Microorganisms demonstrate diversity in their characteristics within two broad classifications: prokaryotes (bacteria and “blue-green algae” cyanobacteria) and eukaryotes (fungi, algae, and protozoa). Both classes of organism must synthesize and regulate all chemicals necessary for maintenance of life and reproduction, which involves biochemical pathways, kinetics, and energetics. Some of those chemicals are clearly ideal for characterization, quantification, control, and exploitation by industry. Natural biological processes at the heart of life have contributed significantly to the well-being of humanity by providing useful products and contributing to processes that maintain our healthy lifestyle (1, 2). Recently, engineered biological processes and products are leading toward highly targeted biopharmaceuticals and biocatalysis in what is termed “green” chemical synthesis (3, 4).

Such processes are noteworthy because, if they are designed and implemented correctly, they can offer considerable environmental and economic advantages over conventional energy-intensive chemical processes (5). However, as with all prospective technologies, a full life-cycle analysis first must be undertaken. The main obstacle to widespread implementation and integration of biocatalytic systems for generating products, or as key industrial process systems in their own right, is their typically low energy efficiency. In nonbiological catalytic systems, extremes of turbulence, temperature, pH, and pressure are often used to achieve the desired enhancement of process efficiency in several different reactor configurations (6). However, turbulence and other conventional intensification tactics are not applicable to biological systems, so much more care must be taken. Use of extremophiles that thrive in harsh environments may reduce these limitations.

It is now evident that environments once thought too hostile for life are the natural habitats of certain microorganisms known as extremophiles. Their discovery stimulated work to define the most extreme conditions compatible with the existence of life (7). Most extremophiles identified to date are members of the Archaea kingdom, although some extremophilic bacteria have also been identified (8).

**Extremophilic Microorganisms**
Extremophiles are microbes that live in conditions that would normally kill other creatures. It was not until the 1970s that such microorganisms were recognized, but the more researchers look, the more they discover that most archaea, some bacteria, and a few protists can survive in strange and harsh environments. The plasma
membranes in archaea are different from those found in other organisms: The cell wall does not contain peptidoglycan; archaea exhibit distinctive characteristics such as glycerol-1-phosphate lipid backbones; but they do share many features with both bacteria (metabolism, biosynthesis, energy generation, and transport) and eukaryotic cells (transcription and translation). Extremophiles are classified in the box on the next page.

Recent developments in the analysis of environmental DNA samples have indicated that only a small fraction (probably 0.1–1%) of organisms can be obtained in pure culture by present methods (9). The range of extremes at which life is found suggests a variety of conditions at which biological activity (e.g., enzymes) might be detected. The greater the diversity of organisms detected in the environment, the greater the potential range of biocatalysts available for bioprocessing applications. The classical route to microbial enzyme production has also been developed to obtain enzymes from extremophiles (extremozymes) (10, 11).

The most significant recent advances in microbiology can be credited to development in the fields of genomics and proteomics. The number of microbial genome sequences available has increased rapidly, including those of several extreme thermophiles, with others reported to be near completion (12, 13). Genome and proteome data include the entire complement of enzymes from each organism, so the selection of extremozymes that may improve bioprocessing efficiency should become more rapid in the future. This and further topics are discussed by Hough and Danson (14).

**Extremophiles Could Provide Solutions to Some Industrial Problems:** As is evident from the isolation and physiological characterization of extremophiles, they present the potential to dramatically improve process performance. The advantages of hyperthermophiles (as an example) over mesophilic microorganisms are summarized in the “Advantages” box. To date, little practical industrial application of such microorganisms has been realized, although considerable potential is often discussed in scientific literature. Understanding the important balance between temperature, pressure, pH, and salinity — and their individual effects on microbial metabolism — may yield information and principles useful to bioprocess engineering. So greater knowledge of the physiological characteristics and transport phenomena of extremophiles could allow industrially relevant biochemical reactions to be intensified through bioprocessing at conditions favoring mass transfer, reduced media viscosity, and less external contamination, for example.

### Biocatalysts From Extremophiles

Use of enzymes as biocatalysts is well established in industry, although most are obtained from mesophilic microbes despite their limited stability to extremes of temperature, pH, and other conditions. Extremophiles are found in environments of extreme temperature (–2 to 15 °C or 60–110 °C), ionic strength (2–5 M NaCl), or pH (<4 or >9). So extremozymes exhibit extreme stability, and their application in bioprocessing is attractive because they are stable and active under conditions previously regarded as incompatible with biological materials. Extremophiles also exhibit diverse metabolic pathways and thus could produce enzymes with unique activities.

Molecular community analysis shows that the diversity of extremophiles is far greater than was initially thought. Pure cultures are infrequently isolated, however, so it is difficult to determine the stability characteristics and substrate specificity of extremozymes. The main thrust of recent work has investigated their stability at a molecular level along with enzyme engineering to create novel biocatalysts with enhanced stability and altered specificity (14).

The role of biocatalysts in the pharmaceutical and fine chemical industries is expanding, although historically biocatalysis has been more evident in the pharmaceutical sector than in the chemical industry. The global trend suggests an increasing commercial interest in biocatalytic processes. Several examples of current commercial applications are shown in Table 1. Some recent advances in the field of biocatalysis include novel cofactor regeneration protocols, one-pot synthesis, directed evolution strategies, enzyme engineering, and combined chemocatalysis and biocatalysis.

The combination of chemocatalysis and biocatalysis provides some exciting new opportunities for using enzymes in chiral synthesis. Transition metal catalysts and enantiospecific lipases can be combined for direct synthesis of chiral alcohols and amines, in which the Ru catalyst interconverts the two enantiomers of a chiral alcohol while an enantiospecific lipase converts one of them to the corresponding ester.
Advantages of Hyperthermophile Use in Industrial Processes

Cost of cooling large-scale thermophilic fermentations is reduced.
Reduced viscosity of media increases efficiency of mixing and centrifugal harvesting rate.
Increase in reactant solubility allows use of higher concentrations of less-soluble components.
Volatile products (such as ethanol) may be removed by application of a mild vacuum, which also stops build-up of inhibitory by-products.
Reactor operation at elevated temperatures reduces risk of contamination by mesophilic microorganisms.
Decreased solubility of oxygen aids cultivation of anaerobic organisms.
Thermophilic enzymes are more resistant to detergents or solvents than mesophilic enzymes.
Immobile enzyme reactors offer longer periods of operation to reduce production costs.
Enzyme isolation and purification may be carried out at room temperature.
Higher enzyme recovery and integration into chemical synthesis processes are possible because of enhanced protein stability.

One advantage that may be of industrial interest is the possibility that cell wall adaptation to high temperatures could make hyperthermophiles extra resistant to mechanical stress in agitated bioreactor systems. Both shear-sensitive and -insensitive hyperthermophiles have been identified.

Table 1: Conventional enzymes used in the pharmaceutical and fine chemicals industries (Adapted from Reference 16)

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Chemical Use</th>
<th>Pharmaceutical Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrile hydratase</td>
<td>Acrylamide</td>
<td>Nicotinamide</td>
</tr>
<tr>
<td>Dehydrogenase</td>
<td>Ethanol, lactic acid,</td>
<td>Omapatrilat, tert-leucine</td>
</tr>
<tr>
<td></td>
<td>high-fructose corn syrup</td>
<td></td>
</tr>
<tr>
<td>Isomerase</td>
<td>High-fructose corn syrup</td>
<td></td>
</tr>
<tr>
<td>Acylase</td>
<td>Amino acids, 6-APA1</td>
<td>Xemilfiban</td>
</tr>
<tr>
<td>Lipase</td>
<td>Cocoa butter</td>
<td>Paroxetine2, 1-DOPA</td>
</tr>
<tr>
<td>Nitirilase</td>
<td>1,5-dimethyl-2-piperidone</td>
<td>Methylphenidate3</td>
</tr>
<tr>
<td>Protease</td>
<td>Aspartame</td>
<td>Abacavir4</td>
</tr>
<tr>
<td>Dehalogenase</td>
<td>Chiral epichlorohydrins</td>
<td>Atorvastatin5</td>
</tr>
<tr>
<td>Hydantoinase</td>
<td>L-methionine</td>
<td>D-phenylglycine, D-amino acids</td>
</tr>
<tr>
<td>Hydroxylase</td>
<td>Niacin</td>
<td>Steroids, metoprolol, atenolol, D-phenylglycine, 6-APA1</td>
</tr>
<tr>
<td>Amidase</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 6-Aminopenicillanic acid 2 Trade name Paxil 3 Trade name Ritalin
4 Trade name Ziagen 5 Trade name Lipitor

Enantioselective lipase-catalyzed hydrolysis of racemic esters has a 50% maximum yield; however, the undesired enantiomer ester can be racemized using chemocatalysis. Examples of lipase-Ru and lipase-Pd combinations are reviewed further by Huisman and Gray (16). One-pot synthesis involving combined chemocatalysis and biocatalysis is a very real option across a wider range of reaction conditions now with the emergence of extremozymes, which often demonstrate greater stability, specificity, and tolerance to a wide range of environmental conditions. The biocatalysis cycle proposed by Schmid et al. (17) shows the number of steps required, once a target reaction has been identified, before a candidate extremozyme may be used in an industrial process (Figure 1).

The industrial chemicals sector is difficult to penetrate for conventional enzyme technology because its manufacturing processes are well established and generally require harsh reaction conditions. Extremophile-derived biocatalysts could break in because of their increased robustness and selectivity throughout a wide range of environments. If new biocatalytic processes are to be accepted, they will have to bring significant improvements, be integrated with chemical and downstream processes, and perform at high concentrations of substrate and product. Enzyme-based processes are already prominent in the manufacture of fine chemicals and pharmaceuticals because such products often include novel and/or chiral compounds, for which biotechnology is often preferable over conventional chemical synthesis. Several commodity chemicals (acrylamide, polylactic acid, 1,3-propanediol, and nicotinamide) have been produced using enzyme technology, demonstrating that biocatalytic technology can be scaled up.

In one manufacturing process, the conversion of nitriles by difficult chemical reactions into a range of useful products (such as acrylamide or ibuprofen) has been successfully demonstrated by a number of companies. Nitriles are also intermediates in the chemical synthesis of nylon polymers and nicotinic acid. Conversion of nitriles to amides and acids requires harsh reaction conditions such as high temperatures and extreme acidity/alkalinity, which can affect the reaction characteristics of sensitive functional molecular groups such as ester linkages. So the potential application of a thermostable nitrilase to convert nitrile groups more selectively in moderate conditions would prove beneficial to industry. A thermostable nitrilase displaying a much greater half-life than the corresponding mesophilic enzyme has been successfully identified from an extremophile, a discovery that has various implications for high-temperature biocatalysis technology and application in the large-scale production of compounds such as acrylamide, nicotinic acid, and ibuprofen (19). Further current examples of extremophile-derived enzymes used in industrial processing are shown in Table 2.

Bioprocess Issues
The viability of extremozyme processes depends on the availability
of a given enzyme in sufficient quantities. Problems instantly arise if the enzyme is isolated directly from a wild strain — or if it is to be used for in situ reactions involving whole cells. The conditions required for extremophile growth include temperatures routinely above 80 °C, an anaerobic environment, at extreme pH or in a medium containing up to 5 M NaCl. Many hyperthermophilic extremophiles are found in superheated water under pressure, and associated barophily is a common trait exhibited by such organisms and their enzymes. Recent work has demonstrated that high pressures (>250 atm) help stabilize enzymes from hyperthermophiles. In fact, halophilic enzymes may be particularly well suited to biotransformations in organic media because the low water activity of high-salt environments makes such enzymes highly functional in that type of environment.

Extreme conditions are not economically feasible within standard industrial fermentation and downstream-processing facilities. Most studies on extremophiles have focused on thermophilic enzymes, so most cited work on large-scale cultivation methods is related to culturing thermophilic and hyperthermophilic microorganisms. For example, three different approaches to the growth of these organisms have been compared, showing that hyperthermophiles and thermoacidophiles could be specifically grown to a high cell density for increased product yields (20). The extremophiles occur at low cell densities in their natural environment, unlike many industrially relevant microbes such as Escherichia coli, so the most successful approach to obtaining significant biomass concentrations has involved membrane bioreactors and dialysed or perfusion fermentation (21). Because most extremophiles were only recently discovered, growth media generally have not yet been optimized or defined for their use in commercial activities. Studies indicate that the majority examined to date require complex media for reasonable cell culturability (22).

Cultivation of extremophiles is associated with many further difficulties: For instance, high-temperature fermentation processes using corrosive media require specially designed equipment and intense maintenance. Of course, gas solubility decreases with temperature increase, and the instability of substrates and reagents are other factors of concern. Under growth conditions at temperatures up to 113 °C, many nutrients may react abiotically, with amino acids decomposed and carbohydrates undergoing Maillard reactions. That could reduce nutrient availability in the growth medium and therefore cause a steady decline in cell number.

Extremophiles generally demonstrate low specific growth rates and significant product inhibition even at very low concentrations, further problems that limit their use as efficient enzyme or metabolite producers. Applications of extremophiles as biotransformation agents are not restricted to situations in which a specific extremozyme has been identified and isolated. There are several reports on in vivo biotransformation or biodegradation processes mediated by growing cultures of extremophiles. It was initially felt that application of extremophilic biomass, enzymes, and biomolecules depended strictly on increasing yields to facilitate further characterization in the laboratory — and to provide essential information for subsequent scale-up. The diverse methods of circumventing the problems discussed above range from optimization of media composition to the use of specialized reactor technology, e.g., gas-lift fermentors or membrane bioreactors (22, 23).

Mode of operation — such as fed-batch, cell-recycling or continuous cultivation techniques — also makes a difference (24–26). Part 2 of this article will look at some innovative bioreactors and fermentation processes, examples of successful industrial implementation of extremozymes, and some new biomedical products coming from extremophiles.

Figure 1: The proposed biocatalysis development cycle (FROM REFERENCES 17 AND 18)
Table 2: Current applications of certain enzymes obtained from extremophiles

<table>
<thead>
<tr>
<th>Classification</th>
<th>Growth Characteristics</th>
<th>Enzymes</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermophiles</td>
<td>&gt;80 °C</td>
<td>Oxidases</td>
<td>Detergents; hydrolysis in food and drink, brewing, and baking</td>
</tr>
<tr>
<td>Hyper-</td>
<td>60–80 °C</td>
<td>Proteases</td>
<td>Starch, cellulose, chitin, and pectin processing; textiles</td>
</tr>
<tr>
<td>thermophiles</td>
<td></td>
<td>Lipases</td>
<td>Chitin modification for food and health products, paper bleaching</td>
</tr>
<tr>
<td>Pychrophiles</td>
<td>&lt;15 °C</td>
<td>Proteases, lipases</td>
<td>Detergents, stereospecific biotransformations</td>
</tr>
<tr>
<td>Halophiles</td>
<td>2–5 M NaCl</td>
<td>Proteases</td>
<td>Molecular biology (PCR)</td>
</tr>
<tr>
<td>Alkaliphiles</td>
<td>pH &gt;9</td>
<td>Dehydrogenases</td>
<td>Diagnostics</td>
</tr>
<tr>
<td>Acidophiles</td>
<td>pH &lt;9</td>
<td>(Gluc-o-)amylases</td>
<td>Detergents, dairy food application, cosmetics</td>
</tr>
</tbody>
</table>

**References**


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