OPTIMIZATION OF SUCCINIC ACID PRODUCTION FROM IMMOBILIZED *ESCHERICHIA COLI*

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Doctor of Philosophy

UNIVERSITI MALAYSIA PAHANG

SUPERVISOR'S DECLARATION

We hereby declare that We have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Doctor of Philosophy in Bio-Process Engineering.

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STUDENT'S DECLARATION

I hereby declare that the work in this thesis is based on my original work 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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LIST OF SYMBOLS

°C	degree celcius
Ca^{2+}	ion calcium
µm	micrometer
%	Percentage
3-D	Three- dimensional
g mol^{-1}	gram per mol
g/l	gram per liter
kPa	kiloPascal
h	hour
L	liter
min	minute
ml	milliliter
n	Variable quantity
NAD^{+}	nicotinamide adenine dinucleotide
nm	nanometer
MT	metrik tons
OD	optical density
Pa.s	Pascal per second
rpm	rotation or revolution per minute
R_t	retention time
v/v	volume per volume
w/v	weight per volume
w/w	mass fraction (mass per mass)

LIST OF ABBREVIATIONS

ATP	adenosine 5’-triphosphate
ZSI	Z source inverter
2FI	two factor interaction
ANOVA	analysis of variance
ATCC	American Type of Culture Collection
CDW	cell dry weight
CFU	colony forming unit
DM	dried matter
DOE	design of experiment
FFD	full factorial design
FTIR	Fourier Transform Infrared Spectroscopy
H_2SO_4	Sulfuric Acid
HPLC	high performance liquid chromatography
MONG	Matter-non-glycerol
NaOH	Sodium Hydroxide
Na_2CO_3	Sodium Carbonate
OD	optical density
OFAT	one-factor-at-a-time
POME	palm oil mill effluent
SD	Standard deviation
RID	refractive index detector
RSM	response surface methodology

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ABSTRAK

Asid suksinik boleh dihasilkan daripada sisa gliserol dengan fermentasi daripada imobilisasi *Escherichia coli*. Kenaikan harga asid succinic adalah salah satu faktor asid succinic dihasilkan daripada sisa gliserol. Sebagai salah satu produk pengeluaran daripada oleochemical, gliserol kini telah menjadi sumber karbon yang banyak dan murah. Untuk mengurangkan kos penghasilan, sisa gliserol merupakan substrat alternatif untuk asid succinic dihasilkan. Selain itu, dengan menggunakan kaedah imobilisasi, sel-sel boleh digunakan semula untuk pengeluaran asid succinic. Oleh yang demikian, kajian ini dibuat bertujuan menghasilkan asid suksinik menggunakan gliserol yang di perolehi semula dari sisa industri oleokimia, dengan *E. coli* yang telah di imobilisasi. Perbandingan antara proses fermentasi menggunakan *E. coli* bebas dan imobilisasi dijalankan dengan mengambil kira faktor jisim substrat, ketumpatan inokulum dan masa fermentasi. Asid suksinik yang telah dihasilkan dari fermentasi sel imobilisasi kemudiannya di optimumkan menggunakan kaedah gerak balas permukaaan. Kajian ini juga telah mengenalpasti ciri-ciri sel imobilisasi termasuk kestabilan sel dan kebolehan kitar semula manik semasa proses fermentasi. Kromatografi cecair prestasi tinggi digunakan bagi menganalisa kepekatan gliserol dan asid suksinik. Manakala, spektroskopi inframerah fourier transformasi digunakan bagi menganaslisa kumpulan berfungsi gliserol. Salah satu kajian utama adalah kajian kinetik dari sel bebas dan sel-sel imobilisasi, yang mana mengkaji kesan substrat ke atas profil pertumbuhan mikroorganisma dan dengan pengeluaran asid suksinik. Keputusan yang diperolehi untuk kadar maksimum tertentu pertumbuhan, μmax (hr^{-1}), adalah 0.031 h^{-1} dan K_S adalah 5.11 g untuk sel-sel imobilisasi, manakala bagi sel-sel bebas kadar pertumbuhan maksimum adalah 0.029 h^{-1} dan K_S adalah 4.03 g. Perbezaan antara sel-sel imobilisasi dan bebas adalah 6.9 % untuk μ_{max} (hr^{-1}) dan 26.8 % untuk K_S. Keputusan kajian membuktikan bahawa kepekatan substrat adalah salah satu faktor yang boleh memberi kesan keatas kadar pertumbuhan mikroorganisma. Dalam usaha untuk mengoptimumkan parameter untuk tujuan skala besar, keadaan optimum asid suksinik untuk proses fermentasi adalah pada masa 3.31 jam, substrat jisim 40 g dan ketumpatan inokulum 15%. Pada peringkat optimum ini, 124.09 g/L asid suksinik telah diperolehi. Jumlah hasil yang dihasilkan ialah 5.95 g succinic acid. Kajian ini telah berjaya dibangunkan untuk menghasilkan dan meningkatkan pengeluaran asid suksinik daripada sisa gliserol yang boleh diperbaharui dengan kaedah sel imobilisasi.Untuk sel-sel imobilisasi, pemilihan kaedah adalah penting untuk pengeluaran asid suksinik yang tinggi. Untuk mengurangkan kos pengeluaran asid suksinik, sel imobilisasi mempunyai kelebihan yang boleh dikitar semula sehingga 6 kitaran pengeluaran asid suksinik. Kajian lanjut perlu diambil untuk mengkaji struktur manik untuk pengeluaran asid suksinik yang tinggi dalam kaedah pemerangkapan.

ABSTRACT

Succinic acid can be produced from glycerol residue by fermentation of immobilized *Escherichia coli*. Increament of the price of succinic acid is one of the factors to produce the succinic acid from the waste of glycerol. As a by product of oleochemical production, glycerol has now become an abundant and cheap source of carbon. To reduce the production cost, the glycerol waste is an alternative substrate for succinic acid production. Besides, by using immobilization method, the cells can be reused for succinic acid production.Therefore, this study was aimed to produced succinic acid using glycerol recovered from industrial waste oleochemicals, with *E. coli* that have been immobilized. Comparison between fermentation process using free and immobilized *E-coli* is carried out by considering the mass of the substrate, the density of inoculum and fermentation time. Succinic acid that has been produced from immobilized cell fermentation was optimized using response surface methodology. The study also identified the characteristics of immobilized cells including cell stability and the ability to recycle beads during the fermentation process. High performance liquid chromatography was used to analyze the concentration of glycerol and succinic acid. While Fourier transform infrared spectroscopy was used to analyze the functional groups of glycerol. One of the main studies was the kinetic study of the free and immobilized cells, which studied the effect of the substrate on the growth profile of the microorganism and thus, the production of succinic acid. The results obtained for the maximum specific growth rate μ_{max} (hr^{-1}) was 0.031 h^{-1} and the K_S was 5.11 g for the immobilized cells, while for the free cells, the maximum growth rate was 0.029 h^{-1} and K_S of 4.03 g. The difference between the immobilized and free cells was about 6.9 % for the μ_{max} (hr^{-1}) and 26.8 % for the K_S. The results proved that the substrate concentration is one of the factors that may affect the microorganism growth rate. To optimize the parameters for scale-up purposes the optimum conditions observed for succinic acid fermentation process were time 3.31 h, mass substrate 40 g, and inoculum density 15%. At these optimum conditions, 124.09 g/L of succinic acid was obtained. The total yield obtained was 5.95 g of succinic acid. This study was successfully developed to produce and improve the succinic acid production from renewable glycerol residue using the immobilized cell method. For the immobilized cells, the selection method was important for high production of succinic acid. To reduce the cost of succinic acid production, the immobilized cells have the advantage of been able to be reused up to 6 cycles for succinic acid production. A further study should be carried out to investigate the structure of the beads for high production of succinic acid using the entrapment method.

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