XYLITOL PRODUCTION OF RECOMBINANT *ESCHERICHIA COLI* IMMOBILIZED ON MULTI WALLED CARBON NANOTUBES

NOOR HIDAYAH BINTI ABD RAHMAN

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To my beloved parents (Abd Rahman bin Hj. Abd Manap and Hjh. Saipah binti Alias), my husband (Muhammad Faris bin Nasir), my daughter (Fardanah Malisa),my mother-in-law, my siblings, brothers and sisters. I dedicated this work in sincere gratitude for their patience, love and support.

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ABSTRACT

Xylitol is currently produced in a large scale by a chemical reduction process that needs high energy and cost. Biological conversion of xylitol utilizing microorganisms could be an alternative methodology that is environmentally friendly and economical. This method has been proven to offer a high-yield and competitive. However, one of the major drawback in xylitol production using bacteria is the low yield. Cell immobilization is a promising solution for the enhancement of xylitol production. This study was carried out to improve the xylitol production, cell stability and performance by immobilizing recombinant *Escherichia coli* (*E. coli*) on untreated multiwalled carbon nanotubes (MWCNT) using optimum cultural condition. The influence of different treatment on MWCNT and cultural environments on xylitol production, xylose reductase activity, cell viability and lysis of immobilized *E. coli* were investigated. The immobilized cells on untreated MWCNT exhibited about 2-8-fold increase in xylitol production compared to free cells. The immobilized cells also demonstrated a 22-315% reduction of β-galactosidase activity, as indication of reduced cell lysis and a 17-401% increase in plasmid stability compared to free cells. The xylitol production was successfully improved using central composite design for the response surface methodology. The optimized cultivation conditions obtained for pH, temperature and isopropyl β-D-1-thiogalactopyranoside concentration were 7.42, 29 ^{o}C and 0.005 mM, respectively. Under the optimized conditions, the xylitol concentration was 6.325 g/L, representing 91.5% of the predicted value (6.905 g/L) and 1.16-fold higher than the value before optimization process (5.467 g/L). This study demonstrated that the immobilized cells system could be a promising approach to improve the productivity of xylitol using recombinant *E. coli*.

ABSTRAK

Xilitol kini dihasilkan dengan skala besar menggunakan proses reduksi kimia yang memerlukan kos dan tenaga yang tinggi. Penukaran biologi xilitol yang menggunakan bakteria adalah kaedah alternatif yang lebih mesra alam dan berekonomi. Pendekatan ini telah dibuktikan dapat menawarkan hasil yang tinggi dan kaedah yang berdaya saing. Walau bagaimanapun, salah satu penghalang terbesar pengeluaran xilitol dengan menggunakan bakteria ialah pengeluaran yang rendah. Imobilisasi sel adalah satu langkah penyelesaian yang boleh menjanjikan peningkatan pengeluaran xilitol. Kajian ini dijalankan bertujuan untuk meningkatkan pengeluaran xilitol, kestabilan dan prestasi sel oleh imobilisasi rekombinan *Escherichia coli* (*E. coli*) ke atas tiub nano karbon pelbagai lapisan (MWCNT) tidak dirawat menggunakan keadaan kultur yang optimum. Kesan rawatan yang berbeza terhadap MWCNT dan persekitaran kultur untuk pengeluaran xilitol, aktiviti reduktase xilosa, kebolehhidupan sel dan lisis oleh *E. coli* yang diimobilisasikan telah dikaji. Sel yang diimobilisasikan pada MWCNT tidak dirawat mempamerkan peningkatan 2-8 kali ganda dalam pengeluaran xilitol berbanding sel bebas. Sel yang diimobilisasikan juga menunjukkan penurunan 22-315% aktiviti β-galaktosidase, merujuk kepada penurunan sel lisis, dan peningkatan 17-401% kestabilan plasmid berbanding sel bebas. Pengeluaran xilitol telah berjaya ditingkatkan menggunakan reka bentuk komposit pusat bagi kaedah permukaan gerak balas. Keadaan kultur yang optimum diperoleh bagi pH, suhu dan kepekatan isopropil β-D-1-tiogalaktopiranosida adalah masing-masing 7.42, 29 ^{o}C dan 0.005 mM. Kepekatan xilitol sebanyak 6.325 g/L, mewakili 91.5% daripada nilai yang diramalkan (6.905 g/L) dan 1.16 kali ganda tinggi daripada nilai sebelum proses pengoptimuman (5.467 g/L) dengan menggunakan keadaan optimum. Kajian ini menunjukkan bahawa sistem sel yang diimobilisasikan merupakan pendekatan yang menjanjikan peningkatan pengeluaran xilitol dengan menggunakan rekombinan *E. coli.*

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LIST OF SYMBOLS

cal	-	Caloric
cm	-	centimeter
g	-	gram
h	-	hour
J	-	Joule
k	-	kilo
l	-	liter
mg	-	miligram
min	-	minute
ml	-	milliliter
mM	-	milimolar
mV	-	milivoltage
^{o}C	-	temperature
R	-	correlation coefficient
U	-	unit
wt	-	weight
Ω.m	-	resistivity

LIST OF ABBREVIATIONS

ANOVA	-	analysis of variance
CCD	-	central composite design
CNT	-	carbon nanotubes
DNA	-	deoxyribonucleic acid
E. coli	-	*Escherichia coli*
FESEM	-	field emission scanning electron microscopy
FTIR	-	Fourier transform infrared spectroscopy
HPLC	-	high performance light chromatography
IPTG	-	isopropyl β-D-1-thiogalactopyranoside
lac	-	lactose
$MgCl_2$	-	magnesium chloride
MWCNT	-	multiwalled carbon nanotubes
OFAT	-	one factor at one time
ONPG	-	o-nitrophenyl-β-D-galactopyranoside
rpm	-	revolution per minutes
RSM	-	response surface methodology
sp	-	species
SWCNT	-	single walled carbon nanotubes
XR	-	xylose reductase

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Xylitol ($C_5H_{12}O_5$), is a sugar alcohol and a natural food sweetener that has many commercial applications especially in food, pharmaceutical and oral health industries (Mohamad *et al.*, 2014; Rafiqul and Sakinah, 2012; Lagoas, 1998; Granström *et al.*, 2007a). High global demand on xylitol, is as a result of its insulin-independent metabolism, anticarcinogenicity, excellent sweetening power and pharmacological properties (Povelainen, 2008). Additionally, xylitol is utilized as moisturizer, cryoprotectant, preservative and an antioxidant (Mohamad *et al.*, 2014). Xylitol has be produced by using solid-liquid extraction, chemical synthesis and biological processes. In solid-liquid extraction, the naturally occurring xylitol is extracted from fruits and vegetables. However, this process often yields low xylitol recovery with less than 9 g/L (Lagoas, 1998). Currently, xylitol is manufactured industrially by reducing pure xylose that is produced by acid-catalysed hydrolysis. The hydrogenation of D-xylose from hemicellsulosic hydrolyzates has been applied to produce xylitol wherein the downstream processing is very expensive (Mohamad *et al.*, 2014; Rafiqul and Sakinah, 2012; Granström *et al.*, 2007a). The production of xylitol by chemical reduction bring other drawbacks, for example, involvement of high pressure high temperature and pressure and the use of an costly compound (Saha, 2003). Hence, it has been useful to discover methods for an efficient xylitol production by microorganisms.

Fermentation approach for the xylitol production is attractive owing to the problems associated with the quality and cost-effective product when chemically production is applied (Rafiqul and Sakinah, 2012; Saha, 2003). In biological production of xylitol, most studies extensively used *Candida* sp. compared to metabolically engineered *Sacharomyces cerevisiae* because they are able to keep the redox balance during the synthesis of xylitol as they are good natural D-xylose consumers (Granström *et al.,* 2007b). In addition, these yeast strains are considered as the best producer of xylitol (Parajo *et al.,* 1995; Winkelhausen and Kuzmanova, 1998; Roberto *et al.,* 1999). Previously, very few literature reports xylitol production by bacteria which is used xylose and xylulose as substrate. There are some studies have been used bacteria for the xylitol production including *Corynebacterium* sp. (Rangaswamy and Agblevor, 2002; Yoshitake *et al.,* 1973), *Enterobacter liquefaciens* (Yoshitake *et al.,* 1976), *Cellulomonas cellulans, Corynebacterium glutamicum, Corynebacterium ammoniagenes, Serratia marcescens* (Rangaswamy and Agblevor, 2002) and *Bacillus coagulans,* and *Mycobacterium smegmatis* (Izumori and Tuzaki, 1988). Engineered *E. coli* is also one of the potential bacteria for the development of efficient industrial-scale production of xylitol from hemicellulose hydrolysate (Zhao *et al*., 2012), although in many engineered *E. coli* has been shown to produce relatively low xylitol yield of recombinant protein (Schein 2010). Moreover, xylitol has been widely produced in free cells. Even though in some cases remarkable yields were gained, but the xylitol productivity were very low.

In an attempt to increase product yield, immobilization of cell has several benefits compared to free cells such as improved stability and productivity, cell reutilization, reduced contamination, continuous operation, and easier downstream processing. As stated in a study by Domínguez (1998), in order to maintain the functionality of microorganisms in biological processes, the immobilization is a preferred technique. The most common techniques of immobilization of cell employed in bioprocesses are adsorption, entrapment in a polymer gel and covalent binding to supports (Kosseva *et al.,* 2009). Immobilization supports such as calcium alginate, polyvinyl alcohol, polyacrylic hydrogel thin films, polyethylene oxide, polymer resins, porous glass spheres, zeolite and porous glass are most commonly

used in xylitol production previously. However, the gel entrapment method that involved the use of calcium chloride during the solidification of gel reduced the pH of mixture and gave impacts on cell growth (Atanasova *et al.*, 2009). Futhermore, the major disadvantages of covalent binding to a matrix are expensive but low yield due to the exposure of the cells to poisonous reagents and severe reaction conditions. Therefore, the immobilization of cell by adsorption on multiwalled carbon nanotubes (MWCNT) has attracted great interest as a result of their special and unique characteristics.

Carbon nanotubes is a new form of carbon that have created a great attention due to their unique tubular structure and excellent properties (Valcarcel and Cardenas, 2007). The hollow and layered nanosizes structure make them as a good absorber because of the high electrical conductivity of carbon nanotubes (Tan *et al.*, 2012). For example, the adsorption of metal ions on MWCNT is a fast process and only takes a few minutes (Li *et al.*, 2002). Generally, for the immobilization cells, there are two methods to improve the interactions between substrate and the cells which are chemical variation of the support surface to have high affinity to the cells (irreversible) and physical attachment of the cells on the support (reversible) (Folch and Toner, 2000). The key factors in immobilization are the choice of support and immobilization method. These crucial factors influence the stability and catalytic activity of the whole cells biocatalysts in order to achieve the goal of immobilization. Innovative studies and research of carbon nanotubes ought be continue to create new technologies and approaches by using carbon nanotubes as immobilization matrix for whole-cell biocatalyst.

In this study, MWCNT was chosen as a support for the cell immobilization. Recombinant *E. coli* were immobilized on MWCNT via adsorption to increase the productivity of xylitol, to decrease the cell lysis and to increase the plasmid stability. The main advantage of immobilization via adsorption is direct contact between nutrients and the matrix. There has been no report on xylitol production by immobilized recombinant *E. coli* on multiwalled carbon nanotubes through adsorption technique. The results presented propose that immobilization of cell is an encouraging method for xylitol production with high plasmid stability.

1.2 Problem Statement

The chemical xylitol production is expensive due to the use of expensive chemicals and materials. As time goes on, demand for the production of xylitol keeps increasing and market is very high, especially in biomedical application. Formerly, the green innovation is introduced to the world, to create an alternative method for the biological xylitol production. Application of recombinant *E. coli* as host organism in xylitol production faced problems such as low xylitol yield and plasmid stability, and high cell lysis due to overexpression limitation. Cell immobilization approach is preferred to overcome the problems. MWCNT is a potential material as immobilization support because of their unique and special characteristics in order to enhance the cell immobilization efficiency. Therefore, the more effective cell immobilization technique, the high cell viability and plasmid stability, thus could improved the xylitol production.

1.3 Objective of Study

The main objective of this study is to improve the xylitol production, cell stability and performance by immobilizing recombinant *E. coli* on multiwalled carbon nanotubes using optimum cultural conditions.

1.4 Scopes of Study

The following are the scopes of this research:

a) Screening the effect of chemical treatment on multiwalled carbon nanotubes for immobilization of recombinant *E. coli*.
b) Screening the effect of cultural conditions (medium, pH, temperature and IPTG concentration) on improvement of xylitol production and plasmid stability by the immobilized cells using one factor at one time method (OFAT).
c) Optimization of the cultural conditions (pH, temperature and IPTG concentration) by central composite design (CCD) toward the achievement of maximum xylitol production.

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