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ORIGINAL PAPER

Mushroom spent straw: a potential substrate for an ethanol-based biorefinery

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Abstract Rice straw (RS) is an important lignocellulosic biomass with nearly 800 million dry tons produced annually worldwide. RS has immense potential as a lignocellulosic feedstock for making renewable fuels and chemicals in a biorefinery. However, because of its natural recalcitrance, RS needs thermochemical treatment prior to further biological processing. Ammonia fiber expansion (AFEX) is a leading biomass pretreatment process utilizing concentrated/liquefied ammonia to pretreat lignocellulosic biomass at moderate temperatures (70–140°C). Previous research has shown improved cellulose and hemicellulose conversions upon AFEX treatment of RS at 2:1 ammonia to biomass (w/w) loading, 40% moisture (dwb) and 90°C. However, there is still scope for further improvement. Fungal pretreatment of lignocellulosics is an important biological pretreatment method that has not received much attention in the past. A few reasons for ignoring fungal-based pretreatments are substantial loss in cellulose and hemicellulose content and longer pretreatment times that reduce overall productivity. However, the sugar loss can be minimized through use of white-rot fungi (e.g. *Pleutorus ostreatus*) over a much shorter duration of pretreatment time. It was found that mushroom spent RS prior to AFEX allowed reduction in thermochemical treatment severity, while resulting in 15% higher glucan conversions than RS pretreated with AFEX alone. In this work, we report the effect of fungal conditioning of RS followed by AFEX pretreatment and enzymatic hydrolysis. The recovery of other byproducts from the fungal conditioning process such as fungal enzymes and mushrooms are also discussed.

Keywords Mushroom spent rice straw · Cellulase enzymatic hydrolysis · AFEX pretreatment · Lignocellulosic biomass

Introduction

There is a growing need to find other alternatives to crude oil as the primary feedstock for the chemicals and fuels industry [1]. Ethanol has many desirable features as a petroleum substitute and could help make a smoother transition from a petroleum-based to a biobased chemical economy [2, 3]. Ethanol is produced in large quantities from natural resources including corn grain and sugarcane juice. However, there is a need to find an inexpensive and widely available lignocellulosic source of biomass [i.e., corn stover, rice straw (RS), wheat straw] to avoid feedstock conflict with the prevalent food industry [3]. Lignocellulosic recalcitrance is one of the primary impediments in the successful implementation of a cellulosic ethanol-based biorefinery. Pretreatment of biomass to reduce this intrinsic recalcitrance is critical to help improve bioconversion.

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therefore has great potential as a lignocellulosic feedstock for making renewable fuels and chemicals. However, RS appears to be more recalcitrant [4] than other agricultural residues like corn stover. For example, about two-thirds of ammonia fiber expansion (AFEX)-pretreated RS glucan was hydrolyzed to glucose [4], while we see almost 95% glucan conversion for AFEX-treated corn stover under similar hydrolysis conditions [3].

Biological conditioning is an area that has been looked at very closely in recent years due to several advantages that it offers to improve the quality of feedstock [5]. Some of the most important advantages of biological pretreatment are the lower energy requirements for processing higher yields and fewer hydrolysis and fermentation inhibitors produced during pretreatment [6, 7]. The most promising microbes for biological pretreatment are white-rot fungi that enhance enzymatic hydrolysis of biomass due to selective degradation of recalcitrant lignin [8, 9]. Fungal growth on lignocellulosics has been known for several centuries and has been used traditionally for producing edible mushrooms [10]. There were over three million metric tons of edible mushroom produced worldwide in 2002 (with over 200 species) using a wide variety of biomass [11, 12]. The world market for the mushroom industry in 2005 was valued at over $45 billion [13, 14]. RS is one of the biomass materials used extensively for growing oyster mushrooms next to composted wood chips [15, 16]. A large amount of mushroom spent straw (MSS) is currently being generated during the process and is used for various applications such as fire wood substitute [17], land-filling materials, compost materials [18], animal feed [19, 20] and bioplastic feedstock [21].

The removal of lignin and/or hemicellulose can substantially reduce the recalcitrance of biomass to enzymatic hydrolysis [1]. Studies have shown that AFEX pretreatment helps improve enzymatic digestibility several fold over untreated lignocellulosic biomass [2, 3]. AFEX pretreatment results in the decrystallization of cellulose, partial depolymerization of hemicellulose, deacetylation of acetyl groups [22], cleavage of lignin carbohydrate complex (LCC) linkages, lignin C–O–C bond cleavage, increase in accessible surface area due to structural disruption [23] and increased wettability of the treated biomass [24]. The AFEX process demonstrates attractive economics compared to several leading pretreatment technologies based on a recent economic model [25] for bioethanol from corn stover.

AFEX has been less effective for biomass such as hardwood and softwood that have much higher lignin content (e.g., 25–30%) compared to agricultural residues (e.g., 5–20%). One possible reason for this is the lignin seal present in recalcitrant woody biomass that prevents effective diffusion and reaction of ammonia with the cell wall ultrastructural components (i.e., lignin–cellulose–hemicellulose matrix). To make the AFEX more effective on such highly recalcitrant biomass, some sort of prestructural modification of the biomass is necessary prior to AFEX pretreatment. Biological pretreatment is one such prospective process due to the several advantages associated with it. In this article, we have demonstrated that treating RS with a white-rot fungus (*Pleurotus ostreatus*) followed by AFEX gives significantly higher glucan conversion at less severe pretreatment conditions than treating RS directly with AFEX. In addition, we have also studied some valuable byproducts produced during fungal pretreatment, such as mushrooms, proteins and organic acids.

Materials and methods

Mushroom spent straw

Pleurotus ostreatus grown on RS patches were purchased from Fungi perfecti, Olympia, WA. These patches were kept at 25°C and at >80% humidity environment chamber with an appropriate flux of light. Oyster mushrooms were harvested ~55 g (dry weight basis) from each patch after 23 days and MSS was either stored in a −20°C freezer or processed the same day.

Water washing of untreated/AFEX-treated biomass

Untreated RS (UT-RS) (same batch used for growing mushrooms) and MSS were presoaked and washed in distilled (deionized) water with a substrate to water loading of 1:10 (w/w). The slurry was mixed for 15 min. The wash liquid was removed from the substrate by squeezing the slurry through a filtration cloth (Calbiochem, CA) and stored in the refrigerator for further analysis. The washed substrates were air-dried to 40% moisture and stored at −20°C until further use. Freezing the biomass for a short period of time did not show any significant effect on pretreatment and hydrolysis. The moisture content of the washed substrate was determined using a moisture analyzer (Model MF-50, A&D). The moisture content was also determined by drying the sample at 110°C till constant weight was reached.

Mass balance for water washing

A mass balance for determining the solids and soluble content of the wash liquid stream was carried out according to the procedure outlined by Chandawat et al. [26]. UT-RS and MSS (200 g dry BM each) were soaked and stirred in distilled (deionized) water with a substrate to water loading of 1:10 for 15 min. The wash liquid was removed from the

substrate by filtering the slurry through a filtration cloth (Calbiochem, CA) and centrifuged at 9,000 rpm to remove fine solid particles from the wash stream, then concentrated using membrane filtration (10 kDa) in a stirred cell from Millipore, Billerica. The retentate (which contains mostly protein >10 kDa, lignin polymers and oligosaccharides) was lyophilized and stored for protein estimation and other analysis. The moisture content of the sample was determined using a lab vacuum oven-operated at 105°C. Small aliquots of the filtrate were stored at −20°C until further liquid chromatography-mass spectrometry (LC-MS) analysis.

Wash stream analysis by liquid chromatography-mass spectrometry

The LC-MS system used in this work consisted of a Shimadzu HPLC (LC-20AD pump) coupled to a Waters LCT Premier Mass Spectrometer Time-of-Flight Mass Spectrometry (TOF-MS). UT-RS- and MSS-washed liquid stream were analyzed by flow injection analysis by direct introduction using a flow of 50% acetonitrile and 50% 0.15% aqueous formic acid. The same solutions were analyzed by LC-MS using a Hypersil Gold C_{18} (50 × 2.1 mm, 1.9 μm) reversed-phase column (Thermo, USA). Gradient elution was carried out using aqueous 0.15% formic acid (v/v; solvent A) and acetonitrile (v/v; solvent B). Gradient conditions were as follows: initial 99%A/1%B, held for 2 min; linear gradient to 75%A/25%B at 4 min with a hold until 6 min, followed by a linear gradient to the initial conditions at 8 min. Injection volume, column temperature and flow rate were 10 μL, 30°C and 0.25 ml/min, respectively. All mass spectra were acquired using electro spray ionization (ESI). Spectra were acquired in both positive and negative ion modes.

Composition analysis

Lignin and sugar content was analyzed using standard analytical LAP protocols. Protein content in the retentate was measured using the Kjeldahl method by Dairy One (Ithaka, NY) [27]. Further analysis was also done for UT-RS and MSS using standard National Renewable Energy Laboratory (NREL) LAP protocols [28] using acid hydrolysis followed by HPLC analysis as discussed below (Table 1).

HPLC sugar analysis

A high performance liquid chromatography (HPLC) system was used for monomeric sugar analysis. The HPLC system consisted of Waters (Milford, MA) Pump and Waters 410 refractive index detector, an Aminex HPX-87P carbohydrate analysis column (BioRad, Hercules, CA) equipped with a deashing guard cartridge (BioRad). Degassed HPLC grade water was used as the mobile phase at 0.6 ml/min at a column temperature of 85°C. The injection volume was 20 μl with a run time of 20 min. Mixed sugar standards were used for quantification of cellobiose and other monosaccharides (glucose, xylose, galactose, arabinose and mannose) in the samples.

Table 1 Composition analysis of untreated rice straw (UT-RS) and mushroom spent straw (MSS)

Components	UT-RS (%)	MSS (%)
Glucan	36.99	28.40
Xylan	20.49	16.38
Galactan	2.08	1.94
Arabinan	3.60	2.72
Mannan	0.00	0.91
Klason lignin	11.63	10.12
Acid-soluble lignin	1.20	1.08
Ash	14.24	25.24
Acetic acid	2.78	1.92
Extractive	7.66	4.52

Acid-soluble lignin of the samples has been calculated by taking absorptivity (30.2) corresponding to corn stover

AFEX pretreatment

UT-RS and MSS were pretreated by the AFEX pretreatment process. The biomass with varying amounts of moisture (40–120%, on dry weight basis) was transferred to a high-pressure Parr reactor and liquid ammonia (1 or 0.5 kg of ammonia/kg of dry biomass) was slowly charged to the vessel. The temperature was raised and maintained at the desired value (e.g., 100°C) for 5 min residence time before explosively relieving the pressure. It took approximately 30–40 min to complete one cycle of the pretreatment process. The instantaneous drop of pressure in the vessel caused the ammonia to vaporize, causing an explosive decompression of the biomass and considerable fiber disruption. The pretreated material was allowed to stand under the hood overnight to remove the residual ammonia and stored in a freezer until further use.

Enzymatic hydrolysis

The NREL standard protocol (LAP-009) was followed for enzymatic hydrolysis of the biomass [28]. Cellulase (Spezyme CP) and xylanase (Multifect) enzymes were a generous gift from Genencor International (Rochester, NY). The substrate was hydrolyzed at a glucan loading of 1% (w:v) in a 0.05 molar citrate buffer solution (pH 4.8) at the desired cellulase enzyme loading (protein concentration 123 mg/ml) of 15 FPU/g glucan and β-glucosidase (Sigma, St Louis,

MO) loading of 64 *p*NPGU/g glucan. Xylanase (protein concentration 42 mg/ml) supplementation was carried out at 10% of the total milligrams of cellulase protein loaded. The protein concentration of the enzymes was determined by the BCA protein assay (Pierce, Rockford, IL). Samples were hydrolyzed at 50°C with gentle agitation (90 rpm) for a period of 72 and 168 h. The hydrolyzed samples were boiled to denature the enzymes and filtered through a 0.2-µm nylon membrane filter at predetermined time periods (72 and 168 h). The samples were frozen until subsequent HPLC sugar analysis.

Results and discussion

White-rot fungi are known to degrade the lignin polymer present in lignocellulosic biomass, while utilizing free sugars and cohydrolyzed oligosaccharides for growth of their fruiting bodies [8]. Substrate processed by fungal conditioning have several advantages that include, forgoing significant particle size reduction, improving susceptibility of cellulosic and hemicellulosic polysaccharides to subsequent hydrolysis, potential to reduce severity of the thermochemical pretreatment step that could save substantial amounts of energy and chemicals. A major reduction in cost of pretreatment would have a significant impact on the overall cost of ethanol production in a biorefinery.

Traditionally, oyster mushrooms are cultivated using sterile RS packed in a polythene bag with spawns distributed at regular intervals followed by storing the bag in humid environment (>80%) at 25°C for a period of 35–40 days. About 100 g of mushroom [dry weight basis (dwb)] can be harvested at three intervals starting from 1 kg RS (dwb) (Fig. 1a). Harvest period and the substrate composition on different days during mushroom growth are shown in Fig. 1b. To conserve both cellulose and hemicellulose for getting maximum sugars yield, in this work, microbial conditioning using *Pleurotus ostreatus* was stopped after 23 days. Proteins, organic acids, soluble lignin compounds and oligosaccharides were extracted from MSS during the washing step. The higher molecular weight components (>10 kDa) namely enzymes, soluble lignin polymer and oligosaccharides were removed using membrane filtration as the retentate fraction, while the low molecular weight components like lignin degradation products, organic acids, lower molecular weight enzymes/proteins were obtained as the filtrate fraction. A detailed mass balance for the above washing protocol is shown in Fig. 2.

There are several advantages in using MSS because it is easily available from the currently well-established edible-mushroom industry. The microbes partially degrade the lignin using various combinations of enzymes (i.e., oxidases, laccases) leaving behind a hemicellulose- and cellulose-

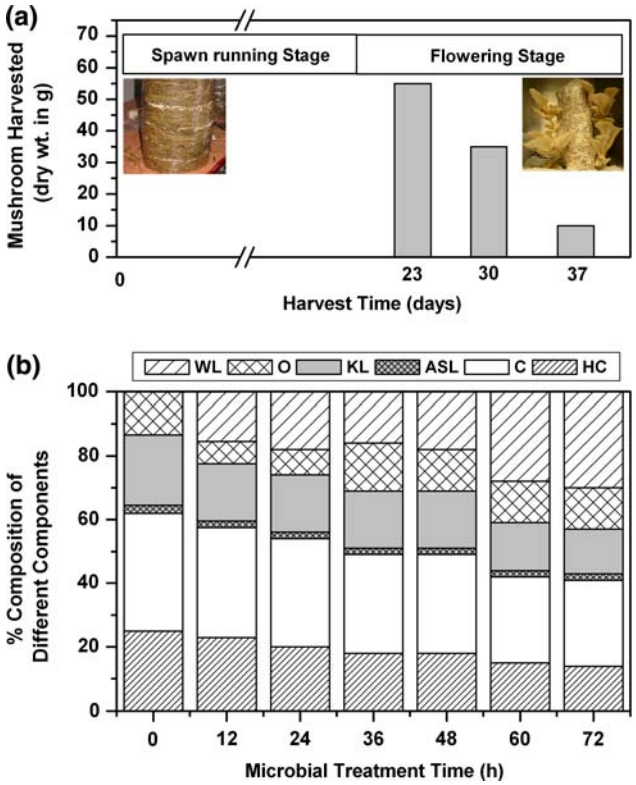

Fig. 1 Harvest period and the substrate composition on different days during mushroom growth. **a** Two different stages of mushroom production, namely, spawn running stage and flowering stage. In addition, the figure shows how mushrooms (dwb) are harvested at three different intervals in a commercial mushroom farm by maintaining sterile rice straw (1 kg on dwb) with mushroom spawns in a polythene bag at 25°C and >80% moisture conditions. **b** Changes in percentage composition of components of rice straw after pretreatment with *P. ostreatus* at different days. Components: *HC* hemicellulose, *C* cellulose, *ASL* acid-soluble lignin, *KL* klason lignin; *O* others (mainly *ash*), *WL* weight loss after pretreatment. (Fig. 1B, reproduced with permission from Taniguchi et al. (2005) J Biosci Bioeng 100:637–643)

enriched fraction that can be easily recovered for subsequent thermochemical pretreatment and enzymatic hydrolysis. Potential for reducing severity of thermochemical pretreatment has been demonstrated for microbial pretreated biomass in previous work [7]. A substantial change in composition (Fig. 1b) was also noticed during the fungal conditioning process [8]. A combination of key enzymes like laccase, manganese peroxidase, lignin peroxidase and phenol oxidase are secreted by white rot fungus that synergistically degrade lignin [5]. Since lignin is a significant barrier to enzymatic hydrolysis, removing it prior to hydrolysis would give higher sugar yields. As expected, when MSS was used without pretreatment, we could get glucan conversion up to 40%, using standard commercial cellulase loading (15 FPU/g glucan), compared to just 20% for UT-RS. This 40% conversion is probably not economical for a biorefinery and further thermochemical pretreatment is necessary for getting higher sugar yields.

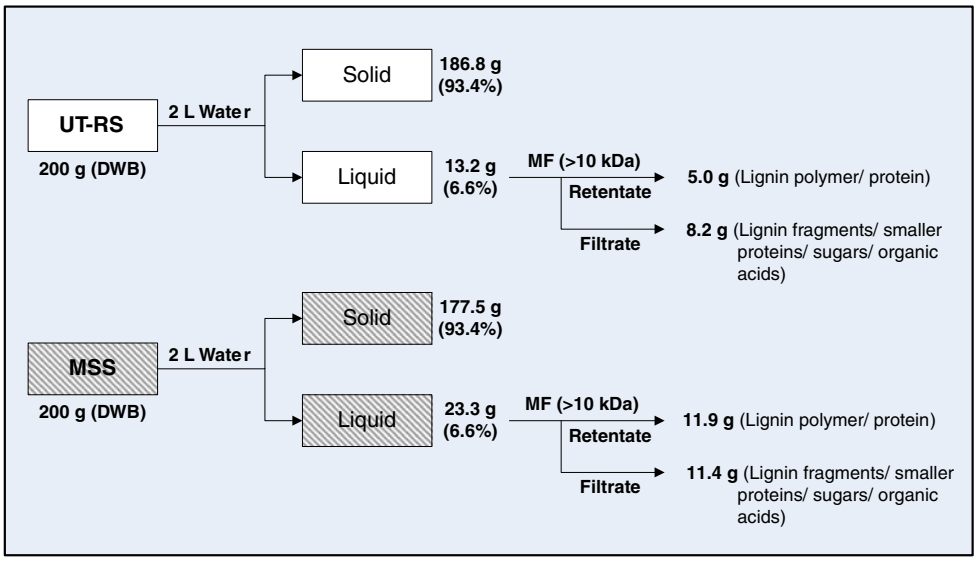

Fig. 2 Overall mass balance during washing step for untreated rice straw (UT-RS) and mushroom spent rice straw (MSS) into liquid and solid fraction (% of initial dry weight). Liquid stream was further separated into two streams namely retentate and filtrate fraction using membrane filtration (MF) with >10 kDa cutoff

The morphology of UT-RS and MSS before and after AFEX treatment is shown in Fig. 3. The MSS and AFEX-MSS are darker, softer and more fragile compared to the UT-RS and AFEX-RS respectively. One possible reason for the darker color of AFEX-MSS is due to solubilization and redeposition of lignin on the surface of the biomass during the AFEX process [26]. The darker color of MSS compared to UT-RS could be due to oxidation of lignin-degraded products during fungal treatment. Less severe AFEX conditions are required to achieve higher glucan conversions for MSS compared to UT-RS. By our approach, the MSS could be easily hydrolyzed at lower pretreatment severities (less ammonia and/or lower temperature) using AFEX.

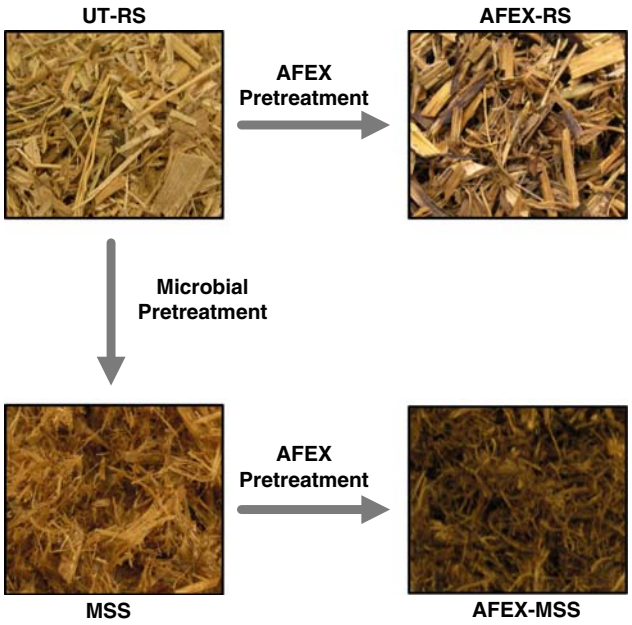

Fig. 3 Picture showing untreated rice straw (UT-RS), AFEX-treated RS (AFEX-RS), mushroom spent substrate (MSS), and AFEX-treated MSS (AFEX-MSS). The MSS and AFEX-MSS are darker (as explained in the text), softer and more fragile compared to the UT-RS and AFEX-RS respectively

In one of our previous studies, pretreatment of RS using AFEX [at 2:1 ammonia to biomass (w/w), 40% moisture, 90°C] could achieve a maximum of 70–80% glucan conversion [4] using a much higher cellulase loading of 75 FPU/g glucan. In the new approach, we fixed the reaction temperature at 100°C for two different ammonia to biomass loadings (i.e., 1:1 or 0.5:1) and varied the moisture between 40, 80 and 120% (dwb). We used both UT-RS and mushroom spent RS (MSS) using *Pleurotus ostreatus* strain for our experiments. For AFEX-treated MSS (optimum conditions, 1:1 ammonia loading, 80% moisture, 100°C), we could achieve close to 92% glucan and 55% xylan conversion using just 15 FPU/g glucan loading of cellulase enzyme after 168 h (Fig. 4). The UT-RS, under similar conditions, gave close to 82% of glucan and 50% xylan conversion. One probable reason for higher glucan conversions could be improved accessibility to chemicals and enzymes due to fungal preconditioning. When the ammonia loadings were lowered from 1 to 0.5, glucan and xylan conversion dropped by 10–15% and 5–8%, respectively, both for UT-RS and MSS, but this loss in conversion could be overcome by adding more cellulases (data not shown).

On the other hand, xylan conversions for both UT-RS and MSS were low compared to our previous experience with AFEX-treated corn stover where we got close to 75% conversion. One possible reason could be due to insufficient hemicellulase activities in the commercial cellulase cocktail [26]. To overcome this problem, we supplemented cellulase with Multifect xylanase (at 10% of cellulase mass loading) and we saw significant increase in both glucan (5–7%) and xylan (20–25%) conversions for AFEX-treated

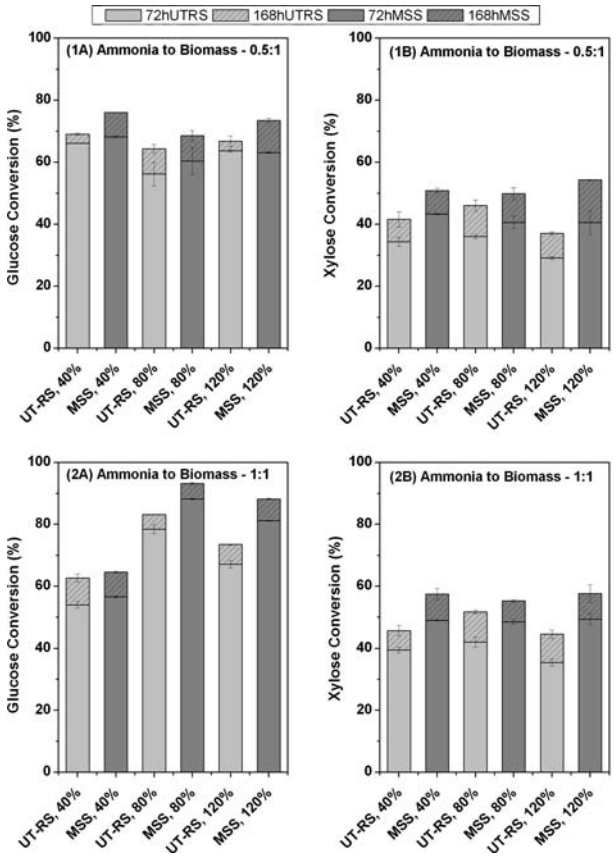

Fig. 4 Enzymatic hydrolysis of untreated rice straw (UT-RS) and mushroom spent rice straw (MSS) for different ammonia to biomass ratio of (1) 0.5:1 and (2) 1:1, respectively, using 15 FPU of cellulase loading/gram of glucan. The glucan and xylan conversion are shown on *left* and *right*, respectively

UT-RS and MSS. The results are shown in Fig. 5. A slightly higher xylan conversion was noticed for MSS compared to UT-RS. At the end of the hydrolysis process, about 10–14% of silica from RS is left behind, which could be a valuable inorganic resources for some other applications [29].

The lignin degrading enzymes (i.e., laccases, peroxidases) produced by *Pleurotus ostreatus* grown on RS have been well studied [5]. Some of the other byproducts such as organic acids are typically found in fungal fruiting body [30]. Results have shown that the most abundant organic acids present in the fungal-treated RS were oxalic, citric, malic, quinic and fumaric acids. We were interested to see how much organic acid could be recovered from the MSS after harvesting the mushrooms. The retentate fraction after membrane filtration had molecular weight components larger than 10 kDa. The protein components present in the retentate are the enzymes secreted by *Pleurotus ostreatus*. Earlier studies have shown that the extracted enzymes [31, 32] have a wide range of applications in degrading xenobiotic compounds and aromatic compounds in dye industry.

The electrospray ionization (ESI) mass spectra of UT-RS and MSS samples were generated using flow injection analysis without chromatographic separation. Figure 6 shows the comparison of ESI mass spectra in negative and positive ion modes. The spectra are normalized such that 100% corresponds to the intensity of the most abundant ion (8.08×10^4 in negative ion mode and 2.62×10^3 in positive ion mode). In subsequent LC-MS analyses, extracted ion chromatograms were generated for each identified

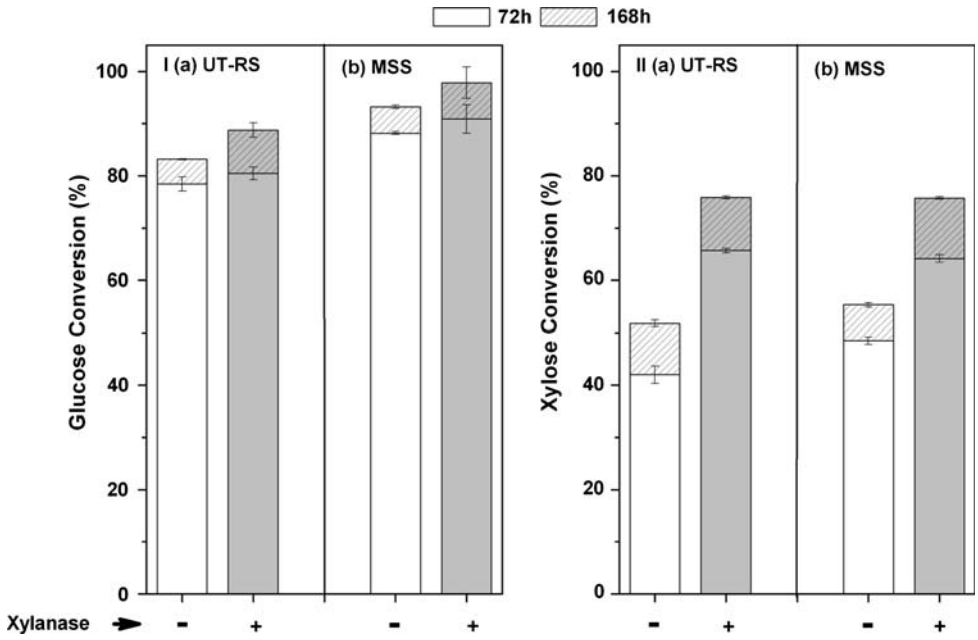

Fig. 5 Enzymatic hydrolysis of (**a**) UT-RS and (**b**) MSS for the best AFEX conditions (100°C, 1:1 ammonia to biomass ratio, 80% moisture) using 15 FPU of cellulase loading/gram of glucan and with Multifect xylanase supplementation (10% as that of cellulase protein loading). Here glucan conversion (I) is shown on the *left* and xylan conversion (II) is shown on the *right*

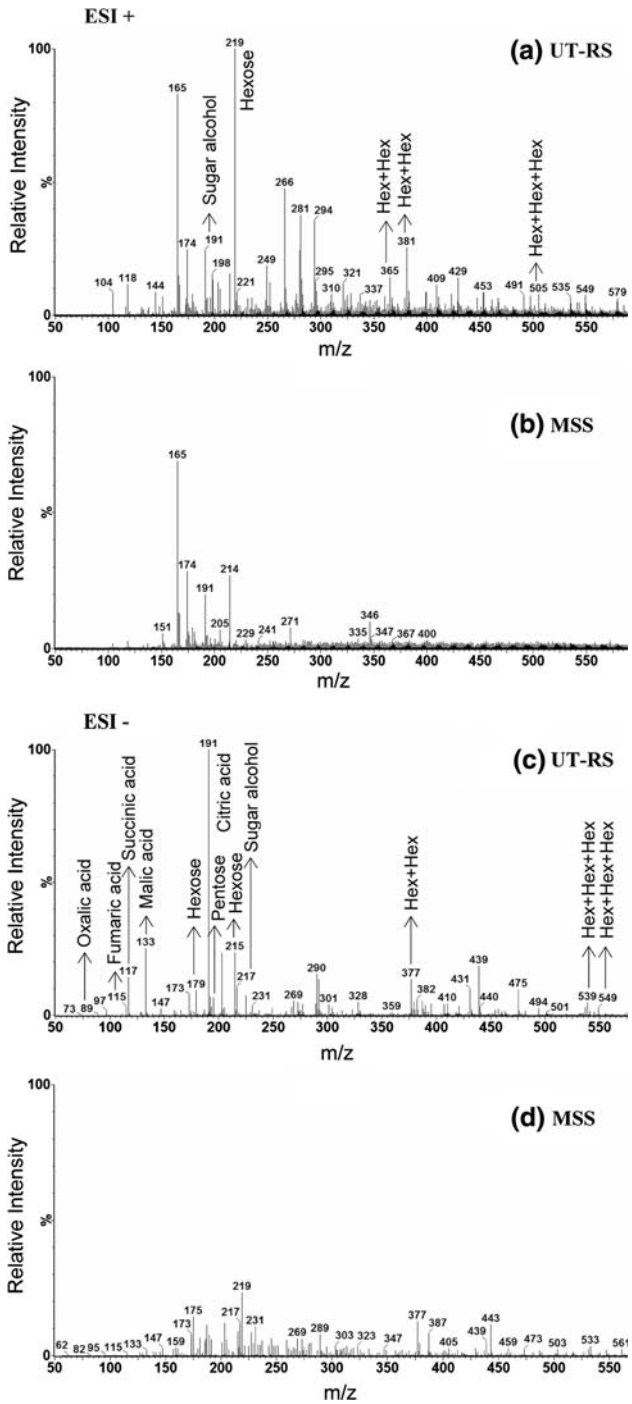

Fig. 6 Comparison of flow injection analysis electrospray ionization mass spectra of rice straw extracts: **a** positive mode spectrum of untreated rice straw (UT-RS), **b** positive mode spectrum of mushroom spent straw (MSS), **c** negative mode spectrum of UT-RS, **d** negative mode spectrum of MSS

compound based on deprotonated ($[M\text{-}H]^-$) ions and in some cases characteristic adduct such as $[M + \text{formate}]^-$. Table 2 tabulates the relative peak areas in treated and untreated samples. Sugars were not retained by the column as was expected because of high polarity.

Table 2 Comparing chromatographic areas of some important organic acids and saccharides in UT-RS and MSS-treated samples

Compound	Area (UT-RS)	Area (MSS)	Area (UT-RS)/(MSS)	% reduction
Sugar alcohol	264.4	348.1	0.76	–
Hex + Hex	478.9	392.9	1.2	17.9
Hexose(Hex)	305.6	92.1	3.3	69.9
Hex + Hex + Hex	136.9	28.5	4.8	79.2
Oxalic acid	37.6	7.6	4.9	79.8
Fumaric acid	65.4	10.6	6.2	83.8
Malic Acid	712.4	97.8	7.3	86.3
Pentose	201.9	17.3	11.7	91.4
Citric acid	2,770.9	111.2	24.9	95.9
Succinic acid	529.4	8.3	63.9	98.4

The positive ion ESI spectra of UT-RS show $[M + K]^+$ at *m/z* of 191, 219 and 381 corresponding to a C5 sugar alcohol (perhaps xylitol), a hexose such as glucose and a dihexose, respectively. Since no chromatographic separation was performed for these analyses, it is impossible to distinguish isomeric compounds. An additional peak at *m/z* of 505 is tentatively assigned as a trisaccharide $[M + H]^+$ ion, and numerous other ions are present. The negative mode mass spectrum of the UT-RS shows major peaks corresponding to $[M-H]^-$ for oxalic, fumaric, succinic, malic and citric acids. In addition, $[M\text{-}H]^-$, $[M + Cl]^-$ and $[M + \text{formate}]^-$ peaks are observed corresponding to 5- and 6-carbon sugars and hexose dimers and trimers. In contrast, all the major peaks observed in spectra of UT-RS are dramatically decreased in the MSS spectra, though the negative ion spectra still reveal a peak at *m/z* of 377, which corresponds to $[M + Cl]^-$ for hexose dimer (Hex–Hex), perhaps cellobiose. As we can see in Fig. 6 and also from the ratio of the UT-RS to MSS peak areas in Table 2, the amount of almost all identified compounds as well as most unidentified compounds are reduced after fungal treatment. It seems that fungal conditioning had greater effect in diminishing levels of organic acids than on carbohydrates. A closer look at the spectra also shows that some new compounds showed up after fungal conditioning, which were not seen in the untreated sample spectrum. Based on their accurate mass measurements (data not shown), it seems that most of these are carbohydrate-related compounds and require a more detailed analysis of chemical structure.

With growing demand for ethanol, a biorefinery using lignocellulosic feed stock will be successfully implemented depending on the large-scale availability and cheap accessibility of biomass. Since corn and cane sugar ethanol industries are well established, waste lignocellulosic biomass generated from these plants will be further utilized for making ethanol in the near future. RS and MSS are other

important feedstocks that are currently being produced in large quantities and are easily available. Logistics of procuring, transporting and storing MSS from a mushroom plant is a major challenge and is currently under investigation. In 2005, the world market for mushroom was $41 billion [13]. Over four to five million tons (per annum) of edible mushrooms are produced world wide using different substrates, generating approximately 10–30 million tons of MSS. Economic analyses have suggested that a 2,000 ton/day feed stock operating biorefinery produces ethanol with maximum ethanol selling price (MESP) from $1.34 to $1.67/gallon depending on different pretreatment technologies [25]. As the technology matures, the MESP will decrease. In the future, MSS generated in several mushroom plants can be readily transported to a nearby biorefinery, which can be used as supplementary lignocellulosics feed stock for producing fuels and chemicals.

Conclusion

Treating RS with *Pleurotus ostreatus* followed by AFEX gave significantly higher glucan and xylan conversions at less severe AFEX conditions than when treating RS with AFEX directly. We have demonstrated that MSS will be an excellent feed stock that can give >98% glucan and 75% xylan conversion after pretreatment and hydrolysis (at 15 FPU/g glucan of commercial cellulase mix + xylanase supplementation). The glucan conversions were always higher for MSS compared to UT-RS under several AFEX conditions. Soluble lignin, which was extracted along with protein and polypeptides, are also considered as one of the important byproducts in the whole process. A mushroom plant coupled with a biorefinery could help generate both food and fuel ultimately benefiting the rural economy. Further experiments on microbial conditioning using different microbes and fermentation of hydrolyzate to ethanol are under progress.

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