BIOETHANOL PRODUCTION BY BAKER'S YEAST IN OPTS - EFFECT OF TEMPERATURE AND PH

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ABSTRACT

This paper presented bioethanol production in oil palm trunk sap (OPTS) by using baker's yeast - effect of temperature and pH. Baker's yeast as known as *Saccharomyces cerevisiae.* Due to rapid growth in population and industrialization, eco-friendly bioethanol demand is rising rapidly worldwide. Bioethanol offers more advantages than fossil fuel since it provides renewable and sustainable sources of energy. The most commonly employed methods for bioethanol generation are fermentation using baker's yeast (Ahmad *et al.*, 2011). Baker's yeast is commonly used because it is the cheapest strain available for conversion of biomass substrate and produces high yield of ethanol. Due to the cost of raw materials, the cheap renewable agricultural wastes are chosen as alternative substrates to produce ethanol. This study is focused on studying the parameters of temperature (27℃-39℃) and pH (3-7) for bioethanol production from oil palm trunk sap (OPTS) by baker's yeast, *Saccharomyces cerevisiae.* The yeast is grown in nutrient broth (NB) and ferment in oil palm trunk sap (OPTS) medium at different conditions. By analyzing temperature and pH parameters, specific growth rate, glucose consumption rate, specific ethanol production and production yield of ethanol can be determined. The analytical technique involved is high-performance liquid chromatography (HPLC) for glucose consumption and ethanol production while cell dry weight (CDW) is determined for growth. From this study, the maximum pH and temperature for bioethanol production were determined to be pH 6 and 30 °C respectively. The maximum concentration of bioethanol for pH was 34.4 g/L while maximum concentration of bioethanol for temperature, it was 43.4 g/L. In addition, the maximum bioethanol production yield was 0.6 g/g at pH 6 and the maximum bioethanol production yield at temperature at 33 °C was 0.46 g/g. The maximum production yield was contrast with to maximum bioethanol production. This is because the total sugar concentration at 30 °C was too high compared to others. This level might exceed sugar critical level and cause substrate inhibition to occur. This factor inhibited the ethanol productivity to be produced in high level. Last but not least, it could be concluded that the optimum pH and temperature for growth and production of bioethanol using *S. cerevisiae* in OPTS were in the range of pH 5-6 and temperature 30 -36°C.

Key words : *S.cerevisae*; Fermentation; Bioethanol; Oil palm trunk sap (OPTS); Kinetic parameter, HPLC.

ABSTRAK

Kertas kerja ini membentangkan mengenai penghasilan bioethanol daripada getah perahan dalam kelapa sawit batang dengan menggunakan yis jenis *Saccharomyces cerevisiae*. Disebabkan penduduk yang ramai dan perindustrian yang pesat membangun, permintaan terhadap bioethanol semakin meningkat di seluruh dunia. Bioethanol menawarkan lebih banyak faedah daripada bahan api fosil kerana bioetanol merupakan sumber yang boleh diperbaharui dan tenaga yang berterusan. Kaedah yang kerap digunakan untuk menghasilkan bioetanol adalah melalui proses fermentasi iaitu proses yang menggunakan yis (Ahmad *et al.*, 2011). Yis jenis *Saccharomyces cerevisiae* biasanya digunakan kerana ia merupakan mikroorganisma yang paling murah berbanding yang lain dan boleh bertukar menghasilkan glukosa dan etanol dalam kuantiti yang banyak. Oleh kerana kos bahan mentah daripada sisa pertanian agak murah, ia boleh diberi perhatian sebagai alternatif substrat untuk menghasilkan etanol. Kajian ini tertumpu kepada kesan suhu (27°C -39°C) dan pH (3-7) yang berbeza untuk penghasilan bioetanol daripada getah perahan dari batang kelapa sawit (OPTS) dengan menggunakan yis, *Saccharomyces cerevisiae*. Yis dilarut di dalam nutrient broth (NB) dan difementasi di dalam getah perahan batang kelapa sawit pada parameter yang berbeza. Dengan menganalisis kesan suhu dan pH, kadar pertumbuhan tertentu, kadar penggunaan glukosa dan produk etanol boleh dicapai. Teknik analisis yang terlibat adalah cecair kromatografi berprestasi tinggi (HPLC) untuk mengkaji penggunaan glukosa dan produk etanol manakala berat sel yang kering dikaji dengan mengeringkan sel daripada fementasi. Daripada kajian ini, pH dan suhu maksimum untuk penghasilan bioethanol ialah pH 6 dan 30 ° C. Kepekatan maksimum bioethanol bagi pH adalah 34.4 g/L manakala kepekatan maksimum bioethanol untuk suhu, ialah 43.4 g/L. Di samping itu, maksimum penghasilan bioethanol ialah pada pH 6 (0.6 g/g) dan suhu pula ialah pada 33°C (0.46 g/g). Hasil pengeluaran maksimum adalah berbeza dengan pengeluaran bioethanol maksimum. Ini kerana jumlah kepekatan gula pada 30°C adalah terlalu tinggi berbanding dengan suhu lain. Tahap ini mungkin melebihi paras kritikal gula dan menyebabkan perencatan berlaku. Faktor ini menghalang penghasila produk etanol yang tinggi. Akhir sekali, hal ini dapat disimpulkan bahawa pH dan suhu yang paling sesuai untuk pertumbuhan sel dan penghasilan bioethanol dengan menggunakan yis, *S. cerevisiae* ialah dalam lingkungan pH 5-6 dan 30 -36 °C.

Kata kunci : *S.cerevisae*; Fermentasi; Bioetanol; getah perahan batang kelapa sawit (OPTS); Kinetik parameter, HPLC

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LIST OF ABBREVIATIONS

μ	- micrometer
h	- hour
no.	- number
HPLC	- High Performance Chromatography
OD	- Optical Density
CDW	- Cell dry weight
OPTS	- Oil palm trunk sap
rpm	- rotation or revolution per minutes
μ	- specific growth rate
P_0	- bioethanol concentrations at the beginning
P_f	- bioethanol concentrations at the end of fermentation
S_0	- the concentrations of fermentable sugars at the beginning
S_f	- the concentrations of fermentable sugars at the end of fermentation
r_s	- sugar consumption
r_p	- bioethanol production
v/v	- volume per volume
v/w	- volume per weight
Y	- Yield

1 INTRODUCTION

1.1 Background of study

Fuel including petroleum oil is a non-renewable resource on human timescale. This fuel has been the world's leading energy source since the mid-1950s because of its high energy density, easy transportability, and relative abundance for many sectors. Nowadays, the world face a crisis of diminishing fossil fuel reserves and the transportation sector worldwide is almost dependent fully on petroleum-based fuels. Nevertheless, transportation sector is one of the factors that contribute to global pollution such as greenhouse gas (GHG) emissions, global warming and climate change. The global population is expected to increase by approximately 3 billion people by 2050. Due to that fact, replacement of fuels should be considered seriously to maintain stability of ecosystems and global climates.

Mustafa (2011) revealed that more than 70 % of global carbon monoxide (CO) emissions and 19% of global carbon dioxide (CO2) emissions are identified in 2011. Bioethanol has the potential to be a sustainable transportation fuel, as well as a fuel oxygenate that can replace gasoline and petroleum. Ethanol has several attractive features as an alternative fuel. It is most environmental friendly, which has high octane with low emission. For example, it is easily transported and can be blended with gasoline to increase the octane rating of fuel. According to Tang *et al.* (2006), the suitable alternative to replace fossil fuels is the production of bioethanol due to its ability in reducing vehicles carbon dioxide (CO2) by 90 %. In order to reduce greenhouse emission and climate change, bioethanol is much needed as the second choice for fuels.

Bioethanol is produced by fermentation process from various raw materials. Saravanan *et al.* (2012) stated that bioethanol production through microbial fermentation provided an economically competitive source of energy. There are two types of raw materials. First is a primary raw material like sugarcane, molasses, and

maize. Second is a secondary raw material which is lignocellulose biomass such as oil palm trunk, wood waste, and banana peel waste as well as rice husk. Lignocellulose biomass represents the most abundant global source of renewable biomass. Hydrolysis using appropriate enzymes represents the most effective method to liberate simple sugar from cellulosic materials. In recent years, oil palm has become a major economic crop in Malaysia and Indonesia. Oil palms trees are replanted approximately every 20-25 years because oil productivity of old trees are decreased. Consequently, the felled trunks are the enormous amount of biomass waste that needs to be discharged due to replantation especially in Malaysia and Indonesia. Instead of destroying or burning the trunk, it is better to be utilized for good use. Hence, because of the trunk sap contains a lot of sugar content, the crops can be used for bioethanol production with the most suitable microorganism, which is *Saccharomyces cerevisiae*.

1.2 Problem statement

Currently, sugarcane is the most efficient raw materials for bioethanol production. Nevertheless, use of sugarcane as biomass is not considered to be sustainable for long times due to its competition with food and animal feed usage. They have their own drawback in such that they are served as staple food in some countries. The increase demand of these crops for bioethanol production will increase global food prices too. Therefore, secondary raw material such as oil palm trunk sap (OPTS) would replace sugarcane due to its potential to generate high glucose for bioethanol production.

Othman (2012) in his current report has indicated that the sap in inner part of trunk contains 85.2 g/L of glucose concentration and 14.8 % of sucrose, fructose, galactose, xylose and rhamnose. Accordingly, oil palm trunk sap (OPTS) is suitable to be used as a carbon source for yeast fermentation in producing bioethanol. Moreover, as mentioned by Akihiko *et al.* (2012), the composition sugar contents in OPTS are nearly the same with the composition of sugarcane while ethanol yield in OPTS is greater than ethanol yield in sugarcane. *Saccharomyces cerevisiae* is the cheapest strain available for the conversion of biomass substrate and it can produce high concentration of ethanol which is preferred for most ethanol fermentations (Chew *et al.*, 2009). Yeast can break

down the starch and water, creating bioethanol and carbon dioxide as end of products. However, there is no collecting data for kinetic parameter of bioethanol production using oil palm trunk sap (OPTS). Therefore, details investigation about the kinetic parameter of bioethanol production is necessary.

1.3 Objectives

The main objective of this experiment was to investigate the effect of temperature and pH in the production of bioethanol by baker's yeast using oil palm trunk sap (OPTS).

1.4 Scope of study

The scope of this study was to examine the effect of temperature and pH for bioethanol production using OPTS medium. Temperature range study was 27°C, 30°C, 33°C, 36°C and 39°C while pH range examined is 3, 4, 5, 6 and 7. Then, time series of yeast's growth was determined by cell dry weight. High-performance liquid chromatography (HPLC) was used to identify glucose consumption and bioethanol production. The specific growth rate (μ) and the kinetic parameters such as specific subtrate consumption rate (q_s) and specific bioethanol production rate (q_p) were determined.

2 LITERATURE REVIEW

2.1 Oil Palm Trunk Sap (OPTS)

Oil palms, also known as *Elaeis guineensis*, are replanted at 20- 25 years intervals to maintain oil productivity. Anon (2011) stated that oil palm is the most important vegetable oil and it contributes the largest in terms of total production quantity. Murata *et al.* (2012) also mentioned that oil palm is the most rapidly expanding equatorial crops in the world and a source of economic life. Furthermore, the world's demands for oils rise steadily over the years such that Malaysia and Indonesia have focused on cultivating oil palm crop. In 2008, Malaysia and Indonesia contributed about 85 % of oil palm production, which is nearly 36 million tonnes. Because of high demand of oil palm, the land for oil palm plantation increased to 5 million hectares in 2011, automatically boost the country economics.

Oil palm trunk (OPT) is one of the lignocellulosic waste materials. Saravanan *et al.* (2012) said currently industries across the world generated huge volumes of lignocellulosic wastes. These wastes have an immense potential to be utilized for the production of several bio-products. They provide a low-cost and uniquely sustainable resource for production of many organic fuels and chemicals, which enhance energy security and improve health quality. Oil palm trunk was found to contain large amount of sugar contents, including glucose, fructose, sucrose and galactose. All these sugars are easily to be converted to ethanol and also to lactic acid. Therefore, the trunk was found to be significant resource especially for ethanol, biochemical and bioplastics production. Glucose was found to be the dominant sugar in all parts, accounting for approximately 86.9 %, 86.3 % and 65.2 % of the total free sugars contained in the inner, middle and outer parts of OPT, respectively (Yamada *et al.*, 2010). Their results of research clearly showed a significant increase of fermentable sugars in the oil palm sap occurred during storage of the trunks after logging as compared to fresh oil palm sap. Other components in the squeezed sap are namely amino acids, organic acids, minerals and vitamins (Akihiko *et al.*, 2010).

Table 2.1 shows the comparison between the OPTS and sugar cane juice, which is the largest current feedstock. Even though the percentage of fermentable sugar and total sugar were lower in OPTS, the yield of ethanol was higher than sugar cane. In addition, Figure 2.1 clearly showed that sugars increased sharply during day 30 and this proved that there were large amount of sugar after logging than the fresh sap. The concentration calculated at Day 0 was 83 mg/ml and it increased to 153 mg/ml at Day 30 and then dropped to 43 mg/ml after 120 days as shown in Figure 2.2. Although dispersion in sugar content was observed among trunk samples, a distinct changing pattern of sugar concentration in sap increased during the first 30 days followed by a decreased was recognized. The sugars contained in the sap were glucose, sucrose, fructose and galactose and all of them were fermentable by ordinary yeast strains. Yamada *et al.*, (2010) strongly indicated that old oil palm trunk becomes a promising source of sugars by proper aging after logging. Thus, its sap could be a good feedstock of bioethanol and bio-plastics production.

Table 2.1: Comparison between the OPTS and Sugarcane Juice (Akihiko et al., 2012)

	Sugar cane	***OPTS at day 60***
Fermentable sugar concentration in juice or sap (%)	14.5	12.8
Moisture content (%)	Approx. 70	68
Amount of sugars contained (g/kg)	102	87
Cane/trunk produced per area (t/ha)	77.6	154-168
Possible ethanol yield (m^3/ha)	6.5	8.7-9.4

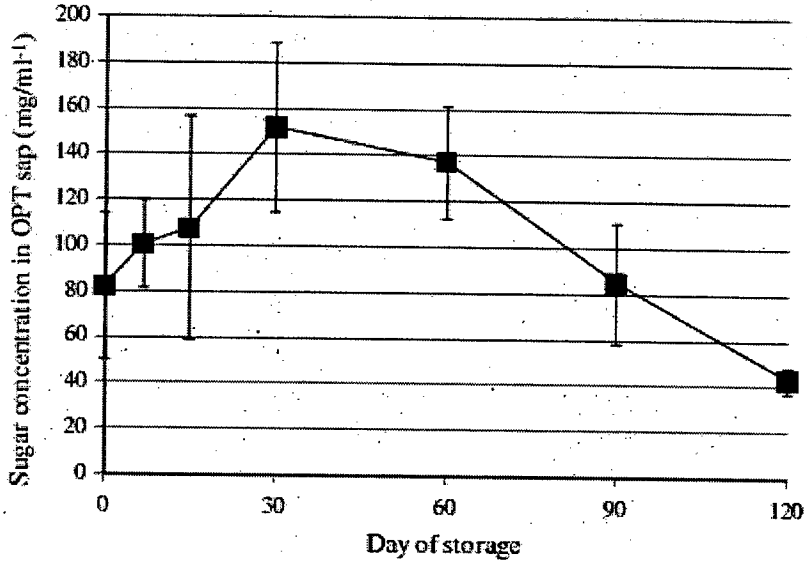

Figure 2.1: Sugar concentration during storage (Yamada et.al., 2010)

On the other hand, OPT sap medium was also used as a carbon source for previous research by using *Bacillus megaterium MC1*. Based on Kumar *et al.* (2012), they also found that OPT sap extracted from younger tree trunks with prolonged storage had higher sugar content and *B. megaterium* was able to utilize all the sugars in oil palm trunk sap (OPTS). They achieved the highest biomass after 16 hours cultivation in shake flask cultures. By comparing between *B. megaterium* growth in OPTS medium and commercially available media such as Luria Broth (LB) and Nutrient Broth (NB), it showed a good growth in OPTS medium.

2.2 *Bioethanol Production*

Ethanol has known as bioethanol because it was produced from simple sugars, starch or lignocelluloses biomass by fermentation process. It is biomass energy source (biofuels) that classified as a second-generation feedstock. Biofuels consist of two parts, which were the primary biofuels (untreated and natural) and the secondary biofuels, which was usually used for combustion, heating, cooking fire, and power consumption. Ethanol, biodiesel and methanol were included in the secondary biofuels (Larson, 2008). Almost all the raw materials of secondary biofuels or second-generation fuels were coming from agricultural waste (residue), wood and grass.

Bioethanol is presently an alternative for fuels and gasoline for automobile. Most ethanol used for fuel is being blended into gasoline at concentrations of 5 to 10 %. In California, ethanol has replaced methyl tertiary butyl ether (MTBE) as a gasoline component. One of the main advantages for ethanol as compared to gasoline is it, anti-knock performance that allows its use in higher compression ratio engines. Then, ethanol powered cars emit less pollution which are reducing more than 50 % of smog forming emissions. As consequences, the ethanol fuel cars help the reduction of greenhouse gases that cause global warming. As shown by Figure 2.2, using ethanol as a vehicle fuel has measurable greenhouse gases (GHG) emissions advantages compared with using gasoline. U.S. Department of Energy's Clean (2013) studied that by using cellulosic biomass, ethanol provided a greater benefit in reducing greenhouse (GHG) emissions by up to 86%. It followed by 78 % by using sugarcane. Recent studies have proved the importance of bioethanol in replacing the gasoline for vehicles fuels.

As ethanol is easy to manufacture, the ethanol powered cars gained good wide acceptance in the green car market. Ethanol powered cars are eco-friendly and deliver power at good fuel efficiency. Comparison of properties for ethanol and gasoline are given in Table 2.2. At high temperature, ethanol produces superior thermal efficiency due to its higher heat of vaporization. As ethanol can burn richer fuel/air mixtures, it allows for higher engine power output in comparison to gasoline. However, due to its lower heating value, the use of ethanol results is in higher fuel consumption (Rodrigo & Jose, 2010).

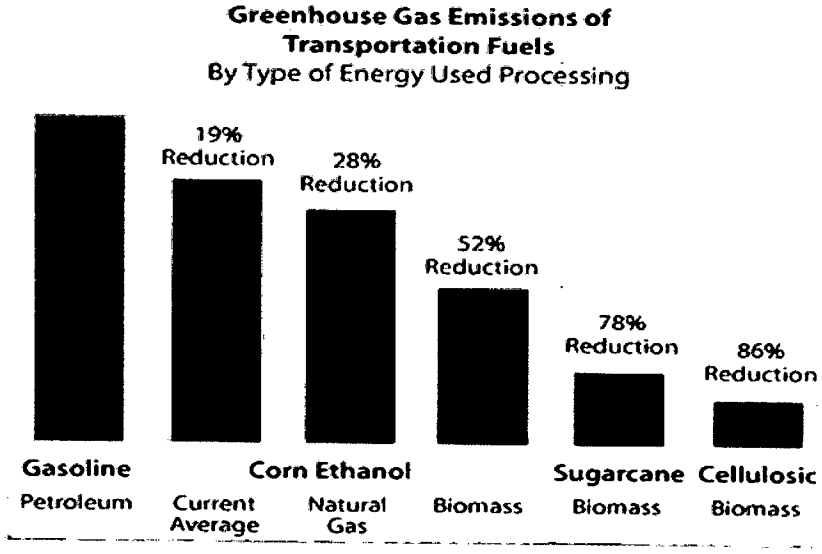

Figure 2.2: Life cycle Energy and Greenhouse Gas Emission Impacts (U.S. Department of Energy's Clean, 2013)

Table 2.2: Comparison between some properties of ethanol and gasoline (Hasan, 2008)

Properties	*Gasoline*	*Ethanol*
Chemical formula	C_4–C_{12}	C_2H_5OH
Molecular weight	100–105	46
Oxygen (mass %)	0–4	34.7
Net lower heating value (MJ/kg)	43.5	27
Latent heat (kJ/L)	223.2	725.4
Stoichiometric air/fuel ratio	14.6	9
Vapor pressure at 23.5 °C (kPa)	60–90	17
MON	82–92	92
RON	91–100	111

Bioethanol is neutral carbon that contains no harmful sulphur and aromatics (Ying *et al.*, 2011). The complete combustion of ethanol only produces carbon dioxide and water and does not contain others harmful substances. Also ethanol does not harm any seals or valves and does not increase corrosion. Besides that, ethanol represents closed carbon dioxide cycle (Figure 2.2) because after ethanol burnt, the released carbon dioxide is recycled back into plants due to absorption of CO_2 to synthesize cellulose during photosynthesis cycle. By this fact, friendly bioethanol will go a long way in protecting next generation from any negative feedback of pollution. Prasad *et al.* (2007)

stated that research on improving ethanol production has been accelerating for both ecological and economic reasons, primarily for its use as an alternative to petroleum based fuels. Using bioethanol, air pollution and CO_2 accumulation, also petrol consumption can be decreased.

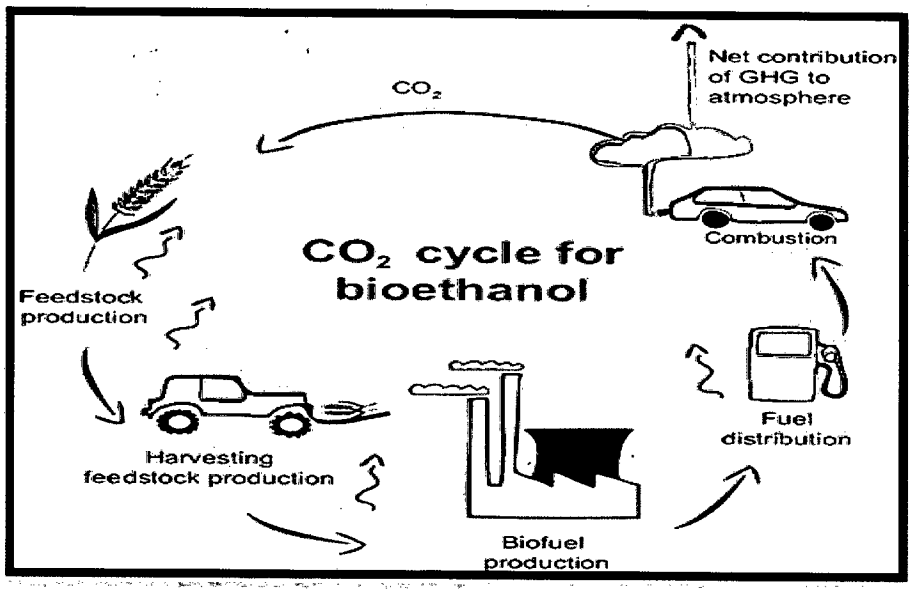

Figure 2.3: Bioethanol presents closed CO2 cycle (BEST, 2009)

Presently, Brazil and the United States lead the industrial world in global ethanol production, accounting together for 70 % of the world's production and nearly 90 % of ethanol is used for fuel. Until this time, almost 40 % of ethanol composition out of total fuels was used by the car in Brazil. Brazil is the world's top ethanol producer, using sugar cane as the feedstock. Meanwhile, the United States and Europe mainly used starch from corn, wheat and barley, respectively (Mustafa, 2011). Sugar cane plantations cover 3.6 million hectares of land for ethanol production with a productivity of 7500 litres of ethanol per hectare. In U.S., more than 3000 litres per hectare of maize was used to produce ethanol. Other countries also increased the production of ethanol fuels and started to choose ethanol for cars because of the fuel efficiency and lesser pollution.

Bioethanol could also be produced from mahula flowers, *Madhuca latifolia L.* by *Saccharomyces cerevisiae* in solid-state fermentation (SSF) (Mohanty *et al.*, 2009). However, these raw materials required more agricultural land for cultivation. This has affected other plants cultivation. In addition, they are also used for human food and animal feed. As a result, they are not sufficient to meet the rising demand for biofuels. In view of the facts above, lignocellulose biomass was utilized to replace the crops. In fact, it is cheaper and has greater availability than sugars and starch. Lignocellulose waste materials obtained from energy crops, wood and agricultural residues represent the most abundant global source of renewable biomass. Olokayode (2012) stated that it can provide clean energy and stable national security for future generations. Ideally, the technology should also foster recycling of agricultural feedstocks and improve soil fertility and human health (Sivakumar *et al.*, 2010).

In Europe, wheat straw is the largest biomass feedstock among the agricultural residues and the second largest in the world after rice straw. About 21 % of the world's food depends on the wheat crop and its global production needs to be increased to satisfy the growing demand of human consumption. Therefore, wheat straw achieved as a good potential feedstock for production of bioethanol in 21st century. Based on the wheat straw pretreatment method by Tablenia (2010), a sugar yield of ethanol production achieved was in the range of 74–99.6 %. On the other hand, rice straw also has potentially produced 205 billion liters of bioethanol per year and it was an attractive lignocellulosic material for bioethanol production in India. Balasubramaniam (2013) stated that rice straw by Separate Hydrolysis and Fermentation (SHF) method using yeast cells, *Saccharomyces cerevisiae* and *Pachysolen tannophilus* produce bioethanol in the high range. The percentage of bioethanol produced was 24.50 % (v/w) in which 19.10 g of bioethanol was produced from 100 g of rice straw. The optimum temperature for both organisms was found to be 30°C and optimum pH for *S. cerevisiae* and *Pachysolen tannophilus* was determined as 5.5 and 6, respectively.

Bioethanol production could be produced by using sap squeezed from old oil palm trunks felled with *S. cerevisiae Kyokai no. 7*. It was supporting by high production of oil palm in Malaysia. According to Akihiko *et al.* (2010), they found that the amount of ethanol produced corresponded to 94.2 % of the theoretical yield calculated based on

consumption of glucose, sucrose, fructose, and galactose in oil palm trunks felled. Figure 2.3 showed the ethanol concentration was 30 g/L and glucose consumption was 55 g/L by using oil palm trunk sap (OPTS) without added nutrients. Reference fermentation was carried out on YPD medium producing 32 g/L of ethanol and consuming 60 g/L glucose. This trend revealed that amount of ethanol production by using squeezed sap with *S. cerevisiae Kyokai no. 7* was near to amount of ethanol production with Yeast extract, Peptone & Glucose (YPD medium). Therefore, squeezed sap has a potential to replace YPD medium in ethanol production.

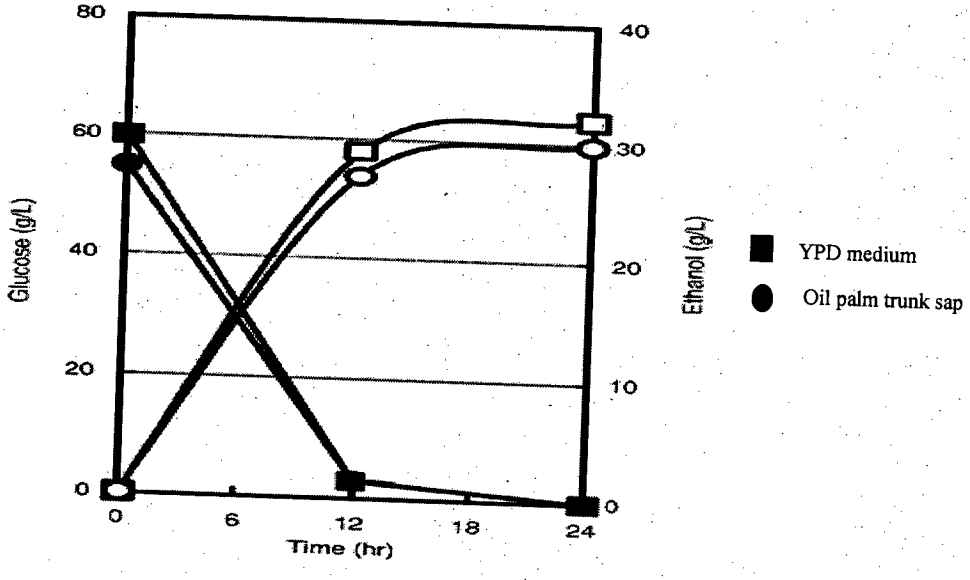

Figure 2.4: Time course of ethanol production using felled oil palm trunk sap with S. cerevisiae Kyokai no.7 at 30°C (Akihiko et al., 2010)

2.3 Baker's Yeast as Microorganism in Bioethanol Production

Saccharomyces cerevisiae is recognized as an ideal eukaryotic microorganism for biological studies (Guthrie, 2004). *S. cerevisiae* is the only yeast that can rapidly grow under aerobic as well as anaerobic conditions. Some of yeast properties are suitable for biological *studies. Saccharomyces cerevisiae*, yeast which need a natural environment is always associated with high sugar content, plant-related environments and displayed

much kind of cellular responses to temperature changes. This yeast strain could be live at range of 25 °C to 30 °C and it could not adapt with high temperature except mutant strain. According to Nonklang *et al.* (2008), they found that *K. marxianus* and *S. cerevisiae* strains depicted similar levels of ethanol production and glucose consumption at 30 °C but, *S. cerevisiae* did not grow and also did not produce ethanol when the fermentation was carried out at 45 °C. In contrast, *K. marxianus* strains produced ethanol with high productivity at high temperature.

Yeast does not required sunlight to grow, but it use sugars as a source of energy. Gnode *et al.* (2009) stated that there are three major pathways for growth on glucose by yeast:

1) Fermentation of glucose: $C_6H_{12}O_6(s) \longrightarrow 2CH_3CH_2OH(l) + 2CO_2(g)$
2) The oxidation of glucose: $C_6H_{12}O_6(s) + 6O_2(g) \longrightarrow 6CO_2(g) + 6H_2O(l)$
3) The oxidation of ethanol: $CH_3CH_2OH(l) + 3O_2(g) \longrightarrow 2CO_2(g) + 3H_2O(l)$

The first pathway can be related to this research because it involves the production of ethanol.

Today, yeast for ethanol production is valuable when combines with innovation and formulation new technologies (Knauf and Kraus, 2006). Bioethanol could be produced by fermentation of simple sugars present in biomass or the sugars obtained by prior chemical or enzymatic treatment of biomass. Halim and Yahya (2013) found that *S. cerevisiae* was the best choice for lignocelluloses-derived substrate because it is particularly suitable for the hexoses fermentation. There are a lot of hexoses sugars such as glucose, fructose and galactose in oil palm trunk sap (OPTS) medium. Hence, it is suitable for fermentation in OPTS medium. During fermentation, baker's yeast was utilized to convert glucose in sap into ethanol.

Hoek (1998) stated that *S. cerevisiae* could produce high sugar concentrations and high specific growth rates even under fully aerobic conditions. Instead, ethanol yield of anaerobic bacteria was low and inhibited at low sugar and ethanol concentration. Liu *et al.* (2009) reported that when oxygen absent, the growth of *S. cerevisiae* would be

inhibited. Particularly, it required a certain supply of elemental oxygen in order to synthesize unsaturated fatty acids and sterols, which were important constituents of its cell envelopes. However, if oxygen was provided too much, yield of production ($Y_{p/s}$) decreased sharply because of aerobic respiration. Under full aeration, yeast would consume more glucose to produce carbon dioxide and water. Investigated by Hosein *et al.* (2013) in recent years, *S. cerevisiae* among several other microorganisms, has attracted considerable attention for the solid state fermentation (SSF) in the production of bioethanol from agricultural wastes. This is owing to its higher tolerance to both ethanol and inhibitors present in hydrolysates of lignocellulosic materials. The possibility of performing fermentation at higher temperatures using thermo-tolerant yeast strains that capable to grow at temperatures compatible with optimal cellulase activities would greatly improve the enzymatic hydrolysis in SSF processes. Thereby, it was making the ethanol production process more economically feasible.

Despite of that, Chandel *et al.* (2010) worked on combination of *Pichia stipitis* with *S. cerevisiae* and found that this co-culture was able to achieve higher final ethanol concentration compared to using only single strain of *Pichia stipitis* or *S. cerevisiae*. *Zymomonas mobilis* which is gram negative species was also considered an alternative microorganism for the industry scale of ethanol production. *Zymomonas mobilis* could give higher sugar uptake and ethanol yield. This species was able to utilize glucose, fructose, and sucrose as the substrates for the ethanol production. But, it has lower biomass production than *Saccharomyces cerevisae* (Halim and Yahya, 2013). They also found that all *S. cerevisiae* strains, which were *S. cerevisiae Kyokai no. 7*, ordinary *S. cerevisiae* and *S. cerevisiae JCM2220,* produced a good amount of final ethanol yield at 30°C. The final yield of each strain was 0.483 g/g, 0.426 g/g and 0.449 g/g respectively.

In another case, a mixed bacterial culture, *Thermoanaerobacterium* and *Caldonaerobacter* could also produce ethanol from wheat straw, but only under extreme thermophilic conditions (Talebnia *et al.,* 2010). He also mentioned that *E.coli* has been tested for ethanol production too and produce high ethanol yield from wheat straw. Various bacteria, yeasts and fungi have been investigated with the ethanol yield ranging from 65 % to 99 % of theoretical value. So far, the best results with respect to ethanol

yield, final ethanol concentration and productivity were obtained with wild type, *S. cerevisiae*.

2.4 Kinetic Study on effect of temperature and pH

This research's objective was to investigate the kinetic study of bioethanol production from oil palm trunk sap (OPTS) by baker's yeast. Kinetics is the study of changes in a physical or chemical system. The parameters were investigated consists of specific growth rate, glucose consumption, yield production and ethanol production. Equation 3, 4, 5, 6 and 7 are used to calculate these kinetic parameters. For this research, Monod model (equation 1 and 2) is one of the important kinetic models to model the growth of cell. Substrate in Monod model was known as growth-limiting substrate. Due to the abundant substrate in this study and it could not be varied. Monod model was not being used.

Biomass growth rate

$$\frac{dX}{dt} = \mu X \text{ ------ (equation 1)}$$

Monod equation

$$\mu = \mu \max \frac{S}{Ks+S} \text{ ------ (equation 2)}$$

Volumetric rate of substrate consumption, r_s

$$r_S = -\frac{dS}{dt} = -\frac{\mu X}{Y_{X/S}} = q_S X \text{ ------ (equation 3)}$$

The specific rate of substrate consumption, q_s

$$q_S = -\frac{1}{X}\frac{dS}{dt} \text{ ------(equation 4)}$$

The volumetric rate of product formation, r_p

$$r_P = \frac{dP}{dt} = q_P X \text{ ------ (equation 5)}$$

The specific rate of product formation, q_P

$$q_P = \frac{1}{X}\frac{dP}{dt} \text{ ------ (equation 6)}$$

The specific rate of product formation, q_P

$$q_P = \frac{1}{X}\frac{dP}{dt} \text{ ------ (equation 7)}$$

Growth yield was the most important consideration in some industrial biotechnology applications, such as enzyme or cellular protein synthesis. Ethanol yield could be calculated with respect to glucose consumed and biomass generated. The specific substrate consumption rate is generally linked with a yield of biomass on substrate ($Y_{X/S}$) to the specific growth rate. Others, the maximum theoretical yield for bioethanol over consumed sugars ($Y_{P/S}$) also could be calculated according to equation 8 and 9 below:

$$Y(x/s) = \frac{-dx}{ds} = \frac{Xf - Xo}{So - Sf} \text{ ---------- equation 8}$$

$$Y(p/s) = \frac{-dp}{ds} = \frac{Pf - Po}{So - Sf} \text{ ---------- equation 9}$$

Where, X_0 and X_f are cell concentration at the beginning and at the end of fermentation (g/L) while P_0 and P_f are the bioethanol concentrations at the beginning and at the end of fermentation (g/l), respectively. S_0 and S_f are the concentrations of fermentable sugars at the beginning and at the end of fermentation, respectively (Dodic, 2012).

According to Groot *et al.* (1992), they suggested that the inhibition of the capacity of the yeast for substrate consumption was less severe than the inhibition of growth on ethanol production by *Saccharomyces cerevisiae* in batch culture and also on flocculent strain of Saccharomyces uvarum in a tower fermenter. On the other hand, Chew (2009) stated that when the specific growth rate decreased, the sugar concentration rate for fermentation process would be high. It occurred due to inhibition of cell growth. While,

when glucose concentration rate was lower, the bioethanol production would be higher because of glucose concentration was not saturated and enhanced the ethanol productivity.

In addition, in Dodic (2012) previous research on ethanol production from sugar beet juice, she compared between sugar beet raw juice and thin juice. She found that biomass formation was slightly higher and maximum specific growth rate of raw juice was larger than thin juice. This led to a slightly lower maximum bioethanol concentration, since the maximum bioethanol production rates were nearly the same for both sugar beet processing intermediates. Therefore, it is possible to conclude that raw juice impurities only have a negative effect on biomass multiplication, which is favorable for this process because bioethanol production starts earlier. Considering the lower price of raw juice, it seems to be a more cost-effective feedstock.

Furthermore, according to Tanaka and Lin (2006), the optimum temperature and pH for *S. cerevisiae* by using glucose as a substrate were at 30°C and pH 5.5 where, the maximum ethanol concentration achieved was 91.8 g/L. For Manikandan *et al.* (2008), their investigated parameters for ethanol production from banana peel waste by *S. cerevisiae* mutant strain are temperature and pH. For the effect of different temperature, as the temperature increased from were 27°C to 39°C, the rate of ethanol production also increased and the maximum yield was 9 g/L at 33°C. This was similar to pH effect where, ethanol production increased when pH increased and the maximum yield was 9.2 g/L at pH 4.5. In contrast, Benigno and Octavio (2010) have indicated that the optimal conditions for ethanol production were pH 3.5 and 30°C with the initial glucose concentration of 150 g/L. In this case, a maximum ethanol concentration of 58.4 g/L, ethanol productivity of 1.8 g/L.h and ethanol yield of 0.41 g/ g were obtained.