Immobilization of cyclodextrin glucanotransferase (CGTase) on hollow fiber membrane: optimization of the immobilization parameters by response surface methodology

N Jamil*, R C Man*, S Suhaimi, S M Shaarani, Z I M. Arshad, S K A Mudalip and S Z Sulaiman

Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Kuantan, Pahang, Malaysia

*email: natasshajamil@gmail.com; rohaida@ump.edu.my

Abstract. Cyclodextrin glucanotransferase (CGTase) is a multifunctional industrial enzyme which undergoes cyclization reaction to converts starch into cyclodextrin (CD). Due to their potential properties, CD has been discovered to have numerous application in food industries, pharmaceuticals, agriculture and environmental engineering. However, the instability of the enzyme during the reaction process result in the low production of CD. Thus, enzyme immobilization process has been used to improve the enzyme stability in order to achieve high production of CD. In this study, CGTase from *Bacillus licheniformis* was immobilized on polyvinylidene difluoride (PVDF) hollow fiber membrane via physical adsorption. The optimization of the immobilization parameters and the performance of the immobilized CGTase were investigated. The adsorption of CGTase on hollow fiber membrane was evaluated by fourier transform infrared spectroscopy (FTIR). Response surface methodology (RSM) was employed to optimize enzyme immobilization by manipulating the immobilization parameters of contact time (15-33 h), immobilization pH (pH 6-8) and immobilization temperature (20-30 °C) on the immobilization yield. The optimized immobilization conditions were 24 °C of immobilization temperature, pH 6.7 and 24 h of contact time, with 88.25% of immobilization yield. Immobilization of CGTase on the hollow fiber membrane was successfully optimized and about 4.6-fold increment of immobilization yield was achieved after the optimization process. Reusability of the immobilized CGTase revealed that the immobilized enzyme could retain 37.7% of its initial activity after the 10th cycles. The cumulative production of CD by the immobilized CGTase after 10 cycles was 26.43 mg/ml. The kinetic parameters of the immobilized CGTase were 9.42 mgml^{-1} h^{-1} and 9.99 mg ml^{-1} for V_{max} and K_m value, respectively. The kinetic studies revealed that the catalytic efficiency of the immobilized CGTase was similar to the free CGTase, demonstrated that upon the immobilization process, adsorption of CGTase on hollow fiber membrane does not cause structural changes to the enzyme. Hence, immobilization of CGTase on the hollow fiber membrane substantially improved the production of CD and suggesting that the hollow fiber membrane appeared as a suitable support for the enzyme immobilization system.

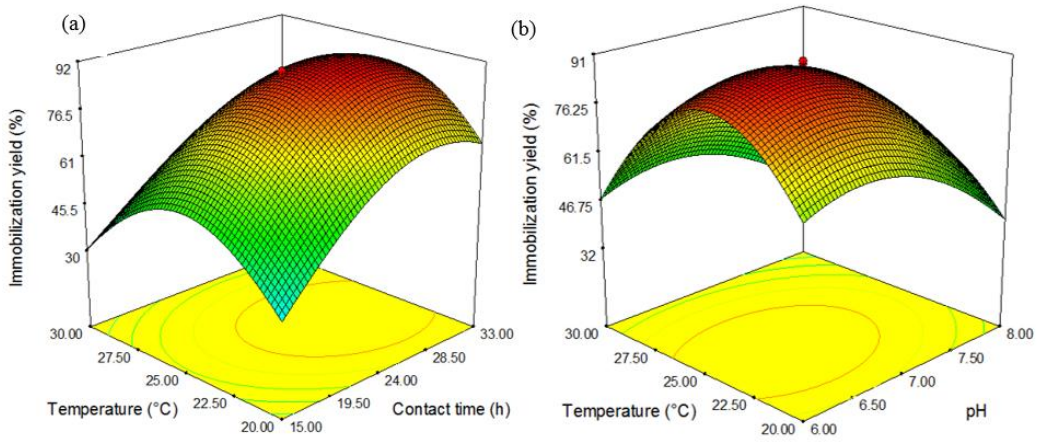

Figure 1. Response surface of plot of immobilization yield as a function of: (a) contact time and temperature at fixed level of pH (pH 7); (b) pH and temperature at fixed level of contact time (24 h).

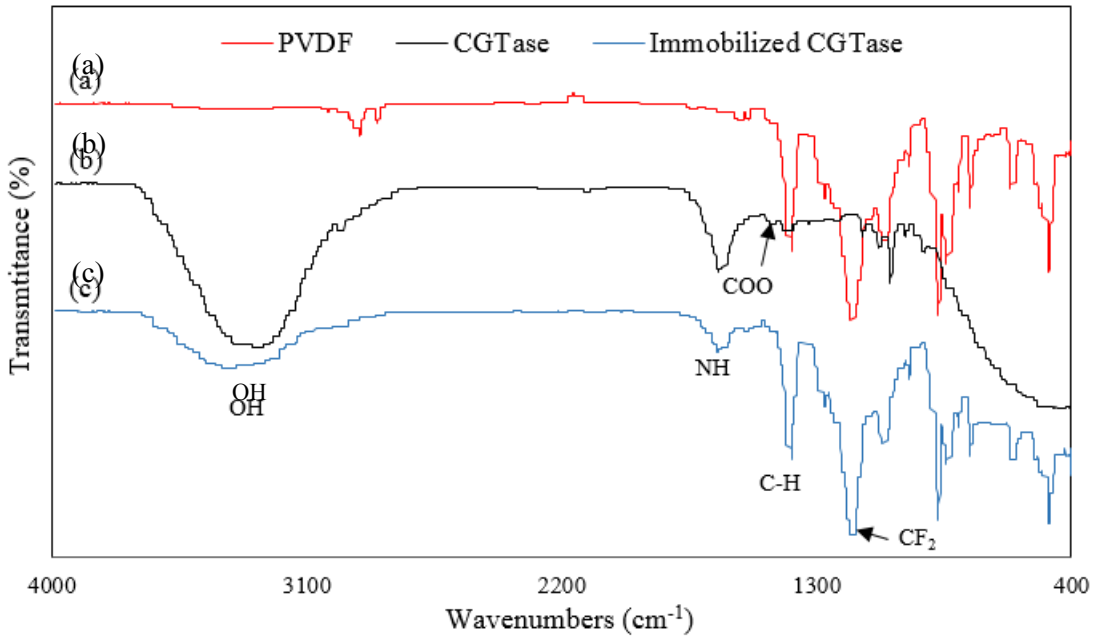

Figure 2. Comparison of FTIR spectra of (a) PVDF hollow fiber membrane; (b) Free CGTase and (c) Immobilized CGTase on PVDF hollow fiber membrane.

Acknowledgments

This study was financially supported by Universiti Malaysia Pahang under Research Grant Scheme (Grant No. RDU160322) and Universiti Malaysia Pahang Postgraduate Research Grant Scheme (Grant No. PGRS170321). The authors are grateful to Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang for the research facilities.

References

[1] Jamil N, Man RC, Shaarani S M, Sulaiman S Z, Mudalip S K A and Arshad Z I M, 2017 *Indian J. Sci. Technol.*, **10**, 1-7.
[2] Kriaa M, Ayadi D Z, Jemli S, Sahnoun M, Bejar S, and Kammoun R, 2012 *Biologia (Bratisl.)*, **67**, 1049–1055.
[3] Biwer A and Heinzle E, 2004 *Enzyme Microb. Technol.*, **34**, 7, 642–650.
[4] Guzik U, Hupert-Kocurek K, and Wojcieszyńska D, 2014 *Mol. Basel Switz.*, **19**, 8995–9018.
[5] Mohamad N R, Marzuki N H C, Buang N A, Huyop F, and Wahab R A, 2015 *Biotechnol. Biotechnol. Equip.*, **29**, 205–220.
[6] Schöffer J N, Klein M P, Rodrigues R C, and Hertz P F, 2013 *Carbohydr. Polym.*, **98**, 1311–1316.
[7] Rehm F B, Chen S, and Rehm B H, 2016 *Molecules*, **21**, 1370.
[8] Algieri C, Donato L, and Giorno L, 2016 *Biotechnol. Appl. Biochem.*
[9] Dror Y, Kuhn J, Avrahami R, and Zussman E, 2008 *Macromolecules*, **41**, 4187–4192.
[10] Hsu C H, Chu Y F, Argin-Soysal S, Hahm T S, and Lo Y M, 2004 *J. Food Sci.*, **69**, 441–448.
[11] Krajewska B, 2004 *Enzyme Microb. Technol.*, **35**, 126–139.
[12] Dwevedi A and Kayastha A M, 2009 *Bioresour. Technol.*, **100**, 2667–2675.
[13] Deng Y, Xue C, and Xu Y, 2010 *Third International Joint Conference on Computational Science and Optimization*, **2**, 233–237.
[14] Li Z, Li B, Gu Z, Du G, Wu J, Chen J, 2010 *Carbohydr. Res.*, **345**, 886–892.
[15] Toropainen T, Jarho P, Lehtonen M, Keski-Rahkonen P, Raatikainen H, and Järvinen T, 2008 *J. Chromatogr. B*, **867**, 90–98.
[16] Ibrahim A S S, Al-Salamah A A, El-Toni A M, El-Tayeb M A and Elbadawi Y B, 2014 *Electron. J. Biotechnol.*, **17**, 55–64.
[17] Chen G J, Kuo C H, Chen C I, Yu C C, Shieh C J and Liu Y C, 2012 *J. Biosci. Bioeng.*, **113**, 166–172.
[18] Ying L, Kang E T, and Neoh K G, 2002 *J. Membr. Sci.*, **208**, 361–374.
[19] Wang C *et al.*, 2016 *Enzyme Microb. Technol.*, **93**, 59–69.

[20] Kim D W, Jang Y H, Kim C S, and Lee N S, 2001 *Bull. Korean Chem. Soc.*, **22**, 716–720.
[21] Liu Y, Jin Z, Meng H, and Zhang X, 2018 *Mater. Res. Express*, **5**, 015402.
[22] Zeng L, Luo K, and Gong Y, 2006 *J. Mol. Catal. B Enzym.*, **38**, 24–30.
[23] Fang S, Chang J, Lee Y S, Hwang E J, Heo J B, and Choi Y L, 2016 *J. Appl. Biol. Chem.*, **59**, 75–81.
[24] Fortes C C S, Daniel-da-Silva A L, Xavier A M R B, and Tavares A P M, 2017 *Chem. Eng. Process. Process Intensif.*, **117**, 1–8.
[25] Sun J, Jiang Y, Zhou L, and Gao J, 2010 *New Biotechnol.*, **27**, 53–58.
[26] Blanco C, Santos F J, Bernardi N S, Jafelicci Júnior M, Monti R, and Contiero J, 2013 *Enzyme Eng.*, **2**, 1–5.
[27] Xie W. and Ma N, 2010 *Biomass Bioenergy*, **34**, 890–896.
[28] Martín M T, Plou F J, Alcalde M, and Ballesteros A, 2003 *J. Mol. Catal. B Enzym.*, **21**, 299–308.
[29] Lei Z and Bi S, 2007 *J. Biotechnol.*, **128**, 112–119.
[30] Seenuvasan M, Kumar K S, Malar C G, Preethi S, Kumar M A, and Balaji N, 2014 *Appl. Biochem. Biotechnol.*, **172**, 2706–2719.
[31] Ibrahim A S S, Al-Salamah A A, El-Toni A M, El-Tayeb M A and Elbadawi Y B, 2013 *Electron. J. Biotechnol.*, **16**, 10–10.
[32] Ibrahim A S S, El-Tayeb M A and Al-Salamah A A, 2010 *Afr. J. Biotechnol.*, **9**, 7550–59.
[33] Tripathi P, Kumari A, Rath P and Kayastha A M, 2007 *J. Mol. Catal. B Enzym.*, **49,** 69–74.
[34] Arya S K and Srivastava S K, 2006 *Enzyme Microb. Technol.*, **39**, 507–510.