TWO-STAGE INTEGRATED CERAMIC MEMBRANE REACTOR SYSTEM FOR THE CONTINUOUS ENZYMATIC SYNTHESIS OF OLIGOSACCHARIDES

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ABSTRACT

A new type of ceramic membrane reactor is proposed for the continuous enzymatic synthesis of oligosaccharides using native enzyme. Beta-Galactosidase (EC 3.2.1.23) catalyses the hydrolysis of lactose to the monosaccharides glucose and galactose and also the transgalactosylation reaction to produce galactosyl-oligosaccharides (GOS). GOS are non-digestible oligosaccharides which are recognized as prebiotics. GOS consist of a number of oligosaccharides with varying ß-glycosidic linkages depending on the enzyme source. In this work, the results for the continuous production of GOS from lactose by means of physically-confined ß-Galactosidase in a novel ceramic membrane reactor system are presented.

KEYWORDS

Ceramic Membrane, Membrane Reactor, Ultrafiltration, Cross-Flow Filtration, Lactose

1. Introduction

Due to the transferase activity of the enzyme during enzymatic hydrolysis of lactose, an additional transfer is caused from galactose to other saccharides, including galactosyl-oligosaccharides (GOS). GOS consist of a galactosyl-galactose chain with a terminating glucose and are the desired by-products of the additional reaction. GOS consist of a galactosyl-galactose chain with a terminating glucose. The composition of the GOS fraction varies in chain length and in the interconnection of the monomer units with varying ß-glycosidic linkages depending on the enzyme source. GOS are very slowly hydrolyzed in contrast to the lactose both *in vivo* and *in vitro*. The GOS produced here are low-molecular, not viscose, water-soluble liquid dietary fibers [1]. Many benefits are ascribed to GOS, including their status as physiologically active functional food; their enhancement of the growth of Bifido bacteria in the intestine and the promotion of health [2, 3].
Recently, a number of chemical and enzymatic methods for the production of galactosyl-oligosaccharides from disaccharide substrates have been developed. The chemical synthesis of GOS requires multiple protection and de-protection steps. This poses a complexity that does not render chemically-driven synthesis attractive for industrial applications. To overcome this challenge, new strategies for the continuous production of GOS in innovative enzymatic membrane bioreactors have been developed [4, 5, 6].

The use of a novel two-stage integrated ceramic membrane reactor system to physically confine (physically immobilize) ß-galactosidase from *Klyveromyces lactis* for the continuous production of galactosyl-oligosaccharides through enzymatic conversion of lactose was investigated. The results of these investigations are reported here.

2. Material and Methods

Chemicals

Deionized water was used. 5 mmol/l potassium phosphate containing 5 mmol/l $MgSO_4$ (pH 7.0) was used as a buffer in all experiments. Food-grade lactose monohydrate was used (99.95% pure, Meggle GmbH, Wasserburg, Germany). Other chemicals all of p.a. quality were purchased from VWR International GmbH (Darmstadt, Germany)

Membranes

Table 1 lists the material and properties of the tubular ceramic membranes used in these investigations. Ceramic membranes (atech innovations GmbH, Gladbeck, Germany) have an asymmetric structure consisting of one support layer (AL_2O_3) with large pores and a low pressure drop and one separation layer (TiO_2) which controls the permeation flux. Membrane (1) exhibits an inner diameter of 16 mm and a total active filtration area of $8.2*10^{-3}$ m². Membrane (2) has an outer diameter of 10 mm and a total active filtration area of $20.7*10^{-3}$ m² (Fig. 1).

Table 1. Ceramic membrane material and properties.

Membrane	MWCO	Membrane material	pH range	Max. temp.
UF-TiO_2	20 kD	AL_2O_3 / TiO_2	0 - 14	121°C

The nominal molecular weight cut-off of both ultrafiltration ceramic membranes was in a range of 20.000 g/mol, indicating that the enzyme (MWCO>117,000 g/mol) should be retained by both membranes.

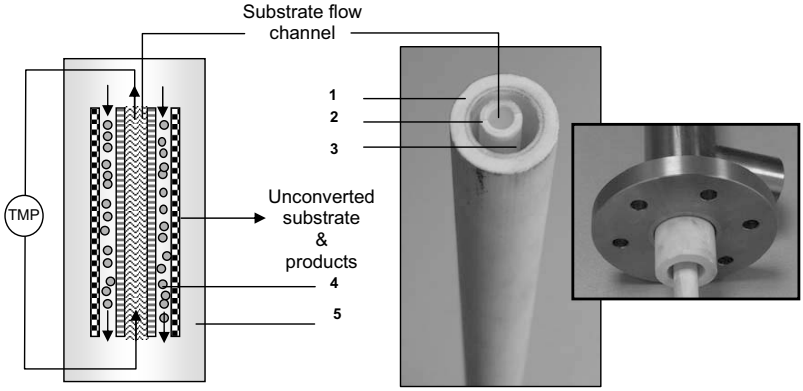

Figure 1. Schematic of the two-stage integrated ceramic membrane reactor system: ceramic membranes (1) and (2); annular space (3); physically immobilized enzyme (4); outer compartment (5).

Enzyme

The enzyme used in this study was a commercially available ß-galactosidases, Maxilact® L 2000 (Gist-Brocades NV, Delft, Holland) which has a declared activity of 2000 NLU/g. One NLU is defined as the quantity of enzyme that liberates 1 µmol of *o*-nitrophenol from *o*-nitrophenyl-ß-D-galactopyranoside (ONPG) per minute under standard conditions. The enzyme has a molecular weight exceeding 117,000 g/mol. The source and properties of the enzyme used in these investigations are shown in Table 2.

Table 2. Source and properties of enzyme.

Trade name	Derived from	Optimum pH	Optimum temp. [°C]	pI	Activity [U/g]
Maxilact L 2000	*Kluyveromyces lactis*	6.8 – 7.0	35 – 40	5.1	2000

Continuous Ceramic Membrane Reactor

A continuous stirred tank reactor (CSTR) was used with a membrane module in a stainless steel housing as shown in Figure 2. The enzymes were confined in the annular space (volume about 43.5 ml) defined by two separating walls (Fig. 1, (4)). The ceramic membrane reactor is tubular and contains two coaxial, cylindrical monoliths with a monoflow channel (Membrane 2) for the substrate.

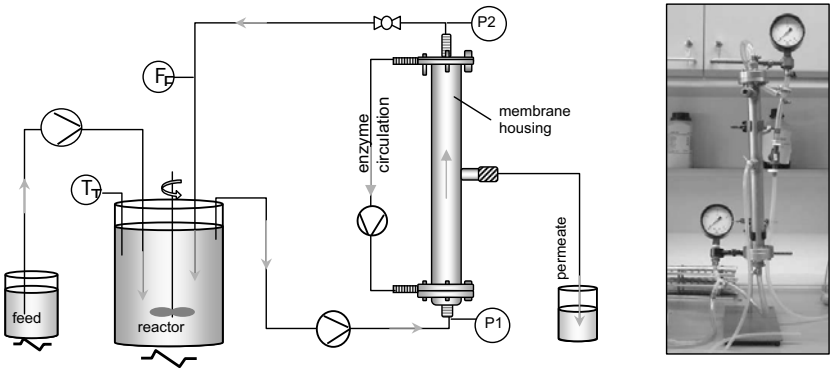

Figure 2. Schematic diagram of continuous laboratory scale reactor/membrane system with native ß-galactosidase and enzyme circulation.

During continuous processes, the lactose solution was poured into the inner compartment of the membrane reactor (substrate flow channel) using a peristaltic pump, as shown in Figure 2. The substrate percolated in the radial direction across the inner membrane (2) to the annular space (3) where enzymatic hydrolysis took place. The permeate containing oligosaccharides, unconverted lactose and some of the by-products was passed through the outer membrane (1) at the controlled trans-membrane pressure (TMP) gradient (Fig. 1). TMP is the driving force for the membrane separations and is defined as the difference between retentate and permeate pressure. The permeate was collected in the outer compartment (5).

Analytical Method

The analysis of the carbohydrates containing products (glucose, galactose, and oligosaccharides) and the amounts of unconverted lactose in permeate were performed with high performance thin-layer chromatography (HPTLC) using a Camag system (Linomat 5, TLC Scanner 3, Camag GmbH, Berlin, Germany).

3. Results and conclusions

Our results show that physically immobilized enzyme used in a newly developed ceramic membrane reactor may perform well in the continuous production of GOS from lactose. Significant product concentrations of oligosaccharide were achieved in the permeate.

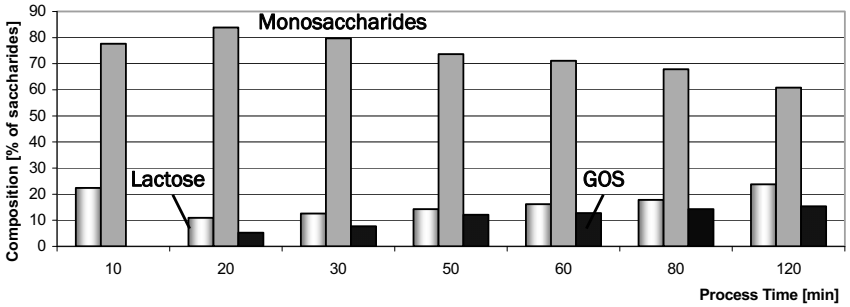

Figure 3. Production of oligosaccharides from lactose in a continuous membrane reactor system (10% initial lactose concentration (feed); 1 bar TMP; enzyme: Maxilact L 2000; 40°C; pH 6.7). 20 kD ceramic membrane was used.

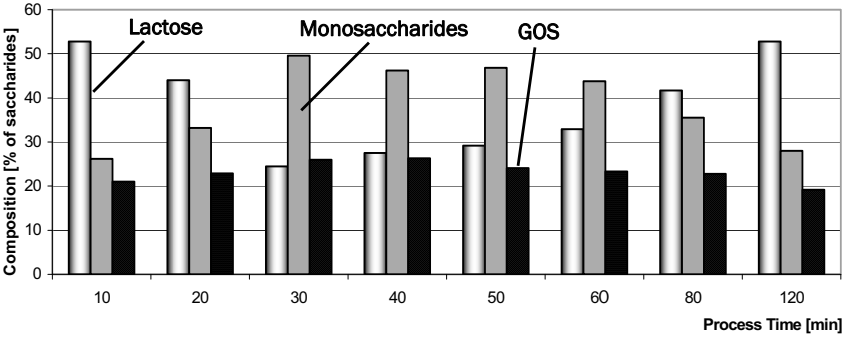

Figure 4. Production of oligosaccharides from lactose in a continuous mode membrane reactor system (20% initial lactose concentration (feed); 1 bar TMP; enzyme: Maxilact L 2000; 40°C; pH 6.7). 20 kD ceramic membrane was used.

High substrate lactose concentration is essential for process economy [6]. The results show an increase in the production of oligosaccharides with a higher initial lactose concentration due to more lactose competing with H_2O to be an acceptor for galatosyl residue at a higher concentration.

Further investigations and optimization for the process parameters of the newly developed ceramic membrane reactor system in continuous mode are necessary. The influence of a number of parameters, such as the use of high initial substrate concentrations (lactose) under varying transmembrane pressure, use of different enzymes and different residence times, have to be investigated if oligosaccharide yield in permeate is to be maximized.

Acknowledgements

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