Separation and Purification Technology 124 (2014) 61–67

Contents lists available at ScienceDirect

Separation and Purification Technology

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

Effect of substrate and enzyme concentration on cyclodextrin production in a hollow fibre membrane reactor system

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ARTICLE INFO

Article history:
Received 31 May 2013
Received in revised form 21 November 2013
Accepted 1 January 2014
Available online 15 January 2014

Keywords:
Cyclodextrins
Ultrafiltration
Enzymatic membrane reactor
Hydraulic resistance
Tapioca starch

ABSTRACT

The batch and continuous production of cyclodextrins (CDs) was assessed by employing an enzymatic membrane reactor (EMR) system. The effects of tapioca starch substrate and cyclodextrin glycosyltransferase (CGTase) concentrations on the yield of CDs were studied. A similar effect on the behaviour of the ultrafiltration membrane in the EMR system (integration system) was also evaluated. The results for the batch process showed that incremental doses of CGTase caused gradual increments in CD yield; however, further addition of CGTase (above 1.0%) showed a 16% reduction in the total CD production. Further incremental in the tapioca starch concentration increased CD concentration (23 g/L). However, addition above 8% w/v resulted in an insignificant yield of CDs. In the case of integration system, tapioca starch feeding rate that is higher than 4.41 g/h caused adverse effects (lower CD yield and membrane flux). In particular at higher tapioca starch feeding rate (5.0 g/h), the hydraulic resistance would reach as high as 1.31×10^{13} m^{-1}. Presumably this phenomenon was due to the unreacted substrates that were adsorbing onto the membrane surface and pores that subsequently led to greater fouling conditions and a severe flux decline. In addition, the weak adsorption (r_{a1}) has been found to be the major fouling mechanism attributed to starch and its by products. Therefore, hydraulic cleaning is highly suggested as the procedure to be used for this EMR system.

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1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides composed of α-1,4-glycosidic-linked glucosyl residues produced from starch or starch derivatives using cyclodextrin glycosyltransferase (CGTase). In other words, CGTase (EC 2.4.1.19) is an enzyme capable of converting starch and related substrates into CDs [1,2]. CDs can solubilise hydrophobic materials and entrap volatile components by forming inclusion complexes with organic compounds, subsequently enhancing their chemical and physical properties [2–6]. These properties have led CDs to a substantial spectrum of commercial applications such as those for the food industry [7,8], the pharmaceutical industry, cosmetics, agricultural and plastic emulsifiers, antioxidants and stabilising agents [9]. In general, conventional production of CDs is performed through a batch process as this technique is simple and easy to control. However, there are several disadvantages of this process: intensive requirement of enzyme concentration, long operating hours, and high labour cost [10].

An alternative method, employing an enzymatic membrane reactor (EMR), which is more practical and economical, has been suggested. The ability to operate an EMR continuously and the reusability of the enzyme leads to greater yield productions of CDs compared with the batch process.

The EMR combines membrane separations and the use of an enzymatic reactor to enable product separation from the enzyme and/or substrates through a semi-permeable membrane. An asymmetric semi-permeable membrane composed of an ultra-thin separation layer has been widely used in enzyme separation. During the separation process, the enzyme is retained by the membrane within the reaction reactor, while the CD products pass through the membrane as permeate. In particular, the membrane would discriminate solutes that have molecular weights (MW) larger than its molecular weight cut-off (MWCO) or pore size. Several types of enzymatic membrane reactors have been classified according to the allocation of their membrane modules. The common

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http://dx.doi.org/10.1016/j.seppur.2014.01.005

configuration of an EMR is that of a stirred tank reactor combined with a separation-membrane unit that recirculates the reaction mixture through a membrane module placed outside the enzymatic reactor. The pressure difference across the membrane, or the transmembrane pressure (TMP), impels the product through the membrane, while the unreacted substrate and free enzyme are recirculated to the enzymatic reactor [11,12]. However, the challenge in using this external membrane system is in the frequency of membrane fouling because of substrate deposition onto the membrane surface and enzyme precipitation within the membrane pores. In practice, substrate deposition on the membrane surface can be eliminated by employing a high crossflow velocity (CFV), but this condition generally requires a large amount of energy and reduces the enzyme activity [10].

The other advantages of EMRs are high production flow rates; reductions in cost, energy, and waste products by the recycling practice; easy reactor operation and control; a straightforward scale-up to large systems; and the high yields of pure material [13]. Nevertheless, the loss of enzyme activity, inappropriate selection of starch type or concentration, and membrane fouling problems are the reported downsides. In the continuous EMR system, enzyme stability is also influenced by its deposition on the membrane surface as this condition may reduce enzyme concentration and activity in the reactor. As the CGTase activity is reduced, CD yield will also decrease.

In a large-scale CD production, greater starch concentration is essential. However, this condition may cause higher hydraulic resistance against membrane flow and may potentially lead to severe membrane fouling. In particular, membrane fouling results in flux decline over time, which occurs because of substance deposition on the membrane surface, subsequently creating greater hydraulic resistance and more TMP requirements. In addition, the fouling layer buildup may completely plug the membrane's pores strongly influencing filtration performance [14–19]. Therefore, the objectives of this research are to study the effects of tapioca starch concentration and CGTase on CD production and membrane performance in an EMR system. A resistance-in-series model was also used to identify the governing fouling mechanisms and to determine a way to address the consequences.

2. Materials and methods

2.1. Production of CDs in the batch process

A 10 L enzymatic reactor was filled with 8 L acetate buffer (10 mM; pH 5.0) and 8% food grade tapioca starch. The starch was heated at 70 °C for an hour with continuous stirring at 200 rpm. Then, a 0.5% heat-stable CGTase (Toruzyme 3.0 l produced by Novozymes A/S, Bagsvaerd, Denmark) was added in free form to the tapioca starch solution and the reaction was carried out for 4 h after the reactor temperature was reduced to 60 °C.

2.2. Production of CDs in the continuous process

The EMR has been set-up based on Mimi Sakinah et al. [20]. In this study, the TMP used was 1.0 bar. The operation of the continuous EMR consisted of reaction and separation processes. The reaction process procedure was similar to that of the batch process; however, an additional process was performed in order to separate the CDs from the reaction mixture. After 4 h of reaction period, the reaction mixture (unreacted tapioca starch, starch-degraded products, and active CGTase) was continuously filtered using an ultrafiltration membrane at a TMP of 1.0 bar and a CFV of 0.32 m s^{-1}.

2.3. Fabrication of hollow fibre ultrafiltration (UF) membranes

The hollow fibre UF membrane was fabricated and characterised using a procedure described by Mimi Sakinah et al. [20].

2.4. Membrane characterisation by molecular weight cut-off

The nominal MWCO of the membrane was determined by ultrafiltration experiments at the operating pressure of 1.4 bar using a series of polyethylene glycols (PEGs) and polyvinylpyrolidones (PVPs) of MWs between 10 and 55 kDa [20]. The feed solution was supplied to the outer skin layer of the hollow fibres. The MWCO of this hollow fibre was found to be 32 kDa.

2.5. Scanning electron microscopy (SEM)

Digital scanning microscopy (SEM) was used to observe the cross-section of the hollow fibre. The membrane samples were placed on the stud and coated with gold–palladium before photographs were taken.

2.6. Analysis of CDs

The concentrations of CDs were determined using an HPLC (Water Assoc.), eluted with acetonitrile: water (70:30) at 1.0 mL/min and a refractive index detector (Waters 410). Column temperature was controlled at 30 °C. All samples were filtered with a Whatman® nylon membrane filter (0.2 μm pore size, 13 mm diameter) before injection.

2.7. CGTase activity by Kaneko method

CGTase activity was determined by using a phenolphthalein assay [21]. The reaction mixture contains 1 ml of 40 mg soluble starch in 0.1 M phosphate buffer (pH 6.0) and 0.1 ml enzyme solution. The mixture was incubated at 60 °C for 10 min in a water bath. The reaction was stopped by adding 3.5 ml of 30 mM NaOH solution. Subsequently, 0.5 ml of 0.02% w/v phenolphthalein in 5 mM Na_2CO_3 solution was then added to the reaction mixture and mixed well (Thermolyne: type 16700 mixer). After 15 min, the reduction in colour intensity was measured at 550 nm. As a standard, the soluble starch and CGTase were replaced by β-CD in 0.1 M phosphate buffer of pH 6.0. One unit of enzyme activity was defined as the amount of enzyme that formed 1 μmol β-CD per minute under the conditions defined above.

2.8. Determination of individual resistances in the resistance-in-series model

The resistance-in-series model is most widely used in determining the various hydraulic resistances in membrane separation [22–26]. There are five resistances (Eq. (1)) in the resistance-in-series model based on Darcy's law as given in Mimi Sakinah et al. [20].

$$J = \frac{\Delta P}{\mu(r_m + r_{cp} + r_g + r_{a1} + r_{a2})} \quad (1)$$

where J is the flux through the membrane (m/h), ΔP is the transmembrane pressure (Pa), μ is the dynamic viscosity (Pa h), r_m is the membrane hydraulic resistance, r_{cp} is the concentration polarisation resistance, r_g is the gel layer resistance, r_{a1} is the weak adsorption resistance and r_{a2} is strong adsorption resistance (all resistance are in m^{-1}).

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