INTRODUCTION

Humic acids (HAs) are complex organic molecules produced by the decomposition of plant and animal remains in soils. The surfactant-like micellar microstructure of HA is thought to accelerate the degradation of polycyclic aromatic hydrocarbons (PAHs) by enhancing PAH solubility, thereby increasing the PAH bioavailability to microorganisms. Despite abundant evidence that HA is important in the bioremediation of several anthropogenic pollutants, its role in the detoxification of PAHs by microbes remains uncertain.

Previous inconclusive results motivate a novel approach to the study of this important biogeochemical process. We used SR-FTIR spectromicroscopy to examine the effects of soil HA on biodegradation of the model PAH pyrene in the presence of a colony of Mycobacterium sp. JLS, on a mineral surface in an unsaturated environment. Infrared spectra measured during the onset and progress of biodegradation constitute the first microscopic study of this process to be made in real time.

PROCEDURE

SR-FTIR spectra were obtained at ALS BL1.4.3 from samples of M. sp. JLS as they degraded pyrene on magnetite surfaces, with and without the addition of Elliott Soil Humic Acid (ESHA). The pyrene-degrading microorganism M. sp. JLS is a gram-positive, rod-shape bacterium (GenBank accession no. AF387804); our samples were recently isolated from PAH-contaminated soil at the Libby Groundwater Superfund Site in Libby, Montana, USA. Our mineral substrates were freshly cleaved and sonicated surfaces of small chips (less than 1 cm in diameter) of magnetite rock from Minerals Unlimited of Ridgecrest, CA.

The time-dependent pyrene biodegradation experiments were began by adding 2.5 ml of cell suspension (~1.5 × 108 cells/milliliter) of M. sp. JLS onto the prepared magnetite chips. A custom IR microscope-stage mini-incubator was used to maintain the proper growth conditions for M. sp. JLS, while allowing in situ FTIR spectromicroscopy measurements. For abiotic controls, no M. sp. JLS was applied. Non-overlapping IR spectral markers were selected to monitor each component.

RESULTS

Figure 1 summarizes the time series of infrared spectra obtained by repeatedly measuring the same location on each pyrene-coated sample for more than a month. Since the sample surface is different for each experiment, the absolute value of absorbance can vary. However by
monitoring the same position on each sample individually, the changes in absorption are quantitative. Over a similar period, the infrared spectra obtained from samples free of pyrene did not show statistically significant changes.

For samples without ESHA, pyrene biodegradation starts very slowly, and about 168 hours elapse before significant changes are observed. Biodegradation then proceeds quickly, and all the observed pyrene is completely degraded within the next 35 hours. As the pyrene peaks in the spectra disappear, we observe an increase in the biomass IR absorption peaks, implying concurrent biomass formation during the consumption of pyrene (Fig. 1, panels (a) and (b)). By contrast, the biodegradation of pyrene on samples with ESHA begins almost immediately (~1 hour) after the introduction of M. sp. JLS (Fig. 1, panels (c) and (d)). The degradation of the observed pyrene is complete by the fourth hour. Again we detect an increase in biomass absorption during the later stage of the pyrene degradation, which implies that biomass formation is concurrent with the consumption of pyrene.

Figure 2 displays pyrene concentration and biomass versus time under three different conditions, as measured by associated spectral absorbances normalized to remove surface effects as described above. Abiotic results show that pyrene remains on the mineral surface, with only slow removal mechanisms. Pyrene degradation by M. sp. JLS without ESHA did not proceed until ~170 hours after the introduction of the bacteria, followed by a rapid decrease of pyrene and a rapid increase of biomass within the next thirty-five hours, as described earlier. After the pyrene was depleted the biomass signal significantly decreased, presumably as the M. sp. JLS bacteria transformed themselves into ultramicrocells, a starvation-survival strategy commonly observed among bacteria in oligotrophic environments. In the presence of ESHA, pyrene biodegradation begins within an hour and the observed pyrene is depleted by the end of the fourth hour, with a concurrent increase of biomass. It is likely that the water-insoluble pyrene is solubilized into cores of ESHA pseudo-micelles and therefore becomes available for bacterial consumption.

Over longer times, IR absorption bands of pyrene on magnetite surfaces showed a slight increase and decrease. The increase is probably due to pyrene diffusing from pyrene trapped in
micropores of the magnetite and/or neighboring surfaces of higher pyrene concentration. Thus the first wave of rapid depletion of pyrene by M. sp. JLS set up a diffusion gradient from the pyrene-containing micropores toward the bacterial colony, leading to a subsequent small increase in pyrene concentration. For the surface containing ESHA, the biomass remained almost constant over a period of more than 200 hours, indicating that the flux of pyrene from the micropores was sufficient to maintain the bacterial colony. For the surface free of ESHA, there is little evidence of the presence of a quasi-steady state biomass.

**DISCUSSION**

Our results have significant implications for the bioremediation of contaminated soils. In many PAH-contaminated sites, bioremediation is specified by the U.S. Environmental Protection Agency as the preferred remedial technology. Bioremediation of PAH-contaminated soils is often limited, however, by the low solubility of PAH, which inhibits microbial uptake. Adding synthetic surfactants to enhance PAH solubility may be toxic to natural microorganisms and further inhibit bioremediation. Based on results reported here, a potential alternative in unsaturated soil environments may be the application of natural HA to accelerate the biodegradation of PAH.

SR-FTIR spectromicroscopy can assess real-time interactions between multiple constituents in contaminated soils. Combined with conventional mineralization measurements, which monitor respiration through carbon dioxide production, SR-FTIR spectromicroscopy is a powerful tool for evaluating bioremediation options and designing bioremediation strategies for contaminated vadose zone environments.

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