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REVIEW ARTICLE

Protein interactions in enzymatic processes in textiles

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Enzymes are the catalysts of all reactions in living systems. These reactions are catalysed in the active sites of globular proteins. The proteins are composed by amino acids with a variety of side chains ranging from non-polar aliphatic and aromatic to acidic, basic and neutral polar. This fact allows to a globular 3D protein to create in the active site all ranges of microenvironments for catalysis. Major advances in microbial technology and genetics allow recently the broad range of enzymatic applications in the industry. Enzymatic processes have been increasingly incorporated in textiles over the last years. Cotton, wool, flax or starches are natural materials used in textiles that can be processed with enzymes. Enzymes have been used for desizing, scouring, polishing, washing, degumming, peroxide degradation in bleaching baths as well as for decolourisation of dyehouse wastewaters, bleaching of released dyestuff and inhibiting dye transfer. Furthermore many new applications are under development such as natural and synthetic fibres modification, enzymatic dyeing, finishing etc. Most of the textile processes are heterogeneous where an auxiliary as a dye, enzyme, softener or oxidant have to be taken from the solution to the fibre. These processes require the presence of surface-active agents, ionic force "balancers", buffers, stabilisers and others, and are characterized with high turbulence and mechanical agitation in the textile baths. In this paper it is intended to understand and discuss the major protein interactions within textile processes and to try to anticipate troubleshooting possibilities when enzymes are used. It can be expected that an enzyme protein can interact with all chemical agents in solution due to the large variety of side chains of the outer-amino-acids in the large 3D structure of the protein. Without the aim of being exhaustive various points will be discussed where protein interactions are important for textile processing.

BACKSTAINING DURING CELLULASE STONE-WASHING

The production of "aged" denim garments (*i.e.* Jeans) with cellulases is the most successful enzyme process that emerged in the textile industry in the last decade. The aged look is obtained by the non homogenous removal of the Indigo dye trapped inside the fibres by the cooperative

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action of enzymatic hydrolysis and mechanical stress such as beating and friction. During enzymatic treatment of denim with cellulases the removed Indigo was found to backstain the cotton fabric, whereas no such behaviour was observed with the traditional stone-wash with pumice stones and without enzymes. The redeposition of Indigo dye on the cotton fibres diminishes the desired contrast between white and blue yarns and can be easily noted on the reverse side of the fabric. Due to commercial interests the phenomenon was studied extensively in the last years and it was found that beside dye-cellulose interactions (Andreaus et al. 2000) mainly dye-cellulase interactions (Clarkson et al. 1994; Cavaco-Paulo et al. 1998; Campos et al. 2000; Gusakov et al. 2001) are the major cause for Indigo backstaining. The presence of *Trichoderma reesei* cellulase proteins in aqueous Indigo dispersions leads to the adsorption of cellulases to Indigo (Campos et al. 2000), the reduction of Indigo particle size (Campos et al. 2000) and results in a better "solubilization" of the insoluble Indigo dye (Andreaus et al. 2000).

Neutral cellulases from *Humicola insolens* at low concentrations and some isopeptides seem to promote Indigo agglomeration (Campos et al. 2000). Gusakov et al. 2001 have shown that protein adsorption on Indigo is much less specific than cellulase adsorption on cellulose. Even albumin and purified cellulase enzymes without a cellulose binding domain (CBD), which do not bind to Avicel cellulose (<1-7 mg mg-1), showed a reasonable binding (57-111 mg mg-1) to Indigo dye. As in the case of the binding of CBDs to cellulosic substrates (Reinikainen, 1994) the Indigo-protein interactions are mainly due to surface hydrophobic and aromatic amino acid residues (Gusakov et al. 2000; Gusakov et al. 2001). The binding forces between Indigo and proteins are not strong and due to hydrophobic interactions and formation of hydrogen bonds. On endoglucanases EG3 and EG5 *from P. verruculosum* and *C. lucknowense*, respectively, Gusakov et al. 2001 identified two possible Indigo binding sites consisting of 2-4 aromatic residues (Tyr, Trp and/or Phe). In both enzymes one of them was located near the active site cleft.

Three principally different mechanisms for backstaining were proposed. Andreaus et al. 2000 and Andreaus et al. 2001b suggested that cellulase proteins interact with Indigo, reduce Indigo particle size and act as carriers of fine Indigo particles, already dispersed in the bulk solution, to the cotton fabric. Since cellulases adsorb and desorb continuously during their hydrolytic activity on cotton cellulose (Azevedo et al. 2000), it can be expected that cellulase proteins function as carriers for the microfine Indigo particles. After enzyme desorption from the cotton fabric Indigo particles remain attached to the cellulosic fibers. In fact, cellulase enzymes can carry up to 250 times their weight to deliver other materials to cellulosic fabrics (Jones and Perry, 1998). The adsorption of Indigo to cellulases and the capacity to carry microfine Indigo particles depends on the type of the enzyme and the presence and the type of the cellulose binding domain of the used enzyme. (Cavaco-Paulo et al. 1998; Andreaus et al. 2000). Whole enzymes (CBH I of *Trichoderma reesei*, EG V of *Humicola insolens* and Cen A of *Cellumonas Fimi*) were found to have more affinity to Indigo and cause more backstaining than the core enzyme without CBD (Andreaus et al. 2000; Gusakov et al. 2001). In the case of CBH I the removal of the CBD, which contains a planar strip of three aromatic Tyr residues, explains the reduced Indigo affinity of the core enzyme.

Gusakov et al. 2001 suggested that the removal of colour from denim is related to the enzymes affinity to Indigo and its capacity to transport the insoluble dye from the fabric into solution. This would mean that no real backstaining occurs, but the dye actually does not leave the fabric without the aid of the enzyme or another solubilizing agent. They found that three endoglucanases (EGII from *Trichoderma reesei*, EG3 from *P. verruculosum* and EG5 from *C. lucknowense*) with the highest Indigo affinity demonstrated the best washing performance (abrasive activity). They supported this idea also by the finding that the purified endoglucanases EG3 and EG5, which do not have a CBD, have a significantly higher content of aromatic and overall non-polar residues than CBH I, CBHII and EG I from *Trichoderma reesei*, the commercially most used enzymes for denim washing, and are able to bind more Indigo. This mechanism would explain, why no correlation between enzyme activity (saccharifying activity) and colour removal (topolytic activity) was found (Gusakov et al. 1999).

A third proposal for a backstaining mechanism, made in the beginning of backstaining research (Clarkson et al. 1994), refers to the presence of proteins with high Indigo affinity in the cellulase preparations, that do not desorb from the cotton fabrics after treatment. The addition of proteases to the cellulase treatment solution or the post-washing formulation was suggested and found to be efficient to reduce this kind of backstaining (Clarkson et al. 1994; Andreaus et al. 2000). Anyhow, due to the reversibility of the binding of cellulases to cellulosic fibres (Linder et al. 1996; Cavaco-Paulo et al. 1999) this mechanism does not seem to play an important role. Several studies demonstrated that severe post-washing procedures reduce backstaining (Andreaus et al. 2001b). Nonionic surfactants and dispersing agents were found to be the most efficient chemical reagents at low concentrations to desorb Indigo from cotton. The same products are also efficient, when applied in the denim treatment solution together with the cellulases (Andreaus et al. 2001a). Another possibility is the after-treatment with laccases, which are able to oxidise insoluble Indigo to water soluble isatin and anthranilic acid (Campos et al. 2001). Similar to cellulase-Indigo interactions, laccases showed, depending on origin, different Indigo affinity and were shown to influence Indigo particle size.

In industry different cellulases and ageing processes for

denim garments are in use. Beside *Trichoderma* cellulases, which still seem to be the most important cellulases, a big variety of cellulases, mainly produced by fungi, are available. For ageing of denim or other dyed textiles monocomponents are preferred over total crude mixtures. Variables such as enzyme concentration, washing time and addition of abrasive material are selected according to the desired ageing effect. Recently cellulases are also used for the ageing of sulphur or pigment dyed garments. The selection of the cellulase enzyme depends mostly on the pretended ageing effect, the level of backstaining and the price of the enzyme.

DYEING IN DYEBATHS PREPARED WITH ENZYMATICALLY RECYCLED TEXTILE EFFLUENTS

High water and energy costs in textile wet processing imposed the implementation of closed-loop processes and recycling of the textile liquors. For the adequate processing of the textile materials it is necessary to apply various additives. The removal of hydrogen peroxide or hydrolysed dyestuff after the bleaching and dyeing processes respectively, are the two major areas where the washing liquors can be recycled by means of an appropriate enzymatic treatment with *i.e.* catalases, laccases, peroxidases, and azoreductases. This treatment could be carried out with enzymes in both free (Tzanov et al. 2001a) and immobilised form. Usually, low concentration of the free enzymes is sufficient to achieve the desired effect, however the catalyst is not recoverable and stability problems may arise from the high temperature and alkalinity of the bleaching, washing or dyeing liquors. The main disadvantage of using free enzymes is that the protein remains in the recycled effluent that is intended to be reused for dyeing. The dyeing is carried out mostly at temperatures where proteins undergo denaturation. The transition from globular to random coil conformation of the protein occurs during denaturation at elevated temperature. This thermally initiated process increases the hydrophobic dye-protein interactions. The denaturated protein precipitates, binds dye from the dyeing solution and thereby decreases its concentration. The presence of denaturated protein does not alter the dye exhaustion kinetics but reduces the final exhaustion level (Tzanov et al. 2001b). The use of immobilized enzymes will avoid the above-described problem and will provide resistant against inactivation catalysts.

EFFECT OF IONIC AND NON-IONIC ADDITIVES IN THE TEXTILE BATHS ON ENZYME ACTIVITY

From another point of view the interactions between dye and protein in recycling dyehouse wastewater and other applications where enzymes and dyes might be used together, can be beneficial in terms of improved enzyme stability at stress temperatures and pHs. Matulis reported (Matulis et al. 1999) that synthetic azoaromatic sulfonate dye anions frequently provide notable protection for enzymes from inactivation by acids and elevated temperature, through a co-precipitation-protection mechanism. These anionic dyes bind to enzyme molecules forming ion pairs between negatively charged sulfonate groups and positively charged protein groups. The ratio of positively and negatively charged amino acids side chains varies, depending on pH. Thus the composition of the protein-dye complex depends on the pH of the medium. Proteins tend also to bind some protons from water to neutralise the excessive negative charge, resulting in increased pH. When the number of bound sulfonate anions is equal to the total positive charge of the protein, the protein dye complex precipitates. However, co-precipitation does not always result in protection. Anionic dyes with small hydrophobic group did not have protecting ability. The addition of increasingly large aliphatic or aromatic groups increased the ability of the sulfonate for enzyme stabilisation. Large organic non-polar groups displace water from near environment of ion pairs, preventing in such a way the denaturant action of the water. On the other hand, a too bulky hydrophobic tail was not effective due to possible steric hindrance during binding. Disulfonates were better protectors than monosulfonate dyes.

All textile processes due to their heterogeneous character require the application of surface-active agents to accomplish various functions – wetting, dispersing and solubilising, levelling, retarding, foaming, defoaming and others. Many of them are more or less complex mixtures, of imprecisely known structures or are subject of trade confidentiality. Furthermore, it is often difficult to differentiate between auxiliaries promoted purely for commercial reasons and those that serve a definite technical need. Ionic surfactants are known to show strong associative behaviour with globular proteins in aqueous solutions. The charged headgroup of a surfactant is electrostatically attracted to an oppositely charged amino acid residue of the protein. Additionally the alkyl chain of the surfactant is hydrophobically attracted to non-polar regions on the surface as well in the interior of the globular protein. At higher concentrations complexes between protein and aggregated surfactants are formed, resulting in precipitation. The protein-surfactant precipitate complex is neutral. Three phases were observed – solution phase, precipitation phase and gel phase (Moren et al. 1997). On further addition of surfactant, the precipitate re-dissolves forming a solution phase. At relatively higher protein concentration the formation of a viscous gel phase was identified. The interactions between anionic surfactants and proteins have been more extensively studied than those between protein and cationic surfactants (Kaneshina et al. 1973; Nozaki et al. 1974; Subramanian et al. 1984; Subramanian et al. 1986; Ericsson and Hegg, 1987; Prieto et al. 1993; Magdassi et al. 1996). It has been observed that cationic surfactants show a weaker primary interaction with proteins compared with anionic surfactants (Nozaki et al. 1974; Ananthapadmanabhan, 1993). This was explained by

the weaker attractive hydrophobic interaction with the negatively charged amino acids, owing to the fact that the hydrophobic part carried by the different negatively charged amino acids residues is shorter than that of the positively charged residues.

The binding isotherm of an ionic surfactant, according to Vermeer and Norde, 2000, is best described by an initial steep part that corresponds to electrostatic binding of the surfactant to oppositely charged sites on the protein molecule. After saturation of the charged sites, at higher concentration, a co-operative association takes place that is driven by hydrophobic interactions. The concentration at which this co-operative binding occurs is lower than the critical micellar concentration (CMC). In contrast to ionic surfactants, non-ionic surfactants exhibit only co-operative binding at higher surfactant concentrations.

Vermeer and Norde, 2000 investigated the effect of low-molecular weight surfactants (sodium dodecyl sulphate - anionic, and polyoxyethylene-sorbitan monolaurate – non-ionic) on the thermal stability of proteins by means of differential scanning calorimetry. At low surfactant concentration the thermal stability of the protein was not affected. With increasing surfactant concentration the surfactant molecules do not only bind to the cationic sites of the protein, but also hydrophobic interactions become increasingly more dominant. The hydrophobic tail of the surfactant will penetrate into the hydrophobic domains of the protein globular molecule in order to reduce their contact with water. Due to such penetration the protein molecule may deform somewhat. The surfactants chain length also influences the stability of proteins (Jones and Manley, 1980; Yamasaki et al. 1996). With increase of chain length the hydrophobic forces become dominant. At higher concentration the surfactant forms a layer of micellar structure on the protein molecule. This surfactant layer or micellar matrix may decrease the conformational mobility of the unfolded protein thereby increasing the stability of the globular state. Such a stabilising effect of the surrounding shell has already been reported several decades ago and in more recent studies related to encapsulation of proteins in reversed micelles (Battistel et al. 1988; Shastry and Eftink, 1996; Rariy et al. 1998). The unfolded protein has less conformational space available to explore inside a given volume and consequently a compact protein structure is stabilised by a confined environment. Some oligomeric enzymes, *i.e.* catalase, are known to lose catalytic activity in the presence of sodium dodecyl sulphate, due to dissociation into their constituent subunits. This is not valid for thermophilic enzymes, where additional stabilization results from increased number of electrostatic interactions within and between subunits (Walker et al. 1980; Jaenicke et al. 1996; Jaenicke, 2000).

Göller showed that addition of compatible solutes (glycine, betaine, hydroxyectoine) shifts the enzyme's activity curve towards higher temperature (Göller and Galinski, 1999). The increase in temperature stability is gained at the expense of a slightly reduced maximal activity. In the presence of $(NH_4)_2SO_4$ enzyme activation as well as stabilisation has been observed, resulting not only in a shift of the activity profile but also in different shape of the curve. The stabilising properties of inorganic ammonium sulphate against both high temperature and urea denaturation were most remarkable. Urea is the most widely used hydrotropic agent for textile dyes while the salts take part in any dyebath composition. A comparison with other salts revealed that the sulphate ion is mostly responsible for this effect. Dötsch et al. 1995 examined salt-stabilization of globular protein structure in very concentrated aqueous urea solutions (6-7 M) and reported 100% stabilization with the addition of 2 M $(NH_4)_2SO_4$ or 2.5 M NaCl. Carpenter and Crowe, 1988 reported a destabilising influence of "salting-out" salts like ammonium sulphate when applied at concentrations lower than 0.5 M. At higher concentrations, however, all tested salts, even NaCl, which is not regarded a "salting-out" salt, displayed enzyme stabilising properties. The ionic strength of the enzymatic solutions is one of the most important factors affecting the biocatalyst performance. The high amount of salt presented in the dyeing effluents increases the ionic strength and enhances the electrostatic coupling of the anionic dyes and the positively charged proteins, thereby forming more stable dye/enzyme aggregates. Apparently, in the case of salts the degree of stabilisation/destabilisation strongly depends on the protein: salt ratio. Most dyeing operations in textile mills are normally preformed with salt concentrations above 0.5 M. It is well known fact that the stabilising properties of solutes strongly depend on the enzyme under investigation (Lippert and Galinski, 1992). Solutes and urea have opposing effects as proposed by Baskakov and Bolen, 1998. While urea, by loosening the structural conformation, facilitates unfolding, any stabilising solute, which increases compactness, will relieve denaturing effects to a certain degree. The more compact conformation of the enzyme requires increased activation energy (higher temperature) to be disrupted and the enzyme remains active over a wider temperature range. The stabilising properties of compatible solutes are not only concentration dependent but also depend on the nature of the enzyme.

Non-polar amino acids constitute about one half of surface area of proteins and are organised as hydrophobic surface clusters (Longo and Combes, 1999). The interaction of these hydrophobic clusters with water is responsible for the heat-induced denaturation of biocatalysts. Hence it is necessary to protect these non-polar clusters from contact with water. Water could be considered as a reactant in inactivation reactions and as a lubrificant in conformational changes associated with protein unfolding. Stabilisation with hydrophilic compound near to the hydrophobic cluster will trap the surrounding water and create a protecting shield to the hydrophobic regions. The hydrophilic molecules however could also attract water and facilitate its contact with enzyme surface instead of protecting it. Thermo stability is mainly ascribed to the high number of

hydrophobic interactions, while decrease in thermal stability appears to be related to hydrophilisation of the biocatalyst (Illanes, 1999).

In general it is believed, that the stabilizing compounds do not strengthen the protein conformation by specific binding as would a substrate or an inhibitor (Wimmer et al. 1997). The stabilizing effect of these compounds has been attributed mainly to their exclusion from the protein surface, hence leading to "preferential hydration" of the protein, or "preferential exclusion" of the additive from the protein surface (Lee and Timasheff, 1981; Timasheff et al. 1989; Gekko and Ito, 1990). There is however no universal cause of exclusion valid for all types of additives. Stabilising and destabilising compounds have been categorized into two groups depending on their mechanism of interaction with proteins (Arakawa et al. 1990; Timasheff, 1993). In the first group preferential interaction between compound and protein are determined solely by the properties of the solvent and are totally independent of the chemical nature of the protein surface; the protein and the additive being inert towards each other. The most widespread mechanism behind exclusion of such an additive is its enhancing effect on the surface tension of water, as in the case of sugars, amino acids and some salts.

The second category comprises compounds exhibiting selective repulsion or attraction with certain part on the protein surface. In the case of exclusion, the additive is repelled from certain groups on the protein leading to the formation of a hydration layer. The predominant cause of exclusion has been suggested to be the solvophobic effect, as with glycerol and other polyols where contacts between water and non-polar sites on the protein are more favourable than those between the additive and these sites. A compound belonging to this category can, for instance, both be excluded from the protein surface due to the solvophobic effect and at the same time be attracted to certain polar groups on the surface. Glycerol is a hydrotropic agent useful for all class of dyes and polyols find application as emulsifiers and defoamers.

Intermediate cases of the two categories can also exist with both mechanisms operating simultaneously. For instance PEG is excluded non-specifically from the globular protein but interacts specifically with the nonpolar residues exposed upon denaturation (Timasheff, 1993), and thus is a weak protein destabilizer. It does not denaturate proteins at room temperature, but does decrease their melting temperature (Timasheff and Inoue, 1968; Pittz and Timasheff, 1978; Arakawa and Timasheff, 1985; Lee and Lee, 1987). PEG is commonly used emulsifier in textile processing. PEG derivatives are applied as softeners and lubrificants.

A strong correlation between solution stabilization and preferential interaction has been observed for many compounds. Numerous experimental results of preferential interaction measurements are now available for a variety of protein stabilizers, including sugars, amino acids, amines, polyols and certain salts (ammonium, sodium, and magnesium sulphate). Although these compounds differ widely in chemical properties, one thing they share in common is preferential exclusion from the surface of native proteins. In the presence of destabilizers, the free energy of denaturation becomes smaller and hence the stability of the protein decreases (Arakawa et al. 2001).

One major difference between the protein stabilizing and destabilizing excluded compounds is in their chemical nature. The first class of compounds is very polar and has almost no hydrophobic groups in the molecule, whereas the second class has hydrophobic character. This implies that co-solutes such as PEG, may have some affinity for the denaturated state of the protein, which has many more exposed hydrophobic groups (Arakawa et al. 2001).

Amar et al. 2000 found that stabilisation against temperature might be achieved by the binding substrate itself due to change in pH and dielectric constant, based on electrostatic forces. The pH and dielectric constant in the microenvironment of the enzyme change with the increase of the temperature (Amar et al. 1998). The addition of counter-parts stabilises the enzyme. Surfactants may facilitate the transfer of hydrophobic substrate through the layer of water molecules to the enzyme-binding site (Khalaf et al. 1996).

Costa et al. 2002 studied the storage stability of native catalase treated with different additives at stress conditions, *i.e.*. pH 10 - 12, and 60 and 70ºC. At pH 10 and 11 the glycerol was the only additive to improve the enzyme stability. The maximum in the pH profile of catalase activity, shifted toward more alkaline pHs in the presence of additives. The high capability of polyols to form hydrogen bonds should play the most important role in catalase stabilization, increasing the degree of organization of water molecules and preserving the water shell around the protein molecule (Yuji et al. 1984; Michiaki et al. 1997; Longo and Combes, 1999; Noriko et al. 1999). The stabilizing effect of structured water suggests that organic solvents could stabilize the proteins acting by analogous mechanism.

EFFECT OF CROSS-LINKING ON ENZYME STABILITY

Cross-linking with bi-functional reagents, *i.e.* glutaraldehyde (Costa et al. 2001) might stabilise the enzyme. Excessive cross-linking may lead to aggregation, precipitation, loss of activity and distortion of the 3D enzyme structure (Chandrika, 1999). Various additives in different concentrations were studied in order to evaluate their effect on the catalase stability at pH 7 and 30ºC (Costa et al. 2001). The increase of additives concentration did not affect significantly the catalase activity at the above conditions, except for the glutaraldehyde. When increasing the concentration of glutaraldehyde, a bifunctional reagent,

from 0.2 to 0.6% v/v, the enzyme activity decreased. Possibly the reaction of the aldehyde groups with the amine groups of catalase promoted cross-linking of the protein chains. The linkers normally provoke changes in the conformation of the protein molecule during the cross-linking itself. On the other hand the number of possible conformations of the polypeptide molecule increases when the linker is longer. The thermally induced movement also causes a number of conformations of the chains, and probably the accumulation of all the displacements of the molecules impeded the accessibility of the active centre of the enzyme. In other words inactivation, without denaturation of the protein occurred. Another explanation could be that the cross-linking limits the thermal movement of the molecules and greater stress, which if is not uniformly distributed along the protein chains, is accumulated in the polypeptide structure, leading to its disruption.

EFFECT OF IMMOBILIZATION ON ENZYME ACTIVITY

The immobilization of enzyme on insoluble supports provides stabilisation effect at elevated temperature and pH. The stabilisation depends on the position of the support attachment to the protein molecule. In general, unfolding of soluble proteins is initiated at their most labile site. The stabilisation is most successful when this unfolding region is strengthened through immobilisation or cross-linking (Ulbrich-Hofmann and Mensfeld, 1999). For the conservation of the functional state of biocatalysts at extreme conditions, a balance between stability and flexibility should be found. The crosslinking and immobilization improve enzyme stability by decreasing the entropy of unfolding or dissociation.

Both free and immobilized catalase, according to Costa et al. 2001, showed activity maximum at pH 7. However a significant distinction can be made based on their pH and temperature sensitivity: the immobilized enzyme had a higher stability at alkaline pHs and higher temperature. The covalent binding of catalase to the support might improve the resistance of the enzyme against inactivation presumably by restricting the protein unfolding process as a result of the introduction of both intra- and inter- molecular crosslinks. The procedure of enzyme immobilization on insoluble supports has a variety of effects on protein conformation as well as on the state of ionisation and dissociation of the enzyme and its environment (Emine and Leman, 1995). It is known that in some cases, when the enzyme is coupled with a polyanionic support the pH optimum usually shifts in alkaline direction whereas if the support is polycationic the shift is in acid direction. The connection between support and biocatalyst can be obtained either by direct linkage between the components or via a link of differing length, a so-called spacer. The spacer molecule gives a greater degree of mobility to the coupled biocatalyst so that its activity can, under certain circumstances, be higher than if it is bound directly to the support. Costa et al. 2002 studied also the effect of different surfactants and peroxide stabilizing agents present in the bleaching liquor on the activity of immobilized catalase. Some anionic surfactant ($C_{10} - C_{13}$) reduced the activity of the immobilized enzyme by 96%. Studies on the influence of detergents and surfactants on the protein conformation reported that some surfactants are strong denaturants, and significantly affect the conformation of the protein (Creagh et al. 1993).

DYE-PROTEIN INTERACTIONS IN IMMOBILISED ENZYME SYSTEMS

When using enzymes immobilized on insoluble supports the interaction of the support with the textile baths additives must also to be taken into consideration. Zille et al. 2003 showed that in the first cycle of decolourization of Reactive Black 5 with immobilized on porous aluminium oxide laccase, the colour removal was mostly due to adsorption on the support and on the protein. In the next cycles partial saturation of the support occurred; the extra dye adsorption, due to the protein itself decreased and the contribution of laccase increased. The enzymatic decolourisation becomes noticeable only after saturation of the support. Even though the support was pre-adsorbed with dye, further adsorption occurs and appears to be an important factor for decolourization. After 24 hrs it was still difficult to distinguish the laccase decolourization from the alumina adsorption of the dye. Decolourization due to adsorption on the support continues even after loss of the enzymatic activity. The decolourization with immobilized laccase is a complex process, which consists of concomitant dye-support adsorption, dye-protein adsorption and enzymatic dye degradation.

CONCLUDING REMARKS

The application of biocatalysts in textile practice requires a careful investigation of the possible protein-textile chemicals interactions. The operational stability of the enzymes depends on the slight balance of stabilizing and destabilizing interactions. In general, the formation of a protecting shell around the protein, the cross-linking and immobilization are some guidelines for preservation of the globular state of the proteins and the favourable microenvironment for enzyme catalysis. The molecular basis of enzyme stabilization or destabilization is not fully understood and great controversy still exists about protein interactions mechanisms.

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