

Nisa Romsomsa 2009: Optimized Conditions for Silk Degumming Protease Production from *Bacillus* sp. C4 by Response Surface Methodology. Master of Science (Microbiology), Major Field: Microbiology, Department of Microbiology. Thesis Advisor: Assistant Professor Patoomporn Chim-anage, Dr.Eng. 152 pages.

Bacillus sp. C4 was isolated from waste water of Thai silk industry, and determined as a potential protease producer for silk degumming process. The objective of this research is to optimize the medium and cultivation condition for growth and protease production from C4. The prior selection of raw materials suited for protease production was employed by simple screening method in BMSM broth as a basal medium. Hydrolyzed cassava starch, soy flour and skim milk were selected to be suitable carbon, nitrogen sources and the enzyme inducer, respectively. Plackett-Burman design was introduced for screening of seven variables for protease production, i.e., hydrolyzed cassava starch, soy flour, skim milk, initial pH, temperature, shaker speed and inoculum size. The results indicated that soy flour and skim milk were significant variables on protease production ($P \leq 0.05$). Hence soy flour, skim milk and shaker speed were further optimized via central composite design (CCD) and response surface methodology for potential use on an industrial scale. The analysis of variance (ANOVA) showed the adequacy of the model ($R^2 = 0.912$) and verification experiments confirmed its validity. According to the model, the predicted maximal protease production was 1,575.5 units/ml and the corresponding concentrations of soy flour and skim milk were 2.0% (w/v), 0.1% (w/v) and 280 rpm of shaker speed, respectively. However the observed value of maximum protease production was 1,536.9 units/ml which was a 2.2-fold increase as compared to that of unoptimized condition in shake flask (729.0 units/ml). To emphasize the aeration necessity, C4 was cultivated in 2 liter stirred tank reactor containing the optimized BMSM medium and controlling pH, agitation rate, temperature, aeration rate and dissolved oxygen at 7.5, 350 rpm, 30°C, 2 vvm and 70% air saturation, respectively. Accordingly, the result obtained was 1,898.1 units/ml of enzyme which was an about 1.2-fold increase as compared to that in the shake flask with optimized BMSM medium (1,575.5 units/ml).

Student's signature

Thesis Advisor's signature

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