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

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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.

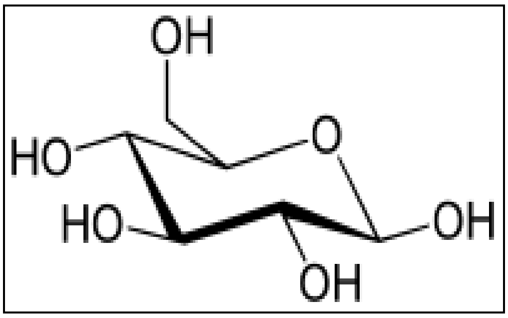

Figure 2.0: Glucose structure in ring form

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%).

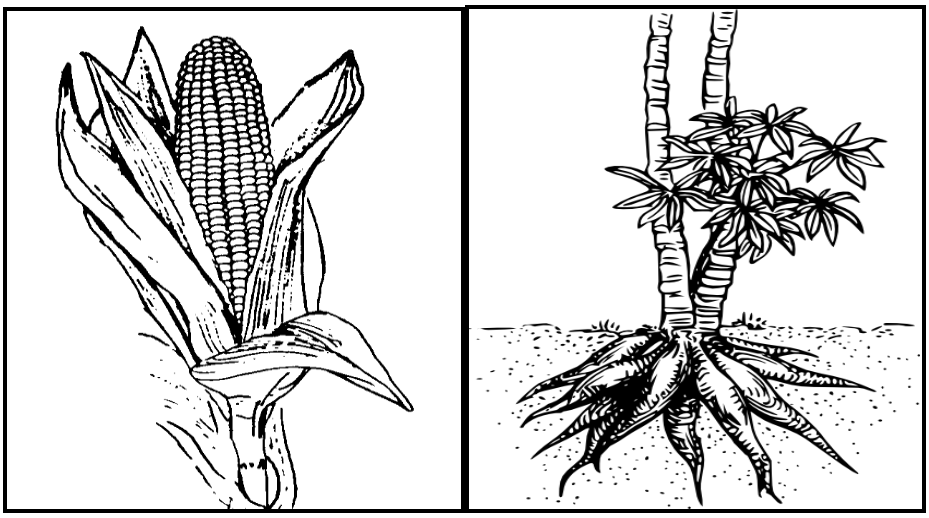

Figure 2.1: The examples of agricultural crops

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).

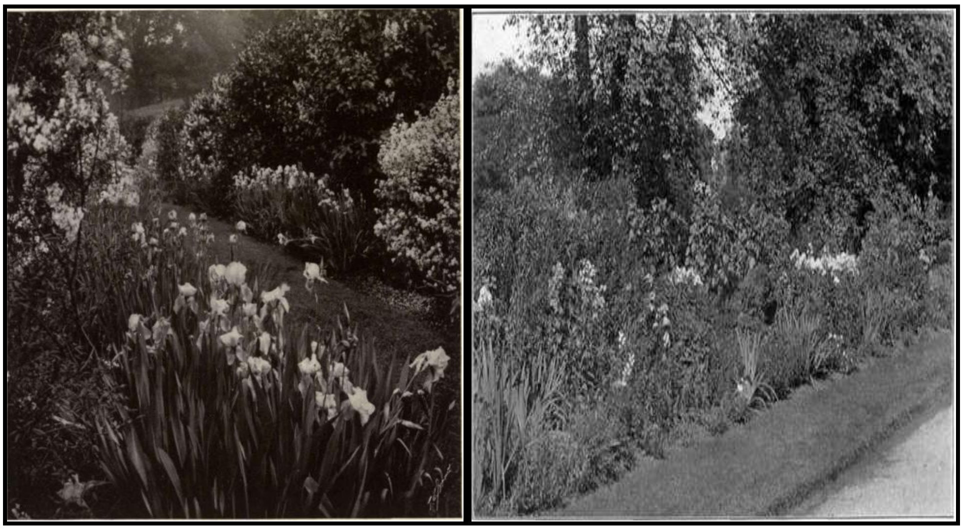

Figure 2.4: The examples of herbaceous residue

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).

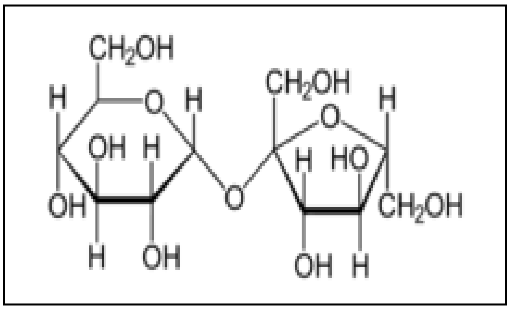

Figure 2.5: Sucrose hydrolysis

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).

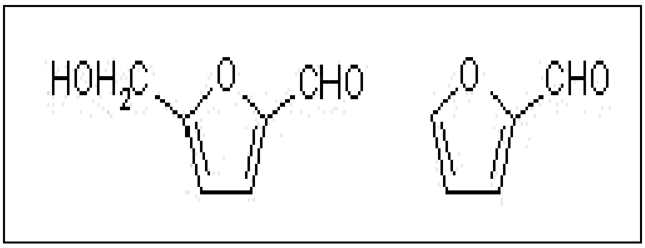

Figure 2.6: Examples of inhibitor structure

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.