



UNIVERSITI PUTRA MALAYSIA

**BIOCONVERSION OF GELATINISED SAGO STARCH TO
FERMENTABLE SUGAR USING RECOMBINANT
*SACCHAROMYCES CEREVISIAE***

AZLIAN MOHAMAD NAZRI

FSMB 2004 16



**BIOCONVERSION OF GELATINISED SAGO STARCH TO
FERMENTABLE SUGAR USING RECOMBINANT
*SACCHAROMYCES CEREVISIAE***

By

AZLIAN MOHAMAD NAZRI

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

March 2004





Specially dedicated to,

mak, ayah
and my family

I love you all

Yang 2004



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

**BIOCONVERSION OF GELATINISED SAGO STARCH TO
FERMENTABLE SUGAR USING RECOMBINANT
*SACCHAROMYCES CEREVISIAE***

By

AZLIAN BINTI MOHAMAD NAZRI

March 2004

Chairman: Suraini Abd-Aziz, Ph.D.

Faculty: Food Science and Biotechnology

Bioconversion of sago starch to fermentable sugar was investigated using three genetically modified *Saccharomyces cerevisiae* strains, YKU107 (expressing α -amylase), YKU131 (expressing glucoamylase) and YKU 132 (expressing α -amylase and glucoamylase). Alpha-amylase (YKU107) and glucoamylase (YKU131) was partial purified using acetone and ammonium sulphate precipitation, respectively before characterisation studies were carried out. The enzymes were purified by about 2.78 and 1.08 fold with recovery of 41.93% and 33.64%, respectively. Through DEAE-cellulose column chromatography, only 26.31% α -amylase and 36.68% glucoamylase were recovered with purification fold of 6.90 and 1.81. Further characterisation showed that both enzymes were stable at pH 5.5, temperature 30°C and ionic strength of 0.05 M, evidenced with residual activity higher than 90%. Optimum pH, temperature and initial starch concentration for glucose production were determined as 5.5, 30°C and 20gL⁻¹, respectively. From influence of various starches studied, potato starch was hydrolysed efficiently, followed by corn, sago, cassava and rice starch. However, the maximum yield of glucose based on utilised

starch followed the sequence: sago > corn > potato > cassava > rice starch. Batch fermentation using 2 L fermenter showed that strains YKU107, YKU131 and YKU132 were able to hydrolyse about 97.82%, 86.86% and 88.06%, respectively during 60 hours cultivation with maximum glucose concentration of 9.32 gL⁻¹, 3.63 gL⁻¹ and 0.85 gL⁻¹, respectively. Based on maximum glucose production, YKU107 was selected for further studies. The influence of rpm examined by this strain indicated that the glucose production consistently increased with rpm. Repeated-batch fermentation at maximum glucose concentration produced 6.91 gL⁻¹ of glucose and 12.35 gL⁻¹ of biomass. The continuous culture was performed in order to increase the glucose production. The maximum glucose concentration of 7.80 gL⁻¹ was obtained at 0.075 h⁻¹ dilution rate and suggested that the optimum operating conditions for glucose production is just at the critical dilution rate. The plasmid was categorised as stable even after 348 hours of continuous cultivation (43 residence times).

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

**BIOPENUKARAN KANJI SAGU KEPADA GULA FERMENTASI
OLEH REKOMBINAN *SACCHAROMYCES CEREVISIAE***

Oleh

AZLIAN BINTI MOHAMAD NAZRI

Mac 2004

Pengerusi: Suraini Abd-Aziz, Ph.D.

Fakulti: Sains Makanan dan Bioteknologi

Biopenukaran kanji sagu kepada gula fermentasi telah dijalankan dengan menggunakan strain rekombinan yis *Saccharomyces cerevisiae* iaitu YKU107 (menghasilkan α -amilase), YKU131(menghasilkan glucoamilase) dan YKU 132 (menghasilkan α -amilase dan glucoamilase). Alpha-amilas (YKU107) dan glucoamilas (YKU131) telah dituliskan pada peringkat pertama menggunakan kaedah pemendakan Aceton dan Sodium sulfat masing-masing sebelum kajian tentang pencirian dilakukan. Setelah dimendakkan, enzim-enzim ini telah dituliskan kira-kira 2.78 dan 1.08 kali indeks dengan peratus pendapatan semula 41.93 dan 33.64, masing-masing. Melalui kromatografi turus DEAE-selulosa, hanya 26.31% α -amilase dan 36.68% glucoamilase didapati semula dengan indeks penulenan masing-masing 6.90 dan 1.81. Pencirian menunjukkan bahawa kedua-dua adalah stabil pada pH, suhu dan kekuatan ion masing-masing 5.5, 30°C dan 0.05 M dibuktikan dengan aktiviti melebihi 90%. PH, suhu dan kepekatan awal kanji optima untuk penghasilan glukosa telah didapati seperti 5.5, 30°C dan 20gL⁻¹ masing-masing. Dari kajian kesan pelbagai jenis kanji, kanji kentang telah dihidrolisis dengan berkesan diikuti oleh jagung, sagu, ubi kayu dan beras. Hasil maksima glukosa berdasarkan kanji yang

telah diguna adalah mengikut turutan sagu > jagung > kentang > ubi kayu > beras. Fermentasi sesekelompok menggunakan fermenter 2 L menunjukkan strain YKU107, YKU131 dan YKU132 masing-masing boleh menghidrolisis sebanyak 92.82%, 86.86% dan 88.06% dalam 60 jam dengan kepekatan glukosa maksima 9.32 gL⁻¹, 3.63 gL⁻¹ dan 0.85gL⁻¹, masing-masing. Berdasarkan kepada keupayaan penghasilan maksimum glukosa, strain YKU107 telah dipilih untuk kajian selanjutnya. Kajian kesan goncangan ke atas strain ini menunjukkan penghasilan glukosa adalah berkadar terus dengan kadar goncangan. Fermentasi sesekelompok-berulang pada kepekatan glucosa maksima menghasilkan 6.91 gL⁻¹ glucose dan 12.35 gL⁻¹ sel. Fermentasi selanjar telah dijalankan bertujuan untuk meningkatkan penghasilan glukosa. Kepekatan glukosa iaitu 7.80 gL⁻¹ telah diperolehi pada kadar dilusi 0.075 h⁻¹ seterusnya mencadangkan bahawa keadaan operasi optima untuk penghasilan glucosa secara selanjar adalah pada kadar dilusi kritikal. Plasmid dikategorikan adalah stabil walaupun setelah menjalani fermentasi selanjar selama 348 jam (43 kali masa residen).

ACKNOWLEDGEMENTS

Bismillahirrahmaanirrahim,

Syukur Alhamdulillah to merciful Allah of giving me the strength to finish my project. I would like to take this opportunity to give special words of thanks to Dr. Suraini Abd. Aziz, my supervisor whom without her supervision, advice, assistance guide, and favourable approval this project might not have been possible. My appreciation is also special extended to my co-supervisors, Assoc. Prof. Dr. Arbakariya Ariff, Dr. Hirzun Mohd. Yusof and Dr. Raha Abd. Rahim. Their many useful suggestions and comments have been great help.

My deepest gratitude goes to my beloved family for their constant support, endless love and cares. My heartfelt thanks to my beloved, Saiful Fizwan for his motivation that has enlightened me during the difficult moments of the project. Thank you so much.

Sincere appreciation to all Fermentation Technology Laboratory staffs especially to Mr. Rosli Aslim, Mrs Aluyah Marzuki, Mrs Renuga a/p Panjamurti and Mrs Latifah Husin. Also to my dear housemate Maizureen and colleagues; Kak Nor, Kak Meah, Rahman, Lisa, Ang, Kak Mai, Kak Chah, Kak Mala, Linn, Kak Zai, Sue, Julia and Kak Zam for their helps, zest and humor that has added more memorable experience. All members in Feed Bioprocess Lab. MARDI, especially Pn. Noraini, Lily and Apai, for the stimulating professional relationship we have had. I sincere my wish them all the best in their future endeavors.

I certify that an Examination Committee met on 5 March 2004 to conduct the final Examination of Azlian binti Mohamad Nazri on her Master of Science thesis entitled “Bioconversion of Gelatinised Sago Starch to Fermentable Sugar using Recombinant *Saccharomyces cerevisiae*” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follow:

Mohamed Ismail Abdul Karim, Ph.D.

Professor
Department of Biotechnology Engineering
Faculty of Engineering,
Universiti Islam Antarabangsa Malaysia
(Chairman)

Suraini Abd-Aziz, Ph.D.,

Associate Professor,
Biotechnology Department,
Faculty of Food Science and Biotechnology,
Universiti Putra Malaysia.
(Member)

Arbakariya Ariff, Ph.D.,

Associate Professor,
Fermentation Technology Centre,
Institute of Bioscience,
Universiti Putra Malaysia.
(Member)

GHULAM RASUL RAHMAT ALI, Ph.D.

Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:



This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

Suraini Abd-Aziz, Ph.D.

Associate Professor
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia.
(Chairman)

Arbakariya Ariff, Ph.D.

Associate Professor
Institute of Bioscience
Universiti Putra Malaysia
(Member)

Raha Abdul Rahim, Ph.D.

Associate Professor
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

Hirzun Mohd. Yusof, Ph.D.

Associate Professor
Biotechnology Division
Sime Darby Technology Centre Sdn. Bhd.
(Member)

AINI IDERIS, Ph.D.

Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:



DECLARATION

I here 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 concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

AZLIAN MOHAMAD NAZRI

Date:

TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGEMENTS	vii
APPROVAL SHEETS	viii
DECLARATION FORM	x
LIST OF TABLES	xv
LIST OF FIGURES	xvi
LIST OF ABBREVIATIONS	xix
 CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	4
2.1 The Yeast and its Properties	4
2.1.1 Strains of <i>Saccharomyces cerevisiae</i>	6
2.1.2 Growth of Yeast	7
2.1.3 Strain Preservation	8
2.2 The Enzyme: Amylases	8
2.2.1 Classification of Amylases	9
2.2.2 α -amylase	10
2.2.2.1 Yeast α -amylase	11
2.2.3 Glucoamylase	12
2.2.3.1 Distribution and Substrate Specificity	12
2.2.3.2 Glucoamylase from Yeasts	15
2.2.4 Effect of Culture Conditions on Amylase Production	17
2.2.4.1 Medium Composition	17
2.2.4.2 Influence of Carbon Source	18
2.2.4.3 Effect of Nitrogen Source	20
2.2.4.4 Influence of pH on Enzyme Expression	23
2.2.4.5 Influence of Temperature	25
2.2.4.6 Influence of Aeration	26
2.2.5 Industrial Importance of Amylases	26
2.3 Starch as a Carbon Source	34
2.3.1 Sago Starch Versus Other Starches	34
2.3.2 Starch Properties	35
2.3.3 Enzymes in Starch Processing	37
2.3.4 Starch Processing Enzyme Produced by Recombinant Yeasts	40
2.4 General Discussion	42
3 MATERIALS AND METHODS	44
3.1 Sago Starch	44
3.2 The Yeast Strains	44
3.3 Culture Medium	47



3.4	Inoculum Preparation	48
3.5	Analytical Assays	49
3.5.1	Starch Concentration	49
3.5.2	Cell Concentration	49
3.5.3	Reducing Sugar Concentration	49
3.5.4	Glucose Concentration	50
3.5.5	α -amylase Activity	50
3.5.6	Glucoamylase Activity	51
3.5.7	Plasmid Stability	51
3.6	Experimental Design	52
3.7	Statistical Analysis	53
4	PARTIAL PURIFICATION AND CHARACTERIZATION OF AMYLOLYTIC ENZYME OBTAINED FROM FERMENTATION OF GELATINISED SAGO STARCH USING RECOMBINANT <i>SACCHAROMYCES CEREVISIAE</i>	56
4.1	Introduction	56
4.2	Materials and Methods	57
4.2.1	Strains and Culture Medium	57
4.2.2	Partial Purification of α -Amylase from Strain YKU107	58
4.2.3	Partial Purification of α -Amylase from Strain YKU131	59
4.2.4	Assays	59
4.2.4.1	Protein Concentration	60
4.2.4.2	Effect of pH	61
4.2.4.3	Effect of Temperature	61
4.2.4.4	Effect of Ionic Strength	61
4.2.4.5	Hydrolysis of Starch	62
4.3	Results and Discussion	62
4.3.1	Crude Extracted	62
4.3.2	Partial Purification of α -amylase	62
4.3.3	Partial Purification of Glucoamylase	63
4.3.4	Factors that Influence α -amylase and Glucoamylase activity	66
4.3.4.1	Effect of pH	66
4.3.4.2	Effect of Temperature	66
4.3.4.3	Effect of Ionic Strength	67
4.3.4.4	Hydrolysis of Starches	70
4.4	General Discussion	72
5	OPTIMIZATION STUDY ON BIOCONVERSION OF GELATINISED SAGO STARCH TO FERMENTABLE SUGAR USING RECOMBINANT <i>SACCHAROMYCES CEREVISIAE</i> STRAIN YKU107 IN SHAKE FLASK	73
5.1	Introduction	73
5.2	Materials and Methods	74
5.2.1	Inoculum Preparation	74
5.2.2	Cultivation Condition	74
5.2.3	Assays	75
5.3	Results and Discussion	75
5.3.1	Effect of Initial Sago Starch Concentration	75

	5.3.1.1 Growth Characteristic	77
	5.3.1.2 Enzyme Accumulation	78
	5.3.1.3 Glucose Formation	79
5.3.2	Effect of pH	80
	5.3.2.1 Growth Characteristic	81
	5.3.2.2 Enzyme Accumulation	82
	5.3.2.3 Glucose Formation	83
5.3.3	Effect of Temperature	85
	5.3.3.1 Growth Characteristic	85
	5.3.3.2 Enzyme Accumulation	86
	5.3.3.3 Glucose Formation	87
5.4	Effect of Initial Starch Concentration, pH and Temperature: Conclusions	89
6	BIOCONVERSION OF GELATINISED SAGO STARCH TO FERMENTABLE SUGAR USING RECOMBINANT <i>SACCHAROMYCES CEREVISIAE</i> UNDER OPTIMAL FERMENTATION STRATEGY	91
6.1	Introduction	91
6.2	Material and Methods	92
	6.2.1 Inoculum Preparation	92
	6.2.2 Cultivation Condition	92
	6.2.3 Assays	93
6.3	Results and Discussion	93
	6.3.1 Glucose Production	93
	6.3.2 Growth Characteristic	96
	6.3.3 Glucoamylase and α –amylase Secretion	98
	6.3.4 Sago Starch Fermentation by <i>S. cerevisiae</i> YKU107	100
	6.3.5 Glucose as a Substrate	103
6.4	Conclusion	104
7	BIOCONVERSION OF VARIOUS STARCHES TO FERMENTATBLE SUGAR USING RECOMBINANT <i>SACCHAROMYCES CEREVISIAE</i>	106
7.1	Introduction	106
7.2	Materials and Method	107
	7.2.1 Inoculum Preparation	107
	7.2.2 Culture Condition	107
	7.2.3 Assays	107
7.3	Results and Discussion	108
	7.3.1 Starch Hydrolysis	108
	7.3.2 Growth Characteristic	110
	7.3.3 α –amylase Secretion	111
	7.3.4 Glucose Formation	112
7.4	Conclusion	113
8	EFFECT OF AGITATION AND MODE OF FERMENTATION OPERATION ON BIOCONVERSION OF GELATINISED SAGO STARCH TO FERMENTATBLE SUGAR USING RECOMBINANT <i>SACCHAROMYCES CEREVISIAE</i> USING STIRRED TANK FERMENTER	114
8.1	Introduction	114

8.2	Materials and Methods	116
8.2.1	Inoculum Preparation	116
8.2.2	Medium	116
8.2.3	Fermentation Condition	116
8.2.3.1	Effect of Agitation	116
8.2.3.2	Repeated-batch	117
8.2.3.3	Continuous Culture	117
8.2.4	Assays	118
8.3	Results and Discussion	118
8.3.1	Effect of Agitation	118
8.3.2	Repeated-batch	122
8.3.3	Continuous Culture	126
8.3.3.1	α –amylase Activity and Starch Hydrolysis Profiles	126
8.3.3.2	Biomass Concentration and Plasmid Stability	127
8.3.3.3	Effect of Dilution Rate	129
8.4	Conclusion	131
9	CONCLUSION AND RECOMMENDATION	132
9.1	Conclusion	132
9.2	Recommendation	134
	REFERENCES	135
	APPENDICES	146
	BIODATA OF THE AUTHOR	158

LIST OF TABLES

Table		Page
2.1	Endoamylase	10
2.2	α -amylase and glucoamylase expressed in yeast	13
2.3	Glucoamylases	14
2.4	Characteristic of some purified amyloglucosidases of yeast	15
2.5	Amylose content of various starches	36
2.6	Some wide used amylases and their products	41
3.1	Sago starch specifications	44
3.2	Recombinant <i>Saccharomyces cerevisiae</i> strains	46
3.3	Selection medium for genetically modified yeast	48
4.1	Partial purification of <i>S. cerevisiae</i> YKU107 α -amylase	64
4.2	Partial purification of <i>S. cerevisiae</i> YKU107 α -amylase	64
5.1	Comparison of fermentation parameters using YKU107 at different initial sago starch concentrations	75
5.2	The effect of pH on 2% sago starch fermentation by YKU 107. Maximal data for biomass concentration, glucose, yield and productivity coefficients	81
5.3	The effect of temperature on 2% sago starch fermentation by YKU107. Maximal data for biomass concentration, glucose, yield and productivity coefficients	85
6.1	Glucose production and glucose production rate of sago starch fermentation using different genetically modified yeast strain	93
6.2	Specific growth rate of <i>S. cerevisiae</i> YKU107, YKU131 and YKU132 on fermentation of sago starch to glucose	97
6.3	Sago starch fermentation by YKU107 using different sizes of bioreactor	101
7.1	Production of glucose by <i>S. cerevisiae</i> YKU107 using different types of starch	109
8.1	Data for maximum glucose, α -amylase and biomass accumulation,	

	Yield $Y_{p/x}$ and $Y_{p/s}$ in 2 L fermentation	121
8.2	Comparison of maximum glucose and cell concentration in repeated-batch at the different on the strategy used	124
8.3	Starch, glucose and biomass concentration, α -amylase activity and Productivity during steady-state of the continuous treatments	130

LIST OF FIGURES

Figure		Page
2.1	The enzymatic hydrolysis of starch and the enzymes used in the starch Industry	29
2.2	Industrial enzymes used for starch transformations and main products of the starch industry	29
2.3	Enzymatic Process involved in starch hydrolysis	31
2.4	Major products resulting from enzymatic transformations of starch	32
3.1	Rice α -amylase expression vector, p739 which contains the yeast <i>PHO84</i> promoter, 2 μ m ori, <i>URA3</i> and a rice α -amylase cDNA fragment	46
3.2	Glucoamylase expression vector pKU122 which contains the yeast <i>PHO84</i> promoter, 2 μ m ori, <i>LEU2</i> and glucoamylase cDNA fragment	46
3.3	Flow diagram of the experimental work	54
3.4	Stirred Tank Fermenter	55
4.1	Calibration curve for protein analysis using BSA as a standard	60
4.2	The elution profile of <i>S. cerevisiae</i> YKU107 α -amylase from DEAE-cellulose column	65
4.3	The elution profile of <i>S. cerevisiae</i> YKU131 glucoamylase from DEAE-cellulose column	65
4.3	Effect of pH on <i>S. cerevisiae</i> YKU107 α -amylase activity and stability	68
4.4	Effect of pH on <i>S. cerevisiae</i> YKU131 glucoamylase activity and stability	68
4.5	Effect of temperature on the activity and stability of <i>S. cerevisiae</i> YKU107 α -amylase	69
4.6	Effect of temperature on the activity and stability of <i>S. cerevisiae</i> YKU131 glucoamylase	69
4.7	Effect of ionic strength (sodium acetate) at 40°C on the enzyme activity of α -amylase and glucoamylase	70
4.8	Hydrolysis of various starches by genetically modified <i>S. cerevisiae</i> YKU107 α -amylase	71

4.9	Hydrolysis of various starches by <i>cerevisiae</i> YKU131 glucoamylase	72
5.1	Sago starch fermentation by recombinant <i>S. cerevisiae</i> YKU107 using various initial starch concentration	76
5.2	Time courses of yeast cell concentration under various initial starch concentrations	77
5.3	Time courses α -amylase secreted by recombinant <i>S. cerevisiae</i> YKU107 under various initial starch concentrations	78
5.4	Glucose profiles during growth of recombinant <i>S. cerevisiae</i> YKU107 in different initial sago starch concentrations	79
5.5	Time courses yeast cell concentration under various pH	82
5.6	Time courses α -amylase activity secreted by <i>S. cerevisiae</i> YKU107 under various pH	83
5.7	Time courses glucose concentration accumulated by <i>S. cerevisiae</i> YKU107 under various pH	84
5.8	Time courses yeast cell concentration obtained under various temperature	86
5.9	Time courses of α -amylase activity secreted by <i>S. cerevisiae</i> YKU107 under various temperature	87
5.10	Time courses glucose concentration accumulated by <i>S. cerevisiae</i> YKU107 under various temperature	88
6.1	Sago starch fermentation by recombinant yeast <i>S. cerevisiae</i> strains. (a) YKU107, (b) YKU131 and (c) YKU132	93
6.2	Growth profile and plasmid stability of <i>S. cerevisiae</i> YKU107, YKU131 and YKU132	98
6.3	The enzyme secreted pattern of <i>S. cerevisiae</i> YKU107, YKU131 and YKU132, during the fermentation process	100
6.4	Fermentation of 2% sago starch by <i>S. cerevisiae</i> strains YKU107 using 2 L fermenter	101
6.5	Biomass and α -amylase formation by genetically modified <i>S. cerevisiae</i> YKU107 in sago starch and glucose containing media	104
7.1	Time courses of starch concentration under various types of starch	109
7.2	Time courses of cell concentration under various types of starch	110

7.3	Time courses α -amylase activity under various types of starch	111
7.4	Time courses glucose concentration under various types of starch	113
8.1	Time course batch fermentation by <i>S. cerevisiae</i> YKU107 in 2 L fermenter using various agitation speed (A) 400 rpm; (B) 500 rpm; (C) 600 rpm and (D) 700 rpm	119
8.2	Profile of repeated-batch culture at substrate depletion.	125
8.3	Profile of repeated-batch culture at maximum glucose production	125
8.4	Continuous sago starch fermentation by genetically modified <i>S. cerevisiae</i> YKU107	128



LIST OF ABBREVIATIONS

E.C.	Enzyme Commission
GA	Glucoamylase
rpm	Rotation per minutes
DEAE	diethylaminoethyl
μ_{\max}	Maximum specific growth rate
$Y_{x/s}$	Yeild of cell on the basis of hydrolysed starch
$Y_{p/s}$	Yeild of glucose on the basis of hydrolysed starch
$Y_{p/x}$	Yield of glucose on the basis of biomass
dS/dt_{\max}	Maximum starch hydrolysis rate during fermentation
P_{\max}	Maximum glucose concentration during fermentation
X_m	Maximum cell concentration
t_m	Fermentation time, the time needed to reach the maximum glucose concentration
DOT	Dissolved oxygen tension
OD	Optical density
pH	Hydrogen potential
$(NH_4)_2 SO_4$	Ammonium sulphate

CHAPTER 1

INTRODUCTION

The use carbohydrates as the carbon sources in microbial fermentation processes are common practice in the industry. In Malaysia, sago starch was reported to have the greatest potential for commercial production of glucose due to its relatively low prices and availability. Sago is an important source of industrial starch for local food industries. Glucose obtained from sago starch is used as a substrates for the fermentation industries as well as for the production of high fructose syrup. In industry, sago starch is also used as an ingredient in the production of monosodium glutamate and caramel. Sago starch is also used in the animal feed industry, the manufacturing of high fructose syrup as an alternative of sucrose and in gasohol fuel production (Zulpilip *et al.*, 1990).

The hydrolysis of starch to glucose has been carried out in many studies and usually is made up of two distinct steps performed by two different enzymatic reactions using different conditions in a batch system (Berghoeer and Sarhaddar, 1988). The present study is to explore the possibility of converting sago starch to fermentable sugar biologically using recombinant yeast and also to determine the physicochemical properties of the starch.

A large variety of starches are used for this production around the world. In Asia it is not uncommon for the industries to use sago or tapioca starches for syrup production, depending on the availability and price, (Schenck and Habeda, 1992).



At present, the use of sago starch in Malaysia has been increasing and has a great potential to be utilized for the production of glucose due to its relatively low prices and abundance. Thus, the possibility of producing glucose from sago starch should be explored.

The yeast, *Saccharomyces cerevisiae*, is recognized as an ideal eukaryotic microorganism for biological studies and has been widely used as a host cell for foreign gene products due to the abundance of information that are available following the early development of recombinant DNA techniques for the microorganism. Furthermore, yeast has an ability to produce mature foreign protein from plants or animals.

The recombinant *Saccharomyces cerevisiae* obtained from the host strain YKU 76 named YKU 107 (expressing α -amylase), YKU 131 (expressing glucoamylase) and YKU 132 (expressing α -amylase and glucoamylase) were used for glucose production from sago starch, due to its abundance in Malaysia and relatively low prices.

Design of fermentation medium for glucose production must take into consideration factors beyond simple nutrition. It is not just the presence of a given nutrient in the medium that is important but also how it acts in terms of cell growth, the microorganism physiology and its ability to produce the enzyme. The medium composition is a communication code used to achieve the objectives of the fermentation processes, its effects must be well understood.

The work reported in this thesis has been aimed at the performance of these recombinant *Saccharomyces cerevisiae* strains using sago starch as substrate and to gain the optimum condition for bioconversion of starch to fermentable sugar by recombinant yeasts. Experiments have been carried out in shake flasks and 2L stirred tank fermenter and results have been obtained relating to the different parameters of cultivation conditions.

The objectives of the study are:

- 1) To investigate the performance of the recombinant yeast to hydrolyze sago starch into fermentable glucose;
- 2) To study the influence of initial starch concentration, pH, temperature, ionic strength and various types of starches on the glucose accumulated, activity and stability of α - as well as glucoamylase secreted by the recombinant yeast;
- 3) To select the best strain from kinetic analysis in relation to cell growth, substrate consumption, enzyme accumulation and glucose production;
- 4) To study the feasibility of using different agitation speeds, mode of fermentation operation, repeated batch and continuous culture for the improvement of sago starch hydrolysis by recombinant yeast.

CHAPTER 2

LITERATURE REVIEW

2.1 The Yeast and Its Properties

The yeast *Saccharomyces cerevisiae*, is recognized as an ideal eukaryotic microorganism for biological studies. Some of the properties that make yeast particularly suitable for biological studies include rapid growth, a budding pattern resulting in dispersed cells, the ease of replica plating and mutant isolation, a well-defined genetic system, and the most importantly, a highly versatile DNA transformation system. Moreover, it has been widely used as a host cell for foreign gene products due to the abundance of genetic information, microbiological and biochemical (Beggs, 1978; Hinnen *et al.*, 1978). Besides, yeast has similar transcription, translation and secretion systems that ability to produce mature foreign protein from plants or animals. Therefore, yeast become an attractive host for production of useful animal or plant proteins, cheaply, maturely and in large amounts (Brunt, 1986; Romanos *et al.*, 1992). Accordingly, protein production by recombinant yeasts is important in bioindustry, because yeasts perform many of the post-translational modifications characteristic of eukaryotes.

The used of *Saccharomyces cerevisiae* begun since prehistoric times in the making of breads and wines, but their cultivation and use in large quantities was put on a scientific basis by the work of the French microbiologist Louis Pastuer in the 19th century. Today they are used industrially in a wide range of fermentation processes, medicinally, as a source of B-complex vitamins and thiamine and as a

