

Formation of Authigenic Minerals During Microbial Degradation in Artificial Marine Media

by

Matthew Albert Henry Joss

Submitted to the Department of Earth, Atmospheric, and Planetary
Sciences

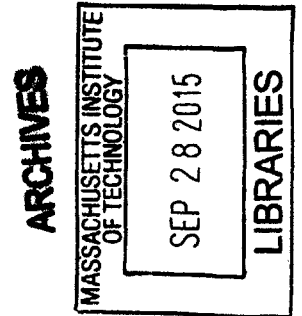
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Abstract

I have conducted incubation experiments exploring microbial degradation of organic matter within an artificial sediment designed to simulate an organic-rich siliciclastic marine sediment. Central questions to my research include: 1) How does the presence of dissolved iron influence the degradation and/or protection of organic matter within sediments? 2) How does dissolved iron influence the development of authigenic minerals? 3) How do microbial metabolisms affect dissolved iron concentrations and authigenic mineral precipitation?

My experimental system consisted of dead cyanobacterial cells that were mixed with acid-washed glass beads, thereby making an artificial sediment essentially free of contaminants. This material was deposited into incubation cultures with artificial marine liquid media with varying concentrations of dissolved Fe(III). The cultures were exposed either to a natural sediment community or to the iron-reducing facultative anaerobe *Shewanella Putrefaciens*, in oxic or in anoxic. These cultures were compared against controls that contained no living microbes. The liquid media had varying initial concentrations of dissolved iron. For cultures with initially 0.2 mg/L of dissolved Fe(III) or no initial dissolved Fe(III), I measure the extent of degradation through loss on ignition gravimetry after 40 days of incubation. I also measured pH levels, dissolved Fe(II), and total Fe over a period of 60 days. Scanning electron microscopy and energy dispersive X-ray spectroscopy were used to identify mineralized organic particles and mineral grains within subsamples of the living cultures. In contrast, the sterile controls had no recognizable mineralization present. Finally, X-ray diffraction spectra were obtained for sediment subsamples of cultures with initial iron concentrations of 0.1 mg/L, revealing spectral peaks indicative of small amounts of authigenic mineral structure present within the oxic living cultures. Iron is identified as a key component of many mineralized structures within the living samples, particularly in the anoxic samples, with iron rich particles usually being mostly amorphous. It is possible that longer incubation times would increase the extent of mineralization and produce divergent rates of organic matter protection and degradation.

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Chapter 1

Introduction

1.1 Earth's Carbon Cycle

Natural cycles including the carbon cycle as envisioned by Joseph Priestley and Antoine Lavoisier (Holmes 2008) help to regulate Earth's climate. Figure 1-1 illustrates the carbon cycle in terms of carbon reservoirs and fluxes. Within this cycle, inorganic carbon in the form of CO_2 is released from various sources and sequestered by various sinks. The CO_2 sources include volcanoes, deep sea thermal vents, burning of fossil fuels, and respiration from organisms. CO_2 sinks include carbonate precipitation which occurs in marine sediments, weathering and oxidation of silicate rocks, and photosynthesis which sequesters and converts CO_2 into solid organic carbon while releasing free oxygen. Once the carbon has been sequestered into organic material the solid carbon can be buried within terrestrial or marine sediments indefinitely. Living organisms will tend to consume organic material within a sediment or soil to obtain energy and organic carbon. However, for various reasons some organic carbon will be particularly difficult for sedimentary organisms to access and/or break down. Therefore some of the preserved organic material will persist within the sediments until the material is resurfaced through bioturbation or in an orogeny or until it is dragged into the mantle of the Earth via plate tectonics. The buried organic material is then physically separated from the free oxygen dissolved in the ocean and upper atmosphere. This separation has been identified as having a key role in the preservation

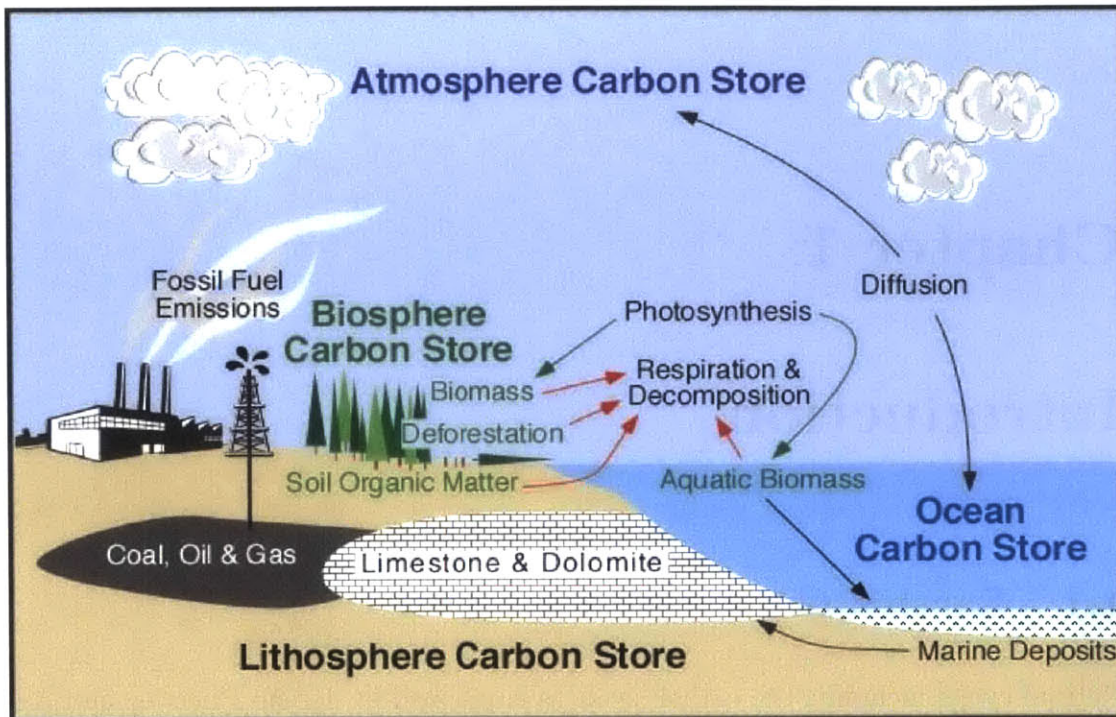


Figure 1-1 Schematic representation of the various carbon reservoirs on Earth, and surface processes cycling Earth's carbon. Various sources and sinks of inorganic carbon (CO₂) are shown, as well as sources and sinks of organic carbon. In my project, I have focused on the degradation of organic material within marine deposits. Source: <http://www.physicalgeography.net>

of organic material due to the elimination of oxygenic respiration within the sediment (Hartnett et al.1998). Furthermore, organic particles are often physically protected due to an intimate association with mineral surfaces (Mayer 1993) where matter adsorbed directly to mineral surfaces limits the matter's accessibility to microbes (Keil et al. 1994). Therefore, by actively sequestering and preserving organic carbon in marine sediments, and through mass balance, the Earth's carbon cycle actively frees oxygen to build up within the atmosphere for later use. The organic burial within sediments on geological timescales is also intrinsically linked to the cycling of other important biogeochemical elements such as S, P, N, Mn, and Fe (Bernier 1982, Hedges 1992, Holland 1978).

Iron is thought to play a key and multifaceted role in the protection and ultimate

preservation of organic material within marine sediments and soils. The biogeochemical cycles of organic carbon and iron in marine sediments are strongly coupled: Iron has been shown to readily complex with and trap dissolved organic matter within redox interfaces (Riedel et al 2013, Johnson 1997). Solid iron phases have been demonstrated to protect and shelter organic carbon within soils (Kaiser et al. 2000). Significant amounts of iron phases have been demonstrated to be bound directly to organic material in marine sediments (Lalonde et al. 2012). It has therefore been suggested that these iron phases serve as an efficient “rusty sink” for organic material within marine sediments. However it is not clear to what extent these bound iron phases protect different forms of organic matter within different sedimentary environments.

Previous laboratory studies have demonstrated preservative effects of organic matter induced by the presence of iron. Synthetic organo-ferric and organo-aluminous complexes were demonstrated to degrade at different rates depending on the relative ratio of metal to complexing functional groups. Protective effects were observed for ratios of one or two times greater than the saturation of their complexing sites (Boudot et al. 1989). Furthermore, in the same study, flocculated metallic hydroxide trapping and coating of the organic molecules is suggested to account for the observed protective effects better than pure chemical binding. In a different study, incubation experiments were conducted using citrate or glucose as the carbon source while mineral phases (illite-mica, kaolinite, mixed soil clays, or ferric hydroxide) were included to test varying degradation rates (Jones et al. 1998). These solid phases, especially ferric hydroxide, were observed to affect the degradation rate of citrate. These studies suggest that when dissolved metals bind to the complexing groups of various organics, or when organics are coated in flocculated iron-hydroxides, organic carbon preservation is expected to increase.

The existence of microbial metabolisms capable of using Fe as an electron donor/acceptor further complicates the role of iron in marine sediments. Various iron reducing bacteria, such as *Shewanella Putrefaciens*, are capable of using Fe(III), as well as other electron acceptors, during the degradation of organic material (Mayer et al. 1988,

1990). In an iron redox reaction, this electron transfer to Fe(III) is said to ‘reduce’ the iron to Fe(II) where Fe(III) acts as the terminal electron acceptor. This suggests that the presence of Fe(III) in anoxic pore waters could, to a limited extent, enhance organic degradation by providing the microbes with an extra source of terminal electron acceptors.

The general fluxes of inorganic carbon into and out of the atmosphere and ocean interest Earth scientists, resulting in the development of various measures of rates of carbon emission and sequestration around the globe. However, specific sedimentary processes and mechanisms central to sediment formation and diagenesis aren’t well understood. In particular, preservation of organic carbon in natural sediments is still an active area of research. Much of this is due to the sheer complexity of diagenetic processes within particular sediments. Typical sedimentary environments are extremely complicated with a massive number of varying inorganic and organic structures, mineralogies, and a diversity of microbes with contrasting metabolisms. These complications are combined with diverse spatio-temporal heterogeneities, all of which make the sedimentary processes difficult to study. For this reason, it is necessary for scientists to choose particular sedimentary systems to study and understand in depth in order to test and identify particular diagenetic processes specific to that sediment and that sedimentary community. I am interested in the Fe-influenced development of authigenic mineral structures and how they could affect the degradation of organics within the sediment. However, in natural sediments, a vast number of contaminating mineral structures and micro-particles makes studying the role of mineral authigenesis in organic matter protection challenging. It can be difficult to tell what is and is not already in a natural sediment before an experiment begins. By inventing a simplified sedimentary system made of lab ingredients low in contaminants, I can simulate a simple sediment without the added complication of also having contaminating mineral structures or micro-particles present.

1.2 Thesis Content

I explored the preservation of organic material in the form of dead cyanobacterial cells mixed with sediment composed of glass beads. It was my goal to study the birth and growth of authigenic mineral grains and clays in close association with organics while focusing on iron and its role in diagenesis and organic carbon preservation.

In the next chapter I describe two lab experiments where I incubated various microbes using my artificial sediment as well as artificial seawater with varying concentrations of dissolved Fe(III), in either oxic or anoxic. I sought to identify any clays or mineral grains that precipitated within the artificial sediment. In particular, I chose to identify newly precipitated mineral grains adsorbed to organic particles which might serve to protect the organic material from further degradation. I measured approximate rates of organic carbon degradation through loss on ignition (LOI) gravimetry after 40 days of incubation. I also measured the pH and concentrations of dissolved Fe(II) and total dissolved Fe using a Ferrozine assay in a spectrophotometer over 60 days. I examined subsamples of my cultures using a scanning electron microscope (SEM) and energy dispersive spectroscopy (EDS), and finally I analyzed sediment samples with X-ray diffraction in the attempt to identify particular mineral structures present.

Chapter 2

Experimental Methods

I explored the degradation of organic carbon using both an existing marine sedimentary community and *Shewanella Putrefaciens st. 200* cultures. Because natural sediments are very complex with many contaminants, it was my goal to produce a system in which I could identify authigenic minerals. This was done by creating an artificial sediment composed of acid-washed glass beads mixed with dead *Spirulina* (cyanobacterial) aggregates. By choosing to use glass beads mixed with dead cyanobacterial cells as a simple siliciclastic sediment, I could effectively differentiate between the un-degraded sediment and a sediment that has undergone degradation where mineral authigenesis should have occurred. This allows me to identify authigenic particles.

The *Spirulina* utilized in this experiment was commercially grown and then freeze-dried for human consumption. Thus the *Spirulina* cells are rich sources of trace metals such as iron, copper, and zinc. As sedimentary organisms begin to degrade the dead *Spirulina*, some or perhaps all of these trace metals would be released and remobilized into the porewaters in aqueous form or as a component of a soluble organic metal complex. This release of trace metals from the organics into the porewaters could encourage a feedback mechanism for the production of new mineralized-organic complexes and in clay authigenesis. By releasing more metals and ions into the pore waters, microbes can then utilize those metals for further degradation of organics. An example of this would be the utilization of more iron reduction reactions. Furthermore, the release of trace metals from rich substrates could encourage a wide variety

of minerals to precipitate. With this experiment we observe effects of trace metals being released from organics without the interference of these same metals being released from nearby mineral structures or from new detrital sediment particles.

2.1 Preparation of Model Sediment

In my attempt to create a simplified sediment, I reduced my model sediment to just two non-living ingredients: Organic material in the form of dead cyanobacterial cells, and inorganic material in the form of relatively large granular glass beads. In particular I utilized freeze-dried, autoclaved, and re-dried at 110° C Spirulina in powder form. I also used 0.2-0.5mm diameter acid-washed glass beads (Sigma-Aldrich, G1277).

Initial combustion experiments of these basic sediment ingredients were conducted in the Parsons Lab at MIT in the combustion furnace at 440° C over 10 hours. The LOI measurements suggest that the processed Spirulina was comprised of about 91.1% organic material by weight and that the glass beads were about 99.9% inorganic material by weight. Using these initial LOI measurements the model sediment was chosen to be 2% organic by weight by adding appropriate proportions of the Spirulina with the beads. The dry sediment was then made by manually mixing different amounts of glass beads and dried Spirulina within the same vessel using the addition of 3 ml of nanopure H₂O for every 10 ml of dry sediment. This helped spatially homogenize the organic material within the sediment.

After preparing about 400g of sediment, I conducted more LOI measurements in the Summons Lab at MIT using 12 subsamples, and the batch was found to contain $1.55\% \pm 0.072\%$ wt organic matter. I used this batch for the degradation experiments discussed in the next section.

The composition of the marine media is listed in Table 2.1. Two different media types were made for the first incubation experiment. The first with no dissolved Fe(III), and the second with added aqueous Fe(III) (0.15 mg/L). Solid aluminum chloride was added to obtain a concentration of 100 nmol/L of Al to provide minerals

Table 2.1. Liquid Media Ingredients for the First Incubation Experiment

Ingredient (1)	Concentration (2)
NaCl	23 g/L
Na ₂ SO ₄	0.71 g/L
KCl	1.48 g/L
NaHCO ₃	0.336 g/L
AlCl ₃	10 ⁻⁵ g/L
MnCl ₃ 4H ₂ O	2.67 mg/L
ZnSO ₄ 7H ₂ O	0.5 mg/L
CuSO ₄ 5H ₂ O	0.03 mg/L
Na ₂ MoO ₄	0.02 mg/L
Co ₂ NO ₃ 6H ₂ O	0.06 mg/L
H ₃ BO ₃	0.5 mg/L
H ₂ SO ₄	0.5 mL/L
KNO ₃	5.15 mg/L
NaNO ₃	34.45 mg/L
Nitrilotriacetic Acid (NTA)	10 mg/L
NaCl	48.2 mg/L
NaH ₂ PO ₄	0.9 mg/L
KCl	7.4 mg/L
Sodium Silicate Solution	65.24 μ L/L
CaCl ₂ 2H ₂ O	0.2565 g/L
MgCl ₂ 6H ₂ O	0.4145 g/L
FeCl ₃ 6H ₂ O	0 -or- 0.6 mg/L

Note. — Nanopure water was used to avoid contaminants, particularly iron. In experiment 1, FeCl₃ concentration was varied for the two media types by adding no Fe to one, and for experiment 2 media was made identically except for the substitution of 0.1 mg/L of Fe(III) instead of 0.2 mg/L (0.3 mg/L FeCl₃6H₂O instead of 0.6 mg/L).

with a source of Al during the degradation phase. This concentration is typical to find in natural sedimentary porewaters (e.g. Mackin et al. 1986, Mackin et al. 1984). Identical liquid medium was prepared for the second incubation experimental samples intended for XRD analysis, however this medium had an Fe(III) concentration of 0.1 mg/L.

2.2 Growth of Microbial Community and *Shewanella Putrefaciens* in Model Sediment

A natural sediment community harvested from near-shore marine waters and the iron reducing bacteria *Shewanella Putrefaciens strain 200* were used to degrade organic matter within my artificial sediment. These microbial cultures were grown on the model sediment as well as marine seawater media with dissolved Fe(III) (0.2 mg/L) prepared in the lab. After 1 week of growth I then extracted model sediment with the living organisms and deposited the subsample into new growth cultures with sterile

model sediment. This procedure diluted the original natural sediment inoculum, thereby reducing the number of potential particle contaminants from the natural sediment. Additionally it allows the selection of organisms capable of degrading dead *Spirulina* cells.

The natural sediment sample in my experiment was originally collected near the shore of Plum Island in Massachusetts. I enriched a community from this sealed sediment jar by pipetting 1 ml of sediment from less than 1 cm deep within the sample sediment, 1 ml of sediment from about 1-2 cm deep, and 1 ml of sediment from about 3-5 cm deep. These 3 ml of natural sediment were then mixed with about 10 grams of model sediment. Once the 10 grams of model sediment had been inoculated within a sterile glass test tube with the inoculate, I manually mixed the slurry within the test tube using a sterile loop to distribute the inoculum evenly throughout the sediment. Once the slurry appeared well mixed, I then added about 17 ml of marine growth media to the slurry, and I used the stylus to mix the sediment in order to release any trapped air pockets and to level out the sediment. In this mixing process, some heterogeneities (especially in the vertical profile for the concentration of organic matter) developed within the sediment due to the large difference in relative densities of the sediment ingredients. This test tube, referred to as the first generation of the sediment community, was allowed to grow under oxic conditions in the fume hood at room temperature for one week with ambient light conditions.

After one week at $\sim 20^{\circ}\text{C}$, a new sterile test tube with a fresh 10 grams of model sediment was used to grow a second generation of the natural community. For the second generation sample, 0.5 ml of surface sediment and 0.5 ml of sediment/ media from about 3cm deep from the first generation was used for inoculate, and then 19 ml of fresh marine media was added. This was the second generation sample which was allowed to grow for one week at $\sim 20^{\circ}\text{C}$ open to the atmosphere. A third generation was grown in a larger test tube using about 20g of sediment and about 45 ml of media, and was inoculated using the same technique as for the second generation. The third generation was allowed to grow for 1 week at $\sim 20^{\circ}\text{C}$. After this time, sediment from the third generation sample was extracted and utilized to inoculate my degradation

experiment samples.

A similar procedure was conducted for growing *Shewanella Putrefaciens strain 200* cultures. Freeze-dried *Shewanella* cultures from the company ATCC were initially grown in tryptic soy broth and incubated for 48 hours at 26 ° C. Then, a first generation culture of *Shewanella* was inoculated using about 10 grams of model sediment, 19 ml of fresh media, and 1ml of *Shewanella* grown on the tryptic soy broth. This culture grew at ~ 20 ° C for 1 week before inoculating a second generation which also grew for 1 week at ~ 20 ° C. Sediment from the second generation was extracted and utilized to inoculate degradation experiment samples. Each living culture became optically dense, as seen by eye, indicative of microbial activity.

2.3 Experiment: Degradation of Model Sediment

Degradation experiment number 1 was prepared by using 48 ~10 cm tall disposable glass test tubes (VWR, 47729-576) which were labelled with a diamond engraver and weighed three times on a Mettler Toledo AG204 digital balance. Each sample tube was prepared with ~ 5g of model sediment and fitted with oxic autoclavable caps. All 48 tubes were then dry autoclaved to ensure they were sterile and to try to remove the nanopure water from the sediment samples. The sediment within each sample expanded due to evaporative loss of water from the sediment, so I vortexed the samples again.

A control set of samples was prepared with no living organisms to test the lone effects of the experimental environmental setup on the organics within the sediment without the added complexity of living components. Additional sample sets with living microbes were also prepared. Each culture was grown in one of two seawater media types having either no added dissolved Fe(III) or 0.2 mg/L of Fe(III). Also during experiments, half of the samples were grown in oxia (open to the atmosphere), and half were grown in anoxia using an artificial atmosphere consisting of 80% N₂ and 20% CO₂. To flush their atmospheres, the anoxic samples were inserted into larger glassware and capped with rubber stoppers. Artificial atmosphere was then

fed into the sealed sample tubes via sterile (0.2 micron filter) syringe for 40 minutes, effectively flushing out most of the original atmosphere and oxygen.

Degradation experiment number 2, which I also refer to as the ‘XRD experiment,’ was designed to degrade larger quantities of the organic material in the attempt to obtain high abundances of crystallites for measurement in the Panalytical X’Pert pro XRD apparatus. The experiment was designed identically to experiment 1 except for two key differences. 18 of the larger 200 ml glass vessels were chosen and acid washed with 0.1M HCl and the liquid media had a concentration of 0.1 mg/L of Fe(III). This placed it right in between the ‘high Fe’ and ‘no Fe’ samples from experiment 1. Due to the larger vessel size, a total mass of ~ 30 g of model sediment and about 150 ml of liquid media was used in each sample. Furthermore these samples were prepped in triplicates so that the 3 sets of samples could be opened in sequence to observe changes in dissolved Fe levels and changes in mineral structure over time.

Inoculum was prepared by pipetting approximately 15 ml of media from either the 3rd generation natural community growth tube or the 2nd gen *Shewanella* growth tube described in the previous section. While pipetting out approximately 15 ml from each sample, I made sure to include sediment pore water in the pipette. The sediment slurry was deposited in a sterile Falcon tube and was then vortexed to agitate the microbes free from any sediment particles. The sediment particles were then allowed to settle and ~ 90% of the liquid media in the water column above the sediment was then pipetted into a new sterile Falcon tube. This process was done in the attempt to free any microbes into the liquid media as well as to leave all of the sediment particles behind.

Inoculation of each sample included nothing for the ‘control’ cultures, 0.5 ml of the prepared community liquid media for each ‘natural community’ sample, and 0.5 ml of the prepared *Shewanella Putrefaciens strain 200* liquid media for each ‘*Shewanella*’ sample. The inocula were inserted into the dried test tubes with the pre-autoclaved 5 g of model sediment. A sterile inoculating loop was used to swirl the slurry around and to evenly distribute the inoculum. Inoculation was done near a flame to avoid contamination, and each tube was given 9.5 ml of fresh liquid media

(either the ‘high Fe’ media or the ‘no Fe’ media). In the case of the samples from experiment 2, approximately 150 ml of media was added. Each tube was then capped with an oxic growth cap or placed into larger glassware with rubber stoppers for atmospheric flushing. Anoxic samples had their atmospheres flushed within 3 hours after inoculation. All samples were placed on a shaker in an incubator with moderate levels of light set up on a 12 hour timer cycle.

2.4 Post-Experiment: Sample processing

Experiment 1: After allowing the cultures to degrade, the liquid media was removed from the water column above the sediment while avoiding any sediment disturbances. One sample per parameter set was chosen for imaging, and the three remaining were placed in a wet oven in the Summons lab set to 110 C for approximately 3 days to dry. Once dry, each sample was weighed three times on a Mettler Toledo AG204 digital balance and masses were recorded in Matlab. The samples were placed in aluminium boats and oriented upright so as to not lose any of the sediment. The samples were sealed in the furnace and combusted at 440° C for 10 hours. While cooling, I placed the samples in a desiccator to avoid water from condensing within the samples. Once cooled, the samples were weighed again. These masses, in addition to the masses of the glass vessels, are all that was required to calculate the loss on ignition as well as the percent loss of organic carbon due to microbial degradation.

Below are the equations used to calculate the total loss of organic material as well as the percent of sediment that is organic material which remains after degradation:

$$LOI = M_{dry} - M_{combusted} \quad (2.1)$$

$$\%ORG = \frac{LOI}{M_{dry} - M_{vessel}} \quad (2.2)$$

The variables ‘ M_{dry} ’ and ‘ $M_{combusted}$ ’ represent the mass of a particular sample after drying, and after combustion, respectively. ‘ M'_{vessel} ’ represents the mass off the

glass vessel from before any sediment was placed into the vessel. '*LOI*' represents the loss on ignition, which is interpreted to be the total mass of organic material within the sample. Last, '*%ORG*' represents the percent mass of the sediment that was organic material. These variables are considered to be semi-quantitative.

In experiment 2, after allowing the cultures to degrade, I opened and extracted liquid media subsamples. This media was then filter-sterilized using 0.2 micron filters affixed to 10 ml syringes. The filtered media generally still had a greenish-yellowish color suggesting the presence of dissolved organics, but the media remained translucent. The pH of this media was measured for each sample.

Each media sample was stored in 15 ml Falcon tubes. Filtered media from each sample (3 ml total each) was then transferred to two labelled 1.5 ml Eppendorf tubes (one for Fe(II) analysis and one for total Fe). These subsamples were then acidified with 10 μ L of HNO₃ (14.7 N) per 1 ml of liquid sample in the attempt to break any Fe-organic complexes. The Eppendorf tubes were heated to approximately 100 ° C for several minutes, and were then allowed to cool to 24 ° C. This heating was also done in the attempt to break any bonds within Fe-organic complexes, as recommended by the Hach company. Once cooled, 0.75 ml of media from each Eppendorf tube was transferred to a new Eppendorf tube, and 100 μ l of Ferrozine reagent (0.1 M) per 1 ml of sample was added to these new samples of 750 μ l. I then had 4 Eppendorf tubes for each sample type: 2 for Fe(II) and 2 for total Fe. For each iron type I had one tube with Ferrozine reagent added, and one without the reagent. A reducing agent (150 μ l hydroxylamine HCL, 1.4M) was then added to the 'total Fe' sample tubes to reduce any dissolved Fe(III) to Fe(II). After 20 minutes, 50 μ l ammonium acetate 5 M with 30% ammonium hydroxide was added to each sample to buffer the pH to 9.5 so that the characteristic pink-purple color could develop. A clean plate for use in the spectrophotometer was then utilized and 200 μ l was extracted from each Eppendorf tube in triplicates and deposited into the plate wells. Media with Ferrozine and without Ferrozine was deposited for direct comparison. The plate was inserted into the apparatus and spectrophotometric analysis (measuring absorbance) was conducted on the plate at 562 nm. This yielded optical density measurements in

Excel format. This Excel data was imported and processed in Matlab. A calibration curve was also prepared on a separate plate using nanopure water mixed with various concentrations of FeCl_2 . Ferrozine reagent, reducing agent (for total Fe), and buffer was also added to these calibration solutions, and the optical density at 562 nm was measured in the spectrophotometer. Using this calibration curve, I was able to infer Fe(II) and total Fe concentrations.

The equations for determining the Fe(II) concentration are:

$$OD_{diff} = OD_{ferr} - OD_{control} \quad (2.3)$$

$$[Fe_{(II)}] = m * OD_{diff} + b \quad (2.4)$$

The variable ' OD_{ferr} ' is the measured optical density (Absorbance) of the pre-heated acidified media sample with the added Ferrozine reagent. The variable ' $OD_{control}$ ' is the measured optical density (Absorbance) of the pre-heated media sample without Ferrozine. The Variables m and b in equation 3.2 are linear fit parameters, and I determined these for both the dissolved Fe(II) and dissolved total Fe calibration data. Using m and b , we can insert the differences in absorbance into equation 3.2 to infer Fe(II) and total Fe concentrations

X-Ray Diffraction (XRD) spectra were gathered from subsamples of organic pellets extracted from samples grown in experiment 2. The spectra were gathered as 3-hour 2θ reflection scans on the Panalytical X'Pert Pro apparatus with the assistance of Charles Settens at the MIT CMSE XRD Shared Experimental Facility. Subsampled organic pellets in Eppendorf tubes left over from SEM preparation were removed and put into a mortar and soaked with ethanol. The pellets were then ground into a powder using a mortar and pestle, and the powdered slurry was poured onto zero background disks. The samples were then allowed to dry to partially to remove excess ethanol, and the powdered sample was then gathered to the center of the zero background disk using a clean utensil. The sample disks were loaded into an automatic sample changer and the Divergent slit was changed to a 2 degree slit to get

a stronger X-ray intensity. Scans were conducted from 10 degrees to 120 degrees on each sample, and each sample was automatically spun slowly to rotate any present crystal faces and to maximize the chances of gathering a measurable signal from the dilute mineral structures.

SEM sample Imaging: Upon ending degradation experiments 1 and 2, the liquid media from the water column was removed from the vessels, and a subsample of the sediment was extracted using a clean plastic spatula. Sediment subsamples were put into Falcon tubes and rinsed 3 times with nanopure water to remove as many salts as possible, avoiding additional precipitation. While rinsing, approximately 10 ml of nanopure water was added to the sample, and a sterile inoculating loop was used to stir and mix the sediment to release any salts from the porewaters. The samples were then placed in the centrifuge and spun at 3,000 RPM for 3 minutes, which forced all of the larger organic mineral particles to the bottom of the Falcon tubes. The water column was then pipetted out and the process was repeated two additional times. While doing this procedure, it was apparent that there were clear differences between my control samples and my microbial samples. The controls had numerous large dark intact organic particles, while the microbial samples had more of a slime that accumulated at the bottom of the Falcon tube. After centrifuging, this slime would readily dissolve back up into the water column. This meant that most of the organic material in the microbial subsamples tended to be washed away more easily than in the control subsamples.

After rinsing, each of the imaging subsamples were then fixed with 1 ml of Dirk's Fixative (sodium cacodylate and glutaraldehyde) and left in the 4 ° C refrigerator overnight. The next day, samples were removed from the fridge and the fixative was pipetted out. Then I added 1 ml of buffer to each sample and let the samples sit in the 4 ° C refrigerator for 20 minutes. The samples were rinsed 3 more times, and an ethanol drying series was implemented next. Various solutions of ethanol and nanopure water were prepared including 30% ethanol, 70% ethanol, and 100% ethanol. Each sample was allowed to sit in 3 ml of each solution for 20 minutes in the following order: 30% solution for 20 minutes, 70% solution for 20 minutes, 100%

solution for 20 minutes, 100% solution for 20 minutes again. The last of the ethanol was removed from the samples and a clean plastic spatula was used to extract a small amount of sediment. The sediment was deposited on a plastic slide prepped with a piece of carbon tape. Samples were sputter-coated at Harvard's CNS facilities using a 5 nm Pt:Pd alloy coating consisting of 80% Pt and 20% Pd.

Imaging took place at Harvard's CNS facilities on SEM-5 the FESEM Supra55VP. Operating EHT was 10.0 KV for all samples so as to be able to measure Fe present within individual particles using the EDS. I attempted to get each EDS scan to occur at a working distance of ~ 8.5 mm as this is optimal working distance for the Supra. Each image below consists of a 10 or 20 second integration line scan. Spectra of certain EDS scans are reported with images of their corresponding particles in Chapter 3.

Chapter 3

Sample Analysis and Results

In experiment 1, the loss on ignition (LOI) samples were combusted and weighed before and after combustion providing semi-quantitative measurements of the total extent of degradation. The difference in mass between the samples before combustion and after combustion is considered to be the total mass of organic material remaining in the samples after the 40 day experiment. In experiment 2, I measured dissolved Fe(II), total Fe, and pH within the water column using a Ferrozine assay and a pH probe over a 60 day period. Scanning electron microscopic (SEM) imaging as well as electron dispersion spectroscopic (EDS) analysis enabled the elemental identification of organic or partially mineralized organic particles within each sample and to semi-quantitatively assess the elemental composition of these sediment particles. Comparison with controls informs us if any mineralization is due to the presence of microbes. XRD analysis of sediment samples provide insight on which mineral structures are taking form within each sediment. With these measurement techniques, we can qualitatively and semi-quantitatively assess the extent of organic degradation as well as the net amount of iron reduction.

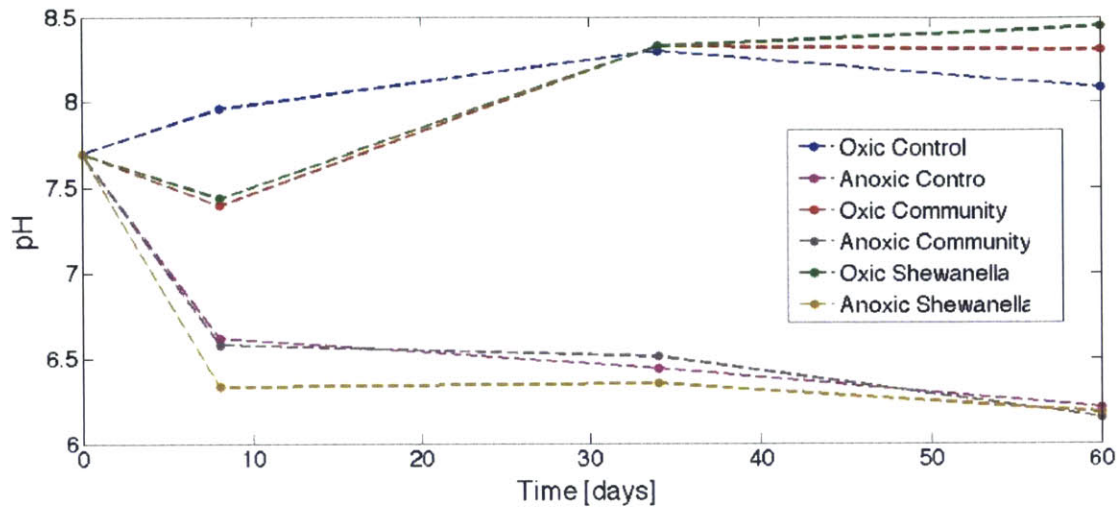


Figure 3-1 The pH values from the water columns of my various samples from experiment 2 over the course of 60 days. The anoxic samples have particularly low pH values due to the high levels of CO₂ added with the anoxic atmosphere.

3.1 Dissolved Fe Concentrations and pH During Degradation

The pH measurements from experiment 2 are plotted in Figure 3-1, and the plots for the dissolved Fe(II) and total Fe concentrations are shown in Figures 3-2 and 3-3. The pH in the oxidic control sample increased from ~ 7.7 at inoculation to ~ 8.1. The anoxic control decreased to 6.5 after flushing with 20% CO₂ atmosphere which acidified the media. The anoxic microbial samples maintained a pH of ~6.3-6.5 and the oxidic microbial samples increased from ~7.4 to ~8.4. Within the first 8 days, microbial activity and growth decreased the pH of the microbial samples compared to the controls, but over the next 52 days the pH of the microbial samples converged with their respective controls.

The dissolved Fe(II) and total Fe concentrations in my oxidic control samples were typically ~ 0 mg/L. However, the anoxic control seemed to have increasing levels of Fe(III) and Fe(II), as seen in Figures 3-2 and 3-3. Apparently, iron from inside of the *Spirulina* within the sediment was leaching out of the organics into the pore waters, and ultimately into the water column. Dissolved organic concentrations are low in

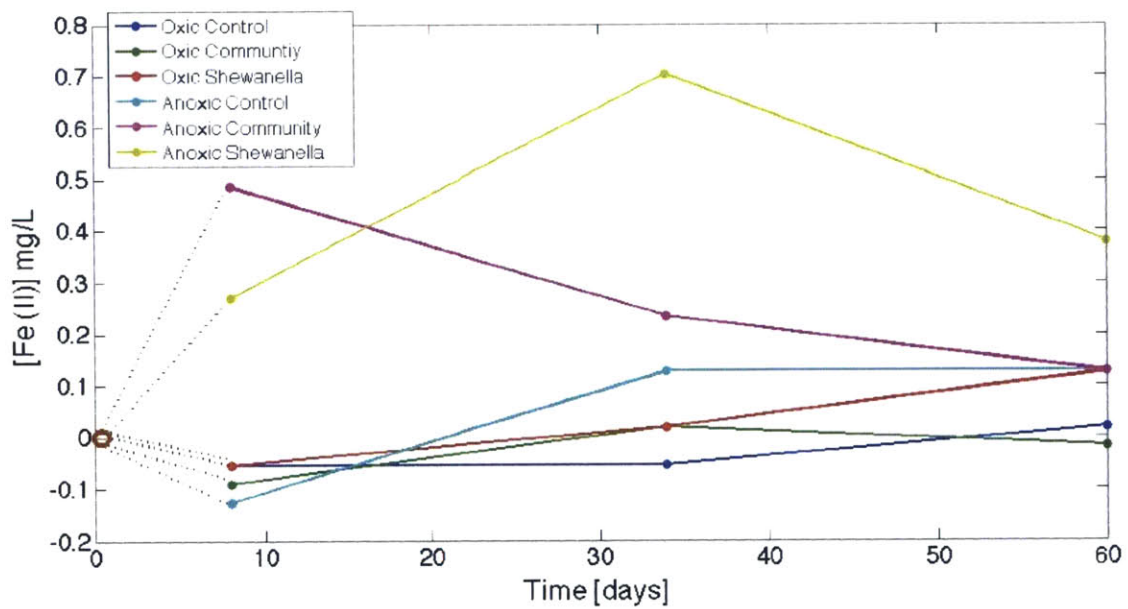


Figure 3-2 Concentration of dissolved Fe(II) measured by the Ferrozine assay from three sets of samples. Samples from the natural community and *Shewanella* cultures in anoxia have high concentrations of Fe(II) early in the experiment. These high concentrations then drop significantly by the end of 60 days. Fe(II) concentration increases in the oxidic *Shewanella* sample and the anoxic control sample over the 60 day period.

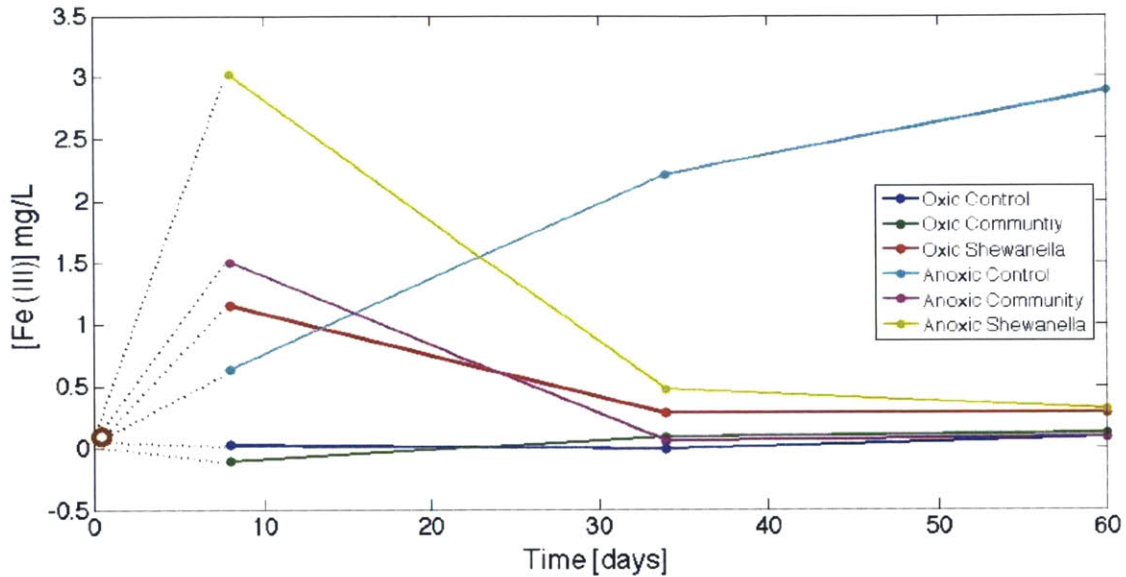


Figure 3-3 Total dissolved Fe concentration in three sets of samples from experiment 2. The concentration of total iron within the anoxic *Shewanella* sample increased to about 3 mg/L within the first 8 days, and the anoxic community reacted similarly increasing to about 1.5 mg/L. The microbial samples decrease in total Fe concentration over the next 52 days, possibly due to rapid sequestration of the Fe into sedimentary particles or due to interference from dissolved Fe-organic complexes. Additionally, the anoxic control sample increased in concentration of total dissolved iron over 60 days, suggesting that iron was leaching out of the organics and into the pore waters. Without the presence of abundant O₂ or dissolved organics within this control, the Fe(III) built up in the solution.

these controls as noted by lack of color in the media, therefore without O₂ in the solution, the Fe is able to increase in concentration over time.

The Fe(II) concentrations were measured to be higher for the anoxic samples and lower for the oxic samples. In particular the *Shewanella Putrefaciens* samples were very interesting, yielding small Fe(II) and total Fe concentrations in oxic conditions and quite high concentrations in anoxic conditions, especially total Fe. The total Fe concentrations were typically very high in the anoxic microbial samples after 8 days of incubation, and even the oxic *Shewanella* sample had considerable concentrations of total Fe. However, over time, the total Fe decreases to zero in the microbial samples. This could be due to Fe sequestration within sedimentary particles, or dissolved organics could be strongly binding to the Fe while the concentration of these organics increase over time.

As can be seen in Figures 3-2 and 3-3, anomalies arose consisting of slightly negative OD differences for some data points. These OD values, when subtracted from one another, normally produced a positive value. But negative OD_{diff} values put into equation 3.2 suggests negative values of iron concentration, which must be false. Organic molecules and organic-Fe complexes (smaller than the 0.2 μm filter) that produced the greenish-yellowish color to the filtered media are causing this interference with the Ferrozine assay. The dissolved organic material complexes with iron and/or with the Ferrozine. This interaction alters the absorbance of the media.

3.2 Net Loss of Organics During Degradation

Loss on ignition was performed on samples from experiment 1 in triplicates, and these measurements are displayed in Figure 3-4. The organic content of the initial model sediment was measured to be %*ORG* = 1.55% ± 0.072%, allowing the conclusion that each microbial culture is within measurement error of the other living cultures. By these measurements I find that each microbial sample is degrading organic material at nearly equal rates. According to the data, having high initial Fe(III) versus no

Loss On Ignition Results: Remaining organic content after degradation

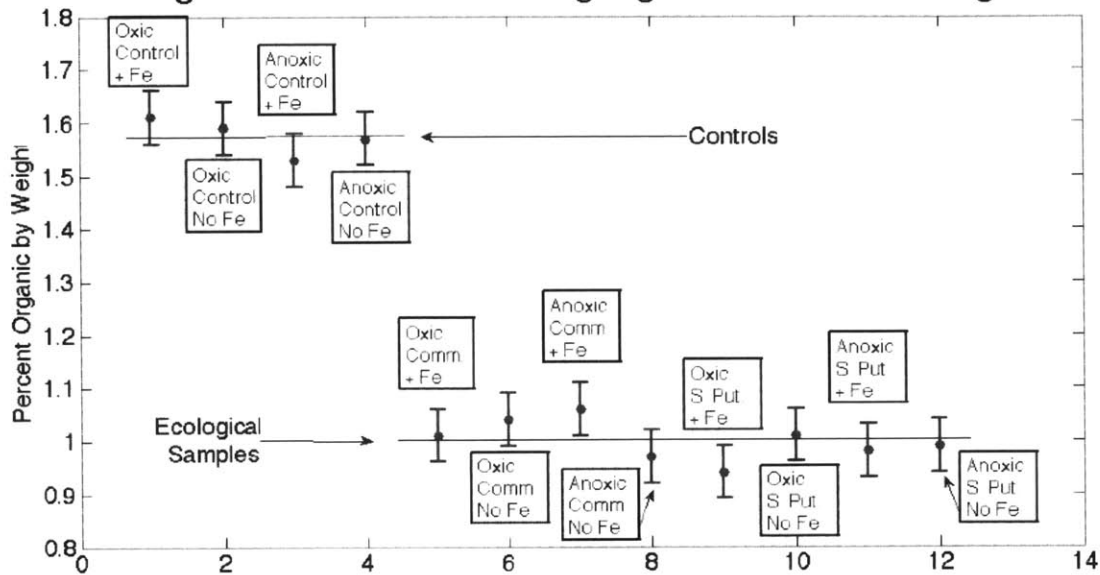


Figure 3-4 The remaining amount of organic matter as measured in triplicates within each sediment parameter type after 40 days of incubation. The organic matter within each microbial sample was measured to have degraded by ~ 35% within measurement error despite contrasting iron concentrations. The controls were measured to have undergone no net degradation from 1.6% organic.

initial Fe(III) in the media, or having oxic versus anoxic conditions was measured to have no differentiating effects on the total extent of degradation for this short time scale. Furthermore, using my results from the end-time dissolved Fe(II) and total Fe concentrations, we can also see that having high Fe concentrations appears to have no measurable effect on organic degradation on this 1-month timescale. This experiment with solid cyanobacterial aggregates is in contrast to other studies of the effects of Fe and/or Al on the degradation rates of dissolved organic matter DOM that demonstrated preservative effects when the metal to complexing functional groups molar ratio (M/CG) was exceeding the saturation of the organic-metal complexing sites (Boudot et al. 1989).

The net extent of degradation of the organics within these samples was ~ 35%. Assuming a constant degradation rate, this would imply that the whole samples would have degraded within about 3-4 months. However if we presume an exponential decay

of the organics, it could take about 10 months to degrade 98% of the organics within a similar system. If significant numbers of recalcitrant particles build up within that time, it could be possible to measure a divergence in sedimentary organic degradation rates.

3.3 SEM Imaging and EDS Analysis of Sediment Particles After Degradation

The sub samples from the controls were abundant in large intact *Spirulina* aggregates and there was no apparent evidence of mineralization or degradation. A representative image of these samples is displayed in Figure 3-5. The organics within the control samples had no compelling evidence of mineral precipitation or microbial activity and appear unchanged from their initial state discussed in section 2.1 of this thesis. Typical EDS scans reported the particles tended to have a very strong carbon peaks, as well as oxygen peaks, and other signals tend to be less than 1%. Furthermore, EDS scans of the glass beads reported no change from the initial scans discussed in section 2.1, having strong silicon and oxygen peaks as well as moderate levels of sodium and calcium. In Figure 3-6 a close up of *Spirulina* aggregates from the anoxic control of experiment 2 is shown, and one aggregate has been ripped open revealing the interior *Spirulina* fragments.

Figure 3-7 is included as a representative image of what the microbial samples looked like: organic aggregates were sparse and generally much smaller than their control counterparts. Some of these organics seemed intact for the most part, maintaining a normal organic EDS signature with high carbon and high oxygen. Some of these particles, however, had organo-mineral EDS signatures with reasonably high carbon levels and elevated levels of silicon or calcium in addition to other trace metals including but not limited to aluminum, iron, magnesium, sodium, and potassium. It was more typical to find individual *Spirulina* fragments unmineralized, and the remaining larger aggregates tended to be a least partially mineralized.

Referring to figures 3-8 through 3-16 for SEM images and EDS reports of various particles found within the natural microbial community samples. Images 3-7 through 3-11 are oxic, and 3-12 through 3-15 are anoxic. The oxic community samples from experiment 1 had several particles with what appear to be triangular structures growing around organic material (Figure-12). The oxic samples from experiments 1 and 2 exhibit a variety of structures including partially degraded aggregates exhibiting microporous structures (Figure 3-8 through 3-10) and semi-amorphous globules of mineralized material (Figure 3-11). The anoxic samples had a variety of structures including a right-angled semi-silicate structure (Figure 3-15), amorphous silicates (Figure 3-14), and partially mineralized Spirulina filaments (not shown), and it was typical to find numerous undegraded filament fragments in these samples. The high abundance of Spirulina fragments suggests that the extracellular polymeric substance (EPS) that was holding the organic aggregates together may have degraded first in these anoxic conditions. Also some tabular structures of organo-minerals were found (e.g. Figure 3-16). Compositions of particles varied from being high in calcium and magnesium and possibly phosphorous to instead being high in silicon and various metals like iron and aluminum.

Refer to figures 3-17 through 3-20 for SEM images and EDS reports of various particles found within the *Shewanella Putrefaciens Strain 200* cultures. Images 3-16 and 3-17 are oxic while 3-18 and 3-19 are anoxic. The oxic samples had several mineralized and partially degraded particles (e.g. Figure 3-17), including large aggregates sometimes more than 100 microns across (not shown). These particles tend to have mostly silicate or possibly carbonate signatures with elevated levels of aluminum or iron. Mineralized Spirulina filaments with iron signals were located (not shown). The anoxic samples had mostly amorphous particles with silicates or carbonates (Figures 3-19 and 3-20).

Overall, the SEM and EDS analysis of sediment subsamples indicate little to no mineralization in the controls and significant levels of degradation and mineralization in all of the other samples. Oxic samples had fewer intact fragments making it easier to locate mineralized and degraded Spirulina aggregates. In contrast, the high

abundance of Spirulina fragments in the anoxic samples made it difficult to find intact or degraded Spirulina aggregates, however some mineralized particles were located, including amorphous organic and metal-rich particles. Considering that Fe and Al have been shown to help precipitate dissolved organic matter into the sediment (Riedel et al. 2013), these amorphous particles likely originated as dissolved organic matter and then precipitated once the matter complexed with enough metals. In some samples particles were identified with clear crystal faces present, e.g. Figures 3-12 and 3-15, however these were typically uncommon and most mineralization appeared mostly amorphous on the micron scale.

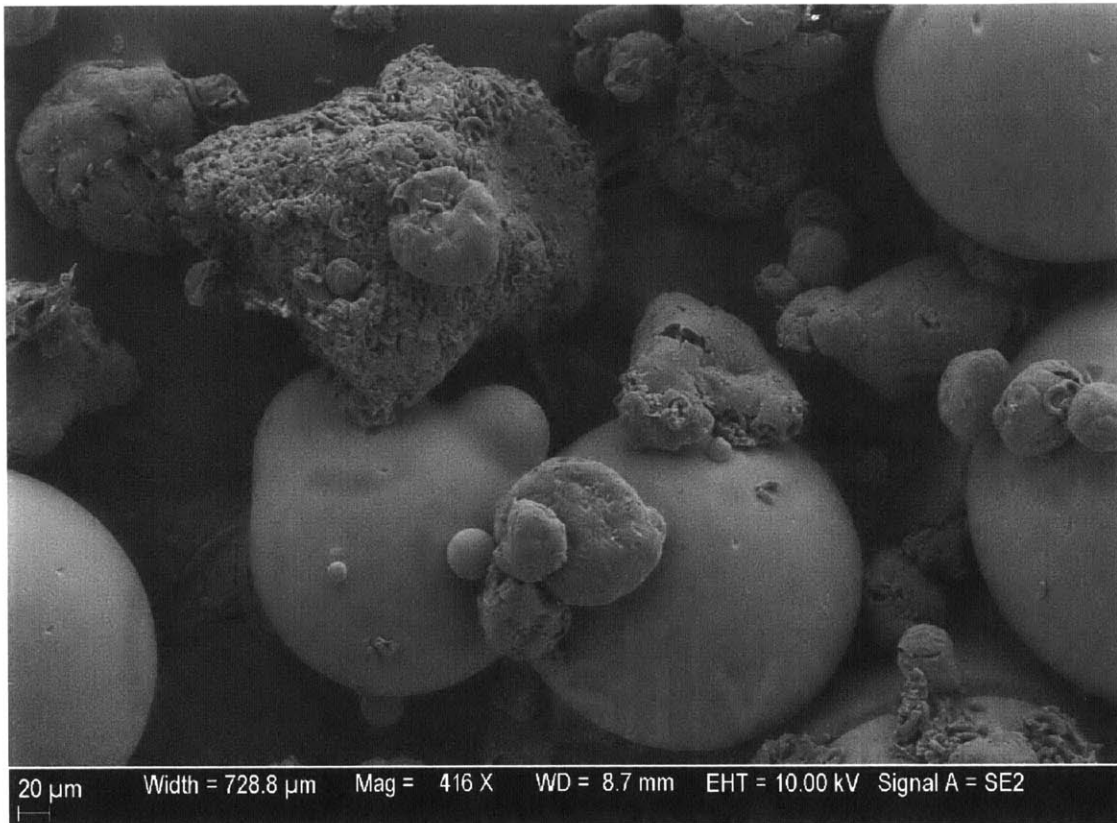


Figure 3-5 A representative image of sterile model sediment in a control sample after 5 weeks on a shaker. Aggregates of organic material composed of Spirulina are observed but clays or mineral precipitates are absent. Large organic aggregates and glass beads are easily identified. Other control samples (anoxic and oxic) had similar appearing organic aggregates. No differences could be identified with SEM imaging or EDS analysis. Typical EDS scans of an organic aggregate show carbon (> 60% by weight, > 75% by atomic count) and oxygen (> 20% by weight and by atomic count). Trace metals (Na, Mg, Si, Ca, S, Cl, K) were also detected (typically < 1% by weight and atomic count).

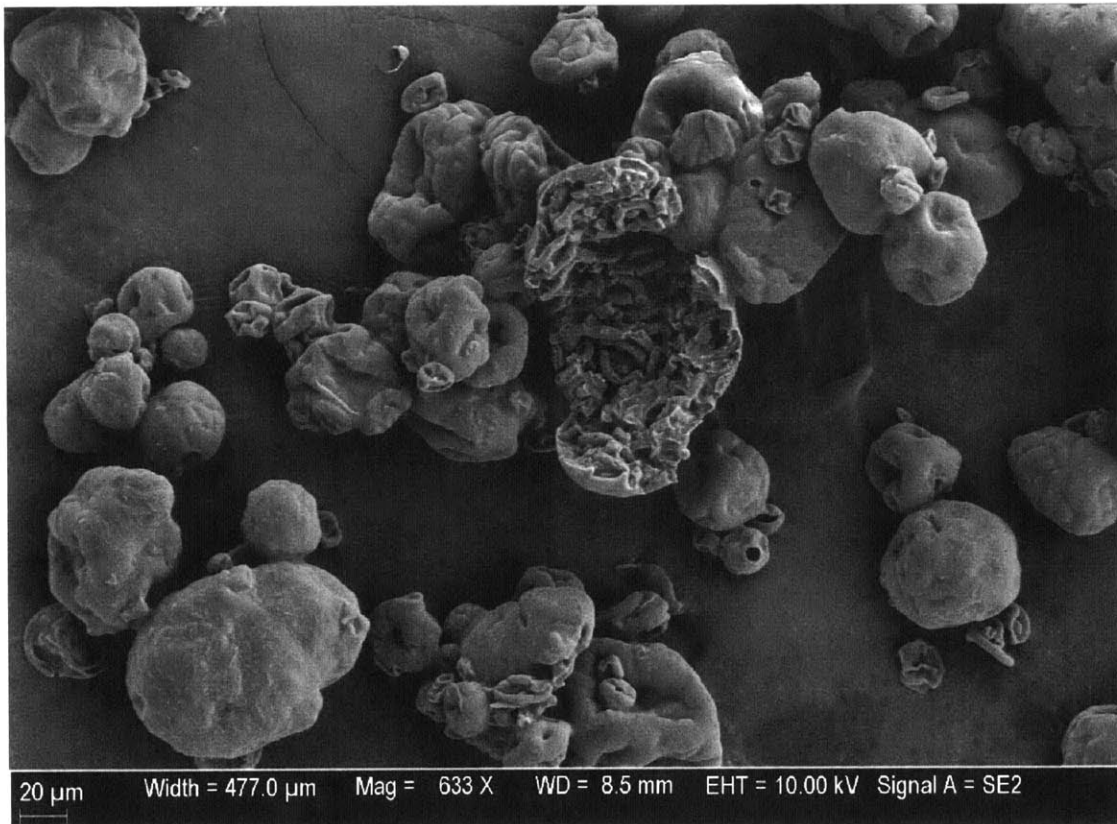


Figure 3-6 Fragments of the Spirulina cells are apparent inside of an aggregate that appears to have been ruptured in this anoxic control sample. These fragments had an identical EDS spectra as the typical intact aggregate with mostly carbon and oxygen.

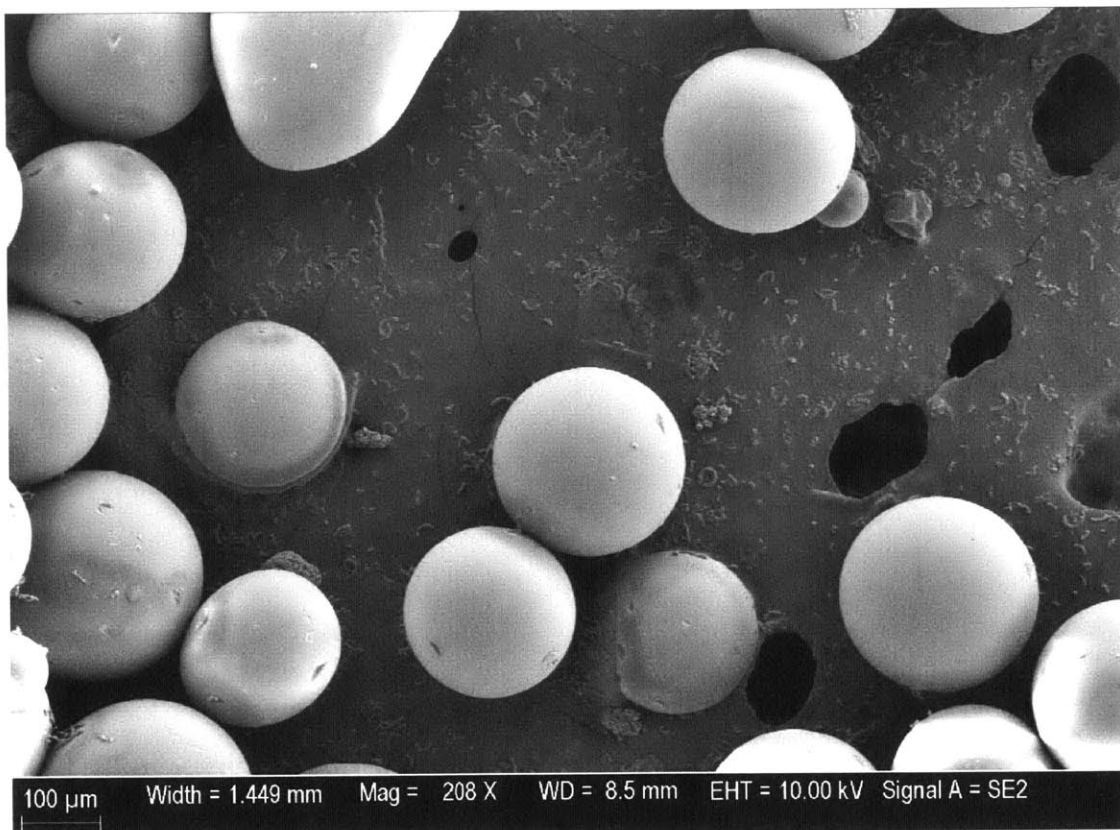


Figure 3-7 A representative image of sediment from a 5 week old culture. Organic particles are not common and large aggregates of organics are difficult to find. This pattern is typical of all of the microbial cultures in my experiment. Some $\sim 20\text{-}40\ \mu\text{m}$ semi-mineralized particles are also visible in this image.

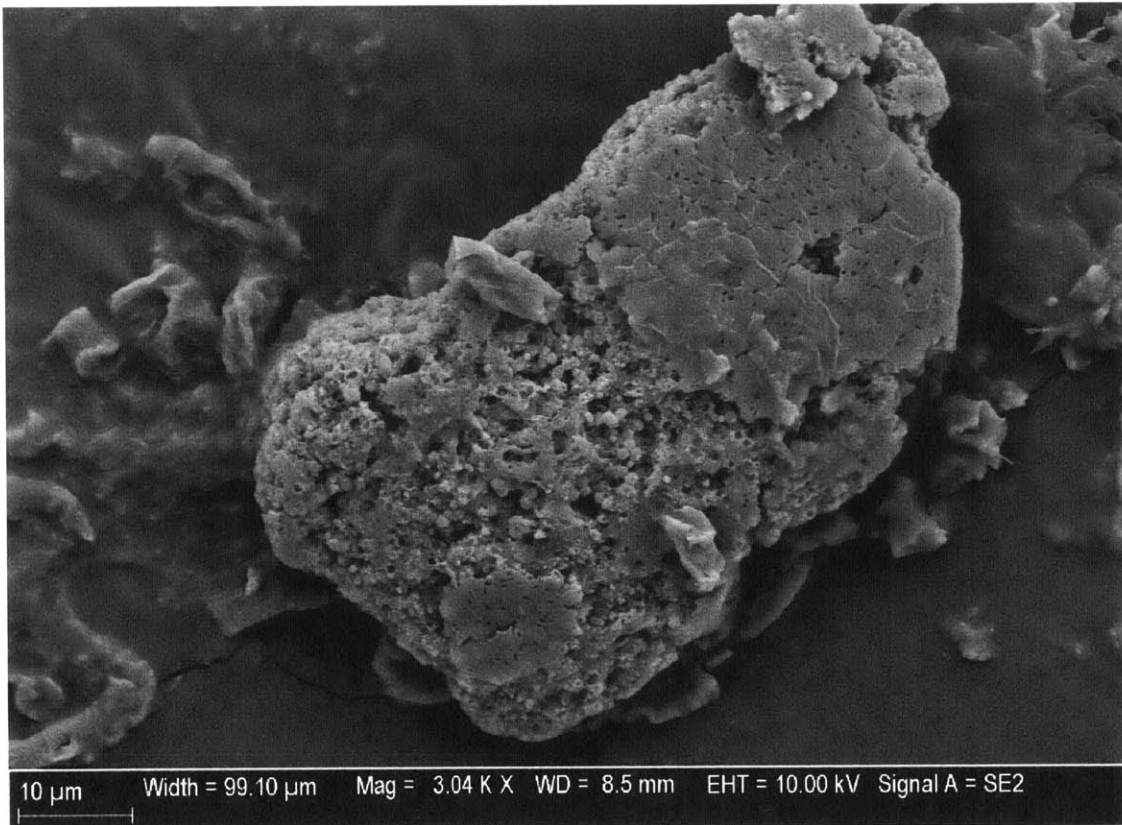


Figure 3-8 A representative image of a partially degraded Spirulina aggregate within the oxic natural community sample from experiment 2. The left hand side of the aggregate has been degraded producing micropores and small pseudo-spherical globules of semi-mineralized organic material.

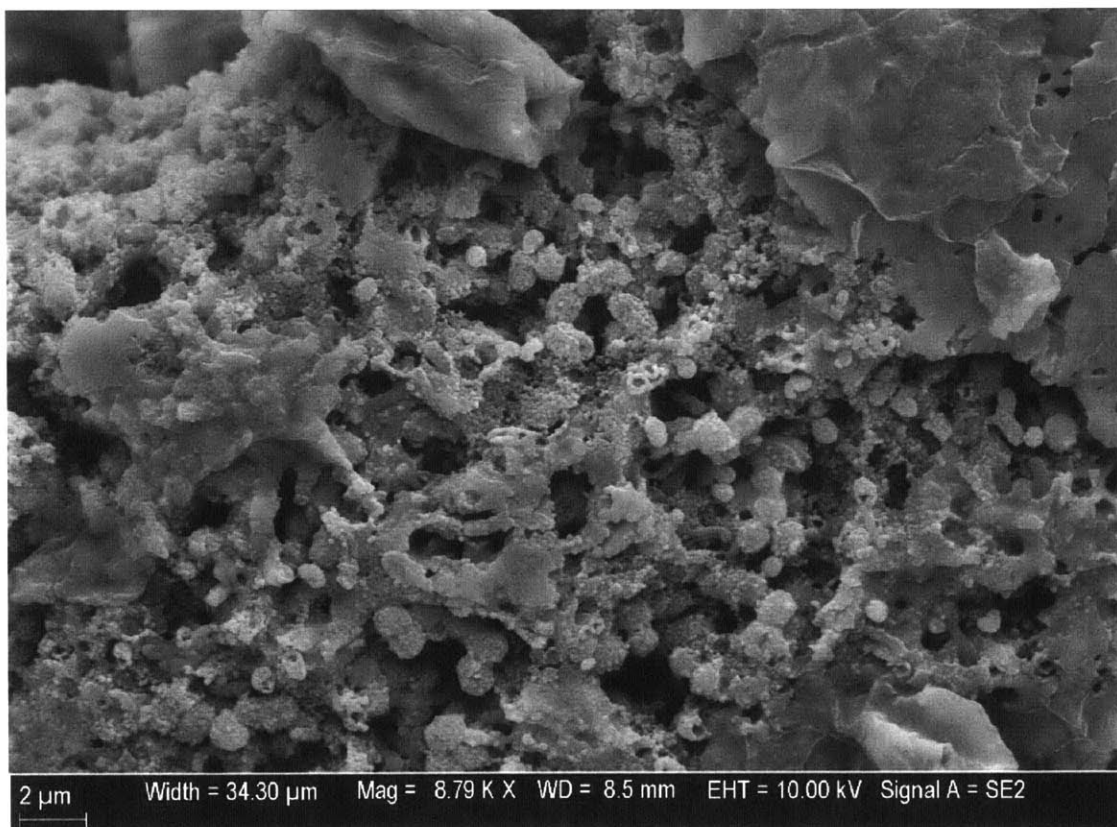


Figure 3-9 A representative image exhibiting a degraded particle from the oxic natural community sample in experiment 2. Microporous structure is visible and micron sized globules of organo-mineral material is apparent. This image is magnified from the previous figure.

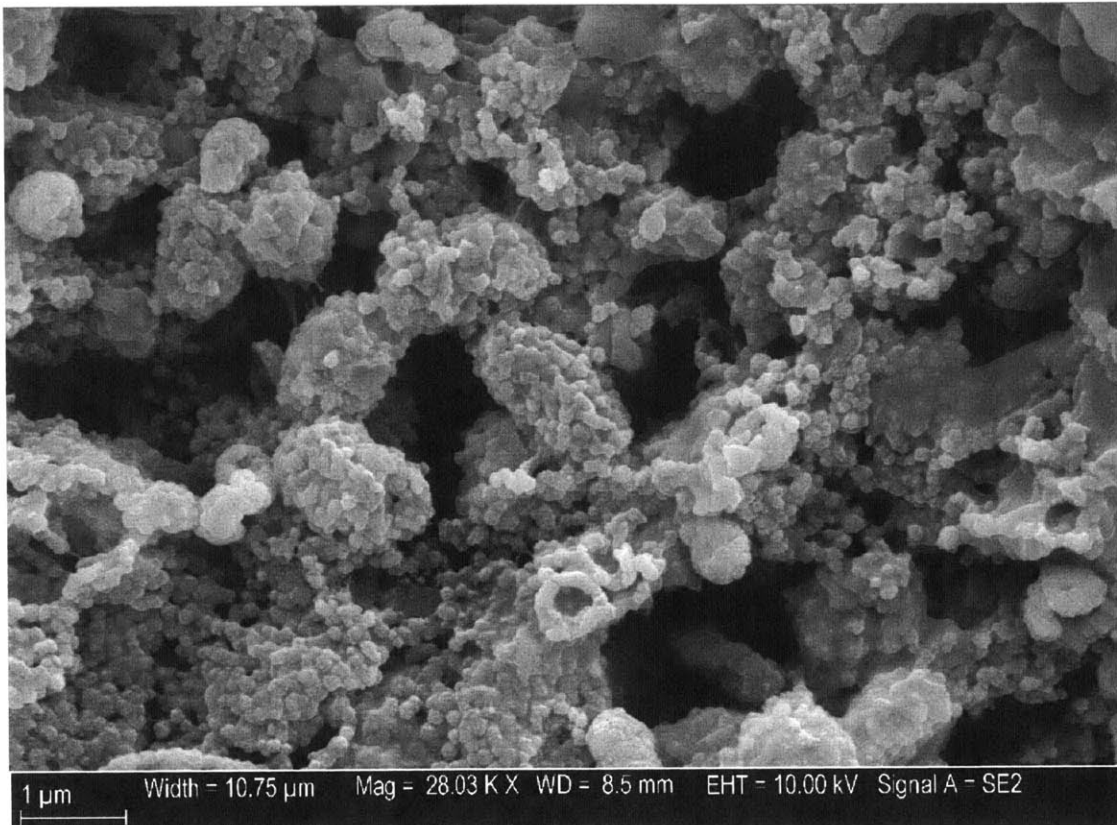


Figure 3-10 A representative image of typical degraded structures from the oxic natural community sample from experiment 2. It is apparent that nano-spheres approximately 100 nm in diameter constitute and/or coat the majority of the microporous structure. The nano-spheres appear to be adsorbed together. A circular structure is visible below the center of the image. These toroidal and/or cylindrical structures were common in other locations of this sample. This image is a magnification of the previous figure.

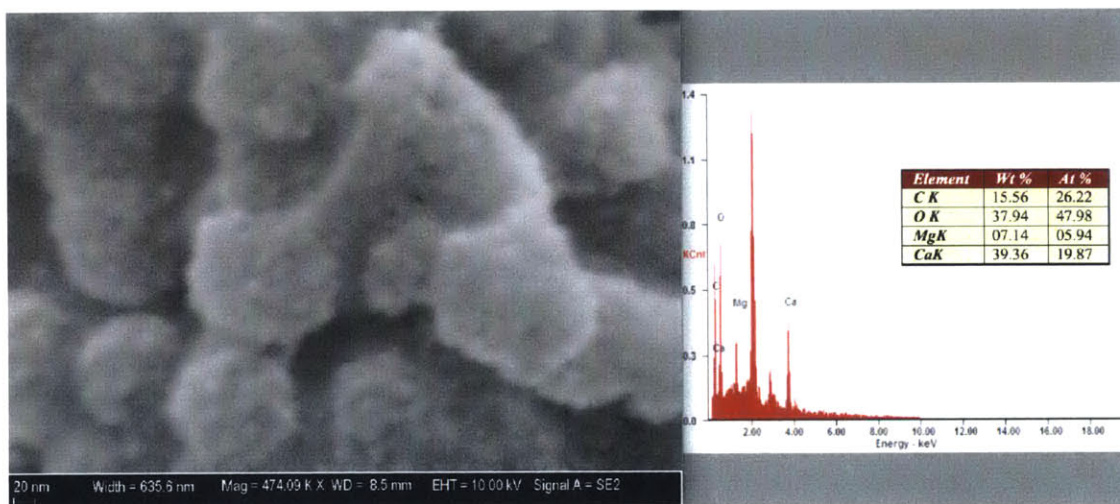


Figure 3-11 A typical EDS scan and corresponding quantification taken from the oxic natural community sample from experiment 2. There may also be significant levels of phosphorous (not shown) considering the relative height of the platinum peak. However for this quantification I omitted P, S, Pt and Pd to avoid their interfering effects. Nanometer scale lumps are apparent on the exterior of the 100 nm sized globules. This image is a magnification of the previous figure.

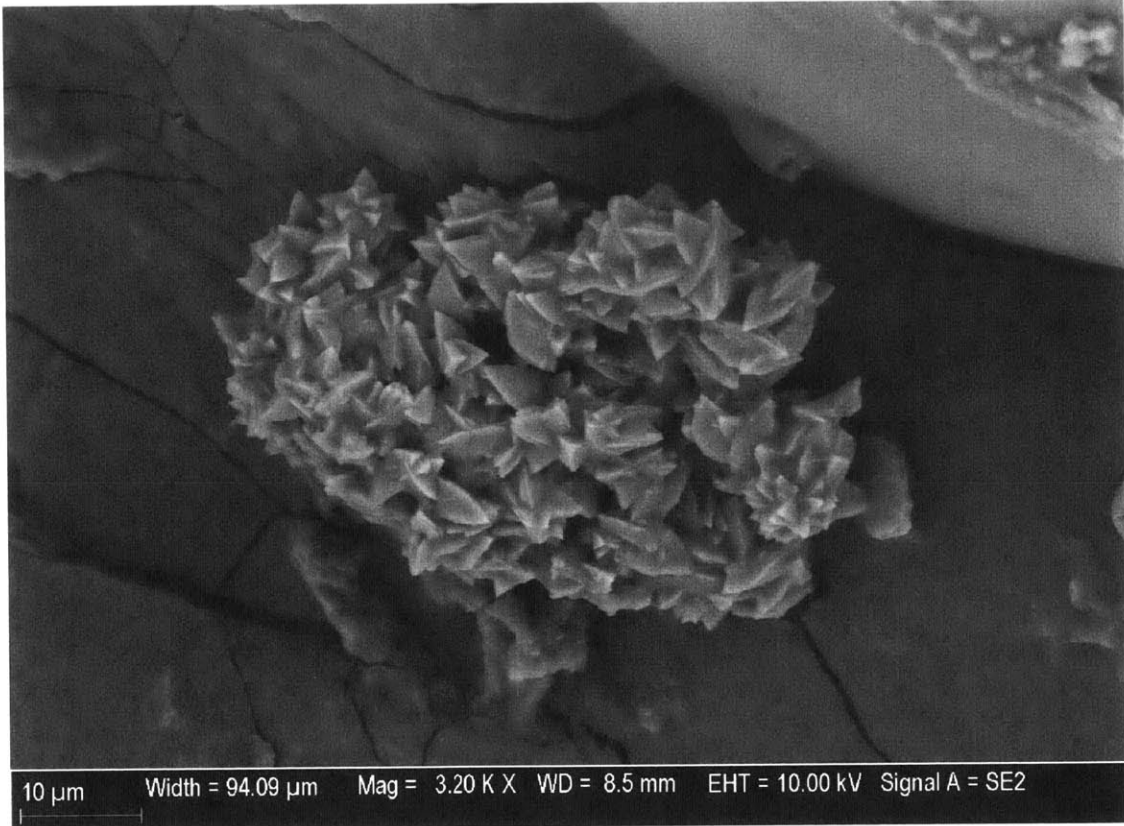


Figure 3-12 Triangular shaped mineralized particles adsorbed to each other were also typical in the oxic natural community samples from experiment 1. Elements present include Ca, O, C, Si, and trace levels of Na, Mg, and Al.

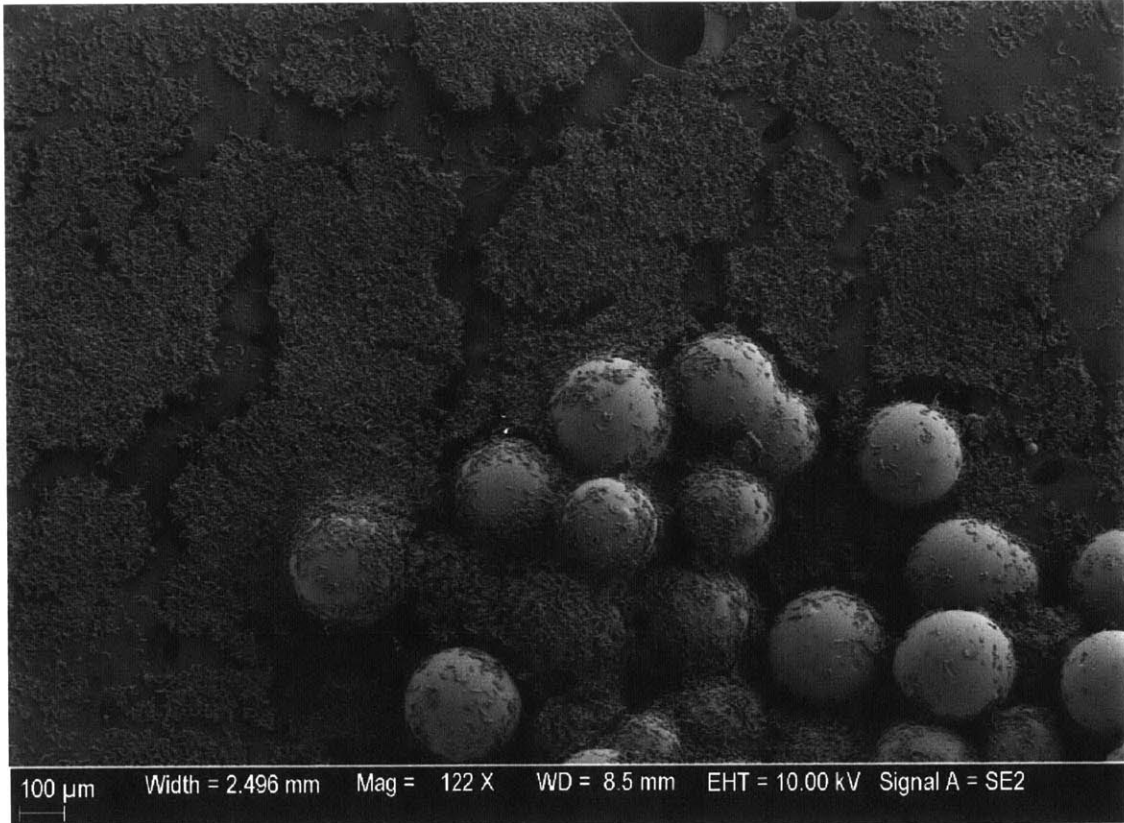


Figure 3-13 This representative image from the living anoxic samples shows many small intact spirulina fragments coating most surfaces with a layer of un-degraded material. This material exhibits little mineralization, suggesting that sticky extracellular polymeric substances holding the control aggregates together was degraded first in anoxic conditions. Despite the abundance of small intact fragments, it was possible to find some mineralized and/or degraded particles in smaller quantities within these samples.

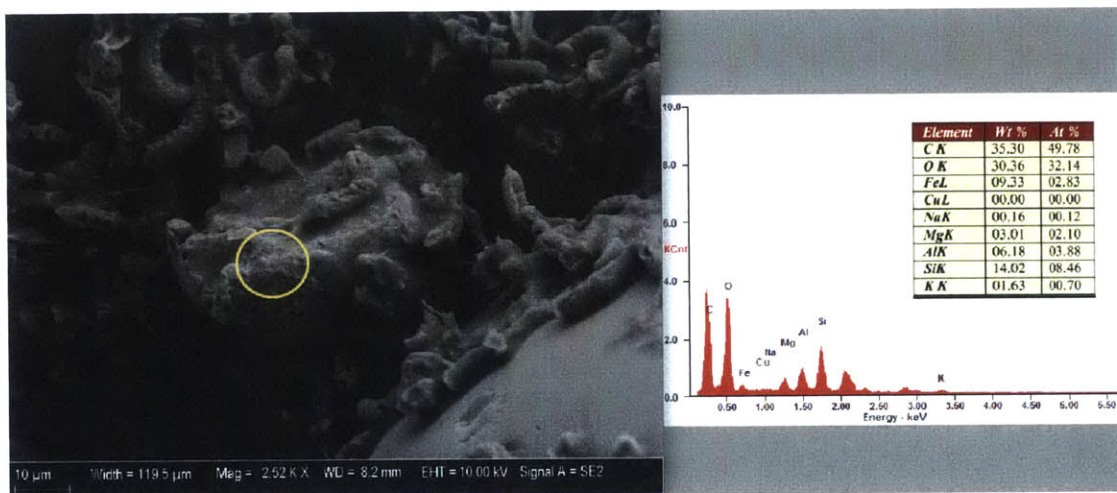


Figure 3-14 A representative image of a typical mineralized particle within the anoxic community samples. This particle has a less definitive form, no obvious crystal faces, and amorphous clumps adsorbed to the exterior. The EDS scan occurred within the yellow circle, suggesting the particle is rich in organics, Fe, Si, and Al, as well as having other trace metals.

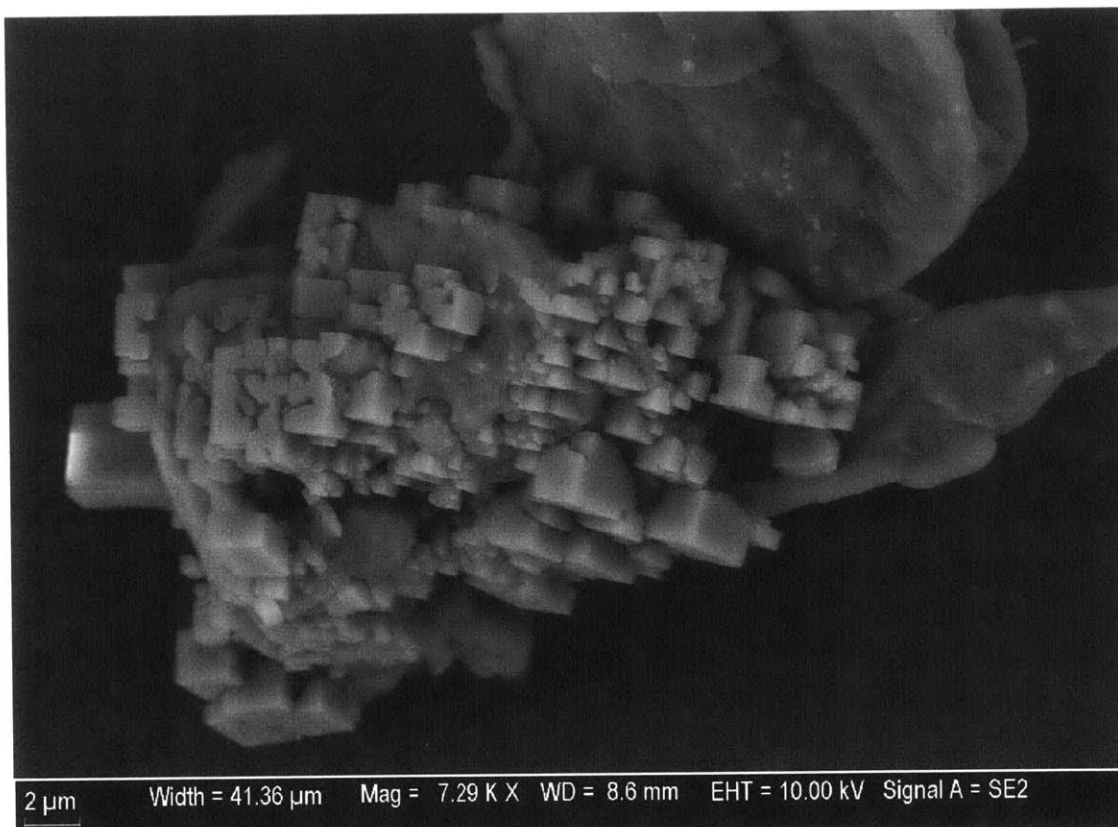


Figure 3-15 Large right-angled granular crystal structures are apparent on the surface of an organic particle found in the anoxic natural community with initially high dissolved Fe(III) from experiment 1. This was the only such particle of this morphology. Micron scale crystal structure combined with the near alignment of the individual lattices suggest this mineral may have geochemically precipitated, possibly in response to distant microbial activity. The *Spirulina* aggregate adsorbed to the crystals maintained a normal organic signature. EDS scans of the large crystal face in the center of this image revealed C, O, Si, and smaller amounts of Na, Al, and Mg. This is an excellent example of authigenic mineral production within the anoxic natural community samples.

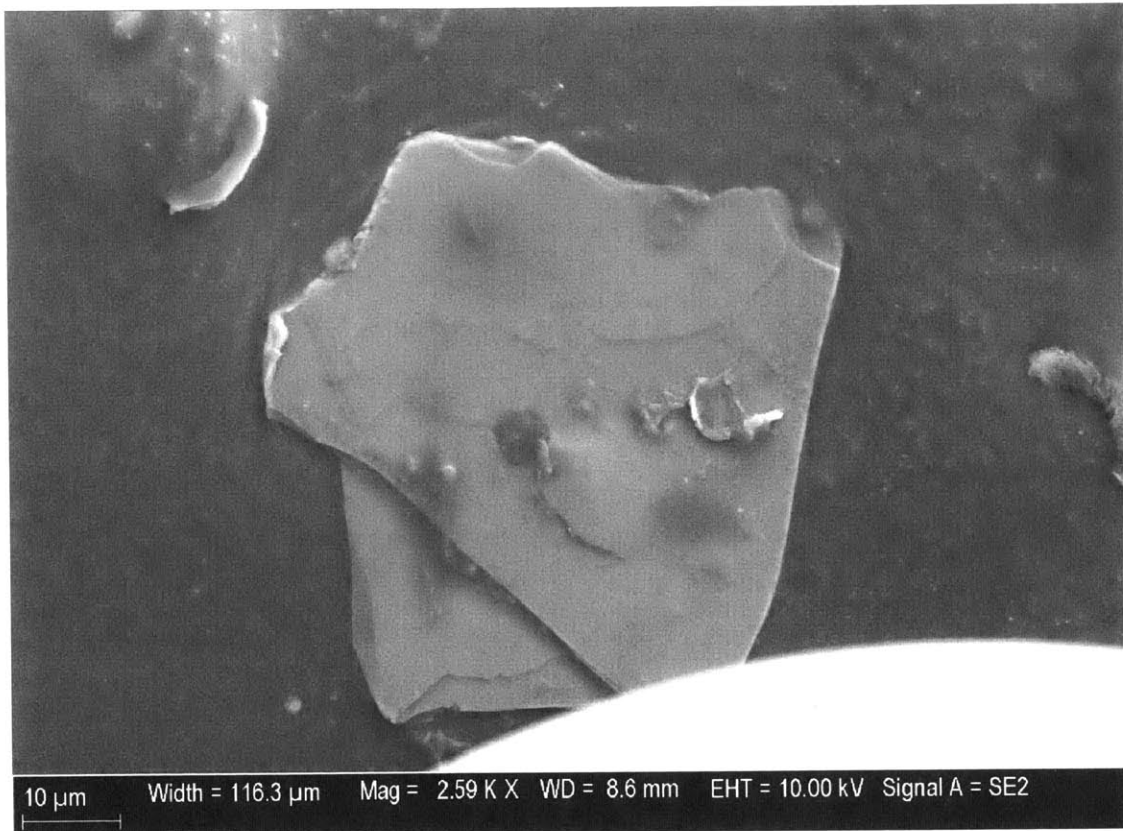


Figure 3-16 An uncommon organo-mineral with tabular structure is apparent. Several particles of this general morphology were found in the anoxic natural community samples. EDS scans of this particle reveal an abundance of C, O, Si, and Al as well as lower levels of P, Fe, Na, and Mg. The tabular structure suggests it could be composed of sheet-silicate material.

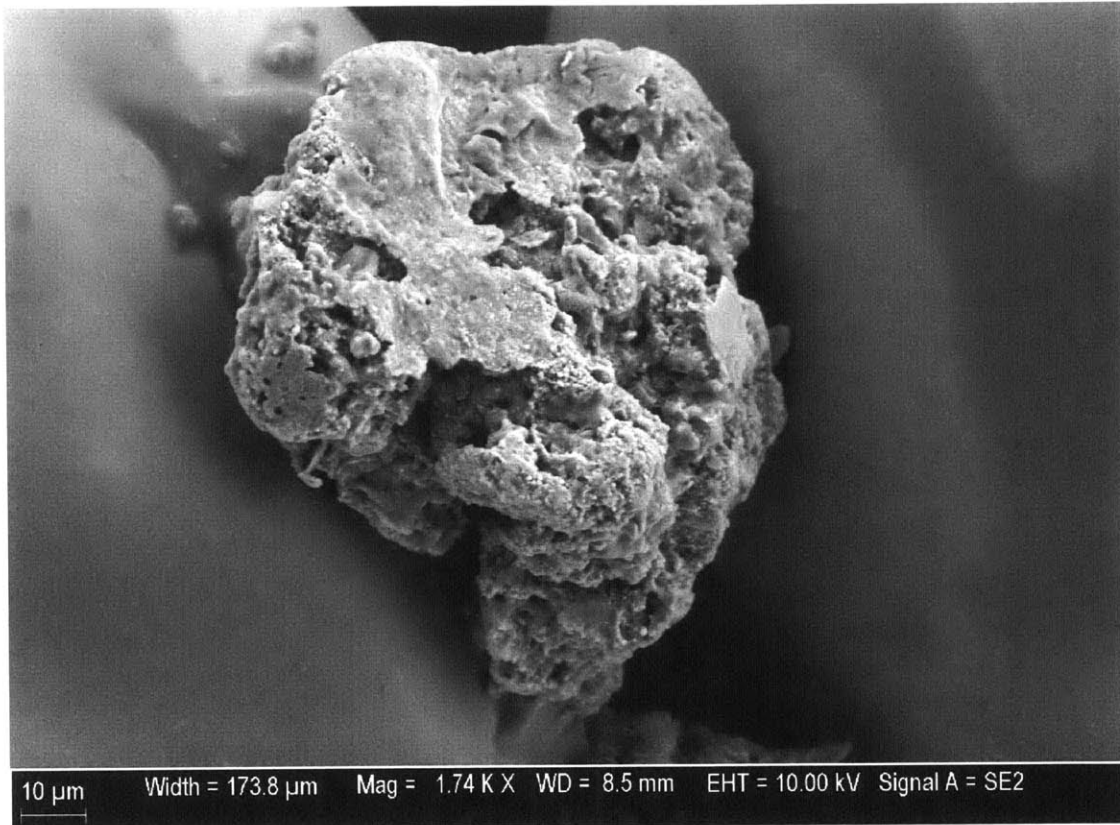


Figure 3-17 A partially mineralized organic aggregate typical of oxic *Shewanella* cultures. The degraded particle has small lumps on the exterior of the particle giving it a crusty appearance. These aggregates resemble the aggregates imaged from the oxic natural community samples. Noticeable degradation has occurred revealing a microporous structure as well as localized mineralized globules, similar to those shown in Figure 3-8. According to the Expert ID and Quant functions on the SEM, EDS scans of the center of this image revealed the presence of C, O, and Ca as well as lower levels of P, Fe, Na, and Mg. Similar particles were identified with varying levels of Fe (up to 10%), Si, P, Al, and Mg.

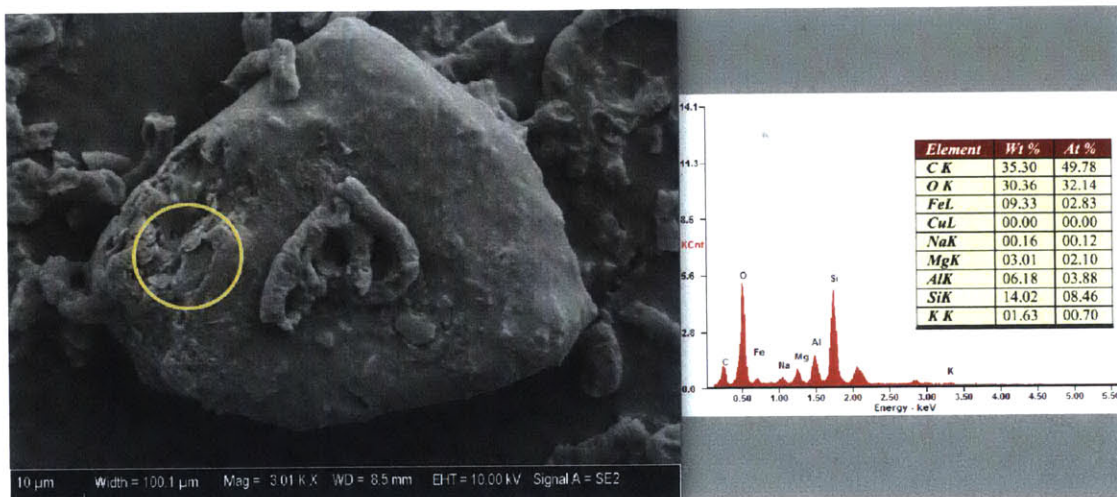


Figure 3-18 A typical mineralized particle from the oxic *Shewanella* sample from experiment 2. Though the overall shape is amorphous, irregularities in surface features as well as a broken corner of the aggregate within the yellow circle reveal semi-mineralized structures. The EDS scan took place in the yellow circle, revealing Fe is present, in addition to Si and Al. Other trace metals are also present. The Pt and Pd peaks were again removed from quantification.



Figure 3-19 A typical organo-silicate particle from the anoxic *Shewanella* samples. Mineralization appears generally amorphous with some small lumps on the exterior. Some similar particles had slightly more structure, but typically clear crystal faces were not present. According to the Expert ID and Quant functions on the SEM, EDS scans of the center of this image revealed abundant C, O, Si, up to 10% Fe, and lower levels of Al, and Mg.

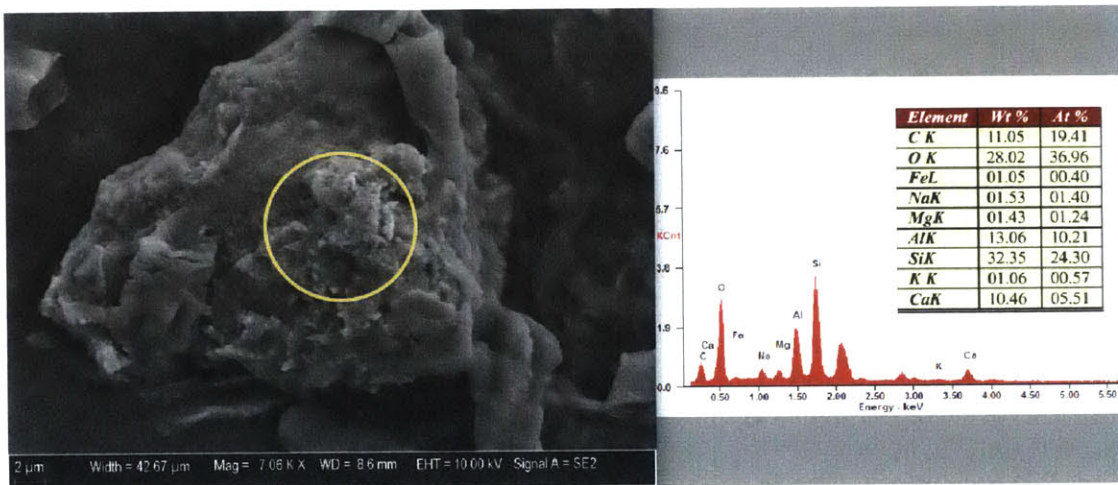


Figure 3-20 A typical mineralized particle from the anoxic *Shewanella* samples. The EDS spectra and quantification gathered from inside of the yellow circle revealed abundant Si and Al. Some similar appearing particles also had high levels of Ca, Mg, or Fe.

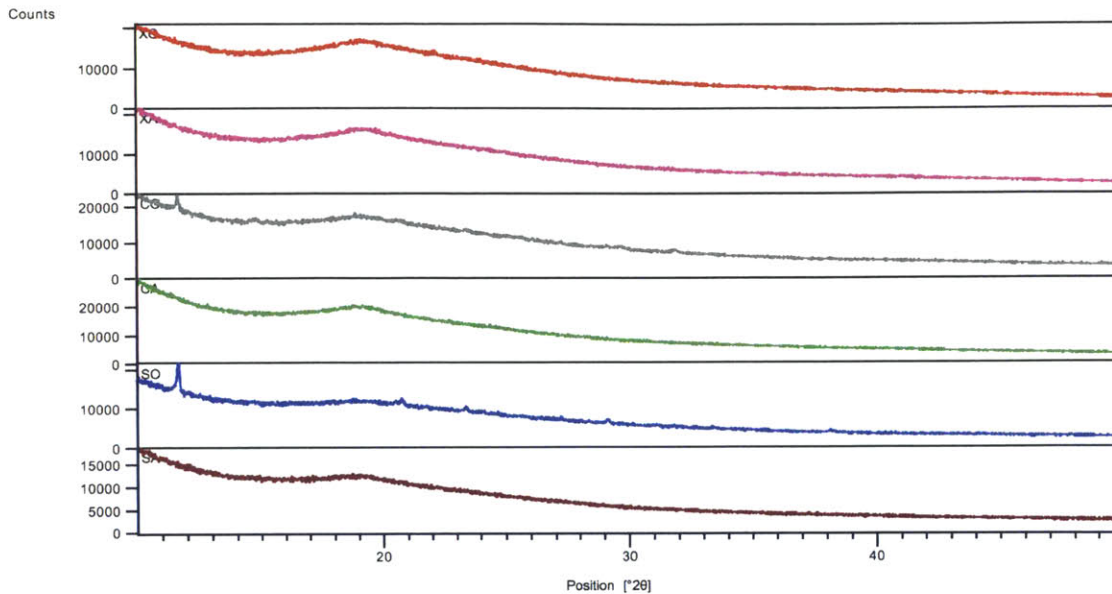


Figure 3-21 A side-by-side comparison of the XRD spectra from the 6 incubation samples grown in experiment 2. Letters in the upper left of each spectra provide the legend. ‘X’ represents control samples, ‘C’ represents the natural community samples, and ‘S’ represents the *Shewanella Putrefaciens* samples. ‘O’ represents oxic samples and ‘A’ represents anoxic samples: the top 2 spectra are controls, the middle 2 are from the community, and the bottom 2 are *Shewanella*. The oxic natural community and the oxic *Shewanella* samples are the only two samples where numerous peaks above the amorphous background were identified.

3.4 X-Ray Diffraction Spectra

A side-by-side comparison of the XRD spectra from the 6 samples incubated for 34 days in experiment 2 is shown in Figure 3-21. A strong amorphous background is present, consistently peaking at about 19 degrees and then this background decreased towards ‘O’ as 2θ increased towards 120 degrees. The oxic natural community sample and the oxic *Shewanella Putrefaciens* sample both had identifiable peaks within their spectra. Comparing these spectra with the controls, there is clear mineral structure developing within these oxic samples. In contrast, no detectable peaks were found in the anoxic community and *Shewanella* samples. This may imply a lack of crystalline structure, or small fragments of un-degraded spirulina fragments could be obscuring the few crystal faces that have begun to grow, just as in the anoxic SEM samples.

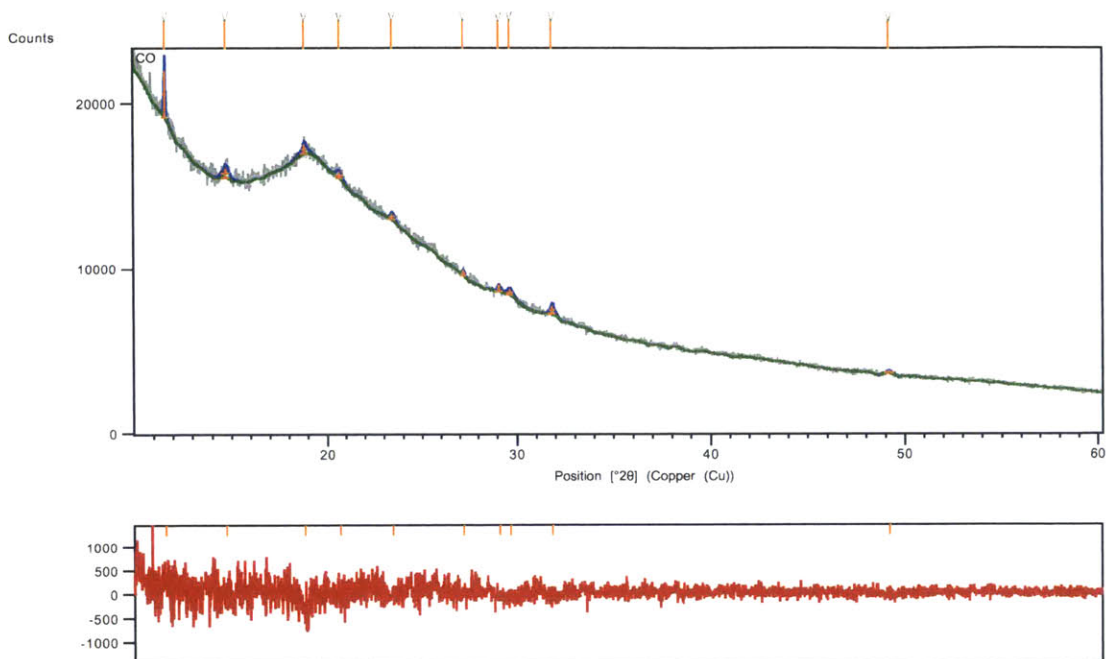


Figure 3-22 The XRD spectrum and collection of fitted peaks from the oxalic natural community sample from experiment 2. Residuals are plotted below the spectra in red. Peak locations are denoted at the top of the figure with yellow lines. A total of 10 peaks were found, refer to table 3.1 for a list and summary of these identified peaks. The intensities of the identified peaks are particularly low compared to the amorphous background.

Figures 3-22 and 3-23 display the profile fits for the oxalic natural community and the oxalic *Shewanella* respectively, and the corresponding lists of the identified peaks are located in Tables 3.1 and 3.2. The given number and relative intensities of these peaks are not sufficient for definitive matches with any particular mineral structures. It is evident that mineral structure is becoming measurable in these oxalic samples. I ran a search and match algorithm using the ‘high score plus’ software in the attempt to find the best matches to the observed peaks. Using this algorithm I compared the most intense peaks from hundreds of reference patterns against my sample data. The best matches I found to these peaks are listed in Tables 3.1 and 3.2. Also, the oxalic control sample appeared to have one anomalous peak at a 2θ of 22.1694 degrees and a D-spacing of 4.00987 angstroms.

Table 3.1. List of Fitted Peaks for the Oxic Natural Community XRD Spectrum

Peak No.	2θ	D-Spacing	Peak Intensity	Potential Match 1	Potential Match 2
(1)	(2)	(3)	(4)	(5)	(6)
1	11.6306	7.60249	2724.49	Brushite	Gypsum
2	14.7411	6.00456	516.16	.	.
3	18.8523	4.70337	508.96	Bayerite	.
4	20.6696	4.29375	294.84	Brushite	Illite
5	23.4351	3.79295	289.87	Brushite	.
6	27.2326	3.27204	298.57	.	.
7	29.1062	3.06553	317.85	Brushite	Calcite
8	29.6400	3.01153	280.34	Ca/Mg Carbonate	.
9	31.8328	2.80891	465.19	Siderite	.
10	49.2114	1.85003	84.57	.	.

Note. — Oxic natural community XRD spectra after 34 days of incubation. 2θ is in degrees, D-spacing is in angstroms, peak intensity is in counts. Mineral structure candidates are listed in columns 5 and 6, however these are not definitive matches

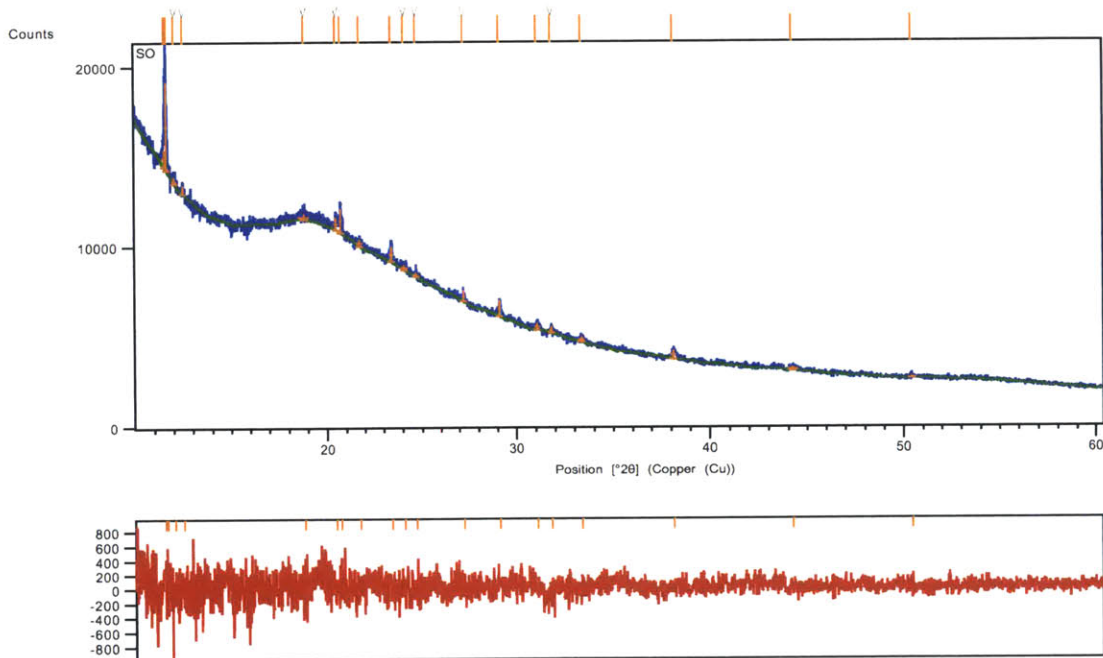


Figure 3-23 The XRD spectrum and collection of fitted peaks from the oxic *Shewanella* sample from experiment 2. Residuals are plotted below the spectra in red. Peak locations are denoted at the top of the figure with yellow lines. A total of 19 peaks were found, refer to table 3.2 for a list and summary of these identified peaks. The intensities of the identified peaks are again particularly low compared to the amorphous background.

Table 3.2. List of Fitted Peaks for the Oxidic *Shewanella Putrefaciens* XRD Spectrum

Peak No.	2θ	D-Spacing	Peak Intensity	Potential Match 1	Potential Match 2
(1)	(2)	(3)	(4)	(5)	(6)
1	11.5475	7.65698	888.51	Brushite	Gypsum
2	11.6485	7.59084	803.43	Brushite	Gypsum
3	12.0893	7.31507	349.31	.	.
4	12.5256	7.06117	430.75	Chamosite	Kaolinite
5	18.8298	4.70893	272.45	Bayerite	.
6	20.5027	4.32833	629.43	Illite	.
7	20.7399	4.27936	1272.13	Brushite	Gypsum
8	21.7562	4.08170	301.28	.	.
9	23.3888	3.80035	800.81	Brushite	.
10	24.0629	3.69539	239.25	Berlinite	.
11	24.6897	3.80298	188.90	.	.
12	27.2466	3.27040	531.58	.	.
13	29.1298	3.06310	824.51	Brushite	Calcite
14	31.0903	2.87427	305.19	Dolomite	Ankerite
15	31.8157	2.81038	300.49	Siderite	.
16	33.3833	2.68190	227.75	Merwinite	.
17	38.1411	2.35758	406.49	.	.
18	44.2681	2.04446	129.75	Gypsum	.
19	50.4547	1.80732	93.92	.	.

Note. — Oxidic *Shewanella* XRD spectra after 34 days of incubation. 2θ is in degrees, D-spacing is in angstroms, peak intensity is in counts. Mineral structure candidates are listed in columns 5 and 6, however these are not definitive matches

Sediment subsamples incubated for 60 days were also processed and XRD analyzed with the hope that the XRD signal would improve with more numerous peaks and of higher intensities, however these samples were extracted and prepared slightly differently. The samples were not fixed with chemical fixative and were only rinsed 3x before ethanol drying. Performing the same X-Ray measurements on these samples overnight revealed similar spectra to Figure 3-21, however these spectra had even weaker intensity peaks. This likely occurred due to my particular technique in preparing the pellets of organics: by selecting the lighter organics with a pipette, saturation of the sample with ethanol ultimately leaves a coating of light non-mineralized *Spirulina* fragments on top of any underlying crystal faces. More research is necessary to duplicate my results from Figure 3-21, and to develop better techniques to quantify the extent of mineralization and authigenic mineral growth.

Chapter 4

Conclusions and Future Work

4.1 Summary

In my experiments I sought to simulate organic degradation within a siliciclastic sediment rich in organics. Specifically, my goal was to observe the growth of iron rich authigenic minerals and active protection of organics from degradation due to the presence of precipitated minerals. I also observed the role of dissolved Fe on the growth of authigenic minerals and the protection of organic matter within my system. I designed an artificial marine sediment consisting of dead cyanobacteria (*Spirulina*) aggregates, glass beads, and liquid marine growth medium with contrasting levels of dissolved Fe(III) with other potential electron acceptors. Using this artificial marine medium, I grew controls and microbial cultures in oxic and anoxic conditions. The control samples had no living organisms, the 'natural community' samples had a sediment community harvested from Plum Island MA, and the '*Shewanella*' samples were inoculated with cultures of *Shewanella Putrefaciens strain 200*. After the 40 days of incubation, samples from experiment 1 which started with 'High Fe' or 'No Fe' were imaged by SEM. Loss on ignition (LOI) gravimetry was conducted in triplicates to quantify the total extent of organic degradation within each sample type. Sediment subsamples were also taken from the samples from experiment 2, and these were analyzed with SEM, EDS, and XRD. This revealed the development of authigenic minerals and amorphous organo-mineral structures within the living microbial sam-

ples. Dissolved Fe(II) and total Fe concentrations, as well as pH were measured from liquid media three times over 60 days.

While the LOI results do not imply any differences between the living samples regarding the extent of degradation, many interesting organic-mineral structures were identified with the SEM. Some had clear and repeated crystal structures such as in Figures 3-12, 3-15. These particles tended to have strong carbon peaks in addition to signatures typical for silicate or carbonate or perhaps phosphate minerals. However P and S signatures in particles measured by the EDS interfered with the Pt and Pd used to sputter coat each sample, and therefore P and S quantification was less reliable. Also, globule structures were common in the oxic samples, particularly in the natural community but also in the *Shewanella* cultures. It is expected that longer experiments of this sort would result in more mineralization and larger quantities of organo-mineral structures. However XRD spectra from samples that incubated for 60 days had less diagnostic peaks than samples incubated identically for 34 days. This signal diminishment might be accounted for by changes in the sub-sampling technique which selected for the lighter non-mineralized organics as opposed to the more dense particles. More experiments are needed to determine if this is the case. From my limited data set, it is not clear as to whether elevated Fe(II) or Fe(III) concentrations help or hinder organic carbon preservation. Some of the examined organic-mineral structures contained abundant iron, particularly in anoxia, indicating that iron was sequestered within these particles. These mineralized particles with an abundance of Fe and other mineralized particles are expected to be more recalcitrant than the original cyanobacterial aggregates of filament fragments. Therefore, it still seems plausible that elevated levels of iron could enhance organic preservation through elevated levels of precipitation of recalcitrant iron-based minerals on or around solid organic material.

Considering that my choice of organic substrate is particularly rich in trace metals, and in Fe in particular, this made it difficult to study the effects of elevated dissolved Fe versus no dissolved Fe on the organics in experiment 1. Fe leaching out of the organics increased Fe concentrations to very high concentrations with or without

initial levels of dissolved Fe. In order to properly study the effects of no dissolved Fe versus higher levels of dissolved Fe, it will be necessary to choose an organic material that is much lower in Fe than *Spirulina*. That being said, the *Shewanella* samples in both experiments 1 and 2 were measured to have very high levels of dissolved Fe. As incubation progressed in experiment 2, dissolved Fe concentrations then tended to decrease within the microbial samples. This decrease in Fe over time was partially attributed to dissolved organics that were being released into the media. This organic matter potentially complexed with the dissolved Fe and interfered with the Ferrozine complexing reagent. This could mean that much of the dissolved iron was bound to complexing sites of dissolved organics, allowing iron to be sequestered into amorphous Fe-organo-mineral aggregates. These iron rich aggregates could also be significantly more recalcitrant than the original dissolved organic matter.

4.2 Conclusions

I set out to observe the development of authigenic mineral structures in a simplified sedimentary system where interference from detrital sediments and contamination from micro particles would not happen. Furthermore my artificial sediment avoided the complication of having many pre-existing mineral grains within the sediment. This allowed for the identification of small abundances of authigenic crystals possible with SEM/EDS and XRD analysis. I was able to do this using a variety of concentrations of Fe as well as using various microbes. Crystal faces were visible via SEM as well as other amorphous and partially mineralized structures and EDS analysis suggest the presence of mineral-making elements like Si, Ca, C, O, P, and trace metals. Furthermore XRD scans of the organic material indicate small amounts of mineral structure developing in the living oxic samples. Crystal structures were identified to be developing in the anoxic microbial samples as well. However these crystals in my subsamples were too few and/or too obscured to be detected with XRD after my sediment processing. Alternative sample processing methods could produce more subsamples with recognizable crystal structure and with fewer interfering organics. I

also sought to observe different degradation rates between samples under contrasting conditions. However the Loss On Ignition (LOI) measurements found indistinguishable rates of degradation between the living microbial samples despite contrasting O₂ concentrations and dissolved Fe concentrations. Better measurement technique could reduce the measurement error of the LOI results, and longer incubation times (6-12 months) could produce measurably different degradation rates and potentially showing different organic preservation rates.

4.3 Future Work

More experiments are needed to be conducted to explore several key questions that have arisen from experiments 1 and 2. These questions are multiple and complex: Exactly what mineral structures are developing in these sorts of conditions, and in what abundances? Does the development of these authigenic minerals affect the degradation of certain organics? Under what conditions are organic aggregates better preserved over time, and under what conditions are they more easily degraded? Are certain microbial metabolisms responsible for enhanced organic protection? How does dissolved Fe affect the preservation or the degradation of the organics?

To answer some of these questions, I would propose a longer term experiment (3-12 months) with precise gravimetric measurements of the initial ingredients as well as precise LOI measurements after degradation to get a better measure of the extent of degradation with a smaller measurement uncertainty. Samples sets in triplicates can be incubated and analyzed on a monthly basis, and thus monthly measurements of dissolved Fe as well as observations with the SEM and EDS and LOI can be made. For subsampling sediments, a broken pipette could be used to extract sediment cores and to help quantify mineralized particle abundances. This sort of experiment could reveal a divergence in organic preservation rates in addition to the possibility of identifying the authigenic minerals associated with organic preservation under these conditions. One could also consider changing other experimental factors. For example, for an extended 6-12 month incubation experiment, it might make more sense to replace

the liquid growth media periodically. Opening and replacing media and re-flushing the anoxic atmospheres would change the experimental conditions. However the pH would be able to remain more constant over long periods of time, and new electron acceptors could be replenished within the system. Furthermore the pH could be kept at a more moderate level. This may be considered more realistic and closer to the actual world being that natural marine waters cycle nutrients constantly. However one will need to take care to account for the lost dissolved organics due to each media change in order to accurately quantify the extent of degradation. It is also worth considering doing these experiments in the dark instead of near a light on a timer, where dark experiments would represent a deeper benthic marine system as opposed to a shallow continental margin. Choosing Spirulina as a carbon source was inconvenient based on the amount of trace metals within the cyanobacterial cells, thus one could explore an alternative source of solid organic carbon without as many trace metals. Furthermore, a modified sediment system could be made with the addition of micro particles to test the effects of clays or the precipitation of nucleated minerals on the degradation of the organic matter.

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