CHAPTER V
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The isolation results of cellulose-degrading bacteria from lower termites, *Schedorhinotermes sp.*, and higher termites, *Microcerotermes sp.* confirmed the results of previous literature, which supported that only the higher termites used bacteria for degrading the cellulose. 57 isolates from the higher termites, which were isolated by three conditions, were preliminarily screened for their ability to degrade cellulose by measuring the HC value. The highest HC value of the bacteria isolated from each condition was identified and determined for the specific cellulase enzyme activities at different temperatures and their tolerance in the presence of 1-butyl-3-methylimidazolium chloride or [BMIM]Cl, which was used to enhance the accessibility of cellulase enzyme to internal surface of cellulose.

The three effective isolates (strain A 002, M 015, and F 018) were determined for their specific cellulase activities—endoglucanase, exoglucanase and β-glucosidase activity—at the different temperatures (37, 40, 45, and 50°C). The results showed that the optimum temperature, at which all strains had the highest specific cellulase enzyme activities, was 37°C. At this temperature, strain M 015 had the highest specific endoglucanase activity; and strain F 018 had the highest specific exoglucanase activity and specific β-glucosidase activity.

All effective strains were investigated for their tolerance and specific growth rates in the presence of [BMIM]Cl. The results showed that strains M 015 and F 018 were able to tolerate [BMIM]Cl in the range of 0.1 to 1.0 vol.% without retarding the lag phase of the growth curve. In contrast, for strain A 002, the growth retardation in the lag phase in the presence of 0.5 to 1.0 vol.% [BMIM]Cl was observed. In addition, the specific growth rates of strains F 018 and M 015 increased in the range of 0.1 to 0.5 vol.% [BMIM]Cl, while that of strain A 002 increased when the concentration of [BMIM]Cl was less than 0.1 vol.%.

The three effective isolates were identified for their genus and species by 2 methods—biochemical methods and 16S rDNA method—and physical properties by

microbiological methods. The results from both identification methods confirmed that strain A 002 and strain M 015 were *Bacillus subtilis* with 99.9% identity, but different identifications were obtained from the biochemical methods and 16S rDNA method for strain F 018. However, with the higher percentage of the identity from the 16S rDNA method, strain F 018 was also believed to be as *Bacillus subtilis.*

5.2 Recommendations

The effective isolates, which were used in this work, were preliminarily selected by comparing the HC value, which is related to the endoglucanase activity of each isolate. However, this method has some limitations in the precision of endoglucanase activity and cannot detect the exoglucanase activity (Sharrock, 1988). Then, the determination of the most effective isolates was determined by measuring the specific cellulase enzyme activity.

For the future work, firstly, the effective isolates shall be cultured in the fermenters for expansion the cells, which would enhance the amount of cellulase enzyme. Secondly, the mixed strain between strain M 015 and F 018 shall be tried to investigate the synergistic effect on degrading cellulose at the optimum temperature. This recommendation is partly from the highest specific endoglucanase activity of strain M 015 and the highest exoglucanase activity of strain F 018. It is expected that there may be a synergistic effect from the two strains.