3

Catalysis and processing

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The function and application of enzymes used in textile processing are discussed in this chapter which is composed of four parts: basic thermodynamics and enzyme kinetics, function of textile processing enzymes, homogenous and heterogeneous catalysis and important applications of enzymes in textile wet processing.

The first part on thermodynamics and kinetics of enzymes describes basic thermodynamics of chemical reactions, including the concepts of free energy, collision theory and catalysed reactions. After a general introduction to enzymes, the second part gives an overview of the catalytic mechanisms of enzymes used in textile processing including amylases, cellulases, pectinolytic enzymes, esterases, proteases, nitrile hydrolysing enzymes, catalases, peroxidases and laccases.

Substrate–enzyme interactions at the active sites of these enzymes belonging to different classes are discussed and parameters influencing the reactions are listed.

Subsequently, the function of enzymes in homogenous and heterogenous enzymatic reactions is discussed more in detail and important models such as those of Michealis–Menten and Briggs–Haldane are presented. Furthermore, parameters influencing the performance of enzymes such as enzyme stability and the presence of inhibitors are discussed based on models. The relevance of these models for the development of industrial processes is shown.

In the last part of this chapter, an introduction is given to classical textile wet processing followed by a description of successful enzyme applications in textile processing such as enzymatic desizing, degradation of hydrogen peroxide in bleaching effluents by catalases, cellulase finishing and enzymes in detergents formulations. Major areas of research and potential future applications of enzymes are also discussed in this part.

3.1 Basic thermodynamics and enzyme kinetics

Enzymes as biocatalysts catalyse many essential chemical reactions taking place in living systems. Reactions catalysed by enzymes proceed faster and at moderate temperatures and pH values (Atkins and Paula, 2001; Palmer, 1995). As with all other reactions, the basic laws of thermodynamics also apply to enzyme-catalysed processes. The first law of thermodynamics states that the change of energy caused by any event in a closed system is zero, i.e. energy cannot be created or destroyed, but it can be converted to other forms of energy to perform work. The second law states that the degree of entropy or degree of disorder is always increasing. Systems with high organisation and low entropy can be maintained by consumption of energy. Basically there are two forms of energy: one that can be used to perform work, also called free energy, and the other, which cannot. In any event, process or chemical reaction only happens spontaneously as a result of the decrease of the free energy, i.e. conversation of energy into work.

Gibbs defined the increase in free energy of a system, ΔG, as $\Delta G = \Delta H - T\Delta S$, where ΔH is the variation of enthalpy and ΔS is the variation of entropy for any event at a constant temperature T. For a process to take place in a spontaneous fashion, i.e. under thermodynamically irreversible conditions, ΔS must be higher than $\Delta H/T$, giving an overall increase in entropy of the system plus surroundings, as required by the second law of thermodynamics.

For any chemical reaction, the change of Gibbs free energy (ΔG) is the energy which is available to perform work as the reaction proceeds towards chemical equilibrium from the initial concentrations of reactants and products. If the sign of ΔG is negative, the system will release free energy to its surroundings as the reaction proceeds towards the equilibrium.

For the reaction:

$$\mathrm{R} \rightleftharpoons \mathrm{P} \qquad [3.1]$$

at a given temperature T, the free energy (ΔG) is given by:

$$\Delta G = -RT \cdot \ln \frac{[\mathrm{P_{eq}}]}{[\mathrm{R_{eq}}]} + RT \cdot \ln \frac{[\mathrm{P_0}]}{[\mathrm{R_0}]} \qquad [3.2]$$

where $[\mathrm{P_{eq}}]$, $[\mathrm{R_{eq}}]$ are the concentrations of the product and reactant at equilibrium, respectively and $[\mathrm{P_0}]$, $[\mathrm{R_0}]$ are the initial concentrations of the product and reactant, respectively. (To simplify the discussion we consider 'concentrations' instead of the more correct 'activities'.)

The equilibrium constant can be defined as $K_{eq} = \dfrac{[\mathrm{P_{eq}}]}{[\mathrm{R_{eq}}]}$ and

$$\Delta G = -RT \cdot \ln K_{\mathrm{eq}} + RT \cdot \ln \frac{[\mathrm{P_0}]}{[\mathrm{R_0}]} \qquad [3.3]$$

When the initial concentration of reactants and products is 1 M,

$$\Delta G = -RT \cdot \ln K_{eq} = \Delta G^{\theta} \quad [3.4]$$

where ΔG^{θ} is also called standard free energy change, i.e. the change of energy acquired from the initial concentrations of reactants and products of 1 M in reaching the equilibrium:

$$\Delta G = \Delta G^{\theta} + RT \ln \frac{[P_0]}{[R_0]} \quad [3.5]$$

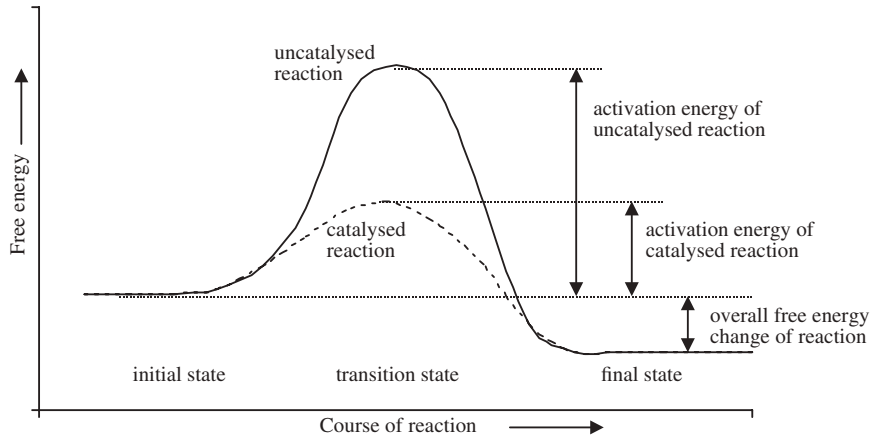
Any reaction taking place will depend on the initial concentrations of products and reactants and also on the standard free energy. The balance between these two parameters will determine in which direction a reaction will run, provided that there is no interference from outside the system. However, in reality, this situation hardly ever exists and even with an overall negative free energy some reactions do not proceed. This can be explained by concepts such as collision theory and potential barrier or free activation energy. Chemical reaction can only occur when molecules collide. However, not all collisions are effective, i.e. not all colliding molecules will react with each other. This is mainly when colliding molecules do not have proper orientation or they do not have enough energy to react. This energy needed to initiate the reaction is called potential barrier or free activation energy.

Eyring postulated that every chemical reaction proceeds via the formation of an unstable intermediate between reactants and products, in the transition state. If the energy available in the system as collision energy is higher than a certain potential barrier, the reaction takes place. If not, the unstable intermediate returns to the initial state.

A catalyst accelerates a chemical reaction without changing its extent and with no overall thermodynamic effect, i.e. the amount of free energy change is the same in the presence or absence of the catalyst. The catalyst only reduces the amount of activation free energy resulting in a more stable transition state. In this fashion a more efficient transition intermediate is formed upon interaction between reactants and the catalyst (Fig. 3.1).

These principles can be applied to enzyme catalysis where an intermediate transition state is formed between a substrate and an enzyme accelerating the conversion of a substrate into a product. In this reaction, the substrate must fit precisely into the active site of the enzyme. Since enzymes are highly specific catalysts, it can be expected that the formation of the enzyme–substrate complex or the binding of the substrate in the active site will require only little energy. Consequently, enzymes are very effective catalysts, enhancing reactions up to 10 000-fold more than the most effective chemical catalysts:

$$\underset{\text{(initial state)}}{E + S} \rightleftharpoons \underset{\text{(intermediate state)}}{ES} \rightarrow \underset{\text{(final state)}}{P} \quad [3.6]$$



3.1 Change in free energy in catalysed and uncatalysed reactions.

3.2 Function of textile processing enzymes

Enzymes are proteins which are composed of folded peptide chains containing a wide range of amino acids. In living systems, mainly 20 different amino acids occur with a structural variety ranging from non-polar (aliphatic and aromatic) to acidic, basic and neutral polar properties. Therefore, depending on the amino acid composition and the three-dimensional (3D) structure of the protein, different microenvironments for catalysis exist at the active sites of enzymes. The high substrate specificity of enzymes is due to the individual architecture of the active site where only certain molecules can 'stereo-fit in'. Only little energy is required for the formation of this enzyme–substrate complex and therefore enzyme-catalysed reactions proceed very fast. Enzymes are generally active at mild temperatures because the enzyme proteins need to maintain their folded state in order to operate. Enzyme-catalysed reactions also proceed at mild pH-values; however, at the catalytic site, extreme acid or basic environments for catalysis can exist even when the reactions are carried out at neutral pH values. Depending on the organisms, some enzymes are also stable at extreme temperatures and pH values such as those from extremophiles living under these conditions.

Ionic bonds are important for the structural stability of a folded enzyme protein; it can be expected that the degree of protonation of the amino acid residues are a major issue and minor changes in pH have great effect on the stability of an enzyme and on its activity. Since the velocity of chemical reactions generally increases with temperature, the optimum temperature of an enzyme will be the highest temperature at which the enzyme protein can be maintained in a folded native state. Analysis of 3D structures and

amino acid homology between thermophilic and non-thermophilic representatives of the same class indicate that enzyme structures with tighter loops, a higher level of glycosylation and/or higher level of crosslinkages have higher temperature optima and stability, and can also tolerate higher levels of agitation (Danson and Hough, 1998). In living systems enzymes catalyse essential chemical reactions under optimum reaction conditions via, for example, acid–base catalysis, covalent bonding or electron transfer mechanisms.

Acid–base catalysis is a common mechanism in enzyme reactions – for example, hydrolysis of ether, ester or peptide bonds, phosphate group reactions, additions to carbonyl groups and others. Acid catalysis usually involves donation of a proton by the catalyst while base catalysis involves abstraction of a proton. The side chains of the amino acids Asp, Glu, His, Cys, Tyr and Lys can be involved in general acid–base catalysis. Covalent catalysis involves rate enhancement by the transient formation of a covalent bond between the substrate and the catalyst, especially involving side chains of His, Cys, Asp, Lys and Ser.

Enzyme systems involving metal ion catalysis accelerate the reaction velocities by binding substrates in the proper orientation, mediating oxidation–reduction reactions and electrostatically stabilising or shielding negative charges. Metalloenzymes contain tightly bound metal ions such as Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+} or Mn^{2+}, while metal-activated enzymes contain loosely bound metal ions such as Na^{+}, K^{+}, Mg^{2+} and Ca^{2+}. Electrostatic catalysis refers to the fact that when a substrate binds to an enzyme, water is usually excluded from the active site. This causes the local dielectric constant to be lower, which enhances charge–charge interactions at the active site. Proximity and orientation effects are also important in enzymatic reactions. The 3D structure of the enzyme can bring several reactive side chains into close proximity to the active site. Binding of the substrate at the active site can orientate the substrate for most efficient interaction with these side chains. Enzymes commonly used in textile processing will be discussed next.

3.2.1 Amylases

Amylases are widely used as desizing agents to remove starch from fabrics after weaving. Starch is a polysaccharide composed of glucose units primarily linked by α (1–4) glucosidic bonds with α (1–6) linked side chains. Depending on the number of branches, two types of polymer, amylose and amylopectin, are distinguished. Amylopectin accounts for around 70–80% of starch, containing branches at about every 20–24th glucose residue, while amylose is a much more linear polymer.

Enzymes involved in the complete degradation of starch are α-amylases, (EC 3.2.1.1), β-amylases (EC 3.2.1.2) and glucoamylase (EC 3.2.1.3).

α-Amylases hydrolyse randomly and are *endo*-acting on the α (1–4) bonds within the starch backbone, while the *exo*-acting glucoamylases cleave-off glucose units from the non-reducing ends of polysaccharides. The industrially less important β-amylases release only maltose units from the chains ends of starch polymer. However, these enzymes are not able to bypass branches. The enzyme mechanism usually involves two acidic amino acid residues, such as aspartic acid and/or glutamic acid along with a basic amino acid residue, e.g. histidine. In the same way as for most glycoside hydrolases there are two basic mechanisms: the α–α retaining mechanism of α-amylases or glucoamylase and the α–β inverting mechanism characteristic of β-amylases.

Since amylases have been naturally designed to act on an insoluble substrate, most amylases have an extra substrate binding domain. The substrate binding domain brings the catalytic domain into the close vicinity of the target substrate, enhancing the catalytic performance of the enzyme (Watanabe *et al.*, 2001 and Horvathova *et al.*, 2001). Studies on pancreatic amylases revealed that chlorine ions could also be essential for hydrolysis of starch. It is believed that chloride is required to increase the acidity at the active site thereby enhancing hydrolysis (Numao *et al.*, 2002).

Most commercial amylases used are crude mixtures of thermostable enzymes of bacterial origin. Amylases are activated by Ca^{2+} ions and it is known that these enzymes perform well in hard water rich in bivalent ions (Cavaco-Paulo, 1998). The presence of Ca^{2+} enhances the enzymatic reaction up to a certain level and is believed to stabilise the catalytic sites through structural organisation. The presence of calcium ions is a very important feature of bacterial thermostable amylases (stable up to 110°C) where they (up to three calcium cations) are believed to enhance the stability of enzyme by crosslinking the folded structure (Machius *et al.*, 1998).

α-Amylase activity is generally measured using starch as a substrate, monitoring the formation of reducing sugars using maltose as a standard (Numao *et al.*, 2002). Advanced activity measurement techniques for α- and β-amylases use oligosaccarides and follow the production of shorter chain sugars by chromatography (Watanabe *et al.*, 2001 and Horvathova *et al.*, 2001). β-amylase activity can be measured towards *p*-nitrophenyl-α-D-maltopentaose monitoring the release of *p*-nitrophenyl (Erkkilä *et al.*, 1998), while glucoamylase activity can also be determined following the release of *p*-nitrophenol from *p*-nitrophenyl-α-D-glucose (Lee *et al.*, 2001).

3.2.2 Cellulases

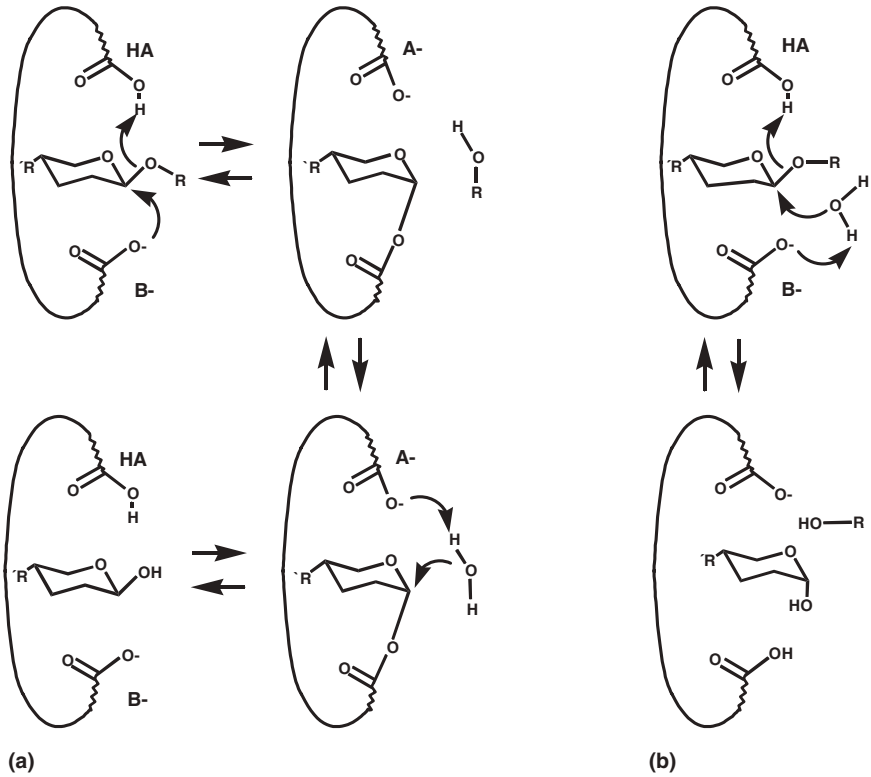
Cellulases are used in textile processing mainly for depilling and to obtain stone washing effects. Cellulases are also used as part of detergent formu-

lations to enhance detergency, to improve brightness and to remove microfibrils (Cavaco-Paulo, 1998). In nature, cellulose, the world's most abundant polysaccharide, is enzymatically hydrolysed by the synergistic action of endo-β-1,4-glucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) and β-glucosidases (EC 3.2.1.21). It has been suggested that endoglucanases (EGs) randomly cleave cellulose into smaller fragments generating new ends which are then hydrolysed endwise by the action of cellobiohydrolases. These latter enzymes are also thought to erode crystalline regions of cellulose making them more susceptible to EG attack (Wood, 1992). However, it is widely recognised that the classification of β-1,4-glucanases into exclusively *endo*- and *exo*-acting enzymes is, in many cases, not strictly definitive, as several enzymes have been isolated exhibiting both types of enzyme activities (Tomme *et al*., 1996).

Like amylases, cellulolytic enzymes also employ an acid–base mechanism for the hydrolysis of their substrates, which involves two acidic amino acid residues such as aspartic acid and/or glutamic acid. However, in contrast to amylases, their catalytic activity and stability are generally independent of the presence of metallic ions. Hydrolysis of cellulose is catalysed via the β–β retaining mechanism or via the β–α inverting mechanism (Fig. 3.2).

Like amylases, cellulases have a catalytic domain and a substrate binding domain. In the past a considerable amount of work has been carried out to classify or group various cellulases and hemicellulases based on the degree of homology of the amino acid sequence of the various catalytic and binding domains of the enzymes. Several fungal cellulases have been grouped with enzyme families that are more closely related to known bacterial enzymes than they are to each other (Henrissat and Bairoch, 1996). Consequently, EGs from different microbial origins have shown similar substrate specificities, both on isolated and synthetic oligo- and polysaccharides and on their 'natural' substrates such as wood. In contrast, some closely related EGs have shown quite different substrate specificities (Tomme *et al*., 1995). Important retaining cellulases belong to families 5, 7 and 12 while inverting cellulases are found in families 6, 8 and 45 (Davies and Henrissat, 1995; Henrissat, 1991; Henrissat and Bairoch, 1993, 1996). Cellulose binding domains (CBDs) of fungal origin are from family I (also called carbohydrate-binding module family 1) and they account for 33–36 amino acid residues, while bacterial cellulose binding domains from family II (also called carbohydrate-binding module family 2) are bigger with 105–120 amino acids. Family I CBDs are present in almost all cellulase preparations commonly used in textile and detergent applications and they bind cellulosic fibres reversibly while family II CBDs bind cellulose more strongly (Cavaco-Paulo *et al*., 1999).

Cellulase activities can be measured towards insoluble cellulose in the form of filter paper, or microcrystalline cellulose eventually swollen in



3.2 (a) β–β Configuration retaining mechanism of cellulose hydrolysis by cellulase enzymes. (b) β–α Configuration inversion mechanism of cellulose hydrolysis by cellulase enzymes.

phosphoric acid. Reducing sugars released can be monitored by, for example, the DNS (dinitro salicylic acid) method (Ghose, 1987). In a commercial mixture, the values obtained with this method reveal the hydrolysis rate caused by the synergistic action of EG and cellobiohydrolase activities. EG activity can be measured towards carboxymethylcellulose (CMC) following the release of reducing sugars or the decrease of viscosity of CMC solutions. Enzyme preparations completely free of EG activity do not show any activity towards CMC solutions (Cavaco-Paulo *et al.*, 1996 and Ghose, 1987).

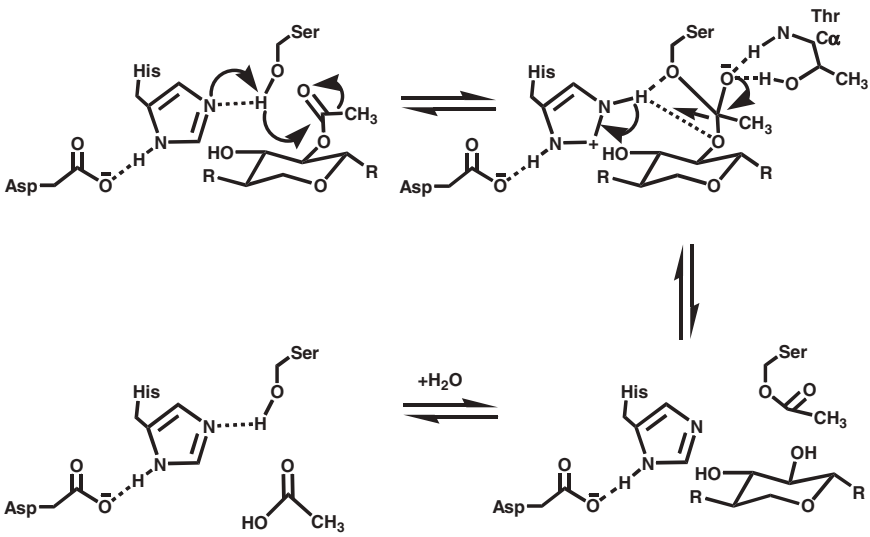
3.2.3 Pectinolytic enzymes

Pectin-degrading enzymes have received much interest for their use in the pretreatment of textile fabrics ('bioscouring') prior to dyeing. The removal of pectin components from the cotton cell wall is claimed to improve

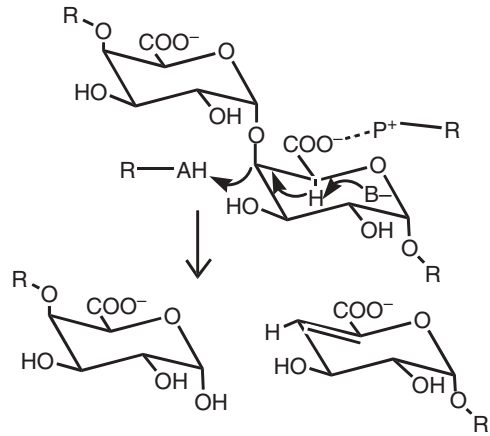
fibre hydrophilicity, to facilitate dye penetration and to contribute to substantial water savings when compared to the traditional alkaline scouring process.

In nature, three major classes of enzymes are involved in the degradation of pectins: pectin esterases, polygalacturonases and pectin lyases. Pectin esterases (EC 3.1.1.11) catalyse the de-esterification of polymethylgalacturonate forming pectic acid (polygalacturonate). Pectin esterases commonly employ a Ser-His-Asp catalytic triad such as acetylxylan esterases to catalyse deacetylation, but other mechanisms such as a Zn^{2+} catalysed deacetylation may also be considered for some families (Fig. 3.3). Polygalacturonases cleave α(1–4) glycosidic linkages in polygalacturonate and can be divided into two groups according to their mode of action on the polymer: endopolygalacturonases (EC 3.2.1.15) hydrolyse randomly within pectic acid while exopolygalacturonases (EC 3.2.1.67) cleave in a sequential fashion generally from the non-reducing end of the pectin chain. Only little is known about the stereochemistry of the hydrolysis reaction but there seem to exist both retaining and inverting endo- and exopolygalacturonases (Biely *et al.*, 1996).

Pectin lyases cleave polygalacturonate or pectin chains via a β-elimination resulting in the formation of a double bond between C4 and C5 at the non-reducing end (Fig. 3.4). There are three major types of lyases: endopolygalacturonate lyases (EC 4.2.2.2) which randomly cleave polygalacturonate chains, exopolygalacturonate lyases (EC 4.2.2.9) which cleave at the



3.3 Mechanism of hydrolysis of the acetyl xylan esterase by the triad Asp-Hist-Serine (Hakulinen *et al.*, 2000).



3.4 Schematic diagram of α-1,4-polygalacturonic acid cleavage by the β-elimination mechanism (Herron *et al.,* 2000).

chain end of polygalacturonate yielding unsaturated galacturonic acid and endopolymethylgalacturonate lyases (EC 4.2.2.10) which randomly cleave pectin (Sakai *et al.*, 1993 and Whitaker, 1989).

Enzymes that solubilise pectin from protopectin are called protopectinases (Sakamoto and Sakai, 1994). Various enzymes including endoarabinases, pectate lyases and polygalacturonases can show this ability and are called protopectinases to distinguish these enzymes from classical endoarabinases, pectate lyases and polygalacturonases without pectin-releasing activity (Ferreyra *et al.*, 2002; Matsumoto *et al.*, 2000).

Pectin esterase activity can be measured with pectin (polymethylgalacturonate) as a substrate monitoring the pH change in the solution caused by the formation of carboxylic acids. Another possibility for detecting pectin esterase activity is to measure methanol released from the substrate (Sakai *et al.*, 1993). It should be noted that at pH values higher than 7, pectin can be hydrolysed. The determination of endo- and exopolygalacturonase activity towards pectin can be followed by the formation of reducing sugars. Since the enzyme catalyses the depolymerisation of a soluble substrate, an alternative assay method measures the decrease in viscosity. To distinguish between *endo* and *exo* enzymes, chromatographic techniques that identify short chain oligosaccharides formed or viscosity methods can be used (Whitaker, 1989). Pectin lyase activity towards pectic acid can be measured by monitoring the absorbance change at 235 nm caused by the formation of the unsaturated product. Another method for determining pectin lyase activity is based on the formation of a red complex caused by the reaction between the unsaturated galacturonic acid and thiobarbituric acid

(Whitaker, 1989). Protopectinase activity is measured using protopectin as substrate (Sakai *et al*., 1993).

3.2.4 Esterases

Esterases have been suggested as useful components of detergent formulations to remove lipid-based stains from textiles while some esterases have been claimed to hydrolyse polyester. Esterases hydrolyse ester bonds and their classification is based on the type of ester bond hydrolysed.

Esterases with applications in textile processing include carboxylesterases (EC 3.1.1.1) which hydrolyse carboxylic esters yielding the corresponding alcohol and carboxyl anion, arylesterases (EC 3.1.1.2) which hydrolyse phenyl acetate to phenol and acetate and triacylglycerolesterases (EC 3.1.1.3) which hydrolyse triacylglycerol giving a diacyl glycerol and fatty acid anion. The latter enzymes are better known as lipases. Many esterases are multifunctional enzymes and they can work as carboxylesterases, lipases and others. In the hydrolysis reaction, the catalytic triad Ser-His-Asp can be involved in the same way as some proteases (Fig. 3.3). Esterases can show activation in water/lipid interfaces which has been described particularly for lipases dependent on the pH of the medium (Petersen *et al*., 2001 and Cambillau *et al*., 1996). Cutinases, a class of acyl esterases, are particularly active on cutin and do not show any interfacial activity even though these enzymes have been described as hydrolysing triglycerides (Cambillau *et al*., 1996).

Carboxylesterase activity is measured, for example towards *o*-nitrophenyl butyrate; arylesterases are measured, for example towards phenyl acetate; and lipases are assayed towards triacylglycerols (e.g. olive oil). These reactions can be monitored via pH change or alternatively via numerous colorimetric methods following the products formed (Bergmeyer, 1974).

3.2.5 Proteases

Proteases are important components of detergent formulations for removing protein stains (egg, blood etc.) from textiles. Additionally, proteases have a useful potential in silk and wool processing. Proteases or, more correctly, peptidases hydrolyse peptide bonds in soluble and insoluble peptides and form the group EC 3.4.X.X. of hydrolases. Peptidases can be divided into endopeptidases and exopeptidases, which cleave peptide bonds within the protein or release amino acids sequentially from either the N- or C-terminus, respectively. Proteases have been grouped into families and clans according to the homology in their catalytic domains. According to the mechanism of hydrolysis these enzymes have been grouped into serine, cysteine, aspartic and metallo-proteases. Representatives of serine pro-

teases are mammalian chymotrypsin and trypsin or the bacterial subtilisin with the catalytic triad consisting of Ser-His-Asp. Cysteine-type proteases include papain with the catalytic triad of Cys-His-Asn while in the catalytic reaction of aspartic type proteases such as pepsin two aspartates are involved. Metallopeptidases such as thermolysin generally contain a Zn atom which is involved in the catalytic reaction.

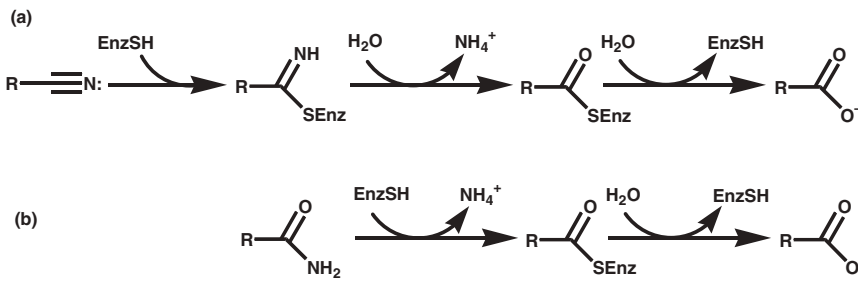
Protease activities can be measured towards proteins such as casein or haemoglobin by following the release of hydrolysis products colorimetrically. Other more specific substrates are used if the hydrolysis of a certain peptide bond is targeted (Beynon and Bond, 1996; Bergmeyer, 1974).

3.2.6 Nitrile-hydrolysing enzymes

Nitrilases have been shown to improve dye uptake and hydrophilicity of acrylic fibres. These improved properties are achieved by enzymatic conversion of nitrile groups into carboxylic acid groups at the surface of acrylic fibres.

In nature, three different groups of enzymes are involved in the microbial hydrolysis of nitriles (Fig. 3.5). Nitrilases (EC 3.5.5.1, EC 3.5.5.7) hydrolyse nitriles to the corresponding carboxylic acids forming ammonia; nitrile hydratases (EC 4.2.1.84) form amides from nitriles which can subsequently be hydrolysed by amidases (EC 3.5.1.4) (Tauber *et al.*, 2000). Formerly, nitrilases were thought to hydrolyse exclusively aromatic substances while aliphatic nitriles were believed to be degraded by a nitrile hydratase/amidase enzyme system. However, recent investigations have shown that this strict rule does not always apply.

The reaction mechanism, regulation and photoactivation of nitrile hydratases, which usually consist of α- and β-sub-units containing either non-heme iron or cobalt atoms have been studied in detail (Kobayashi and Shimizu, 1998). Hydrolysis of nitriles and amides by nitrilases and amidases,



3.5 (a) Enzymes hydrolysing nitriles are classified to branch 1 of the nitrilase superfamily. (b) The amidase reaction is the most frequently observed activity of enzymes classified to other branches of the nitrilase superfamily.

respectively, is catalysed via a thiol acyl enzyme intermediate involving the Glu-Lys-Cys catalytic triad (Pace and Brenner, 2001). Nitrile-hydrolysing enzymes can be assayed towards nitriles such as to acetonitrile and/or benzeno nitrile methods yielding the respective amides and carboxylic acids and the reaction is generally followed by chromatography (Tauber *et al.*, 2000).

3.2.7 Catalases, peroxidases and catalase-peroxidases

Catalases (EC 1.11.1.6) can be used in textile processing for the removal of residual hydrogen peroxide after bleaching while peroxidases (EC 1.11.1.7) have a potential for dye decolourisation after dyeing (Gudelj *et al.*, 2001).

Catalases convert hydrogen peroxide into water and oxygen showing first order kinetics. This loop reaction starts by oxidation of the catalase to compound I by one molecule of hydrogen peroxide yielding water and regeneration via production of oxygen from the second molecule of H_2O_2 (see reactions [3.7] and [3.8]). Usually catalases have heme-containing prosthetic groups. Bifunctional catalase-peroxidases can oxidise substrates other than H_2O_2 (Zamocky *et al.*, 2001). In the first step catalase-peroxidase compound I is formed because of oxidation by peroxide. Compound I is situated two oxidation equivalents higher and has a porphyrin-π-cation radical with an iron (IV) centre and can be reduced to the starting form by hydrogen peroxide. Alternatively compound I can be reduced by a one-electron reduction to Compound II, which is the peroxidase reaction. Compound II has an amino acid radical ($R^{\bullet}$) and iron (III). Finally, Compound II is reduced to the starting form by a second one-electron reduction.

$$\underset{\text{Ferric enzyme}}{\text{Fe(III)}\ldots\text{R}} + \text{H}_2\text{O}_2 \rightarrow \underset{\text{Compound I}}{[\text{Fe(IV)}{=}\text{O}\ldots\text{R}]^{\bullet+}} + \text{H}_2\text{O} \qquad [3.7]$$

$$\underset{\text{Compound I}}{[\text{Fe(IV)}{=}\text{O}\ldots\text{R}]^{\bullet+}} + \text{H}_2\text{O}_2 \rightarrow \underset{\text{Ferric enzyme}}{\text{Fe(III)}\ldots\text{R}} + \text{O}_2 + \text{H}_2\text{O} \qquad [3.8]$$

$$\underset{\text{Compound I}}{[\text{Fe(IV)}{=}\text{O}\ldots\text{R}]^{\bullet+}} + \text{AH}_2 \rightarrow \underset{\text{Compound II}}{[\text{Fe(III)}{=}\text{O}\ldots\text{R}]^{\bullet+}} + \text{AH}^{\bullet} \qquad [3.9]$$

$$\underset{\text{Compound II}}{[\text{Fe(III)}{=}\text{O}\ldots\text{R}]^{\bullet+}} + \text{AH}_2 \rightarrow \underset{\text{Ferric enzyme}}{\text{Fe(III)}\ldots\text{R}} + \text{AH}^{\bullet} \qquad [3.10]$$

Catalases catalyse reactions [3.7] and [3.8] and catalase-peroxidases catalyse reactions [3.7], [3.9] and [3.10] (Zamocky *et al.*, 2001).

During lignin degradation, fungi employ so-called manganese-peroxidases (EC 1.11.1.13) requiring the presence of manganese ions:

$$2\text{Mn(II)} + 2\text{H}^{+} + \text{H}_2\text{O}_2 \rightarrow 2\text{Mn(III)} + 2\text{H}_2\text{O} \qquad [3.11]$$

These enzymes and other peroxidases can also be used for textiles dye degradation.

Catalase and peroxidase activities can be measured spectrophotometrically following the degradation of hydrogen peroxide at 240 nm and the colour change during the oxidation of various substrates, respectively (Gudelj *et al.*, 2001).

3.2.8 Laccases

Laccases in combination with redox mediators are used in textile processing to bleach denim fabrics, decolourising indigo. Research efforts have been made to use laccase as a bleaching and/or oxidative coupling agent for dyeing animal fibres and human hair.

Laccases (1.10.3.2) are unspecific oxidoreductases which catalyse the removal of a hydrogen atom from the hydroxyl group of *ortho* and *para*-substituted mono- and polyphenolic substrates and from aromatic amines by one-electron abstraction while the cosubstrate oxygen is reduced yielding water. Free radicals formed in this reaction from the substrates are capable of undergoing further depolymerisation, repolymerisation, demethylation or quinone formation. The rather broad substrate specificity of laccases may be additionally expanded by addition of redox mediators such as ABTS [2,2′-azinobis(3-ethylbenzthiazoline-6-sulphonate)]. These blue oxidases typically contain four copper atoms per polypeptide chain distributed in three different copper binding sites (types I, II and III). It is believed that the initial oxidation of the enzyme by oxygen occurs at the T2/T3 site followed by an electron transfer from T1 to T2/T3 site and further oxidation of the substrate (Gianfreda *et al.*, 1999). Laccases are assayed following the oxidation of various substrates such as dimethoxyphenol, ABTS or syringaldizine spectrophotometrically (Abadulla *et al.*, 2000).

3.3 Homogeneous and heterogeneous enzyme catalysis and kinetics

3.3.1 Enzyme kinetics of homogenous systems

Kinetics is the study of reaction rates measured by the change in quantity of reactants with time. Chemical kinetics is ruled by the law of mass action. This law states that the rate of reaction is proportional to the product of the activities of the reactants (A,B) considering the stoichiometric constants (a,b) of each reactant:

$$a\mathrm{A} + b\mathrm{B} \rightarrow \text{production} \qquad [3.12]$$

$$v = k[\mathrm{A}]^a \cdot [\mathrm{B}]^b \qquad [3.13]$$

For practical proposes activity can be replaced by concentration measured in molarity. The order of the reaction is a for the reactant A and b for the reactant B and of general order $a + b$. The rate v of the reaction:

$$\mathrm{A} \rightarrow \mathrm{P} \qquad [3.14]$$

can be described as:

$$v = -\frac{\mathrm{d[A]}}{\mathrm{d}t} = +\frac{\mathrm{d[P]}}{\mathrm{d}t} = k[\mathrm{A}] \qquad [3.15]$$

where k is a rate constant and [A] and [P] are the concentrations of reactant A and the product P at the time t.

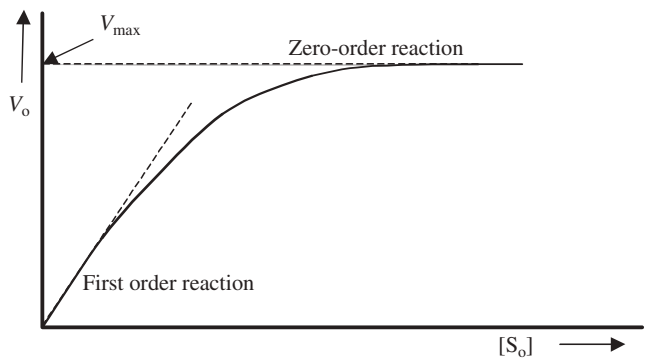
$-\frac{\mathrm{d[A]}}{\mathrm{d}t}$ and $+\frac{\mathrm{d[P]}}{\mathrm{d}t}$ describe the rate of decrease of A and increase of P, respectively. The rate of reaction at various times can be found by taking tangents in a plot of concentration change versus time and calculating their gradients. The reaction orders for each reactant are experimentally determined by measuring the initial reaction rates at different initial concentrations of this reactant.

These rules can be also applied to enzymatic reactions. Enzyme-catalysed reactions occurring in homogenous media where both the substrate(s) and the enzyme are in solution show a general trend: the initial rates are first order at low substrate concentrations and zero order at very high substrate concentrations (Fig. 3.6).

This behaviour can be explained by the formation of an enzyme–substrate complex:

$$\mathrm{E} + \mathrm{S} \underset{k_b}{\overset{k_a}{\rightleftharpoons}} \mathrm{ES} \overset{k_c}{\rightarrow} \mathrm{E} + \mathrm{P} \qquad [3.16]$$

(initial state) (intermediate state) (final state)



3.6 Typical initial rates – substrate dependence.

During the reaction all the enzyme is usually present in the form of the enzyme–substrate complex ES if the concentration of the enzyme is much lower than the concentration of the substrate. A quasi-steady state for the enzyme–substrate complex can be assumed:

$$-\frac{\mathrm{d}[\mathrm{ES}]}{\mathrm{d}t} = k_a[\mathrm{E}][\mathrm{S}] - k_c[\mathrm{ES}] - k_b[\mathrm{ES}] = 0 \quad [3.17]$$

Using the mass balance for the enzyme in free or associated form:

$$[\mathrm{E}] = [\mathrm{E}_0] - [\mathrm{ES}] \quad [3.18]$$

From the equation:

$$k_a[\mathrm{E}][\mathrm{S}] = (k_c + k_b)[\mathrm{ES}] \quad [3.19]$$

the concentrations of ES can be determined to give:

$$k_a[\mathrm{E}_0][\mathrm{S}] - k_a[\mathrm{ES}][\mathrm{S}] = (k_c + k_b)[\mathrm{ES}] \quad [3.20]$$

or

$$[\mathrm{ES}] = \frac{[\mathrm{E}_0][\mathrm{S}]}{k_m + [\mathrm{S}]} \quad [3.21]$$

where:

$$k_m = \frac{k_b + k_c}{k_a} \quad [3.22]$$

With the reaction rate for the dissociation of the enzyme–substrate complex and formation of the product:

$$v = k_c[\mathrm{ES}] \quad [3.23]$$

the result is the Michaelis–Menten equation:

$$v = \frac{k_c[\mathrm{E}_0][\mathrm{S}]}{k_m + [\mathrm{S}]} \quad [3.24]$$

For the maximum reaction rate:

$$v_{\max} = k_c[E_0] \quad [3.25]$$

we obtain:

$$v = \frac{v_{\max}[\mathrm{S}]}{k_m + [\mathrm{S}]} \quad [3.26]$$

k_m gives the substrate concentration $[S_0]$ at v_0 as 1/2 $v_{\max}$. k_m is also called the Michaelis–Menten constant.

For an enzymatic reaction, k_c is also called the turnover number k_{cat}, which represents the maximum number of substrate molecules that can be

Table 3.1 Classical linearisation methods for Michaelis–Menten equation

Equation	Plot
$\frac{1}{v_0} = \frac{k_m}{v_{max}} \frac{1}{[S_0]} + \frac{1}{v_{max}}$	Lineweaver–Burk
$v_0 = -k_m \frac{v_0}{[S_0]} + v_{max}$	Eadie–Hofstee
$\frac{[S_0]}{v_0} = \frac{1}{v_{max}} [S_0] + \frac{k_m}{v_{max}}$	Hanes–Woolf

converted by a unit of time. In more complex enzymatic reactions involving several steps and various intermediates following Michealis–Menten kinetics k_{cat} can be seen as a function of several individual reaction rates.

k_{cat}/k_m can be regarded as the catalytic efficiency of an enzyme. Comparing the values of k_{cat}/k_m for different substrates and one enzyme, this value can be regarded as the specificity of an enzyme towards a substrate. k_m can be regarded as the affinity of an enzyme towards a substrate, or the stability of enzyme substrate complex, i.e. higher k_m, lower affinity and lower stability.

The determination of v_{max} and k_m may involve the determination of initial reaction rates for several substrate concentrations at a given enzyme concentration. The classical linearisation methods of the Michealis–Menten equation have been employed over the years to determine the parameters while some of them give considerable errors (Table 3.1). Nowadays Michealis–Menten parameters can be estimated directly by non-linear regression methods using computer programs.

Although many enzyme-catalysed reactions can be described by this simple Michealis–Menten model, some enzymes like catalases show very high turnover numbers where the enzyme can never be saturated with its substrate hydrogen peroxide because, for example, H_2O_2 destroys the catalase at very high concentrations. On the other hand, a lot of biological reactions involve more than one substrate and complex enzyme systems. In several reactions involving more than one substrate, the Michealis–Menten model can still be applied to one individual substrate provided that the other substrates are present in excess. However, no information can be obtained about the exact multisubstrate reaction mechanism (e.g. random, ordered and ping-pong).

3.3.2 Enzyme catalysis in heterogeneous systems

In heterogeneous systems at least the catalyst or one of the reactants or products is present in a different phase from the others. An example of the

application of an insoluble enzyme used to convert soluble substrates related to textile processing is the application of immobilised enzymes such as laccases or catalases for the treatment of dyeing and bleaching effluents, respectively. Most of the enzyme applications in textile processing, however, involve heterogeneous systems consisting of soluble enzymes and insoluble substrates in the form of textile materials or their components. Classical examples of heterogeneous enzymatic catalysis are the enzymatic hydrolysis of insoluble polymers like wool or silk by proteases and cotton or synthetic fibres by cellulases. Most carbohydrolases such as cellulases, pectinases and amylases are known to have substrate binding domains. These enzymes have been designed by nature with a special peptide binding to the substrate which is the driving force of the soluble enzyme in attacking an insoluble substrate. It is believed that substrate binding domains increase the concentration of the enzyme nearby the substrate and that they are essential for efficient enzymatic hydrolysis of insoluble polymers.

Often there is a limitation in terms of accessibility of the insoluble substrate to the soluble enzyme. The enzyme can only access the outer parts of the substrate at the liquid–solid interface while inner parts are only accessible when the outer parts are removed. Interestingly, the synergistic action between several cellulase components during hydrolysis of crystalline cellulose has only been observed at lower concentrations, i.e. when there was no competition between the different cellulase components for the hydrolytic substrate sites (Woodward *et al.*, 1988). These facts are of particular importance for industrial applications using solubles enzyme for the modification of insoluble substrates, since sometimes very high enzyme concentrations are used. It is most likely that in soluble enzyme–insoluble substrate systems, enzymes are saturating the few available substrate sites. It is obvious that classical Michealis–Menten kinetics cannot be applied to these systems because of the simple fact that a solid concentration cannot be determined. In Michealis–Menten kinetics, saturation of the enzyme by the substrate is verified, but in soluble enzyme–insoluble substrate systems it is the substrate that is saturated by enzyme; therefore a relationship has been suggested (Bailey, 1989) between v_0 and $[E_0]$:

$$v_0 = \frac{v_{max}[E_0]}{k_e + [E_0]} \qquad [3.27]$$

In a similar fashion to classical kinetics, interchanging S_0 by E_0, the parameters would have similar significance but could not be interchanged, since enzyme concentration could barely be expressed in molar units. Empirically these expressions have been verified and the estimated parameters are of prime importance to characterise different enzymes systems and different process conditions (Cavaco-Paulo *et al.*, 1998).

The former dependence of initial enzyme rate on enzyme concentration can be extended to all conversion times and since the performance of an

enzyme is directly dependent on reaction rates, a performance (P) benefit can also be measured as a function of enzyme dosage (D_e).

$$P = \frac{P_{max} D_e}{D_{e,0.5P_{max}} + D_e} \quad [3.28]$$

P_{max} is the maximal performance and $D_{e,0.5P_{max}}$ is the enzyme dosage for half of maximal performance. This is of prime importance for optimisation of industrial enzyme treatments of soluble enzyme–insoluble substrate systems (Ee *et al.*, 1997).

Turnover numbers for soluble substrates are usually much higher than for insoluble substrates. For cellulases from *Humicula insolens,* it is known that turnover numbers for soluble substrates such as carboxymethylcellulose are 20 times higher than for insoluble substrates such as acid-swollen cellulose (Schulein, 1997). These low turnover numbers might allow the interaction of enzyme and the substrate almost in a quasi-reversible fashion. The number of enzyme sites on the insoluble substrate surface can be determined using typical surface adsorption isotherms, such as the monolayer Langmuir type model (Cavaco-Paulo, 1998):

$$\frac{E_{ads}}{E_{max}} = \frac{KC_e}{1 + C_e} \quad [3.29]$$

where E_{ads} is the amount of adsorbed enzyme per substrate mass, E_{max} is the maximum amount of adsorbed enzyme, K is the adsorption constant of the enzyme on the substrate and C_e is the free enzyme concentration in solution. Comparative values of E_{ads} and K can explain important characteristics about the individual enzyme substrate interaction (Cavaco-Paulo, 1998).

The adsorption of enzymes at the surface of an insoluble substrate only follows the Langmuir isotherm law when a monolayer of enzymes is formed. This is not the case when enzymes agglomerate on the substrate surface or the enzyme penetrates into a porous substrate.

Kinetic models for immobilised enzymes strongly depend on the immobilisation method. Enzymes can be attached to solid materials (glass, alumina, synthetic and natural polymers) via a range of different approaches from entrapment to covalent linking. Provided that the carrier material does not influence diffusion of the reactants and the enzyme, kinetic models for soluble enzymes can be used. However, the nature of the carrier material can lead to higher or lower concentrations of the substrate in proximity to the immobilised enzyme. Interaction of charged carrier materials and charged substrates lead to changes in the k_m values and can be described by models based on the Maxwell–Boltzmann distribution of the charged substrate between the polyelectrolyte phase and the solution.

On the other hand, the accessibility of the enzyme active site can be decreased.

The reaction rate for the immobilised enzyme can be described by extension of the Michaelis–Menten model with an efficiency factor η (Bisswanger, 2002):

$$v' = \eta v = \eta \frac{v_{\max}[\mathrm{S}]}{k_m + [\mathrm{S}]} \qquad [3.30]$$

The factor η is dependent on the substrate concentration and for η = 1 the reaction obeys the Michaelis–Menten model for the soluble enzyme while for lower values the reaction is predominantly diffusion controlled. A number of models have been developed to describe enzyme reactions controlled by external diffusion phenomena on the enzyme carrier layer and internal diffusion within porous carrier materials to the enzyme (Bisswanger, 2002). Kinetic models for immobilised enzymes usually do not consider changes in the enzyme itself which are especially likely during covalent modification.

3.3.3 Enzyme activity

We have learned in the previous section that enzymes are specific to a limited number of substrates. Especially for dosing enzymes in industrial applications, it is very important to know the exact activity of enzymes in commercial preparations which usually cannot be deduced from protein concentrations or other parameters. Assays for the determination of the activity of a certain enzyme are standardised and use well-defined substrates and reaction conditions (pH, temperature etc.). Enzyme activity is expressed as katals where one katal (kat) is defined as the amount of enzyme transforming one mole of substrate per second under standard conditions of temperature, optimal pH and optimal substrate concentration ($v_{\max}$). Previously, enzyme activity was expressed as International Units (IU) corresponding to the transformation of 1 micromole of substrate per minute ($1\,\mathrm{IU} = \mu\mathrm{mol\,min^{-1}} \approx 16.67\,\mathrm{nkat}$):

$$-\frac{\mathrm{d}[\mathrm{S}]}{\mathrm{d}t} = v_{\max} = K[\mathrm{E}] \qquad [3.31]$$

3.3.4 Enzyme inhibition or enhancement

Inhibition or enhancement of the rate of enzyme-catalysed reactions involves specific interaction of agent (inhibitors or enhancers) with catalytic or regulatory sites on the enzyme or the enzyme substrate intermediate. There are three types of reversible inhibition including competitive, non-

competitive and uncompetitive inhibition. Competitive inhibitors usually have a structure similar to the substrate and they bind in competition with the actual substrate at the substrate binding site without being transformed. At high substrate concentrations v_{max} remains unchanged while higher k_m values result. A non-competitive inhibitor does not influence the binding of the substrate but it prevents the enzyme–substrate complex from dissociating. In this case v_{max} is reduced while k_m remains the same. The non-competitive inhibitor can bind both to the free enzyme and to the enzyme–substrate complex while so-called uncompetitive inhibitor can only react with the enzyme–substrate complex changing both k_m and v_{max}. The rate equations for the different types of inhibition based on dissociation constants k_I of the enzyme (E)–inhibitor (I) complexes are presented in equations [3.32] to [3.34]:

$$\text{competitive} \quad v_0 = \frac{v_{max} \cdot [S_0]}{k_m\left(1 + \frac{[I]}{k_i}\right) + [S_0]} \qquad [3.32]$$

$$\text{non-competitive} \quad v_0 = \frac{v_{max} \cdot [S_0]}{\left(1 + \frac{[I]}{k_i}\right)(k_m + [S_0])} \qquad [3.33]$$

$$\text{uncompetitive} \quad v_0 = \frac{v_{max} \cdot [S_0]}{k_m + [S_0]\left(1 + \frac{[I]}{k_i}\right)} \qquad [3.34]$$

Both strong non-covalent binding (binding constants of $>10^{-10}$ M) and covalent binding of the inhibitor to the enzyme can lead to irreversible inhibition. A time-dependent decrease of the enzyme activity is characteristic of irreversible inhibition.

3.3.5 Stability of enzymes and half-life times

For the industrial application of enzymes both the stability of the enzymes in the process and during storage is of great interest. At extreme pH values drastic changes in the charge on the enzyme molecule can cause irreversible destruction of the native protein structure. Usually this so-called denaturation shows a first order exponential decrease in the enzyme activity. Similarly, the native structure of enzymes can be destroyed at high temperatures, by detergents and other substances. In textile applications particularly, a number of auxiliaries could potentially interact with enzymes. As an example it has been shown that sequestering agents can affect the activity of laccase chelating copper which is essential for the enzyme function. Also, multimeric enzymes like catalases are deactivated by surfactants separat-

ing the individual units. These important issues are discussed in more detail in Chapter 5 where techniques for the stabilisation of enzymes are also presented. Usually enzyme stabilities are described as half-life times of the enzyme activity. The deactivation of an enzyme generally follows a first order reaction. Based on the first order deactivation of the enzyme

$$-\frac{\mathrm{d}[\mathrm{E}]}{\mathrm{d}t} = k_\mathrm{d}[\mathrm{E}] \qquad [3.35]$$

where k_d is the deactivation constant. After integration

$$\ln\frac{[\mathrm{E}_0]}{[\mathrm{E}]} = k_\mathrm{d} \cdot t \qquad [3.36]$$

The half-life time $t_{1/2}$ is defined as:

$$t_{1/2} = \frac{\ln 2}{k_\mathrm{d}} \qquad [3.37]$$

3.4 Major enzymatic applications in textile wet processing

Enzymes can be applied in several steps of textile wet processing and in formulation of detergent powders. Since the major textile finishing process is coloration, classical finishing processes can be divided into preparation for coloration and after-coloration steps. Coloration might be done during fibre extrusion of synthetic fibres, on a bundle of fibres, on yarns, on fabrics or on garments. The sequence of processes depends on the demands of the market for the characteristics of a final product but depends essentially at which stage the coloration process is done. (If fashion or market regulations demand materials in the raw state, they are supplied unfinished.) Preparation for coloration steps generally involves the removal of impurities, natural coloured pigments, sizes and lubricants. Preparation of synthetic fibres also involves thermal treatments for uniform dyeing. After coloration, processes include chemical and mechanical processes. Industrial laundering and home washing of garments can be also included in the after-coloration processes. To give an overview of enzymatic applications in textiles a brief characterisation of major wet processing steps before and after coloration and during coloration itself will be presented.

3.4.1 Overview of traditional wet processing

Enzymes can be applied in several steps of textile wet processing and in formulations of detergent powders.

3.4.1.1 Preparation for coloration

Preparation for coloration processes aims to prepare the textile materials to receive dyes or pigments with high fastness properties. In preparation, all impurities and natural colored pigments have to be removed. Generally preparation for coloration is similar for all colours, but is more stringent for whites and lighter shades. Major processes during preparation are singeing, desizing, scouring, washing-off, bleaching, mercerising, carbonisation and thermal treatments.

Singeing consists of treatment with flames to burn out fuzz fibres directly from fabrics and is applied mainly on cellulosic materials and their mixtures. Desizing is the removal of sizes that are added to yarns to prevent breaks and stops during the weaving process. Desizing is only done on woven fabrics. Depending on the chemical nature of the size, removal could be effected by hydrolysis or oxidative processes or both. Scouring is the removal of natural impurities of natural fibres and can be applied to fibres, yarns, knitted or woven fabrics and garments. Scouring is done by neutral or alkaline washing with detergents. Washing-off is the removal of lubricants added during the spinning, knitting or weaving process to reduce friction and electrostatic energy. Washing-off is also done with detergents. Both the scouring and washing-off processes improve the hydrophilicity of the textile material and help the dyes to penetrate the fibres. Scouring is usually applied to natural fibres and washing-off is usually applied to synthetic fibres.

Carbonisation is a process applied to wool fibres to remove the vegetal soils, by treatment with sulfuric acid. Digested cellulosic impurity residues are removed from the fibres by brushing and suction. Bleaching is the removal of naturally coloured pigments in natural fibres. Nowadays it is done with hydrogen peroxide in alkaline conditions and it applied to fibres, yarns, fabrics or garments. Bleaching treatments are performed in more gentle alkaline conditions on wool and in very caustic conditions in linen. Bleaching of bast fibres most of the time involves a double bleaching process to achieve good whiteness results. Bleaching can be combined with scouring for cellulosic knitted fabrics and combined with desizing and scouring for cellulosic wovens using more concentrated alkaline conditions where sizes and natural impurities are removed along with natural pigments.

Mercerisation is the treatment of cellulosic fibres with highly concentrated solutions of caustic soda (300 g/L) under tension. Mercerisation induces intercrystalline swelling of cellulose, changing the crystal structure of cellulose I to mixture of cellulose I and II, the changes in the microstructure of cellulose being responsible for improved properties such as fibre strength, dye uptake brightness and hydrophilicity.

Thermal treatment, also called thermosetting, is used for all synthetic fibres with the aim of giving the same thermal history to the textile in order to achieve even results in further dyeing.

3.4.1.2 Coloration

Coloration is a major process in textile finishing and consists of the fixation of dyes and pigments in textile materials with high fastness properties. There are several classes of dyes, depending on the process of application and on the chemical nature of the fibre. Major classes of dyes for cellulosic fibres are direct, vat and reactive dyes. Major classes for protein fibres are acid and reactive dyes. Disperse dyes can be used mainly for polyester fibres, cationic dyes for acrylics and acid or disperse dyes for polyamides.

All dyes for cellulosic fibres are applied under neutral to alkaline conditions, since only at high pH values are cellulosic fibres charged. Direct dyes are large molecules which can have high affinity for the cellulosic fibres. The presence of sulphonic groups in their structure enhances solubility but necessitates the use of salts to balance negative charges on the fibre and on the dye molecules. Reactive dye molecules have a reactive head (vinylsulphonic or halotriazine groups) which reacts with cellulose at high pH, but like direct dyes, they have a similar need for salts. Vat dyes are insoluble and must be reduced to be soluble in water, at which stage they can be adsorbed onto the fibres with high salt concentrations and later reoxidised by air or hydrogen peroxide, as they are trapped inside the fibres.

Dyeing protein fibres is performed at neutral to acidic pHs. Acid dyes are easily adsorbed and fixed in wool fibres but with low fastness. Increased fastness can be achieved with metallic dyes that have large structures or with mordent dyes containing chromium which creates extra bonds between the dye and the fibre. Reactive dyeing of wool is performed under acid conditions using reactive groups similar to those in the reactive dyes for cotton.

Dyeing synthetic fibres is performed at temperatures above the glass transition, when dyes can penetrate better inside the fibre. The mechanism could be called ‘solubilisation’ of the dye inside the fibre, with high fastness resulting. Cationic dyes are linked to acrylics owing to the existence of negative groups on acrylics such as comonomers with sulphonic groups. Polyamides can be dyed with acid dyes because of the existence of amide groups that charge positively and can fix anionic dyes. If a white colour is demanded the fabrics can be delivered with double bleaching only, but if super whites are desired, optical brighteners can also be added to the fabric.

3.4.1.3 After coloration

Processes after coloration may include a variety of chemical and mechanical treatments where an effect can be added to or removed from the fabric: dimensional stability treatments, anti-crease finishing, softening, sanforisation, calendering, lamination, carding and others.

3.4.2 Desizing cotton with amylases

The use of α-amylases for desizing starch and their derivatives from woven fabrics was introduced almost 100 years ago. The enzymes used are mainly of bacterial origin such as *Bacillus subtilis*. Owing to advances in biotechnology a range of amylases acting at different temperatures from 20°C up to 115°C is available today. The optimum pH of the treatment lies between 5 and 7, depending on the enzymes used. All kinds of techniques can be used for the treatment ranging from padding to exhaustion methods. Amylases are used to desize fabrics made of dyed yarns, where oxidative desizing agents cannot be applied. Enzymatic desizing is the method of choice in wetting processing routes prior to dyeing when high levels of dye fastness are demanded, owing to the fast and very efficient removal of starch. Incomplete removal of starch might cause friction fastness problems.

3.4.3 Enzymatic removal of H_2O_2

Catalases were successfully introduced to the textile industry for the removal of hydrogen peroxide after bleaching and prior to dyeing at the beginning of the 1990s. The fast decomposition of hydrogen peroxide by catalases leads to a reduction in water consumption during washing the bleached cotton and prevents problems in further dyeing. For some catalases the pH of bleaching or washing liquors has to be adjusted to neutral values. Catalases are multimeric enzymes that might lose their activity in the presence of some surfactants by denaturating the fourth level structure of the enzyme, which should be considered in the choice of bleaching compositions (Costa *et al*., 2001).

3.4.4 Cellulase finishing

Cellulases are the most successful enzymes used in textile processing. They can be used to obtain an aged or renewed look for cotton fabric. Cellulase systems include the individual enzymes endoglucanases (EGs) and cellobiohydrolases. For the generation of ageing effects EGs or EG-rich mixtures are used, while for renewal and depilling effects complete mixtures can be applied. Commercially available cellulases are mainly pro-

duced from the fungi *Humicola insolens* (optimum activity at pH 7) and *Trichoderma reesei* (optimum activity at pH 5). Although monocomponent EGs and EG-enriched products have been made available recently and have proved to be successful in many applications, for economic reasons mainly cellulase mixtures are still used (Cavaco-Paulo, 1998).

3.4.4.1 Depilling/cleaning effects

Fabric or garment depilling is usually carried out after heavy processing where pills are raised. Cellulases are used for pilling removal from fabric surfaces in machinery with high levels of mechanical agitation like jets, winches or drum washing machines. The most likely mechanism of enzymatic depilling/cleaning is the action of the enzyme (adsorption/hydrolysis) on easily accessible pills (or fibrils) at the surface of a fabric (or fibre). The pills become weaker after partial hydrolysis by cellulase and they are removed from the fabric by mechanical action. This mechanism is supported by the fact that depilling effects only take place at higher levels of mechanical agitation.

3.4.4.2 Ageing effects

The action of cellulases and mechanical agitation, simultaneously or sequentially, will abrade fibre surfaces, releasing cotton powder and causing defibrillation at the surface. In denim fabrics, because of enzymatic abrasion dye or dye aggregates with cotton will be released from yarns giving contrasts in the blue colour. The fibrillation produced during the ageing process is a result of the synergistic action of cellulases and mechanical action, and therefore the aged look is produced by less abrasive methods than traditional washing with pumice stones. This is the main advantage of the enzymatic washing process.

3.4.4.3 Key features of cellulase processing

In both applications mechanical agitation is very important as it seems to create more sites for cellulase attack either because of increased diffusion into the fabric or due to the increased surface area after defibrillation. Prior to direct and reactive dyeing, hard water and high ionic strength buffers negatively influence the performance of cellulases. Similarly, ionic surfactants inhibit cellulases. Dyeability and moisture recovery are not expected to change after cellulase treatment, since no changes occur in crystallinity of cellulase-treated cotton. However, owing to defibrillation, water retention has been shown to increase. Sometimes, slightly deeper shades are apparently obtained after cellulase treatment that cleans fibre surfaces.

Experimental evidence using complete crude mixtures and EG-enriched compositions suggest that strength loss is mainly produced by EG activity.

3.4.4.4 Indigo backstaining during enzymatic washing

The redeposition of the removed indigo dye by washing on the reverse side of denim is commonly known as backstaining. A mechanism responsible for backstaining has been proposed (Andreaus *et al.*, 2000) which suggests that cellulase proteins interact with indigo, reducing indigo particle size and acting 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 of microfine indigo particles. After enzyme desorption from the cotton fabric indigo particles remain attached to the cellulosic fibres. In fact, cellulase enzymes can carry up to 250 times their weight in delivering other materials to cellulosic fabrics (Jones and Perry, 1998). The adsorption of indigo onto cellulases and the capacity for carrying microfine indigo particles depends on the type of the enzyme and the presence and type of the cellulose binding domain of the enzyme used. The best way to reduce backstaining is to perform a good wash after stone-washing, independent of the enzyme used.

3.4.4.5 Cellulosic fibres

Cellulosic fibres are currently the only 'synthetic' fibres treated with enzymes. Cellulase dosages applied to regenerated cellulose fibres are lower than for cotton as the former fibres are more susceptible to enzyme attack. This is mainly due to the fact that regenerated cellulose is present as cellulose II. In the area of synthetic fibres, cellulases are mainly used for the treatment of lyocell fabrics having a high pilling tendency after processes with strong mechanical agitation. Cellulases are essential finishing agents when used in a processing route to obtain a peach-skin feeling. When lyocell fabrics are subjected to a process with strong mechanical action, so-called primary fibrillation is produced (with raised longer fibres and fibrils). Cellulases can be used to clean fabric and fibre surfaces; thereafter another treatment with high mechanical action is applied and a secondary and uniform fibrillation is produced with very short fibrils, giving the peach-skin feeling.

3.4.5 Enzymes in detergents

Detergents are one of the most important markets for industrial enzymes (Ee *et al.*, 1997, Cavaco-Paulo, 1998). The function of the enzymes in deter-

gents is to enhance the removal of soil particles by breaking them into smaller particles which can be more efficiently washed off.

Proteases have been used since the late 1960s in fabric washing products. Unspecific enzymes are used to work on a variety of protein soils. This implies that wool and silk fabrics cannot be washed with detergent formulations containing proteases. However, under mild washing conditions and short treatment times, little or no damage is produced in these fabrics. Lipases are also used in some detergent formulations to hydrolyse fats, improving detergency of fat soils. However, the benefit of these enzymes is still under discussion. Lipases seem to adsorb on fat soils and degradation occurs between the washing steps, giving complete removal in the subsequent wash. Amylases are also part of a few detergents that remove starch soils.

Cellulases were claimed to aid detergency during fabric washing more than 30 years ago. The known effect of microfibril removal by cellulases will help to liberate entrapped soils at disrupted fibre surfaces. The cleaning of fibre surfaces from soils and loss of microfibrils will give a brighter effect to fabrics and garments making garments look renewed. However, the first cellulases available were not active enough at alkaline pH values during washing. Nowadays, alkaline cellulase preparations containing mainly EGs are available and are used in detergents.

3.5 Promising areas of enzyme applications in textile processing

3.5.1 Enzymatic scouring of cotton

Scouring cotton with enzymes is one of the areas where considerable research effort has been expended resulting in the release of a commercial product. In these studies, lipases, pectinases, proteases, cellulases and their mixtures were used to improve cotton properties. Contradictory statements are reported in the literature about the efficiency of a new bioscouring formulation based on a pectin lyase. The major advantages feasible with this product seem to be savings in water and energy consumption, since the process is carried out at milder pH values and at lower temperatures when compared with traditional boiling scouring processes.

3.5.2 Bleaching

Bleaching processes for cotton have been proposed based on the application of glucose–oxidase for controlled production of hydrogen peroxide during oxidation of glucose released during enzymatic desizing. The resulting gluconic acid has been reported to serve as a sequestering agent for metal ions (Fe III) (Tzanov *et al.*, 2001).

Laccases have been suggested as a pretreatment step for subsequent peroxide bleaching to achieve high levels of whiteness. In the future, this process might replace two consecutive peroxide bleaching steps for bleaching cotton or flax fibres (Tzanov *et al.*, 2002a). Furthermore, a laccase mediator system has been launched on the market recently for bleaching denim fabrics. However bleaching levels are still low when compared with traditional agents like hydrogen peroxide.

3.5.3 New finishing enzymes for cotton

Permanent-press finishing with crosslinking agents generally induces fabric strength loss. With crosslinking agents such as polycarboxylic acids and *N*-hydroxymethyl acryl amide, ester and amide bonds are formed after curing. A controlled enzymatic hydrolysis with lipases and proteases has shown an increase in fabric strength without the loss of the permanent-press proprieties (Tzanov *et al.*, 2002b and Stamenova *et al.*, 2003). New antiflammatory properties have also been induced on cotton by a treatment with hexokinases and adenosine triphosphate (ATP), with phosphate groups being attached at the C6 of the glucosidic units at the surface of cellulose (Tzanov *et al.*, 2002c).

3.5.4 Lignocellulosic fibres

Bast fibers (flax, hemp, jute, kenaf and others) are composed of cellulose (over 50%), hemicelluloses, lignin, pectins, fats, waxes and others substances. Bast fibres are extracted from the plant stem by a process called 'retting' as mentioned before. The purpose of retting is the partial degradation of the fibre materials, in such a way that fibres can be obtained from the plant stems. Former retting processes of flax were based on incubation with bacteria and moisture (the stem in an open grass field) or in water (immersing the stem in slow rivers); nowadays retting is more often carried out in tanks of water at 30°C. Despite being an old process, much attention has been recently given to retting. The use of enzymes like hemicellulases and pectinases for retting allows a more controlled degradation of the fibres and a reduction of effluents. The up-grading of bast fibres is based on the use of cellulases for cleaning and softening, simplifying further processing. However, in retting or further softening treatments, care should be taken since the removal of fibrous material may yield unacceptable levels of strength loss (Cavaco-Paulo, 1998).

3.5.5 Wool processing with enzymes

Most of the wet processing steps of wool are carried out under very mild agitation owing to the tendency of wool to felt. This tendency is a result of

the presence of the 'scales' of the cuticles on the wool surface. The removal or modification of these scales by oxidation and addition of polymers causes antishrinking behaviour. Most of the chemicals used for oxidation (halogen derivatives) are environmentally harmful and therefore, intensive research has been made to develop more environmentally friendly processes. The investigation of enzymatic processes for antishrink finishing of wool dates back to 1910, when trypsin and pepsin were used to clean skin scales. The first studies showed that the preswelling of the fibre could determine the extent of proteolysis. It was also stated that, if the cystine disulphide bonds remained intact, the proteolysis was slow. However, when some of the crosslinks were broken, the reaction rate increased. Several processes (already patented in the 1940s) based on an oxidative treatment followed by proteolysis had been suggested, but none was applied to the industry because of the high enzyme costs and the unacceptable weight losses obtained. Reports about the use of papain and commercial proteases after oxidative treatment, showed a good 'descaling' effect but high fibre damage. Various studies suggest that enzymes affect mainly the inner part of wool, confirming that the enzymes seem to diffuse inside the fibre, 'retting' it (Cavaco-Paulo, 1998).

The use of protein disulphide isomerase has been reported to improve the shrinkage behaviour of wool fabrics. This enzyme rearranges disulphide bonds with the aid of a cofactor in a reduced form, such as glutathione or dithiothreitol. The use of transglutaminase has also been reported to improve shrinkproofing of wool, by a rather different mechanism, with the formation of new crosslinks (*N*6-(5-glutamyl)-lysine)) and the liberation of ammonia. Attempts to replace carbonisation of wool by enzyme treatments have been made using a range of different enzymes to remove vegetable matter, reducing the amount of sulphuric acid used. However, the enzymatic degradation of vegetable materials by hydrolases such as cellulases and hemicellulases is a slow process (Cavaco-Paulo, 1998).

3.5.6 Enzymes on silk

Silk fibres are composed mainly of a double filament of fibroin surrounded by a layer called sericin. Both sericin and fibroin are proteins that have almost no cysteine residues after hydrolysis. The amount of sericin, in terms of weight loss after degumming varies between 17–38%. Sericin is mainly composed of serine (33%), aspartic acid (17%), glycine (14%) and minor quantities of other residues. Fibroin is mainly composed of glycine (44%) and alanine (29%). Sericin is more accessible to chemicals than fibroin and it is removed during preparation. An ideal degumming agent would specifically attack peptide bonds near serine residues. However, several methods have been developed for degumming silk, such as extraction with water, boiling with detergent, with alkali, acids and enzymes. Degumming with

commercially available bacterial proteases is more effective than using trypsin and papain. Proteases can also be used to alter the silk fibroin surface to give an aged look in a similar way to enzyme washing of denim garments. It has also been recently reported that proteases may provide improved softness and wetability of silk fabrics.

3.5.7 Synthetic fibres

Several enzymes can catalyse the modification of synthetic polymers. The modification of polyacrylonitrile with enzymes (nitrile hydratase) increases the number of amide groups on the fibre surface giving improved dyeability and hydrophilicity. The same enzymes are also used for production of precursors of polyamide 6,6. This could be the beginning of the application of enzymes in the production of synthetic textile fibres. Esterases and peptidases are good candidates for treatment of polyester and polyamide, but no processes have been introduced so far in the textile industry.

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