UNIVERSITI PUTRA MALAYSIA

HYDROLYSIS OF SAGO STARCH BY *ASPERGILLUS AWAMORI* FOR THE PRODUCTION OF A GENERIC FERMENTATION MEDIUM

SOBRI BIN MOHD AKHIR

IB 2004 5

HYDROLYSIS OF SAGO STARCH BY *ASPERGILLUS AWAMORI* FOR THE PRODUCTION OF A GENERIC FERMENTATION MEDIUM

By

SOBRI BIN MOHD AKHIR

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

December 2004

Specially dedicated

Alhamdulillah

To my most beloved parents

Mohd Akhir b. Awang Isa and Fatimah bt. Hanafiah

Your care, support and endless love
"My success is only for you"

To my wife

Che Norma bt. Mat Taib

"Thanks for your caring and loving support"

To my son

Muhammad Danish Irfan b. Sobri

"Anak baba"

To my father and mother in-law and all my family

"Thanks for your support"

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

HYDROLYSIS OF SAGO STARCH BY *ASPERGILLUS AWAMORI* FOR THE PRODUCTION OF A GENERIC FERMENTATION MEDIUM

By

SOBRI BIN MOHD AKHIR

December 2004

Chairman: Professor Arbakariya Ariff, Ph.D.

Institute: Bioscience

The efficiency of several amylolytic-enzyme-producing fungi such as *Aspergillus niger*, *Aspergillus awamori* and *Aspergillus flavus* to hydrolyse cooked sago starch has been carried out in shake flask cultures. Subsequently, the performance of microbial hydrolysis of sago starch by *A. awamori*, which was an industrial strain used for glucoamylase production, into fermentable sugar was studied in shake flask culture and 2 L stirred tank fermenter. The influence of medium composition (different types and concentrations of starch and nitrogen sources) on the rate of hydrolysis and fermentable sugar production was first carried out using shake flask cultures. The performance of microbial hydrolysis was also carried out in different modes of bioreactor operation (batch, repeated batch and continuous operation) using freely suspended cell system. Beside rate and degree of starch conversion, the enzymes produced during growth were also determined. For comparison, two-steps enzymatic hydrolysis which involve liquefaction and saccharification steps, was also carried out in batch and continuous processes.

The microbial hydrolysis of gelatinised sago starch to fermentable sugar by three strains of amylolytic-enzyme-producing fungus (*A. awamori*, *A. flavus* and *A. niger*)

was successfully carried out at low temperature (30^{o}C) in a single step process. However, the performance of the hydrolysis by *A. awamori*, in term of yield (0.59 g glucose/g starch) and overall productivity (0.42 g glucose/L.h), was higher than other strain. In addition, the yield of microbial hydrolysis of the different starches (sago, potato, corn and tapioca starch) by *A. awamori* was more or less the same, ranged from 0.56 to 0.59 g glucose/g starch. From this study it was also found that the individual concentration of nitrogen and starch influenced glucoamylase production, and thus the efficiency of starch hydrolysis to fermentable sugar, to a greater extent than the carbon to nitrogen (C/N) ratio. The optimized medium formulation for high performance of starch hydrolysis was by using sago starch ranged from 50 to 100 g/L, yeast extract as the sole nitrogen source and C/N ratio of 17.13.

The yield of glucose produced based on starch used in continuous microbial hydrolysis using 2 L stirred tank fermenter was lower than that obtained in batch hydrolysis (0.58 and 0.92 g/g, respectively) but the overall productivity was higher than that obtained in batch hydrolysis (1.44 and 0.58 g/L.h, respectively). The yield and overall productivity for microbial hydrolysis were lower than for enzymatic hydrolysis (0.75 g/g and 4.4 g/L.h, respectively). However, substantially high amount of biomass was also produced during microbial hydrolysis which can be used as nitrogen sources for media formulation. The optimum dilution rate and starch concentration for enzymatic hydrolysis using stirred tank was obtained at 0.125 h^{-1} and 300 g/L, respectively. On the other hand, the optimum dilution rate and starch concentration for microbial hydrolysis was obtained at 0.022 h^{-1} and 30 g/L, respectively. In addition, microbial hydrolysis was carried out at lower temperature (30^{o}C) than that required in enzymatic hydrolysis (60-95^{o}C). Thus, large reduction of

operating cost is possible. Once, sterilised the glucose produced in outflow of the continuous microbial hydrolysis can be used as a generic medium for various fermentation processes.

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

HIDROLISIS KANJI SAGU OLEH *ASPERGILLUS AWAMORI* UNTUK PENGHASILAN MEDIUM FERMENTASI

Oleh

SOBRI BIN MOHD AKHIR

Disember 2004

Pengerusi: **Profesor Arbakariya Ariff, Ph.D.**

Institut: **Biosains**

Keberkesanan beberapa kulat penghasil-enzim-amilolitik seperti *Aspergillus niger, Aspergillus awamori dan Aspergillus flavus* untuk menghidrolisis kanji sagu yang dimasak telah dijalankan di dalam kultur kelalang goncang. Seterusnya, hidrolisis kanji sagu secara proses mikrobial oleh *A. awamori* yang merupakan sejenis strain industri yang digunakan untuk penghasilan glukoamilase, kepada gula dijalankan di dalam kultur kelalang goncang dan fermenter berpengaduk 2 L. Sebagai permulaan, kesan komposisi media (jenis dan kepekatan kanji dan sumber nitrogen yang berbeza) ke atas kadar hidrolisis dan penghasilan gula dijalankan di dalam kelalang goncang. Hidrolisis secara mikrobial juga dijalankan pada mod operasi bioreaktor yang berbeza (sesekumpul, sesekumpul berulang dan selanjar) menggunakan sistem sel bebas terampai. Di samping kadar dan darjah penukaran kanji, enzim yang terhasil semasa pertumbuhan kulat juga telah ditentukan. Sebagai perbandingannya, hidrolisis kanji secara enzimatik dua-peringkat yang melibatkan proses likuifikasi dan sakarifikasi juga dijalankan secara sesekumpul dan selanjar.

Hidrolisis kanji sagu kepada gula secara proses mikrobial oleh ketiga-tiga strain kulat penghasil enzim amilolitik (*A. awamori, A. flavus* dan *A. niger*) dijalankan pada suhu

yang rendah (30^{o}C) menggunakan proses satu peringkat. Walau bagaimanapun, hidrolisis kanji oleh *A. awamori* berdasarkan kepada hasil (0.59 g glukosa/g kanji) dan produktiviti keseluruhan (0.42 g glukosa/L.j), adalah lebih tinggi berbanding strain yang lain. Malahan, hasil daripada hidrolisis secara mikrobial ke atas kanji yang berlainan (sagu, kentang, jagung dan ubikayu) oleh *A. awamori* adalah lebih kurang sama, dalam julat 0.56 hingga 0.59 g glukosa/g kanji). Daripada kajian ini, di dapati bahawa kepekatan nitrogen dan kanji mempengaruhi penghasilan glukoamilase dan keberkesanan hidrolisis kanji kepada gula pada tahap yang lebih besar daripada nisbah karbon kepada nitrogen (C/N). Formulasi media yang dioptimakan untuk hidrolisis kanji yang tinggi perlaksanaannya adalah dengan menggunakan kanji sagu yang berkepekatan antara 50 hingga 100 g/L, ekstrak yis sebagai sumber nitrogen dan nisbah C/N pada 17.13.

Glukosa yang terhasil berasaskan kanji yang digunakan bagi hidrolisis kanji secara mikrobial selanjar di dalam fermenter berpengaduk 2 L adalah lebih rendah daripada yang diperolehi dari hidrolisis secara sesekumpul (masing-masing 0.58 dan 0.92 g/g) tetapi produktiviti keseluruhannya adalah lebih tinggi daripada hidrolisis secara sesekumpul (masing-masing 1.44 dan 0.58 g/L.j). Hasil dan produktiviti keseluruhan proses hidrolisis secara mikrobial adalah lebih rendah daripada hidrolisis secara enzimatik (masing-masing 0.75 g/g dan 4.4 g/L.j).

Walau bagaimanapun, amaun biomas yang agak tinggi yang dihasilkan semasa proses hidrolisis secara mikrobial boleh digunakan sebagai sumber nitrogen untuk formulasi media fermentasi. Kadar pencairan yang optimum dan kepekatan kanji untuk hidrolisis secara enzimatik di dalam tangki berpengaduk adalah diperolehi

pada kadar 0.125 j^{-1} dan 300 g/L. Manakala, kadar pencairan yang optimum dan kepekatan kanji untuk hidrolisis secara mikrobial adalah diperolehi pada kadar 0.022 j^{-1} dan 30 g/L. Malahan, hidrolisis kanji secara mikrobial boleh dijalankan pada suhu yang lebih rendah (30^{o}C) berbanding suhu yang diperlukan untuk hidrolisis secara enzimatik (60-90^{o}C). Oleh itu, kos operasi untuk menjalankan proses hidrolisis dapat dikurangkan dengan banyaknya. Apabila disterilkan, glukosa yang dihasilkan dari hidrolisis selanjar kanji secara mikrobial boleh digunakan sebagai medium untuk pelbagai proses fermentasi.

ACKNOWLEDGEMENTS

I wish to express my deepest appreciation and sincere gratitude to my family members, without their support and instinctive love, I could not become what I am today. My deepest appreciations go to my father, mother and my wife, Che Norma for their enormous support and sacrifices that given to me willingly. I would like to express my hearty thanks to my dear Chairman Committee Supervisors: Prof. Dr. Arbakariya Ariff, Prof. Dr. Mohammed Ismail Abdul Karim and Assoc. Prof. Dr. Suraini Abd. Aziz for their financial support, numerous patient, constructive suggestions and understanding throughout the years of my master study.

My special thanks go to members and staffs in Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences – Encik Rosli, Kak Aluyah and Renuga. Last but not least, special thanks goes to all my friends, wherever you are I will always remember you, Insya-Allah.

I certify that an Examination Committee met on 16th December 2004 to conduct the final examination of **Sobri b. Mohd Akhir** on his **Master of Science** thesis entitled **"Hydrolysis of Sago Starch by *Aspergillus awamori* for the Production of a Generic Fermentation Medium"** in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

RAHA ABDUL RAHIM, Ph.D.
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

ROSFARIZAN MOHAMAD, Ph.D.
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

LING TAU CHUAN, Ph.D.
Faculty of Engineering
Universiti Putra Malaysia
(Member)

SAHAID MOHD KHALIL, Ph.D.
Associate Professor
Faculty of Engineering
Universiti Kebangsaan Malaysia
(Independent Examiner)

_____
GULAM RUSUL 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 fulfilment of the requirements for the degree of Master of Science. The members of the Supervisory Committee are as follows:

ARBAKARIYA ARIFF, Ph.D.
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

MOHAMMED ISMAIL ABDUL KARIM, Ph.D.
Professor
Kuliyyah of Engineering
International Islamic University Malaysia
(Member)

SURAINI ABD AZIZ, Ph.D.
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

_____
AINI IDERIS, Ph.D.
Professor / Dean
School of Graduate Studies
Universiti Putra Malaysia

Date :

DECLARATION

I hereby 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 UPM or other institutions.

_____

SOBRI B. MOHD AKHIR

Date :

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AG	Novo amyloglucosidase unit
BSPs	Biomass support particles
C	Carbon
C/N	Carbon/Nitrogen
$CaCl_2$	Calcium chloride
$conc^n$	Concentration
D	Dilution
DE	Dextrose equivalent
DNS	3,5 - dinitrosalicylic acid
DOT	Dissolved oxygen tension
DP	Degree of polymerization
DW	Dry weight
Eq.	Equation
F	Flow rate
$FeSO_4.7H_2O$	Ferum sulfate heptahydrate
GA_{max}	Maximum glucoamylase concentration
G_{max}	Maximum glucose concentration
Glu	Glucose
HCl	Hydrochloric acid
HFCS	High fructose corn syrup
KH_2PO_4	Kalium dihydrogen phosphate
Km	The concentration of substrate that gives “half maximal activity”
M	Molar
$MgSO_4.7H_2O$	Magnesium sulfate heptahydrate
MW	Molecular weight
N	Nitrogen
NaOH	Sodium hydroxide
$(NH_4)_2SO_4$	Ammonium sulfate
NU	Novo α-amylase unit
P	Productivity
PU	Polyurethane
SCP	Single cell protein
SSF	Solid-state fermentation
t	Time
μ	Specific growth rate
V	Volume
x	Cell concentration
X_{max}	Maximum cell concentration
YE	Yeast extract
$Y_{G/S}$	Ratio of glucose to substrate
$Y_{X/S}$	Ratio of cell to substrate

CHAPTER 1

INTRODUCTION

One of the problems in the development of fermentation process for the production of various valuable products is the availability of cheap local carbon sources. Starch is a polymer of glucose (Kennedy *et al*., 1988) and can be used as an alternative for less expensive carbon sources. Several types of starch such as sago, tapioca, corn, barley, wheat, potato and rice starches are available in the market. Sago palm can produce 25 tonnes of starch/hectare in swampy areas that unsuitable for any other crop (Doelle, 1998). The production of sago starch in Peninsular Malaysia is about 11, 000 tonnes which is from an area of only 4, 400 hectares. While Sarawak's export is 43, 000 tonnes of quality flour from an area of 19, 720 hectares (Chew and Shim, 1993). The total area planted with sago in Malaysia is estimated at 35, 000 hectares (mainly in Sarawak and Sabah) and the yield is about 20 tonnes of dry starch per hectare of sago between the age of 10-15 years (Westphal and Jansen, 1986). At present, amongst other similar agricultural feedstock (eg. cellulose), starch is most readily available and most readily convertible to fermentable sugars (Reczey *et al*., 1986).

Hydrolysis of starch can be carried out by enzymatic and/or chemical routes. With the availability of commercial amounts of starch-degrading enzymes, traditional methods using hydrochloric and sulfuric acid have decreased considerably. The availability of enzymes has been followed by a rapid growth in the starch industry. Starch-degrading enzymes and their role in the starch industry have already been

well reviewed (Fogarty, 1983; Kennedy *et al.*, 1987; Maldonado and Lopez, 1995). Existing processes were improved with greater efficiency and better quality of products and the production of a wide variety of starch hydrolysates with well-defined physical properties and carbohydrate profiles was also made possible (Slominska, 1993). Biological-based technique is one of the oldest and most powerful processing tools ever known. So, it is common practice to use carbohydrates as the carbon sources in microbial fermentation processes and convert it to a variety of chemicals.

Products of starch hydrolysis, such as maltodextrins, corn, glucose and high-fructose syrups have wide application in the food, textile, brewing and pharmaceutical industries. These products are derived mainly from corn, barley, wheat or potato. It should be possible to obtain similar products from sago starch in addition to other functional ingredients. One possible area that interesting is in the use of raw sago starch for the production of glucose. This product can be further converted to fermentation medium (fructose, gluconic acids, fatty acid esters or polyols) or high value added products (penicillin).

Glucose syrups are conventionally produced from gelatinised starches by acid and/or enzyme hydrolysis (Hakulin *et al.*, 1983). The main disadvantages of acid hydrolysis are the relatively low yield and the excessive formation of by-products. On the other hand, enzymatic hydrolysis requires starch to be gelatinised first, usually in a jet cooker, producing a highly viscous slurry. Recent reports highlighted the use of twin screw extrusion technology as a bioreactor (Chouvel *et al.*, 1983; Hakulin *et al.*, 1983; Roussel *et al.*, 1991). But these techniques required simultaneous high

temperature for enzymatic liquefaction of sago starch and then followed by subsequent saccharification. So, this technique may require higher operational cost and in some cases may not economically viable. To overcome such problems, the microbial hydrolysis of sago starch by amylolytic enzyme-producing fungus such as *Aspergillus awamori* and *Aspergillus oryzae* to fermentable sugar is one of the possible approaches.

The mold such as *A. awamori* is able to grow in cooked starch by secreting amylolytic enzymes (α-amylase and glucoamylase) at 30^{o}C and as a result glucose is accumulated in the culture broth. Limitation of nitrogen supply inhibits growth, so that glucose produces is not consumes for further growth. The activity of this mold can be exploited in continuous hydrolysis of cooked starch at room temperature for the production of fermentable sugars. The outflow from the continuously operated bioreactor, once sterilized, is an ideal fermentation medium. In addition, the biomass produced during the process can also be autolysed chemically or by heat treatments to extract nitrogen which can be used as nitrogen source in the formulation of the fermentation medium.

In this study, the feasibility of using several amylolytic enzyme-producing fungus, such as, *A. awamori*, *A. niger* and *A. flavus* in microbial hydrolysis of gelatinised sago starch into fermentable sugars was investigated. Further study was also carried out to find the optimize medium (starch concentration, type of nitrogen and C/N ratio) for improvement of the microbial hydrolysis rate of gelatinised sago starch and other starches (corn, potato and tapioca) by *A. awamori* in a single step batch process. The use of continuous process for the improvement of microbial hydrolysis

in terms of yield and productivity was also explored. For comparison, two steps enzymatic hydrolysis, liquefaction and saccharification processes, was also carried-out.

The objectives of this study were as follows:

a) To study the feasibility of using amylolytic enzyme producing mold (*A. awamori, A. flavus and A. niger*) in hydrolysing gelatinised sago starch for the production of a generic fermentation medium.

b) To investigate the effect of sago starch concentration, type of nitrogen source and C/N ratio on the performance of sago starch hydrolysis by *A. awamori*.

c) To compare the performance between microbial technique in hydrolyzing starch to fermentable sugars with enzymatic technique.

d) To study the influence of the different modes of bioreactor operation (batch and continuous) on the performance of microbial hydrolysis of sago starch by *A. awamori.*

CHAPTER 2

LITERATURE REVIEW

2.1 Properties of Starch

In fermentation, the cost of the substrate is an important economic parameter. Instead of using pure glucose, starch can be served as a cheap source of substrate for fermentation processes. According to Andrzejczuk *et al*. (1985), various kinds of starch such as potato, corn, wheat, rice and tapioca proved uniform as a carbon source for microbial hydrolysis. Starch is formed as a reserve carbohydrate in plants by the enzymatic condensation of glucose. It is the most abundant utilizable resource in plant biomass and can be hydrolysed enzymatically into fermentable sugar available to microorganisms. Pure starch is a white, amorphous, relatively tasteless solid, which possesses no odour and naturally is water-insoluble granules. Starches are polymers of anhydroglucose units with the general formula of $(C_6H_{10}O_5)_n$ in which these units are joined by α-glucosidic linkages.

Normally natural starches consist of two main polysaccharides, amylose and amylopectin in a ratio of 20-30% to 70-80%, respectively (Shannon and Garwood, 1984). Both of which are based on 1,4-linked α-D-glucose chains. Amylose is an essentially linear polymer having primarily α-1,4 linkages whereas amylopectin is highly branched polymer which is also mainly α-1,4-linked units, but about 5% of the bonds are α-1,6 branch points. Amylose is very unstable in water even in dilute solutions of 1% or less. It is usually defined, on the basis of its interaction with