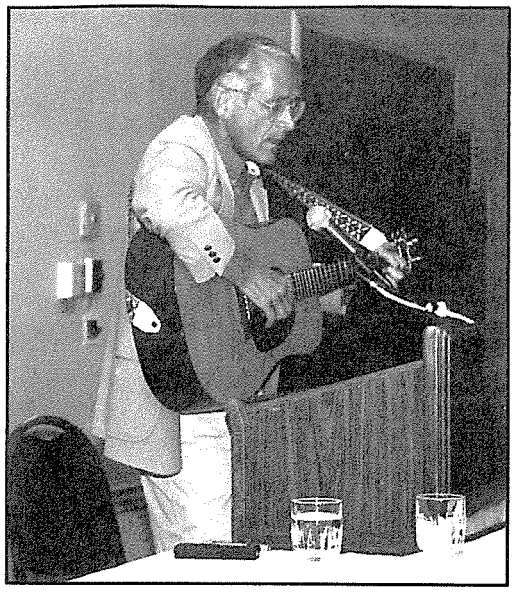
James (Skipper) Botsford graduated in 1964 with a degree in bacteriology from the University of Idaho. He went on to Oregon State University to obtain a Ph.D. in microbiology in 1968. Skipper then held a postdoctoral fellowship at the University of Illinois before coming to New Mexico State University in 1970. He has been interested in how Rhizobium meliloti, the bacterium that grows symbiotically with alfalfa and fixes atmospheric nitrogen, deals with salt stress. After talking with a colleague about a method to measure toxic chemicals, he thought of a method to do this using R. meliloti. He ran an experiment and the very first one worked. Skipper has continued to work with the assay for toxic chemicals for about three years, has received a patent, and is seeking a firm to market the method.



An Assay for Toxic Chemicals Using Microorganisms

Three years ago I learned of the Microtox test for toxic chemicals. This test uses a bioluminescent marine bacterium. The bacterium emits light. Toxic chemicals inhibit the production of light. This method of determining toxicity consists of mixing different concentrations of toxic chemicals with the bacteria and measuring the light produced with a luminometer. The Microbics Corporation will sell you a kit including a luminometer and a refrigerated water bath, a computer program to handle the data, and some cells for $25,000. The supplies are expensive. Still, this method of testing for toxic chemicals is much cheaper than any other method.

I work with the bacterium *Rhizobium meliloti*. This bacterium can reduce tetrazolium dyes readily. I thought perhaps toxic chemicals could inhibit reduction of the dye. Like bioluminescence, reduction of tetrazolium dyes is thought to be dependent on electron transport. After learning of Microtox, I ran an experiment with *R. meliloti*, a tetrazolium dye, and a couple of toxic chemicals. It worked. The bacterium could reduce the dye readily. The toxic chemicals inhibited the reduction. I could follow the changes with an inexpensive spectrophotometer. I have tested 11 bacteria, 8 tetrazolium dyes, determined the optimal incubation time, the optimal pH, and the optimal concentration for the various components. More than 160 chemicals have been tested. The test has been compared with 20 tests from the literature. It is a good test. It is simple—high school students have learned to use it. It is fast—it takes about an hour to run the test and analyze the data (this can be done on a pocket calculator—it doesn't require a computer). It is inexpensive—no special equipment not found in a typical laboratory is required and the chemicals are cheap.

This is simple third-world technology that can be exported to Mexico and contribute to their battles with environmental contamination.

Two years ago I obtained some funding from the Water Resources Research Institute. We determined the toxicity of 30 herbicides used on the University farm, and we followed the degradation of three of the herbicides in soil. We determined the toxicity of the soil periodically. Of the three herbicides, one was degraded or complexed with clay particles immediately. One was degraded in 3 or 4 days as would be expected if bacteria can readily degrade the compound. One was not degraded after two weeks. This third herbicide could be a problem for water contamination in shallow wells.

I have a good way to measure toxicity. I have the assay patented. I would like to sell this method to someone to market the assay in this country, in Europe, and in third-world situations.

Note: For more details on Botsford's work, see WRRI Technical Completion Report No. 301.