

UNIVERSITI PUTRA MALAYSIA

PRODUCTION OF XYLITOL FROM SAGO TRUNK HYDROLYSATE USING CANDIDA TROPICALIS

NURUL LINA BINTI MOHAMAD

FK 2011 60

PRODUCTION OF XYLITOL FROM SAGO TRUNK HYDROLYSATE USING CANDIDA TROPICALIS



By

NURUL LINA BINTI MOHAMAD

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

DEDICATION



For my beloved parent, husband and children,

Who offered me unconditional love and support throughout the course of this thesis

Abstract of thesis presented to senate of University Putra Malaysia in fulfillment of the requirement for the degree of Master Science

PRODUCTION OF XYLITOL FROM SAGO TRUNK HYDROLYSATE USING CANDIDA TROPICALIS

By

NURUL LINA BINTI MOHAMAD

May 2011

Chairman: Associate Professor Siti Mazlina binti Mustapa Kamal

Faculty: Faculty of Engineering

Xylitol is natural alternative sweetener and used in some foods because of a number of advantageous natural properties. Production of xylitol from agricultural waste, sago trunk cortex using *Candida tropicalis* was performed. Many researches have been carried out to increase the production of xylitol from various plant sources. Xylose, an intermediate carbon source for the xylitol production, was obtained from hemicellulose fraction of sago trunk cortex lignocellulosic compound. The diluted acid hydrolysis method was used to hydrolyze the xylose component. The influence of acid concentration and reaction time to obtain the optimum condition for xylose production was determined using Response Surface Methodology (RSM). It was found that xylose production was 22.78 g L⁻¹ when operated with 8% sulfuric acid concentration for 60 minutes reaction time. Results showed the formation of toxic compounds which are furfural and phenolic compound from hydrolysis process, inhibited the microorganism performance and subsequently reduced the product formation. Further improvement of xylitol production by *Candida tropicalis* was

observed when using detoxification method. Detoxification of sago trunk hydrolysate was done using activated charcoal and overliming method. The activated charcoal method was found to be the best detoxification method with the highest xylitol concentrations were achieved when 2.5% (w/v) charcoal and adsorption time of 60 minutes was employed. In this condition, the xylitol concentration, volumetric productivity and yield were found 19.53 g L⁻¹, 0.37 g L⁻¹h⁻¹, 0.78 g g⁻¹, respectively. The fermentation conditions for Candida tropicalis were again obtained using RSM by varying the pH, temperature and agitation speed. The responses from RSM study for xylitol concentration, xylitol yield and volumetric productivity were found to be 19.23 g L⁻¹, 0.79 g g⁻¹ and 0.4 g L⁻¹h⁻¹, corresponding to the temperature of 34°C, pH 4 and agitation speed of 250 rpm. Based on the optimization parameter, an inoculum preparation was done in the sago trunk hydrolysate medium without adding nutrient in shake flasks. By performing the fermentation process in the bioreactor in attempt to further improve xylitol production by Candida tropicalis, it was found that the application of fermentation conditions has resulted in increased by 6% of xylitol concentration and 10% of volumetric productivity when compared to the results obtained under the shake flasks. The specific growth rate was found to be higher when fermentation was done in bioreactor compared to shake flasks. This indicating that the cell growth was favored by controlled conditions in the bioreactor than in the shake flasks.

iv

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

PENGHASILAN XILITOL DARIPADA KULIT BATANG SAGU MENGGUNAKAN CANDIDA TROPICALIS

Oleh

Nurul Lina binti Mohamad

Mei 2011

Pengerusi: Profesor Madya Siti Mazlina binti Mustapa Kamal

Fakulti: Kejuruteraan

Xilitol adalah gula gantian semulajadi dan digunakan didalam beberapa penyediaan makanan disebabkan oleh kelebihan pada sifat semulajadinya. Penghasilan xilitol dari sisa pertanian iaitu kulit batang sagu telah dijalankan. Pelbagai kajian telah dilakukan bagi meningkatkan hasil xilitol daripada pelbagai sumber pertanian. Xilosa, adalah sumber karbon bagi penghasilan xylitol, yang diperolehi daripada gentian hemiselulosa a kulit batang sagu. Kaedah hidrolisis asid digunakan bagi menghidrolisis komponen xilosa daripada kulit batang sagu. Kesan kepekatan asid dan tindakbalas masa dijalankan bagi mendapatkan kepekatan xilosa yang optimum dengan menggunakan kaedah tindakbalas permukaan (RSM). Hasil ujian ini, kepekatan xilosa adalah 22.85 g L^{-1} apabila menggunakan 8% kepekatan asid sulfurik yang bertindakbalas selama 60 minit. Hasil ujikaji menunjukkan terhasilnya komponen toksik yang akan menyebabkan perencatan kepada pertumbuhan mikrob dan menghadkan penghasilan xylitol. Komponen toksik didalam media kulit batang sagu dapat dikurangkan dengan menggunakan kaedah menggunakan kaedah penyahtoksik menggunakan

penyerapan karbon teraktif dan pengalkalian media. Kaedah penyerapan karbon teraktif telah dilihat sebagai kaedah yang paling berkesan bagi menyahtoksik berbanding kaedah pengalkalian dengan menghasilkan kepekatan xylitol paling tinggi apabila diserapkan dengan 2.5% karbon dan bertindakbalas selama 60 minit. Keadaan ini menghasilkan kepekatan xylitol, produktiviti dan hasil xylitol adalah 19.53 g L⁻¹, 0.37 g L⁻¹ dan 0.78 g g⁻¹. RSM digunakan sekali lagi untuk mengoptimumkan keadaan fermentasi dengan menggunakan parameter berubah, pH, suhu dan kelajuan putaran. Hasil daripada kaedah ini, kepekatan xilitol, hasil xilitol dan produktiviti yang didapati adalah 19.23 g L⁻¹, 0.79 g g⁻¹ dan 0.4g L⁻¹j⁻¹, sesuai dengan suhu 34°C, pH 4 dan 250 rpm bagi kelajuan putaran. Berdasarkan parameter ini, penyediaan inokulum didalam media batang sagu tanpa nutrisi dilakukan di dalam kelalang kon dan proses itu dilanjutkan didalam bioreactor bagi meningkatkan pengeluaran xylitol. Hasil fermentasi di dalam bioreactor menghasilkan peningkatan sebanyak 6% bagi kepekatan xilitol dan 10% bagi produktiviti apabila dibandingkan dengan hasil yang diperolehi bagi fermentasi di dalam kelalang kon.

ACKNOWLEDGEMENTS

I express my deepest grateful to Allah S.W.T for guidance and strength that enables me to complete this thesis.

I would like to thank my major advisor, Associate Professor Dr. Siti Mazlina Mustapa Kamal for her expertise, advice and guidance, during my Master study and this research. I would especially like to thank her for the opportunity provided for me to continue the pursuit of my goals, and her patients and hard work during my studies and research. I would like to thank En. Abdul Ghani Liew Abdullah, the project leader for his suggestions and assistance throughout my research. I would also like to thank Associate Professor Dr. Norhafizah Abdullah, a member of my committee, for her contributions to my research.

I would also like to thank to the staff and fellow graduate studies in Department of Process and Food Engineering, UPM for their valuable technical help during conducting experiments and the sample analysis.

Lastly a special thanks and sincere appreciation to my family especially Allahyarham Abah, Mama, Abang, Harris, Balqis and Haiqal for their encouragement, inspiration and love has carried me through the good and the bad. I certify that an Examination Committee has met on **6 May 2011** to conduct the final examination of **Nurul Lina binti Mohamad** on her degree thesis entitled **"Production of xylitol from sago trunk hydrolysate by using** *Candida tropicalis***" 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 of Science.**

Members of the Thesis Examination Committee were as follows:

Ling Tau Chuan, PhD

Professor Faculty of Engineering Universiti Putra Malaysia (Chairman)

Yus Aniza Yusof, PhD

Faculty of Engineering Universiti Putra Malaysia (Internal Examiner)

Chin Nyuk Ling, PhD

Associate Professor Faculty of Engineering Universiti Putra Malaysia (Internal Examiner)

Firdausi Razali, PhD

Associate Professor Department of Bioprocess Engineering Faculty of Chemical and Natural Resources Engineering, Universiti Teknologi Malaysia Malaysia. (External Examiner)

> BUJANG KIM HUAT Professor/Deputy Dean School of Graduate Studies Universiti Putra Malaysia

Date:

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 Science. The members of the Supervisory Committee were as follows:

Siti Mazlina Mustapa Kamal, PhD

Associate Professor Faculty of Engineering Universiti Putra Malaysia (Chairman)

Norhafizah Abdullah, PhD

Associate Professor Faculty of Engineering Universiti Putra Malaysia (Member)

> HASANAH MOHD GHAZALI 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 Universiti Putra Malaysia or at any other institution.

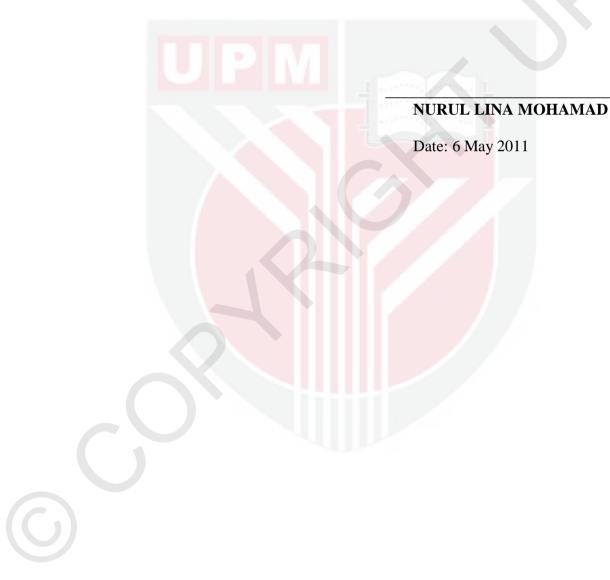


TABLE OF CONTENTS

	Page
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGEMENTS	vii
APPROVAL	viii
DECLARATION	x
LIST OF TABLES	xiv
LIST OF FIGURES	XV
LIST OF ABBREVIATIONS	xvii

CHAPTER

1	INTR	ODUCTION	
	1.1	Xylitol	1
	1.2	Sago waste	2
	1.3	Problem statement	3
	1.4	Objectives	4
	1.5	Experimental design	5
	1.6	Scopes of research	6
•			
2		RATURE REVIEW	0
	2.1	Xylitol background	8
	2.2	Xylitol	9
		2.2.1 Xylitol as sugar substitute	10
		2.2.2 Xylitol in food applications	10
	2.2	2.2.3 Xylitol in pharmaceuticals industry	11
	2.3	Xylose	12
	2.4	Lignocellulosic materials, sources for xylitol production	13
	2.5	2.4.1 Characteristics of lignocellulosic materials	15
	2.5	Sago palm	19
		2.5.1 Sago production statistics	21
		2.5.2 Sago starch	21
	2.6	2.5.3 Utilization of sago bark waste	22
	2.6	Lignocellulose pretreatment	24
	0.7	2.6.1 Acid hydrolysis	25
	2.7	Detoxification	27
		2.7.1 Biological detoxification methods	28
		2.7.2 Physical detoxification methods	28
	2.0	2.7.3 Chemical detoxification methods	29
	2.8	Fermentation process for xylitol production	31
	2.9	Factors affecting xylitol production in fermentation proces	
		2.9.1 pH and temperature	33
	2 10	2.9.2 Aeration	34
	2.10	Kinetic Model	35
		2.10.1 Specific growth rate	35
		2.10.2 Monod model	35
	0.11	2.10.3 Lineweaver-Burk method	36
	2.11	Response Surface Methodology	37

GEN	ERAL MATERIALS AND METHODS		
3.1	Chemical reagents	38	
3.2	Microorganisms and maintenance		
3.3	Inoculum preparation and medium compositions	39	
3.4	General experimental plan 3		
3.5	Bioreactor		
3.6	Analytical procedures	44	
	3.6.1 Cell concentration	44	
	3.6.2 Dry cell weight determinations	44	
	3.6.3 Determination of sugars and by-products	45	
OF S	RACTERIZATION AND ACID HYDROLYSIS AGO TRUNK CORTEX FOR PRODUCTION YLOSE		
4.1	Introduction	47	
4.2		48	
	4.2.1 Raw materials	48	
	4.2.2 Drying	49	
	4.2.3 Milling	49	
	4.2.4 Determination of total cellulose	50	
	4.2.5 Determination of total sugar	51	

OF X	YLOSE	
4.1	Introduction	
4.2	Materials and methods	

3

4

5

4.3

4.2.6 Determination of lignin 52 4.2.7 Determination of ash 52 4.2.8 Dilute acid hydrolysis 53 4.2.9 Experimental design 53 **Results and Discussions** 55 4.3.1 Sugar analysis technique 55 4.3.2 Composition of sugar trunk cortex 57 133 Effects of Sugar and by product fo 60 ...

4.3.3	Effects of Sugar and by-product formation	00
4.3.4	Statistical analysis	62
435	Effects of response surface on vylose concentration	63

oncentration 63 ects of i +.J.. copu s 4.4 Conclusion 67

DETOXIFICATION OF SAGO TRUNK HYDROLYSATE

5.1	Introd	uction	68
5.2	Mater	Materials and method	
	5.2.1	Dilute acid hydrolysis conditions	70
	5.2.2	Detoxification procedures	70
	5.2.3	Microorganism	71
	5.2.4	Inoculum preparation and production media	72
	5.2.5	Fermentation	72
	5.2.6	Analysis	73
5.3	Results and discussions		73
	5.3.1	Effects of detoxification on the chemical	
		composition of the hydrolysate	73
	5.3.2	Effects of detoxification on biomass	
		and xylitol production	76
5.4	Concl	usions	80

FER	MENTA	ATION CONDITIONS USING	
CAN	DIDA T	ROPICALIS FOR XYLITOL PRODUCTION	
6.1	Introd	uction	81
6.2	Mater	ials and methods	82
	6.2.1	Sago trunk hydrolysate and detoxification	82
	6.2.2	Microorganisms	82
	6.2.3	Inoculum preparation and production medium	82
	6.2.4	Fermentation in shake flasks	83
	6.2.5	Fermentation in bioreactor	83
	6.2.6	Analysis	84
	6.2.7	Experimental design	84
6.3	Result	s and discussions	86
	6.3.1	Statistical analysis	86
	6.3.2	Effects of fermentation conditions on xylitol	
		production	87
	6.3.3	Effects of fermentation conditions on xylitol	
		yield on consumed xylose	91
	6.3.4	Effects of fermentation conditions on volumetric	
		productivity	93
	6.3.5	Optimum conditions of xylitol	98
	6.3.6	Inoculum preparation and xylitol fermentation	
		in shake flasks and bioreactor	99
	6.3.7	Growth kinetics of Candida tropicalis	106
6.4		usions	110
SUM	MARY	GENERAL CONCLUSIONS AND	

7 SUMMARY, GENERAL CONCLUSIONS AND RECOMMENDATION FOR FUTURE RESEARCH

6

7.1	Introduction	111
7.2	Summary and conclusion of Research	112
7.3	Future Prospects	113

REFERENCES115APPENDICES124BIODATA OF STUDENT145LIST OF PUBLICATIONS146