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**ADAPTATION OF ENVIRONMENTAL TRANSMISSION ELECTRON MICROSCOPY (ETEM)
AND ELECTRON ENERGY LOSS SPECTROMETRY (EELS)
FOR STUDIES OF MICROBIOLOGICALLY INFLUENCED CORROSION**

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Microbiologically influenced corrosion (MIC) is of wide concern in marine and non-marine environments. Biofilms and corrosion products associated with microorganisms cause numerous problems in aqueous environments, such as increased fluid frictional resistance, reduced heat transfer, and many types of corrosion, all of which can lead to failure of materials. Corrosion of metals has been extensively examined using TEM, but examination of MIC with TEM has only just begun.^{1,2} Previous studies examining microbial colonization of copper surfaces and distribution throughout corrosion products demonstrate copper immobilization by bacterial biofilms.³ In the current study, *Pseudomonas putida* attachment to corroding iron particles was examined in a sealed environmental cell in a JEOL 3010 scanning transmission electron microscope (STEM).

Iron filings were produced from carbon steel (C1010) using 600 grit sandpaper, collected with a teflon coated magnet, degreased in acetone and sterilized in ethanol. Filings were incubated in distilled water until corrosion was visible under a dissecting microscope. *Pseudomonas putida* was maintained in brain-heart-infusion broth at room temp. Log phase cells were washed in distilled water (dH₂O) two times and incubated with corroding filings overnight on a rotator. After incubation, samples were rinsed in dH₂O to remove excess biopolymer and soluble ions, and examined in the STEM. Additional ports on the environmental cell allow injection of two separate liquids (Figure 1). Ports were filled with dH₂O to wash excess sample from the viewing windows. Ports also can be used to inject chemical solutions to view reactions directly in the environmental chamber.

Traditional examination of non-fixed, hydrated bacteria on coated grids in the TEM demonstrate collapse of extracellular polymers into stringy bridges between cells, and general collapse of cell structure (Figure 2). Fixation and dehydration preserve some of the structure, but buffer and organic solvent washes remove soluble ions from extracellular and intracellular sources. The environmental cell allows examination of unfixed, hydrated specimens directly in the TEM. Residual water in the specimen lowers resolution and scatters electrons, but enough information is transmitted to observe reactions, obtain electron diffraction patterns, and compose good micrographs (Figure 3). A thick layer of extracellular polymer surrounding the bacterium retains moisture. The polymer serves several purposes, including water retention to protect from dehydration and harsh environments, and as a region to store enzymes, and potential food sources. The layer of extracellular polymer on the bacterium surface in this micrograph has bound iron from the corroding iron particle, as demonstrated by EELS analysis of the hydrated specimen (Figure 4). After hydrated samples have been examined, flowing water vapor can be changed to dry gas to remove residual extracellular water. This improves surface resolution, while allowing the intracellular space to remain hydrated (Figures 5-6).

Current studies indicate that it may be possible to obtain energy-filtered images of hydrated specimens in the environmental cell, however this capability has yet to be demonstrated. The background signal, while not high enough to inhibit viewing of EELS spectra, may inhibit collection of valuable energy filtered images. The ability to collect individual EELS spectra and potentially energy-filtered images of hydrated specimens will help in determining the location of metal species in bacteria and associated biopolymers. These techniques also will contribute to our understanding of transport of ions, and other materials across biofilms.

REFERENCES

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4. This work was performed under Program Element 0601153N, NRL Contribution Number NRL/AB/7303--00-0023. Robert K. Pope was supported by a CORE/NRL Postdoctoral Fellowship.

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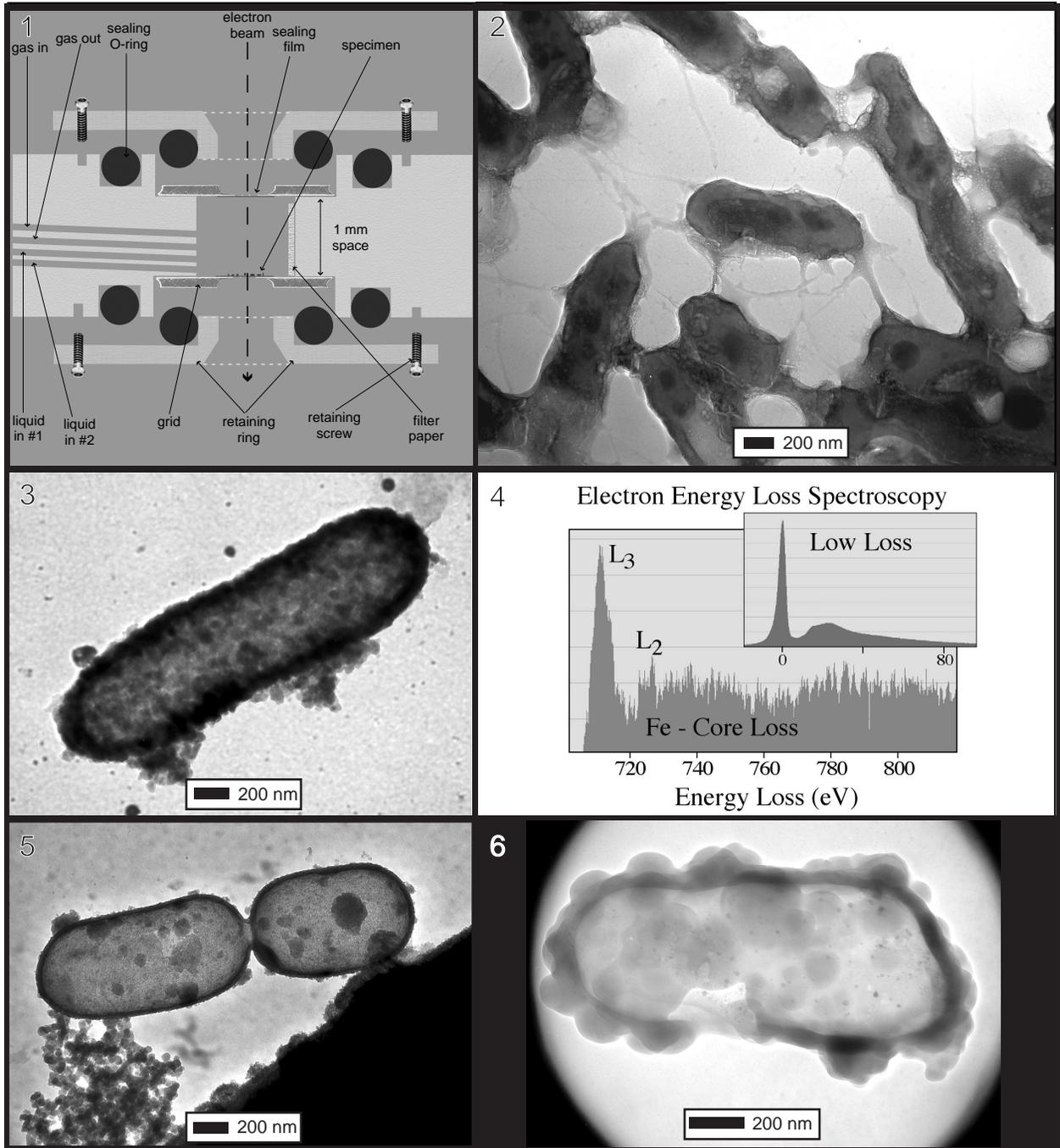


Fig. 1. Diagram of environmental cell chamber, gas circulation lines, and liquid injection lines.
 Fig. 2. Unfixed *Pseudomonas putida* on a coated grid placed directly into the TEM column.
 Fig. 3. Unfixed hydrated *Pseudomonas putida* in the environmental cell in the TEM.
 Fig. 4. EELS spectrum from bacterial extracellular polymer in Fig. 3 demonstrating the presence of iron from corroding iron particles.
 Fig. 5. Hydrated *Pseudomonas putida* in the environmental cell after removal of excess moisture by circulation of dry air through the chamber.
 Fig. 6. Hydrated *Pseudomonas putida* in the environmental cell after removal of excess moisture by circulation of dry air through the chamber.