Faculty of Resource Science and Technology

Evaluation of *Candida glabrata* for Simultaneous Saccharification and Fermentation (SSF) of Total Sago Effluent

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Evaluation of *Candida glabrata* for Simultaneous Saccharification and Fermentation (SSF) of Total Sago Effluent

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DECLARATION

I hereby declare that the dissertation entitled, "Evaluation of *Candida glabrata* for Simultaneous Saccharification and Fermentation (SSF) of Total Sago Effluent", is based on my original work except for quotations and citations which have duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted in candidature for any degree.

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ABSTRACT

Sago palm plantations are mostly found in Sarawak and the sago industries have produced tons of wastes every year. These sago wastes could be potential biomass substrate for bioethanol production as it can be found abundantly. In bioethanol production, the ability of microorganisms to ferment substrate is important to achieve high ethanol yield. *Candida glabrata* is commonly found in fermented foods such as *ragi* and can potentially be used as a fermentative organism in bioethanol production. Hence, in this study, *C. glabrata* was evaluated to convert carbohydrate substrates to ethanol. The first objective of the study was evaluation of *C. glabrata* in production of ethanol from simple sugar such as glucose, maltose and sucrose. Fermentation process was carried out for 5 days, with 150 rpm at ambient temperature. Results indicated that *C. glabrata* only consumed glucose and sucrose with the maximum ethanol production of approximately 20.4 g/l and 1.64 g/l, respectively. Among these sugars, *C. glabrata* yielded the highest ethanol content in glucose substrate. This showed that *C. glabrata* could be a potential fermentative organism since it can ferment glucose efficiently. Next, the fermenting capability of *C. glabrata* was evaluated on complex sugars such as soluble starch and cellulose powder. Results showed that *C. glabrata* capable of fermenting soluble starch and cellulose powder only with the aid of enzymes (amylases and cellulases). In the absence of enzymes, there was no ethanol produced. This indicated that *C. glabrata* does not produce amylolytic enzymes that breakdown starch. *C. glabrata* also unable of breaking down cellulose and requires extracellular cellulase to convert cellulose into fermentable sugar. In the last objective, *C. glabrata* was subjected to Simultaneous Saccharification and Fermentation (SSF) using total sago effluent as a biomass feedstock. Prior to the SSF, compositional analysis steps were conducted. Based on the results, total sago effluent was comprised 97.14 ± 0.09% sago hydrolysate and 2.86 ± 0.09%

solid component (sago hampas). Further compositional analysis was carried out, and it was found that sago hydrolysate was containing 2.23 ± 0.86% starch while sago hampas was composed of 52.03 ± 1.11% starch, 27.82 ± 0.62% cellulose, 5.32 ± 0.96% hemicellulose, 3.00 ± 0.05% lignin and 1.95 ± 0.03% ash. SSF was conducted on total sago effluent for 5 days with the aid of enzymes (amylases and cellulases) and *C. glabrata* as a fermenting organism in a working volume of 150 ml. Results showed that the highest ethanol production was detected at 24 h, with a yield of 5.44 g/l. This indicated that *C. glabrata* could be used in bioethanol production because it can grow on lignocellulosic substrate such as total sago effluent. During the SSF, *C. glabrata* was found to be incapable of fermenting xylose and arabinose, thus resulting in low ethanol yield. Overall, *C. glabrata* has high fermentative activity for glucose, with 79.84% of theoretical yield which makes it highly suitable candidate for glucose fermentation. However, *C. glabrata* was incapable of fermenting other substrates efficiently as glucose since it only yielded low ethanol content for both sucrose and total sago effluent. Therefore, it can be concluded that *C. glabrata* is inefficient and unsuitable for industrial bioethanol processes. The efficiency and productivity of ethanol can be enhanced by performing genetic modification to increase the range of consumed sugars by *C. glabrata*.

Keywords: *Candida glabrata*; bioethanol; biomass feedstock; total sago effluent; fermentation; simultaneous saccharification and fermentation (SSF)

Penilaian tentang Candida glabrata dalam Sakrafikasi dan Fermentasi Serentak (SSF) Jumlah Efluen Sagu

ABSTRAK

Ladang sagu kebanyakannya terdapat di Sarawak dan industri sagu telah menghasilkan banyak sisa buangan setiap tahun. Sisa sagu ini berpotensi menjadi substrat bahan mentah untuk penghasilan bioetanol kerana ia boleh didapati dalam jumlah yang banyak. Dalam penghasilan bioetanol, keupayaan mikroorganisma melakukan proses fermentasi ke atas substrat adalah penting untuk mencapai hasil etanol yang tinggi. Candida glabrata biasanya terdapat dalam makanan yang ditapai seperti ragi, yang berpotensi untuk digunakan sebagai organisma fermentasi dalam penghasilan bioetanol. Oleh itu, dalam kajian ini, C. glabrata digunakan untuk proses penapaian untuk menghasilkan etanol. Objektif pertama kajian ini adalah tentang keupayaan C. glabrata dalam penghasilan etanol daripada gula mudah seperti glukosa, maltose dan sukrosa. Penapaian telah dijalankan selama 5 hari, dengan 150 rpm pada suhu ambien. Hasil kajian menunjukkan bahawa C. glabrata hanya menggunakan glukosa dan sukrosa dengan penghasilan etanol maksimum, 20.4 g/l dan 1.64 g/l. C. glabrata telah menghasilkan kandungan etanol tertinggi dalam substrat glukosa. Ini menunjukkan bahawa C. glabrata berpotensi menjadi fermentasi organisma kerana ia boleh menukar glukosa kepada etanol dengan cekap. Seterusnya, keupayaan C. glabrata dalam penapaian dinilai pada gula kompleks seperti kanji larut dan serbuk selulos. Hasil kajian menunjukkan bahawa C. glabrata boleh melakukan penapaian terhadap kanji larut dan serbuk selulos hanya dengan bantuan enzim. Tiada etanol dapat dihasilkan tanpa bantuan enzim. Ini menunjukkan bahawa C. glabrata tidak menghasilkan enzim amilolitik yang memecahkan struktur kanji larut. C. glabrata juga tidak boleh memecahkan struktur selulos dan memerlukan enzim selulase untuk menukar selulos kepada

gula. Dalam objektif akhir, C. glabrata diguna pakai untuk proses sakrafikasi dan fermentasi serentak (SSF) menggunakan jumlah efluen sagu sebagai bahan mentah. Sebelum SSF, beberapa langkah analisis telah dilaksanakan. Berdasarkan analisis, jumlah efluen sagu terdiri daripada 97.14 ± 0.09% sisa cecair (hidrolisat sagu) dan 2.86 ± 0.09% komponen pepejal (sagu hampas). Analisis yang lebih terperinci telah dijalankan, dan mendapati bahawa hidrolisat sagu mengandungi 2.23 ± 0.86% kanji manakala sagu hampas mengandungi 52.03 ± 1.11% kanji, 27.82 ± 0.62% selulosat, 5.32 ± 0.96% hemiselulosa, 3.00 ± 0.05% lignin dan 1.95 ± 0.03% abu. SSF telah dijalankan menggunakan jumlah efluen sagu selama 5 hari dengan bantuan enzim (amilase dan selulase) dan C. glabrata sebagai organisma fermentasi dalam jumlah 150 ml. Keputusan menunjukkan bahawa penghasilan etanol tertinggi dikesan pada jam ke-24, dengan hasil 5.44 g/l. C. glabrata boleh digunakan dalam penghasilan bioethanol kerana ia boleh hidup dalam substrat lignoselulosat seperti jumlah efluen sagu. Semasa SSF, C. glabrata didapati tidak dapat melakukan proses penapaian terhadap xylosa dan arabinosa, sekaligus meyebabkan penghasilan etanol yang rendah. Secara keseluruhannya, C. glabrata mempunyai aktiviti yang tinggi untuk glukosa, dengan 79.84% daripada hasil teori dan menjadikan ia berpotensi dan sangat sesuai untuk proses penapaian glukosa. Walau bagaimanapun, C. glabrata kurang efisien terhadap substrat lain kerana ia hanya menghasilkan kandungan etanol yang rendah untuk sukrosa dan jumlah efluen sagu. Kesimpulannya, C. glabrata adalah tidak cekap dan tidak sesuai untuk proses industri bioetanol. Kecekapan dan produktiviti etanol boleh dipertingkatkan dengan melaksanakan pengubahsuaian genetik untuk mempelbagai rangkaian gula yang digunakan oleh C. glabrata.

Kata kunci*: *Candida glabrata; bioetanol; bahan mentah biojisim; jumlah efluen sagu; sakrafikasi dan fermentasi serentak (SSF)

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LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
ADH	Alcohol dehydrogenase
ANOVA	One-way analysis of variance
ATP	Adenosine triophosphate
C_2H_5OH	Ethanol
CH_3CH_2OH	Ethanol
$C_6H_{12}O_6$	Glucose
CBD	Carbohydrate binding domain
CBH	Cellobiodehydrase
C. glabrata	*Candida glabrata*
CO_2	Carbon dioxide
EMP	Embden-Meyerhof-Parnas
EtOH	Ethanol
FPU	Filter paper unit
g	Gram
g/cm^3	Gram per centrimetre
GHG	Green-house gases
g/l	Gram per liter
h	Hour
HPLC	High performance Liquid Chromatography
Kg	Kilogram
Kton/year	Kiloton per year

M	Molar
mg/g	Miligram per gram
MJ/dm^3	Megajoule per cubic decimetre
MJ/Kg	Megajoule per kilogram
mm	Milimetre
ml	Mililitre
ml/min	Mililitre per minute
min	Minutes
NDF	Neutral Detergent Fibre
nm	Nanometer
PDC	Pyruvate decarboxylase
pH	Potential of hydrogen
PSA	Phenol sulfuric acid
p-value	Probability value
RBDC	Rose Bengal Chloramphenicol Agar
RFA	Renewable Fuels Association
RI	Revolutions per minute
RID	Refractive index
rpm	Refractive index
S.cerevisiae	*Saccharomyces cerevisiae*
SD	Standard deviation
SHF	Separate hydrolysis and fermentation
SSF	Simultaneous saccharification and fermentation
TEY	Theoretical ethanol yield

tons	Tonnes
µl	Microlitre
µm	Micrometre
v/v	Volume per volume
w/v	Weight per volume
w/w	Weight per weight
YM	Yeast Malt
^{O}C	Degree Celsius

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background of the study

The demand for energy continues to rise due to population growth and developing countries (Robak & Balcerek, 2018). The continuous consumption of fossil fuel would lead to a shortage of energy resources. Furthermore, the intensive use of fossil fuels has caused greenhouse gases (GHG) emissions like carbon dioxide (Okkerse & van Bekkum, 1999; Solomon & Qin, 2013). Therefore, it is crucial to find other alternatives to sustain our energy resources. Biofuel has been introduced as one of the alternatives to substitute non-renewable energy. In fact, biofuels have been used for years with the purpose to increase energy self-sufficiency, minimize import costs and strengthen domestic agricultural development (Kovarik, 2013; Araújo, 2017). Bioethanol (CH_3CH_2OH) from biomass is one of the most widely used biofuels across the world. Bioethanol has been used as prime renewable biofuel for transportation sector in some countries such as Brazil, US other European countries (Hahn-Hägerdal et al., 2006).

According to Vohra et al. (2014), bioethanol is a liquid biofuel produced from biomass feedstocks and conversion technologies. The important key-point in the production of bioethanol from biomass is the conversion of the residual materials (biomass) into advantageous products like fermentable sugars (Mezule et al., 2015) and bioethanol derived from sugar fermentation (Shahsavarani et al., 2013). Biomass refers to any organic matter that comes from biogenic sources and is available on a renewable basis (Ahorsu et al., 2018). In bioethanol production, the suitable biomass should allow the growth of the microorganism