CHAPTER 5
CONCLUSION AND RECOMMENDATION

5.1 The recombinant chitosanase from *Bacillus subtilis* 168 was successfully cloned into pMAL-p5X expression vector and expressed in *E. coli* TOP10. The pure chitosanase can be obtained via Maltose Binding Protein (MBP) fusion and one-step purification using amylose beads.

5.2 The optimal temperature of recombinant Maltose Binding Protein- *Bacillus subtilis* Chitosanase fusion (MBP-*Bs*CsnA) was 55°C and stable up to 50°C after incubation for 30 min at pH 6.0, without substrate. The optimal pH was 6.0 and stable within pH 2-9 after incubation at 30°C for 24 hrs. The thermal inactivation kinetics at 50°C showed that the enzyme was more stable in the presence and absence of substrate than *Bs*CsnA-10xHistidine tag. MBP-*Bs*CsnA can cleave GlcN-GlcN links, which is common to all known chitosanases.

5.3 The chitosan-oligosaccharide (CHOS) generated by MBP-*Bs*CsnA and *Bs*CsnA-10-His constructs showed similar anti-inflammatory activity suggesting that MBP-*Bs*CsnA is an attractive format for industrial valorization of chitosan.

5.4 Recombinant MBP-*Bs*CsnA fusion is safe, efficient, and suitable for bioconversion of chitosan into value-added chitosan-oligosaccharide.