ASM MEETING

BETTER BIOTECH VIA COMPARTMENTALIZATION

ATLANTA—Recasting the physical and chemical constraints of a microbe-based bioreactor can dramatically increase output efficiencies, according to J. Gregory Zeikus and his collaborators at the nonprofit Michigan Biotechnology Institute in Lansing. Analogously, putting enzymes in reverse micelles allows these valuable proteins to be separated efficiently from complex mixtures using organic solvents that ordinarily are inappropriate for handling such molecules, according to Kiran Kadam of Miles Laboratories (Elkhart, IN).

Such efforts are part of the recurrent theme of "better biotechnology through appropriate compartmentalization" in evidence at the 87th annual meeting of the American Society for Microbiology (ASM), held here early in March. Kadam reviewed how enzymes can be encapsulated in protective reverse micelles—spherical aggregates of surfactants in which polar groups face inward and nonpolar groups face outward—making the whole entity compatible with organic solvents. Because the interior phase is aqueous, reverse micelles provide a relatively gentle means for carrying enzymes into organic media without drastically altering the immediate physical-chemical surroundings of the protein.

Some research groups are eyeing reverse micelles as a way of conducting catalysis, particularly for steroids and cholesterols that are soluble in organic media. Kadam, however, is more interested in protein separations: "For separations, reverse micelles are something new, and definitely can be used.... It's really not a purification technique, but a recovery method that still requires further purification downstream. The most important advantage is ease of scale-up."

The focus of Zeikus' effort has been a prototype waste treatment operation, with its starting point the type of system now used widely for treating municipal wastes. The object is to treat organic materials in complex mixtures with a medley of microbes, which eventually produce methane gas. The process involves microbes in "three different feeding groups—two that work together and one working independently," he says. " 'Ecoengineering' is important [for ensuring] that the organisms are viable in the reactor; otherwise your bugs won't stay there."

Zeikus' research group has now separated the two processes by engineering a two-phase reactor system that coordinates and thereby optimizes the physical and chemical environments for both steps. In the first phase of the bioreaction, complex organic materials are metabolized aerobically into simpler organic acids. In the second phase, they are converted anaerobically into methane.

One of the chief new tricks developed by the Michigan group is to transfer organic acids from the first phase to the second by means of a solid-phase anionic resin, with bicarbonate used to discharge the organic acids in the second fluid phase while regenerating the resin. This cycling procedure controls pH, increases the rate of production in the first phase by drawing off products, and stimulates the second phase's rate by ensuring that only the proper materials reach it. "Methanogens are particularly sensitive to inhibitors," Zeikus points out, referring to the bacteria that catalyze the second phase reactions. Thus, the use of anion resins and of membranes for preventing particles and other contaminants from being transferred diminishes the likelihood of fouling between the two.

The overall effect is to "increase production of methane by 2,000 percent" compared to more conventional systems, Zeikus reports. Moreover, "pipeline quality" methane is produced because "most carbon dioxide is removed."

The design of the system leads to substantial decreases in the size of the reactor, meaning that small volumes of waste material may be efficiently treated. One goal is to make pilot-sized reactors that could be used economically for whey treatment because the current market for dried whey from dairy operations is so bad. The reactor might allow processors at least to use the material for producing energy. Also, a system for controlling the cycling of the two-phase system by microprocessors is under development.

—Jeffrey L. Fox

ENVIRONMENTAL REGULATION

EPA PANEL PONDERS 'PATHOGEN'

WASHINGTON, D.C.—In the continuing effort to implement federal biotechnology regulatory policies, the Environmental Protection Agency (EPA) recently asked a panel of outside experts to help refine its proposed definition for "pathogen." So far, however, there is little agreement—except on obvious examples at the extremes of the pathogen-nonpathogen spectrum.

What makes this definition so important is that EPA requires "pathogens" to be reviewed before they are released into the environment. As requests continue to come in to field-test genetically engineered organisms, EPA is seeking a clearer definition to determine how widely it must cast its regulatory net.

EPA proposed the following controversial definition in the June 26, 1986, *Federal Register*:

> ...a pathogen is...a virus or organism (including its viruses and plasmids, if any) that has the ability to cause disease in other living organisms (i.e., humans, animals, plants, or microorganisms). A disease is an abnormal physiological function...occurring as a consequence of the activity of proliferating microorganisms...or due to biologically active substances...produced by the [micro]organism....

Against that backdrop, EPA's panel of outside experts found themselves faced with a difficult task, one that they certainly did not resolve on the spot. The panel has, however, strongly recommended certain refinements—insisting, for example, that most microorganisms be dropped from consideration as *targets* of pathogens. Although researchers speak casually of "bugs getting sick," the notion that EPA would try to regulate genetically engineered organisms on the basis of their potential for "infecting" other microbes in the wild disturbed several panel members. The panel also favored formulating lists of pathogens to guide both EPA and those it regulates.

"The underlying issue before EPA is whether genetically engineered organisms should be handled differently from other organisms," said a participant who asked not to be identified. "If EPA were taking a strictly product-based approach, then a manipulated organism should be of less concern than a frank pathogen. But the release of these pathogens is not regulated in any rigorous way.... We haven't come to terms with this underlying issue, and the lack of understanding led people on the panel to go around and around."

—JLF