# Novel enzyme-based detergents: An Indian perspective

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*Enzyme-based detergents also known as 'green chemicals' find a wide range of applications in laundry, dishwashing, textile and other such industries. The enzyme preparations like proteases, amylases, lipases and cellulases are considered as indispensable ingredients in these detergents. These components account for the major portion of the market for various cleaning applications. The cleaning ability of these formulations is mainly due to the synergistic action exhibited by the different detergent ingredients and the enzyme-preparations. Nowadays, the use of enzyme-based detergents is preferred over the conventional synthetic ones in view of their better cleaning properties, performance with respect to lowering of washing temperatures and the alleviation of pollution. This aspect is more relevant to India in view of the rapid population explosion and industrialization taking place now.*

IN dairy and food processing units, cleaning is very important and it is imperative that all food-contact surfaces of processing and handling equipment be clean and hygienic, to prevent microbial contamination and to produce quality food products. To achieve this goal, the cleaning system should include a specific sequence of cleaning agents and sanitizers applied by defined time–temperature combinations after the use of the equipment. In practice, much attention is given to the cleaning and sanitizing operations within the cleaning system. These are complementary processes which together help achieve the desired results. However, in some situations, periodic sanitation has been recommended rather than combined cleaning and sanitation. Similarly appropriate cleaning agents with or without sanitizers are used for washing soiled clothes. The exploitation of the synergistic action between various detergent components ushered a phenomenal growth of synthetic detergents. Various builders, silicates, surfactants and other detergent adjuvants are the main components of detergent formulations. For many years, sodium tripolyphosphate (STPP) has been considered to be one of the most versatile and preferred builder of choice for detergents[1].

Since their advent, synthetic detergents are being increasingly used in bulk quantities worldwide. In India, synthetic detergents were introduced three decades ago. The production of these synthetic detergents in 1980–89 varied between 12 and14 lakh tonnes/annum which reached 13.35 lakh tonnes in 1990 and rose to 18.48 lakh tonnes in 1995. It was further projected that the production would increase to 25.50 lakh tonnes by the turn of the century indicating an extremely fast growth[2]. The overall growth of the detergent market has been about 15% per annum and this has been mainly due to the increase in population, spread of education, higher urbanization and rising levels of income and consumption. In addition, a new washing segment that has emerged in the last two or three years is the washing machine market. The market sources predict that the synthetic detergents would increase by the next decade at a growth rate of 25% per annum. In the recent past, due to the liberalization of the national policy regarding import and export, a large number of multinational companies have entered Indian markets thus competing with the domestic detergent market. This has led to the emergence of alternate detergents including zeolites, citrates, polycarboxylates, polyacrylates and the enzyme-based preparations. The advent of these detergents and the associated advertisement blitz has further brought to the fore the inadequacies of the conventional detergents hitherto unknown to the consumer. The detergents that are marketed at present in India have undergone a transformation and have assumed various forms, viz. powders, liquids, bars/cakes, etc. Surprisingly, more recently, like in Western countries, pastes and gels have also emerged and have gained widespread popularity. Due to their ease in handling, most of these cater to the lucrative domestic market.

Until recently, the emphasis in the formulation of an ideal detergent preparation was to achieve a synergistic action between various components of the synthetic detergent to give it a balanced activity with regard to its various desirable properties. However, during the past few years, there has been serious public concern about the ecological problems arising from the use of such

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synthetic detergents on a large scale. Owing to the fact that these synthetic detergents are corrosive, toxic and exhibit a slow rate of biodegradation, their extensive usage leads to the formation of slumps, creating unhygienic conditions in the surroundings. However, the latest cleaning technologies include enzyme-containing detergent formulations and zeolite-based detergents. Of these, the enzyme detergents are proving extremely useful in keeping a check on environmental pollution. They offer a suitable option to the synthetic detergents with regard to their biodegradability, low toxicity, non-corrosiveness, environment-friendliness, enhanced cleaning properties as well as increased efficiency and stability in different formulations. In this context, these are also referred to as *green chemicals* and are becoming an ideal consumer choice. In addition, the use of enzyme-based detergents enables the lowering in washing temperatures with the increased use of synthetic fabrics.

## Developments in enzyme-based detergents

The idea of using enzymes is certainly not new. In 1913, Otto Rohm's patent, indicated the use of pancreatic enzymes as washing aids for laundry cleaning and the product was marketed as a presoak detergent under the brand name *Burnus*. It enjoyed moderate commercial success and it was sold for about 50 years in the European markets[3].

However, several new detergent enzymes have emerged ever since Novo Industri A/S introduced, in the mid-1960s, an alkaline protease preparation produced by *Bacillus licheniformis*, that was stable and active at an alkaline pH of 8–10 and trade named it as *Alcalase*. Later, in 1970s, there was a temporary setback in the use of proteases in detergents because some producers and users developed allergic reactions. These problems were overcome by developing dust-free granules, which prevent dust formation and protect the enzymes against damage by other detergent components during storage. In 1985, Novo introduced a revolutionary type of cellulase detergent enzyme, *Cellulase*, produced by *Humicola insolens* for soil removal, softening and colour restoration of cotton textiles[4].

During the last decade, new developments in enzymology with the application of recombinant DNA technologies, viz. genetic and protein engineering led to the current era of bioengineered enzymes. Moreover, Novo in 1988 came out with a major breakthrough by introducing a detergent enzyme, lipase in *Lipolase*, the first bioengineered industrial enzyme product of its type. The lipases and cellulases have been mainly introduced in the detergents to supplement the proteases. In 1989, two more detergent enzymes based on these technologies were introduced – a cloned *Savinase* type, *Maxacal* from Gist-brocades, Delft, Netherlands and a genetically engineered *Subtilisin Novo* from Genencor International of California. Subsequently, enzyme detergent manufacturers explored new generation enzymes for better wash performance and stability towards oxidative bleaching agents such as perborates. *Maxapem*, an alkaline protease and *Durazym*, a protein engineered detergent enzyme were developed by Solvay Enzymes, Germany and Novo Nordisk, Denmark, respectively. These exhibited improved stability in the presence of oxidative bleaching agents while maintaining better cleaning performance[5,6].

## Status of the enzyme industry

Generally, the commercial preparations of various enzymes are employed in the formulation of different types of enzyme-based detergents. The proteases, in particular, represent about 60% of all the industrial enzyme sales in world[7]. Of these, alkaline proteases used primarily as detergent additives, represent a major fraction. In 1992, alkaline proteases worth $56 million were transacted in US alone[8]. However, in the year 1994, the total market for industrial enzymes (Figure 1) accounted for around $400 million, of which enzymes worth $112 million were used for detergent production[9]. As far as the Indian scenario is concerned, the picture is rather different where the respective figures for enzymes transacted and finding their way into the detergent industry for the corresponding year were Rs 500 million and Rs 20 million, only. Nevertheless, there is expected to be an upward trend in the use of enzymes in the domestic market and by the turn of the decade, the total value is likely to touch Rs 3000 million or more[10].

Presently, two companies actively engaged in the production and handling of a variety of industrial enzymes are Advanced Biochemicals Ltd. and Biocon (India) Ltd., located in Thane (Maharashtra) and Bangalore, respectively. Both these companies share the top bracket in the area and have an annual turnover of around

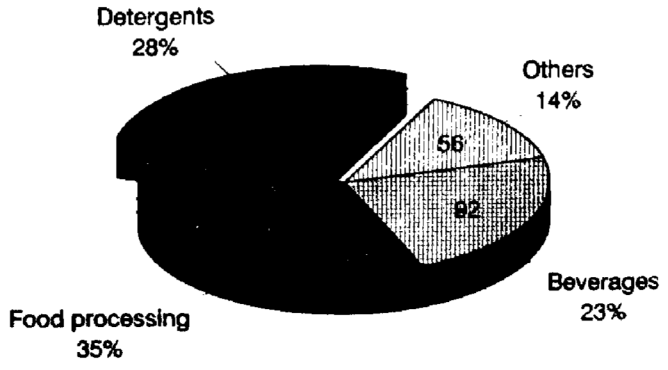


**Figure 1.** Global enzyme market (in million dollars). Source: ref. 9.

**Table 1.** Commercial enzymes used in detergent formulations and other applications

| Trade names | Source organism | Optimum pH | Optimum temperature (°C) | Manufacturer |
|---|---|---|---|---|
| **Alkaline proteases/Subtilisins** | | | | |
| Alcalase | *Bacillus licheniformis* | 8–9 | 60 | Novo Nordisk Bagsvaerd, Denmark |
| Savinase | Alkalophilic *Bacillus* sp. | 9–11 | 55 | Novo Nordisk Denmark |
| Esperase | Alkalophilic *Bacillus* sp. | 9–11 | 60 | Novo Nordisk Denmark |
| Maxacal | Alkalophilic *Bacillus* sp. | 11 | 60 | Gist-brocades, Delft, The Netherlands |
| Maxatase | Alkalophilic *Bacillus* sp. | 9.5–10 | 60 | Gist-brocades, The Netherlands |
| Opticlean | Alkalophilic *Bacillus* sp. | 10–11 | 50–60 | Solvay Enzymes GmbH, Hannover, Germany |
| Optimase | Alkalophilic *Bacillus* sp. | 9–10 | 60–65 | Solvay Germany |
| Protosol | Alkalophilic *Bacillus* sp. | 10 | 50 | Advanced Biochemicals Ltd., Thane, India |
| Alkaline protease 'Wuxi' | Alkalophilic *Bacillus* sp. | 10–11 | 40–50 | Wuxi Synder Bioproducts Ltd., China |
| Proleather | Alkalophilic *Bacillus* sp. | 10–11 | 60 | Amano Pharmaceuticals Ltd., Nagoya, Japan |
| Protease P | *Aspergillus* sp. | 8 | 40 | Amano, Japan |
| Durazym | Protein engineered variant of Savinase™ | 10–10.5 | 50 | Novo Nordisk, Denmark |
| Maxapem | Bleach-resistant, protein engineered variant of alkalophilic *Bacillus* sp. | 11–12 | 60 | Solvay, Germany |
| Purafect | Recombinant enzyme Donor – *B. lentus* Expressed in *Bacillus* sp. | 10 | 40–65 | Genencor International Inc., Rochester, USA |
| **Amylases** | | | | |
| BAN | *Bacillus amyloliquefaciens* | 6–7 | 70 | Novo Nordisk, Denmark |
| Termamyl | Recombinant enzyme Donor – *Humicola* sp. Expressed in *Aspergillus* sp. | 7–8 | 70–75 | Novo Nordisk, Denmark |
| Maxamyl | Alkalophilic *Bacillus* sp. | 6–8.5 | 100 | Gist-brocades, The Netherlands |
| Solvay amylase | Thermostable *Bacillus licheniformis* | 5–8 | 75–90 | Solvay, Germany |
| **Lipases** | | | | |
| Lipolase | Rocombinant enzyme Donor – *Humicola lanuginosa* Expressed in *Aspergillus oryzae* | 10.5–11 | 40 | Novo Nordisk, Denmark |
| Lumafast | Recombinant enzyme Donor – *Pseudomonas mendocina* Expressed in *Bacillus* sp. | 7.5–9 | 60 | Genencor, USA |
| Lipofast | NA* | 8.5 | 50 | Advanced Biochemicals, India |
| **Cellulases** | | | | |
| Celluzyme | *Humicola insolens* | 6–7 | 50–55 | Novo Nordisk, Denmark |

*Not available.

Rs 100 million each. The remaining amount of enzyme requirement of the country is catered by a number of other small units of manufacturers and still a sizeable amount of it is being met through imports. Currently, Advanced Biochemicals Ltd. is in the process of setting up a new plant at Sinnar, near Nasik, Maharashtra, in technical collaboration with a renowned South Korean firm, Pacific Corporation, the largest manufacturer of industrial enzymes in that country[10]. Further, many world premier industries are involved in the manufacture and trade of detergent enzymes (Table 1). These enzymes are mostly derived from either the submerged or solid state fermentation process by using different microorganisms, in particular, the various strains of alkalophilic *Bacillus* sp. or the use of genetic and/or protein engineered organisms. Another alkaline protease preparation has been developed by Central Leather Research Institute, Madras under the trade name *Clarizyme*. This enzyme was produced by an *Aspergillus flavus* isolate using a solid substrate fermentation (SSF) process.

## Production of enzyme-based detergents

The manufacture of an enzyme detergent is not very different from that of the conventional synthetic one. The prime step for the formulation of an enzyme-based detergent is the compatibility of the enzyme(s) with various detergent ingredients. In general, the suitability of an enzyme preparation mainly depends on its compatibility with the detergents at moderately higher temperatures. An ideal enzyme for detergent preparation should be effective at low levels (0.4–0.8%) in the detergent solution. It should also be compatible with various detergent components along with oxidizing and sequestering agents and possess adequate temperature stability to be active in a wide range of cleaning temperatures. It must also have a long shelf life[11]. Moreover, the very low use concentration is due to the fact that the enzymes added to the product are *biocatalysts*. In this context, the term *biocatalyzation* is implied wherein the enzymes themselves are not being consumed during the cleaning process and a single enzyme triggers numerous chemical reactions. As a result, the disadvantages of the conventional detergents are eliminated.

The early use of enzyme powders in detergents led to dust problems in the production process. In addition, the reduced stability of the enzymes due to autolysis and detrimental effects by the other detergent ingredients in the presence of moisture were encountered. These problems led to the use of granulation techniques and enzyme *prilling* with enzymes being encapsulated in an inert water soluble waxy substance. In powder detergents, the enzymes are mixed with the finished powders as granulates or prills. Currently, wax-coated enzyme detergent granules are being offered in colours identical to the non-coloured detergent granules. The coloured granules are termed as *signal granules* by the detergent manufacturers which symbolize the presence of an extra-added active ingredient in the detergent preparations.

In case of enzyme-based automatic dishwashing detergents, citrate and other polyacrylate builders are added. Moreover, perborates and percarbonates are also used. These peroxybleach generating systems are not too harmful for enzymes and through the action of activators such as tetraacetylethylene diamine (TAED), enable the acceptable bleaching action at low temperature[12].

## Enzyme stabilization

Most early enzyme products such as detergent proteases were just powders. Almost all of them were granulated and further protected by coatings. Another method to prevent enzyme dust in the air is liquid formulations. Today a lot of research work is being done in the different formulations and stabilization techniques in many enzyme detergent production facilities.

The enzymes used in various detergent formulations are subject to proteolytic and autolytic degradation on storage and sudden exposure to harsh operating conditions results in rapid inactivation of enzyme activity. Loss of enzyme activity is also encountered during storage in the factory, shipment to client(s) and/or storage in client(s) facilities. Hence, storage stability is of prime concern to enzyme manufactures. The rate of enzyme inactivation is largely dependent on temperature, pH and other detergent components such as surface active agents, sequestrants and bleaching agents. Moreover, the higher the temperature and alkalinity, the less stable is the enzyme[3].

The loss of the enzyme activity is mainly due to the partial unfolding of the polypeptide chain, since the inactivating agent breaks down the delicate balance of noncovalent bonds which maintain the native conformation[13,14]. The ideal approach to stabilize the enzyme would be to identify the mechanism of inactivation and then design a procedure which would prevent that mechanism[14].

In order to protect the enzyme against denaturation, addition of stabilizers like calcium salts, sodium formate, borate, polyhydric alcohols and protein preparations have proved successful[13,15–17]. To prevent contamination of the final commercial crude preparation during storage, addition of sodium chloride at 18–20% concentration has been suggested[18,19]. These processes maintain the enzyme activity and improve storage stability. In certain cases, for the purpose of convenience in handling and storage, liquid enzyme preparations are often brought to powder form by vacuum or air drying which are milder and less expensive than lyophilization.

The stabilization of enzymes has also been made possible through use of protein engineering to design tailor-made enzymes with specific enzyme properties and stability[20,21] and this technique is leading new insights into the process of biocatalysis[22]. Protein engineering is rapidly emerging today as a new science and is basically an art of modifying an existing protein or creating *de novo*, a protein of pre-specified properties[23]. From a commercial viewpoint, this technology is inherently complex, costly and time consuming. Despite these inherent drawbacks, commercial detergent enzyme producers adopt this technology for producing novel and/or superior enzymes with stable, new and/or improved properties like stain removing ability, improved stability due to resistance to oxidizing agents (oxygen-based bleaching), etc.[24].

## Applications of enzyme-based detergents

Data published up to now indicate that the enzyme detergents are being mainly explored for their application in laundry, dishwashing, textile and other such industries. Of late, in view of their advantages and increased potentiality, some researchers have tried to use them in the food and dairy industries. The different applications wherein the enzyme detergents are being currently used are:

### *In laundry*

The microbial enzymes which have found application so far in laundry are the proteases, amylases and lipases. More recently, the cellulases have also been employed in the detergent industry with an added dimension. The proteases hydrolyse the proteinaceous residues of blood, egg, grass and sweat to form soluble peptides which are subsequently easily removed by detergent suds. The amylases degrade the residues of starchy foods like porridge, potatoes, gravies, custard, chocolate, etc. to dextrins, while the lipases catalyse the hydrolysis of salad oil, sauces, lipstick, etc. The cellulases in the detergents degrade mainly the microfibrils which are generated during continuous use and repeated washings of the garment and also help in restoring the original shine and colour of the garment.

The washing performance of the enzyme detergent depends on many factors to achieve better results. These are detergent composition and dosage, pH and buffer capacity, water hardness, washing time and temperature, mechanical handling, soiling agents, textile types to name a few. In addition, the specificity of the enzyme is another most important parameter. As a general opinion, it is considered that a detergent enzyme should have as wide a specificity as possible. For example, a protease should be capable of degrading as many proteins as possible. However, a reasonably good wash performance can be achieved by a specific protease, in comparison to a non-specific protease (Figure 2). As the hydrolysis proceeds, small peptide fragments are formed by the action of an unspecific protease, which are rather difficult to remove as they are not very much soluble in detergent solutions. On the other hand, larger protein or peptide fragments are formed on hydrolysis with a specific protease due to the breakdown of very few peptide bonds which can be easily removed during the washing process[31]. Presently, use of dual enzymes in detergent formulations is practised, wherein the enzymatic hydrolysis and degradation can be broadened considerably in comparison to a single enzyme approach.

Recently, workers of the Genencor International Inc., USA have developed enzymes called endoglycosidases which deglycosylate biopolymers like glycoproteins which are widely distributed in living organisms. They employed rDNA technology to develop *Endo-β-N-acetyl glucosaminidase H* (*Endo H*) as a cleaning agent. *Endo H* has a unique property to remove bacteria (Staphylococci and *E. coli*) from glass and cloth surfaces in buffer and detergent solutions[25].

At present, most of the advanced countries like Japan, United States and some European countries almost invariably use the detergents incorporated with enzymes. Interestingly, in Japan, all detergent brands contain enzymes. In India, a few premium detergent brands presently available in the market like *Ariel* (Procter and Gamble (India) Ltd.), *Surf Ultra, Rin Biolites, Revel Plus* (Hindustan Lever Ltd.) and *Zymo* (Henkel) contain enzymes in their formulations. Recently, Procter and Gamble (P&G) has introduced a new cellulase enzyme in the detergent powder, *Ariel*, presently marketed in India, that eliminates the fuzz formed during washing and tumble-drying, particularly of the cotton fabrics. The manufacturers claim that use of this product retains the colour and improves the texture of the fabric on repeated washings.

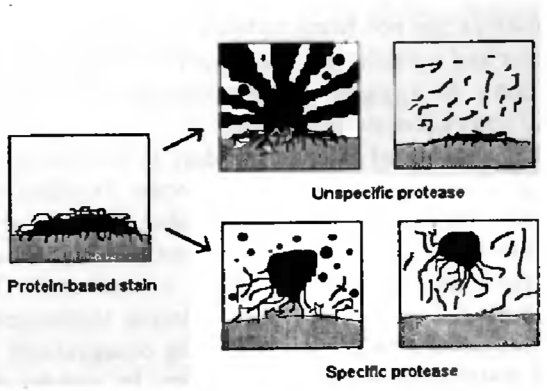


**Figure 2.** Schematic representation of the mechanism of the action of specific and non-specific protease on protein-based stains[31].

*In dishwashing*

Enzymes have been successfully used in laundry detergents for many years as an aid to remove tough stains. However, the interest in using enzymes in automatic dishwashing detergents (ADDs) has increased recently. Both laundry and dishwashing detergents share similar functions such as removal of stains from egg, milk and starch-based soilings, etc. The performance of the enzymes in the ADDs are strongly influenced by the ADD formulation and the conditions of the automatic dishwashing. At present, proteases and amylases are the only two enzymes which have found major application in dishwashing detergents[26]. In particular, enzyme-based dishwashing detergents are less abrasive in function and thus are suitable for use on delicate chinaware; they prevent the erosion of designs and colours. This application was first exploited in Japan where the use of richly decorated chinaware and wooden kitchen utensils is widespread.

Enzymatic ADDs have gained widespread usage since the last decade. In the past 2–3 years, ADDs with enzymes were launched in several European countries, viz. Austria, Germany, Switzerland, Denmark and the United Kingdom. In Japan, all major ADD brands contain enzymes, whereas only one brand in the US market currently contain enzymes. However, at present, there are no enzymatic dishwashing detergents available in India.

*In the textile industry*

Currently, in the textile industry, there is a widespread demand for faded jeans. This involves subjecting such clothes to amylases – a process commonly referred to as *biowashing* or *biobleaching*, an alternative to the term, *enzyme-fade*. This allows elegant softness and unique shades to be given to the cloth which overcomes the traditional methods of bleaching by sodium hypochlorite or tumbling with pumice stones, and also offers better safety as well as economy.

*In food and dairy industries*

With the better understanding of such enzymes, more and more areas of their application are emerging, such as in dairy, food and beverage industries. The use of enzymes in these industries in the cleaning operations helps in creating the required hygienic conditions in such plants. Probably, the use of enzyme-based detergents in the in-place cleaning of membranes of ultrafiltration (UF) and reverse osmosis (RO) equipments proves promising and forms one of the most important aspects of modern dairy and food industries. The UF and RO membranes are put to a variety of uses including concentration, clarification and/or sterilization of liquid foods like skim milk, whey, egg white, fruit juices and beverages[27].

Despite their diverse applications, these two membrane processes have some inherent disadvantages. The membrane filters come in contact with the feed stock during use. Even a small degree of adsorption causes pore blockage resulting in clogging of filters, a phenomenon called fouling, and thereby cause a reduction in the permeate flux rate and loss in the product quality with increase in production costs. In general, the proteins, inorganic salts and fat residues along with bacteria constitute the common and important fouling agents responsible for lowering the flux and affecting the product quality[28].

Depending on the type of application, the precise formulations are made; for instance, proteases are used for fouled dairy filters, $\alpha$-amylases and $\beta$-glucanases in yeast and cereal, and cellulases and pectinases for wines and fruit juices. The enzyme detergent preparations presently marketed for cleaning of membrane systems are *Terg-a-zyme* (Alconox, Inc, New York, USA) and *Ultrasil 53* (Henkel KGaA, Dusseldorf, Germany). These enzyme-based cleaners that have been marketed rely very much on the proteases to cleave and solubilize the protein foulant. The use of alkaline proteases from *Bacillus* sp. strain MK5-6 have also proved successful in our laboratory[29]. Pilot scale evaluation of the enzymes at plant level operations for UF membrane cleaning indicated the enzyme preparation to be highly effective and restored 100% flux in comparison to *Terg-a-zyme*, a commercial preparation which resulted in only 80% restoration of the flux. The use of proteases and lipases to degrade and solubilize protein and fat foulants has also proved beneficial.

*Other uses*

The application of enzyme-cleaners in the optical industry is important, enabling one to give 100% safe and efficient cleaning to lenses. In India, presently one such enzyme-based optical cleaner in the form of tablets containing *Subtilopeptidase A* is being marketed by M/s Bausch and Lomb (India) Ltd. Enzyme detergents have also found application in hospitals. *Promod 153L*, a protease enzyme-based cleaner, has been used to clean surgical instruments fouled by blood proteins[30].

## Conclusions

Thus to conclude, cleaning forms an important aspect for the maintenance of hygiene and safety of foods in the food processing industry. Improperly cleaned food-contact surfaces lead to the accumulation of food particulates which favour the formation of biofilms, i.e. attachment of microorganisms. These cause post con-

tamination and spoilage of foods. It is, therefore, necessary to understand the interactions of the biotic and abiotic entities in the food-processing operations and further effectively analyse the impacts of cleaning and sanitation from a microbiological viewpoint. The use of enzyme-based detergents as biocleaners can also serve as a viable option to overcome the biofilm problem in the food industry.

Further, the technology and production of these enzymes and the enzyme-based detergents is mostly patent-protected. As such most of the enzymes used in the detergent industry in India are being imported. Even the large scale detergent manufacture seems highly technical requiring specific know-how and infrastructure. Work has been going on in the recent past in order to develop an indigenous technology on different enzyme systems in certain well reputed laboratories, viz. National Dairy Research Institute, Karnal, National Chemical Laboratory, Pune and Institute of Microbial Technology, Chandigarh. Due to their high efficiency and safety, it is assumed that the enzyme detergents will eventually capture a bulk of the Indian detergent market.


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