CHAPTER I
INTRODUCTION

Nowadays, petroleum oil is depleted faster because of the continuous overconsumption of the reserved oil. Therefore, there are several attempts to search for an alternative energy source. Among a variety of potential candidates, bioethanol, which is renewable and environmental friendly, is of great interest because it can be directly used as a fuel or blended with gasoline in order to get "gasohol". In addition, bioethanol also increases the octane number of gasoline. For the purposes of creating the energy security and decreasing environmental issues, many technologies have been developed to increase the ethanol production yield.

Bioethanol is mainly produced via a fermentation process by using sugar, agriculture crops, or lignocellulosic biomass as a carbon source. To enhance fermentation productivity, the genetically modified microorganisms is often used for the bioethanol production. Nonetheless, the long-term effects of genetic modification microorganisms are still doubted. Regardless of the use of the genetically modified microorganisms, a continuous fermentation strategy which incorporates the engineering process to improve the fermentation step is also employed, but this method requires high investment and appropriate system control. Thus, another approach, known as cell immobilization, has been developed.

Cell immobilization is a technique involving the attachment of yeast cells to the polymeric matrices via physical attachment, covalent bonding, or cell entrapment (Fukui and Tanaka., 1982; Nunez and Lema., 1987) Among these various immobilization techniques, cell entrapment has been widely used due to its many advantages, such as inexpensive, high cell densities, high volumetric productivity and elimination of the inhibition caused by a high concentration of substrate or product which normally found during the fermentation process (Pilkington *et al.,* 1997; Nedovic *et al.,* 2001; Kourkoutas *et al.,* 2003).

Silk fibroin (SF) — a natural protein fiber obtained from the cocoons of the larvae of the mulberry silkworm *Bombyx mori* — can be regenerated into different morphologies, such as films, fibers, and scaffolds or sponges. The three dimensional porous SF-based materials are found to be a suitable matrix for cell entrapment. SF was not only used in textile fields but also in biomedical applications, including tissue engineering, enzyme immobilization, and cell culture, due to its good biological compatibility as well as biodegradability (Li *et al.*, 2002; Vepari and Kaplan., 2007) and high cytocompatibility (Minoura *et al.*, 1995; Motta *et al.*, 2004). Nevertheless, after being regenerated, SF predominantly possesses a random coil conformation which is not stable in water. The post treatment, like methanol treatment, is usually required to convert the random coil conformation to the β-sheet structure which is a water-stable conformation. However, this conformation transition induced by the post treatment causes the shrinkage of the obtained SF materials and the reduction of their dimensional stability (Wongpanit *et al.*, 2007) Therefore, nanofillers, such as cellulose whiskers (CLWs), have been used in many researches to improve the dimensional stability of the SF materials.

The purpose of this study is to prepare the porous matrix used in yeast immobilization for bioethanol production. The matrix is prepared by fabricating SF and CLWs, which extracted from banana rachis, by freeze drying technique and treating the SF-based nanocomposite with methanol to achieve structural integrity. The CLWs were used as reinforcements in the present study because cellulose is the most abundant of all naturally occurring polymers, is degradable, and is inexpensive. The presence of CLWs was expected to decrease the shrinkage of the nanocomposite sponge after the methanol treatment and to improve its mechanical properties. In addition, this study was also focused on the effect of SF-to-CLWs blended ratio on the chemical structure and morphology of the resultant bionanocomposite sponge. The potential use of the bionanocomposite sponge as a supporting material used in cell entrapment was evaluated in terms of viability of cell, residual sugar content, and ethanol yield.