CHAPTER 1

GENERAL INTRODUCTION AND THESIS OUTLINE

The most common renewable fuel today is ethanol produced from fermentation of corn grain (starch) and sugar cane (sucrose). It is expected that there will be limits to the supply of these raw materials in the near future (Kevin *et al*., 2006). Therefore, lignocellulosic biomass has long been known as a potential low cost source of mixed sugars for fermentation to fuel ethanol and it seen as an alternative raw material for future supplies for ethanol production. World-wide lignocellulosic residue generation every year results in pollution of the environment and in loss of valuable materials that can be bioconverted to several value-added products. Lignocellulosic biomass resulting complex composition, cellulose, hemicelluloses, and lignin which presense in wood represents a major problem for the development of biomass conversion process. Therefore, the biological process of ethanol fuel production utilizing lignocelluloses as substrate requires: (1) delignification (pretreatment) to liberate cellulose and hemicelluloses from their complex with lignin, (2) depolymerization (hydrolysis) of the carbohydrate polymers (cellulose and hemicellulose) to produce free sugars, and (3) fermentation of mixed hexose and pentose sugars to produce ethanol (Jeewon, 1997).

There have been many processes developed for substrates pretreatment such as acid hydrolysis, alkaline treatment, and steam treatment with SO_2. However, acidic pretreatments result in high concentrations of furfurals in the liquid phase (Eggeman and Elander, 2005). Furfural had a great negative effect on specific growth rate. Moreover, furfural and catechol showed a synergistic effect on toxicity with respect to ethanol yield and biomass yield (José *et al*, 2006). The alkaline methods may result in high concentrations of ferulate and acetate in the hydrolysate. These compounds present in the sugar stream and have deleterious effects on the fermentative microorganism (Eggeman and Elander, 2005). Many white rot basidiomycetes have been employed for production of lignin degrading enzymes and for delignifying which used as biological method for lignocellulosic materials. The advantages of biological delignification over the previous methods may include mild reaction condition, higher product yields and fewer side reactions, and less energy demand and less reactor resistance to pressure and corrosion (Jeewon, 1997).

After pretreatment, cellulose and hemicelluloses are hydrolyzed to soluble monomeric sugar using cellulases and hemicellulases, respectively. Recently, many fungal species were reported as hydrolyzed enzyme producer such as *Trichoderma* sp., *Penicillium* sp. and *Aspergillus* sp. (Dashtban *et al*., 2009). Moreover, thermostable enzymes have been studied. There are several advantages including higher specific activity and higher stability which improve the overall hydrolytic performance. *Thermoascus aurantiacus* has been proposed as good candidates for bioconversion of lignocellulosic biomass (Viikari *et al*., 2007). Then, the major components in enzyme hydrolysates, glucose and xylose released from cellulose and hemicelluloses, are used for ethanol fermentation by using microorganisms, for example, *Saccharomyces cerevisiae* (Sanjeev *et al*, 2002) and *Pichia stipitis* (Sonali and Banwari, 2007).

This study will focus on the use of fungi in the biological delignification (pretreatment) and bioconversion (hydrolysis) of lignocellulosic biomass, mainly agricultural residues for bio-ethanol production.

The objectives of the research are as follow;

1. To screen randomly selected white rot fungi for the production of laccase enzyme, using for lignocellulosic biomass pretreatment.
2. To find out optimum condition for laccase production from agricultural residues using selected strain.
3. To use cellulose and hemicelluloses degrading enzymes from selected fungi for lignocellulosic biomass hydrolysis.
4. To determine optimum condition for reducing sugar and bio-ethanol production from agricultural residues.

For setting the background to this work, Chapter 2 reviews the ethanol production from lignocellulosic biomass. The attractive of fungal pretreatment and hydrolysis were presented. Moreover, the knowledge of lignocellulosic biomass, solid state cultivation (SSC) and statistical experiment were included.

Chapter 3 Isolation and identification of laccase producing white-rot fungi

Chapter 4 Optimization condition for laccase production

Chapter 5 Purification, characterization and properties of laccase from *Trametes polyzona* WR710-1

Chapter 6 Morphological and chemical composition of pretreated lignocellulosic biomass

Chapter 7 Optimization condition for cellulase and xylanase production

Chapter 8 Enzymatic hydrolysis and preliminary of bio-ethanol production