GLUCOSE PRODUCTION FROM OIL PALM TRUNK

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"I hereby declare that I have read this thesis and in
my opinion this thesis is sufficient in terms of scope and
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Bachelor of Chemical Engineering (Biotechnology)"

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GLUCOSE PRODUCTION FROM OIL PALM TRUNK

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A thesis submitted in fulfillment of the requirements for the award of the degree of Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
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May 2011

I declare that this thesis entitled "Glucose Production from Oil Palm Trunk" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Date : 19 MAY 2011

Special dedication to

<u>My parent</u>

Ab Majid @ Aziz bin Abdullah and Asiah bt Ludin

<u>My beloved brother and sister</u>

Izatie,Azizi, Azura, Aminata, Ahiran, Azmazilawanie

and all my friends

for give support and motivation to me

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ABSTRACT

Oil palm trunk is one of the agricultural wastes which can be used as a raw material for production of glucose through fermentation process. It can be as another alternative way to reduce cost and also more environmental friendly. The main objective of this research is to produce glucose from oil palm trunk fibre using biological method. In biological method, the conversion of cellulose to glucose with the help of mix culture from oil palm sap. Twenty runs of experiment were carried out by using Design Expert Software based on three factors which are reaction time (0 – 24 hours), temperature (25°C - 40°C) and agitation speed (100 rpm – 300 rpm). Glucose was estimated by 3, 5-dinitrosalicylic acid (DNS) method and the effect of three factors on glucose concentration employing oil palm trunk fibre were evaluated using Response Surface Methodology (RSM) two level, three-variable central composite rotatable design (CCRD). The optimum conditions derived via RSM were 10.41 hours of reaction time, temperature value of 30.45°C and agitation of 225.20 rpm. The result show that, the best condition to produce optimum glucose were achieved at 12.50 hours of reaction time, temperature value of 32.50°C and agitation speed of 300 rpm. There are also produce interaction between reaction time and temperature in order to produce glucose. After optimization, the glucose concentration was increased to 0.983454 mg/mL to give value of percentage of error which is 47.4%. As a conclusion, the objective of the research has been achieved. Glucose can be produced from oil palm trunk using biological method.

ABSTRAK

Batang kelapa sawit adalah salah satu sisa pertanian yang boleh di gunakan sebagai bahan mentah untuk pengeluaran glukosa melalui proses fermentasi. Ianya boleh dijadikan sebagai cara alternatif untuk mengurangkan kos dan juga lebih mesra alam.Tujuan utama kajian ini adalah untuk menghasilkan glukosa dari serat batang kelapa sawit menggunakan kaedah biologi. Dalam kaedah biologi, penukaran selulosa menjadi glukosa dengan bantuan kultur campuran dari sap kelapa sawit. Eksperimen dilakukan sebanyak dua puluh kali dengan menggunakan Perisian Design Expert berdasarkan tiga faktor iaitu masa tindak balas (0 - 24 jam), suhu (25°C - 40°C) dan kelajuan pengadukan (100 rpm - 300 rpm). Glukosa di tentukan dengan dengan menggunakan 3, 5-dinitrosalisilat asid (DNS) dan kesan tiga faktor pada kepekatan glukosa menggunakan minyak serat batang sawit dinilai menggunakan Kaedah Tindak Balas Permukaan (RSM), dua tahap, tiga pembolehubah desain rotatable komposit pusat (CCRD). Keadaan optimum diperolehi melalui RSM adalah 10.41 jam waktu tindak balas, nilai suhu 30.45°C dan kelajuan pengadukan 225.20 rpm. Keputusan kajian menunjukkan bahawa, keadaan terbaik untuk menghasilkan optimum glukosa di perolegi pada 12.50 jam waktu tindak balas, nilai suhu 32.50°C dan kelajuan tindakan 300 rpm. Ianya juga menghasilkan interaksi antara waktu tindak balas dan suhu untuk menghasilkan glukosa. Setelah pengoptimuman, kepekatan glukosa meningkat menjadi 0.983454 mg/mL untuk memberikan nilai peratusan kesalahan yang 47.4%. Kesimpulannya, objektif kajian telah tercapai. Glukosa boleh dihasilkan dari batang kelapa sawit dengan menggunakan kaedah biologi.

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LIST OF SYMBOLS/ABBREVIATIONS

°C	-	Degree Celcius
ABS	-	Absorbance
ANOVA	-	Analysis Of Variance
CCRD	-	Central Composite Rotatable Design
DNS	-	Dinitrosalicylic acid
g	-	Gram
OD	-	Optical Density
RMSE	-	Root Mean Square Error
rpm	-	revolutions per minute
RSM	-	Response Surface Methodology
UV	-	Ultra Violet
vs	-	Versus

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Glucose is a reducing sugar that classified as a monosaccharide carbohydrate, the chemical formula is $C_6H_{12}O_6$. Glucose is one of the primary molecules which serve as energy sources for plants and animals. The long polymer chains of glucose units will produce cellulose which is the main polymeric component of the plant cell wall (Maijala, 2000).

The main sources for the production of glucose are such as corn, wheat crops, sawdust and sorghum plants (Ibeto, 2011). Currently, the agricultural waste can be as the alternative method to produce glucose. Oil palm trunk fibre is one of the agricultural waste which is the by product periodically left in the field on replanting (Sun, 2001) and generated from palm-oil upstream industry. It is rich in cellulose and hemicelluloses which could be of interest to produce glucose because more environmentally friendly materials for industries (Suhaimi and Ong, 2001).

Oil palm trunks contain high glucose content sap and there are various microorganisms especially the bacteria and yeasts presence in oil palm sap (Kosugi *et*

al., 2010). The mix culture of microorganisms that already exists is responsible to produce glucose as intermediate product using oil palm trunk fibre as substrate by fermentation method. Fermentation begins as the growing population of microorganism produces enzymes to break two-molecule sugars into single molecule sugars and then convert the single molecule sugars into the commercial chemicals and by products (Shide, 2004). There are two types of fermentation which are aerobic and anaerobic fermentation. In aerobic condition, the productivity of cell was achieved in the presence of oxygen compare to anaerobic condition which is the cell most productivity in the absence of oxygen (Klein *et al.,* 2005)

There are several types of hydrolysis process that was occur in fermentation process to produce glucose which are enzymatic, chemical and biological hydrolysis. The chemical hydrolysis is performed by attacking the cellulose with an acid, enzymatic hydrolysis of cellulose is a reaction carried out by cellulose enzyme, which are highly specific (Beguin and Aurbert, 1994). In biological hydrolysis, the microorganism was used to degrade the lignocelluloses into glucose. The microorganisms will produce its own enzyme to degrade cellulose to become simple sugar (Castellanos, 1995). Based on these finding, the study is to produce glucose from oil palm trunk fibre using biological methods.

1.2 Problem statement

Production of glucose from oil palm trunk fibre using biological method can be another alternatives way to reduce cost in process to produce of glucose because the cost of using microorganisms is cheaper than using enzymes and chemical. The difficulty in separating or recovering used enzymes has continued to be a problem too. Because of the relatively high cost of enzymes, it is desirable to recover them for re-use in subsequent reactions (Ehrenthal *et al.*, 1980).

Limited researches have been done in order to use biological methods. Earlier studies have indicated that production of glucose using oil palms trunk fibre is usually using enzymes as catalyst to convert oil palm trunk fibre to glucose. In this study, it is using the biological methods which mean using mix culture of microorganisms that had already growth in oil palm sap. An advantage in using mix culture of microorganisms is easier to handle with low cost because microorganisms are getting with the natural way of life microorganisms.

Oil palm is needs to be replanted at an interval of 20 to 25 years in order to maintain oil productivity. Consequently, the felled palm trunks can be regarded as one of the most important biomass resources in Malaysia (Kosugi *et al.,* 2010) that can be a benefit for this economy's country. The use of biological is also means have greater advantages over the use of chemicals for degradation because biotechnological synthesized products are less toxic and environmentally friend (Shide, 2004). Indirectly, the usage of agricultural waste will give the high impact to the environment which prevent from the abundant of waste and from pollutant by burning wastes.

1.3 Objectives

To produce glucose from oil palm trunk fibre using biological method

1.4 Scope of studies

There are certain scopes have been identified in order to achieve the objective. Firstly, the studies are using three factors on production of glucose which is reaction time, reaction temperature and agitation speed. The reaction of time are varied from 0 hour until 24 hour, reaction temperature is varied from 25°C - 40 °C and agitation speed is varied from 100 rpm – 300 rpm. Based on these three factors analysis, the experimental design was done with Design Expert for 20 runs to observe the response of glucose. Secondly, the microorganisms used are a facultative anaerobe which is called mix culture of microorganisms from oil palm sap. The substrate used in this study is oil palm trunk fibre which are felled and squeezed at plantation in Risda Ulu Cheka, Jerantut, Malaysia. The biological method was done by aerobic fermentation using shake flask as a batch reactor. Then the analysis of glucose was done by using Miller Method (dinitrosalicyclic acid assay) to get optical density (OD). Finally, the production of glucose was determined based on standard curve that was done previously.

1.5 Rational & significant

There are two major rational and significant to study production of oil palm trunk fibre using biological method. Firstly, production of glucose from oil palm trunk fibre will turn the waste of the palm oil into profit and give income to this economy's country. This agricultural residue is also an ideal inexpensive, renewable and abundantly available resource (Ho & Brainard, 1998; Sun & Cheng, 2002). Secondly, the production of glucose using mix culture of microorganisms can be as another alternatives way in glucose production with low cost, saving time and more environmental friendly.

CHAPTER 2

LITERATURE REVIEW

2.1 Glucose overview

2.1.1 Properties of Glucose

Glucose with molecular formula $C_6H_{12}O_6$ is contains six carbon atoms and an aldehyde group and is therefore referred to as an aldohexose as in **Figure 2.1**. The aldohexose sugars contains two isomers are known as glucose and only one of which (D-glucose) is biologically active. This form (D-glucose) is often referred to as dextrose (*dextrose monohydrate*). The mirror-image of the molecule, L-glucose, cannot be used by cells. Aldohexose sugars have 4 chiral centers and these are split into two groups, L and D, with 8 sugars in each. Glucose is one of these sugars, and L and D-glucose are two of the stereoisomers. Only 7 of these are found in living organisms, of which D-glucose (Glu), D-galactose (Gal) and D-mannose (Man) are the most important.

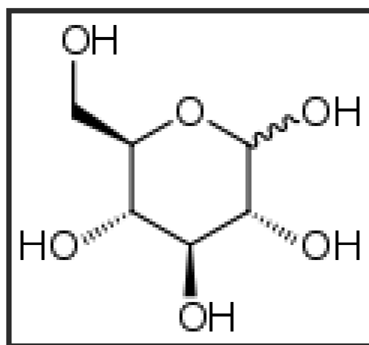

Figure 2.1: Glucose structure in ring form

2.1.2 Application of Glucose

There are many applications of glucose. In natural, glucose is used in photosynthesis for plant cell and it stores the energy. Glucose is widely used in evolution, the ecosystem and metabolism compare the other monosaccharide such fructose. It is due to the ability of glucose which can be form from formaldehyde under antibiotic conditions, so it may well have been available to primitive biochemical systems.

Glucose has wide application in the Maillard reaction which a typical chemical reaction in food processing and flavor chemistry. It has far reaching implications in the productions of flavors and aromas, nutrition, toxicology, human pathology and technology of food processing (Ikan, 1996; Yaylayan, 1997). Furthermore, in the pharmaceutical industry, glucose is used in the medicine preparations, tablet coating and drug formulation, for the example the manufacturing of antibiotic drugs and penicillin (Riddhi Siddhi, 2007). Another application of glucose is as a precursor in the production of proteins and in lipid metabolism.

2.2 Substrate for glucose production

There are several substrates that can be used in order to produce glucose which are oil palm trunk, sago palm trunk, oak trunk and poplar trunk. These trees is considering as hardwood. Hardwood is wood that have a more complex structure than softwoods. The dominant feature separating hardwoods from softwoods is the presence of pores, or vessels. Hardwoods have a higher proportion of cellulose, hemicelluloses and extractives than softwoods, but softwoods have a higher proportion of lignin and hardwoods are also denser than softwoods (Ayhan, 2003). Meanwhile, wood debris is byproduct of wood processing, pollutes the environment even though these debris are materials suited for biodegradation (Shide, 2004). Wastes and their disposal have become enough substances of environmental concern worldwide especially when these wastes are biodegradable to useful goods and services.

2.2.1 Oil palm trunk

Oil palm trunk is lignocellulosic biomass which includes as agricultural residues that were found after old palm trunks felled for replanting. After squeezed, it will produce oil palm sap and the residues considering as an oil palm trunk fibre. Bioconversion of this waste material to commercial value such as glucose is low cost and widespread in nature. Malaysia is well known for its potential in renewable resource such oil palm waste and this country is largest exporter of palm oil in the international market. Based on research in year 2007, the plantation area in Malaysia was 4,304,913 ha and nearly 7 million ha, consider 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, it's means on average 64 million to 80 million old palm trees will be felled every year in this countries, as approximately 142 oil palms are usually planted in one hectare. Based on this situation, the waste produced is most abundant and can be the important

biomass resources in Malaysia to produce commercial value as increase this economy's country (Kosugi *et al.,* 2010).

2.2.2 Sago palm trunk

The sago palm trunk waste produced by the sago starch industries is also one type of lignocellulosic waste material and it is an available in large quantities but of no commercial value. Earlier studies have indicated that sago waste produced by the sago starch mill is still rich in starch (Haryanto *et al.,* 1991) and the residue from starch extraction is a very strong pollutant because of its cellulosic fibrous material. Sago palm trunk can be an alternative cheap carbon source for fermentation process to produce glucose which that is attractive out of both economic, geographical considerations and also environmental friendly. Unfortunately, sago is now only a minor crop in Peninsular Malaysia, occupying less than 1% of the total agricultural land. The largest sago-growing areas in Malaysia are to be found outside the Peninsula, in the state of Sarawak, which is now the world's biggest exporter of sago (Suraini, 2002).

2.2.3 Oak trunk

An oak is a tree or shrub in the genus *Quercus* of which about 600 species exist on earth. Oak may also appear in the names of species in related genera, notably *Lithocarpus*. The genus is native to the northern hemisphere, and includes deciduous and evergreen species extending from cold latitudes to tropical Asia and the Americas. Oak wood is mainly composed of three large polymers which are cellulose about 50%, hemicelluloses about 20% and the other large polymer is lignin about 30%. Due to the high temperature reached during the toasting process, the chemical bonds between polymers are disrupted and the hemicelluloses and lignin in particular are degraded since

they are less structured than cellulose. Their degradation gives rise to a great number of new compounds which play an important role in the development of wine flavours (Singleton, 1995) and produced glucose as intermediate product.

2.2.4 Poplar trunk

A poplar is a type of tree in the genus *Populus* which is is a genus of 25–35 species of deciduous flowering plants in the family *Salicaceae*, native to most of the Northern Hemisphere. Previous research shows that poplar trunk contains cellulose as a major component and it can be converting to produce glucose by thermal degradation process (Ayhan, 2003).

2.2.5 Selection of substrate for glucose production

Based on the above types of substrates, the oil palm trunk has been chosen in order to produce glucose. The main reasons are because this substrate can find with most abundant in this country compare to the others substrate. It also cheap and their use will yield economic as environmental friendly.

2.3 Hydrolysis Process

Hydrolysis is a chemical reaction in which a chemical compound decomposes by reaction with water and the reacting water molecules are split into hydrogen (H^+) and hydroxide (OH^-) ions, which react with and break up (or "lyse") the other reacting compound. The term *hydrolysis* is also applied to the electrolysis of water to produce hydrogen and oxygen. In biochemistry, hydrolysis is considered the reverse or opposite of dehydration synthesis and it has same meaning as in chemistry. In hydrolysis, a water molecule (H_2O) is added, whereas in dehydration synthesis, a molecule of water is removed. The use of biological means have greater advantages over the use of chemicals for degradation because biotechnological synthesized products are less toxic and environmentally friend (Shide *et al.*, 2004) In a polysaccharide molecule, monosaccharide subunits are linked together by glycosidic bonds and this bond can be cleaved by hydrolysis to yield monosaccharides such as glucose. There are two types of biological hydrolysis which are enzymatic and microbial hydrolysis.

2.3.1 Enzymatic Hydrolysis

Enzymatic hydrolysis is one of the methods which common use in order to hydrolyze cellulose to glucose with high efficiency (Li *et al.,* 2009). It is carried out using cellulase enzymes (Vlasenko *et al.,* 1996). Cellulase can be refers to a class of enzymes produced chiefly by fungi, bacteria, and protozoans that catalyze the cellulolysis or hydrolysis of cellulose. However, there are also cellulases produced by other types of organisms such as plants and animals. Several different kinds of cellulases are known, which differ structurally and mechanistically (Chapin *et al.,* 2002). Currently, most commercial cellulases (including β- glucosidase) are produced by Trichoderma species and Aspergillus species (Cherry and Fidantsef, 2003). Cellulases

are used in food applications and also in the textile, laundry as well as in the pulp and paper industries. (Li *et al.,* 2009)

Production of glucose which catalyzed by enzymatic hydrolysis produces better yields compare to the chemical hydrolysis. However, the main challenges are to enhancing the activity of cellulase enzyme in order to increase glucose production. Besides that, the high cost of cellulase enzymes often restricts the large-scale application of these enzymes in the bioconversion of lignocellulosic biomass (Chen *et al.,* 2007). There is need to find another better way to solve this problem, and now it is use genetic techniques which are to clone the cellulase coding sequences into bacteria, yeasts, fungi, plants and animals to create new cellulase production systems. It is very important to improve enzyme production and activities (Li *et al.,* 2009) as increasing hydrolyze of cellulose into glucose.

2.3.2 Microbial Hydrolysis

In recent years, metabolic engineering for microorganisms used in glucose production has shown significant progress. Due to the complex nature of the carbohydrates present in lignocellulosic biomass such as oil palm trunk fibre, a significant amount of glucose present can be determined. Microorganisms are unicellular, meaning they contain only a single cell. Recently, the study indicated oil palm sap contains microorganisms which are including a complex mixture of wild yeasts and bacteria. The presence more than one kind species of microbes in oil palm sap is called mix culture. In this study, by using mix culture of microbes which include bacteria and yeasts, there are responsible to changes in biochemical composition of lignocellulosic during microbial hydrolysis (Kosugi *et al.,* 2010). A majority of microbes can degrade modified cellulose since microbes itself can produce enzymes. Microbial hydrolysis is an attractive technique for enzyme production because it

presents many advantages which can reduce the cost of production because simple design machinery and less energy usually are required (Hong, 2011). Cultivation of enzymes for degradation of lignocellulosic materials has been reported through fermentation process (Shide, 2004). Another advantage is the production process is more nature because using the microbes that already has in environment.

2.3.3 Selection of Biological Hydrolysis

Based on that two chosen of biological hydrolysis process, the microbial hydrolysis has been chosen to study in produce glucose from oil palm trunk fibre. It is because, in microbial hydrolysis, the production of enzymes is produced by the microbe itself with nature condition but in enzymatic hydrolysis, the enzymes used are more specific for certain substrates due to several conditions needs to take in consideration which PH and temperature. Besides that, the major disadvantage is the enzyme is very high cost compare to the microbial hydrolysis.

2.4 Factors effecting on glucose production

There are three factors to study on production of glucose from oil palm trunk fibre using biological hydrolysis which are reaction time, temperature and agitation speed.

2.4.1 Reaction time

Reaction time is also referred as fermentation time. The effect of fermentation time is one of the important factors to study on production of glucose. It is because the all cases analysis of variance revealed statistically significant using lignocellulosic hydrolysates has been showed the differences in fermentation time. The formation of products on fermentation time is also depending on types of lignocellulosic hydrolysates. Earlier studies indicated that in ethanol fermentation process which glucose as intermediate product, short fermentation time is desirable by using oil palm trunk as a substrate (Chin *et al.,* 2010). Based on these finding, the fermentation time used in this study is start from 0 hour until 24 hour. It is also possible range because in this study only using shaker flask as a bioreactor too. The selection of reaction time as one of the factor is also important to analysis the glucose production at certain time. Based on fermentation time, the highest glucose yield can be determined at appropriate times as conditions too. It is because, depending on the conditions of fermentation such as temperature of reaction, the fermentation time is also change as glucose produced is also difference (Chin *et al.,* 2010).

2.4.2 Temperature

Temperature changes have profound effects upon living thing and it is one of the most crucial variables to monitor during the fermentation. In fermentation process, the temperature has an exponential effect on the biological reaction and others such as bacteria growth. It is also similar to the effect of temperature on chemical reactions such as enzyme-catalyzed reactions which are especially sensitive to small changes in temperature. Previous research indicated the influence of temperature on the fermentation has yielded a complex mixture of products (Chin *et al.,* 2010). The selection of reaction temperature in this study as one of the factors is because very little is known on how fermentation temperature affects the fermentation on lignocellulosic hydrolysates such as oil palm trunk fibre using biological method. Therefore, there is a need to understand and evaluate the effect of fermentation temperature on the fermentation efficiency. The reaction temperature used in this study is between 25°C to 40 °C. The appropriate fermentation conditions for lignocellulosic hydrolysates can be determined by evaluating the effect of different temperature on the glucose yield from fermentation process. Indirectly, the maximum yield of glucose can be determined too.

2.4.3 Agitation speed

Agitation speed has been also chosen as one important factor to study since it will increase the amount of dissolved oxygen in the cultivation medium along the fermentation process. It is also give effect to the enzymes activity and growth of microbes. Agitation speed of the culture broth has a variety of effects on microorganisms, including rupture of the cell wall, change in the morphology of filamentous microorganisms, variation in the efficiency and rate of growth and also variation in the rate of formation of the desired product (Purwanto *et al.,* 2009). Agitation speed should be evaluated on the glucose yield from fermentation process

because it is well known that agitation speed creates turbulence and shear force in the cultivation process which will influence both cell growth and product formation (Chin *et al.,* 2010). The maximum production of glucose can be determined by biological method based on the variation of agitation speed used in this study which is between 100 rpm to 300 rpm.

2.5 Bioreactors

A bioreactor also call fermentation tanks may refer to a vessel in which a chemical process is carried out which involves organisms or biochemically active substances derived from such organisms and the process can either be aerobic or anaerobic. These bioreactors are commonly cylindrical, ranging in size from liters to cubic meters, and are often made of stainless steel for the large scale. Laboratory scale fermentations are carried out in shaker flasks and flat bed bottles. The shaker flasks are conical vessels made of glass and are available in different sizes. The typical volume of these flasks is 250 ml. There are different types of shaker flasks, such as baffled, unbaffled or Erlenmeyer flask, and flying saucer. Shaker flasks are used for the screening of microorganisms and cultivation of them for inoculation. Baffled flasks are used to increase the oxygen transfer. Shaker flasks need to be plugged to prevent contamination with other microorganisms. Cotton-wool, polyurethane foam, glass, and synthetic plugs are commonly used. A fermentation vessel should be cheap, not allow contamination of the contents, be non-toxic to the microorganism used for the process, be easy to sterilize, be easy to operate, be robust and reliable, allow visual monitoring of the fermentation process, allow sampling, and be leak proof. Fermentation can be occurring in batch, fed batch or continuous process.

2.5.1 Batch bioreactor

The type of fermentation in batch bioreactor is also called a closed culture system because nutrients and other components are added in specific amounts at the start of the process and are not replenished once the fermentation has started. At the end of the process the product is recovered; then, the fermenter is cleaned, sterilized, and used for another batch process. In the initial stages microorganisms grow at a rapid rate in the presence of excess nutrients but as they multiply in large numbers they use up the

nutrients. This fermentation vessel is very cheap and it is also easy to sterilize and operate too.

2.5.2 Fed-batch Bioreactor

In fed batch bioreactor process, the nutrients and substrates are added at the start of the process and at regular intervals after the start. This is called controlled feeding. Inoculum is added to the fermentation vessel when microorganisms are in exponential growth phase. Fed-batch culture is controlled by feed-back control and control without feed-back. Feed-back control is the fermentation process is controlled by monitoring process parameters like dissolved oxygen content, carbon dioxide to oxygen ratio, pH, concentration of substrate, and concentration of the product. Control without feed-back is the substrates and nutrients are added at regular intervals. Fed-batch culture requires special equipment such as a reservoir which holds the nutrients, pH modifiers so that they can be added to the fermenter at regular intervals, and pumps to deliver culture medium aseptically to the fermenter.

2.5.3 Continuous Bioreactor

This method of continuous bioreactor prolongs the exponential growth phase of microbial growth as nutrients are continually supplied and metabolites and other wastes are continually removed thus promoting continual growth of the microorganisms. Continuous culture fermentation is advantageous because of its high productivity. Two control methods are used in continuous culture fermentation, namely, chemostat and turbidostat.

2.5.4 Selection of bioreactor

Based on the above bioreactor, the batch bioreactor is chosen. In fed-batch bioreactor, it is not easy to manage because the nutrients and substrates need to add at the start and at regular intervals after the start and the continuous bioreactor is more difficult than that because it is run continuously which meaning that the nutrients are continually supplied. These two conditions can give contaminant of the content. So that it will disturb the fermentation process. Batch bioreactor is more suitable in the study is because considering the cost of bioreactor which is very cheap and it is also easy to sterilize and operate too.

2.6 Response Surface Methodology (RSM)

Optimization is the conditions under which a certain process attains the optimal results. The purpose is to determine the levels of the design parameters at which the response reaches its optimum. The optimum could be either a maximum or a minimum of a function of the design parameters. One of methodologies for obtaining the optimum is response surface technique (Raissi, 2009) which is used in this study.

Response Surface Methodology (RSM) was utilized to optimize the biological hydrolysis process which is to determine the optimum operational conditions for the system or to determine the region that satisfies the operating specifications. It is widely used especially in determining optimum conditions for chemical investigations and maximizing yields in biocatalyzed syntheses (Bidin *et al.,* 2009). Optimization studies are carried out by varying one parameter at a time while keeping others constant (Roberto *et al.,* 2001).

The effects of different variables on glucose production employing oil palm trunk fibre were simultaneously using a two level, three-variable central composite rotatable design (CCRD). The central composite rotatable design (CCRD) is one of the common experimental designs for fitting quadratic polynomial. It is also used to design an experimental program to model the effects of reaction time, temperature and agitation speed on glucose production (Obeng, 2005)

The full quadratic second – order polynomial equation was found to explain the glucose production by applying multiple regression analysis on the experimental data as shown in **Equation 2.1.**

$$Y = a_0 + \sum_{i=1}^{3} a_i X_i + \sum_{i=1}^{3} a_{ii} X_i^2 + \sum_{i}^{2} \sum_{j=i+1}^{3} a_{ij} X_i X_j$$

$$= a_0 + a_1 X_1 + a_2 X_2 + a_3 X_3 + a_{11} X_1^2 + a_{22} X_2^2 + a_{33} X_3^2 + a_{12} X_1 X_2 + a_{13} X_1 X_3 + a_{23} X_2 X_3$$

(Equation 2.1)

From the equation, Y is glucose concentration which also known as predicted value, and X_1 is the coded value for reaction time, X_2 is the coded value for temperature, X_3 is coded value for agitation speed. The linear coefficients a_1, a_2 and a_3 express the linear effect of each variable; the a_{11}, a_{22} and a_{33} coefficients express the quadratic effect; a_{12} a_{13} and a_{23} coefficients express interactive effects between the variables and a_0 is a constant corresponding to the central point of experimental variables (Deyhimi *et al.,* 2006).

The mathematical models were evaluated for each response by means of multiple linear regression analysis. The modeling was started with a quadratic model including linear, squared and interaction terms. The significant terms in the model were found by analysis of variance (ANOVA) for each response. Significance was judged by determining the probability level that the F-statistic calculated from the data is less than 5% (Raissi, 2009). The goodness of fit of the model was checked by the determination coefficient (R^2). The R-squared value provided a measure of the variability in the actual response values that could be explained by the experimental factors and their interactions. A value of one represents the ideal case at which 100% of the variation in the observed value can be explained by the model (Aminah, 2006). Maximization and minimization of the polynomials thus fitted was usually performed by desirability function method, and mapping of the fitted responses was achieved using computer software such as Design Expert (Raissi, 2009)

CHAPTER 3

METHODOLOGY

3.1 Overview of Research Methodology

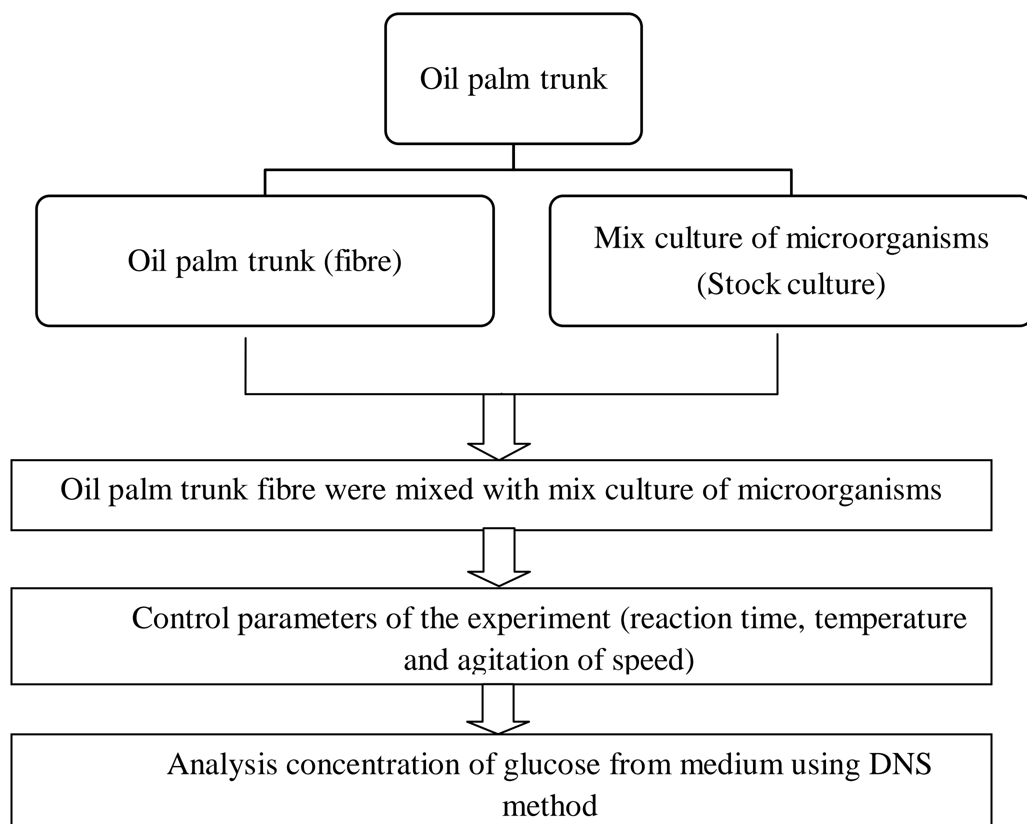

Figure 3.1: Glucose Production from oil palm trunk using biological methods

3.2 Material

3.2.1 Raw materials

The substrate used for this study was oil palm trunk as the part of oil palm biomass is cheap and readily available sources of lignocellulosics. The oil palm trunk was felled from plantation in Risda Ulu Cheka, Jerantut, Malaysia as in **Figure 3.2**.Sap was collected by squeezing the oil palm trunk and the residue considered as oil palm trunk fibre. Oil palm trunk fibre and oil palm sap were selected for this study as raw materials.

Figure 3.2: Oil palm trunk felled in Risda Ulu Cheka, Jerantut, Malaysia

3.2.2 Microorganisms and condition

The study is using mix culture of microorganisms which are including bacteria and yeasts. The mix culture of microorganisms is facultative anaerobic, obtained from oil palm sap, and was cultivated for 24 hours in incubator at 30°C.

3.3 Methods of Analysis

3.3.1 Standard Calibration Curve Preparation

The concentration of glucose was determined by using a method of standard calibration curve. A standard calibration curve for determine glucose concentration is done by comparing the unknown to a set of glucose of known concentration. The standard calibration curve was prepared in glucose concentration sample which are 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 g/L. The readings for absorbance glucose are taken for each concentration and the graph of absorbance readings against theglucose concentration was plotted. The measurement of absorbance was taken by using UV-Visible spectrophotometer as in **Figure 3.3**.

Figure 3.3: UV-Visible spectrophotometer

3.3.2 Preparation of Nutrient Agar

Nutrient agar is a microbiological growth medium commonly used for the routine cultivation of non-fastidious bacteria. It is useful because it remains solid even at relatively high temperatures. Also, bacteria grown in nutrient agar grows on the surface, and is clearly visible as small colonies. Nutrient agar is considered as a complex medium because it contains ingredients with contain unknown amounts or types of nutrients. It was prepared by adding the distilled water to the agar powder in the Schott bottle and swirled the flask by magnetic stirrer to dissolve the mixture. For 23 grams of nutrient agar powder, 1.0 liter of distilled water is needed. The pH of solution is checked, it should be 7.0. Before undergo the autoclave process, the capped of Schott is covered with aluminum foil and tape. Then, it was sterilized at 121°C of temperature. The autoclave process is about 2 hours. After that, it is cooled to 50°C of temperature before taking out from autoclave. Next, the agar is poured about 15-20 mL in each petri dish and cooled to obtain solid nutrient agar plate as in **Figure 3.4**. Finally, after the agar became solid, the lid of petri dish is sealed and transferred to the chiller.

Figure 3.4: The nutrient agar is poured and cooled in petri dish

3.3.3 Preparation of Nutrient broth

Nutrient Broth is used for the cultivation of a wide variety of microorganisms. The broth is prepared by mixture of nutrient broth with distilled water. For 8.0 grams of nutrient broth powder, 1.0 liter of distilled water is needed. Preparation of nutrient broth is using 1.0 liter Schott bottle. Firstly, 1.0 liter of distilled water was added in a 1.0 liter Schott bottle. Put magnetic bar into the bottle and place on the hotplate. The powder was dissolved completely in the water. After that, 150 mL dissolved nutrient broth was poured into 500 mL Erlenmeyer flask. The mouth of flask was covered with cotton wool and aluminum foil. Next, it is sterilized at 121°C and the autoclave process is about 2 hours. Then, it is cooled to 50°C of temperature before taking out from autoclave. Finally, nutrient broth is transferred to chiller before using in the next process.

3.3.4 Transfer of Culture via Streaking Method

The main objective of the streaking method is to transfer a stock of mix culture to nutrient agar plate aseptically. It is also to grow microorganisms on nutrient agar plate. The microbial culture is started when the inoculation loop is flamed to redness and cool for few minutes. Streak gently the inoculating loop on the plate. Since the microorganism used is mix culture, there no need to isolate to get pure culture. The streaking method was repeating with do not let the loop touch any of the previously streaked areas. All the process must be done near the Bunsen burner in order to reduce contamination to the microbe growth. After the cultural process done, the petri dish is incubated at 30°C for 24 hours and placed in an inverted position in incubator. The colony of bacteria was obtained after 24 hours.

3.3.5 Transfer of Mix Culture (Agar Plate - Nutrient Broth)

The transferring process of mix culture from agar plate to the nutrient broth is called as inoculums development. Inoculums development of mix culture was done to active state of growth microorganisms and to get a solution with a high cell concentration to start the fermentation. Firstly, the inoculating loop is flamed until it is red and let it to cool. Then, aseptically take a few loops of 24 hours-incubated culture and place into the sterilized 500 mL Erlenmeyer flask which contains 150 mL nutrient broth as in **Figure 3.5**. After that, the flask was placed in the incubator shaker. Set the temperature and speed of the incubator at 30°C and 150 rpm, respectively. Incubate the culture for 24 hours as in **Figure 3.6**.

Figure 3.5: Transfer of mix culture from agar plate to nutrient broth

Figure 3.6: The inoculum developments was incubated in shaker

3.3.6 Preparation of cell

The mix culture from the inoculums developments need to centrifuge to get constant the optical density (OD) of cell which is 2.0. Firstly, the inoculums development was transferred to sterile centrifuge tubes and starts to centrifuge it for five minutes at 5000 rpm. After that, decant the supernatant and re-suspend the cell with 100 ml of sterile normal saline solution, 0.85% (w/v) NaCl. This step is known as cell washing. Perform the cell washing step twice. For the final cell suspension, set the optical density (OD) value of 2.0 at 660nm. The measurement of absorbance was taken by using UV-Visible spectrophotometer. If the OD value exceeds 2.0, add more saline solution. If the OD value less than 2.0, add more cell. Then, use the final cell suspension with OD value of 2.0 as the inoculums for run of the experiment.

3.3.7 Substrate Preparation

To be used as substrate, the oil palm trunk fibre was thoroughly dried in oven (60°C – 70°C) at 4 hours to give constant weight. Then, it was cut to 2 cm in order to give uniform value of length and to give proper condition as size of Erlenmeyer flask. 0.25 grams of oil palm trunk fibre was then weighed and placed into a 100 mL Erlenmeyer flask.

3.3.8 Experimental Set Up

There are three parameters that had been chosen in order to produce glucose from oil palm trunk fibre which are reaction time, temperature and agitation speed. From the literature review, the range of reaction time is start from 0 hour until 24 hour. For the reaction temperature, the range used in this study is start from 25°C until 40 °C. The last one parameter is agitation speed with range is start from 100 rpm until 300 rpm. Based on these range, the experimental design was done by using three factor analyses in Design Expect. The table of the experimental design as showed in **Table 3.1** below.

Table 3.1: The experimental design of the reaction time, temperature and agitation

Run	Block	Factor 1 A: Reaction time (hour)	Factor 2 B: Temperature (°C)	Factor 3 C: Agitation (rpm)
1	Block 1	12.50	40.00	200.00
2	Block 1	1.00	32.50	200.00
3	Block 1	5.66	36.96	140.54
4	Block 1	12.50	32.50	100.00
5	Block 1	5.66	28.04	259.46
6	Block 1	5.66	36.96	259.46
7	Block 1	12.50	32.50	300.00
8	Block 1	5.66	28.04	140.54
9	Block 1	24.00	32.50	200.00
10	Block 1	12.50	32.50	200.00
11	Block 1	12.50	32.50	200.00
12	Block 1	19.34	28.04	140.54
13	Block 1	12.50	32.50	200.00
14	Block 1	19.34	28.04	259.46
15	Block 1	12.50	32.50	200.00
16	Block 1	12.50	32.50	200.00
17	Block 1	12.50	25.00	200.00
18	Block 1	19.34	36.96	259.46
19	Block 1	12.50	32.50	200.00
20	Block 1	19.34	36.96	140.54

3.3.9 Fermentation Procedure

The fermentation is run in batch with 20 runs of experiments was running one by one. Each run have the same and also differ value of reaction time, temperature and agitation speed. For each runs, used the inoculums at 10.0 % (v/v) of the basal medium. The working volume for each run was 25 ml from 100 ml of Erlenmeyer flask and each runs have 0.25 gram of substrate. After the cell, substrates, and water were added in the flask, cover the mouth of flask with cotton wool for the aerobic condition. After that, all the samples were placed into the stackable incubator shaker at temperature 40°C, revolution per minute (rpm) at 200 and with the reaction time is 12.50 hour as in **Figure 3.7**. After 12.50 hour incubated, the samples were collected and proceeds to filtering process by using filter paper to separate substrate and liquid. Then, 2 mL of liquid that already filtered was taken for reducing sugar analysis. The procedures were repeated for each run follow as Table 3.1. The summary of the fermentation procedure and analysis of glucose showed as in **Figure 3.8** below.

Figure 3.7: The sample was placed in stackable incubator shaker

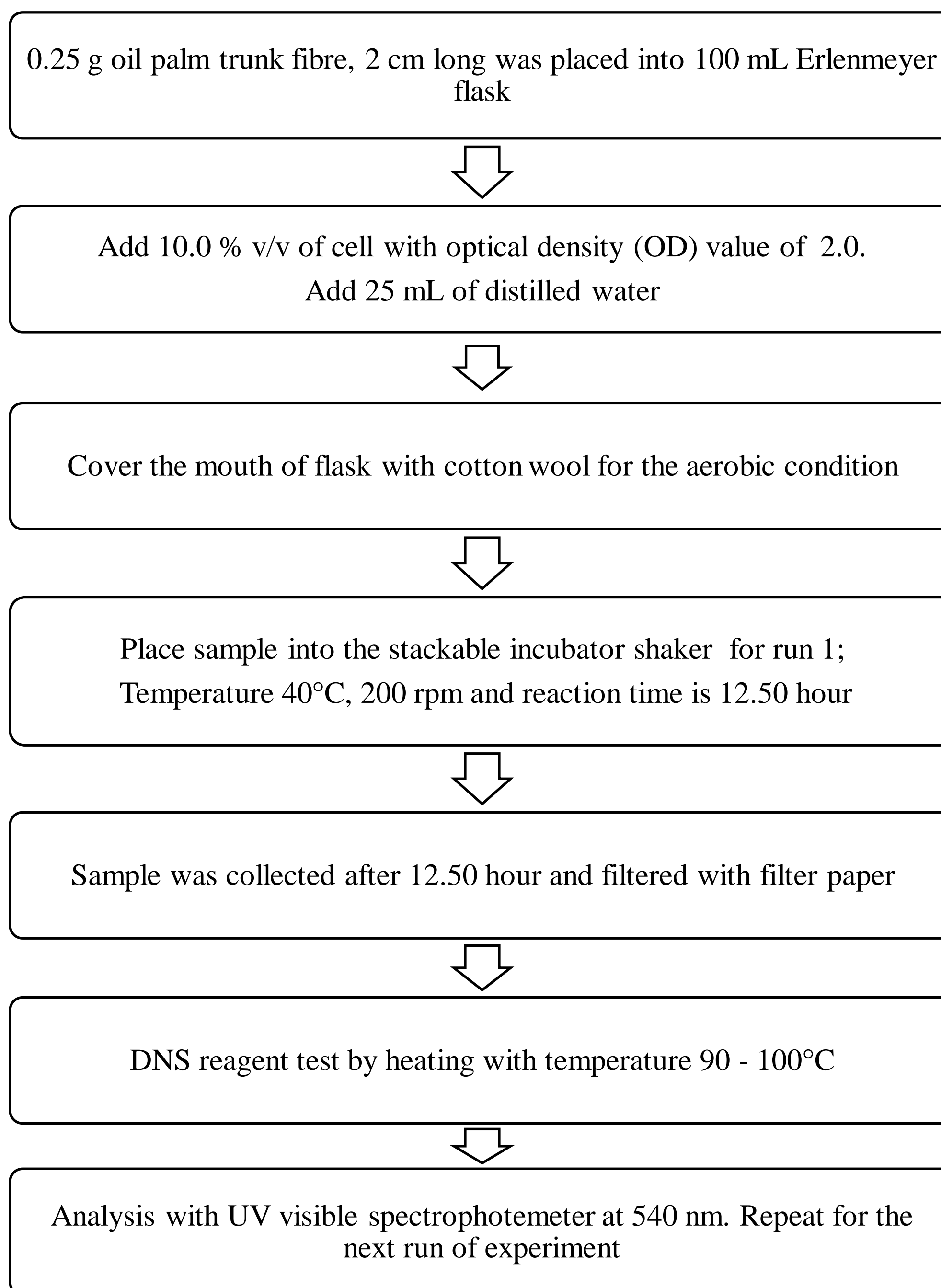

Figure 3.8: Overview of fermentation procedure and analysis of glucose

3.3.10 Dinitrosalicylic Acid Assay

Glucose (reducing sugar) production was measured and determined using dinitrosalicyclic acid (DNS) method (Miller, 1959). DNS reagent was prepared by mixture of 3,5- dinitrosalicyclic, sodium hydroxide, sodium potassium tartrate and water. For one liter of DNS reagent, 10g of 3,5- dinitrosalicyclic, 16 gram of sodium hydroxide, and 300g of sodium potassium tartrate and water were needed. After the glucose sample was collected, 2 mL of DNS reagent was added to 2 mL of glucose sample. After that, the samples in test tubes were heated at 90°C for 10 minutes to develop the red brown color as in **Figure 3.9**. After the sample is cool to room temperature, the absorbances of samples were recorded using UV visible spectrophotometer at 540 nm.

Figure 3.9: The samples were heated in water bath at 90°C -100°C

CHAPTER 4

RESULT & DISCUSSIONS

4.1 Factors of Reaction Time, Temperature and Agitation on Glucose Production

Studies on the factors of reaction time, temperature and agitation that affect the production of glucose are performed in Design Expert Software by using Response Surface Methodology (RSM). For this study, 20 run of experiments were required to complete the aerobic fermentation**. Table 4.1** below is the results which concentration of glucose is come from optical density (OD) of standard calibration curve. The reading of the optical density (OD) is determined from UV Visible spectrophotometer.

Table 4.1: Concentration of glucose produced from three factors of analyses

Run	Block	Factor 1 A: Reaction time (hour)	Factor 2 B: Temperature (°C)	Factor 3 C:Agitation (rpm)	Concentration of glucose (mg/mL)
1	Block 1	12.50	40.00	200.00	0.646326
2	Block 1	1.00	32.50	200.00	0.576091
3	Block 1	5.66	36.96	140.54	0.794458
4	Block 1	12.50	32.50	100.00	0.558213
5	Block 1	5.66	28.04	259.46	0.642495
6	Block 1	5.66	36.96	259.46	0.809782
7	Block 1	12.50	32.50	300.00	0.913219
8	Block 1	5.66	28.04	140.54	0.498194
9	Block 1	24.00	32.50	200.00	0.223639
10	Block 1	12.50	32.50	200.00	0.408804
11	Block 1	12.50	32.50	200.00	0.507133
12	Block 1	19.34	28.04	140.54	0.430513
13	Block 1	12.50	32.50	200.00	0.427959
14	Block 1	19.34	28.04	259.46	0.404973
15	Block 1	12.50	32.50	200.00	0.450945
16	Block 1	12.50	32.50	200.00	0.475208
17	Block 1	12.50	25.00	200.00	0.430513
18	Block 1	19.34	36.96	259.46	0.433067
19	Block 1	12.50	32.50	200.00	0.545443
20	Block 1	19.34	36.96	140.54	0.411358

From the Table 4.1, it is showed that Run 7 gave the highest production of glucose which produced 0.913219 mg/mL. The optimum conditions of Run 7 were 12.50 hours of reaction time, temperature value of 32.50°C and agitation speed of 300 rpm.

The lowest production of glucose found at Run 9 which produced 0.223639 mg/mL. The minimum conditions of Run 9 were 24 hours of reaction time, temperature value of 32.50°C and agitation speed of 200 rpm.

According to the results, the conditions of 12.50 hours of reaction time with temperature value of 32.50°C and agitation speed of 300 rpm is the best of condition to give maximum yield of glucose. The production of glucose is in maximum at these condition because of the microbial hydrolysis process occurs to degrade lignocellulosic of oil palm trunk to glucose (Chin *et al.,* 2010).

At 12.50 hours, glucose produced is at maximum yield to show that most of the cellulose has been fully converted to glucose by microbial hydrolysis. The production of glucose were become decreased in longer time because the glucose produced has been converted to alcohol in fermentation process.

At 32.50°C, glucose produced is at maximum yield to show that this temperature is the best temperature for growth rates of microbes for enhances mix culture of microbe to degrade of oil palm trunk to glucose (Chin *et al.,* 2010).

Besides that, agitation speed is a very important factor in the fermentation process since it will increase the amount of dissolved oxygen in the cultivation medium.

Maximum yield of glucose is produced at 300 rpm showed that increasing of agitation speed will increasing of dissolved oxygen. By increasing of agitation speed will give effects to microorganism in term of high rate of growth microbe to increasing production of glucose (Purwanto *et al.,* 2009)

4.2 Determination of the Glucose Production Using Response Surface Methodology (RSM)

Two-dimensional plot were drawn to investigate the effect of different parameters towards glucose production. The purpose of this plotting is to convince and comprehends the interaction between three parameters and also to locate their optimum levels. The response surface representing the glucose production activity was a function of two parameters with the other one parameter being at their optimal levels.

The result was analyzed by using analysis of variance (ANOVA) as appropriate to the experimental design used. The full quadratic second-order polynomial equation was found to explain the glucose production by applying multiple regression analysis on the experimental data. From the design expert, the model for glucose production is given as **Equation 4.1** below:

$$Y = +1.06290 + 0.067139*A - 0.028124*B - 7.60591E\text{-}003*C - 5.71030E\text{-}004*A^2$$

$$+1.12064E\text{-}003*B^2 + 2.60332E\text{-}005*C^2 - 1.86353E\text{-}003*A*B - 5.02525E\text{-}005*A*C$$

$$-3.85269E\text{-}005*B*C$$

(Equation 4.1)

From the equation, Y is glucose concentration which also known as predicted value. A is the coded value for reaction time, B is the coded value for temperature and C is coded value for agitation speed. **Table 4.2** showed that results from actual values and predicted values. The actual values are determined from the experiment and the predicted values are come from the equation 4.1. From the actual and predicted value, the percentage of error has been calculated.

Table 4.2: Comparison between actual value and predicted value

Order	**Actual Value (mg/mL)**	**Predicted Value (mg/mL)**	**Percentage of Error (%)**
1	0.50	0.47	6.0
2	0.43	0.39	9.3
3	0.79	0.73	7.6
4	0.41	0.42	2.4
5	0.64	0.65	1.6
6	0.40	0.48	20.0
7	0.81	0.86	6.2
8	0.43	0.46	7.0
9	0.58	0.60	3.4
10	0.22	0.19	13.6
11	0.43	0.43	0
12	0.65	0.64	1.5
13	0.56	0.64	14.3
14	0.91	0.82	10.0
15	0.51	0.47	7.8
16	0.55	0.47	14.5
17	0.41	0.47	14.6
18	0.43	0.47	9.3
19	0.45	0.47	4.4
20	0.48	0.47	2.1

4.3 ANOVA analysis

Table B.1 shows the ANOVA and regression analysis for the concentration of glucose. The precision of a model can be checked by determination coefficient (R^2). As a rule, a regression model having an R^2 value higher than 0.9 is considered to have a very high correlation. The value of R indicates better correlation between the experimental and predicted values (Fang *et al.,* 2010). According to the table B.1, the value of R^2 was 0.9137. Meanwhile the lack of the fit was insignificant but the R^2 value (0.9137) was high indicating that the model was well adapted to the response (Fang *et al.,* 2010).

The value of "Prob > F" of the model is 0.0003 which less than 0.05 indicating that the model have a significant effect on the response. The value "Prob> F" of A, B, C, C^2 are also demonstrating that they were the most significant factors than the other ones influencing the response. Besides that, the value "Prob > F" of AB was also a significant factors with value 0.0367 which manifesting that there was interaction existing between reaction time and temperature as Figure 4.1 (b). Other model terms can be said to be not significant. These insignificant terms can be removed and may result in an improved model.

The Standard Error of the Estimate (also known as the Root Mean Square Error) is the square root of the Residual Mean Square. It is the standard deviation of the data about the regression line, rather than about the sample mean. Based on calculation, the value of root mean square error is 0.07.

4.4 Interaction between Temperature and Reaction Time on Glucose Production

Based on the **Figure 4.1(a)**, results showed that the glucose production is increased when the reaction time is decreased with the increase of temperature. The production of glucose has a maximum yield in reaction time started from 5.66 hour to 9.08 hour. **Figure 4.1(b)** show the relation of reaction time and temperature towards glucose production. It is concluded that reaction time and temperature has an interaction in order to produce glucose.

The results showed that short reaction time is needed to produce maximum yield of glucose. Based on the previous research, the production of reducing sugars as well as percent biological hydrolysis rate increased as decreasing of reaction time. It is might be due to the inhabitation of the microbial hydrolysis reaction which produced by by the accumulated hydrolysis products (Xu *et al.,* 2007).

The formation of products on reaction time is also depending on types of lignocellulosic hydrolysates. Earlier studies also indicated that in ethanol fermentation process which glucose as intermediate product, short reaction time is desirable by using oil palm trunk as a substrate (Chin *et al.,* 2010). In fermentation, glucose was thoroughly consumed after 24 hour (Kosugi *et al.,* 2010) which meaning that production of glucose will be decrease as increasing of reaction time because most of the glucose has been convert to ethanol.

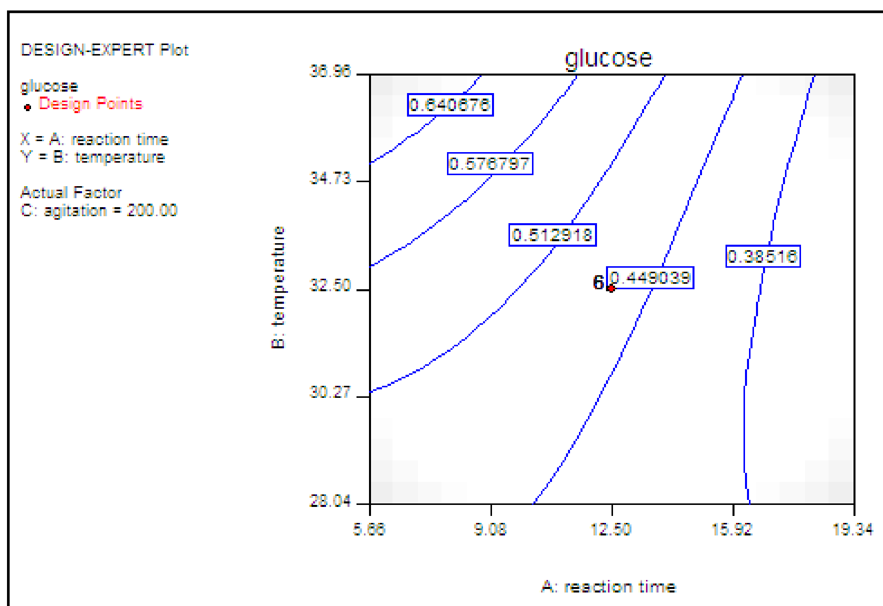

Figure 4.1 (a): The effect of reaction time and temperature towards glucose production

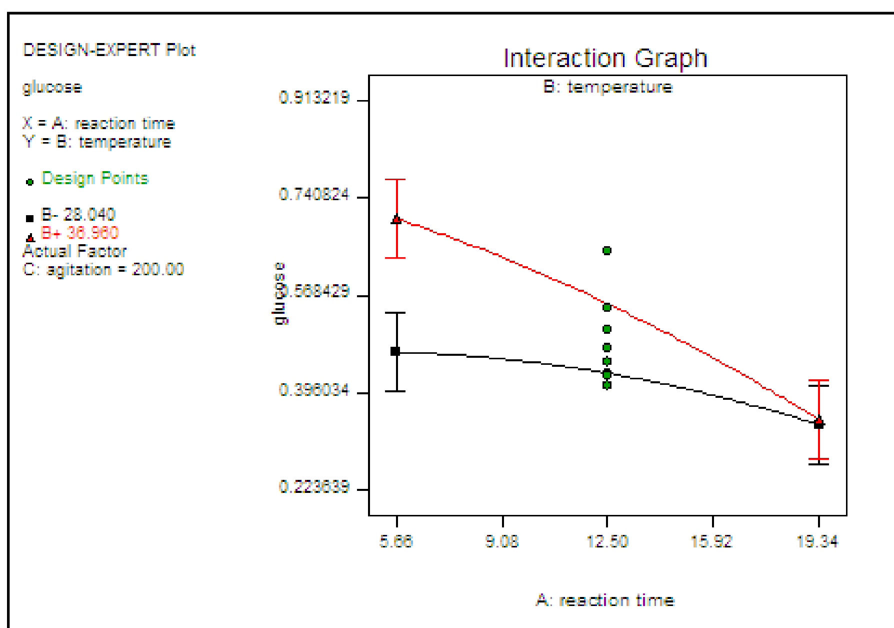

Figure 4.1(b): Relation of Reaction time and Temperature towards glucose production

4.5 Interaction between Temperature and Agitation on Glucose Production

Based on the **Figure 4.2(a)** and **Figure 4.2(b)**, results showed that the effect and relation of temperature and agitation towards glucose production. Figure 4.2 (a) concluded that when the temperature and agitation speed were increased, the production of glucose is also increased. Production of glucose is directly proportional to both parameters. Figure 4.2(b) concluded that there is no interaction between temperature and agitation speed in order to produce glucose.

The maximum yield of glucose produced is in temperature range of 34.73°C to 36.96°C. It is showed that, these temperature ranges are the best temperature for the mix culture of microbe to degrade of oil palm trunk to glucose. The production of glucose has a maximum yield with the increasing of temperature is also indicated that greater temperatures increased growth rates of microbes. In fermentation process, the temperature has an exponential effect on the biological reaction and others such as bacteria growth. However, microbes may behave differently in different lignocellulosic biomass hydrolysate at different temperatures (Chin *et al.,* 2010)

Since the increasing of growth rates of microbes were occurred, it will increase the microbial hydrolysis of oil palm trunk to produce more glucose. Previous research indicated the influence of temperature on the fermentation has yielded a complex mixture of products (Chin *et al*., 2010).

From another research which utilized of palm kernel cake for the production of mannanase by indigigeneous filamentous fungus, *Aspergillus Niger* through fermentation, the effect of temperature showed that microbial activity increases in

temperature range start from 30°C – 40°C (Rashid *et al.*, 2011). It is closely to this research which is showed that these ranges of temperature (34.73°C - 36.96°C) are significant for the microbial activity to produce maximum yield of glucose.

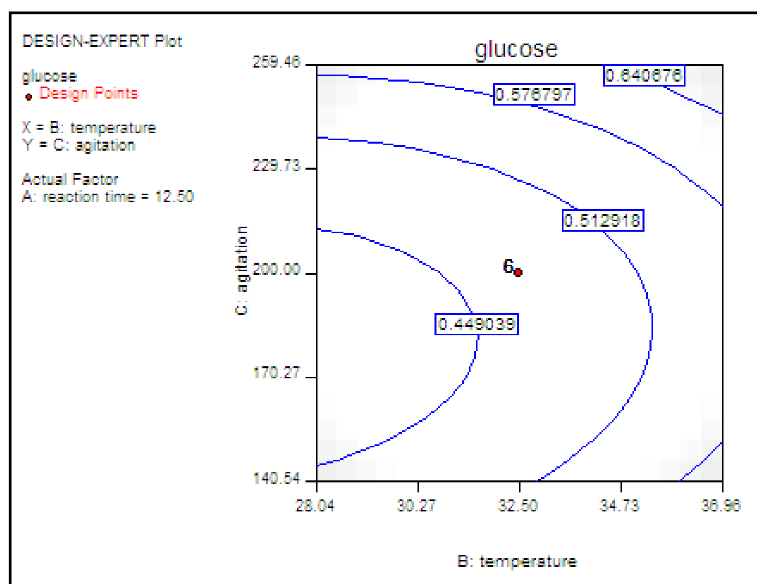

Figure 4.2(a): The effect of Temperature and Agitation towards glucose production

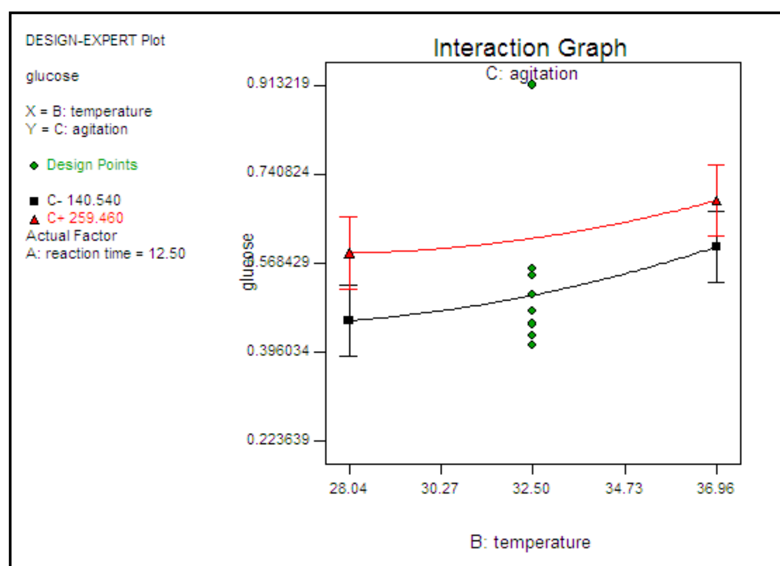

Figure 4.2(b): Relation of Temperature and Agitation towards glucose production

4.6 Interaction between Agitation and Reaction Time on Glucose Production

According to the **Figure 4.3(a)**, the production of glucose is increased when the reaction time is decreased as agitation is increased. The maximum yield of glucose is produced with the short of reaction time as mentioned before. Based on the **Figure 4.3(b)**, there are no interaction between reaction time and agitation in order to produce glucose. The most influence for the agitation to give maximum yield of glucose is value of revolution per minute whether it is high or low.

The production of glucose has a maximum yield in agitation speed range above 229.73 rpm. Production of glucose increases as agitation speed increases. The previous study has been showed that the amount of dissolved oxygen in the cultivation medium along the fermentation process will be increased as agitation increased. It is also giving effect to the microbial activity and growth of microbes which that condition gives maximum growth of microbes to increases microbial activity (Purwanto *et al.,* 2009). Indirectly, the production of glucose is also increased too.

Another research which study about the influence of agitation speed on production of glucose oxidase of *Asp.niger* has found that rate of growth and glucose oxidase production was higher when agitation speed increased. It means that as increase of agitation speed will be give maximum yield of product (Zetelaki, 2004) such as glucose.

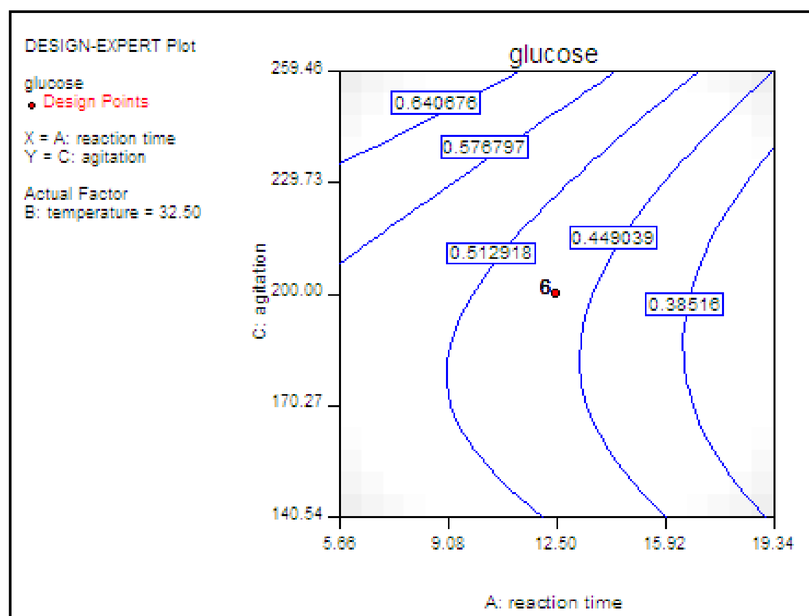

Figure 4.3(a): The Effect of Reaction Time and Agitation towards glucose production

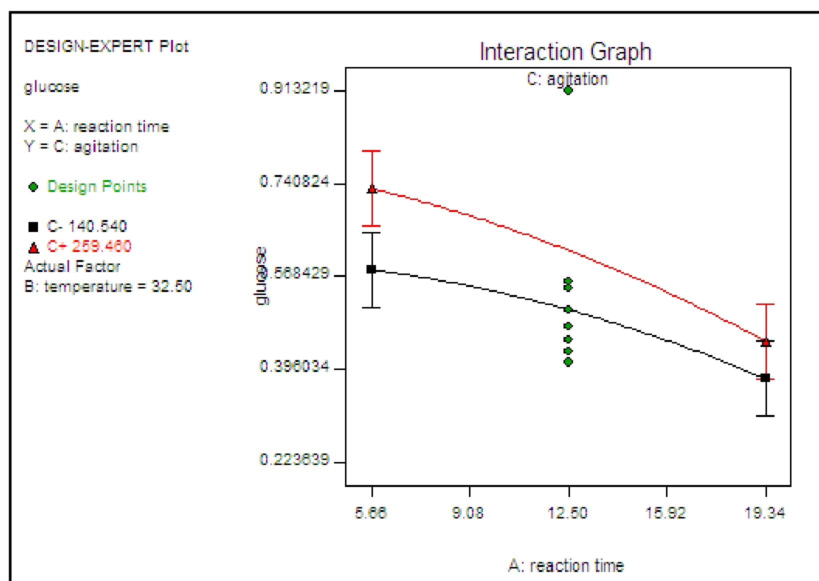

Figure 4.2(b): Relation of Reaction time and Agitation towards glucose production

4.7 Optimization of Glucose Production by Experimental Design

All the parameters were set up with lower limit and upper limit in order to identify the optimum parameters for production of glucose. The reaction time range is set up from 5.66 h - 19.34 h, temperature is set up from 28.04°C - 36.96°C and agitation is set up from 140.54 rpm - 259.46 rpm. The solutions from experimental design showed that the optimum condition were 10.41 hours of reaction time,temperature value of 30.45°C and agitation of 225.20 rpm.

From the experiment of optimization based on optimum condition, the production of glucose is 0.983454 mg/mL. **Table 4.3** is showed comparison of predicted and actual value of optimization condition from equation 4.1 and experiment respectively.

Table 4.3: Comparison of predicted and actual value of optimization condition

<table>
<tr><th colspan="2">Value of parameters</th><th>Predicted value</th><th>Actual value</th></tr>
<tr><td>Reaction Time (hour)</td><td>10.41</td><td rowspan="3">0.517333 mg/mL</td><td rowspan="3">0.983454 mg/mL</td></tr>
<tr><td>Temperature (°C)</td><td>30.45</td></tr>
<tr><td>Agitation (rpm)</td><td>225.20</td></tr>
</table>

After optimization and validation, the glucose production was increased to 0.983454 mg/mL. The value of percentage of error produced is 47.4%. The value of percentage of error from the optimization conditions is quite high. It is showed that the actual value is quite different from the predicted value, it is maybe because of the some error has been occurred during the experiment.

It might be caused by the surrounding factor. During the preparation of the mixed culture of the microbe to get constant cell concentration, it maybe exposes to the bacteria or other pollutant from the surrounding and air so which also give effect to the microbial hydrolysis that was analysis.

Beside that, the equipment which being used, the UV Visible spectrophotometer is might not be the most suitable equipment to measure the absorbance. As a solution to overcome this problem, maybe another equipment should used and the equipment which is propose is the glucose analyzer which is has higher efficient for the process.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The objective of the research to produce glucose from oil palm trunk using biological method was successfully achieved. There are three factors studied which are reaction time, agitation speed and temperature. The best condition to produce optimum glucose were achieved at 12.50 hours of reaction time, temperature value of 32.50°C and agitation speed of 300 rpm. There are also produce interaction between reaction time and temperature in order to produce glucose.

After optimization, the glucose concentration was increased to 0.983454 mg/mL to give value of percentage of error which is 47.4. Although some error occurred based on the high value percentage of error produced, the result can be concluded that glucose can be produced from oil palm trunk using biological method.

Glucose can be produced from oil palm trunk using biological method and it's a new potential as alternatives way in production of glucose in order to reduce cost because more economically and also more environmental friendly.

5.2 Recommendation

In order to make this study more interesting, the research can be carried on the other parameter should be study to observe the effect towards yield of glucose

It also recommended to analyzing the glucose production using glucose analyzer that was more effective than UV Visible spectrophotometer. It can give more accurate result

REFERENCES

Akmar, P.F. and Kennedy, J.F. (2001). The potential of oil and sago palm trunk wastes as carbohydrate resources. Wood Science and Technology. 35: 467 – 473.

Alañón, M.E., Rubio, H., Díaz-Maroto, M.C. and Pérez-Coello, M.S. (2010). Monosaccharide anhydrides, new markers of toasted oak wood used for ageing wines and distillates. *Food Chemistry*. 119 (2): 505 – 512.

Ayhan Demirba. (2004). Linear equations on thermal degradation products of wood chips in alkaline glycerol. *Energy Conversion and Management.* 45(7-8): 983-994.

Beguin, G., and Aurbert J.P. (1994). The biological degradation of cellulose, *FEMS Microbiology*. 13: 8 – 25.

Castellanos, O.F., Sinitsyn, A.P., and Vlasenko, E.Y. (1995). Comparative Evaluation of hydrolytic efficiency toward microcrystalline cellulose of *Penicillium* and *Trichoderma* cellulases. *Bioresource Technology*. 52: 119 – 124.

Chapin, F.S., Matson, P. and Mooney, H. (2002). *Principles of terrestrial ecosystem ecology.* New York: Springer – Verlag.

Chen, M., Zhao, J., Xia, L. (2008). Enzymatic hydrolysis of maize straw polysaccharides for the production of reducing sugars. *Carbohydrate Polymers*. 71: 411–415

Cherry, J.R. and Fidantsef, A.L. (2003). Directed evolution of industrial enzymes. Current Opinion in Biotechnology. 14: 438 – 443.

Chin, K.L., H'ng, P.S., Wong, L.J., Tey, B.T., Paridah, M.T. (2010). Optimization study of ethanolic fermentation from oil palm trunk, rubberwood and mixed hardwood hydrolysates using *Saccharomyces cerevisiae. Bioresource Technology.* 101: 3287–3291.

Darah S.I. and Omar I. (2011). Utilization of palm kernel cake for the production of mannanase by an indigenous filamentous fungus, *Aspergillus Niger* USM f4 under solid substrate fermentation. *The Internet Journal of Microbiology.*9(1).

Ehrenthal, Irving, Miner, Keith, E. (1980). Immobilization of glucose isomerase. United States Patent 4208482 enzyme and biomass utilization. *African Journal of Biotechnology*. 8 (11): 2418-2422.

Fang, H., Zhao, C., Song, X.S. (2010). Optimization of enzymatic hydrolysis of steam exploded corn stover by two approaches. *Bioresource Technology.* 101: 4111 – 4119.

Farzad, D., Rahman, S.A., Massood, A. and Lida P. (2006). Application of response surface methodology for modeling the enzymatic assay of hydrogen peroxide by Emerson – Trinder reaction using 4-iodophenol. *Intern. J. Environ. Anal. Chem.* 86(15): 1151 – 1163.

Hasmah, B., Mahiran, B., Salina, M.R., Arbakariya, A., Raja Noor Zaliha, R.A.M., Abu Bakar, S. (2009). Optimization of lipase-catalyzed synthesis of palm amino acid

Hong, L.S., Ibrahim, D. and Omar, I.C. (2011). Lignocellulolytic materials – as a raw materials for the production of fermentable sugars via solid state fermentation. *Asian Journal of Scientific Research*. 4(1): 53 – 61.

Ibeto, C.N., Ofoefule, A.U. and Agbo, K.E. (2011). A global overview of biomass potentials for bioethanol production: A renewable alternative fuel. *Trends in Applied Sciences Research.* 6(5): 410 – 425.

Klein, J., Maia, J., Vicente, A.A., Domingues, L., Teixeira J.A., Juraŝĉĩk, M. (2005). Relationships between hydrodynamics and rheology of flocculating yeast suspensions in a high cell density airlift bioreactor. *Biotechnology Bioengineering*. 89: 393 – 402.

Kosugi, A., Tanaka, R., Magara, K., Murata, Y., Arai, T., Othman, S., Rokiah, H., Zubaidah Aimi, A.H., Mohd Khairul, A.Z., Mohd Nor, M.Y., Wan Asma, I., Mori, Y. (2010). Ethanol and lactic acid production using sap squeezed from old oil palm trunks felled for replanting. *Journal of Bioscience and Bioengineering*. 110 (3): 322–325.

Li, X.H., Yang, H.J., Roy, B., Wang, D., Yue, W.F., Jiang, L.J., Park, E.Y., and Miao, Y.G. (2009). The most stirring technology in future: Cellulase

Liao, X., Raghavan, G.S.V., Dai, J. and Yaylayan, V.A. (2003). Dielectric properties of α-D-glucose aqueous solutions at 2450 MHz. *Food Research International* 36(5): 485-490.

Miller, G.L. (1959). Use of dinitrosalicyclid acid reagent for determination of reducing sugar. *Analytical Chemistry.* 31: 426.

Obeng, D.P., Morrell, S. and Napier-Munn, T.J. (2005). Application of central composite rotatable design to modeling the effect of some operating variables on the performance of the three-product cyclone. *International Journal of Mineral Processing*. 76(3): 181 – 192.

Pekka Maijala. (2000). Heterobasidion annosum and wood decay: Enzymology of cellulose, hemicelluloses, and lignin degradation. *Department of Biosciences, Division of Plant Physiology University of Helsinki.*

Purwanto, L.A., Ibrahim, D. and Sudrajat, H. (2009). Effect of agitation speed on morphological changesin *Aspergillus niger* hyphae during production of tannase. *World Journal of Chemistry.* 4 (1): 34-38.

Raissi, S and Farsani R.E. (2009). Statistical process optimization through multi-response surface methodology. *World Academy of Science, Engineering and Technology*. 51.

Riddhi, S. (2007). Glucose usage in industry. *Scribd Collection*. In published. 1 – 45.

Roberto, I.C., Mussatto, S.I., Rodrigues, R.C.L.B. (2003). Dilute acid hydrolysis for optimization of xylose recovery from rice straw in a semi pilot reactor. *Industrial Crops and Products*. 17: 171 – 176.

Shide, E.G., Wuyep, P.A., and Nok, A.J. (2004). Studies on the degradation of wood sawdust by *Lentinus squarrosulus* (Mont.) Singer. *African Journal of Biotechnology* . 3 (8): 395-398.

Siti Aminah, S., Aidil, A.H. and Wan Mohtar, W.Y. (2006). Medium optimization for the production of lipidless biomass by *Cunninghamella* sp. 2A1 using response surface methodology. *Malaysian Journal of Microbiology*. 2(1): 40 – 45.

Suhaimi, M. and Ong, H.K. (2001). Composting Empty Fruit Bunch of oil palm. Retrieved from *http://www.fftc.agnet.org*.

Sun, R.C., Fang, J.M., Tomkinson, J., Geng, Z.C., Liu, J.C. (2001). Fractional isolation, physico-chemical characterization and homogeneous esterification of hemicelluloses from fast-growing poplar wood. *Carbohydrate Polymers*. 44: 29–39.

Suraini Abd Aziz (2002). Sago starch and its utilisation, *Journal of Bioscience and Bioengineering*. 94 (6): 536 – 529.

Vlasenko, E.Y., Ding, H., Labvitch, J.M. (1996). Enzymatic hydrolysis of pretreated rice straw. Bioresource Technology. 59: 109 – 119.

Xu, Z., Wang, Q., Jiang, Z., Yang, X. (2007). Enzymatic hydrolysis of pretreated soybean straw. *Biomass and Bioenergy* 31: 162-167.

Zetelaki, K. and Vas K. (1970). The role of aeration and agitation in the production of glucose oxidase in submerged culture. *Biotechnology and Bioengineering*. 12(3): 379 -97.

APPENDIX A

METHODOLOGY

Standard Calibration Curve Preparation

Table A.1: Reading from UV-Visible Spectrophotometer

Concentration, mg/mL	Optical density, OD
0	0
0.2	0.057
0.4	0.279
0.6	0.395
0.8	0.429
1	0.779

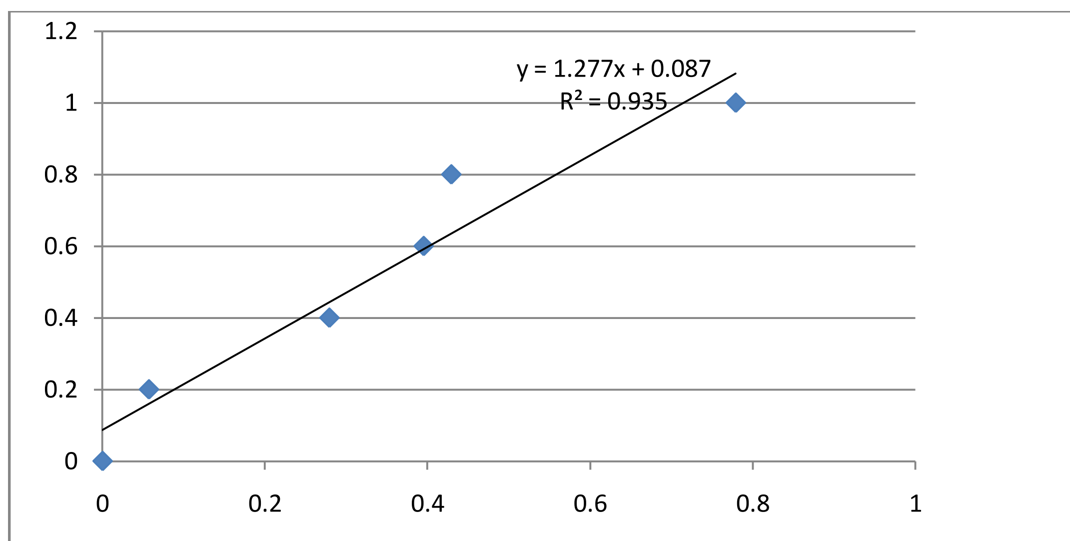

Figure A.1: Graph of OD versus concentration of glucose

APPENDIX B

RESULT AND DISCUSSIONS

ANOVA analysis

Table B.1: ANOVA for response surface quadratic model

Source	Sum of squares	Degree of Freedom	Mean Square	F Value	Prob > F	
Model	0.47	9	0.052	11.70	0.0003	Significant
A	0.20	1	0.20	45.24	<0.0001	
B	0.051	1	0.051	11.49	0.0069	
C	0.042	1	0.042	9.33	0.0122	
A^2	0.010	1	0.010	2.31	0.1596	
B^2	7.158E-003	1	7.158E-003	1.61	0.2334	
C^2	0.12	1	0.12	27.44	0.0004	
AB	0.026	1	0.026	5.81	0.0367	
AC	3.340E-003	1	3.340E-003	0.75	0.4065	
BC	8.349E-004	1	8.349E-004	0.19	0.6740	
Residual	0.044	10	4.449E-003			

Lack of Fit	0.032	5	6.303E-003	2.43	0.1761	Not significant
Pure error	0.013	5	2.594E-003			
Correlation Total	0.51	19				

Calculation of R^2 value

$$R^2 = 1 - \frac{\text{Residual Sum of Squares}}{\text{Correlation Total}}$$

$$= 1 - \frac{0.044}{0.51}$$

$$= 0.9137$$

Calculation of Root Mean Square Error (RMSE)

$$\text{RMSE} = \sqrt{\text{Residual Mean Square}}$$

$$= \sqrt{4.449\text{E-}003}$$

$$= 0.07$$

Optimization of Glucose Production by Experimental Design

Table B.2: Optimization condition for glucose production

Name	**Goal**	**Lower Limit**	**Upper Limit**
Reaction time	is in range	5.66	19.3379
Temperature	is in range	28.04	36.96
Agitation	is in range	140.54	259.46
Solutions Numbers	**Reaction time***	**Temperature***	**Agitation***
1	10.41	30.45	225.20

$$\text{Percentage of error} = \frac{\text{Actual value} - \text{Predicted value}}{\text{Actual value}}$$

$$= \frac{0.983454 \text{ mg/mL} - 0.517333 \text{ mg/mL}}{0.983454 \text{ mg/mL}} \times 100\%$$

$$= 47.4\%$$