'THE TREATMENT OF SUGAR CANE BAGASSE FOR SUBSEQUENT USE AS SUBSTRATE FOR CELLULASE PRODUCTION BY *ASPERGILLUS TERREUS SUK-1*'

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ABSTRACT

As bio-product such as ethanol replace oil refineries, society and the environment will benefit from a switch from renewable resources as a source of energy, materials and chemicals. Biomass-based ethanol technologies are rapidly evolving are being identified that need to be overcome to achieve widespread commercialization. Current research is driven by the need to reduce the cost of biomass-ethanol production. The preferred method is enzymatic hydrolyzing the pretreated material to fermentable sugars that can then be converted to ethanol. That enzyme is cellulase. In this project, sugar cane waste was used as a substrate for the production of cellulase enzyme from *Aspergillus terreus SUK-1* according to continued our government effort to converting the agricultural waste into something more valuable. Pretreatment research was focused on developing processes that would result in increased cellulase enzyme yields. Cellulase research efforts are focused on developing a cost-effective, highly thermostable, synergistically acting enzyme mixture that would meet the end user's needs. Strong fermentation microorganisms are also being developed for conversion of biomass sugars to production of cellulase enzyme and other bio-products.

CHAPTER I

INTRODUCTION

1.1 Introduction

Biomass offers an abundant and inexpensive source of renewable resources. For example, sugarcane residue, called bagasse. Bagasse is the highly fibrous residue remaining after sugar-cane is pressed to remove sucrose. This situation happens when the growth of industries related to these raw materials such as sugar-cane mills. After remove their product, a few commercial uses for the excess bagasse have been developed and its accumulation presents a waste problem for the sugar industry. This waste is plentiful in tropical and subtropical regions such as Brazil, India, Thailand, Hawaii and the southern U.S. Alternative lignocellulosic feedstock include agricultural residues such as wheat and rice straw, forestry residue, industrial residue such as pulp and paper processing waste, and energy crops such as switch grass but in this project, we are more focused on sugar cane bagasse as our substrate.

The bioconversion of cellulosic materials has been receiving attention in recent years. It is now a subject of intensive research as a contribution to the development of a large-scale conversion process beneficial to mankind. Agricultural wastes and in fact all lignocellulosics can be converted into products that are of commercial interest such as ethanol, glucose, and single cell protein (Solomon *et al*, 1996). Cellulase enzyme has been reported (Fan *et al*, 1986) is one of the commercialize product from the bioconversion of lignocellulosics materials. This production from the renewable lignocellulosic resources can give us many benefits especially to decrease air pollution,

use for the production of biofuel (ethanol) and gives macro-economic benefits for rural communities and society at large. Despite realizing these benefits at bench scale, commercialization and widespread application of lignocellulosic biomass utilization must be developed and enlarged.

Feedstock availability, its location and transport to the site of treatment, pretreatment strategies, efficient hydrolytic agents, availability of strong fermentative microorganisms and process options all impact to the production cost of cellulase or other commercialize bio-products. Recent technology developments have the potential to remove these economic performance obstacles and make commercialization possible. Many government, university and industry partnerships have been formed to develop compatible processes and enable an integrated approach towards biomass conversion to achieve the required cost reduction.
(Fan *et al*, 1986)

Since the production of cellulase enzyme is a major factor in the hydrolysis of cellulosic materials, it is important to make the process economically viable. Although much work has been done on the production of cellulase from lignocellulosics (Solomon *et al,* 1996), emphasis has been placed much on bagasse. This work focused at improving its yield by using the various pre-treatment of sugar cane waste as a substrate.

Some features of natural cellulosic materials are known to inhibit their degradation/bioconversion (Solomon *et al*, 1996). These are degree of crystallinity and lignification and the capillary structure of cellulose. The crystallinity and lignification limit the accessibility and susceptibility of cellulose to cellulolytic enzymes and other hydrolytic agents (Fan *et al*, 1986). However, many physical, chemical and microbial pre-treatment methods for enhancing bioconversion of cellulosic materials have been reported. Pre-treatment of cellulose opens up the structure and removes secondary interaction between glucose chains (Fan *et al*, 1986). So this project may compare which

pre-treatment gives the maximum yield of cellulase enzyme instead to enhancing bioconversion of cellulosic materials.

1.2 Objective

The objective of this research is to study the effect of substrate treatment method on production of cellulase enzyme which is CMCase and xylanase by *Aspergillus terreus SUK-1*.

1.3 Scope

The scope of this research is to use an agricultural by-product like cereal straws and sugar-cane bagasse which is contains high in ligno-cellulose as a substrate to produce cellulase enzyme. Then, this undergraduate project also is to know the production of cellulase enzyme at different substrate concentration where 1g, 3g, and 5g of bagasse wiil be used and operate at different cultivation temperature (in incubator) by measuring xylanase and carboxymethylcellulase (CMCase). From the experimental also, the treatment activity method using NaOH and H_2SO_4 will be defined to compare treatment which gives optimum production of cellulase enzyme.

1.4 Problem statement

Although feedstock is available in large quantities, the main challenge for commercialization is to reduce the major operating costs of biomass conversion processes, primarily pretreatment and enzymes. The focus of my project is to investigate the most efficient and cost effective pretreatment technology to substrate which is sugar

cane bagasse (SCB). Pretreatment research is focused on developing processes that would result in reduced bioconversion time, high cellulase enzyme production, and/or higher ethanol yields. This pretreatment is important to ensure the optimum of cellulase production because these enzymes are depends on the successful pretreatment that we have done.

Current pretreatment research and development activities are geared towards identifying, evaluating, developing, and demonstrating promising approaches that primarily support the subsequent enzymatic hydrolysis of the treated biomass. Universal pretreatment process is difficult to predict due to the different nature of biomass. Thus, several physical, chemical and biological treatments are under evaluation. The resulting composition of the treated material is dependent on the source of the biomass and the type of treatment, but in general, is much more satisfying to enzymatic hydrolysis by cellulase and related enzymes than native biomass. (Bailey *et al*, 1986)

CHAPTER II

LITERATURE REVIEW

2.1 Sugar cane

Sugarcane or Sugar cane (*Saccharum*) is a genus of between 6 and 37 species of tall grasses. This plant is grown in warm temperate or tropical regions. It is a fibrous stalk which is about 2 m – 6 m tall and its juice rich of sugar. (Helen *et al*, 1939)

Figure 2.1: Sugar cane (Helen, 1939)

2.1.1 Sugar cane bagasse

This lignocellulosic waste material is the most abundant in this world. Bagasse is the highly fibrous residue remaining after sugar-cane is pressed to remove sucrose. This situation happens when the growth of industries related to these raw materials such as sugar-cane mills. After remove their product, a few commercial uses for the excess bagasse have been developed and its accumulation presents a waste problem for the sugar industry. One potential use of bagasse is a production of commercial product based on cellulose such as ethanol.

The reason why bagasse is choosing in my project is because it's easy to get and the process to turn from the waste of lignocellulosic resources can give us many benefits especially to decrease air pollution.

2.1.2 Composition of sugar cane bagasse

Agricultural by-products like cereal straws and sugar-cane bagasse are high in ligno-cellulose. Roughly three-quarters of straw is cellulose plus hemicellulose. According to the sources, bagasse composition consist of approximately contains around 50% cellulose, 27.9% hemicellulose, 9.8% lignin and 11.3% cell contents. All of these contains is combined together in a complex structure called lignocellulosic biomass and this materials are used to be a substrate in my project (Pandey, 1992).

2.1.3 Products from bagasse

Increasingly, agricultural residues are being used to manufacture something more valuable products. One potential use of bagasse is a production of commercial product

based on cellulose such as ethanol. However, (Spano *et al*, 1992) showed that cellulase production was the most expensive step during ethanol production from cellulosic biomass, which is accounted for approximately 40 % of the total cost.

2.1.4 Substrate pretreatment and method.

Pretreatments are designed to open the structure of lignocellulosic biomass prior to enzyme hydrolysis, to allow efficient production of C5 and C6 sugars. Hydrolysis of the major component, cellulose has received the most attention, as it can be used to produce cellulase and ethanol by fermentation. Cellulose exists in nature as a compact and complex matrix with lignin and hemicellulose. The cellulose is highly ordered and crystalline with amorphous regions. It is surrounded by lignin which acts as a physical barrier and is associated with the hemicellulose. Obviously, reduction of crystallinity of cellulose and removal of lignin and hemicellulose are important goals for any pretreatment process.

According to (Tsao, 1994) the β-1, 4-glucosidic bonds in cellulose are easier to cleave than the α-1, 4-glucosidic bonds of starch. He makes clear that the main problem in cellulose hydrolysis of biomass cellulose is due to the secondary and tertiary structures, not the primary linkage structure. A number of pretreatment methods have been developed to improve cellulose hydrolysis from lignocellulose. They include mechanical pulverization, pyrolysis, concentrated acid, dilute acid, alkali, hydrogen peroxide, autohydrolysis, ammonia fiber explosion (AFEX), wet-oxidation, lime, CO_2 explosion, organic solvent treatment, etc. Each method, in some way, decreases the size of the biomass and opens its physical structure.

A summary of pretreatment methods is given in Table 2.1. Each pretreatment method has distinct advantages and disadvantages.

Table 2.1: Pretreatment method for lignocellulosic materials. (Tsao *et al*, 1994)

Pretreatment Method		Example
Physical	Mechanical pulverization	Chipping, grinding, milling, shearing, extruder
	Pyrolysis	
Physico-chemical	Autohydrolysis	Steam pressure, steam explosion, supercritical carbon dioxide explosion
	Ammonia fiber explosion (APEX)	
Chemical	Ozonalysis	
	Acid hyrolysis	Sodium hydroxide, ammonia, alkaline hydrogen peroxide
	Oxidative delignification Organosolve process	Methanol, ethanol, butanol, phenol
Biological	Brown, white and Soft-rot fungi	

2.1.4.1 Mechanical Pulverization.

Lignocellulosic biomass can be pulverized by chipping, grinding, shearing, or milling. The goal of mechanical pulverization is to reduce the particle sizes of the biomass, as increased surface area leads to improved cellulose hydrolysis. Some of the crystalline structure of cellulose is also destroyed using these methods, though the amount varies according to the type of biomass and power applied in milling or grinding. A vibration ball mill is the most effective mechanical tool for breaking the crystalline structure of cellulose (Simpson, 1996). Mechanical pulverization methods generally are high cost and do not remove the lignin or hemicellulose.

2.1.4.2 Pyrolysis

Lignocellulosic biomass can be rapidly decomposed to gas, bio-oil, and char when heated to temperatures above 300^{o}C in the absence of oxygen. Biomass pyrolysis is conducted at 400-600°C. At these temperatures, the biomass structure separates randomly. Presence of oxygen, zinc chloride and sodium carbonatecan accelerate the decomposition of cellulose, reducing temperature requirements but producing CO_2 and H_2O. However, pyrolysis does not allow recovery of sugars that can be used to make products such as ethanol.

2.1.4.3 Ammonia Fiber Explosion (AFEX)

AFEX treatment is similar to the autohydrolysis process described below. AFEX is usually conducted at 90^{o}C for 30min. It is simple and has a short process time. It is effective for the treatment of corn stover. However, against aspen chips, which contain higher lignin content than sugarcane bagasse, the AFEX process was less effective. The AFEX process requires efficient ammonia recovery to be economical due to the high cost ammonia. A possible approach is to recover the ammonia after the pretreatment by evaporation (Morikawa, 1994).

2.1.4.4 Autohydrolysis (Steam Explosion)

Autohydrolysis is an efficient pretreatment method for some lignocellulosics. Biomass is heated to 205°C, for 10min and then the pressure is released rapidly. Hemicellulose and lignin degradation increase the ability to enzymatically hydrolyze the cellulose. Temperature, moisture content, and treatment time all affect steam explosion. Even though autohydrolysis requires less energy than mechanical pulverization and does

not require recycling, unlike AFEX, it may produce aliphatic acids, furaldehydes, and phenolic side products that are inhibitors of fermentation (Bacher, 1990). To reduce deleterious by-product formation and increase enzymatic hydrolysis yield, catalysts like sulfur dioxide or sulfuric acid have been added prior to steam explosion. Autohydrolysis is considered to be one of the most effective of the pretreatment processes.

2.1.4.5 Ozonolysis

Ozone is a powerful oxidizing agent. Lignin can be removed very effectively using ozone, without by-product formation. Ozonolysis can be conducted at room temperature and atmospheric pressure, and removes about 60% of the lignin from wheat straw. However, this process has a high costs and requires large amounts of ozone.

2.1.4.6 Acid Treatment

There are many methods for acid treatments including use of phosphoric acid, sulfuric acid, hydrochloric acid or peracetic acid (Buchhol, 1983). Reacting biomass with dilute sulfuric acid alters the crystalline nature of the cellulose structure by expanding the surface area of the biomass, allowing water penetration into the crystalline structure. Dilute sulfuric acid treatment improves ease of solubilization of biomass and formation of glucose. Concentrated sulfuric acid pretreatment solubilizes cellulose by breaking down the hydrogen bonds. According to (Lai, 1968), peracetic acid can also be use to treat lignocellulosic biomass. Peracetic acid is a powerful oxidizing agent that removes lignin, in a manner similar to ozone treatment. It can also cleave the aromatic molecules in lignin.

2.1.4.7 Alkali Treatment

Alkali treatment reduces the lignin and hemicellulose content in biomass, increases the surface area, allowing penetration of water molecules to the inner layers, and breaks the bonds between hemicellulose and lignin-carbohydrate. Dilute sodium hydroxide is usually used for alkali treatment. On the other hand, alkaline based methods are generally more effective at solubilizing a greater fraction of lignin while leaving behind much of the hemicellulose in an insoluble and polymeric form (Beldman, 1985).

2.1.4.8 Biological Treatment

Microorganisms, including the brown, white, and soft-rot fungi, attack lignocellulosic biomass, breaking down both lignin and hemicellulose. White and soft rot fungi attack cellulose and lignin. On the other hand, brown rot fungi usually attack only cellulose. The white rot fungi produce powerful lignin degrading enzymes. *Phanerochaete chrysosporium,* a species of white rot fungi produces both lignin peroxidases and manganese-dependent peroxidases for lignin degradation. Polyphenol oxidases, laccases, H_2O_2 producing enzymes and quinosine-reducing enzymes also degrade lignin (Bergmeyer, 1983). Biological treatment requires low energy and normal environmental conditions but the hydrolysis yield is low and requires long treatment times.

2.2 *Aspergillus terreus* SUK-1

Aspergillus terreus is a cosmopolitan fungus which is primarily isolated from compost, plant material, and from soil. *Aspergillus terreus* is more common in tropical or sub – tropical areas.

2.2.1 Macroscopic features

The major macroscopic features remarkable in species identification are the growth rate and the color of the colony. The growth rate of *Aspergillus terreus* is rapid and texture of colonies varies from soft to powdery. Besides that, the surface colony color is light yellow to brown with yellow soluble pigments that are frequently present.

2.2.2 Microscopic features

Hyphae are septate and hyaline. Conidial heads are biseriate (containing metula that support phialides) and columnar (conidia form in long columns from the upper portion of the vesicle). Conidiophores are smooth-walled and hyaline, 70 to 300µm long, terminating in mostly globose vesicles. Conidia are small (2-2.5 µm), globose, and smooth. Globose, sessile, hyaline accessory conidia (2-6 µm) frequently produced on submerged hyphae.

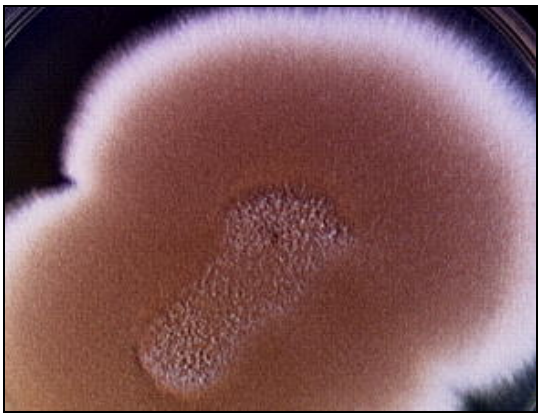

Figure 2.2: *Aspergillus terreus* head (Campbell, 1996).

2.2.3 Advantage and disadvantage using *Aspergillus terreus* SUK-1

The advantage of *Aspergillus terreus* is its can grown at the lower pH. As a result, this advantage indirectly can minimize the contamination problem. Then, mycotoxins are produced only by a few organisms in stationary phase as secondary metabolisms and they are synthesizing only at a particular stage in the life cycle of the organism. Hence, evening fermentations with such organisms, it may be possible to inhibit the production of toxins by growing the organism at fast growth rates (Gray, 1970). Then the disadvantage of *Aspergillus terreus* is its might be produced mycotoxins during the cultivation process.

2.3 Microbial growth cycles

The observation of microbial cell when it's inoculated into batch reactor in different medium is taken to monitor the changes that happens to that cells such as the increasing in cell number, increase in cell population mass and others.

That changes then was plotted as phases of growth as a Figure 2.3:

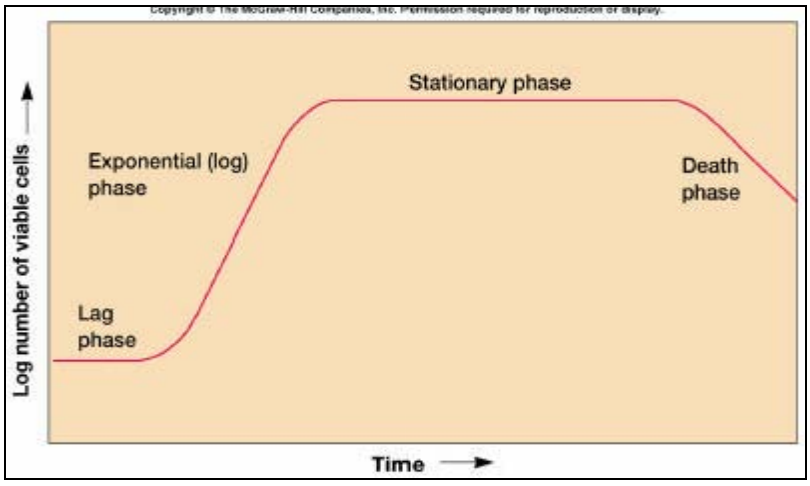

Figure 2.3: Graph of phase of microbial growth cycle (Black, 1996)).

There are 4 different phases of microbial growth cycle which is lag phase, exponential phase, stationary phase and death phase.

a. Lag phase:

At a lag phase, the metabolically of the cells are active but there are no increasing in number of cells. So at this initial phase, it's only stress in increasing size.

b. Logarithmic (Exponential) phase:

Exponential growth is a physiological state marked by back-to-back division cycles such that the population doubles in number every generation time. This exponential phase is shown the growth of population is increasing rapidly.

c. Stationary Phase

Stationary phase is classically defined as a physiological point where the rate of cell division equals the rate of cell death, hence viable cell number remains constant. Mathematically this stationary phase means that when cell division = 0 and cell death = 0, then the rate of cell division = rate of cell death. In other words, when cells stop dividing but have not yet started dying, so they are in *stationary phase*.

d. Death Phase

Microorganism's cell death is the point at which the division of cells is no more happens.

2.4 Cellulase enzyme

Cellulase is an enzyme that breaks down cellulose, the carbohydrate that is the main part of the cell walls of plants. It is also can breaks down cellulose to beta-glucose. It is produced mainly by fungal or microorganism using the lignocellulosic materials such as sugar cane waste. At least two steps in cellulose degradation by microorganisms begin with the preparatory prehydrolytic first step involving an enzyme (C1) which swells and/or hydrates anhydroglucose chains. The second step uses hydrolytic enzymes (Cx) and beta glucosidase (cellobiase).

Trichoderma reesei and *Aspergillus terreus* has an extensively studied cellulase enzyme complex. This complex converts crystalline, amorphous, and chemically derived celluloses quantitatively to glucose (Henrissat, 1985).

2.4.1 Physical and chemical properties

Most cellulase studied has similar pH optima, solubility and amino acid composition. Thermal stability and exact substrate specificity may vary. However, it should be remembered that cellulase preparations generally contain other enzymatic activities besides cellulase, and these may also affect the properties of the preparations. The optimum pH for cellulase preparations is effective between pH 3 and 7 but the

optimum pH generally lies between 4 and 5. Besides that, the optimum temperature for cellulase production is between 40 - 50°C (Henrissat, 1985).

2.4.2 Stability and storage

The activity of cellulase preparations has been found to be completely destroyed after 10-15 minutes at 80°C. Solutions of cellulase at pH 5-7 are stable for 24 hours at 4°C. These products should be stored at 4°C, in a dry place in tightly closed containers. If stored in this manner, lyophilized preparation is stable for several months without significant loss of activity (Henrissat, 1985).

2.4.3 Applications

Cellulase enzyme is used in production of ethanol. The greatest potential for ethanol production from biomass lies in enzymatic hydrolysis of cellulose using cellulase enzymes. Then the other application of cellulase enzyme is various industries such as in alcoholic beverages industries to produce wine. It is also important in chemicals and food industries.

Because cellulase is an enzyme that can breaks down cellulose, which is the carbohydrate that is the main part of the cell walls of plants, so it is important for ruminant's animals such as cows and buffalos. This is the reason why they can get nutrition from plants such as grasses (Funke, 1995).

2.4.4 Economic feasibility

In general, the adoption and implementation of bioconversion technology will depend largely upon the economic feasibility of integrating these practices into existing agricultural and agro-industrial residue management schemes. In most cases of rural agricultural and agro-industrial operations, collection of residues in a large enough quantity to be economical to process is a major problem. Transportation adds to raw material costs and leads to problems such as need for adequate storage facilities and deterioration in quality. In many instances, transportation facilities are not adequate. Where recovery or treatment of residues is necessary largely for environmental reasons, potential economic benefit is rarely realized. In such cases, it is anticipated that at least operating costs can be recovered.

A survey was conducted in Thailand to identify socio-economic issues surrounding the practical feasibility of wide-scale promotion of biogas generation. It was found that most farmers were generally aware of the technology, but economics were the primary concern. They were found to be understandably conservative regarding additional capital expenditure. This attitude is also true among industrial entrepreneurs in the country, with the exception of those operating joint ventures with foreign counterparts (Chamley, 1990).

2.5 Lignocellulosic

Lignocellulosic biomass is the most abundant material in the world. Its sources range from trees to agricultural residues. Long ago, these materials were used as firewood, building materials and animal food. Nowadays, lignocellulosic materials are not just used in their old ways but their applications have expanded into the high level as in enzymatic and bio fuel products. In some cases, the use of lignocellulosics is proceeding to the level of the chemical component itself. For example, cellulose, which

is a major chemical constituent of lignocellulosic materials, can be used for cellulase production.

2.5.1 Bio processing of lignocellulosic materials

To turn the lignocellulosic materials into something more profitable products, normally have several steps to follow as below:

- Pretreatment of lignocellulosic materials with eg. physically, chemically, biologically and naturally.
- Hydrolysis of the polymers to produce readily metabolized molecules.
- Bio-utilization of these molecules to support microbial growth.

Figure 2.4 illustrate the normal procedure to twist the raw materials into marketable products such as cellulase enzyme and ethanol (bio-fuel).

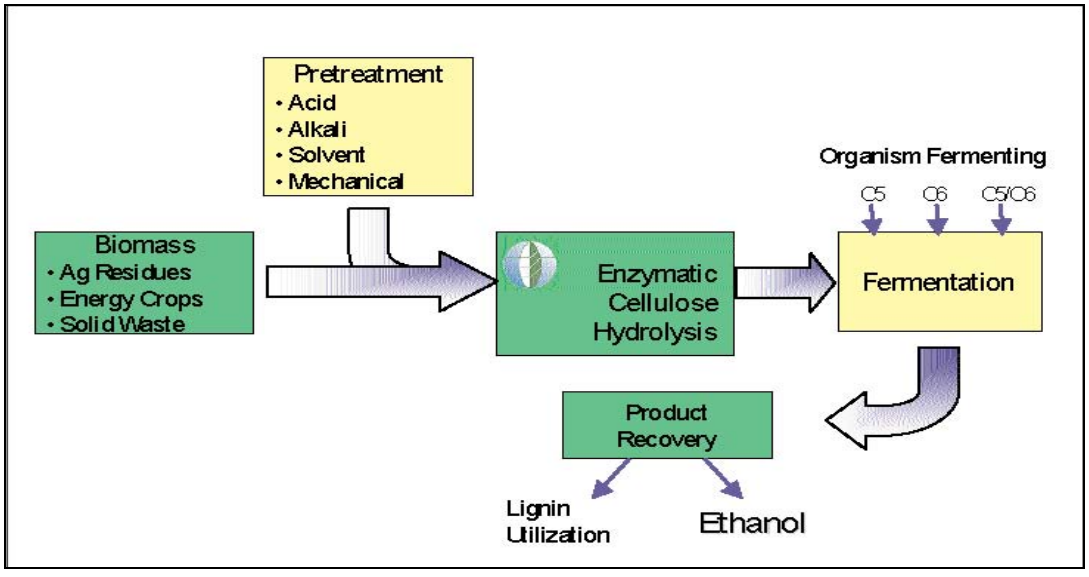

Figure 2.4: Generalized process stages in lignocellulosic materials conversion into bio products (Brown, 2003).

2.6 Cellulose

Cellulose is often said to be the most abundant polymer on earth. It is certainly one of the most important structural elements in plants and other living systems. In nature it is synthesized as slender rod-like crystalline micro fibrils. One of the key features of cellulose is that each of its monomers bears three hydroxyl groups. It is these hydroxyl groups and their hydrogen bonding ability that not only play a major role in directing crystalline packing but also in governing important physical properties of cellulose materials (Evans, 1984).

2.6.1 Structural unit of cellulose.

Cellulose is an insoluble molecule consisting of between 2000 - 14000 residues with some preparations being somewhat shorter. It is a linear polymer chain which is formed by joining the anhydroglucose units into glucan chains. These anhydroglucose units are bound together by β-(1, 4)-glycosidic linkages. Due to this linkage, cellobiose is established as the repeat unit for cellulose chains (Kolpak, 1994) as shown in Figure 2.5.

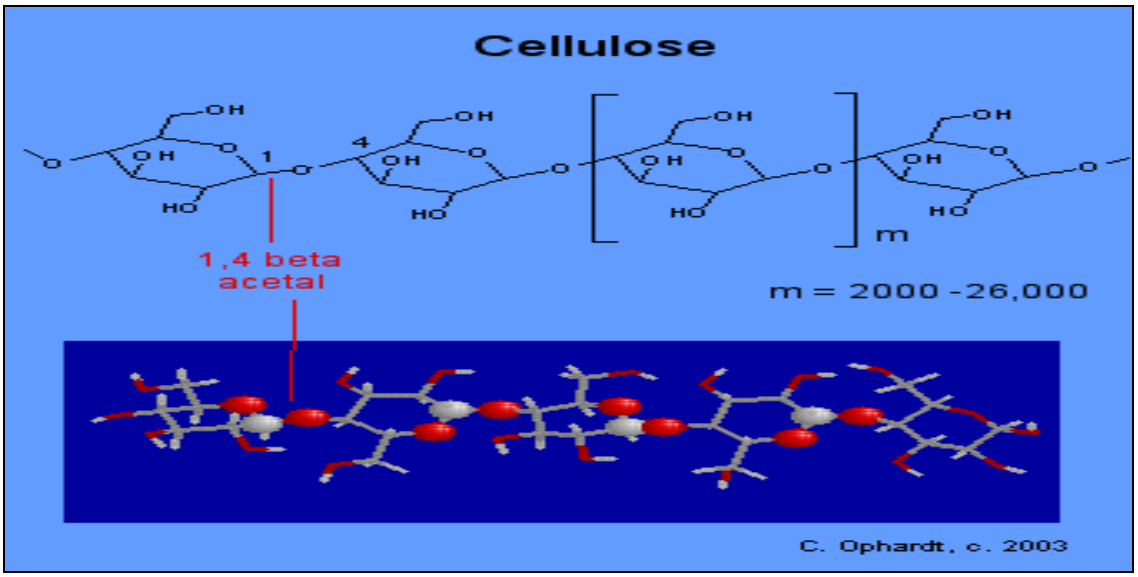

Figure 2.5: Structural unit of cellulose (Ophardt, 2003)

2.6.2 Functionality

Cellulose has many uses as an anticake agent, emulsifier, stabilizer, dispersing agent, thickener, and gelling agent but these are generally subsidiary to its most important use of holding on to water. Water cannot penetrate crystalline cellulose but dry amorphous cellulose absorbs water becoming soft and flexible. Some of this water is non-freezing but most is simply trapped. Less water is bound by direct hydrogen bonding if the cellulose has high crystallinity but some fibrous cellulose products can hold on to considerable water in pores and its typically straw-like cavities, water holding ability correlating well with the amorphous (surface area effect) and void fraction (*i.e.* the porosity). As such water is supercoolable, this effect may protect against ice damage. Cellulose can give improved volume and texture particularly as a fat replacer in sauces and dressings but its insolubility means that all products will be cloudy (Martin Chaplin, 2004)

2.7 Enzymes

According to (Chahal, 1981), enzymes are proteins that speed up biochemical reactions without being consumed or changed by the reaction. They are found throughout nature such as in our bodies, in the environment, and in all living things. Without enzymes, life would not be possible. Enzymes speed up chemical and biochemical reactions, and this process is called catalysis. The substances upon which enzymes work when performing catalysis are known as substrates, and each enzyme will only fit and act upon a specific set of substrates.

2.7.1 Xylanase enzyme

Enzymes are biological catalysts produced by all living things. The enzyme named xylanase deconstructs plant structural material by breaking down hemicellulose, a major component of the plant cell wall. Plant cell walls are necessary to prevent dehydration and maintain physical integrity. They also act as a physical barrier to attack by plant pathogens. In nature, some plant consumers or pathogens use xylanase to digest or attack plants. Many microorganisms produce xylanase, but mammals do not. Some herbivorous insects and crustaceans also produce xylanase.

Xylanase consists of 190 amino acids. Xylanases belong to the glucanase enzyme family and are characterized by their ability to break down various xylans to produce short-chain xylo-oligosaccharides. Xylanase readily crystallizes in ammonium sulfate and sodium/potassium phosphate across pH 3.5 to 9.0. Xylanase can also be crystallized with other salts, polymers, and organic solvents. Xylanase solubility increases with increasing temperature in moderate concentrations of ammonium sulfate. Xylanase solubility in phosphate pH 9 decreases in the temperature range of 0 to 10 degrees Celsius but remains constant in the range of 10 through 37^{o}C. Xylanase has been extracted from many different fungi and bacteria. It is commonly used in animal feeds, paper production, and food production.

2.7.2 Carboxymethylcellulase enzyme (CMC)

Carboxymethylcellulase is a derivative of cellulase formed by its reaction with alkali and chloroacetic acid.

2.7.2.1 Structural unit of CMC

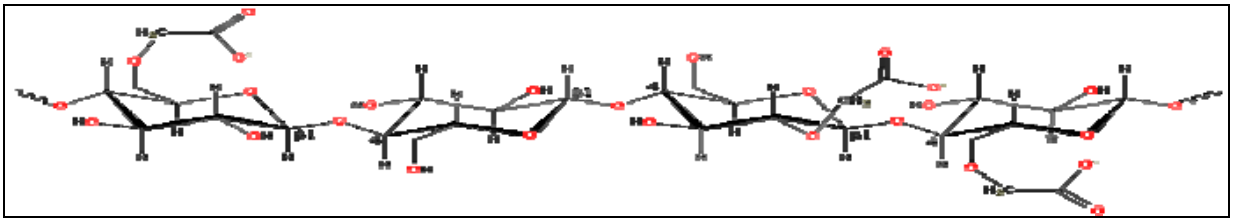

Figure 2.6: Structural unit of carboxymethylcellulase (Chaplin, 2004)

The CMC structure is based on the β-(1→4)-D-glucopyranose polymer of cellulose. Different preparations may have different degrees of substitution, but it is generally in the range 0.6 - 0.95 derivatives per monomer unit.

2.7.2.2 Molecular structure

CMC molecules are somewhat shorter, on average, than native cellulose with rough derivatization giving areas of high and low substitution. This substitution is mostly 2-O- and 6-O-linked, followed in order of importance by 2, 6-di-O- then 3-O-, 3, 6-di-O-, 2, 3-di-O- lastly 2, 3, 6-tri-O-.linked. It appears that the substitution process is a slightly cooperative (within residues) rather than random process giving slightly higher than expected unsubstituted and trisubstituted areas. CMC molecules are most extended (rod-like) at low concentrations but at higher concentrations the molecules overlap and coil up and then, at high concentrations, entangle to become a thermo reversible gel. Increasing ionic strength and reducing pH both decrease the viscosity as they because the polymer to become more coiled (Mitchell, 1992).

2.7.2.3 Functionality

CMC dissolves rapidly in cold water and mainly used for controlling viscosity without gelling (CMC, at typical concentrations, does not gel even in the presence of calcium ions). As its viscosity drops during heating, it may be used to improve the volume yield during baking by encouraging gas bubble formation. Its control of viscosity allows use as thickener, phase and emulsion stabilizer (*e.g.* with milk casein), and suspending agent. CMC can be also used for its water-holding capacity as this is high even at low viscosity; particularly when used as the Ca^{2+} salt. Because of that, it is used for retarding, staling and reducing fat uptake into fried foods (Mitchell, 1992).

2.8 Mechanism of cellulase enzyme

The enzymatic mechanism whereby certain microorganisms can quite rapidly and completely degrade cellulose is not yet understood. Reese *et al*, (1950) proposed that at least two steps are involved. Firstly is prehydrolytic step where anhydroglucose chains are swollen or hydrated and secondly, hydrolytic cleavage of the now susceptible polymers either randomly or endwise. The first step would involve an enzyme designated C1 and the second, hydrolytic enzymes termed Cc. A third type of enzyme is β-glucosidase (cellobiase).

The C1 component attacks highly ordered (crystalline) cellulose, such as cotton fibers or Avicel, but have little effect on soluble derivatives such as carboxymethyl cellulose (CMC). According to Spano *et al*. (1975), C1 "decrystallizes" or hydrates cellulose chains whereas Cc consists of exo and endo β-1, 4 glucanases that attack

soluble derivatives or cellulose that has been acid or alkali swollen (Wood and Philips, 1969.

2.9 Fermentation

Fermentation typically refers to the conversion of sugar to alcohol using yeast. The process is often used to produce wine and beer, but fermentation is also employed in production of cellulase enzyme. The science of fermentation is known as zymology. Fermentation (formerly called zymosis) is the anaerobic metabolic breakdown of a nutrient molecule, such as glucose, without net oxidation. Fermentation does not release all the available energy in a molecule because it merely allows glycolysis (a process that yields two ATP per glucose) to continue by replenishing reduced coenzymes. Fermentation yields lactate, acetic acid, ethanol, or other reduced metabolites. Fermentation is also used much more broadly to refer to the bulk growth of microorganisms on a growth medium. No distinction is made between aerobic and anaerobic metabolism when the word is used in this sense. Fermentation usually implies that the action of the microorganisms is desirable.

According to encyclopedia, fermentation also can define as process by which the living cell is able to obtain energy through the breakdown of glucose and other simple sugar molecules without requiring oxygen. Fermentation is achieved by somewhat different chemical sequences in different species of organisms. Two closely related paths of fermentation predominate for glucose. When muscle tissue receives sufficient oxygen supply, it fully metabolizes its fuel glucose to water and carbon dioxide. However, at times of strenuous activity, muscle tissue uses oxygen faster than the blood can supply it. During this anaerobic condition, the six-carbon glucose molecule is only partly broken down to two molecules of the three-carbon sugar called lactic acid. This process, called lactic acid fermentation, also occurs in many microorganisms and in the cells of higher animals. In alcoholic fermentation, such as occurs in brewer's yeast and