ORNL/CP-99087

CONF-980812--

Extremozymes for Bioprocessing

Non-Technical Summary

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To be presented as a poster at the
1998 Fall Meeting
of the American Chemical Society
Boston, August 23-27, 1998

[1]Managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract DE-AC05-96OR22464.

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For many years, people have been seeking to employ enzymes, proteins that act as biocatalysts, as environmentally friendly replacements for many currently used industrial processes, as well as for the production of fuels and chemicals from biomass sources such as waste paper and agricultural residues. Current applications of enzymes include enzyme-assisted bleaching of wood pulp, preparation of textiles, design of biosensors, enzyme diagnostic kits, and bioremediation of toxic metals and chlorinated chemicals. However, until fairly recently, most of these enzymes came from mesophilic sources--organisms that grow at moderate temperatures (20-50°C) and pH 5 to 8. Frequently, these enzymes are not robust enough to preform well in industrial processes, have little tolerance for organic solvents or toxic chemicals, lose activity rapidly during catalysis, and cannot be used at high temperatures and alkaline or acidic conditions. All of these factors increase the cost and lower the effectiveness of industrial use of enzymes. But there are microorganisms that are known to grow under extreme conditions such as high temperature (thermophiles), low temperature (psychrophiles), acidic pH (acidophiles), and alkaline pH (alkalinophiles). The enzymes, extremozymes, from such microorganisms are active under the extreme conditions of temperature and pH at which the extremophiles grow. Use of extremozymes extends the potential temperature range for efficient enzymatic reactions to between 4°C and 100°C. Many extremozymes exhibit increased stability and faster reaction rates compared to their mesophilic counterparts. For these reasons, microorganisms that grow under extreme conditions, extremophiles, are being intensively investigated for enzyme activities that can be used in commercial applications.

For the past two years, our multidisciplinary group at Oak Ridge National Laboratory (ORNL) has been studying extremozymes. These extremozymes are obtained from extremophiles of the

ORNL subsurface culture collections, the *Methanococcus jannaschii* genome clones available from the American Type Culture Collection, and biotechnology companies. Our research studies include the identification, isolation, purification, characterization, and structural studies of extremozyme versions of enzymes potentially useful for bioprocessing and energy production.

Production of fuels from biomass sources requires the hydrolysis of the biomass to its component sugars, glucose and xylose, by enzymes that act on specific components. These enzymes include cellulase and xylanase that, respectively, hydrolyze cellulose and xylan in plant fibers, amylase to digest starch, and invertase (sucrase) to hydrolyze sucrose. Based on the discovery by one of our co-authors (J. Woodward) of a method for enzymatic production of hydrogen, the sugar glucose obtained by enzymatic digestion of biomass can be used to produce hydrogen, the cleanest fuel yet found. To increase the yield of hydrogen from sugars, enzymes of the pentose phosphate pathway, a natural metabolic pathway common to many organisms, can be used to cycle the sugar and obtain a yield of six hydrogens per glucose.

Screening of the ORNL collection of subsurface thermophiles has found that these microorganisms can digest cellulose and xylan, as well as producing sucrase, galactosidase, and amylase. These extremozymes were partially purified and characterized, then employed in hydrogen production experiments. An extremozyme version of an enzyme, transaldolase from *Methanococcus jannaschii*, from the pentose phosphate pathway, is being purified and characterized for application to hydrogen production. Subsurface psychrophiles are being investigated for useful extremozymes. Purified extremozymes will be crystallized and used for structural studies to determine why they are stable under extreme conditions.