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Continuous ultrafiltration membrane reactor coupled with nanofiltration for the enzymatic synthesis and purification of galactosyl-oligosaccharides

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ABSTRACT

Continuous enzymatic production of galactosyl-oligosaccharides (GOS) from a lactose substrate in an ultrafiltration membrane reactor (UMR) coupled with a nanofiltration separation system (CPNSS) was investigated. The overall rate of production over 4 h of continuous production was 80–104 mg GOS formed/U with an average residence time of 66 min, an initial lactose concentration of 300 g/L, an inlet pressure of 2.0 bar inlet pressure, and an outlet pressure of 1.5 bar. In the continuous diafiltration (CD) process, the concentration of various sugars and the relationship between the yield and purity of oligosaccharides was well predicted by mathematical models, the increased rate of sugar rejections was less than 10%, and the decreased rate of concentrations in the tank was less than 15%. In the CPNSS, 33.4 wt.% GOS was obtained in the UMR, and 1.24 kg of high-purity GOS (specifically, GOS purity of 57.2% and lactose content less than 20%) was achieved. This final yield was 80.1% GOS, which meets industry standards.

1. Introduction

In recent years, some of the most significant developments in functional food science have been related to the development of dietary supplements that beneficially affect the microbial composition of the gut [1,2]. Modulation of the ecology of the gut to improve the well-being of the host, by administering probiotics and prebiotics, is attracting increasing attention [3,4].

Prebiotics are non-digestible oligosaccharides (NDOs) which have been found to reach the human colon without being hydrolyzed or absorbed in the upper part of the gastrointestinal tract [5,6]. The key aspect of a prebiotic is that it is selectively metabolized by benign or health-positive species, such as *bifidobacteria* and *lactobacilli*, at the expense of less desirable groups, such as *clostridia* [7,8].

Galactosyl-oligosaccharides (GOS) are NDOs, which are recognized as prebiotics [9]. GOS selectively stimulate the growth of *bifidobacteria* in the lower part of human intestine. Increase in the growth of *bifidobacteria* is usually accompanied by the suppression of potentially harmful bacteria, such as the *Clostridium* and *Bacteroides* genera, in the intestine [10].

GOS consist of galactosyl-galactose chains with a terminating glucose and are the desired by-products of the enzymatic catalysis [11,12]. The composition of the GOS fraction varies in chain length and in the interconnection of the monomer units with varying β-glycosidic linkages depending on the enzyme's source [13]. In contrast to the hydrolyzation of lactose, GOS are very slowly hydrolyzed both *in vivo* and *in vitro*. The GOS produced by β-galactosidases are low in molecular weight, not viscous, and water-soluble liquid dietary fibers that are stable at elevated temperatures and at low pH [14].

There have been several investigations on the synthesis of GOS by β-galactosidases from various sources. Among these sources, β-galactosidase from *Kluyveromyces lactis* has been extensively studied [15]. The enzyme was reported to have stronger hydrolytic activity than transferase activity and produced a high proportion of trisaccharide in the synthetic GOS mixtures [16,17]. There have been many studies on the immobilization of the enzyme, and various substrates have been investigated [18–20]. Continuous synthesis of galacto-oligosaccharide from lactose using β-galactosidase

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Nomenclature

J	the permeate flux (L (m^2 h))
V	volume (L)
A	the membrane effective area (m^2)
t	time (h)
τ	the average residence time (h)
v	the average permeate flow rate (L/h)
PR	productivity (g of GOS formed/U)
$\overline{P}$	the average product output (g/h)
E	the enzyme concentration (U/L)
LAC	the average conversion of lactose (%)
TMP	the transmembrane pressure (bar)
R	the rejection coefficient (%)
L	the concentration of lactose (g/L)
P	pressure (bar)
C	concentration (g/L)
y	the yield value
Pu	purity (%)
Y	yield (%)

Superscript

A	micro-molecular solute
B	macro-molecular solute

Subscript

up	the ultrafiltration membrane permeate
np	the nanofiltration membrane permeate
p	permeate
r	reaction mixture
in	inlet
F	feed
out	outlet
f	final
i	initial

from *K. lactis* in an ultrafiltration membrane reactor (UMR) has been reported [15,21,22]. However, to our knowledge, the continuous synthesis of GOS from lactose using β-galactosidase and a nanofiltration membrane to produce high concentrations of GOS has not yet been investigated.

In this study, continuous production of GOS is achieved using a cross-flow UMR. We also investigated the optimization of the yield by selectively lowering the concentration of the product from the reaction mixture. Experiments evaluating the dependence on pressure, temperature, and concentration were conducted to determine the performance of the nanofiltration membrane based on the permeate flux and the apparent rejection coefficient. Then, the purification of a commercial GOS mixture was performed with the nanofiltration membrane using a continuous diafiltration (CD) procedure. Finally, we investigated the continuous synthesis of GOS from lactose using β-galactosidase coupled with nanofiltration membrane to achieve a high yield of GOS.

2. Experimental

2.1. Chemicals and materials

Deionized water, 5 mmol/L potassium phosphate (pH 6.5) containing 5 mmol/L $MgSO_4$ as a buffer were used in all experiments. β-Lactose was kindly provided by the Lactose Company of NEW Zealand Limited (NEW Zealand). The enzyme Lactozym Pure 6500L from *K. lactis* with an activity of 6500 LAU/g was kindly provided by Novozymes (Denmark). Other chemicals were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd (China).

2.2. Enzyme

The commercially available enzyme used was β-galactosidases by Lactozym Pure 6500L(Novozymes, Denmark). The enzyme was highly purified, liquid, and prepared from *K. lactis* with an optimum temperature of 25–40 °C and an activity of 6500 LAU/g, where one LAU is defined as the quantity of enzyme that liberates 1 μmol o-nitrophenol from o-nitrophenyl-β-D-galactopyranoside (ONPG) per minute under standard conditions. The enzyme had a molecular weight exceeding 100,000 Da [23].

2.3. Membranes

Table 1 lists the membranes used in this study. The membranes include an ultrafiltration (UF) membrane and a nanofiltration (NF) membrane.

The UF membrane module (UOF4, Tianjin Motian Membrane Technology Co., Ltd., Tianjin, China) is tubular and contains a shell of UPVC material and 5000 roots membrane silks (350 μm i.d. × 450 μm o.d.) of composite regenerated cellulose material. The ultrafiltration membrane had a 50-kDa nominal molecular weight cut-off (NMWCO) and an effective area of 0.12 m^2. Maximum operating pressure and temperature for the membrane were stated to be 3.0 bar and 45 °C, respectively.

The NF membrane is the patented three-layer composite film membrane obtained from General Electric Company (United States). The membrane from the inside to the outside is divided into three layers: the first layer is a thin separation layer made of a polyethylene piperazine material with a thickness of about 0.2 μm and a pore diameter of about 0.1–0.2 nm; the second layer is a porous intermediate support layer made of polysulfone with a thickness of about 40 μm and a pore diameter of about 15 nm; the third layer is a non-woven fabric made of polyester material with a thickness of about 120 μm. The NF membrane has a total active filtration area of 0.32 m^2. The maximum operating pressure and temperature for the membrane were stated to be 10.0 bar and 50 °C, respectively.

2.4. Membrane properties towards enzyme

A volume of 800 mL of enzyme (1 U/mL) diluted in the synthesis buffer was placed in the reactor vessel, passed through the membrane, and recycled to the vessel. The experiment was run at room temperature for four hours maintaining a 2.0 bar inlet pressure and a 0.5 bar outlet pressure. Every 30 min, samples (1 mL) were taken from both the retentate and permeate streams.

2.5. Continuous synthesis of GOS in an UMR

Continuous production of oligosaccharides from lactose was performed using the laboratory equipment shown schematically (Fig. 1). The UMR contains a 1.5 L stirred-tank, an ultrafiltration membrane, and an electronic diaphragm pump. The reactor volume was 800 mL. The flow rate of the feed was controlled by an