THE USE OF DESIGN OF EXPERIMENT IN MODELING THE BIOETHANOL PRODUCTION FROM OIL PALM TRUNK SAP

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ABSTRACT

Bioethanol, a biomass product, is one of the renewable energy sources that can be used as an automotive fuel. Therefore, bioethanol is essential in replacing the decreasing fossil fuels. The modeling of the experimental design for bioethanol production from oil palm trunk sap is conducted using Design Expert software version 7.1.6 (Stat Ease). This design of experiment is conducted to screen out the significant parameters that affecting the bioethanol production. The parameters that are being screened are the temperature, pH, inoculum percentage and agitation. The microbe involved is *Saccharomylesn Cerevisiae Kyokai 7* since it accumulates bioethanol more than any other type of microbes. The *Saccharomylesn Cerevisiae Kyokai 7* is cultured in the inoculums preparation and being fermented in the oil palm trunk sap for 48 hours. By using the 2-Level Full Factorial Design and Response Surface Methodology, the optimal parameters levels are determined. The best range value for temperature is in the range of 25^{o}C, pH around 3, agitation speed in the range 250 rpm and inoculums percentage around 10 % for the maximum concentration of bioethanol.

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ABSTRAK

Bioetanol ialah sumber biojisim adalah salah satu daripada sumber tenaga yang boleh diperbaharui untuk menggantikan bahan bakar fosil yang semakin berkurangan. Perisian Design Expert versi 7.1.6 (Stat Ease) digunakan untuk merangka model penghasilan bioethanol dari cecair getah dari empulur kepala sawit tua. Objektif eksperimen ini adalah untuk mengelaskan parameter penting yang mempengaruhi pengeluaran bioetanol dan mengenalpasti kadar optimum bagi setiap parameter untuk penghasilan maksima bioethanol. Parameter yang digunakan adalah suhu, pH, peratusan inokulum dan kelajuan tindakan. Mikrob yang terlibat adalah *Saccharomylesn cerevisiae Kyokai* 7 kerana ia menghasilkan bioetanol lebih daripada jenis mikrob yang lain. *Saccharomylesn cerevisiae Kyokai 7* dikulturkan untuk penyediaan inokulum dan ditapaikan dalam cecair getah empulur kelapa sawit getah selama 48 jam. Dengan menggunakan ‘2-Level Full Factorial Design’ dan ‘Response Surface Methodology’ dari perisian ‘Design Expert’ tahap optimum setiap parameter dapat ditentukan. Nilai rentang terbaik untuk suhu ialah menghampiri 25^{o}C, pH menghampiri 3, kelajuan tindakan menghampiri 250 putaran seminit dan peratusan dalam lingkungan inokulum 10% untuk kepekatan maksimum bioetanol.

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LIST OF ABBREVIATION

^{o}C	-	Celcius
DNS	-	Di-Nitro Salicylic Acid
g	-	gram
g/L	-	gram per liter
K	-	potassium
L	-	liter
min	-	minutes
mL	-	mililiter
nm	-	nanometer
µm	-	micrometer
mm	-	millimeter
m	-	meter
OD	-	optical density
rpm	-	rotation or revolution per minute
ha	-	hectare
uv-vis	-	ultraviolet-visible spectroscopy

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

This chapter will discuss the background of study, objectives, scopes, problem statement and also rationale and significance of the research.

1.1 Background of Study

Nowadays, petroleum and diesel are highly demands because they are non-renewable energies. It takes million of years to develop the fossil fuels. Days to days, the prices of them are keeping increased due to limited source of the energy. Humankind is currently facing the huge challenge of securing sustainable supply and use of energy (Kullander, 2009). The depletion of fossil fuels and sustained high world oil prices have encouraged several researchers to do some researches to find an alternative way to replace the fossil fuels by introducing biodiesel and bioethanol, especially in the transportation sector. Bioethanol is an agricultural-based fuel and is one of the renewable energies that can be used to replace the depleted fossil fuels.

Generally, bioethanol is produced by the sugar fermentation process. During 1970's, the production of bioethanol grown from sugarcane and corn was started by Brazil and the USA respectively. Meanwhile Spain, France and Sweden started the smaller scale production of bioethanol from wheat and sugar beat. Ethanol or ethyl alcohol (C_2H_5OH) is a clear colourless liquid which is biodegradable, low in toxicity and causes little environmental pollution if spilt. Ethanol can be derived from the chemical reaction of ethylene and other petroleum products but these sources are not considered renewable. The keep increasing demands of bioethanol production from year to year from 13,100 millions of gallons in 2007 to 17,300 millions of gallons in 2008 (Guillen-Gosalbez *et al*., 2010) making the world becomes eager to produce bioethanol from variables of sources. The main sources of sugar required to produce ethanol come from fuel or energy crops, such as corn, maize and wheat crops, waste straw, willow and popular trees, sawdust, reed canary grass, cord grasses, Jerusalem artichoke, myscanthus and sorghum plants.

Many factors contribute in producing the ethanol from biomass such as pH, temperature, agitation speed, type of microorganism used and inoculums percentage. All these factors need to be screened out before performing the production of bioethanol in the large scale. To minimize the time consumption for the fermentation process, each of the factors designing of the experiment is being implemented. Design of experiment (DOE) has been proposed in order to control the experimental quality, cost and time consuming. It is designed to help in improving the design and interpretation of multifactor experiments. The 2-level full factorial design and response surface methodology are used to model the production of bioethanol from oil palm trunk sap.

1.2 Objectives

The objectives of this research are to screen out the significant parameters that affecting the bioethanol production and to find the value of growth parameter that will optimize the production of bioethanol from oil palm trunks sap.

1.3 Scopes of Study

In this research, the scopes of study are to find the significant parameters that will affect the bioethanol production and to find the value of growth parameter that will optimize the production of bioethanol from oil palm trunks sap using 2-level full factorial design and response surface methodology

. The parameters that are to be tested in this study are:

- pH
- Temperature
- Agitation speed
- Inoculum percentage

1.4 Problem Statement

The serious shortage of fossil resource has put great pressure on our society to find renewable fuel alternatives such as bioethanol and biodiesel. The increased concern and awareness among the people for the negative impacts of fossil fuel towards the environment have made the bioethanol and biodiesel essential in replacing the decreasing of the fossil fuels. However, the production of bioethanol from fermentation process is very expensive and time consuming. In order to cut out the costs, energy and time consuming the modeling of the production needs to be implemented.

Previous study has used ‘One-Factor at a Time’ (OFAT) to screen out the optimum condition for bioethanol production. However, the used of OFAT design can vary only one factor or variable at a time while keeping others fixed. The traditional ‘one-factor at a time’ technique used for optimizing a multivariable system is not only time consuming but also often easily misses the alternative effects between components. Furthermore, this method requires carrying out a number of experiments to determine the optimum levels, which is untrue (Bandaru *et al*., 2006).

In order to overcome the problem, Design of Experiment (DOE) is being used. Design of experiments (DOE) provides a powerful means to achieve breakthrough improvements in product quality and process efficiency. This is because statistically designed experiments that vary several factors simultaneously are more efficient when studying two or more factors (Anderson & Kraber, 2008).

1.5 Rationale and Significance of Study

Bioethanol is an environmental friendly because of its properties such as biodegradable, low in toxicity and has low emission profile. It is a clean fuel that can be used in today's cars. One of the many attributes of bioethanol is, it does not contribute net carbon dioxide to the atmosphere. Bioethanol fuels are one of the cost-effective ways to reduce carbon dioxide emissions. The most attractive feature of bioethanol fuels is, it is one of the lowest-cost, consumer-friendly ways to reduce gasoline consumption and carbon dioxide emissions from vehicles. It causes little environmental pollution if spilt compare to the conventional ethanol. Ethanol from biomass gives no net contribution to global warming since the carbon dioxide produced by the combustion of ethanol is consumed by the growing raw material.

The aim of using oil palm trunk sap as the raw material in producing bioethanol is to convert the waste to wealth. Everything in this world has its own beneficial side including the wastes. It only needs to be discovered and researches need to be done in order to develop the potential product. More money can be generated from the waste. The use of oil palm trunk sap is significant for rural development impact and important environmental benefits in Malaysia. Moreover, using oil palm trunk sap as the raw material for bioethanol production can avoid “food and energy crisis” caused by the use of corn and wheat as the raw materials.

This study aims to perform value of growth parameter in order to reduce the cost, energy and time consumption in producing bioethanol from oil palm trunk sap. The optimum parameters will be pointed out using design of experiment. This is important to produce the bioethanol in economically ways whereby the cost for conducting the experiments is quite expensive and minimizing the time for fermentation process of the sugar.

CHAPTER 2

LITERATURE REVIEW

2.1 History

Ethanol has been used by human being since prehistory as the intoxicating ingredient in alcoholic beverages. Dried residues on 9000-year-old pottery found in northern China implied the use of alcoholic beverages even among Neolithic peoples. Ethanol which is derived from biomass is called as bioethanol. Chemically, bioethanol is identical to ethanol and can be represented by either the formula C_2H_6O or C_2H_5H. Bioethanol and synthetic ethanol are chemically indistinguishable; they are both the same compound, C_2H_5OH. The only difference between these two is the isotopic composition of their carbon atoms. Synthetic ethanol comes from fossil raw materials, while bioethanol comes from contemporary materials.

The history of bioethanol as the additive fuel started during late 1800s, when Henry Ford first designed his Model T. He was intended to run it by using ethanol that was derived from corn. Henry Ford, Nicholas Otto and others built engines and cars that could run by using ethanol. Ford equipped his Model T in 1908 as a flexible fuel vehicle, with carburettors that could be adjusted to use alcohol, gasoline, or a ‘‘gasohol’’ mix. The need for fuel during World War I increased the demand of ethanol in US from 0.19–0.23 hm^3 (550–60Mgal) per year .The discovery of the anti knock properties of tetraethyl lead in 1921 dampened some of the enthusiasm for ethanol, and despite persistent health concerns, sales of leaded gasoline increased dramatically in subsequent years (Solomon *et al.,* 2007).

During the World War II, Standard Oil Co. had made a deal with Germany to cut off the supply of petroleum to U.S. Felt uneasy and anxious; the country looked elsewhere for a solution to the petroleum shortage. So, American farmers quickly began to divert their energies to growing corn for ethanol-based synthetic rubber. The industry grew and about 600 million gallons of corn-based ethanol was produced during the World War II (Abebe, 2008) .

2.2 Bioethanol

The processes by which ethanol can be produced are diverse as from sugarcane, wheat, corn, sugar beet, sweet sorghum, rice, cassava, and potato (Gopinathan & Sudhakaran , 2009). However to avoid the competition with the food industry, the other initiative of using waste such as molasses, oil palm trunk sap, sawdust, empty fruit bunch has been proposed. It is also to promote the waste-to-wealth strategy in producing valuable products.

Ethanol production has grown dramatically in the last few years as the demand for this clean-air fuel has escalated. It is used as a petrol substitute for road transport vehicles. Bioethanol fuel is mainly produced by the sugar fermentation process, although it can also be manufactured by the chemical process of reacting ethylene with steam. Ethanol burns to produce carbon dioxide and water. Ethanol is a high octane fuel and has replaced lead as an octane enhancer in petrol. By blending ethanol with gasoline, we can also oxygenate the fuel mixture so it will burn more completely and reduces polluting emissions. In a large number of countries, ethanol obtained a predominant position among biofuels as a blending agent with petrol because of its oxygenation properties, energy balance, environmentally friendly nature, possible employment benefits in the rural sector, and contribution to energy security at the national level (Gopinathan & Sudhakaran, 2009). Global production of fuel ethanol increased by 18% over 2006 to 46 billion liters in 2007, marking the sixth consecutive year of double-digit growth (Worldwatch Institute, 2009).

According to FOA (2008) biofuels can be classified according to source, type, and technological process of conversion under the categories of first, second, third, and fourth generation of biofuels. The first generation of biofuels are the biofuels that made of biomass, consisting of sugars, starch, vegetable oils, animal fats, or biodegradable output wastes from industry, agriculture, forestry, and households using conventional technologies. Second generation biofuels are derived from lignocellulosic biomass to liquid technology, including cellulosic biofuels from nonfood crops such as the stalks of wheat, corn, wood, and energy-dedicated biomass crops, such as miscanthus. Many second generation biofuels are under development such as biohydrogen, biomethanol, dimethyl furan, dimethyl ether, Fischer–Tropsch diesel, biohydrogendiesel, mixed alcohols, and wood diesel. Third generation biofuels are in the nascent stage of development and are derived from low input/high output production organisms such as algal biomass. Fourth generation biofuels are derived from the bioconversion of living organisms (microorganisms and plants) using biotechnological tools.

2.3 Oil Palm Trunk Sap.

The oil palm trees were first introduced in Africa on 1911. The oil palm treess (*Elaeis guineensis jacq*) were grew wildly and later was developed as an agricultural crop, where it is used in a wide variety of ways by the local population. Oil palm plantations start to produce fruit after four to five years. It composed of specially selected and cloned varieties and reaches the highest rate of productivity when the trees are 20 to 25 years old. The fruit bunches, each weighing between 15 and 25 kgs, are made up of between 1000 and 4000 oval-shaped fruits, measuring some three to five centimeters long. Once harvested, the fleshy part of the fruit is converted into oil through a series of processes, while the palm kernel oil is extracted from the nut itself. (Carrere, 2006)

By 2005, from all around the world, the area occupied by oil palm plantations had grown to 12 million hectares and palm oil production had reached 30 million tons. In Asia, the two largest producers of palm oil are Malaysia, with a total of 4 million hectares of plantations in 2005, and Indonesia, with 5.3 million hectares in 2005. These two countries combined account for 85% of the palm oil produced worldwide. (Carrere, 2006)

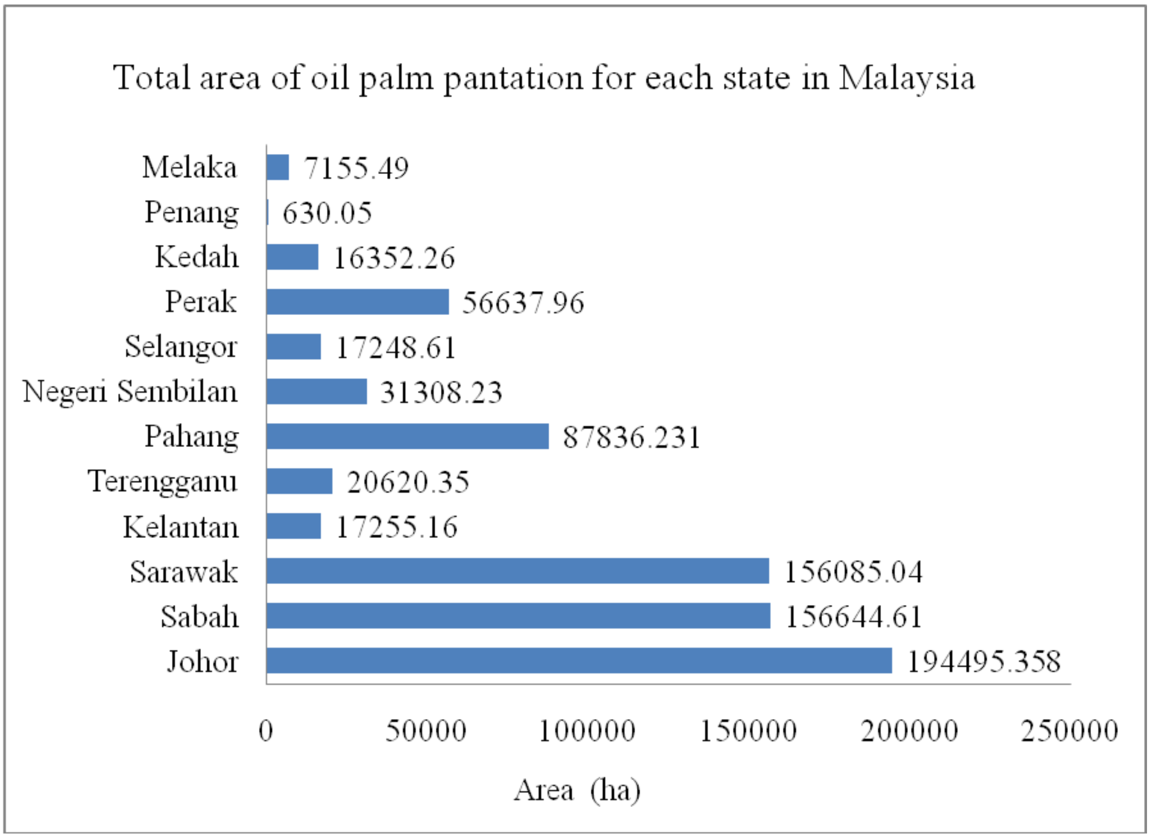

Figure 2.1:The Total Area Of Oil Palm Plantation For Each State In Malaysia. (FRIM.,2010)

Figure 2.1 shows that the total area of oil palm plantation for each state in Malaysia. Johor has the largest area of oil palm plantation in Malaysia followed by Sabah and Sarawak. Even though Johor is not the largest state in Malaysia compared to Sarawak and Sabah, the state has the largest oil palm plantation because of its geographical itself that suitable to plant the oil palm trees and near to the port so that it is easy to be export outside the Malaysia. Meanwhile Penang has the smallest area of oil palm plantation in Malaysia because Penang is a small state and has high density in terms of human population.

Today, 4.49 million hectares of land in Malaysia is under oil palm cultivation; producing 17.73 million tons of palm oil and 2.13 tons of palm kernel oil. Malaysia is one of the largest producers and exporters of palm oil in the world, accounting for 11% of the world's oils & fats production and 27% of export trade of oils & fats. The industry provides employment to more than half a million people and livelihood to an estimated one million people (Malaysian Palm Oil Council, 2010).

Since the highest rate of productivity is when the trees are at the age of 20 to 25years old, the trees need to be replanted to maintain its productivity. The plantation areas in Malaysia and Indonesia in 2007 were 4,304,913 ha and nearly 7 million ha *(Kosugi et al*., 2010), respectively. Considering the replanting interval, 450,000 ha to 560,000 ha of the oil palm plantation area is expected to be replanted annually during the next 25 years. This means, on average, about 64 million to 80 million old palm trees will be felled every year in the two countries, with approximately 142 oil palms trees are usually planted in one hectare. Consequently, the felled palm trunks can be regarded as one of the most important biomass resources in Malaysia and Indonesia. (Kosugi *et al*., 2010)

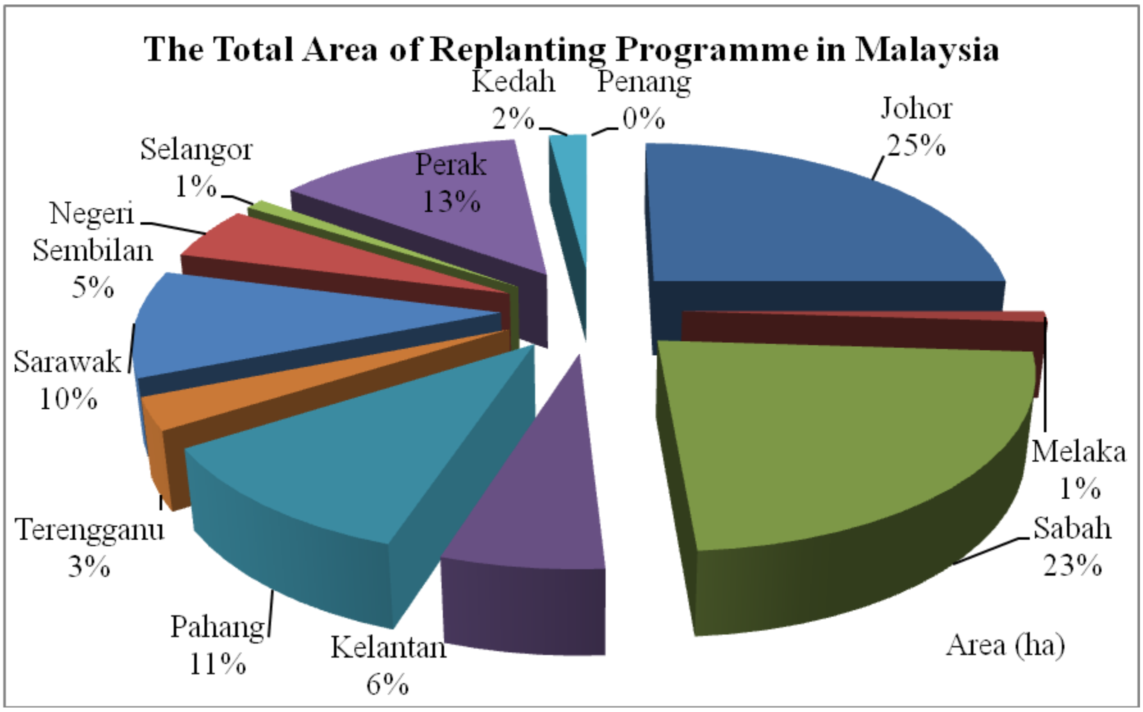

Figure 2.2: The Total Area of Replanting Programme in Malaysia (FRIM., 2010)

Figure 2.2 shows that the total area of replanting programme in Malaysia for year 2010. Johor has the highest percentage with 25% of the oil palm plantation areas that need to be replant after 25 years followed by Sabah and Perak. Meanwhile Penang has 0% of oil palm plantation areas that need to be replant. This means that the oil palm plantation areas in Penang are still new and the trees still have high productivity.During the replanting process of oil palm trees, the old palms will be cut off and burned at the site. This is not the environmental friendly method because it involves open burning. More efficient and environmental friendly ways are to utilize the old palm trunks. The outer part of the old palm trunk is utilized as the plywood in manufacturing industry for its strong ability. During the plywood production, the inner part of the palm trunk will be thrown away in large amounts because of its weak physical properties. In order to make the old palm trunks are fully useful especially the the inner part, some studies on converting the inner part to the bioethanol has been carried out. The inner part of palm trunk contains high moisture content, thus it is not strong enough to be used for different purpose such as lumber. Meanwhile, there are some studies attempted to produce lactic acid, the material for bio-plastics, from felled trunks. (Kosugi *et al.*, 2010)

Figure 2.3: The replanting the old palm trunk. (FRIM., 2010)

Figure 2.4: The inner part of the palm trunk. (FRIM., 2010)

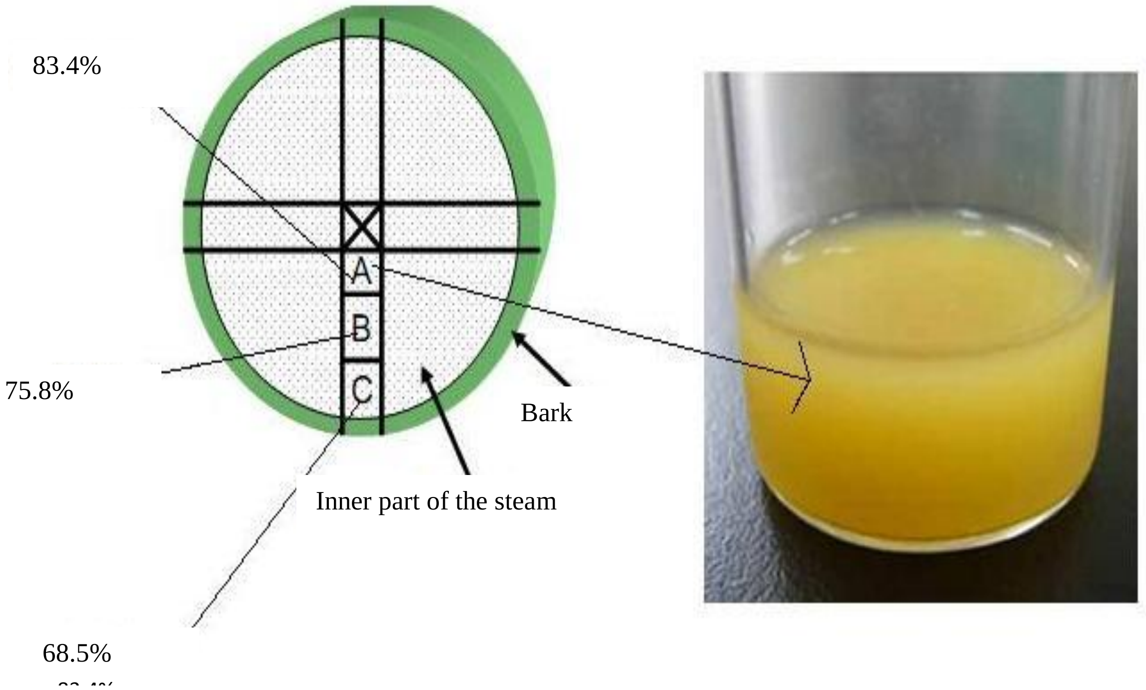

Figure 2.5: The oil palm trunk sap from the inner part (FRIM., 2010)

Figure 2.5 shows that the moisture content in the inner part of the palm trunk. The A (core) part has the highest moisture content with 83.4% of the moisture, the B (middle) part has 75.8% moisture and the C (outer) part has the lowest moisture content with 68.5%.Oil palm trunk sap contains very high glucose. It 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 fiber contains 4.9%-7.8% sugars, which correspond to 2.1%-3.4% sugars in the sap assuming that moisture content of oil palm trunk is 70%. The ratio of sugars in the methanol extract of the pulverized trunk is significantly different from the one in the tapped sap. (Yamada *et al.,* 2010)

2.4 Design of Experiment (DOE)

Design of experiments is defined as a methodology for systematically applying statistics to experimentation. More precisely, it can be defined as a series of tests in which purposeful changes are made to the input variables of a process or system so that one may observe and identify the reasons for these changes in the output response (Tanco *et al.,* 2008). Nowadays, the statistical experimental design has been widely used in the optimization of fermentation processes and media. It is a proven technique that is used extensively in many industrial-manufacturing processes. Their applications of the design of experiments also well documented. The application of experimental design which uses more than a single design in sequence is less frequent (Sullivan & Cockshott 2001).

Normally, a full application of experimental design would include the screening of a large number of media components followed by the optimization of a subset of those components. Previously, the traditional One-Factor at a Time (OFAT) technique was used in determining the optimum variable. However, it is a time consuming process and will easily miss the alternative effects between components because it is consist of varying one variable while others remain fix. Studying one variable at a time might be appropriate in some cases, but normally it fails to consider the combined effects of multiple factors involved in physiological process (Coninck *et al*., 2004). The other disadvantage of OFAT is, the presence of many potential important factors will increase the risk of locating a local maximum and thereby it can cause a lost of its actual best condition (Chambers & Swalley., 2009).

Recently, many statistical experimental design methods have been employing in bioprocess optimization (Bandaru *et al*., 2006). During fermentation, where operation variables interact and influence the respective effects on response, it is essential that the experimental method accounts for these interactions so that a set of optimal research conditions can be determined (Coninck *et al.,* 2004).

The advantage of using design of experiments is, it would increase the amount of information in every experiment way better than the ad hoc approach. Secondly, it also can provide an organized approach towards analysis and interpretation of results, thus facilitate communication. The ability of DOE in identifying the interaction among the factors, leads to the more reliable prediction of response in the areas which are not directly covered by experimentation. DOE also gives benefits in the assessment of information reliability in light of experimental and analytical variation (Chambers & Swalley, 2009). Through DOE technique, the total number of the experiments can be reduced by evaluating the more relevant interaction among variables.

2.4.1 Full Factorial Design.

In 1930's, the fractional factorial designs was first proposed and in 1940's, the full factorial designs were proposed in response to the overwhelming number of experiments involved (Tanco *et al*., 2008). It was employed to reduce the number of experiments in order to achieve the best overall optimization of the process. The objective of full factorial design is to identify the significant factors and interactions involved in maximizing soluble expression. A full factorial experiment will contain all possible combinations of factors represents not only the most conservative approach, but also the most costly in terms of experimental resources (Chambers & Swalley., 2009).

This design will help in screening out the factors to discover the vital few, and perhaps how they interact with each of the factors. It uses all the combinations of the levels of factors. Interaction effects of different factorial could be attained using designs of experiments only. Factorial designs comprise the greater precision in estimating the overall main factors effects and interactions of difference factors.

2.4.2 Response Surface Methodology (RSM)

The full application of experimental design would include the screening of a large number of media components followed by the optimization of a subset of those components using Response Surface Methodology (Sullivan & Cockshott, 2001). Response Surface Methodology is a collection of statistical and mathematical techniques; it is a useful tool for the development, improvement, and optimization of processes. It is used to examine the relationship between one or more response variables and a set of quantitative experimental variables (Onsekizoglu *et al*., 2010).

The development of RSM began in 1950's and was evolved by Box and Wilson. Their methodology allowed the DOE to be applied in the chemical industry and afterwards, in other industries as well (Tanco *et al*., 2008). The response surface designs are used to model the response of a curved surface to a range of continuous variables. The non-inclusion of categorical variables is a limitation to RSM; hence, it is used in optimization and not in the initial screening. RSM provides a more complete understanding of the significant factors involved and is capable to identify whether minimum or maximum responses exist within the model (Chambers & Swalley, 2009).

Response-surface methods also involve some unique experimental-design issues, due to the emphasis on iterative experimentation and the need for relatively sparse designs that can be built-up piece-by-piece according to the evolving needs of the experimenter (Lenth., 2010).

CHAPTER 3

METHODOLOGY

3.1 Procedures of Modeling the Design of Experiments (DOE).

In this research, the procedures have been divided into five steps in order to achieve the objectives of the research. Firstly, the selection of the response of the variable was made. Next, the choice of factors, levels and range of the parameters was decided. Thirdly, experiment was designed by using 2-Level Full Factorial design software Design Expert version 7.1.6 (Stat Ease). Next procedure was performing the experiments which involved inoculums preparation, fermentation process of oil palm trunk sap for 48 hours and analyzing the result. Lastly, the data obtained was analyzed using 2-Level Full Factorial with six center points to estimate the error and curvature. If curvature is significance to the design, the fermentation process needs to be done once again according to the conditions designed by the response surface methodology.