BIOTECHNOLOGY OF ARCHAEA

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Contents

Summary

Archaea are unique microorganisms that are adapted to survive in ecological niches such as high temperatures, extremes of pH, high salt concentrations and high pressure. They produce novel organic compounds and stable biocatalysts that function under extreme conditions comparable to those prevailing in various industrial processes. Some of the enzymes from Archaea have already been purified and their genes successfully cloned in mesophilic hosts. Enzymes such as amylases, pullulanases, cyclodextrin glycosyltransferases, cellulases, xylanases, chitinases, proteases, alcohol dehydrogenase, esterases, and DNA-modifying enzymes are of potential use in various biotechnological processes including in the food, chemical and pharmaceutical industries.

1. Introduction

The industrial application of biocatalysts began in 1915 with the introduction of the first detergent enzyme by Dr. Röhm. Since that time enzymes have found wider application in various industrial processes and production (see *Enzyme Production*). The most important fields of enzyme application are nutrition, pharmaceuticals, diagnostics, detergents, textile and leather industries. There are more than 3000 enzymes known to date that catalyze different biochemical reactions among the estimated total of 7000; only 100 enzymes are being used industrially. The world market for industrial enzymes, which includes enzymes for research and diagnostic purposes, is estimated to be around

1 billion US dollars. The products derived from these enzymes are estimated to represent a value of more than 100 billion US dollars. For various industrial applications, there is a great demand for enzymes of high specificity and stability. Extreme environments provide a unique resource of microorganisms and novel biocatalysts. Microorganisms that live under extreme conditions are defined as extremophiles. Many parts of the world are considered extreme—geothermal environments, polar regions, acid and alkaline springs, and cold pressurized depths of the oceans. As conditions become increasingly demanding, extreme environments become exclusively populated by microorganisms belonging to Archaeal domains. It is very likely that higher organisms are unable to survive under extreme conditions due to their cellular complexity and compartmentation. The realization that extreme environments harbor different kinds of prokaryote lineage has resulted in a complete reassessment of our concept of microbial evolution and has given considerable impetus to extremophile research.

It is worth mentioning that modern biotechnology, which provides a whole new repertoire of methods and products, still tries to mimic nature, thus demanding continuous efforts in the isolation and characterization of novel microorganisms. In this review, we will focus on biocatalysts that are produced by Archaea living under extreme conditions. These unusual microorganisms have unique biochemical features which can be exploited for use in the biotechnological industries. The extreme molecular stability of their enzymes, membranes and the synthesis of unique organic compounds and polymers make extremophilic Archaea interesting candidates for industrial applications.

1.1. Archaea Living at the Boiling Point of Water

Microorganisms that are adapted to grow optimally at high temperatures (60-108 °C) have been isolated from high-temperature terrestrial and marine habitats. The most common biotopes are volcanically and geothermal heated hydrothermal vent systems such as solfataric fields, neutral hot springs, and submarine hot vents. Submarine hydrothermal systems are situated in shallow and abyssal depth. They consist of hot fumaroles, springs, sediments, and deep-sea vents with temperatures up to 400 °C ("black smokers"). Shallow marine hydrothermal systems are located at the beaches of Vulcano, Naples, and Ischia (Italy), Sao Miguel (Azores) and Djibouti (Africa). Examples of deep-sea hydrothermal systems are the Guaymas Basin (depth 1500 m) and the East Pacific Rise (depth 2500 m), both off the coast of Mexico, the Mid-Atlantic Ridge (depth 3700 m), and the Okinawa Trough (depth 1400 m). Because of their ability to convert volcanic gases and sulfur compounds at high temperatures, hyperthermophilic communities living in such hydrothermal vents are expected to play an important role in marine ecological, geochemical and volcanic processes. Shallow as well as deep-sea hydrothermal systems harbor members of various Archaeal genera including *Pyrococcus, Pyrodictium, Igneococcus, Thermococcus*, *Methanococcus*, and *Archaeoglobus*. So far, members of the genus *Methanopyrus* have been found only at greater depths, whereas *Aquifex* has been isolated exclusively from shallow hydrothermal vents. Recently, interesting biotopes of extreme and hyperthermophiles were discovered in deep, geothermally heated oil reservoirs around 3500 m below the bed of the North Sea and in the permafrost soil of North Alaska.

Microorganisms capable of growing optimally at temperatures between 50 and 60 °C are designated as moderate thermophiles. Most of these microorganisms belong to the many different taxonomic groups of eu- and prokaryotic microorganisms such as protozoa, fungi, algae, streptomycetes, and cyanobacteria, which comprise mainly mesophilic species. It can be assumed that moderate thermophiles, which are closely related phylogenetically to mesophilic organisms, may be secondarily adapted to life in hot environments.

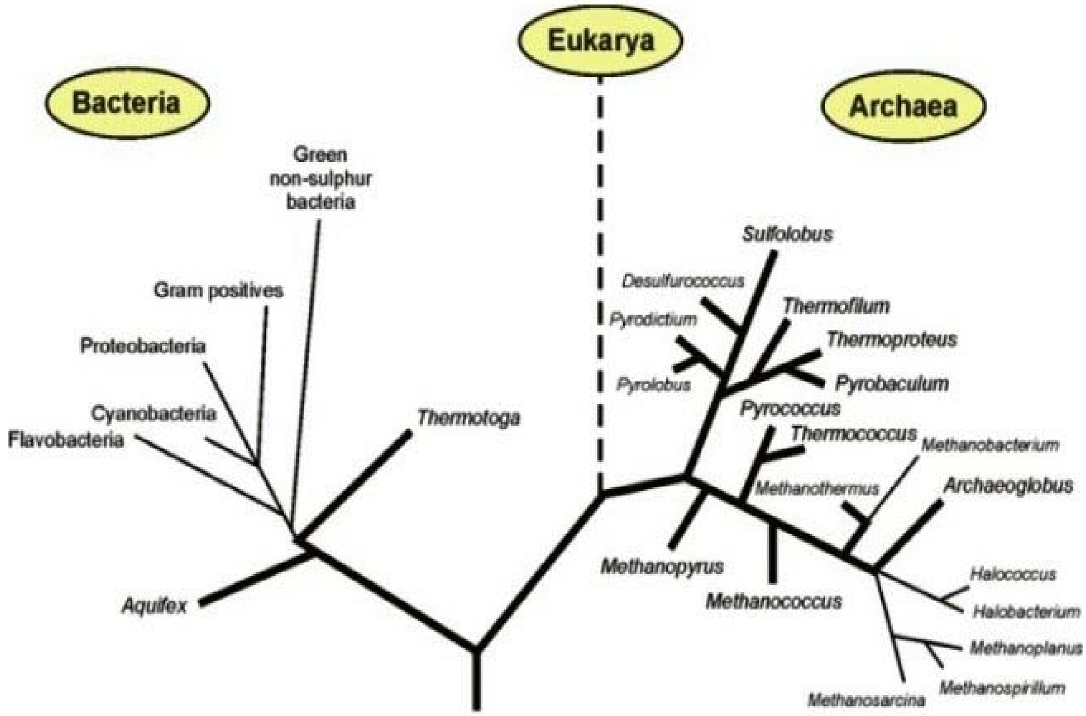

Figure 1. The phylogenetic tree of organisms. Hyperthermophilic species are highlighted by bold branches. Branching order and branch lengths are based upon 16/18S rDNA sequence comparisons [modified version of Blöchl et al. 1995]

Most of the hyperthermophiles on the other hand, grow optimally between 80 and 108 °C. It is of note, as shown in Figure 1, that the majority of the hyperthermophiles isolated to date belong to the Archaeal domain of life, and no eukaryotic organism has been found that can grow at the boiling point of water. A few strains of bacteria belonging to the genera *Aquifex* and *Thermotoga* are able to grow at 90 °C. A 16S rDNA-based universal phylogenetic tree shows a tripartite division of the living world consisting of the domains Bacteria, Archaea and Eukarya. The Archaea consists of two major kingdoms: the Crenarchaeota (some genera are *Sulfolobus*, *Picrophilus*, *Pyrodictium*, *Pyrolobus*, *Pyrobaculum*, and *Thermoproteus*) and the Euryarchaeota which include hyperthermophiles (some genera are *Thermococcus* and *Pyrococcus*), methanogenes (for example, *Methanococcus*, *Methanobacterium*, and *Methanosarcina*), sulfate-reducers (*Archaeoglobus*) and halophiles (including genera such as *Halobacterium* and *Halococcus*). Short phylogenetic branches indicate a rather slow clock of evolution. Deep branching points are evidence for early separation of the two groups. The separation of the bacteria from the Eukarya-Archaea lineage is the deepest and earliest branching point known so far. Hyperthermophiles are represented among all the deepest and shortest lineages, including the genera *Aquifex* and *Thermotoga* within the bacteria and *Pyrodictium*, *Pyrobaculum*, *Thermoproteus*, *Desulfurococcus*, *Sulfolobus*, *Methanopyrus*, *Pyrococcus*, *Thermococcus*, *Methanococcu,s* and *Archaeoglobus* within the Archaea (Figure 1).

The relative abundance of Archaea and Bacteria in high-temperature environments was, until recently, mainly studied by cultivation-based techniques. Because of the frequent isolation of Archaea from these habitats, it was assumed that Archaea dominate the high-temperature biotope. Recently, the application of molecular-biological methods revealed a quite different picture. Slot-blot hybridizations of rRNA utilizing oligonucleotide probes targeting the 16S rRNA of Archaea and Bacteria revealed that Bacteria seem to be the major population of the microbial community along a thermal gradient at a shallow submarine hydrothermal vent near Milos Island. Bacteria made up at least 78 percent (mean 95 percent) of the prokaryotic rRNA. Along the steepest temperature gradient, the proportion of Archaeal rRNA increased. Nevertheless, even in the hottest sediment layer Archaeal rRNA made up only around 12 percent of the prokaryotic rRNA. These results suggest that Archaea may generally be of lower abundance in hot environments than could be assumed from cultivation-based experiments. However, the factors that allow Bacteria to dominate in high temperature habitats, that were once believed to be the realm of Archaea, remain unknown. Most of these microorganisms that can be found in low-salinity and submarine environments are strict anaerobes. Terrestrial solfataric fields as they can be found in Italy or Iceland, harbor members of the genera *Pyrobaculum*, *Thermoproteus*, *Thermofilum*, *Desulfurococcus*, and *Methanothermus*. *Pyrobaculum islandicum* and *Thermoproteus tenax* are able to grow chemolithoautotrophically, gaining energy by anaerobic reduction of S^0 by H_2. In contrast to these strictly anaerobic microorganisms, *Pyrobaculum aerophilum* and *Aeropyrum pernix* are able to use oxygen as a final electron acceptor (see *Cell Thermodynamics and Energy Metabolism*). *Methanothermus fervidus*, on the other hand, is highly sensitive towards oxygen and can only survive in low redox environments at temperatures between 65 and 97 °C. Some microorganisms from marine environments such as members of the genera *Archaeoglobus*, *Methanococcus* and *Methanopyrus* are able to grow chemolithoautotrophically, gaining energy by the reduction of SO_4^{2-} by H_2 (*Archaeoglobus lithothrophicus* and *A. fulgidus*) or by the reduction of CO_2 by H_2 (*Methanococcus jannaschii*, *Methanopyrus kandleri*). Other members of the hyperthermophilic genera, *Staphylothermus*, *Pyrococcus*, *Thermococcus*, and *Pyrodictium* are adapted to marine environments (Sodium Chloride (NaCl) concentration: about 30 gL^{-1}). Most of them gain energy by fermentation of polysaccharides, peptides, amino acids, and sugars. Consequently, such thermophilic microorganisms have been found to be producers of polymer degrading enzymes of industrial relevance.

1.2. Archaea Growing at Extremes of pH

Solfataric fields are the most important biotopes of microorgansms that prefer to live under both thermophilic and acidic conditions. Solfataric soils consist of two different layers which can be easily distinguished by their characteristic colors: the upper, aerobic layer has an ochre color due to the presence of ferric iron. The layer below, which is anaerobic, appears rather blackish-blue owing to the presence of ferrous iron. According to the chemical parameters of the two layers, different kinds of microorganisms can be isolated from these habitats. Thermophilic acidophiles belonging to the genera *Sulfolobus*, *Acidianus*, *Thermoplasma,* and *Picrophilus*, with growth optima between 60 and 90 °C and pH 0.7 to 5.0, are commonly found in the aerobic upper layer, whereas slightly acidophilic or neutrophilic anaerobes such as *Thermoproteus tenax* or *Methanothermus fervidus* can be isolated from the lower layer. Species of *Thermoplasma* (growth optima: pH 2 and 60 °C) have been found in hot springs, solfataras, and coal refuse piles. Their closest known phylogenetic relatives, also found in solfataras, are species of the genus *Picrophilus*, which are so far the most extreme acidophiles with growth close to pH 0. *Picrophilus oshimae* and *P. torridus* are both aerobic, heterotrophic Archaea that grow optimally at 60 °C and pH 0.7 and utilize various polymers such as starch and proteins as sole carbon source.

Members of the genus *Sulfolobus* are strict aerobes growing either autotrophically, heterotrophically or facultatively heterotrophically. During autotrophic growth, S^0, S^{2-} and H_2 are oxidized to sulfuric acid or water as end products. *Sulfolobus metallicus* and *S. brierley* are able to grow by oxidation of sulfidic ores. A dense biofilm of these microorganisms is responsible for the microbial ore leaching process, in which heavy metal ions such as Fe^{2+}, Zn^{2+} and Cu^{2+} are solubilized. During heterotrophic growth, a range of sugars and proteinaceous substrates are utilized.

In contrast, the alkaliphiles that grow at high pH values are widely distributed throughout the world. They have been found in carbonate-rich springs and alkaline soils, where the pH can be around 10.0 or even higher, although the internal pH is maintained around 8.0. The two Archaeal thermoalkaliphiles identified to date are *Thermococcus alcaliphilus* and *Thermococcus acidoaminivorans*, both growing at 85 °C and pH 9.0. The main industrial application of alkali-active enzymes is in the detergent industry, where they account for approximately 30 percent of the total world-wide enzyme production. Alkaline enzymes have been also used in the hide-dehairing process, where dehairing is carried out at pH values between 8.0 and 10.0. (Table 1b)

(a) Microbial life at the boiling point of water

Microorganism	Optimal growth (°C)
Extreme thermophiles (60 – 80 °C)	
Sulfolobus acidocaldarius	65
Hyperthermophiles (80 – 110 °C)	
Archeoglobus fulgidus	83
Methanopyrus kandleri	88
Sulfolobus sulfataricus	88
Thermococcus aggregans	88
Pyrobaculum islandicum	100
Pyrococcus furiosus	100
Pyrodictium occultum	105
Pyrolobus fumarii	106

(b) Archaea growing at extreme pH

Acidophilic ^	**Optimal growth**	
	(°C)	**pH**
Picrophilus oshimae	60	0.7
Picrophilus torridus	60	0.9
Thermoplasma acidophilum	60	2.0
Sulfolobus acidocaldarius	75	2.5
Acidianus infernus	75	2.0
Alkaliphilic		
Thermococcus alcaliphilus	85	9.0
Thermococcus acidoaminivorans	85	9.0

(c) Halophilic Archaea

Halophilic microorganism	**Salinity (M NaCl) required for growth**		
	Minimum	**Optimum**	**Maximum**
Haloferax vulcanii	1.0	1.5	3.0
Methanohalobium evestigatum			4.3

Table 1.Some representatives of Archae living at extreme conditions. (a) Microbial life at the boiling point; (b) Archae growing at extreme pH; and (c) Halophilic Archae.

1.3. Halophilic Microorganisms

The halophiles comprise bacteria and Archaea that grow optimally at NaCl concentrations above those of seawater (>0.6 M NaCl). In general, halophilic microorganisms are classified as moderate halophiles if they can grow at salt concentrations between 0.4 and 3.5 M NaCl, and as extreme halophiles if they require NaCl concentrations above 2 M for growth. Halophiles have been mainly isolated from saline lakes, such as the Great Salt Lake in Utah (salinity >2.6 M) and from evaporitic lagoons and coastal salterns with NaCl concentrations between 1 and 2.6 M. Saline soils are less well explored. Bulk salinity measurements of 1.7-3.4 M NaCl have been reported for saltern soils. Saline soils constitute less stable biotopes than hypersaline waters since they are subjected to periodic significant dilution during rainy periods. It can be assumed that microbial survival under these oscillating conditions would be even more difficult. There is no doubt that almost all hypersaline habitats harbour significant populations of specifically adapted microorganisms. However, it remains unclear what substrates for growth might be available in these biotopes. Hypersaline lakes often contain up to 1 g L^{-1} of dissolved organic carbon. In many of these lakes, primary producers such as cyanobacteria, anoxygenic phototrophic bacteria, and algae may be the main source of organic compounds.

It has been speculated that organic compatible solutes, produced by many of the phototrophs as a means of counterbalancing osmotic stress, contribute significantly to the input of carbon sources. It is noteworthy that, despite the typically large surface-to-volume ratios, hypersaline environments are low in dissolved oxygen (<2 mg/L) and might be essentially anaerobic.

In one study of aerobic heterotrophs in a marine saltern, it was shown that bacterial halophiles were predominant up to 2 M NaCl. Above this concentration, Archaeal halophiles become predominant, almost to the exclusion of Bacteria. Halophilic primary producers mainly belong to the cyanobacteria and anoxygenic phototrophic sulfurbacteria. The former often thrive in eutrophic salterns forming large floating mats. The latter group, on the other hand, grows either in anaerobic sediments or in the water column where they are responsible for the characteristic red color of high-salinity habitats. The range of heterotrophic bacteria comprises proteobacteria, actinomycetes, and Gram-positive rods and cocci. Fermentative anaerobes as well as sulphur oxidizers, sulphate reducers, and nitrate reducers are also present and give rise to the assumption that all kinds of metabolic features may be found in high-salinity environments (Table 1c). Halophilic Bacteria do not belong to one homogeneous group but rather fall into many bacterial taxa in which the capability to grow at high salt concentrations is a secondary adaptation.

Most halobacteria require 1.5 M NaCl in order to grow and to retain the structural integrity of the cell. Halobacteria can be distinguished from halophilic bacteria by their Archaeal characteristics, in particular the presence of ether-linked lipids. Most halobacteria are colored red or orange due to the presence of carotenoids, but some species are colorless, and those with gas vesicles form opaque, white or pink colonies. A purple hue may be seen in halobacteria that form the bacteriorhodopsin-containing purple membrane. Halobacteria are the most halophilic organisms known so far and form the dominant microbial population when hypersaline waters approach saturation. Interestingly, the reddening caused by halobacterial blooms has an impact on the evaporation rates in salterns. It is known that the carotenoid pigments of halobacteria trap solar radiation, thus increasing the ambient temperature and evaporation rates.

The singular physiology of halophilic microorganisms that have to cope with a 4 M ion concentration inside and outside of cells has theoretically evolved enzymes that might be capable of working under conditions of low water activity which could be imposed by substances other than salts, for example solvents, and would thus be of interest. The reality is that, despite a range of potentially exploitable properties, halophiles have not yet had much of an impact on commerce. However, there is still considerable interest, as evidenced by about 20 percent of all patent applications for extremophiles to date being concerned with halophiles in one form or another. Interestingly, halophilic and marine halotolerant bacteria produce and/or accumulate organic osmolytes (compatible solutes) for osmotic equilibrium. These metabolically compatible hygroscopic compounds not only protect living cells in a low-water environment but also exhibit an enzyme-stabilizing effect *in vitro* against a variety of stress factors such as heating, freezing, urea, and other denaturants. The ectoine-type osmolytes (2-methyl-1,4,5,6-tetrahydropyrimidine derivatives) represent the most abundant class of stabilizing solutes, typical for aerobic chemoheterotrophic halophilic and/or halotolerant bacteria. The extrinsic stabilization effect of ectoines and compatible solutes is most likely based on the solvent-modulating properties of these compounds. The osmolytes already referred to have considerable potential as effective stabilizers of the hydration shell of proteins, and hence could be highly efficient stress protectants and stabilizers of biomolecules, suitable for vaccines where refrigeration might be not available, or industrial enzymes functioning under extreme conditions. A number of enzymes have been shown to be totally protected against heating and freeze-thaw cycles in the presence of a range of compatible solutes. However, there was a significant variation in the degree of freeze and heat protection, dependent on compatible solute and enzyme under investigation. Recently, it has been shown that a number of hyperthermophilic Archaea are also able to produce a variety of compatible solutes that have been found to be effective in enzyme stabilization.

2. Cultivation of Extremophilic Archaea

Extremophilic Archaea are receiving increasing interest because they provide a unique source of biocatalysts and cell components. However, until recently only low cell yields could be obtained, making application studies very difficult. This is mainly due to the difficulties related to producing and purifying large quantities of biocatalysts and cell components. Moreover, extremophilic microorganisms require special equipment to reach and maintain their optimal cultivation temperatures and extreme pH. There are two different approaches to overcoming this problem: recombinant DNA technique for increasing enzyme production in mesophilic hosts; or innovative bioreactor design to improve biomass yield. Because the accumulation of toxic compounds is thought to be responsible for low biomass yields, dialysis fermentations with a number of extremophiles have been performed for effective removal of low-molecular-mass components from fermentation broth. Applying dialysis membrane reactors, a dramatic increase in cell yields was achieved. The cultivation of the hyperthermophilic Archaeon *Pyrococcus furiosus* (growth at 90 °C), the thermoacidophile *Sulfolobus shibatae* (growth at 75 °C, pH 3.5) and the halophile *Marinococcus* M52 (growth at 35 °C, pH 7.5 and 10 percent NaCl) resulted in cell yields of 2.6 g L^{-1}, 114 g L^{-1} and 132 g L^{-1} (cell dry weight), respectively. For *P. furiosus* the optimum stirrer speed was 1800 rpm and neither hydrogen nor the metabolic products were found to be responsible for the comparatively low cell yield. In the case of *S. shibatae*, the choice of an appropriate membrane was crucial. Cuprophan membrane, which consists of regenerated cellulose and polyamide membrane, was damaged after 2 days of operation, probably due to enzyme action. A porous, non-transparent polyethersulphonic membrane was found to be stable. The fermentation processes can be scaled-up from 3 L over 30 L up to 300 L (see Figure 2). The reactor (total volume 4 liters) contains inner (1 liter) and outer (3 liters) chambers, which are separated by a membrane (Cuprofan or polyamide). The inhibitory compounds that are produced by the cell growing in the inner chamber are diffused into the outer chamber This pilot scale plant demonstrates the possibilities for transferring the fermentation performance into industrial standards. In recent experiments it was shown that even the results of the dialysis reactor can be reproduced in the 30 L reactor using external dialysis modules.

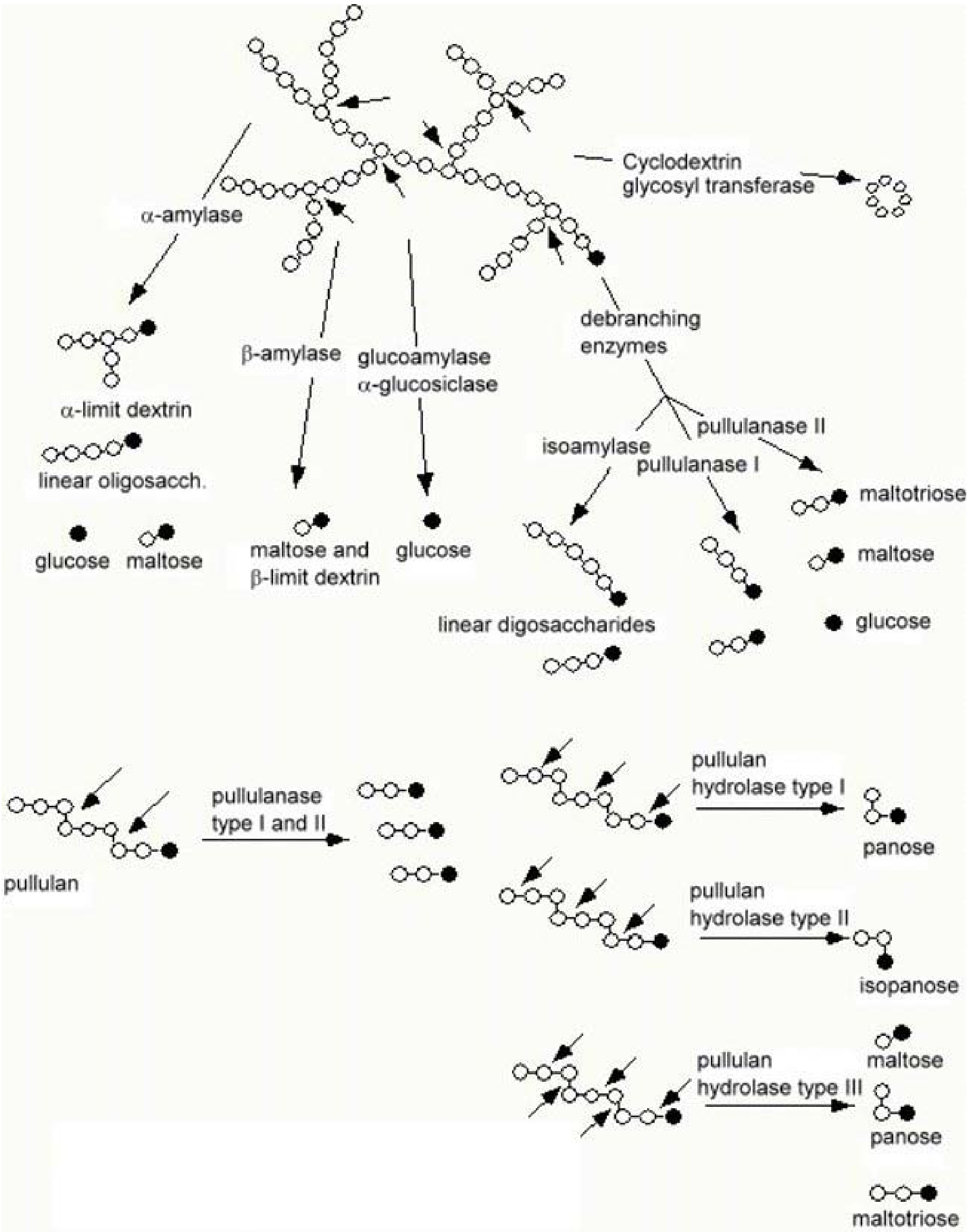

Figure 2. Schematic representation of a dialysis membrane fermentor used for the cultivation of extreme Archaea. [The figure was kindly provided by D.Koster, C.Fuchs and H.Märkl of the Technical University of Hamburg-Harburg, Germany]

In addition to the dialysis fermentation technique, the application of a novel microfiltration (MF) bioreactor, based on a microfiltration hollow-fiber module located inside the traditional fermentation vessel, has been designed for improving both biomass yield and enzyme productivity. Using the cultivation of the thermoacidophilic archeon *S. solfataricus* as a model, a biomass of 35 g L^{-1} dry weight was obtained, almost 20-fold higher than results obtained in batch fermentors.

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Bibliography

Antranikian G. (1992). Microbial degradation of starch. *Microbial degradation of natural products*, Vol. 2 (ed. G. Winkelman*)*, pp. 28–50. Weilheim (Germany): VCH Verlaggsgesellschaft mbH . [This review describes the different mechanisms of starch hydrolysis from microorganism.]

Bertoldo C. and Antranikian G (2000). Amylolytic enzymes from hyperthermophiles. *Methods of Enzymology*, Vol. 330, pp. 269–289. Academic Press. [This review describes some methods for the screening of pullulanase and amylase activity in agar plates and in the acrylamide gels.]

Bragger J. M., Daniel R M, Coolbear T. and Morgan H.W. (1989). Very stable enzyme from extremely thermophilic archaebacteria and eubacteria. *Applied Microbiological Biotechno*logy **31**: 556–561. [This review describes some examples of stable enzymes from archaea and bacteria.]

Breed R. S., Murray E.G.D. and Smith N.R. (1957). *Bergey´s Manual of Determinative Bacteriology*, 7th edition. Baltimore: Williams and Wilkins Company. [This is the most widespread manual of systematic bacteriology.]

Brock T. D. (1986). *Thermophiles. General, molecular and applied microbiology*. New York: John Wiley and Sons. [A book describing the properties of thermophilic microorganisms in the earlier times after their discovery.]

Ciaramella M., Cannio R., Moracci M., Pisani F.M. and Rossi M. (1995). Molecular biology of extremophiles. *World Journal of Microbiology and Biotechnology* **11**: 71–84. [A comprehensive review about the molecular biology of extremophiles.]

Cohen J. (1994). “Long PCR” leaps into larger DNA sequences. *Science* **263**: 1564–1565 [A scientific article about PCR.]

Cohen–Kupiec R. and Chet I. (1998). The molecular biology of chitin digestion. *Current Opinion in Biotechnology* **9**: 270–277. [A review about the molecular biology of chitin digestion.]

Coolbear T., Daniel R.M. and Morgan H.W. (1992). The enzymes from extreme thermophiles: bacterial sources, thermostabilities and industrial relevance. *Advances in Biochemical Engineering Biotechno*logy **45:**57–98. [An article about the industrial application of thermoactive enzymes.]

Costa J. C. Duarte and Williams R.A.D. (eds.), *Microbiology of Extreme Environments and Its Potential for Biotechnology*. London and New York: Elsevier Applied Science. [A complete book about the potential application of enzymes from extremophilic microorganisms.]

Cowan D. (1996). Industrial enzyme technology. *Tibtech.* **14**:177–178. [A mini review on industrial enzyme technology.]

Crabb W.D. and Mitchinson C. (1997). Enzymes involved in the processing of starch to sugars. *Tibtech* **15**: 349–352 [A good review describing industrial starch processing in detail: liquefaction, saccharification, and isomerization.]

De Costa M.S., Santos H. and Galinski E.A. (1998). An overview of the role and diversity of compatible solutes in Bacteria and Archaea. *Advances in Biochemical Engineering Biotechnology* **61**: 117–153. [A complete review describing the role of the compatible solutes in archaea and bacteria.]

DeRosa M. and Gambacorta A. (1994). Archaeal lipids. *Chemical methods in prokaryotic systematics* (eds. M. Goodfellow and A.G. O´Donnell), pp. 199–264. New York: John Wiley. [A complete review describing the structure and the role of the lipids in archaea.]

Erlich H.A., Gelfand D.H. and Saiki R.K. (1988). Specific DNA amplification–product review. *Nature* **331**: 461–462. [A scientific article about the amplification of DNA by PCR.]

Feller G., Narinx E., Arpigny J.L., Aittaleb M., Baise E., Genicot S. and Gerday C. (1996). Enzymes from extremophilic mocroorganisms. *FEMS Microbiological Rev*iew **18**: 189–202. [A review describing various examples of extremophilic enzymes and their potential application.]

Forterre P., Bergerat A. and Lopez–Garcia P. (1996). The unique DNA topology and DNA topoisomerases of hyperthermophilic Archaea. *FEMS Microbiological Rev*iew **18**: 237–248. [A review describing the properties of topoisomerase from hyperthermophilic archaea.]

Galinski E. A. and Tindall B.J. (1992). Biotechnological prospects for halophiles and halotolerant microorganisms. *Molecular Biology and Biotechnology of Extremophiles* (eds. R. A. Herbert and R. J. Sharp), pp. 76–114. Glasgow: Blackie. [One of the best reviews about the characteristics of the halophilic microorganisms and their potential application in industry.]

Gooday G.W. (1994). Physiology of microbial degradation of chitin and chitosan. *Biochemistry of microbial degradation* (ed. C. Ratledge), pp. 279–312. Dordrecht: Kluwer. [Describes the mechanism of the hydrolysis of chitin]

Grant W.D., Gemmell R.T. and McGenity T.J. (1998). Halophiles. *Halophiles (eds.* K. Horikoshi and W.D. Grant), pp. 93–133. New York: Wiley Liss. [Another review about the characteristics of the halophilic microorganisms and their potential application in industry.]

Grayling R. A., Sandman K. and Reeve N.J. (1994). Archaeal DNA binding proteins and chromosome structure. *Molecular Biology of Archaea* (eds. F. Pfeifer, P. Palm and K. H. Schleifer), pp. 82–90. Stuttgart, Jena, New York: Gustav Fischer Verlag. [The histones of archaea and their properties of DNA binding are described.]

Gyllensten U.B. (1989). PCR and DNA sequencing. *Biotechniques* **7**: 700–708. [The use of PCR and the description of DNA sequencing.]

Horikoshi K. and Grant W.D. (eds.) (1998). *Extremophiles—Microbial Life in Extreme Environments*. New York: Wiley–Liss. [This represents the most modern and best book about the different extremophilic microorganisms: from thermophiles to halophiles, from alkaliphiles to acidophiles; every kind of extremophilic microorganisms as well their characteristics, metabolism, physiology and enzymes are fully described.]

Keohavong P. and Thilly W.G. (1989). Fidelity of PCR polymerases in DNA amplification. *Proceedings of the National Academy of Sciences USA* **86**: 9253–9257. [The fidelity of PCR is studied.]

Kletzin A. (1992). Molecular characterization of a DNA ligase gene of the extremely thermophilic Archaeon *Desulfurolobus ambivalens* shows close phylogenetic relationship to eukaryotic ligases. *Nucleic Acids Research* **20**: 5389–96. [One of the few archaeal ligase is studied here in detail.]

Krahe M., Antranikian G. and Märkl H. (1996). Fermentation of extremophilic microorganisms. *FEMS Microbiological Review* **18**: 271–285. [In this review results concerning the fermentation of the archaeon *Pyrococcus furiosus* in dialysis fermentor show how is possible to obtain large biomass of themophilic archaea.]

Kristjansson J. K. and Hreggvidsson G.O. (1995). Ecology and habitats of extremophiles. *World Journal of Microbiology and Biotechnology* **11**:17–25. [A good review about the habitats of extremophilic microorganisms: from cold adaptation to the sulphataric vents.]

Ladenstein R. and Antranikian G. (1998). Proteins from hyperthermophiles: stability and enzymatic catalysis close to the boiling point of water. *Advances in Biochemical Engineering and Biotechnology* **61:** 37–85. [A good review describing how thermostable enzymes gain their adaptation at high temperature.]

Landegren U., Kaiser R., Sanders J. and L. Hood (1988). A ligase–mediated gene detection technique. *Science* **241**: 1077–80.

Leuschner C. and Antranikian G. (1995). Heat–stable enzymes from extremely thermophilic and hyperthermophilic microorganisms. *World Journal of Microbiology and Biotechnology* **11**: 95–114. [An overview providing some examples of enzymes from thermophilic microorgansims.]

M.R., Hagemans M.L.D., v. Gorcom R.F.M., Hessing J.G.M., v.d. Hondel C.A.M.J.J. and v. Rotterdam C. (1992). Xylanases and their application in bakery. *Progress in Biotechnology*, Vol. 7 (eds. J. Visser et al), pp. 349–360. Amsterdam: Elsevier. [An overview providing some microbial xylanases and their application.]

Niehaus F., Bertoldo C., Kähler M. and Antranikian G. (1999). Extremophiles as a source of novel enzymes for industrial applications. *Applications in Microbiological Biotechnology* **51**:711–729. [One of the most complete reviews about enzymes from thermophilic microorgansims with potential biotechnology application.]

Norris P. R., and Johnson D.B. (1998). Acidophilic microorganisms. *Extremophiles – Microbial Life in Extreme Environments* (eds. K. Horikoshi and W. D. Grant), pp. 133–153. New York: Wiley–Liss. [One of the most complete reviews about acidophilic microorganisms and their enzymes.]

Perler F.B., Kumar S. and Kong H. (1996). Thermostable DNA polymerases. *Advances in Protein Chemistry* **48**: 377–435. [One of the most complete review about DNA polymerases and their revolutionary aspects.]

Ruttersmith L.D., Daniel R.M. and Simpson H.D. (1992). Cellulolytic and hemicellulolytic enzymes functional above 100 °C. *Ann NY Acad Sci* **672:** 137–141. [A scientific article regarding the cellulolytic enzymes and their applications.]

Schiraldi C., Marulli F., Di Lernia I., Martino A. and De Rosa M. (1999). A microfiltration bioreactor to achieve high cell density in *Sulfolobus solfataricus* fermentation. *Extremophiles* **3:**199–204. [A scientific article describing a new technique in order to obtain large biomass of archaea.]

Schönheit P. and Schäfer T. (1995). Metabolism of hyperthermophiles. *World Journal of Microbiology and Biotechnology* **11:** 26–57. [A review that provides different examples of the metabolism of hyperthermophiles, which differs from the metabolism of mesophilic microorganisms.]

Stetter K. O. (1999). Extremophiles and their adaptation to hot environments. *FEBS Lett*ers **452**: 22–25. [K.O. Stetter is the most famous pioneer of archaea. The review describes the properties of the thermophilic microoganisms, their characteristics (optimum temperature, pH growth, duplication time, etc.) and their adaptation at high temperature.]

Stetter K. O. (1996). Hyperthermophilic procaryotes. *FEMS Microbiology Rev*iew **18:** 149–158.

Stetter K. O. and Völkl P. (1995). Isolation, taxonomy and phylogeny of hyperthermophilic microorganisms. *World Journal of Microbiology and Biotechnology* **11**: 9–16.

Stetter, K.O., Fiala, G., Huber, G., Huber, R. and Segerer, A. (1990). Hyperthermophilic microorganisms. *FEMS Microbiology Review* **75:** 117–124.

Sunna A., Moracci M., Rossi M. and Antranikian G. (1996). Glycosyl hydrolases from hyperthermophiles. *Extremophiles* **1:** 2–13. [A review about the starch hydrolizing enzyme from extreme thermophilic microorganisms.]

Takagi M., Nishioka M., Kakihara H., Kitabayashi M., Inoue H, Kawakami B., Oka M. and Imanaka T. (1997). Characterization of DNA polymerase from *Pyrococcus* sp. strain KOD1 and its application to PCR. *Applied Environmental Microbiology* **63:** 4504–4510. [A scientific article about one of the most thermoactive DNA polymerases from a hyperthermophilic microorganism.]

Ventosa A. and Bieto J.J. (1995). Biotechnological applications and potentialities of halophilic microorganisms. *World Journal of Microbiology and Biotechnology* **11**:85–94. [A review about the biotechnological aspects of microorganisms living at high salt concentrations.]

Viikari L., Kantelinen A., Sundquist J. and Linko M. (1994). Xylanases in bleaching. From an idea to industry. *FEMS Microbiology Letters* **13**: 335–350. [The xylanases and their application in bleaching.]

Woese C. R., Achenbach L., Rouviere P. and Mandelco L. (1991). Archaeal phylogeny: reexamination of the phylogenetic position in light of certain composition–induced artifacts. *System. Appl. Microbiol.* **14**:364–371. [A review which provides the new tree of life according to the discovery of archaea and their evolutionary implications.]

Biographical Sketches

Dr. Costanzo Bertoldo has a degree in Biology Summa cum Laude from the University of Naples (1988); his thesis was on the presence of antibiotic substances in Briophytae. After postgraduate studies at the Botanical Institute of the University of Naples, he became a Fellow at the Institute of Proteins and Enzymology, Arco Felice, Italy, and then at the Department of Biochemistry of Macromolecules, Faculty of Medicine, II University of Naples, where his PhD (1996) was on "5´–Methylthioadenosine phosphorylase: a model enzyme for the study of molecular basis of thermophilicity and thermostability of proteins". He currently holds a post–doctoral position in Biotechnology at the Technische Universität Hamburg Harburg. His research has included studies on: antibiotic substances from vegetable organisms; purification and characterization of enzymes from thermophilic microorganisms; the effect of microwave radiation on the stability of enzymes; cloning and sequence of genes from microorganisms; and cloning and sequencing of a thermophilic protease and pullulanase.

Professor G.Antranikian gained a BSc and MSc in Biology at the American University of Beirut in the 1970s, followed by study at the Goethe–Institut in Freiburg and a PhD (1980) in Microbiology at the Georg–August–University Göttingen, Institute for Microbiology, where he subsequently carried out post–doctoral research and teaching. In 1989, he became Professor of Microbiology at the Technical University Hamburg–Harburg and, subsequently, leader of the Technical Microbiology team there. He was coordinator of the 39–partner European Network Project "Biotechnology of Extremophiles" from 1993 to 1996, and then coordinator of the 58–partner European Network Project "Extremophiles as Cell Factories" from 1997 to 1999. Since April 2000, he has coordinated the 30–partner Network Project "Biocatalysis", supported by the German Federal Environmental Foundation