

**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

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**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

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March 2005

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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 ((NH₄)₂HPO₄, (NH₄)H₂PO₄, NaNO₃, NH₄NO₃, (NH₄)₂SO₄, (NH₄)₂S₂O₈, (NH₄)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 [K₂HPO₄ (2.5 g/L), KH₂PO₄ (2.5 g/L), MgSO₄.7H₂O (1.0 g/L), KCl, (0.5 g/L), ZnSO₄.7H₂O (0.01 g/L), FeSO₄.7H₂O (0.01 g/L) and MnSO₄.H₂O (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 UA₅₀₀ and 13.2 g/L) and using shake flask (9.26 UA₅₀₀ and 11.425 g/L) with overall productivity (P) was 0.122 UA₅₀₀/h and 0.055 UA₅₀₀/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

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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 ((NH₄)₂HPO₄, (NH₄)₂H₂P₄, NaNO₃, NH₄NO₃, (NH₄)₂SO₄, (NH₄)₂S₂O₈, (NH₄)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 [K₂HPO₄ (2.5 g/L), KH₂PO₄ (2.5 g/L), MgSO₄.7H₂O (1.0 g/L), KCl, (0.5 g/L), ZnSO₄.7H₂O (0.01 g/L), FeSO₄.7H₂O (0.01 g/L) dan MnSO₄.H₂O (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 persamaan 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) $0.122 \text{ UA}_{500}/\text{j}$ dan $0.055 \text{ UA}_{500}/\text{j}$, 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 metabolisme 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 strength 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:

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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.

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TABLE OF CONTENTS

	Page
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL DECLARATION	xii
LIST OF TABLES	xvii
LIST OF FIGURES	xix
LIST OF ABBREVIATIONS	xxiii
CHAPTER	
1. INTRODUCTION	1
2. LITERATURE REVIEW	8
2.1 The Microorganism: <i>Monascus purpureus</i>	8
2.2 Pigments	9
2.3 Production	10
2.3.1 Solid State Cultivation	11
2.3.2 Submerged Cultivation	15
Effect of carbon source	15
Effect of nitrogen source	19
Effect of pH	23
Effect of other medium components	25
Effect of inoculum size	27
Effect of temperature	28
Effect of agitation and aeration	31
Effect of other physical factors	32
2.3.3 Cultivation Medium for Red Pigment Production	34
2.3.4 Inoculant Production in Submerged Culture	41
2.4 Pigment Analysis Methods and Amounts Produced	43
2.5 Batch Culture	45
2.6 Concluding Remarks	47
3. GENERAL MATERIALS AND METHODS	49
3.1 The Microorganism	49
3.2 Inoculum Preparation	51
3.3 Fermentation	51
3.4 Experimental Plan	53

3.5	Fermenter	56
3.6	Analytical Procedures	59
	3.6.1 Determination of Cell Concentration	59
	3.6.2 Glucose and Other Reducing Sugar Analysis	59
	3.6.3 Determination of Red Pigment	60
	3.6.4 Determination of Ethanol	61
	3.6.5 Determination of Residual Nitrogen	61
3.7	Statistical Analysis	62
4.	IDENTIFICATION AND CHARACTERIZATION OF MORPHOLOGY AND STRUCTURE OF NEWLY ISOLATED RED-PIGMENT-PRODUCING FUNGUS (<i>MONASCUS PURPUREUS</i> FTC 5391)	63
4.1	Introduction	63
4.2	Microorganism and Maintenance	64
4.3	Strain Identification	65
4.4	Examination of Structure and Morphology	66
4.5	Results and Discussion	67
	4.5.1 Strain Identification using MIS Method	67
	4.5.2 Structure and Growth Morphology	69
5.	IMPROVEMENT OF RED-PIGMENT PRODUCING FUNGAL STRAIN (<i>MONASCUS PURPUREUS</i> FTC 5391) USING MONOSPORE ISOLATION TECHNIQUE	76
5.1	Introduction	76
5.2	Materials and Methods	78
	5.2.1 Strain	78
	5.2.2 Monospore Isolation	78
	5.2.3 Media Composition, Inoculum Preparation and Fermentation	79
5.3	Analytical Determination	80
	5.3.1 Determination of Cell Concentration	80
	5.3.2 Determination of Starch	81
	5.3.3 Determination of Glucose	81
	5.3.4 Determination of Red Pigment	82
	5.3.5 Determination of α -amylase Activity	82
	5.3.6 Determination of Glucoamylase Activity	82
5.4	Calculation of Growth Kinetic Parameter Values	84
	5.4.1 Abbreviation	86
5.5	Statistical Analysis	86
5.6	Results	86
	5.6.1 Fermentation using Glucose as a Carbon Source	87
	5.6.2 Fermentation using Starch as a Carbon Source	87
5.7	Discussion	93
5.8	Conclusions	95

6.	NITROGEN AND TRACE ELEMENTS REQUIREMENT FOR GROWTH OF <i>MONASCUS PURPUREUS</i> FTC 5391 AND RED PIGMENT PRODUCTION	96
6.1	Introduction	96
6.2	Materials and Methods	98
6.2.1	Strain and Media	98
6.2.2	Shake Flask Culture	98
6.2.3	Analytical Determination	99
6.3	Statistical Analysis	99
6.4	Results and Discussion	100
6.4.1	Effect of Initial pH in Submerged Fermentation	100
6.4.2	Effect of Inoculum Size in Submerged Fermentation	104
6.4.3	Effect of Nitrogen Source in Submerged Fermentation	107
6.4.4	Effect of Different Concentrations of Monosodium Glutamate	113
6.4.5	Effect of Trace Elements	117
6.5	Conclusions	120
7.	KINETICS AND MODELLING OF RED PIGMENT FERMENTATION IN 2 L STIRRED TANK FERMENTER USING DIFFERENT TYPES AND CONCENTRATIONS OF CARBON SOURCE	121
7.1	Introduction	121
7.2	Materials and Methods	124
7.2.1	Microorganism and Medium	124
7.2.2	Fermentations	124
7.2.3	Analytical Methods	125
7.2.4	Development of Mathematical Model	126
7.2.5	Mathematical Method	134
7.3	Results and Discussion	135
7.3.1	Comparison of Batch Fermentation of <i>M. purpureus</i> FTC 5391	135
	Shake flask	135
	2 L Stirred tank fermenter	137
7.3.2	Effect of Different Carbon Sources	142
7.3.3	Effect of Glucose Concentration	147
7.3.4	Relationship Between Red Pigment Production and Ethanol Accumulation	151
7.4	Conclusions	152
8.	AERATION AGITATION STRATEGIES FOR IMPROVEMENT OF RED PIGMENT PRODUCTION BY <i>MONASCUS PURPUREUS</i> FTC 5391	154
8.1	Introduction	154

8.2	Materials and Methods	157
	8.2.1 Microorganism and Medium	157
	8.2.2 Fermentation in 2 L Stirred Tank Bioreactor	157
	8.2.3 Analytical Methods	158
	8.2.4 Mathematical Model	160
8.3	Results and Discussion	162
	8.3.1 Effect of Agitation Speed	162
	8.3.2 Effect of Aeration Rate	171
8.4	Conclusions	178
9.	GENERAL DISCUSSION, CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK	180
	BIBLIOGRAPHY	189
	APPENDICES	200
	BIODATA OF THE AUTHOR	216