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Mineralization of Surfactants by Microbiota of Aquatic Plants

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The biodegradation of linear alkylbenzene sulfonate (LAS) and linear alcohol ethoxylate (LAE) by the microbiota associated with duckweed (*Lemna minor*) and the roots of cattail (*Typha latifolia*) was investigated. Plants were obtained from a pristine pond and a pond receiving wastewater from a rural laundromat. Cattail roots and duckweed plants were incubated in vessels containing sterile water amended with [^{14}C]LAS, [^{14}C]LAE, or ^{14}C-labeled mixed amino acids (MAA). Evolution of $^{14}CO_2$ was determined over time. The microbiota of cattail roots from both ponds mineralized LAS, LAE, and MAA without lag periods, and the rates and extents of mineralization were not significantly affected by the source of the plants. Mineralization of LAS and LAE was more rapid in the rhizosphere than in nearby root-free sediments, which exhibited differences as a function of pond. The microbiota of duckweed readily mineralized LAE and MAA but not LAS. The rate and extent of mineralization were not affected by the source of the duckweed.

Many aquatic environments are characterized by the presence of floating, submerged, and emergent plants. These plants provide food and habitat for a variety of animals, and their surfaces are colonized by a range of microorganisms. Aquatic plants are known to take up xenobiotic compounds from solution and biotransform them in conjunction with their associated microbiota. For example, Pignatello et al. (6) observed that *Lemna minor* and *Potamageton crispus* contributed to the removal of pentachlorophenol from stream environments through a combination of degradative activities of epiphytic microbes and uptake and biotransformation by the plants themselves. With rooted plants, the rhizosphere also represents an important site for degradation of xenobiotics. Hsu and Bartha (3) have shown accelerated mineralization of diazinon and parathion in the rhizosphere of bush beans compared with that in root-free soils. Microbial populations in the rhizosphere are 1 to 2 orders of magnitude larger than those in adjacent soil without roots (11). Rhizosphere microbes also tend to have faster growth rates and to differ in their nutritional requirements and metabolic capabilities. Some aquatic macrophytes transport oxygen from the shoots in the roots, thereby creating aerobic conditions in otherwise anaerobic environments (4). Much interest exists currently for using artificial wetlands and aquatic macrophytes for treating wastewater (1, 5, 12).

Investigations of a pond system in north-central Wisconsin formed almost entirely by wastewater from a rural laundromat revealed that oxygen levels were higher and concentrations of surfactants were lower in the water and sediments of portions of the pond colonized by macrophytes, including cattails (*Typha latifolia*) and duckweed (*L. minor*). The objectives of this work were (i) to determine the ability of microbial communities associated with cattail roots and duckweed plants to mineralize surfactants and amino acids and (ii) to establish whether a history of exposure to surfactants affected the biodegradative capabilities of these communities. The surfactants tested were an anionic surfactant, linear alkylbenzene sulfonate (LAS), and a nonionic surfactant, linear alcohol ethoxylate (LAE). Approximately, 3 to 4 million metric tons of synthetic surfactants are produced yearly in Western Europe, Japan, and the United States (10). LAS and LAE account for 28 and 13% of this total, respectively.

Cattail and duckweed plants were collected from two ponds in northern Wisconsin. One pond was artificially formed by discharged wastewater from a rural laundromat operating since 1962 (2). The other pond (control) was naturally formed and unaffected by any anthropogenic inputs. Cattail plants and nearby root-free sediments were recovered from both ponds. Sediment was gently washed from the roots with sterile water. Intact cattail plants were incubated in natural light by using the apparatus shown in Fig. 1, which is a modification of that described by Hsu and Bartha (3). The roots were submerged in 400 ml of sterile water containing 1 mg of the radiolabeled test compounds per liter in a side-arm flask. $^{14}CO_2$ was trapped in 1.5 N KOH contained in a sidearm test tube attached to the flask by rubber tubing. Three plants from each site were utilized for each compound. Triplicate samples of root-free sediment (20 g) were amended with 1 μg of the test compounds per g and incubated in 40-ml vials sealed with stoppers possessing suspended reservoirs containing fluted filter papers soaked with 1.5 N KOH to trap $^{14}CO_2$. Ten duckweed plants were placed in 25 ml of sterile water amended with 1 mg of the test compounds per liter in 40-ml vials as described above. Triplicate vials were sealed and incubated in natural light. $^{14}CO_2$ evolution was measured with time by analyzing KOH or KOH-soaked filter papers for radioactivity by liquid scintillation counting.

[U-^{14}C ring]sodium tridecylbenzene sulfonate (LAS) with a phenyl isomer distribution similar to that of commercial LAS and a specific activity of 8.69 mCi/mmol was obtained from New England Nuclear Corp. (Boston, Mass.). Purity was 98% based upon thin-layer chromatography on silica gel G with chloroform-methanol-water-formic acid (80:25:3:1). Radiolabeled LAE was synthesized by Procter and Gamble and had the following formula: $CH_3(CH_2)_{13}{}^{14}CH_2O(CH_2CH_2O)_7H$. Its specific activity was 1.84 mCi/mmol, with a purity >97% based upon thin-layer chromatography on silica gel with methyl ethyl ketone-water (95:5). The L-[U-^{14}C]amino acid mixture (specific activity, 50 mCi/mmol) was obtained from ICN Pharmaceuticals Inc. (Irvine, Calif. with a purity >98%.

Data were expressed as the cumulative percentage of the radiolabeled compounds recovered as $^{14}CO_2$. The data were

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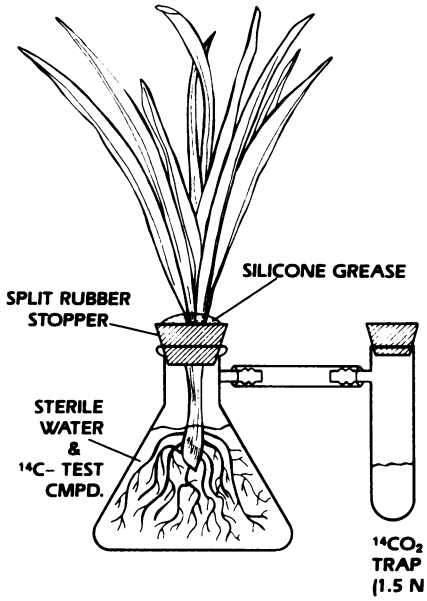

FIG. 1. Apparatus used to determine mineralization in the rhizosphere of cattails.

corrected utilizing controls containing sterile water treated as above but without plants or in the case of sediments, abiotic controls treated with Formalin. Figure 2 shows the mineralization of LAS, LAE, and mixed amino acids in the rhizosphere of cattails and root-free sediments of the laundromat wastewater pond and control pond. LAS was mineralized without a lag and at a similar rate and to a similar extent by cattails from both sites. Mineralization in root-free sediments was slower and less extensive and affected by the source of the sediments. LAE likewise was mineralized without a lag and at a similar rate and to a similar extent by cattails from both sites. Mineralization of LAE by root-free sediments from the control pond was minimal. LAE mineralization by root-free sediments from the laundromat pond was more rapid and extensive but was slower than in the rhizosphere and exhibited an S-shaped pattern. Amino acid mineralization did not differ significantly in the rhizosphere or root-free sediment of either pond.

Recovery of radiolabel was $^{14}CO_2$ from the cattails averaged 17% for LAS, 36% for LAE, and 26% for mixed amino acids. Of the total radioactivity added, 1 to 5% was associated with the roots and 0.4 to 14% was still in solution at the termination of the experiment. This poor mass recovery indicates that radiolabel was being lost from the system, probably as a result of translocation into the shoots and transpiration. Therefore, although this test system is useful for demonstrating the potential for mineralization in the rhizosphere, it is only semiquantitative, and its utility is likely to decrease with longer incubations.

Despite these methodological limitations, the data indicate that the rhizosphere of cattails represents a potentially important site for removal of surfactants from the sedimentary compartment of some aquatic environments. This observation is consistent with the lower sediment concentrations of surfactants in the weed bed compared with those in open-water zones of the laundromat wastewater pond. Furthermore, biodegradation in this habitat is of practical significance in the laundromat pond because approximately one-third of its total area is colonized by macrophytes and

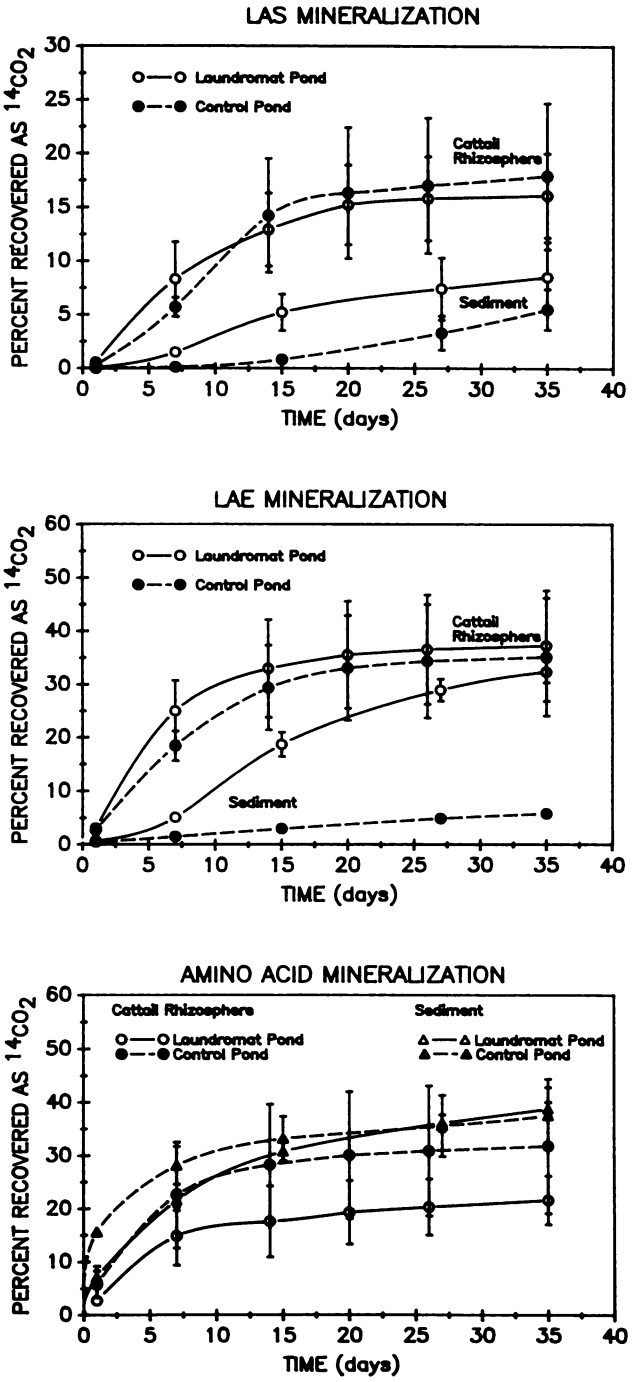

FIG. 2. Mineralization of LAS, LAE, and mixed amino acids in the rhizosphere of cattails and sediments from a laundromat wastewater pond and pristine control pond.

the colonized portion is connected hydraulically with an underlying aquifer, which is used as a source of drinking water. The data also indicate that mineralization in the rhizosphere is not determined by prior exposure to surfactants. Accelerated mineralization in the rhizosphere could result from both the elevated oxygen levels near the roots as well as the composition and activity of the rhizosphere inhabitants. Although incubated with oxygen in the head space, the root-free sediments were anaerobic based upon

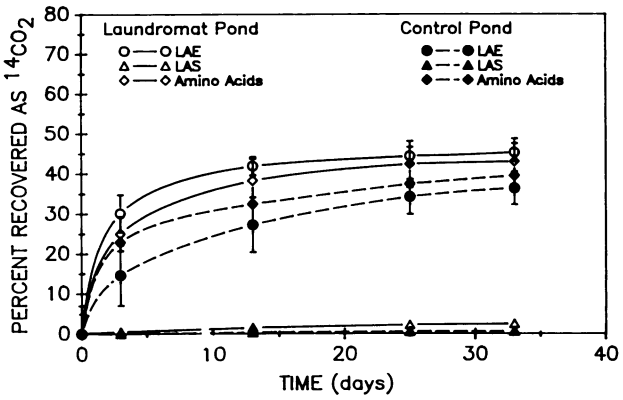

FIG. 3. Mineralization of LAS, LAE, and mixed amino acids incubated with duckweed from a laundromat wastewater pond and pristine control pond.

their low redox potentials. The low level of LAS mineralization by root-free sediments, even those with a long exposure history to LAS, was likely related in part to oxygen limitation. Current evidence indicates that oxygenases play a pivotal role in LAS degradation (8). In contrast, recent reports (7, 9) indicate the existence of anaerobic pathways for LAE degradation.

Figure 3 shows mineralization of LAS, LAE, and mixed amino acids by duckweed from the laundromat and control ponds. LAS was not mineralized by duckweed from either pond. LAE and amino acids were rapidly mineralized without lags by duckweed from both ponds. No significant differences existed as a function of pond. These data indicate that duckweed epiphytes play a role in the degradation of LAE but not LAS, even though plants in the laundromat pond have been exposed to LAS for nearly 25 years and LAS degraders are present in the pond water. This observation suggests that factors other than those in the environment control the composition of epiphyte communities on duckweed. It also raises a question about the efficacy of using duckweed for secondary treatment of wastewater.

In the case of LAS and LAE, rhizosphere microbes mineralized these compounds at similar rates and to similar extents independent of the prior exposure of the community to these compounds. Likewise, the duckweed community mineralized LAE but not LAS independent of the past history of the community with these compounds. These observations suggest that the plant itself rather than previous exposure to surfactants determines the composition and, therefore, the biodegradative capabilities of the microbiota associated with its surfaces. Hence, the microbiota associated with a plant is defined by ecological interactions with the plant rather than with the surrounding environment, decreasing the importance of adaptation in this key environmental compartment.

In summary, this study demonstrates the potential for surfactants to be mineralized in the rhizosphere of cattails and by duckweed plants. It illustrates a potential role for rooted macrophytes in accelerating the biodegradation of xenobiotics in sediments, possibly through their maintenance of large diverse microbial communities and by translocating oxygen into otherwise anaerobic sediments. Furthermore, the plants alone could have a role in the biotransformation of surfactants. In this study, no attempt was made to elucidate the relative roles of the plant and its associated microbiota in degradation.

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