Faculty of Resource Science and Technology

Evaluation of Strategies for Lysing Spent Baker's Yeast Generated Upon Production of Sago Bioethanol

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Signature

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ABSTRACT

Spent Baker's yeast (*Saccharomyces cerevisiae*), a by-product of the ethanol production industry, is mainly discarded as a waste. The spent yeast can be converted to yeast extract by the lysis process, in which the yeast cells are disintegrated under specific conditions to release significant bioactive components. To date, studies on the lysis strategies of spent *S. cerevisiae* produced after sago bioethanol synthesis is still limited. The present work was aimed to evaluate the feasibility of autolysis and enzymatic hydrolysis of spent *S. cerevisiae* produced following sago bioethanol fermentation. The autolysis of spent *S. cerevisiae* was studied according to the effect of initial pH (3, 5, and 7) and incubation time (24, 48, 72, and 96 hours). Secondly, the enzymatic hydrolysis was investigated using two different enzyme types (alcalase and cellulase) with varying enzyme doses (0.1, 0.2, 0.3, 0.4, and 0.5 %, v/v). The resulting lysates were analysed for protein and carbohydrate concentrations using Lowry assay and phenol-sulphuric acid assay, respectively. The lysed yeast cells were observed for their morphological characteristics using a scanning electron microscope. The results indicated that after 72 hours of incubation, the autolysis of spent Baker's yeast at an initial pH of 3 produced the maximum concentration of protein and carbohydrate in the autolysate. These were 2.5 and 2-fold higher than the concentrations of protein and carbohydrates in the control samples, respectively. Meanwhile, in enzymatic hydrolysis, the highest protein and carbohydrate concentration of lysate were achieved from the hydrolysis of spent Baker's yeast using cellulase (0.5%, v/v) and alcalase (0.4%, v/v), respectively. The enhancement of protein and carbohydrate concentrations were 8.6-fold and 11.4-fold, respectively as compared to that achieved by the control experiment. The surface morphology analysis of lysed yeast cells resulting from autolysis and enzymatic hydrolysis of spent *S. cerevisiae* under the aforementioned conditions were parallel with the lytic events occurred during both

processes. The best strategies for lysing spent Baker's yeast generated upon production of sago bioethanol was enzymatic hydrolysis, considering to the high release of protein and carbohydrate concentration as compared to autolysis. The current work provides early insights into the potential strategies of valorising spent *S. cerevisiae,* generated from sago bioethanol production, where the resulting yeast extract can be used for various industrial applications.

Keywords: Autolysis, enzymatic hydrolysis, *Saccharomyces cerevisiae,* spent Baker's yeast, yeast extract

Penilaian terhadap Strategi untuk Memecahkan Sisa Yis Roti yang Terhasil daripada Produksi Bioetanol Sagu

ABSTRAK

*Sisa yis roti (*Saccharomyces cerevisiae*), iaitu produk sampingan daripada industri fermentasi etanol kebanyakannya dibuang sebagai hasil buangan. Sisa yis boleh ditukar menjadi yis ekstrak melalui proses lisis, di mana sel-sel yis dipecahkan dalam keadaan yang spesifik untuk merembeskan kandungan-kandungan bioaktif yang penting. Sehingga kini, kajian mengenai strategi lisis terhadap sisa* S. cerevisiae *yang dihasilkan berikutan produksi bioetanol sagu masih terhad. Penyelidikan ini bertujuan untuk menyiasat kebolehlaksanaan autolisis dan hidrolisis enzim terhadap sisa* S. cerevisiae *daripada fermentasi bioetanol sagu. Autolisis yis roti dikaji berdasarkan kesan pH awal (3,5, dan 7) dan tempoh inkubasi (24, 48, 72, dan 96 jam). Kedua, hidrolisis enzim telah dikaji menggunakan dua jenis enzim yang berbeza (alcalase dan cellulase) dan kepekatan enzim yang berbeza (0.1, 0.2, 0.3, 0.4, dan 0.5%, v/v. Produk lisis yang terhasil dianalisa untuk kepekatan protein dan karbohidrat masing-masing menggunakan ujian Lowry dan ujian asid fenol-sulfurik. Ciri-ciri morfologi sel yis yang dipecahkan, dicerap menggunakan mikroskop elektron pengimbas. Hasil kajian menunjukkan selepas 72 jam inkubasi, autolisis sisa yis roti pada pH permulaan 3 menghasilkan kepekatan protein dan karbohidrat tertinggi dalam produk autolisis. Ini adalah 2.5 dan 2 kali ganda lebih tinggi daripada kepekatan protein dan karbohidrat, masing-masing dalam sampel kawalan. Sementara itu, dalam hidrolisis enzim, kepekatan protein dan karbohidrat tertinggi dalam produk hidrolis enzim telah tercapai daripada hidrolisis sisa yis roti masing-masing menggunakan cellulase (0.5%, v/v) dan alcalase (0.4%, v/v). Peningkatan untuk kepekatan protein dan karbohidrat masing-masing adalah 8.6 kali ganda dan 11.4 kali ganda berbanding yang telah dicapai daripada eksperimen*

kawalan. Analisa morfologi permukaan sel yis yang telah dipecahkan hasil daripada autolisis dan hidrolisis enzim terhadap sisa S. cerevisiae *menggunakan keadaan yang telah dinyatakan adalah selari dengan keadaan lisis yang berlaku daripada kedua-dua proses. Strategi terbaik untuk memecahkan sis yis Baker yang dihasilkan selepas penghasilan bioetanol sagu ialah hidrolisis enzim, berikutan penghasilan kepekatan protein dan karbohidrat yang tinggi berbanding dengan autolisis. Kajian ini memberikan pemahaman awal tentang strategi yang berpotensi dalam meningkatkan penggunaan sisa* S. cerevisiae *yang dihasilkan daripada produksi bioetanol sagu, di mana ekstrak yis yang terhasil boleh digunakan untuk pelbagai aplikasi perindustrian.*

Kata kunci: *Autolisis, hydrolisis enzim,* Saccharomyces cerevisiae*, sisa yis roti, ekstrak yis*

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LIST OF ABBREVIATIONS

β	Beta
µm	Micrometer
µg/mL	Microgram per mililitre
AN	Amino nitrogen
ANOVA	Analysis of variance
AU	Avson unit
BSA	Bovine serum albumin
CAZyme	Carbohydrate active enzyme
CBD	Cellulose binding domain
CBM	Carbohydrate binding module
CD	Catalytic domain
CG	Commercial glucose
DNA	Deoxyribonucleic acid
EC	Enzyme commission
ER	Endoplasmic reticulum
FAO	Food and agricultural organisation
g/L	Gram per litre
GHs	Glycoside hydrolases
GPI	Glycosylphosphatidylinositol
GRAS	Generally recognised as safe
GTs	Glycosyltransferases
HPLC	High performance liquid chromatography
hr	Hour

Leu	Leucine
M	Molarity
Met	Methionine
MOS	Mannanoligosaccharides
mg/g	Miligram per gram
mg/mL	Miligram per mililitre
mg/L	Miligram per litre
min	Minute
mm	Milimetre
mM	Milimolar
MSG	Monosodium glutamate
nm	Nanometre
N	Normality
OD	Optical density
OH	Hydroxyl group
PDA	Potato dextrose agar
Phe	Phenylalanine
PLs	Polysaccharide lyases
RNA	Ribonucleic acid
rpm	Revolutions per minute
S. cerevisiae	*Saccharomyces cerevisiae*
SEM	Scanning electron microscopy
SFH	Sago fibre hydrolysate
SFP	Sago fibre powder
TN	Total nitrogen

Tyr	Tyrosine
U/g	Unit per gram
v/v	Volume per volume
w/v	Weight per volume
w/w	Weight per weight
YCW	Yeast cell wall
YDE	Yeast distillery effluent
YE	Yeast extract
YMB	Yeast malt broth

CHAPTER 1

INTRODUCTION

1.1 Study Background

The exploitation of Baker's yeast (*Saccharomyces cerevisiae*) in the fermentation sector has been widely utilised. This in turn, generates large amount of waste products, including spent yeast (Jacob et al., 2019a). The spent yeast in general, is typically used either as a low-cost protein source in animal feed formulations or discarded to the environment causing severe ecological impacts (Podpora et al., 2015; Jaeger et al., 2020). However, the waste constitutes of many valuable compositions such as proteins, essential amino acids, RNA, vitamins B, and minerals which essentially serves as a raw material for the production of yeast extract (Vieira et al., 2016; Jacob et al., 2019a).

Yeast extract refers to soluble portions of yeast cells after the separation of the insoluble components after the lysis of yeast cells (Vukašinović-Milić, 2007). Spent yeast extracts show promise as a source of valuable nutrients such as bio-active components and amino acids for use in functional food, nutritional supplements, and as a component for microbial growth medium. In addition, yeast extract has also been widely used as a flavouring agent and flavour enhancer in food industry (Boonraeng et al., 2000). It is possible to recover the extracts by disrupting the yeast cells by the processes of autolysis or enzymatic hydrolysis, which involve the utilisation of either endogenous or exogenous enzymes (Li et al., 2015; Takalloo et al., 2020).

1.2 Problem Statement

Previously, studies on the autolysis and enzymatic hydrolysis of spent Baker's yeast have commonly utilised either fresh yeast or yeast generated from fermentations, supplemented with synthetic feedstocks. Currently, the information regarding the exploitation of *S. cerevisiae* obtained from the fermentation of bioethanol employing agricultural waste, such as sago fibre is limited. Bioethanol generation from sago fibre has been documented in the literature (Awg-Adeni et al., 2010). While sago bioethanol has the potential to meet expanding industrial demand, its growth will almost certainly be accompanied by an increase in waste output. The possible fate of the waste produced after sago bioethanol production is necessary to be investigated to ensure the sustainability of the entire process in sago bioethanol biorefinery.

One of the sources of spent Baker's yeast is bioethanol fermentation. The spent Baker's yeast is discarded from the fermentation as a solid waste. Following bioethanol production, the wastes generated were discarded to landfill or as wastewater. Previously, utilisation of sago bioethanol liquid waste for production of an industrial biocatalyst has been reported by Mamat et al. (2021). However, there is no work reported for the autolysis and enzymatic hydrolysis of spent *S. cerevisiae* generated from sago bioethanol fermentation. Bioethanol solid waste is currently used as a component of animal feeds in ruminants and aquaculture for its cheap sources of protein, B vitamins, and minerals (Oliveira et al., 2016; San Martin et al., 2020). Additionally, it was reported that spent *S. cerevisiae* before the primary fermentation in a brewing process was recycled in small amounts to start the following batch of fermentation before being discarded (Vieira et al., 2012; Marson et al., 2019). Therefore, appropriate techniques to manage and exploit the wastes created following the bioethanol production should be proposed in order to ensure that the whole process is

both cost-effective and sustainable. A fundamental inquiry of the characteristics and uses of the sago bioethanol waste stream, as investigated in this research, will help to illuminate the potential application of the waste stream.

1.3 Aim and Objectives

The aim of this work is to establish effective strategies for lysing spent Baker's yeast generated after the production of sago bioethanol. The objectives of the work are as follow:

i. To evaluate the effectiveness of different lysis strategies (autolysis and enzymatic hydrolysis) for lysing spent Baker's yeast generated after the production of bioethanol from sago fibre.

ii. To determine the properties of the resulting yeast lysates obtained from autolysis and enzymatic hydrolysis with particular emphases on its protein and carbohydrate contents, and surface morphology of the lysed yeast cells.

CHAPTER 2

LITERATURE REVIEW

2.1 Baker's Yeast (*Saccharomyces cerevisiae*)

In biological terms, *Saccharomyces cerevisiae* is classified as a unicellular eukaryote under kingdom fungi. It was the first eukaryotic organism that was sequenced in 1996 (Goffeau et al., 1996), and to date has proven to be the most ideal organism for study in biology. It is ellipsoid in shape and the size of an individual cell has a diameter ranging from 5–10 µm and 1-7 µm for large and smaller diameter, respectively (Montes de Oca et al., 2016). *S. cerevisiae* is ubiquitous and may be isolated from variety of sources, including soil, water, plants, animals, and even insects (Starmer & Lachance, 2011). The structural components of *S. cerevisiae* include a rigid cell wall, nucleus, mitochondria, endoplasmic reticulum (ER), Golgi apparatus, vacuoles, microbodies, and secretory vesicles with a complex network of membranes within and outside of the cell (Montes de Oca et al., 2016). Figure 2.1 shows the surface morphology of *S. cerevisiae*.

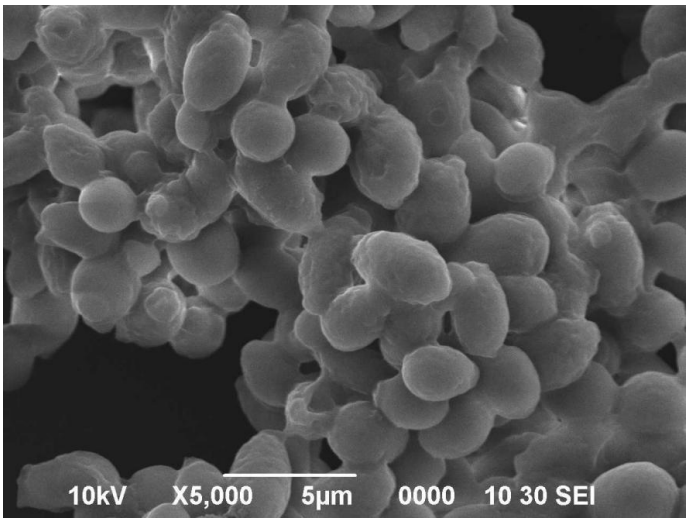

Figure 2.1: Scanning electron micrograph of *S. cerevisiae* (×5000).
Adapted from Mahmod et al. (2021)

The cell reproduces both vegetatively and sexually through the process of ascospore production and multilateral budding, respectively (Stewart, 2014). *S. cerevisiae* thrives in environments with a pH between 4.5 and 6.5 and a temperature range of about 28 to 33°C for optimal growth due to its acidophilic nature (Jach et al., 2022). In addition to water and oxygen, *S. cerevisiae* requires sufficient amounts of macronutrients including carbon, free amino nitrogen, sulphur, phosphorus potassium, and magnesium to meet its nutritional needs. Apart from that, calcium, copper, iron, manganese, and zinc are required serving as micronutrients, known as trace elements for the yeast cells (Walker & Stewart, 2016). According to Ljungdahl and Daignan-Fornier (2012), yeast cells prefer nitrogen sources derived from amino acids alanine, arginine, asparagine, aspartate, glutamate, glutamine, and serine.

Among all yeast strains, *S. cerevisiae*, referred to as Baker's yeast, is most recognised for its role in fermentation processes for the food, beverage, and biofuel production industries (Parapouli et al., 2020). In biotechnological application, *S. cerevisiae* is the world's most exploited microorganism, responsible for the development of ethanol production, one of the most valuable biotechnological products, by converting carbohydrates (glucose or maltose) into ethanol and carbon dioxide (Chang et al., 2018). Moreover, the ethanol fermentation by *S. cerevisiae* involves in anaerobic respiration through glycolysis pathway. In the absence of oxygen, glucose provides the cell with essential molecules such as carbon, hydrogen and oxygen. During fermentation of ethanol ($C_3H_4O_3$), two molecules of pyruvic acid are formed from a single molecule of glucose ($C_6H_{12}O_6$). Two molecules of acetaldehyde (CH_3CHO) are produced by further decarboxylating pyruvic acid, and these molecules are then reduced to ethanol (C_2H_5OH). Finally, two molecules of ATP are gained for every molecule of glucose converted into two molecules of ethanol and two molecules of carbon dioxide (CO_2)

during the process (Akhtar et al., 2017). Figure 2.2 shows the glycolysis pathway during ethanol fermentation by *S. cerevisiae.*

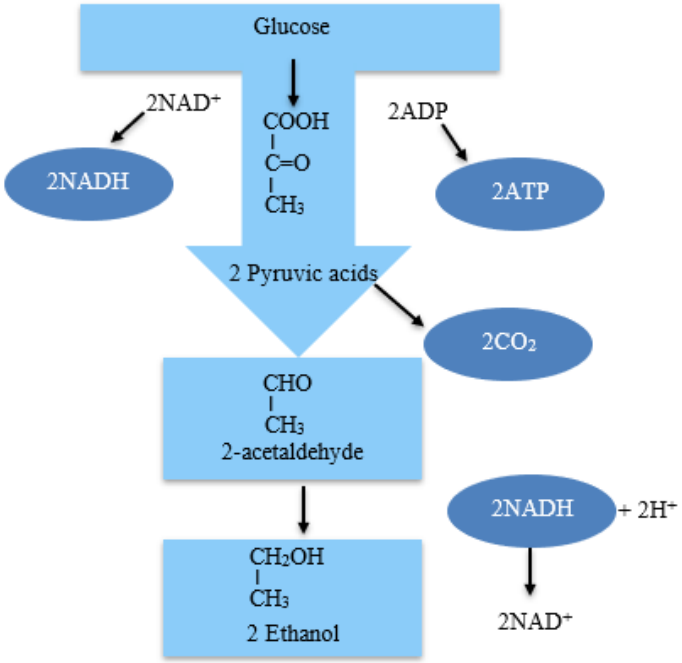

Figure 2.2: Ethanol fermentation pathway by *S. cerevisiae*. Adapted from Akhtar et al. (2017)

This well-studied organism dominates in fermentation process in ethanol production due to its high fermentation ability, fast growth rates, high ethanol yield as well as keeping low cost of distillation. *S. cerevisiae* is an ideal microorganism for industrial-scale fermentation due to its ability to survive high temperatures, acidic environment, and a high concentration of substrates and ethanol (Kasavi et al., 2012; Lin et al., 2012; Morales et al., 2015). There are many strains that can thrive in an environment with 8–12% ethanol (v/v), including Variant M1 and Yeast distillery effluent (YDE) strain (Tikka et al., 2013; Qiu & Jiang, 2017). Moreover, it was reported that a UV-C mutagenesis mutant strain, UVNR56 and genetically modified industrial fuel ethanol CAT-1 strain, exhibited considerably higher viability during ethanol fermentation in the presence of 15% and 16% (v/v), respectively