Coimmobilization of Detergent Enzymes onto a Plastic Bucket and Brush for Their Application in Cloth Washing

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ABSTRACT: A mixture of detergent enzymes, α-amylase, cellulase, protease, and lipase has been coimmobilized covalently onto an inner wall of a plastic beaker and bristles of a plastic brush. The coimmobilized α-amylase, cellulase, protease, and lipase retained 66.7, 54.2, 44.6, and 62.8% on the beaker and 44.01, 66.23, 33.9, and 45.8% on the brush of their initial activity in free/native form. The conjugation yield of the enzymes/proteins onto beaker and brush was 0.02 mg/cm² and 0.016 mg/cm², respectively. The scanning electron micrographs (SEM) and Fourier transform infrared (FTIR) spectra of PVC sheet surfaces before and after coimmobilization of enzymes confirmed their coimmobilization. There were slight changes in optimal pH, incubation temperature and time for maximum activity and Km of enzymes after immobilization. The combination of any nonenzymic (cheaper) detergent and immobilized enzymes gave equal/better washing of cotton cloths than that by enzymic detergent (costly) in distilled water. Similar results were obtained with other types of water.

1. INTRODUCTION

The most effective detergent powders being sold in the market is a mixture of chemical detergent and free/native enzymes (α-amylase, cellulase, protease, and lipase), which constitute approximately 90% and 10% of the powder, respectively. The enzymes are being mixed in these detergents as cleaning and fabric care agents. The following classes of enzymes are known to improve the laundry process: Proteases which are known to break down long protein/peptide chains into smaller chains, act on soils and stains containing proteins, for example, collar and cuff soil-lines, grass, and blood. Amylases, which break down starch chains into smaller sugar molecules, remove starch-based soils and stains, such as sauces, ice-creams, and gravy. Lipases are effective in removing oil/greasy body and food stains. Cellulases provide general cleaning benefits, especially on dust and mud and also work on garments made from cellulosic fibers, minimizing pilling to restore color and softness. Thus enzymes breakdown the large, water-insoluble soil and stains attached to fabrics into smaller, more water-soluble pieces. Subsequently, the smaller molecules are removed, by the mechanical action of the washing machine or by the interaction of other detergent ingredients. The enzymes do not lose their functionality after having worked on one stain and continue to work on the next one. Enzymes also deliver fabric care benefits by maintaining whiteness or keeping colors bright in better ways. The most important reasons to use enzymes in detergents are (i) a very small quantity of these inexhaustible biocatalysts replace a very large quantity of man-made chemicals, (ii) enzymes can work at very low temperature at which traditional chemicals are quite often no longer effective, and (iii) enzymes are fully biodegradable. However, the high cost of enzymes leads to almost double the cost of an enzymic detergent over the cost of chemical detergents. Nevertheless, if the enzymes are used in immobilized form in a chemical detergent, the repeated use of the enzymes leads to reduce the cost of washing. Further an immobilized enzyme is easily removed from the reaction mixture, making it easy to recycle.

Immobilized enzymes typically have greater thermal and operational stability than the soluble form of the enzyme. The utility of immobilized lipase and α-amylase in the removal of oil and starch stain from cotton cloth by various detergents was tested by a chemical method. All the detergents gave better washing (removal of oil/starch stain) in the presence of immobilized lipase/α-amylase than that by detergent alone. The washing by non-enzymic detergents in the presence of immobilized enzyme was similar to that by enzymic detergent. Lipase, trypsin, and α-amylase have been coimmobilized onto the surface of nonwoven polyester material to achieve a uniform distribution of the various enzyme species, where the different enzyme activities were bound on the support. Polyvinyl chloride (PVC) sheets are a promising material for enzyme immobilization owing to the PVC’s properties such as being chemically inert, corrosion free, weather resistant, tough, lightweight, and maintenance free and having ease in molding to various shapes and size due to high strength-to-weight ratio. We have immobilized covalently a number of enzymes onto PVC sheet and studied their properties individually.

However, all the four detergent enzymes have not been coimmobilized onto PVC/plastic sheet, which is used in the manufacturing of plastic buckets and brushes. We report herein for the first time, the covalent coimmobilization of commercial α-amylase, cellulase, protease (partially purified from soybean seeds), and lipase onto the inner side of a plastic beaker and bristles of a plastic brush, their properties, and use in removal of stain from cloth.

Received: September 8, 2011
Revised: January 10, 2012
Accepted: February 10, 2012
Published: February 10, 2012
2. EXPERIMENTAL SECTION

Chemicals and Reagents. Glutaraldehyde (25%) from Sigma St. Louis, USA, and cellulase from Trichoderma viridae, α-amylase from Aspergillus niger, lipase from porcine pancreas (40–70 U/mg protein), sodium potassium tartrate, dinitrosalicylic acid (DNS), anthrone, TCA, and starch from Dr. Reddy’s Laboratories Pvt. Ltd., Mumbai, India. Tris-base, calcium chloride, and sodium benzoate from Qualigen Mumbai, Mumbai, were used. All other chemicals were of analytical reagent (AR) grade. White PVC/plastic beaker (capacity 100 mL) and brush, commercial enzyme detergents and non-enzymatic detergents, olive-oil, and seeds of soybean (Glycine max var.Ogden) were purchased from the local market. Well, canal, and ground/handpump water samples were collected from the nearby rural region of Rohtak.

Extraction and Partial Purification of Protease from Soybean Seeds. Preparation of Crude Enzyme. Seeds of soybean were ground to powder in a chilled Waring blender with pauses every 2 min. The powder (100 g) was mixed in 1.0 L of chilled distilled water in a chilled blender and blended for 6 min with pauses at 2 min intervals to prevent overheating. The resulting suspension was centrifuged at 15000g for 10 min at 4 °C. A thin, white oily layer was skimmed off. The pellet was discarded, and the supernatant was collected and treated as crude enzyme. It was tested for activity16 and protein16 and stored at 4 °C until use.

Assay of Protease. The activity of protease was measured using the method of Nam Sun Wang16 with modifications and based on the quantification of amino acids produced from the hydrolysis of casein by protease using a color reaction of ninhydrin. In a 15 mL test tube, the reaction mixture contained 3.8 mL of 0.05 M sodium phosphate buffer (pH 6.3), 0.1 mL of 1% casein in reaction buffer, and 0.1 mL of enzyme in a total volume of 4.0 mL. After incubation at 50 °C for 90 min, under continuous stirring, 0.5 mL of ninhydrin (0.2% in acetone) was added to the reaction mixture, and the mixture was kept in a boiling water bath for 10 min to develop yellow color. Turbidity, if any was settled down by centrifugation at 850g for 10 min. A355 nm recorded against control (run in similar manner as described above except that protease was replaced by the reaction buffer). The concentration of the casein was determined from a standard curve between the casein concentration and A355 (figure not given).

One unit of enzyme is defined as the amount of enzyme required to liberate 1 mg of amino acids from casein/min/mL under standard assay conditions.

Purification of Protease. The crude enzyme was purified by 5% TCA precipitation and ion exchange chromatography on a DEAE-cellulose column (1.5 x 10) preincubated in 0.01 M sodium phosphate buffer of pH 6.8 and using 0.01 M sodium acetate buffer at pH 5.6 for elution of the enzyme at a flow rate of 0.5 mL/min. The enzyme was partially purified as it showed more than one band in PAGE (figure not shown). The partially purified enzyme had a specific activity of 24.88 U/mg.

Assay of Free Enzymes. α-Amylase Assay. The assay of α-amylase (EC 3.2.1.1) was based on the fact that it cleaves internal α-1,4-glycosidic linkages in starch to produce glucose (reducing sugar), maltose, or dextrins. The reducing sugar was measured by dinitrosalicylic acid (DNS) assay.7 To 1.9 mL of 0.05 M acetate buffer (pH, 5.6) containing 2% starch in a test tube, 0.1 mL of enzyme solution was added. For a blank, 2 mL of reaction buffer containing 2% starch was taken in a test tube. Both blank and assay tubes were incubated at 37 °C under continuous stirring in a water bath. After incubation for 10 min, 0.1 mL of 2 N NaOH and 0.9 mL of dinitrosalicylic acid (DNS) reagent (5.0 g DNS in 100 mL of 2 N NaOH) were added to both test tubes. The test tubes were placed in a boiling water bath for 5 min, cooled to room temperature, and A540 of red color was read. The amount of glucose generated in the reaction was interpolated from the standard curve between glucose concentration and A540 (figure not given).

One unit of amylase is defined as the amount of enzyme required to liberate 1 μmole of glucose from starch per min under the standard assay conditions.

Assay of Free Cellulase. The assay of cellulase was based on the measurement of glucose generated from the hydrolysis of cellobiose by cellulase using a DNS reaction. To 1.9 mL of 0.05 M sodium phosphate buffer, pH 7.0, containing 4.0 mg of cellobiose in a test tube was added 0.1 mL of dissolved enzyme in reaction buffer (1 mg/mL). For a blank, 2.0 mL of reaction buffer containing 4.0 mg of cellobiose was taken. Both assay and blank tubes were incubated at 40 °C for 30 min in a water bath. After incubation, 0.1 mL of 2 N NaOH and 0.9 mL of DNS reagent were added to both the tubes. The tubes were placed in a boiling water bath for 10 min and cooled to room temperature. A540 of red color was read against the blank, and the amount of glucose generated in the assay was interpolated from the standard curve between glucose concentration vs A540 (figure not given).

One unit of cellulase is defined as the amount of enzyme required to liberate 1 μmole of glucose from starch per min under the standard assay conditions.

Assay of Free Lipase. The activity of lipase was assayed according to Naher17 with modifications. In a 100 mL conical flask, 5.0 mL of olive oil emulsion was added to 5.0 mL of 0.1 M tris buffer (pH 8.0) and incubated at 35 °C for 10 min. Lipase solution (5 mg/mL) (1 mL) was added and incubated at 35 °C for 20 min. The reaction mixture was then kept at room temperature for 20 min. A 10 mL aliquot of an acetone and methanol mixture (1:1) was added to stop the reaction, and the mixture was titrated against 0.025 N NaOH using 1% phenolphthalein as an indicator. A control was run for each sample to correct any drop in pH due to any factor other than lipase or incomplete termination of the reaction by acetone and methanol mixture. In the case of control, 1.0 mL of lipase solution was kept in a boiling water bath for 5 min to get it heat denatured. The remainder of the procedure was similar to that described for the test.

One unit of lipase is defined as the amount of enzyme required to liberate 1 μmole of free fatty acids from olive oil/min under standard assay conditions.

The protein content of various enzyme preparations was determined by the method of Lowry15 using bovine serum albumin (BSA) as standard protein.

Co-immobilization of α-Amylase, Cellulase, Protease, and Lipase onto PVC/Plastic Beaker and Bristles of Brush. A 1 mg portion of each enzyme (α-Amylase, cellulase, protease and lipase) was dissolved in 1.0 mL of 0.05 M sodium phosphate buffer pH 7.0 separately and then mixed together in a unit ratio of 20:60:1.3:1.28. The enzyme mixture was coimmobilized onto the inner side of a PVC/plastic beaker and bristles of brush through covalent coupling using the method of Pundir et al., 2008.12 Nitric acid (50 mL of mixture of concentrated...
onto the PVC beaker was also measured. The activity of enzymes after coimmobilization were calculated as follow:

\[
\text{retention} \% = \frac{\text{specific activity of immobilized enzyme}}{\text{specific activity of native/free enzyme}} \times 100
\]

Surface Characterization of PVC Beaker/Brush. The SEM images and FTIR spectra of plastic beaker and bristles of brush with and without enzymes were taken at the Electron Microscopy Facility, AIIMS, N. Delhi, and with a FTIR spectrometer (model iS10, Thermoelectron, USA), respectively, to confirm the coimmobilization.

Assay and Kinetic Properties of Coimmobilized Enzymes. Assay of Coimmobilized Enzymes. The assay of coimmobilized enzymes was carried out in the same plastic beaker in which these were immobilized. The beaker was termed as “reaction beaker” and for the brush, it was carried out in a 100 mL glass beaker containing a plastic brush on which enzymes were coimmobilized. The assay procedure of coimmobilized enzymes was carried out in the similar manner as described for their free form, except free enzymes were replaced by plastic-sheet-bound enzymes, the reaction buffer was increased by 0.1 mL, and the reaction mixture was kept under constant stirring during incubation. After incubation, the reaction mixture was transferred to a test tube or flask. In the case of a plastic brush, the brush was taken off from the reaction mixture after incubation.

Kinetic Properties of Coimmobilized Enzymes on PVC. The following kinetic properties of coimmobilized enzymes were studied and compared with those of kinetic properties of free enzyme: Optimum pH, temperature, incubation period, and effect of substrate concentration and calculation of \( K_m \) and \( V_{\text{max}} \).

To determine optimum pH of coimmobilized enzymes, the pH of the reaction buffer was varied from 4.0 to 9.0 using different buffer systems within their effective pH ranges, for example, 0.05 M sodium acetate for pH 4.0–7.5, and 0.05 M Tris—HCl for pH 7.5–9.0. Similarly, for optimum temperature of coimmobilized enzymes, the reaction mixture was incubated at different temperatures ranging from 25 to 70 °C at an interval of 5 °C. The optimum time of coimmobilized enzymes was studied from 5 to 100 min at an interval of 5 min. To study the effect of substrate concentration on the initial velocity of coimmobilized enzymes, assays were performed at different concentrations ranging from 0.1 to 3.5% starch for \( \alpha \)-amylase, 25–250 mM cellobiose for cellulase, 0.1 to 3.5% casein for protease, and 30–100% olive oil for lipase. \( K_m \) and \( V_{\text{max}} \) values for coimmobilized enzymes were calculated from the Lineweaver–Burk plot between the reciprocal of substrate concentration [1/S] and reciprocal of initial velocity of the reaction (1/\( v \)).

Applications of Coimmobilized Enzymes in Cloth Washing. Washing Performance of Coimmobilized Enzyme. Rectangular pieces of white cotton cloth (size, 4.5 × 4.5 cm²) were used for the test and therefore termed as test clothes. These cloth pieces were stained with 0.2 mL of 2% starch, grass stain, 2% egg albumin, and 0.2 mL of mustard oil individually. The coimmobilized \( \alpha \)-amylase, cellulose, protease, and lipase were used to remove starch, grass, egg albumin, and oil stain, respectively. The commercial enzymic and non-enzymic detergent powders were dissolved in different waters (distilled water, canal water, groundwater/hand pump water, and well water) at a concentration of 2 g/L individually. A 50 mL portion of detergent solution was transferred to a plastic beaker containing coimmobilized enzyme. For each washing performance, 4 test cloth pieces were taken. The first piece was washed with water alone. The second piece was washed with non-enzymatic detergent, the third piece was washed with enzymatic detergent, and the fourth piece was washed with non-enzymatic detergent in a reaction beaker containing coimmobilized enzymes. In the case of washing by beaker, the cloth was dipped into the reaction beaker containing 50 mL of detergent solution, and in the case of washing by brush a stained cloth was dipped into detergent solution for 2 min and then rubbed 7–8 times with the help of the brush consisting of coimmobilized enzymes. The washing was done at 35 °C for 20 min under continuous shaking, after which the cloth was rinsed 2 times manually with water. The washing performance was judged by quantifying the residual stain (starch/cellulose/protein/oil) after washing.

Determination of Residual Starch Content in Test Cloth after Washing. The washed test cloth was dipped into 5.0 mL of hot distilled water and squeezed into a separate beaker to collect the residual starch. This was repeated three times. All the fractions were combined, and the volume was made up to 100 mL with distilled water. A 5 mL aliquot of this diluted extract was taken into a 25 mL test tube and 10 mL of freshly prepared anthrone reagent (2% in 95% H₂SO₄) was added. Tubes were placed in boiling water bath for 10 min and cooled to room temperature. \( A_{540} \) was recorded, and the glucose content was interpolated from a standard curve between the glucose concentration and \( A_{540} \). The value of glucose was multiplied by 0.9 to get the starch content.

Determination of Residual Cellulose in Test Cloth after Washing. To determine the residual cellulose in washed test cloth, it was dipped into 10 mL 5% H₂SO₄ for 2 h at 90 °C in a water bath. The hydrolysis reaction was stopped by neutralizing the acid adding small quantity of concentrated KOH solution. The glucose content in the hydrolyzed cellulose was determined by DNS reaction as described earlier. Glucose/
cellulose content was interpolated from standard curve between glucose concentration and $A_{540}$.

**Determination of Residual Casein in Test Cloth after Washing.** To determine the residual protein content in the test cloth after washing, it was dipped into 10 mL of (1 N KOH) solution for 20 min under gentle shaking so that the residual casein of cloth got extracted into the solvent. Then the protein content in the solution was measured by Lowry’s method.

**Determination of Residual Oil Content in Test Cloth.** To determine the residual oil content of test cloth after washing, it was dipped into 10 mL of petroleum ether for 20 min with gentle shaking so that oil retained in the test cloth is extracted into the fat solvent (petroleum ether). Then this fat solution was transferred to a 100 mL round-bottom distillation flask. A 25 mL aliquot of 0.5 M alcoholic potassium hydroxide was added to it. The flask was attached to a reflux condenser and the mixture was refluxed in a boiling water bath for 30 min. The flask was removed and cooled to room temperature, and the mixture was titrated against 0.5 M HCl using 1% phenolphtheline as an indicator. The blank was set up similarly but no oil was taken in it. The volume of HCl consumed in the titration was noted.

### 3. RESULTS AND DISCUSSION

Commercial cellulase from *Trichoderma viridae*, $\alpha$-amylase from *Aspergillus niger*, lipase from porcine pancreas, and protease, partially purified from soybean seeds, were coimmobilized covalently onto the inner surface of a plastic beaker and bristles of brush with a conjugation yield of 0.02 and 0.016 mg/cm², respectively. The higher conjugation yield on the brush

![Diagram](https://via.placeholder.com/150)
compared to that on beaker wall might be due to more surface area of brush than beaker wall. The coimmobilized enzyme retained about 66.7%, 54.2%, 44.64%, and 62.8% on the beaker wall and 44.01%, 66.23%, 33.9%, and 45.8% on the brush bristles. During the immobilization, the vinyl polymers of PVC material were broken down after treatment with a HNO₃ and H₂SO₄ mixture, which introduced nicks in the long-chain polymer and generated free ends protruding from the polymer surface. This reaction of strong oxidizing agents removes chlorine molecules from the damaged ends of a polymer in a zipper action and introduces a double bond at the ends of these short-chain polymers. When the free ends of treated polymer are reacted with the aldehyde group of a bifunctional cross-linking agent such as glutaraldehyde, one aldehyde group of a glutaraldehyde reacts with the free end of the vinyl chain to form a −C═CH− bond between the PVC sheets of glutaraldehyde and thus leads to activation of surface for covalent immobilization of the enzymes. The −NH₂ group on the surface of the enzyme is attached covalently onto another −CHO group of glutaraldehyde, already attached to PVC sheet through Schiff base formation of a Schiff (Figure 1 panels A and B). This leads to covalent linkage of protein/enzyme with PVC membrane surface.

Evidences for Coimmobilization on PVC Sheet. The SEM of a chemically modified PVC sheet surface with the immobilized enzyme under high resolution resolved folds and clusters along some beaded structures, which were not observed in membranes without enzymes (Figure 2). This change in surface morphology of the support after the immobilization confirmed the enzyme immobilization. The formation of folds instead of globular beaded structures may be due to a high concentration of coimmobilized enzymes on the surface of the PVC membrane.

Figure 3 (curve i) showed the FTIR spectra of the PVC membrane. The characteristic bands of PVC can be classified into three regions. The first is called the C−Cl stretching region in the range from 600 to 700 cm⁻¹. The second region is called C−C stretching in the range from 900 to 1200 cm⁻¹. The third region is 1250−2970 cm⁻¹ in PVC (numerous CH modes). Figure 3 (curve ii) shows the PVC membrane with glutaraldehyde and enzyme.

The peak at 1725 cm⁻¹ shows the −CO− bond of aldehyde, while the no peak at 1725 cm⁻¹ in curve ii confirms that the −C O− group of glutaraldehyde got combined with −NH₂ groups on the surface of the enzyme to form the −N═C− bond. Curve ii shows a peak of −NC− bond at 1630 cm⁻¹, which also revealed that there was no free aldehyde group of glutaraldehyde, as it got cross-linked with enzyme.

These studies confirmed that the enzyme was immobilized through covalent/glutaraldehyde coupling on the surface of PVC membrane.

Changes in Kinetic Parameters. A comparison of various kinetic properties of coimmobilized enzymes with those of free enzymes is given in Table 1. The results showed that the enzymes underwent minor changes after coimmobilization, which revealed the stability of the enzyme and no interference from the PVC membrane support (Table 1). A slight increase in the optimum pH of the enzyme after immobilization could be attributed to a change in H⁺ concentration in the enzymatic microenvironment, due to loss of −NH₂ groups on the surface of enzyme during immobilization. The increase in optimum temperature of enzyme after immobilization might be due to the slow transfer of heat from the surroundings to the catalytic system due to the immobilization support barrier. Because of this reason, the temperature of the system and surrounding varies, and the catalytic system needed a slightly higher temperature of the surroundings to maintain an optimum catalytic temperature in the system. A change in the optimum temperature had also been observed in earlier immobilization studies. The change in $K_m$ and $V_{max}$ was also observed after immobilization. $K_m$ was slightly increased in the case of $\alpha$-amylase, cellulose, and protease, but decreased slightly in the case of lipase. The rate of enzyme catalysis was measured in
There was either a slight decrease in $V_{\text{max}}$ for lipase and protease or a slight increase for $\alpha$-amylase and cellulose. The change in $K_m$ and $V_{\text{max}}$ of an enzyme after immobilization depends upon the change in the microenvironment and product inhibition. Because of a change in the microenvironment of enzyme after immobilization, diffusibilities of substrate and products were different from that for native enzyme, so a change in $K_m$ and catalytic efficiency were generally observed.20

**Application of Coimmobilization of Enzymes onto Beaker and Brush in Cloth Washing.** The starch, grass, egg albumin, and oil-stained cotton cloth pieces were washed with detergents alone and in the presence of coimmobilized enzymes in a plastic beaker. The content of the residual of stain in the cloth was calculated, and the results are shown in Table 2.
bleach, bleach activators, and special additives, such as washing was also observed when it occurred with a brush. nonenzymatic detergent alone (Tables 2, 3, 4, 5). The better performance. The lesser the content of residual stain there

cloth piece was determined as the criteria of washing performance. The lesser the content of residual stain there was, the better was the washing. Two types of detergents were tested, expensive (enzymic) detergents and cheaper (non-enzymic) detergents. This washing was carried out in four types of water, distilled water, canal water, ground water, and well water. The combination of any detergent plus immobile enzyme (containing no detergent only water), enzymatic detergent, and

<table>
<thead>
<tr>
<th>detergent used</th>
<th>well water</th>
<th>canal water</th>
<th>ground water</th>
<th>distilled water</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>0.084 ± 0.56</td>
<td>0.964 ± 0.45</td>
<td>0.884 ± 0.43</td>
<td>0.884 ± 0.51</td>
</tr>
<tr>
<td>non-enzymatic</td>
<td>0.071 ± 0.42</td>
<td>0.867 ± 0.34</td>
<td>0.431 ± 0.40</td>
<td>0.721 ± 0.22</td>
</tr>
<tr>
<td>enzymatic</td>
<td>0.032 ± 0.51</td>
<td>0.533 ± 0.37</td>
<td>0.235 ± 0.25</td>
<td>0.468 ± 0.46</td>
</tr>
<tr>
<td>non-enzymatic</td>
<td>0.017 ± 0.43</td>
<td>0.369 ± 0.28</td>
<td>0.297 ± 0.53</td>
<td>0.405 ± 0.51</td>
</tr>
</tbody>
</table>

“*The values in this table represent residual content of cellulose in cloth (mg/cm2). b Control: Contained no detergents but only water.

Table 4. A Comparison of Washing Performance (Egg Albumin Stain Removal from Cotton Cloth) of Non-enzymatic Detergent, Enzymatic Detergent, and Non-enzymatic Detergent in a Plastic Beaker or with a Plastic Brush Bound to Detergent Enzymes (α-Amylase, Cellulase, Protease, and Lipase)\(^a\)

<table>
<thead>
<tr>
<th>detergent used</th>
<th>well water</th>
<th>canal water</th>
<th>ground water</th>
<th>distilled water</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>1.220 ± 0.45</td>
<td>1.067 ± 0.42</td>
<td>0.854 ± 0.47</td>
<td>0.970 ± 0.46</td>
</tr>
<tr>
<td>non-enzymatic</td>
<td>0.600 ± 0.39</td>
<td>0.932 ± 0.36</td>
<td>1.244 ± 0.31</td>
<td>0.901 ± 0.36</td>
</tr>
<tr>
<td>enzymatic</td>
<td>0.531 ± 0.37</td>
<td>0.631 ± 0.27</td>
<td>0.743 ± 0.29</td>
<td>0.932 ± 0.54</td>
</tr>
<tr>
<td>non-enzymatic</td>
<td>0.282 ± 0.41</td>
<td>0.442 ± 0.52</td>
<td>0.690 ± 0.49</td>
<td>0.784 ± 0.41</td>
</tr>
</tbody>
</table>

“*The values given in this table represent the residual content of egg albumin after washing (mg/cm2). b Control: Contained no detergents but only water.

Table 5. A Comparison of Washing Performance (Oil Stain Removal from Cotton Cloth) of Non-enzymatic Detergent, Enzymatic Detergent and Non-enzymatic Detergent in a Plastic Beaker or with a Plastic Brush Bound to Detergent Enzymes (α-Amylase, Cellulase, Protease, and Lipase)\(^a\)

<table>
<thead>
<tr>
<th>detergent used</th>
<th>well water</th>
<th>canal water</th>
<th>ground water</th>
<th>distilled water</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>28.70 ± 0.23</td>
<td>20.70 ± 0.36</td>
<td>31.25 ± 0.45</td>
<td>22.62 ± 0.37</td>
</tr>
<tr>
<td>non-enzymatic</td>
<td>18.75 ± 0.34</td>
<td>19.44 ± 0.37</td>
<td>15.12 ± 0.41</td>
<td>17.13 ± 0.39</td>
</tr>
<tr>
<td>enzymatic</td>
<td>12.50 ± 0.40</td>
<td>17.80 ± 0.73</td>
<td>7.50 ± 0.67</td>
<td>8.52 ± 0.35</td>
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<tr>
<td>non-enzymatic</td>
<td>7.60 ± 0.42</td>
<td>12.20 ± 0.25</td>
<td>8.75 ± 0.44</td>
<td>8.48 ± 0.27</td>
</tr>
</tbody>
</table>

“*The values given in this table represent the residual content of oil (μmole equivalent/cm2) after washing. b Control: Contained no detergents but only water.
after immobilization, which does not allow the binding of surfactant with its active site.

**Storage stability and reusability.** The coimmobilized enzymes were used for 200 times during the span of 3 months at 4 °C. In such washings without any considerable loss of enzymes activity was observed when stored in the cold (4–10 °C). Generally enzymes in free form are not safe as they might be attacked by proteases and inhibited by surfactant. Thus, the use of beaker-bound enzymes in the washing of different stained cloths by detergents not only increases their washing efficiencies without consuming them in the process but also makes cheaper detergents better than expensive detergents for washing purposes. The half-life (t1/2) of coimmobilized enzyme was 3 months (Figure 4).

![Figure 4. Effect of storage stability on PVC sheet bound enzymes.](image)

### 4. CONCLUSION

The enzymes used in expensive commercial detergents such as α-amylases, cellulase, protease (partially purified) and lipase were coimmobilized covalently onto a PVC/plastic beaker and brush and used for washing by both nonenzymic and enzymic detergents. Our results show that washing produced by nonenzymic detergents in the presence of coimmobilized enzyme was better than that by enzymic detergent in distilled water, canal water, hand pump, and well water. The important fact of this innovative work is covalent coimmobilization of four detergent enzymes on the surface of a plastic beaker and brush, which provided equal/better washings by non-enzymic (cheaper) detergent than that by costly enzymic detergents. The washing performance of plastic beaker/brush was also better than that by a nonenzymic detergent.

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### REFERENCE


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