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Separation of xylose and glucose using an integrated membrane system for enzymatic cofactor regeneration and downstream purification

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ABSTRACT

Mixtures of xylose, glucose and pyruvate were fed to a membrane bioreactor equipped with a charged NF membrane (NTR 7450). Value-added products were obtained in the reactor via enzymatic cofactor-dependent catalysis of glucose to gluconic acid and pyruvate to lactic acid, respectively. The initial cofactor (NADH) concentration could be decreased to 10% of the stoichiometric value (relative to glucose) without compromising process time and substrate conversion via i) efficient cofactor regeneration and ii) high retention of cofactor (R=0.98) in the membrane bioreactor. Furthermore, accumulation of xylose (R < 0.1), lactic acid (R=0.38) and gluconic acid (R=0.63) was minimized. After separation of the cofactor in the membrane bioreactor, xylose, lactic acid and gluconic acid were separated based on charge repulsion and size exclusion in a sequence of two NF steps. Broad substrate specificity of glucose dehydrogenase (EC 1.1.1.47) (GDH) lead to partial conversion of xylose to xylonic acid, causing some loss of xylose, but the results obtained nevertheless showed that it is possible to build a robust system for conducting enzyme reactions by sequentially regenerating the cofactor and at the same time obtaining valuable products of high purity.

1. Introduction

Lignocellulosic biomass is an abundant and renewable feedstock with the potential to supplement or substitute fossil resources for production of valuable chemicals and fuels [1]. In order to exploit the full potential of lignocellulose as a feedstock for biorefining, effective utilization of all biomass constituents must be accomplished [2]. One major challenge of lignocellulose biorefining is to achieve separation of different types of monosaccharides, notably glucose and xylose. Recently, we demonstrated that xylose-glucose separation could be greatly improved through enzyme-assisted nanofiltration (NF), in which one component (glucose) was enzymatically converted to another value-added chemical (gluconic acid) whereby the purification of the remaining component (xylose) was significantly enhanced [3]. Glucose oxidase (EC 1.1.3.4) (GOD)/catalase (EC 1.11.1.6) (CAT) was the enzyme system used for obtaining gluconic acid and pure xylose; however, gluconic acid may also be produced via glucose dehydrogenase (EC 1.1.1.47) (GDH) catalysis which requires the cofactor NAD^+– rather than oxygen – as the electron acceptor (r1). As cofactors are expensive molecules, GDH should preferably be combined with a cofactor regenerating enzyme to enable reuse of the cofactor [4]. L-lactic dehydrogenase (EC 1.1.1.27) (LDH) is a suitable candidate for such cofactor regeneration since it uses NADH during reduction of pyruvate to L-lactic acid (r2) and operates at the same optimal temperature (37 °C) and pH as GDH (GDH: pH 8 [5]; LDH: pH 7.5 [6]):

$$\text{D–glucose} + \text{NAD}^+ \xrightarrow{\text{GDH}} \text{D–gluconic acid} + \text{NADH} \quad \text{(r1)}$$

$$\text{Pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{L–lactic acid} + \text{NAD}^+ \quad \text{(r2)}$$

In order to obtain a high efficiency in (r1, r2), the cofactor must first of all reach a high total turnover number (TTN) – here defined as number of moles of product(s) formed per mole of cofactor added in the system [7]. If the cofactor is to be used in consecutive reaction cycles, separation of the cofactor from the reaction products is moreover required. Since membranes allow high retentions of both the cofactor and the enzymes, membrane bioreactors have shown great potential in such applications. For instance, Wichmann et al. [8] used a 5 kDa UF membrane to retain L-leucine dehydrogenase/formate dehydrogenase and NAD(H) linked to 10 kDa polyethyleneglycol (PEG).

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Covalent attachment of the enzymes and the cofactor to PEG significantly improved the size exclusion of the cofactor without altering the enzyme kinetics. Obón et al. [9] studied the retention of GDH/LDH and NAD(H) by a 10 kDa uncharged UF membrane. Prior to the filtration, polyethyleneimine (PEI), a cationic polymer, was added to the substrate solutions to induce electrostatic attraction between the negatively charged cofactor at pH 7.0 and the positively charged 50 kDa PEI, which resulted in a cofactor retention above 80% and TTN around 14,000; however the study did not reveal how the retention of charged products (gluconic acid and lactic acid) was affected. Nidetzky et al. [10] used a charged 1 kDa NF membrane to retain xylose reductase/xylose dehydrogenase/glucose dehydrogenase and NAD(H) while removing the neutral (xylitol) and the charged (xylonic acid or gluconic acid) products from the membrane bioreactor. Since the retention of cofactor was governed by charge repulsion, reaction conditions favoring the reduced form of the cofactor (NADH) were preferred, enabling 98% cofactor retention and a TTN around 10,000. In the present study, we expanded the application of enzyme assisted membrane separation to a system generating two charged products (gluconic acid and lactic acid). We conducted the cofactor regeneration reactions (r1 and r2) and the downstream product purification using an integrated NF system (Fig. 1) for the purpose of enhancing the separation of xylose from glucose. Different feed and cofactor concentrations were investigated, while five different membranes were applied for the separation of cofactor as well as the downstream purification. The GDH/LDH system studied here was compared with GOD/CAT [3,11] and the base case separation of xylose from glucose. The evaluation was based on a quantitative comparison of the purity and yield of xylose and the value-added stream(s), and the biocatalytic productivity rate (mass of product per mass of enzyme per time) of the three systems. Furthermore, qualitative and practical considerations regarding the system designs were included in the assessment.

Even if the main purpose of the study is just to present the proof of concept for the viability of a sequential cofactor regeneration-purification system, some of the basic principles of green chemistry have been inherently considered in the design of the sequence. Indeed, the ultimate objective of using a system for cofactor regeneration is maximizing the product yield while minimizing the use of resources (in this case cofactor), and also minimizing byproduct formation, which was attained by selecting a cofactor regeneration system from which value-added chemicals, i.e. gluconic acid and lactic acid could be achieved. Furthermore, membrane separation enables, as compared to the competing purification technologies available e.g. chromatography, to achieve high throughput without use of potentially harmful solvents.

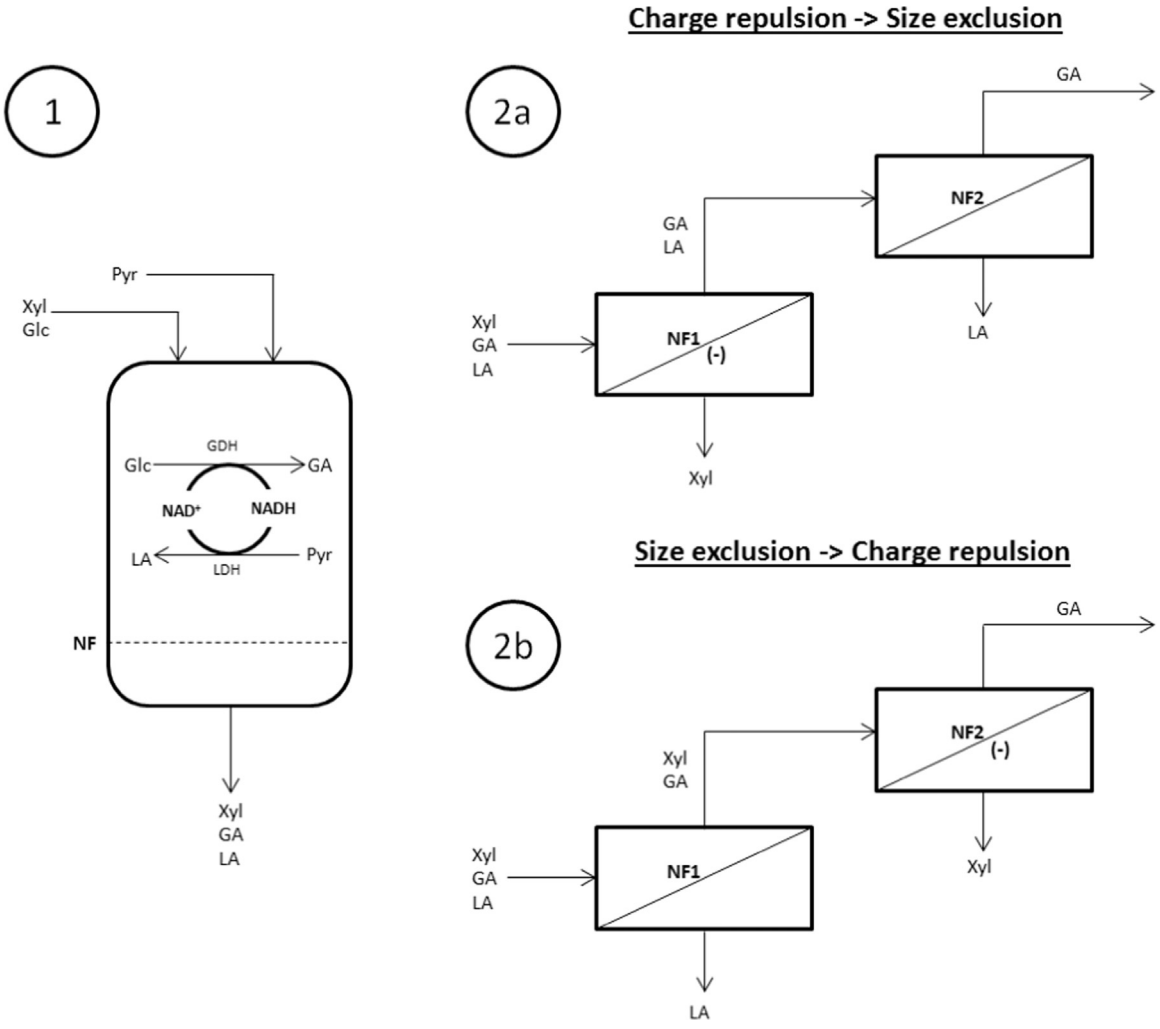

Fig. 1. Separation of xylose from xylose-glucose mixtures using an integrated membrane system comprising 1) a membrane bioreactor (equipped with a NF membrane) where consecutive cofactor regeneration reactions take place and 2) a downstream purification sequence (2a or 2b) consisting of two NF steps. Mixtures of xylose and glucose are fed to the reactor together with pyruvate, while xylose leaves the reactor together with gluconic acid and lactic acid. In sequence 2a, the purification of xylose, gluconic acid and lactic acid is based on charge repulsion (NF1) and size exclusion (NF2). In sequence 2b, the purification is based on size exclusion (NF1) and then charge repulsion (NF2). Xyl=xylose; Glc=glucose; Pyr=pyruvate; GA=gluconic acid; LA=lactic acid; GDH=glucose dehydrogenase; LDH=l-lactic dehydrogenase; NF=nanofiltration.