THE LOST COMPONENT IN BIOREMEDIATION: HABITAT SUPPORTS ABUNDANCE, DIVERSITY, AND SUSTAINABLE MICROBIAL POPULATIONS

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Every ecological system is location based



Soil microaggregates develop through interactions among soil fauna, microorganisms, roots, inorganic materials, and physical processes. This is also true of aquifer materials.

Ecosystem: a biological community of interacting organisms and their physical environment.

Habitats are subcomponents of an ecosystem.

Image From: Totsche, K.U., Amelung, W., Gerzabek, M.H., Guggenberger, G., Klumpp, E., Knief, C., Lehndorff, E., Mikutta, R., Peth, S., Prechtel, A. and Ray, N., 2018. Microaggregates in soils. Journal of Plant Nutrition and Soil Science, 181(1), pp.104-136.

Rough areas and cavities are habitats that favor colonization

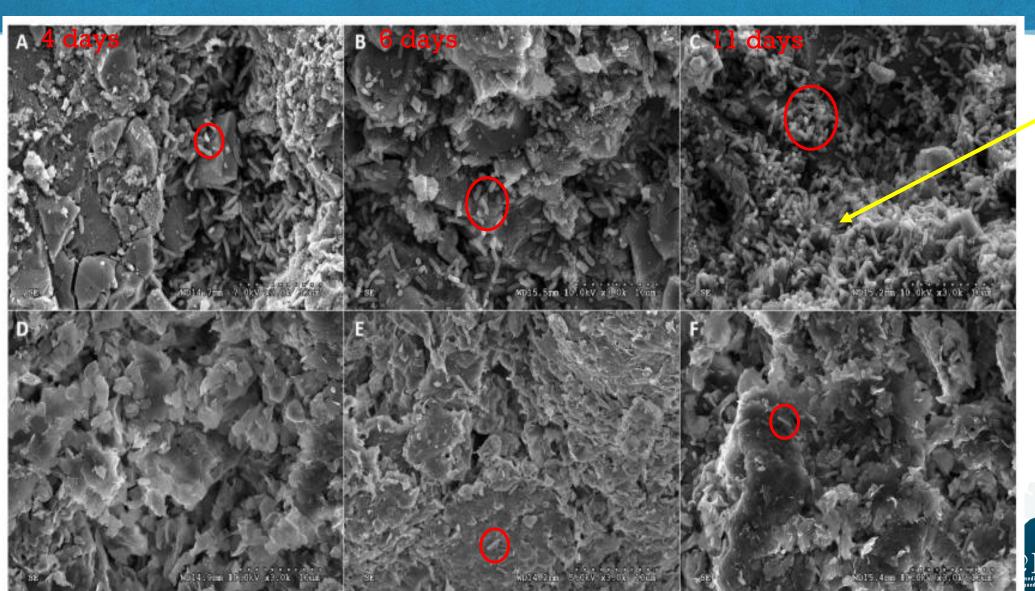


Microbial colonies growing on granular activated carbon three days after incubation. During the early stages of colonization, growth occurs primarily in rough areas and within cavities (Or, 2007).

I've circled a single bacterium in red.

Smoother elevated areas are circled in blue.

Microbial growth on Powder Activated Carbon (PAC) is more abundant than on clays. PAC provides habitat!



PAC

Clay

Microbes

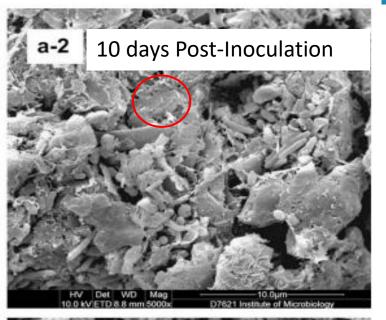
Pagnozzi, 2020

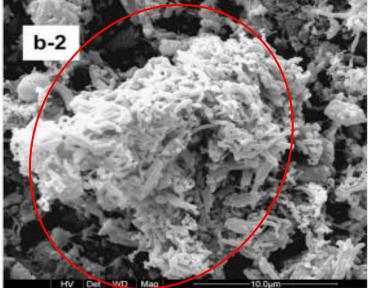
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nediation Products, Inc

Microbial growth on PAC is more abundant than on zeolite

Zeolite

Control Material





High microbial colonization of both zeolite and activated carbon were observed by SEM (Fig. 3).

The biofilm was about 5 mm thick on the zeolite and about 20 mm thick on the activated carbon

Biofilm 4X thicker on PAC to Zeolite

Liang, Y., Zhang, X., Dai, D. and Li, G., 2009. Porous biocarrier-enhanced biodegradation of crude oil contaminated soil. International Biodeterioration & Biodegradation, 63(1), pp.80-87.

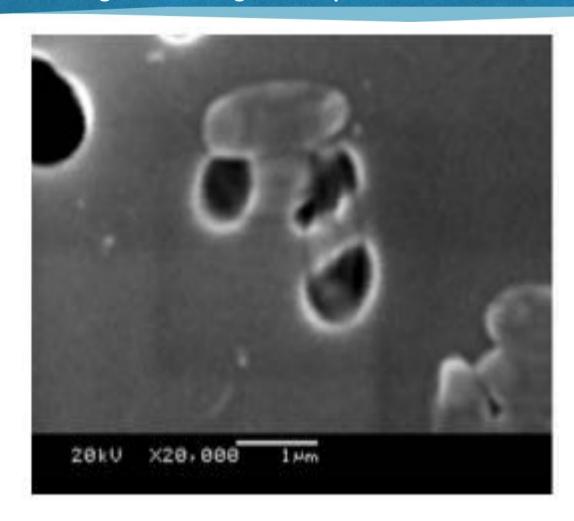


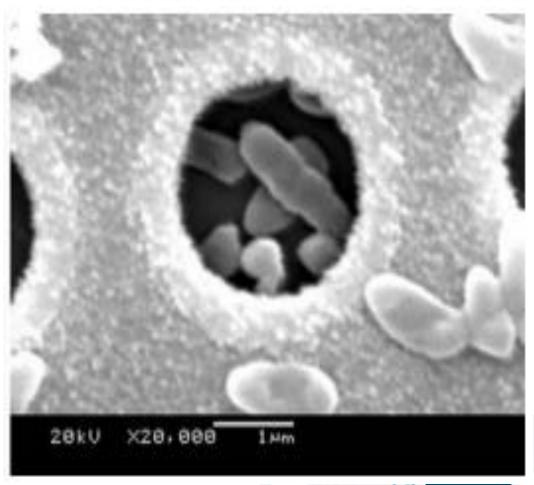
Activated Carbon

Bacteria-sized niches support growth and diversity

P. aeruginosa too large for 0.5 μm niche

P. aeruginosa "housed" in a 2 μm niche

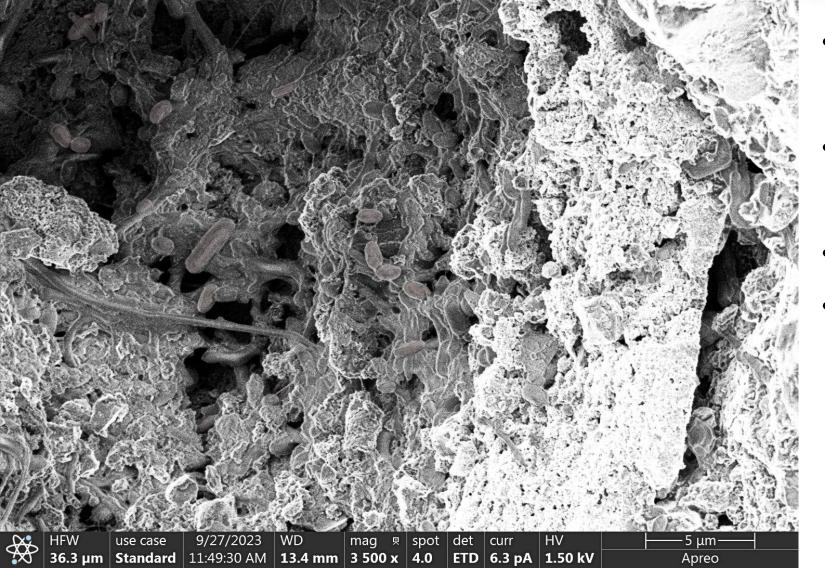




Pseudomonas aeruginosa grown of titanium oxide—Figure from Whitehead and Verran, 2006. As a range, most soil bacteria are found in pores smaller than 10 μm and are absent in pores smaller than 0.8 μm (Wang et al., 2005).

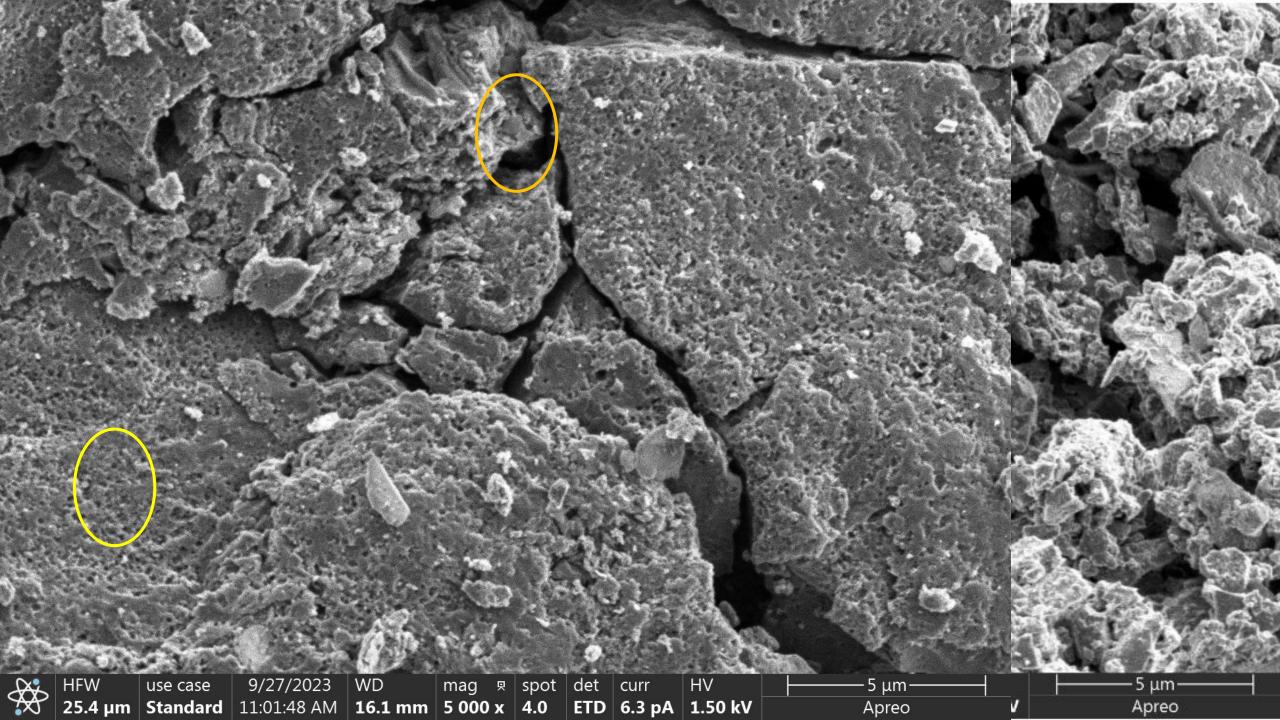


What makes good habitat? Activated Carbon



- Surface Area:
 >500 m²/g AC vs. <10 m²/g clays
- Protected environments
 Wang et al. (2005).
- Attachment-friendly roughness
- Concentrates organic materials





Bacteria-sized niches support growth and diversity

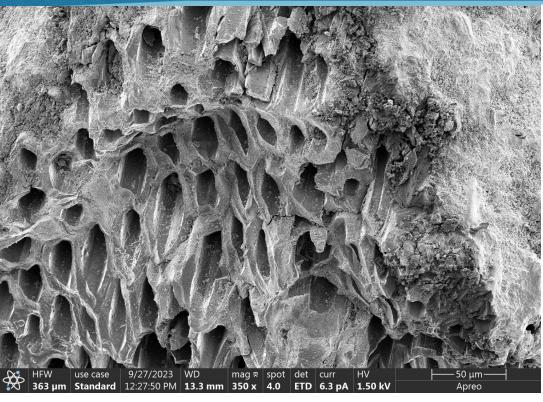


The colorized scanning electron microscope picture shows a mixed microbial population on F400 activated carbon 72 hours after inoculation. The bacteria reside almost exclusively in surface niches. The yeast had been colored yellow, and the bacteria are reddish.

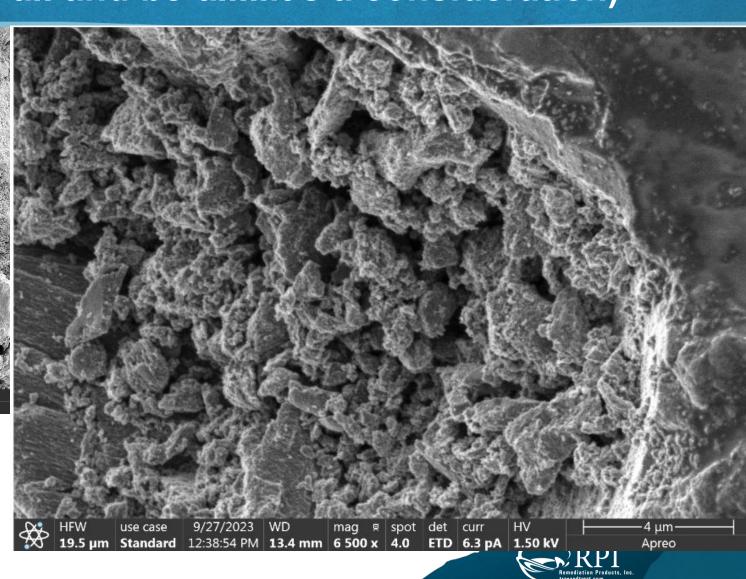
It is well published that initial adhesion is substrate-dependent (Berne et al., 2013).



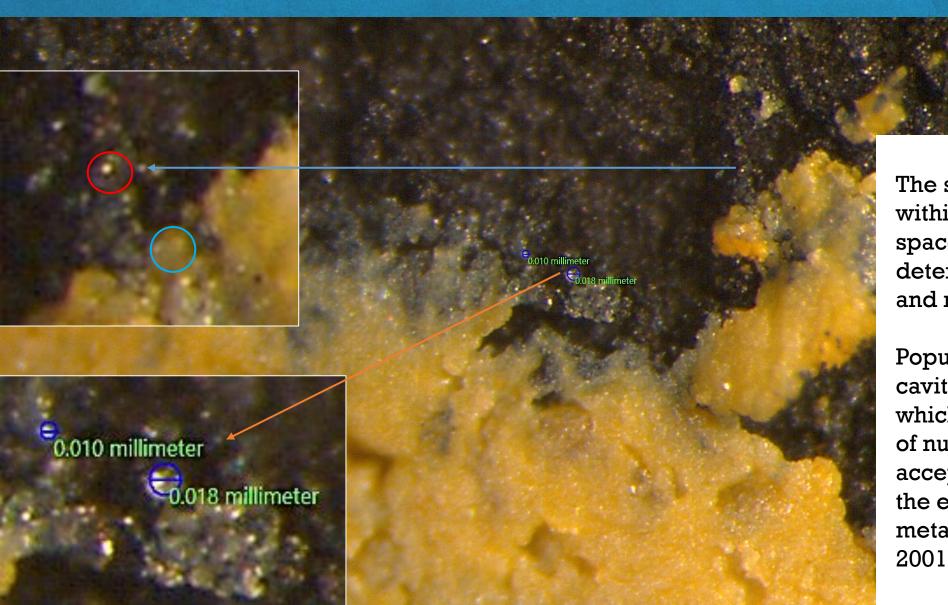
Wood-based activated carbon has large openings. (The space is not the end-all and be-all...it's a consideration)



Bacteria are not apparent at the same level of magnification as used to identify bacteria on the activated carbon. So, space is not the only important consideration.



How might spaces play out in the field - cavities and interstitial spaces in a core taken from a BOS 200® in situ injection site



The size and shape of the cavities within the media and interstitial space between media grains determine microbial size, form, and mobility (Luckner, 1991).

Population density is linked to the cavities and interstitial spaces, which are essential to the supply of nutrients, electron donors and acceptors, carbon sources, and the elimination of microbial metabolic wastes (Fredrickson, 2001).

New Flow Paths and Niches Due to Emplacement of BOS 200®



The subsurface is an ecosystem that provides the essential elements of microbial life, including moisture, chemical factors such as electron donors, and media factors like granularity. These elements are linked to the transport of water in the subsurface. It follows that microbial populations reflect groundwater flow pathways (Maamar, 2015) (Graham, 2017) (Danczak, 2018).

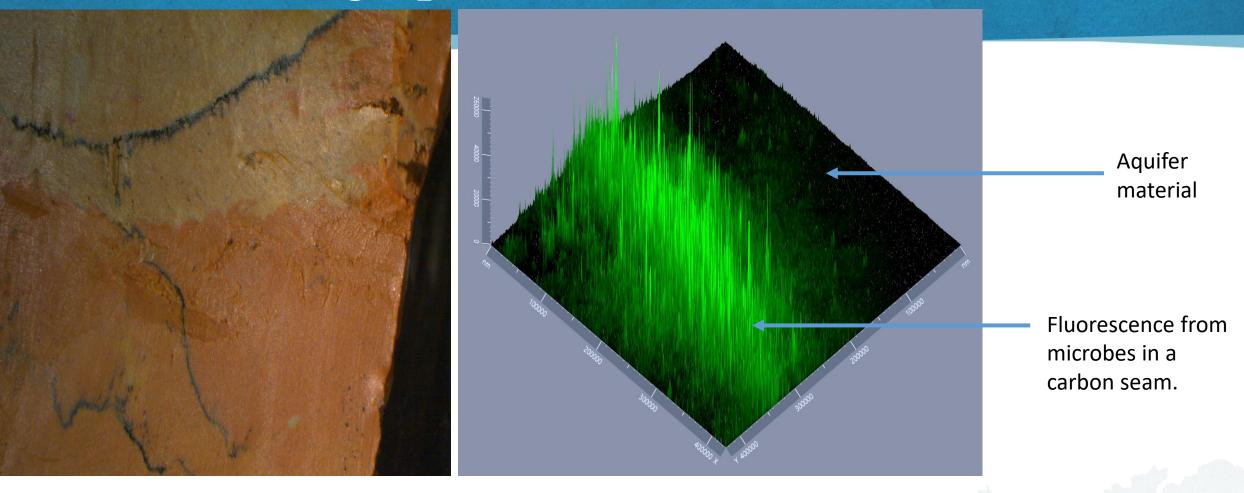
Establishment of flow paths even on a micrometer scale



The picture shows petite seams within a very fine-grained clay. If one looks closely, branching can be seen. The red "man" reflects the branching image to the right. The branching circled in green is toward the viewer.

These seams and surfaces should support contaminant dispersion, movement of nutrients and terminal electron acceptors, the interchange of microbial metabolic products, and dispersal and colonization in the aquifer.

Microbes in a carbon seam are more abundant than in the surrounding 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.



A simple experiment: Where would microbial growth initiate from in field cores?

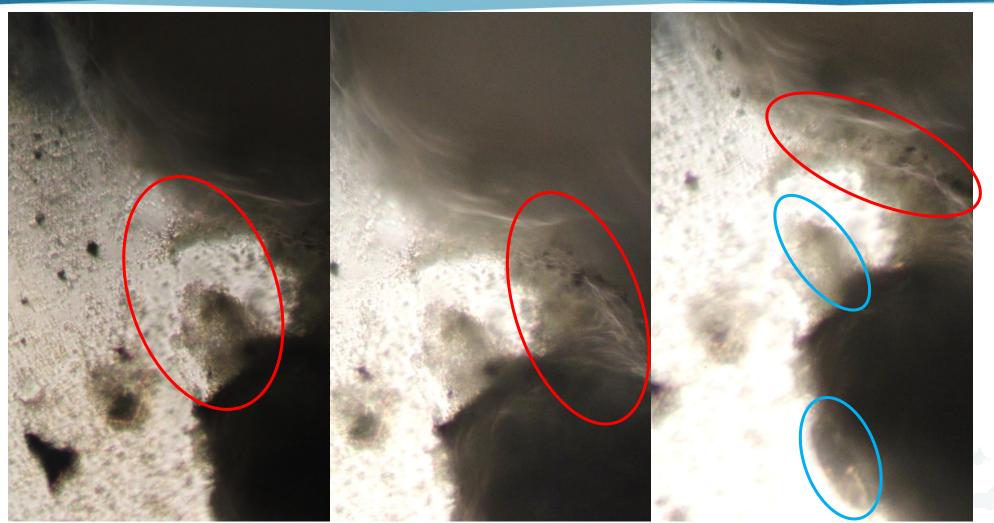




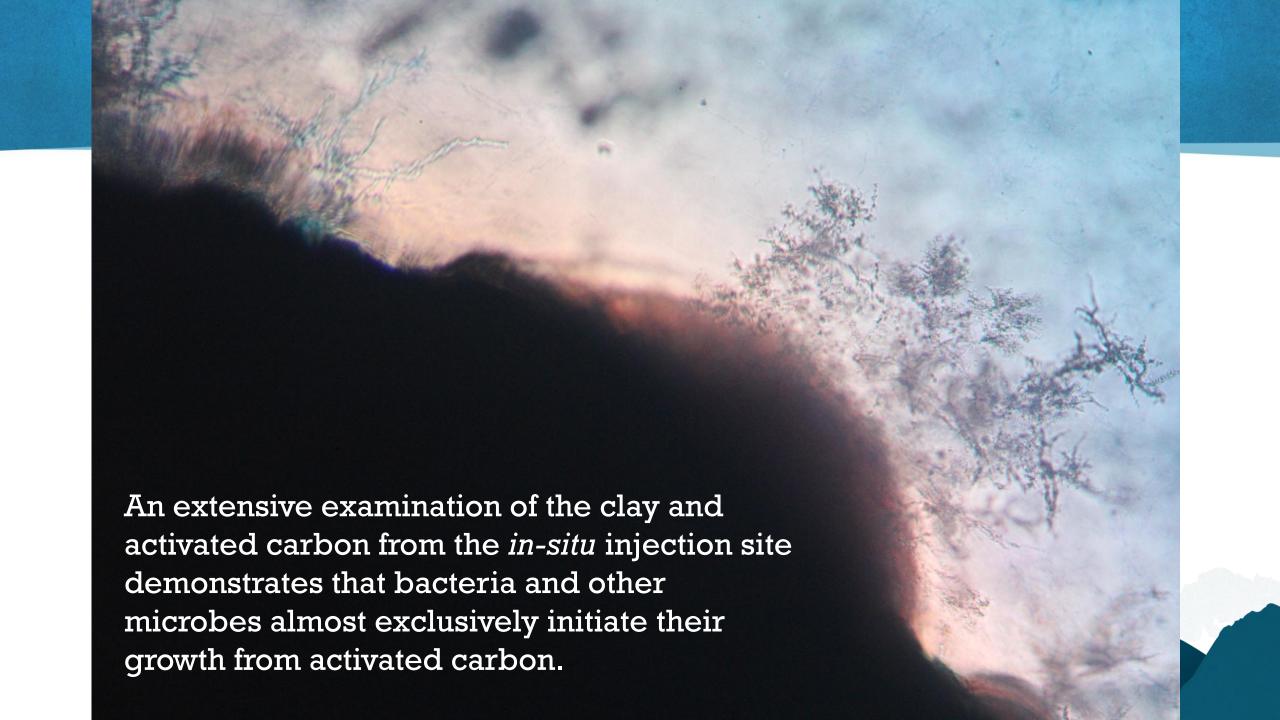


Cores of aquifer solids collected from a CAT 100 in situ injection site were embedded in LB agarose. The site injection occurred two years prior to the core collections. Incubation occurred at approximately 13 C for two weeks. Bright and darkfield microscopy examined the interface between the agar and the clay and activated carbon base of CAT 100.

The microbial population is heavy on the activated carbon base and poor on the nearby clay.



The clay is at the top of the picture, and the activated carbon is in the lower right corner. In the first picture, the bacterial mass is circled in red. In the middle picture, we focus on a clay bridge to the activated carbon, where very little bacterial growth is noted. The third picture focuses on a ridge in the clay—again, microbial growth is not evident.



Summary

- Habitat Structure is Critical for Microbial Growth
- Activated Carbon Provides a Superior Microbial Habitat
- Field Applications Confirm Laboratory Findings
- Habitat Engineering Creates New Treatment Opportunities
- Habitat is the lost component in bioremediation

Physical habitat is crucial to the success of bioremediation, and activated carbon provides a superior habitat for this process.

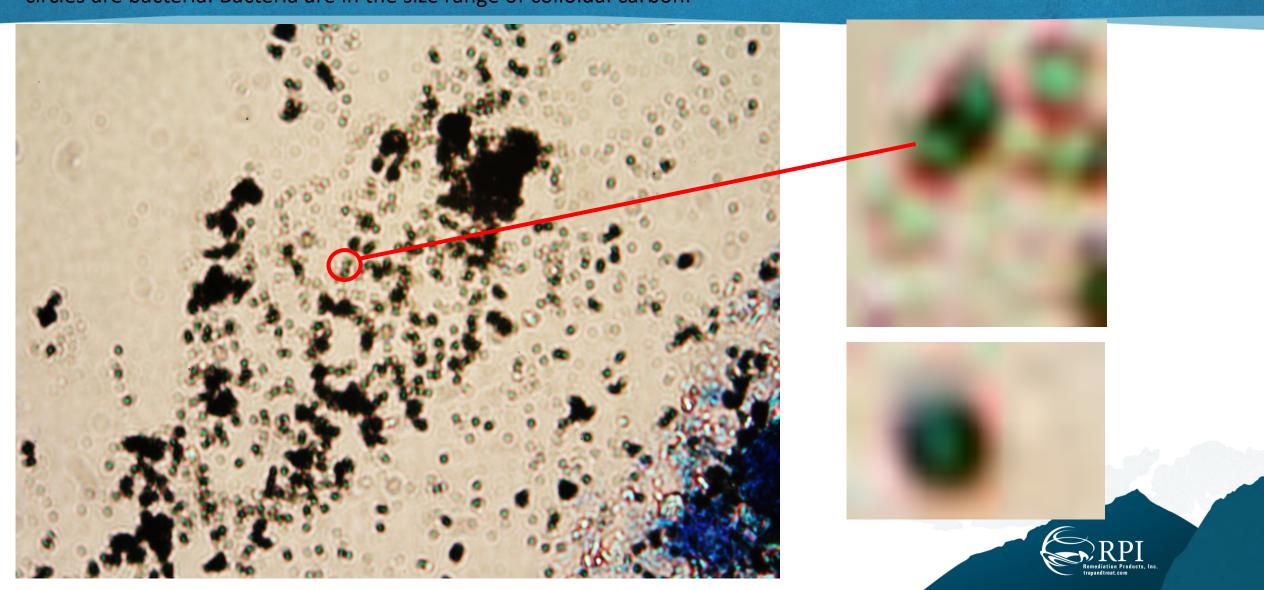
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Bacteria mixed with Colloidal Carbon (1-2 µm)

Figure 8. Bacteria were mixed with colloidal carbon to illustrate the size of the colloidal carbon versus bacteria. There was no opportunity for bacterial growth. This is just mixing. The black spots are colloidal carbon, while the green circles are bacteria. Bacteria are in the size range of colloidal carbon.



Bacteria were grown for 48 hours then

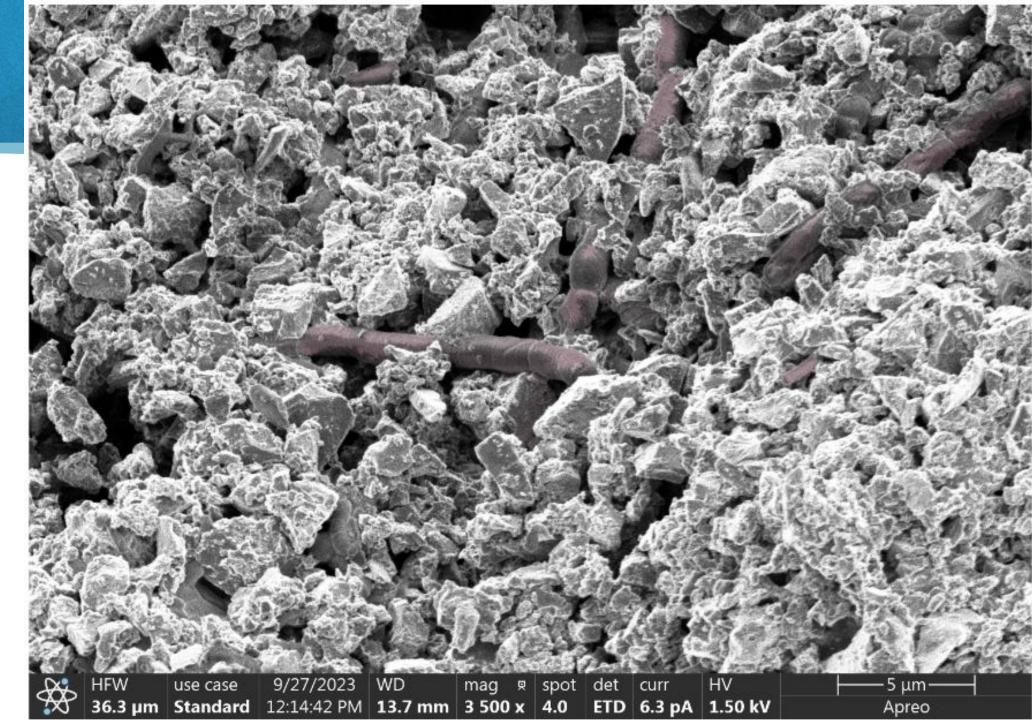
fixed on particulate AC.

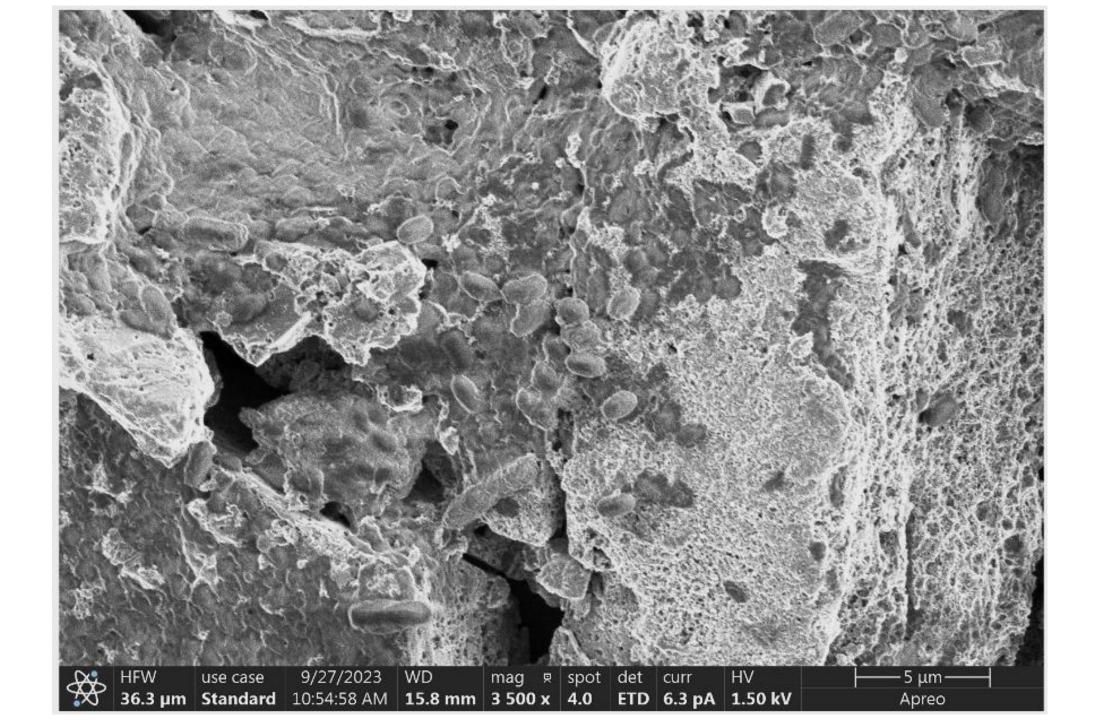
How does this picture look different from the previous pictures?

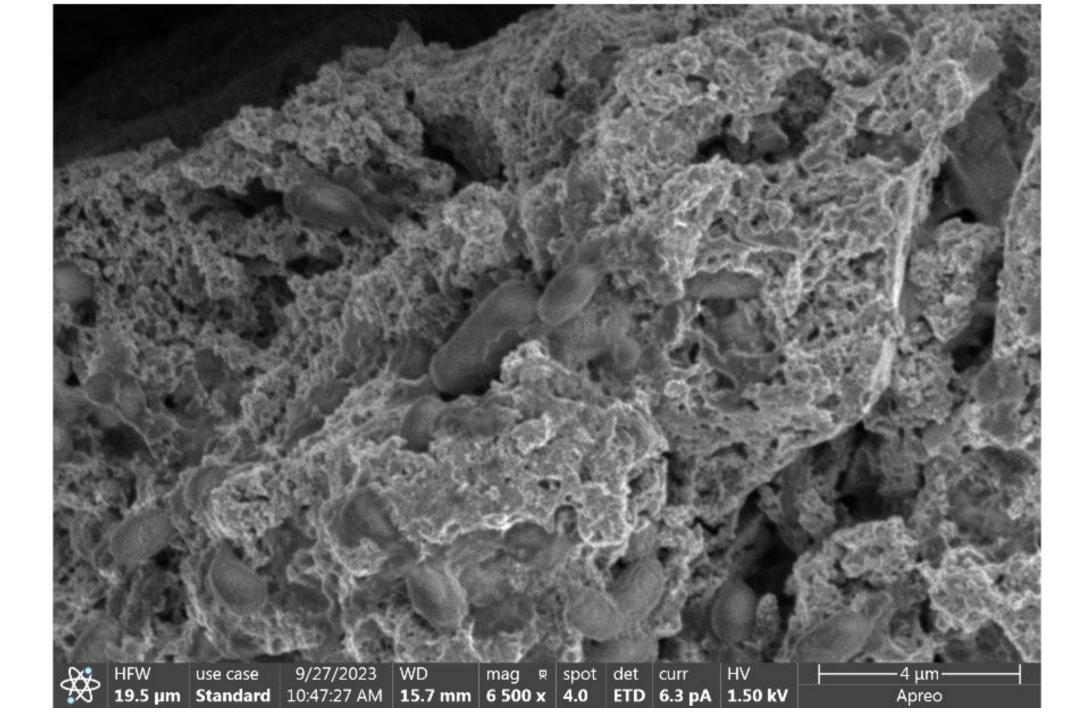
No extracellular matrix

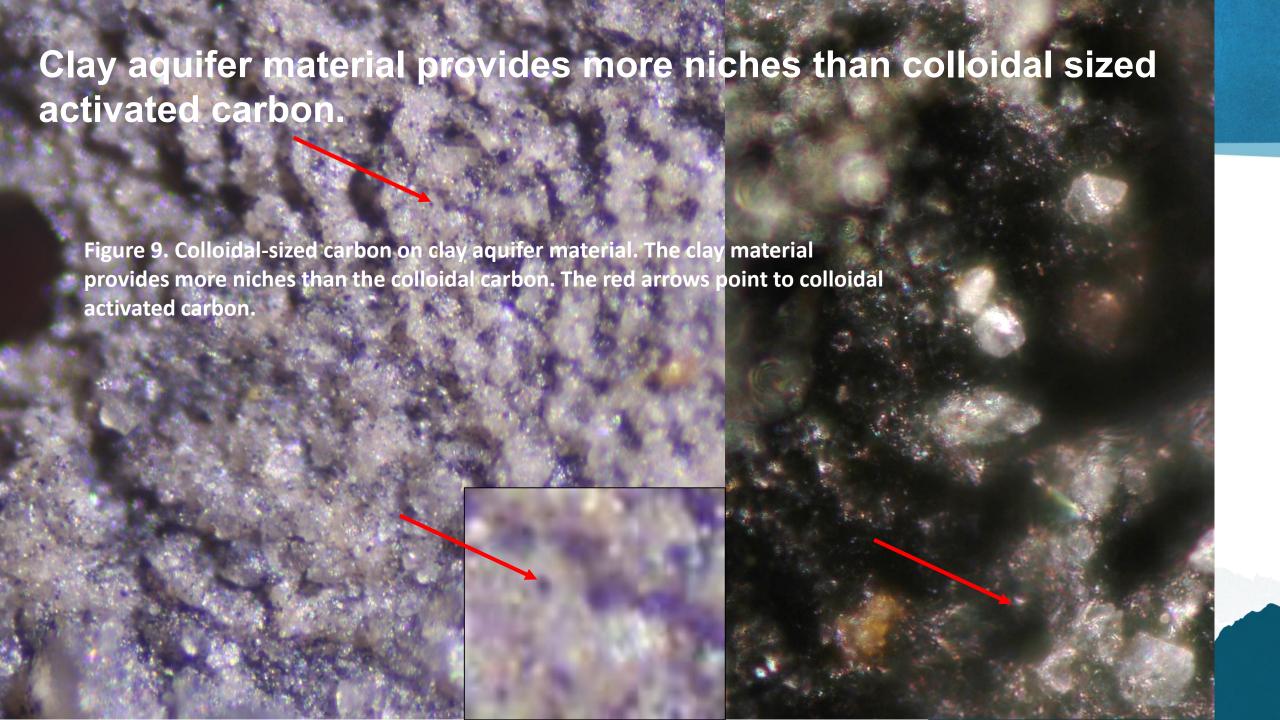
Note the material "on" the microbes

One type of large filamentous bacteria



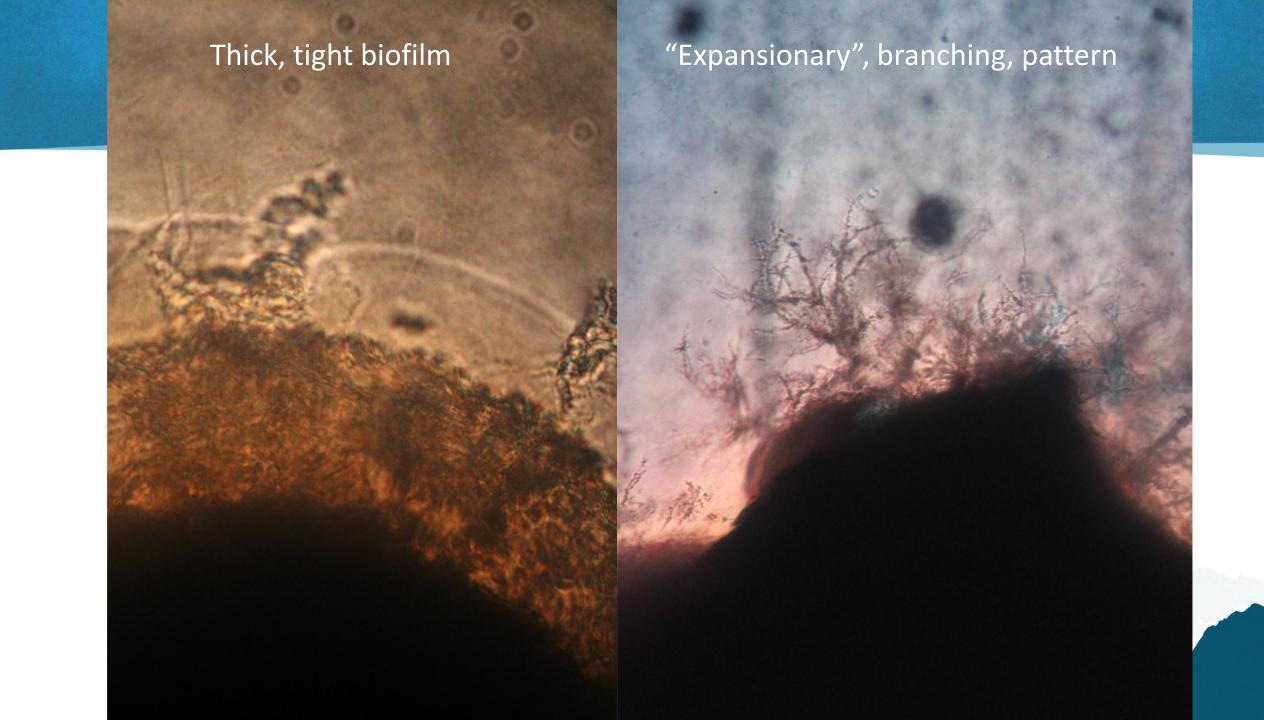






Under the same growth conditions, colloidal-size carbon does not support microbial growth, while larger AC grains do support microbial growth.

Figure 10. Colloidal activated carbon in solid LB agar with bacteria No growth is noted.



ABSTRACT OBJECTIVE:

To demonstrate the expansion of habitat in the subsurface leads to sustainable bioremediation

ABSTRACT:

The subject(s) covered are Biostimulation, bioaugmentation, abundance, richness, niche characterization and expansion, bioremediation success.

Method of engaging the audience: classroom-style presentation

Preferred presentation format: conference presentation (The images need a large screen to be appreciated. A poster does not provide the space needed to enlarge the pictures.)

Background/Objectives

Bioremediation through bioaugmentation or biostimulation often underperforms expectations. This is a problem because, in petroleum remediation, natural biological activity is often responsible for more petroleum removal over time than active remedial efforts. Is there a barrier to increasing the rate of biodegradation that is not presently being considered?

This presentation will present the thesis that the **best niches in the subsurface are filled**. The need for microbially desirable physical space must also be met to support increased bacterial abundance and diversity. This presentation will demonstrate that physical spaces, conducive to exchanging metabolic products, protecting from predation, segregating incompatible species, and building microbial communities are indispensable to sustained bioremediation.

Approach/Activities

Key elements from the peer-reviewed literature, laboratory experiments, and field samples are presented. Images from confocal microscopy, scanning electron microscopy, and brightfield microscopy will be used to demonstrate microbial abundance and bacteria location preferences. NexGen sequencing is used to characterize changes in microbial populations. Functional assays demonstrate changes in activity due to changes in physical habitat. This is a very visual presentation!

Results/Lessons Learned

The audience will understand that a successful, sustainable effort at bioremediation requires that microbes be accommodated with the appropriate and expanded habitat. They will appreciate that most soil and aquifer materials have a limited capacity to support microbial abundance significantly greater than that found before intervention by biostimulation and bioaugmentation. They will see that the failure to provide physical space that meets the needs of the microbial population they hope to sustain will result in low abundance, poor performance, and the need for ongoing intervention. This presentation will mention RPI products as activated carbon-based. It is based on information from peer-reviewed literature, laboratory studies, and field samples. It is not specifically, a product presentation. The information is broadly applicable to bioremediation.