THE UNIVERSITY of ADELAIDE

Preparation and Characterization of β-Galactosidase Nanobiocatalysts and Its Application for Galacto-Oligosaccharides Production

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For my husband,

Johnes Julait

And precious daughter,

Michelle Andrea Joanne Johnes

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Preface

The doctoral thesis is prepared in "Publication" style according to the "specifications for Thesis (2015)" of the University of Adelaide. It includes publications that have been published or readyto be submitted for publication:

1. Misson, M., Zhang, H., Jin, B. (2015). Nanobiocatalyst Advancements and Bioprocessing Applications. Journal of the Royal Society Interface. 12 (102): 1-20.

2. Misson, M., Du, X., Jin, B., Zhang, H. (2016). Dendrimer-Like Nanoparticles Immobilized β -Galactosidase For Enhancing Galacto-Oligosaccharide Production. Enzyme and Microbial Technology. 84: 68-77.

3. Misson, M., Dai, S., Jin, B., Chen, B., Zhang, H. (2016). Manipulation of Nanofiber-Based β -Galactosidase Nanoenvironment for Enhancement of Galacto-Oligosaccharide Production. Journal of Biotechnology. 222: 56-64.

4. Misson, M., Jin, B., Chen, B., Zhang, H. (2015). Enhancing Enzyme Stability and Metabolic Functional Ability of β-Galactosidase through Functionalized Polymer Nanofiber Immobilization. Bioprocess and Biosystems Engineering. 38(10):1915-23.

5. Misson, M., Jin, B., Zhang, H. (2016). Characterization of β-Galactosidase/Polymer-Nanofibers Nanobiocatalysts and Application for Galactooligosaccharides Production. In preparation for submission.

6. Misson, M., Jin, B., Zhang, H. (2016). Recirculating Spiral Reactor for Galactooligosaccharide Production from Nanofiber-supported Nanobiocatalysts. In preparation for submission.

Some relevant components of the work that have been presented in conferences and symposiums:

1. Misson, M., Jin, B., Zhang, H. (2015). Immobilized β-Galactosidase on Functionalized Nanoparticles and Nanofibers: A Comparative Study. International Proceedings of Chemical, Biological and Environmental Engineering. DOI 10.7763/IPCBEE. 90: 1-7. (In 4th International Conference on Environment, Chemistry and Biology 2015, Auckland, New Zealand, 19th-21st November 2015) – Oral Presentation.

2. Misson, M., Jin, B., Dai, S., Zhang, H. (2014). Enzyme Immobilization of Functionalized Polystyrene Nanofibers for Bioprocessing Applications. International Journal of Bioengineering and Life Sciences. 1(12): 680. (In 16th International Conference on Biological and Bioprocess Engineering 2014, Sydney, Australia, 15th-16th December 2014) – Oral Presentation.

3. Misson, M., Jin, B., Zhang, H. (2015). Nano-Biocatalyst for Conversion of Dairy Industry Waste into Valuable Product. Spring Symposium MyPSA. Pp 11. Adelaide, Australia. (17th October 2015) – Oral Presentation.

4. Misson, M., Jin, B., Dai, S., Zhang, H. (2014). Enzyme Immobilization onto Acid-treated Electrospun Polystyrene Nanofibers. RACI SA Student Polymer & Bionanotechnology Symposium 2014. Pp 21. Adelaide, Australia. (28th July 2014) – Oral Presentation.

5. Misson, M., Jin, B., Zhang, H. (2014). Nanobiocatalysts for Bioconversion of Dairy Industry Wastes into Functional Products. Bioprocess Network Conference 2014. Melbourne, Australia (21st-23rd October 2014) – Oral Presentation.

6. Misson, M., Jin, B., Dai, S., Zhang, H. (2013). Optimization of Electrospun Polystyrene Nanofibers Synthesis for Enzyme Immobilization. RACI SA Student Polymer & Bionanotechnology Symposium 2013. Pp 11. Adelaide, Australia. (4th October 2013) – Poster Presentation.

In addition, some awards were achieved during PhD candidature:

1. Best Presentation in 4th International Conference on Environment, Chemistry and Biology (ICECB 2015), Auckland, New Zealand.

2. Best Presentation in Postgraduate Research Seminar in 2014, School of Chemical Engineering, The University of Adelaide, Adelaide, Australia.

3. Malaysian Government Scholarship (*Hadiah Latihan Di Bawah Skim Latihan Akademik IPTA* (SLAI) 2012-2016.

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"The works of the Lord are great, studied by all who have pleasure in them"

(*Psalm 111:2*)

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Abstract

Enzyme immobilization has been recognized as a promising technique to enhance enzyme stability, activity and reusability for the development of cost-effective, green and sustainable biotechnological processes. Recent development in nanotechnology has opened a new frontier for diverse nano-scale enzyme carriers. The immobilization of enzyme onto nanomaterials produces a nanobiocatalyst assembly, which maximizes reaction efficiencies by favoring desirable chemical reaction kinetics and selectivity for substrates, while the unique properties of nanocarriers offer a revolution of biocatalyst applications in the bioprocessing field. Nevertheless, the issues of enzyme leakage and conformational changes make the translation of the biocatalyst technology into commercial practices technically challenging and economically infeasible. Hence, investigating new technologies for fabricating the nanobiocatalyst with promising biocatalytic activities and functionalities is of great importance.

In this PhD research, nanoparticle- and nanofiber-based enzyme carriers were developed and explored to immobilize β-galactosidase for conversion of lactose from dairy industry wastes into galacto-oligosaccharide (GOS) as a high value product. The structure-function relationship for the nanocarrier, the enzyme-nanocarrier microenvironment and the enzyme-nanocarrier nanobiocatalyst structure were extensively evaluated, aiming to enhance the bioengineering performance of the nanobiocatalysts.

Dendrimer-like silica nanoparticles (HPSNs) with hierarchical pores were synthesized, characterized and functionalized with amino (NH_2) and carboxyl group (COOH) to facilitate enzyme binding. Our findings revealed that surface functionalization can promote enzyme affinity towards the nanomaterial interface and selectively enhance enzyme reusability and its catalytic activity for improving the GOS production yield.

A systematic synthesis of polystyrene nanofibers (PSNFs) was executed by optimizing key fabrication parameters using the electrospinning technique, including polymer concentration, electric voltage and distance between discharge needle tips and the collector. Surface modification of the PSNF was found to improve enzyme loading and activity. In addition, the local microenvironment of the nanobiocatalysts was able to

optimize the enzyme selectivity and specificity, resulting in favouring transgalactosylation over hydrolysis for the lactose bioconversion.

Further investigation to enhance the enzyme stability and catalytic activity at various operating conditions was conducted. PSNFs were chosen as the enzyme carrier owing to their scaling up potential in a manufacturing reactor system with their excellent mechanical and structural properties. Immobilizing β-galactosidase on the modified PSNF surface facilitated formation of stable enzyme binding and exhibited distinguished catalytic performance. Thermal and pH stability were improved significantly while the recyclability was enhanced from four to nine cycles. The evaluation of lactose conversion performance showed an improved GOS yield from 14 to 28% in comparison to free β-galactosidase.

To advance the knowledge of understanding β-galactosidase binding on the PSNF surface, the β-galactosidase/nanofiber nanobiocatalyst structure were comprehensively analyzed. Characterizations on the nanobiocatalyst properties were performed before and after biocatalyst immobilization. The analysis using scanning electron microscope (SEM), fluorescence microscope, Fourier transform infrared spectroscopy (FTIR), and Raman spectroscopy demonstrated successful biocatalyst attachment, homogenous distributions and no conformation changes. The effectiveness factor for lactose conversion into galacto-oligosaccharides (GOS) in a disc-stacked column reactor indicated distinguished biocatalyst performance in comparison to the free counterpart.

Finally, a scalable recirculating spiral reactor was designed in-house and operated for a continuous GOS production using the nanofibers-β-galactosidase nanobiocatalyst. The PSNF-β-galactosidase performed better in GOS production yield by exceeding the free counterpart about 1.5 to 3.7-fold. The variable parameters of the bioreactor system such as reaction time, feed flow rate and initial substrate concentration were found to have a profound effect in optimizing GOS synthesis. The best GOS production yield was determined at 159 g/l with 86% lactose conversion under the optimal operating conditions of 24 h reaction time, 15 ml/min flow rate and 400 g/l initial lactose concentration.

Overall, nanoparticles- and nanofibers-immobilized β-galactosidase nanobiocatalysts were successfully developed and assessed for conversion of lactose into GOS in this study. The nanobiocatalyst assembly demonstrated remarkable selectivity towards transgalactosylation to produce GOS from lactose.Comparing with free enzyme, the immobilized β-galactosidase significantly enhanced enzymatic activities, leading to

excellent bioconversion performance. The distinguished bioengineering perfomance of nanofiber-immobilized β-galactosidase in a scalable recirculating spiral bioreactor indicates their great potential for a large scale and continuous process application. Furthermore, the understanding of the binding mechanism for the enzyme and its nanoscale support surface and the nanobiocatalyst structure can be a key driver for fabricating biocatalyst-nanomaterial hybrids and improving biocatalyst efficiency. In summary, the findings of this study provide new insights into the development of economically and industrially viable nanobiocatalysts for industry-scale bioprocesses.

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