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High performance separation of xylose and glucose by enzyme assisted nanofiltration

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ABSTRACT

An integrated membrane system was investigated for the separation of mixtures of xylose and glucose. Separation of these sugars is extremely challenging due to their similar structure, size and charge. In order to enhance the xylose separation factor in nanofiltration (NF), we present an enzymatic process for converting glucose to gluconic acid followed by separation of xylose from gluconic acid by nanofiltration. Process conditions which favored the negative charge repulsions between gluconic acid and the NF270 membrane were examined. At the best conditions (9:1 feed molar ratio of xylose to gluconic acid, 0.15 M total feed concentration, pH 9.5, 25 °C and 4 bar), we achieved a xylose separation factor of 34 and a throughput of 18.7 L m^{-2} h^{-1}. In comparison, the separation factor was only 1.4 for solutions of xylose and glucose at the same process conditions, thus demonstrating the huge potential of the integrated system. Full conversion of glucose to gluconic acid assisted by glucose oxidase (GOD) could be achieved by coupling a parallel reaction catalyzed by catalase (CAT), where H_2O_2 (GOD-inhibitor formed in the first reaction) was decomposed to water and oxygen. GOD has a high oxygen-demand and it was demonstrated that sufficient oxygen could be obtained by controlling the CAT-catalyzed reaction through initial H_2O_2 addition. The new strategy suggested in this study, integrating reaction and nanofiltration to enhance separation while obtaining another value-added stream, presents new options for separating compounds with similar molecular weights by nanofiltration.

1. Introduction

Mixtures of pentoses and hexoses are obtained from enzymatic or chemical treatment of lignocellulosic biomass. Designing processes and technologies to separate these mixtures is a prerequisite for shifting to a biobased economy, since such compounds are used as building blocks for subsequent production of a wide range of industrial derivatives. In the current study, different model solutions of xylose (pentose) and glucose (hexose) are considered with the aim of obtaining pure streams of xylose. Attainment of highly pure xylose streams is e.g. very important in the chemical production of xylitol where purification of xylose comprises the most costly process step [1]. In most cases, separation of monosaccharides is carried out by column liquid chromatography due to its high selectivity [2–4]. Nanofiltration, with properties ranging between reverse osmosis and ultrafiltration, could offer a high throughput and easy-maintenance alternative. However, the molar masses of xylose and glucose only differ by a factor 1.2, which inevitably makes separation by nanofiltration extremely difficult. Several studies have already reported a very limited separation of xylose and glucose when nanofiltration was applied; Mah et al. [5] achieved xylose separation factors from 0.7–2 using a self-fabricated nanofiltration membrane, while Sjöman et al. [6] obtained xylose separation factors up to around 3.3 with the Desal-5 DK membrane. Several operational parameters besides the type of membrane used can influence the nanofiltration performance. According to Goulas et al. [7], the retention of monosaccharides can be increased by increasing the pressure/flux and decreasing the feed concentration. Furthermore, solute retention can be decreased by increasing the temperature due to reduced viscosity and increased diffusivity. According to Sharma et al. [8], increases in temperature at the same time lead to changes in the membrane structure, resulting in larger pore sizes which additionally contribute to reducing the solute retention. The ratio of xylose to glucose in the feed also has an influence on the filtration. Sjöman et al. [6] reported a higher xylose separation factor when the proportion of xylose in the feed was increased. Mah et al. [5] contradictorily observed that the xylose separation factor decreased when more xylose was added to the feed solution. Since the previous investigations have not led to significant separation factors, we present a new strategy for separating xylose from xylose–glucose mixtures with the aim of obtaining a stream with high purity of xylose. We suggest an enzymatic process for converting glucose to gluconic acid followed by separation of xylose from gluconic

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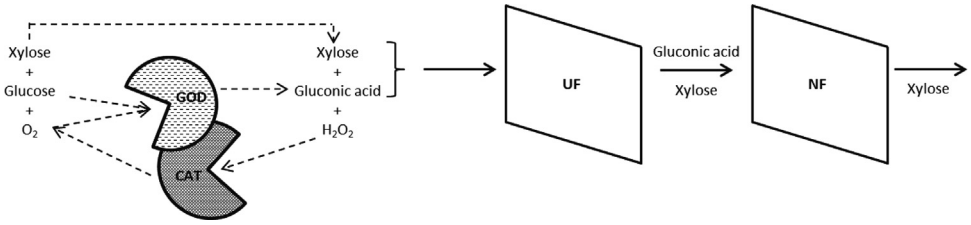

Fig. 1. Separation of xylose from xylose–glucose mixtures in a membrane bioreactor. Glucose is first oxidized to gluconic acid and hydrogen peroxide by glucose oxidase (GOD). Catalase (CAT) simultaneously decomposes the GOD-inhibitor H_2O_2 to oxygen. The enzymes can be separated from xylose and gluconic acid by UF (not conducted in this work) and then xylose is separated from gluconic acid by NF. Although gluconic acid in solution at a pH above its pKa is correctly termed "gluconate", "gluconic acid" is used for simplicity throughout the study.

acid using nanofiltration (Fig. 1). The separation mechanism is based on negative charge repulsions – rather than merely sieving effects – between gluconate (conjugate base of gluconic acid) and the surface of the membrane, thus allowing only xylose to pass through. Like xylose, gluconic acid ($C_6H_{12}O_7$) finds various applications in the food industry. Its inner ester (glucono-δ-lactone) brings out a sweet and sour taste which makes it a valuable ingredient in dairy products, wines and pickled goods [9]. Furthermore, the National Renewable Energy Laboratory (NREL) [10] has identified gluconic acid as one of the top 30 building blocks for derivation of high-value chemicals. Gluconic acid is naturally found in fruit juices and honey but can be produced from glucose ($C_6H_{12}O_6$) via a simple oxidation reaction catalyzed by glucose oxidase (GOD):

$$D-\text{glucose}+O_2+H_2O \xrightarrow{GOD} D-\text{gluconic acid}+H_2O_2 \quad \text{(r1)}$$

The hydrogen peroxide formed in the reaction is highly reactive and can inactivate GOD, so in order to avoid this inactivation, a peroxide-decomposing reaction is required [11]. Hydrogen peroxide can be decomposed to water and oxygen through the catalytic action of catalase (CAT):

$$2H_2O_2 \xrightarrow{CAT} 2H_2O+O_2 \quad \text{(r2)}$$

The decomposition of hydrogen peroxide has the additional advantage of producing oxygen for r1. Otherwise, oxygen had to be provided by bubbling air or pure oxygen into the glucose solution [12–14].

The present work was undertaken to verify the hypothesis of using a previous step of enzymatic reaction of glucose to gluconic acid to subsequently ease the separation of xylose from glucose in nanofiltration. The influence of temperature, pressure, pH, concentration and composition of the feed solution was systematically examined with respect to the xylose separation factor (versus gluconic acid) in the nanofiltration. The influence of enzyme concentration (GOD and CAT) and agitation was investigated with respect to the conversion of glucose to gluconic acid in the enzymatic reaction.

2. Materials and methods

2.1. Chemicals and membranes

D-xylose (≥99%) and D-gluconic acid sodium salt (≥99%) were purchased from Sigma-Aldrich (Steinheim, Germany). D(+)-Glucose was purchased from EMD Millipore (Darmstadt, Germany). The main characteristics of these components are shown in Table 1 [15–17]. Glucose oxidase from *Aspergillus niger* and catalase from bovine liver were purchased from Sigma-Aldrich (Steinheim, Germany). The main properties of these enzymes are shown in Table 2 based on information from the manufacturer [18,19]. NF270 polyamide flat sheet membranes (DOW-Filmtec) with a molecular weight cut-off of 150–200 Da were purchased from Sterlitech (Kent, USA).

Table 1
Main characteristics of the feed components adapted from the Human Metabolome database.

	Xylose	Glucose	Gluconic acid
Abbreviation	Xyl	Glc	GA
Molecular formula	$C_5H_{10}O_5$	$C_6H_{12}O_6$	$C_6H_{12}O_7$
Molecular weight (Da)	150	180	196
pKa	11.3	11.3	3.4
Reference	[15]	[16]	[17]

Table 2
Main characteristics of the enzymes adapted from Sigma-Aldrich.

	Glucose oxidase	Catalase
Abbreviation	GOD[a]	CAT[b]
Source	*Aspergillus niger*	Bovine liver
Molecular weight (kDa)	160	250
pI	4.2	5.4
Activity	136 U/mg enzyme	4540 U/mg enzyme
Reference	[18]	[19]

[a] For GOD 1 U converts 1 μmole ß-D-glucose per min. at pH 7, 25 °C (air, atm. pressure 0.25 mM O_2).
[b] For CAT 1 U converts 1 μmole H_2O_2 per min. at pH 7, 25 °C (10 mM H_2O_2).

2.2. Experimental set-up and procedure

The enzymatic reactions and 4 bar-nanofiltration experiments were conducted in a magnetically stirred dead-end cell (Amicon 8050, Millipore, USA) with a working volume of 50 mL and effective membrane area of 13.4 cm^2. High-pressure nanofiltration experiments (10 and 15 bar) were conducted in a stainless steel stirred dead-end cell (Sterlitech HP4750, USA) with a working volume of 300 mL and effective membrane area of 14.6 cm^2. A constant pressure was provided by filling nitrogen gas into the cells, while permeate was collected in a tube placed on an electronic scale in order to calculate the permeate flux. When the reactions or filtrations were carried out at elevated temperatures, heat was supplied by circulating hot water through an isolated tube wrapped around the stirred cell. The reactions were performed in an open cell (no lid) in order to let in as much oxygen as possible and to allow for regular sampling. The enzymatic reaction and subsequent nanofiltration were examined as two separate process steps.

2.2.1. Water permeability measurements

The virgin membranes were soaked in 50% ethanol solution for 15 min before flushing them with deionized water at 3 bar for