INDUSTRIAL ENZYMES AND THEIR APPLICATIONS

HELMUT UHLIG, PH.D.

Translated and Updated by

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PREFACE

Until quite recently, industrial enzymes were of interest only to scientists, engineers, and others directly involved in enzyme research and development; now, with the widespread commercial advertisement (in television and other media) of the relative efficiency and safety of enzymes in everyday products such as household detergents and foods, enzymes are of increasing concern to the general public. The consumer's decision whether to buy a specific product is often based on close perusal of the contents or of the ingredients' list on the package label; for instance, one may question whether eating a food treated with a certain ingredient (viz., enzyme) might pose a health risk or whether that ingredient will improve the quality of the food.

This book describes the current state of technology in industrial enzyme preparations and applications, especially those in which enzymes have replaced chemical reagents. It also discusses how enzymes can be used to reduce costs in low-energy processes, improve raw materials utilization, and enhance the quality of foods and other products. The book begins with an overview of the most important industrial enzymes, describing their microbiological origin and such parameters as pH, temperature dependence, and reaction stability of each specific enzyme. Throughout the book, the frequent descriptions of enzyme properties and activities are intended to help food technologists, engineers, students, and others find new applications for industrial enzymes, and should be of value to microbiologists in the development of enzymes with modified characteristics.

During my 30 years of work on enzymes, I have often been asked by students, clients, technicians, and marketing representatives to recommend an easily understandable book on industrial enzymes. Since a comprehensive treatise on this subject was not available in German, my Röhm colleagues and I collected our practical experience and observations on enzymes in a book entitled *Enzyme arbeiten für uns,* which was published in 1991 by Carl Hanser in Munich and was intended as a reference source for industrial enzyme chemists at all levels, from beginning students to R&D technologists, as well as for nonchemists involved in industrial enzyme marketing.

The previous manuscript has been updated by including discussion of some recently developed industrial enzymes and progress in enzyme applications. Especially updated are the chapters on baking and fruit juice; in particular, new amylases and xylanases have been added. Chapter 4, on enzyme immobilization, was completed by Dr. D. Frank. New references have been added, and errors in the literature references of the German edition were eliminated by Dr. Linsmaier-Bednar.

I thank my colleague Mr. Reinhold Schmitt, a world-class computer expert, for producing the figures and for his patience in teaching me how to handle a computer. I also thank Prof. Dr. W. Pilnik, Wageningen; Prof. Dr. K. Girschner, Hohenheim; Prof. Dr. K. Wünscher, Lemgo; and Prof. Dr. H. Ruttloff, Potsdam-Rehbrücke for critical discussions and information. Dr. Don Scott, of Scott Biotechnology Inc., Chicago, helped greatly with his long experience in the enzyme field. He provided me with the motivation for this English edition. I am grateful to the enzyme-producing companies Alko, Amano, Cultor, Daiwa, Gist Brocades, Grindsted, Meiji, Novo, Röhm, Solvey, Shin Nihon, Stern-Enzyme, and Ueda for permission to use their technical information.

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CONTENTS

1 Introduction

1.1 WHAT ENZYMES DO

Life on earth is based on constant change. Inorganic matter forms the complicated structures that make up the living world, which will again decay into lifeless matter. The lives of plants, animals, and human beings are included in this great cycle. The inert substances that form the foundation of life have a very simple structure. Water, carbon dioxide, and nitrogen are the basic ingredients that, with the energy supplied by sunlight, permit plants to synthesize the molecular moieties that support life. These compounds are then used to provide more elaborate compounds required by animal life.

Chemical experiments have shown how specific compounds, namely, the essential amino acids, can be generated from simple mixtures of carbon, nitrogen, oxygen, and hydrogen when subjected to extreme pressures and temperatures. Once formed, less extreme conditions can cause the degradation of these complex structures as well. Under the current global conditions, such processes proceed only very slowly.

What, then, causes the rapid synthesis of these compounds that are essential for life, and the degradation of the high-molecular-weight and highly organized structures that make up life? The catalysts for this assembly and disassembly were for a long time thought to be life itself, or rather a fundamental principle of living cells.

The first observation of an enzymatic degradation reaction was in 1783 by Spallanzani (1729–1799), a priest and naturalist from Padua, Italy. After placing meat in small porous capsules, he examined the regurgitated pellets of the hawks who had eaten this material and found the capsules to be empty, proving that the meat had been rapidly liquefied by the stomach juices of these birds of prey.

In 1814, Kirchhoff (1815) found that barley contained a substance that was capable of liquefying starch paste into sugar. He assumed that the reaction was caused by the gluten protein of the barley. Subsequently, Payen and Persoz (1833) termed the working principle of this saccharification *diastase* (Greek for *separation*), a term still used for the amylases in the brewing industry. The degradation of starch, which is composed of sugar moieties, and the subsequent process of alcoholic fermentation of the sugar into carbon dioxide and alcohol occupied much of this nineteenth-century chemist's time.

In 1857 Pasteur showed that fermentation is closely associated with live yeast. He distinguished between the actions of "organized ferments" (cellular) and the "unorganized ferments" (soluble). These soluble "ferments," which are not bound to the living cell, were labeled *enzymes* by Kühne (1878). This term is derived from the

Greek *en zyme*, meaning "in sour dough." Concrete evidence for this assumption was provided by E. Buchner in 1897, as he showed that the cell-free extract from yeast cells could also produce alcohol from sugars (Buchner, 1897, 1898). The enzyme active in fermentation was termed *zymase*. The first book summarizing the "wonderful mechanism" of the enzymes known at that time and outlining the history of their discovery was written by Green (1901) and is still read with interest today.

1.2 ENZYMES DEFINED AS CATALYSTS

Friederich Wilhelm Ostwald first coined the now-familiar definition of a catalyst, specifically, that a catalyst is a substance that alters the rate of a chemical reaction without being present in the reaction products. Thus, Ostwald recognized enzymes as catalysts.

Today, *catalysts* and *catalysis* are familiar terms. The catalysts in internal-combustion engines accelerate the conversion of the pollutants carbon monoxide and nitrogen oxides into the less polluting carbon dioxide and nitrogen, without any change in the catalysts during the process. According to the basic research of Tammann (1892) and Van't Hoff (1898), a catalyst must be able to regenerate the initial substrate from its products. Enzymatic reactions also have an equilibrium. An example is the formation and scisson of an ester that forms on reaction between an alcohol with an acid (Ammon and Dirscherl, 1959):

$$\text{Acetic acid} + \text{alcohol} \underset{\text{hydrolysis}}{\overset{\text{synthesis}}{\rightleftarrows}} \text{acetate ester} + \text{water}$$

If acetic acid and alcohol are mixed in equimolar amounts, some of the molecules will react to form an ester and water (synthesis). Yet, there still will be significant quantities of acetic acid and alcohol in the reaction mixture as well as the products generated, ester and water. A mixture of the same composition can also be obtained when starting with water and an ester (hydrolysis). Even in the absence of a catalyst, the reactions reach equilibrium between ester and synthesis and ester hydrolysis.

When forming and splitting butyl ester, it was found that the equilibrium was rapidly established from either direction on the addition of porcine pancreatic powder (pancreatic lipase). Thus, for life to exist, the biochemical equilibria of essential and exceedingly complex reaction systems must be established rapidly. Therefore, enzymes are the vital catalysts in all life processes.

1.3 SOME HISTORICAL USES OF ENZYMES

The use of enzymes and microorganisms in processing raw materials from plants and animals has been practiced for a long time. At one time, living microorganisms were used predominantly. Traditional processes, such as the production of alcoholic beverages and yeast-fermented doughs in baking bread, are displayed in Egyptian wall paintings. Further examples are the processes for preserving food, such as vegetable conservation by fermentation with lactobacilli (Sauerkraut), or preserving milk by making cheese.

"Bread and wine in ancient times" is a contribution by Reisner (1981). He provides an interesting description of the historical development of food processing biotechnology:

> The oldest baking utensils and ovens are 5000 years old and were used in Jericho (Canaan), one of the most ancient cities in the world. The simplest way of baking was to place a flat loaf of dough in glowing ashes. However, around 3500 BC, the variety of breads produced at that time which were mainly flat breads, were baked in the first cylindrical ovens. A dough was made from coarse milled kernels without addition of leavening agents. Such breads were tasty only when fresh. Sourdough was rarely used except when extended storage was desired. Ovens for baking bread were installed in the kitchens of the temple area of Ur, the capital of the Sumerians. Near Abu Kemal, where the Damaskus–Mossul line crosses the Euphrates, lie the high walls of the palace of Mari. Here the cuneiform writings relate the marshalling of men for military conscription. On such occasions, government officials distributed free beer and bread.
>
> A large bakery dating from around 2000 BC has been excavated in the palace of Sargon in Babylon. Clay pots were found in which pieces of dough were still stuck to the inner walls and apparently were baked in a quickly kindled fire. In Nebuchadnezzar's Babylon, baking with sourdough was already known. In the palace bakery of Ramses III, cylindrical baking ovens were used, as shown in a wall drawing in the side-chamber of El Amarna [Fig. 1.1].
>
> In this picture the dough is not hand kneaded but trodden on while employing two plots to help balance the worker. Above this scene a basket with sourdough is suspended. Two other bakers carry liquids in jugs to a table upon which a firm dough is formed. In the middle row, a heated oil bath with lid can be seen. By utilizing tongs, a spiral shaped pastry is fried in the oil. A baker removes the finished, round baked goods from a cooled baking oven. In the bottom row, flour is run through a sieve and on the right, a drum containing fresh sourdough can be seen. It is not known whether the Egyptians sometimes used malt in baking. They were familiar with the production of malt by wetting and sprouting barley.
>
> Wine had already been mentioned in the book of Genesis as a special fermentation product. "And Noah, a farmer, started to till the soil and planted a vineyard. And then he drank the wine and became drunk." In Babylon drinking was a favorite pastime; 16 different beer varieties were known, of which black beer was the most popular. The first word deciphered from the hieroglyphics by Champollion was "Erep" meaning wine. At least six types of wine could be distinguished, including white, red, black and lower Egyptian. After the grape harvest, the baskets were carried to a wine press, in this case a long stone trough, above which a man-sized wooden scaffold was erected [Figs. 1.2 and 1.3].
>
> The men supported themselves with the upper beams of the scaffolding and crushed the grapes with their feet. Later, round wine presses were built; the workers held onto ropes and the juice was discharged from the trough into a container [as shown in Fig.

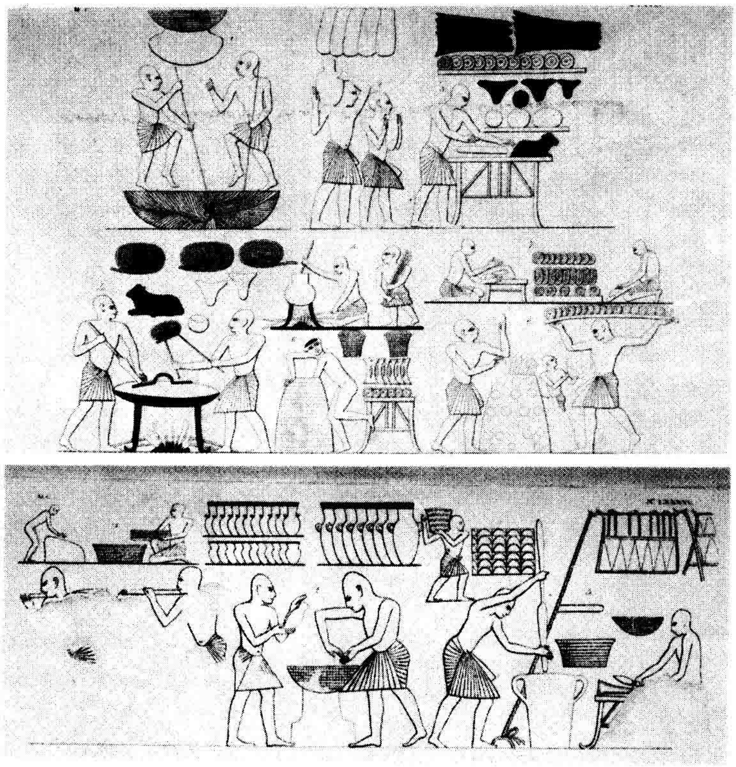

Figure 1.1 Court bakery of pharaoh Ramses III. Tomb painting from El Amarna. [*Source:* I. Rosellini, *I Monumenti dell'Egitto,* Pisa, Italy, 1832 (Reisner, 1981).]

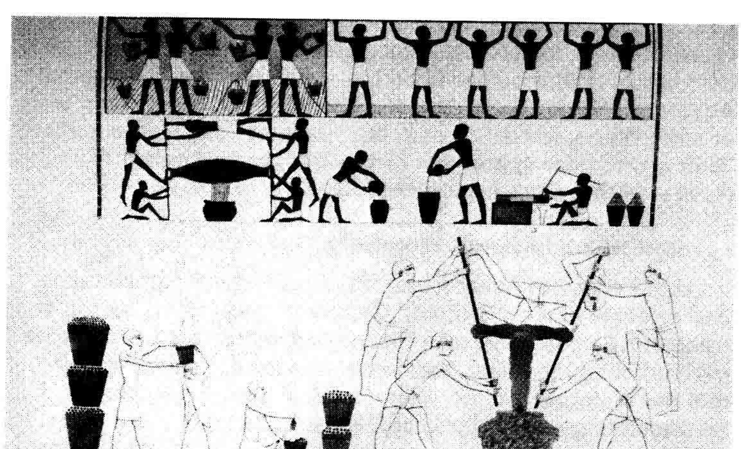

Figure 1.2 Harvesting and making wine. From a Theban tomb painting. [*Source:* I. Rosellini, *I Monumenti dell'Egitto,* Pisa, Italy, 1832 (Reisner, 1981).]