# CHAPTER 4

# CONCLUSION

## 4.1 Conclusion

1. Palm pressed fiber (PPF) compositions are 32.06% cellulose, 25.83% hemicelluloses and 17.28% lignin. The delignified palm pressed fiber (dPPF) contained 37.70% cellulose, 34.67% hemicelluloses and 7.31% lignin (based on 1 g PPF).
2. Extraction of hemicellulose from dPPF by alkaline method was optimized by the response surface methodology (RSM). The optimum condition was 28.8% (w/v) KOH concentration, the PPF to KOH ratio of 1:20 (w/v), and reaction time of 20 min, giving the highest hemicellulose yield of 38.67 ± 1.21% with 99.25% extraction. Under these conditions, the hemicellulose was composed of 80.8% xylose, 15.7% glucose, 3.2% acetic acid and <0.38% furfural.
3. The optimum condition of one-stage process for furfural production from extracted hemicelluloses was 150°C, sulfuric acid to hemicellulose ratio (liquid to solid, L/S ratio) of 8 ml/g, sulfuric acid concentration of 5% v/v for 90 min reaction time. The maximum furfural yield was 0.86 g/l or 3.44 wt%.

   Two-stage process for furfural production was consisted of hydrolysis followed by dehydration process. In the acid hydrolysis step, the optimum conditions were 120°C, 5.7% sulfuric acid, L/S ratio of 8.5 ml/g for 31 min ($R^2$ = 0.90). The maximum yield of xylose was 12.32 ± 2.42 g/l. In the dehydration process, the optimum reaction temperature was 135°C and reaction time of 90 min ($R^2$ = 0.93). The maximum furfural production was 8.67 ± 0.62 g/l.
4. After hemicelluloses extraction, the cellulose was used for glucose production by cellulase (*Trichoderma reesei*). The 7.9 g/l of maximum reducing sugar mainly glucose (60% saccarification) was achieved when incubated 12 g/l of

the extracted cellulose with cellulase of 4,166 U/g substrate under the optimum condition at pH 4.8 incubated for 50 °C for 900 min.

5. The glucose production from the extracted cellulose by acid hydrolysis was conducted by two-stage process, firstly it was treated by 72% sulfuric acid and followed by 4% sulfuric acid hydrolysis using solid/liquid ratio (SLR) of 1:16 (w/v) at 120 °C for 86 min. The 0.54 g/l of glucose was produced under those conditions.
6. Xylose production by dilute acid hydrolysis was achieved by RSM. The optimum condition was the 2% (v/v) $H_2SO_4$ at 120 °C for 30 min, giving the highest xylose and glucose yields of 27.23 and 2.3 g/l. In addition, the concentrations of acetic acid and furfural, which are inhibitors in the fermentation, were 5.99 g/l and 0.42 g/l, respectively.

   The ethanol production in synthetic medium by *Candida shehatae* TISTR5843 was studied. When cultured the yeast cell in the synthetic medium containing glucose as a sole carbon source, the optimum concentration of glucose and xylose were found to be 24 g/l and 20 g/l, respectively. The optimum glucose to xylose ratio in the medium was 2:8 w/w with the initial pH of 5. Incubation conditions were achieved at room temperature (30°C) with the shaking speed of 180 rpm. The highest ethanol yields and ethanol productivities at optimum condition were 0.42-0.45 g ethanol/ g sugar, and 0.103-0.343 g/l/h, respectively.
7. *Saccharomyces cerevisiae* TISTR5017 was found to be the best strain for ethanol production in cellulosic hydrolysate. The highest ethanol yield and ethanol productivity were 0.34 g/g sugar, and 0.118 g/l/h, respectively.
8. The optimum condition for ethanol production in cellulosic hydrolysate by *S. cerevisiae* TISTR5017 was the initial pH of 5.40, shaking speed at 137 rpm and initial cells concentration of 0.56 g/l. The ethanol production was 3.98 g/l with the ethanol yield of 0.48 g ethanol/g sugar, and the productivity of 0.167 g/l/h.
9. The optimum nitrogen source and concentration was 3 g/l peptone, and C/N ratio of 9.3. The inhibitory compounds in PPF hydrolysate for ethanol

production by *Candida shehatae* TISTR5843 were acetate, furfural, and vanillin, should be less than 2.5, 0.5, and 0.5 g/l, respectively. The highest ethanol production was 4.75 g/l. The optimum dilution factor of PPF hydrolysate in ethanol production by *C. shehatae* TISTR5843 was 1/2 dilution giving the maximum ethanol yield of 0.32 g ethanol/g sugar and ethanol productivity of 0.125 g/l/h.

10. The optimum conditions for ethanol production in PPF hydrolysate by *C. shehatae* TISTR5843 was the initial pH of 5.25, shaking speed of 135 rpm and initial cells concentration of 1.08 g/l. The ethanol production was 5.25 g/l with the ethanol yield of 0.40 g ethanol/g sugar, and the productivity of 0.146 g/l/h.
11. The maximum ethanol production of fed batch and semi-continuous process in 1/2 dilution PPF hydrolysate conducted in 3 cycles by *C. shehatae* TISTR5843 were 3.92 and 4.02 g/l, respectively, which were similar ethanol production from batch process (4.07 g/l).
12. The pretreatment of PPF, milling and delignification increased surface area of natural support, which enhances cell adsorption and ethanol production. The ethanol concentrations, ethanol yields and ethanol productivities of free and immobilized cells increased in repeated batch fermentation in the range of 10.78-30.12%, 9.52-22.22% and 11.90-32.35%, respectively. The immobilized cells on sPPF can be reused 4 times with retaining the activity of 93%. Therefore, PPF has a potential as a natural support in the immobilization system.

## 4.2 Suggestion

1. Study the lignin removal by steam explosion because of (i) chemicals cost reduction, (ii) pretreatment cost reduction after lignin removal (pH adjustment), and (iii) environmental friendly.
2. Study the lignin removal by white rot fungi.
3. Study how to reuse the acid in the process of furfural production.
4. Study the immobilized cells in PPF hydrolysate.
5. Produce ethanol from cellulose by fungi.
6. Study the down stream process of ethanol production by the process of evaporation (evaporation and permeation) which used for obtaining the ethanol when it's produced.
7. Apply the knowledge from this study to produce value added products from lignocellulosic materials.
8. Study the xylose production by using xylanase in order to no any inhibitory compounds generation.
9. Study how to reduce the cost of xylanase in xylose production.
10. Study the investment cost for each process, enzymatic hydrolysis and acid hydrolysis.