In a Ferment

An independent inventor hopes to catalyze biochemical reactions with his newly patented rotating fiber fermenter

By SARAH STEINBERG

Lord, I fall upon my knees
And pray that all my syntheses
May no longer be inferior
To those conducted by bacteria.
—Synthetic Chemist's Ode

Robert A. Clyde is trying to make this plea futile with equipment that speeds up naturally occurring reactions. A chemical engineer turned entrepreneur, Clyde has spent the seven years since he retired inventing and patenting such devices. "I keep thinking I'm going to settle down and play tennis every day," he says. "But I just keep getting these ideas."

Clyde's latest idea ventures into the realm of industrial microbiology, a mutli-billion-dollar industry that takes advantage of nature's smallest alchemists — the microbes. These microscopic organisms produce enzymes that catalyze the myriad of biochemical reactions that turn sugar water into wine, cow's hide into leather, and toxic chemicals into less toxic ones, to name but a few. Clyde hopes to facilitate some of these microbe-mediated transformations with a stack of fiber discs and a rotary motor.

To construct his basic bioreactor, or "fiber fermenter," Clyde arranged 80 silver-dollar sized polyester discs along a 4 inch long segment of a thin metal rod, separating each paper-thin disc with a small cardboard washer. The shaft of fibers fits inside a glass pipe that contains the reaction medium, or "broth."

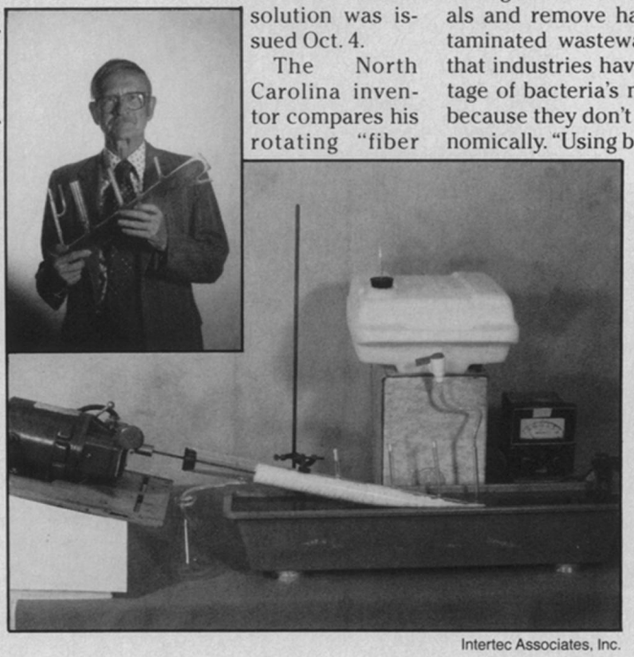
Clyde's bioreactor involves two main concepts: immobilizing bacteria and moving them relative to the medium. When floating freely, micron-sized cells are difficult to separate from solution, whereas immobilized bacteria are relatively easy to manipulate. "First, you attach bacteria to the fibers and run the reaction," says Clyde, referring to an apparatus and methodology he patented a year ago. "Then you can either drain off the product and leave the bacteria attached inside the reactor or you can take the carrier out."

Researchers have immobilized living cells on or in various substances since the early sixties. But according to Clyde, reaction broths diffuse too slowly through conventional carriers — such as ceramic, charcoal, or polymeric gels — which also occupy too much volume for the amount of bug-clinging surface area they provide. In his search for a better carrier, Clyde tried cotton string, soon followed by acrylic and polyester discs. "The bacteria go right on the fibers and stay on until you knock them off," says Clyde, who's not sure if the cells are entrapped among the fibers or held by electrostatic attraction (cells are slightly positive). "Frankly, I think it's a little bit of both."

Soon after he designed an apparatus to hold bacteria to fibers in a biological reactor, Clyde began testing the device for its practical application. One day, while "tinkering around" with a basic sugar-to-ethanol fermentation, he began rotating the shaft of bacteria-clad fibers with respect to the reaction broth. The result? "I almost fell off my lab stool," he told scientists at the meeting last month in Washington, D.C., of the American Chemical Society (ACS). "Within ten minutes, carbon dioxide began foaming right out of the fermenter." Clyde's new patent for moving the fiber substrate relative to the reaction solution was issued Oct. 4.

The North Carolina inventor compares his rotating "fiber fermenter" to the innards of a car. "If you stop while the engine's running, your car would overheat if it weren't for the fan, which reduces the fluid film of air on the radiator," Clyde says. "The same goes for bacteria." A layer of stagnant fluid surrounds the surface of every cell, preventing fresh reaction broth access to the bacteria's catalytic enzymes. By moving the broth relative to the bacteria, Clyde theorizes he's reducing that fluid film layer, thereby speeding up the reaction, sometimes as dramatically as 150 percent. "It used to take me 25 hours to get an 80 percent alcohol yield," he says. "When I rotate the fibers, it only takes 10 minutes."

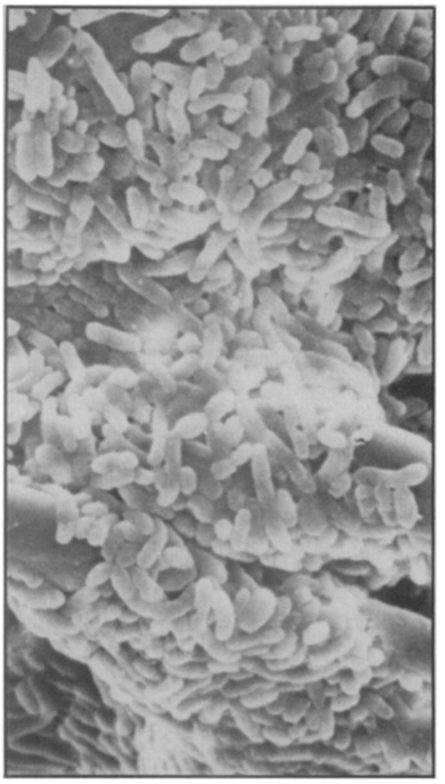
Clyde has also turned his fiber-bound bacteria through solutions of uranium, chromium, silver and other ionic metals. Because many microbes synthesize negatively charged metal-binding proteins, such as metallithionein, the organisms quickly accumulate positively charged metals. For years, scientists have used microorganisms to recover valuable minerals and remove harmful ones from contaminated wastewater. But Clyde claims that industries have not taken full advantage of bacteria's metal-leaching abilities because they don't know how to do it economically. "Using bacteria immobilized on



Courtesy of Robert Clyde

Intertec Associates, Inc.

Clyde and his bioreactor. The shaft of bacteria-clad fibers fits inside a hand-blown glass tube, which rests in a cooling bath. As a rotary motor turns the fiber shaft, sugar solution flows into the lower end of the reactor, carbon dioxide escapes through exhaust vents, and alcohol drains out the upper end.



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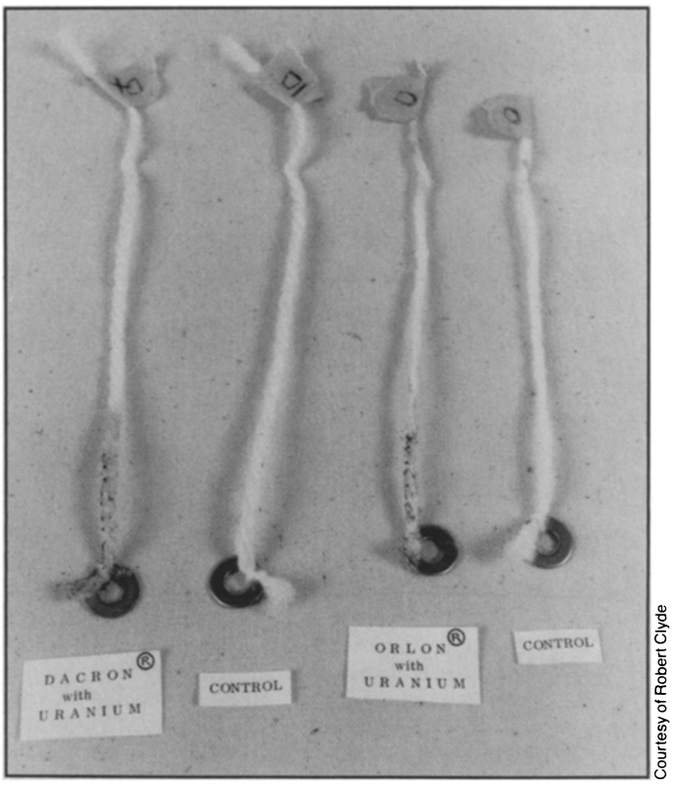
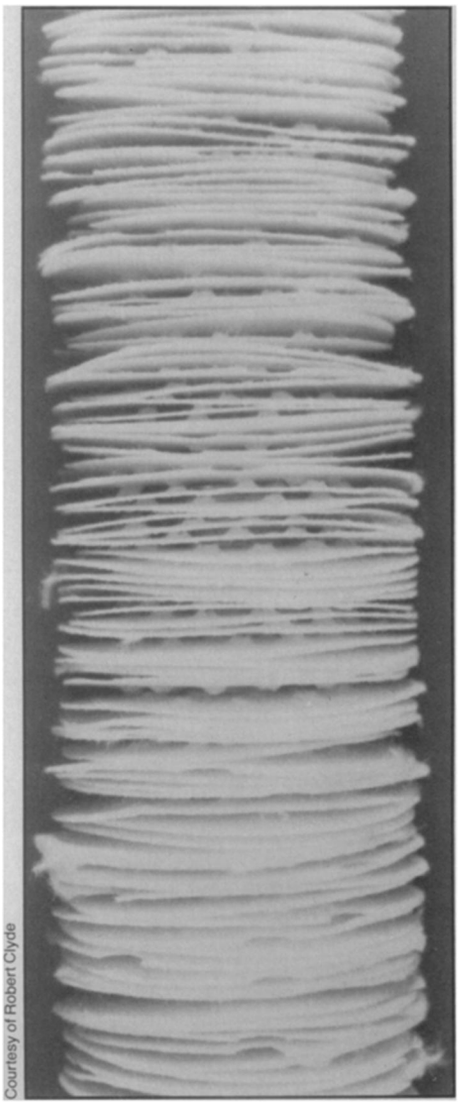


Courtesy of Robert Clyde

Left: Bacteria of the species Zymomonas Mobilis *immobilized on cotton fibers for manufacturing ethanol. Though fermenters usually use yeast to convert carbohydrates to alcohol, some researchers now believe that these bacteria catalyze the reaction more efficiently. Below: Polyester discs arranged in parallel along a rotatable shaft. According to Clyde, fiber provides more surface area per unit volume than other microbe immobilizers. Right: Uranium picked up by* Pseudomonas aeruginosa *attached to strings of Dacron (polyester) and Orlon (acrylic).*



Courtesy of Robert Clyde

fibers," he says, "they could just lift the metals right out of solution."

But bacteria can rid contaminated wastewater of more than metals — other industrial by-products, pesticides and defoliants also appear on bacteria's hit lists. Researchers have discovered — or more recently, engineered — various species that can partially degrade chlorinated hydrocarbons, including Agent Orange, dioxins and polychlorinated biphenyls (PCBs), by breaking the chemicals down into smaller, less-toxic components.

John F. Quensen, of Michigan State University in East Lansing, for example, has been working with *Bacillus megaterium*, a species that can dechlorinate naphthalene, dioxin and DDT, among other things. *B. megaterium* contains a number of plasmids, small circular pieces of extrachromosomal DNA that contain between 2 and 250 genes. By transferring some plasmids from *B. megaterium* to *B. subtilis*, Quensen has also transferred the microbes' ability to degrade naphthalene. "Our preliminary data indicate that plasmids contain genes that code for some of the degradative enzymes," says Quensen, who believes that plasmid transfer has more immediate promise than other recombinant DNA techniques for bioengineering strains to break down toxins. "If plasmids are involved," he says, "you could use them to transfer entire degradative pathways."

After Quensen presented his findings at the recent ACS meeting, Clyde offered to send the microbiologist a rotating fiber fermenter. Quensen, who has been performing his experiments using bacteria "swirling around in the broth," says that while *B. megaterium* degrades a large number of chlorine compounds, the bacteria work too slowly to be practical on a large scale. "That's why Clyde's technique is intriguing," says Quensen. "I don't see why it won't make the reaction go faster."

Other scientists, however, are not so optimistic about Clyde's work. "I don't see any serious industrial applications," says Harvey W. Blanch, a chemical engineer at the University of California at Berkeley. For a number of years, he's been immobilizing bacteria *inside* (rather than on the surface of) hollow fibers and then moving the reaction fluid past the fibers, rather than vice versa. "Clyde's technique is not really very much different from others," says Blanch.

Alexander M. Klibanov, of the Massachusetts Institute of Technology in Cambridge, agrees, adding "I don't see that it solves any problems that cannot be solved by other methods, of which there are about 150." Klibanov also complains that because Clyde fails to quantify his results in the usual scientific manner, "it's very difficult to judge the merit of what he proposes."

In the face of such criticism, Clyde remains undaunted, preferring to patent his work, rather than publish it. He's spoken at a number of recent meetings, hoping to interest scientists, both academic and industrial, in his rotating fiber fermenter, which he feels could be used on a large scale, with any type of bacteria. If other researchers are able to reaffirm Clyde's claim that such an invention can speed up bacterially mediated transformations, sometimes as much as 150 per cent, then perhaps synthetic chemists' reactions may never be superior to those conducted by bacteria. □