Eukaryote-Dominated Microbial Communities that Build Iron-Stromatolites in Acid Mine Drainage, Western Indiana: An analog for Proterozoic Banded Iron Formations and Oxygenation of the Early Atmosphere?

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Introduction: Biologically stratified benthic mats dominated by an acidophilic, photosynthetic eukaryote, *Euglena mutabilis*, are found in acid mine drainage (AMD) systems in western Indiana. The metabolic activity of this species contributes to the formation of iron-rich stromatolitic deposits—features commonly attributed to the activity of prokaryotes (bacteria and cyanophytes). *E. mutabilis* also exhibits aerotactic (moves toward oxygen) and phototactic (moves toward sunlight) behavior by moving through the colloidal precipitates of Fe and Al that form due to rainfall events and changes in biologically induced changes in pH. The activity of AMD microbial communities results in (1) accretionary organosedimentary structures, (2) composed of megascopic, thinly layered, biogenically derived laminae (3) formed by microbial mat-building communities.

These deposits are analogous in composition and structure to Neoproterozoic superior-type banded iron formations (BIF). We can learn a great deal about the precipitation of metal ions from solution due to allogenic and autogenic controls, as well as the oxygenation of the early atmosphere.

Environmental Setting: At the Green Valley mine site, AMD effluent drains from about 5 million tons of coal waste-rock that were re-contoured and slurry ponds backfilled to form one large hill [1]. Rip-rap-lined drainage channels were constructed at the western edge of the hill to facilitate the removal of AMD into West Little Sugar Creek. The pH of the AMD water is between 2.5-4.5 and contains extreme concentrations of Fe (up to 12,100 mg/l), SO_4 (up to 7100 mg/l), Al (up to 1847 mg/l), and Cl (up to 629 mg/l) [2]. The toxicity of the AMD systems excludes all macroscopic life forms.

The channel bottom and margins are covered by iron-rich stromatolites that range in thickness from 5-60 cm. Stromatolite samples, with and without living biofilm, were collected from the main AMD channel at the Green Valley mine site.

Composition of the Biofilm: *Euglena mutabilis* (Protista: Euglenaphyta) is the most abundant and physiologically dominant organism that comprises the biofilm. To a much lesser extent (<1%) the biofilm also contains filamentous algae, yeasts, and gram positive and negative bacteria. *E. mutabilis* has an elongate cell that is typically 100-125 microns long and 25-50 microns wide, with a prominent red stigma (eyespot) situated off-center [1]. A centrally located nucleus appears as a large colorless area and is surrounded on either side by evenly distributed chloroplasts that extend to the ends of the cell. *E. mutabilis* is distinguished from other euglenoids by its considerably larger size and the absence of a prominent functional flagellum. It possesses a flexible cell membrane, allowing it to change length with a distinctive amoeboid-like motion that creates cytoplasmic flowage in a forward direction.

This protozoan also exhibits highly oxygenic photosynthesis [1]. Dissolved oxygen studies show that oxygen concentrations are consistent higher (8.8-32.1 mg/l) in areas of containing *E. mutabilis*. The upper amounts are in excess of 200+% of oxygen dissolved in water. Ongoing studies show that individuals of *E. mutabilis* sequester Fe from the effluent in the channels. The Fe appears as orange-black granules in the cytoplasm that are 3-5 microns and number from 15-25+ [1]. As the organism increases in age, the granules increase in size and number.

Stromatolitic Structures: The stromatolites are composed of an uppermost emerald green biofilm (or mats) that overlies iron-rich alternations of thin, wavy, and sponge-like laminations from 0.5-2.3 mm thick; the overall preserved thickness of these stromatolites ranges from 5-60 cm. The biofilm is relatively smooth, with some pustular textures. The underlying iron-rich laminations extend the either fairly continuous along the entire width of the channel or form localized, small terraces.

Observations in field and laboratory reveals the iron-rich laminations are produced by the aerotactic and phototactic behavior of biofilm by moving through the colloidal precipitates of Fe and Al that form due to changes in pH caused by rainfall events and biological oxygenation of the water. Most of the microorganisms move through the precipitants 6-12 hours after an event.

Microscopic analysis so far reveals that in freshly collected biofilm, there is little evidence of undermat communities. More interestingly, only three types of Gram-negative bacteria (mostly rods) are tentatively identified and appear to co-occur with *E. mutabilis* in extremely low numbers. Thin section analysis of the iron-rich stromatolites reveals no visible remains of , but instead profusely abundant Fe colloids, Fe granules, diatoms, fungal hyphae and cell septae, and few strands of filamentous algae (in decreasing order of abundance). Much of the material is heavily coated in

Fe-precipitate and is also obscured by Fe and Al colloids.

These structures meet the criteria established for the biogenicity of stromatolite-like deposits because they are (1) an accretionary organosedimentary structure, (2) composed of megascopically thinly layered, biogenically derived laminae that are (3) formed by the activity of microbial mat-building communities. Stromatolites, in general, are commonly attributed to the activity of bacteria, cyanophytes, and other prokaryotes, but the low pH excludes these types of mat-building microbes from the AMD effluent. Thus, the Green Valley iron-rich stromatolites appear to be quite unique.

Implications: The physiological and behavioral activity of *E. mutabilis* in the upper most layer of the stratified mats positively impacts the local environment by elevating the oxygen and removing contaminants from the AMD system. The granules represent compartmentalized and/or converted Fe into more innocuous forms as a method of detoxification. As a result, Fe is removed from the system via intracellular sequestration and oxygen stripping via oxygenic photosynthesis by *E. mutabilis*. The stromatolites are representative of these activities and can serve as indicators of similar eukaryotic physiological and behavior processes in the geologic record.

Significance: The biolaminates in these AMD environments are be used as analogs for the formation of Proterozoic iron-rich laminated deposits and the oxygenation of the early atmosphere. AMD systems are good Proterozoic analogs because they 1) contain elevated concentrations of Fe and total dissolved solids, 2) are composed exclusively of microbial communities with no higher metazoans, and 3) support both planktic and benthic microbial populations.

It may be possible that microbial activity of early eukaryotes via oxygenic photosynthesis and mediation of Fe species likely contributed to the genesis of Fe formations and oxygenation of the atmosphere between 2.7-1.9 Ga. These dates span the time of the earliest chemical [3] and paleontological [4] evidence of simple protozoans. Early eukaryotes with flexible cell membranes may have metabolically adapted to elevated concentrations of Fe in their environment by evolving the ability to internally sequester Fe from the ocean. They may have also preferred areas with slightly higher pH and elevated concentrations of metals that might exclude competition from cyanophytes and bacteria for space and light. Fe used for metabolic activity or that diffused into the cytoplasm was likely removed via intracellular precipitation as a detoxification mechanism. These granules were later expelled or released after death to form concentrated layers of Fe precipitants and as nucleation sites for Fe precipitation.

References: [1] Brake et al. (2001) *Environmental Geology*, in press. [2] Brake et al. (2001) *Applied Geochemistry*, in press. [3] Brocks et al. (1999) *Science* 285, 1033-1036. [4] Hans and Runnegar (1992) *Science* 257, 232-235.