UNIVERSITI PUTRA MALAYSIA

PRODUCTION OF SUGARS FROM SAGO HAMPAS BY TRICHODERMA SP. DURING SOLID SUBSTRATE FERMENTATION

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

March 2006

GOD
You are my Supreme Love

My beloved parents
who brought me to this world, thanks for your love and care

My husband, Mohd. Nasir
my love, my inspiration

My lecturers
guidance and knowledge

My friends
thanks for your support.

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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Chairman : Professor Mohd. Ali Hassan, PhD

Faculty : Biotechnology and Biomolecular Sciences

Advances in industrial biotechnology offer potential opportunities for economic utilisation of agro-industrial residues such as sago *hampas*. Sago *hampas*, which is a complex material, is one of the major by-product of the sago starch industry. It contains about 69.82% of starch and 13.88% lignocellulosic materials on dry weight basis. Due to its abundant availability, it can serve as an ideal substrate for microbial processes for the production of sugars. Application of solid substrate fermentation technology is an attractive possibility for such bioconversions.

In this study, solid substrate fermentation (SSF) of sago *hampas* by indigenous isolated *Trichoderma sp.* was carried out. In laboratory scale, SSF was conducted in a 250 mL Erlenmeyer flask contains 5g of *hampas* was used as solid substrate and 10% (v/w) of

mycelia suspension was used as inoculum. Parameters optimised includes the initial moisture content (60, 65, 70, 75 and 80%), mineral salts solution (10, 20 and 30% v/w), urea concentration (0.5, 1.0 and 2.0% w/v), inoculum density (10, 20 and 30% v/w), incubation temperature (25, 30, 35, 40, and 45 °C), incubation time (0, 12, 24, 36, 48, 60, 72, 84, 96, 108 and 120 h) and homogenisation speed (8,000, 9,500 and 13,500 rpm) and time (1, 3 and 5 min) on reducing sugars recovery. Maximum reducing sugars obtained after optimisation was 460 mg/g substrate on 96 h incubation with 80% of initial moisture content, 10% (v/w) of inoculum density, 1.0% of urea concentration in 20% (w/v) of mineral salts solution and incubated at 30 $\pm$ 2 °C. The solid culture was homogenised at 9, 500 rpm for 3 minutes for reducing sugars recovery. Meanwhile, the maximum enzyme activities obtained were 3.19 U/mL, 2.22 U/mL, 1.66 U/mL, 1.11 U/mL and 1.48 U/mL for α-amylase, glucoamylase, carboxymethyl cellulase, filter paperase and ß-glucosidase respectively.

Bioconversion of sago *hampas* using a rotary drum was conducted by using a modified cement mixer. Operating parameters such as temperature, moisture, agitation and aeration via SSF were studied to achieve higher production of reducing sugar. After 96 h of fermentation, maximum reducing sugar obtained was 380 mg/g substrate. Maximum enzyme activities achieved were 2.74 U/mL, 2.19 U/mL, 1.33 U/mL, 1.12 U/mL and 1.07 U/mL for α-amylase, glucoamylase, carboxymethyl cellulase, filter Paperase and ß-glucosidase, respectively.

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

PENGHASILAN GULA DARIPADA HAMPAS SAGU OLEH *TRICHODERMA SP.* SEMASA PENAPAIAN SUBSTRAT PEPEJAL

Oleh

ZAIZUHANA BINTI SHAHRIM

Mac 2006

Pengerusi : Profesor Mohd. Ali Hassan, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Kejayaan dalam industri bioteknologi telah membuka peluang ekonomi melalui penggunaan sisa agroindustri seperti hampas sagu. Hampas sagu ialah sejenis bahan yang kompleks, antara sisa buangan terbesar dalam industri penghasilan kanji daripada sagu. Ia mengandungi 69.82% kanji dan 13.88% bahan lignoselulos dalam berat kering. Oleh kerana penghasilan hampas sagu yang berlebihan, ianya amat sesuai dijadikan substrat dalam proses mikrobial untuk penghasilan gula. Justeru itu, penggunaan teknologi penapaian substrat pepejal amat berpotensi bagi menjayakan proses biopenukaran ini.

Dalam kajian ini, penapaian substrat pepejal (SSF) oleh kulat tempatan yang telah dipencilkan iaitu *Trichoderma sp.* telah dijalankan. Kajian diperingkat makmal, SSF dilakukan dengan menggunakan kelalang *Erlenmeyer*, 250 mL. 5g hampas sagu

digunakan sebagai substrat dan ampaian spora berusia tujuh hari pengkulturan digunakan sebagai inokulum. Parameter yang dioptimumkan termasuk lembapan awal (60, 65, 70, 75 and 80%), larutan garam mineral (10, 20 dan 30% b/i), kepekatan urea (0.5, 1.0 dan 2.0% b/i), ketumpatan inokulum (10, 20 dan 30% b/i), suhu inkubasi (25, 30, 35, 40, dan 45°C), masa inkubasi (0, 12, 24, 36, 48, 60, 72, 84, 96, 108 and 120 h) kelajuan homogenasi (8,000, 9,500 dan 13,500 rpm) dan masa homogenasi (1, 3 and 5 min). Gula penurun maksima yang diperolehi pada 96 jam inkubasi ialah 460 mg/g substrat dengan lembapan awal 80%, 10% (v/w) ketumpatan inokulum, 1.0% (b/i) kepekatan urea dalam 20% (b/i) larutan garam dan diinkubasi pada 30 $\pm$ 2 °C. Pepejal kultur dihomogenasi pada 9,500 rpm selama 3 minit. Aktiviti enzim maksima yang diperolehi ialah 3.19 U/mL, 2.22 U /mL, 1.66 U/mL, 1.11 U/mL dan 1.48 U/ml untuk α-amilase, glukoamilase, karboksimetil selulase, filter paperase dan β-glukosidase masing-masing.

Biopenukaran hampas sagu menggunakan drum berputar dijalakan dengan menggunakan pengadun simen yang telah diubahsuai. Parameter operasi seperti suhu, lembapan, pengadukan, pengudaraan dalam SSF dikaji untuk memperolehi hasil gula penurun yang tinggi. Setelah 96 jam fermentasi, gula penurun maksima yang diperolehi ialah 380 mg/g substrat. Aktiviti enzim maksima yang diperolehi ialah 2.74 U/mL, 2.19 U/mL, 1.33 U/mL, 1.12 U/mL and 1.07 U/mL untuk α-amilase, glukoamilase, karbosimetil selulase (CMCase), Filter Paperase (FPase) and β-glukosidase (BGDase) masing-masing.

ACKNOWLEDGMENTS

All praise to supreme Almighthy Allah s.w.t the only creator, whose blessing and kindness have enabled me to accomplish this project successfully.

I would like to convey my deepest appreciation and thanks to Prof. Dr. Mohd. Ali Hassan from the Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, chairman of my supervisory committee for his endless advise, guidance, patience and encouragement that lead to the completion of this thesis.

I would also like to express my thanks and gratitude to Prof. Dr Mohamed Ismail Abdul Karim (UIA), Prof. Dr. Vikineswary Sabaratnam from the Institute of Biological Sciences, Faculty of Science, Universiti Malaya and Assoc. Prof. Dr. Suraini Abdul Aziz from the Faculty of Biotechnology and Biomolecular Sciences (UPM) for their constructive suggestions and guidance, which has helped to sharpen my thinking.

In addition, I would like to express my appreciation to all my lab-mates and fermentation laboratory staffs for your moral support, cooperation and willingness to help me throughout this study. My heartiest thanks go to my husband Mohd. Nasir Abd. Aziz and both of my parents for their endless love, support and encouragement.

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LIST OF ABBREVIATIONS

SSF	Solid Substrate Fermentation
SmF	Submerged Fermentation
DNS	Dinitrosalicylic acid
PDA	Potato Dextrose Agar
CMC	Carboxymethyl Cellulose
RSS	Raw Sago Starch
RSH	Raw Sago *Hampas*
SEM	Scanning Electron Microscopy

CHAPTER I

INTRODUCTION

Solid substrate fermentation (SSF) has been known for centuries and successfully used for the production of oriental foods. SSF stimulates the growth of microorganisms in nature on moist solids. More recently, it has gained much importance in the production of microbial enzymes due to several economic advantages over conventional submerged fermentation (SmF), because of its advantages such as less requirement of water, smaller volumes of fermentation mash, less physical energy requirement, smaller capital investment, fewer operating costs and lower space requirement furthermore reduced reactor volumes, easier product recovery, less liquid water to dispose of and hence less pollution problems. Several reports on SSF have been published in recent years on the production of fine chemicals (Roukas, 1999; Vandenberghe, 2000) enzymes antibiotics and immunosuppressant (Leangon et al., 1999).

Lack of knowledge about the fundamental processes is the major limitation of successful application of SSF for the large scale production, especially on the transport phenomena occurring during SSF, and also effects of design and operation of bioreactors on SSF performance itself (Conti et al., 2001). The intermittent mixed bed bioreactor has become a popular choice for large-scale operation. It is a promising solution since many fungal processes can tolerate infrequent mixing events without unduly deleterious effects on the growth kinetics, if the mixing periods are relatively short. In fact, various authors have

worked successfully with intermittently mixed bioreactors at pilot scale, indicating the potential of this operating strategy (Meien et al., 2004).

In Malaysia, the use of sago starch has been increasing, and it is presently being used for the production of glucose (Suraini, 2002), on the other hand the sago starch processing industry is now being confronted with solid and liquid waste disposal problems. One of the major byproduct is sago *hampas*. Sago *hampas* (the fibrous pith residue) obtained after starch extraction from the rasped sago pith consists of about 66% starch, 15% crude fiber and 1% crude protein on a dry weight basis (Shim, 1992). The development of appropriate technology for converting sago *hampas* into value-added product has been undertaken by several researches (Tuen, 1994). *Hampas*, which contains large amounts of trapped starch granules, has been studied by Rifat et al. (2003) and Vikineswary and Nadaraj (1992) for its utilisation by amylolytic and cellulolytic fungi. Study by Shim (1992) revealed high activity of cellulase and α-amylase during the growth of *Myceliopthora thermophila* on sago *hampas*. None of these researches concentrate on the production of sugars. Based on these findings, sago *hampas* is a preferable choice as solid substrate for fermentable sugars production. This so called 'sugars syrup' as a product in this study is an ideal substrate for all microorganisms, and thus can be used in making a variety of other products such as biopolymers, antibiotic, acetone, butanol, citric acid, lactic acid etc.

Fungi play a key role in solid substrate fermentation. Their hyphal development allows them to effectively colonise and penetrate the solid matrix (Biesebeke et al., 2002). The hyphal mode of growth gives a major advantage to filamentous fungi over unicellular

microorganisms in the colonization of solid substrate and for the utilisation of available nutrients. Considering that fungi are suitable for wide range of industrial applications (e.g. organic acids and enzymes) their exploitation to their full potential many times associated with the use of heterogeneous fermentation media (Koutinas et al., 2003) such as sago *hampas*. Members of the fungal genus *Trichoderma* have been extensively studied, particularly due to their ability to secrete cellulose degrading enzymes (Zaldivar et al., 2001). Most of the work has been carried out on strains of *T. viride, T. reesei, T. harzianum*. These strains have been extensively studied in their ability to produce extracellular cellulolytic enzymes, namely endoglucanases, exoglucanases and cellobiase, which act synergistically in the conversion of cellulose to glucose (Zaldivar et al., 2001). The use of *Trichoderma sp.* in SSF for the production of lytic enzymes such as cellulose has tremendous impact for an industrial scale production (Nampoothiri et al., 2004). *Trichoderma sp.* is also known of its amylases production as well and few studies on its ability to produce amylases have been reported and among them by Azevedo et al. (2000).

The objectives of this study were:

a. to isolate and screen sago *hampas* degrading fungus and to study its growth profile via SSF.
b. to optimise the production of reducing sugars from sago *hampas via* solid substrate fermentation by *Trichoderma* EB001.
c. to study the bioconversion of sago *hampas* to reducing sugars *via* SSF by *Trichoderma* EB001 in rotary drum bioreactor.