IMMOBILIZATION OF *Escherichia coli* PRODUCING β-CYCLODEXTRIN GLUCANOTRANSFERASE ON HOLLOW FIBER MEMBRANE FOR β-CYCLODEXTRIN PRODUCTION

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We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Master of Science.

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I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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Thesis submitted in fulfillment of the requirements
for the award of the degree of
Master of Science

Faculty of Chemical and Process Engineering Technology

UNIVERSITI MALAYSIA PAHANG

FEBRUARY 2023

ACKNOWLEDGEMENTS

First and foremost, all praise to Almighty Allah for His blessing that I received throughout my study. Upon completion of this study, I felt lucky and honoured to have so many people who guided and supported me to finish my research.

First of all, I would like to express my deepest gratitude to my supervisor, Dr. Rohaida Che Man, for your patience, encouragement, guidance and endless support in helping me to complete my study. I have greatly benefited from your wealth of knowledge and meticulous editing. I am extremely grateful that you took me on as your student and continued to have faith in me over these years. Also, many thanks to Dr. Shariza Jamek, my co-supervisor who always been there when I needed a second opinion.

Besides, my sincere thankfulness goes to my research mate, Nur Nadia Mohd Zakaria for the knowledge and discussion about this research. Your encouraging words and thoughtful have been very important to me. I would like to thanks to all my fellow friends (Mey, Farah, Chang, Fathin, Suhaily, Fier) for their sharing during this study.

Thank you to my husband, parents and family members for their encouragement, support and love. All of you have always stood behind me, and this was no exception.

Lastly, this research would not have been possible without the support from the administrative (Encik Hilmy) and lab assistants (Encik Zainal and Encik Zulhabri) for their guidance in the lab. My appreciation also dedicated to those who have directly or indirectly lent their hands in this research.

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ABSTRAK

β-siklodekstrin adalah kitaran oligosakaride yang mendapat permintaan tinggi dalam pelbagai industri kerana mempunyai banyak aplikasi. Ia dihasilkan daripada kanji melalui penukaran enzim β-siklodekstrin glukanotransferase (β-CGTase). Walaupun penyelidik telah menemui kaedah untuk menghasilkan kepekatan CGTase yang tinggi dalam medium melalui perembesan oleh rekombinan *Escherichia coli* (*E. coli*), lisis sel kekal sebagai masalah kerana tekanan hidrostatik membina dalam ruang sitoplasma dan periplasmik *E. coli*. Imobilisasi sel adalah penyelesaian yang menjanjikan untuk mengurangkan lisis sel dan meningkatkan perembesan enzim. Dalam kajian ini, rekombinan *E. coli* diimobilisasikan menggunakan membran gentian berongga. Pengeluaran β-CD dioptimumkan dan penggunaan semula sel-sel imobilisasi telah dinilai. Kesan kepekatan substrat, suhu, kadar pergolakan, pH, dan masa pada hasil β-CD, perembesan CGTase, dan lisis sel oleh sel-sel imobilisasi ditentukan dengan menggunakan kaedah satu faktor pada satu masa (OFAT). Hasilnya menunjukkan bahawa sel-sel imobilisasi dapat menghasilkan 11-14 kali lebih banyak β-CD berbanding dengan sel bebas. Sel-sel imobilisasi juga menunjukkan peningkatan 17-19 kali ganda dalam perembesan β-CGTase dengan pengurangan 64-92% lisis sel berbanding sel bebas. Selepas itu, parameter operasi telah disaring untuk mengenalpasti parameter-parameter yang penting menggunakan reka bentuk faktorial penuh (FFD). Keputusan menunjukkan kepekatan kanji, suhu dan kadar pergolakan adalah parameter operasi terpenting dan digunakan untuk proses pengoptimuman menggunakan Reka Bentuk Komposit Pusat (CCD) di bawah Metodologi Gerak Balas Permukaan (RSM). Di bawah keadaan optimum (3.9% kepekatan kanji, 44 ° C suhu tindak balas, dan kadar pergolakan 170 rpm), pembentukan β-CD dan perembesan β-CGTase adalah masing-masing 8-kali ganda dan 7-kali ganda lebih tinggi berbanding sebelum proses pengoptimuman. Selain itu, sel imobilisasi menunjukkan pembentukan β-CD dan perembesan β-CGTase adalah masing-masing 3-kali ganda dan 2.5-kali ganda lebih tinggi dengan penurunan sel lisis sebanyak 54% berbanding dengan sel bebas. Sel imobilisasi berjaya mengekalkan sehingga 62% daripada aktiviti awal dan boleh digunakan semula untuk 5 kitaran untuk pengeluaran β-CD. Oleh itu, imobilisasi *E. coli* pada membran gentian berongga sesuai untuk meningkatkan pengeluaran β-CD dengan perembesan β-CGTase dan kestabilan sel yang tinggi.

ABSTRACT

β-cyclodextrin is a cyclic oligosaccharide that has been in high demand in various industries due to its numerous applications. It is produced from starch via the enzymatic conversion of β-cyclodextrin glucanotransferase (β-CGTase). While researchers have discovered a method to produce a high concentration of β-CGTase in the medium through excretion by recombinant *Escherichia coli* (*E. coli*), cell lysis remains an open problem as hydrostatic pressure builds up in the cytoplasm and periplasmic space of the *E. coli*. Cell immobilization is a promising solution to reduce cell lysis and enhance enzyme excretion. In the present study, the recombinant *E. coli* was immobilized using hollow fiber membrane. The production of β-CD was optimized and the reusability of the immobilized cells were evaluated. The effects of substrate concentration, temperature, agitation rate, pH, and time on β-CD production, β-CGTase excretion, and cell lysis by the immobilized cells were determined by using the one factor at a time (OFAT) method. The results revealed that the immobilized cells could produce 11-14-fold more β-CD compared to the free cells. The immobilized cells also exhibited a 17-19-fold increase in β-CGTase excretion with a 64-92% reduction of cell lysis compared to free cells. Then, the operating parameters was screened to identify the significant parameters using Full Factorial Design (FFD). It showed that the substrate concentration, temperature and agitation rate were the significant operating parameters and were used for optimization process using Central Composite Design (CCD) under Response Surface Methodology (RSM). Under the optimized conditions (3.9% of starch concentration, 44°C of reaction temperature, and 170 rpm agitation rate), the β-CD production and β-CGTase excretion was 8-fold and 7-fold, respectively higher than before the optimization process. Moreover, the immobilized cells showed 3-fold and 2.5-fold of β-CD production and β-CGTase excretion, respectively with 54% reduction of cell lysis in comparison with the free cells. The immobilized cells successfully retained up to 62% of the initial activity and can be reused for 5 cycles for the production of β-CD. Therefore, the immobilization of *E. coli* on hollow fiber membrane was suitable for enhancing β-CD production with high excretion of β-CGTase and high cell stability.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

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