†Deep Surface Bacteria Responses to Contaminants

Terry C. Hazen
Savannah River Laboratory, Westinghouse Savannah River Company,
Aiken, SC.

Abstract Deep subsurface communities have some unique characteristics that will make bioremediation more difficult in some cases and easier in others. One serious drawback of subsurface remediation will be our inability to readily identify bacteria present and the necessity for deep sediment sampling. Sediment samples, when compared with groundwater from the same geological zone, have higher densities, physiological activities, and species diversity. DNA analysis of sediment samples and bacteria isolates reveal that these communities are unlike anything on the surface. However, these same methods have demonstrated that these communities have great intrinsic potential for use in bioremediation. A high frequency of plasmid-containing isolates has been found and most isolates show a much broader range of carbon assimilation capabilities than surface bacteria. They also have a unique bimodal chemotactic response to nutrients and very strong attraction to trichloroethylene (TCE), even at concentrations that are toxic. The deep subsurface has hinted that it can be bioremediated, but with problems that must be addressed carefully.

†Oral presentation.

Introduction

This presentation looks at the potentials and the problems with doing bioremediation beneath the surface, as well as some of the specific data in response to contaminants. Some of the other work that has been presented in these proceedings will be reviewed.

Discussion

To obtain an idea of what the problem is in the United States, it would be appropriate then to first discuss the problems. The office of Technology Assessment has recorded that 1-6% of all groundwater in the United States is contaminated and this number is constantly being revised upwards. Transient groundwater withdrawals have been well documented to be increasing exponentially for domestic, industrial, and agricultural uses, and a reliance of the groundwater as a drinking water source has also increased dramatically. This illustrates the urgency of the problem and also, as the shallower aquifers become more and more contaminated, our reliance upon deeper aquifers is going to increase.

Take, for example, underground storage tanks. The United States Environmental Protection Agency (EPA) estimates that there are more than 5 million underground storage tanks in the U.S. and at least 1.1 million are going to be subject to regulation. In one study with 12,000 tanks, it was revealed that 30% were leaking. Through extrapolation, it was evident that almost 500,000 tanks could be leaking now or will be shortly, and 50,000-100,000 of these sites are expected to require mandatory action. This number is constantly increasing and it does not include landfills and other waste sites, only leaking underground storage tanks.

McCormick (1985) estimated that it is going to cost at least $100 billion to clean up the 93,000 dumping sites that are known to exist, and this number is increasing daily. Therefore, the urgency in finding methods that are going to save money is real. This is also why some of the extreme activity that is occurring in bioremediation has been seen.

Surveys done by the EPA (reported by McCormick in 1984) provide an idea of some of the compounds that involve the United States. One reason a lot of field work is being done with TCE and chlorinated hydrocarbons is that it is one of the most common compounds found in groundwater. It has been detected in 35% of 25 communities samples. Of course, these compounds are all either experimental carcinogens or known human carcinogens. Furthermore, the Resource Conservation and Recovery Act (RECRA) prohibits land disposal of many hazardous substances and it, as well as other laws, mandates that these sites be cleaned up. Contaminates have gotten into groundwater environments and there are a large number of possibilities as to how these contaminates infiltrated the groundwater (slide). Infiltration sometimes occurs even by direct well injection.

At the Savannah River Site, all of these problems exist, including some instances in which chlorinated hydrocarbons were directly dumped into clay-lined seepage basins. They have since penetrated into some deep aquifers, though most of it is confined to the shallow areas because of the large amount of clay in the soils. A lot of soil, microbial survival, and groundwater factors have been identified that

may affect contaminate removal. Knowing that bacteria do exist below the surface sheds light on the doctrine of infallibility. People sometimes do not like the strength of this statement, but it is of course of one of the selling points of bioremediation. Simply put, there is no compound, man-made or natural, that bacteria cannot degrade given the right conditions.

Bioremediation of the deep subsurface. While aquifers continue to become contaminated, they are also becoming a major source of water for many industrial processes and agricultural water. Because of the depth, slow turnover times and large volumes involved, *in situ* bioremediation, in many cases, will be the only possibility for some sites. *In situ* bioremediation has been around for quite a while, and the degradation of hydrocarbon was actually first established by Dick Ramond in the early 1970s. A full adoption of this technique was not seen until the early 1980s, but a very dramatic increase can be seen now. It is probably more realistic to look at bioremediation, not in terms of biorestoration, or taking a toxic site and changing it into a rain forest. Most of the time, the best one can hope for is removal or transformation of some of the toxic components and achieving a less toxic environment. Bioremediation companies will quite often try to sell the biorestoration scenario.

In the Deep Subsurface Science Program, boreholes have been drilled at the Savannah River Site on the border of South Carolina and Georgia (slide). They were deep holes, with the last one going down to a depth of over 1700 feet. David Balkwill, Bill Ghiorse, and others have recorded that there were many organisms in the sediment profile and they represented quite a number of different types (slide). The number of colonies or biotypes and the number of API-NFT were identified by Dr. Balkwill, as well as the PTYG counts versus the number of viable counts on 1% PTYG (in borehole P29). The numbers were fairly similar, quite surprisingly, and the number of types (colony diversity) was also quite high (slide). The same thing occurred in P24 and P28. A large number of types existed, either by counting or colony diversity. There was also a large number of organisms with really no decrease as one went down the borehole.

There was also a strong interest in how good the identifications were and how different the microorganisms really were. They may have been identified by API-NFT at the 99% confidence interval, but were they really that particular species since the database that was used to identify the microorganisms was not clinical in origin? Therefore, in light of this question, actual DNA melts were done. For those of you not familiar with this, it is a way to separate bacteria, at least exclusionary. Of course, this technique does not look at the DNA structure, but instead at the amount of G+C in the particular bacterium. Therefore, it can only be used as an exclusionary technique because if the measurements are different, the bacteria have to be different. If they are the same, it does not mean that the same bacteria has been obtained.

However, using this type of technique along with the G+C and the API identification code, the data showed that all of these organisms (in the slide) from

the subsurface had identical phenotypes. There was a large range of % G+C, so even though they had identical phenotypes, the genotype was not the same. Thus, they could not have been the same bacterium. What should be emphasized here is the high diversity that was evident by the API-NFT tests and by the other types of physiological measurements. Furthermore, colony morphology was probably even much greater.

These bacteria were quite different and DNA homologies verified this point. From the data, it was assumed that 70% homology was the same organism. The probe used in these analyses was ATCC P. putida. However, using that probe and cross matching showed that none of these bacteria were similar even though they were API-NFT identification.

A number of probes were also made against deep subsurface bacteria and DNA was extracted from the bacteria. A probe was made by attaching the bacteria to a filter, and the similarity between bacteria was measured. Some of the data collected by one of the graduate students, Luis Jimenez, showed that only the homologous system proved a match at one site. None of the data indicated that even the same species were present. In a few cases, they may have been similar, but there was actually quite a difference among all of the bacteria even at one site.

The frequency of plasmids, in general, seems to increase with depth, which suggests that there is greater possibilities for remediation. Furthermore, as the environment becomes more recalcitrant, the bacteria pick up a higher plasmid load, which allows them to degrade a wider variety of substances. In general, the studies that have been run, along with the information David Balkwill has provided, have shown that the plasticity of the bacteria in terms of their ability to assimilate different compounds, also increases as depth increases. This certainly provides hope that there is quite a bit of potential at depth for any type of bioremediation of toxic compounds.

Carl Fliermans and Tommy Phelps have also shown that in surface and subsurface environments contaminated with TCE, there is a lot of activity in these environments that is contaminated and that the microorganism there can degrade the compound. At very high concentrations of TCE, very little activity can be seen, possibly due to toxicity. However, in one particular zone of activity, the ability to mineralize both above and below the plume was observed. This provided more evidence that a site could be remediated, especially at the Savannah River Site. Fliermans et al. also reported that one could stimulate bacteria from the Savannah River Site to degrade TCE cometabolically and they presented a number of different electron donors like propane, methane, and methanol to stimulate TCE degradation.

As a part of that work, a link was made with the Gas Research Institute, and their interest in methane is obvious. They were interested in how we might be able to use a methanotrophic bioreactor or use methane in remediation, using the methanotrophic organism's ability to degrade TCE. The fatal flaw analysis has been done by Radian Corporation for the Gas Research Institute. They also did a cost analysis which showed that the technology was possible and feasible. The cost analysis was very

helpful as well because an effort was made to put this into a real world situation to determine if it was really better than the conventional technology. The analyses showed that the conventional technology centers on direct carbon sorption from the water and stripping of the air stream by granulated activated carbon. If one looks at the capital cost for a variety of the same scenarios, including either 100 or 1000 ppm TCE in the groundwater and flow rates of either one or ten million gallons per day, then one can see that the costs are lower for the methanotrophic system than both the carbon sorption system and the air-stripping system (slide). The major cost is the carbon. However, the methanotrophic system was 40-60% cheaper just for capital costs, except at the very highest flow rates and at the very highest TCE concentrations (slide). At those rates and concentrations, a much larger system would have been needed, one that exceeded the costs of the airstripping and granulated activated carbon. When the operating costs of these systems were actually looked at based upon a conservative analogy from three years ago (because the rates of degradation of TCE that are now being found are much higher than what was used in this particular model), a 40-60% difference in costs was once again found between the methanotrophic system and any type of airstripping or carbon sorption system. Furthermore, the air-stripping system prevailed as well, whether the water ratio of 85:1 or 300:1 was used (slide). Therefore, it appears that it will really be a cost effective way to remediate TCE.

We went back to some bioremediation bioreactors that have actually run to see if we were in a real world situation, since the methanotrophic system was hypothetical. In 1988, Nyer published data on a toulic acid bioreactor, and from it one can see that the off-site disposal cost was at 20 cents per gallon and activated carbon cost was 80 cents per gallon. However, when it went to a bioreactor, the cost was less than one cent per gallon. This was a very substantial savings for the site; therefore, development began for a methanotrophic bioreactor system. This system is being developed with a research program between the Savannah River Laboratory and the Gas Research Institute. It is a four-year program.

Drs. Phelps and White have taken Savannah River Site bacteria and put them into an expanded fluidized bed design bioreactor. They put the Savannah River consortium of bacteria into the reactor system and added either propane or methane. There was a significant reduction in the amount of TCE when the methane was added, and with propane there was an even better degradation rate. Two different consortia were used to ensure the results of the technique could work. We are in the process of scaling up with a trickle filter system, but we are going to have to overcome the mass transfer problems that we might have with the fluidized, expanded bed bioreactor for the pump-and-treat system.

The work done by Dr. Phelps shows some of the most interesting things that might apply to pump-and-treat systems. Dr. Phelps put very contaminated Oak Ridge water through his system and looked at the controls versus the two microbial consortia from the Savannah River Site. The consortium actually degraded everything else that was in there, including benzene, toluene, and xylene. In some cases, it

actually degraded the benzene, toluene, and xylene a little bit better than it did the TCE. Therefore, an added bonus was obtained. Not only were all of the volatile compounds being removed, but so were a lot of the less volatile compounds. This suggests that these bioreactors may be really useful for direct treatment of water streams in a pump-and-treat scenario. A methanotrophic system may operate in a similar manner, with interceptor wells pulling up a contaminated plume, mixing with methane in air, putting it into your methanotrophic bioreactor, and then recharging it back into the subsurface aquifer.

One of the problems that will be encountered with any type of pump-and-treat scenario is that contaminate recovery will have a diminishing return as one tends to encounter more clay and more subsurface pockets. Once a certain depth is reached, fewer returns will be seen. This may be a good area to concentrate on *in situ* bioremediation so the microorganism can be delivered to those areas, or filtrate nutrients into those sites and degrade the compounds that are already there *in situ*. Therefore, the pump-and-treat scenario may work best for uniform sand and gravel aquifers, especially in areas like the site at Traverse City, Michigan. However, it may not work as well in areas that have a lot of clay lenses.

Jack Corey indicated earlier that some horizontal wells have been installed as a method of extraction and *in situ* airstripping. This method is an adaption from technology used by oil companies. Our wells have been placed at approximately 125 feet deep, where the water table is at 100 feet and the upper horizontal well is at 75 feet. Injection of air is expected to begin within the next two months. Air will be injected in the lower well and a vacuum will be applied to the upper well. Hopefully, movement of air through the system will be obtained, which will provide actual *in situ* airstripping. It will probably affect microbial activity as well, and it will be measured. The single horizontal well may also be a way to reduce the cost associated with deep aquifer remediation. With the single horizontal well, only a single hole is needed, allowing further expansion in the plume (instead of drilling a variety of verticle holes just to get into the contaminate plume). Therefore, this may be a way to get around some of the problems that are encountered with deep subsurface systems.

At the Savannah River Site, there were a large number of cluster wells at each of the boreholes that were used to sample bacteria in the sediment. Therefore, I had the opportunity to go in and sample at very discreet interval and to compare that to the sediment that was found in another site. This other site, which was originally thought to have been very contaminated, was found to have all of its contamination on the soil surface. The bulk water from very discreet screen zone regions was sampled and compared in terms of numbers and types of bacteria with the sediment sample that was found. This comparison provided some very interesting results (slide). The numbers were fairly consistent between AODC's and viable counts, indicating fairly high activities (except in some of the clay areas). A different picture was seen with the groundwater. Densities from the AODC and PTYG were three to four orders of magnitude lower, and the differences between the AODC and the

PTYG were also much greater. This indicated that these bacteria were under a much greater stress. Some of the other measurements that were made would indicate the same thing. Measurements of diversity and G+C, and a variety of other measurements, showed that what was in the water was quite different from what was in the sediments. These results showed quite well that these bacteria are fairly strongly attached, especially as one gets deeper and deeper into the subsurface. A comparison was also made between the groundwater and sediment. Again, there was a five orders-of-magnitude difference in some cases and barely detectable concentrations in others.

Typically, groundwater is used to monitor biodegradation rates and microbial population changes. However, this may produce erroneous results, especially in the deep subsurface, where indigenous microorganisms are strongly attached to the sediment. This strong attachment allows only stressed, dead, or maladapted organisms to be observed in the groundwater, which is quite different from what is actually present in the sediments. In addition, pore water microorganisms are often associated with these wells, which can provide false indications as to what is occurring in bioremediation.

How bacteria move, that is to say their ability to transport between wells and their ability to move themselves, is of particular interest. Chemotraction, which is movement of a bacteria toward a nutrient gradient, is one way that they can move. A capillary tube technique was used to observe the chemotaxis to a variety of amino acids and sugars (slide). This work was done principally by one of my other graduate students, Geryalyne Lopez. She developed 10 different sugars and 10 different amino acids, along with TCE for chemotaxtic responses over a wide range of concentrations. The results showed what one would typically see in a chemotaxis experiment with chemotactically positive substrates. As the concentration increases, a threshold is reached, which triggers a positive movement from the bacterium. As the concentration continues to increase, the movement of the bacterium is always toward the attractant, never away (slide).

When deep subsurface bacteria were observed in our study, something quite unusual was found. In general, a negative chemotaxis or repellent response in high concentrations was seen with most of the bacteria. A fairly strong negative chemotaxis was observed at some high concentrations and a fairly strong positive chemotaxis, sometimes double that amount, was observed over very low concentrations of substrates (slide). Motility controls were run to make sure it was not just random motility due to increased activity of the substrate. The response range was a bit wider than what is normally seen for bacteria for similar substrates. However, I have never observed this bimodal phenomenon with surface strains and various other bacteria that have been checked.

Some of the adaptions that these microorganisms have to low nutrient environments, very oligitrophic environments, are reflected in their response to some of the amino acids. There were a couple of differences with respect to the type of bacteria. However, very different responses were seen for TCE. As the concentration of TCE was diluted to 10^{-10} Molar, no negative responses were ob-

served, only positive chemotaxis. Again, however, some of the highest chemotaxis indexes that have ever been recorded were four to five times greater than water alone. This was a very unusual response.

It is believed that this response was due in part to the fact that TCE affects the chemo-receptors, which are methylated proteins. Of course, this TCE response needs to be studied a lot more. It is believed that there is actually a binding of the TCE to conformation changes in the receptor proteins by turning them on quite nonspecifically even if the concentrations were toxic for the bacterium. This was evident when observing the bacterium even with acridine orange-stained bugs. The bacteria go screaming into the TCE in a response which is called the "fatal attraction", but it may have some significant implications. This was not universal, but it was common in almost all of the bacterial screens that were tested, and it may have some applications to the terms of bioremediation and the controlling bioremediation processes. It may provide some idea of what could happen in a TCE-contaminated aquifer if the attracting of microorganisms does not specifically destroy them. We have actually filed for a patent on some of these observations.

To see if this technique was applicable to the real world, a crude model was developed that used sediment samples from the Myrtle Beach Site and the Middendorf aquifer at the C10 borehole. A water reservoir was placed at one end of the column and then the whole system was autoclaved. A small number of bacteria were introduced at one end and then observations were made to see how long it took for the bacteria to break into the reservoir. When a very small amount of TCE was put into the reservoir, the bacteria literally went through the sediments twice as fast. Sterile and killed controls were done and no breakthrough was observed over the 3-4 week period. It was a crude system, but it provided an idea of what might happen in the real world.

A few calculations were also done in regard to this model. With the distance between the preliminary hole and the C10 hole being 190 feet, it would take 49 days for a bacteria that was introduced in the preliminary hole to get to the investigators hole, that is assuming it was traveling at the fastest rate. At the lowest rate, it would take 444 days. We were on the site 49 days, so if there was something strongly attractive being introduced into the C10 hole, it is possible that we could have seen some of the bacteria breaking through the investigators hole from the preliminary hole.

Conclusion

From the various presentations of investigators in these proceedings and from what I have discussed, many of the problems concerning bioremediation of the subsurface have been brought forth. One problem could be the unique microflora that exists in the deep subsurfaces. The subsurface microbial community is largely prokaryotic. The monitoring of groundwater provides little information about changes in activity and numbers that are very relevant for *in situ* bioremediation. Concentration of nutrients, slow water flow, slow recharge rates, and a recalcitrant nature of deep subsurface environments may make conventional nutrient manipula-

tions fairly difficult. Perhaps things such as the horizontal well will help overcome these problems, but it certainly is going to be difficult. The epilithic nature of these environments will make any strategic infusion of nutrients more difficult.

On the positive side, the good news is that the microflora is Gram-negative. There are a lot of plasmids present, and it looks like they can degrade many compounds. This has already been seen from work done in various laboratories for these particular bacteria at the Savannah River Site. They have a lot of plasmids, which leads to a lot of potential. Deep subsurface microorganisms also show great diversity in the types of organics that they can assimilate. Large proportions of plasmids in the communities suggest a high potential for plasmid degradation of contaminants. This may be fairly important.

Some studies have been recently published. In *Biotechnology Journal*, Amgen Corp. reported the extraction of genes from a Pseudomonas strain that coded for the degradation of TCE and put it into E. coli K12. In doing so, they uncoupled it from the cometabolic process, allowing the bacteria to degrade TCE in a very simple cultured system. The TCE was degraded from 40,000 ppb down to less than 10 ppb in four hours. This finding raises all sorts of possibilities for genetically engineered microorganisms and for introducing them into contaminated environments. However, one must first look at the transferability of genetic elements and some of the problems that might be associated with it. In addition, chemotaxic behavior in subsurface bacteria may be used to help control or enhance remediation. Probably the best thing to say right now is do not pollute. Thank you.

Q and A

J. Bauld: You and other people have stressed the probable epilithic nature of the communities present downhole. Do you think that it is probable that when you crank up the nutrient levels down in the hole when you are attempting bioremediation, that there may be significant changes in the distribution of organisms between the epilithic and planktonic populations and in fact, that it may be a significant problem in bioremediation?

T. Hazen: That certainly could be a problem. David Balkwill presented some data in Plenary Session 5 that showed such a problem with nutrient addition. With the deepest sediment, they did not seem to switch that much, and after the nutrient was withdrawn, they switched back fairly quickly. Is that a fair synopsis David? That is probably some of the best evidence that we have that such a shift may not occur in deep subsurface.

D. Balkwill: I think it is a little hasty to say whether they shift back swiftly. I think Ron Harvey has some very interesting information in terms of distribution of the organism being attached to those kinds of nutrient levels.

B. Russell: I want to say that I really appreciate seeing the diagrams that showed the information characteristics of breakthrough curves. Could you elaborate on the conditions that were associated with running the experiment in a test tube? Did you create any sort of hydraulic head associated with the experiments? Did you look at any variations of flow velocity through that material? Then, did you try varying formation characteristics just for the heck of it to see what happens when you introduce clays or other sorts of things, or do you plan to do that if you have not already?

T. Hazen: I have not done that yet and it was a very crude experiment. I am waiting for the next graduate to come along to continue some of that work. We also are building a chamber system that Carl and I have worked out where sediment and water can actually be taken from the subsurface and it can be pumped into sterile Middendorf sediment to see if the communities can be reestablished there and observed that way. This appears to be a better way to examine some of those problems. Those are very good things to look at and that is why I kept qualifying that this was a crude experiment, because I do not know what some of those parameters would have done. However, since the differences were great enough, they were mentioned.

B. Benoit: I want to compliment you on the chemotactic experiment. The question I have is do you think the organisms were responding to factors besides substrates? The reason I ask the question is the microaerophiles we have isolated in the Middendorf are responding to oxygen and substrates. If you look at the literature, there is also evidence that some of the microaerophiles are quite sensitive when you increase the phosphate concentration.

T. Hazen: Clearly they responded to things other than their substrates, because TCE is not a substrate. I am sure that they were responding both for avoidance and for attraction to various compounds, which may have been metabolic by-products or things like that. It has been well shown that certain fatty acids or similar compounds can be an attractant or a repellant depending on what that bacteria likes. How significant chemotaxis is in subsurface environments is pretty speculative right now. It could be fairly important and the unusual thing is the bimodal response that was seen on the subsurface bacterium negative and positive for the same substrate. What a great strategy for a bacteria that was adapted to a fairly oligotrophic environment like you have seen presented by a lot of other people.

C. Litchfield: I would like to make one comment on the epiphytic versus planktonic nature of the bacteria. This may well have something to do with substrate as well as sediment type. From the studies that we have done on the chlorobenzene-degrading organisms, we have found more chlorobenzene degraders in the groundwater that we recovered from our monitoring wells than we did from adjacent boreholes. This was not true with hydrocarbons; we found more organisms in the soil than we found in the groundwater. Therefore, we may have to be careful about how this is interpreted depending upon substrate and soil types and not just look a for a simple, single answer.

R. Dietz: Given what we have seen about the techniques for cleaning up the subsurface environment, whether it be biochemical or stripping, or whatever, and the diversity of contaminated subsurface environments that probably exist, and the cost of cleanup with the exception of removing free-standing pollutants, has anyone really thought about assessing whether or not the direction that we seem to be heading in is the most viable direction? Namely, cleaning up all the subsurface environments down to drinking water qualities, rather than just getting rid of free-standing subsurface contamination and then cleaning up using a lot of these technologies? Rather than cleaning the water to drinking water standards? Rather than cleaning up 99.9% of what will never be extracted? Where do we stand in terms of the legal aspects? Has the scientific community responded in this direction?

T. Hazen: I have talked to some EPA personnel about the same thing. They said that sooner or later we are going to realize that the United States Government cannot afford to absolutely clean all the sites. We are going to have to start thinking more, at some sites, in terms of containment, preventing it from spreading rather than absolute total cleanup. This probably will become more realistic as the study moves along. Right now, with the regulatory environment such as it is, there is a lot of contamination to get rid of. That is probably unrealistic given the monetary resources that even the United States has.

G. Matthess: I think you are right. The point is very important if you consider the whole cost of the cleaning procedure and the containment procedure. These indeed are very expensive. I think from the standpoint of the geochemist, I will say that we have to try to define acceptable levels of emissions into the environment. If one does not accept any emission, it is not possible. Even if there is containment, there are emissions—that is a natural law. So we have to define what is acceptable for the environment with respect to chemicals; they are not natural products. One could say that they do not want any man-made chemicals in the environment. This is a very poor approach here in the United States.

C. Fliermans: I think the concept in aquatic microbiology may have applicability here and that is assimilative capacity of streams. When one gets into subsurface environments, is there such a thing as assimilative capacity that will allow one to, as was already mentioned, remove free products so that there are organisms that are capable of an assimilative capacity that can be utilized? I think assimilative capacity is a concept that needs to be part of our ongoing research.

P. Strom: It seems as though there may be some contradiction in thinking of organisms as both epiphytic and motile, at least at the same time. In fact, may not motility be a response to contamination?

T. Hazen: I have difficulty dealing with that too. These organisms in the deep subsurface are uniquely different than anything that has been on the surface. Another comment I would like to make concerning the horizontal wells is that in our next phase this coming year, we are planning to inject methane directly through one of the horizontal wells, trying to encourage methanotrophs. We think that is a plausible way to remediate a site in the near surface. Furthermore, using methane to try to stimulate quite specific components of the community might allow us to avoid some of the plugging problems and things that may be encountered with a general nutrient addition, since the methanotrophs are a fairly small component of the system. By adding methane, the increases of every bacterium under the sun that might be seen with the addition of phosphates, nitrates, and types of hydrogen peroxide will never be seen. We have also shown, from direct DNA extraction of sediments of the C10 hole, that TOL 1 plasma bacteria are present. Probing can actually be done with the TOL 1 plasma, which indicates a hit in some of the lower sediments that have been directly extracted. Thus, it is in high enough concentration that it can be detected. This has provided even more evidence that there is a significant potential in there for degradation for a lot of compounds such as TCE.

M. Fletcher: I want to comment on the previous question about the apparent paradox between attachment and the ability to be chemotactic. I think if any microorganism is going to be successful, it must contain some options. It is not going to go down the evolutionary trail that will trap it into a particular habitat. It is always living on

particular sites on the surface. It may be untrue to speculate here, but it may in fact be that chemotaxis is more important to the organism that is adapted to the epilithic mode of growth, because it is not in an environment where it can move about by water flow.