CHAPTER I
INTRODUCTION

Nowadays, the fluctuation of oil prices has directly effect on everyday living then seeking for less dependent fossil fuel is the inspect route to avoid this problem. One of the alternative fuels keeping more popular is ethanol because it can be blended with gasoline to reduce the fossil fuel consumption, also improve the octane value of the blend, and lower the emission. Especially, for the agricultural-base country that often has problems concerning with harvest's price. However, ethanol itself has many limitations such as the azeotrope which found during the separation process and high corrosion to the conventional infrastructure including the internal combustion engine. Then butanol is promising to be the next generation biofuel due to its advantages over ethanol.

Butanol is the four-carbon alcohol that can be produced from fermentation, the same process as ethanol. For 1 gallon, butanol can produce energy up to 15,500 British thermal unit, BTU (25% higher than ethanol). Compared with ethanol, butanol has lower Reid Vapor Pressure (RVP, safety) and it can be blended at the higher concentration for standard vehicle engine (11.5% v/v in US gasoline) and less corrosive; therefore, it does not need to change the existing infrastructure of gas station. The combustion of butanol has no presence of SO_X, NO_X, or CO thus they are considered to be green. In the butanol recovery process, they do not require a lot of energy for dehydration as ethanol.

Butanol can be produced via Acetone–Butanol–Ethanol (ABE) fermentation, named after Chain Weizmann who discovers the *Clostridium acetobutylicum*. This method defined the fermentation process using bacteria to break down sugar to be butyric, propanoic, lactic, acetic acid and shift through metabolic pathway to isopropanol, acetone, butanol, and ethanol. The conventional ABE fermentation produces the solventogenic products—acetone, butanol, ethanol—in the ratio of 3:6:1 and only 13 g/L (1.3%) that quite suite for cell growth because butanol over this range can act as toxic to the fermentation.

The carbon sources from ABE fermentation are xylan, levan, pectin, starch, and other polysaccharide that different from the ethanol production utilizing only C6 sugar. Nevertheless, using sugar or molasses as a substrate for ABE fermentation called first generation biofuels is not practical anymore since they threatened food supplies. For this reason, using pretreated lignocellulosic biomass as a carbon source considered as second generation biofuels was detailed in this work since biomass has abundantly and not concern in food competition. Various types of biomass has been used in the study of ABE fermentation focusing on the conventional method pretreatment and directly apply as a substrate. Each biomass shows different results in butanol production since it contains various composition of cellulose, hemicellulose, and lignin. In Thailand, corncobs are considered agricultural waste, with over 35 million kilograms generated each year. Corncobs consist mainly of cellulose (39%), hemicellulose (43%), and lignin (7.6%), with xylose being the major constituent of hemicellulose. Then they were chosen as a substrate in this study to extract fermentable sugars to ABE fermentation via the combination steps of pretreatment process and enzymatic hydrolysis since there is no detail in the study which use pretreated corncob as a substrate for ABE fermentation.

The pretreatment process aims for alternate the structure of biomass suite for enzymatic hydrolysis. In this work, acid and base pretreatment applied separately to investigate its advantages. The acid pretreatment took place mainly to solubilize hemicellulose in biomass then the hydrolysate contained xylose, other trace elements and small amount of glucose. It also damages surface made the advancement in enzymatic hydrolysis since it can penetrate deeply. Base pretreatment was different from acid pretreatment since it was mainly solubilize lignin which acts as sticky glue in the structure of biomass then there is no sugars contained in the hydrolysate and ready to wash and used in the enzymatic hydrolysis. To hydrolyse biomass with enzyme, a variation of brand and batch was used and controlled in the same loading. These lead to the problem of optimization since there is no study of the combination of pretreatment and enzymatic hydrolysis using corncob as a substrate for ABE fermentation. For instance, the vital condition of acid pretreatment bears high amount of sugars and inhibitors which toxic to microbial. Type of acids was also studied since sulfuric acid commonly used in acid pretreatment has major effect on microbial growth. At last, even there is a conclusion in the optimization condition for the pretreatment and enzymatic hydrolysis, the limitation in ABE fermentation still consists since the microbial were toxic by their products. Then, the fermentation process simultaneous with the separation process should be further studied to eliminate the toxicity or develop the strain, which can grow under high concentration of alcohol products.

In this study, *C. beijerinckii* TISTR 1461 and *C. acetobutylicum* TISTR 1462 was chosen for used in the ABE fermentation. They were compared in the potential of ABE fermentation. The chosen strain, which produce the highest ABE, was further studied to investigate the regulation of ammonium acetate uptake in production medium and D–xylose uptake in preculture medium. Then the studied conditions were applied to the hydrolysate obtained from sulfuric pretreatment of corncobs instead of synthetic medium.

Sodium hydroxide was used as a base pretreatment aiming for solubilize lignin. The microwave oven was used as a reactor. The varied parameters (temperature, time and base concentration) were optimized by monitor total sugars obtained after enzymatic hydrolysis.

Sulfuric pretreatment of corncobs was studied in the detail of temperature, time and acid concentration under the stainless tube which has heating coil and thermocouple for temperature control. The three chosen parameters were optimized to inspect high concentration of sugars using RSM then subject to enzymatic hydrolysis to evaluate the theoretical yield of sugars from corncob. The different scenarios of hydrolysate were used as a substrate for ABE fermentation.

Since sulfuric acid did not show the satisfied result using hydrolysate as a substrate due to the inhibitors from acid pretreatment at high temperature. Other type of acids (nitric acid and phosphoric acid) was selected to compare the result of ABE fermentation including the optimization of enzyme loading and time used in enzymatic hydrolysis by RSM for economic feasible. Hydrolysate from all acids combined with enzymatic hydrolysis was used as a substrate. Then the selected acid was used and combined with supplements to promote the hydrolysate as synthetic medium.