CHAPTER IV

CONCLUSIONS

Two enzymes, cellulase *Ac* from *Acremonium cellulolyticus* and Chi 60 from *Serratia* sp., were investigated for their potential use in a preparation of *N,N'*-diacetylchitobiose by hydrolysis of β-chitin from squid pens. In the hydrolysis of chitin with cellulase *Ac*, two forms of β-chitin were used as substrates, powder and fibrous chitin. The difference between both substrates were the optimum chitin concentration, 80 mg/mL for powder chitin and 60 mg/mL for fibrous chitin. The optimum hydrolysis condition for cellulase *Ac* was pH 3 (0.2 M McIlvaine buffer) and 37 °C. The enzyme affinity technique increased the $(GlcNAc)_2$/ GlcNAc mole ratio from 1 to 3, that indicated the presence of both chitinase and β-N-acetylhexosamini dase enzymes in cellulase *Ac*. These two enzymes possessed different affinity to the chitin surface.

The hydrolysis of chitin with Chi 60 was only conducted on fibrous chitin substrate which is readily available in Thailand. The optimum condition was pH 6 (0.05 to 0.1 M McIlvaine buffer) and 37 °C. The effective concentration of chitin and enzyme for hydrolysis were 30 mg/mL and 150 mU/mL, respectively. Unlike cellulase *Ac*, the enzyme affinity technique with Chi 60 could not improve the products mole ratio implying that Chi 60 consisted of only one enzyme possessing both chitinase and *β-N*-acetylhexosaminidase activities. To obtain a higher product mole ratio, the low concentration of enzyme (less than 30 mU/mL) should be used. With Chi 60 (30 mU/mL), a production of $(GlcNAc)_2$ was achieved in 42% HPLC yield with 93/7 selectivity of $(GlcNAc)_2$/GlcNAc in 6 days for hydrolysis of fibrous β-chitin (2 g). In literature, the colloidal chitin was hydrolyzed with *Streptomysis griseus* for 83 days and gave 61% yield of $(GlcNAc)_2$.[47]

For future work, purification of $(GlcNAc)_2$ should be conducted. The purification may be done by crystallization or chromatography of $(GlcNAc)_2$ crude product or its peracetylated derivative.