KINETICS OF RED-PIGMENT FERMENTATION BY *MONASCUS PURPUREUS* FTC 5391 IN SHAKE FLASK CULTURE AND STIRRED TANK FERMENTER USING DIFFERENT CARBON AND NITROGEN SOURCES

By

MUSAALBAKRI ABDUL MANAN

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

March 2005

The microbe is so very small
You cannot make him out at all
But many sanguine people hope
To see him through a microscope

His jointed tongue that lies beneath
A hundred curious rows of teeth
His seven tufted tails with lots of lovely pink and purple spots
On each of which a pattern stands
Composed of forty separate bands
His eyebrows of a tender green
All these have never yet been seen
But Scientist who ought to know
Assure us that they must be so

Oh, let us never, never doubt
What nobody is sure about

Hilaire Belloc
1972

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

KINETICS OF RED-PIGMENT FERMENTATION BY *MONASCUS PURPUREUS* FTC 5391 IN SHAKE FLASK CULTURE AND STIRRED TANK FERMENTER USING DIFERENT CARBON AND NITROGEN SOURCES

By

MUSAALBAKRI ABDUL MANAN

March 2005

Chairman : Professor Arbakariya Ariff, PhD

Institute : Bioscience

A newly isolated red-pigment producing fungus from local source was identified using Microbial Identification System based on fatty acids profiles. Growth morphology and structure of the fungus were also identified using Scanning Electron Microscope. Monospore isolation technique was performed to obtain the improved strain that has high and consistent ability to produce red pigment. Kinetics of red-pigment fermentation by this local strain, identified as *Monascus purpureus* FTC 5391, in 500 mL shake flask culture and 2 L stirred tank fermenter using different carbon and nitrogen sources were also investigated.

From scanning electron microscope examination, *M. purpureus* FTC 5391 exhibited reproduction characteristics by sexually formation of cleistothecium with ascospores and also asexual formation of conidia. The ability of *M. purpureus* FTC 5391 wild

strain in producing red pigment was successfully improved using monospore isolation technique. By using this approach of improvement, three different monospore isolates of *M. purpureus* FTC 5391 (MP 3, MP 4 and MP 5) were obtained as the best red-pigment producers when glucose, potato starch and rice starch were used as carbon source, respectively.

For subsequent experiments on the development of red-pigment fermentation using glucose as a basal medium, *M. purpureus* FTC 5391 MP 3 was employed. Among the different nitrogen sources investigated ($(NH_4)_2HPO_4$, $(NH_4)H_2PO_4$, $NaNO_3$, NH_4NO_3, $(NH_4)_2SO_4$, $(NH_4)_2S_2O_8$, $(NH_4)Cl$, peptone, yeast extract, monosodium glutamate, urea and tryptone, where monosodium glutamate was found to be the preferred nitrogen source for growth of *M. purpureus* FTC 5391 and red pigment production. From medium optimization studies, medium consisted of 50 g/L glucose and 12 g/L of monosodium glutamate with the addition of trace elements [K_2HPO_4 (2.5 g/L), KH_2PO_4 (2.5 g/L), $MgSO_4.7H_2O$ (1.0 g/L), KCl, (0.5 g/L), $ZnSO_4.7H_2O$ (0.01 g/L), $FeSO_4.7H_2O$ (0.01 g/L) and $MnSO_4.H_2O$ (0.03g/L)] was found optimal for red-pigment production by *M. purpureus* FTC 5391. On the other hand, optimal fermentation conditions for red pigment production in 2 L stirred tank fermenter were obtained at initial pH 6.5 with agitation speed of 600 rpm, and dissolved oxygen tension (DOT) was maintained at high level (>30% saturation) throughout the fermentation.

The experimental data from batch fermentation were analysed in order to form a kinetic model of the process. The unstructured model based on logistic and Leudeking-Piret equations was found suitable to describe growth, substrate consumption and red pigment production by *M. purpureus* FTC 5391. The maximum specific growth rate (μ_{max}) of 0.055 h^{-1} and 0.065 h^{-1} were obtained from simulated modeling of *M. purpureus* FTC 5391 during growth in shake flask and 2 L stirred tank fermenter, respectively. The maximum red pigment and cell concentrations obtained in batch fermentation using 2 L stirred tank fermenter (20.63 UA_{500} and 13.2 g/L) and using shake flask (9.26 UA_{500} and 11.425 g/L) with overall productivity (P) was 0.122 UA_{500}/h and 0.055 UA_{500}/h, respectively. The production of red-pigment by *M. purpureus* FTC 5391 appeared to be a non-growth associated process; where by rapid red-pigment production occurred during non-growth phase after the depletion of glucose in the medium and the on-set of ethanol accumulation. It seemed that the red-pigment was formed from the metabolism of ethanol accumulated in the culture.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KINETIK FERMENTASI PIGMEN MERAH OLEH *MONASCUS PURPUREUS* FTC 5391 DI DALAM KULTUR KELALANG BERGONCANG DAN FERMENTER BERPENGADUK MENGGUNAKAN SUMBER-SUMBER KARBON DAN NITROGEN YANG BERBEZA

Oleh

MUSAALBAKRI ABDUL MANAN

Mac 2005

Pengerusi : Profesor Arbakariya Ariff, PhD

Institut : Biosains

Sistem Pengenalpastian Mikrob yang berdasarkan kepada profil asid lemak telah digunakan bagi mengenalpasti fungus pengeluar pigmen merah yang diasingkan daripada sumber tempatan. Morfologi pertumbuhan dan struktur fungus tersebut telah dikenalpasti dengan menggunakan Mikroskop Scanning Elektron. Teknik pengasingan monospora juga telah dibangunkan bagi mendapatkan strain yang mempunyai keupayaan yang tinggi untuk penghasilan pigmen merah. Kinetik fermentasi pigmen merah dari strain tempatan yang telah dikenali sebagai *Monascus purpureus* FTC 5391 di dalam kultur kelalang bergoncang berkapasiti 500 mL dan fermenter berpengaduk bersaiz 2 L telah dijalankan dengan menggunakan sumber-sumber karbon dan nitrogen yang berbeza.

Dari kajian mikroskop scanning electron, didapati bahawa *M. purpureus* FTC 5391 menunjukkan penghasilan secara seksual iaitu melalui pembentukan "cleistothecium" bersama askospora dan juga secara aseksual melalui pembentukan konidia. Teknik pengasingan spora ini telah berjaya mempertingkatkan keupayaan strain liar *M. purpureus* FTC 5391 dalam penghasilan pigmen merah. Dengan kaedah ini, tiga (3) jenis monospora (MP 3, MP 4 dan MP 5) telah berjaya diasingkan sebagai penghasil pigmen merah terbaik apabila glukosa, kanji kentang dan kanji beras digunakan sebagai sumber karbon utama.

Eksperimen seterusnya dijalankan dengan menggunakan medium berasaskan glukosa dan monospora 3 (MP 3) *M. purpureus* FTC 539. Dari kajian penggunaan sumber nitrogen yang berbeza ($(NH_4)_2HPO_4$, $(NH_4)H_2P)_4$, $NaNO_3$, NH_4NO_3, $(NH_4)_2SO_4$, $(NH_4)_2S_2O_8$, $(NH_4)Cl$, peptone, ekstrak yis, monosodium glutamate, urea dan tryptone), didapati monosodium glutamate merupakan sumber nitrogen terbaik bagi pertumbuhan dan penghasilan pigmen merah oleh *M. purpureus* FTC 5391. Hasil kajian pengoptimuman medium menunjukkan medium asas yang menggunakan 50 g/L glukosa dan 12 g/L monosodium glutamate dengan penambahan unsur-unsur surih [K_2HPO_4 (2.5 g/L), KH_2PO_4 (2.5 g/L), $MgSO_4.7H_2O$ (1.0 g/L), KCl, (0.5 g/L), $ZnSO_4.7H_2O$ (0.01 g/L), $FeSO_4.7H_2O$ (0.01 g/L) dan $MnSO_4.H_2O$ (0.03g/L)] adalah keadaan medium optima untuk penghasilan pigmen merah oleh *M. purpureus* FTC 5391. Keadaan fermentasi yang optima untuk penghasilan pigmen merah di dalam fermenter berpengaduk 2 L pula telah diperolehi dengan pH permulaan 6.5 dengan

kadar pemutaran 600 rpm, dan kepekatan oksigen terlarut dikekalkan pada kadar yang tertinggi (> 30% tepu) sepanjang proses fermentasi.

Daripada data eksperimen proses fermentasi sesekelompok, satu model kinetik proses telah dibentuk. Model tidak berstruktur ini berdasarkan persaman Logistik dan Leudeking-Piret didapati sesuai untuk menerangkan pertumbuhan, penggunaan substrat dan penghasilan pigmen merah dalam sistem sesekelompok oleh *M. purpureus* FTC 5391. Nilai kadar pertumbuhan spesifik (μ) iaitu 0.055 j^{-1} dan 0.065 j^{-1} diperolehi dari model pertumbuhan *M. purpureus* FTC 5391 dalam kelalang bergoncang 500 mL dan fermenter berpengaduk 2 L. pigmen merah dan kepekatan sel maksimum juga diperolehi dalam fermentasi sesekelompok menggunakan fermenter berpengaduk 2 L (20.63 UA_{500} and 13.2 g/L) dan menggunakan kelalang bergoncang (9.26 UA_{500} and 11.425 g/L) dengan produktiviti keseluruhan (P) 0.122 UA_{500}/j dan 0.055 UA_{500}/j, masing-masing. Penghasilan pigmen merah oleh *M. purpureus* FTC 5391 ditunjukkan sebagai proses pertumbuhan tidak berkait, di mana penghasilan pigmen merah lebih pantas semasa fasa tiada pertumbuhan iaitu selepas glukosa di dalam kultur semakin berkurangan dan etanol bertambah. Ianya seolah-olah pigmen merah terbentuk daripada kesan metabolisma ke atas etanol di dalam kultur.

ACKNOWLEDGEMENTS

All praise to Allah S.W.T. who has showed 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 strengthen to finish the study.

I wish to express my deepest appreciation, honour and gratitude to my supervisor, *Professor Dr Arbakariya Ariff* for his invaluable guidance, constant encouragement and constructive suggestions throughout the course of this study. My appreciations and gratitude also go to the members of my supervisory committee, **Dr Rosfarizan Mohamad** and **Professor Dr Mohammed Ismail Abdul Karim** for their guidance, valuable comments and encouragement throughout this study.

To the Malaysian Agricultural Research and Development Institute (MARDI) for providing me an opportunity to further study and financial support during my study.

Heartfelt appreciation is also due to all faculty members, staffs and fellow graduate students of Fermentation Technology Unit, Institute of Bioscience for their kindly co-operation and assistance during the period of this study.

Finally, a special appreciation and deepest gratitude is extended to my family for their love and support to encourage me to continue my endeavor, and to make this step in my life possible. Without love and support no work can be done. This work is dedicated to *Emak, Bapa, Adik Mala,* and *Adik Sarah,. Thank You.*

I certify that an Examination Committee met on 2nd March, 2005 to conduct the final examination of Musaalbakri Abdul Manan on his Master of Science thesis entitled "Kinetics of Red-Pigment Fermentation by *Monascus purpureus* FTC 5391 in Shake Flask Culture and Stirred Tank Fermenter using Different Carbon and Nitrogen Sources" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidates be awarded the relevant degree. Members of the Examination Committee are as follows:

Norhani Abdullah, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Ling Tau Chuan, PhD
Faculty of Engineering
Universiti Putra Malaysia
(Member)

Radzali Muse, PhD
Associate Professor
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

Mohd Sahaid Hj Kalil, PhD
Associate Professor
Faculty of Engineering
Universiti Kebangsaan Malaysia
(Independent Examiner)

_____
GULAM RUSUL RAHMAT ALI, PhD
Professor / Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

Arbakariya Ariff, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Rosfarizan Mohamad, PhD
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Mohammed Ismail Abdul Karim, PhD
Professor
Department of Biotechnology Engineering
Faculty of Engineering
International Islamic University Malaysia
(Member)

_____
AINI IDERIS, PhD
Professor / Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I hereby declare that the 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 currently submitted for any other degree at Universiti Putra Malaysia or other institutions.

_____

MUSAALBAKRI ABDUL MANAN

Date:

TABLE OF CONTENTS