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Improvement of the fermentability of oxalic acid hydrolysates by detoxification using electrodialysis and adsorption

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HIGHLIGHTS

- The removal efficiency of acetic acid reached 100% by electrodialysis.
- Most of non-ionizable inhibitors were removed by XAD-4 resin adsorption process.
- The ethanol productivity was high at 0.27–0.35 g/L h in detoxified hydrolysates.
- A continuous detoxification process was efficient in ethanol production.

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ABSTRACT

A two-step detoxification process consisting of electrodialysis and adsorption was performed to improve the fermentability of oxalic acid hydrolysates. The constituents of the hydrolysate differed significantly between mixed hardwood and softwood. Acetic acid and furfural concentrations were high in the mixed hardwood, whereas 5-hydroxymethylfurfural (HMF) concentration was relatively low compared with that of the mixed softwood. The removal efficiency of acetic acid reached 100% by electrodialysis (ED) process in both hydrolysates, while those of furfural and HMF showed very low, due to non-ionizable properties. Most of the remaining inhibitors were removed by XAD-4 resin. In the mixed hardwood hydrolysate without removal of the inhibitors, ethanol fermentation was not completed. Meanwhile, both ED-treated hydrolysates successfully produced ethanol with 0.08 and 0.15 g/L h ethanol productivity, respectively. The maximum ethanol productivity was attained after fermentation with 0.27 and 0.35 g/L h of detoxified hydrolysates, which were treated by ED, followed by XAD-4 resin.

1. Introduction

The production of liquid fuels and industrial chemicals from lignocellulosic biomass depends on the utilization of cellulose, hemicelluloses, and lignin. Lignocellulosic biomass is available, abundant, and renewable, compared to the first general feedstocks. In addition, it may be converted into products that are identical, or functionally equivalent, to current petroleum-base liquid fuels and industrial chemicals (Lin and Huber, 2009). Therefore, it provides great potential for reducing the global dependence on fossil fuels.

Polysaccharide has to be converted into monosaccharides by thermochemical or enzymatic hydrolysis, to produce liquid fuels, and chemicals. However, lignocellulosic biomass cannot be efficiently degraded into monosaccharides, due to its recalcitrant structure. Therefore, an appropriate pretreatment process is required to improve bioconversion. Numerous pretreatment processes have been suggested, including the physical, chemical, physic-chemical, and biological (Ferraz et al., 2001; Lloyd and Wyman, 2005; Pan et al., 2005). Among the pretreatment processes, dilute acid pretreatment has been considered as an effective pretreatment process that can enhance biomass sugar release performance (Himmel et al., 2007; Yang and Wyman, 2008).

In general, dilute acid pretreatment degrades most of the hemicelluloses, and some of the lignin. The hydrolysate contained pentose and hexose sugars, aliphatic acids (acetic, formic, and levulinic acid), furan aldehydes (furfural and 5-hydroxymethylfurfural; HMF), aromatic compounds (phenolics), and extractives (Jonsson et al., 2013). Those are widely known as fermentation inhibitors except for sugars. The fermentation inhibitors affect microorganism growth, and sugar uptake during fermentation, as well as cellulose-degrading enzymes, under certain concentration (Palmqvist and Hahn-Hägerdal, 2000). In most dilute acid

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pretreatment, however, fermentation inhibitors occur, thus decreasing the fermentability of hydrolysate.

To reduce the inhibitor's toxicity during the fermentation, the development of effective removal strategies for these inhibitors from the biomass hydrolysate is attractive. The effect of detoxification differs depending on the type of hydrolysate and the concentration of inhibitors. Lignocellulosic hydrolysate contains different types of inhibitors, with different concentration. Therefore, it is difficult to remove most of inhibitors from hydrolysate by one detoxification process. Several techniques for hydrolysate detoxification have been proposed to overcome these inhibitory effects on the yeast, and bacterial metabolism, such as physical methods (evaporation, extraction, and adsorption), chemical methods (neutralization, overliming, alkaline detoxification, and ion exchange), biological methods (enzymatic, and microbial detoxification), electrochemical method (electrodialysis), and combined treatments (Cavka and Jonsson, 2013; Kim et al., 2013; Ludwig et al., 2013).

Adsorption is a convenient and effective technique to remove low concentrations of chemicals from water (Sainio et al., 2011). Inhibitors should be removed effectively without removal of the valuable hydrolysates, such as monosaccharide, and soluble oligosaccharide, which are the source to be converted into ethanol in the fermentation (Jonsson et al., 2013). Among fermentation inhibitors, furfural and HMF, and total phenolic compounds (TPC) can be removed effectively in adsorption. Meanwhile, the removal efficiency of ionizable inhibitors, acetic acid, and formic acid, is low in adsorption. Electrodialysis (ED) shows high removal efficiency, due to ionizable properties. ED is an electrochemical separation process with cation and anion exchange membranes, using an electric potential as the driving force, which has been considered for the separation and purification containing ionic species (Lee et al., 1998).

Adsorption of XAD-4 resins was evaluated for the ED treated hydrolysates. The hydrophobic polymeric adsorbents, XAD-4, was considered to adsorb fermentation inhibitors. Amberlite XAD-4, one of the best commercially available polymeric adsorbents of the second-generation styrene–divinylbenzene copolymers, is found to be one of the excellent hydrophobic adsorbents for adsorption of small organic molecules dispersed in aqueous media (Huang et al., 2013; Li et al., 2013).

However, aliphatic acids and furan aldehydes were not completely removed by one step detoxification process in the previous study (Lee et al., 2013). In this study, two-step detoxification process was applied to remove the fermentation inhibitors from oxalic acid hydrolysates of woody biomass. First of all, ED was used to remove aliphatic acids (acetic acid), and then Amberlite XAD-4 resin was sequentially used to remove furan aldehydes (furfural and HMF) and aromatic compounds (TPC) from the hydrolysates. The removal efficiency of fermentation inhibitors in a lignocellulosic hydrolysate was investigated by the two-step detoxification process, and identified fermentation inhibitors in each hydrolysate by mass spectrometry. Furthermore, the influence of detoxification on ethanol fermentation performance was evaluated between the detoxified hydrolysates, and the untreated hydrolysates.

2. Methods

2.1. Biomass and pretreatment

Mixed hardwood (*Quercus mongolica*, *Robinia pseudoacacia L*, and *Castanea crenata*) and softwood (*Pinus rigida* and *Pinus densiflora*) chips were used in this study. The biomass was milled to pass a 40 mesh, and stored at 4 °C, until further use.

The pretreatment of biomass was performed in cylindrical stainless steel reaction vessels. The biomass and oxalic acid solution was placed in 500 mL stainless steel vessels that were placed into a larger tumbling digester, heated to the reaction temperature, and then rotated to keep the liquor in contact with the material during pretreatment. Each vessel was loaded with 50 g (dry weight basis) of biomass and sufficient oxalic acid/water mixture to give a total solid/liquid ratio of 1:4 (w/w). Pretreatment was performed at 160 °C for 118 min with oxalic acid catalyst solution of pH 1.34. Fig. 1 shows a schematic illustration of the removal of fermentation inhibitors, consisting of ED and adsorption.

2.2. Removal of fermentation inhibitors from hydrolysates by electrodialysis

ED was used to remove ionizable fermentation inhibitors from the hydrolysate. A stack consisting of ten cell pairs (diluate and concentrate) was assembled in a CJ-S3 electrodialysis stack, having a total effective membrane area of 550 cm^2 (Changjo Techno, Korea). A commercial cation exchange membrane, NEOSEPTA® CMX, and an anion exchange membrane, NEOSEPTA® AMX (ASTOM Corp., Tokyo, Japan) were used to prepare the stack.

The hydrolysate was fed through the membrane module in the diluate as an initial feed. Distilled water was used as the initial concentrate for the ED experiments of the hydrolysate. The volume of the three solutions (diluate, and concentrate, and electrode rinse solution) was set to 300 mL each. The flow rate of the solution was maintained at 1.56 L/min. A constant electrical potential (10 V) was supplied through ED experiments. The ED-treated hydrolysate was used for ethanol fermentation, and the adsorption experiment for the removal of furan aldehydes (Fig. 1).

2.3. Removal of fermentation inhibitors from hydrolysates by XAD-4 resins adsorption

For the removal of fermentation inhibitors, XAD-4 resin was packed in a glass column (ID 0.9 cm, height 15 cm) from iso-propyl alcohol suspension. Firstly, water (200 mL) was pumped through the column, and then the ED-treated hydrolysates (100 mL) were fed to the column, and the column effluent was collected periodically. To separate furfural, and HMF from the adsorbents, water (200 mL), and ethanol (200 mL) were supplied through the column. Water was fed to the column, to clean ethanol from the column.

2.4. Sugars, furans and organic acids in the hydrolysate

The concentrations of fermentable sugars, furan aldehydes (furfural and HMF), organic acids, and ethanol were determined using an HPLC (Waters 2695 system, MA, USA) outfitted with a refractive index detector (Waters 2414 system; Alliance, MA, USA). An Aminex HPX-87P column (300 × 7.8 mm, Bio-Rad, Hercules, CA, USA) was used for the analysis of fermentable sugars. The analysis was performed with de-ionized water as the mobile phase, at an isocratic flow rate of 0.6 mL/min for 55 min. The furan aldehydes, organic acids, and ethanol were analyzed by Aminex HPX-87H column (300 × 7.8 mm, Bio-Rad, Hercules, CA, USA). The analysis was performed with 5 mM H_2SO_4 as the mobile phase, at an isocratic flow rate of 0.6 mL/min for 55 min.

TPC were estimated colorimetrically, by the Folin–Ciocalteu method (Scalbert et al., 1989).

2.5. LC/MS/MS analysis of hydrolysate

The analysis of hydrolysates was performed using a HPLC (Waters Alliance 2695 system, MA, USA) and mass spectrometer (Waters Quattro micro AP, MA, USA I). A Waters SunFire C-18 column (2.1 × 150 mm, 3.5 μm) was used for the peak separation. Elution was performed with a mixture of solutions 0.1% of formic