

**USING STABLE ISOTOPE PROBING AND BIO-TRAPS TO IDENTIFY  
MICROBES INVOLVED IN THE BIODEGRADATION OF TBA**

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We have demonstrated that biofilms characteristic of aquifer conditions can be rapidly and efficiently collected using *in situ* microcosms or "bio-traps" containing Bio-Sep® beads. Bio-Sep® beads are 3-4 mm in diameter and composed of 25% aramid polymer and 75% powdered activated carbon (PAC). They have a porous, sponge-like structure with 74% porosity and 600 m<sup>2</sup>/g of surface area surrounded by an ultrafiltration-like membrane that contains holes and tears of 1-10 microns in size. Bio-Sep® beads are heated to 300 °C for sterilization and to render the beads free of fossil biomarkers.

When bio-traps are deployed in groundwater, indigenous bacteria enter through the outside membrane and migrate into the porous internal matrix. Microbes then attach to this internal structure and reproduce to form biofilms. In this manner, microbes can be concentrated for analysis despite their relatively low density of microbes in the sampled groundwater. Microorganisms must grow and reproduce within the bead to be detected. Thus the beads will collect only those organisms which are active under the specific subsurface conditions. Those microorganisms that are not capable of growth under *in situ* conditions due to a nutrient limitation, unfavorable redox potential, or adverse environmental conditions will not be collected in the beads at detectable levels.

Bio-Sep® beads can also be "baited" with a variety of organic compounds by vapor phase adsorption onto the PAC component of the beads. The adsorbed organic has been shown to be bioavailable to bacteria that form biofilms in the beads during incubation in a contaminated aquifer. If the compound is labeled with <sup>13</sup>C, polar lipids may be extracted from bead biofilms and the derived fatty acid methyl esters analyzed for <sup>13</sup>C incorporation using GC-IRMS. Since the beads are clean of biomarkers and sterile when deployed, incorporation of <sup>13</sup>C in phospholipids provides proof of *in situ* biodegradation of a target compound by indigenous microorganisms under actual aquifer conditions.

We have successfully used bio-traps amended with  $^{13}\text{C}_6$ -benzene,  $^{13}\text{C}_5$ -MTBE, and  $^{13}\text{C}_4$ -TBA to demonstrate the *in situ* biodegradation potential of these compounds at several sites. One such site has been the Vandenberg AFB site where bio-traps amended with  $^{13}\text{C}_4$ -TBA were deployed in an anaerobic TBA plume. Several phospholipid fatty acids extracted from bead biofilms were found to be labelled with  $^{13}\text{C}$  indicating biodegradation of TBA. Fatty acid and DNA labelling patterns suggest the participation of sulfate-reducing bacteria in the degradation process.

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