VI. SUMMARY

The optimal conditions for cultivation of *Myrothecium verrucaria* TISTR 3112 and TISTR 3225 were examined. In order to obtain the maximum bilirubin oxidase production, these two strains should be cultured aerobically at 25 °C, in a pH 8.0 potato-glucose medium for 48 hrs.

After filtration, the culture filtrate was purified by either purification technique; ammonium sulfate precipitation, clarification by an activated charcoal, DEAE-Cellulose or DEAE-Sepharose column chromatography. The most powerful technique observed was the clarification using the activated charcoal treatment which the recoveried yield was obtained, 60-70 % of an original enzyme in the starting culture filtrate. By Mini Prep Cell SDS-PAGE electrophoresis, two peaks of protein eluted from the culture filtrate of both strains were identical to that obtained from a crude commercial bilrubin oxidase which used as a control for identification.

The isolated enzyme was evaluated for molecular weight by Sephadex G-100 gel filtration chromatography. The molecular weight of isolated bilirubin oxidase was approximately 49,000 Da for both strains. The enzyme kinetics and the inhibitory effect of some metal ion and compounds such as Zn^{2+}, $CaCl_2$ and BSA were also observed. Finally the isolated enzyme treated with activated charcoal was applied to develope the enzymatic methods for determination of total and conjugated billirubin in serum in a Beckman Synchron CX5 autoanalyzer.