Chapter 11

Microbial Enzymes of Use in Industry

Xiangyang Liu[1] and Chandrakant Kokare[2]
[1]UNT System College of Pharmacy, University of North Texas Health Science Center, Fort Worth, TX, United States [2]Department of Pharmaceutics, Sinhgad Technical Education Society, Sinhgad Institute of Pharmacy, Pune, Maharashtra, India

11.1 INTRODUCTION

Many microbes such as bacteria, actinomycetes, fungi, and yeast extracellularly or intracellularly produce a group of versatile and attractive enzymes with a wide variety of structures and commercial applications. Many microbial enzymes, such as amylases, proteases, pectinases, lipases, xylanases, cellulases, and laccases, are extracellularly produced. Some enzymes such as catalase from *Saccharomyces cerevisiae* and *Aspergillus niger* are intracellular enzymes (Fiedurek and Gromada, 2000; Venkateshwaran et al., 1999). As biocatalytic molecules, microbial enzymes are ecologically effective and highly specific, which can result in the formation of stereo- and regio-chemically defined reaction products with a rate acceleration of 10^5 to 10^8 (Gurung et al., 2013; Koeller and Wong, 2001). Among the industrial enzymes, 50% are made by fungi and yeast, 35% from bacteria, while the remaining 15% are from plants (Saranraj and Naidu, 2014). When compared to animal and plant enzymes, microbial enzymes have several advantages. First, the microbial enzymes are more active and stable than plant and animal enzymes. Through the development of fermentation processes, particularly selected strains are able to produce purified, well-characterized enzymes on a large scale. Second, enzymes produced by microorganisms have high yield and are easy for product modification and optimization owing to the biochemical diversity and susceptibility to gene manipulation. Engineering techniques have been applied to microorganisms in order to improve the production of enzymes and alter the properties of enzymes by protein engineering (Gurung et al., 2013). Third, microbes represent a rich source for the discovery of microbial enzymes by many modern techniques such as

Biotechnology of Microbial Enzymes. DOI: http://dx.doi.org/10.1016/B978-0-12-803725-6.00011-X

metagenome screening, genome mining, and exploring the diversity of extremophiles (Adrio and Demain, 2014; Zhang and Kim, 2010).

Currently, approximately 200 types of microbial enzymes from 4000 known enzymes are used commercially. However, only about 20 enzymes are produced on a truly industrial scale. The world enzyme demand is satisfied by about 12 major producers and 400 minor suppliers. Nearly 75% of the total enzymes are produced by three top enzyme companies, that is, Denmark-based Novozymes, US-based DuPont (through the May 2011 acquisition of Denmark-based Danisco) and Switzerland-based Roche. The market is highly competitive, has small profit margins and is technologically intensive (Li et al., 2012). With the improved understanding of the microbial recombination, metagenome mining, fermentation processes, and recovery methods, an increasing number of industrial enzymes can be supplied. For example, the recombinant DNA technology can be applied to microorganisms to produce enzymes commercially that could not be produced previously. Approximately 90% of industrial enzymes are recombinant versions (Adrio and Demain, 2014).

The industrial applications for microbial enzymes have grown immensely in recent years. For example, the estimated value of worldwide sales of industrial enzymes for the years 2012, 2013, and 2015 are $1 million, $3 billion, and $3.74 billion, respectively (Deng et al., 2010; Godfrey and West, 1996a,b). Protease sales represent more than 60% of all industrial enzyme sales in the world (Rao et al., 1998) and still constitute the largest product segment in the 2015 global industrial enzymes market. Amylases comprise about 30% of the world's enzyme production. Lipases represent the other major product segment in the market. Geographically, demand for industrial enzymes in matured economies, such as the United States, Western Europe, Japan, and Canada, was relatively stable during the recent times, while the developing economies of Asia-Pacific, Eastern Europe, and Africa and Middle East regions emerged as the fastest growing markets for industrial enzymes. On the basis of the application, commercial applications of enzymes can be divided in nine broad categories including food and feed, detergents, etc. (Sharma et al., 2010). About 150 industrial processes use enzymes or whole microbial cell catalysts (Adrio and Demain, 2014). Food and feed represents the largest segment for industrial enzymes. Detergents constitute the other major segment for industrial enzymes. This chapter covers the classification, resource, production, and applications of biotechnologically and industrially valuable microbial enzymes.

11.2 CLASSIFICATION AND CHEMICAL NATURE OF MICROBIAL ENZYMES

Based on the catalyzed reaction, microbial enzymes can be classified into six types: Oxidoreductases (EC 1, catalyze oxidation/reduction reactions), Transferases (EC 2, transfer a functional group), Hydrolases (EC 3, catalyze

the hydrolysis of various bonds), Lyases (EC 4, cleave various bonds by means other than hydrolysis and oxidation), Isomerases (EC 5, catalyze isomerization changes within a single molecule), and Ligases (EC 6, join two molecules with covalent bonds) (http://www.chem.qmul.ac.uk/iubmb/enzyme/). Currently, there are 510 commercial useful microbial enzymes in the metagenomics database (Sharma et al., 2010). Of the industrial enzymes, 75% are hydrolytic (Li et al., 2012).

11.2.1 Amylases

Amylases are a class of enzymes that catalyze the hydrolysis of starch into sugars such as glucose and maltose (Sundarram and Murthy, 2014). Amylases are divided into three sub classes—α-β-γ-amylase according to the type of bond/link they are able to cleave (Fig. 11.1). α-Amylases (EC 3.2.1.1) catalyze the hydrolysis of internal α-1,4-*O*–glycosidic bonds in polysaccharides with the retention of α-anomeric configuration in the products. Most of the α-amylases are metalloenzymes, which require calcium ions (Ca^{2+}) for their activity, structural integrity, and stability. They belong to family 13 (GH-13) of the glycoside hydrolase group of enzymes. β-Amylases (EC 3.2.1.2) are exohydrolase enzymes that act from the nonreducing end of a polysaccharide chain by hydrolyzing α-1, 4-glucan linkages to yield successive maltose units. Since β-amylases are unable to cleave branched linkages present in branched polysaccharides, such as glycogen or amylopectin, the hydrolysis is incomplete and dextrin units remain. γ-Amylases (EC 3.2.1.3) cleave α(1-6) glycosidic linkages, in addition to cleaving the last α(1-4) glycosidic linkages at the nonreducing end of amylose and amylopectin, unlike the other forms of amylase, yielding glucose. α-Amylase is produced by several bacteria, fungi, and genetically modified species of microbes. The most widely used source among the bacterial species are *Bacillus* spp.—*B. amyloliquefaciens* and *B. licheniformis*. Fungal sources of α-amylase are mostly limited to *Aspergillus* species and to only a few species of *Penicillium*, *P. brunneum* being one of them.

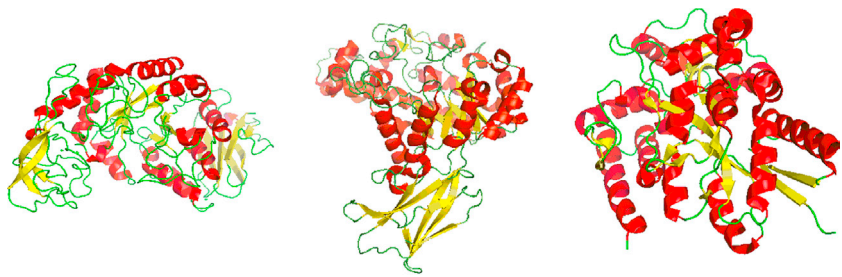

FIGURE 11.1 Structures of selected microbial amylases.

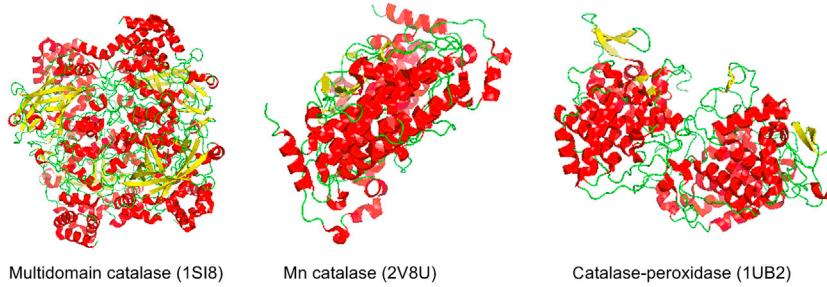

FIGURE 11.2 Structures of selected microbial catalases.

11.2.2 Catalases

Catalases (EC 1.11.1.6) are antioxidant enzymes that catalyze the conversion of hydrogen peroxide to water and molecular oxygen. According to the structure and sequence, catalases can be divided into three classes (Fig. 11.2): monofunctional catalase or typical catalase, catalase-peroxidase, and pseudocatalase or Mn-catalasee (Zhang et al., 2010). Currently, there are at least eight strains that can produce catalases (Zhang et al., 2010): *Penicillum variable*, *A. niger*, *S. cerevisiae*, *Staphylococcus*, *Micrococcus lysodeiktious*, *Thermoascus aurantiacus*, *Bacillus subtilis*, and *Rhizobium radiobacte*. Catalases are used in several industrial applications such as food or textile processing to remove hydrogen peroxide that is used for sterilization or bleaching.

11.2.3 Cellulases

Cellulases are enzymes that hydrolyze β-1,4 linkages in cellulose chains. The catalytic modules of cellulases have been classified into numerous families based on their amino acid sequences and crystal structures (Henrissat, 1991). Cellulases contain noncatalytic carbohydrate-binding modules (CBMs) and/or other functionally known or unknown modules, which may be located at the N- or C-terminus of a catalytic module. In nature, complete cellulose hydrolysis is mediated by a combination of three main types of cellulases (Juturu and Wu, 2014; Kuhad et al., 2011; Sukumaran et al., 2005; Yang et al., 2013) (Fig. 11.3). These are endoglucanases (EC 3.2.1.4), exoglucanases (EC 3.2.1.91), and glucosidases (EC 3.2.1.21). Endoglucanases hydrolyze glycosidic bonds at the amorphous regions of the cellulose generating long chain oligomers (nonreducing ends) for the action of exoglucanases or cellobiohydrolases, which cleave the long chain oligosaccharides generated by the action of endoglucanases into short chain oligosaccharides. There are two types of exoglucanases, acting unidirectionally on the long chain oligomers either from the reducing (EC 3.2.1.176) or nonreducing ends (EC 3.2.1.91) liberating cellobiose, which is further hydrolyzed to glucose by β-glucosidases (EC 3.2.1.21) (Juturu and Wu, 2014). Cellulases are inducible

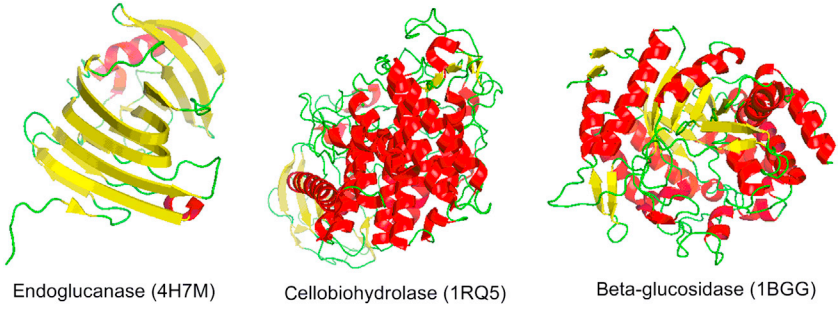

FIGURE 11.3 Structures of selected microbial cellulases.

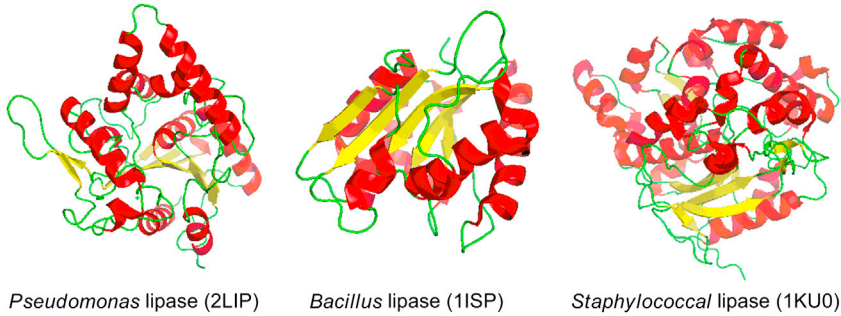

FIGURE 11.4 Structures of selected microbial lipases.

enzymes synthesized by a large diversity of microorganisms including both fungi and bacteria during their growth on cellulosic materials (Ma et al., 2013; Quintanilla et al., 2015). These microorganisms can be aerobic, anaerobic, mesophilic, or thermophilic. Among them, the genera of *Clostridium*, *Cellulomonas*, *Thermomonospora*, *Trichoderma*, and *Aspergillus* are the most extensively studied cellulose producers (Kuhad et al., 2011).

11.2.4 Lipases

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) catalyze the hydrolysis of triacylglycerols to glycerol, diacylglycerols, monoglycerols, and free fatty acids (Treichel et al., 2010). Bacterial lipases are classified into eight families (families I–VIII) based on differences in their amino-acid sequences and biological properties (Arpigny and Jaeger, 1999). Family I of true lipases is the most represented one and can be further divided into *Pseudomonas* lipase subfamily, *Bacillus* lipase subfamily, *Staphylococcal* lipase subfamily, etc. (Fig. 11.4). Lipases belong to the class of serine hydrolases. Therefore, lipases do not need any cofactor. Lipases catalyze the hydrolysis of ester bonds at the

interface between an insoluble substrate phase and the aqueous phase where the enzymes remain dissolved. Lipases do not hydrolyze dissolved substrates in the bulk fluid. In nature, lipases have considerable variations in their reaction specificities. From the fatty acid side, some lipases have an affinity for short-chain fatty acids (C2, C4, C6, C8, and C10), some have a preference for unsaturated fatty acids (oleic, linoleic, linolenic, etc.) while many others are nonspecific and randomly split the fatty acids from the triglycerides. Some of the most commercially important lipase-producing fungi belong to the genera *Rhizopus* sp., *Aspergillus*, *Penicillium*, *Geotrichum*, *Mucor*, and *Rhizomucor* (Gupta et al., 2004; Treichel et al., 2010). The main terrestrial species of yeasts that were found to produce lipases are (Treichel et al., 2010): *Candida rugosa*, *Candida tropicalis*, *Candida antarctica*, *Candida cylindracea*, *Candida parapsilopsis*, *Candida deformans*, *Candida curvata*, *Candida valida*, *Yarrowia lipolytica*, *Rhodotorula glutinis*, *Rhodotorula pilimornae*, *Pichia bispora*, *Pichia mexicana*, *Pichia sivicola*, *Pichia xylosa*, *Pichia burtonii*, *Saccharomycopsis crataegenesis*, *Torulaspora globosa*, and *Trichosporon asteroids*. Among bacterial lipases being exploited, those from *Bacillus* exhibit interesting properties that make them potential candidates for biotechnological applications (Gupta et al., 2004; Treichel et al., 2010). *Bacillus subtilis*, *Bacillus pumilus*, *Bacillus licheniformis*, *Bacillus coagulans*, *Bacillus stearothermophilus*, and *Bacillus alcalophilus* are the most common lipase producing strains. In addition, *Pseudomonas* sp., *Pseudomonas aeruginosa*, *Burkholderia multivorans*, *Burkholderia cepacia*, and *Staphylococcus caseolyticus* are also reported as bacterial lipase producers (Gupta et al., 2004).

11.2.5 Pectinases

Pectinases are a group of enzymes that catalyze pectic substance degradation through depolymerization (hydrolases and lyases) and deesterification (esterases) reaction (Pedrolli et al., 2009). According to the cleavage mode and specificity, pectic enzymes are divided into three major types (Fig. 11.5): pectinesterases (PE), depolymerizing enzymes and cleaving (Tapre and Jain,

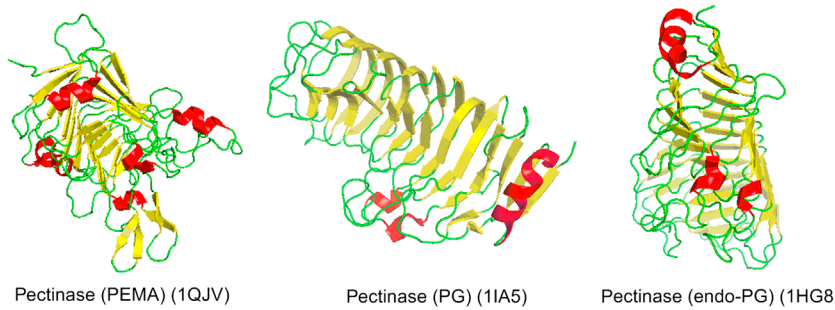

FIGURE 11.5 Structures of selected microbial pectinases.

2014). These types can be further divided into 13 groups, protopectinases, pectin methyl esterases (PME), pectin acetyl esterases (PAE), polymethylgalacturonases (PMG), polygalacturonases (PG), polygalacturonate lyases (PGL), pectin lyases (PL), rhamnogalacturonan rhamnohydrolases, rhamnogalacturonan galacturonohydrolases, rhamnogalacturonan hydrolases, rhamnogalacturonan lyases, rhamnogalacturonan acetylesterases, and xylogalacturonase (Pedrolli et al., 2009). For example, PME or pectinesterase (EC 3.1.1.11) catalyzes deesterification of the methoxyl group of pectin forming pectic acid and methanol. The enzyme acts preferentially on a methyl ester group of galacturonate unit next to a nonesterified galacturonate unit (Kashyap et al., 2001). PAE (EC 3.1.1) hydrolyzes the acetyl ester of pectin forming pectic acid and acetate (Shevchik and Hugouvieux-Cotte-Pattat, 1997). PG catalyzes hydrolysis of α-1,4-glycosidic linkages in polygalacturonic acid producing D-galacturonate. Both groups of hydrolase enzymes (PMG and PG) can act in an endo- or exo- mode. Endo-PG (EC 3.2.1.15) and endo-PMG catalyze random cleavage of substrate, whereas exo-PG (EC 3.2.1.67) and exo-PMG catalyze hydrolytic cleavage at the substrate nonreducing end producing monogalacturonate or digalacturonate in some cases (Kashyap et al., 2001). Among those enzymes, homogalacturonan degrading enzymes are well known (Pedrolli et al., 2009). It has been reported that microbial pectinases account for 25% of the global food enzymes sales and 10% of global industrial enzymes produced (Ceci and Lozano, 1998; Jayani et al., 2005; Saranraj and Naidu, 2014). Pectinase production has been reported from bacteria including actinomycetes, yeast, and fungi (Murad and Azzaz, 2011; Saranraj and Naidu, 2014). However, almost all the commercial preparations of pectinases are produced from fungal sources (Singh et al., 1999). *Aspergillus niger* is the most commonly used fungal species for the industrial production of pectinolytic enzymes (Gummadi and Panda, 2003).

11.2.6 Proteases

Proteases (EC 3:4, 11-19, 20-24, 99) (peptidase or proteinase) constitute a very large and complex group of enzymes that catalyze the hydrolysis of covalent peptide bonds. Proteases can be classified on the basis of pH, substrate specificity, similarity to well characterized enzymes, and the active site amino acid (Ellaiah et al., 2002). Based on the pH optima, they are referred to as acidic, neutral, or alkaline proteases (Rao et al., 1998). On the basis of their site of action on protein substrates, proteases are broadly classified as endo- or exo-enzymes (Rao et al., 1998). They are further categorized as serine proteases, aspartic proteases, cysteine proteases, or metallo proteases-depending on their catalytic mechanism (Jisha et al., 2013) (Fig. 11.6). Microorganisms account for a two-third share of commercial protease production in the enzyme market across the world. Alkaline serine proteases are the most dominant group of proteases produced by bacteria, fungi, yeast, and actinomycetes. Currently,

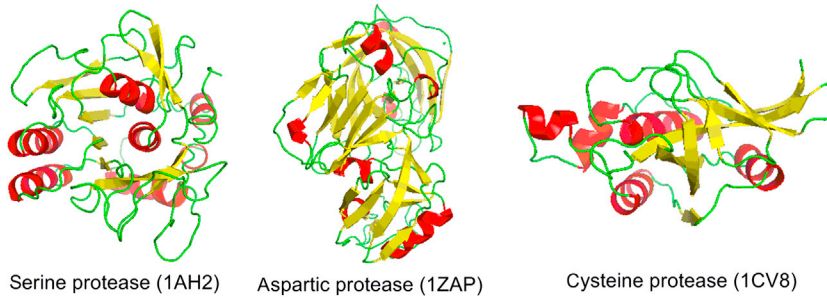

FIGURE 11.6 Structures of selected microbial proteases.

there are at least 29 *Bacillus* species and 17 fungal producers that have been reported to produce alkaline proteases (Jisha et al., 2013). Commercial producers of alkaline proteases include protein engineered *Bacillus licheniformis*, alkalophilic *Bacillus* sp., and *Aspergillus* sp. (Ellaiah et al., 2002).

11.2.7 Xylanases

Xylanases are among the xylanolytic enzyme system that include endoxylanase, β-xylosidase, α-glucuronidase, α-arabinofuranosidase, and acetylxylan esterase (Juturu and Wu, 2012). Xylanases are a group of glycoside hydrolase enzymes that degrade the linear polysaccharide xylan into xylose by catalyzing the hydrolysis of the glycosidic linkage (β-1,4) of xylosides. Xylanases have been classified in at least three ways: based on the molecular weight and isoelectric point (Wong et al., 1988), the crystal structure (Jeffries, 1996) and kinetic properties, or the substrate specificity and product profile (Motta et al., 2013). The favorable system for the classification of xylanases is based on the primary structure and comparison of the catalytic domains (Collins et al., 2005; Henrissat and Coutinho, 2001). According to the CAZy database (http://www.cazy.org), xylanases (EC3.2.1.8) are related to glycoside hydrolase (GH) families 5, 7, 8, 9, 10, 11, 12, 16, 26, 30, 43, 44, 51, and 62. Among those, xylanases in GH 10 and 11 are the two families that have been thoroughly studied (Fig. 11.7). GH family 10 is composed of endo-1,4-β-xylanases and endo-1,3-β-xylanases (EC 3.2.1.32) (Motta et al., 2013). Members of this family are also capable of hydrolyzing the aryl β-glycosides of xylobiose and xylotriose at the aglyconic bond. Furthermore, these enzymes are highly active on short xylo-oligosaccharides, thereby indicating small substrate-binding sites. Family 11 is composed only of xylanases (EC 3.2.1.8), leading to their consideration as "true xylanases," as they are exclusively active on D-xylose-containing substrates. Among all xylanases, endoxylanases are the most important due to their direct involvement in cleaving the glycosidic bonds and in liberating short xylooligosaccharides (Collins et al., 2005). Although several *Bacillus* species secrete high levels of extracellular xylanase (Beg et al., 2001), filamentous fungi secret

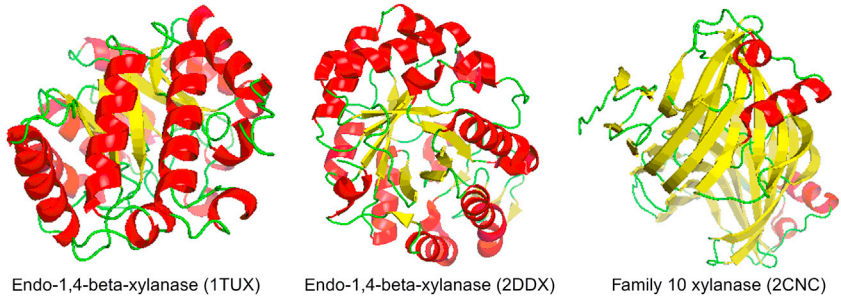

FIGURE 11.7 Structures of selected microbial xylanases.

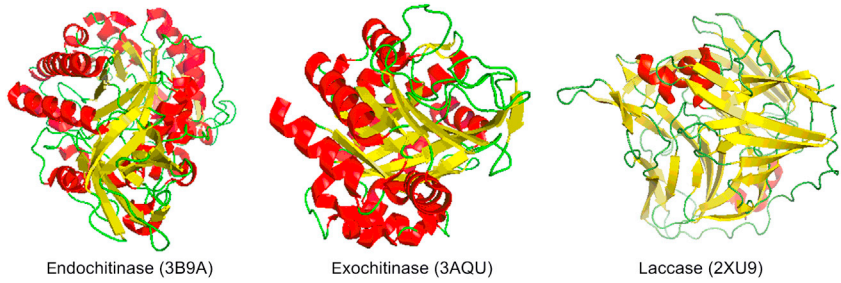

FIGURE 11.8 Structures of selected microbial chitinases and laccase.

high amounts of extracellular xylanase, which are often accompanying cellulolytic enzymes—for example as in species of *Trichoderma*, *Penicillium*, and *Aspergillus* (Kohli et al., 2001; Polizeli et al., 2005; Wong and Saddler, 1992).

11.2.8 Other Enzymes

Chitinases have been divided into two main groups: endochitinases (EC 3.2.1.14) and exo-chitinases (Fig. 11.8). The endochitinases randomly split chitin at internal sites, thereby forming the dimer di-acetylchitobiose and soluble low molecular mass multimers of GlcNAc, such as chitotriose and chitotetraose. The exochitinases have been further divided into two subcategories: chitobiosidases (EC 3.2.1.29), which are involved in catalyzing the progressive release of di-acetylchitobiose starting at the nonreducing end of the chitin microfibril, and 1-4-β-glucosaminidases (EC 3.2.1.30), cleaving the oligomeric products of endochitinases and chitobiosidases, thereby generating monomers of *N*-acetylglucosamine (GlcNAc). Chitinases (EC 3.2.1.14) can catalyze the hydrolysis of chitin to its monomer *N*-acetyl-D-glucosamine. Chitinases are widely distributed in bacteria such as *Serratia, Chromobacterium, Klebsiella*, *Pseudomonas*, *Clostridium*, *Vibrio, Arthrobacter*, *Beneckea*, *Aeromonas*, and *Streptomyces*. They are also found in fungi like *Trichoderma*, *Penicillium*,

Lecanicillium, *Neurospora*, *Mucor*, *Beauveria*, *Lycoperdon*, *Aspergillus*, *Myrothecium*, *Conidiobolus*, *Metharhizium*, *Stachybotrys*, and *Agaricus* (Felse and Panda, 2000; Islam and Datta, 2015; Matsumoto, 2006).

Laccases (benzenediol:oxygen oxidoreductase, EC 1.10.3.2) are multicopper oxidases that participate in cross-linking of monomers, degradation of polymers, and ring cleavage of aromatic compounds. These enzymes contain 15–30% carbohydrate and have a molecule mass of 60–90 kDa (Shraddha et al., 2011) (Fig. 11.8). Laccases contain four copper atoms termed Cu T1 (where the reducing substrate binds) and trinuclear copper cluster T2/T3 (electron transfer from type I Cu to the type II Cu and type III Cu trinuclear cluster/reduction of oxygen to water at the trinuclear cluster) (Gianfreda et al., 1999). These four copper ions are classified into three categories: Type 1 (T1), Type 2 (T2), and Type 3 (T3). Laccases carry out one electron oxidation of phenolic and its related compound and reduce oxygen to water. When substrate is oxidized by a laccase, it loses a single electron and usually forms a free radical which may undergo further oxidation or nonenzymatic reactions including hydration, disproportionation, and polymerization. Most laccases are extracellularly produced by fungi (Agematu et al., 1993; Brijwani et al., 2010; Chandra and Chowdhary, 2015; Mougin et al., 2003). The production of laccase can also be seen by soil and some freshwater Ascomycetes species (Banerjee and Vohra, 1991; Junghanns et al., 2005; Rodríguez et al., 1996; Scherer and Fischer, 1998). In addition, laccases are also produced by *Gaeumannomyces graminis*, *Magnaporthe grisea*, *Ophiostoma novo-ulmi*, *Marginella*, *Melanocarpus albomyces*, *Monocillium indicum*, *Neurospora crassa*, and *Podospora anserina* (Binz and Canevascini, 1997; Edens et al., 1999; Froehner and Eriksson, 1974; Iyer and Chattoo, 2003; Kiiskinen et al., 2002; Molitoris and Esser, 1970; Palonen et al., 2003; Thakker et al., 1992).

Cytochromes P450 (CYPs) catalyze various types of reactions, such as hydroxylation, epoxidation, alcohol and aldehyde oxidation, *O*-dealkylation, *N*-dealkylation, oxidative dehalogenation, and oxidative C–C bond cleavage (Sakaki, 2012). Among these, regio- and enantioselective hydroxylation by P450 is quite attractive as a bioconversion process. There are 10 classes of CYPs (Kelly and Kelly, 2013). Most bacterial CYPs are class I and driven by ferredoxin and ferredoxin reductases. CYPs have potential applications on bioconversion processes, biosensors, and bioremediation due to their regio- and enantioselective hydroxylation which is difficult for chemical synthesis. *Streptomyces carbophilus* CYP105A3 is a CYP that are successfully applied in industry for bioconversion to produce pravastatin (Watanabe et al., 1995).

11.3 PRODUCTION OF MICROBIAL ENZYMES

Fermentation is the technique of biological conversion of complex substrates into simple compounds by various microorganisms. It has been widely used for the production of many microbial enzymes (Aehle, 2007). Much work has been

focused on the screening of enzyme-producing microorganisms, physiological optimizations for substrates, carbon source and nitrogen source, pH of the media, and the cultivation temperature during the fermentation process (Ellaiah et al., 2002; Juturu and Wu, 2014; Sundarram and Murthy, 2014).

11.3.1 Fermentation Methods

There are two types of cultivation methods for all microbial enzymes: submerged fermentation (SmF) and solid state fermentation (SSF). Submerged fermentation involves the nurturing of microorganisms in high oxygen concentrated liquid nutrient medium. Viscosity of broth is the major problem associated with the fungal submerged fermentations. When fungal cells grow and a mycelium is produced, this hinders impeller action, due to this limitation occurring in oxygen and mass transfer. SSF is suitable for the production of enzymes by using natural substrates such as agricultural residues because they mimic the conditions under which the fungi grow naturally.

Since SSF involves relatively little liquid when compared with SmF, downstream processing from SSF is theoretically simpler and less expensive. During the past 10 years, a renewed interest in SSF has developed due, in part, to the recognition that many microorganisms, including genetically modified organisms (GMO), may produce their products more effectively by SSF (Singh et al., 2008). SSF has three major advantages: (1) high volumetric productivity, (2) relatively higher concentration of the products, and (3) less effluent generation, requirement for simple fermentation equipment, etc. Moreover, the biosynthesis of microbial enzymes in SmF process is of economic importance because it is strongly affected by catabolic and end product repressions. The amenability of SSF technique to use up to 20–30% substrate, in contrast to the maximum of 5% in SmF process, has been documented (Pamment et al., 1978).

11.3.2 Purification Methods

Enzymes are manufactured in bioreactors for commercial use. These enzymes are in the crude form and have to be purified for further use. The extraction methods are followed by the purification processes. There are mainly three major purification methods for microbial enzymes: (1) based on ionic properties of enzymes, (2) based on the ability to get adsorbed, and (3) based on difference in size of molecules. Special procedures employed for enzyme purification are crystallization, electrophoresis, and chromatography. The main applications of industrial-scale chromatography were the desalination of enzyme solutions by use of highly cross-linked gels such as Sephadex G-25 and batch separations by means of ion exchangers such as DEAE-Sephadex A-50. The stability and hydraulic properties of chromatographic media have been improved so that these techniques are now used on a production scale. Important parameters for the scale-up of chromatographic systems are the height of the column, the

linear flow rate, and the ratio of sample volume to bed volume. Zone spreading interferes with the performance of the column. Factors that contribute to zone spreading are longitudinal diffusion in the column, insufficient equilibration, and inadequate column packing. Longitudinal diffusion can be minimized by using a high flow rate. On the other hand, equilibration between the stationary and the mobile phases is optimal at low flow rates. Because good process economy depends to a large extent on the flow rate, a compromise must be made. In addition, the flow rate is also dependent on particle size; the decisive factor is usually the pressure drop along large columns. Although optimal resolution is obtained only with the smallest particles, the gel must have a particle size that favors a good throughput and reduces processing times. The use of segmented columns prevents a large pressure drop in the column. Above all, the column must be uniformly packed so that the particle-size distribution, porosity, and resistance to flow are the same throughout the column. If this is not done, viscous protein solutions can give an uneven elution profile, which would lead to zone bleeding. The design of the column head is important for uniform distribution of the applied sample. This is generally achieved by symmetrical arrangement of several inlets and perforated inserts for good liquid distribution. The outlet of the column must have minimal volume to prevent back-mixing of the separated components (Aehle, 2007).

11.4 APPLICATIONS OF MICROBIAL ENZYMES

Microbial enzymes are of great importance in the development of industrial bioprocesses. The end use market for industrial enzymes is extremely widespread with numerous industrial applications (Adrio and Demain, 2014). Over 500 industrial products are being made using different microbial enzymes (Kumar and Singh, 2013). The demand for industrial enzymes is on a continuous rise driven by a growing need for sustainable solutions.

Microbes are one of the largest and most useful sources of many enzymes (Demain and Adrio, 2008). A large number of new enzymes have been designed with the input of protein-engineering and metagenomics. Various molecular techniques have also been applied to improve the quality and performance of microbial enzymes for their wider applications in many industries (Chirumamilla et al., 2001; Nigam, 2013). Many microorganisms including bacteria, actinomycetes, and fungi have been globally studied for the synthesis of economically viable preparations of various enzymes for commercial applications (Pandey et al., 1999). The special characteristics of enzymes are exploited for their commercial interest and industrial applications (Table 11.1), which include thermotolerance, tolerance to a wide range of pH, and stability of enzyme activity over a harsh reaction conditions.

The majority of currently used industrial enzymes are hydrolytic and they are used for the degradation of various natural substances. Proteases are one of most important classes of enzymes for the detergent and dairy industries.

TABLE 11.1 Microbial Enzymes and Their Applications

Industry	Name of enzymes	Applications	References
Food, dairy and beverage	Protease, lipase, lactase, pectinase, amylase, laccase, amyloglucosidase, phospholipase	Degradation of starch and proteins into sugars, Production of low caloric beer, Fruit juice processing, Cheese production, Glucose production from lactose, Dough stability and Conditioning.	Gurung et al. (2013) and Nigam and Singh (1995)
Detergents	Amylase, cellulase, lipase, protease, mannanase	Remove protein after staining, Cleaning agents, Removing insoluble starch, fats and oils, To increase effectiveness of detergents.	Pandey et al. (2000a) and Wintrode et al. (2000)
Textiles	Amylase, cellulase, pectinase, catalase, protease, peroxidase, keratinase	Fabric finishing in denims, Wool treatment, Degumming of raw silk (biopolishing), Cotton softening.	Liu et al. (2013) and Saha et al. (2009)
Animal feed	Phytase, xylanase	Increase total phosphorus content for growth, Digestibility	Mitidieri et al. (2006) and Tomschy et al. (2000)
Ethanol production	Cellulase, ligninase, mannanase	Formation of ethanol	Jolly (2001)
Paper and pulp	Amylase, lipase, protease, cellulase, hemicellulase, esterase, ligninase, xylanase	Degrade starch to lower viscosity, aiding sizing, deinking, and coating paper. Cellulase and hemicellulase smooth fibers, enhance water drainage, and promote ink removal. Lipases reduce pitch and ligninase remove lignin to soften paper.	Kirk et al. (2002), Kohli et al. (2001), and Polizeli et al. (2005)
Leather	Protease, lipase	Unhearing, bating, depicking	Parameswaran et al. (2013) and Saha et al. (2009)
Pharmaceuticals	Penicillin acylase, peroxidase	Synthesis of semisynthetic antibiotics, Antimicrobials	Neelam et al. (2013) and Roberts et al. (2007)
Molecular biology	DNA ligase, restriction enzymes, polymerase	Manipulate DNA in genetic engineering. DNA restriction and the polymerase chain reaction. Important in forensic science.	Nigam (2013) and Roberts et al. (2010)

Carbohydrases, primarily amylases and cellulases, used in industries such as the starch, textile, detergent, and baking industries, represent the second largest group (Underkofler et al., 1958). The fastest growth over the past decade has been seen in the baking and animal feed industries, but growth is also being generated from applications established in a wealth of other industries spanning from organic synthesis to paper and pulp and personal care. The use of enzymes in animal nutrition is important and growing, especially for pig and poultry nutrition.

Enzymes play key roles in numerous biotechnology products and processes that are commonly encountered in the production of food and beverages, detergents, clothing, paper products, transportation fuels, pharmaceuticals, and monitoring devices (Gurung et al., 2013). As the industrial enzyme market has expanded at a rate of about 10% annually, microbial enzymes have largely replaced the traditional plant and animal enzymes. DNA technology has been used to modify substrate specificity and improve stability properties of enzymes for increasing yields of enzyme-catalyzed reactions. Enzymes can display regional stereospecificity, properties that have been exploited for asymmetric synthesis and racemic resolution. Chiral selectivities of enzymes have been employed to prepare enantiomerically pure pharmaceuticals, agrochemicals, chemical feedstock, and food additives.

11.4.1 Food and Beverage

In the 20th century, enzymes began to be isolated from living cells, which led to their large-scale commercial production and wider application in the food industry. Food and beverage enzymes constitute the largest segment of industrial enzymes with revenues of nearly $1.2 billion in 2011 which is expected to grow to $2.0 billion by 2020. Enzymes used in food can be divided into food additives and processing aids. Most food enzymes are considered as processing aids used during the manufacturing process of foodstuffs (Saha et al., 2009) with only a few used as additives, such as lysozyme and invertase. The applications of different enzymes in the food industry are shown in Table 11.2.

Amylases are the most important enzymes in the industrial starch conversion process. Amylolytic enzymes act on starch and related oligo- and polysaccharides (Pandey et al., 2000a). The application of these enzymes has been established in starch liquefaction, paper, food, sugar, and pharmaceutical industries. In the food industry, amylolytic enzymes have a large scale of applications, such as the production of glucose syrups, maltose syrup, reduction of viscosity of sugar syrups, to produce clarified fruit juice for longer shelf-life, solubilization of starch in the brewing industry (Pandey et al., 2000b). The baking industry uses amylases to delay the staling of bread and other baked products.

The major application of proteases in the dairy industry is for the manufacture of cheese. Calf rennin (chymosin) had been preferred in cheese making due to its high specificity, but microbial proteases are also used. Chymosin is

TABLE 11.2 Applications of Enzymes in the Food Industry

Process	Enzyme	Applications	References
Baking	Amylase, protease	Conversion of sugar into ethanol and CO_2. To prepare bread	Collar et al. (2000)
Brewing	Amylase, protease	Conversion of sugar into ethanol and CO_2. To prepare alcoholic drink	Pandey et al. (2000a)
Corn syrup	Amylase	Preparation of low dextrose equivalent syrups	Kirk et al. (2002)
Cheese making	Rennin, lipases	Milk clotting, Flavor production	Okanishi et al. (1996)
Baby foods	Trypsin	Digestion	Parameswaran et al. (2013)
Coffee	Pectinase	Coffee bean fermentation	Kirk et al. (2002)
Dairy industry	Protease, lactase lactoperoxidase	Preparation of protein hydrolysates, preparation of milk and ice cream, Cold sterilization of milk	Tucker and Woods (1995)
Fruit juices	Glucose oxidase, pectinase	Oxygen removal, clarification of fruit juices	Godfrey and West (1996,b)
Soft drinks	Glucose oxidase	Stabilization	Kirk et al. (2002)
Meat and fish industries	Proteinase	To tenderize meat and solubilize fish products	Saha and Demirjian (2000)

an aspartic acid protease which causes the coagulation of milk. The primary function of these enzymes in cheese making is to hydrolyze the specific peptide bond that generates para-k-casein and macropeptides (Rao et al., 1998). Production of calf rennin (chymosin) in recombinant *A. niger* var. *awamori* amounted to about 1 g/L after nitrosoguanidine mutagenesis and selection for 2-deoxyglucose resistance. Four recombinant proteases have been approved by FDA for cheese production (Bodie et al., 1994; Pariza and Johnson, 2001).

The application of enzymes (proteases, lipases, esterases, lactase, and catalase) in dairy technology is well established. Rennets (rennin) are used for coagulation of milk in the first stage of cheese production. Proteases of various kinds are used for acceleration of cheese ripening, for modification of functional properties and for modification of milk proteins to reduce the allergenic properties of cow milk products for infants. Lipases are used mainly in cheese ripening for development of lipolytic flavors. Lactase is used to hydrolyze lactose to

glucose and galactose as a digestive aid and to improve the solubility and sweetness in various dairy products. Lactose hydrolysis helps these lactose-intolerant people to drink milk and eat various dairy products (Tucker and Woods, 1995). Lactases have also been used in the processing of dairy wastes and as a digestive aid taken by humans in tablet form when consuming dairy products. Recently three novel xylanases thermophilic in nature (XynA,B,C) have been characterized (Du et al., 2013); these were produced by *Humicola* sp. for their potential applications in the brewing industry. This XynA also possessed higher catalytic efficiency and specificity for a range of substrates.

Proteinases, either indigenous (cathepsin) or those obtained from plants and microorganisms, are used in the meat and fish industries to tenderize meat and solubilize fish products. Tenderization of meat can be achieved by keeping the rapidly chilled meat at 1–2°C to allow proteolysis by indigenous enzymes. Enzymes are also used to facilitate separation of hemoglobin from blood proteins and removal of meat from bones. For the preparation of pet food, minced meat or meat by-products are hydrolyzed by proteases to produce a liquid meat digest or slurry with a much lower viscosity (Saha and Demirjian, 2000). Fish protein concentrates are generally prepared by treating ground fish parts with a protease.

In the baking industry, there is an increasing focus on lipolytic enzymes. Recent findings suggest that phospholipases can be used to substitute or supplement traditional emulsifiers, as the enzymes degrade polar wheat lipids to produce emulsifying lipids. Also, research is currently devoted towards the further understanding of bread staling and the mechanisms behind the enzymatic prevention of staling in presence of α-amylases and xylanases (Andreu et al., 1999). Lipases are commonly used in the production of a variety of products, ranging from fruit juices, baked foods, and vegetable fermentations. Fats, oils, and related compounds are the main targets of lipases in food technology. Accurate control of lipase concentration, pH, temperature, and emulsion content is required to maximize the production of flavor and fragrance. The lipase mediation of carbohydrate esters of fatty acids offers a potential market for use as emulsifiers in foods and pharmaceuticals. There are three recombinant fungal lipases currently used in the food industry, one from *Rhizomucor miehi*, one from *Thermomyces lanuginosus*, and another from *Fusarium oxysporum* (Mendez and Salas, 2001).

Enzymes can play important roles in preparing and processing various fruit and vegetable juices, such as apple, orange, grapefruit, pineapple, carrot, lemon etc. Fruits and vegetables are particularly rich in pectic substances. Pectin, a hydrocolloid, has a great affinity for water and can form gels under certain conditions. The addition of pectinases, pectin lyase, pectin esterase, and polygalacturanase, reduces viscosity and improves pressability as the pectin gel collapses (Tucker and Woods, 1995). For complete liquefaction of fruits and vegetable juices, hemicellulases and amylases can be used with pectinases. Flavoprotein glucose oxidase, is used to scavenge oxygen in fruit juice and beverages to

prevent color and taste changes upon storage. Glucose oxidase is produced by various fungi such as *A. niger* and *Penicillium purpurogenum* (Godfrey and West, 1996b).

11.4.2 Detergents

The detergent industry occupies about 30% of the entire industrial enzyme market. The application of enzymes in detergents enhances the detergents ability to remove tough stains and also makes detergent ecofriendly. Constantly, new and improved engineered versions of the "traditional" detergent enzymes, proteases and amylases, are developed. These new second- and third-generation enzymes are optimized to meet the requirements for performance in detergents. Over half of the laundry detergents contain enzymes such as protease, amylase, lipase, and cellulase. These enzymes must be very efficient in laundry detergent environments, work at alkaline pH conditions and high temperatures, be stable in the presence of chelating agents and surfactants, and possess long storage stability. Proteases are the most widely used enzymes in the detergent industry. DNA technology has been used extensively to modify the protein catalysts, primarily for increasing stability properties (Bisgaard-Frantzen et al., 1999; Wintrode et al., 2000). These detergent enzymes (serine proteases) are produced by fermentation of *B. lichiniformis*, *B. amyloliquefaciens*, or *Bacillus* sp.

Novo Industri A/S produces and supplies three proteases, Alcalase, from *B. licheniformis*, Esperase, from an alkalophilic strain of a *B. licheniformis*, and Savinase, from an alkalophilic strain of *B. amyloliquefaciens*. GistBrocades produce and supply Maxatase, from *B. licheniformis*. Alcalase and Maxatase are recommended for use at 10–65°C and pH 7–10.5. Savinase and Esperase may be used at up to pH 11 and 12, respectively.

Amylases are the second type of enzymes used in the detergent formulation, and 90% of all liquid detergents contain these enzymes (Mitidieri et al., 2006). These enzymes are used for laundry and automatic dishwashing to clean up residues of starchy foods such as mashed potato, custard, oatmeal, and other small oligosaccharides. The α-amylase supplied for detergent use is Termamyl, the enzyme from *B. licheniformis*, which is also used in the production of glucose syrups. α-Amylase is particularly useful in dishwashing and destarching detergents.

Lipases facilitate the removal of fatty stains, such as lipsticks, frying fats, butter, salad oil, sauces, and tough stains on collars and cuffs. Recently, an alkali-stable fungal cellulose preparation has been introduced for use in washing cotton fabrics. Treatment with these cellulose enzymes removes the small fibers extending from the fabric, without apparently damaging the major fibers, and restores the fabric by improving color brightness and enhancing softness feel. Cellulases are used in textile manufacturing to partially remove dye (indigo) from denim, producing a stone-washed appearance. Bleach-stable enzymes (amylase, protease) are now available for use in automatic dishwashing

detergents. The most commercially important field of application for hydrolytic lipases is their addition to detergents, which are used mainly in household and industrial laundry and in household dishwashers. To improve detergency, modern types of heavy duty powder detergents and automatic dishwasher detergents usually contain one or more enzymes, such as protease, amylase, cellulase, and lipase (Ito et al., 1998).

11.4.3 Textiles

Enzymes are being used increasingly in textile processing, mainly in the finishing of fabrics and garments. Some of the more important applications are desizing and jeans finishing. The use of enzymes in the textile industry allows the development of environmentally friendly technologies in fiber processing and strategies to improve the final product quality. The consumption of energy as well as increased awareness of environmental concerns related to the use and disposal of chemicals into landfills, water, or release into the air during chemical processing of textiles are the principal reasons for the application of enzymes in finishing of textile materials (O'Neill et al., 2007).

Enzymes have been used increasingly in the textile industry since the late 1980s. Many of the enzymes developed since the late 1990s are able to replace chemicals used by mills. The first major breakthrough was when enzymes were introduced for stonewashing jeans in 1987. Within a few years, the majority of denim finishing laundries had switched from pumice stones to enzymes.

The main enzymes used in textile industry are hydrolases and oxidoreductases. The group of hydrolases includes amylases, cellulases, proteases, pectinases, lipases and esterases. Amylases were the only enzymes applied in textile processing until the 1980s. These enzymes are still used to remove starch-based sizes from fabrics after weaving. Nowadays, amylases are commercialized and preferred for desizing due to their high efficiency and specificity, completely removing the size without any harmful effects on the fabric (Etters and Annis, 1998). Cellulases have been employed to enzymatically remove fibrils and fuzz fibers, and have also successfully been introduced to the cotton textile industry. Further applications have been found for these enzymes to produce the aged look of denim and other garments. The potential of proteolytic enzymes was assessed for the removal of wool fiber scales, resulting in improved antifelting behaviour. Esterases have been successfully studied for the partial hydrolysis of synthetic fiber surfaces, improving their hydrophilicity and aiding further finishing steps. Besides hydrolytic enzymes, oxidoreductases have also been used as powerful tools in various textile-processing steps. Catalases have been used to remove H_2O_2 after bleaching and to reduce water consumption. In the textile industry lipases are used for the removal of size lubricants, which increases fabrics absorbance ability for improved levelness in dyeing (Raja et al., 2012). In the denim abrasion systems, it is used to lessen the frequency of cracks and streaks.

11.4.4 Animal Feed

Animal feed is the largest cost item in livestock and poultry production, accounting for 60–70% of total expenses. To save on costs, many producers supplement feed with enzyme additives, which enable them to produce more meat or to produce the meat cheaper and faster. Found in all living cells, enzymes catalyze chemical processes that convert nutrients into energy and new tissue. They do this by binding to substrates in the feed and breaking them down into smaller compounds. For example, proteases break down proteins into amino acids, carbohydrases split carbohydrates into simple sugars, and lipases take apart lipids into fatty acids and glycerol.

Animal feed is composed mainly of plant materials, such as cereals, agricultural and grain milling byproducts, and agricultural waste residues. These contain nonstarch polysaccharides, protein, and phytic acid. Monogastric animals generally cannot fully digest and utilize the fiber-rich feedstuffs. Due to the complex nature of the feed materials, starch sequestered by β-glucans and pentosans is also not digestible. Feed enzymes can increase the digestibility of nutrients, leading to greater efficiency in feed utilization (Choct, 2006). Currently, feed enzymes commercially available are phytases, proteases, α-galactosidases, glucanases, xylanases, α-amylases, and polygalacturonases (Selle and Ravindran, 2007). The use of enzymes as feed additives is restricted in many countries by local regulatory authorities (Pariza and Cook, 2010) and applications may therefore vary from country to country.

During recent years focus has been on the utilization of natural phosphorus bound in phytic acid in cereal-based feed for monogastrics. Phytic acid forms complexes with metal ions such as calcium, magnesium, iron, and zinc, thus preventing their assimilation by the animal. Better utilization of total plant phosphorus, of which 85–90% is bound in phytic acid, is only obtained by adding the enzyme phytase to the feed. Microbial phytase liberates part of the bound phosphorus and makes it possible to reduce the amount of supplements (phosphorus, calcium, and other nutrients) added to the animal diet. Phytase in animal feed can alleviate environmental pollution from bound phosphorous in animal waste and development of dietary deficiencies in animals (Lei and Stahl, 2000). The most common source of microbial phytase is *Aspergillus ficuum*.

Protein utilization from vegetables can be enhanced by using microbial proteases. Thus, feed utilization and digestion by animals can be enhanced by adding enzymes to the feed (Lehmann et al., 2000). Various microbial enzymes are now used as feed enhancers and hold the prospect of serving larger roles in animal and poultry production. Commercially-available enzymes can be derived from plants and animals (eg, actinidin from kiwi and rennet from calf stomachs) as well as microorganisms (eg, amylase from *Bacillus* and lactase from *Aspergillus*).

11.4.5 Ethanol Production

In the alcohol industry, the use of enzymes for the production of fermentable sugars from starch is also well established. The process of making ethanol from starch involves the basic steps of preparation of the glucose feedstock, fermentation of glucose to ethanol, and recovery of ethanol. Enzymes have an important role in preparing the feedstock, that is, in converting starch into the fermentable sugar, glucose. Corn kernels contain 60–70% starch, and it is the dominant source (97%) of starch feedstock used for ethanol production.

Over the past decade, there has been an increasing interest in fuel alcohol as a result of increased environmental concern and higher crude oil prices. Therefore, intense efforts are currently being undertaken to develop improved enzymes that can enable the utilization of cheaper and partially utilized substrates such as lignocellulose, to make bioethanol more competitive with fossil fuels (Wheals et al., 1999; Zaldivar et al., 2001).

Two methods are used industrially to process corn for making starch accessible to enzymes in subsequent treatment. In the wet-milling process, corn is steeped in acidic water solutions and the oil, protein, and fiber fractions are successively removed as products leaving the starch fraction. Enzymatic liquefaction and saccharification of the starch fraction are then carried out for the production of glucose. Microbial enzymes have replaced the traditional hydrolytic enzymes formerly supplied by adding malt. Glucose is fermented by the traditional yeast *S. cerevisiae* to ethanol, which can be recovered by distillation. In beverage ethanol processes, the beer may be treated with acetolactate decarboxylase from *Bacillus brevis* or *Lactobacillus* sp. to convert acetolactate into acetoin via nonoxidative decarboxylation. Saccharification and fermentation steps can also be carried out concurrently in a process known as simultaneous saccharification and fermentation In the United States, most ethanol (over 80%) from corn is produced from corn processed through dry grind facilities because of the lower capital investment required in comparison to that of wet mills. In the typical dry grind process, corn is mechanically milled to coarse flour. Following liquefaction, enzymatic saccharification using glucoamylase and fermentation using the conventional yeast are carried out simultaneously (Taylor et al., 2000, 2001). The addition of protein-splitting enzymes (proteases) releases soluble nitrogen compounds from the fermentation mash and promotes growth of the yeast, decreasing fermentation time. The residue left after fermenting the sugars is known as distiller's grains, which is used as animal feed. Typically, large-scale industrial fermentation processes provide 12–15% (v/v) ethanol with an ethanol yield as high as 95% of theoretical, on the basis of starch feedstock.

11.4.6 Other Applications

In recent years, tremendous research efforts have been made to reduce the amount of chlorine used for bleaching of kraft pulp after the pulping processes.

Environmental regulatory pressures have prompted the pulp and paper industry to adapt new technology to eliminate the presence of various contaminants in the bleaching plant effluents. The main constituents of wood are cellulose, hemicellulose, and lignin. Research in the use of enzymes in pulp manufacture involves the degradation or modification of hemicellulose and lignin without disturbing the cellulose fibers.

Xylanase preparations used for wood processing in the paper industry should be free of cellulose activity. Cellulase-free xylanase preparations have applications to provide brightness to the paper due to their preferential solubilization of xylans in plant materials and selective removal of hemicelluloses from the kraft-pulp. The production of cellulase free extracellular endo-1,4-β-xylanase has been studied at a higher temperature of 50°C and at pH 8.5 employing a strain of *Thermoactinomyces thalophilus* (Kohli et al., 2001). The paper and pulp industry requires a step of separation and degradation of lignin from plant material, where the pretreatment of wood pulp using ligninolytic enzymes is important for a milder and cleaner strategy of lignin removal compared to chemical bleaching. Bleach enhancement of mixed wood pulp has been achieved using coculture strategies, through the combined activity of xylanase and laccase (Dwivedi et al., 2010). The ligninolytic enzyme system is used in biobleaching of craft pulp and in other industries. Fungi are the most potent producers of lignin degrading enzymes. The use of laccase to promote degradation of lignin and bleaching of pulp has attracted considerable interest as a cost-effective replacement for chlorine bleaches. Thermophilic laccase enzyme is of particular use in the pulping industry. Recently, the biophysical characterization of thermophilic laccase isoforms has been reported (Kumar and Srikumar, 2013).

The removal of pitch by chemical pulping or bleaching is not efficient. Pitch is the sticky resinous material in wood. Treatment with lipases has been found to be useful in reducing pitch deposits since lipases hydrolyze the triglycerides in the wood resin to fatty acids and glycerol making the material less viscous. The enzyme does not affect the cellulose quality. Removal of ink is an important part of waste paper processing. Conventional deinking involves pulping of the paper in highly alkaline solution. It has been reported that cellulase enzymes can increase the efficiency of the deinking process. The coating treatment makes the surface of paper smooth and strong to improve the writing quality of the paper. For paper sizing the viscosity of the natural enzyme is too high, and this can be changed by partially degrading the polymer with α-amylases in a batch or continuous processes (van der Maarel et al., 2002). Starch is considered to be the good sizing agent for the finishing of paper, improving the quality and reusability, besides being a good coating for the paper.

In the leather industry, skins are soaked initially to clean them and to allow rehydration. Proteolytic enzymes effectively facilitate the soaking process. Lipases have also been used to dissolve and remove fat. Dehairing is then carried out using alkaline protease, such as subtilisin. Alkaline conditions swell the

hair roots, easing removal of the hair by allowing proteases to selectively attack the protein in the hair follicle. Conventional dehairing processes require harsh chemicals, such as slaked lime and sodium sulfide, which essentially swell the hide and loosen and damage the hair (Godfrey and West, 1996b). Enzyme-based dehairing has led to much lower pollution emissions from tanneries.

Enzymes are used in various analytical methods, both for medical and non-medical purposes. Immobilized enzymes are used as biosensors for the analysis of organic and inorganic compounds in biological fluids. A glucose biosensor consists of a glucose oxidase membrane and an oxygen electrode, while a biosensor for lactate consists of immobilized lactate oxidase and an oxygen electrode. The lactate sensor functions by monitoring the decrease in dissolved oxygen which results from the oxidation of lactate in the presence of lactate oxidase (Saha et al., 2009). The amperometric determination of pyruvate can be carried out with the pyruvate oxidase sensor. A bioelectrochemical system for total cholesterol estimation was developed, based on a double-enzymatic method. In this system, an immobilized enzyme reactor containing cholesterol esterase and cholesterol oxidase is coupled with an amperometric detector system. An amino acid electrode for the determination of total amino acids has also been developed using the enzymes L-glutamate oxidase, L-lysine oxidase, and tyrosinase. Enzyme electrodes are used for continuous control of fermentation processes.

Successful application of enzymatic processes in the chemical industry depends mainly on cost competitiveness with the existing and well-established chemical methods (Tufvesson et al., 2011). However, new scientific developments in genomics, as well as in protein engineering, facilitate the tailoring of enzyme properties to increase that number significantly (Jackel and Hilvert, 2010; Lutz, 2010). An enzymatic conversion was devised to produce the amino acid L-tyrosine. Phenol, pyruvate, pyridoxal phosphate and ammonium chloride are converted to L-tyrosine using a thermostable and chemostable tyrosine phenol lyase obtained from *Symbiobacterium toebii* (Kim do et al., 2007; Sanchez and Demain, 2011).

The numerous biocatalytic routes scaled up for pharmaceutical manufacturing have been recently reviewed (Bornscheuer et al., 2012), showing the competitiveness of enzymes versus traditional chemical processes. Enzymes are useful for preparing beta-lactam antibiotics such as semisynthetic penicillins and cephalosporins (Volpato et al., 2010). The semisynthetic penicillins have largely replaced natural penicillins and about 85% of penicillins marketed for medicinal use are semisynthetic. 6-Aminopenicillanic acid is obtained by the hydrolysis of the amide bond of the naturally occurring penicillin with the enzyme penicillin amidase, which unlike chemical hydrolysis does not open the β-lactam ring. The most important applications in biocatalysis are the synthesis of complex chiral pharmaceutical intermediates efficiently and economically. Esterases, lipases, proteases, and ketoreductases are widely applied in the preparation of chiral alcohols, carboxylic acids, amines, or epoxides (Zheng and Xu, 2011). Kinetic resolution of racemic amines is a common method used in

the synthesis of chiral amines. Acylation of a primary amine moiety by a lipase is used by BASF for the resolution of chiral primary amines in a multithousand ton scale (Sheldon, 2008). Atorvastatin, the active ingredient of Lipitor, a cholesterol-lowering drug can be produced enzymatically. The process is based on three enzymatic activities, such as a ketone reductase, a glucose dehydrogenase, and a halohydryn dehalogenase. Several iterative rounds of DNA shuffling for these three enzymes led to a 14-fold reduction in reaction time, a sevenfold increase in substrate loading, a 25-fold reduction in enzyme use, and a 50% improvement in isolated yield (Ma et al., 2010).

Therapeutic enzymes have a wide variety of specific uses such as oncolytics, thrombolytics, or anticoagulants and as replacements for metabolic deficiencies. Enzymes are being used to treat many diseases like cancer, cardiac problems, cystic fibrosis, dermal ulcers, inflammation, digestive disorders etc. Proteolytic enzymes serve as good anti-inflammatory agents. Collagenase enzyme, which hydrolyzes native collagen and spares hydrolysis of other proteins, has been used in dermal ulcers and burns. Papain has been shown to produce marked reduction of obstetrical inflammation and edema in dental surgery. Deoxyribonuclease is used as a mucolytic agent in patients with chronic bronchitis. Trypsin and chymotrypsin have been successfully used in the treatment of athletic injuries and postoperative hand trauma. Hyaluronidase has hydrolytic activity on chondroitin sulphate and may help in the regeneration of damaged nerve tissue (Moon et al., 2003). Lysozyme hydrolyzes the chitins and mucopeptides of bacterial cell walls. Hence, it is used as antibacterial agent usually in combination with standard antibiotics. Lysozyme has also been found to have activity against HIV, as the RNase A and urinary RNase U present selectively degrade viral RNA (Lee-Huang et al., 1999) showing possibilities for the treatment of HIV infection.

Cancer research has some good instances of the use of enzyme therapeutics. Recent studies have proved that arginine-degrading enzyme (PEGylated arginine deaminase) can inhibit human melanoma and hepatocellularcarcinomas (Ensor et al., 2002). Currently, another PEGylated enzyme, Oncaspar1 (pegaspargase), is showing good results for the treatment of children newly diagnosed with acute lymphoblastic leukemia. The further application of enzymes as therapeutic agents in cancer is described by antibody-directed enzyme prodrug therapy (ADEPT). A monoclonal antibody carries an enzyme specific to cancer cells where the enzyme activates a prodrug and destroys cancer cells but not normal cells. This approach is being utilized for the discovery and development of cancer therapeutics based on tumor-targeted enzymes that activate prodrugs. Certain enzymes such as L-asparaginase have been found to be useful in treating cancer. L-asparaginase, by lowering the concentration of asparagine, retards the growth of cancer cells. It has proven particularly useful in treating lymphoblastic leukemia and certain forms of lymphomas.

Genetic engineering basically involves taking the relevant gene from the microorganism that naturally produces a particular enzyme (donor) and inserting

it into another microorganism that will produce the enzyme more efficiently (host). The first step is to cleave the DNA of the donor cell into fragments using restriction enzymes. The DNA fragments with the code for the desired enzyme are then placed, with the help of ligases, in a natural vector called a plasmid that can be transferred to the host bacterium or fungus. In recombinant DNA technology, restriction enzymes recognize specific base sequences in double helical DNA and bring out cleavage of both strands of the duplex in regions of defined sequence. Restriction enzymes cleave foreign DNA molecules. The term *restriction endonuclease* comes from the observation that certain bacteria can block virus infections by specifically destroying the incoming viral DNA (Adrio and Demain, 2014). Such bacteria are known as restricting hosts, since they restrict the expression of foreign DNA. Certain nicks in duplex DNA can be sealed by an enzyme-DNA ligase which generates a phosphodiester bond between a 5′-phosphoryl group and a directly adjacent 3′-hydroxyl, using either ATP or NAD^+ as an external energy source.

11.5 FUTURE OF MICROBIAL ENZYMES

Enzymes are some of the most important biomolecules, which have a wide range of applications in industrial as well as biomedical field. Today enzymes are some of the most important molecules that are widely used in every sector, whether that may be dairy, industrial, agriculture, or pharmaceutical fields. The global market for industrial enzymes is estimated at $3.3 billion in 2010 and is expected to reach $5 billion by 2020. The market segmentation for various areas of application shows that 34% of the market is for food and animal feed, followed by detergent and cleaners (29%). Paper and pulps share an 11% market while 17% of the market is captured by the textile and leather industries (Parameswaran et al., 2013).

The ongoing progress and interest in enzymes provide further success in areas of industrial biocatalysis. There is the need for exciting developments in the area of biotransformation and molecular biology. There are many factors influencing the growing interest in biocatalysts, which include enzyme promiscuity, robust computational methods combined with directed evolution, and screening technologies to improve enzyme properties to meet process prospects (Adrio and Demain, 2014).

Recent advances in genomics, proteomics, efficient expression systems, and emerging recombinant DNA techniques have facilitated the discovery of new microbial enzymes from nature or by creating enzymes with improved catalytic properties. A future trend is to develop more effective systems that use much smaller quantities of chemicals and less energy to attain maximum product yield. Modern biotechnology will lead to the development of enzyme products with improved effects with diverse physiological conditions. Biotechnology offers an increasing potential for the production of goods to meet various human needs. Enzyme technology is a subfield of biotechnology where new processes

had been developed and are still developing to manufacture both bulk and high added value products utilizing enzymes as biocatalysts, in order to meet needs in food, fine chemicals, agricultural, and pharmaceuticals, etc.

Enzymes contribute to more environmentally-adapted clean and green technology due to their biodegradable nature. They can be used in order to develop environmentally friendly alternatives to chemical processes in almost all steps of textile fiber processing (Araujo et al., 2008). Further research is required for the implementation of commercial enzyme-based processes for the biomodification of synthetic and natural fibers. An active field of research is the search for new enzyme-producing microorganisms and enzymes extracted from extremophilic micro-organisms (Schumacher et al., 2001).

Since the late 1990s, enzyme applications have been continuously increasing with highly research and development oriented activity covering various scientific and technological issues. Many enzymes need rigorous research and development to explore commercially through basic research in enzymology and process engineering. It is likely that the functional understanding of different enzyme classes will provide new applications in the future. Multidisciplinary research involving industry is required to develop application-oriented research on enzymes. Over the past 10 years, major advances in DNA technologies and in bioinformatics have provided critical support to the field of biocatalysis. These tools have promoted the discovery of novel enzymes in natural resources and have substantially accelerated the redesign of existing biocatalysts. Next-generation DNA sequencing technology has allowed parallel sequence analysis on a massive scale and at dramatically reduced cost (Bornscheuer et al., 2012).

New and exciting enzyme applications are likely to bring benefits in other areas like less harm to the environment, greater efficiency, lower cost, lower energy consumption, and the enhancement of product properties. New enzyme molecules capable of achieving this will be developed through protein engineering and recombinant DNA techniques. Industrial biotechnology has an important role to play in the way modern foods are processed. New ingredients and alternative solutions to current chemical processes will be the challenge for the enzyme industry. When compared with chemical reactions, the more specific and cleaner technologies made possible by enzyme-catalyzed processes will promote the continued trend towards natural processes in the production of food.

11.6 CONCLUDING REMARKS

The enzyme industry is one of the major industries of the world and there exists a great market for enzymes. Enzymes are used in many different industrial products and processes and new areas of applications are constantly being added because of advances in modern biotechnology. Microorganisms provide an impressive amount of catalysts with a wide range of applications across many industries, such as food, animal feed, technical industries, paper, fine chemicals, and pharmaceuticals. The unique properties of enzymes, such as high

specificity, fast action, and biodegradability, allow enzyme-assisted processes in industry to run under milder reaction conditions, with improved yields and a reduction in waste generation. Naturally occurring enzymes are often modified by molecular biology techniques to redesign the enzyme itself in order to fine-tune substrate specificity activity and thermostability. Enzyme technology offers a great potential for many industries to meet challenges in the future with the help of recombinant technology.

REFERENCES

Adrio, J.L., Demain, A.L., 2014. Microbial enzymes: tools for biotechnological processes. Biomolecules 4, 117–139.

Aehle, W., 2007. Enzymes in Industry – Production and Applications. Wiley-VCH Verlag GmbH & Co., Weinheim, Germay.

Agematu, H., Tsuchida, T., Kominato, K., Shibamoto, N., Yoshioka, T., Nishida, H., et al., 1993. Enzymatic dimerization of penicillin X. J. Antibiot. (Tokyo) 46, 141–148.

Andreu, P., Collar, C., Martínez-Anaya, M.A., 1999. Thermal properties of doughs formulated with enzymes and starters. Eur. Food Res. Technol. 209, 286–293.

Araujo, R., Casal, M., Cavaco-Paulo, A., 2008. Application of enzymes for textile fibers processing. Biocatal. Biotransformation 26, 332–349.

Arpigny, J.L., Jaeger, K.E., 1999. Bacterial lipolytic enzymes: classification and properties. Biochem. J. 343 (Pt 1), 177–183.

Banerjee, U.C., Vohra, R.M., 1991. Production of laccase by *Curvularia sp*. Folia Microbiol. (Praha) 36, 343–346.

Beg, Q., Kapoor, M., Mahajan, L., Hoondal, G., 2001. Microbial xylanases and their industrial applications: a review. Appl. Microbiol. Biotechnol. 56, 326–338.

Binz, T., Canevascini, G., 1997. Purification and partial characterization of the extracellular laccase from *Ophiostoma novo-ulmi*. Curr. Microbiol. 35, 278–281.

Bisgaard-Frantzen, H., Svendsen, A., Norman, B., Pedersen, S., Kjaerulff, S., Outtrup, H., et al., 1999. Development of industrially important α-amylases. J. Appl. Glycosci. 46, 199–206.

Bodie, E.A., Armstrong, G.L., Dunn-Coleman, N.S., 1994. Strain improvement of chymosin-producing strains of *Aspergillus niger* var. awamori using parasexual recombination. Enzyme Microb. Technol. 16, 376–382.

Bornscheuer, U.T., Huisman, G.W., Kazlauskas, R.J., Lutz, S., Moore, J.C., Robins, K., 2012. Engineering the third wave of biocatalysis. Nature 485, 185–194.

Brijwani, K., Rigdon, A., Vadlani, P.V., 2010. Fungal laccases: production, function, and applications in food processing. Enzyme Res. 2010, 149748.

Ceci, L., Lozano, J., 1998. Determination of enzymatic activities of commercial pectinases for the clarification of apple juice. Food Chem. 61, 237–241.

Chandra, R., Chowdhary, P., 2015. Properties of bacterial laccases and their application in bioremediation of industrial wastes. Environ. Sci. Process Impacts 17, 326–342.

Chirumamilla, R.R., Muralidhar, R., Marchant, R., Nigam, P., 2001. Improving the quality of industrially important enzymes by directed evolution. Mol. Cell. Biochem. 224, 159–168.

Choct, M., 2006. Enzymes for the feed industry. World Poult. Sci. 62, 5–15.

Collar, C., Martinez, J.C., Andreu, P., Armero, E., 2000. Effects of enzyme associations on bread dough performance. A response surface analysis. Food Sci Technol Int. 6, 217–226.

Collins, T., Gerday, C., Feller, G., 2005. Xylanases, xylanase families and extremophilic xylanases. FEMS Microbiol. Rev. 29, 3–23.

Demain, A.L., Adrio, J.L., 2008. Contributions of microorganisms to industrial biology. Mol. Biotechnol. 38, 41–55.

Deng, A., Wu, J., Zhang, Y., Zhang, G., Wen, T., 2010. Purification and characterization of a surfactant-stable high-alkaline protease from *Bacillus* sp. B001. Bioresour. Technol. 101, 7111–7117.

Du, Y., Shi, P., Huang, H., Zhang, X., Luo, H., Wang, Y., et al., 2013. Characterization of three novel thermophilic xylanases from *Humicola insolens* Y1 with application potentials in the brewing industry. Bioresour. Technol. 130, 161–167.

Dwivedi, P., Vivekanand, V., Pareek, N., Sharma, A., Singh, R.P., 2010. Bleach enhancement of mixed wood pulp by xylanase-laccase concoction derived through co-culture strategy. Appl. Biochem. Biotechnol. 160, 255–268.

Edens, W.A., Goins, T.Q., Dooley, D., Henson, J.M., 1999. Purification and characterization of a secreted laccase of *Gaeumannomyces graminis* var. tritici. Appl. Environ. Microbiol. 65, 3071–3074.

Ellaiah, P., Srinivasulu, B., Adinarayana, K., 2002. A review on microbial alkaline proteases. J. Sci. Ind. Res. 61, 690–704.

Ensor, C.M., Holtsberg, F.W., Bomalaski, J.S., Clark, M.A., 2002. Pegylated arginine deiminase (ADI-SS PEG20,000 mw) inhibits human melanomas and hepatocellular carcinomas *in vitro* and *in vivo*. Cancer Res. 62, 5443–5450.

Etters, J.N., Annis, P.A., 1998. Textile enzyme use: a developing technology. Am. Dyestuff Reporter 5, 18–23.

Felse, P.A., Panda, T., 2000. Production of microbial chitinases – a revisit. Bioprocess Eng. 23, 127–134.

Fiedurek, J., Gromada, A., 2000. Production of catalase and glucose oxidase by *Aspergillus niger* using unconventional oxygenation of culture. J. Appl. Microbiol. 89, 85–89.

Froehner, S.C., Eriksson, K.E., 1974. Purification and properties of *Neurospora crassa* laccase. J. Bacteriol. 120, 458–465.

Gianfreda, L., Xu, F., Bollag, J.M., 1999. Laccases: a useful group of oxidoreductive enzymes. Biorem. J. 3, 1–26.

Godfrey, R., West, M., 1996a. Industrial Enzymology. Macmillan Publishers Inc., New York.

Godfrey, T., West, S.I., 1996b. Introduction to industrial enzymology. In: Godfrey, T., West, S. (Eds.), Industrial Enzymology. Macmillan Press, London, pp. 1–8.

Gummadi, S.N., Panda, T., 2003. Purification and biochemical properties of microbial pectinases—a review. Process Biochem. 38, 987–996.

Gupta, R., Gupta, N., Rathi, P., 2004. Bacterial lipases: an overview of production, purification and biochemical properties. Appl. Microbiol. Biotechnol. 64, 763–781.

Gurung, N., Ray, S., Bose, S., Rai, V., 2013. A broader view: microbial enzymes and their relevance in industries, medicine, and beyond. Biomed. Res. Int. 2013, 1–18.

Henrissat, B., 1991. A classification of glycosyl hydrolases based on amino acid sequence similarities. Biochem. J. 280, 309–316.

Henrissat, B., Coutinho, P.M., 2001. Classification of glycoside hydrolases and glycosyltransferases from hyperthermophiles. In: Michael, W.W., Adams, R.M.K. (Eds.), Methods in Enzymology. Academic Press, pp. 183–201.

Islam, R., Datta, B., 2015. Diversity of chitinases and their industrial potential. Int. J. Appl. Res. 1, 55–60.

Ito, S., Kobayashi, T., Ara, K., Ozaki, K., Kawai, S., Hatada, Y., 1998. Alkaline detergent enzymes from alkaliphiles: enzymatic properties, genetics, and structures. Extremophiles 2, 185–190.

Iyer, G., Chattoo, B.B., 2003. Purification and characterization of laccase from the rice blast fungus, *Magnaporthe grisea*. FEMS. Microbiol. Lett. 227, 121–126.

Jackel, C., Hilvert, D., 2010. Biocatalysts by evolution. Curr. Opin. Biotechnol. 21, 753–759.

Jayani, R.S., Saxena, S., Gupta, R., 2005. Microbial pectinolytic enzymes: a review. Process Biochem. 40, 2931–2944.

Jeffries, T.W., 1996. Biochemistry and genetics of microbial xylanases. Curr. Opin. Biotechnol. 7, 337–342.

Jisha, V.N., Smitha, R.B., Pradeep, S., Sreedevi, S., Unni, K.N., Sajith, S., et al., 2013. Versatility of microbial proteases. Adv. Enzyme Res. 1, 39–51.

Jolly, L., 2001. The commercial viability of fuel ethanol from sugar cane. Int. Sugar. J. 103, 17–143.

Junghanns, C., Moeder, M., Krauss, G., Martin, C., Schlosser, D., 2005. Degradation of the xenoestrogen nonylphenol by aquatic fungi and their laccases. Microbiology 151, 45–57.

Juturu, V., Wu, J.C., 2012. Microbial xylanases: engineering, production and industrial applications. Biotechnol. Adv. 30, 1219–1227.

Juturu, V., Wu, J.C., 2014. Microbial cellulases: engineering, production and applications. Renew. Sust. Energ. Rev. 33, 188–203.

Kashyap, D.R., Vohra, P.K., Chopra, S., Tewari, R., 2001. Applications of pectinases in the commercial sector: a review. Bioresour. Technol. 77, 215–227.

Kelly, S.L., Kelly, D.E., 2013. Microbial cytochromes P450: biodiversity and biotechnology. Where do cytochromes P450 come from, what do they do and what can they do for us? Philos. Trans. R. Soc. Lond. B. Biol. Sci. 368, 20120476.

Kiiskinen, L.L., Viikari, L., Kruus, K., 2002. Purification and characterisation of a novel laccase from the ascomycete *Melanocarpus albomyces*. Appl. Microbiol. Biotechnol. 59, 198–204.

Kim do, Y., Rha, E., Choi, S.L., Song, J.J., Hong, S.P., Sung, M.H., et al., 2007. Development of bioreactor system for L-tyrosine synthesis using thermostable tyrosine phenol-lyase. J. Microbiol. Biotechnol. 17, 116–122.

Kirk, O., Borchert, T.V., Fuglsang, C.C., 2002. Industrial enzyme applications. Curr. Opin. Biotechnol. 13, 345–351.

Koeller, K.M., Wong, C.H., 2001. Enzymes for chemical synthesis. Nature 409, 232–240.

Kohli, U., Nigam, P., Singh, D., Chaudhary, K., 2001. Thermostable, alkalophilic and cellulase free xylanase production by *Thermoactinomyces thalophilus* subgroup C. Enzyme. Microb. Technol. 28, 606–610.

Kuhad, R.C., Gupta, R., Singh, A., 2011. Microbial cellulases and their industrial applications. Enzyme Res. 2011, 280696.

Kumar, A., Singh, S., 2013. Directed evolution: tailoring biocatalysts for industrial applications. Crit. Rev. Biotechnol. 33, 365–378.

Kumar, G., Srikumar, K., 2013. Biophysical characterization of thermophilic laccase from the xerophytes: *Cereus pterogonus* and *Opuntia vulgaris*. Cellulose 20, 115–125.

Lee-Huang, S., Huang, P.L., Sun, Y., Kung, H.F., Blithe, D.L., Chen, H.C., 1999. Lysozyme and RNases as anti-HIV components in beta-core preparations of human chorionic gonadotropin. Proc. Natl Acad. Sci. U.S.A. 96, 2678–2681.

Lehmann, M., Kostrewa, D., Wyss, M., Brugger, R., D'Arcy, A., Pasamontes, L., et al., 2000. From DNA sequence to improved functionality: using protein sequence comparisons to rapidly design a thermostable consensus phytase. Protein Eng. 13, 49–57.

Lei, X.G., Stahl, C.H., 2000. Nutritional benefits of phytase and dietary determinants of its efficacy. J. Appl. Anim. Res. 17, 97–112.

Li, S., Yang, X., Yang, S., Zhu, M., Wang, X., 2012. Technology prospecting on enzymes: application, marketing and engineering. Comput. Struct. Biotechnol. J. 2, e201209017.

Liu, B., Zhang, J., Li, B., Liao, X., Du, G., Chen, J., 2013. Expression and characterization of extreme alkaline, oxidation-resistant keratinase from *Bacillus licheniformis* in recombinant *Bacillus subtilis* WB600 expression system and its application in wool fiber processing. World J. Microbiol. Biotechnol. 29, 825–832.

Lutz, S., 2010. Biochemistry. Reengineering enzymes. Science 329, 285–287.

Ma, L., Li, C., Yang, Z., Jia, W., Zhang, D., Chen, S., 2013. Kinetic studies on batch cultivation of *Trichoderma reesei* and application to enhance cellulase production by fed-batch fermentation. J. Biotechnol. 166, 192–197.

Ma, S.K., Gruber, J., Davis, C., Newman, L., Gray, D., Wang, A., et al., 2010. A green-by-design biocatalytic process for atorvastatin intermediate. Green Chem. 12, 81–86.

Matsumoto, K.S., 2006. Fungal chitinases. In: Guevara-González, R.G., Torres-Pacheco, I. (Eds.), Advances in Agricultural and Food Biotechnology Research Signpost, Kerala, India, pp. 289–304.

Mendez, C., Salas, J.A., 2001. Altering the glycosylation pattern of bioactive compounds. Trends Biotechnol. 19, 449–456.

Mitidieri, S., Souza Martinelli, A.H., Schrank, A., Vainstein, M.H., 2006. Enzymatic detergent formulation containing amylase from *Aspergillus niger*: a comparative study with commercial detergent formulations. Bioresour. Technol. 97, 1217–1224.

Molitoris, H.P., Esser, K., 1970. The phenoloxidases of the ascomycete *Podospora anserina*. V. properties of laccase I after further purification. Arch. Mikrobiol. 72, 267–296.

Moon, L.D., Asher, R.A., Fawcett, J.W., 2003. Limited growth of severed CNS axons after treatment of adult rat brain with hyaluronidase. J. Neurosci. Res. 71, 23–37.

Motta, F.L., Andrade, C.C.P., Santana, M.H.A., 2013. A Review of xylanase production by the fermentation of xylan: classification, characterization and applications. In: Chandel, A.K., Silvério da Silva, S. (Eds.), Sustainable Degradation of Lignocellulosic Biomass – Techniques, Applications and Commercialization. InTech, pp. 284.

Mougin, C., Jolivalt, C., Briozzo, P., Madzak, C., 2003. Fungal laccases: from structure-activity studies to environmental applications. Environ. Chem. Lett. 1, 145–148.

Murad, H.A., Azzaz, H.H., 2011. Microbial pectinases and ruminant nutrition. Res. J. Microbiol. 6, 246–269.

Neelam, G., Sumanta, R., Sutapa, B., Vivek, R., 2013. A broader view: Microbial enzymes and their relevance in industries, medicine, and beyond. BioMed Res. Int. 2013, 1–18.

Nigam, P., Singh, D., 1995. Enzyme and microbial systems involved in starch processing. Enzyme. Microb. Technol. 17, 770–778.

Nigam, P.S., 2013. Microbial enzymes with special characteristics for biotechnological applications. Biomolecules 3, 597–611.

O'Neill, A., Araújo, R., Casal, M., Guebitz, G., Cavaco-Paulo, A., 2007. Effect of the agitation on the adsorption and hydrolytic efficiency of cutinases on polyethylene terephthalate fibres. Enzyme. Microb. Technol. 40, 1801–1805.

Okanishi, M., Suzuki, N., Furuta, T., 1996. Variety of hybrid characters among recombinants obtained by interspecific protoplast fusion in *Streptomycetes*. Biosci. Biotechnol. Biochem. 60, 1233–1238.

Palonen, H., Saloheimo, M., Viikari, L., Kruus, K., 2003. Purification, characterization and sequence analysis of a laccase from the ascomycete *Mauginiella* sp. Enzyme. Microb. Technol. 33, 854–862.

Pamment, N., Robinson, C.W., Hilton, J., Moo-Young, M., 1978. Solid-state cultivation of *Chaetomium cellulolyticum* on alkali-pretreated sawdust. Biotechnol. Bioeng. 20, 1735–1744.

Pandey, A., Nigam, P., Soccol, C.R., Soccol, V.T., Singh, D., Mohan, R., 2000a. Advances in microbial amylases. Biotechnol. Appl. Biochem. 31 (Pt 2), 135–152.

Pandey, A., Soccol, C.R., Nigam, P., Soccol, V.T., Vandenberghe, L.P.S., Mohan, R., 2000b. Biotechnological potential of agro-industrial residues. II: cassava bagasse. Bioresour. Technol. 74, 81–87.

Pandey, A., Selvakumar, P., Soccol, C.R., Nigam, P., 1999. Solid-state fermentation for the production of industrial enzymes. Curr. Sci. 77, 149–162.

Parameswaran, B., Piyush, P., Raghavendra, G., Nampoothiri, K.M., Duggal, A., Dey, K., et al., 2013. Industrial enzymes – present status and future perspectives for India. J. Sci. Ind. Res. 72, 271–286.

Pariza, M.W., Cook, M., 2010. Determining the safety of enzymes used in animal feed. Regul. Toxicol. Pharmacol. 56, 332–342.

Pariza, M.W., Johnson, E.A., 2001. Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century. Regul. Toxicol. Pharmacol. 33, 173–186.

Pedrolli, D.B., Monteiro, A.C., Gomes, E., Carmona, E.C., 2009. Pectin and pectinases: production, characterization and industrial application of microbial pectinolytic enzymes. Open Biotechnol. J. 3, 9–18.

Polizeli, M.L., Rizzatti, A.C., Monti, R., Terenzi, H.F., Jorge, J.A., Amorim, D.S., 2005. Xylanases from fungi: properties and industrial applications. Appl. Microbiol. Biotechnol. 67, 577–591.

Quintanilla, D., Hagemann, T., Hansen, K., Gernaey, K.V., 2015. Fungal morphology in industrial enzyme production-modelling and monitoring. In: Krull, R., Bley, T. (Eds.), Filaments in Bioprocesses. Springer International Publishing, Switzerland, pp. 29–54.

Raja, K.S., Vasanthi, N.S., Saravanan, D., Ramachandran, T., 2012. Use of bacterial lipase for scouring of cotton fabrics. Indian J. Fibre Text Res. 37, 299–302.

Rao, M.B., Tanksale, A.M., Ghatge, M.S., Deshpande, V.V., 1998. Molecular and biotechnological aspects of microbial proteases. Microbiol. Mol. Biol. Rev. 62, 597–635.

Roberts, R.J., Vincze, T., Posfai, J., Macelis, D., 2007. REBASE—enzymes and genes for DNA restriction and modification. Nucleic Acids Res. 35, D269–D270.

Roberts, R.J., Vincze, T., Posfai, J., Macelis, D., 2010. REBASE – a database for DNA restriction and modification: enzymes, genes and genomes. Nucleic Acids Res. 38, D234–D236.

Rodríguez, A., Falcón, M.A., Carnicero, A., Perestelo, F., la Fuente, G.D., Trojanowski, J., 1996. Laccase activities of *Penicillium chrysogenum* in relation to lignin degradation. Appl. Microbiol. Biotechnol. 45, 399–403.

Saha, B.C., Demirjian, D.C., 2000. Applied biocatalysis in specialty chemicals and pharmaceuticals. American Chemical Society, Washington, DC.

Saha, B.C., Jordan, D.B., Bothast, R.J., 2009. Enzymes, industrial (overview). Encyclopedia of Microbiology. Academic Press, Oxford.

Sakaki, T., 2012. Practical application of cytochrome P450. Biol. Pharm. Bull. 35, 844–849.

Sanchez, S., Demain, A.L., 2011. Enzymes and bioconversions of industrial, pharmaceutical, and biotechnological significance. Org. Process Res. Dev. 15, 224–230.

Saranraj, P., Naidu, M.A., 2014. Microbial pectinases: a review. Global J. Trad. Med. Sys. 3, 1–9.

Scherer, M., Fischer, R., 1998. Purification and characterization of laccase II of *Aspergillus nidulans*. Arch. Microbiol. 170, 78–84.

Schumacher, K., Heine, E., Hocker, H., 2001. Extremozymes for improving wool properties. J. Biotechnol. 89, 281–288.

Selle, P.H., Ravindran, V., 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol. 135, 1–41.

Sharma, V.K., Kumar, N., Prakash, T., Taylor, T.D., 2010. MetaBioME: a database to explore commercially useful enzymes in metagenomic datasets. Nucleic Acids Res. 38, D468–D472.

Sheldon, R.A., 2008. E factors, green chemistry and catalysis: an odyssey. Chem. Commun. (Camb), 3352–3365.

Shevchik, V.E., Hugouvieux-Cotte-Pattat, N., 1997. Identification of a bacterial pectin acetyl esterase in Erwinia chrysanthemi 3937. Mol. Microbiol. 24, 1285–1301.

Shraddha, Shekher, R., Sehgal, S., Kamthania, M., Kumar, A., 2011. Laccase: microbial sources, production, purification, and potential biotechnological applications. Enzyme Res. 2011, 217861.

Singh, S., Sczakas, G., Soccol, C., Pandey, A., 2008. Production of enzymes by solid-state fermentation. In: Pandey, A., Soccol, C., Larroche, C. (Eds.), Current Developments in Solid-State Fermentation. Springer, New York, pp. 183–204.

Singh, S.A., Ramakrishna, M., Appu Rao, A.G., 1999. Optimisation of downstream processing parameters for the recovery of pectinase from the fermented bran of *Aspergillus carbonarius*. Process Biochem. 35, 411–417.

Sukumaran, R.K., Singhania, R.R., Pandey, A., 2005. Microbial cellulases-production, applications and challenges. J. Sci. Ind. Res. 64, 832–844.

Sundarram, A., Murthy, T.P.K., 2014. α-amylase production and applications: a review. J. Appl. Environ. Microbiol. 2, 166–175.

Tapre, A.R., Jain, R.K., 2014. Pectinases: Enzymes for fruit processing industry. Int. Food Res. J. 21, 447–453.

Taylor, F., Kurantz, M.J., Goldberg, N., McAloon, A.J., Craig Jr., J.C., 2000. Dry-grind process for fuel ethanol by continuous fermentation and stripping. Biotechnol. Prog. 16, 541–547.

Taylor, F., McAloon, A.J., Craig Jr., J.C., Yang, P., Wahjudi, J., Eckhoff, S.R., 2001. Fermentation and costs of fuel ethanol from corn with Quick-Germ process. Appl. Biochem. Biotechnol. 94, 41–49.

Thakker, G., Evans, C., Rao, K.K., 1992. Purification and characterization of laccase from *Monocillium indicum* Saxena. Appl. Microbiol. Biotechnol. 37, 321–323.

Tomschy, A., Tessier, M., Wyss, M., Brugger, R., Broger, C., Schnoebelen, L., et al., 2000. Optimization of the catalytic properties of *Aspergillus fumigatus* phytase based on the three-dimensional structure. Protein. Sci. 9, 1304–1311.

Treichel, H., de Oliveira, D., Mazutti, M., Di Luccio, M., Oliveira, J.V., 2010. A review on microbial lipases production. Food Bioprocess Technol. 3, 182–196.

Tucker, G.A., Woods, L.F.J., 1995. Enzymes in Food Processing. Blackie Academic, Bishopbriggs, Glasgow, UK.

Tufvesson, P., Lima-Ramos, J., Nordblad, M., Woodley, J.M., 2011. Guidelines and cost analysis for catalyst production in biocatalytic processes. Org. Process Res. Dev. 15, 266–274.

Underkofler, L.A., Barton, R.R., Rennert, S.S., 1958. Production of microbial enzymes and their applications. Appl. Microbiol. 6, 212–221.

van der Maarel, M.J., van der Veen, B., Uitdehaag, J.C., Leemhuis, H., Dijkhuizen, L., 2002. Properties and applications of starch-converting enzymes of the alpha-amylase family. J. Biotechnol. 94, 137–155.

Venkateshwaran, G., Somashekar, D., Prakash, M.H., Agrawal, R., Basappa, S.C., Joseph, R., 1999. Production and utilization of catalase using *Saccharomyces cerevisiae*. Process Biochem. 34, 187–191.

Volpato, G., Rodrigues, R.C., Fernandez-Lafuente, R., 2010. Use of enzymes in the production of semi-synthetic penicillins and cephalosporins: drawbacks and perspectives. Curr. Med. Chem. 17, 3855–3873.

Watanabe, I., Nara, F., Serizawa, N., 1995. Cloning, characterization and expression of the gene encoding cytochrome P-450sca-2 from *Streptomyces carbophilus* involved in production of pravastatin, a specific HMG-CoA reductase inhibitor. Gene 163, 81–85.

Wheals, A.E., Basso, L.C., Alves, D.M., Amorim, H.V., 1999. Fuel ethanol after 25 years. Trends Biotechnol. 17, 482–487.

Wintrode, P.L., Miyazaki, K., Arnold, F.H., 2000. Cold adaptation of a mesophilic subtilisin-like protease by laboratory evolution. J. Biol. Chem. 275, 31635–31640.

Wong, K.K., Tan, L.U., Saddler, J.N., 1988. Multiplicity of beta-1,4-xylanase in microorganisms: functions and applications. Microbiol. Rev. 52, 305–317.

Wong, K.K.Y., Saddler, J.N., 1992. Trichoderma xylanases, their properties and application. Crit. Rev. Biotechnol. 12, 413–435.

Yang, S.T., El-Enshasy, H.A., Thongchul, N., 2013. Cellulases: characteristics, sources, production and applications. In: Yang, S.T., El-Enshasy, H.A., Thongchul, N. (Eds.), Bioprocessing Technologies in Biorefinery for Sustainable Production of Fuels, Chemicals, and Polymers. John Wiley & Sons, Inc., Hoboken, NJ.

Zaldivar, J., Nielsen, J., Olsson, L., 2001. Fuel ethanol production from lignocellulose: a challenge for metabolic engineering and process integration. Appl. Microbiol. Biotechnol. 56, 17–34.

Zhang, C., Kim, S.K., 2010. Research and application of marine microbial enzymes: status and prospects. Mar. Drugs. 8, 1920–1934.

Zhang, D.X., Du, G.C., Chen, J., 2010. Fermentation production of microbial catalase and its application in textile industry. Chin. J. Biotech. 26, 1473–1481.

Zheng, G.W., Xu, J.H., 2011. New opportunities for biocatalysis: driving the synthesis of chiral chemicals. Curr. Opin. Biotechnol. 22, 784–792.