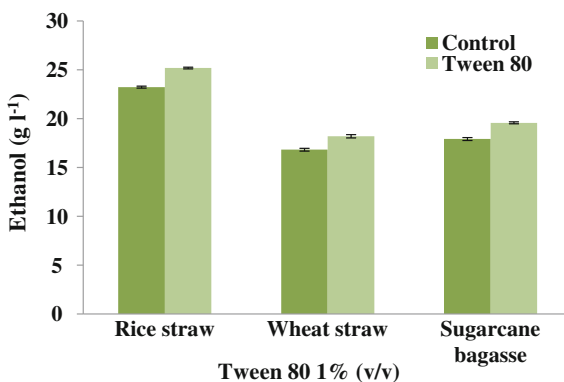


depends on the ratios of substrate to enzyme load (Wang et al. 2011; Xin et al. 2010). The present results also revealed that higher ethanol yields at lower solid loads may be due to lower glucose accumulation compared to higher solid loads. The current results were in accordance with Naveen et al. (2011) that at 8 % solid loading, higher ethanol yields were observed compared to 12 % solid loading when pretreated switchgrass was used for SSF studies by *Kluyveromyces marxianus* IMB3.

Enhanced ethanol yields were achieved by the addition of 1 % Tween 80 to the reaction mixture in comparison to the control (Fig. 4). The yields were increased by 8.39, 9.26, and 8.14 % with 10 % solid loading at 60 h from RS, WS, and SB, respectively. Maximum ethanol yield from RS, SB, and WS were found to be 25.18, 19.57, and 18.19 g l<sup>-1</sup>, respectively. Kaar and Holtzapple (1998), Zhu et al. (2014) similarly have found that addition of surfactant enhanced the rate and extent of hydrolysis as the surfactants could have changed the nature of the substrate either by increasing the available cellulose surface area or by eliminating inhibitory lignin.

The present results have shown that 1 kg untreated RS, WS, and SB contained 410, 370, 380 g, and 252, 205, 207 g cellulose and hemicelluloses, respectively. This amount can be theoretically enough for production of 232, 217, 215 g and 129, 105, 106 g ethanol from cellulose and hemicellulose, respectively. Based on the best yields attained in the current work, the ethanol production from RS, WS, and SB through SSF was found to be 201, 162, 156 g kg<sup>-1</sup> raw biomass, respectively. Whereas, the ethanol production from RS, WS, and SB through SHF was found to be 95, 75, 82 g kg<sup>-1</sup> raw biomass, respectively (Table 3). The present results revealed that SSF could prove enhanced ethanol production from delignified biomass compared to SHF. The reason could be that the optimum condition for separate enzymatic hydrolysis might be higher than 42 °C, and secondly the product inhibition exerted by saccharification might have occurred during the process. Whereas in case of SSF, pre-hydrolysis was carried out at 42 °C for 6 h followed by yeast addition to ease the problems caused by production inhibition.

**Fig. 4** Influence of Tween 80 on SSF of RS, WS, and SB. Substrate load 10 % (w/v), enzyme load 9 FPU g<sup>-1</sup> substrate, pH 4.8, time 60 h, rpm 16



**Table 3** Comparison of ethanol yields from SSF and SHF of different lignocellulosic biomass

Process	SSF			SHF		
Lignocellulosic substrates	RS	WS	SB	RS	WS	SB
Optimum conditions	SSF: Temperature: 42 °C, substrate load: 10 % (w/v), enzyme load: 9 FPU g <sup>-1</sup> substrate, duration: 48 h, Tween 80: 1 % (v/v)			SH: Temperature: 42 °C, substrate load: 10 % (w/v), enzyme load: 9 FPU g <sup>-1</sup> substrate, duration: 48 h, Tween 80: 1 % (v/v) Fermentation: Temperature: 30 °C, duration: 24 h, sugar concentration: 2 %		
<i>Theoretical ethanol yield, g kg<sup>-1</sup> substrate</i>						
Cellulosic substrate	232	217	215	232	217	215
Hemicellulosic substrate	129	105	106	129	105	106
<i>Ethanol yield, g kg<sup>-1</sup> substrate</i>						
Cellulosic substrate	129	108	95	23	21	21
Hemicellulosic substrate	72	54	61	72	54	61

## 4 Conclusion

Cellulosic ethanol is considered to be a globally accepted alternative fuel. The current results demonstrated that SSF could ascertain improved ethanol production from delignified lignocellulosic biomass compared to SHF in terms of total ethanol production time and ethanol yield. RS yielded higher amount of ethanol followed by WS and SB using in-house cellulases produced by *Aspergillus terreus* and in-house thermotolerant yeast strain *Kluyveromyces* sp.

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## References

- Borbala E, Mats G, Guido Z (2013) Simultaneous saccharification and co-fermentation of whole wheat in integrated ethanol production. *Biomass Bioenergy* 56:506–514
- Chen HZ, Xu J, Li ZH (2007) Temperature cycling to improve the ethanol production with solid state SSF. *Appl Biochem Microbiol* 43:57–60
- Faveri DD, Torre P, Perego P, Convati A (2004) Statistical investigation on the effects of starting xylose concentration and oxygen mass flow rate on xylose production from RS hydrolysate by response surface methodology. *J Food Eng* 65:383–389
- Goering K, Van Soest PJ (1975) Forage fiber analysis-agriculture research series. Handbook 379

- Hemantnejad N, Vahabzadeh F, Koredestani SS (2002) Effect of surfactant on enzymatic hydrolysis of cellulosic fabric. *Iranian Poly J* 11:333–338
- Kaar WE, Holtzapfel MT (1998) Benefits from tween during enzymatic hydrolysis of corn stover. *Biotechnol Bioeng* 419–427
- Kaya F, Heitmann JA, Thomas WJ (2000) Influence of lignin and its degradation products on enzymatic hydrolysis of xylan. *J Biotechnol* 80:241–247
- Kuhad RC, Gupta R, Khasa YP, Singh A (2010) Bioethanol production from *Lanata Camara* (red sage): pretreatment, saccharification and fermentation. *Bioresour Technol* 641–654
- Kumar R, Mago G, Balan V, Wyman CE (2009) Physical and chemical characterization of corn stover and poplar solids resulting from leading pretreatment technologies. *Bioresour Technol* 100:3948–3962
- Lu Y, Yang B, Gregg D, Saddler J, Mansfield S (2002) Cellulase adsorption and an evaluation of enzyme recycling during hydrolysis of steam-exploded soft wood residues. *Appl Biochem Biotechnol* 98–100
- Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Anal Chem* 31:426–428
- Modig T, Liden G, Taherzadeh MJ (2002) Inhibition effects of furfural on alcohol dehydrogenase, aldehyde dehydrogenase and pyruvate dehydrogenase. *Biochem J* 363:769–776
- Narra M, Dixit G, Madamwar D, Shah AR (2012) Production of cellulases by solid state fermentation with *Aspergillus terreus* and enzymatic hydrolysis of mild alkali-treated RS. *Bioresour Technol* 121:355–361
- Narra M, James J, Balasubramanian V (2015) SSF of delignified lignocellulosic biomass at high solid loadings by a newly isolated thermotolerant *Kluyveromyces* sp. for ethanol production. *Bioresour Technol* 179:331–338
- Naveen KP, Hasan KA, Mark RW, Danielle DB, Ibrahim MB (2011) SSF of Kanlow switchgrass by thermotolerant *Kluyveromyces marxianus* IMB3: The effect of enzyme loading, temperature and higher solids. *Bioresour Technol* 102:10618–10624
- Nigam JN (2001) Ethanol production from WS hemicellulose hydrolysate by *Pichia stipitis*. *J Biotechnol* 87:17–27
- Nopparat S, Khatiya W, Navadol L, Verawat C (2013) Optimize simultaneous saccharification and co-fermentation of RS for ethanol production by *Saccharomyces cerevisiae* and *Scheffersomyces stipitis* co-culture using design of experiments. *Bioresour Technol* 142:171–178
- Scordia D, Cosentino SL, Jeffries TW (2013) Enzymatic hydrolysis, simultaneous saccharification and ethanol production of oxalic acid pretreated giant reed (*Arundodonax* L.). *Ind Crops Prod* 49:392–399
- Wang W, Kang L, Wei H, Arora R, Lee Y (2011) Study on the decreased sugar yield in enzymatic hydrolysis of cellulosic substrate at high solid loading. *Appl Biochem Biotechnol* 164:1139–1149
- Xin F, Geng A, Chen ML, Gum MJM (2010) Enzymatic hydrolysis of sodium dodecyl sulphate (SDS) pre-treated newspaper for cellulosic ethanol production by *Saccharomyces cerevisiae* and *Pichia stipitis*. *Appl Biochem Biotechnol* 162:1052–1064
- Zhu JQ, Lie Q, Li BZ, Yuan YJ (2014) Simultaneous saccharification and co-fermentation of aqueous ammonia pretreated corn stover with an engineered *Saccharomyces cerevisiae* SyBE005. *Bioresour Technol* 169:9–18

# Liquid Hot Water Pretreatment of Paddy Straw for Enhanced Biomethanation

Abhinav Trivedi, Virendra Kumar Vijay and Ram Chandra

**Abstract** Indian agriculture produces nearly 150–160 million tons of paddy straw per annum. 70–80 % of total produced rice straw is burned in the open field for cultivation of the next crop. Proper disposal of paddy straw in an economical and environment friendly manner is a serious concern in India, where rice is the major agricultural crop in some states. The work presented in the paper is focused on pretreatment of paddy straw using liquid hot water treatment followed by biomethanation. Results observed during batch process are encouraging and work is being carried out for bigger models. Under batch condition with  $\sim 5\text{--}7$  kg of paddy straw can yield  $1.0\text{ m}^3$  of biogas with average methane content of 60.0 and 25–30 % carbon dioxide content during retention period of 40 days.

**Keywords** Agricultural residue · Lignocellulosic biomass · Biomass pretreatment · Liquid hot water pretreatment · Biogas from paddy straw · Biomethane production

## 1 Introduction

Paddy straw is among ubiquitous lignocellulosic agricultural waste biomass which has been rarely utilized as a source for biofuel production. For the year 2013, annual production of rice was reported as 159.200 million metric tons in India (FAOSTAT 2013). With such colossal production of rice, around 238.50 million metric tons of paddy straw is generated per annum with an average of 1.0–1.5 kg of paddy straw per kilogram of the rice grain harvested (Maiorella 1985) of which two-third is burnt in the field itself (Phutela 2011). Some amount of unburnt residue is used as a fuel for modern biomass-based power plant, while some amount is used

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to fuel biomass cookstoves in rural area for cooking and heating applications (Gadde et al. 2009).

The portion of the paddy straw residue that remains in the field as uncollected is subsequently plowed back into the soil (residue incorporation into soil), which serve as biofertilizer for upcoming crops. This is widely practised across the country and it provides a nutrient source for upcoming crops but also conducive to crop diseases (Hrynychuk 1998). This activity stimulates methane emissions due to decomposing paddy straw in the field (Yuan 2013).

The research paper published in recent years has been perceived that open field burning of paddy straw residues contributes toward emissions of polycyclic aromatic hydrocarbons, i.e., PAHs (Korenaga et al. 2001), as well as polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs), referred to as dioxins (Gullett and Touati 2003). It has also been reported that open burning of one ton of paddy straw emits 3 kg particulate matter, 60 kg CO, 1460 kg CO<sub>2</sub>, 199 kg ash, and 2 kg SO<sub>2</sub> (Jenkins and Bhatnagar, 2003a, b). These compounds are toxic and carcinogenic for humans and have a long term impact on human health (Meesubkwang 2007).

Anaerobic digestion of rice straw for methane production is considered as the best option among all thermochemical and biochemical processes for providing energy in form of methane, thus reducing air pollution up to considerable limits and at the same time producing digestate solids, which is a potential biofertilizer for land application (Nand 1999). The anaerobic digestion technology is a most efficient way in term of energy output/input ratio for handling of biomass resources to produce energy and biofertilizer. Furthermore, raw biogas after enrichment is equivalent to natural gas and can be alternatively used in mobile and static internal combustion engines.

The present research focused on evaluation of liquid hot water (LHW) pretreatment method for delignification of paddy straw and subsequently anaerobic digestion of the substrates. In this method of pretreatment the destruction of lignocellulosic structure occurs by super-heated water at temperature 200 and 240 °C. The work was carried out using nonstirred reactor having volume of 2.0 liter, which can be operated to maximum working temperature of 300 °C. The degraded paddy straw samples were evaluated for its biomethane potential under laboratory conditions.

## 2 Materials and Methods

### 2.1 Characterisation of Paddy Straw

The characterization of paddy straw was carried out using proximate and ultimate analyses. Initially paddy straw was dried at 105 °C using hot air oven and ground to nearly 1.0 mm size using grinder. Percentage of moisture content, total solids, volatile solids, and ash contents (proximate analysis) in paddy straw was estimated using standard methods as described by APHA while basic elements such as carbon, hydrogen, and nitrogen (ultimate analysis) were determined using 'Vario EL'



elemental analyzer (Perkin Elmer, USA Made). 20 mg of homogenized samples were packed in tin foil and placed into the carousel of the automatic sample feeder of the analyzer and analyzed as per procedure described in the instruction manual of the instrument supplied by the manufacturer of CHN elemental analyzer.

## **2.2 Pretreatment of Paddy Straw**

Fresh paddy straw samples were pretreated in a hydrothermal reactor at reactor operating temperatures of 200 and 240 °C for pretreatment time duration of 15–20 min. During pretreatment the water-to-paddy straw ratio was maintained as 8:1, this ratio is maintained in order to get nearly 10 % of total solids concentration in the digesting substrate. Appropriate amount of NaOH was added in the pretreated paddy straw substrate to maintain pH around 7.5–7.8. To maintain the homogeneity of the substrates in reactor bottles, the bottles were shook daily manually. The prepared substrates were kept at 37 °C temperature in an incubator for stabilization of pH before being fed to anaerobic digester (Chandra et al. 2012).

## **2.3 Experimental Setup**

The biogas production potential of pretreated paddy straw samples was estimated using 1.0 L glass bottles (Schott Duran). Each reactor consisted 30 g of hydrothermally pretreated paddy straw and 240 mL of process water along with 480 mL of fresh slurry taken from cow dung-based biogas plant. The C/N ratio of substrate was adjusted in a range of 20–25 by addition of 0.75 g of urea in the substrate. Biogas production was monitored for 40 days after which biogas production had been found ceased. The reactor bottles contained the desired substrates along with inoculum were placed in an incubator and maintained constantly at 37 °C operating temperature. The daily biogas volume was measured by using inverted water displacement system. The biogas produced from various anaerobic reactors was analyzed for methane as well as carbon dioxide contents using a gas chromatograph (Agilent 7890) equipped with Porapak Q column and thermal conductivity detector (TCD).

# **3 Results and Discussion**

## **3.1 Properties of Paddy Straw**

The results of proximate and ultimate analysis of untreated paddy straw are shown in Table 1. The proximate analysis revealed that untreated paddy straw contains up

**Table 1** Proximate and ultimate analysis of nontreated paddy straw

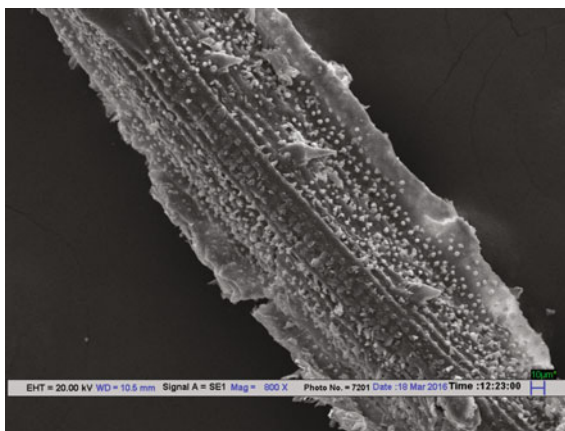
Sl. No.	Properties parameter	Observed value (%)
<i>Proximate properties</i>		
1	Moisture content (wet basis)	08.00
2	Total solids (wet basis)	92.00
3	Volatile solids (dry basis)	81.50
4	Ash content (dry basis)	18.50
<i>Ultimate properties</i>		
5	Carbon	35.20
6	Hydrogen	5.596
7	Nitrogen	0.745
8	<i>C/N</i> ratio	47.248

to 8.0 % moisture and 92.0 % total solids on wet weight basis, while 81.50 and 18.50 % volatile solid matter and ash matter, respectively, on dry weight basis. The ultimate analysis resulted into 35.2 % carbon, 5.5 % hydrogen, and 0.745 % nitrogen contents on dry weight basis. Upon elemental analysis, it has been found that the amount of nitrogen content present in rice straw biomass is very low (*C/N* ratio = 47.2) to maintain an appropriate range of *C/N* ratio (20–30) for effective methane fermentation.

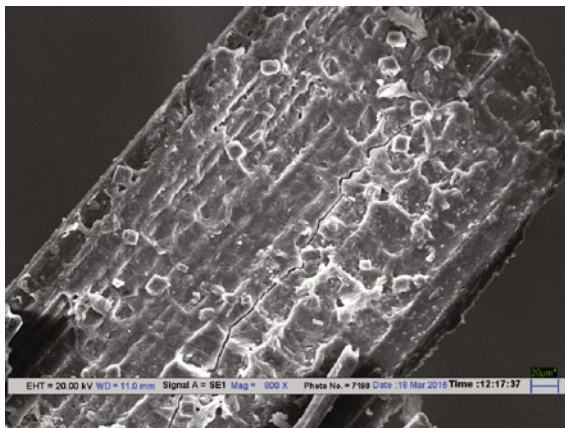
### 3.2 *Effect of Liquid Hot Water Pretreatment on Structure of Paddy Straw*

Figs. 1, 2, 3, and 4 show the scanning electron microscopic images of nontreated paddy straw and various hydrothermal pretreated paddy straw such as 200 and

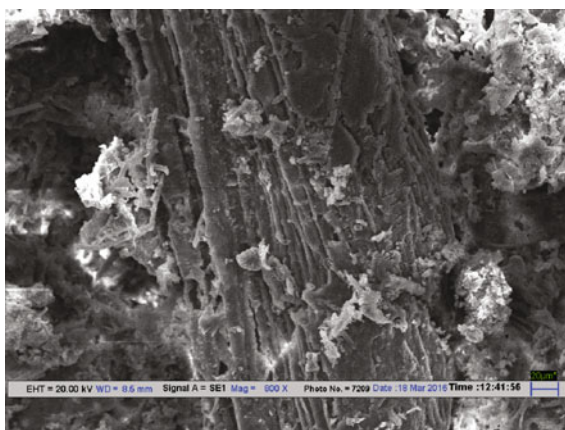
**Fig. 1** SEM image of nontreated paddy straw



**Fig. 2** SEM image of pretreated paddy straw (200 °C)



**Fig. 3** SEM image of pretreated paddy straw (240 °C)



240 °C, respectively. It is clearly evident from Fig. 1 that the microfibrils are very tightly packed in case of nontreated paddy straw. However, in case of hydrothermal pretreated paddy straw the original structure of paddy straw has been visibly disrupted depending upon the level of pretreatment temperature. The microfibrils are loosed and open for microbial degradation. The brightness in the various images is due to charging effect during SEM imaging of volatile fatty acids released during the pretreatment process.