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Improved Wet Air Oxidation Pretreatment for Enhanced Enzymatic Hydrolysis of Rice Husk for Bioethanol Production

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ABSTRACT

Pretreatment of rice husk by the Alkaline Peroxide Assisted Wet Air Oxidation (APAWAO) approach enhanced the enzymatic convertibility of cellulose in APAWAO-pretreated rice husk. The present work describes the structural changes in rice husk brought about by APAWAO pretreatment by means of Scanning Electron Microscopy (SEM). The SEM images illustrate the extensive loss of biomass integrity following APAWAO pretreatment. X-ray diffraction (XRD) studies indicated the loss of amorphous lignin following APAWAO to be a factor contributing to the enhanced enzymatic digestibility of pre-treated rice husk.

Keywords: enzymatic saccharification, rice husk, SEM, wet air oxidation, XRD
Abbreviations: APAWAO, Alkaline Peroxide Assisted Wet Air Oxidation; **CrI**, Crystallinity Index; **DM**, Dry matter; **ECC**, Enzymatic cellulose convertibility; **FPU**, Filter Paper Units; **HPLC**, High Performance Liquid Chromatography; **IU**, International Units; **SEM**, Scanning Electron Microscopy; **WAO**, Wet Air Oxidation; **XRD**, X-ray diffraction

INTRODUCTION

Rice husk is an agricultural residue that represents 20% dry weight of the harvested rice, and can serve as a low cost abundant feedstock for production of fuel alcohol. About 19–24 T g of rice husk is produced annually in India, as a result of the various cleaning and polishing practices (Ghosh and Ghose 2003), which can potentially produce more than 2.7 GL per year of ethanol even if only 30% rice husk is utilized at 60% efficiency (Bhojvaid 2006; U.S. Department of Energy 2012). Rice husk is not generally used as animal feed due to its low digestibility, peculiar size, low bulk density, high ash/silica contents, and abrasive characteristics (Saha and Cotta 2007). Rice husk can be obtained in bulk quantities from rice mills, thereby easing out the process of biomass collection (Bharadwaj *et al.* 2004). However, the high quantities of lignin (16–18 wt%) and ash (20 wt %) complicate the use of rice husk for ethanol production. Previous works on the use of rice husk for ethanol production have indicated the inherent difficulties in pretreating and hydrolyzing rice husk (Saha and Cotta 2007; Martin and Thomsen 2007; Saha and Cotta 2008). In our previous studies, we have reported that Wet Air Oxidation (WAO) and Alkaline Peroxide Assisted Wet Air Oxidation (APAWAO) pretreatment of rice husk lead to an improvement in its cellulose content, and enhanced the enzymatic digestibility of rice husk (Banerjee *et al.* 2009). This work builds a deeper insight using Scanning Electron Microscopy (SEM) and X-ray Diffraction (XRD) tools to visualize the structural changes caused due to the two pretreatment methods.

MATERIALS AND METHODS

Raw material

The rice husk (obtained from *Oryza sativa* L. 'Pusa Basmati,' that was harvested in early October and the husk separated using a mechanical paddy dehusker), was acquired from local producers (Tumsar, District Bhandara, Maharashtra, India). The fresh raw material was dried at 45°C for 48 h in an oven (Bio-Technics, India) to a dry matter (DM) content of 90.8%, and ground (Mixer Grinder, Philips, India) to pass through -20/+80 mesh sieves (BSS specification). 75% by weight of the initial rice husk was retained after the milling and sieving process. The ground and sieved raw material was stored in glass bottles capped tightly and kept at room temperature. The materials were used shortly after milling.

Pretreatment, analysis, and enzymatic convertibility

The experimental setup and procedure for WAO and APAWAO have been described elsewhere (Banerjee *et al.* 2011). The analysis of the glucan and lignin contents of rice husk before and after pretreatment was performed as per the method of Sluiter *et al.* (2008) by a two-step acid hydrolysis procedure using 72% H_2SO_4 in the primary hydrolysis step at 30°C and then diluting to 4 wt% for secondary hydrolysis step at 121°C. The concentrations of sugars (glucose, xylose, etc.) were determined by High Performance Liquid Chromatography (HPLC). The conditions for HPLC and other details have been provided elsewhere (Banerjee *et al.* 2011). Studies on enzymatic convertibility were performed using a commercial preparation of *Trichoderma reesei* cellulases (EC Number 3.2.1.4) kindly provided by M/s Zytex, Mumbai, India and β-glucosidase enzyme (EC Number 3.2.1.21) (extra pure, HiMedia, India), added at a loading of 25 FPU/g DM and 12.5 IU/g DM, respectively, to biomass at 2% DM. The percentage of cellulose enzymatically converted to glucose (% enzymatic cellulose convertibility) was calculated as:

$$\%ECC = \frac{grams\ glucose\ formed}{grams\ cellulose\ added} \times 0.9 \times 100$$

The factor of 0.9 is the correction factor for hydration of cellulose polymer during hydrolysis.

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Short Communication

Scanning electron microscopy

Imaging by Scanning Electron Microscopy (SEM) was performed for untreated and pretreated rice husk solids (dried powder of particle size <180 µm) at different magnifications using an Analytical SEM (JSM-6380A, JEOL, Japan) operated at 15 kV in the centralized facility at the Indian Institute of Technology Kharagpur, WB, India. The samples were platinum-coated with a JEOL JFC-1600 Auto Fine Coater before imaging.

X-ray diffraction

Biomass crystallinity was measured by X-ray diffraction using a D8 Advance Powder X-ray Diffractomer (Brucker Company, Zurich, Switzerland) operated at 40 kV and 200 mA. Biomass sample was dried at 45°C for 72 h prior to measurement. The sample (-80 mesh) was scanned at a scan speed of 2°/min from 2θ = 10° to 26° with a step size of 0.05°. Biomass crystallinity index (CrI) was determined as the percentage of crystalline material in biomass (Segal *et al*. 1959).

$$CrI = \frac{I_{002} - I_{am}}{I_{002}} \times 100$$

where I_{002} is the intensity of the 002 peak at 2θ = 22.5° and I_{am} is the intensity of the peak for the amorphous portion at 2θ = 18.7°s.

RESULTS AND DISCUSSION

Wet air oxidation has been shown to increase the cellulose content of rice husk, by solubilizing hemicelluloses and lignin (Banerjee *et al*. 2009). The effect of coupling alkaline peroxide pretreatment with WAO on the structure of rice husk, as well as its enzymatic convertibility have also been reported earlier by our group (Banerjee *et al*. 2011). Alkaline peroxide is known to cause delignification of lignocellulosic biomass (Gould 1984). Up to 86 wt% of cellulose present in APAWAO-pretreated rice husk could be converted into glucose, which is the best reported for rice husk so far (Banerjee *et al*. 2011) and a brief comparison of results is given in **Fig. 1**. This work discusses the structural changes that might have been responsible for the extensive increase in the enzymatic convertibility.

Scanning electron microscopy

An insight into the major structural changes to rice husk following WAO and APAWAO can be obtained from Scanning Electron Microscopy (SEM) (**Fig. 2**). **Fig. 2A** shows a SEM image of untreated rice husk. The structure of rice husk resembles that of a composite material with cellulose fibers regularly interspaced in the matrix with silica as the base and hemicellulose and lignin as the "cementing" material. The transverse section is dense, intact and ordered structure, with no evidence of pores. The untreated rice husk, which also shows the presence of flanges on the outer surface of rice husk that are similar to the corn kernels of the corncob are nothing but the outer bristles of the rice husk (Lynam 2011). **Fig. 2B** shows that WAO pretreatment created holes that are not evident in untreated rice husk particles. The holes could be a result of the volatile constituents escaping from the surface as a result of the high temperature and pressure conditions of WAO (Bharadwaj *et al*. 2004). In addition, the holes on the biomass surface and loss of biomass integrity can be attributed to solubilization of hemicellulose and lignin (Kumar *et al*. 2009). Thus, the formation of holes is consistent with the removal of lignin and hemicellulose during the pretreatment. Observations on SEM images of APAWAO pre-treated rice husk in **Fig. 2C** suggest that the APAWAO process extensively disintegrates the compact biomass structure, and liberates the cellulose fibers from the tightly woven lignocellulosic structure. The same matrix structure as seen for untreated and WAO-pretreated rice husk is evident, but with an apparent reduction in consistency. The black arrows in **Fig. 2C** point to the cellulose fibers that are exposed during the pretreatment.

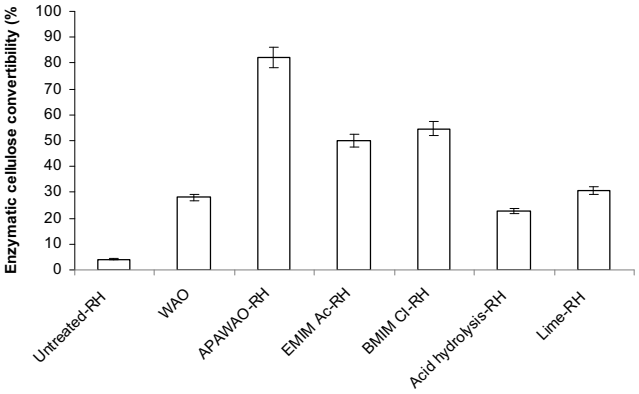

Fig. 1 Enzymatic saccharification of untreated and pre-treated rice husk (RH) acid hydrolysis-RH (Saha *et al*. 2005); lime-RH (Saha and Cotta 2007). EMIM Ac and BMIM Cl are ionic liquid namely 1-ethyl-3-methylimidazolium acetate and 1-butyl-3-methylimidazolium chloride.

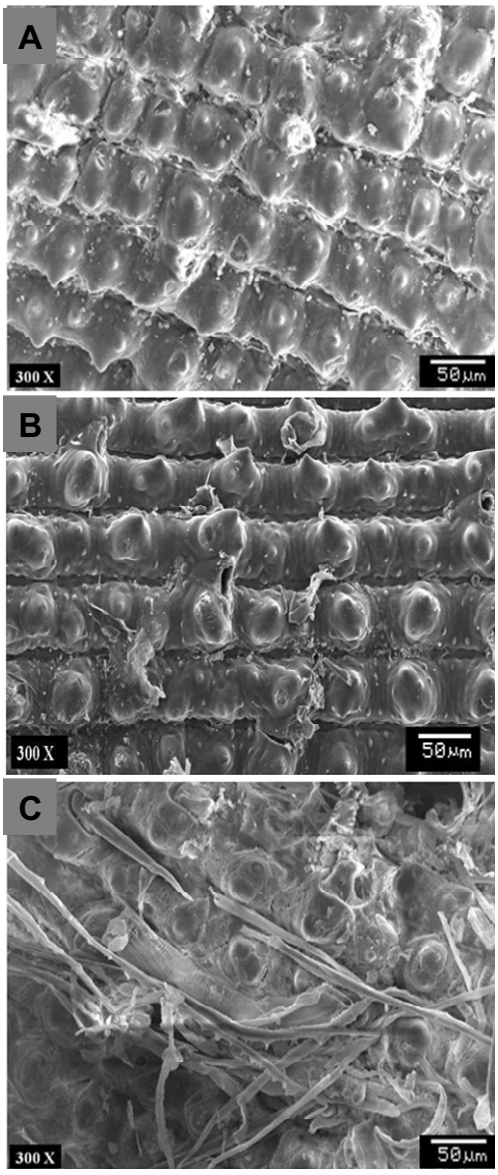

Fig. 2 SEM images showing alterations in untreated rice husk structure (**A**) following WAO (**B**) and APAWAO (**C**).

This exposure of cellulose fibers from within the lignin and hemicellulose barriers improves the accessibility of cellulase enzymes, and reduces unproductive and irreversible binding to other groups.

Table 1 Crystallinity indices of untreated and pretreated rice husk and comparison to crystalline cellulose.

Substrate	Crystallinity Index (CrI)
Untreated rice husk	48.70
WAO pre-treated rice husk	30.98
APAWAO pre-treated rice husk	56.95
Crystalline cellulose	73.82

Effect of biomass crystallinity on biomass digestibility

Biomass digestibility is described as a two-step process wherein the enzymes first pass through the lignin barrier to gain access to cellulose. Upon accessing the cellulose, crystallinity of cellulose dictates the extent of enzymatic digestibility. Thus, biomass digestibility is a function of the extent of cellulose exposed for enhanced enzyme accessibility, as well as cellulose crystallinity. The crystallinity of biomass was determined by means of X-ray diffraction. The measurement of true cellulose crystallinity by X-ray diffraction method is difficult due to the presence of the other amorphous materials such as hemicellulose and lignin, apart from the amorphous cellulose and hence, the crystallinity measured by X-ray diffraction method is an apparent value of cystallinity of the biomass. The crystallinity indices (CrI) of raw rice husk, WAO and APAWAO pretreated rice husk, and crystalline cellulose are reported in **Table 1**. The CrI for untreated rice husk is lower than that of crystalline cellulose. This is because rice husk has other "impurities" such as lignin and hemicellulose, which are amorphous. A reduction in crystallinity after WAO pretreatment is apparent from **Fig. 1**. However, APAWAO seems to increase the crystallinity of rice husk. This may be due to the extensive delignification of biomass caused by APAWAO. Since lignin is mostly amorphous, biomass delignification led to slight increase in biomass crystallinity (Kim and Holtzapple 2006). However, as indicated by Zhu *et al*. (2008), extensive delignification is sufficient to achieve near-complete hydrolysis, regardless of biomass crystallinity. Thus, the enhancement in enzymatic digestibility of APAWAO pretreated rice husk (as reported earlier by Banerjee *et al*. 2011) can be attributed to biomass delignification rather than biomass crystallinity (**Fig. 1**). The effect of delignification on biomass digestibility may be explained as follows: (1) it reduces non-specific, irreversible adsorption of enzyme on lignin, thereby increasing the access of enzymes to cellulose and (2) it increases internal surface area and pore volume, thereby reducing steric hindrance (Zhu *et al*. 2008). Thus, the increased availability of cellulose fibers for enzymatic attack is the key contributing factor that led to the increase in the enzymatic convertibility of rice husk following APAWAO pretreatment.

CONCLUSIONS

An intensive structural breakdown of rice husk was evident when APAWAO pretreatment was applied. These structural changes were revealed by SEM analysis. The increased exposure of cellulose fibers due to extensive delignification led to reduction in non-specific binding and improved the attachment of cellulase enzymes over the fiber surface, thereby providing enhanced conversion of cellulose into glucose.

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