Journal of Biotechnology 203 (2015) 89–96

Contents lists available at ScienceDirect

Journal of Biotechnology

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

Influences of operating conditions on continuous lactulose synthesis in an enzymatic membrane reactor system: A basis prior to long-term operation

Azis Boing Sitanggang[a,c,*], Anja Drews[b], Matthias Kraume[a]

[a] Chair of Chemical and Process Engineering, Technische Universität Berlin, Ackerstraße 76, 13355 Berlin, Germany
[b] HTW Berlin - University of Applied Science, Engineering II, School of Life Science Engineering, Wilhelminenhofstraße 75A, 12459 Berlin, Germany
[c] Department of Food Science and Technology, Bogor Agricultural University, Raya Darmaga St, Kampus IPB Darmaga Bogor 16680, West Java, Indonesia

ARTICLE INFO

Article history:
Received 22 September 2014
Received in revised form 9 March 2015
Accepted 18 March 2015
Available online 30 March 2015

Keywords:
Enzymatic membrane reactor (EMR)
Lactulose
Transgalactosylation
β-galactosidase
Process intensification

ABSTRACT

Lactulose synthesis was performed in a continuous stirred enzymatic membrane reactor. Each investigated operating condition (agitation, pH, feed molar ratio of lactose to fructose (m_L/m_F ratio), hydraulic residence time (HRT)) had an influence on reaction performances, in terms of lactulose concentration, productivity and selectivity. Lactulose concentration was maximum at an m_L/m_F ratio of 1/2. Higher than this ratio, synthesis of galactooligosaccharides was promoted rather than lactulose. At m_L/m_F ratios lower than 1/2, enzyme inhibition was pronounced to the detriment of lactulose production. At 7 or 9 h HRT, higher lactulose concentrations were obtained than at shorter HRTs. Applying an m_L/m_F ratio of 1/2 and an HRT of 9 h in a long-term operation, nearly constant lactulose concentration was reached after 23 h and lasted up to 32 h with a mean concentration of 14.51 ± 0.07 g/L and a reaction selectivity of 0.075–0.080 $mol_{lactulose}/mol_{cons.lactose}$. After 7 d, lactulose concentration reduced by 31%. A continuous synthesis of lactulose at lab-scale was shown to be amenable using a membrane reactor process. Moreover, for process evaluation, this study can bridge the gap between batch laboratory scale and continuous full-scale operation regarding lactulose synthesis.

1. Introduction

Lactulose (4-*O*-β-D-galactopyranosil-D-fructofuranose) that is built from one molecule of galactose and one molecule of fructose has received increasing attention due to its role in the dairy industry, acting as a prebiotic (Panesar and Kumari, 2011). Lactulose can be produced either by chemical isomerization (using acid or base) or enzymatic synthesis. The chemical isomerization generally possesses several drawbacks, such as colored by-products, waste management issues, and poorly specific reaction (Aider and Halleux, 2007; Guerrero et al., 2011; Schuster-Wolff-Bühring et al., 2010). Several time-consuming steps in the chemical isomerization-based lactulose synthesis are neutralization, catalyst separation and deionization (Schuster-Wolff-Bühring et al., 2010). In contrast, the enzymatic-based lactulose synthesis has been reported to be more environmentally friendly and to require less laborious steps in product purification (Panesar and Kumari, 2011). Considering these facts, enzymatic-based lactulose synthesis is more preferred.

There are two enzyme classes that can catalyze lactulose synthesis, such as glycosyltransferases and glycosidases (Mayer et al., 2004; Van Rantwijk et al., 1999). Glycosidases are more relevant for industrial applications (e.g., β-galactosidase for hydrolysis of lactose) as they are commercially available and relatively inexpensive (Perini et al., 2013). In addition to that, glycosyltransferases generally need cofactors to maintain the enzymes' catalytic activities.

Studies have been reported on enzyme-catalyzed lactulose synthesis using the bi-substrate lactose and fructose within the last decade (Schuster-Wolff-Bühring et al., 2010). However, most of the reported lactulose productions were run in batch procedures with typical working volumes of 1–500 mL (Hua et al., 2010; Kim et al., 2006; Mayer et al., 2004; Panesar and Kumari, 2011; Schumann, 2002; Seki and Saito, 2012). In batch operation, there is a problematic issue during the enzyme-catalyzed lactulose synthesis, namely secondary hydrolysis. As lactose is hydrolyzed, the concentration of the produced lactulose will peak when the probability of fructose as a galactosyl acceptor is higher than that of water (Fig. 1). Thus, the products of transgalactosylation will accumulate. As secondary

* Corresponding author. Chair of Chemical and Process Engineering, Technische Universität Berlin, Ackerstraße 76, 13355 Berlin, Germany. Tel.: +49 030 314 72693; fax: +49 030 314 72756.
E-mail addresses: azis.b.sitanggang@mailbox.tu-berlin.de, boing.lipan@ipb.ac.id (A.B. Sitanggang).

http://dx.doi.org/10.1016/j.jbiotec.2015.03.016
0168-1656/

hydrolysis is kinetically controlled (Mayer et al., 2004), lactulose at its highest concentration is prone to undergo an increased rate of secondary hydrolysis by the action of the pertinent enzyme (i.e., β-galactosidase) resulting again in galactose and fructose (Van Rantwijk et al., 1999, see Fig. 1). Hence, the yield of lactulose is determined by the availability of fructose and the possibility of continuous removal of lactulose during the reaction.

Continuous syntheses of lactulose using whey/lactose in the presence of fructose have been studied in a packed-bed reactor (PBR) using immobilized β-glycosidase or β-galactosidase (Mayer et al., 2010; Song et al., 2013). The stability of the immobilized β-glycosidase from *P. furiosus* was constant during14 d operation (Mayer et al., 2010). Up to now, it is the longest reported continuous synthesis of lactulose. Besides an extra cost for the immobilization, with a reported duration of 30 h, the enzyme preparation was found to be tedious. It is worth mentioning that the enzyme is not commercially available. For the enzyme production, the gene encoding β-glycosidase (CelB) from *P. furiosus* (DSM 3638) was over expressed in *E. coli* BL21 (DE3) by using the vector pLUW511 (Petzelbauer et al., 1999). Continuous synthesis of lactulose was also reported in a novel micro-channels system with activated walls where the β-galactosidase from *K. lactis* was immobilized (Song et al., 2012). However, the preparation of the activated walls of the micro-reactors was likely intricate and eventually led to a still time consuming process. The reported maximum concentration of lactulose was only 1.29 g/L.

A newly developed enzymatic membrane reactor (EMR) system (Lyagin et al., 2010; Sitanggang et al., 2014a) was used for lactulose synthesis in a continuous process. Supported with a PI/D controller, the EMR system could be operated at constant flux operation (thus constant HRT). In addition to that, two parallel reactors in the EMR system were shown to have a high level of similarity (about 95%) during continuous lactulose production (Sitanggang et al., 2014a).

The EMR system was chosen for continuous synthesis of lactulose to minimize the reduction of the enzyme's activity due to immobilization and the unproductive time during the enzyme preparation, as well as the extra cost for immobilization (Panesar and Kumari, 2011). Recovered activities of the immobilized enzymes can be as little as 10% (Kamrat and Nidetzky, 2007; Mateo et al., 2007). A freely-dissolved enzyme system generally negates the mass-transfer limitations of substrates to contact with the enzyme molecules which are normally encountered in immobilized enzyme systems. Through a combination of a continuous stirred tank reactor (CSTR) and an UF membrane in this EMR system, a continuous enzymatic process with a freely-dissolved enzyme system can be realized. When the molecular weight cut-off (MWCO) of the membrane is smaller than the molecular weight of the enzyme molecules, enzyme molecules can be retained inside the reactor during a continuous operation while the smaller products can be continuously withdrawn.

In a batch operation, Guerrero et al. (2011) reported an optimum m_L/m_F ratio of 1/8 for lactulose synthesis using *A. orizae* β-galactosidase. At an m_L/m_F ratio of 1/15, immobilized β-glycosidase from *P. furiosus* (CelB) produced the maximum concentration of lactulose, up to 16 g/L (Mayer et al., 2004). Using β-galactosidase from *S. solfataricus*, Kim et al. (2006) reported that high concentrations of lactulose were obtained at m_L/m_F ratios in a range of 2/1 to 1/2. At ratios over 4/1 and under 1/4, lactulose concentrations were

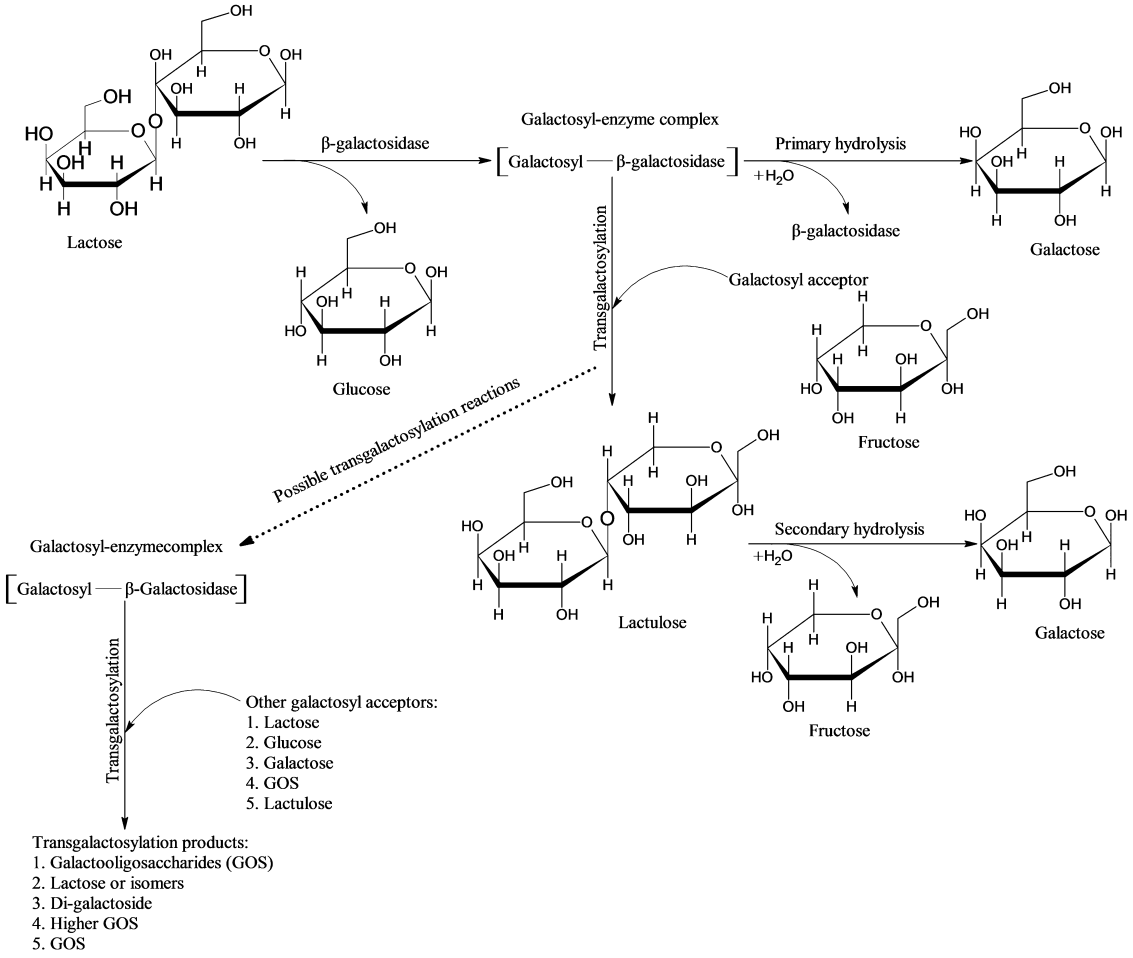

Fig. 1. Possible reactions during lactulose transgalactosylation in the presence of the bi-substrate (lactose and fructose) using β-galactosidase.