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Optimized membrane process to increase hemicellulosic ethanol production from pretreated rice straw by recombinant xylose-fermenting *Saccharomyces cerevisiae*

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HIGHLIGHTS

- Rice straw was pretreated by hot water.
- NF, dilution, UF, and enzymatic hydrolysis were optimized.
- Oligomeric and monomeric sugars were recovered.
- High and low molecular weight fermentation inhibitors were removed.
- Lignocellulosic ethanol was produced by xylose-fermenting *Saccharomyces cerevisiae*.

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ABSTRACT

Oligomeric sugars in the liquid fraction of hot water-pretreated rice straw are more amenable to membrane process than monomeric sugars, as lower pressure is required. Following membrane process was employed: nanofiltration (NF) concentration → (dilution → NF concentration) × 2 times → enzymatic hydrolysis (EH) → ultrafiltration (UF) permeation [Implication: NF for recovery of oligomeric sugars, dilution and NF for removal of low molecular weight fermentation inhibitors, UF for removal of high molecular weight fermentation inhibitors and recovery of monomeric sugars after EH]. This process provided the liquid fraction containing 111.4 g L^{-1} of sugars, corresponding to 681.0 mM as monomeric sugars, from the original liquid fraction (181.1 mM monomeric sugars). Concentrations of low molecular weight fermentation inhibitors, acetic and formic acids, were decreased to 24% and 48%, respectively. Xylose-fermenting recombinant *Saccharomyces cerevisiae* produced 34.5 ± 2.2 g L^{-1} ethanol from the 0.8 times liquid fraction (76% of theoretical yield).

1. Introduction

Bioethanol, a second generation biofuel, is produced from abundant renewable lignocellulosic biomass such as crop wastes, forestry and agricultural residues, and the organic fraction of municipal solid wastes (Sims et al., 2010; Farrell et al., 2006). Bioethanol is a promising alternative to fossil fuels. Among agricultural residues, rice straw is the largest biomass feedstock in the world (Kim and Dale, 2004). Bioethanol production from lignocellulosic biomass comprises mainly the following steps: pretreatment, hydrolysis, sugar fermentation, and final recovery and purification of ethanol. These multi steps are required due to the structural complexity of rice straw, which contains hemicellulose (in general, 15–35% of the biomass), cellulose and lignin as its three main components (Alvira et al., 2010; Gírio et al., 2010; Lynd et al., 2008; van Zyl et al., 2007). In most cases, pretreatment produces water-insoluble solids containing cellulose and lignin, and a liquid fraction containing hemicellulose and lignin (Fig. 1) (Geddes et al., 2011; Klinke et al., 2004). Depending on the process and conditions

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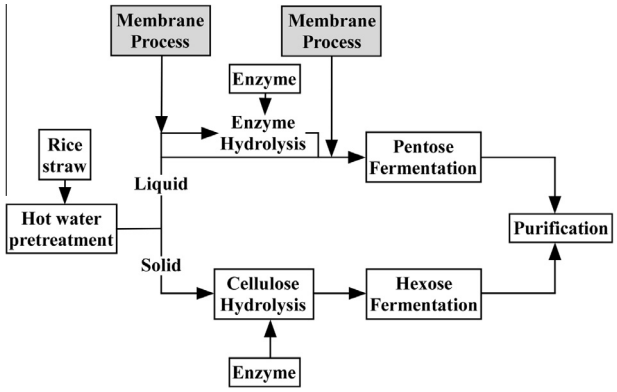

Fig. 1. Configuration of the ethanol process from hot water-pretreated rice straw. The membrane process can be applied to the liquid fraction.

for pretreatment, acids such as acetic acid or formic acid, furan derivatives, and phenolic compounds are released in the liquid fraction, which inhibit fermentation (Klinke et al., 2004).

Requirements for commercializing cellulosic ethanol technology include increasing the ethanol concentration after fermentation in order to decrease energy consumption in downstream purification processes. To achieve this, a high sugar concentration is required for fermentation. However, sugars derived from hemicellulose are at low concentration in the liquid fraction after pretreatment (Qi et al., 2012). Membrane technologies that are simple to operate and ease to scale-up (He et al., 2012) have been used to remove fermentation inhibitors and/or concentrate sugars in the liquid fraction of pretreated lignocellulosic biomass (Sasaki et al., 2013; Maiti et al., 2012; Qi et al., 2012; Weng et al., 2010). In these previous studies, the pressure-driven membrane processes, ultrafiltration (UF) and/or nanofiltration (NF), were utilized. A UF membrane was used to recover macromolecules with molecular weights ranging from 1000 to 100,000 g mol^{-1} in order to recycle the enzyme (Qi et al., 2012). An NF membrane with a 150 g mol^{-1} molecular weight cutoff, proved effective for separating inhibitors such as acetic acid and furfural (Maiti et al., 2012). To obtain high concentration of total sugars, the presence of oligomeric sugars and the minimization of monomer formation are advantageous for membrane concentration process under low pressure because the osmotic pressure required is proportional to the molar concentration in the solute, as given by the van't Hoff equation (Cheryan, 1998). Most previous research has been aimed at recovering oligomeric sugars from lignocellulosic biomass, such as solid wood, in pulp process (Ahsan et al., 2014; He et al., 2012). It has been reported that liquid hot water pretreatment is effective for the high recovery of hemicellulose-derived sugar from the liquid fraction of wheat straw, a lignocellulosic material (Pérez et al., 2008). Moreover, hot water pretreatment retained hemicellulosic sugars mainly in their oligomeric form (Alvira et al., 2010). Therefore, membrane separation was applied to the liquid fraction of hot water-pretreated rice straw in this study.

Hemicelluloses are a heterogeneous class of polymers that yield pentoses and hexoses upon hydrolysis (Gírio et al., 2010). A major pentose sugar, xylose, cannot be fermented by wild type strains of *Saccharomyces cerevisiae*, the most promising platform for large-scale bioethanol production (van Maris et al., 2006). However, *S. cerevisiae* that have been engineered to ferment xylose is favorable for utilization of hemicelluloses (Hasunuma and Kondo, 2012; Van Vleet and Jeffries, 2009). In addition, a diploid xylose-fermenting *S. cerevisiae* strain, in the presence of inhibitors such as acetic acid, formic acid, and furfural, has been exploited (Hasunuma et al., 2014; Sanda et al., 2011). However, there has only been limited research into evaluating the combination of membrane separation and ethanol fermentation (Kawa-Rygielska et al., 2013; Sasaki et al., 2013). Therefore, the above diploid xylose-fermenting *S. cerevisiae* strain was utilized in ethanol fermentation studies in this investigation.

The aim of this investigation was threefold. The first was to optimize a membrane separation process to efficiently concentrate oligomeric and, to a lesser extent, monomeric sugars in the liquid fraction of hot water-pretreated rice straw. The second aim was to minimize the concentration of fermentation inhibitors with a wide range of molecular weights. Thus, the sequence of NF, dilution, UF, and enzymatic hydrolysis processes was optimized. The third aim was to evaluate membrane separation and subsequent ethanol fermentation process using a xylose-fermenting *S. cerevisiae* strain to allow better utilization of hemicellulose.

2. Methods

2.1. Feed material

Rice straw pretreated by liquid hot water (Alvira et al., 2010) at determined temperature (130–300 °C) at a pressure below 10 MPa was purchased from Mitsubishi Heavy Industries, Ltd. (Tokyo, Japan). The liquid fraction (pH: 4.4) was separated from the solid fraction by filtration through a mesh filter and used as a source of hemicellulosic material. The liquid fraction of hot water-pretreated rice straw with a pH of 2.7 was produced by acidifying with 5 N HCl.

2.2. Membrane separation

Polyvinylidene fluoride UF membrane (RS50) and polyamide NF membrane (ESNA3) were obtained from Nitto Denko Corporation (Osaka, Japan). The membranes were cut into circles (diameter: 7.5 cm; effective are: 32 cm^2). RS50 was soaked in 50% (v v^{-1}) ethanol solution for 15 min, then in deionized water for 15 min, and was soaked in deionized water overnight before use.

Membrane separation experiments were carried out using a flat membrane test cell (diameter: 104 mm, height: 147 mm) (model C40-B; Nitto Denko Corporation) at 25 °C as described previously (Sasaki et al., 2013). The cell was placed on a magnetic stirrer and feed solution (maximum working volume: 380 mL) was stirred at 400 rpm by a magnetic spin bar fitted into the cell. Pressure (2.8 or 0.4 MPa) was applied using nitrogen gas and a pressure control valve. The permeate was collected; the permeation rate was determined by measuring the permeate weight at 25 °C. The observed rejection (R) was calculated from the following equation,

$$R = \left(1 - \frac{C_p}{C_f}\right) \times 100\%$$

where C_f and C_p are the concentration in the feed solution and permeate, respectively, of monomeric sugar and carboxylic acid. The concentration before and after membrane separation is averaged for the calculation of C_f.

The following membrane separation processes were used prior to ethanol fermentation: (1) 5.0 times NF concentration at 2.8 MPa → enzymatic hydrolysis, (2) UF permeation at 0.4 MPa → 5.4 times NF concentration at 2.8 MPa → enzymatic hydrolysis, (3) UF permeation at 0.4 MPa → 5.4 times NF concentration at 2.8 MPa → [5 times dilution → NF concentration at 2.8 MPa (final concentration of 5.4 times)] × 2 → enzymatic hydrolysis, (4) 5.1 times NF concentration at 2.8 MPa → [5 times dilution → NF concentration at 2.8 MPa (final concentration of 5.3 times)] × 2 → enzymatic hydrolysis → UF permeation at 2.8 MPa. The NF concentrate was diluted with Milli-Q water. In addition, NF and UF separation processes to check the rejections of