LIVING ROOM, TRANSPORTATION, AND COMMUNITY: THE OVERLOOKED INFRASTRUCTURE IN SUBSURFACE MICROBIAL BIODEGRADATION

EDWARD WINNER, PH.D. VICE PRESIDENT, RPI GOLDEN, COLORADO

ABSTRACT

It is commonly thought that if one can identify a microbe, usually a bacterium, which degrades a contaminant of interest, then introducing that microbe into the subsurface will result in persistent contaminant degradation. But bacteria are not little superheroes. They are biological creatures and have metabolic needs. Bioaugmentation and biostimulation efforts typically supply nutrients, sources of nitrogen, and electron acceptors as appropriate. However, these same efforts often fail to consider that subsurface is already inhabited.

If a new sustainable microbial community is going to be established, accommodations must be made. To investigate the thesis that physical space is necessary to build microbial communities capable of sustained bioremediation, we searched the scientific literature, conducted initial observations, and examined field data. The presented material is intended to open the broad topic of microbial ecology in bioremediation by encouraging the reader to consider the space a microbe might call home.

EVERY ECOLOGICAL SYSTEM STARTS WITH PLACE

While microbes grow on all subsurface materials, it is well documented (1. 2. 3.) that microbial growth on powdered activated carbon (PAC) is more abundant than on clays, silts, and other geomaterials.



Scanning electron microscope images show microbial growth on capping material amended with PAC. The top images (A-C) include PAC while the bottom images (D-F) are clay. The samples were collected after 4 (A,D), 6 (B,E) and 11 days (C,F) (4.).

As microbes are abundant on PAC material, we examined a PAC-based injectate, BOS200[®], by light microscope. The image on the left is of a 57mm (2.25 inch) diameter core log laying horizontally. Core slices from similar logs are on the right. These logs were selected because they show BOS200[®] seams and inclusions. BOS200[®], seams are not always observed, and the distribution of the BOS200[®] is geology depend.



AVAILABLE CAVITIES AND INTERSTITIAL SPACES

The size and shape of cavities within the media and interstitial space between media grains in the subsurface are essential to the supply of nutrients, electron donors and acceptors, carbon sources, and the elimination of microbial metabolic wastes (5.). Thus, population density, size, form, and mobility depend on these spaces (6.).



The light microscope picture is of a mixed sandy clayey media from below the water table. The two sand grains circled in blue are 10 to 18µm in diameter. The black material is BOS 200[®] having a diameter range of 0.5 to 200µm with an average particle diameter of 24µm (median 18µm). The addition of the BOS 200[®] increases granularity adding to the available cavities and interstitial spaces.

NEW FLOW PATHS AND NICHES[®]

As an ecosystem, the subsurface must provide the essential elements of microbial life: moisture, chemical factors such as electron donors, and media factors such as granularity. These elements are linked to the transport of water in the subsurface. Thus, microbial populations reflect groundwater flow pathways (7. 8. 9.) which reflect granularity.



The above picture is of a fine-grained clay, aquifer material, with most of the grain size below 5µm in diameter. The black line running top to bottom is BOS 200[®], which has opened a pathway through the clay. This pathway is anticipated to facilitate the movement of nutrients, electron acceptors, etc. in a manner superior to that of the clay prior to BOS 200[®] injection.

The BOS 200[®] is injected using sufficient pressure to facilitate localized soil lifting and propagation from the injection tip. Injection pressures typically vary from 200 to 600 psig as measured at the discharge end of the injection pump. In fine-grained sediments (clays and silts) there is typically a soil lifting pressure that is momentarily sustained after which the pressure drops off to a propagation pressure. In coarse-grained sediments (sands and fine gravels), a steady progression pressure is typically displayed as the lithology near the injection tip is fluidized and turbulent flow is created.

ESTABLISH FLOW PATHS ON A MICROMETER SCALE

Just as activated carbon accentuates water movement (10. 11.) due to the addition of three-dimensional networks of carbon seams and surfaces (12. 13. 14.), BOS 200[®] should also support contaminant dispersion, movement of nutrients and terminal electron acceptors, the interchange of microbial metabolic products, and the dispersal and colonization on newly accessible soil and aquifer compartments.



The picture shows petite seams within a very fine-grained clay. If one looks closely, branching can be seen. Branching is circled blue. Note also that PAC seams are also directed toward the viewer. A couple of examples are circled in green.

FLOW PATHS TWIG AND BRANCH PATTERN

BOS 200[®] distributed in a branching pattern which is pictured ideally in schematic (A-C). The injection force opens the initial pathway (A.). Branching occurs due to weakness in the clay (B.) and avoidance of physical interference, e.g., two carbon seams traveling in parallel must compact the clay between the parallel lines (C.). This interference is avoided by branching at right angles.





Picture from 15.

Although not so idealized, branching patterns are demonstrated in these photos. The pattern demonstrates the integration of BOS 200[®] into the media and is consistent with both observed and theoretical distribution patterns.

MICROBES IN A BOS 200[®] SEAM IN CLAY

A confocal microscope image of a core log was collected from a BOS 200[®] emplacement site 2 years post-injection. A carbon seam runs through a finegrained clay. Microbes' fluorescence as compared to the adjacent clay. The image illustrates that microbial life is sustained on the BOS 200[®] superior to the clay aquifer material.



The image presented was made by alternating excitation light between 405 and 550nm wavelengths. The green column height represents intensity and is a qualitative indication of microbial density.

MICROBES GROW ABUNDANT & BUILD BIOFILMS ON AC

Brightfield microscope images show microbes originating from CAT 100 (GAC), growing as a biofilm, and invading the media. The CAT 100 was in a soil core collected from 30 - 37 ft bgs. These pictures are slices from these soil cores embedded in agar. CAT 100 appears as black areas. The blue structures are bacteria stained with methylene blue. The pink structures are bacteria stained with carbol fuchsin. Due to the overlapping of bacterial cells and the fact that microscopes have a limited depth-of-field, some areas look purple, or orange and some areas are out of focus.



The "structures" marked by the arrows are biofilm growing on CAT 100 (GAC). The further away from the GAC, the lower the microbial density. 100X water immersion lens.



The "tree-like" structures are bacterial biofilms growing from the CAT 100 (GAC) into the media. 40x lens. The GAC was completely covered in biofilm. Each area must be focused on individually.





The same "tree-like" structure using a 100X lens

SUMMATION

A review of the published literature, examination of cores drawn from BOS 200[®] emplacement sites, and the examination of a soil core containing a BOS 200[®] seam have been examined. The emplacement process used has produced BOS 200[®] seams within fine-grained clay. These seams appear to branch even at a micrometer scale. Multiple published sources support the contention that such seams should be hydraulically active. When examined from microbial density, the seams are richer in microbial life than surrounding clay media.

An examination of the CAT 100 (GAC) demonstrates that it supports biofilm development and that the CAT 100 demonstrates more abundant microbial growth than surrounding aquifer material.

REFERENCES

- Liang, Y. Z. (2008). Porous biocarrier-enhanced biodegradation of crude oil contaminated soil.
- International Biodeterioration & Biodegradation, 80-87.
- Semenyuk, N. N. (2014). Effect of activated charcoal on bioremediation of diesel fuel-contaminated soil. Microbiology, 589-598. Bonaglia, S. B. (2020). Activated carbon stimulates microbial diversity and PAH biodegradation under anaerobic conditions in oil-polluted sediments, Chemoshere, 126023
- Pagnozzi, G., 2020. Evaluating the influence of capping materials on composition and biodegradation activity of benthic microbial communities: Implications for designing bioreactive sediment caps (Doctoral dissertation), Texas Tech University, Civil Engineering. Lubbock: Texas Tech.
- Fredrickson, J. F. (2001). Subsurface Microbiology and Biochemistry. New York: Wiley-Liss, Inc.
- 6. Luckner, L. S. (1991). Migration process in soil and groundwater zone. Leipzig: Bundesrepublik Deutschland.
- Maamar, S. A.-C.-A. (2015, December). Groundwater isolation governs chemistry and microbial community structure along hydrologic flowpaths. Front. Microbiol., 6, 13.
- . Graham, E. C. (2017). Deterministic influences exceed dispersal effects on hydrologically connected microbiomes Environmental Microbiology, 19(4), 1552-1567.
- Danczak, R. E. (2018). Microbial Community Cohesion Mediates Community. mSystems, 3(4), e00066-18.
- 231 A Demonstration of In Situ Remediation of DNAPL Compounds in Low Permeability Media by Soil Fracturing with Thermally Enhanced Mass Recovery or Reactive Barrier Destruction. Oak Ridge: Oak Ridge National Laboratory and Collaborators. Retrieved from https://clu-in.org/download/techfocus/fracturing/X231A-3445604441067.pdf
- Sorenson, K. N. (2019). Use of Permeability Enhancement Technology for Enhanced In Situ Remediation of Low-permeability Media. Alexandria, VA : Department of Defense Environmental Security Technology Certification Program (ESTCP). Retrieved from file:///C:/Users/ winne/Downloads/ER-201430%20Guidance%20Document%20(2).pdf
- 2. Murdoch, L. (1995). Forms of hydraulic fractures created during a field test in fine-grained glacial drift. Q. J. Eng. Geology, 28, 23-35. 13. Bradner, G. M. (2005). Effects of skin and hydraulic fractures on the performance of an SVE well.
- Journal of Contamination Hydrology, 77, 271-297.
- 14. Or. D. S. (2007). Physical constraints affecting bacterial habitats and activity in unsaturated porous media a review. Advances in Water resources, 30, 1505-1527.
- . Rahimi-Aghdam, S., Chau, V. T., Lee, H., et al., (2019). Branching of hydraulic cracks enabling permeability of gas or oil shale with closed natural fractures. Proceedings of the National Academy of Sciences, 116(5), 1532-1537.