

CONTENTS

	Page
ACKNOWLEDGEMENT	iii
ABSTRACT IN THAI	iv
ABSTRACT IN ENGLISH	vii
CONTENTS	x
LIST OF TABLES	xv
LIST OF FIGURES	xviii
ABBREVIATIONS AND SYMBOLS	xxi
STATEMENTS OF ORIGINALITY IN THAI	xxiv
STATEMENTS OF ORIGINALITY IN ENGLISH	xxv
CHAPTER 1	1
Introduction	
1.1 Statement and significant of the study	1
1.2 Objectives	3
CHAPTER 2	4
Literature review	
2.1 Biodiesel production	4
2.2 Crude glycerol from biodiesel production	6
2.3 Bioconversion of crude glycerol into high value products by oleaginous red yeast	9
2.4 Lipids production	10
2.4.1 Lipids accumulation and fatty acid synthesis in oleaginous red yeast	10
2.4.2 Lipids compositions in oleaginous red yeast	12

CONTENTS (CONTINUED)

	Page
2.5 Carotenoids production	16
2.5.1 Carotenoids biosynthesis in oleaginous red yeast	19
2.5.2 Carotenoids compositions in oleaginous red yeast	21
2.6 Optimization of carotenoids and lipids productions by oleaginous red yeast	22
2.6.1 Optimization of medium composition and cultural conditions in shaking flask level	23
2.6.2 Scale up of carotenoids production in bioreactor	27
CHAPTER 3	30
Screening of oleaginous red yeast producing lipids and carotenoids and its optimization production by using crude glycerol as carbon source	
3.1 Introduction	30
3.2 Materials and methods	33
3.2.1 Microorganisms	33
3.2.2 Inoculum preparation	33
3.2.3 Raw materials	33
3.2.4 Screening of carotenoids and lipids producing oleaginous red yeasts	34
3.2.5 Effect of impurity in crude glycerol	34
3.2.6 Screening of factors affecting on carotenoids and lipids productions	35
3.2.7 Optimization of significant variables using response surface methodology (RSM)	36
3.2.8 Analytical methods	38
3.3 Results and discussion	40
3.3.1 Screening of carotenoids and lipids producing oleaginous red yeasts	40
3.3.2 Effect of methanol in crude glycerol on lipids and carotenoids productions from <i>Spolobomyces pararoseus</i> TISTR5213	42
3.3.3 Screening of significant variables using the Plackett-Burman design	44

CONTENTS (CONTINUED)

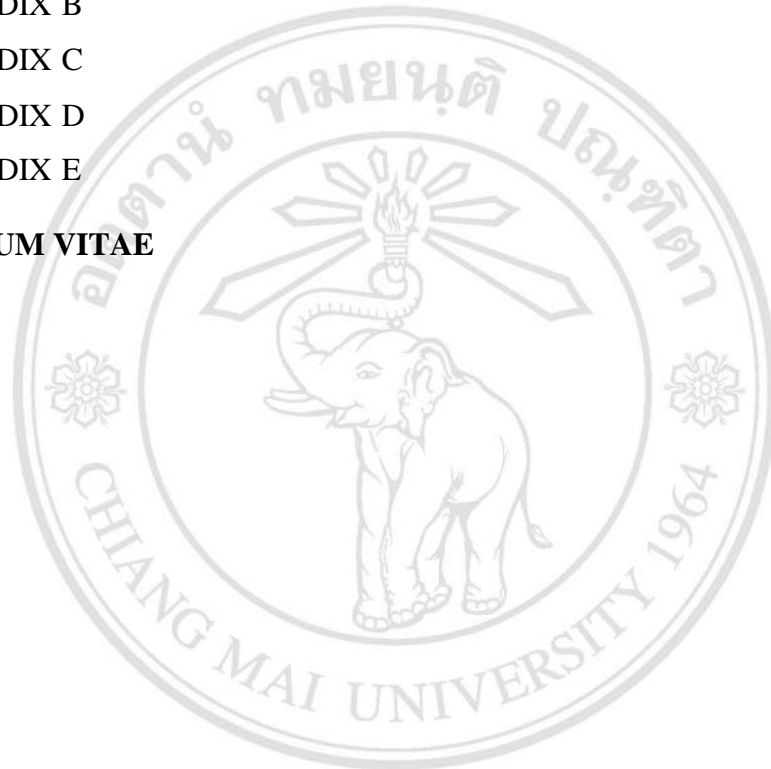
	Page
3.3.4 Optimization of significant variables using response surface methodology (RSM)	52
3.3.5 Validation of CCD optimization model	73
3.4 Conclusions	76
CHAPTER 4	77
Effect of additive agent on carotenoids and lipids productions by <i>Sporidiobolus pararoseus</i> KM281507	
4.1 Introduction	77
4.2 Material and methods	79
4.2.1 Identification of oleaginous red yeast strain	79
4.2.2 Microorganisms and medium	80
4.2.3 Raw materials	80
4.2.4 Effect of additive agents	81
4.2.5 Analytical methods	81
4.3 Results and discussion	83
4.3.1 Identification of oleaginous red yeast strain	83
4.3.2 Effect of additive agents	85
4.3.3 Fatty acid composition	89
4.4 Conclusions	91
CHAPTER 5	92
Bioconversion of crude glycerol into lipids and carotenoids by <i>Sporidiobolus pararoseus</i> KM281507 in an airlift bioreactor	
5.1 Introduction	92
5.2 Material and methods	93
5.2.1 Microorganism and culture conditions	93
5.2.2 Bioconversion of crude glycerol in stirred tank and airlift bioreactors	94
5.2.3 Factors affecting on bioconversion of crude glycerol in airlift bioreactors	94

CONTENTS (CONTINUED)

	Page
5.2.4 Analytical methods	95
5.3 Results and discussion	96
5.3.1 Bioconversion of crude glycerol in stirred tank and airlift bioreactors	96
5.3.2 Effect of aeration rate on DCW, lipids, β -carotene and carotenoids productions of strain KM281507	101
5.3.3 Effect of light irradiation and dissolved oxygen on DCW, lipids, β -carotene and carotenoids productions of strain KM281507	103
5.3.4 Fatty acid profile of lipids from strain KM281507	108
5.4 Conclusions	110
CHAPTER 6	111
Mutation of oleaginous red yeast <i>Sporidiobolus pararoseus</i> KM281507 for carotenoids and lipids productions	
6.1 Introduction	111
6.2 Materials and Methods	114
6.2.1 Microorganism and culture conditions	114
6.2.2 Mutagenesis	114
6.2.3 Analytical methods	115
6.3 Results and discussion	116
6.3.1 UV mutagenesis	116
6.3.2 EMS mutagenesis	119
6.3.3 5-Bromouracil mutagenesis	121
6.3.4 Carotenoids and lipids productions by mutant strains	124
6.4. Conclusions	125
CHAPTER 7	126
Conclusions	
REFERENCES	129

CONTENTS (CONTINUED)

	Page
APPENDICES	143
APPENDIX A	144
APPENDIX B	147
APPENDIX C	150
APPENDIX D	153
APPENDIX E	156
CURRICULUM VITAE	158



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

LIST OF TABLES

Table		Page
2.1	Bioconversion of crude glycerol to high value added products	7
2.2	Number of carbons, double bonds and structural formula of fatty acids	14
2.3	Fatty acid composition of various types of microbial lipids	15
2.4	Structure and major commercial utility of carotenoids	21
3.1	Experiment variables at various levels used in the lipids and carotenoids productions by <i>Sporobolomyces pararoseus</i> TISTR5213 using the Plackett-Burman design	36
3.2	Experimental codes, ranges and levels of independent variables in the response surface methodology experiment	38
3.3	DCW, β -carotene, total carotenoids and lipids productions yield of nine red yeasts cultivated in pure glycerol (BMP) and crude glycerol (BMC)	41
3.4	Twelve-trial Plackett-Burman design matrixes for eight variables and the predicted DCW	44
3.5	Estimated effects, linear regression coefficients and corresponding F -ratio and p -values for the DCW for eight variables using the Plackett-Burman experiment design	45
3.6	Twelve-trial Plackett-Burman design matrixes for eight variables and the predicted lipids production yields	46
3.7	Estimated effects, linear regression coefficients and corresponding F -ratio and p -values for the lipids production yield for eight variables using the Plackett-Burman experiment design	47
3.8	Twelve-trial Plackett-Burman design matrixes for eight variables and the predicted β -carotene production yields	48

LIST OF TABLES (CONTINUED)

Table		Page
3.9	Estimated effects, linear regression coefficients and corresponding F -ratio and p -values for the β -carotene production yield for eight variables using the Plackett-Burman experiment design	49
3.10	Twelve-trial Plackett-Burman design matrixes for eight variables and the predicted total carotenoids production yields	50
3.11	Estimated effects, linear regression coefficients and corresponding F -ratio and p -values for the total carotenoids production yield for eight variables using the Plackett-Burman experiment design	51
3.12	The CCD matrixes for the experiment design and predicted responses of DCW	53
3.13	Analysis of variance (ANOVA) of the quadratic model for response variables. The probability values (p -values) of parameter and regression of estimated coefficients of the second order polynomial for response variables are shown for DCW	55
3.14	The CCD matrixes for the experiment design and predicted responses of lipids production yield	57
3.15	Analysis of variance (ANOVA) of the quadratic model for response variables. The probability values (p -values) of parameter and regression of estimated coefficients of the second order polynomial for response variables are shown for lipids production	59
3.16	The CCD matrixes for the experiment design and predicted responses of β -carotene production yield	62
3.17	Analysis of variance (ANOVA) of the quadratic model for response variables. The probability values (p -values) of parameter and regression of estimated coefficients of the second order polynomial for response variables are shown for β -carotene production	64

LIST OF TABLES (CONTINUED)

Table		Page
3.18	The CCD matrixes for the experiment design and predicted responses of total carotenoids production yield	67
3.19	Analysis of variance (ANOVA) of the quadratic model for response variables. The probability values (<i>p</i> -values) of parameter and regression of estimated coefficients of the second order polynomial for response variables are shown for total carotenoids production	69
4.1	Effect of various types of additive agents on DCW and lipids productions, lipids content and fatty acid composition of <i>Sporidiobolus pararoseus</i> KM281507	90
5.1	Kinetic parameters of batch fermentation of <i>Sporidiobolus pararoseus</i> KM281507 in a stirred tank and airlift bioreactors under uncontrolled and controlled pH regimes	100
5.2	Kinetic parameters of batch fermentation of <i>Sporidiobolus pararoseus</i> KM281507 operated in airlift bioreactor with different light irradiation and dissolved oxygen levels	105
5.3	The fatty acid profiles of crude lipids from <i>Sporidiobolus pararoseus</i> KM281507 under different batch fermentation conditions operating in airlift bioreactor	109
6.1	Comparison of DCW, total carotenoids and total lipids produced by the wild-type and mutant strains of <i>Sporidiobolus pararoseus</i> KM281507 using crude glycerol as the carbon source	124

LIST OF FIGURES

Figure		Page
2.1	Transesterification reaction for production of biodiesel	5
2.2	Schematic flow chart for the productions and utilization of lipids and carotenoids from crude glycerol by the effective oleaginous red yeast	9
2.3	Pathway of triacylglycerol synthesis in the oleaginous yeasts. Enzyme: GK, glycerol kinase; PD, pyruvate dehydrogenase; Ac, acotinase; ICDH, iso-citrate dehydrogenase; MD, malate dehydrogenase; ME, malic enzyme; ACL, ATP-citrate lyase; FAS, fatty acid synthetase	11
2.4	The orientation of astaxanthin and β -carotene in phospholipid bilayer	17
2.5	Biosynthesis of carotenoids from glycolysis pathway to carotenogenic pathway by oleaginous red yeast	20
3.1	The effect of methanol on dry cell weight, β -carotene, total carotenoids and lipids productions of <i>Sporobolomyces pararoseus</i> TISTR5213	42
3.2	Dry cell weight in three-dimension for quadratic response surface optimization. The comparison was made between demethanolized crude glycerol and pH, temperature and demethanolized crude glycerol, temperature and pH	56
3.3	Lipids in three-dimension for quadratic response surface optimization. The comparison was made between demethanolized crude glycerol and pH, temperature and demethanolized crude glycerol, temperature and pH	60
3.4	β -carotene in three-dimension for quadratic response surface optimization. The comparison was made between demethanolized crude glycerol and pH, temperature and demethanolized crude glycerol, temperature and pH	65

LIST OF FIGURES (CONTINUED)

Figure		Page
3.5	Total carotenoids in three-dimension for quadratic response surface optimization. The comparison was made between demethanolized crude glycerol and pH, temperature and demethanolized crude glycerol, temperature and pH	70
3.6	Time course of dry cell weight, residual glycerol, pH, lipids, β -carotene and total carotenoids by <i>Sporobolomyces pararoseus</i> TISTR5213 under optimal conditions	75
4.1	Phylogenetic tree constructed using the 26S rRNA gene sequence of <i>Sporidiobolus pararoseus</i>	84
4.2	Effect of formic acid, acetic acid, citric acid and succinic acid on dry cell weight, lipids, β -carotene and total carotenoids of <i>Sporidiobolus pararoseus</i> KM281507 cultivated with demethanolized crude glycerol as a carbon source under optimal condition	86
4.3	Effect of Tween 20, Tween 40, Tween 60, Tween 80, oleic acid (C18:1) and olive oil on dry cell weight, total lipids, β -carotene and total carotenoids of <i>Sporidiobolus pararoseus</i> KM281507 cultivated with demethanolized crude glycerol as a carbon source under optimal condition	88
5.1	Time course of dry cell weight, pH, residual glycerol, lipids, β -carotene and total carotenoids of <i>Sporidiobolus pararoseus</i> KM281507 in an stirred-tank bioreactor with an uncontrolled pH regime, with a controlled pH regime of 5.63 and airlift bioreactor with an uncontrolled pH regime and with a controlled pH regime of 5.63	98
5.2	Effect of aeration rate at 2 vvm, 4 vvm and 6 vvm on the production of dry cell weight, residual glycerol, lipids, β -carotene and total carotenoids of <i>Sporidiobolus pararoseus</i> KM281507 in airlift bioreactor	102

LIST OF FIGURES (CONTINUED)

Figure		Page
5.3	Effect of irradiation and dissolved oxygen (DO) on dry cell weight, lipids, β -carotene and total carotenoids on batch fermentation of <i>Sporidiobolus pararoseus</i> KM281507, when cultured under natural light, dark, light 1,000 Lux, light 10,000 Lux, pure oxygen and light 10,000 Lux plus pure oxygen	106
6.1	Effect of UV irradiation time on the survival of <i>Sporidiobolus pararoseus</i> KM281507	117
6.2	Effect of different carbon sources on total carotenoids and dry cell weight produced by the wild-type and UV-induced mutant strains of <i>Sporidiobolus pararoseus</i> KM281507	118
6.3	Effect of EMS concentration and incubation time on the survival of <i>Sporidiobolus pararoseus</i> KM281507	119
6.4	Effect of different carbon sources on total carotenoids and dry cell weight produced by the wild-type and EMS-induced mutant strains of <i>Sporidiobolus pararoseus</i> KM281507	120
6.5	Effect of time and 5BU concentration on the survival of <i>Sporidiobolus pararoseus</i> KM281507	121
6.6	Effect of carbon source on total carotenoids and dry cell weight produced by the wild-type and 5BU-induced mutant strains of <i>Sporidiobolus pararoseus</i> KM281507	123
B1	Chromatogram of glycerol analyzed by HPLC	147
C1	Chromatogram of β -carotene analyzed by HPLC	151
C2	The β -carotene concentration standard curve	152

ABBREVIATIONS AND SYMBOLS

g	gram
L	liter
mg	milligram
mL	milliliter
μ g	microgram
μ m	micrometer
μ L	microliter
M	molarity
mM	millimolar
mm	millimeter
m	meter
Hz	hertz
Lux	luminous intensity
etc.	et cetera
C	carbon
h	hour
min	minute
rpm	round per minute
pH	power of hydrogen
vvm	volume air per volume medium per minute
ppm	parts per million
amu	atomic mass unit
FAME	fatty acid methyl ester
DO	dissolved oxygen
BMP	basal medium supplemented with pure glycerol
BMC	basal medium supplemented with crude glycerol
DCW	dry cell weight
CCD	central composite design

RSM	response surface methodology
TLC	thin layer chromatography
HPLC	high performance liquid chromatography
GC	gas chromatography
GC-MS	gas chromatography–mass spectrometry
GC-FID	gas chromatography–flame ionization detector
EI	electro ionization
TISTR	Thailand Institute Scientific and Technological Research
AOAC	Association of Official Analytical Chemist
ANOVA	analysis of variance
OD ₆₀₀	optical density at 600 nm
CO ₂	carbon dioxide
O ₂	oxygen
<i>g</i>	g force
<i>p</i> -value	probability value
<i>F</i>	Fisher's
<i>Y</i>	response value
<i>k</i>	number of input factors
<i>R</i> ²	coefficient of determination
e.g.	example gratia
°C	degree Celsius
%	percent
/	per
±	deviation
<i>α</i>	alpha
<i>γ</i>	gamma
<i>β</i>	beta
v/v	volume by volume
w/v	weight by volume
w/w	weight by weight
<	less than

X_{\max}	Maximum dry cell weight (g/L)
μ	Specific growth rate (h^{-1})
μ_{\max}	Maximum specific growth rate (h^{-1})
$Y_{x/s}$	Biomass yield (g/g)
Q_s	Glycerol consumption rate (g/L/d)
C_{\max}	Maximum volumetric productivity of total carotenoids (mg/L)
$Y_{C/S}$	Total carotenoids yield (mg/g glycerol)
$Y_{C/X}$	Specific total carotenoids production yield (mg/g DCW)
Q_C	Total carotenoids productivity (mg/L/d)
β_{\max}	Maximum volumetric productivity of β -carotene (mg/L)
$Y_{\beta/S}$	β -carotene yield (mg/g glycerol)
$Y_{\beta/X}$	Specific β -carotene yield (mg/g DCW)
Q_{β}	β -carotene productivity (mg/L/d)
L_{\max}	Maximum volumetric productivity of lipids (g/L)
$Y_{L/S}$	Lipids yield (g/g glycerol)
$Y_{L/X}$	Specific lipids yield (g/g DCW)
Q_L	Lipids productivity (g/L/d)

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
 Copyright© by Chiang Mai University
 All rights reserved

ข้อความแห่งการริเริ่ม

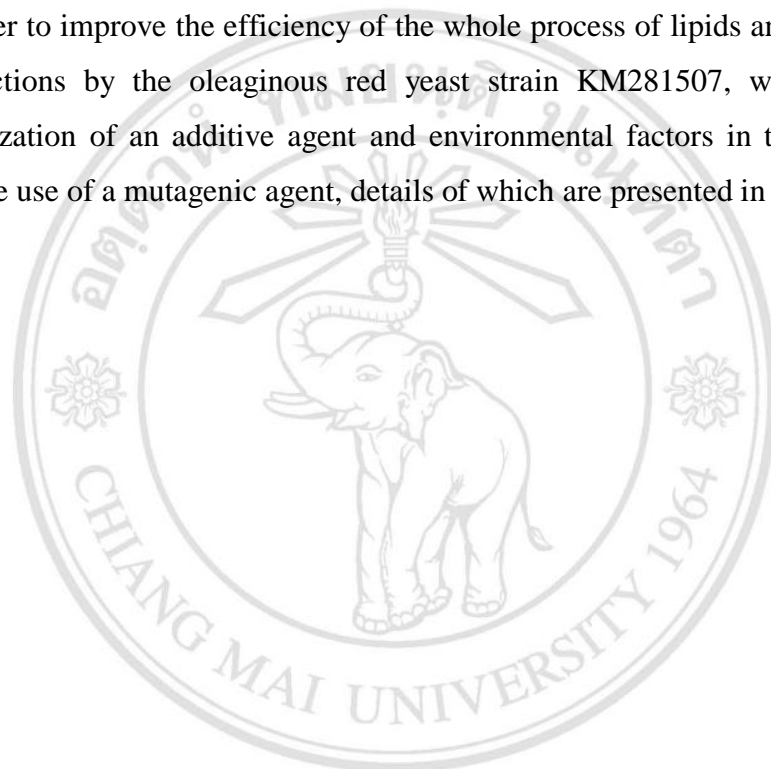
- 1) วิทยานิพนธ์นี้ได้เสนอวิธีการเพิ่มประสิทธิภาพในการผลิตลิพิดเพื่อใช้เป็นสารตั้งต้นในการผลิตไบโอดีเซลและการผลิตแคโรทีนอยด์เพื่อใช้เป็นแหล่งวิตามินและสารสีตามธรรมชาติจากยีสต์โอดิจีนัสสีแดง *Sporidiobolus pararoseus* KM281507 โดยใช้กลีเซอรอลดิบที่ได้จากการผลิตไบโอดีเซลเป็นแหล่งคาร์บอน
- 2) เพื่อการเพิ่มประสิทธิภาพของกระบวนการผลิตลิพิดและแคโรทีนอยด์จากยีสต์โอดิจีนัสสีแดงสายพันธุ์ KM281507 ได้ทำการศึกษาหาสภาวะที่เหมาะสม สารเติมแต่ง ปัจจัยทางกายภาพในถึงปฏิบัติการชีวภาพ และการใช้สารก่อกลายพันธุ์ ซึ่งรายละเอียดต่าง ๆ เหล่านี้ได้นำเสนอไว้ในวิทยานิพนธ์ฉบับนี้



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

STATEMENTS OF ORIGINALITY

- 1) This thesis proposes a method for increasing the efficiency of lipids production for biodiesel feedstock and carotenoids for use as a natural source of vitamins and pigments from an oleaginous red yeast *Sporidiobolus pararoseus* KM281507, by using crude glycerol as a carbon source.
- 2) In order to improve the efficiency of the whole process of lipids and carotenoids productions by the oleaginous red yeast strain KM281507, we studied the optimization of an additive agent and environmental factors in the bioreactor, and the use of a mutagenic agent, details of which are presented in this thesis.



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved