THE CENTRALITY OF PHYSICAL SPACES TO SUSTAINED, ABUNDANT MICROBIAL POPULATIONS IN BIOREMEDIATION

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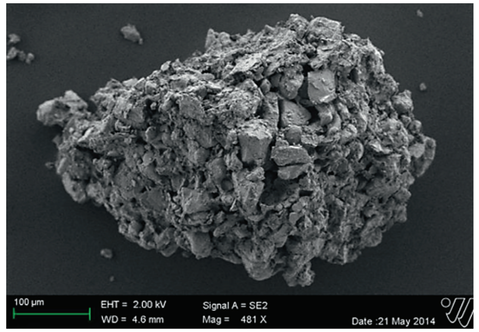
EVERY ECOLOGICAL SYSTEM IS LOCATION BASED

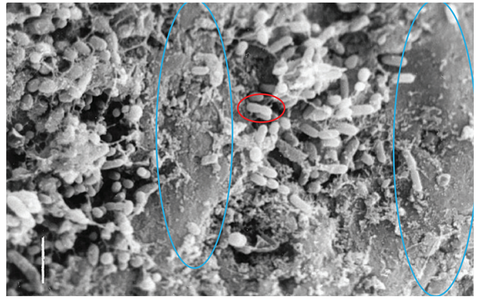


Microaggregate in soil developed from the interaction of soil fauna, microorganisms, roots, inorganics, and physical processes. This is also true of aquifer materials.

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

Habitat in an ecosystem.

ROUGH AREAS AND CAVITIES FACILITATE BACTERIAL COLONIZATION

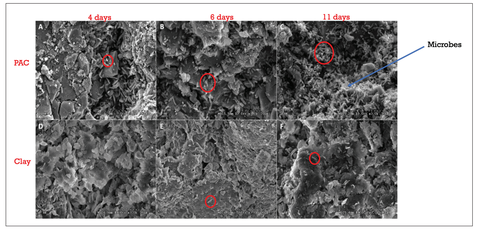


Microbial colonies growing on granular activated carbon three days after incubation. During the early stages of colonization, growth occurs primarily in rough areas and within cavities (Or, 2007).

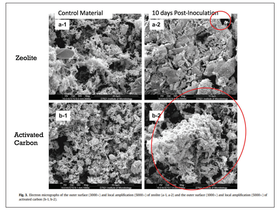
I've circled a single bacterium in red.

Smoother areas are circled in blue.

MICROBIAL GROWTH ON PAC IS MORE ABUNDANT THAN ON CLAYS, SILTS, AND OTHER GEOMATERIALS



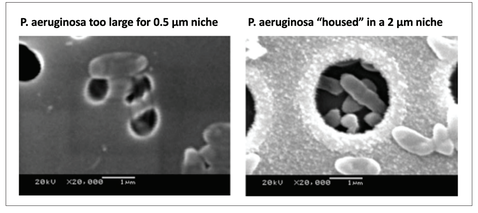
MICROBIAL GROWTH ON PAC IS MORE ABUNDANT THAN ON ZEOLITE



High microbial colonization of both zeolite and activated carbon were observed by SEM (Fig. 3). The immobilized microbes were chiefly rod and spherical shaped. 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

BACTERIA-SIZED NICHES SUPPORT GROWTH AND DIVERSITY

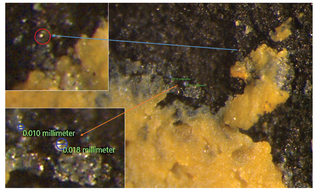


Pseudomonas aeruginosa grown of titanium oxide illustrates that features of microbial dimension retain microbes and thus provide a niche to initiate growth and support microbial diversity. Figure from Whitehead and Verran, 2006.

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.

AVAILABLE CAVITIES AND INTERSTITIAL SPACES IN A CORE TAKEN FROM A BOS200® IN SITU INJECTION SITE



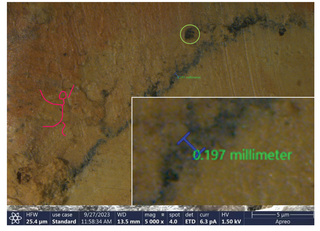
The size and shape of the cavities within the media and interstitial space between media grains determine microbial size, form, and mobility (Luckner, 1991).

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: 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. It follows that microbial populations reflect groundwater flow pathways (Maamar, 2015) (Graham, 2017) (Danczak, 2018).

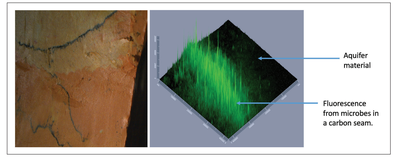
ESTABLISHMENT OF FLOW PATHS EVEN ON A MICROMETER SCALE



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.

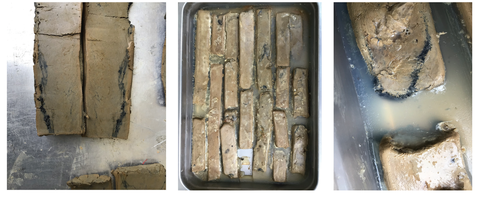
These seams and surfaces should support contaminant dispersion, movement of nutrients and terminal electron acceptors, the interchange of microbial metabolic products, and dispersal and colonization in the aquifer.

MICROBES IN A CARBON SEAM ARE MORE ABUNDANT THAN IN THE SURROUNDING AQUIFER MATERIAL



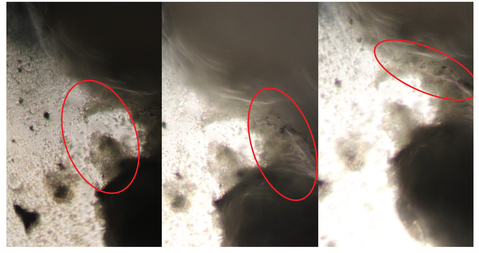
The image presented was made by alternating excitation light between 405 and 550nm wavelengths. The green column height represents intensity and is a qualitative indication of microbial density.

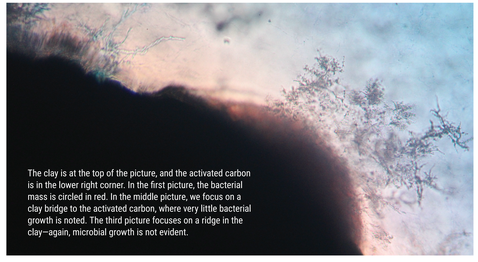
A SIMPLE EXPERIMENT: WHERE WOULD MICROBIAL GROWTH INITIATE FROM IN FIELD CORES?



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.

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