CHAPTER 1 INTRODUCTION

1.1 Back ground and rationale

Cellulose is the main constituent component of photosynthesis in the plant biomass composed of fibrous and woody material such as straw, weeds, grass, leaves, stems and branches of plants (Sutedja et al., 1991; Jarvis, 2003; and Zhang et al., 2004). Cellulose is a linear polymer consisting of D-anhydroglucopyranose molecules are joined together by the bond of ß-1, 4 glycosidic of the degree of polymerization (Lynd et al., 2005 and Zhang et al., 2007). According to Schwarz (2001), cellulose is difficult degraded because it crystalline and insoluble in water. Besides that cellulose in nature is rarely found in pure form but together with lignin and hemicellulose.

As plant biomass, cellulose often found in the biomass lignocellulosic materials such as biomass agricultural, forestry, and agro-industrial wastes that are abundant, renewable and inexpensive energy sources. At this time, most of energy sources are from non-renewable resources, such as oils, gas and coals, so there is some other alternative source of energy which is changed utilization energy non-renewable resources to renewable resource such as biomass of agricultural or forestry waste (Yanni et al., 2010). Biomass of agricultural or forestry waste include a variety of lignocelulosic materials such as sawdust, oil palm empty fruit bunch, poplar trees, sugarcane bagasse, waste paper, brewer’s spent grains, switchgrass, and straws, stems, stalks, leaves, husks, shells and peels from cereals like rice, wheat, corn, sorghum and barley, among others. Lignocellulose wastes are accumulated every year in large quantities, causing environmental problems. However, due to their chemical composition based on sugars and other compounds of interest, they could be utilized for the production by-products, such as ethanol, food additives, organic acids, enzymes, and others. Utilization of agricultural waste will be tackling pollution problems. Agricultural waste is a major component of lignocellulose. Lignocellulose consists of three polymers are cellulose, hemicellulose, and lignin (Klemm et al., 1998 and Perez et al., 2002).

Cellulose is a glucose polymer-bonded β-1 ,4-glycosidic (Kim et al., 2004). Hemicellulose is a polymer of linear and branched heterogeneous usually consists of five different sugars - Larabinose, D-galactose, D-glucose, D-mannose, and D-xylose - as well as other components such as acetic, glucuronic, and ferulic acid (Fengel et al., 1989). Lignin is a very complex molecule constructed of phenylpropane units linked in a three-dimensional structure of the major. Lignin is closely bound to cellulose and hemicellulose and its function is to provide rigidity and cohesion in the cell wall material, to provide water impermeability to xylem vessels, and to establish the physical-chemical barrier against microbial attack (Fengel et al., 1989).

Cellulose degrading organisms have been used to convert cellulose to sugars dissolved components, some of which have been applied in biotechnology and industry (Cheryl et al., 2003 and Kotchoni et al., 2003). Cellulose can be degraded easily and quickly just by specific organisms such as bacteria, fungi, actinomycetes, and the lower animals. Experts classify cellulose decomposers organisms are aerobic bacterial, myxobacteria, anaerobic bacteria, thermophilic group, actinomycetes, filamentous fungi, mushroom, protozoa and insects. Success in breaking down cellulose depends on the nature or circumstances of cellulose degrading microorganisms and environmental factors such as humidity, aeration, temperature, and nitrogen availability of adequate and nutritional elements (Sutedja et al., 1991). More recently, some bacteria such as *Clostridium thermocellum*, *Clostridium cellulolyticum*, *Clostridium cellulovorans, Clostridium Josui, Ruminocoocus albus,* while the other group of actinomycetes is *Thermoactinomycetes sp*., *Thermonospora curvata* and *Streptomyces sp*., they have been reported as a producer of cellulase using a different substrate such as cellulose, carboxymethylcellulose, starch, and glucose as the carbon source (Schwarz, 2001; Belaich et al., 2002; Lopez-Contreras et al., 2004; and Keller et al., 2005).

When degrading cellulose, microorganisms produce an enzyme called cellulase. Cellulase (1,4-ß-D-glucan glucanohydrolase) is a multienzyme complex consisting of three main components, namely endo-ß-glucanase, exo-ß-glucanase and ß-glucosidase which has demonstrated synergistic activity in hydrolyzing cellulose (Emert et al., 1974 and Ryu et al., 1980). Since the first, cellulase was used as animal feed (Ishiruko, 1993). In industry, the utilization produces organic acids (Luo et al., 1997), detergents and chemicals, besides cellulases are also used in the pulp and paper industry (Oksanen et al., 2000), in the fermentation of sugar and ethanol products (levy et al., 2002 and Van-Wyk et al., 2003), in the textile industry (Cavaco-Paulo et al., 2003; Miettinen-Oinonen et al., 2004; Nierstrasz et al., 2003), in the food industry (Urlaub, 2002; Pentilla et al., 2004), for the processing of paper and cellophane, cellulosic waste biotransformation to ferment sugars (Van-Wyk et al., 2003). As a lytic enzyme, cellulase was also instrumental in producing protoplast (Bhat, 2000; Davis, 1985; and Mandels et al., 1974).

Oil palm empty fruit bunch (OPEFB), a lignocellulosic material is generated as a waste product. Here is needed the most attention in the utilization of lignocellulosic wastes as raw materials that have low cost value if harnessed through the fermentation process produces high-value products. That requires microorganisms that can degrade lignocellulose from OPEFB induction using extra-cellular enzymes such as cellulase, hemicellulase (xylanase), and lignin peroxidase. Chemical and enzymatic methods are the most commonly applied methods for hydrolyzing cellulosic materials. In the chemical method, the hydrolysis of sugar polymers in lignocellulose is catalyzed by an acid, whereas in the enzymatic process, enzymes are used for hydrolyzing cellulose and hemicellulose to monomeric sugars. In this study, oil palm empty fruit bunches as the raw material performed pretreatment hydrolysis using mild sulfuric acid to obtain residue from acid hydrolysis processed of OPEFB. OPEFB and acid hydrolysis residue is then performed fermentation using microorganisms to produce glucose as saccharification process and subsequently fermented using *Saccharomyces cerevisiae* to produce ethanol.

1.2 Objective

1.2.1 To isolate and identify the cellulolytic microorganisms from several places at the location associated with the oil palm plantation and industry.

1.2.2 To obtain microorganisms that can degrade cellulose, have a high cellulase activity and produce reducing sugars by using CMC, oil palm empty fruit bunch fiber and residue from acid hydrolysis of oil palm empty fruit bunch as substrates.

1.2.3 To find the optimal conditions for growth and cellulase production of the selected microbial isolate in CMC, OPEFB, and the acid hydrolysis residue.

1.2.4 To compare saccharification between OPEFB and its acid hydrolysis residue by the selected microbial isolate.
1.2.5 To determine and characterize enzyme cellulases of the selected cellulolytic microorganism.
1.2.6 To convert sugars from a saccharification process into ethanol using *Saccharomyces cerevisiae.*

1.3 Scope of work

1.3.1 Preparation of acid hydrolysis of OPEFB by hydrolyzing the fiber with mild acid and steaming under pressure.
1.3.2 Isolation of the cellulolytic microorganisms from several places at the location associated with the oil palm plantation and industry.
1.3.3 Selection of the most suitable cellulolytic microorganism for using in saccharification of OPEFB and its acid hydrolysis residue.
1.3.4 Ethanol production from saccharified products of OPEFB and its acid hydrolysis residue by *Saccharomyces cerevisiae.*