Supplementary Material For ES&T Manuscript ES9808696: ***Sequential Anaerobic Dechlorination Of Pentachlorophenol: Competitive Inhibition Effects And A Kinetic Model***

Victor S. Magar*, H. David Stensel, Jaakko A. Puhakka, and John F. Ferguson

TEM Examination of Biofilm Depth on the Celite® Support Matrix.

Transmission electron microscope (TEM) studies were conducted to evaluate the thickness of the biofilm surrounding and contained within the spherical Celite® (World Minerals, Inc.; Lompoc, CA) particles in the FBRs. The Celite® used in these experiments (Type R-633) had a 30/50 mesh size (i.e., 80% of the particles are between 300 and 600 μm in diameter) and a specific surface area of 1.3 m^2/g (World Minerals, Inc.; Lompoc, CA).

Celite® particles from FBR-1 were prepared for examination by TEM by fixing the biomass with gluteraldehyde, followed by an osmium (OsO_4) fix to enhance TEM resolution. The Celite® particles were suspended in 3% gluteraldehyde in reduced anaerobic medium (RAM) for 1 hour; RAM maintained ionic and anaerobic conditions. The gluteraldehyde-fixed Celite® particles were rinsed four times with RAM followed by a one-hour fix in 4% OsO_4 in RAM. The samples were dehydrated with ethanol and dried with a Samdri-780 critical point dryer (Tousmins Research Corp, Rockville, NY).

The critical-point dried Celite®/biofilm particles were embedded in resin (Epon 812 resin; Electron Microscopy Sciences, PA), cut into thin sections (less than 100 Å), and viewed with a Philips Electronics (NV Eindhoven, The Netherlands) CM 100 TEM, equipped with a model 690 wide-angle, slow-scan CCD camera (Gatan, Inc., Pleasanton, CA). Photographs were taken of two samples and are shown in Figures 1 through 4. The black areas represent Celite®; loss of Celite® occurred when the samples were cut due to its brittle characteristics, indicated by the white areas immediately surrounding the Celite®. Bacteria are located between the Celite® mass and are spherical or cylindrical in shape.

* Corresponding author address Battelle, 505 King Avenue, Columbus, OH, 43201; tel: (614) 424-4604; fax: (614) 424-3667; e-mail: magarv@battelle.org.

Figures 1 and 2 show two photographs taken of a single Celite® particle, photographed at 5800 times magnification. Figure 1 shows the outer 15 μm of biofilm Celite® particle, where all the pores appear to be occupied by bacteria. Figure 2 was taken from the same sample, deeper into the biofilm, and overlaps approximately 5 μm with Figure 1. In Figure 2, the density of the bacteria is substantially reduced, showing that the bacteria did not significantly penetrate the Celite® particle beyond 15μM and most of the bacteria occupied the outer surface of the particle.

Figures 3 and 4 were taken from a second Celite® particle at 5800 and 10,500 times magnification, respectively. Similar to Figure 1, Figure 3 shows that the pore spaces in the outer 18 μm of the particle are densely populated with bacteria. Figure 4 was taken approximately 100 μm from the outer edge of the Celite® particle. Although bacteria are present, and in spite of the higher magnification, Figure 4 shows that the concentration of bacteria 100 μm deep in the particle was much lower than in the outer 18 μm show in Figure 3. These results confirm that most of the bacteria occupied the outer surface of the Celite® particles. For the second particle, the density of bacteria dropped off at approximately 40 μm from the outer edge of the particle.

Although the Celite® used in the FBRs had a reported specific surface area of 1.3 m^2/g Celite®, the TEM results demonstrate that the entire surface area was not occupied by bacteria and that the bacteria tended to colonize the outer surface of the spherical Celite® particles. Based on empirical observation of numerous samples, the biofilm depth appeared to range from greater than 15 μm to less than 50 μm, suggesting a relatively thin biofilm in the FBRs.

A thin biofilm located on the outer surface of the Celite® particles should result in a relatively low biomass density in the FBRs. The total Celite® external surface area in the FBRs was calculated based on the following Celite® specifications provided by World Minerals, Inc. (Lompoc, CA).

- The interstitial pore volume for compact Celite® is 0.3758 cc/g Celite®, and the total volume is 2.83 cc/g Celite®; thus the Celite® volume excluding the interstitial pore volume but including the internal pore volume is 2.45 cc/g.

- Assuming an average particle diameter of 0.45 mm (this equates to an average particle volume of 4.77×10^{-5} cc and external surface area of 6.36×10^{-3} cm^2), the Celite® contained approximately 51,400 particles/g Celite® with an external surface of 327 cm^2/g Celite®.
- On the basis of the above calculation, the specific surface area (1.3 m^2/g Celite®) was approximately 40 times greater than the external surface area.

During the batch kinetic tests, the Celite® in the FBRs contained approximately 10 mg VS/g Celite®. Using the 327 cm^2 for the surface area, the VS concentration per unit surface area was or 3.06×10^{-2} mg VS/cm^2 Celite®. Assuming a biofilm density of 4% (40 g VS/L), the biofilm depth is calculated as follows:

$$\text{Depth, L} = \frac{0.0306 \text{ mg VS/cm}^2}{40 \text{ mg/cm}^3 \text{ VS}} = 7.6 \times 10^{-4} \text{ cm} = 7.6\,\mu\text{m}$$

The value of 7.6 μm compares fairly well with the TEM. If the specific surface area (1.3 m^2/g Celite®) were used instead of the calculated outer surface area of the particles, the calculated biofilm thickness would be approximately 0.2 μm, which is significantly less than the TEM-measured thickness. This analysis confirms that the bacteria colonized the outer surface of the Celite® particles and not the internal pore surfaces, and that a thin biofilm (less than 50 μm) was present.

The fact that the biofilm occupied the outer surfaces of the Celite® particles and did not penetrate them indicates that the internal pores were not used optimally. It also suggests that diffusion limitations for CP diffusion into the Celite® particles could be ignored. Furthermore, because the biofilm was less than 50 μm, a thin film could be and CP diffusion into the biofilm also could be ignored.

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Figure 1. Transmission electron micrograph of a Celite® particle from the outer surface to a depth of 15 μm, with bacteria colonizing the pore spaces. Black regions are Celite® embedded into the resin; gray regions are pores that contain bacteria; white regions are regions that lost Celite® during cutting.

Figure 2. Transmission electron micrograph of a Celite® particle, beginning approximately10 μm from the outer surface to a depth of approximately30 μm. Same particle as Figure 1. Note that the bacteria did not significantly penetrate the Celite® particle.

Figure 3. Transmission electron micrograph of a Celite® particle, from the outer surface to a depth of approximately18 μm, with bacteria colonizing the pore spaces.

Figure 4. Transmission electron micrograph of same particle as Figure 3 at a magnification of 10,500 and 100 μm from the outer edge of the Celite® particle.

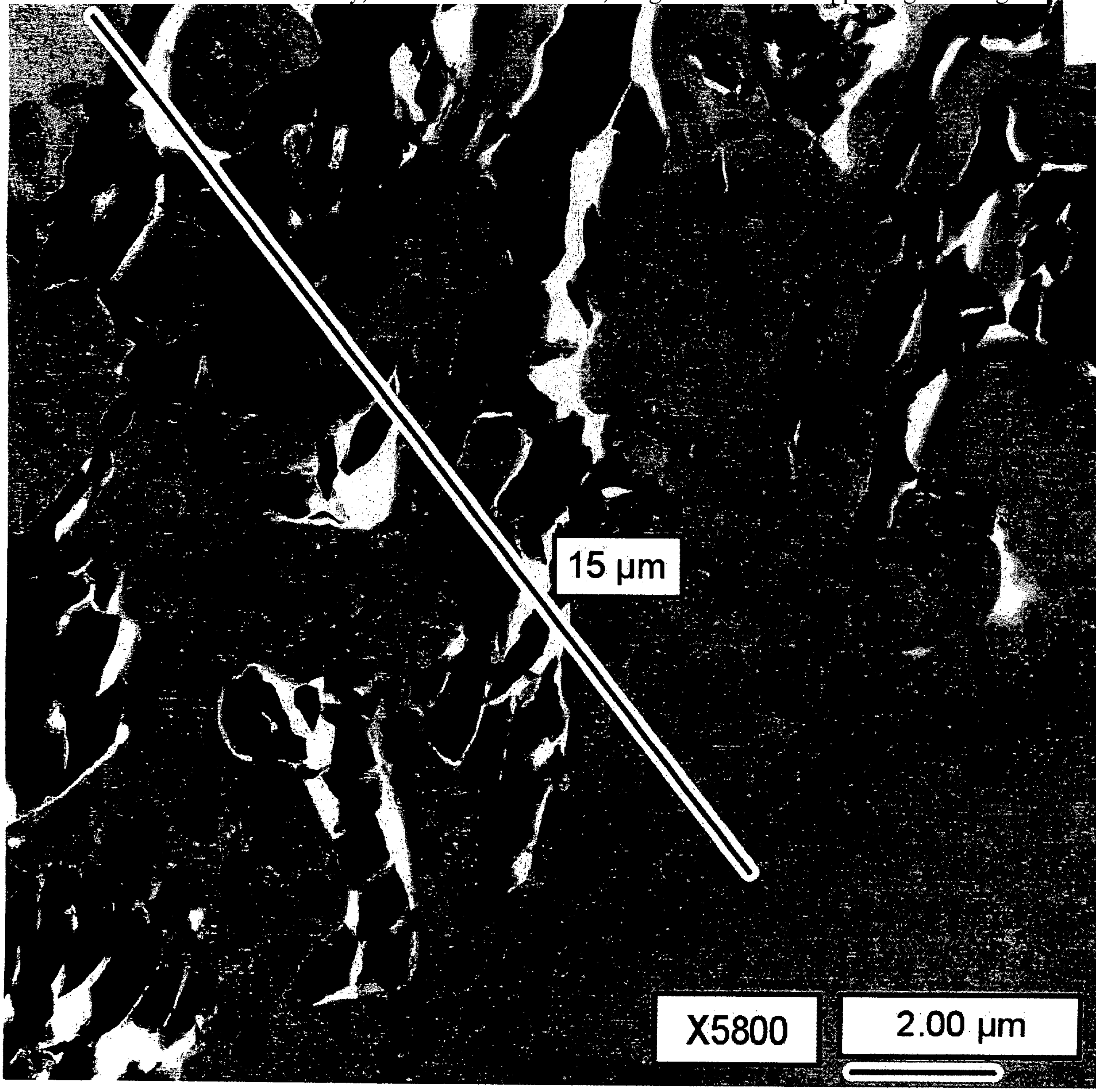

Figure 1. Transmission electron micrograph of a Celite® particle from the outer surface to a depth of 15 µm, with bacteria colonizing the pore spaces. Black regions are Celite® embedded into the resin; gray regions are pores that contain bacteria; white regions are regions that lost Celite® during cutting.

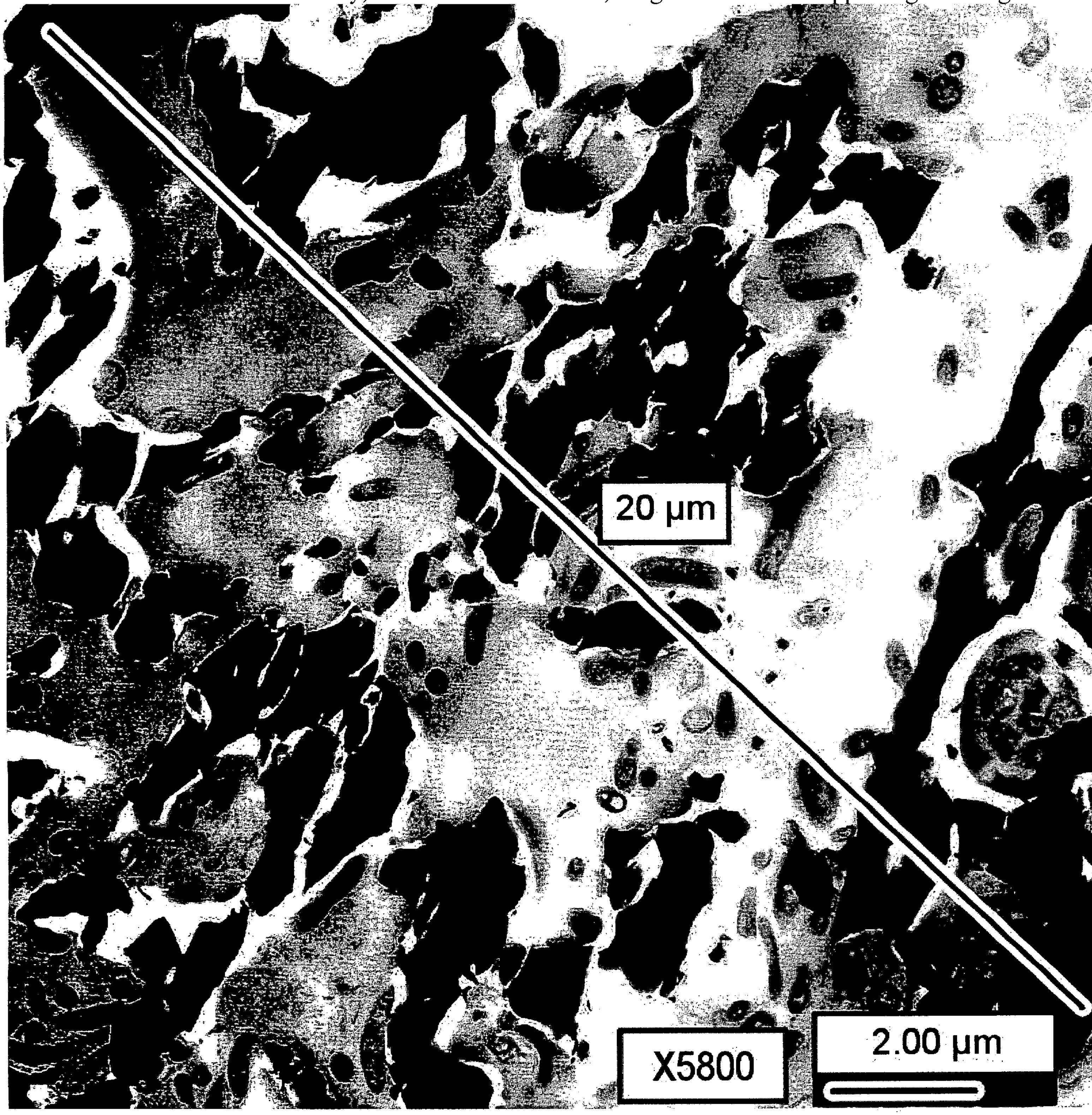

Figure 2. Transmission electron micrograph of a Celite® particle, beginning approximately 10 µm from the outer surface to a depth of approximately 30 µm. Same particle as Figure 1. Note that the bacteria did not significantly penetrate the Celite® particle.

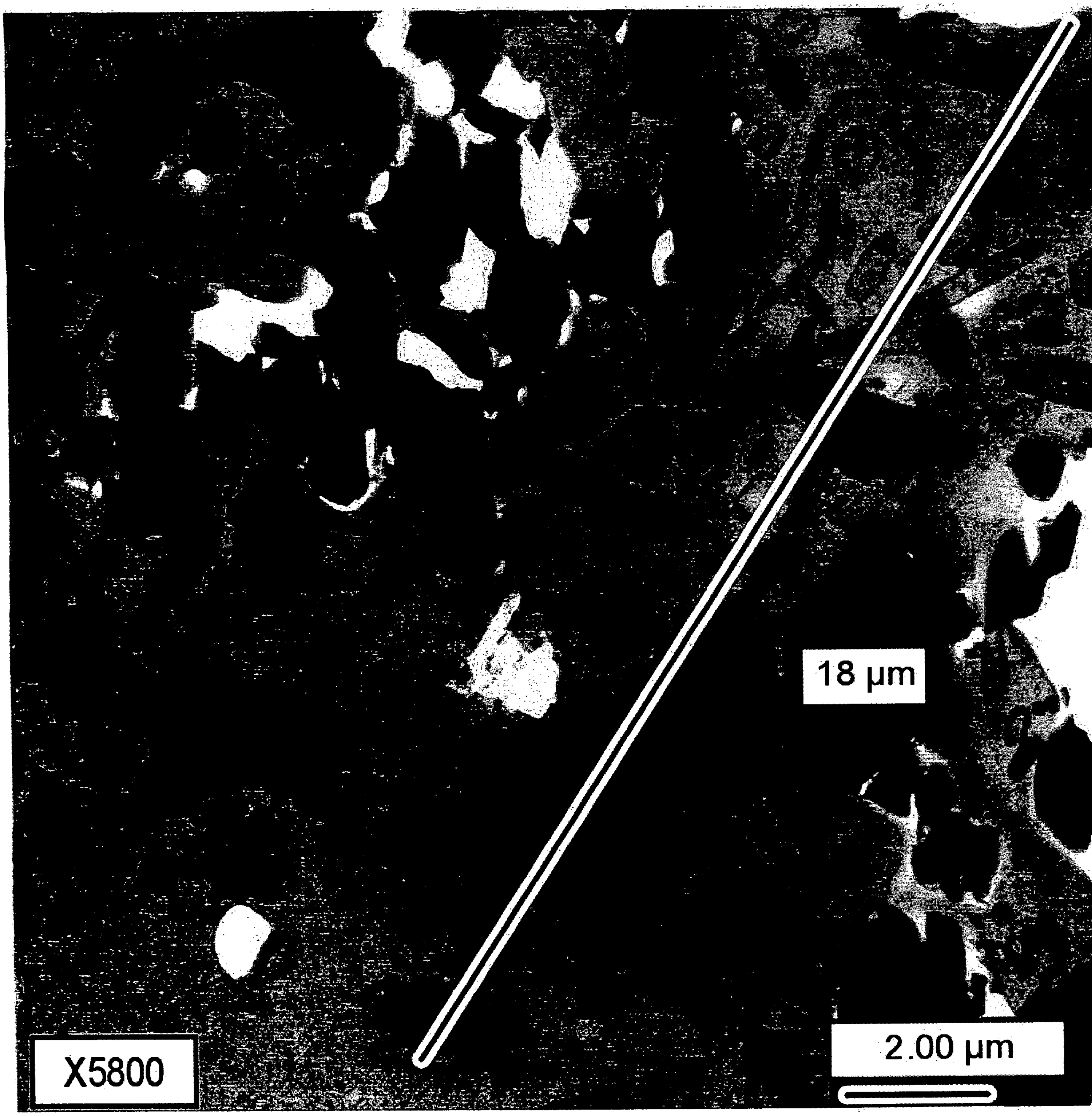

Figure 3. Transmission electron micrograph of a Celite® particle, from the outer surface to a depth of approximately 18 μm, with bacteria colonizing the pore spaces.

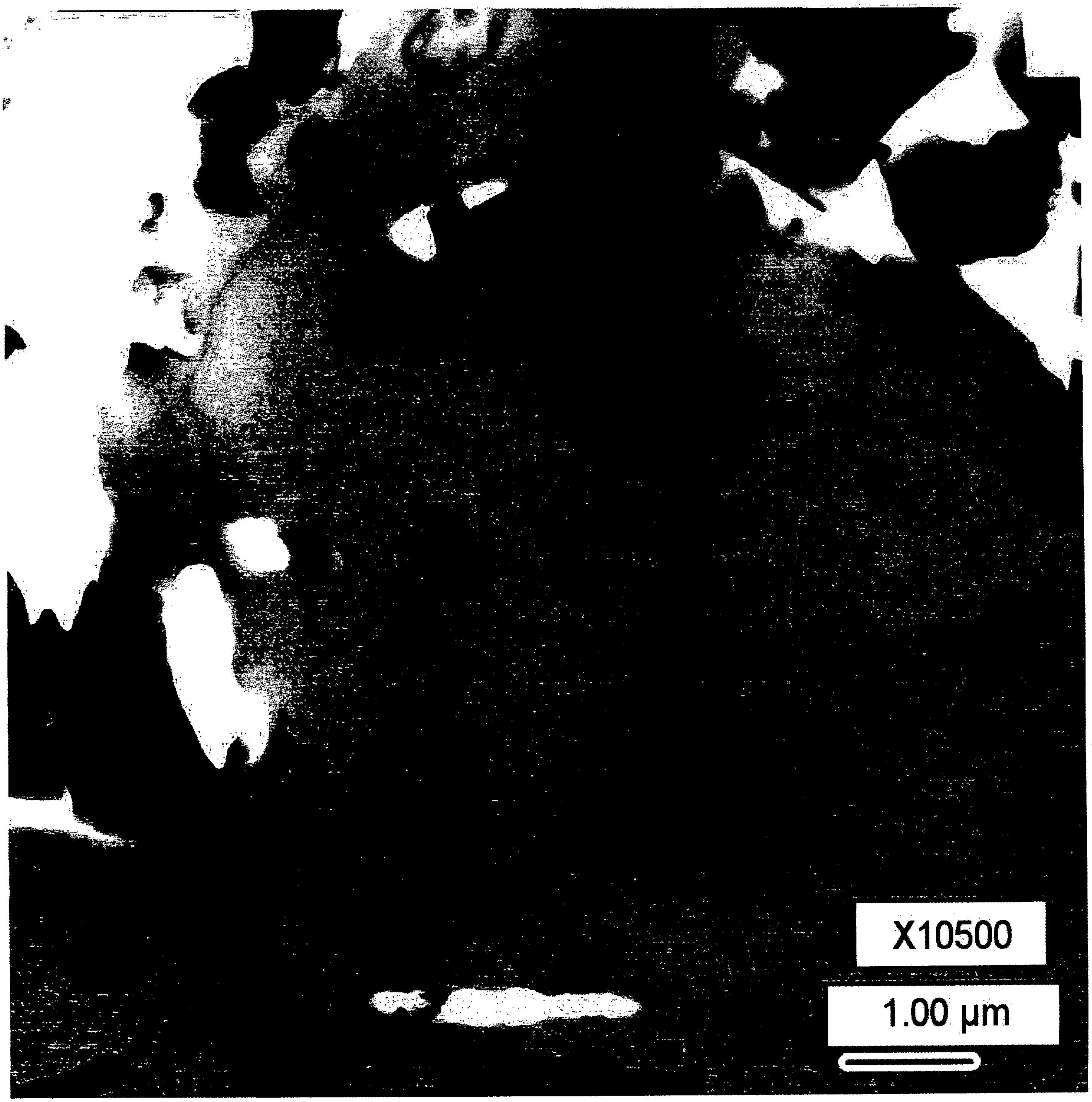

Figure 4. Transmission electron micrograph of same particle as Figure 3 at a magnification of 10,500 and 100 µm from the outer edge of the Celite® particle.