ABSTRACT

A culture medium was designed for maximal production of maltooligosaccharide-forming amylase by *Bacillus circulans* GRS-313, isolated from the local soils of IIT-Kharagpur. The influence of carbon and nitrogen sources, mineral salts and surfactants were characterized. In presence of 4% (w/v) soybean meal along with other nutrients (2% yeast extract, 2% wheat bran, 0.001% $CaCl_2$ and 0.01%, TritonX-100), a maximum activity of 61.1 U/ml was recorded. Growth conditions for amylase production were optimal at 40°C temperature under static fermentation condition with an initial pH of 5.5.

Response surface methodology (RSM) was employed to study the cumulative interactive effect of the macronutrients of the media and to optimize their concentration to enhance the production of maltooligosaccharide-forming amylase from *Bacillus circulans* GRS 313. A 2^3 factorial central composite experimental design was used to study the combined effect of the nutritional constituents-soybean meal (X_1) yeast extract (X_2) and wheat bran (X_3). The optimal combination of the media constituents for amylase production from the contour plots were; soybean meal 4.84 g/100ml, yeast extract 1.58 g/100 ml, wheat bran 2.84 g/100 ml. The optimization of the media increased the amylase yield by 1.25 times.

RSM involving central composite experimental design and regression analysis was also employed for optimization of the fermentation conditions viz, temperature (X_1), pH (X_2), incubation time (X_3) and inoculum volume (X_4), for production of amylase. The environmental conditions were optimized at 46°C, pH 5.8, 24 hrs incubation time and 0.5 ml inoculum volume. The yield of the enzyme was increased by optimization of the process parameters, to 104.2 Uml^{-1}, resulting in an improvement of 1.36 fold and an overall improvement of 2.6 folds with respect to the amylase yield before optimization (61.1U/ml).

The amylase that was found in the culture filtrate of a strain of *B circulans* GRS 313 was purified and characterized. The enzyme was purified by organic solvent fractionation, Sephadex G-100 gel filtration and CM-Sephadex column chromatography. The molecular weight of the purified enzyme was found to be around 38 kD. The optimum pH and temperature were evaluated using response surface methodology and were found to be 4.9 and 48°C, respectively. The enzyme was stable upto 60°C and its pH stability was in the range of 5.0-8.0. The K_m and V_{max} of the amylase was 11.66 mg/ml and 68.97 U/ml, respectively and the energy of activation, E_a was 75200 cal/mole. Dextrin inhibited the enzyme competitively,

with a K_i of 6.1 mg/ml and glucose caused non competitive inhibition with K_i of 9.5 mg/ml. The enzyme was inhibited by Hg^{2+}, Mn^{2+}, Fe^{3+} and Cu^{2+} and enhanced by Co^{2+} and Mg^{2+}. EDTA reversed the inhibitory effect of the metals. Paper chromatographic analysis of the products with the enzyme showed the presence of maltotriose, maltopentaose, maltose and small amounts of glucose.

A simple and inexpensive method for immobilizing amylase on coconut fibre was developed. The immobilization conditions for highest efficiency was optimized with respect to immobilization pH 5.5, 30°C, contact time of 4 hr and enzyme to support ratio of 1:1 containing 0.12 mg/ml protein. The catalytic properties of the immobilized enzyme were compared to the free enzyme. The activity of amylase adsorbed on coconut fibre was 38.7 U/g of fibre at its optimum pH 5.7 and 48°C, compared with the maximum activity, 40.2 U/ml, for free enzyme at the optimum pH 4.9 at 48°C. The reutilization capacity of the immobilized matrix was upto three cycles.

In the present study an attempt was also made to immobilize the amylase by entrapment. The immobilized enzyme activity was affected by the size of the bead. A bead size of 2mm was found to be most effective for hydrolysis. Kinetics constants, K_m and V_{max}, were estimated and were found to be also affected by the bead size. The K_m of the immobilized amylase increased with the size of the beads. The catalytic activity of the enzyme was studied in presence of various starchy residues and metal ions. $HgCl_2$, $CuSO_4$ and $FeCl_3$ caused inhibition of the enzyme. The reaction conditions, pH and temperature, were optimized using response surface methodology. At the optimum pH and temperature of 4.9 and 57°C, the apparent activity was 25.6U/g of beads. The immobilized enzyme showed a high operational stability by retaining 83% of the initial activity after seventh use.

Keywords: *Bacillus circulans* GRS313, maltooligosaccharide, amylase, soybean meal, wheat bran, media selection, optimization, central composite design, response surface methodology, fermentation, purification, coconut fibre, adsorption, calcium alginate, entrapment.