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# Nanofiltration potential for the purification of highly concentrated enzymatically produced oligosaccharides

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## ABSTRACT

The performance of five commercial nanofiltration (NF) membranes was evaluated in the fractionation of enzymatically produced galacto-oligosaccharides (GOS). Filtration experiments were performed by modifying the solute concentration (10.5–40°Brix) and effective transmembrane pressure ( $TMP_e$ ) from 5 to 40 bar.

In terms of flux and apparent rejection ( $R_{ap}$ ), thin film composite membranes (ATF and NFA) resulted inadequate. Polyamide membrane (GE) showed a better performance in terms of low  $R_{ap}$  for mono and disaccharides, as well as a negligible fouling; however, a strong reduction of flux was observed when increasing the solute concentration.

Polyethersulphone (PES) membranes (NP030 and NP010) showed a very good performance in terms of low  $R_{ap}$  values for monosaccharides and disaccharides at  $TMP_e$  values lower than 25 bar. Membrane NP010 showed the highest flux at all the operating conditions investigated, producing total rejection of GOS at 40 bar with high potential for their concentration; however, selectivity could not be controlled because of the high  $R_{ap}$  in lactose. At an operating  $TMP_e$  of 20 bar, GOS were fractionated with sustainable fluxes (28 kg/m<sup>2</sup>/h) and a good selectivity, even when highly concentrated solutions (40°Brix) were treated, demystifying the limitation of nanofiltration as a downstream operation for treating highly concentrated solutions.

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## 1. Introduction

Due to the alarming figures associated to bad or poor nutrition, the last decade has witnessed how the population perception about the importance of functional food consumption has increased, prebiotics having an important role

as functional food ingredients (Pérez-Conesa et al., 2004). This situation has prompted the development of new formulations generating new business opportunities because of the increased demand and acceptance of this kind of products (Annunziata and Vecchio, 2013), which represents a most dynamic area in the food industry. In this category,

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non-digested oligosaccharides, such as fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) have gained interest because of their health promoting properties, which are mainly associated to the stimulation of specific microbial activities, enabling the regulation of vitamins and cholesterol adsorption, being helpful for intestinal health and for colon cancer prevention (Torres et al., 2010). In addition, the production of these prebiotics by bioprocess technology, such as enzyme biocatalysis, is an opportunity to add value to currently underutilized agriculture and agroindustrial by-products (Choi et al., 2015). The bioconversion of lactose into GOS by enzymatic transgalactosylation is a kinetically controlled reaction, which means that the biocatalyst ( $\beta$ -galactosidase) presents both transgalactosylation (GOS formation) and hydrolytic activity on the substrate yielding also glucose and galactose as reaction products, while a significant fraction of lactose remains unreacted so that product yield is rather low, not exceeding 40% (Park et al., 2008; Neri et al., 2009; Splechtna et al., 2006; Chockchaisawasdee et al., 2005; Gekas and Lopez-Leiva, 1985). It is well known that the use of high substrate concentrations (lactose) depresses the hydrolytic potential of the enzyme favoring the reaction of synthesis over hydrolysis; anyhow, under such conditions undesired monosaccharides and lactose still remain at the end of reaction (Gosling et al., 2010; Vera et al., 2012).

Since the main application of non-digested oligosaccharides is for human consumption, product purity is a key aspect, so that purification operations are indispensable (Pinelo et al., 2009). Thus, if undesired monosaccharides and disaccharides are removed from the reaction product (raw GOS), purified GOS will be produced amenable for use in special food formulations for those segments of the population that cannot benefit from the health promoting effects associated to their consumption, such as diabetics, lactose intolerants and weight watchers (Hernandez et al., 2009).

Purification of enzymatically synthesized oligosaccharides has been conducted by selective adsorption on activated charcoal (Lee et al., 2004), size exclusion chromatography (Hernandez et al., 2009), nanofiltration (NF) with continuous diafiltration (Feng et al., 2009) or selective fermentation (Goulas et al., 2007). An important issue related to the first three methodologies is the high volume of water required, while the use of selective fermentation implies the addition of nutritional supplements and removal of metabolites that may contaminate the product (Cheng et al., 2006; Rabiou et al., 2001). However, membrane NF is attractive for the purification and concentration of GOS mixtures because their scale-up is straightforward, it is energy efficient, and the operational parameters can be easily optimized (Patil et al., 2014).

Despite the progress made in the application of NF to the purification of oligosaccharides, one of the main limitations is the difficult separation of highly concentrated streams (Cheryan, 1998). In fact, previous research on GOS fractionation by NF has been done at low solute concentrations (Feng et al., 2009; Goulas et al., 2002; Michelson et al., 2014) but, as stated above, one of the main strategies to increase GOS yield in the enzymatic transgalactosylation of lactose is the use of high substrate concentrations in order to favor transgalactosylation over hydrolysis (Vera et al., 2012; Huerta et al., 2011). Thus, in terms of economy and operational simplicity, it is convenient to process the raw GOS stream at a condition as close as possible to the one leaving the bioreactor.

Considering these facts, the objective of this work was to evaluate the real potential of different commercial NF

membranes as a sound strategy for the purification of enzymatically produced oligosaccharides at very high concentrations, by characterizing their performance in terms of the apparent rejection coefficient, as well as determining a level of transmembrane pressure and flux feasible for the operation.

## 2. Materials and methods

### 2.1. Reagents

$\beta$ -Galactosidase from *Aspergillus oryzae* (Enzeco® Fungal Lactase) was kindly donated by Enzyme Development Corporation, EDC (New York, USA). The enzyme had a pH optimum between 4.5 and 5.0 and an optimum temperature of 55 °C with respect to its hydrolytic activity. Lactose was purchased from Aiesi Hospital Service (Napoli, Italy). Glucose and sucrose ( $\geq 99\%$  purity), sodium phosphate dibasic, citric acid, *o*-nitrophenol (*o*-NP) and *o*-nitrophenyl  $\beta$ -D-galactopyranoside (*o*-NPG) were supplied by Sigma Aldrich (Milano, Italy).

### 2.2. NF membranes and equipment

Different commercial NF membranes with different active layer material (polyethersulfone (PES); composite polyamide; thin film composite) and different average molecular weight cut-off (MWCO) were used. The MWCO was selected according to the molecular size of the compounds typically present in oligosaccharides solutions: monosaccharides (180 Da), disaccharides (360 Da) and oligosaccharides (540–1000 Da). Table 1 shows the characteristics of the tested membranes.

NF experiments were performed by using a laboratory bench plant supplied by Three-Es Srl (Milano, Italy). The plant consists of a feed tank, a positive displacement pump (Cut pump, pressure range 7–140 bar, maximum operational flow 16 L/min), a stainless steel cross-flow cell for flat-sheet membranes with a filtration area of 0.0032 m<sup>2</sup>, two pressure gauges (0–60 bar) for measuring of the inlet and outlet pressures, a digital flow meter, a pressure control valve and a cooling coil fed with tap water used to maintain the feed temperature constant.

All experimental runs were performed by fixing the axial velocity at 0.362 m/s. The pumping velocity was controlled with an inverter connected to the pump. An analytical balance (Radwag, WTB 2000, Radom, Poland) was used to record the mass variation at the output of the permeate stream. The permeate flux (*J*) was determined by measuring the collected permeate weight at a given time through the membrane surface area as stated elsewhere.

### 2.3. Experimental procedure

#### 2.3.1. Preparation of model solutions

In order to evaluate the rejection of individual sugars, membranes were characterized by using model solutions of monosaccharides, disaccharides and mixtures of them, at different solute concentrations, but at the same ratio in which they are present in the original raw GOS solution, which is (on wet basis): 10% (w/w) monosaccharides (glucose or galactose), 18% (w/w) disaccharides (lactose) and a mixture of mono and disaccharides (28%, w/w). All these model solutions were also prepared in 30 mM citrate buffer pH 4. Table 2 summarizes the composition of each model solution as well as the raw GOS solution used for membranes characterization.

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