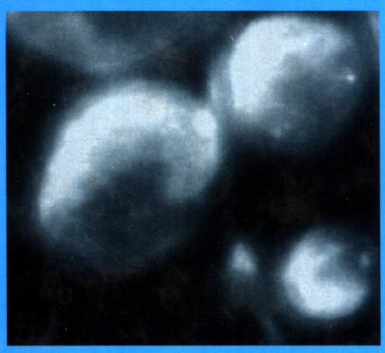
Textile processing with enzymes



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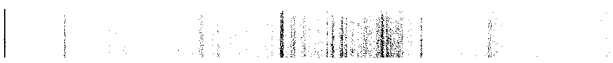

Preface

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The first use of enzymes in textile processing was reported in 1857 when starch-sized cloth was soaked with liquor containing barley. Later, in 1900, this process was slightly improved using malt extract, but only in 1912 with the use of animal and bacterial amylases was the process of enzymatic desizing introduced into many textile factories. Interestingly, amylases remained the only enzymes applied in textile wet processing for almost 70 years. In the late 1980s, cellulases were introduced with great success for depilling and defuzzing cellulose-based fabrics as well as to age garments made from materials like denim to obtain the stone-washed look. Since the early 1990s, catalases have been introduced to destroy hydrogen peroxide after bleaching, reducing the consumption of water. Pectin degrading enzyme products have been commercialised for cotton processing to replace traditional alkaline scouring. Intense investigations are being conducted on new enzyme applications for almost all cotton processing steps and for modification of cellulosic, proteic and synthetic fibres. Textile processing with enzymes is therefore a new emerging and multidisciplinary area. Engineers with knowledge and basic understanding in both textile technology and enzymology will help to introduce these environmentally-friendly processes more extensively to the industry. However, only little information about enzymes for textile processing can be found in educational programmes or in the literature.

This book was put together to generate a basic understanding of enzymes, textile materials and process engineering. It can serve as a textbook for everyone interested in the subject; students, scientists and engineers alike with a basic background in either textiles, biotechnology, chemistry or engineering. The book covers all relevant aspects of textile processing with enzymes, from the chemical constitution and properties of textile materials as potential substrates for enzymes, to processing of these materials, and

from basic biochemistry and enzymology to industrial application of these biocatalysts.

Chapter 1 deals with the fundamental aspects of enzymes determining catalytic properties. It is intended to provide a basis for the understanding of many aspects related to the application of enzymes considered in subsequent chapters. Chapter 2 gives an overview of non-fibrous and fibrous materials as substrates for enzymes. Included is a discussion on dyes, sizes, textile fibres and textile auxiliaries that might influence enzymatic reactions. Chapter 3, about catalysis and processing, gives an overview about the function and application of enzymes used in textile processing. Basic thermodynamics and enzyme kinetics, function of textile-processing enzymes, homogenous and heterogeneous catalysis and important applications of enzymes in textile wet processing are addressed. Chapter 4 gives insights into process engineering and describes major problems in the industrial applications of enzymes in textiles. Important facts about the influence of mass transfer are described. Chapter 5 discusses practical aspects of handling enzymes, like enzyme activity. Operational and storage stabilities are discussed in detail as well as health and safety issues. The last chapter, Chapter 6, deals with effluent treatment and the potential use of enzymes therein.

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Contents

1

Enzymes

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1.1 Introduction

Enzymes are biological catalysts that mediate virtually all of the biochemical reactions that constitute metabolism in living systems. They accelerate the rate of chemical reaction without themselves undergoing any permanent chemical change, i.e. they are true catalysts. The term 'enzyme' was first used by Kühne in 1878, even though Berzelius had published a theory of chemical catalysis some 40 years before this date, and comes from the Greek *enzumé* meaning 'in (*en*) yeast (*zumé*)'. In 1897, Eduard Büchner reported extraction of functional enzymes from cells. He showed that a cell-free yeast extract could produce ethanol from glucose, a biochemical pathway now known to involve 11 enzyme-catalysed steps. It was not until 1926, however, that the first enzyme (urease from Jackbean) was purified and crystallised by James Sumner of Cornell University, who was awarded the 1947 Nobel Prize. The prize was shared with John Northrop and Wendell Stanley of the Rockefeller Institute for Medical Research, who had devised a complex precipitation procedure for isolating pepsin. The procedure of Northrop and Stanley has been used to crystallise several enzymes. Subsequent work on purified enzymes, by many researchers, has provided an understanding of the structure and properties of enzymes.

All known enzymes are proteins. They therefore consist of one or more polypeptide chains and display properties that are typical of proteins. As considered later in this chapter, the influence of many chemical and physical parameters (such as salt concentration, temperature and pH) on the rate of enzyme catalysis can be explained by their influence on protein structure. Some enzymes require small non-protein molecules, known as cofactors, in order to function as catalysts.

Enzymes differ from chemical catalysts in several important ways:

1. Enzyme-catalysed reactions are at least several orders of magnitude faster than chemically-catalysed reactions. When compared to the

corresponding uncatalysed reactions, enzymes typically enhance the rates by 10^6 to 10^{13} times.

2. Enzymes have far greater reaction specificity than chemically-catalysed reactions and they rarely form byproducts.
3. Enzymes catalyse reactions under comparatively mild reaction conditions, such as temperatures below 100°C, atmospheric pressure and pH around neutral. Conversely, high temperatures and pressures and extremes of pH are often necessary in chemical catalysis.

1.1.1 In this chapter

This chapter is concerned mainy with the fundamental aspects of enzymes that determine their properties and catalytic capabilities. It is intended to provide a sound basis for understanding of many of the applied aspects of enzymes considered in subsequent chapters in this text. Given the wealth of fundamental knowledge on enzymes, it is only possible here to provide a perspective on each of the topics. Some of the topics will be considered in more detail, or from a different perspective, later on in the text.

Section 1.2 deals with the classification and nomenclature of enzymes. It considers some of the rules that form the basis of a rational system classification and naming enzymes, and provides examples of enzymes in each of the six main classes. Much of the chapter is devoted to protein structure (Section 1.3) because this ultimately defines the properties of enzymes, such as substrate specificity, stability, catalysis and response to physical and chemical factors. Protein structure is considered at all levels of organisation, from the 'building blocks' (amino acids) of proteins, through backbone conformations and three-dimensional shapes, to enzymes having more than one sub-unit. Consideration of the forces that stabilise protein molecules follows (Section 1.4) and the strengths of the various bonds are compared in relation to level of protein structure. Section 1.5 briefly describes some of the basic properties of proteins, such as chemical reactions with reactive amino acid groups, the acid–base properties of enzymes and some other factors (temperature and pH) that influence protein solubility and catalytic activity. Cellular biosynthesis of proteins is described in Section 1.6, with the emphasis very much on the process of reading the genetic code to synthesising a chain of amino acids in the correct predetermined sequence. This is followed by a section (1.7) on enzymatic modification of proteins within cells *after* they have been synthesised. Such post-translational modification influences the structural stability or activity of enzymes. Section 1.8 considers enzymatic catalysis, with the emphasis on enzyme substrate specificity and the requirement of some enzymes for the presence of non-proteinaceous compounds for catalytic activity. Comments on future trends (Section 1.9) and recommendations for further reading (Section 1.10) are

also included. Papers from the primary literature have not been referred to; rather, a list of relevant books and review articles are provided at the end of the chapter.

1.2 Classification and nomenclature of enzymes

Organisms – whether animal, plant or microorganism – are both complex and diverse. In biological systems, thousands of different types of reactions are known to be catalysed by different enzymes; many more are yet to be discovered. The diversity of enzymes is, therefore, enormous in terms of type of reaction(s) they catalyse, and also in terms of structure. Enzymes range from individual proteins with a relative molecular mass (RMM) of around 13000 catalysing a single reaction, to multi-enzyme complexes of RMM several million catalysing several distinct reactions.

Given such diversity, it is essential to have a rational basis for classification and naming of enzymes. Currently, it is the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB) that considers these matters and gives recommendations to the international scientific community.

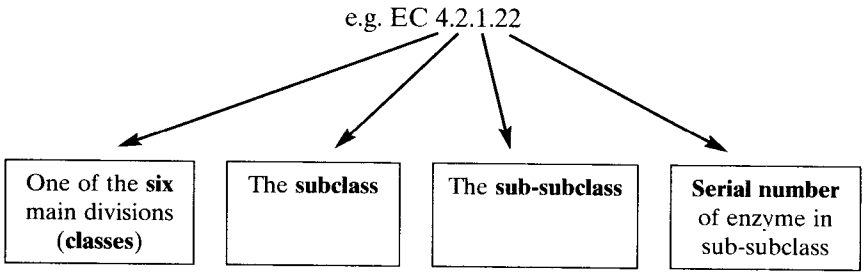
1.2.1 General rules

Enzymes are principally classified and named according to the **chemical reaction** they catalyse, as this is the specific property that distinguishes one enzyme from another. It is the observed chemical change produced by the complete enzyme reaction that is used for this purpose, i.e. the overall reaction, rather than the formation of intermediate complexes of the reactants with the enzyme. Some notable consequences of this system are:

- A systematic name cannot be given to an enzyme until the chemical reaction is known. This applies, for example, to enzymes that catalyse an isotopic change to a molecule that indicates one step in the overall reaction, but the reaction as a whole remains unknown.
- An enzyme name is assigned not to a single enzyme protein but to a group of proteins with the same catalytic property. Some exceptions exist, where more than one name is assigned to enzymes with the same catalytic property because the reaction is so different in terms of substrate specificity or mechanism. Other exceptions include acid and alkaline phosphatases. These enzymes carry out the same reaction but at widely different pH values.
- Enzymes from different sources – such as animal, plant and microorganisms – are classified as one entry.

- To classify an enzyme it is occasionally necessary to choose between alternative ways of regarding the chemical reaction. In general, the alternative selected should reduce the number of exceptions.
- The direction of the chemical reaction needs to be considered, since all reactions catalysed by enzymes are reversible. For simplicity, the direction chosen should be the same for all enzymes in a given class even if this direction has not been shown for all of the enzymes.

The Enzyme Commission of the International Union of Biochemistry, in its report of 1961, devised a rational system for classification of enzymes and assigning code numbers to them based on the reaction catalysed. The code numbers, prefixed by EC, are now used widely and contain four elements separated by points:



There are six classes of enzymes that are distinguished by the first digit of the EC code (Table 1.1). The second and third digits describe further the type of reaction catalysed. These digits are defined for each of the separate main classes of enzymes and there is no general rule that applies to their meaning. Enzymes that catalyse very similar reactions, e.g. enzymes that cleave C—O bonds in a substrate molecule, will have the same first three digits in their EC code. They will, however, have different fourth digits that define the actual substrate for the reaction.

A consequence of enzymes being classified according to the chemical reaction they catalyse is that **isoenzymes** (different enzymes catalysing identical reactions) carry the same four digit EC classification number. There are, for example, five different isoenzymes of lactate dehydrogenase in the human body and the EC code does not provide a means of distinguishing between them. Rather, the particular isoenzyme and its source (e.g. mammalian heart) have also to be specified.

1.2.2 Recommended and systematic names

The Enzyme Commission has recommended that there should be 'systematic' as well as 'trivial' (working) nomenclatures for enzymes; examples for

Table 1.1 Classification and nomenclature for the six classes of enzymes

1. **Oxidoreductases**: enzymes that catalyse oxidoreductase reactions
   2nd EC digit: indicates group in the hydrogen donor (substrate oxidised), e.g. —CHOH—, aldehyde, keto
   3rd EC digit: indicates type of acceptor involved, e.g. a cytochrome, molecular oxygen, an iron–sulphur protein, etc
   Systematic name: *donor:acceptor oxidoreductase*
   Recommended name: *donor:dehydrogenase* (*reductase* as alternative; *oxidase* where O_2 is acceptor)
   e.g. alcohol dehydrogenase (trivial); alcohol NAD^+ oxidoreductase (EC 1.1.1.1)
2. **Transferases**: enzymes transferring a group
   2nd EC digit: indicates group transferred, e.g. methyl, glycosyl, phosphate
   3rd EC digit: further information on group transferred, e.g. hydroxymethyl
   Systematic name: *donor:acceptor grouptransferase*
   Recommended name: *acceptor grouptransferase*
   e.g. glucokinase; ATP glucose phosphotransferase (EC 2.7.1.2)
3. **Hydrolases**: enzymes that catalyse cleavage of C—O, C—N, C—C and some other bonds
   2nd EC digit: indicates nature of bond hydrolysed, e.g. ester, glycosyl
   3rd EC digit: indicates nature of substrate, e.g. carboxylic ester, thiolester
   Systematic name: *substrate:hydrolase*
   Recommended name: *substrate* with suffix *-ase*
   e.g. carboxypeptidase A (EC 3.4.17.1)
4. **Lyases**: enzymes that cleave C—C, C—O, C—N and other bonds by elimination, leaving double bonds or rings, or add groups to double bonds
   2nd EC digit: indicates the bond broken
   3rd EC digit: further information on group eliminated, e.g. CO_2, H_2O
   Systematic name: *substrate group-lyase* (hyphen included)
   Recommended names: e.g. *decarboxylase, dehydratase* (in case of elimination of CO_2 and H_2O); *synthase* used if reverse reaction described
   e.g. pyruvate decarboxylase; pyruvate-lyase (EC 4.1.1.1)
5. **Isomerases**: enzymes that catalyse geometric or structural changes within one molecule
   2nd EC digit: indicates type of isomerism, e.g. racemase, epimerase, *cis-trans* isomerase
   3rd EC digit: indicates type of substrate
   Systematic name: *substrate:type of isomerism*
   Recommended name: *substrate:isomerase*
   e.g. maleate isomerase; maleate *cis-trans* isomerase (EC 5.2.1.10)
6. **Ligases**: enzymes catalysing the joining of two molecules coupled with hydrolysis of a diphosphate bond in ATP (or similar triphosphate)
   2nd EC digit: indicates the bond formed, e.g. C—O, C—S, C—N
   3rd EC digit: (only used in the C—N ligases)
   Systematic name: *X:Y ligase (ADP-forming)*
   Recommended name: *X:Y ligase* (previously *synthetase* was used)
   e.g. pyruvate carboxylase (trivial); pyruvate carboxyligase (ADP forming) (EC 6.4.1.1)

each of the six classes of enzymes are given in Table 1.1. The systematic name describes the action of an enzyme as exactly as possible, whereas the trivial name is sufficiently short for general use and is often a name already in common use. The Enzyme Commission-recommended trivial names for new enzymes are often condensed versions of systematic names.

Since enzymes are divided into groups according to the type of reaction catalysed, this and the name(s) of the substrate(s) are the basis for systematic naming of individual enzymes. It is also the basis for classification and code numbers. Names of enzymes, especially those ending in *ase*, generally refer to single enzymes and are not applied to systems containing one or more enzymes. When an overall reaction involving more than one enzyme is named, the word 'system' is included in the name. For example, the 'succinate oxidase system' is used to describe the enzymatic oxidation of succinate involving succinate dehydrogenase, cytochrome oxidase and several intermediate carriers.

General rules for systematic names and guidelines for recommended names, as well as rules and guidelines for particular classes of enzymes, are available at Enzyme Nomenclature Database at the Swiss Institute of Bioinformatics (http://www.espasy.ch/enzyme).

1.3 Protein structure

1.3.1 Overview

Proteins consist of one or more polypeptides and each polypeptide is a chain of amino acids linked together by peptide bonds. A different gene codes for each polypeptide and determines the sequence of amino acids of the polypeptide. Polypeptide chains fold up when synthesised to form a unique three-dimensional shape (conformation), determined by their amino acid sequences. Multiple weak interactions stabilise the conformation of polypeptides and factors (such as pH, heat and chemicals) that disrupt these interactions distort the polypeptide's conformation. Enzymes lose their functional activity when their three-dimensional conformation is distorted in this manner, through enzyme denaturation. This demonstrates a clear dependence of enzyme functioning upon protein structure.

There are two main types of proteins: 'fibrous' and 'globular'. **Fibrous** proteins normally have a structural role in biological systems. They are insoluble in water and are physically durable/strong. The three-dimensional structure of fibrous proteins is relatively simple and usually elongated. Examples of fibrous proteins are:

- α-Keratin: the main protein of hair, nails, wool, horn and feathers
- β-Keratin: the main structural component of silk and spider's web

- Collagen: a major protein of cartilage, tendons, skin and bones
- Elastin: a protein found in ligaments in the walls of arteries.

Globular proteins are generally soluble in water and can often be crystallised from solution. They have a more complex three-dimensional structure and tend to adopt an approximate spherical shape in which the amino acid chain is tightly folded. Globular proteins have functional roles in biological systems and all enzymes are globular proteins. Proteins are also categorised as 'simple' or 'conjugated'. **Simple** proteins are composed entirely of amino acids, while **conjugated** proteins contain one or more other materials bound to one or more of the amino acid residues. Examples of conjugated proteins and their bound components are:

- Glycoproteins – carbohydrate
- Metalloproteins – metal ions
- Lipoproteins – lipids
- Nucleoproteins – nucleic acids
- Flavoproteins – flavin nucleotides

1.3.2 Amino acids – the 'building blocks' of proteins

Amino acids are organic molecules that contain an amino group (primary —NH_2; secondary >NH) and a carboxyl group (O=C(—OH) or —COOH). There are 20 commonly occurring amino acids. All except one has a central (α) carbon atom, to which is attached a primary amino group (—NH_2), a carboxyl group (—COOH), a hydrogen atom and a side group or chain (R); the side groups are different in all amino acids. Proline is unique because it lacks a primary amino group; instead it contains a secondary amino group (>NH). In proline the side group is curled round so that the nitrogen and the α-carbon atoms form part of a non-polar and fully saturated five-membered imino ring; proline is termed an imino acid. Representations of the generalised structure of amino acids are shown in Fig. 1.1.

Side chains (R-groups) of α-amino acids are polar or non-polar. The structure of the polar molecules may be stabilised by hydrogen bonding in aqueous solution; they display ionic character and are therefore hydrophilic and soluble in water. Conversely, non-polar molecules are relatively insoluble in water, but more soluble in organic solvents. The categorisation of amino acids according to the hydrophobic or hydrophilic character of the side chains is shown in Fig. 1.2. Phenylalanine, tryptophan and tyrosine are termed aromatic amino because the R-group has a six-membered aromatic benzene ring, whereas hystidine has a five-membered imidazole ring. The double-ringed R-group of tryptophan is called indole and in tyrosine the ring is linked to —OH to form a phenolic group. As mentioned earlier,