Studying the interface between cyanobacteria and biotite mineral surfaces using FIB and TEM

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Studying the interface between cyanobacteria and biotite mineral surfaces using FIB and TEM

M B Ward and A P Brown

Institute for Materials Research, School of Process, Environmental and Materials Engineering, University of Leeds, LS2 9JT, UK

m.b.ward@leeds.ac.uk

Abstract. Recent analysis of the bioweathering of minerals has highlighted the challenges for investigating the interface between fungi or bacteria and the surface of the mineral that they live on. Transmission electron microscopy (TEM) with its ability to gather imaging information and collect elemental data at high spatial resolution is the ideal technique to analyse such interfaces. Further to this, a dual beam scanning electron and focused ion beam (FIB) microscope is an ideal instrument to prepare specimens for TEM because of its ability to simultaneously cut through hard and soft materials from specific sites of interest. There are however precautions that must be taken when analysing such mineral systems. The electron beam sensitive nature of most sheet silicate minerals means that consideration has to be made as to whether the structure and/or chemistry of the material is being altered during (S)TEM analysis. Here, results from a study of cyanobacteria grown on the surface of biotite are discussed. Particular reference is given to the methods used to determine an electron beam intensity threshold, below which STEM-EDX analysis could be performed without detrimental alteration to the mineral.

1. Introduction

The bioweathering of minerals by organisms such as fungi and bacteria requires a suitable analysis method to characterize the interface chemically and structurally at a high spatial resolution. Transmission electron microscopy (TEM) with Focused ion beam (FIB) sample preparation has been shown to be a useful combination of techniques for the productive study of such interfaces [1, 2].

Dual beam SE-FIB microscopy has the advantage of being a versatile, site-specific technique, and is routinely used for the preparation of thin specimens for TEM [3]. Further to this, TEM analysis of geological samples such as zoned minerals, allows for accurate determination of chemistry/structure at high spatial resolutions, within the limits of the materials' resistance to damage from the electron beam [4]. For example, the weak interlayer bonds of sheet silicate minerals make them prone to radiation damage from the electron beam. Irradiation results in the mobilisation of certain atomic species, mass loss and structural rearrangement, that have a rate dependency on the crystal orientation to the electron beam [5].

The identification and minimization of electron beam damage is therefore an important part of any electron microscope studies that are to be carried out on such mineral materials. Ideally the amount and rate of any beam damage can be quantified to enable the pristine structure of the mineral to be inferred. This can be achieved by the identification of a characteristic or threshold electron dose,

below which there is minimal specimen alteration. Here, we have established an electron intensity threshold for the sheet silicate mineral biotite, irradiated with 200 kV electrons by STEM. .

2. Methods

The filamentous cyanobacterial strain of *Hassallia byssoidea* was isolated from biotite granite in the Czech Republic and incubated on a freshly cleaved biotite surfaces in modified BBM medium (BBM-mod3) for 95 days. 60W fluorescent lamps were used to provide 16/8 hour intervals of light/dark, and a temperature of 22± 1ºC was maintained throughout [1].

TEM samples were prepared by FIB using a FEI Nova200 Nanolab dual beam SEM/FIB fitted with a Kleindiek micromanipulator for ex situ lift-out. The instrument was operated at 30 kV, and at beam currents between 0.1 and 5 nA. Lamella cross sections were taken across the cyanobacteria so that the contact interface with the mineral beneath could be studied (figure 1). Samples were attached to copper support grids (Omniprobe) ready for TEM examination. TEM was carried out using a FEI Tecnai F20 FEGTEM (200 kV) and a Philips CM200 FEGTEM (200kV) fitted with an Oxford Instruments UTW EDX detector and a Gatan GIF200 imaging filter.

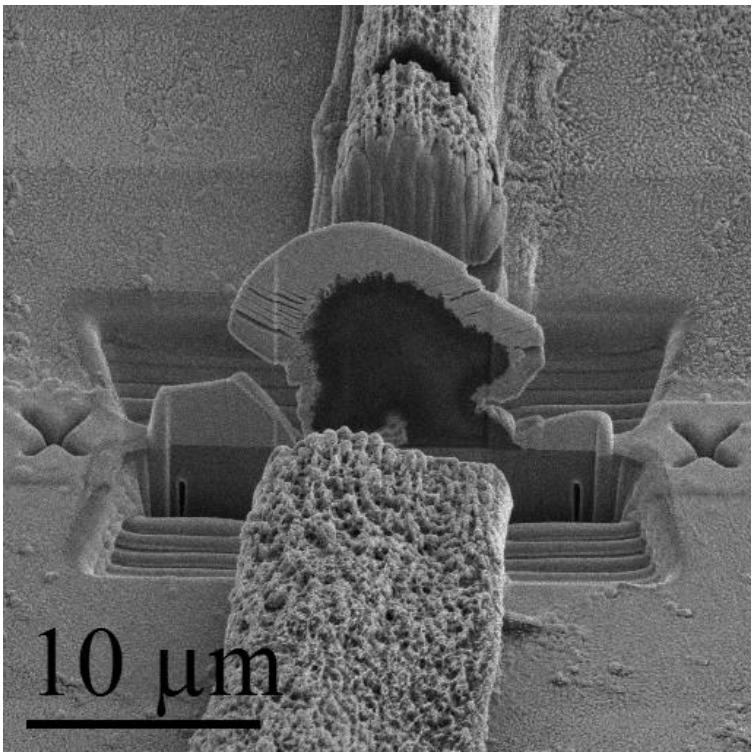

Figure 1. A secondary electron SEM image showing a TEM lamellar cross-section specimen from a cyanobacterial filament attached to a biotite surface, prepared by FIB and prior to lift-out onto a TEM grid.

3. Results and discussion

Scanning (S)TEM combined with Energy dispersive X-ray spectroscopy across the cyanobacteria/mineral interface was used to characterize elemental diffusion between the bacteria and minera]. HAADF images were used to identify the interface and measure the size of the electron probe (as will be described later), and STEM-EDX linescans were used to measure any compositional variations at distance from the interface, however the linescans induced significant damage of the

biotite [1]. This damage results from the relatively high electron dose within a very focussed electron probe of high current density and with relatively long dwell times that is required for high spatial resolution EDX analysis by STEM.

The TEM samples were oriented such that the viewing direction was normal to the cyanobacteria/mineral interface (Figure 2). Several STEM probes were scanned across the interface using different beam conditions (extraction voltage, spot size and C2 aperture) resulting in linescans of different probe dimensions and beam currents, and thus different fluence rates. The interface scans could be seen to mark the biotite (figure 2 lines A, B and C). Beam currents were measured using brightness readings taken from the phosphor viewing screen following the procedure described in Pan et al [6], and the probe dimensions were measured using a HAADF image taken of the interface using each respective probe setting. For these images, the sample was oriented such that the viewing direction was normal to the interface, resulting in the 002 planes of the biotite being on axis. This gave an atomically abrupt mineral surface, and so any blurring of the interface in the image would be a result of the probe width. By taking an integrated intensity profile across the image of the interface, the probe width could be measured (figure 3). The total fluence per dwell point measured for probes A,B and C in figure 2 were 1.2×10^4, 3.0×10^4 and 1.3×10^4 electrons nm^{-2} respectively [1].

A clear transition can be seen between probes of increasing fluence A, B and C; from contamination build-up (A) to hole-drilling (B, C) (figure 2). We suggest that below this electron intensity threshold the rate of hydrocarbon contamination induced by electron beam heating exceeds the rate of ionization induced sputtering or mass loss, providing a coating on the specimen that inhibits further mass loss [7]. Thus STEM-EDX data can be collected using probe setting A without significant alteration of the structure or chemistry of the biotite and has shown potassium depletion of biotite by fungal hypha [2] but no significant elemental alteration of biotite by cyanobacteria [1].

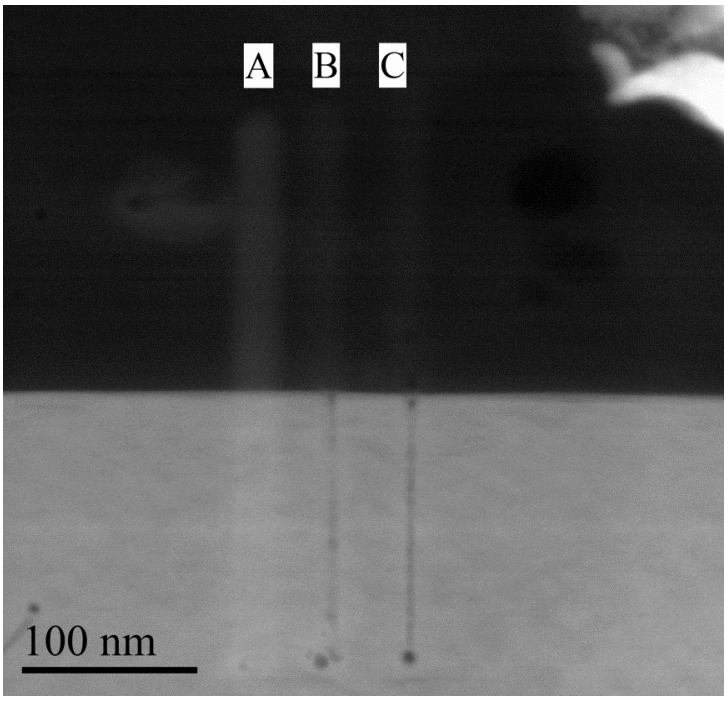

Figure 2. A HAADF image of a cyanobacteria/mineral interface showing three electron probe linescans produced using different beam conditions. A transition from hydrocarbon contamination build-up to hole-drilling in the mineral can be seen between line A and the higher electron fluence lines B and C.

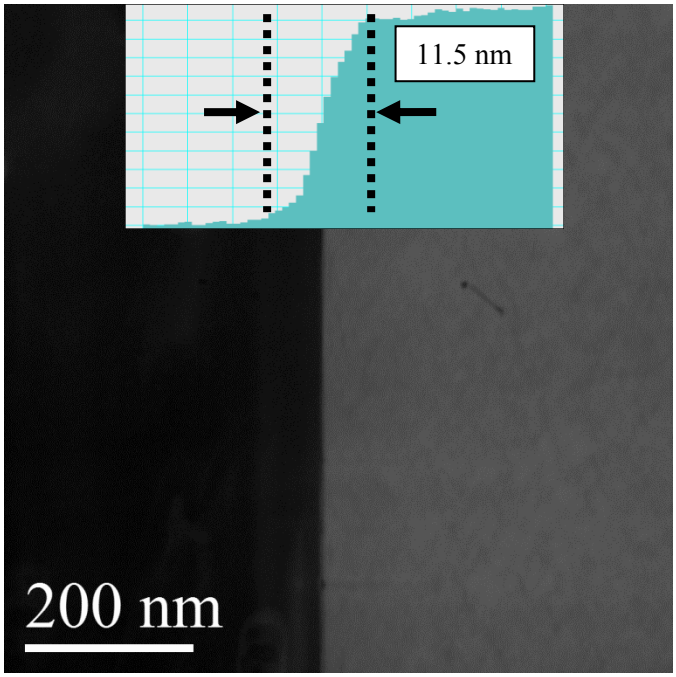

Figure 3.An example HAADF image of the cyanobacteria/mineral interface with an intensity profile across the interface inset. The intensity profile was integrated across 200 pixels, and the width of the profile transition was taken to be the electron probe width for the beam conditions the image was taken at.

4. Conclusions

This paper describes a method to characterize electron beam damage induced in biotite by 200kV STEM analysis. The purpose was to determine STEM operating conditions for EDX elemental analysis of biotite without the artefactual loss of material from the specimen.

An electron probe intensity for a transition between hydrocarbon contamination build-up and mass-loss was demonstrated. Representative analytical STEM studies were conducted below this electron intensity threshold for biotite and reported elsewhere. In general analytical STEM analysis of similar layered minerals should only be carried out once a thorough investigation into beam damage has been performed.

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