FERMENTATIVE PRODUCTION OF BUTANOL FROM OIL PALM TRUNK (OPT) SAP USING *CLOSTRIDIUM ACETOBUTYLICUM* AND *CLOSTRIDIUM BEIJERINCKII*

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ABSTRACT

Malaysia is the one of world's largest producer of palm oil. Waste from palm oil production factories has been increasing rapidly and one of the serious problems in the palm oil fruit processing is managing the waste generated by the processes. Recent studies showed that butanol can be used as excellent renewable liquid transportation fuel. In this study, oil palm trunk sap is used as the substrate. The objective of this study is to compare the production of biobutanol from biomass waste (oil palm trunk sap) via fermentation process using different microorganisms. The fermentation type done was submerged fermentation. Fermentation was conducted for five days at 37 ^{0}C. Samples were taken for every 6 hours for 3 days, then for every 12 hours. Samples taken were analyzed using gas chromatography-flame ionization detector (GC-FID) and high performance liquid chromatography (HPLC) was used to analyze sugar composition in OPT sap. UV Spectrophotometer is used to analyze biomass. Oil palm trunk (OPT) sap contains sucrose as its dominant sugar followed by glucose, fructose and galactose. Based on the analysis, acetone, butanol and ethanol were produced. Comparisons between the two strains *Clostridium acetobutylicum* and *Clostridium beijerinckii* were made, and *C. beijerinckii* showed higher production of acetone, butanol and ethanol. The highest yield for butanol production by *C. acetobutylicum* and *C. beijerinckii* were 13.910 g/L and 15.301 g/L and highest production of ethanol were 11.17 g/L and 12.09 g/L. 4.13 g/L and 4.49 g/L acetone was produced by *C. acetobutylicum* and *C. beijerinckii*. Based on this study, it is concluded that OPT sap is preferable substrate to produce butanol by using *C. beijerinckii*.

PENGHASILAN BUTANOL DARI PERAHAN GETAH BATANG KELAPA SAWIT MELALUI PROSES PENAPAIAN MENGGUNAKAN *CLOSTRIDIUM ACETOBUTYLICUM* DAN *CLOSTRIDIUM BEIJERINCKII*

ABSTRAK

Malaysia merupakan salah satu pengeluar kelapa sawit yang terbesar di dunia. Sisa dari kilang pengeluaran kelapa sawit makin meningkat dan salah satu masalah serius dalam pemprosesan bauak kelapa sawit adalah pengendalian sisa yang terhasil dari proses tersebut. Kajian terbaru menunjukkan butanol boleh digunakan sebagai bahan api pengangkutan yang boleh diperbaharui. Dalam kajian ini, getah perahan dari batang kelapa sawit digunakan sebagai subsrat untuk menghasilkan butanol. Pokok kelapa sawit yang berusia 25 tahun akan ditebang untuk penanaman semula. Objektif kajian ini adalah untuk membanding penghasilan butanol dari perahan getah batang kelapa sawit melalui proses penapaian menggunakan mikroorganisma yang berlainan. Proses penapaian dikendalikan selama 5 hari pada suhu 37 ^{0}C. Sampel diambil setiap 6 jam sekali bagi 3 hari yang pertama, dan kemudian diambil setiap 12 jam sekali. Sampel yang diambil dianalisis menggunakan GC-FID dan HPLC digunakan untuk analisis kandungan gula dalam perahan getah batang kelapa sawit. Perahan getah kelapa sawit mengandungi empat jenis gula iaitu sukrosa, glukosa, fruktosa dan galaktosa. Berdasarkan analisis yang dilakukan, acetone, butanol dan ethanol telah terhasil melalui proses penapaian. Perbandingan antara dua jenis mikroorganisma iaitu *Clostridium acetobutylicum* dan *Clostridium beijerinckii* dan *Clostridium beijirinckii* menunjukkan penghasilan acetone, butanol dan ethanol yang lebih tinggi. *Clostridium acetobutylicum* telah menghasilkan 13.910 g/L butanol, 11.17 ethanol dan 4.13 g/L acetone manakala *Clostridium beijerinckii* telah menghasilkan 15.301 g/L butanol, 12.09 g/L ethanol dan 4.49 g/L acetone. Berdasarkan kajian ini, kesimpulan yang dibuat mengatakan perahan getah kelapa sawit merupakan substrat yang bagus untuk penghasilan butanol menggunakan *Clostridium beijerinckii*.

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LIST OF ABBREVIATIONS

ABE	acetone-butanol-ethanol
FID	flame ionization detector
GC-FID	gas chromatography flame ionization detector
HPLC	high performance liquid chromatography
OPT	oil palm trunk
RCM	reinforced clostridia medium
UV-Vis	ultraviolet visible

LIST OF SYMBOLS

α	alpha
ºC	degree Celcius
µm	micrometer
%	percent
g/cm	gram per centimeter
g/L	gram per litre
hr	hour
kJ/kg	kilojoule per kilogram
L	litre
min	minute
ml	milimeter
MW	megawatt
nm	nanometer
v/v	volume per volume

CHAPTER 1

INTRODUCTION

1.1 Introduction

Butanol or butyl alcohol (sometimes also called biobutanol when produced biologically), is a primary alcohol with a 4 carbon structure and the molecular formula of C_4H_9OH. Butanol is the fourth member of the aliphatic alcohol series. The aliphatic alcohols are a series of homologous series organic compounds containing one or more hydroxyl groups [-OH] attached to an alkyl radical. Butanol is a colourless liquid with a distinct odour and its vapour has an irritant effect on mucous membranes and a narcotic effect in higher concentrations (Lee S.P., *et a.l.,* 2008). Butanol is completely miscible with organic solvents and partly miscible with water. Butanol can be used as an intermediate in chemical synthesis and as a solvent for a wide variety of chemical and textile industry applications.

Biobutanol is synthesized from biomass and it is renewable. Recent studies have shown that biobutanol can be used as excellent renewable liquid transportation fuel and is recognized to be a 'superior fuel' (Jang Y.S., *et al.,* 2011). Biomass is usually comprised of lignocellulosic material and is comprised of high contents of cellulose, hemicelluloses, lignin and proteins (Demirbas M.F., *et al*., 2011). The biomass materials are usually readily available as wood residues, agricultural wastes and food wastes.

In this study, oil palm trunk is used as the raw material. Oil palm is widely planted in tropical countries like Malaysia and Indonesia. The palm starts bearing oil-contained fruits in 25 years after planted and its productivity becomes lower after 20-25 years (Yamada H., *et al.*, 2010). Therefore, the old palms are cut in order to replant new seedling in the plantations. This is an efficient way in utilizing the oil palm trunks.

1.2 Objective

The objective of this research is to compare the production of biobutanol from biomass waste (oil palm trunk sap) via fermentation process using different microorganisms.

1.3 Scope of study

The scopes of this study are:

i. To determine the possibility of butanol production that can be obtain from acetone-butanol-ethanol (ABE) fermentation.
ii. To study the suitability of oil palm trunk sap in butanol production.
iii. To compare the production of butanol from different microorganisms.

1.4 Problem of statement

Since the last decade there has been an increasing interest for the production of fuels from the renewable sources like biomass waste. The reason for this trend is the increasing price of fossil fuels. This is because fossil fuel is not renewable. At current rate, the world uses fossil fuels 100,000 times faster than they can form. It is estimated that the current sources of fossil fuels can last for around 80-90 years. Hence producing butanol from biomass waste is an absolute option in prevailing over this problem. Beside this, carbon dioxide emission from combustion of fossil fuels can lead to greenhouse effect. When the concentration of carbon dioxide in earth's atmosphere increase, heat is prevented from being radiated out into space. This will cause the Earth's surface, ocean and temperature to heat up which will lead to global warming. Biomass waste is chosen as the better raw material since it is renewable and cheap. Biomass that is used in this research is oil palm trunk. No paper has been discussed on production of biobutanol from the oil palm trunk sap.

1.5 Rationale and Significant

The arguments are in favour of biobutanol replacing gasoline as transportation fuel. The fuel properties of biobutanol are considered to be superior to bioethanol because of higher energy content and better air-to-fuel ratio (Demirbas M.F., *et al*., 2011). Biobutanol is safer to handle since it has high flash point and lower vapour pressure. Furthermore, isobutanol (a branched isomer of straight chain butanol) may be blended up to 15% on volumetric basis to petrol versus 10% for ethanol (Demirbas M.F., *et al.*, 2011). In addition, biobutanol provides positive environmental benefits in the form of reductions in greenhouse gas emissions (CO_2). As the biomass grows, it consumes as much carbon dioxide as it forms during the combustion of bioethanol, which makes the net contribution to the greenhouse effect zero. Furthermore, oil palm trunk is chose as biomass for this research because Malaysia is one of the largest producers of oil palm. Oil palm trunk is abundant, cheap and can be obtain easily.

CHAPTER 2

LITERATURE REVIEW

2.1 Background of biobutanol

2.1.1 History of biobutanol production

The formation of butanol in the microbial fermentation was reported first by Louis Pasteur in 1861 (Gabriel C.L. and Crawford F.M, 1930). In the following years Albert Fitz worked actively in the field of fermentation and obtained butanol from glycerol using a mixture of two bacteria. In the beginning of the 20th century, the research was focused on producing acetone, amyl alcohol or butanol by fermentation to use them for the manufacture of synthetic rubber (Gabriel C.L. and Crawford F.M, 1930). In the summer of 1912, Chaim Weizmann succeeded in isolating a bacterium strain, later named *Clostridium acetobutylicum*, which was capable of using starch as a substrate in the butanol production process (Jones D.T. and Woods D.R, 1986). This process exhibited higher product yields of butanol and acetone. The need of cordite (smokeless gunpowder) during the First World War (Gabriel C.L, 1928), made acetone the desired product of the fermentation process since the production of cordite requires acetone. At that time, butanol was considered as by product and was stored in containers.

However, the expanding automobile industry needed a solvent for the quick drying lacquers for the finishing of cars (Garcia V., *et al*., 2010). Butanol and butyl acetate showed good properties as solvents for this purpose and in 1920 butanol became the main product of the fermentation process (Gabriel C.L. and Crawford F.M, 1930). The ABE process was successful prior to the actual development of large scale, aseptic, submerged industrial fermentation technology. This can be explained by the nature of the anaerobic fermentation and the products (toxic acids and solvents). For the processes, locally isolated *clostridium* strains were used (Garcia V., *et al.*, 2010). Since 1911 new discoveries are continuously patented until present. New strains were isolated and novel substrates and more effective preparation methods were developed. The fermentation step was improved by a better equipment design and by using parallel batteries of reactors in series (Garcia V., *et al*., 2010). Continuous processes and exploitation of by products were carried out as well. Nevertheless, many plants were forced to be closed during the 1960s due to the increased price of substrates, low solvent yields and a more competitive process based on fossil fuels. The industrial biobutanol production ceased in the early 1990s (Jones D.T. and Woods D.R, 1986).

Research and development of the ABE process continued and during the 1980s and 1990s studies at pilot plants were carried out in Soustons, France and in Lower, Austria. In France, the research focused on evaluating the commercial potential of solvent production for gasoline substitute fuels by using hydrolysates of cereal straw, corn stover and sugar beet in batch fermentation (Garcia V., *et al.*, 2010). Potatoes were used as raw material for batch, fed batch and continuous fermentation modes in Austria. China developed its ABE process industry from *Clostrodium acetobutylicum* strains using mainly starch based feedstocks (Ni Y. and Sun Z., 2009). The first plants were built in the 1960s reaching an annual production of 170,000 tonnes. However, the facilities were closed at the end of the 20th century due to the rapid development of petrochemical synthesis processes (Garcia V., *et al.*, 2010). In the past years China has restarted the ABE production. At least 11 production plants are in operation and others are under construction or at the start up stage (Ni Y. and Sun Z., 2009).

2.1.2 Butanol as a fuel

Butanol is better than ethanol as a source of energy as it tolerates water contamination better and is less corrosive than ethanol. Butanol also need not be stored in high pressure vessels like natural gas nor does it have to be blended with fossil fuel to reap its benefits. It is possible to transport butanol through existing pipelines for its distribution purposes. Morever, butanol can be easily reformed for its hydrogen content, so that it is distributed with the require purity for fuel cells or vehicles at existing gas stations.

Besides, butanol can be easily blended with conventional gasoline at higher concentrations than ethanol for use in unmodified engines (Gable C. and Gable S., 2010). Experiments have shown that butanol can run in an unmodified conventional engine at 100 percent. Not only is butanol a higher-grade more energy dense fuel, it is also less explosive than ethanol. Butanol is safer to use than ethanol and gasoline as a result of its lower vapour pressure. It is difficult to ignite and it burns slowly.

Like diesel, a match has to be held to it for ignition; butanol is combustible but not flammable, whereas ethanol and gasoline are flammable and potentially explosive (Ramey D.E., 2010). In addition, butanol is can be dissolve with vegetable oils at any ratio (Stoeberl M., *et al.,* 2011). Vegetable oils mostly consist of triglycerides, which are inherently viscous. However, high viscosity and poor volatility are the major challenges to the running of modern diesel on vegetable oils.

2.1.3 Advantages of Butanol

Butanol has one third higher energy density (36 vs 27 kJ/kg) than ethanol (Demain, 2009) and its energy is similar to gasoline (Ha *et al*., 2010). Besides, butanol use in cars does not require engine modification until it reaches 40% of total fuel; ethanol requires it at concentration of over 15%. At one time, ABE fermentation was used commercially to produce the solvents acetone and butanol, but the fermentation was replaced by less expensive chemical procedures. Today, there is renewed interest in this fermentation to produce biobutanol (Demain, 2009). Furthermore, it could be used as a solvent of quick-drying lacquer for automobile coating (Ni and Sun, 2009).

Butanol is also widely used as a solvent for acid-curable lacquers and baking finishes. Other important applications of butanol and its derivatives include paint thinners, solvent for dyes, brake fluids and as an extractant in the production of drugs and natural substances such as antibiotics hormones, vitamins, etc. in a similar vein of interest in ethanol and biodiesel, and important application has emerged for butanol as a renewable energy carrier since it can be used directly as a liquid fuel.

Furthermore, the bioethanol industry in Brazil was criticized as environmentally hazardous, as large area is being used for monocultures. A similar discussion was sparked in North America and Europe where starch production for biofuel competes over land with the food industry and environmental issues. The sky rocketing price for starch is already hindering the start-up of new bioethanol plants (Antoni *et al*., 2007).

2.2 Overview of Biomass

Biomass is usually comprised of lignocellulosic materials and, as such, is comprised of high contents of cellulose, hemicellulose, lignin and proteins, thus constituting renewable natural resources for a plethora of inexpensive eco-friendly and sustainable materials (Demirbas M.F., *et al.*, 2010). According to Oliveira and Franca (2009), these biomass materials are usually readily available as wood residues, municipal solid wastes (MSW), agricultural and food wastes, and dedicated energy crops. The average majority of biomass energy is produced from wood and wood wastes (64%), followed by MSW (24%), agricultural waste (5%), and landfill gases (5%), (Demirbas M.F., *et al.*, 2010). The basic structure of all woody biomass consists of three basic polymers: cellulose $(C_6H_{10}O_5)_x$, hemicelluloses such as xylan $(C_5H_8O_4)_m$, and lignin $[C_9H_{10}O_3(OCH_3)\ 0.9–1.7]_n$ in trunk, foliage, and bark. The proportion of these wood constituents varies between species, and there are distinct differences between hardwoods and softwoods. Generally, hardwoods contain about 43–47% cellulose, 16–24% lignin, 25–35% hemicelluloses, and 2–8% extractives while softwoods contain about 40–44% cellulose, 25–31% lignin, 25–29% hemicelluloses, and 1–5% extractives (Balat M. and Demirbas F.S., 2009).

Cellulose is a homopolysaccharide composed of β-D-glucopyranose units linked together by 1,4-glycosidic bonds. The basic repeating unit of the cellulose polymer consists of two glucose anhydride units, called a cellobiose unit. A second major wood chemical constituent is hemicellulose, which is also known as polyose. Hemicelluloses are related to plant gums in composition, and occur in much shorter molecule chains than cellulose. Hemicelluloses are derived mainly from chains of pentose sugars, and act as the cement material holding together the cellulose micells and fibre (Demirbas M.F., *et al*., 2010).

Among the most important sugar of the hemicelluloses component is xylose. Lignin is an aromatic polymer synthesised from phenylpropanoid pre-cursors. The basic chemical phenylpropane units of lignin (primarily syringyl, guaiacyl and p-hydroxy phenol) are bonded together by a set of linkages to form a very complex matrix (Demirbas M.F., *et al*., 2010). This matrix comprises a variety of functional groups, such as hydroxyl, methoxyl and carbonyl, which impart a high polarity to the lignin macromolecule. The table below summarize the production of butanol from various type of biomass using *Clostridia* microorganism.

Table 2.1 Biomass type and butanol production

Biomass	Microbes	Butanol (g/L)	References
Potato starch	C.Acetobutylicum	0.17	Yen et al.(2011)
Whey	C.Acetobutylicum	12.0	Stoeberl et al.(2011)
Barley straw	C.Beijerinckii	13.62	Qureshi et al.(2010)
Wastewater algae	C.Saccharoperbutyl-acetonium	5.61	Ellis et al.(2012)
Seaweed extract	C.Acetobutylicum	10.4	Huesemann et al.(2012)
Corn fiber	C.Beijerinckii	13.	Qureshi et al.(2007)
Maple Wood	C.Acetobutylicum	0.2	Sun and Liu (2010)
Wheat straw	C.Beijerinckii P260	5.5	Qureshi et al (2007)
Palm empty fruit bunches	C.Acetobutylicum	0.8	Noomtin et al.(2011)
Date palm fruits	C.Acetobutylicum	7.90	Abd-Alla & Elsadek El-Enany (2012)

2.2.1 Biomass in Malaysia

Malaysia has abundant biomass waste resource coming mainly from its palm oil, wood and agro-industries. A total of about 665 MW capacities can be expected if the estimated overall potential of about 20.8 million tone of biomass residues from this main source in addition to 31.5 million m^3 of palm oil mill effluent (POME) is used for power generation and cogeneration In addition, there is a substantial amount of unexploited biomass waste resources in the form of logging wood residues, rice straw, palm kernel trunks and other residues. This biomass residue could further supplement future biomass-based power generation in the country if necessary.

Biomass fuels currently account for about 16% of the energy consumption in the country, of which about 51% is palm oil biomass waste and about 22% is wood waste. The present installed biomass-based power generation capacity in the country is 138 MW, about 100 MW of which are in the palm oil industry.

2.2.2 Oil Palm

The oil palm originated from West Africa and was introduced to Malaysia as an ornamental plant in early 1870s. The oil palm botanical name *Elaeis guineensis* was derived from Greek *elaion* (oil) and *guineensis* (palm) showed its origin, which is came from Guinea coast (Henderson and Osborne, 2000). In 1917, Henri Fauconnier, a French rubber and coffee planter saw its potential as a cash crop and commercially planted oil palm in Tennamaran Estate, Batang Berjuntai, Selangor (Malaysian Oil Palm Council, 2007). This oil palm can be cultivated easily in Malaysia since it was tropical palm tree and accommodate very well with local climax.

Oil palm trees are replanted at an interval of approximately 25 years because of decreased oil productivity of old trees. The production of palm oil is 39 Mt per year in 2007, which is the most produced plant oil in the world (Yamada H. *et al*., 2010). Generally, the palm starts bearing oil-contained fruits in 25 years after planted and its productivity becomes lower after 20-25 years. Oil palm biomass contains quite significant amount of organic nutrient like phosphorus, kalium and magnesium which contributes to its fertilizer values. Table 2.3 below shows several types of products that are produced by oil palm trunk.

Table 2.2 Oil palm trunk and oil palm sap products

Biomass	Products	References
Oil palm trunk	Bioethanol	Jung et al. (2011)
	Bioplastic	Jung et al. (2011)
	Lactic acid	Mori et al. (2007)
	Glucose	Chin et al. (2011)
	Hydrogen rich gas	Mohammed et al. (2011)
Oil palm sap	Lactic acid	Kosugi et al. (2010)
	Biohydrogen	Noparat et al. (2012)
	Bioethanol	Kosugi et al. (2010)

Fundamentally, the oil palm biomass contains about 18–21% of lignin, and 65-80% of holocellulose (α-cellulose and hemicellulose), which are more or less comparable with that of other wood or lignocellulosic materials. Oil palm sap is obtained by squeezing the oil palm trunk using presser. Oil palm sap was reported to contain approximately 11% sugars with sucrose as a major component accounting for approximately 90% of total sugar. Table 2.4 below show the sugar contain in oil palm sap. A, B and C represent the bottom, middle and upper part of the trunk. From this table, it can be conclude that the bottom part of the trunk has the highest content of sugar.

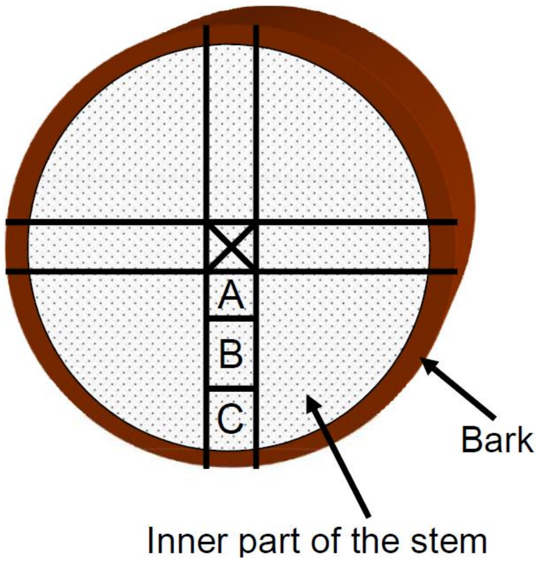

Figure 2.1: Inner part of OPT (Source: Mori, 2007)

Table 2.3 Sugar contain in oil palm sap (g/L)

Sugar	A	B	C
Sucrose	68.4	60.8	54.7
Glucose	23.3	27	30.9
Fructose	20.6	23.6	22.1
Total	113	112	108

(Source: Mori, 2007)

2.3 Fermentation

Fermentation is the process of extracting energy from the oxidation of organic compounds, such as carbohydrates, using an endogenous electron acceptor, which is usually an organic compound (John H., *et al.*, 2006). Alcoholic fermentation is a biological process in which sugars such as glucose, fructose, and sucrose are converted into cellular energy.