Chapter 19
Biotechnological Aspects of Cold-Active Enzymes

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Abstract Cold-adapted enzymes produced by organisms inhabiting permanently low temperature environments are typically characterized by a high activity at low to moderate temperatures and a poor thermal stability. Such characteristics make these enzymes highly attractive for various applications where they can enable more efficient, cost-effective, and environmentally friendlier processes than higher temperature-adapted enzymes. In this chapter, the biotechnological aspects of cold-adapted enzymes and their application in industry are reviewed and discussed with a focus on cleaning/detergents, food and beverages, molecular biology, biomedicine, pharmaceuticals, cosmetics, textiles, biofuels, and materials applications.

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19.1 Introduction

Enzymes are highly specific biological catalysts that accelerate the rate of chemical reactions in the cells of living organisms. These natural catalysts are biodegradable, fast, efficient, and selective, and produce low amounts of by-products while also

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being less demanding with respect to process energy, raw materials and toxic components than many traditional chemical catalysts. Their exquisite catalytic power, specificity of action and reduced environmental footprint makes them seemingly ideal tools for numerous biotechnological applications. Indeed, they have already found application in many diverse industries including food, beverages, pharmaceuticals, detergents, textiles, leather, chemical, biofuels, animal feed, personal care, pulp and paper, diagnostics, and therapy, and are continuously developing into new areas. They are employed to enable improved and/or more economic and eco-friendly end-products and bioprocesses and can even facilitate the development of novel products and processes. The current market value of industrial enzymes is estimated at almost USD 5 billion (BCC Research 2017), up from USD 3.3 billion in 2010 (Blamey et al. 2017), and is expected to reach almost USD 6.3 billion by 2021 (BCC Research 2017).

The International Union of Biochemistry and Molecular Biology (IUBMB) and the International Union of Pure and Applied Chemistry (IUPAC) have classified enzymes into six main classes (Enzyme Commission, EC, numbers 1–6) based on the types of reactions catalyzed. Enzymes belonging to all six classes have found application, but the hydrolases (EC number 3) are the most widespread used, with lipases, proteases, glycosidases, and other hydrolytic enzymes accounting for almost 90% of the total industrial enzymes market share (Blamey et al. 2017). These are mainly used as high volume, commodity, or bulk enzymes in the manufacture of food, beverage, cleaning agents, textiles, biofuels, and pulp and paper. On the other hand, specialty enzymes, for use in high value, low volume products such as in diagnostics, research and development, and as biocatalysts, currently have a small market share but have shown strong growth in recent years. Improved health care and the adaption of a "personalized" medicine approach drive this growth in diagnostics. Similarly, the development of "Green chemistry," making use of the high substrate-, regio-, and enantio- selectivity of enzymes for the sustainable production, modification, and/or functionalization of pharmaceuticals, fine chemicals, flavors, fragrances, etc., drives growth in biocatalysis. Indeed, the current societal shift towards greener technologies and a more sustainable low carbon resource-efficient economy as well as the expansion of biotechnology into new fields previously dominated by petroleum-based chemicals will continue to drive growth of enzymes in the future. In fact, currently only about 11% of all chemicals are made from renewable raw materials, with the remaining being obtained from crude oil, natural gas, and coal (Blamey et al. 2017), and it is believed that enzymes will play a major role in the shift towards a greater utilization of renewables in the future and in the development of efficient, improved, environmentally friendly, and sustainable bio-based processes.

For successful integration into a particular application, the ideal enzyme needs to appropriately catalyze the desired reaction with the desired specificity under the conditions used. That is to say, the enzyme needs to have the required specificity and selectivity as well as a high activity and stability in the process environment. Unfortunately, and due mainly to the harsh conditions frequently used in industrial processes, few naturally occurring enzymes fulfill all of these requirements

(Bommarius and Paye 2013; Sarmiento et al. 2015). High temperatures, as used, for example, in pulp and paper manufacture and in bioconversion, are often required in processes to enable a better breakdown and improved solubility of substrates and products as well as reduced viscosity, higher mass transfer rates, easier separation of volatile products, reduced contamination, and a shift in the equilibrium of endothermic reactions towards products (Siddiqui 2015). Conversely, low temperatures are more suited for processes involving heat-sensitive or/and volatile components or/and where undesirable chemical side-reactions occurring at high temperatures and contamination problems are to be avoided, such as in the manufacture of many foods, beverages, fine chemicals, and pharmaceuticals. Furthermore, in addition to extremes of temperature, many industrial processes are also carried out under extremes of pH, pressure, salinity, and/or in the presence of detergents, non-aqueous solvents, etc. (Bommarius and Paye 2013; Sarmiento et al. 2015; Barroca et al. 2017). Hence, standard, naturally occurring enzymes, which are typically stable and active over a narrow range near moderate physical and chemical conditions, are unsuited for use in numerous industrial processes in which "unnatural" conditions are used. In an attempt to overcome this, scientists have turned to extremophilic enzymes (Liszka et al. 2012; Elleuche et al. 2014; Littlechild 2015; Siddiqui 2015) and protein engineering (Liszka et al. 2012; Bommarius and Paye 2013). The use of rational design or directed evolution to fine-tune or engineer the properties of a protein for a particular application has achieved some success, but the use of extremophilic enzymes already adapted to extreme conditions offers a more direct route. Indeed, extremophilic enzymes, produced by organisms inhabiting and adapted to extreme environments, have already been shown to be valuable tools for processes in which extreme conditions prevail. A large number of extremophilic enzymes are already commonly used in various diverse applications with the vast majority being thermophiles, these being active and stable at high temperatures and frequently also in the presence of harsh chemicals and detergents. In contrast, use of enzymes from other extreme environments appears much less developed. As an example, a search of patenting databases for patents on enzymes with the keywords "cold-active," "cold-adapted," "cold-resistant," or "psychrophilic" in the title or abstract identified 53 patents whereas a similar search using the keywords "thermostable," "heat-stable," "heat-resistant," and "thermophilic" gave over 60-times more hits. Interestingly, while currently being much less employed than thermophilic enzymes, cold-adapted enzymes have an enormous potential as highly valuable tools for various biotechnological applications, and this review will focus on these enzymes and their biotechnological aspects.

19.2 Cold-Adapted Enzymes

As discussed in Chap. 10 of this book and in numerous previous review papers on the subject (Santiago et al. 2016; Fields et al. 2015; Siddiqui 2015; Gerday 2013, 2014; Collins et al. 2002a, 2007, 2008; Huston 2008; D'Amico et al. 2006), cold-adapted or psychrophilic enzymes, produced by organisms inhabiting permanently low temperature environments, are typically characterized by a high activity at low temperatures and a reduced stability as compared to their mesophilic and thermophilic homologs. They are believed to have overcome the low temperature challenge and maintained high activities at low temperatures by increasing the flexibility of specific regions of their molecular edifice. This increased flexibility enables a continued mobility of those regions important for enzyme activity, even in the low energy environment characteristic of low temperatures, and is achieved via a reduction in the number and/or strength of stabilizing interactions in the protein structure which in turn leads to the observed reduced structural stability of cold-adapted enzymes. Importantly, this balancing of flexibility, activity, and stability is believed to be key in enzyme adaptation to temperature, with thermophilic enzymes, in contrast to those from psychrophiles, being generally characterized by a high stability, low flexibility, and reduced low temperature activity.

The intrinsic attributes of a high activity at low temperatures and reduced stability of cold-adapted enzymes offer many advantages for use in a variety of commercial applications. Low temperature processes are common in the food and beverages industries and cold-adapted enzymes, highly active under these conditions, offer obvious benefits for such processes. Also, it is important to note that cold-adapted enzymes are not only more highly active than their mesophilic and thermophilic homologs at low temperatures, but frequently also show a higher activity at moderate temperatures. Hence, processes can be carried out at ambient temperatures without the need for energy input (for heating or cooling) and with lower quantities of cold-adapted enzyme being required as compared to enzymes adapted to higher temperatures. That is to say that these enzymes can be instrumental in developing processes with an improved economic and environmental impact. Furthermore, the thermolability of these enzymes offers solutions for those processes where a greater control is required and where a simple selective inactivation of the enzyme can be achieved by mild heat treatment (Margesin et al. 2003). Such a characteristic may prove beneficial for preserving product quality in the food and beverages industry and in biocatalysis or for sequential multi-step processes such as those used in molecular biology (Huston 2008). In fact, cold-adapted enzymes have already found application in these industries and their now commonplace application in cleaning/detergents applications as well as development into new markets underscores the potential and market value of these enzymes. In effect, market research by the Freedonia Group (The Freedonia Group 2016) has indicated that, in the mature commodity enzymes markets where competition among enzyme makers is intense, novel enzymes presenting efficient performance at lower temperatures will play an important role in allowing for market expansion in the future. In addition, while little explored at present, it is believed that in the future, psychrophilic enzymes will offer valuable tools as specialty enzymes in the preparation of temperature-sensitive pharmaceutical ingredients, fine-chemicals, flavors, and fragrances. In the following sections, the application of cold-adapted enzymes in various industrial enzymes markets will be discussed.

19.3 Cleaning/Detergents

This is probably the best developed market for cold-adapted enzymes with a large number of different hydrolytic enzymes (EC 3) being commercialized for this application by various companies throughout the world. See Sarmiento et al. (2015) for an in-depth review.

Enzymes are used as cleaning agents in household and industrial scale laundry and dishwashing, as well as for cleaning-in-place in the food, dairy, and brewing industries and even in the cleaning of buildings, carpets, contact lenses, etc. (Cavicchioli et al. 2002; Damhus et al. 2013; Sarmiento et al. 2015; Siddiqui 2015). This market currently accounts for approximately 20–25% of total industrial enzymes sales (BCC Research 2017). Enzymes break down stains, soiling, and deposits into more soluble products, thereby allowing for improved cleaning performance in, for example, laundry and dishwashing as well as for the deblocking and cleaning of filters and equipment in the food and beverages industries. Lipases hydrolyze lipids as found, for example, in grease, butter, oil, sauces, tears, molds, and biofilms; proteases break down proteins common in grass, blood, egg, milk, cheese, yoghurt, sweat, tears, molds, etc., and amylases are used for breakdown of starch soiling from cereals, pasta, potatoes, molds, biofilms, etc. Cellulases have also been used and act on oat products, such as cereals and snack bars. Furthermore, in laundry detergents, cellulases have an added benefit of contributing to fabric care; they degrade accessible broken cotton fibers (known as fuzz or pills) and thereby remove any captured dirt but at the same time reduce fuzz build up and hence also increase the softness and color brightness of cotton fabrics. Recently, attention has also been turned to mannanases and pectinases for use in cleaning detergents for removal of difficult stains due to gum, fruit products, juices, mayonnaise, tomato sauce, salad dressing, body lotions, personal care products, etc. Pullulanases have also been shown to have potential for biofilm removal (Antranikian et al. 2004). The action of enzymes allows for improved cleaning performance and in turn this improved effectiveness enables a reduction in the use of other more hazardous components, e.g., detergents, surfactants, polymers, alkaline builders (in laundry), phosphates (in dishwashing), and organic solvents (for cleaning-in-place), as well as reduced water consumption (Damhus et al. 2013; Sarmiento et al. 2015). Indeed, up to 25–50% reduction in a laundry detergent surfactant system has even been demonstrated upon use of enzymes (Damhus et al. 2013; Siddiqui 2015). Such modifications obviously lead to environmentally friendlier wash water wastes and more sustainable wash processes.

The major benefit of using cold-adapted enzymes in cleaning/detergents is that the process temperature and hence energy input can be reduced and thus enables an improved economic and environmental impact. It has been estimated that a reduction in wash temperature from 40 to 30 °C allows for a 30% saving in energy, corresponding to 100–300 g of CO_2 per wash (Cavicchioli et al. 2011; Siddiqui 2015), and already over 50% of laundry machine washes are carried out at low temperatures (Sarmiento et al. 2015). Furthermore, in laundry washing, low temperatures also extend garment life by being less aggressive, reducing garment degradation, and lessening shrinkage and/or dye bleeding. Currently, the focus is on further reducing temperatures to approx. 20 °C and leading to further savings, and a continued growth in the use of cold-adapted enzymes in this application is thus forecast. In cleaning in-place applications in the food and beverages industries, the use of cold-adapted enzymes would avoid the need for warm cleaning washes and the poor stability of these enzymes would give a greater assurance of complete enzyme inactivation following heat treatment, a desirable characteristic for the cleaning of food, dairy, and brewing industry equipment. Additionally, cold-adapted enzymes could offer advantages in the cleaning of large immovable objects where heating is not viable, and indeed glucose oxidases, proteases, amylases, and lipases have already been shown to be effective in mold and biofilm removal from building surfaces (Webster and May 2006; Valentini et al. 2010). These were shown to enable effective building cleaning and conservation while also reducing the use of more aggressive cleaning agents. Similarly, enzymes have been shown to be effective in contact lens cleaning, and a thermolabile fish waste isolate protease has been shown to have potential as an efficient, non-hazardous cleaning agent for the removal of tear films and proteinaceous deposits on contact lens (Pawar et al. 2009).

19.4 Food and Beverages

Industrial enzymes for use in the food and beverages industries represent a relatively well developed market with current sales of nearly USD 1.5 billion (BCC Research 2017). They are used as food additives and processing aids in such diverse areas as the more traditional processes of cheese manufacturing, wine making, brewing, and bread making to the more recent applications in the preparation of functional foods and nutraceuticals (Chandrasekaran 2015). Enzymes are used in the manufacture, processing, preparation, and treatment of foods and beverages. They can enable improved process efficiency, reduced processing costs, and reduced environmental impact and, frequently also, enhance the flavor, nutritional value, appeal, digestibility, texture, and/or shelf life of the final product. Growing consumer preference for more natural, healthier, and flavorsome foods as well as an improved awareness of environmental issues and food safety is driving continued growth in the use of enzymes in this sector, and a compound annual growth rate of 4.7% through 2021 has been forecasted (BCC Research 2017).

Cold-adapted enzymes are particularly attractive in food and beverages preparation due to their high catalytic activity at temperatures that minimize spoilage, alterations in taste, and loss of nutritional value as well as their ease of inactivation (Huston 2008). They have found application in the dairy, baking, beverages, meat, and fish processing industries and in the production of functional foods.

In the dairy industry, a number of cold-adapted β-galactosidases, or lactases, have been developed for the production of lactose free milk and treatment of the waste by-product whey. Approximately 65% of the human population has a reduced ability to digest lactose after infancy, with Asian and African populations being the most affected. β-Galactosidases hydrolyze lactose to glucose and galactose and hence can be used to remove lactose from dairy products and improve product digestibility while also enhancing sweetness. A variety of β-galactosidases are currently being marketed, but cold-adapted β-galactosidases offer the advantage of efficient hydrolysis at refrigeration temperatures which minimize problems associated with contamination and alteration of product organoleptic properties (Hoyoux et al. 2001; Ghosh et al. 2012; Stougaard and Schmidt 2012; Pawlak-Szukalska et al. 2014). In the valorization of whey, a by-product of cheese production and a waste disposal problem, the glucose and galactose produced by β-galactosidase treatment can be used as sweeteners in soft drinks and confectionary products, in the production of functional foods/nutraceuticals (Van de Voorde et al. 2014), and in biofuel production (Huston 2008). A recent study showed the potential of using a cold-adapted β-galactosidase for the initial steps of preparation of tagatose, a novel, low-calorie sweetener (Van de Voorde et al. 2014). Similarly, cold-adapted β-galactosidases have also been shown to be useful in the preparation of other low calorie sweeteners, namely, galactooligosaccharides (Karasova-Lipovova et al. 2003; Nakagawa et al. 2007; Schmidt and Stougaard 2010; Pawlak-Szukalska et al. 2014). In effect, in addition to hydrolysis, many β-galactosidases also display transglycosylation activities where monosaccharides are transferred to oligosaccharides with the production of di-, tri-, tetra-, and pentasaccharides. These can be produced in the milk or from whey, and in addition to functioning as low calorie sweeteners, they have also been found to be effective prebiotics, selectively stimulating the growth of beneficial intestinal microorganisms (Pawlak-Szukalska et al. 2014). Proteases are another family of enzymes which play an important role in the dairy industry and in particular during clotting and ripening in cheese making. Lipases and phospholipases are also used and due to the low temperatures employed, cold-adapted variants have been suggested for this (Huston 2008).

Amylases, xylanases, oxidases, asparaginases, and lipases are all commonly used in baking applications so as to improve product quality (dough machinability, bread texture, and shelf life), reduce the use of chemical additives (e.g., potassium bromate, emulsifiers, etc.), and reduce the production of acrylamide (in baked or fried products including biscuits, crisps, crackers, etc.). As yet, the majority of enzymes used appear to be of mesophilic or thermophilic origin whereas dough preparation and proofing is typically carried out at moderate temperatures at which cold-adapted enzymes offer considerable cost and efficiency advantages. Nevertheless, it appears that currently only one cold-adapted enzyme has found application in baking, namely, a cold-adapted xylanase (Collins et al. 2002b, 2006, 2012; Dornez et al. 2011; Dutron et al. 2012). Xylanases improve dough machinability, giving rise to a more flexible, easier-to-handle dough, larger loaf size, and improved crumb structure, and the cold-adapted xylanase was shown to be more effective than the other commercial xylanases studied (Collins et al. 2006, 2012; Dutron et al. 2012).

In the brewing, wine, and fruit and vegetable processing industries, pectinases (polygalacturonases, pectin lyases, and pectin methylesterase) and hemicellulases such as xylanases are used to increase extraction yield, improve clarification, reduce viscosity, and enhance color and flavor (Tu et al. 2013; Adapa et al. 2014). Rhamnogalacturonases, galactanases, and arabinanases have also been recently developed for these applications. Apparently no cold-adapted enzymes have been commercialized in this sector as yet but low temperature active enzymes are available, e.g., Lallzyme EX (Lallemand) is active between 5 and 20 °C (Sarmiento et al. 2015). Pectin esterases can also be used in the manufacture of fruit preparations composed of intact fruit pieces, and a cold-active pectin methylesterase was found to increase gelling and enhance fruit integrity during processing (Pan et al. 2014). Finally, pectinases, in addition to other glycoside hydrolases (EC 3.2.1), lipases, and proteases, can likewise be used for the treatment of food and beverage industry wastes with cold-adapted enzymes allowing for a more effective ambient temperature waste management (Margesin et al. 2005; Naga Padma et al. 2011; Tsuji et al. 2013).

In meat and fish processing, cold-active proteases can be used for tenderization and taste enhancement as well as improving the nutritional and functional properties of refrigerated products (He et al. 2004; Bjarnason and Benediktsson 2010; Venugopal 2016). They can be employed in the preparation of soluble protein hydrolysates for use as flavor enhancers, meat extracts, emulsifiers, and foaming agents and which have also been shown to exert health benefits such as antihypertensive, antioxidant, and immunoregulatory activity (Cazarin et al. 2015). A study by He et al. (2004) showed how a cold-adapted protease released more taste amino acids and essential amino acids from meat than a mesophilic protease during cold storage. Similarly, a marine protease was shown to be effective in the preparation of protein hydrolysates for use as flavor enhancers in foods for human consumption and animal feed (Bjarnason and Benediktsson 2010). Finally, the use of cold-adapted enzymes (proteases, lipases, chitinase etc.) in seafood processing (fish descaling, skin removal and degreasing, waste treatment, oil extraction, etc.) has also been discussed (Shahidi and Janak Kamil 2001; Junpei et al. 2016; Venugopal 2016).

19.5 Molecular Biology

Cold-adapted alkaline phosphatases (Kobori et al. 1984; Sullivan et al. 1988; Rina et al. 2000; Nilsen et al. 2008; Muller-Greven et al. 2012), both single and double stranded nucleases (Awazu et al. 2011) and uracil-DNA *N*-glycosylases (Lanes et al. 2002), are currently being commercialized as molecular biology tools by various companies (New England Biolabs Inc., ArcticZymes, Takara-Clontech, Affymetrix, Inc.). Alkaline phosphatases are most commonly used in the dephosphorylation of the 5′ end of DNA or RNA during cloning and end-labeling procedures. Nucleases, depending on their specificity, degrade single and/or double stranded DNA and/or RNA and are used, e.g., in removing contaminating

DNA/RNA from RNA preparations, PCR master mixes, and protein solutions. Uracil-DNA *N*-glycosylases are used in PCR, RT-PCR, site-directed mutagenesis, and SNP genotyping procedures to release free uracil from uracil-containing DNA (Sarmiento et al. 2015). In all these cases, in addition to a high activity at low temperatures being beneficial, the instability of cold-adapted enzymes is a determining factor in their successful application. This latter characteristic enables for simplified enzyme inactivation by moderate heat treatment as opposed to the time consuming chemical treatments or column purifications required with mesophilic or thermophilic variants which often also lead to sample loss and downstream contamination problems.

Recently, a cold-adapted polymerase has been commercialized by Arcticzymes for use in gene sequencing, molecular diagnostics, and other markets. Furthermore, cold-adapted ligases, recombinases, and proteinase k have been called for (Huston 2008; Sarmiento et al. 2015).

19.6 Biomedicine, Pharmaceuticals, and Cosmetics

Many pharmaceuticals, active pharmaceutical ingredients, fine chemicals, flavors, and fragrances are heat sensitive or/and volatile and hence must be synthesized at low temperatures at which cold-adapted enzymes are most active. In addition, it has been proposed that as a result of their proposed high structural flexibility, cold-adapted enzymes can operate at low water activity, such as in the aqueous/organic and non-aqueous solvent systems frequently used during organic synthesis of complex molecules (Huston 2008; Karan et al. 2012). In this market sector, hydrolases, oxidoreductases, lyases, transferases, reductases, carboxylases, etc. are becoming more commonly used but only a few cold-adapted enzymes have been investigated and below an overview of these is given.

The most widely used cold-adapted enzymes in this sector are lipases and esterases for the synthesis of optically pure intermediate compounds of synthetic value. In fact, lipases (mainly CALB) from *Candida antarctica* are among the most extensively and diversely used enzymes in organic synthesis. They are used in a broad range of surprisingly diverse applications, including the modification of sugars and sugar-related compounds, desymmetrization of complex prochiral drug intermediates, and resolution of racemic alcohols and amines (Huston 2008; Kirk and Christensen 2002; Suen et al. 2004) during the synthesis of various pharmaceuticals (e.g., calcium antagonists as antihypertensive drugs, NK1/NK2 antagonist for asthma treatment), cosmetics (e.g., iso-propyl myristate, a skin emollient), flavors, and fragrance esters.

As discussed above (Sect. 19.4), cold-adapted ß-galactosidases are suited to the production of tagatose (an antihyperglycemic agent) and galactooligosaccharides (prebiotics). Moreover, these have also been shown to catalyze the synthesis of heterooligosaccharides such as lactulose (for treatment of constipation and hepatic encephalopathy, use as a prebiotic, and use in diagnostics), galactosyl-xylose (use in diagnostics), and alkyl glycosides (foaming agents) as well as glycosylated salicin (antiinflammatory agent) from lactose (Pawlak-Szukalska et al. 2014). Also, as discussed previously, cold-adapted proteases can be used for the preparation of bioactive peptides for use as antihypertensive, antioxidant, and immunoregulatory agents (Cazarin et al. 2015).

Cold-adapted proteases are currently being marketed as therapeutic agents against bacterial (biofilm breakdown) and viral (virus infectivity reduction) infections and in oral health care (plaque removal) and cosmetics (frown line reduction and dead or dried skin removal) (Fornbacke and Clarsund 2013).

Other cold-adapted enzymes with potential in biomedical applications include a marine α-galactosidases which was shown to be capable of converting B red blood cells into the universal blood type O cells for use in transfusion therapy (Balabanova et al. 2010) and a cold-active nitroreductase as a cancer prodrug activating enzyme using low temperature therapy for activation (Çelik and Yetiş 2012).

19.7 Other Applications

In the textiles industry, cold-adapted amylases, cellulases, and laccases have been developed for the rapid desizing, or starch removal, of woven fabrics, bio-finishing of cellulosic fabrics, and less abrasive enzymatic stonewashing and bleaching of denim (Sarmiento et al. 2015).

Cold-adapted enzymes, namely, cell wall degrading enzymes, amylases, laccases, lipases, and phospholipases, have been suggested for improving the energy efficiency and costs of biofuel (bioethanol, biodiesel, and biogas) production processes. In particular, they should find application in cold-cook or no-cook processes, simultaneous saccharification and fermentation (Festersen et al. 2005; Huston 2008; Gohel and Duan 2012; Ji et al. 2014; Wen et al. 2015), and low temperature biogas production (Akila and Chandra 2010).

Marine silicatein enzymes have been shown to be central in biomineralization and in the synthesis of biosilicates found in marine diatoms, radiolaria, and sponges (Shimizu et al. 1998; Wang et al. 2012). This has important implications in materials science and indicates the potential of cold-adapted enzymes for the synthesis of a variety of nanostructured mineral/organic composite materials under low temperature and mild chemical conditions. Examples of such materials include silica and siloxane polymers, bimetallic alloy nanoparticles, bimetallic perovskite-like materials, zirconia nanoparticles, spinel gallium oxide, etc. See Huston (2008) for a review.

19.8 Conclusions

The initial development of cold-adapted enzymes for use in industry was somewhat delayed as compared to mesophilic and thermophilic enzymes. Nowadays, however, they have found application in almost all sectors of the industrial enzymes markets. A continued growth in their use is envisaged in the future as novel cold-adapted enzymes with unique properties are isolated from the vast and varied cold environments in the world and as techniques for their isolation (Cavicchioli et al. 2011), efficient production (Cavicchioli et al. 2011), purification, engineering (Liszka et al. 2012; Bommarius and Paye 2013), stabilization, and immobilization (Mateo et al. 2007) are further developed. In addition, the current shift towards a more environmentally friendly and sustainable economy and the development of enzymes for biocatalysis will enable expansion into new application areas and drive growth further.

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