CHAPTER IV

CONCLUSIONS

Five stains of fungi, *Aspergillus fumigatus*, *Trichoderma viride*, *Trichoderma aureoviride*, *Trichoderma reesei* and *Mucor sp.* could be induced to produce of extracellular chitinolytic enzymes by cultivation in colloidal chitin minimum medium (CCMM) as a carbon source and urea as a nitrogen source. *Aspergillus fumigatus* TISTR 3045 was the most active to be induced to produce extracellular chitinolytic enzymes. Fed-batch technique could further improve the production of chitinolytic enzyme. The optimum cultivating temperature for *Aspergillus fumigatus* was 40 °C where the fungi produced 438 mU/mL of chitinolytic enzymes. The crude enzyme preparation contained 1.70 mg protein per milliliter.

The chitinolytic enzymes produced from *Aspergillus fumigatus* had potential to be used in the preparation of *N*-acetyl-D-glucosamine from the hydrolysis of squid pen β-chitin. The effective enzyme/chitin ratio was 1-4 mU/mg at the chitin concentration of 20 mg/mL. The optimum pH range for the enzyme was 3.0-5.0 buffered with McIlvaine buffer solution (0.05-0.1 M). The hydrolysis could also be performed without buffer to provide approximately 85% enzyme efficiency. The optimum reaction temperature was 45 °C. The hydrolysis at the optimum condition gave 1.61 g of GlcNAc from 2 g of chitin, corresponding to 74% HPLC yield in 1 day when the enzyme/substrate ratio of 4 mU/mg was used.

In the hydrolysis, toluene could also be used as a preservative in place of the highly toxic sodium azide. In a fed- batch preparative scale using swollen chitin gave GlcNAc 54% HPLC yield in 7 days.