

Combined Atomic Force and Fluorescence Microscopy of Bioleaching/Biocorrosion Biofilms

Stefanie Mangold, Kerstin Harneit, Thore Rohwerder, and Wolfgang Sand

University of Duisburg-Essen, Biofilm Centre Duisburg, Germany
www.biofilm-centre.de, wolfgang.sand@uni-due.de

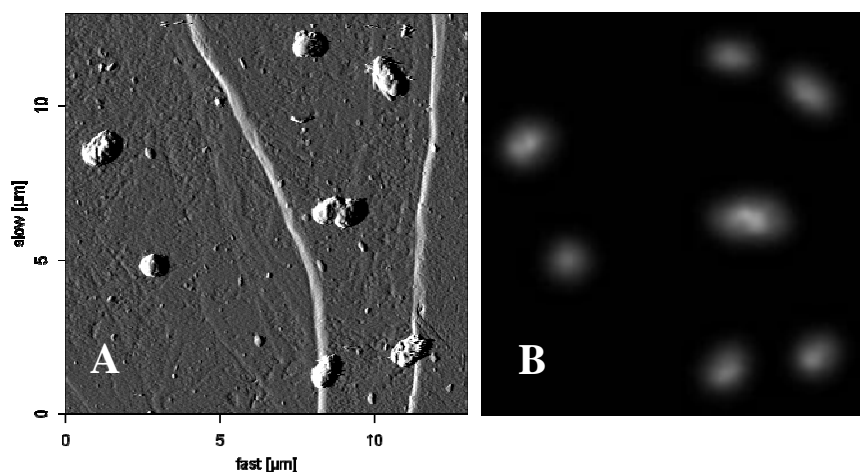
The attachment of the bioleaching bacterium *Acidithiobacillus ferrooxidans* A2 on pyrite was visualized using atomic force and epifluorescence microscopy (AFM and EFM, respectively). A novel system by JPK instruments developed in cooperation with us, the BioMaterial WorkstationTM, allows the investigation of the same location on opaque samples with AFM and EFM. Sessile cells on pyrite coupons were stained with 4',6-diamidino-2-phenylindol (DAPI) and visualized by EFM as well as AFM. Scans were performed in contact mode in air as well as in intermittent contact mode in fluid. To avoid the dislocation of microorganisms by the AFM probe in intermittent contact mode the sample was dried in air for 1 h prior to scanning in fluid.

The investigation of attachment and biofilm formation of bacteria causing bioleaching/biocorrosion is of great importance for understanding the processes associated with those microorganisms. Leaching bacteria, like the chemolithoautotroph *Acidithiobacillus ferrooxidans*, attach to their substrates and dissolve them by the so called contact mechanism [1, 2]. Metal ions are solubilized from mineral sulfides and serve together with the sulfur moiety as substrate. A similar mechanism causes biocorrosion of metals. As the close interaction of bacteria with their substratum plays an important role for these processes, the elucidation of surface-related mechanisms is needed. A powerful tool for the investigation of surfaces and surface interaction is the atomic force microscope (AFM) [3, 4]. A major advantage of AFM against other imaging methods with comparable resolution is that biological material can be investigated in its physiological conditions [5, 6]. For a few years a combination of AFM with epifluorescence microscopy (EFM) is available, but it is applicable only to translucent samples. For the investigation of opaque substrates, like mineral sulfides, the new system combining AFM and EFM has recently been developed by JPK instruments. This system, the BioMaterial WorkstationTM, whose main innovation is the shuttle stage, allows the observation of the same location on opaque samples.

To achieve this purpose pyrite plates were incubated in bacterial cultures for 1 to 4 days. Cells of *Acidithiobacillus ferrooxidans* A2 attached to these coupons, as shown by combined imaging with AFM and EFM. Figure 1 displays bacteria immobilized on the pyrite surface after four days of incubation. The AFM image (Figure 1A) presents the bacteria as topographic features with the typical size of 0.5 μm x 1 to 3 μm [7]; additionally, steps of the

natural pyrite structure are visible in the AFM scan. The EFM figure (Figure 1B) shows DAPI-stained bacteria. The spatial agreement between the (DAPI-stained) cells with the bacteria-shaped structures in the AFM-image prove that the same location was imaged by both microscopes.

Bacteria attached to pyrite were scanned by AFM with contact mode in air and with intermittent contact mode in fluid without observing any problem. Imaging in air was performed for the whole range of cantilever spring constants tested and up to 60 nN acting on the microorganisms, the highest force applied in the experiments. Imaging bacteria in fluid was only possible after drying the samples for 1 h in air prior to scanning. Intermittant contact mode in fluid yielded the best results. When contact mode in fluid was used without drying, the lateral forces were often exceeding the adhesive forces causing a relocation of the cells.



AFM- & EFM-images of cells of *Acidithiobacillus ferrooxidans* A2 attached to the surface of pyrite after four days of incubation. **Fig.A** is the vertical deflection image of an AFM scan performed in contact mode in air. Typical surface characteristics of the pyrite structure are visible. **Fig.B** is the EFM-image of the same bacterial cells at the identical location stained with DAPI.

Thus, AFM in combination with EFM is a powerful tool for the investigation of attachment, cell surface location, and biofilms formed by bioleaching/biocorrosion bacteria. Fluorescence staining with lectins of extracellular polymeric substances (EPS) produced by bacteria and probing of characteristics like softness and stickiness of bacteria by AFM will help to deepen the knowledge about attachment and biofilm formation of these industrially important bacteria.

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