CHAPTER I
INTRODUCTION

Environmental impacts and inevitable depletion of fossil fuels have led researchers to find alternative energy sources. The interesting fuels are biofuels (bioethanol, biohydrogen, and methane), which are a group of promising energy that can substitute fossil fuels in the future, because they can be produced from renewable resources as cellulose.

Cellulose is a glucan polymer of D-glucopyranose units, which are linked together by β-1,4-glucosidic bonds. Cellulose molecules are oriented and formed by both intra- and inter-molecular hydrogen bonds. These interactions cause packing density of cellulose to increase, and then crystalline regions are formed (Rowell *et al.*, 2000). Therefore, using cellulose as a feedstock for direct biofuel productions is non-effective because these crystalline regions inhibit the cellulose conversion. To improve the production effeciencies, cellulose should be converted to reducing sugar, glucose.

An effective way, which can convert cellulose to glucose, is to use cellulase enzyme. Cellulase or cellulolytic enzyme is a group of enzyme, which can hydrolyze β-1,4-glycosidic linkage in cellulose via synergistic actions by three different enzymes: endoglucanase (1,4-β-D-glucan-4-glucanohydrolase, E.C. 3.2.1.4), exoglucanase (1,4-β-D-glucan cellobiohydrolase, E.C. 3.2.1.91), and β-glucosidase (E.C. 3.2.1.21) (Zhang *et al.*, 2006). Cellulase enzyme can be produced by various microorganisms, such as fungi, protozoa in lower termites, and bacteria in higher termites.

Typically, the hydrolysis rate of cellulase enzyme usually remains constant during the first few hours. However, the reaction rate eventually slows down, resulting in lower glucose yields, longer processing time, and some accumulated recalcitrant residue due to incomplete hydrolysis. Then, for reaction rate enhancement and product recovery, pretreatment methods are required (Chandra *et al.*, 2007). Solvent pretreatments are one type of effective pretreatment methods, which are usually used for cellulose pretreatment because they are cleaner and less energy-consumption. Nowadays, an effective solvent for dissolving cellulose is 1-butyl-3-

methylimidazolium chloride, [BMIM]Cl. [BMIM]Cl is classified as one type of ionic liquids, which has a low melting point (< 100°C) and low vapor pressure. Thus, it is environmental friendly because it has possibility to be regenerated and reused by distillation or membrane separation. Normally, most studies on reducing-sugar production from cellulose always involve with [BMIM]Cl as a solvent for increasing the hydrolysis rate of commercial cellulase enzyme (Dadi *et al.*, 2007; Liyig *et al.*, 2006). However, because the cost of cellulase enzyme is still expensive, applying it for reducing-sugar production in commercial scale is not feasible (Zhang *et al.*, 2006).

In this work, isolating cellulose-degrading bacteria from termites was the first aim of this research. Afterwards, specific cellulase enzyme activities at different temperatures were determined. Furthermore, various concentrations of [BMIM]Cl were added to the broths for culturing isolated bacteria in order to determine their tolerance.