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Separation of phenolic acids from monosaccharides by low-pressure nanofiltration integrated with laccase pre-treatments

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ABSTRACT

Separation of phenolic acids from monosaccharides is required for detoxification of lignocellulosic hydrolysates. For the first time, a low-pressure nanofiltration (NF) process was used to retain phenolic acids (vanillic acid, *p*-coumaric acid and ferulic acid) and at the same time permeate monosaccharides (xylose, arabinose, glucose). Four commercial NF membranes (NF270, NP030, NTR7450 and NP010) were evaluated at different pH values and with various laccase pre-treatments (for polymerization of phenolic acids). The results showed that with increasing pH, the retentions of phenolic acids by NF increased, reaching 86–88% for NTR7450 and 90–94% for NF270 at pH 9.55. The retentions of monosaccharides kept almost constant ($<10\%$) for NP030, NTR7450 and NP010 membranes at different pH but significantly increased at pH 9.55 for the NF270 membrane due to enhancement of solute interactions. Phenolic acids could be polymerized by laccase and then completely retained by the NF membranes via size exclusion at pH 5.15. The formation of large polymeric products by laccase could alleviate the irreversible fouling in/on a NF membrane and decrease the monosaccharide retention, while the small polymeric products (e.g. dimers and trimers) were mainly responsible for the adsorption fouling. Free laccase treatment was preferred since it was prone to produce large polymeric products while the biocatalytic membrane with immobilized laccase was not suitable as it generated smaller polymers by in-situ product removal. Furthermore, the NF membranes with more charge and higher hydrophilicity were more resistant to the irreversible fouling caused by hydrophobic adsorption of phenolic acids and their polymers. This work not only provides fundamental data for removal of phenolic acids from lignocellulosic hydrolysates, but also opens a new gate for separation of small solutes with similar molecular weight by NF integrated with enzymatic conversion.

1. Introduction

Biorefinery has gained considerable momentum as a solution to the serious energy and environmental issues. Shifting society's dependence from petroleum-based to biomass-based resources is commonly believed to be a key for development of a sustainable society, energy independence, and effective control of greenhouse gas emissions [1–4]. Lignocellulosic biomass is an abundant and carbon-neutral renewable resource for the production of biofuels and valuable chemicals [1]. Lignocellulose-derived ethanol can be used as an environmentally friendly liquid fuel. However, rapid and efficient fermentation of the hydrolysates to ethanol is limited because besides monosaccharides, various toxic compounds, which have a potential inhibitory effect on the enzymatic hydrolysis and subsequent fermentation [5], are generated during pre-treatment and pre-hydrolysis of lignocelluloses [2,6–9]. The detoxification of hydrolysates can be accomplished by physico-chemical (e.g. evaporation, membrane extraction, solvent extraction, over-liming, activated charcoal adsorption and ion-exchange) and biological (e.g. microbiology, enzyme, adaption of fermenting microorganism) methods [6,7,10,11].

Nanofiltration (NF), as a powerful tool for separation of small molecules and salts, has attracted growing attention in removal of inhibitors and concentration of fermentable sugars from lignocellulosic hydrolysates [2,3,12–19]. It is widely accepted that the inhibitors are divided into three groups, namely, furan derivatives (furfural and 5-hydroxymethylfurfural (HMF)), aliphatic acids (formic acid, acetic acid and levulinic acid) and phenolic compounds (phenolic acids and aldehydes) [6,10]. Generally, monosaccharides (i.e. xylose, arabinose and glucose) are retained while inhibitors pass through the NF membranes. Weng et al. found that the maximum separation factor of xylose and acetic acid was 5.4 when a Desal-5 DK membrane was operated at pH 2.9 and

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24.5 bar [12]. Qi et al. claimed that with a NF90 membrane, the recovery of xylose and glucose in the retentate was higher than 98.5%, while the removal of furfural reached 66.2% [13]. Maiti et al. reported that a separation factor of 3 was obtained for the inhibitors (acetic acid, HMF, ferulic and vanilic acids) over sugars using a NovaSep NF membrane with a molecular weight cut-off (MWCO) of 150 Da at pH 3, while the retention of monosaccharides was lower than 65% [14]. Zhou et al. found that with a Desal-5DK membrane, the separation factors were 8.87 and 56.5 for acetic acid over xylose and glucose at 30 bar and pH 2.93, respectively [15]. Brás et al. investigated the detoxification of hemicellulosic hydrolysates from extracted olive pomace by diafiltration using NF270 membrane, showing that 99.7% of acetic acid and 100% of formic acid and furfural could be removed while around 50% of arabinose and xylose together with 74% of glucose were recovered, respectively [16]. Gautam and Menkhaus evaluated the separation performance and fouling behavior of various NF membranes during processing of lignocellulosic biomass hydrolysates, and found that the sugar retentions were much higher for the lignocellulosic hydrolysates, compared with the pure sugar mixture, which was probably caused by component interactions and a higher degree of fouling [17]. Based on these examples from the literature, it can be concluded that most studies focused on the removal of furan derivatives and aliphatic acids from monosaccharides, and that NF has been able to achieve this goal. However, very little research has been devoted to the separation of monosaccharides and phenolic compounds, especially phenolic acids (i.e. vanillic acid, *p*-coumaric acid and ferulic acid) which share a similar molecular weight with monosaccharides (i.e. xylose, arabinose and glucose). It can be anticipated that during the removal of furan derivatives and aliphatic acids, monosaccharides are concentrated by dense NF membranes and at the same time, the phenolic acids will accumulate in the concentrated lignocellulosic hydrolysate and induce a negative effect on the ethanol production [8].

It is well known that the variation of the solute retention with permeate flux by NF follows an exponential cumulative distribution function [20–23]. That is, the real solute retention normally increases with permeate flux due to the increment of solvent convective transport (dilution effect) [20]. For instance, the real retention of glucose by a Desal-5 DK membrane increased from 30% to 70% when the permeate flux increased from 9 to 90 L m^{-2} h^{-1} [21]. Inspired by this mechanism, in the present report we attempt to separate monosaccharides and phenolic acids using a low-pressure NF process (low permeate flux), where the monosaccharides pass through the NF membrane and the phenolic acids are retained. Such a process offers two advantages: first, monosaccharides transmission can avoid enormous increase of osmotic pressure in the retentate and save energy (sugars are major components of the concentrated lignocellulosic hydrolysates); second, operating at low pressure (below threshold flux) can minimize the fouling formation [24]. As illustrated in Fig. 1, the charges on both phenolic acids and NF membrane can be increased by manipulating solution pH, leading to a high retention of phenolic acids due to enhanced electrostatic repulsions; on the other hand, it was reported that phenolic acids could be polymerized by laccase at acid pH [25–27], and then the polymeric products of laccase-catalyzed phenolic acids are supposed to be retained by the NF membrane via size exclusion. If these hypothetic strategies can be verified, high purity of monosaccharide streams can be obtained by a multi-stage membrane filtration of lignocellulosic hydrolysates.

To the best of our knowledge, this is the first attempt to detoxicate (i.e. removal of inhibitors) lignocellulosic hydrolysate by letting monosaccharides pass through a NF membrane. Xylose, arabinose, glucose, vanillic acid, *p*-coumaric acid and ferulic acid were used as model solutes and the performance (i.e. retention, permeate flux and fouling) of four commercial NF membranes (i.e. NF270, NP030, NTR7450 and NP010) were assessed at different pH values and with various pre-treatment methods (under a constant pressure of 4 bar). For enzymatic treatment, both free and immobilized enzymes were used. By comparing the NF results from different strategies, the retention and fouling mechanisms of this low-pressure NF process were discussed and the most suitable membrane was also selected together with the desirable detoxification strategy.

2. Materials and methods

2.1. Chemicals, membranes and enzyme

D-Xylose ($\geq$99.0%), L-arabinose ($\geq$99.0%), D-glucose (99.5%), vanillic acid (analytical standards), *p*-coumaric acid (98.0%), ferulic acid (99.0%) and dopamine hydrochloride were purchased from Sigma Aldrich. Their main characteristics and concentrations used in this study are listed in Table 1 [14,28]. The phenolic acids were first dissolved in 1 mL 50% acetonitrile solution. All the model solutions were prepared using a 10 mM acetate buffer (pH=5.15). Sodium hydroxide (5 M) was used to adjust pH. Dopamine hydrochloride was prepared freshly using a 10 mM Tris–HCl buffer (pH 8.5). Four commercial NF membranes were used in this work, and their main properties are summarized in Table 2, on the basis of the manufacturers' information, literature [29] and our own measurements. A commercial ultrafiltration membrane, RC70PP (Alfa Laval, regenerated cellulose/polypropylene, 10 kDa) was used for enzyme immobilization. Laccase (EC 1.10.3.2, 60–70 kDa, 26.0 U mg^{-1}) from *Trametes versicolor* was purchased from Fluka. The enzyme solution was prepared using a 50 mM phosphate buffer.

2.2. Experimental set-up and procedure

2.2.1. Low-pressure NF process

NF experiments were performed in a stirred cell (Amicon 8050, Millipore, USA). Descriptions of the equipment can be found in our previous work [30]. A fresh membrane was used for each set of experiments. The NF membranes were first soaked in 50% ethanol solution for 30 s and then washed with deionized water. After the membranes were pre-pressured at 4 bar for 30 min, the water permeability of the membranes was measured in the stirred cell at 4 bar with Millipore pure water. Subsequently, 11 mL model

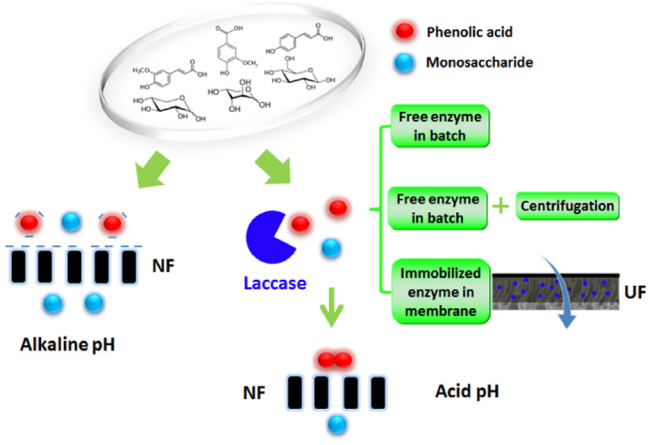

Fig. 1. Schematic diagram of the strategies for separation of phenolic acids from monosaccharides. Strategy A: single nanofiltration (NF) using charge effect (left); Strategy B: laccase polymerization of phenolic acids followed by NF using size exclusion (right).