STUDY ON BIOETHANOL PRODUCTION FROM OIL PALM TRUNK SAP

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ABSTRACT

Old oil palm trunk becomes a promising source of sugars by proper aging after logging and, thus, its sap can be a good feedstock for bioethanol. It aims to develop alternative resources waste to wealth for bio-ethanol production using available biomass in this country. To produce high production of bioethanol, determine fermentation conditions are important in culture fermentation to predict the optimum temperature and optimum inoculums size. The process selection includes preparation of pure culture, yeast activation, fermentation profile, and validation between two types of yeast, batch fermentation and analysis of the data. The overall process performance is measured by the productivity and quality of bioethanol produced. Glucose was thoroughly nearly consumed after 48 hour for the fermentation profile. Validation experiment between showed that *Saccharomyces cerevisiae* Kyokai 7 produce high yield of ethanol. The effects of temperature (25- 40 ^{o}C),and percentage inoculums (5- 15 %v/v) on ethanol yield were assessed by using 2^4 full factorial design (FFD) and validated statistically by analysis of variance (ANOVA). The optimum temperature is 25.29 celcius and optimum inoculum size is 10.54 % .*Saccharomyces cerevisiae* Kyokai 7 produced 41.43% higher ethanol compared to S.cerevisiae. The mean percentage error is 10.32 in screening parameter experiment. The influence of temperature was found to be more pronounced on ethanol yield compare to others parameter

ABSTRAK

Batang pokok kelapa sawit yang tua boleh menjadi sumber gula dari penuaan sempurna selepas ditebang,kemudian menjadi sumber bahan mentah untuk bioetanol. Ini bertujuan untuk membentuk sumber sisa alternatif kepada kekayaan untuk penghasilan bioetanol menggunakan biomas yang sedia ada di negara ini. Untuk menghasilkan penghasilan bioetanol yang tinggi, penentuan keadaan fermentasi adalah sangat penting dalam fermentasi kultur untuk menjangka suhu optimum dan saiz inokulum. Pemilihan proses meliputi penyediaan kultur tulen, pengaktifan yis, profil fermentasi dan pengesahan diantara dua jenis yis, fermentasi dan analisis data.Keseluruhan proses diukur dengan produktiviti dan kualiti bioetanol yang terhasil. Glukosa hampir digunakan sepenuhnya selepas 48 jam untuk profil fermentasi. Eksperimen pengesahan menunjukkan S.cerevisiae Kyokai 7 menghasilkan etanol yang tinggi. Kesan suhu (25-40 ^{o}C) dan peratus inokulum (5- 15 %v/v) terhadap hasil etanol dihubungkan menggunakan "2^4 *full factorial design (FFD*)" dan disahkan secara statistic menggunakan analisis varians.Suhu optimum adalah 25.29 ^{o}C dan saiz inokulum optimum adalah 10.54 % v/v. *Saccharomyces cerevisiae* Kyokai 7 menghasilkan 41.43% etanol lebih banyak berbanding S.cerevisiae. Peratus purata ralat adalah 10.32% dalam memilih parameter eksperimen. Suhu lebih mempengaruhi hasil etanol berbanding parameter yang lain.

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LIST OF SYMBOLS/ABBREVIATIONS

^{o}C		Celcius
DNS	-	Di-Nitro Salicylic Acid
DI	-	deionized
g	-	gram
g/L	-	gram per liter
L	-	liter
mg/L	-	milligram per liter
min	-	minutes
mL	-	mililiter
nm	-	nanometer
OD	-	optical density
rpm	-	rotation or revolution per minute
%		percentage
Ha		hectar

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CHAPTER 1

INTRODUCTION

1.0 Research Background

In recent years, a new round of enthusiasm in biomass and bioenergy has been initiated with the recognition that the global crude oil reserve is finite, and its depletion is occurring much faster than previously predicted. In addition, the environmental deterioration resulting from the over-consumption of petroleum-derived products, especially the transportation fuels, is threatening the sustainability of human society (Bai, *et* al., 2007). Fig.1 shows the energy demand in Malaysia that indicates a rapid increase in demand. For year 2030, energy demand is expected to reach almost 100 Mtoe (million tonne of oil equivalent) (APEC, 2006)

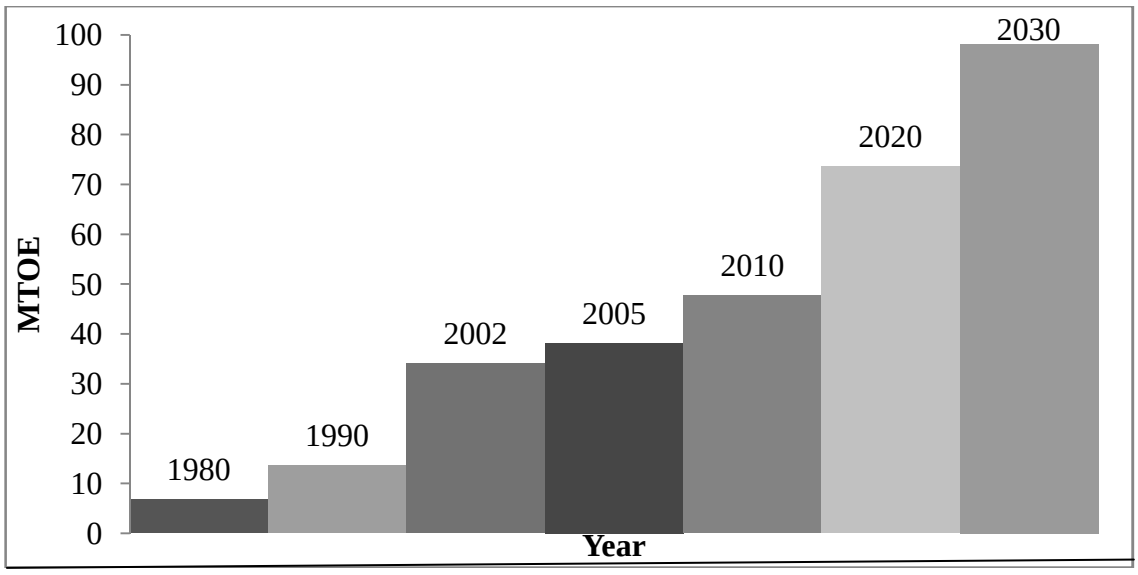

Fig. 1 Energy demand in Malaysia

Although extensive researches of renewable energy (RE) are being carried out throughout the world, the focus on renewable liquid biofuels are restricted to biodiesel and bio-ethanol only while 40% of total energy consumption in the world is in the form of liquid fuels (Tan *et* al., 2008). Research shows that if bio-fuels like bio-ethanol and biomethanol are blended with conventional diesel or bio-diesel, this can help reduce the emission of CO2 by almost 80% compared to using petroleum diesel (Sairan and Aman, 2003). Bioethanol is an attractive alternative fuel because it is a renewable bio-based resource and it is oxygenated thereby provides the potential to reduce particulate emissions in compression–ignition engines (Hansen *et* al, 2005). Ethanol made biologically by fermentation from a variety of biomass sources is widely recognized as a unique transportation fuel with powerful economic, environmental and strategic attributes (Brethauer and Wyman, 2009)

1.1 Objective

This research is aiming to produce high production of bio-ethanol from local biomass which is oil palm trunks sap as substrates. It aims to:

- To develop batch fermentation system for bio-ethanol production from oil palm trunks sap
- To obtain effect temperature and inoculums size for fermentation bio-ethanol process

1.2 Scope

Scope research includes studying optimum parameter for fermentation which is optimum temperature and optimum inoculums size. Moreover, screening fermentation profile of *Saccharomyces cerevisiae* Kyokai 7 is important to determine the length of time for the yeast fully consumed the glucose to produce bioetanol. Next, the validation experiment between *S.cerevisiae* and *S.cerevisiae* Kyokai 7 is essential to determine yeast that produced high yield of ethanol

1.3 Problem Statement

- Serious shortage of fossil resource and increased concern for the negative impact of fossil fuel on the environment has put great pressure on society to find renewable fuel alternatives

- Optimal conditions are required to ensure high yield of the fermentation product and shorter fermentation time for cost effieciency of producing ethanol from lignocellulosic biomasss

1.2 Rational and Signification

Malaysian Palm Oil Council (MPOB) state that energy palm oil crop provides direct and indirect employment to 860,000 people excluding other multiplying effects and spin-offs activities. The exports of reasonable, healthy, nourishing and high-yielding Malaysian palm oil now feed some 1.3 billion people in 150 countries (MPOB, 2006).Lignocelluloses ethanol production is attractive because the nonfood portion of the plant can be used to produce ethanol; hence, there is no competition for feedstock with the food industry (Li *et* al, 2009). Indeed a key trend in the market today is a move away from food crops to nonfood oilseed crops (Li *et* al, 2009). The potential for using lignocellulosic materials in bioethanol production is well recognized.

CHAPTER 2

LITERATURE REVIEW

2.1 Oil Palm Trunk Sap as Raw Material

The oil palm is native to West and Central Africa. Its botanical classification, *Elaeis guineensis*, Jacq., is derived from the Greek *elaion* (oil) and the specific name of guineensis is indicative of its origin from the equatorial Guinea coast.

The chemical characteristics of oil palm trunks were investigated to find out the best utilization method. Oil palm trunk tissue mainly consists of vascular bundles and parenchyma cells, which are separated easily and discriminately from each other by mechanical crush. The starch content was remarkably high in parenchyma cells. Xylose and glucose were the main sugar components in both tissues, indicating that the polysaccharide consists of xylan, starch, and cellulose. The lignin content was less than 20% in both fractions. The lignin of oil palm contained *p*-hydroxybenzoic acid as an ester group which could easily be removed by alkaline treatment

In general, the palm starts bearing oil-contained fruits in 2.5 years after planted and its productivity becomes lower after 20-25 years. Therefore it is necessary to cut the old palms and to replant new seedlings at plantation sites. In Malaysia, about 120,000 ha of oil palm are estimated to be replanted annually from 2006 to 2010 for maintaining the oil productivity (Basiron and Chan, 2006). When replanting, old palms are cut and most of them are discarded or burnt at the plantation site. Therefore, efficient ways for utilizing oil palm trunks is desired for ideal oil palm plantation and sustainable palm oil industry.

Oil palm sap was reported to contain approximately 11% sugars with sucrose as a major component accounting for approximately 90% of total sugar .Meanwhile, it has been reported that the 75% methanol extracts of the dried oil palm trunk (OPT) fiber contains 4.9%-7.8% sugars, which correspond to 2.1%-3.4% sugars in the sap assuming that moisture content of OPT is 70%. There are three part in the oil palm trunk (A, B, C) which have 83%, 75% and 68% moisture content respectively as shown in Fig 2.0.

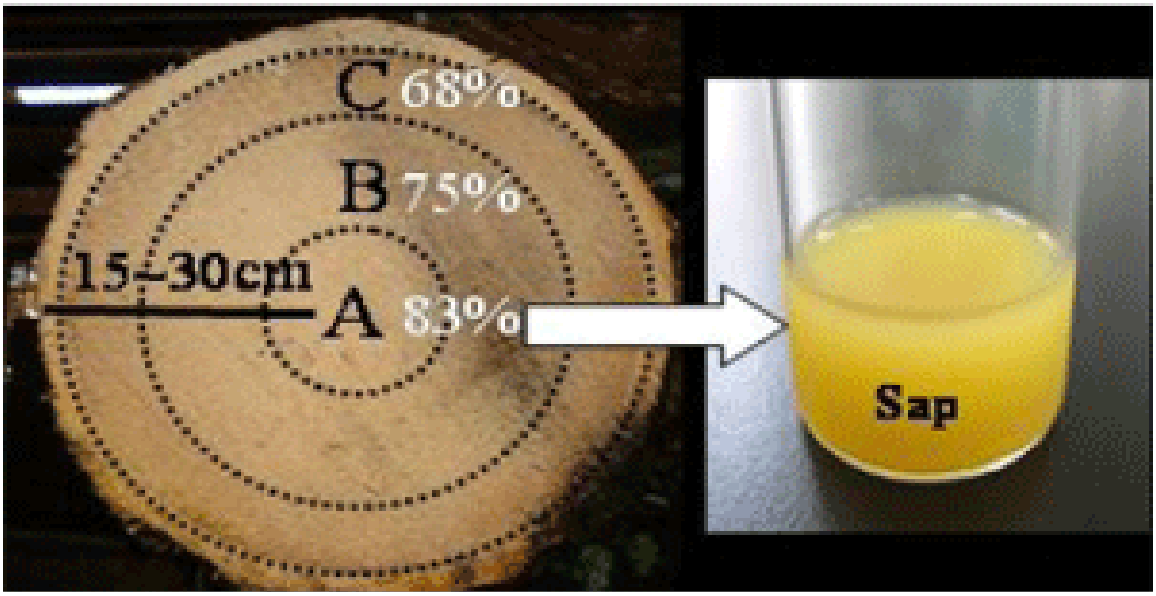

Fig 2.0: Inner, middle and outer of oil palm trunk

2.2 Potential of ethanol from OPT

Every 25 years, the palm oil trees are replanted because of oil productivity of old trees decreasing. It has high moisture content lead it potential to produce biofuel. It contains sap that can be converted into bioethanol. They found that the felled oil palm trunk contains large quantity of sap, which accounts for approximately 70% of the whole trunk weight, and that sugars existing in the sap increased remarkably during storage after logging. Total sugar in the sap increased from 83 mg ml^{-1} to 153 mg ml^{-1}, the concentration comparable to that of sugar cane juice, after 30 days of storage, followed by the gradual decrease. The sugars contained in the sap were glucose, sucrose, fructose and galactose, all of which are fermentable by ordinary

industrial yeast strains. The oil palm sap was found to be rich in various kinds of amino acids, organic acids, minerals and vitamins. The results indicate that old oil palm trunk becomes a promising source of sugars by proper aging after logging and, thus, its sap can be a good feedstock for bioethanol. These results indicate that oil palm trunks felled for replanting are significant resources for producing fuel ethanol and lactic acid in palm oil-producing countries such as Malaysia and Indonesia.

2.3 Ethanol production by fermentation

Fermentation is one of the oldest biochemical processes known. It is used to produce a variety of products, including foods, flavorings, beverages, pharmaceuticals, and value-added chemicals like ethanol. The future of the fermentation industry with respect to bioethanol production depends on three major strategies. First, its ability to exploit a variety of microorganisms that is capable of efficient ethanol production by fermentation; second, to utilize various substrates such as sugars, starches or celluloses derived from a variety of different sources; and third, since utilizing starches and celluloses requires enzymes, to locate, develop and investigate relatively inexpensive sources of enzymes.

2.4 Microorganisms related to Ethanol Fermentation

One of the criteria for an ideal ethanol-producing microorganism is high optimum temperature (Bender, 1999; Subramanian *et al.*, 2005). High temperature tolerance simplifies fermentation cooling (Mohammad and Keikhosro, 2008).Among the ethanol-producing yeasts, the “industrial working horse” *S. cerevisiae* is by far the most well-known and most widely used yeast in industry and research for ethanol fermentation (Saha , 2003; Schneider, 1989). In this work, batch fermentation runs were performed to produce bioethanol using two strains of *Saccharomyces cerevisiae* (baker’s yeast), and *Saccharomyces cerevisiae* Kyokai 7 were used in this study. There are difference in term of maximum ethanol concentration and substrate of different type of microorganism.

Table 2.1 Different in Characteristic between Sake yeasts and other industrial yeasts of *Saccharomyces Cerevisiae*

Microorganism	Maximum Ethanol Concentration	Substrate
Saccharomyces cerevisiae CBS 8066	147 g/L	glucose
K.fragilis NRRL Y-2415	64 g/L	concentrated whey permeate
K. marxianus UFV-3	80 g/L	cheese whey permeate
S.cerevisiae K-7	more than 20 %	rice
S.cerevisiae Baker's yeast	96.71 g/L	sucrose
K.marxianus strain DMKU 3-1042	8.70%	sugar cane juice
K.lactis NBRC 1903	63.7 g/L	lactose

Yeast cells (*Saccharomyces cerevisiae*) are facultative anaerobes and under anaerobic conditions can ferment glucose to ethanol. *S. cerevisiae* is ideal for ethanol production due to several properties including fast growth rates, efficient glucose repression, efficient ethanol production and a tolerance for environmental stresses, such as high ethanol concentration and low oxygen levels. *Saccharomyces cerevisiae* Kyokai 7 discovered by Masumi of Nagano, is the single most commonly used yeast in the country, with its mellow fragrance and robust strength during fermentation. The sake yeast *Saccharomyces cerevisiae* Kyokai no. 7 (K-7) is capable of producing more than 20% (v/v) ethanol in culture (Ohbuchi, 1996). The comparison in characteristic between sake yeasts and other useful yeasts shown in the Fig 2.2.

Table 2.2 Differences in characteristics between sake yeasts and other industrial yeasts of Saccharomyces Cerevisiae

Characteristic	Sake yeasts	Other useful yeasts
Fermentation ability for maltose	weak	Moderate-strong
Growth in vitamin-free medium	growth	Variable
growth in biotin-deficient medium	growth	Variable
Aggregation with Lactobacillus casei	non-aggregation	Aggregation
Charge on cell surface at pH 3.0	positive	Negative
Ethanol tolerance	20-21%	16-19%

2.5 Optimum parameter need to produce higher yield of ethanol

Yeasts are active in a very broad temperature range from 0 to 50° C, with an optimum temperature range of 20° to 30° C. The temperature of fermentation is usually from 25 to 30°C, and the duration of the fermentation process may extend from a few days to two weeks.

Inoculum size is one of the most important factors that influence the industrial fermentation, including lag phase duration, specific growth rate, biomass yield and the final product quality (Sen and Swaminathan, 2004). To carry out steady and complete ethanol fermentation, in part, it is important to achieve an adequate yeast cell population. However, the metabolic regulation against the variation of inoculum size is still elusive and less characterized, especially the changes of yeast intercellular metabolite contents in fermentations with different initial cell densities (Verbelen *et al*., 2009). Obtaining quantitative data concerning the impact of inoculation size on yeast growth and metabolism is of great importance for industrial ethanol fermentation process. Taken together, it is necessary to gain insights into the mechanism of how yeast metabolism is affected by inoculums size in detail.

2.6 Advantages of Production Ethanol

There are a number of advantages in using palm oil for the production of bioethanol. Unlike fossil fuels, the combustion of palm oil bioethanol does not increase the level of carbon dioxide in the atmosphere as the oil is merely returning carbon dioxide obtained earlier from the atmosphere through photosynthesis. As such, bioethanol is regarded as carbon neutral. An added benefit of photosynthesis is the release of oxygen to the atmosphere. The quantity of oxygen released by oil palm, a perennial crop, far exceeds that produced by annual crops such as soybean or rapeseed. The cultivation of palm trees is therefore a huge contributing factor in the reduction of global warming.

Bioethanol, not only reduces the reliance on oil imports and alleviates uncertainties caused by the fluctuations of oil price, but also secures reductions in

environmental pollution problems due to its high oxygen content (Huang *et al*., 2008). Ethanol is an attractive alternative fuel since that it can be blended with gasoline or used as neat alcohol in dedicated engines, taking advantage of the higher octane number and higher heat of vaporization (Hahn-Hägerdal *et al*., 2006). When biofuel production and use should allow a minimum reduction of GHG emissions, when compared with the use of fossil fuels. Then, large scale biofuels should not impact natural ecological systems nor contribute to reduction of of water availability. The location of biofuels pilot plant will positively impact the region where it takes place.

CHAPTER 3

METHODLOGY

This chapter will listed all method use starting from medium and reagent preparation, transferring pure culture, yeast activation, fermentation profile experiment, validation experiment between *S.cerevisiae* and *S. cerevisiae* Kyokai 7 and screening of parameter as shown in Fig 3.0. The method of analysis to fulfill the scope and objectives is also discussed. Basically experiment is divided into two parts. The first part involved fermentation is done in the shake flask to determine *S.cerevisiae* Kyokai 7 fermentation profile. For the second part, validation experiment and screening parameter done.

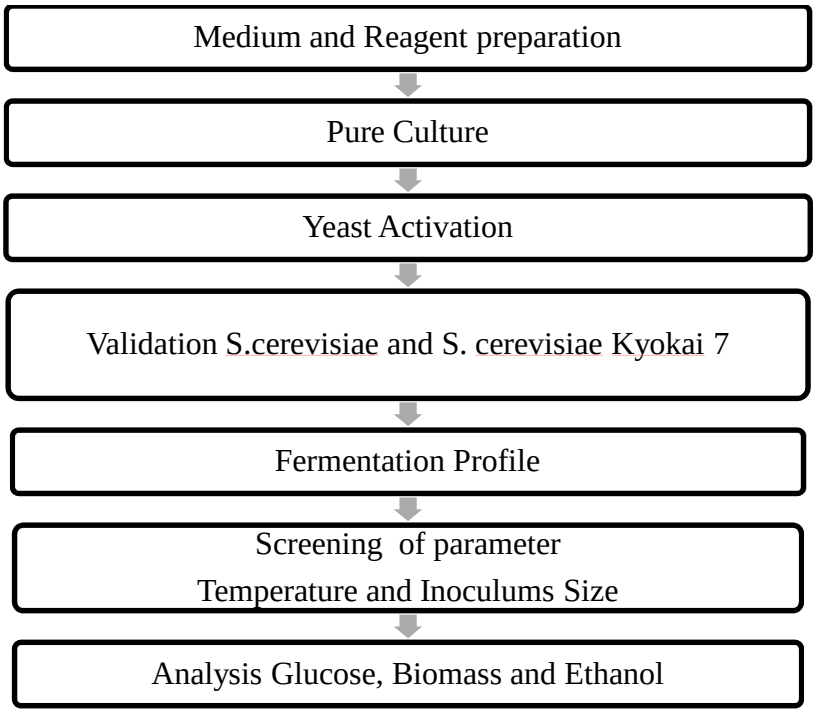

Fig 3.0: The work flow diagram of fermentation bioethanol

3.1 Medium and Regent Preparation

3.1.1 Medium Preparation

Nutrient agar plates (NA) is prepared by mixing the nutrient ingredients in proportion to the amount required in flask. Before that, nutrient agar powder is weighed out about 23 grams. Then, 1.0 liter of distilled and deionized water is added in a 1.0 liter Schott bottle and sterilized at 121^{o}C for 20 minutes. Flask is covered with aluminum foil to avoid any contamination. After sterilization process finished, the mixture of nutrient agar is cool until the temperature reached 50 ^{o} C. After temperature has lowered, agar is pour into sterilized petri plate. The plates are leaved undisturbed until the agar solidifies. All plates are closed and seal before kept in refrigerator at 4 ^{o}C. Streaking method is used for transferring a culture of *Saccharomyces cerevisiae* Kyokai 7 to a petri plate in order to grow yeast on nutrient agar plate.

Nutrient broth (NB) is prepared by mixing the nutrient ingredients glucose (dextrose) 10, peptone 5, yeast extracts 5, agar 20 to make YPD medium agar (g/L). Dextrose is added in NB after both dextrose solution and NB are autoclave separately to avoid Maillard reaction (brownization). NB also need to sterilize at 121 ^{o}C for 20 minutes. All NB is kept in refrigerator at 4 ^{o}C.

The palm oil trunks were freshly obtained from Jerantut Plantation, Pahang, Malaysia. Palm oil trunks sap is collected by squeezing palm oil trunks to obtain sap using sugar cane press machines within less than 24 hour after trees was cut down. Palm oil trees age more than 25 years is chosen. It is cut into several pieces (suitable with ability of sugar cane press machine) and outer layer is removed. The sap is collected into big container and mix well before kept in smaller container (make usage and keeping are easier).The sap was immediately kept at -20 ^{o}C to avoid any/further microbial reaction before use.

3.1.2 Reagent Preparation

DNS reagent is prepared by dissolving the 10 g Sodium Hydroxide (NaOH), 182 g Sodium Potassium Tartarate, 2 g Phenol, 0.5 g Sodium Sulphite and 10 g Dinitrosalicylic acid in 600mL distilled water. Then, after all the material fully dissolved, add up to 1 liter in amber bottle or cover with aluminium foil. If it not dissolved, used stirred and hot. Then it is stir overnight. It is keep at 4 ^{o}C in refrigerator (Miller, 1959).

3.2 Fermentation

Culture of *Saccharomyces cerevisiae* Kyokai 7 is in freeze dry condition. Thus, culture is activated by rehydration process. Rehydration is process of adding water to. *Saccharomyces cerevisiae* Kyokai 7 until it can growth. Then, yeast suspensions are streaked on new agar plate and incubate 2-3 days at 30 ^{o}C. The strain was maintained at 4 ^{o}C and sub-cultured every month (4 weeks) on YPD medium agar (g/L): glucose (dextrose) 10, peptone 5, yeast extracts 5, agar 20. The main purposes of sub-cultured strain are to activate and maintain good conditions of strain.

For inoculums preparation, about a few loops of culture S.cerevisiae Kyokai 7 transfers to the nutrient broth. Then, the culture incubated about 24 hours in the incubator shaker. It is essential to place the culture into the sterilized 500 mL Erlenmeyer flask to avoid any contamination and any other microbial growth. The flask contains 250 mL nutrient broth which is same as working volume. All procedures are done aseptically and experiment is run duplicate for all parts. Shake flask is shaked at 150 rpm and 30 ^{o}C for 12-18 hours (to reach exponential phase).

3.3 Fermentation profile

For the fermentation process, a 500 ml of shake flask was used. The fermentation medium was prepared by filtering palm oil trunk sap by 2.0 micron filter. Then, the fermentation medium was adjusted to the 30 ^{o}C, 150 rpm,pH 5 and 10% of inoculums size, before proceed with the fermentation process. The

fermentation process was started by transferring 25 mL of the prepared inoculums into the shake flask containing 225ml palm oil trunk sap. Nitrogen gas (100 ml/min) was sparged through the system to create an anaerobic environment for the yeast. The data collected at 0, 2, 4, 12, 24, 36 and 48 hour.

Table 3.0: Parameter That Control In Fermentation Process

Parameter	Lower	Centre	Upper
pH	3	5	7
Agitation rate (rpm)	110	180	250
Temperature (^{o} C)	25	32.5	40
Inoculums Size (%)	5	10	15

In this part, sampling is done at certain interval for total 48 hours (experiment will be extended if glucose concentrations still high or, ended immediately when all glucose is consumed).1.5-mL of sample is taken at t=0, 24, 36, 48 hours. Sample is analyzed for glucose content, ethanol concentration and cell optical density.

3.4 Validation between *S.cerevisiae* and *S.cerevisiae* Kyokai 7

Initial concentration is maintained value of optical density by adding saline solution. Different type of yeast was cultured in the inoculums which are S.cerevisiae and *S.cerevisiae* Kyokai 7. Shake flask is incubated on the 30 ^{o} C, 180 rpm, 10% inoculums size and no change of pH .

3.5 Effect of parameter to *Saccharomyces cerevisiae* Kyokai 7 performance

A four-factor two-level full factorial design (2^4) augmented with 4 center point; FFD was used for the modeling of fermentation process. Factorial design allowed determination whether interactions between the factors occurred and also to obtain quantitative cause-effects relationships (Santos *et al*., 2010).