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Improvement of ethanol fermentation from lignocellulosic hydrolysates by the removal of inhibitors

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ABSTRACT

In this study, the removal efficiency of fermentation inhibitors in a lignocellulosic hydrolysate by electrodialysis (ED) and the ethanol performance of ED-treated hydrolysate were investigated. The fermentable sugars and inhibitors concentrations in the hydrolysate differed significantly depending on the kind of biomass and acid catalysts. In the mixed hardwood, acetic acid and furfural in the hydrolysate were high as 8.41–8.57 g/L and 2.68–4.23 g/L, respectively, but 5-hydroxymethylfurfural (HMF) concentration was relatively low compared with that of mixed softwood. The ED process showed the high effectiveness for removing acetic acid and total phenolic compounds in the hydrolysate without loss of fermentable sugars. However, most of the HMF and furfural remained in the hydrolysate after ED. Ethanol fermentation was not completed in untreated and mixed hardwood ED-treated hydrolysates due to the high concentration of furfural. Meanwhile, ethanol fermentation was successfully performed in a mixed softwood ED-treated hydrolysate pretreated with dicarboxylic acid. The maximum ethanol concentration attained after fermentation with an initial fermentable sugar level of 27.78 g/L was 10.12 g/L after 48 h.

1. Introduction

Lignocellulosic biomass has received attention as a resource for biofuels because it is renewable and abundant. Moreover, lignocellulosic biomass does not compete with a food source; therefore, it has great potential as a substrate for biofuel production [1]. In particular, research on bioethanol production from lignocellulosic biomass is being carried out for its direct use in converted car engines or for anhydrous ethanol to add to gasoline as a fuel enhancer [2].

The bioconversion process to produce ethanol from a lignocellulosic biomass consists of three steps, including pretreatment, enzymatic hydrolysis, and fermentation. Pretreatment of a lignocellulosic biomass is required to improve bioconversion. Pretreatment is the process by which the biomass surface area is opened up for the subsequent enzymatic attack. The fibrous structure of the biomass is destroyed as a result of pretreatment. Various pretreatment processes have been suggested, including chemical, physical, physicochemical, and biological [3–5]. However, most pretreatment processes induce fermentation inhibitors in the lignocellulosic hydrolysate. These fermentation inhibitors are degradation products of sugars and lignin that may have a potential inhibitory effect on microorganisms during fermentation. Fermentation inhibitors in lignocellulosic hydrolysates are widely known as aliphatic acids (acetic, formic and levulinic acid), furaldehydes (furfural and 5-hydrolsymethylfurfural), aromatic compounds (phenolics), and extractives [6,7].

As generating inhibitors is unavoidable during acid pretreatment, a number of technologies have been employed to remove fermentation inhibitors from lignocellulosic hydrolysate. The techniques include lime treatment, ion exchange resin, organic solvent extraction, and adding adsorbents such as activated carbon and zeolite [8–10]. In particular, treating ion exchange resins effectively removes weak acidic inhibitors and can lead to an inhibitor-free hydrolysate, but it has shortcomings such as the release of exchanged ions and the periodical chemical regeneration of ion exchange resins [11].

Electrodialysis (ED) is one of ion exchange membrane process using an electrical potential as a driving force. Its system typically consists of a cell arrangement with a series of alternating anion and

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cation exchange membranes between an anode and a cathode to form individual cells having a volume with two adjacent membranes [12]. ED has been widely applied to bioseparation processes to separate organic acids such as lactic acid, citric acid, acetic acid, and their salts including conventional applications to mineralize water, desalinate saline solutions, produce table salt, and treat wastewater [13,14]. However, membrane fouling, which takes place due to deposition of organics on the membrane surface, is one of the most significant considerations in ED. In this system, organics including organic acid, furaldehydes, and TPC are considered as foulants. Therefore, we evaluated the removal efficiency of fermentation inhibitors in a lignocellulosic hydrolysate by ED and evaluated the ethanol performance of the ED-treated hydrolysate.

2. Experimental

2.1. Biomass and pretreatment

Mixed hardwood (*Quercus mongolica*, *Robinia pseudoacacia L.*, and *Castanea crenata*) and softwood (*Pinus rigida* and *Pinus densiflora*) chips were purchased from Poong Lim Inc. (Daejeon, Korea). The biomass was milled and screened to a size of 40–60 mesh using a J-NCM Wiley mill (JISICO, Seoul, Korea) and stored at 4 °C at <10% moisture content. Oxalic, maleic, and sulfuric acid were each used as acid catalyst for pretreatment.

The pretreatment was conducted in 500 mL cylindrical stainless steel reaction vessels. Each biomass portion and acid solutions were placed in 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 acid/water mixture to give a total solid/liquid ratio of 1:4 (w/w). Pretreatment was performed at 170 °C for 60 min with each acid catalyst solution of pH 1.34, corresponding to combined severity factor (CSF) of 2.5.

2.2. Sugar and inhibitor analysis in the hydrolysate

The concentrations of fermentable sugars in the hydrolysate were determined using an HPLC (Waters 2695 System, MA, USA) outfitted with an Aminex HPX-87P column (Bio-Rad, Hercules, CA, USA). The concentrations of inhibitors such as acetic acid, furfural, and 5-hydroxymethylfurfural (HMF) in the hydrolysate were determined with an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) and a refractive index detector (Waters 2414 System, MA, USA). All samples were properly diluted and filtered through a 0.45 μm spin-filter to remove particles before analysis.

Total phenolic compounds (TPC) were estimated colorimetrically by the Folin–Ciocalteu method [15]. Inductively coupled plasma analysis of metal ions was carried out using a Nexion 300X instrument (Perkin Elmer, MA, USA). Total organic carbon (TOC) was analyzed with a TOC Muti N/C (Analytic Jena AG, Munster, Germany).

2.3. Removal of fermentation inhibitors from hydrolysate by electrodialysis

ED experiments were performed using a hydrolysate obtained from following acid pretreatment. Ten pairs of cell structures were assembled in a CJ-S3 ED stack with a total membrane effective area of 550 cm^2 (Changjo Techno, Seoul, Korea). A commercial cation exchange membrane, NEOSEPTA® CMX, and an anion exchange membrane, NEOSEPTA® AMX (ASTOM Corp., Tokyo, Japan) were used to prepare of the stack. The hydrolysate was fed through the membrane module in the diluate as an initial feed and the treated hydrolysate in the diluate was fermented to produce ethanol. Desalting experiments with NaCl were carried out after chemical cleaning with distilled water, NaOH solution, and distilled water between the ED experiments of hydrolysate to observe the fouling effect on ED process performance. The ED-treated hydrolysate was analyzed by HPLC as described in Section 2.2.

2.4. Fermentation of hydrolysate

Pichia stipitis CBS 6054 was used for the fermentation. Yeast cells were grown in 1000 mL Erlenmeyer flasks containing 400 mL of YPD (10 g/L yeast extract, 10 g/L peptone, and 20 g/L glucose) in a shaking incubator for 24 h, after which the cell cultures were harvested and washed with sterile deionized water. The hydrolysate was adjusted to pH 6.0 with sodium hydroxide. Washed cells at a dry cell weight of 2.0 g/L were transferred to hydrolysate with 5 g/L yeast, 5 g/L urea, 0.5 g/L $MgSO_4$ $7H_2O$ and 1 g/L KH_2PO_4. Fermentation was performed at 30 °C in an orbital shaker at 150 rpm. Samples were taken at 24, 48, 72, and 96 h, and the amount of monosaccharides remaining and the ethanol produced were analyzed by HPLC as described in Section 2.2.

3. Results and discussion

3.1. Sugars and inhibitors in the hydrolysate following acid pretreatment

The compositional analysis of the hydrolysate obtained from each pretreatment is shown in Tables 1 and 2. Xylose was the most abundant sugar in the mixed hardwood hydrolysate of the pretreatment conditions studied, whereas glucose released from cellulose was relatively low. The mixed softwood hydrolysate mainly contained mannose and glucose. The sugars and inhibitors concentrations differed significantly depending on the kind of biomass and acid catalyst.

In the mixed hardwood, the highest fermentable sugar reached 35.27 g/L, which was obtained from the oxalic acid pretreatment.

Table 1
Sugars, inhibitors and total organic carbon (TOC) in hydrolysate during acid pretreatment of lignocellulosic biomass.

Biomass	Acid catalyst	Sugar (g/L)			Inhibitor (g/L)				TOC (g/L)
		Glucose	Xylose	Mannose	Acetic acid	HMF	Furfural	TPC	
Mixed hardwood	Oxalic acid	10.10	25.17	NA	8.41	1.31	2.68	5.04	19.54
	Maleic acid	12.71	18.92	NA	8.57	1.37	3.99	7.98	25.65
	Sulfuric acid	8.12	15.49	NA	8.52	1.46	4.23	5.51	17.65
Mixed softwood	Oxalic acid	9.31	6.38	15.10	1.84	2.24	0.47	2.80	16.82
	Maleic acid	12.41	5.22	12.18	1.60	1.82	0.90	4.40	22.11
	Sulfuric acid	7.99	1.44	3.82	1.84	5.42	1.26	4.05	13.89

NA is data not available. Pretreatment was performed at 170 °C for 60 min with each acid catalyst solution of pH 1.34, corresponding to combined severity factor (CSF) of 2.5.