Thesis Title Immobilization of Cells and Properties of Extracellular Proteases of a Thermophilic Bacterium Isolated from San Khampaeng Hot Spring

Author Ms. Sumonthip Janphuk

M.S. Chemistry

Examining Committee :

Assoc. Prof.Dr. Pawinee Kanasawud **Chairman**

Assoc. Prof.Dr. Suree Phutrakul **Member**

Assist. Prof.Dr. Siddhichoke Sangsoda **Member**

Abstract

Protease production from thermophilic bacteria Thermus 2S at temperature 65 °C in a medium contained nitrilotriacetic acid as nitrogen source, yeast extract, tryptone and essential mineral salt in solution of pH 7.2 gave the maximum activity 8.5 U/ml and wet cells 3.02 g/l after 32 hours The optimum pH and temperature of protease from Thermus 2S were 7.0 and 65 °C respectively. When casein was used as a substrate, K_m and V_{max} of the protease were 0.35 µg/ml and 5.6 µg/min respectively. The effciency of casein hydrolysis in the presence of Ca^{2+} was faster than in the absence of cofactor, and also faster than hydrolysis

of haemoglobin and gelatin respectively. Protease was expected as an allosteric enzyme consisted of 2 subunits by Hill equation. The result from SDS-PAGE showed that the molecular weight of protease was approximately 13,700 dalton The precipitation of protein by acetone, ethanol, ammonium sulphate and pH was unsuccessful for protease from Thermus 2S, less than 2% yield was obtained and protease activity was highly decreased. Concentrated protease could be obtained by ultrafiltration with lowering of activity to 16-17% of initial activity.

Studied on cell immobilization of Thermus 2S for production of protease using five supports; calcium alginate, alginate-agar, polyacrylamide, PAAH-alginate and crosslinked-PAAH by entrapment method providing the immobilized beads indicated that the stability of cells entrapment with calcium alginate at 65 °C was more than 2 months and higher than the immobilized cells with other supports. Although entrapped cells leaked from calcium alginate immobilized beads more than from the other supports, maximum protease activity was produced in the system containing 20% wet cells, 8.9 and 8.2 U/ml for batch and continuous reactor respectively. Crosslinked-PAAH was the best support for protection of cell leaking from immobilized beads. However, the stability at 65 °C was only 25 days. Production of protease from this immobilized cells system was 6.3 and 5.6 U/ml in batch and continuous reactor respectively.

Production of protease by immobilized cells from Thermus 2S in calcium alginate beads depended on cells content in immobilized beads. When the immobilized cells contained 5% wet cells, protease of 7.1 U/ml culture was found in the system. This production yield quite approached to free cell system with 10% innocula, 9.1 U/ml Wherease the immobilized cells system containing 10 and 20% wet cells produced protease 15.9 and 31.8 U/ml respectively. In batch system, the reused immobilized cells yielded only 50% of protease production wherease in continuous system, protease activity was close to the first use. The microscopic study of cells distribution in immobilized beads a microscope confirmed the leakage of cells during protease production in the reactor. The problem of leakage of immobilized cell should be studied furthermore for the improvement of protease production from immobilized Thermus 2S system.