STUDY ON BIOETHANOL PRODUCTION BY USING OIL PALM TRUNK (OPT) SAP

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ABSTRACT

Fossil fuels were used in large quantities as a solvent and chemical feedstock in various industries. Due to limited stock of fossil fuel and rapid increase of energy demand in Malaysia, bioethanol has become a very important and necessary alternative energy source in order to replace the limited fossil fuel stock. Hence, this paper presents the study on bioethanol production by using oil palm trunk (OPT) sap which mainly focusing on substrate concentration effect toward the yield, productivity rate, and kinetic behaviour of cell growth in different substrate concentration This research was carried out by using *Saccharomyces cerevisiae Kyokai No.7* in 200mL conical flask, under 30^{o}C anaerobic condition. The research are focusing on the inoculums culture preparation, fermentation process, sample preparation, and analyzing methods. It was found that bioethanol production is increase when substrate concentration increase, from 14g/L in 60% substrate concentration, followed by 18g/L in 80% OPT sap to 22g/L in 100% OPT sap. With the highest sugar content in the medium, *S. cerevisiae* can produce faster ethanol production within 30 hours of fermentation duration. However, high bioethanol concentration in the process will cause the cell growth rate falls due to the product inhibition. Besides, glucose consumption rate were found out that inversely proportional to the substrate concentration whereas ethanol production rate and cell growth rate were directly proportional to the substrate concentration. Moreover, specific bioethanol production yield was found out that exceed 0.482, 0.516, and 0.465 for 60%, 80%, and 100% of substrate concentration respectively. These results will be useful in the future scale up processes.

ABSTRAK

Bahan api fosil telah digunakan dalam kuantiti yang besar sebagai pelarut dan bahan mentah kimia dalam industri. Oleh kerana stok terhad, bahan api fosil telah meningkatkan permintaan tenaga yang tinggi di Malaysia, manakala bioetanol telah menjadi satu sumber tenaga alternatif yang sangat penting bagi menggantikan stok bahan api fosil yang terhad. Oleh itu, kertas kerja ini keutamanya membentangkan kajian mengenai pengeluaran bioetanol daripada jus batang kelapa sawit (OPT) dan memberi tumpuan kepada kesan kepekatan substrak ke arah penghasilan, kadar produktiviti , dan kinetik pertumbuhan sel dalam proses tersebut. Kajian ini dijalankan dengan menggunakan *Saccharomyces cerevisiae Kyokai No.7* di bawah keadaan anaerobik 30^{o}C . Kajian ini memberi tumpuan dalam penyediaan inoculums mikroorganisma, proses penapaian, penyediaan sampel , dan kaedah menganalisis . Ia didapati penghasilan bioetanol telah meningkat apabila kepekatan substrat meningkat, dari 14g / L dalam kepekatan substrat 60 %, diikuti oleh 18g / L di 80 % kepekatan jus OPT dan 22g / L dalam 100% jus OPT digunakan. Dengan kandungan gula yang tertinggi, *S. cerevisiae* boleh menghasilkan produk etanol lebih cepat dalam tempoh masa 30 jam proses penapaian. Walau bagaimanapun, kepekatan bioethanol tinggi dalam proses ini akan menyebabkan kadar pertumbuhan sel jatuh yang disebabkan oleh perencatan produk. Selain itu, kadar penggunaan glukosa telah didapati bahawa berkadar songsang dengan kepekatan substrat manakala kadar penghasilan etanol dan kadar pertumbuhan sel adalah berkadar terus dengan kepekatan substrat. Selain itu, hasil penghasilan bioethanol bergantung kepada keseluruhan gula telah mendapati bahawa melebihi 0.482 , 0.516 , dan 0.465 dalam 60% , 80%, dan 100% daripada kepekatan substrat masing-masing . Keputusan ini akan berguna pada masa hadapan dalam proses peningkatan penghasilan bioetanol dalam industri.

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LIST OF SYMBOLS

°C	degree celcius
μ	specific growth rate
μl	microliter
μ_{max}	maximum specific growth rate
μm	micrometer
cm	centimetre
e	ethanol
g/cm	gram per centimetre
g/mol	gram per mol
g/g	gram per gram
g/l	gram per liter
g/l.h	gram per liter per hour
kPa	kiloPascal
ha	hectare
h	hour
Ks	growth constant
L	liter
m	meter
mg/l	miligram per liter
min	minute
ml	mililiter
ml/min	mililiter per minutes
nm	nanometer
P	product concentration
Pa.s	Pascal per second
Pm	maximum product concentration above cell ceases
r_c	cell growth rate
r_e	ethanol production rate
r_g	glucose consumption rate
rpm	rotation per minute
R_t	retention time
r_x	cell growth rate
S	Substrate concentration
v/v	volume per volume
w/v	weight per volume
w/w	weight per weight
X	cell concentration
Yp/s	Yield factor of gram product per gram substrate
Yp/x	Yield factor of gram product per gram cells
Yx/s	Yield factor of gram cells per gram substrate

LIST OF ABBREVIATIONS

ATCC	American Type of Culture Collection
BO	Bottom
GC	gas chromatography
FID	flame ionized detector
HPLC	high performance liquid chromatography
RID	refractive index detector
LM	Lower middle
LSD	least significant difference
Mtoe	millions tonnes of oil equivalent
OPT	oil palm trunk
TO	Top
UM	Upper middle

1 INTRODUCTION

1.1 BACKGROUND OF STUDY

In recent years, the energy demand in Malaysia indicates a rapid increase, which will be expected to reach almost 100 Mtoe in year 2030. This will lead to the serious depletion of the fossil fuel energy sources due to the more and more intense in global energy requirement. (Nurul Ain, 2010) Besides, global environmental problem had become the serious concerns nowadays and for new generations. Therefore, the limited fossil fuel stock had made the alternative energy source become more important and necessary. (Chandel et al., 2007)

Bioenergy is a kind of alternative energy in solving these problems. It is a special form of renewable energy from biomass-rich resources. The example bioenergy sources include wood and sawmill waste, molasses, oil palm waste, charcoal, biogas resulting from the anaerobic decomposition of waste, as well as liquid biofuels, such as bioethanol and biodiesel. It had been proved to be a clean and highly essential bioenergy substitution for fossils today.

Bioenergy such as bioethanol can be directly used as a fuel or can be blended with petrol or gasoline to form blend fuel. It has been long considered as a suitable alternative to fossil fuels either as a sole fuel in cars with dedicated engines. Bioethanol, is a kind of alcohol. According to Rao D.G. (2010), industrial solvent such as alcohol, were being extensively produced in fermentation route. Mostly, the alcohol produced by fermentation process is used for human consumption, for example in production of wine; and for the use in pharmaceutical industry.

1.2 MOTIVATION

For a decade, there are many efforts have been done on upgrading the utility of lignocellulosic biomass, for example oil palm trunk, rubber wood, and mixed hardwood hydrolysate due to the growing demand for petrochemicals usage. (Malherbe and Cloete, 2002) However, due to the rapid in depletion of world petroleum reserves, new and alternative source must be found in order to replace the fossil fuel and energy needs. (Pramanik K., 2003)

Regarding to this oil palm trunk sap research, there are numerous studies have been conducted, for example: pH control, optimum temperature used, feasibility study and strain used (Nurul Ain, 2010; Norhazimah et.al., 2013). Recent works by Norhazimah et.al. (2013) suggested that, *S. cerevisiae* is the strain that can have the highest productivity of bioethanol. Unfortunately, their work focuses more on the different strain used in the fermentation process. Besides, Amenaghawon et.al. (2012) carried out a study on kinetic modelling of ethanol inhibition during alcohol fermentation of corn stover by using *S. cerevisiae*, however, they more focus on the kinetic modelling instead of substrate concentration profile. Last but not least, Nurul Ain (2010) discussed the effect of pH and temperature towards the alcohol production yield by using OPT sap.

According to all the research as mention as example in above, it is required to ensure a highest yield of the fermentation product, within a very short period of time, even lower the cost efficiency of producing ethanol. (Chin et. al., 2010) Therefore, a specific study on substrate concentration effect toward OPT sap is needed for the further research in the future.

1.3 PROBLEM STATEMENT

Oil palm trunk sap had covered over a large area of 12,000 ha per year in Malaysia. Due to the serious shortage of fossil fuels had led society to find alternative renewable fuel to replace fossil fuels used. Therefore, various type of crop had been used over the world in bio-fuel production, especially bioethanol production. For example, rice straw, cotton stalks, corn, and sugar cane. Bioethanol production by using oil palm trunk (OPT) had also been carried out. (Sanjeev et.al., 2004) There are various types of factor that can affect the fermentation process. The affecting factors are included temperature, substrate concentration and pH value. Although the effect of substrate concentration for fermentation process is well understood, however, no research has been performed for substrate concentration effect and kinetic study cell growth on OPT sap, *Elaeis Guineensis,* and hence, this is the aim of this study.

1.4 OBJECTIVE

The aim of this research is to study the effects of substrate concentration in bioethanol production by using oil palm trunk (OPT) sap.

1.5 SCOPES OF RESEARCH

The following are the scopes of this research, in order to achieve the stated objectives:

(i) To study the effect of substrate concentration for bioethanol production by using OPT sap. .

(ii) To determine the bioethanol production and characterized the sugar content in OPT sap.

(iii) To study the microbial growth kinetics and their effect on bioethanol productivity.

(iv) To identify the optimum yield of bioethanol production based on different substrate concentration.

2 LITERATURE REVIEW

2.1 BIOETHANOL

Bioethanol, CH_3CH_2OH is a kind of alcohol which contains a hydroxyl group (-OH) that bonded to carbon atom. It is also said to be a renewable energy source because it always been tightly coupled with the growth of yeast cells in fermentation process of agricultural sources such as sugarcane, and corn. The characteristics and properties of ethanol are shown in the table below.

Table 2-1: Characteristic and properties of ethanol

Parameters	**Units**	**Properties/values**
Molecular formula	-	C_2H_6O
Molar mass	g/ mol	46.07
Density, ρ	g/cm	0.789
Melting point	°C	-114
Boiling point	°C	78
Flash point	°C	13-14
Vapor pressure, P_v	kPa	5.95 (at 20 °C)
Viscosity, µ	Pa.s	0.0012 (at 20 °C)
Acidity (pK_a)	-	15.9
Basicity (pK_b)	-	-1.9
Colour	-	Colourless

Ethanol can be produced from two main processes: via synthetic process such as hydration of ethene, and via biological pathway such as fermentation. From ethene, ethanol is manufactured by reacting ethene with steam as in equations below.

$$\underset{\text{(ethene)}}{C_2H_4} + H_2O \rightarrow CH_3CH_2OH$$

Compared to the synthetic processes, ethanol is commonly produced by fermentation of sugar by employing yeast *Saccharomyces cerevisiae* which will consume available sugar to the bioethanol under certain conditions.

$$C_6H_{12}0_6 \xrightarrow[\text{(yeast)}]{} 2CH_3CH_2OH + 2CO_2$$

Ethanol is a typical primary metabolite, which had provides economic strategic benefits. (Solomons & Fryhle, 2011; Bai et al., 2008; Chandel et al., 2007) According to Chandel et al. (2007), bioethanol is considered as a kind of safe and cleanest fuel which is the alternative to fossil fuels nowadays. This is because no net carbon dioxide is added to the atmosphere since the ethanol production process only involved renewable energy sources. A large scale biofuels pilot plant should not impact natural ecological systems. (Nurul Ain, 2010) However, burning of fossil fuel had created a global environmental crisis, and green house gas emissions. (Chandel et al., 2007)

Therefore, bioethanol was the most important biofuel in worldwide industry. (Nina Farhana & Mohd Jamaludin, 2010). It was used in large quantities as a solvent and chemical feedstock in various industries. (James et.al., 2001) Also, it can be used as an automotive fuel or to form an ethanol-petrol blend by mixing with petrol, increase octane levels and extend the supply of gasoline. Bioethanol, as a fuel additive and also as a non-renewable fuel replacement, in addition reduce the significant amount of oil imported. (Suraini, 2002) Nowadays, research had been focused on different types of biomass resources in order to produce bioethanol in alternative way due to energy crisis since 1970s. (James et.al., 2001) This paper presents the experimental studies of bioethanol fermentation by using different substrate concentration, 60%, 80%, and 100% of OPT sap.

2.2 BIOMASS POTENTIAL

Bioethanol production can be synthesized from various of biomass, such as sweet sorghum, corn, sugar cane, sugar beet, OPT sap and etc. (Nina Farhana, 2010) The capacity of bioethanol production from different type of biomass are shown.

Table 2-2: Bioethanol production capacity by using different raw material

Raw material	Bioethanol production (l/ha)
Forage sorghum (stalk juice)	770
Sweet sorghum (stalk juice)	924-1051
Wheat	1075-1730
OPT sap (stalk juice)	1758
Corn	2011-3700
Sugar beet	5145
Sugar cane	6641

Source: Norhazimah et.al. (2013)

In this research paper, oil palm trunk sap is chosen as the biomass to produce bioethanol. This is because Malaysia recently accounts for 41% of palm oil production in the world, which also counted for the biggest producer and exporter of palm oil industry. (Nina Farhana, 2010) Besides, according to the data in table 2-1, the bioethanol production capacity of oil palm trunk sap is much higher than forage sorghum, sweet sorghum stalk juice, and wheat, while slightly lower than corn, sugar beet and sugar cane. Therefore, oil palm trunk sap has the big potential in the production of bioethanol.

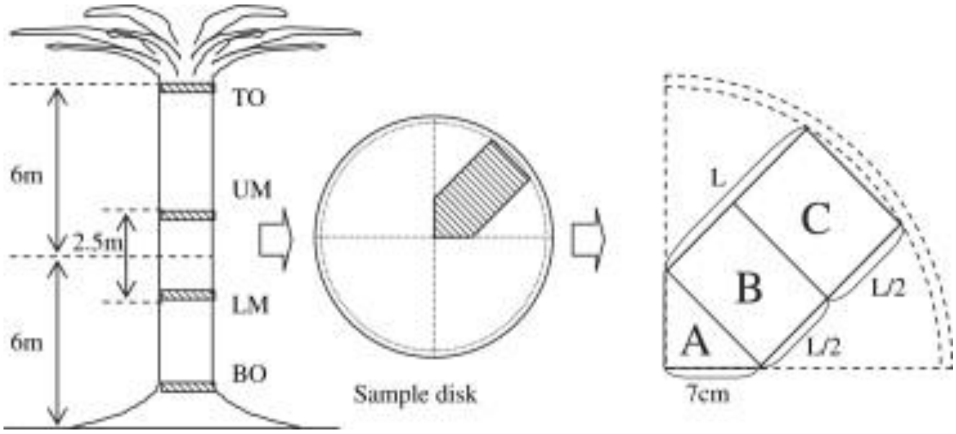


Figure 2-1: Oil palm trunk (OPT) samples

Table 2-3: Total sugar content (mg/ml) in the sap from different part of oil palm trunk

Part	**TO**	**UM**	**LM**	**BO**
Inner	111.8	129.9	129.2	93.0
Intermediate	72.7	118.0	94.2	102.8
Outer	71.1	103.6	81.6	107.7
Average	85.2	117.2	101.7	101.2

Source: Yamada (2010)

According to Yamada (2010), it showed that inner part of oil palm trunk has the higher sugar content than intermediate and outer part. Besides, the average sugar content in upper middle part of oil palm trunk is the highest among other part.

Table 2-4: Moisture content in different parts of oil palm trunk

	Part		
Moisture % (w/w)	**Inner (A)**	**Middle (B)**	**Outer (C)**
	82.4 ± 1.2	75.9 ± 4.7	67.7 ± 6.9

Source: Kosugi et. al. (2010)

From the research shown by Kosugi et. al. (2010), the moisture content of oil palm trunk was normally higher compared to wood timber, which normally around 40% to 50%. Hence, oil palm trunk contains a high quantity of sap especially the inner part.

2.3 RAW MATERIAL

Elaeis Guineensis, is a kind of oil palm that originates from West Africa, and widely planted in Malaysia since early 1870s. (Nina Farhana, 2010) There are about 120,000 ha of oil palm is replanted every year, which mainly used in the food related industries, besides maintaining the oil productivity. Therefore, a lot of research studies also carried out for bio-fuels production study by using the oil palm nowadays. Generally, oil palm has 25 years of life. In replanting stage, old oil palms tree were cut and discarded, directly burnt at the plantation site, or been smashed into small pieces and left to be rotten naturally in plantation area. (Yamada, 2010) High sugar present in OPTs will then promotes unwanted microorganism grow. (Norhazimah et.al., 2013) Therefore, felled palm trunks are a kind of troublesome waste material especially in the plantation sites. (Murata, 2012) Besides, it increased the possibility of plant diseases and infected those new young oil palm trees that had replanted.

Felled trunk contains a large amount of sap, which is approximately 70% from the total weight of whole oil palm trunk, and it consist of a large quantity of sugars in its trunk sap, for example glucose, fructose and sucrose, and these sugars can be converted easily into bioethanol. (Yamada, 2010; Murata, 2012) Oil palm trunk (OPT) sap is a kind of sap that squeezed from old oil palm trunks. OPT sap is identified as the important alternative feedstock for bioethanol production because they do not need extensive pre-processing such as extraction process. (Sanjeev et.al., 2004; Yamada, 2010) The middle part of the whole log of oil palm tree is taken. 15cm thickness disc slice from each log after 0 and 120 days of storage while it storage temperature was 28-32^{o}C and 70-80% of humidity. 5cm of the hard cover layer are normally removed before slicing process in order to avoid microbial contamination happens. The inner most part will be used due to the highest sugar contents. Then, the OPT is cut into smaller pieces with 15cm length and 2cm width for mechanical pressing. This mechanical pressing process has to carry out within 12 hour in order to control the quality of liquid sap. (Yamada, 2010; Norhazimah et.al., 2013) The procedure involved during the preparation of OPT sap were shown clearly in the figures below.

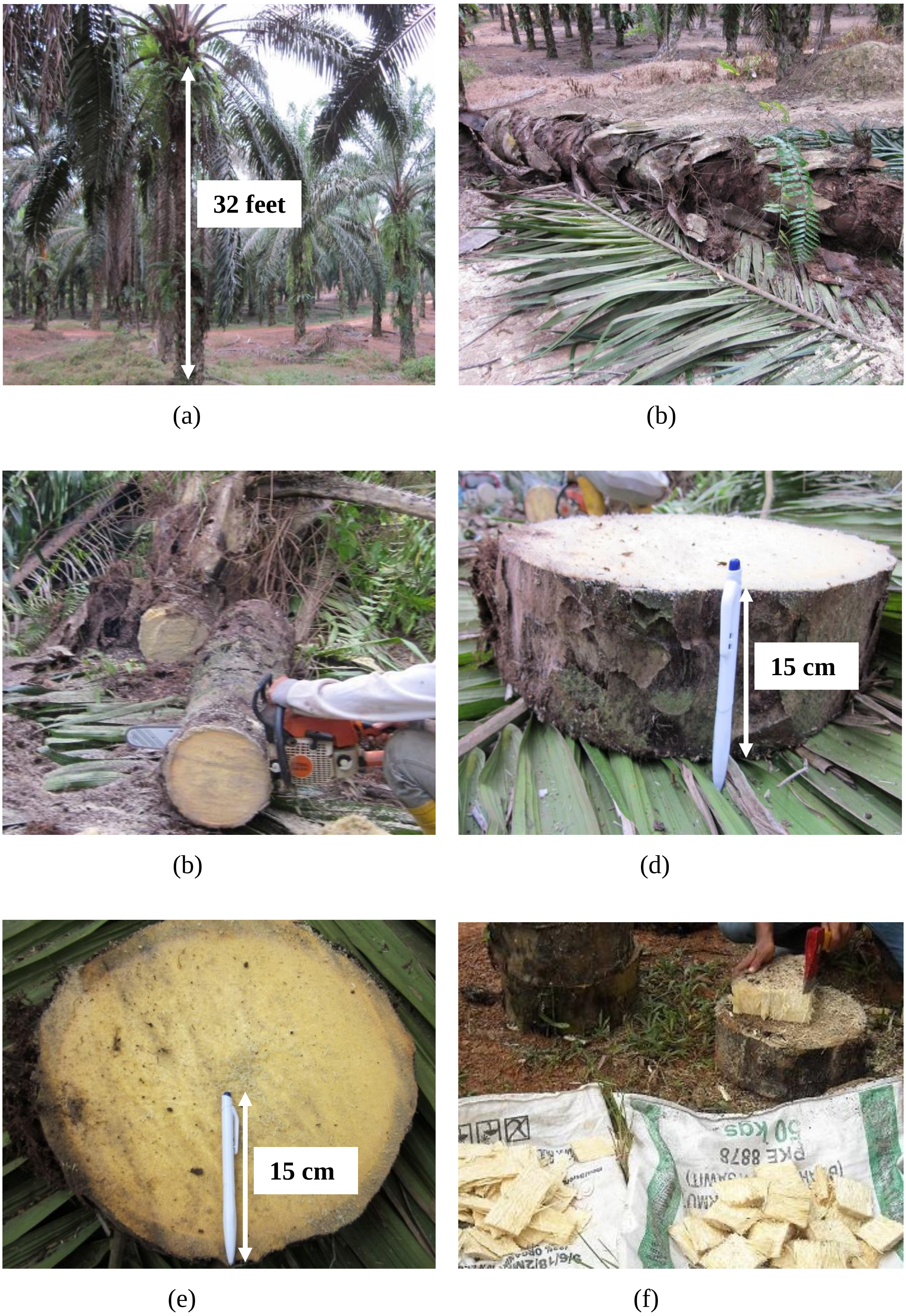


Figure 2-2: Preparation of OPT sap

(a) oil palm tree, (b) frond was removed, (c) OPT was cut into several pieces, (d) OPT with 15 cm length, (e) cross section at the middle of OPT, and (f) OPT was chopped into smaller pieces.

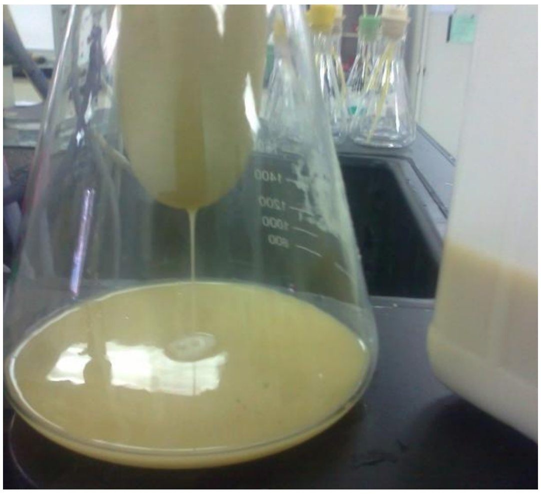
(a)

(b)

(c)

(d)

(e)



(f)

Figure 2-3: Continued of preparation of OPT sap

(a) OPT ready to be squeezed, (b) sugar-cane press machine for OPT squeezing, (c) fiber remains after pressing, (d) OPT was mixed well in a container before storage (e) OPT kept at -20 ^{o}C (f) OPT was filtered prior to use.

In this research, sap of OPT, from RISDA Plantation in Pahang, Malaysia was obtained freshly within 12h, and it was processed within 15h after felled of oil palm trunk, so that glucose will be the dominant sugar in OPT sap. (Yamada, 2010; Norhazimah et.al., 2013) Felled palm trunk was found to be an essential resource for the production of biofuel ethanol, since it can be easily available and obtained free from oil palm plantation sites. (Murata, 2012)

2.4 MICROORGANISM RELATED TO BIOETHANOL PRODUCTION

Bioethanol can be produce by either using traditional yeast or by using new developed bacterial, *Zymomonasmobilis* which isolated from tropical fruits. (Suraini, 2002) Regarding to Bailey and Ollis (1986), yeast is the only important microbes in alcoholic beverages production industries to supplying the consumer market. Generally, the yeasts that commonly used in alcohol production industries were included *Saccharomyces cerevisiae* (glucose, fructose, maltose, maltoriose), *Saccharomyces uvarum* (carlbergensis), *Saccharomyces diataticus* (dextrins), *Kluyveromyces fragilis* and *Kluyveromyces lactus* (lactose) (Kun, 2003).

In this research, Yeast cells, named *Saccharomyces cerevisiae Kyokai no.7* was used. It is facultative anaerobes, which obtained from American Type Culture Collectionis chosen due to its high growth rates, efficient ethanol production, efficient glucose repression, and tolerance for environmental stresses. Besides, it is believed that capable in producing more than 20% (v/v) bioethanol. (NurulAin, 2010; Blanch and Clark, 1996).

2.5 BIOETHANOL FERMENTATION

In the fermentation process, aerobic and anaerobic process can be used. Fermentation process by using yeast to produce alcohol required a small amount of oxygen for cell to multiply, but afterwards, no air is required. (Rao D.G., 2010) Anaerobic process can have a very well mixed for the reaction than aerobic process. In anaerobic fermentation process, less heat is generated per unit of glucose consumed, and it can be removed externally. Furthermore, *S. cerevisiae* is facultative anaerobes and it can ferment glucose to ethanol under anaerobic conditions. (Bakker, Lee, & Charles. 2007)

Besides, bioethanol fermentation is a kind of submerged fermentation, where the microorganisms and substrate are present in the liquid form. In submerged fermentation, the efficiency of mass and heat transfer is high. Moreover, it is amenable for process modeling and easy in study of kinetics. (Rao D.G., 2010)

By using OPT sap, selection of an appropriate sterilization method can be ignored. It was well-known that the barrier to commercialization of biomass waste to bioethanol production is high cost for pre-treatment. But, OPT sap fermentation can take place in the heat and cold sterilization instead of pre-treatment, and heat sterilization is generally cheaper than cold sterilization.

2.6 PREVIOUS WORK ON BIOETHANOL FERMENTATION

Recently, researchers have been focused on the feasibility of new application on converting biomass to alternative energy carriers, for example, fuel ethanol, butanol, and acetone by using different types of biomass such as sugar cane molasses, sunflower hulls, cassava mash, soybean molasses and etc. (Kaylen et. al., 2000) There are variety of biomass had been chosen in the research of bioethanol production. However, some of the biomass need pre-treatment or extraction in order to gain the sugar for bioethanol fermentation. The previous works that had been carried out in recent year were shown in the table below.

Table 2-5: Screening on previous works

Biomass	Microorganism	Parameters	References
Sugarcane molasses	*S. bayanus*	Substrate concentration	Pradeep and Reddy (2008)
	Z. mobilis	pH	Maiti et al. (2011)
	Z. mobilis	Agitation	Cazetta et al. (2007)
	Z. mobilis	Temperature	Cazetta et al. (2007)
Mango fruit juice	*S. cerevisiae*	-	Veeranjaneya (2007)
Sunflower hulls	*S. cerevisiae* var. *ellipsoideus*	pH	Sanjeev et al. (2004)
	S. cerevisiae var. *ellipsoideus*	Temperature	Sanjeev et al. (2004)
Cassava mash	*S. cerevisiae*	pH	Yingling et al. (2010)
	S. cerevisiae	Agitation	Yingling et al. (2010)
Soybean molasses	*S. cerevisiae*	pH	Siqueira et al. (2008)
Sweet sorghum stalk juice	mutant strain baker yeast	pH	Yu et al. (2009)
	S. cerevisiae	Agitation	Liu and Shen (2008)
	S. cerevisiae	Temperature	Liu and Shen (2008)
Soft drink waste water	*S. cerevisiae*	Kinetic parameters	Miguel et al. (2013)
Corn Stover	*S. cerevisiae*	Kinetic parameters	Amenaghawon et al. (2012)
Banana peel waste	*S. cerevisiae*	Kinetic parameters	Manikandan et al. (2008)
OPT sap	*S. cerevisiae*	-	Kosugi (2010)
		Temperature	Chin et al., 2010

2.7 KINETIC PARAMETERS

The chemical factors that will affect the fermentation process are normally the substrate concentration, pH control agents, and medium quality. In the other hand, physical factors that will affect the process include tank configuration, pressure, mixing condition, and temperature.

In general, temperature and pH are the most studied factors for high production of bioethanol. Temperature is essential to the microbial growth which mostly related to the energy requirement for cell maintenance that can affect yield coefficient (Shuler and Kargi, 2002). The factor of pH also important as the pH can affect the activity of enzymes in the cell thus affects microbial growth rate due to the higher maintenance energy requirement needed if the pH differs from the optimal value (Shuler and Kargi, 2002). Furthermore, agitation is used in the fermentation mainly to reduce the diffusion barriers by improving mass transfer characteristic. This helps to shorten fermentation time as the fermentation broth in the tank is well mixed. (Cazetta et al., 2007; Maiti et al., 2011). Shorter fermentation time was favourable in term of profit however the longer fermentation time usually can increase the ethanol concentration as more time is given for sugar utilization. (Chin et al., 2010)

From the previous study done, temperature and pH studied on OPT sap fermentation process had been carried out in lab scale. Among the factors, substrate concentration has the great effect on fermentation process of *S.cerevisiae* by using oil palm trunk sap. (Bakker et al., 2007) The batch experiment was performed with various substrate concentrations to develop bioethanol production since sugar content is one of the main influences of the bioethanol production. According to Pramanik (2003), in a reasonable time, the fermentations which conducted with the sugar concentration range from 50 to 250g/L able to obtain high yield of bioethanol. In this research, the substrate concentration were decided to carried out at 60%, 80% and 100% tested at 30^{o}C, over 72 hours of fermentation period, where it sugar concentration was determined in the range.

2.8 KINETIC MODELLING

To analyze the biological process effectively, kinetic model of the process need to be understood well. During the study of bioethanol production, it is important to explore the kinetic parameters that are involved in fermentation process. Fermentation in a relatively equitable and similar environment should produce almost similar and constant multiplication rate of microbes. Therefore, kinetic models were used to describe the microbial behaviour and mechanism of the process. Several phases of cell growth can be observed during batch fermentation, which including lag phase, acceleration phase, exponential phase stationary and death phase, as shown in the figure below. Maximum specific growth rates, half saturation constant and biomass yield can be calculated from the end of exponential phase until decline phases.

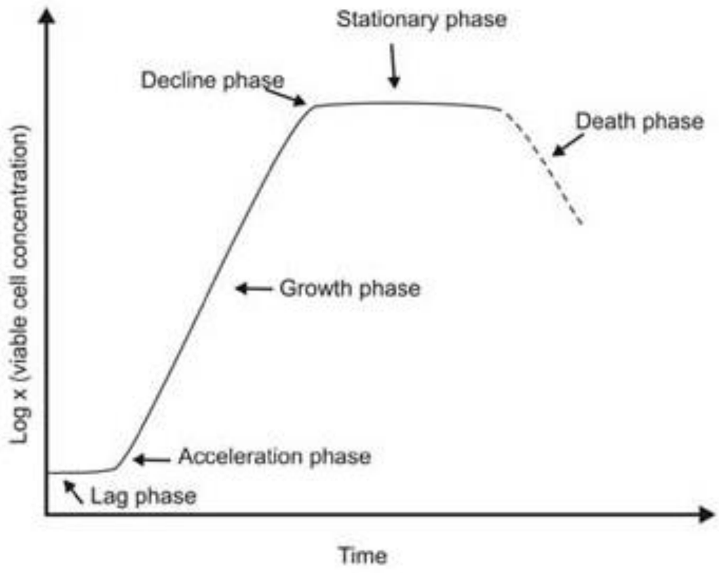


Figure 2-4: Typical growth curve for a microorganism population.
Source: Shuler & Kargi (2002)

Monod and Hinshelwood model is used in this research to understand the productivity of bioethanol, and the cell growth in different substrate concentration. It is one of the simplest models which had included the effect of nutrient concentration. The Monod equation is derived from the basis that a single enzyme system with Michaelis-Menten kinetics is responsible for substrate uptake and catalytic activity is low enough from to be growth rate limiting. (Blanch and Clark, 1996)

In the substrate limiting growth condition, the relationship between specific growth rates to substrate concentration often assumes the saturation kinetics form. According to Shuler and Kargi (2002), by assuming substrate, S, is growth-rate limiting chemical species, which means increase in substrate will influences rate of cells growth, while others nutrient changes will have no effect. This kinetic study is similar to the Langmuir-Hinshelwood kinetics in the traditional chemical kinetics. The Monod equation can be described as the following equations.

$$\mu = \frac{\mu_m S}{K_s + S} \tag{2.1}$$

Where μ_m is the maximum specific growth rate when S>>Ks, and other nutrients concentrations are unchanged, which mean high substrate concentration, $\mu=\mu_{max}$. Ks is the value of the limiting nutrient concentration which is equal to one half of the maximum (Ks=S when $\mu=1/2\mu_{max}$). The term S/(Ks+S) can be simply ad a deviation for μ from μmax. This relation suggested that specific growth rate is finite ($\mu \neq 0$) for any finite concentration of rate limiting component. Hence, this behaviour is not well tested for S<<Ks. (Bailey and Ollis, 1986; Blanch and Clark, 1996; Rao D.G., 2010)

At low substrate concentration, S<<Ks,

$$\mu = \frac{\mu_{max}}{K_s} \cdot S \tag{2.2}$$

According to Blanch and Clark (1996), typical values of Ks for various types of yeasts are as shown in the table below.

Table 2-6: Different characteristics of yeast cells

Microorganisms	Growth temperature, ^oC	Limiting nutrient	Ks (mg/L)
Escherichia coli	37	Glucose	2-4
Escherichia coli	37	Glycerol	2
Escherichia coli	37	Lactose	20
Saccharomyces cerevisiae	30	Glucose	25
Candida tropicalis	30	Glucose	25-75

For the and Hinshelwood model equation,

$$\mu = \mu_m \left(\frac{S}{K_s + S} \right) \left(1 - \frac{P}{P_m} \right) \tag{2.3}$$

Where Pm is the maximum ethanol concentration above cell growth ceases. Hinshelwood model is developed for describing cell growth during fermentation process, and the validation of this model able to have a dynamic response prediction of the cell growth during the bioprocess. (Shuler & Kargi, 2002; Amenaghawonet.al., 2012)