IMMOBILIZATION OF CYCLODEXTRIN GLUCANOTRANSFERASE ON POLYVINYLIDENE FLUORIDE HOLLOW FIBER MEMBRANE FOR CYCLODEXTRIN PRODUCTION

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SUPERVISOR'S DECLARATION

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STUDENT'S DECLARATION

I hereby declare that the work in this thesis is based on own research 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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ABSTRAK

Siklodekstrin glukanotransferas (CGTase) adalah enzim perindustrian pelbagai fungsi yang terlibat dalam tindak balas siklisasi untuk penghasilan siklodestrin (CD) daripada kanji. CD adalah sebatian sakarida yang mempunyai permukaan hidrofobik di dalam dan hidrofilik di luar. Oleh itu, CD dapat membentuk kompleks inklusif dengan molekul hydrofobik yang membolehkan ia mengubah sifat fizikal dan kimia molekul tersebut. Dengan ciri-ciri potensi mereka, CD telah diaplikasikan dalam pelbagai industri termasuk industri makanan, farmaseutikal, pertanian dan kejuruteraan alam sekitar. Walau bagaimanapun, ketidakstabilan enzim semasa proses reaksi telah menyebabkan penghasilan CD yang rendah. Oleh sebab itu, teknik imobilisasi enzim telah diperkenalkan untuk meningkatkan kestabilan enzim bagi mencapai pengeluaran CD yang tinggi. Objektif kajian ini adalah untuk mengoptimakan proses imobilisasi CGTase di atas membran gentian berongga poliviniliden fluorida (PVDF) dengan memanipulasikan parameter imobilisasi dan bandingkan prestasi enzim yang terlekat dan bebas terhadap penghasilan CD. Dalam kajian ini, kaedah satu faktor pada satu masa (OFAT), reka bentuk faktorial pecahan (FFD) dan reka bentuk komposit pusat (CCD) telah diadaptasikan bagi memeriksa dan memoptimakan kesan keadaan imobilisasi terhadap kadar imobilisasi. Keupayaan penggunaan semula dan kajian kinetik terhadap CGTase yang telah diimobilisasi juga dijalankan bagi mengkaji prestasi enzim yang terlekat. Aktiviti siklisasi dan kestabilan CGTase dari *Bacillus licheniformis* terhadap suhu dan pH telah dikaji. Aktiviti siklisasi CGTase yang tertinggi telah dicatatkan pada suhu 40 °C dan pH 6.0. CGTase juga menunjukkan kestabilan pada suhu sehingga 60 °C dan kestabilan pada pH antara pH 6.0 hingga pH 8.0, menunjukkan bahawa enzim ini mampu mengekalkan kestabilan pada suhu yang tinggi dan pH yang sederhana. Imobilisasi CGTase di atas membran gentian berongga PVDF berjaya dilaksanakan melalui kaedah penjerapan. Kesan kadar kepekatan enzim, suhu, kadar agitasi, masa pelekatan dan pH, terhadap proses imobilisasi enzim telah dikaji menggunakan kaedah OFAT. Imobilisasi CGTase telah mencatatkan kadar imobilisasi sebanyak 19.21% pada keadaan 100 U kepekatan enzim, 25 °C suhu, 100 rpm agitasi, 24 jam masa pelekatan dan pH 4.0. Imobilisasi CGTase di atas membran gentian berongga juga dioptimakan menggunakan kaedah gerak balas permukaan (RSM). Dengan menggunakan kadar optimum [100 U kepekatan enzim, 24 °C suhu pelekatan, 100 rpm agitasi, 24 jam masa pelekatan dan pH 6.7], mencatatkan 88.25% kadar imobilisasi. Ini menunjukkan peratusan imobilisasi adalah meningkat sebanyak 4.6 kali ganda berbanding dengan kadar imobilisasi sebelum dioptimakan. Keupayaan penggunaan semula enzim yang telah diimobilisasi menunjukkan bahawa CGTase yang terlekat dapat mengekalkan 37.7% daripada aktiviti awalnya selepas digunapakai sebanyak 10 kitaran. Jumlah penghasilan CD yang terkumpul daripada CGTase yang terlekat setelah digunapakai sebanyak 10 kitaran adalah 26.43 mg/ml. Kajian kinetik terhadap CGTase yang telah diimobilisasi dan bebas, mendapati bahawa proses pelekatan tidak mengubah sifat intrinsik enzim, sekaligus menunjukkan bahawa membran gentian berongga adalah sokongan yang sesuai dalam teknik imobilisasi enzim. Pendekatan ini membuktikan bahawa CGTase yang terlekat pada membran gentian berongga dapat meningkatkan penghasilan CD disebabkan keupayaan enzim untuk digunakan semula.

ABSTRACT

Cyclodextrin glucanotransferase (CGTase) is a multifunctional industrial enzyme that undergoes cyclization reaction to converts starch into cyclodextrin (CD). CD is a non-reducing maltooligosaccharides with a hydrophobic inside and hydrophilic surface outside. With these properties, CD able to form inclusion complexes with many hydrophobic molecules, changing their physical and chemical properties. Due to their potential properties, CD has been discovered to have numerous application in food industries, pharmaceutical, agricultural and environmental engineering. However, the instability of the enzyme during the reaction process resulted in the low production of CD. Therefore, enzyme immobilization technique is a promising solution to improve the enzyme stability in order to achieve high production of CD. The aims of this study are to optimize the immobilization of CGTase on polyvinylidene fluoride (PVDF) hollow fiber membrane by manipulating the immobilization parameters and to investigate the performance of the immobilized enzyme compared to the free CGTase on CD production. In the present study, one-factor-at-one-time (OFAT), fractional factorial design (FFD) and central composite design (CCD) were employed to screen and optimize the effect of immobilization conditions towards the immobilization yields. The reusability and kinetic study of the immobilized enzyme were also performed in order to study the performance of the immobilized CGTase. The free CGTase from *Bacillus licheniformis* was characterized to determine their optimum temperature and pH for CD production. The enzymatic activity was highest at the temperature of 40 °C and pH 6.0. Immobilization of CGTase on the PVDF hollow fiber membrane was successfully performed via adsorption technique. The effects of enzyme concentration, temperature, agitation rate, contact time and pH on the enzyme immobilization yield were investigated by OFAT method. The immobilized CGTase exhibited an immobilization yield of 19.21% under the conditions of 100 U of enzyme concentration, 25 °C of immobilization temperature, 100 rpm of agitation rate, 24 h contact time and pH 4.0. The immobilization of CGTase on hollow fiber membrane was further optimized by using response surface methodology (RSM). Under the optimized conditions [100 U of enzyme concentration, 24 °C of immobilization temperature, 100 rpm of agitation, 24 h of contact time and pH 6.7], 88.25% of CGTase immobilization yield was recorded. This illustrated that 4.6-fold increment of the immobilization yield was achieved compared to before optimization process. The reusability of the immobilized CGTase revealed that the immobilized enzyme could retain 37.7% of its initial activity after 10 cycles of reusability. The cumulative production of CD by the immobilized CGTase after 10 cycles was 26.43 mg/ml. The kinetic study of the immobilized and free CGTase discovered that the immobilization process not relatively altered the intrinsic characteristic of the enzyme, suggesting that the hollow fiber membrane appeared as a suitable support for enzyme immobilization system. Hence, immobilization of CGTase on the hollow fiber membrane substantially improved the production of CD by allowing the reusability of the enzyme.

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LIST OF SYMBOLS

cm	centimeter
g	gram
h	hour
l	liter
min	minute
ml	mililiter
mg/ml	milligram/mililiter
U	unit (enzyme activity)
µl	microliter
µmol	micromole
M	molar
w/v	weight solute per volume solution
v/v	volume solute per volume solution
K_m	michealis-menten constant
V_{max}	maximum reaction rate
V	volt
S	substrate
°C	degree celcius
%	percentage

LIST OF ABBREVIATIONS

ANOVA	analysis of variance
ATR	attenuated total reflectance
BSA	bovine serum albumin
CCD	central composite design
CD	cyclodextrin
CGTase	cyclodextrin glucanotransferase
CLEA	cross-linking aggregation enzyme
CLSM	confocal laser scanning microscopy
COO-	carboxylic bonds
Da, KDa	dalton, kilodalton
DOE	design of experiment
FDA	food and drug administration
FESEM	field emission scanning electron microscopy
FFD	fractional factorial design
FTIR	fourier transform infrared spectroscopy
GRAS	generally recognized as safe
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
MW	molecular weight
NaOH	sodium hydroxide
NH	amine group
OD	optical density
OFAT	one factor at one time
OH	hydroxyl group
PEI	polyethyleneimine
POS-PVA	polysiloxane-polyvinyl alcohol
PVDF	polyvinylidene fluoride
R^2	coefficient of determination
RID	refraction index detector
rpm	revolution per minute
RSM	response surface methodology

Sp.	species
SEM	scanning electron microscopy
UMP	University Malaysia Pahang
UV	ultraviolet

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