CHAPTER VII
CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

In this work, the comparative study of *Clostridium beijerinckii* TISTR 1461 and *Clostridium acetobutylicum* TISTR 1462 was firstly conducted to investigate the possibility of ABE fermentation since these strains have been maintained in the strain collection at Thailand Institute of Scientific and Technological Research (TISTR). The result shows that *C. acetobutylicum* TISTR 1462 tended to produce mainly acids under regular synthetic medium (P2) and conditions used in the shake flask scale of ABE fermentation studies (anaerobic condition, 150 rpm, 37 °C). It suggested that *C. acetobutylicum* TISTR 1462 has no ability to shift to the solventogenic phase while *C. beijerinckii* TISTR 1461 produced acetone, butanol, and ethanol comparable with wild type in other studies. Then *C. beijerinckii* TISTR 1461 was chosen for further study in the enhancement of ABE fermentation by changing medium in the pregrown culture and adding ammonium acetate in the production medium (P2). The positive results show in both parameters. Then the optimal value of additions was applied to corncob hydrolysate obtained from dilute sulfuric pretreatment with overliming process to detoxify inhibitors. It was found that the addition of 2 parameters could enhance ABE production approximately 2 times compared to the control experiment using corncob hydrolysate.

Since the main objective of this study is the substitution of corncob to the substrate of ABE fermentation, further study in the pretreatment and enzymatic hydrolysis of corncob was branched in 2 parts (acid and base). The NaOH pretreatment by microwave irradiation was studied under the parameters of retention times, temperatures, and NaOH concentration. Alkali pretreatment is the method mainly removes lignin content then the pretreatment was followed by the enzymatic saccharification and then total sugars were evaluated the optimal condition of NaOH pretreatment. The morphology and composition of corncob were also studied to assure the enhancement of enzyme accessibility obtained from alkali pretreatment. However, the hydrolysate was not a suitable medium for ABE fermentation since no

microbial growth of *C. beijerinckii* TISTR 1461 was found. Thus, this study, as a fermentation substrate, was applied to ethanol production using *Saccharomyces cerevisiae* compared to YPD medium. The result shows that the hydrolysate could use as a substrate for *S. cerevisiae* but inhibition of microbial growth occurs at a certain time.

Dilute sulfuric pretreatment was a promising method because it was widely use in the pretreatment prior ABE fermentation. The optimal conditions (retention times, temperatures, and H_2SO_4 concentration) of sulfuric pretreatment were found using response surface methodology. The sulfuric pretreatment was done in the specific type of stainless steel reactor to prevent the corrosion. The hydrolysate was subjected to enzymatic hydrolysis and used as a substrate for ABE fermentation. Corncob morphology changed from sulfuric pretreatment was also investigated as an alkali pretreatment. The different conditions of substrate were studied since the overliming process cannot detoxify the hydrolysate from enzymatic saccharification. It was found that using simultaneous saccharification and fermentation (SSF) was the alternative route for ABE fermentation.

To conquer the problem of inhibitors contained in the hydrolysate from acid pretreated corncob and enzymatic hydrolysis, type of acids was studied in comparison of fermentability of *C. beijerinckii* TISTR 1461 between H_2SO_4, HNO_3, and H_3PO_4. Corncob morphology and crystallinity index after each of acid pretreatment was also conducted. It was found that phosphoric acid has a prominent result in bacterial growth and ABE production while sulfuric acid was effective in hydrolysing corncob. Then the conditions (enzyme loading, and time) for enzymatic hydrolysis after phosphoric pretreatment were investigated using RSM to optimize cost of enzyme and operation time. The cellobiase enzyme loading was also studied to reduce a cost of enzyme. The optimal condition of enzymatic hydrolysis after phosphoric acid pretreatment of corncob was applied in different conditions of substrate. It was found that adding 10 g/L glucose initially can enhance the ABE fermentation comparable to the synthetic medium. Then phosphoric pretreatment was practically used as a substrate for *C. beijerinckii* TISTR 1461 without applying detoxification methods.

7.2 Recommendations

The recommendations for future work are as follows:

1. Other chemicals used in the pretreatment like ionic liquid should be studied since it may enhance the enzymatic saccharification and lower inhibitors.
2. Simultaneous saccharification and fermentation was a promising route for ABE fermentation then the compromising condition of enzyme and microorganism should further investigated.
3. To overcome the problem of butanol toxicity, some materials which has ability in butanol adsorption and immobilization of cultures should provided.