16 Why Settle for Mediocre, When Extremophiles Exist?

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Abstract

The ever-increasing uses of microorganisms and enzymes in the food, medical, pharmaceutical, detergent, leather, and textile industries has triggered a great amount of research in “extreme” enzymology. In areas of research that are based on solving environmental problems, by methods such as bioremediation, considerable attention has been paid to enzymes/microorganisms that can survive in extreme environments. Such entities include thermostable and organic solvent-tolerating microorganisms/enzymes. The study of enzymes (such as amylases, proteases, lipases, and nitrilases) that can tolerate high organic solvent concentrations has revolutionized the way science and industry work together and evolve. Organic solvent-rich environments provide an edge with respect to enzyme behavior and applications as compared with aqueous environments. These behavioral attributes in organic solvent-rich environments include thermal stability, a positive shift in the thermodynamic equilibrium, simple removal of solvent from the system, and enhanced enantio-recognition and stereo-stability. Non-aqueous biocatalysis is a key area of research that has led us in various directions through the exploration of the stated properties of such enzymes. The applications of non-aqueous biocatalysis include the biocatalytic synthesis of cardiovascular drugs and anti-inflammatory agents, the resolution of racemic acids and alcohols, and fatty acid ester synthesis.

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This chapter narrates the journey of these extremists—these solvent-tolerant microorganisms/enzymes—from the initial need for their identification to their multifarious applications in solving environmental, industrial, and biotechnological issues.

Keywords
Organic solvent tolerance · Biocatalysis · Organic synthesis · Bioremediation

16.1 Introduction

This chapter outlines the present and forecasted applications of extreme environment-tolerating microorganisms and their enzymes in the field of biotechnology, particularly in the production and processing of chemicals in textiles, biocatalytic synthesis, and agriculture. Microbial biotechnology can offer environmental benefits, process efficiency, product quality, and economic benefits in the production of high value commercials, along with bio-eco-friendly waste management. Microbial biotechnology/engineering assists in the invention/discovery of sustainable technologies that offer a number of process and market benefits.

The sustainable production of existing and novel products is a major advantage of microbial biotechnology, and this technology also offers reduced dependence on nonrenewable fuels and other resources, improving the economics of production. Since the 1970s, biotechnology, particularly enzymology, has substantially affected healthcare and pharmaceuticals; food and agriculture; environmental protection; and the production of various materials and chemicals. “White biotechnology” was primarily dependent on aqueous enzymology until enzymes that could withstand organic and biphasic environments were explored. Organic solvent-rich environments, which are lethal for most organisms/enzymes, enhance hydrolytic activity in a few useful extremophiles. The study of enzymes that can tolerate high organic solvent concentrations has revolutionized the way science and industry work together and evolve. Organic solvent-rich environments provide an edge with respect to enzyme behavior and applications as compared with aqueous environments. These behavioral attributes in organic solvent-rich environments include thermal stability, a positive shift in thermodynamic equilibrium, simple removal of solvent from the system, and enhanced enantio-recognition and stereo-stability. Non-aqueous biocatalysis is a key area of research that has led us in various directions through the exploration of the stated properties of such enzymes. The applications of non-aqueous biocatalysis include the biocatalytic synthesis of cardiovascular drugs and anti-inflammatory agents, the resolution of racemic acids and alcohols, and fatty acid ester synthesis (Martinkova et al. 2017).

Interest in exploring the benign applications of enzymes was promoted by the successful production of nicotinic acid (Lonza, Guangzhou, China) and (*R*)-(−)-mandelic acid (Mitsubishi Rayon, Tokyo, Japan; BASF, Ludwigshafen, Germany) and this has motivated multiple studies in the field to take the findings from the laboratory to scaled-up products/processes in the market.

16.2 Adequate Process Design

Traditionally, primary screening for microorganism/s producing extreme environment-tolerating enzyme/s was done in a selective enrichment medium. But it takes more than screening to take the product/enzyme to the market. In modern chemistry, a primary established mainstream discipline, various non-organic catalysts are used, but the industry is now embracing green catalysis (also known as biotransformation). This is the biochemical conversion/alteration of a substance by the use of biocatalysts, with a compound being transformed into a relevant form via the action of biological agents. The biological agents can be plants, plant products, microorganisms, or microbial products.

With an effect on the global economy, enzymes such as nitrilases now have proven prominence in the field of green chemistry, with an advantageous ratio of waste produced and product obtained (Gong et al. 2012). The major requirement is to develop/discover shorter/smarter/faster alternatives to the conventional methods to produce relevant products. Therefore, research is now directed toward green energy.

16.3 Biological Conversion of Hazardous Compounds

The hazardous nature of compounds in the environment such as nitriles and cyanide has led to the exploration of nitrile-hydrolyzing enzymes by industries and by enzymologists, who are investigating biotransformation and the widespread applications of biologically transformed hazardous product/s. Various studies have reported the increased research in the area (e.g., Ludmila and Křen 2010; Gong et al. 2012). There are multiple approaches for the conversion of an undesirable product into a desirable one. With chemical (acid-catalyzed or base-catalyzed) hydrolysis posing environmental and economic threats, biological processes have gained importance and present a better solution.

16.4 Industrial Tenability

The term ‘industrial tenability’ relates to the achievement of sustainability with regard to production, processing, and economic landmarks. Bioprocessing and biotechnology engineering, as a fourth discipline, along with genetic engineering, protein engineering, and metabolic engineering, is required for the commercial production of biotechnology products and their delivery. The industrial chemical method of production and commercialization relies excessively on nonrenewable energy and resources and on environmentally damaging production processes that can be unsafe. These processes produce toxic products and waste and products that are not readily recyclable and degradable after their useful life. Further, the industrial chemical method involves excessive regional concentration of production so that the social benefits of production are limited.

16.5 Advantages of Organic Solvents Over Aqueous Media for Biotransformation

Apart from their action in eliminating microbial contamination, which is an underrated advantage, the organic solvent-tolerating enzymes that assist in efficient biotransformation present a number of other advantages. These advantages, compared with processes in aqueous media, include better solubility of the substrate and/or product, the shifting of thermodynamic equilibrium toward the synthesis of the product, simpler removal of solvent (most organic solvents have lower boiling points than that of water), and a reduction in the water-dependent side of the reaction (hydrolysis of acid anhydrates). Furthermore, the organic solvents offer better enzyme thermal stability, since aqueous media inactivate enzymes at higher temperatures.

A new study has presented ionic liquids as an alternative to organic solvents, with these liquids overcoming the problem of the volatile nature of organic solvents. These designer solvents are environmentally friendly and have a defined set of properties. The properties and aspects of ionic liquids in catalytic organic transformation are well discussed by Vekariya (2017).

As well as the class of ionic liquids, there is another important and promising solvent class: Bronsted acidic ionic liquids (BAILS). This newly studied, versatile, and fast-evolving category is employed in various essential organic reactions, such as hydrogenation and dehydrogenation, oxidation, transesterification, esterification, and alkylation. The great potential of BAILs in various reactions has led to what has been called a new era in acid-catalyzed transformation (Vafaeezadeh and Alinezhad 2016).

With particular focus on biomass conversion processes, alkyl phenols have also managed to enter the organic solvent race (Jérôme et al. 2017). β-Sitosterol, cholesterol, and campesterol are some of the steroids that can be converted into industrially important compounds by *Mycobacterium* sp. (de Carvalho 2017). The introduction of an organic phase, in contrast to water, limits the possible slow solubility of steroids. The employment of an organic solvent instead of aqueous media in the biocatalysis of β-sitosterol, 4 androstadiene-3, 17 (AD), and 1,4 androstadiene-3, 17 (ADD) leads to better chances of exploring potentially useful enzymes (de Carvalho 2017).

Better yields, such as that seen in the increased production of ethyl lactate through enzyme esterification in green solvents, have involved the use of heterologous *Rhizopus oryzae* and *Candida rugosa*, which retain high activity in organic solvents such as chloroform (Koutinasa et al. 2018). Another cost-effective method was the immobilization of a lipase from *C. rugosa*; its reusable stability was confirmed, and its esterification activity was retained for up to 60% of its maximum activity after five reuse cycles in solvents such as cyclohexane (V_{max} = 26.4 mMol/min) (Lidija et al. 2017).

Reports on organic solvents suggest that the appropriate optimization of the type of enzyme, e.g., lipase, and the use of an appropriate reaction medium lead to an enantiomerically pure product (Sikora et al. 2017). Lipo amino acids were synthesized using enzymes from *Pseudomonas stutzeri* (lipase) and *Bacillus subtilis* (protease) and the selectivity and synthesis were dependent upon the reaction conditions (Bernal et al. 2018). Low-cost mass cultivation of organic solvent-tolerant enzymes in *Pseudomonas sp*. BCNU 106 in toluene supplemented with glycerol can be employed in biotransformation and biodegradation (Choi et al. 2017).

16.6 Applications of Organic Solvent-Tolerant Enzymes

Basic chemicals or commodity chemicals include the products of industries that are generally involved in processing applications (e.g., pulp and paper, oil refining, metal recovery), as well as the raw materials used for producing other basic chemicals, specialty chemicals, and consumer products, including manufactured goods.

Specialty chemicals are derived from basic chemicals, but are more technologically advanced and are used in lower volumes than the basic chemicals. Examples of specialty chemicals include adhesives and sealants, catalysts, coatings, and plastic additives. Specialty chemicals command higher profit margins and their demand is less cyclic than that of basic chemicals. Specialty chemicals have a higher value-added component because they are not easily duplicated by other producers or are protected from competition by patents. Consumer-care products, including soaps, detergents, bleaches, laundry aids, hair care products, skin care products, and fragrances, are one of the oldest segments of the chemical industry. Other products include pharmaceuticals, products for crop protection, and products of modern biotechnology.

C. cylindracea lipase, a thermostable lipase with potential for use in the oleochemical industry for soap production, has been studied and reported on. Processes such as hydrolysis and glycolysis are catalyzed by this enzyme. Similarly, lipases from bacteria such as *Pseudomonas thermomyces* sp. have been used in detergent making (Choudhary and Bhunia 2015).

Other microorganisms, especially fungi such as *Aspergillus niger*, *C. antarctica*, *C. rugosa, C. viscosum, Mucor miehei, P. fluorescens, P. cepacia*, *P. lypolyticum, and Thermomyces lanuginosus* have been well studied in the production of biodiesel (Choudhary and Bhunia 2015). A novel organo solvent-tolerant esterase, from *Monascus purpureus* strain M7, retained 99%–110% relative activity (minimum 20%) in hydrophilic organic solvents such as methanol and ethanol (Kang et al. 2017). As well as bacteria and fungi, microalgae are potential candidates for use in molecular- to industrial-scale biocatalysis (Miazek et al. 2017). In the past decade there has been an exponential increase in microbiologists' interest in studying the potential of microalgae for biocatalysis; this can be ascertained by the huge number of reports in the field, covering diverse microalgae (Bayat et al. 2015; Hunt et al. 2010). The target product range is wide, ranging from lipids to pigments. The microalgae studied tolerate high concentrations of organic solvents such as ethylene glycol, benzene, xylene, acetaldehyde, chloroform, waste organic solvents, and ionic liquids. The microalgae studied include *Chlorella minutissima, Chlamydomonas reinhardtii, Chlorella sorokiniana, Euglena gracilis, Botryococcus braunii*, and *Dunaliella tertiolecta*; the growth of these was promoted by methanol (Miazek et al. 2017). Active aggregates of an organic solvent-tolerant lipase from *Marinobacter* *sp.* EMB5 have also been reported (Hemamalini and Khare 2016); this lipase from this halophilic bacteria is stable for long incubation periods in organic solvents. These studies are usually linked with studies of the bioremediation of undesired and/or toxic material.

The applications of the extreme microorganisms noted above, and their enzymes and products, are well established, as evidenced by diverse reports from all over the globe (Fernandes et al. 2003; Li et al. 1998). There are also reports and discussions on the mechanisms underlying the causes/effects of the microorganisms' actions. Manefield et al. (2017) discuss mechanisms such as efflux pumps in bacterial resistance to antimicrobial compounds, in terms of organic solvent-tolerance. Other mechanisms of microorganisms' resistance to antimicrobial compounds include biofilm formation, motility, and the formation of endospores.

Pan-genome studies of *P. putida,* a microbe generally recognized as organic solvent-tolerant, have revealed 30% of genes belong to *Pseudomonas.* A highly organic solvent-tolerant *Pseudomonas* strain, dot-t1e, has also been identified (Molina-Santiago and Udaondo 2017). With biofilm formation as one of the "favorite" mechanisms of organic solvent-tolerant microorganisms, biomass quantification of *P. taiwanensis* VLB120ΔC biofilm was done in the presence of n-butanol; this study showed a robust organism capable of tolerating and adapting to increased concentrations of reactants and products that can be toxic to microorganisms (Halan 2017). In another study, an ethylene glycol-tolerant lignolytic ascomycete strain, *Pseudo Cochliobolus verruculosa* NFCCI3818, was investigated for its utility in waste management (Nikama et al. 2017).

In biotechnology, biocatalysis and metabolic engineering are the two fast-evolving fields that have the potential to replace and drive transformation in the conventional chemical industry (Martinkova et al. 2017). Genetic engineering and molecular biology techniques have been used to obtain many modified enzymes with enhanced properties compared with their natural counterparts. Some well established biotechnology products include bioethanol, L-glutamic acid (MSG), citric acid, L-lysine, lactic acid, food-processing enzymes, vitamin C, gluconic acid, antibiotics, feed enzymes, xanthan, L-threonine, L-dihydroxyphenylalanine, 6-aminopenicillanic acid, nicotinamide, D-p-hydroxyphenylglycine, vitamin F, 7-aminocephalosporanic acid, aspartame, L-methionine, dextran, vitamin B12, and provitamin D2.

16.7 Enzymatic Processes

Enzymes are being increasingly used in the chemical industry as catalysts for numerous reactions. The global microbial identification market alone is estimated to reach 1194 million $US by 2019 (de Carvalho 2017). Millions of years of evolution have provided enzymes with an unparalleled capacity for facilitating life reactions in ways that are sustainable. Compared with conventional chemical catalysis, enzyme catalysis is highly specific and it functions under temperatures, pressures, and pH conditions that are compatible with life. Unlike many processes in conventional synthetic chemistry, enzymes require nontoxic and noncorrosive conditions.

About 75% of enzyme use by value is accounted for by the detergent, food, and starch-processing industries. These industries mostly use hydrolytic enzymes such as proteases, amylases, lipases, and cellulases. Specialty enzymes account for around 10% of the enzyme market and they are finding numerous analytical uses, as well as increasing uses in the development of new drugs and medical diagnostics. Modern biotechnology has contributed to more than 60% of commercialized products and/or enzymes such as Novozymes' Cellic®, Shire's Velaglucerasealfa VPRIV™, and Taliglucerasealfa Elelyso™ (Li et al. 2012).

Some industrial enzymes and their various substrates include proteases-proteins, carbohydrases-carbohydrates, lipases-fats and oils, amylases-polysaccharides, cellulases-cellulose, pectinases-pectin, and nitrilases-nitriles. The reactions include proteolysis, hydrolysis of carbohydrates to sugars, hydrolysis of fats to fatty acids and glycerol, hydrolysis of pectin, hydrolysis of cellulose, hydrolysis of starch to sugars, and hydrolysis of hazardous nitriles to high-value commercial products (Martinkova et al. 2017).

The industries that primarily require/explore such enzymes include the detergent, food, pharmaceutical, synthetic food, feed, pulp and paper, sugar, and textile industries. Analytical applications include the development of enzymes for the production, degradation, and biotransformation of chemicals, foods and feeds, agricultural produce, and textiles. For example, isomalto-oligosaccharides, produced using glucosyl transferases, are used to suppress tooth decay and prevent baked goods from becoming stale; cellulases, which synergistically break down cellulose, are used because of their potential for providing fuel, food, and other chemicals from widely available cellulose. Enzymes such as amylases and proteases are added to animal feed to improve digestibility by supplementing the animals' own enzymes. The addition of enzymes such as beta-glucanases and arabino-xylanase to feed cereals breaks down non-starch polysaccharide anti-nutritional factors, aiding the digestion and absorption of nutrients.

16.8 Pharmaceuticals: Exploring Biotransformation

Pharmaceutics, chiral intermediates, enantiomers, and precursors are some of the terms used by the pharmaceutical industry today to describe their products. Many pharmaceutical companies adopt chemical methods for the synthesis of chiral intermediate, enantiomer, and precursor compounds such as α-hydroxy acids, α-hydroxyl amides, α- and β-amino acids, and mono/di acids. These chemical methods, apart from being environmentally unfriendly, raise global issues when employed on a large scale. They also lead to reduced overall product yields because of the formation of nonspecific and unwanted by-products. The process is expensive owing to the addition of chemical substances for enhancing enantiomeric selectivity; in contrast, enantiomeric selectivity is naturally provided by some microorganisms. Some shrewder manufacturers in the field employ smart microorganisms that show "extremophilicity" in more than one aspect, be it organo-solvent tolerance, thermophilicity, halophilicity, or alkalophilicity.

These microorganisms present the most desirable trait in the industry; that is, enantio-selectivity and/or enantio-retentivity. These traits reduce the downstream processing cost, bringing down the overall cost of the process by selecting and/or retaining the wanted enantiomer. These factors help the industry not only in regard to reducing costs, but also in regard to overcoming the regulatory pressures that every pharmaceutical company faces today. Justifying the regulatory pressure, the final product must contain the active pharmacological enantiomer of the desired compound and not the racemic mixture. The complex key intermediates can be synthesized in an environmentally friendly, cost-effective manner; as noted previously, such synthesis has been exemplified by the successful production of nicotinic acid (Lonza) and (*R*)-(−)-mandelic acid (Mitsubishi Rayon; BASF). This success has motivated multiple studies in the field to take the laboratory findings to a scaled-up product/process in the market on a large scale (Yamada and Kobayashi 2014).

This section presents some microorganisms recently used in studies across the globe; the enzymes from these organisms include lipases, nitrilases, nitrile hydrolases, amidases, and laccases. Different *Aspergillus* spp. secrete lipases that show multiple characteristics of extremophilicity, including thermostability, organic solvent tolerance, enantio-selectivity, and pH stability (Contesini et al. 2016).

Of note, Li et al. (2017) have reported the use of structure-guided saturation mutagenesis to produce high-quality mutant libraries. Also, other authors have discussed examples of stereoselectivity in enzymes overcoming the distinct traditional limitations of the processes (Maksimova et al. 2017; Mazmouza et al. 2018). The asymmetrical synthesis of chiral intermediates has now reached a point of resolution as a result of these studies. Gurung et al. (2013) have reported that lipases from *Candida rugosa* synthesize lovastatin, a drug that lowers serum cholesterol level. Lipase from *Serratia marcescens* has been reported to asymmetrically hydrolyse trans-3-phenylglycidic acid ester, the key intermediate in the synthesis of diltiazem hydrochloride (Matsumae et al. 1993; Singh and Banerjee 2005).

Sun et al. (2018) have described reductases, oxidases, hydrolases, lyases, isomerases, and transaminases in relation to expression of enzyme activity, specificity, thermostability, and solvent-tolerance. For example, reduction of 4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro-[1,2,4] triazolo [4,3-a] pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-one (OTPP) by *Pseudomonas pseudoalcaligenes* XW-40 (Wei et al. 2016). The challenging nature of the enzymatic synthesis of complex natural compounds, such as by smart single-step conversion followed by cascade reactions, has been highlighted in a study by Classen and Pietruszka (2017). Another class of enzymes, the nitrilases, have proven to be valuable for their potential use in the biotransformation of various hazardous nitrile compounds to useful intermediates and corresponding carboxylic acid, for example, acrylonitrile to acrylic acid, etc. (Sharma and Vashist 2017). One of the most inspiring success stories in this regard is the biosynthesis of acrylamide using nitrilase on a commercial scale. Some nitrilases have also been successfully applied to practical production in food industries, chemical manufacturing, pharmaceutical processes, wastewater treatment, and textile industries.

From the production of (R)-mandelic acid from (R,S)-mandelonitrile through *Aspergillus niger* reported by Vesela et al. (2015) and the production of (R)-mandelic acid through *Burkholderia cenocepacia* reported by Ni et al. (2013) and Wang et al. (2015a), there is evidence that establishes nitrilases as promising biocatalysts. Other examples include the production of (R)-o-chloromandelic acid from (R,S)-o-chloro-mandelonitrile, using *Burkholderia cenocepacia* (nitrilase mutant I113M/Y199G) (Wang et al. 2015b), the production of (R)-phenylglycine from (R,S)-2-amino-2-phenylacetonitrile, using *Sphingomonas wittichii* (Qiu et al. 2014a, b), and the production of β-alanine from 3-aminopropionitrile, using *Bradyrhizobium japonicum* (Han et al. 2015). Further examples include the production of 1-cyanocyclohexylacetic acid, (s)-2-cyano-2-methylpentanoic acid, and iminodiacetic acid from 1-cyanocyclohexylacetonitrile (Xue et al. 2015), 2-methyl-2-propylmalononitrile (Yoshida et al. 2013), and iminodiacetonitrile (Cai et al. 2014; Liu et al. 2013), respectively, using the microorganisms *Acidovorax facilis* (nitrilase mutant F168V), *Rhodococcus rhodochrous*, *Arthrobacter aurescens*, and *Acidovorax facilis* (nitrilase mutant F168V/L201N/S192F).

Apart from these examples, glucose isomerases have been employed in the pharmaceutical industry to convert aldoses and ketoses from *Streptomyces rubiginosus*, and their crystal structures have been elucidated by Eun Bae et al. (2017). With potential applications in l-ribose production, Tseng et al. (2017) studied the overproduction and characterization of a recombinant l-ribose isomerase from *Actinotalea fermentans* ATCC 43279.

Martínez et al. (2017) showed that oxidoreductases were potential candidates for use in biotransformation, reporting reactions such as 1 naphthol, 2,5-hydroxyvitamin D3 drug metabolism catalyzed by peroxygenases, copper oxidases, and laccases, hence elucidating the characteristics of peroxidases from fungi, including Basidiomycetes, along with their limitations. Truppo (2017), in a study that showed a dramatic increase in protein engineering, reported excellent multiple contact of enzymes with substrates, with increased selectivity.

The development of biocatalysts that, in comparison with chemical catalysts, are faster, less expensive, and more versatile in their selection and preference for substrates, that can catalyze an increased range of reactions, and that have higher temperature stability and improved solvent compatibility is promising for the sustainability of various products/processes in the market today.

16.9 Agricultural Chemicals

Agricultural chemicals, mainly fertilizers and pesticides, are used in massive amounts worldwide to sustain the productivity of land. Because of their widespread use, agrochemicals are an important source of pollution, pose health risks, and consume large amounts of resources in their production. Enzymology can present useful products that can replace conventional agrochemicals and the methods used to degrade agrochemicals (Malik et al. 2017). In addition, biotechnology can provide animal feed with enhanced nutritional and storage characteristics, to improve the sustainability of animal production.

16.10 Fiber, Textiles, Pulp, and Paper Processing and Other Applications

Through biotechnology and improved silviculture, trees and other bioresources used in papermaking can be specifically tailored to match the properties required in cellulose fibers for different product applications, thus showing potential to increase the paper yield and product quality. Producing optimal fibers for papermaking through genetic engineering is an important long-term objective that requires a better understanding than we have at present of fiber biosynthesis in plants. Furthermore, the use of engineered microorganisms and enzymes can displace many of the environmentally adverse practices used in pulp processing. Some of these developments are discussed next.

In kraft pulping, bleaching of the pulp remains one of the most expensive operations and is a prime target for cost reduction. Because of the polluting potential of chlorine bleach, pulp mills are mostly changing to bleaching methods that do not require elemental chlorine. The use of low-molecular weight xylanase from *Trichoderma viride* VKF3 has recently been reported for the bio-bleaching of newspaper pulp (Nathan et al. 2017). Oxidative enzymes such as laccase provide other promising options for reducing costs in pulp mills. Other processing improvements have been achieved by using lipases to control pitch deposits; cellulases to improve rates of pulp dewatering; and pectinases for digesting pectins. Ongoing developments will provide engineered enzymes that are better suited to the needs of pulp processing and cost less than the enzymes used at present. In future, it may be possible to manufacture unique paper products by developing enzymes that can be used to control the properties of the pulp fibers and, therefore, the end product.

The production of paper consumes huge amounts of water. Extensive research is underway for the treatment of wastewater from paper mills, the aim being total recycling. Pulp and paper mills in Canada are aiming for total effluent reuse after secondary and tertiary biotreatment. Wastewater recycling potentially saves on the expense of treating any freshwater entering the mill and greatly reduces the environmental impact of effluent disposal.

In the processing of textiles, cellulose pulp is usually bleached with hydrogen peroxide, which must be removed before the fibers are colored. The traditional method for the removal of hydrogen peroxide relied on extensive washing in hot water and the use of inorganic salts. The enzymatic process saves water and energy and the effluent is ecologically harmless. Of note, *Aspergillus oryzae* lipase is capable of modifying polyethylene terephthalate fabrics, improving their hydrophilicity and anti-static capacity, while the immobilization of porcine-pancreas lipase on zirconia-coated alkylamine glass beads by glutaraldehyde coupling improved washing properties in cotton cloth. Fungi such as *M. miehei* has been reported for esterification in the presence of pentane (Bloomer et al. 1992). Other fungi, such as *C. rugosa*, *Penicillium roqueforti*, and *Humicola lanuginosa* have the ability to grow in medium supplemented with organic solvents such as cyclohexane and hexane. These fungi have industrial applications in the field of organic synthesis as well as in the textile industry (Mehta et al. 2017).

Enzyme options in the textile industry range from lipases to amylases. Lipases, together with alpha amylase, are used for the desizing of denim and other cotton fabrics on a commercial scale. Nippon Paper Industries (Tokyo, Japan) developed a pitch control method that used a fungal lipase from *C. rugosa* to hydrolyse up to 90% of wood triglycerides. *Rhizomucor meihei* lipase is used as a biocatalyst in personal care products such as skin and sun-tan creams and bath oils. *C. antarctica* lipase B-synthesized amphiphilic compounds are important in the cosmetic industry as they have a range of beneficial properties for the skin. The lipase component increases detergency and prevents scaling. Recently, lipase from *Rhizopus nigricans* showed maximum lipolytic activity, as well as bio-emulsification activity, indicating high bio-surfactant production in kerosene A lipase obtained from *C. cylindracea* considerably reduced pitch problems and talc consumption of triglyceride in groundwood pulp. *C. antarctica* lipase A was also used in pitch control in the paper industry (Mehta et al. 2017).

Bacillus subtilis is one of the most widely used bacteria for the production of industrial enzymes. *Bacillus* spp., especially *B. subtilis* and *B. licheniformis*, are the sources of most extracellular proteases (Kamal et al. 2016). These enzymes sourced from *B. subtilis* (6381.75 U/mg), *B. altitudinis* (MCCB0014) (7407.5 U/mg), *B. circulans* MTCC 7906 (3147.33 U/mL), and *B. alcalophilus* ATCC 21522 (18,000 U/mg) were reported to exhibit high activity (Kamal et al. 2016).

Enzymes such as alcalase and savinase from *B. licheniformis* and other *Bacillus* spp. are also used in the detergent industry and the textile industry. Enzymatic degradation using alkaline proteases with keratinolytic activity (keratinases) is an attractive method for hydrolysis of proteins and keratins and also helps to reduce the biological oxygen demand (BOD) for aquatic macro and micro flora. *Bacillus* spp. are extensively reported as the bacterial source of keratinases for the degradation of feathers (Kamal et al. 2016). A novel *Chryseobacterium* sp. was screened for cold-active protease production in the presence of a high concentration of NaCl, and its tolerance to several organic solvents, surfactants, and detergents was reported. Classical optimization for enhanced protease production, of 18 U/mg to 26 U/mg, was studied and reported (Mageswari et al. 2017). A protease from *B. licheniformis* K-3 showed remarkable tolerance to detergents such as cetrimonium bromide, sodium dodecyl sulfate, and Tween-20, suggesting its industrial applications for the de-gelatinization of X-ray films and the dehairing of animal hide (Singh and Bajaj 2017). Studies also suggest the application of a thermostable and pH-stable protease from *B. licheniformis* K-3, using agroindustrial/forestry residues as an inexpensive substrate for cost-effective enzyme production. A serine protease from a newly isolated *Bacillus* sp. was reported to show efficient silk-degumming, sericin-degrading, and color-bleaching activities (Suwannaphana et al. 2017).

Cellulose, hemicellulose, pectin (carbohydrate), and lignin (noncarbohydrate) polymers are the main substrates of lignocellulose-degrading enzymes. These polymers are present in large amounts in the primary cell walls and dietary fibers of major fruits and vegetables. During the processing of fruits and vegetables to the corresponding final food products, lignocellulosic substrates are hydrolyzed by different lignocellulolytic enzymes (Toushik et al. 2017).

The biological treatment of textile wastewater varies widely, ranging from bacterial, fungal culture (*Armillaria* sp. F022), yeast to any consortia. Reports of the use of enzymes in textile wastewater treatment started in 1970 with the isolation of three microbial strains, viz. *B. subtilis, Aeromonas hydrophila*, and *B. cereus*. A wide range of aerobic and anaerobic bacteria, such as *Pseudomonas sp.*, *B. subtilis*, *Geobacillus sp.*, *Escherichia coli*, *Rhabdobacter sp.*, *Enterococcus sp.*, *Staphylococcus sp.*, *Corneybacterium sp.*, *Lactobacillus sp.*, *Xenophilus sp.*, *Clostridium sp.*, *Acinetobacter sp.*, *Micrococcus sp.*, *Dermacoccus sp.*, *Rhizobium sp.*, *Proteus sp.*, *Morganella sp.*, *Aeromonas sp.*, *Alcaligenes sp.*, *Klebsiellla sp.*, *Shewanella sp.*, and *Alishewanella sp.*, have been extensively reported to show good, nonspecific biodegradation of azo dyes. *Pseudomonas sp.* is widely used in decolorization studies because of its capacity to degrade a variety of azo dyes (Red HE7B, Reactive Blue 172, Reactive Red 22, Reactive Red 2, and orange I and II). *Pseudomonas sp.* has shown its potential for the degradation of commercial azo dyes used in textile wastewaters (Sarkar et al. 2017). The use of microbial enzymes in the degradation of synthetic azo dyes in the textile industry is a sustainable methodology that can be employed by industry on a large scale. An alkali-tolerant EG gene of *B. subtilis* Y106 was homologously overexpressed to obtain a suitable enzyme for pulp modification (Wang et al. 2017). For eliminating textile waste from the environment, the co-plantation of *Typha angustifolia* and *Paspalum scrobiculatum* has shown enhanced removal of dye such as Congo red from textile effluent (Chandanshive et al. 2017).

Enzymes have been strongly accepted as a green alternative for use in many textile processes. These biocatalysts are not consumed and immobilization has been adopted as the most promising tool for their efficient recovery and reuse. Smart polymers and nanoparticle materials have been used for textile applications (Madhu and Chakraborty 2017).

In regard to efficient process techniques, the immobilization of lignin-modifying enzymes (LMEs), including lignin peroxidase, manganese peroxidase, and white-rot fungi laccase, has also been studied. The successful use of immobilized LMEs in the decolorization and/or detoxification of industrial dyes and dye-based industrial wastewater effluents has also been reported (Bilal et al. 2017).

16.11 Environmental Biotransformation and Bioremediation

Historically, the treatment of municipal wastewater by the activated sludge method has represented a major use of microorganisms in environmental care and bioremediation applications. The use of microbial extremozymes has made its mark in the field of environmental biotransformation and biodegradation, with a long history of applications. From the mid-1990s to 2017, many applications and reports have suggested the use of microorganisms in bioremediation and degradation—from environmentally friendly biotransformation in the pharmaceutical industry to the employment of microbial enzymes such as lipases in biosensors for the detection of specific pollutant levels; these applications have shown high efficiency, with wide diversity in the field, including the removal of nitrates and phosphates from wastewaters. A very interesting study at the laboratory-scale batch level used *B. cereus* AKG1 and AKG2 to treat wastewater, investigating BOD, chemical oxygen demand, and total organic carbon (Nikama et al. 2017). Hyper phenol-tolerant microorganisms from oil refineries and oil exploration sites were investigated for their potential to biotransform phenol by Sarkar et al. (2017). Biodegradation and detoxification of dyes is also possible through consotia of *Providencia rettgeri* strain HSL1 and *Pseudomonas* sp. SUK1 (Lade et al. 2015). *Pseudo Cochliobolus verrucosus* NFCC 3818, an ethyl glycol-tolerant lignolytic *Ascomycete* strain, has shown capacity for the detoxification and degradation of azo dyes.

Enhanced solvent tolerance of a psychrophilic phthalate esterase in an arctic bacterium, *Sphingomonas glacialis* PAMC 26605, was seen after the cloning and characterization of this esterase (Hong et al. 2017). For soil bioremediation, bacterial associations with plants have been observed for *Azotobacter* and *Lepidium sativum*, with tolerance for heavy metals being observed (Sobariu et al. 2017).

16.12 Conclusion

Economic and biotechnological benefits with respect to cost, productivity, reduction of environmental hazards, and sustainability are the well reported and evident advantages of enzymology. In the production of critical and chiral molecules, microbial enzymes have unrivalled precision, owing to their enantio-, regio-, and substrate selectivity, and this selectivity has supported the use of nitrile-hydrolyzing enzymes in industry today (Xue et al. 2015). Industrial-friendly enzyme-producing organisms, including bacteria, filamentous fungi, yeasts, and plants have been well studied for their possible use in the commercial production of carboxylic acids and amides on an industrial scale. Key properties of such organisms, such as enantioselectivity and enantio-retentivity, come with supporting traits such as thermostability, halostability, pH stability, and organic solvent tolerance. These characteristics occur in various microbial enzymes that assist in the production of specific molecules at lower cost and better yield than what is seen with conventional methods. The use of these enzymes has reduced hazardous impacts on the environment. It is time for us to focus on the commercial-scale production of such enzymes and their products in order to increase the overall bioeconomy.

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