Faculty of Resource Science and Technology

BIOETHANOL PRODUCTION FROM OFFICE WASTE USING *Saccharomyces cerevisiae* VIA SIMULTANEOUS SACCHARIFICATION FERMENTATION (SSF)

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Bachelor of Science with Honours
(Biotechnology Resource)
2012

ACKNOWLEDGEMENT

Alhamdulillah, all praises to Allah for the strength and His blessing in completing this thesis. Special appreciation goes to my supervisor, Dr Micky Vincent and my co-supervisor Pn. Dayang Salwani Awang Adeni for their supervision and constant support. Their valuable help and constructive comments and suggestions throughout the experimental and thesis work have contributed to the success of this research.

I would like to express my appreciation to the all the lecturers and the technician of Faculty of Resource Science and Technology especially Mr. Azis Bin Ajim and Mr. Leo Bungin for their co-operation. A million thanks to Christy, Vel and Ziana as my senior lab mates and loyal advisor in the Microbiology Lab for your patient and guidance on my lab works and reports.

My deepest gratitude goes to my parent, Mr Hambali Bin Jamaluddin, Mrs Mahazan binti Abdul Mutalib and also to my sisters Nor Hamizah and Nor Hairina for their endless love, prayers and encouragement. Sincere thanks to all my course mates and laboratory mates for their kindness and moral support during my study. Thank you for the friendship and memories. My appreciation also goes to my beloved boyfriend Wan Al-Junaidi Bin Jamil for his assistance and accompany in completing my research.

Lastly, it is impossible to list the entire individuals that have been with me in this research, thus, I dedicate my utmost gratitude to all of them. To those who indirectly contributed in this research, your kindness means a lot to me. Thank you very much.

DECLARATION

I hereby declare that no portion of the work referred in this project has been submitted in support of an application for another degree qualification of this or any other university or institution of higher learning.

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LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
cm	centimetre
DNA	Deoxyribonucleic acid
DNS	Dinitrosalicylic
FPU	Filter Paper Unit
g	gram
hr	Hour
HPLC	High Performance Liquid Chromatography
IUPAC	International Union of Pure Applied Chemistry
Kg	Kilogram
LB	Luria Bertani
M	Molar
mg	milligram
ml	millilitre
NaOH	Sodium Hydroxide
NREL	National Renewable Energy Laboratory
rpm	Revolution per Minute
SPSS	Statistical Package for the Social Science
SSF	Simultaneous Saccharification Fermentation
TYE	Theoretical Yield Ethanol

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Production of Bioethanol from Office Waste using *Saccharomyces cerevisiae* via Simultaneous Saccharification Fermentation (SSF)

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ABSTRACT

Bioethanol is an attractive and sustainable energy source to fuel transportation. It is seen as a good alternative fuel as it emits zero net carbon output into the atmosphere. In this study, bioethanol was produced from office waste by using *Saccharomyces cerevisiae.* Prior to the laboratory experimentations, a mini survey was conducted to collect and determine the approximate amount of the office waste being used weekly by respondents. Simultaneous saccharification and fermentation (SSF) experiments were then performed to utilize the enzymatic bond breaking of the office waste into fermentable sugar in order to produce ethanol. The final fermentation products were analysed via DNS reducing sugar assay and phenol-sulphuric total carbohydrate assays and High Performance Liquid Chromatography (HPLC). This study has shown that office waste can be converted to bioethanol from *S. cerevisiae* via SSF. From the result obtained, 5% for 50 FPU/g paper give an effective production of ethanol which is 70.99% as it utilized the glucose faster with low amount of lactic acid and acetic acid.

Keywords: Bioethanol, DNS, High Performance Liquid Chromatography, Office waste, Phenol-sulphuric, *Saccharomyces cerevisiae*.

ABSTRAK

Bioetanol merupakan salah satu tarikan dan sumber tenaga yang seimbang terhadap minyak kenderaan. Ia dilihat sebagai minyak alternatif kerana ia tidak membebaskan karbon ke atmosfera. Dalam kajian ini, kami menghasilkan bioetanol daripada bahan buangan pejabat dengan menggunakan Saccharomyces cerevisiae*. Satu mini kaji selidik telah dijalankan bagi mengumpul dan mengenalpasti jumlah bahan buangan pejabat yang telah digunakan oleh responden. Teknik SSF telah dijalankan bagi melihat pemecahan jalinan enzim bahan buangan pejabat kepada gula dan menghaslkan etanol. Produk fermentasi yang terakhir dianalisa menggunakan ujian DNS, phenol-sulfurik dan Kromatograf Cecair Berprestasi Tinggi (KCBT). Kajian ini telah menunjukkan bahan buangan pejabat boleh menghasilkan bioetanol menggunakan* S. cerevisiae *melalui kaedah SSF. Daripada kajian ini, 5% bagi 50 FPU/g kertas menghasilkan bioetanol dengan efektif iaitu sebanyak 70.99% kerana ia menghabiskan glukos secara pantas dan menghasilkan asid laktik serta asid asetik pada kadar yang rendah.*

Kata kunci: Bioetanol, DNS, Kromatografi Cecair Berprestasi Tinggi (KCBT), Bahan buangan pejabat, Phenol-sulfirik, Saccharomyces cerevisiae.

CHAPTER 1

INTRODUCTION

1.1 Introduction

In recent years, environmental problems have become very controversial issues. The usage of fossil fuels in the transportation sector is causing the emission of greenhouse gases that pollute the environment. Thus, biofuel such as bioethanol as an alternative fuel is attracting a lot of interest because of its zero net carbon output into the atmosphere as it is recycled through photosynthesis (Ohgren et al., 2006; Araque et al., 2008; Yamashita et al., 2010). According to the literatures, ethanol can be describe as an exotic synthetic oxygen-containing organic chemicals because of its unique combination of properties as a solvent, germicide, antifreeze, fuel, a depressant and especially of its versatility as a chemical intermediate for other chemicals (Ingledew, 1999; Pramanik, 2003; Favela et al. 2005 and Pramanik, 2005).

Ethanol is traditionally produced from a liquid or a fluid mash via submerged microbial fermentation (Hang et al., 1981). *Saccharomyces cerevisiae* which is also known as brewer's yeast is the most widely used fermentation microbe for ethanol production (Gunasekaran and Chandra, 1999; Roehr, 2001; Michika, 2007) besides *Zymomonas mobilis* and *Escherichia coli*. Mature technologies for bioethanol production are crop-based, utilizing substrates such as sugar cane, cane juice and cornstarch. However, the cost of raw material can be as high as 40% of the bioethanol cost (von Sivers et al., 1994; Wyman, 1999). Recent effort concentrate on utilizing lignocelluloses as this natural and

potentially cheap and abundant polymer is found as agricultural waste, industrial waste, and forestry residue, municipal solid waste as well as office waste (Wiselogel et al., 1996).

In this study, we performed bioethanol production from office waste by using *S. cerevisiae.* This study focused on the effects of the different enzyme concentration and effect of the feedstock loading on the saccharification and fermentation rate of the office waste.

1.2 Problem Statements

The environment and sustainability are pressing matters today. Tackling the global warming and environmental issues while supplying energy to meet the biofuels demand has become central to the problem. Thus, in this study we are going to produce bioethanol from the office waste using *S. cerevisiae.* According to Global Environment Centre (n.d.), over 23,000 tonnes of waste is produced each day in Malaysia. This amount is expected to rise to 30,000 tonnes by the year 2020. Thus, producing bioethanol from these wastes can reduce environmental problems as well as provide cheap raw materials for the bioethanol production.

The simultaneous saccharification and fermentation (SSF) method is a method that consolidates hydrolyses of cellulose with the direct fermentation of the produced glucose. This method is very desirable as it lowers contamination; decrease the initial osmotic stress of yeast by avoiding the usage of concentrated glucose solution and generally more energy-efficient (Xiaojian et al., 2008). In this study, we will carry out the SSF method to produce the bioethanol from office waste.

1.3 Objectives

The objectives of this research are:-

1. To conduct a mini survey on the approximate amount of paper being generated by the UNIMAS's academic staff, admin staff and students, and also gather the perception of bioethanol production and utilization among the correspondents.
2. To produce bioethanol from office waste by using *Saccharomyces cerevisiae.*
3. To study the effect of different enzyme concentration on the saccharification fermentation rate of office waste.
4. To study the effect of feedstock loading on the saccharification fermentation rate.

CHAPTER 2

LITERATURE RIVIEW

2.1 Office Waste

According to the United Nations Statistics Division (UNSD) (n.d.), wastes are the materials that are not prime products for which the generator has no further use in terms of their own purposes of production, transformation or consumption. Waste may be generated during the extraction of raw materials, the processing of raw materials into intermediate and final products. Apart from that, waste also includes all the items that people no longer have any use for which they intend to dispose.

The Global Environment Centre (n.d.) indicates that over 23,000 tonnes of waste is produced each day in Malaysia. However, this amount is expected to rise to 30,000 tonnes by the year 2020. A middle-income country such as Indonesia, Malaysia and Thailand generates 0.52 and 1.0 kg of municipal solid waste (MSW) per capita per day. This MSW is mainly made up of waste coming from offices, households, shops and other institutions. The major components of MSW are office waste (papers), plastics, metal and glass. Paper products are considered to be the largest group in municipal solid waste which is about 55% of the total waste (Muttamara et al., 1994). The approximate composition of municipal solid waste in selected cities was shown in Figure 1.

In Kuala Lumpur in 2010, the percentage of office waste being produce is about 15% (anon, n.d.). The most common office waste is paper based. This cellulosic material can be broken down into glucose and converted into ethanol by fermentation process (anon, n.d.). And because of its abundance and low costs, this waste makes a very ideal feedstock for ethanol production.

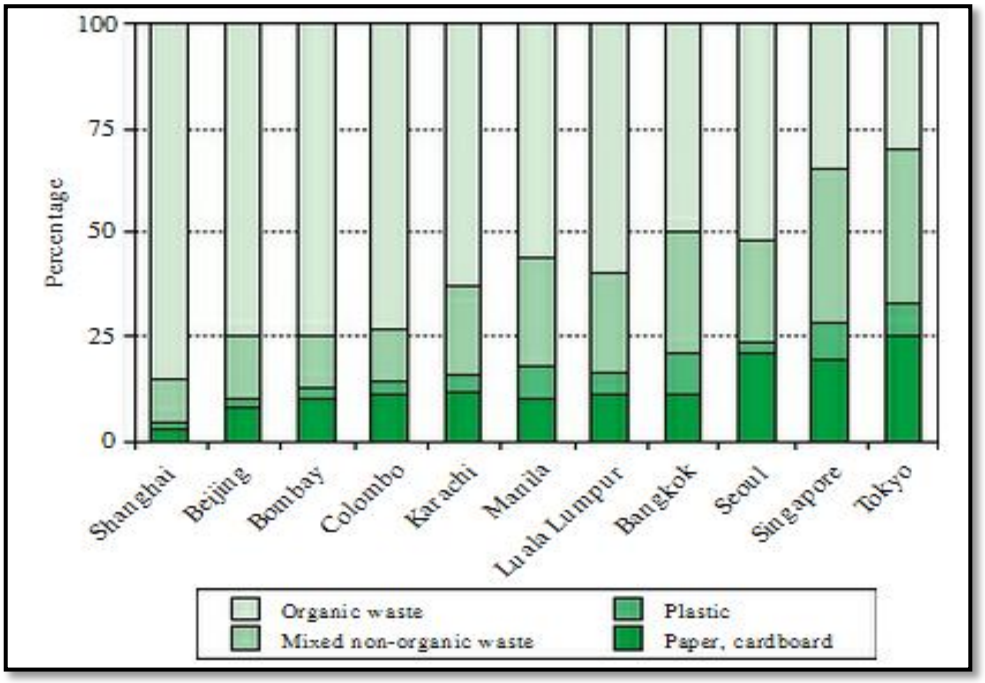

Figure 1: The approximate composition of municipal solid waste in selected area (United Nation, 1995)

2.2 Bioethanol

Ethanol or ethyl alcohol is a clear colourless liquid which is biodegradable, low toxicity and causes little environment pollution. Ethanol burns completely to produce carbon dioxide and water. It is a high octane fuel and has replaced leads as an octane enhancer in

petrol (Altıntas et al., 2002). Bioethanol is an environmentally friendly fuel for vehicles. It is a renewable source of energy and can reduce the demand on fossil fuels. Furthermore, it burns more cleanly and also reduces the overall emission of carbon dioxide (Bawa, 2008).

Bioethanol can be described as the principle fuel used as a petrol substitute for road transport vehicles. It is mainly produced by the sugar fermentation process, although it can also be manufactured by the chemical process of reacting ethylene with steam. The main sources of sugar required to produce ethanol come from starchy plants or energy crops.

Bioethanol is seen as a good alternative fuel. This is because the feedstock crops can be grown renewably and in most climates around the world. Bioethanol is generally considered carbon dioxide neutral because in the growing phase of the feedstock crops, carbon dioxide absorbed by the plant and oxygen released is in the same volume. This is an advantage over fossil fuel which only emits carbon dioxide as well as other poisonous emissions (Olfert et al., 2007).

2.3 *Saccharomyces cerevisiae* (ATCC 24859)

Saccharomyces cerevisiae is a eukaryotic microbe which is globular shaped, yellow-green yeast that belong to the fungi kingdom (Landry et al., 2007). Its cell wall is made of chitin, with no peptidoglycan and its lipids are ester linked. It is considered a yeast because it is a unicellular organism. *S. cerevisiae* has adapted in several important ways. They are able to break down their food through both aerobic respiration and anaerobic fermentation. They

can also survive in an oxygen deficiency condition for an extended period (Madigan et al., 2006). *S. cerevisiae* is used extensively in batch fermentation to convert sugar to ethanol for the production of beverages and biofuels as it is capable of very rapid rate of glycolysis under optimal conditions, producing over 50 mol of ethanol per h per g of cell protein (Casey et al., 1986).

2.4 Lignocellulosic Biomass

Developing ethanol as both an additive and an alternative to fuel will require developing lignocellulosic biomass as a feedstock because of its abundance and low cost (Badal et al., 2005). Lignocellulosic biomass have been recognised as potential feedstocks for ethanol production due to its abundance which include wood, grass, grains, or indigestible plants (Zhang et al., 2009). Lignocellulose is a non-digestible substance which is comprised of cellulose, hemicellulose, and lignin. Of the three components in lignocellulose, cellulose and hemicellulose can be broken down to glucose and converted by yeast to produce ethanol.

2.5 Cellulase

Cellulose is the major components in all lignocellulosic wastes that can be hydrolysed to glucose by using a group of enzymes called cellulases. Cellulase consists of at least three types of enzymes which are endoglucanases, exoglucanases and β-glucosidases. The

cellulase enzymes hydrolyze cellulose to D-glucose, which in turn is fermented to ethanol by yeast (Krishna et al., 2001).

2.6 Simultaneous Saccharification and Fermentation (SSF)

Simultaneous saccharification and fermentation (SSF) is a process that utilizes the enzymatic saccharification of cellulose by cellulosic enzymes and fermentation of resulting sugars to ethanol by a fermentation microorganism that occurs at the same time. This process has been studied for several decades and shows great potential for the economic production of bioethanol (Takagi et al., 1977). SSF combines two steps in the same vessel to generate ethanol which the first step is enzymatic break down of the complex sugars into glucose and followed by fermentation of the glucose into ethanol by yeast (Asli et al., 2008).

According to Dowe et al. (2008), SSF is the chosen method to produce ethanol from lignocellulosic biomass as this process alleviates end-product inhibition of the enzymes, and is also less capital intensive than separate hydrolysis and fermentation (Wingren et al., 2003). Furthermore, SSF has been shown to be superior to SHF in terms of overall ethanol yield (Galbe et al., 2005). There are few advantages of SSF. The primary advantage is cost saving resulting from the reduction in the quantity of reactor vessels required (Chadha, 1995). Apart from that the advantage of SSF is the increase of hydrolysis rate by conversion of sugars inhibiting the cellulose (Barron, 1995). Furthermore, the presence of

ethanol in the culture medium leads to reduced potential for microbial contamination (Wu et al., 1998).

2.7 Reducing Sugar Assay (Dinitrosalycylic Acid Assay)

Dinitrosalicylic assay is one of the most common method used for reducing sugar assay. It is also recommended by the IUPAC commision on biotechnology for measuring standard cellulase activities against filter paper. The DNS assay also function to measure amylases, pectinases, xyloglucanases and xylanase activities.

2.8 High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) is a highly improved form of column chromatography. It is used increasingly in the analysis of sample to separate and detect additives and contaminants. This method breaks down the complex mixtures into individual compounds on the basis of their polarity, which in turn are identified and quantified by detectors and data handling systems (Angelika et al., 2001). In addition, HPLC is also used to purify and quantify the compounds.

2.9 Filter Paper Unit (FPU) Assay

Filter Paper Unit (FPU) assay is a specific enzyme activity assay that is performed using the protocol described by the official National Renewable Energy Laboratory (NREL) procedure (Adney and Baker, 2008). This method is based on the International Union of Pure and Applied Chemistry (IUPAC) guidelines to determine cellulase activity in terms of "filter-paper units" (FPU) per milliliter (FPU/mL) of an original enzyme solution (Ghose, 1987). In this method, the glycosidic bond cleavage is detected by the parallel and identical treatment of three categories of experimental tubes which are assay mixtures, blanks and controls, and glucose standard. According to NREL (2008), the substrate used is a 50 mg Whatman No. 1 filter paper strip (1.0 x 6.0 cm).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

The materials used in this study were:-

1. Office waste (paper)
2. Cellulase enzyme (Accelerase 1500, Gnencore Company, United States)
3. *Saccharomyces cerevisiae* (ATCC 24859)
4. 10X YP Solution
    i. 10 g yeast
    ii. 20 g peptone
    iii. 1000 ml citrate buffer
5. DNS reagent
    i. 1 g dinitrosalicylic acid
    ii. 200 mg crystalline phenol
    iii. 50 mg sodium sulphite
    iv. 100 ml 1% NaOH
6. 1 M Citrate buffer
    i. Citric acid monohydrate
    ii. Deionised water
    iii. Sodium Hydroxide
7. Distilled water
8. Phenol 5%

9. Sulphuric acid 96%
10. Standard glucose stock

3.2 Methods

3.2.1 Mini Survey

A survey regarding the office paper usage and waste was conducted on 80 respondents which include 60 students, 10 lecturers and 10 support staff of University Malaysia Sarawak (UNIMAS). The mini survey was conducted to assess the respondents' knowledge on bioethanol and possibilities of converting office waste to bioethanol. Besides that, the objective of the questionnaire was also to determine the amount of office waste being generated by the respondents. A sample of the questionnaire is shown in Appendix A.

3.3 Cellulase Activities Determination-Filter Paper Unit (FPU)

The cellulose activity assay was performed according to the International Union of Pure Applied Chemistry (IUPAC).