Introduction

Archaea are prokaryotic micro-organisms which are able to live only in extreme environments. Based on state-of-the-art those micro-organisms can be divided into three groups: methanogens, thermocacidophiles and halophiles [1]. Those micro-organisms are considered to be pioneer organisms, inhabiting most adverse environments, such as ocean bottoms, hot springs, acid waters or salt lakes [2]. Archaea can survive in conditions similar to those in archaic era, at the dawn on Earth. Those micro-organisms are relatively poorly investigated, among others due to complexity of their culturing and difficult observation. At first archaea were considered evolutionary older than bacteria proper (eubacteria), but in the course of development of molecular techniques in late 1970s, the following division was postulated: archaea, eubacteria and eukaryotes [3].

Until 1970s, when only classical microbiological techniques were used, it was considered that archaea occur exclusively in extreme environments. However, with progress in molecular methodology (i.a. amplifications of genes 16S rRNA in the polymerase chain reaction (PCR)) [4], it was discovered that multiple species of archaea are capable of growth and multiplication also in seas, lakes and soils they inhabit [5]. Such research allowed discovery of over 20000 sequences of genes 16S rRNA originating from cells of archaea inhabiting variety of environments [6–8]. Thus, better understanding of genetics, biochemistry and physiology of archaea will help raising new research questions, and better understanding of archaea evolution in such extreme conditions is a great challenge faced by biotechnology. Recent research activities focus on investigation of archaea structure and adaptability allowing them to inhabit multiple environments. Their specific properties make them a valuable resource with potential application in new sectors of industry. They can help reduce the quantity of waste, energy and material consumption, thus making the technology more environmentally-friendly. Extremozymes are used in the synthesis of up to now unprofitable pharmaceutics, polymer semi-products, pesticides or salt lakes [2]. Archaea can survive in conditions similar to those in archaic era, at the dawn on Earth. Those micro-organisms are relatively poorly investigated, among others due to complexity of their culturing and difficult observation. At first archaea were considered evolutionary older than bacteria proper (eubacteria), but in the course of development of molecular techniques in late 1970s, the following division was postulated: archaea, eubacteria and eukaryotes [3].

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Purpose of this publication was analysis of literature on application of archaea in various areas of biotechnology. Particular attention was paid to extremozymes, enzymes produced by archaea cells, and their potential use in pharmaceutical, cosmetic, food and chemical industry and environmental biotechnology.

Microbiological characteristics of archaea

What differentiates archaea from other cells of bacteria proper are, among others, different structure of cell wall and partially monolayer structure of cell membrane [11]. In addition, cell membrane in eubacteria and eukaryotes contains mostly glycerol esters and fats, whereas in archaea it contains glycerol ethers and fats. Such differences may affect adaptability to live under extreme ambient conditions [12]. Organisms of archaea, similar to bacteria, have flagella, which differ however in composition and how they are developed: archaea, eubacteria and eukaryotes [3].

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is availability of those compounds [9]. This difficulty is solved by cloning of genes coding extremozymes using mesophiles (of medium temperatures). Purpose of such a treatment is to achieve overproduction of enzyme and modification of its properties for specific commercial application. Micro-organism species *Escherichia coli*, *Bacillus subtilis* and yeast were used successfully as mesophilic hosts, where the expression of genes coding extremozymes was achieved [4]. Techniques of genetic engineering are very valuable and useful as they allow production of new biocatalysts that may improve the efficiency of bioprocesses and support innovative biotransformations. Using techniques of molecular biology can overcome the difficulty with availability of enzymes and help design new industry process specific biocatalysts.

**Enzymes of thermophilic archaea**

As of present thermophilic enzymes are the best investigated group of extremozymes [24]. Extremozymes resistant to high temperatures are used not only as ideal models which facilitate understanding of protein stability but also they demonstrate significant bioengineering potential. Results of structural and biophysical examinations of those enzymes evidenced significant differences as compared with their mesophilic counterparts, such as increased number of salt bridges, increased hydrophobic properties and increased number of hydrogen bonds [25]. Due to unique properties of those biocatalysts their bioengineering potential is significant, ranging from biodegradation of toxic compounds to production of medicinal substances. The advantage of using thermophilic extremozymes is in the fact that running bioengineering processes at higher temperatures is often beneficial from production efficiency viewpoint. For example, in chemical reactions in the presence of organic solvents, decrease in viscosity and increase in diffusion coefficient that occur at higher temperatures is accompanied by increase in reaction rate. Such actions are suitable for various processes, including those with hydrophobic compounds demonstrating low solubility at lower temperatures. Increase in temperature can also improve the availability of substance in biodegradation processes [25]. In addition, in reactions held at higher temperature undesired by-products, often resulting from contamination of reaction environment, are less likely to occur. In addition to improved high temperature stability, thermophilic enzymes produced by archaea cells demonstrate significant resistance under high pressure and in the presence of detergents and solvents, thus further extending the range of industrial applications. Thermophilic extremozymes are particularly desirable in exothermic processes in which energy is generated. In the event of processes held at lower temperatures the need to heat the system might be a challenge. Hence the use of thermophiles when the enzymatic reaction is compatible with the process that progresses at high temperature. Thermostable enzymes are isolated from thermophiles which optimum temperature of growth exceeds 60°C, of from hyperthermophiles which optimum growth is observed above 90°C. Thermophiles occur in the domain of bacteria and archaea, whereas the vast majority of hyperthermophiles occur in the *Archaea* domain. *Pyrococcus* and *Thermococcus* genus receive the most attention among all thermophilic archaea species [25]. Division of enzymes produced by cells of extremophilic archaea, including their applications, is presented in Table 1.

The first group of enzymes discussed in this publication are amylases. Those enzymes can be used in the process of starch hydrolysis. Starch is a complex polymer which processing requires amylases and also many other enzymes, which shall be presented further in the document. Endoenzyme, such as α-1,4 amylase, randomly hydrolize α-1,4 bonds in starch polymer, resulting in the polysaccharide releasing glucose particles accompanied by linear and branching oligosaccharides. α-Amylases produced by *Pyrococcus woesei* and *Pyrococcus furiosus* species of archaea demonstrate optimum activity at temp. 100°C [25]. In addition, it is possible to produce recombinated α-amylase. To that end genes coding the production of this enzyme in *Pyrococcus furiosus* are used, and then they are cloned and expressed in mesophilic host. Examples of such micro-organisms are *Escherichia coli* or *Bacillus subtilis*. Thus obtained α-amylase demonstrates maximum activity at pH 5.6 and temp. 93°C. After two-hour incubation at temp. 120°C it maintains 24% activity [10]. *Thermococcus profundus* DT5432 is also capable of producing thermostable α-amylases. One of them (S α-amylase), following purification, demonstrates optimum activity at 80°C, and its stability at increased temperature is based on the presence of calcium ions. Fully of partially purified thermophilic α-amylase can be also produced from cells of the strain *Sulfolobus solfataricus* [25]. The main application of thermophilic amylases is production of glucose and fructose used in production of sweeteners [24].

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Micro-organisms</th>
<th>Temperature of activity</th>
<th>Application</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-Amylases</td>
<td><em>Pyrococcus woesei</em></td>
<td>Topt. = 100°C</td>
<td>Sugar industry – acquisition of glucose and fructose</td>
<td>[10, 25–26]</td>
</tr>
<tr>
<td></td>
<td><em>Pyrococcus furiosus</em></td>
<td>Topt. = 80°C</td>
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<td></td>
<td><em>Thermococcus profundus</em> DT5432</td>
<td>Topt. = 90 – 105°C</td>
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<tr>
<td>Pullulanases</td>
<td><em>Pyrococcus woesei</em></td>
<td>Topt. = 90 – 105°C</td>
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<tr>
<td></td>
<td><em>Pyrococcus furiosus</em></td>
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<td></td>
<td><em>Thermococcus TSY</em></td>
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<td><em>Thermococcus TV</em></td>
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<tr>
<td></td>
<td><em>Desulfurococcus mucosus</em></td>
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<td></td>
<td><em>Staphylothermus marinus</em></td>
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<tr>
<td>Glucoamylases</td>
<td><em>Thermoplasma acidiphilum</em></td>
<td>Topt. = 90°C</td>
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<td></td>
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<tr>
<td></td>
<td><em>Pyrodictium occultum</em></td>
<td></td>
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<tr>
<td></td>
<td><em>Pyrococcus furiosus</em></td>
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<tr>
<td></td>
<td><em>Picrophilus torridus</em></td>
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<td></td>
<td><em>Picrophilus oshimae</em></td>
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<td></td>
<td><em>Sulfolobus solfataricus</em></td>
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</table>

Pullulanases are the next group of enzymes used in hydrolysis of starch. The majority of thermophilic and hyperthermophilic pullulanases produced by archaea cells belongs to class II, capable of hydrolysis of both α-1,4 bond and α-1,6 glycoside bond in branching polymers. Class I, splitting only α-1,6 bonds, is rarely seen in archaea.
Those enzymes produce the mixture of maltotriose, maltose and glucose [10]. Their optimum temperature of activity is in the range 90°C to 105°C [24]. Genes coding production of this enzyme, originating from Pyrococcus woesei and Pyrococcus furiosis can be used in production of recombinant pullulanase. Cloning and expression of genes responsible for production of this enzyme occurs in cells of Escherichia coli. Following purification, the enzyme demonstrates optimum activity at temperature 100°C, and pH 6.0 [25]. Class II pullulanases is also produced by Thermococcus TSY and Thermococcus TY and such species as Desulfurococcus mucosus and Staphylococcus marinus [25]. This group of enzymes is very interesting as it can be used in optimization of starch bioconversion process – by triggering saccharification process at lower pH and higher temperature.

Based on the analysis of literature on such enzymes as α-amylases, pullulanases, β-amylases, glucoamylases and α-glucosidases, it can be stated that their contribution to starch processing is significant. Using the same extremozymes allows conversion of this polysaccharide to more valuable products, such as dextrose, fructose, glucose or trehalose [24]. For example, purified glucoamylases obtained from cells of species Thermoplasma acidophilum, Picrophilus torridus and Picrophilus shibatae demonstrate optimum activity at pH 2.0 and temp. 90°C [26].

In all starch conversion processes high temperature is required for fluidisation of this polysaccharide and making it available for enzymatic hydrolysis. Contribution of thermophilic amylase, pullulanase and α-glucosidase optimizes industrial process of starch processing, reducing production costs of sugar syrup [25]. In addition, the use of amylolytic enzymes can lead to production of other valuable products that are innovative starch-based materials, such as linear dextrine, used as fat substitutes, fragrance stabilisers or probiotics [24].

Tests on β-galactosidase obtained from cells of Pyrococcus woesei [27] gave new examples of application of thermophilic enzymes. Results of those tests demonstrated that the enzyme is suitable for production of milk with reduced lactose and whey content. β-Galactosidase remains active in the broad range of pH (4.3 to 6.6) and demonstrates high thermal stability, which suggests that it can be used in processing of milk products at temperatures that with longer operation of continuous reactor result in decrease in activity of mesophilic enzymes. Recombined β-galactosidase can be obtained by cloning and expression of genes coding this enzyme from cells of Pyrococcus woesei – using cells of Escherichia coli as a host [10].

Synthesis of trehalose is a very interesting application of extremozymes. Trehalose is a non-reducing disaccharide used as stabiliser in a number of industry sectors. Trehalose can also be used to stabilize vaccines stabilizer when stored at room temperature [9]. Due to high cost of trehalose extraction from baking yeasts new possibilities of biosynthesizing this disaccharide were sought. In recent years enzymes synthesizing trehalose were discovered in cells of archaean species Sulfolobus shibatae. Discovery of archaean capable of synthesizing trehalose may revolutionize industrial production of this disaccharide and expand its range of application [25].

Considering the fact that cellulose is the most important biopolymer on Earth, extremozymes produced by archaean cells, that are capable of its decomposition, are worth mentioning [24]. Cellulose can comprise as many as 14000 glucose residues combined with β-1,4-glycoside bonds. This polysaccharide can be hydrolyzed to glucose by simultaneous reaction of endoglucanase, exoglucanase and β-glucosidase. β-Glucosidase was detected, among others, in cells of Pyrococcus furiosis. This enzyme is active and stable even at 103°C [6]. In addition, a number of β-glucosidases was identified in cells of the genus Sulfolobales sp. [26] The mixture of those enzymes (produced by cells of Pyrococcus furiosis and Sulfolobales sp.) can be used in the process of enzymatic synthesis (at ultra-high temperatures), leading to production of new oligosaccharides from lactose [9]. Thermostable cellulases are in high demand as they are more and more commonly used in various industries sectors. Those enzymes are used in production of alcohol, process optimisation in fruit industry, and they allow efficient extraction of dyestuff from fruits. In addition, cellulases are used as an additive to detergents, to lighten colours and soften fabrics [6].

Another group of extremozymes with high biotechnological potential are enzymes hydrolyzing xylans. Xylans are a heterogeneous group of polysaccharides (heteropolysaccharides), where major components of the chain are xylopirose residues combined with β-1,4-glycoside bonds [25]. Xylans are the major component of hemicellulose, and they are the component of cell walls in plants [24]. Thermostable xylans are more and more thoroughly investigated in their application offers multiple advantages. Those enzymes can decompose event hard and decomposable hemicellulose. They are used in enzymatic bleaching of paper – attractive alternative to current bleaching methods utilizing chlorine [25]. Thermostable xylanases used in the process of biological bleaching of pulp and paper disintegrate cell wall, facilitating removal of lignin and making the technology more environmentally friendly [28]. In archaean domain xylans are produced, among others, by Pyrococcus abyssi and Thermococcus zillii AN1. Optimum temperatures of such xylanases activity are 110°C and 100°C, respectively [9, 29].

Due to their application in organic synthesis, thermostable esterases are very intensively studied extremozymes. They contribute to multiple chemical processes, such as transesterification, stereospecific hydrolysis and other organic biosynthesis reactions of organically purse compounds [25]. Esterases produced by cells of Aeropyrum pernix, Pyrococcus calidifontis and Sulfolobus tokodaii are thermostable and thermally active. In addition they remain active in the mixture of buffer solution with organic solvents (such as acetonitrile or dimethyl sulfoxide). Thanks to those properties they can be used in numerous reactions of chemical synthesis [24]. Esterase secreted by Pyrococcus furiosus is the most thermostable of all enzymes in this class (its optimum activity is at 100°C). Esterase originating from Archaeoglobus fulgidus and Sulfolobus shibatae are used in dairy industry. Lipases also play an important role in chemical processes. Esterases and lipases are biocatalysts that are commonly used in chemical processes, since reactions catalysed by those enzymes lead to production of optically pure compounds [18].

Another group of enzymes discussed in this publication are chitinases. Chitin is a non-soluble linear biopolymer comprised of N-acetylglucosamine residues combined with β-1,4-glycoside bonds. It is produced in large quantities in marine environments [24]. The first identified enzyme originating from archaea and capable of decomposing chitin was N-acetylgulcosaminidase produced by hyperthermophilic species Thermococcus chitonophagus [30]. Chitinases were also identified in Thermococcus kodakarenosis KOD1 [31, 32] and Pyrococcus furiosus [33]. Thermostable chitinase produced by Thermococcus kodakarenosis KOD1 in addition maintains activity in the presence of detergents and organic solvents, thanks to which it can be used in industry as a catalyst [32].

Yet another group with multiple potential applications in chemical industry are alcohol dehydrogenates. They constitute a very important group of biocatalysts due to their significant contribution to synthesis of optically active alcohols [24]. In addition, alcohol dehydrogenates can catalyse the reaction of alcohol oxidation to ketones and reverse reaction [25]. In the archaean domain there are only a few species that are capable of producing those enzymes. Hyperthermophilic enzymes were identified, among others, in cells of Thermococcus stetteri and Sulfolobus solfataricus. Unlike dehydrogenates produced by cells of eukaryotes and bacteria, the enzyme produced by Thermococcus stetteri is thermostable and free from metal ions [25].
Enzymes produced by psychrophilic archaea

In context of biotechnological processes enzymes produced by psychrophilic archaea (Tab. 3) are as interesting as thermophilic enzymes. Optimum temperature of psychrophilic micro-organisms development are temperatures below 15°C [34]. Natural habitats in which temperature does not exceed 5°C can be the abundant source of those extremozymes. Archaea living in low temperatures (i.e. in oceans) are capable of producing multiple psychrophilic enzymes which due to reduced demand for energy are suitable for a number of biotechnological applications [10]. Proteins of psychrophilic micro-organisms feature a number of specific properties, setting them apart from their mesophilic counterparts. Those properties are, among others, smaller number of disulphide bonds and salt bridges, smaller number of hydrogen bonds, decrease in hydrophobic property of proteins [10]. All those modifications make protein structure more flexible which is the reason for higher catalytic activity in cold environments.

Table 3

<table>
<thead>
<tr>
<th>Extremophiles</th>
<th>Enzyme/group</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychrophiles</td>
<td>Amylases, β-glucanase</td>
<td>Detergents, paper industry, bioremediation</td>
</tr>
<tr>
<td></td>
<td>Pectinases</td>
<td>Maturing cheeses, milk products, processing of fruit juices</td>
</tr>
<tr>
<td></td>
<td>Methanogens</td>
<td>Production of methane, low temperature sewage purification</td>
</tr>
</tbody>
</table>

Psychrophilic enzymes have numerous applications in various sectors of industry. Addition of psychrophilic extremozymes (such as amylases or β-glucanases) to detergents offers the possibility of washing in cold water. Such enzymes are further used in paper industry, where they support manipulation of pulp, or in bioremediation processes [10]. Food industry can use pectinases active at low temperatures in such areas as processing of fruit juices, dairy products or maturing of cheeses [25].

Due to large variety of methanogenic archaea in cold environments and of their properties, such micro-organisms as Methanogenium frigidum, Methanococcales burtonii and Halorubrum lacustrefundi are used in production of methane and in the process of low temperature sewage purification [35].

**Table 4**

<table>
<thead>
<tr>
<th>Extremophiles</th>
<th>Environment</th>
<th>Enzyme</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaliphiles</td>
<td>High pH</td>
<td>Proteases</td>
<td>Production of cleaning agents, food industry</td>
</tr>
<tr>
<td></td>
<td></td>
<td>α-Amylase</td>
<td>Beer industry, paper industry, food and baking industry</td>
</tr>
<tr>
<td>Acidophiles</td>
<td>Low pH</td>
<td>-</td>
<td>Coal desulphurization, biomedetallurgy</td>
</tr>
</tbody>
</table>

Considering tolerance to salt, metals and organic pollutants, halophilic archaea can be used in purification of salty industrial sewage [36]. Immobilized nitrates and nitrites, isolated from haloarchaea, can be used to remove nitrogen compounds from high salinity sewage. They are also a good example of enzymes used in construction of potentiometric ion-selective electrodes used to detect nitrates and nitrites. Recent research evidenced that a few species, among others Halobacterium salinarum, demonstrate the capability of detoxicating inorganic forms of arsenic. Such compounds are transformed into volatile forms or converted to less toxic forms [36].

Proteases identified in cells of species Natronomonas pharaonis demonstrate optimum of activity at temperature 61°C and pH 10.0. Thanks to such properties they can be used as detergent additives [25].

Another group of extremozymes produced by cells of haloarchaea and broadly used throughout industry are α-amylases. Due to stability and activity of those enzymes at high concentrations of salt, they are used in production of starch syrup (containing glucose and fructose). Further uses of α-amylases include improvement of flour properties in baking industry, production of modified starch for paper industry and beer industry [37].

Acidophilic micro-organisms in archaea domain inhabiting low pH environments also play an important role in industry, as they can be used in desulphurization of coal and retrieving precious metals.
process. Table 5 presents comparison of thermophilic and mesophilic bioleaching of chalcopyrite developed for industrial application in 1995. Attempts to bioleach this mineral using *Acidithiobacillus ferrooxidans* bacteria were not completely satisfactory. Bioleaching at higher temperatures is therefore far more beneficial, and application of archaea improves process efficiency. Table 5 presents comparison of thermophilic and mesophilic bioleaching process.

**Table 5**

<table>
<thead>
<tr>
<th>Bioleaching duration, days</th>
<th>Bioleached copper at adequate temperature conditions, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>22°C</td>
</tr>
<tr>
<td>30</td>
<td>10.9</td>
</tr>
<tr>
<td>60</td>
<td>12.7</td>
</tr>
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</table>

The analysis of data in Table 5 demonstrates that the use of archaea in bioleaching of copper from chalcopyrite is more efficient than those micro-organisms remain active and stable activity at high temperatures, which allows much higher production of metal. Due to a number of studies on acidophilic archaea oxidizing iron, they can be now used in industrial processes. A BioCop™ process was developed by BHP Billiton, which successfully utilizes acidophilic archaea oxidizing iron in bioleaching of chalcopyrite concentrate in aerated stirred reactors. Micro-organisms used in this process belong, among others to genus *Sulfobolus*, *Metallophaga* and *Acidianus*. The process is held at temp. 78°C and pH 1.5, with 98% copper recuperation rate. Based on results of thermophilic bioleaching efficiency test of covellite, it was demonstrated that it is also higher as compared with mesophilic process [38].

**Enzymes produced by piesophilic archaea**

In aquatic environments, at large depths, where average water pressure is 38MPa, there live piesophilic micro-organisms. Among them there are also numerous strains of archaea [25]. Those ecosystems are very rich source of many precious compounds. Piesophilic enzymes (e.g. hydrogenases and α-glycosidase) can be isolated, among others, from *Methanococcus jannaschii* and *Methanococcus igneus*. Extremozymes produced by cells of those micro-organisms demonstrate stability at high pressures and high temperatures, and this is why they are used in various sectors of industry (e.g. food industry). Despite high potential of those piesophilic enzymes, there are difficulties preventing their use on industrial scale. The main difficulty is complex culturing of those micro-organisms [25].

**Summary**

Based on the analysis of literature it can be stated that archaea, their extremozymes and other metabolites, can be used in many different sectors of industry. In addition, they constitute an alternative to multiple industrial processes, since their use allows improving efficiency, lowering production costs, optimising processes, and many more benefits. Thermophilic enzymes are the most interesting ones, although there are more and more processes employing extremozymes from other extreme environments. The examples are acidophiles, alkaliphiles, psychrophiles or piesophiles. Application of archaea is not limited to chemical industry – they can be used as well, among others, in environmental biotechnology, pharmaceutical industry, cosmetic industry, food industry and dairy industry. Many enzymes demonstrate also potential of application in processes that with the progress in science shall be possible to implement on an industrial scale. Due to properties of extremozymes (activity in a broad range of temperatures and pH, stability in organic solvents and enantioselectivity) biocatalysts made of extremophilic micro-organisms can be playing a critical role in multiple industrial processes. Their significance shall increase in a number of areas, such as production of food additives, detergents, chemical and pharmaceutical products. Slow incorporation of metabolites and enzymes secreted by archaea cells to industrial application is mostly due to difficulties related to culturing of archaea. However, with the aid of genetic engineering, those difficulties can be overcome, allowing large scale production of those valuable biomaterials.

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**Literature**

Sze zagrożenie zdrowotne w perspektywie najbliższych lat. Dlatego śla on choroby cywilizacyjne, takie jak nowotwory złośliwe, choroby dla państwa kierunki badań naukowych i prac rozwojowych. Określena podstawie Krajowego Programu Badań, wyznaczający strategiczne ty poświęcone chorobom cywilizacyjnym.

220 mln PLN Centrum przeznaczy dla polskich naukowców na projekty strategicznego programu badań naukowych i prac rozwojowych


