

THE ROLE OF SECONDARY PRECIPITATES AND SULFATE REDUCING
BACTERIA IN MICROBially INFLUENCED STEEL CORROSION:
IMPLICATIONS FOR FUEL TANK DEGRADATION

by

Christine Anne Maday

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Earth Sciences

MONTANA STATE UNIVERSITY
Bozeman, Montana

May 2013

©COPYRIGHT

by

Christine Maday

2013

All Rights Reserved

APPROVAL

of a thesis submitted by

Christine Anne Maday

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citation, bibliographic style, and consistency and is ready for submission to The Graduate School.

Dr. David Mogk

Approved for the Department of Earth Sciences

Dr. David Mogk

Approved for The Graduate School

Dr. Ronald W. Larsen

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library.

If I have indicated my intention to copyright this thesis by including a copyright notice page, copying is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Requests for permission for extended quotation from or reproduction of this thesis in whole or in parts may be granted only by the copyright holder.

Christine Anne Maday

April 2013

ACKNOWLEDGEMENTS

I would like to extend my gratitude and appreciation to my advisor and committee chair, Dr. David Mogk, for his advice, assistance and thoughtful critique of this project. Dr. Mogk accepted me as his graduate student with the understanding that I did not have the traditional geology background, but instead, was more focused on chemical and analytical techniques. Dr. Mogk was dedicated to helping me find my niche at Montana State University and introduced me to Dr. Recep Avci of the Physics Department and the Imaging and Chemical Analysis Laboratory (ICAL). I would like to extend special thanks to Recep and I am indebted to him for his help with experimental design, thoughtful input, and all around support of my project. I would also like to acknowledge the staff of ICAL, Laura Kellerman, for help with my endless questions, Mark Wolfenden, for his help with data collection, Killeen Lucas, for his help with experimental design, and Linda Loetterle, for her work with the bacterial cultures. Thanks, also to Iwona Beech for her inspiration. I would also like to thank my committee member Lowell Miyagi for his assistance with mineralogy and his encouragement throughout my project. I would also like to extend thanks to Jacob Thacker for his friendship, support with MAUD software and intellectual curiosity that helped me gain perspective.

This project would not have been possible without the support of the Multi University Research Initiative (MURI), the Office of Naval Research, the Department of Earth Sciences, and again the wonderful people of ICAL.

TABLE OF CONTENTS

1. INTRODUCTION	1
2. BACKGROUND INFORMATION AND PREVIOUS STUDIES	4
Economic Losses; United States Navy Corrosion Problems	4
MIC is Complex System	5
Desulfovibrio indonensis	5
Secondary Mineral Formation	7
Mineralogy of Goethite, Pyrrhotite and Pyrite	11
Goethite Crystal Chemistry and Formation	11
Pyrrhotite Crystal Chemistry and Formation	12
Pyrite Crystal Chemistry and Formation	13
3. MATERIALS AND METHODS.....	17
Polishing Mineral Samples	17
Preparation of Samples for Exposure to SRB.....	17
The Anaerobic Glove Box	18
Instrumentation Housed in the Imaging and Chemical Analysis	
Laboratory (ICAL) at Montana State University.....	19
X-ray Diffraction (XRD)	19
Scanning Electron Microscope (SEM)	20
Field Emission Scanning Electron Microscope (FE SEM).....	21
X-ray Photoelectron Spectroscopy (XPS)	21
4. RESULTS	23
Goethite.....	23
Mineral Phase Identification with XRD	23
Characterization of Goethite Using SEM with EDS.....	23
Characterization of Goethite Using FE SEM.....	26
Characterization of Goethite Surface with XPS	31
Before Exposure to SRB – a Baseline Signal for Goethite.....	31
After Exposure to SRB	34
Goethite Discussion	40
Pyrrhotite.....	40
Mineral Phase Identification with XRD	40
Characterization of Pyrrhotite Using SEM with EDS	41
Characterization of Pyrrhotite Using FE SEM with EDS.....	46

TABLE OF CONTENTS - CONTINUED

Characterization of Pyrrhotite Surface with XPS	49
Before exposure to SRB – a Baseline Signal for Pyrrhotite	49
After Exposure to SRB	52
Pyrrhotite Discussion	60
Pyrite	61
Mineral Phase Identification with XRD	61
Characterization of Pyrite Using SEM with EDS	62
Characterization of Pyrite Using FE SEM with EDS	64
Characterization of Pyrite Surface with XPS	69
Before Exposure to SRB – a Baseline Signal for Pyrite	69
After Exposure to SRB	72
Pyrite Discussion	83
5. DISCUSSION	84
REFERENCES CITED	89
APPENDICES	94
APPENDIX A: SEM Images and EDS Spectra of Goethite Before and After Exposure to SRB	95
APPENDIX B: SEM Images and EDS Spectra of Pyrrhotite Before and After Exposure to SRB	130
APPENDIX C: SEM Images and EDS Spectra of Pyrite Before and After Exposure to SRB	171
APPENDIX D: FE SEM Images and EDS Spectra of Goethite, Pyrrhotite and Pyrite After Exposure to SRB	181
APPENDIX E: XPS Data for Goethite, Pyrrhotite and Pyrite After Exposure to SRB	221

LIST OF TABLES

Table	Page
1. 16S rRNA sequence for <i>Desulfovibrio indonensis</i>	6
2. Constituents of Widdel Medium for seawater	7
3. Dates and exposure time of minerals	19
4. Values of XPS data for iron from goethite	32
5. Values of XPS data for oxygen from goethite	33
6. Values of XPS data for oxygen from goethite after 7 days exposure	36
7. Values of XPS data for iron from goethite after 7 days exposure	37
8. Values of XPS data for iron from pyrrhotite	51
9. Values of XPS data for sulfur from pyrrhotite	52
10. Values of XPS data for sulfur from pyrrhotite after 7 days exposure	56
11. Values of XPS data for oxygen from pyrrhotite after 7 days exposure	57
12. Values of XPS data for iron from pyrite	70
13. Values of XPS data for sulfur from pyrite	71
14. Values of XPS data for iron from pyrite after 7 days exposure	75
15. Values of XPS data for sulfur from pyrite after 7 days exposure	77
16. Values of XPS data for oxygen from pyrite after 7 days exposure	79

LIST OF FIGURES

Figure	Page
1. Schematic of SRB biofilm on steel tank	9
2. Schematic of secondary mineral precipitates	10
3. Breaking of S-S bond in pyrite	14
4. Mechanisms for localized iron oxidation on pyrite	15
5. Schematic for pyrite formation.....	16
6. XRD analysis of goethite.....	23
7. EDS spectra of goethite	24
8. Backscatter and secondary electron images of goethite	25
9. EDS spectrum of goethite after 7 days exposure.....	26
10. FE SEM secondary electron image of goethite	27
11. Before and after FE SEM images of goethite.....	28
12. EDS spectrum of goethite surface crystals	29
13. Before and after FE SEM images of control sample for goethite.....	30
14. EDS spectrum of crystals on control sample for goethite	31
15. XPS spectrum of iron from goethite.....	32
16. XPS spectrum of oxygen from goethite.....	33
17. XPS survey scan of goethite after 7 days exposure	34
18. XPS spectrum of carbon from goethite after 7 days exposure	35
19. XPS spectrum of oxygen from goethite after 7 days exposure	36
20. XPS spectrum of iron from goethite after 7 days exposure.....	37

LIST OF FIGURES – CONTINUED

Figure	Page
21. Goethite XPS signal after 1 minute sputter	38
22. XPS survey scan of goethite after 30 days exposure	39
23. XPS survey scan of goethite after 30 days exposure 1 minute sputter	39
24. XRD data for pyrrhotite.....	41
25. SEM image of pyrrhotite	42
26. Backscatter image of pyrrhotite.....	43
27. EDS spectrum of pyrrhotite	43
28. EDS spectrum of pyrrhotite	44
29. Elemental mapping of pyrrhotite	45
30. Elemental mapping of pyrrhotite	46
31. FE SEM secondary electron image of cells on pyrrhotite	47
32. FE SEM image of cells on pyrrhotite after 30 days exposure	47
33. FE SEM image of precipitate on pyrrhotite.....	48
34. FE SEM EDS of precipitate on pyrrhotite.....	48
35. Backscatter and spot analysis of pyrrhotite	49
36. XPS survey scan of pyrrhotite	50
37. XPS spectrum of iron pyrrhotite.....	51
38. XPS spectrum of sulfur	52
39. XPS survey scan of pyrrhotite after 7 days exposure	54
40. XPS spectrum of iron from pyrrhotite after 7 days exposure	55

LIST OF FIGURES – CONTINUED

Figure	Page
41. XPS spectrum of sulfur from pyrrhotite after 7 days exposure	56
42. XPS spectrum of oxygen from pyrrhotite after 7 days exposure.....	52
43. XPS spectrum of carbon from pyrrhotite after 7 days exposure	58
44. XPS survey scan of pyrrhotite after 7 days exposure 1 minute sputter	59
45. XPS survey scan of control sample pyrrhotite after 7 days exposure	60
46. XRD data for pyrite	61
47. SEM secondary electron image of pyrite.....	62
48. SEM backscatter electron image of pyrite.....	63
49. EDS spectrum of pyrite	63
50. EDS spectrum of pyrite after 7 days exposure	64
51. FE SEM secondary electron image of pyrite after 7 days exposure.....	65
52. FE SEM secondary electron image of pyrite after 7 days exposure.....	66
53. FE SEM secondary electron image of pyrite control after 7 days exposure	66
54. FE SEM secondary electron image of pyrite after 14 days exposure.....	67
55. FE SEM secondary electron image of pyrite after 14 days	67
56. FE SEM backscatter electron image of pyrite after 14 days	68
57. FE SEM InLens image of pyrite after 14 days exposure.....	68
58. FE SEM EDS spectrum of precipitate on pyrite.....	69
59. XPS spectrum of pyrite.....	70
60. XPS spectrum of sulfur from pyrite.....	71

LIST OF FIGURES – CONTINUED

Figure	Page
61. Depth profile for pyrite exposed for 7 days	73
62. XPS spectrum for carbon from pyrite depth profile cycle -1	73
63. Depth profile for carbon from pyrite after 7 days exposure	74
64. Depth profile for iron from pyrite after 7 days exposure.....	74
65. XPS spectrum for iron peaks from pyrite depth profile cycle -1.....	75
66. Depth profile for sulfur from pyrite after 7 days exposure.....	76
67. XPS spectrum for sulfur peaks from depth profile cycle -1	77
68. Depth profile for oxygen from pyrite after 7 days exposure	78
69. XPS spectrum for oxygen peaks from pyrite depth profile cycle -1	78
70. XPS survey scan of control pyrite sample after 7 days exposure.....	79
71. Depth profile for pyrite control.....	80
72. Depth profile for pyrite after 14 days exposure	81
73. Depth profile for carbon from pyrite after 14 days exposure	81
74. Depth profile for iron from pyrite after 14 days exposure.....	82
75. Depth profile for sulfur from pyrite after 14 days exposure.....	82

ABSTRACT

Microbially influenced corrosion causes wide reaching economic impacts. One example is the United States Navy's recent setbacks after converting to biofuels. The presence sulfate reducing bacteria in fuel tanks has caused contamination of biofuel and accelerated corrosion processes although the exact mechanism is not well understood. Steel corrosion processes include precipitation of secondary minerals in the form of iron sulfides and oxides. Interaction of sulfate reducing bacteria with secondary precipitates has not been previously investigated. The purpose of this study is to document microbe-mineral interaction and characterize products of such interaction.

One iron oxide, goethite, and two iron sulfides, pyrrhotite and pyrite, were chosen as analogues to alteration products associated with steel corrosion. These minerals were polished and characterized with an array of analytical techniques prior to and after exposure to sulfate reducing bacteria, *Desulfovibrio indonensis*. X-ray diffraction was performed to confirm mineral phase. Standard scanning electron microscopy and field emission scanning electron microscopy were used to obtain secondary and backscatter electron images to display cell attachment and discriminate phases. Spot analyses using energy dispersive spectroscopy was used to obtain elemental information to help identify phase of mineral substrate, mineral inclusions, and secondary precipitate on the surface. Surface chemistry was further investigated with x-ray photoelectron spectroscopy to identify chemical states of elements present.

The x-ray diffraction data confirmed goethite and pyrite to be free of significant contamination. Pyrrhotite, however, had other minerals associated with the sample. Backscatter images of the sample confirmed goethite to be slightly heterogenous, pyrrhotite to have greater heterogeneity and pyrite to be completely homogenous. After exposure to *Desulfovibrio indonensis* for a period of 7, 14, and 30 days, the samples were analyzed again. Goethite was found to be inert with respect to bacteria while pyrrhotite and pyrite had cell attachment and overlying precipitate indicating microbe-mineral interaction.

The results of this study indicate the negative feedback mechanism associated with sulfate reducing bacteria and goethite. Sulfide minerals can create a positive feedback mechanism for the corrosion process if localized sites of oxidation occur on the secondary precipitates to allow for sulfate reducing bacteria interaction.

INTRODUCTION

The United States economy loses ~3.1% of the \$15.1 trillion gross domestic product to the corrosion of steel products and is estimated to be over \$500 billion by June 2013 (NACE, accessed April 18, 2013). Steel products are used in almost every industry and the impacts of corrosion are wide reaching. The national infrastructure (e.g. electric power lines), environmental agencies (e.g. corroded oil pipelines), and the national defense (e.g. naval vessels) are affected by corrosion. The deterioration of steel may occur through both biotic and abiotic pathways. Microbially influenced corrosion (MIC) is of particular interest to the United States Navy, as the biocorrosion of fuel tanks leads to souring of fuel, premature clogging of filters, and degradation of turbines (Avci, personal communication).

Common products of steel corrosion are iron sulfides and iron oxides. These secondary minerals may form as direct precipitation from saturated solutions or as the product of microbial metabolic processes (White, 2007; Banfield and Hamers, 1997). Sulfate reducing bacteria (SRB) may play an important role in MIC as they are a class of heterotrophic anaerobic bacteria that obtain energy from organic carbon sources and use sulfate as a terminal electron acceptor reducing SO_4^{2-} to H_2S (White, 1997). The presence of SRB in anaerobic solutions accelerates the corrosion process although exact mechanisms are not well understood (Lee et al., 1995). The role of iron sulfide/oxide precipitates in the system has not been previously studied and it is unknown if these secondary minerals are active participants in MIC or become inert bystanders. To determine the role in which these minerals play in the corrosion process, this study

exposed goethite ($\text{FeO}\cdot\text{OH}$), pyrrhotite (FeS), and pyrite (FeS_2) to SRB, *Desulfovibrio indonensis*, for periods of 7, 14, and 30 days in experimental conditions that served as analogues of primary alteration products of steel corrosion.

Characterization of the mineral substrates was done prior to and after exposure to SRB to document changes in mineralogy, morphology, composition and surface chemistry. The samples were analyzed with X-ray Diffraction (XRD) to verify the identity of mineral phases present and to confirm the homogeneity of the samples. Before and after a duration of bacterial-mineral interaction, samples were imaged with a Scanning Electron Microscope (SEM) to document any changes in morphology and imaged with a Field Emission Scanning Electron Microscope (FE SEM) to reveal microbial cell attachment. Back scatter electron (BSE) imaging was used for phase discrimination. Energy Dispersive Spectroscopy (EDS) on the SEMs was used to perform area and spot analyses in order to obtain elemental information used to identify the mineral substrate phases, impurities in the samples such as mineral inclusions, and secondary precipitates on the surface. X-ray Photoelectron Spectroscopy (XPS) was used for surface analyses of precipitates to show oxidation state of elements present and aid in identification of any alteration products. The results of this study provide insight into reaction pathways related to MIC. The significance of this work is to aid in the understanding of secondary mineral precipitates and their influence on MIC.

The role of the iron sulfide and iron oxide secondary mineral precipitates in the overall corrosion process is not well understood. The alteration products may be host to localized sites of oxidation that create sulfates to enhance SRB growth. Secondary

reactions involving the alteration products and SRB may result in a positive feedback mechanism that further promotes the corrosion processes, or conversely, a negative feedback mechanism that shuts down the corrosion reactions.

BACKGROUND INFORMATION AND PREVIOUS STUDIES

Economic Losses; United States Navy Corrosion Problems

Corrosion of metal surfaces occurs in both natural and manufactured environments and has consequences such as degradation of steel tanks and pipes that lead to huge economic loss. Corrosion problems have hindered the United States military's attempt to convert to alternative fuels. The United States Navy recently experienced huge financial losses due to their commitment of using biodiesel in their fleets (Avci, 2011, personal communication). Currently, the fleets are using a petroleum/biofuel blend in which seawater is pumped into the fuel tanks as ballast to maintain stability of the ship. The fuel sits atop the seawater with a relatively large interface between the two layers, creating a perfect environment for aerobic and anaerobic microbes to thrive. The bacteria are introduced by the seawater and the biofuel serves as a viable food source (Avci, 2011, person communication). At the bottom of the tanks, an oxygen depleted zone provides conditions for anaerobic bacteria to thrive at the steel/water interface. Often, iron is a limiting nutrient in marine environments (Schlesinger and Bernhardt, 1997) and the dissolution of the carbon steel tanks provides an abundance of iron. The growth of the microbes enhances corrosion and can contaminate fuels with metabolic by products (Avci, 2011, personal communication). As the popularity of biofuel increases, it becomes even more important to understand MIC.

MIC is a Complex System

The electrochemical process by which corrosion deteriorates steel involves an anodic reaction where the metallic surface is oxidized and a cathodic reaction where some chemical species are reduced (Beech and Gaylarde, 1999). The presence of microbes such as anaerobic SRB (Hamilton, 1998), metal reducing bacteria (MRB) and/or acid producing bacteria (APB) along with many other types of microbes and combinations of species have been found to aid in the corrosion process (Beech and Gaylarde, 1999). There are many mechanisms in which bacteria affect corrosion. The presence of H₂S corrodes steel in an abiotic process, meanwhile, SRB produce H₂S which can accelerate the anode/cathode behavior (Lee et al., 1995). The local area of cell attachment was found to initiate pits on carbon steel at the anodic site (Franklin et al., 1991). Gaylarde and Beech (1996) investigated lipopolysaccharide (LPS) composition of *Desulfovibrio* cell wall based on evidence that LPS from SRB is involved in adhesion to metal surfaces but found no conclusive evidence that LPS was the cause for MIC (Beech and Gaylarde, 1999). Beech and Gaylarde (1999) found that biofilms and suspended microbes interact with vast microbial consortium and accelerate the corrosion process thus adding to the complexity of the system.

Desulfovibrio indonensis

The microbe used in this study was *Desulfovibrio indonensis*, a SRB and known alkane degrader that was isolated from a biofilm found in a severely corroded carbon steel oil storage vessel off the coast of Indonesia (Feio et al., 1998). It was characterized

by Feio et al. (1998) and found to be a gram negative curved rod 3-5 μm long and 0.5-1.2 μm wide. The 16S rRNA gene sequence identified it as a Proteobacteria of the genus *Desulfovibrio* and most closely related to *Desulfovibrio ganonensis* with a 97% similarity (Table 1). *D. indonensis* was chosen for this thesis because of its strong survival rate in the lab. Pure cultures were diluted in Widdel seawater medium (Table 2). This medium was used by Davidova et al., (2006) for isolating a strain of n-alkane-degrading SRB. It is a sulfate containing synthetic seawater medium developed by Widdel and Bak (1992) with added RST trace metal solution and RST vitamins. Hexane and decane were added in excess. The medium was used to keep consistency with collaborators on the Multi University Research Initiative (MURI) project that has conducted research on corrosion problems for the Office of Naval Research (ONR).

Strain	Similarity value (%)
<i>D. gabonensis</i>	97.0
<i>D. desulfuricans</i>	90.0
<i>D. vulgaris</i>	90.0
<i>D. gigas</i>	90.0
<i>D. salexigens</i>	88.7
' <i>D. piger</i> '	82.6
<i>D. hydrogenophylus</i>	81.8

Table 1. 16S rRNA sequence similarity for *D. indonensis* compared with other *Desulfovibrio* species (taken from Feio et al., 1998).

Widdel Medium for Seawater	g/L	RST Trace Elements	g/L	RST Vitamins (Use 10mL/L)	mg/L
NaCl	20.0	Nitroloacetic acid	2.0	Biotin	2.0
MgCl x 6H ₂ O	3.0	MnSO ₄ x H ₂ O	1.0	Folic Acid	2.0
CaCl ₂ x 2H ₂ O	0.15	Fe(NH ₄) ₂ (SO ₄) ₂	0.8	Pyridoxine-HCL	10
NH ₄ PO ₄	0.2	CoCl ₂ x 6H ₂ O	0.2	Thiamine-HCL	5.0
KCl	0.5	ZnSO ₄ x 7H ₂ O	0.2	Riboflavin	5.0
RST Trace Elements	10 mL	CuCl ₂ x 2 H ₂ O	0.02	Nicotinic Acid	5.0
Resazurin	1.0 mL	NiCl ₂ x 6H ₂ O	0.02	Calcium pantothenate	5.0
Na ₂ SO ₄	40.0	Na ₂ MoO ₄ x 2 H ₂ O	0.02	Cyanocobalamin	5.0
pH of 7.2 -7.4		Na ₂ SeO ₄	0.02	p-Amonobenzoic Acid	5.0
		N ₂ WO ₄	0.07	Lipoic Acid	5.0

Table 2. Constituents of Widdel Medium used to grow *D. indonensis*. This medium was developed by Widdel and Bak, 1992 and is used for experiments conducted by MURI.

Secondary Mineral Formation

The corrosion process is driven by an electrochemical reaction in which iron is locally oxidized and brought into solution. Anodic and cathodic reactions are involved.

Reactions 1-3 are the inorganic processes that occur during corrosion (Avci, personal communication).

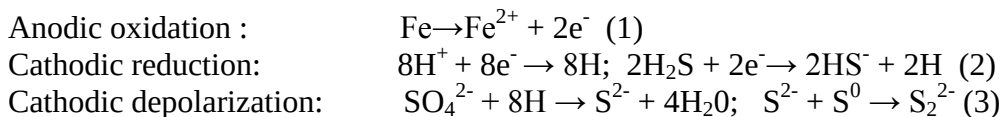
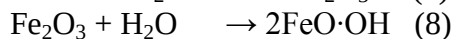
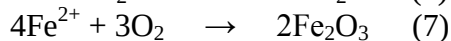
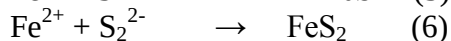
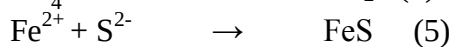
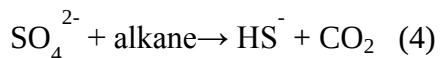


Figure 1 represents a simplified environment analogous to the inside of the naval fuel tanks. In the natural environment, many different kinds of prokaryotes and eukaryotes live in this system. The schematic drawing (Figure 1) demonstrates the processes around an SRB biofilm. It is important to note that although these microbes are anaerobes, and

are living in an oxygen-depleted zone, dissolved oxygen does exist outside the biofilm. As seen in the schematic the anodic site releases Fe^{2+} into solution forming the initial pit and drives electrons to the cathodic site. This process can be abiotically controlled. SRB increase the dissolution of iron as they excrete metabolites. Sulfate is their terminal electron acceptor and is reduced to sulfide (reaction 4). The sulfide ions are then able to bond with the dissolved ferrous iron to form framboidal, disordered and/or amorphous minerals such as pyrrhotite (FeS) (reaction 5) and pyrite (FeS_2) (reaction 6 is simplified; actually mechanism for pyrite formation is discussed later). In addition, ferrous iron is oxidized to ferric iron outside the SRB biofilm in a region with low levels of dissolved oxygen. Ultimately, the ferric iron precipitates as iron oxide and ferri-oxy-hydroxide minerals such as hematite (reaction 7) and goethite (reaction 8). Based on these observed occurrences of secondary minerals in corroded fuel tanks, pyrrhotite, pyrite and goethite were chosen for experimental studies to determine their role in MIC. Other iron oxides and sulfides can be present in the system but are omitted, as the three phases used in this study are volumetrically dominant in observed corroded fuel tanks. The energetics of microbially mitigated reactions such as the following have been described by Amend and Shock (2006) and Shock (2009).



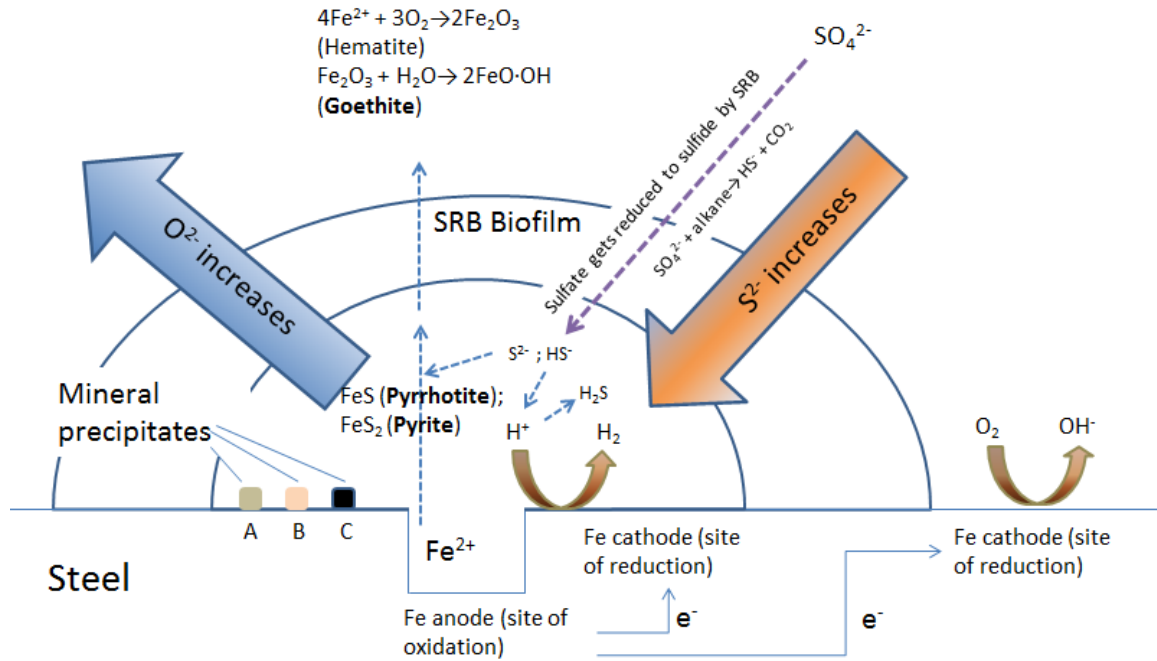


Figure 1. Schematic of SRB biofilm with relevant precipitates in bold. Three type of mineral precipitates A, B, and C are shown. SRB reduced sulfate to HS^- (reaction 4) that can disassociate to sulfide ions or bond with a proton to form H_2S gas. Protons in solution also act as a reducing agent and pick up electrons at the cathode to form hydrogen gas. Dissolved oxygen outside of the anaerobic biofilm can also be reduced by the flow of electrons to the cathode. Dissolved iron and sulfide bond to form the secondary minerals pyrrhotite (reaction 5) and pyrite (Reaction 6). Dissolved ferrous iron is oxidized to ferric iron outside the biofilm by low levels of dissolved oxygen and is converted to hematite (reaction 7). Hematite then adsorbs water to form goethite (Reaction 8) (modified from Avci, personal communication).

As the secondary minerals become present, they are also becoming bioavailable to SRB to some extent (Figure 2). Secondary minerals A, B, and C represent goethite, pyrrhotite, and pyrite. The bacteria cells attach to the surface of these minerals and may recycle them back into the system. This thesis is addressing the ability of the iron sulfide/oxide precipitates to react in the system and determine the role in feedback mechanisms they may play.

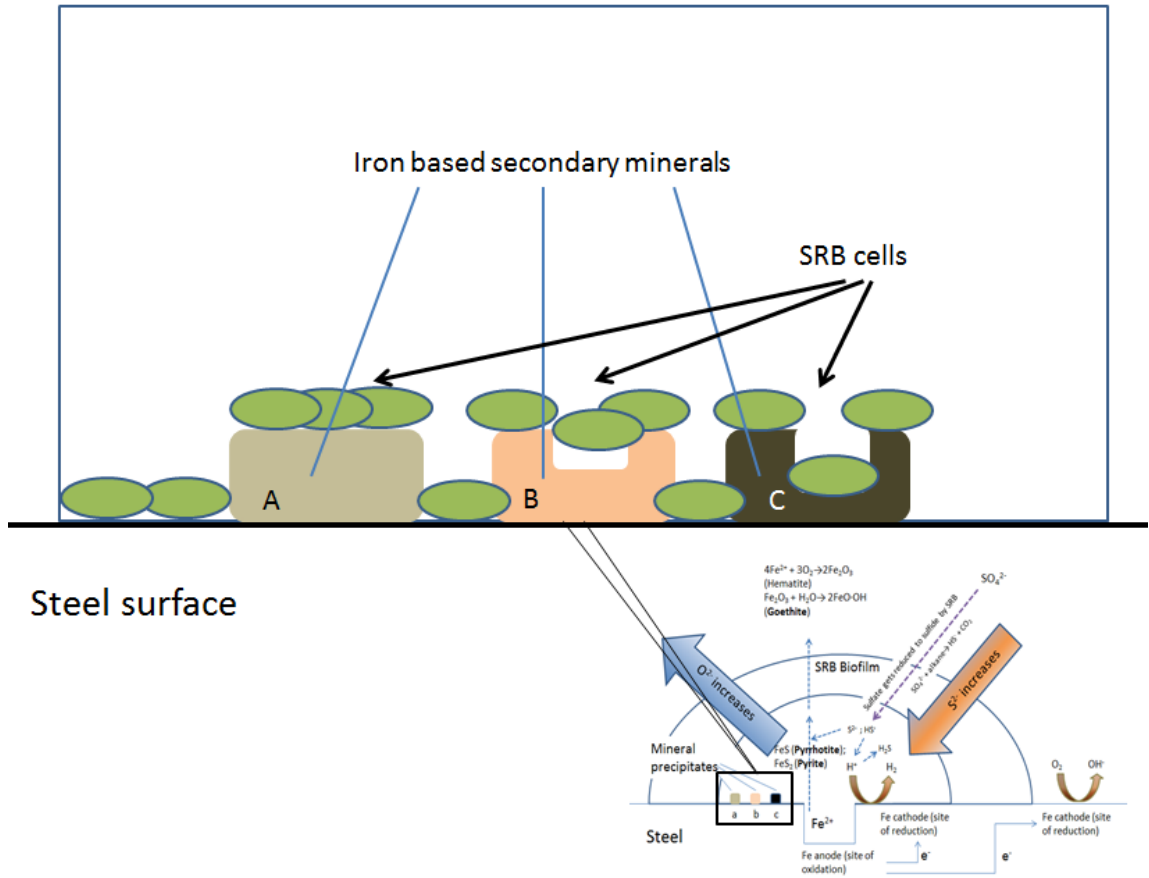


Figure 2. Schematic of secondary mineral precipitates and SRB cells as in inset of Figure 1. A), B) and C) are different types of minerals with varying degree of reactivity $A < B < C$. The SRB attach to the secondary minerals and may induce positive or negative feedback mechanisms. A) this mineral represents an alteration product that is inert in SRB metabolic processes, thus a negative feedback to the corrosion process; B) and C) represent alteration products that SRB are able to utilize and in turn create more mineral precipitates, thus a positive feedback mechanism to enhance corrosion.

Mineralogy of Goethite, Pyrrhotite and Pyrite

Goethite Crystal Chemistry and Formation

Goethite, iron oxy-hydroxide ($\text{FeO}\cdot\text{OH}$), is orthorhombic and can be found as tabular or prismatic crystals but most commonly found in botryoidal or stalactitic forms (Bowles et al., 2011). Its atomic structure consists of layers of oxygen and hydroxyl ions in hexagonal close-packed arrangement. Fe^{3+} ions occupy half the octahedral sites with alternating vacant sites (Bowles et al., 2011). It exhibits perfect {010} cleavage and moderate {100} cleavage where corner sharing oxygen bridges are broken (Manceau et al., 2000).

Goethite is the most common form of Fe (III) oxy-hydroxides and most polymorphs revert to this phase upon weathering (Bowles et al., 2011). It is a very common secondary mineral for weathering of most iron-bearing minerals and often found with corrosion products associated with the Fe(II)-Fe(III) hydroxide salts known as “green rust”(Usman et al., 2012). Goethite is the most abundant iron oxide in soils (Rakovan et al., 1999) and can directly precipitate out of marine and meteoritic waters by abiotic or biotic processes (Bowles et al., 2011). The hydration of hematite (Fe_2O_3) generally forms goethite and biological activities increase the amount of hematite available for hydration. The small grain size of goethite particles correspond to a large surface area to volume ratio that allows for a significant reactive site in both abiotic and biotic reactions (Echigo et al., 2012). The presence of the hydroxyl in the mineral structure makes it active in adsorption of anions and cations and the abundance of the hydroxyl group coincides with its geochemical capacity (Echigo et al., 2012).

The sample used as the feedstock in this study was obtained from Ward's Scientific. It had a massive habit, showing no distinct crystal form. It was extremely difficult to polish due to the very fine grain structure. XRD powder studies of the material used for polished goethite samples confirmed the material to be goethite and with minor amounts of quartz which is inert in the proposed MIC reactions (Figure 5).

Pyrrhotite Crystal Chemistry and Formation

Pyrrhotite is the name of an iron mono-sulfide mineral that includes a range of compositions Fe_{1-x}S where $0 < x < 0.125$. The pyrrhotite family can have hexagonal, orthorhombic or monoclinic symmetry. The crystal structure has vacancies in the octahedral sites and can be missing up to one-eighth of the iron atoms. In the case of low temperature formed magnetic monoclinic Fe_7S_8 , the vacancies occur in alternating layers of iron atoms and in alternating rows within the layer (Bowles et al., 2011).

At $\text{pH} > 7$, sulfide can react with Fe^{2+} , forming a transient intermediate $\text{Fe}(\text{HS})^+$ that will quickly decompose to FeS . Aqueous Fe-S clusters, also decompose to FeS (Figure 6).

Pyrrhotite is commonly found with pentlandite ($(\text{Fe},\text{Ni})_9\text{S}_8$), pyrite (FeS_2), and chalcopyrite (FeCuS_2). Complete characterization of pyrrhotite crystal class and determination of impurities is often difficult (Bowles et al., 2011). The sample used in this study was obtained from Ward's Scientific. It is magnetic which indicates monoclinic 4C structure and matched XRD data from the Sudbury formation, Ontario, Canada. Magnetic pyrrhotite from Sudbury comes from the Gertrude West Ni-Cu mine (Becker et al., 2010). XRD data demonstrated the presence of pentlandite (FeNiS_2),

quartz (SiO_2), biotite, $(\text{K}(\text{Mg},\text{Fe})_3\text{AlSi}_3\text{O}_{10}(\text{F},\text{OH})_2)$, titanite (CaTiSiO_5) and an unidentified phase associated with this pyrrhotite sample (Figure 24).

Pyrite Crystal Chemistry and Formation

Pyrite, iron disulfide (FeS_2) is the most abundant sulfide mineral and is isometric with $2/m\bar{3}$ symmetry with common crystal forms as cubes $\{001\}$ and pentagonal dodecahedron (pyritohedrons) $\{102\}$. Its structure is similar to face-centered halite where Fe replaces the Na sites and dumbbell shaped pairs of sulfur anions replace the Cl sites. The sulfur pairs are oriented with their center of gravity in the equivalent Cl site in such a manner that each corner of the unit cell and each face center contain a dumbbell pair (von Oertzen et al., 2005). The nature of the sulfur bonds becomes significant when interpreting XPS spectra (following sections). Pyrite has poor $\{001\}$ cleavage along the Fe-S bond where the bond energy is greater than 400 kJ/mol. The S-S bonds have a much lower bond energy of $245 \pm$ kJ/mol and although the process requires breaking more bonds than along the cleavage plane, the energy saved by fracturing the S-S bonds could be favorable (Nesbitt et al., 1998). Thus, the exposed S atoms on fractured surfaces become activated sites that may initiate either biotic or abiotic corrosion processes.

It is also important to note that SRB use sulfate as their terminal electron acceptor to create sulfide and precipitates such as FeS and FeS_2 . The sulfur atom in pyrite is a reduced form (S^{2-} or S_2^{2-}) so it would seem that no reaction would occur between the SRB and the secondary mineral pyrite. However, the S-S bonds tend to cleave regularly

(Figure 3) and these broken bonds provide S^- that pulls an electron from Fe^{2+} by autooxidation to create Fe^{3+} and S^{2-} (reaction 9) (Nesbitt et al., 1998).

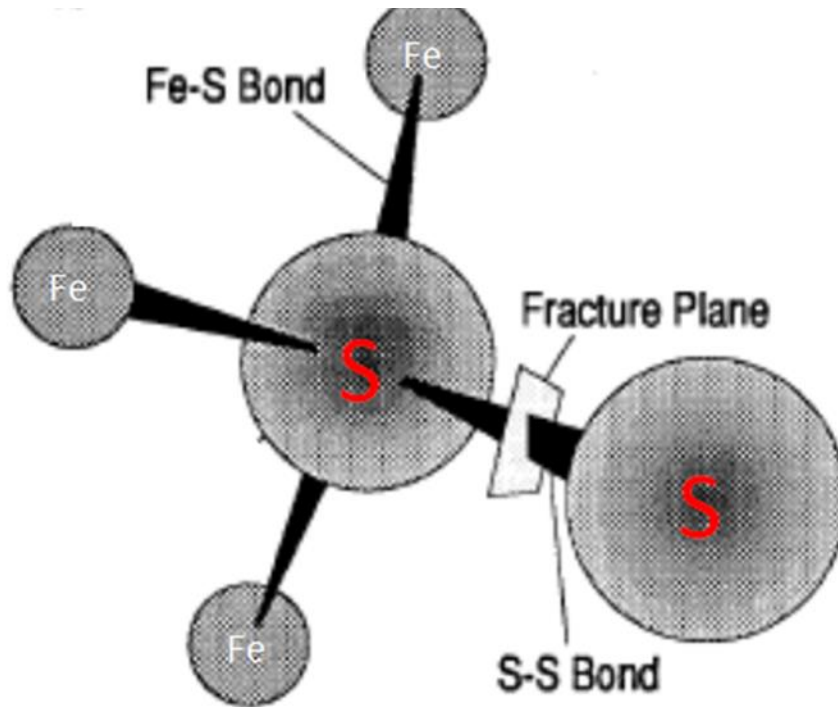
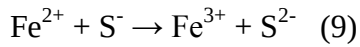


Figure 3. A Fe-S₂ cluster depicting the coordination of the S-S bonds and the S-Fe bonds in pyrite. Both S atoms are bonded to 3 Fe atoms although additional Fe atoms are not shown here. Modified from Nesbitt et al., 1998.

The local oxidized site of Fe^{3+} then can adsorb water by a mechanism put forward by Schafuss et al. (1998) where, in turn, S^{2-} quickly reacts with water and dissolved oxygen to form sulfate, returning sulfide to its oxidized state (Figure 4). Pyrite scavenges dissolved oxygen, creates localized regions of oxidation, and then provides sulfate for SRB to utilize.

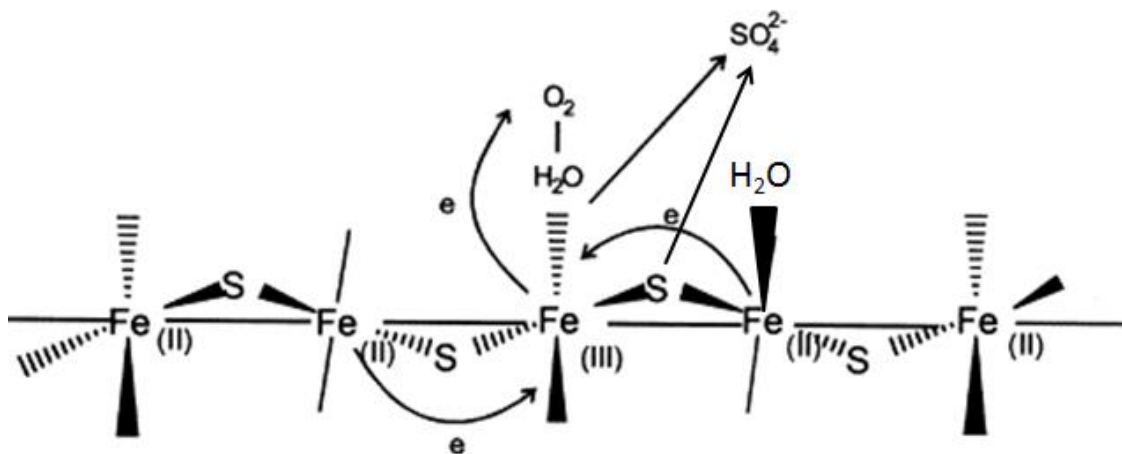


Figure 4. Proposed mechanism for localized site of iron oxidation that adsorbs water. Electrons move from Fe(II) sites via sulfur bridges toward O₂. The sulfur atom is displaced by competitive adsorption of an additional water molecule. The sulfur reacts with water and oxygen to form sulfate. The localized regions of sulfate are then available for SRB (modified from Schauffuss et al., 1998).

Pyrite can form numerous ways both natural and artificial. It has been prepared synthetically by heating pyrrhotite (Fe_{x-1}S_x) in H₂S in a weakly acidic or neutral solution. This same reaction is thought to be responsible for pyrite by alteration of pyrrhotite in the presence of H₂S in volcanic sites (Deer et al., 1966). Disordered mackinawite (Fe_{1+x}S) has been suggested as a precursor to and nucleation site for pyrite growth in both abiotic and biotic systems (Herbert Jr. et al., 1998). Iron disulfide is a common secondary mineral precipitate from dissimilatory bacterial reduction of sulfate. The exact mechanism by which pyrite is formed via bacterial metabolic processes has yet to be determined but there are currently a few proposed pathways involving: 1) FeS oxidation by polysulphide species (reaction 9) (Luther, 1991); (2) FeS oxidation by H₂S (reaction 10)(Rickard, 1997); and (3) conversion of FeS by iron loss through intermediate phases such as greigite (Fe₃S₄) (reactions 11 and 12) (Wilkin and Barnes, 1996) (Figure 5).

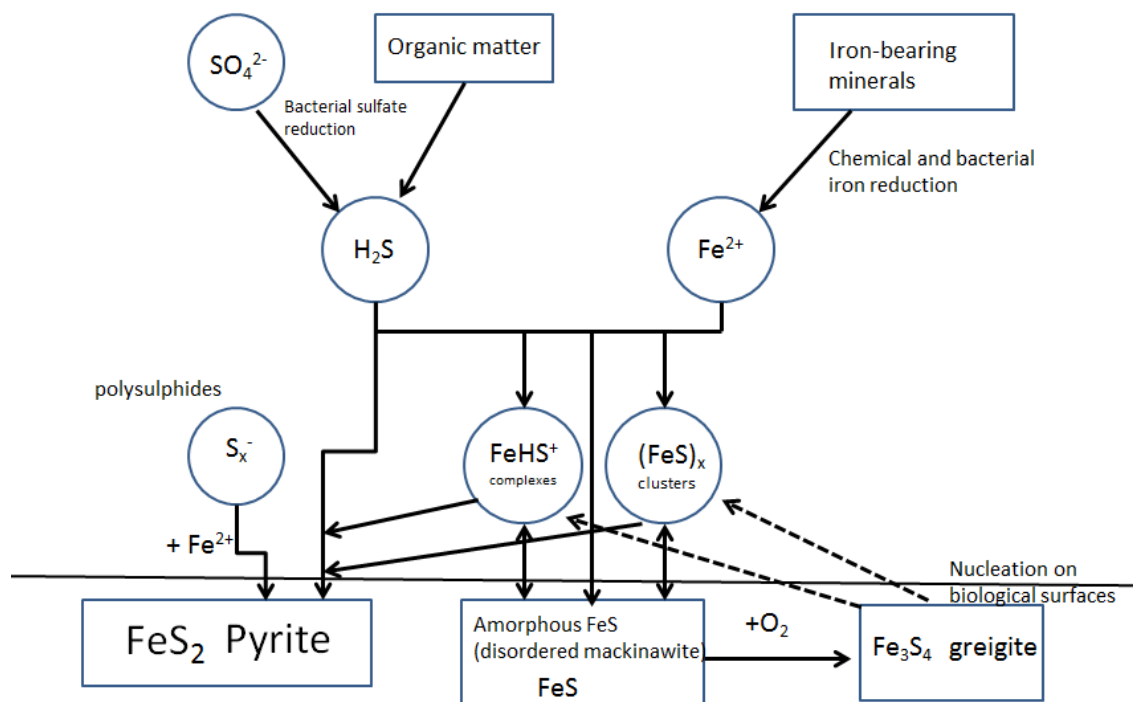
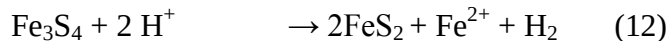
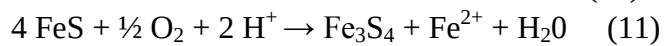
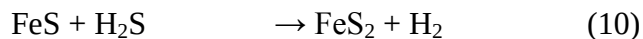
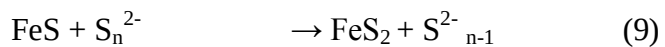


Figure 5. Schematic representing possible pathways for biologically mediated pyrite and other iron sulfide formation, dashed arrows represent reactions thought to be present but by unknown mechanisms (modified from Bowles et al., 2011).

The sample used in this study was procured from Ward's Scientific. XRD powder studies of the material used for the polished pyrite samples confirmed the only mineral phase present was pyrite (Figure 47).

MATERIALS AND METHODS

Polishing Mineral Samples

Samples of goethite, pyrrhotite, and pyrite were polished on three grinding wheels starting with 600 grit polish, followed by 1000 grit polish and finished with 1 micron aluminum oxide “alpha” polish at high speed (460 rpm). The samples were then cut with a diamond embedded rock saw to approximately 1 cm in width. While the 1 cm width is acceptable for SEM, XPS analysis required thinner and more highly polished samples. The polished side of each sample was fixed with silicon glue to glass slides. The samples were then shaved down to approximately 1.5 mm thickness with an Allied Prep polisher with polishing paper grits of 120, 200, 320, 480, 600, 800, 1200, and then buffed to a mirror finish with 1 micron colloidal diamond and the appropriate polishing cloth for 30 minutes. The samples were then removed from the glass slides and residual silicon glue was scraped off with a razor blade. The polished minerals were then cut up into 12 pieces with a rock saw and a diamond etching tool was used to orient an x, y axis to identify regions of interest for SEM imaging and EDS analysis before and after exposure to microbes. The mineral samples were oriented such that the longest straight edge of each sample was the bottom helping to replicate the exact same spot for every analysis.

Preparation of Samples for Exposure to SRB

Prior to inoculation, all vials and stoppers were wet-heat autoclaved for 25 minutes. The mineral samples were rinsed with 100% ethanol and dried with N₂ gas. To insure

proper exposure of the polished face of the mineral samples to the medium and microbes, it was determined (Avci, 2012, personal communication) that the samples should be suspended in the medium, rather than lying flat in the sample container. This procedure was chosen because previous experiments had polished steel coupons lying flat in the container only to observe corrosion on the unpolished underside. The mineral pieces were tied with Teflon coated quartz string and the string was pushed through the rubber stopper of the vial by the following method: a 16 gauge needle was held with needle nose pliers and clamped to the counter surface; the needle was pushed through the rubber stopper and then the string slipped into the needle tube; the stopper was removed from the needle leaving the silica string running through the rubber stopper and allowing the minerals to be suspended inside the vials.

The Anaerobic Glove Box

The organisms of interest, *Desulfovibrio indonensis*, is anaerobic bacteria so all work was done inside a glove box with a maintained oxygen level of less than 25 ppm at all times. Mineral samples tied to silica string and suspended from rubber stoppers, a rubber stopped flask of *D. indonensis* in Widdell medium for seawater, (12) 10ml vials, a test tube rack, (1) syringe, (1) 16 gauge needle, and (3) 0.02 micron syringe filters were put into the input chamber. The chamber was pumped out by vacuum and filled with CO₂ and hydrogen gas. One sequence equals the chamber being pumped down and filled three times then equalized with nitrogen gas. Two sequences were completed before opening the inner chamber door.

The syringe with the 16 gauge needle was inserted into the rubber stopper of the flask with the bacteria. Four ml of media and bacteria were drawn and put into samples 1-3, 5-7, 9-11. The syringe was then filled to 5 ml of media and the needle removed. The filter was screwed on the syringe (the extra ml was drawn into the syringe to compensate for the loss on the filter paper). Four ml of the filtered media was put into vials containing samples 4, 8, and 12. This filtered media in samples 4, 8, and 12 acted as the experimental control.

Samples 1-4 were left in the glove box exposed to *D. indonensis* for a duration of one week. The vials were opened inside the glove box and rinsed with nanopure water to remove salt residue and allowed to dry in the anaerobic chamber. Samples 5-8 were allowed exposure to microbes for 2 weeks. Samples 9-12 were allowed exposure to microbes for one month (Table 3). This procedure was the same for all minerals.

Mineral Type	Date of exposure to microbes	Date of removal (1 week)	Date of removal (2 weeks)	Date of removal (4 weeks)
Goethite	08/01/2012	08/08/2012	08/15/2012	08/29/2012
Pyrrhotite	08/29/2012	09/05/2012	09/12/2012	09/26/2012
Pyrite	09/26/2012	10/03/2012	10/10/2012	10/24/2012

Table 3. Mineral samples types and the dates on which they were removed from the media and rinsed for analysis.

Instrumentation Housed in the Imaging and Chemical
Analysis Laboratory (ICAL) at Montana State University

X-ray Diffraction (XRD)

Powder XRD was performed on a SCINTAG X1 Diffraction System to verify the mineral phases used in this experiment. The mineral samples were powdered with a

mortar and pestle and made into a slurry with ethanol. The slurries were allowed to dry on an XRD slides leaving the powdered minerals in a random orientation. Samples were scanned from 3° - 73° for a full scan at $1^{\circ}/\text{min}$. The instrument was operated at 40 kW, 45 mA, using $\text{CuK}\alpha$ radiation. Data were reduced using background subtraction and peak identification referenced from International Center for Diffraction Data (ICDD) library. Results are presented with Material Analysis Using Diffraction (MAUD) software (Lutterotti et al. 1997). All three of the mineral samples were analyzed with XRD to insure that the phases were homogeneous and without significant contaminant phases before the experiments.

Scanning Electron Microscope (SEM)

The polished mineral samples were imaged with a JEOL JSM 6100 standard SEM at 20kV. Magnification of 700 X was used to determine an area within the etched coordinate system based on degree of polish and least amount of surface defects. The coordinate system on the outer part of the SEM was recorded for the region of interest from a reference point i.e. starting position was $x = 338.00$, $y = 660.00$, and position of point of interest was $x = 336.00$ and $y = 662.00$. The scale for the system is such that the number immediately to the left of the decimal represents hundreds of microns. In general, the point of interest was 200 microns to the left or right of the origin and 200 microns up or down from the origin. SEM images were captured at 700x and 7000x magnification at the point of interest. Backscatter electron images were also obtained at these magnifications. EDS was used to obtain spectra of elements present in the samples and their relative abundance. Elemental mapping of the mineral constituents was

performed with the RONTEC Flash Map EDS system x-ray detector. This procedure was followed both before and after exposure to SRB and was the same for all mineral types.

Field Emission Scanning Electron Microscope (FE SEM)

The FE SEM was used to image cell attachment after exposure to SRB. The gun was set to 1kV and magnification up to 30,000x with a working distance (WD) of 3-5 mm images were obtained for each sample using the secondary electron detector. InLens images and backscatter electron images were obtained at 10 kV with a WD of 8-15 mm and spot analysis with EDS was performed on areas of interest. The InLens sits higher up in the column and biological cells appear very dark in the images. This was useful for locating cells.

X-ray Photoelectron Spectroscopy

X-ray Photoelectron Spectroscopy (XPS) was performed on a 5600 model Physical Electronics spectrometer. Data were collected in survey mode to identify components on the substrate surfaces and multiplexes were used to examine narrow binding energy windows of interest to determine the chemical state of specific elements. The source used was a monochromatized $AlK\alpha$. The system was operated at 15 kV, 300 W and kept at ultra-high vacuum levels of $\sim 10^{-9}$ Torr. Pass energy was 46.95 eV for survey scans and 23.5 eV for multiplexes. Values are reported within ± 0.4 eV accuracy for survey scans and ± 0.05 eV accuracy for multiplexes and depth profiles. Carbon calibration was done to align the sample in the z direction by maximizing counts with the z goniometer and aligning the peak to 284.5 eV (Moulder et al., 1993) by adjusting emission control

(mA) and electron energy (%) on the flood gun. Before exposure to microbes, samples were sputtered with an argon ion gun to remove ~1 nm of any potential sorbed environmental contaminants (e.g. adventitious carbon). Goethite samples were relatively porous and required leaving the sample in the sample exchange chamber to be pumped down over 2 days before it could be put into the chamber at ultra-high vacuum (greater than 10^{-9} Torr). Liquid nitrogen was used to cool the sample and the detector so the integrity of the vacuum system was not compromised. A survey scan was used over a range of 0 eV to 1200 eV for pyrite and goethite and 0 eV to 1400 eV for pyrrhotite to determine elements present. Then a multiplex routine was used to provide more detailed information of the oxidation states for iron, sulfur, oxygen and carbon. Depth profiles were also obtained for the pyrite samples with a pass energy of 23.5 eV to determine chemical stratigraphy of monolayers of alteration products. Argon gas was used to remove 1 nanometer at a time of the surface with a sputtering rate of 1 nanometer per minute.

Fe 2p_{3/2}, O 1s, S 2p, and C 1s spectra were analyzed by curve fitting multiplets using AugerScan software. After integrated (Shirley) baseline subtraction, curves were fit by combinations of Lorentzian and Gaussian line shapes. Full width at half maximum (FWHM) and all line shape parameters were set equal for curve fitting. Sp₂ peaks were fitted by doublets with a separation of 1.18 eV to account for spin orbit coupling (Moulder et al., 1993; Avci, personal communication).

RESULTS

GoethiteMineral Phase Identification with XRD

XRD analysis confirmed mineral phase to be goethite with minor presence of quartz.

No other impurities were present (Figure 6).

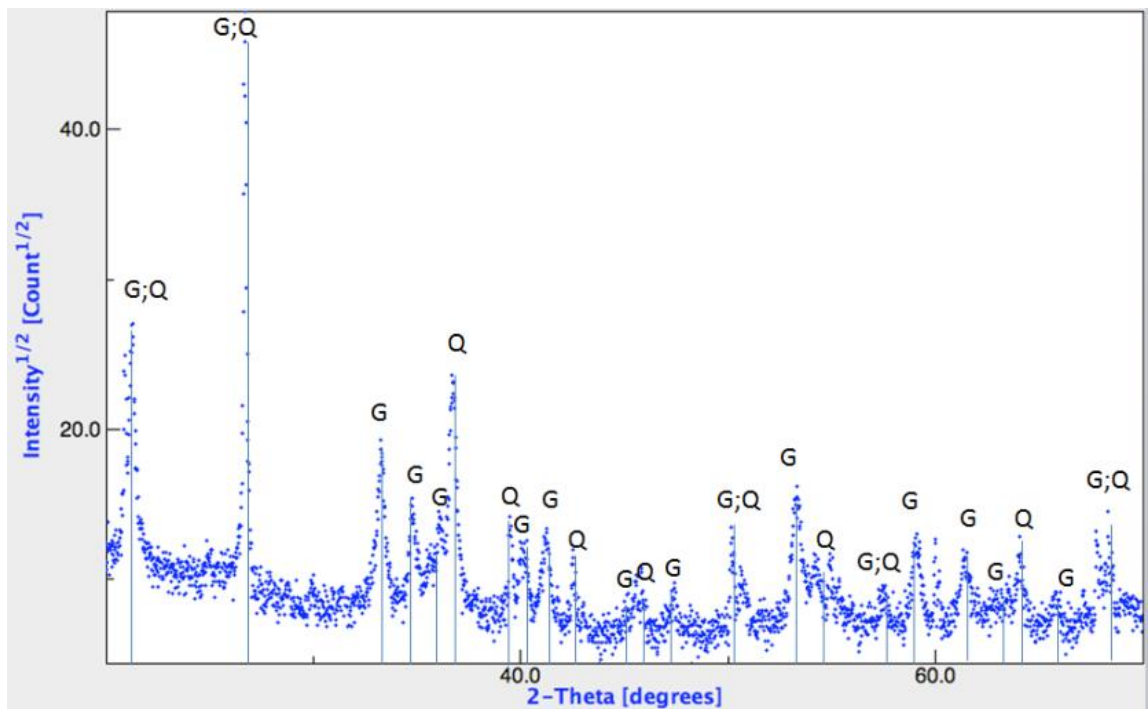


Figure 6. XRD analysis of the goethite sample used in this study. Major peaks are due to goethite and quartz. Analysis confirmed material is iron oxyhydroxides with silica.

Characterization of Goethite
Using SEM with EDS

EDS of goethite samples before exposure to SRB confirmed iron oxide with small inclusions of quartz present (Figure 7). The surface topography was not uniform. Irregularity in composition could be seen when secondary electron images were

compared with backscattered images (Figure 8). The heterogeneous nature of the sample could be due to goethite's varying concentration of the hydroxyl group (Echigo et al., 2012), its reported occurrence as coatings on smaller grains (Rakovan et al., 1999) and/or the presence of silicon.

After exposure to SRB, EDS revealed the same iron, oxygen and silicon peaks with trace amounts of carbon, sodium, magnesium, aluminum, phosphorus and sulfur (Figure 9). The trace elements were due to residue from the media. Goethite was the only mineral of the three studied to have enough media retained to appear on the EDS spectra. The samples used were extremely porous and the most likely reason for trace elements is media in the pore spaces.

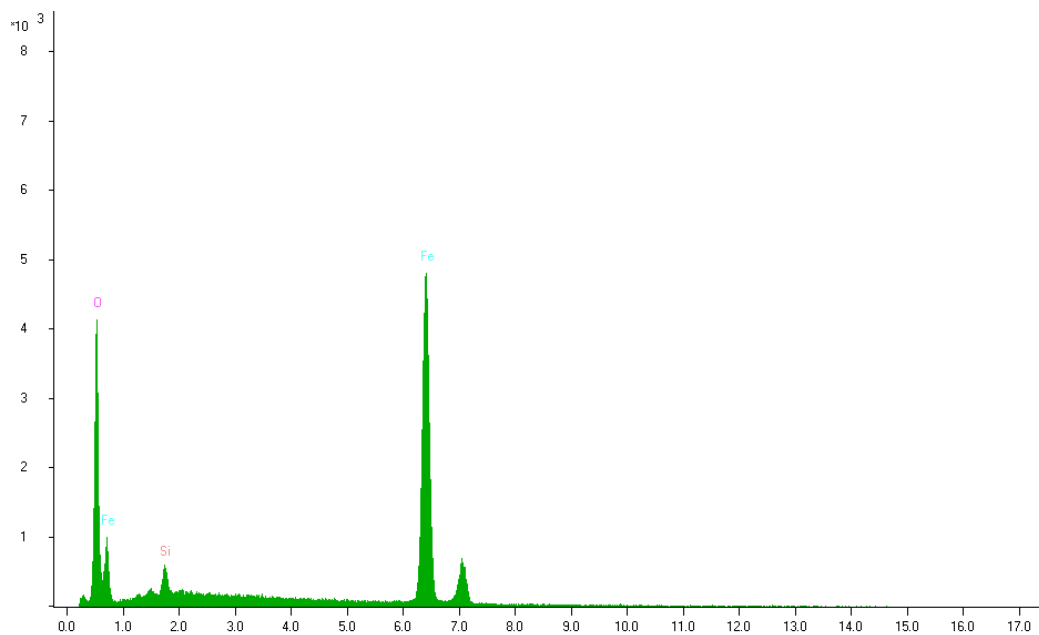


Figure 7. EDS spectrum of goethite sample 5 before exposure to SRB. Spectra shows only the presence of iron, oxygen and very small amount if silicon.

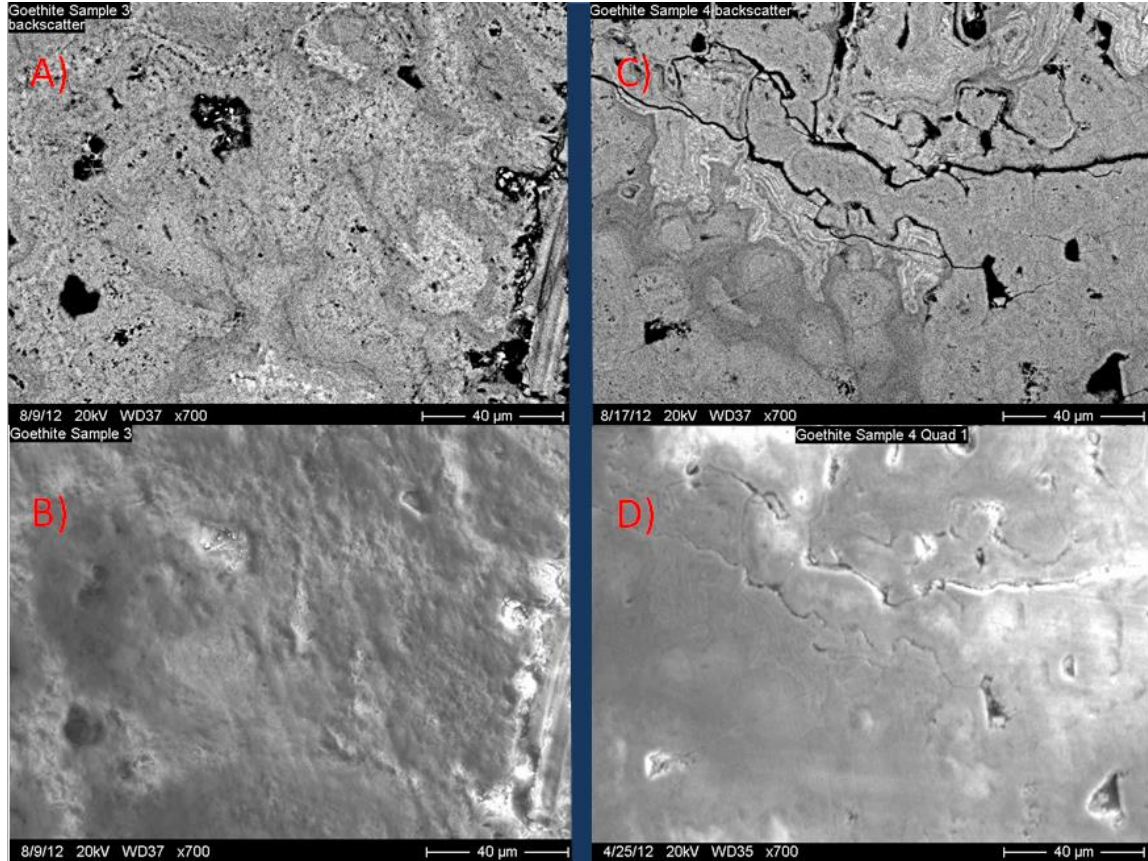


Figure 8. A) and B) are backscatter and secondary electron images of sample 3 respectively. C) and D) are backscatter and secondary electron images of sample 4 respectively. Backscatter images A) and C) show grain morphologies as concentric layers with variation on composition as seen with the light and dark areas.

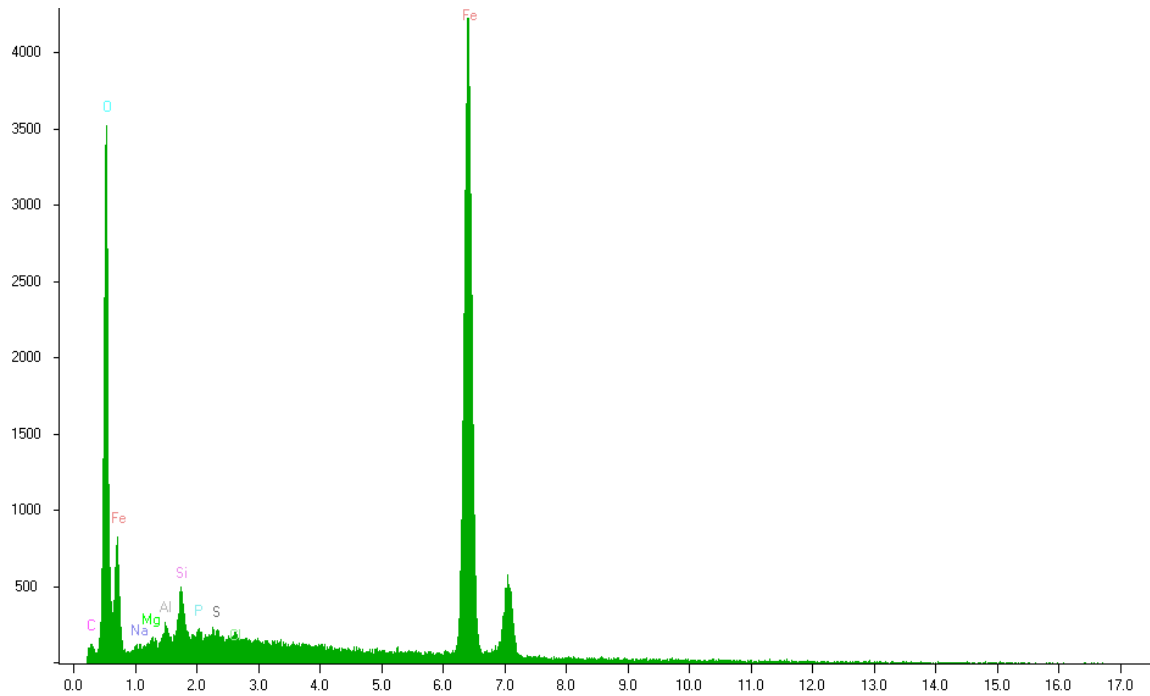


Figure 9. EDS spectrum of goethite sample 5 after exposure to SRB for 14 days. Peaks show trace elements that can be attributed to media retention in pore spaces.

Characterization of Goethite Using FE SEM with EDS

The images of goethite show few to zero cells attached to the sample surface (Figure 10). The SRB were not shown to have significant interaction with this mineral; however, many acicular, prismatic crystals did appear on the surfaces of the samples exposed to SRB (Figure 11) as well as the controls (Figure 13). Spot analyses with EDS suggested iron oxide as the composition with very small amount of Si present (Figures 12 and 14). The crystals may have been a result of dissolution and recrystallization of goethite as the EDS, and shape of the crystals indicate.

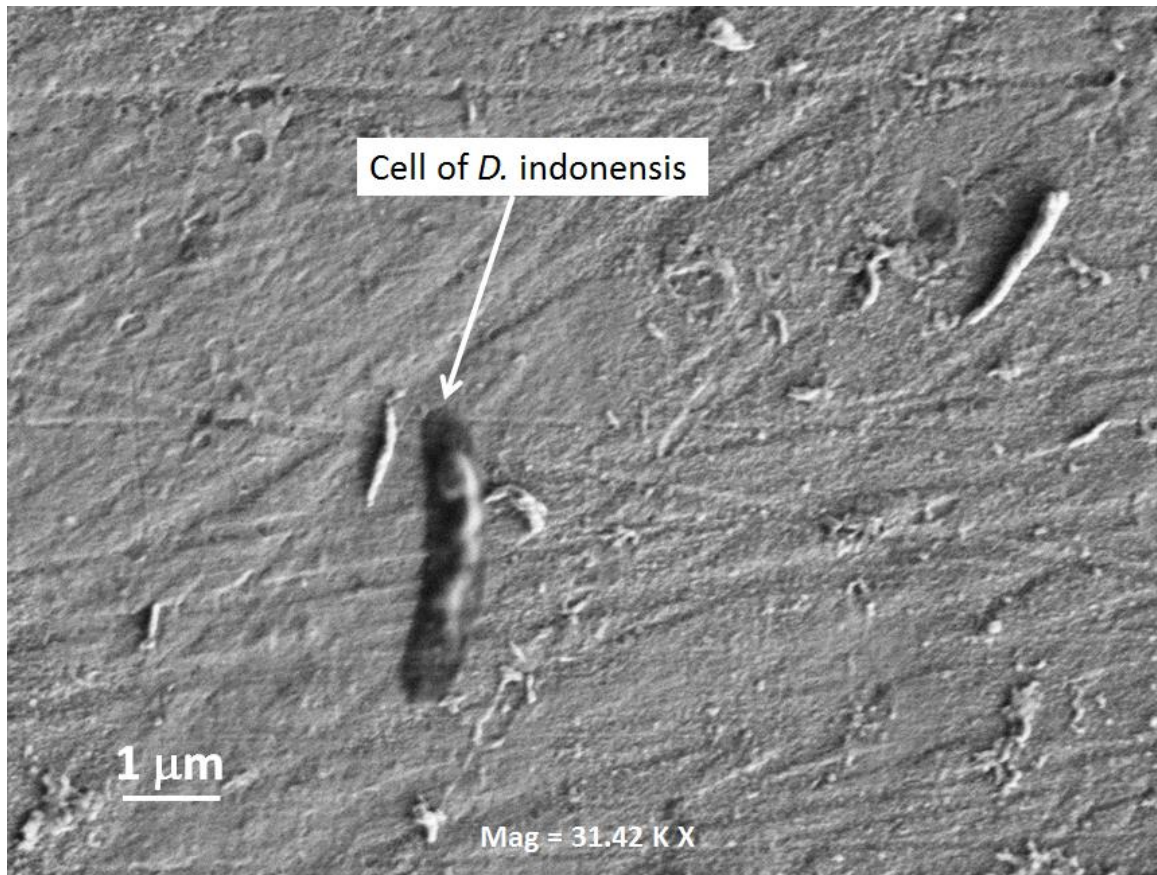


Figure 10. FE SEM secondary electron image of a single cell of SRB on goethite sample 1 after 7 days of exposure. Very few cells were seen on these samples.

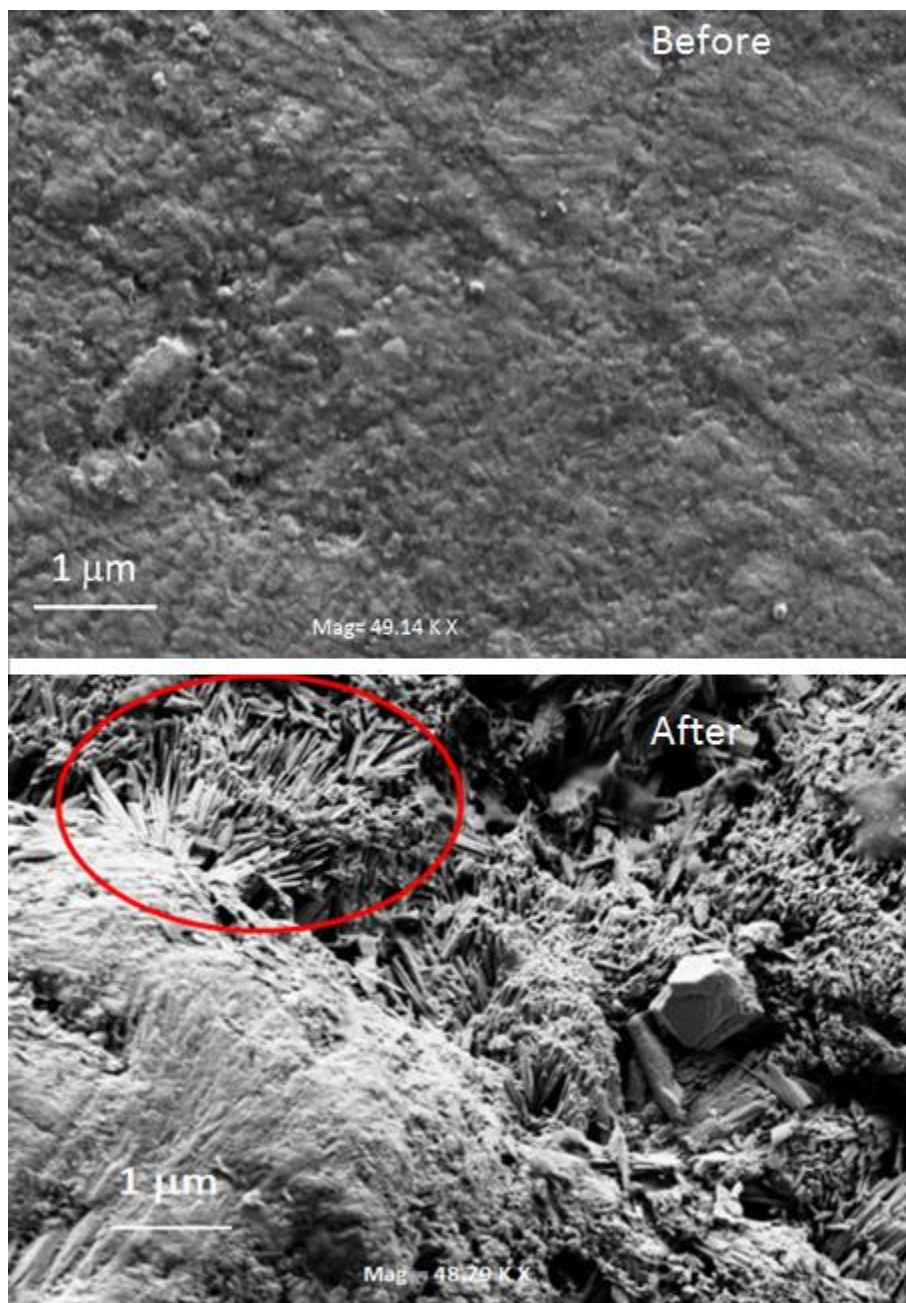


Figure 11. Goethite sample 6 before and after exposure to SRB for 15 days. Prismatic crystals were not seen on the surface prior to exposure to medium. Red circle indicates area that was analyzed by EDS. The results of that spot analysis are shown in Figure 12. SRB cells were rarely observed on the surface of goethite samples; however prismatic crystals covered the surface. Region imaged both before and after exposure to SRB was located with x, y axis etching 200 μm east and 200 μm north of origin.

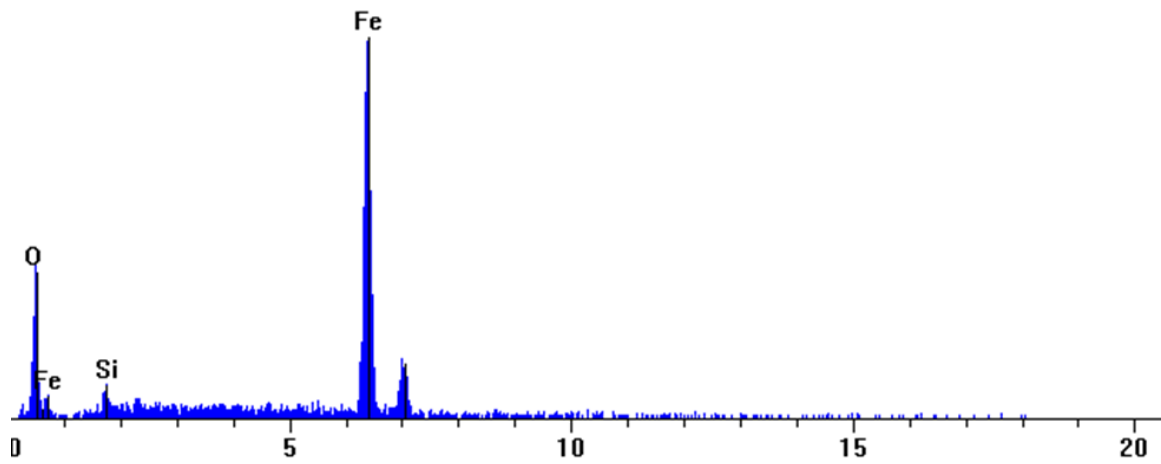


Figure 12. EDS analysis of crystals in Figure 11 goethite sample 6. Amount of Si present was extremely small.

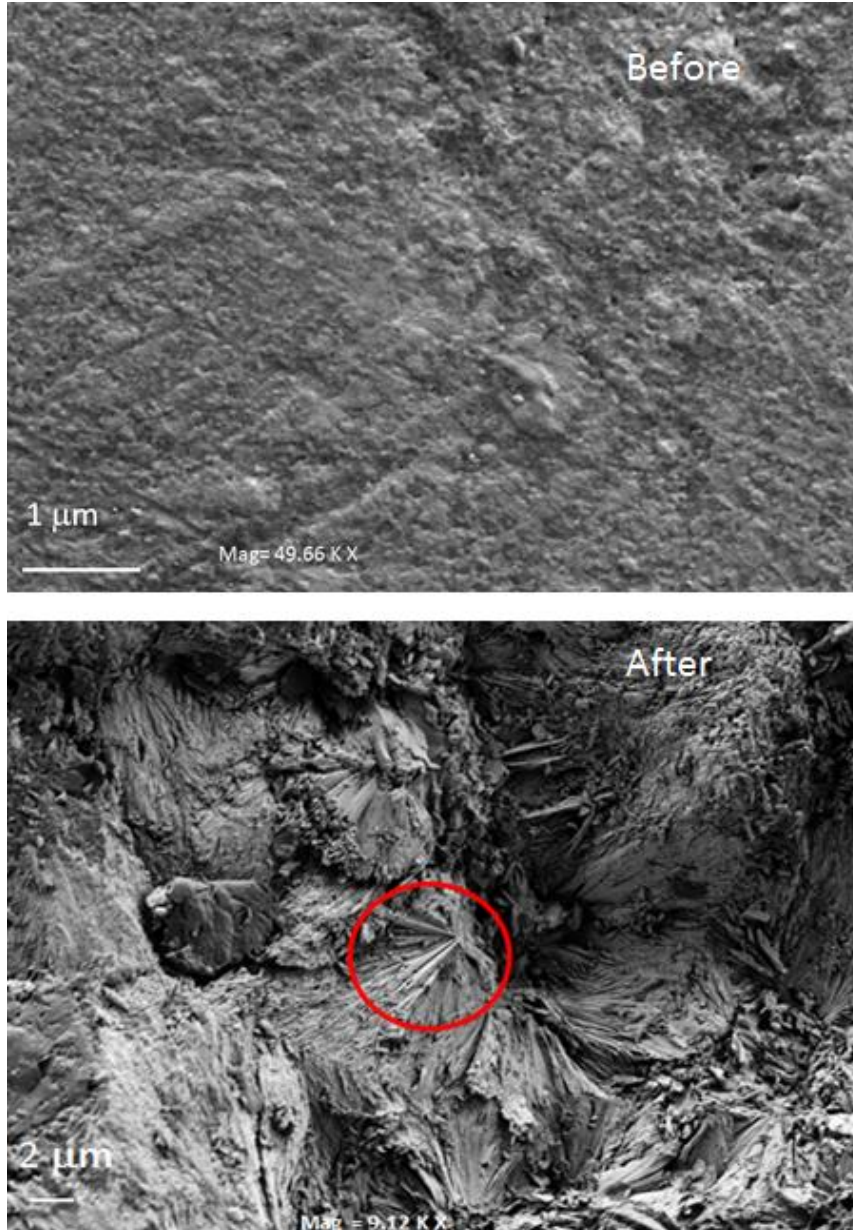


Figure 13. Goethite control sample 8 before after 15 days of exposure to SRB. Concentric acicular crystals are seen similar to the ones shown in Figure 11. This sample, however, was a control and was exposed only to filtered medium. Surfaces prior to exposure to media did not have crystals. Red circle indicates area analyzed by EDS. The alteration of surface morphology was observed in both the experimental and control samples implying that the recrystallization was an abiotic process but may have been influenced by microbial metabolites present in the filtered medium.

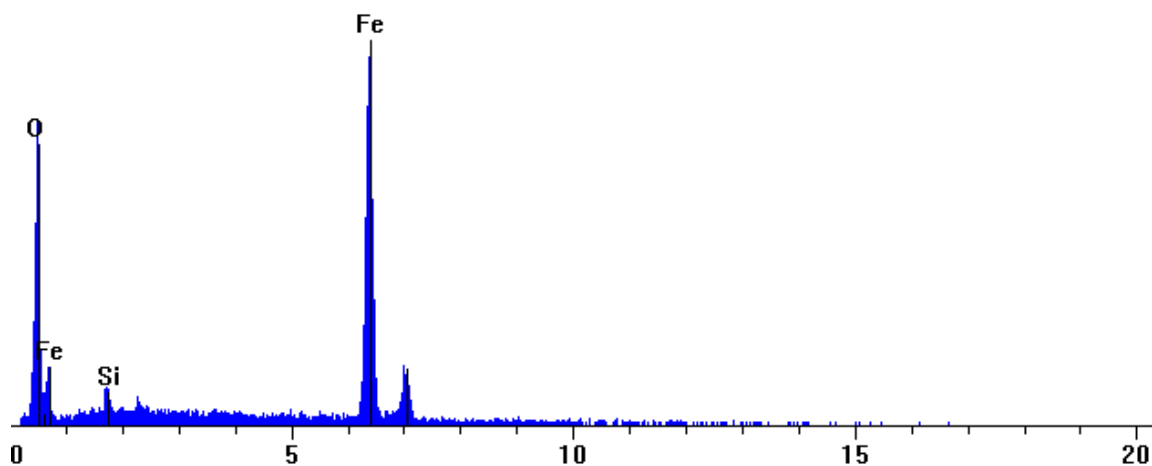


Figure 14. EDS spot analysis of crystals on goethite sample 8 as depicted in Figure 12. Amount of Si present was extremely small.

Characterization of Goethite Surface with XPS

Before Exposure to SRB – a Baseline Signal for Goethite. The goethite samples prior to exposure show iron Fe^{3+} peaks in XPS data (Figure 15) (Table 4). The weighted average of the peaks based on area gives is equal to 709.51 eV. This is a slightly lower binding energy than suggested by previous works, which is approximately 711 eV (Echigo et al., 2011). This is most likely due to the variable content of hydroxyl ions. The oxygen spectra show peaks for the oxide and the hydroxide bonds at 529.35 eV and 530.97 eV respectively (Figure 16) (Table 5). These binding energies are only slightly lower than the previously reported values of 529.8 eV and 531.0 eV (Echigo et al., 2011). Carbon peak was 284.4 eV and all values were shifted appropriately to compensate for charging on surface. For the deconvolution of data, a minimum number of peaks were used to fit the raw data. It was assumed that all iron present was Fe^{3+} but slight variation

in binding energy should be seen between the Fe-O and the Fe-OH bond with the Fe-O bond having a slightly higher binding energy.

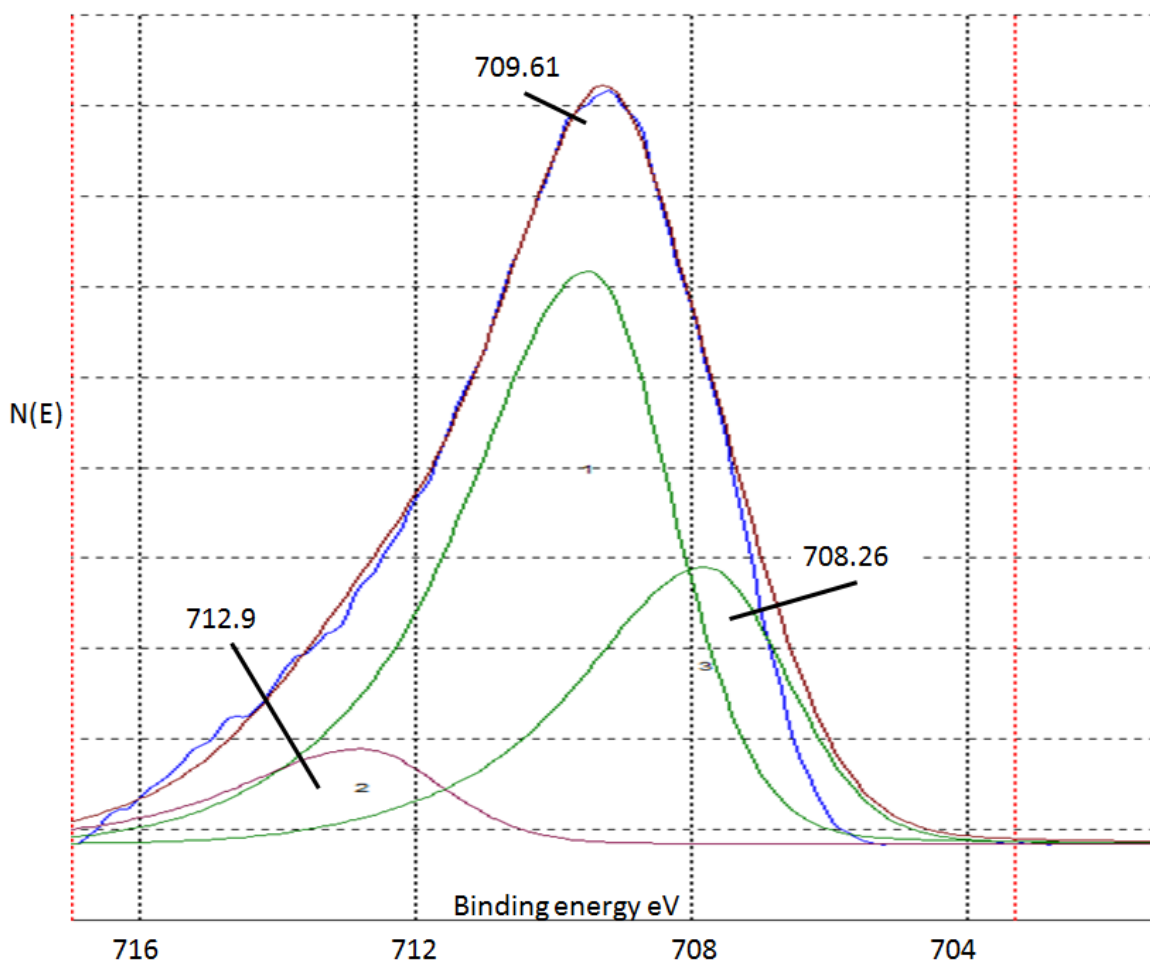


Figure 15. XPS spectrum of iron on goethite sample 1 prior to exposure to SRB. Peaks 1, 2 and 3 indicate Fe^{3+} . Blue line is XPS data, brown is curve fitted data.

Peak	area	position	intensity	FWHM	chemistry
1	22927	709.61	5576	2.8	Fe-OH; Fe-O
2	3816	712.9	928	2.8	Fe-O
3	11094	708.16	2698	2.8	Fe-OH

Table 4. Values of curve fitted data ± 0.05 eV for iron peaks from goethite sample 1 (Figure 15).

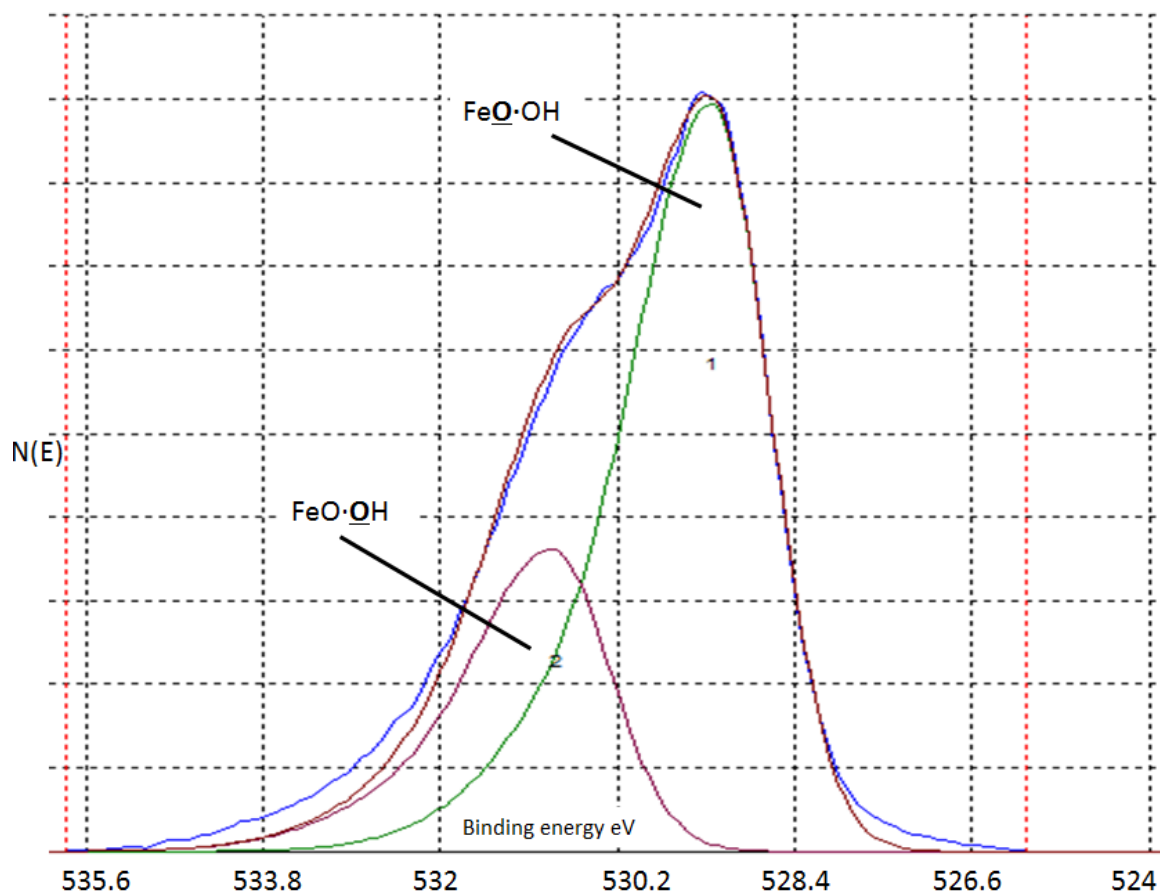


Figure 16. XPS spectrum of oxygen peaks from goethite sample 1 prior to exposure to SRB. The oxygen represented by individual peaks is in bold and underlined. Blue line is XPS data; brown line is curve fitted data

Peak	area	position	intensity	FWHM	Chemistry
1	26506	529.35	13449	1.39	oxide
2	10696	530.97	5427	1.39	hydroxide

Table 5. Values of curve fitted data ± 0.05 eV for oxygen peaks from goethite sample 1 (Figure 16).

After Exposure to SRB. After seven days of exposure to SRB, a survey scan showed a significant peak for carbon and oxygen and a small peak for iron and sulfur (Figure 17). The carbon peak indicated the presence of organic metabolites with C-O and C=O bonds as seen with the high energy bonds (Figure 18). The carbon peak value was 284.6 and all peaks all values were shifted appropriately to compensate for charging on surface. The oxygen peak indicated the carbon oxygen bonds found in metabolites along with small amounts of iron oxide and hydroxide (Figure 19) (Table 6). The amount of oxygen bonded to carbon was much greater than amount bonded to iron. The iron peak showed only Fe^{3+} , with the weighted average of the peak based on area and position equal to 711.38, which was most likely from the original goethite substrate (Figure 20) (Table 7). Sulfur signal was too weak and distorted to reduce data.

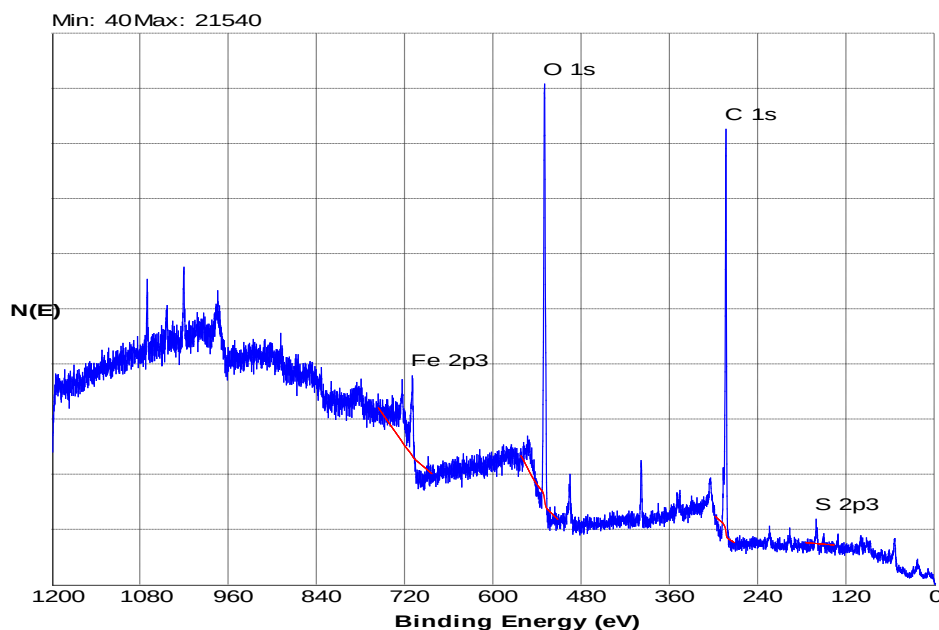


Figure 17. XPS survey scan of goethite sample 1 after 7 days exposure to SRB. Spectrum shows large peaks for oxygen and carbon, small peaks for iron and sulfur. Values have an accuracy of ± 0.4 eV

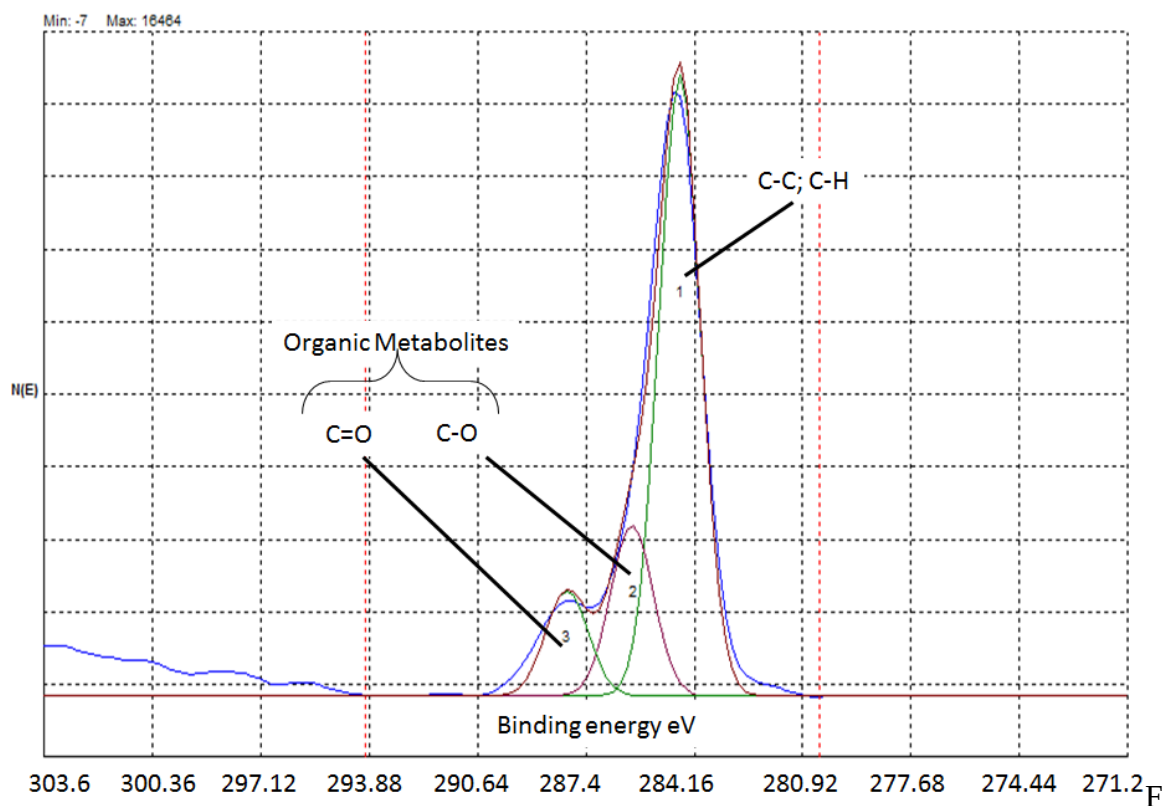


Figure 18. XPS spectrum for carbon on goethite sample 1 after 7 days exposure to SRB. Blue line is raw XPS data; Brown line is curve fitted data. Peaks 2 and 3 represent organic metabolites on the surface.

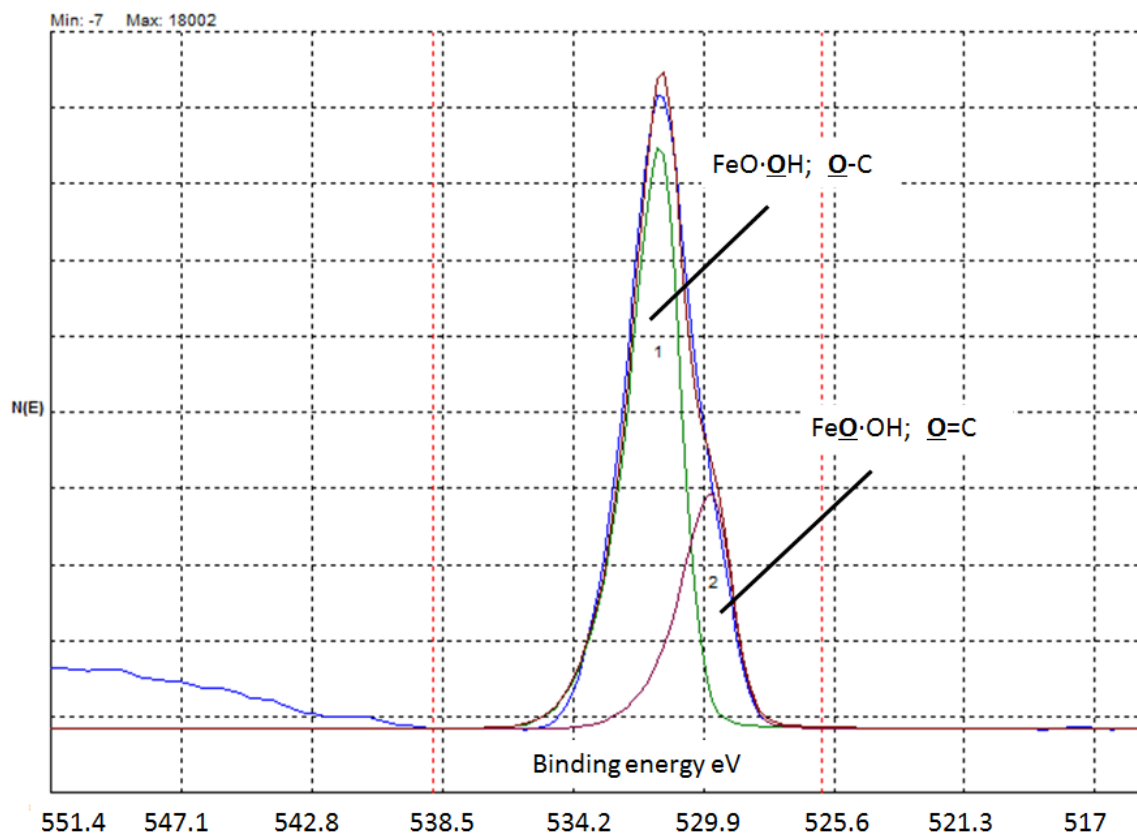


Figure 19. XPS spectrum of oxygen from goethite sample 1 after 7 days exposure to SRB. Oxygen atoms corresponding to peaks are in bold and underlined. Blue line is raw XPS data; brown is curve fitted data.

Peak	area	position	intensity	FWHM	Chemistry
1	38471	531.22	16581	1.56	FeO· <u>O</u> H; <u>O</u> -C
2	14805	529.55	6721	1.56	Fe <u>O</u> ·OH; <u>O</u> =C

Table 6. Values of curve fitted data ± 0.05 eV for oxygen peaks in Figure 19.

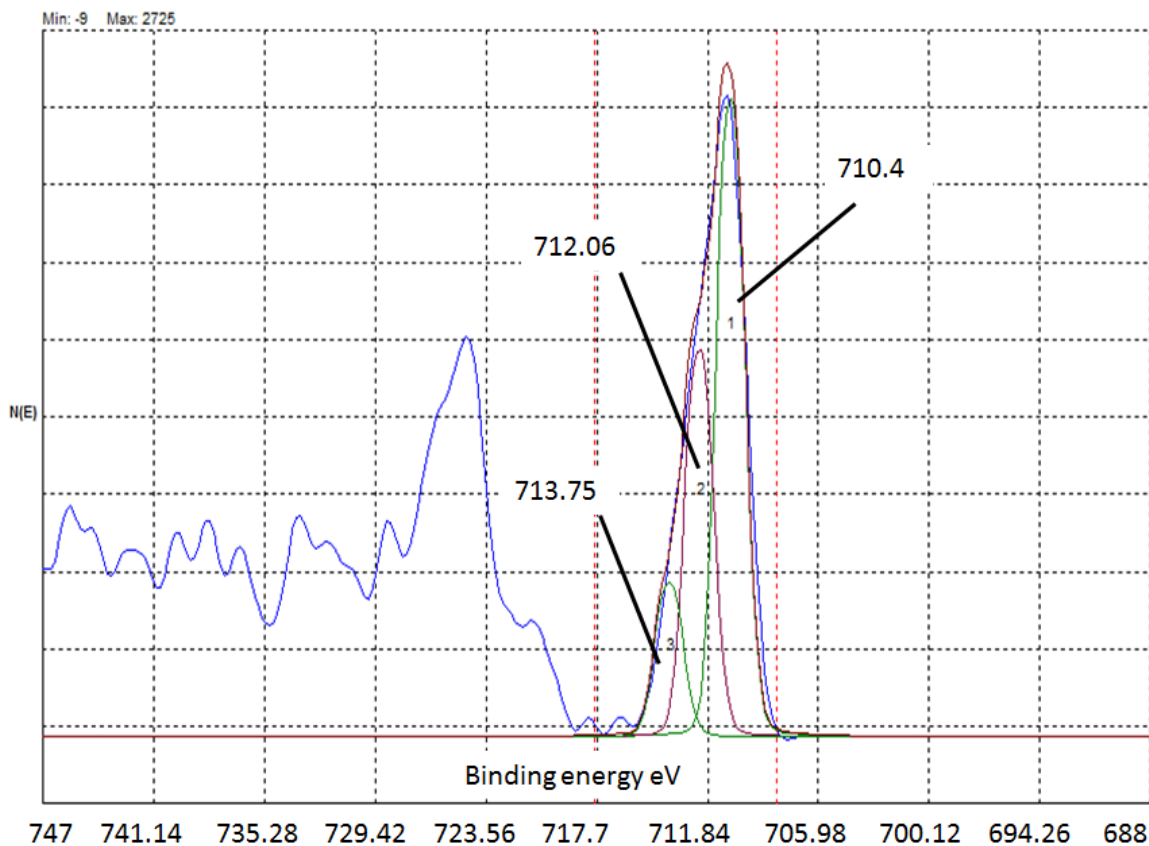


Figure 20. XPS spectrum of iron on goethite sample 1 after 7 days exposure to SRB. Weighted average of peaks equals 711.38. Blue line is XPS data; brown is curve fitted data.

Peak	area	position	intensity	FWHM	chemistry
1	5162	710.4	2706	1.5	Fe-O;Fe-OH
2	3136	712.06	1644	1.5	Fe-O
3	1249	713.75	655	1.5	Fe-O

Table 7. Values for curve fitted data ± 0.05 eV for iron peaks in Figure 20. The binding energy values indicate presence of only Fe^{3+} (Moulder et al., 1993).

The surface was sputtered with argon for one minute at rate of one nanometer/min.

The survey scan showed the carbon peak was significantly reduced, indicating a removal of the organic layer. The iron signal had amplified, with a weighted average of 710.22 eV. Oxygen signal remained significant; however, the peak was shaped differently than

the previous surface analysis. Comparison of baseline goethite signal to signal of sample 1 sputter depth of one nanometer suggests iron oxy-hydroxide substrate (Figure 21). The control for 7 days suggested the same results where the organic metabolites covered the surface to a depth of no more than 1 nm. Samples exposed for 15 days and 30 days (Figures 22-23) indicated the same results: one nanometer of organic metabolites on the surface on samples with SRB and one nanometer of organic on controls. The control mineral samples were exposed to filtered medium thus had the presence of metabolites but without living SRB cells.

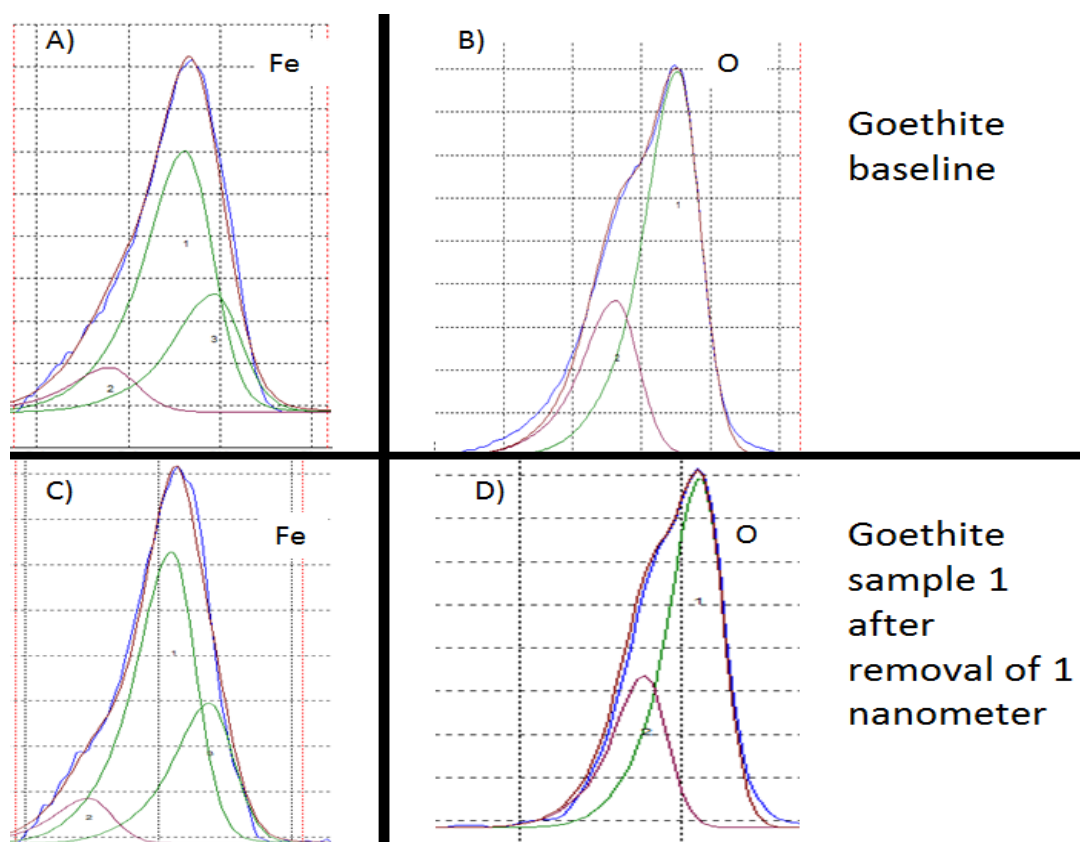


Figure 21. A) and B) represent Fe and O peaks from goethite sample prior to exposure to microbes. C) and D) represent Goethite sample 1 after 7 days exposure to SRB and removal of the top 1 nm of surface. Similarity of iron peaks and oxygen peaks from samples before and after exposure suggest all organics were removed.

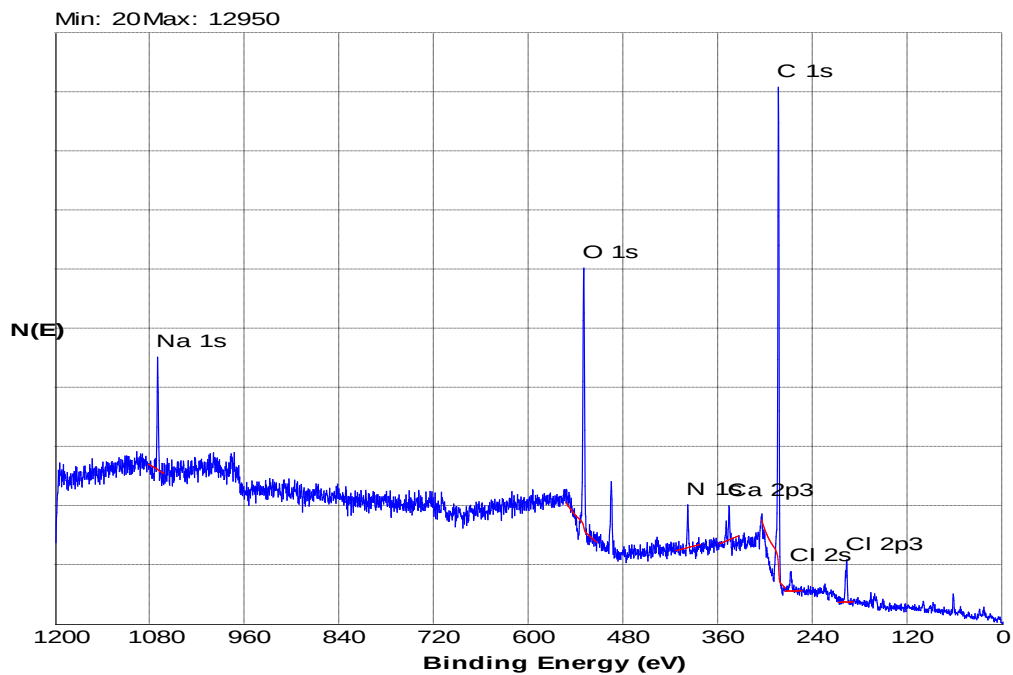


Figure 22. Initial survey scan of sample 9 after 30 days exposure to SRB. No iron peak is seen. Peak values are accurate within ± 0.4 eV.

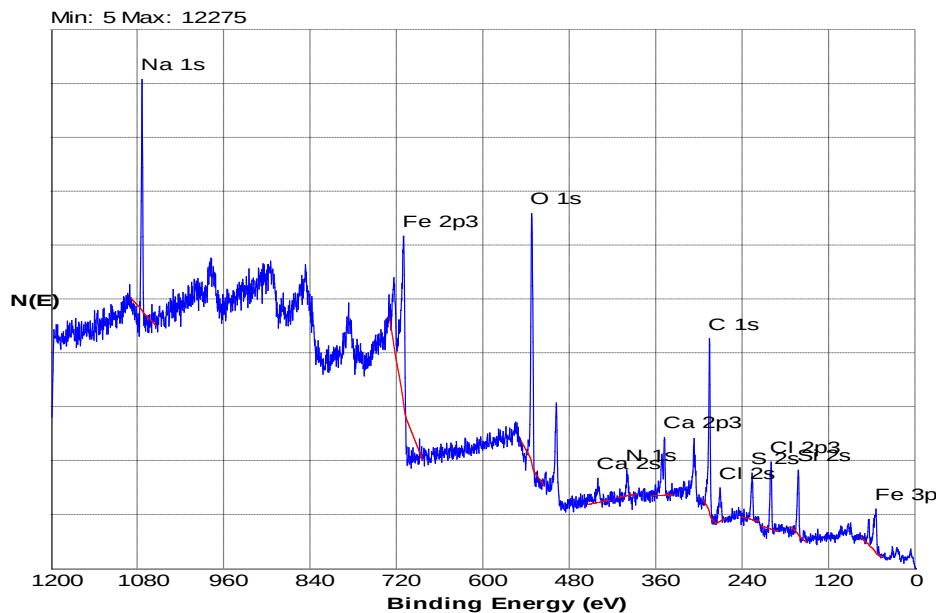


Figure 23. Survey scan of sample 9, 30 days exposure time after 1 minute of sputtering. Significant iron peak indicates removal of no more than 1 nanometer of organic material on surface. Peak values are accurate within ± 0.4 eV.

Goethite Discussion

FEM images of goethite samples exposed to *D. indonensis* showed few to no cells attached to the surface. Acicular, prismatic crystals of an iron oxide did appear on surfaces of both the experimental and control samples, possibly from dissolution and reprecipitation. These crystals suggest abiotic formation due to the presence on both the experimental and control samples but may have been influenced by microbial metabolites present, as the control medium was filtered to be free of cells. XPS data confirmed no interaction of cells with minerals, however the presence of metabolites in media did leave a >1 nm layer on all samples as apparent in XPS survey scans. Results of bacteria experimental and control samples were identical independent of exposure time. The null result indicates a negative feedback mechanism in which goethite acts as an inert byproduct of the corrosion process. These results suggest that iron fixed as goethite is not accessed by *D. indonensis* and can act as an armoring layer, thus can create limits to further MIC.

Pyrrhotite

Mineral Phase Identification by XRD

Natural pyrrhotite like the sample used in this experiment, often has impurities in the form of Ni, Cu, and Zn sulfides (Bowles et al., 2011). Some studies have shown unidentified phases when trying to reduce XRD data (Thomas et al., 2001). Analysis of this sample indicated pyrrhotite with inclusions of pentlandite, biotite, titanite, and quartz (Figure 24). There was also an unidentified phase.

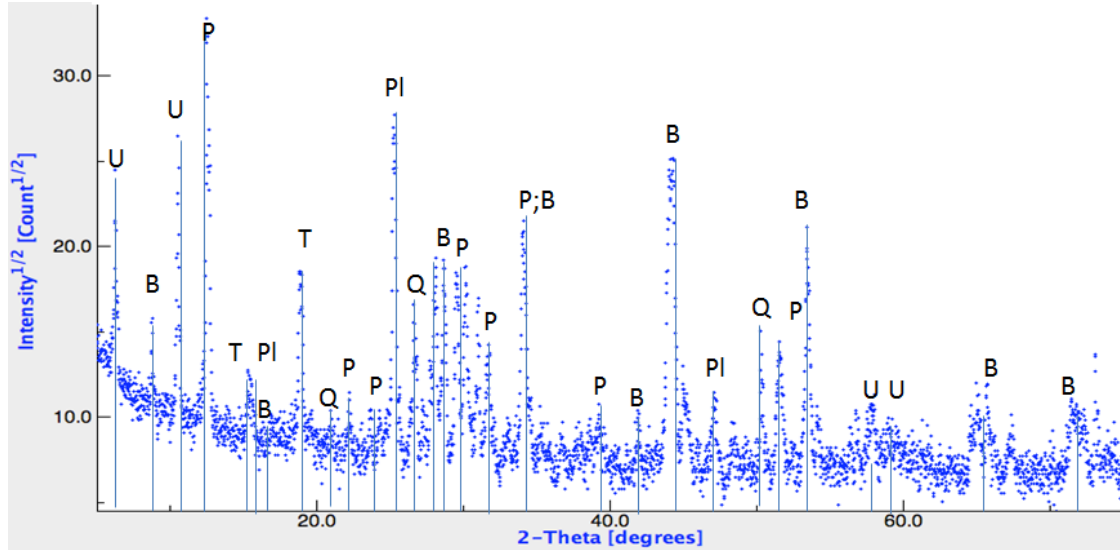


Figure 24. XRD of pyrrhotite indicating pyrrhotite (P), pentlandite (PI), biotite (B), quartz, (Q), and unidentified phase (U).

Characterization of Pyrrhotite Using SEM with EDS

The SEM images demonstrate the complex nature of this sample. Figure 25 shows areas of the highly polished pyrrhotite surface that exhibit charging due to low conductance. This gives the impurities an oily luster and a false appearance of relief. The backscatter image (Figure 26) reveals the impurities are less dense than the surrounding material. Figure 26 also suggests regions of more dense phases represented by the lighter colored areas. EDS analyses averaged over entire area demonstrate the presence of iron, sulfur and oxygen with small amounts silicon, magnesium, calcium, copper, zinc and sodium (Figure 27). EDS analysis averaged over entire area at magnification of 7000x in a region with no inclusions showed only iron and sulfur (Figure 28). Elemental mapping of the samples imply the less dense inclusions that experience charging do not have iron but instead consisted of calcium, magnesium and possibly silicon (other elements may have been present as well; however EDS is not an

effective tool for measuring light elements) (Figure 29). Elemental mapping also indicates the more dense areas to have copper (Figure 30) and/or zinc present with iron and sulfur as would be commonly seen in pyrrhotite (Bowles et al., 2011; Thomas et al., 1998). BSE imaging and EDS spot analyses to identify phases is presented in more detail in the next section. Although complex in chemistry, SEM images and EDS analysis of the pyrrhotite samples were the same before and after exposure to SRB. There were also no unusual changes seen in the elemental mapping.

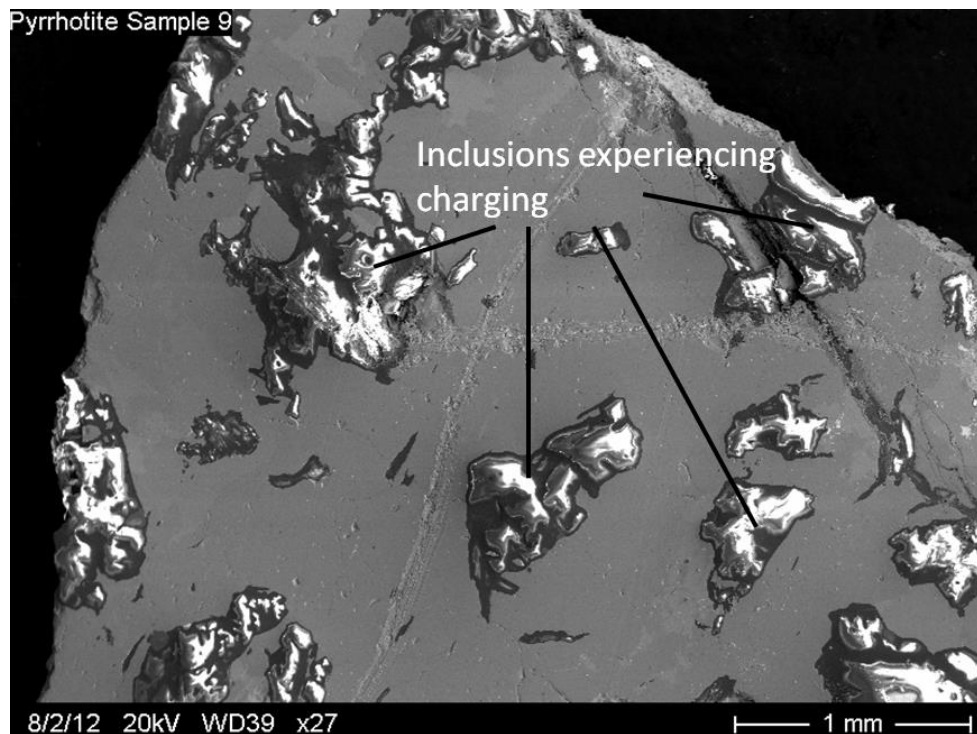


Figure 25. SEM secondary electron image of pyrrhotite sample 9 before exposure to SRB. Regions of charging are indicated.

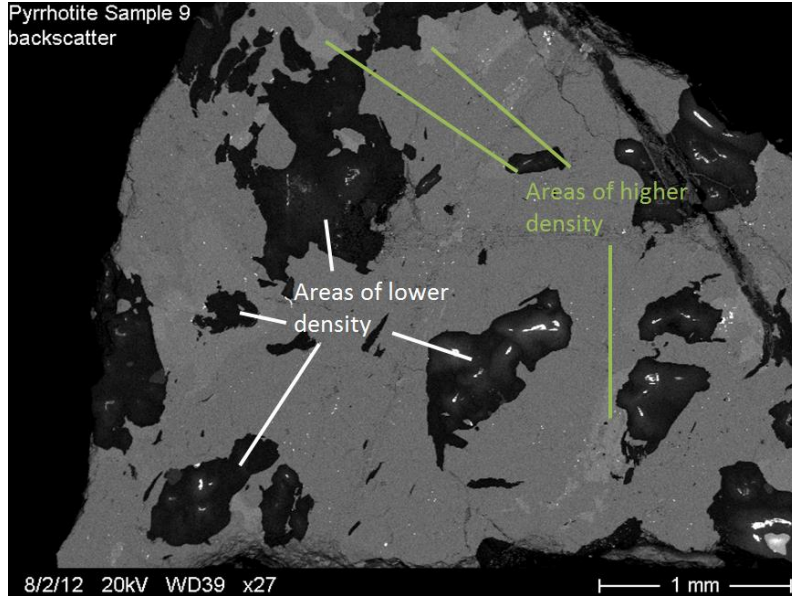


Figure 26. SEM backscatter electron image of pyrrhotite sample 9 showing areas of charging that were highlighted in Figure 25 have a lower density than surrounding material. More dense regions are also noted.

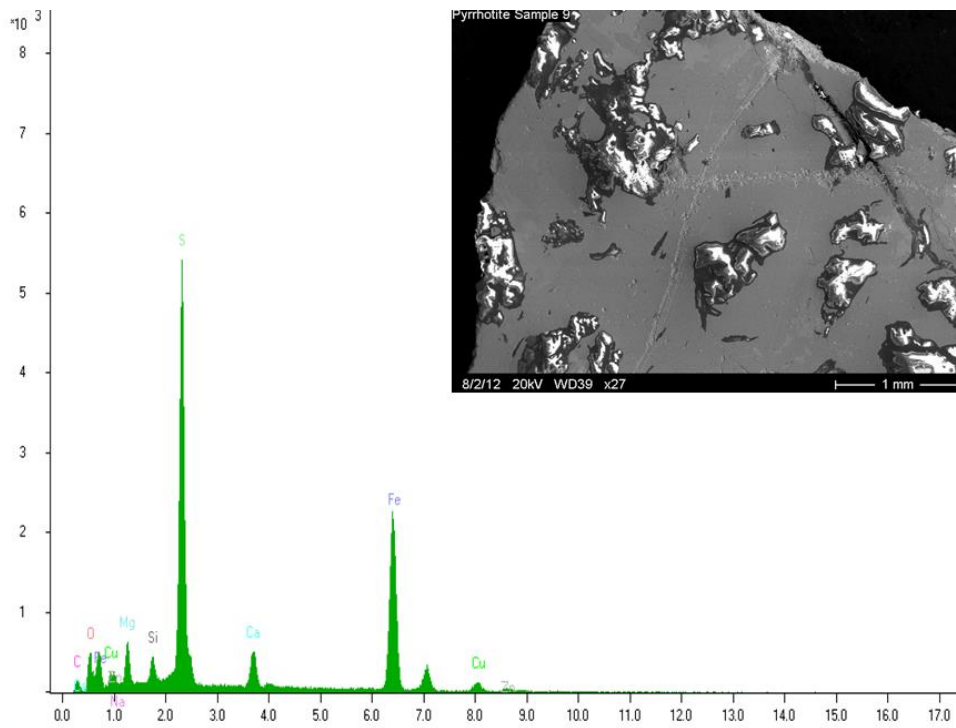


Figure 27. EDS spectrum of pyrrhotite sample 9 representing entire sample. Spectra was obtained at 27x magnification. Inset shows secondary electron image of sample 9 at 27 x magnification reflecting heterogeneous nature of sample. This spectrum represents an average of the entire sample.

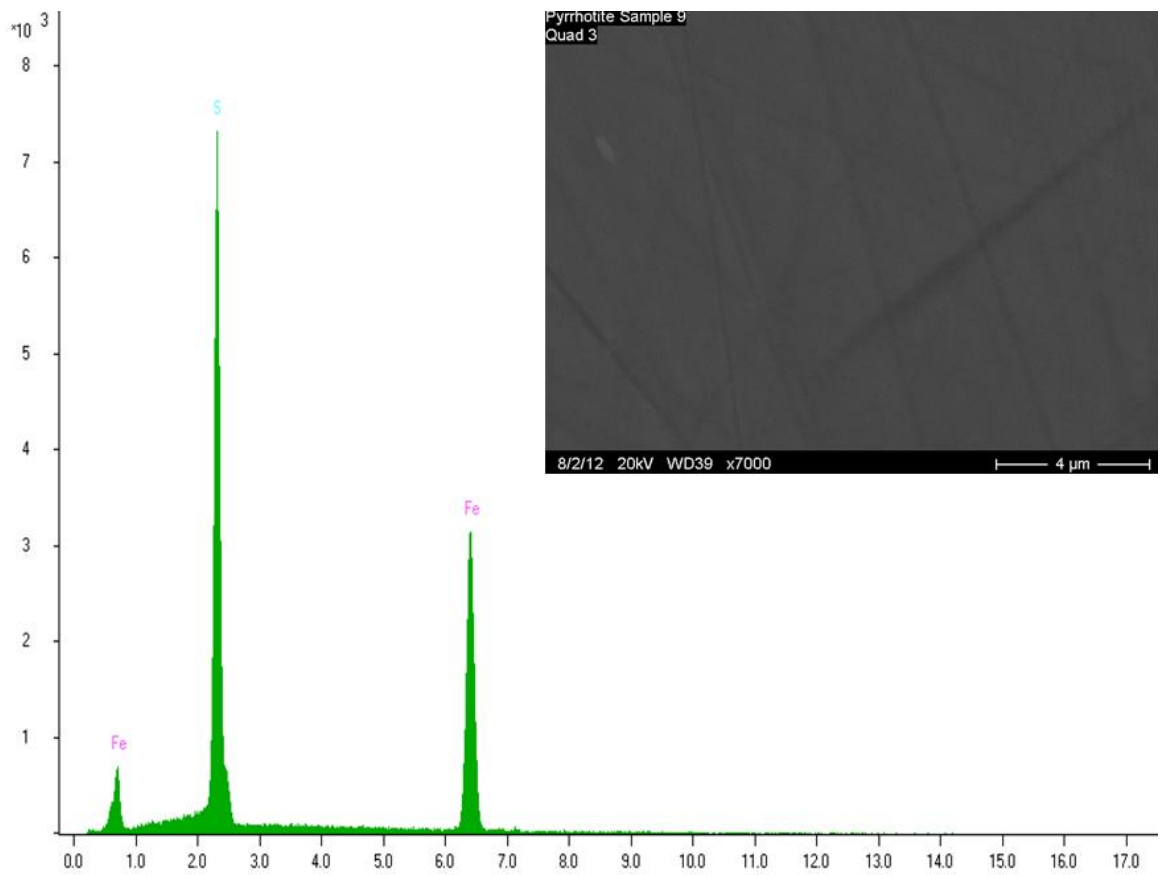


Figure 28. SEM EDS spectrum of pyrrhotite sample 9 obtained at 7000x representing region of sample without inclusion. Inset is secondary electron image of sample 9 at 7000x reflecting homogeneous nature of that portion of sample.

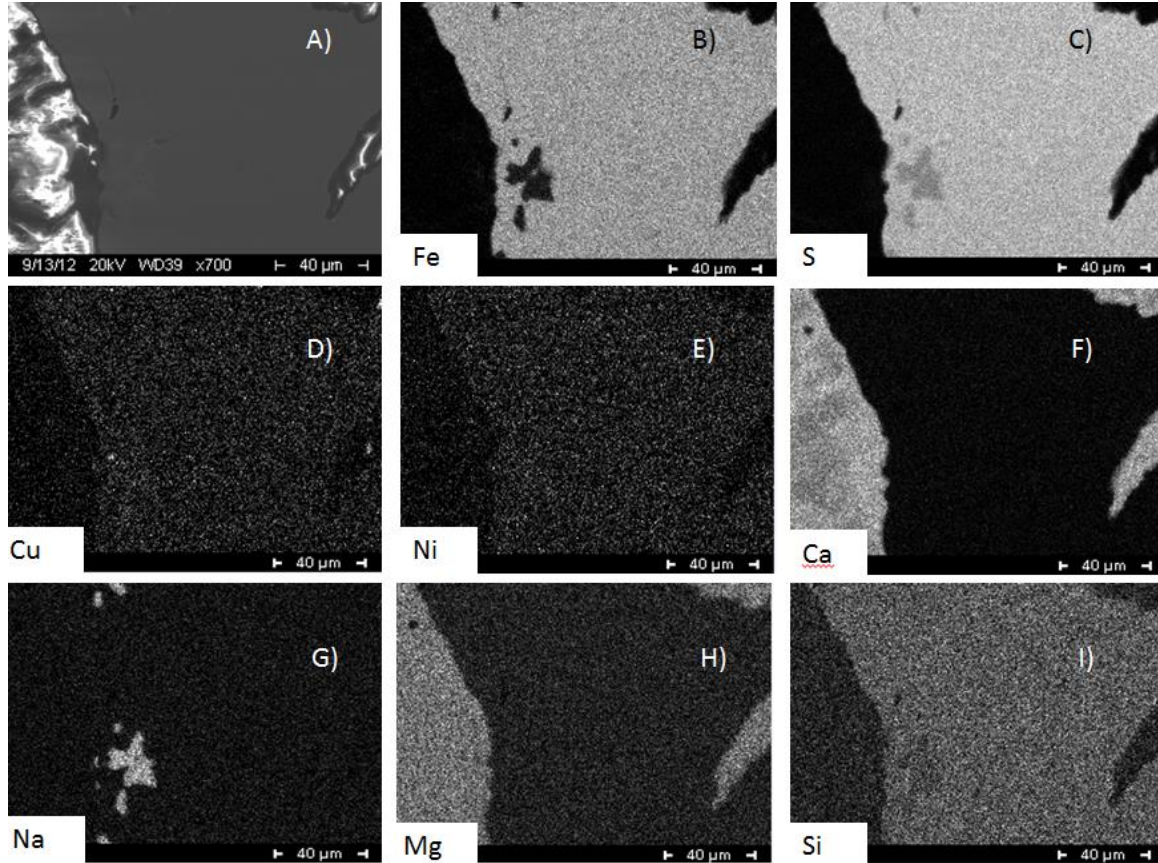


Figure 29. Elemental mapping of pyrrhotite sample 1 at 700 x magnification. Lighter areas represent the element in question and dark areas represent an absence of the element. A) secondary electron image of region mapped, inclusions shown with oily appearance due to charging; B) elemental map of iron showing depleted zones corresponding to the inclusions show in A); C) map of sulfur showing depleted zones in the inclusions; D) map of copper indicating little to none in this area; E) map of nickel indicating little to none in this area; F) map of calcium indicating presence in the inclusions; G) map of sodium indicating a small presence; H) map of magnesium indicating presence in inclusions; I) map of silicon indicating depleted zones in inclusions but small amount in surrounding material.

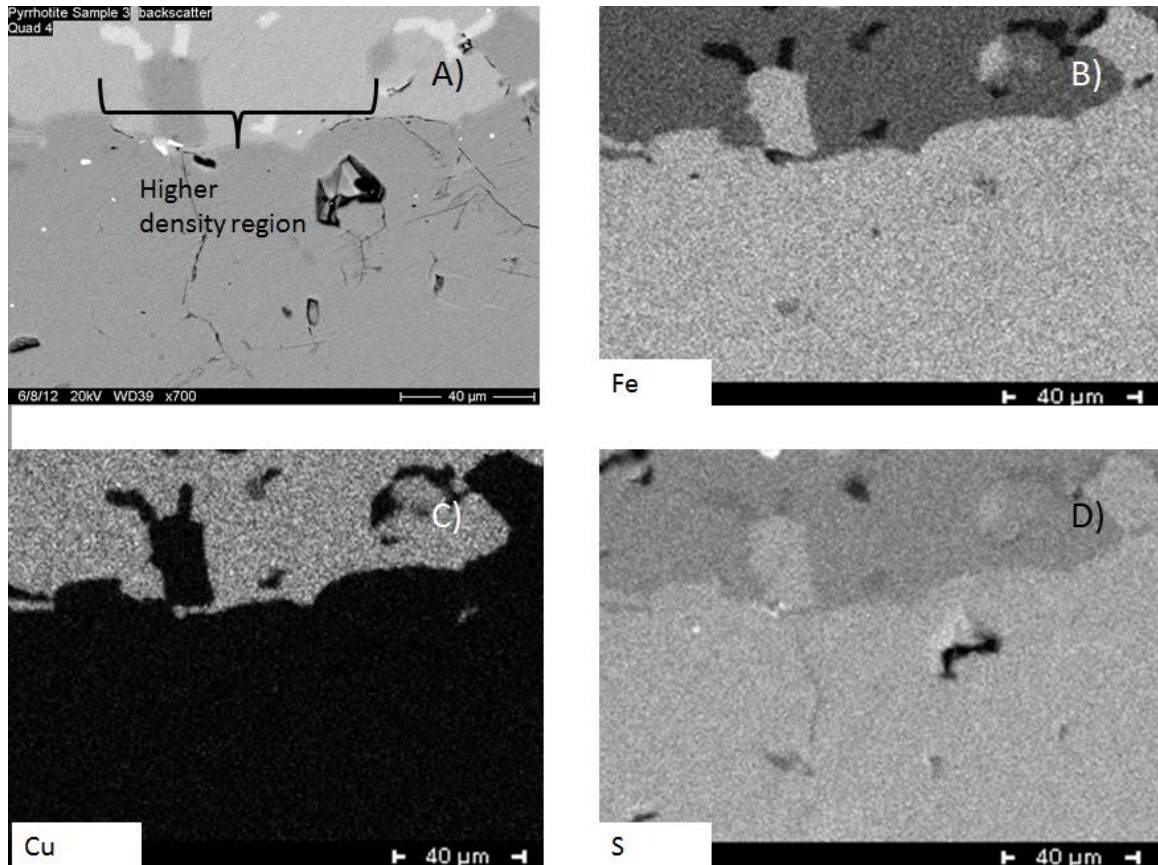


Figure 30. Elemental mapping of sample 3 at 700 x magnification. A) backscatter electron image of mapped region demonstrating area of higher density; B) map of iron showing slightly depleted zone in higher density region; C) map of copper showing presence in higher density region; D) map of sulfur showing presence in all areas but lower abundance in areas of higher density.

Characterization of Pyrrhotite Using FE SEM with EDS

The images of pyrrhotite after 7, 15 and 30 days exposure time to SRB revealed cell attachment (Figures 31-32). The cells after 30 days appeared to encircle mounds of precipitate (Figure 32). A region greater than 60 µm in diameter, that represented precipitate (Figure 33) was analyzed with EDS indicating the presence of iron, sulfur, oxygen and trace amounts of potassium (Figure 34). The relative intensities of the peaks suggest FeS and possibly some iron oxide. Figure 35 displays two backscatter electron

images of sample 9 with ten points of spot analysis. The spectra indicate the areas of high density (points 1, 2, 5, and 9) consist mainly of iron, nickel and sulfur, most likely representing pentlandite. Areas of lower density (points 3, 4, 6, and 7) are silicates, biotite, quartz and titanite. Point 10 represents pyrrhotite.

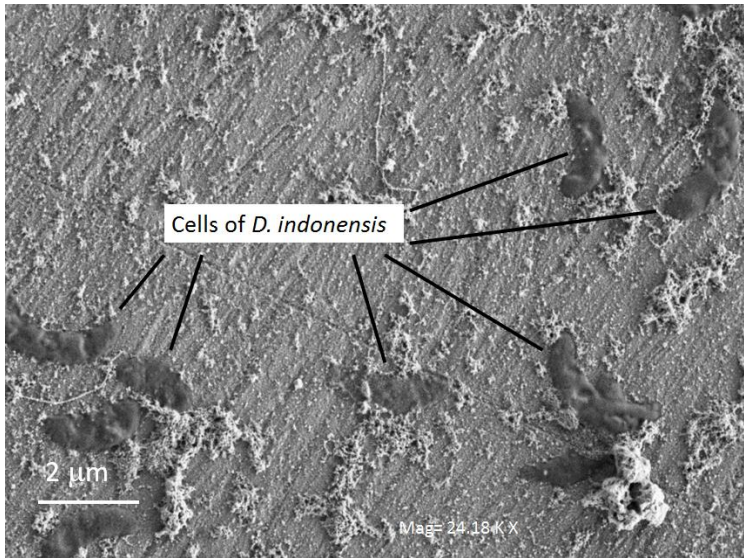


Figure 31. FE SEM secondary electron image of pyrrhotite sample 1 exposed to SRB for 7 days.

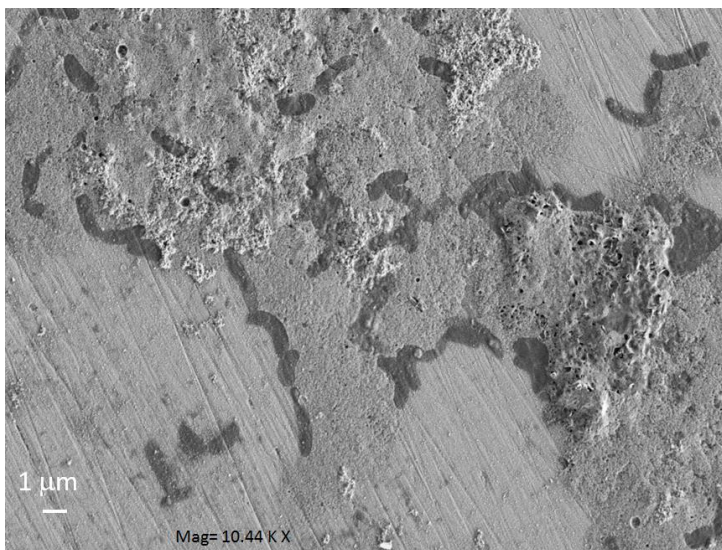


Figure 32. FE SEM secondary electron image of pyrrhotite sample 11 after 30 days exposure to SRB.

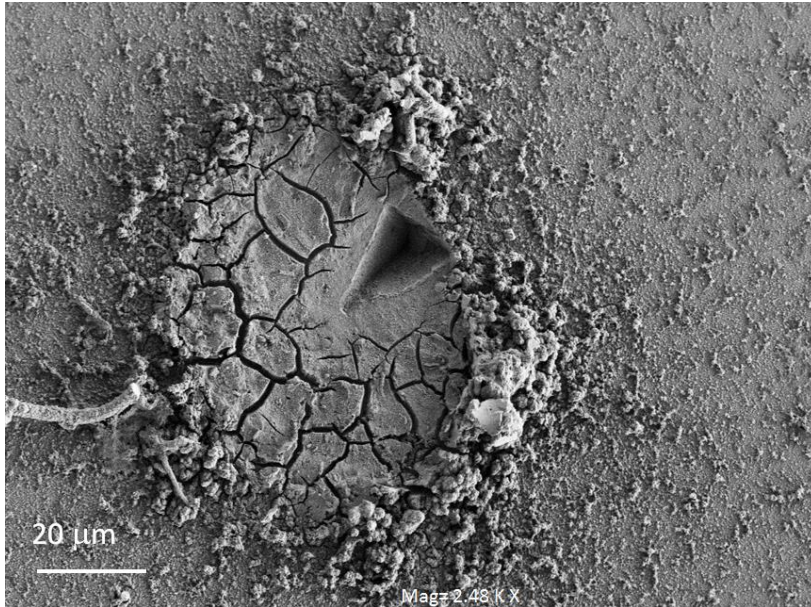


Figure 33. FE SEM secondary electron image of pyrrhotite sample 1 after 7 days exposure. This image denotes a large (diameter greater than 60 μm) structure of precipitate. Spot analysis of the structure revealed iron, sulfur, oxygen and trace potassium (Figure 34).

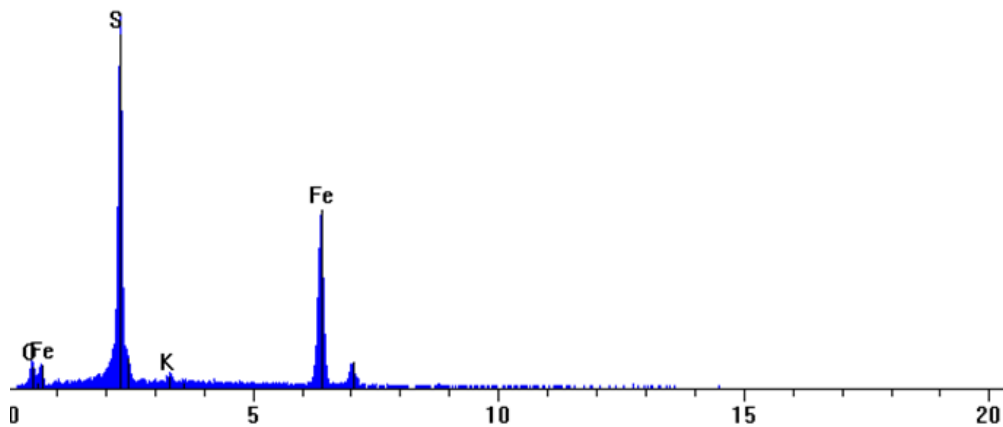


Figure 34. FE SEM EDS spectrum of precipitate structure seen in Figure 33. Peaks suggest presence of iron, sulfur, oxygen and trace potassium.

Characterization of Pyrrhotite Surface with XPS

Before Exposure to SRB – a Baseline Signal for Pyrrhotite. XPS survey scan indicated the presence of iron, sulfur, nickel, zinc, oxygen, carbon, calcium, magnesium, aluminum, titanium and rhodium (Figure 36) consistent with the description of the Sudbury deposit (Becker et al., 2010; Mertie, 1969). The iron peak implies the presence of Fe^{2+} and Fe^{3+} (Figure 37)(Table 8). Possible sources of the curve fitted peak at 707.92

eV are pyrrhotite and pentlandite (Legrand et al., 2005). The signal at 709.4 eV implies Fe-O and/or Fe(III)-S (Legrand et al., 2005). The higher energy peaks could represent Fe(III)-S (Legrand et al., 2005) and/or ilmenite (FeTiO_3) (Yang-ge et al., 2011). Carbon peak was 284.55 eV and data was adjusted for charging.

The sulfur peak (Figure 38)(Table 9) showed only the presence of sulfide with oxidation state of -2. This most likely represents the both the pentlandite and pyrrhotite signal and possibly a signal from zinc sulfide (sphalerite).

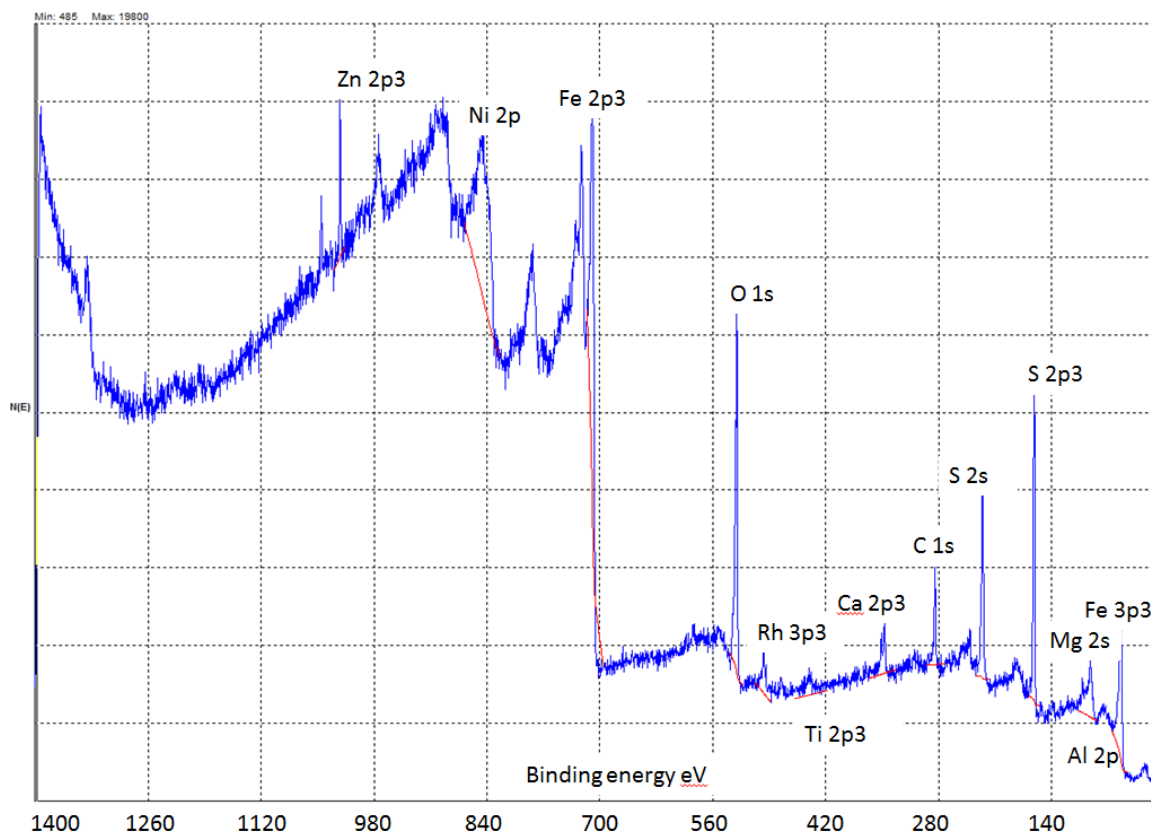


Figure 36. XPS survey scan of pyrrhotite surface prior to exposure to SRB. This scan represents the heterogeneous nature of the sample.

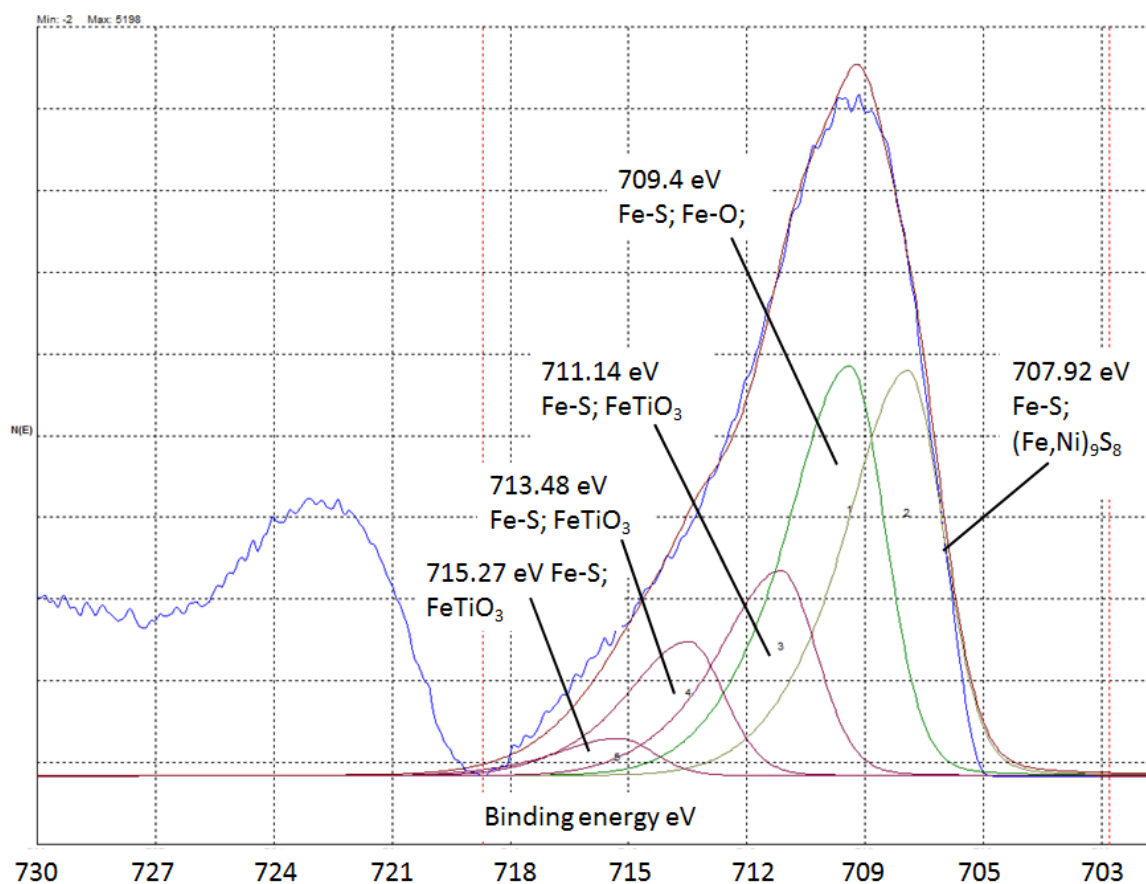


Figure 37. XPS spectrum of iron on pyrrhotite sample 3 prior to exposure to SRB. Blue line is raw XPS data; brown is curve fitted. Peaks are labeled with possible iron bearing minerals.

Peak	area	position	intensity	FWHM	chemistry
1	10548	709.4	3130	2.15	Fe-S; Fe-O
2	10433	707.92	3096	2.15	Fe-S; (Fe,Ni) ₉ S ₈
3	5274	711.14	1565	2.15	Fe(III)-S; FeTiO ₃
4	3458	713.48	1026	2.15	Fe(III)-S; FeTiO ₃
5	967	715.27	287	2.15	Fe(III)-S; FeTiO ₃

Table 8. Values for curve fitted data ± 0.05 eV in Figure 37.

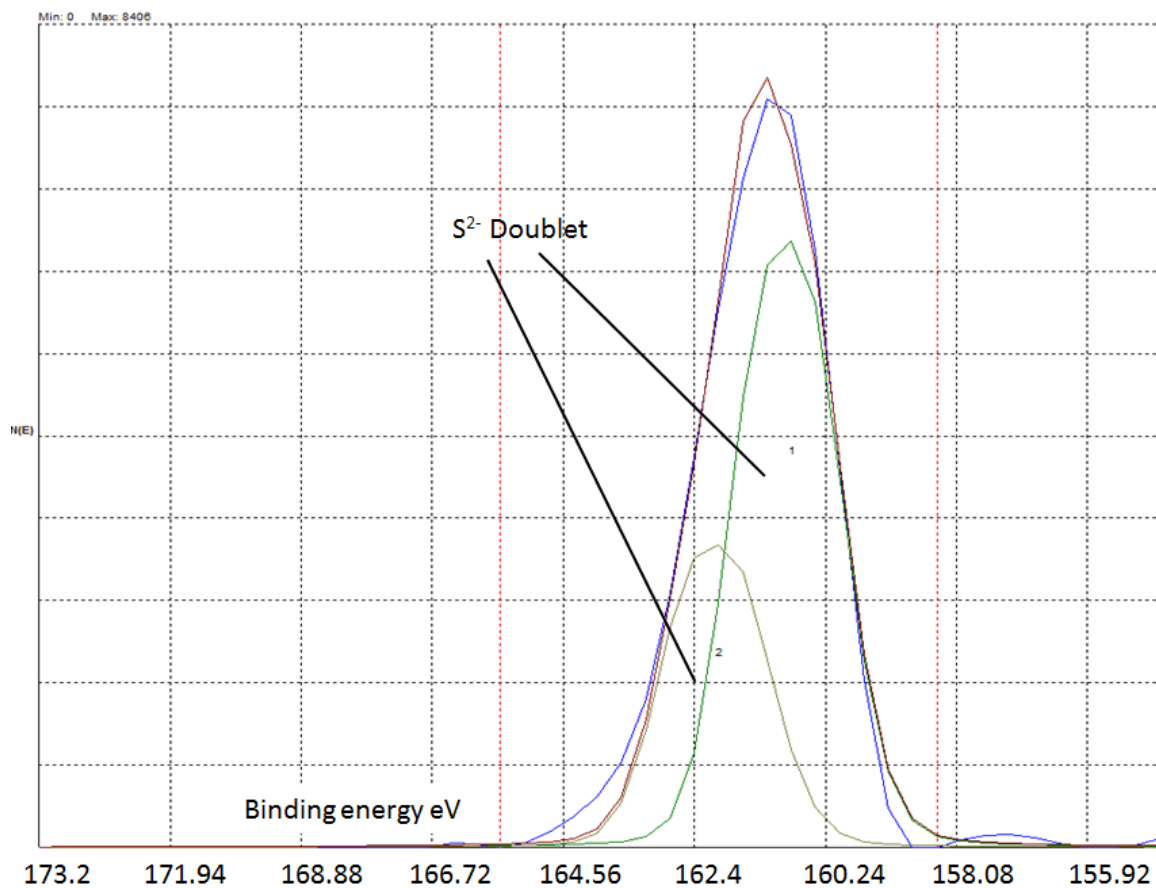


Figure 38. XPS sulfur peak for pyrrhotite sample 3 prior to SRB exposure. Doublet shows presence of sulfide oxidation state -2.

Peak	area	position	intensity	FWHM
1	160.73	6806	6806	1.72
2	161.91	3403	3403	1.72

Table 9. Values for curve fitted data ± 0.05 eV in Figure 38.

After Exposure to SRB. After 7 days exposure to SRB, the initial survey scan indicated sodium, iron, oxygen, rhodium, nitrogen, carbon, sulfur and silicon (Figure 39). The nitrogen signal is most likely a biogenic product and part of an organic metabolite. The iron signal indicates the presence of both Fe²⁺ and Fe³⁺ (Figure 40) on the surface consistent with values from Legrande et al., 2005 and Moudler, 1993. The sulfur peak is

weak and distorted; however, signal was strong enough to reduce data (Figure 41) (Table 10). The curve fitted data suggests four oxidation states for sulfur. The largest doublet (peaks 3 and 4) implies sulfide, oxidation state -2. The second largest doublet (peaks 1 and 2) implies disulfide, oxidation state -1. The third largest doublet (peaks 5 and 6) implies sulfate, sulfur oxidation state +6. The smallest doublet (peaks 7 and 8) implies a polysulfide, sulfur oxidation state greater than -1. These values are consistent with Legrande et al., 2005 and Moulder, 1993.

Oxygen peak indicates possible iron oxide, hydroxide, sulfate and organic metabolites (Figure 42) (Table 11). Carbon peak supports the evidence of organic metabolites on the surface (Figure 43). These values are consistent with Legrande et al., 2005 and Moulder, 1993.

Iron sulfide, iron disulfide, and iron oxide/hydroxide are the most likely phases of secondary precipitates seen on the surface of pyrrhotite after 7 days exposure to SRB.

XPS data collected after one minute of sputtering (~1 nm) produced spectra that are comparable between the baseline survey and the survey obtained after 7 days exposure to SRB (Figure 44). This implies the alteration products were less than 1 nanometer thick on the surface.

The control sample also had less than 1 nanometer of material overlying the surface. The initial survey scan (Figure 45) was similar to the survey scan of the experimental sample (Figure 39). Iron and sulfur signals were too weak and distorted to reduce data.

The results from 14 days and 30 days exposure time also revealed 1 nanometer of material on the surface. Reduced data indicated the presence of sodium, iron, oxygen,

rhodium, nitrogen, carbon, sulfur and silicon that corresponded to the same elements present in the initial survey of the sample exposed for 7 days. Results were same independent of exposure time.

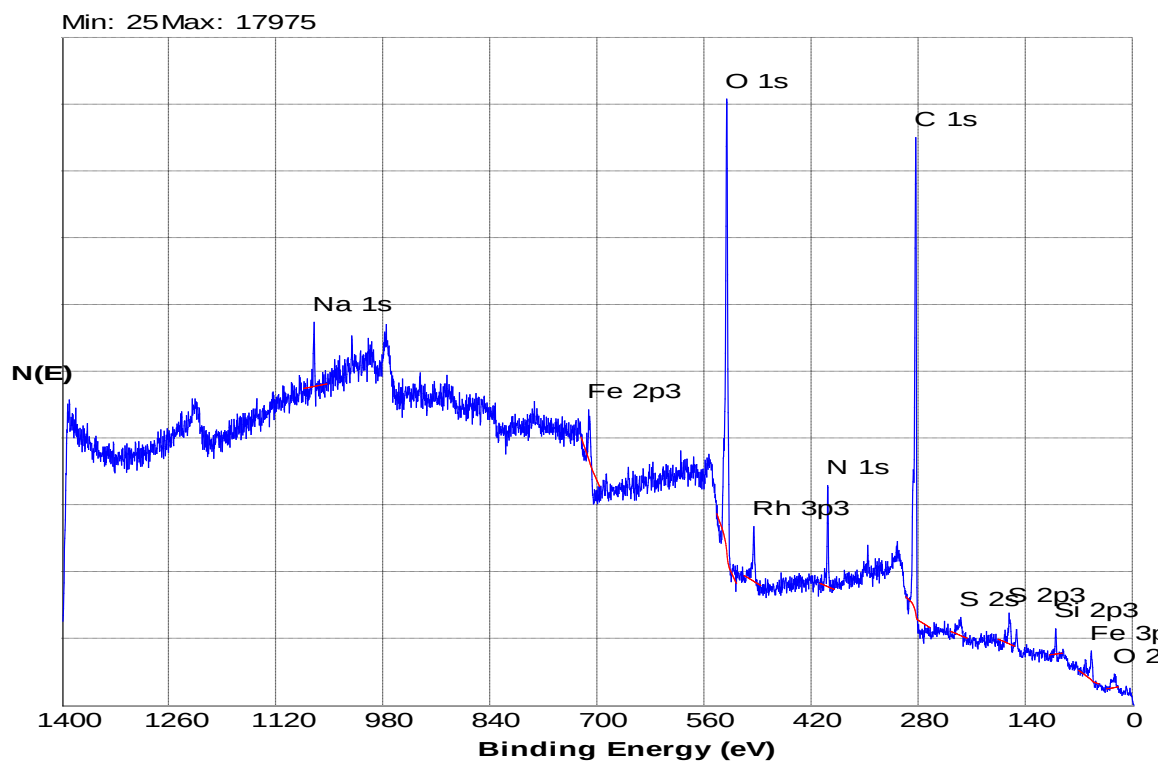


Figure 39. XPS survey scan of pyrrhotite after 7 days exposure to SRB. Peaks indicate sodium, iron, oxygen, rhodium, nitrogen, carbon, sulfur and silicon on the surface.

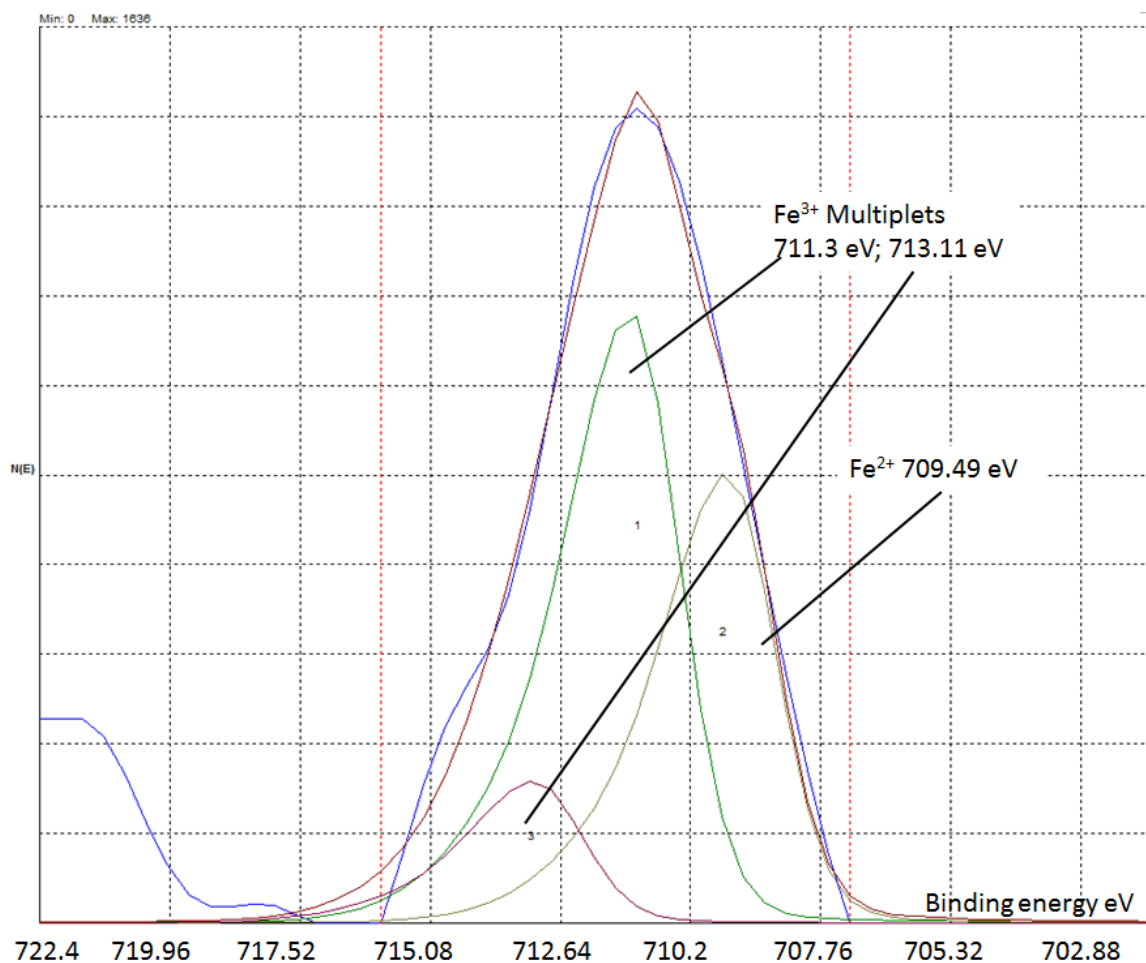


Figure 40. XPS spectrum representing iron on the surface of exposed pyrrhotite sample. Curve fitted data indicates presence of both Fe²⁺ and Fe³⁺. Blue line is XPS data; brown line is curve fitted data.

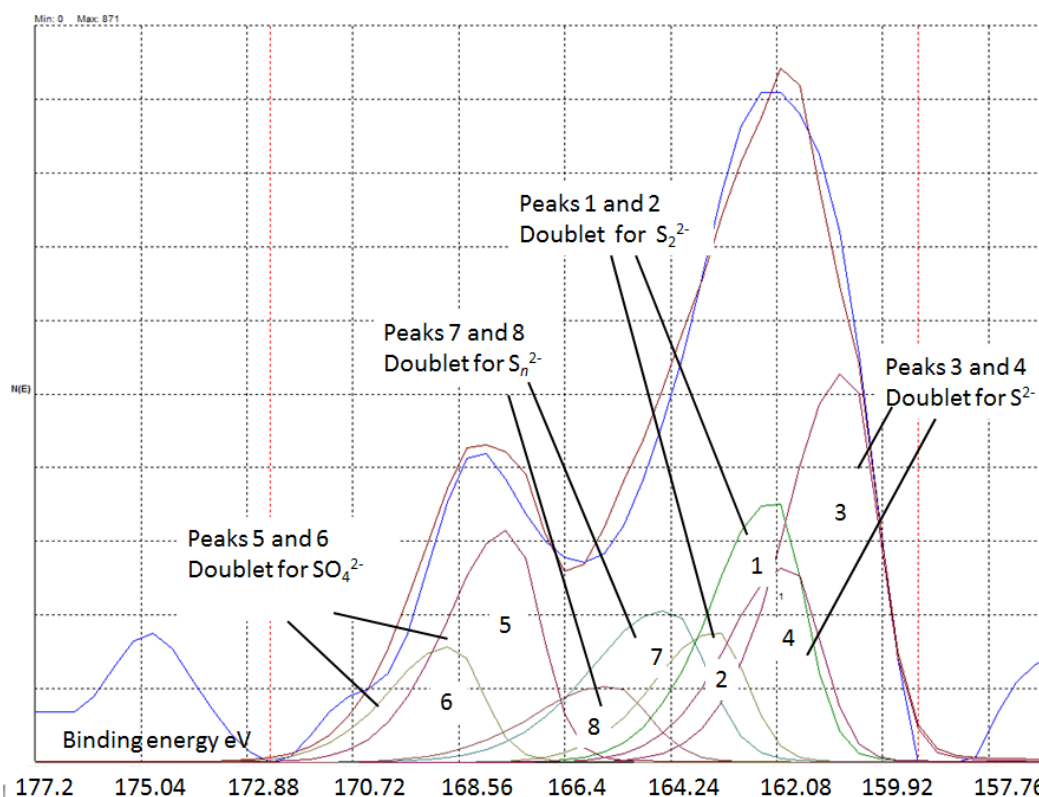


Figure 41. XPS spectrum for sulfur on the surface of pyrrhotite sample 1 exposed to SRB for 7 days. Signal was weak and distorted so curve fitted data did not exactly match, however four distinct oxidation states were observed. Probable chemistry of sulfur is indicated. Blue line is XPS data; Brown line is curve fitted data.

	Peak	area	position	intensity	FWHM	chemistry
	1	849	162.11	341	1.45	S_2^{2-}
fixed to 1	2	423	163.29	170	1.45	
	3	1269	160.61	510	1.45	S^{2-}
fixed to 3	4	634	161.79	255	1.45	
	5	751	167.51	302	1.45	SO_4^{2-}
fixed to 5	6	376	168.69	151	1.45	
	7	611	164.26	198	1.45	S_n^{2-}
fixed to 7	8	306	165.44	99	1.45	

Table 10. Values for curve fitted data from pyrrhotite sample 1 after 7 days exposure to SRB. Doublets for sulfur 2p3 and 2p1 peaks are represented by doublet with a fixed spacing of 1.18 eV and a 1:0.5 area ratio. Probable chemistry of sulfur is indicated for each doublet. Values are accurate within ± 0.4 eV.

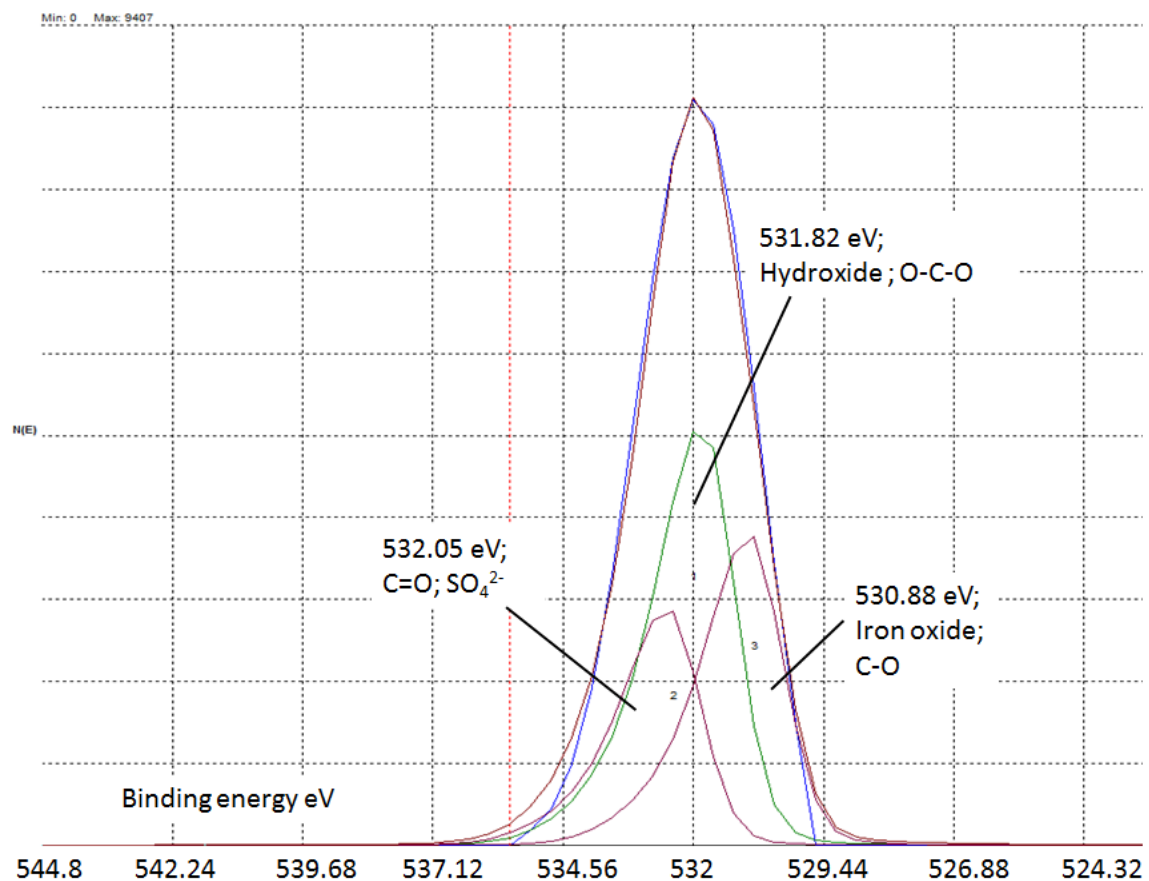


Figure 42. XPS spectrum representing oxygen on the surface of pyrrhotite sample exposed for to SRB for 7 days. Curve fitted data suggests presence of iron oxide/hydroxide, and carbon based metabolites. Blue line is XPS data; brown is curve fitted data.

Peak	area	position	intensity	FWHM	chemistry
1	11753	531.82	5336	1.5	OH-; O-C-O
2	6615	532.5	3002	1.5	SO ₄ ²⁻ , C=O
3	8666	530.88	3933	1.5	O=C; iron oxide

Table 11. Values for curve fitted data for oxygen peaks on pyrrhotite sample 1 exposed to SRB for 7 days. Probable chemistry is indicated for each peak. Values are accurate within ± 0.4 eV.

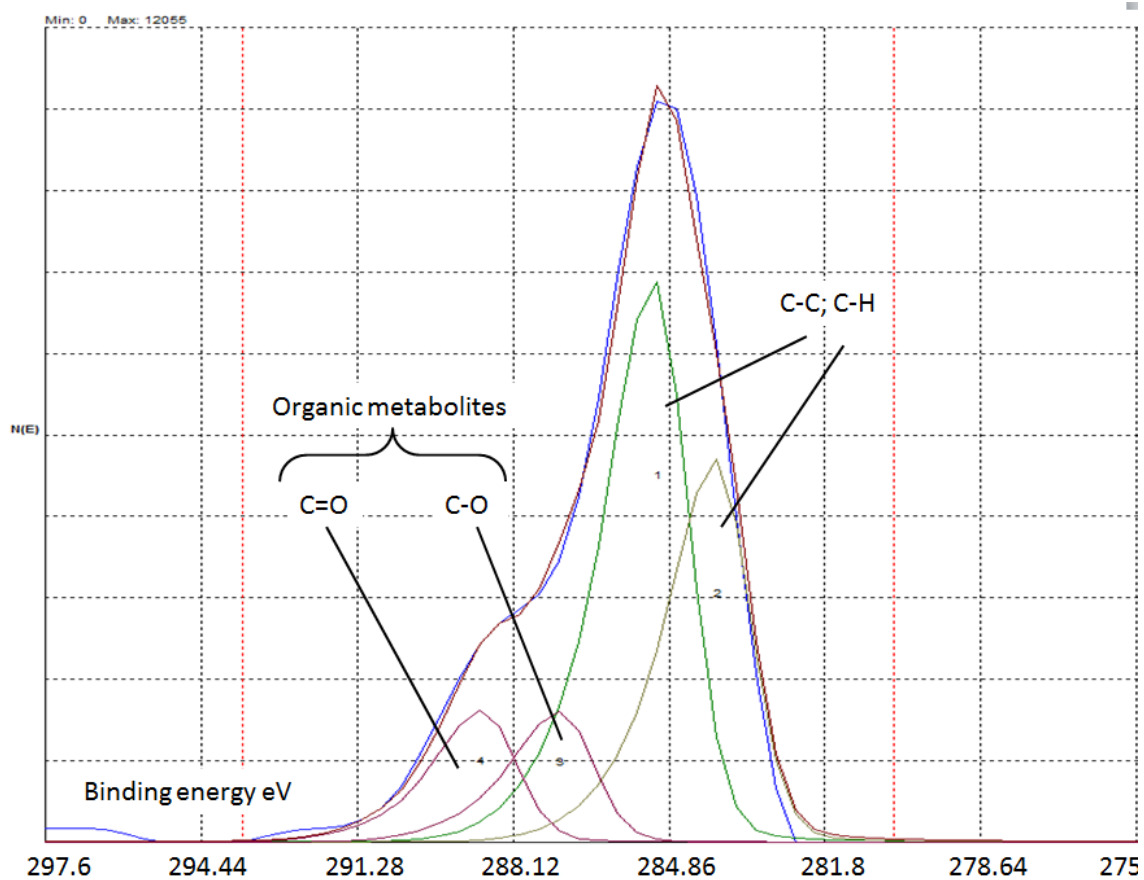


Figure 43. XPS spectrum for carbon on surface of pyrrhotite sample 1 after 7 days exposure to SRB. High-energy shoulder indicates presence of organic metabolites. Blue line is XPS data; brown is curve fitted. Values are accurate within ± 0.4 eV.

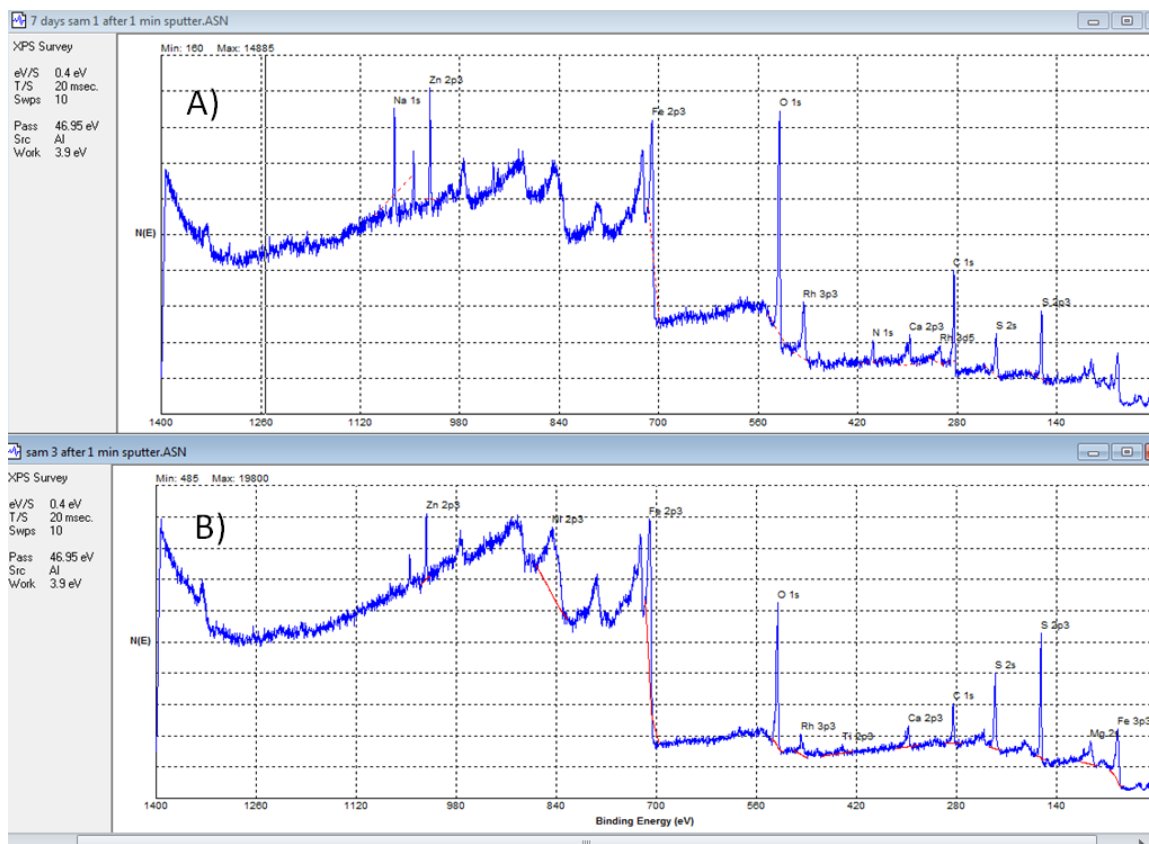


Figure 44. A) Survey scan of pyrrhotite sample 1 after exposure to SRB for 7 days with 1 nanometer of material removed. B) Survey scan of pyrrhotite sample 1 prior to exposure to SRB. The similarities between the two scans demonstrate that the substrate was reached in A) after removal of 1 nanometer. Values are accurate within ± 0.4 eV.

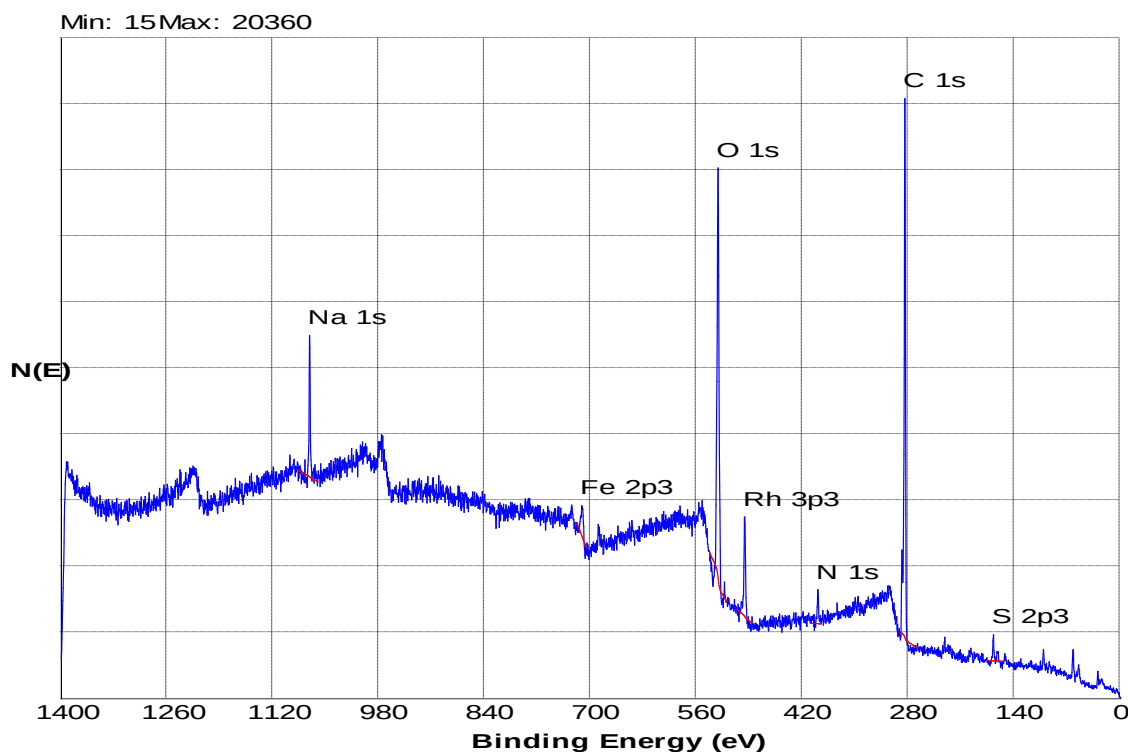


Figure 45. XPS survey scan of pyrrhotite sample 4, the control sample. Rhodium peak is most likely enlarged due to sodium auger electron signal.

Pyrrhotite Discussion

Natural pyrrhotite, like the sample used in this study, is complex. Often when iron sulfide is formed from biologic activity, it is amorphous and disordered (Bowles et al., 2011). Owing to the amount of impurities and complexity of these samples, it is difficult to quantify the results. From FE SEM images with EDS, it appears the SRB attached to the surface of the mineral samples and did create iron sulfide/oxide in the form of a disordered thin film. XPS analysis of the surface suggested iron sulfide and iron oxide precipitates as well. The high surface area to volume ratio of the thin film alteration products would suggest that these precipitates would be bioavailable. This would create a positive feedback mechanism in which pyrrhotite locally propagates more pyrrhotite as

long as there were areas of local oxidation to initiate SRB interactions. The presence of increased rhodium on the surface is interesting because it appears on both the experimental and the control samples. The control samples however did not have a significant iron and sulfur signal. The medium used in the control was filtered to remove cells but metabolites were present. The rhodium may have interacted with these metabolites to increase dissolution and reprecipitation. Sodium auger electrons are also observed at 493 eV and may account for enlargement of peak seen after exposure. Baseline signals indicated rhodium but did not have sodium present. More research is needed to confirm rhodium and SRB interaction on the surface of pyrrhotite.

Pyrite

Mineral Phase Identification with XRD

XRD analysis of pyrite sample confirmed mineral phase identification to be only pyrite. The sample was free of contamination (Figure 46).

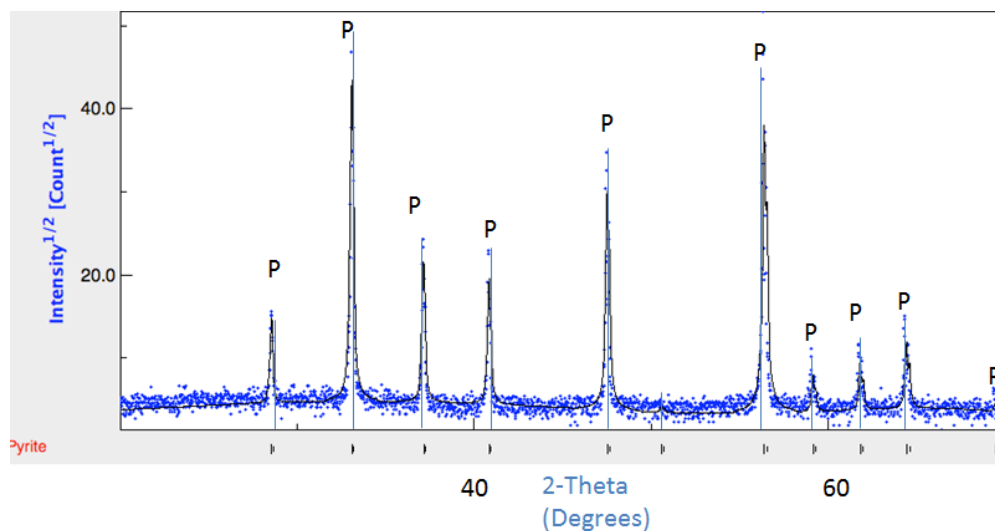


Figure 46. XRD analysis of pyrite sample. All peaks indicate pyrite.

Characterization of Pyrite Using SEM with EDS

SEM secondary electron (Figure 47) and backscatter images (Figure 48) confirmed a homogenous sample. EDS spectra indicated samples 1-12 were pure iron disulfide (Figure 49) and spectra taken after exposure to SRB confirmed that bulk analysis was pure iron disulfide after 7 days, 14 days and 30 days (Figure 50). There were also no unusual changes seen in the elemental mapping of the pyrite samples. These results were the same for all pyrite samples.

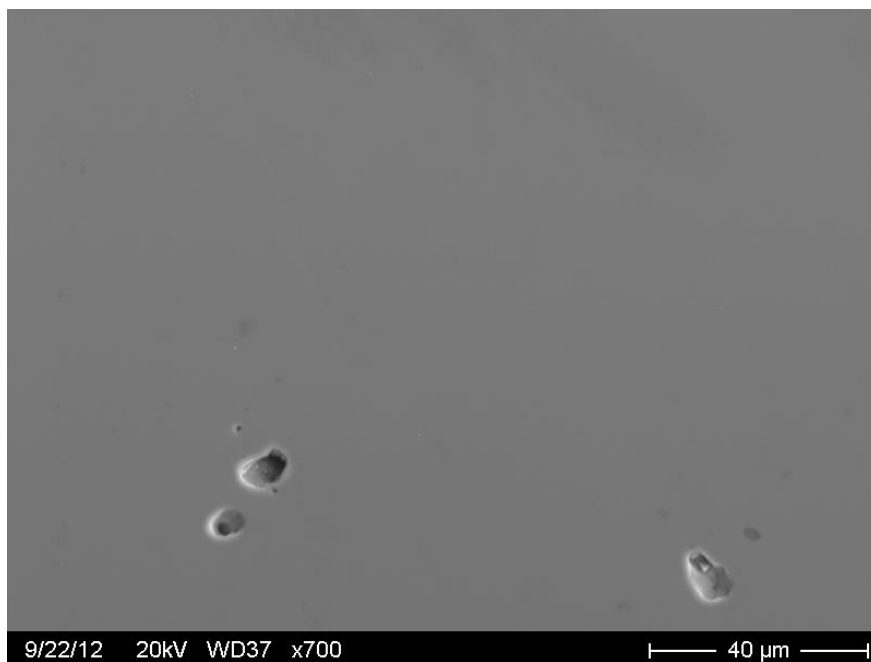


Figure 47. SEM secondary electron image of sample 1 showing small surface defects and homogeneous nature of sample.

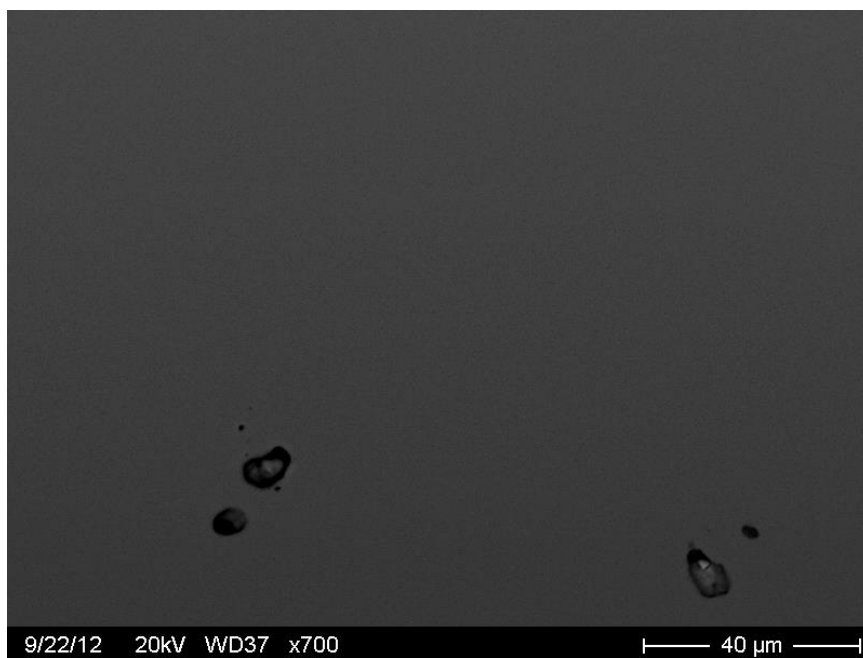


Figure 48. SEM backscatter electron image of sample 1 showing surface defects and homogenous nature of sample.

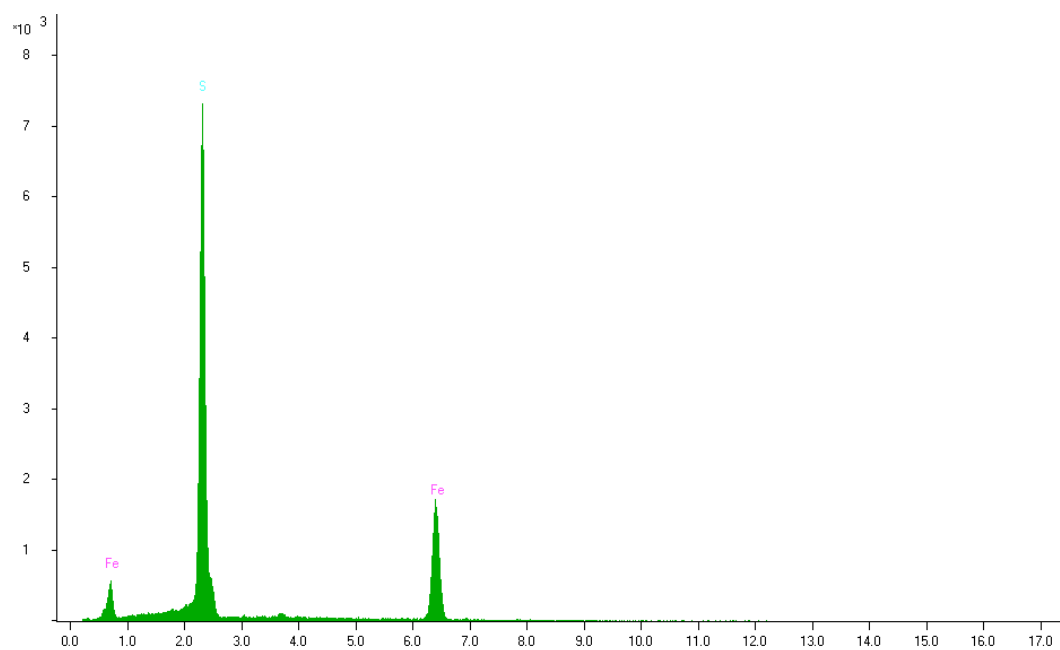


Figure 49. EDS of pyrite sample 1 at 7000x before exposure to SRB. Peaks show presence of sulfur and iron only.

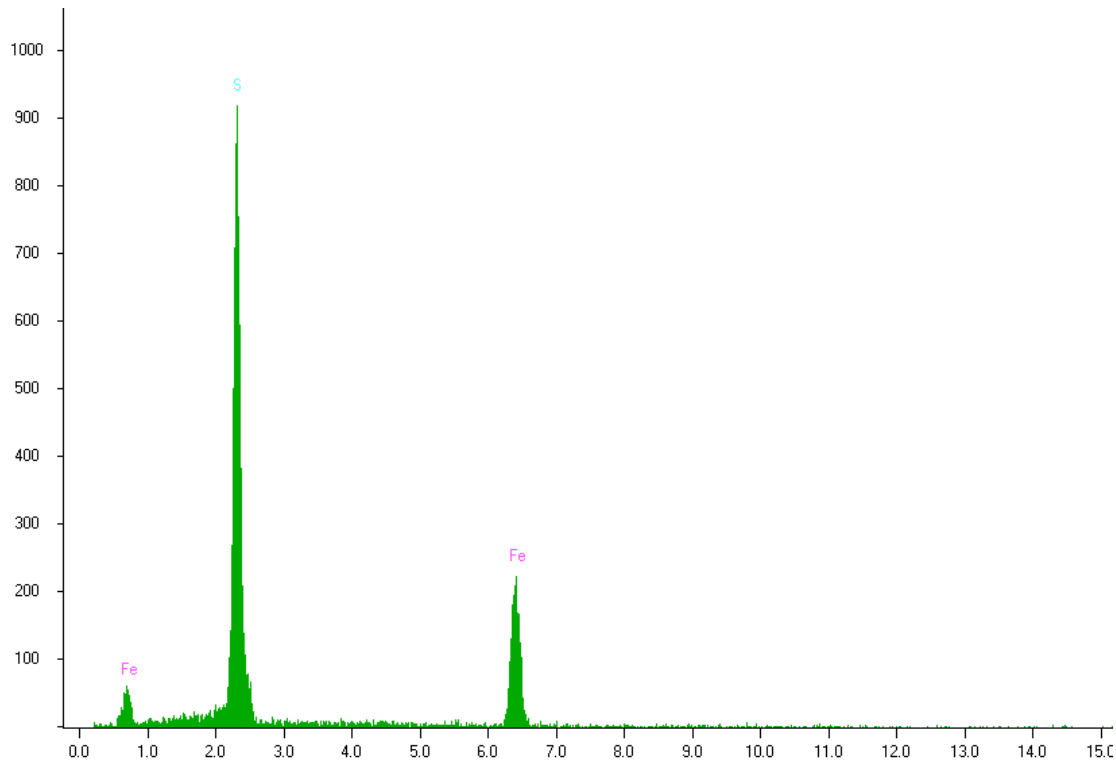


Figure 50. EDS spectrum of sample 1 at 700x after exposure to SRB for 7 days. Peaks show presence of sulfur and iron only showing no change in bulk composition after 7 days.

Characterization of Pyrite Using FE SEM with EDS

After 7 days exposure time to SRB, FEM images of pyrite showed significant cell attachment. Sample 2 had slipped out of silica string and fallen to the bottom of the vial while samples 1, 3, and 4 (the control) remained suspended in the medium. Images of cells on samples 1 and 3 showed curved rods with flagella (Figure 51) and sample 2 (Figure 52) showed mat-like structures of biofilms indicative of the local environment in which the samples had been. Possible precipitates were seen on the surface of the biofilms (Figure 52). The control sample was free of contamination (Figure 53).

The samples exposed to SRB for 14 days also had significant cells attachment.

Sample 5 (Figure 54) had fallen to the bottom of vial and experienced similar results to sample 2. Secondary electron (Figure 55), backscattered electron (Figure 56), and InLens images (Figure 57) were obtained for cells and precipitate on sample 5. The backscatter electron image has a slight variation in tint that reflects the morphological differences in the overlaying precipitate and there is a lack of defining phase discrimination (Figure 58).

Samples exposed for 30 days did not show significant cell attachment in any of the triplicate samples. The bacteria may have perished. This result is strange considering all the samples were inoculated at the same time with the same cultures under the same experimental parameters.

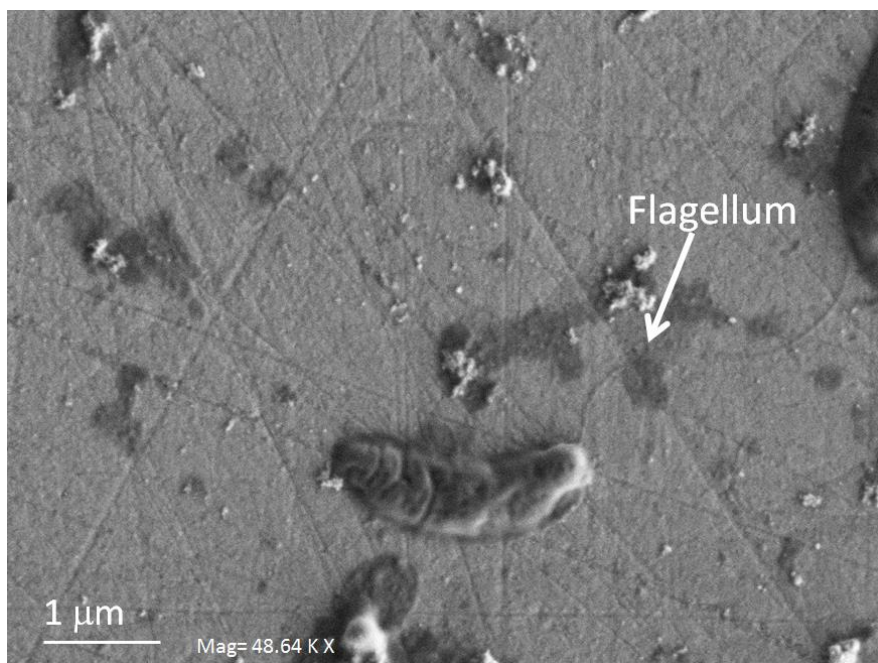


Figure 51. FE SEM secondary electron image of sample 1 showing *D. indonensis* cell with flagellum. This sample was suspended in the media.

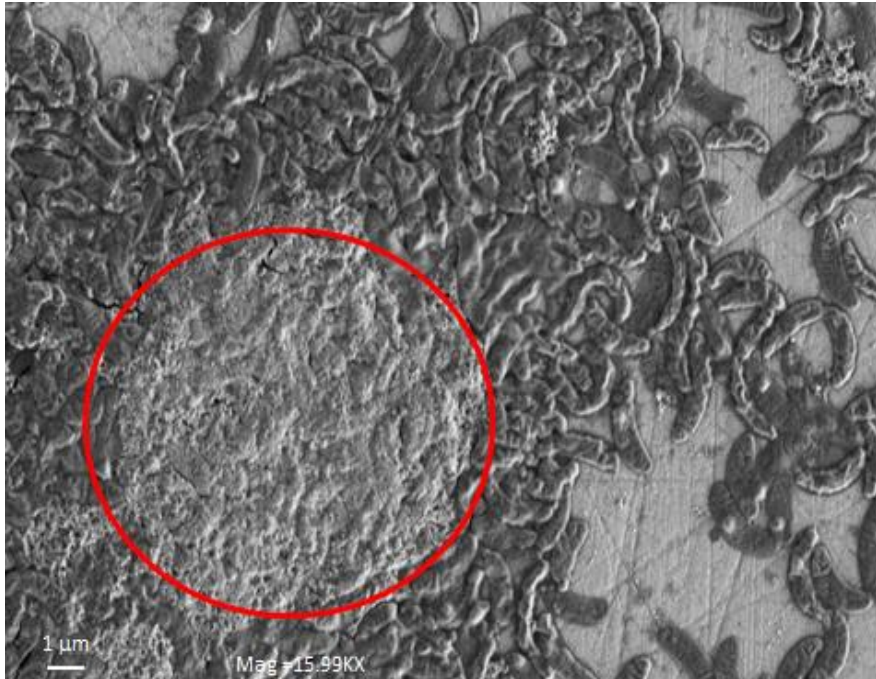


Figure 52. FE SEM secondary electron image of sample 2 showing mat-like biofilm structures. Red circle indicates possible precipitate.

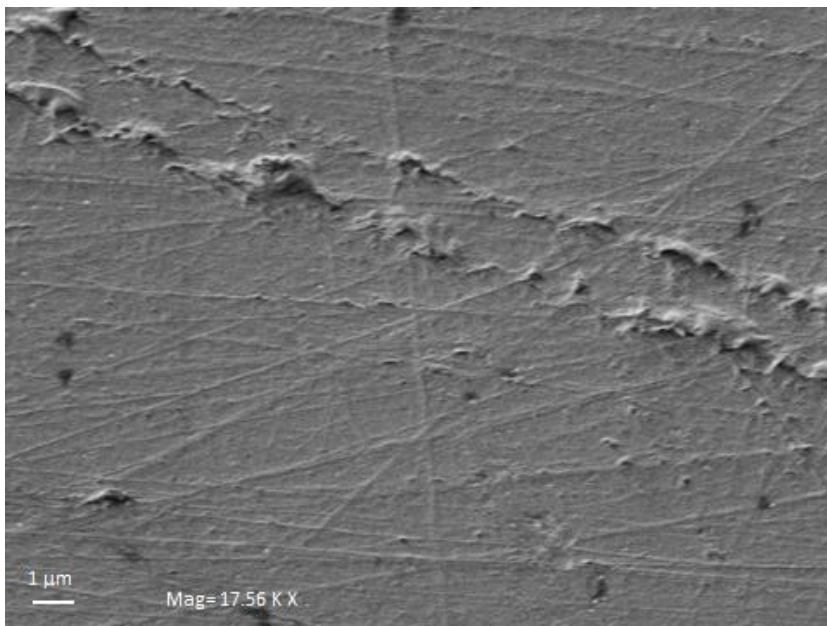


Figure 53. FE SEM secondary electron image of the control sample 4. Image shows no indication of contamination.

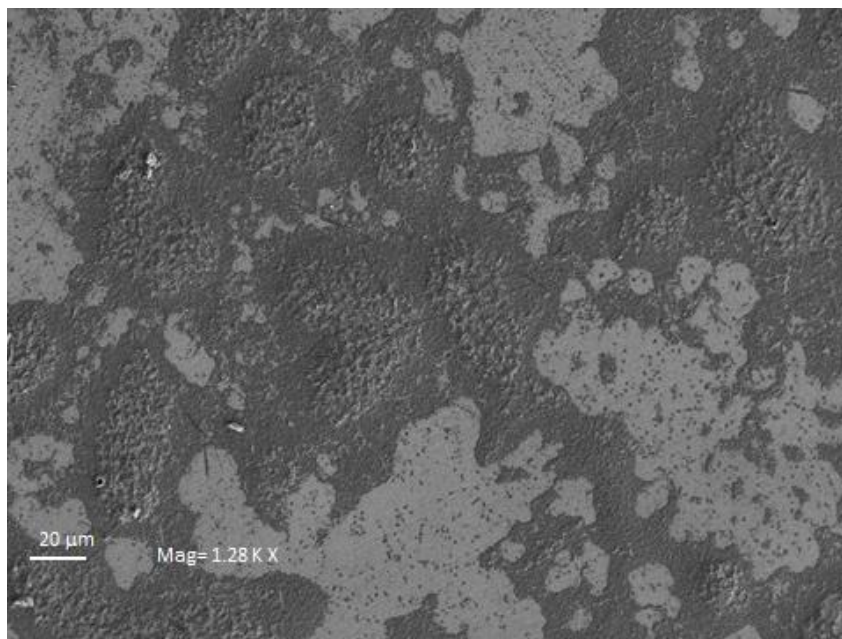


Figure 54. FE SEM secondary electron image of cells on sample 5 after 14 days exposure. The sample had fallen to the bottom of container and indicated significant cell attachment.

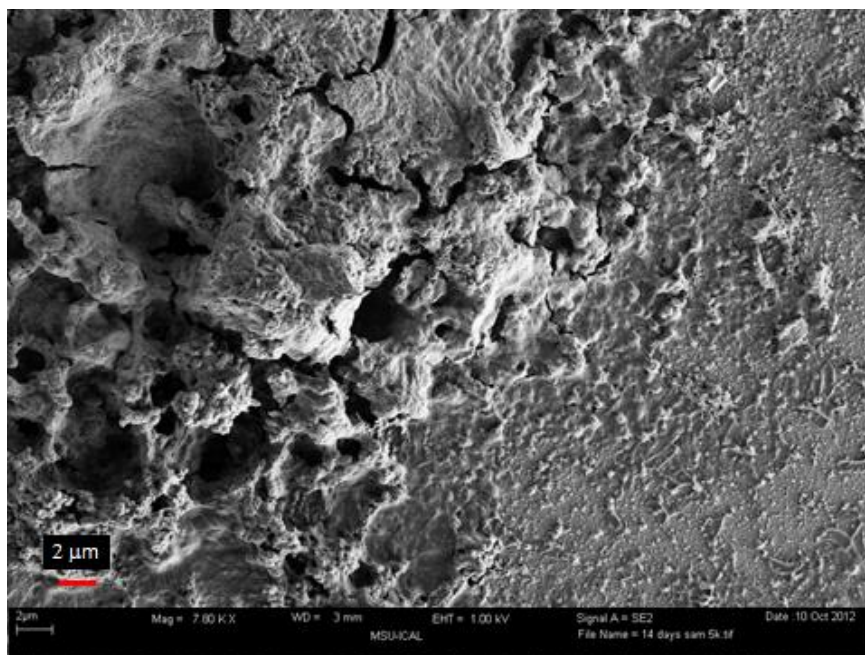


Figure 55 . FE SEM secondary electron image of sample 5 at 7.80 K x magnification. Precipitate on left side of image. EDS spectrum of the precipitate shown in Figure 58. Bacterial cells are on right side of image. Cells become apparent with InLens detector (Figure 57).

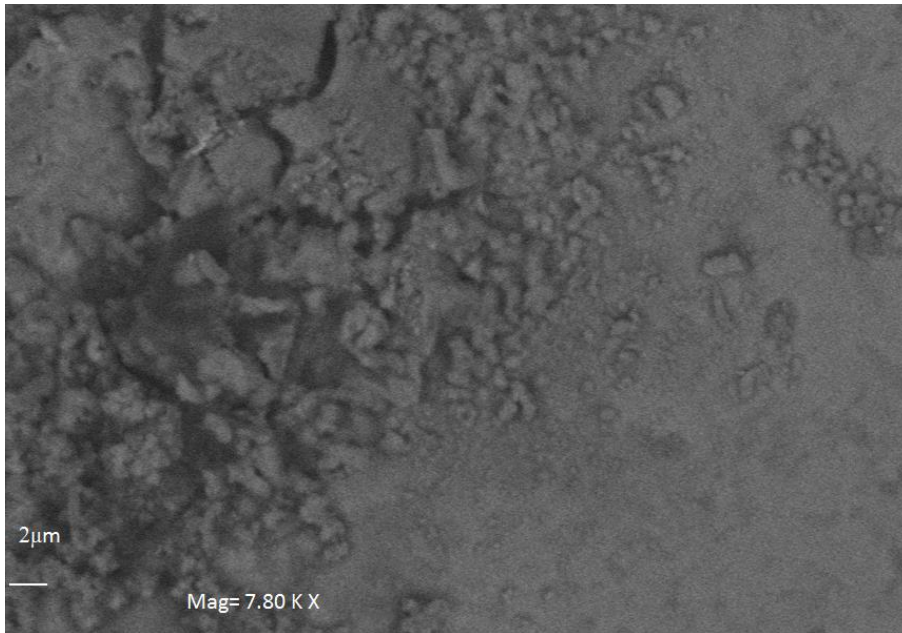


Figure 56. FE SEM backscatter electron image of sample 5 at same location of images in Figures 55 and 57. The lack of defining phase discrimination implies the variation in tint is most likely due to morphological features as opposed to differences in phases.

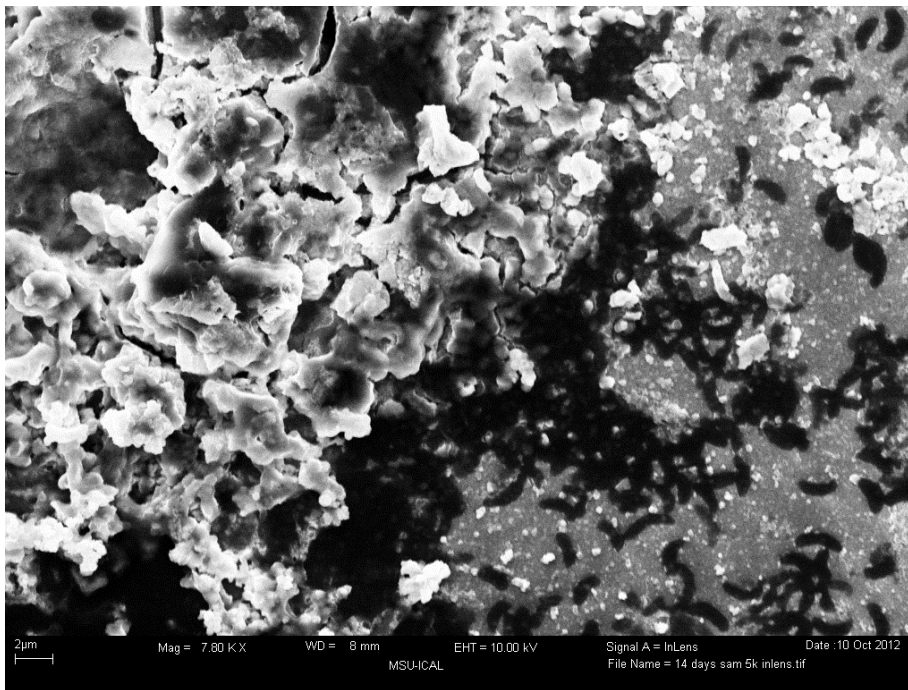


Figure 57. FE SEM InLens image of sample 5 at same location of images in Figures 55 and 56. Precipitate is on left side of image. Dark curved rods are bacteria cells on right side of image. EDS spectrum of precipitate is shown in Figure 58.

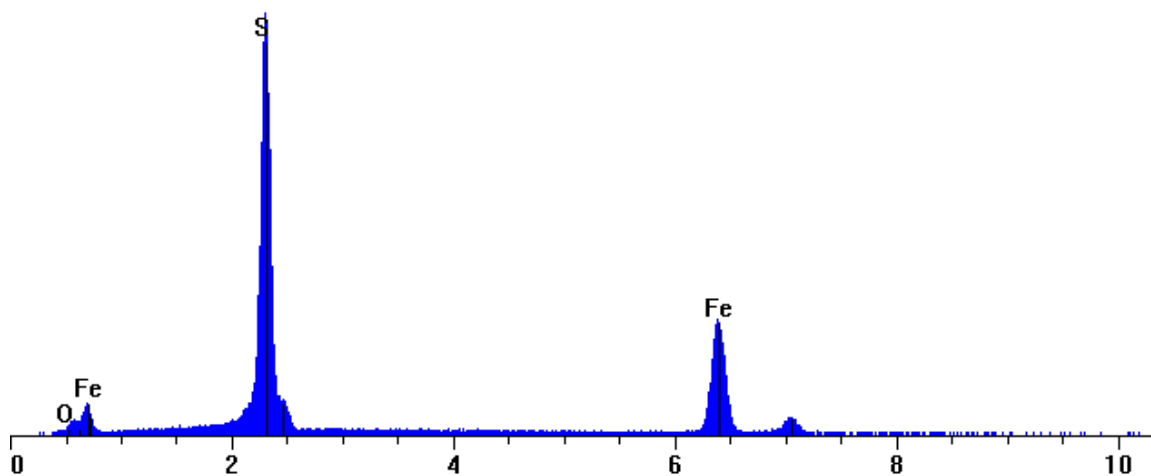


Figure 58. FE SEM EDS spectra of participate see in in Figures 48-50. Relative peak heights suggest iron disulfide pyrite with a small amount of oxide.

Characterization of Pyrite Surface with XPS

Before Exposure to SRB – a Baseline Signal for Pyrite. Pyrite surfaces before exposure to SRB were expected to have the presence of both Fe^{2+} and Fe^{3+} as previously discussed. XPS analysis provided evidence of two iron oxidation states seen by the high- energy tail (Figure 59) (Table 12). Based on the area of curves fit to the data, Fe^{3+} accounts for ~17% of the total iron signal. Pyrite's baseline signal indicated the presence of sulfur with oxidation state of -1 and -2 corresponding to the iron sulfur bonds previously described (Figure 60)(Table 13). Sulfur with oxidation state of -2 accounted for ~ 21% of the signal. Carbon peak was 284.42 and all peaks were shifted accordingly to compensate for charging.

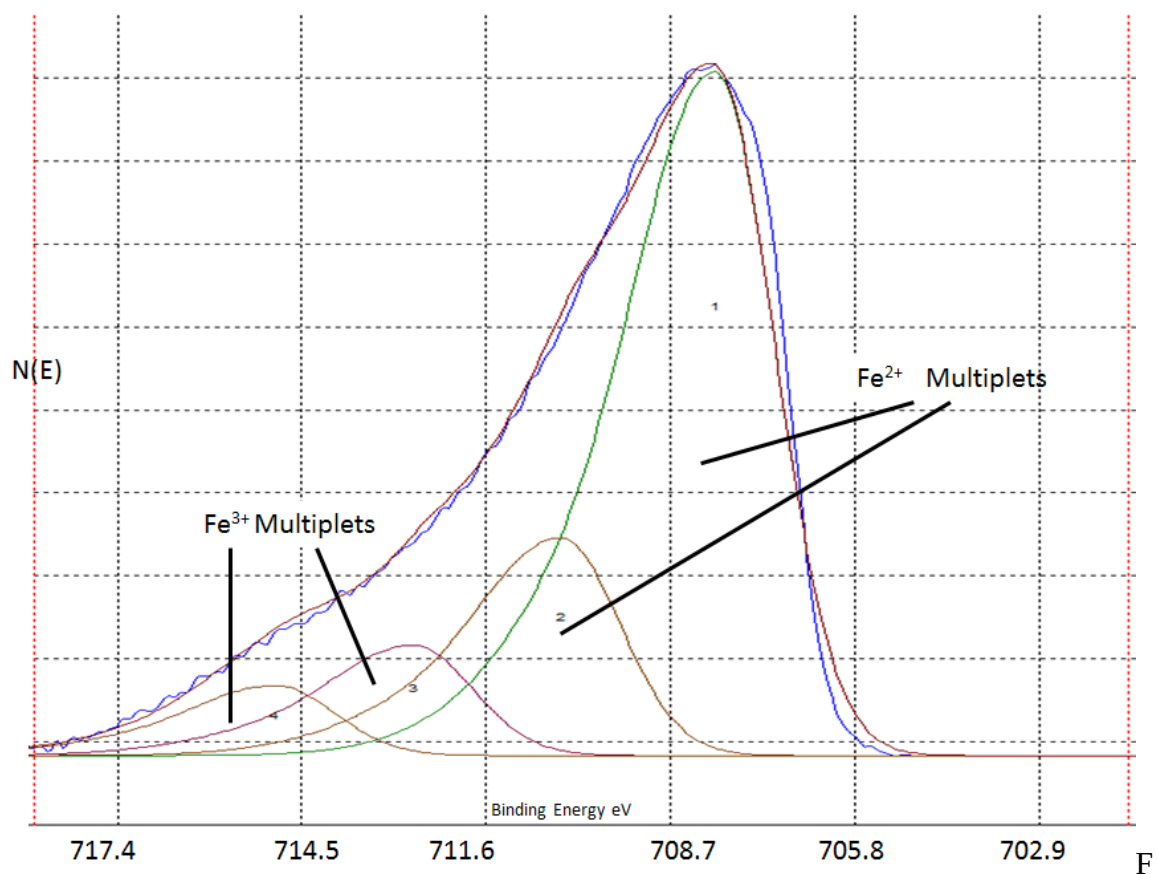


Figure 59. XPS spectra of polished pyrite sample 1 before exposure to microbes. Blue line is XPS data, brown line is curve fitted data.

peak	area	position	Intensity	FWHM
1	19657	708	6060	2.2
2	6257	710.43	1929	2.2
3	3169	712.75	977	2.2
4	2008	714.92	619	2.2

Table 12. Values of curve fitted data for Fe peak before exposure to microbes (Figure 59). Peaks 1 and 2 represent Fe^{2+} . Peaks 3 and 4 represent Fe^{3+} that was a result from the breaking of the S-S bond and the autooxidation of Fe^{2+} .

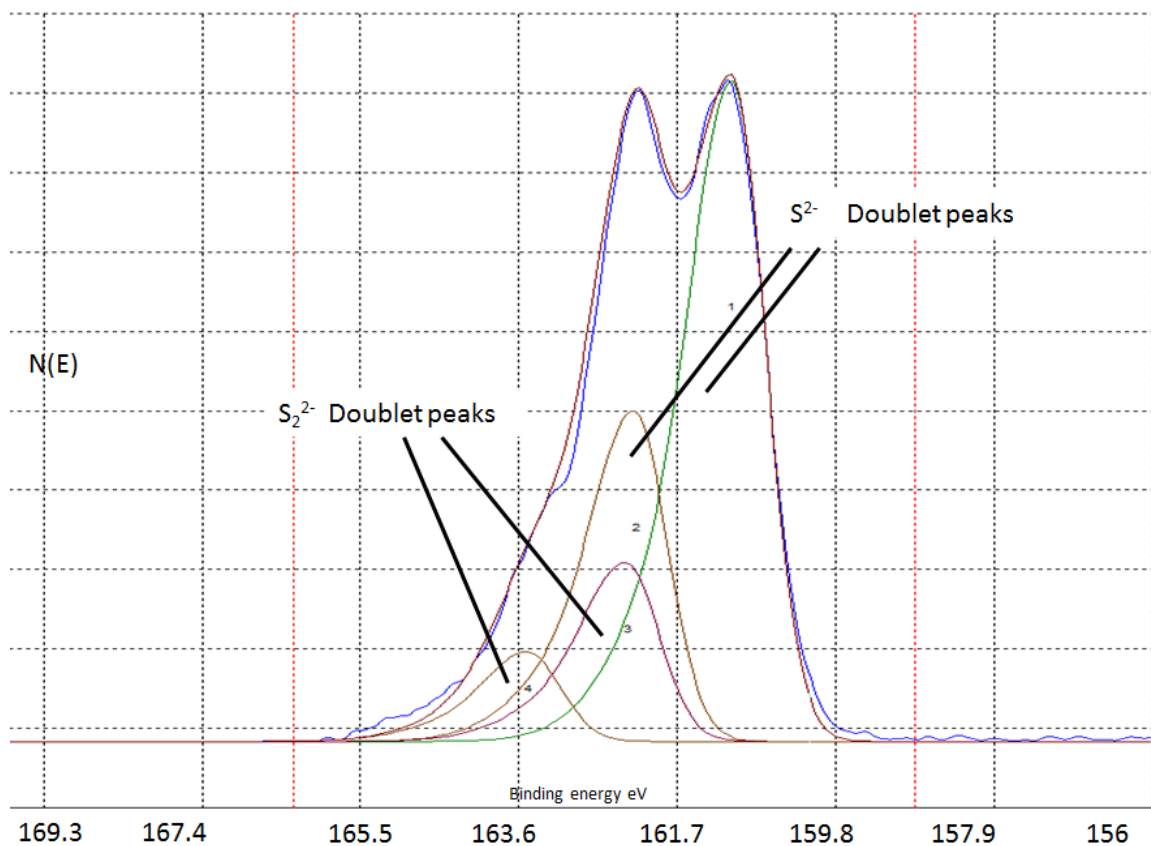


Figure 60. XPS spectra of sulfur on the surface of pyrite sample 1 before exposure to SRB. Blue line is XPS data and brown line is curve fitted data.

Peak	area	position	intensity	FWHM
1	7276	161.04	5459	0.94
2	3637	162.22	2729	0.94
3	1970	162.32	1478	0.94
4	985	163.5	739	0.94

Table 13. Values of curve fitted data for sulfur peak from pyrite sample 1 (Figure 60). Peaks 1 and 2 represent the doublet for S^{2-} with and oxidation state of -2. Peaks 3 and 4 represent the doublet for S_2^{2-} with an oxidation state of -1.

After Exposure to SRB. A depth profile was obtained for elements carbon, oxygen, sulfur and iron (Figure 61). After each cycle, one minute of sputtering removed a nanometer of material. Figure 61 shows carbon and oxygen decreased with depth indicating removal of the organic layer while iron and sulfur signals increased with depth.

The carbon peak showed high binding energy peaks representing organic metabolites on the surface (Figure 62). The metabolites did not appear in the spectra after the first two cycles indicating their thickness to be less than 2 nanometers (Figure 63). The carbon peak was 284.93 and all peaks were adjusted to compensate for charging.

The depth profile also revealed a significant change in the iron signal after the first two cycles (Figure 64). Once the top 2 nanometers were removed, the baseline pyrite peaks were seen in cycles 1, 2 and 3 (Figure 64). This progression seems to provide evidence that the initial iron peaks represent the precipitate overlaying the surface as seen in Figures 52, 55, 56 and 57. Iron peaks from depth profile cycle -1 show two distinct iron phases (Figure 65)(Table 14). This was the only signal in which curve fitted parameters were not set equal to one another because the shape of the curves indicate two separate iron signals associated with two different phases of minerals (Table 14). The other elements present in the XPS scan provide evidence of the types of iron mineral precipitates that occurred on the surface.

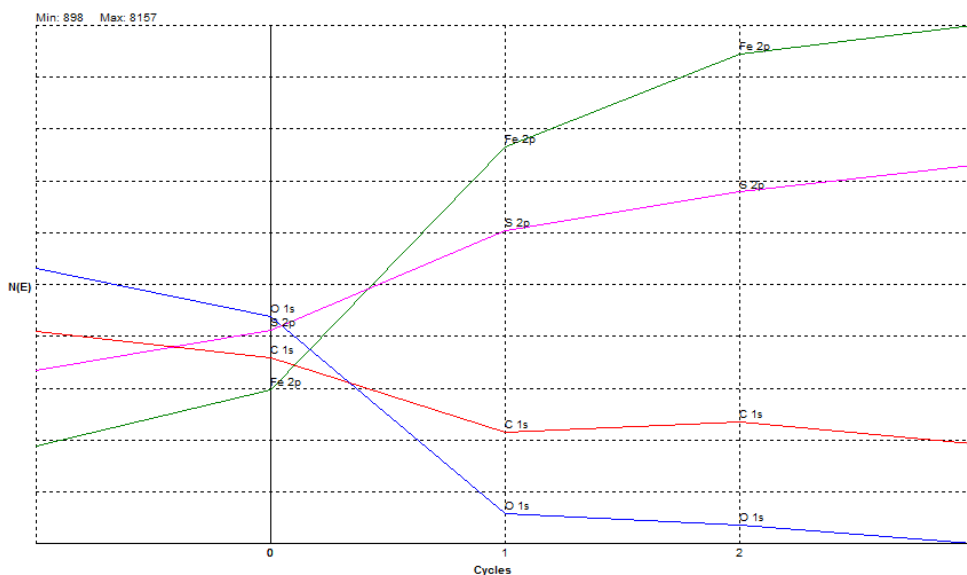


Figure 61. Depth profile for pyrite sample 2 after 7 days of exposure to SRB.

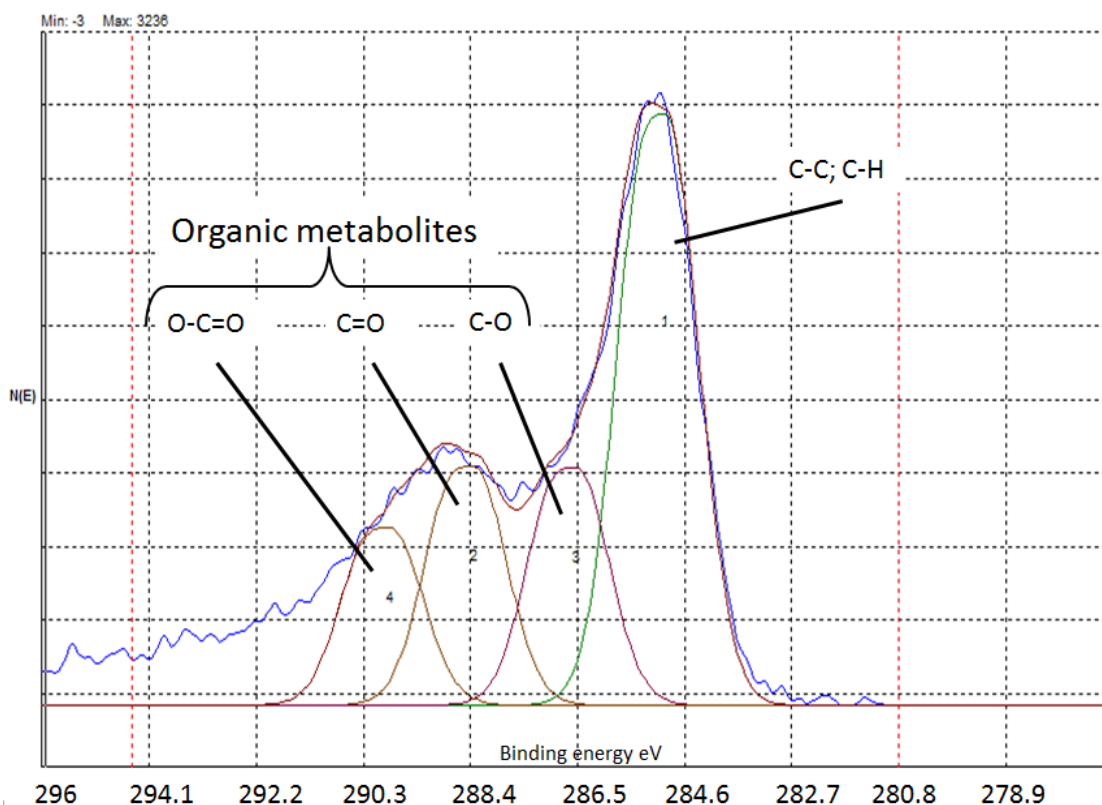


Figure 62. XPS data from depth profile cycle -1 for carbon on pyrite sample 2 after 7 days of exposure to SRB. High-energy peaks represent bacterial metabolites. Blue line is XPS data; brown is curve fitted data.

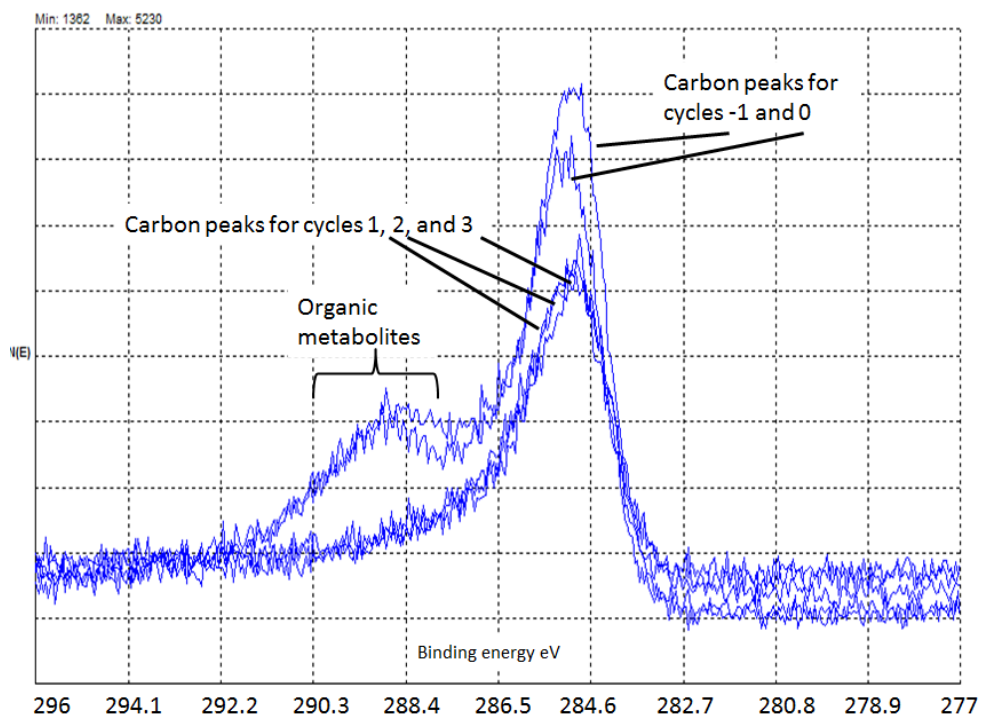


Figure 63. Carbon peaks for all cycles of depth profile for pyrite sample 2 decreasing with depth. Peaks for cycles 1,2 and 3 do not show organic metabolite shoulder.

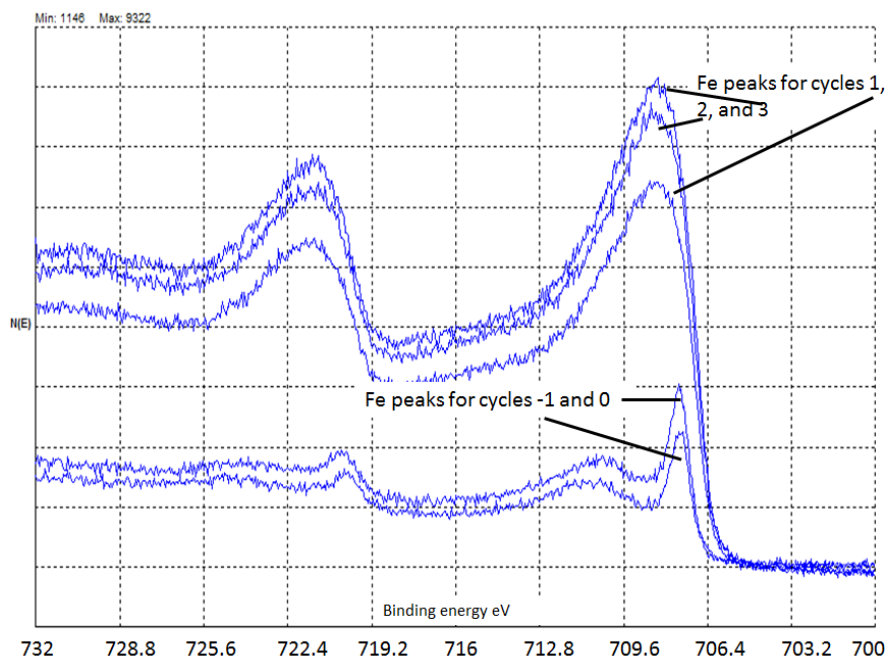


Figure 64. Fe peaks for all cycles of depth profile for pyrite sample 2 increasing with depth. Peaks from cycles 1, 2, and 3 represent the baseline signal of pyrite while the peaks from cycles -1 and 0 represent precipitate on surface.

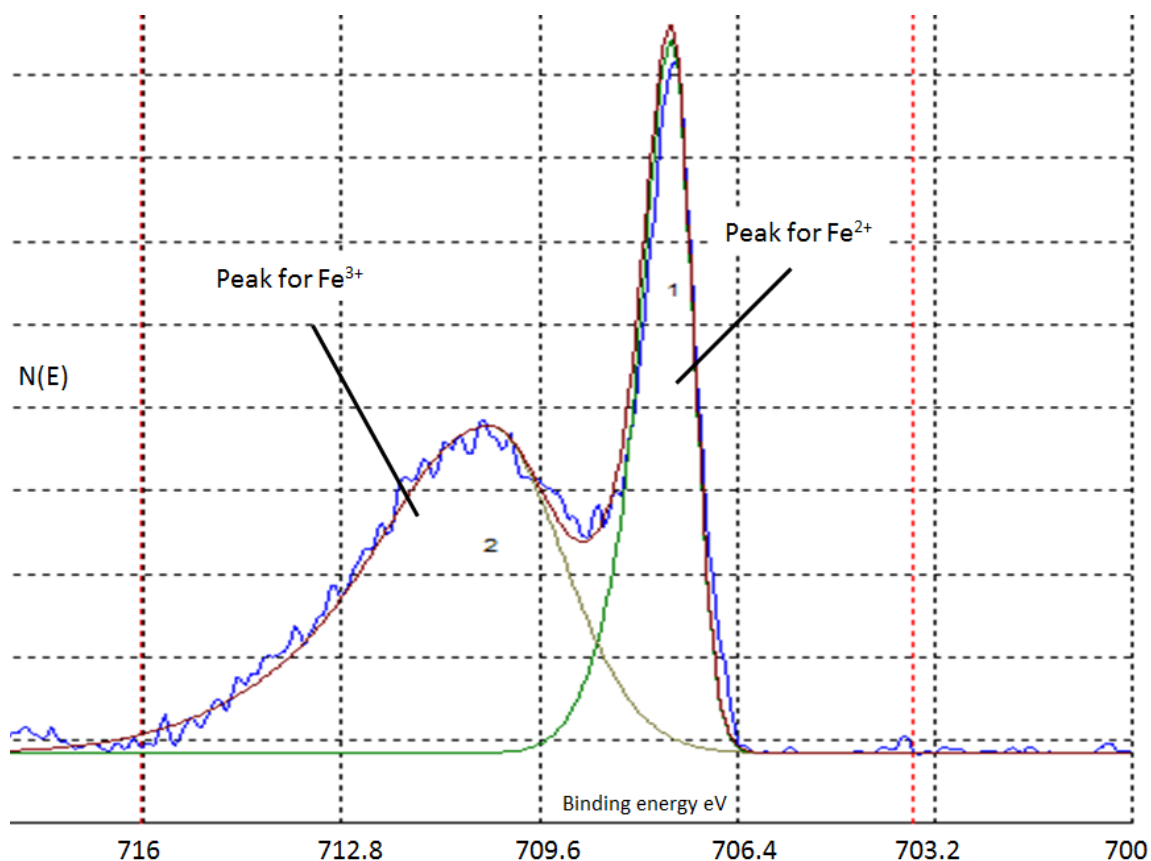


Figure 65. Iron peaks from depth profile cycle -1.

Peak	area	position	intensity	FWHM	Chemistry
1	3143	707.44	2548	0.87	Fe(II)
2	5219	710.17	1199	3.07	Fe(III)

Table 14. Values of curve fitted data for iron peak from depth profile cycle -1 (Figure 65). FWHM and other line shape parameters were not set equal due to the two distinct peaks seen in the spectra revealing two different phases of iron minerals.

The depth profile for pyrite sample 2 shows the sulfur peak significantly change after two sputtering cycles. The sulfur peaks from cycles -1 and 0 suggest an overlaying phase separate from the bulk pyrite signal that appears in cycles 1-3 (Figure 66). The curve fitted data for cycle -1 indicates S_2^{2-} with an oxidation state of -1 and is the only species present on the outer most surface layer (Figure 67) (Table 15). Fe^{2+} is most likely

bonded to the disulfide indicating one of the precipitates is FeS_2 . It is important to note that the iron disulfide is a layer of thin film. This type of precipitate is often small grained and disordered. These characteristics can increase reactivity due to large surface area to volume ratio.

The depth profile for oxygen is similar to the carbon profile. The signal decreases and changes significantly after the first two cycles (Figure 68). Some of the curve fitted peaks for cycle -1 are part of the organic metabolites. The larger peak at 532.06 could indicate hydroxide or O-C bonds and the peak at 530.02 may represent iron oxides or O=C bonds (Figure 69) (Table 16). This provides evidence that the Fe^{3+} present on the surface is possibly goethite or hematite precipitate.

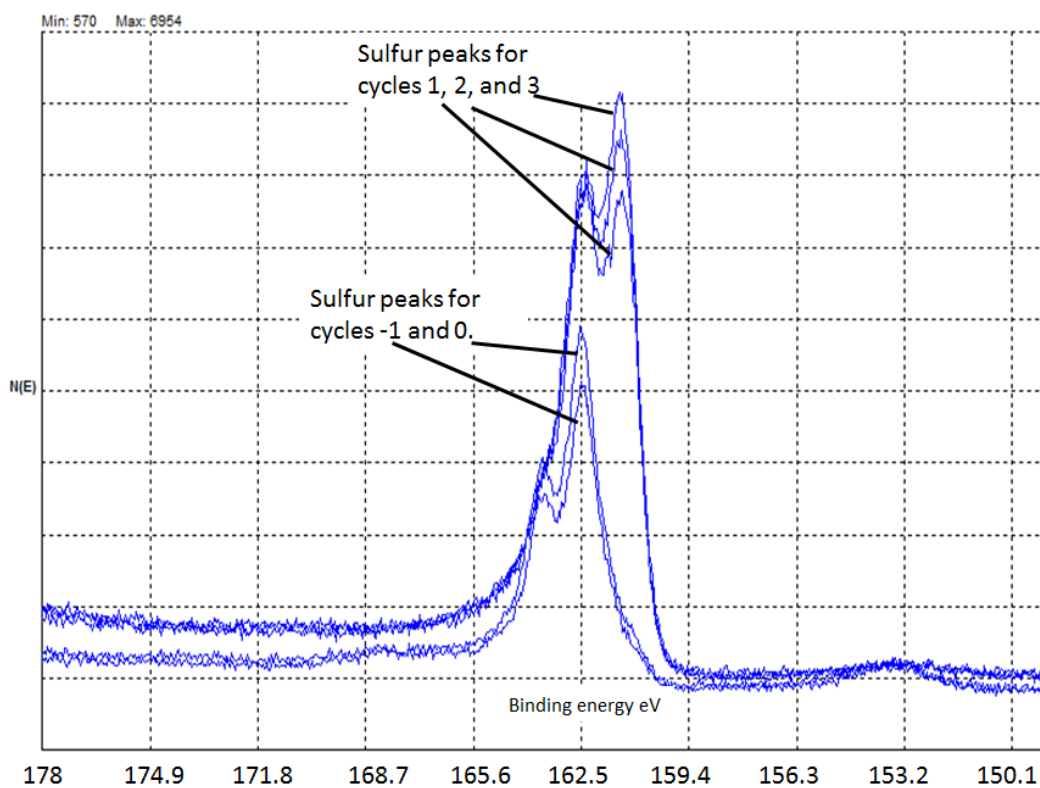


Figure 66. Sulfur peaks for all cycles of depth profile for pyrite sample 2.

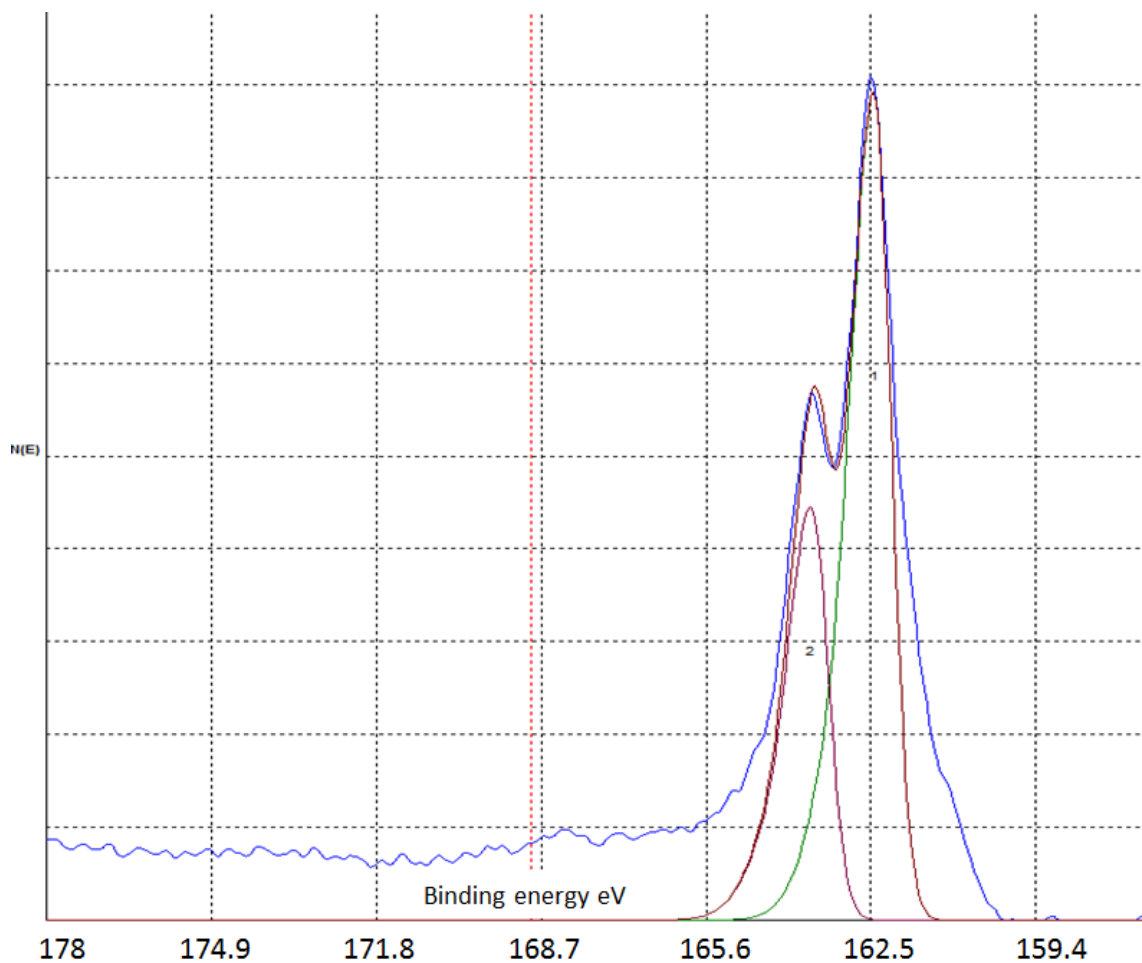


Figure 67. Sulfur peak in cycle -1 from depth profile for pyrite sample 2. Blue line is XPS data; brown is curve fitted data.

Peak	area	position	intensity	FWHM
1	3956	162.45	3671	0.76
2	1978	163.63	1836	0.76

Table 15. Values of curve fitted data for sulfur peak in cycle -1 from depth profile for sample 2. The doublet reveals only one oxidation state of -1 indicating presence of disulfide.

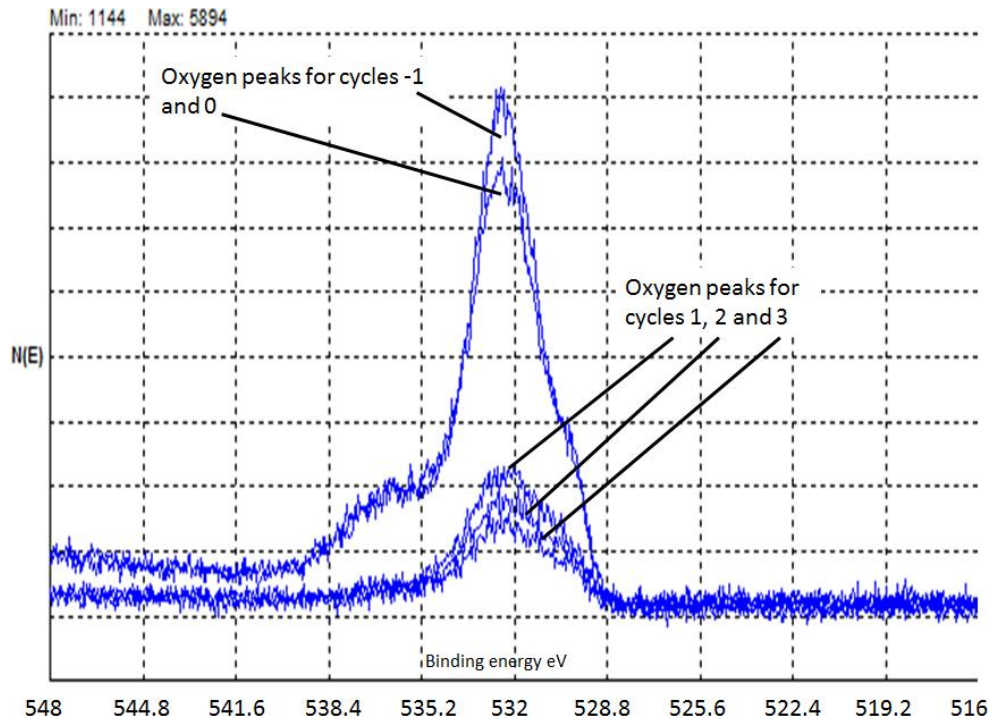


Figure 68. Oxygen peaks for all cycles of depth profile from pyrite sample 2. Peaks show a decreasing and changing signal.

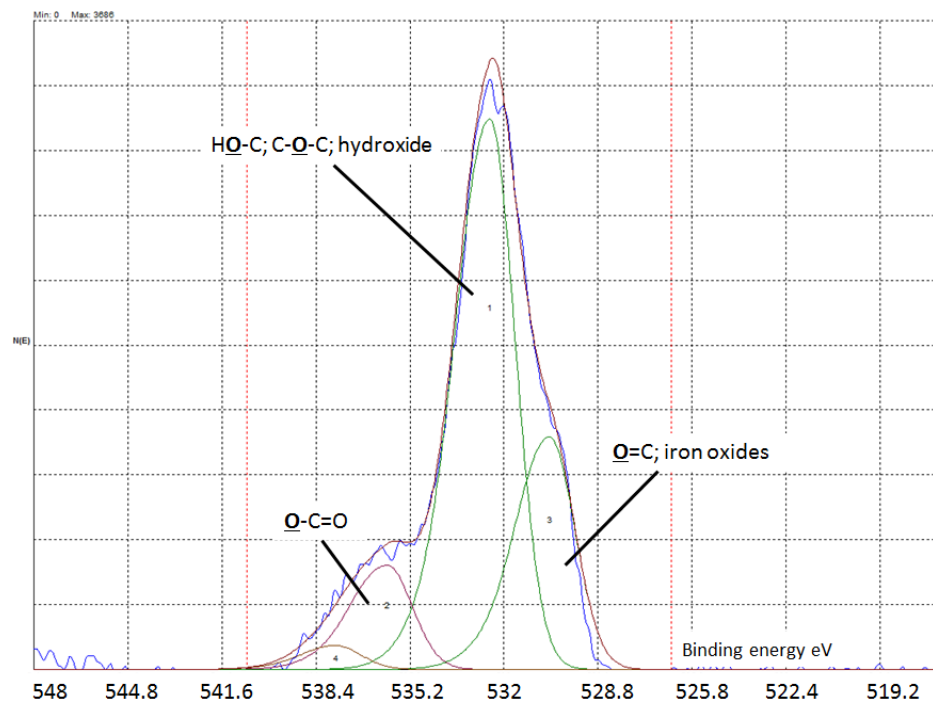


Figure 69. Oxygen peaks for cycle -1 in the depth profile for pyrite sample 2. Oxygen represented in peak is bold and underlined.

Peak	area	position	intensity	FWHM
1	9052	532.06	3441	2.03
2	1774	535.55	651	2.03
3	3825	530.02	1454	2.03
4	392	537.31	149	2.03

Table 16. Values of curve fitted data for oxygen peaks from cycle -1 in the depth profile for pyrite sample 2 (Figure 69).

The initial survey scan of the control sample after 7 days showed no iron peak and one minute of argon sputtering was performed (Figure 70). After removal of one nanometer from the surface, a depth profile for the control sample demonstrated little change in composition with depth (Figure 71). These results were analogous to the goethite samples that had less than one nanometer of organic on the surface with no other reactions.

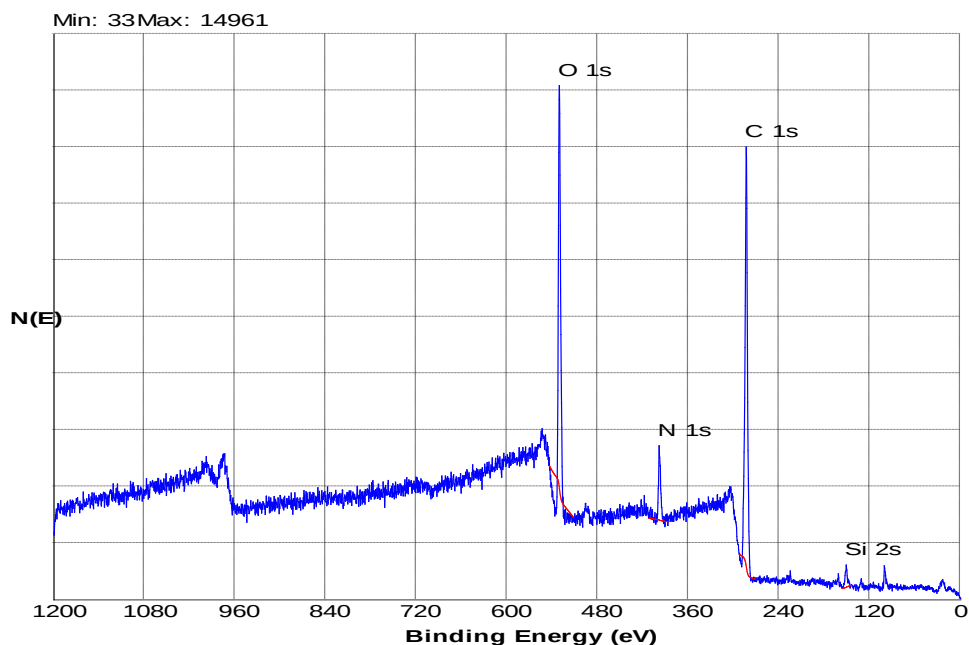


Figure 70. Survey scan of control sample 4. No iron peak was seen on initial scan.

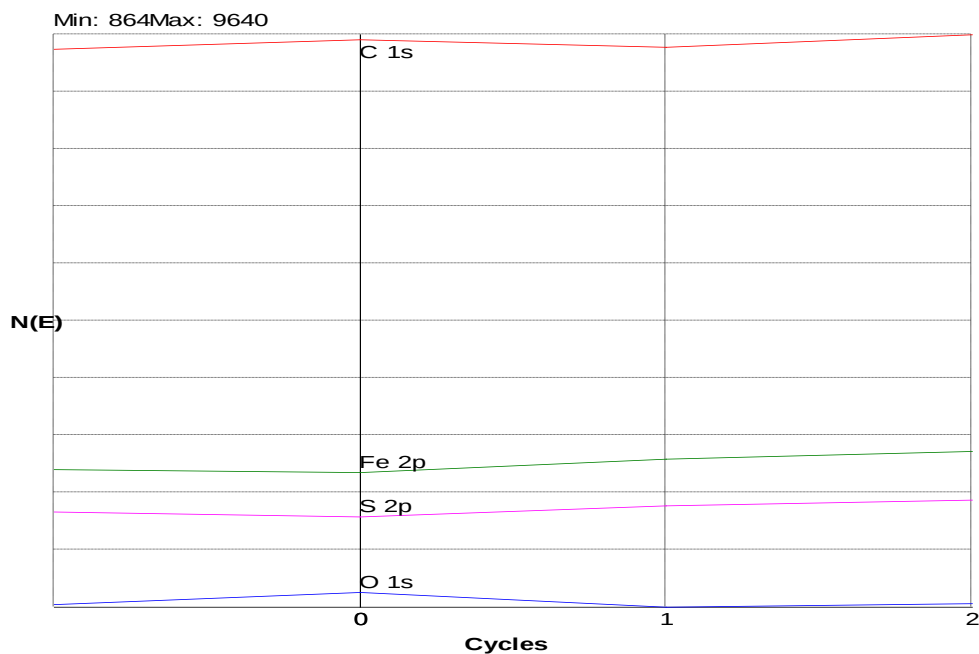


Figure 71. Depth profile of control sample 4 after 7 days exposure to SRB and after removal of 1 nanometer layer. No significant changes were seen in any of the signals.

The sample exposed for 14 days showed very similar results to the samples exposed for 7 days. The depth profile indicated increasing iron and sulfur peaks with depth and decreasing oxygen and carbon peaks with depth (Figure 72). Carbon peaks suggest the presence of 2 nanometers of organic material (Figure 73). Depth profile for iron (Figure 74) and depth profile for sulfur (Figure 75) both indicated the same results as sample 2 exposed for 7 days. Identical to the results for sample 2, the precipitate had Fe^{2+} and Fe^{3+} , S_2^{2-} , and oxygen. Possible mineral phases for precipitate were FeS_2 , $\text{FeO}\cdot\text{OH}$ and/or Fe_2O_3 .

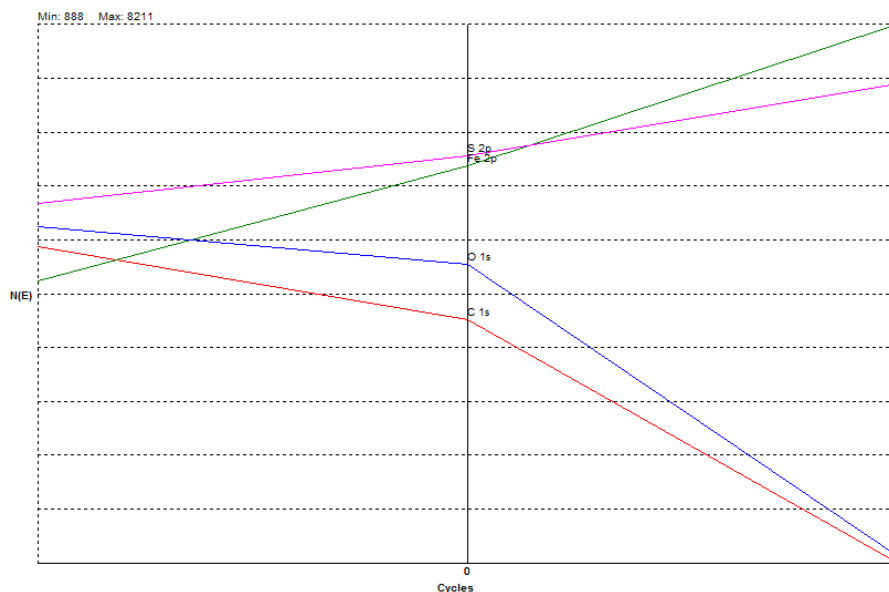


Figure 72. Depth profile for sample 5 after 14 days exposure to SRB. The signals show an increasing trend for iron and sulfur and a decreasing trend for oxygen and carbon similar to results for sample 2 exposed for 7 days.

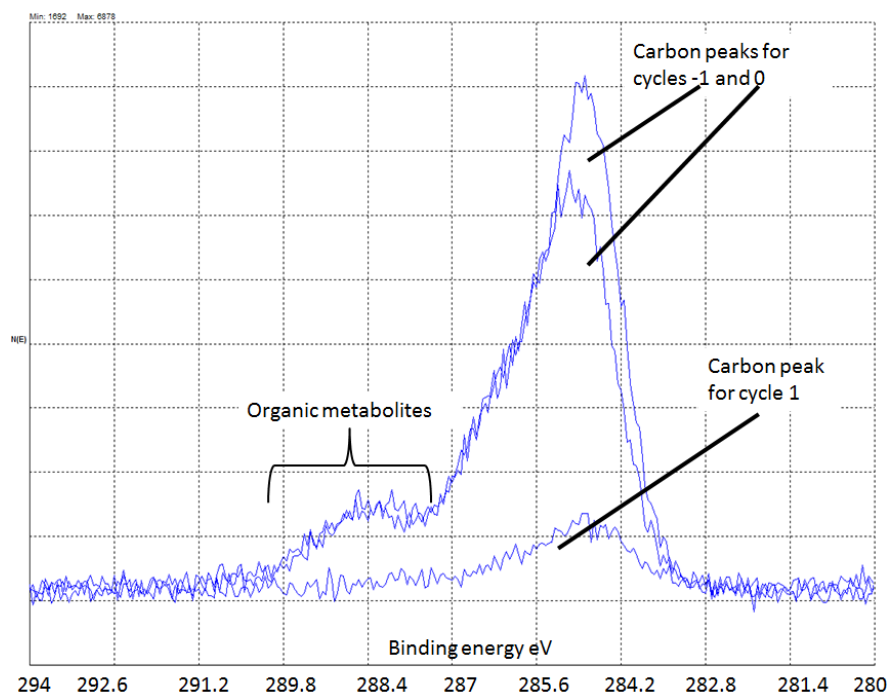


Figure 73. XPS depth profile for carbon in sample 5 after 14 days exposure to SRB. The high-energy tail on cycles -1 and 0 suggests the presence of organic metabolites. These were removed after 2 cycles indicating the thickness was 2 nanometers.

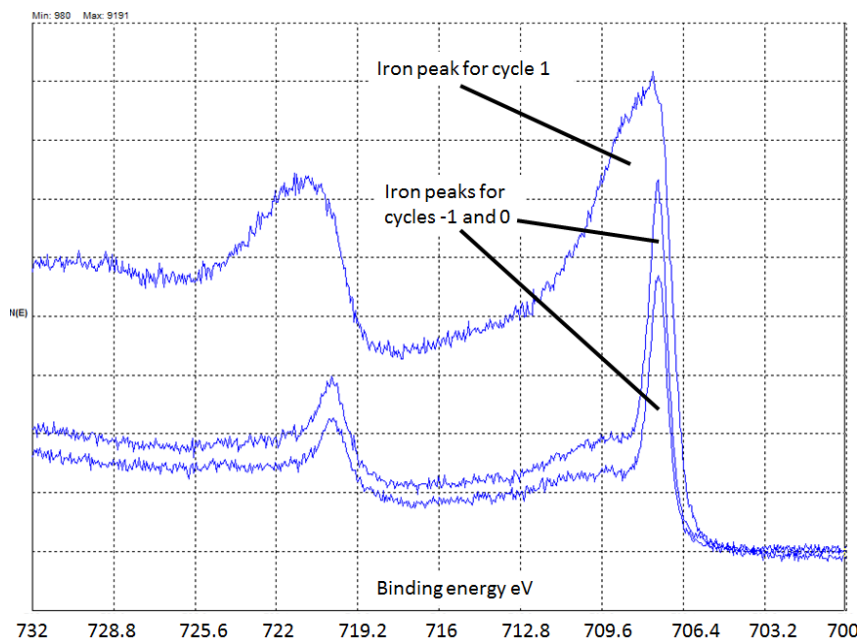


Figure 74. XPS depth profile for iron in sample 5 after 14 days exposure to SRB. Cycles -1 and 0 represent precipitate on the top surface of sample. Depth profile part two indicated no change in iron peak after cycle 1 indicating surface layers had been removed down to host pyrite substrate.

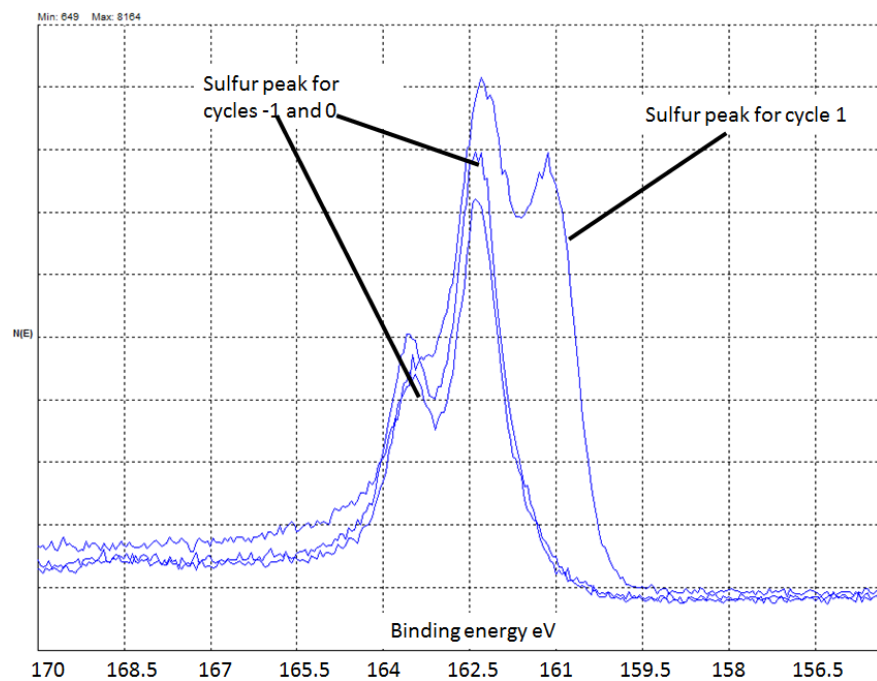


Figure 75. Depth profile part 1 for sulfur on sample 5. Cycles -1 and 0 represent precipitate on surface. Cycle 1 represents substrate.

Pyrite Discussion

Both pyrite sample 2, exposed for 7 days, and pyrite sample 5, exposed for 14 days, had fallen out of the silica string that suspended them in the medium. Biofilm grew extensively on the surface of these samples. FE SEM images revealed precipitate overlaying the surface. SEM w/EDS indicated only the presence of iron and sulfur with no unusual changes in elemental mapping. The EDS spectra and XPS depth profiles suggested precipitate composition to be iron disulfide and iron oxide/hydroxide. The thickness of the precipitate layer was less than 2 nanometers independent of exposure time. In this case, pyrite is not an inert by product of corrosion and appears to be an active participant in the metabolic pathways of the SRB. Pyrite also becomes a secondary product of this interaction – pyrite is recycled back into the system where it can be utilized again as long as there are sites of local oxidation. Areas where the S-S bond is broken and autooxidation of Fe^{2+} to Fe^{3+} can occur will adsorb water. The sulfide quickly reacts with the water to form sulfate that the SRB can utilize. These areas of localized sulfate production give the SRB a purpose for attaching to pyrite surfaces. The production of pyrite from pyrite creates a positive feedback mechanism that will accelerate the corrosion processes until a rate limiting step is achieved.

DISCUSSION

The study of corrosion has made important advances in recent years but there is still a lack of thorough understanding of the mechanisms and reaction pathways associated with MIC. The results of this present study provide insights into the role of secondary mineral precipitates as products and reactants produced by MIC. All of the experiments in this study involved exposure of expected secondary minerals (goethite, pyrrhotite, pyrite) to *D. indonensis*, which served as a proxy for the larger family of SRB.

The results of experiments run at 7, 14 and 30 days of incubation with SRB did not provide any evidence of interaction with iron oxy-hydroxide (goethite), as suggested by the lack of cells observed in the FE SEM images. Bladed goethite crystals were observed in both the inoculated and sterile control batches, indicating that an abiotic reprecipitation mechanism controlled crystal growth on the substrate surface. Goethite was observed to have a less than a one nanometer thick layer of organic metabolites on the surface as determined by XPS multiplex data of carbon and oxygen. The goethite substrate was present in the survey scans after the removal of one nanometer of the surface via argon sputtering. The results were the same, independent of exposure time and independent of the presence/absence of cells. Since the SRB did not interact with goethite, it seems plausible that a thin layer of iron oxy-hydroxide as an armoring surface in fuel tanks could shut down the microbially influenced corrosion process. Goethite in polycrystalline form, however, is quite porous so it may not be the best iron oxide to use as a passivating layer. Further research is needed in a longer time exposure if our model SRB and other anaerobic and/or aerobic bacteria interact with this mineral or if it is inert

to the vast marine microbial consortia and understand the mechanisms of why bacteria avoids colonizing this mineral surface.

D. indonensis did appear to interact with the pyrrhotite samples. The FE SEM images revealed significant cell attachment; however, no pitting was seen on the surface as would be observed in steel corrosion. Previous studies have shown corrosion to occur at the metal/mineral boundaries of manganese sulfide inclusions in steel. This is the result of development of areas of instability and localized strain due to stress exerted on the Fe atoms in the matrix by the presence of inclusion materials such as MnS (Williams et al., 2010). The interfaces between sulfide minerals and steel, whether as inclusions or as precipitated overgrowths, appears to activate sites where general corrosion including MIC may be nucleated. However, in this study no surface degradation such as pitting was observed on the surface of the pyrrhotite samples. The SRB cells in this study seemed to congregate on the pyrrhotite substrate not the observed mineral inclusions (e.g. chalcopyrite, pentlandite, quartz, titanite or biotite) or on the grain boundaries. It is important to understand this work in context. The presence of sulfides as inclusions in steel have been observed to initiate pitting by changing electrochemical potentials (Williams et al., 2010; Ryan et al., 2002) but no studies have been done on SRB interaction with secondary precipitates. In this work, the iron sulfides were not part of an anodic/cathodic system within a steel tank, they were the entire substrate. In order for the SRB to interact with and metabolize pyrrhotite, areas of local oxidation are needed on the surface of the substrate. This holds true for the layer of precipitates seen on the

surface; localized areas of oxidation are needed for SRB to recycle the material through their metabolism.

Identification of precipitates and/or thin film layer based on EDS analysis and XPS data, suggest SRB-pyrrhotite interactions propagated more iron sulfide and iron oxide. The layer of precipitates was averaged to be ~1 nanometer thick independent of exposure time. The control sample exposed only to filtered medium did not generate the same iron sulfide or iron oxide precipitates.

The interaction of SRB and pyrrhotite did create iron disulfide on the surface; although further research is needed to determine if the product was crystalline or amorphous. As described earlier, pyrrhotite converts to iron disulfide (pyrite) in the presence of H_2S and heat, in laboratory procedures as well as at volcanic sites (Deer et al., 1966). Since the SRB produce copious amount of H_2S , the presence of iron disulfide as an alteration product was not surprising, however, it is unclear if the product was crystalline or a thin film. This chemical reaction may have occurred at a slower rate because of the lower temperature of the experimental system or the quick diffusion of H_2S away from the substrate (Deer et al., 1966). The presence of pyrite in the system also accelerates the corrosion process (mechanisms discussed later).

The recycling of pyrrhotite to iron sulfide, disulfide and polysulfide represents a positive feedback mechanism in which pyrrhotite creates active sites for SRB nucleation and growth. The importance of this study lies with the positive feedback mechanism observed with iron sulfides in the presence of a localized site of oxidation (Figure 4). This creates small domains of oxidized iron that then propagate localized sulfate and

enable SRB growth. The smaller grain sized particles would supply a larger reactive site that could accelerate the corrosion process. Further research is needed to investigate the extent of the alteration products' reactivity. Future studies also need to investigate if iron is being stripped off the mineral surface during the creation of sulfate. Iron is often a limiting nutrient in marine environments (Schlesinger and Bernhardt, 2013) and SRB need both the iron and the sulfate to survive. In addition to iron sulfides, XPS data indicated iron oxides as an alteration product on the surface of pyrrhotite. As bacteria interact with pyrrhotite, more iron sulfides and oxides are created. Since the oxides are non-reactive, eventually the positive feedback loop will reach a rate-limiting step when enough iron oxides have entered the system and interrupt the feedback loop.

SRB cell attachment to pyrite was significant on samples that had fallen to the bottom of the container as well as the samples that were suspended in the medium. EDS, and XPS depth profiles indicate the precipitation of secondary pyrite and iron (III) oxide, most likely goethite, in a layer about 2 nanometers thick in all experiments independent of exposure time. Pyrite was also observed as a reactant in abiotic corrosion processes in a study by Starosvetsky et al. (2010). It was determined that iron sulfide precipitates act as a cathodic reduction site, thus furthering the anodic oxidation of the steel. Iron sulfides are good electrical conductors and act as conduits of electron transfer pathways from the steel to the locations where the cathodic reactions take place on the surface. Starosvetsky et al. (2010) also concluded that "various forms of iron sulfides detected among corrosion products, accumulated on the steel surface in SRB media are actually cathodic reduction products of primarily precipitated ferrous sulfides." This mechanism

supports the role of pyrite accelerating corrosion in both biotic and abiotic reactions. To mitigate the effects of pyrite, future research could investigate interactions with sulfur oxidizing bacteria and the possibility of a push-pull symbiotic relationship, where pyrite could be a product of SRB and a substrate for sulfur oxidizers.

The previous work by Starovetsky identify the potential of iron sulfides to abiotically accelerate corrosion. In addition, the results of the current study provide evidence that corrosion of steel is further enhanced by MIC related to production of secondary sulfides. The experimental results demonstrate that pyrite is more reactive with SRB than pyrrhotite. Goethite may be a product of either abiotic or biotic reactions, but has been shown to be non-reactive with SRB. The production of goethite can ultimately poison the surface of steel and sulfides and limit further chemical reactions. Further research is needed to investigate the role of iron oxides in corrosion and if the possibility of an insulating and/or armoring layer, protecting the carbon steel tanks from SRB, could end the impact of corrosion. It is also important to address the difference between crystalline mineral structures and, the more likely present, thin film of disordered iron-sulfur complexes. Future studies could focus on rate limiting steps, such as the production of goethite as a co-precipitate of iron sulfides, and develop ways in which to accelerate the formation of goethite. It is also important to direct future research to help understand the conducting properties of the alteration products. If the conductance is broken, the cathodic reactions will be disrupted. The MIC system is complex and much work is needed for a thorough understanding in order to direct the bacteria and reactions in a way that is favorable.

REFERENCES CITED

Amend, J. P., and Shock, E. L., 2001, Energetics of overall metabolic reactions of thermophilic and hyperthermophilic Archaea and Bacteria: *Fems Microbiology Reviews*, v. 25, no. 2, p. 175-243.

Banfield, J., and Hamers, R., 1997, Processes at Minerals and Surfaces with Relevance to Microorganisms and Prebiotic Synthesis, Washington DC, The Mineralogical Society of America, *Reviews in Mineralogy, Geomicrobiology: Interaction Between Microbes and Minerals*.

Becker, M., de Villiers, J., and Bradshaw, D., 2010, The Mineralogy and Crystallography of Pyrrhotite from Selected Nickel and PGE Ore Deposits: *Economic Geology*, v. 105, no. 5, p. 1025-1037.

Beech, I. B., and Gaylarde, C. C., 1999, Recent advances in the study of biocorrosion - An overview: *Revista De Microbiologia*, v. 30, no. 3, p. 177-190.

Bowles, J. F. W., Howies, R. A., Vaughan, D. J., and Zussman, J., 2011, Rock Forming Minerals Non-silicates; Oxides, Hydroxides and Sulphides, Bodmin, UK, The Geological Society of London.

Davidova, I. A., Duncan, K. E., Choi, O. K., and Suflita, J. M., 2006, *Desulfoglaeba alkanexedens* gen. nov., sp. nov., an n-alkane-degrading, sulfate-reducing bacterium: *International Journal of Systematic and Evolutionary Microbiology*, v. 56, p. 2737-2742.

Deer, W. A., Howie, R. A., and Zussman, J., 1966, An introduction to the rock forming minerals, New York, Wiley, x, 528 p. p.:

Echigo, T., Hatta, T., Nemoto, S., and Takizawa, S., 2012, X-ray photoelectron spectroscopic study on the goethites with variations in crystallinity and morphology: their effects on surface hydroxyl concentration: *Physics and Chemistry of Minerals*, v. 39, no. 9, p. 769-778.

Feio, M. J., Beech, I. B., Carepo, M., Lopes, J. M., Cheung, C. W. S., Franco, R., Guezennec, J., Smith, J. R., Mitchell, J. I., Moura, J. J. G., and Lino, A. R., 1998, Isolation and characterisation of a novel sulphate-reducing bacterium of the *Desulfovibrio* genus: *Anaerobe*, v. 4, no. 2, p. 117-130.

Franklin, M. J., and White, D. C., 1991, BIOCORROSION: Current Opinion in Biotechnology, v. 2, no. 3, p. 450-456.

Gaylarde, C. C., and Beech, I. B., 1996, Lipopolysaccharide composition of *Desulfovibrio* cell wall: *World Journal of Microbiology & Biotechnology*, v. 12, no. 1, p. 113-114.

- Gusek, J.J., 2002, Sulfate-reducing bioreactor design and operating issues: Is this the passive treatment technology for your mine drainage? *In Proceedings, National Association of Abandoned Mine Land Programs: Park City, Utah.*
- Hamilton, W. A., 1998, Sulfate-reducing bacteria: Physiology determines their environmental impact: *Geomicrobiology Journal*, v. 15, no. 1, p. 19-28.
- Herbert, R. B., Benner, S. G., Pratt, A. R., and Blowes, D. W., 1998, Surface chemistry and morphology of poorly crystalline iron sulfides precipitated in media containing sulfate-reducing bacteria: *Chemical Geology*, v. 144, no. 1-2, p. 87-97.
- Lee, W., Lewandowski, Z., Nielsen, P. H., and Hamilton, W. A., 1995, Role of sulfate reducing bacteria in corrosion of mild steel- a review: *Biofouling*, v. 8, no. 3, p. 165-&.
- Legrand, D. L., Bancroft, G. M., and Nesbitt, H. W., 2005, Oxidation/alteration of pentlandite and pyrrhotite surfaces at pH 9.3: Part 1. Assignment of XPS spectra and chemical trends: *American Mineralogist*, v. 90, no. 7, p. 1042-1054.
- Luther, G. W., 1991, Pyrite synthesis via polysulfide compounds: *Geochimica Et Cosmochimica Acta*, v. 55, no. 10, p. 2839-2849.
- Lutterotti, L., S. Matthies, H.-R. Wenk, A. S. Schultz, and J. W. Richardson. "Combined Texture and Structure Analysis of Deformed Limestone from Time-of-flight Neutron Diffraction Spectra." *Journal of Applied Physics* 81, no. 2 (1997): 594. doi:10.1063/1.364220.
- Manceau, A., Schlegel, M. L., Musso, M., Sole, V. A., Gauthier, C., Petit, P. E., and Trolard, F., 2000, Crystal chemistry of trace elements in natural and synthetic goethite: *Geochimica Et Cosmochimica Acta*, v. 64, no. 21, p. 3643-3661.
- Mertie, J., 1969, *Economic Geology of Platinum Metals*, Washington DC, United States Department of Interior.
- Moulder, J., Stickle, W., Sobol, P., and Bomben, K., 1993, *Handbook of X-ray Photoelectron Spectroscopy*: Eden Prairie, MN, Perkin-Elmer Corporation.
- NACE, Corrosion Costs and Preventative Strategies in the United States: <http://www.nace.org/uploadedFiles/Publications/ccsupp.pdf> (April 2013).
- Nesbitt, H. W., Bancroft, G. M., Pratt, A. R., and Scaini, M. J., 1998, Sulfur and iron surface states on fractured pyrite surfaces: *American Mineralogist*, v. 83, no. 9-10, p. 1067-1076.

- Rakovan, J., Becker, U., and Hochella, M. F., 1999, Aspects of goethite surface microtopography, structure, chemistry, and reactivity: *American Mineralogist*, v. 84, no. 5-6, p. 884-894.
- Rickard, D., 1997, Kinetics of pyrite formation by the H₂S oxidation of iron (II) monosulfide in aqueous solutions between 25 and 125 degrees C: The rate equation: *Geochimica Et Cosmochimica Acta*, v. 61, no. 1, p. 115-134.
- Ryan, M. P., Williams, D. E., Chater, R. J., Hutton, B. M., and McPhail, D. S., 2002, Why stainless steel corrodes: *Nature*, v. 415, no. 6873, p. 770-774.
- Schaufuss, A. G., Nesbitt, H. W., Kartio, I., Laajalehto, K., Bancroft, G. M., and Szargan, R., 1998, Incipient oxidation of fractured pyrite surfaces in air: *Journal of Electron Spectroscopy and Related Phenomena*, v. 96, no. 1-3, p. 69-82.
- Schlesinger, W. H., Bernhardt, J., 2013, *Biogeochemistry : an analysis of global change*, San Diego, Calif., Academic Press.
- Shock, E. L., 2009, Minerals as Energy Sources for Microorganisms: *Economic Geology*, v. 104, no. 8, p. 1235-1248.
- Starosvetsky, D., Starosvetsky, J., Armon, R., and Ein-Eli, Y., 2010, A peculiar cathodic process during iron and steel corrosion in sulfate reducing bacteria (SRB) media: *Corrosion Science*, v. 52, no. 4, p. 1536-1540.
- Thomas, J. E., Jones, C. F., Skinner, W. M., and Smart, R. S., 1998, The role of surface sulfur species in the inhibition of pyrrhotite dissolution in acid conditions: *Geochimica Et Cosmochimica Acta*, v. 62, no. 9, p. 1555-1565.
- Thomas, J. E., Skinner, W. M., and Smart, R. S., 2001, A mechanism to explain sudden changes in rates and products for pyrrhotite dissolution in acid solution: *Geochimica Et Cosmochimica Acta*, v. 65, no. 1, p. 1-12.
- Usman, M., Hanna, K., Abdelmoula, M., Zegeye, A., Faure, P., and Ruby, C., 2012, Formation of green rust via mineralogical transformation of ferric oxides (ferrihydrite, goethite and hematite): *Applied Clay Science*, v. 64, p. 38-43.
- von Oertzen, G. U., Skinner, W. M., and Nesbitt, H. W., 2005, Ab initio and x-ray photoemission spectroscopy study of the bulk and surface electronic structure of pyrite (100) with implications for reactivity: *Physical Review B*, v. 72, no. 23.
- White, D., 2007, *The Physiology and Biochemistry of Prokaryotes*, New York, Oxford Press.

Widdel, F., and Bak, F., 1992, Gram-negative mesophilic sulfate-reducing bacteria, *in* The Prokaryotes, New York, Springer.

Wilkin, R. T., and Barnes, H. L., 1996, Pyrite formation by reactions of iron monosulfides with dissolved inorganic and organic sulfur species: *Geochimica Et Cosmochimica Acta*, v. 60, no. 21, p. 4167-4179.

Williams, D. E., Kilburn, M. R., Cliff, J., and Waterhouse, G. I. N., 2010, Composition changes around sulphide inclusions in stainless steels, and implications for the initiation of pitting corrosion: *Corrosion Science*, v. 52, no. 11, p. 3702-3716.

Zhu, Y. G., Zhang, G. F., Feng, Q. M., Yan, D. C., and Wang, W. Q., 2011, Effect of surface dissolution on flotation separation of fine ilmenite from titanite: *Transactions of Nonferrous Metals Society of China*, v. 21, no. 5, p. 1149-1154.

APPENDICES

APPENDIX A

SEM IMAGES AND EDS SPECTRA OF GOETHITE
BEFORE AND AFTER EXPOSURE TO SRB

Appendix A contains SEM secondary electron backscatter electron images with EDS spectra of goethite prior to and after exposure to SRB. 1) Secondary electron images of samples 1-12 before exposure. 2) Back scattered electron images before exposure 3) EDS spectra before exposure 4) secondary electron images after exposure 5) Backscatter electron images after exposure 6) EDS after exposure.

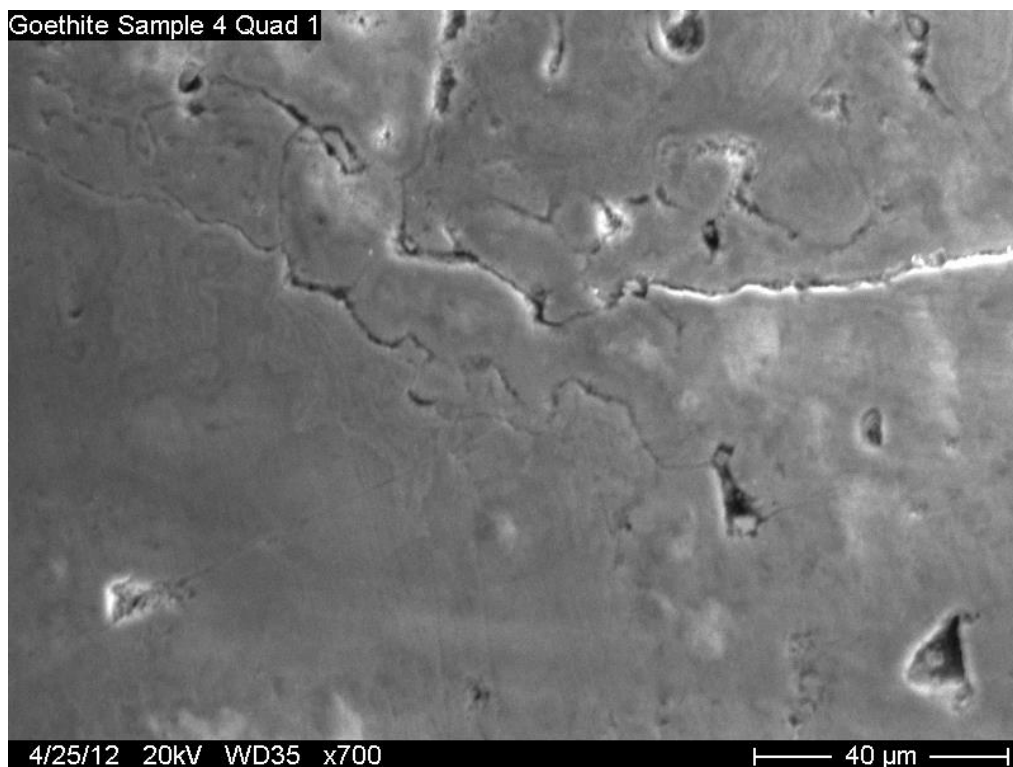
- 1) Secondary electron images of samples 1-12 prior to exposure.



Goethite Sample 2

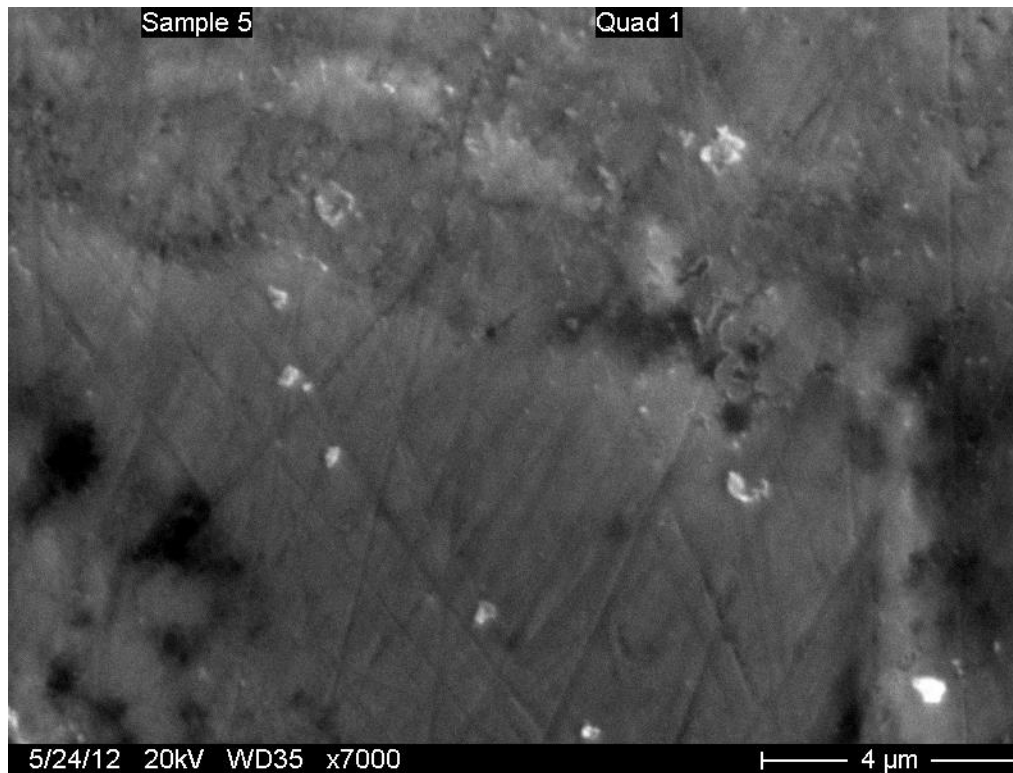
Sample 3 Quad 1
Goethite

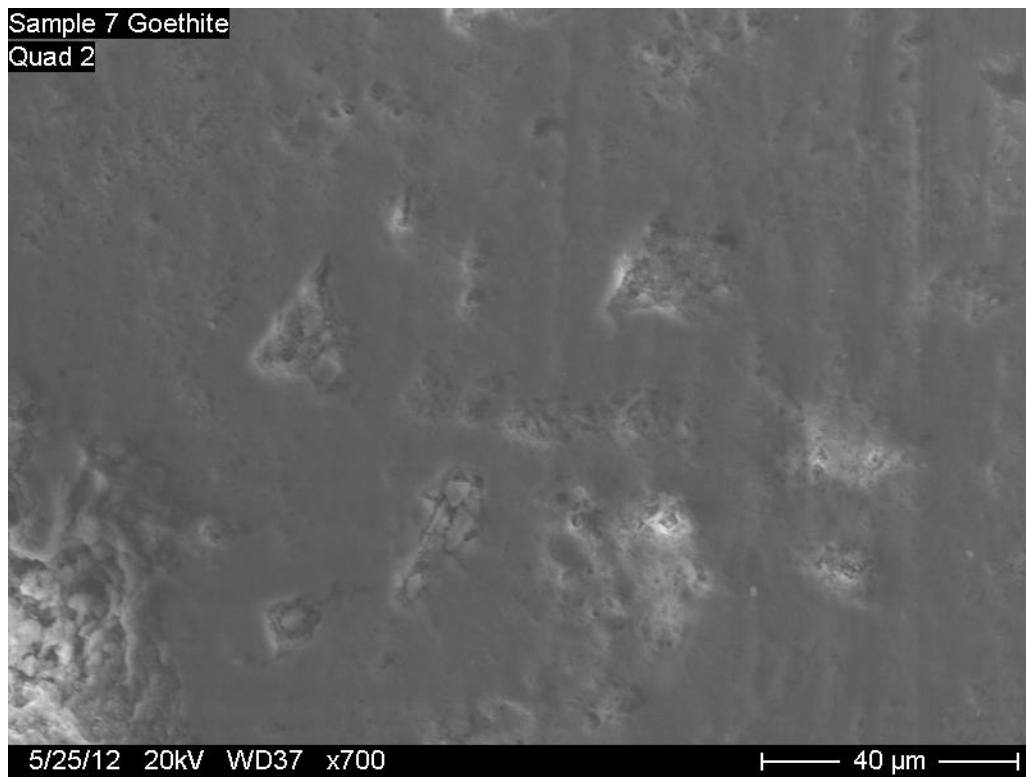
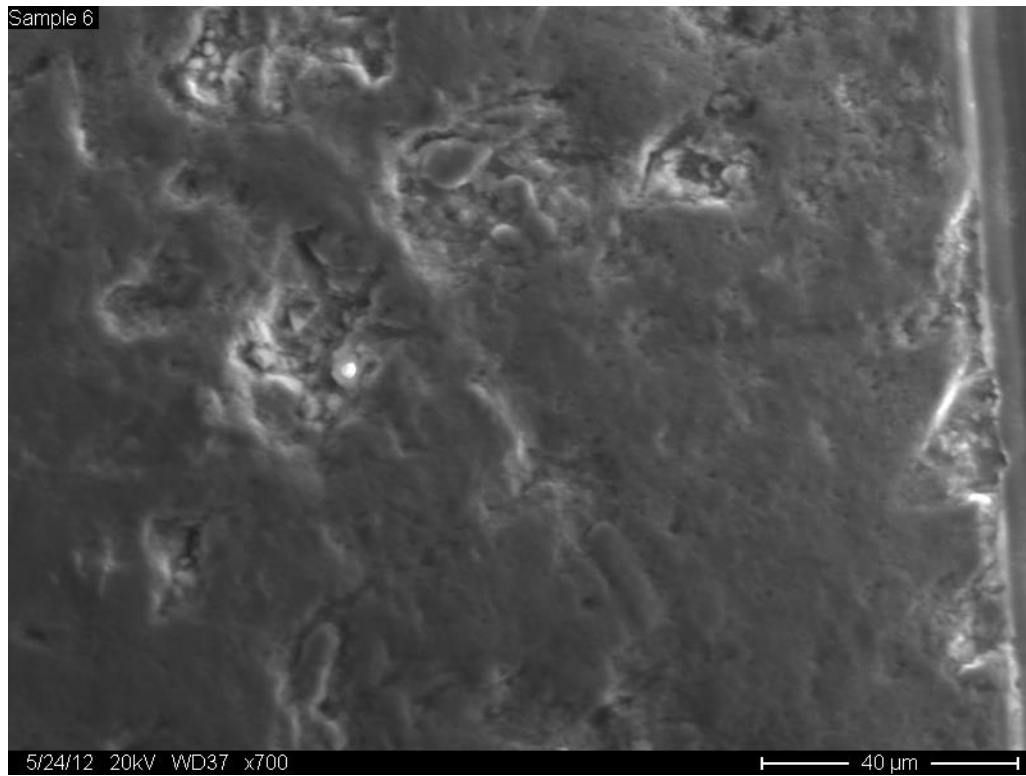
Goethite Sample 4 Quad 1

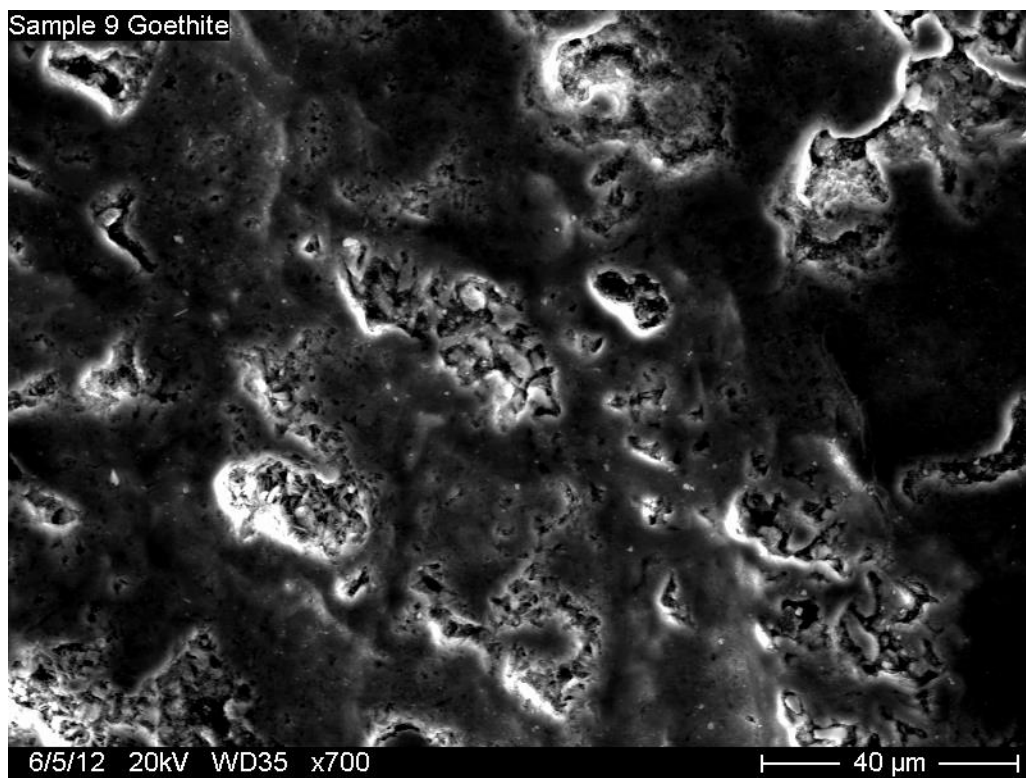
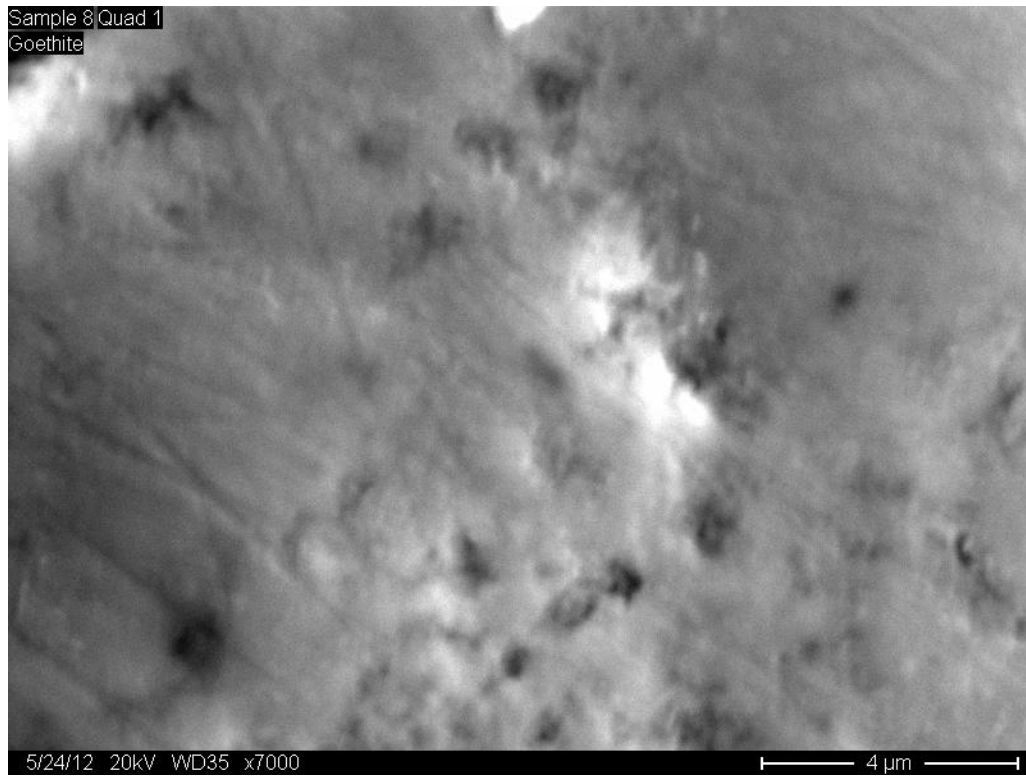


Sample 5

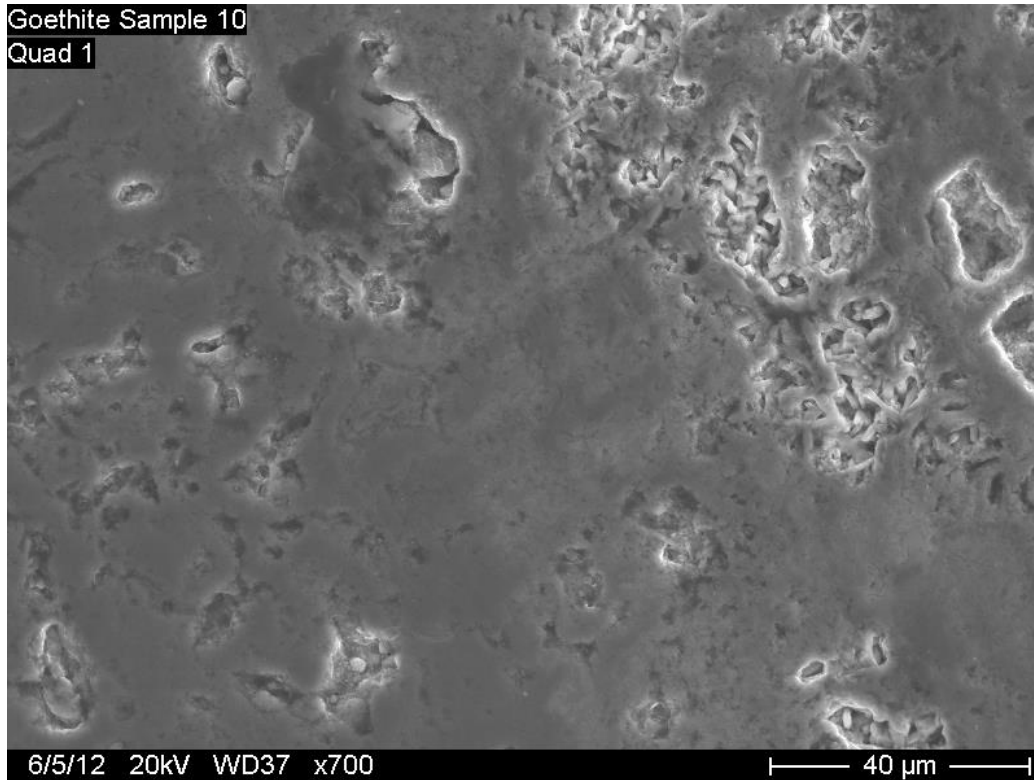
Quad 1





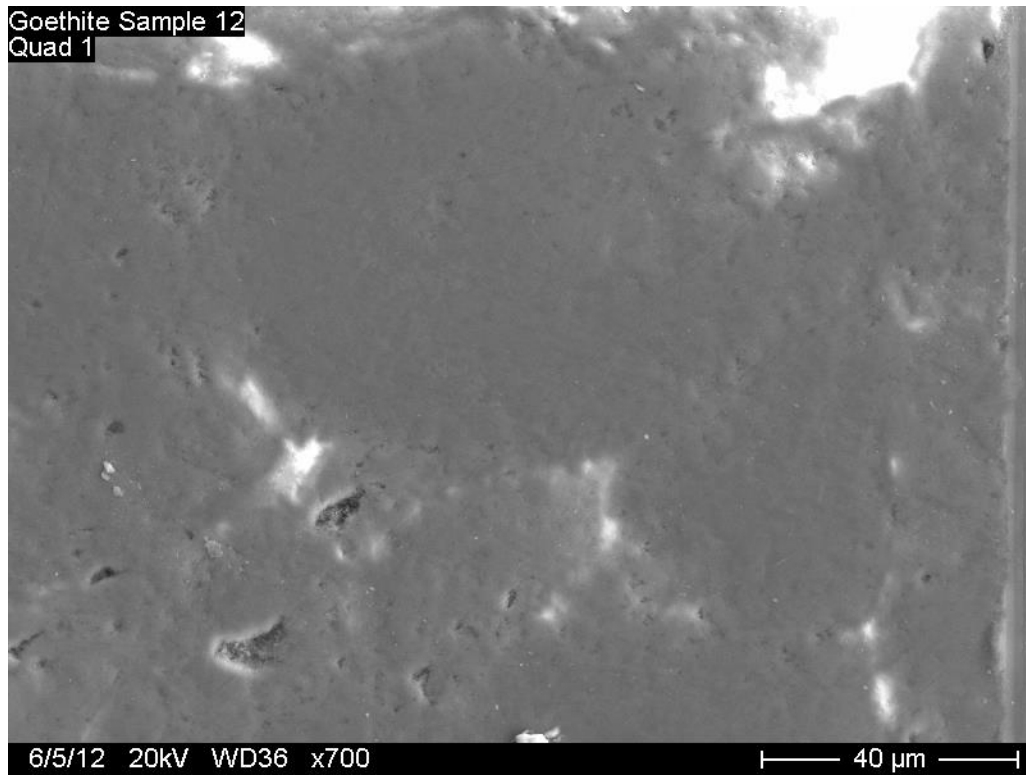


Goethite Sample 10
Quad 1

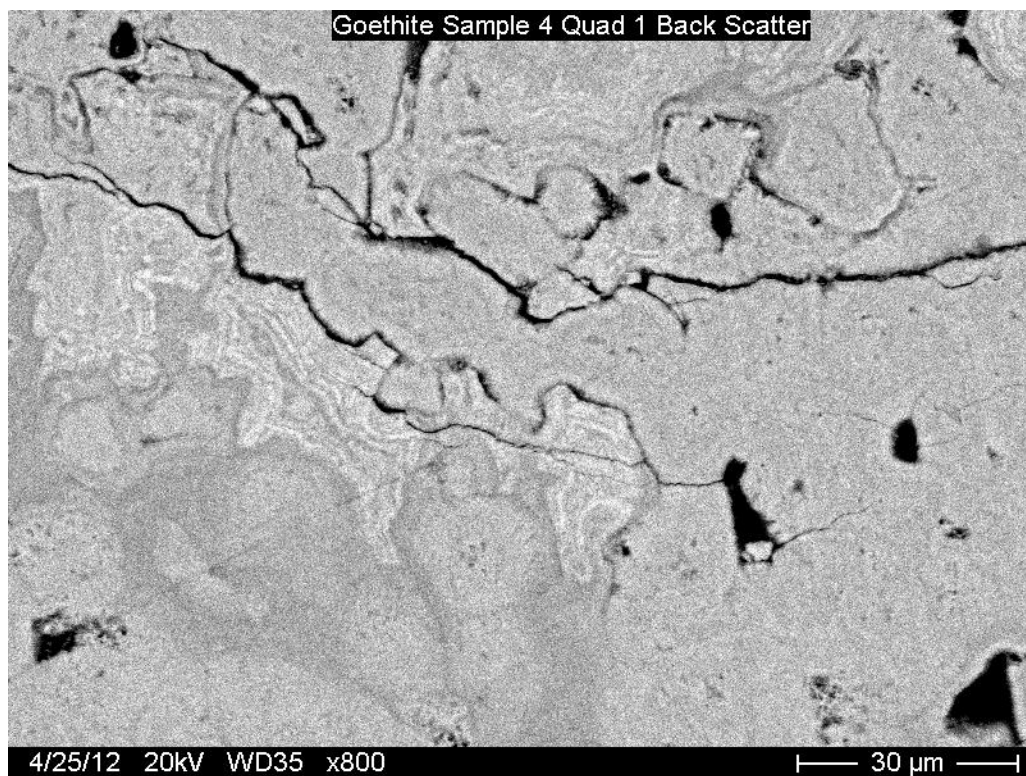
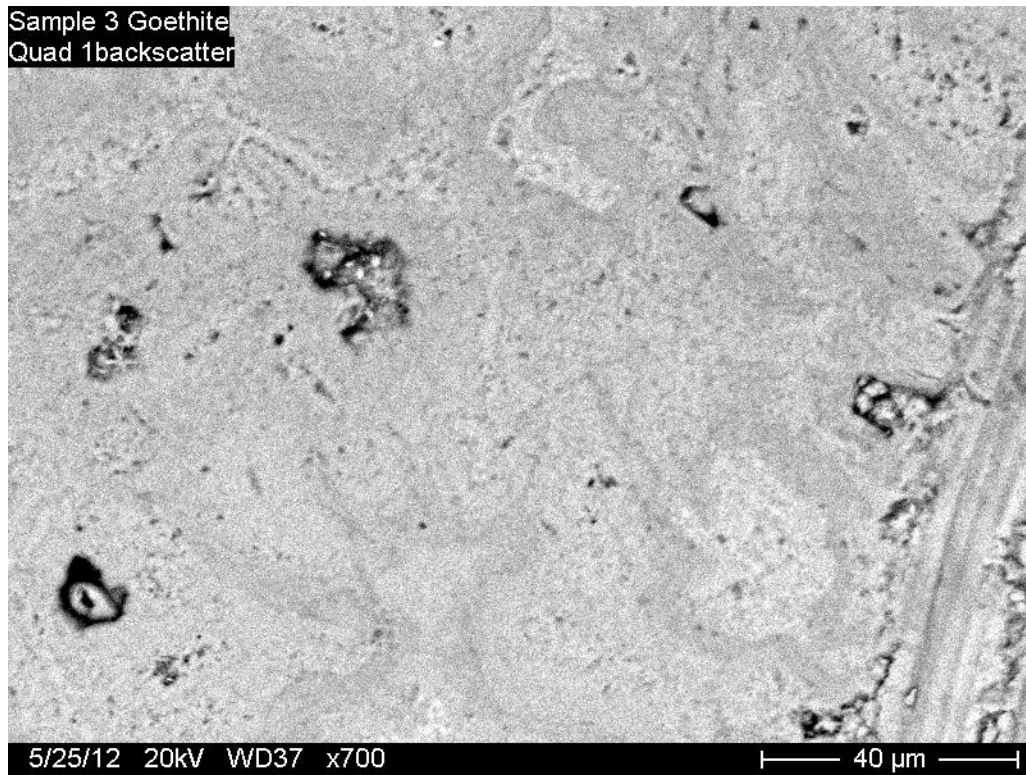


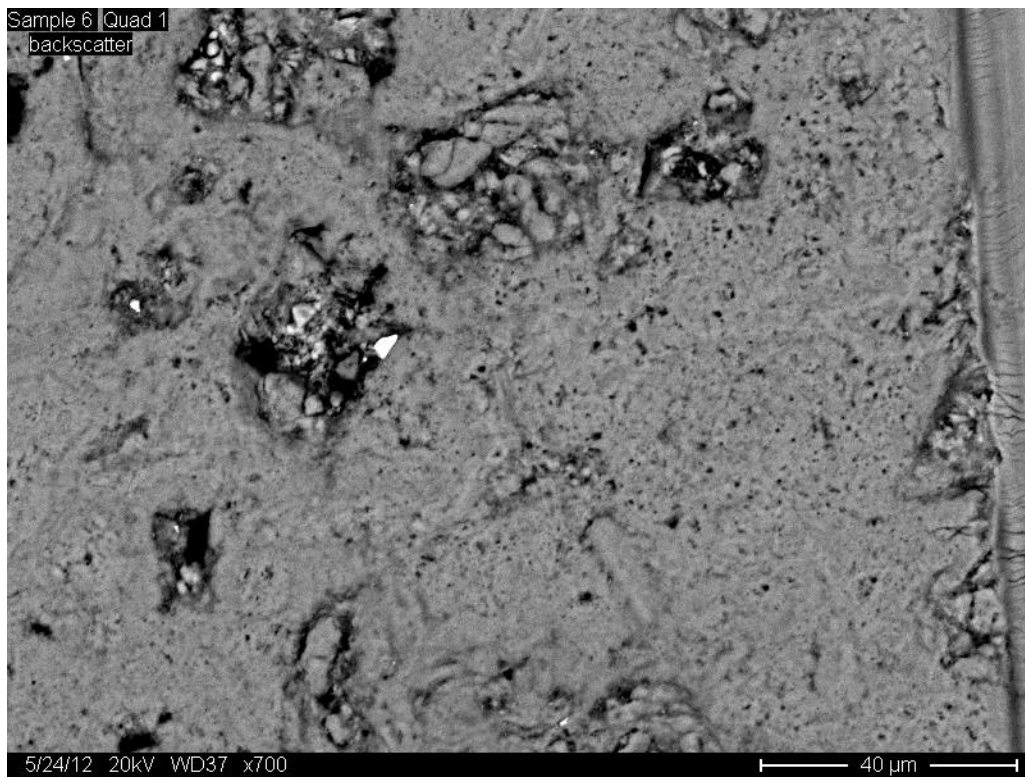
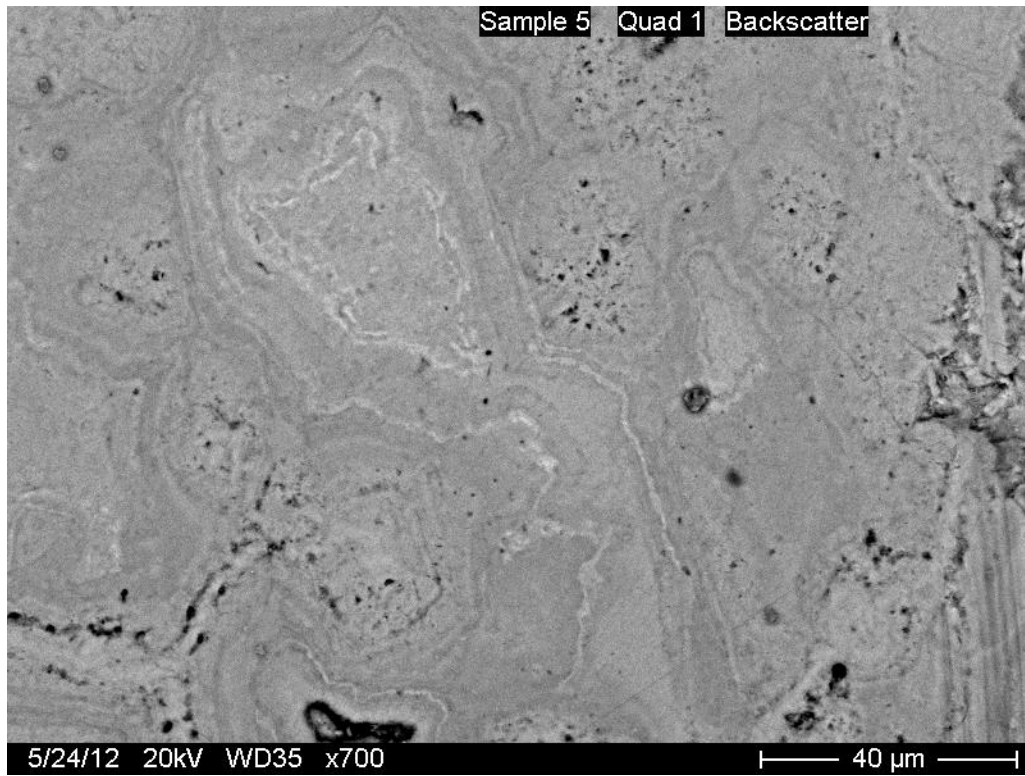
Goethite Sample 11
Quad 1



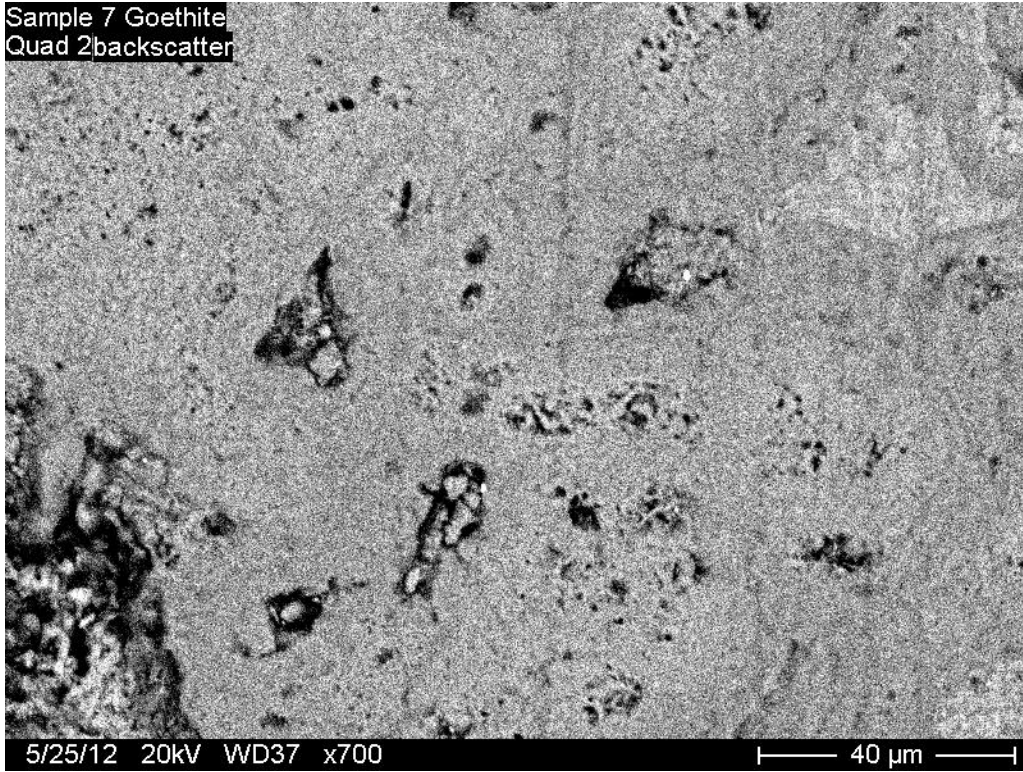


2) Backscatter electron images of goethite samples 3-12 prior to exposure to SRB.

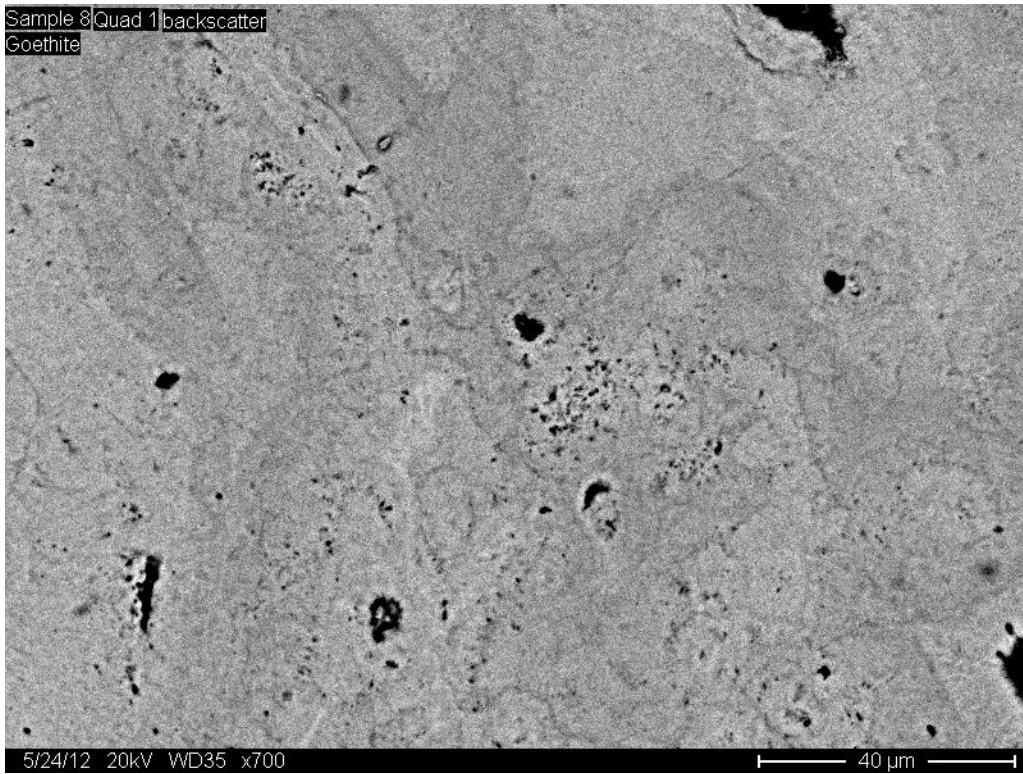


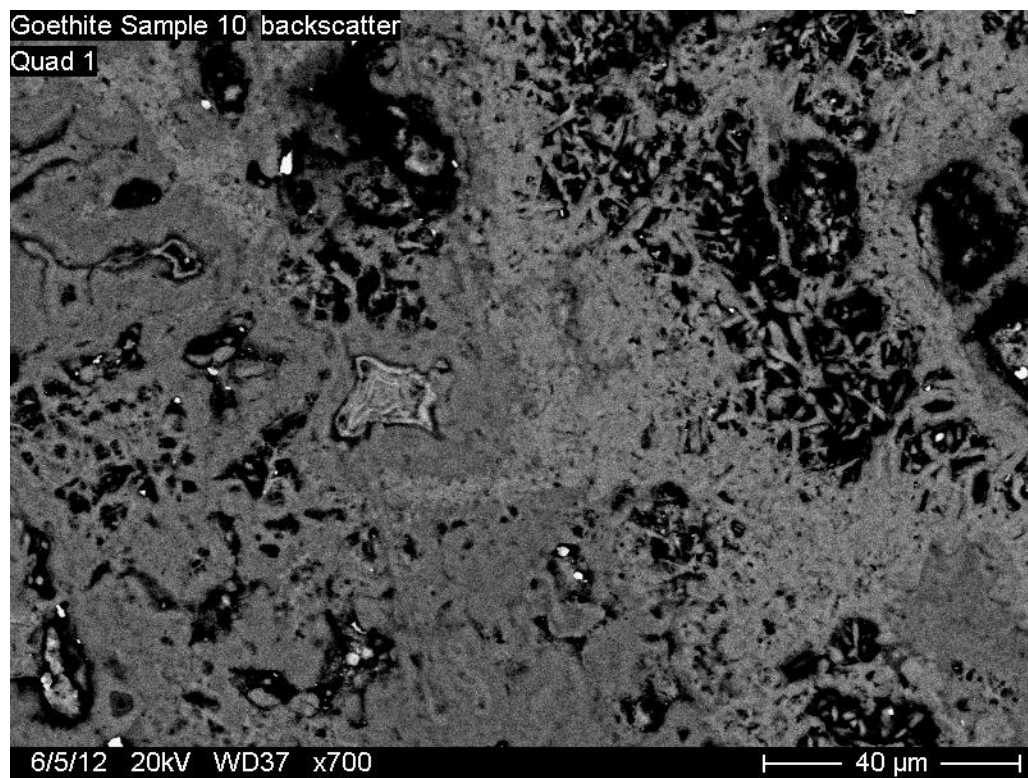
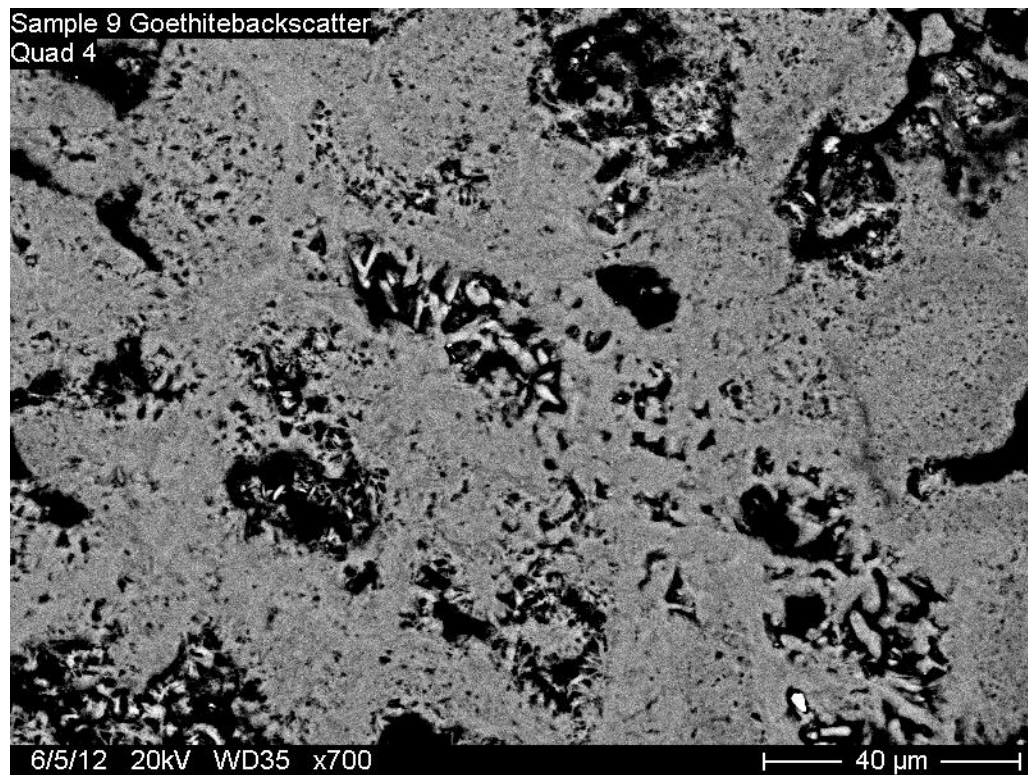


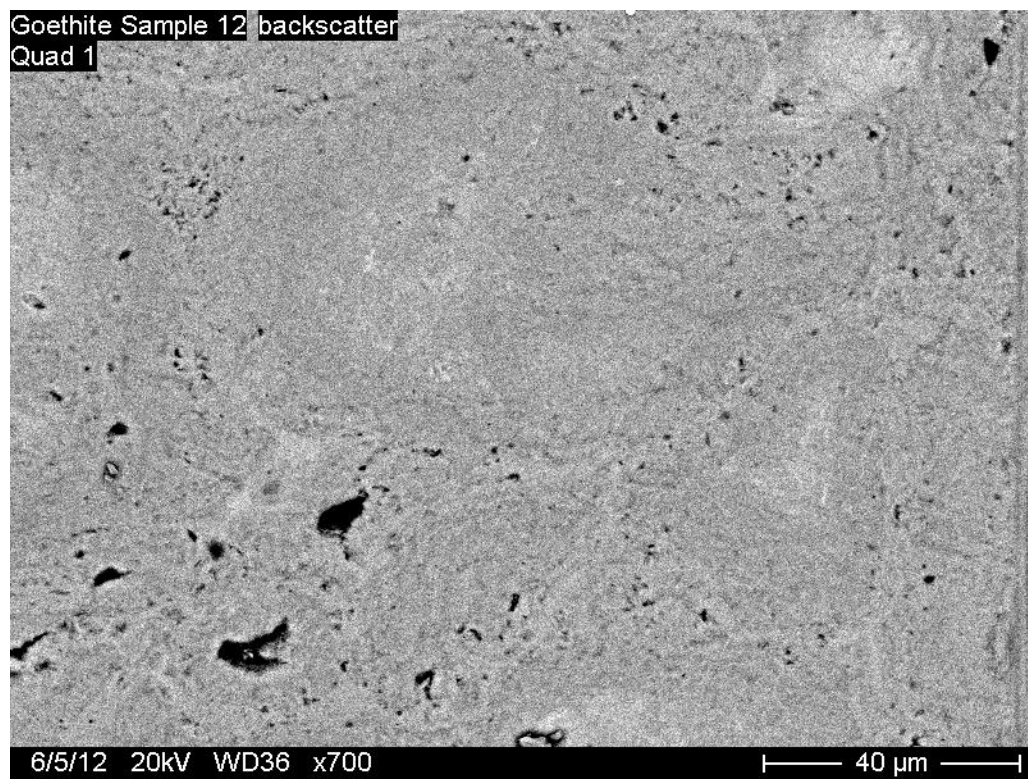
Sample 7 Goethite
Quad 2backscatter



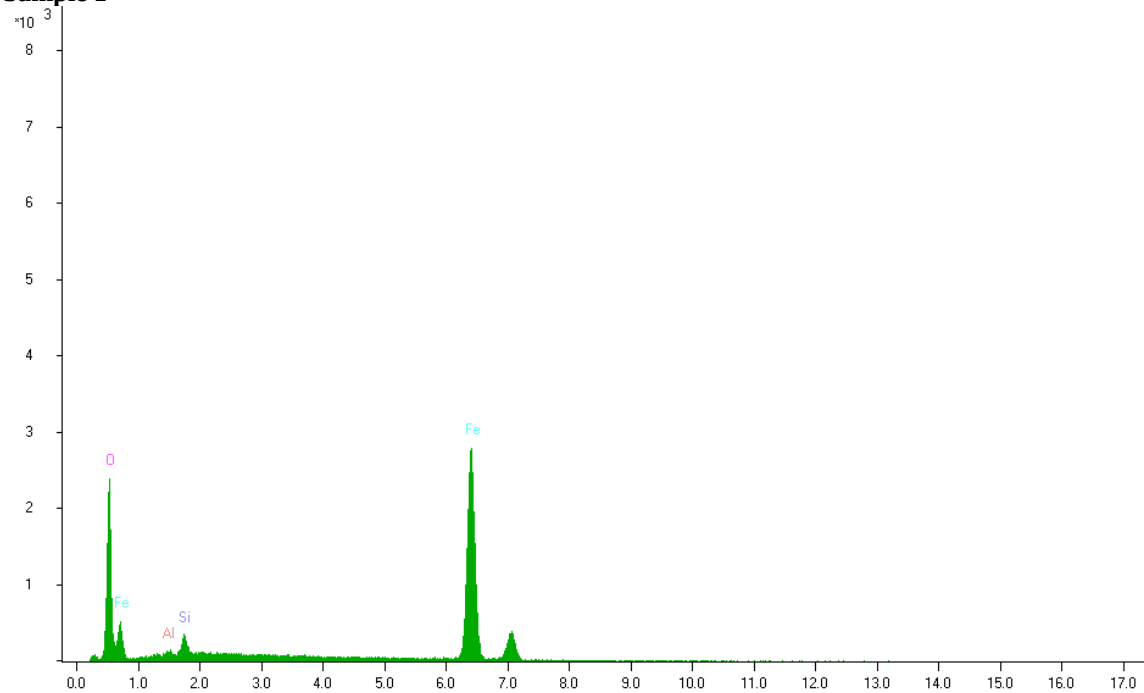
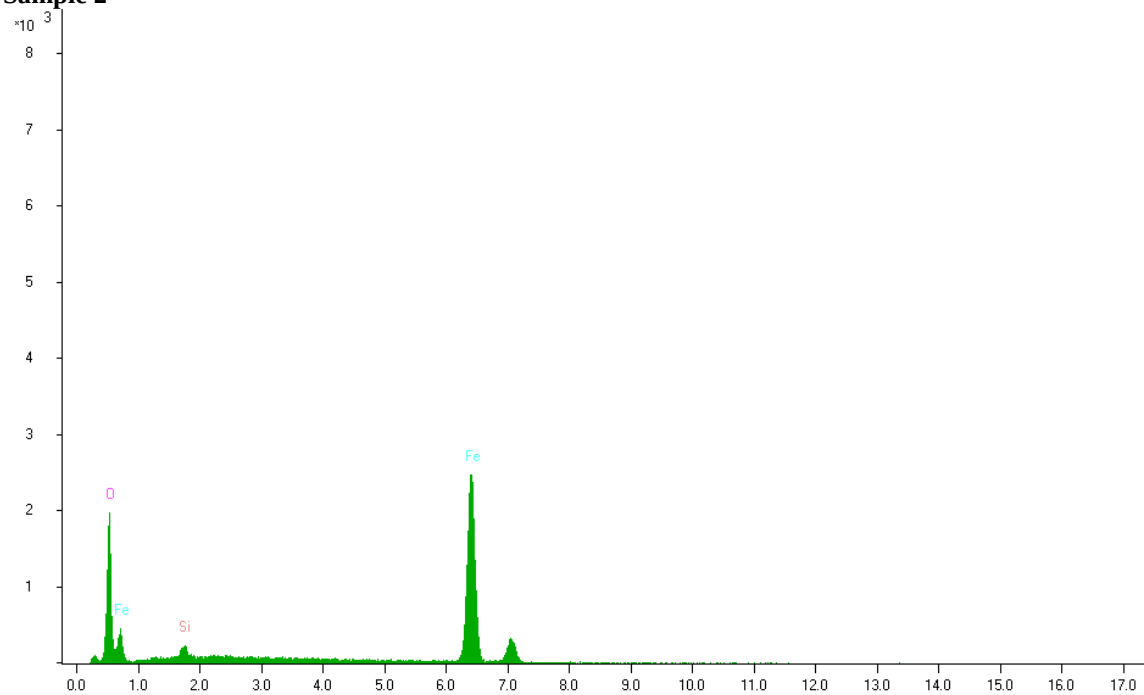
Sample 8 Quad 1backscatter
Goethite

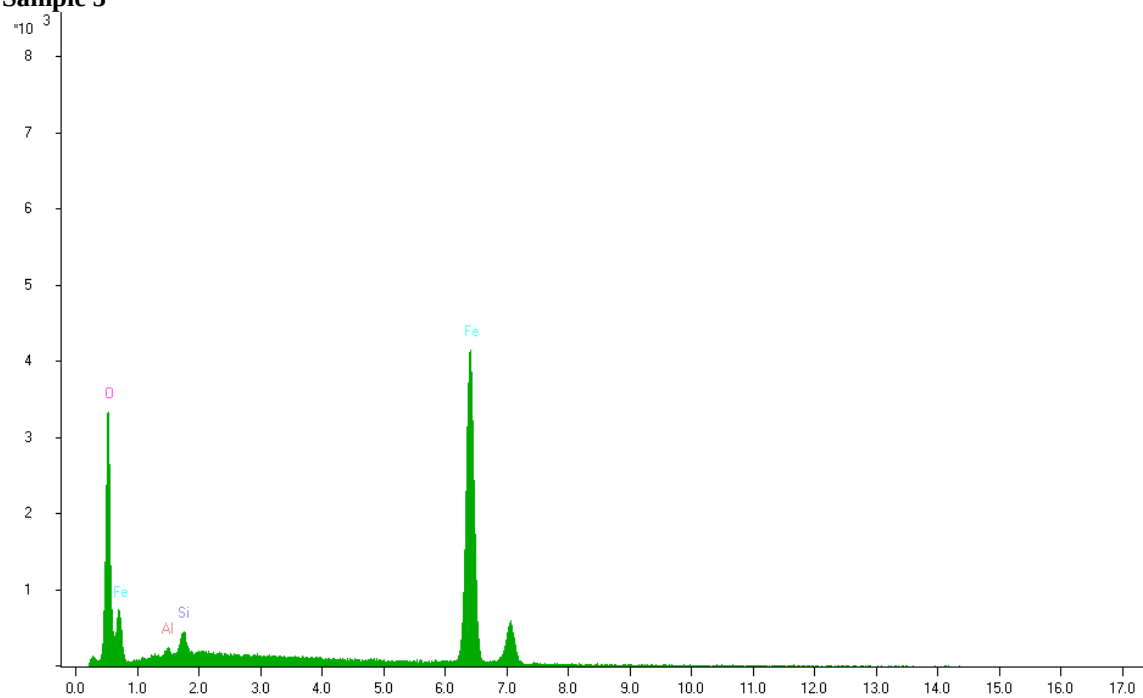
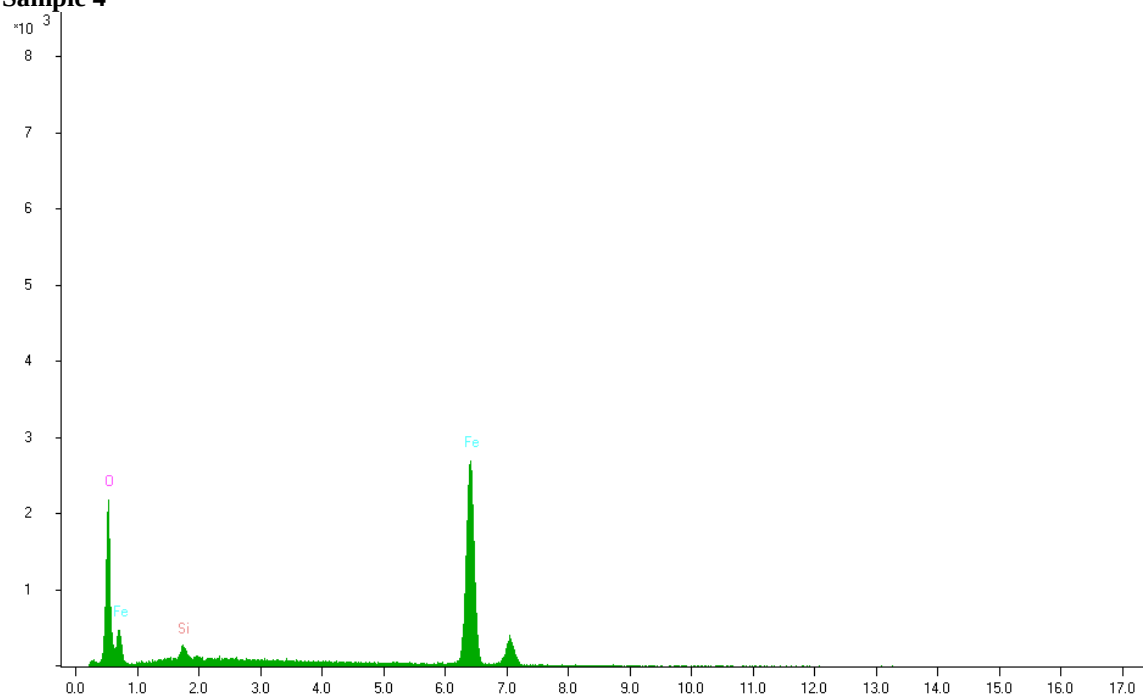


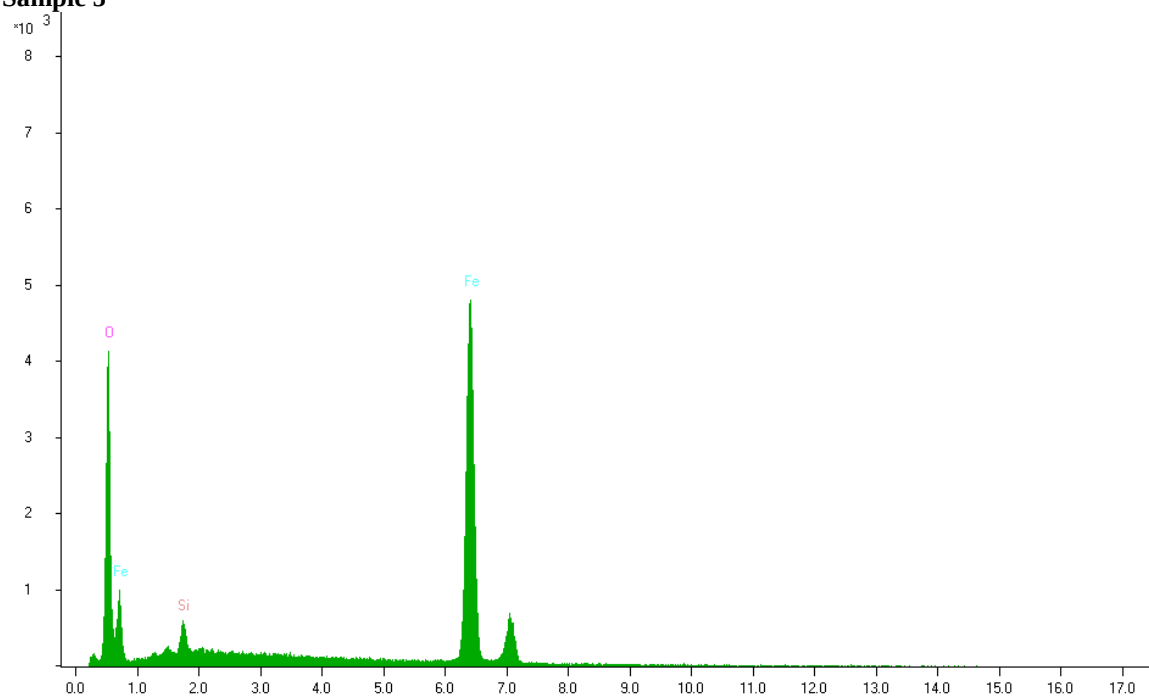
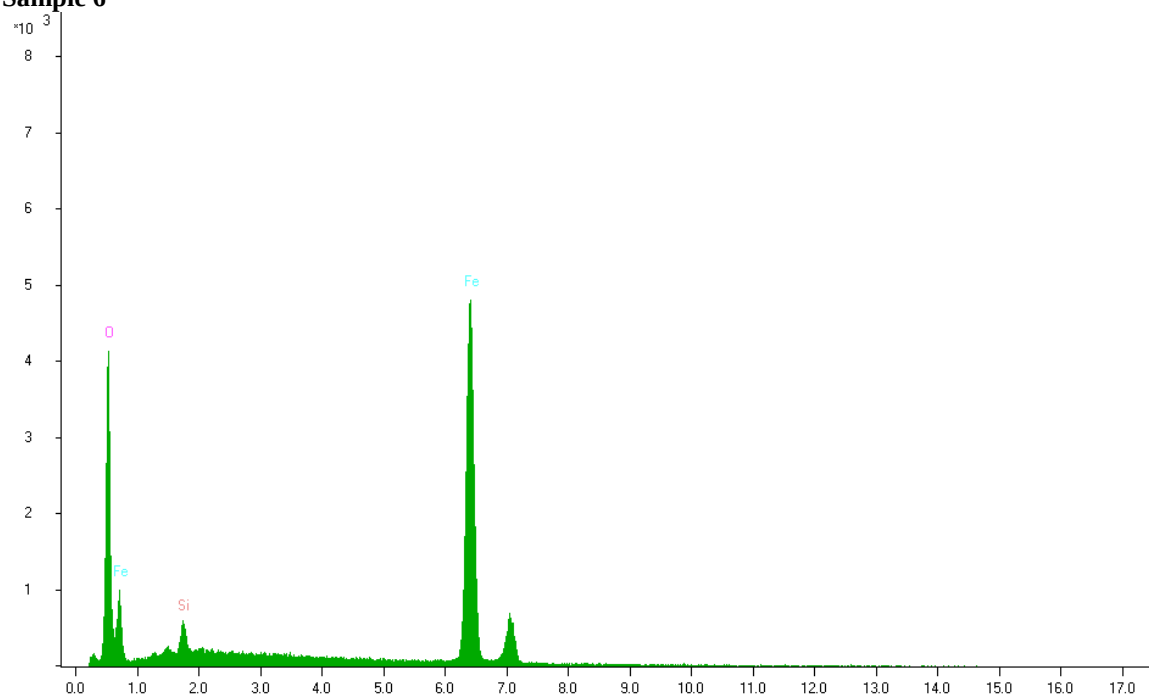


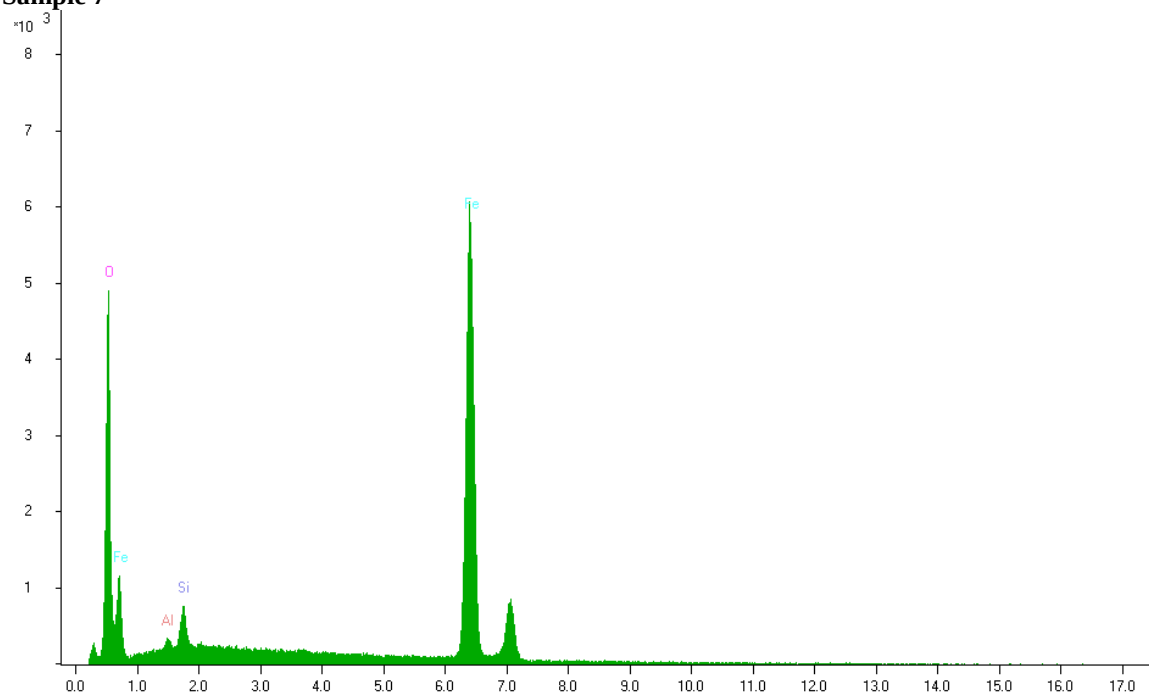
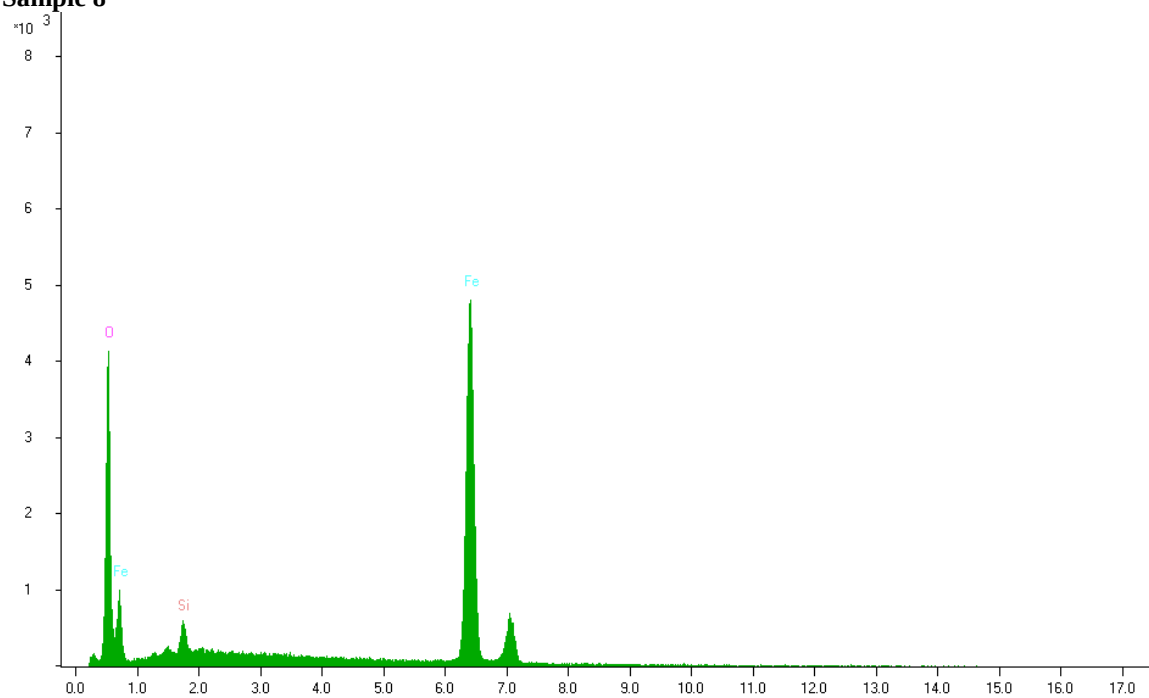


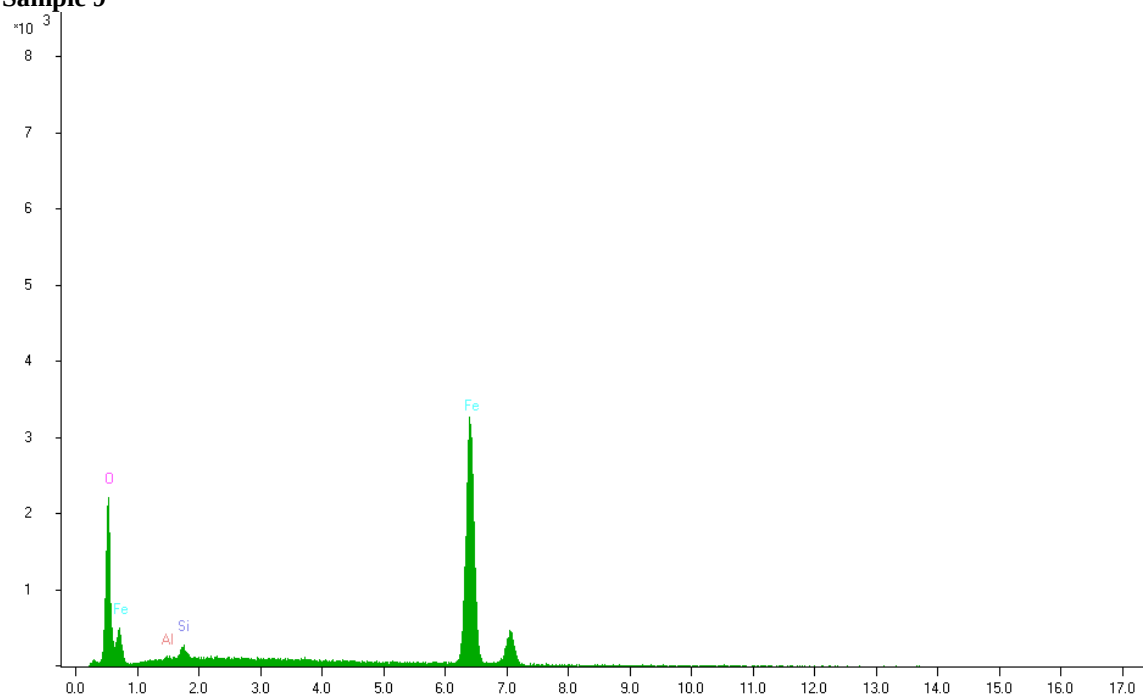
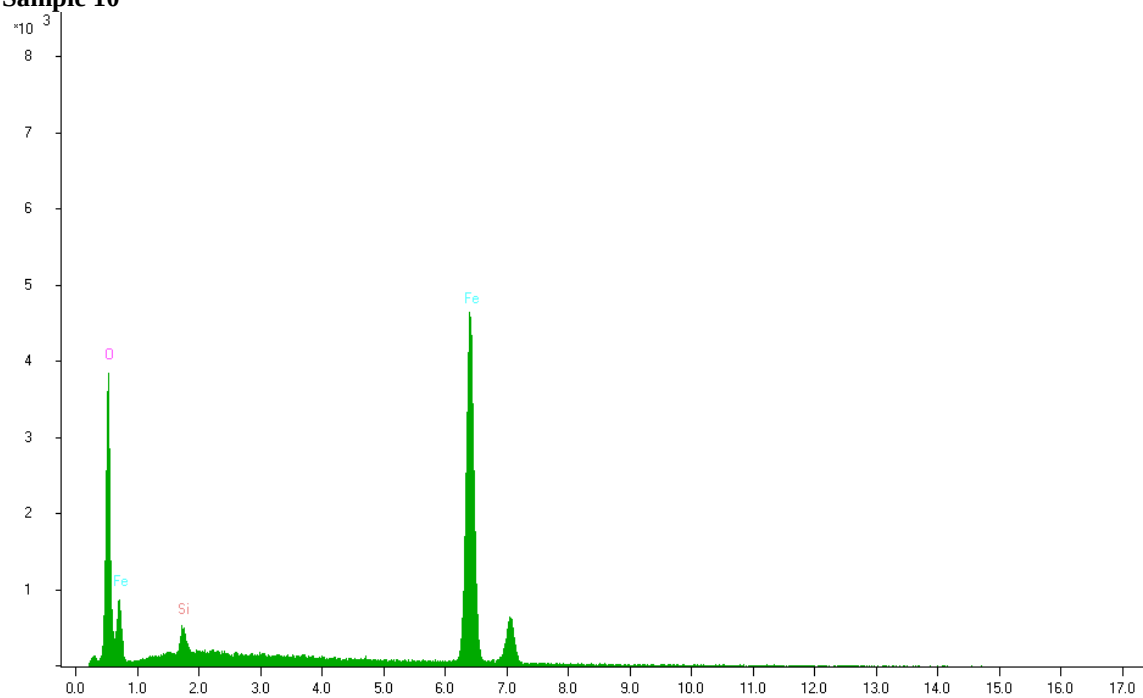
3) EDS spectra of goethite samples 1-12 prior to exposure to SRB

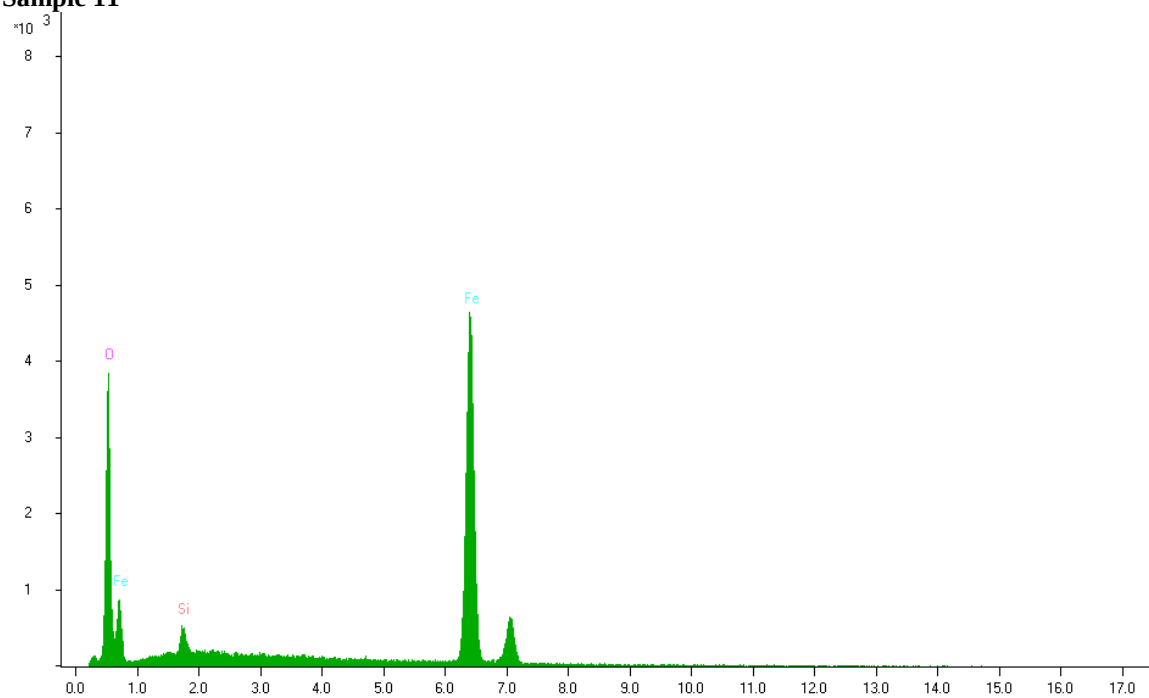
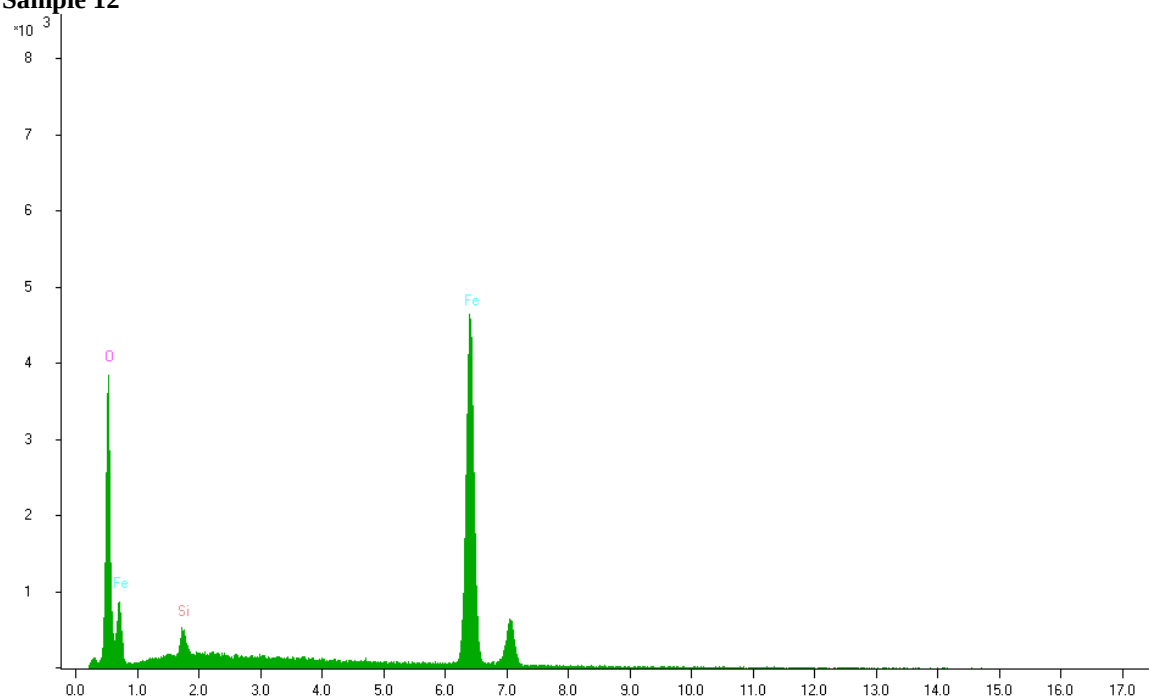
Sample 1**Sample 2**

Sample 3**Sample 4**

Sample 5**Sample 6**

Sample 7**Sample 8**

Sample 9**Sample 10**

Sample 11**Sample 12**

4) Secondary electron images of goethite samples 1-10 after exposure to SRB. Samples 1-4 were exposed for 7 days; samples 5-8 were exposed for 14 days; samples 9-10 were exposed for 30 days.

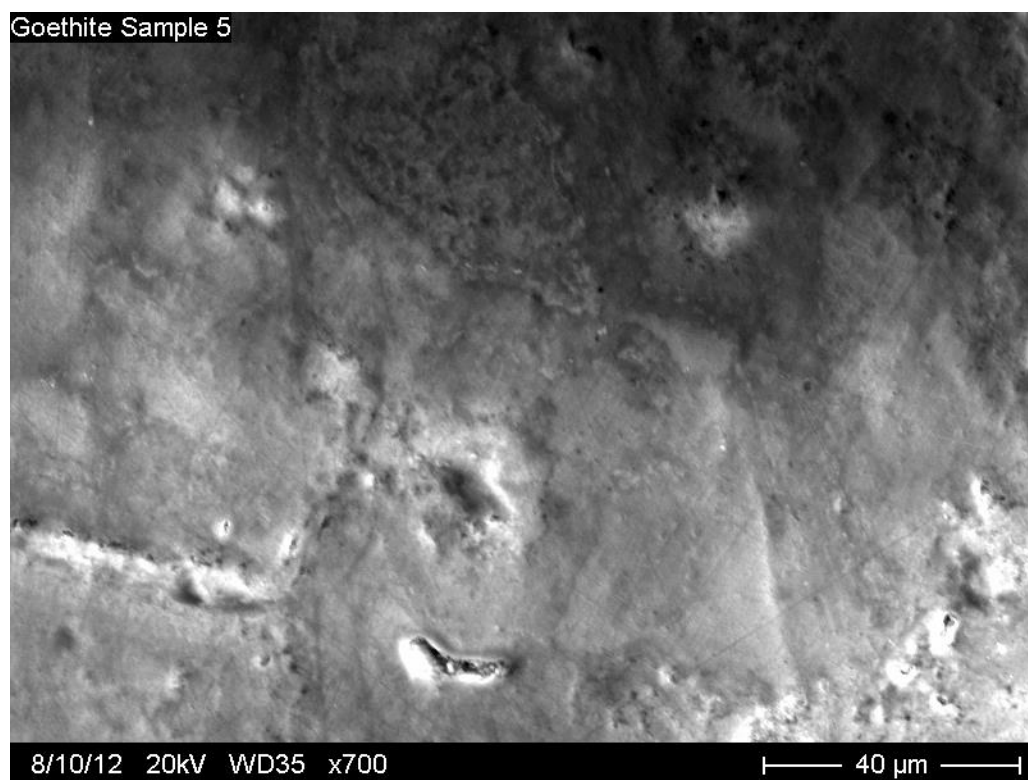
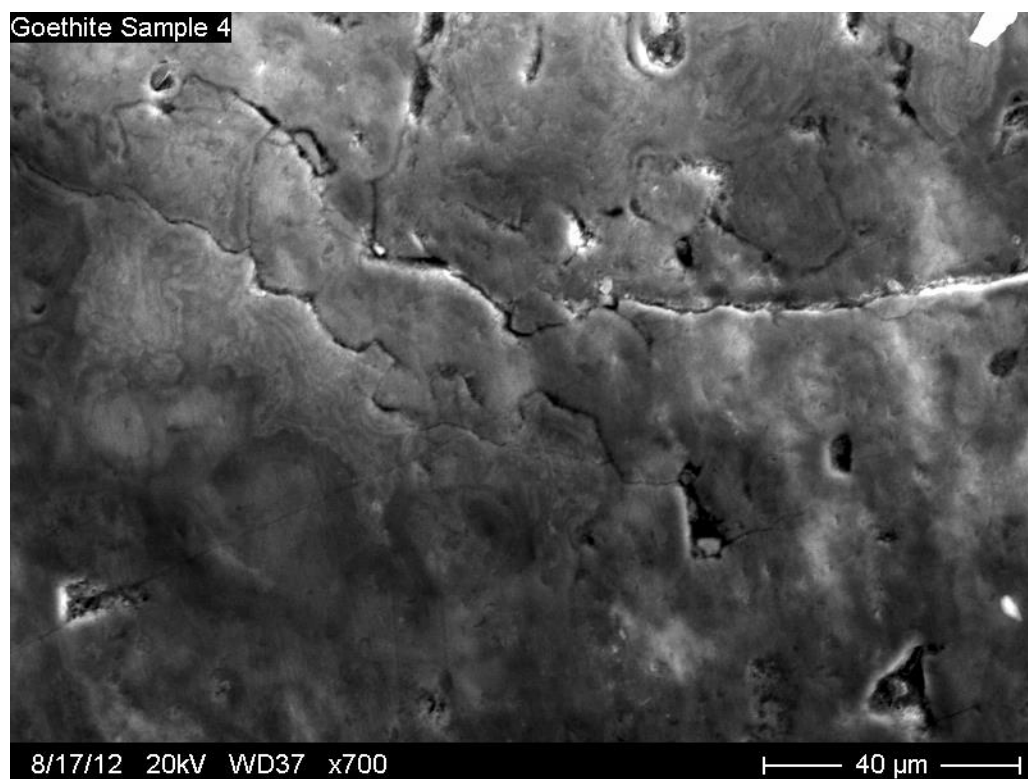


Goethite Sample 2

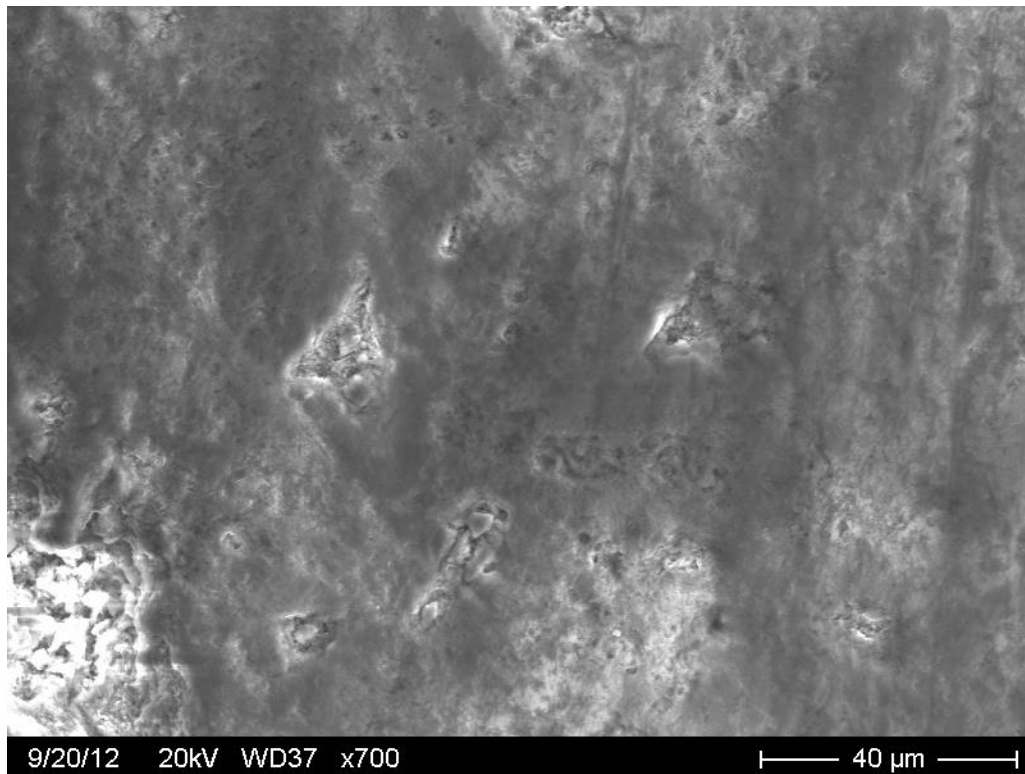
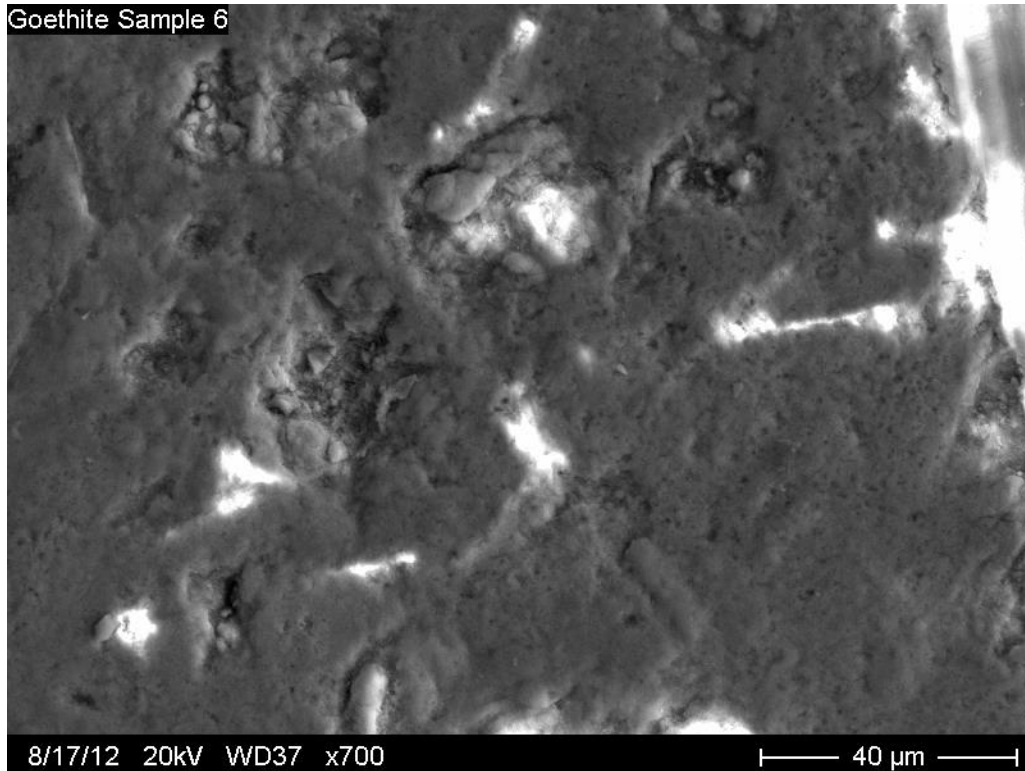


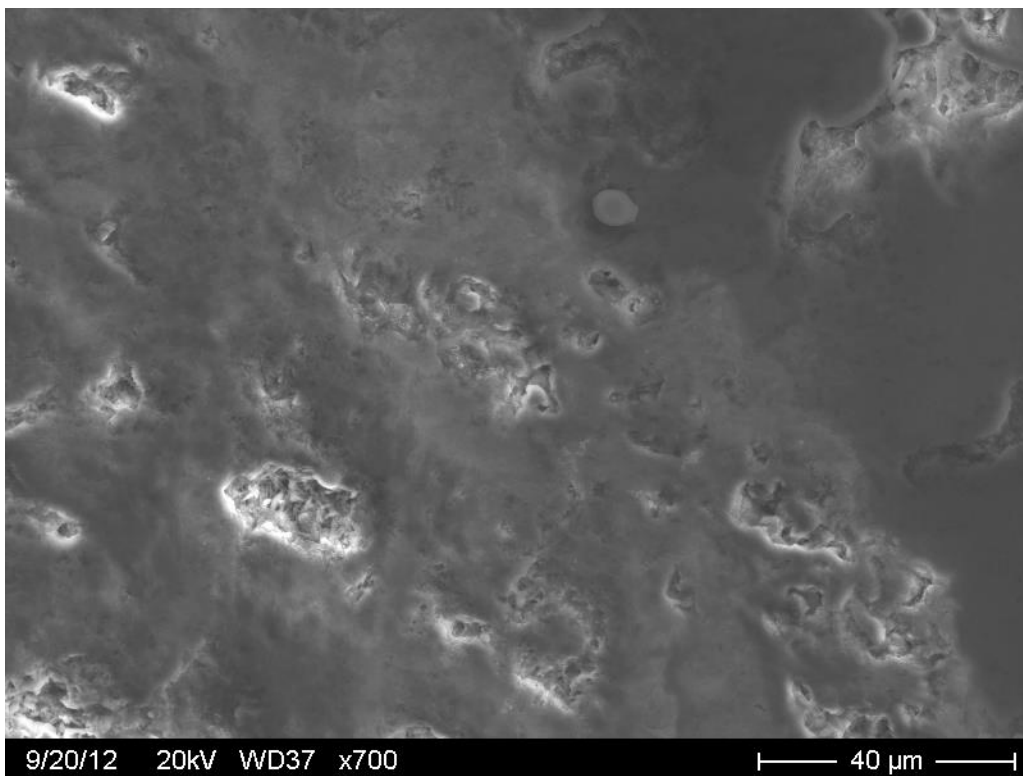
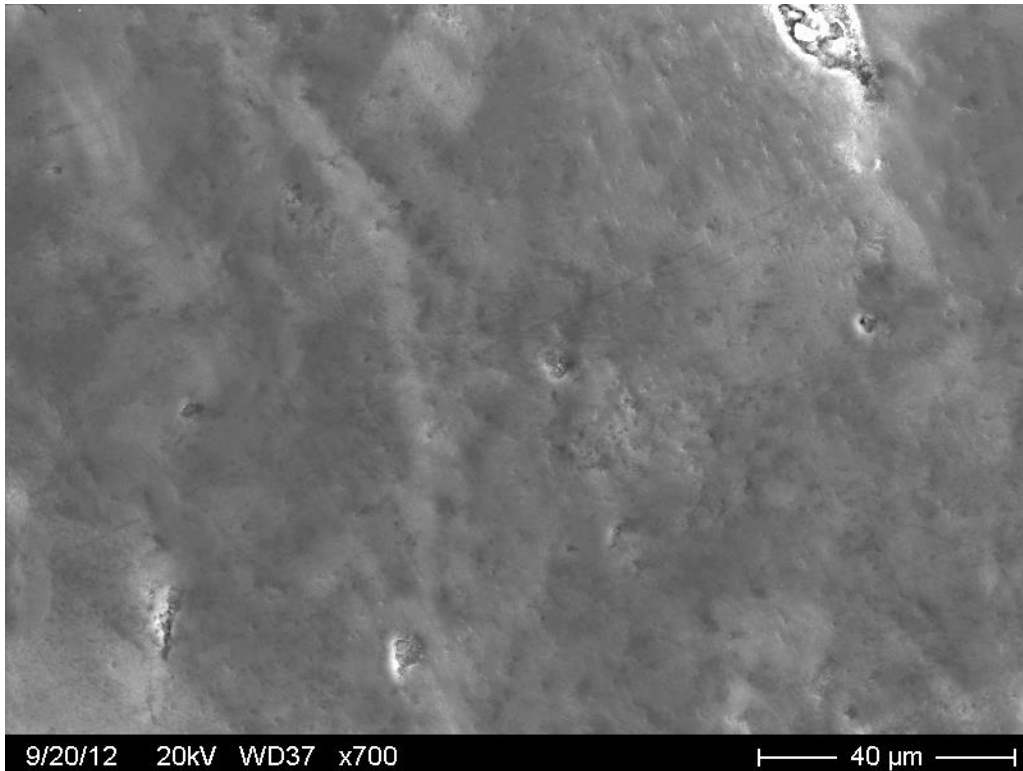
Goethite Sample 3

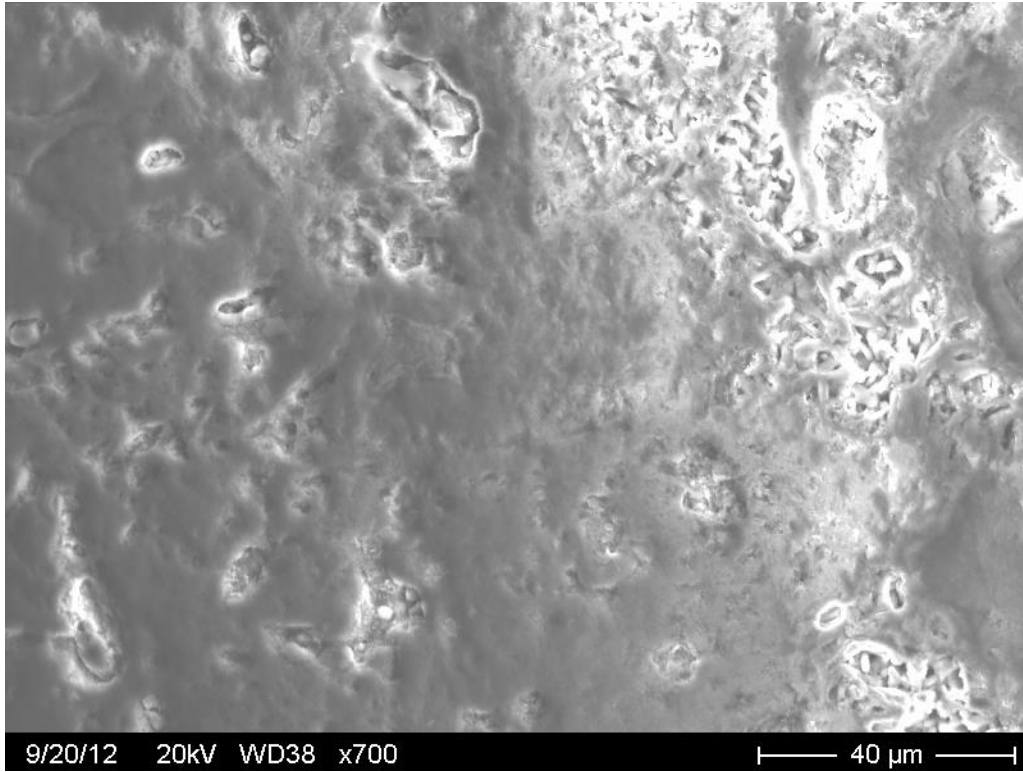




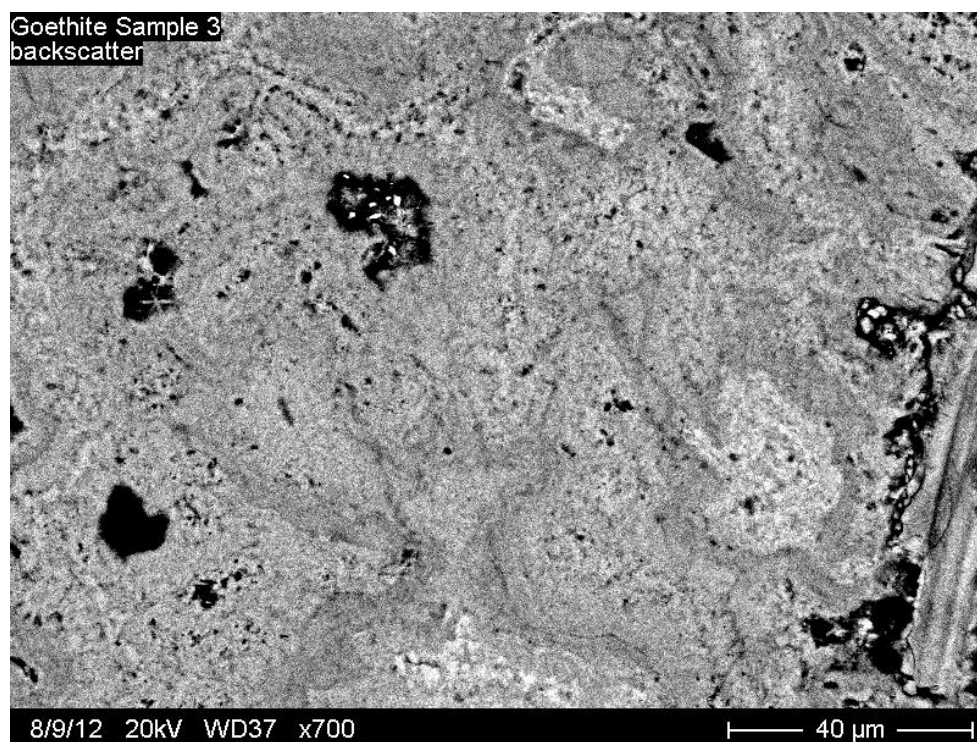
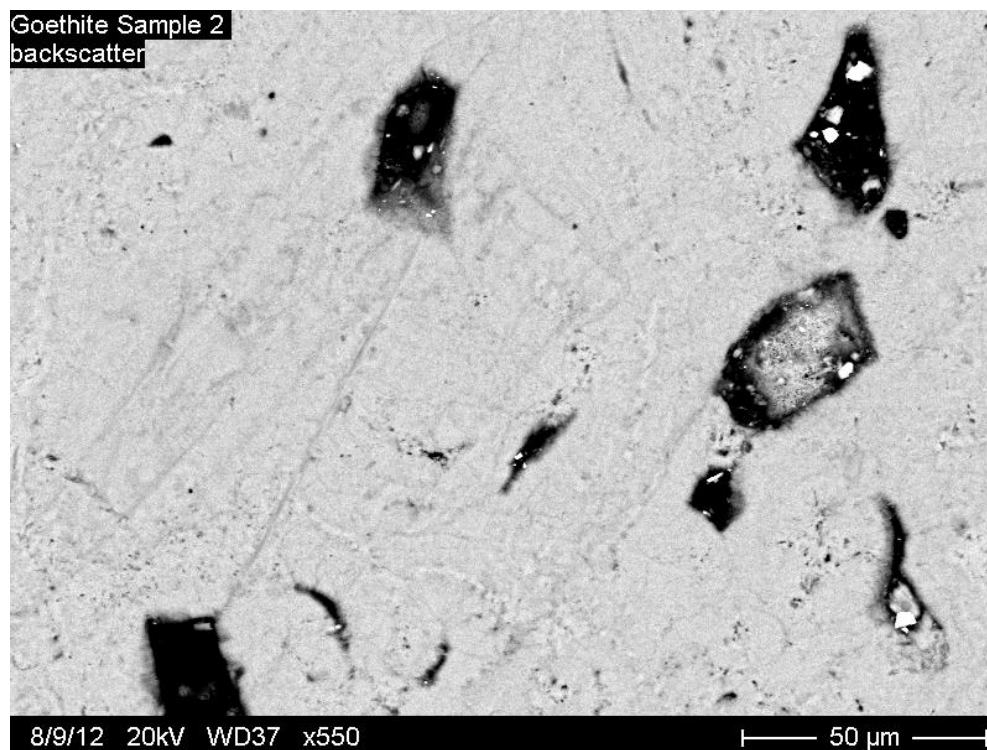
Goethite Sample 6

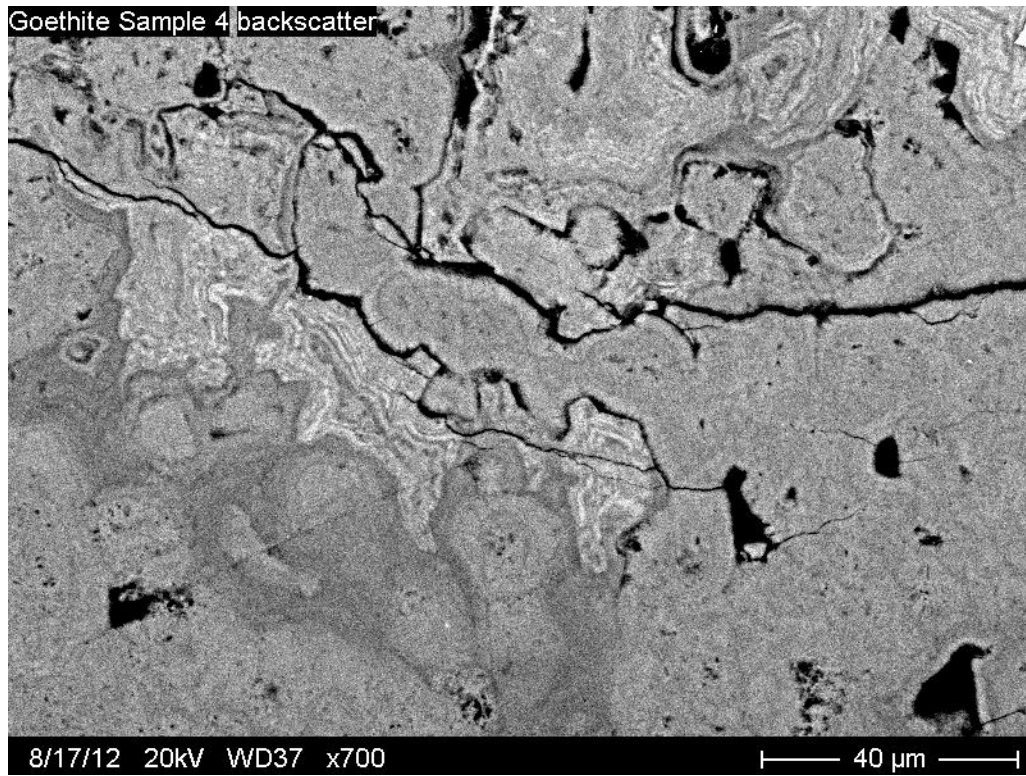




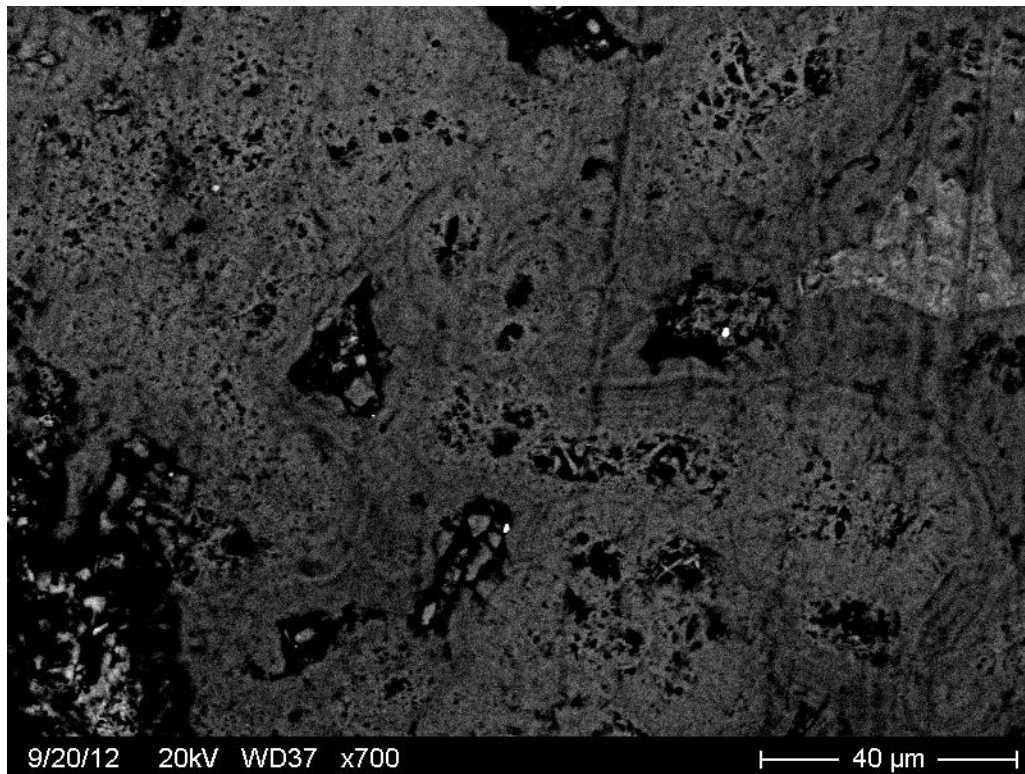
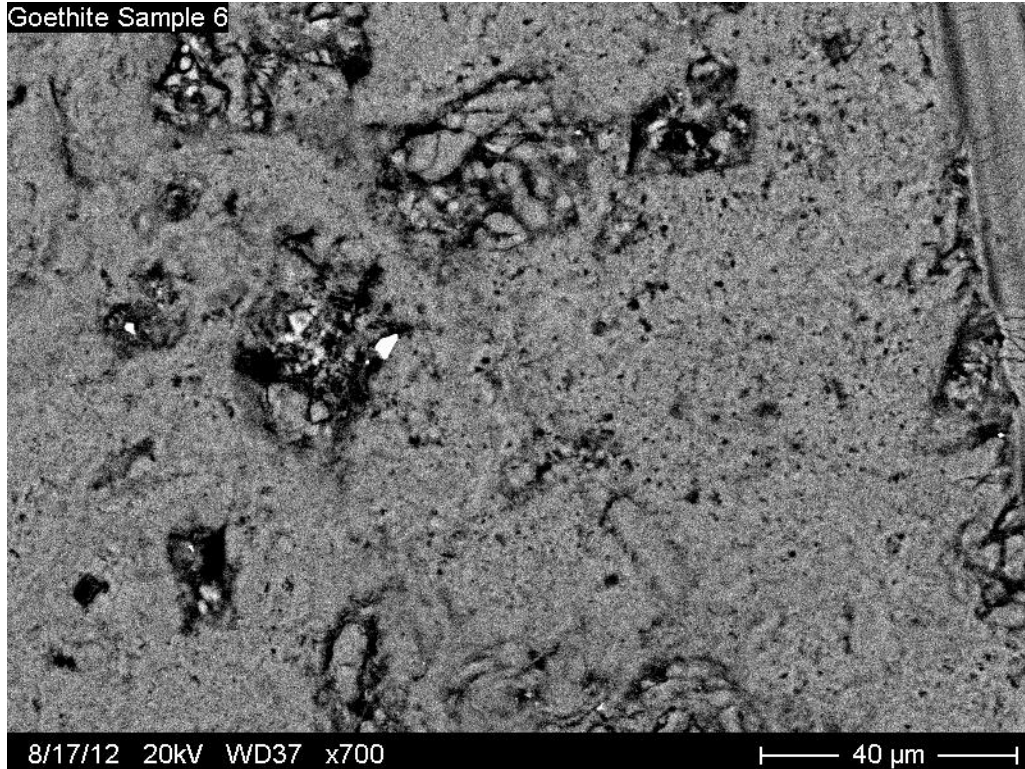


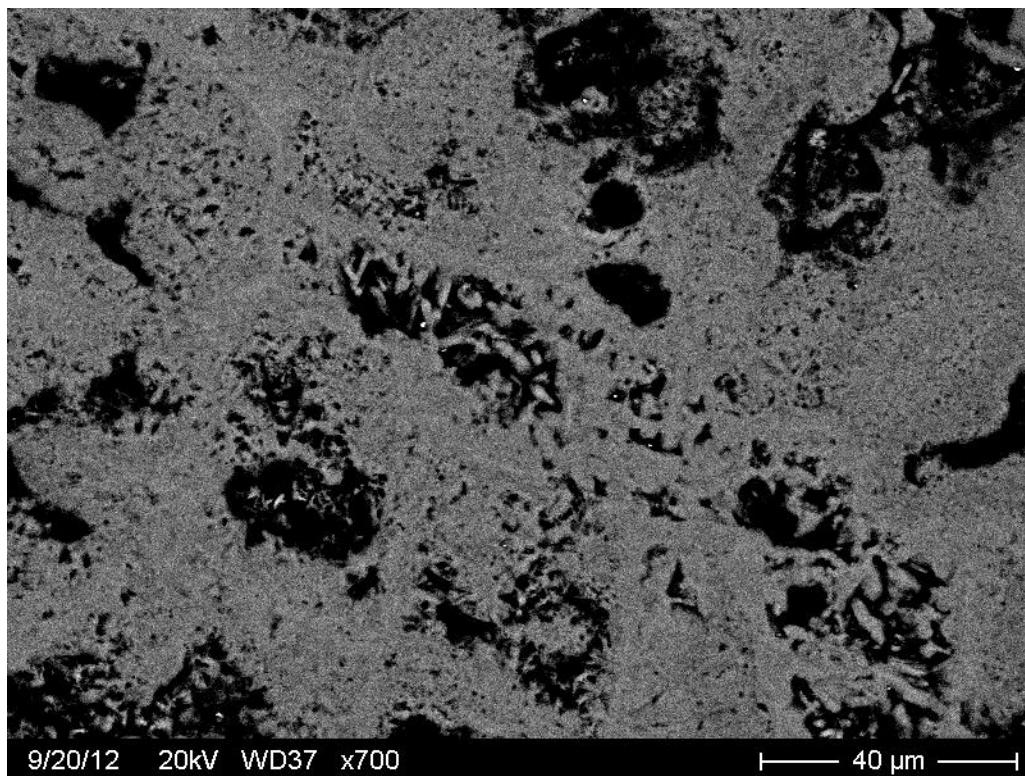
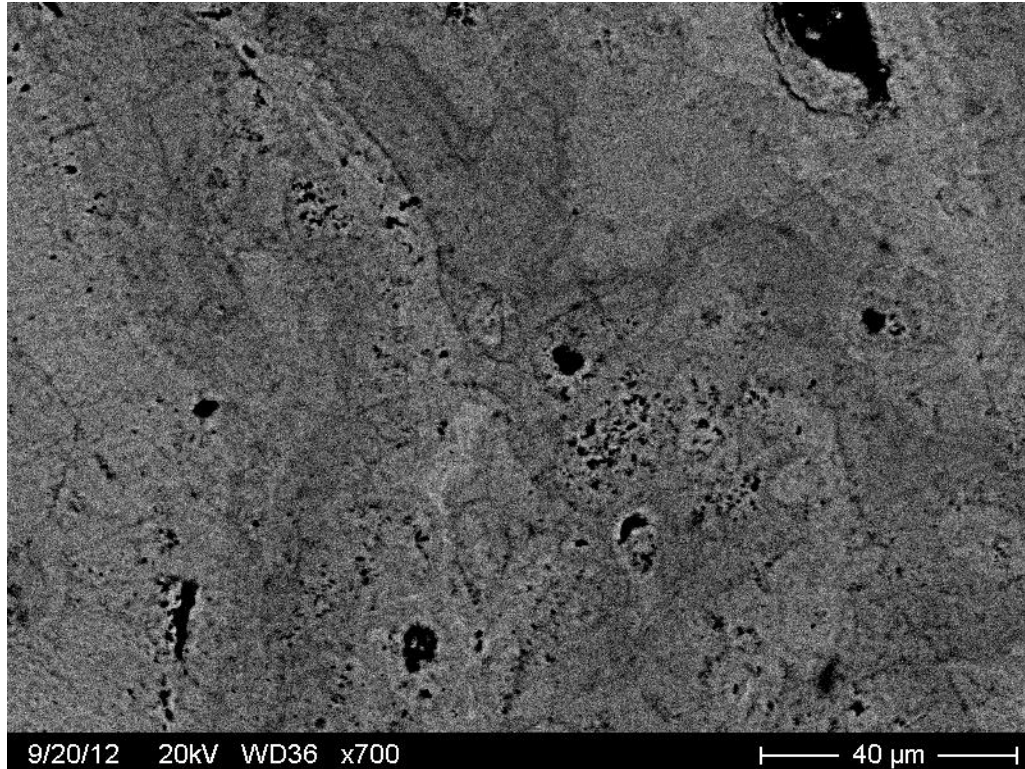
5) Backscatter electron images of goethite samples 1-10 after to exposure to SRB





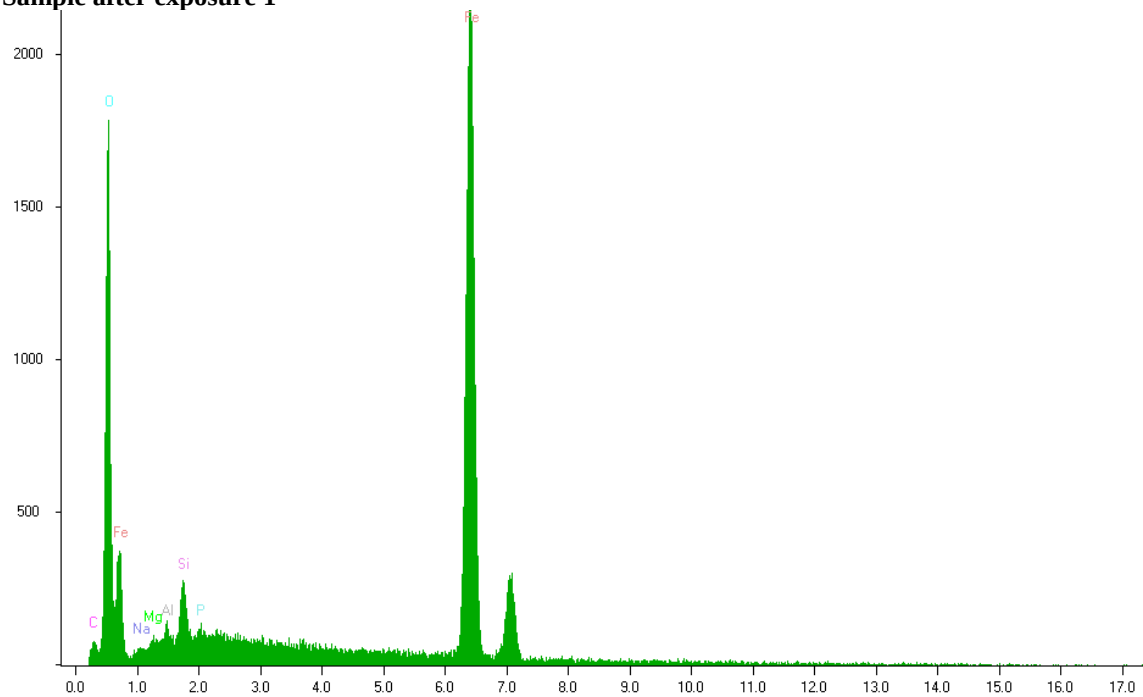
Goethite Sample 6



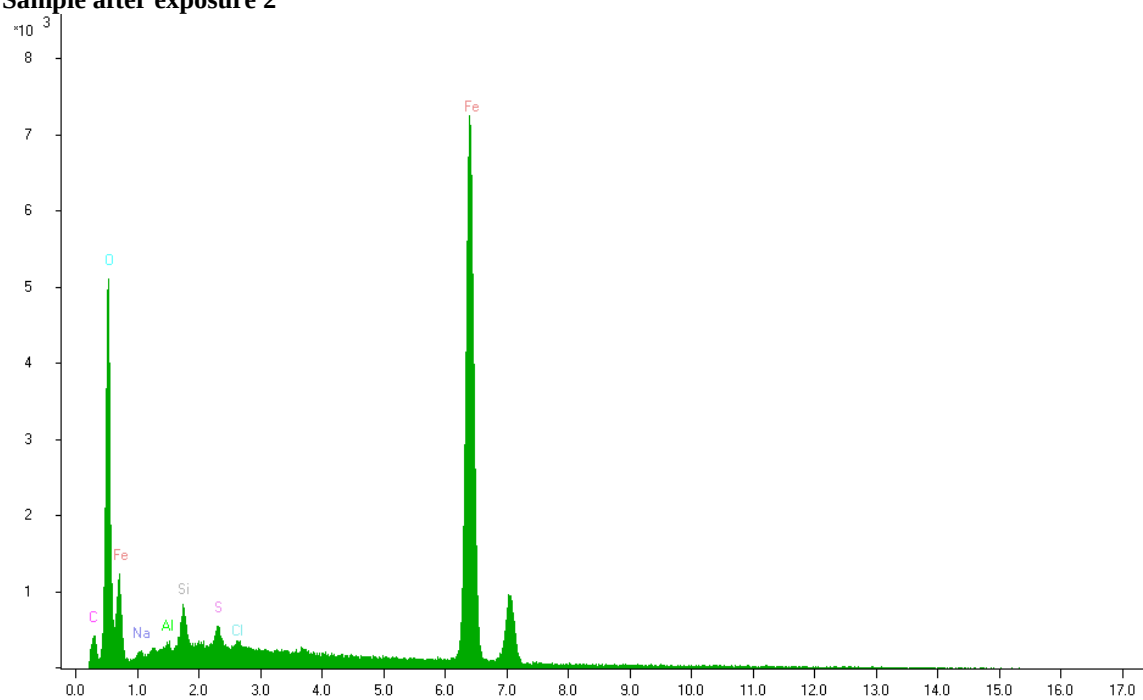


6) EDS spectra of goethite samples

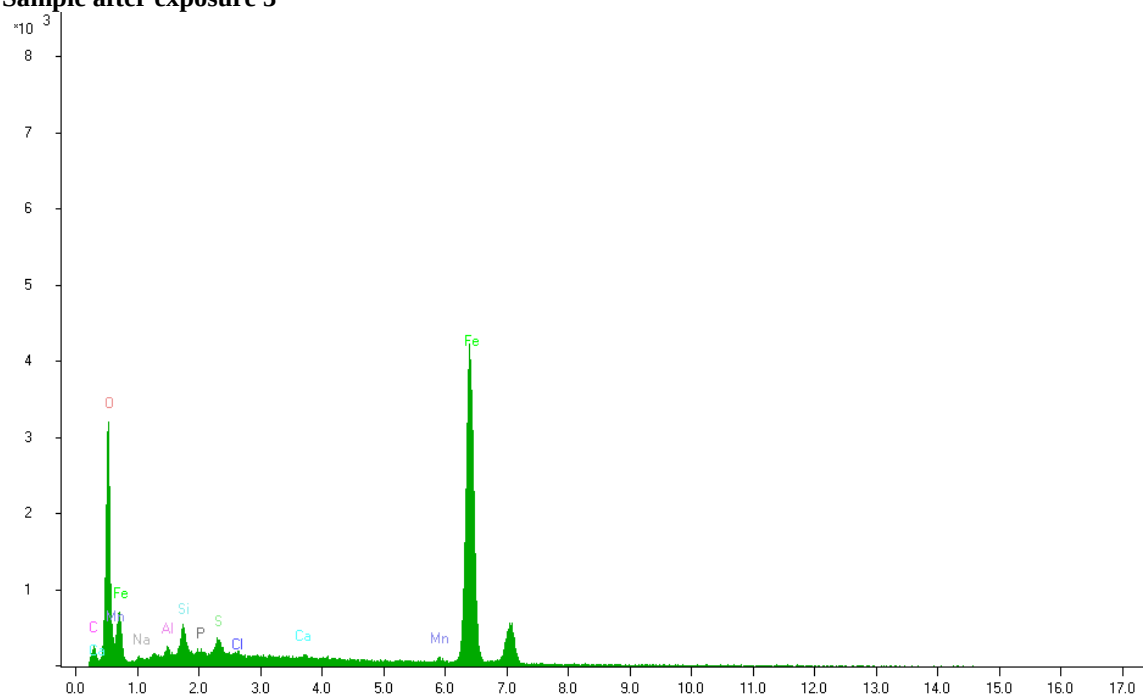
Sample after exposure 1



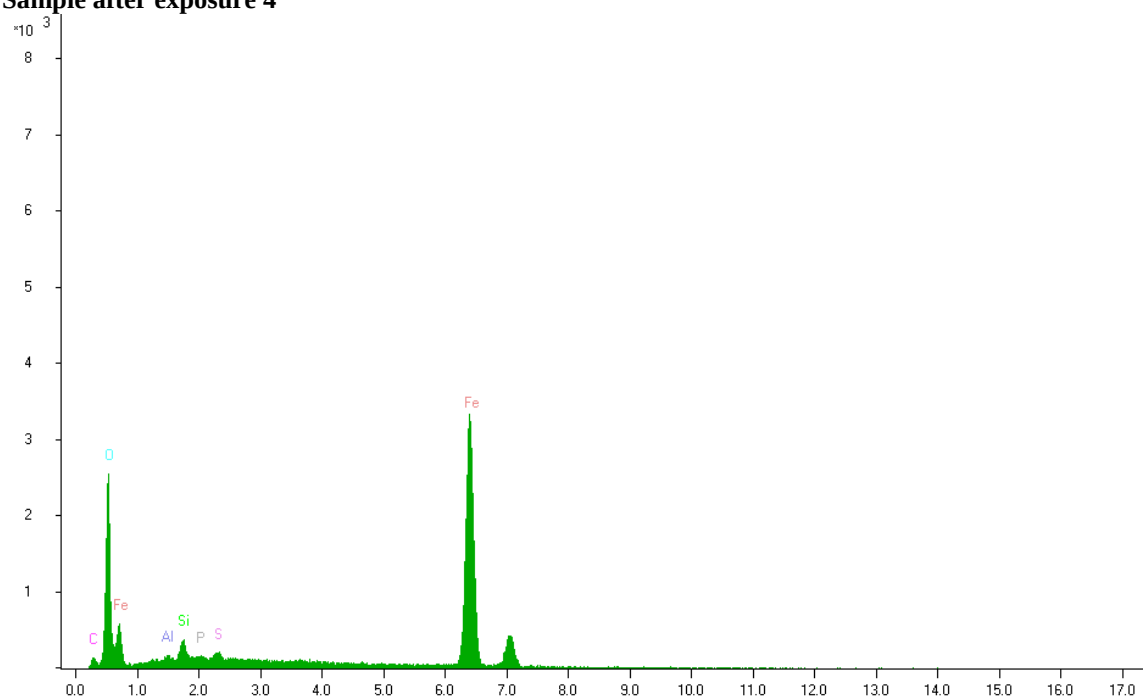
Sample after exposure 2

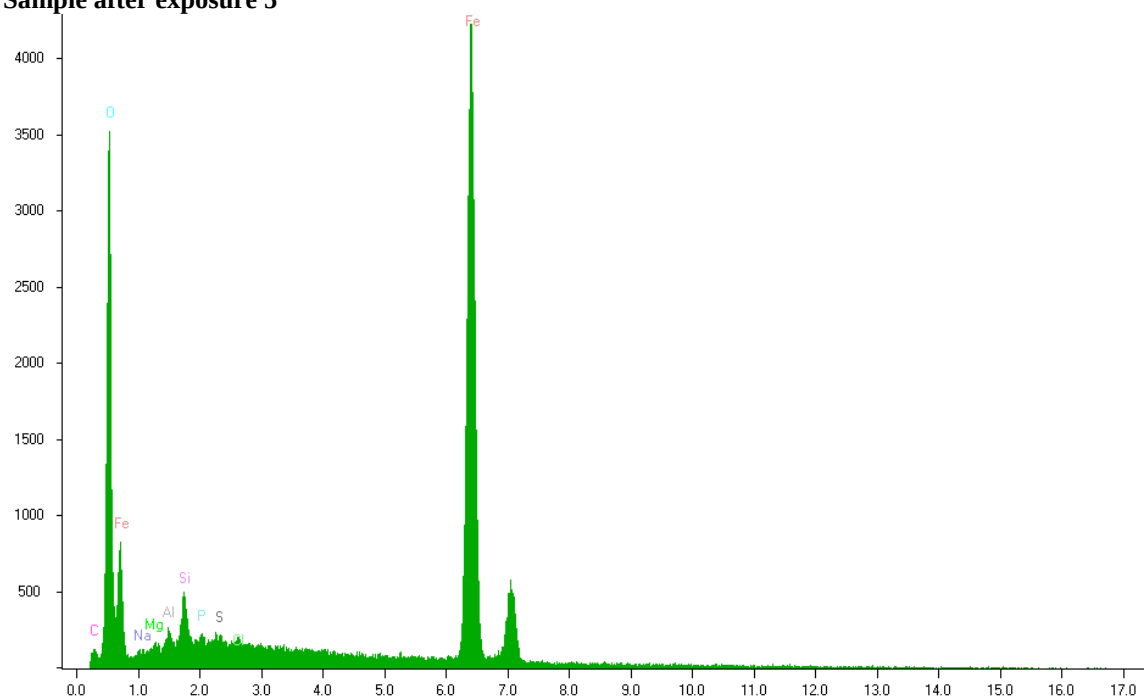
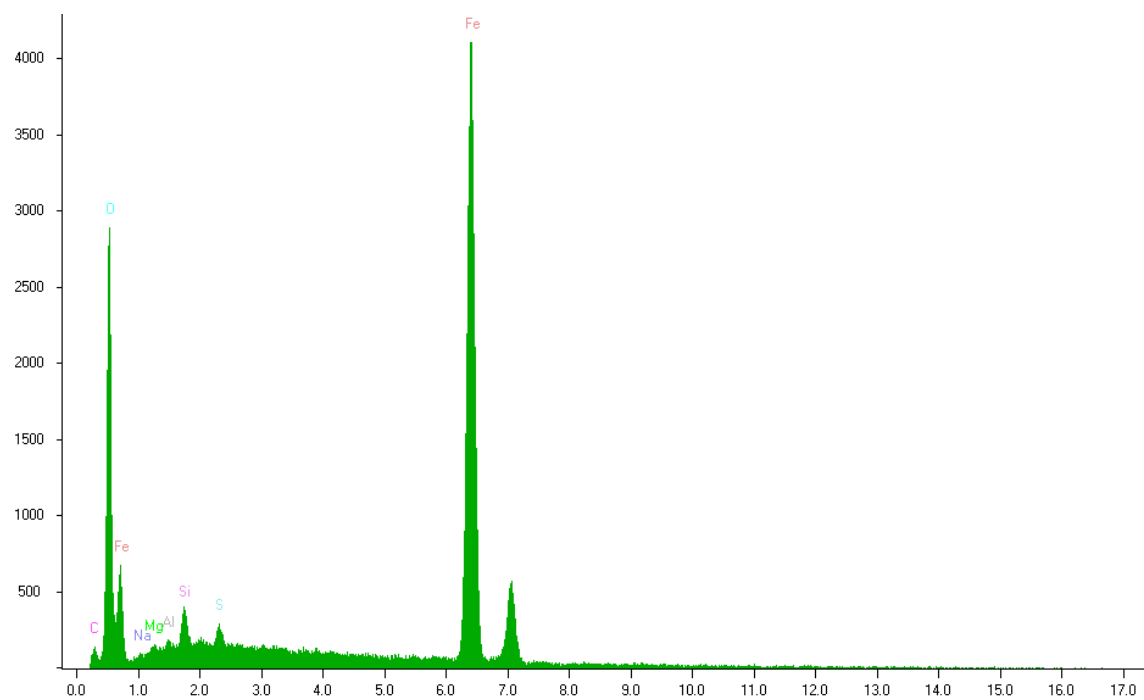


Sample after exposure 3

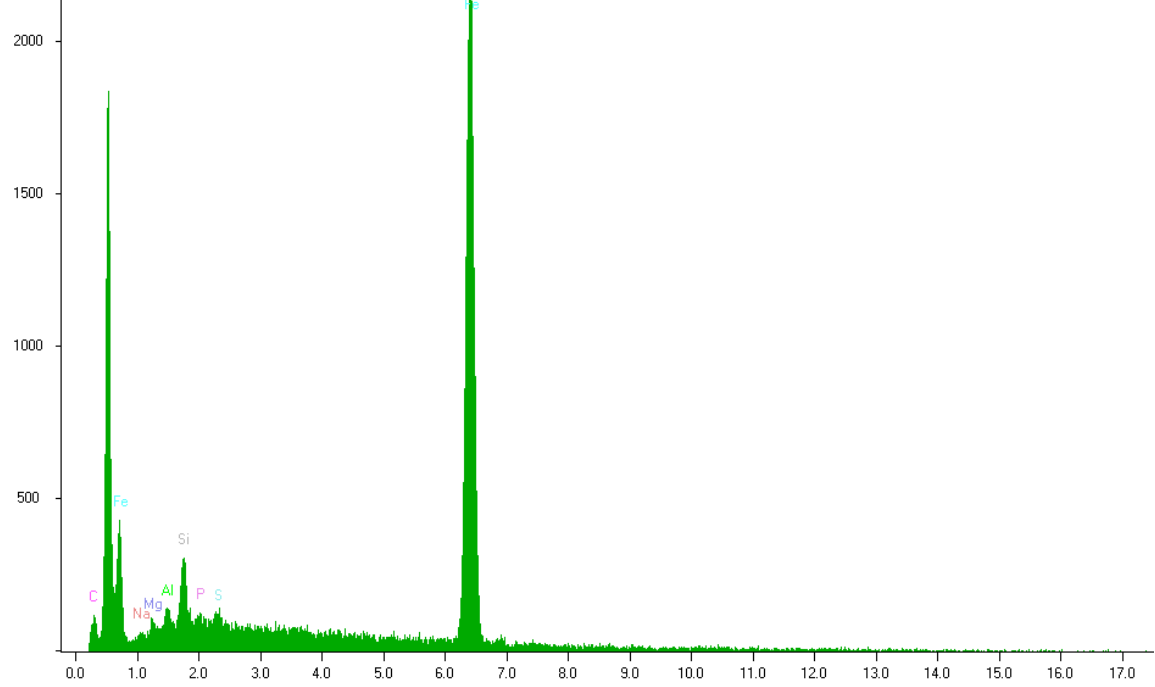


Sample after exposure 4

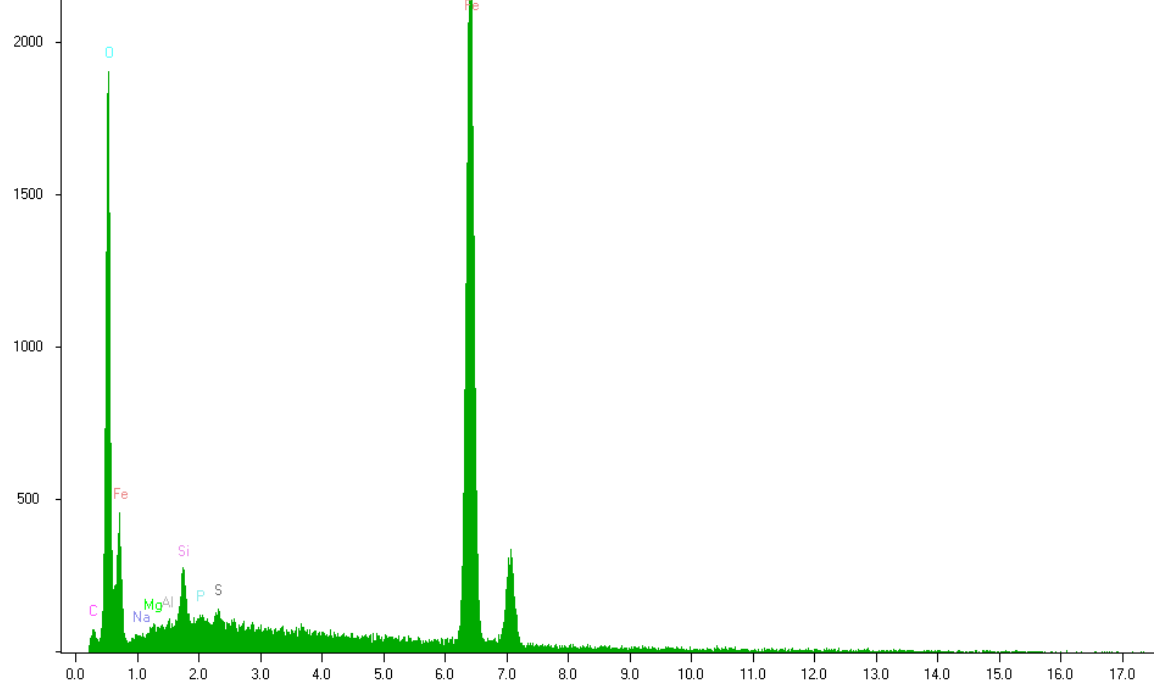


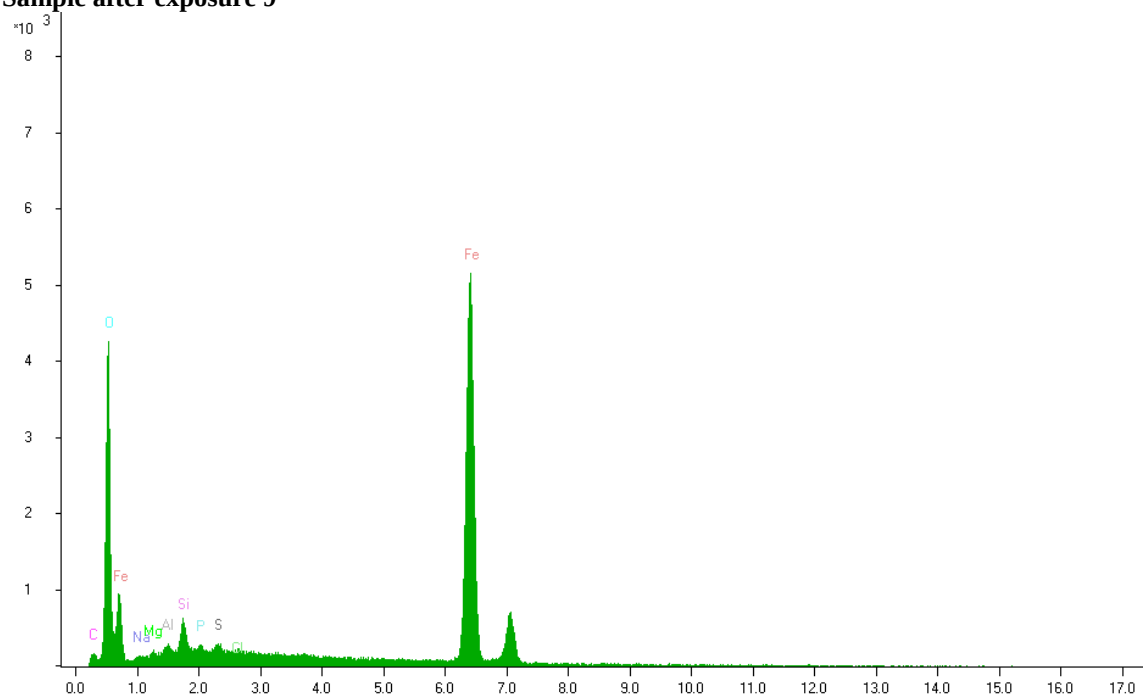
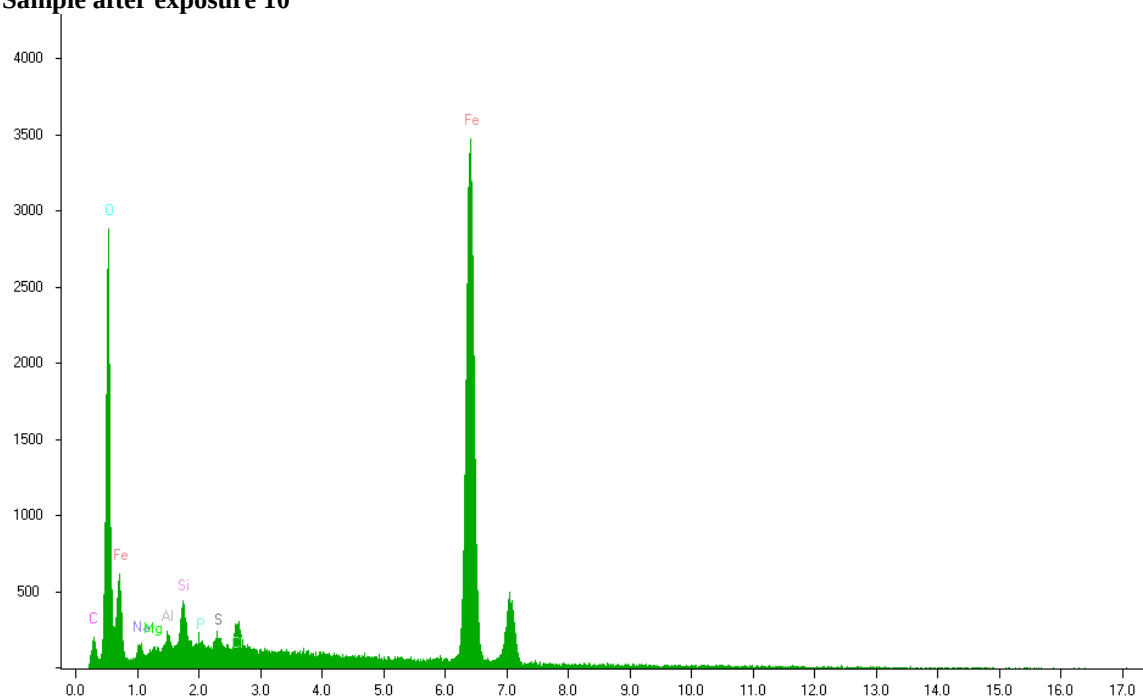
Sample after exposure 5**Sample after exposure 6**

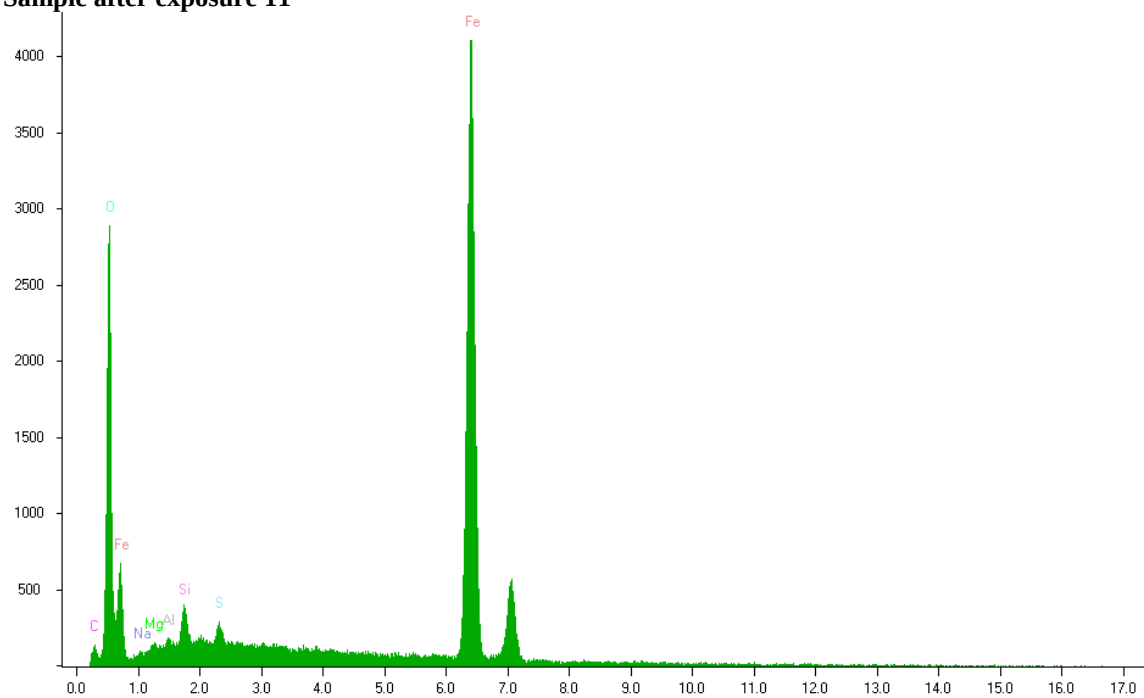
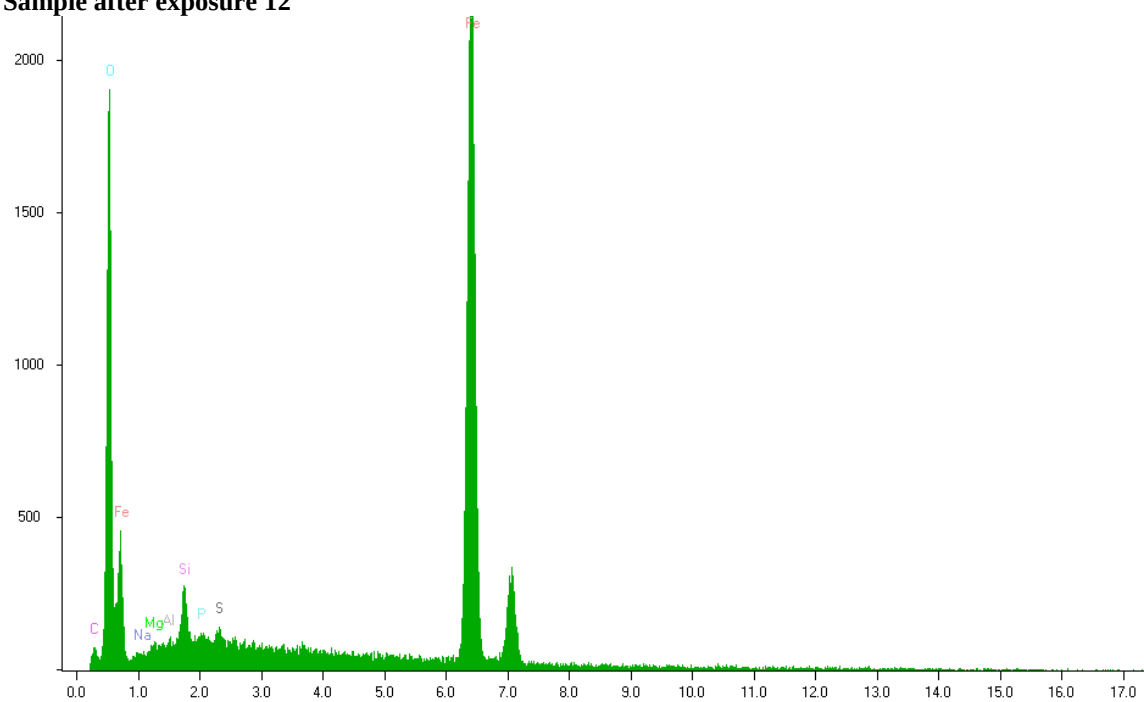
Sample after exposure 7



Sample after exposure 8



Sample after exposure 9**Sample after exposure 10**

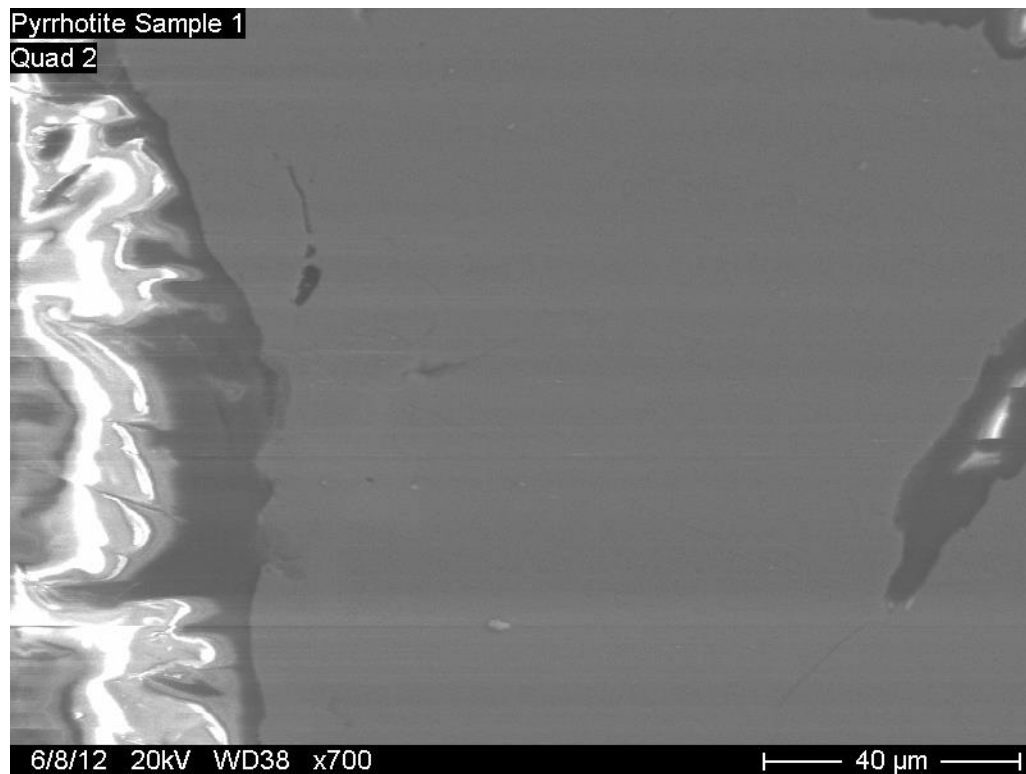
Sample after exposure 11**Sample after exposure 12**

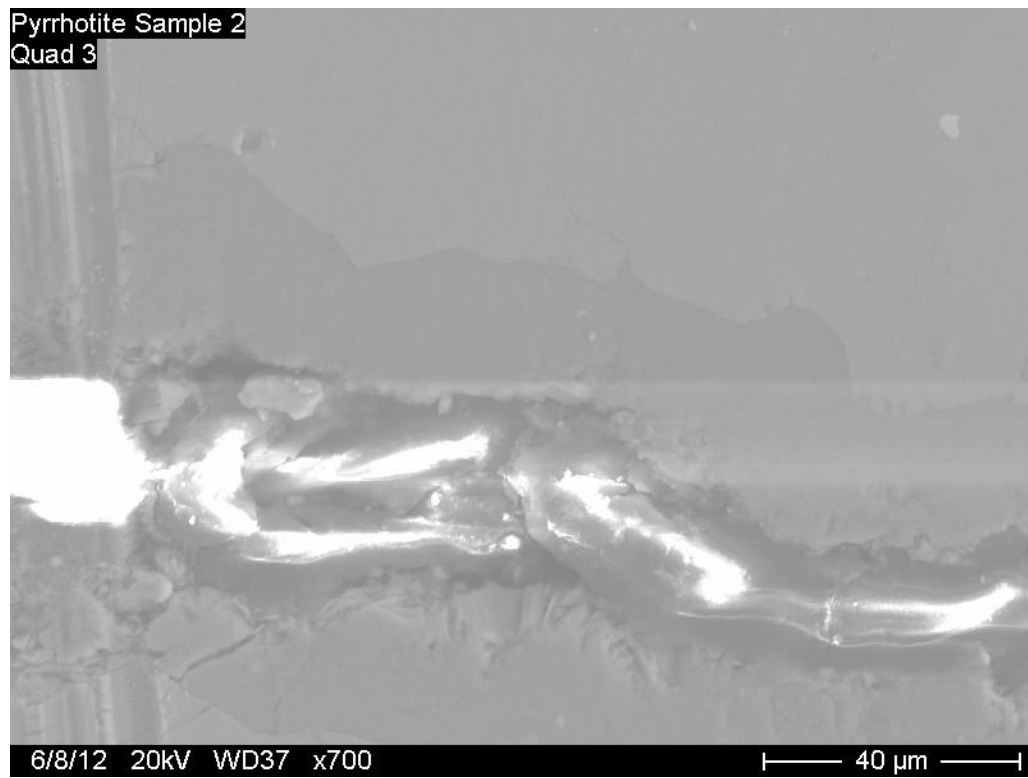
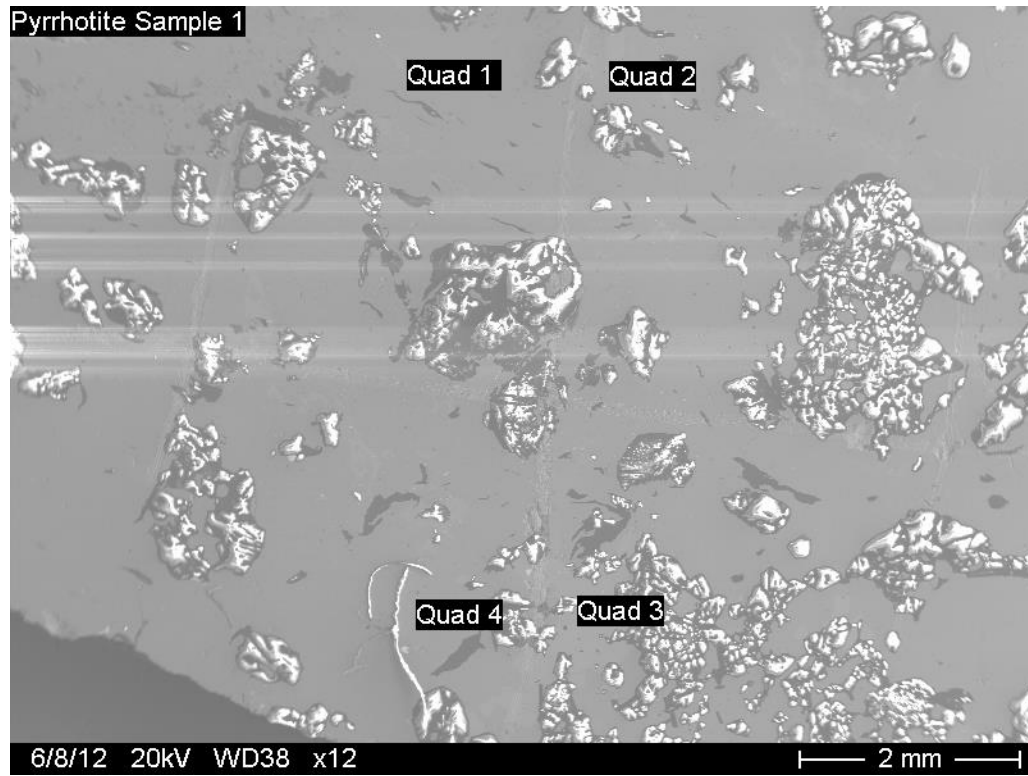
APPENDIX B

SEM IMAGES AND EDS SPECTRA OF PYRRHOTITE
BEFORE AND AFTER EXPOSURE TO SRB

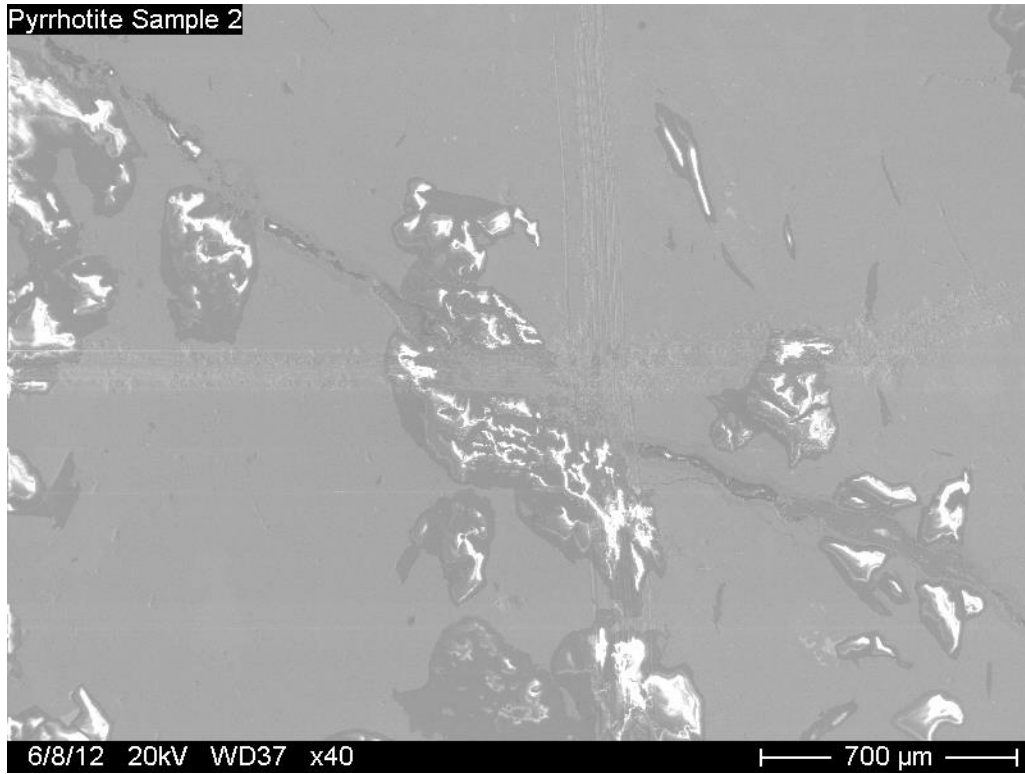
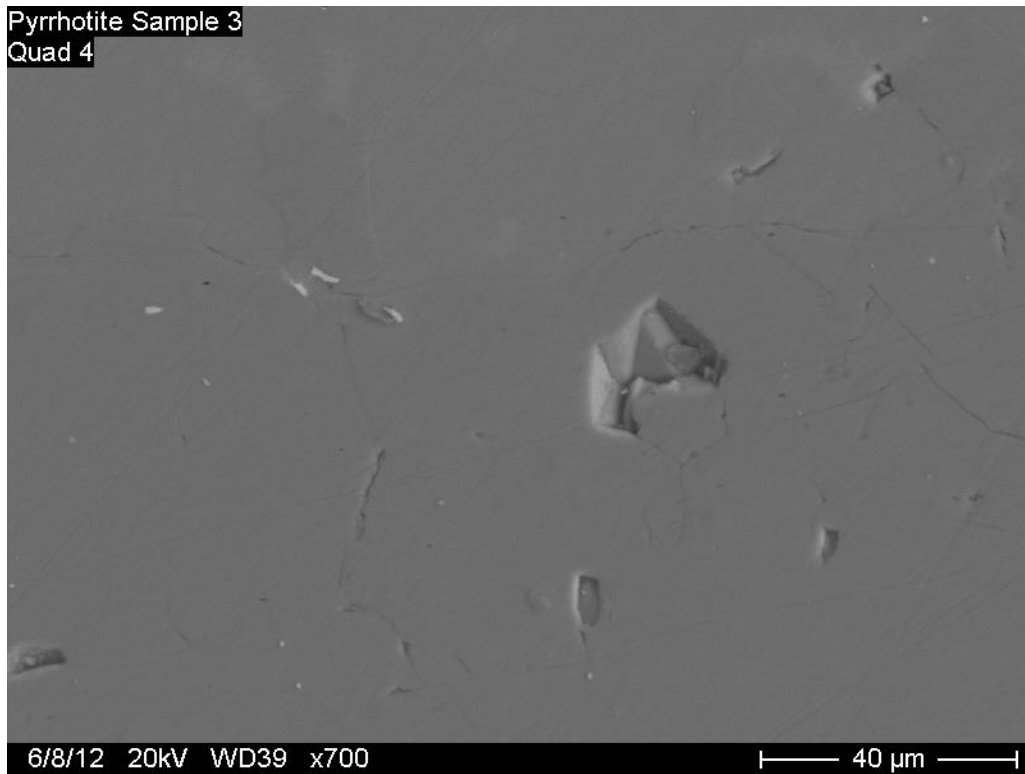
Appendix B contains SEM secondary electron backscatter electron images with EDS spectra of pyrrhotite prior to and after exposure to SRB. 1) Secondary electron images before exposure; 2) Back scattered electron images before exposure; 3) EDS spectra before exposure; 4) secondary electron images after exposure; 5) Backscatter electron images after exposure; 6) EDS after exposure.

1) SEM secondary electron images of pyrrhotite samples 1-12 before exposure to SRB.

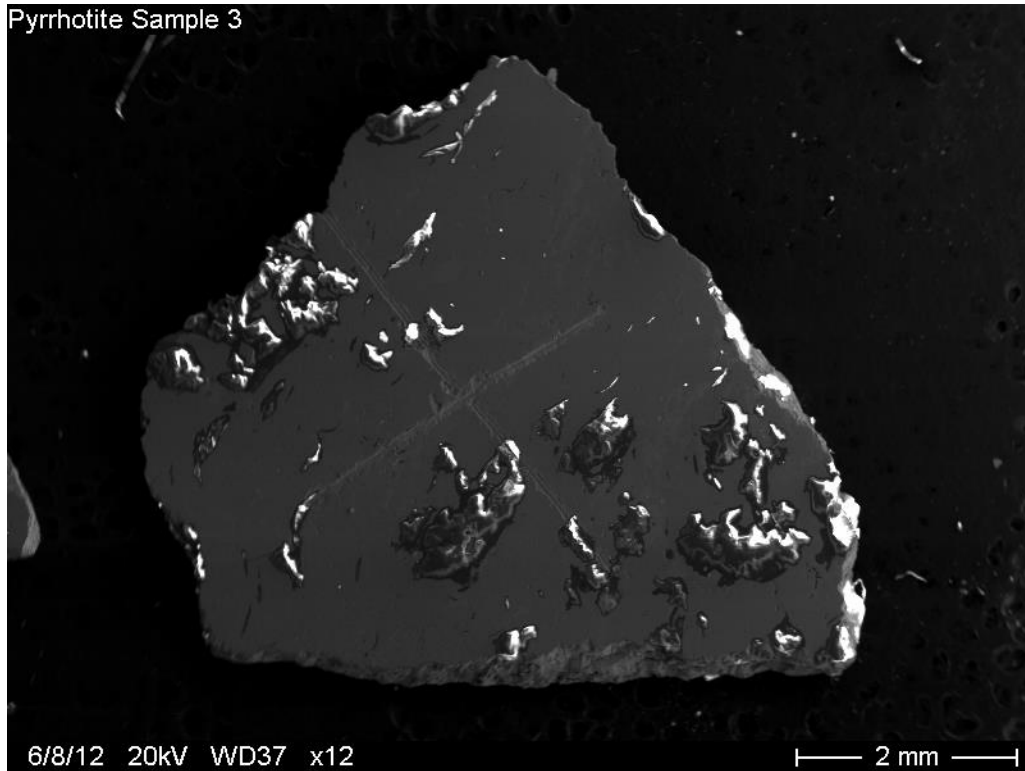


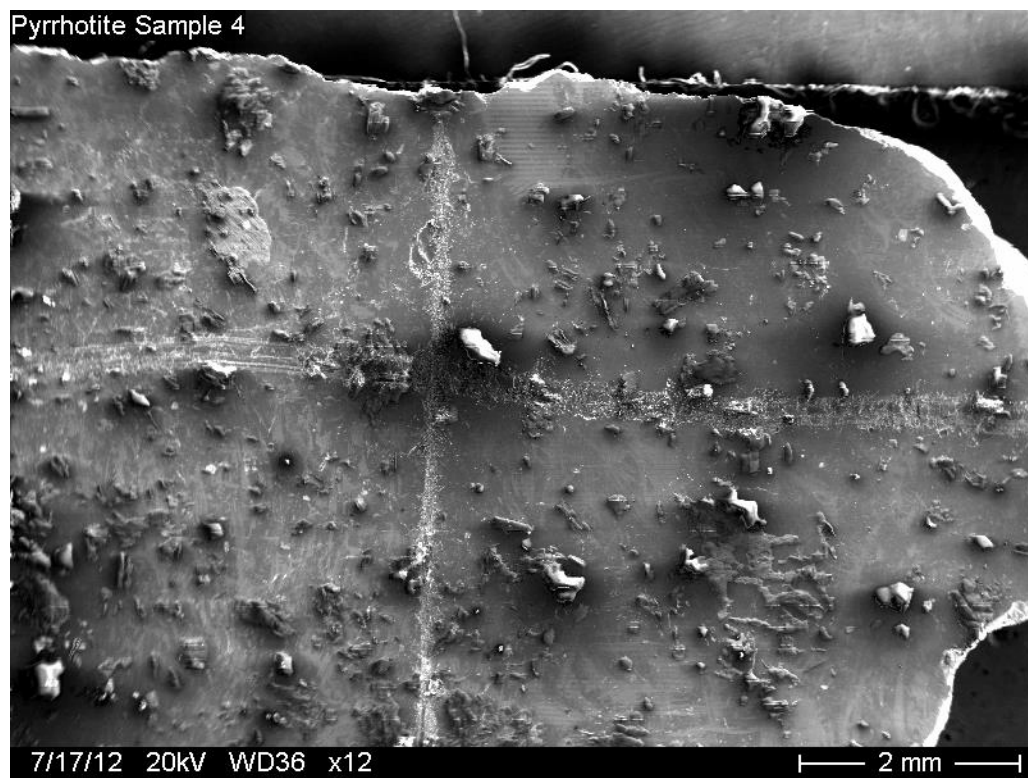
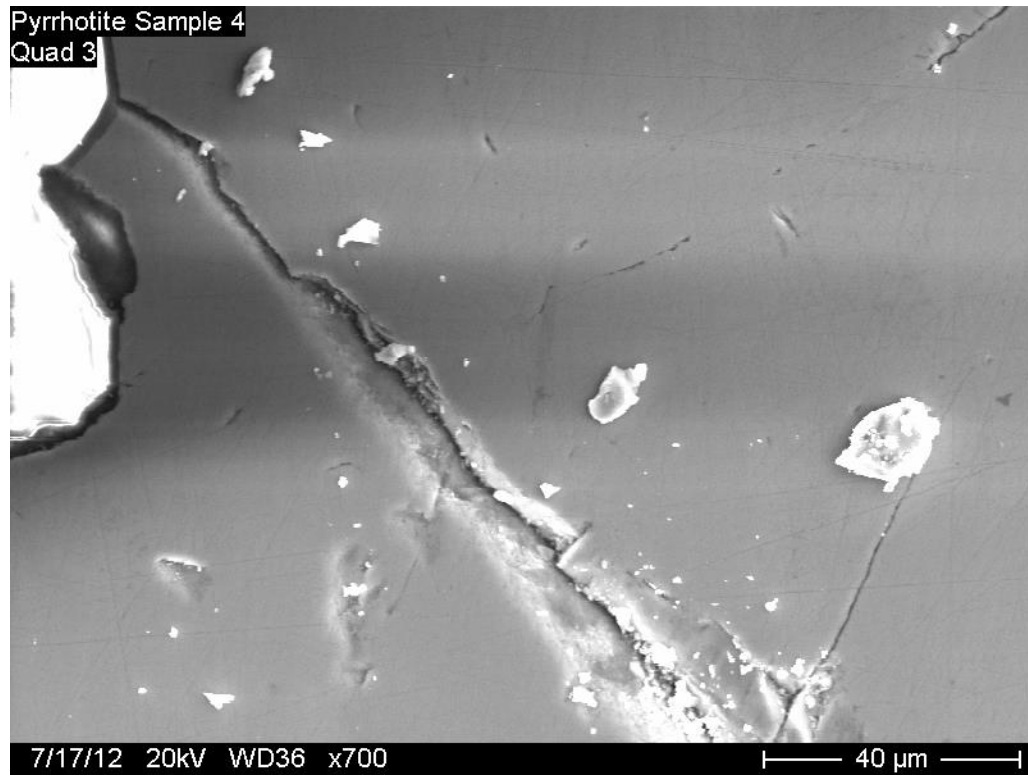


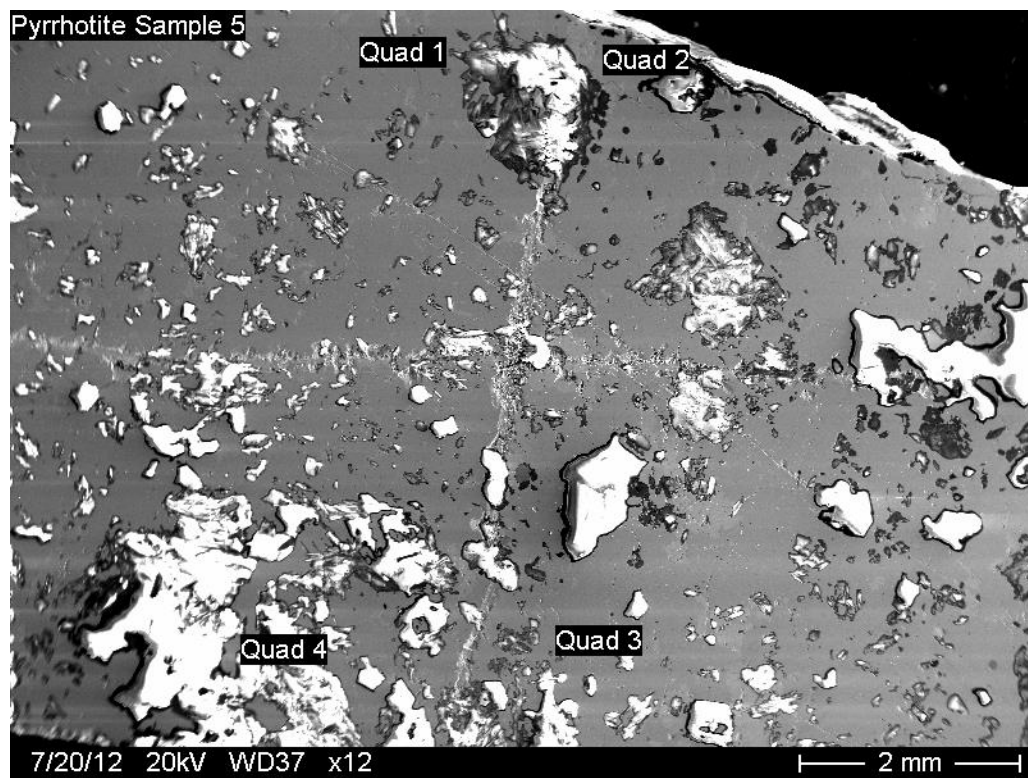
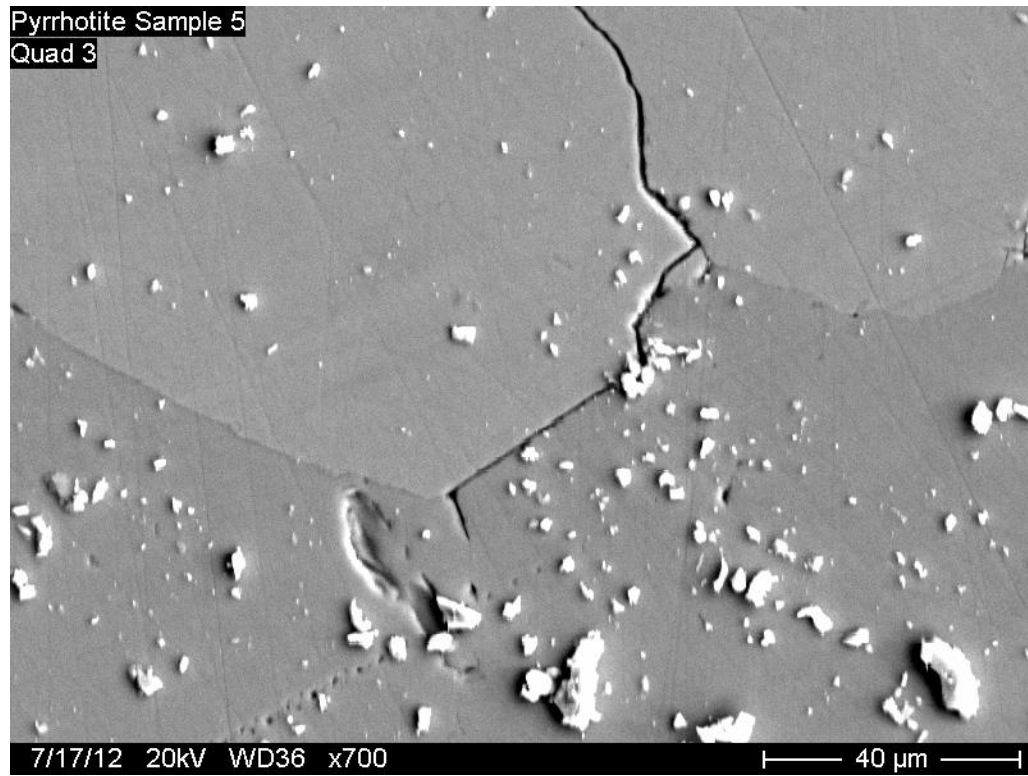
Pyrrhotite Sample 2

Pyrrhotite Sample 3
Quad 4

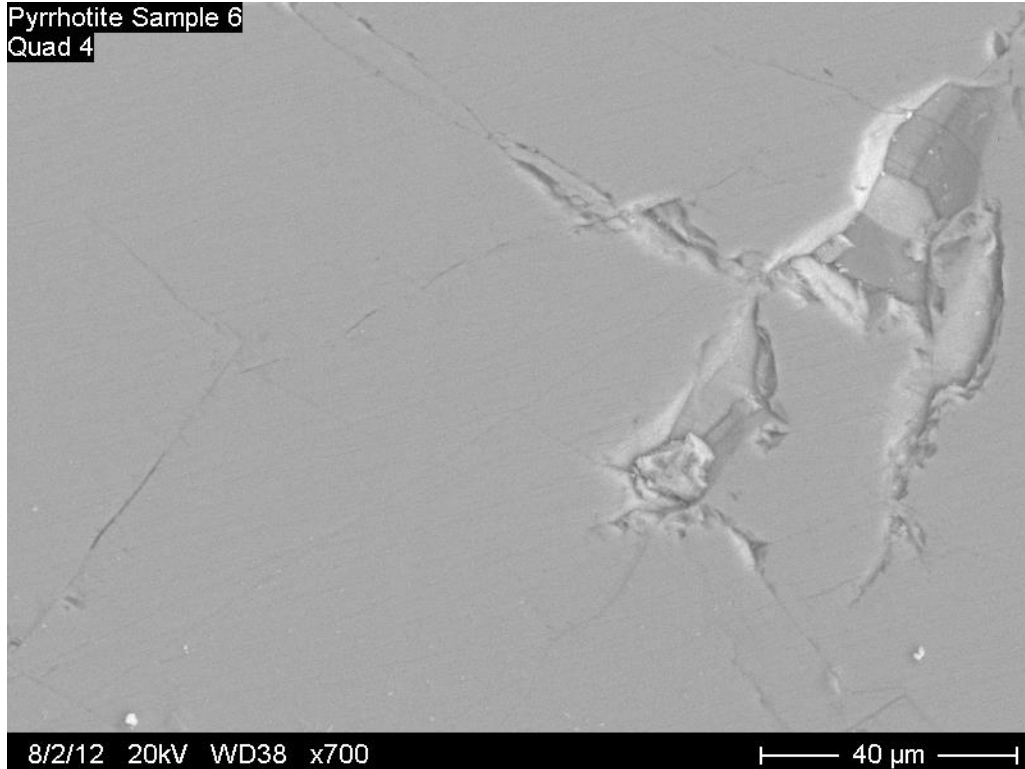
Pyrrhotite Sample 3



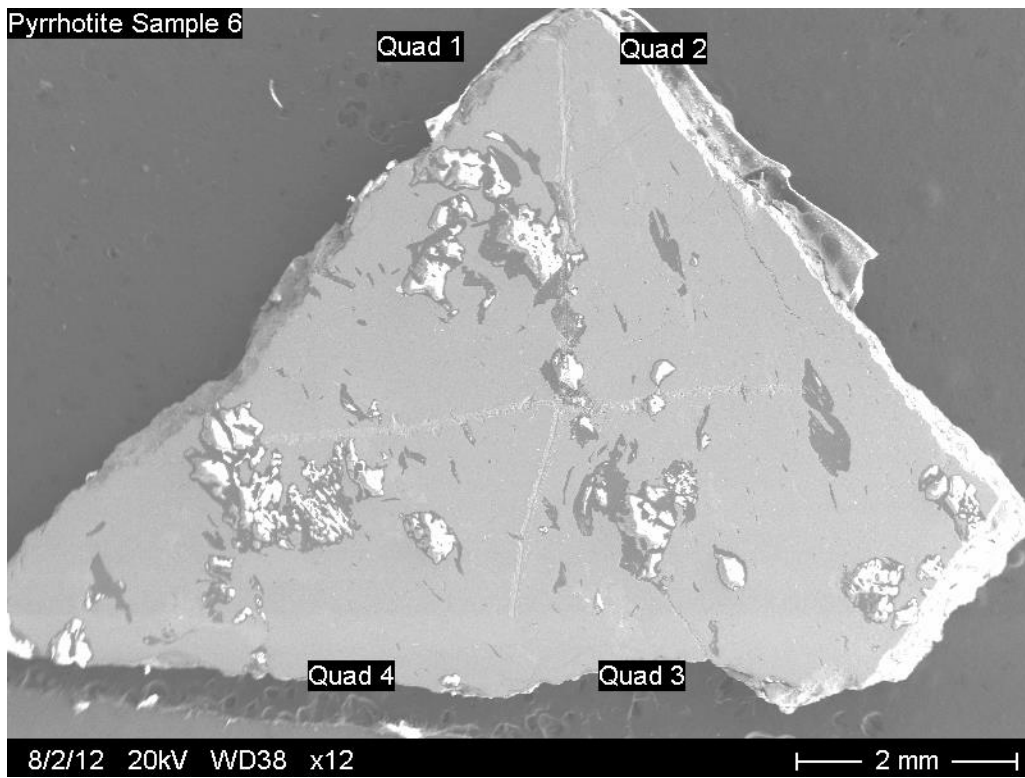


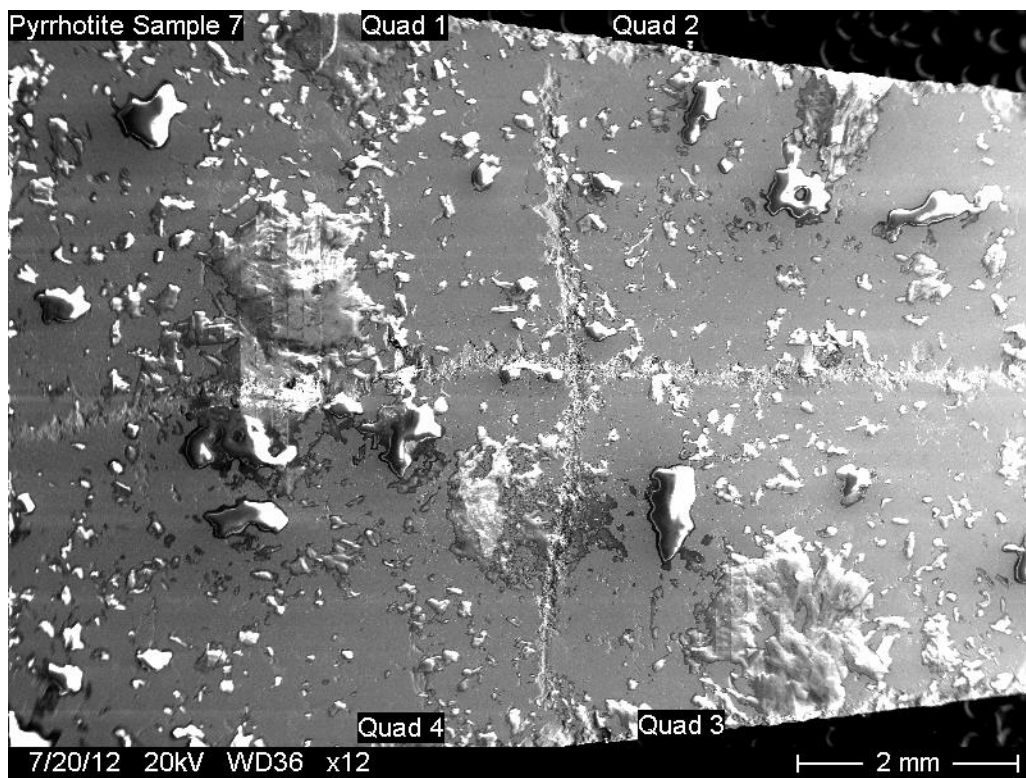
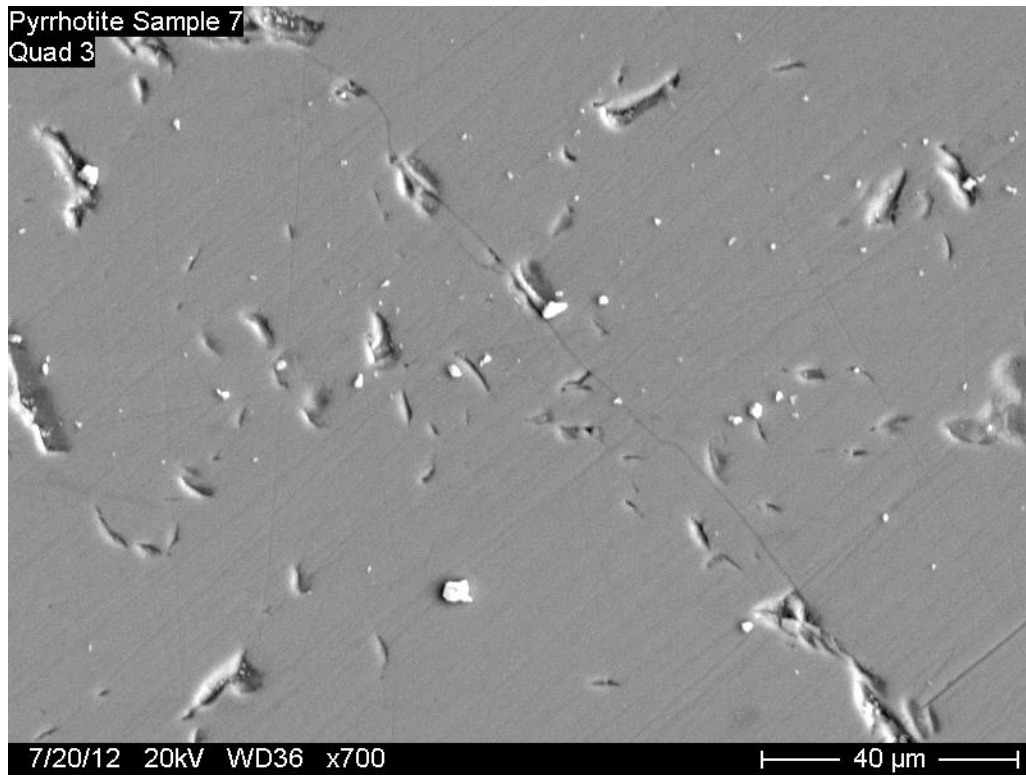


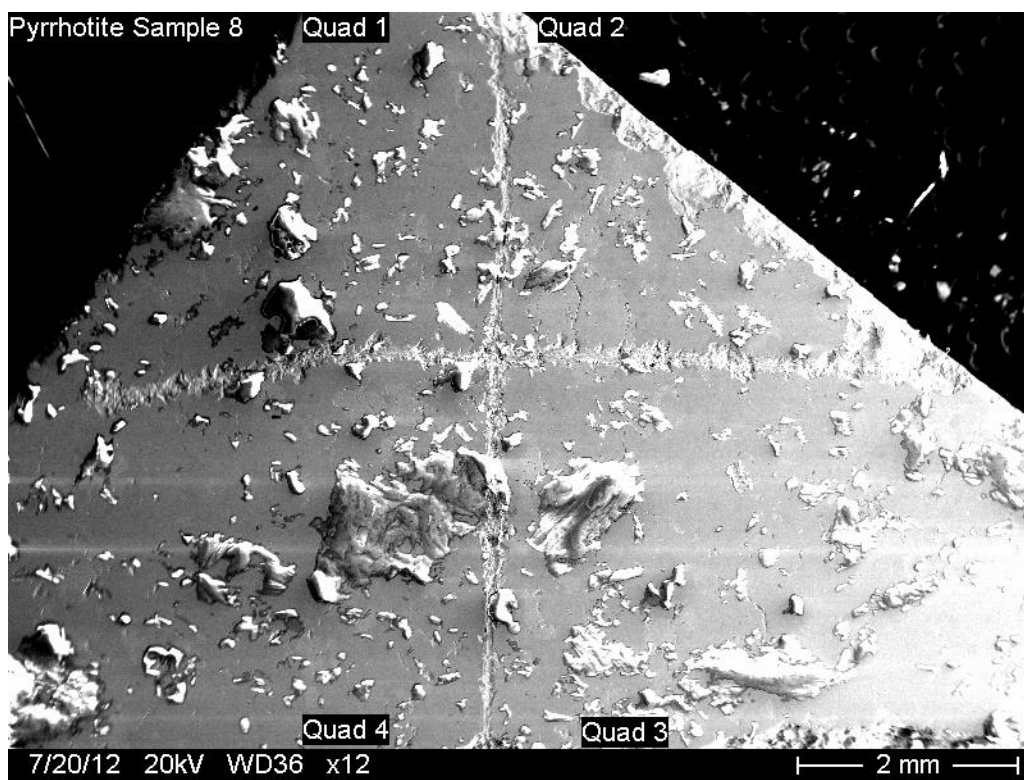
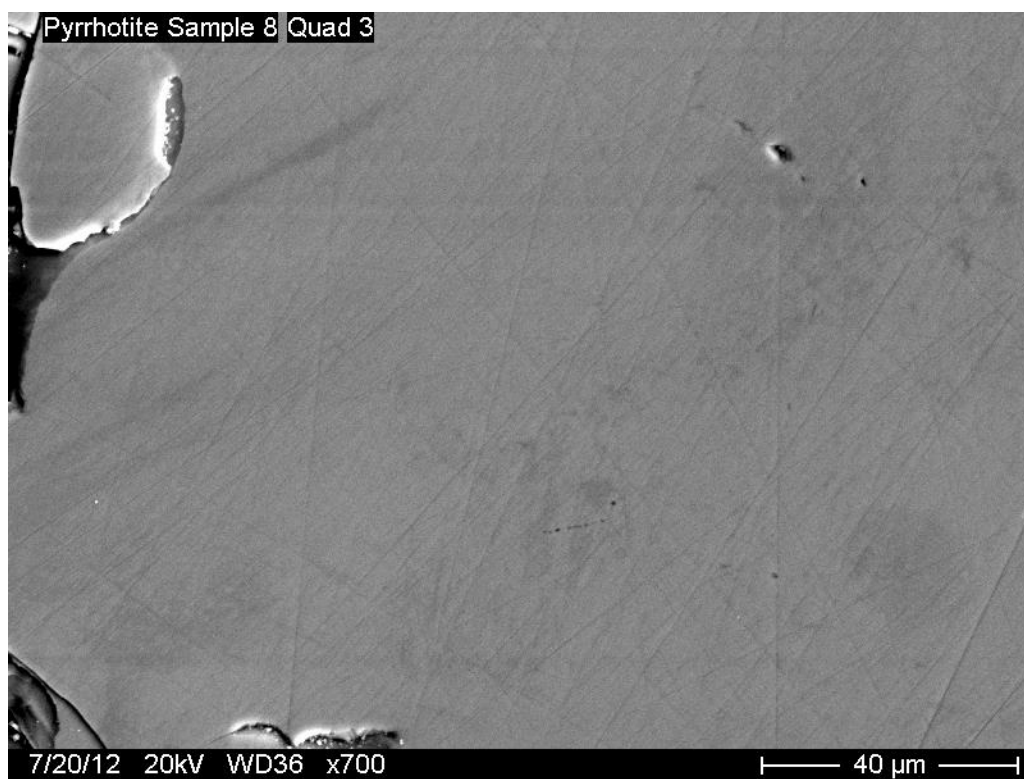
Pyrrhotite Sample 6
Quad 4



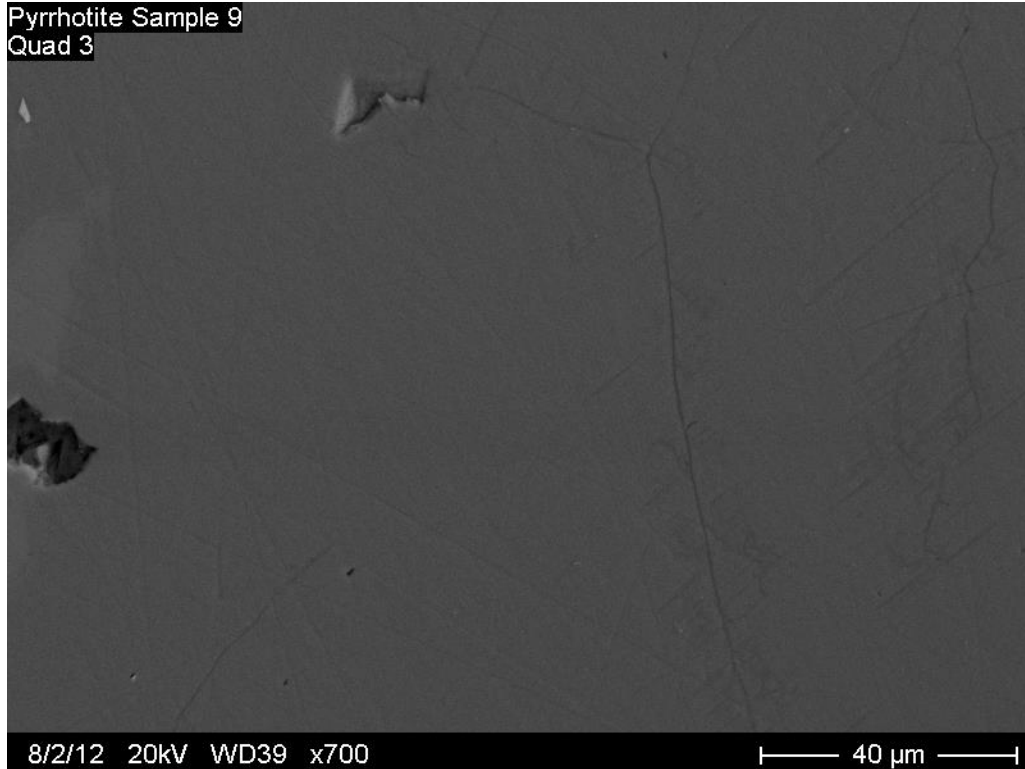
Pyrrhotite Sample 6



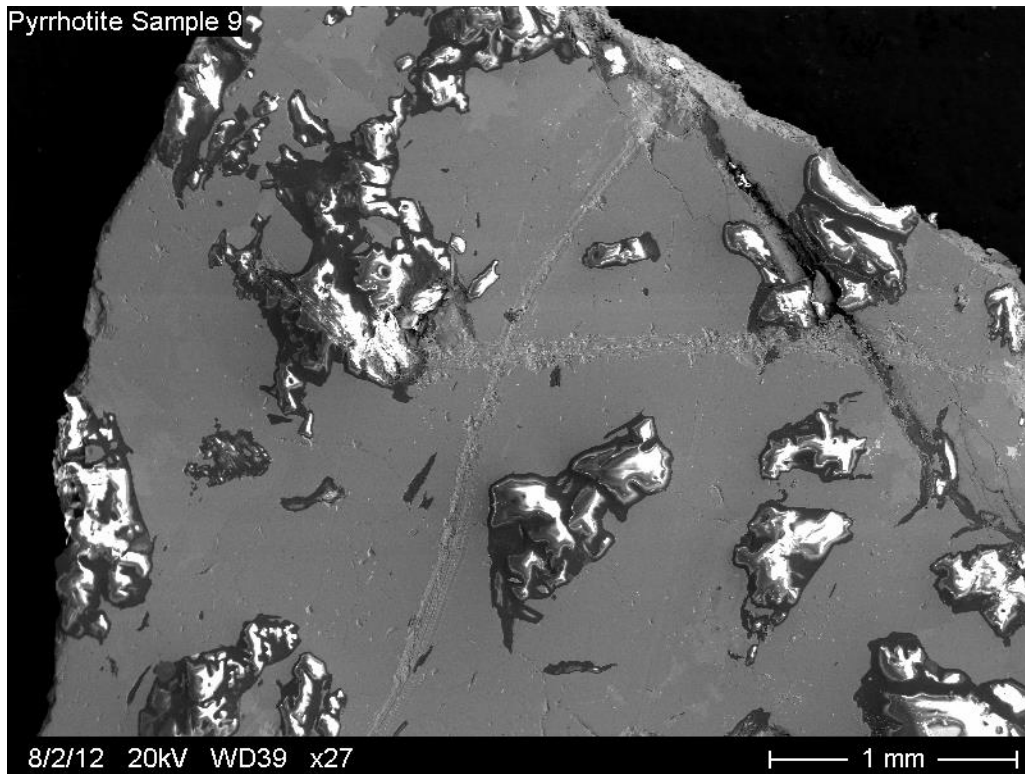




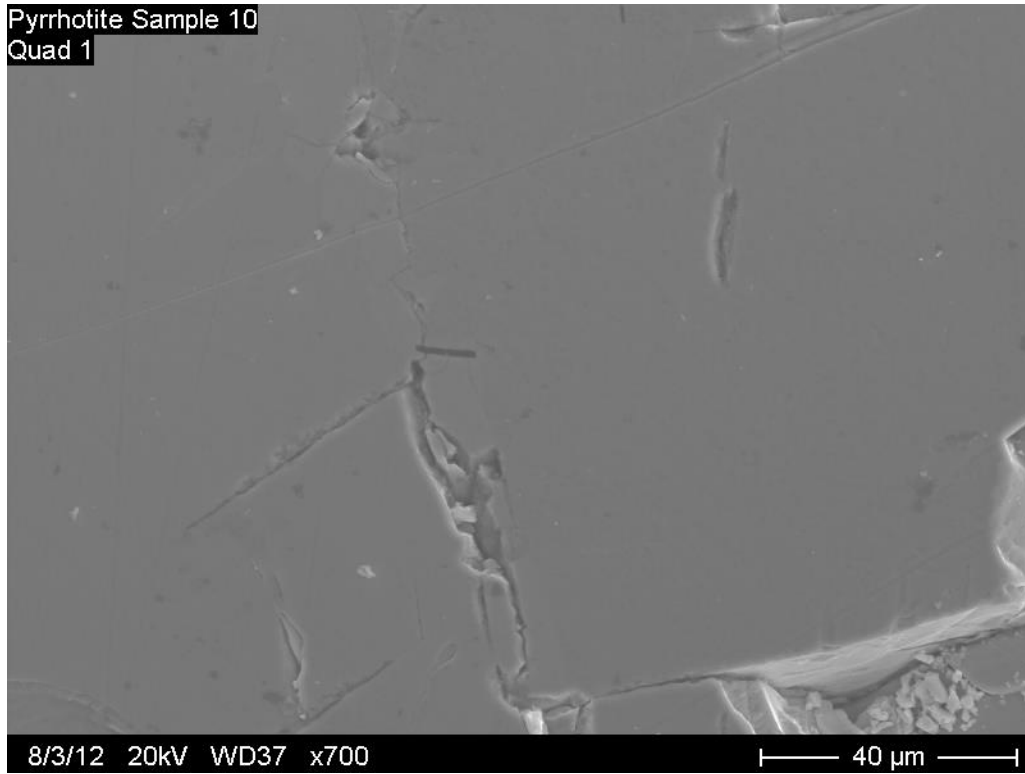
Pyrrhotite Sample 9
Quad 3



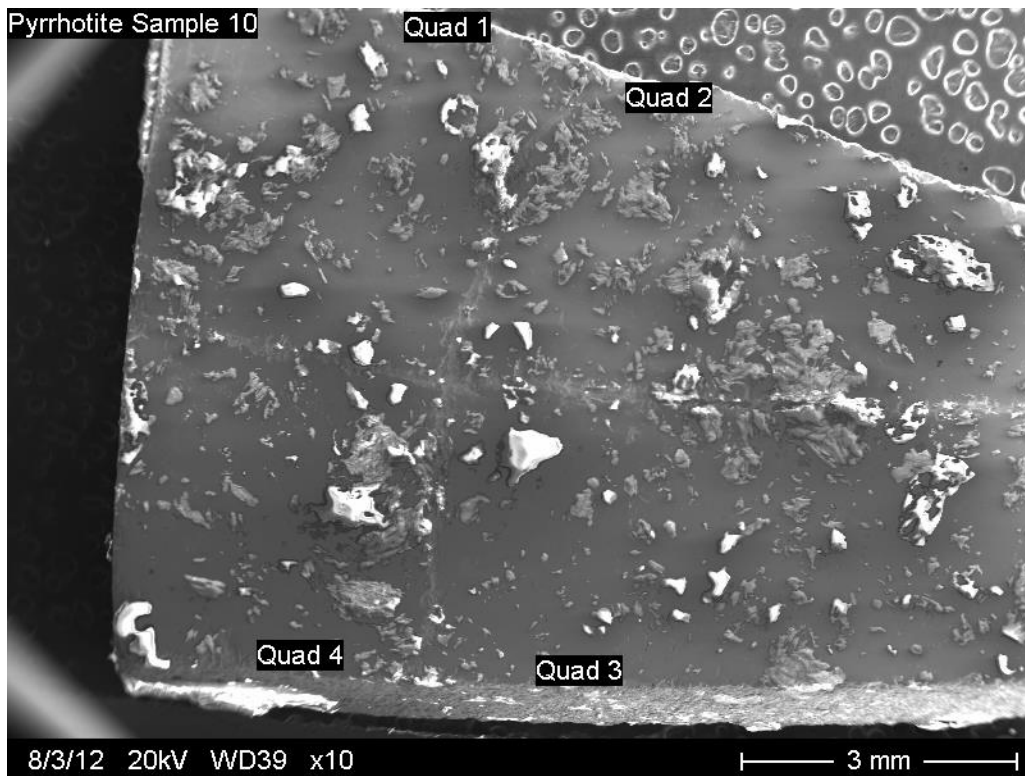
Pyrrhotite Sample 9

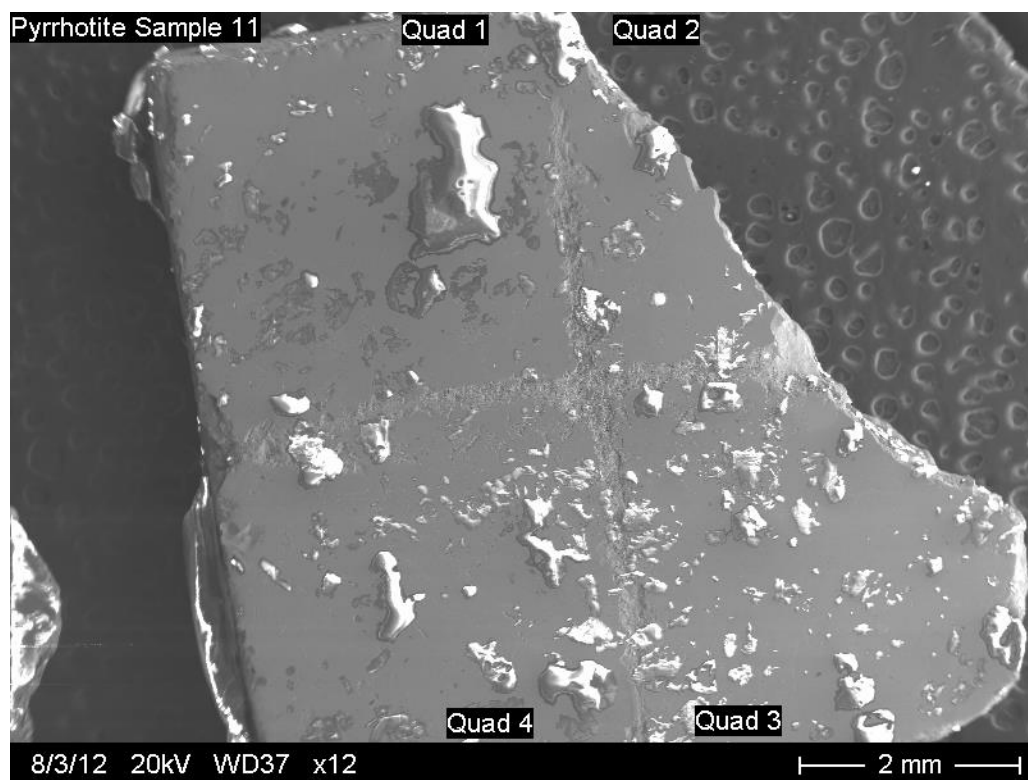


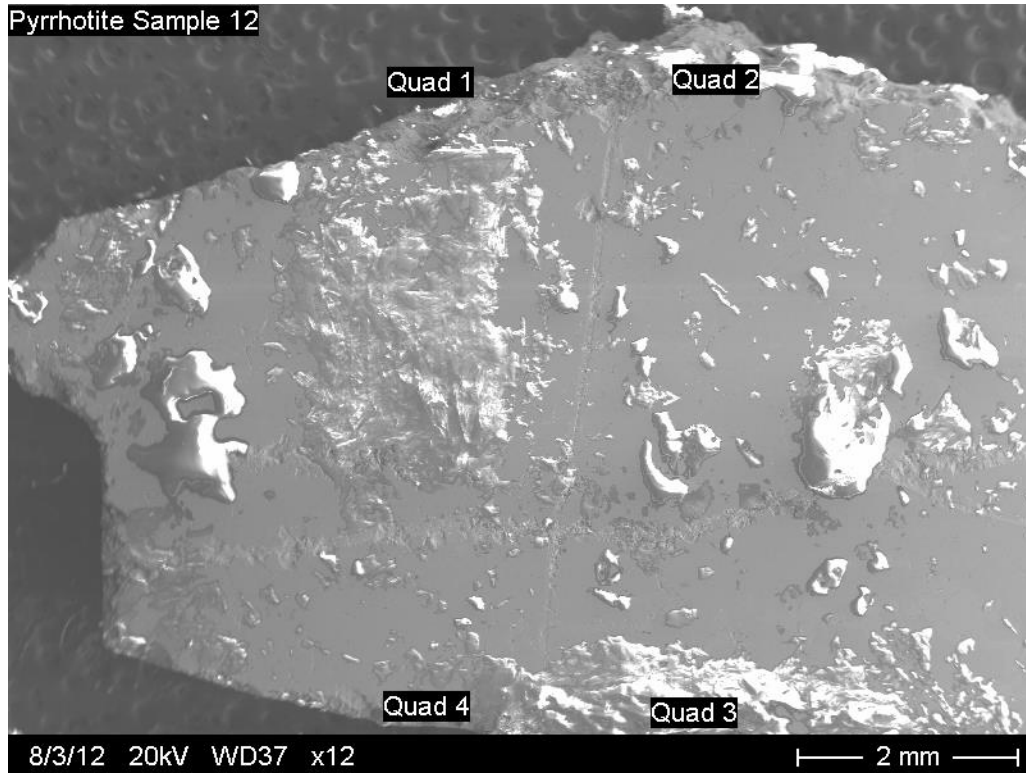
Pyrrhotite Sample 10
Quad 1



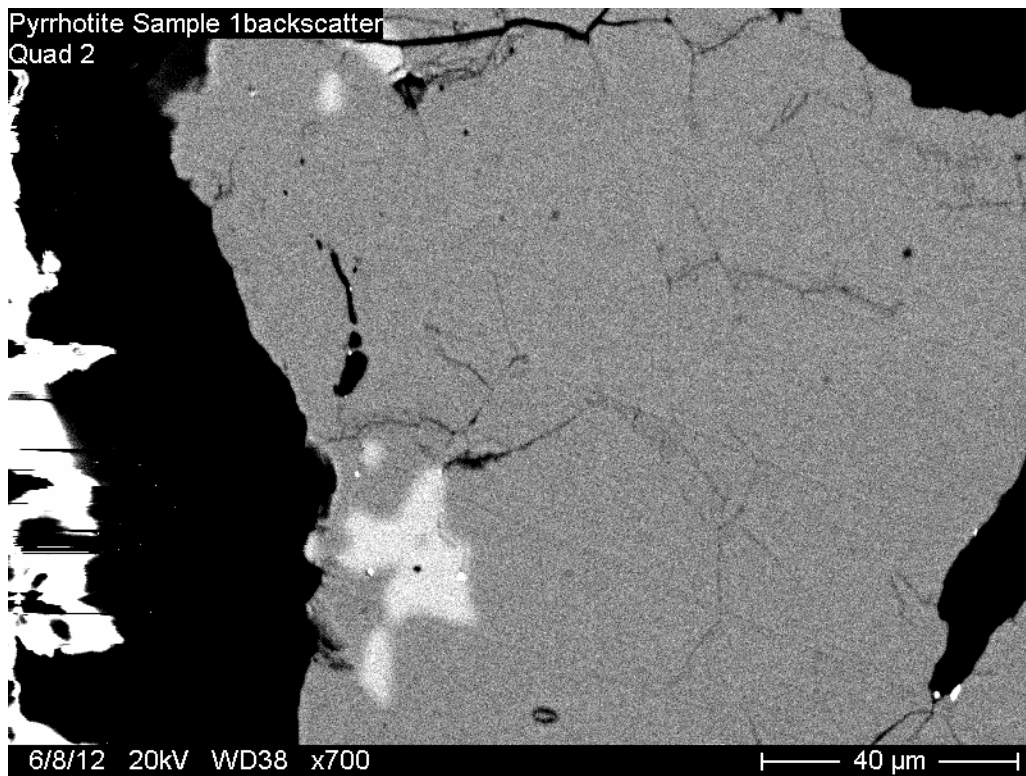
Pyrrhotite Sample 10

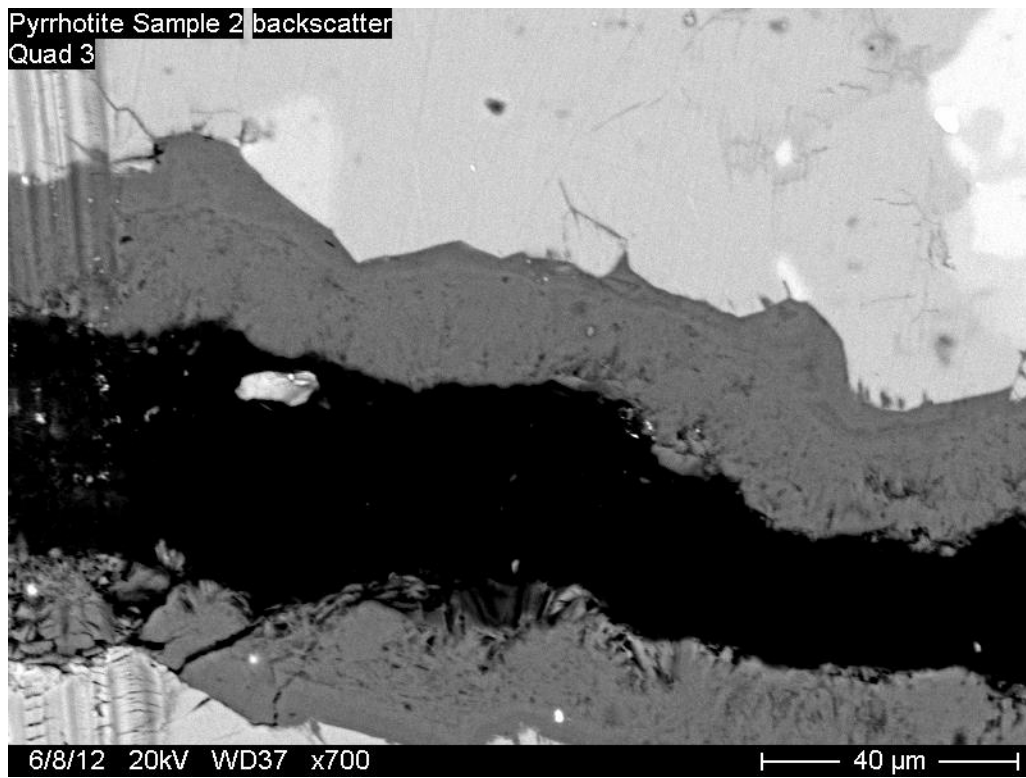
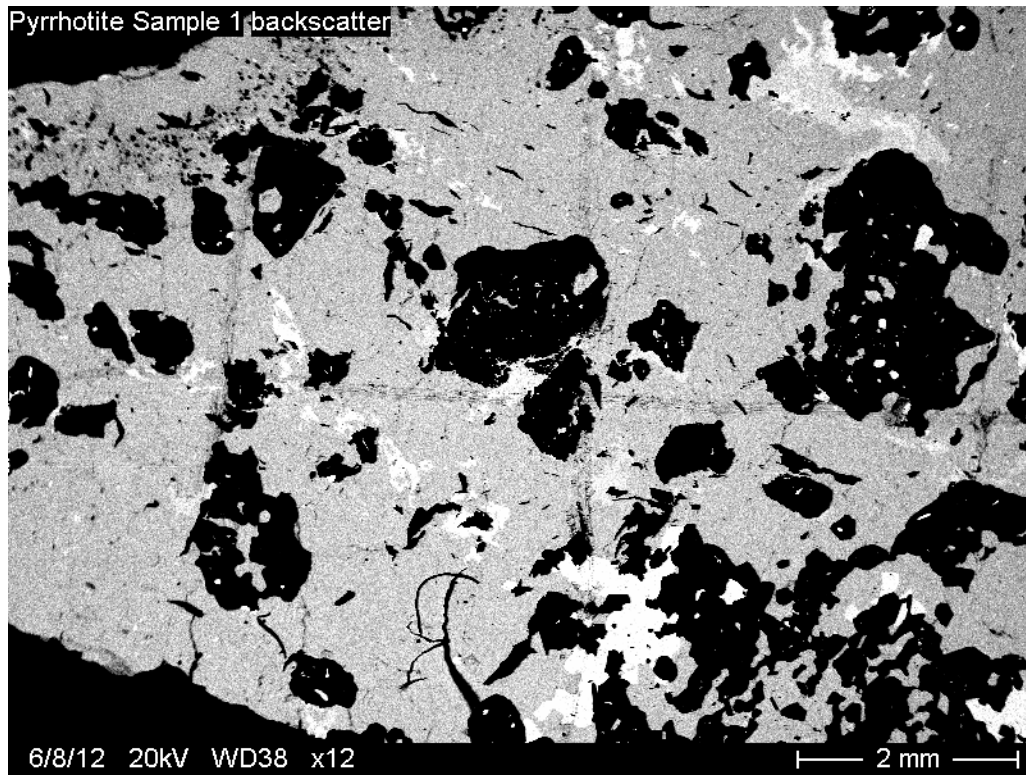




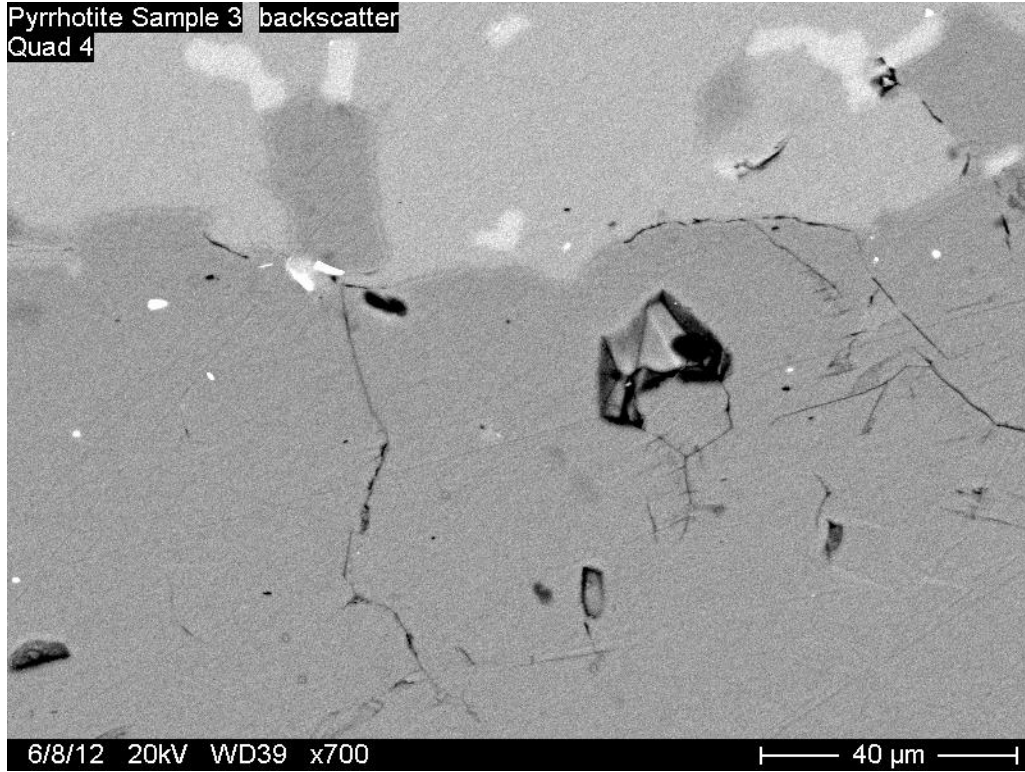


2) SEM backscatter electron images of pyrrhotite samples 1-12.

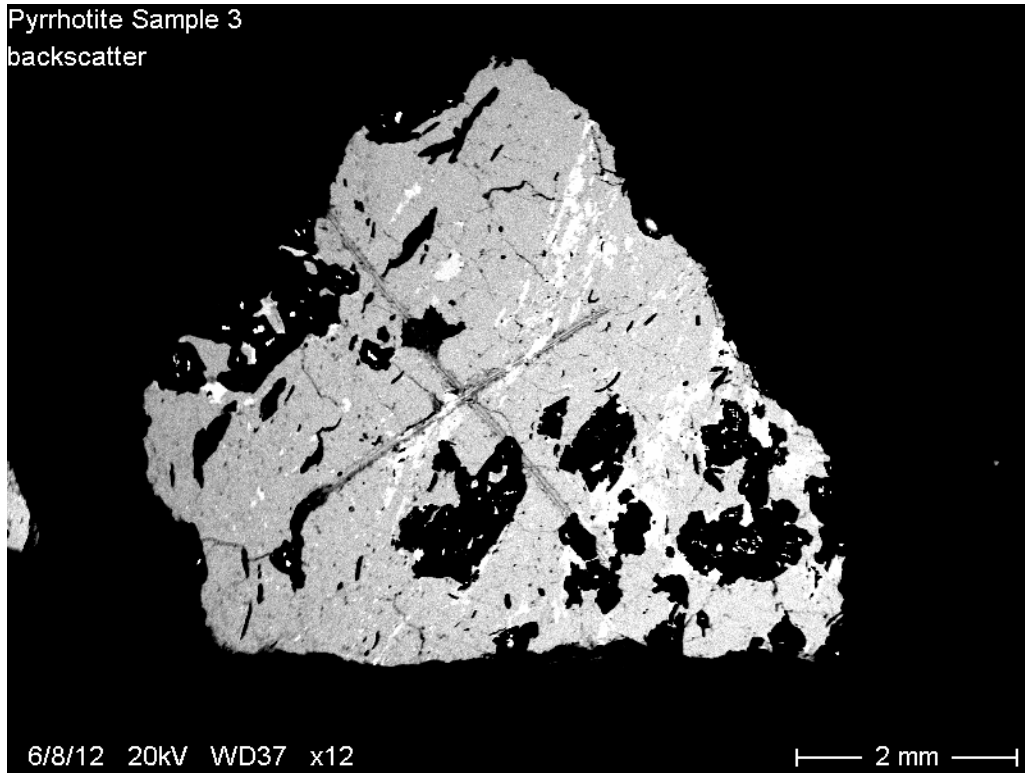


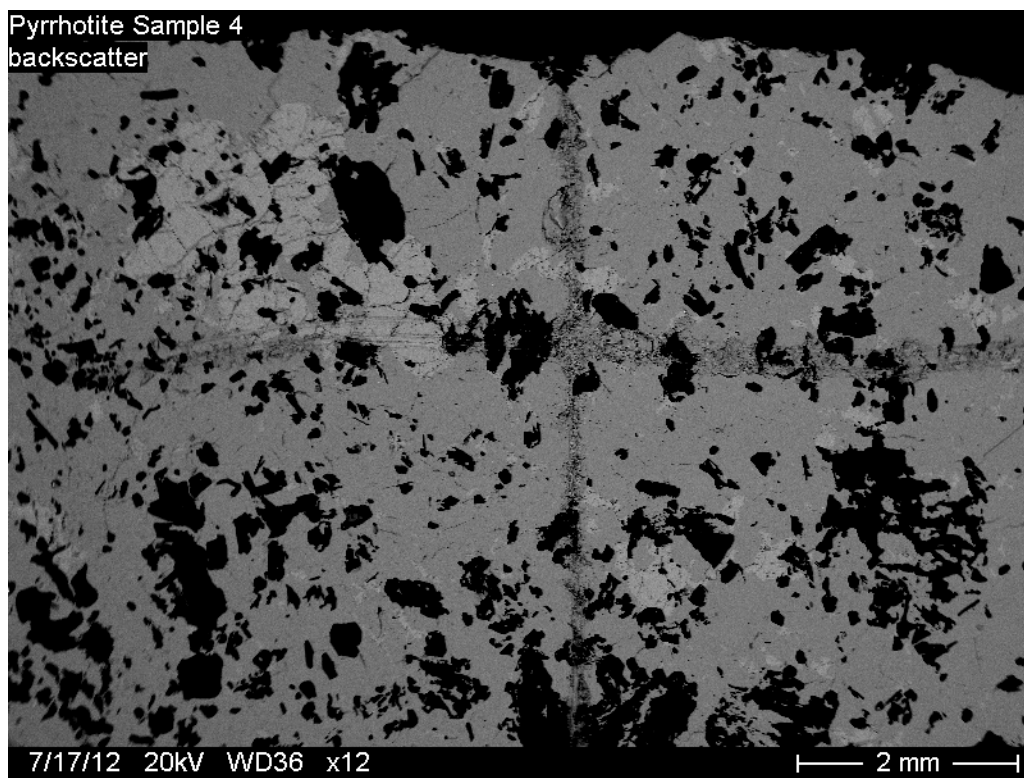
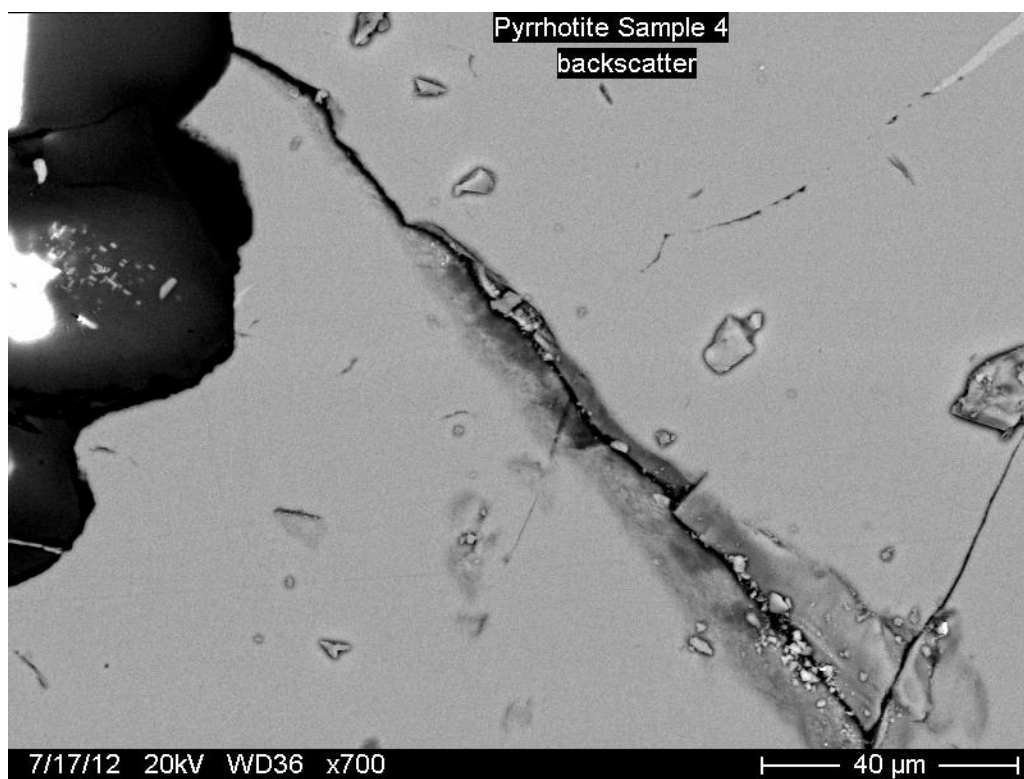


Pyrrhotite Sample 3 backscatter
Quad 4

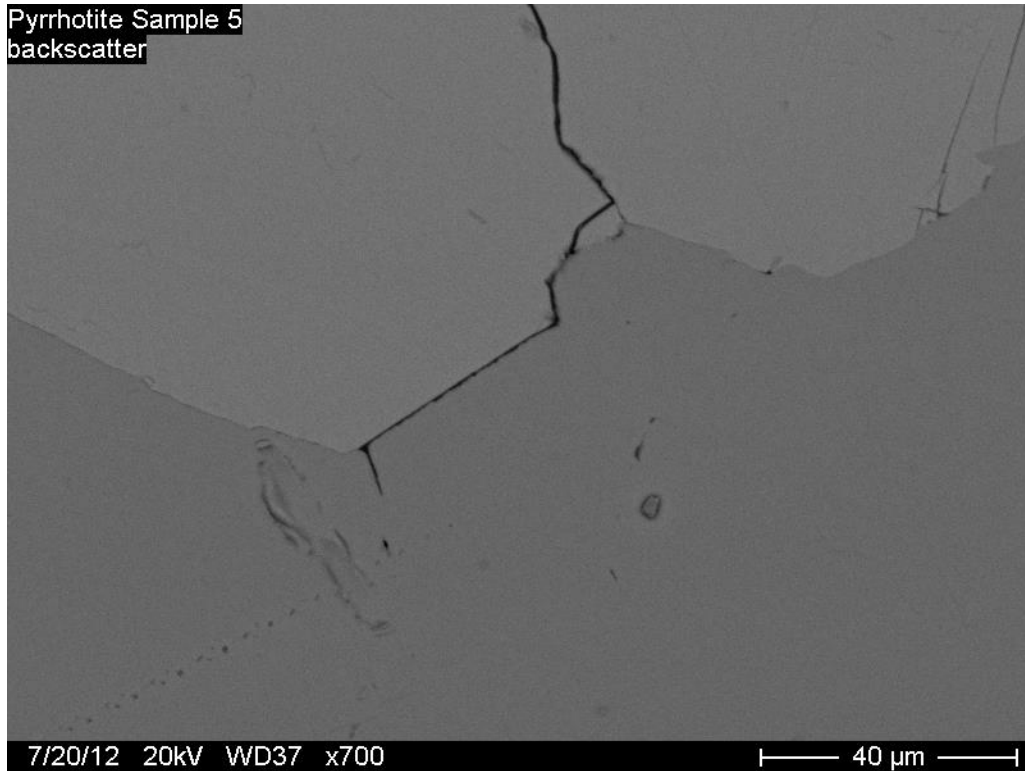


Pyrrhotite Sample 3
backscatter

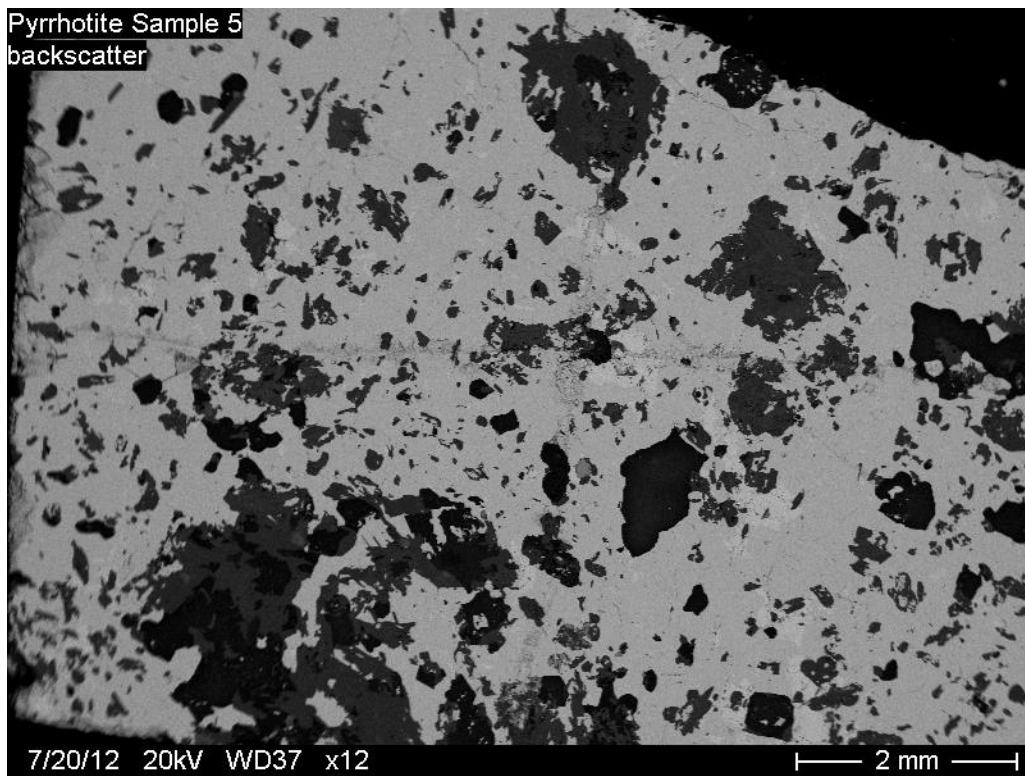




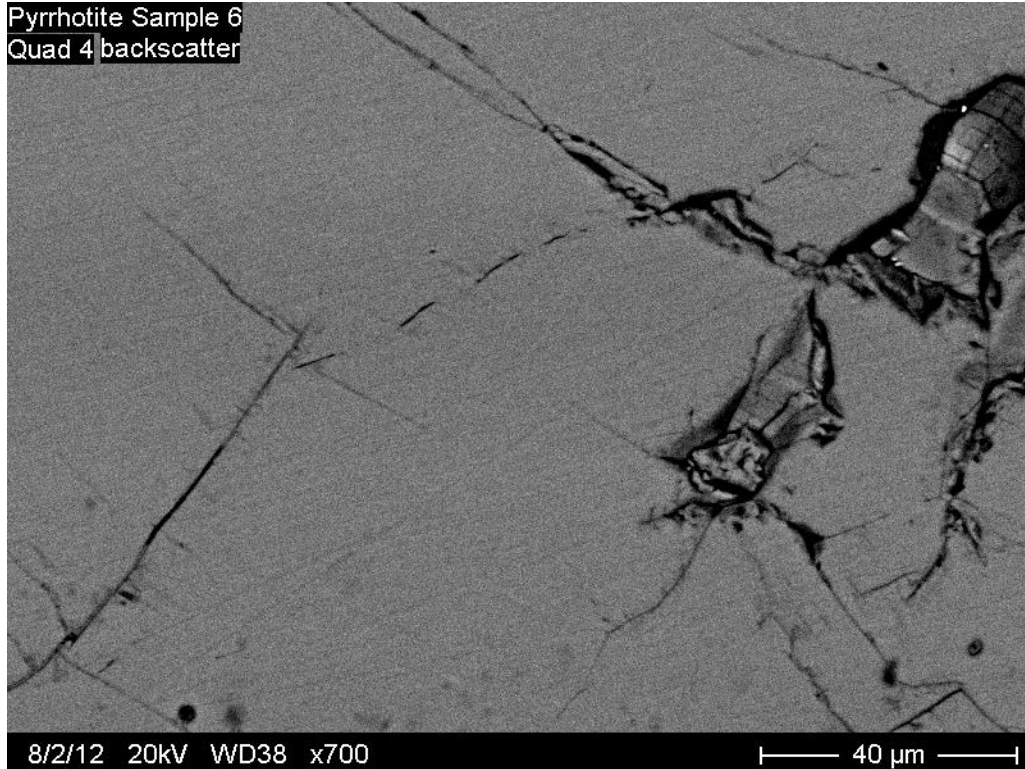
Pyrrhotite Sample 5
backscatter



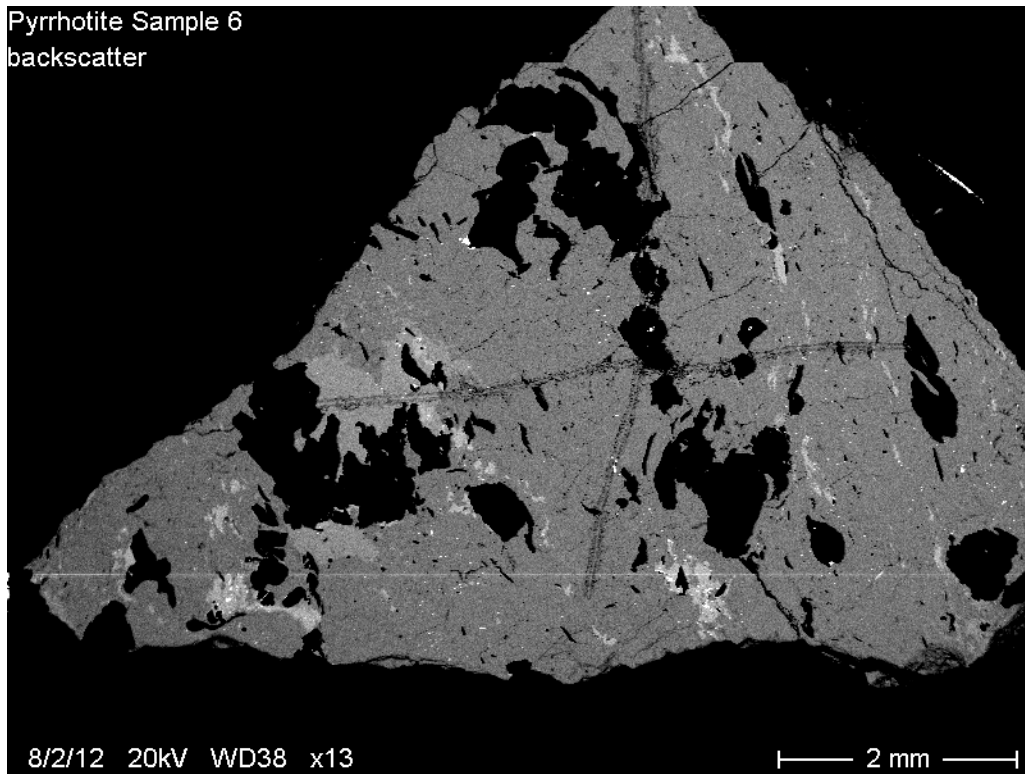
Pyrrhotite Sample 5
backscatter

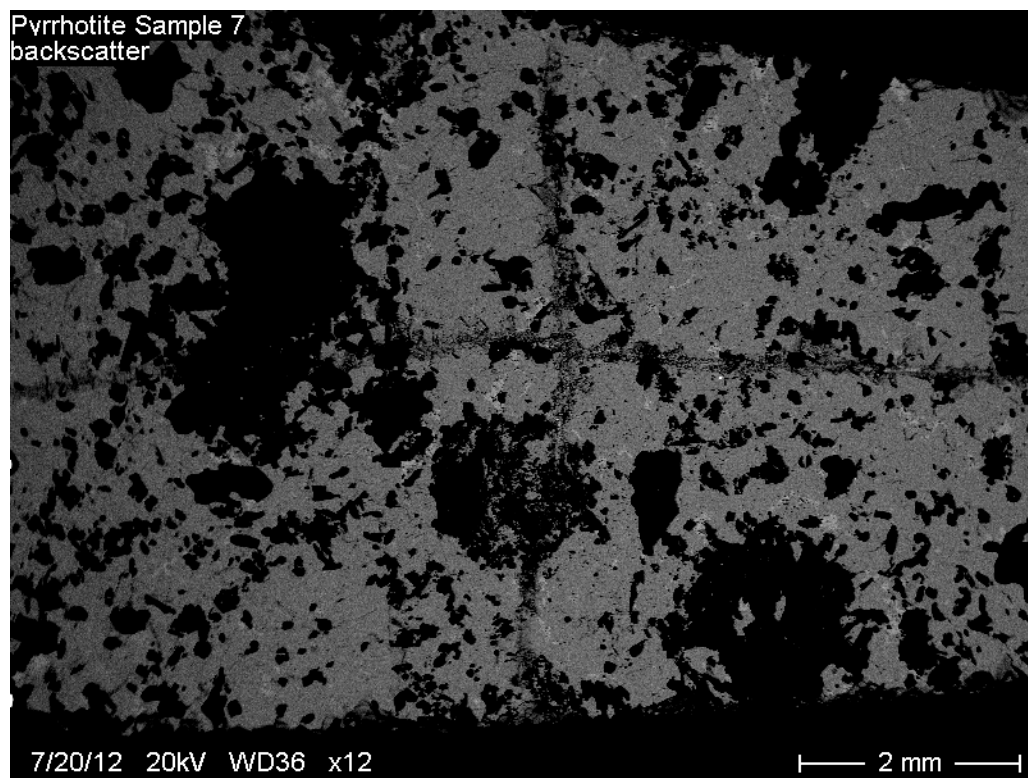
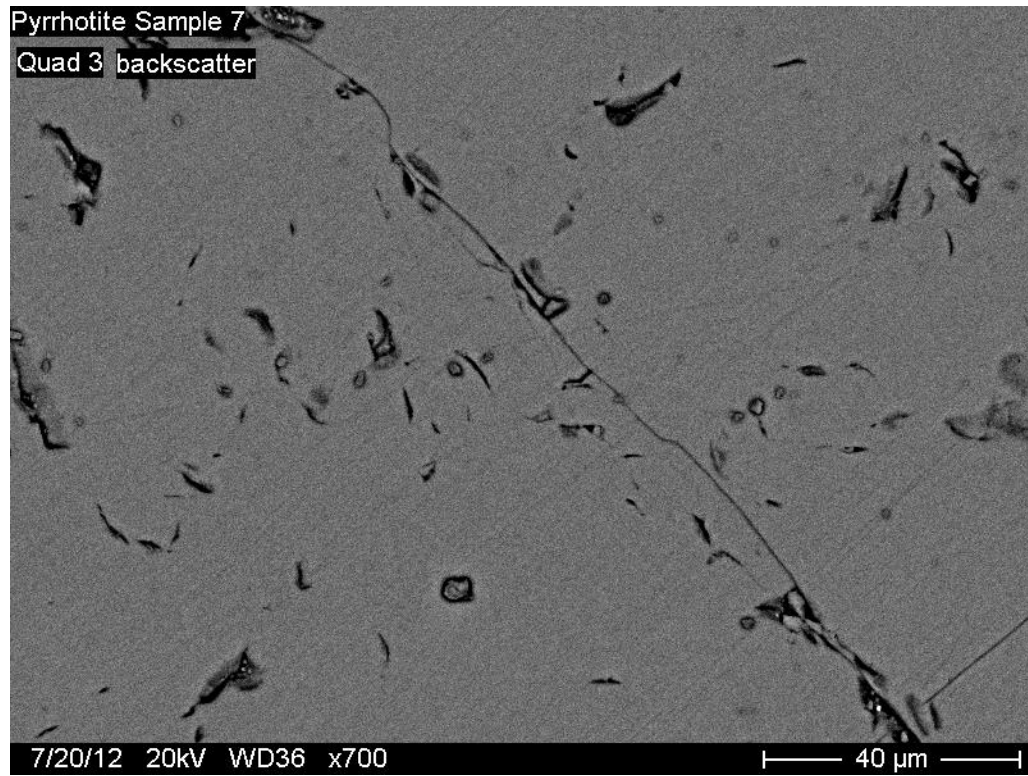


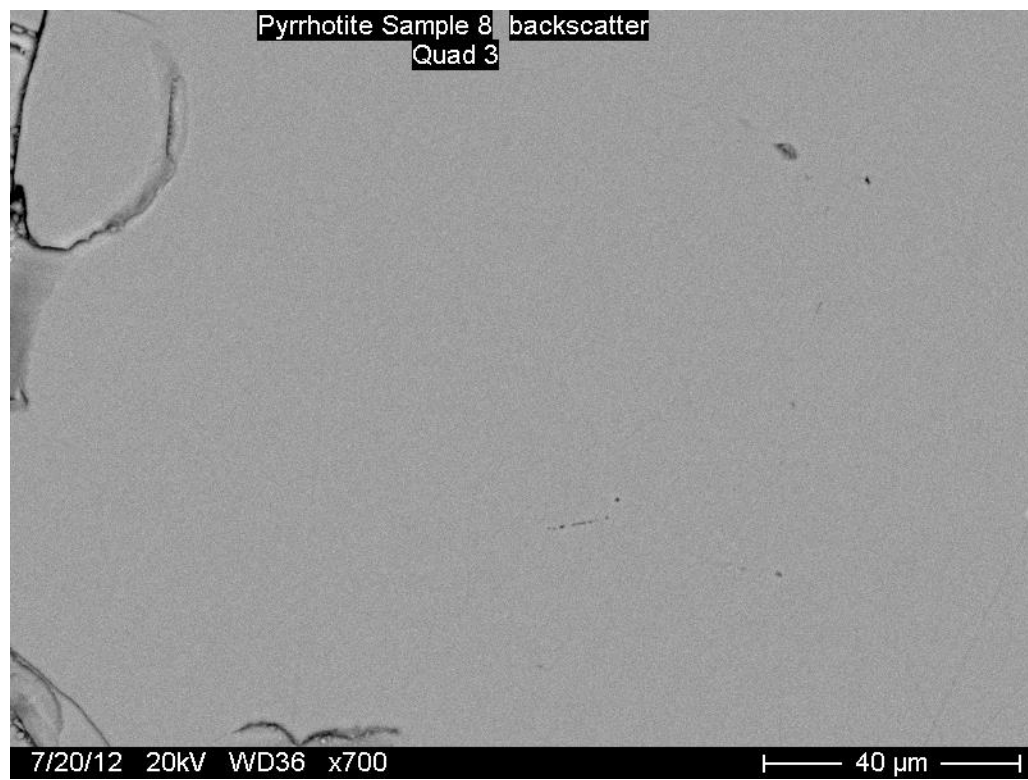
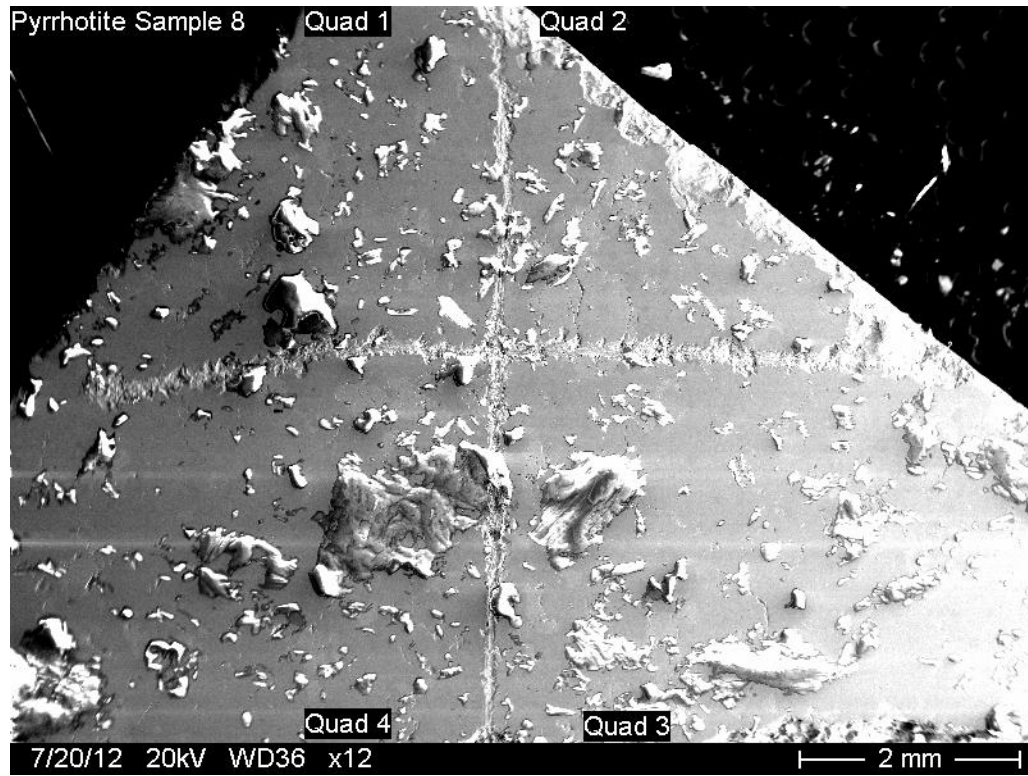
Pyrrhotite Sample 6
Quad 4 backscatter

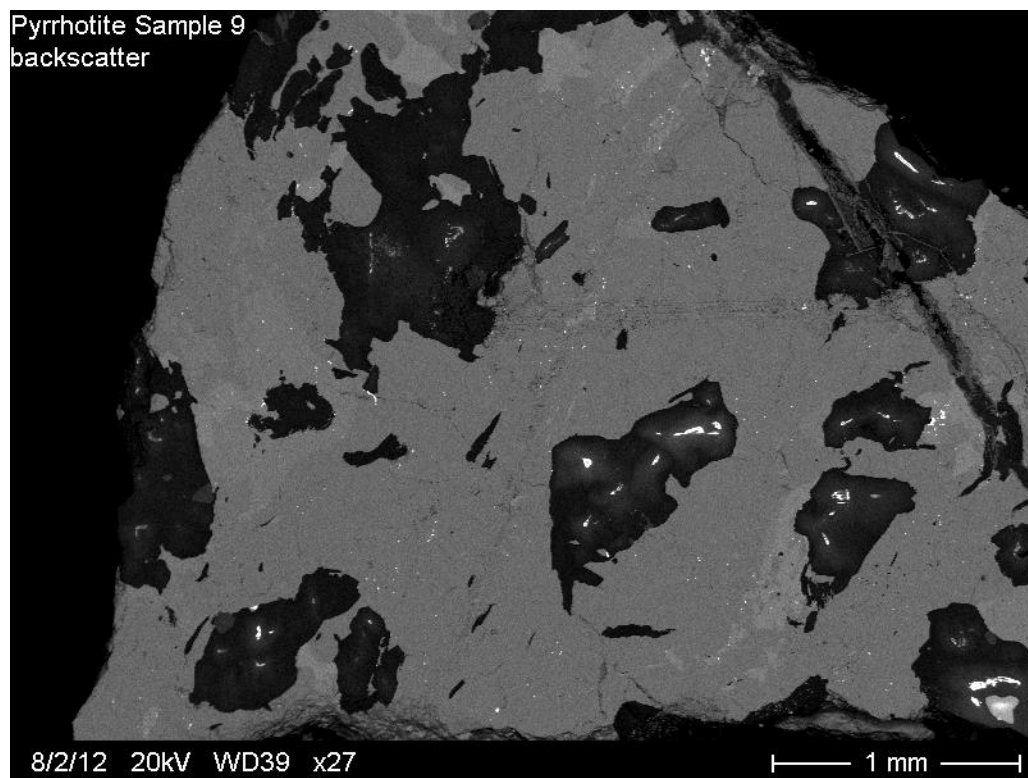
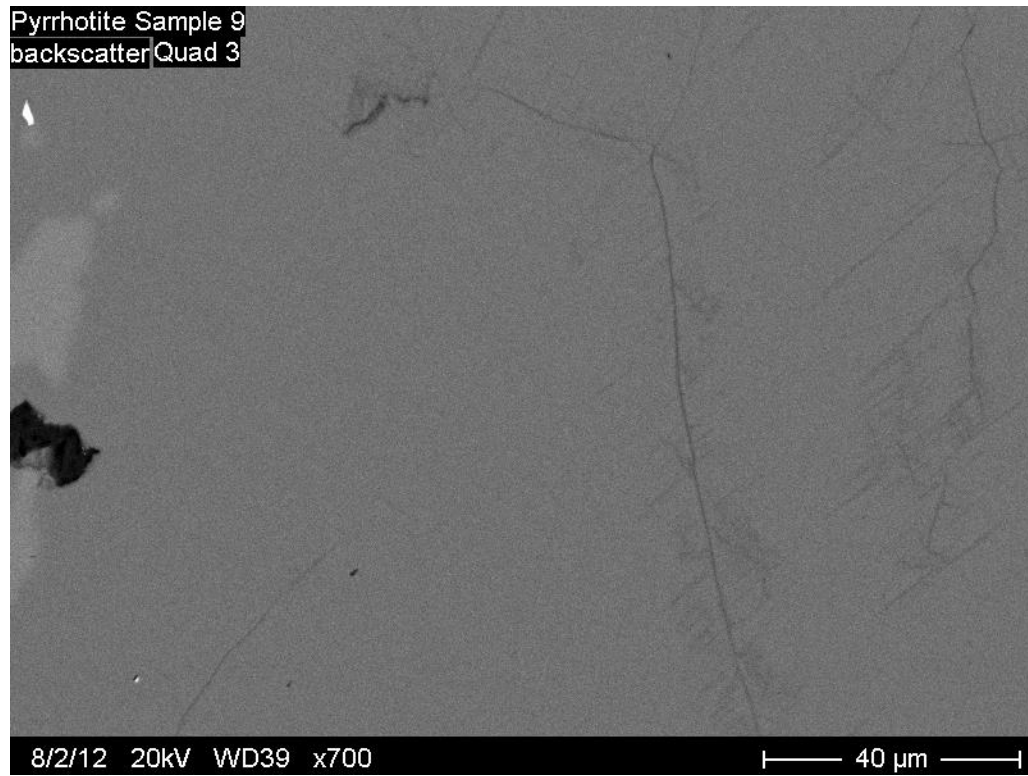


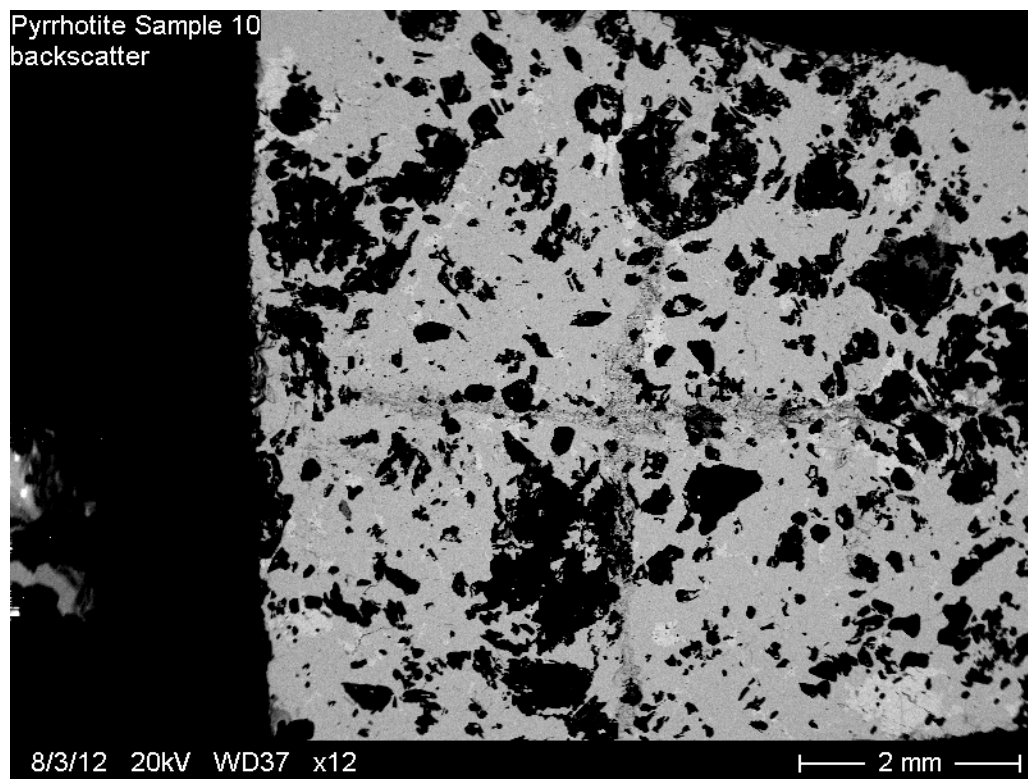
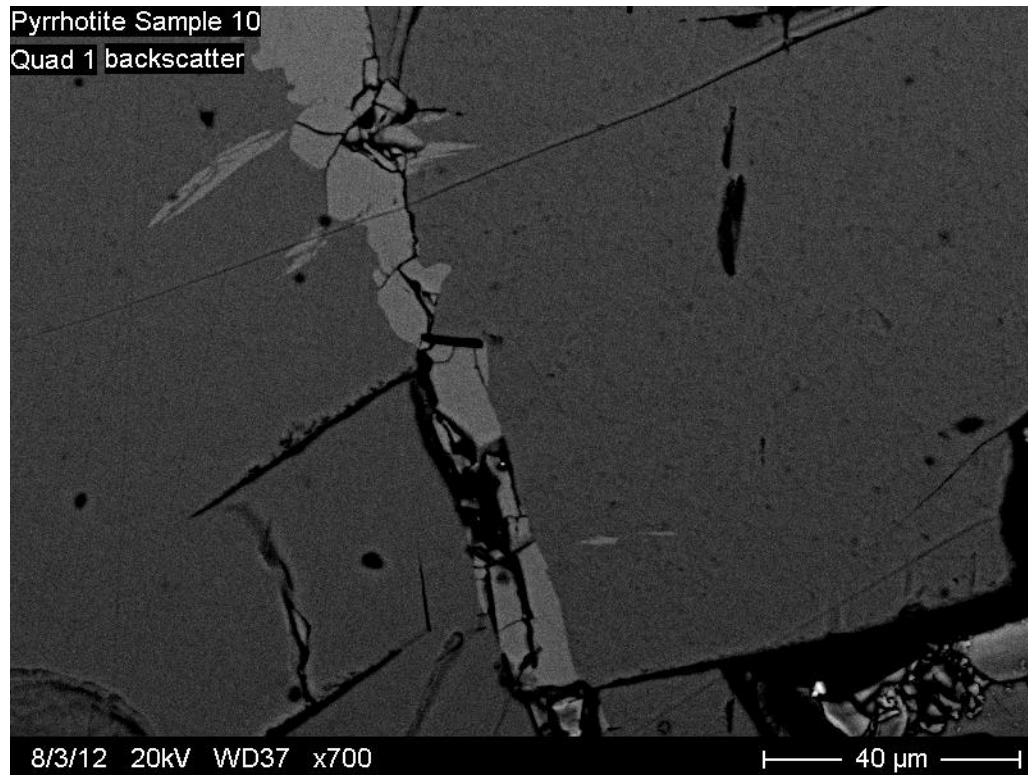
Pyrrhotite Sample 6
backscatter



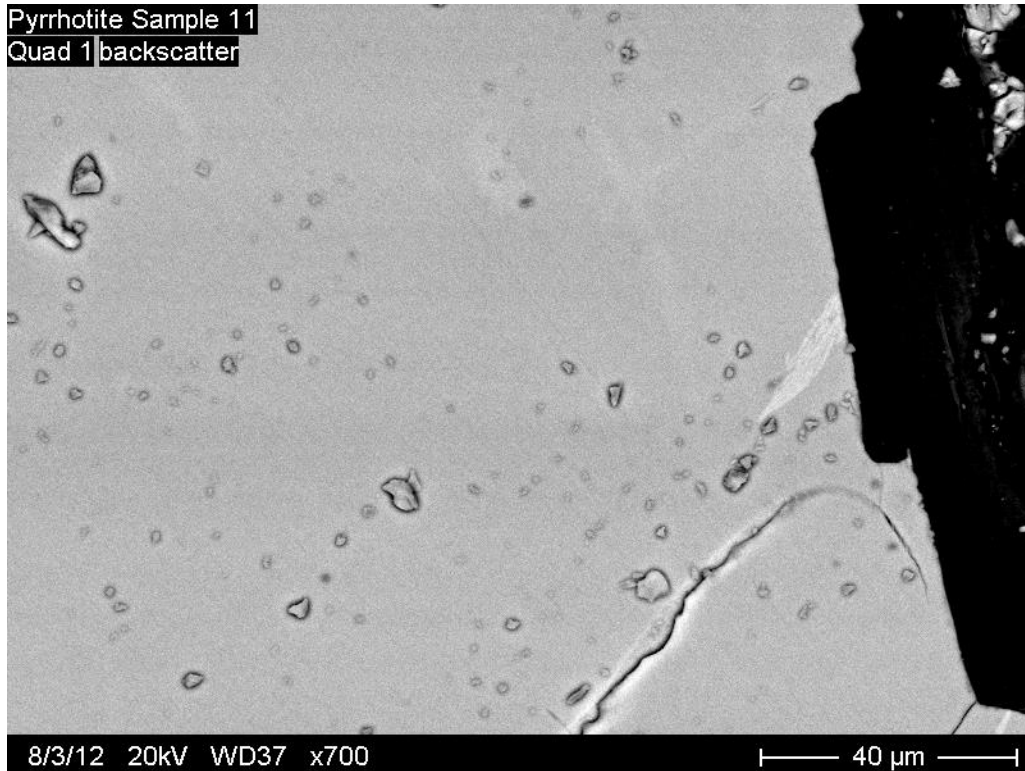




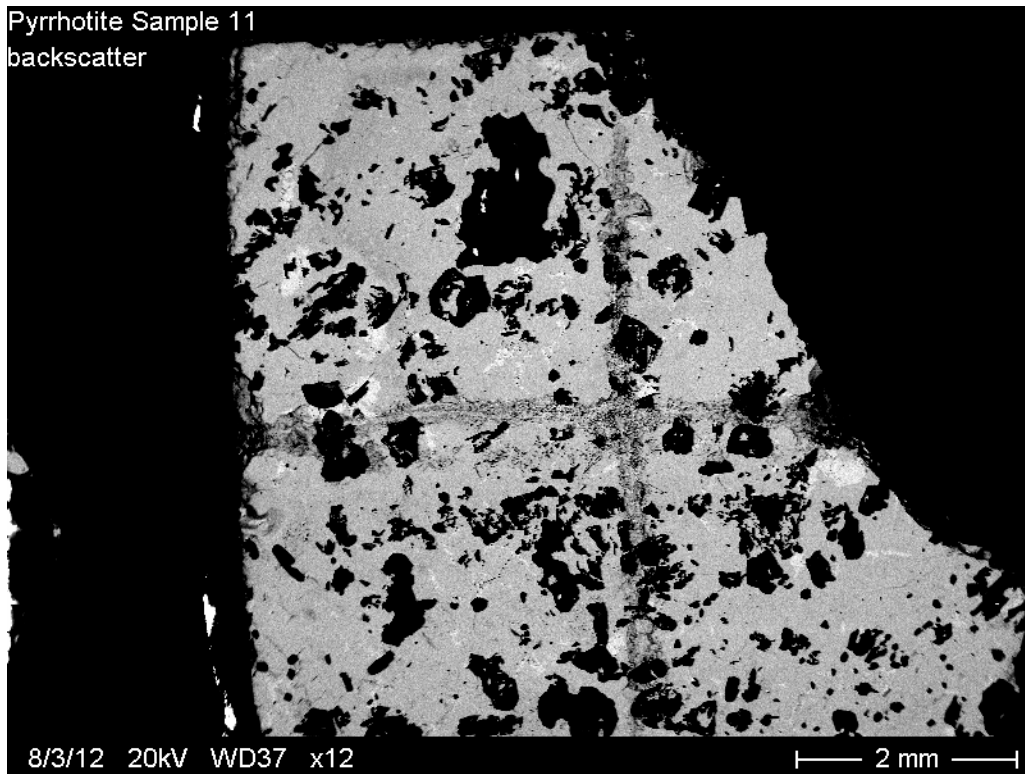


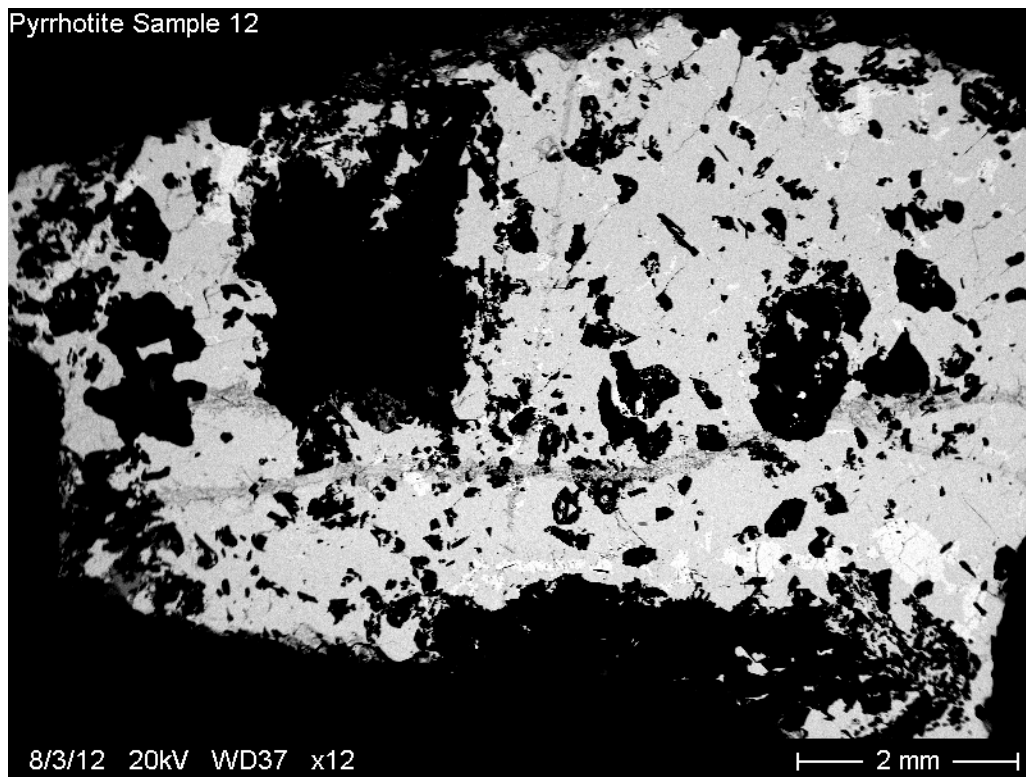


Pyrrhotite Sample 11
Quad 1 backscatter



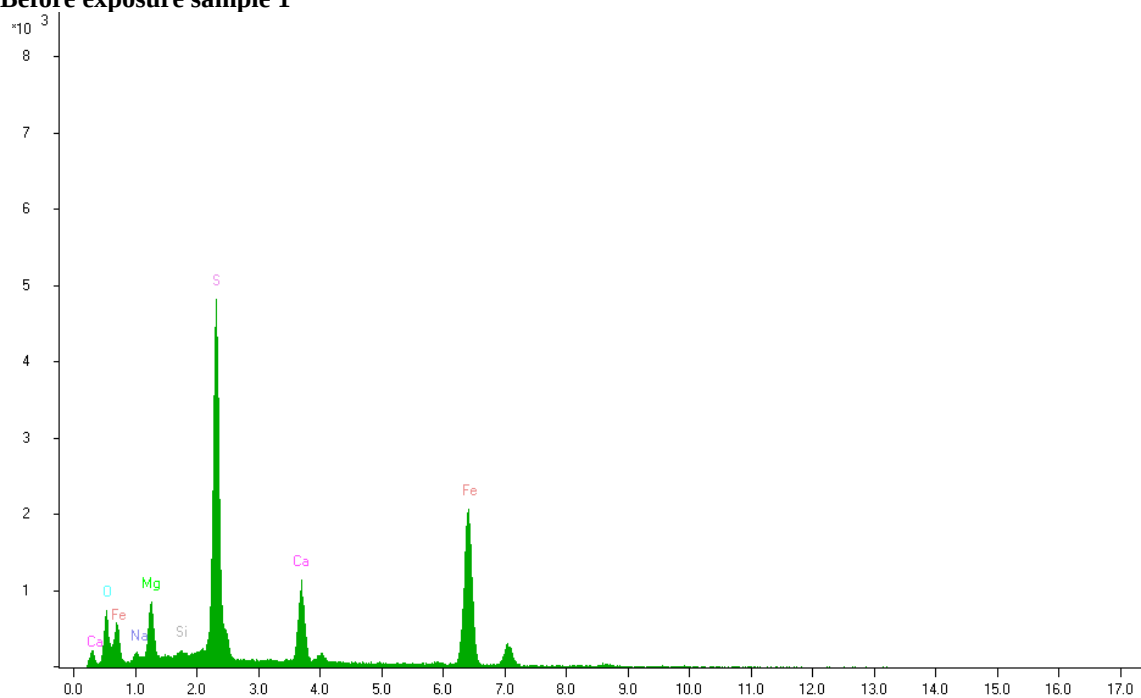
Pyrrhotite Sample 11
backscatter



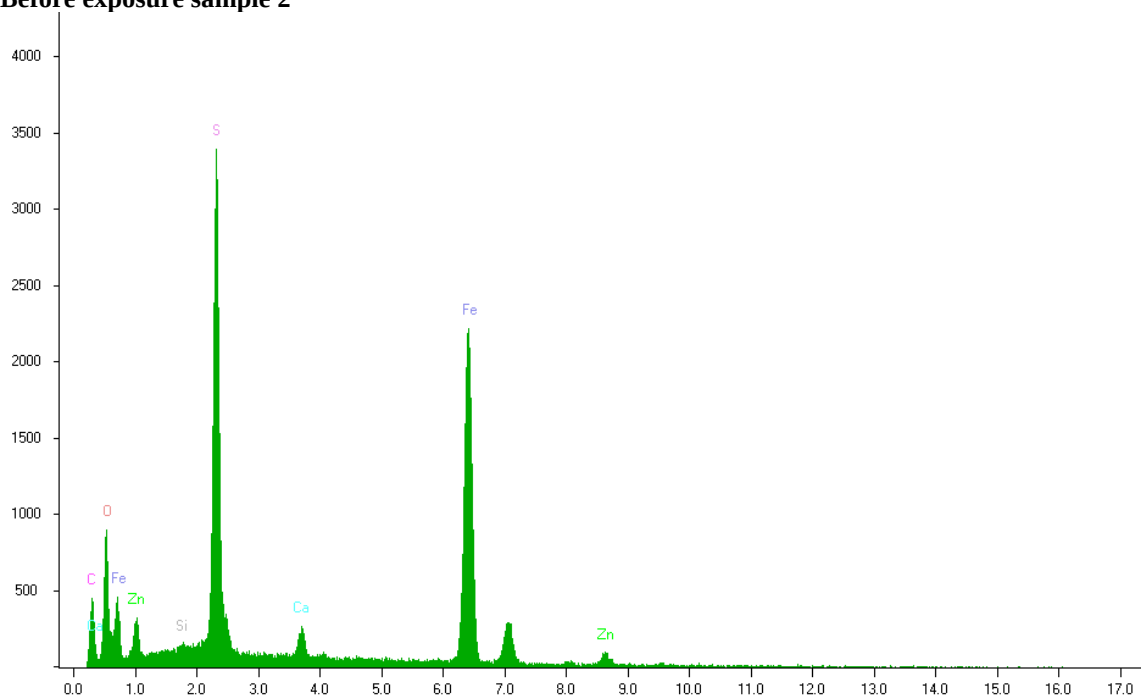


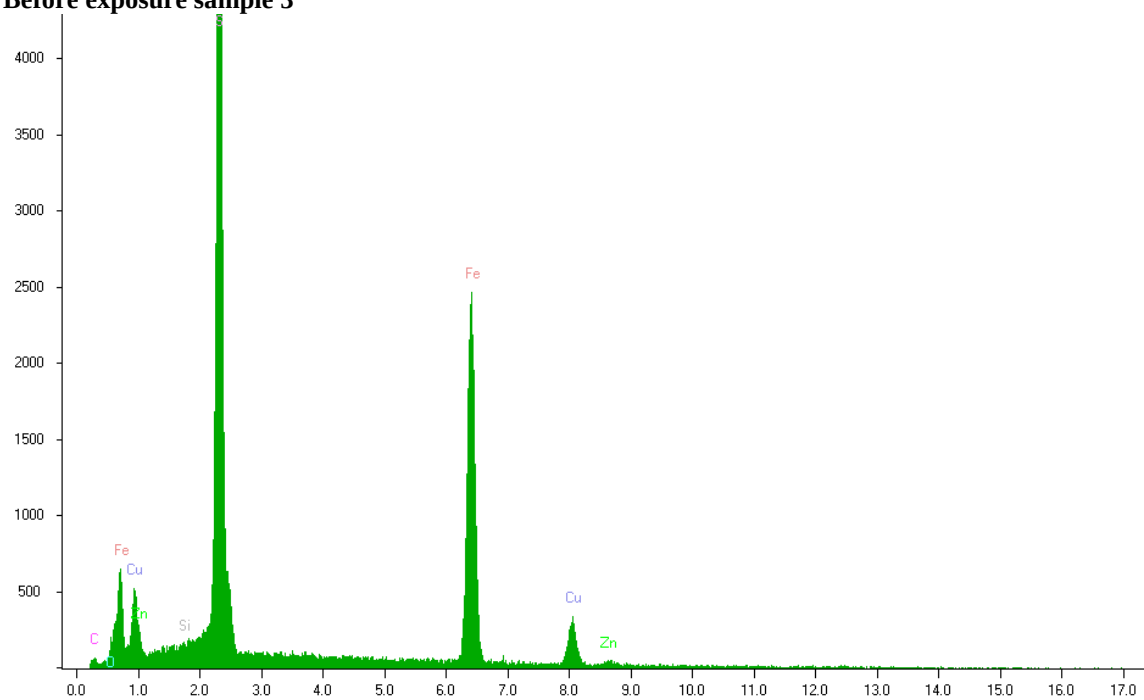
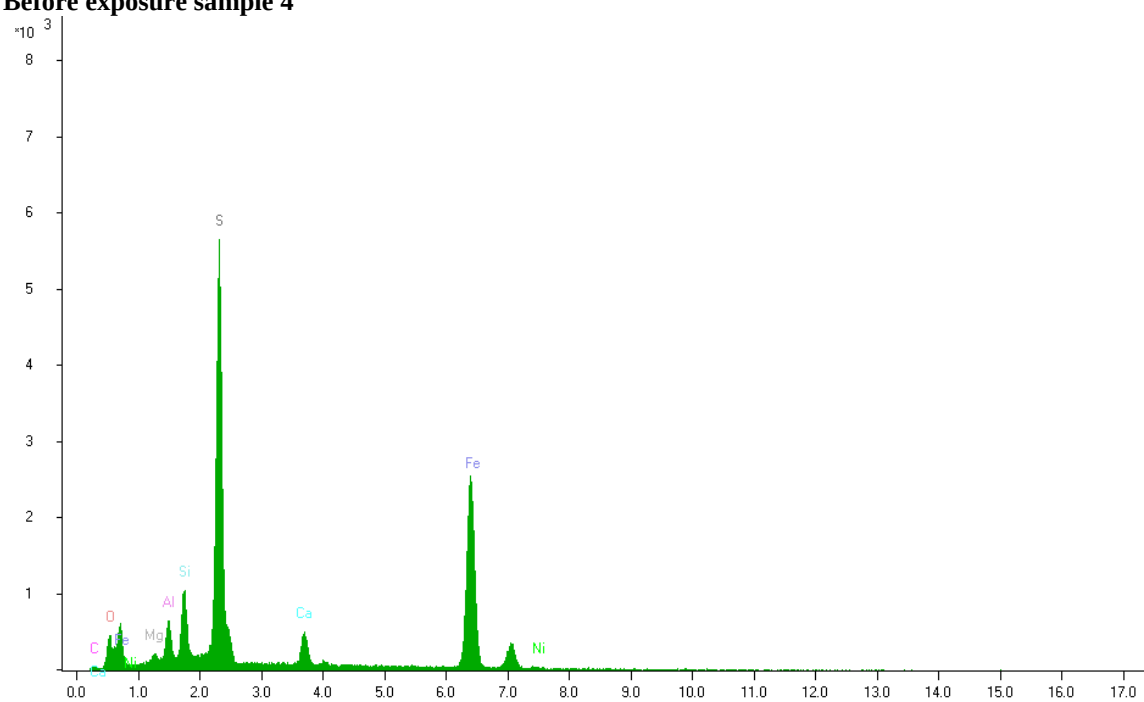
- 3) EDS spectra of pyrrhotite averaged over entire sample at magnification 700x for pyrrhotite samples 1-12 before exposure to SRB.

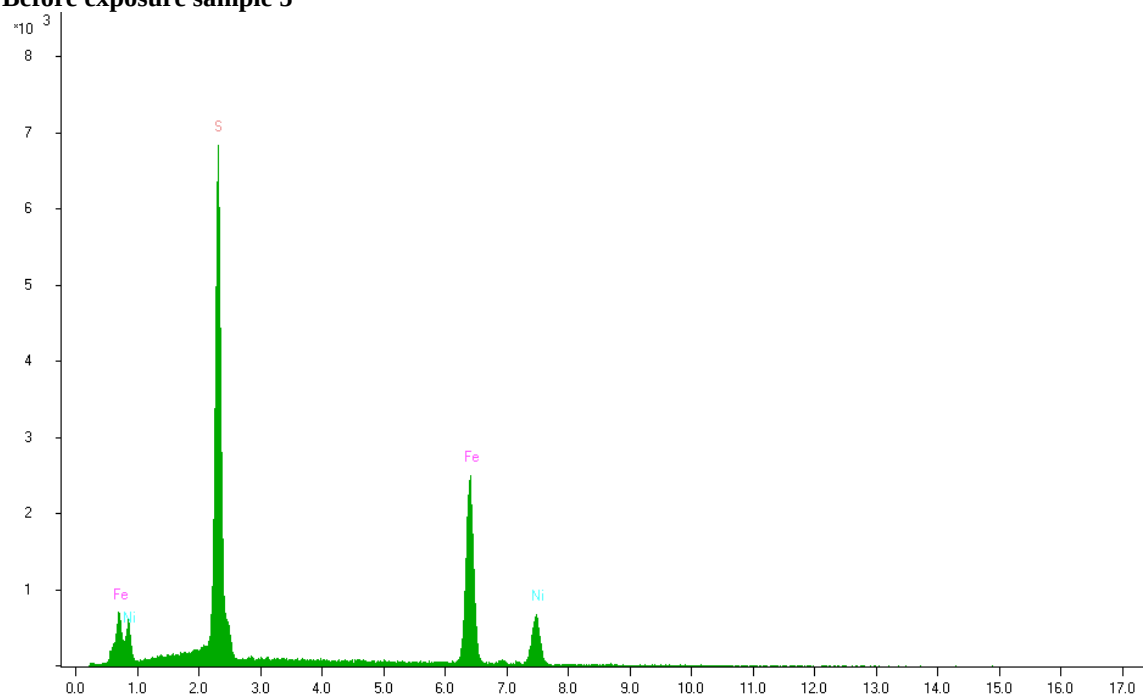
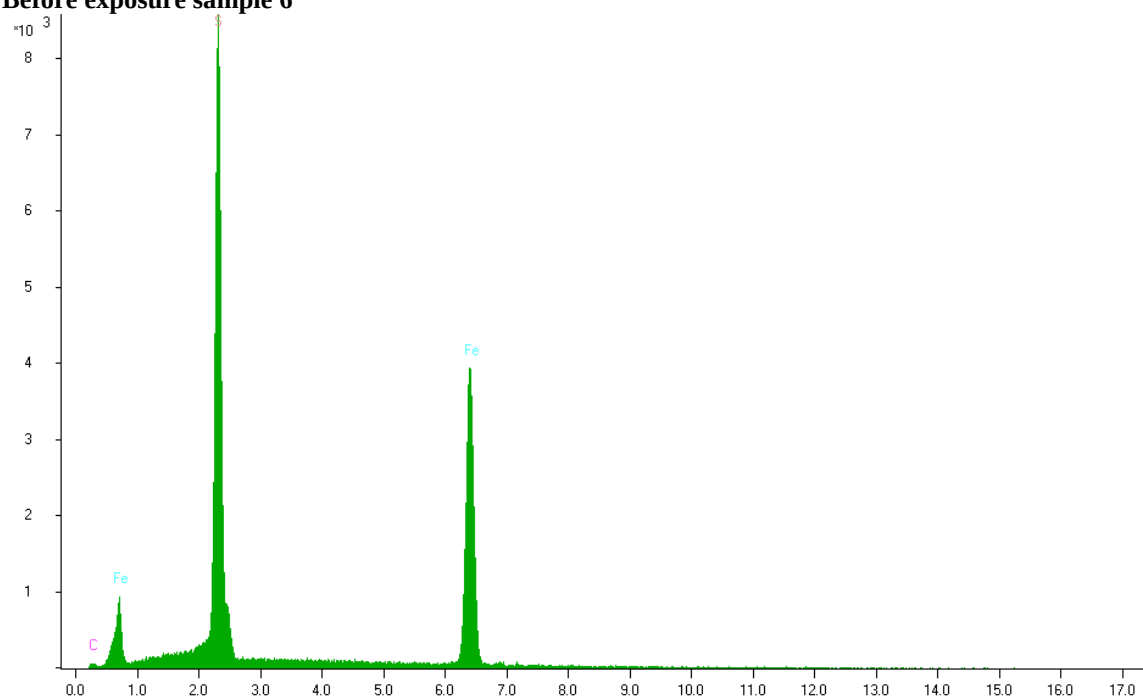
Before exposure sample 1

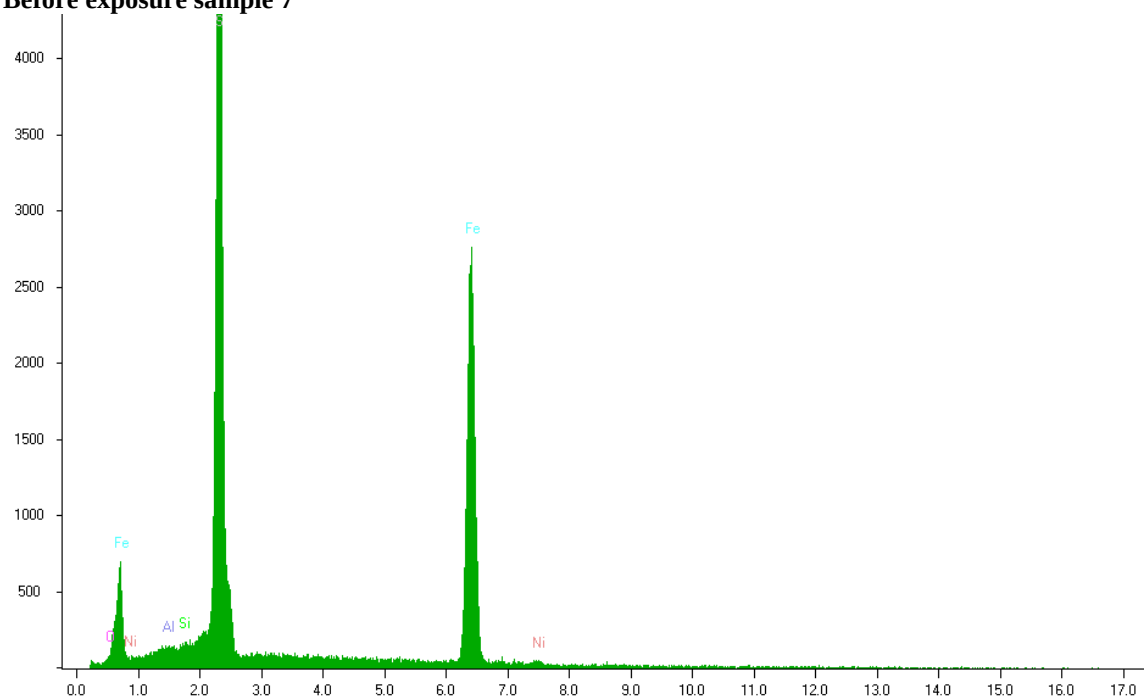
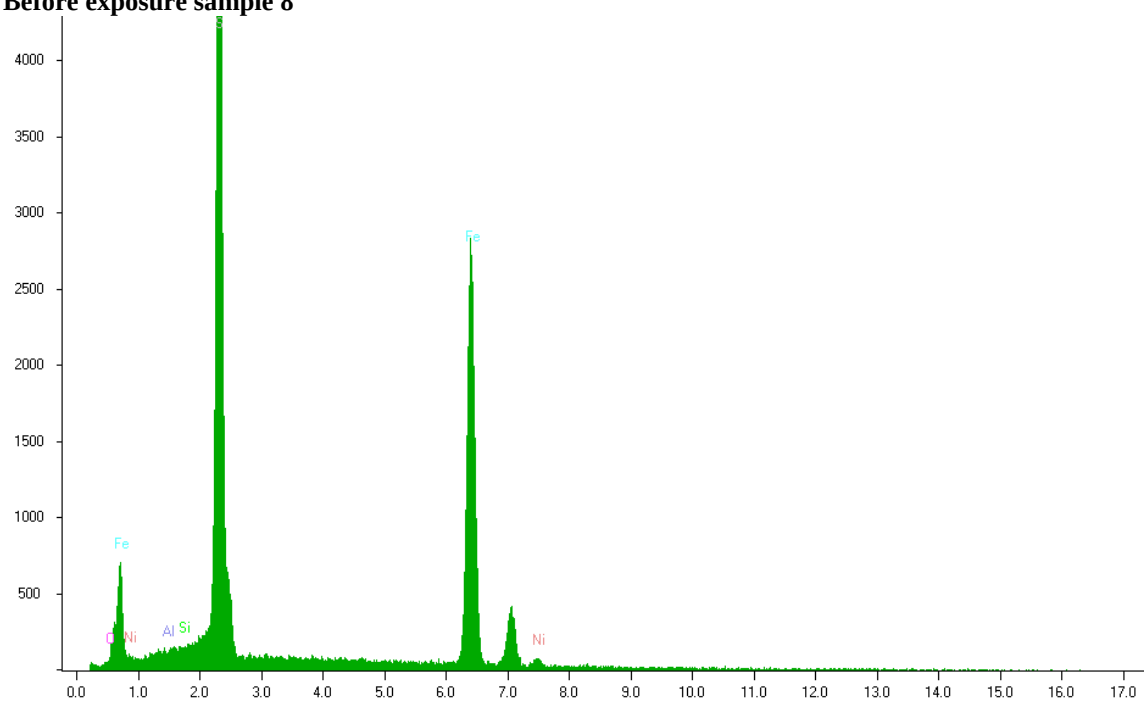


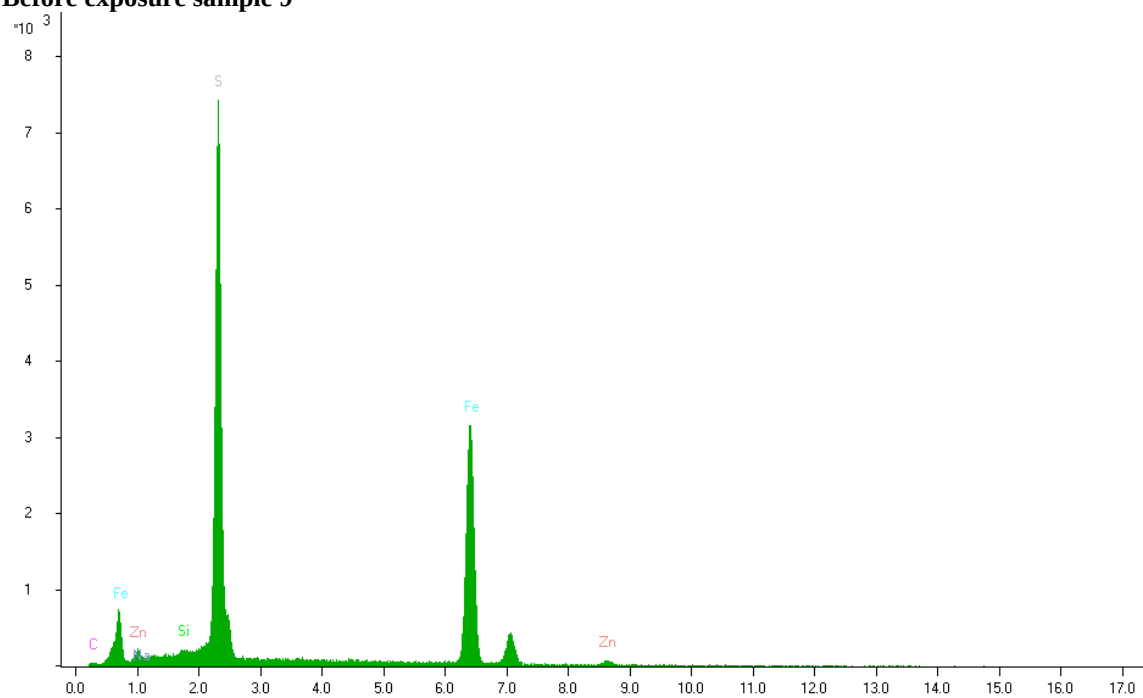
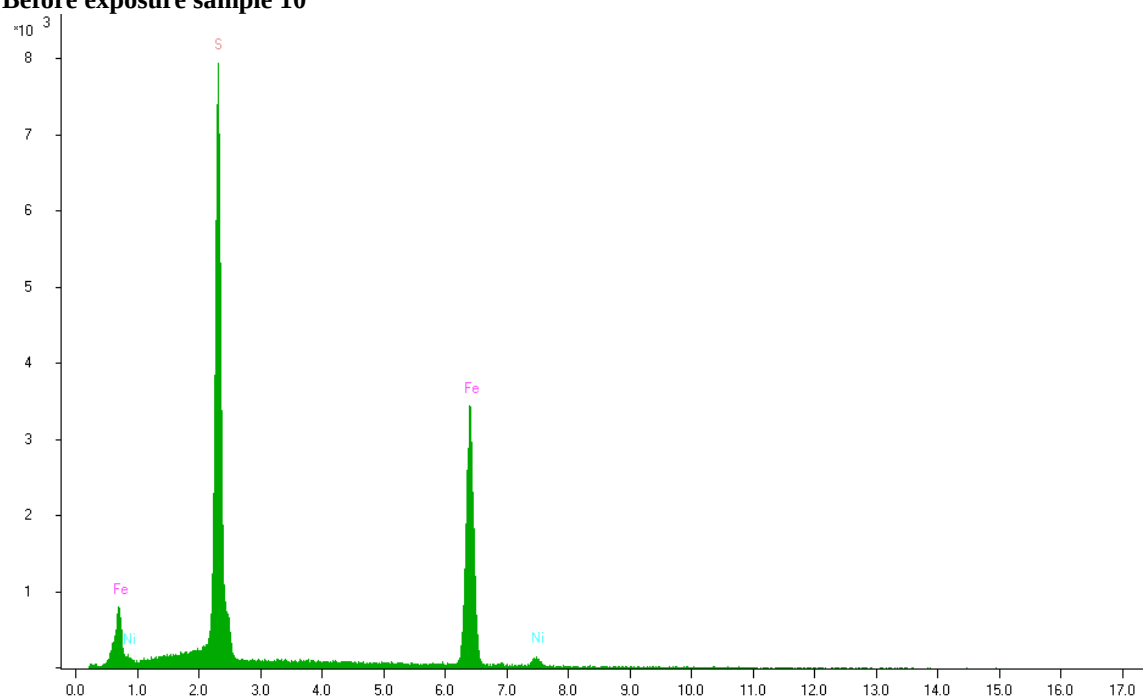
Before exposure sample 2

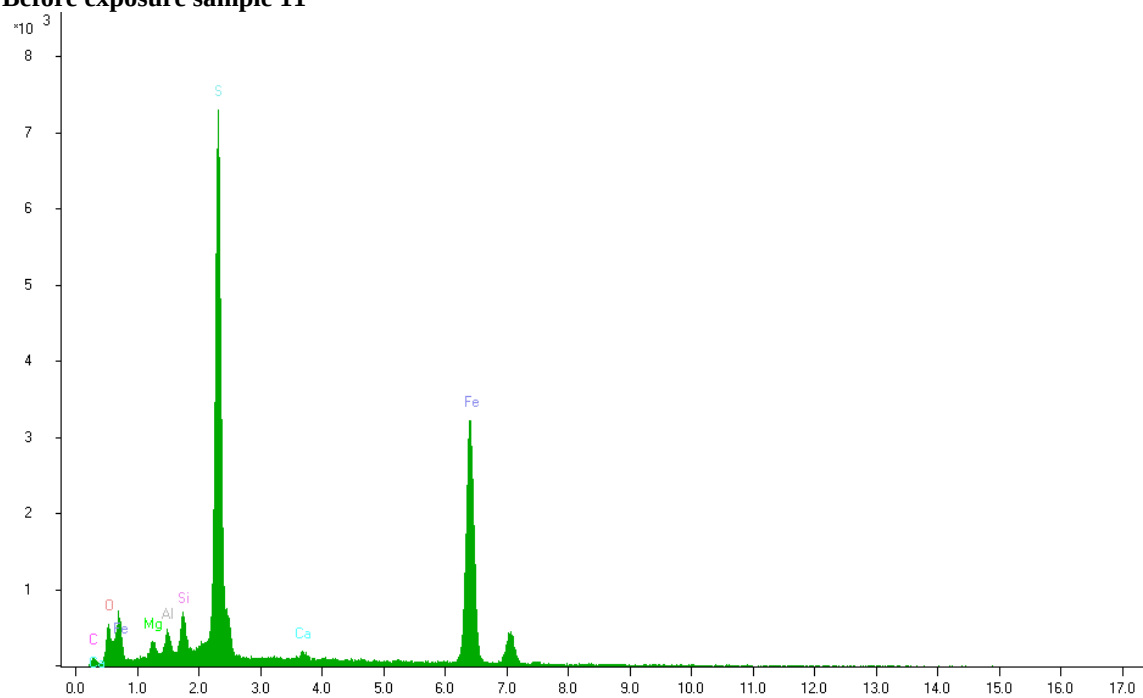
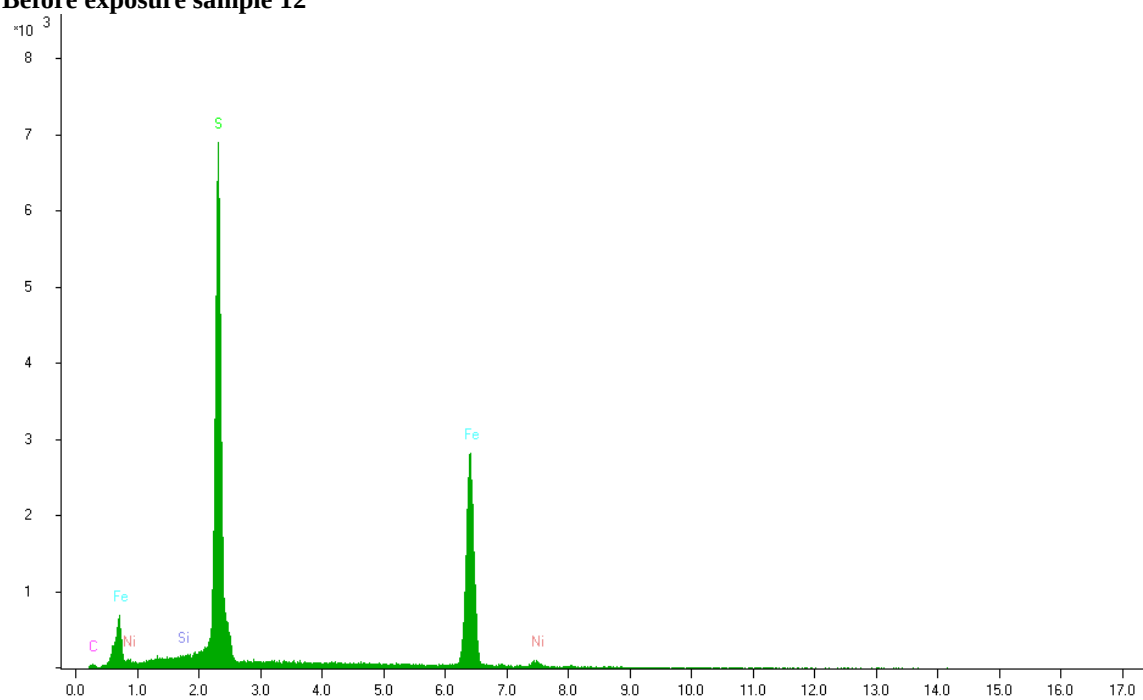


Before exposure sample 3**Before exposure sample 4**

Before exposure sample 5**Before exposure sample 6**

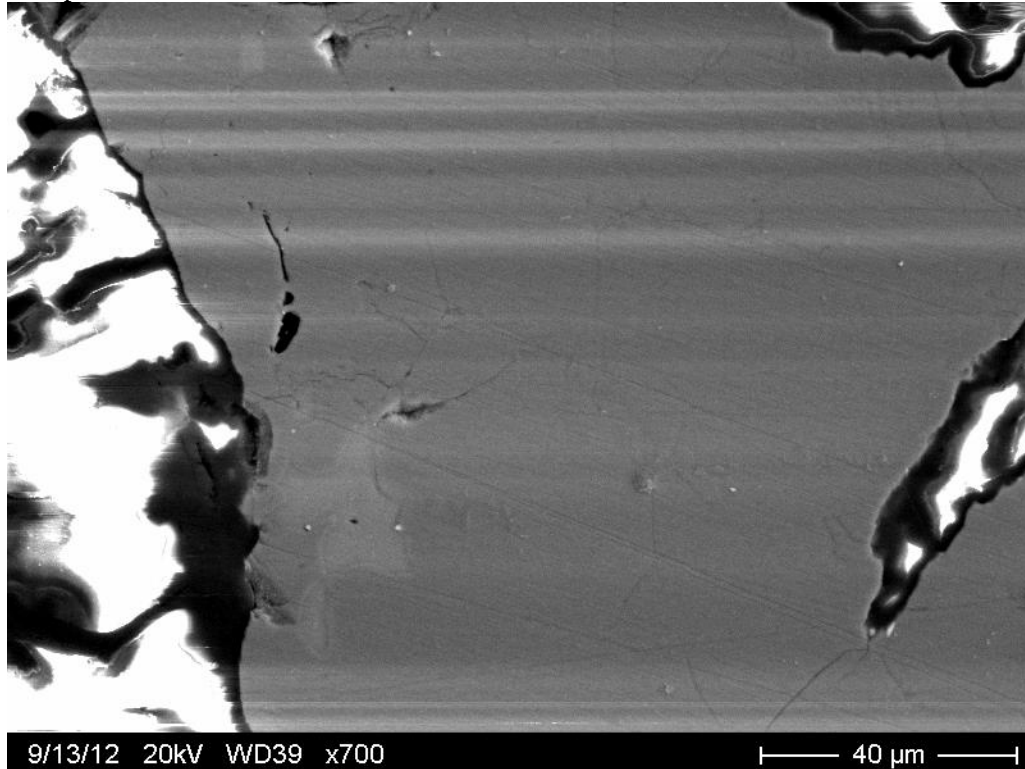
Before exposure sample 7**Before exposure sample 8**

Before exposure sample 9**Before exposure sample 10**

Before exposure sample 11**Before exposure sample 12**

- 4) SEM secondary electron images of pyrrhotite samples 1, 2, 3, 5, and 10 after exposure to microbes. Samples 1-4 were exposed to SRB for 7 days; samples 5-8 were exposed for 14 days; samples 9-12 were exposed for 30 days.

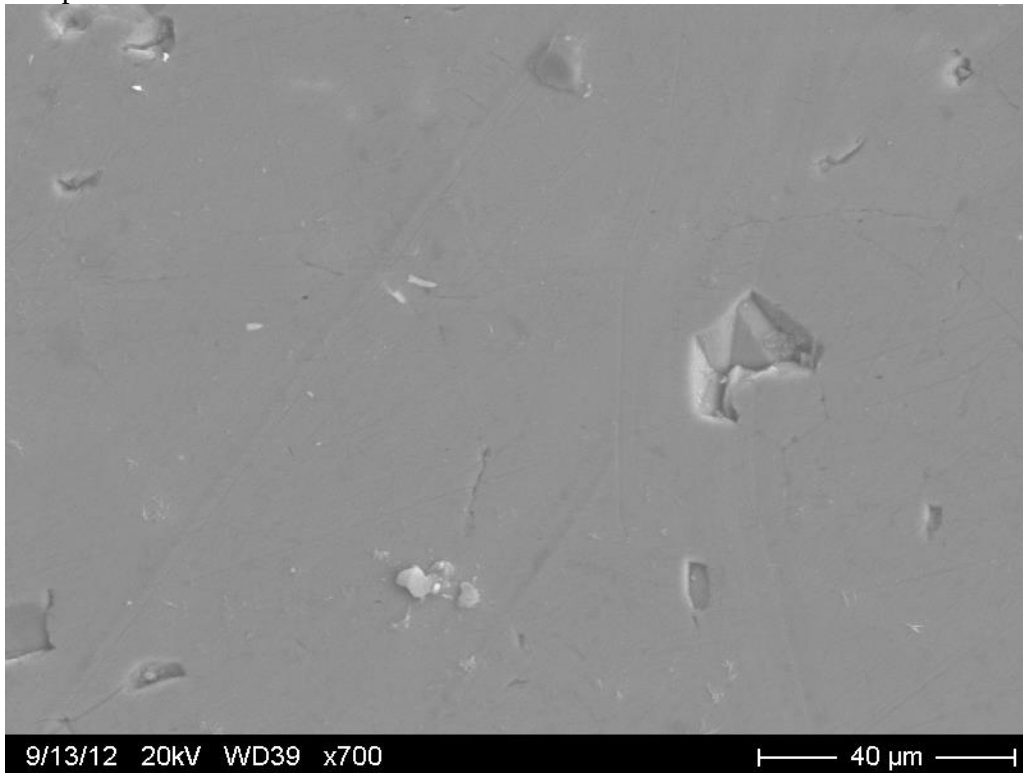
Sample 1

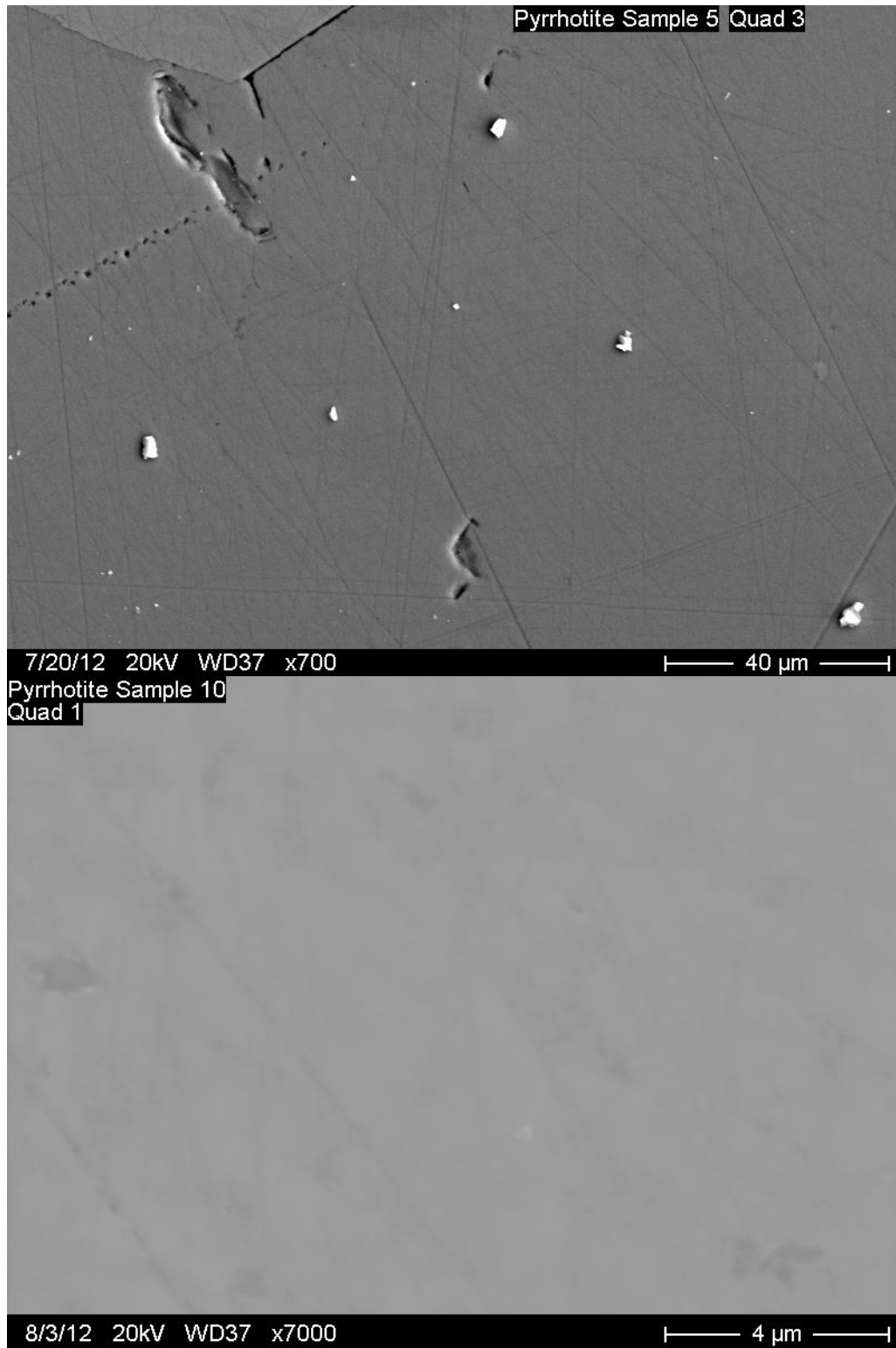


Pyrrhotite Sample 2
Quad 3



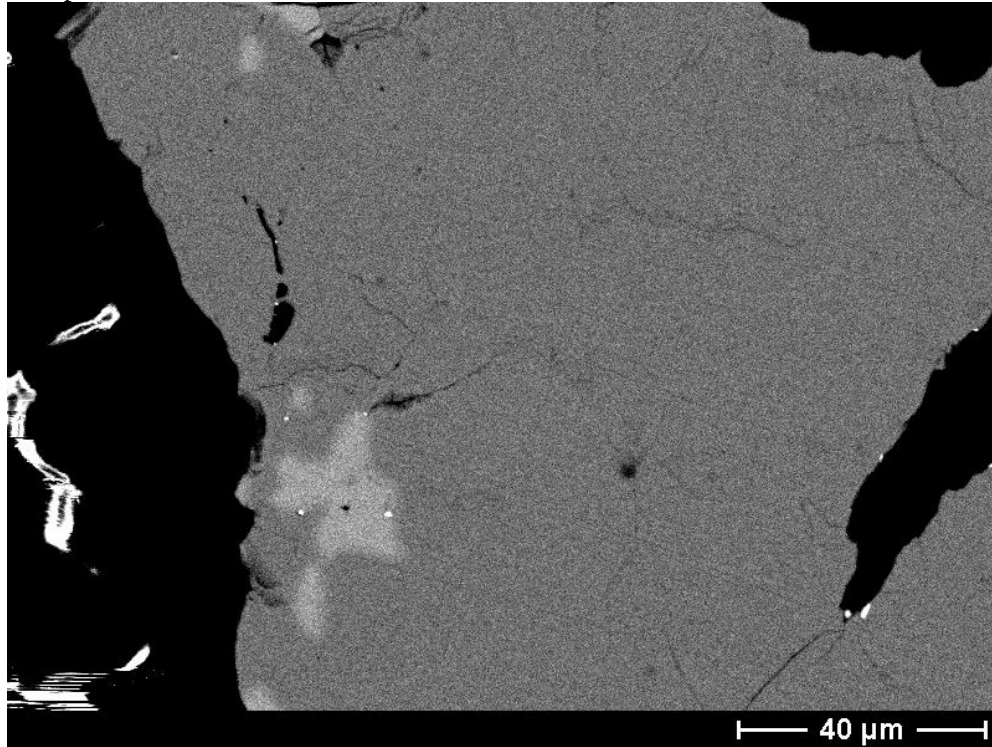
Sample 3



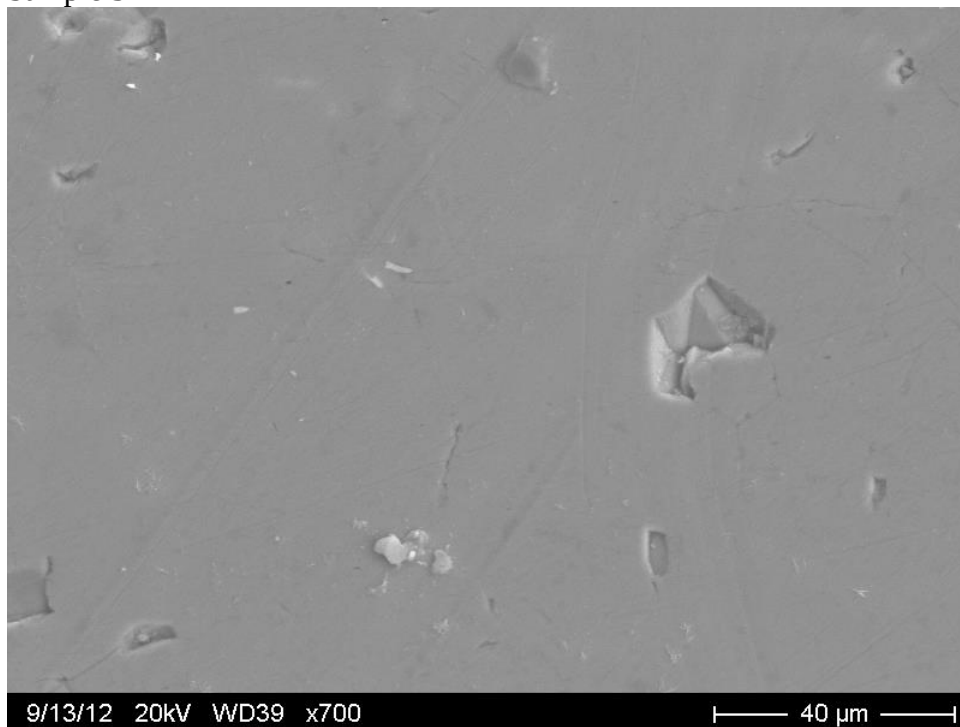


- 5) SEM backscatter electron images of pyrrhotite samples 1 and 3 after exposure to SRB for 7 days

Sample 1

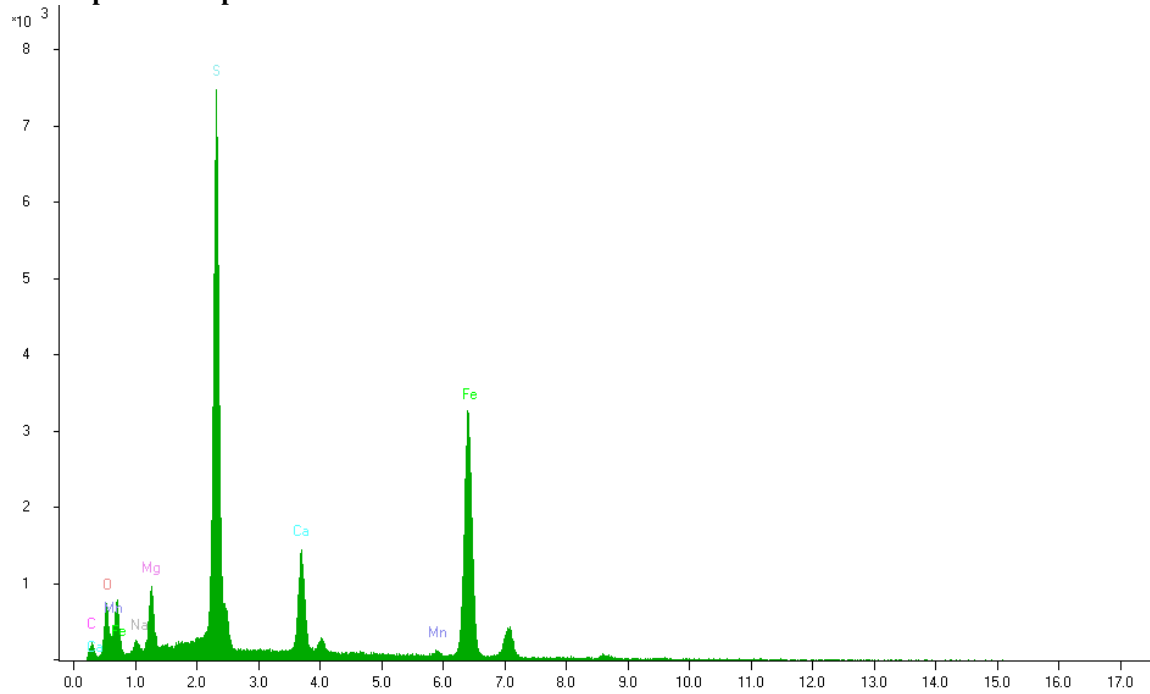


Sample 3

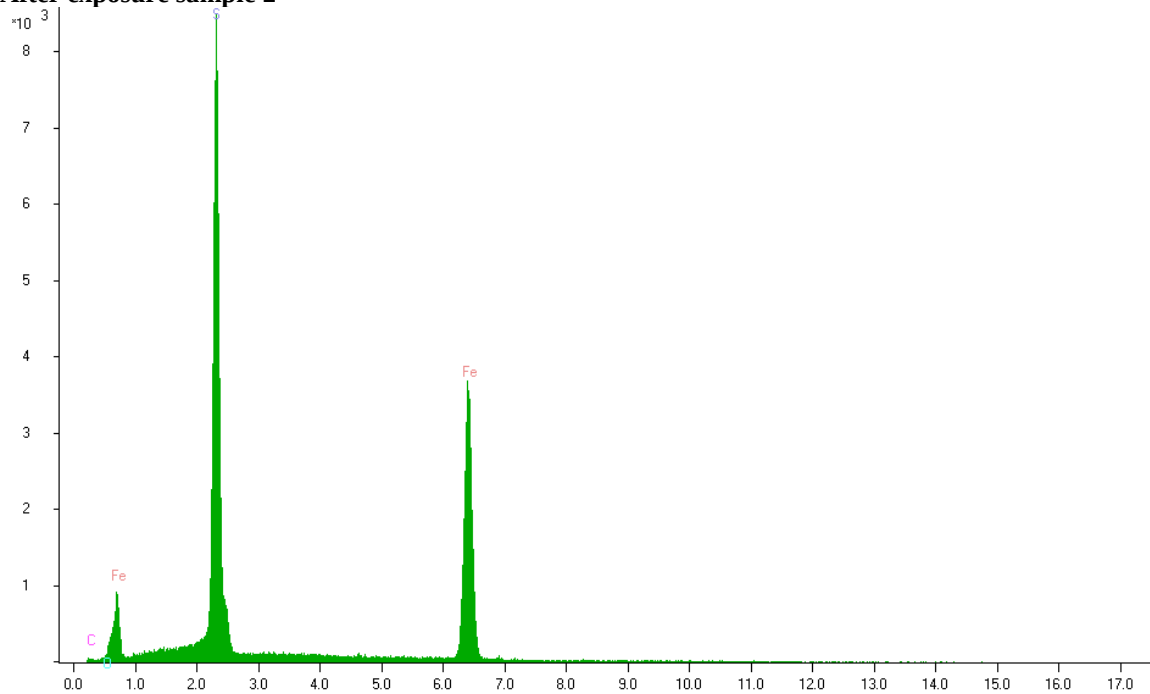


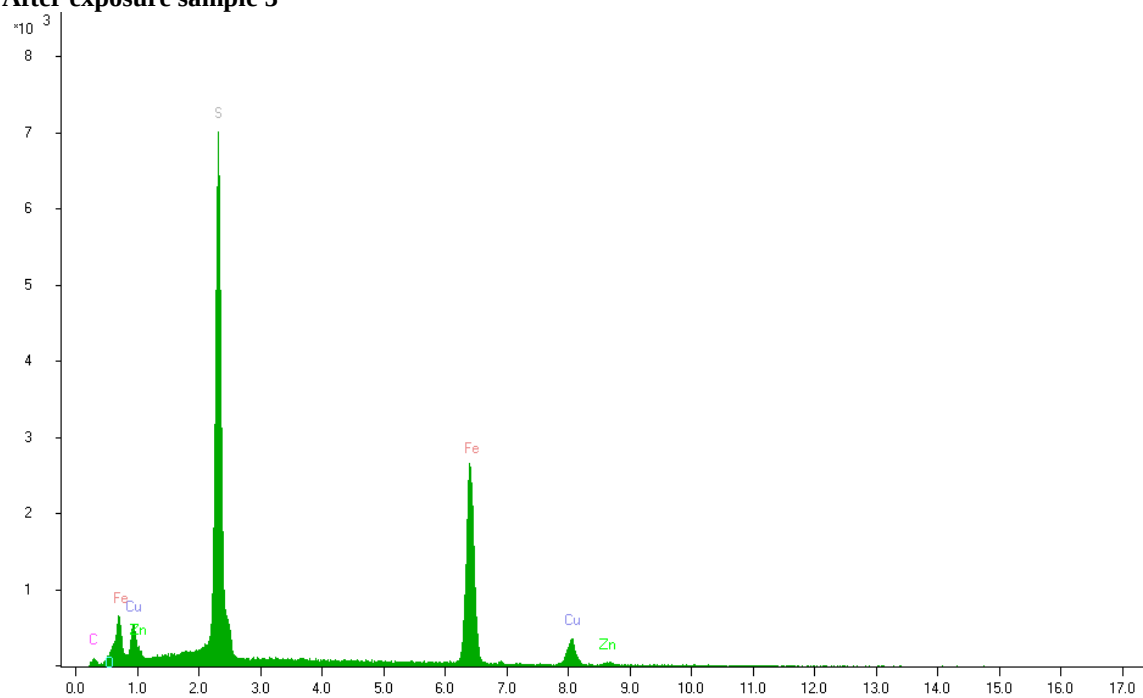
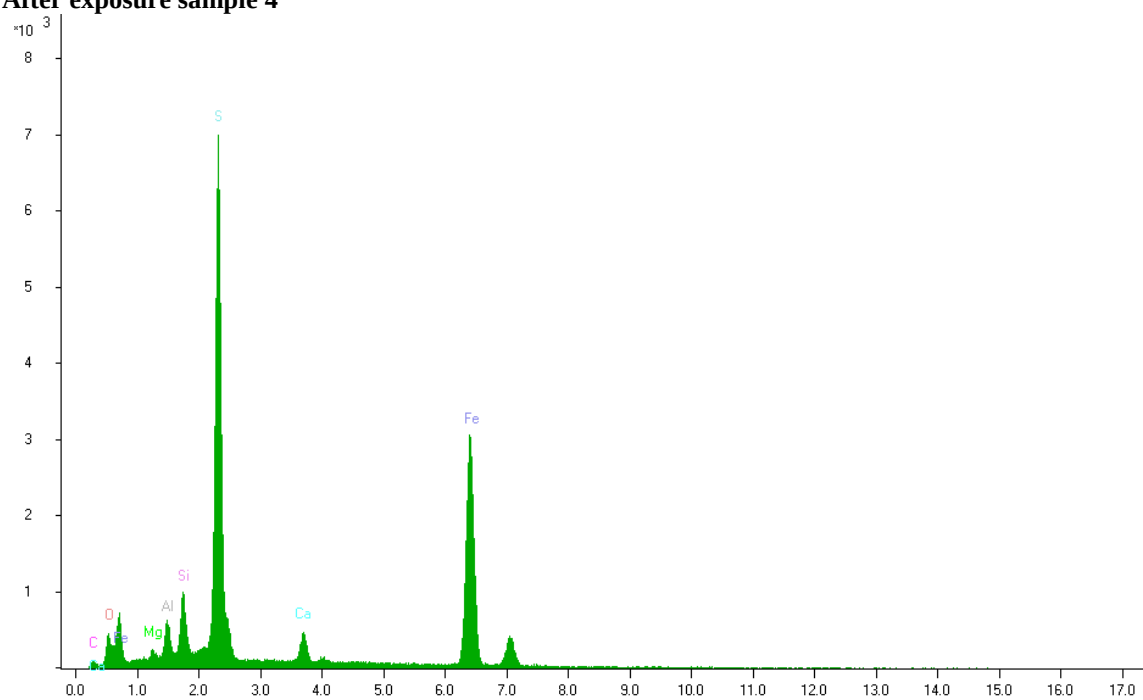
- 6) EDS spectra of pyrrhotite samples 1-12 at 700 x magnification averaged over entire area after exposure to SRB. Samples 1-4 were exposed for 7 days; samples 5-8 were exposed for 14 days; samples 9-12 were exposed for 30 days.

After exposure sample 1

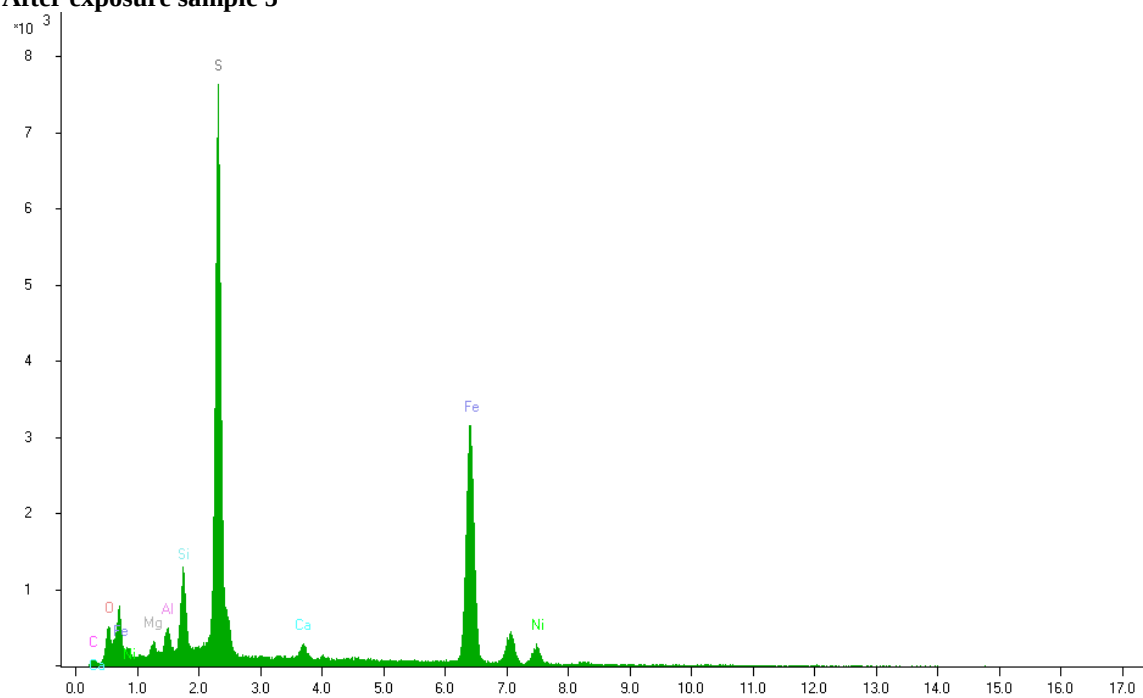


After exposure sample 2

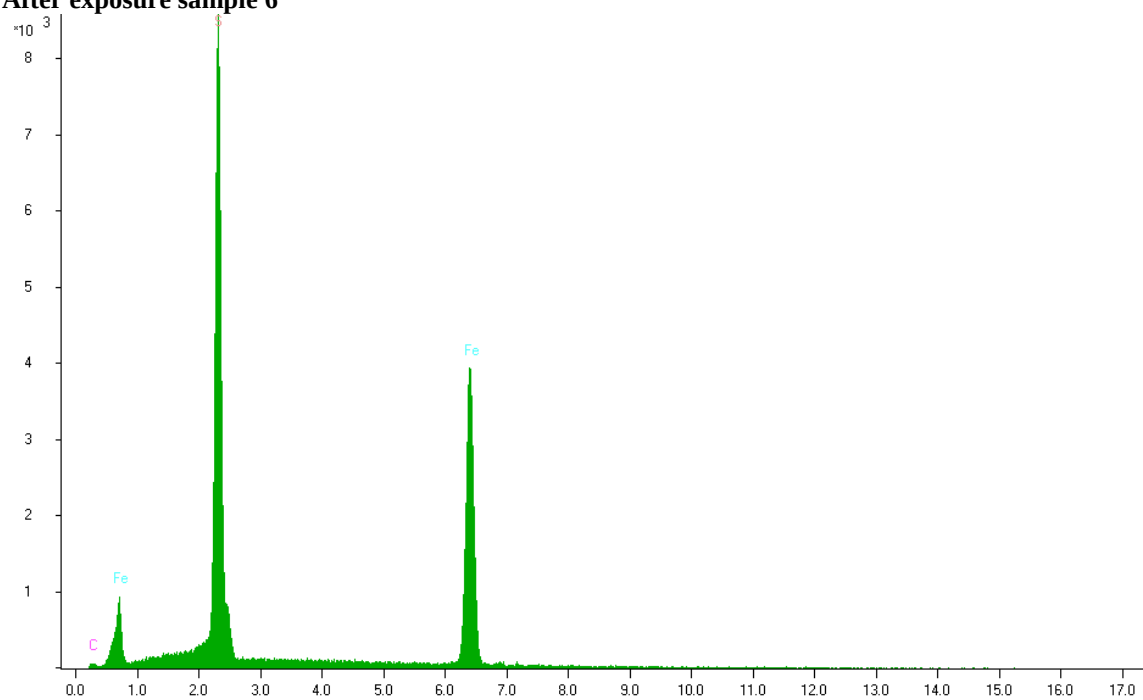


After exposure sample 3**After exposure sample 4**

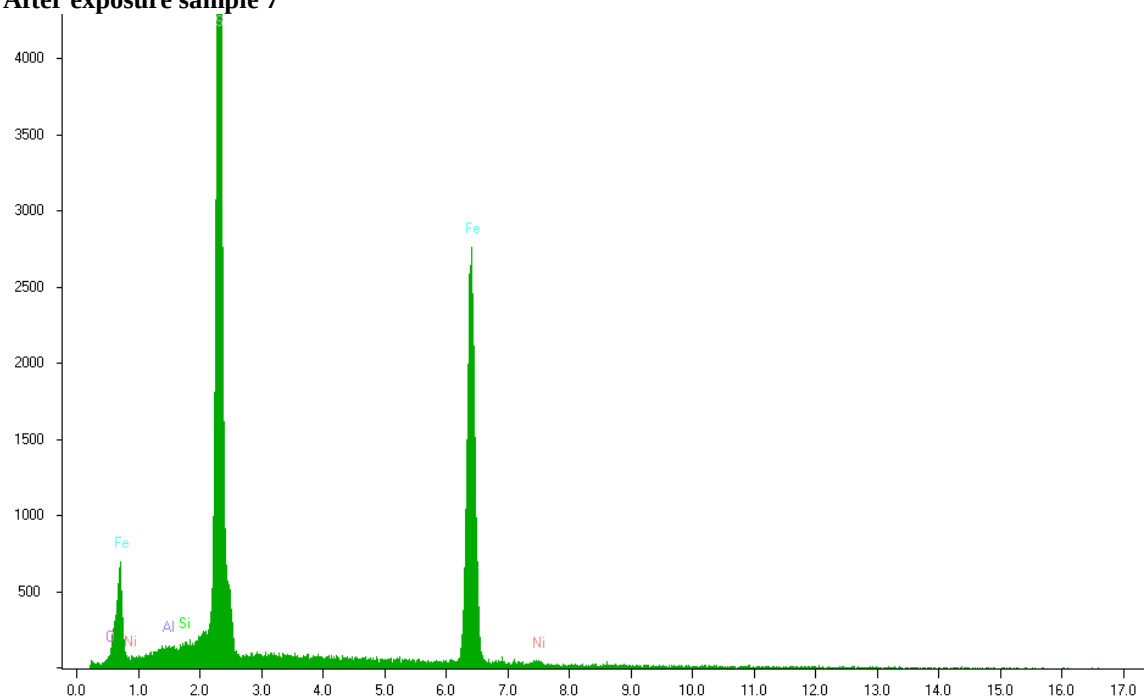
After exposure sample 5



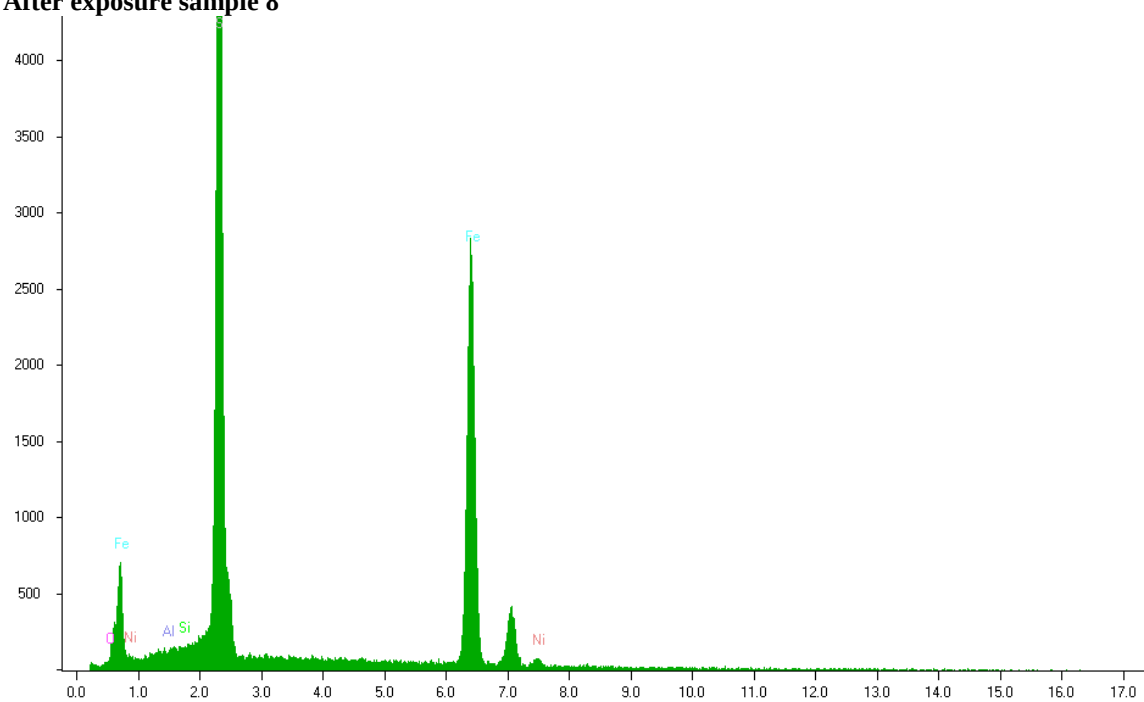
After exposure sample 6

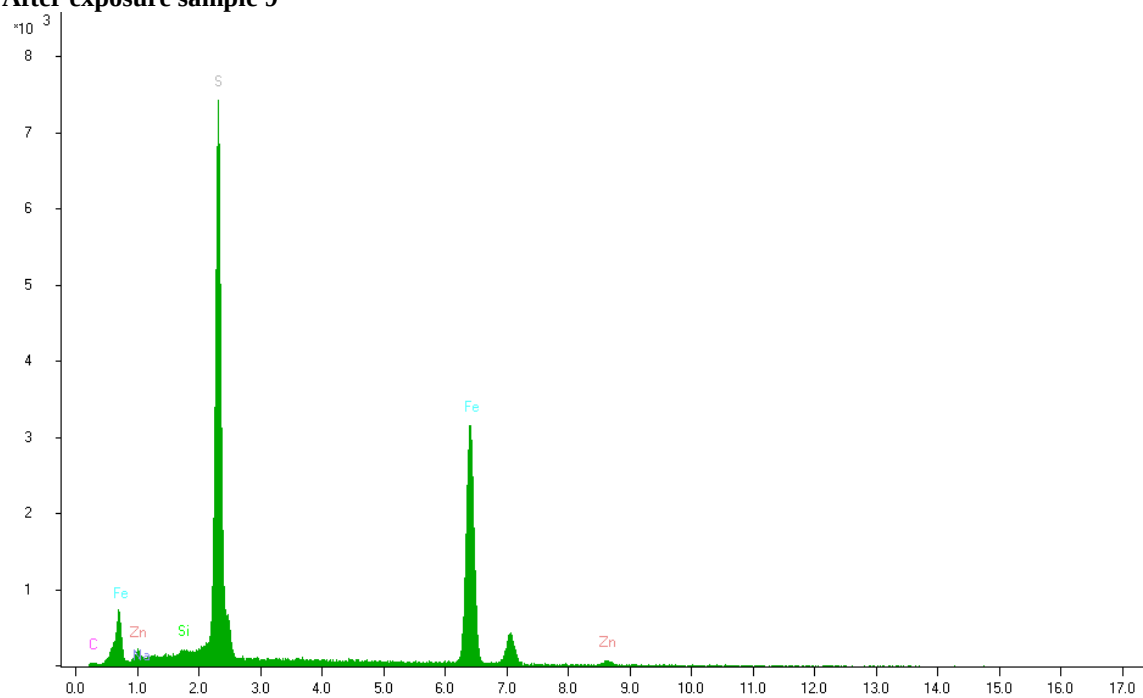
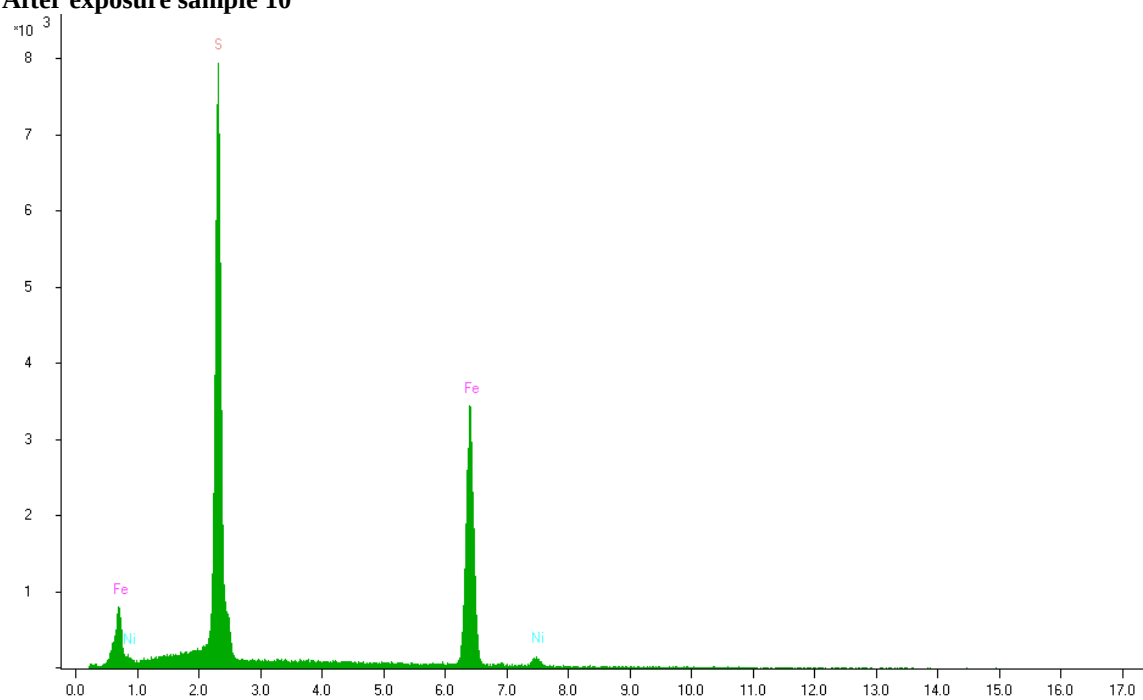


After exposure sample 7

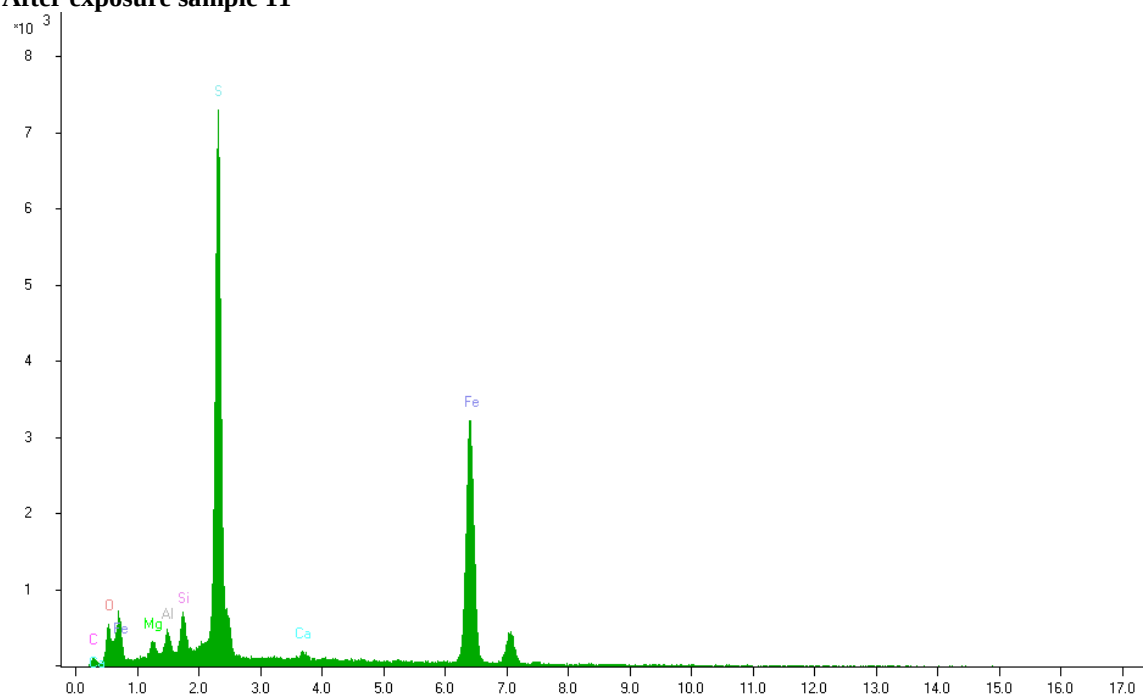


After exposure sample 8

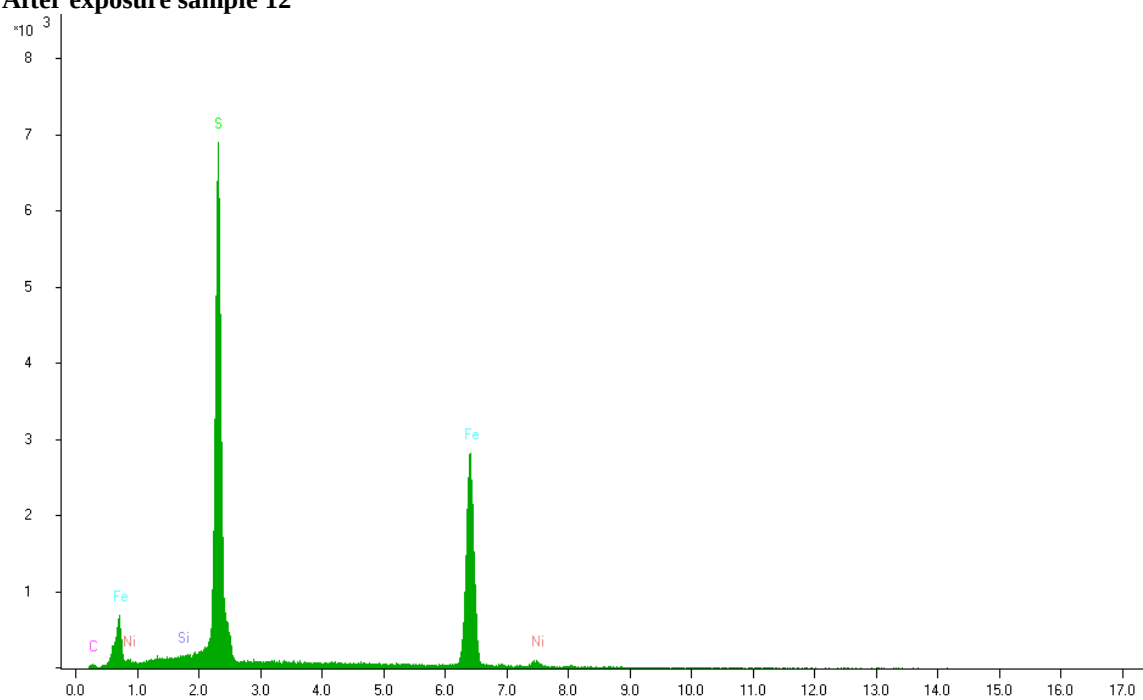


After exposure sample 9**After exposure sample 10**

After exposure sample 11



After exposure sample 12

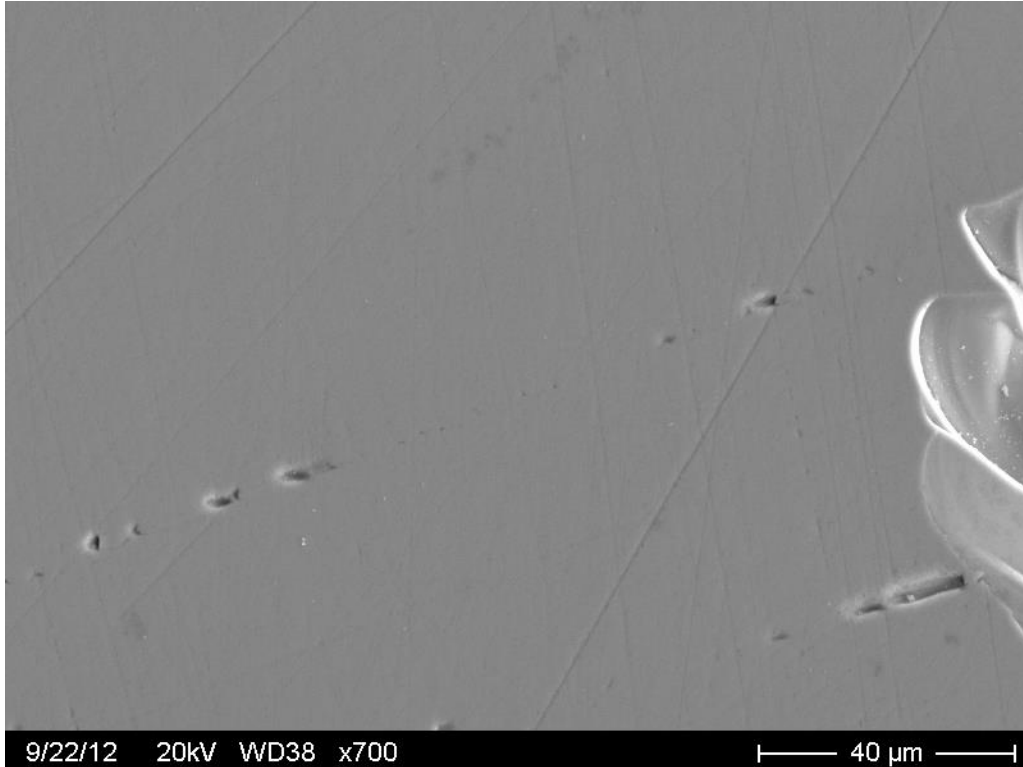


APPENDIX C

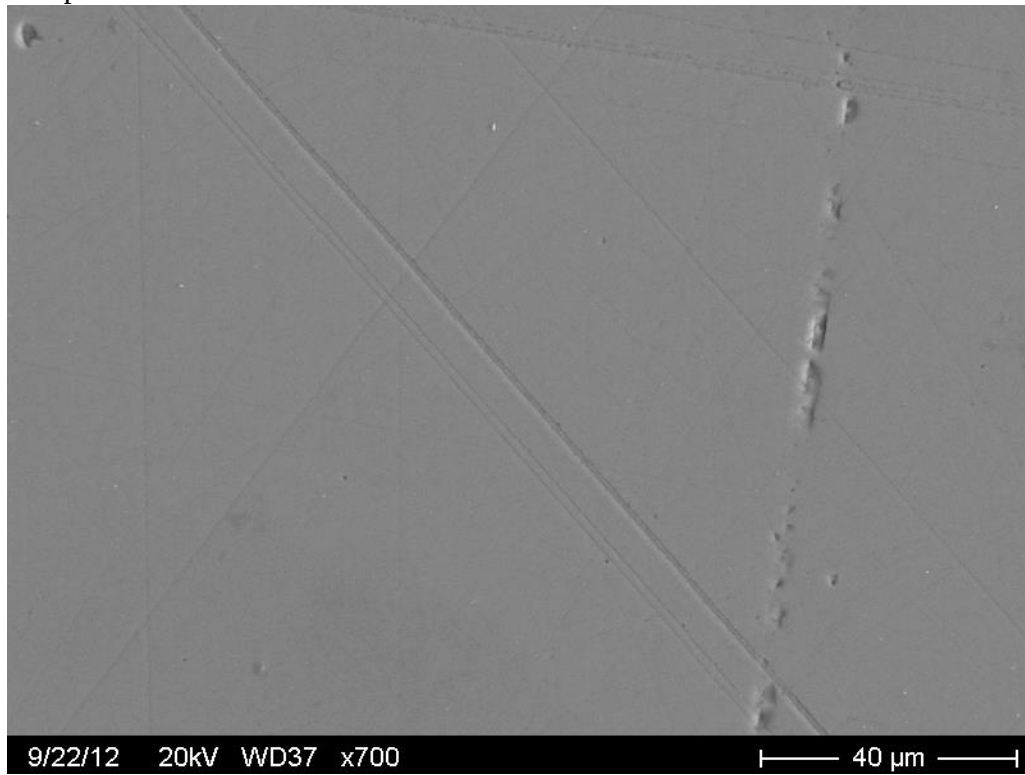
SEM IMAGES AND EDS SPECTRA OF PYRITE
BEFORE AND AFTER EXPOSURE TO SRB

Appendix C contains SEM secondary electron backscatter electron images with EDS spectra of pyrite prior to and after exposure to SRB. 1) Secondary electron images before exposure; 2) EDS spectra before exposure; 3) secondary electron images after exposure;; 4) EDS after exposure.

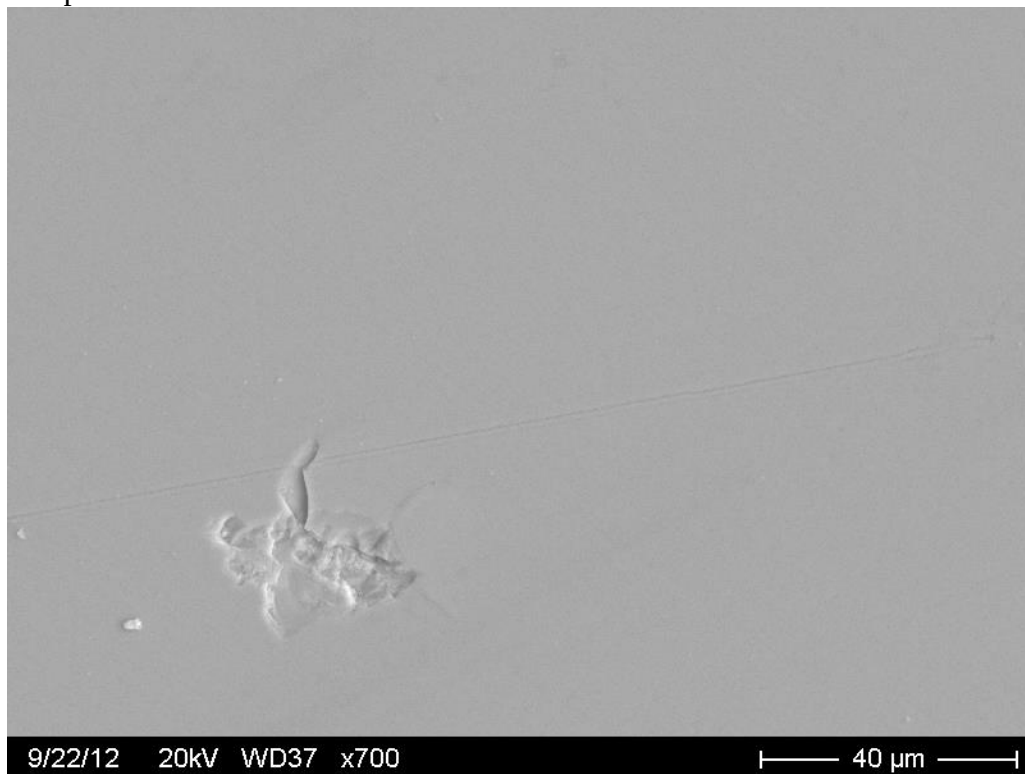
1) Secondary electron images of pyrite samples 2,3,5,6,7, and 9
Sample 2



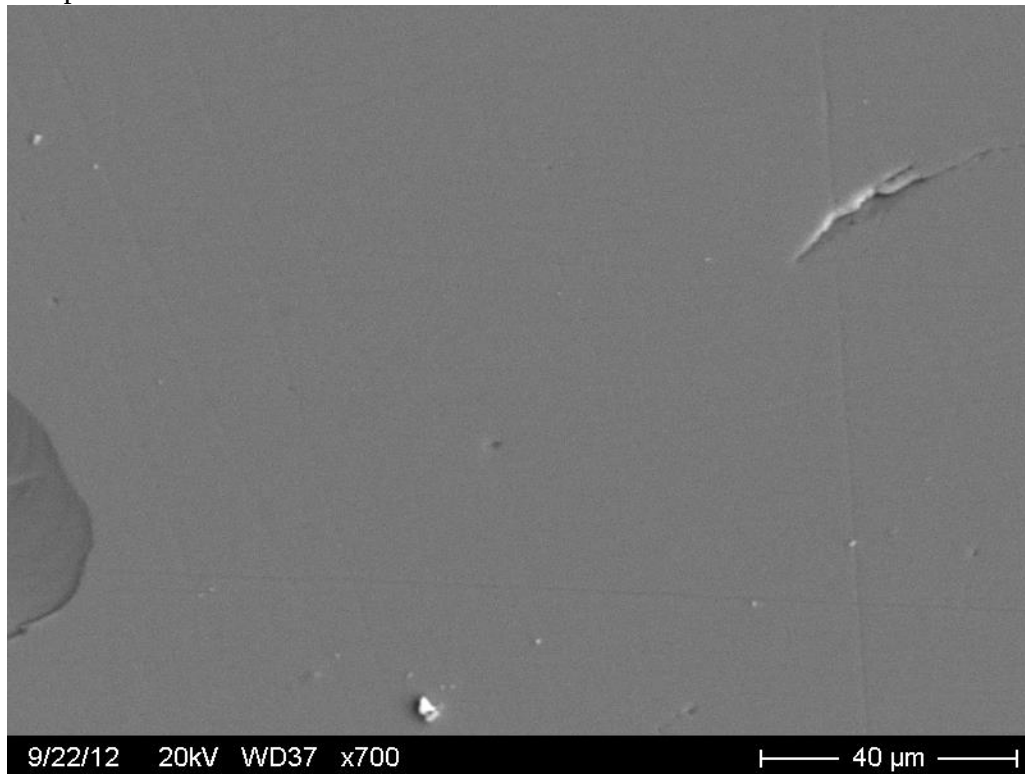
Sample 3



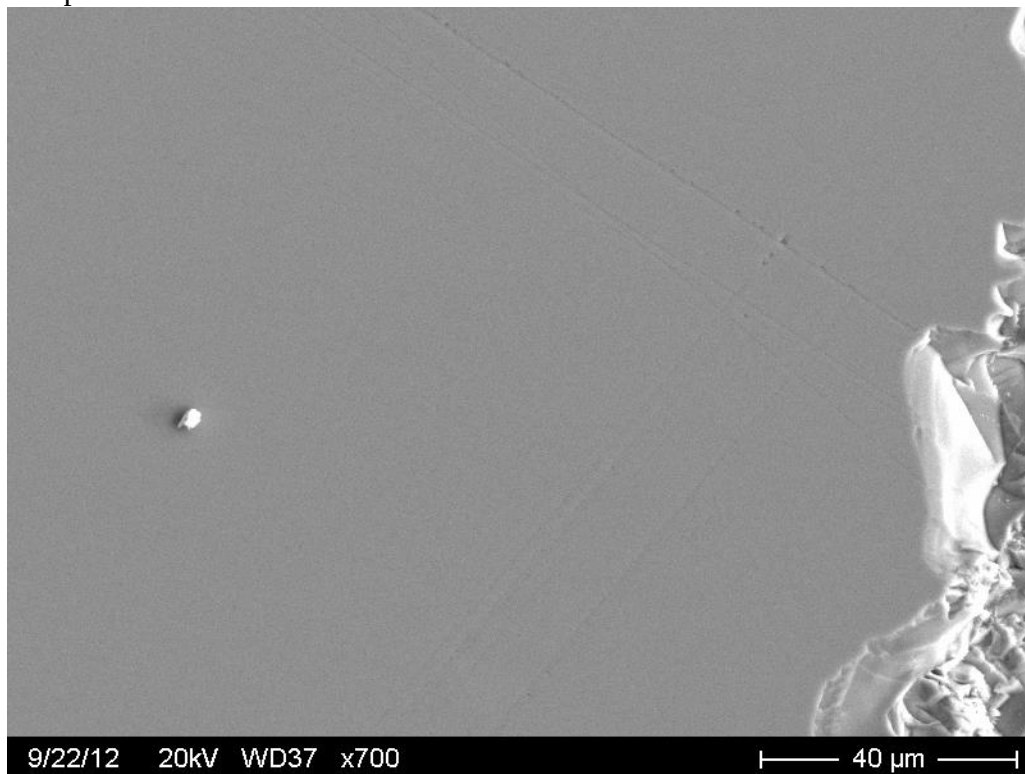
Sample 5



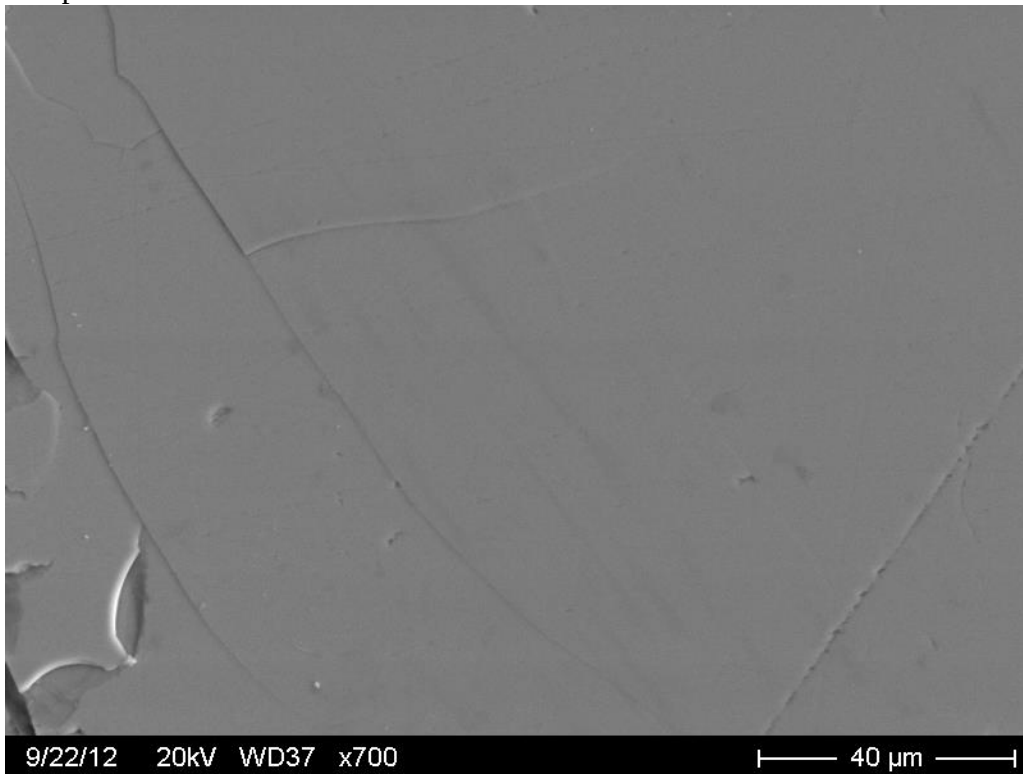
Sample 6



Sample 7

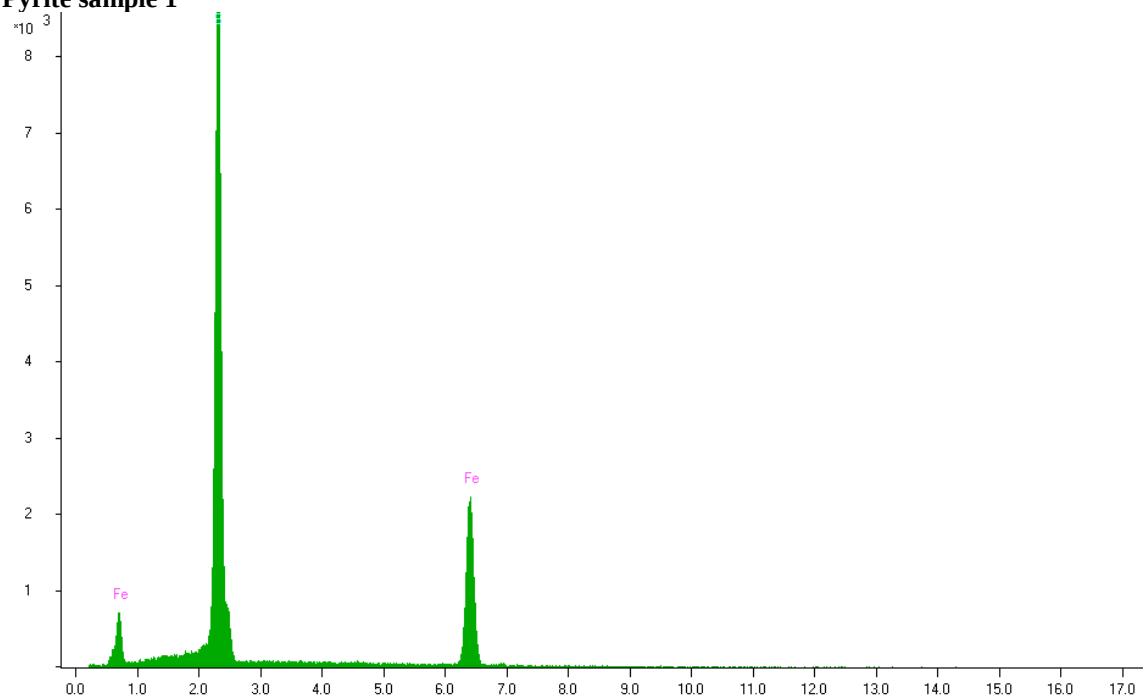


Sample 9

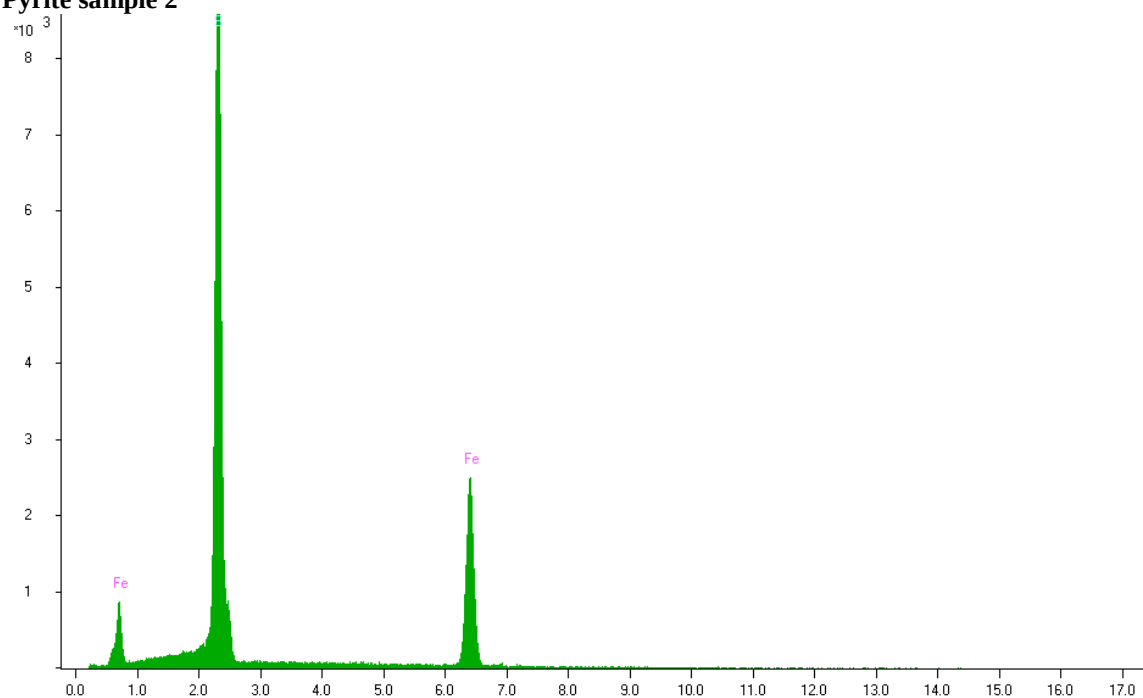


2) EDS of pyrite samples 1 and 12 before exposure to SRB. All spectra were nearly identical for samples 1-12.

Pyrite sample 1

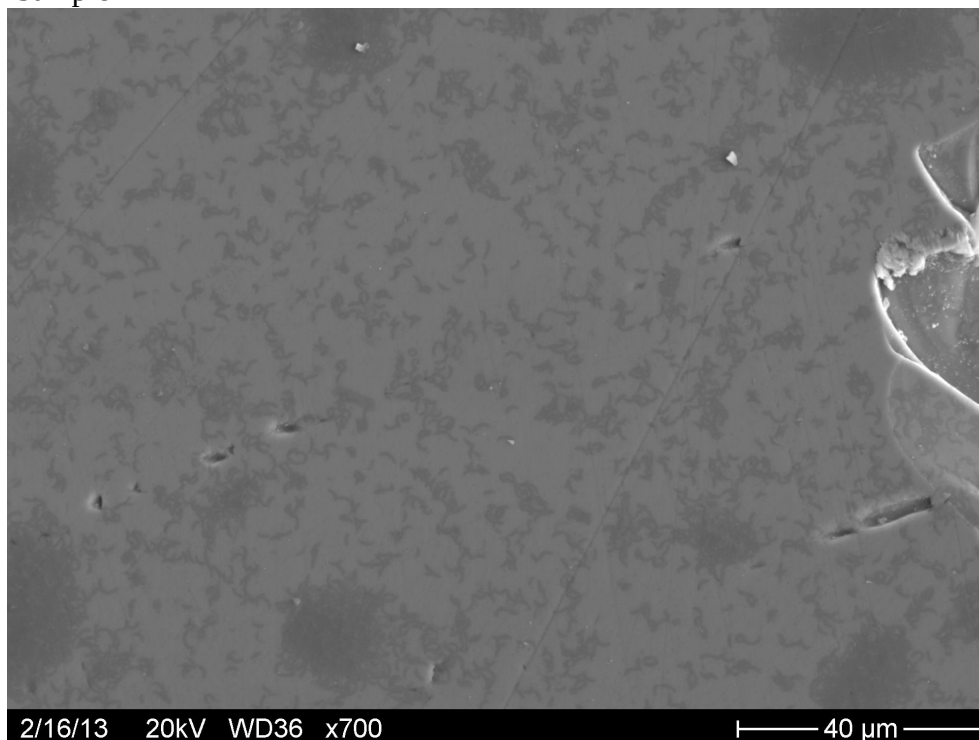


Pyrite sample 2

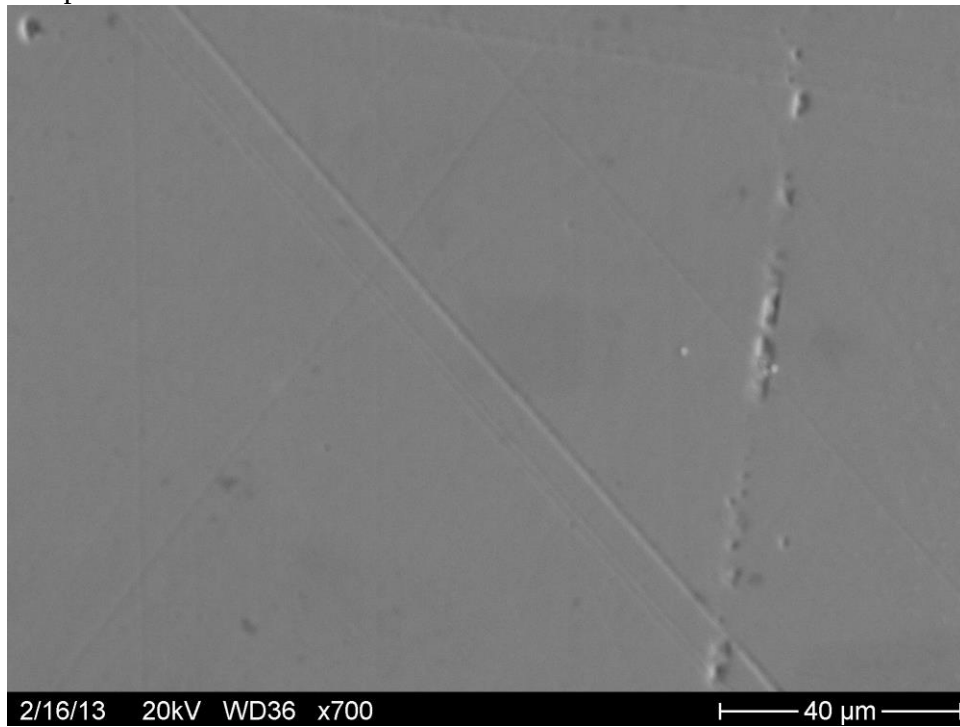


- 3) SEM secondary electron images of pyrite samples 2,3,6,7, and 9 after exposure to SRB. Samples 1-4 were exposed for 7 days; samples 5-8 were exposed for 14 days; samples 9-12 were exposed for 30 days.

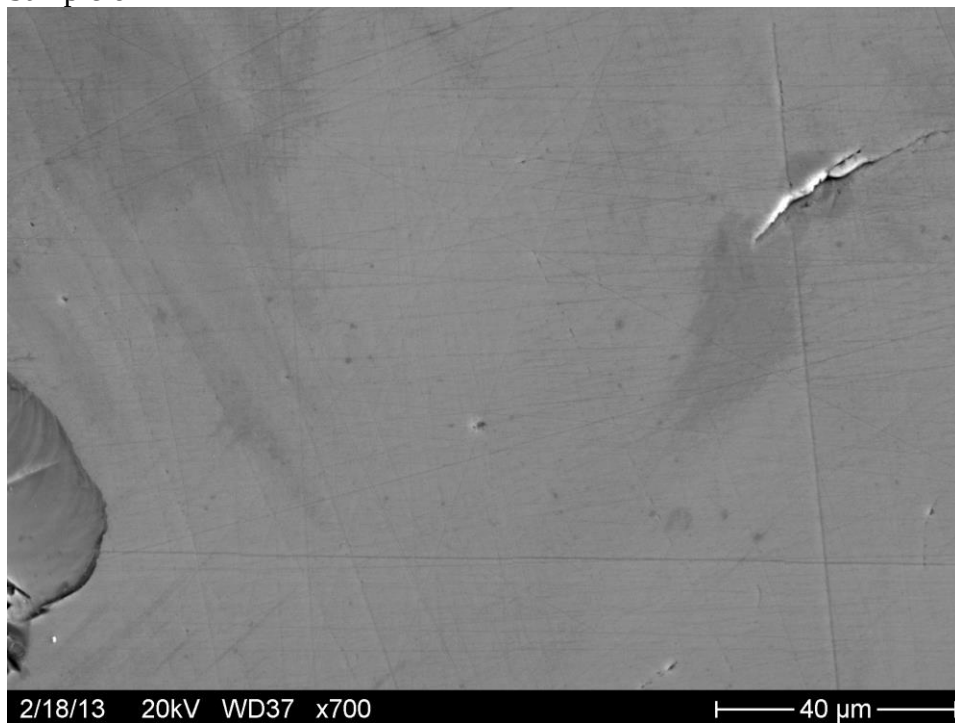
Sample 2



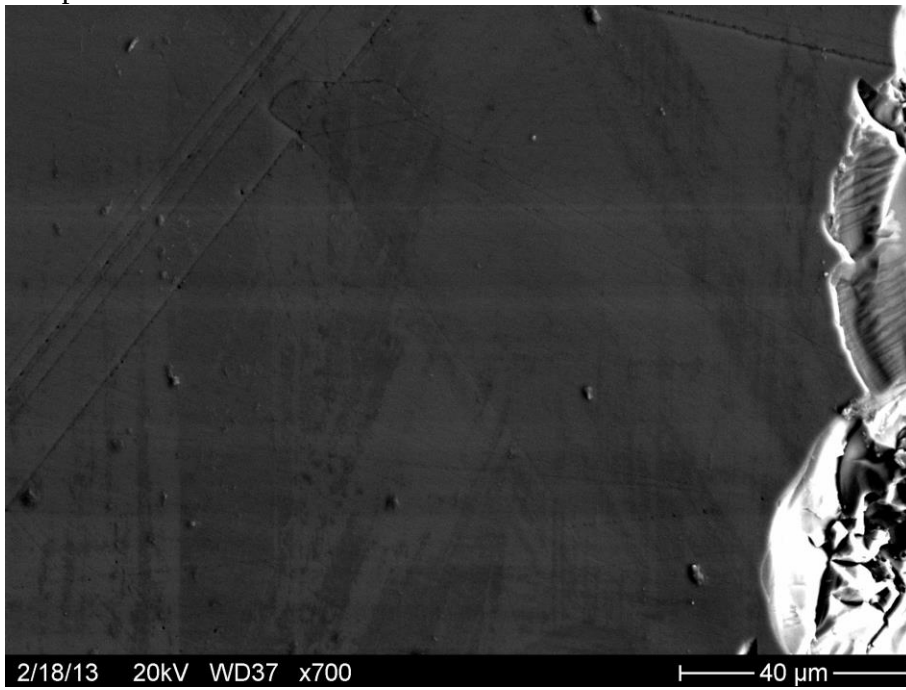
Sample 3



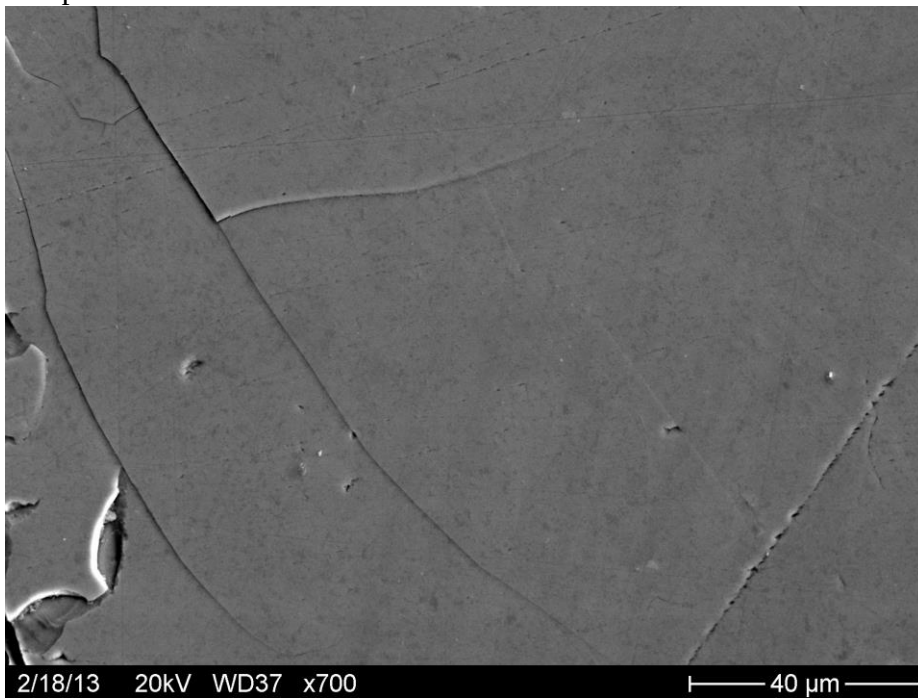
Sample 6



Sample 7

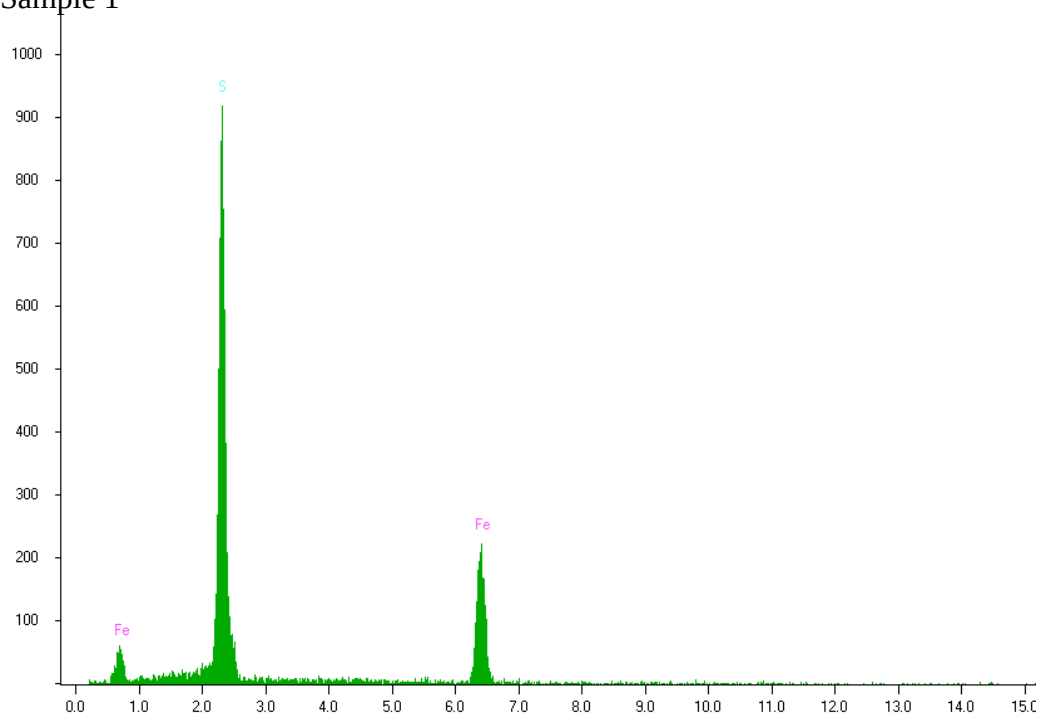


Sample 9

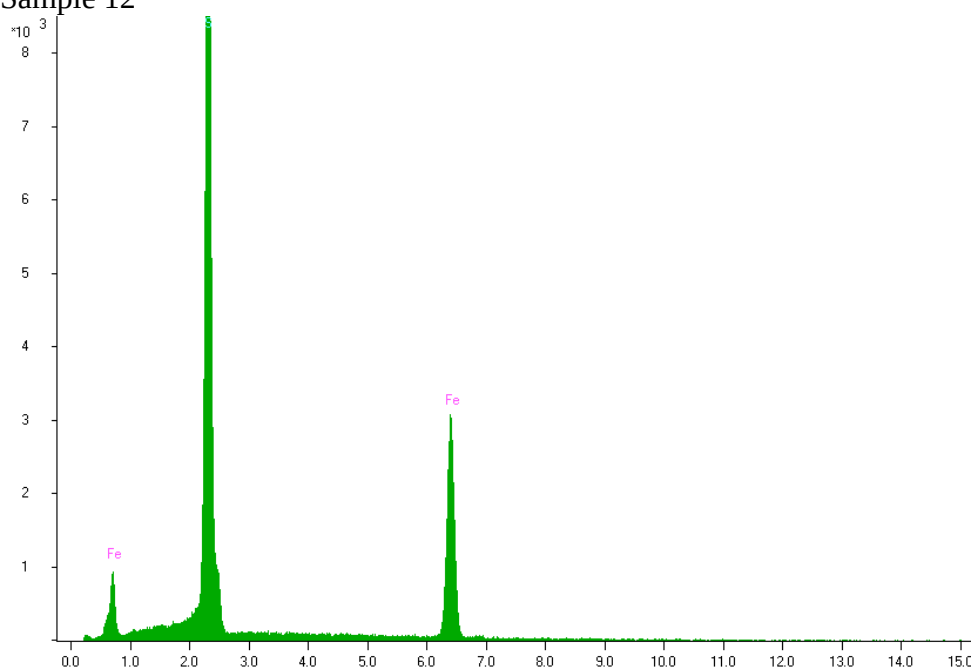


- 4) SEM EDS spectra of pyrite samples 1 and 12 after exposure to SRB. All spectra were nearly identical for samples 1-12.

Sample 1



Sample 12



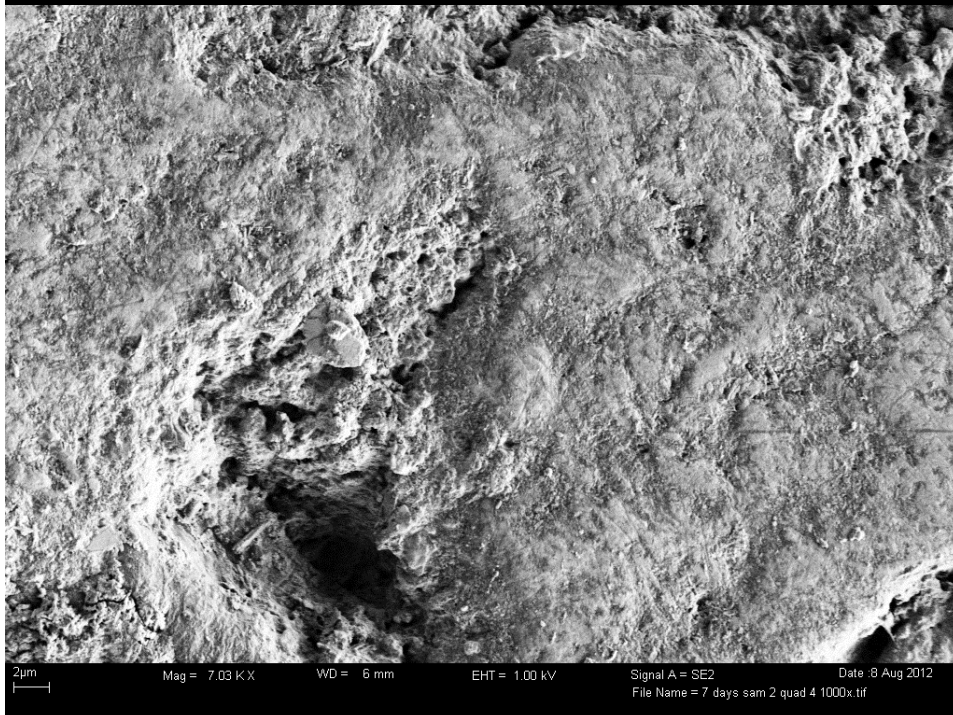
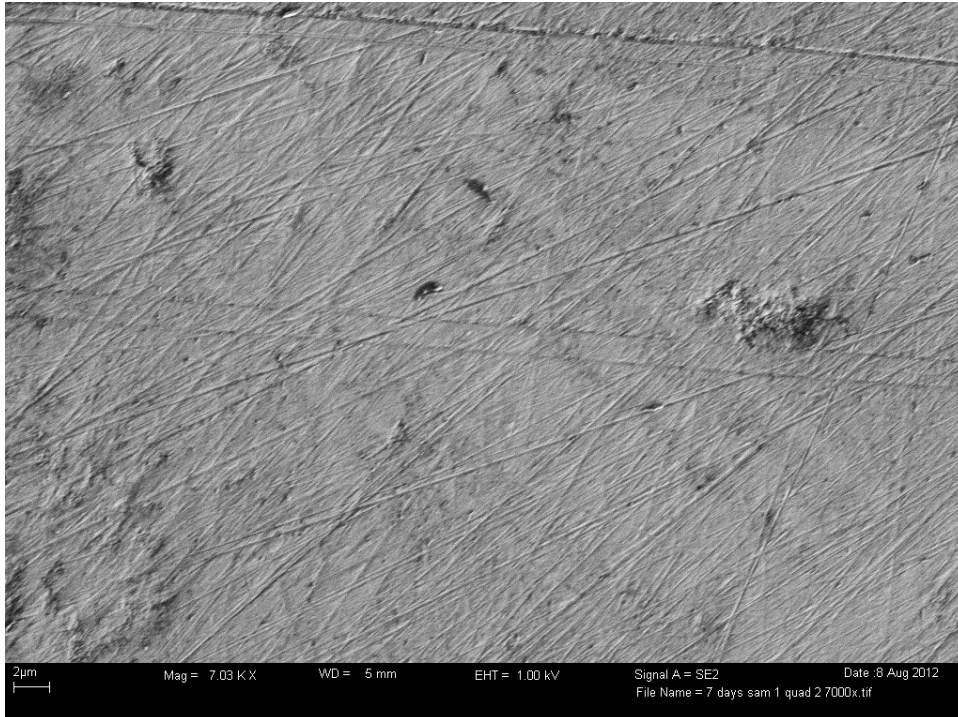
APPENDIX D

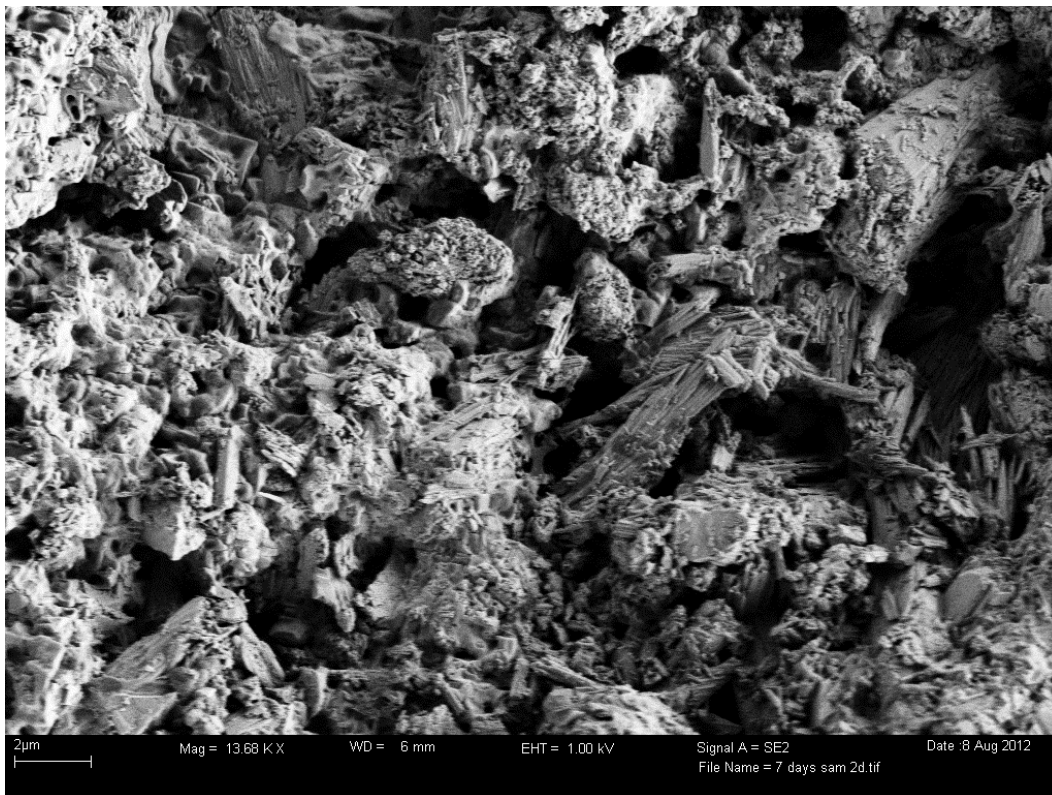
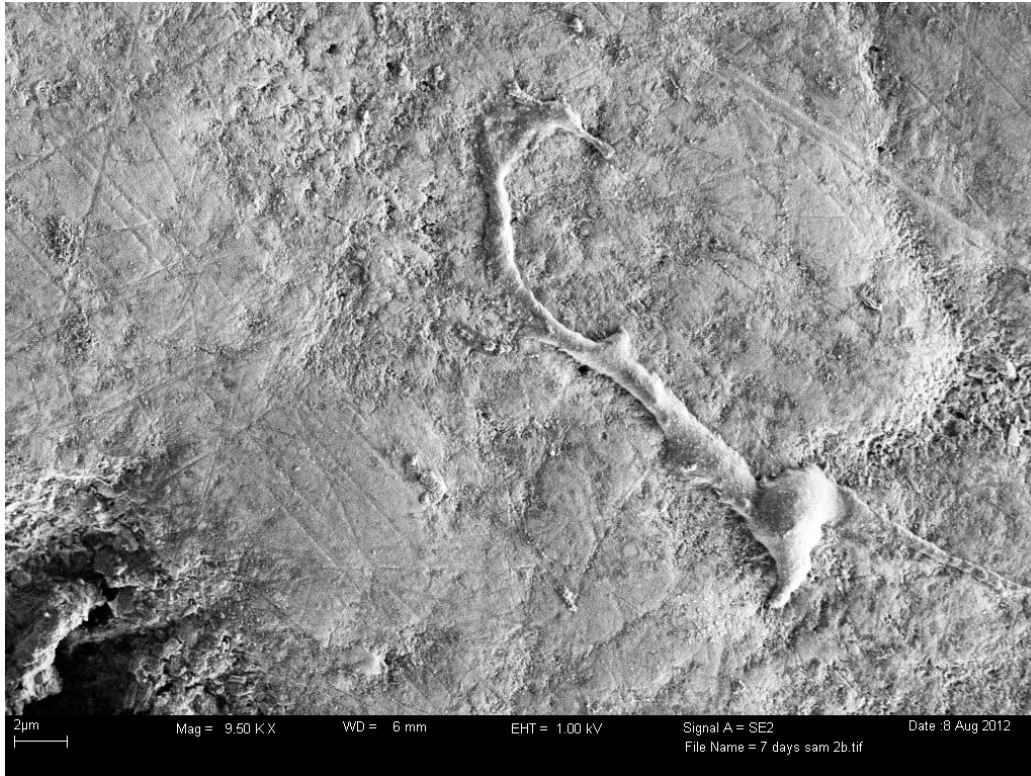
FE SEM IMAGES AND EDS SPECTRA OF GOETHITE
PYRRHOTITE AND PYRITE
AFTER EXPOSURE TO SRB

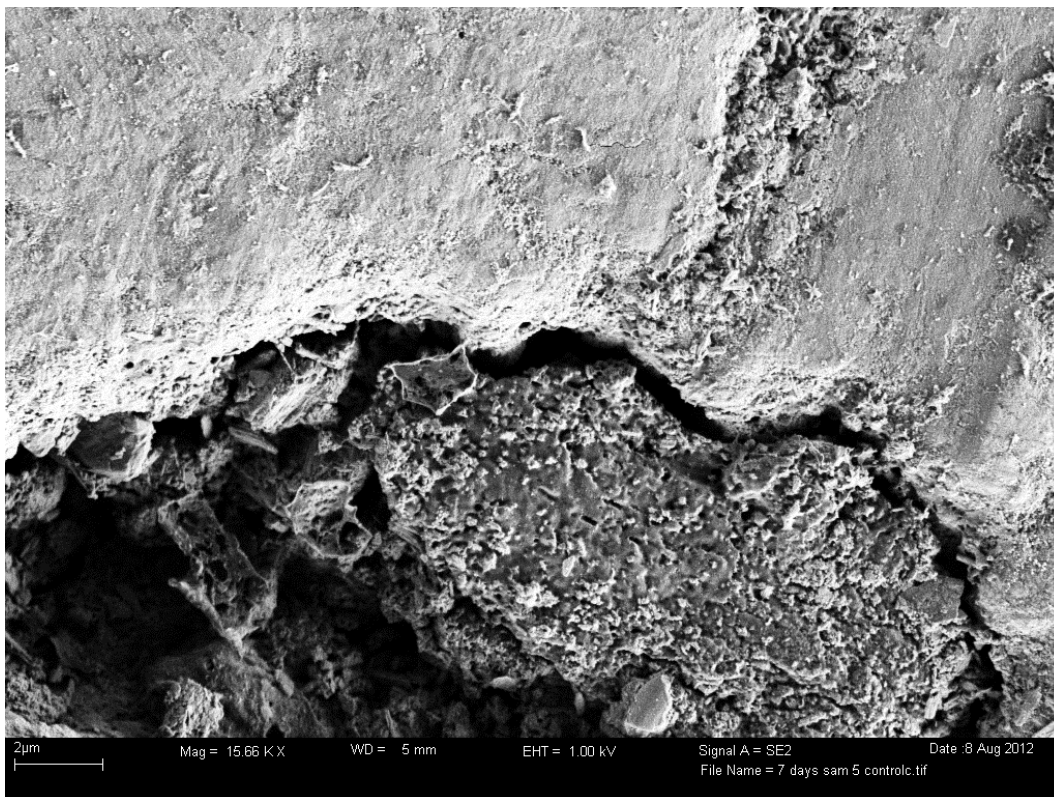
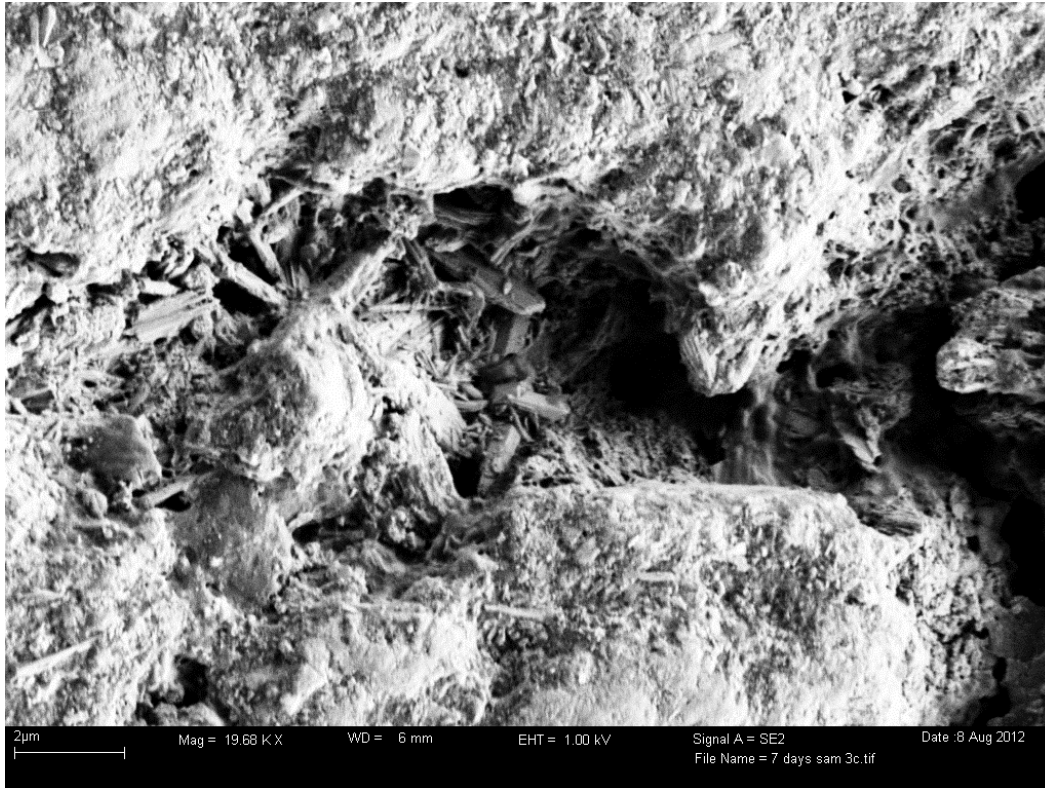
Appendix D contains FE SEM secondary electron, backscatter electron and InLens images of goethite, pyrrhotite and pyrite after exposure to SRB. 1) Secondary electron images of goethite samples after exposure with EDS spectra of spot analyses; 2) Secondary electron and InLens images of pyrrhotite with EDS spot analyses; 3) Secondary electron, backscatter electron and Inlens images of pyrite with EDS spot analyses. All spectra for pyrite were nearly identical.

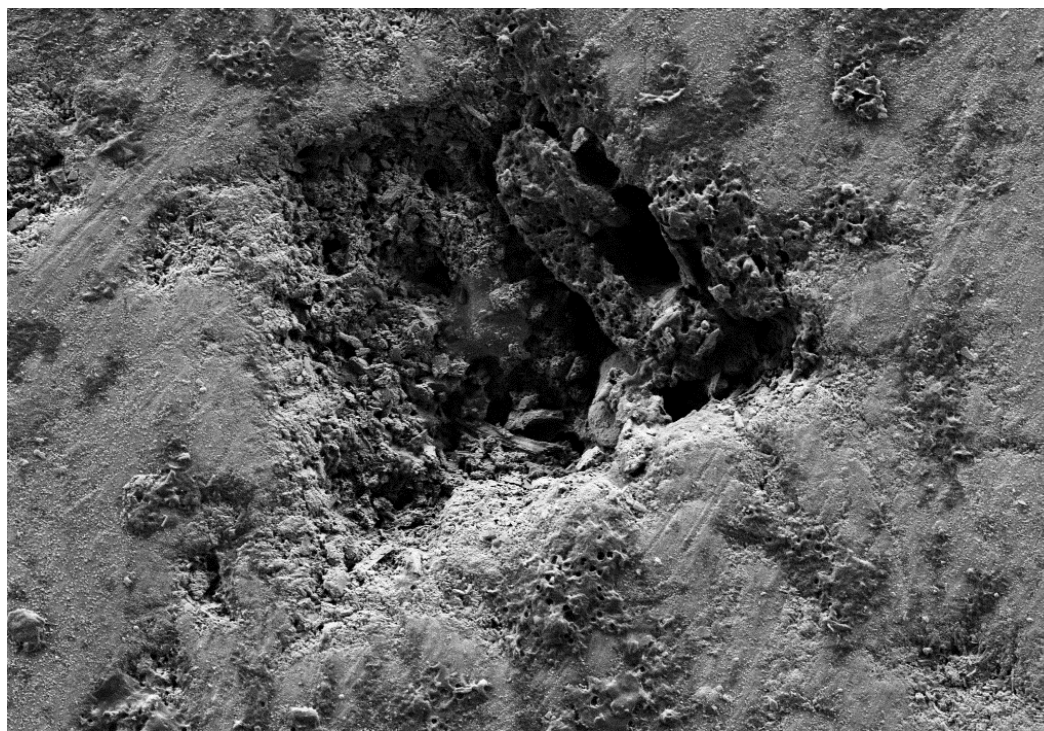
- 1) Secondary electron images of goethites samples after exposure to SRB. Samples 1-4 were exposed for 7 days; samples 5-8 were exposed for 14 days; samples 9-12 were exposed for 30 days.











1 μ m

Mag = 11.26 KX

WD = 5 mm

EHT = 1.00 kV

Signal A = SE2

Date :17 Aug 2012

MSU-ICAL

File Name = 15 days sam 4c.tif



10 μ m

Mag = 5.50 KX

WD = 5 mm

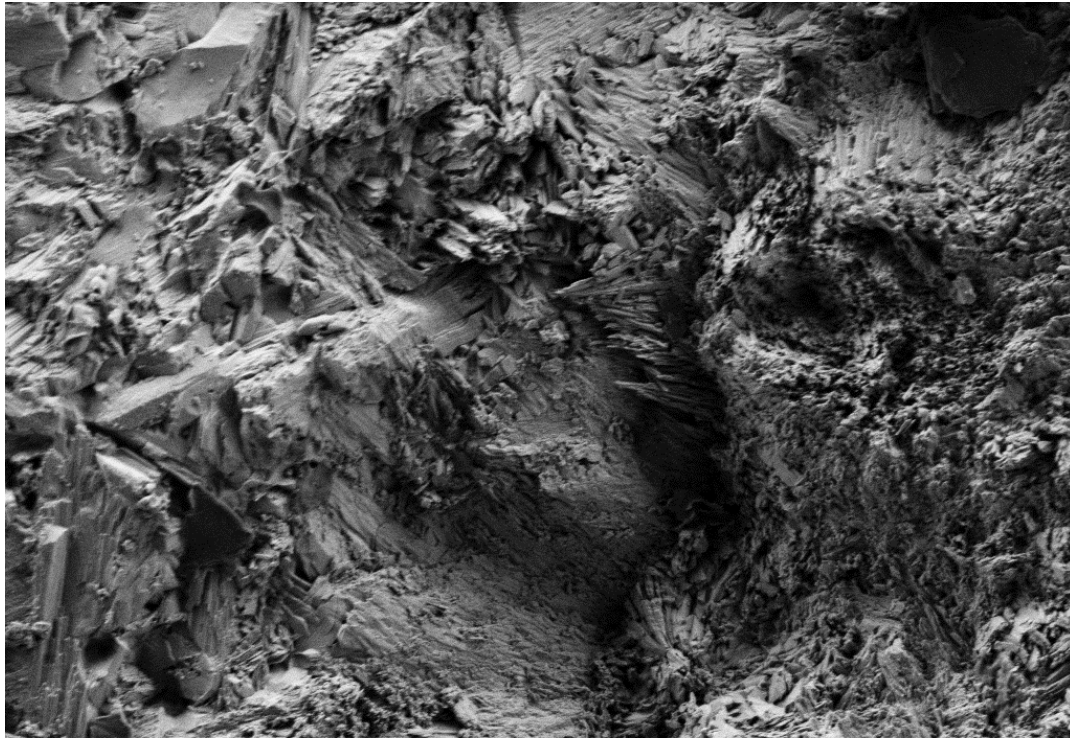
EHT = 1.00 kV

Signal A = SE2

Date :17 Aug 2012

MSU-ICAL

File Name = 15 days sam 4f.tif

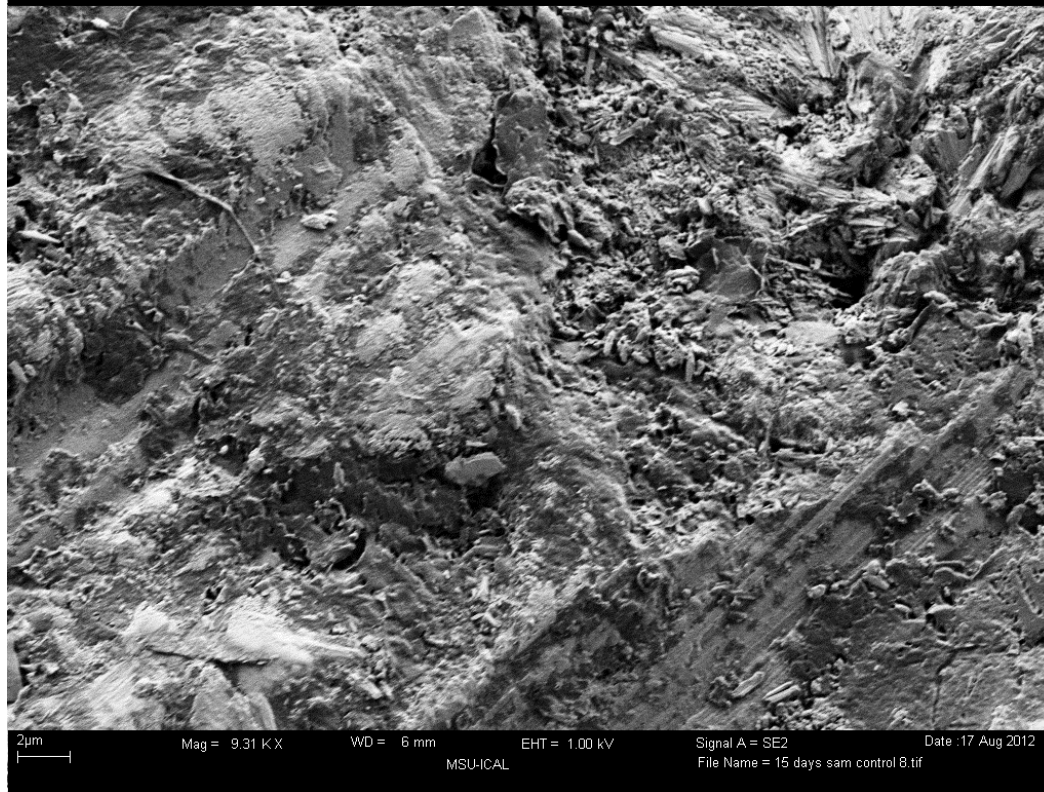
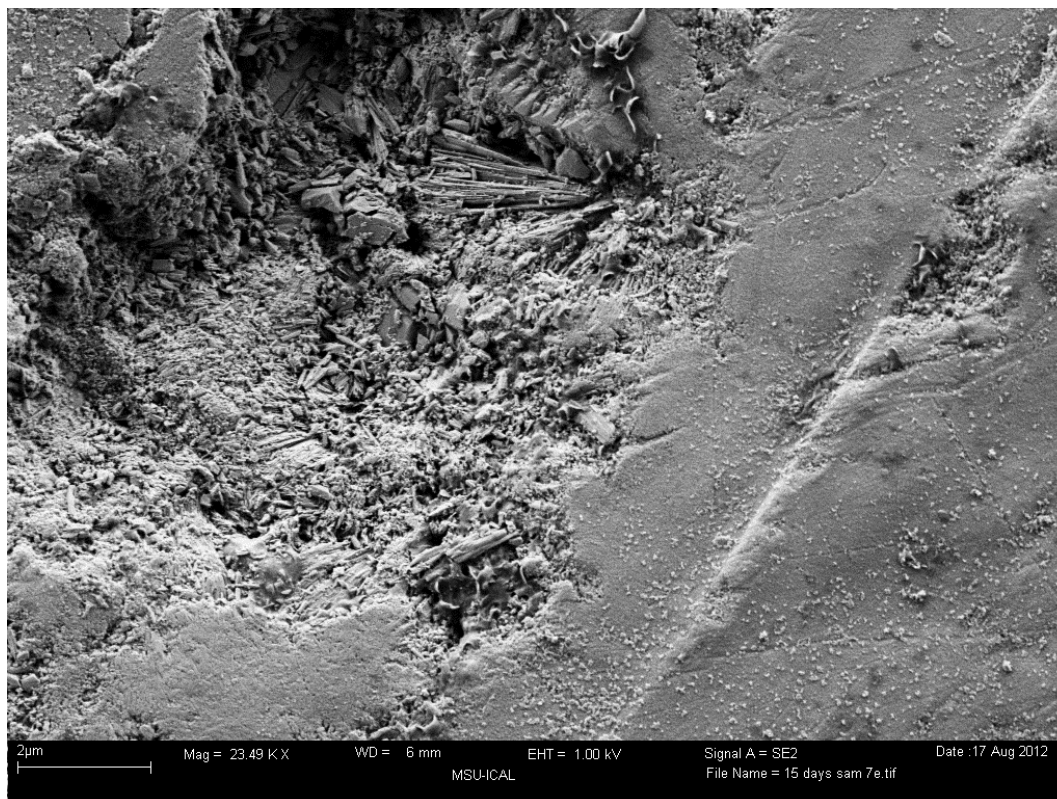


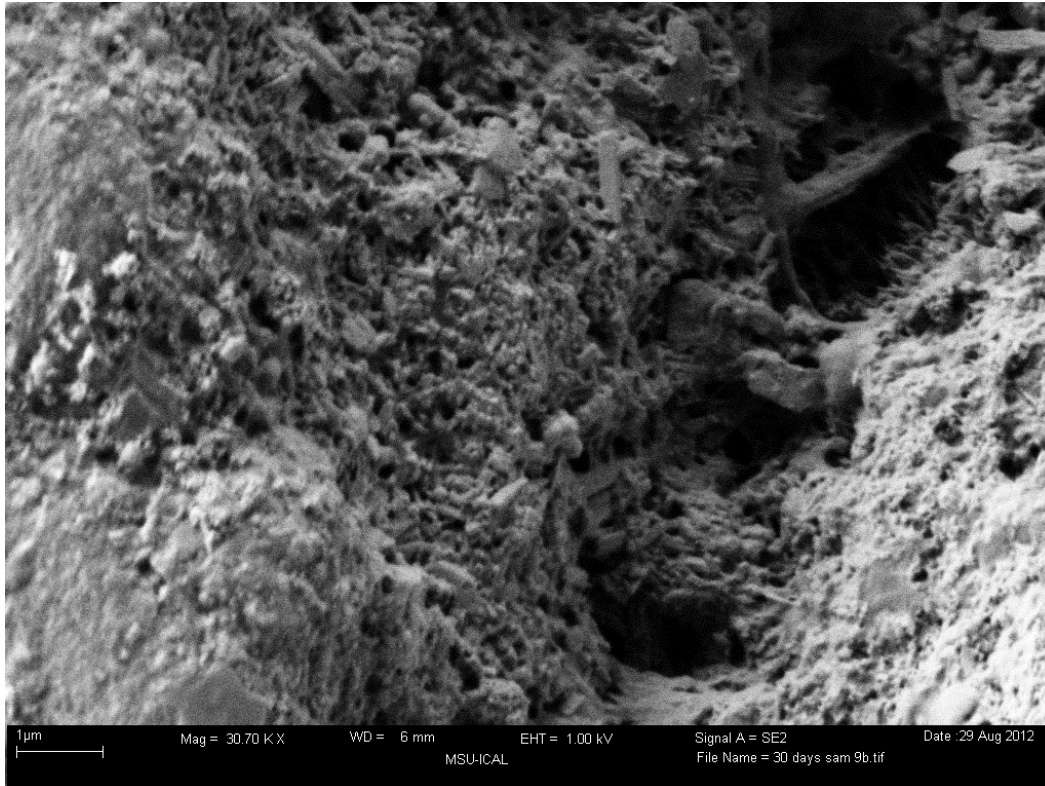
2μm Mag = 21.17 KX WD = 5 mm EHT = 1.00 kV Signal A = SE2
File Name = 15 days sam 4i.tif Date :17 Aug 2012
MSU-ICAL

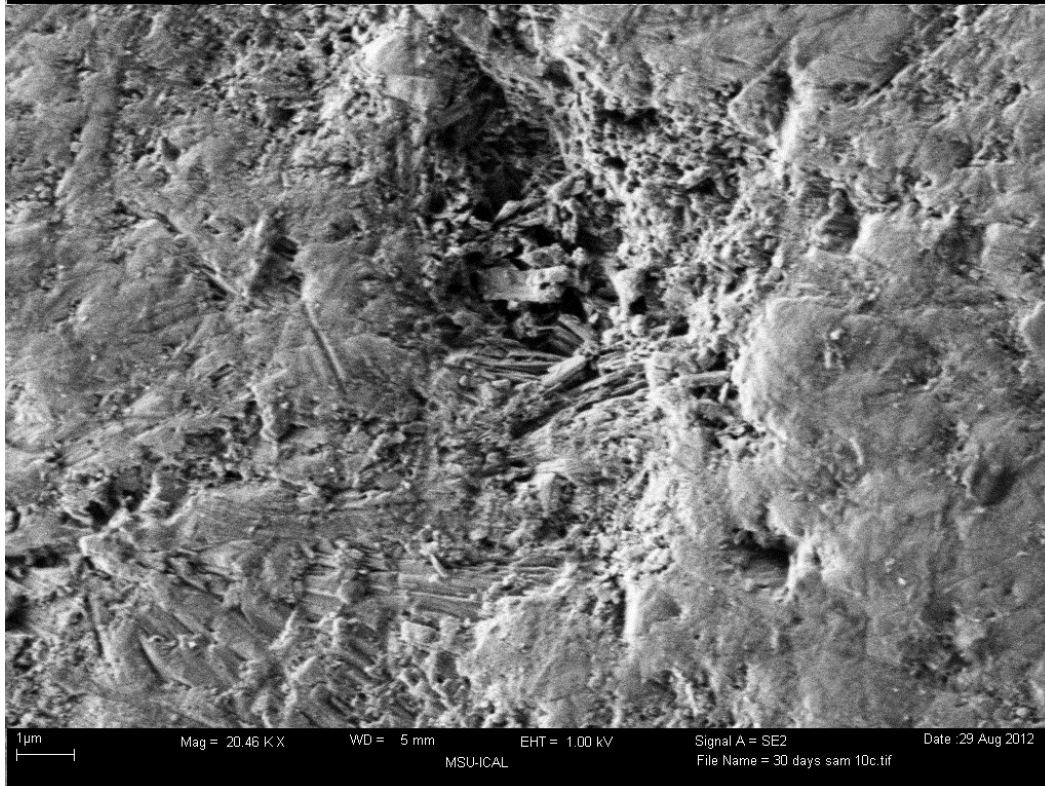
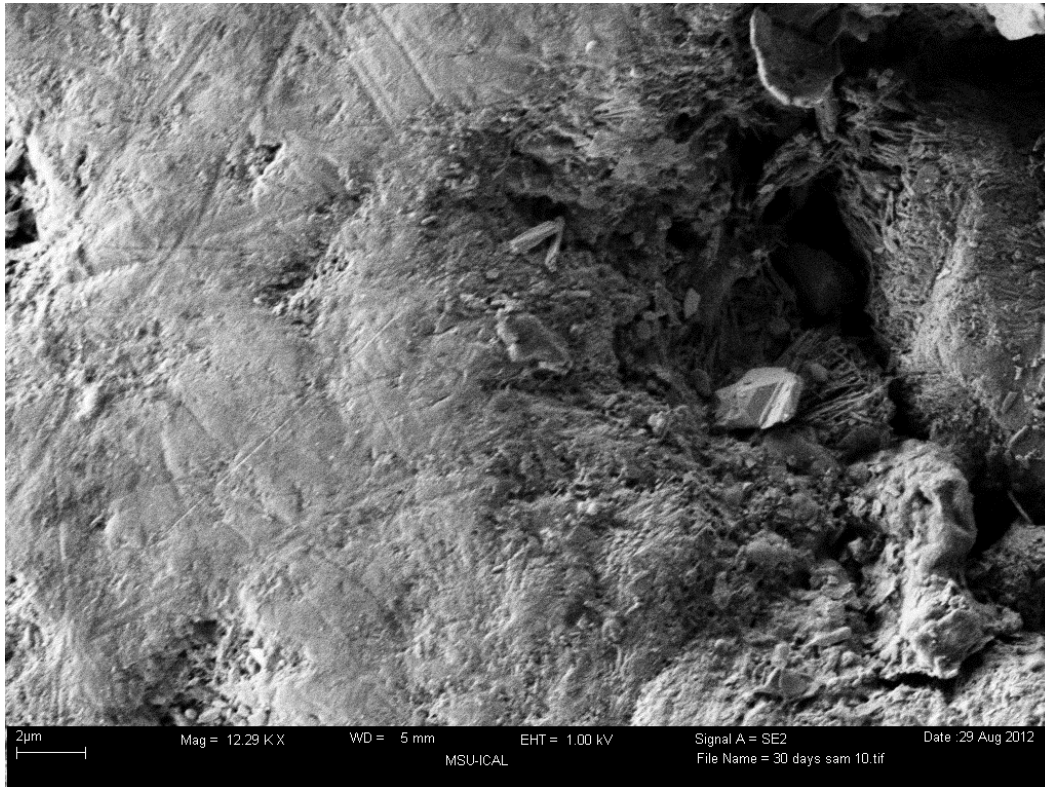


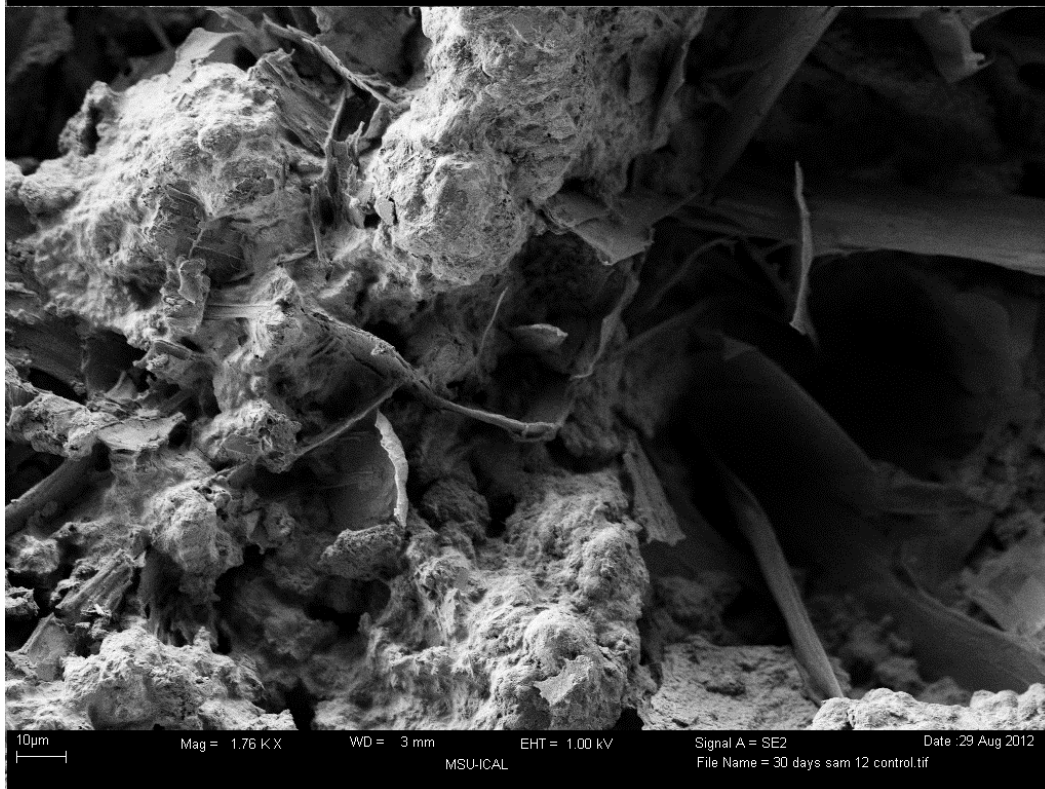
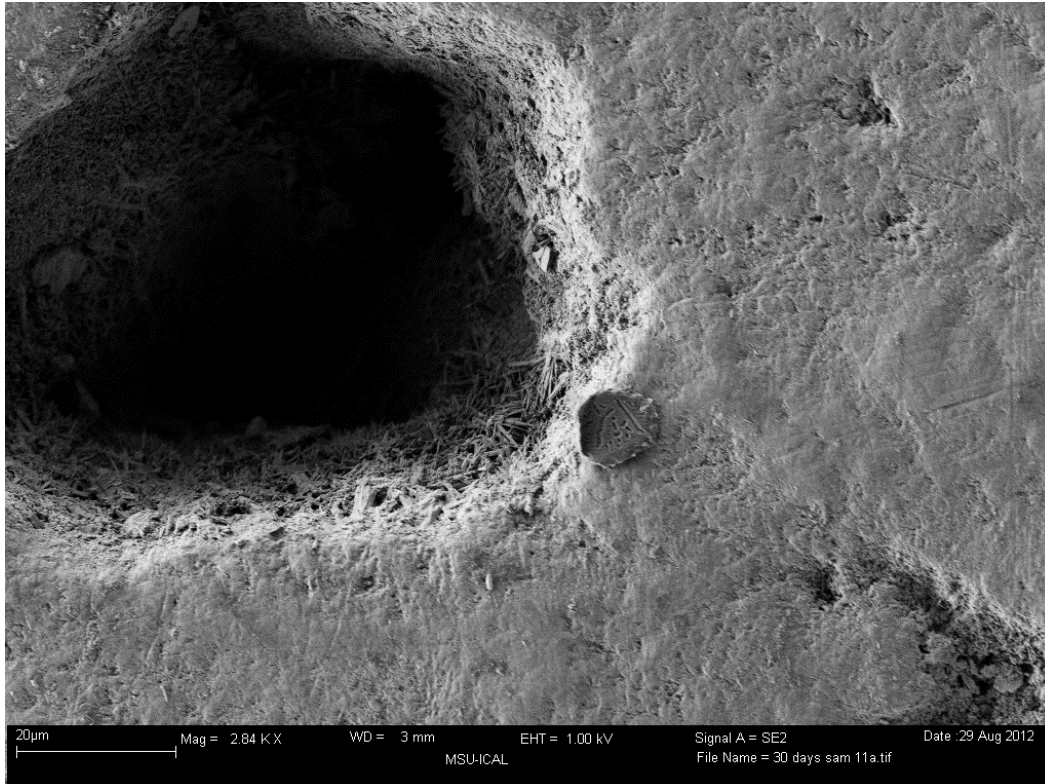
2μm Mag = 13.87 KX WD = 6 mm EHT = 1.00 kV Signal A = SE2
File Name = 15 days sam 6.tif Date :17 Aug 2012
MSU-ICAL

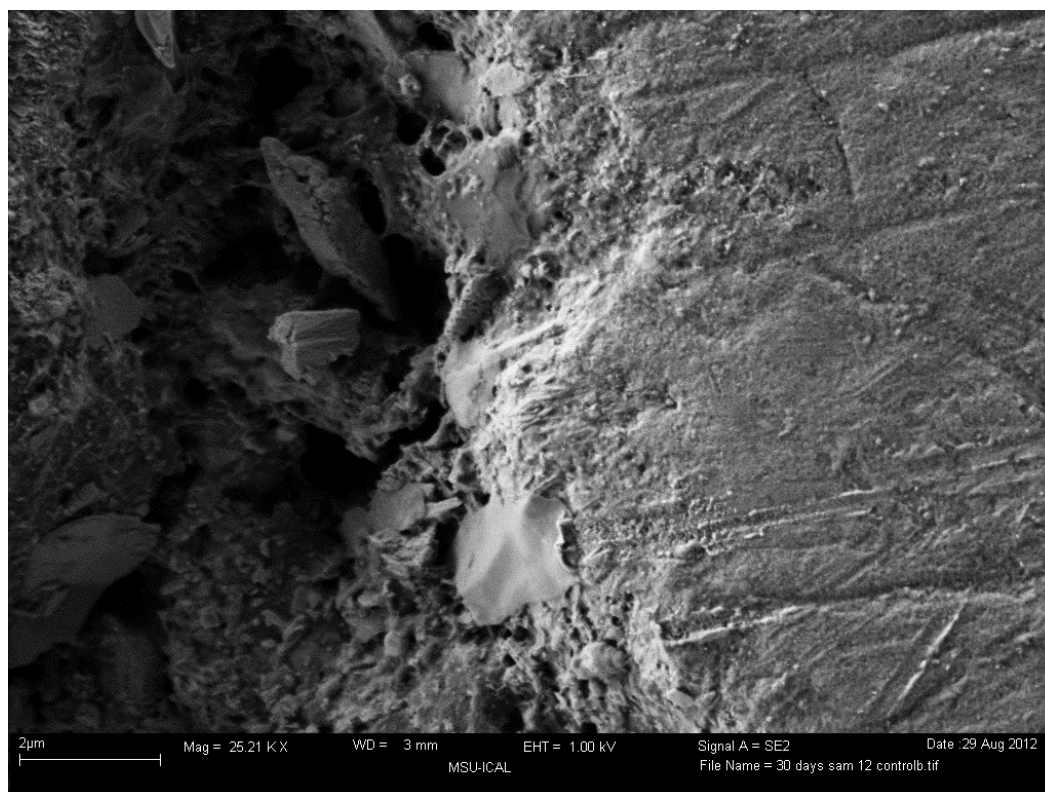






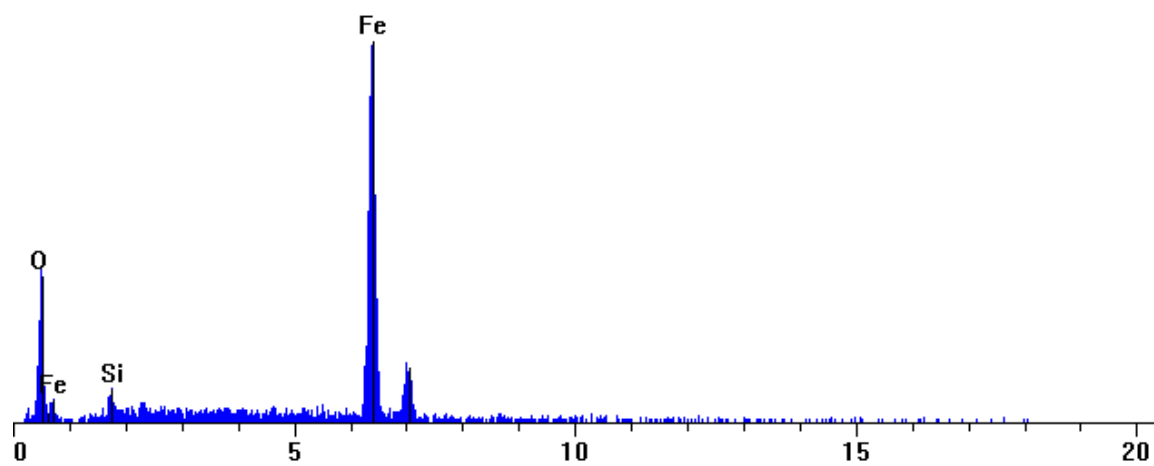






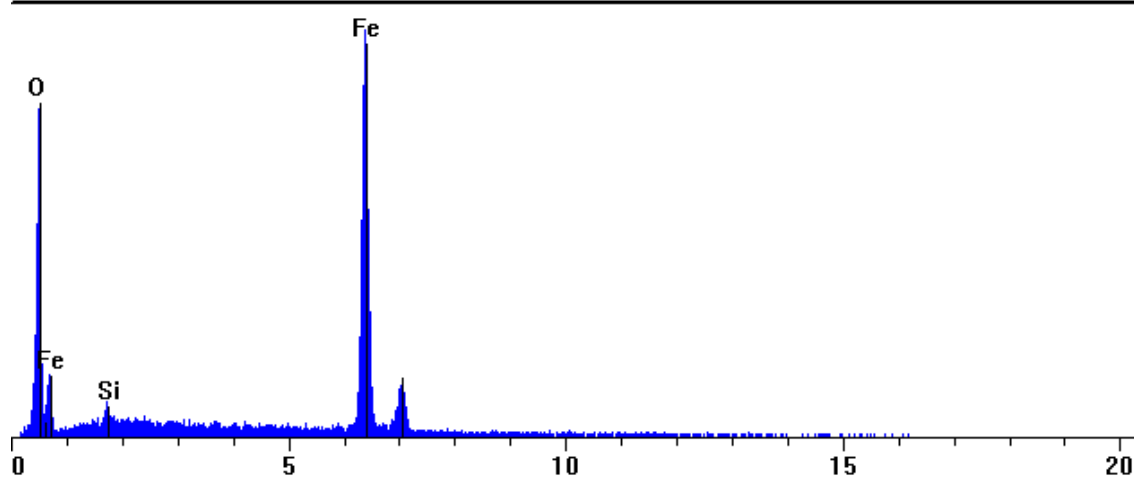
■ crystal 4i.pgt

FS: 400



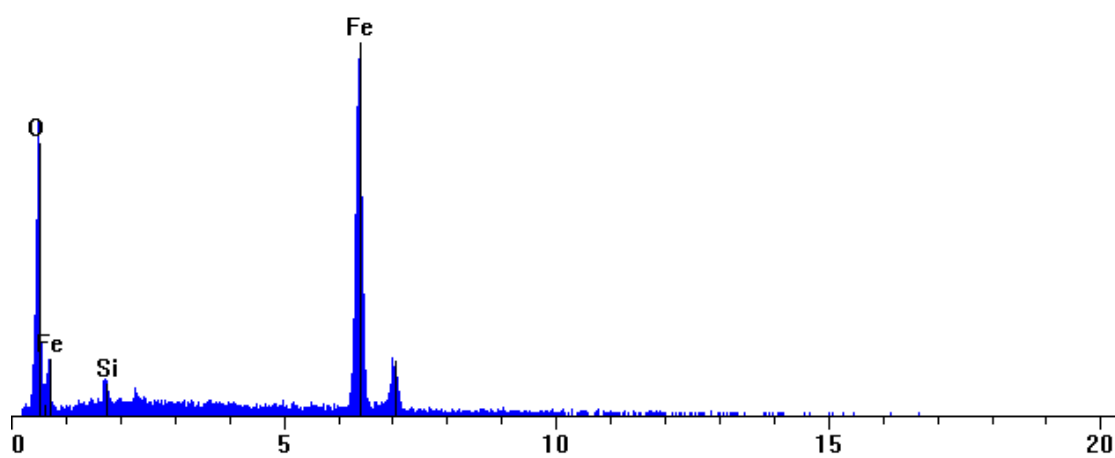
■ crystal from 7e.pgt

FS: 1400



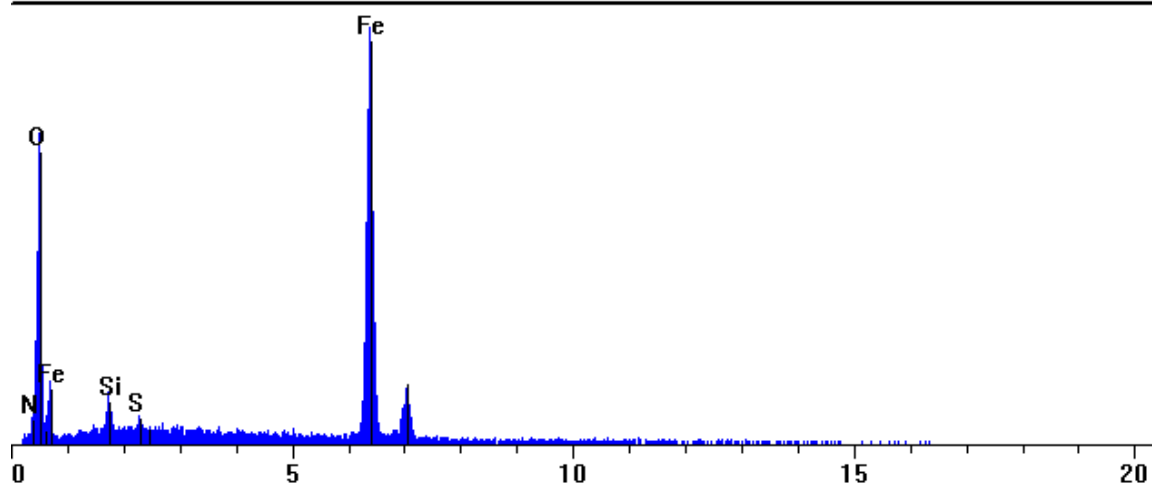
■ crystal 8a.pgt

FS: 1000



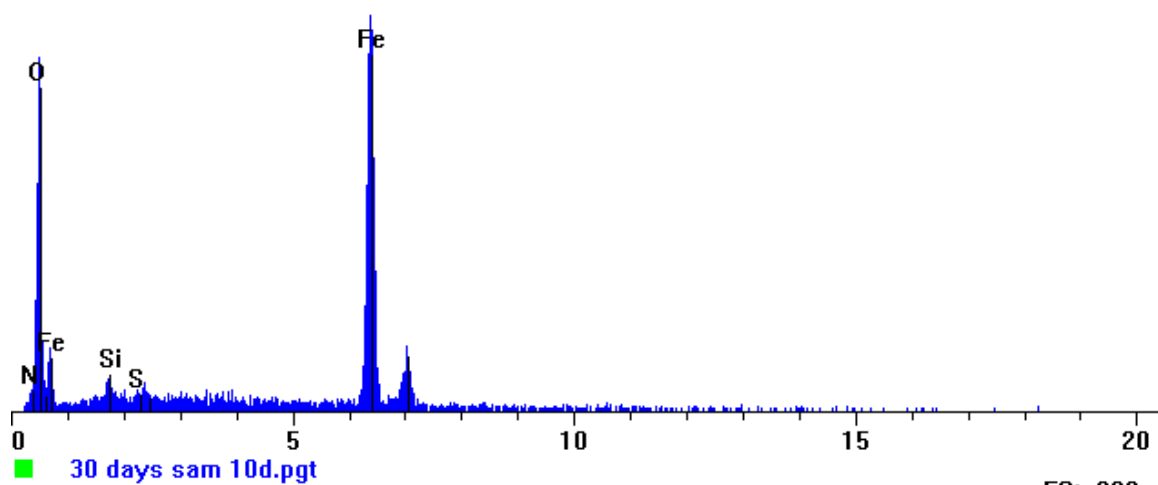
■ 30 days sam9.pgt

FS: 1200



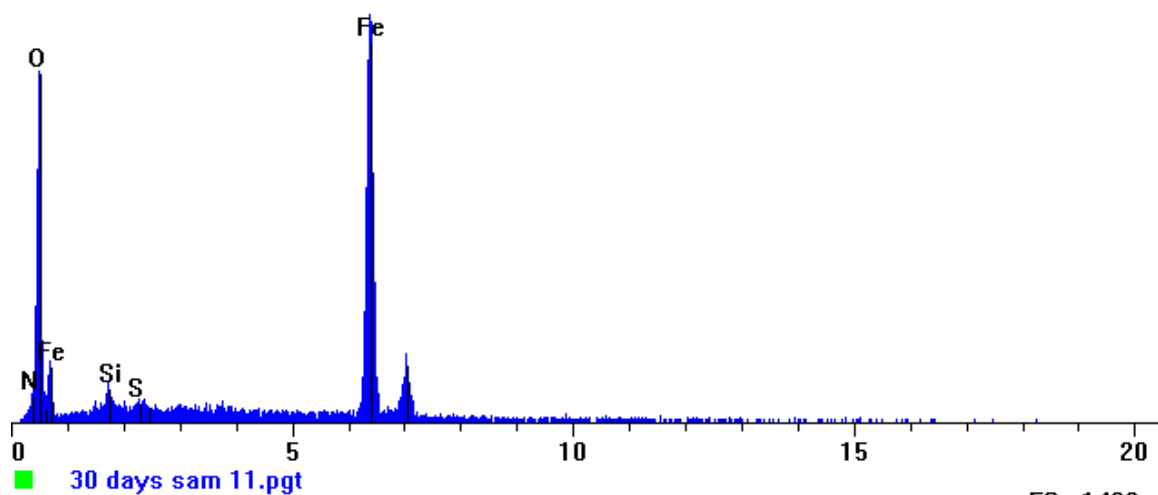
■ 30 day sam 10 c.pgt

FS: 540



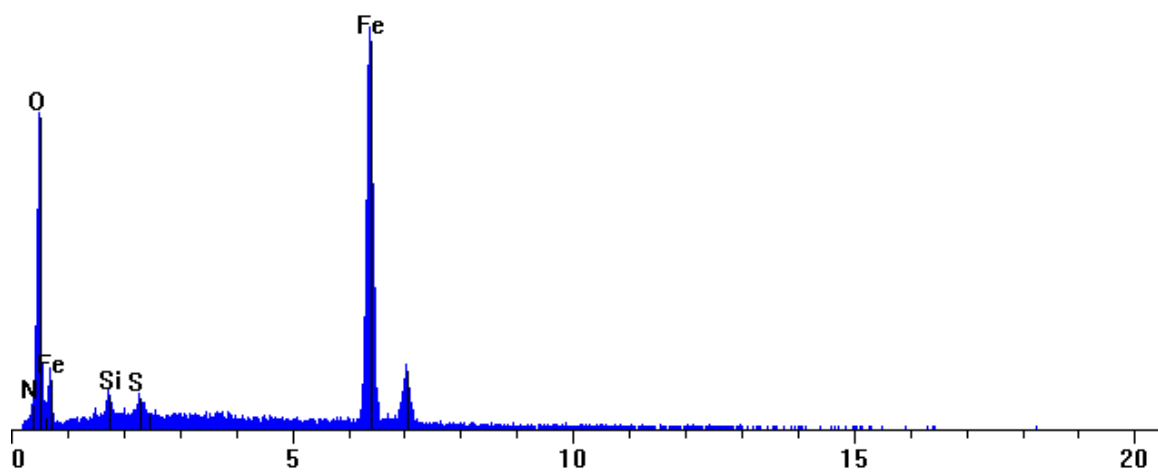
■ 30 days sam 10d.pgt

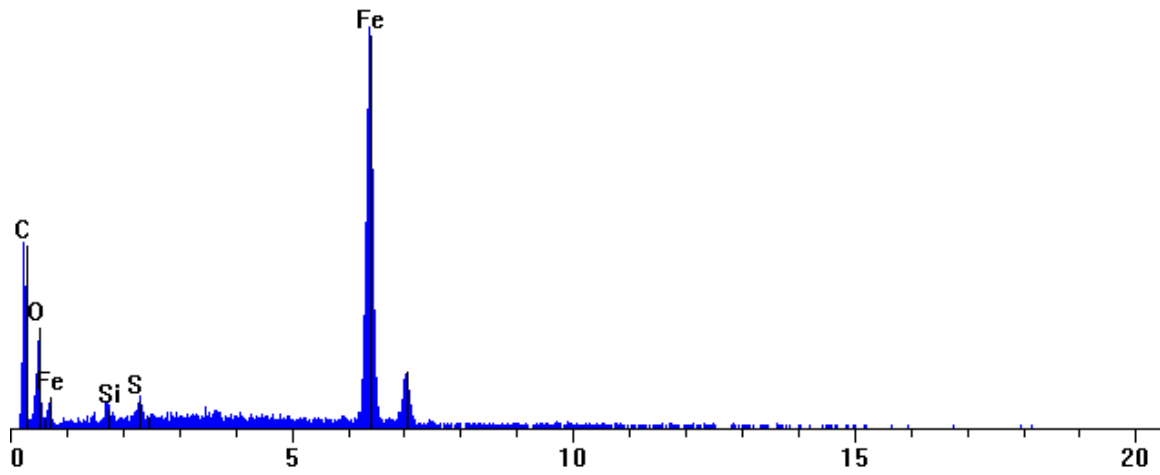
FS: 900



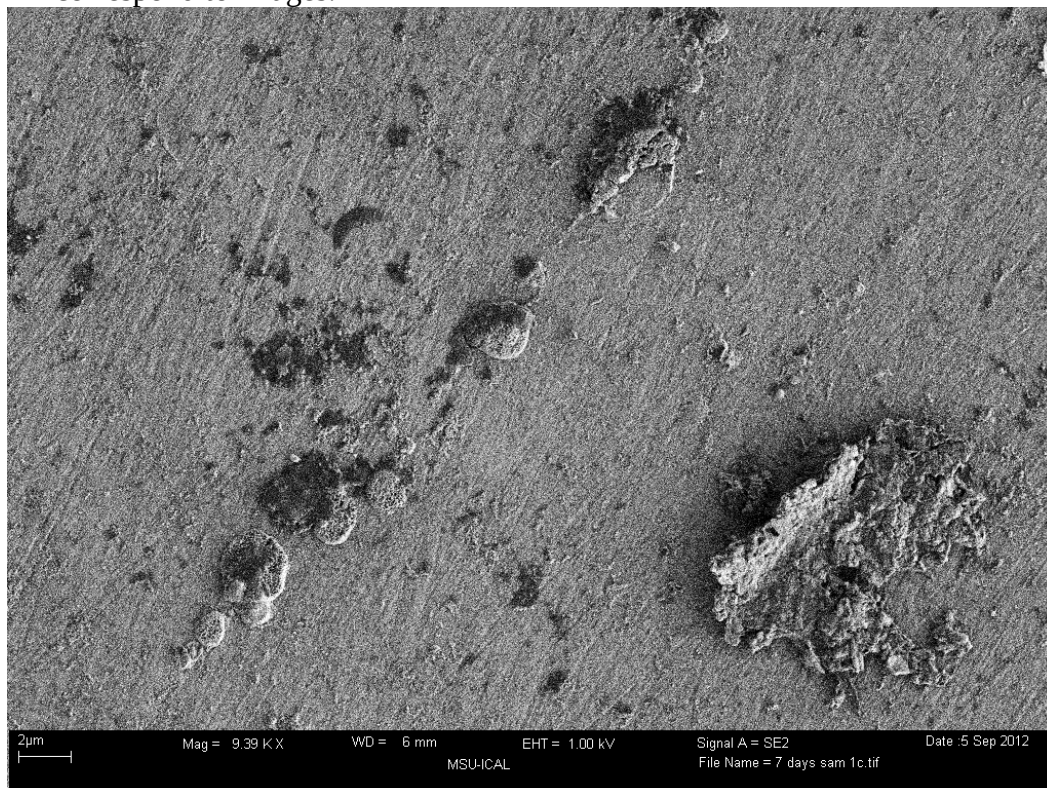
■ 30 days sam 11.pgt

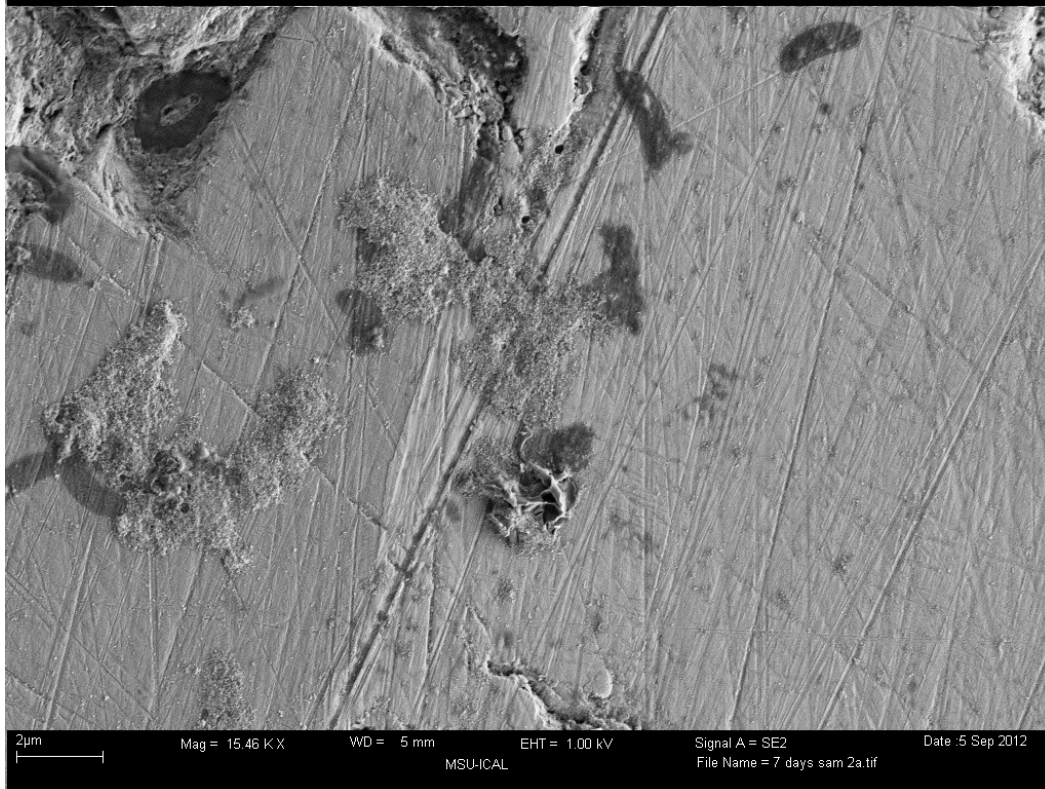
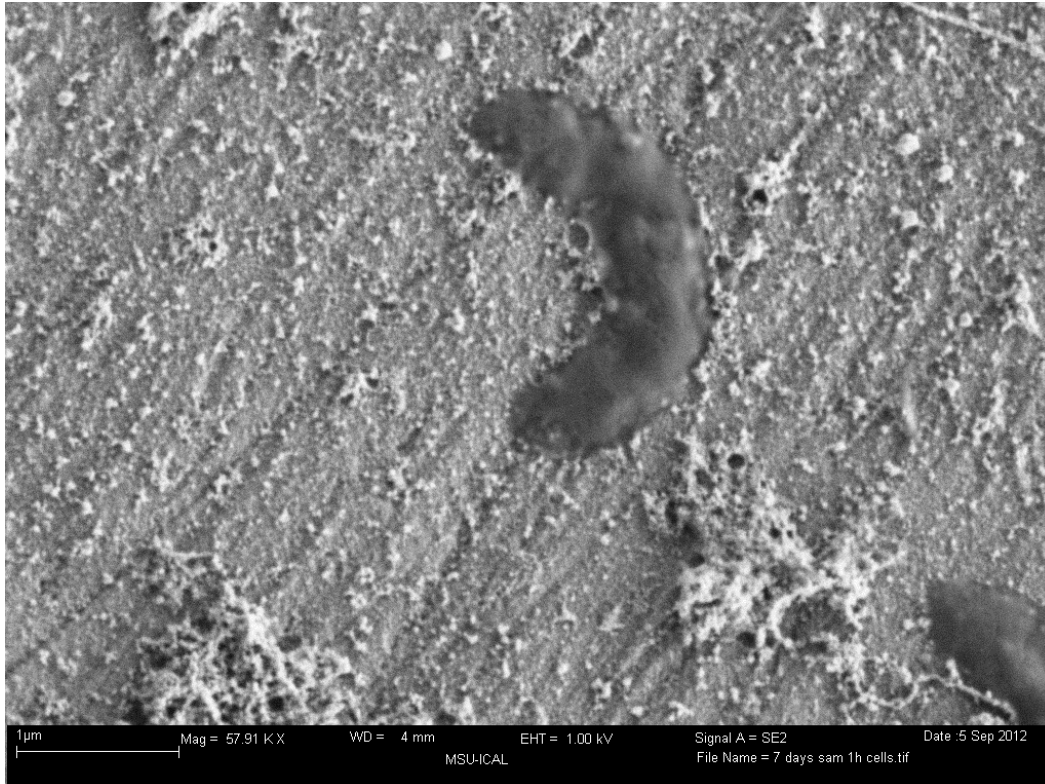
FS: 1400

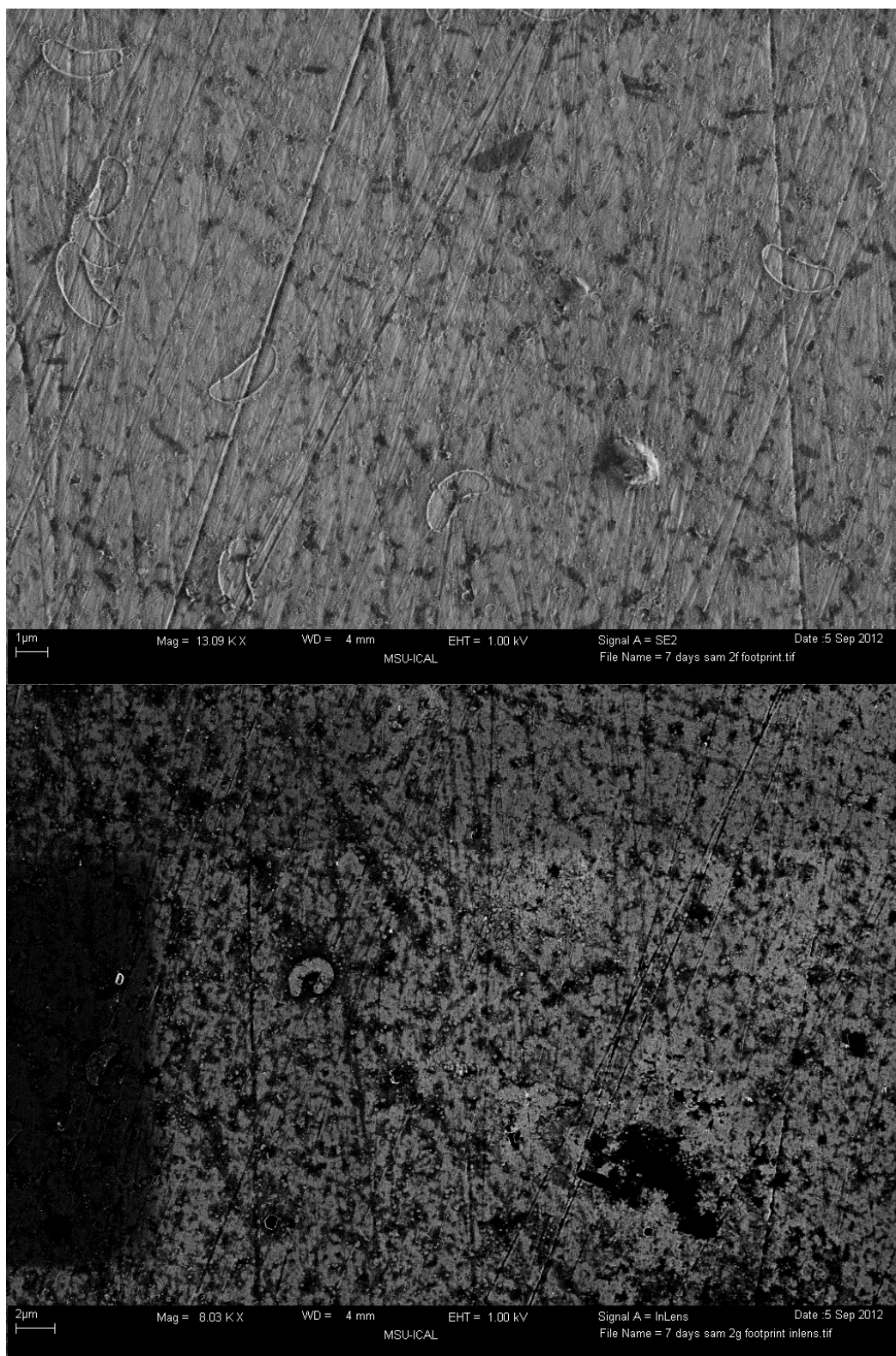




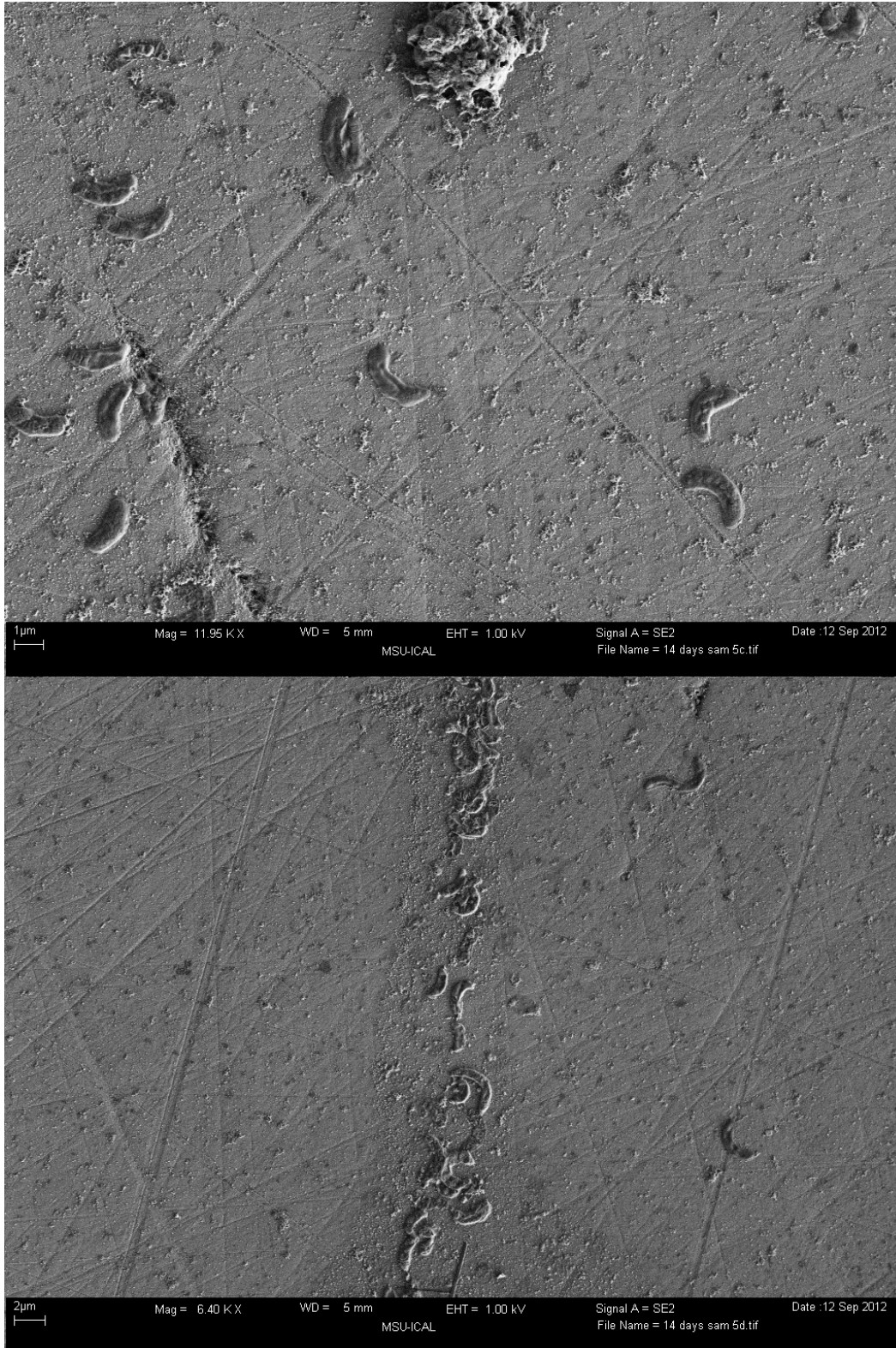
- 2) FE SEM secondary electron and InLens images of pyrrhotite with EDS spot analyses. Sample and detector type are labeled in images. EDS spectra are labeled to correspond to images.

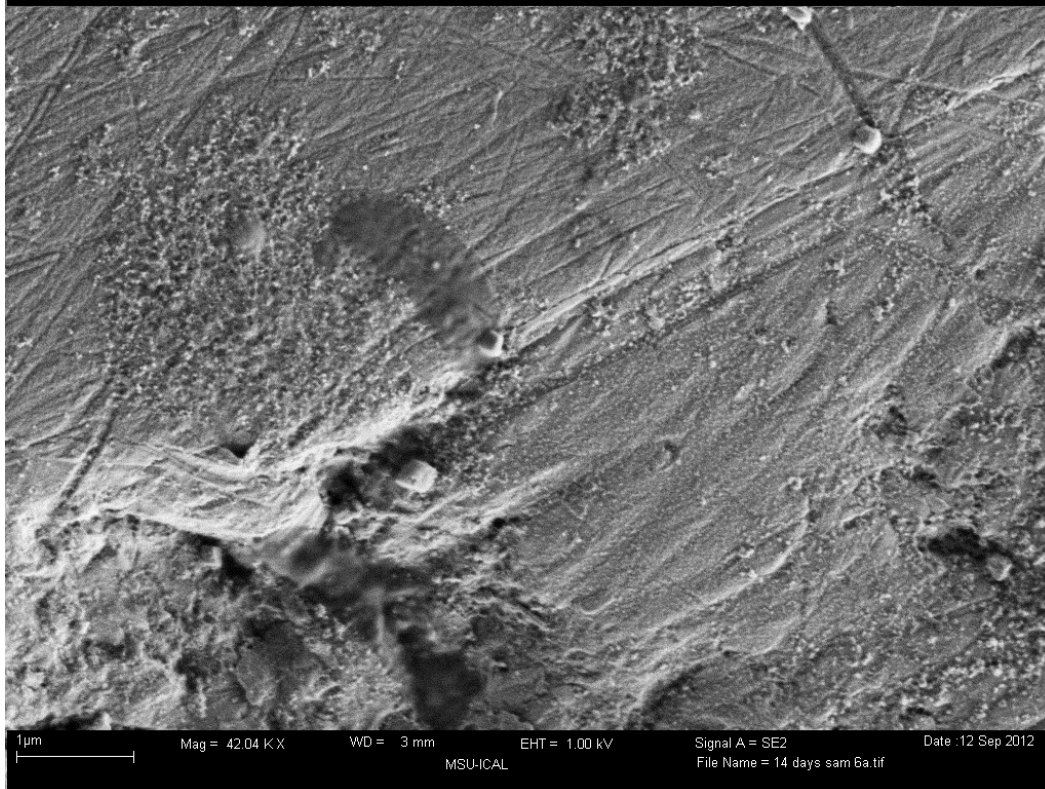
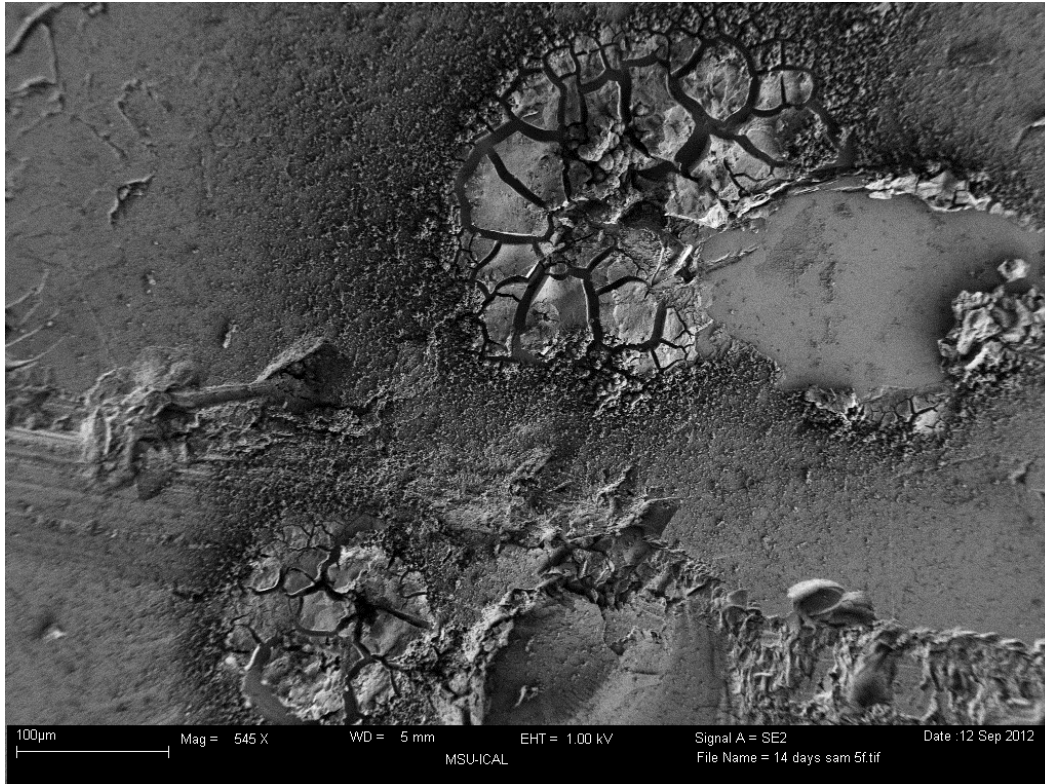


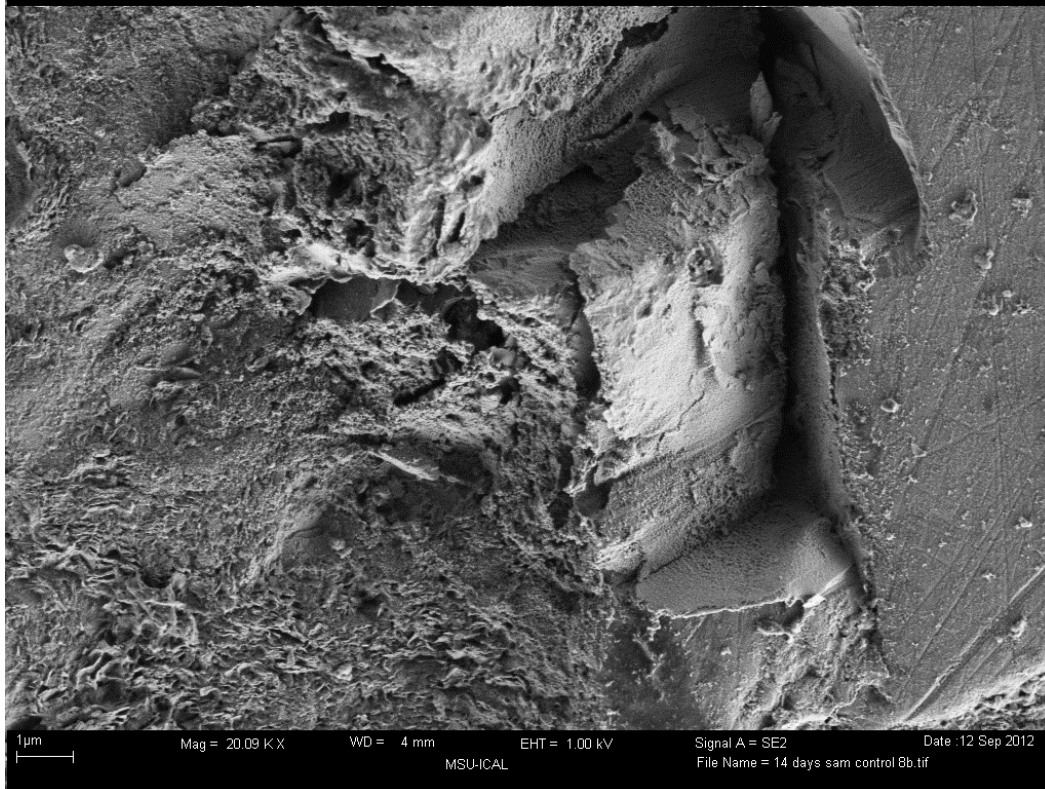
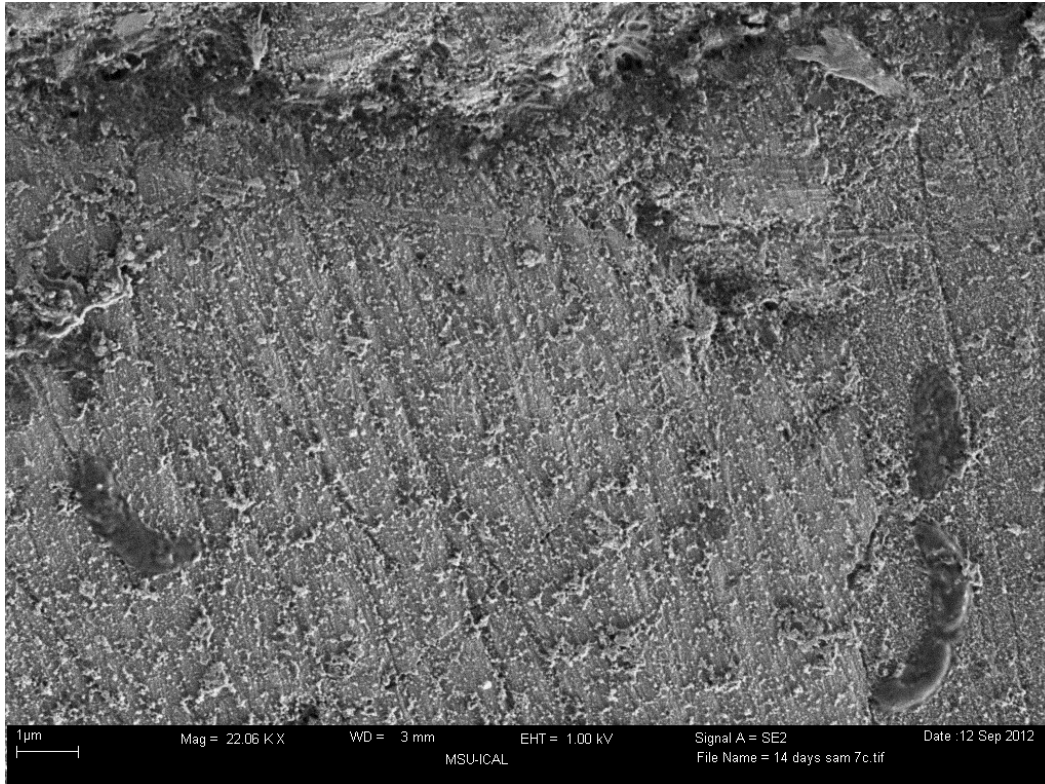


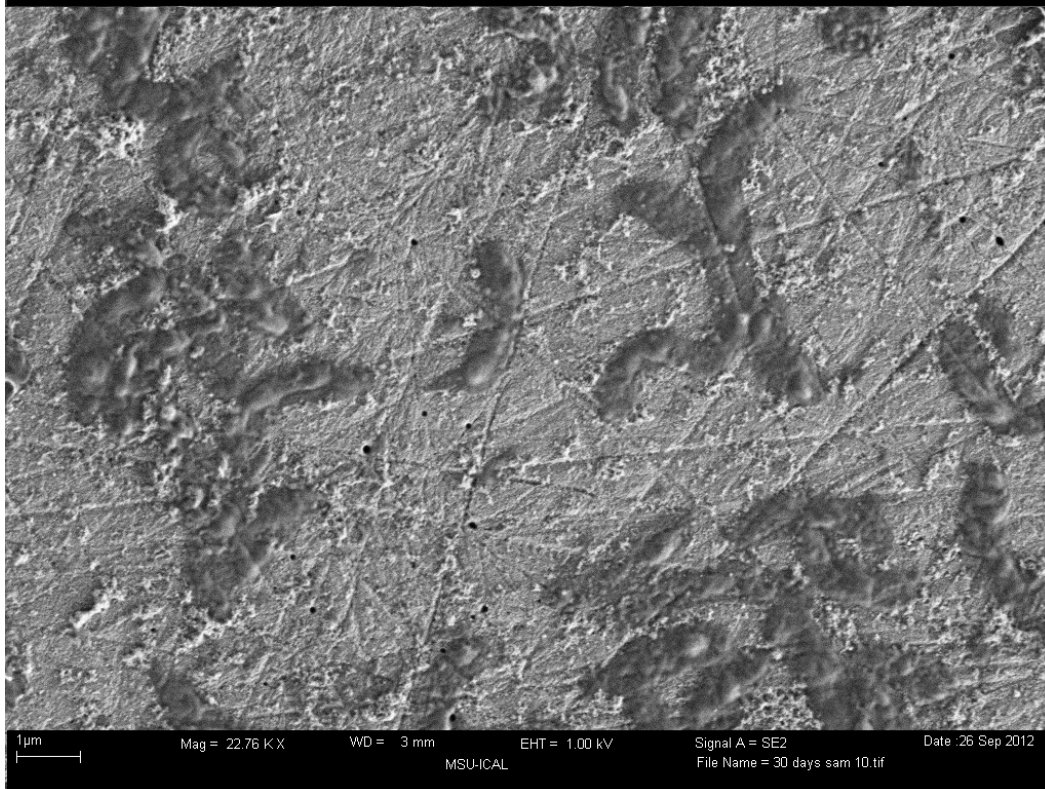
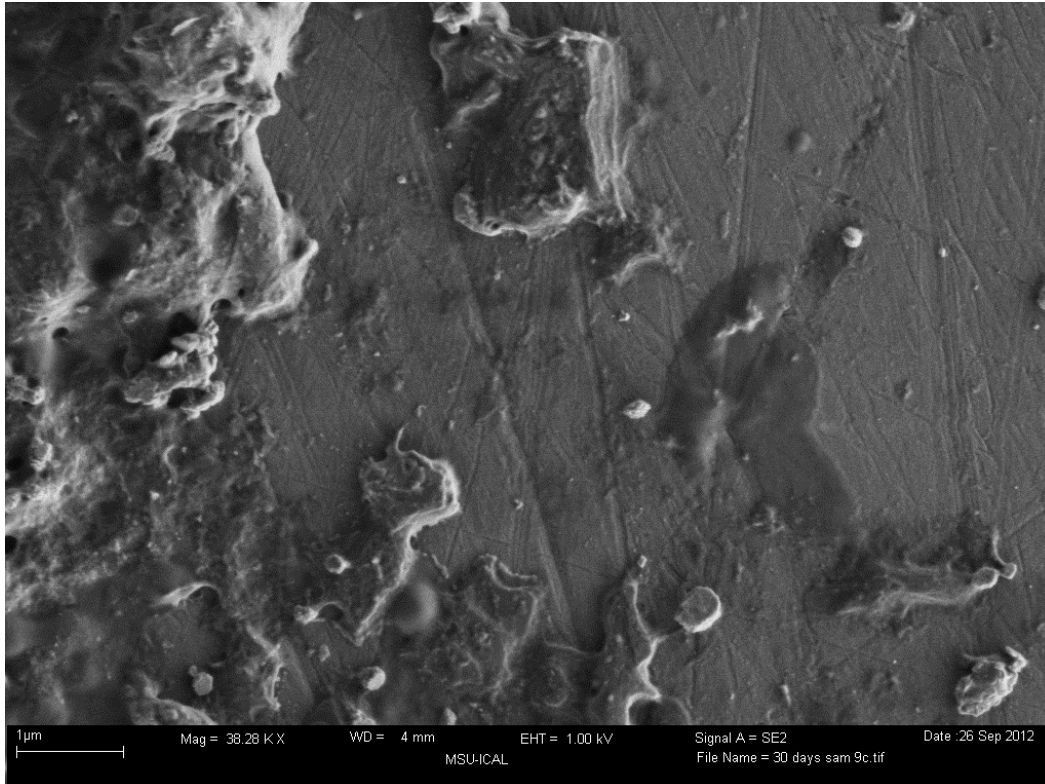


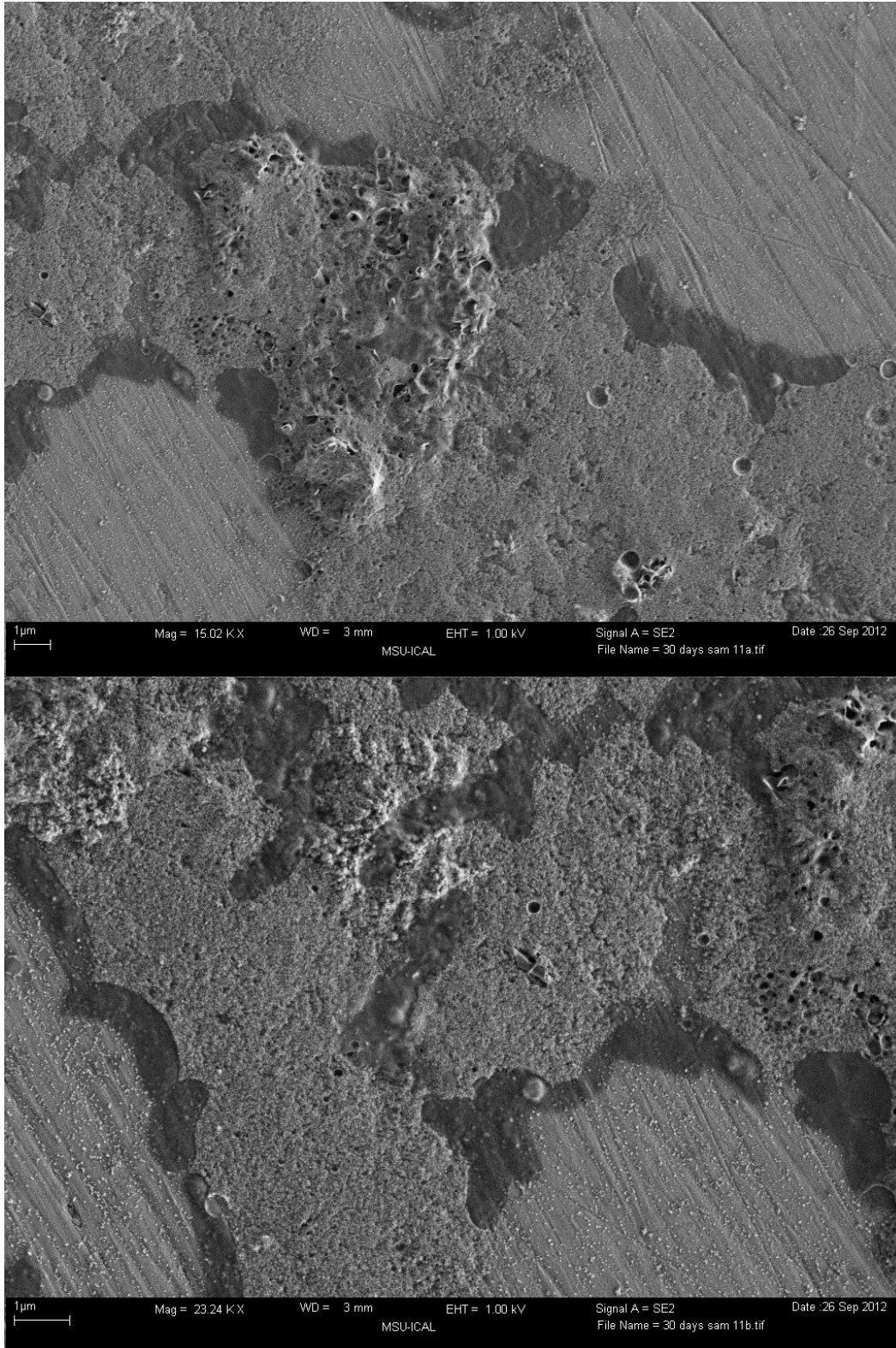


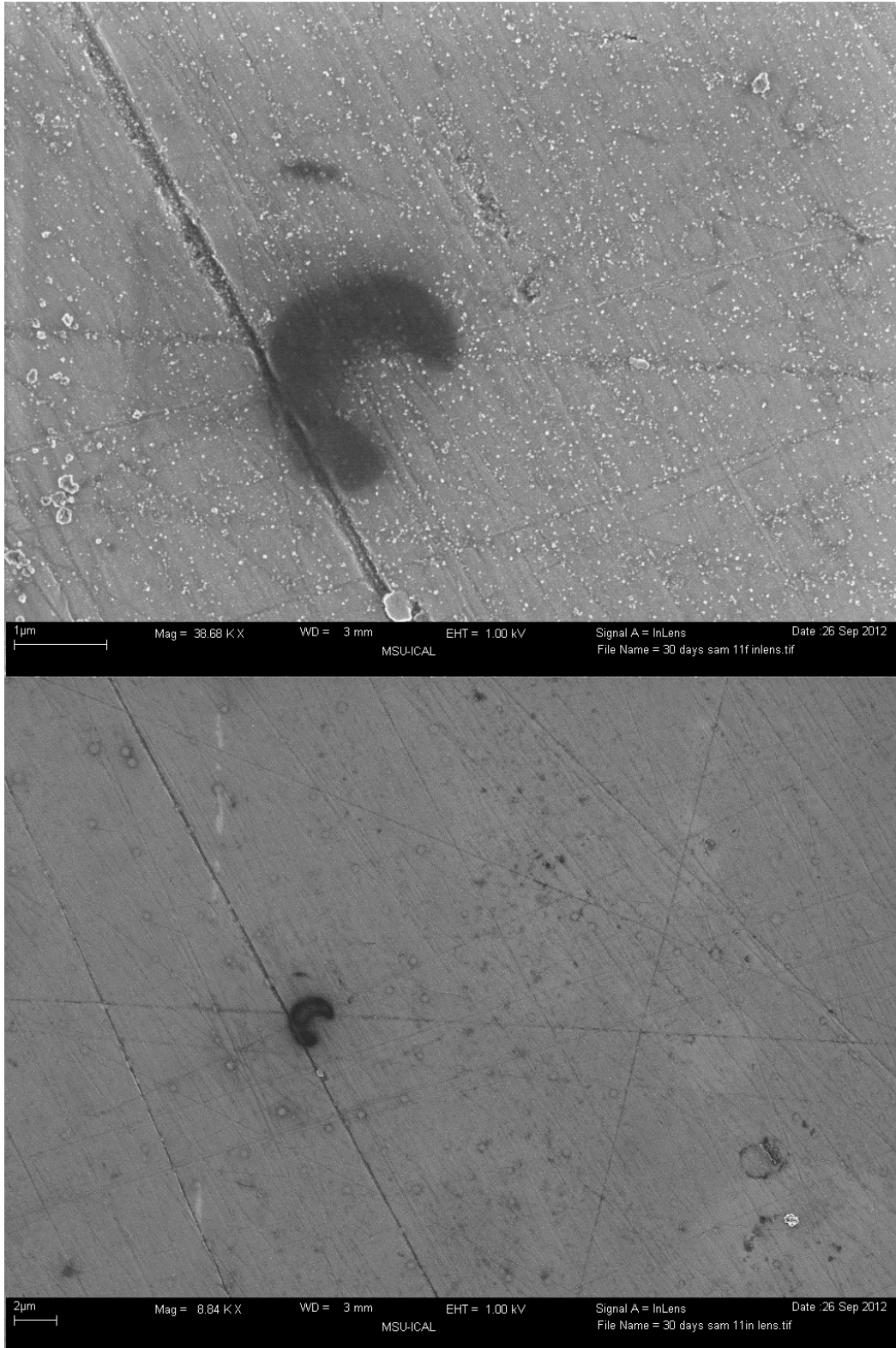


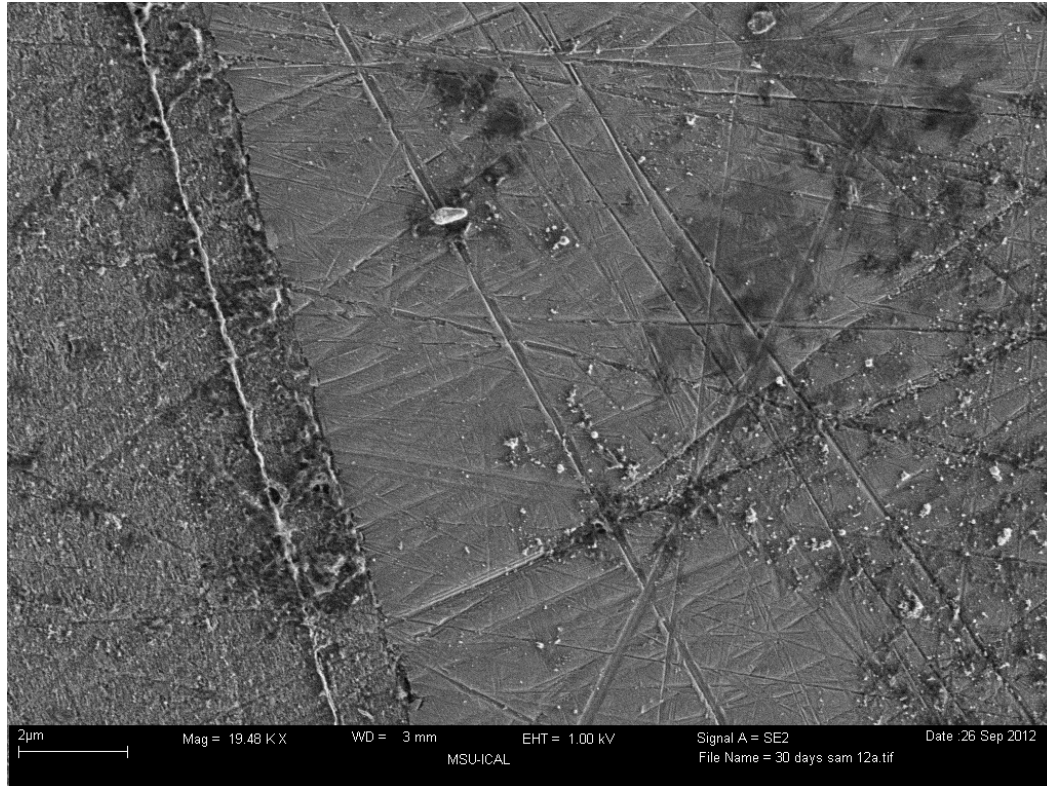






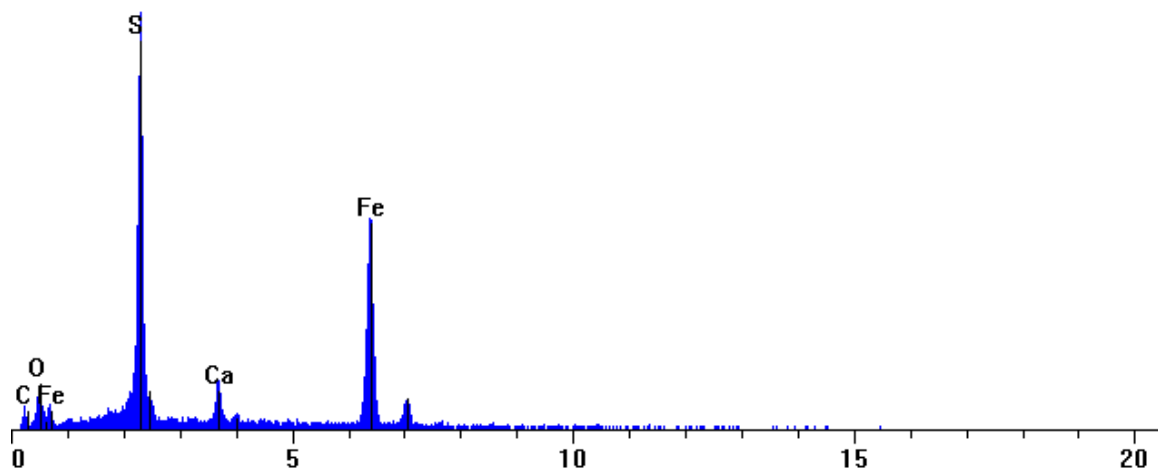






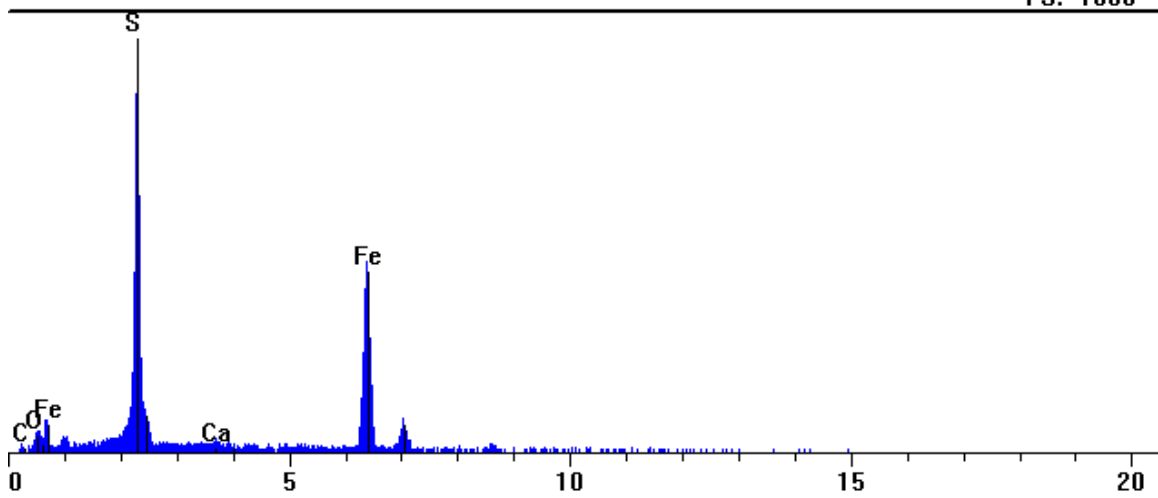
■ blob on right 7 days sam 1c.pgt

FS: 1200



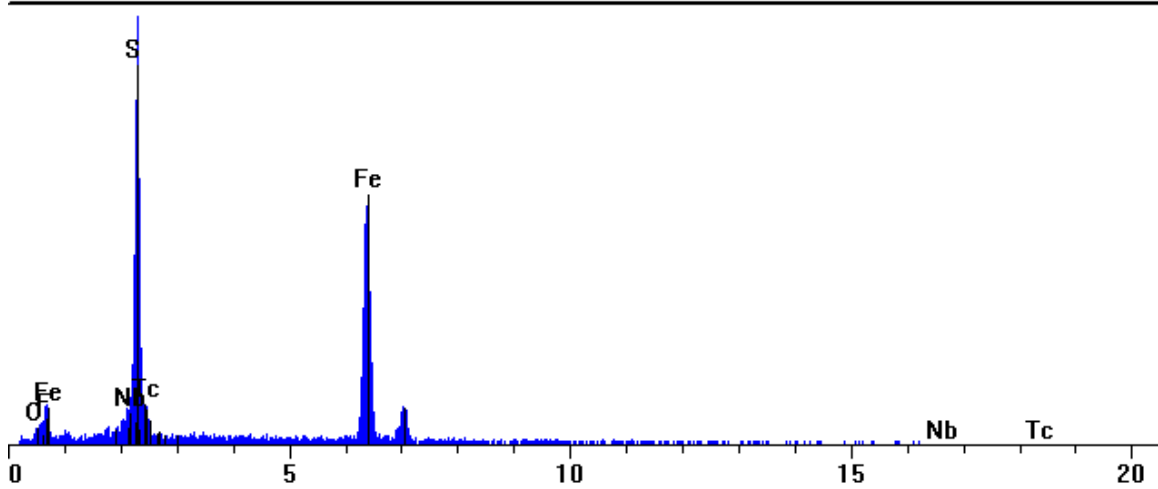
■ circles on 7 days sam 1c.pgt

FS: 1000



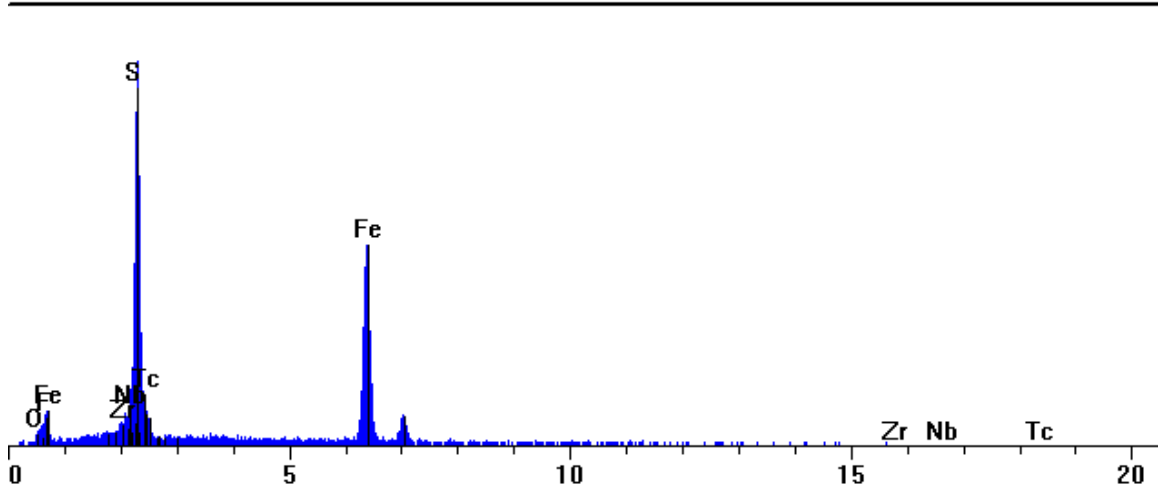
■ crystals on 14 days sam 5a.pgt

FS: 900

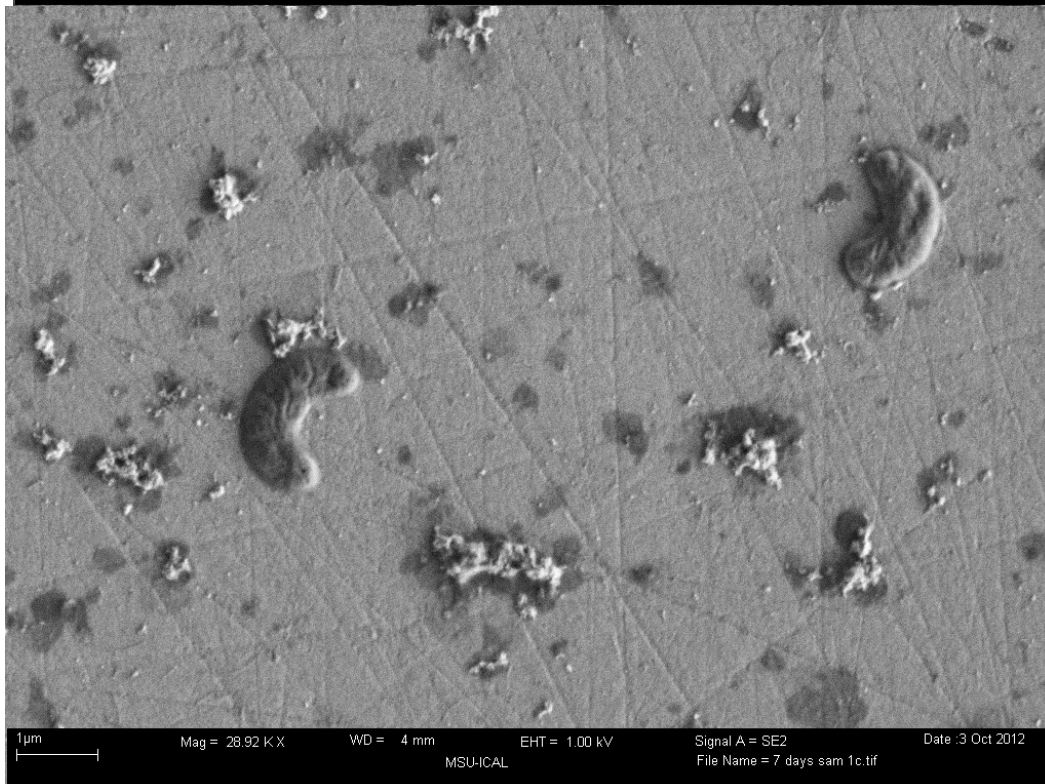
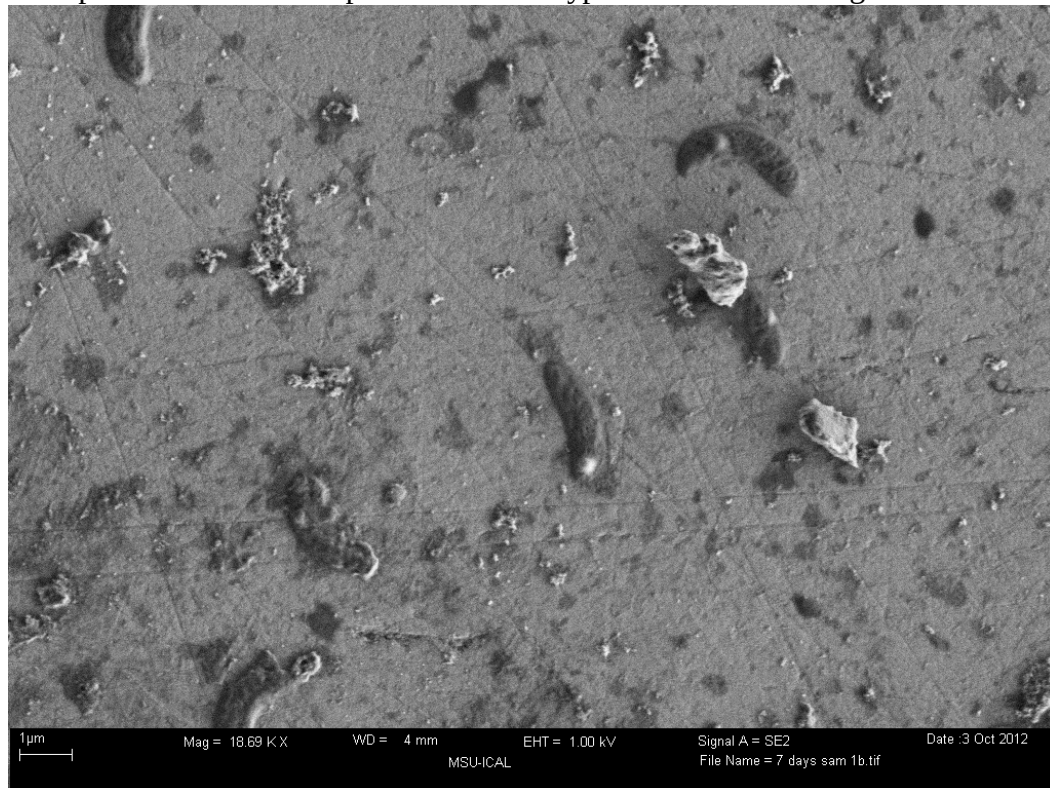


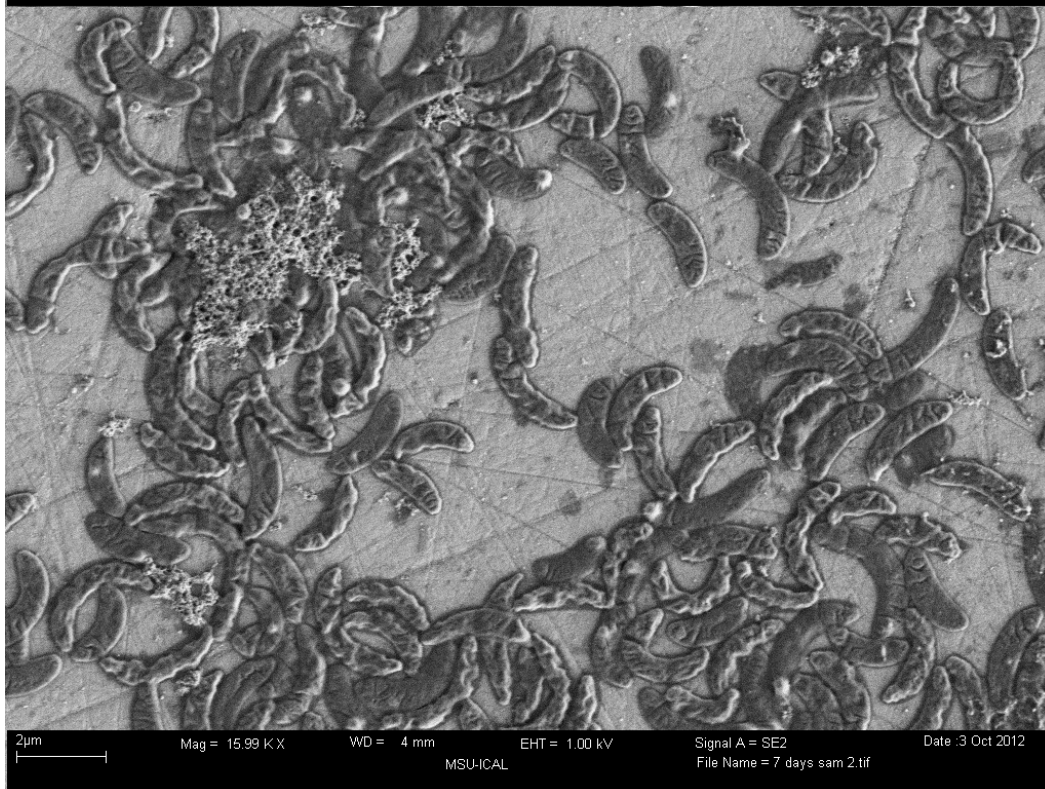
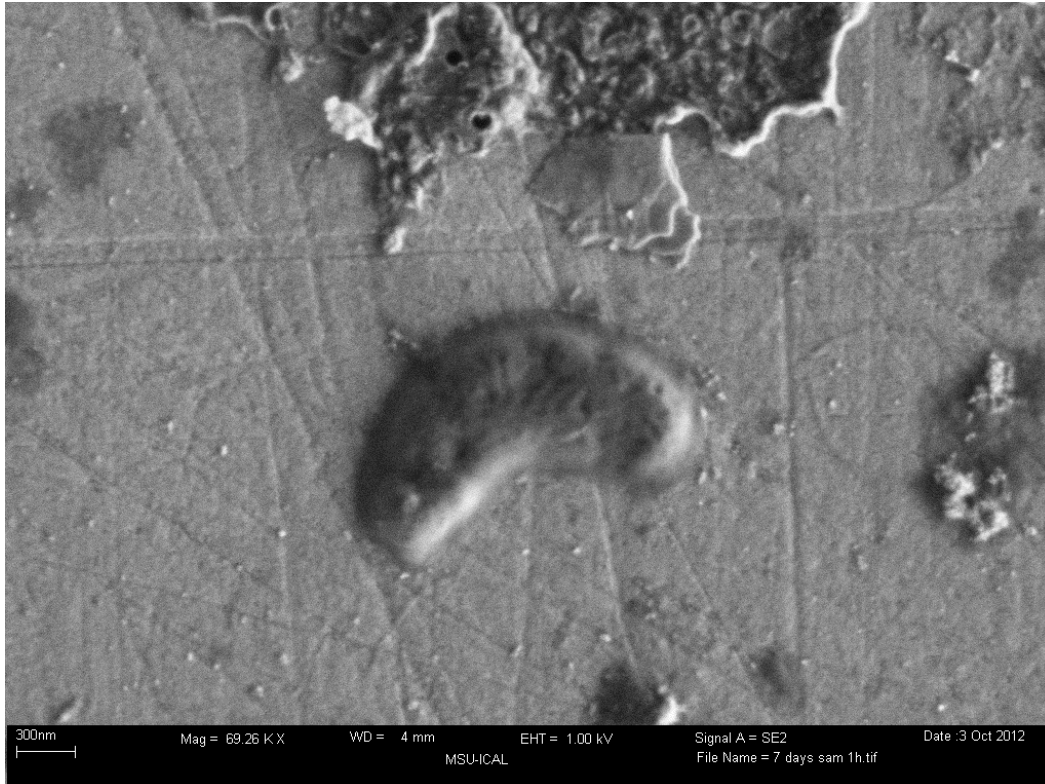
■ background on 11c.pgt

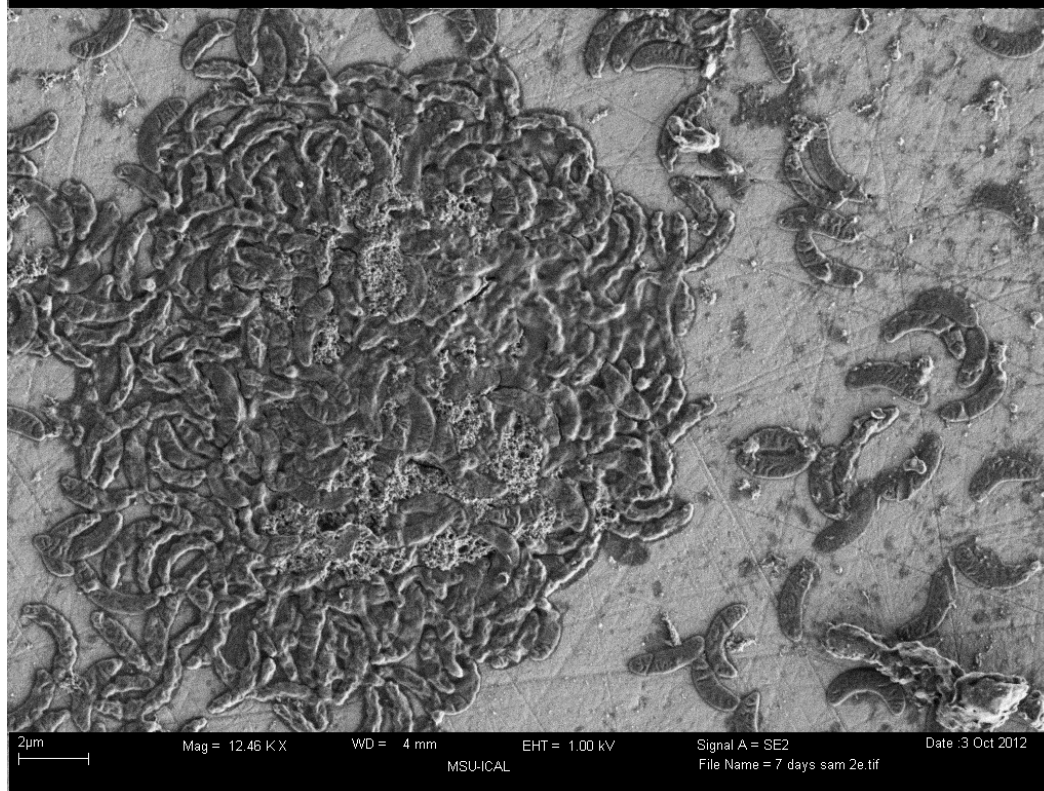
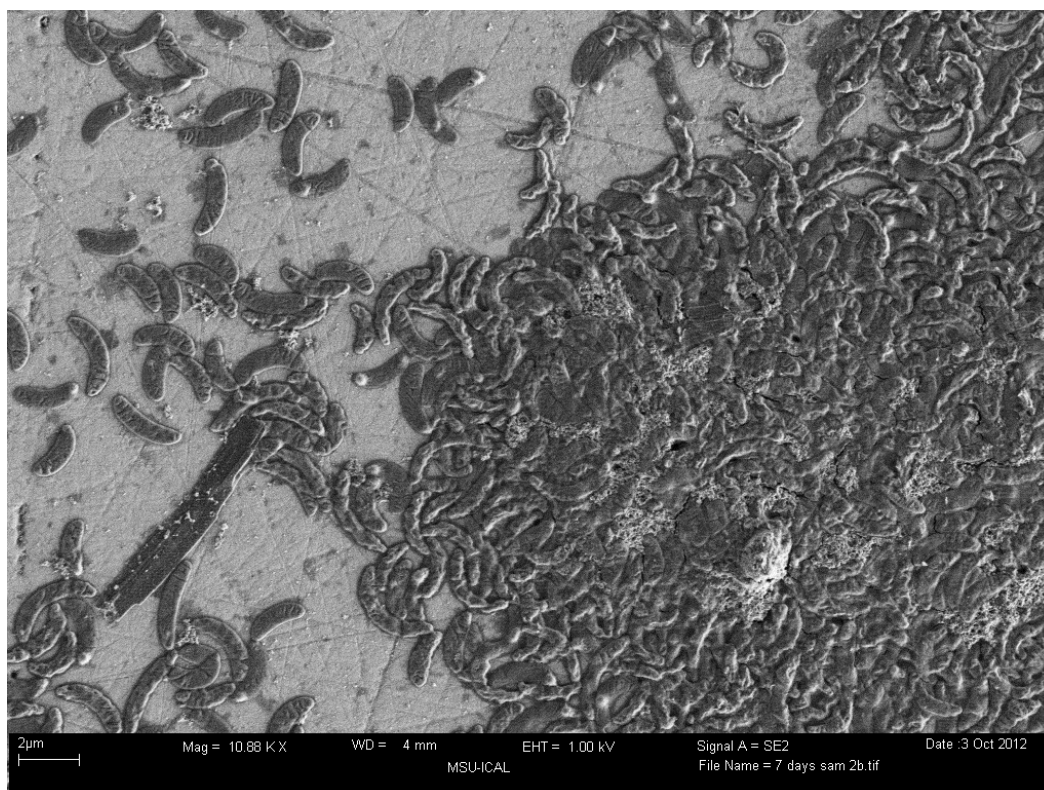
FS: 1400

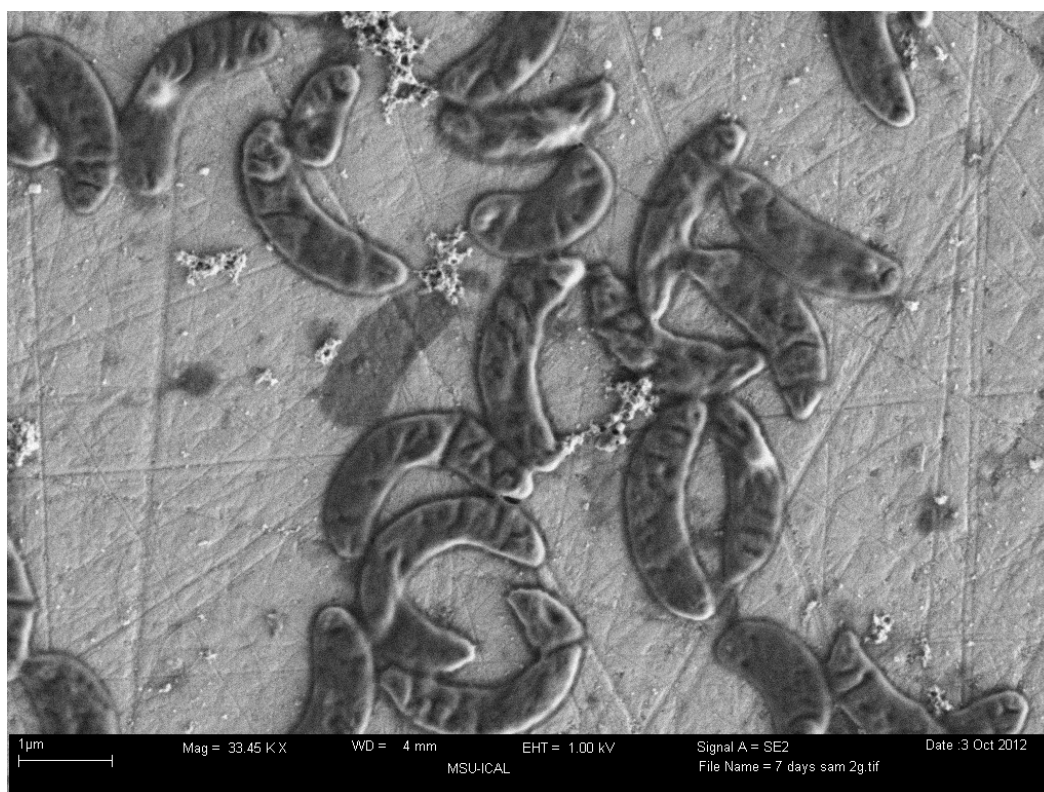
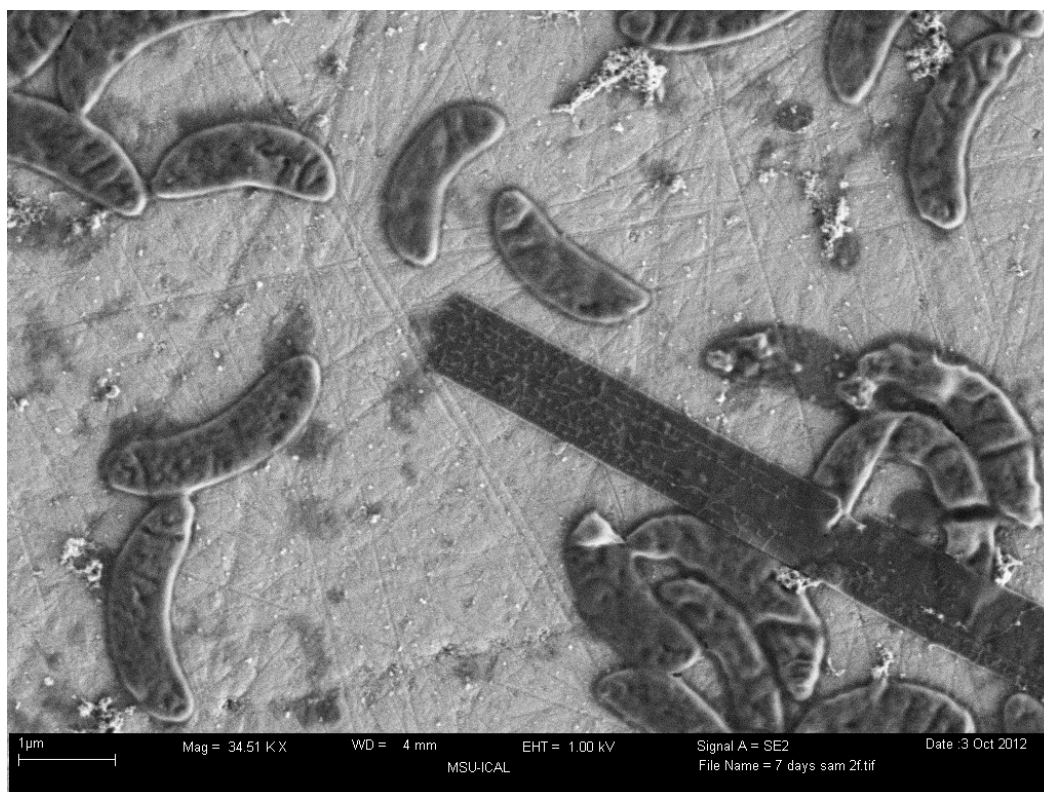


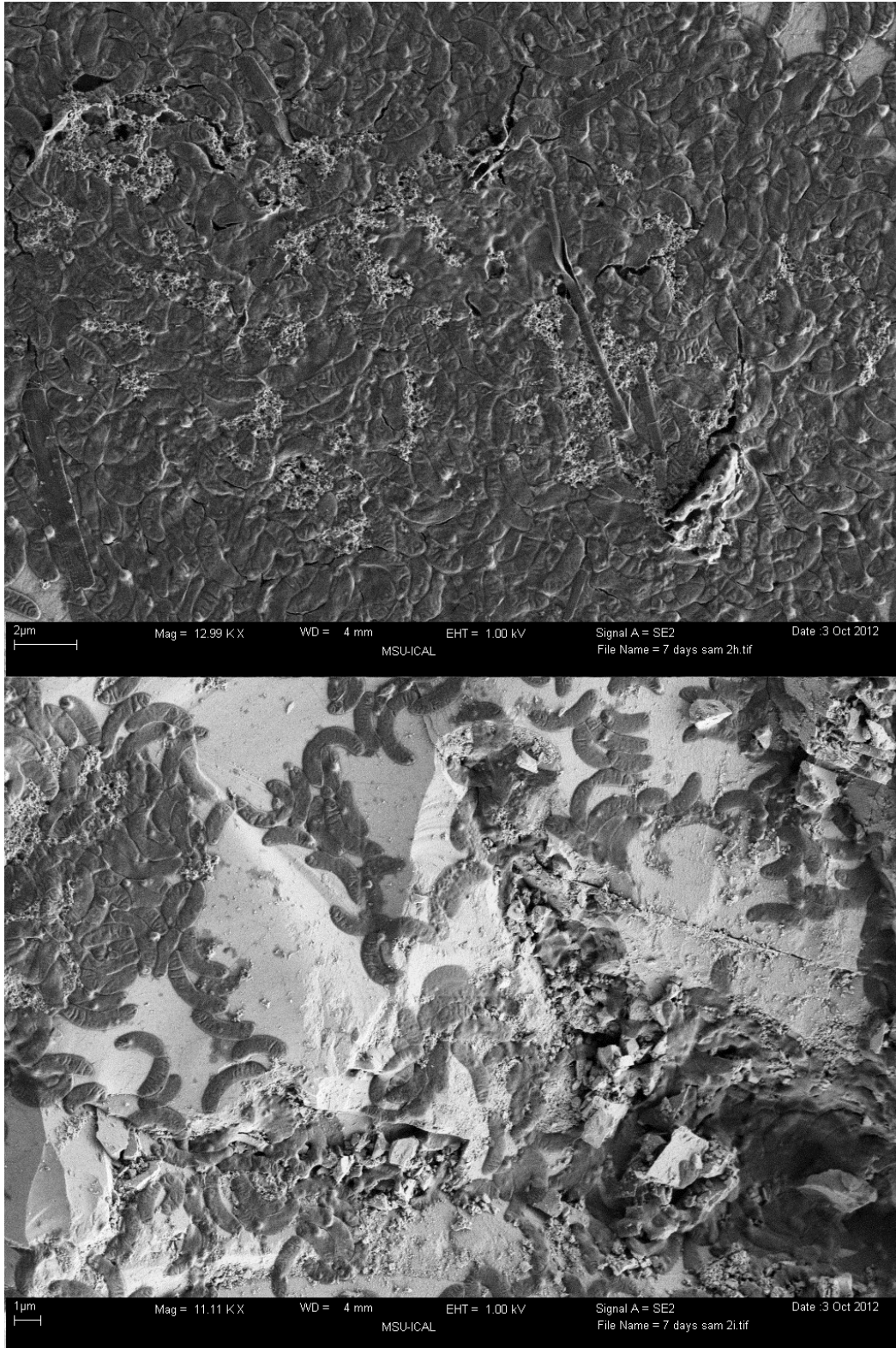
- 3) FE SEM secondary electron, backscatter electron and InLens images of pyrite after exposure to SRB. Sample and detector type are labeled in image.

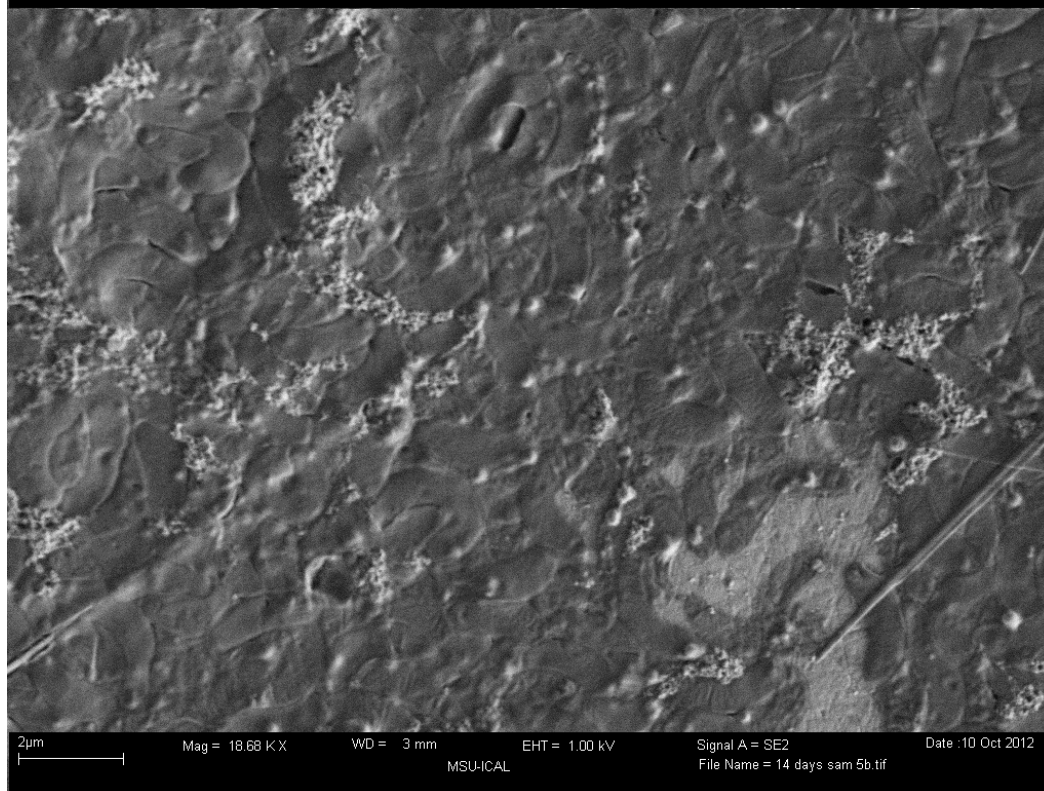
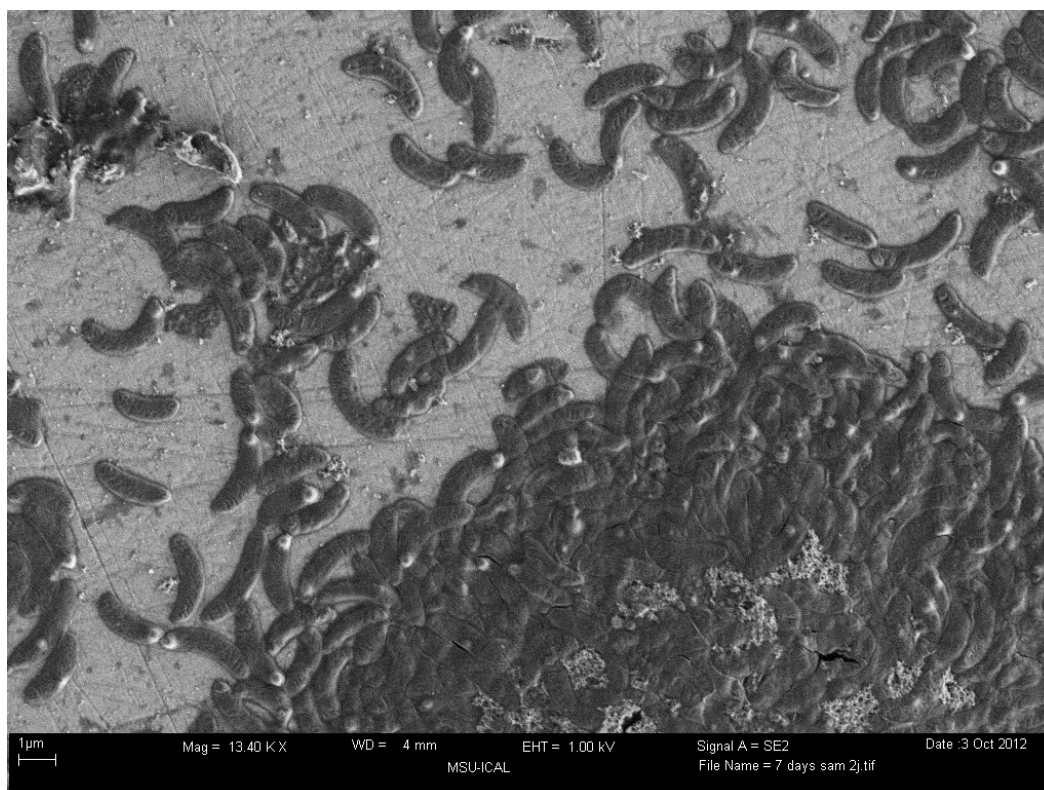


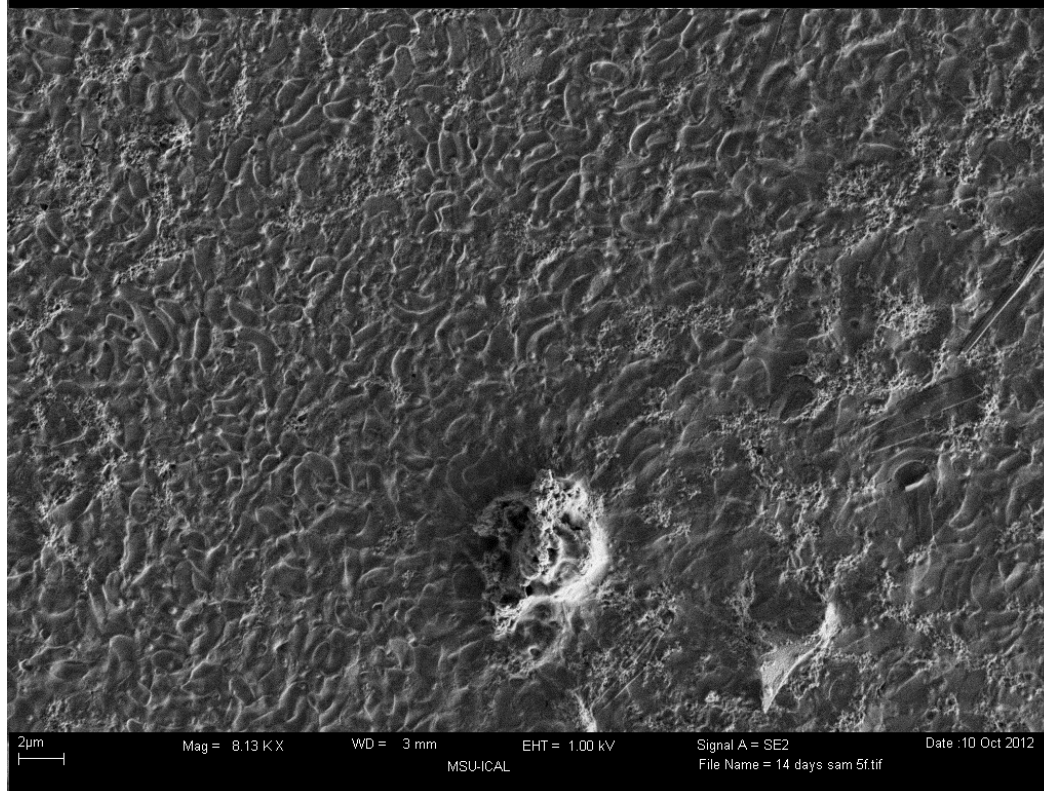
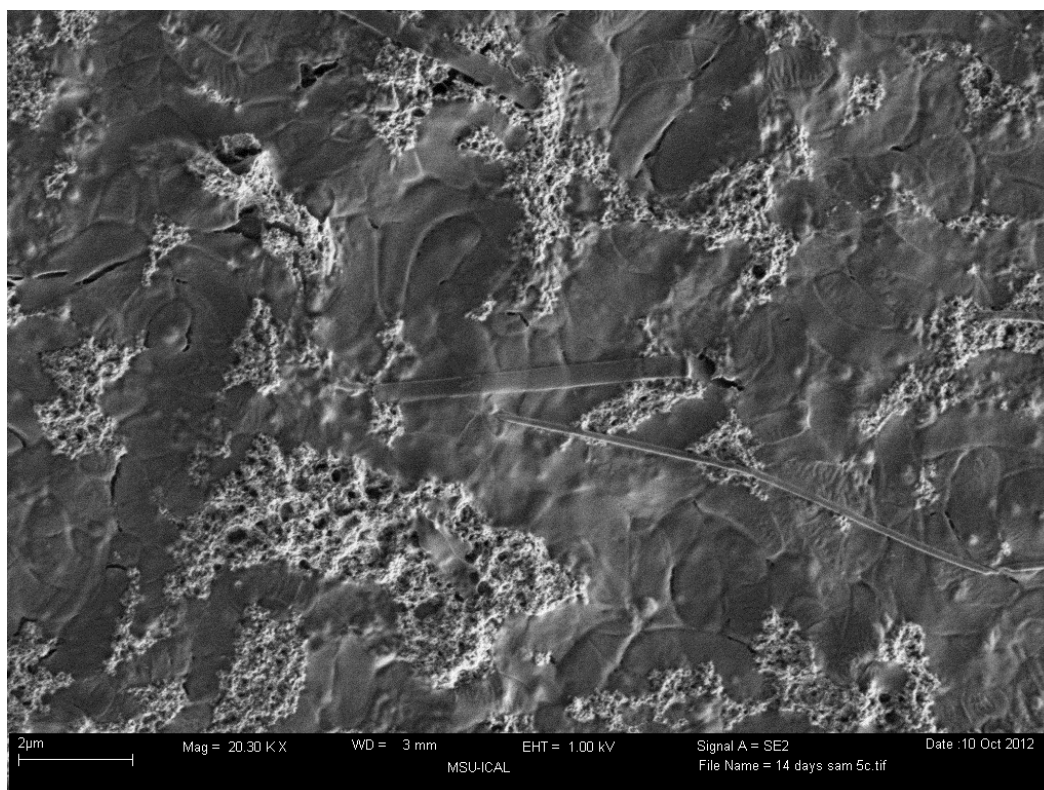


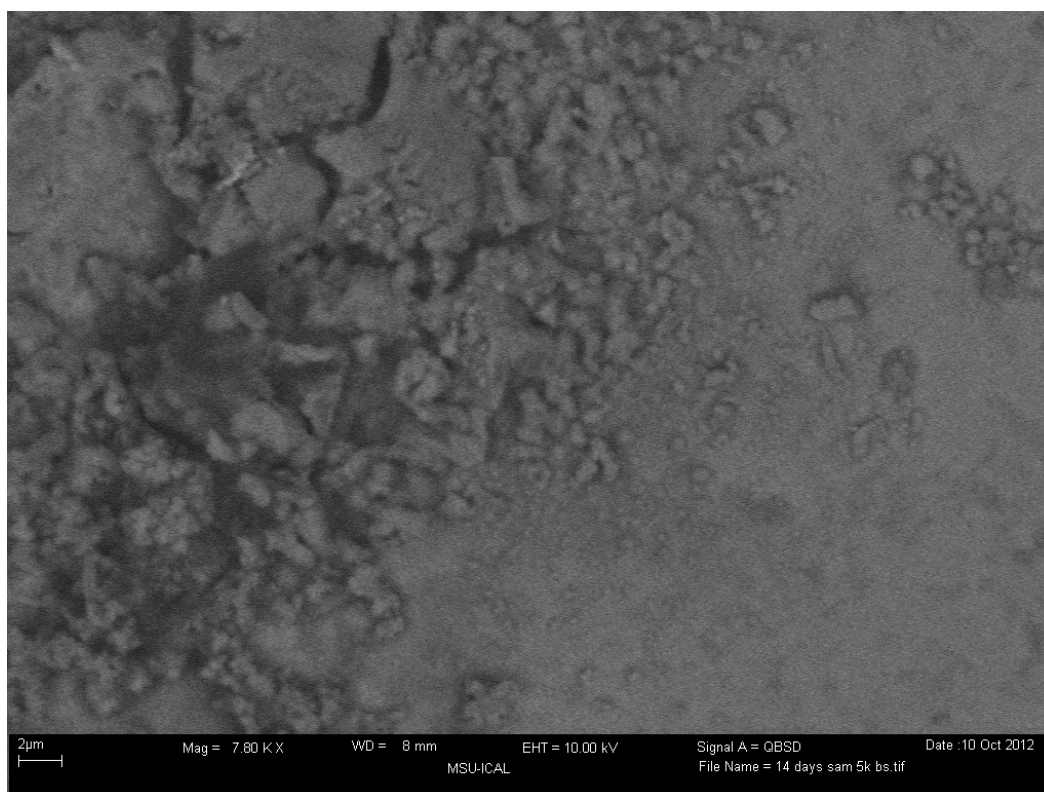
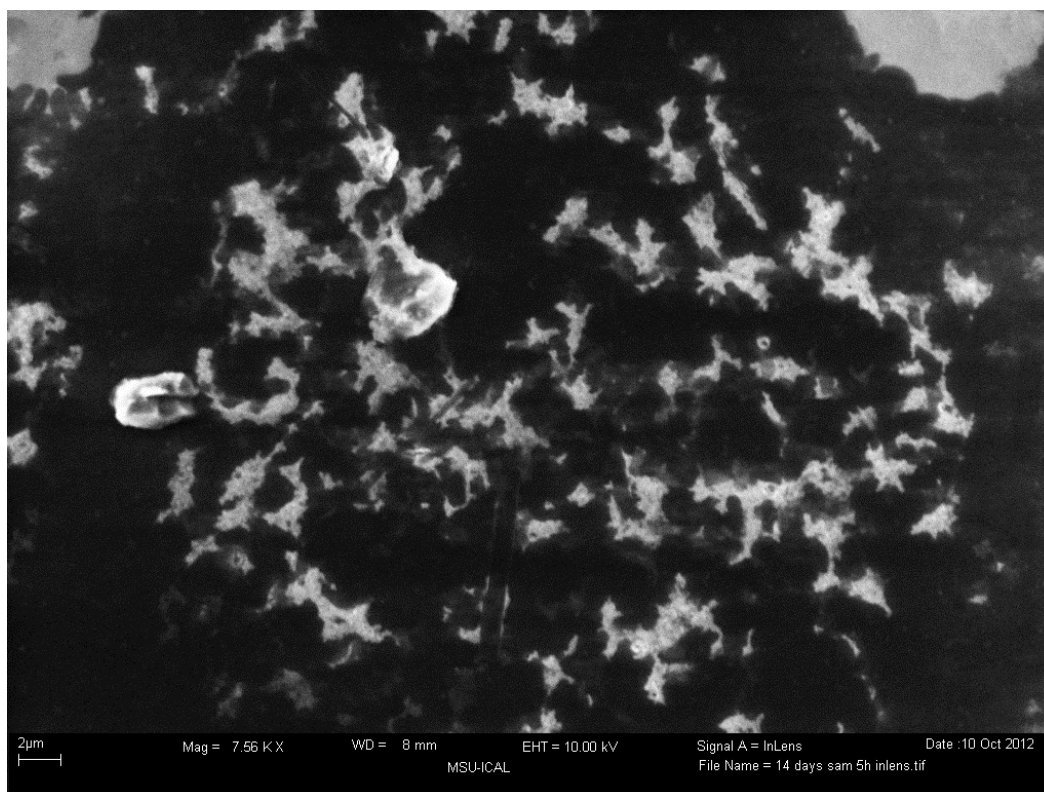


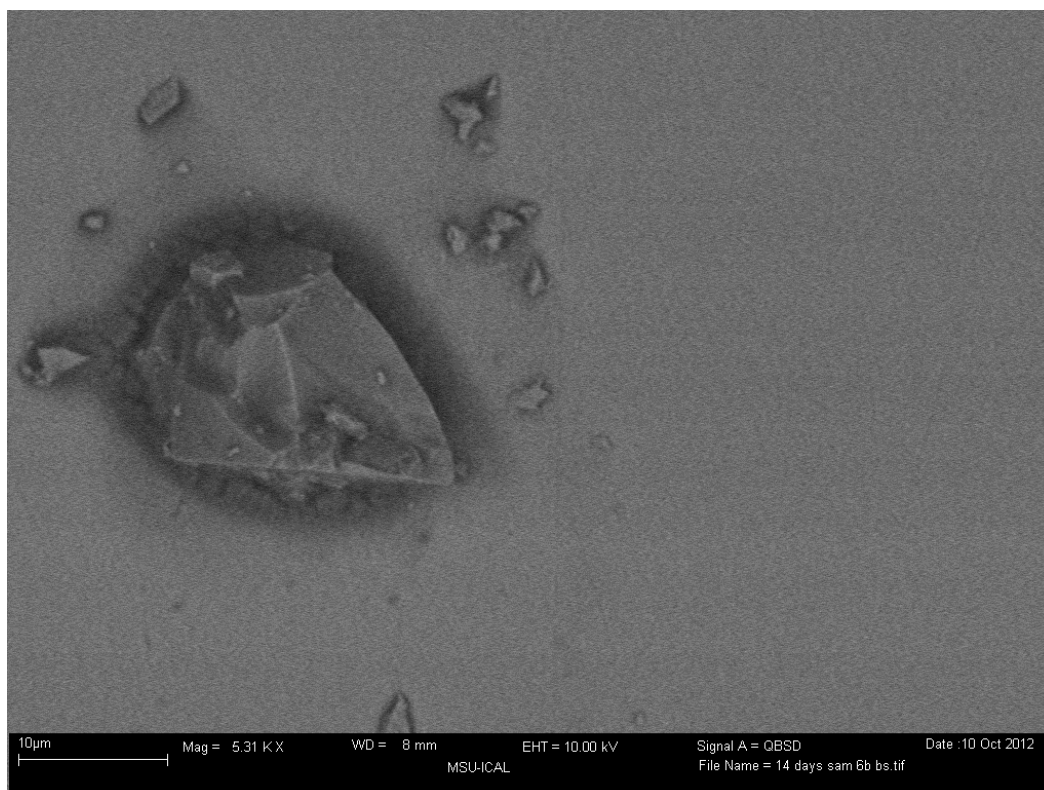


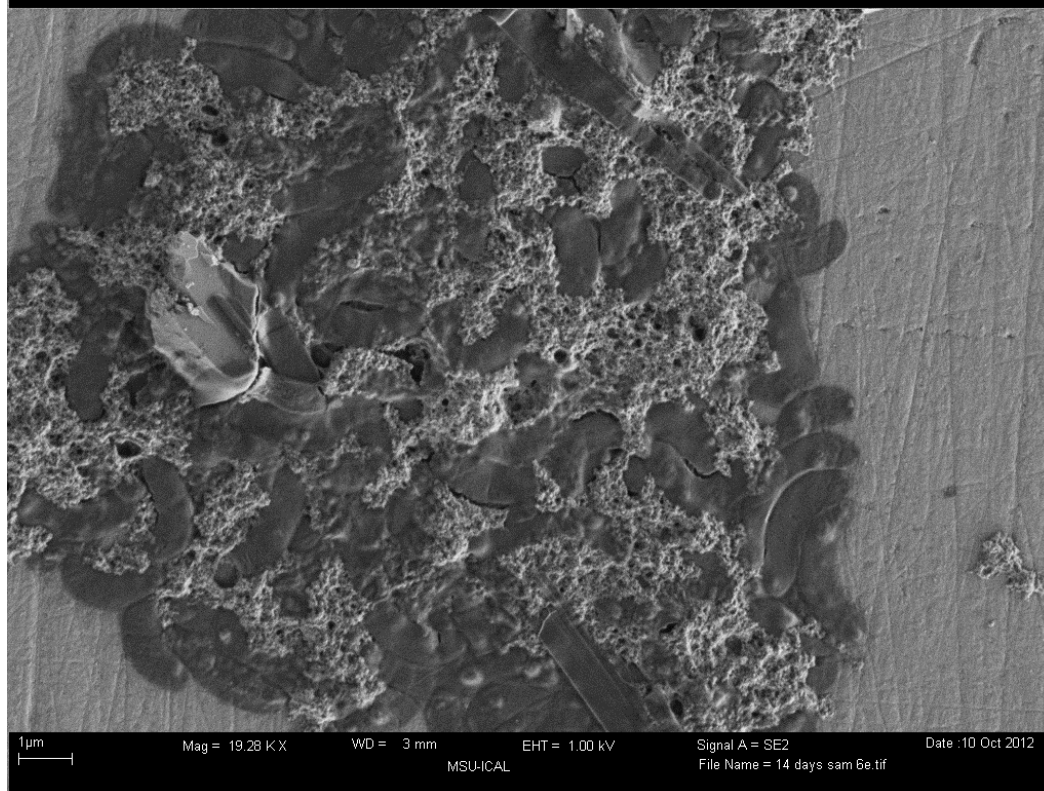
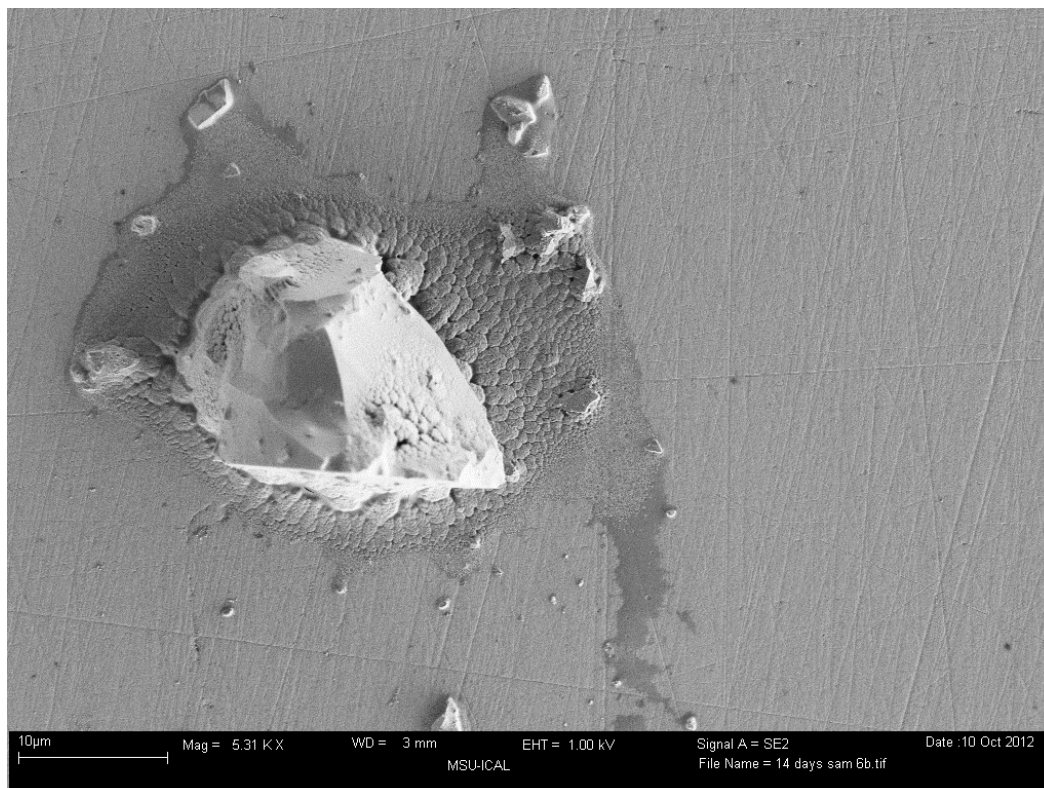


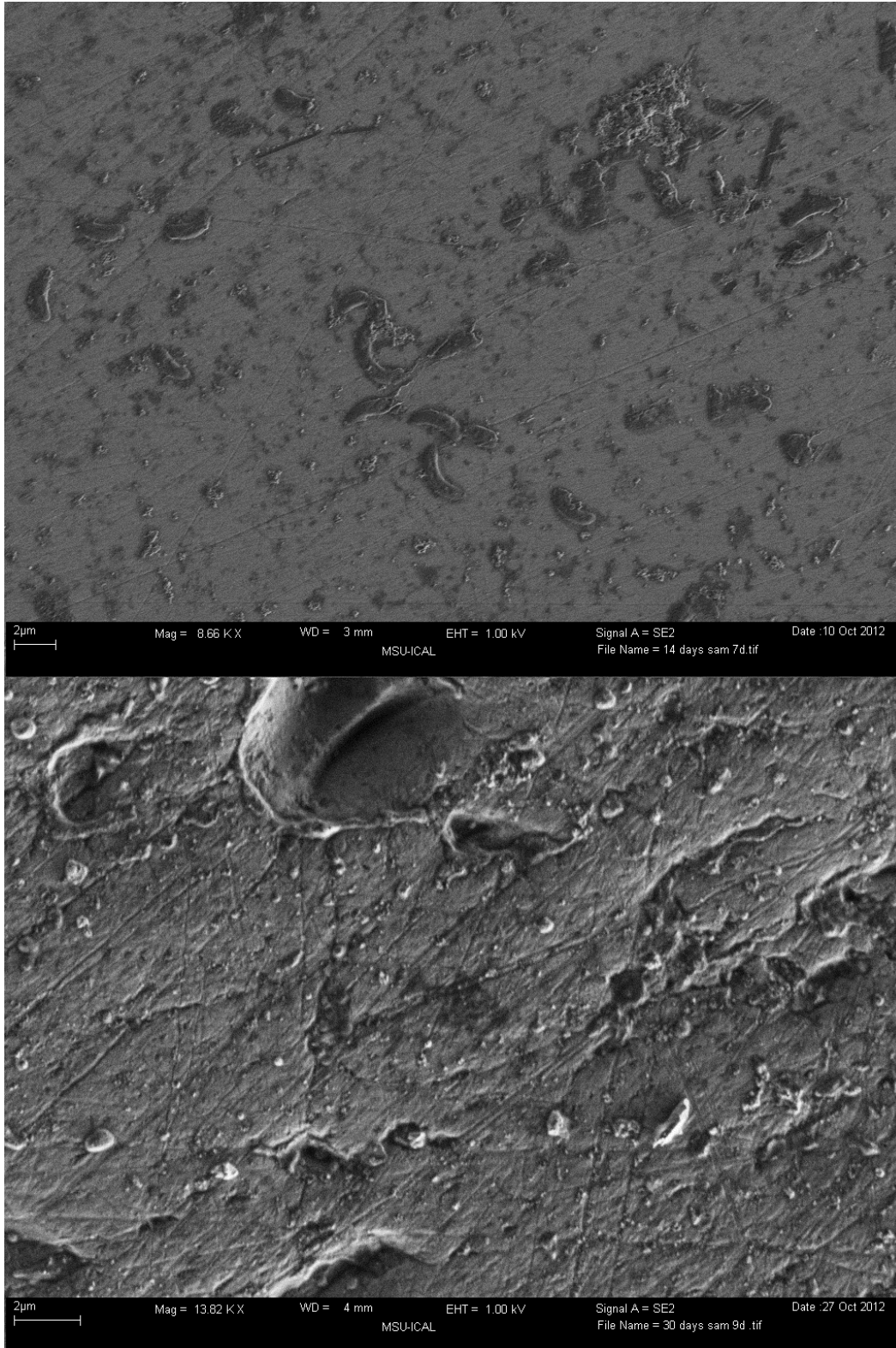


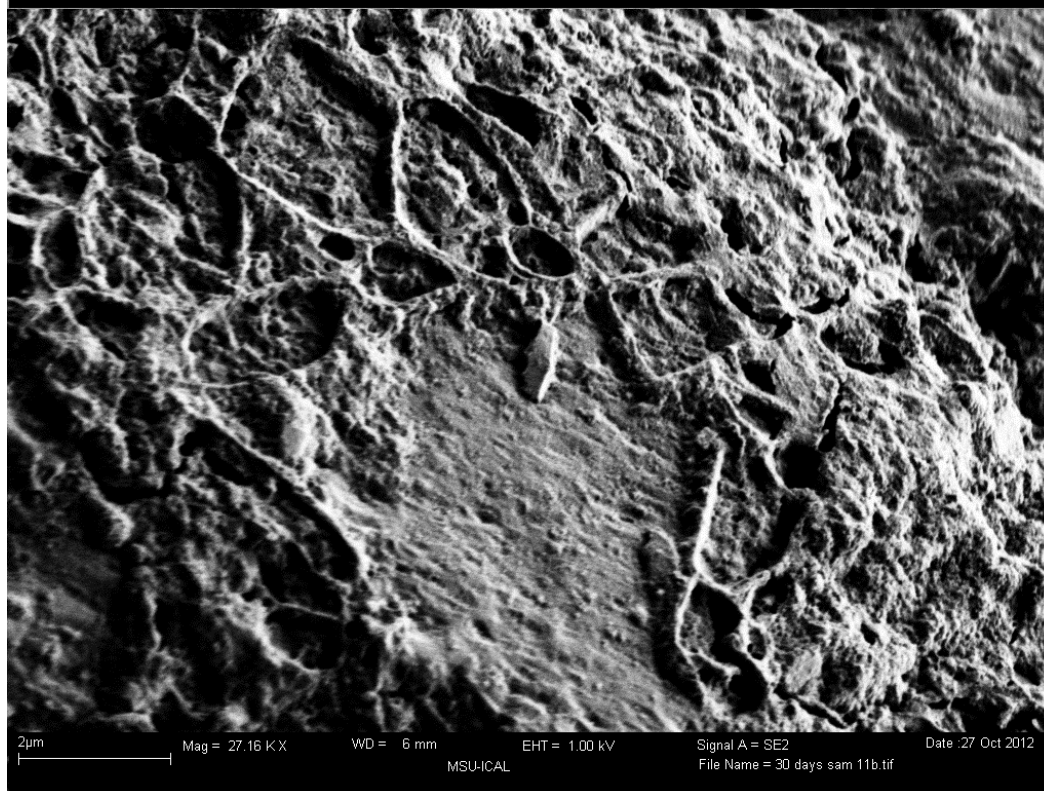
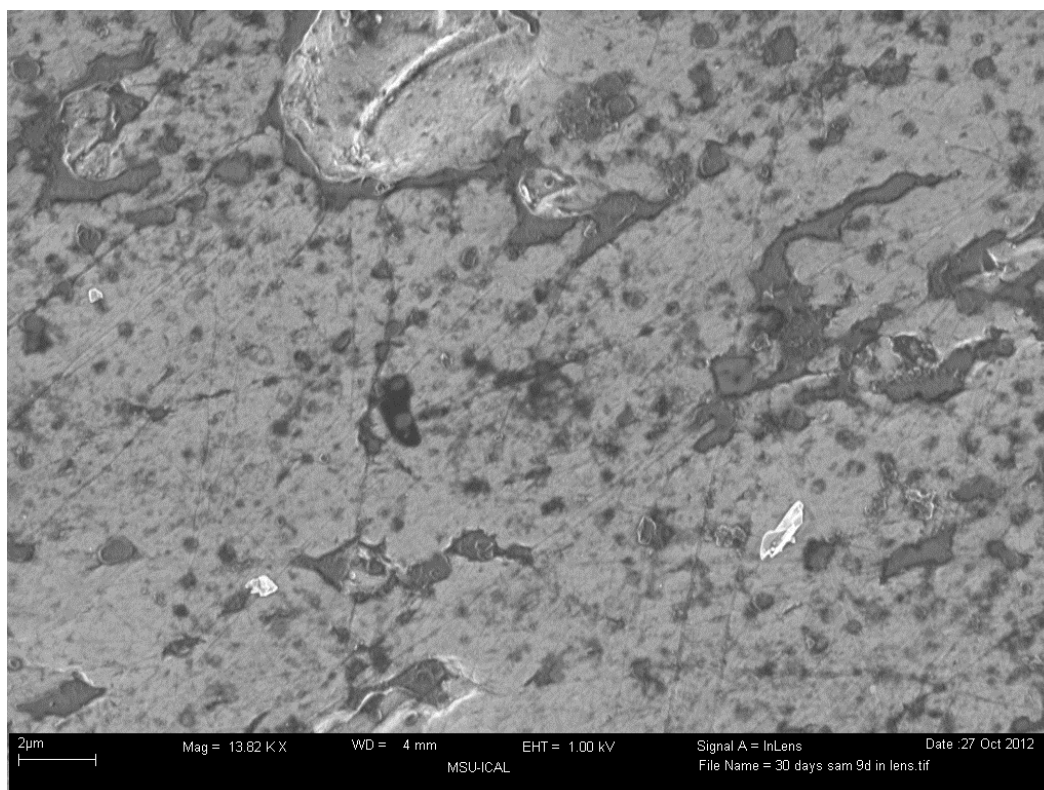






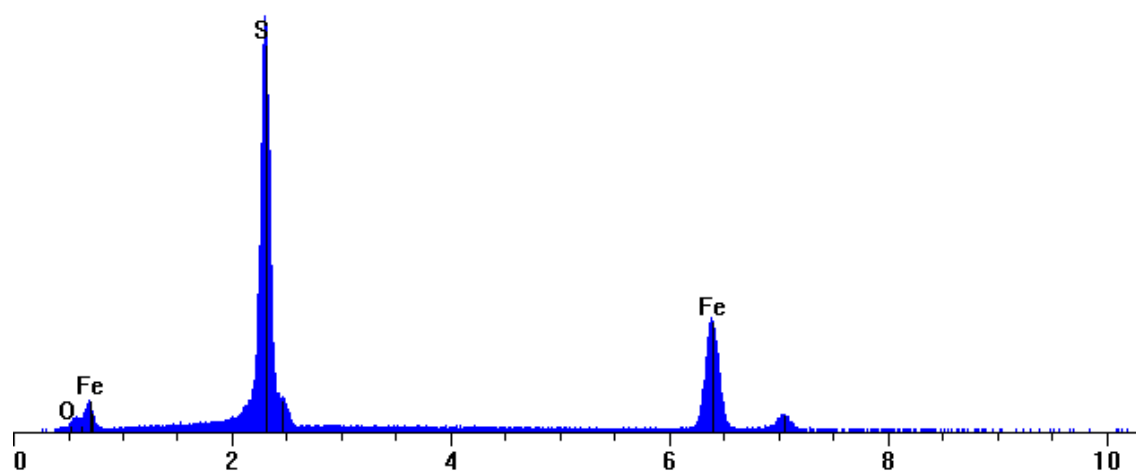






■ pyrite after spectra.pgt

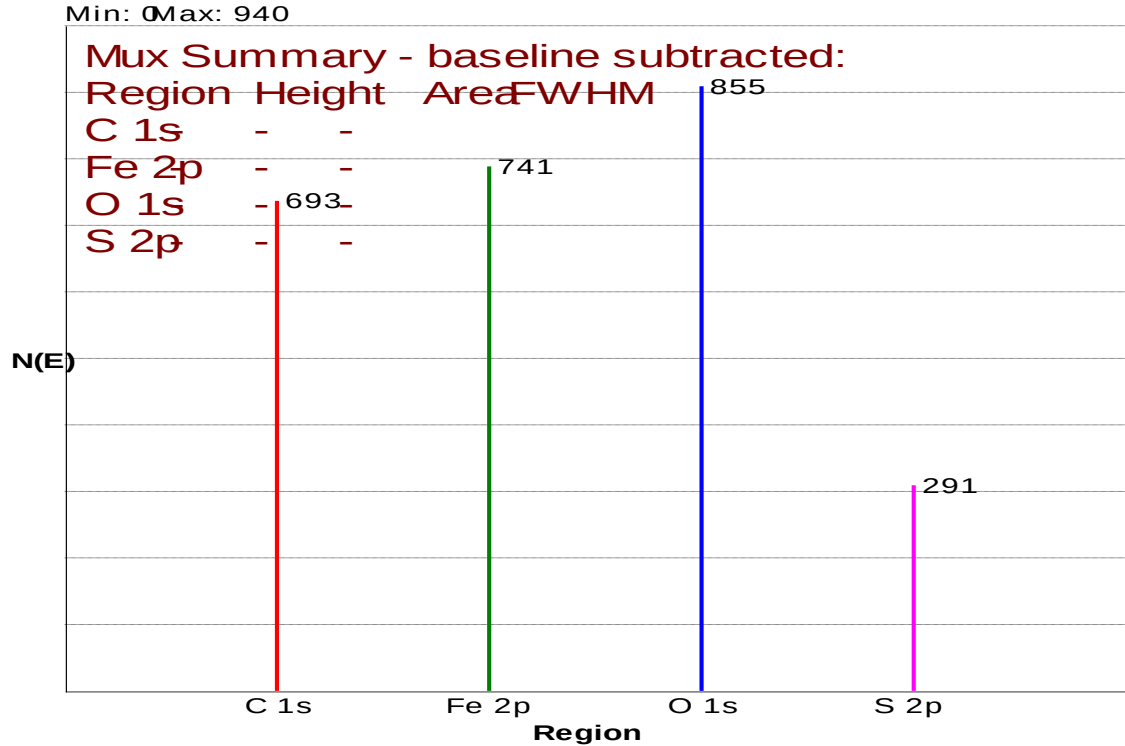
FS: 4800

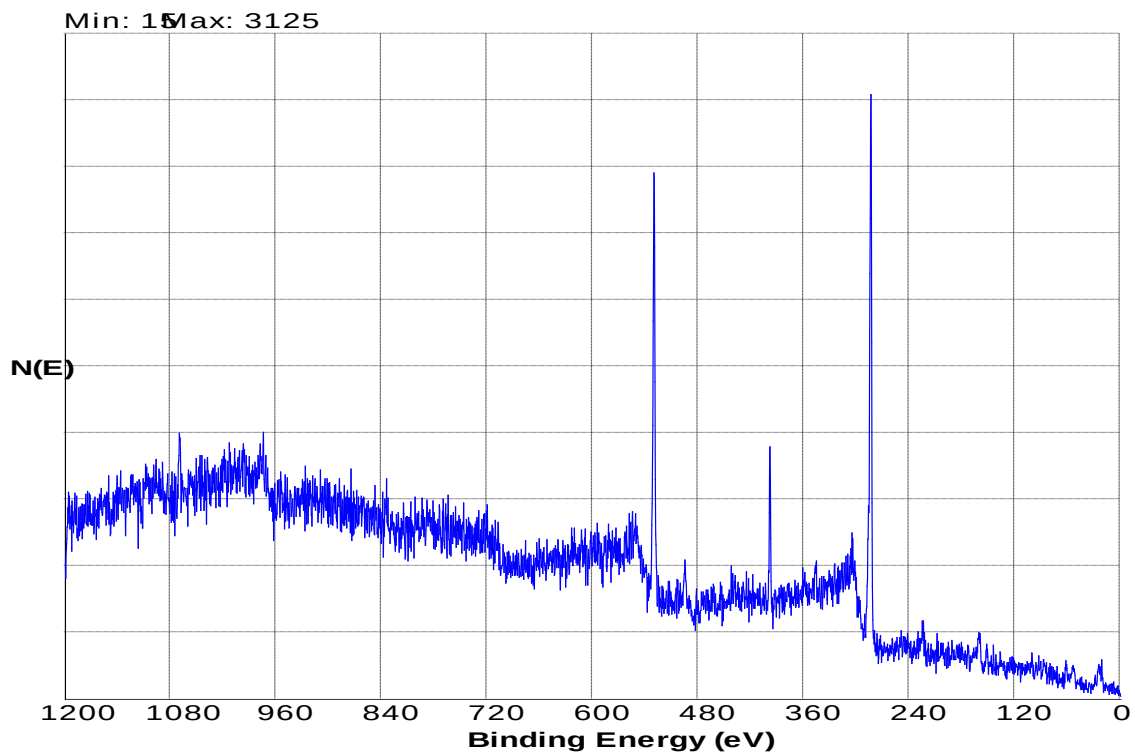


APPENDIX E

XPS DATA FOR GOETHITE, PYRRHOTITE AND PYRITE
AFTER EXPOSURE TO SRB

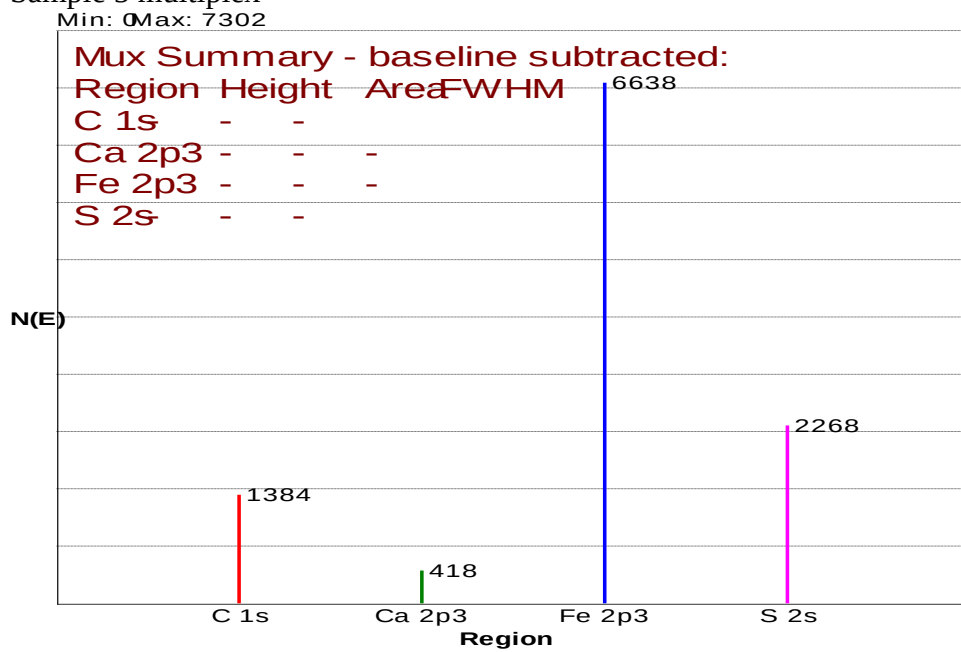
Appendix E contains XPS survey scans and multiplexes from goethite, pyrrhotite and pyrite after exposure to SRB. 1) XPS multiplex data and survey scan from goethite control sample 8 after exposure for 7 days; 2) XPS multiplexes and survey scan for pyrrhotite samples 5 and 11; 3) XPS depth profile and survey scan of pyrite control sample 4.



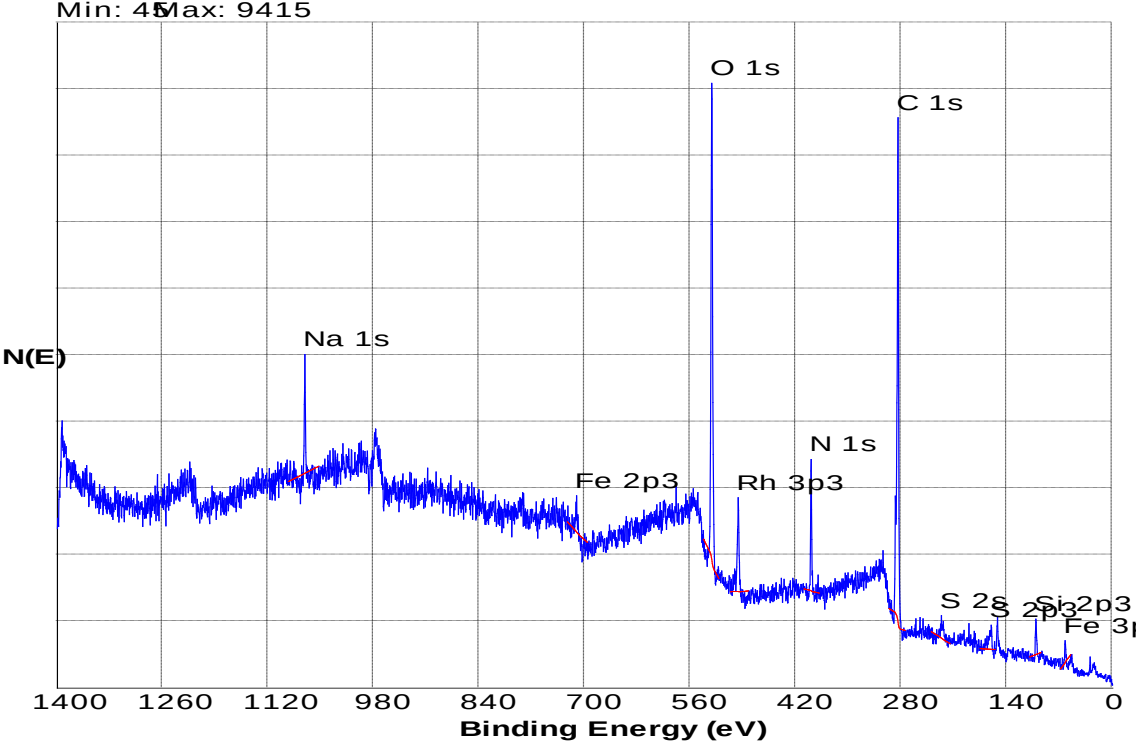


- 2) XPS multiplex and survey scan for pyrrhotite sample 5 after 14 days exposure to SRB. Multiplex and survey scan for pyrrhotite sample 11 after 30 days exposure.

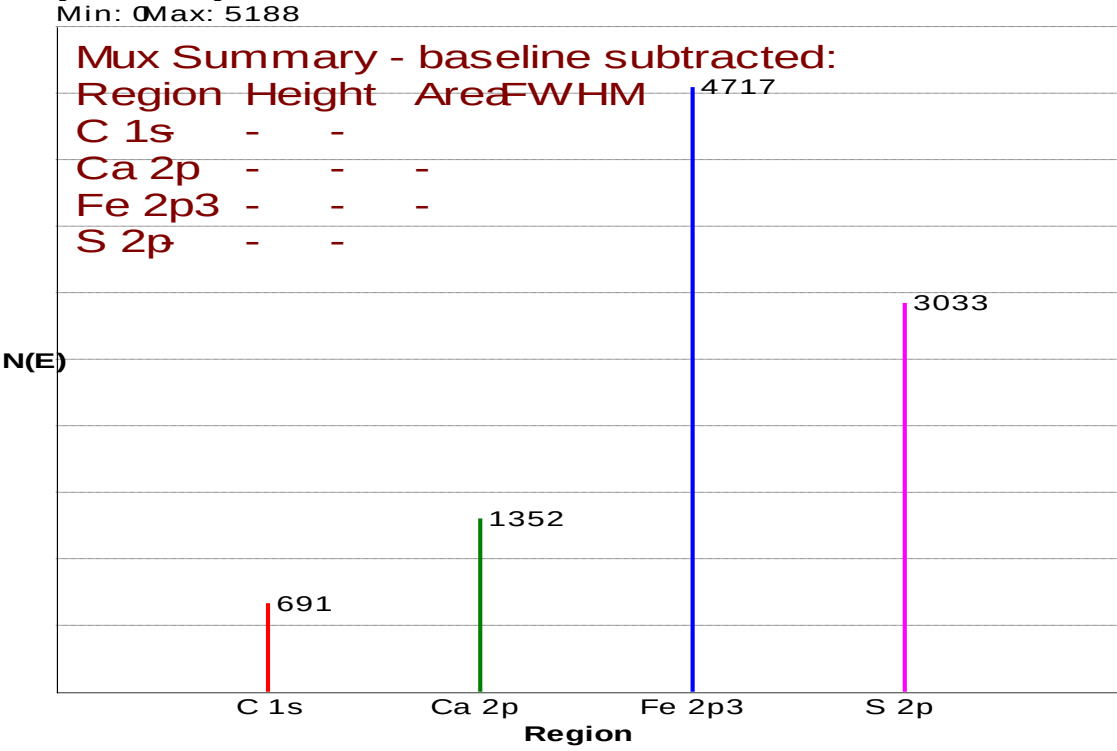
Sample 5 multiplex



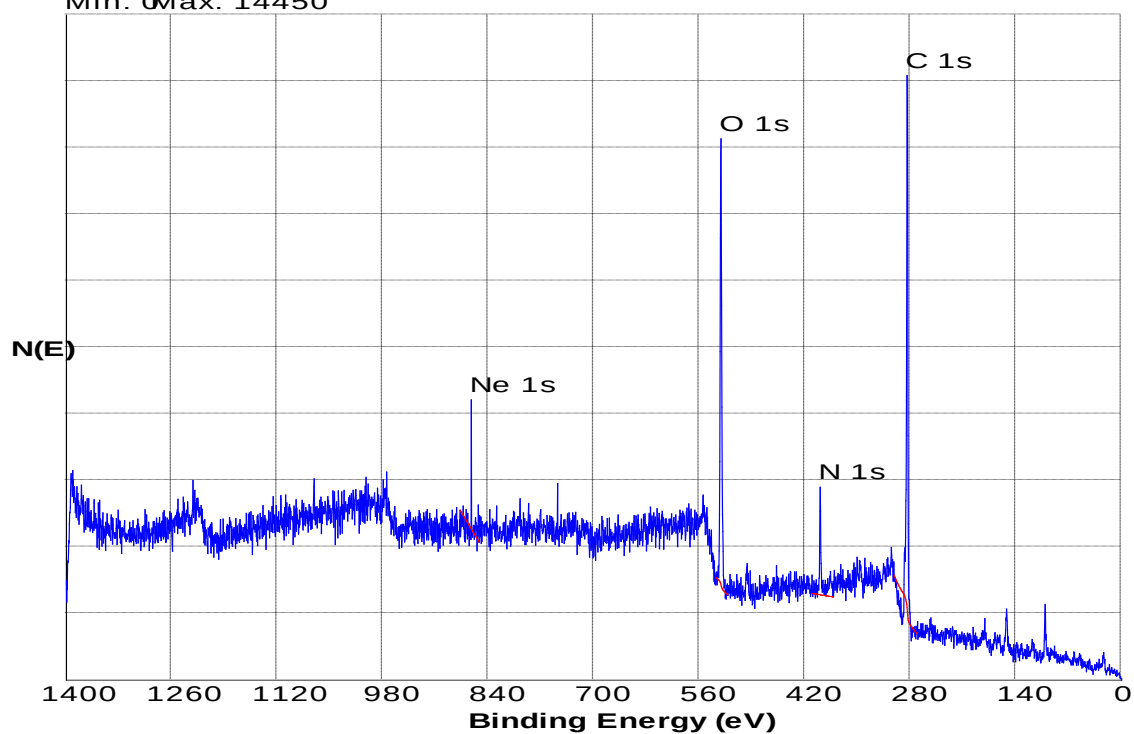
Sample 5 survey scan



Sample 11 multiplex



Sample 11 survey scan
Min: 0 Max: 14450



3) XPS depth profile and survey scan for pyrite control sample 4.

Min: 880 Max: 8211

