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Preserving Anaerobic Conditions of Biogeochemical Samples for Electron and X-ray Chemical Imaging

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High resolution electron microscopy imaging correlated with a suite of X-ray methodologies such as STXM and XANES spectroscopy provides valuable information on the processes in biogeochemistry, including microbial association with soil minerals, photosynthetic organisms' responses to environmental perturbations and microbial dissimilatory reduction of metals and radionuclides related to bioremediation of contaminated soils [1]. However, many metabolic processes occur under anaerobic conditions, such as anaerobic phototrophy of a photosynthetic *Rhodobcter sphaeroides*, as well as processes induced by dissimilatory metal reducing bacteria *Shewanella* or *Geobacter* sp. that can yield energy for cellular maintenance by coupling the oxidation of organic matter or hydrogen to the reduction of metal oxides during anaerobic respiration. This process often results in biomineralization – newly formed crystals in the nano- to micrometer size that are vulnerable to transformation and reoxidation. Other redox-sensitive samples include a wide variety of geological samples with a biological component, such as microbial biofilms, soil organic matter or microbial necromass. In this fashion, the traditional sample preparation in ambient conditions would compromise the system redox situation.

A novel approach for anaerobic sample preparation for TEM was developed in our lab to identify sites of mineral formation, enabling us to examine the cell-mineral interface in detail, under their native conditions. To preserve cell morphology and the accurate composition of newly formed biominerals, we use a strictly anoxic environment for embedding and microtomy (a glove bag setup) excluding most fixatives and all heavy metal stains. Traditional sample preparation of biological samples for TEM includes osmium tetroxide. OsO_4 is a strong oxidation agent, and although it's a superior lipid stain due to the production of a high contrast in its reduced form it must not be used for any redox-sensitive sample preparation. Samples that contain a biological portion need to be fixed in an anaerobic aldehyde, made up in anaerobic water which can be prepared by purging with nitrogen or argon gas. Ascending ethanol series for the dehydration are also prepared in anoxic water. Often, larger equipment like a centrifuge needs to be brought into a glovebag if cell pelleting is necessary. The choice of resins falls into two categories: multi component epoxy polymers such as Spurr's or araldite, and acrylic-based resin monomers such as LR White or LR Gold. Low viscosity resin is very important for a faster, more thorough infiltration of material, and the ability to process samples in the anoxic conditions. In fact, LR White won't polymerize in the air, as the oxygen molecules prevent it from crosslinking even at the required temperature, making it a good indicator of anaerobic processing. This presents a difficulty for samples where a flat embedding is required but for samples processed anaerobically, this a great advantage. The polymerization process should also be carried out in anaerobic conditions, by transfer in an anaerobic container to a 50C oven outside of the glovebag. Once polymerized, the samples are considered locked in its anaerobic condition inside of a resin block. When sectioned, the outer surfaces of a sections will be exposed to O_2, unless an anaerobic shuttle carrier is used for a grid transfer. However, the entire bulk of the section remains presumably anaerobic, and its projection image and spectrum represent its preserved state.

The above scheme has been proven to preserve the anaerobic situation of the samples sensitive to the oxygen, as Fe^{2+} and U^{2+} species vulnerable to reoxidation if processed aerobically were commonly identified in the samples processed this way.

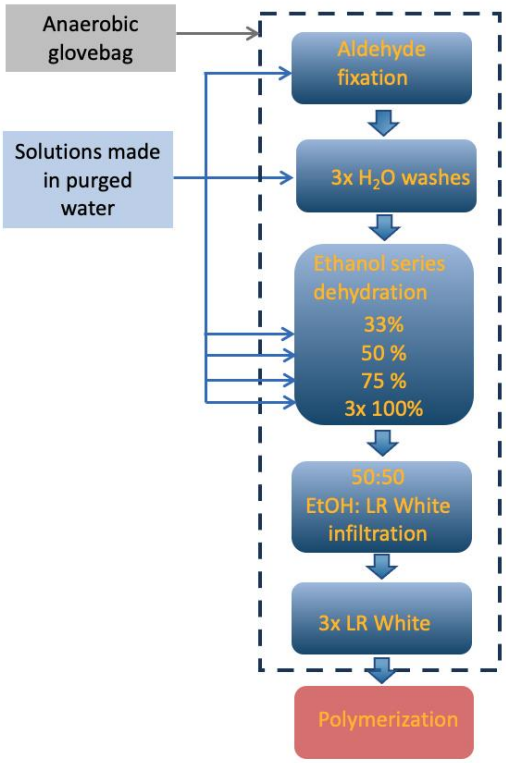

Figure 1. Processing scheme for anaerobic sample resin embedding

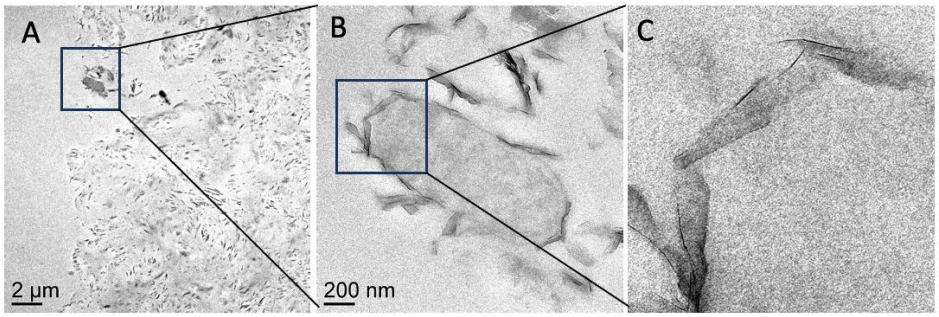

Figure 2. *S. putrefaciens* CN32 culture grown anaerobically with iron mineral nontronite and lignin (A), showing a cell with specific extracellular nontronite associations (B-inset of A), (C-inset of B). Sample was processed according to the protocol for preservation of anaerobic redox conditions.

References

[1] Lovley DR (1993) Dissimilatory metal reduction. Annu. Rev. Microbiol. 47, 263-290

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