

UNIVERSITI PUTRA MALAYSIA

OPTIMIZATION OF MICROBIAL PROCESS FOR THE CONVERSION OF KITCHEN WASTE INTO ORGANIC ACIDS USING RESPONSE SURFACE METHODOLOGY

HALIMATUN SAADIAH HAFID

FBSB 2011 12

OPTIMIZATION OF MICROBIAL PROCESS FOR THE CONVERSION OF KITCHEN WASTE INTO ORGANIC ACIDS USING RESPONSE SURFACE METHODOLOGY



HALIMATUN SAADIAH HAFID

MASTER OF SCIENCE

UNIVERSITI PUTRA MALAYSIA

2011

OPTIMIZATION OF MICROBIAL PROCESS FOR THE CONVERSION OF KITCHEN WASTE INTO ORGANIC ACIDS USING RESPONSE SURFACE METHODOLOGY



By

HALIMATUN SAADIAH HAFID

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfillment of the requirements for the Degree of Master of Science

May 2011

Abstract of a thesis to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

OPTIMIZATION OF MICROBIAL PROCESS FOR THE CONVERSION OF KITCHEN WASTE INTO ORGANIC ACIDS USING RESPONSE SURFACE METHODOLOGY

By

HALIMATUN SAADIAH HAFID

May 2011

Chairman: Nor 'Aini Abdul Rahman, PhD Faculty: Biotechnology and Biomolecular Sciences

Model kitchen waste was formulated in this study to overcome the problem of kitchen waste variation composition. The performance of organic acids production was compared between the model kitchen waste and actual kitchen waste. Both substrates were subjected to anaerobic treatment by indigenous mixed microflora from fermented kitchen waste in a 250 mL shake flask. The parameters conditions used were agitation at 200 rpm, adjusted pH 5 and 7 and temperature of 30°C, 37°C and 40°C. The highest organic acid produced in real kitchen waste and model kitchen waste were 48.64 g/L and 37.49 g/L, respectively after 6 days of incubation at pH 5 and 37°C. For both

kitchen waste fermentation, lactic acid was dominant (76.2%) followed by acetic acid (17.7%) and butyric acid (6.1%).

Organic acids production from model kitchen waste fermentation was further improved by optimization of fermentation procedures using response surface methodology (RSM). Central composite design (CCD) was employed to determine the maximum organic acids production for several parameter variables which include temperature, pH adjusted intermittently and inoculum size. The source of inoculum was originated from kitchen waste being used after 15 days of fermentation. The optimum temperature, pH and inoculums size for the acidogenic fermentation of organic acids from kitchen waste were 35°C, adjusted pH 6.0 and 20%, respectively. The predicted value for the final organic acids produced in this optimal fermentation was 78.20 g/L. The verification study has been carried out and the maximum organic acid produced was 77.28 g/L. Organic acids production was significantly affected by pH and temperature and the interaction between them. Lactic acid showed the main organic acids detected in the kitchen waste fermentation (more than 80%) followed by acetic acid and butyric acid. The organic acids produced have potential to be used as substrate for the production of biopolymer.

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

PENGOPTIMUMAN PROSES MIKROORGANISMA BAGI PENGHASILAN ASID ORGANIK DARIPADA SISA MAKANAN MENGGUNAKAN KAEDAH PERMUKAAN TINDAK BALAS



Oleh

HALIMATUN SAADIAH HAFID

Mei 2011

Pengerusi: Nor 'Aini Abdul Rahman, PhD Fakulti: Bioteknologi dan Sains Biomolekul

Model sisa makanan untuk penghasilan asid organik telah diformulasi bagi mengatasi masalah variasi komposisi sisa makanan. Prestasi penghasilan asid organik bagi keduadua substrat sisa makanan sebenar dan model sisa makanan telah dibandingkan. Rawatan anaerobik bagi kedua-dua substrat oleh mikroflora semulajadi daripada sisa makanan telah diuji di dalam kelalang kon 250 mL dengan kelajuan mengaduk 200 rpm. Keadaan parameter bagi rawatan anaerobik adalah kelajuan campuran pada 200 rpm, pH terlaras masing-masing kepada pH 5 dan 7 dengan suhu 30°C, 37°C and 40°C. Penghasilan asid organik paling tinggi bagi kedua-dua substrat ialah masing-masing



48.64 g/L dan 37.49 g/L, pada pH 5 dan suhu 37°C. Asid laktik dihasilkan paling banyak dalam kedua-dua substrat iaitu 37 g/L (76.2%) diikuti oleh asid asetik (17.7%) dan asid butirik (6.1%).

Penghasilan asid organik daripada model sisa makanan ditingkatkan lagi melalui prosedur pengoptimuman fermentasi menggunakan kaedah permukaan tindak balas (RSM). Rekabentuk komposit pusat (CCD) digunakan untuk menentukan penghasilan asid organik maksimum untuk parameter suhu, pH kawalan dan saiz inokulum. Inokulum yang digunakan diperolehi dari sisa makanan yang difermentasikan selama 15 hari. Suhu, pH terlaras dan saiz inokulum yang paling sesuai untuk penghasilan asid organik dalam fermentasi asid ialah masing-masing pada suhu 35°C dan kawalan pH 6 menggunakan sejumlah 20% inokulum. Nilai anggaran asid organik yang dihasilkan ialah sebanyak 78.20 g/L. Kajian untuk menentukan kesahihan keputusan telah dijalankan dan jumlah asid organik paling tinggi dihasilkan ialah 77.28 g/L. Penghasilan asid organik sangat dipengaruhi oleh faktor pH, suhu dan interaksi antara kedua-duanya. Asid laktik ialah asid yang paling banyak dihasilkan melalui fermentasi sisa makanan iaitu merangkumi 80% dari jumlah keseluruhan asid berbanding dengan asid asetik dan asid butirik. Asid organik yang dihasilkan berpotensi untuk digunakan sebagai substrat bagi penghasilan biopolimer.

ACKNOWLEDGEMENTS

بسمشالرحمن الرحيم

Alhamdulillah thanks a lot to Merciful Allah for giving me the strength to finish this project. I would like to express my deepest appreciation to my main supervisor and supervisory committee Dr Nor 'Aini Abdul Rahman and Assoc. Prof. Dr Suraini Abd

Aziz

My sincere thanks to Dr Phang Lai Yee and Dr Hidayah Arifin for their guidance, advice and encouragement throughout my studies. I also gratefully thanked to the financial support by Ministry of Science, Technology and Innovation, Malaysia and Universiti Putra Malaysia.

My gratitude to all of the laboratory staff such as Mr Rosli Aslim, Mr Azman, Mrs. Renuga A/P Panjamurti and Mrs Aluyah Marzuki and all of my postgraduate's colleagues; Farah Nadia Omar, Nor Farina, Noor Azman, Siti Balkhis and others. Thank you for your help and moral support whenever I needed.

Special thanks to my family for all the sacrifices, time, money, understanding and encouragement's from them. Thank you for always be by my side. Acknowledgement is also due to those who are involved directly and indirectly in the completion of this study.

الحمدلله رب العلمين

I certify that a Thesis Examination Committee has met on 18th May 2011 to conduct the final examination of Halimatun Saadiah hafid on her thesis entitled "Optimization of microbial process for the conversion of kitchen waste into organic acids using Response Surface Methology" in accordance with the Universiti Putra Malaysia [P.U (A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Mohd Shukuri bin Mohamad Ali, PhD

Senior Lecturer Faculty Biotechnology and Biomolecular Sciences Universiti Putra Malaysia (Chairman)

Arbakariya binAriff, PhD

Professor Faculty Biotechnology and Biomolecular Sciences Universiti Putra Malaysia (Internal Examiner)

Mohd Yunus bin Abd. Shukor, PhD

Associate Proffessor Faculty Biotechnology and Biomolecular Sciences Universiti Putra Malaysia (Internal Examiner)

Jamaliah binti Md. Jahim, PhD

Associate Proffessor Faculty of Chemistry and Built Engineering Universiti Kebangsaan Malaysia (External Examiner)

NORITAH OMAR, PhD

Associate Professor and Deputy Dean School of Graduate Studies Universiti Putra Malaysia Date: 26 July 2011 This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows;

Nor 'Aini Abdul Rahman, PhD

Senior Lecturer Faculty of Biotechnology and Biomolecular Sciences Universiti Putra Malaysia. (Chairman)

Suraini Abd. Aziz, PhD

Professor Faculty of Biotechnology and Biomolecular Sciences Universiti Putra Malaysia. (Member)

HASANAH MOHD GHAZALI, PhD

Professor and Dean School of Graduate Studies Universiti Putra Malaysia Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at University Putra Malaysia or at any other institution.



TABLE OF CONTENT

ABSTRA ABSTRA ACKNO APPROV DECLAI LIST OF LIST OF	ACT AK WLEDGEMENTS VAL RATION TABLES FIGURES FABBREVIATIONS	ii iv vi vii ix xii xiii xiii xiv
CHAPTI	ZK	
1 I Y	NTRODUCTION	1
2 I	ITEDATIDE DEVIEW	4
2 L 2.	1 Municipal Solid Waste (MSW) 2.1.1 Kitchen Waste	4 4 9
2.	 Anaerobic Digestion 2.2.1 Stages involves in anaerobic digestion 2.2.2 Microbes involves in anaerobic digestion 2.2.3 Important operating parameters in anaerobic digestion 2.2.4 Anaerobic fermentation product and the commercial 	11 12 16 20
2	Interest in industry	25
2.	A Response Surface Methodology (RSM)	33 37
2.	2.4.1 Theory and Steps	37
	2.4.2 Symmetrical second-order experimental design (CCD)2.4.3 Graphical presentation of the model equation and	40
	determination of optimal operating condition	43
	2.4.4 Advantages and limitations of RSM	45
	5 Concluding Remark	46

3 A COMPARATIVE STUDY OF ORGANIC ACIDS PRODUCTION FROM ACTUAL KITCHEN WASTE AND MODEL KITCHEN WASTE

3.1	Introduction	
3.2	Materials and Methods	50
	3.2.1 Kitchen waste	50
	3.2.2 Proximate analysis	50
	3.2.3 Inoculum preparation	55
	3.2.4 Organic acids production	55
	3.2.5 Analytical methods	56
3.3	Results and Discussion	61
	3.3.1 Characteristic of kitchen waste	61
	3.3.2 Anaerobic digestion	63
3.4	Conclusions	71

4 ENHANCEMENT OF ORGANIC ACIDS FROM MODEL KITCHEN WASTE USING RESPONSE SURFACE METHODOLOGY (RSM)

4.1	Introd	uction	72
4.2	Mater	ials and Methods	74
	4. <mark>2.</mark> 1	Kitchen waste, inoculums preparation and	
		culture conditions	74
	4 <mark>.2.2</mark>	Analytical methods	75
	4. <mark>2.3</mark>	Central composite design (CCD)	75
4.3	Result	s and Discussion	78
	4.3.1	Characteristic of model kitchen waste and inoculums	78
	4.3.2	Optimization of the key culture conditions for organic	
		acids production	80
	4.3.3	Verification of the predicted results in the	
		optimal conditions	93
4.4	Concl	usions	94

5

SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORKS

5.1	Summary and Conclusions	95
5.2	Suggestions for future works	98

REFERENCES	99
APPENDICES	111
LIST OF PUBLICATIONS	116
BIODATA OF STUDENT	117