EFFECT OF TEMPERATURE AND pH ON GLUCOSE PRODUCTION USING ENZYMATIC HYDROLYSIS

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ABSTRACT

Enzymatic hydrolysis is one of the effective methods in glucose production due to enzyme properties which are highly specific and very sensitive. Therefore, this paper was designed to study the effect of temperature and pH on glucose production using enzymatic hydrolysis. By using enzymatic hydrolysis, cellulase from *Trichoderma reesei* was used and supplemented with cellobiase from *Aspergillus niger* for increasing the glucose production. Cellulose as a substrate was hydrolyzed by these enzymes to produce glucose and the amount of glucose production was determined by high-performance liquid chromatography (HPLC). The result showed that the highest glucose concentration was produced at the temperature of 50°C and pH 5.0 while the lowest glucose production was produced at 30°C and pH 6.5 which were 21.09 mg/ml and 3.55 mg/ml, respectively. By changes the temperature and pH above or below 50°C and pH 5.0 results in low glucose production because the enzyme was lose their three-dimensional functional shape due to disruption from unstable condition of the environment system. As a conclusion, all the requirement for complementary in the configuration of cellulose and enzyme must be considered seriously for the highest glucose production.

ABSTRAK

Hidrolisis enzim merupakan salah satu kaedah berkesan dalam penghasilan glukosa disebabkan oleh sifat enzim yang sangat khusus dan sensitif. Oleh itu, tujuan kajian ini dilaksanakan untuk mengetahui kesan suhu dan pH kepada penghasilan glukosa dengan menggunakan kaedah hidrolisis enzim. Dengan menggunakan kaedah ini, enzim selulase daripada *Trichoderma reesei* dan dilengkapi dengan selobiase daripada *Aspergillus niger* telah digunakan untuk meningkatkan penghasilan glukosa. Selulosa yang digunakan sebagai bahan mentah dihidrolisis oleh enzim untuk menghasilkan glukosa dan jumlah penghasilan glukosa dianalisis menggunakan sistem kromatografi (HPLC). Keputusan kajian menunjukkan bahawa penghasilan glukosa tertinggi adalah pada suhu 50°C dan pH 5.0 manakala penghasilan glukosa terendah adalah pada suhu 30°C dan pH 6.5. Jumlah kepekatan tertinggi yang dihasilkan ialah 21.09 mg/ml dan jumlah kepekatan terendah yang dihasilkan pula ialah 3.55 mg/ml. Perubahan suhu dan pH di atas atau di bawah nilai maksimum akan menyebabkan penghasilan glukosa rendah kerana enzim kehilangan fungsinya disebabkan oleh gangguan dari sistem persekitaran yang tidak stabil. Sebagai kesimpulan, semua keperluan dalam konfigurasi enzim dan selulosa, harus dipertimbangkan secara serius untuk menghasilkan glukosa yang tertinggi.

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LIST OF ABBREVIATIONS/SYMBOLS

%	-	Percent
mg/ml	-	Milligram per milliter
°C	-	Degree celsius
µm	-	Micro meter
α	-	Alpha
β	-	Beta
MPa	-	Mega Pascal
FPU	-	Filter paper unit
CBU	-	One Cellobiase Unit
HPLC	-	High-performance Liquid Chromatography
ml	-	Milliliter
w/v	-	Weight per volume
rpm	-	Rotation per minute
M	-	Molarity
cm	-	Centimeter
µL	-	Microliter
ml/min	-	Milliliter per minute
nRIU*s	-	Unit area of chromatogram peak

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Glucose is a commercially important product widely used by the food and pharmaceutical industries (Johnson *et al*., 2009). In general, glucose is used in the food industry as a partial or complete substitute for sucrose. Glucose is the common name for the syrup which is used in large quantities in fruit canning, confectioneries, jams, jellies, preserves, ice cream, bakery products, pharmaceuticals, beverages and alcoholic fermentation. The functional purpose of glucose in the confectionery industry is to prevent crystallization of the sucrose while in the bakery products industry it is to supply fermentable carbohydrates. In the ice-cream and fruit-preserves, it used to increase the solids without causing an undue increase in the total sweetness. In pharmaceutical industry, glucose is used as a precursor to make vitamin C in the Reichstein process, to make citric acid, gluconic acid, polylactic acid and sorbitol. Currently, glucose is utilized as an intermediate raw material for bio-ethanol production.

Commonly, glucose is prepared commercially via the enzymatic hydrolysis of starch instead of acid hydrolysis. Many crops can be used as the source of the initial starch. Maize, rice, wheat, potato, cassava, arrowroot and sago are all used in various parts of the world. Nevertheless, using the starch needs to compete with their primary use as food crops. Due to the abundant of non-food energy crops like cellulosic material, they are use to reduce the utilization of starch as raw material for production of glucose. Cellulosic materials including agricultural, agro-industrial and forestry lignocellulosic residues have potential as cheap and renewable feedstocks for large scale production of fuels and chemicals. Currently, bio-processing of lignocellulosics is focused on enzymatic hydrolysis of the cellulose fraction to glucose, followed by fermentation to fuel-grade ethanol. However, enzymatic hydrolysis of cellulosic materials to produce fermentable sugars has also enormous potential in meeting global food and energy demand via biological route (Gan *et al*., 1994).

In lignocellulosic materials cellulose is physically associated with hemicellulose, and physically and chemically associated with lignin. The presence of these two fractions is reported to make the access of cellulase enzymes to cellulose difficult, thus reducing the efficiency of the hydrolysis (Mussatto *et al*., 2008). There are several kinds of pretreatment able to disrupt the lignocellulosic structure for increasing the efficiency of the hydrolysis which have been investigated but are not within the scope of this study. However, the effect of temperature and pH are also significant in cellulose hydrolysis which will be studying in this study. The temperature and pH influence the efficiency of cellulase to degrade the cellulose for producing glucose.

1.2 Problem Statement

In order to produce glucose, cellulose is essential to break it down first. By using acid hydrolysis, conversion of cellulose to glucose only produces low glucose concentration because acid is no selectivity. Furthermore, by using acid causes the cost production of glucose is high due to demand of neutralization after hydrolysis which can contribute to corrosion problem if there is no neutralization process. Other that, the need of high temperature during acid hydrolysis process also contributes to the cost of production because high energy is consumed. Waste from acid hydrolysis also gives the bad effect to the environment which is using high concentration of acid can cause harmful to the environment. Therefore, the investigation attempted to determine the glucose production by using enzymatic hydrolysis process in order to replace acid hydrolysis process.

Nevertheless, by using enzymatic hydrolysis need highly specific and very sensitive. Their environmental condition such temperature and pH influence the activity of the enzymes in the system. Hence, the effect of temperature and pH is investigated to determine the maximum conditions of enzymatic hydrolysis process in glucose production.

1.3 Research Objective

The main purpose of this study is to study the effect of temperature and pH on glucose production using enzymatic hydrolysis.

1.4 Scope of Study

The scope of this study includes studying the effect of temperature at 30°C, 40°C, 50°C, 60°C and 70°C which control by incubator. For the effect of pH at 4.5, 5.0, 5.5, 6.0 and 6.5 are control by citrate buffer. This study also includes analyzing the glucose concentration by using high performance liquid chromatography (HPLC).

1.5 Significant of Study

The significant of this study is to increase glucose yield from enzymatic hydrolysis by the maximum temperature and pH. Furthermore, by using enzymatic hydrolysis can reduce the cost production of glucose due to less energy consumption because the temperature consume in this process is low. Besides that, this process is green technology because it will not produce harmful waste.

CHAPTER 2

LITERATURE REVIEW

2.1 Glucose Overview

Glucose is a simple monosaccharide sugar also known as grape sugar, blood sugar or corn sugar which is a very important carbohydrate in biology. The living cell uses it as a source of energy and metabolic intermediate. Glucose is one of the main products of photosynthesis and starts cellular respiration in both prokaryotes and eukaryotes. Glucose ($C_6H_{12}O_6$) contains six carbon atoms, one of which is part of an aldehyde group (Figure 2.1). Therefore, glucose is an aldohexose. Glucose is commonly available in the form of a white powder or as a solid crystal, called dextrose. It can also be dissolved in water as an aqueous solution, glucose syrups. Its solubility level is very high (McMurry, 1988).

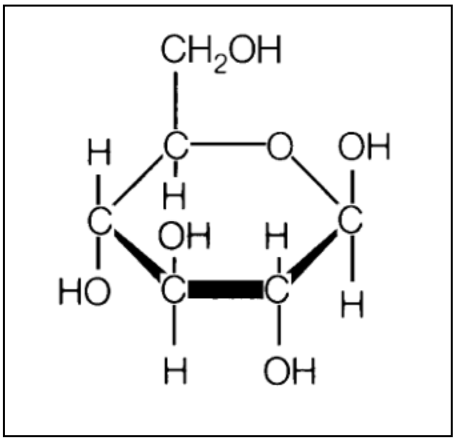

Figure 2.1 Structure of Glucose

Glucose can be forms disaccharide when two of monosaccharide are linked together such sucrose, the combination of glucose with fructose (Figure 2.2). Sucrose is the most common sweetener in the modern, industrialized world, although it has been displaced in industrial food production by some other sweeteners such as glucose syrups or combinations of functional ingredients and high intensity sweeteners.

In lactose, another important disaccharide, glucose is joined to galactose (Figure 2.3). It used as the predominant sugar in milk. For maltose, a product of starch digestion is glucose-glucose disaccharide (Figure 2.4). Glucose also can be forms polysaccharides when the units (either mono- or di-saccharides) are repeated and joined together by glycosidic bonds like cellulose. Cellulose is yet a third polymer of the monosaccharide glucose (Carpi, 2003).

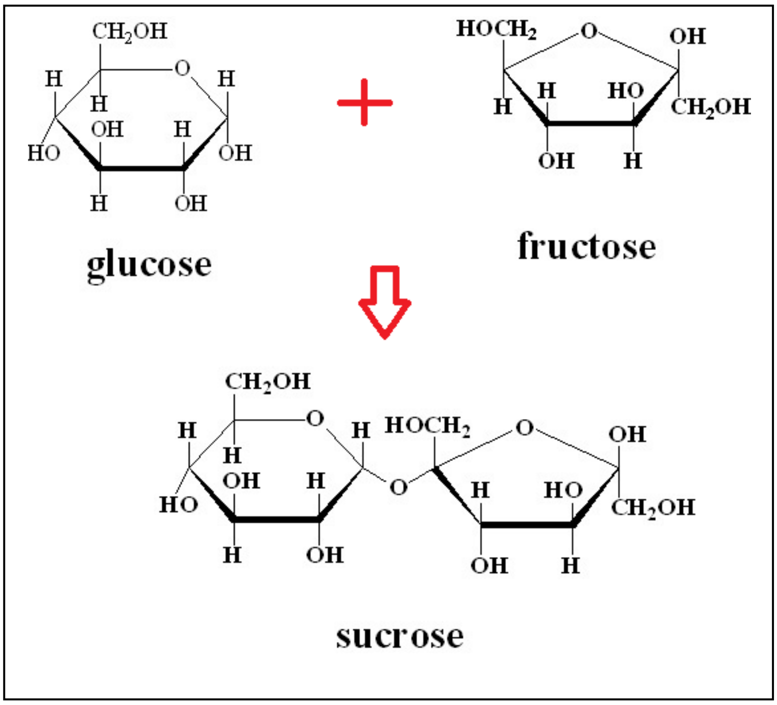

Figure 2.2 Structure of Sucrose

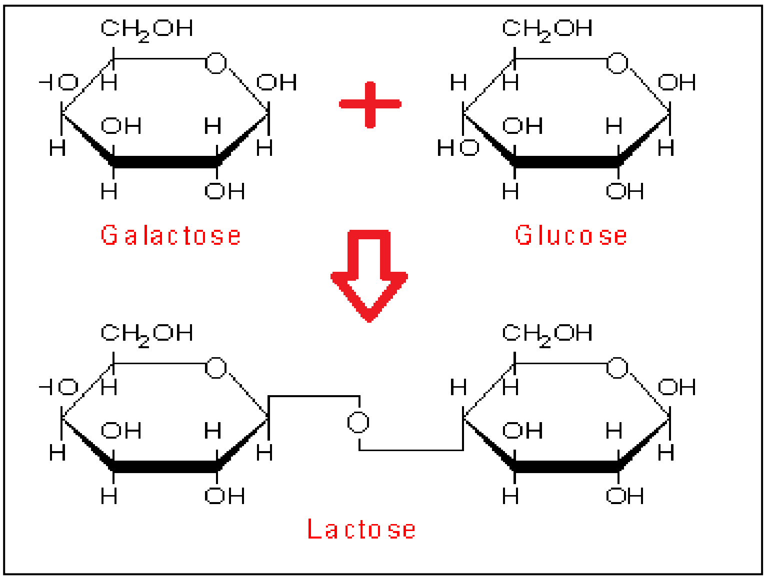

Figure 2.3 Structure of Lactose

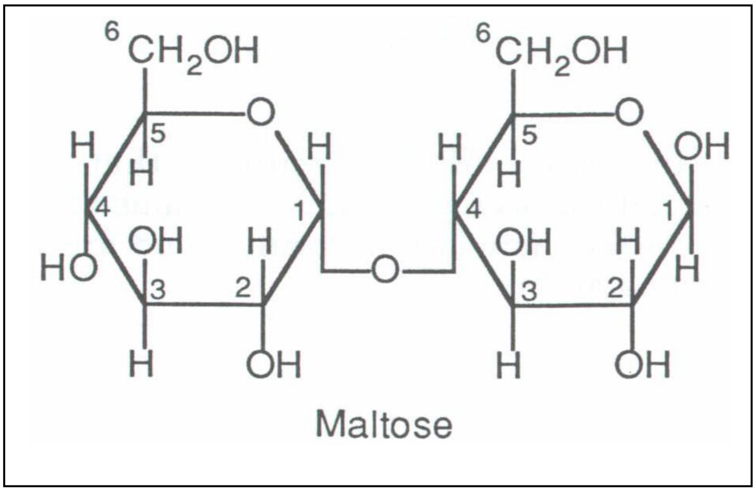

Figure 2.4 Structure of Maltose

2.1.1 Glucose as Energy Source in Living Cell

Glucose used as an energy source in most organisms from bacteria to human. In human, glucose is the main source of energy for the body, especially for the brain and the red blood cells. It is utilized by the cells to generate energy that is needed to carry out important cellular functions. If the glucose level becomes very low (hypoglycemia), the cells cannot function normally and it results in headache, confusion, nervousness, convulsions and coma in extreme cases (Gailliot *et al*., 2007). Since the brain is so sensitive to the glucose level in the blood, a number of mechanisms are in place to ensure that the blood glucose remains more or less constant. If the blood glucose level increases, it is promptly converted into glycogen in the liver and stored. On the other hand, if the glucose level falls, the stored glycogen is readily converted back to glucose and released in the blood stream.

Glucose may come directly from dietary carbohydrates or from glycogen stores in the liver and the muscles. Glucose is the results of the breakdown of glycogen. Several hormones, including insulin, work rapidly to regulate the flow of glucose to and from the blood to keep it at a steady level. While in animal, glucose is synthesized in the liver and kidneys from non-carbohydrate intermediates, such pyruvate and glycerol, by a process of gluconeogenesis. For plant, glucose is produced from photosynthesis process which is used as an energy source in cells via aerobic or anaerobic respiration.

2.1.2 Commercial Production of Glucose

Nowadays, glucose is valued in almost all industrial countries for its unique properties. It is used in the manufacture of a number of products in food industries, pharmaceuticals and industrial fermentations. In food industries, glucose is extremely popular in the sweet manufacturing business (Figure 2.5). It is extensively used in confectionery as a doctoring agent to prevent crystallization. Being a non-crystallizing substance, it helps produce homogenous confectionery like chewing gums and chocolates. It provides a smooth texture, possesses good preservative qualities for a longer shelf life and has several desirable organoleptic properties. In processed foods like jams and jellies, glucose syrup is used to prevent crystallization of sugar. It acts as a good preservative and prevents spoilage of the product without unduly increasing its sweetness. It is used in the preparation of common syrups as it is easily digestible and provides an instant source of energy. Baby foods and baby syrups also favour glucose syrup as it serves as a rich source of carbohydrates.

Furthermore, glucose syrup adds body, bulk and optimum sweetness to bakery products. This is why it is so often used by bakery houses in pie and cream fillings. It also prevents crystallization, enhances shelf-life and its non-crystallizing and hygroscopic properties keep the preparations fresh and longer ice creams. Nobody likes ice creams that crystallize, melt soon or are rough to the tongue. This is exactly what glucose syrup prevents. It prevents crystallization, gives a smooth texture and ensures that ice creams do not melt soon. It prevents sucrose crystallization and lends a creamy, soft mouthful to the ice cream, lending it homogenous sweetness. There is no undesirable taste and it can even replace expensive ingredients like non-fat milk solids.

Figure 2.5 Usage of Glucose in Food Industries

Otherwise, in pharmaceuticals, glucose is a valuable vehicle for cough syrups and vitamin-based tonics and may be used as a granulating agent for tablet coating. Cough lozenges also use glucose syrup as one of the principal ingredients. It provides body, consistency, a good mouth feel and balanced sweetness when used with other carbohydrate sweeteners like sucrose and sorbitol. Glucose syrup adds flavour to tobacco and lends it a smooth texture. When used in the preparation of chewing tobacco, it enhances the shelf-life. It also helps in the dressing of cigarette tobacco. Others usage, glucose syrup also finds use as a preservative in pan masalas, besides helping in the brewing and fermentation industries. It is also used in the traditional oil extraction industries for its gumming properties. It is used to improve stability in adhesives, as a setting retardant in concrete, as humectants in air fresheners and for evaporation control in cologne and perfumes.

2.1.3 Substrate for Glucose Production

Glucose is produced from many plant sugars. Cellulose and starch are just two examples but in no matter what sources, glucose production process takes polysaccharides or complex sugars from the plant and breaks them into single sugar, glucose. Nevertheless, there have some difference process between cellulose and starch because the structure of cellulose and starch is different. By using the starch, the structure is easy to breakdown because it joined together by α- (1-4) acetal linkage and the enzyme that used in the breakdown process is α-amylase. While for cellulose, the structure is difficult to breakdown because it joined together by β- (1-4) acetal linkage and the enzyme that used in the breakdown process is cellulose (Figure 2.6). Therefore, it makes sense that starch is easier to convert to glucose and production process is faster and not complicated compare to cellulose which the production process is slower and more complicated.

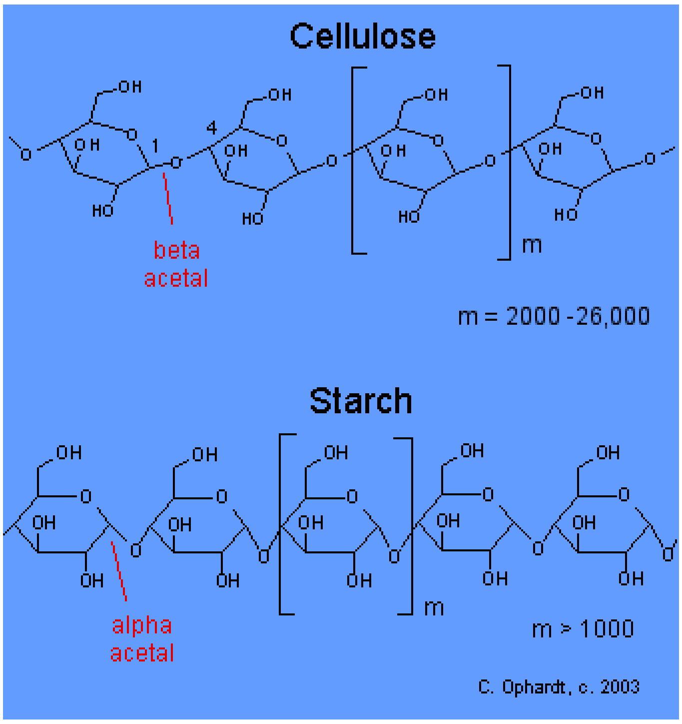

Figure 2.6 The Comparison Structure of Starch and Cellulose

Due to the starch advantages, glucose is prepared commercially via the enzymatic hydrolysis of starch. Starch, the ubiquitous storage carbohydrate of plants like corn, potato, rice, sorghum, wheat and cassava are becomes the primary raw material for the production of glucose (Aschengreen *et al*., 1979). But it creates a problem when starch becomes the primary raw material because starch needs to compete with their primary use as food crops. Therefore, due to the abundant of non-food energy crops like cellulosic material, cellulose is use to reduce the utilization of starch as raw material for production of glucose. Cellulosic materials including agricultural, agro-industrial and forestry lignocellulosic residues have potential as cheap and renewable feedstocks for large scale production of fuels and chemicals. Currently, bioprocessing of lignocellulosics is focused on enzymatic hydrolysis of the cellulose fraction to glucose, followed by fermentation to fuel-grade ethanol. However, enzymatic hydrolysis of cellulosic materials to produce fermentable sugars has also enormous potential in meeting global food and energy demand via biological route (Gan *et al*., 1994).

2.2 Cellulose

The major component in the rigid cell walls in plants is cellulose. Cellulose is an organic compound with the formula $(C_6H_{10}O_5)n$, which is a linear polysaccharide polymer with many glucose monosaccharide units are linked together by β-(1→4)-glycosidic bonds (Figure 2.7). A linear chain of (1-4) linked β-D-glucopyranose units aggregated to form a highly ordered structure due to its chemical constitution and spatial conformation. The highly order structure and crystallinity of cellulose makes it recalcitrant to hydrolysis which it should be disrupted in a pretreatment step in order to hydrolyze cellulose efficiently (Kua and Lee, 2009).

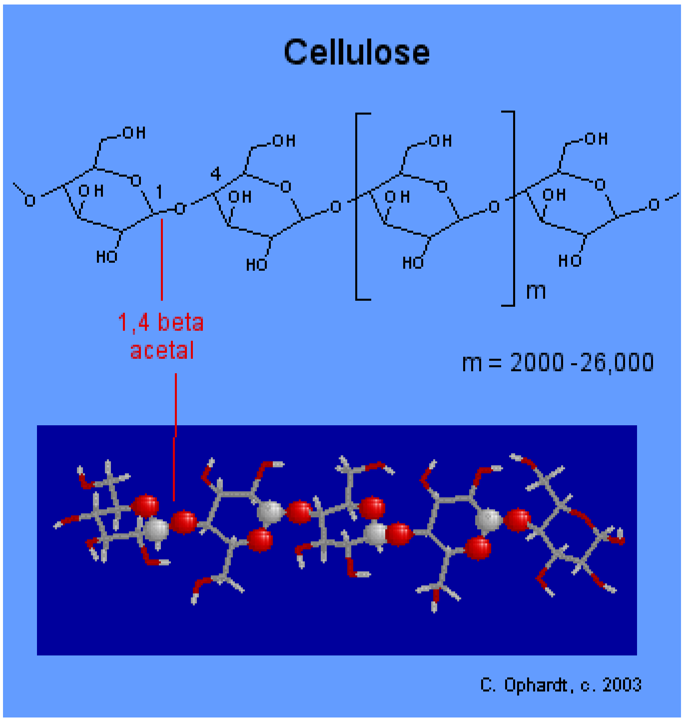

Figure 2.7 Structure of Cellulose

On the other hand, the beta acetal linkage makes cellulose different from starch, glycogen and other carbohydrates linkages. This peculiar difference in acetal linkages results in a major difference in digestibility in humans. Humans are unable to digest cellulose because the appropriate enzymes to breakdown the beta acetal linkages are lacking. Animals such as cows, horses, sheep, goats, and termites have symbiotic bacteria in the intestinal tract. These symbiotic bacteria possess the necessary enzymes to digest cellulose in the GI tract. They have the required enzymes for the breakdown or hydrolysis of the cellulose, the animals do not, not even termites, and have the correct enzymes. No vertebrate can digest cellulose directly.

Cellulose has no taste, is odourless, is hydrophilic in water and most organic solvents, is chiral and is biodegradable. Commercially, it can be broken down chemically into its glucose units by treating it with concentrated acids at high temperature. Due to the highly order structure and crystallinity, cellulose requires a temperature of 320°C and pressure of 25 MPa to become amorphous in water (Deguchi *et al*., 2006). Many properties of cellulose depend on its chain length or degree of polymerization, the number of glucose units that make up one polymer molecule.

2.2.1 Sources of Cellulose

Cellulose is the main component of higher plant cell walls and one of the most abundant organic compounds on earth. It can be derived from a number of sources using a number of techniques that are considered synthetic, and some that might be considered non-synthetic (natural). Cellulose from such major land plants as forest trees and cotton is assembled from glucose which is produced in the living plant cell from photosynthesis. These are macroscopic, multi-cellular photosynthetic plants with which we are all familiar.

In the oceans, however, most cellulose is produced by unicellular plankton or algae using the same type of carbon dioxide fixation found in photosynthesis of land plants. In fact, it is believed that these organisms, the first in the vast food chain, represent Nature's largest resource for cellulose production. Without photosynthetic microbes, all animal life in the oceans would cease to exist. Several animals, fungi, and bacteria can assemble cellulose; however, these organisms are devoid of photosynthetic capacity and usually require glucose or some organic substrate synthesized by a photosynthetic organism to assemble their cellulose. Some bacteria can utilize methane or sulfur substrates to produce glucose and other organic substrates for cellulose (Brown, 1979).

Nowadays, due to the abundant of biomass wastes, it becomes major resources to obtain the cellulose. Biomass wastes are including agricultural residues such as straws, corn stalks and cobs, bagasse, cotton gin trash and palm oil wastes. Paper such recycled newspaper, paper mill sludge's and sorted municipal solid waste. For wood wastes are prunings, wood chips and sawdust while for green wastes are leaves, grass clippings, vegetable and fruits wastes. By utilizing the biomass wastes for the production of value-added product, it becomes environmental-friendly alternative to the disposal of solid waste.