ENHANCING THE IMMOBILIZATION OF CYCLODEXTRIN GLUCANOTRANSFERASE PRODUCING *Escherichia coli* FOR DIRECT CONVERSION OF STARCH TO β-CYCLODEXTRIN

SAMSON PACHELLES

UNIVERSITI TEKNOLOGI MALAYSIA

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SAMSON PACHELLES

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Faculty of Engineering
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ABSTRACT

Enzyme can be considered as one of the most used and effective biocatalyst both in research and industry. However, some of the major drawbacks of using enzyme are their cost and the unavoidable one-time-usage. This problem has been temporary solved by enzyme immobilization. Nevertheless, there are still disadvantages especially in terms of the enzyme stability and reduction of catalytic activity. Therefore, whole-cell immobilization, also known as whole-cell biocatalyst, can provide a better solution as the immobilized whole-cell can provide fresh enzyme for each reaction and has high reusability. In this study, *Escherichia coli* (*E. coli*) harbouring the enzyme cyclodextrin glucanotransferase (CGTase) was immobilized to a commercial activated charcoal (ACh) for the direct conversion of starch to β-cyclodextrin (β-CD). The immobilization of the *E. coli* was enhanced by employing two methods; first, treatment to the ACh, and second, manipulating the appendages of the *E. coli* called curli. This is done because cell immobilization can become problematic in terms of the amount of cell successfully immobilized. The treatment of the ACh was done through chemical treatment, using four different chemicals, sodium hydroxide (NaOH), hydrochloric acid (HCl), ammonium hydroxide (NH_4OH), and acetic acid (CH_3COOH). The treatment was done relatively at mild conditions. Treatment with NaOH showed the highest increment in cell immobilized with more than 120 % increase. This is mainly attributed by the higher surface area and pore volume resulted from the NaOH treatment. The manipulation of the curli was done by the addition of nickel (II) chloride during the immobilization process. The added nickel triggered the natural response of the cell, forcing the cell to produce its curli and making the cell sticky and easily attached to surfaces. This in turn further increased the cell immobilized by at least 50 %. The resulting enhanced-immobilized cell increases CGTase activity by 10 % and can be reused up to 10 cycles for CGTase expression and showed lower cell lysis compare to both the free cell and to the cell immobilized without any treatment. The optimization of starch direct conversion using the immobilized cell was done using the Box-Behnken design in the design expert software. Under the optimized condition, the highest yield of 15.45 mg/mL β-CD was obtained, which was analysed using high performance liquid chromatography, and the immobilized cell was managed to be reused up to 6 cycles. Based on the results of this study, it can be concluded that the immobilization of *E. coli* was greatly enhanced by the combination of the two methods, ACh treatment and curli manipulation. Also, the enhancement resulted in less cell lysis and stronger cell attachment to the support material. The immobilized cell was successful in directly converting starch to β-CD.

ABSTRAK

Enzim boleh dianggap sebagai biomangkin yang paling banyak digunakan dan yang paling efektif dalam penyelidikan dan industri. Walaubagaimana pun, salah satu kekangan menggunakan enzim yang paling besar adalah kos dan penggunaannya yang hanya sekali sahaja. Masalah ini dapat diselesaikan secara imobilisasi enzim. Namun begitu, masih terdapat kekurangan terutamanya dalam kestabilan enzim dan penurunan aktiviti bermangkin. Oleh itu, imobilisasi keseluruhan-sel, juga dikenali sebagai biomangkin keseluruhan-sel, boleh memberikan penyelesaian yang lebih baik di mana imobilisasi keseluruhan-sel boleh memberikan enzim yang baharu untuk setiap tindak balas dan mempunyai kebolehgunaan semula yang tinggi. Di dalam kajian ini, *Escherichia coli* (*E. coli*) yang mengandungi enzim siklodekstrin glukanotransferase (CGTase) telah diimobilisasikan kepada arang teraktif (ACh) komersial untuk penukaran langsung kanji kepada β-siklodekstrin (β-CD). Imobilisasi *E. coli* telah dipertingkatkan dengan menggunakan dua kaedah; yang pertama, rawatan kepada ACh, dan yang kedua, memanipulasi protein luaran *E. coli* yang dikenali sebagai kurli. Kaedah ini digunakan disebabkan imobilisasi sel boleh menjadi masalah dari segi jumlah sel yang berjaya diimobilisasikan. Rawatan kepada ACh dibuat melalui rawatan kimia, di mana empat jenis bahan kimia digunakan iaitu natrium hidroksida (NaOH), asid hidroklorik (HCl), ammonium hidroksida (NH_4OH), dan asetik asid (CH_3COOH). Rawatan kimia tersebut dibuat dalam keadaan yang ringan. Rawatan menggunakan NaOH menunjukkan peningkatan yang paling tinggi dalam imobilisasi sel dengan peningkatan lebih daripada 120 %. Ini dikaitkan dengan peningkatan luas permukaan dan isipadu pori hasil daripada rawatan NaOH tersebut. Manipulasi kurli dibuat dengan penambahan nikel (II) klorida semasa proses imobilisasi. Penambahan nikel akan mencetuskan tindak balas semula jadi sel, di mana ia akan memaksa sel menghasilkan kurli dan menjadikan sel itu melekit dan memudahkan sel melekat pada mana-mana permukaan. Ini turut meningkatkan lagi imobilisasi sel sekurang-kurangnya 50 %. Sel imobilisasi-dipertingkatkan yang terhasil dapat meningkatkan aktiviti CGTase sebanyak 10 % dan boleh digunakan semula sebanyak 10 kitaran untuk ekspresi CGTase dan menunjukkan lisis sel yang lebih rendah berbanding dengan sel bebas dan dengan sel yang diimobilisasi tanpa sebarang rawatan. Pengoptimuman bagi penukaran langsung kanji menggunakan sel yang diimobilisasi dilakukan menggunakan rekabentuk Box-Behnken dalam perisian *design expert*. Dalam keadaan yang optimum, hasil tertinggi β-CD yang diperoleh adalah 15.45 mg/mL, di mana ia dianalisis menggunakan kromatografi cecair prestasi tinggi, dan sel yang diimobilisasi ini mampu diguna semula sebanyak 6 kitaran. Berdasarkan hasil daripada penyelidikan ini, boleh disimpulkan bahawa imobilisasi *E. coli* telah dipertingkatkan dengan banyaknya melalui gabungan dua kaedah, iaitu rawatan ACh dan manipulasi kurli. Peningkatan tersebut juga mengurangkan sel lisis dan menyebabkan perlekatan sel yang lebih kuat kepada bahan sokongan. Sel yang diimobilisasi telah berjaya menukarkan kanji secara langsung kepada β-CD.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Aa-AC	-	Acetic acid treated Activated Carbon
Am-AC	-	Ammonia treated Activated Carbon
AC	-	Activated Carbon
ACh	-	Activated Charcoal
ANOVA	-	Analysis of Variance
BBD	-	Box-Behnken Design
CD	-	Cyclodextrin
CFU	-	Colony Forming Unit
CGTase	-	Cyclodextrin glucanotransferase
CR	-	Congo Red
DF	-	Dilution Factor
EC	-	Enzyme Commission
FESEM	-	Field Emission Scanning Electon Microscopy
FTIR	-	Fourier Transfrom Infrared Spectroscopy
H-AC	-	Hydrochloric acid treated Activated Carbon
HPLC	-	High Performance Liquid Chromatography
IPTG	-	Isopropylthio-β-galactoside
LB	-	Luria Bertani
M9	-	Minimal medium
N-AC	-	Sodium hydroxide treated Activated Carbon
OD	-	Optical Density
OFAT	-	One-Factor-At-a-Time
ONP	-	Ortho-Nitrophenyl
ONPG	-	Ortho-Nitrophenyl-β-galactoside
rpm	-	Revolutions per minute
RSM	-	Response Surface Methodology
SDS	-	Sodium Dodecyl Sulfate
SOB	-	Super Optimal Broth
TAC	-	Treated Activated Carbon
TB	-	Terrific Broth

TEM	-	Transmission Electron Microscope
TLC	-	Thin Layer Chromatography
UTAC	-	Untreated Activated Carbon
v/v	-	Volume per volume
w/v	-	Weight per volume
XRD	-	X-ray Diffraction
2xYT	-	Two times yeast extract and tryptone medium

LIST OF SYMBOLS

α	-	Alpha
β	-	Beta
γ	-	Gamma
°C	-	Degree Celsius
θ	-	Theta
μ	-	Micro
M	-	Molar
%	-	Percentage

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Enzyme is a protein which serves as a biocatalyst and accelerate the rate of certain reaction. Initially, enzymes were obtained from plants and animals. This had created a huge problem in attaining the enzymes as the process of obtaining it from plants and animals were difficult, in low amount and availability, and costly which in turn made the enzyme quite expensive. Therefore, through genetic and protein engineering, enzyme was able to be produced easier through selected host, which then dubbed the enzyme as recombinant protein or recombinant enzyme. Enzyme can be produced using several hosts such as bacteria, yeast and mammalian cell. Each of these hosts have its advantages and disadvantages. Bacteria, such as *Escherichia coli* (*E. coli*), offer the easiest way to produce enzyme, however, has limitation on the host itself as well as in its inability to produce complex enzyme. Yeast may be able to be used to express more complex enzyme but inadequate in its handling and production process. Mammalian cell can offer the expression to the utmost quality of enzyme, however, possessing major drawback by being too expensive.

In order to address the mentioned disadvantages, cell immobilization was one of the most promising solution. The immobilization process can be done involving the process of attachment or entrapment. Immobilization of bacteria came about, essentially, as an alternative for enzyme immobilization. With the immobilization of bacteria, which often involves enzymes produced on the surface of the bacteria or expressed extracellularly, does not need the long and expensive procedure for enzyme separation and purification. Moreover, this process also enable the procurement of products and the recovery of the biocatalyst with ease (Junter and Jouenne, 2004; Stolarzewicz *et al.*, 2011). So far there have been four established method for cell

immobilization: covalent bonding or cross linking, encapsulation, entrapment and adsorption (Mallick, 2002).

Support material for cell immobilization exist in many shapes, forms and sizes. The selection of support material heavily depends on the cell to be immobilized and the desired product. One of the potentially suitable support material is activated carbon (AC). AC is best known for its porous internal structure and adsorptive capability which are the most preferable qualities in cell immobilization process. Moreover, AC can be utilized as candidate for such task because AC can be obtain in abundant at a low cost, structurally and thermally stable as well as having the resistance towards microbial degradation (Cassidy *et al.,* 1996; Verma *et al.,* 2006). On top of that, AC is an inert material which will not impaired the growth of bacteria and preserves their physiological activity (Martins *et al.,* 2013). In order to improve cell immobilization process, certain modification can be done to the AC in order for the AC to accommodate more in the immobilization process. Modification to AC can be done through multiple ways which are thermal, chemical or biological modification.

Other than modification to the AC, modification to the *E. coli* itself may also be done. This modification involves the manipulation of an outer appendages of *E. coli* which is called curli. Curli are included in the category of fimbriae which are thin and aggregative produced by many Enterobacteriaceae including *E. coli* (Chapman *et al.,* 2002) and *Salmonella typhimurium* (Collinson *et al.,* 1991). Curli constitutes as the major protein component of the extracellular matrix and they possessed multiple functions including cell adhesion and biofilm formation (Kikuchi *et al.,* 2005), host cell invasion (Wang *et al.,* 2006), immune system activation (Gophna *et al.,* 2001) and protection from environmental stresses (Smith *et al.,* 2017). Enhancing the production of curli may increase the attachment of bacteria to the AC. Thus far, combining the manipulation of AC and the surface of bacteria to enhance its attachment has not been done yet.

1.2 Problem Statement

The greatest limitation of enzyme is its unavoidable one-time-use-only feature as well as its full-of-hassle procurement (Junter and Jouenne, 2004). This problem can be identified in the cell used in this study. The *E. coli* BL21 (DE3) harboring the enzyme CGTase used in this study was constructed by Ling (2018) in which the enzyme was expressed extracellularly by the signal peptide GAP. This problem in general was initially solved by enzyme immobilization. However, this was only a temporary solution where another problems arose which were the drop in enzyme activity and stability. In order to tackle these issues, cell immobilization was concocted to create a whole cell biocatalyst. A whole cell biocatalyst created through cell immobilization are able to be use repeatedly, easily produced and handled, easier to collect the end product, faster and require less cost, and most importantly fresh enzyme will be able to be produced for every use. However, there are still a limitations to immobilization process where the cell might be difficult to be immobilized, less amount of cell immobilized, and the cell immobilized could be loosely attached. Therefore, certain modification can be made to the AC and *E. coli* in order to solve these problems.

1.3 Objectives of the Study

There objectives of this study are as follow:

1. To study the enhancement of *E. coli* immobilization through chemical treatment of activated charcoal and surface modification of *E. coli*.
2. To study the production of CGTase using the immobilized cell.
3. To study the application of recombinant immobilized cell for starch direct conversion to β-cyclodextrin (β-CD).

1.4 Scopes of the Study

The approaches used to achieve the objectives of this study are as follows:

1. Screening the parameters for cell immobilization including the duration process of cell immobilization, agitation rate, and medium.
2. Optimization of the medium compositions for cell immobilization enhancement.
3. Determine the effect of the chemical treatments towards the commercial activated charcoal and to the immobilization process.
4. Determine the effect of nickel towards the surface of *E. coli* and to the immobilization process.
5. Optimization of the expression conditions for CGTase using the immobilized cells.
6. Optimization of the conditions for direct conversion of starch to cyclodextrin including reaction temperature, time, substrate concentration and reusability.

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LIST OF PUBLICATION

1. **Pachelles, S**., Fuzi, S. F. Z. M., Man, R. C., Abdullah, A. A., and Illias, R. M. (2021). Combine strategy of treated activated charcoal and cell surface protein curli induction for enhanced performance in *Escherichia coli* immobilization. *Process Biochemistry*. 110, 26 – 36. (**Q2, IF: 3.757**)