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ORIGINAL ARTICLE

Application of enzymes for textile fibres processing

RITA ARAÚJO[1,2], MARGARIDA CASAL[1], & ARTUR CAVACO-PAULO[2]

[1]*CBMA Centre of Environmental and Molecular Biology, Department of Biology, University of Minho, Campus of Gualtar, Braga and* [2]*Department of Textile Engineering, University of Minho, Campus of Azurém, Guimarães, Portugal*

Abstract
This review highlights the use of enzymes in the textile industry, covering both current commercial processes and research in this field. Amylases have been used for desizing since the middle of the last century. Enzymes used in detergent formulations have also been successfully used over the past 40 years. The application of cellulases for denim finishing and laccases for decolourization of textile effluents and textile bleaching are the most recent commercial advances. New developments rely on the modification of natural and synthetic fibres. Advances in enzymology, molecular biology and screening techniques provide possibilities for the development of new enzyme-based processes for a more environmentally friendly approach in the textile industry.

Keywords: *Enzymes, biotechnology, textile fibres, textile processing*

Biotechnology in the textile industry

The use of enzymes in the textile industry is an example of white/industrial biotechnology, which allows the development of environmentally friendly technologies in fibre processing and strategies to improve the final product quality. The consumption of energy and raw-materials, as well as increased awareness of environmental concerns related to the use and disposal of chemicals into landfills, water or release into the air during chemical processing of textiles are the principal reasons for the application of enzymes in finishing of textile materials (O'Neill et al. 1999).

Production of enzymes: searching for efficient production systems

Commercial sources of enzymes are obtained from any biological source – animal, plants and microbes. These naturally occurring enzymes are quite often not readily available in sufficient quantities for industrial use, but the number of proteins being produced using recombinant techniques is exponentially increasing. Screening approaches are being performed to rapidly identify enzymes with potential industrial application (Korf et al. 2005). For this

purpose, different expression hosts (*Escherichia coli*, *Bacillus* sp., *Saccharomyces cerevisiae*, *Pichia pastoris*, filamentous fungi, insect and mammalian cell lines) have been developed to express heterologous proteins (Makrides 1996; Huynh & Zieler 1999; Chelikani et al. 2006; Ogay et al. 2006; Silbersack et al. 2006; Li et al. 2007). Among the many systems available for heterologous protein production, the enteric Gram-negative bacterium *E. coli* remains one of the most attractive. Compared with other established and emerging expression systems, *E. coli*, offers several advantages including its ability to grow rapidly and at high density on inexpensive carbon sources, simple scale-up process, its well-characterized genetics and the availability of an increasingly large number of cloning vectors and mutant host strains (Baneyx 1999). However, the use of *E. coli* is not always suitable because it lacks some auxiliary biochemical pathways that are essential for the phenotypic expression of certain functions, so there is no guarantee that a recombinant gene product will accumulate in *E. coli* at high levels in a full-length and biologically active form (Makrides 1996). In such circumstances, the genes have to be cloned back into species similar to those from which they were derived. In these cases bacteria from the

Correspondence: A. Cavaco-Paulo, University of Minho, Textile Engineering Department, 4800-058 Guimarães, Portugal. Tel: +351 253 510271. Fax: +351 253 510293. E-mail: artur@det.uminho.pt

unrelated genera *Bacillus,* (Silbersack et al. 2006, Biedendieck et al. 2007) *Clostridium* (Girbal et al. 2005) *Staphylococcus* and the lactic acid bacteria *Streptococcus* (Arnau et al. 2006) *Lactococcus* (Miyoshi et al. 2002) and *Lactobacillus* (Miyoshi et al. 2004) can be used.

If heterologous proteins require complex post-translational modifications and are not expressed in the soluble form using prokaryotic expression systems, yeasts can be an efficient alternative once they provide several advantages over bacteria for the production of eukaryotic proteins. Among yeast species, the methylotrophic yeast *Pichia pastoris* is a particularly well suited host for this purpose. The use of this organism for expression offers a number of important benefits:

- high levels of recombinant protein expression are reached under the alcohol oxidase1 gene (*aox1*) promoter;
- this organism grows to high cell densities;
- scaled-up fermentation methods without loss of yield have been developed;
- efficient secretion of the recombinant product together with a very low level of endogenous protein secretion represents a very simple and convenient pre-purification step;
- some post-translational modifications are feasible (such as proteolytic processing and glycosylation).

Furthermore, the existence of efficient methods to integrate several copies of the expression cassette carrying the recombinant DNA into the genome, eliminating problems associated with expression from plasmids, is making this yeast the micro-organism of choice for an increasing number of biotechnologists (Hollenberg & Gellissen 1997; Cereghino & Cregg 2000).

Role of enzymes in textile industry

Textile processing has benefited greatly in both environmental impact and product quality through the use of enzymes. From the 7000 enzymes known, only about 75 are commonly used in textile industry processes (Quandt & Kuhl 2001).

The principal enzymes applied in textile industry are hydrolases and oxidoreductases. The group of hydrolases includes amylases, cellulases, proteases, pectinases and lipases/esterases. Amylases were the only enzymes applied in textile processing until the 1980s. These enzymes are still used to remove starch-based sizes from fabrics after weaving. Cellulases have been employed to enzymatically remove fibrils and fuzz fibres, and have also successfully been introduced to the cotton textile industry. Further applications have been found for these enzymes to produce the aged look of denim and other garments. The potential of proteolytic enzymes was assessed for the removal of wool fibre scales, resulting in improved anti-felting behaviour. However, an industrial process has yet to be realized. Esterases have been successfully studied for the partial hydrolysis of synthetic fibre surfaces, improving their hydrophilicity and aiding further finishing steps. Besides hydrolytic enzymes, oxidoreductases have also been used as powerful tools in various textile-processing steps. Catalases have been used to remove H_2O_2 after bleaching, reducing water consumption. Lenting (2007) contains an excellent chapter dealing with enzyme applications in the textile processing industry. A more detailed description of the most common groups of enzymes applied in the textile industry and the processes where they are applied will be given in this review.

Amylases

Amylases hydrolyse starch molecules to give diverse products including dextrins and progressively smaller polymers composed of glucose units (Windish & Mhatre 1965). Starch hydrolysing enzymes are classified according to the type of sugars produced: α-amylases and β-amylases. α-Amylases are produced by a variety fungi, yeasts and bacteria, but enzymes from filamentous fungal and bacterial sources are the most commonly used in industrial sectors (Pandey et al. 2000). Microbial α-amylases range from 50 to 60 KDa, with a few exceptions, like the 10 KDa α-amylase from *Bacillus caldolyticus* and a 210 KDa α-amylase from *Chloroflexus aurantiacus* (Grootegoed et al. 1973; Ratanakhanokchai et al. 1992). α-Amylases from most bacteria and fungi are quite stable over a wide range of pH from 4 to 11. *Alicyclobacillus acidocaldarius* α-amylase has a pH optimum of 3, while those from alkalophilic and extremely alkalophilic *Bacillus* sp. have pH optima of 9–10.5 and 11–12, respectively (Krishnan & Chandra 1983; Lee et al. 1994; Schwermann et al. 1994; Kim et al. 1995). Optimum temperature for the activity of α-amylases is usually related to growth of the producer micro-organism (Vihinen & Mantsala 1989). Temperatures from 25 to 30°C were reported for *Fusarium oxysporum* α-amylase (Chary & Reddy 1985) and temperatures of 100 and 130°C for *Pyrococcus furiosus* and *Pyrococcus woesei,* respectively (Laderman et al. 1993; Koch et al. 1991). Addition of Ca^{2+} can, in some cases, enhance thermostability (Vallee et al. 1959; Vihinen & Mantsala 1989). They are severely inhibited by

heav metal ions, sulphydryl group reagents, EDTA and EGTA (Mar et al. 2003; Tripathi et al. 2007).

In general, microbial α-amylases display the highest specificity towards starch followed by amylase, amylopectin, cyclodextrin, glycogen and maltotriose (Vihinen & Mantsala 1989).

Textile desizing

For fabrics made from cotton or blends, the warp threads are coated with an adhesive substance know as 'size' to lubricate and protect the yarn from abrasion preventing the threads to break during weaving. Although many different compounds have been used to size fabrics, starch and its derivatives are the most common because of their excellent film forming capacity, availability and relatively low cost (Feitkenhauer et al. 2003). After weaving, the sizing agent and natural non-cellulosic materials present in the cotton must be removed in order to prepare the fabric for dyeing and finishing. Before the discovery of amylases, desizing used to be carried out by treating the fabric with acid, alkali or oxidizing agents at high temperatures. The chemical treatment was not totally effective in removing the starch, leading to imperfections in dyeing, and also resulted in a degradation of the cotton fibre destroying the natural, soft feel of the cotton. Nowadays amylases are commercialized and preferred for desizing due to their high efficiency and specificity, completely removing the size without any harmful effects on the fabric (Etters & Annis 1998; Cegarra 1996). The starch is randomly cleaved into water soluble dextrins that can be then removed by washing. This also reduced the discharge of waste chemicals to the environment and improved working conditions.

Pectinases

Pectin and other pectic substances are complex polysaccharides present in plant cell walls as a part of the middle lamella. Pectinases are a complex group of enzymes involved in the degradation of pectic substances. They are primarily produced in nature by saprophytes and plant pathogens (bacteria and fungi) for degradation of plant cell walls (Bateman 1966; Lang & Dörenberg 2000). There are three major classes of pectin degrading enzymes: pectin esterases (PEs), polygalacturonases (PGs) and polygalacturonate lyases (PGLs).

Pectin esterases are mainly produced in plants such as banana, citrus fruits and tomato, but also by bacteria and fungi (Hasunuma et al. 2003). They catalyze hydrolysis of pectin methyl esters, forming pectic acid. The enzyme acts preferentially on a methyl ester group of a galacturonate unit next to a non-esterifed galacturonate unit. The molecular weight of most microbial and plant PEs varies between 30–50 kDa (Christensen et al. 2002; Hadj-Taieb et al. 2002). The optimum pH for activity varies between 4.0 and 7.0. The exception is PE from *Erwinia* with an optimum pH in the alkaline region. The optimum temperature ranges between 40 and 60°C, and pI between 4.0 and 8.0.

Polygalacturonases are a group of enzymes that hydrolyze α-1,4 glycosidic linkages in pectin using both exo- and endo-splitting mechanisms. Endo-PGs are widely distributed among fungi, bacteria and yeast. These enzymes often occur in different forms having molecular weights in the range of 30–80 kDa, and pI between 3.8 and 7.6. Their optimum pH is in the acidic range of 2.5–6.0 and the optimum temperature between 30 and 50°C (Takao et al. 2001; Singh & Rao 2002). Exo PGs are found in *Aspergilus niger*, *Erwinia* sp. and some plants, such as carrots, peaches, citrus and apples (Pressey & Avants 1975; Pathak & Sanwal 1998). The molecular weight of exo-PGs vary between 30 and 50 kDa, and their pI ranges between 4.0 and 6.0.

Polygalacturonate lyase cleaves polygalacturonate or pectin chains via β-elimination that results in the formation of a double bond between C4 and C5 at the non-reducing end and elimination of CO_2. Endo-polygalacturonate lyase cleaves polygalacturonate chains arbitrarily and exo-polygalacturonate lyase splits at the chain end of polygalacturonate which yields unsaturated galacturonic acid (Sakai et al. 1993). The molecular weight of PGLs varies between 30 and 50 kDa except in the case of PGL from *Bacteroides* and *Pseudoalteromonas* (75 kDa; McCarthy et al. 1985; Truong et al. 2001). The optimum pH ranges between 8.0 and 10.0, although PGL from *Erwinia* and *Bacillus licheniformis* were still active at pH 6.0 and 11.0, respectively. The optimum temperature for PGL activity is typically between 30 and 40°C, although PGL from thermophiles have an optima between 50 and 75°C. The potential of some pectate lyases for bioscouring has been exploited.

Enzymatic scouring

Greige or untreated cotton contains various non-cellulosic impurities, such as waxes, pectins, hemi-celluloses and mineral salts, present in the cuticle and primary cell wall of the fibre (Batra 1985; Etters et al. 1999). These are responsible for the hydrophobic properties of raw cotton and interfere with aqueous chemical processes on cotton, like dyeing and finishing (Freytag & Dinze 1983). Therefore, before cotton yarn or fabric can be dyed, it needs to be pretreated to remove materials that inhibit dye

binding. This step, named scouring, improves the wetability of the fabric and normally uses alkalis, such as sodium hydroxide. However, these chemicals also attack the cellulose, leading to reduction in strength and loss of fabric weight. Furthermore, the resulting wastewater has a high COD (chemical oxygen demand), BOD (biological oxygen demand) and salt content (Buschle-Diller et al. 1998). Enzymatic or bioscouring, leaves the cellulose structure almost intact, preventing cellulose weight and strength loss. Bioscouring has a number of potential advantages over traditional scouring. It is performed at neutral pH, which reduces total water consumption, the treated yarn/fabrics retain their strength properties, the weight loss is reduced or limited compared with processing in traditional ways, and it increases cotton fibre softness. Several types of enzyme, including pectinases (Li & Hardin 1997; Karapinar & Sariisik 2004; Tzanov et al. 2001; Choe et al. 2004; Ibrahim et al. 2004), cellulases (Li & Hardin 1997; Karapinar & Sariisik 2004), proteases (Karapinar & Sariisik 2004), and lipases/cutinases, alone or combined (Deganil et al. 2002; Sangwatanaroj & Choonukulpong 2003; Buchert et al. 2000; Hartzell & Hsieh 1998) have been studied for cotton bioscouring, with pectinases being the most effective.

Despite all the research on bioscouring, it has yet to be applied on industrial scale. There is a need for pectinases with higher activity and stability at high temperatures and alkaline conditions. A new pectate lyase from *Bacillus pumilus BK2* was recently reported, with optimum activity at pH 8.5 and around 70 °C (Klug-Santner et al. 2006), and assessed for bio-scouring of cotton fabric. Removal of up to 80% of pectin was demonstrated by ruthenium red dyeing and HPAEC, and the hydrophilicility of the fabric, evaluated by liquid porosimetry (Bernard & Tyomkin 1994), was also dramatically enhanced. Solbak et al. (2005) developed a novel pectate lyase, by Directed Evolution, with improved thermostability. The new enzyme contained eight point mutations (A118H, T190L, A197G, S208K, S263K, N275Y, Y309W and S312V) and had a 16°C higher melting temperature than the wild-type, giving better bioscouring performance at low enzyme dosage in a high temperature process. More recently, Agrawal et al. (2007) performed a wax removal step prior to enzymatic scouring of cotton. The authors hypothesized that removal of outer waxy layer would allow access and efficient reaction of pectinase with the substrate. They demonstrated that pre-treatment of fibres with *n*-hexane (for wax removal) improved alkaline pectinase performance in terms of hydrophilicity and pectin removal (Agrawal et al. 2007).

Characterization of chemical and physical surface changes of fabrics after bioscouring and identification of suitable methods for surface analysis, are essential to better understand the bioscouring mechanism and evaluate its effects on fabrics. Fourier-transform infrared (FT-IR) attenuated total reflectance (ATR) spectroscopy was used for the first time, by Chung and collaborators, for fast characterization of cotton fabric scouring process (Chung et al. 2004). Later, Wang combined FT-IR ATR spectroscopy with scanning electron microscopy (SEM) and atomic force microscopy (AFM) to characterize bioscoured cotton fibres (Wang et al. 2006). SEM had been used before for this purpose (Li & Hardin 1997); however, this technique did not provide information about the height and roughness of the sample surface. The authors demonstrated that AFM, which can generate fine surface topographies of samples at atomic resolution, is a useful supplement to SEM in characterizing cotton surfaces (Wang et al. 2006).

Cellulases

Cellulases are hydrolytic enzymes that catalyse the breakdown of cellulose to smaller oligosaccharides and finally glucose. Cellulase activity refers to a multicomponent enzyme system combining at least three types of cellulase working synergistically (Teeri 1997). Endoglucanases or endocellulases cleave bonds along the length of cellulose chains in the middle of the amorphous region. Cellobiohydrolases or exo-cellulases start their action from the crystalline ends of cellulose chains, producing primarily cellobiose. Cellobiohydrolases act synergistically with each other and with endoglucanases, thus mixtures of all these types of enzymes have greater activity than the sum of activities of each individual enzyme alone. Cellobiose and soluble oligosaccharides, produced by exo-cellulases, are finally converted to glucose by β-4-glucosidase (Teeri 1997). These enzymes are commonly produced by soil-dwelling fungi and bacteria, the most important being *Trichoderma*, *Penicillium* and *Fusarium* (Verma et al. 2007; Jorgensen et al. 2005; Kuhad et al. 1999). Many of the fungal cellulases are modular proteins consisting of a catalytic domain, a carbohydrate-binding domain (CBD) and a connecting linker. The role of CBD is to mediate the binding of the enzyme to the insoluble cellulose substrate (Mosier et al. 1999). Cellulases are active in a temperature range from 30 to 60°C. Based on their sensitivity to pH, they are classified as acid stable (pH 4.5–5.5), neutral (pH 6.6–7) or alkali stable (pH 9–10). The application of cellulases in textile processing started in the late 1980s with denim

finishing. Currently, in addition to biostoning, cellulases are also used to process cotton and other cellulose-based fibres.

Denim finishing

Many garments are subjected to a wash treatment to give them a slightly worn look, e.g. stonewashing of denim jean, in which the blue denim is faded by the abrasive action of pumice stones on the garment surface. Thanks to the introduction of cellulases, the jeans industry can reduce or even eliminate the use of stones, resulting in less damage to the garment and machine, and less pumice dust in the laundry environment. Productivity can also be increased because laundry machines contain fewer stones or none at all, and more garments. Denim garments are dyed with indigo, which adheres to the surface of the yarn. The cellulase hydrolyses exposed fibrils on the surface of the yarn in a process known as 'Bio-Stonewashing', leaving the interior part of the cotton fibre intact. Partial hydrolysis of the surface of the fibre removes some of the indigo is creating light areas. There are a number of cellulases available, each with their own special properties. These can be used either alone or in combination in order to obtain a specific look. Heikinhemo et al. (2000) demonstrated that *Trichoderma reesei* endoglucanase II was very effective in removing colour from denim, producing a good stonewashing effect with the lowest hydrolysis level. Later Miettinen-Oinonen & Suominen (2002) developed new genetically engineered *T. reesei* strains able to produce elevated amounts of endoglucanase activity. Production of endoglucanase I and II was increased four-fold above that of the host strain, without any production of cellobiohydrolases. Cellulase preparations derived by the new *T. reesei* over-production strains proved to be more efficient for stonewashing than those produced by the parental strain. The prevention or enhancement backstaining, ie the redeposition of released indigo onto the garments, is a current focus of research. Cavaco-Paulo et al. (1998) attributed backstaining to the high affinity between indigo and cellulase and proved that the strong binding of cellulases to cotton cellulose is the major cause of backstaining (Cavaco-Paulo et al. 1998). Later, the affinity of cellulases from different fungal origins for insoluble indigo dye in the absence of cellulose was compared. The authors reported that acid cellulases from *T. reesei* have a higher affinity for indigo than neutral cellulases from *Humicola insolens* (Campos et al. 2000). The same group studied the interactions of cotton with CBD peptides from family I and family II, and highlighted the fact that truncated cellulases without CBDs caused less back-staining than complete enzymes (Cavaco-Paulo et al. 1999; Andreaus et al. 2000). These authors had previously studied the effect of temperature on the cellulose binding ability of cellulases from *T. reesei* and the influence of agitation level on the processing of cotton fabrics with cellulases having CBDs from different families (Cavaco-Paulo et al. 1996; Andreaus et al. 1999).

In order to overcome the lack of methods to access the performance of small quantities of enzymes, Gusakov et al (2000) developed a model microassay to test the abrasive and backstaining properties of cellulases on a 'test-tube scale', using it to identify an endoglucanase from *Chysosporium lucknowense* with a high washing performance and a moderate level of backstaining (Sinitsyn et al. 2001).

Knowing that backstaining could be significantly reduced at neutral pH, neutral cellulases started to be screened in order to minimize backstaining. Miettinen-Oinonen et al. (2004) reported the purification and characterization of three novel cellulases from *Melanocarpus albomyces* for textile treatment at neutral pH: a 20 and 50 KDa endoglu-canases, and a 50 KDa cellobiohydrolase. The 20 KDa endoglucanase had good biostoning perfor-mance. Combining the 50 KDa endoglucanase or the 50 KDa cellobiohydrolase with the 20 KDa endoglucanase, it was possible to decrease the level of backstaining. The respective genes were cloned in *T. reesei* and efficiently expressed at adequate levels for industrial applications by the same group (Haakana et al. 2004; Pazarlioglu et al. 2005; Anish et al. 2007). Nowadays due to the availability of effective anti-backstaining agents based on chemi-cals or enzymes, like proteases and lipases, back-staining problems can be minimized. The combination of new looks, lower costs, shorter treatment times and less solid waste have made abrasion with enzymes the most widely used fading process today.

Pilling and fuzz fibre removal

Besides the 'biostoning' process, cotton, and other natural and man-made cellulosic fibres can be improved by an enzymatic treatment called 'biopol-ishing'. The main advantage of this process is the prevention of pilling. A ball of fuzz is called a 'pill' in the textile trade. These affect garment quality since they result in an unattractive, knotty fabric appear-ance. Cellulases hydrolyse the microfibrils (hairs or fuzz) protruding from the surface of yarn because they are most susceptible to enzymatic attack. This weakens the microfibrils, which tend to break off from the main body of the fibre and leave a smoother yarn surface. After treatment, the fabric shows a

much lower pilling tendency. Other benefits of removing fuzz are a softer, smoother feel and superior colour brightness. Unlike conventional softeners, which tend to be washed out and often result in a greasy feel, the softness-enhancing effects of cellulases are washproof and non-greasy.

Optimization of biofinishing processes has been an important area of research. Azevedo et al. (2001) studied the desorption of cellulases from cotton, for recovering and recycling of cellulases. Lenting & Warmoeskerken (2001) came up with guidelines to minimize and prevent loss of tensile strength that can result from cellulase application. The choice of enzyme, enzyme concentration and incubation time, as well as application of immobilized enzymes, use of liquids with different viscosities, use of foam ingredients and hydrophobic agents to impregnate clothes can minimize the drawbacks of cellulases action. Yamada et al. (2005) reported the action of cellulases on cotton dyed with reactive dyes, which have an inhibitory effect on cellulase activity. The use of ultrasound has been shown to be an efficient way to improve enzymatic action in the bioprocessing of cotton (Yachmenev et al. 2002).

For cotton fabrics, polishing is optional for upgrading the fabric. However, this step is essential for the fibre lyocell, invented in 1991. It is made from wood pulp and is characterized by a tendency to fibrillate easily when wet (fibrils on the surface of the fibre peel up). If they are not removed, finished garments made from lyocell will end up covered with pills. Lyocell fabric is treated with cellulases during finishing, not only to avoid fibrillation, but also to enhance its silky appearance. There are several reports describing lyocell treatment with cellulases and elucidation of their mechanism of action (Morgado et al. 2000; Valldeperas et al. 2000). Cellulases are also used for viscose type regenerated celluloses like viscose and modal (Carrillo et al. 2003).

Serine proteases: subtilisins

Subtilisins are a family of alkaline serine proteases, generally secreted by a variety of *Bacillus* species (Siezen & Leunissen 1997). They catalyse the hydrolysis of peptide and ester bonds through the formation of an acyl-enzyme intermediate. Subtilisins are made as preproprotein precursors (Wells et al. 1983). The NH_2-terminal prepeptide, of 29 amino acid residues is the signal peptide required for secretion of prosubtilisin across the plasma membrane. The propeptide of 77 amino acids, located between the prepeptide and mature sequence, acts as an intramolecular chaperone required for the correct folding of mature enzyme in active form (Stahl & Ferrari 1984; Wong & Doi 1986; Ikemura et al. 1987;

Ikemura & Inouye 1988). Subtilisins are characterized by a common three-layer $\alpha/\beta/\alpha$ tertiary structure. The active site is composed of a catalytic triad of aspartate, histidine and serine. Molecular masses of subtilisins are generally between 15 and 30 KDa, but there are a few exceptions, like the 90 KDa subtilisin from *Bacillus subtilis* (*natto*) (Kato et al. 1992). The optimum temperature of alkaline proteases ranges from 50 to 70°C, but these enzymes are quite stable at high temperatures. The presence of one or more calcium binding sites enhances enzyme thermostability (Paliwal et al. 1994). Phenyl methyl sulphonyl fluoride (PMSF) and diisopropyl-fluorophosphate (DFP) are able to strongly inhibit subtilisins (Gold & Fahrney 1964; Morihara 1974). Most subtilisin protein engineering has focused on enhancement of catalytic activity (Takagi et al. 1988; Takagi et al. 1997), and thermostability (Takagi et al. 1990; Wang et al. 1993; Yang et al. 2000a,b), as well as, substrate specificity and oxidation resistance (Takagi et al. 1997).

Enzymatic treatment of wool

Raw wool is hydrophobic due to the epicutical surface membranes containing fatty acids and hydrophobic impurities like wax and grease. Harsh chemicals are commonly used for their removal— alkaline scouring using sodium carbonate, pretreatment using potassium permanganate, sodium sulphite or hydrogen peroxide. Wool fabric has the tendency to felt and shrink on wet processing. The shrinkage behaviour of wool can be regulated by various chemical means. The most successful commercial shrink-resistant process available is the chlorine-Hercosett process developed more than 30 years ago (Heiz 1981). Although this is a beneficial method (good antifelt effect, low damage and low weight loss) there are some important drawbacks (limited durability, poor handling quality, yellowing of fibres, difficulties in dyeing and environmental impact of the release of absorbable organic halogens; Julia et al. 2000; Schlink & Greeff 2001). Several authors have suggested the use of benign chemical processes such as low temperature plasma to treat wool (Kan et al. 1998, 1999, 2006a,b; El-Zawahry et al. 2006). Plasma treatment is a dry process, in which the treatment of wool fibre is performed by electric gas discharges (plasma). It is regarded as an environmentally friendly process, as no chemicals are used and it can modify the surface properties of wool without much alteration of the interior part of the fibre. However, costs, compatibility and capacity are obstacles to commercialization of a plasma treatment process, and the shrink-resist properties

obtained do not impart a machine-washable finish, which is one of the main objectives (McDevitt & Winkler 2000). The subsequent application of a natural polymer, such as chitosan, has been investigated to improve wool shrink-resistance or anti-felting properties (Onar & Sariisik 2004). More recently, and mainly for environmental reasons, proteases of the subtilisin type have been studied as an alternative for chemical pre-treatment of wool. Several studies reported that pretreatment of wool fibres with proteases improved anti-shrinkage properties, removed impurities and increased subsequent dyeing affinity (Levene et al. 1996; Parvinzadeh 2007).

However, due to its small size, the enzyme is able to penetrate into the fibre cortex, which causes destruction of the inner parts of the wool structure (Shen et al. 1999). Several reports show that increasing enzyme size by chemical cross-linking with glutaraldehyde or by the attachment of synthetic polymers like polyethylene glycol, can reduce enzyme penetration and the consequent reduction of strength and weight loss (Silva et al. 2004; Schroeder et al. 2006). Some of these processes have been tested on industrial process scale (Shen et al. 2007). Pretreatment of wool fibres with hydrogen peroxide, at alkaline pH in the presence of high concentrations of salts, also targets enzymatic activity to the outer surface of wool, by improving the susceptibility of the cuticle to proteolytic degradation (Lenting et al. 2006).

Some authors describe methods to improve the shrink resistance of wool by pretreating with a gentler oxidizing agent, like H_2O_2, instead of the traditional oxidizers, NaClO or $KMnO_4$ and then with a protease (Yu et al. 2005). The strong oxidation power of NaClO and $KMnO_4$ are always difficult to control. Besides, reaction of NaClO with wool produces halides. However, H_2O_2 provides a more controlled, cleaner and moderate oxidation. Zhang et al. (2006) used an anionic surfactant to promote the activities of proteases on wool. Other authors refer to processes to achieve shrink-resistance by treating wool with a protease followed by a heat treatment (Ciampi et al. 1996). The screening for new protease producing micro-organisms with high specificity for cuticles is being investigated as an alternative for the existing proteases (Erlacher et al. 2006).

Cysteine proteases: papain

Cysteine proteases (CP's) catalyse the hydrolysis of peptide, amide, ester, thiol ester and thiono ester bonds. More than 20 families of cysteine proteases have been described (Barrett 1994). The CP family can be subdivided into exopeptidases (e.g. cathepsin X, carboxypeptidase B) and endopeptidases (papain, bromelain, ficain, cathepsins). Exopeptidases cleave the peptide bond proximal to the amino or carboxy termini of the substrate, whereas endopeptidases cleave peptide bonds distant from the N- or C-termini (Barrett 1994). CPs have molecular masses in the range of 21–30 kDa. They are synthesized as inactive precursors with an N-terminal propeptide and a signal peptide. Activation requires proteolytic cleavage of the N-terminal propeptide that also functions as an inhibitor of the enzyme (Otto & Schirmeister 1997; Grzonka et al. 2001).

Papain is the best known cysteine protease. It was isolated in 1879 from the fruits of *Carica papaya* and was the first protease with a crystallographic structure (Drenth et al. 1968; Kamphuis et al. 1984). Papain has 212 amino acids with a molecular mass of 23.4 kDa. The enzyme has three internal disulphide bridges and an isoelectric point of 8.75. The optimal activity of papain occurs at pH 5.8–7.0 and at temperature 50–57°C, when casein is used as the substrate (Light et al. 1964; Kamphuis et al. 1984). The general mechanism of action has been very well studied. The catalytic triad is formed by Cys25, His159 and Asn175 residues. Asn175 is important for orientation of the imidazolium ring of the histidine in the catalytic cleft. The reactive thiol group of the enzyme has to be in the reduced form for catalytic activity. Thus, the cysteine proteases require a rather reducing and acidic environment to be active (Theodorou et al. 2007). Generally, papain can cleave various peptide bonds and, therefore, have fairly broad specificity.

Degumming of silk

Papain is used for boiling off cocoons and degumming of silk. Raw silk must be degummed to remove sericin, a proteinaceous substance that covers the fibre. Degumming is typically performed in an alkaline solution containing soap, a harsh treatment that also attacks fibrin structure. Several alkaline, acidic and neutral proteases have been studied as degumming agents since they can dissolve sericin, but are unable to affect silk fibre protein. Alkaline proteases seem to be the best for removing sericin and improving silk surface properties like handle, shine and smoothness (Freddi et al. 2003; Arami et al. 2007), although this is not in commercial use.

In the past, papain was also used to 'shrink-proof' wool. A successful method involved the partial hydrolysis of the scale tips. This method also gave wool a silky lustre and added to its value. The method was abandoned a few years ago for economic reasons.

Transglutaminases (TGs)

Transglutaminases are a group of thiol enzymes that catalyse the post-translational modification of proteins mainly by protein to protein cross-linking, but also through the covalent conjugation of polyamines, lipid esterification or the deamidation of glutamine residues (Folk & Cole 1966; Folk et al. 1968; Folk 1969, 1980; Lorand & Conrad 1984). Transglutaminases are widely distributed among bacteria, plants and animals. The first characterized microbial transglutaminase (MTG) was that of the bacterium *Streptomyces mobaraensis* (Ando et al. 1989). This enzyme is secreted as a zymogen that is sequentially processed by two endogenous enzymes to yield the mature form (Zotzel et al. 2003). The mature enzyme is a monomeric protein with a molecular weight of 38 kDa. It contains a single catalytic cysteine residue (Cys-64) and has an isoelectric point (pI) of 9 (Kanaji et al. 1993; Pasternack et al. 1998). The optimum pH for MTGase activity is between 5 and 8. However, MTGase showed some activity at pH 4 or 9, and was thus considered to be stable over a wide pH range (Ando et al. 1989). The optimum temperature for enzymatic activity is 55°C; it maintained full activity for 10 min at 40°C, but lost activity within a few minutes at 70°C. It was active at 10°C, and retained some activity at near-freezing temperatures. MTG does not require calcium for activity, shows broad substrate specificity and can be produced at relatively low cost. These properties are advantageous for industrial applications.

Treatment of wool and leather

The use of TGs for the treatment of wool textiles has been shown to improve shrink resistance, tensile strength retention, handle, softness, wetability and consequently dye uptake, as well as reduction of felting tendency and protection from damage caused by the use of common detergents (Cortez et al. 2004, 2005).

Treatment of leather with TG, together with keratin or casein, has a beneficial effect on the subsequent dyeing and colour properties of leather (Collighan et al. 2002). The application of TG for leather and wool treatment seems to be a promising strategy, but is still at the research level.

Lipases/esterases: cutinase

Esterases represent a diverse group of hydrolases that catalyse the cleavage and formation of ester bonds. They are widely distributed in animals, plants and micro-organisms. These enzymes show a wide substrate tolerance, high regio- and stereo-specificity, which make them attractive biocatalysts for the production of optically pure compounds in fine-chemicals synthesis. They do not require cofactors, are usually rather stable and are even active in organic solvents (Bornscheuer 2002). Two major classes of hydrolases are of most importance: lipases (triacylglycerol hydrolases) and 'true' esterases (carboxyl ester hydrolases). Both classes of enzymes have a three-dimensional structure with the characteristic α/β-hydrolase fold (Ollis et al. 1992; Schrag & Cygler 1997). The catalytic triad is composed of Ser-Asp-His (Glu instead of Asp for some lipases) and usually also a consensus sequence (Gly-x-Ser-x-Gly) is found around the active site serine (Ollis et al. 1992).

The mechanism for ester hydrolysis or formation is essentially the same for lipases and esterases and is composed of four steps: first, the substrate is bound to the active serine, yielding a tetrahedral intermediate stabilized by the catalytic His and Asp residues. Next, the alcohol is released and an acyl-enzyme complex is formed. Attack of a nucleophile (water in hydrolysis, alcohol or ester in transesterification) reforms a tetrahedral intermediate, which after resolution yields the product (an acid or an ester) and free enzyme (Stadler et al. 1995). Lipases can be distinguished from esterases by the phenomenon of interfacial activation (which is only observed for lipases). Esterases obey classical Michaelis–Menten kinetics; lipases need a minimum substrate concentration before high activity is observed (Verger 1998). Structure elucidation revealed that this interfacial activation is due to a hydrophobic domain (lid) covering the lipase active site and only in the presence of a minimum substrate concentration, (a triglyceride phase or a hydrophobic organic solvent) will the lid open, making the active site accessible (Derewenda et al. 1992). Furthermore, lipases prefer water-insoluble substrates, typically triglycerides composed of long-chain fatty acids, whereas esterases preferentially hydrolyse 'simple' esters (Verger 1998). Lipases and esterases were among the first enzymes tested and found to be stable and active in organic solvents, but this characteristic is more apparent with lipases (Schmid & Verger 1998).

A comparison of the amino acid sequences and 3D-structures of both enzymes showed that the active site of lipases displays a negative potential in the pH-range associated with their maximum activity (typically at pH 8); esterases show a similar pattern, but at pH values around 6, which correlates with their usually lower pH-activity optimum (Fojan et al. 2000).

Cutinases are extracellular esterases secreted by several phytopathogenic fungi and pollen that catalyse the hydrolysis of ester bonds in cutin, the

structural polyester of plant cuticles (Soliday & Kolattukudy 1975). Cutinases are also able to hydrolyse a wide variety of synthetic esters and triacylglycerols, as efficiently as lipases, without displaying interfacial activation (Martinez et al. 1992; Egmond & Van Bemmel 1997). Therefore, cutinases are suitable for application in the laundry industry, dishwashing detergents for removal of fats, in the synthesis of structured triglycerides, polymers and agrochemicals, and in the degradation of plastics (Murphy et al. 1996; Flipsen et al. 1998; Carvalho et al. 1999).

Among cutinases, that from the phytopathogenic fungus *Fusarium solani pisi* is the best studied example of a carboxylic ester hydrolase. *F. solani* cutinase is a 22 KDa enzyme shown to be present at the site of fungal penetration of the host plant cuticle (Purdy & Kolattukudy 1975a,b; Shaykh et al. 1977). Specific inhibition of cutinase was shown to protect plants against fungal penetration and consequently infection (Koller et al. 1982). The enzyme belongs to the family of serine esterases containing the so-called α/β hydrolase fold. The active site of cutinase is composed of a catalytic triad involving serine, histidine and aspartate. *Fusarium solani pisi* cutinase has an isoelectric point of 7.8 and an optimum pH around 8. The enzyme contains two disulfide bonds which are essential for structural integrity and catalytic activity (Egmond & de Vlieg 2000).

Surface modification of synthetic fibres

Synthetic fibres represent almost 50% of the world-wide textile fibre market. Polyethyleneterephthalate (PET), polyamide (PA) and polyacrylonitrile (PAN) fibres show excellent features like good strength, high chemical resistance, low abrasion and shrinkage properties. However, synthetic fibres share common disadvantages, such as high hydrophobicity and crystallinity, which affect not only wearing comfort (making these fibres less suitable to be in contact with human skin), but also processing of fibres, impeding the application of finishing compounds and colouring agents. Most of the finishing processes/agents are water-dependent, which require an increase in hydrophilicity of fibre surface (Burkinshaw 1995; Jaffe & East 1998; Yang 1998; Frushour & Knorr 1998). Currently, chemical treatments with sodium hydroxide are used to increase hydrophilicity and improve flexibility of fibres. However, chemical treatment is hard to control, leading to unacceptable losses of weight and strength, and to irreversible yellowing in the case PAN and PA fibres. Besides, this is not an environmentally appealing process since it requires large amounts of energy and chemicals. A recently identified alternative is the use of enzymes for the surface modification of synthetic fibres (Gübitz & Cavaco-Paulo 2003). The use of cutinase on vinyl acetate (a co-monomer in acrylic fibre) was described by Silva et al. (2005), while lipases and esterases are mainly used for biomodification of PET. Enzymatic hydrolysis of PET fibres with different lipases increased hydrophilicity, measured in terms of wetability and absorbent properties (Hsieh et al. 1997; Hsieh & Cram 1998). A polyesterase was reported by Yoon et al (2002), for surface modification of PET and polytrimethyleneterephthalate (PTT). The authors reported that formation of terephthalic acid, (a hydrolysis product), could be monitored at 240 nm. The enzymatic treatment resulted in significant depilling, efficient desizing, increased hydrophilicity and reactivity with cationic dyes and improved oily stain release (Yoon et al. 2002). The production of polyester-degrading hydrolases from a strain of *Thermomonospora fusca* was investigated and optimized (Gouda et al. 2002). Later, Alisch et al (2004) reported biomodification of PET fibres by extracellular esterases produced by different strains of actinomycete. Fischer-Colbrie and collaborators found several bacterial and fungal strains able to hydrolyse PET fibres, after screening using a PET model substrate (bis-benzoyloxyethyl terephthalate; Fischer-Colbrie et al. 2004). O'Neill & Cavaco-Paulo (2004) came up with two methods to monitor esterase hydrolysis of PET fibres surface, as alternatives to the detection of terephthalic acid release at 240 nm. Cutinase hydrolysis of PET, will cleave ester bonds, releasing terephthalic acid and ethylene glycol, leaving hydroxyl and carboxyl groups at the surface. The terephthalic acid is quantified, after reaction with peroxide, by fluorescence determination of the resulting hydroxyterephthalic acid. Colouration of PET fibres with cotton reactive dyes, specific for hydroxyl groups, allows direct measurement of hydroxyl groups that remain on the fibre surface (O'Neill & Cavaco-Paulo 2004). Given the promising results obtained with cutinase and other PET degrading enzymes, several authors performed comparisons between different class/activity types of enzymes. All of the studies confirmed that cutinase from *F. solani pisi* exhibits significant hydrolysis on PET model substrates, as well as on PET fibres, resulting in an increased hydrophilicity and dyeing behaviour (Vertommen et al. 2005; Alisch-Mark et al. 2006; Heumann et al. 2006).

Despite the potential of cutinase from *F. solani* to hydrolyse and improve synthetic fibres properties, these fibres are non-natural substrates of cutinase and consequently turnover rates are quite low. By

the use of site-directed mutagenesis, recombinant cutinases with higher specific activity to large and insoluble substrates like PET and PA, were developed (Araújo et al. 2007). The new cutinase, L181A mutant, was the most effective in the catalysis of amide linkages of PA and displayed remarkable hydrolytic activity towards PET fabrics (more than 5-fold compared to native enzyme; Araújo et al. 2007). This recombinant enzyme was further used to study the influence of mechanical agitation on the hydrolytic efficiency of cutinase on PET and PA in order to design a process for successful application of enzymes to synthetic fibres (Silva et al. 2007; O'Neill et al. 2007). The use of cutinase opens up new opportunities for targeted enzymatic surface functionalization of PET and PA, polymers formerly considered as being resistant to biodegradation.

Recently, Nechwatal et al. (2006) have tested several commercial lipases/esterases for their ability to hydrolyse oligomers formed during manufacture of PET. These low-molecular-weight molecules are insoluble in water and can deposit themselves onto the dye apparatus, resulting in damage. The authors found that lipase from *Triticum aestivum* removed 80 wt% of oligomers from the liquor bath treatment, but the observed decrease seems to be more related to adsorption of oligomers on the enzyme than with catalytic hydrolysis of ester groups (Nechwatal et al. 2006).

Nitrilases and nitrile hydratases

Nitrilase was the first nitrile-hydrolysing enzyme described some 40 years ago. It was known to convert indole 3-acetonitrile to indole 3-acetic acid (Thimann & Mahadevan 1964; Kobayashi & Shimizu 1994). The nitrilase superfamily, constructed on the basis of the structure and analyses of amino acid sequence, contains 13 branches. Members of only one branch are known to have true nitrilase activity, whereas 8 or more branches have apparent amidase or amide condensation activities (Pace & Brenner 2001; Brenner 2002). All the superfamily members contain a conserved catalytic triad of glutamate, lysine and cysteine, and a largely similar α-β-β-α structure. Nitrilases are found relatively infrequently in nature. This enzyme activity exists in 3 out of 21 plant families (*Gramineae*, *Cruciferae* and *Musaceae*; Thimann & Mahadevan 1964), in a limited number of fungal genera (*Fusarium*, *Aspergillus*, *Penicillium*; Harper 1977; Šnajdrová et al. 2004; Vejvoda et al. 2006; Kaplan et al. 2006), but it is more frequently found in bacteria. Several genera such *Pseudomonas*, *Klebsiella*, *Nocardia* and *Rhodococcus* are known to utilize nitriles as sole sources of carbon and nitrogen (Bhalla et al. 1995; Hoyle et al. 1998; Dhillon et al. 1999; Kiziak et al. 2005; Bhalla & Kumar 2005). Manly due to the biotechnological potential of nitrilases, different bacteria and fungi capable of hydrolysing nitriles were isolated (Singh et al. 2006). Most of the nitrilases isolated consisted of a single polypeptide with a molecular mass of 30–45 kDa, which aggregate to form the active holoenzyme under different conditions. The prevalent form of the enzyme seems to be a large aggregate composed of 6–26 subunits. Most of the enzymes show substrate dependent activation, though the presence of elevated concentrations of salt, organic solvents, pH, temperature or even the enzyme itself may also trigger subunit association and therefore activation (Nagasawa1 et al. 2000).

Nitrile hydratase (NHase) is a key enzyme in the enzymatic pathway for conversion of nitriles to amides, which are further converted to the corresponding acid by amidases. Several micro-organisms (*Rhodococcus erythropolis*, *Agrobacterium tumefaciens*) having NHase activity have been isolated and the enzymes have been purified and characterized (Hirrlinger et al. 1996; Stolz et al. 1998; Trott et al. 2001; Okamoto & Eltis 2007). NHases are composed of two types of subunits (α and β) complexed in varying numbers. They are metalloenzymes containing either cobalt (cobalt NHases) or iron (ferric NHases).

Surface modification of polyacrylonitrile (PAN)

PAN fibres exhibit excellent properties such as high chemical resistance, good elasticity and natural-like aesthetic properties, which contribute to the increased use of these fibres, currently about 10% of the global synthetic fibre market. However, the hydrophobic nature of PAN fabrics confers undesirable properties resulting in a difficult dyeing finishing process (Frushour & Knorr 1998). Chemical hydrolysis of PAN fibres at the surface generally leads to irreversible yellowing of fibres. Thus, as with other synthetic fibres, selective enzymatic hydrolysis of PAN could represent an interesting alternative. The surface of PAN was modified by nitrile hydratase and amidase from different sources (*Rhodococcus rhodochrous* and *A. tumefaciens*). After enzymatic treatment the fabric became more hydrophilic and the adsorption of dye was enhanced (Tauber et al. 2000; Fischer-Colbrie et al. 2006). Similarly, in a work by Battistel et al (2001) treatment of PAN with nitrile hydratases from *Brevibacterium imperiale*, *Corynebacterium nitrilophilus* and *Arthrobacter* sp. resulted in an increase of amide groups on the PAN surface giving increased hydrophilicity and

dyeability. In another study, a *Micrococcus luteus* strain *BST20* was shown to produce membrane-bound nitrile hydrolysing enzymes. By determining the NH_3 release from PAN powder and measuring the depth of shade of enzyme treated fabric after dyeing with a basic dye, the enzymes were shown to hydrolyze nitrile groups on the PAN surface (Fischer-Colbrie et al. 2007).

The biomodification of acrylic fibres using a nitrilase, instead of nitrile hydratases/amidases, was demonstrated by Matamá et al (2006). Addition of 1 M sorbitol and 4% *N,N*-dimethylacetamide to the treatment media enhanced catalytic efficiency.

Although there is no industrial application yet, the results of research demonstrate that enzymatic treatment of PAN would give advantages in the quality of treated fibres, as well as in energy saving and pollution control.

Laccases

Laccases are extracellular, multicopper enzymes that use molecular oxygen to oxidize phenols, and various aromatic and non-aromatic compounds by a radical-catalysed reaction mechanism (Thurston 1994). They belong to a larger group of enzymes termed the blue-multicopper oxidase family. Laccases have been found in plants, insect, bacteria, but are most predominant in fungi (Benfield et al. 1964; Claus 2004; Baldrian 2006). Laccase activity has been demonstrated in more than 60 fungal strains (Gianfreda et al. 1999). Typical fungal laccase is a protein of approximately 60–70 KDa with a pH optimum in the acidic pH range, and optimal temperature range between 50 and 70°C. Few enzymes with optima temperature below 35°C have been described, an example being the laccase from *Ganoderma lucidum* with its highest activity at 25°C (Ko et al. 2001). The range of substrates with which laccases can react is very broad, showing a remarkable lack of specificity towards their reducing substrate.

Decolourization of dyes and textile bleaching

Laccases are widely researched for the decolourization of textile effluents. Due to their ability to degrade dyes of diverse structures, including synthetic dyes, laccases are an environmentally friendly tool to treat dye wastewater (Abadulla et al. 2000; Hou et al. 2004; Couto et al. 2006; Salony et al. 2006; Hao et al. 2007). They have also been studied for textile bleaching. Bleaching of cotton is achieved by the decolourization of natural pigments giving cotton fibres a white appearance. The most common industrial bleaching agent is hydrogen peroxide usually applied at temperatures close to boiling. However, high temperatures and alkaline pH can cause severe damage to the fibres, and large amounts of water are needed to subsequently remove the hydrogen peroxide from fabrics. Laccases can improve whiteness of cotton by oxidation of flavonoids. The substitution or combination of chemical bleaching with an enzymatic bleaching system leads not only to less fibre damage, but also to significant water economy (Tzanov et al. 2003a). Pereira and collaborators isolated a new strain of *Trametes hirsuta* for cotton bleaching. Laccases of this organism were responsible for oxidation of the flavonoids morin, luteolin, rutin and quercetin. The authors reported that pretreatment of cotton with *T. hirsuta* laccases resulted in an increase of whiteness (Pereira et al. 2005). Later, ultrasound was used to intensify the efficiency of enzymatic bleaching. The authors found that low intensity ultrasound improved diffusion of the enzyme from the liquid phase to the fibre surface, acting synergistically with the enzyme in the oxidation of natural pigments (Basto et al. 2007). In denim finishing, there are already some successful industrial applications of laccases like DeniLite® commercialized by Novozyme (Novo Nordisk, Denmark) and Zylite from the company Zytex (Zytex Pvt. Ltd, Mumbai, India).

The application of laccases for the coating of natural and synthetic fibres is under study. Tzanov et al. (2003b,c) developed a laccase-assisted dyeing process for wool, using low temperatures without dyeing auxiliaries, which permits saving water and energy. More recently, Kim and collaborators (2007) described the use of natural flavonoids to dye cotton by an enzymatic process catalysed by laccases (Kim et al. 2007).

Catalases

Catalases (CATs), more correctly hydroperoxidases, catalyse the degradation of H_2O_2 to H_2O and O_2. They are produced by a variety of different microorganisms including bacteria and fungi (Mueller et al. 1997) and most have optima at moderate temperatures (20–50°C) and neutral pH. CATs from animal sources (bovine liver) are generally cheap; therefore, the production of microbial CATs will only be economically advantageous when recombinant strains and cheap technology is used, or for CATs with special properties such as thermostability or operation at alkaline or acidic pH.

Treatment of bleach liquor

In the textile industry, bleaching with H_2O_2 is performed after desizing and scouring, but before

dyeing. Historically, a reducing agent was used to destroy the hydrogen peroxide, or water to rinse out the hydrogen peroxide bleach, but CAT can now be used to decompose excess H_2O_2 (Fraser 1986). This eliminates the need for a reducing agent and minimizes the need for rinse water, resulting in less polluted wastewater and lower water consumption. The cost of enzyme for degradation of hydrogen peroxide in bleaching effluents could be reduced by the introduction of immobilized enzymes, allowing not only the recovery of enzyme, but also the reuse of treated bleaching effluents for dyeing (Costa et al. 2001; Paar et al. 2001; Fruhwirth et al. 2002).

Enzyme use in related markets: the detergent industry

Most of the enzymes previously reported can be used in detergent formulations. In fact, the most successful and largest industrial application of enzymes is in detergents. The first use of enzymes in detergents goes back to the use of pancreatic extracts by Roehm in 1913. However, the use of enzymes from animal sources had limited success, as those enzymes were not suited to prevailing washing conditions. The first detergent containing a bacterial enzyme was introduced into the market in the 1960s (Maurer 2004). Due to environmental concerns, since the early 1980s detergent manufacturers have replaced phosphate with other detergent builders, such as zeolite and silicates, and developed and incorporated bleach activators. New proteases that were stable at alkaline pH, showed good washing performance at low temperatures, in the presence of sequestering agents, bleach and surfactants were sought. The bacterial subtilisins were identified as being the most suitable for detergent applications (Saeki et al. 2007).

At present only proteases and amylases are commonly used, to remove proteinaceous and starchy stains, respectively. More recently, cellulases have been incorporated in detergents to remove pills, reducing the fuzzy appearance and restoring lustre. Lipases are under research and can be used to remove fatty stains, especially at low temperatures and on blends of cotton/polyester. The most recent introduction of a new class of enzyme into detergent formulation is the addition of a mannanase. This enzyme helps removal of various food stains containing guar gum, a commonly used stabilizing agent in food products (Bettiol & Showell 1999). The most recent innovation in the detergent industry is the use of psychrophilic enzymes able to work effectively in cold water, allowing the save of energy (Cavicchioli et al. 2002). Currently, the majority of enzymes used in detergents are subtilisins isolated from *B. licheniformis, B. lentus, B. alcalophilus* or *B. amyloliquefaciens*. They can now be generated by recombinant techniques (heterologous expression) and engineered in any aspect, as already described. Products like Purafect xP (Genencor), Everlase, Savinase, Esperase (Novozymes), were created and have been used as detergent additives for several years (Maurer 2004).

Conclusions and future prospects

Enzymes can be used in order to develop environmentally friendly alternatives to chemical processes in almost all steps of textile fibre processing. There are already some commercially successful applications, such as amylases for desizing, cellulases and laccases for denim finishing, and proteases incorporated in detergent formulations. Further research is required for the implementation of commercial enzyme based processes for the biomodification of synthetic and natural fibers. An active field of research is the search for new enzyme-producing micro-organisms and enzymes extracted from extremophilic micro-organisms (Schumacher et al. 2001).

There is still considerable potential for new and improved enzyme applications in future textile processing.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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