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Review

Enzymes used in detergents: Lipases

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Microbial lipases are an important group of biotechnologically valuable enzymes, because of the versatility of their applied properties and ease of mass production. Lipases of microbial origin are widely diversified in their enzymatic properties and substrate specificity, which make them very attractive for industrial applications. This review describes the applications of microbial lipases in detergents. Enzymes can reduce the environmental load of detergent products as the chemicals used in conventional detergents are reduced; they are biodegradable, non-toxic and leave no harmful residues. Besides lipases, other enzymes are widely used in household cleaning products, in laundering, medical, agriculture, etc. This article also reviews the use of enzymes, especially lipases as detergents and different types of lipase containing detergents available in the market.

Key words: Detergents, enzymes, lipases.

INTRODUCTION

Lipases constitute the most important group of biocatalysts for biotechnological applications (Benjamin and Pandey, 1998). Lipases have been isolated from many species of plants, animals, bacteria, fungi and yeast. The enzymes from microorganisms are used in various industries such as dairy, food, detergents, textile, pharmaceutical, cosmetic and biodiesel industries, and in synthesis of fine chemicals, agrochemicals and new polymeric materials (Saxena et al., 1999; Jaeger and Eggert, 2002). Research on microbial lipases, has increased due to their great commercial potential (Silva et al., 2005).

Lipases are added to detergents such as household and industrial laundry (Kumar et al., 1998) and in household dishwashers, where their function is in the removal of fatty residues and cleaning clogged drains (Vulfson, 1994). The cleaning power of lipase (or other enzyme containing) detergents increases markedly. The enzymes such as proteases, amylases, cellulases and lipases are added to the detergents to improve their efficiency (Ito et al., 1998).

Laundry detergents are becoming more and more popular because of their increasing use in washing machine, where it impart softness, resiliency to fabrics, antistaticness, dispersible in water and mild to eyes and skins. There are many different brands or types of laundry detergents, and mostly they claim some special qualities (Bajpai and Tyagi, 2008). The detergents industry, regulated by the European Commission (EC) 648/2004, Detergent Directive, is considered as the largest enzyme consuming sector. Concerns over persistence of detergent chemicals in the environment and its possible contamination of ground water, other fresh sources and their subsequent health related issues has raised speculation over biodegradability. This has acted as an impetus for greater consumption of enzymes as Europe is witnessing increasing preference for greener detergents.

The need for greater control over microorganisms and enzymes for industrial purposes has led to a greater focus on Genetic Engineering and Recombinant DNA technology. This technology, allows genetic modification of microorganisms to produce the desired enzyme under specific conditions. This helps either to produce a particular type of enzyme or enhance the quantity of enzyme produced from the single recombinant microorganism. Understanding the structure of enzymes and modifying them to extract benefits is categorized as Protein Engineering. Studies are being conducted on ways to improve or modify protein structure and its function thus finally the enzyme. Biotechnology is therefore, being increasingly viewed as a possible solution against traditional chemicals processes. The production of enzymes from natural sources and their environment friendly characteristics has led the industry to believe that enzymes are

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Table 1. Four classes of enzymes are generally used in detergents.

Proteases	Most widely used enzymes in the detergent industry remove protein stains such as grass, blood, egg and human sweat which have a tendency to adhere strongly to textile fibers.
Amylases	Used to remove residues of starch-based foods like potatoes, spaghetti, custards, gravies and chocolate.
Lipases	Decompose fatty material. Lipase is capable of removing fatty stains such as fats, butter, salad oil, sauces and the tough stains on collars and cuffs.
Cellulases	Modify the structure of cellulose fiber on cotton and cotton blends. When it is added to a detergent, it results in; color brightening, softening and soil removal.

indeed a sustainable alter-native to chemicals in industrial processes. The factors that aid their increased application is the availability of wide enzyme types, environmental friendly behaviour, minimised energy consumption, easily controlled processes, possible modification of enzyme characteristics, environ-mentally friendly by-products, minimal greenhouse gas emissions, decreased use of non-renewable sources and proliferation of chemicals in environment. The multi faceted benefits availed through using enzymes is bound to convert into a greener world (Kumar, 2009).

ENZYMES USED IN DETERGENTS

Biotechnology based cleaning agents such as enzyme-based agents, are widely used in industries. The biotechnology based cleaning agents (ETBPP, 1998) are cheaper and less harmful to the environment. They have specific cleaning action and can also be used at lower temperatures. They produce effluents with lower COD and non-corrosive nature. Enzyme-based cleaners are becoming increasingly popular in the food industry as compared to caustic or acid cleaning regimes (D'Souza and Mawson, 2005).

The enzyme based detergents have better cleaning properties as compared to synthetic detergents. They are active at low washing temperatures and environment friendly also (Kumar et al., 1998). The enzymes in the detergents do not lose their activity after removing stain. The enzyme containing detergents also improve the fabric quality and keeping color bright.

The enzyme based detergents are used in small quantity as compared synthetic chemicals. They can work at very low temperature, environment friendly and completely biodegradable.

Four classes of enzymes are generally used in detergents as given in Table 1 (Gormsen et al., 1991).

Removal of proteins by non-enzymatic detergents can result in permanent stains due to oxidation and denaturing caused by bleaching and drying. Proteases hydrolyze proteins and break them down into more soluble polypeptides or free amino acids. As a result of the combined effect of surfactants and enzymes, such hard to remove stains can be removed from fibers.

An alkaline extracellular enzyme obtained from alkaliphilic *Bacillus* strains is used in detergents. Other enzymes like proteases, alpha amylases and cellulases are produced on large scale and have been used in detergents (Ito et al., 1998). The domestic dishwashing detergents contain different chemicals like chelating agents, surfactant, polycarboxylates (PCA), phosphonates, active chlorine compounds etc. These substances have high environmental risks, particularly via sewage biosolids, poor biodegradability, increased organic load to sewage works, toxicity (CEEP-phosphates in dishwasher detergents, 2007).

Sodium hydroxide or caustic soda (NaOH) is the most widely used alkaline detergent in the food and beverage industry due to its low price and high cleaning efficiency for fatty-type and protein soils. Nitric acid and phosphoric acid are most commonly used by the food and beverage industries which lead to the increased level of TDS and sodium discharge in effluent. To avoid any damage to soil and vegetation, it is important to reduce the use of traditional chemicals in food and beverage industries (Palmowski et al., 2005).

Enzymes are used in detergents to increase the cleaning ability of detergents. Enzymes can be used instead of chlorine bleach for removing stains on cloth. The enzyme protease was produced from alkaliphilic *Bacillus clausii* KSM-K16 and strain KP-43 and *Bacillus* sp. strain KSM-KP43 and have been incorporated into laundry detergents. Subtilisin-like serine proteases belonging to family A of subtilase super family has been used in laundry and dishwashing detergents (Saeki et al., 2007). A number of alkaliphilic *Bacillus* produce alkaline cellulase (carboxymethylcellulase) that is used as an additive for improving the cleaning effect of detergents. Enzymatic properties of some cellulases fulfilled the essential requirements for enzymes to be used practically in laundry detergents (Ito, 1997).

Among other enzymes used in some detergents are Guardzyme (a peroxidase) which inhibits dye transfer and Carezyme removing the fuzz that builds up on cotton clothes. Enzymes used in detergents must be effective at low levels, compatible with various detergent components and to be active at wide range of temperatures (Kumar et al., 1998).

A number of detergent formulations such as anionic or nonionic surfactants and the powdered lipase are used

Table 2. Compositions of an enzyme detergent.

Constituent	Composition (%)
Sodium tripolyphosphate (water softener, loosens dirt)	38.0
Sodium alkane sulphonate (surfactant) (cationic/ anionic/ non-inonic/ amphoteric)	25.0
Sodium perborate tetrahydrate, sodium percarbonate (oxidising agent, oxygen bleach)	25.0
Soap (sodium alkane carboxylates)	3.0
Sodium sulphate (filler, water softener)	2.5
Sodium carboxymethyl cellulose/sodium polyacrylate/ polyethylene glycol (dirt-suspending agent/ (Antiredeposition agents)	1.6
Sodium metasilicate (binder, loosens dirt)	1.0
Bacillus protease (3% active) (any other enzyme)	0.8
Fluorescent brighteners	0.3
Foam-controlling agents	Trace
Perfume	Trace
Water	100%

Source: Jain, 2008.

for the removal of fatty soils from fabrics. These include sodium nitrilotriacetate, sodium tripolyphosphate, sodium silicate, sodium citrate, and potassium diphosphate. Optional ingredients include a detergent builder such as, foam boosters, alkalies (e.g. sodium carbonate); optical brighteners (e.g. stilbene derivatives); stabilizers (e.g. triethanolamine); fabric softeners (e.g. quaternary ammonium salts) together with bleaching agents and systems (such as sodium perborate and ethylene diaminetetra acetate). Different fragrances, dyes, foam depressors and corrosion agents are also used additionally (Thom et al., 1990). Use of liquid laundry detergents has been increasing over the years (Bajpai and Tyagi, 2007). For more than forty years, enzymes in encapsulated form have been used in detergent products, such as laundry formulations. They have increasing importance as they are biodegradable and function at low temperatures and offer environmental benefits (Basketter et al., 2008). A very small amount of the enzymes is used in detergent preparations and it must withstand the other components of the detergents, such as surfactants, oxidants, optical brighteners and also stable at pH values between 8 and 10.5. (Table 2) (Jain, 2008).

Alkaline yeast lipases are preferred because they can work at lower temperatures as compared to bacterial and fungal lipases (Ahmed et al., 2007; Amino et al., 1998; Saxena et al., 1998). Cold active lipase detergent formulation is used for cold washing which reduces the energy consumption and wear and tear of textile fibers (Feller and Gerday, 2003). Enzymes can reduce the environmental load of detergent products because they save energy by enabling a lower wash temperature to be used (Vakhlu and Kour, 2006). Addition of cold active lipase in detergent become biodegradable, leaves no harmful residues, have no negative impact on sewage treatment processes and do not have risk for aquatic life (Joseph et al., 2007).

Various factors affect the performance of enzymes in detergents; its composition, type of stains to be removed, washing temperature, washing procedure and water hardness. Some of the additives of the detergents include; builders (Sequestering builders- polyphosphates, citrate, precipitating builders- sodium carbonate, sodium silicate), zeolite, alkaline agents- sodium carbonate, sodium silicates, corrosion inhibitor- sodium silicate, processing aids- sodium sulfate, water, alcohol, xylene sulphonate, colorants, suda control agents, Opacifiers, bleaching agents-sodium percarbonate, sodium perborate, hydrogen peroxide (Bajpai and Tyagi, 2007).

Enzymes in detergent formulations are stable at high pH and temperature and remove protein and lipid stains. Enzymatic detergent requires low workable temperature, low mechanical energy and is less toxic and non-corrosive (Sekhon and Sangha, 2004). Thermostable enzymes are usually stable in solvents and detergents; therefore, they also have considerable potential for many biotechnological and industrial applications (Haki and Rakshit, 2003).

The first industrial extremozymes used in textile detergents, were the alkaline cellulases produced by bacteria isolated from an east African soda lake, which was the first industrial extremozymes for use in textile detergents, and were commercialized by Genencor (Antranikian et al., 2005). Enzymes used in household detergents have stability at extreme temperatures and pH. The enzymes must fulfill different requirements such as activity and stability, substrate specificity and enantioselectivity, for their application in industry (Podar and Reysenbach, 2006).

The cold active lipases will have a larger share of enzymes of industrial importance in the coming years with the increasing interest in psychrophiles (Joseph et al., 2007). These enzymes offer a great industrial and biotechnological potential due to their capability to catalyze reactions at low temperature (Gomes and Steiner, 2004).

LIPASES IN DETERGENTS

There has been a tremendous increase in the significance of the biotechnological applications of lipases since the last two decades (Jaeger and Eggert, 2002; Jaeger and Reetz, 2000; Jaeger et al., 2001), due to the versatile catalytic behavior of lipases (Bosley, 1997). Lipases form an integral part of the industries ranging from food (Jaeger et al., 1994), pharmaceuticals (Chang et al., 1999) and detergents (Venegas, 1993) to oleo-chemicals (Houde et al., 2004), tea industries (Saxena et al., 1999) agriculture, cosmetics, leather and in several bioremediation processes. Because of the vast applications, newer microbes are to be screened for production of lipases having desirable properties. Molecular analysis of the lipase gene helps to understand the structure and activity relationship of the enzyme, which will help the researchers to construct new lipases for biotechnological applications (Tripathi, 2003) according to the demand and need of industry.

Detergents are common commercial products used in dish washing, bleaching compositions (Nakamura and Nasu, 1990), in dry cleaning solvents (Abo, 1990), leather cleaners (Kobayashi, 1989), cleaning of contact lens (Bhatia, 1990), clogged drain clearing in domestic/ industrial effluent or food processing treatment plants (Bailey and Ollis, 1986), in degradation of organic wastes from exhaust pipes, toilet bowls, etc. (Moriguchi et al., 1990). Lipases and cellulases in certain detergents remove dirt/ cattle manure from domestic animals (Abo et al., 1990). Lipases are also used in degreasing and water reconditioning in combination with oxido-reductases that require lesser amount of surfactants and work at low temperatures (Novak et al., 1990).

The world market for enzymes is expected to reach $7 billion in 2013, with average annual increase of 6.3%, mostly in the pharmaceutical and biocatalyst enzymes. Biotechnology will continue to be of importance, helping to sustain demand for research and biotechnology enzymes (World Enzymes, 2009). Whereas, according to another report by Global Industry Analysts, Inc., world market for industrial enzymes is expected to exceed $2.9 billion by 2012. Another report by San Jose, California (PRWEB) (2008), states that United States is going to be the fastest growing market for industrial enzymes with a CAGR of 5% in 2001 - 2010 while Europe was reported as the largest regional market having an estimated share of 30.93% in 2008. Sale of industrial enzymes in Asia-Pacific was estimated at US$327 million in 2008. Lipases (for their use in the detergent and cosmetics markets), were expected to have a CAGR of 9.13% in 2001 - 2010 (http://www.prweb.com/releases/industrial_enzymes/carbohydrases_proteases/prweb1569084.htm).

It is estimated that every year, about 1000 tons of lipases are added to approximately 13 billion tons of detergents (http://www.aukbc.org/beta/bioproj2/introduction.html).

Lipases at present account for less than 5% of the market that is expected to increase because of wide range of different applications (Vakhlu and Kour, 2006). China's demand for detergent has grown in the past decade and both production and demand will continue to grow in the next five years (Detergent Market Research, 2006).

Rohm in 1913 patented the use of pancreatic enzymes for laundry cleaning and marketed as a presoak detergent under the brand name ‘Burnus’. It was sold for about 50 years in the European markets. Rohm developed the first detergent, containing enzymes as well as the method for washing protein stained cloth in it. Protein degrading enzyme (trypsin) was extracted from pancreatic glands and developed a detergent formulation known as Pancreatin. Pancreatin preparation also contains enzymes having protease, lipase and amylase activity (http://home.intekom.com/pharm/alcon/polyzym.html). In 1959, a Swiss chemist Dr. Jaag developed a product called Bio-40 that instead of trypsin contained a protease obtained from bacterial. In 1965 - 1970, use and sale of detergent enzymes grew very fast, and slowed down as the workers started to develop allergies. In 1975, encapsulation of the granules of enzyme started (Jain, 2008).

BACTERIAL LIPASES IN DETERGENTS

Lipases for detergents are specifically selected on the basis of low substrate specificity, stability under alkaline (pH 10 - 11, 30 - 60°C) conditions, in the presence of surfactants (linear alkyl benzene sulfonates) and enzymes (proteases), constituents of many detergent formulations. Continuous screening and protein engineering leads to the discovery of lipases with the desired properties (Wang et al., 1995; Kazlauskas and Bornscheuer, 1998; Cardenas et al., 2001). Detergent enzymes should be cost-effective and safer to use. Bacterial lipases added to household detergents reduces or replaces synthetic detergents, which have considerable environmental problems (Jaeger et al., 1994).

Normally fat stains are not easy to remove at low temperatures using conventional detergents, therefore lipases are required which are active at lower temperatures and can be used in detergent formulations. The use of lipase active at low temperature in detergent formulation, reduces the energy consumption as well as the wear and tear of textile fibers (Feller and Gerday, 2003, Chaplin, 2004) and maintain the texture and quality of fabrics (Bjorkling et al., 1991). Lipases from *Candida* (Nishioka et al., 1990) and *Chromobacterium* (Minoguchi and Muneyuki, 1989) are also used in detergents.

Lipases which are stable and work at alkaline pH (8 to 11), suitable wash conditions for enzymated-detergent powders and liquids have good potential for use in detergent industry (Jaeger et al., 1994; Gerhartz, 1990; Hasan et al., 2006). Lipase produced by *Acinetobacter radioresistens* was found to be optimally active at pH 10 and showed stability in the range of pH 6 - 10; therefore, it has a greater potential to be used in the detergent industry (Chen et al., 1998). Hasan et al. (2007) reported 100% stability of lipase produced by *Bacillus* sp. FH5, at pH 10. This enzyme showed promising results when used in combination with different commercially available conventional detergents (Javed, 2007). *A. radioresistens* produced lipase having optimum activity at pH 10 and stability in the range of pH 6 - 10, and showed a great potential to be used in the detergent industry (Chen et al., 1998).

Extracelluar lipase produced by *Micrococcus* sp. ML-1 was commonly used in combination with various commonly used detergents, to enhance the removal of oily stains from various types of fabrics. Soybean oil-dye stain, followed by dye stain in mustard oil, groundnut oil and coconut oil were removed quite easily. The detergents; Surf, Nirma and Wheel, differed in the oil-dye removes potential stains from cotton, terrrycot and polyester (Neeru and Gupta, 2001). The ability of detergents to wash, remove fatty food stains and sebum from fabrics, is enhanced by addition of lipases (Andree et al., 1980; Hemachander and Puvanakrishnan, 2000).

SYSCO makes cleaning products for foodservice industry. Sysco® Action Suds with enzymes removes tough stains and makes the fabric white and gives brightness. This enzyme product has low-alkalinity and works with optical brighteners, soil re-deposition agents and color-safe bleach to give longer life to fabric. Sysco® enzyme grease and waste digester is a natural, multi-strain bacterial enzyme formulation, effective in both hard and soft water, unclogs drain lines and digests any accumulated grease and waste (Cleaning and sanitation Catalog, 2004).

Olin Corporation (Cheshire, UK) invented a detergent composition comprising the microbial lipase SD2, dodecylbenzene sulfonate and gelatin (Holmes, 1991). Novozymes has also marketed lipases (Lipex® and Lipolase ®), used in detergent industry (Novozymes report 2006). US Agricultural Research Service scientists obtained an enzyme from a bacterium isolated from shipworm glands that digests proteins in common laundry stains (Hardin, 1992).

Commercial detergent formulations with high-temperature optima have been produced from *P. mendocina* (Lumafast) and *Pseudomonas glumae* are used (Jaeger et al., 1994). In 1995, Lumafast and Lipomax, lipases from *P. mendocina* and *Pseudomonas alcaligenes*, respectively, were produced by Genencor International, AU-KBC Research Center, Life Sciences, Anna University, Chennai, India (http://www.au-kbc.org/beta/bioproj2/uses.htm). Gerritse et al. (1998) reported an alkaline lipase from *P. alcaligenes* M-1, capable of removing fatty stains when used in a washing machine.

European Patent Office (EPO) Solvay Enzyme Products, Inc. 1992-01-29/1990-07-25, is a nonionic and/or anionic detergent formulation that contains lipase from *Pseudomonas plantarii* (Bycroft and Byng, 1992).

Composition of a U.S. Patent 4,707,291, a detergent, comprise a mixture of an anionic and a nonionic detergent-active compound in combination with a lipase similar to the enzyme produced by *Pseudomonas fluorescens* IAM 1057, P. gladioli or *Chromobacterium viscosum* (Detergent formulations containing alkaline lipase, 1992). Lion has released an improved version of its powder type laundry detergent "Top" with two different acting enzyme cleansing agents (http://www.accessmylibrary.com/comsite5/bin/pdinventory.pl?pdlanding=1andreferid=2930andpurchase_type=ITManditem_id=0286-23617629).

FUNGAL LIPASES IN DETERGENTS

During 1980-1990, many different enzymes containing detergents were developed for softening of clothes. In 1988, Novo Nordisk developed a lipase produced by the fungus *Humicola*. Commercial detergent formulations with high-temperature optima have been produced from *Humicola lanuginosa* (Lipolase) (Jaeger et al., 1994). As the quantity of this enzyme was less than required for its commercial application, therefore, the yield of enzyme was increased by cloning the gene coding for this lipase in *Aspergillus oryzae*. This fungus produced increased quantity of the enzyme that can be used commercially in detergents(http://www.efbweb.org/topics/genetic/menu4_3.htm). The first commercial lipase, Lipolase, was introduced by Novo Nordisk In 1994. This enzyme was produced from *Trichoderma lanuginosus* and was expressed in *A. oryzae* (Hoq et al., 1985). They also produced a lipase containing detergent 'LipoPrime®'.

Laundering with lipase containing detergents is generally carried out in alkaline conditions; therefore, alkaliphilic lipases are preferred for example, lipase obtained from the *A. oryzae* (Gerhartz, 1990; Umehara et al., 1990). A presoak formulation was developed using an alkaline lipase produced by *Trichosporon asahii* MSR 54, used for removing oil stains at ambient temperature (Kumar et al., 2009).

ABS fungal lipase is used in a many sanitary and waste treatment liquid formulations. This product exhibits activities in a wide range of pH. Enzymatic methods are preferred to chemical methods in many sanitary applications such as grease trap applications; other applications include detergents, pre-spotters and industrial cleaning compounds (http://www.americanbiosystems.com/products/abs_fungal_lipaseL.php).

OTHER LIPASE CONTAINING DETERGENTS

SEBrite-L detergent containing lipase helps in removing cosmetics, sebum and fatty food stains from garments, salad oil, animal fat and butter, suitable to use at lower washing temperatures and neutral pH. Enzyme-containing detergents are also used in disinfecting and cleaning hard surfaces in food manufacturing plants and buildings, contaminated by bacteria and fungi. SEBalase-M and SEBrite-M detergents with protease and lipase enzymes, respectively, are used to effectively remove proteinaceous debris and fat lubricating oils from medical instruments (http://www.specialtyenzymes.com/detergents.shtml).

Lipases that deal with greasy soil on cloths leave residual "sick" odors on the cloth. Some detergent compositions are produced which have fragrances and have the ability to counter the bad odors due to lipase in detergent products (Yet2.com).

Enzyme containing laundry detergents marketed by Procter and Gamble (P&G) includes; tide, ivory snow, dreft, cheer, and era, perform well. The other benefits to consumers are better performance, the abilities to remove fatty stains at low wash temperatures, improve whiteness, soften fabrics, brighten their colors and perform in the presence of chemicals (bleach, builder, surfactant, etc.). P&G searches for microorganisms/ genes which can fulfill consumer need, e.g. an alkaline cold wash enzyme by a limited number of cycles of directed evolution is a major challenge in using mutagenically improved strain of microorganisms/a gene. Thousands of mutants have to be tested to find the best strain (http://www.scienceinthebox.com/en_UK/glossary/ Enzymes2_en.html).

Immobilized lipase (Patent # 6,265,191, issued 07/24/2001) is used on surfaces for easy removal of oil and to change the wettability of fabric surfaces. Lipase in detergents forms a fabric-lipase complex on the surface of cloth and removes oil stain and makes them resist denaturation in the presence of surfactants and deactivation by heat. This complex resists the removal of enzyme from the surface of fabric during washing and it remains active stains after drying of fabric at higher temperatures, during storage and wear of fabric. Lipase impedes the oil re-deposition during laundering of fabric. Oil hydrolysis by-products are removed during washing of fabric at a basic pH or in the presence of a surfactant (http://www.kstate.edu/tech.transfer/macc/LaundryEnzymes.htm).

Two laundry products (a liquid and a solid enzyme) by Gateway ProClean Inc. USA, breaks down soil into simpler forms which can then be easily removed. Lipase in these products remove fat-containing stains of frying fats, salad oils, butter, fat-based sauces, soups, human sebum or certain cosmetics (http://www.gatewayproclean.com/GatewayProCleanInc.USA).

During manufacturing beverages such as wine, fruit juice, beer and milk are commonly filtered through ultra- and nano-filters. These filters are fouled with protein, cellulose, starches, fats, and pectin residues and are cleaned and disinfected with caustic and chlorine. These can be effectively cleaned using enzyme-containing detergents like SEB-Prolase P or SEBalase-PB (protease), CelluSEB-T (carbohydrase), SEBamyl-B (alpha-amylase), SEBrite-L (lipase), and ExtractSEB-R (pectinase) (http:// www.specialtyenzymes.com/detergents.shtml).

Novozyme launched a low-temperature detergent enzyme suitable that can be used in the detergents usually used for hand-washing in developing countries like China and India. Another product, Polarzyme, can clean clothes in cold water, and also it has attractive price (Press Release, 2005).

Enzymes and detergents are used to clean medical instruments since the last two decades. Multiple enzyme/ detergent instrument cleaners can also remove the microbial biofilms (Enzymatic instrument cleaners in health care) (http://www.highbeam.com/doc/1G1-83061938.html).

Another common application of enzymatic detergents is cleaning of gastrointestinal endoscopes before disinfection. Misuses of enzyme detergents include failure to dilute the detergent, over dilution, use of expired detergent, inadequate exposure time, and failure to rinse the detergent properly from the instrument (http://www.highbeam.com/doc/1G1-83061938.html).

ISI enzymatic detergents contain the enzymes that can remove the stubborn organic materials. A pre-cleaning enzymatic foam developed by ISI, Hemaway enzymatic foam, is ideal for use in the immediate cleaning of surgical instruments. Another product by ISI, Hemaway Tri-Max contain three enzymes for enhanced cleaning and removing tough organic materials from surgical instruments (Hutchisson and LeBlanc, 2005). A unique enzyme formulation, Endozime® AW Plus, can rapidly remove fat, blood, starch, carbohydrates and protein from scopes and surgical instruments, cleans the most difficult instruments (that is, orthopaedic, laproscopic) and is safer to use on the most delicate (that is, ophthalmic, microsurgical) instruments. It is a mixture of enzymes (protease, amylase, lipase and carbohydrase), buffers and nonionic detergents (http://www.ruhof.com/CatalogProducts.asp?nProductsID=79).

Protease and lipase in the lens cleaning system considerably enhances the removal of proteinaceous and lipid material that accumulates on the contact lens (http://www.enzymetechnicalassoc.org/benefits_paper.pdf). POLYZYM® enzymatic cleaning tablets loosen organic deposits on the lens surface and remove it easily. Clear-Lens (TM) Lipo is also a lipase preparation available for cleaning contact lenses (http://www.novonordisk.com/ Reports/investors/annualreports/ar1997/eb/ prodintros.htm).

Besides increasing detergency, lipase component in detergents also prevents scaling. The cost of production of these enzymes is very high. This limitation may be overcomed by employing molecular techniques, thus enhancing the production of these enzymes at high levels and in purified form (Houde et al., 2004).

Table 3. Commercial enzymes used in detergent formulations and other applications.

Trade Names	Source organism	Manufacturer
Alkaline proteases/ subtilisins		
Alclase	*Bacillus licheniformis*	Novo Nordisk , Denmark
Savinase	Alkalophilic *Bacillus* sp.	Novo Nordisk, Denmark
Esperase	Alkalophilic *Bacillus* sp.	Novo Nordisk, Denmark
Liquanase		Novo Nordisk, Denmark
Everlase		Novo Nordisk, Denmark
Ovozyme		Novo Nordisk, Denmark
Polarzyme		Novo Nordisk, Denmark
Maxacal	Alkalophilic *Bacillus* sp.	Gist-brocades, Delft, The Netherlands
Maxatase	Alkalophilic *Bacillus* sp.	Gist-brocades, Delft, The Netherlands
Opticlean	Alkalophilic *Bacillus* sp.	Solvay Enzymes GmbH, Hannover, Germany
Optimase	Alkalophilic *Bacillus* sp.	Solvay Germany
Protosol	Alkalophilic *Bacillus* sp.	Advanced Biochemicals Ltd., Thane, India
Alkaline protease “Wuxi”	Alkalophilic *Bacillus* sp.	Wuxi Synder Bioproducers Ltd., China
Proleather	Alkalophilic *Bacillus* sp.	Amano Pharmaceuticals Ltd., Nagoya, Japan
Protease P	*Aspergillus* sp.	Amano, Japan
Durazym	Protein engineered variant of Savinase	Novo Nordisk, Denmark
Maxapem	Bleach-resistant, protein engineered variant of alkalophilic *Bacillus* sp.	Solvay, Germany
Purafect	Recombinant enzyme donor *Bacillus lentus* Expressed in *Bacillus* sp.	Genencor International Inc., Rochester, USA
Amylases		
BAN	*Bacillus amyloliquefaciens* Recombinant enzyme	Novo Nordisk, Denmark
Termamyl	Donor- *Humicola* sp. Expressed in *Aspergillus* sp.	Novo Nordisk, Denmark
Stainzyme		Novo Nordisk, Denmark
Duramyl		Novo Nordisk, Denmark
Maxamyl	Alkalophilic *Bacillus* sp.	Gist-brocades, Delft, The Netherlands
Solvay amylase	Thermostable *Bacillus licheniformis*	Solvay, Germany
Lipases		
Lipolase	Recombinant enzyme Donor-*Humicola lanuginosa* Expressed in Aspergillus oryzae	Novo Nordisk, Denmark
Lumafast	Recombinant enzyme Donor-*Pseudomonas mendocina* Expressed in *Bacillus* sp.	Genencor, USA
Lipofast	NA	Advanced Biochemicals, India
Cellulases		
Celluzyme	*Humicola insolens*	Novo Nordisk, Denmark
Endolase		Novo Nordisk, Denmark
Mannanase		
Mannaway		Novo Nordisk, Denmark

(Novozyme report, 2006; Kumar et al., 1998).

Extensive and continued research is going on to develop lipases, which will work under various suitable conditions as fat removers. There is a lot to be done to extend and increase the use of enzymes in detergents globally. Unfortunately, the enzyme detergents are not used much in developing countries which are comparatively more hot and dusty, which requires frequent washing of clothes. Enzymes such as glucose oxidase, lipoxygenase and glycerol oxidase can generate hydrogen peroxide *in situ*. Peroxidases may help enhance the bleaching efficacy of peroxide (Chaplin, 2004).

There is still a need of extensive and continuous screening for new microorganisms that have the ability to produce lipolytic enzymes with enhanced activities at low temperature, have stability at high temperatures, active at alkaline conditions, higher stabilities in the presence of other additives usually present in a detergent. The most important thing among all is to create awareness in the developing world about the 'pros' of enzyme detergents and 'cons' of the chemical detergents they are continuously exposed to.

Developments in enzymology and recombinant DNA technologies, led to the current era of bioengineered enzymes. Many industries of the world are involved in the manufacture and trade of enzymes used in detergents. Presently, three major giants, Novo Nordisk (Novozymes A/S, North America), Genencor International Inc. (California, USA) and DSM N.V. Enzymes (Netherlands), are leading production of industrial enzymes. Novo Nordisk (Novozymes A/S) markets a range of enzymes for various industrial purposes (Table 3) (Kumar et al., 1998), mostly derived from microorganisms, such as various strains of alkalophilic *Bacillus* sp. or genetic and/or protein engineered organisms (Kumar et al., 1998). They also produce enzymes, other than lipases, which are used in detergent industry. Many of these enzymes are produced by fermentation of genetically modified microorganisms (Table 3).

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