CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Optimization by RSM based on CCD plays a key role in identifying the optimum values of independent variables in medium composition and fermentation conditions efficiently. In this experiment, RSM proved to be a useful tool in establishing optimum production for both responses of surfactin and biomass. Furthermore, OFAT approach was applied first in order to investigate the effect of medium composition and fermentation conditions on surfactin and biomass production.

One-factor-at-a-time (OFAT) optimization was first introduced to identify the optimal concentration of critical nutritional components. The experiments of OFAT optimization suggested an optimal concentration of nutrients, which were found at 30 g/L glucose, 0.05 M NH_4NO_3, 120 µM $FeSO_4$ and 2.0 mM $MnSO_4$. With these conditions, the concentration of surfactin and biomass produced from the fermentation could reach up to 179.4 mg/L and 4.0 mg/L respectively.

Thereafter, the optimization was employed by an RSM experimental design to obtain medium compositions for Cooper's media, thus enhancing surfactin and biomass production. The optimization results show that glucose, ammonium nitrate, ferrous sulfate and manganese sulfate have a significant effect on surfactin production. However, only glucose, ferrous sulfate and manganese sulfate have a significant effect on biomass production. The optimal condition of the system for surfactin production can be achieved for the concentrations of glucose, ammonium nitrate, ferrous sulfate and manganese sulfate of42.3 g/L, 0.051 M, 131 μM and 1.64 mM respectively. Under these conditions, surfactin was produced at 190.8 mg/L by the local isolates of *B. subtilis* 3M.

Consequently, enhancement of fermentation conditions was performed by exploitation of the results obtained from the RSM optimization of media composition. In this case, one-factor-at-a-time (OFAT) optimization technique was recurrently applied to enhance the production of surfactin and biomass by *B. subtilis* 3M. The OFAT optimization of fermentation conditions suggested an optimal condition to obtain the highest surfactin and biomass production, which were found at 72 h of fermentation time, 5% of inoculum volume and temperature of 30 °C. Under these conditions, the production of surfactin and biomass from the fermentation process could reach up to 203.0 mg/L and 4.2 mg/L respectively.

From the OFAT optimization of fermentation conditions, a desirable quadratic polynomial mathematical model developed by RSM that could improve both surfactin and biomass production was obtained. Other than that, validation experiments were also carried out to verify the availability and the accuracy of the models, and the results showed that the predict values agreed with experimental values well. In this experiment, the optimum production of surfactin by *B. subtilis* 3M could be achieved by addition of 7% inoculum at 25 °C for 95 hours. The conditions had resulted in surfactin production of 229.2 mg/L, closer to the predicted value of 221 mg/L. At the end of this study, surfactin yield by local isolate of *B. subtilis* 3M was subjected to MALDI-ToF for mass determination. From the analysis, six peaks were observed which explained the existence of six different types of surfactin isoforms.

5.2 Recommendations for further study

Findings in this work are directive towards several ideas which requisite further investigation, some of which are listed below:

1. Scaling up surfactin production from shake flask fermentation into bioreactor by using media composition and fermentation condition which were optimized from this study.

2. Utilization of waste or cheaper substrate as the medium and nutrient for fermentation. This way, less cost of production is required to produce surfactin.

3. Fermentation of standard *Bacillus* sp that showed better production of surfactin (e.g. ATCC 21332) as substitution for local isolate of *B. subtilis* 3M using media composition and fermentation condition which were optimized from this study.

4. Instead of response surface methodology (RSM), artificial neural network (ANN) can be applied as an attractive tool for multivariate modeling. At some point, ANN has more advantages than RSM.

5. Since surfactin from this study is produced by new type local isolate of *B. subtilis*, antimicrobial and antifungal study can be explored to determine the extent of its activity against several bacteria and fungi.

6. Beside of using MALDI-ToF, more instrument can be employed to determine the characteristic of surfactin and its isoforms such as nuclear magnetic resonance (NMR) and fourier transform infra-red (FTIR).

7. For post-fermentation of surfactin, more efficient and economic recovery procedures can be developed to enhance surfactin yield by using cost-effective separation processes.