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BORANG PENGESAHAN STATUS TESIS♦

JUDUL : PRODUCTION OF GLUCOSE FROM BANANA STEM WASTE USING *STRAIN A*

SESI PENGAJIAN : 2009/2010

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PRODUCTION OF GLUCOSE FROM BANANA STEM WASTE USING *STRAIN A*

NURHASIKEN BINTI RAMAN

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

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UNIVERSITI MALAYSIA PAHANG

APRIL 2010

I declare that this thesis entitled "*Production of Glucose from Banana Stem Waste Using Strain A*" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : ……..
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To my beloved parent, lecturer and myself

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ABSTRACT

Fermentable sugars are the largest feedstock available to support bio-based chemicals industry. Growth of a bio-based chemicals industry will depend on production of fermentable glucose. Production of this glucose from banana stem waste can help to reduce the environmental problem. This study is studying glucose production from banana stem waste using *Strain A*. While, the purposes of this study are to study the effect of organic loading rate (OLR) during production of glucose, to optimize the glucose production using banana stem waste and to study suitability of banana stem waste as substrate for glucose production using *Strain A*. Organic loading rate for this study is determined by using Design Expert. The banana stem waste is used as substrate and contains 27.64% of total solid. The experiment used fed batch fermentation. The fermented mixture were removed 50 mL and after that, 50 mL of fresh substrate is added back to shake flask. This experiment be done for eight runs with different values of OLR (g L^{-1} d^{-1}); 5, 11.25, 17.5, 23.75, and 30. The experiment was also carried out for 30 days. The concentration of glucose is analyzed using DNS assay. The optimization of glucose production is determined using Design Expert through One Factor Analysis. The increasing yield of glucose is affected by decreasing the value of OLR. The optimum OLR is 5 g L^{-1} d^{-1} was produced the maximum of yield of glucose and it is 0.0734 g/g substrate. The banana stem waste is suitable substrate in production of glucose using biological hydrolysis process and *Strain A*.

ABSTRAK

Glukosa yang diperolehi daripada fermentasi adalah bahan utama yang tersedia untuk menyokong industri bahan kimia berasaskan bio. Pertumbuhan industri bahan kimia berasaskan bio bergantung pada penghasilan glukosa. Penghasilan glukosa ini dari sisa batang pisang dapat membantu mengurangkan masalah pencemaran. Kajian ini mengkaji penghasilan glukosa dari sisa batang pisang menggunakan *Strain A*. Manakala, tujuan kajian ini adalah untuk mengkaji kesan kadar beban organik (OLR) terhadap penghasilan glukosa, mengoptimumkan penghasilan glukosa menggunakan sisa batang pisang dan mengkaji kesesuaian sisa batang pisang sebagai substrat untuk penghasilan glukosa menggunakan *Strain A*. Kadar beban organik untuk kajian ini ditentukan dengan menggunakan Design Expert. Sisa batang pisang yang digunakan sebagai substrat mengandungi 27.64% dari jumlah keseluruhan pepejal. Eksperimen ini mengunakan teknik penapaian secara suapan berkelompok. Substrat yang telah ditapai dikeluarkan sebanyak 50 mL dan selepas itu, 50 mL substrat yang baru akan dimasukkan kembali ke dalam tempat penapaian. Penapaian ini dijalankan sebanyak lapan kali dengan nilai kadar beban organik (OLR) yang berbeza-beza iaitu 5 g L^{-1} h^{-1}, 11.25 g L^{-1} h^{-1}, 17.5 g L^{-1} h^{-1}, 23.75 g L^{-1} h^{-1}, dan 30 g L^{-1} h^{-1}. Kajian ini juga dijalankan selama 30 hari. Kepekatan glukosa dianalisa menggunakan kaedah DNS. Penghasilan glukosa yang optimum ditentukan dengan menggunakan Design Expert melalui Analisis Satu Faktor. Peningkatan dalam penghasilan glukosa dipengaruhi oleh penurunan nilai OLR. Nilai OLR yang optimum adalah 5 g L^{-1} h^{-1} telah menghasilkan maksimum glukosa dan nilainya adalah 0.0734 g / g substrat. Sisa batang pisang adalah substrat yang sesuai dalam penghasilan glukosa melalui proses hidrolisis secara biologi dan *Strain A*.

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LIST OF ABBREVIATIONS/ SYMBOLS

[glucose]	-	Concentration of glucose
[substrate]	-	Concentration of substrate
˚ C	-	Degree Celsius
CSTR	-	Continuous Stirrer Reactor
d	-	Day
DNS	-	Dinitrosalicyclic Acid Reagent
DP	-	Degree of Polymerization
g	-	Gram
h	-	Hari
hr	-	Hour
HRT	-	Hydraulic retention time
L	-	Liter
mL	-	Milliliter
nm	-	Nanometer
OD	-	Optical density
OLR	-	Organic loading rate
TS	-	Total solid
w/v	-	Weight per volume

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CHAPTER 1

INTRODUCTION

1.1 Background

Glucose is an organic compound and known as monosaccharide (a simple sugar). It is also known as grape sugar, blood sugar, or corn sugar. Its molecular formula is $C_6H_{12}O_6$ and contains aldehyde group. When glucose units in long chains form, it is called polysaccharides. The types of polysaccharides are cellulose, glycogen and starch. Glucose is commonly available in the form of a white substance or as a solid crystal. It can also be dissolved in water as an aqueous solution.

Glucose is produced commercially via the hydrolysis, enzymatic hydrolysis of starch or biomass waste (Moshe and Reese, 1968) and fermentation. The sources of starch are sugarcane bagasse (Martin *et al*., 2007), rice (Yanez *et al*., 2006), wheat, cassava, corn husk (Ohgren *et al.*, 2007), sago and all source of organic. Agriculture waste also widely used in the production of glucose as alternative sources.

In nature, many microorganisms such fungus or bacteria can degrade the cellulosic materials. Microorganisms (single culture) that used to degrade the cellulosic material have been well studied (Hayashi *et al.*, 1999, Del Re *et al.*, 2003, Lo *et al.*, 2008). Mixed cultures also have been well studied to degrade the cellulose (Lewis *et al.*, 1988). The process to degrade the cellulose by microorganisms is called bacterial hydrolysis or biological hydrolysis. This hydrolysis could degrade the cellulosic materials aerobically and anaerobically (Lo *et al.*, 2008).

Fermentation is the process formed of energy by the process of oxidation of organic compounds like carbohydrates and sugars. Anaerobic fermentation is fermentation that carried out without the presence of oxygen. While, when the fermentation is carried out with oxygen, it is commonly known as aerobic fermentation. Each microorganism has its condition of fermentation. For example, when use microbe from soil, the anaerobic fermentation must be applied because the soil fungi are facultative anaerobic organisms able to change their energy metabolisms depending on aeration conditions (Tetsubin *et al.*, 2003).

1.2 Problem Statement

Nowadays, many people and industries were aware about environmental problem. The agriculture waste or biomass is one of the problems to environmental. The amount of agriculture waste was increases by days and causes the serious problem to environment. With increasing environmental awareness, the conversion of biomass and agriculture waste into chemical is receiving an increased interest.

Biomass and agriculture waste can convert into any bio- product, but before that, the waste need convert to glucose. Fermentable sugars are the largest feedstock available to support bio-based chemicals industry. Growth of a bio-based chemicals

industry will depend on production of fermentable glucose. Fermentable glucose is also used in foods, medicine, brewing, and wine making and as the source of various other organic chemicals.

Production of this glucose from banana stem waste is the good chosen. It is because banana stem waste is cheap (low cost) and easy to find in Malaysia. Banana is covering about 26,000 hectares with a total production is 530,000 metric tones in Malaysia. Banana is a most popular fruit and has received demand for food industries. But, banana stem from banana tree will be the waste and became the environmental problem.

1.3 Objective of The Study

The main objectives of this research are:-

1. To study the effect of Organic Loading Rate (OLR) during production of glucose.
2. To optimize the glucose production using banana stem waste.
3. To study suitability of banana stem waste as substrate for glucose production using *Strain A*.

1.4 Scopes of Study

In order to achieve the objectives, the equation of organic loading rate has been identified. The equation of OLR is related to concentration of substrate (banana stem waste) and hydraulic retention time (HRT). The equation of HRT contains

volume of reactor and flow rate. The type of reactor that use in this study is sequencing batch reactor (fed-batch reactor). The value of volume of reactor is 5 liters will used and flow rate will fix, so that HRT will be fixed. The value of OLR is varied. Then, the value of concentration of substrate will get with using equation OLR and the other method is using the Design Expert. Total solid (TS) test will used to make sure the substrate has similar moisture for each run of experiment. The other scope is type of substrate. The banana stem waste will use as substrate in this study. The method to measure concentration of glucose in this study is DNS assay. Design Expert also used to optimize the glucose production using method One Factor Analysis. The suitability of banana stem waste as substrate is determined from glucose that produced and compared it with substrate that used from previous study.

CHAPTER 2

LITERATURE REVIEW

2.1 Fermentable Glucose/ Fermentable Sugar

2.1.1 Properties of Fermentable Glucose

Fermentable glucose is known as glucose or simple sugar. Glucose is an example of a carbohydrate. Molecular formula and molar mass of glucose is $C_6H_{12}O_6$ and 180.16 g/mol. Figure 2.1 shows the structure of glucose that contains six carbons and aldehyde group.

According Forest Encyclopedia (2008), fermentable sugars can be produced using crops and wastes from agriculture and forestry. The types of crop that always used to produce fermentable sugar are corn, wheat, potato, sugar beet, and sugarcane. Besides that, potato-processing residues, cane molasses, and apple pomace (Polman 1994).

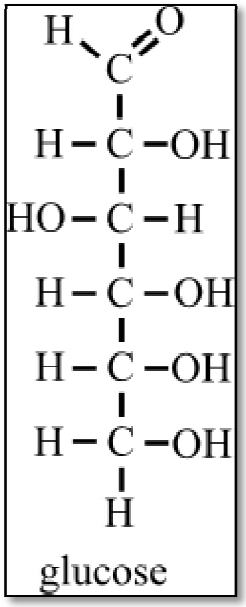

Figure 2.1: The Structure of glucose.

Sugars can be produced directly or derived from polysaccharides such as cellulose and starch and then, via microbial fermentation to produce a wide range of others chemicals. Glucose is produced commercially via the enzymatic hydrolysis of starch. Besides that, cellulose can be hydrolyzed by acid to glucose, although much of the glucose is destroyed during this process.

2.1.2 Application of Fermentable Glucose

Glucose is used as a precursor for the synthesis of several important substances. According Wikipedia, glucose is a precursor for vitamin C (ascorbic acid) production in plants and most animals. In the industry, glucose is also used as a precursor to make vitamin C in the Reichstein process, to make citric acid, gluconic acid, bio-ethanol, polylactic acid and sorbitol.

Besides that, fermentable glucose is used to produce bio-chemical product. It is proven by Forest Encyclopedia (2008) that stated fermentable sugars are the largest feedstock available to support a bio-based chemicals industry. Existing commercial fermentation primarily utilizes glucose to produce ethanol, acetic acid, amino acids, antibiotics, and other chemicals.

2.2 Source of Substrate

Fermentable glucose can be derived by fermentation process from any material that contains celluloses and hemicelluloses. The many and varied raw materials used in the manufacture of fermentable glucose via hydrolysis and fermentation. The most substrate that used to produce fermentable glucose is starch, food waste and agriculture waste.

2.2.1 Starch

Starches are complex sugars that can break down into one of the simple sugars (maltose). Since starches do not taste very sweet, they do not jump to mind when sugar is mentioned, but they quickly become the simple sugar maltose, and then the simple sugar glucose because the breakdown of starch from the complex sugar form to the simple sugar form is quick and easy. Essentially, starches are sugars that merely require a few more steps to make them into glucose.

Figure 2.2 was shown the structure of starch that made up of repeated structure of glucose.

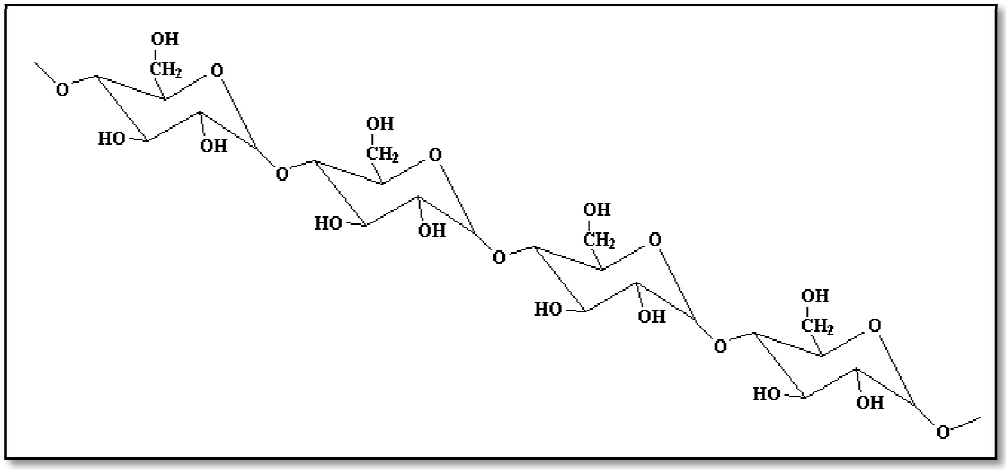

Figure 2.2: The structure of starch.

Starch is found in potatoes, and in grains such as corn and wheat. Besides that, many crops can be used as the source of starch. The example crops that can be used as the source of starch are maize, rice, wheat, potato, cassava, arrowroot, and sago. In the United States, corn starch (from maize) is used almost exclusively. But in Malaysia, cassava (tapioca) starch and sago starch is always used as substrate to produce fermentable glucose and bio-chemical.

Starch also used as source of food to human and animal. Besides that, starch is used in pressing clothes to keep them from wrinkling and to make a foam packing.

2.2.2 Food Waste

Food waste is a kind of organic waste discharged from households, cafeterias and restaurants, and accounts for a considerable proportion of municipal solid garbage. Figure 2.3 shown seven percent of waste is municipal solid waste. It is still be a problem to environmental.

Many researches were done to study the conversion of food waste. Kim *et al*. (2003) researched about conversion of food waste into lactic acid and reported that the lactic acid concentration in the medium of food waste could reach 80 g/L after 48 hr under the catalysis of a commercial enzyme mixture. In previous works, Kim *et al*. had developed a bioprocess for the lactic acid production from food waste, but the lactic acid concentration was still below 30 g/L when the process did not use any commercial enzyme.

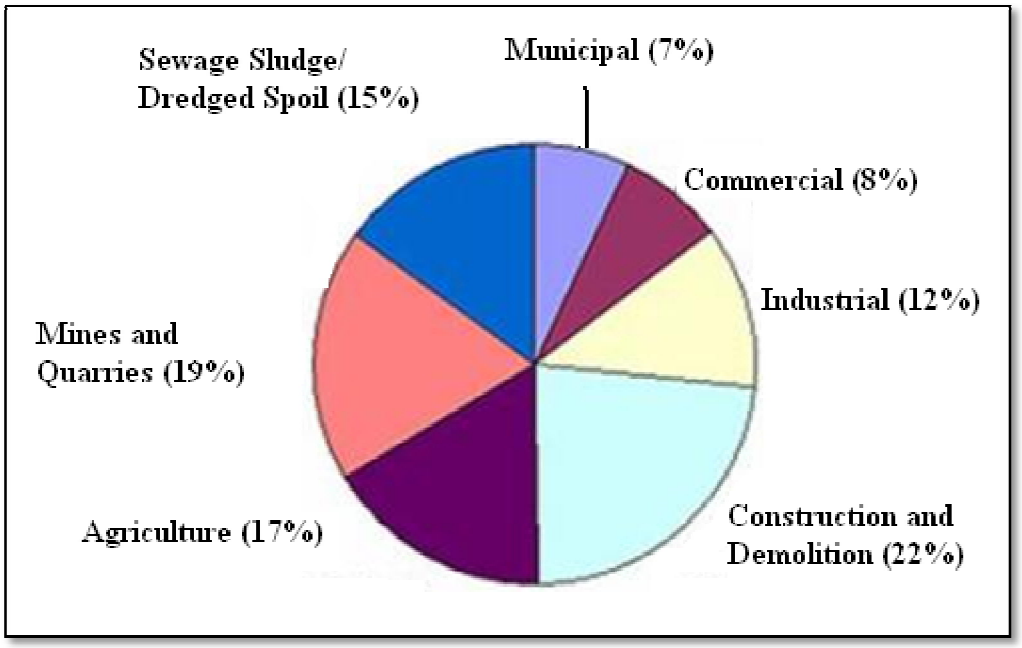

Figure 2.3: Type of waste in global.

2.2.3 Agriculture waste

Agricultural waste is one of the largest segments of the nationwide waste problem. Figure 2.3 was shown the 17% of waste in global is agriculture waste. Agricultural wastes include both natural (organic) and non-natural. The large volumes of agricultural waste threaten surface water and groundwater quality in the event of waste spills, leakage from waste storage facilities, and run off from fields on

which an excessive amount of waste has been applied as fertilizer. Agriculture waste also is an environmental problem issues that need to solve quickly.

The organic in agricultural waste contains cellulose and hemicelluloses that can convert into the fermentable glucose and bio-chemical product. Many researches were being done to study the conversion of agriculture waste into valuable product.

Saccharification of banana agro waste by cellulases of *Trichoderma lignorum* was investigated by Baig *et al*. (2004). Banana is major cash crop of this region generating vast agricultural waste after harvest. The agro waste including dried leaves and psuedostem after harvest was used as substrate for the release of sugars (Baig *et al.*, 2004**).** Banana fruit stalk abundantly available in banana production fields and markets appears to be a favorable substrate as it is cheaply available in the tropical and subtropical countries and has a cellulose content of 23.85% (Krishna, 1996).

2.2.4 Selection of Substrate

Over the long term, new sources of glucose will be required to meet the demands of a bio-based industry. Growth of a bio-based chemicals industry will depend on substrate that contains cellulose. Based on list of substrate, agricultural waste (banana waste) has been chosen as source of substrate in this study. Banana waste was been chosen because it is easy to find in Malaysia. Selection of banana waste is based on to reduce environmental problem. Besides that, it is better not to disturb source of food such as starch.

2.3 Hydrolysis Process

2.3.1 Enzymatic Hydrolysis

Enzymatic hydrolysis is widely use in production of fermentable glucose. This hydrolysis uses enzymes that produced by a variety of microorganisms. Those enzymes must capable to break down lignocellulosic material and convert it to fermentable glucose. The advantage of this hydrolysis is does not having the same problem with the acid hydrolysis that causes corrosion of equipment. Besides that, enzymatic hydrolysis using mixtures of enzymes, such as cellulase and hemicellulaces, is used to avoid the destruction of sugars associated with acid treatments (hydrolysis) of lignocellulosic material. These enzymes, when combined with effective pretreatment of lignocellulosics, provide high yields of glucose, xylose, and other fermentable sugars with minimal sugar losses.

In addition, this hydrolysis has low utility cost compared to acid hydrolysis and low environmental conditions (Sun and Cheng, 2002 and Puwardi, 2006). However, these enzymes are currently too costly to use in large-scale conversion of lignocellulosic materials to fermentation substrates. This hydrolysis also requires longer retention time and the rate of hydrolysis is very slow (Puwardi, 2006 and Kumar *et al*., 2009).

Basically, there are two major processes involve in enzymatic hydrolysis; liquefaction and saccharification. Aggarwal *et al*. (2001) use this hydrolysis to convert starch to glucose. Aggarwal *et al* liquefaction under pressurized steam and found that technique more effective than using water bath.

While, saccharification is refers to production of fermentable sugar from polysaccharides (Dunson *et al*., 2007). The saccharification was improved with the increasing enzymes unit. Effect of addition of divalent ions on the process of

saccharification was studied by the addition of calcium chloride, magnesium and zinc sulphate to provide these ions in the range of 25-250 mg/l. Results obtained for the level of saccharification in presence of these ions (Aggarwal *et al*., 2001).

In many cases relatively high doses of glucoamylases and other maceration enzymes besides amylases such as xylanase, cellulose and pectinase are necessary to saccharify various starches containing substrates efficiency. Moreover, the efficiency of an enzymatic starch saccharification process depends on the activity of the glucoamylase and also on the purity of enzyme (Aggarwal *et al*., 2001).

2.3.2 Acid Hydrolysis

Initially, acid hydrolysis appears to be a relatively efficiently means of accessing and breaking down cellulose. The hydrogen ion, therefore, does not face the problem of accessibility compared to cellulose enzymes.

Initial hydrolysis rates are typically very rapid performed experiments to show that in the initial stages of the hydrolysis reaction, larger pore volumes do correspond to faster reaction rates. However, after limited hydrolysis, the reaction rate slows down considerably. The glycosidic bonds most susceptible to hydrolysis are those either at the surfaces or in the amorphous region of cellulose. Rapid hydrolysis rates reflect hydrolysis activity in these regions and can be seen as a decrease in the degree of polymerization (DP) from several thousand to about 200.

The cellulose can then be rapidly hydrolyzed at low temperature to avoid degradation, making almost quantitative yield of glucose attainable. However, in the process, high capital cost is an avoidable because of expensive corrosion resistant

equipment, acid recovery plants and higher operation costs. Moreover, one of the major problems with hydrolyzates produced by acid hydrolysis is the poor ferment ability caused by the presence of inhibitors in the hydrozylates. Furfural is known to be one of the most important of these inhibitors. It is a breakdown product from pentose and is formed in a browning reaction during hydrolysis in the presence strong acids. It therefore may be impossible to completely avoid furfural formation in a chemical hydrolysis process designed to give a high sugar yield (Taherzadeh *et al*., 1999).

2.3.3 Biological Hydrolysis

Waren (1996) was written in his paper that microorganisms are efficient degraders of starch, chitin, and the polysaccharides in plant cell walls. Biological hydrolysis uses the microorganisms to degrade, so, it different with enzymatic hydrolysis. This hydrolysis is always used in waste treatment. While, Chen *et al*. (2009) use this hydrolysis to degrade the starch so that, it can convert starch to glucose.

Biological treatment using various types of rot fungi, a safe and environmentally friendly method, is increasingly being advocated as a process that does not require high energy for lignin removal from a lignocellulosic biomass, despite extensive lignin degradation (Okano *et al*., 2005). In biological pretreatment processes, microorganisms such as brown-, white-, and soft-rot fungi are used to degrade lignin and hemicellulose in waste materials (Galbe and Zacchi, 2007).

Brown rots mainly attack cellulose, whereas white and soft rots attack both cellulose and lignin. Lignin degradation by white-rot fungi occurs through the action of lignin-degrading enzymes such as peroxidase and laccase (Lee *et al*., 2007).

These enzymes are regulated by carbon and nitrogen sources. White-rot fungi are the most effective for biological pretreatment of lignocellulosic materials. Hatakka *et al*. (1983) studied the pretreatment of wheat straw by 19 white-rot fungi and found that 35% of the straw was converted to reducing sugars by *Pleurotus ostreatus* in five weeks.

The advantage of this hydrolysis is only requiring low energy to degrade the starch, lignin and hemicelluloses (Kumar *et al*., 2009). This hydrolysis also has mild environmental condition.

Biological hydrolysis is defined as the rate limiting step in anaerobic digestion because this hydrolysis can reduce the impact of rate limiting step, pretreatment systems such as thermal, alkaline, ultrasonic and mechanical disintegration systems (Park *et al*., 2005, and Tiehm *et al*., 2001). Besides that, this hydrolysis have a low rate of hydrolysis but this method involves relatively cheaper maintenance costs and suitable for large scale treatment (Park *et al*., 2005).

2.3.4 Selection of Hydrolysis Process

This study will be use biological hydrolysis. This process has been chosen because it is can reduced the cost and suitable for large scale treatment. Enzymatic hydrolysis will give the high yield of sugar but this process is very expensive. Besides that, biological hydrolysis is efficient to degrade the starch, chitin, and the polysaccharides in plant cell walls. It can reduce the usage of other pretreatment method.

2.4 Bioreactor

Fermentations can be operated in batch, fed-batch or continuous bioreactors.

2.4.1 Batch Bioreactor

In batch bioreactor all components, except gaseous substrates such as oxygen, pH-controlling substances and antifoaming agents, are placed in the reactor in the beginning of the fermentation. During process there is no input nor does output flow. Batch bioreactors have several advantages over continuous flow reactors. The advantage is the fermentation can be stopped between batches, so the production rate is flexible and can be varied if economically desirable.

The other advantages are batch bioreactors are also more flexible, in that one can easily use different compositions in different batches to produce products with different specifications and if the reactants are stirred, a batch bioreactor can often achieve better quality than a plug flow reactor and better productivity than a continuous flow reactor.

Batch bioreactor cannot achieve the steady state condition. It will cause wrong interpretation of the results for the full scale implementations (Ucisik and Henze, 2008).

2.4.2 Continuous Stirrer Bioreactor

Continuous stirrer bioreactor always operated at steady state. The characterization continuous stirrer bioreactor (CSTR) is run at steady state with continuous flow of reactants and products; the feed assumes a uniform composition throughout the reactor, exit stream has the same composition as in the tank.

This reactor commonly used in industry processing. Besides that, this type of reactor is used in the real life fermentation application (Ucisik and Henze, 2008). The advantages of CSTR are easy to clean, low operating (labor) cost, easily adapts to two phase runs and it is good temperature control. The disadvantages of CSTR are poor agitation and lowest conversion per unit volume (Fogler, 2006).

2.4.3 Fed Batch Bioreactor

Bushan and Joshi (2004) use this type of bioreactor for produce baker's yeast. This reactor is very popular in the ethanol production. In fed-batch process, the substrate or medium or product will removed from the reactor for few days. Then, the fresh substrate is added in order to control the reaction rate by its concentration. The remove and added process is based on hydraulic retention time (HRT). There are both input and output flows in a continuous process, but the reaction volume is kept constant. In addition, fed batch is using to enhance the biological hydrolysis (Chen *et al*., 2009).

Fed-batch bioreactors are widely used in industrial applications because they combine the advantages from both batch and continuous processes. Process is at first started as a batch process, but it is exhibited from reaching the steady state by

starting substrate feed once the initial glucose is consumed. Besides that, this type of reactor can create anaerobic condition and recover higher concentration of fermentable glucose.

Fed-batch offers many advantages over batch and continuous cultures. The production of by-products that are generally related to the presence of high concentrations of substrate can also be avoided by limiting its quantity to the amounts that are required solely for the production of the biochemical.

When high concentrations of substrate are present, the cells get overloaded, this is, the oxidative capacity of the cells is exceeded, and due to the Crabtree effect, products other than the one of interest are produced, reducing the efficacy of the carbon flux. Moreover, these by-products prove to even contaminate the product of interest, such as ethanol production in baker's yeast production, and to impair the cell growth reducing the fermentation time and its related productivity.

2.4.4 Selection of Bioreactor

Fed batch bioreactor has been chosen as bioreactor in this study. It is because this reactor is combination concept of batch reactor and continuous flow reactor. So, fed batch reactor can reduce the contamination, less time for fermentation and low cost. Besides that, fed batch widely used in industrial application. Fed batch process always has constant reaction volume and can avoid the problem of limited substrate.

CHAPTER 3

METHODOLOGY

3.1 Substrate

Banana stem wastes used in this study were collected from a local farmer at banana plantation in Sabak Bernam, Selangor, Malaysia. This waste has 27.6% of total solid. That stem was dried and it was formed in powder using blender. That stem was going through total solid test to determine concentration of substrate. After that, the banana stems were stored in the bottle and close it tightly to avoid contamination. Before used in fermentation, the banana stem wastes need to autoclave first.

Figure 3.1: The banana stem waste.

Figure 3.2: Step of blending the banana stem.

Figure 3.3: The banana stem after autoclave.

3.2 Microorganisms and Inoculums Preparation

Strain A was used for production of glucose or reducing sugar is got from the previous research. That strain was isolated from soil at banana plantation in Pahang, Malaysia. It was maintained and stored on agar slants at four degree Celsius. It was cultured using nutrient agar. After cultured for a week at 30°C, the *Strain A* was collected and diluted to be suspension by sterile water (Wang *et al*., 2008). After that, the strain suspension was used as inoculums. Inoculums (10^2-10^3 cells/mL) was added at the rate of one percent to initiate the fermentation (Bushan and Joshi, 2005).

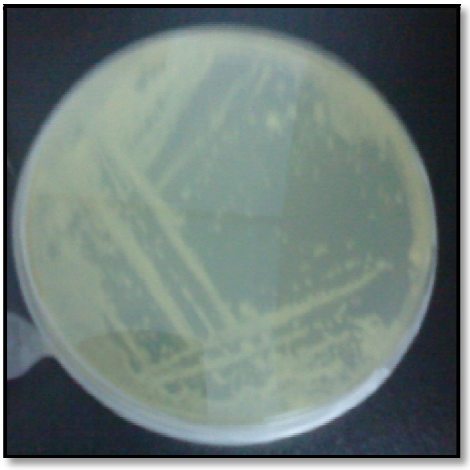

Figure 3.4: *Strain A* on nutrient agar.

Figure 3.5: Inoculums of *Strain A.*

3.3 Material Characterization

The moisture of substrate (banana stem waste) was determined by Total Solid (TS) test and fermentation glucoses were estimated by the dinitrosalicylic acid method using D-glucose as the standard. Organic loading rate (OLR) and concentration of substrate is determined using the equation below:-

$$OLR = \frac{\text{Concentration of substrate}}{\text{Hydraulic retention time (HRT)}} \quad (3.1)$$

$$HRT = \frac{\text{Working volume of bioreactor}}{\text{Flow rate}} \quad (3.2)$$

$$\text{Concentration of substrate} = \frac{\text{Mass of substrate}}{\text{Working volume}} \quad (3.3)$$

3.4 Preparation of Dinitrosalicylic Acid Reagant

The 3,5-dinitrosalicylic acid (DNS) reagent were prepared with weigh and put ten grams of 3,5-dinitrosalicylic acid into a 2000 mL beaker. Then, 16 g sodium hydroxide, 300 g of potassium tartrate and 500 mL of water were added in the beaker that contains the DNS. The solution in the beaker was stirred to dissolve all compounds. Then, the contents of the beaker were transferred into 1000 mL volumetric flask. The water is added until the volume of reagent was reached 1000 mL. The reagent was stored at ambient temperature and can be use for ten weeks (Miller, 1959).

3.5 DNS Assays

Dinitrosalicylic acid (DNS) reagent of three milliliters was added to three milliliters of sample. Then the mixture was heated at 90 - 100°C for 15 minutes to develop the red brown color. After cooling to room temperature, the absorbance was recorded with UV-Vis (U-1800) spectrophotometer Hitachi at 540 nm. Calibration curve was got from standard was used to determine the fermentable sugar. The fermentable glucose yield is the amount of fermentable glucose measured in weight percent of the substrate.

Figure 3.6: Sample before DNS assay.

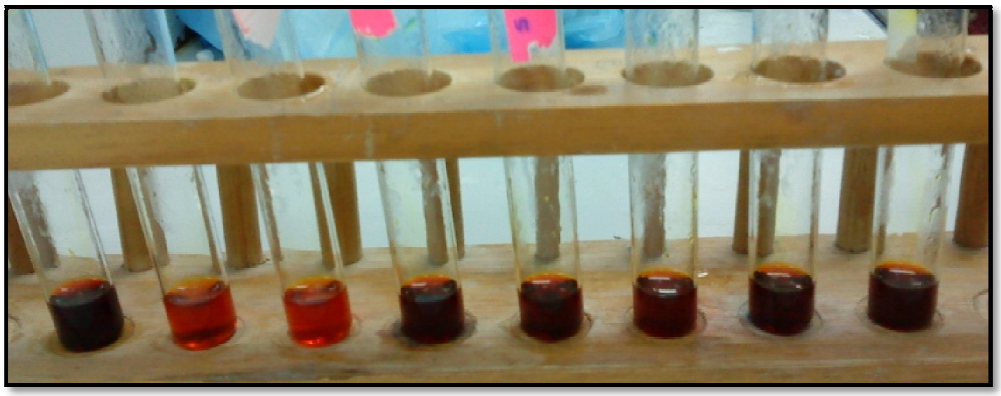

Figure 3.7: Sample after DNS assay.

Figure 3.8: UV-Vis (U-1800) spectrophotometer Hitachi

3.6 Total Solid Test

Total solid test were used to determine the amount of solids (or moisture) present in a solid biomass. The convection oven procedure was followed. Accurately weigh the pan and the weight of the pan was recorded. The sample was chopped and weighed into the weighing pan. The weight of the sample with pan was recorded. The sample was placed into a convection oven at 105°C ± 3°C and dry to constant weight for three hours. The sample was removed from the oven and cold it to the room temperature. The pan containing the oven-dried sample was weighed and the weight is recorded (Sluiter *et al.*, 2008). Each sample was run two or three times. The total solid and moisture of substrate was calculated by using the following formula:-

$$\text{mg Total Solids} = (\text{Weight}_{\text{dry pan plus dry sample}} - \text{Weight}_{\text{dry pan}}) \qquad (3.4)$$

$$\%\ \text{Total Solids} = \frac{(\text{Weight}_{\text{dry pan plus dry sample}} - \text{Weight}_{\text{dry pan}})}{\text{Weight}_{\text{sample before dry}}} \times 100 \qquad (3.5)$$

% Moisture = 100 - % Total Solids (3.6)

3.6 Experimental Procedure

Fermentation was performed in 250 mL Erlenmeyer flask containing a 150 mL mixture of 5-10% (w/v) of banana stem waste, tap water and *Strain A* and this fermentation was occurred in fermentative anaerobic condition. The amount of banana stem waste in that mixture was depend on organic loading rate (OLR) which were determined before the experiment using Design Expert. The range of OLR (g L^{-1} d^{-1}) is 5 until 30. After that, it was incubated in a rotary shaker at 150 rpm and 30°C. The mode of fermentation is fed batch. After that, the mixture was fermented were let a day, the mixture were remove 50 mL. Then, 50 mL of fresh substrate where have same concentration with the remove mixture were added to that flask. The mixture that taken out was collected three milliliters to doing the DNS assays. Lastly, the all step of experimental procedure were repeated for 29 cycles.

Cloned View

View

	Std	Run	Block	Factor 1 A:OLR g/l.d	Response 1 glucose mg/l
	8	1	Block 1	30.00	
	2	2	Block 1	5.00	
	1	3	Block 1	5.00	
	4	4	Block 1	17.50	
	6	5	Block 1	23.75	
	3	6	Block 1	11.25	
	5	7	Block 1	17.50	
	7	8	Block 1	30.00	

Figure 3.9: Organic loading rate (OLR) for each run.

Figure 3.10: Step to transfer inoculums into shake flask that contains substrate.

Figure 3.11: Banana stem, water and inoculums in shake flask.

Figure 3.12: Step of purging nitrogen into shake flask.

Figure 3.13: Shake flasks in incubator shaker.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Calibration Curve of Concentration of Glucose

Calibration curve is using to determine the concentration of a glucose in a sample of banana stem waste after fermentation by comparing the unknown to a set of standard, D-(+)-glucose that known concentration. The concentration of standard is determined in range 0 g/L to 1.0 g/L. Every standard concentration determined has their distinctive value of optical density.

After the value of OD is known for each concentration of standard, the graph was plotted like as Figure 4.1. That figure shows the value of optical density is increasing when the concentration of glucose is increase. When the glucose was mix with DNS reagent, it will change colour whether be brown and dark brown. The more concentration of glucose will change to dark brown colour. UV-Vis Spectrophotometer will detect the colour of standard based on their intensity of transmitted radiation and the result were transfer as optical density or absorbance.

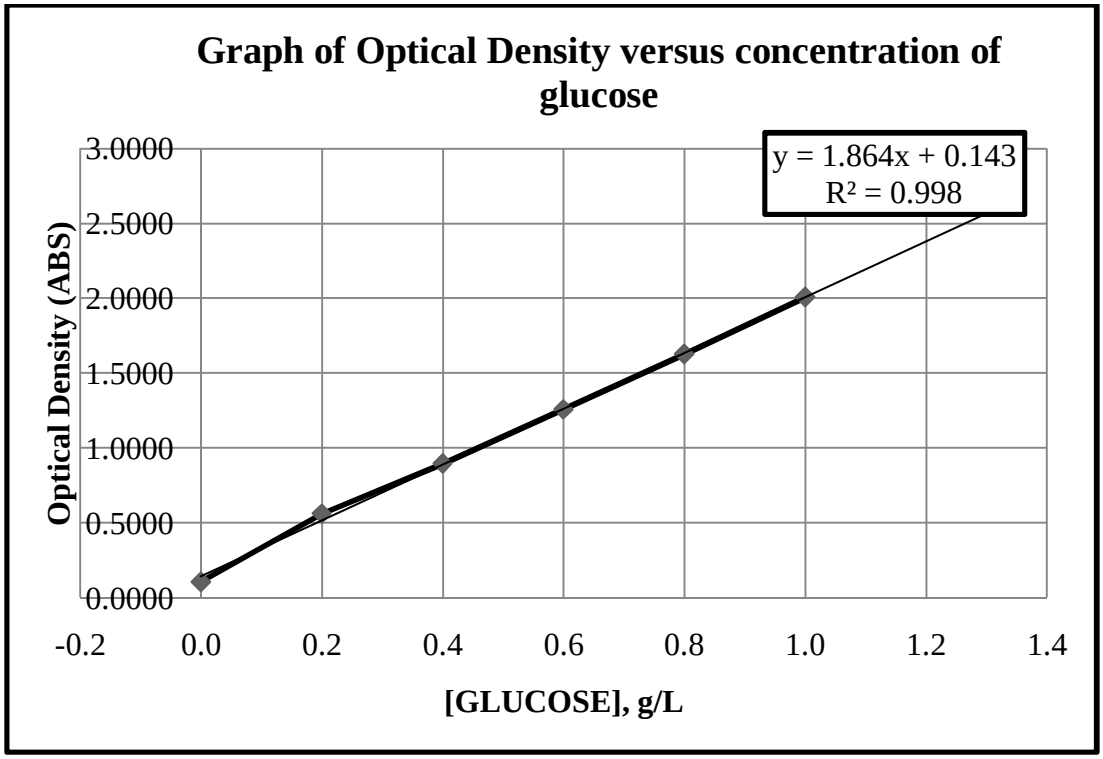

Figure 4.1: Calibration curve of glucose.

4.2 The Effect of Organic Loading Rate (OLR).

The experiment was started as batch fermentation. After 24 hr or 1 day, the fed batch fermentation was stated. Fifty milliliters of fermentation medium was removed and 50 L of fresh banana stem waste was added to the fermentation medium every 24 h for 29 days. The fed batch process is done based on hydraulic retention time. This experiment is done for 8 runs with different values of OLR. The results of this experiment are presented in Figure 4.2.

At OLR (g $.L^{-1}.d^{-1}$) of 5 (run 2), 5 (run 3), 11.25 (run 6), 17.5 (run 4), 17.5 (run 7), 23.75 (run 5), 30 (run 1) and 30 (run 8), the yield of glucose 0.0757 g/g substrate, 0.0709 g/g substrate, 0.0589 g/g substrate, 0.0494 g/g substrate, 0.0469 g/g

substrate, 0.0461 g/g substrate, 0.05 g/g substrate and 0.0461 g/g substrate were obtained after 30 day fed batch fermentation, respectively.

The yield of glucose was increase when the OLR is decrease. The OLR is decrease was affect by the concentration and mass of substrate. Every run has similar quantity of *Strain A*. This strain can more degrade the substrate at minimum OLR than other OLR. At highest OLR, the substrate cannot well degrade by that strain because quantity of substrate is excess and quantity of the strain is limit. This can be explained by which loss of activity during immobilization and diffusion between *Strain A* and substrate is limit (Caylak and Vardar Sukan, 1998). Besides that, when the amount of *Strain A* was fixed, it will make more intact cellulose was present in the system, which led to a low glucose yield (Wen *et al*, 2004).

The yield of glucose is highest at OLR is 5 g/L. At OLR is 17.5 g $.L^{-1}.d^{-1}$, the production of glucose is minimum after 30 days. The yield of glucose at all OLR for ninth day has almost the same value that is in the range.0.02 g/g substrate until 0.04 g/g substrate. The trend of graph is fluctuations for all OLR. This graph is like that because the banana stem waste does not degraded very well by microbe. The yield of glucose is decrease because the *Strain A* is at the log phase which it starts to multiply doubling and need more food to growth. So, the fermentable glucose also utilized by microbe as the food to growth an convert it to other product (Chen *et al*., 2008, Badger, 2002, and He *et al*., 2005). Then, the yield of glucose is increase because the *Strain A* already at stationary phase which the number of microbe is stabilizing, less to competing in dwindling the glucose and only degrade the banana stem waste to be the glucose.

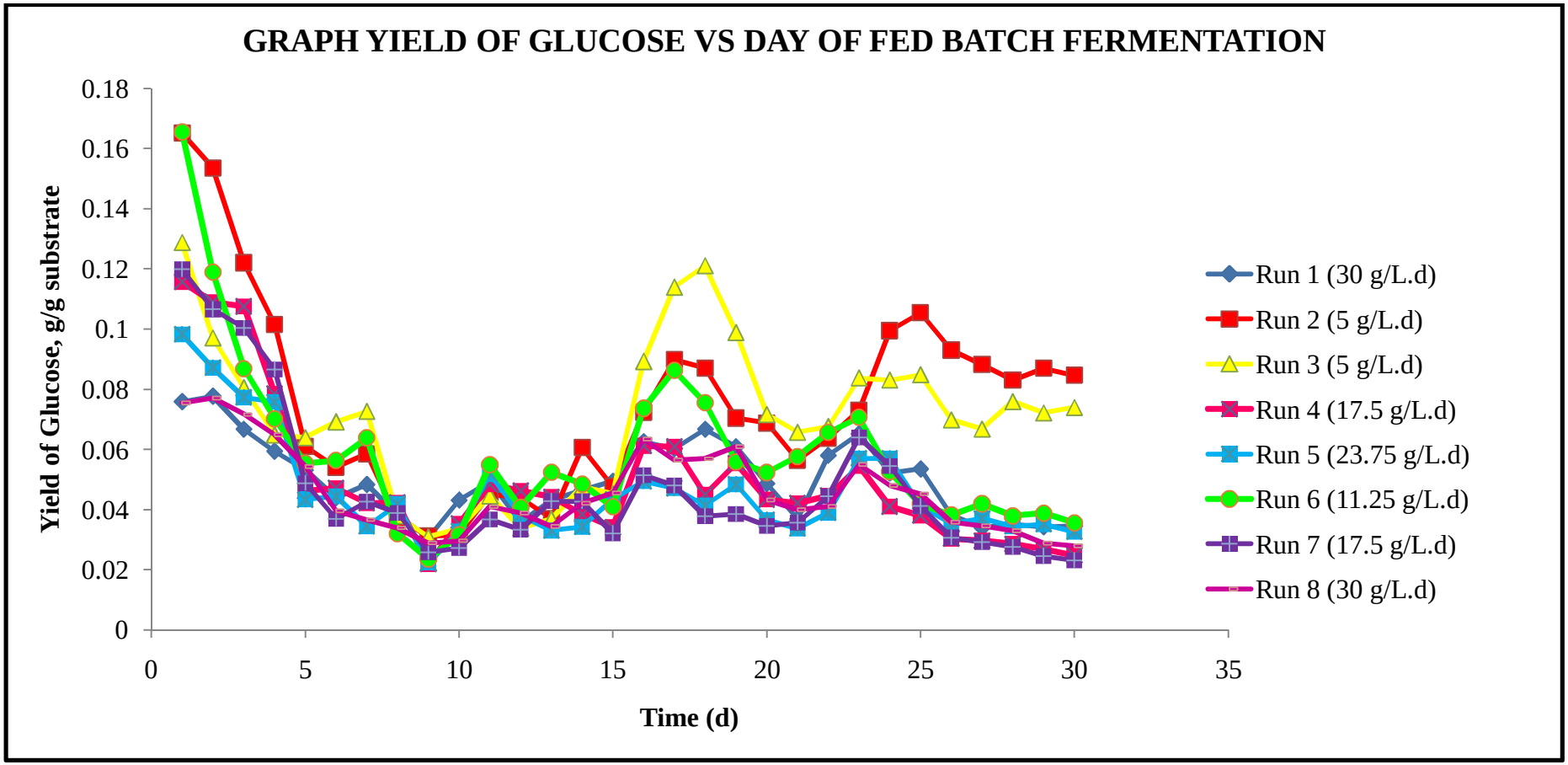

Figure 4.2: Effect of organic loading rate on yield of glucose for 30 days

4.3 The Optimization of Yield of Glucose.

Design Expert is used to optimize the glucose production. This optimization is using One Factor Analysis. Figure 4.2 presents the effect of OLR on glucose production by Design Expert. At OLR of 5 g $.L^{-1}.d^{-1}$, 11.25 g $.L^{-1}.d^{-1}$, 17.5 g $.L^{-1}.d^{-1}$, 23.75 g $.L^{-1}.d^{-1}$and 30 g $.L^{-1}.d^{-1}$, the yield of glucose is 0.073 g/g substrate, 0.058 g/g substrate, 0.048 g/g substrate, 0.45 g/g substrate and 0.048 g/g substrate were obtained from that graph, respectively.

The graph shows decreasing of glucose production by increasing of the OLR. But at OLR is 30 g $.L^{-1}.d^{-1}$, the yield of glucose was few increasing of the OLR. At the optimum OLR is 5 g $.L^{-1}.d^{-1}$, the glucose production is maximum because the concentration of banana stem waste is easy to microbe degrade it. At OLR is 5 g $.L^{-1}.d^{-1}$, the quantity of substrate is less than at other OLR. The diffusion between *Strain A* and substrate at minimum OLR is unlimited (Caylak and Vardar Sukan, 1998). So, the strain can be degrading the substrate very well.

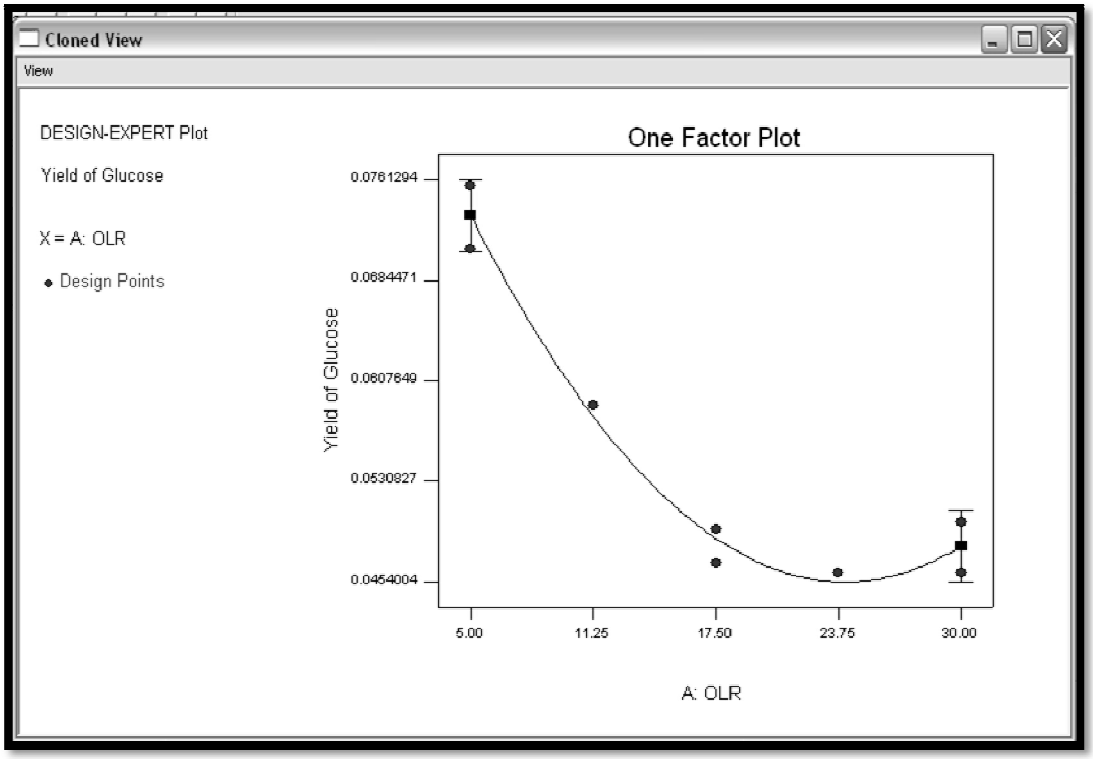

Figure 4.3: Effect of the organic loading rate on yield of glucose by Design Expert.

4.4 The Study Suitability of Banana Stem Waste as Substrate.

Design Expert also used to study suitability of banana stem waste as substrate. Organic loading rate is determined their goal at Design Expert for optimization as in range at lower and upper limit. Its range is 5 g $.L^{-1}.d^{-1}$ to 30 g $.L^{-1}.d^{-1}$. While, the yield of glucose is determined as maximize. The maximum yield of glucose is 0.0734 g/g substrate at the optimum OLR, 5 g $.L^{-1}.d^{-1}$. The desirability is 0.922. The result for this study is presented in Figure 4.4.

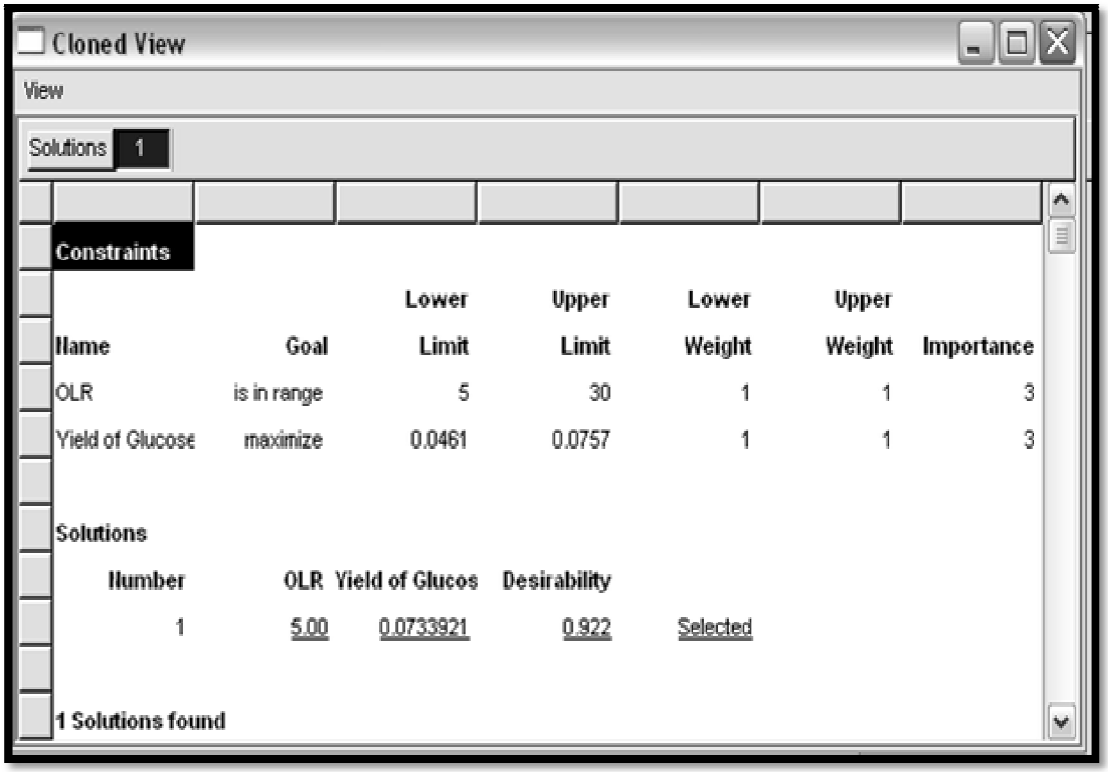

Figure 4.4: The optimization of glucose production

Table 4.1 shows comparison between production of glucose from banana stem waste and other agriculture wastes. That table presents the production of glucose from banana stem waste using biological hydrolysis and *Strain A* is highest than production of glucose from other agriculture wastes that use different type of hydrolysis or different microorganisms. So, the banana stem waste is suitable to be substrate in production of glucose with using biological hydrolysis method and the *Strain A* as the microbe. Based on production of glucose in this experiment and banana production in Malaysia and neighboring countries, banana stem waste is suitable uses as substrate for large scale glucose production.

Table 4.1: Comparison the glucose production using different agriculture wastes.

Substrate	**Method**	**Yield of Glucose (g/g substrate)**	**Study**
Wheat straw	Enzymatic hydrolysis	0.03125	Singh *et al*., 2009
Orange baggase	Solid state fermentation	0.04125	Giese *et al*., 2008
Melon seed shell	Enzymatic hydrolysis	0.04667	Okeke and Obi, 1994
Potato waste	Biological hydrolysis (*Rhizopus oligosporus*)	0.025	Del Re *et al*., 2003
Banana stem waste	Biological hydrolysis (*Strain A*)	0.0734	This study

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Decreasing of organic loading rate (OLR) will effect the increasing concentration of glucose produced from banana stem waste. So, the first objective in this research, the effect of (OLR) during production of glucose is studied.

Besides that, the banana stem waste was optimized for production of glucose. The optimization of glucose production is using Design Expert. The maximum yield of glucose was obtained from optimum OLR which is 5 g/L.d that contain less concentration of banana stem waste. The maximum yield of glucose is 0.0734 g/g substrate.

The suitability of banana stem waste as substrate for glucose production using *Strain A* was also studied. Based on result, the banana stem waste is suitable used as substrate in production of glucose using *Strain A*. This production from banana stem waste using *Strain A* has higher than previous study.

5.2 Recommendation

Future research should continue with using different agriculture waste such as rice hulls, rice straws or corn cobs to investigate the production rate of glucose based on OLR. Besides that, this experiment should be continuing with investigate more about kinetic model. In addition, the characteristic and type microorganism of *Strain A* need to investigate at the future study.

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APPENDIX A

TOTAL SOLID TEST

Aluminium pan = 2.5437 g

Aluminium pan + fresh banana stem = 20.2302 g

Aluminium pan + dry banana stem = 7.4327 g

Use Eq. 3.5,

$$\% \text{ Total solid} = \frac{(7.4327-2.5437)}{(20.2302-2.5437)} \times 100$$

$$= 27.6426\ \%$$

Use Eq. 3.6,

$$\% \text{ Moisture} = 100 - 27.6426$$

$$= 72.3574\%$$

APPENDIX B

CALIBRATION CURVE OF STANDARD GLUCOSE

B.1 Preparation of Stock Solution

$$10 \text{ mg/mL} = \frac{\text{mass}}{\text{Volume}}$$

Mass of glucose = (10 mg/mL) x (100 mL)
= 1000 mg
= 1 g

B.2 Preparation of Standard Solution

$M_1V_1 = M_2V_2$

Prepare 6 standard solution of glucose. The standard is prepared using equation above.

i) Standard glucose = 0 mg/mL

$$M_1V_1 = M_2V_2$$

(10 mg/mL) V_1 = (0.0 mg/mL) x (10 mL)

V_1 = 0.0 mL of stock solution and 10 mL of distilled water

ii) Standard glucose = 0.2 mg/mL

$$M_1V_1 = M_2V_2$$

(10 mg/mL) V_1 = (0.2 mg/mL) x (10 mL)

V_1 = 0.2 mL of stock solution and 9.8 mL of distilled water

iii) Standard glucose = 0.4 mg/mL

$$M_1V_1 = M_2V_2$$

(10 mg/mL) V_1 = (0.4 mg/mL) x (10 mL)

V_1 = 0.4 mL of stock solution and 9.6 mL of distilled water

iv) Standard glucose = 0.6 mg/mL

$$M_1V_1 = M_2V_2$$

(10 mg/mL) V_1 = (0.6 mg/mL) x (10 mL)

V_1 = 0.6 mL of stock solution and 9.4 mL of distilled water

v) Standard glucose = 0.8 mg/mL

$$M_1V_1 = M_2V_2$$

(10 mg/mL) V_1 = (0.8 mg/mL) x (10 mL)

V_1 = 0.8 mL of stock solution and 9.2 mL of distilled water

vi) Standard glucose = 1.0 mg/mL

$$M_1V_1 = M_2V_2$$

(10 mg/mL) V_1 = (1.0 mg/mL) x (10 mL)

V_1 = 1.0 mL of stock solution and 9 mL of distilled water

B.3 Calibration Curve of Glucose

Table B.1: Optical density of each standard solution.

Concentration of glucose (mg/mL)	**OD (Absorbance)**			
	1	**2**	**3**	**Average**
0.0	0.109	0.106	0.106	0.1070
0.2	0.561	0.562	0.563	0.5620
0.4	0.897	0.897	0.894	0.8960
0.6	1.251	1.255	1.266	1.2573
0.8	1.621	1.622	1.637	1.6267
1.0	2.003	2.007	2.009	2.0063

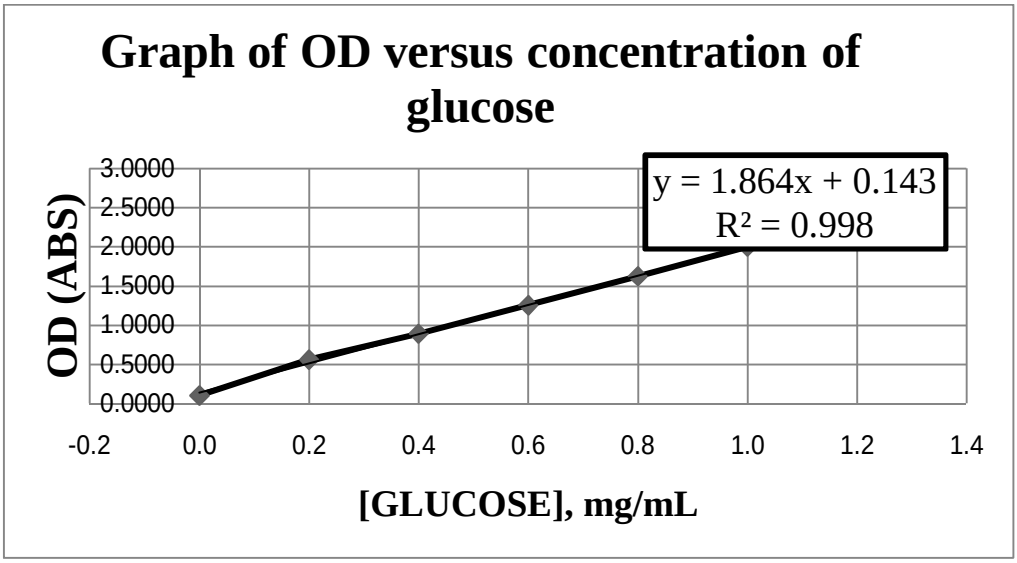

Figure B.1: Calibration curve of standard glucose.

APPENDIX C

PREPARATION OF SUBSTRATE

C.1 Preparation of Substrate for Batch Fermentation

Cloned View

View

	Std	Run	Block	Factor 1 A:OLR g/l.d	Response 1 glucose mg/l
	8	1	Block 1	30.00	
	2	2	Block 1	5.00	
	1	3	Block 1	5.00	
	4	4	Block 1	17.50	
	6	5	Block 1	23.75	
	3	6	Block 1	11.25	
	5	7	Block 1	17.50	
	7	8	Block 1	30.00	

Figure C.1: Organic loading rate that determine by Design Expert.

i) For Run 1 and 8, OLR = 30 g/L.d

$$OLR = \frac{[substrate]}{HRT}$$

$$[substrate] = (30\ g/L.d) \times (3\ d)$$
$$= 90\ g/L$$

$$\text{Mass of substrate} = \frac{(90\ g/L) \times (150\ ml)}{1000\ mL}$$
$$= 13.5\ g$$

ii) For Run 2 and 3, OLR = 5 g/L.d

$$OLR = \frac{[substrate]}{HRT}$$

$$[substrate] = (5\ g/L.d) \times (3\ d)$$
$$= 15\ g/L$$

$$\text{Mass of substrate} = \frac{(15\ g/L) \times (150\ ml)}{1000\ mL}$$
$$= 2.25\ g$$

iii) For Run 4 and 7, OLR = 17.50 g/L.d

$$OLR = \frac{[substrate]}{HRT}$$

$$[Substrate] = (17.50\ g/L.d) \times (3\ d)$$
$$= 52.50\ g/L$$

$$Mass\ of\ substrate = \frac{(52.50\ g/L) \times (150\ ml)}{1000\ mL}$$
$$= 7.875\ g$$

iv) For Run 5, OLR = 23.75 g/L.d

$$OLR = \frac{[substrate]}{HRT}$$

$$[substrate] = (23.75\ g/L.d) \times (3\ d)$$
$$= 71.25\ g/L$$

$$Mass\ of\ substrate = \frac{(71.25\ g/L) \times (150\ ml)}{1000\ mL}$$
$$= 10.6875\ g$$

v) For Run 6, OLR = 11.25 g/L.d

$$OLR = \frac{[substrate]}{HRT}$$

$$[substrate] = (11.25\ g/L.d) \times (3\ d)$$
$$= 33.75\ g/L$$

$$\text{Mass of substrate} = \frac{(33.75\ g/L) \times (150\ ml)}{1000\ mL}$$
$$= 5.0625\ g$$

C.2 Preparation of Substrate for Fed Batch Fermentation

i) For Run 1 and 8, OLR = 30 g/L.d

$$OLR = \frac{[substrate]}{HRT}$$

$$[substrate] = (30\ g/L.d) \times (3\ d)$$
$$= 90\ g/L$$

$$\text{Mass of substrate} = \frac{(90\ g/L) \times (50\ ml)}{1000\ mL}$$
$$= 4.5\ g$$

ii) For Run 2 and 3, OLR = 5 g/L.d

$$OLR = \frac{[substrate]}{HRT}$$

$$[substrate] = (5\ g/L.d) \times (3\ d)$$
$$= 15\ g/L$$

$$\text{Mass of substrate} = \frac{(15\ g/L) \times (50\ ml)}{1000\ mL}$$
$$= 0.75\ g$$

iii) For Run 4 and 7, OLR = 17.50 g/L.d

$$OLR = \frac{[substrate]}{HRT}$$

$$[Substrate] = (17.50\ g/L.d) \times (3\ d)$$
$$= 52.50\ g/L$$

$$\text{Mass of substrate} = \frac{(52.50\ g/L) \times (50\ ml)}{1000\ mL}$$
$$= 2.625\ g$$

iv) For Run 5, OLR = 23.75 g/L.d

$$OLR = \frac{[substrate]}{HRT}$$

$$[substrate] = (23.75\ g/L.d) \times (3\ d)$$
$$= 71.25\ g/L$$

$$\text{Mass of substrate} = \frac{(71.25\ g/L) \times (50\ ml)}{1000\ mL}$$
$$= 3.5625\ g$$

v) For Run 6, OLR = 11.25 g/L.d

$$OLR = \frac{[substrate]}{HRT}$$

$$[substrate] = (11.25\ g/L.d) \times (3\ d)$$
$$= 33.75\ g/L$$

$$\text{Mass of substrate} = \frac{(33.75\ g/L) \times (50\ ml)}{1000\ mL}$$
$$= 1.6875\ g$$

APPENDIX D

ANALYSIS OF SAMPLE

D.1 Determine of Concentration of Glucose

Concentration of glucose from the sample was determined by their absorbance and calibration curve of glucose.

D.2 Calculation of Yield of Glucose

Yield of Glucose, g/g substrate = [glucose]/ [substrate]

D.3 Analysis Concentration and Yield of Glucose From Sample

i) Run 1(OLR = 30 g/L.d)

Run	Date	Adsorbance				[glucose], mg/mL	Day	Yield of Glucose, g/g substrate
		1	2	3	average			
1	6-Mar	12.84	12.840	12.925	12.8683	6.8269	1	0.0759
	7-Mar	13.10	13.100	13.290	13.1633	6.9852	2	0.0776
	8-Mar	11.345	11.215	11.425	11.3283	6.0007	3	0.0667
	9-Mar	10.155	10.090	10.065	10.1033	5.3435	4	0.0594
	10-Mar	9.065	9.065	9.080	9.0700	4.7892	5	0.0532
	11-Mar	7.620	7.280	7.615	7.5050	3.9496	6	0.0439
	12-Mar	8.250	8.250	8.260	8.2533	4.3510	7	0.0483
	13-Mar	6.545	6.550	6.550	6.5483	3.4363	8	0.0382
	14-Mar	5.290	5.265	5.290	5.2817	2.7568	9	0.0306
	15-Mar	7.530	7.455	7.185	7.3900	3.8879	10	0.0432
	16-Mar	8.410	8.445	8.410	8.4217	4.4413	11	0.0493
	17-Mar	6.250	6.175	6.075	6.1667	3.2316	12	0.0359
	18-Mar	7.385	7.360	7.370	7.3717	3.8780	13	0.0431
	19-Mar	7.950	7.935	7.935	7.9400	4.1829	14	0.0465
	20-Mar	8.420	8.445	8.410	8.4250	4.4431	15	0.0494
	21-Mar	10.655	10.625	10.625	10.6350	5.6288	16	0.0625
	22-Mar	10.300	10.230	10.205	10.2450	5.4195	17	0.0602
	23-Mar	11.340	11.340	11.300	11.3267	5.9998	18	0.0667
	24-Mar	10.355	10.355	10.355	10.3550	5.4785	19	0.0609
	25-Mar	8.320	8.290	8.290	8.3000	4.3761	20	0.0486
	26-Mar	6.365	6.330	6.320	6.3383	3.3237	21	0.0369
	27-Mar	9.880	9.880	9.880	9.8800	5.2237	22	0.0580
	28-Mar	11.075	11.075	11.075	11.0750	5.8648	23	0.0652
	29-Mar	8.900	8.900	8.900	8.9000	4.6980	24	0.0522
	30-Mar	9.120	9.120	9.120	9.1200	4.8160	25	0.0535
	31-Mar	6.560	6.550	6.545	6.5517	3.4381	26	0.0382
	1-Apr	5.845	5.845	5.845	5.8450	3.0590	27	0.0340
	2-Apr	6.045	6.050	6.045	6.0467	3.1672	28	0.0352
	3-Apr	5.895	5.895	5.900	5.8967	3.0867	29	0.0343
	4-Apr	5.955	5.945	5.945	5.9483	3.1144	30	0.0346
	Total							**1.5022**
	Average							**0.0501**

ii) Run 2 (OLR= 5g/L.d)

Run	Date	Adsorbance				[glucose], mg/mL	Day	Yield of Glucose, g/g substrate
		1	2	3	average			
2	6-Mar	4.725	4.765	4.790	4.7600	2.4769	1	0.1651
	7-Mar	4.455	4.440	4.410	4.4350	2.3026	2	0.1535
	8-Mar	3.905	3.370	3.390	3.5550	1.8305	3	0.1220
	9-Mar	3.020	2.985	2.945	2.9833	1.5238	4	0.1016
	10-Mar	1.880	1.830	1.835	1.8483	0.9149	5	0.0610
	11-Mar	1.650	1.655	1.660	1.6550	0.8112	6	0.0541
	12-Mar	1.785	1.785	1.770	1.7800	0.8782	7	0.0585
	13-Mar	1.185	1.165	1.165	1.1717	0.5519	8	0.0368
	14-Mar	1.020	1.035	0.995	1.0167	0.4687	9	0.0312
	15-Mar	1.050	1.010	1.010	1.0233	0.4723	10	0.0315
	16-Mar	1.420	1.395	1.395	1.4033	0.6761	11	0.0451
	17-Mar	1.385	1.365	1.365	1.3717	0.6592	12	0.0439
	18-Mar	1.180	1.180	1.175	1.1783	0.5554	13	0.0370
	19-Mar	1.840	1.830	1.840	1.8367	0.9086	14	0.0606
	20-Mar	1.475	1.495	1.460	1.4767	0.7155	15	0.0477
	21-Mar	2.165	2.190	2.135	2.1633	1.0839	16	0.0723
	22-Mar	2.660	2.655	2.650	2.6550	1.3476	17	0.0898
	23-Mar	2.570	2.570	2.590	2.5767	1.3056	18	0.0870
	24-Mar	2.115	2.110	2.110	2.1117	1.0562	19	0.0704
	25-Mar	2.050	2.055	2.090	2.0650	1.0311	20	0.0687
	26-Mar	1.720	1.720	1.720	1.7200	0.8460	21	0.0564
	27-Mar	1.925	1.925	1.925	1.9250	0.9560	22	0.0637
	28-Mar	2.175	2.195	2.180	2.1833	1.0946	23	0.0730
	29-Mar	2.920	2.930	2.920	2.9233	1.4916	24	0.0994
	30-Mar	3.090	3.090	3.090	3.0900	1.5810	25	0.1054
	31-Mar	2.740	2.745	2.745	2.7433	1.3950	26	0.0930
	1-Apr	2.610	2.610	2.610	2.6100	1.3235	27	0.0882
	2-Apr	2.460	2.470	2.465	2.4650	1.2457	28	0.0830
	3-Apr	2.575	2.575	2.575	2.5750	1.3047	29	0.0870
	4-Apr	2.510	2.510	2.510	2.5100	1.2698	30	0.0847
	Total							**2.2719**
	Average							**0.0757**

iii) Run 3(OLR= 5g/L.d)

Run	Date	Adsorbance				[glucose], mg/mL	Day	Yield of Glucose, g/g substrate
		1	2	3	average			
3	6-Mar	3.84	3.845	3.54	3.74167	1.93062	1	0.12871
	7-Mar	2.875	2.85	2.845	2.85667	1.45583	2	0.09706
	8-Mar	2.505	2.18	2.495	2.39333	1.20726	3	0.08048
	9-Mar	2.005	1.94	1.925	1.95667	0.973	4	0.06487
	10-Mar	1.92	1.94	1.93	1.93	0.95869	5	0.06391
	11-Mar	2.185	2.015	2.03	2.07667	1.03737	6	0.06916
	12-Mar	2.16	2.21	2.15	2.17333	1.08923	7	0.07262
	13-Mar	1.22	1.175	1.21	1.20167	0.56795	8	0.03786
	14-Mar	1.02	0.995	1.01	1.00833	0.46423	9	0.03095
	15-Mar	1.12	1.08	1.06	1.08667	0.50626	10	0.03375
	16-Mar	1.385	1.395	1.39	1.39	0.66899	11	0.0446
	17-Mar	1.085	1.1	1.09	1.09167	0.50894	12	0.03393
	18-Mar	1.175	1.175	1.175	1.175	0.55365	13	0.03691
	19-Mar	1.475	1.48	1.465	1.47333	0.7137	14	0.04758
	20-Mar	1.415	1.41	1.4	1.40833	0.67883	15	0.04526
	21-Mar	2.635	2.645	2.635	2.63833	1.3387	16	0.08925
	22-Mar	3.355	3.305	3.325	3.32833	1.70887	17	0.11392
	23-Mar	3.53	3.525	3.525	3.52667	1.81527	18	0.12102
	24-Mar	2.91	2.905	2.905	2.90667	1.48265	19	0.09884
	25-Mar	2.145	2.145	2.145	2.145	1.07403	20	0.0716
	26-Mar	1.99	1.98	1.97	1.98	0.98552	21	0.0657
	27-Mar	2.045	2.03	2.02	2.03167	1.01323	22	0.06755
	28-Mar	2.495	2.48	2.48	2.485	1.25644	23	0.08376
	29-Mar	2.47	2.465	2.46	2.465	1.24571	24	0.08305
	30-Mar	2.51	2.515	2.515	2.51333	1.27164	25	0.08478
	31-Mar	2.085	2.13	2.07	2.095	1.04721	26	0.06981
	1-Apr	2.01	2.01	2.01	2.01	1.00161	27	0.06677
	2-Apr	2.26	2.265	2.27	2.265	1.13841	28	0.07589
	3-Apr	2.16	2.16	2.16	2.16	1.08208	29	0.07214
	4-Apr	2.21	2.21	2.21	2.21	1.10891	30	0.07393
	Total							**2.12566**
	Average							**0.07086**

iv) Run 4(OLR= 17.5g/L.d)

Run	Date	Adsorbance				[glucose], mg/mL	Day	Yield of Glucose, g/g substrate
		1	2	3	average			
4	6-Mar	11.38	11.505	11.505	11.4633	6.07314	1	0.11568
	7-Mar	10.855	10.78	10.8	10.8117	5.72353	2	0.10902
	8-Mar	10.625	10.685	10.685	10.665	5.64485	3	0.10752
	9-Mar	7.885	7.805	7.78	7.82333	4.12035	4	0.07848
	10-Mar	4.645	4.655	4.7	4.66667	2.42686	5	0.04623
	11-Mar	4.735	4.78	4.765	4.76	2.47693	6	0.04718
	12-Mar	4.315	4.24	4.24	4.265	2.21137	7	0.04212
	13-Mar	4.35	4.26	4.25	4.28667	2.223	8	0.04234
	14-Mar	2.32	2.275	2.265	2.28667	1.15004	9	0.02191
	15-Mar	3.58	3.63	3.575	3.595	1.85193	10	0.03527
	16-Mar	4.87	4.87	4.87	4.87	2.53594	11	0.0483
	17-Mar	4.68	4.66	4.66	4.66667	2.42686	12	0.04623
	18-Mar	4.485	4.465	4.45	4.46667	2.31956	13	0.04418
	19-Mar	3.9	3.91	3.91	3.90667	2.01913	14	0.03846
	20-Mar	3.525	3.485	3.445	3.485	1.79292	15	0.03415
	21-Mar	6.13	6.13	6.13	6.13	3.21191	16	0.06118
	22-Mar	6.09	6.12	6.105	6.105	3.1985	17	0.06092
	23-Mar	4.555	4.55	4.54	4.54833	2.36338	18	0.04502
	24-Mar	5.58	5.57	5.55	5.56667	2.90969	19	0.05542
	25-Mar	4.385	4.385	4.365	4.37833	2.27217	20	0.04328
	26-Mar	4.265	4.27	4.26	4.265	2.21137	21	0.04212
	27-Mar	4.51	4.51	4.51	4.51	2.34281	22	0.04462
	28-Mar	5.47	5.475	5.485	5.47667	2.86141	23	0.0545
	29-Mar	4.16	4.155	4.16	4.15833	2.15415	24	0.04103
	30-Mar	3.865	3.865	3.86	3.86333	1.99589	25	0.03802
	31-Mar	3.1	3.115	3.115	3.11	1.59174	26	0.03032
	1-Apr	3.045	3.05	3.04	3.045	1.55687	27	0.02965
	2-Apr	2.945	2.945	2.935	2.94167	1.50143	28	0.0286
	3-Apr	2.765	2.765	2.76	2.76333	1.40576	29	0.02678
	4-Apr	2.57	2.57	2.57	2.57	1.30204	30	0.0248
	Total							**1.48334**
	Average							**0.04944**

v) Run 5(OLR= 23.75g/L.d)

Run	Date	Adsorbance				[glucose], mg/mL	Day	Yield of Glucose, g/g substrate
		1	2	3	average			
5	6-Mar	13.19	13.19	13.19	13.19	6.99946	1	0.09824
	7-Mar	11.735	11.685	11.735	11.7183	6.20994	2	0.08716
	8-Mar	10.405	10.4	10.415	10.4067	5.50626	3	0.07728
	9-Mar	10.2	10.205	10.2	10.2017	5.39628	4	0.07574
	10-Mar	6.005	5.865	5.805	5.89167	3.08405	5	0.04328
	11-Mar	6.055	6.005	6.05	6.03667	3.16184	6	0.04438
	12-Mar	4.715	4.715	4.715	4.715	2.45279	7	0.03443
	13-Mar	5.7	5.695	5.695	5.69667	2.97943	8	0.04182
	14-Mar	3.12	3.11	3.12	3.11667	1.59531	9	0.02239
	15-Mar	4.485	4.54	4.49	4.505	2.34013	10	0.03284
	16-Mar	7.035	7.005	7.025	7.02167	3.69027	11	0.05179
	17-Mar	5.205	5.215	5.22	5.21333	2.72014	12	0.03818
	18-Mar	4.84	4.405	4.355	4.53333	2.35533	13	0.03306
	19-Mar	4.695	4.69	4.69	4.69167	2.44027	14	0.03425
	20-Mar	5.9	5.895	5.89	5.895	3.08584	15	0.04331
	21-Mar	6.705	6.705	6.7	6.70333	3.51949	16	0.0494
	22-Mar	6.395	6.405	6.405	6.40167	3.35765	17	0.04712
	23-Mar	5.63	5.63	5.63	5.63	2.94367	18	0.04131
	24-Mar	6.58	6.57	6.55	6.56667	3.44617	19	0.04837
	25-Mar	5.015	5	5	5.005	2.60837	20	0.03661
	26-Mar	4.645	4.62	4.615	4.62667	2.4054	21	0.03376
	27-Mar	5.31	5.31	5.31	5.31	2.772	22	0.03891
	28-Mar	7.725	7.72	7.695	7.71333	4.06134	23	0.057
	29-Mar	7.82	7.66	7.66	7.71333	4.06134	24	0.057
	30-Mar	5.615	5.61	5.6	5.60833	2.93205	25	0.04115
	31-Mar	4.85	4.84	4.83	4.84	2.51985	26	0.03537
	1-Apr	5.065	5.06	5.065	5.06333	2.63966	27	0.03705
	2-Apr	4.715	4.715	4.715	4.715	2.45279	28	0.03443
	3-Apr	4.815	4.82	4.81	4.815	2.50644	29	0.03518
	4-Apr	4.48	4.48	4.48	4.48	2.32672	30	0.03266
	Total							**1.38344**
	Average							**0.04611**

vi) Run 6(OLR= 11.25g/L.d)

Run	Date	Adsorbance				[glucose], mg/mL	Day	Yield of Glucose, g/g substrate
		1	2	3	average			
6	6-Mar	10.51	10.625	10.54	10.55833	5.587625	1	0.165559
	7-Mar	7.645	7.615	7.615	7.625	4.013948	2	0.118932
	8-Mar	5.47	5.675	5.68	5.608333	2.932046	3	0.086875
	9-Mar	4.56	4.545	4.565	4.556667	2.367847	4	0.070158
	10-Mar	3.63	3.645	3.6	3.625	1.868026	5	0.055349
	11-Mar	3.66	3.695	3.695	3.683333	1.89932	6	0.056276
	12-Mar	4.175	4.15	4.145	4.156667	2.153255	7	0.0638
	13-Mar	2.13	2.185	2.16	2.158333	1.081187	8	0.032035
	14-Mar	1.635	1.62	1.63	1.628333	0.796853	9	0.02361
	15-Mar	2.175	2.115	2.105	2.131667	1.066881	10	0.031611
	16-Mar	3.605	3.615	3.58	3.6	1.854614	11	0.054952
	17-Mar	2.72	2.705	2.695	2.706667	1.375358	12	0.040751
	18-Mar	3.445	3.44	3.425	3.436667	1.766989	13	0.052355
	19-Mar	3.195	3.19	3.2	3.195	1.637339	14	0.048514
	20-Mar	2.715	2.715	2.72	2.716667	1.380722	15	0.04091
	21-Mar	4.79	4.775	4.76	4.775	2.484979	16	0.073629
	22-Mar	5.575	5.575	5.57	5.573333	2.913269	17	0.086319
	23-Mar	4.985	4.855	4.85	4.896667	2.55025	18	0.075563
	24-Mar	3.55	3.46	3.955	3.655	1.88412	19	0.055826
	25-Mar	3.42	3.44	3.44	3.433333	1.7652	20	0.052302
	26-Mar	3.805	3.765	3.74	3.77	1.945815	21	0.057654
	27-Mar	4.265	4.265	4.29	4.273333	2.215844	22	0.065655
	28-Mar	4.585	4.59	4.59	4.588333	2.384835	23	0.070662
	29-Mar	3.44	3.46	3.44	3.446667	1.772353	24	0.052514
	30-Mar	2.775	2.775	2.77	2.773333	1.411123	25	0.041811
	31-Mar	2.545	2.545	2.54	2.543333	1.287732	26	0.038155
	1-Apr	2.78	2.78	2.78	2.78	1.4147	27	0.041917
	2-Apr	2.53	2.53	2.52	2.526667	1.278791	28	0.03789
	3-Apr	2.58	2.59	2.585	2.585	1.310086	29	0.038817
	4-Apr	2.38	2.38	2.38	2.38	1.200107	30	0.035559
	Total							**1.765962**
	Average							**0.058865**

vii) Run 7(OLR= 17.5g/L.d)

Run	Date	Adsorbance				[glucose], mg/mL	Day	Yield of Glucose, g/g substrate
		1	2	3	average			
7	6-Mar	11.83	11.885	11.885	11.8667	6.28952	1	0.1198
	7-Mar	10.595	10.485	10.625	10.5683	5.59299	2	0.10653
	8-Mar	9.965	9.965	9.95	9.96	5.26663	3	0.10032
	9-Mar	8.625	8.615	8.605	8.615	4.54506	4	0.08657
	10-Mar	4.98	4.875	4.89	4.915	2.56009	5	0.04876
	11-Mar	3.98	3.955	3.345	3.76	1.94045	6	0.03696
	12-Mar	4.35	4.315	4.29	4.31833	2.23999	7	0.04267
	13-Mar	3.98	3.915	3.935	3.94333	2.03881	8	0.03883
	14-Mar	2.68	2.68	2.66	2.67333	1.35747	9	0.02586
	15-Mar	2.875	2.705	2.85	2.81	1.43079	10	0.02725
	16-Mar	3.725	3.73	3.74	3.73167	1.92525	11	0.03667
	17-Mar	3.36	3.42	3.42	3.4	1.74732	12	0.03328
	18-Mar	4.35	4.335	4.33	4.33833	2.25072	13	0.04287
	19-Mar	4.335	4.325	4.31	4.32333	2.24267	14	0.04272
	20-Mar	3.28	3.28	3.285	3.28167	1.68383	15	0.03207
	21-Mar	5.17	5.165	5.17	5.16833	2.69599	16	0.05135
	22-Mar	4.855	4.835	4.83	4.84	2.51985	17	0.048
	23-Mar	3.845	3.845	3.845	3.845	1.98605	18	0.03783
	24-Mar	3.875	3.91	3.935	3.90667	2.01913	19	0.03846
	25-Mar	3.525	3.52	3.52	3.52167	1.81259	20	0.03453
	26-Mar	3.64	3.64	3.635	3.63833	1.87518	21	0.03572
	27-Mar	4.48	4.505	4.505	4.49667	2.33566	22	0.04449
	28-Mar	6.39	6.415	6.415	6.40667	3.36034	23	0.06401
	29-Mar	5.47	5.47	5.465	5.46833	2.85694	24	0.05442
	30-Mar	4.17	4.175	4.175	4.17333	2.1622	25	0.04118
	31-Mar	3.145	3.15	3.15	3.14833	1.6123	26	0.03071
	1-Apr	2.995	2.995	2.995	2.995	1.53004	27	0.02914
	2-Apr	2.845	2.835	2.84	2.84	1.44689	28	0.02756
	3-Apr	2.545	2.545	2.545	2.545	1.28863	29	0.02455
	4-Apr	2.41	2.41	2.41	2.41	1.2162	30	0.02317
	Total							**1.40628**
	Average							**0.04688**

viii) Run 8(OLR= 30 g/L.d)

Run	Date	Adsorbance				[glucose], mg/mL	Day	Yield of Glucose, g/g substrate
		1	2	3	average			
8	6-Mar	12.84	12.84	12.685	12.7883	6.78398	1	0.07538
	7-Mar	13.1	13.09	13.05	13.08	6.94045	2	0.07712
	8-Mar	12.055	12.125	12.275	12.1517	6.44242	3	0.07158
	9-Mar	11.015	11.005	11.015	11.0117	5.83083	4	0.06479
	10-Mar	9.18	9.38	9.215	9.25833	4.8902	5	0.05434
	11-Mar	6.795	6.79	6.77	6.785	3.5633	6	0.03959
	12-Mar	6.28	6.295	6.29	6.28833	3.29685	7	0.03663
	13-Mar	5.775	5.91	5.825	5.83667	3.05454	8	0.03394
	14-Mar	5.03	4.96	4.99	4.99333	2.60211	9	0.02891
	15-Mar	5.11	5.1	5.135	5.115	2.66738	10	0.02964
	16-Mar	7.01	7.01	7.01	7.01	3.68401	11	0.04093
	17-Mar	6.665	6.655	6.635	6.65167	3.49177	12	0.0388
	18-Mar	5.845	5.935	5.935	5.905	3.0912	13	0.03435
	19-Mar	7.215	7.21	7.2	7.20833	3.79041	14	0.04212
	20-Mar	7.78	7.785	7.785	7.78333	4.09889	15	0.04554
	21-Mar	10.77	10.795	10.8	10.7883	5.71102	16	0.06346
	22-Mar	9.605	9.605	9.585	9.59833	5.0726	17	0.05636
	23-Mar	9.68	9.74	9.64	9.68667	5.11999	18	0.05689
	24-Mar	10.35	10.35	10.355	10.3517	5.47675	19	0.06085
	25-Mar	7.375	7.37	7.375	7.37333	3.87893	20	0.0431
	26-Mar	6.855	6.84	6.845	6.84667	3.59639	21	0.03996
	27-Mar	7.03	7.035	7.035	7.03333	3.69653	22	0.04107
	28-Mar	9.38	9.38	9.38	9.38	4.95547	23	0.05506
	29-Mar	8.2	8.2	8.19	8.19667	4.32064	24	0.04801
	30-Mar	7.68	7.755	7.755	7.73	4.07028	25	0.04523
	31-Mar	6.165	6.165	6.155	6.16167	3.2289	26	0.03588
	1-Apr	5.94	5.94	5.94	5.94	3.10998	27	0.03456
	2-Apr	5.675	5.675	5.675	5.675	2.96781	28	0.03298
	3-Apr	4.975	4.975	4.975	4.975	2.59227	29	0.0288
	4-Apr	4.825	4.82	4.825	4.82333	2.51091	30	0.0279
	Total							**1.38374**
	Average							**0.04612**