Nathida Ari 2008: Optimization of Recombinant Mutant Beta-glucosidase Production by Taguchi Approach. Master of Science (Biotechnology), Major Field: Biotechnology, Department of Biotechnology. Thesis Advisor: Assistant Professor. Pramuk Parakulsuksatid, Ph.D. 150 pages.

The cultivation of recombinant *Pichai pastoris* for mutant (A454N) β-glucosidase expresstion using a shaking flask was inhibited by high methanol concentration (5 g/l) known as substrate inhibition. The maximum methanol concentration was 4 g/l for further study.

The optimization of conditions to produce recombinant β-glucosidase was investigated in a fermentor and by the Taguchi approach. Dissolved oxygen, pH and temperature were optimized in 2 levels using L_4 (2^3) Orthogonal Array. The results were shown that the condition of 50 % dissolved oxygen, pH 5.5 and 28 °C was the optimum condition for maximum enzyme activity and enzyme productivity (Q_p), whereas the condition of 25 % dissolve oxygen, pH 5.5 and 28 °C was the optimum condition for yield coefficient of product from methanol ($Y_{P/m}$). The study was also indicated that pH was a leading factor in maximizing β-glucosidase activity, enzyme productivity and yield, while dissolved oxygen had higher effect than temperature to maximize β-glucosidase activity and β-glucosidase productivity. Temperature had more effect than dissolved oxygen when optimizing yield.

The study of methanol feeding strategy using stepped intermittent pattern under the optimum condition from the Taguchi approach was shown that the maximum β-glucosidase activity, β-glucosidase productivity and yield coefficient of product from methanol were 2,681 U/l 36 U/l.h and 6 U/l.g, respectively. By comparison to the constant intermittent pattern at the same cultivation time, the stepped intermittent pattern had higher β-glucosidase activity, β-glucosidase productivity, yield and specific productivity by 7, 7, 3 and 3 fold, respectively. Therefore, the Taguchi approach is an effective experimental design for optimization including use of the stepped intermittent feeding strategy which further improves mutant β-glucosidase production.

Nathida Ari — Student's signature

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