CHAPTER VI

Application of enzyme as a detergent additive for cold washing
6.1. INTRODUCTION

6.1.1. Detergent

A detergent or washing powder is not a pure compound rather than a mixture of many components and it is made by mixing of different ingredients in the form of a slurry, and then spray-dried to yield free-flowing granules. The composition of two brands of detergent is not exactly same. Even the same brand name in different countries has a different composition. Typically detergents have some or all of the following major components, in a different proportion;

**Surfactants (8-18%):** These are surface-active agent, key ingredient of any washing powder, improves the wetting ability of water (reduce surface tension), helps to suspends soil in the water and prevents their redeposition, e.g. ABS (alkyl benzene sulfonate).

**Builders (20-45%):** These are the second major component which enhances the effect of the surfactant by deactivating calcium and magnesium ions, which would otherwise use up surfactant molecules. The most common builders used today are synthetic zeolites, which trap the divalent ions inside the solid particles.

**Bleaches (15-30%):** These are usually compounds of hydrogen peroxide, added to bleach colored stains, need high temperature (60°C) to work properly, e.g. sodium perborate, but detergents particularly for colored cloth contains no bleach.

**Fluorescers (0.1%):** These are organic molecules having fluorescent properties. They bind with the fabrics and absorb UV light and finally re-emit white light, thus brightening the fabric. Also known as optical brighteners, 'whiter than white'.
Fillers (5-45%): These are added to alter the physical properties of the detergents. Sodium sulfate is added to make the materials flow freely. Alcohols used to liquid detergents to keep everything in solution.

Others: Antifoam agent, corrosion inhibitor, water softener, perfume, etc. It also contains 4-20% water.

Approximately eighty percent of all industrial enzymes are hydrolytic in nature and are used for depolymerization of natural substances i.e. the breaking down of complex molecules into simpler ones. Of these enzymes, sixty percent enzymes are used by the detergent, dairy and leather industries (Rao et al., 1998). Detergents that contain chemicals don't break down easily in waste-water and cause pollution and eutrophication in the rivers/water bodies so the same may be replaced by enzyme which enhances the detergents ability to remove tough stains and making the detergent environmentally safe. The targeted benefit of enzyme inclusion in detergents is due to much milder conditions than with enzyme free detergents. The early automatic dishwashing detergents were very harsh, toxic when ingested and were not suitable for delicate china and wooden dishware. This forced the detergent industries to search for milder and more efficient solutions (Van et al., 1992).

Enzymes have been known to be used for improving the cleaning efficiency of detergents and are now well accepted as ingredients in powder as well as liquid detergents and industrial/institutional cleaning products. Detergent enzymes account for about 30% of the total worldwide enzyme production and represent one of the largest and most successful applications of modern industrial biotechnology. Alpha- amylases have been used in powder laundry detergents since 1975. Amylases are the second type of
enzymes used in the formulation of enzymatic detergent, and 90% of all liquid detergents contain these enzymes (Gupta et al., 2003; Hmidet et al., 2009; Mitidieri et al., 2006). These enzymes are used in detergents for laundry and automatic dishwashing to degrade the residues of starchy foods to dextrins and other smaller oligosaccharides which are water soluble (Mukherjee et al., 2009; Olsen and Falholt, 1998). Removal of starch from surfaces is also important in providing a whiteness benefit, since starch can be an attractant for many types of particulate soils.

6.1.2. Performance of enzyme in detergent

The performance of enzyme in detergents depends on a number of factors, viz the detergent’s composition, type of stains, wash temperature, washing procedure and hardness of water. Further a good detergent enzyme should be alkaline and should be stable in the presence of detergent additives such as bleaching agents, bleach activators, surfactants, perfumes, etc. (Kumar and Takagi, 1999). Washing with detergents also uses a lot of energy particularly when done at high temperature (peroxide-based bleaches need higher temperature, 60°C, to work properly). So, lowering the wash temperature, by using biocatalysts, can saves lots of energy. Cold-active amylase is a good alternative when used as one of the component of detergents to degrade starch containing stains (chocolate, tomato sauce, mashed potatoes, baby food, ready cooked meals, grass sap and gravy) from clothing materials at low temperature.

Among the various enzymes, bacterial enzymes are the most significant, compared with animal and fungal sources. Selection of the enzyme is to be done on the basis of its stability and its wash performance. Need of enzyme in immobilized form for
industrial purpose offer further advantages like repeated use of the enzyme, ease of product separation and improvement in stability (Ahmed et al., 2007).

Recently scientists from the two major detergent enzyme suppliers Novozymes and Genencore International have used protein engineering to improve the bleach stability of the amylases (Svendsen and Bisgaard, 1994; Tierny et al., 1995; Bisgaard et al., 1995). They independently replaced oxidation sensitive amino acids with other amino acids. The replacement of ‘met’ at position 197 by ‘leu’ in *B. licheniformis* amylase resulted in an amylase with improved resistance against oxidative compounds. This improved oxidation stability resulted in better storage stability and performance of the mutant enzyme in the bleach containing detergent formulations. Genencore International and Novozyme have introduced these new products in the market under the trade names Purafect OxAm® and Duramyl®, respectively.
6.2. MATERIALS AND METHODS

6.2.1. Enzyme compatibility with commercial detergents

The compatibility of cold-adapted GA6 α-amylase with commercially available laundry detergents was studied. Detergents used were Ariel (Procter and Gamble, India), Ghari (Rohit Surfactant Pvt. Ltd., India), Surf Excel (Hindustan Lever Ltd, India), Tide (Procter and Gamble, India) and Wheel (Hindustan Lever Ltd, India). The detergent solution (1% w/v) was prepared in double distilled water and the solutions were boiled at 100ºC for 10 min to destroy any enzyme already present and then cooled at room temperature. The detergent solutions were incubated with purified α-amylase enzyme (1% w/v) for different time intervals (0.5 to 3 hours) at 20ºC and the residual activity was determined. The enzyme activity of a control sample (without any detergent) was taken as 100 percent.

6.2.2. Wash performance analysis of enzyme for cold washing

Use of purified cold-active α-amylase in laundry detergent formulations was studied on small square white cotton cloth pieces (5x5 cm) stained with gravy and a mixture of baby food & chocolate. The stained cloth pieces were allowed to sit overnight and taken in separate flasks. The following sets were prepared and studied:

i) Flask with distilled water (100 ml) + stained cloth (cloth stained with gravy and a mixture of baby food & chocolate, independently)

ii) Flask with distilled water (100 ml) + stained cloth + 2 ml Tide detergent (1% w/v)
iii) Flask with distilled water (100 ml) + stained cloth + 2 ml purified cold-active α-amylase

iv) Flask with distilled water (100 ml) + stained cloth + 2 ml Tide detergent (1% w/v) + 2 ml purified cold-active α-amylase

The above flasks were incubated at 20°C for 30 minutes. After incubation, cloth pieces were taken out, rinsed with cold tap water and dried. The wash performance was analyzed on reflectance meter (Digital Reflectance Meter, Aimil Ltd., New Delhi India). The relative reflectance of all pieces was examined to test the efficiency of enzyme to remove the stains. Untreated cloth pieces stained with gravy and a mixture of baby food & chocolate were taken as control (Adinarayana et al., 2003; Beg and Gupta, 2003). All the experiments were repeated thrice and the values were average of these three independent set-ups.
6.3. RESULTS AND DISCUSSION

6.3.1. Enzyme compatibility with commercial detergents

The tolerance capacity of pH and stability with enzyme inhibitors are very important aspects for amylase to act as an ideal additive for detergent formulations to be used at comparatively low washing temperatures. These characteristics were exactly fulfilled by α-amylase of *B. cereus* GA6, so was further studied for this application. Moreover, a good detergent amylase is also expected to be stable in the presence of commercial detergents. In order to confirm the potential of this cold-active amylase as a detergent additive, its compatibility and stability was examined at low temperature (20±1°C) towards the popular, commercial Indian detergents such as Ariel, Ghari, Surf excel, Tide and Wheel.

The α-amylase from *B. cereus* GA6 showed excellent stability and compatibility with a wide range of locally available commercial detergents (1% w/v) such as Ariel, Ghari, Surf excel, Tide and Wheel, at low temperature (20°C). It showed highest compatibility with ‘Tide’ detergent retaining 92.27% activity after 0.5 hour incubation at 20°C. However, it retained 86%, 78% and 72% activity after 1.0, 2.0 and 3.0 hours of incubation at 20°C, respectively (Table 6.1). Subsequent to Tide detergent the enzyme was more compatible with ‘Ghari’ detergent exhibiting 89% activity after 0.5 hour incubation, and retained 81.8%, 74.9% and 70.7% activity after 1.0, 2.0 and 3.0 hours of incubation, respectively at 20°C. Remaining activity of the enzyme was more than 64% with rest of the tested detergents even after 3.0 hours of incubation. Therefore, it may be
suggested that the cold-active α-amylase from *B. cereus* GA6 is appropriate for commercial detergents as an additive.

**Table 6.1. Compatibility of α-amylase from *B. cereus* GA6 with commercial detergents at 20±1ºC**

<table>
<thead>
<tr>
<th>Detergents</th>
<th>Relative residual amylase activity (%)</th>
<th>0.5 hour</th>
<th>1.0 hour</th>
<th>2.0 hour</th>
<th>3.0 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surf Excel</td>
<td></td>
<td>88.27</td>
<td>80.20</td>
<td>70.5</td>
<td>65</td>
</tr>
<tr>
<td>Wheel</td>
<td></td>
<td>85.55</td>
<td>79</td>
<td>71.38</td>
<td>66.22</td>
</tr>
<tr>
<td>Tide</td>
<td></td>
<td><strong>92.27</strong></td>
<td><strong>86.11</strong></td>
<td><strong>78</strong></td>
<td><strong>72</strong></td>
</tr>
<tr>
<td>Ariel</td>
<td></td>
<td>80.91</td>
<td>77.50</td>
<td>70</td>
<td>64.50</td>
</tr>
<tr>
<td>Ghari</td>
<td></td>
<td>89.07</td>
<td>81.80</td>
<td>74.92</td>
<td>70.70</td>
</tr>
</tbody>
</table>

A similar compatibility result was obtained by Kiran and Chandra (2008), where a partially purified α-amylase of moderately halophilic alkalitolerant *Bacillus* sp. strain TSCVKK isolated from a salt-manufacturing industry showed high stability with various surfactants and detergents at 30ºC. Similar result was also found by Mukherjee et al. (2009) where crude α-amylase enzyme from *B. subtilis* DM-03 strain demonstrated excellent stability and compatibility with the laundry detergents that favors its inclusion in commercial laundry detergent formulations. Alpha-amylase purified from the culture filtrate of *Penicillium janthinellum* NCIM 4960 retained 90% of its activity when tested with Henko, Vim and White giant commercial detergents after 30 minutes of incubation at 40ºC (Sindhu et al., 2011).
The compatibility of *Bacillus amyloliquefaciens* MTCC (610) amylase was shown to be good, as the enzyme retained 96.4%, 94% and 86% of its activity after 1 hour of incubation at 40°C in the presence of the detergent brands Surf Excel Blue, Surf Excel and Fena Bar, respectively. The wash performance of Surf excel blue detergent (7 mg/ml) prepared with amylase showed maximum stain removal for chiffon samples when studied on cotton, terrycloth and chiffon fabric stained with potato curry sample (Dahiya et al., 2010). Same compatibility finding was also reported but with a purified metalloprotease of 75 kDa obtained from a psychro-tolerant bacterium *Stenotrophomonas maltophilia* (MTCC 7528) showed excellent stability and compatibility with Wheel detergent retaining 92% and 90% activity after 0.5 hour and 72% and 68% activity after 3.0 hours of incubation at 15°C, respectively (Kuddus and Ramteke, 2009). Further comparison of the detergent compatibility of the present cold-active α-amylase enzyme could not be drawn owing to the limited availability of such data in literature. The enzyme stability and compatibility was reported mostly at higher temperature by various workers. However, literature reports suggests, this is for the first (to the best of my knowledge) the cold-active α-amylase of *B. cereus* GA6 was tested with different detergents and was found significantly more stable in commercial detergents at low temperature (20°C).

Since this alkali-tolerant, SDS-resistant, cold-active α-amylase of *B. cereus* GA6 was found to be good detergent additive (i.e. Tide), so next approach was to test to remove different kinds of stains to improve the washing performance of the detergent.

**6.3.2. Wash performance analysis at low temperature**
The wash performance analysis was carried out to determine the efficiency of α-amylase for the removal of starchy stains from the fabrics. Analysis was carried out with new white cotton cloth stained with two different stains, first used stain was mixture of baby food and chocolate and second stain was food gravy. Alkaline α-amylase from B. cereus GA6 exhibited high efficiency for the removal of both stains in combination with commercial detergent (Tide) i.e., mixture of baby food and chocolate and food gravy stains at 20ºC. The wash performance analysis of mixture of baby food and chocolate stains on cotton fabric showed an increase in reflectance from 56% to 86% with detergent and enzyme as compared to detergent only. Reflectance for control was 32% and it was 35% and 50% with water and enzyme, respectively (Figure 6.1). Increase in reflectance was also observed for removal of stain of food gravy from white cloth, when washed with detergent supplemented with enzyme. This was from 52% to 76% in comparison to detergent alone. However, 25%, 31% and 45% reflectance was obtained with control, water and enzyme alone, respectively (Figure 6.2).
Figure 6.1. Removal of baby food and chocolate stain at low temperature (20°C) by cold-active *B. cereus* GA6 α-amylase (reflectance analysis).

![Figure 6.1](image1.png)

Figure 6.2. Removal of food gravy stain at low temperature (20°C) by cold-active *B. cereus* GA6 α-amylase (reflectance analysis).

![Figure 6.2](image2.png)

One more noticeable feature was observed after washing with this enzymatic-detergent, as the fabric becomes softer on touch which is not commonly experienced after removing starchy stains from cotton cloth washed only with detergent. Thus, it may be concluded that the supplementation of cold-active α-amylase enzyme from *B. cereus* GA6 in Tide detergent at 20°C could significantly improve the cleaning of the starchy stains resulted in complete stain removal and thereby can be successfully used in detergents for laundry and automatic dishwashing to degrade the residues of starchy foods (Figure 6.3 and Figure 6.4).
Figure 6.3. Wash performance (against baby food and chocolate stain) of alkaline \( \alpha \)-amylase from \textit{B. cereus} GA6 in combination with commercial detergent (Tide) at 20°C.

(A) Cloth stained with baby food and chocolate

(B) Stained cloth washed with water only

(C) Stained cloth washed with enzyme only

(D) Stained cloth washed with detergent only
(E) Stained cloth washed with detergent and enzyme

Figure 6.4. Wash performance (against food gravy stain) of alkaline α-amylase from *B. cereus* GA6 in combination with commercial detergent (Tide) at 20°C.

(A) Cloth stained with food gravy
(B) Gravy-stained cloth washed with water only
(C) Gravy-stained cloth washed with enzyme only
(D) Gravy-stained cloth washed with detergent only
(E) Gravy-stained cloth washed with detergent and enzyme.
6.3.3. Conclusion

For these applications, the present investigation provided a novel cold-active alkaline $\alpha$-amylase for laundry and automatic dishwashing to degrade the residues of starchy foods such as potatoes, gravies, custard, chocolate, etc. and thereby starchy stains on clothes of hotels and restaurants can profitably be removed. The present enzyme could be promising for its application in detergents for cold washing as it is active at low temperature. The detergent amylase work best by hydrolyzing large insoluble starch fragments to dextrins and other smaller oligosaccharides in the bulk wash liquor. These fragments are initially removed from the fabric surface either by component of the detergent matrix, or by water alone. Depending upon the size of the resulting fragments, they are either solubilized into bulk solution, or they deposit themselves back to the fabric. Hence, the best detergent amylase provides improved starch hydrolysis, resulting in better stain removal and anti-deposition benefits. In the present investigation, the treatment of stains with ‘cold-active $\alpha$-amylase + Tide detergent’ combination gave the best stain removal of all the treatments.

The results suggest that *B. cereus* GA6 $\alpha$-amylase serve a multifunctional role by first hydrolyzing starch absorbed to the fabric, into smaller components, thus making the starch more easily removed by the detergent, and thus prevent starch and their fragments re-deposition to the clean fabric. Thus, in brief it can be summarized that the supplementation of *B. cereus* GA6 $\alpha$-amylase in detergent (i.e. Tide) could significantly improve the cleansing of the starchy stains (gravy, chocolate and baby food) and may serve as a potential candidates to use as a detergent additive for cold washing.
Washing with detergents having cold-active biocatalyst not only increases the washing efficiency but also lowers the wash temperature and thereby saves lots of energy. Moreover, there is no damage for the fabric so shelf life increases and the fabric becomes softer and brighter.

“Biodetergents may be used to protect environment and save energy because it is biodegradable and can work effectively at low temperature”