Journal of Membrane Science 378 (2011) 471–478

Contents lists available at ScienceDirect

Journal of Membrane Science

journal homepage: www.elsevier.com/locate/memsci

Feasibility study of enzyme immobilization on polymeric membrane: A case study with enzymatically galacto-oligosaccharides production from lactose

Dwaipayan Sen[a], Ankur Sarkar[a], Aaron Gosling[b,c], Sally L. Gras[b,c], Geoff W. Stevens[b], Sandra E. Kentish[b], P.K. Bhattacharya[d], Andrew R. Barber[e], Chiranjib Bhattacharjee[a,*]

[a] Department of Chemical Engineering, Jadavpur University, Kolkata 700032, India
[b] The Department of Chemical and Biomolecular Engineering, The University of Melbourne, Victoria 3010, Australia
[c] The Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Victoria 3010, Australia
[d] Department of Chemical Engineering, Indian Institute of Technology Kanpur, Kanpur 208016, India
[e] Innovative Food and Plants Division, South Australian Research and Development Institute, Regency International Centre, Days Road, Regency Park, SA 5010, Australia

ARTICLE INFO

Article history:
Received 16 February 2011
Received in revised form 22 April 2011
Accepted 17 May 2011
Available online 23 May 2011

Keywords:
Immobilization
Carbohydrates
Polymeric membrane
Galacto-oligosaccharides
Lactose

ABSTRACT

Present work primarily deals with an exhaustive investigation on the effect of β-galactosidase (EC 3.2.1.23) immobilization on polymeric polyether sulfone, cellulose triacetate and thin film composite polyamide membrane to produce galacto-oligosaccharides from lactose. Fouling is one of the key issues that control any membrane separation process to obtain the desired product. Especially, this issue with membrane becomes multiplied after making any attachment of immobilization chemicals on its surface, i.e. in case of enzymatic membrane reactor. Present work thus aims to identify the insights of carbohydrate interactions with the membrane surface after immobilization and how far it controls the production of galacto-oligosaccharides in this membrane reactor.

1. Introduction

Integrating membrane separation techniques with biochemical reactions to produce several products are the most coveted techniques implemented commercially in various industrial sectors [1] nowadays. Application of a membrane separation process is quite common in food industry. Mainly, in dairy industry ultrafiltration process [2] is a widely accepted technique, which is applied to manufacture yoghurt and different categories of cheeses [3]. Enzymatic conversion of lactose, present in milk, to glucose, galactose and prebiotic galacto-oligosaccharides (GOS), is another common food grade application in dairy based industry. The global retail market for prebiotic products was estimated an annual 167,000 ton and 390 million Euro market [4] in current days showing an increasing demand for prebiotics. GOS is one of such prebiotic food ingredients, manufactured from lactose that promotes the proliferation of bifidobacteria in the gastrointestinal microflora [5]. Thus it acts as one of the essential food stuffs to the people those who are suffering from lactose intolerance [6]. However, GOS production is limited by the preference of transgalactosylation activity over hydrolytic activity determined by the source of enzyme. Another retarding effect on the production of GOS is enzyme inhibition by monosaccharides [7] produced during the enzymatic conversion of lactose. Therefore, to reduce the inhibition impact on the reaction yield as well as to enhance the purity of GOS, online elimination of monosaccharides from the reaction mixture is always considered to be the primary concerns for the food engineers. Immobilizing enzyme on membrane of suitable molecular weight cut-off (MWCO) thus provides an efficient separation tool for GOS from mono- and disaccharides along with the production of it [8]. Moreover, enzyme immobilization offers reutilization of enzyme and product recovery without catalyst contamination [9]. In agro-food and dairy industry, application of biocatalyst immobilization shows a remarkable footstep [10] that encouraged food scientists to explore the idea of GOS production using immobilized enzyme.

Engel et al. [11] had made an investigation on membrane assisted GOS production where they had done adsorption based immobilization of enzyme β-galactosidase (EC 3.2.1.23) on an anion exchange polyether sulfone (PES) membrane. During their study, they had noticed around 82% lactose conversion and 24% GOS yield after 3600s (1 h) of the reaction period. Ulbricht and Papra [12] made a comparative study between adsorption based and cross-linking based immobilization of both amyloglucosidase and invertage enzyme on polyacrylonitrile (PAN) and

* Corresponding author. Tel.: +91 33 2414 6203; fax: +91 33 2414 6203; mobile: +91 98364 02118.
E-mail addresses: c.bhatta@gmail.com, cbhattacharyya@chemical.jdvu.ac.in, c_bhatta@rediffmail.com (C. Bhattacharjee).

0376-7388/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.memsci.2011.05.032

carboxyl-modified polyacrylonitrile (PAN-AA) ultrafiltration membranes. Reusability of the membrane matrix for future immobilization of enzyme is always a fundamental concern for any industry based immobilization application. Adsorption based immobilization provides that opportunity of reusing those membranes after removing the adsorbed enzyme layer from the membrane surface because of loose binding and at the same time it increases the chances of enzyme leakage [13]. Thereby, operational stability of the immobilized enzyme layer on membrane should be considered also. Ulbricht and Papra [12] noticed that although the cross-linking method performs irreversible immobilization, but it yields better enzyme stability compared to the adsorption technique and thus made it more acceptable to industrial application. Present study offers a comparative investigation on the applicability for GOS production with an enzyme immobilized polyamide thin film composite (TFC) nanofiltration (NF) with both polyether sulfone (PES) and cellulose triacetate (CTA) ultrafiltration (UF) membrane. Both adsorption and cross-linking based immobilization techniques were employed in this investigation. Fundamentally, the current work aims an attempt to understand the applicability of a membrane reactor equipped with an UF membrane and a NF membrane in GOS production from lactose.

Complying with the molecular weight distribution of the carbohydrate mixture obtained after enzymatic reaction with lactose, nanofiltration membrane could be considered as a separating medium for GOS from the reaction medium [14]. Goulas et al. [14] noticed that the continuous diafiltration accompanied nanofiltration yields 98% pure GOS. Based on this encouraging outcome with NF, β-galactosidase enzyme was immobilized on nanofiltration membrane to design a membrane reactor where production and purification of GOS will be taken place simultaneously. However, the study made by Tang et al. [15] showed that immobilization on membrane leads to a partial pore blocking on the membrane surface. In their study, they attempted to modify the surface property of hydrophobic polypropylene membrane after immobilizing α-allyl glucoside with a 58% decrease in the water contact angle exhibiting increased hydrophilicity of the membrane. In contrast, according to their study with an increased extent of immobilization, it also effectively reduces the water flux after certain value of immobilization degree and thus manifests to pore blocking phenomenon. As a result immobilization of enzyme on nanofiltration membrane leads to an application of high process pressure more than that of required for normal nanofiltration and thereby makes the process more energy intensive. The requirement of this high pressure with immobilized nanofiltration membrane insists to understand the applicability of enzyme immobilization on ultrafiltration membrane in the production of purified GOS.

2. Materials

2.1. Chemicals

β-Galactosidase enzyme (Market name: Biolacta FN5; EC 3.2.1.23) extracted from *Bacillus circulans*, with initial lactose activity, 4500 LU g^{-1}, was procured from Burra Foods, Australia. 1LU, abbreviated form of 'Lactose Unit' [16] refers to the amount of enzymes required to produce 1.67×10^{-11} kmol of glucose/s from lactose. Selection of enzyme source is one of the difficult tasks that need to be considered during any biochemical reaction. In this present work selection of enzyme from *B. circulans* was mainly because of its pronounced transgalactosylation activity at a high temperature (313–323 K) compared to other sources of enzymes for this particular reaction [17]. Glutaraldehyde, one of the main constituents for immobilization, enhanced the transgalactosylation activity of this particular enzyme from *B. circulans* by making conformational changes near the active site of the enzyme [17] through binding with the enzyme. Lactose was purchased from SISCO Research Laboratory Pvt. Ltd., Mumbai, India. Polyethyleneimine (50%, w/v, average molecular weight: 750 kg mol^{-1}) and glutaraldehyde (250 kg m^{-3} of aqueous solution) were purchased from Hi-Media Laboratories Pvt. Ltd., Mumbai, India. The commercial enzyme preparation has protein content of 0.041 kg per kg of solid powder. Enzymatic protein content was measured by Folin–Lowry [18] method using Folin–Ciocaltaeu's Phenol reagent (2 N, AR Grade) was supplied by SISCO Research Laboratory Private Limited, Mumbai, India. 98% sulfuric acid, sodium carbonate, caustic soda, anhydrous copper sulfate and sodium potassium tartarate were procured from Merck India Ltd.

2.2. Membranes

5 kg mol^{-1} polyether sulfone (PES) (procured from: Pall Omega™ 76 mm; P/N: OM005076; LOT: A10640107), 5 kg mol^{-1} cellulose triacetate (CTA) (procured from: Sartorius AG Gottingen, LOT: 0601 14529 9901863) and 50 kg mol^{-1} PES (procured from: Pall Omega™ 76 mm; P/N: OM050076; LOT: A1031386) circular disc membranes of diameter 0.076 m and effective diameter 0.056 m were used in the present study. TFC®-SR2 membrane (0.4 kg mol^{-1}) procured from Koch Membrane Systems (San Diego, CA) was basically polyamide thin-film composite with a microporous polysulfone supporting layer. 50 kg mol^{-1} PES UF cross-flow membrane module (Vivascience Vivaflow 200; overall length/height/width: 0126/0.138/0.038 m; channel width/height: 0.01/0.0004 m) of effective surface area 0.02 m^2 was purchased from Sartorius AG Gottingen. Immobilized polymeric membrane was fitted in a rotating disc membrane module to carry out both the GOS production and purification through enzymatic reaction with lactose on the membrane surface [19]. Concentration polarization is one of the key issues in membrane separation process. Here the effect of concentration gradient became pronounced as because of immobilised enzyme and thereby, restricts the passage of solutes towards the enzyme immobilised membrane surface. Therefore, the reaction will be restricted and hence the production of GOS. Rotating disc membrane [19] creates turbulence on the membrane surface because of the rotating membrane and thus reduces the effect of concentration polarization.

2.3. Analytical instruments

De-ionized (DI) water was collected from ultrapure water system (Model-Arium 611) of Sartorius AG, Germany. Primarily, tap water was purified through iron filters and resin beds followed by a reverse osmosis (RO) system (Model-Arium 61315, make: Sartorius AG Germany). Permeate from RO unit was practically pure water having no total dissolved solids (TDS). This TDS free water was fed to the Arium 611 unit to prepare de-ionized water used in this particular work. VARIAN UV–visible spectrophotometer (Cary50 Bio) was used for protein concentration measurement at 750×10^{-9} m (750 nm) [18]. HPLC (Perkin Elmer, Series 200) with, RI detector and Spheri 5 amino column (5×10^{-6} m, 0.046×0.220 m) (Perkin Elmer), was used with mobile phase of 75:25 (v/v) acetonitrile:water at a flow rate of 8.33×10^{-9} m^3 s^{-1} (0.5 ml min^{-1}) for sugar analysis. Column oven temperature maintained at 298 K.

3. Experimental

3.1. Membrane compaction and water run

Prior to any experimental run with the polymeric membranes, both NF and UF circular disk (0.076 m diameter) membranes were subjected to a trans-membrane pressure of 1.4 MPa and 0.9 MPa,