PRODUCTION OF GLUCOSE FROM BANANA STEM WASTE BY USING *STRAIN B*

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JUDUL **PRODUCTION OF GLUCOSE FROM BANANA STEM WASTE BY USING *STRAIN B***

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NORHIDAYU BINTI ALIMAN

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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APRIL 2010

I declare that this thesis entitled "*Production of Glucose from Banana Stem Waste by Using Strain B*" 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.

Signature : .....

Name : Norhidayu binti Aliman

Date : 30 April 2010

"Special dedication to my beloved mother, family members, supervisor, staffs of FKKSA laboratory and all my beloved friends"

For all your love and support towards this study. Thank you.

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ABSTRACT

Banana is a major cash crop of many regions generating good amount of waste after harvest. This agricultural waste used as substrate for the fermentation process to produce organic products such ethanol and acetic acid. Production of glucose from agro waste is one way to reduce the abundant of waste on the plantation floor. The objectives of this research are to study the effect of organic loading rate (OLR) during glucose production, to optimize the glucose production by using banana stem waste and to study the suitability of banana stem waste as substrate for glucose production. In this study, eight experimental runs were carried out simultaneously at OLRs of 30, 5.0, 5.0, 17.50, 23.75, 11.25, 17.50 and 30 g/L.d respectively which have the same hydraulic retention times, three days. The effect of OLR was measured by total average yield of glucose produced for each run while the optimization of glucose production was done by using One Factor Analysis. Then, the suitability of banana stem in producing glucose was determined by doing comparison with other lignocelullose waste. The results reveal that, the OLR was affected to the glucose production. At range 5-23.75 g/L.d the yield was decreased as the OLR increased while at 30 g/L.d which the highest OLR the yield of glucose was higher compare to 23.75 g/L.d. This was possible due to the accumulation of inhibitor in the high influent substrate concentration. As the OLR increase, the accumulation of inhibitor increased. At 5 g/L.d, those OLR was selected as the optimum OLR for this study by using One Factor Analysis due to the maximum glucose production within thirty days experiment. As conclusion, banana stem waste was successfully degraded by *strain B* and produced glucose.

ABSTRAK

Pisang ialah satu tanaman yang banyak ditanam dan menjana sejumlah sisa yang banyak selepas tuaian. Sisa pertanian ini digunakan sebagai substrat dalam proses fermentasi untuk mengeluarkan produk organik seperti etanol dan asid asetik. Penghasilan glukosa daripada sisa pertanian adalah satu cara untuk mengurangkan pengumpulan sisa tersebut di ladang penanaman. Objektif penyelidikan ini adalah untuk mengkaji kesan kadar beban organik (OLR) semasa pengeluaran glukosa, mengkaji kadar optimum pengeluaran glukosa dan mengkaji kesesuaian sisa batang pisang sebagai substrat dalam pengeluaran glukosa. Dalam kajian ini, lapan eksperimen telah dikaji serentak mengikut nilai OLR yang ditetapkan iaitu 30, 5.0, 5.0, 17.50, 23.75, 11.25, 17.50, dan 30 g/L.h untuk setiap eksperimen mengikut urutan, di mana masa penahanan hidraulik (HRT) ditetapkan iaitu tiga hari. Kesan OLR terhadap penghasilan glukosa diukur mengikut jumlah purata glukosa yang terhasil selama 30 hari fermentasi. Manakala, kadar optimum pengeluaran glukosa pula dianalisis menggunakan Analisis Satu Faktor. Kesesuaian batang pisang untuk menghasilkan batang pisang pula telah diukur dengan membandingkan dengan sisa lignoselulosa yang lain. Hasil daripada kajian menunjukkan bahawa OLR memberi kesan terhadap penghasilan glukosa. Di dalam julat OLR 5-23.75 g/L.h, menunjukkan kadar glukosa semakin menurun sekiranya OLR meningkat. Manakala pada OLR 30 g/L.h didapati bahawa kadar glukosa meningkat sedikit berbanding pada OLR 23.75 g/L.h. Ini kemungkinan berpunca daripada pengumpulan perencat yang terhasil daripada kepekatan substrat masuk yang tinggi. Semakin meningkat OLR, semakin banyak pengumpulan perencat. 5 g/L.h, terpilih sebagai OLR yang optimum kerana telah menghasilkan glukosa yang banyak berbanding OLR lain. Kesimpulannya, *pencilan B* mampu menghuraikan sisa batang pisang kepada glukosa.

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LIST OF ABBREVIATIONS/SYMBOLS

ABS	-	Absorbance
CSTR	-	Continuous Stirred Tank Reactor
DNS	-	Dinitrosalicyclic Acid
g	-	gram
Gal	-	Galactose
Glu	-	Glucose
hr	-	hour
h	-	hari
HMF	-	Hydroxymethyl Furfural
HRT	-	Hydraulic Retention Time
Man	-	Mannose
Nm	-	Nanometer
^{o}C	-	Degree Celcius
OD	-	Optical Density
OLR	-	Organic Loading Rate
rpm	-	revolutions per minute
UV	-	Ultra Violet
VFA	-	Volatile Fatty Acid
vs	-	Versus

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CHAPTER 1

INTRODUCTION

1.1 Background

Glucose is a monosaccharide carbohydrate which usually exists in the fruits and honey, the chemical equation is $C_6H_{12}O_6$. Glucose is composed of carbohydrate, which is stored as an important energy in the plant, cellulose as a unit and glycogen which is store in the vertebrate animal. Cellulose cannot digest by Eukaryotes, but only Prokaryotes, bacteria and algae can digest it and over three thousand of glucose units made up to a unit of cellulose.

The common primary raw materials for the production of glucose are such corn, rice, sorghum, wheat and cassava (Aschengreen *et al.*, 1979). Nowadays, the agricultural waste had been focused as the alternative method to produce glucose. Agricultural wastes are materials left in an agricultural field or orchard after the crop has been harvested. These residues include stalks and stubble (stems), leaves, and seed pods which contain the largest source of cellulose such as banana stem, rice straw and pineapple leaf (Bhaduri *et al.*, 1995).

By using fermentation method, glucose can be produced as the intermediate product. Fermentation begins as the growing population of microorganisms produces enzymes to break two-molecule sugars into single molecule sugars and then convert the single molecule sugars into the commercial chemicals and byproducts. Fermentation can be divided into two major condition which aerobic and anaerobic. In aerobic condition, the productivity of cell was achieved in the presence of oxygen, compare to anaerobic condition which the cell most productive in the absence of oxygen (Klein *et al.*, 2005).

In fermentation process, the hydrolysis process was occurred which the process to convert the polysaccharide molecule into monosaccharide compound such glucose. There are several method in hydrolysis had been practiced such as enzymatic hydrolysis and biological hydrolysis. Enzymatic hydrolysis of cellulose is a reaction carried out by cellulase enzymes, which are highly specific (Beguin and Aubert, 1994). It is an important reaction in nature for it marks the first step in the decay of cellulose, the most abundantly occurring organic materials. Besides the hydrolysis of cellulose molecules into glucose monomers, gut microbes play an important role in the synthesis of acetate, CO_2 and H_2 by the fermentation of each glucose monomers. In biological hydrolysis, the bacterium was used to degrade the lignocelluloses into simple sugar like glucose. The bacterium was isolated from the same area with waste substrate in order to get the similarities in characteristic of bacterium and substrate. The bacterium will undergo fermentation process with substrate which the bacterium will produce its own enzyme to degrade cellulose to become simple sugar (Castellanos, 1995).

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1.2 Problem Statement

Currently, the production of glucose is widely by using the potato, corn, rice and wheat as the raw material (Aschengreen et al., 1979). As we know, all these material are the food source for human living and important for energy source. If the food sources are used continuously for glucose production, the depletion of that source will occur thus, starvation will started.

In order to overcome this situation from happen, the alternative method have been applied which by using agricultural waste as the raw material for production. Banana stem waste is the most suitable agricultural waste for the glucose production. In Malaysia, there is a large plantation of banana which about 34 thousands hectare, in 2001. Thus, large quantities of cellulosic and non cellulosic raw material are generated during harvesting. Furthermore, from the previous study showed that the banana stem waste contain the lower percentage of lignin which 18.6% while have the abundant of cellulosic which 63.9% (Abdul Khalil *et al.*, 2006). The high contain of cellulosic make the banana stem the most appropriate raw material of glucose production. As we know, glucose is widely used for food industries as additive and also for pharmaceutical industries for bio-product production. Thus, enormous production of glucose will obviously make benefit for this economy's country.

Then, the explosive expansion of plantation in this country has generated enormous amount of vegetable waste, creating problem in replanting operation and tremendous environmental concerns. 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 Objective of The Study

1. To study the effect of organic loading rate (OLR) during the glucose production.
2. To optimize the glucose production by using banana stem waste.
3. To study the suitability of banana stem waste as substrate for glucose production by using *strain B*.

1.4 Scopes of Study

In order to achieve the objective, certain scopes have been identified. Firstly, the organic loading rate (OLR) are varied in ranges 5 g/L.d to 30 g/L.d to determine the optimal amount of glucose produced for eight runs. The organic loading rate is related to the substrate concentration with the hydraulic retention time (HRT), while the HRT is related to reactor volume and flow rate. The substrate concentration is directly proportional to organic loading rate value. Furthermore, substrate concentration was the parameter observed from the OLR. Microorganism used is a facultative anaerobe and namely *strain B*, which isolated from the mix culture into single strain. The substrate used in this study is banana stem waste, which collected at plantation in Sabak Bernam, Malaysia. Then, the analysis of glucose was done by using Miller Method (dinitrosalicyclic acid assay). The optimization of glucose production was measured by using One Factor Analysis of Design Expert due to only one factor was studied in this research. Then, the suitability of banana stem to produce glucose was measured by doing comparison with other lignocellulosic material to produce glucose by refer to the literature review.

CHAPTER 2

LITERATURE REVIEW

2.1 Glucose Overview

2.1.1 Properties of Glucose

Glucose ($C_6H_{12}O_6$) as in Figure 2.0 (McMurry and John, 1988) contains six carbon atoms and an aldehyde group and is therefore referred to as an aldohexose. Aldohexose sugars have 4 chiral centers giving 16 optical stereoisomers. 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.

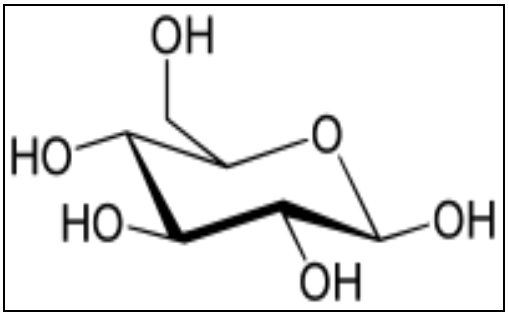

Figure 2.0: Glucose structure in ring form

2.1.2 Application of Glucose

Glucose works important part in the living creatures, such as, cellular function and regulating metabolism. Glucose is used in photosynthesis and it stores the energy. In the plant, the energy stored as the cellulose and glycogen in the vertebrate animal. Glucose is widely used in evolution, the ecosystem and metabolism compare the other monosaccharide such fructose due to the ability of glucose which can form from formaldehyde under abiotic conditions, so it may well have been available to primitive biochemical systems.

Glucose has wide application in the food, textile, brewing and pharmaceutical industries. In food industry, glucose syrups are a vital ingredient in confectionery as they are used to control crystallization, rheology, hygroscopicity, color development, and sweetness. While in the pharmaceutical industry, it mainly used in the medicine preparations, tablet coating and drug formulation, for the example the manufacturing of antibiotic drugs and penicillin (Riddhi Siddhi, 2007).

2.2 Substrate for Glucose Production

2.2.1 Agricultural Crops

The common primary raw materials for the production of glucose are such corn, rice, sago, wheat and cassava as in Figure 2.1 (Aschengreen *et al.*, 1979). Products from hydrolysis of starch such as maltodextrin, corn syrup, glucose syrup and high glucose syrup have a wide application in the food, textile, brewing, and pharmaceutical industries (Griffin and Brooke, 1989). Starch is a polymer of glucose found in most plants and organized in 1-140 µm granules in plants.

In Malaysia, sago starch is considered as one of the most important sources of starch. Wang *et al.* (1996) reported that about 60 million tonnes of sago starch extracted from sago palms are produced per annum in South-east Asia. Attempts have been made to produce glucose from direct conversion of raw starches using the novel raw starch-degrading enzyme to replace conventional methods in glucose syrup production (Yetti *et al.*, 2000). However, the raw sago starch exists as large granules with compact crystalline structure. As a result, the enzyme reaction rate and yield of products from raw sago starch was reported to be too low for industrial application (Wang *et al.*, 1995) and the rate conversion of sago starch to glucose is below 53.3%.

Cassava and sweet potato (Ipomoea batatas L.) are popular root crops of the tropical countries (FAO, 2006). Although, their primary use is as food crops, both the crops are widely used for the production of starch and of late, their role has been increasingly recognized as industrial crops for the production of bioethanol, glucose, HFS (Baskar *et al.*, 2008; Berghofer and Sarhaddar, 1988; Gorinstein, 1993 and Shetty *et al.*, 2007). Glucose was higher in cassava (22–25%) than in the sweet potato (14.0–15.7%).

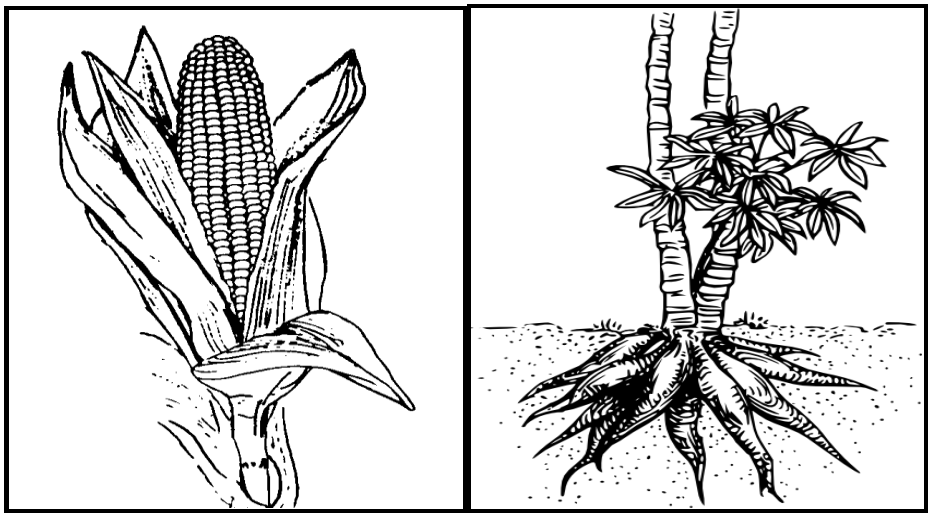

Figure 2.1: The examples of agricultural crops

2.2.2 Agricultural Residue

Agricultural residues are a promising alternative to virgin wood fiber as an industrial feedstock. Residues are abundant, cheap, and their use will yield economic as well as environmental dividends. The most significant division is between those residues that are predominantly dry such as straw and those that are wet such as animal slurry. Biomass, whether as sugar crops, starch crops, or cellulosic materials, provides a unique resource for sustainable production of organic fuels and chemicals that are now primarily made from petroleum. Furthermore, cellulosic materials including agricultural (e.g. banana stem) and forestry (e.g. sawdust) residues and herbaceous (e.g. switch grass) and woody (e.g. poplar trees) crops can be sufficiently abundant to provide a major resource for making commodity products (Charle *et al.*, 2005). The agrowaste including dried leaves and psuedostem after harvest was used as substrate for the release of sugars (Baig *et al.*, 2004) such as oil palm frond, pineapple leaf and banana stem as in Figure 2.2 (Abdul Khalil *et al.*, 2006).

Bananas (Musaceae) are produced in large quantities in tropical and subtropical areas. The total planted area of banana in Malaysia (2001) was 33,704.2 hectares (MAO, 2006). Banana plants range in height from 0.8m to more than 15m. Each contains a flattened, modified stem, called a pseudostem consisting of concentric layers of leaf sheath and crown of large leaves (Ennos *et al*, 2000). After harvesting fruit, the banana stem (pseudostem) is traditionally wasted, as it usually left in the soil plantation to be used as the organic material. From statistic showed that banana stem contains 63.9% of cellulose and less lignin composition which (18.6%) (Abdul Khalil *et al.*, 2006).

Figure 2.2: The examples of agricultural residue

2.2.3 Forestry Residue

Wood is among the most abundant and widely distributed biomass resources. However, due to its complex multi-component structure it is difficult to use it directly as a chemical feedstock. Normally it is first separated into its main components, cellulose, lignin, and hemicelluloses, which are then further processed, while transformations of unseparated wood are less common (Andrej, *et al.*, 2009).

Forestry residue like sawdust is composed of fine particles of wood. This material is produced from cutting with a saw, hence its name. It has a variety of practical uses, including serving as a mulch, or as an alternative to clay cat litter, or as a fuel, or for the manufacture of particleboard. Until the advent of refrigeration, it was often used in icehouses to keep ice frozen during the summer. Historically, it has been treated as a byproduct of manufacturing industries and can easily be understood to be more of a hazard, especially in terms of its flammability. It has also been used in artistic displays and as scatter. It sometimes used in bars in order to soak up spills, allowing the spill to be easily swept out the door. Figure 2.3 (Andrej, *et al.*, 2009) showed the examples of forestry residue.

Figure 2.3: The examples of forestry residue

2.2.4 Herbaceous Residue

Herbaceous energy crops are mostly types of grasses, which are harvested like hay. Perennial grasses, such as switch grass, miscanthus, bluestem, elephant grass, and wheatgrass could all potentially be grown as energy crops as in Figure 2.4. These grasses re-grow from their roots and therefore do not require replanting for long periods of time (15 years or more). Switch grass has become a main focus for research over other types of energy crops because yields are higher and production costs lower. One reason switch-grass has lower production cost is that standard farming equipment can be used for cutting and baling. Another benefit of switch grass over other types of energy crops is its drought tolerance and adaptability to many types of soils and climates (Kelly Launder, 2002).

The main components of these types of biomass are the cellulose, hemicelluloses and the lignin. Cellulose is the most common form of carbon in biomass, accounting for 40%-60% by weight of the biomass, depending on the biomass source. It is a complex sugar polymer, or polysaccharide, made from the six-carbon sugar, glucose. Its crystalline structure makes it resistant to hydrolysis, the chemical reaction that releases simple, fermentable sugars from a polysaccharide. Hemicellulose is also a major source of carbon in biomass, at levels of between 20% and 40% by weight. It is a complex polysaccharide made from a variety of five- and six-carbon sugars. It is relatively easy to hydrolyze into simple sugars but the sugars are difficult to ferment to ethanol. Then, lignin is a complex polymer, which provides structural integrity in plants. It makes up 10% to 24% by weight of biomass. It remains as residual material after the sugars in the biomass have been converted to ethanol. It contains a lot of energy and can be burned to produce steam and electricity for the biomass-to-ethanol process (Kelly Launder, 2002).

Unlike many traditional crops, switchgrass is a perennial so it does not need to be planted each year. Once established it can be harvested up to twice a season. Switchgrass reaches full yield capacity after 3 years. Its permanent root system can extend over 10 feet into the ground and coupled with its large temporary root system it can improve soil quality through increased water infiltration and "nutrient-holding capacity" (David Bransby, 1999).

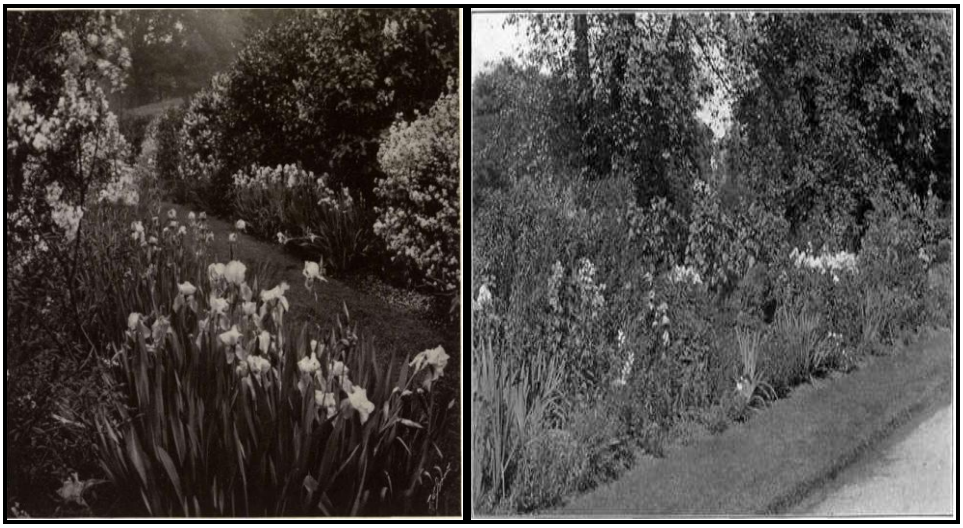

Figure 2.4: The examples of herbaceous residue

2.2.5 Selection of Substrate for Glucose Production

Based on the above type of substrates, the agricultural residue has been chosen in glucose production. The main reasons are because mostly the agricultural residue is non-edible part of plant by human and their cellulose and hemicelluloses is high. Then, the residues also are abundant, cheap, and their use will yield economic as well as environmental dividends.

2.3 Hydrolysis Process

Hydrolysis (Figure 2.5) is a chemical process in which a molecule is cleaved into two parts by the addition of a molecule of water. One fragment of the parent molecule gains a hydrogen ion (H+) from the additional water molecule. The other group collects the remaining hydroxyl group (OH−) (Victor Gold,1987).

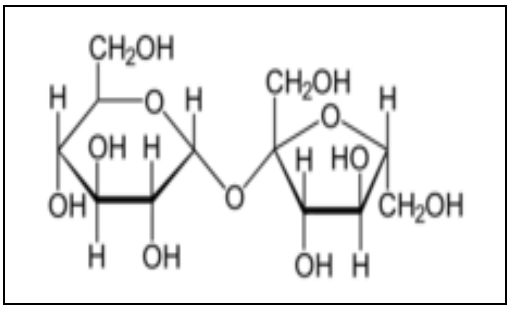

Figure 2.5: Sucrose hydrolysis

During the hydrolysis not only free sugars are formed, but also inhibitors. Examples of inhibitors are: furfural, 5-hydroxymethyl furfural (HMF), carboxylic acids and phenolic compounds. Two of the most important inhibitors are furfural and HMF as in Figure 2.6 (Taherzadeh *et al*., 1999).

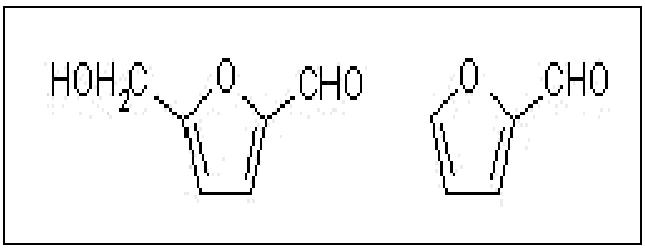

Figure 2.6: Examples of inhibitor structure

2.3.1 Acid or Alkali Hydrolysis

Acid-base-catalyzed hydrolyses are very common; one example is the hydrolysis of amides or esters. Their hydrolysis occurs when the nucleophile which a nucleus-seeking agent, such water or hydroxyl ion attacks the carbon of the carbonyl group of the ester or amide. In an aqueous base, hydroxyl ions are better nucleophiles than dipoles such as water. In acid, the carbonyl group becomes protonated, and this leads to a much easier nucleophilic attack. The products for both hydrolyse are compounds with carboxylic acid groups (Victor Gold, 1987).

2.3.2 Enzymatic Hydrolysis

Cellulase 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). Cellulases are relatively costly enzymes, and a significant reduction in cost will be important for their commercial use in biorefineries. Cellulase based strategies that will make the biorefinery processing more economical include; increasing commercial enzyme volumetric productivity, producing enzymes using cheaper substrates, producing enzyme preparations with greater stability for specific processes, and producing cellulases with higher specific activity on solid substrates.

Currently, most commercial cellulases (including β- glucosidase) are produced by *Trichoderma* species and *Aspergillus* species (Cherry and Fidantsef, 2003). Cellulases are used in the textile industry for cotton softening and denim finishing, in the detergent market for color care, cleaning, and anti-deposition, in the food industry for mashing, and in the pulp and paper industries for deinking drainage improvement, and fiber modification (Cherry and Fidantsef, 2003). The cellulase market is expected to expand dramatically when cellulases are used to hydrolyze pretreated cellulosic materials to sugars, which can be fermented to commodities such as bioethanol and bio-based products on a large scale (Cherry and Fidantsef, 2003).

2.3.3 Biological Hydrolysis

Recently, most of the bio-waste product such as banana stems was degraded through fermentation. The bacterium was isolated from the same area with the bio-waste product in order to produce polymer, monomer, and other intermediates from cellulose materials. The recent thrust in bioconversion of agricultural and industrial wastes to chemical feedstock has led to extensive studies on cellulolytic enzymes produced by fungi and bacteria. The investigations into ability of microbes to degrade native and modified cellulose so far have revealed that only a few fungi possess ability to degrade native cellulose. A majority of microbes can however degrade modified cellulose. The cost of carbohydrate raw material influences the economy of many fermentation processes, hence the cost play a decisive role in future and scope of industries employing fermentation processes (Castellanos *et al.*, 1995).

2.3.4 Selection of Hydrolysis Process

Based on the above hydrolysis process, the biological hydrolysis has been chosen due to the potential for the production of enzymes by the microbe itself. Compare to the other acid or alkaline hydrolysis, it will disrupt the cell in the medium and make the fermentation less efficient. Then, in enzyme hydrolysis, the process will become more sensitive or specific due to several conditions needs to take in consideration which pH and temperature, and the most disadvantage is the enzyme is very costly to be obtained while in the biology hydrolysis, the process is more natural which mean without the addition of enzyme and lesser cost.

2.4 Bioreactor

Fermentation tanks, also called bioreactors, used for industrial fermentation processes are glass, metal or plastic tanks, equipped with gages and settings to control aeration, stir rate, temperature, pH and other parameters of interest. Units can be small enough for bench-top applications 5-10 L or up to 10,000 L in capacity for large-scale industrial applications. Fermentation units such as these are used in the pharmaceutical industry for the growth of specialized pure cultures of bacteria, fungi and yeast, and the production of enzymes and drugs. The fermentation can be done in a batch, fed-batch or continuous process.

2.4.1 Batch Bioreactor

In this type of bioreactor, the medium and inoculums are loaded in the beginning and the cells are allowed to grow. There is no addition or replacement of medium and the entire cell mass is harvested at the end of incubation period. The characteristic features of the bioreactor system such continuous depletion of medium, accumulation of cellular wastes, alterations in growth rate and continuous change in the composition of cells.

2.4.2 Fed Batch Bioreactor

Fed-batch offers many advantages over batch and continuous cultures. From the concept of its implementation it can be easily concluded that under controllable conditions and with the required knowledge of the microorganism involved in the fermentation, the feed of the required components for growth and other substrates required for the production of the product can never be depleted and the nutritional environment can be maintained approximately constant during the course of the batch.

Then, in a fed-batch fermentation, no special piece of equipment is required in addition to that one required by a batch fermentation, even considering the operating procedures for sterilization and the preventing of contamination. Fed-batch might be the only option for fermentations dealing with toxic or low solubility substrates. Additionally, the process also allows the replacement of water loss by evaporation and decrease of the viscosity of the broth such as in the production of dextran and xanthan gum, by addition of a water-based feed (McNeil and Harvey,1990).

The use of fed-batch culture by the fermentation industry takes advantage of the fact that the concentration of the limiting substrate may be maintained at a very low level, thus avoiding repressive effects of high substrate concentration and controlling the organism's growth rate and consequently controlling the oxygen demand of the fermentation (Mcneil and Harvey, 1990).

2.4.3 Continuous Bioreactor

The continuous bioreactor generally starts off like a batch reactor. When it reaches a critical point (usually determined by cell mass), it is switched to a continuous mode of operation. Nutrients are added and product is harvested continuously. This provides a higher yield, since the reactor is running at full capacity for longer. But it does bring some risks for contamination, and some added complexity to the operation and automation (Buckbee and Alford, 2008). The continuous stirred-tank reactor (CSTR) or backmix reactor, is a common ideal reactor type in chemical engineering. A CSTR often refers to a model used to estimate the key unit operation variables when using a continuous agitated tank reactor to reach a specified output.

2.4.4 Selection of Bioreactor

Based on the above bioreactor, fed batch reactor has been chose for the glucose production. From the previous study showed that, the productivity of fed-batch fermentation per cell biomass inoculated was at least 30 % higher than that of batch fermentation (Seong and Cha, 1982). In batch, the accumulation of cellular wastes, will effect to the product, so it is no efficient. In fed batch process, the final fermentor volume will reduce, thus contribute to enhance the productivities of glucose. Then, the advantage of fed batch process is that high concentration of inhibitors can be avoided and there is no loss of substrate or microorganisms compare to continuous reactor which allow the flow out of the substrate of microorganism.

CHAPTER 3

METHODOLOGY

3.1 Material

3.1.1 Raw Material

Banana stem waste was obtained from the banana plantation at Sabak Bernam, Malaysia. The total solid and moisture were determined by using the standard formula.

3.1.2 Microorganism and Conditions

The *strain B* was isolated from the mix culture which isolated from fields where banana was cultivated .The bacterium is a facultative anaerobic microbe and was cultivated for two days in incubator at 30 ^{o}C.

3.2 Method of Analysis

3.2.1 Standard Calibration Curve Preparation

A standard calibration curve is a method that can determine the unknown concentration glucose in a glucose solution sample by comparing the unknown standard sample of known glucose concentration sample. The calibration curve was prepared in glucose concentrations which are 0.0 g/L, 0.2 g/L, 0.4 g/L, 0.6 g/L, 0.8 g/L, 1.0 g/L. The readings for absorbance glucose are taken for each concentration and the graph of absorbance readings was plot against the glucose concentration. The measurement of absorbance was taken by using UV-visible spectrophotometer as in the figure below.

Figure 3.1: UV-visible spectrophotometer

Figure 3.2: The absorbance readings of glucose sample

3.2.2 Preparation of Nutrient Agar

Nutrient agar is a complex medium because it consists of ingredient with unknown amount or types of nutrients. The advantage of complex media is that they support the growth of a wide range of microbes. The nutrient agar is prepared as the nutrient media for the growth of microbes. 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 500 ml of agar solution, 10 g of agar is needed. The pH of solution is checked, it should be 7.0. Then, with using the Bunsen burner, the solution medium is heated to dissolve the agar well. While boiling the agar, keep rotating the flasks to prevent the agar from cooking onto the bottom of the flasks and also make sure the capped of Schott bottle is loosen. Before undergo the autoclave

process, the capped of Schott is covered with aluminum foil and tape. The autoclave process is about 2 hours with 120 - 121 ^{o}C of temperature. As soon as it autoclaved, the agar is poured about one-fourth into the petri dish. After the agar became solid, the lid of petri dish is sealed and transferred to the chiller.

3.2.3 Preparation of Nutrient Broth

The broth is prepared by mixture of nutrient broth with distilled water. For one liter broth solution, 8 g of nutrient broth is needed. Firstly, distilled water is pour into the flask about one half of flask. Then the nutrient powder was transferred into the flask and the distilled water was added until become one liter of solution. The mouth of the flask was covered by using cotton wool. The solution was heated to dissolve the solution well and enhanced the stirring by using magnetic stirrer. After heating, the solution was left cooled and prepared for autoclave process. Duration of the autoclave is about two hours at 121^{o}C. Then, the broth bottle will transfer to chiller for the next step.

3.2.4 Transfer of Culture via Streaking Method

Bacteria must be cultured in order to facilitate identification and to examine their growth and metabolism. Bacteria is inoculated, or introduced, into various forms of culture media in order to keep them alive and to study their growth. The microbial culture is started when the inoculation loop is flamed to redness and cool for few minutes. The inoculating loop is gently hold and touch the surface of plate as in the Figure 3.3. Then, one edge of petri dish cover is lifted to streak the plate and the first sector is streaked by making as many streaks as possible without overlapping

previous streaks. After the first sector finished, the second sector is started which the loop is flamed to redness and let it cool. Then, the plate is turned again and the loop is streaked through one area of second sector. The third sector is proceeding after the second sector by streak through one area of the third sector and also the remaining area of the agar surface. Then, all the process must be done near the bunsen burner in order to reduce contamination to the microbe growth (*strain B*). After the cultural process done, the petri dish is incubated at 30 ^{o}C for 24 to 48 hours and placed in an inverted position in incubator. After two days, the colony of bacteria was obtained as in Figure 3.4.

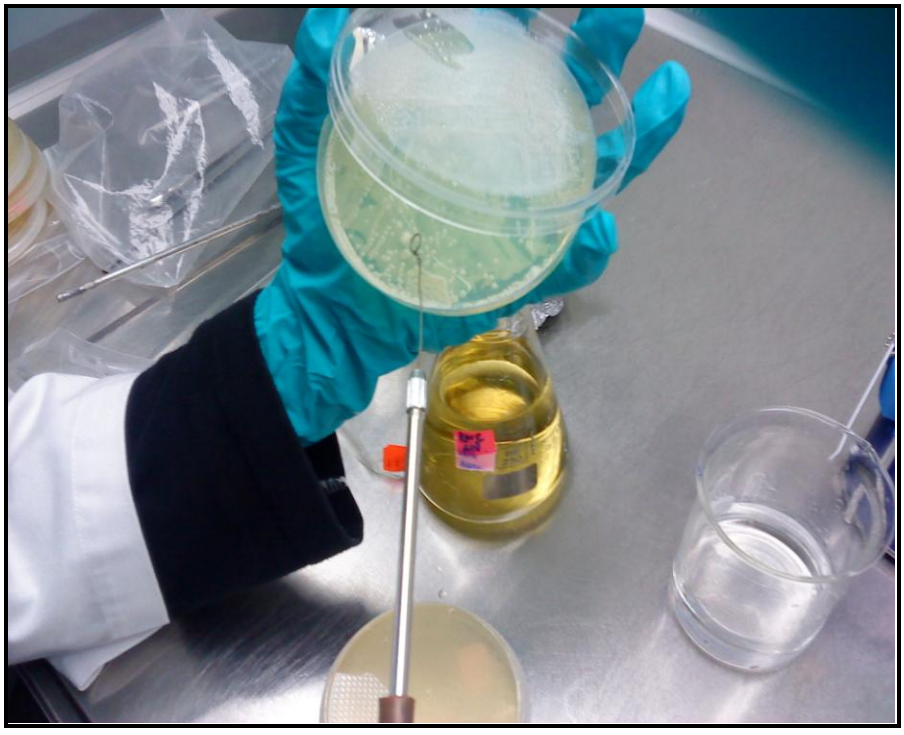

Figure 3.3: Process of streaking bacteria

Figure 3.4: Growth of *strain B* after two days

3.2.5 Transfer of Culture from Agar Plate to Broth Bottle

The microbial culture was transferred with careful in order to avoid the contamination to the microbe growth. Firstly, the inoculating loop is flamed until it is red and let it cool. Then, aseptically a loop of broth culture is obtained from the Petri dish of broth culture of bacteria. The inoculating loop must be hold in the dominant hand, while the other hand is opened the broth bottle cap and the mouth of the bottle is flamed to avoid contamination and recap it. Again, the broth bottle cap is opened and flamed the mouth of the bottle. By using the inoculating loop, the slant is inoculated by moving the loop gently across the agar surface from the bottom of the slant to the top. While doing this step, make sure the agar is not gouge and the mouth of bottle is flamed and the cap is replaced. Then, the broth bottle is incubated at 30 ^{o}C for 24 to 48 hours as in Figure 3.5.

Figure 3.5: Broth media placed in incubator at 30 ^{o}C

3.2.6 Estimation of Total Solid Content in Banana Stem Waste

Total solid test is to determine the amount of solids remaining after heating the sample at 103 ^{o}C – 105 ^{o}C to constant weight. The total solid test was started when the weighing dish is placed in the drying oven (103 ^{o}C–105 ^{o}C) for a minimum of four hours; the dish is cooled after heating, weighed and keeps in a desiccators. Then, thoroughly mix the sample (chopped banana stem) and weigh out an appropriate amount to the nearest 0.1 mg, into the weighing dish. The weight of sample plus weighing dish is recorded. Then, the sample was placed into the convection oven for a minimum of four hours. After that, the sample is removed from the oven and allowed it cool in the room temperature in the desiccators. The dish containing the oven-dried sample is weighed to the nearest 0.1 mg and the weight is recorded. Then, the sample is placed back into the convection oven and

dries to constant the weight of sample. Figure 3.6 and Figure 3.7 showed the structure before and after heating banana stem for 24 hours.

Determine the total solid is using the following formula:

$$\% \text{ Total solid} = \frac{\text{Weight dry pan + dry sample} - \text{Weight dry}}{\text{Weight sample as received}} \times 100 \quad (3.1)$$

$$\text{mg total solids/L} = \frac{(A - B) \times 1000}{\text{Sample volume, ml}} \quad (3.2)$$

Where;

A = weight of dried residue + dish, mg, and

B = weight of dish, mg.

Figure 3.6: Banana stem waste before heating at 105 ^{o}C

Figure 3.7: Banana stem waste after heating at 105 ^{o}C

3.2.7 Influent Substrate Preparation

Banana stem waste was chopped into small pieces and blended into small particles as in Figure 3.8. After that, banana stem was weighed depend on each run usage. The mass of substrates of each run was 4.5, 0.75, 0.75, 2.625, 3.562, 1.687, 2.625 and 4.5 g respectively. Then, the substrates were autoclaved for 2 hours at 121 ^{o}C as in Figure 3.9. The contact volume for the first day influent of substrate is 150 ml, while for the second day until thirty days the contact volume used is 50 ml.

The formula used to calculate the mass of substrate;

$$\text{Mass Biomass} = [\text{Biomass}] \times \text{Contact Volume} \tag{3.3}$$

The value of biomass concentration is measured by using formula;

$$\text{Organic Loading Rate} = \frac{[\text{Biomass}]}{\text{Hydraulic Retention Time}} \tag{3.4}$$

Figure 3.8: Banana stems being blended to the fine particle

Figure 3.9: Banana stem after weighed and autoclaved

3.2.8 Experimental Set Up

Before running the experiment, the review on optimum organic loading rate in fermentation was done. From the literature review, the range organic loading rate chose was 5 g/L.d until 30 g/L.d. Based on the range, the experimental design was done by using One Factor Analysis in Design Expert. Below is the table of results from the experimental design;

Table 3.1: The experimental design of organic loading rate

Run	Block	Factor 1: OLR (g/L.d)
1	Block 1	30
2	Block 1	5.0
3	Block 1	5.0
4	Block 1	17.50
5	Block 1	23.75
6	Block 1	11.25
7	Block 1	17.50
8	Block 1	30.0

3.2.9 Fermentation Procedure

Mode of this fermentation is fed batch which 8 fermentation runs were performs simultaneously for thirty days continuously. Each run have the differ value of organic loading rate (OLR). For each runs, used the inoculums at 1.0 % (v/v) of the basal medium. The working volume for each run was 150 ml from 250 ml of

shake flask and each runs have their own substrate concentration. After the cell, substrates, and water were added in the flask, the nitrogen was purged into the flask about 10 minutes for the anaerobic condition. After that, all the samples were placed into the stackable incubator shaker at temperature 30 ^{o}C with revolutions per minute (rpm) at 150 as in Figure 3.10. After one day incubated, the sample was collected about 50 ml in order to proceed with fed batch fermentation. Then, new 50 ml solution contains differ substrate concentration was added into each flask. From 50 ml sample collected, 1 ml was taken for reducing sugar analysis. Then, the sample in flask was purged again with nitrogen before incubated back. The procedures were repeated for 30 days.

Figure 3.10: The shake flask incubated at 30^{o}C and 150 rpm

3.2.10 Dinitrosalicyclic Acid (DNS) Assay

A dinitrosalicylic acid (DNS) assay has been available since 1955 and is still useful for the quantitative determination of reducing sugars. DNS reagent was prepared by mixture of 3,5-dinitrosalicyclic, sodium hydroxide, sodium potassium tartrate and water. For one liter of DNS reagent, 10 g of 3,5-dinitrosalicyclic, 16 gram of sodium hydroxide, and 300 g of sodium potassium tartrate and water were needed. After the glucose sample was collected, 1 ml of DNS reagent was added to 1 ml of glucose sample in lightly capped test tube, in order to avoid the loss of liquid due to evaporation, the test tube was covered with a piece of paraffin film. After that, the samples in test tubes were heated at 90^{o}C for 10 minutes to develop the red brown color as in Figure 3.10 and Figure 3.11. After the sample is cool to room temperature, the absorbances of samples were recorded using UV visible spectrophotometer at 540 nm.

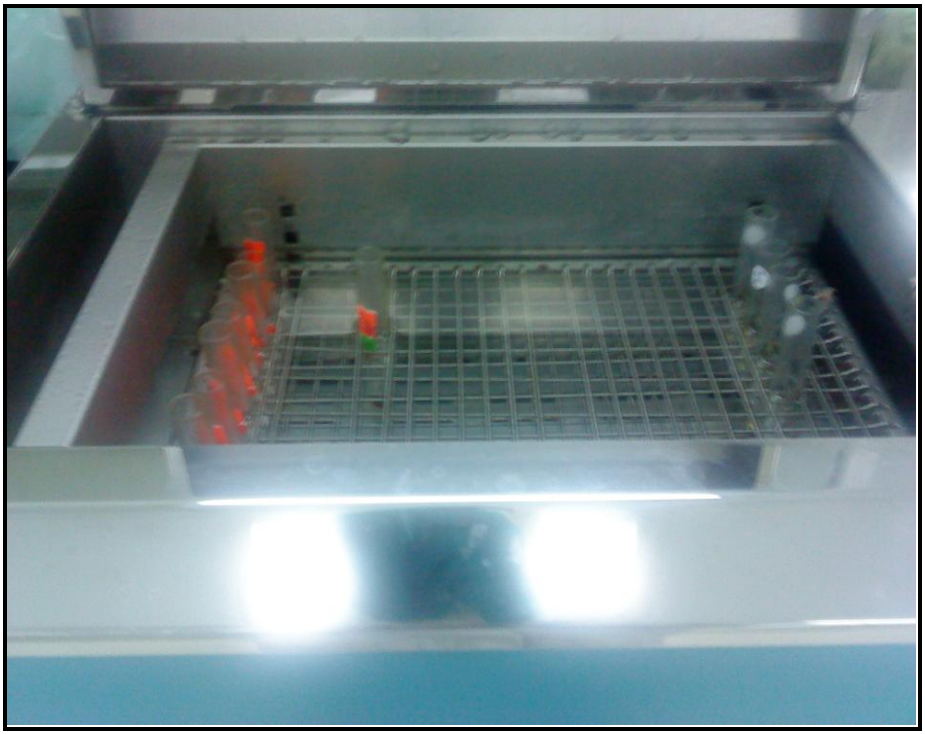

Figure 3.11: The samples were heated in water bath at 90-100 ^{o}C

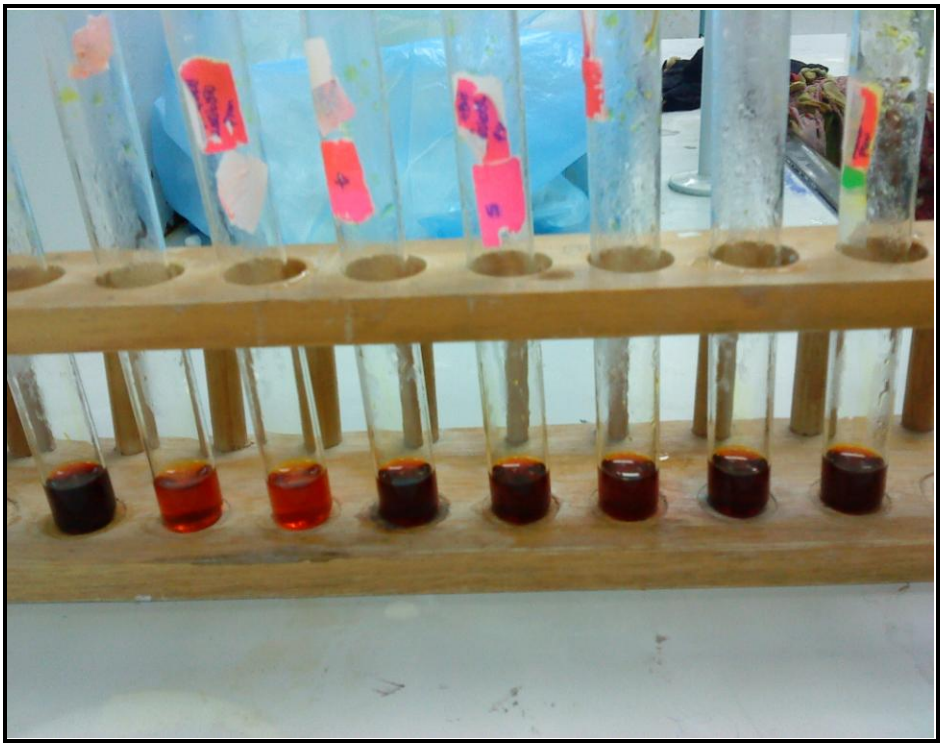

Figure 3.12: Reddish brown color was appeared after heated

CHAPTER 4

RESULT AND DISCUSSION

4.1 The Standard Calibration Curve for Glucose

Based on the Figure 4.1, it is shown that as the glucose concentration is increased, the reading of the absorbance or optical density (OD) also directly increased. The graph pattern is linear with correlation 0.998. This standard curve is important in observation the unknown concentration glucose in a glucose solution sample by comparing the unknown sample glucose with the prepared standard calibration glucose as below.

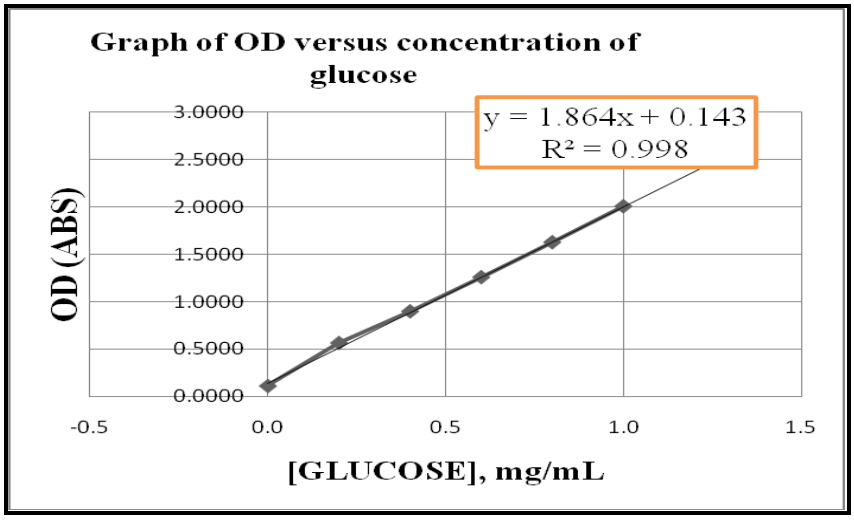

Figure 4.1: The optical density versus glucose concentration

4.2 Total Solid Test

Based on the data at Table 4.1, the calculated total solid amount in banana stem is 27.6 % with the moisture content 72 %. From this test, the total solid and moisture of substrate can be fixed to avoid error in glucose yield.

Table 4.1: Data for total solid test

Item	Amount (gram)
Weight dry pan	2.5437
Weight dry pan + fresh sample	20.2302
Weight dry pan + dried sample	7.4327

4.3 Effect of Organic Loading Rate towards Glucose Production

From the Figure 4.1 shown that, at the lowest value of organic loading rate at 5.0 g/L.d, the yield of glucose is the highest with 0.0593 g.glucose/g.substrate produced. Then, at the second 11.25 g/L.d OLR seems that the yield of glucose is reduced to 0.0446 g.glucose/g.substrate. Next, for the OLR at 17.5, the yield of glucose produce is 0.0455 g.glucose/g.substrate while at the 23.75 g/L.d the yield of glucose is also reduced with increasing of OLR value, which yield 0.0426 g.glucose/g.substrate. Lastly, at the highest value of OLR seems that the yield of glucose is increased to 0.0492 g.glucose/g.substrate produced for the thirty days experimental. Organic loading rate (OLR = influent substrate concentration/HRT) can influence glucose yield in fermentation but there is controversy in the literature as to whether higher yields can be achieved with lower or higher OLRs (Shen *et al.*, 2009). It was assumed that the accumulation of fermentation products at high OLR inhibited the yield of production. Several studies have found that great amount of Volatile Fatty Acids (VFAs) such as acetic acid, propionic acid, butyric acid and CO_2 produced from highly concentrated influent inhibited metabolic activities of anaerobic bacteria (Taguchi *et al.*, 1995; Zoetemeyer *et al.*, 1982; van den Heuvel *et al.*, 1988). Since glucose is intermediate product of fermentation, it is reasonable that glucose yield became lower when the metabolic activities of bacteria got inhibited at high OLR.

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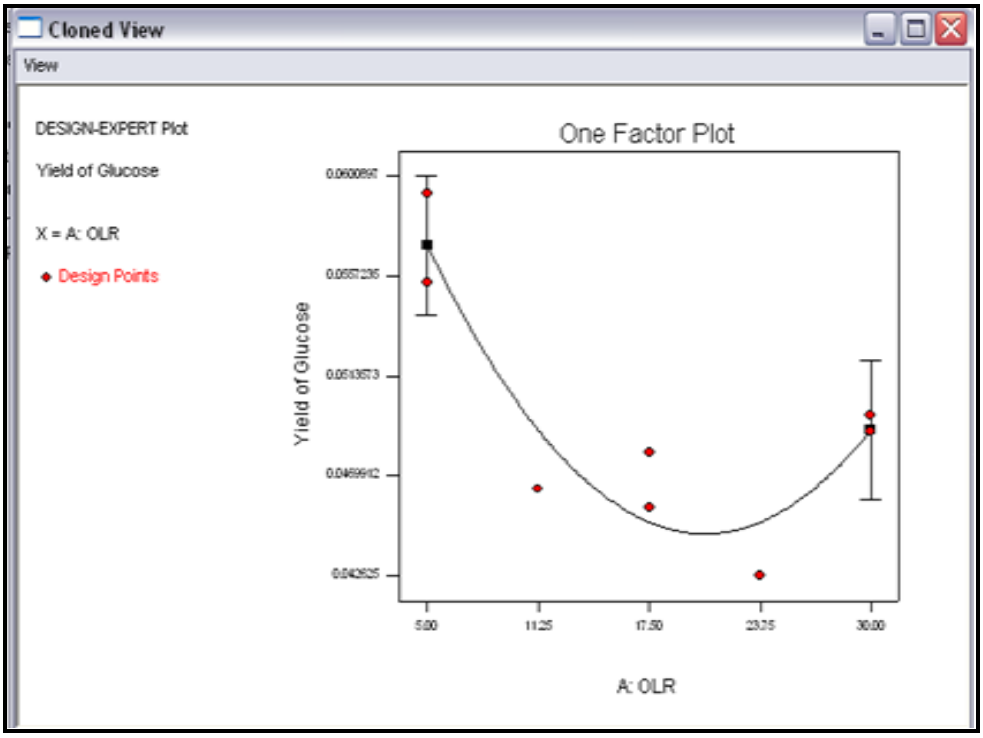

Figure 4.2: The effect of OLR towards yield of glucose

4.3.1 Effect of Yield Glucose towards Time

From Figure 4.3, at run 1 and run 8 the value of their OLR are the same which 30 g/L.d. At run 1, the highest yield of glucose is at 0.076 g.glucose/g.substrate and the lowest is at 0.034 g.glucose/g.substrate. While for the run 8, the highest glucose can produced is 0.072 g.glucose/g.substrate and the lowest is at 0.036. From the theoretical, the value of yield glucose for run 1 and 8 are supposedly same due to the similar OLR. But then, from this research seems that run 1 is much efficient compare to run 8. However, both of run have the close value of yield, as can sees the yield of glucose is almost same at last six days for both run. By increasing the days, the yield of glucose is more stable which no more high fluctuation on the last days of experiment. The stability of yield glucose is observed at 0.042 g.glucose/g.substrate.

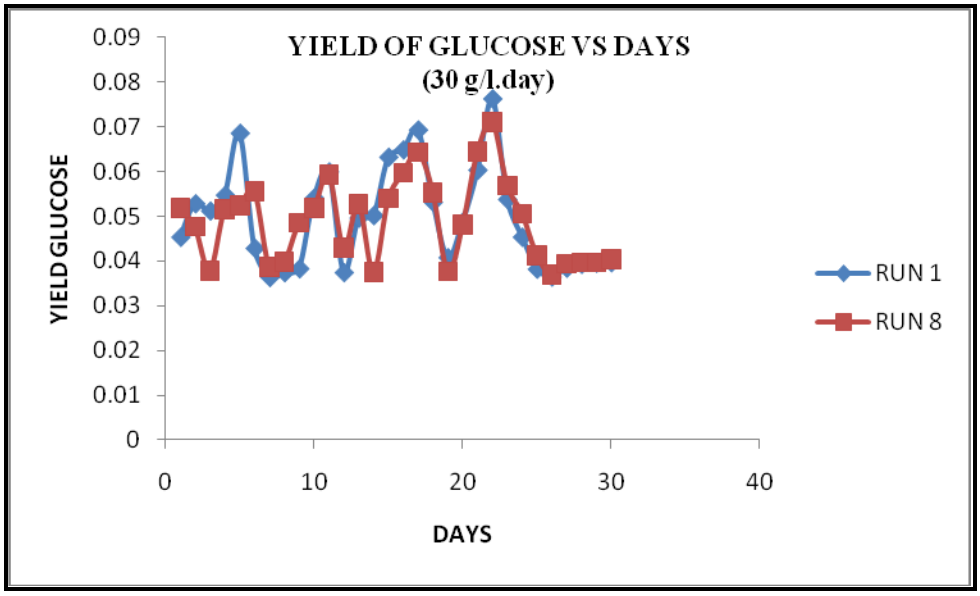

Figure 4.3: Yield of glucose for OLR:30 g/L.d versus days

From the Figure 4.4, the graph pattern is same as the first OLR. In run 5, the value of OLR is 23.75 which the second highest of OLR in this experiment. The highest yield can produce at this OLR is about 0.06 g.glucose/g.substrate and the lowest yield is about 0.03 g.glucose/g.substrate. By increasing the days, the stability in the yielding of glucose is obviously with there is no large fluctuation on the last six days. The stability of yield glucose is observed at the 0.03 g.glucose/g.substrate.

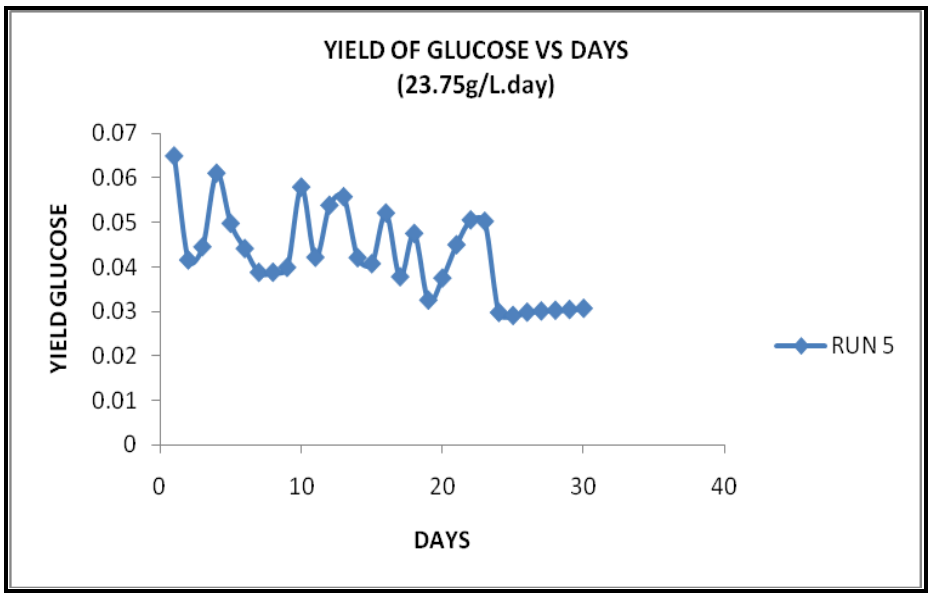

Figure 4.4: Yield of glucose for OLR: 23.75 g/L.d versus days

Based on the Figure 4.5, run 4 and run 7 have the similar value of OLR 17.50 g/L.d which the third highest OLR in this experiment. As we can sees, the highest glucose can produced for the run 4 is 0.12 g.glucose/g.subtrate while the lowest can yield is 0.024 g.glucose/g.substrate. Then, for run 7 the highest glucose can produce is at 0.088 g.glucose/g.substrate while the lowest yield is at 0.02 g.glucose/g.substrate. The trend of glucose yield is not follow the theoretical which supposedly the same amount of OLR should have the same amount of yield glucose. As increasing the days, the pattern of glucose produce is much more stable with no high fluctuation in the process and give yield about 0.038 g.glucose/g.substrate at the end of fermentation process.

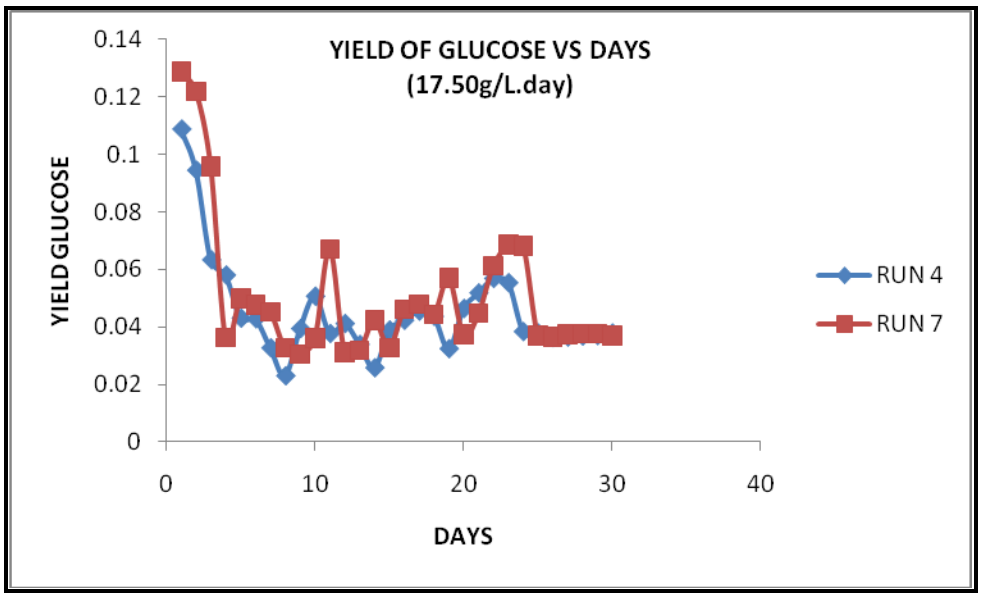

Figure 4.5: Yield of glucose for OLR:17.50 g/L.d versus days

From Figure 4.6, shown the effect of glucose yield with 11.25 g/L.d OLR as time increased. This value 11.25 g/L.d is the second lowest OLR in this experiment. The highest glucose can produced at this OLR is 0.064 g.glucose/g.substrate on the day 22 while the lowest yield is at 0.024 g.glucose/g.substrate on the day 12. The fluctuation trend of the glucose yield is due to the process not reach the stability yet. By increasing the days, seems that the large fluctuate is not exist anymore. The yield of glucose almost reached the stability at the end of process which gives yield 0.042 g.glucose/g.substrate produced.

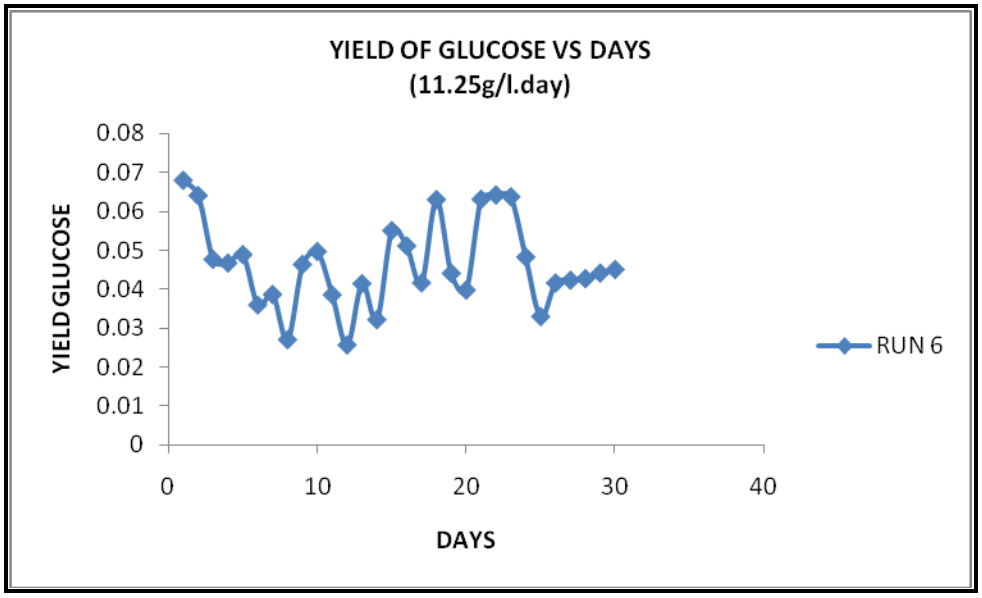

Figure 4.6: Yield of glucose for OLR:11.25 g/L.d versus days

From Figure 4.7, the OLR used is 5 g/L.d for run 2 and 3 which the lowest range of OLR in this experiment. Then, the highest glucose can produced at run 2 is 0.110 g.glucose/g.sbstrate while the lowest yield for run 2 is at 0.02 g.glucose/g.substrate. In the run 3, the highest glucose produced is at 0.082 g.glucose/g.substrate while the lowest at 0.004 g.glucose/g.substrate. By increasing the days, the yield of run 2 and 3 are become more similar and stable. The yield at the end of process give yield about 0.08 g.glucose/g.substrate.

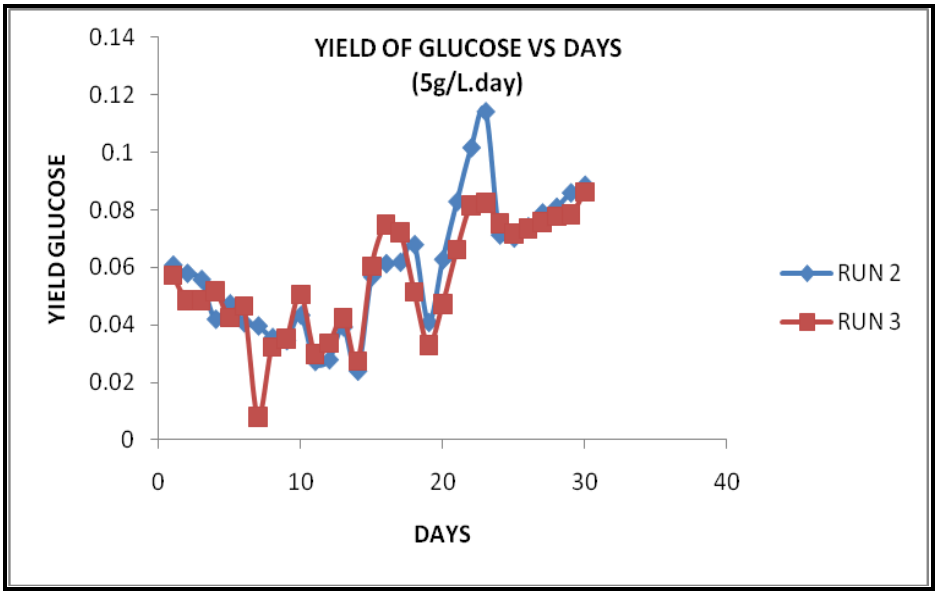

Figure 4.7: Yield of glucose for OLR 5 g/L.d versus days

Refer to the overall graph in Figure 4.8, seems that the pattern of the yield glucose is change as time increased. Then, the most amount glucose yield for OLR at range 5-30 g/L.d is in 0.03 g.glucose/g.substrate – 0.07 g.glucose/g.substrates. At the ends of days limit, the yield of glucose for each OLR had shown the differences among each other. For the 5 g/L.d (run 1 and 8) the yield of glucose is the highest and reduce to 11.25 g/L.d (run 6), 30 g/L.d (run 1 & 8), 17.50 g/L.d (run 4 & 7) and 23.75 g/L.d (run 5) respectively. The trend of this graph is due to the activity of bacteria itself. At the initial day, yield of glucose is high, due to availability of food source such peptone and beef extract in broth media. When the bacteria are introduced to fresh medium, usually no immediate increase in cell number occurs, so the bacteria at the initial consume the available food source itself until the food source limited (Eshwaranv, 2007). Thus, that is why the yield of glucose high at the initial day.

As the day increased, bacteria are growing and dividing at the possible maximal rate which called exponential phase of bacteria growth. In this stage, the bacteria are doubling in number at regular interval. As the population of bacteria increased and no carbon source is supplied, the bacteria use some of the glucose

yield from the hydrolysis process in order to growth. Badger (2002) also claims that some of glucose was used by bacteria in order to growth due to yield production of ethanol is less than 100 % compare to theoretically which supposed can yield 100% ethanol. This statement can support on the pattern of reducing glucose yield as the time increased.

At the last day seems that the yield of glucose increased back, this is probably due to the sufficiently high nutrient levels consume by microbe and also the total microorganism remains constant. This growth phase called as stationary phase. .Within this phase, the population growth also may cease due to the accumulation of toxic waste product such as carbon dioxide (Eshwaranv, 2007). When the population of bacteria decreased, the glucose yield can be increased. This support the trend which glucose yield is little increase as the days increased.

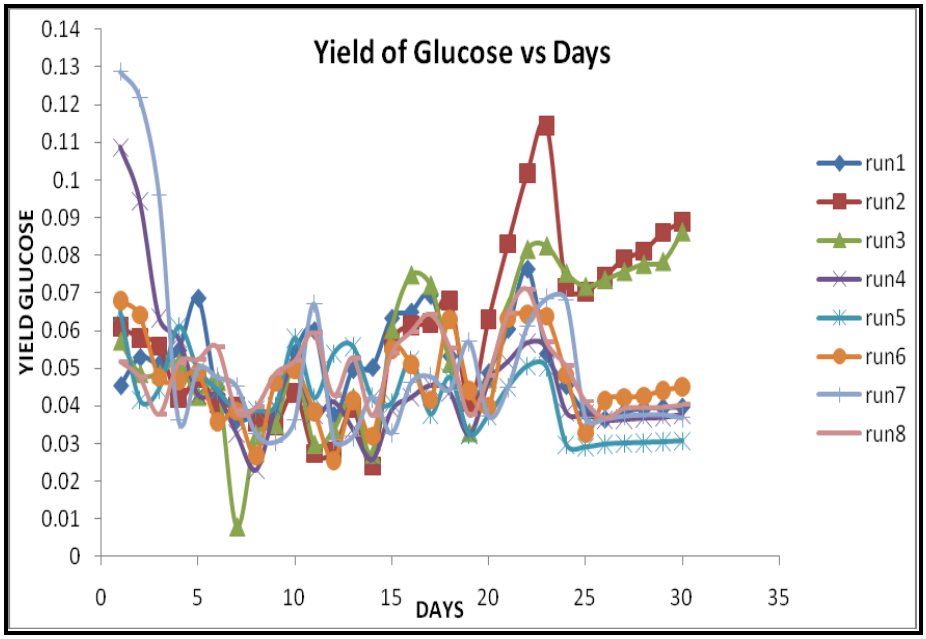

Figure 4.8: Yield of glucose for overall run versus days

4.4 Optimization of Glucose Production by Experimental Design

From Figure 4.9, the OLR value and yield glucose were set up in order to identify the optimum OLR within production of glucose The OLR range is set from 5g/L.d -30 g/L.d while the range of yield of glucose is 0.0426 g.glucose/g.substrate to 0.0593 g.glucose/g.susbstrate. The solutions from the experimental design showed that the optimum OLR,is at 5.00 g/L.d with desirability 86%. The glucose at 5.00 g/L.d is at the maximum; hence those OLR is selected as the optimum value.

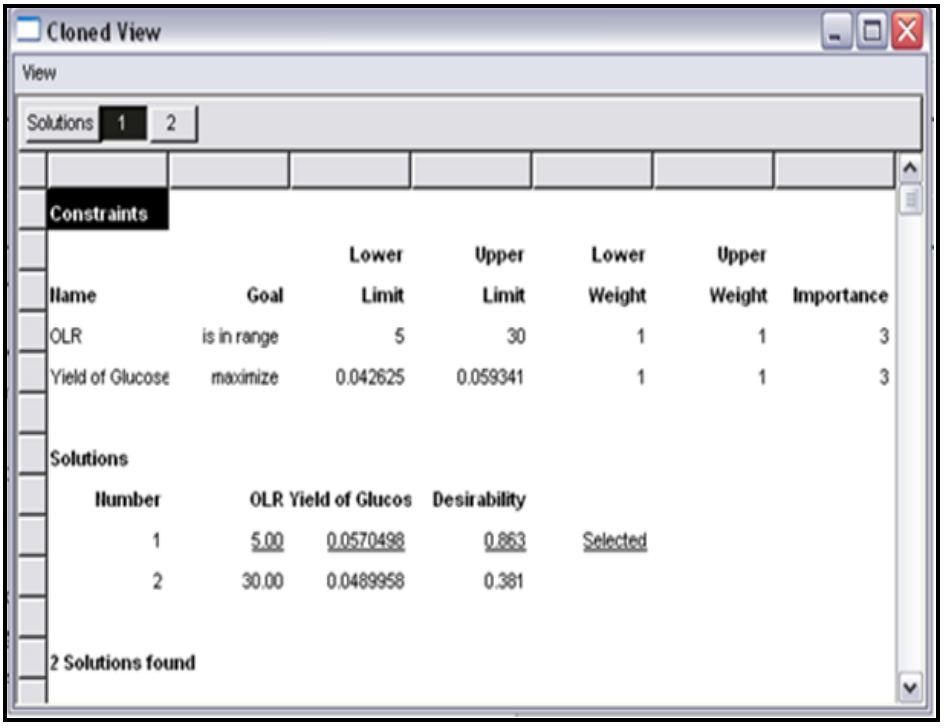

Figure 4.9: Optimization of yield glucose

4.5 Determination the Suitability of Banana Stem

Based on the Table 4.2, showed that the yields of glucose when variety of agricultural waste used as the substrate in fermentation process such wheat straw, sugarcane bagasse, corn cob, and rice hull. Their yield of glucose are 0.031 g.glucose/g.substrate , 0.0765 g.glucose/g.substrate, 0.08 g.glucose/g.substrate and 0.099 g.glucose/g.substrate respectively. However, in this research the yield of glucose when using banana stem waste as the substrate is 0.0593 g.glucose/g.subtrate. This yield is in the range of the other substrate. Hence, the suitability of banana stem waste with using *strain B* can be considered as suitable.

Table 4.2: Summary of yield glucose from variety agro waste

Types of Substrate	**Process**	**Substrate Concentration (g/l)**	**Glucose Concentration (mg/ml)**	**Yield Glucose [glucose] / [substrate]**	**Study**
Wheat Straw	Submerged Fermentation	40	1.25	0.031	Singh *et al*.,2009
Sugarcane Bagasse	Saccharification using acid pretreatment and followed by enzymatic	400	30.6	0.0765	Geddes *et al*.,2009
Corn Cob	Saccharification by using enzymatic	13.5	1.1	0.08	Okeke and Obi,1994
Rice Hull	Saccharification by using enzymatic	13.5	1.34	0.099	Okeke and Obi,1994

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The difference value of OLR affects the glucose yield. In this research, the OLR at the lowest range which 5 g/L.d give the highest yield of glucose, while the highest range of OLR which 30 g/L.d give the less yield of glucose. It was assumed that the accumulation of fermentation products at high OLR inhibited the yield of production. That is why the high OLR have low yield glucose. The high concentration of influent substrate at high OLR cause production of high amount VFAs were inhibited the metabolic of anaerobic bacterial. Thus, reduced to yield glucose and vice versa happen to the less concentration influent substrate. The optimum of the OLR for this research is at 5 g/L.d, due to high yield produced within experiment. Then, the suitability of banana stem waste degraded by strain B is proved by obtained the yield as similar the other lignocellulosic materials. As conclusion, all the objectives of this research were achieved.

5.2 Recommendation

Based on the result and conclusion of this study, some recommendations for future work compromise of certain aspect. In order to make this study more interesting, more parameter should be study to observe the effect towards yield of glucose such as particle size, cell concentration and other related. From this, the variety of yield glucose can obtained and recorded. Then, it is also recommended for the future study to longer the duration times of experiment to get the best graph.

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APPENDIX A

Calculation on Preparation of Glucose Calibration Curve

By using the formula;

10 mg/ml = mass / Volume

When Volume= 100 ml, Thus, Mass of glucose= 1000 mg=1gram

a) When, M_2=0 mg/ml

$M_1V_1 = M_2V_2$

10 mg/ml (V1) = 0 mg/ml (10 ml)

V_1= 0 ml

b) When M_2=0.2 mg/ml

$M_1V_1 = M_2V_2$

10 mg/ml (V1) = 0.2 mg/ml (10 ml)

V_1= 0.2 ml

c) When M_2=0.4 mg/ml

$M_1V_1 = M_2V_2$

10 mg/ml (V1) = 0.4 mg/ml (10 ml)

V_1= 0.4 ml

d) When M_2=0.6 mg/ml

$M_1V_1 = M_2V_2$

10 mg/ml (V1) = 0.6 mg/ml (10 ml)

V_1= 0.6 ml

e) When M_2=0.8 mg/ml

$M_1V_1 = M_2V_2$

10 mg/ml (V1) = 0.8 mg/ml (10 ml)

V_1= 0.8 ml

f) When M_2=1.0 mg/ml

$M_1V_1 = M_2V_2$

10 mg/ml (V1) = 1.0 mg/ml (10 ml)

V_1= 1.0 ml

Table A.1: The readings of sample by using UV-vis spectrophotometer

Concentration (mg/ml)	Optical Density (OD)			
	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

APPENDIX B

Calculation of Mass Substrate for the Start Up Experiments

By using the formulas;

$$\text{Organic Loading Rate} = \frac{[\text{Biomass}]}{\text{Hydraulic Retention Time}}$$

$$\text{Mass Biomass} = [\text{biomass}] \times \text{Contact Volume}$$

Table B.1: Table of OLR

Run	Factor:OLR (g/L.day)
1	30
2	5
3	5
4	17.5
5	23.75
6	11.25
7	17.5
8	30

Hydraulic retention time fixed= 3 days ; Contact Volume= 150 ml

Thus, [biomass@substrate] and mass biomass;

Table B.2: Data of mass biomass

Run	[biomass] (mg/ml)	Mass biomass (gram)
1	90	13.5
2	15	2.25
3	15	2.25
4	52.5	7.88
5	71.25	10.69
6	33.75	5.063
7	52.5	7.88
8	90	13.5

Mass Substrate Calculation for the each day usage (Contact Volume= 50 ml)

Mass Biomass= [biomass] x Contact Volume

Table B.3: Data for mass Biomass

Run	[biomass] (mg/ml)	mass biomass (gram)
1	90	4.5
2	15	0.75
3	15	0.75
4	52.5	2.625
5	71.25	3.5625
6	33.75	1.6875
7	52.5	2.625
8	90	4.5

APPENDIX C

Calculation of Total Solid in Banana Stem Waste

By using the formula;

$$\% \text{ Total Solid} = \frac{(\text{Weight dry pan + dry sample} - \text{Weight dry pan})}{\text{Weight sample as recieved}} \times 100$$

Table C.1: Data for total solid calculation

ITEM	AMOUNT (gram)
Weight dry pan	2.5437
Weight dry pan + fresh sample	20.2302
Weight dry pan + dried sample	7.4327

$$\% \text{ Total Solid} = \frac{7.4327-2.5437}{20.2303-2.5437}$$

Thus; Total solid= 27.6 %

APPENDIX D

Data for Glucose Concentration and Yield for 30 days Experiment

Yield=[Glucose]/[Substrate]

TABLE D.1: Data for run 1

RUN	Date	Absorbance				[glucose]	Yield
		1	2	3	average	, mg/mL	
1 (OLR:30)	07/Mar	7.900	7.725	7.665	7.7633333	4.088162	0.045424022
	08/Mar	9.080	8.985	8.995	9.02	4.762339	0.052914878
	09/Mar	8.740	8.750	8.750	8.7466667	4.615701	0.051285567
	10/Mar	9.365	9.335	9.315	9.3383333	4.933119	0.05481243
	11/Mar	11.260	11.735	11.990	11.661667	6.179542	0.06866158
	12/Mar	7.350	7.300	7.385	7.345	3.863734	0.042930377
	13/Mar	6.440	6.115	6.200	6.2516667	3.277182	0.03641313
	14/Mar	6.955	6.310	6.040	6.435	3.375536	0.037505961
	15/Mar	6.605	6.610	6.530	6.5816667	3.45422	0.038380226
	16/Mar	9.285	9.225	9.195	9.235	4.877682	0.054196471
	17/Mar	10.230	10.230	10.180	10.213333	5.402539	0.060028215
	18/Mar	6.470	6.415	6.420	6.435	3.375536	0.037505961
	19/Mar	8.475	8.485	8.485	8.4816667	4.473534	0.049705929
	20/Mar	8.62	8.575	8.53	8.575	4.523605	0.050262279
	21/Mar	10.745	10.775	10.775	10.765	5.698498	0.063316643
	22/Mar	11.04	11.04	11.04	11.04	5.84603	0.064955889
	23/Mar	11.785	11.785	11.785	11.785	6.245708	0.069396757
	24/Mar	9.12	9.05	9.05	9.0733333	4.790951	0.053232793
	25/Mar	6.975	6.965	7.045	6.995	3.675966	0.040844063
	26/Mar	8.35	8.33	8.34	8.34	4.397532	0.048861469
	27/Mar	10.28	10.28	10.28	10.28	5.438305	0.060425608
	28/Mar	12.84	13.01	13.01	12.953333	6.872496	0.076361071
	29/Mar	9.18	9.18	9.18	9.18	4.848176	0.053868622

	30/Mar	7.765	7.765	7.765	7.765	4.089056	0.045433953
	31/Mar	6.565	6.565	6.565	6.565	3.445279	0.038280877
	01/Apr	6.27	6.27	6.27	6.27	3.287017	0.036522413
	02/Apr	6.605	6.605	6.605	6.605	3.466738	0.038519313
	03/Apr	6.75	6.75	6.75	6.75	3.544528	0.039383643
	04/Apr	6.79	6.79	6.79	6.79	3.565987	0.039622079
	05/Apr	6.81	6.81	6.81	6.81	3.576717	0.039741297

TABLE D.2: Data for run 2

RUN	Date	Absorbance				[glucose], mg/mL	Yield
		1	2	3	average		
2 (OLR:5.0)	07/Mar	1.97	1.785	1.785	1.846667	0.913984	0.060932267
	08/Mar	1.76	1.76	1.78	1.766667	0.871066	0.058071054
	09/Mar	1.705	1.7	1.715	1.706667	0.838877	0.055925131
	10/Mar	1.34	1.31	1.31	1.32	0.631438	0.042095851
	11/Mar	1.5	1.475	1.435	1.47	0.71191	0.047460658
	12/Mar	1.27	1.295	1.29	1.285	0.612661	0.040844063
	13/Mar	1.265	1.255	1.245	1.255	0.596567	0.039771102
	14/Mar	1.155	1.135	1.14	1.143333	0.53666	0.035777301
	15/Mar	1.11	1.105	1.12	1.111667	0.519671	0.034644731
	16/Mar	1.365	1.355	1.355	1.358333	0.652003	0.043466857
	17/Mar	0.91	0.915	0.9	0.908333	0.410587	0.027372437
	18/Mar	0.925	0.925	0.925	0.925	0.419528	0.027968526
	19/Mar	1.25	1.24	1.24	1.243333	0.590308	0.039353839
	20/Mar	0.81	0.825	0.81	0.815	0.360515	0.024034335
	21/Mar	1.71	1.81	1.685	1.735	0.854077	0.056938484
	22/Mar	1.86	1.86	1.86	1.86	0.921137	0.061409156
	23/Mar	1.865	1.9	1.86	1.875	0.929185	0.061945637
	24/Mar	1.995	2.075	2.065	2.045	1.020386	0.068025751
	25/Mar	1.29	1.285	1.29	1.288333	0.614449	0.040963281
	26/Mar	1.9	1.92	1.885	1.901667	0.943491	0.06289938
	27/Mar	2.465	2.465	2.465	2.465	1.245708	0.08304721
	28/Mar	2.99	2.99	2.99	2.99	1.527361	0.101824034
	29/Mar	3.34	3.34	3.34	3.34	1.715129	0.114341917
	30/Mar	2.14	2.14	2.14	2.14	1.071352	0.071423462
	31/Mar	2.11	2.11	2.11	2.11	1.055258	0.070350501
	01/Apr	2.22	2.22	2.22	2.22	1.11427	0.074284692
	02/Apr	2.355	2.355	2.355	2.355	1.186695	0.079113019
	03/Apr	2.41	2.41	2.41	2.41	1.216202	0.081080114
	04/Apr	2.55	2.55	2.55	2.55	1.291309	0.086087268
	05/Apr	2.625	2.625	2.625	2.625	1.331545	0.088769671

TABLE D.3: Data for run 3

RUN	Date	Absorbance				[glucose]	Yield
		1	2	3	average	, mg/mL	
3(OLR:5.0)	07/Mar	1.73	1.78	1.725	1.745	0.859442	0.057296133
	08/Mar	1.585	1.61	1.315	1.503333	0.729793	0.048652837
	09/Mar	1.51	1.5	1.495	1.501667	0.728898	0.048593228
	10/Mar	1.64	1.545	1.58	1.588333	0.775393	0.051692895
	11/Mar	1.31	1.36	1.335	1.335	0.639485	0.042632332
	12/Mar	1.455	1.46	1.425	1.446667	0.699392	0.046626133
	13/Mar	0.364	0.364	0.374	0.367333	0.120351	0.008023367
	14/Mar	1.075	1.03	1.035	1.046667	0.4848	0.032319981
	15/Mar	1.11	1.155	1.12	1.128333	0.528612	0.03524082
	16/Mar	1.545	1.55	1.57	1.555	0.757511	0.050500715
	17/Mar	0.99	0.98	0.97	0.98	0.449034	0.029935622
	18/Mar	1.07	1.11	1.065	1.081667	0.503577	0.033571769
	19/Mar	1.31	1.335	1.335	1.326667	0.635014	0.042334287
	20/Mar	0.9	0.915	0.905	0.906667	0.409692	0.027312828
	21/Mar	1.81	1.855	1.835	1.833333	0.906831	0.060455412
	22/Mar	2.25	2.23	2.225	2.235	1.122318	0.074821173
	23/Mar	2.165	2.165	2.15	2.16	1.082082	0.07213877
	24/Mar	1.57	1.61	1.56	1.58	0.770923	0.05139485
	25/Mar	1.08	1.06	1.06	1.066667	0.495529	0.033035289
	26/Mar	1.445	1.46	1.49	1.465	0.709227	0.047281831
	27/Mar	1.995	1.995	1.995	1.995	0.993562	0.066237482
	28/Mar	2.425	2.425	2.425	2.425	1.224249	0.081616595
	29/Mar	2.45	2.45	2.45	2.45	1.237661	0.08251073
	30/Mar	2.25	2.25	2.25	2.25	1.130365	0.075357654
	31/Mar	2.15	2.15	2.15	2.15	1.076717	0.071781116
	01/Apr	2.2	2.2	2.2	2.2	1.103541	0.073569385
	02/Apr	2.26	2.26	2.26	2.26	1.13573	0.075715308
	03/Apr	2.315	2.315	2.315	2.315	1.165236	0.077682403
	04/Apr	2.34	2.335	2.33	2.335	1.175966	0.078397711
	05/Apr	2.555	2.555	2.555	2.555	1.293991	0.086266094

TABLE D.4: Data for run 4

RUN	Date	Absorbance				[glucose]	YIELD
		1	2	3	average	, mg/mL	
4(OLR:17.50)	07/Mar	10.715	10.805	10.805	10.775	5.703863	0.10864501
	08/Mar	9.365	9.380	9.380	9.375	4.95279	0.094338851
	09/Mar	6.345	6.335	6.320	6.333333	3.320994	0.063257034
	10/Mar	5.885	5.800	5.765	5.816667	3.043813	0.057977383
	11/Mar	4.39	4.35	4.315	4.351667	2.257868	0.043007017
	12/Mar	4.275	4.355	4.35	4.326667	2.244456	0.04275155
	13/Mar	3.33	3.335	3.36	3.341667	1.716023	0.03268615
	14/Mar	2.36	2.48	2.345	2.395	1.208155	0.023012467
	15/Mar	4.085	3.965	3.94	3.996667	2.067418	0.039379386
	16/Mar	4.285	5.5	5.5	5.096667	2.657546	0.050619933
	17/Mar	3.83	3.835	3.825	3.83	1.978004	0.037676272
	18/Mar	4.18	4.21	4.115	4.168333	2.159514	0.041133592
	19/Mar	3.46	3.455	3.46	3.458333	1.778612	0.03387833
	20/Mar	2.67	2.665	2.66	2.665	1.353004	0.02577151
	21/Mar	3.95	3.95	3.95	3.95	2.042382	0.038902514
	22/Mar	4.29	4.285	4.255	4.276667	2.217632	0.042240616
	23/Mar	4.625	4.59	4.555	4.59	2.38573	0.045442469
	24/Mar	4.36	4.385	4.485	4.41	2.289163	0.043603106
	25/Mar	3.32	3.31	3.31	3.313333	1.700823	0.032396621
	26/Mar	4.695	4.675	4.67	4.68	2.434013	0.04636215
	27/Mar	5.21	5.21	5.21	5.21	2.718348	0.05177805
	28/Mar	5.7	5.7	5.7	5.7	2.981223	0.056785203
	29/Mar	5.555	5.555	5.555	5.555	2.903433	0.055303495
	30/Mar	3.895	3.895	3.895	3.895	2.012876	0.038340486
	31/Mar	3.845	3.845	3.845	3.845	1.986052	0.037829552
	01/Apr	3.69	3.695	3.69	3.691667	1.903791	0.036262688
	02/Apr	3.71	3.71	3.71	3.71	1.913627	0.036450031
	03/Apr	3.745	3.745	3.745	3.745	1.932403	0.036807684
	04/Apr	3.76	3.76	3.76	3.76	1.940451	0.036960965
	05/Apr	3.845	3.845	3.845	3.845	1.986052	0.037829552

TABLE D.5: Data for run 5

RUN	Date	Absorbance				[glucose]	YIELD
		1	2	3	average	, mg/mL	
5(OLR:23.75)	07/Mar	8.75	8.765	8.785	8.766667	4.626431	0.064932365
	08/Mar	5.745	5.76	5.475	5.66	2.959764	0.041540547
	09/Mar	5.98	6.065	6.105	6.05	3.168991	0.044477073
	10/Mar	8.575	8.155	8.03	8.253333	4.351037	0.061067189
	11/Mar	6.77	6.8	6.69	6.753333	3.546316	0.049772858
	12/Mar	6.19	5.96	5.855	6.001667	3.143062	0.044113144
	13/Mar	5.11	5.66	5.095	5.288333	2.760372	0.038742063
	14/Mar	5.35	5.265	5.26	5.291667	2.76216	0.038767161
	15/Mar	5.525	5.485	5.3	5.436667	2.83995	0.039858946
	16/Mar	7.835	7.925	7.755	7.838333	4.128398	0.057942424
	17/Mar	5.765	5.725	5.725	5.738333	3.001788	0.042130362
	18/Mar	7.305	7.3	7.28	7.295	3.83691	0.053851367
	19/Mar	7.565	7.545	7.545	7.551667	3.974607	0.055783952
	20/Mar	5.755	5.715	5.715	5.728333	2.996423	0.042055066
	21/Mar	5.555	5.56	5.535	5.55	2.900751	0.040712296
	22/Mar	7.04	7.055	7.095	7.063333	3.712625	0.05210702
	23/Mar	5.17	5.2	5.105	5.158333	2.690629	0.037763221
	24/Mar	6.42	6.455	6.485	6.453333	3.385372	0.047513992
	25/Mar	4.425	4.46	4.495	4.46	2.315987	0.032505082
	26/Mar	5.12	5.11	5.125	5.118333	2.66917	0.037462039
	27/Mar	6.115	6.115	6.115	6.115	3.203863	0.044966493
	28/Mar	6.86	6.845	6.865	6.856667	3.601753	0.050550912
	29/Mar	6.82	6.81	6.82	6.816667	3.580293	0.05024973
	30/Mar	4.09	4.09	4.09	4.09	2.117489	0.029719148
	31/Mar	4	4	4	4	2.069206	0.029041488
	01/Apr	4.1	4.1	4.1	4.1	2.122854	0.029794443
	02/Apr	4.135	4.135	4.135	4.135	2.141631	0.030057978
	03/Apr	4.1575	4.1575	4.1575	4.1575	2.153702	0.030227393
	04/Apr	4.18	4.18	4.18	4.18	2.165773	0.030396807
	05/Apr	4.215	4.215	4.215	4.215	2.184549	0.030660342

TABLE D.6: Data for run 6

RUN	Date	Absorbance				[glucose], mg/mL	YIELD
		1	2	3	average		
6(OLR:11.25)	07/Mar	4.45	4.415	4.405	4.423333	2.296316	0.06803899
	08/Mar	3.955	4.27	4.3	4.175	2.16309	0.064091559
	09/Mar	3.155	3.125	3.135	3.138333	1.606938	0.047612992
	10/Mar	3.12	3.035	3.085	3.08	1.575644	0.046685742
	11/Mar	3.255	3.21	3.185	3.216667	1.648963	0.048858157
	12/Mar	2.375	2.39	2.43	2.398333	1.209943	0.035850156
	13/Mar	2.605	2.56	2.54	2.568333	1.301144	0.038552429
	14/Mar	1.83	1.845	1.83	1.835	0.907725	0.026895565
	15/Mar	3.15	3.09	2.925	3.055	1.562232	0.046288348
	16/Mar	3.23	3.295	3.275	3.266667	1.675787	0.049652943
	17/Mar	2.585	2.61	2.495	2.563333	1.298462	0.038472951
	18/Mar	1.745	1.75	1.75	1.748333	0.86123	0.025517936
	19/Mar	2.74	2.73	2.765	2.745	1.395923	0.041360674
	20/Mar	2.15	2.16	2.165	2.158333	1.081187	0.032035183
	21/Mar	3.62	3.61	3.59	3.606667	1.85819	0.05505749
	22/Mar	3.35	3.36	3.365	3.358333	1.724964	0.051110051
	23/Mar	2.74	2.73	2.8	2.756667	1.402182	0.041546124
	24/Mar	4.11	4.11	4.11	4.11	2.128219	0.063058337
	25/Mar	2.905	2.93	2.9	2.911667	1.485336	0.044009961
	26/Mar	2.74	2.605	2.575	2.64	1.339592	0.039691623
	27/Mar	4.115	4.115	4.115	4.115	2.130901	0.063137816
	28/Mar	4.24	4.14	4.175	4.185	2.168455	0.064250517
	29/Mar	4.156	4.156	4.156	4.156	2.152897	0.063789541
	30/Mar	3.177	3.177	3.177	3.177	1.627682	0.048227627
	31/Mar	2.21	2.21	2.21	2.21	1.108906	0.032856462
	01/Apr	2.755	2.755	2.755	2.755	1.401288	0.041519631
	02/Apr	2.8	2.8	2.8	2.8	1.425429	0.042234939
	03/Apr	2.825	2.825	2.825	2.825	1.438841	0.042632332
	04/Apr	2.916	2.916	2.916	2.916	1.487661	0.044078843
	05/Apr	2.975	2.975	2.975	2.975	1.519313	0.045016691

TABLE D.7: Data for run 7

RUN	Date	Absorbance				[glucose]	YIELD
		1	2	3	average	, mg/mL	
7(OLR:17.50)	07/Mar	12.220	12.100	11.890	12.07	6.398605	0.1287819
	08/Mar	9.465	9.570	9.535	9.523333	5.032368	0.121878193
	09/Mar	3.715	3.68	3.675	3.69	1.902897	0.095854622
	10/Mar	5.035	5.055	5.005	5.031667	2.622675	0.036245657
	11/Mar	4.81	4.805	4.805	4.806667	2.501967	0.049955719
	12/Mar	4.63	4.545	4.52	4.565	2.372318	0.047656516
	13/Mar	3.435	3.345	3.235	3.338333	1.714235	0.045187002
	14/Mar	3.275	3.000	3.085	3.12	1.597103	0.032652088
	15/Mar	3.730	3.705	3.605	3.68	1.897532	0.03042101
	16/Mar	6.700	6.830	6.610	6.713333	3.524857	0.03614347
	17/Mar	3.305	2.965	3.300	3.19	1.634657	0.067140132
	18/Mar	3.26	3.24	3.285	3.261667	1.673104	0.031136317
	19/Mar	4.485	4.495	3.900	4.293333	2.226574	0.031868656
	20/Mar	3.35	3.35	3.355	3.351667	1.721388	0.042410927
	21/Mar	4.67	4.65	4.665	4.661667	2.424177	0.032788337
	22/Mar	4.825	4.83	4.82	4.825	2.511803	0.046174808
	23/Mar	4.475	4.495	4.49	4.486667	2.330293	0.047843859
	24/Mar	5.71	5.72	5.745	5.725	2.994635	0.044386539
	25/Mar	3.795	3.795	3.795	3.795	1.959227	0.05704067
	26/Mar	4.53	4.525	4.54	4.531667	2.354435	0.037318618
	27/Mar	6.13	6.13	6.13	6.13	3.21191	0.044846379
	28/Mar	6.86	6.845	6.865	6.856667	3.601753	0.061179236
	29/Mar	6.82	6.82	6.82	6.82	3.582082	0.06860481
	30/Mar	3.75	3.75	3.75	3.75	1.935086	0.068230125
	31/Mar	3.725	3.725	3.725	3.725	1.921674	0.036858778
	01/Apr	3.8	3.8	3.8	3.8	1.96191	0.036603311
	02/Apr	3.825	3.825	3.825	3.825	1.975322	0.037369712
	03/Apr	3.835	3.835	3.835	3.835	1.980687	0.037625179
	04/Apr	3.75	3.75	3.75	3.75	1.935086	0.037727366
	05/Apr	4	4	4	4	2.069206	0.036858778

TABLE D.8: Data for run 8

RUN	Date	Absorbance				[glucose], mg/mL	YIELD
		1	2	3	average		
8(OLR:30.0)	07/Mar	9.12	8.995	8.39	8.835	4.66309	0.051812111
	08/Mar	8.27	8.045	8.1	8.138333	4.289342	0.047659355
	09/Mar	6.48	6.505	6.5	6.495	3.407725	0.037863615
	10/Mar	8.8	8.785	8.765	8.783333	4.635372	0.051504133
	11/Mar	8.81	8.985	8.985	8.926667	4.712268	0.052358528
	12/Mar	9.255	9.68	9.48	9.471667	5.004649	0.055607217
	13/Mar	6.515	6.68	6.695	6.63	3.48015	0.038668336
	14/Mar	6.85	6.765	6.84	6.818333	3.581187	0.039790971
	15/Mar	8.37	8.24	8.28	8.296667	4.374285	0.048603163
	16/Mar	8.41	9.12	9.025	8.851667	4.672031	0.051911461
	17/Mar	10.155	10.065	10.045	10.08833	5.335479	0.059283103
	18/Mar	7.34	7.34	7.34	7.34	3.861052	0.042900572
	19/Mar	8.97	9.01	9.01	8.996667	4.749821	0.052775791
	20/Mar	6.445	6.44	6.435	6.44	3.378219	0.037535765
	21/Mar	9.195	9.195	9.195	9.195	4.856223	0.053958035
	22/Mar	10.065	10.16	10.255	10.16	5.373927	0.0597103
	23/Mar	10.935	10.935	10.935	10.935	5.7897	0.064329995
	24/Mar	9.445	9.43	9.395	9.423333	4.97872	0.055319107
	25/Mar	6.42	6.465	6.495	6.46	3.388948	0.037654983
	26/Mar	8.22	8.22	8.22	8.22	4.333155	0.048146161
	27/Mar	10.97	10.97	10.97	10.97	5.808476	0.064538627
	28/Mar	12.05	12.05	12.05	12.05	6.387876	0.070976395
	29/Mar	9.686	9.686	9.686	9.686	5.119635	0.056884835
	30/Mar	8.63	8.63	8.63	8.63	4.553112	0.050590129
	31/Mar	7.065	7.065	7.065	7.065	3.713519	0.041261326
	01/Apr	6.335	6.335	6.335	6.335	3.321888	0.036909871
	02/Apr	6.75	6.75	6.75	6.75	3.544528	0.039383643
	03/Apr	6.79	6.79	6.79	6.79	3.565987	0.039622079
	04/Apr	6.8	6.8	6.8	6.8	3.571352	0.039681688
	05/Apr	6.906	6.906	6.906	6.906	3.628219	0.040313543