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PHYSICAL STABILIZATION OF LIPASE ON ACTIVATED CHARCOAL

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Lipase enzyme was immobilized by adsorption on charcoal. The enzymatic activity of free and immobilized enzyme was compared. It has been observed that optimum conditions for maximum activity with respect to pH and temperature are the same for free and immobilized lipase. The immobilized enzyme does not dissociate and can be reused in the fresh substrate solution.

INTRODUCTION

Enzymes are highly efficient catalysts, but at present are not used at household or in industrial processes. The main problems are (i) their high costs of production, (ii) difficulties in their recovery from reaction products for eventual recycling, (iii) instability towards temperature, pH, storage etc., and (iv) biodegradability. These problems can be overcome if the enzymes are immobilized (Yaqub, M. 1974). The present investigations are aimed to explore the feasibility of immobilization of lipase for its application on industrial scale economically.

MATERIALS AND METHODS

Lipase (wheat germ) (Kotch Light Biochemical Co.) was procured through the courtesy of department. The enzyme has been labelled as commercial preparation. A stock solution was prepared by dissolving enzyme (50 mg/5 ml) in phosphate buffer of pH 6.5. Serial dilutions of this stock solution were further made. Lipase assayed for its lipolytic activity by using (5 gm per cent) of commercial grade olive oil (A. SABATER ESTEVES A REUSE) (ESPANA) in substrate mixture. The substrate homogenized emulsion was prepared by mixing 5 gm olive oil (gm per cent), guma cacia 2.7 gm, calcium chloride 0.06 gm, sodium chloride 2.3 gm and 4 ml of 0.2 M phosphate buffer of 6.5 pH. Homogenized substrate emulsion was prepared freshly whenever required. Activated charcoal (E. MERCK Biochemical Co. of Germany) was used as a

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biomaterial support throughout the investigation. Lipase activity was determined by following Khundu and Paul, (1970) assay method.

The assay conditions were employed for the determination of optimum H ion concentration, ranging the change in pH from 5–8. The lipase activity was estimated employing the temperature range from 30–60°C using water bath shaker. The incubation time period was kept 60 minutes for all variable temperatures.

Physical Stabilization

Lipase was immobilized by using activated charcoal as biomaterial support, following the general procedure of Mosbach (1971). Two grams of activated charcoal was added in a 100 ml Pyrex beaker, containing 40 mg/5 ml enzyme solution. The mixture was stirred over-night at room temperature using a magnetic stirrer. The charcoal mixture was centrifuged and finally decanted and was thoroughly washed with phosphate buffer of 6.5 pH until no lipase enzyme activity was detected in the washings. The adsorbed charcoal was dried, keeping in desicator. Free and immobilized lipase was assayed for optimum temperature and pH.

Reuse of Immobilized Enzyme

After the completion of reaction, there action mixture containing immobilized enzyme was centrifuged. Charcoal binding the enzyme was dried and again checked was activity This enzyme was recycled three times.

RESULTS AND DISCUSSION

In the preliminary studies, commercially available lipase was studied to establish its characteristics. The maximum activity of the lipase enzyme was found when the concentrations of the enzyme and olive oil substrate solution were 3 ug/5 ml and 20 ml respectively. The optimum temperature and pH for lipase activity has been found to be 37°C and 6.5 respectively. The optimum conditions remained unchanged on immobilization. The stability of enzyme against temperature and pH increased on immobilization as the loss in the activity of free enzyme at 60°C is 40.6 per cent as compared to immobilized

enzyme which was 36.4 per cent as compared to the immobilized enzyme that was 68.2 per cent. These results have shown that stability of enzyme against temperature and pH has been increased on immobilization. The fact that bound enzyme can be reused is evident from the table 1. It can be seen that after the completion of the reaction in the prescribed assay system, if the bound enzyme is removed from reaction mixture by centrifugation and again added to the fresh substrate mixture, the catalytic activity is almost the same. This indicated that bound enzyme did not dissociate from the matrix support as has also been shown by Smiley (1971).

Table 1. *Reuse of bound enzyme after centrifugation.*

No.	Nature of enzyme	Centrifuged material	Units of activity
1	Immobilized enzyme	Supernatent	No activity
		Residue	15.4 Units
		1st Cycle	15.4 Units
		2nd Cycle	15.4 Units
		3rd Cycle	15.4 Units

As reported that lipase, have been used as preservatives, in the baking products, pharmaceutical preparations, in food processing and in chemical detergents on commercial scale for several decades (Somkute and Bable, 1969), Kobayashi (1971), so the present basic studies enables us to overcome the existing limitations of use of lipase in industries.

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