CHAPTER 5

Conclusions

This research was investigated for the optimized medium composition for achieving the higher amount of β-mannanase from *Bacillus subtilis* M7, a lipase defective mutant of *B. subtilis* MR10. In addition, purification and characterization of β-mannanase from *B. subtilis* M7 were also investigated. The overall studies can be concluded as follows:

1. The most proper inorganic nitrogen and organic nitrogen source were ammonium sulfate and soybean meal, respectively.
2. The result obtained from Plackett and Burman design indicated that only copra meal was found to be the positive significant factor at *P*-value less than 0.05. The similar results were found when locust bean gum and konjak flour was used as the sole carbon source.
3. The activity obtained from the optimal medium with copra meal as a carbon source was approximately 1.3 folds comparing to the activity obtained from the basal medium.
4. The β-mannanase activity obtained from the optimal medium with either locust bean gum or konjac flour as the sole carbon source was higher than that from copra meal approximately 2.3 folds. Moreover, both locust bean gum and konjac flour were of interest to be used for the enzyme production, however, the cost of carbon source use in practical enzyme production has to be considered.
5. The optimum condition for β-mannanase production from *B. subtilis* M7 was an initial pH range of 6.8-7.0 and temperature at 37ºC.
6. The activity of β-mannanase produced in a 5-L bioreactor 30 h using the previous optimized medium and conditions from laboratory scale was approximately 1.3 folds comparing to the activity obtained from laboratory scale.

7. The addition of sodium chloride to β-mannanase solution positively influenced the enzyme stability as 94% of its initial activity was remained after storage at 4°C for 42 days.
8. β-Mannanase from *B. subtilis* M7 was purified to homogeneity and the enzyme was concluded to be a monomeric protein with the molecular weight of 42 kDa, approximately.
9. The enzyme was active efficiently between 50-60°C and stable for 1 h up to 60°C. It was stable over a broad pH range of 4.0 to 9.0 for 24 h at 4°C, and pH optimum was at pH 5.0-7.0.
10. The β-mannanase was found to be the metal-dependent enzyme and Co^{2+}, Mn^{2+}, Fe^{3+}, Al^{3+} ions and mercaptoethanol played an important role as the enhancing factor of the enzyme activity.
11. The Michaelis-Menten constants (K_m), and maximum velocity (V_{max}) values were 30.34 mg/ml and 1347.76 µmole/min/ml, respectively.
12. The pattern of MOS from copra meal hydrolysis by purified enzyme was similar to MOS obtained from locust bean gum hydrolysis. However, the density of MOS products from both substrates was different which was suggested to be due to the difference in β-mannan content in the substrates used.