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ORIGINAL PAPER

Degradation of native wheat straw lignin by *Streptomyces viridosporus* T7A

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Abstract Lignin is one of the major contributing factors toward the recalcitrance of lignocellulosic biomass. Understanding the process of lignin degradation in natural biological processes will provide useful information to develop novel biomass conversion technologies. Functional group changes in the lignin entities during the process may contribute to the cellulose degradation (utilization) by the microorganisms. In this study, compositional and advanced Fourier transform infrared, pyrolysis gas chromatography/mass spectrometry and ^{13}C cross polarization/magic angle spinning nuclear magnetic resonance analysis were performed to explore the mechanism of biodegradation of wheat straw by *Streptomyces viridosporus* T7A. The results indicated that *S. viridosporus* T7A removed lignin and hemicelluloses as indicated by the increased carbohydrate/lignin ratio. Significant modification of carbonyl and methoxyl groups in the complex lignin structure was also evident. Most importantly, the quantitative results showed that lignin degradation was featured by deduction of guaiacyl unit. The results provide new insight for understanding lignin degradation by bacteria.

Keywords Nuclear magnetic resonance · Fourier transform infrared · Pyrolysis gas chromatography/mass spectrometry · Lignin bio-degradation

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Introduction

Lignocellulosic materials are well recognized as a potential sustainable source of mixed sugars for the production of biofuels and biochemicals (Himmel et al. 2007). Due to the complex structure of cellulose, hemicellulose and lignin within the lignocellulosic matrix, there lies a critical challenge for utilization of these major carbohydrates from the biomass. One of the reasons contributing to the barrier of lignocellulosic biomass' saccharification is the lignin content of the plant cell wall with increased degree of polymerization (Chen et al. 2010; Himmel et al. 2007). The lignin polymers were basically constituted by three types of phenylpropanoid with various proportions: hydroxyphenyl (H), guaiacyl (G) and syringyl (S) (Fig. 1). Evidences point out that lignin embraces and protects cellulose by building physically matrix (Chundawat et al. 2011). Various pretreatment processes, chiefly thermal or chemical, have been developed for delignification to reduce biomass recalcitrance and/or enhance enzymatic hydrolysis efficiency (Mosier et al. 2005; Yang and Wyman 2008). These pretreatments mainly break apart or weaken the lignin and hemicelluloses complex providing the access for cellulases. However, due to the release of heterogenous lignin derivatives and xylooligomers during the thermal or chemical pretreatment processes, the enzyme catalysis becomes inefficient as a result of non-productive hydrophobic absorption of these compounds in cellulases (Kristensen et al. 2007; Yang and Wyman 2006) and subsequent deactivation of cellulases (Pan 2008; Ximenes et al. 2011). Interestingly, recent studies provide evidence that variation in compositional distribution of hydroxyl (H), syringyl (S) and guaiacyl (G) units and functional groups in lignin complex may affect the cellulose hydrolysis more than absolute amount of residual lignin (Li et al. 2010; Studer

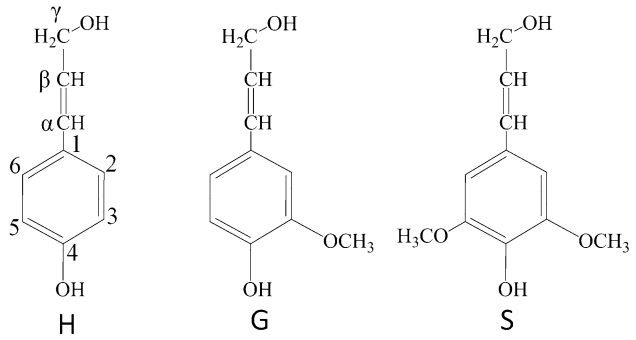

Fig. 1 The primary lignin phenylpropanoids: H, G and S

et al. 2010). It has been revealed that the phenolic hydroxyl groups from lignin complex are found to be more critical than its methoxyl groups during cellulose hydrolysis (Pan 2008). Moreover, a strong negative correlation between sugar release and lignin content was only found for pretreated *Populus* with an S/G ration <2.0, while for higher S/G ratios, negative influence of lignin content was less pronounced (Studer et al. 2010). However, the detailed mechanism about the effects of lignin units' distribution on cell wall and interior chemical bondages on cellulase accessibility are not clear right now.

Unlike thermochemical pretreatment processes, biological degradation essentially overcame the physical and chemical barriers of lignocellulosic complex in order to facilitate the utilization of carbohydrates in lignocellulosic biomass. To unlock that complex, microbes expressed various biological catalysts such as carbohydrate hydrolyzing enzymes (CHE) and ligninolytic enzymes (Chen et al. 2010). The previous studies in *Phanerochaete chrysosporium* revealed that the majority of the proteins secreted during the wheat straw degradation were related to cellulose digestion in addition to lignin degradation and hemicellulose utilization (Singh et al. 2011). It was found that the lignin degrading enzymes were detected at the beginning and CHE were observed subsequently. It suggested that these enzymes worked in a synergetic strategy: ligninases initially targeted phenolic and non-phenolic lignin; the collapse of lignin further facilitated CHE attacking later; finally polysaccharides converted into monomeric sugars and were utilized as a carbon source by the fungus; the consumption of carbohydrate further accelerated the lignin decomposition (Leonowicz et al. 1999). Basically, the degradation and or modification in the functional groups of lignin entities during the biological lignin degradation and subsequent cellulose hydrolysis may provide an insight to develop a better biomass catalysis technology. The biomass enzyme catalysis is one of the costly processes in the biomass-based biorefineries. Investigation of natural biodegradation process will provide useful information to researchers for good understanding the relationship among recalcitrance of lignin structure, the lignin degradation and/or modification and cellulose hydrolysis.

The lignin bio-degradation mechanisms of biological system have been mainly focused on white rot basidiomycetes for many years (Bugg et al. 2011; Odier et al. 1992). The cleavage of *β-O*-4 via breakdown of C_α–C_β linkages was predominant characteristic in the fungal degradation process as main functional results of lignin peroxidase (LiP; EC 1.11.1.14) and Mn peroxidase (MnP; EC 1.11.1.13). It has been found that the ligninolytic enzymes from *Phanerochaete chrysosporium* decomposed lignin substructure model compounds as well as lignin (Tien and Kirk 1983, 1984). The reactions in lignin are oxidative, involving demethylation (or demethoxylation), side-chain oxidation at C_α, propyl side-chain cleavage between C_α and C_β (Chen et al. 1983). Brown-rot fungi are able to remove the hemicellulose and cellulose with only minor modification to the lignin. Consequently, lignin remains a major component of the degraded plant cell wall (Green and Highley 1997). The resulting lignin is demethylated on aryl methoxy groups and contains a greater number of ring hydroxyl groups (Kirk and Highley 1973).

In contrast to white rot and brown-rot fungi, several bacterial species belonged to actinomycetes, α-proteobacteria and γ-proteobacteria also have capability to degrade lignin (Bugg et al. 2011). The catabolic lignin degradation pathway and the corresponding gene clusters were well studied in *Sphingomonas paucimobilis* SYK-6 using various lignin derived biaryls and monoaryls (Masai et al. 2007). It was clear that bacteria mineralized lignin via protocatechuic acid 4, 5-cleavage pathway and the multiple 3MGA catabolic pathways which may be more significant on lignin degradation than previous thought (Bugg et al. 2011). Considering the other advantages, including fast doubling time and easier gene manipulation, the bacterial system could be a better candidate in bio-conversion of lignocellulosic biomass. However, only a few reports addressed the performance of bacterial degradation on the raw lignocellulosic biomass.

Among lignin degrading bacterial species the genus *Streptomyces* showed better performance on lignocellulosic biomass degradation (Crawford 1978; Crawford et al. 1983). *Streptomyces viridosporus* T7A degrades both lignin and carbohydrate. A number of single-ring aromatic intermediates released during the degradation of hardwood, softwood, and grass lignins by *S. viridosporus* also have been identified (Crawford et al. 1983). *S. viridosporus* T7A transformed 30 % of the initial lignin of corn lignocellulose into water soluble acid-precipitated polymeric lignin (APPL) (Crawford et al. 1983). It was also found that this species can highly degrade biologically resistant lignosulfonated compounds at certain cultural condition (Hernandez-perez et al. 1998a, b, 1999). Lignin degradation by

these bacteria was suggested via the oxidation route of both aromatic rings and propyl side-chain lignin carbons (Phelan et al. 1979). It is proven that *S. viridosporus* T7A produces a lignin degrading peroxidase (ALiP-P3) (Crawford 1978) and ALiP-P3 may involve a random-binding bi-reactant system, which differs from the ping pong bi-reactant system typically adapted by the lignin peroxidases originating from the fungus *P. chrysosporium* (Yee and Wood 1997). Based on the model compounds degradation, this bacterial LiP was capable of C_α-oxidation as well as C_β–C_β cleavage of lignin and lignin substructure model compounds. The C_β–C_β cleavage of C_α carbonyl-containing compounds by the bacterial lignin peroxidase was in direct contrast to the action of lignin peroxidase of *P. chrysosporium*, which readily cleaved only C_α-hydroxyl containing compounds (Crawford and Ramachandra 1993). Besides that, three peroxide-induced gene homologs were identified from *S. viridosporus* T7A which may involve in regulating the oxidative lignin biodegradation (Ramachandran et al. 2000). Within the completion of the genome information, its special capability on biodegradation of lignocelluosic biomass will be revealed in the future.

Although the lignin products APPL produced by *S. viridosporus* T7A was characterized in many aspects, researchers have yet to demonstrate the depolymerization of lignin by *S. viridosporus* T7A directly on lignocellulosic substrates degradation. Detailed understanding of biomass chemistry after *S. viridosporus* T7A cultivation can provide crucial information on mimicking an efficient biocatalysis of the lignocellulosic biomass. Therefore, in this study, the biodegradation process of wheat straw by *S. viridosporus* T7A was investigated. The *S. viridosporus* T7A spent biomass was subjected to comprehensive chemical compositional analysis, Fourier transform infrared (FTIR), pyrolysis gas chromatography/mass spectrometry (Py-GC/MS) and ^{13}C cross polarization magic angle spinning (CP-MAS) solid state nuclear magnetic resonance (NMR). Results obtained from this study provide insight into the detailed process of lignocellulosic degradation by the bacterial system. Experiments were carried out at Washington state university of Pullman campus in 2011.

Materials and methods

Streptomyces viridosporus T7A cultivation and solid state fermentation (SSF)

Streptomyces viridosporus T7A was provided by Dr. Lee Deobald (University of Idaho). 20 g of wheat straw (*Triticum sativum*, grown in Moscow, Idaho) in 500-mL Erlenmeyer flasks was autoclaved. Before inoculation, *S. viridosporus* T7A spores were suspended by ISP 4 medium supplemented with 0.6 % yeast extract. The SSF experiments were conducted at 37 °C for a period from 1 to 3 weeks.

FTIR spectroscopy analysis

Surface chemical analysis was conducted using a FTIR spectrometer (Shimadzu) to compare the changes of the samples during different SSF conditions. 32 scans were taken for each sample from 4,000 to 800 cm^{-1} at a resolution of 4 cm^{-1}.

Solid state ^{13}C NMR

Solid state NMR was used to study the native form of the substrate without fractionation or isolation of components for determining the associated chemical changes in the structure. Finely ground samples were used for solid state NMR analysis. 200 mg sample were mixed with 100 µL 20 mg/ml 3-(trimethylsilyl) propionic-2,2,3,4-d_4 acid (TSP) and freeze dried. Thereafter, samples were packed in a 5.0-mm rotor and ^{13}C CP-MAS NMR spectra were recorded at ambient temperature in Bruker DMX 400 spectrometer (NMR center, Washington State University). The techniques of proton-carbon cross polarization (CP), high-power proton decoupling, and magnetic angle spinning (MAS) were combined in solid state CP-MAS NMR analysis. The integrals for each peak were normalized with reference to the internal standard.

Analysis of chemical composition of the biomass

The control and pretreated straw samples were water washed and freeze dried then individually ground. 0.5 g of biomass was extracted with toluene:ethanol (2:1) (at room temperature). The resulting samples were characterized by the two-stage acid hydrolysis method described by Standard Biomass Analytical Procedures (NREL) for determination of lignin and carbohydrate content (Zeng et al. 2010).In briefly, the biomass samples were mixed with 72 % w/w H_2SO_4 at 30 °C for 30 min. After diluting the samples to 4 %, 1 h autoclave was carried out at 121 °C. The solid residue was reported as acid insoluble lignin. The sugar content was determined by ion chromatography using an ion exchange chromatography apparatus (Dionex ICS-300 DC IC). Acid soluble lignin was also measured by UV absorbance at 205 nm with an extinction coefficient of 110 l/g cm (Zimbardi et al. 1999).

Acetyl bromide (AcBr) analysis

The control and *S. viridosporus* T7A pretreated wheat straw (1 g each) were individually frozen (liq. N_2), ground

to powder in a Waring blender, with the resulting powder subjected to successive extraction at room temperature for 8 h each with toluene–EtOH (1:1,100 ml g^{-1}), EtOH (100 ml g^{-1}) and H_2O (100 ml g^{-1}), respectively, and then freeze dried. The resulting extractive-free freeze-dried cell wall residues (CWR) were ball milled for 2 h individually to fine powder with a Fritsch planetary mill (Pulverisette) using agate bowls and balls, and then subjected to acetyl bromide analysis. The AcBr method was performed as described earlier (Iiyama and Wallis 1990) to estimate the lignin content of extractive-free CWR samples for the control and *S. viridosporus* pretreated wheat straw (1, 2 and 3 weeks), respectively.

Pyrolysis GC–MS

To determine the compositional changes, samples were subjected to pyrolysis GC–MS (Py-GC–MS). Py-GC–MS was carried out using a CDS pyroprobe 5000 connected inline to an Agilent GC–MS 6890N. Samples were loaded into a quartz tube and gently packed with quartz wool prior to pyrolysis. The samples were kept briefly in the oven (210 °C) for 1 min to ensure adequate removal of oxygen prior to pyrolysis and were pyrolyzed by heating nearly instantaneously to 600 °C for 1.0 min. The inlet temperature was maintained at 250 °C. The resulting pyrolysis vapors were separated by means of a 30 m × 0.25 µm inner diameter (5 %-phenyl)-methylpolysiloxane non-polar column, with a split ratio of 50:1. The gas flow rate was 1 ml min^{-1}. Linear heating (3 °C min^{-1}) from 40 to 280 °C was designated for the oven program, and to ensure that no residuals were retained, the oven was held at 280 °C for 10 min. The gas was then sent into a mass spectrometer (Agilent Technologies Inert XL MSD) to be analyzed. Carbon dioxide was used as an internal standard. The abundance area (%) of each lignin related compound was referenced against the area (%) of internal standard in each sample (Zeng et al. 2010).

Enzymatic hydrolysis of bio-treated wheat straw

The enzymatic hydrolysis was carried out in 2 % solid loading with 50 mM sodium citrate buffer (pH 5.0) 60 FPU/g cellulase and 120 CBU/g glucosidase were added. The definition of on unit FPU (filter paper unit) is that the dilution of cellulase preparation to a point where 2.0 mg of reducing sugar equivalents is released from filter paper in 1 h at 50 °C and pH 4.8 (Decker et al. 2003). The definition of one unit CBU (cellubiose unit) is based on the international unit: the amount of enzyme to convert 1 µmol/min. The flask was put in a shaking incubator at 50 °C at for 72 h. After hydrolysis, supernatants were collected and used for the sugar analysis. Determination of sugar conversion rate was done by calculating the percentage of released sugars of the samples after enzymatic hydrolysis to respond sugar content in biomass. The enzymes used for enzymatic hydrolysis were cellulase from Trichoderma reesei ATCC 26921 (Sigma) and Novozym 188 (Sigma) as β-glucosidase.

Results and discussion

FTIR spectroscopy

Figure 2 shows the FTIR spectrum of wheat straw after growth with *S. viridosporus* T7A for 1–3 weeks. The FTIR analysis clearly indicates that significant changes of lignin associated functional groups occurred initially during the first week of solid state fermentation. In this regard, the peaks of the spectrum represent the distribution of functional groups and were assigned to the three major components: hemicelluloses, cellulose and lignin in biomass. Most of the peaks were well defined as compared with previous studies (Buta et al. 1989; Faix and Bottcher 1992; Lin and Dence 1992; Pandey and Pitman 2003). The major peaks during the biological degradation have been marked and listed as: (1) 1,120 cm^{-1} for cellulose ring stretch, (2) 1,250 cm^{-1} for vibration of guaiacyl (G) and syringyl ring (S), (3) 1,320–1,330 cm^{-1} for skeletal of syringyl ring, (4) 1,460 cm^{-1} for C–H deformation in lignin and carbohydrates, (5) 1,500 cm^{-1} and (6) 1,595 cm^{-1} for aromatic skeletal in lignin, (7) 1,650 cm^{-1} for C=O conjugated ketone stretch, and (8) 1,730 $cm^{-1}$for unconjugated C=O stretch.

A visible shoulder at around 1,120 cm^{-1} was observed in the spectrum of bio-pretreated wheat straw accompanied with the reduced intensity at 1,730 cm^{-1} of carbonyl groups with increase in pretreatment time. This indicates that the *S. viridosporus* T7A possibly consumed some hemicellulose in the biomass which could result in increased exposure of the cellulosic counterpart. In addition to the vibration of carbohydrate, the lignin related chemical groups were also observed as expected. The intensity of 1,250, 1,330, 1,460, 1,500, 1,595 and 1650 cm^{-1} reflected the notable changes on lignin complex. Since the 1,250 cm^{-1} (2) is attributed to G and S lignin units, the decrease of this peak could signify the unlocking of the lignin cross-links through removal of lignin subunits. Meanwhile, it was also observed that the peak representing the skeleton of the S ring (3) increased which implied the relative increase of S composition in the total lignin complex. Therefore, the vibration at 1,250 cm^{-1} would mainly result from the changes in G related unit. Furthermore, *S. viridosporus* T7A degraded wheat straw had relatively lower conjugated carbonyl

Fig. 2 FTIR spectrum of *Streptomyces viridosporus* T7A bio-degraded wheat straw for 3 weeks

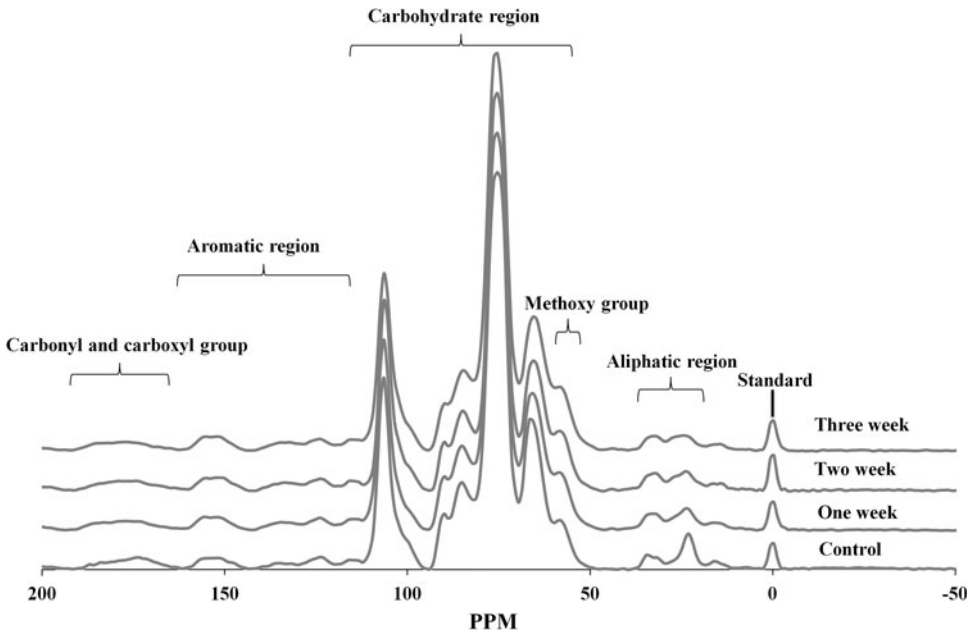

Fig. 3 The solid state ^{13}C CP/MAS spectrum of untreated control and *Streptomyces viridosporus* T7A bio-degraded 1, 2 and 3 week's wheat straw

vibration around 1,650 cm^{-1} compared to the untreated control which suggested the conversion of C=O at α position of lignin. Thus, it can be speculated that *S. viridosporus* T7A produces an aromatic aldehyde oxidase to oxidize aldehyde groups in lignin into corresponding acidic groups (Deobald and Crawford 1989).

^{13}C CP-MAS solid state NMR analysis

The normalized ^{13}C CP-MAS NMR spectrum of control and bio-degraded wheat straws are depicted in Fig. 3. The most predominant assignments for each peak have been listed in Table 1 (Almendros et al. 1992; Gilardi et al. 1995; Sun et al. 2005; Zimbardi et al. 1999). In general, the spectrum was composed of two strong signal regions: carbohydrate region (110–162 ppm) and aromatic region (60–110 ppm). The relative fluctuation of carbohydrate/aromatic ratio reflects the three major chemical compositional changes (Cellulose, hemicelluloses and lignin). Besides, the chemical shift resonances of carbonyl and carboxyl group (162–200 ppm), methoxyl group (52–55 ppm) and carbon in etherified and/or non-etherified

Table 1 The solid state NMR chemical shift assignments

δ (ppm)	Assignment
162–200	Carbonyl and carboxyl group
198	Aromatic carbonyl group (–CO–)
170–178	Aliphatic carbonyl/carboxyl group (–COOR, –COO–)
166	Aromatic carboxyl group (–COO–)
110–162	Aromatic region
157–160	C4 in *p*-coumarate ester/*p*-hydroxyphenl unit
149–152	C3, C5 in etherified syringyl lignin/C3 in etherified guaiacyl lignin
147–149	C3, C5 in non-etherified syringyl lignin/C3 in non-etherified guaiacyl lignin
136–140	C1 in etherified lignin unit
132–134	C1 in non-etherified lignin unit
103–130	C2, C6 in lignin unit/C3, C5 in *p*-hydroxyphenyl lignin unit/C5 in guaiacyl unit
60–110	Carbon in carbohydrate
52–60	Methoxyl in lignin unit
4	TSP (internal standard)

region (132–152 ppm) acts as indicators to determine the structural characteristics of the biomass. Thus, the solid state NMR spectroscopic analysis of both control and bio-degraded wheat straw suggested that pretreatment with *S. viridosporus* T7A led to obvious changes in chemical composition and structural characteristics. Specifically, the gradually diminishing shoulder at a chemical shift of 100 ppm refereed to the well ordered hemicelluloses. In addition, the loss of carbonyl and carboxyl group compared to the untreated control sample strongly pointed out the removal of hemicelluloses and side-chain alterations in lignin complex. Meanwhile, the significant changes of C4 carbon at crystalline cellulose/amorphous cellulose (90–80 ppm) and C6 carbon at crystalline cellulose/amorphous cellulose (70–60 ppm) was observed and suggested the deconstruction of cellulose structure which could be explained as a result of the breakdown of β (1–4) linkages and hydrogen bonds in cellulose complex. This indicated that *S. viridosporus* T7A not only degraded lignin but also released cellulose structures. However, because the background of hemicelluosic carbon widely interfering the spectrum of cellulose, it was very difficult to quantify the content of each component in solid state NMR analysis of biomass. For the lignin part, the chemical shift of 110–130 ppm represented the C2, C6 in syringyl/guaiacyl units and C3, C5 in the *p*-hydroxyphenyl units. And the C3, C5 in non-etherified and/or etherified lignin contributed to the spectrum of 130–160 ppm. The results about aromatic region of spectrum after *S. viridosporus* T7A degradation indicated similar distribution compared with control which meant that the bio-treated samples still kept the basic structure of lignin polymers. However, slight changes were observed around 150 ppm suggesting the modification on etherified lignin. Furthermore, in consistence with FTIR analysis, the enhanced signal intensities of methoxyl group supported the hypothesis of preferential G deduction during *S. viridosporus* T7A-mediated biological degradation process (Table 2). The relative content of carbohydrate component and lignin were measured (Gilardi et al. 1995) and normalized by integration value of internal standard. The decreasing carbohydrate/aromatic ratio of bio-biodegraded sample (Table 2) implied relatively reduced amounts of carbohydrate in comparison to the control wheat straw.

Chemical composition analysis

In order to quantitatively determine the extent of lignin degradation in wheat straw on pretreatment by *S. viridosporus* T7A, acetyl bromide (AcBr) analysis was carried out individually on the control and pretreated wheat straw tissues (1, 2 and 3 weeks). In this regard, AcBr analysis is widely accepted method for the estimation of lignin contents in various plant cell wall residues (CWR) (Iiyama and Wallis 1990). Therefore, extractive-free CWRs of the control and pretreated wheat straw samples (1, 2 and 3 weeks) were individually treated with a reaction mixture consisting of 25 % AcBr by volume in glacial acetic acid containing 4 % of perchloric acid, with the corresponding solubilized materials being individually measured for UV absorptivity (k, 280 nm). An extinction coefficient of 20.09 l g^{-1} cm^{-1} (Iiyama and Wallis 1988, 1990) was employed for lignin content estimation. On applying the standard extinction coefficient to the AcBr analyses, the lignin contents were found to be $\sim$17.65 % (176 mg/g of CWR) for the control and $\sim$16.23 % (162 mg/g of CWR), $\sim$14.83 % (148 mg/g of CWR) and $\sim$14.71 % (147 mg/g of CWR) for the 1, 2 and 3 weeks pretreated wheat straw tissues, respectively. These results indicated that in comparison to the control wheat straw ($\sim$17.65 % of CWR), a gradual decrease in the lignin content ($\sim$16.23 %–14.71 %

Table 2 ^{13}C CP-MAS NMR quantitative analysis of wheat straw after biodegradation

	Aromatic	Carbohydrate	Carbohydrate/aromatic	Methoxy/aromatic	C=O/biomass
Control	8.41	75.29	8.95	0.46	0.06
1 week	7.10	60.08	8.46	0.54	0.03
2 week	7.22	61.57	8.53	0.60	0.03
3 week	7.23	61.28	8.47	0.61	0.03

of CWR) as a result of pretreatment by *S. viridosporus*. This can only be possible if efficient degradation and utilization of lignin occurred in contrast to cellulosic counterpart during the pretreatment process.

To confirm such a possibility, chemical composition analysis (two-stage acid hydrolysis) on both the control and 3-week pretreated wheat straw tissues was further carried out for evaluating the relative amount of cellulose, hemicellulose and acid soluble and acid insoluble Klason lignin, respectively (Table 3). The estimated/putative monomeric sugar and Klason lignin content for the control and 3 week pretreated wheat straw is summarized in Table 3. As can be seen, the glucose release of the 3-week pretreated sample under acid hydrolysis conditions increased in comparison to the control wheat straw. This elevated level of glucose release indicated the relative increase in cellulose content in the wheat straw samples after pretreatment. The decreased level of corresponding cleavable products obtained from acid hydrolysis i.e., mannose, xylose, arabinose and galactose monomers, which are released from the hemicellulosic counterpart, also provided evidence for removal of hemicellulose as same as the results of FTIR and solid state NMR.

Py-GC/MS analysis

Pyrolysis is a thermochemical technique that can deconstruct lignocellulosic complex into small organic molecules at a certain temperature. GC/MS allows for separation and identification of these organic molecules through selected *m/z* ratio and retention time. As an advanced analytical technique, Py-GC/MS is widely used to characterize the chemical distribution changes of lignocellulosic biomass (Camarero et al. 1994; Zeng et al. 2011). In this study, PyGC/MS was applied to analyze the lignin compositional changes in (A) untreated wheat straw control and (B) *S. viridosporus* T7A spent wheat straw (Fig. 4). The major lignin derivative compounds were generated from hydroxyphenyl (H), guaiacyl (G) and syringyl (S) phenylpropanoid including: phenol; phenol, 2-methyl; phenol, 4-methyl-; phenol, 2-methoxy-; *p*-cresol, 2-methoxy-; phenol, 4-ethyl-2-methoxyl-; 2-methoxy-4-vinylphenol; phenol, 2,6-dimethoxy-; isoeugenol; 3′,5′-dimethoxyacetophenon; phenol, 2,6-dimethoxy-4-[2-propenyl]-; methoxyeugenol and acetosyringone. The pyrogram showed that the biopretreated wheat straw shared a similar chemical distribution with untreated wheat straw control. However, the quantitative analysis suggested that *S. viridosporus* caused significant changes in lignin and carbohydrate composition (Table 4). Improvement of the levoglucosan/lignin ratio (from 0.716 to 1.02) was in agreement with chemical composition analysis (Table 3) which supported the idea that relative enhancement of cellulose content resulted from removal of lignin and hemicellulose during *S. viridosporus* T7A-mediated biopretreatment. In addition, the S/G ratio of the three week sample was increased from 0.358 to 0.620 (>40 %). The degradation of *P. chrysosporium* on wheat straw also showed a similar trend of G unit reduction (Singh et al. 2011; Zeng et al. 2010). It needed to note that *S. viridosporus* T7A was proven to produce water soluble acid-precipitable polymeric lignin with increased phenolic G content and decreased methoxy group compared with native lignin (Crawford et al. 1983; Hernández-Coronado et al. 1997; Rodriguez et al. 1997). Therefore, the conversion of G units to soluble lignin derivatives could explain the loss in solid residues after pretreatment. The results showed here were distinguished with popular understandings: (1) S unit were more easily degraded than G unit due to the lower redox potential and (2) The linear arrangement of S units by *β-O*-4′ linkages were more attackable than G units which provided one more position for connecting. The reasonable explanations could come to the structural differences of lignin on cell wall among various lignocellulosic biomasses. The straw lignin of herbaceous crops contained all three lignin units with certain degree of acetylation which distinguished with the structure of woody biomass (Buranov and Mazza 2008). The quantitative analysis revealed that 20 % *β-O*-4′ linkage of wheat straw lignin was contributed by G type free phenolic lignin (Camarero et al. 1994; Lapierre et al. 1988). Compared with S units, the phenolic G lignin has less redox potential and was easily oxidized by ligninolytic enzymes. Furthermore, the ALiP-P3 produced by *S. viridosporus* T7A oxidized phenolic compounds rather than oxidizing non-phenolic substrates (Spiker et al. 1992). In addition, the enzymatic hydrolysis of *S. viridosporus* T7A

Table 3 Chemical composition (%) of biologically treated and untreated wheat straw samples

	Glu	Mann/xyl	Ara	Gala	Lignin		Ash
					AIL	ASL	
Control	38.24 ± 0.7	21.20 ± 1.2	4.84 ± 0.5	1.02 ± 0.1	16.6 ± 0.5	1.2 ± 0.2	3.7 ± 0.8
3 week	42.01 ± 0.3	15.69 ± 0.7	3.4 ± 0.6	0.51 ± 0.4	14.5 ± 1.1	1.1 ± 0.3	4.3 ± 0.5

The data presents triplicate experiments

Glu glucose, *Mann/xyl* mannose/xylose, *Ara* arabinose, *AIL* acid insoluble lignin, *ASL* acid soluble lignin, *Gala* galactose

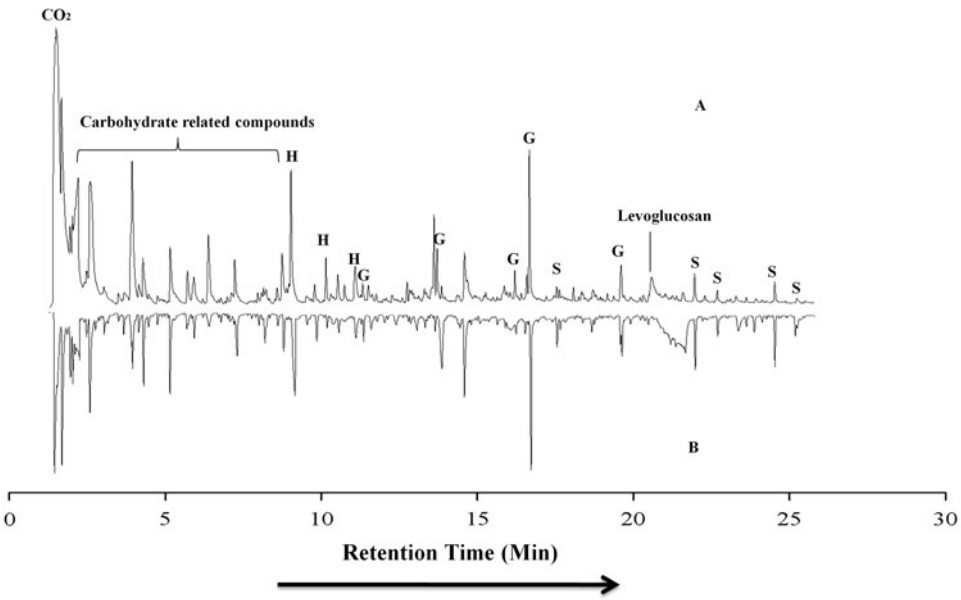

Fig. 4 Pyrograms of untreated control (**a**) and *Streptomyces viridosporus* T7A bio-degraded 3 week's wheat straw (**b**). The markers of *H*, *G*, *S* represented hydroxylphenyl, guaiacyl and syringyl derivative lignin unit

Table 4 Py-GC/MS analysis of lignin and carbohydrate derived compounds in *Streptomyces viridosporus* degraded wheat straw

Number	Retention time	Name	Control	Sample	MW	Lignin type	Ion Pair
1	1.58	Carbon dioxide	1.000	1.000	44		44
2	8.91	Phenol	0.015	0.067	94	H	94,66
3	10.71	Phenol, 2-methyl	0.009	0.015	108	H	108,107
4	11.26	Phenol, 4-methyl-	0.017	0.032	108	H	108,107
5	11.53	Phenol, 2-methoxy-	0.005	0.024	124	G	124,109
6	14.05	*P*-cresol, 2-methoxy-	0.003	0.018	138	G	123,138
7	16.06	Phenol, 4-ethyl-2-methoxy-	0.003	0.014	152	G	152,137
8	16.87	2-Methoxy-4-vinylphenol	0.032	0.115	150	G	135,150
9	17.72	Phenol, 2,6-dimethoxy-	0.004	0.030	154	S	154,139
11	18.93	Isoeugenol	0.007	0.028	164	G	164,77
10	21.02	Levoglucosan	0.078	0.444	162	C	57,60
12	22.15	3′,5′-Dimethoxyacetophenone	0.004	0.021	180	S	165,180
13	22.87	Methoxyeugenol	0.006	0.046	194	S	194,91
15	25.36	Acetosyringone	0.003	0.025	196	S	181,196
		Approximate total lignin ratio	0.109	0.435			
		Approximate total carbohydrate/lignin ratio	0.716	1.02			
		Approximate total H%	37.9	26.1			
		Approximate total G%	45.7	45.6			
		Approximate total S%	16.4	28.3			
		Approximate S/G ratio	0.358	0.620			

The data presents as average number by triplicate experiments

H para-hydroxyl lignin, *G* guaiacyl lignin, *S* syringyl lignin, *C* carbohydrate

treated wheat straw showed that conversion rate of glucose was increased 80 % while the xylose's conversion rate was similar (Fig. 5). This result, in consistence with FTIR, NMR, demonstrated that the consumption of hemicelluloses and modification on cellulose. It is speculated that the deconstruction of G unit lignin on wheat straw was the results of oxidation phenolic G lignin units which further facilitated the utilization of hemicelluose and celluose. However, due to the compositional complexity in lignocellulosic biomass, the heterogeneously distributed thermal reaction with interaction of cellulose, hemicelluloses and lignin, will definitely influence the accuracy of Py-GC/MS

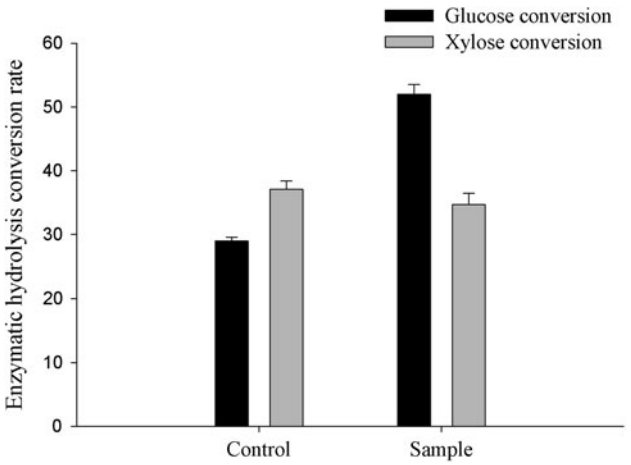

Fig. 5 The enzymatic hydrolysis of *Streptomyces viridosporus* T7A bio-degraded 3 week's wheat straw

analysis. Current results was also not able to answer the G deconstruction location happened in the lignin complex. Therefore, comprehensive structural analysis at the sub-structural level of isolated lignin therefore needs to be done and is currently underway to further verify the observed critical modification within the lignin assembly.

Conclusion

In this study, we investigated the degradation pattern of wheat straw by *S. viridosporus* T7A. Significant changes on lignin structures after bio-pretreatment were measured by FTIR, Py-GC/MS and Solid state NMR analysis. Besides the relative reduction in content, the degradation and/or modification on lignin units were reflected by an increased S/G ratio, reduction of carbonyl groups and enhancement of methoxyl groups. This study provided new information towards elucidation of the mechanisms involved in biological degradation processes on wheat straw.

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