UNIVERSITI PUTRA MALAYSIA

KINETICS, MODELLING AND SCALING-UP OF KOJIC ACID FERMENTATION BY ASPERGILLUS FLAVUS 44-1 USING DIFFERENT CARBON SOURCES

ROSFARIZAN MOHAMAD

KINETICS, MODELLING AND SCALING-UP OF KOJIC ACID FERMENTATION BY *ASPERGILLUS FLAVUS* 44-1 USING DIFFERENT CARBON SOURCES

ROSFARIZAN MOHAMAD

DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA

2000

KINETICS, MODELLING AND SCALING-UP OF KOJIC ACID FERMENTATION BY *ASPERGILLUS FLAVUS* 44-1 USING DIFFERENT CARBON SOURCES

By

ROSFARIZAN MOHAMAD

Thesis Submitted in Fulfilment of the Requirement for the Degree of Doctor of Philosophy in the Faculty of Food Science and Biotechnology Universiti Putra Malaysia

December 2000

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

KINETICS, MODELLING AND SCALING-UP OF KOJIC ACID FERMENTATION BY *ASPERGILLUS FLAVUS* 44-1 USING DIFFERENT CARBON SOURCES

BY

ROSFARIZAN MOHAMAD

December 2000

Chairman : Assoc. Prof. Dr. Arbakariya Ariff

Faculty : Food Science and Biotechnology

Kojic acid production by *Aspergillus flavus* strain 44-1 using different types of carbon source (glucose, starch hydrolysate from enzymatic hydrolysis of sago starch, sucrose, fructose and gelatinized sago starch) was carried out in 250 mL shake flask, 2 L, 8 L and 50 L stirred tank fermenters. The experimental data from batch fermentation and resuspended cell system were analysed in order to form the basis for a kinetic model of the process. Unstructured model based on logistic and Luedeking-Piret equations was found suitable to describe growth, substrate consumption and kojic acid production by *Aspergillus flavus* in batch and also resuspended cell system using either glucose or sucrose. From the modelling, it was found that kojic acid production by *A. flavus* was non-growth associated process. The kinetic parameter values for each fermenter were calculated from the modelling

and they can be used to verify the experimental data using various types and concentration of carbon source.

Kojic acid production (23.5 g/L) using 100 g/L sago starch in a shake flask was comparable to fermentation of glucose (32.5 g/L) and starch hydrolysate (27.9 g/L) but in the 8 L and 50 L fermenter kojic acid production was greatly reduced due to non-optimal aeration conditions. Fed-batch fermentation with intermittent feeding of concentrated sago starch (140 g/L) can be employed to improve direct fermentation of sago starch to kojic acid by about 4 times higher as compared to batch fermentation. *A. flavus* was also capable to utilise sucrose for kojic acid fermentation where the highest production (40.23 g/L) in 2 L fermenter was obtained at 150 g/L sucrose. Kojic acid production (10.25 g/L) was greatly reduced in fermentation using fructose as the sole carbon source. Scaling-up based on a constant impeller tip speed (1.65 m/s) together with optimal DOT and pH control strategies was successfully used for kojic acid fermentation in 50 L fermenter using glucose and sucrose as carbon sources.

Kojic acid fermentation by *A. flavus* can be divided into two phases; growth and production phase. The culture pH during growth phase influenced the performance of kojic acid fermentation to a further extend than did the pH during the production phase. The fermentation without pH controlled (started with an initial culture pH 3) showed higher kojic acid production than single-phase pH controlled fermentation at a range of pH 2.2 – 4.0. Comparable kojic acid production to fermentation without pH controlled was obtained in two-phase pH controlled fermentation (started with initial culture pH, without control during growth phase and switched to 3 during production phase).

Efficient conversion of glucose to kojic acid was achieved in a resuspended cell system, in a solution containing only glucose with citrate buffer at pH 3.5 and 30°C. The rate of glucose conversion to kojic acid was increased with increasing glucose concentration up to 100 g/L, suggesting that the biotransformation of glucose to kojic acid by the cell-bound enzymes followed the Michaelis-Menten enzyme kinetic models. The value of K_m and V_{max} for the reaction, as determined by using Langmuir plot, was 10.042 g/L glucose and 0.076 g/L.h, respectively.

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

KINETIK, PERMODELAN DAN PENINGKATAN SKALA FERMENTASI ASID KOJIK OLEH *ASPERGILLUS FLAVUS* 44-1 MENGGUNAKAN SUMBER-SUMBER KARBON YANG BERBEZA

oleh

ROSFARIZAN MOHAMAD

Disember 2000

Pengerusi : Assoc. Prof. Dr. Arbakariya Ariff

Fakulti : Sains Makanan dan Bioteknologi

Penghasilan asid kojik oleh *Aspergillus flavus* strain 44-1 menggunakan pelbagai sumber karbon (glukosa, hidrolisat kanji daripada hidrolisis berenzim kanji sagu, sukrosa, fruktosa dan kanji sagu) telah dijalankan menggunakan kelalang bergoncang 250 mL dan pelbagai saiz fermenter berpengaduk (2 L, 8 L dan 50 L). Data-data eksperimen daripada proses fermentasi sesekelompok dan sistem sel terampai telah dianalisa untuk membentuk asas bagi model proses kinetik. Model tidak berstruktur berdasarkan persamaan-persamaan logistik dan Luedeking-Piret didapati sesuai untuk menerangkan pertumbuhan *Aspergillus flavus*, penggunaan substrat dan penghasilan asid kojik dalam sistem sesekelompok dan sel terampai menggunakan glukosa ataupun sukrosa. Daripada permodelan, penghasilan asid

kojik oleh *A. flavus* telah ditunjukkan sebagai proses pertumbuhan tidak berkait. Nilai-nilai parameter kinetik bagi setiap proses fermentasi telah dikira daripada permodelan dan ianya boleh digunakan untuk menguji benar tidaknya data eksperimen menggunakan pelbagai jenis dan kepekatan sumber-sumber karbon.

Penghasilan asid kojik (23.5 g/L) menggunakan 100 g/L kanji sagu di dalam kelalang bergoncang adalah setara dengan fermentasi menggunakan glukosa (32.5 g/L) dan hidrolisat kanji (27.9 g/L) tetapi di dalam fermenter 8 dan 50 L, penghasilan telah berkurang kerana keadaan pengudaraan yang tidak optima. Fermentasi suapan sesekelompok dengan penambahan bersela kanji sagu pekat (140 g/L) boleh digunakan untuk meningkatkan prestasi fermentasi asid kojik menggunakan kanji sagu sebanyak empat kali ganda lebih tinggi berbanding dengan fermentasi sesekelompok. *A. flavus* juga berupaya menggunakan sukrosa bagi fermentasi asid kojik di mana penghasilan tertinggi (40.23 g/L) di dalam fermenter 2 L diperolehi pada kepekatan sukrosa 150 g/L. Penghasilan asid kojik (10.25 g/L) menurun dengan banyaknya bagi fermentasi menggunakan fruktosa sebagai sumber karbon. Peningkatan skala berdasarkan halaju hujung pengaduk yang tetap (1.65 m/s) bersama-sama strategi kawalan kepekatan oksigen terlarut dan pH yang optima telah berjaya digunakan untuk fermentasi asid kojik dalam 50 L fermenter menggunakan glukosa dan sukrosa sebagai sumber karbon.

Fermentasi asid kojik boleh dibahagikan kepada dua fasa, fasa pertumbuhan dan fasa penghasilan. pH kultur semasa fasa pertumbuhan lebih banyak mempengaruhi proses fermentasi asid kojik berbanding pH semasa fasa penghasilan. Fermentasi tanpa kawalan pH (dimulakan dengan pH 3) menunjukkan penghasilan asid kojik yang lebih tinggi berbanding fermentasi kawalan pH satu fasa pada julat pH 2.2 – 4.0. Penghasilan asid kojik yang setara dengan fermentasi tanpa kawalan pH telah diperolehi bagi fermentasi kawalan pH dua-fasa (dimulakan dengan pH 3, tanpa kawalan pH semasa fasa pertumbuhan dan ditukarkan kepada pH 3 semasa fasa penghasilan).

Penukaran glukosa kepada asid kojik yang berkesan telah diperolehi dalam sistem sel terampai, dalam larutan mengandungi hanya glukosa dengan penimbal sitrat pada pH 3.0 dan suhu 30^{o}C. Kadar pertukaran glukosa kepada asid kojik adalah meningkat dengan peningkatan kepekatan glukosa sehingga 100 g/L, menunjukkan yang proses biotransformasi ini mengikuti model enzim kinetik Michaelis-Menten. Untuk tindakbalas ini, nilai K_m adalah 10.042 g/L glukosa dan nilai V_{max} adalah 0.076 g/L.j, seperti yang ditentukan menggunakan plot Langmuir.

ACKNOWLEDGEMENTS

All praise to Allah S. W. T. who has showered me with kindness and affection during the course of my study that I cannot adequately thank for. His endless grace and love have provided me with the strength to finish this study.

I wish to express my deepest appreciation, honour and gratitude to my supervisor, Associate Professor Dr. Arbakariya Bin Ariff for his invaluable guidance, constant encouragement and constructive suggestions throughout the course of this study. My appreciation and gratitude also go to the members of my supervisory committee, Professor Dr. Mohamed Ismail Abdul Karim and Associate Professor Dr. Mohd. Ali Hassan for their guidance, valuable comments and encouragement throughout this study.

Sincere thanks are also extended to Professor Dr. Suteaki Shioya, Associate Prof. Dr. Hiroshi Shimizu and members of Ecosystem Technology Laboratory, Graduate School of Engineering, Osaka University, Japan for their assistance and help in setting up the 8 L fed-batch fermenter. Thanks also extended to the Government of Malaysia and Universiti Putra Malaysia for 3 years PASCA fellow sponsorship offered.

Heartfelt appreciation is also due to all faculty members, staffs and fellow graduate and undergraduate students of Department of Biotechnology, Faculty of

Food Science and Biotechnology for their kindly co-operation and assistance during the period of this study.

Last but not least, a special appreciation and gratitude to all my family members and friends for their understanding, caring and moral support. My deepest appreciation is recorded to my sister for her enormous support and sacrifices that given during the period of this study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

α	Growth-associated rate constant for glucose consumption (g glucose/g cell)
β	Non-growth-associated rate constant for glucose consumption (g glucose/g cell.h)
C/N	Carbon to nitrogen ratio of medium in mM basis
D	Dilution rate
D_i	Impeller diameter
DOT	Dissolved oxygen tension
μ_{max}	Maximum or initial specific growth rate (h^{-1})
m	Growth associated rate constant for kojic acid production (g kojic acid/g cell)
n	Non-growth associated rate constant for kojic acid production (g kojic acid/g cell.h)
P_o	Initial kojic acid concentration (g/L)
P	Kojic acid concentration (g/L)
P_{max}	Maximum kojic acid concentration (g/L)
S_o	Initial substrate concentration (g/L)
S	Substrate concentration (g/L)
t	Time (h)
X	Cell concentration (g/L)
X_o	Initial cell concentration (g/L)
X_{max}	Maximum cell concentration (g/L)
$Y_{p/s}$	Yield of kojic acid based on glucose consumed (g/g)