CHAPTER I
INTRODUCTION

Nowadays, the energy demand is increasing everyday but petroleum resource is running out, which resulted in the price of crude oil is rapidly increasing. Moreover, the increased concern about the toxic and greenhouse gases from combustion of petroleum are driving factors make the alternative fuel is an interesting fuel. Ethanol had been used as a biofuel for several years by blending with gasoline. Biobutanol, which can be produced by microorganism called *Clostridia,* is another alternative fuel that is a four-carbon primary alcohol. It has an energy density closer to gasoline but it is less volatile, hygroscopic and corrosive than ethanol.

Clostridium sp. is a microorganism producing the biofuels by Acetone-Butanol-Ethanol (ABE) fermentation with a typical ratio 6:3:1, respectively. The metabolic pathway of *Clostridia* (e.g., *C. acetobytylicum, C. beijerinckii, C. butyricum, and C. sporogenes*) is an anaerobic fermentation which glucose is consumed. The microbial cells in initial phase, which known as acidogenic phase, have an active growth and produce some organic acids (acetate and butyrate), hydrogen, and carbon dioxide. These generating needed for cell growth and metabolism. Solventogenesis, which is the second phase of the ABE fermentation, will shift from acidogenesis phase by using these organic acids to produce acetone, butanol, and ethanol. For example, *Clostridium beijerinckii* BA101 can produce 17-21 g butanol per liter of fermentation broth (Pfromm *et al.*, 2010). However, the concentration of butanol is severely limited by some factors, such as high cost of substrate, substrate inhibitors (furfual, hydroxymethyl furfural or HMF, acetic, ferulic, glucuromic, and phenolic compounds) and low cell density that can reduce the productivity yield. Another critical problems of ABE fermentation is butanol toxicity which microbial cell cannot tolerate more than 2% butanol (Liu *et al.*, 2009). So, many technics of downstream have been applied to separate and relieve broth fermentation including adsorption, liquid-liquid extraction, perstraction (membrane permeation operates with extraction), reverse osmosis, pervaporation gas stripping.

Batch fermentation is a simple process but fed-batch fermentation give higher concentration and productivity than batch reactor. Continuous is another popular model that was used in industry. However, the continuous model has a limitation of cell-washout that is a critical effect to butanol productivity in the fermentation step. The fermentation with high cell density cultivation can overcome this problem by applying cell immobilization and cell recycling that will produce more productivity than batch, fed-batch and free cell continuous fermentation. Cell immobilization is a popular method of fermentation which improves the period the product yield and volumetric productivity. This technic protects the microbial cell from shear forces and imparts a special stability to the microbial cell against environmental stresses such as pH. temperature, organic solvents, salts, inhibitors, and toxics (Zhu, 2007). Thus, this system can operate for a long time period with better operational stability. There are many types of immobilized materials including celite, sand, porous brick, glass beads, clay, ceramics, wood chips, ion exchange resins, plastic materials, fibrous matrials, organic materials such as cellulosics material and activated carbon (Zhu, 2007). Among the immobilized materials studied, activated carbon has a high porous material with a large adsorption capacity that might be used as immobilized material for fermentation process. However, the results from the previous work found that the ABE production is not as high compared to the zeolite immobilized system. Because the pH surface of zeolite was slightly base buffer while activated carbon surface was high acidity which is not suitable for *Clostridium* culture growth. Therefore, this work will focus on the activated carbon treatment as immobilized material. The chemical treatment will adjust the suitable pH at activated carbon surface for microbial cells. In addition, other important variables during fermentation will be studied.