Abstract

In the present study, lipase was produced from *Rhizopus oligosporus* NRRL 5905 through solid state fermentation. Evolutionary operation factorial design technique was adopted as a suitable statistical tool to find the optimum fermentation conditions for production of maximum lipase taking into account the interaction between the process variables in a fermentation system. The best combination of process parameters that gave maximum lipase was incubation period of 5 days, fermentation temperature 30 °C, liquid to solid ratio of 1.5:1, initial fermentation pH 6 and spore concentration of 10^8 spores/ml.

Lipases from *R. oligosporus* NRRL 5905 was partially purified by ammonium sulphate precipitation (60 % saturation). The purified lipase was stable in the pH range 4 to 11, exhibiting maximum activity at pH 8. The optimum temperature was 30 °C, at which the enzyme was stable for 1 day.

Immobilization of *R. oligosporus* lipase was done following two techniques: (a) Adsorption on un-activated silica gel 60, (b) Covalent linking on activated silica gel 60. A new activation technique was developed which comprised two steps: amination of silica gel 60 with ethylene diamine followed by cross linking with glutaraldehyde. The activated matrix showed higher immobilization yield (80 %) compared to un-activated silica (42 %). The covalently linked lipase (CLL) was thermally most stable followed by adsorbed lipase.

Enzymatic synthesis of flavor and fragrance esters (geranyl acetate, n-butyl acetate and n-propyl acetate) was carried out in a solvent free system. The maximum molar conversion (67 %) for geranyl acetate was achieved at 25 % (w/v) (15 U/ml) CLL, 30 °C and shaking at 200 rpm for 48 h. For synthesis of n-butyl acetate and n-propyl acetate, molar conversion reached up to ~50 % and ~56 % respectively at an enzyme concentration of 25 % (w/v) (15 U/ml) after 24 h of reaction. Pseudo first order kinetic model was adopted to estimate the kinetic parameters (apparent K_m and V_{max}) of the enzymatic synthesis of the flavor esters.

Keywords:

Lipase, *Rhizopus oligosporus* NRRL 5905, EVOP, Immobilization, Synthesis, Geranyl acetate, n-Butyl acetate, n-Propyl acetate.