Biodelignification and hydrolysis of rice straw by novel bacteria isolated from wood feeding termite

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Received: 23 April 2018 / Accepted: 4 October 2018 / Published online: 11 October 2018
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Abstract

In this study, two bacterial strains capable of degrading lignin, cellulose, and hemicellulose were isolated from wood feeding termite. The isolates were identified by 16S rRNA gene sequencing. A bacterium *Ochrobactrum oryzae* BMP03 capable of degrading lignin was isolated on alkali lignin medium and *Bacillus* sp. BMP01 strain capable of degrading cellulose and hemicellulose were isolated on carboxymethyl cellulose and xylan media. The efficiency of bacterial degradation was studied by evaluating the composition of rice straw both before and after degradation. The appearance of new cellulose bands at 1382, 1276, 1200, and 871 cm⁻¹, and the absence of former lignin bands at 1726, 1307, and 1246 cm⁻¹ was observed after biodelignification. This was further confirmed by the formation of channeling and layering of the microstructure of biodelignified rice straw observed under electron microscope. Maximum lignin removal was achieved in separate biodelignification and hydrolysis process after the 14th day of treatment by *Ochrobactrum oryzae* BMP03 (53.74% lignin removal). Hydrolysis of the biodelignified rice straw released 69.96% of total reducing sugars after the 14th day hydrolysis by *Bacillus* sp. BMP01. In simultaneous delignification and hydrolysis process, about 58.67% of total reducing sugars were obtained after the 13th day of biotreatment. Separate delignification and hydrolysis process were found to be effective in lignin removal and sugar released than the simultaneous process. The bacteria, *Bacillus* sp. BMP01, has the ability to degrade hemicellulose and cellulose simultaneously. Overall, these results demonstrate that the possibility of rice straw bioconversion into reducing sugars by bacteria from termite gut.

Keywords Biodelignification · Microbial hydrolysis · Wood feeding termite · Rice straw · Lignin

Introduction

Fossil fuel remains one of the dominant sources of energy in the world. The sustainability of its supply to the fast growing population of the world and its environmental concern forced the scientific community to think and search for alternate energy sources. In the year 2016 alone, about

13,903 million tons of oil equivalent (5.82×10^{20} J) of energy was consumed in the world in which fossil fuels account for a major share (World Energy Statistics 2017). Therefore, the use of alternate energy sources is mandatory to solve the current problems caused by the use of fossil fuels. Currently, lignocellulose biomass attracts the attention of many researchers for biofuel production due to their sustainability and relative abundance (Ma et al. 2015; Zhang et al. 2017; Yao et al. 2018).

Lignocellulose biomass is one of the most abundant biopolymers in the world. Hemicellulose, cellulose, and lignin are the three main components of lignocellulose biomass (Himmel et al. 2007). Cellulose is a homogenous chain of glucose monomers. Hemicellulose is heterogeneous monomers of a pentose sugar (arabinose and xylose) and hexose sugar (galactose, glucose, and mannose). Ester and/or ether bond joins lignin and hemicellulose molecules together. Lignin is a heterogeneous chain of phenylpropane

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bonded together by ether and carbon–carbon bonds (Feldman et al. 1986).

In lignocellulosic biomass, the polysaccharides (hemicellulose and cellulose) are wrapped by lignin, which protects it from degradation by microorganisms and enzymes (Chabannes et al. 2001; Wout et al. 2003; Studer et al. 2011). Therefore, modification of lignocellulose biomass is mandatory and required for hydrolyzing it into its monomers (Lorenz et al. 2009; Gao et al. 2017). The modification of biomass is performed using different pretreatment methods. The most common pretreatment methods are alkali pretreatment, acid pretreatment, liquid hot water pretreatment, ammonia fiber expansion method, physicochemical pretreatment, biological pretreatment, steam explosion method, ionic pretreatment method, and organosolv pretreatment methods (Wang et al. 2016; Xu et al. 2007; Ayed et al. 2017; Tsegaye et al. 2017; Wu et al. 2017; Zhang et al. 2018).

After lignin removal by different pretreatment methods, enzymes and/or microorganisms are easily hydrolyzing the biomass. Microbial hydrolysis of pretreated lignocellulose biomass was shown as a better alternative compared to enzymatic hydrolysis. To degrade cellulose to its monomers (glucose), three combined activities of enzymes in the cellulolytic complex are required (Mao et al. 2018; Ke et al. 2011; Shelomi et al. 2014). The first enzyme is endo- β -1, 4-glucanases (EG; EC. 3.2.1.4) which hydrolyzes the inner β -1, 4-bonds of cellulose chains. The second enzymes are exo- β -1, 4 cellobiohydrolases (CBH; EC. 3.2.1.91) which release cellobiose from the non-reducing ends of cellulose. The third is β glucosidases (EC. 3.2.1.21) which hydrolyzes cellobiose or longer cellulose chains to release glucose (Ke et al. 2013; Albuquerque et al. 2014; Shelomi et al. 2014).

Biological conversion of lignocellulose biomass to bioenergy (biofuels) is gaining much research focuses than other pretreatment and hydrolysis technologies mostly because of the associated cost. Abilities to prohibit the formation of inhibitory compounds, specificity, requirements of no chemicals, eco-friendly, cheap, and advantage of low energy requirements are some of the relative advantages of biological pretreatments (Beauchet et al. 2012; Cianchetta et al. 2014; Kothari et al. 2017). Many scholars reported that various species of bacteria and fungi have abilities of delignifying and hydrolyzing lignocellulose biomass (Kumar et al. 2009; Sun and Scharf 2010; Cianchetta et al. 2014; Torre et al. 2017; Shirkavand et al. 2017; Waghmare et al. 2018). Among the microorganisms, fungi are the most widely studied microbes in biological delignification and hydrolysis process (Cianchetta et al. 2014). The efficiency of biological delignification mainly depends upon the type of microorganism used, nature and type of substrate used, and the cultivation methods and conditions.

Nature gives the ultimate gifts of utilizing wood as the food sources for wood feeding termites and they efficiently

degrade wood within the ecosystems (converts 65–99% of carbohydrates in wood within 24 h) (Esenther and Kirk 1974). This makes termites as a model organism for biological pretreatments of lignocellulose biomass under natural conditions (Sun and Scharf 2010; Scharf et al. 2011). The mechanisms by which termites utilize lignocellulose biomass as their energy sources are not fully understood and are currently a center of studies in scientific research institutes (Ni and Tokuda 2013; Tarayre et al. 2014).

Termite guts are considered as a reservoir of different species of bacteria capable of degrading lignocellulose biomass (Auer et al. 2017). Therefore, isolation of lignocellulose degrading bacteria from termite gut may lead to the discovery of new bacteria that plays a vital role in lignocellulose bioconversion. Cellulose degrading bacteria, *Serratia marcescens* and *Bacillus cereus*, have been isolated from wood feeding termites (Thayer 1978). Various enzymes, such as cellobiohydrolase, carboxymethylcellulase, beta-glucosidase, and beta-xylosidase, which degrades cellulose and hemicellulose chains into reducing sugars, have been isolated and characterized from termite gut bacteria (Droge et al. 2006; Cho et al. 2010). Lignin-degrading enzymes such as lignin peroxidase and laccase enzymes have been also detected in bacteria isolated from termite guts (Tsegaye et al. 2018). However, fewer studies have been conducted on harnessing and exploring the huge advantage of gut microbiomes for biotechnological applications despite their natural competence to produce relevant chemicals (Agler et al. 2011). Therefore, isolating and characterizing bacteria from termite gut give some insight into the mechanism and it might open the biological way to convert lignocellulose biomass to biofuel.

The main objective of this study was to study the delignification and hydrolysis abilities of bacteria isolated from wood feeding termite on rice straw samples. The extent of degradation and hydrolysis capabilities of the isolated bacteria was also studied. It was attempted to characterize the biologically treated and untreated rice straw samples using Fourier Transform Infrared Spectroscopy (FTIR), Field Emission Scanning Electron Microscopy (FE-SEM), and powder X-ray diffraction (XRD) analysis. The efficiencies of delignification and hydrolysis were also studied.

Materials and methods

Chemicals and preparation of raw materials

Xylan was purchased from Sisco research laboratories Pvt. Ltd, Mumbai, India, and alkali lignin from Sigma Aldrich Chemicals Pvt. Ltd, Bangalore, India. All other chemicals were purchased from Himedia Laboratories Pvt. Ltd, Mumbai, India.

Wood feeding termites (*Cryptotermes brevis* species) and rice straw were collected from the village around Roorkee, Uttarakhand, India (29.87°N latitude, 77.89°E longitude, 276 msl elevation). The termites were sterilized in petri dish with 70% alcohol for about a minute. After sterilization, termite's guts were pulled out using forceps and smeared in Eppendorf tubes to obtain internal fluids of termite guts.

Rice straw was dried to a moisture content of less than 10%. Then, it was ground and powdered to the size of 200 microns by laboratory grinder (Sunflame style DX). Samples were taken to analyze the chemical composition as well as to characterize the straw before treatments. Rice straw samples were kept in airtight bags until used.

Isolation of bacteria from termite gut

The gut content of wood feeding termite was collected in Eppendorf tubes, which contains Basal salt medium in a sterile condition. After extraction, Eppendorf content was transferred to three different round-bottom flasks; each contains 100 mL of basal salt medium. Basal salt medium (NaNO₃ 2.5 g; KH₂PO₄ 2 g; MgSO₄ 0.2 g; NaCl 0.2 g; CaCl₂·6H₂O 0.1 g in a liter) were used as culture medium. The medium was enriched with 2% CMC for isolating cellulose degrading bacteria, 2% xylan for isolating hemicellulose bacteria, and 2% alkali lignin to isolate lignin-degrading bacteria. The media were kept in both aerobic and anaerobic conditions to identify oxygen requirement for the growth of the isolates. Growth was detected only in an anaerobic condition and no growth was found in aerobic condition. The medium was kept in the anaerobic condition in shaker incubator maintained at a temperature of 30 °C and rotational speed of 120 rpm. The sample was taken every 12 h to monitor the growth and change in components. After 6 days of incubation, 100 µL of each medium were inoculated to their respective agar medium (CMC agar, xylan agar, and alkali lignin agar media). Pure colonies of each culture medium were isolated and subcultured in their respective media. After subsequent subculturing, pure bacteria were isolated on alkali lignin agar medium, xylan agar medium, and CMC agar medium.

16S rRNA identification

The pure cultures of bacterial isolates were sent to national centers for microbial resources, Pune, India, for 16S rRNA sequencing. The identification was performed by characterizing 1200 bp sequences. The sequences were analyzed for the closest neighbor by comparing the sequences with available information in National Center for Biological Information (NCBI). The sequences were submitted to NCBI Genbank for deposition and for searching the closest neighbor to identify the strains.

Biological delignification/biopretreatment

Biological pretreatments were done in 250 mL round-bottom flasks in a shaker incubator at a temperature of 30 °C and rotational speed of 120 rpm. 3 g of powdered rice straw (particle size of about 200 microns) was added to 100 mL of basal salt medium and 5 mL of pure bacterial culture from alkali lignin medium was added. A sample of rice straw without bacteria was used as a control. After the bacteria reaches maximum growth rate, the residue of rice straw samples was taken for the characterization and analysis of morphological and compositional changes. Comparisons of the amount of lignin before and after pretreatments were used to identify the performance of the delignification process. Compositional changes caused due to biodelignification of rice straw were calculated according to the following equations:

$$\text{Biodelignification (\%)} = 1 - \frac{\text{Lignin in biotreated sample}}{\text{Lignin in untreated sample}} \times 100\% \quad (1)$$

$$\begin{aligned} \text{Hemicellulose(\%)} \\ = 1 - \frac{\text{Hemi/Cellulose in biotreated sample}}{\text{Hemi/Cellulose in untreated sample}} \times 100\% \end{aligned} \quad (2)$$

$$\text{Cellulose(\%)} = 1 - \frac{\text{Hemi/Cellulose in biotreated sample}}{\text{Hemi/Cellulose in untreated sample}} \times 100\%. \quad (3)$$

Microbial hydrolysis of biologically pretreated rice straw

The biopretreated samples were subjected to hydrolysis by pure bacterial isolates on CMC medium. Total reducing sugar was measured to detect and evaluate the hydrolysis process. Reducing sugars were measured using 3,5 dinitrosalicylic acid method (Miller 1959). The efficiency of hydrolysis was evaluated by the following equation (Mohanram et al. 2015):

$$\begin{aligned} \text{Sacharification efficiency } (\eta) \\ = \frac{\text{Total reducing sugar released} \times 0.9 \times 100}{\text{Carbohydrate content in pretreated rice straw}}. \end{aligned} \quad (4)$$

Simultaneous microbial delignification and hydrolysis

Pretreatment and hydrolysis were performed in 250 mL round-bottom flasks in a shaker incubator at a temperature of 30 °C and rotational speed of 120 rpm. 3 g of powdered rice straw was added into 100 mL of basal salt medium for microbial delignification and hydrolysis process. Simultaneous pretreatment and hydrolysis were carried out by

inoculating 10 mL of pure culture from alkali lignin and CMC media. The efficiency of the process was evaluated by measuring the amount of reducing sugar released. Total reducing sugars was measured using 3,5 dinitrosalicylic acid method (Miller 1959).

Compositional analysis of treated and untreated rice straws

The chemical composition (polysaccharides and lignin) of biologically pretreated and controlled rice straw samples were analyzed according to the standard method of NREL laboratory procedure (Sluiter et al. 2008). After drying and grinding, 3 mL of 72% sulphuric acid were mixed with 300 mg of rice straw samples. After an hour of treatment, it was diluted to 4% and autoclaved for an hour at 121 °C. Acid insoluble lignin was measured by weight difference and acid soluble by UV spectrophotometry (Hach DR-5000). Moisture content was determined by the conventional oven drying method (Sluiter et al. 2008). Glucose and xylose were determined by HPLC system equipped with an RI detector.

Characterization of treated and untreated rice straws

Microstructural and morphological changes in rice straw due to microbial pretreatments were analyzed by Field Emission Scanning Electron Microscopy (Carl Zeiss Ultra Plus FE-SEM, Germany). Different areas and magnifications were taken for careful examination of the effect of microbial pretreatment on surface morphology rice straw. The change in chemical composition and functional groups were analyzed by Fourier Transform Infrared Spectroscopy (FTIR, Thermo Nicolet 6700, USA). The spectra of rice straw samples were generated with a scanning range of 4000–500 cm^{-1} . The crystallinity and removal of the amorphous part were studied by powdered X-ray diffractometer (Bruker D8). Peak intensity method was used to determine the crystallinity index (Segal et al. 1959):

$$\text{CrI} = \left(\frac{I_{002} - I_{\text{am}}}{I_{002}} \right), \quad (5)$$

where I_{am} is the intensity of the peak for the amorphous portion at $2\theta = 18$ and I_{002} is the maximum intensity at $2\theta = 22$.

Apparent crystallite sizes (L) were calculated using the Scherrer equation (Scherrer 1918):

$$L = K\lambda / \beta \cos \theta, \quad (6)$$

where k dimensionless shape factor (0.94), β the full width at half maximum intensity of the peak in radians, and L

apparent crystalline size, θ Bragg angle, and λ X-ray wavelength (0.1542)

The degree of polymerization was calculated based on the area under crystalline peaks and non-crystalline region using the following equation:

$$X_c = \left(\frac{F_c}{F_a + F_c} \right) \times 100, \quad (7)$$

where X_c is a degree of polymerization, F_c is an area of the crystalline region, and F_a area of non-crystalline regions.

Results and discussion

Isolation of lignin-degrading and polysaccharide-hydrolyzing bacteria

After subsequent subculturing, two different bacteria were isolated and identified from wood feeding termites gut (*Cryptotermes brevis* species). One bacterium was isolated on a lignin model compound (alkali lignin medium) which has lignin-degrading ability and the other strain was isolated on CMC and Xylan media. This polysaccharide-degrading bacterial isolate has both cellulose and hemicellulose-degrading ability. The lignin-degrading bacteria were grown on alkali lignin medium and reached maximum growth rate on the 8th day of incubation. The cellulose and hemicellulose-degrading bacteria reached maximum growth on the 6th day on their respective medium. The growths were determined by direct counting the number of viable cells on hemocytometer under a microscope.

Morphological analysis of isolates

Different micrographs of isolated bacteria were taken at different magnifications for careful analysis of size and shape of bacteria. Figure 1 shows images of bacterial isolates from wood feeding termites. Lignin-degrading bacteria have a round shape (cocci) and have a size between 1.25 and 3.5 μm . These bacteria are shiny when seen under a microscope. CMC and xylan-degrading bacterial isolates have a rod shape. CMC and Xylan hydrolyzing bacteria have a size between 1 and 2.5 μm .

Identification of isolates by 16S rRNA gene sequencing

Taxonomic identities of bacterial isolates from wood feeding termites' guts were determined by 16S rRNA gene sequence analysis. Basic Local Alignment Search Tool was used to search for similarity by comparing the nucleotide

Fig. 1 SEM micrographs of bacterial isolates: **a** alkali lignin-based isolates at $\times 1000$ magnification; **b** alkali lignin-based isolates at $\times 5000$ magnification; **c** CMC-based isolates at $\times 5000$ magnification; **d** CMC-based isolates at $\times 20000$ magnification

sequences of the isolates to NCBI Genbank (National Center for Biological Information) database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). NCBI Genbank database similarity search revealed that the bacteria isolated on lignin model compound belonged to *Ochrobactrum* and *Ochrobactrum oryzae*. The homology between the isolated strain and *Ochrobactrum oryzae* reached 99% similarity. The isolated bacteria were designated as *Ochrobactrum oryzae* BMP03 and deposited in Genbank repository under accession number MG786919 (<https://www.ncbi.nlm.nih.gov/nuccore/MG786919>). The strain isolated on cellulose and xylan media belonged to *Bacillus* sp. The homology between this isolate and *Enterococcus gallinarum* was 97%. The 16S rRNA gene sequence data of this isolate were designated as *Bacillus* sp. BMP01 and deposited in Genbank

under accession number MG800701 (<https://www.ncbi.nlm.nih.gov/nuccore/MG800701>). The biodelignification and hydrolysis capabilities of the isolates were evaluated on rice straw substrates.

Biodegradation of rice straw by bacterial isolates, *Ochrobactrum oryzae* BMP03

Change in chemical composition of biopretreated rice straw samples is given in Table 1. Rice straw samples were analyzed for change in the composition after the 14th day of pretreatment. A significant change was seen in the chemical composition of biotreated samples. As shown in Table 1, *Ochrobactrum oryzae* BMP03 strain significantly degrades and mineralize lignin and released the trapped cellulose

Table 1 Chemical composition of biologically treated and untreated rice straw samples

Treatment	Lignin content (%)	Cellulose (%)	Hemicellulose (%)	Moisture content (%)
Untreated	20.80	37.89	17.95	9.80
Control	20.32	38.20	18.40	9.50
Biologically treated	9.40	52	21.5	9.60

and hemicellulose in the rice straw. The depolymerization ability of *Ochrobactrum oryzae* BMP03 strain is clearly seen from morphological changes observed in Fig. 2. In

addition to compositional and morphological changes, It was evident from XRD and FTIR results that lignin was broken down and solubilized after pretreatments by bacteria strain, *Ochrobactrum oryzae* BMP03. Maximum lignin removal of about 53.74% was achieved after 14th days of pretreatment. Lignin degradation was monitored by measuring the maximum growth rate of bacteria. Zeng et al. (2012) reported 29% of lignin removal from bamboo by *E. taxodii* 2538 after 30 days of pretreatment. Chandra et al. 2007 reported about 37% of kraft lignin degradation by bacteria species of *Novosphingobium* sp. B-7. 41.5% of kraft lignin was removed after 7 days of pretreatment by *Cupriavidus basilensis* B-8 (Shi et al. 2017). Medina-Morales et al.

Fig. 2 SEM micrograph of **a** controlled rice straw samples at a magnification of $\times 500$; **b** controlled rice straw samples at a magnification of $\times 1000$; **c** biotreated rice straw samples at a magnification of $\times 1000$; **d** biotreated rice straw samples at a magnification of $\times 2000$, respectively

(2017) reported around 62% saccharification efficiency for *agave atrovirens*. Compared to the other lignolytic bacteria, *Ochrobactrum oryzae* BMP03 isolated from termite guts has higher lignin-degrading capacity. Removal of lignin is a key factor for lignocellulose bioconversion process, since its existence hinders the availability of sugar monomers. The breaking of bonds that links polysaccharides to lignin leads to an easy way for microbial hydrolysis of the biotreated rice straw (Gao et al. 2017). This, in turn, enhances the hydrolysis process.

The contents of cellulose were increased by 36.12% after biotreatment. This change is due to structural disruption of rice straw caused by lignin depolymerization by *Ochrobactrum oryzae* BMP03. In addition, 16.85% of hemicellulose was released after pretreatment. These bacteria reserve the cellulose and hemicellulose chains by selectively cutting lignin bonds (Costa et al. 2016). Compared to a controlled sample, a change in the major chemical composition of rice straw was observed after pretreatment. This change is mainly due to the solubilization of lignin by *Ochrobactrum oryzae* BMP03. In this study, we found that these bacteria have a potential lignin depolymerization ability to a greater extent and plays a major role in bioconversion of lignocellulose biomass to biofuels.

Hydrolysis of cellulose and hemicellulose by *Bacillus* sp. BMP01

The efficiencies of bacterial hydrolysis of biotreated rice straw were studied by evaluating total reducing sugars released from biotreated and untreated/controlled rice straw samples. The results of total reducing sugar obtained during microbial hydrolysis of biotreated and controlled (uninoculated) rice straw samples are given in Table 2. The maximum amount of total reducing sugar was released with the 14th day of hydrolysis (440 mg/g of biotreated rice straw). Saccharification efficiency of 69.96% was achieved. The effect of microbial treatment of rice straw on total reducing sugars is given in Fig. 3. Significant changes are observed between treated and controlled samples. Jagannathan et al. (2017) reported 515 mg/g of biomass conversion after enzymatic hydrolysis. Mohanram et al. (2015) achieved 71.6% of glucose conversion from rice straw after pretreating with by *M. roridum*. 400 mg/g of sugar was obtained after pretreating corn stover with fungi strains of basidiomycete species *Cya. stercoreus* NRRL-6573 (Saha et al. 2016). In this study, the hydrolysis efficiency is significantly increased due to the high capabilities of the bacterial isolates to hydrolyze hemicellulose and cellulose to their respective monosaccharides. Moreover, the absence of inhibitory compounds from lignin degradation products contributes to the increment. The use

Table 2 Total reducing sugar obtained from biotreated rice straw hydrolysis

Hydrolysis time (days)	Total reducing sugar concentration (mg/g of biotreated rice straw)		Efficiency of saccharification, η (%)	
	Control	Biotreated	Control	Biotreated
1	18	43	2.86	6.84
2	23	68	3.66	10.81
3	27	92	4.30	14.63
4	29	125	4.61	19.87
5	32	168	5.09	26.71
6	39	216	6.20	34.35
7	46	249	7.31	39.60
8	51	273	8.11	43.41
9	58	313	9.22	49.77
10	64	359	10.18	57.08
11	67	384	10.65	61.06
12	73	408	11.61	64.87
13	82	438	13.04	69.64
14	86	440	13.68	69.96
15	91	438	14.47	69.50
16	90	436	14.32	69.32
17	89	435	14.15	69.17

Fig. 3 Effect of rice straw biotreatment on total reducing sugar released a by bacterial hydrolysis of biotreated rice straw samples and b simultaneous delignification and hydrolysis by *Ochrobactrum oryzae* BMP03 and *Bacillus* sp. BMP01 strains; c control rice (uninoculated) straw samples

of this microbial hydrolysis process may replace the dependence on the commercial enzyme and reduces the total cost of bioethanol production.

Fig. 4 Saccharification efficiencies of biotreated rice straw samples; a bacterial hydrolysis; b simultaneous delignification and hydrolysis by bacteria; c control (uninoculated) rice straw samples

Simultaneous biodelignification and hydrolysis of rice straw by *Ochrobactrum oryzae* BMP03 and *Bacillus* sp. BMP01

Biodelignification and microbial hydrolysis were carried out by inoculating *Ochrobactrum oryzae* BMP03 and *Bacillus* sp. BMP01 into rice straw samples. The percentage of total reducing sugar obtained after simultaneous delignification and hydrolysis of rice straw are given in Fig. 4. The maximum amount of sugars were obtained at 13th day of pretreatment (58.67%). The hydrolysis efficiency of simultaneous delignification and hydrolysis process is lower than that of separate delignification and hydrolysis process. This is more probably because of inhibition effects from lignin degradation product on the hydrolytic bacteria. The simultaneous biodelignification and hydrolysis process requires lower time than separate hydrolysis and delignification process. Employing microbial consortium, which consumes lignin degradation products, might improve the efficiency and yield of reducing sugars. Hence, it has an advantage of cost reduction by reducing space and time requirement relative to separate hydrolysis and delignification process.

Characterization of pretreated untreated rice straw samples

SEM analysis

The SEM micrographs of controlled and biotreated rice straw samples are shown in Fig. 2. Different images at different magnification (1000×, 2000×) were taken for careful examination. Morphological and microstructural changes

Fig. 5 XRD spectra of biotreated and controlled rice straw samples

were observed on biotreated rice straw samples. Continuous and rigid surfaces structure was observed in controlled rice straw samples (Fig. 2a, b), whereas significant morphological changes were observed on biotreated samples exhibiting layering and channeling on the surfaces (Fig. 2c, d). The separation of microfibrils from the surface exposed the inner surfaces causing significant microstructural changes between the control and biotreated rice straw samples. These microstructural changes caused by biotreatment of rice straw by *Ochrobactrum oryzae* BMP03 bacterial strain increase accessibility and enhance efficient hydrolysis of polysaccharides by reducing the barriers. Many researchers reported microstructural changes on rice straw and wheat straw samples caused by different microbes (Ma et al. 2015; Zhang et al. 2017; Yao et al. 2018). This morphological observation strengthens the results of compositional analysis and confirms rice straw lignin degradation components by *Ochrobactrum oryzae* BMP03 bacteria strain.

XRD analysis

XRD profile of biotreated and controlled rice straw samples are given in Fig. 5. Similar patterns and shapes are observed in both controlled and biotreated rice straw samples but with different diffraction peak intensity. The crystalline index value increased from 0.324 to 0.488 due to biotreatment. The partial degradation of lignin during biotreatment contributed to the increment leading to higher sugar proportion in biotreated rice straw samples (He et al. 2008; Udeh and Erkurt 2017). Cellulose and hemicellulose are interlinked with lignin in raw rice straw biomass. When bacteria attack lignin bonds, it releases the entangled sugars, which leads to loss of its reluctant nature. This increases the length and degree of polymerization of crystalline cellulose. The crystalline size was changed

Table 3 Overall XRD profiles of biotreated and controlled rice straw samples

XRD parameters	Control	Biotreated
Crystalline index	0.324	0.488
Crystalline length (nm)	2.018	4.354
Degree of polymerization (%)	48.25	67.81

from 2.018 to 4.354 nm and the degree of polymerization was increased from 48.25 to 67.81%. The increment of both length and degree of polymerization articulates increment in cellulose chain length, which further confirms the depolymerization of rice straw biomass into its constituents. The overall XRD profile of biotreated and controlled rice straw samples is given in Table 3. The previous studies on rice straw and corn stover pretreatment of lignin removal showed higher crystallinity value after pretreatment (Wang et al. 2016; Udeh and Erkurt 2017; Yao et al. 2018; Zhang et al. 2018). These results confirm again depolymerization of rice straw lignin by *Ochrobactrum oryzae* BMP03 bacterial strain isolated from wood feeding termite gut.

FTIR analysis

FTIR analysis was used to observe changes in functional groups caused by *Ochrobactrum oryzae* BMP03 strain during biotreatment. The FTIR spectra and characteristics peaks of rice straws are illustrated in Fig. 6 and Table 4, respectively. The appearance of new bands at 1382, 1276, 1200, and 871 cm^{-1} , and the absence of former bands at 1726, 1307, and 1246 cm^{-1} was observed after biotreatment. Beside this, increasing in the percentage of transmittance (decrease in absorbance) was observed in all the other peaks after biotreatment. The absorption band at 3424 cm^{-1}

Fig. 6 FTIR spectra of biotreated and controlled rice straw samples**Table 4** Major FTIR characteristics peaks of biotreated and controlled rice straw samples

Vibrations	Functional groups	Band positions (cm^{-1})	
		Control	Biotreated
O–H stretching	Lignin, cellulose, and hemicellulose	3424	3437
C–O stretching	Lignin	1726	
C–H bending	Cellulose	1482	1500
C–H bending	Cellulose	1430	1452
C–H bending	Cellulose		1382
C–O bending	Lignin	1307	
Acetylated hemicellulose	Hemicellulose		1276
C–O stretching	Lignin	1246	1240
Weak C–O stretching	Cellulose		1200
Glucose ring stretch	Cellulose	1167	1162
C–O stretching	Cellulose		871

obtained from O–H stretching of cellulose, hemicellulose, and aliphatic lignin showed a decrease in absorbance after biotreatment indicating partial solubilization of lignin and release of polysaccharide from rice straw (Wu et al. 2017). Removal of lignin rings was observed in the syringyl ring vibration of C–O stretching at absorption band of 1307 cm^{-1} (Hyun et al. 2003) in the side chain cleavage of lignin at 1726 cm^{-1} of C–O stretching (Xu et al. 2007) and in the guaiacyl ring of C–O stretching at 1246 cm^{-1} (Elisabeth et al. 2007). Besides this, the absorption band at 1517 and 1531 cm^{-1} , which represents aromatic skeletal vibration showed lower absorbance after biotreatment, confirming the partial degradation of lignin from rice straw (Pandey 1999). The b- (1/4) glycosidic linkage at 871 cm^{-1} (He et al. 2008) with characteristics band of cellulose was increased after biotreatment indicating the exposure of cellulose after biotreatment. New bands of cellulose at 1380 cm^{-1} and 1200 cm^{-1} were observed as a result of lignin removal. These results are coherent with XRD, SEM, and compositional analysis results. From this study, we confirmed that *Ochrobactrum oryzae* BMP03 bacteria strain has the capability of degrading lignin and released the entangled cellulose and hemicellulose from rice straw biomass matrix, which enhances the efficiency of hydrolysis and minimizes the reluctant nature of lignin.

Conclusion

The study conducted on biodelignification and hydrolysis of rice straw showed that the significant capacity of bacteria isolated from wood feeding termite guts to remove rice straw

lignin and increase hydrolysis efficiency. These showed the possibilities of biological conversion of lignocellulose biomass to its monomers by bacteria from termite guts. The microstructure of biotreated rice straw samples was modified after lignin removal. About 53.74% of rice straw lignin was removed after the 14th day of biotreatment. In addition, about 69.96% of total reducing sugar was converted to glucose and xylose. Lignin-degrading bacteria preserve polysaccharides (cellulose and hemicellulose). The cellulose and hemicellulose-degrading abilities of the hydrolytic bacteria have the advantage of increasing biofuel yield. The separate delignification and hydrolysis were found to be a good combination to remove lignin and to release the trapped cellulose and hemicellulose from rice straw.

Acknowledgements The authors are thankful to Indian Institute of Technology Roorkee, India, for providing necessary facilities and technical support. Our gratitude will extend to the National Center for Microbial Resources, Pune, India, for performing 16S rRNA sequencing. One of the authors, Bahiru Tsegaye, would like to thank the Ministry of Education of Ethiopia for providing fellowship.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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