CHAPTER IV

CONCLUSIONS

N-Acetyl-D-glucosamine (GlcNAc) and *N-N'*-diacetylchitobiose ($(GlcNAc)_2$) can be prepared selectively from the hydrolysis of squid pen β-chitin with enzymes from *Aspergillus fumigatus* fungi and cloned *Serratia sp.* bacteria, respectively. To avoid using ionic salts, acetic acid can be used for pH adjustment instead of a buffer and the ethanol can be used as a preservative in place of $NaNO_3$.

The optimum condition for the preparation of GlcNAc is 30 mg/mL chitin, 4 U of crude enzyme from *Aspergillus fumigatus* per 1 g of chitin, pH = 3 and 40 °C. The isolation of GlcNAc can be accomplished by the ethanol precipitation from the extremely concentrated solution of crude product containing over 50% w/v of GlcNAc to give the sugar product as a light yellow solid in 70% yield with 90% purity. The purity of the product may be improved by.decolorization with the activated charcoal to give pure GlcNAc (100% w/w) in 92% recovery with careful washing of activated charcoal with water during filtration.

The optimum condition for the preparation of $(GlcNAc)_2$ is 30 mg/mL chitin 5 U of crude enzyme from cloned *Serratia sp.* per 1 g of chitin pH 6 and 37 °C in 40% isolated yield and GlcNAc as a by-product in 4% isolated yield within 6 days. The isolation of GlcNAc can be accomplished by a column chromatography using activated charcoal as a stationary phase and gradient water to 30% aqueous ethanol as an eluting system. The minimum activated charcoal/sugar ratio is 30/1 (w/w). The elution of GlcNAc should be completed with 10% ethanol before eluting $(GlcNAc)_2$ with 20% ethanol. The loading volume of the sugar solution does not significantly affect the separation between GlcNAc and $(GlcNAc)_2$. Pure $(GlcNAc)_2$ can be obtained by this method in 40% yield.