THE POTENTIAL IMPACT OF ACTIVATED CARBON GRAIN SIZE ON BIOREMEDIATION

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

ABSTRACT

The particle size (grind) of activated carbon-based remediation products may affect remedial performance. It has been proffered that particle size is a mere convenience and a benefit that allows low-flow injection techniques to be utilized for subsurface installation. While activated carbon particle size does dictate installation techniques and equipment, it is critical to understand that particle size may impact the ability of microbial populations to thrive and sustain biodegradation.

The purpose of an activated carbon emplacement in the subsurface is not simply to adsorb contaminants from groundwater. Activated carbon is also a platform for microbial growth. Activated carbon grind affects activated carbon's value as a platform for microbial growth. Grind influences activated carbon's effectiveness in initiating biofilm formation, its value as a niche for growth, its capacity to act as a resource, and its biodegradation performance.

The collected data illustrates the impact of activated carbon particle size on microbial growth. This is an initial, observational study.

ROUGH AREAS AND CAVITIES FACILITATE BACTERIAL COLONIZATI

Figure 1. Microbial colonies growing on granular activated carbon three days after incubation. During the early stages of colonization, growth occurs primarily in rough areas an within cavities (Or, 2007).



WHAT SIZE NICHE IS SUFFICIENT FOR **A SINGLE BACTERIUM?**



0.5 to 0.8 µm deep and wide



1.5 to 3.0 µm long

Figure 2a. illustrates a niche sufficient to accommodate a typical bacillus. A single niche should be deep enough to protect the bacillus from predation, that is, the bacillus should be below the surface. A niche 0.5 to 0.8 µm deep and wide and 1.5 to 3.0 µm long could accommodate a typical bacillus. This estimate presumes that the niche is cuboid.



Figure 2b. In a V-shaped groove or niche, the depth of the niche would be related to the angle between the walls such that the bacillus is sheltered yet has sufficient contact with the sides of the niche to adhere to the surface. The angles are relative to the overall size of the niche. Using the range of 30° to 135°, the depths of the niches would be approximately 1.9 to 1.2 µm, respectively.

ESTIMATION OF MINIMAL SMOOTH SURFACE PEAKS FOR BACTERIAL NICHES

Figure 3. A surface that has a peak of about 0.110 µm and a 10° angle of incline to the peak, gives a periodicity of 1.134 µm. This periodicity allows an average bacillus to lay within the trough with significant contact to the surface. The peak-totrough ratio must be balanced such that the microbe of interest maintains sufficient contact with the surface. Steep inclines and close-spaced peaks inhibit microbial attachment and, as such, are antimicrobial (Luo, 2020) (Siddiquie, 2020).

Figure 4. 3D printed polylactic acid polymer surfaces Observations:

- Biofilm first filled the grooves.
- Later, the biofilm bridged the grooves.

Point:

Figure from Hall et al., 2021.

ESTIMATION OF MINIMAL GRAIN SIZE TO SUPPORT BACTERIAL GROWTH

Figure 5. If a niche is approximately 0.8 µm, as in Figure 2a,b., and the niche is half the depth of the grain, then the grain is 1.6 µm in diameter. If four AC grains are grouped around a central space or atrium that can also hold a bacillus, then the distance across the three grains would be 4.8 µm or about 5 µm. By this, we estimate the minimal grain size relevant to microbial interaction.

BACTERIA ON TITANIUM OXIDE ILLUSTRATES MICROBES IN 2 µm NICHE

Figure 6. Pseudomonas aeruginosa grown of titanium oxide illustrates that features of microbial dimension retain microbes and thus could provide a niche in which to initiate growth and support microbial diversity. Figure from Whitehead and Verran, 2006.



GROOVES AS BACTERIA-SIZED NICHES

Biofilm formation is significantly impacted by surface opography at the micrometric scale, comparable with the size of prokaryotic cells. Thus, to build subsurface biofilms, we should look to materials that have topologies that provide bacteria-sized niches



A MODEL-BASED **ARGUMENT FOR NICHES THAT** HOLD MULTIPLE BACTERIA **AND THUS SUPPORT MICROBIAL DIVERSITY**

Figure 7. Two microbial species Sp1 and Sp2 are grown together in either a homogeneous niche (A & B) or in a heterogeneous niche (D & C). From Or, 2007.

- In a homogenous niche a more competitive species (Blue) circles) will dominate the niche.
- In a heterogeneous group of niches, some species may locate in niches isolated from competing species.
- Thus, greater availability of niches translates into greater microbial diversity.

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.





P. aeruginosa too large for 0.5 µm niche

20kU X20,000 1.4m

P. aeruginosa "housed" in a 2 µm niche



CLAY AQUIFER MATERI PROVIDES MORE NICHES THAN COLLOIDAL SIZED ACTIVATED CARBON

Figure 9. Colloidal-sized carbon on clay aquifer material. The clay material provides more niches than the colloidal carbon. The red arrows point to colloidal activated carbon.

COLLOIDAL-SIZE CARBON DOES NOT APPEAR TO SUPPORT MICROBIAL GROWTH

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





GRANULAR ACTIVATED CARBON IN THE SAME GROWTH ENVIRONMENT AS THE COLLOIDAL-SIZE ACTIVATED CARBON IN FIGURE 10

Figure 11. Bacterial growt is evident. The red arrows

Figure 12. Same time

period and environment as

Figures 11 and 12 show rich

bacterial growth. The black

in the lower right corner is

granular activated carbon.







Figure 13. The left picture, lower left corner, shows a granular activated carbon (GAC) grain. The grain is covered in microbial growth. The smaller grains (yellow and green circles) are in the powdered activated carbon range. Both show microbial growth. The smallest grains are in the colloidal range (1-2 µm)(blue circle) and demonstrate no discernable bacterial growth.

Figure 14. Microbial growth on granular activated carbon-sized grains. Growth was also visible on the grains in the left corner. Due to shallow focal depth, all grains cannot be in focus together. The smallest grains show no growth.









CONSIDERATIONS

Activated carbon (AC) grains in the smallest size range (X< \approx 5 µm) do not visually demonstrate bacteria growth at one month, 3 months, or one year. The time periods examined. Time data are not specifically presented.

To function as microbial habitat, AC must have sufficient surface features to allow bacterial adhesion (Lu et al., 2020). The consideration of bacterial sizes to potential niche sizes provides logic to the potential minimal grain size for activated carbon that can act as a platform for microbial growth.

Creating new niches can shelter communities, restrict diffusion to support thriving communities, and enhance bacterial diversity (Torsvik and Ovreas, 2008).

Obvious bacterial growth on the larger grain sizes may be due to larger grain sizes providing superior niches and/or holding more adsorbed resources. This was not tested. This is an observation consistent with published literature in that biodegradation on AC depends on biomass, growth rate, and microbial community structure (Lu et al., 2020; Gagnon and Huck, 2001; Oh et al., 2018).

Supporting the mechanisms that promote and maintain microbial diversity is the central challenge for bioremediation. These observations and the published literature support a contention that activated carbon grain size should be a consideration.

REFERENCES

- Gagnon, G.A. and Huck, P.M., 2001. Removal of easily biodegradable organic compounds by drinking water biofilms: analysis of kinetics and mass transfer. Water Research, 35(10), pp.2554-2564.
- Hall Jr, D.C., Palmer, P., Ji, H.F., Ehrlich, G.D. and Król, J.E., 2021. Bacterial biofilm growth on 3D-printed materials. Frontiers in Microbiology, 12, p.646303.
- 3. Lu, Z. S. (2020). Effect of granular activated carbon pore-size distribution on biological activated carbon filter performance. Water Research, 177(15), 115768.
- 4. Luo, X. Y. (2020). Biocompatible nano-ripples structured surfaces induced by femtosecond laser to rebel bacterial colonization and biofilm formation. Optics & Laser Technology, 124, 105973. 5. Oh, S., Hammes, F. and Liu, W.T., 2018. Metagenomic characterization of biofilter microbial communities in a full-scale drinking water treatment plant. *Water*
- *research*, 128, pp.278-285. Or, D., Smets, B.F., Wraith, J.M., Dechesne, A. and Friedman, S.P., 2007. Physical constraints affecting bacterial habitats and activity in unsaturated porous media
- a review. Advances in Water Resources, 30(6-7), pp.1505-1527. Siddiquie, R. Y. (2020). Anti-biofouling properties of Femtosecond laser-induced submicron topographies on elastomeric surfaces. Langmuir, 36, 5349-5358.
- 8. Torsvik, V. and Øvreås, L., 2008. Microbial diversity, life strategies, and adaptation to life in extreme soils. *Microbiology of extreme soils*, pp.15-43.
- 9. Whitehead, K.A. and Verran, J., 2006. The effect of surface topography on the retention of microorganisms. *Food and bioproducts processing*, 84(4), pp.253-259.