PRODUCTION OF XYLONIC ACID BY RECOMBINANT *Escherichia coli* IMMOBILIZED ON MAGNETIC NANOPARTICLES

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This research project is dedicated to those who have endured a great deal of pain and joy for just being around me, especially to my beloved parents, Zahari Bin Ali and Norazimah Binti Idris, my supportive family members, lecturers and friends.

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ABSTRACT

Conversion of xylose to xylonic acid has obtained growing interest over the years. As the native metabolic pathway of *Escherichia coli* (*E. coli*) was unable to convert xylose to xylonic acid, native *E. coli* has been genetically modified to charter for the production of xylonic acid. However, production of xylonic acid by free cells has encountered some drawbacks that include low yield, lack of stability and reusability of using free cell. Thus, cell immobilization was used for the production of xylonic acid because it can counter the drawbacks of using free cells and elevate the production of xylonic acid. In this study, the parameters affecting the production of xylonic acid by recombinant *E. coli* immobilized on magnetic nanoparticles were studied. The effect of post induction temperature, Isopropyl β-D-1-thiogalactopyranoside (IPTG) concentration, xylose concentration, medium pH and expression media towards xylonic acid production, cell density and plasmid stability of the immobilized recombinant *E. coli* were investigated. The parameters were further optimized by using response surface methodology with the optimum condition of 0.6 mg/mL magnetic nanoparticles at post induction temperature of 30 ˚C, 0.1 mM IPTG concentration and 50 gL^{-1} of xylose concentration in expression medium. The immobilized recombinant *E. coli* produced up to 24.58 gL^{-1} xylonic acids during one factor at a time screening with the productivity of 1.024 $gL^{-1}h^{-1}$ and yield of 0.492 g g^{-1}, which has 3-fold increment as compared to free cell. The cells immobilized on the magnetic nanoparticles exhibited a 47 % increment in stability of the plasmid as compared to free cells and can be used for up to 4 times while retaining xylonic acid productivity more than 50 %. Hence, the immobilization of recombinant *E. coli* using magnetic nanoparticles was demonstrated to increase xylonic acid production, cell stability and reusability.

ABSTRAK

Penukaran xilosa kepada asid xilonik telah mendapat perhatian yang meningkat sejak beberapa tahun lalu. Oleh kerana *Escherichia coli (E. coli)* tidak dapat menukarkan xilosa kepada asid xilonik secara metabolik semulajadi, *E. coli* telah diubah suai secara genetik untuk membolehkan pengeluaran asid xilonik. Penghasilan asid xilonik oleh sel bebas mempunyai beberapa kelemahan, antaranya adalah hasil yang rendah, ketidakstabilan sel dan kesukaran penggunaan semula sel bebas. Oleh itu, teknik imobilisasi sel telah digunakan untuk menghasilkan asid xilonik kerana ia dapat mengatasi kelemahan sel bebas dan meningkatkan pengeluaran asid xilonik. Dalam kajian ini, faktor-faktor yang mempengaruhi pengeluaran asid xilonik oleh *E. coli* rekombinan yang telah diimobilisasikan pada nanopartikel magnetik telah dikaji. Kesan suhu pasca induksi, kepekatan Isopropyl β-D-1-thiogalactopyranoside (IPTG), kepekatan xilosa, pH media dan jenis media pertumbuhan terhadap tahap pengeluaran asid xilonik, ketumpatan sel dan kestabilan plasmid rekombinan *E. coli* telah dikaji. *E. coli* rekombinan telah diimobilisasikan dengan 0.6 mg /mL nanopartikel magnetik pada keadaan optimum iaitu suhu pasca induksi 30 ˚C, kepekatan IPTG 0.1 mM dan kepekatan xilosa sebanyak 50 gL^{-1} dalam media pertumbuhan menggunakan kaedah gerak balas permukaan. *E. coli* rekombinan yang telah diimobilisasi telah menghasilkan sehingga 24.58 gL^{-1} asid xilonik melalui kaedah tapisan satu faktor pada satu masa dengan produktiviti 1.024 $gL^{-1}h^{-1}$ dan kadar penghasilan sebanyak 0.492 g g^{-1}, yang mempunyai kenaikan 3 kali ganda berbanding dengan sel bebas. Sel-sel yang diimobilisasi pada nanopartikel magnetik menunjukkan peningkatan sebanyak 47 % dalam kestabilan plasmid berbanding dengan sel-sel bebas dan sel imobilisasi boleh digunakan sehingga 4 kali sambil mengekalkan produktiviti asid xilonik lebih daripada 50 %. Oleh itu, *E. coli* rekombinan yang telah diimobilisasikan dengan nanopartikel magnetik telah menunjukkan peningkatan dari segi penghasilan asid xilonik, kestabilan sel serta boleh digunakan semula.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	-	Percent
˚C	-	Degree Celsius
2xYT	-	2 x Yeast Tryptone
E. coli	-	*Escherichia coli*
FESEM	-	Field Emission Scanning Electron Microscopy
HPLC	-	High Performance Liquid Chromatography
LB	-	Luria Bertani
RPM	-	Rotation per minute
SOB	-	Super Optimal Broth
TB	-	Terrific Broth
UTM	-	Universiti Teknologi Malaysia
ZP	-	Zeta Potential

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

These days, processing of biomass for the production of value-added chemicals has become important to both biological and chemical engineering research (FitzPatrick *et al.,* 2010). These value-added chemicals can be produced through chemical synthesis route and microbial conversion (Cao *et al*., 2013). Whole cell has been used for functional sensing in various fields such as biocatalysis (Zhang *et al.,* 2015), environmental monitoring (Ben-yoav *et al.,* 2009), pharmacology and food industry (Gao *et al.,* 2009).

Free cell can act as a mini-reactor as it contains all the necessary cofactors and enzymes (Ni *et al.,* 2012). Therefore, free cell is frequently used as the biocatalyst for preparation of value added product. However, free cell is unstable and difficult to be recycled, which affects the separation and purification of the subsequent product (Yu *et al.*, 2016). Thus, immobilization technique was used to immobilize the free cell, as it is one of the effective methods to overcome these drawbacks and can facilitate its reuse and simplify the product purification (Zhuang *et al.,* 2017).

Xylonic acid, a five-carbon organic acid has gained outstanding interest due to its potential as an important platform chemical. It has extensive applications in food, pharmaceutical and chemical industries. Xylonic acid can be produced by oxidation of xylose with the microorganism. Recently, xylonic acid has been produced by the recombinant *Escherichia coli (E. coli), Saccharomyces cerevisiae*

and *Kluyveromyces lactis* (Toivari *et al.,* 2013). *E. coli* has native metabolic pathways for xylose consumption, unfortunately, it is incapable of converting xylose directly to xylonic acid. Liu *et al.,* (2012) in their studies, genetically modified *E. coli* to produce xylonic acid by introducing a xylose dehydrogenase gene from *Caulobacter crescentus.* Both xylose and xylonic acid catabolic pathways were blocked to prevent the conversion from xylose or xylonic acid to biomass.

The production of this sugar acid can be increased by immobilization of cell. The strategy to use the immobilized cell as compared to free cells would give a lot of benefits such as improved stability and reusability. Travieso *et al.,* (1999), stated in his study that in order to maintain the microbial cell functionality in biological processes, the use of immobilization is a preferable technique. There are various types of method used to immobilize the biological cells that include adsorption, entrapment and covalent binding (Kosseva, 2011).

The choices of support for immobilization are also one of the important factors that need to be considered when choosing immobilization technique. Nanomaterials have been known to be novel and remarkable matrices for immobilization technique. The high surface to volume ratio of nanomaterial is an obvious advantage (Gupta *et al.,* 2011). Ensafi *et al*., (2014), mentioned in their studies that there are various nanomaterials that can be found in the current market including nano-wires, nanofibers and nanoparticles. These nanomaterials are known to be important for scientific works.

In this study, magnetic nanoparticles have been chosen as an immobilization matrix due to several merits, including nontoxicity, large surface area, and the ability to produce desired magnetic properties so that it can ease separation by using magnets and can be reused (Zang *et al.,* 2014).

Various approaches have been previously studied in order to improve the production of recombinant proteins in *E. coli.* These include improving culture conditions, such as varying the medium composition, nutritional feeding design,

induction mode and utilization of media additives (Cheng *et al.*, 2011; Fang *et al.*, 2011).

1.2 Problem Statement of the Study

Xylonic acid has been known to have extensive use as an important sugar acid in the chemical industries. Since the native metabolic pathway of *E. coli* was unable to convert xylose to xylonic acid, thus *E. coli* was genetically modified to charter for xylonic acid production. However, the use of free cell was known to have comparable disadvantages than immobilized cell as it has lack of stability and no possibility to be recycled, that in turns causes low yield of xylonic acid. Therefore, immobilization technique was used in this study as it can encounter the drawbacks of using free cell.

The immobilization matrix used to immobilize the free cell is important as it can affect the separation and cell attachment. Nanomaterial was used as the immobilization matrix as it has high surface area and has been used widely for immobilization. Nevertheless, it is a concern whether the genetically modified *E. coli* have the ability to attach onto the nanomaterial and become immobilized.

Besides that, the effect of cultural conditions towards immobilization of recombinant *E. coli* on xylonic acid production, cell density and plasmid stability was also a concern in this study. Parameters such as post induction temperature, xylose concentration, IPTG concentration, pH and expression medium have been known to affect the productivity of immobilized cell. Therefore, the study was conducted to analyse the effect of immobilization of recombinant *E. coli* on magnetic nanoparticle towards the production of xylonic acid.

1.3 Objective of the Study

The objectives of this study are stated as below;

1. To determine the effect of cultural conditions on the production of xylonic acid by recombinant *E. coli* immobilized on magnetic nanoparticles.
2. To optimize and evaluate the effect of immobilization on xylonic acid concentration, plasmid stability, cell density and reusability of recombinant *E. coli* immobilized on magnetic nanoparticles.

1.4 Research Scope

In achieving the objectives of this research, the scopes of this work have been identified and stated as below:

1. Preparation and characterization of recombinant *E. coli* cell immobilized with magnetic nanoparticles.
2. Screening and optimization of the effect of cultural conditions (post induction temperature, IPTG concentration, xylose concentration, medium pH and expression medium) on xylonic acid concentration, cell density and plasmid stability using one factor at a time method (OFAT).
3. Determination of mathematical model and interaction between parameters by Historical Data Design (HDD).
4. Evaluation of growth kinetics and reusability of immobilized cells.

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PUBLICATION AND AWARD

1. Farrah Noor Izzati Zahari, Rosli Md Illias, Zanariah Hashim. Production of xylonic acid by recombinant *Escherichia coli*. (2017) 12th Colloquium.