"We hereby declare that we have read this thesis and in our opinion this thesis is sufficient in terms of scope and quality for the award of the degree of Doctor of Philosophy (Bioprocess Engineering).

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BAHAGIAN A – Pengesahan Kerjasama*

Adalah disahkan bahawa projek penyelidikan tesis ini telah dilaksanakan melalui kerjasama antara _____ dengan _____

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* *Jika penyediaan tesis/projek melibatkan kerjasama.*

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IMPROVEMENT OF CYCLODEXTRIN GLUCANOTRANFERASE EXCRETION AND CELL VIABILITY OF RECOMBINANT *Escherichia coli* IMMOBILIZED ON HOLLOW FIBER MEMBRANE

ROHAIDA BINTI CHE MAN

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Doctor of Philosophy (Bioprocess Engineering)

Faculty of Chemical and Energy Engineering
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MARCH 2016

I declare that this thesis entitled "*Improvement of Cyclodextrin Glucanotranferase Excretion and Cell Viability of Recombinant Escherichia coli Immobilized on Hollow Fiber Membrane*" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : .....

Name : Rohaida binti Che Man

Date : 2/3/2016

To my beloved parents (Hj. Che Man bin Mamat and Hjh. Hasmah binti Mohd Noh), my husband (Saiful Aflah bin Abdol Karim), daughter (Nur Alya Safiah), son (Muhammad Aiman Rafiqin), sisters (Fauziah, Rohaniah, Rohaniza and Rohaina) and brothers (Mohd Hatta, Mohd Lutffi, Mohd Asri, Mohd Nizam, Muhammad and Mohd Hafizullah). I dedicated this work in sincere gratitude for their patience, love and support.

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ABSTRACT

The excretion of a recombinant enzyme into culture medium presents significant advantages over cytoplasmic expression. However, cell lysis is one of the major drawbacks during the excretion of recombinant enzyme when using *Escherichia coli* (*E. coli*) as a host. Cell immobilization is a promising solution for the enhancement of enzyme excretion with reduction of cell lysis. In the present study, a recombinant *E. coli* was immobilized using hollow fiber membrane to improve the enzyme excretion, cell viability and plasmid stability. The effects of different polymers of hollow fiber membrane and culture conditions on the cyclodextrin glucanotransferase (CGTase) excretion, cell lysis and plasmid stability of immobilized *E. coli* were investigated. The immobilized cells on a polyvinylidene fluoride polymer exhibited a 2.0–4.5-fold increase in the CGTase excretion with 18-95% reduction of cell lysis and over 100% increment of plasmid stability compared to the free cells. The CGTase excretion was successfully optimized by response surface methodology. Under the optimized conditions [25 °C of post- induction temperature, 0.011 mM isopropyl β-D-1-thiogalactopyronoside and pH 8.8], the CGTase excretion was 3.8-fold higher with 80% reduction of cell lysis compared to the value before optimization process. The use of low tryptone concentration (5 g/l) reduced the occurrence of cell lysis (90% reduction) and increased the plasmid stability (86% increment) without significant change in CGTase excretion in comparison with initial tryptone concentration (20 g/l). This approach (5 g/l) produced an approximately two times higher CGTase excretion (compared with 20 g/l during) recycle process. The membrane bioreactor also showed 2.5-fold increase in the CGTase excretion (473 x 10^3 U/ml) with 75% reduction of cell lysis compared to shake flask culture (190 x 10^3 U/ml of CGTase activity). Hence, the immobilization of *E. coli* on hollow fiber membrane proved to be valuable for the excretion of recombinant proteins in *E. coli* with high cell stability.

ABSTRAK

Perembesan enzim rekombinan ke dalam media kultur adalah pendekatan yang lebih baik berbanding pengungkapan sitoplasmik. Walau bagaimanapun, lisis sel adalah salah satu masalah utama dalam perembesan enzim rekombinan apabila menggunakan *Escherichia coli* (*E. coli*) sebagai perumah. Imobilisasi sel adalah penyelesaian yang baik untuk peningkatan perembesan enzim dengan pengurangan kadar lisis sel. Dalam kajian ini, *E. coli* rekombinan telah diimobilisasikan menggunakan polimer membran gentian berongga bertujuan untuk meningkatkan perembesan enzim, bilangan sel hidup dan kestabilan plasmid. Kesan polimer membran gentian berongga yang berbeza dan keadaan pertumbuhan untuk perembesan siklodekstrin glukanotransferase (CGTase), kadar lisis sel dan kestabilan plasmid bagi sel imobilisasi telah dikaji. Sel imobilisasi pada poliviniliden fluorida polimer mempamerkan 2.0-4.5 kali ganda peningkatan dalam perembesan CGTase dengan 18-95% pengurangan kadar lisis sel dan peningkatan kestabilan plasmid melebihi 100% berbanding dengan sel bebas. Perembesan CGTase berjaya dioptimakan dengan menggunakan kaedah gerak balas permukaan. Dengan menggunakan keadaan optimum [25 °C suhu induksi, 0.011 mM isopropil β-D-1-thiogalaktopiranosida dan pH 8.8], perembesan CGTase adalah 3.8 kali ganda tinggi dengan pengurangan kadar lisis sel sebanyak 80% berbanding dengan nilai sebelum proses pengoptimuman. Penggunaan kepekatan tripton yang rendah (5 g/l) mengurangkan kadar lisis sel dengan 90% pengurangan dan meningkatkan kestabilan plasmid (86% peningkatan) tanpa perubahan perembesan CGTase yang ketara berbanding dengan kepekatan tripton yang asal (20 g/l). Pendekatan ini (5 g/l) membuktikan penghasilan perembesan CGTase dengan kira-kira dua kali ganda berbanding 20 g/l sepanjang proses berulang. Bioreaktor membran juga menunjukkan peningkatan perembesan CGTase (473 x 10^3 U/ml) sebanyak 2.5 kali ganda dengan pengurangan lisis sel sebanyak 75% berbanding kelalang kon (190 x 10^3 U/ml aktiviti CGTase). Oleh itu, sel *E. coli* imobilisasi pada membran gentian berongga berguna untuk tujuan perembesan protein rekombinan dengan kadar sel hidup yang tinggi.

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LIST OF SYMBOLS

g	-	gram
h	-	hour
l	-	liter
min	-	minute
ml	-	milliliter
sec	-	second
v/v	-	volume solute per volume solution
w/v	-	weight solute per volume solution

LIST OF ABBREVIATIONS

A_{600}	-	absorbance at wavelength 600 nm
ANOVA	-	analysis of variance
BRP	-	bacteriocin release protein
CCD	-	central composite design
CD		cyclodextrin
CGTase	-	cyclodextrin glucanotranferase
Da, kDa	-	dalton, kilodalton
DNA	-	deoxyribonucleic acid
E. coli	-	*Escherichia coli*
EDTA	-	ethylenediaminetetra-acetate
FESEM	-	field emission scanning electron microscopy
FFD	-	full factorial design
HCl	-	hydrochloric acid
IM	-	inner membrane
IPTG	-	isopropyl β-D-1-thiogalactopyranoside
lac	-	lactose
LB	-	luria bertani
LPS	-	lipopolysaccharide
MgCl	-	magnesium chloride
MW	-	molecular weight
NMP	-	1-methyl-2-pyrolidon
OD	-	optical density
OFAT	-	one factor at one time
OM	-	outer membrane

ONPG	-	*ortho*-nitrophenyl-β-galactoside
PEG	-	polyethylene glycol
PES	-	polyethersulfone
rpm	-	revolution per minutes
RSM	-	response surface methodology
SDS-PAGE	-	sodium dodecyl sulfate polyacrylamide gel electrophoresis
Sp.	-	species

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