

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Global energy consumption has increased 17-fold in the last 10 decades due to industrialization and population expansion. The depletion of fossil fuel resources, catalysed by a high rate of consumption of fossil fuel, contributed to the increase in price of crude oil and the reduction in energy security (Hammond *et al.*, 2008). These factors have strengthened worldwide interest in renewable sources of energy. Renewable energy has much greater advantages compared to non-renewable energy that are derived from fossil fuels in terms of availability, sustainability and environment friendliness (Tan *et al.*, 2008). The use of biomass to produce bioethanol to substitute fossil fuels has been given much attention world-wide. Bioethanol, aptly named as the carbon-neutral fuel, is the most viable way to ensure future sustainability of energy (Andrietta *et al.*, 2008).

Another advantage on the use of bioethanol compared to fossil fuels is that bioethanol has a higher oxygen content and a zero sulphur content, thereby reducing the emission of acidic gases during the combustion of fuel (Demirbas and Balat, 2006). Bioethanol has a low net carbon dioxide emission because the carbon content in the ethanol is derived from carbon dioxide in the atmosphere through photosynthesis by plants. The carbon dioxide is eventually released back into the atmosphere during combustion,

theoretically giving a net zero carbon cycle. Additional benefit of bioethanol development includes creating job opportunities in rural areas (Demirbas, 2009).

There are two main types of biofuels, namely bioethanol and biodiesel. Biofuels have similar characteristics as liquid fossil fuels except that biofuels are produced from biomass. Biodiesel is produced by transesterification of feedstock into methyl esters. The feedstock is mainly waste vegetable oils that are extracted from seeds, nuts or animal fats (Puppan, 2002). Bioethanol is produced by the fermentation of crops that are high in sugar or starch content or from lignocellulosic biomass. The biomass could be in the form of grass, agricultural crop residues and timber waste (Demirbas, 2007). Biofuels are often used as 5-25% blends in gasoline, or used neat in modified engines (Hammond *et al.*, 2008). Bioethanol is the most common liquid transportation biofuel used for engines in the world today. Bioethanol derived from renewable sources such as sugar crops or lignocellulosic biomass is non polluting, locally available, highly accessible, sustainable and reliable (Demirbas, 2009).

A biological feedstock that contains a substantial amount of carbohydrates can be converted into glucose and be fermented to produce bioethanol (Malça and Freire, 2006). The biological feedstock includes starch-based grains such as corn, or sugar from sugarcane (Hamelinck *et al.*, 2005). The second option is to convert cellulose or lignocellulose obtained from woody parts of plant residues into glucose, followed by fermentation to yield bioethanol (Verdin, *et al.*, 2009).

First generation bioethanol is produced from agricultural crops that are rich in starch such as wheat, corn, sorghum grains and cassava, or from sugar crops such as

sugarcane, sugar beet and fruits. Second generation bioethanol is derived from cellulose in plant stems and wood or from marine algae that are cellulose-rich (Polycarpou, 2009). Starch-based crops are part of the food chain and are also used as feedstock for poultry and cattle. Bioethanol derived from food crops may lead to many socio-economic problems such as shortages of food, increases in food price and a growing competition for land (Mol, 2007). Furthermore, cultivation of feedstock may also create more environmental pollution such as soil pollution and excessive use of fertilizers (Crutzen et al., 2007). The Royal Society and The House of Commons Audit Committee commented on the sustainability of bioethanol supply and addressed the issues of price rise, destruction of ecosystem and claims that carbon balance of bioethanol from maize may be worse than that of fossil fuels once land displacements are taken into account. On the contrary, bioethanol produced from biomass waste does not pose similar risks. On the other hand, bioethanol production can save landfills for waste disposal, utilize the waste, and reduce green house gas emission from disposal sites (Stichonothé and Azapagic, 2009). These studies have spotlighted the advantages of second generation bioethanol which is produced from lignocellulosic biomass compared to the first generation bioethanol depending on the energy balance and sustainability (Taylor, 2008).

Among all biological feedstocks, lignocellulosic residues in the form of agricultural residues and forest residues are the most abundant and attractive sources because they are non-food portions of plants and do not compete with human for food (Li *et al.*, 2009). On the other hand, the price of feedstock contributes the most in production cost, hence there is a need to substitute the use of energy crops that are grown merely for this purpose and the utilization of lignocellulosic biomass from unwanted parts of plants or agricultural

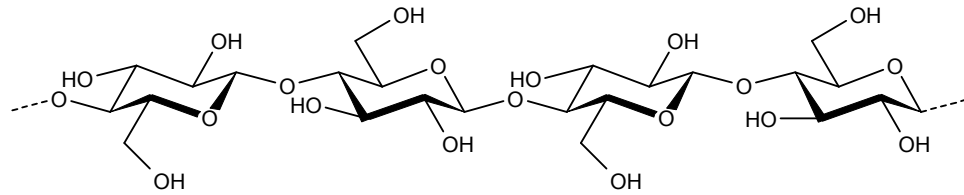
waste will cut the production cost of bioethanol and make bioethanol more competitive in the market (Sanchez and Cardona, 2008). These lignocellulosic wastes can be used as the feedstock for production of bioethanol instead of being disposed. However, to derive sugar monomers from cellulose and hemicellulose is a far more challenging task compared with that from starch (Linde, *et al.*, 2008).

1.2 Production of Bioethanol from Lignocellulosic Biomass

Lignocellulosic biomass consists of 40-50% cellulose, 23-35% of hemicelluloses and 15-20% of lignin. Due to the complex structure of cellulose, hemicellulose and lignin, the activity of enzymes is inhibited in hydrolysis and often, a pretreatment is required in order to increase the efficiency of enzymes (Mabee and Saddler, 2010).

Starch and cellulose are polymeric forms of glucose. In cellulose, glucose units are bonded by β -1,4-linkages that generate a linear and a more rigid structure than starch in which the glucose units are bonded via α -1,4-linkages (Mabee and Saddler, 2010), the hydrolysis of cellulose is more challenging than that of starch.

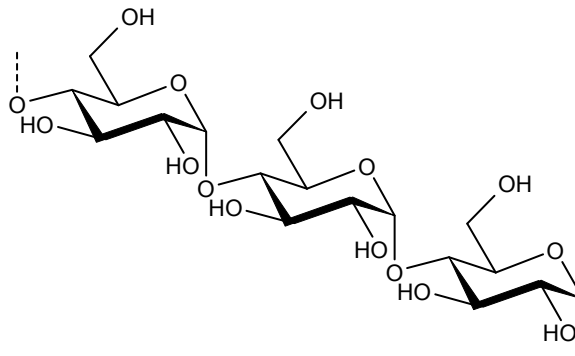
The following diagram shows the structure of cellulose. Glucose monomers are bonded by a more rigid β -1,4-glycosidic bonding making the production of monomeric glucose from cellulose more challenging.



--- β -glucose-(1 \rightarrow 4)- β -glucose-(1 \rightarrow 4)- β -glucose-(1 \rightarrow 4)- β -glucose---

Figure 1.1: Partial structure of cellulose

The following figure shows the structure in starch. Glucose monomers are bonded by α -1,4-glycosidic linkages that can be hydrolysed easily. Enzymes such as α -amylase and amyloglucosidase can hydrolyse starch easily but not cellulose or hemicelluloses that have a linear and more rigid structure (Linde et al., 2008).



--- α -glucose-(1 \rightarrow 4)- α -glucose-(1 \rightarrow 4)- α -glucose---

Figure 1.2: Partial structure of starch

Xylan, glucomannan and xyloglucan are the three main types of hemicelluloses found in hardwood. Hemicellulose is made up of many types of monomers unlike cellulose and starch that are made up of one type of monomer only (Laine, 2005).

The following figure shows the structure of xylan, the major hemicelluloses found in plants. It consists of mainly xylose.

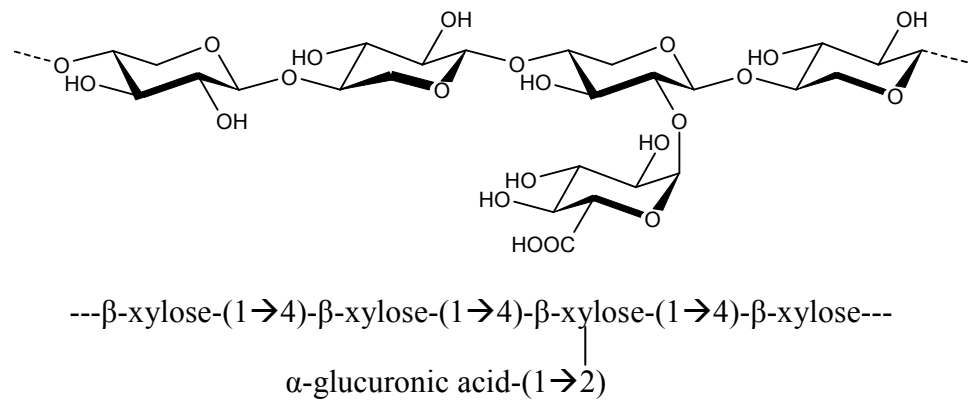
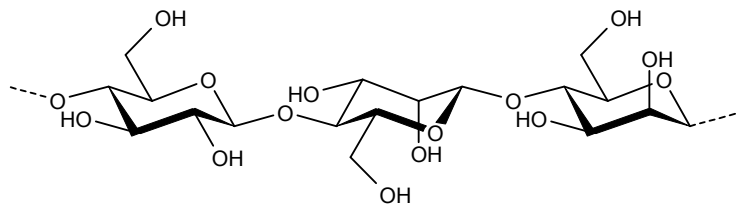


Figure 1.3: Partial structure of xylan.

The following figure shows the structure of glucomannan, another type of hemicellulose found in plants, consisting of glucose and mannose.



--- β -glucose-(1 \rightarrow 4)- β -mannose-(1 \rightarrow 4)- β -mannose---

Figure 1.4: Partial structure of glucomannan

The following figure shows the structure of xyloglucan, another type of hemicelluloses found in plants, consists of mainly xylose and glucose.

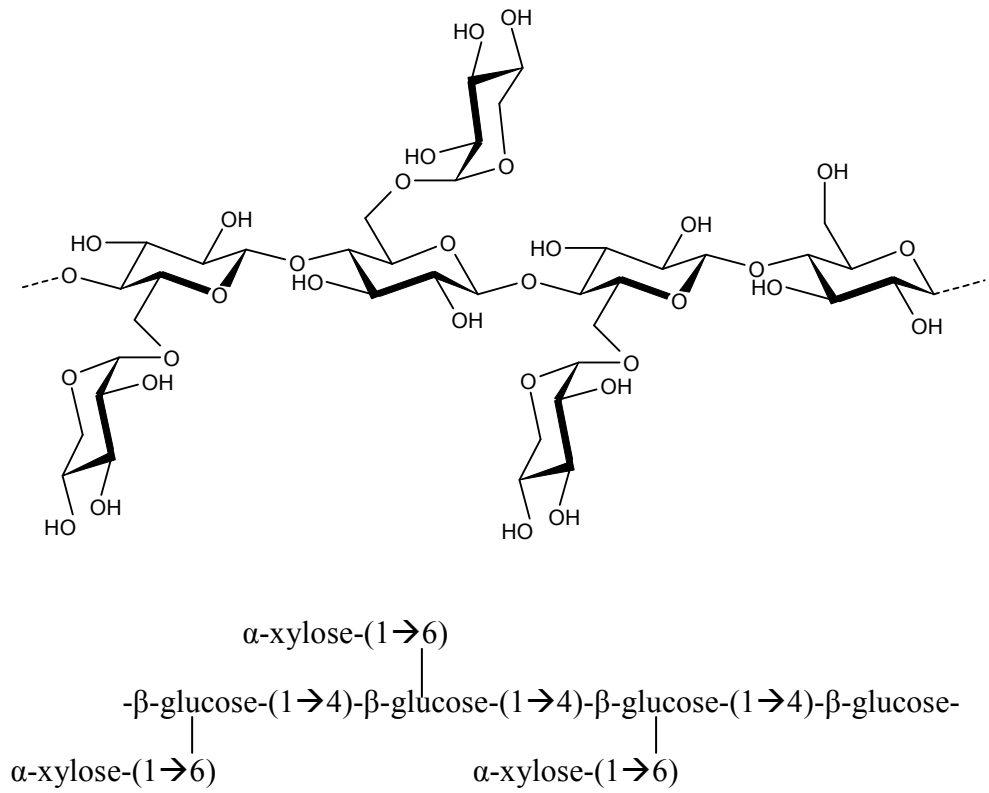


Figure 1.5: Partial structure of xyloglucan

Lignin is a stubborn material that inhibits the hydrolysis of cellulose and hemicelluloses (Mabee and Saddler, 2010) that is a polymer consisting of phenylpropane units. Monomers of lignin are bonded by ether bonds or carbon-carbon bonds. Carboxyl groups are found in the lignin structure.

The following figure shows a part of lignin. Lignin is a polymer that made up from phenylpropane units.

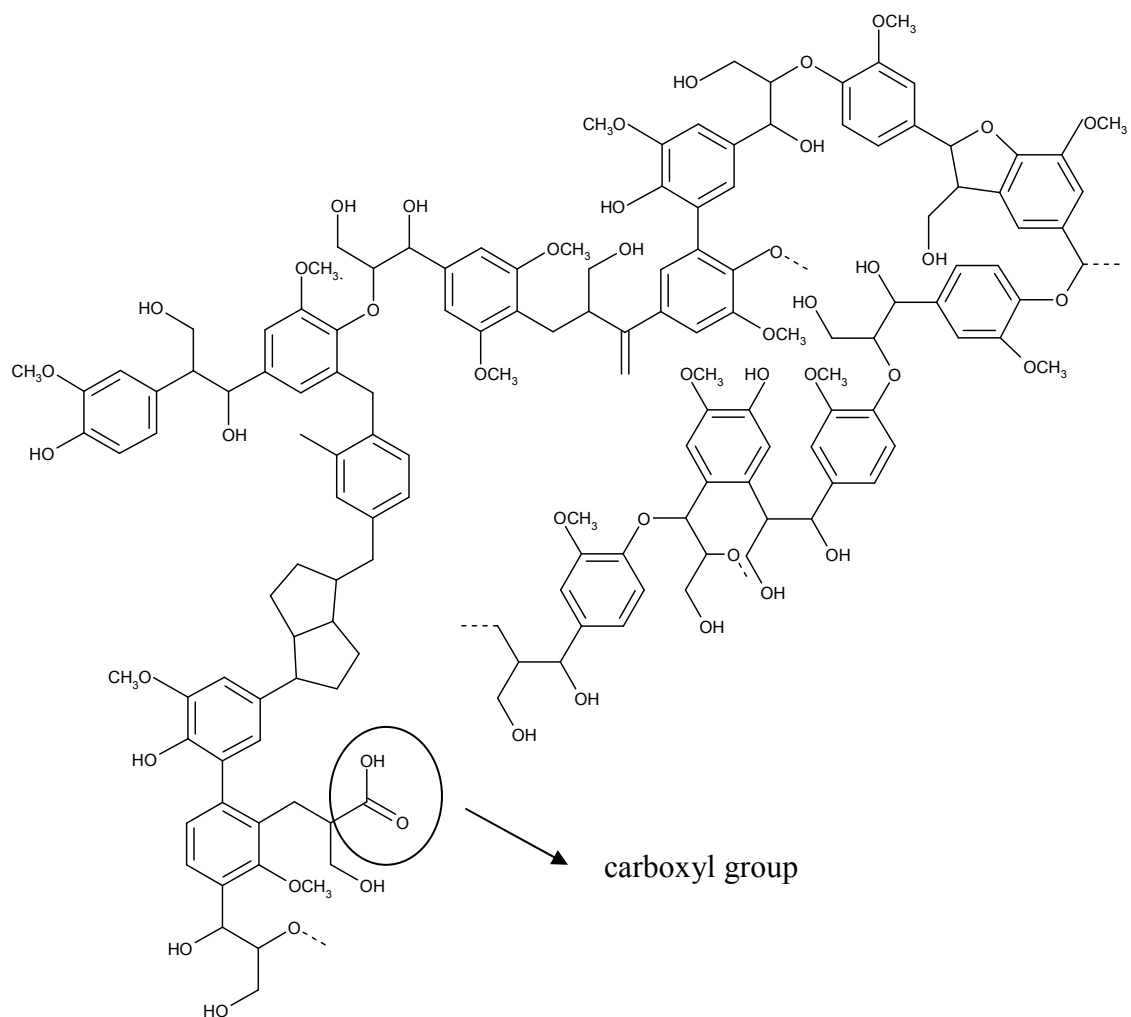


Figure 1.6: Partial structure of lignin

Functional groups present on lignin are methoxyl, phenolic hydroxyl, benzyl alcohol, carboxyl and carbonyl groups. It has been proposed that the lignin and

hemicellulose are bonded by different types of bonding such as glycosidic bond and ester bond between the functional groups on hemicellulose and lignin.

The following diagram shows that a hydroxyl group in the cellulose is bonded to a carboxyl group in the lignin forming an ester bond in the plant cell wall.

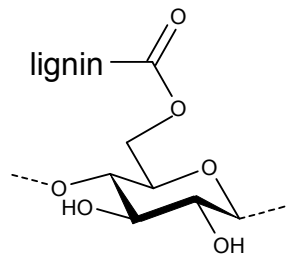


Figure 1.7: Ester bond between lignin and cellulose

The first step in bioethanol production is the pretreatment of the lignocellulosic biomass which separates hemicelluloses and cellulose from lignin. Pretreatment increases the total surface area of the wood fibres by water-based, acidic, alkaline or organic solvent pulping methods (Wyman *et al.*, 2005). Pretreatment results in two portions, a solid fraction of cellulose that is made up of glucose and a liquid fraction of hemicellulose that is made up of mainly xylose and arabinose (Kaparaju *et al.*, 2009). In the pretreatment, the destruction of cellulose and hemicellulose, the formation of the inhibitor for fermenting yeast, the use of high energy and the use of expensive chemicals should be avoided in order to reduce the cost of production and to increase the yield of ethanol (Balat, 2011).

The following figure shows the effect of pretreatment on lignocellulosic biomass (Hsu, *et al.*, 1980). Lignin is separated from the hemicellulose and the cellulose, this helps to increase the efficiency of hydrolysis.

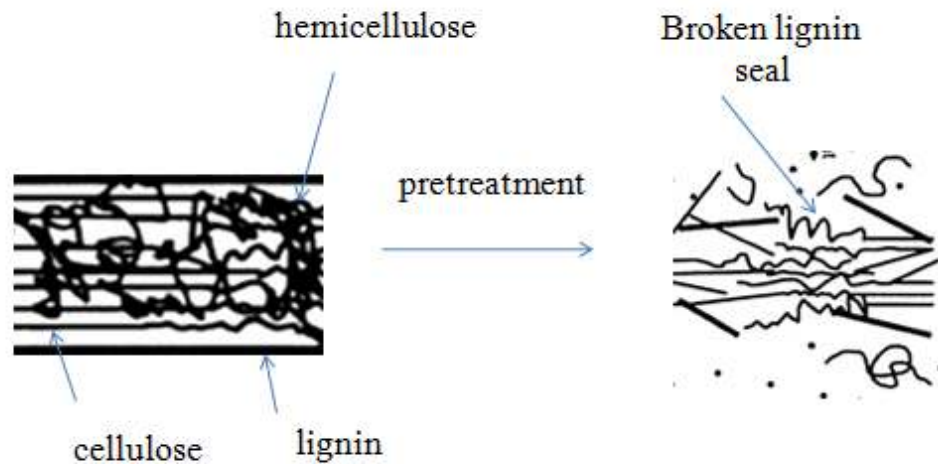
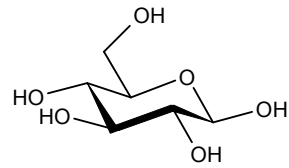


Figure 1.8: Effect of pretreatment on lignocellulosic biomass

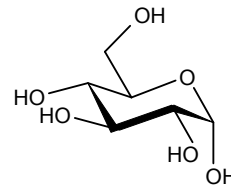
Pretreatment is followed by the hydrolysis of hemicellulose and cellulose into monosaccharides such as into glucose, xylose, galactose, mannose, and arabinose. Hydrolysis may be done by means of enzymes such as cellulases that are produced by microorganisms or by chemical means by using mineral acids. The six-carbon sugars are then fermented into ethanol by using suitable strains of yeast (Mabee and Saddler, 2010). *Saccharomyces cerevisiae* is the most commonly used yeast in the fermentation to give high yields of bioethanol (Watanabe *et al.*, 2009). Hemicellulose contains pentose sugars that are not fermentable by ordinary yeast. However, some studies have shown that some

pentose-fermenting microorganisms are capable to ferment pentose sugars in a large scale (Linde *et al.*, 2008).

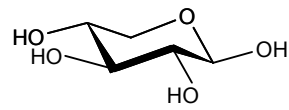
The following figure shows the products of hydrolysis of cellulose and hemicelluloses.



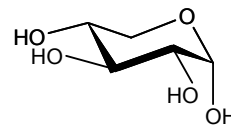
β -glucose



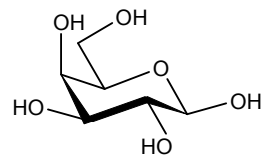
α -glucose



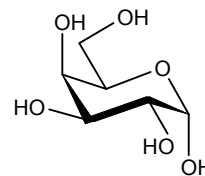
β -xylose



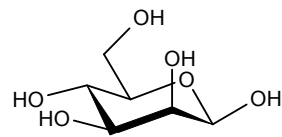
α -xylose



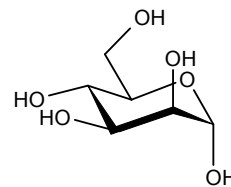
β -galactose



α -galactose



β -mannose



α -mannose

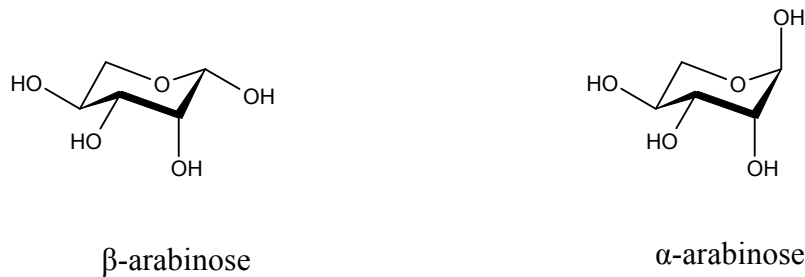


Figure 1.9: Examples of monosaccharide produced by hydrolysis

The final step in the production of bioethanol is the distillation of ethanol from the fermented sugar solution. Ethanol forms azeotropic solution with water, hence the maximum percentage of ethanol that can be obtained is 95%. Absolute ethanol can be obtained by drying agents such as calcium oxide or molecular sieve that absorbs water from ethanol-water azeotrope.

1.3 Problem Statement and Significance of the Study

Energy crops such as corn and sugar cane are the main sources of bioethanol currently. Starchy crops and sugary crops are commonly used because the fermentable sugars such as glucose or sucrose can be obtained easily by the hydrolysis or directly from the juice. One problem with the production of bioethanol from energy crops is the suitability of the raw materials for the production. The feedstocks for bioethanol may not be available throughout a whole year and they also depend on geographic locations. The volatility of the price of the raw materials can highly affect the production cost as the cost of the raw materials is the major cost (Yoosin and Sorapipatana, 2007). On the other hand, the production of bioethanol at current stage is at a high cost because firstly, the yeast

Saccharomyces cerevisiae is not capable of converting starch into ethanol, therefore, a large amounts of amylolytic enzymes, namely, glucoamylase and α -amylase, need to be added; secondly, the process requires a large amount of heat energy as the starchy materials need to be cooked at a high temperature (413–453 K), in order to obtain a good yield of ethanol; thirdly, the hydrolysis and the fermentation are biological processes, making the production more time consuming.

In this study, we utilize a waste from a mushroom farm, which is the spent mushroom sawdust substrate (SMSS) in bioethanol production. The production of ethanol by SMSS will not only save the costs of raw materials but also helps to preserve the beauty of our environment by reducing the area of landfills for waste disposal. For the pretreatment, we utilized aqueous potassium hydroxide as it is a cheap and easily available chemical. In addition, the pretreatment is done at room temperature in order to reduce the usage of energy for heating. Compared to other methods, such as microwave, a large amount of energy is needed; acetone, is unfriendly to the environment and to the human health. For the hydrolysis, we used 70% (v/v) perchloric acid at 80°C. The temperature of the perchloric acid is kept low, this helps to save heat energy and to ensure that the acid is not overheated.

The conversion of cellulose and hemicellulose into fermentable sugar is harder than that of the starch (Ohgren et al., 2006). During the chemical pretreatment and the hydrolysis, due to the presence of the lignin, more potential inhibitory products will be formed (Sun and Cheng, 2002). In order to produce bioethanol by lignocellulosic biomass in large scale, we need to address some of the problems:

- The efficiency of the pretreatment in helping to increase the ethanol yield.
- The optimum conditions of the hydrolysis in order to achieve higher yield.
- The suitable conditions for the fermentation.
- The survivor of the microorganism in the presence of potential inhibitory compounds such lignin and the side products formed in the pretreatment and the hydrolysis.
- The minimum usage of chemicals and energy in the production.

1.4 Objectives of the Study

The objectives of this study are:

- To find the optimum conditions from various hydrolysis parameters.
- To hydrolyse the spent mushroom sawdust substrate (SMSS), delignified spent mushroom sawdust substrate (DSMSS) and cellulose by perchloric acid at optimum conditions.
- To ferment hydrolysates of spent mushroom sawdust substrate, delignified spent mushroom sawdust substrate and cellulose by *Saccharomyces cerevisiae* to obtain optimum yield of bioethanol.