UNIVERSITI PUTRA MALAYSIA

MODELING AND OPTIMIZATION OF LIPASE-CATALYZED SYNTHESIS OF ADIPATE ESTERS USING RESPONSE SURFACE METHODOLOGY AND ARTIFICIAL NEURAL NETWORK

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By

NAZ CHAIBAKHSH LANGROODI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Doctor of Philosophy

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March 2010

Chairman: Professor Mohd Basyaruddin Abdul Rahman, PhD

Faculty: Science

Optimized Novozym 435 (*Candida antarctica* lipase B immobilized on acrylic resin)-catalyzed esterification of adipic acid and various monohydric alcohols was successfully performed. Solvent-based synthesis of adipate esters was carried out in small scale reaction using 30 mL screw-capped vials. The synthetic reaction was optimized by Response Surface Methodology (RSM) based on central composite rotatable design (CCRD) to evaluate the interactive effects of reaction parameters including temperature, time, enzyme amount and alcohol/acid molar ratio. A high percentage yield (>96.0%) using optimum conditions was obtained using a minimum amount of enzyme, which matched well with the predicted values.

Artificial Neural Network (ANN) approach was also employed for the estimation of esterification yield in enzymatic synthesis of adipate esters. Various feedforward neural networks were performed using different learning algorithms. The best algorithm was

found to be Levenberg–Marquardt (LM) for a network composed of seven hidden nodes with hyperbolic tangent sigmoid transfer function. ANN showed better prediction ability compared to RSM. A high coefficient of determination (R^2) (>0.9) and a low mean absolute error (MAE) and root mean squared error (RMSE) for training, validating and testing data implied the good generalization of the developed models for predicting the reaction yield.

In order to develop an efficient enzyme catalyzed process, alcohol specificity of enzyme in terms of chain length and structure in the synthesis of adipate esters was determined. Methanol, n-butanol, octanol, dodecanol, octadecanol, isobutanol, sec-butanol and tert-butanol were the alcohols used for this study. The results demonstrated that alcohol chain length and structure were determining factors that affect the optimum condition of the reaction parameters for the enzymatic synthesis of adipate esters. Minimum reaction time for achieving maximum ester yield was obtained for isobutanol. The initial rates of synthesis of adipate esters for primary and secondary alcohols were nearly the same.

Kinetic study of the lipase-catalyzed adipate ester synthesis in solvent-based system was carried out as a preliminary step for future industrial scale bioreactor design. The reaction was found to obey the ping-pong bi-bi mechanism with alcohol inhibition. The coefficient of determination (R^2) and MAE values between the simulated and experimental initial rates were determined as 0.9904 and 2.4×10^{-4} which shows a good quality of fit between the simulated and experimental values.

In order to make the synthesis process more environmentally friendly and improve the productivity of the reactor to the highest amount, the reaction was performed in a solvent-free system using 0.5-L batch and 4-L continuous stirred tank reactors. Due to low solubility of the substrate and high viscosity of the reaction mixture, a continuous stirred tank reactor was used for continuous production of the ester. A high percentage conversion was achieved (about 96%) using minimum amount of enzyme (2.5%w/w) indicating the high efficiency of solvent free-system for synthesis of adipate ester. Continuous production of adipate ester was successfully performed with an average yield of 92.7% and high operation stability of enzyme for 28 hours, which is indicative of performing a successful process for the ester synthesis.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PERMODELAN DAN PENGOPTIMUMAN SINTESIS ESTER ADIPAT BERMANGKINKAN LIPASE MENGGUNAKAN KAEDAH RESPON PERMUKAAN DAN RANGKAIAN SARAF TIRUAN

Oleh

NAZ CHAIBAKHSH LANGROODI

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Keadaan optimum tindak balas esterifikasi asid adipik dengan pelbagai alkohol monohidrik bermangkin telah diperolehi menggunakan Novozyme 435 (*Candida antarctica* lipase B tersekatgerak pada resin akrilik). Sintesis ester adipat berasaskan pelarut telah dijalankan dalam skala kecil dengan menggunakan botol tertutup berisipadu 30 mL. Keadaan optimum tindak balas sintesis telah diperolehi melalui pendekatan Kaedah Respon Permukaan (RSM) berdasarkan kaedah komposit putaran pusat (CCRD) untuk menilai kesan interaktif parameter tindakbalas seperti suhu, masa, jumlah enzim dan nisbah molar alkohol/asid. Hasil peratusan yang tinggi (> 96,0%) menggunakan keadaan optimum telah diperolehi dengan menggunakan jumlah enzim yang minimum di mana ianya berpadanan dengan nilai yang dijangkakan.

Pendekatan Rangkaian Saraf Tiruan (ANN) juga digunakan untuk anggaran hasil esterifikasi dalam sintesis berenzim ester adipat. Pelbagai rangkaian saraf suapan terus

dilakukan dengan menggunakan algoritma pembelajaran yang berbeza. Algoritma terbaik adalah Levenberg-Marquardt (LM) untuk rangkaian yang terdiri daripada tujuh nod tersembunyi dengan fungsi pemindahan tangen hiperbolik sigmoid. ANN menunjukkan keupayaan ramalan yang lebih baik berbanding dengan RSM. Nilai penentuan koefisien yang tinggi (R^2) (> 0.9) dan nilai ralat mutlak (MAE) dan sisihan punca min kuasa dua (RMSE) yang rendah untuk latihan, pengesahan dan data pengujian secara keseluruhannya menghasilkan model yang dapat dikembangkan untuk menjangka hasil tindak balas.

Dalam usaha untuk mengembangkan proses pemangkinan berenzim yang cekap, spesifikasi alkohol terhadap enzim dari segi panjang rantai dan struktur dalam sintesis ester adipat telah ditentukan. Dalam kajian ini, metanol, n-butanol, oktanol, dodekanol, oktadekanol, isobutanol, sekunder-butanol dan tertier-butanol adalah alkohol yang digunakan. Hasil menunjukkan rantai panjang dan struktur alkohol merupakan faktor yang mempengaruhi keadaan optimum parameter tindak balas sintesis berenzim ester adipat. Masa minimum tindak balas bagi mencapai hasil ester maksimum telah diperolehi dengan isobutanol. Kadar permulaan sintesis ester adipat untuk alkohol primer dan sekunder adalah hampir sama.

Kajian kinetik sintesis ester adipat bermangkinkan enzim lipase telah dilakukan sebagai langkah awal untuk penggunaan bioreaktor berskala industri pada masa hadapan. Tindak balas ini didapati mematuhi mekanisma ping-pong bi-bi dengan perencatan alkohol. Penentuan nilai koefisien (R^2) dan nilai-nilai MAE di antara simulasi dan kadar awal

kajian telah ditentukan sebagai 0.9904 dan 2.4×10^{-4} yang menunjukkan kualiti yang baik dan sesuai di antara nilai simulasi dan nilai sebenar eksperimen.

Bagi mencapai proses sintesis yang lebih mesra alam dan meningkatkan produktiviti reaktor kepada jumlah tertinggi, tindak balas telah dilakukan dalam suatu sistem yang bebas pelarut menggunakan isipadu bekerja 0.5-L dan 4-L menggunakan tangki reaktor berpengaduk secara berterusan. Oleh kerana kelarutan substrat yang rendah dan kelikatan campuran tindak balas yang tinggi, reaktor tangki pengacauan berterusan telah digunakan untuk penghasilan ester secara berterusan. Peratusan penukaran ester yang tinggi telah dicapai (sekitar 96%) menggunakan jumlah enzim minimum (2.5% w/w) di mana ianya menunjukkan keberkesanan yang tinggi melalui sistem bebas pelarut bagi sintesis ester adipat. Penghasilan berterusan produk ester adipat telah berjaya dijalankan dengan hasil purata 92.7% dan kestabilan operasi enzim yang tinggi selama 28 jam, yang merupakan penunjuk kepada proses sintesis ester yang berjaya.

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I certify that a Thesis Examination Committee has met on 24 March 2010 to conduct the final examination of Naz Chaibakhsh Langroodi on her thesis entitled "Modeling and Optimization of Lipase-Catalyzed Synthesis of Adipate Esters using Response Surface Methodology and Artificial Neural Network" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the Degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Date: 10 June 2010

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

NAZ CHAIBAKHSH LANGROODI

Date: 22 June 2010

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PVC	Poly Vinyl Chloride
RSM	Response Surface Methodology
ANN	Artificial Neural Network
AEs	Adipic acid di-Esters
VOCs	Volatile Organic Compounds
SFS	Solvent-Free System
S	Substrate
P	Product
E	Free Enzyme
E_m	Modified Enzyme
EA	Enzyme-Acid Complex
TAG	Triacylglycerol
FFA	Free Fatty Acids
OLS	Ordinary Least Square
CCRD	Central Composite Rotatable Design
PPL	Porcine Pancreas Lipase
KDD	Knowledge Discovery in Databases
STR	Stirred Tank Reactor
CSTR	Continuous Stirred Tank Reactor
CISTR	Continuous Ideal Stirred Tank Reactor
CPBR	Continuous Packed Bed Reactor