BIOREMEDIATION

FINNS FOCUSING ON BUGS TO FIGHT PHENOLS...

BRAUNSCHWEIG, F.R.G.—Actinomycetes deserve far more attention than they currently receive as inoculants to clean up polluted sites, according to Mirja Salkinoja-Salonen of the University of Helsinki (Finland). Speaking at a symposium held jointly by the Braunschweig-based National Research Centre for Biotechnology (GBF) and the European Environmental Research Organization (EERO), which has recently been established at Wageningen in Holland (*Bio/Technology* **8**:283, Apr. '90), she reported success in using *Rhodococcus chlorophenolicus* for the bioremediation of sawmill sites and groundwater heavily polluted with polychlorinated phenols (PCPs).

Between 1930 and 1984, when PCPs were banned, 25,000 tons of them were used in Finland. This has left persistent soil pollution in many places, with 100–10,000 mg of 2,4,6-trichlorophenol and 2,3,4,6,-tetrachlorophenol per kg of soil even at sites that were abandoned over 10 years ago. In addition, discharges from paper pulp bleaching factories account for around 10,000 tons of organically bound chlorine per year entering the country's lakes and rivers.

"We have thoroughly investigated the possibility of attacking this problem by using nutrients and other measures to promote the growth and biodegrading activities of existing organisms, but now believe that this is not the most appropriate strategy," said Salkinoja-Salonen. "There is far too high a risk of metabolising toxic chemicals to produce equally or more toxic substances. For example, tri- and tetrachloroethylene can be converted into vinyl chloride. Chlorophenols may be transformed into chloroanisols or polychlorinated dibenzeno-p-dioxins, and chlorobenzyl alcohol into chlorobenzoic acid."

Salkinoja-Salonen believes that biochemically and genetically characterised degraders have many advantages, particularly the speed at which they can work and the predictability of reaction products, as compared with "spontaneous unknown degraders." Following laboratory tests which established that *R. chlorophenolicus* can mineralise PCPs completely to carbon dioxide and chlorine, she tried inoculating the organism into naturally peaty soils and sandy loam containing PCPs at concentrations of 30 and 600 mg per kg (dry weight).

A single inoculum of 10^5 to 10^8 cells per quart of peaty soil, and as little as 500 cells in sandy loam, caused mineralisation at a rate of 12–18 mg per kg dry soil over four months in the lightly contaminated soils and at a rate of 130–250 mg per kg in the heavily contaminated soils. There were no detectable PCP-mineralising bacteria in the soils before inoculation.

Viable numbers of *R. chlorophenolicus* also remained constant in the soils within an order of magnitude, for over a year. Salkinoja-Salonen believes that the survivability of such inoculants, reflecting in part their resistance to predation by protozoa, can be improved further by strain selection and genetic manipulation. She and her co-workers have also characterised the enzymes by which *R. chlorophenolicus* dechlorinates PCPs by two hydroxylation reactions followed by the reductive removal of the remaining chlorine atoms. They are now purifying the enzymes, and plan tests to determine whether adding enzymes, rather than organisms, to contaminated land can achieve even more predictable and thus safer bioremediation. **—Bernard Dixon**

WATER PURIFICATION

...AND GERMAN BIOFILM HELPS GRAB GASES

BRAUNSCHWEIG, F.R.G.—Peter Wilderer and co-workers at the Technical University of Hamburg-Harburg (F.R.G.) have developed a novel bioreactor to remove volatile organic substances from polluted waters. Reported during the GBF-EERO symposium, the reactor has been evolved to treat leachate from the Georgswerder landfill in Hamburg, which contains benzene, toluene, and xylenes (BTX). Preliminary tests confirmed that these substances can be biodegraded aerobically. The challenge was to provide aerobic conditions in a bioreactor without bubbling in oxygen and thus losing at least some of the volatile BTX compounds (and at the same time causing air pollution) by stripping effects.

Wilderer and his colleagues decided to transfer oxygen into the leachate by fitting their bioreactor not with a conventional bubble aeration system but with a gas-permeable membrane through which oxygen could diffuse from a gas component into a liquid compartment. Silicone rubber, being highly permeable to oxygen, was a promising candidate as the material for the membrane. However, BTX compounds permeate 15 times more quickly than oxygen through silicone rubber.

The trick devised to prevent the volatiles from escaping was to allow microorganisms capable of degrading these substances to colonise the surface of the membrane and thus form a biofilm. The reactor had a working volume of 2 litres; its gas compartment was a 4.6 m coil of silicone tubing (diameter 4 mm, wall thickness 0.5 mm) through which carbon dioxide-free air or pure oxygen passed. The sole carbon sources provided in the liquid compartment were BTX compounds and the reactor, which operated at 24° C, was seeded initially with activated sludge from a municipal waste-water treatment plant. When the oxygen concentration in the liquid was maintained close to zero, a biofilm formed rapidly on the surface of the tubing and became the only site in the reactor where aerobic metabolism could take place.

The loss of volatile substances into the gas compartment fell substantially as the biofilm increased in thickness. After four days, the organisms were converting over 90 percent of the incoming BTX load into biomass (cells and extracellular polymeric substances) and carbon dioxide. The highest degradation rate was that of benzene, followed by ethylbenzene and the isomers of xylene (o-, m-, p-xylene and ethylbenzene). Wilderer and his co-workers were able to improve the overall efficiency of the system by using pure oxygen in the gas compartment and by applying a high sheer stress in the liquid compartment. Vigorous agitation (by means of a magnetic stirrer) helped to detach the outer layers of the biofilm, producing a thinner, more compact and more strongly attached film and increasing the overall metabolic conversion rate.

Very recent work by the Hamburg group suggests that the same technique can be used to degrade other pollutants such as dibenzofurans. A species of Brevibacterium, immobilised on a polyetherumide membrane permeable to oxygen, has been used to remove dibenzofurans from landfill leachate. **—BD**