†Spatial Distribution of Microbial Biomass, Activity, Community Structure and Xenobiotic Biodegradation in the Subsurface

T. W. Federle,[1] R. M. Ventullo,[2] and D. C. White[3]
[1]Procter and Gamble Company, Cincinnati, OH
[2]University of Dayton, Dayton, OH
[3]University of Tennessee, Knoxville, TN.

Abstract

The vertical distribution of microbial biomass, activity, and community structure, as well as the mineralization of xenobiotic chemicals, was examined in two 20-m soil profiles in northern Wisconsin. One profile was impacted by infiltrating wastewater from a laundromat, while the other served as a control. Biomass and community structure were determined by measuring concentrations of phospholipid derived fatty acids (PLFA) and by acridine orange direct counts (AODC). Microbial activity was estimated by measuring fluorescein diacetate (FDA) hydrolysis, thymidine incorporation into DNA, and mixed amino acid (MMA) mineralization.

Mineralization kinetics of linear alkylbenzene sulfonate (LAS) and linear alcohol ethoxylate (LAE) were determined at each depth. Except for MAA mineralization rates, measures of microbial biomass and activity exhibited similar patterns with depth and correlated with each other. PLFA concentration and rates of FDA hydrolysis and thymidine incorporation decreased 10- to 100-fold below 3 m and then exhibited little variation with depth. Fungal fatty acid markers were found at all depths and represented from 1 to 15% of the total. The relative proportion of tuberculostearic acid (TBS), an actinomycete marker, declined with depth and was not detected in the saturated zone. The profile impacted by wastewater exhibited higher levels of PLFA, but a lower proportion of TBS than the control profile. This profile also exhibited faster rates of FDA hydrolysis and amino acid mineralization at most depths. LAS was mineralized in the upper 2 m of the saturated zone, but not in the vadose zone (2-14 m) of both profiles. LAE was mineralized at all depths in both profiles and the mineralization rate exhibited the same general pattern with depth as biomass and activity measurements.

†Oral presentation.

Introduction

Among the xenobiotic chemicals that are produced in the greatest volume and have received the most widespread use worldwide are in fact those which are ingredients of household cleaning products. Two of the most common are LAS and LAE. LAS is an anionic surfactant, and in 1987 alone, approximately 1.8 million metric tons were consumed worldwide. It is a major ingredient in just about every leading brand of laundry detergent. LAEs are non-ionic surfactants and their annual consumption is approximately 1/2 million metric tons. These cleaning compounds are discarded down the drain and removed by a combination of absorption and biodegradation at sewage treatment plants. However, they commonly enter subsurface environments through leach fields that serve home septic tanks and on some occasions, leach fields that serve rural laundromats. Therefore, in the work described in this paper, the four objectives were as follows: to determine the vertical distribution of microbial communities in soil profiles that were affected and unaffected by infiltrating waste water from a laundromat; (2) to examine the ability of subsurface communities to mineralize mixed amino acids, LAS, and LAE; (3) to compare biodegraded activity of subsurface soil and groundwater that was obtained from the same depths as impacted and nonattached sites; and (4) to establish whether biodegraded activities correlated with measures of microbial biomass activity.

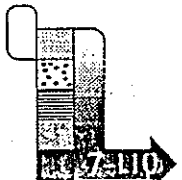
Materials and Methods

The study site was located in northern Wisconsin in the town of Summit Lake. It consisted of a laundromat of 24 washing machines which discharged their wastewater through a cycling tank through a perculation field, through a sluce way, and then to a waste water pond. Monitoring wells were located upgradient and downgradient from the pond in the perculation field. In addition, soil samples were taken at various depths from two sites: the leach field site, which was located adjacent to the perculation field, and the control site, which was located upgradient. The depth to the water, to the underlying aquifer, was approximately 14.5 m.

Various measures of water quality to the upgradient and downgradient wells were gathered. Conductivity, alkalinity, and hardness, as well as the concentration of a variety of inorganic ions, including phosphate, were all elevated in the downgradient well. Notably, the water was oxygenated in both wells, and methylene-blue acid substances, including LAS, and cobalt thiocyanate substances, including LAE, were not detected in either well.

Soil samples were obtained by drilling through the desired depth with a hollow-stem auger and then driving in a coring device. The outer surfaces of the cores were pared using a device similar to that developed by the Ada Group to generate aseptically obtained subsurface material.

Various techniques were used for characterizing microbial communities. Microbial activity was estimated by measuring the rates of FITC-diacetate hydrolysis (FDA) and thymidine incorporation into DNA. FDA is a chromogenic substrate that is cleaved by a wide range of intercellular esterases and proteases. Bacterial numbers were estimated using the AODC procedure and total biomass was estimated by measuring the concentration of phospholipid fatty acids. Community structure was



assessed by looking at the relative abundance of surface fungal fatty acids, protozoan fatty acids, and fatty acids, which are unique markers for actinomyces in mycobacterial.

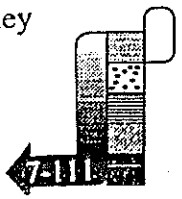
Results

FDA hydrolosis rates, the leach field soil profiles, and the rate of thymidine incorporation, which is a function of depth in the control, were used to obtain activity measures (slide). Both activity measures exhibited a 10- to 1000-fold decline at a depth of 2-3 m. This drop occurred in both profiles and appeared to be independent of the presence of infiltrating waste water. FDA activity, but not the thymidine incorporation rate, was consistently higher in the leach field compared to the controlled soil profile. The bacterial numbers and phospholipid concentrations, as a function of depth, showed that the same discontinuity that was seen with the activity measures was also present with the measures of biomass. Bacterial numbers declined five-fold while phospholipid concentrations declined 10- to 100-fold, depending upon the profile. While bacterial numbers were the same in both profiles, phospholipid concentrations in the leach field profile were up to 10 times higher than what was observed in the controlled profile.

The relative abundance of fungas, actinomycete and protozoan fatty acid were shown as a function of depth in the soil (slide). Fungal fatty acids were present at every depth in both profiles. Their relative abundance tended to be a bit higher in the upper vadose zone compared to the lower vadose zone and the saturated zone. The greatest concentration was at the 3 m depth within the leach field zone. The relative abundance of actinomycetes fatty acids decreased with depth in both profiles and the rate of this decrease was faster in the leach field profile. None of these fatty acids were detected in the saturated zone of either site. Protozoan fatty acids were detected in the upper vadose zone and in the saturated zone of the control site. In general, the relative abundance of protozan fatty acids was a bit higher in the controlled site.

Bacterial numbers in groundwater and in soil from the upgradient and downgradient sites showed that the counts in the soil were similar. At the upgradient site, bacterial numbers in groundwater were about an order of magnitude less than in the soil. In the downgradient site, the difference was two orders of magnitude. Notably, groundwater from the upgradient site had approximately five times more bacteria than that from the downgradient site.

Mineralization or biodegradtion was determined by incubating uniformerly-labelled fatty acids ^{14}C-LAE to ^{14}C ring labelled LAS with groundwater in subsurface soil to follow the evolution of radiolabelled CO_2 over time. First order rate constant K and the yield of CO_2 was estimated from the data using nonlinear regression. The first order rate constants and the CO_2 yield were used to describe the mineralization of amino acids in soil as a function of depth(slide). Amino acid mineralization did not exhibit a consistent pattern of depth when compared to other measures of microbial activity, and biomass actually varied a little with depth. In the control profile, rates were highest in the upper vadose zone and then they decreased. In the leach field profile, the rates were lowest in the upper vadose zone and then they



decreased. Contrasting the mineralization of amino acids with soil and water from the upgradient and downgradient sites showed that mineralization of amino acids in the soil was similar at both sites; however, while mineralization of amino acids in groundwater were comparable with soil in the upgradient site, the mineralization was reduced in the downgradient site, which was consistent with the lower bacterial counts at this site(slide).

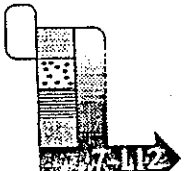
The rate and yield of CO_2 were used to describe the mineralization of LAE in soil(slide). LAE was mineralized at every depth with a maximum lag of approximately five days. Once again, there was a sharp discontinuity in the rate of LAE degradation at the depth of 2-3 m. Interestingly enough, the rates of LAE mineralization tended to be higher in the control profile as compared to the leach field profile. Another look at water and soil from the saturated zone shows that LAE mineralization was faster and more extensive in soil compared to groundwater. In the upgradient site, mineralization was proceeded by a seven-day lag with groundwater. In the downgradient site, this lag was not apparent.

Unlike LAE and mixed amino acids, LAS mineralization did not occur at every depth in the soil(slide). LAS was mineralized fairly rapidly in the upper vadose zone, then not mineralized again until the saturated zone where the rate was about 1/10 of that in the upper vadose zone. Both profiles pretty much exhibited the same general pattern. LAS mineralization in soil was extensive; however, no LAS was mineralized in the groundwater. Hence, it appears that LAS biodegraded activity is isolated in the aquifer solids rather than present in the groundwater. This is not surprising given the fact that LAS is a vary sorbtive material. Mineralization in soil from the upgradient site was proceeded by a seven-day lag. Again, this lag was not nearly as apparent in the downgradient site.

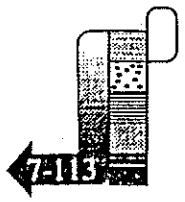
There were some correlations among the various parameter measures (slide). Measures of biomass and activity tended to correlate pretty well with one another. The rate of LAS mineralization correlated somewhat, but not real well with phospholipid concentrations, the rate of FDA hydrolysis, and the rate of thymidine incorporation. There was no correlation with direct count. The rate of LAE mineralization correlated, although not really well, with thymidine incorporation and it actually correlated best with the rate of LAS mineralization.

Conclusion

In summary, there was a marked discontinuity in the vertical distribution o microbial biomass activity in the biodegradation of LAS and LAE below 2.5 m. Th presence of infiltrating waste water affected the magnitude of discontinuity, but it di not affect its existence or depth. The soil profile that was impacted by infiltratin waste water was characterized by higher biomass and activity and also, by a lowe diversity (particularly in the upper 5 m), which appeared to be the zone of maximur impact. Groundwater had lower bacterial numbers and exhibited less biodegrade activity than soil obtained from the same site and depth. LAE and amino aci biodegradative activity was present in groundwater and at every depth in the so profile. In contrast, LAS biodegradative activity was limited to soil in the upp



vadose zone and the saturated zone. Finally, despite the patchiness of the distribution of biodegradative activity, neither of these compounds were detected in groundwater. Thank you.



Q and A

P. Strom: From the plot plans, it looks like that was an open pond. Is that correct?

T. Federle: Yes. In fact, this system has been there since 1962. It was a little tourist community, and the laudromat was built next to the road. In general, the wastewater was running out the back door and creating an ecosystem of suds in the pond.

P. Strom: Were there any kind of analysis of the pond itself?

T. Federle: Yes. In fact, an integrated study looking at fate processes in the surface and subsurface has been done.

P. Strom: And that was where it was degraded?

T. Federle: It is degraded in many different places, depending on the compound. A lot depended upon the availability of oxygen because some of the compounds required molecular oxygen for their initial degradation step.

A Konopka: I have a question about the thymidine incorporation. It seem like a number of investigators use thymidine incorporation in the sediments. It creates more problems than in planktonic samples because it starts getting incorporation into some macro molecules. Did you look at that at all?

T. Federle: Yes, In fact, the thymidine incorporation work was done by Roy Ventullo at the University of Dayton. He just had a paper in *Microbial Ecology* not too long ago, describing the methodologies he used, including data from this site. They used proteases and RNAses to look for labels in other components of biomass. There were steps in the extraction and the purification procedure to hydrolyze the RNA and so forth. Therefore, we have pretty good data that this is thymidine incorporation into the DNA and not just thymidine incorporation into TCA precipitable materials.

A Mills: I have a comment on the thymidine. We found that a lot of the anaerobes in the sediments take up thymidine and incorporate it at different rates. I do not think that it changes the shape of the curve too much, since it is probably a systematic error. However, you might want to take a look at it if you are going to publish productivity numbers.

J. Wilson: Why was the deeper, unsaturated zone not acclimated to remove LAS?

T. Federle: That is a good question.

J. Wilson: I will entertain speculations if you do not know.

T. Federle: I thought a lot about this. In the vadose zone, which is very sandy, did things just shoot through there very fast? Was the loading intermittent because it was based on a cycling tank that possibly you never really do select in that zone, but where water collects in the saturated zone we do select, we do see acclimation there.

J. Wilson: Did you look for any kind of chemical tracer that would indicate that the pond had actually drained past the particular point in the saturated zone from which you acquired your core, such as elevated or extractable phosphorous, iron leaching, or something like that?

T. Federle: In the groundwater, there was real good evidence that we were definitely in communication. In the soil profile, there were very high, elevated levels of organic carbon at the site where perculation was occurring. Some LAS determinations were done, but they were not very good. However, the levels were elevated relative to the blanks although the blanks were also high, yet we feel confident that we were in the perch zone with our samples.