



UNIVERSITI PUTRA MALAYSIA

***PRETREATMENT AND OPTIMISATION OF OIL PALM DECANTER
CAKE FOR OPTIMISED BIOBUTANOL PRODUCTION***

MOHAMAD NAFIS BIN ABDUL RAZAK

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PALM DECANter CAKE FOR OPTIMISED
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**MASTER OF SCIENCE
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By

MOHAMAD NAFIS BIN ABDUL RAZAK

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

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

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MOHAMAD NAFIS BIN ABDUL RAZAK

July 2013

Chairman : Professor Suraini Abd. Aziz, PhD

Faculty : Biotechnology and Biomolecular Sciences

Oil palm industry generated massive amount of lignocellulosic biomass that became a major problem in oil palm mills. Oil palm decanter cake (OPDC) is one of the lignocellulosic biomass that was managed by open dumping in the mills. Utilization of OPDC for the production of crude cellulase cocktail, fermentable sugars and biobutanol were a great approach to manage the abundant biomass, thereby adding value to the waste. In this study, the characteristics and pretreatments of OPDC were investigated. The OPDC with high lignin content (30.66% (w/w)) required an appropriate pretreatment prior to cellulase production and saccharification process. Due to pretreatment process using 1% NaOH and autoclaved at 121°C for 20 minutes, the increment of hydrolysis percentage up to 12-fold higher than untreated OPDC was achieved. The lignin percentage was reduced by 14.1% (w/w) and cellulose and hemicellulose percentages increased by 26.6% (w/w) after the pretreatment.

Production of crude cellulase cocktail using OPDC as the substrate by *Trichoderma asperellum* UPM1 and *Aspergillus fumigatus* UPM2 was successfully investigated. The optimum conditions of these fungi to produce high activity of cellulose using untreated OPDC were 120 hours incubation period, 1×10^7 spores/mL, temperature at 30°C and initial pH of 7.0 -7.5. *T. asperellum* UPM1 produced 17.53 U/mL CMCase, 0.53 U/mL β -glucosidase and 0.28 U/mL FPase while *A. fumigatus* UPM2 produced 10.93 U/mL CMCase, 0.76 U/mL β -glucosidase and 0.24 U/mL FPase. The crude cellulase cocktail with the ratio of 1:1 for *T. asperellum* UPM1 and *A. fumigatus* UPM2 improved the fermentable sugar concentration from 3.17 g/L (1.27 g/g) to 5.08 g/L (2.03 g/g) and hydrolysis percentage increased from 50.69% to 81.29% compared to the single crude cellulase.

Optimization of biobutanol production using OPDC hydrolysate by *Clostridium acetobutylicum* ATCC824 was evaluated statistically using response surface methodology (RSM). The analysis of variance (ANOVA) using 2-level factorial successfully screened three significant variables that influenced the biobutanol yield which were glucose concentration in OPDC hydrolysate, inoculum size and initial pH value. The batch fermentation analyzed using central composite design (CCD) gave the predicted optimum conditions of 70 g/L OPDC hydrolysate, 16.20% inoculum size and initial pH of 5.2. The predicted yield of biobutanol was 0.09 g/g with 70 g/L utilization of glucose. The optimum condition was validated and the actual biobutanol yield was 0.11 g/g with 6.04 g/L biobutanol concentration. The biobutanol production using synthetic glucose produced 15.38% higher biobutanol concentration compared to OPDC hydrolysate probably due to the presence of inhibitor and impurities during the physiochemical pretreatment of the substrate.

Abstrak tesis yang dikemukakan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PRA-RAWATAN DAN PENGOPTIMUMAN KEK DEKANTER KELAPA SAWIT UNTUK PENGHASILAN BIOBUTANOL YANG OPTIMA

Oleh

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Industri kelapa sawit telah menghasilkan sejumlah besar biomas dan ia menjadi suatu masalah kepada kilang sawit. Kek dekanter kelapa sawit (OPDC) merupakan salah satu sisa biomas lignoselulosa yang hanya diurus secara pembuangan terbuka di kilang sawit. Penggunaan OPDC untuk penghasilan koktel selulase mentah, gula fermentasi dan biobutanol adalah pendekatan yang terbaik bagi mengurus sisa biomas yang berlebihan serta memberi nilai tambah kepada sisa tersebut. Dalam kajian ini, pencirian dan pra-rawatan yang sesuai kepada OPDC telah berjaya dilakukan. OPDC mempunyai kandungan lignin yang tinggi (30.66%) dan memerlukan pra-rawatan yang sesuai sebelum penghasilan selulase untuk proses pensakaridaan. Dalam proses pra-rawatan, OPDC yang telah diautoklaf pada suhu 121°C selama 20 minit bersama 1% NaOH menunjukkan peningkatan peratusan hidrolisis yang jelas sehingga 12 kali ganda lebih tinggi berbanding OPDC yang

tidak dirawat. Kandungan peratusan lignin telah menurun sebanyak 14.1% dan peratusan sellulosa bersama hemisellulosa telah meningkat sebanyak 26.6%.

Penghasilan koktel selulase mentah daripada OPDC menggunakan *Trichoderma asperellum* UPM1 dan *Aspergillus fumigatus* UPM2 telah berjaya diselidik. Keadaan optimum kulat-kulat ini untuk menghasilkan aktiviti selulase yang tinggi daripada OPDC yang tidak dirawat adalah selama 120 jam waktu inkubasi, 1×10^7 spora/mL, pada suhu 30°C dan pH permulaan pada 7.0-7.5. *Trichoderma asperellum* UPM1 menghasilkan 17.53 U/mL CMC_{ase}, 0.53 U/mL β-glucosidase dan 0.28 U/mL FPase. *Aspergillus fumigatus* UPM2 telah menghasilkan 10.93 U/mL CMC_{ase}, 0.76 U/mL β-glucosidase dan 0.24 U/mL FPase. Nisbah koktel selulase mentah 1:1 (*T. asperellum* UPM1: *A. fumigatus* UPM2) telah meningkatkan kepekatan gula fermentasi daripada 3.17 g/L kepada 5.08 g/L dan meningkatkan peratusan hidrolisis daripada 50.69% kepada 81.29% berbanding sistem selulase mentah tunggal.

Pengoptimuman penghasilan biobutanol menggunakan hidrolisat OPDC oleh *Clostridium acetobutylicum* ATCC824 telah diuji secara statistik menggunakan kaedah respons permukaan (RSM). Analisis varian (ANOVA) menggunakan analisis faktor 2-peringkat telah berjaya menyaring tiga faktor signifikan yang mempengaruhi penghasilan biobutanol yang terdiri daripada kepekatan glukosa di dalam OPDC hidrolisat, saiz inokulum dan bacaan pH permulaan. Fermentasi kelompok dianalisa menggunakan reka bentuk komposit berpusat (CCD) menunjukkan bahawa keadaan jangkauan optimum adalah 70 g/L gula fermentasi dalam OPDC, 16.20% saiz inokulum dan pH permulaan pada 5.2. Jangkaan hasil biobutanol adalah 0.09 g/g dengan 70 g/L penggunaan glukosa OPDC. Keadaan

optimum telah disahkan dan hasil biobutanol sebenar adalah 0.11 g/g dengan 6.04 g/L kepekatan biobutanol. Penghasilan biobutanol menggunakan glukosa sintetik menghasilkan 15.38% kepekatan biobutanol lebih tinggi berbanding hidrolisat OPDC disebabkan kehadiran perencat dan bendasing ketika pra-rawatan kimiafizikal kepada substrat.



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APPROVAL SHEET 1

I certify that a Thesis Examination Committee has met on 22nd July 2013 to conduct the final examination of Mohamad Nafis Bin Abdul Razak on his thesis entitled "Pretreatment And Its Conditions Optimisation of Oil Palm Decanter Cake for Biobutanol Production" 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 Master Degree of Science.

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DECLARATION

Declaration by Graduate Student

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
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TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	iv
ACKNOWLEDGEMENTS	vii
APPROVAL	viii
DECLARATION	x
LIST OF TABLES	xiv
LIST OF FIGURES	xvi
LIST OF ABBREVIATIONS	xviii
LIST OF APPENDICES	xx
CHAPTER	
1. INTRODUCTION	1
2. LITERATURE REVIEW	5
2.1. Oil Palm Industry	5
2.2. Oil Palm Biomass	7
2.2.1. Oil Palm Decanter Cake (OPDC)	7
2.2.2. Others Oil Palm Biomass	8
2.3. Lignocellulosic Biomass	11
2.3.1. Cellulose, Hemicellulose and Lignin	12
2.3.2. Biosugar	15
2.4. Pretreatment	16
2.4.1. Chemical Pretreatment	17
2.4.2. Physical Pretreatment	20
2.4.3. Physicochemical Pretreatment	20
2.4.4. Biological Pretreatment	21
2.5. Cellulases	22
2.5.1. Endoglucanases	23
2.5.2. Cellobiohydrolase	23
2.5.3. β -glucosidase	24
2.5.4. Problems in Cellulase Production	25
2.6. Saccharification Mechanism	29
2.6.1. Factors Effect Saccharification	33
2.6.2. Crude Cellulase Cocktail	33
2.7. Biobutanol	34
2.7.1. Characteristic of Biobutanol	35
2.7.2. Microorganism Producing Butanol	36
2.7.3. Biochemistry of ABE Production	37
2.7.4. Factors Affecting Biobutanol Production	41
2.7.5. Application of Biobutanol	45
2.7.6. Optimization of Biobutanol Production	47
2.8. Summary	49

3.	MATERIALS AND METHODS	51
3.1.	General Plan of Experimental Work	52
3.2.	Chemicals and Reagents	53
	3.2.1. Substrate	53
	3.2.2. Microorganism	53
	3.2.3. Cellulase Production Media	55
	3.2.4. Biobutanol Production Media	56
3.3.	Methodology	58
	3.3.1. Pretreatment of Substrate	58
	3.3.2. Evaluation on the Pretreatment Efficiency by Hydrolysis Process	59
	3.3.3. Crude Cellulase Production	59
3.4.	Development of Crude Cellulase Cocktail	63
3.5.	Optimization Biobutanol Production	64
	3.5.1. Oil Palm Decanter Cake Sugar Preparation	65
	3.5.2. Two-Level Factorial Design	65
	3.5.3. Central Composite Design (CCD) Experimental Design	68
	3.5.4. Biobutanol Production	69
3.6.	Analytical Method	72
	3.6.1. Biomass Characterization	72
	3.6.2. Cellulase Activity Analysis	79
	3.6.3. Determination of Protein	82
	3.6.4. Determination of Total Reducing Sugar	83
	3.6.5. Biobutanol Analysis	84
4.	RESULTS AND DISCUSSION	87
4.1.	Characterization of OPDC Prior to Pretreatment Process	87
4.2.	Effects of Different Pretreatments on the Chemical Composition of OPDC	91
4.3.	Evaluation on the Pretreatment Efficiency by Hydrolysis Process	96
	4.3.1. Effects of NaOH Concentrations on the OPDC Saccharification	100
4.4.	Effects on OPDC Structure after Pretreatment	104
4.5.	Cellulase Production and Formulation of Crude Enzyme Cocktail	108
	4.5.1. Effects of Fermentation Time on Cellulase Production	108
	4.5.2. Effects of Substrate Pretreatment on Cellulase Production	114
	4.5.3. Effects of Inoculum Size on Cellulase Production	120
	4.5.4. Effects of Temperature on Cellulase Production	124
	4.5.5. Effects of Initial pH on Cellulase Production	127
4.6.	Development of Crude Cellulase Cocktail	129
	4.6.1. Determination of Optimum Enzyme Loading	133

4.7.	Optimization of Biobutanol Production from OPDC Hydrolysate by <i>Clostridium acetobutylicum</i> ATCC 824 via Response Surface Methodological Approach	137
4.7.1.	Initial Screening of Significant Parameters for Biobutanol Production	137
4.7.2.	Central Composite Design for Optimization of Biobutanol Production	140
4.7.3.	Validation of Biobutanol Optimization	146
4.7.4.	Comparison to Others Substrate and Strains	148
5.	SUMMARY, CONCLUSIONS AND SUGGESTION FOR FUTURE RESEARCH	152
5.1.	Summary	152
5.2.	Conclusions	153
5.3.	Suggestions for Future Work	155
	REFERENCES	157
	APPENDICES	176
	BIODATA OF STUDENT	188
	LIST OF PUBLICATIONS	189