



Short Communication

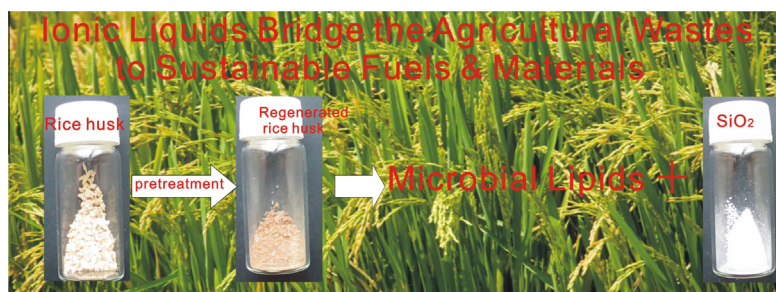
The integrated production of microbial lipids and bio-SiO₂ from rice husks by an organic electrolytes pretreatment technologyXue Yu^{a,b,1}, Jing Tian^{a,1}, Haibo Xie^{b,c,*}, Hongwei Shen^b, Qian Wang^c^a School of Biological Engineering, Dalian Polytechnic University, Dalian 116034, China^b Bioenergy Research Division, Dalian National Laboratory for Clean Energy, 457#, Zhongshan Rd., Dalian 116023, China^c Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457#, Zhongshan Rd., Dalian 116023, China

HIGHLIGHTS

- A full dissolution of rice husks was achieved in mixed organic electrolyte.
- Enzymatic hydrolysates were used as carbon resources for microbial lipid production.
- Nano-SiO₂ materials were prepared by using residuals after the enzymatic hydrolysis.
- Mixed organic electrolyte showed better performance than that of pure ionic liquids.

GRAPHICAL ABSTRACT

The dissolution pretreatment of rice husk (RH) results in an enhanced subsequent enzymatic saccharification of RH, with a total reducing sugars (TRSs) yield of 0.70 g g⁻¹, and a glucose yield of 0.43 g g⁻¹. The hydrolysates were used as carbon resources for cultivation of *R. toruloides* Y4 achieving a cell biomass yield and lipid content of 13.3 g L⁻¹, and 32.5%, respectively. Silica material with uniform spherical structure and particle size around 150 nm and a surface area of 179.3 m² g⁻¹ was achieved through pyrolysis of the residuals after the enzymatic hydrolysis.



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ABSTRACT

In this study, a full dissolution behavior of rice husks (RHs) in ionic liquids-based organic electrolytes was achieved, and physicochemical effect of the dissolution pretreatment on the structures of RHs was elucidated. The physicochemical changes led to an enhanced subsequent enzymatic saccharification of RHs, and a total reducing sugars (TRSs) yield of 0.70 g g⁻¹, and a glucose yield of 0.43 g g⁻¹ were obtained. The hydrolysates could be used as carbon sources for the cultivation of *Rhodospiridium toruloides* Y4 for the production of microbial lipids with a satisfactory productivity of cell biomass (13.3 g L⁻¹) and lipid content (32.5%) after 100 h cultivation. Further pyrolysis of the residuals after the enzymatic hydrolysis at 600 °C for 3 h resulted in new uniform, spherical silica powder materials with particle size around 150 nm, and surface area of 179.3 m² g⁻¹.

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* Corresponding author at: Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457#, Zhongshan Rd., Dalian 116023, China. Tel./fax: +86 0411 84379852.

E-mail address: hbxie@dicp.ac.cn (H. Xie).¹ These authors contributed to the manuscript equally.

1. Introduction

Shifting society's dependence away from petroleum to renewable biomass resources is generally regarded as an important contributor to building up a sustainable society and effective management of greenhouse gas emissions (Ragauskas et al., 2006). Rice husks (RHs) are abundantly available waste material in all rice producing countries, and it is estimated that, in the Asia-Pacific region alone, approximately 1.2 billion tons of RHs are generated annually. So far, many of which are underutilized along with a variety of low value products (Chandrasekhar et al., 2003). Sometimes, RHs have to be disposed with additional cost, and even leading to pollution issues. The major constituents of RHs mainly consists of cellulose (~40%), hemicellulose (~18%), lignin (~22%) and significant content of silica (~20%) comparing with other biomass. These constituents are potential sources for production of biofuels, biochemicals, and materials (Garcia et al., 2011; Pajonk, 2003).

The large quantities of RHs and high content of hydrated silicon, and the naturally and uniformly dispersion by molecular units in RHs offer an opportunity for the production of various silica materials for industrial applications from a sustainable source (Chen et al., 2013). However, due to the tough and complex three-dimensional physical structure of RHs, and high content of ash, it is very recalcitrant to chemicals and microbial destruction, thus leading to a low efficient components fractionation and chemical or biological conversion. Therefore, an efficient pretreatment is necessary for the subsequent application of RHs (Mochidzuki et al., 2001; Ang et al., 2013). Recently, ionic liquids-based dissolution pretreatment technologies have emerged as one of efficient technologies (Ang et al., 2012; Lynam et al., 2012; Teck Nam et al., 2011). However, only partial dissolution of RHs in pure ionic liquids was observed. Bearing the significant progress and the increasing interests in this area, the shortages, such as the high price of ILs, high energy consumption of ILs' recycle and purification, as well as the need of high quality of ionic liquids and biomass (water tolerant) for an efficient dissolution and pretreatment are highly criticized from a practical point of view. Recent studies showed that a mixture of ionic liquids with organic solvents can be used to pretreat lignocellulosic materials efficiently (Xie et al., 2012), and the introduction of traditional solvents into the pure ionic liquids system can release the disadvantage issues aforementioned to some extent.

In this study, the full dissolution behavior of RHs in ionic liquids-based mixed low cost organic electrolytes, and in depth understanding the physicochemical effect of the dissolution pretreatment on the structure of RHs were investigated, on which the integrated production of microbial lipids and nano-silica materials from RHs were investigated.

2. Methods

2.1. Materials

The ILs EmimAc was supplied by Lanzhou Greenchem ILs, LIPC, CAS (Lanzhou, China) and used without any further purification. N-methyl-pyrrolidone (NMP) was purchased from Damao Chemical Reagents Ltd (Tianjin, China) and used without further purification. RHs were collected from Hunan Province, Loudi City, China. The composition (cellulose, hemicellulose and lignin) of the RHs was determined according to the analytical procedures of the National Renewable Energy Laboratory (NREL, http://www.nrel.gov/biomass/analytical_procedures.html). The RHs were ground and placed through a 40 mesh particle size sieve plate before pretreatment, and then the sample was stored under air without any wash. The cellulase was purchased from Zesheng Ltd, China. The activity

was reported to be 7500 IU mL⁻¹. The IUPAC Filter Paper Assay was performed on the cellulose and found to be 175 FPU mL⁻¹ using the protocol of NREL LAP-006. The β -glucosidase and xylanase were purchased from Imperial Jade Ltd, China. The β -glucosidase activity was reported to 10000 000 U g⁻¹. One unit of β -glucosidase (U) was defined as the amount of enzyme (1 g), which produces 1.0 μ g of glucose from 1% β -glucan solution at pH 5.0 and 50 °C within 1 min. Xylanase activities was reported to be 5000000 U g⁻¹, and one unit of xylanase (U) was defined as the amount of enzyme (1 g) which produces 1.0 μ g of xylose from 1% xylan solution at pH 5.0 and 50 °C within 1 min.

2.2. Dissolution of RHs in ionic liquids and regeneration of biomass

A mixed solution was prepared by adding ionic liquid EmimAc/NMP ($X_{ILs} = 0.2$, mole fraction of ILs in the mixed solution, 20.0 g) into a 50 mL two-necked glass flask equipped with a mechanical stirrer. The flask was placed in an oil bath at 140 °C, followed by addition of RHs (2.0 g, 10 wt%) under stirring for dissolution. The pretreated sample was obtained after adding anhydrous methanol (1:30 w/v) into the solution, and subsequently washed by methanol and water for 30 min two times under magnetic stirring to remove the ILs and NMP residues. The regenerated sample (1.3 g) was obtained after freeze-drying for 24 h with a 65% regeneration yield.

2.3. Methods

2.3.1. Enzymatic hydrolysis regenerated biomass

The pretreated sample was suspended in a buffer solution (0.1 mol L⁻¹ sodium citrate and 0.1 mol L⁻¹ citric acid) with the solid to liquid ratio of 5% (w/v), and the cellulose enzyme loading of 0.1 mL g⁻¹ solid substrate, β -glucosidase enzyme and xylanase enzyme loading of 0.02 g g⁻¹ solid substrate, respectively. The initial pH of the mixture was 4.8 \pm 0.1. The regenerated RHs were hydrolyzed in a rotating shaker for 48 h with constant temperature of 50 °C and agitation rate of 200 rpm. Samples were withdrawn and kept at -20 °C until analyzed.

2.3.2. Preparation of silica powders

Residual of RHs from the enzymatic hydrolysis process was dried by a vacuum freeze drier. The residual was weighted and put into a porcelain crucible, which was put in an electric muffle furnace for pyrolysis at different temperatures varying from 600 to 800 °C for 2 or 3 h. The final product, in the form of white solid, was collected, sealed in a 1.5 mL centrifuge tube and stored at room temperature for the following analysis.

3. Results and discussion

3.1. The dissolution of RHs in NMP/EmimAc ($X_{ILs} = 0.2$)

The bristles on the outside of the hull consist of a layer of silicified cuticle and the inside concave surface is similarly protected by a coating of silica (Ouyang, 2001), which will block the interaction of ionic liquids with cellulose, hemicellulose and lignin inside the RHs, and thus decreasing the penetration ratio of ionic liquids into the RHs. Therefore, only partial dissolution behavior was observed in pure ionic liquids under identical conditions (Ang et al., 2012; Lynam et al., 2012). With current in-depth understanding of the dissolution mechanism of ionic liquids to cellulose and lignocellulosic materials, this may be correlated to the different structure of RHs and other lignocellulosic biomass. The results demonstrated that the addition of co-solvent NMP into EmimAc ($X_{ILs} = 0.2$) could significantly enhance the solubility of RHs, and

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