The Role of Enzymes in Modern Detergency

Hans Sejr Olsen* and Per Falholt

Enzyme Development & Application, Enzyme Business, Novo Nordisk A/S, DK-2880 Bagsvaerd, Denmark

ABSTRACT: Enzymes have effectively assisted the development and improvement of modern household and industrial detergents. The major classes of detergent enzymes—proteases, lipases, amylases, and cellulases—each provide specific benefits for application in laundry and automatic dishwashing. Historically, proteases were first to be used extensively in laundry detergents. In addition to raising the level of cleaning, they have also provided environmental benefits by reducing energy consumption through shorter washing times, lower washing temperatures, and reduced water consumption. Today proteases are joined by lipases and amylases in improving detergent efficacy especially for household laundering at lower temperatures and, in industrial cleaning operations, at lower pH levels. Cellulases contribute to overall fabric care by rejuvenating or maintaining the new appearance of washed garments. Enzymes are produced by fermentation technologies that utilize renewable resources.

JSD 1, 555–567 (1998).

KEY WORDS: Amylases, automatic dishwashing, cellulases, enzymes in detergents, industrial cleaning, laundry, lipases, proteases, storage stability, wash performance.

The field of enzymology is an important branch of bioprocess technology. Developments in submerged fermentation processes and genetic engineering have resulted in great progress in the manufacture of industrial enzymes.

Enzymes find use as functional ingredients in detergents and contribute to cleaning of laundry and dishes in an efficient, environmentally mild, and energy-saving manner. This largest of all industrial enzyme applications began slowly in the early 1930s, based on Röhm's 1913 patent of the use of pancreatic enzymes in presoak solutions (1). Pancreatic enzymes include protease (trypsin and chymotrypsin), carboxypeptidase, α-amylase, lactase, sucrase, maltase, and lipase. Thus, with the exception of cellulases, the foundations were already laid in 1913 for the commercial use of enzymes that continues to be important today.

Today the most widely used industrial enzymes are hydrolases, which remove soils based on proteins, lipids, and polysaccharides. Cellulolytic enzymes are another class of hydrolases that provide fabric care through selective reactions not previously possible on fabrics. Research is currently underway into the possibility of using redox enzymes—oxidases or peroxidases—for bleaching colored components (2).

To support the 18–19-million -ton global annual market for laundry and dishwashing detergents (3), the worldwide consumption of detergent enzymes amounted to *ca.* U.S. $500 million in 1995 (4). The principal producers of enzymes are Novo Nordisk A/S (headquartered in Bagsvaerd, Denmark) and Genencor International Inc. (headquartered in Rochester, NY), serving the market with more than 90% of the total volume of detergent enzyme products.

THE ROLES PLAYED BY ENZYMES IN DETERGENTS

Over the years, enzymes have been an important factor in the development and improvement of detergent products. In laundering, dishwashing, and in industrial and institutional (I&I) cleaning, they have contributed to shortening washing times, reducing energy and water consumption by lowering washing temperatures, providing environmentally friendlier wash-water effluents, lowering pH levels in wash liquors, and providing fabric care. Enzymes themselves are environmentally attractive since they are derived from renewable sources. They are also highly space-efficient and are thus of particular advantage in concentrated detergent formulations. Developments in genetic and protein engineering have contributed to long-needed improvements in the stability, economy, specificity, and overall potential of industrial enzyme products.

*To whom correspondence should be addressed at Enzyme Development & Application, Enzyme Business, Novo Nordisk A/S, Novo Allé, DK-2880 Bagsvaerd, Denmark. E-mail: hso@novo.dk

TABLE 1
Consumption During the Washing of Fabrics (European data) (Refs. 5–9)

Consumption/kg dry fabric	1943–44 (manual)	1965 (machine)	1975 (machine)	1985 (machine)	1990–95 (machine)
Chemicals (g)					
Conventional	109	*ca.* 50	*ca.* 50	38–48	32
Compact	0	0	0	0	16–21
Water (L)	31	37	31	21	10–12
Energy for heating (kWh)	1.45 (to heat water)	0.6	0.4	0.25	0.2
Energy for motors and pumps (kWh)	0	0.4	0.2	0.15	0.1

Detergent mechanism based on enzymes. From an enzyme point of view, detergents on the international market contain principal ingredients that operate by almost identical detergency mechanisms. Soil and stains are removed by mechanical action assisted by surfactants, builders, and enzymes. Alkaline proteases, amylases, or lipases in heavy-duty detergents hydrolyze and solubilize substrate soil attached to fabrics or hard surfaces (e.g., dishes). Cellulases clean by hydrolysis of glycosidic bonds which removes particulate soils attached to cotton microfibers. Key effects of cellulases are to soften and improve the color brightness of worn textile surfaces. Surfactants lower the surface tension at interfaces and enhance the repulsive force between the original soil, enzymatically degraded soil, and the fabric. Builders act to chelate, precipitate or ion-exchange calcium and magnesium salts, to provide alkalinity, to prevent soil redeposition, to provide buffering capacity, and to inhibit corrosion.

Laundry. Over the past 35 yr the role of enzymes in detergents has changed from one of a minor additive to becoming a key ingredient. The largest application in which the use of enzymes has grown is household laundry.

Before the introduction of modern detergent formulations, soap and sodium carbonate were the principal detergent constituents, and the cleaning effect in laundering was dependent mainly on mechanical action. Table 1 shows a comparison of the consumption of chemicals, water, and energy over the last 50 yr. The advent of washing machines brought a considerable reduction in the consumption of chemicals over that in manual washing. In the period of 1967–1970 (5), a reduction in energy consumption of about 40% was brought about by the successful introduction of enzymatic detergents. In 1969, at least 50% of European detergents were offering "biological attack" (proteolytic enzymes), which resulted in the energy-savings shown (3). The move away from 95°C washes to 60°C washes, and later to 40°C washes became more common. Furthermore, prewashes and overnight soaking could be eliminated. The additional energy reduction resulted from a combination of more diverse enzyme ingredients (cellulases, amylases, and lipases), more mechanically efficient washing machines (jet-spray and cascade systems), better-managed machine electronics, and compact detergents. In Japan, proteolytic enzymes were introduced by Lion Corp. in 1979, and in the United States the resurrection of enzymes began in the 1980s after health problems with factory workers had arrested the initial introduction in 1971 (3).

Throughout the period covered in Table 1, the development of new ingredient combinations (builders and bleach systems) and machines was optimized in conjunction with the improved cleaning properties offered by enzyme systems. At the same time, the development of new enzyme systems was influenced by the new compositions of detergent mixes.

A comparison of calculated cost distributions for washes from 1943 (5) and 1997 (10) reflects many dramatic changes in society and also indicates the benefits of new developments. Table 2 shows the annual cost of washing on a family basis for the 2 yr. Interestingly, the proportion of the costs attributable to the detergents has not changed. The energy part of the washing costs has been reduced significantly, but the costs for water have increased considerably due to the increasing shortage of clean water and heavy taxation of environmental resources.

Table 2 explains, to some degree, the developments seen in laundry technology over the years from 1943 to 1997. New detergent compositions and enzymes made possible a lowering of washing temperatures and hence of energy consumption. Thus it is easy to understand the current trend of washing machine design heading in the direction of reducing water consumption.

Dishwashing. Automatic dishwashing machines have been in use for almost as long as washing machines but not as widely. Today, automatic dishwashers are found in 50–60% of households in the United States, West Germany,

TABLE 2
Yearly Costs (DKK—Danish crowns) and Cost Distribution for Washing in a Danish Model Family

	1943 (Ref. 5)		1997 (Ref. 10)	
Component	DKK	% distribution	DKK	% distribution
Electricity	14.5	12	368	27
Fuel	47	39		
Water	9.7	8	415	31
Detergents	48.8	41	565	42

Norway, Sweden, and Denmark, and in *ca.* 30% of the households in France and Italy, while in other European countries like The Netherlands and Spain distribution is less than 20% (4,11). In Japan, automatic dishwashers are found in fewer than 5% of domestic households (4).

In manual dishwashing, the removal of food residues is mainly dependent on surfactants and mechanical energy. In machine dishwashing, cleaning performance is dependent on the complete detergent, the temperature, and mechanical energy. Mechanical energy is delivered by the pump, which circulates the wash water. The chemical contribution of the detergents must therefore be high. The most severe cleaning problem is encountered when food items coat tableware—porcelain plates, cups, cutlery, and glasses—with a film. Food may be dried-on, baked-on, or even burned-on and forms complex compounds from carbohydrates, proteins, and lipids. Vegetable pigments or tea scum consisting of Ca-tannin compounds—in brewed tea—also demand considerable attention. In addition, combinations of polyphenols and carbohydrates—from coffee—are considered difficult. Thus, dishwashing is considered successful if tea and coffee stains have been completely removed. Complex compounds based on proteins, polysaccharides, and lipids require hydrolytic treatment that causes swelling and degradation of the soil and thus releases it from the surface. Bleach systems that oxidize polyaromatics are also needed to remove fruit, coffee, tea, wine, and vegetable stains.

The combination of short washing times, high temperature, and the need for avoiding attack on dishware calls for chemicals with special properties. Highly alkaline-oxidizing chemicals are needed to ensure the hydrolysis and oxidative hydrolysis of food compounds and to disperse them into the washing liquor without foaming. Effective binding of hardness ions to prevent lime deposits and protective agents for glass and on-glaze decorations are also needed.

Before enzyme products were introduced in Europe in 1990, automatic dishwashing detergents (ADD) were formulated on the basis of strongly alkaline, strong complexing agents [sodium triphosphate (20–30%), sodium metasilicate (40–70%), and sodium carbonate, 0–10%)] oxidizing compounds, usually di- or trichloroisocyanurate (1–3%), and nonionic surfactants (0–2%; pH in 1% solution = 12.0–12.5; typical dosages = 60–80 g per wash.

At a pH of 12–13, rinsing to remove chemicals must be very effective. This is the reason why large volumes of water were used in dishwashers in the past. Although glass is chemically reasonably resistant, its silicate structure is destroyed by hydrolytic cleavage of Si–O–Si bonds under highly alkaline conditions, resulting in a cloudy surface appearance (12). Disilicates were tested and found to be effective in preventing glass weight loss (12).

Over the last 20 yr, water consumption was reduced from 60 to 15 L per dishwashing cycle, detergent consumption from 80 to 30 g per wash, and energy consumption from 2.8 to 1.5 kWh (13). Chlorine or hypochlorite-releasing bleach compounds (e.g., chloroisocyanurates) have been deemed essential to remove certain stains, e.g., tea stains in cups.

In the United States, most of the ADD market is still based on the traditional chlorine-type ADD although some enzymatic products have been available for a number of years. Efforts to introduce enzymatic low-alkaline ADD in the United States have been ineffective to date. Unlike European dishwashing machines, their U.S. counterparts lack both an ion exchange water softener and a built-in water heater. This places the main burden of cleaning on the detergent and does not favor mild detergent formulations (3).

As with laundry detergent formulations, the introduction of new ADD with enzymes (amylases and proteases) has made it possible to replace harsh chemicals like bleaches and alkalis. Since hypochlorite in low concentrations will degrade enzymes, peroxygen/activator bleach systems based on the combination of sodium perborate or percarbonate with activators (TAED, etc.) that oxidize polyaromatics from fruit, coffee, tea, wine, and vegetables are used instead. At the reduced pH in chlorine-free ADD, the hydrolytic swelling needed to remove food residues from surfaces is supported by the action of respectively starch- and protein-degrading enzymes. These systems are widely used in Europe in compact ADD powders and tablets.

I&I laundry. The application of enzymes in I&I laundry, institutional dishwash, and cleaning in the food industry has grown substantially (14).

Textiles treated under I&I laundry conditions include hospital bed linen, coats and uniforms, workers overalls and white coats from slaughterhouses, and tablecloths and napkins from hotels and restaurants.

Machines used can be washer–extractors having capacities of 120 kg dry cloth per load or they can be tunnel washers with several compartments and capacities of 550 kg per hour.

In washer–extractors a prewash at low temperature (e.g., 35°C) and pH = 11 permit the use of enzymes within a time period of 8–10 min. The main wash is usually carried out for 15 min at 85ºC, pH = 11.5. In order to permit enzymes to work, this temperature can be reduced to 60°C. Usually, the last bleach process is a treatment with active chlorine in cold water.

In tunnel washers the holding time in each compartment is 4–5 min. During prerinse and first wash, enzymes may be added followed by bleaching steps with chlorine or hydrogen peroxide.

Institutional dishwashing. In a traditional institutional dishwashing process, the dishware is placed vertically in trays and then washed with 60°C wash water including 0.5–5 g/L of a detergent containing sodium hydroxide, potassium hydroxide, phosphonate, polycarboxylate, and sodium EDTA. In the absence of enzyme, the pH normally is 13–14. The washing process with detergent normally lasts 60–90 s followed by a rinsing process lasting 20 s at

75–85°C. The dishware is then dried either in the machine or outside.

In large institutional dishwashers, trays are placed on a conveyor belt which pulls the dishware through the above treatments in separate chambers.

In institutional dishwashing processes, the use of enzymes provides cleaning effects as in household usage. With a low-alkaline detergent system without bleach, a preferable temperature range is 45–65°C. Very often a two-component liquid detergent system is used. One component is a liquid detergent containing, e.g., sodium hydroxide, a calcium binder like phosphonate, amphoteric or nonionic surfactant, and polycarboxylate. Typical dosages may be about 2–4 g/L of wash liquid, resulting in a pH of 8.5–9 of the cleaning solution. The second component may be starch- and protein-degrading enzymes. The enzymes are dosed from a separate container directly into the washing chamber.

Cleaning in the food industry. For many years, proteases have been used as minor functional ingredients in formulated detergent systems for cleaning reverse-osmosis membranes. The requirements and dosages of enzymes are similar to those for laundry detergents and ADD. Enzymes are now also used for cleaning processes in the dairy (15) and in the brewing industries for cleaning microfiltration and ultrafiltration membranes (16). Enzymatic cleaning is also being used for membranes used in fruit juice processing (17). Both organic and inorganic soils are removed by cleaning-in-place (CIP). In CIP plants, the cleaning medium is normally 0.5–1% NaOH (± surfactants and EDTA) at 75–85°C followed by rinsing and 0.5–1% HNO_3 (± surfactant) or "acid sulfate."

In dairies, the most difficult soil to remove from hard surfaces is "burnt-on milk" from the fouling of heating surfaces, e.g., on heat exchangers or evaporator tubes. This substrate is a kind of gel assumed to be a complex formed by Maillard-type reactions between protein, lactose, and fat. Milkstone (a calcium phosphate protein complex) may also be involved. For this application, an enzymatic CIP system based on protease and lipase has proven effective for cleaning spiral-wound ultrafiltration modules that are very sensitive to harsh chemicals and are generally difficult to clean with traditional cleaning products (18).

The combination of protease and lipase in a pure enzyme cleaning process of plate heat exchangers used for high pasteurization of sweet milk surfactants (soaps) leads to the *in situ* formation of emulsifiers and foaming agents from the fat and proteins. This is illustrated schematically in Figure 1.

Regulatory aspects and quality assurance of detergent enzymes. In most countries, the regulatory status, classification, and labeling are determined in accordance with existing schemes for chemicals. Many enzyme types are listed on chemical inventories, e.g., EINECS in the European Union and TSCA in the United States. In some cases, enzymes are considered natural substances exempt from listing or are regulated by specific biotechnology products legislation.

The Association of Manufacturers of Fermentation Enzyme Products has defined Good Manufacturing Practice for microbial food enzymes. This practice is generally followed for detergent enzymes also. The most important element is to ensure a pure culture of the production organism.

Commercial enzyme products are usually formulated in aqueous solutions or processed to dry nondusting granulates.

Safety. Like many other proteins foreign to the human body, enzymes are potential inhalation allergens. Inhalation of even small concentrations of a foreign protein in the form of dust or aerosols can stimulate the body's immune system to produce antibodies. In some individuals, increased concentrations of antibody–enzyme protein complexes can trigger increased concentrations of histamine. The latter compounds can cause hay fever-like symptoms such as watery eyes, running nose, and a sore throat. When exposure ceases, these effects cease also.

Enzymes must be inhaled in order to present a risk of causing sensitization, which may lead to an allergic reaction. Working environments where enzymes are used are therefore subjected to extensive monitoring to confirm that

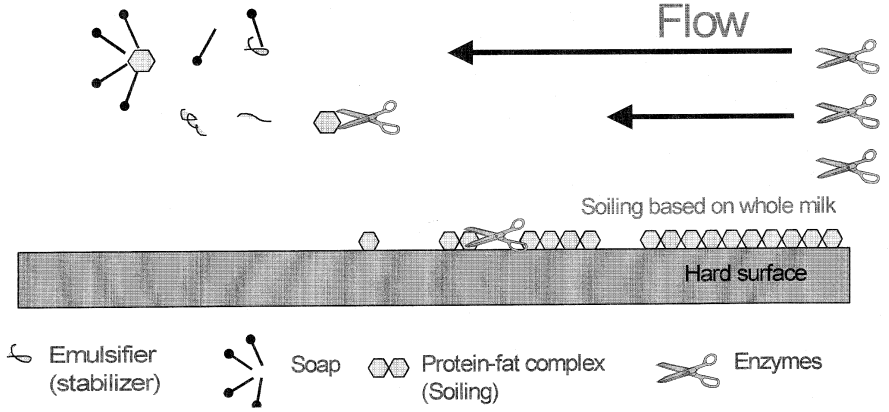


FIG. 1. *In situ* cleaning effects of protease + lipase.

threshold limit values (TLV) for atmospheric enzymes are not exceeded. In many countries, the TLV for enzymes is based on the proteolytic enzyme subtilisin and is stated 0.00006 mg/cu.m of pure crystalline subtilisin in air (19).

Successful experience in controlling enzyme exposure and protecting workers' health has been reported by Procter & Gamble (20). Already in 1971 a National Research Council report had concluded that consumers did not develop respiratory allergies from the use of enzyme-containing laundry products (21). Further studies over the years have confirmed that enzyme-containing laundry and dishwashing detergents are safe for consumer use.

DETERGENT ENZYMES IN USE

Manufacture. The production of microbial enzymes represents a significant part of today's industrial biotechnology. The most significant industrial enzyme products are produced by aerobic batch or continuous fermentation in fermentors with volumes ranging from 20–1,000 m^3. Fermentation processes are carried out on sterilized nutrients based on renewable raw materials like corn starch, various sugars, and soy grits in the presence of various added salts to provide basic elements.

Most industrial enzymes are secreted from selected microorganisms into the fermentation medium in order to break down the carbon and the nitrogen source.

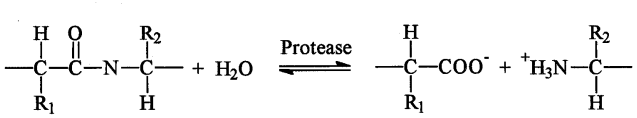
The enzyme activity is harvested from the fermentation broth by removing insoluble products and the biomass produced, usually by filtration or centrifugation. Enzyme in the solution is then concentrated by evaporation, membrane processes, and crystallization. Depending on the specific application, the enzyme product is posttreated. For solid products, such as detergent powders, nondusting granulated enzyme formulations are manufactured (22). For use in liquid detergents, liquid or encapsulated formulations are easy to handle and dose in a factory.

Proteases. Proteases are the most widely used enzymes. In laundry detergents, protein stains such as grass, blood, egg, and human sweat are removed through proteolysis. In ADD, proteases secure the removal of proteinaceous food films, which are a particular problem with glassware and cutlery. Proteases are classified according to their source of origin (animal, plant, microbial), their catalytic action (endopeptidase or exopeptidase), and the nature of the catalytic site (active site). They are characterized by common names and trade names, typical pH ranges, and preferential specificity. Based on a comparison of active sites, catalytic residues, and three-dimensional structures, four major protease families are recognized: serine, thiol, aspartic, and metalloproteases. The serine protease family contains two subgroups: chymotrypsin-like and subtilisin-like. The latter is the most important group for detergent applications.

Examples of thiol (or cysteine) proteases are plant proteases from papain latex (Papain), from pineapple stem (Bromelain), and from fig latex (Ficin). These enzymes are not used in detergents.

The characteristics of a number of commercial enzyme products used for industrial detergents or food protein products are shown in Table 3. The enzymes Alcalase®, Esperase®, Savinase®, and Trypsin are serine proteases, while the enzyme called Neutrase® is a metalloprotease with Zn^{2+} in its active site. Furthermore, this enzyme is stabilized by Ca^{2+}. Durazym® and Everlase™ are protein-engineered variants of Savinase® and mainly used in detergents containing bleach. The development of selected proteases has been guided by factors like oxidation stability, temperature optima, autoproteolysis, denaturation, water hardness, alkalinity, and economy. Thus detergent proteases have been categorized in types for uses within alkaline, high alkaline, high bleach stability, and cold-water applications (3). Table 4 is an attempt to show the status of today's merchant market. Practical selections of proteases for specific detergents are made on the basis of performance tests and stability tests carried out under certain standard conditions chosen in conjunction with formulators.

Protein hydrolysis in detergency. Proteases catalyze the hydrolytic cleavage of the peptide chain, as shown for reactants and products in Scheme 1.



SCHEME 1

TABLE 3
Some Commercial Proteolytic Enzymes (Novo Nordisk A/S, Bagsvaerd, Denmark)

Product names	Microorganism or other origin	State of product	Practical application range (pH)	Practical application range (°C)
Alcalase®	*Bacillus* spp.	Liquid or granulate	6–10	10–80
Esperase®	*Bacillus* spp.	Liquid or granulate	7–12	10–80
Everlase™	*Bacillus* GMO (engineered)	Liquid or granulate	8–11	15–80
Savinase®	*Bacillus* GMO	Liquid or granulate	8–11	15–75
Durazym®	*Bacillus* spp. GMO (engineered)	Liquid or granulate	8–11	15–70
Neutrase®	*Bacillus* spp.	Liquid or granulate	6–8	10–65
Protamex™	*Bacillus* spp.	Microgranulate	6–8	10–65
Flavourzyme™	*Aspergillus* spp.	Liquid or granulate	4–8	10–55
Trypsin	Pancreas	Granulate	7–9	10–55

TABLE 4
Merchant Market Detergent Proteases[a]

Producer	Alkaline	High alkaline	High bleach stability	Cold water
Genencor International (Rochester, NY)	Maxatase Optimase	Purafect Maxacal Opticlean	Maxapem Purafect OxP Opticlean plus	Properase
Novo Nordisk A/S	Alcalase®	Esperase® Savinase®	Durazym® Everlase	New developments

[a]See Table 3 for other producer's location.

The most important parameters for the hydrolysis reaction are surface-available substrate S (percentage protein for the reaction), E/S (enzyme–substrate ratio in activity units per kg protein), pH, reaction time, and temperature. Together with the specificity and properties of the enzyme itself, these parameters are responsible for the course of reaction on a given protein stain. The quantitative criterion for a proteolytic reaction is the degree of hydrolysis (DH) or hydrolytic activity (HA). DH is calculated by determining the number of peptide bonds cleaved and the total number of peptide bonds in the intact protein molecule. HA is here defined as milliequivalent (meq) NaOH consumed in a pH-stat/mass of substrate for a given time.

The DH of enzyme-treated protein is proportional to the molecular size of degraded proteins. Therefore measurement of the DH is important. Only in this way is it possible to optimize the reactions and to study the mechanisms in detail (23). For laundry or dishwashing applications, no estimates of this parameter have been reported, presumably because the substrate concentration is very low and difficult to measure.

However, when sufficient substrate is available, the relatively simple pH-stat technique can be applied directly as an analytical tool during the reaction. For the standard test textile swatches, EMPA 116 (cotton) or EMPA 117 (cotton + polyester), containing blood, milk, and carbon black, Figures 2 and 3 show examples of hydrolytic activity as a function of time. Different detergents, enzyme dosages, and temperatures were tested. The protease is added in dosages relevant to the application, but Figures 2 or 3 do not, as such, represent state of the art with regard to maximal performance under Japanese or European washing conditions. The reaction is carried out at fixed temperature in a 1-L reaction vessel held under an N_2 atmosphere with sufficient stirring to keep the swatches floating. The pH-stat technique can be used as a quick and nonlabor-intensive method for evaluating the effects of some washing parameters. When a simultaneous solubilization of protein occurs as an effect of the protein hydrolysis, measurements of soluble nitrogen can be made. After rinsing and drying, the reflectance can be measured (see Washing performance evaluations—laundry section). These trials cannot fully substitute for machine washing trials because mechanical effects and temperature profiles are different, and the effects of rinsing are not measured.

Some kinetic aspects of protein hydrolysis. The pH-stat technique was utilized to study the proteolytic degradation of hemoglobin in solution and to understand the reaction mechanism (24).

In the discussions of initial proteolysis, Linderstrøm-Lang (25) referred to the reaction of a protease on native hemoglobin molecules as "one-by-one," indicating that a particular protease molecule degraded one substrate molecule at a time. No appreciable amounts of intermediary products were present. The reaction mixture consisted of native proteins and end-products only.

In another reaction mechanism referred to, the native protein molecules were rapidly converted into intermediary forms, which degraded more slowly to end-products ("Zipper reaction").

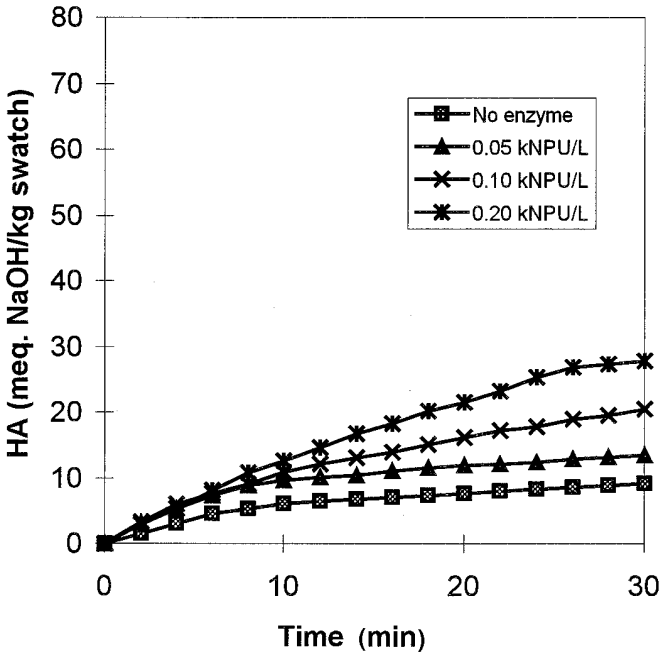


FIG. 2. Example of a pH-stat trial on EMPA 116 swatches—Japanese detergent. HA, hydrolytic activity. Reaction conditions: temperature, 15°C; concentration, 6.5 g EMPA 116 in 800 g wash; detergent, 0.67 g/L Japanese detergent, pH 10.5; water hardness, 3° dH (German degrees hardness); enzyme, Savinase 12 T; activity dosages, 0–0.20 kNPU/L, where kNPU is the proteolytic activity in *k*ilo *N*ovo *P*rotease *U*nits measured as the hydrolysis rate of dimethyl casein and compared with a standard reference protease preparation.

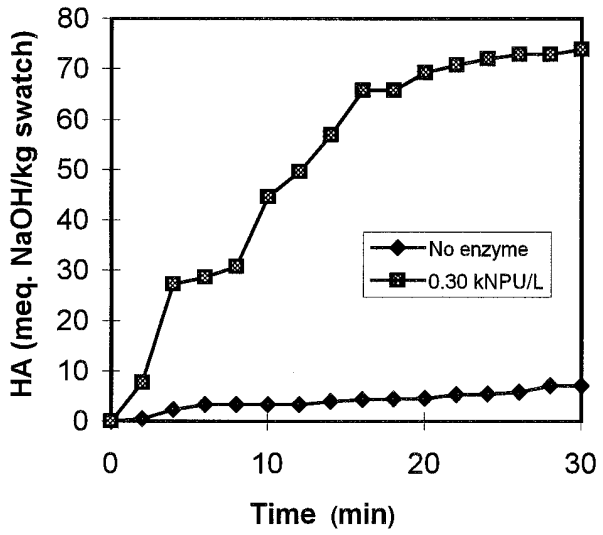


FIG. 3. Example of a pH-stat trial on EMPA 117 swatches—European detergent. Reaction conditions: temperature, 40°C; concentration, 6.5 g EMPA 117 in 800 g wash; detergent, 4 g/L European (bleach-containing) detergent, pH 9.8; water hardness, 18° dH; enzyme, Savinase 12 T; activity dosage, 0–0.3 kNPU/L. See Figure 2 for abbreviations.

In Figure 4, the slight bending in the hydrolysis curve at low DH values indicates a thorough initial degradation to small peptides (a zero-order reaction). This degradation is close to the ideal one-by-one reaction. Hydrolysis proceeds rapidly with little decrease in velocity to the stage where the substrate concentration is so low that first-order kinetics take over.

Proteolysis of proteins improves cleaning of fibers and hard surfaces by increasing the solubility of soils, promoting emulsification, foaming properties, reducing surface tension and redeposition of degraded protein material.

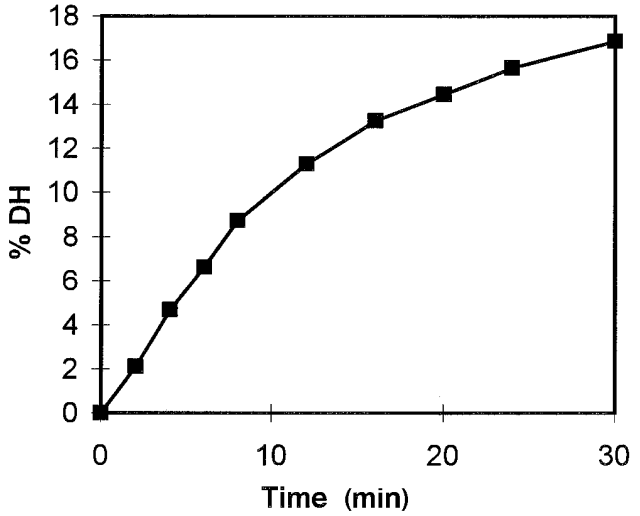


FIG. 4. The pH-stat hydrolysis curve for a hemoglobin in an automatic dishwashing detergent with Savinase. DH, degree of hydrolysis. Reaction conditions: temperature, 50°C; concentration, 0.5% protein (N × 6.25); detergent, 4.5 g/L European dishwashing detergent (pH 10.3); enzyme, Savinase 16 L; activity dosage, 0.75 kNPU/L. See Figure 2 for other abbreviation.

Protease inhibitors. In detergent powders where proteases are granulated and encapsulated, all enzymes are stable in the absence of water. In liquid detergents [more than 40% of the U.S. market (4)], the aqueous environment is inimical to enzyme stability and inhibitors are necessary to prevent autodigestion of the proteases themselves or to prevent the degrading of other enzymes. Polyols such as glycerin, propylene glycol, and polyethylene glycol have been used in combination with boric acid to inhibit protease activity and reduce autodigestion (4,26). Borate works as a reversible protease inhibitor by interacting with groups in the active site of the enzymes. Once in the wash water, these inhibitors are diluted and enzyme activity increases (4). Protein hydrolysate products and amino acids are also known to be able to stabilize proteases by binding to the active site of the enzyme (27).

Amylases. Native starch is only slowly degraded by α-amylases. Gelatinization and swelling are needed to make the starch susceptible to enzymatic breakdown. For most foods, various degrees of gelatinization result from cooking. Therefore, in detergents for laundry and automatic dishwashing, amylases facilitate the removal of starch-containing stains, e.g., pasta, potato, gravy, chocolate, and baby food. Amylases also prevent swollen starch from adhering to the surface of laundry and dishes that may otherwise act as a glue for particulate soiling. Complexes or reaction products between protein, starch, and/or fat are usually found in prepared foods. In such cases, enzyme synergy effects make it possible to remove soil even more efficiently than with single enzyme systems (22). If the food processes have not exceeded the starch gelatinization temperature, the starch may be in the form of partly or nongelatinized granules, or it may be partly retrograded. Such starch may be amorphous and usually difficult to remove from surfaces without cooking. Presence of an amylase renders cooking superfluous.

Industrially important α-amylases are made from *Bacillus* species and *Aspergillus* species. Table 5 shows some application conditions and a few comparative characteristics for amylases.

During enzymatic liquefaction, the α-1,4-linkages in starch (amylose and amylopectin) are hydrolyzed at random, thus reducing the viscosity of the gelatinized starch and increasing the solubility of attached starch, which is converted to water-soluble dextrins and oligosaccharides. Therefore, Termamyl®, Duramyl®, and BAN are often referred to as "liquefying amylases." Fungamyl is a fungal exo-amylase, which also hydrolyzes the α-1,4-linkages in liquefied starch. A prolonged reaction results in the formation of large amounts of maltose. Fungamyl is relevant in detergents for use at low pH levels in industrial cleaning tasks. Duramyl® was developed to achieve increased oxidation stability in the humid environment of detergent powders containing moisture (28). In practice, amylases

TABLE 5
Some Commercial Starch-Degrading Enzymes[a]

Product name	Microorganisms	State of product	Practical application range (pH)	Practical application range (°C)
Termamyl®	*Bacillus* spp. (GMO)	Liquid or granulate	6–11	25–100
Duramyl®	*Bacillus* spp. (GMO)	Liquid or granulate	6–10	25–100
BAN	*Bacillus* spp.	Liquid or granulate	5–8	15–90
Fungamyl®	*Aspergillus* spp.	Liquid or granulate	4–7	15–60

[a]GMO, genetically modified organism.

for detergent applications are selected on the basis of performance and stability tests in specific detergents.

Gelatinized starch may form a film on fabric that can result in an increased pick-up of particulate soil after washing (29). Starch stains, combined with particulate soiling, are more difficult to remove than starch alone. As a result white laundry items turn increasingly gray after repeated wash cycles, an effect that has been demonstrated by adding about 0.5 g starch/kg cotton fabric (30). Starches may react differently depending on their amylose content, which is thought to be the film-forming component of the starch. Film formation is favored under European conditions where the temperature may be closest to the starch gelatinization temperature. In laundry detergents, amylases may maintain or even contribute to increased whitening of dingy fabrics (29) and inhibit the graying of white fabrics resulting from a combination of starch and particulate soiling (29,30).

Cellulases. Cellulases cleave β-1,4-glucosidic bonds in cellulose and operate directly on the natural cotton fibers or cotton/flax blends and on the cellulose portion in synthetic fibers. This enzyme class is divided into endo-cellulases (endo-glucanase = EG) and exo-cellulases (cellobiohydrolase = CBH). Table 6 shows some application conditions and a few comparative characteristics for cellulases.

Celluzyme® and Carezyme® (Novo Nordisk) are "color clarification cellulases," which are applied in detergents to make cotton fabrics regain and maintain clear colors, a smooth surface, and softness. Cellulases provide these effects by shaving off the fuzz and pills of cotton fibrils that are generated on the fabric by normal wear and washing. Cellulases are unique in providing these effects.

The cellulase molecule is composed of up to three types of functionally different domains: the core, which is a large, spherical, catalytic domain; a linker, which is an elongated and flexible spacer; and a small spherical cellulose-binding domain (CBD) (Fig. 5).

Some cellulases consist of a core domain only, others comprise a core domain plus one or two linkers + CBD extending from either the C-terminal or the N-terminal of the core. The nature of the core determines catalytic properties such as endo-activity vs. exo-activity, substrate specificity, and the type of reaction products that are formed. The presence of a CBD is of particular importance for binding on insoluble and crystalline cellulose and for hydrolytic effects (31). Both EG and CBH can contain linkers and cellulose-binding domains. The cellulase products in Table 6 are composed as follows: Celluzyme®: A complex mixture of at least seven cellulases: *Humicola* CBH I, CBH II, EG I, EG II, EG III, EG V, and EG VI; and Carezyme®: mono-component *Humicola*. These cellulases can be used in bleach-containing detergents and are used in a number of "Color" and compact detergent powders.

Extremely high dosages of "color clarification cellulases" can inflict fabric damage in some cotton products after repeated launderings. Damage may appear as loss of fabric strength and excessive softening of the mechanically exposed parts of laundry items, such as hems and edges. These effects may be eliminated by balancing the dosage to manage the desired benefits. Application tests include small-scale laundering in Terg-O-tometers and full-scale multicycle laundering in commercial washing machines (32).

Lipases. Because of their strong hydrophobicity, fats and oils (triglycerides) are difficult to remove from laundry at low temperatures. Lipases hydrolyze triglyceride to more hydrophilic mono- and diglycerides, free fatty acids, and glycerol. These hydrolysis products are all soluble in alkaline conditions. At pH >8 the hydrolysis reaction may be favored by small amounts of free Ca ions due to the formation of Ca soap, although lipases are effective also at low free calcium levels.

The first commercial lipase for detergent application was Lipolase®, which was introduced in 1988 and used at once in Japanese detergents. Leading brands in the United States and Europe included lipase from 1990/91 (33). Lipolase was first isolated from the fungus *Humicola lanuginosa* from where the genetic coding for the lipase was trans-

TABLE 6
Cellulose-Degrading Enzymes for Detergents[a]

Product name	Microorganisms and enzyme type	State of product	Practical application range (pH)	Practical application range (°C)
Celluzyme®	*Humicola* spp. 5 EG + 2 CBH	Liquid or granulate	4–10	25–70
Carezyme®	*Humicola* spp.	Liquid or granulate	5–10.5	25–70

[a]EG, endo-glucanase; CBH, cellobiohydrolase. Product names from Novo Nordisk (Bagsvaerd, Denmark).

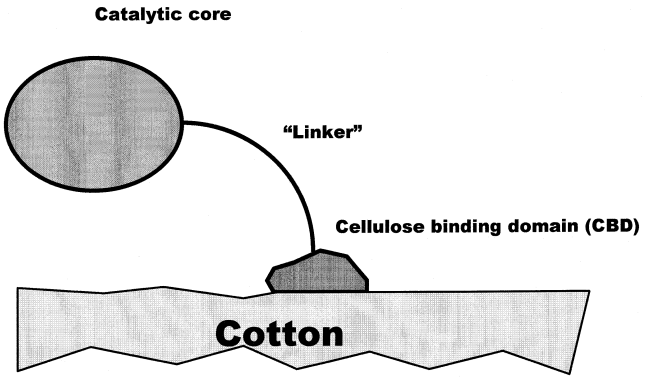


FIG. 5. Cellulase domains.

ferred to a host organism, *Aspergillus* spp., which is used in production (34). In laundering, the effects of lipases are seen only after several wash cycles ("multicycle wash performance"). Further engineering was carried out to develop first-wash effects in laundry usage: one of the 269 amino acids in the Lipolase molecule was changed (in amino acid position no. 96 aspartic acid was substituted with leucine) to make the active site more hydrophobic so that the affinity to a lipid contact zone on the textile surface was improved. For cold washing, this effect was improved considerably. The new lipase is produced by Novo Nordisk as Lipolase Ultra. Further efforts are now being made to develop "first-wash lipases" (35).

The delayed cleaning efficacy of lipases is attributed to increased activity when the moisture concentration during the drying process is reduced. Thus, the activity of Lipolase vs. the moisture content was found to be optimal at 20–40% water content on fabric in a line-drying process at room temperature (36).

In testing lipase, three consecutive washing/drying cycles are usually employed. Washing temperature, time, and Ca^{2+} level depend on the geographical area where the detergent is used. After washing and drying, the reflectance of the colored, lard-containing test swatches is measured and the remaining lard may be extracted for quantitative determination.

Redox enzymes. Redox enzymes, mainly peroxidases and oxidases (including laccases), are being researched as potential components of novel bleaching systems that—apart from utilizing less harsh chemicals than current bleaching systems—might even provide bleaching effects at low washing temperatures (down to 5–10°C). This would provide energy savings and also ensure less overall wear of garments being washed (37).

Until now a peroxidase from *Coprinus cinereus* has been developed for use in dye transfer inhibition (DTI) in combination with a mediator substance (2). This peroxidase (Guardzyme™) can bleach dye material that is released from colored laundry items during the wash (38).

Guardzyme™ is a hem-containing enzyme. The DTI system consists of three components: the enzyme, a mediator, and hydrogen peroxide. The oxidized form of the mediator is the actual oxidizing agent. The dye (the colored form) released from the fabric will be oxidized by the enzymatically oxidized mediator and become colorless. After intensive screening of mediators, phenothiazine-10-propionic acid was found useful under practical conditions in a detergent system (2,38).

FACTORS AFFECTING ENZYME PERFORMANCE IN HOUSEHOLD DETERGENTS

Enzyme performance is influenced by factors like detergent pH, ionic strength, wash temperature, washing time, detergent composition, and mechanical handling. Both performance and stability are influenced by some detergent surfactants and some bleach systems.

To illustrate the reaction conditions under which enzymes have to operate, Table 7 shows the components of household detergents in very broad terms. Heavy-duty powdered detergent formulations may vary by geographic regions, with a tendency for higher levels of builder in North America than in Europe and Japan (9). The ratio of anionic to nonionic surfactant may also vary from region to region. In Japan, surfactant levels are higher than elsewhere (9). These variations between regions should be seen in connection with the laundry practices common in North America, Europe, and Japan. Table 8 shows in broad terms some parameters in laundry conditions in the three principal geographical areas. Worth noting are the different washing machine types which dominate in each region. The ratio of wash liquor to fabric load, temperatures, the usual dosage of detergents, and water hardness around the world are significantly different—as are detergent compositions. Most of these parameters may influence the detergency of enzymes in a laundry washing machine or in automatic dishwashing. In laundry, particularly, the water hardness (i.e., the calcium concentration) is of concern. Cations are known to stabilize some enzymes and destabilize others. Usually sufficient amounts of enzymes are present in the powders to secure the desired detergency power.

Performance. Evaluation of detergency is divided into two steps. (i) "Primary washing effects" (cleaning efficacy) refer to the removal of soil and stains after one wash. Testing is carried out using either artificially soiled test fabrics or naturally soiled laundry. (ii) "Secondary washing effects" (effects after repeat washing) refer to the detection of damage such as loss of tear strength, incrustations (ash residues), and graying. The latter evaluations are usually based on 25 or 50 washes, including a control fabric.

In the evaluation of dishwashing performance, "primary washing effects" are usually tested on soiled plates or test pieces. "Secondary washing effects" in dishwashing refer mainly to the appearance of cloudiness and weight loss (13).

(i) Washing performance evaluations—laundry. Laboratory washing performance evaluations are carried out at rele-

TABLE 7
General Composition of Detergents for Laundry and Dishwashing[a]

Raw materials	Functions in general	Type of compounds	Contents (w/w%) Laundry	Dishwash
Builders	Washing alkalis Water softening Buffer action Stabilizers Corrosion protection	Phosphates (sodium triphosphate) Phosphonates Na-citrates Zeolites Soda Silicates Complex formers (NTA, etc.)	30–60	10–40
Surfactants	Emulsify particles interfacial activity Reduce surface tension	Surfactants: Anionics Nonionics Cationics	10–30	1–4
Soap	Prevents overfoaming in the machine	Soap	1–5	0
Bleach—as peroxygen/activator	Oxidize polyphenolics from, e.g., fruits, coffee, tea, wine, and vegetables	Na-perborates Na-percarbonates Activators (TAED, etc.)	0–25	4–15
Enzymes	Removal of starch-based stains	Amylases	0.4–1	1–3
	Removal of protein complexes	Proteases	0.4–2	0.5–2
	Removal of fat stains	Lipases	0.2–1	0.5
	Textile color brightening, softening, soil removal, whiteness maintenance	Cellulases	1–3	0
Salt	Process aid to promote flow and loading properties (powders)	Na-sulfate	Balance to 100	Balance to 100
Perfume	To add a pleasant smell to the fabric	Various esters	0.1–0.5	0–0.1
Other organics	Graying inhibitors	Carboxy methyl cellulose, polycarboxylates	0.5–5	0–1
	Optical brighteners Solvents	Alcohols		

[a]Data from general brochures, package information, encyclopedias, and other general literature, e.g., Reference 9.

vant temperatures using the Terg-O-tometer, which simulates the top-loading U.S.-type of washing machine, and the Launder-O-meter, which simulates the European drum-type machines. These "-O-meters" simulate to some degree washing mechanics and washing temperatures, including initial temperatures, heating rates, and maximum temperatures. Dose-response trials may be carried out in ordinary washing machines with ballast laundry and artificially soiled fabrics. A sufficient number of "standard" swatches may be fixed onto the ballast cloth.

Assessment of washed test pieces is done visually or instrumentally, the latter by measuring the reflectance of light remitted at 460 nm. The intensity of the reflected light, % R, (% remission) can thus be measured. The value $\Delta R = R_{washed} - R_{unwashed}$ is a measure of the total detergency. $\Delta R_{Enz} = R_{washed} - R_{washed\ without\ enzyme}$ reflects the contribution of the enzyme.

Detailed washing data have been published on many occasions. Such data are used to inform about the performance of new enzymes or as studies of the influences of particular surfactants, builders, or effect of design of washing machines (39). Several of the references cited contain such information.

(ii) Washing performance evaluation—ADD. A standard laboratory procedure for evaluating the cleaning efficacy of enzymes, mainly proteases and amylases, has been published (28):

To test the efficiency of proteases, stainless steel plates are

TABLE 8
Laundry Washing Conditions and Procedures by Region[a]

Conditions	United States/Canada	Japan	Europe
Machine type	Top-loading Vertical axis Agitator	Top-loading Vertical axis Impeller/pulsator	Front-loading Horizontal axis Drum type
Fabric load (kg)	2–3	1–1.5	3–5
Wash liquor (L)	35–80	30–45	15–25
Wash temperature (°C)	20–50	10–30	30–95
Washing time (min)	10–15	15–35	40–60
Water hardness (ppm $CaCO_3$) (simplified picture)	100	50	250
Detergent dosage levels (g/L)			
Powder	1–5	0.5–1.5	5–10
Liquid	2–4	0.75–1.0	7.5–10

[a]Data from general brochures, package information, encyclopedias, and other general literature, e.g., Reference 9.

soiled with a baked egg–milk film. To test amylases, porcelain plates are soiled with a gelatinized starch solution, which is dried overnight at room temperature. After washing, light reflectance values R are measured directly for protein film or for starch films, after staining with iodine (KI/I_2). Calculations of % removed film—RPF% for protein or RSF% for starch—can be as described in the formula:

RPF(%) *[or RSF(%)]*

$$= [R_{\text{after wash}} - R_{\text{before wash}}]/[R_{\text{clean plate}} - R_{\text{before wash}}] \cdot 100\%$$

For protein, especially, measured soil removal values are affected by the temperature at which the film has been baked. Removal of starch film is dependent on film thickness, water hardness, and pretreatment of the porcelain plates (acid or alkaline).

Storage stability. Average storage stability curves for formulated detergents stored at a relative humidity of 55% in an open vial simulate the storage of an opened package in the household. In open packages, detergents may absorb humidity while bleaches like percarbonate or perborate release small amounts of hydrogen peroxide that can oxidize the enzyme (14). The measured loss of enzyme activity after several weeks of storage is a useful measure of enzyme stability.

Storage in a closed vial simulates the storage of a commercial product in the original closed package. Thermostated chambers at 30°C are used for both tests. The enzyme activities are measured after 1, 2, 6, and 8 wk of storage.

Storage stability of the enzymes in detergent formulations and wash performance go hand in hand. Storage stability of enzymes in activated bleach systems has been improved significantly in recent years by using genetic engineering. By such techniques, oxidation-sensitive regions of amino acids in an enzyme molecule may be replaced by more stable amino acids (14).

The dosage and selection of enzymes for incorporation into a detergent formulation may be based on washing trials, model tests or machine tests, and on the results of storage tests. Based on these data, a "minimum task value" for the user can be defined for the enzyme detergency performance for the last wash out of a given package.

The factors affecting enzyme stability in formulated detergents are summarized in Table 9.

FUTURE POSSIBILITIES FOR BIOTECHNOLOGY IN DETERGENTS

Although enzymes have contributed to more environmentally friendly washing and cleaning, the process still requires large quantities of chemicals, energy, and water. Past developments have clearly shown that detergent formulations can be optimized based on biotechnological systems. Supported by further development of enzyme systems, this trend may continue toward development of effective detergent systems that use substantially smaller quantities of chemicals and require less water and energy to attain maximal washing or cleaning performance. One possibility is the development of special dosing techniques that add active ingredients when they are needed at a particular stage in the washing or cleaning cycle and thus enhance their performance.

Continued development of new enzymes through modern biotechnology may yield functional compounds with considerable and sufficient cleaning effects at low temperatures, with low water consumption, at a reasonable price, and without creating problems for the environment.

CONCLUSION

The first development of submerged fermentation techniques for industrial protease production resulted in the creation and maturation of an entire field, and the process is no doubt set to continue. Enzymes have contributed to

TABLE 9
Some Experience of the Effects of Detergent Compounds on the Stability of Enzymes

Compounds	Powder detergents	Liquid detergents
Water	Humidity decreases stability of nonbleach-stable enzymes	Decreases stability.
Proteases	Proteolysis is usually no problem in dry products	Proteases must be inhibited and recovered during dilution in the washing process.
Surfactants	Usually no problem in dry products	Anionics denature. Nonionic or amphoteric surfactants stabilize most enzymes in water-free systems.
O_2-bleach	Many conventional types of enzymes are affected by oxidation when humidity is present.	Bleaches are usually not used in combination with enzymes. Exceptions are found when the bleach is kept completely encapsulated.
Builders	Usually no problem in dry products	Binding or sequestering of Ca may reduce stability of proteases, lipase, and amylase. Free Ca^{2+} may stabilize most enzymes. The pH should be around neutral.

the development of detergency and improvements in household and industrial detergents. In compact detergent formulations, enzymes have also provided significant environmental benefits by reducing energy consumption through shorter washing times, lower washing temperatures, reduced water consumption, and reduced chemical load. Proteases are now supported by lipases and amylases in further increasing these benefits, especially by increasing cleaning effects at lower temperatures in household laundering, and at lower pH levels in industrial cleaning. Cellulases have contributed to overall fabric care by rejuvenating or maintaining the new appearance of washed garments.

ACKNOWLEDGMENTS

We are grateful for help and advice during the preparation of the manuscript from colleagues at Novo Nordisk A/S and at Novo Nordisk BioChem North America, Inc. In particular the following have offered us valuable challenges, critical and well-meaning corrections to improve our circulating drafts for internal approval: Erik Gormsen, Sonja Salmon, Ture Damhus, Tina Sejersgård Jakobsen, Marianne Thellersen, and Morten Birket Andersen.

REFERENCES

1. Röhm, O., German Patent DE 283 923 (1913).
2. Conrad, L.S., T. Damhus, O. Kirk, and P. Schneider, Enzymatic Inhibition of Dye Transfer, *INFORM 8*:950–957, 1997.
3. Houston, J.H., in *Enzymes in Detergency*, edited by J.H. van Ee, O. Misset, and E.J. Baas, Marcel Dekker Inc., New York, 1997, Surfactant Science Series, Vol. 69, pp. 11–21.
4. Krawczyk, T., Detergent Enzymes, *INFORM* 8:7–14 (1997).
5. Braae, K. (ed.), Dansk Husmoder Leksikon, *Standard Forlaget,* Copenhagen, 1954, pp. 560–569.
6. Rieck, H.-P., in *New Horizons. An AOCS/CSMA Detergent Industry Conference*, edited by R.T. Coffey, AOCS Press, Champaign, 1996, pp. 23–36.
7. Hloch, H.G., Tendenzen im Bereich Wasch-und Spülmaschinen, in *The 37th International WFK Detergency Conference, Proceedings*, wfk-Forschungsinstitute für Reinigungstechnologie e.V., D-47798 Krefeld, 1996, pp. 8–14.
8. Kemna, R., in *Ibid.*, 1996, pp. 22–27.
9. Showell, M.S., in *Powdered Detergents*, edited by M.S. Showell, and E.J. Baas, Marcel Dekker Inc., New York, 1998, Surfactant Science Series, Vol. 71, pp. 1–19.
10. Andersen, M., and H. Lundsgaard, Vaskemaskiner in *Rad & Resultater,* Forbrugerstyrelsen, Copenhagen, 1997, Vol. 37. pp. 2–7.
11. Härer, J., Geschirrspülen von Hand und in der Maschine—ein Systemvergleich, in *The 37th International WFK Detergency Conference, Proceedings*, wfk-Forschungsinstitute für Reinigungstechnologie e.V., D-47798 Krefeld, 1996, pp. 156–158.
12. Buchmeier, W., H. Andree, P. Jeschke, J. Härer, and R. Sorg, Corrosion of Tableware in Machine Dishwashing, *Henkel-Referate 33*:76–84 (1997).
13. Gutzschebauch, C., J. Härer, P. Jeschke, F.R. Schröder, and D. Zaika, Dishwashing by Hand and by Machine—a System Comparison, *Ibid. 33*:69–75 (1997).
14. Eriksen, N., Detergents in *Industrial Enzymology—Second Edition*, edited by T. Godfrey, and S. West, Stockton Press, New York and Macmillan Press Ltd., London, 1996, pp. 189–200.
15. Potthoff, A., W. Serve, and P. Macharis, The Cleaning Revolution, in *Dairy Industries 62*:25–29, 1997.
16. Krack, R., and H. Manners, Patent Anmeldung, WO 96/23579 (PCT/EP96/00258) (Henkel-Ecolab GmbH & Co.) (1996).
17. Stutz, Chr., The Use of Enzymes in Ultrafiltration, in *Fruit Processing* 7, 1993, pp. 1–4.
18. Olsen, H.S., PCT/WO 97/02753 (Novo Nordisk A/S) (1997).
19. *American Conference of Governmental Industrial Hygienists*, Documentation of the threshold limit values, 5th edn., 1986, pp. 540–541.
20. Peters, G., and D.P. Mackenzie, in *Enzymes in Detergency*, edited by J.H. van Ee, O. Misset, and E.J. Baas, Marcel Dekker Inc., New York, 1997, Surfactant Science Series, Vol. 69, pp. 327–340.
21. National Research Council (NRC), Enzyme-Containing Laundering Compounds and Consumer Health, Report of the *ad hoc* Committee on Enzyme Detergents, Division of Medical Sciences, National Academy of Sciences-National Research Council, Report PB 204 118, Washington, DC, 1971, pp. 1–31.
22. Gormsen, E., E. Marcussen, and T. Damhus, in *Powdered Detergents*, edited by M.S. Showell, and E.J. Baas, Marcel Dekker Inc., New York, 1998, Surfactant Science Series, Vol. 71, pp. 137–163.
23. Adler-Nissen, J., and H.S. Olsen, The Influence of Peptide Chain Length on Taste and Functional Properties of Enzymat-

ically Modified Soy Protein, *American Chemical Society Symposium Series 92*, pp. 125–146, 1979.
24. Adler-Nissen, J., *Enzymic Hydrolysis of Food Proteins*, Applied Science Publishers, London, 1986, pp. 25–43, 170–186.
25. Linderstrøm-Lang, K., *Proteins and Enzymes. III. The Initial Stages in the Breakdown of Proteins by Enzymes*, *Lane Medical Lectures*, Stanford University Press, California, Vol. VI, 1952, pp. 53–72.
26. Eilertsen, J.H., A.D. Fog, and K. Gibson, U.S. Patent 4,497,897 (Novo Nordisk) (1982).
27. Eilertsen, J.H., A.D. Fog, and K. Gibson, U.S. Patent 4,543,333 (Novo Nordisk) (1982).
28. Jensen, G., The Development and Application of a New Stable Detergent Amylase, *SÖFW-J. 123*:723–731 (1997).
29. Baeck, A.C., T.W. Horner, R. Ohtani, K. Pramod, and S. Rai, Patent WO9704067-A1 (1997).
30. Gibson, K., The Importance of Detergent Amylases for Whiteness Maintenance, Paper Presented at CID, Genoa, October 22–24, 1997 (available from Novo Nordisk A/S as A-6506).
31. Grime, J.K., Laundry Technology Trends in the Americas, *3rd World Conference and Exhibition on Detergents: Global Perspectives*, edited by A. Cahn, AOCS Press, Champaign, 1993, pp. 64–71.
32. Jakobsen, T.S., P. Lindegaard, and M. Chan, Colour Care Cellulases, *INFORM 9*:788–792 (1998).
33. Nielsen, P.H., Development of New Detergent Enzymes, Paper Presented at ISSD96, China, 1996 (available from Novo Nordisk A/S as A-6457).
34. Gormsen, E., and H. Malmos, *Lipolase™—a Lipase for the Detergent Industry*, The Soap and Detergent Association, 1991 Annual Convention, Boca Raton, Florida, February 2, 1991.
35. Gormsen, E., *New Detergent Enzymes Based on Biotechnology*, JOCS/AOCS Conference in Kuala Lumpur, Malaysia, September 1997 (available from Novo Nordisk A/S as A-6537).
36. Aaslyng, D., E. Gormsen, and H. Malmos, Mechanistic Studies of Proteases and Lipases for the Detergent Industry, *J. Chem. Tech. Biotechnol. 50*:321–330 (1991).
37. Hagen, H.A., in *New Horizons. An AOCS/CSMA Detergent Industry Conference*, edited by R.T. Coffey, AOCS Press, Champaign, 1996, pp. 57–62.
38. Damhus, T., L.S. Conrad, and T.T. Hansen, An Enzymatic System for Dye Transfer Inhibition in Laundry Detergents, Presented at the 88th AOCS Annual Meeting & Expo, Seattle, Washington, May 11–14, 1997.
39. Fleuren, R., P. Bauditz, S. Schløsler, and H. Bodenhoff, Paper presented at the 88th AOCS Annual Meeting, Seattle, Washington, May 14–18, 1997.

[Received February 9, 1998; accepted June 30, 1998]

Dr. Hans Sejr Olsen joined the enzyme application research of Novo Nordisk A/S in 1977 as a research scientist. He was head of Enzyme Process Development Pilot Plant 1980–1993 and has worked as application technologist with detergent enzymes mainly for hard-surface cleaning, automatic dishwashing, and industrial cleaning the last few years. He holds several patents and over the years has published many papers mainly within the area of enzymes for food processing. He received his M.Sc. in chemical engineering from the Technical University of Denmark in 1971 and his Ph.D. from the same university in 1980.

Per Falholt was born in 1958 and graduated with a M.Sc. in chemical engineering from the Technical University of Denmark in 1983. He has worked for Novo Nordisk A/S as a research scientist, manager, and director primarily within development of detergent enzymes, but lately also personal care, textile, pulp and paper and food enzymes. Currently he is working out of Novo Nordisk US, NC.