**Table 1** *Effect of addition of algal slurry to cow-dung on biogas production*

| Bottle | Starter (ml) | Fresh cow-dung slurry (ml) | Algal slurry (ml) | Total volume of biogas evolved (ml) | Duration of biogas production (days) |
|---|---|---|---|---|---|
| I | 100 | 260 | 0 | 4600 | 26 |
| II | 100 | 260 | 150 | 7859 | 31 |
| III | 100 | 260 | 300 | 10893 | 42 |
| IV | 100 | 260 | 450 | 14183 | 53 |

digesters with the algae as higher amount of digestible organic matter is present in these digesters than in those containing cow-dung alone.

The cow-dung slurry was maintained under anaerobic conditions and the pH was noted daily. After four weeks, the slurry contains large numbers of methanogenic bacteria, which convert all organic matter to methane under anaerobic conditions. At this stage the cow-dung slurry is called starter. A small amount of starter was mixed with fresh slurries of cow-dung and algae in different proportions in batch digesters. The digesters were kept at room temperature ($25 \pm 2^{\circ}C$) in anaerobic conditions and the volume of biogas evolved (measured as volume of water displaced) was noted daily. The results are presented in table 1.

The amount of biogas produced from algae is twice that obtained from cow-dung (on dry weight basis 1 g of dry algae produces 344 ml whereas 1 g of dry cow-dung produces 179 ml). The duration of gas evolution increased with increase in the proportion of algal slurry. The calorific value of the gas was 4800 kcal/$m^3$ and the percentage of methane was 55.43.

It can therefore be suggested that algae can be added to cow-dung in biogas plants, especially during winter, when production of gas from cow-dung alone is low. The digested sludge can be used as a good manure.

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## IMMOBILIZATION OF EXTRACELLULAR LIPASE PRODUCED BY *ASPERGILLUS JAPONICUS* IN RESPONSE TO PLANT LATEX

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THE power of enzymes as catalysts for organic chemical reactions is diverse and remarkable. Among such biocatalysts are extracellular microbial lipases (glycerol ester hydrolase EC 3.1.1.3), classified among the hydrolases; lipases catalyse the hydrolysis of fats and oils to give diacylglycerols, monoacylglycerols, glycerols and fatty acids[1]. They have been used in hydrolysis of oils and fats[2], flavour development in dairy products[3], ripening of cheddar cheese and improving the flavour of processed and blue cheese[4-7]. The reversibility of the lipase reaction allows use of the enzyme as a catalyst in the formation of esters from alcohols and fatty acids[8,9], and also to change the physical properties of mixtures of fats and oils by altering the distribution of fatty acyl groups among triglycerides.

In our laboratory, a hydrocarbon-degrading strain of *Aspergillus japonicus* was found to produce extracellular lipase[10]. Induction of lipase was possible on an easily available and cheap carbon source, like latex of *Calotropis gigantea*. The enzyme was purified 77-fold in two steps of purification. The lipase (molecular weight 156,000) was stable at room temperature for a month[10]. Keeping in view the extensive applications of lipases, and to further its industrial applicability, we report the immobilization of latex-induced lipase from *A. japonicus* and the properties of the immobilized enzyme.

*Immobilization of lipase on Dowex*(R)

The growth conditions for *A. japonicus* and

induction of lipase by latex were the same as described earlier[10]. DEAE-cellulose-purified (41-fold) enzyme was used for immobilization. A strongly basic anion exchange resin, Dowex(R) 1-x8, was used for our studies. The enzyme (at a protein concentration of 1 mg/g of resin) was loaded onto 3 g of resin taken in a glass column. An equal volume of buffer A (0.01 M Tris-Cl pH 7 + 1 mM dithiothreitol) was added to eluate unbound protein. The eluate was repeatedly passed through the resin. Protein in the eluate was estimated by the method of Bradford[11]. The resin-bound enzyme was assayed after washing with buffer A. The assay system was the same as described earlier[10], and 0.1 g of resin was used per assay system. Control consisted of uncharged resin washed with buffer A. Protein solution containing 112 units of lipase (specific activity = 32.4) was loaded onto 3 g of resin (table 1). Enzyme that bound to the resin had 69 units of activity, but surprisingly the specific activity was approximately 3-fold higher than that of the free enzyme. This high specific activity could be explained if the amounts of protein loaded and eluted were taken into consideration. Of the 3.47 mg of protein loaded only 0.82 mg, or about 23%, bound to the resin, but resin-bound lipase activity was about 62% of that loaded. In other words purification of lipase was achieved, although that was not the intention of this particular experiment.

To standardize conditions for optimum protein binding, different aliquots of protein were loaded onto a fixed amount of resin (1 g). Maximum protein bound was 1 mg/g of resin, at which, apparently, the binding sites got saturated (figure 1). The activity was in perfect correlation with the amount of protein bound. Lipases are known to act at the oil–water interface. An enhancement in activity with stirring was seen for the immobilized enzyme (data not presented). This increase may be due to delocalization and homogenization of the immobilized enzyme upon stirring, giving it more surface area to act and also increasing the diffusion across the unstirred layer around the immobilized enzyme.

**Table 1** *Immobilization of lipase on Dowex(R) 1-x8 resin*

| | Total protein (mg) | Total activity (units) | Specific activity (U/mg protein) |
|---|---|---|---|
| Free enzyme (loaded) | 3.474 | 112.2 | 32.4 |
| Eluate | 2.65 | 33.3 | 12.3 |
| Resin (bound enzyme) | 0.824 | 69 | 82.1 |

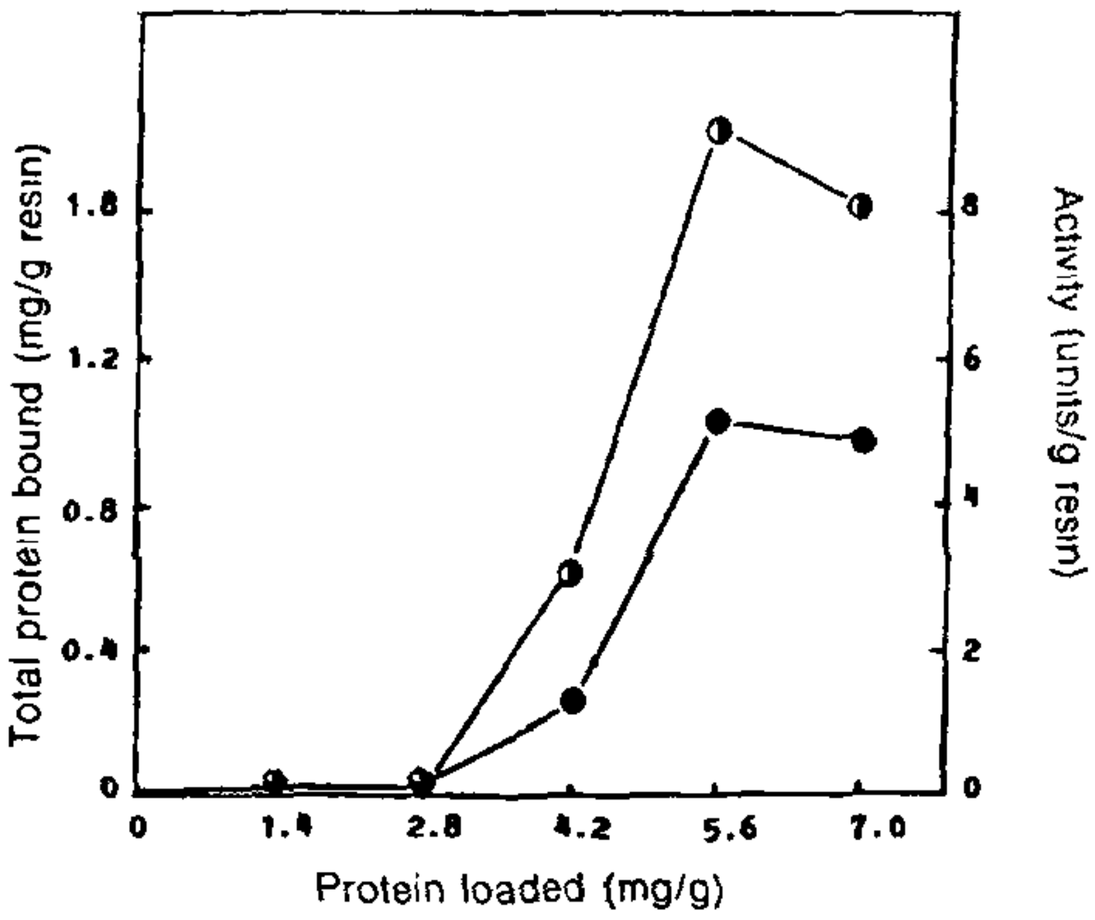


**Figure 1.** Binding of *A. japonicus* lipase to Dowex(R) 1-x8 resin (●, Protein bound; ◑, activity).

Immobilization increases the operational and storage stabilities of bound enzyme[12]. Entrapment of *Rhizopus delamar* lipase with photo-crosslinkable resin prepolymers was seen to enhance operational period from 5 days to 12 days. Ninety per cent of the initial activity was retained at the end of 12 operations[13]. Considering one assay as a single operation cycle, the lipase immobilized on Dowex could be used for 6 cycles without any loss of activity (figure 2). Fifty per cent loss of activity had occurred at the end of 18 cycles. The immobilized enzyme was stable in storage for over 12 weeks whereas the free enzyme was inactivated within 4 weeks, at room temperature (data not presented). This marked increase in stability and reusability are the main advantages of the immobilized enzyme over the free enzyme.

The kinetic constants of the immobilized lipase were also determined using the same procedure as for the free enzyme[10]. The resin-bound enzyme was assayed with different concentrations of olive oil as substrate. The kinetic constants were determined from a Lineweaver–Burk plot. The apparent $K_m$ was found to be 10% olive oil and the apparent $V_{max}$ 119 U/mg (table 2). Comparison of these values with those for the free enzyme shows an increase in $K_m$ and $V_{max}$ upon immobilization. The increase in $K_m$ could be explained on the basis of conformational effects: amino acids which form part of the active site may be involved in binding to the resin[14]. Steric and microenvironmental effects can also be responsible

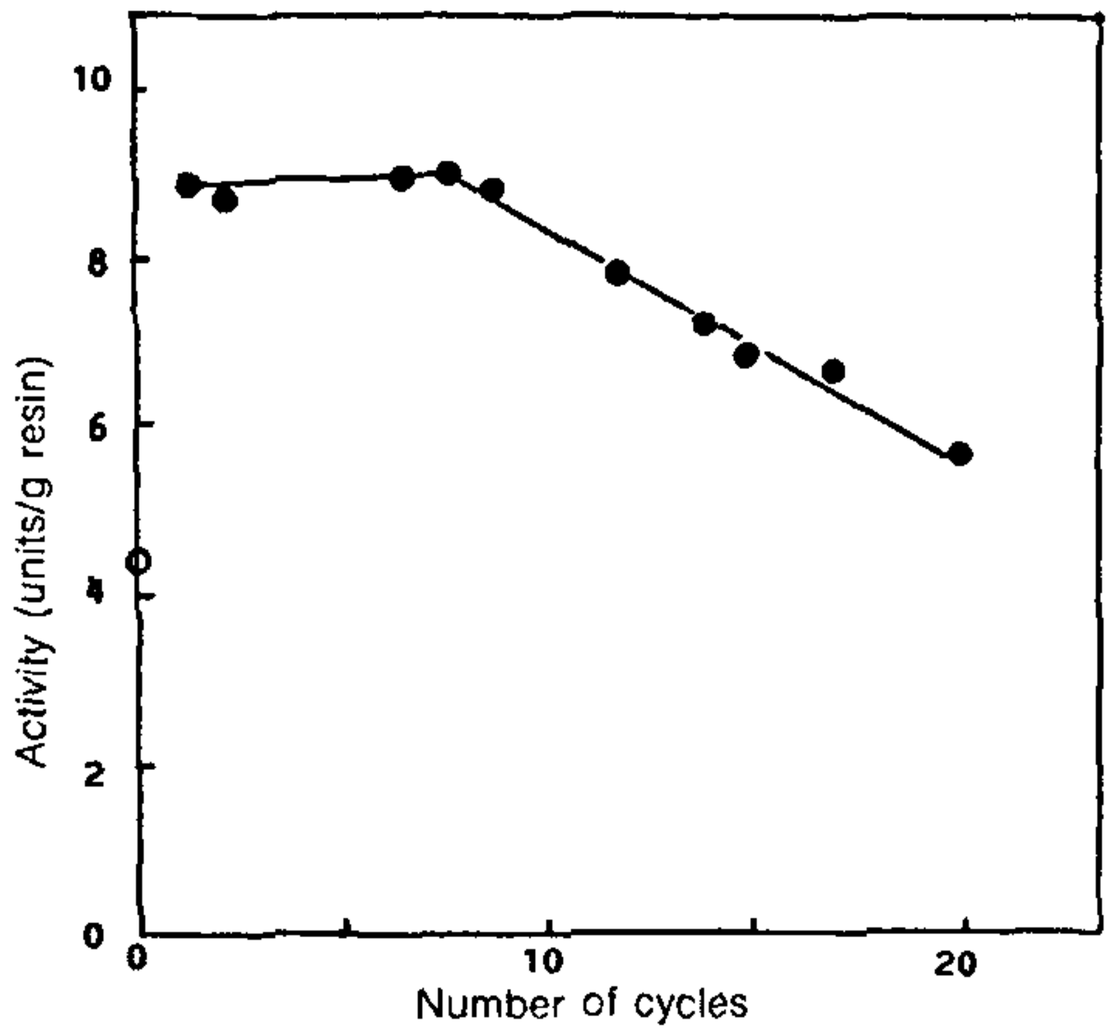


**Figure 2.** Operational stability of immobilized lipase (○, Activity of free enzyme loaded onto the resin; ●, resin-bound immobilized enzyme).

**Table 2** *Kinetic constants of free and resin-bound lipase*

| | $K_m$ (% olive oil, v/v) | $V_{max}$(U/mg protein) |
|---|---|---|
| Immobilized enzyme | 10.0 | 119.0 |
| Free enzyme | 1.54 | 26.0 |

for the increase in $K_m$. However, it should be noted that increase in the $K_m$ of an enzyme upon immobilization may have little effect on its performance because high substrate concentrations are often used in practice. Immobilization of the enzyme provided a larger surface area for it to act, and this is reflected in the increase in $V_{max}$ upon immobilization.

Enhanced thermal stability has been reported for numerous covalently bound enzymes[15]. In our system also, enhancement in thermal stability upon immobilization was observed. Fifty per cent inactivation of the resin-bound lipase occurs at 70°C, but free enzyme is inactivated to the same extent at only 43°C, when heated for 10 min. Full activity was retained when the immobilized enzyme was heated at up to 40°C for 10 min, but free enzyme loses activity when heated above 30°C. Enhancement in thermal stability has been reported for amyloglucosidases[16] and cholinesterases[17] immobilized on DEAE-cellulose.

Immobilized lipases have been subjected to extensive studies recently[13, 18-20]. The lipase from *A. japonicus* has distinct advantages over the other lipases reported earlier. *Calotropis* latex is easily available at relatively low cost and it would be economical to use it as an inducer of lipase, replacing the conventional expensive olive oil. Since the enzyme is secreted, recovery is easier. This enzyme can catalyse both the forward and the reverse reactions, and hence can be used for the upgradation of low-grade oils like rice bran oil (unpublished results). Immobilization of the enzyme on ion-exchange resins not only increases its storage, operational and thermal stabilities, but, surprisingly, a 3-fold purification was also achieved. Immobilized lipases are better biocatalysts than the free enzymes as more surface area is available for their action. If the resin could be recharged with fresh lipase, processes involving immobilized lipases as biocatalysts would become much more cost-effective.

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# ANNOUNCEMENT

## INSA MEDAL FOR YOUNG SCIENTISTS—1990

Instituted by the Indian National Science Academy in 1974 the Medal is awarded annually in recognition of outstanding work of scientists below the age of 32 (as reckoned on 31st December preceding the year of award). Only those born on or after January 1, 1958 are eligible for consideration in 1990. The work done in India by the nominee will be taken into consideration for the award.

The awardee is presented a medal and a cash award of Rs. 5,000/-. In addition, the recipient is considered for a research grant by the Academy not exceeding Rs. 20,000/- per year, including stipend for a JRF for a period of three years for continuing research work, provided the research proposal is considered worthy of such support. Preferential consideration may be given under partial travel grant scheme for attending international conferences.

Nominations for the awards for 1990 may be made by Fellows of the Academy, established scientific societies of all-India character, University faculties and departments, or research institutions.

The last date for the receipt of nominations in the Academy is November 15, 1989.

Nomination Proforma can be obtained from the Indian National Science Academy, Bahadur Shah Zafar Marg, New Delhi 110 002, by sending a self-addressed envelope of 28 cm × 12 cm size.