

AZIS BOING SITANGGANG*, ANJA DREWS**, MATTHIAS KRAUME***

AUTOMATED MEMBRANE REACTORS FOR THE EARLY PROCESS DEVELOPMENT OF ENZYME PROMOTED BIOCONVERSION

ZAUTOMATYZOWANE REAKTORY MEMBRANOWE DLA PROCESU WCZESNEGO ROZWOJU ENZYMU WSPOMAGANEGO BIOKONWERSJĄ

Abstract

In general, there is always a trade-off between the information output and the process throughput. The automation of micro- to small-scale bioreactors is considered necessary as this can facilitate a straightforward bioprocess development to reach its commercial success. A small-scale, dead-end enzymatic membrane reactor system has been realised. Reactor system was tested for the continuous transgalactosylation of lactulose using commercial β -galactosidase.

Keywords: enzymatic membrane reactor, bioprocess, transgalactosylation, enzyme

Streszczenie

Zawsze mamy do czynienia z kompromisem pomiędzy informacją wyjściową a przepustowością procesu. Jeżeli skala maleje, dostępne jest mniej informacji z powodu zmniejszonego monitorowania i kontroli. Automatyzacja mikro- do mini- bioreaktorów jest konieczna aby osiągnąć sukces komercyjny. Zbudowano reaktor membranowy w skali mini, w którym badano ciągłą transgalaktozylację laktulozy z użyciem komercyjnej beta-galaktozydazy.

Słowa kluczowe: enzymatyczny reaktor membranowy, proces biologiczny, transgalaktozylacja, enzym

DOI:

* MSc. Azis Boing Sitanggang, Chair of Chemical & Process Engineering, Faculty of Process Sciences, Technical University of Berlin and Bogor Agricultural University, Indonesia.

** Prof. PhD. DSc. Eng. Anja Drews, Hochschule für Technik und Wirtschaft Berlin.

*** Prof. PhD. DSc. Eng. Mathias Kraume, Chair of Chemical & Process Engineering, Faculty of Process Sciences, Technical University of Berlin.

1. Introduction

Small-scale reactors are generally used to reduce the amount of reactants, samples and biocatalysts. It is worth mentioning that small reactors possess a number of benefits in terms of reaction performances, as high surface area to volume ratios allow the chemical reactions to be more rapid [1]. However, according to Betts and Baganz [2], there is a trade-off between the information output and the experimental throughput at different reactor sizes during enzyme-promoted bioconversions. As the size of the reactor gets smaller, less information is available due to reduced monitoring and control. Therefore, automated micro- to small-scale reactor is of importance as this can facilitate a straightforward bioprocess development to reach its commercial success. This study aimed to establish a small-scale enzymatic membrane reactor (EMR) system which is also able to generate more information output. Several industrially relevant parameters, such as continuous substrate dosing with a constant flow rate (flux), online pH and temperature evaluation, and the control of enzyme activity could be performed. The reactor system was intended for bioprocess characterisations of enzyme-promoted bioconversions where continuous long-term operation (> 100 h) and product separations can be realized. To show the applicability of this developed reactor, a long-term continuous synthesis of lactulose using commercial *A. oryzae* β -galactosidase in the presence of lactose and fructose was performed at a maintained enzyme activity.

2. Materials and methods

2.1. Experimental rig

Two parallel reactors were built in an enzymatic membrane reactor (EMR) system [3, 4]. Each reactor consisted of a pressure-stable glass container and a body (holder) which was modified from the XFUF-047 dead-end stirred cell produced by Merck Millipore Darmstadt, Germany. The maximum working volume of the reactor was 90 mL. The reactor was equipped with a pressure-stable pH electrode produced by ProSense BV, The Netherlands. For the permeate measurement, a Kern EW 620-3NM precision balance was used (Kern & Sohn GmbH, Germany). In addition, a proportional pressure regulator (PPR) MPPE with a precision of ± 20 mbar from Festo AG & Co. KG, Germany was installed. For the realisation of the enzyme dosing, micro-solenoid valves from ASCO Numatics GmbH, Germany were used. A *normally closed* (2/2 NC) valve was used to control the flow of the enzyme into the reactor, whereas a *normally open* (2/2 NO) valve was installed for the substrate feed. The used membranes (diameter = 47 mm, surface area = $12.38 \cdot 10^{-4}$ m²) were polyethersulfone (PES) with molecular weight cut-off (MWCO) of 5, 10 and 20 kDa (Microdyn-Nadir GmbH, Germany), polysulphone (UFX10), cellulose acetate (RC70PP) with a MWCO of 10 kDa (Alfa Laval Mid Europe GmbH, Germany). A constant flux operation (thus providing a constant hydraulic residence time HRT) and the maintenance of enzyme activity (through the addition of “fresh” enzymes) were realised by a proportional-integral-derivative (PID) controller, developed by using Laboratory Virtual Instrument Engineering Workbench (LabVIEW) software [5].

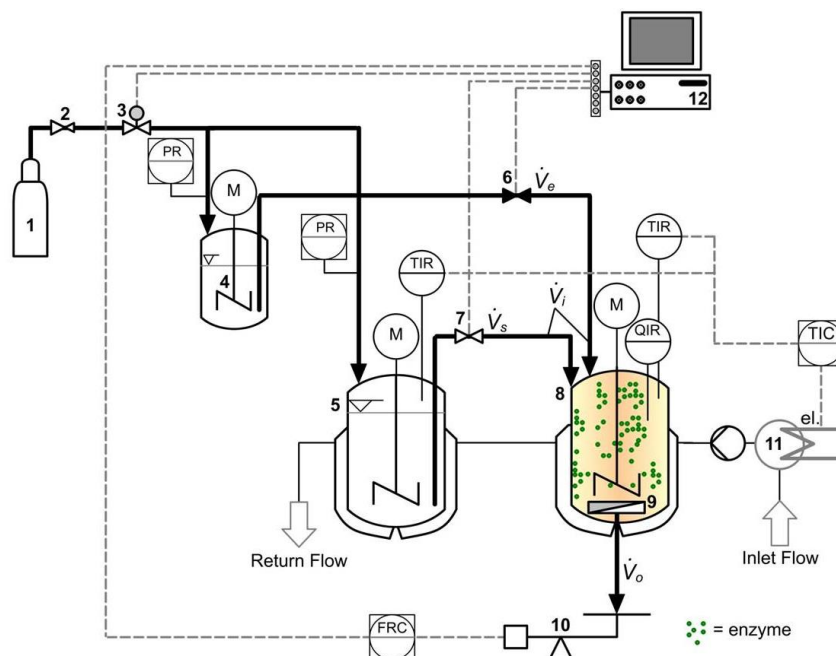


Fig. 1. Process flowsheet of a single reactor in the EMR system with an automatic procedure of enzyme dosing: (1) N₂ bottle, (2) pressure reducer, (3) PPR, (4) enzyme tank, (5) substrate tank, (6) 2/2 NC valve, (7) 2/2 NO valve, (8) reactor, (9) flat-sheet UF membrane, (10) precision balance, (11) heating system, (12) PC. Q = quality parameter, pH [5].

2.2. Chemicals

The enzyme β -galactosidase from *Aspergillus oryzae* (G5160), acetonitrile (271004), lactulose (61360), D-fructose (F0127), lactose (17814), 2-nitrophenyl β -D-galactopyranoside (ONPG, 73660) and 2-nitrophenol (ONP, 19702) were purchased from Sigma-Aldrich, Germany.

2.3. Continuous synthesis of lactulose

Lactose and fructose were used as substrates for lactulose synthesis using a commercial *A. oryzae* β -galactosidase. The total sugar concentration C_s was 500 g/L, with a molar ratio of lactose to fructose $m_L/m_F = 1/8$ or $1/4$, dissolved in 150 mM phosphate-citrate buffer pH 4.5 and with an enzyme concentration $[E] = 3$ or 10 U/mL. The continuous synthesis was performed at 40°C and agitated at 350 rpm. A long-term operation was carried out by dosing the “fresh” enzymes.

2.4. Enzyme activity determination and analytical method

A. oryzae β -galactosidase activity was determined according to Sigma–Aldrich enzymatic assay of β -galactosidase using ONPG as the substrate. One unit of enzyme activity is defined as the amount of enzyme required for hydrolysing 1 μ mol ONPG to ONP and D-galactose per minute at pH 4.5 and 30°C [5]. Lactose and lactulose were analysed by means of HPLC [6].

3. Results and discussion

3.1. Constant flux operation and enzyme dosing

As shown in Fig. 2a, two types of PID controller (with normal and fast setting according to Kuhn [7]) were used to control the flux (e.g., process variable of flux J_{PV}) during the filtration of ultrapure water at a set point of flux $J_{SP} = 25 \text{ L}/(\text{m}^2\text{h})$. The PID controller with a fast setting had a smaller over-shoot response (Fig. 2a). Therefore, the control error ($\text{error} = (|J_{SP} - J_{PV}|/J_{SP}) \cdot 100\%$) of fast setting PID controller was smaller than that of normal one. To have a more stable J_{PV} , the influence of the number of averaged points in the filter of moving average n was investigated for the fast setting PID controller. As can be seen in Fig. 2b, a higher value of n led to a poorly reactive response. Within this study, $n = 50$ points was selected to filter the flux data points.

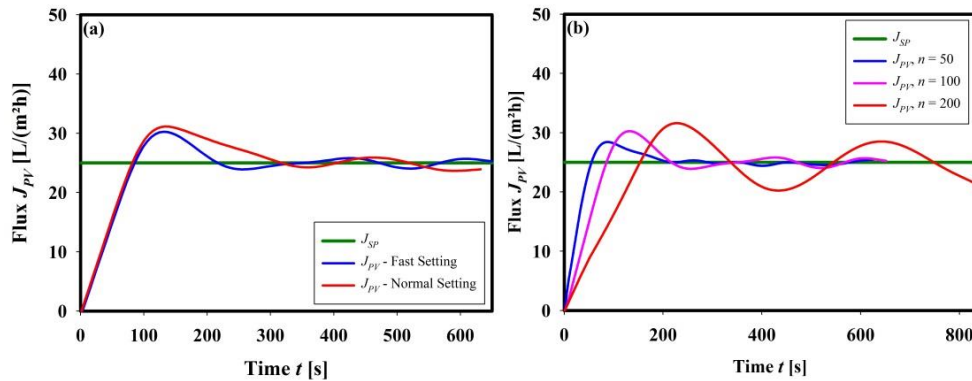


Fig. 2. (a) Testing PID controllers – normal setting vs. fast setting during ultrapure water filtration with $n = 100$ data points and (b) The influence of number of data points being averaged n on the stability of J_{PV} with the PID controller of fast setting: $J_{SP} = 25 \text{ L}/(\text{m}^2\text{h})$, PES membrane with MWCO 5 kDa, agitation = 350 rpm, $T = 40^\circ\text{C}$.

To maintain the enzyme activity during a long-term operation, an open-loop control (i.e., due to off-line product analysis) with automatic procedure of enzyme addition was also established. To have a constant flux operation during the enzyme dosing, the enzyme valve (2/2 NC) is opened whereas the substrate valve (2/2 NO) is closed [5]. Through the opening, the enzyme tank still experiences the same pressure as the substrate tank since the N_2 inlet gas for the substrate and enzyme tank are connected with a T-pipe joint (see

Fig. 1). The dosing accuracy was evaluated by using ultrapure water to replace both the enzyme and the substrate solution with the set dosing volume V_{sD} in the range of 0.5-10 mL. During the dosing, the enzyme tank was placed on the balance. The reduced weight of the enzyme tank should be due to some amount of ultrapure water pumped (or dosed) into the reactor (actual dosing volume V_{aD}). As shown in Fig. 3, the average inaccuracy of the developed dosing system was less than 2%.

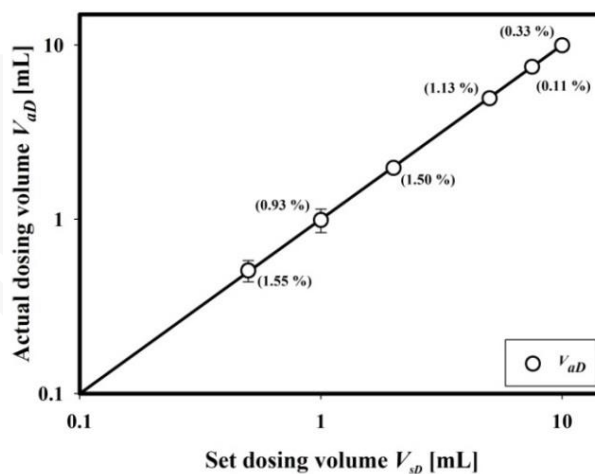


Fig. 3. Evaluation of dosing accuracy in the developed EMR with the averaged errors in parentheses

3.2. Continuous synthesis of lactulose

3.2.1. Membrane selection

The suited membrane was selected from three membrane types, PES (10, 20 kDa), UFX10 (10 kDa) and RC70PP (10 kDa). As can be seen in Fig. 4(a), lactulose concentrations between PES 10 and 20 kDa were almost identical. It indicates that by a higher membrane MWCO for PES membrane, the enzyme permeation did not occur. There was a slight difference between lactulose concentrations for those three membranes where the PES membrane clearly prevailed. However, the transmembrane pressure ΔP increased rapidly for both cut-offs of the PES membrane as reflected by their higher fouling rates. Although, the smallest fouling rate was found for the RC70PP membrane, its initial ΔP was the highest one compared to the other membranes (870 mbar). Hence, the UFX10 membrane was selected for further study as it had the smallest initial ΔP (257 mbar) with a compromised fouling rate.

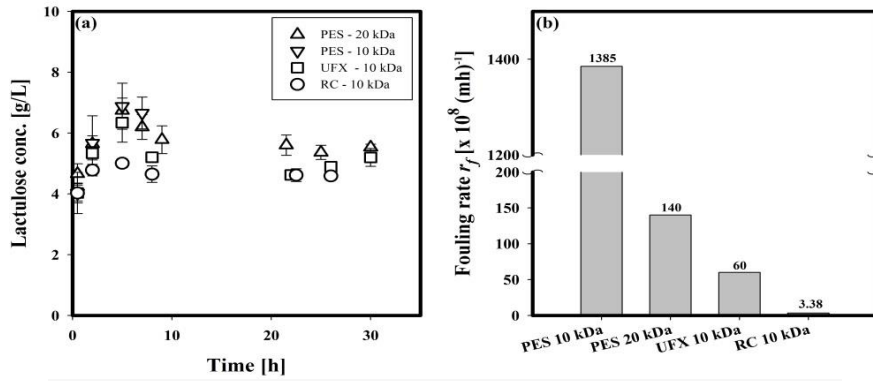


Fig. 4. (a) Lactulose concentrations of different membrane types and (b) fouling rates r_f of different membranes used for continuous lactulose synthesis using *A. oryzae* β -galactosidase in an EMR system with conditions: $C_S = 500$ g/L, $m_L/m_F = 1/8$, $[E] = 3$ U/mL, $HRT = 7$ hour, 150 mM citrate-phosphate buffer pH 4.6, agitation = 350 rpm, $T = 40^\circ\text{C}$. Fouling rate was calculated as

$$r_f = \frac{\Delta P}{\Delta t} \cdot \frac{1}{\eta_{(T)} \times J} \quad \text{with } \eta_{(T)} = 0.0393 \text{ Pa}\cdot\text{s}$$

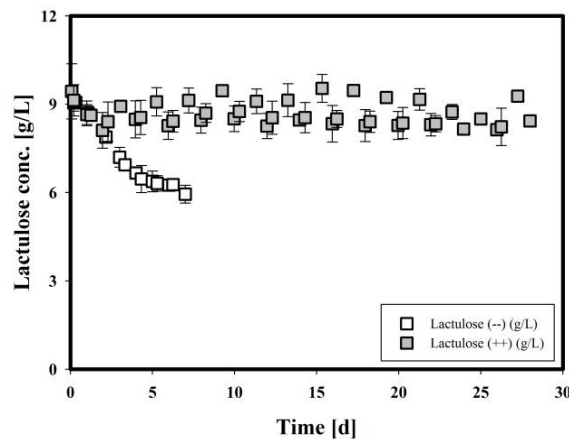


Fig. 5. Long-term continuous synthesis of lactulose by maintaining *A. oryzae* β -galactosidase activity in the EMR system (enzyme dosing: 10% of initial enzyme amount per 48 hours); (-) experiment without enzyme addition and (++) with enzyme addition. Both experiments were carried out with the same operating conditions: $C_S = 500$ g/L, $m_L/m_F = 1/4$, $[E] = 10$ U/mL, $HRT = 9$ hours, 150 mM citrate-phosphate buffer pH 4.6, agitation = 350 rpm, $T = 40^\circ\text{C}$, UFX membrane MWCO = 10 kDa.

3.2.2. Long-term operation of continuous lactulose synthesis

In Fig. 5, a prolonged reaction was carried out for one week without the addition of enzymes into the reactor. At 48 hours, 100 hours and 168 hours, the reductions of lactulose concentration were about 10%, 28.7% and 34.4%, respectively. In our previous study [6], using *K. lactis* β -galactosidase, after 168 hours of reaction, lactulose concentration was reduced by 31 %. Mayer et al. [8] also reported the production of lactulose in an EMR using free β -glycosidase from *P. furiosus* (CelB). The lactulose concentration decreased to almost zero after 96 hours. Another prolonged reaction was carried out with the addition of enzymes as much as 10% of the initial enzyme amount every 48 hours. Herein, a lactulose outlet concentration of 8.67 g/L could be kept constant for 28 days (with the number of enzyme additions was 13 times (see Fig. 3)).

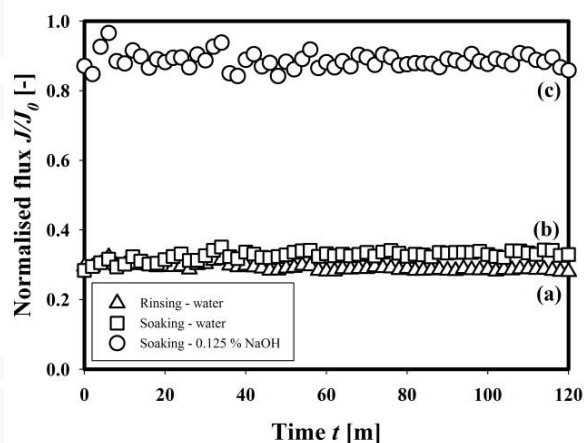


Fig. 6. The efficiencies of serial cleaning for the UFX membranes used in a long-term continuous synthesis of lactulose (see Fig. 5 for operating conditions).

The effectiveness of physical and chemical cleaning were also investigated for the used UFX membranes in the long-term operation of continuous lactulose synthesis (Fig. 5). The cleaning was done successively from rinsing \rightarrow soaking (with water) \rightarrow chemical soaking (0.125% NaOH). In Fig 6, through rinsing, the recovered flux was only 29.14%. Moreover, by soaking the membranes overnight in ultrapure water was not efficient as the recovered flux was only enhanced by 3% from the previous cleaning. Through overnight chemical soaking, the recovered flux was about 88.57%.

4. Conclusions

An automated small-scale EMR system was realised. Both constant flux operation (thus HRT) and maintenance of the enzyme activity could be performed (with a control error of less than 2%) besides online temperature and pH monitoring. Through the long-term

continuous synthesis of lactulose with a maintained enzyme activity, the applicability of the developed reactor was proven. Conclusively, the automated membrane reactor is claimed to be a useful bioreactor for studying the engineering aspects of enzyme-promoted bioconversions at lab-scale.

References

- [1] Jensen K.F., *Microreaction engineering – is small better?*, Chemical Engineering Science, vol. 56(2), 2001, 293-303.
- [2] Betts J., Baganz F., *Miniature bioreactors: Current practices and future opportunities*, Microbial Cell Factories, vol. 5(21), 2006, 1-14.
- [3] Sitanggang A.B., Drews A., Kraume M., *Rapid transgalactosylation towards lactulose synthesis in a small-scale enzymatic membrane reactor (EMR)*, Chemical Engineering Transactions, 38, 2014, 19-24
- [4] Sitanggang A.B., Drews A., Kraume M., *Continuous synthesis of lactulose in an enzymatic membrane reactor reduces lactulose secondary hydrolysis*, Bioresource Technology, vol. 167, 2014, 108-115.
- [5] Sitanggang A.B., Drews A., Kraume M., *Development of a continuous membrane reactor process for enzyme-catalyzed lactulose synthesis*, Biochemical Engineering Journal, vol. 109, 2016, 65-80.
- [6] Sitanggang A.B., Drews A., Kraume M., *Influences of operating conditions on continuous lactulose synthesis in an enzymatic membrane reactor system: A basis prior to long-term operation*, Journal of Biotechnology, vol. 203, 2015, 89-96.
- [7] Kuhn U., *Eine praxisnahe Einstellregel für PID-Regler: Die T-Summen-Regel*, Automatisierungstechnische Praxis, vol. 5, 1995, 10- 16.
- [8] Mayer J., Kranz B., Fischer L., *Continuous production of lactulose by immobilised thermostable β -glycosidase from *Pyrococcus furiosus**, Journal of Biotechnology, vol. 145, 2010, 387- 393.