

**Investigating the Susceptibility of Jarosite Minerals to Reductive Dissolution by a
Dissimilatory Metal Reducing Bacterium (*Shewanella putrefaciens* CN32)**

by

Christina M. Smeaton

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DECLARATION OF CO-AUTHORSHIP / PREVIOUS PUBLICATION

I. Co-Authorship Declaration

I hereby declare that this thesis incorporates material that is the result of joint research, as follows:

This thesis incorporates the outcome of joint research undertaken in collaboration with Karen Hudson-Edwards, Adrian Smith, William Dubbin, Andrew Beale under the supervision of Christopher Weisener and Brian Fryer. The collaboration is covered in Chapter 3 of this dissertation. In all cases, the key ideas, primary contributions, experimental designs, data analysis and interpretation, were performed by the author, and the contribution of co-authors was primarily through the provision of samples and comments on earlier drafts of the manuscript. The only exception is the EXAFS methodology and data analysis presented in the Supporting Information Section in Chapter 3, which was provided by Andrew Beale to provide background information regarding the experimental sample (2nd paragraph of S3.4, S3.6, Figure S3.11).

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Thesis Chapter	Publication title/full citation	Publication status*
Chapter 2	Smeaton, C. M.; Fryer, B. J.; Weisener, C. G., Intracellular Precipitation of Pb by <i>Shewanella putrefaciens</i> CN32 during the Reductive Dissolution of Pb-Jarosite. <i>Environ. Sci. Technol.</i> 2009 , 43, (21), 8091-8096.	Published
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Chapter 4	C.M. Smeaton, G.E. Walshe, B.J. Fryer and C.G. Weisener. Reductive dissolution of Tl(I)-jarosite by <i>Shewanella putrefaciens</i> : Providing new insights into Tl biogeochemistry	Submitted to Environmental Science & Technology

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ABSTRACT

Jarosite-group minerals ($A\text{Fe}_3(\text{TO}_4)_2(\text{OH})_6$) typically form under acidic ($\text{pH} < 3.5$), oxidizing, ferric and sulfate rich conditions and commonly occur in the oxidized portion of sulfide deposits, hydrometallurgical wastes, acid sulfate soils and environments contaminated by acid rock or acid mine drainage. Jarositic wastes are typically stored in disposal ponds under circum-neutral conditions, thereby rendering the mineral phase unstable and susceptible to reductive dissolution through Fe(III) reduction by microbial populations under reducing conditions. The primary goal of this dissertation was to examine the susceptibility of a variety of jarosite-group minerals to microbial Fe(III) reduction and to examine the potential for enhanced metal release as compared to control samples during dissolution.

Experiments were conducted by incubating a variety of synthetic and natural jarosites with the facultative anaerobe, *Shewanella putrefaciens* strain CN32 using lactate as the sole electron donor. Incubations ranged from 336 to 900 hours and solution chemistry, including elemental concentrations, Fe and As oxidation state, Eh and pH were monitored over time. Changes in solid phase, including secondary precipitation, were characterized using environmental scanning electron microscopy (ESEM) and transmission electron microscopy (TEM) coupled with energy dispersive spectroscopy (EDS). X-ray absorption spectroscopy (XAS) was also used to examine simultaneous Fe and As reduction during the reductive dissolution of a Pb-As jarosite.

Results revealed that abiotic and biotic dissolution of jarosites are both incongruent reactions with the former consuming H^+ and the latter producing H^+ . Fe(II) production was used as a proxy for Fe(III) reduction and showed that the susceptibility of

jarosites to microbial Fe(III) reduction was influenced primarily by structure with susceptibility increasing in monovalent A-site substituted jarosites (K, Tl and Na). Moreover, parallel experiments with synthetic and natural jarosites demonstrated that reductive dissolution was greater in the synthetic samples. Enhanced release of structural constituents, such as Tl, Sr and As, was observed in inoculated samples as compared to control samples. Based on the instability of jarosite-group minerals under anaerobic circum-neutral conditions, however, it is expected that both abiotic and biotic processes will contribute to the release of structural constituents such as Tl and As into the environment.

DEDICATION

This dissertation is dedicated to my wonderful husband, Steve, who has seen the first year of our marriage consumed by my thesis writing turmoil. His unconditional love, sense of humour, support and patience has provided me with the strength and perseverance to finish this thesis.

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CHAPTER 1

Introduction

1.1 JAROSITE STRUCTURE

Jarosite, $\text{KFe}_3(\text{SO}_4)_2(\text{OH})_6$, was first described in 1852 by the German mineralogist August Breithaupt in Barranco del Jaroso (Jaroso Ravine) in the Sierra Almagrera mountain range along the southeastern coast of Spain.¹ Jarosite is an iron hydroxysulfate mineral and is a member of the isostructural alunite-jarosite mineral super group with a general formula of $\text{AB}_3(\text{TO}_4)_2(\text{OH})_6$. The A sites are occupied by monovalent (e.g. K^+ , Tl^+ and Na^+) and divalent cations (e.g. Ca^{2+} and Pb^{2+}) with coordination numbers ≥ 9 . B represents cation sites with octahedral (O) coordination, typically Al^{3+} or Fe^{3+} , while the T position corresponds to tetrahedral (T) coordination (e.g. SO_4^{2-} , AsO_4^{3-}) (Figure 1.1).^{2,3}

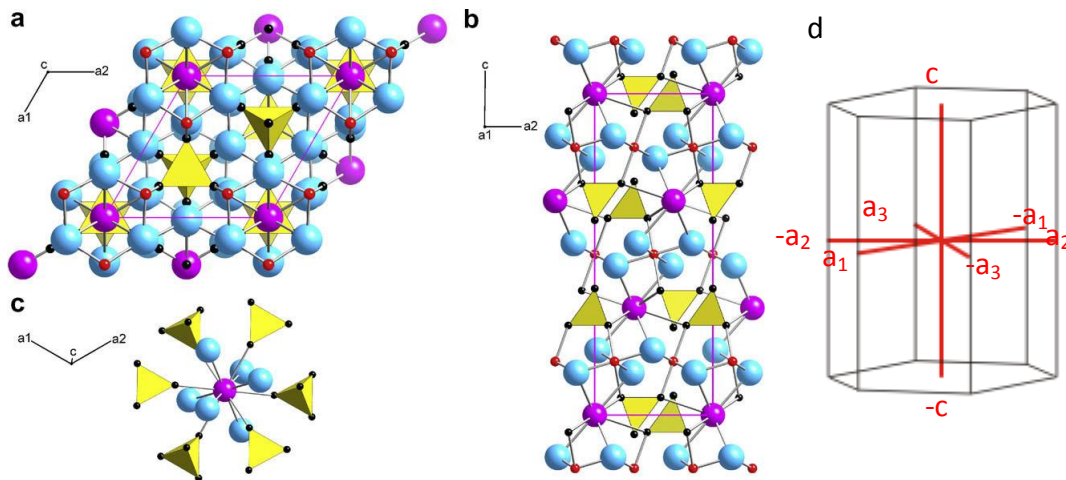


Figure 1.1. Crystal structure of K-jarosite.

The A site is represented by magenta (K) atoms, B site by red (Fe^{3+}) atoms, T site by yellow (SO_4^{2-}) tetrahedra. Oxygen atoms are represented by black and hydroxyl groups are blue spheres. The unit cell is outlined in magenta. (a) Projection down the c -axis, (b) Projection down the a -axis, (c) The A site, separated from the crystal structure, (d)

Jarosite trigonal-pyramidal crystal system and axial configurations. Modified from Burger et al. (2009)⁴ and webmineral.com.

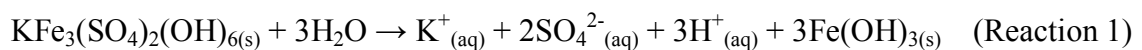
Jarosite is commonly referred to as an elemental "garbage bucket" because it can incorporate a large number of elements into its structure and can undergo solid phase substitution with respect to its chemical composition. With these attributes, it is often expected to reflect the chemical signature of the fluids from which it was formed.¹ The relative environmental stability of synthetic jarosites over other potential waste mineral hosts, such as apatites and pyrochlores, has made synthetic jarosites a potential candidate for the long term disposal of toxic metals such as As, Pb, Bi, Hg, Tl, Sb, Cr, Se and radioactive isotopes of K, Sr, Th, U and rare earth elements (REE).^{5,6} A list of several jarosite-group minerals and their synthetic counterparts are listed in Table 1.2. Jarosite minerals exhibit a rhombohedral symmetry, space group $R\bar{3}m$, with a hexagonal cell and parameters $a \sim 7 \text{ \AA}$, $c \sim 17 \text{ \AA}$.⁷

The stability and reactivity of jarosites is related to the degree of substitution in the A and B structural sites. Deficiencies in the A (e.g., K^+) and B (e.g., Fe^{3+}) sites result in a lack of positive charge. Therefore, deficiency in the A site is balanced by incorporation of hydronium ions (H_3O^+) and is reported to occur in most natural potassium and sodium jarosites.⁸ Incorporation of the larger H_3O^+ cation increases solubility in jarosite minerals by increasing the c -parameter of the crystallographic structure thereby decreasing stability and increasing reactivity.^{9,10} Other A-site substitutions include but are not limited to: Rb, Pb, Ca, Sr, and the REE. Typically, substitution of larger monovalent cations into the A-site results in expansion of the c parameter but has little effect on the a parameter (Figure 1.1 D).¹¹ The general

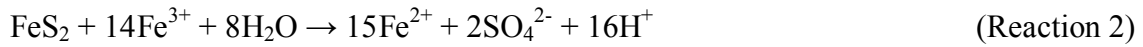
relationship between A site cations and dissolution rates in synthetic jarosites increases in the order K(Fe, Cr)- > K- > Na- > H₃O-jarosite. Deficiency in the B site is balanced by protonation of the hydroxyl groups in the structure to form water molecules and is described by the following chemical formula developed by Kubisz (1970): A_{1-x}(H₃O)_x Fe_{3-y} (OH)_{6-3y}(H₂O)_{3y} (SO₄)₂.^{12,13} Substitution of divalent cations such as Pb²⁺ and Cu²⁺ into the A-site also requires charge balancing. For example, for every K⁺ cations in the K-jarosite formula there are 0.5 Pb²⁺ cations in the Pb-jarosite formula. The Pb²⁺ cation and the A site vacancies are ordered resulting in the doubling of the unit cell.¹⁴ Moreover, arsenate (AsO₄³⁻) will substitute for SO₄²⁻ in the T-site in K-jarosite minerals to charge balance Pb²⁺ substitution in the A-site.^{7,15} Despite extensive elemental substitution into jarosites, the relative stability of these substitutions is not well studied from both a biotic or abiotic perspective.

1.2 JAROSITE FORMATION

Figure 1.2 shows the pe/pH diagram for K-jarosite, which illustrates that jarosites form under oxidizing, ferric and sulfate rich acidic (pH < 2.5) conditions.^{11,16} At higher pH, jarosites dissolve to form goethite (FeO(OH)) or metastable phases, such as schwertmannite (Fe₈O₈(OH)₆SO₄) or ferrihydrite.^{17,18} Consequently, jarosite-group minerals commonly form in acid-sulfate soils, such as on the north coast of New South Wales, Australia, whereby the dissolution of jarosite during events such as flooding represents a major potential source of acidity to the region through the following reaction:¹⁹



Moreover, dissolution of jarosite under acidic conditions ($\text{pH} > 3$) will produce soluble ferric iron instead of iron (III) hydroxides and catalyze pyrite oxidation and enhance H^+ production, as demonstrated in the following reaction:



Jarosites are also found in hypersaline lakes such as Lake Tyrell in Australia and in the Antarctic.^{20,21} Jarosites are also common constituents in mine wastes across the globe with some of the best studied areas such as Rio Tinto, Spain and Kidd Creek, Timmins, Canada.^{7,22-24}

1.3 JAROSITES AND THE ZINC INDUSTRY

Over the past 30 years, the Zn industry has capitalized on the stability of jarosite minerals and synthetic jarosites, which are purposely precipitated to remove unwanted impurities during processing, such as Fe, sulfates, alkalis and other metals (e.g., Pb, Ag). Precipitates are easily filtered and give low losses of Zn metal, therefore, this process accounts for 80% of all Zn extracted worldwide (8 Mt/yr).⁷ While the jarosite process is a highly efficient and economical method to optimize Zn recoveries, it also produces large volumes of waste. For example, a plant annually producing 150,000 tonnes of metallic Zn will generate approximately 125,000 tonnes of jarosite.⁷ Jarositic wastes are not easily recyclable and may contain elevated concentrations of toxic metals such as Pb, As, Tl, and therefore the safe disposal of these wastes is of paramount concern.

Past disposal methods have included deep sea disposal, underground storage and co-surface tailing impoundments, all of which have proven ineffective at containing jarositic wastes. For example, from 1973 to 1997, Pasminco EZ Metals disposed of 4 million tonnes of Zn smelting wastes containing mostly jarosite with elevated

concentrations of Zn (6.5%), Pb (2.4%) and As (0.95%) at an offshore disposal site off the southern coast of Tasmania at depths of 200 m. Consequently, Harris et al. (1999) demonstrated downslope dispersal of these sediments at depths greater than 3000 m.²⁵ In Odda, Norway disposal of jarosite residues in a granitic mountain cavern resulted in metal seepage near the containment area.^{7,26} Recent advances in the hydrometallurgical industry in collaboration with the Canadian government (CANMET) has led to a promising long-term jarosite disposal process called Jarofix, whereby precipitates are stabilized using a mixture of Portland cement, lime and water and may serve to alleviate some of the issues surrounding disposal.²⁷

1.4 MARTIAN JAROSITE

Overall, the use of jarosite precipitation in the Zn industry led to an increase in hydrometallurgical-focused jarosite research in the 1970s, which continues to the present and is led by J.E. Dutrizac (CANMET), whereby the stability and factors affecting the synthesis of various jarosites were studied extensively.² However, it was NASA's 2004 announcement of the discovery of jarosite on Mars, as detailed in the seminal Science paper by Klingelhöfer et al. (2004), that brought the relatively unknown mineral to the headlines and to the forefront of mineralogical research mandates.²⁸ The Mars discovery created a major surge in jarosite research. An ISI Web of Science Survey conducted in April 2012 of the 38 years (1965-2003) prior to the Mars announcement showed that there were only 177 journal articles published featuring "jarosite" in the title. In the last 8 years, however, since the announcement there were 138 articles published accounting for 44% of all jarosite titled publications over the last 48 years.

The discovery of jarosite on Mars had two important astrobiological implications: 1) as an OH^- and SO_4^{2-} anion bearing mineral, jarosite provided mineralogical evidence for aqueous acid sulfate processes under oxidizing conditions, and 2) as a K-bearing mineral, jarosite could potentially be used as a reliable geochronometer using the $^{40}\text{Ar}/^{39}\text{Ar}$ method to identify when water was present on Mars.²⁹⁻³¹ Moreover, evidence of amino acid preservation of glycine and other organics in terrestrial jarosite samples has rendered the mineral a potential preserver of biological signatures of life on Mars.³¹⁻³³

The potential influence of microbial processes on jarosite formation is not a new concept. In 1973, Ivarson was the first to demonstrate jarosite and ammoniojarosite formation through the oxidation of ferrous sulfate by *Thiobacillus ferrooxidans*.³⁴ Indeed, since 1973 several studies have documented the role of microbial processes on jarosite formation through the oxidation of both aqueous and solid phase Fe^{2+} and sulfide by bacteria, such as *T. ferrooxidans*, *Acidiphilium rubrum* and *Acidithiobacillus ferrooxidans*.³⁵⁻³⁹

The focused attention and resulting publications on jarosite spurred by the Mars announcement also benefited other fields of jarosite research and on acid mine wastes in particular. Increased interest into jarositic mine waste and their respective formation locations was partially motivated by the search for terrestrial Mars analogues, such as at Rio Tinto, Spain but it also served to indirectly increase awareness of the potential environmental problems associated with jarosite formation and disposal.⁴⁰

1.5 JAROSITE AND THE ENVIRONMENT

During the late 1990s and early 2000s, studies had already demonstrated formation and incorporation of toxic metals such as Pb and As into jarosites at several acid mine drainage (AMD) sites (including Rio Tinto) and suggested that jarosites served as sinks and helped immobilize metals, particularly Pb and As.^{24,41-43} Therefore, current jarosite research is focused on determining the stability of jarosites and the geochemical conditions influencing the structural release of potentially toxic metals, such as Pb, As and Tl, into the environment.^{19,22,44-48} Recent studies using laboratory batch tests demonstrated that abiotic dissolution of K-jarosite, Pb-jarosite and Pb-As jarosite is an incongruent reaction controlled by selective dissolution at the A and T sites. During dissolution and in the absence of Fe(III) complexation, Fe(III) hydroxides will precipitate at the surface, which may serve to resorb metals such as Pb and As.^{46,49,50}

Many of the underlying mechanisms regulating metal release and uptake onto secondary precipitates associated with jarosites, however, remain to be elucidated. For example, co-disposal of natrojarosite, $\text{NaFe}_3(\text{OH})_3(\text{SO}_4)_2$, and base-metal sulphide tailings at Kidd Creek, Timmons, Canada at neutral pH (6.5 to 6.8) and low Eh (94 to 117 mV) demonstrated increased concentrations of Zn, Pb and As in pore waters associated with the jarosite disposal zone of the tailings pile which was attributed to enhanced dissolution of jarosites.²² In mine waste tailings ponds, Fe minerals such as jarosites may also form crusts or hardpans. In Crimea, Ukraine metals such as Cr, As and Ti are immobilized in a 10 to 50 cm natrojarosite layer 42 km² wide.^{51,52} The natrojarosite layer sealed the sediment-water interface and limits oxygen penetration to

underlying materials, thereby promoting anoxic conditions and creating the potential for dissimilatory metal reduction.

Despite the potential for the reductive dissolution of jarosites by natural microbial consortia, surprisingly few studies have evaluated the potential for microbial dissolution by dissimilatory metal reducing bacteria.^{46-48,53,54} Therefore, the focus of this dissertation is to examine the susceptibility of several synthetic and natural jarosites under circumneutral, anaerobic conditions to reductive dissolution by the dissimilatory metal reducing bacterium, *Shewanella putrefaciens* strain CN32.

1.6 DISSIMILATORY METAL REDUCTION

Dissimilatory metal reduction refers to an enzymatic process whereby a metal such as Fe or Mn is reduced but is not incorporated into the cell for the purpose of biosynthesis during respiration.⁵⁵ During this process, bacteria gain energy for growth by coupling the oxidation of a carbon source to the reduction of a terminal electron acceptor such as Fe(III). Microbial Fe(III) reduction results in release of aqueous Fe(II) and the subsequent precipitation of Fe(II)-bearing minerals, including siderite (FeCO₃), vivianite (Fe₃PO₄·8H₂O), and mixed-valence Fe(II)-Fe(III) minerals, such as magnetite and green rusts.⁵⁶ Based on thermodynamic energy yield, bacteria generally use terminal electron acceptors in the following sequence of decreasing energy yield: O₂ > NO₃⁻ > MnO₂ > FeOOH > SO₄²⁻.^{57,58} The *Shewanella sp.* and *Geobacter sp.* genera are the most commonly studied iron reducing bacteria. In 1988, *Geobacter metallireducens* GS-15 was the first bacterial species reported to show dissimilatory Fe reduction by coupling the oxidation of acetate to the reduction of an amorphous Fe(III) oxide.⁵⁹ Reports of manganese and iron oxide reduction by *Shewanella oneidnesis* MR-1 soon followed and

the era of *Shewanella sp.* research began.^{60,61} As a facultative anaerobe, *Shewanella sp.* has shown remarkable anaerobic versatility and is capable of reducing a variety of other terminal electron acceptors such as nitrate, nitrite, thiosulfite, sulfite, arsenate, uranium, chromate, iodate, technetium, neptunium, plutonium, selenite (see ref. 46 and all refs. therein).^{55,62}

The insolubility of Fe(III) at pH > 4 creates a metabolic quandary for bacteria utilizing Fe(III) minerals as terminal electron acceptors. Various microbial strategies have been proposed to facilitate the transfer of electrons to Fe(III) oxide minerals and include: 1) direct contact; 2) electrically conductive nanowires; 3) electron shuttles; and 4) metal chelators/siderophores.⁵⁶ Direct contact between *Shewanella sp.* and Fe-oxide surfaces is considered the prevalent strategy to ensure effective electron transfer (see 55 and all refs. therein).⁶³ The ability of dissimilatory metal reducing bacteria to reduce iron oxides is well documented and is dependent on several factors including particle size, surface area, and crystallinity.^{64,65} Generally, the rate of Fe reduction increases with increasing surface area, but is inversely related to particle size and crystallinity. Accordingly, Munch and Ottow (1983) showed that reactivity towards microbial iron reduction decreased in the following order: amorphous Fe(III) hydroxides > lepidocrocite ($\gamma\text{FeO(OH)}$) > hematite (Fe_2O_3) > goethite ($\alpha\text{FeO(OH)}$).⁶⁶ More recently, Bonneville et al. (2004, 2009) demonstrated that Fe(III) reduction by *S. putrefaciens* was related to mineral solubility, whereby Fe(III) reduction was positively correlated to the solubility product.^{67,68}

1.7 REDUCTIVE DISSOLUTION OF JAROSITES

In comparison to Fe(III) hydroxides, the reductive dissolution of jarosites are not well-studied. The presence of both Fe(III) and SO_4^{2-} presents two terminal electron acceptors available for reduction. Ivarson and Hallberg (1976) were the first to demonstrate the reductive dissolution of biogenic K-jarosite followed by the formation of mackinawite (FeS) using the sulfate-reducing bacterium, *Desulfovibrio desulfuricans*, under circumneutral anaerobic conditions.⁶⁹ Twenty-four years later, Fe(III) reduction by *Acidiphilium* SJH (pH 2 -3) was investigated across a suite of Fe(III) minerals including biogenic jarosite and natrojarosite.⁵³ Results showed that *Acidiphilium* SJH was able to reductively dissolve all of the minerals tested except hematite in the following order of decreasing reactivity: soluble Fe^{3+} > amorphous $\text{Fe}(\text{OH})_3$ > magnetite > goethite > natrojarosite > akageneite > jarosite. To examine jarosites from a mine waste perspective, Jones et. al (2006) used *Geobacter metallireducens* GS15 and a mine site isolate, *Geobacter* sp. ENN1, to examine Fe(III) reduction at neutral pH from synthetic schwertmannite and two natural jarosite samples. One of the jarosite samples originated from an AMD site, while the other was purchased as a lithified mineral specimen. Both strains were able to reduce Fe(III) from the 2 jarosites, however, neither bacterium reduced Fe(III) from schwertmannite. Results also showed that the lithified mineral specimen was more resistant to Fe(III) reduction and corroborated mineralogical studies, which demonstrate that mine waste jarosites are less stable as opposed to older, stoichiometric jarosites.^{9,10}

To examine the potential for structural metal release from the A-site, Weisener et. al. (2008) examined the reductive dissolution of synthetic Ag-jarosite

($\text{AgFe}_3(\text{SO}_4)_2(\text{OH})_6$) and showed reduction of both Fe and Ag by *Shewanella putrefaciens* CN32.⁵⁴ To examine sulfate reduction, Gramp et al. (2009) used *Acidithiobacillus ferrooxidans* to precipitate K-jarosite and schwertmannite ($\text{Fe}_8\text{O}_8(\text{OH})_6\text{SO}_4$).⁷⁰ The solid phases were then used as electron acceptors at pH 6.5 for mesophilic (22 °C) and thermophilic (45 and 60°C) sulfate reducing bacteria isolated from an AMD mine site and a commercial compost pile, respectively. After 2 weeks, greigite (Fe_3S_4) was formed at 60 °C in both schwertmannite and K-jarosite, however, the greigite formed from the reduction of jarosite was less abundant. The greater S reduction from schwertmannite was likely due to its poor crystallinity and relative instability as compared to the more crystalline jarosite. Most recently, Coggon et al. (2012), demonstrated the reduction of Fe(III) and sulfate in AMD sediment amended with natrojarosite using a natural microbial consortia from the AMD site and also showed increased pH relative to controls due to H^+ consumption during Fe/S reduction.⁷¹ The increase in pH suggests the potential for jarosite to be used to attenuate AMD environments. Aside from the Weisener (2008) study, there are no other studies that examine the potential role that reductive dissolution of jarosites may play in the mobilization of metals, such as As and Tl, into the environment.

1.8 THESIS OUTLINE

This dissertation examines the relative influence of jarosite structure on the abiotic and reductive dissolution of synthetic and natural jarosites using batch incubations under anaerobic circum-neutral conditions. *Shewanella putrefaciens* was used as the metal reducing bacterium because it is a well characterized, model chemoautotrophic anaerobe capable of using structural Fe(III) and As(V) during

dissimilatory metal reduction.^{65,72-74} *S. putrefaciens* was originally isolated from a subsurface core sample (250 m) obtained from the Morrison Formation, a shale-sandstone sequence in north western New Mexico mined extensively for uranium.⁶⁰

The central hypothesis that forms the basis of this dissertation is: *Shewanella putrefaciens* CN32 will reductively dissolve jarosites and the degree of dissolution will be influenced by elemental substitution within the jarosite structure. The results comprise 4 Chapters (2 to 5) (in manuscript format) and encompass the reductive dissolution of several synthetic and natural jarosites.

Chapter 2 investigates the reductive dissolution of synthetic Pb-jarosite, $\text{PbFe}_6(\text{SO}_4)_4(\text{OH})_{12}$, by *S. putrefaciens*. The objective of this study was to examine the influence of the divalent Pb^{2+} cation in the A-site on the susceptibility of jarosite phase to Fe(III) reduction. Pb-jarosite was used because it is suggested to control aqueous Pb mobility in AMD environments and is also a common waste product of the Zn mining industry.^{24,41,75} In addition to solution chemistry, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to examine the intracellular accumulation of Pb demonstrated by *S. putrefaciens* during the experiment.

Chapter 3 examines the relative order and extent of As(V) and Fe(III) reduction in a synthetic Pb-As jarosite $(\text{H}_3\text{O})_{0.68}\text{Pb}_{0.32}\text{Fe}_{2.86}(\text{SO}_4)_{1.69}(\text{AsO}_4)_{0.31}(\text{OH})_{5.59}(\text{H}_2\text{O})_{0.41}$ by *S. putrefaciens*. In this structure, Pb is at the A site and AsO_4^{3-} partially substitutes for SO_4^{2-} in the T site. Solid phase As and Fe speciation was examined using X-ray Absorption Spectroscopy (XAS) at Argonne National Laboratory. Additionally, we examined the fate of As and Pb released during both the abiotic and reductive

dissolution of Pb-As jarosite using solution chemistry, geochemical modeling, HR-TEM and SEM.

In **Chapter 4**, the reductive dissolution of Tl-jarosite, $(\text{H}_3\text{O})_{0.29}\text{Tl}_{0.71}\text{Fe}_{2.74}(\text{SO}_4)_2(\text{OH})_{5.22}(\text{H}_2\text{O})_{0.78}$ was examined. This chapter differs from the previous 2 chapters because Tl^+ is a monovalent cation occupying the A site and the structure is expected to be less stable. Although highly toxic and mobile in the environment, Tl has received very little scientific attention as compared to other metals such as Pb, Hg and Cd. The role of *S. putrefaciens* in Tl sorption was also examined in order to provide greater insight into Tl + bacteria interactions, which is currently lacking in the scientific literature.⁷⁶⁻⁷⁹

The primary objective of **Chapter 5** was to determine if there are any differences between the reductive dissolution of synthetic and natural jarosites. Synthetic jarosite is often used as a proxy for natural jarosites, which may potentially underestimate or overestimate dissolution. Therefore, the reductive dissolution of 2 synthetic K-jarosites and 2 natural jarosites was compared. By using different jarosite synthesis methods, the influence of particle size and surface area on reductive dissolution was also examined. Lastly, the potential influence of *S. putrefaciens* on the release of metals from the natural jarosite phases was evaluated.

In **Chapter 6**, the data from the preceding chapters is amalgamated and the implications on jarosite research and suggestions for future research are discussed.

Formula	Mineral Name	Synthetic Equivalent
$\text{KFe}_3(\text{SO}_4)_2(\text{OH})_6$	jarosite	potassium jarosite
$\text{NaFe}_3(\text{SO}_4)_2(\text{OH})_6$	natrojarosite	sodium jarosite
$(\text{H}_3\text{O})\text{Fe}_3(\text{SO}_4)_2(\text{OH})_6$	hydronium jarosite	hydronium jarosite
$\text{RbFe}_3(\text{SO}_4)_2(\text{OH})_6$	no mineral equivalent	rubidium jarosite
$\text{AgFe}_3(\text{SO}_4)_2(\text{OH})_6$	argentojarosite	silver jarosite
$(\text{NH}_4)\text{Fe}_3(\text{SO}_4)_2(\text{OH})_6$	ammoniojarosite	ammonium jarosite
$\text{TlFe}_3(\text{SO}_4)_2(\text{OH})_6$	dorallcharite	thallium jarosite
$\text{PbFe}_6(\text{SO}_4)_4(\text{OH})_{12}$	plumbojarosite	lead jarosite
$\text{HgFe}_6(\text{SO}_4)_4(\text{OH})_{12}$	no mineral equivalent	mercury jarosite
$\text{Pb}(\text{Fe,Cu})_3(\text{SO}_4)_2(\text{OH,H}_2\text{O})_6$	beaverite	lead-copper-jarosite
$\text{PbFe}_3(\text{SO}_4)(\text{AsO}_4)(\text{OH,H}_2\text{O})_6$	beaudantite	lead arsenic jarosite

Table 1.1. Minerals of the jarosite subgroup and their corresponding synthetic analogues

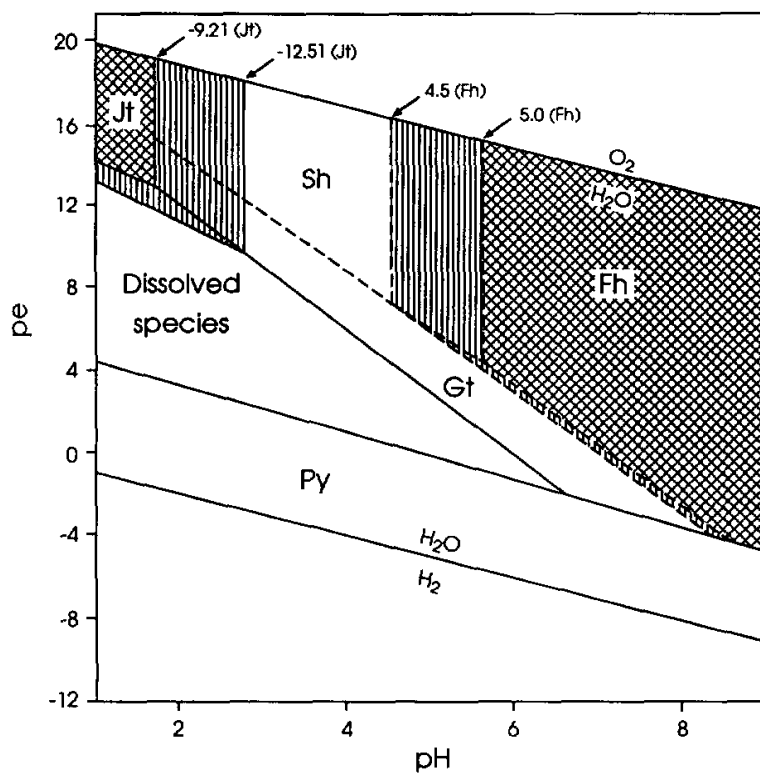


Figure 1.2. pe-pH diagram for Fe-S-K-O-H system at 25°C. Total log activities of $\text{Fe}^{2+} = -3.47$; $\text{Fe}^{3+} = -3.36$ or -2.27 ; $\text{SO}_4^{2-} = -2.32$, $\text{K}^+ = -3.78$. $\text{pe} = \text{Eh}(\text{mV})/59.2$

Where Jt = jarosite, Sh = schwertmannite, Fh = ferrihydrite, Gt = goethite and Py = pyrite.

Single-hatched areas demonstrate expansion of K-jarosite and ferrihydrite fields if lower solubility products are used. Source: Bigham et al. (1996)

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CHAPTER 2

Intracellular precipitation of Pb by *Shewanella putrefaciens* CN32

during the reductive dissolution of Pb-jarosite.

2.1 INTRODUCTION

Plumbojarosite ($\text{PbFe}_6(\text{SO}_4)_4(\text{OH})_{12}$) was first described in 1902 at Cook's Peak, New Mexico and often forms during the oxidation of sulphide deposits.^{1,2} In these environments, Pb mobility is limited by the low solubility of anglesite (PbSO_4 , $\log K_{\text{sp}} = -7.7$) and cerrusite (PbCO_3 , $\log K_{\text{sp}} = -12.8$), consequently plumbojarosite ($\log K_{\text{sp}} = -16.8$) is often the last mineral to form.^{1,3} Plumbojarosite is thermodynamically stable up to pH 5 under high Fe and sulphate activities typical of acid mine drainage (AMD) environments and is suggested to control aqueous Pb mobility at these sites.²⁻⁴ At pH values above 5.0, plumbojarosite is subject to dissolution/reprecipitation reactions and may form Fe oxides or hydroxides.⁵ Under these conditions, Fe hydroxides such as ferrihydrite become important scavengers of Pb.

Structurally, plumbojarosite or Pb-jarosite (i.e., synthetic analog) is a member of the alunite-jarosite mineral super group, with the general formula $\text{AB}_3(\text{TO}_4)_2(\text{OH})_6$. The A sites are occupied by monovalent (e.g., K and Na) and divalent cations (e.g., Ca(II), Pb(II) and Ag(II)). The B position corresponds to cation sites with octahedral (O) coordination, typically Al(III) or Fe(III), while the T site corresponds to tetrahedral (T) coordination (e.g. S(VI), As(V)).⁶ Over the past 20 years, the Zn mining industry has capitalized on the stability of jarosite minerals and during the metal extraction process, synthetic jarosites are purposely precipitated to remove unwanted impurities such as Fe, sulphates, alkalis and other metals (e.g. Pb, Ag). Jarosite precipitation is advantageous because the precipitates are easily filtered and give low losses of Zn metal, therefore, this process accounts for 80% of all Zn extracted worldwide (8 Mt/yr).¹ The majority of the precipitates contain Na and K jarosite, however, Pb-jarosite will form during the acid

pressure leaching of Pb-containing Zn concentrates.¹ While the jarosite process is a highly efficient and economical method to optimize Zn recoveries, it also produces large volumes of wastes. For example, a plant annually producing 150,000 tonnes of metallic Zn will generate approximately 125,000 tonnes of jarosite. Jarositic wastes are not easily recyclable and may contain elevated concentrations of toxic heavy metals such as Pb, therefore the safe disposal of these wastes is of paramount concern.

One of the most widely used storage methods is disposal of jarosite in lined ponds under circumneutral conditions.¹ However, despite the environmental relevance of jarosite minerals, few studies have evaluated the abiotic or biotic dissolution of jarosites under these storage conditions.^{5,7-10} Therefore, the primary objective of this study was to assess the effects of a model metal reducing bacterium, *Shewanella putrefaciens* CN32, on the dissolution of synthetic Pb-jarosite under circumneutral anaerobic conditions. *S. putrefaciens* CN 32 was chosen because it is a well characterized subsurface chemoautotrophic anaerobe capable of using Fe(III) during dissimilatory iron reduction.¹¹⁻¹³ A secondary objective of this study was to evaluate the fate and toxicological effects of the Pb released during the dissolution experiment on *S. putrefaciens* CN32. To the best of our knowledge, this is the first study to assess the fate of Pb during Pb-jarosite/microbe interactions.

2.2 MATERIALS AND METHODS

2.2.1 Preparation of the Pb-jarosite/ cell suspensions and sampling procedures. Pb-jarosite was synthesized as per the method used by Dutrizac et al. (see Supporting Information (SI) Section 2.1 for more details).^{14,15} Pure cultures of *S.*

putrefaciens CN32 were grown, harvested and inoculated into a modified M1 minimal media (SI Section S2.2). The inoculated minimal media was transferred to an anaerobic chamber (96% N₂/4% H₂) and 16 mL aliquots were added to 20 mL polypropylene test tubes containing 0.0500 ± 0.0013 g of Pb-jarosite (33 samples). Experimental controls contained 16 mL of sterile minimal media in 20 mL polypropylene tubes containing 0.0500 ± 0.0013 g of Pb-jarosite (33 samples). To monitor background elemental concentrations over time, an additional set of test tubes was prepared containing only the inoculated minimal media (10 samples) and sterile minimal media (10 samples). All samples were capped, sealed and rotated end-over-end in the anaerobic chamber. Samples containing Pb-jarosite were removed in triplicate from the rotator at 0, 6, 16, 24, 38, 48, 72, 120, 192, 408 and 768 hours, while minimal media samples (i.e., in the absence of Pb-jarosite) were removed from the rotator in duplicate at 0, 48, 192, 408 and 768 hours. All samples were monitored for Eh and pH using semi-micro electrodes and aqueous concentrations of Pb, Fe and S were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES) (SI Section S2.3). Duplicate volumes of the remaining filtrate (100 μ L) were used to immediately determine Fe(II) and total Fe concentrations via the ferrozine method.^{16,17} Fe(III) concentrations were determined by subtracting Fe(II) concentrations from total Fe concentrations. The pH, Eh, minimal media composition and measured aqueous concentrations of Fe(II), Fe(III) and Pb were used to calculate equilibrium aqueous activities and saturation indices for the both the control and inoculated dissolution experiments using the Geochemist's Workbench® (GWB, version 7.01).

Slurries from each sample were collected at each sampling period and imaged immediately (i.e., usually within 2 h) using Field Emission-Environmental Scanning Electron Microscopy (FE-ESEM) (SI Section 2.4). An additional set of 1.0 mL slurries were collected for TEM preparation and analysis (SI Section S2.5).

2.2.2 Cell Viability. A 1.0 mL slurry from each sample (i.e., including controls) was collected and analyzed for cell viability using the Promega BacTiter-Glo™ Microbial Cell Viability Assay and reported as relative luciferase units (RLU) (SI Section S2.6).

2.3 RESULTS AND DISCUSSION

2.3.1 Control Experiments. Aqueous Fe(II), Fe(III) and Pb concentrations were below detection limits in all sterile and inoculated samples (i.e., in the absence of Pb-jarosite). In the control samples containing Pb-jarosite, aqueous Fe(III) release was minimal and reached a maximum at 48 h of $18.01 \pm 0.80 \mu\text{M}$ (Figure 2.1A). Due to the poor solubility of Fe(III) ($10^{-9} \mu\text{M}$) under circumneutral conditions, the low Fe concentrations are likely the result of colloidal Fe(III) hydroxide precipitates that passed through filtration ($< 0.2 \mu\text{m}$), and dissolved during acidification for ICP-OES sample preparation.¹⁸ The concentration of S was 56.0 mM due to the PIPES buffer, which dissociates during acidification of ICP-OES samples, and therefore remained constant throughout the experiment in all samples and could not be used to evaluate Pb-jarosite dissolution. Aqueous Pb concentrations in the control samples reached a maximum at $0.70 \pm 0.41 \mu\text{M}$ at 6 h followed by a steady decrease at a rate of $1.3 \times 10^{-3} \pm 1.0 \times 10^{-3} \mu\text{M/h}$ to 198 h (Figure 2.1B). Beyond 198 h, all samples were below the instrumental Pb

detection limit (0.09 μM). Aqueous Fe and Pb concentrations reported by Smith et al. (2006) for the abiotic dissolution of Pb-jarosite under comparable conditions were mass normalized and showed lower concentrations of Fe (3.90 μM) and slightly higher concentrations of Pb (3.3 μM) at 750 h.⁵

The minimal aqueous Fe and Pb concentrations likely resulted from the precipitation of a secondary mineral phase. Secondary electron SEM images of the Pb-jarosite control samples at 408 h (SI Figure S2.2A) showed spherical precipitates on the surface of the Pb-jarosite, similar in morphology to those previously observed and identified as $\text{Fe}(\text{OH})_3$ during Pb-jarosite dissolution⁵. Energy dispersive x-ray (EDX) spectra measured at 20 kV demonstrated a change in relative elemental concentrations from Fe=24.0 wt %, Pb =13.8 wt %, S=13.4 wt %, O=48.7 wt % on the surface of the Pb-jarosite to Fe=46.1 wt %, Pb= 28.2 wt %, S=11.7 wt %, O=14.1 wt % on the thickest part of the precipitate, thereby suggesting possible Fe and Pb remineralization (SI Figure S2.3). React® (GWB 7.01) calculated saturation indices ($\log Q/K$) at 0 and 48 hours based on experimental data (SI Table S2.1) and predicted the precipitation of hematite, goethite and $\text{Fe}(\text{OH})_3$ (SI Table S2.2). Hematite was discounted as a potential precipitate because it is a highly crystalline phase, typically formed over longer durations.¹⁹ The predicted formation of goethite or $\text{Fe}(\text{OH})_3$ is significant because these phases represent important sinks for Pb sorption in the environment and may account for the enrichment of Pb observed in the EDX measurements.

2.3.2 Microbial reduction of Pb-jarosite. Aqueous Fe(II) and Fe(III)

concentrations were monitored over time to evaluate the susceptibility of Pb-jarosite to microbial Fe reduction. A maximum Fe(III) concentration of $93.0 \pm 7.94 \mu\text{M}$ at a rate of

$12.1 \pm 1.28 \mu\text{M/h}$ was reached at 48 h followed by a gradual decrease to $12.4 \pm 1.89 \mu\text{M}$ at 776 h and reflected Fe(III) colloid precipitation (Figure 2.1A). The later decrease in Fe(III) was likely due to aggregation of the precipitates, which did not pass through filtration. The reduction of Fe(III) within these samples was first observed during the second sampling interval (6 h) with a release of $16.4 \mu\text{M Fe(II)}$, which continued to increase to $197.1 \pm 2.92 \mu\text{M}$ at a rate of $3.9 \times 10^{-1} \mu\text{M/h}$ until the termination of the experiment (768 h) (Figure 2.1A). The first observation of Fe(III) reduction also corresponded to a maximum increase of aqueous Pb concentrations from $0.57 \pm 0.34 \mu\text{M}$ at time 0 to $1.35 \pm 0.26 \mu\text{M}$ at 6 h, likely due to structural Pb release from the Pb-jarosite during dissimilatory Fe reduction (Figure 2.1B). The increase in Pb concentrations was followed by a sharp decrease to $0.097 \mu\text{M}$ at a slow release rate of $6.0 \times 10^{-4} \pm 7.0 \times 10^{-4} \mu\text{M/h}$ to 198 h at which point aqueous Pb concentrations were below detection limits (Figure 2.1B). The rate of Fe reduction increased substantially between 192 and 400 h when aqueous Fe(II) concentrations increased from $37.5 \pm 2.35 \mu\text{M}$ at 198 h to $98.9 \pm 23.4 \mu\text{M}$ at 400 h (Figure 2.1A). Fe reduction in the inoculated Pb-jarosite samples also coincided with a decrease in redox potential from $+84.46 \text{ mV}$ at 192 h to -84.26 mV at 408 h which was not observed in the inoculated minimal media control samples in the absence of Pb-jarosite (SI Figure S2.4).

Secondary electron images of secondary precipitates observed on the surface of the Pb-jarosite at 408 hours showed spherical precipitates similar to those seen in the control samples (SI Figure S2.2 B). Geochemical modelling was divided into 2 parts (i.e., 0-48 h and 48-768 h) to reflect the changes in Fe(II) and Fe(III) concentrations over time. Based on reaction path modelling, React® (GWB 7.01) calculated saturation

indices (log Q/K) over time using the minimal media composition and experimental data collected at 0, 48 and 768 hours (SI Table S2.3) and predicted the initial precipitation of hematite > goethite > magnetite > carbonate green rust (SI Table S4). However, as the reaction progressed and Fe(III) decreased and Fe(II) increased, React® predicted the precipitation of carbonate green rust > sulfate green rust > chloride green rust > hematite > magnetite (SI Table S4). Green rusts are mixed Fe(II)/Fe(III) hydroxides that typically form under weakly acidic and alkaline suboxic environments and were formed in previous studies by *S. putrefaciens* CN32 during the dissimilatory Fe reduction of ferrihydrite and lepidocrocite.^{20,21} The secondary precipitates formed in this study do not exhibit the characteristic hexagonal platelet morphology typical of green rusts. Moreover, Fredrickson et al. (1998) demonstrated magnetite formation over the formation of green rusts in the absence of P by *S. putrefaciens* CN32 during dissimilatory Fe reduction.¹³ Therefore, it is unlikely that carbonate green rust formed during these experiments and it is more likely that magnetite or an Fe hydroxide such as goethite was formed.

The absence of P in the minimal media and the presence of aqueous Pb may have played an important role in the low concentrations of aqueous Fe(II) observed throughout the experiment as both have previously demonstrated inhibitory effects on Fe reduction in Fe hydroxides by *S. putrefaciens*.^{22,23} While dissimilatory iron reduction of various synthetic Fe hydroxides has been studied extensively, few studies have examined the dissimilatory Fe reduction of jarosite minerals. Recently, Weisener et al. (2008) demonstrated Fe reduction during silver jarosite, $(\text{AgFe}_3(\text{SO}_4)_2(\text{OH})_6)$ dissolution in the presence of *S. putrefaciens* CN32 under comparable conditions and showed a

maximum aqueous Fe(II) concentration of 167.0 μM at 168 h.²⁴ Similarly, Jones et al. (2006) also demonstrated Fe reduction from jarosite ($\text{KFe}_3(\text{SO}_4)_2(\text{OH})_6$) using *Geobacter metallireducens* GS-15 and *Geobacter sp.* ENN 1.²⁵

To evaluate the potential effects of the Pb released during Pb-jarosite dissolution on *S. putrefaciens*, cell viability was monitored in inoculated samples in both the presence and absence of Pb-jarosite and reported as luminescence (RLU). Initially, luminosity was higher in the inoculated Pb-jarosite samples ($1.49 \times 10^6 \pm 7.81 \times 10^4$ RLU) compared to the inoculated minimal media samples ($8.81 \times 10^5 \pm 1.62 \times 10^4$ RLU) after 48 h, thus suggesting greater cell viability at the beginning of the experiment in samples containing Pb-jarosite (SI Figure S 2.5). The greater cell viability initially observed in the inoculated samples containing Pb-jarosite corresponded with increased Fe(III) release and subsequent Fe(II) production and was likely due to increased metabolic activity due to dissimilatory Fe reduction (Figure 2.1A). However at 72 h, cell viability in the inoculated samples containing Pb-jarosite ($7.91 \times 10^6 \pm 2.23 \times 10^5$ RLU) decreased while cell viability in the inoculated samples without Pb-jarosite ($6.92 \times 10^6 \pm 2.63 \times 10^4$ RLU) demonstrated larger luminescence (i.e., cell viability) and continued to increase until 198 h (SI Figure S 2.5). The decrease in cell viability observed earlier in the inoculated samples containing Pb-jarosite was likely due to the presence of aqueous Pb released during Pb-jarosite dissolution (Figure 2.1).^{22,23}

2.3.3 Fate of Pb. Back scattered electron (BSE)-SEM images taken within 2 h of sampling demonstrated progressive nanoparticle formation over time indicated by high contrast spots associated with the cell surface (Figure 2.2B, D, F) not observed in the

control experiments (Figure 2.2 A, C, E). The diameter of the electron dense particles associated with the cell surface was measured using the Scandium SEM Image Platform and the mean diameter of each particle was 113.20 nm (n=13, SD= 29.59 nm). While BSE-SEM images suggest extracellular precipitation, TEM images collected on a cross section of an individual cell at 406 h (Figure 2.3A) demonstrated intracellular accumulation of electron dense granules within the cytoplasm which were observed in many of the cells imaged (SI Figure S2.6A). The appearance of extracellular precipitation of the electron dense nanoparticles during BSE-SEM not seen in the TEM images is likely the result of cellular dehydration under low vacuum (80 Pa). The progressive increase in electron dense nanoparticles over time associated with the cells (Figure 2.2A, C, E) coincided with decreased aqueous Pb concentrations, thus suggesting the precipitation of an Pb enriched phase (Figure 2.1B). In addition to typical cellular components such as C and S, TEM-EDS analyses of the electron dense granules (i.e. spots 1 to 3) showed enrichment of Pb (90.38 - 92.74 wt%) and P (1.76 - 2.26 wt%) (SI Table S5). EDS analyses also showed residual Ca (0.46-0.56 wt%) not seen in any other cellular components. The cytoplasm (i.e., spot 4) in the absence of electron dense particles was primarily composed of C (95.58 wt%) and showed residual Pb (0.98 wt%) thus demonstrating transport of Pb into the cytoplasm likely followed by precipitation of the electron dense Pb rich granules. EDS analysis of the cell wall (i.e. spot 5) showed Pb enrichment (5.71 wt%) and is likely due to the formation of adsorption complexes with carboxyl and phosphoryl functional groups commonly reported at neutral pH values.^{26,27} In addition to Pb, the cell wall (i.e. spot 5) was also enriched in C (89.38 wt%) and Fe (11.67 wt%) with lower concentrations of O (0.95 wt%) and P (2.85 wt%). The

enrichment of Fe in the cell wall is likely the result of dissimilatory Fe reduction occurring at the outer membrane by metal-reducing proteins called cytochromes, while the P signal is contributed by typical cellular constituents such as DNA, RNA and phospholipids.²⁸ Therefore, the immediate precipitation of Pb enriched nanoparticles associated with *S. putrefaciens* CN32 at the start of the experiment (Figure 2.2B) coupled with the presence of Pb in the cell wall and cytoplasm suggests a simultaneous mechanism involving rapid Pb sorption onto the cell wall and intracellular uptake and precipitation.^{27,29,30}

While *Shewanella putrefaciens* CN32 has previously demonstrated intracellular precipitation of mixed-valence iron and manganese oxides, this is the first report of intracellular Pb precipitation.^{11,31,32} Several bacterial species such as *Citrobacter sp.* and *Staphylococcus aureus* have demonstrated intracellular Pb precipitation, however, due to the analytical limitations imposed by the small size of the Pb inclusions (< 100 nm), detailed analyses of intracellular Pb precipitates are rare.^{30,33,34} Aside from one reported case of PbS intracellular precipitation by *Klebsiella sp.*, the majority of studies show enrichment of Pb and P within the intracellular granules and are identified as either amorphous Pb-polyphosphate or crystalline Pb phosphate.^{29,30,33,33,35,36}

Polyphosphates (poly P) are linear orthophosphate polymers that serve as energy and phosphorus reservoirs in many microorganisms and are implicated to play a key role in the detoxification of metals such as Cd, Cu, Pb and U.³⁷⁻³⁹ While potassium polyphosphate formation was recently suggested in *Shewanella putrefaciens* CN32, to the best of our knowledge it has not been confirmed microscopically.^{11,40} In contrast to other studies demonstrating Pb-polyphosphate formation, HR-TEM images of the

precipitates in our study reveal a crystalline structure thereby suggesting inorganic lead phosphate formation rather than amorphous polyphosphate formation (Figure 2.3B and SI Figure S 2.6). Moreover, the relative concentration of P is lower than the amount of Pb thus conflicting with similar analyses of bacterial polyphosphate inclusions where P is high (SI Table S5).⁴¹ Finally, while previous studies illustrate polyphosphate degradation under anaerobic conditions, our study demonstrates intracellular Pb phosphate formation under anaerobic conditions thereby eliminating the possibility for polyphosphate formation in our bacteria.³⁹

A potential explanation for Pb phosphate precipitation by *Shewanella putrefaciens* CN32 may be attributed to heavy metal translocating P_{IB}-type ATPases (COG2217P), a superfamily of enzymes responsible for metal ion efflux and resistance in cells. P_{IB}-type ATPases bind divalent metals such as Pb, Cd and Zn to the N-terminal amino acid domain of the enzyme and also catalyze ATP hydrolysis. The energy produced during ATP hydrolysis is used to actively transport the metal complex out of the cytoplasm. Contained within the genome of *Shewanella putrefaciens* CN32 is ZntA (Sputcn32_1954), a metal-translocating ATPase, previously shown to expel Pb(II), Cd(II) and Zn(II) from cells of *Escherichia coli*. Also found within the genome of *Shewanella putrefaciens* CN32 is ZntR (Sputcn32_3400), a member of the MerR family of transcriptional regulators (COG0789K) encoded to activate *zntA* expression in the presence of Pb, Cd and Zn(II).⁴² Previous results showed that soft metal complexes stimulated the ATPase activity of purified ZntA in the order Pb(II) > Cd(II) ~ Zn(II) ~ Hg(II) thus suggesting that ZntA was a Pb(II)-dependent ATPase evolved specifically to mediate resistance to toxic concentrations of environmental Pb.⁴² It may be expected

that in the presence of high Pb concentrations within the cell, ZntR will induce ZntA synthesis and Pb will be transported out of the cell. However, in this study the Pb remained within the cytoplasm which is likely due to complexation with the P released from ATP hydrolysis followed by rapid precipitation of a highly insoluble Pb phosphate phase ($K_{sp}=10^{-54}$).⁴³

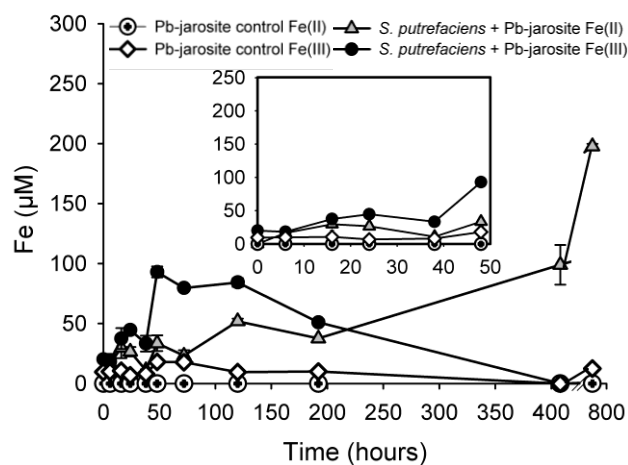
The absence of P in the minimal media may also have played an important role in the intracellular Pb mineralization observed in *S. putrefaciens* CN32. A previous study using *Pseudomonas fluorescens* examined Pb accumulation under P limited and rich conditions and demonstrated intracellular Pb accumulation in P limited media and extracellular Pb accumulation in P rich media.⁴⁴ Due to the insolubility of Pb phosphate and the potential for P surface complexation onto the Pb-jarosite, P was not added to the minimal media. However, it may be possible that due to the reduced ATP production as a result of Pb toxicity and P starvation, the energy released during ATP hydrolysis may have been insufficient to expel the Pb out of the cytoplasm. P is of particular interest because it is often limiting to microbial growth in natural aqueous environments. However, due to the absence of reaction vessels containing P, the interplay between cellular P nutrition, Fe reduction and Pb detoxification by *S. putrefaciens* CN32 remains to be delineated.

Interestingly, the anaerobic dissolution of Ag-jarosite by the same stock culture of *S. putrefaciens* CN32, demonstrated a different mechanism for silver detoxification.⁴⁵ During Ag-jarosite dissolution, reduced Ag nanoparticles precipitated along the inner plasma membrane of the cell rather than inside the cytoplasm as was illustrated in this

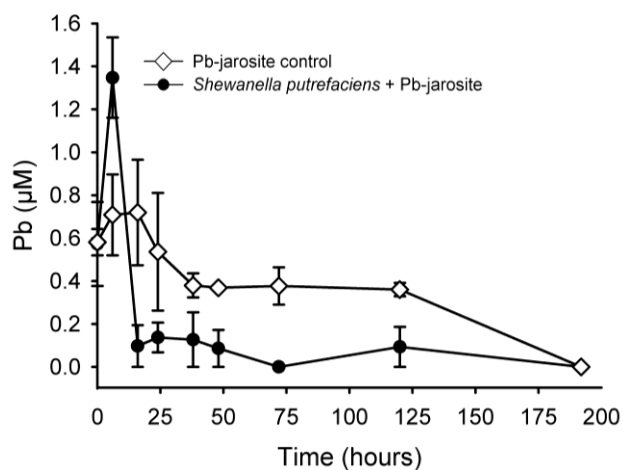
study. A potential explanation for this difference may be in the genome as the *Shewanella putrefaciens* CN32 genome also encodes for a putative silver efflux pump (Sputcn32_0149), a member of the CzcA family of heavy metal efflux proteins. Gene expression was not measured in this study, therefore the role of P-type ATPase expression or other metal efflux pumps responsible for Pb and Ag detoxification by *Shewanella putrefaciens* CN32 remains to be elucidated.

The results of this study demonstrate enhanced dissolution of Pb-jarosite by *S. putrefaciens* CN32 through dissimilatory Fe reduction under anaerobic circumneutral conditions. The dissolution of the control and inoculated Pb-jarosite samples both showed minimal Pb release and immobilization either onto the secondary precipitate or within the bacteria. This study demonstrates the first case of intracellular precipitation of an enriched Pb and P mineral phase by *Shewanella putrefaciens* CN 32. Intracellular Pb-polyphosphate formation was recently observed for the first time in nature in unidentifiable bacteria collected from a vineyard and forest site in France.³⁰ Therefore, this newly reported Pb biomineralization pathway in *S. putrefaciens* CN32 may represent a significant sink for Pb in the environment and should be considered when evaluating future remediation options in Pb contaminated sites. In the absence of samples supplemented with P, the effect of P limitation on the uptake and intracellular accumulation of Pb remains to be delineated and should be the focus of future research. Lastly, while *S. putrefaciens* CN32 is a well-characterized and commonly used bacterium to model dissimilatory Fe reduction, future studies should assess the reductive dissolution of Pb-jarosite using other environmentally relevant bacterial strains. It is expected that experiments using a suite of other environmentally relevant bacteria such

as *Geobacter sp.* would provide a more comprehensive and accurate assessment of the potential for Pb release from Pb-jarosite into the environment.



A



B

Figure 2.1. (A) Aqueous Fe(II) and Fe(III) release in the control and inoculated Pb-jarosite samples as a function of time; (B) Aqueous Pb release in the control and inoculated Pb-jarosite samples as a function of time. Error bars represent standard error (n=3).

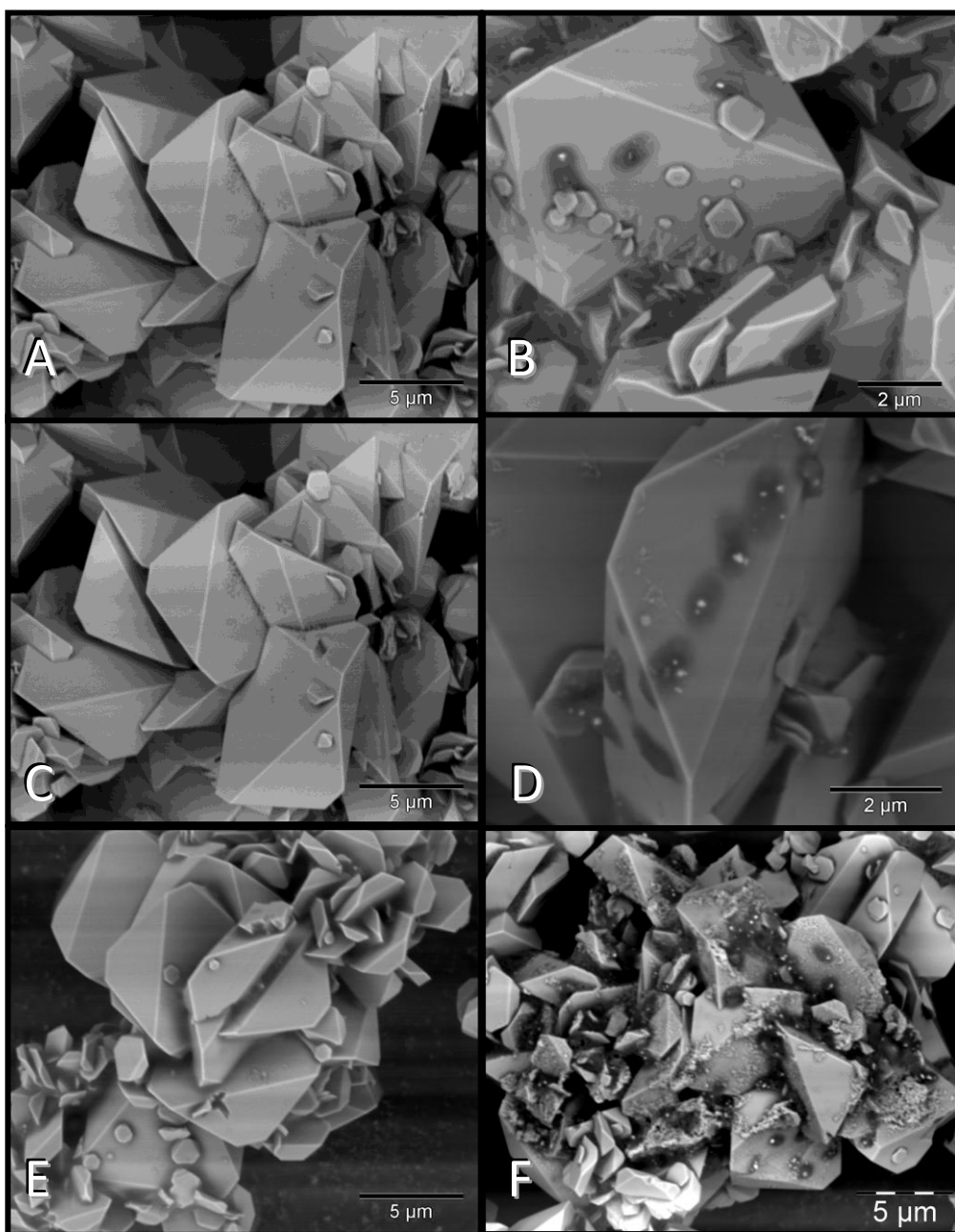


Figure 2.2. BSE-SEM images of control Pb-jarosite (left) and inoculated Pb-jarosite (right) as a function of time; (A) and (B) = 0 h, (C) and (D) = 16 h, (E) and (F) = 768 h.

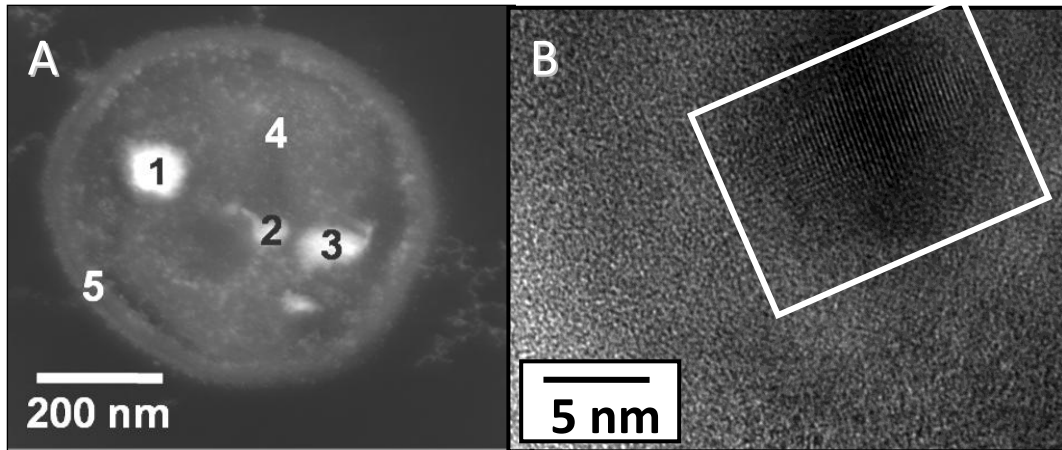


Figure 2.3. A) Darkfield TEM images of a cross section of *Shewanella putrefaciens*. Numbers 1-5 denote areas of EDS analysis. B) HR-TEM image of an electron dense intracellular precipitate. Square denotes area of crystallinity.

2.4 SUPPORTING INFORMATION

S2.1. Synthesis of Pb-jarosite

Pb-jarosite was synthesized using a 0.50 L solution containing 40.98 g FeCl₃, 84.65 g LiCl, 16.83 g Li₂SO₄ and 10.89 g of PbCl₂. The solution was heated to 90 °C in an Erlenmeyer flask and upon dissolution of all reagents the pH was adjusted to 0.840 using a saturated Li₂CO₃ solution. The resulting solution was transferred to a 1.0 L round bottom flask connected to a condenser to minimize evaporation. The apparatus was submersed in a closed thermostatic water bath maintained at 98 ± 0.5 °C for 72 h after which a yellow precipitate was formed. The resulting product was vacuum filtered and washed with 250 mL of hot 4.0 M NaCl solution (98 °C) to prevent PbSO₄ and/or PbCl₂ precipitation caused by dilution. Following the hot NaCl wash, 4000 mL of ultrapure Milli-Q water (18 MΩ cm⁻¹, 98 °C) was applied in 100 mL aliquots to eliminate any dissolved salts and the Pb-jarosite was dried at 110 °C for 24 h. The material was confirmed as synthetic Pb-jarosite using powder X-ray diffraction (XRD) analysis at 25 °C with a D8 Discover Powder X-ray Diffractometer utilising Cu Kα radiation at 40 kV and 40 mA (see Figure S1 for corresponding XRD pattern). Precipitates examined using scanning electron microscopy exhibited rhombohedral (pseudo-cubic) morphology common to Pb bearing jarosite precipitates.

S2.2. Bacteria cultivation method

Cultures were prepared from a 5.0 mL frozen glycerol stock maintained at -80 °C, transferred to Trypticase Soy Agar plates and grown aerobically for 24 h. Single colonies were inoculated into 25 mL tubes of sterile Trypticase Soy Broth (TSB) and

incubated aerobically at 32 °C for 18 h. The 25 mL seed cultures were used to inoculate 100 mL volumes of sterile Luria-Bertrani (LB) media and were incubated aerobically at 32 °C until the culture reached late log phase (18 h). Bacteria were harvested by centrifugation at 3000 g and washed twice using sterile 0.01M NaNO₃, decanting the supernatant at each step. The wet biomass was washed once using 100 mL of aerobic minimal media containing: 1.34 mM KCl, 28 mM, NH₄Cl, 0.68 mM CaCl₂, 50 mM, NaClO₄, 24 mM Na-lactate (60% syrup), 20 mM PIPES (1,4-piperazine diethanesulfonic acid) buffer and adjusted to pH 7.3 using 0.01 M NaOH. The minimal media was decanted and the final wet mass of the bacteria was 1.03 g. Bacteria were transferred to 1.00 L of degassed minimal media. All samples containing bacteria originated from the same inoculated minimal media solution and contained approximately 0.016 g of wet bacteria/test tube at the beginning of the experiment. All experimental media were prepared from reagent grade materials and were either filter sterilized (0.2 µm) or autoclaved. The minimal media used was chosen because it supports growth and metabolism for *S. putrefaciens* CN32.

S2.3. Aqueous Sampling Procedures and Analysis

Semi-micro electrodes sterilized with ethanol were used to measure the pH (Thermo Ross Sure-flow semi micro pH probe) and Eh (Thermo Ross Sure Flow combo redox/ORP) *in situ* before each sampling. Using acid washed sterile pipette tips, 4.0 mL of each sample was filtered through a 0.20 µm nylon syringe filter. A portion of the filtrate (3.0 mL) was diluted and acidified with sub-boiling distilled 0.016 N HNO₃ made from 8.0 N stock HNO₃ and stored at 4 °C until analysis. Aqueous concentrations

of Pb, Fe and S of these samples were determined using inductively-coupled plasma optical emission spectroscopy (ICP-OES). Duplicate volumes of the remaining filtrate (100 μ L) were used to immediately determine Fe(II) and total Fe concentrations via the ferrozine method using a GENESYS 20 UV-VIS Spectrophotometer at 562 nm. Fe speciation was reported as Fe(II) and Fe(III).

S2.4. SEM sample preparation and analysis procedures:

A subset of 1.0 mL slurries from each sample were collected during each sampling period and were sealed with parafilm to prevent oxygen contamination. To avoid sample preservation and storage artefacts, samples were imaged immediately following the entire sampling procedure (i.e. usually within 2 h) using Field Emission-Environmental Scanning Electron Microscopy (FE-ESEM) (FEI Quanta 200F). Samples were examined under low vacuum ($\sim$ 80 Pa) at 2, 5 and 10 kV. The abundance and spatial distribution of bacteria with respect to the Pb-jarosite was examined using a backscattered electron (BSE) detector and enhanced surface detail was investigated using a secondary electron (SE) detector. Energy dispersive x-ray spectroscopy (EDX) spectra were collected to examine changes in elemental composition of the Pb-jarosite and any secondary precipitates formed over time.

S2.5. TEM Sample Collection, Preparation and Analysis

An additional set of 1.0 mL slurries from each sample were collected in 1.5 mL microcentrifuge tubes during each sampling period, treated with 2.5% glutaraldehyde and stored at 4 °C. At the termination of the experiment, samples were sent to McMaster University, Hamilton, ON, Canada, for TEM preparation and analysis. The primary

fixative used was 0.2M glutaraldehyde (2% v/v) in 0.1M phosphate buffer pH 6.8. The samples were rinsed 2X in the buffer solution, then post-fixed in 1% osmium tetroxide in 0.1M phosphate buffer for 1 hour. Samples were dehydrated through a graded ethanol series (50%, 70%, 70%, 95%, 95%, 100%, 100%) and final dehydration was done in 100% propylene oxide (PO). Samples were infiltrated with Spurr's resin in a series (2:1 PO:Spurr's, 1:1 PO:Spurr's, 1:2 PO:Spurr's, 100% Spurr's, 100% Spurr's, 100% Spurr's) with rotation of the samples in between solution changes. The samples were transferred to embedding moulds and were filled with fresh 100% Spurr's resin and polymerized overnight at 60°C. Thin sections were cut on a Leica UCT Ultramicrotome and placed onto both uncoated grids and some Formvar-coated grids. Samples were lightly C coated (10-15 Å). Specimens were examined using field emission-transmission electron microscopy (FE-TEM) (JEOL 2010F). Bright field (BF) images and high angle annular dark field (HAADF) scanning transmission electron microscopy (STEM) images were collected at 200 kV. Energy dispersive x-ray spectra (EDX) were collected in STEM mode with a probe size of 1 nm or less.

S2.6. Cell Viability Analysis

Prior to analysis, all BacTiter-Glo™ reagents were stored at -80°C to prevent degradation and were thawed immediately before use. To prepare the BacTiter-Glo™ reagent, lyophilized BacTiter-Glo™ substrate was reconstituted using the BacTiter-Glo™ buffer and equilibrated at room temperature for 10 minutes. Samples were removed from the refrigerator and 150 µL of the sample slurry was transferred to a sterile 1.5 mL microcentrifuge tube. An equal volume (150 µL) of the BacTiter-Glo™

reagent was added to the sample and shaken for 5 minutes. Luminescence was measured using a GloMax[®] 20/20 Single Tube Luminometer (λ 350-650 nm) at an integration time of 2 seconds and the output was reported in relative luciferase units (RLU). Control samples were also analyzed to determine the intensity of the luminescent signal produced by the minimal media and Pb-jarosite in the absence of bacteria. To maintain identical analytical conditions, all samples were analyzed in groups of five and all samples were analyzed within a span of 80 minutes.

	0 hours		48 hours	
	Concentration (μM)	log activity *	Concentration (μM)	log activity*
Fe³⁺	9.64	-6.1840	18.01	-5.9126
Pb	0.58	-9.5723	0.369	-10.2071
Eh (mV)	210	----	300	---

Table S2.1. Measured chemical parameters used in React (GWB 7.01) for the control experiments at Time 0 and 48 hours.

Mineral	0 hours log Q/K	48hours log Q/K
Hematite	14.2612	17.7137
Goethite	6.6253	8.3508
Fe(OH) ₃	1.9864	3.2249

Table S2.2. Predicted saturation indices (log Q/K) of hematite, goethite and Fe(OH)₃ during the abiotic dissolution of Pb-jarosite at 0 and 48 hours.

	0 hours		48 hours		768 hours	
	Conc. (μM)	log activity	Conc. (μM)	log activity	Conc. (μM)	log activity
Fe²⁺	0	n/a	33.60	-5.2038	197.72	-4.4655
Fe³⁺	20.22	-7.7815	93.02	-7.0902	1.90	-5.9126
Pb	0.573	-7.2278	0.086	-10.0240	0.00	-15.000
Eh (RmV)	90	n/a	86	n/a	-18	n/a

Table S2.3. Measured chemical parameters used in React (GWB 7.01) for the biotic experiments at 0, 48 and 768 hours.

Predicted mineral phase	0 hours log <i>K</i>	48 hours log <i>K</i>	768 hours log <i>K</i>
Hematite	14.9	16.23	13.80
Goethite	6.94	7.61	6.39
Magnetite	7.45	15.30	13.68
Fe(II) ₃ Fe(III)(OH) ₈ Cl, GR1 (Cl ⁻)	-1.90	18.32	19.53
Fe(II) ₄ Fe(III) ₂ (OH) ₁₂ CO ₃ , GR1(CO ₃ ²⁻)	5.59	33.00	33.80
Fe(II) ₄ Fe(III) ₂ (OH) ₁₂ SO ₄ , GR1(SO ₄ ²⁻)	-1.07	26.33	27.14

Table S2.4. Predicted saturation indices (log Q/K) during the microbial dissolution of Pb-jarosite at 0, 48 and 768 hours.

Element	Locations of spectra collected during TEM-EDX analysis. Relative elemental concentrations reported in wt %.				
	1	2	3	4	5
C K	1.77	4.53	4.83	95.58	89.38
O K	1.45	1.34	1.15	5.94	0.95
P K	2.26	1.77	1.76	1.18	2.85
S K	0.14	0.21	0.06	0.76	0.86
Fe K	1.22	1.64	1.82	0.88	11.67
Ca K	0.56	0.41	0.46	n/a	n/a
Pb L	92.74	90.94	90.38	0.98	5.71

Table S2.5. Relative elemental concentrations (wt %) corresponding to locations denoted as 1-5 in Figure 2.3A.

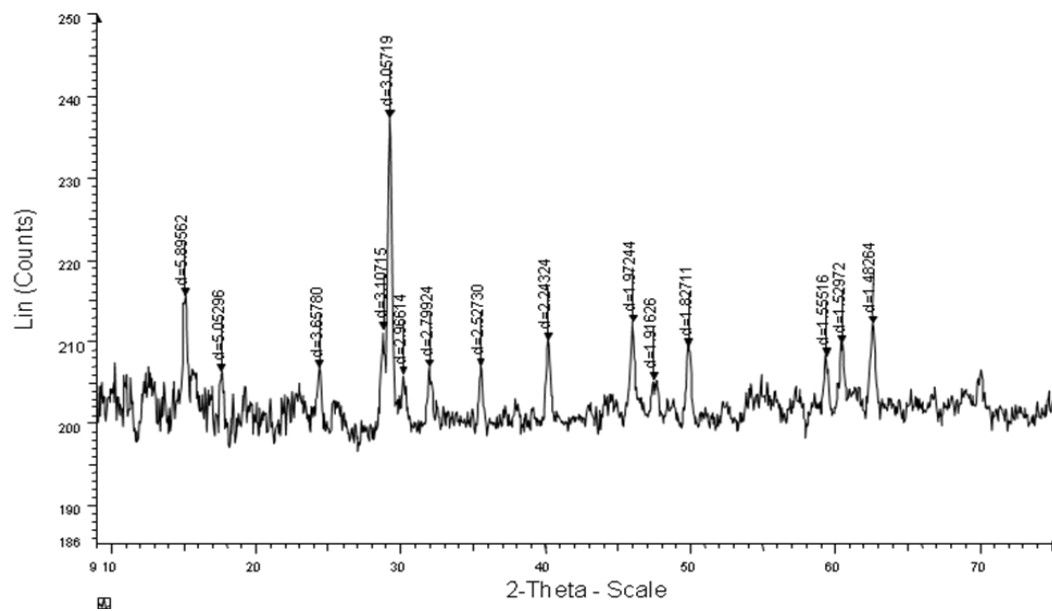


Figure S2.1. XRD pattern for synthesized Pb-jarosite. The reference D-spacings for synthetic Pb-jarosite (ICCD PDF 37-0759) are indicated on the graph.

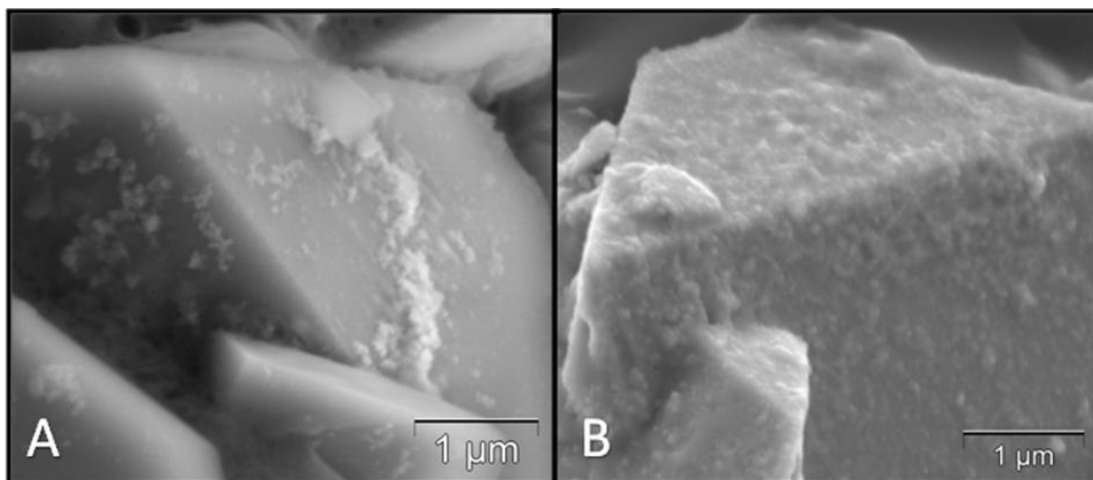


Figure S2.2. BSE image of secondary mineral precipitates associated with A) control Pb-jarosite and B) inoculated Pb-jarosite at 408 hours.

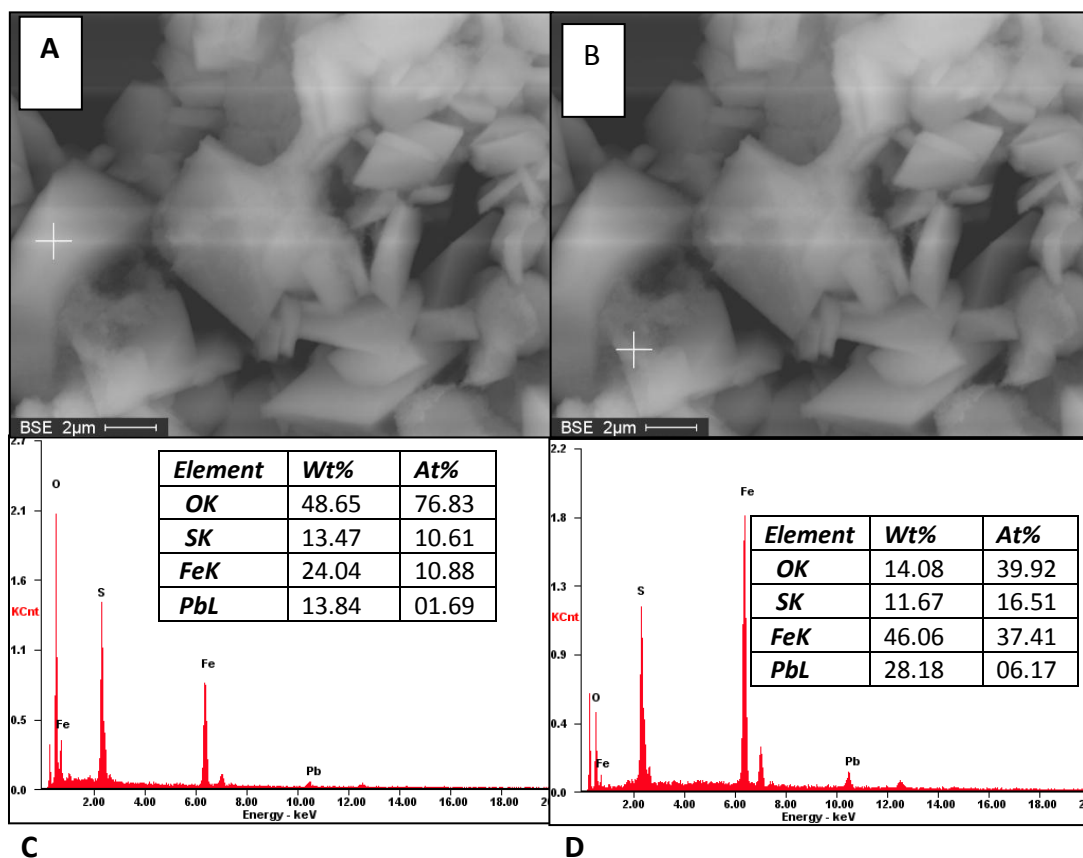


Figure S2.3. BSE-SEM image of inoculated Pb-jarosite at 408 h taken at 25 kV; (A) Cross hair denotes location of EDX analysis and is representative of the surface of the Pb jarosite (i.e. in the absence of secondary precipitate); (B) Cross hair denotes location of EDX analysis and was collected on the thickest part of the secondary precipitate; (C) EDX spectra of chosen area (cross hair) in A, inlaid table is the relative wt% and at% elemental concentrations of O, S, Fe and Pb. (D) EDX spectra of chosen area (cross hair) in B, inlaid table is the relative wt% and at% elemental concentrations of O, S, Fe and Pb.

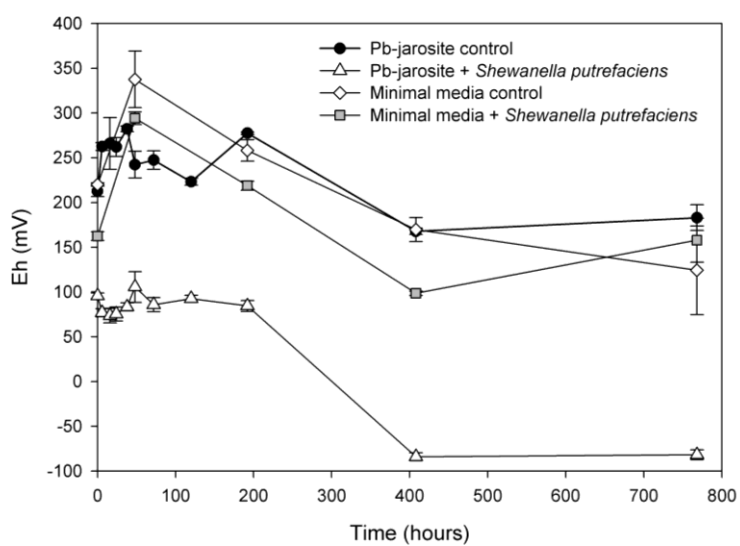


Figure S2.4. Redox potential (Eh) as a function of time. Error bars represent standard error. n = 3 for samples containing Pb-jarosite. n = 2 for control and inoculated media sample

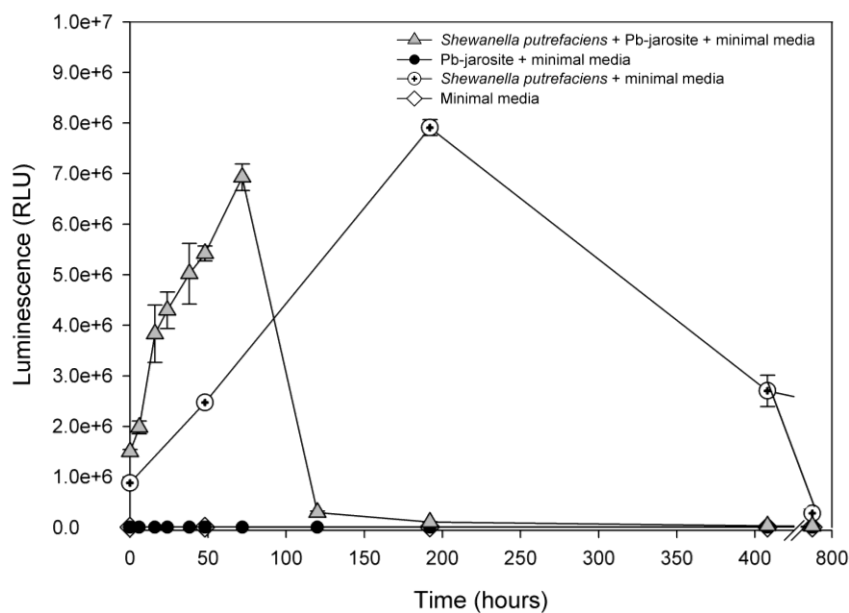


Figure S2.5. Luminescence (RLU) as a function of time. Luminescence is used as a proxy for cell viability (see S4 in Supplemental Information for more details). Error bars represent standard error. $n = 3$ for samples containing Pb-jarosite. $n = 2$ for control and inoculated media samples.

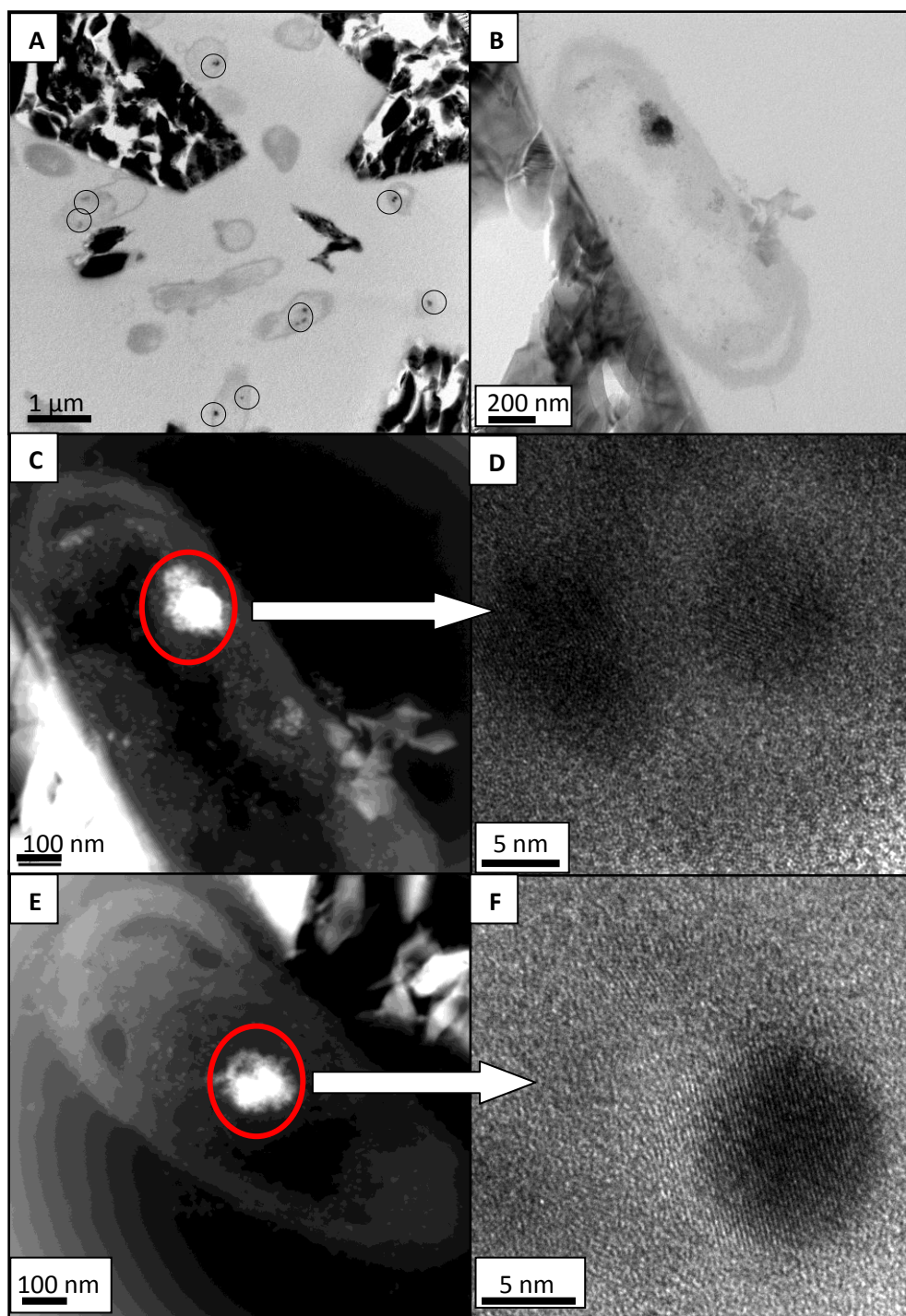


Figure S2.6. A) TEM image of *S. putrefaciens* CN32 associated with Pb-jarosite at 48 hours. Black circles highlight intracellular Pb precipitates; B) TEM image of *S. putrefaciens* associated with the surface of Pb-jarosite; C) Corresponding STEM image of image B; D) HR-TEM image of the electron dense crystalline intracellular precipitate denoted with a circle in image C; E) STEM image of *S. putrefaciens* associated with the surface of Pb-jarosite; F) HR-TEM image of the electron dense intracellular precipitate denoted with a circle in image E.

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CHAPTER 3

Simultaneous reduction of Fe and As during the reductive dissolution of Pb-As jarosite by *Shewanella putrefaciens* CN32

3.1 INTRODUCTION

Elevated concentrations of arsenic (As) in groundwater aquifers continue to threaten the health of millions of people worldwide in places such as Bangladesh and West Bengal.¹ Redox potential (Eh) and pH are the most important factors controlling As speciation and under circumneutral oxidizing conditions below pH 6.8, diprotonated arsenate H_2AsO_4^- dominates while monoprotated HAsO_4^{2-} becomes dominant above pH 6.8. Under reducing conditions below pH 9.2, the uncharged arsenite species H_3AsO_3 is predominant. Arsenite is more toxic than arsenate and reversibly combines with thiol groups to disrupt cellular process, while arsenate mimics phosphate and enters the cell via transporters meant for PO_4^{3-} and interferes with phosphate based energy-generating processes.² Microorganisms mediate As cycling through redox processes and reduce As(V) to As(III) during either detoxification (*ars* system) (As >100 μM) or respiration (*arr* system) (As >100 nM).^{2,3} As(V) is commonly associated with iron (Fe) minerals in sediments through adsorption onto Fe(III) hydroxide (HFO) surfaces, and the reductive dissolution of these minerals are considered the primary pathway of As solubilization in most surface and subsurface environments.⁴⁻⁶ As(V) is also incorporated into secondary As minerals such as scorodite ($\text{FeAsO}_4 \cdot 2\text{H}_2\text{O}$), jarosite ($\text{KFe}_3(\text{SO}_4)_2(\text{OH})_6$) and Pb-As jarosite (i.e. beaudantite, $\text{PbFe}_3(\text{SO}_4, \text{AsO}_4)_2(\text{OH})_6$) through the oxidation of As sulfide minerals such as arsenopyrite (FeAsS) during metallurgical processing, natural weathering and/or acid mine drainage. The susceptibility of these secondary minerals to microbial reductive dissolution via reduction of Fe(III) from scorodite ($\text{Fe(III)AsO}_4 \cdot 2\text{H}_2\text{O}$) and As(V) from symplectite [$\text{Fe(II)}_3(\text{AsO}_4)_2 \cdot 8\text{H}_2\text{O}$] was previously demonstrated.⁷⁻¹⁰

The structure of As substituted minerals is extensively studied, yet As release during dissolution from jarosite-group minerals is less investigated.¹¹⁻¹⁶ Jarosite-group minerals have the general formula: $AB_3(TO_4)_2(OH)_6$, where the A site is occupied by monovalent or divalent cations (e.g. K or Pb), the B site is occupied by cations with octahedral (O) coordination (e.g. Fe(III) or Al(III)) and the T sites correspond to tetrahedral coordination (T) (e.g. S(IV) and As(V)).^{17,18} Jarosites typically form under oxidizing, ferric and sulfate rich and acidic (pH < 3.5) conditions and at pH > 3.5 will dissolve to form goethite or meta stable phases such as schwertmannite or ferrihydrite.¹⁸⁻²⁰ Jarosite precipitation is also widely utilized in the Zn industry to remove impurities such as Fe, sulfate, alkalis and other heavy metals (e.g. Pb, Tl and As) from processing solutions.¹⁴

Consequently, large volumes of jarosites are produced per year and typically stored in disposal ponds under circumneutral conditions, thereby resulting in dissolution/re-precipitation reactions that may potentially release toxic metals into disposal environments.²¹ However, the relative environmental stability of synthetic jarosites over other potential waste mineral hosts such as apatites and pyrochlores has made jarosite-type precipitates a potential candidate for long term disposal of toxic metals such as As, Pb, Bi, Hg, Tl, Sb, Cr, Se and radioactive isotopes of K, Sr, Th, U and REE.^{22,23} Therefore, determining the biogeochemical stability of jarosites is important to evaluate the potential mobility of metals such as As and Pb from disposal sites. To date, research has demonstrated microbial Fe and S reduction in various jarosites including K-jarosite, Pb-jarosite and Ag-jarosite, yet the biogeochemical stability of jarosites as it relates to As reduction remains to be elucidated.²⁴⁻²⁶

Theoretically, Fe(III) and As(V) reduction are expected to occur at similar redox potentials but the reduction sequence may vary. For example, incubations of Bengal Delta sediments with native microbial consortia showed Fe(III) reduction prior to As(V) reduction.⁴ In contrast, incubations of As(V) adsorbed onto the surface of hydrous ferric oxides with a native microbial consortia or *Shewanella* sp. ANA-3 demonstrated As(V) reduction simultaneously or prior to Fe(III) reduction.²⁷ While kinetic factors may drive the reduction of As(V) and Fe(III), ultimately thermodynamic favorability will control whether the reaction will proceed through respiration (*arr* system).²⁸

This study addresses the relative order and extent of microbial As(V) and Fe(III) reduction in a synthetic Pb-As jarosite by *Shewanella putrefaciens* CN32 using batch experiments under circumneutral anaerobic conditions. *S. putrefaciens* was used because it is a well characterized dissimilatory metal reducing bacteria capable of both As(V) and Fe(III) reduction.^{5,28-32} Additionally, we examine the fate of As and Pb released during both the abiotic and reductive dissolution of Pb-As jarosite.

3.2 MATERIALS AND METHODS

3.2.1 Preparation of Pb-As-jarosite/Cell Suspensions and Sampling Protocol.

Smith et al.(2006) previously reported the synthesis, characterization and abiotic dissolution of the Pb-As jarosite sample

$(\text{H}_3\text{O})_{0.68}\text{Pb}_{0.32}\text{Fe}_{2.86}(\text{SO}_4)_{1.69}(\text{AsO}_4)_{0.31}(\text{OH})_{5.59}(\text{H}_2\text{O})_{0.41}$ used in this study.¹² Pure cultures of *Shewanella putrefaciens* CN 32 cultures (ATC# BAA-453) were grown, harvested, and inoculated into a modified M1 minimal media (see Supporting

Information (SI) Section 3.1 for more details). All minimal media were transferred to an anaerobic chamber (95% N₂/5% H₂) and 15 mL of the cell suspension was dispensed into 20 mL polypropylene test tubes containing 0.0498 ± 0.0013 g of Pb-As jarosite (21 samples). Experimental controls contained 15 mL of sterile minimal media and 0.0498 ± 0.0011 g in 20 mL polypropylene tubes (21 samples). An additional set of control samples containing 15 mL of the same minimal media/cell suspension in polypropylene tubes were prepared to examine background pH, Eh, cell viability and elemental concentrations over time (21 samples). All samples were capped, sealed with parafilm, covered with aluminum (Al) foil and rotated end-over-end at 20 rpm in the anaerobic chamber using a bench top rotator (Glas Col) at 27°C and sampled in triplicate at 0, 12, 24, 48, 72, 168 and 336 hours.

At each sampling period, pH (Thermo Ross Sure-flow semi micro pH probe) and Eh (Thermo Ross Sure Flow combo redox/ORP) was measured in each sample. *S. putrefaciens* metabolism was monitored immediately using the Promega BacTiter-Glo™ Microbial Cell Viability Assay, an ATP-based luminescent technique (SI Section 2). Starting ATP concentrations were converted to cell counts using an average ATP-per-biovolume concentration of 2.95×10^{-9} nmol/cell ATP and the starting cell concentration were 1.3×10^8 cells/mL.³³

3.2.2 Solid phase characterization. Sample slurries were collected and imaged under low vacuum at a low accelerating voltage (5-10 kV) using field emission-environmental scanning electron microscopy (FE-ESEM, FEI-Quanta 200F). Additional sample slurries (1.0 mL) were collected and preserved in 2.5% glutaraldehyde and stored at 4°C for transmission electron microscopy (TEM) preparation and analysis (FEI

Titan 80-300) (SI Section S 3.3). Solid phase samples for X-ray absorption spectroscopy (XAS) and X-ray diffraction (XRD) analysis were collected via filtration onto nylon 0.45 μm filter paper (Whatman®, 25 mm) using reusable polypropylene syringe filter holders (Millipore Swinnex®, 25 mm). Samples and reference standards for XAS were sealed between 2 layers of Kapton tape and stored in an anaerobic chamber to minimize oxidation of As and Fe and analysis was carried out at the Pacific Northwest Consortium X-ray Science Division (PNC/XSD) facility bending magnet beamline (20-BM) at the Advanced Photon Source (SI Section S3.4). Data reduction and analysis was performed using the Athena XAS data analysis program.³⁴ The speciation and bonding environments of Fe and As in the unreacted Pb-As-jarosite sample were also determined by EXAFS analysis carried out at the CLRC Synchrotron Radiation Source at Daresbury Laboratory, UK (SI Section S3.4).

3.2.3 Aqueous phase analysis. Samples were filtered through a 0.2 μm nylon syringe filter and subsamples (100 μL) were analyzed immediately for Fe(II) and total Fe concentrations via the Ferrozine method.^{35,36} A 1.0 mL aliquot of the filtrate was acidified immediately to a final concentration of 1% HCl and stored at $-20\text{ }^{\circ}\text{C}$ for As(III) and As(V) speciation determination using a modified molybdenum method (SI Section S3.5).³⁷⁻³⁹ The remaining filtrate was diluted, acidified and aqueous elemental concentrations were determined using inductively-coupled plasma optical emission spectroscopy (ICP-OES).

3.2.4 Thermodynamic Modeling. Eh, pH and measured aqueous concentrations of Fe(II), Fe(III), Pb(II) and As(V or III) were used to calculate equilibrium aqueous activities and saturation indices for the control and inoculated dissolution experiments

using The Geochemist's Workbench® Software Package (GWB, version 7.06).⁴⁰ GWB used the LLNL thermodynamic database and the Dzombak-Morel reaction dataset (DLM) for hydrous ferric oxide binding constants in the reaction model.^{41,42} The DLM database was amended to include additional constants for weak and strong ternary Pb sulfate ($>\text{FeOHPbSO}_4$) complexes and stronger binding constants for weak and strong $>\text{FeOPb}^+$ sites.⁴³

3.2.5 Calculation of ΔG°_f for Pb-As jarosite, $\Delta G^\circ_{\text{rxn}}$ and ΔG for the redox couples. The Gibbs free energy of formation (ΔG°_f) is not available for beaudantite (i.e. Pb-As jarosite).^{13,16} Therefore, the ΔG°_f of Pb-As jarosite was calculated using the method developed by Gaboreau and Viellard (2004), based on an empirical parameter: $\Delta_{\text{GO}}\text{M}^{\text{Z}+}_{(\text{c})}$, which characterizes the oxygen affinity of structural cations ($\text{M}^{\text{Z}+}$) within the jarosite structure.^{44,45} To examine the thermodynamic constraints on Fe, As and S reduction in Pb-As jarosite in inoculated samples, the standard state Gibbs free energy of reactions ($\Delta G^\circ_{\text{rxn}}$) for aqueous and structural arsenate, Fe and sulfate reduction coupled to lactate oxidation were calculated from compiled values of Gibbs free energy of formation (ΔG°_f) for relevant reaction constituents (SI Tables S3.1). To determine the thermodynamic driving force (i.e. ΔG) for aqueous As(V), aqueous Fe(III) and structural Fe(III) reduction, experimental concentrations of aqueous As(III), As(V), Fe(II) were used and concentrations of lactate, acetate and HCO_3^- were estimated assuming a 4:1 and 2:1 stoichiometry for Fe(II) and As(III) production. Due to the high background S concentrations contributed by the PIPES buffer (2 mM) during acidification for ICP-OES preparation, S concentrations were estimated assuming an Fe(II): S ratio of 1: 1.7. Low concentrations of Fe(III) and Pb were chosen (10^{-6} μM Fe and 1 μM Pb) to account

for non-detection in experimental samples. Minimal media component concentrations, measured and estimated aqueous concentrations of Fe(II), Fe(III), As(V), As(III) and SO_4^{2-} were used to calculate equilibrium aqueous activities using the extended form of the Debye-Huckel equation using the React program within The Geochemist's Workbench® Software Package (GWB, version 7.06) (SI Table S3.2).⁴⁰ The estimated ΔG°_f for Pb-As jarosite and species activities were incorporated in the Lewis equation (Eqn. 1) and the non standard state Gibbs free energy of reaction (ΔG) was determined at 298K and 1 atm pressure:

$$\Delta G = \Delta G^\circ + RT(\ln 10)\log(Q) = \Delta G^\circ + 5.7081 \times \log(Q) \quad (\text{Eqn. 1})$$

The reaction quotient, Q , is defined for a reaction as:

$$a(A) + b(B) = c(C) + d(D) \quad (\text{Eqn. 2})$$

$$Q = \frac{\{C\}^c\{D\}^d}{\{A\}^a\{B\}^b} \quad (\text{Eqn. 3})$$

3.3 RESULTS AND DISCUSSION

3.3.1 Control Samples. S concentrations over time could not be used to evaluate Pb-As jarosite dissolution due to the background contribution of S (2 mM) by the PIPES buffer which destabilizes upon acidification of samples during ICP-OES preparation and contributes to higher sulfate concentrations during analysis. In control Pb-As jarosite samples, Pb remained below ICP-OES detection limits (10.5 μM) and As release was minimal, reaching a plateau at 20 μM at 72 hours (Figure 3.1). Aqueous As speciation analysis confirmed the oxidation state of As as As(V). Fe(II) was undetected in control samples and total aqueous Fe concentrations (ICP-OES) were low and

remained between 10-19 μM throughout the experiment (Figure 3.1). Based on the poor solubility of Fe(III) (10^{-9} μM) under circumneutral conditions, the low Fe concentrations are likely the result of Fe oxide colloids precipitated during dissolution that passed through filtration (< 0.2 μm), and dissolved during acidification for ICP-OES sample preparation.⁴⁶ XANES analysis of the control bulk solid phase over time at the Fe-K and As-K edge confirmed that Fe and As was present as Fe(III) and As (V) (SI Figure S3.1). The pH remained buffered at 7.3 throughout the experiment while the redox potential (Eh) reached a plateau of 227- 243 RmV between 168 and 336 hours (Figure 3.1). X-ray diffraction patterns collected on the solid phase at 72 and 336 hours were similar to the unreacted Pb-As jarosite and showed no extra peaks (SI Figure S3.2). However, TEM images of the sample at 336 hours show secondary precipitation and EDS analyses of precipitates showed enrichment of Fe (25.15 wt.%), O (39.33 wt.%), As (19.64 wt.%) and Pb (15.88 wt.%) (SI Figures S3.3 and S3.4, SI Table S3.5).

Mineral saturation indices (log Q/K) calculated by The Geochemist's Workbench® (GWB) predicted the formation of hematite> schwertmannite> goethite> ferrihydrite and undersaturation (log Q/K < 0) of anglesite (SI Table S3.3). Hematite was discounted as the potential phase because it is expected to form over longer durations, and schwertmannite was discounted because it typically forms between pH 2.8-4.5.^{19,47} Based on TEM analyses the secondary precipitate is likely goethite or ferrihydrite. The association of Pb (15.88 wt.%) with the precipitate and the lack of detectable aqueous Pb suggests surface complexation with $\text{Fe}(\text{OH})_3$, which may be enhanced in the presence of the sulfate released during dissolution through ternary sulfate surface complexes.⁴³ Similarly, the association of As with the precipitate may the

result of complexation of AsO_4^{3-} with the Fe-oxyhydroxide surface through the formation of inner-sphere bidentate binuclear corner and edge-sharing complexes.^{48,49}

To account for Pb and As sorption onto hydrous ferric hydroxides (HFO), the Dzombak and Morel (1990) diffuse layer complexation model (DLM) was incorporated into the reaction model. GWB predicted Pb sorption (60.44%) onto HFO surfaces with greatest binding to the weak (low affinity) $>(\text{w})\text{FeOPb}^+$ (58.40%) site. The remaining aqueous Pb (39.54%) was predicted as free Pb^{2+} (22.85 %) and PbCl^+ (16.10%) complexes (SI Table S3.4). GWB predicted minimal As sorption (5.11%) onto HFO surfaces with greatest complexation (4.85%) on the weak $>(\text{w})\text{FeOHAsO}_4^{3-}$ site. The remaining aqueous As (94.93%) was predicted as HAsO_4^{2-} (56.49%) and H_2AsO_4^- (38.44%). The predicted Pb and As sorption and speciation should be interpreted qualitatively rather than quantitatively because the predictions were not confirmed experimentally. Nevertheless, the predicted higher sorption (i.e. removal) of Pb (60.44%) versus As (5.11%) onto ferric oxides corroborates with solution data because arsenic was detectable (20 μM) while Pb remained below detection limits (10.5 μM). The smaller amount of Pb (15.88 wt.%) shown in the secondary precipitates during TEM-EDS analysis as compared to As (19.64 wt.%) may also be due to the precipitation of another Pb phase such anglesite (PbSO_4) not observed during TEM analysis but observed in the previous abiotic dissolution study.¹²

3.3.2 Inoculated Samples. Inoculated Pb-As jarosite samples remained buffered at pH 7.3 throughout the experiment. The redox potential (Eh) increased from -222.73 RmV at Time 0 to -134. 2 RmV by 24 hours and remained between -134 and -81 RmV for the rest of the experiment (Figure 3.1). As observed in the control samples, Pb

remained below detection limits (10.5 μM) in all samples. Higher concentrations of aqueous As(V) were released from inoculated samples than in the controls at a rate of $4.66 \pm 2.24 \mu\text{M}\cdot\text{h}^{-1}$ ($r^2 = 0.948$) until 72 hours where concentrations reached a maximum of $328.22 \pm 12.52 \mu\text{M}$ (Figure 3.1). After 72 hours, As(V) concentrations decreased at a rate of $0.71 \pm 0.41 \mu\text{M}\cdot\text{h}^{-1}$ to a final concentration of $191.13 \pm 12.52 \mu\text{M}$. During the last sampling interval, $49.89 \pm 1.46 \mu\text{M}$ of As(III) was detected (D.L= 6.25 μM) (Figure 3.1). Aqueous As(V) reduction/release may have occurred earlier but the $12.5\times$ dilution of arsenic speciation samples would have precluded As(III) detection. EXAFS modeling of the un-reacted (original) Pb-As jarosite suggests that it contains Fe(III) in octahedral co-ordination with O, and As(V) in tetrahedral co-ordination with O substituting for S in the structure (SI Section S3.6). These interpretations are confirmed by As K-edge and Fe K-edge XANES spectra and linear fitting (see below) at 0 h (Figure 3.2). A comparison of the normalized absorption As K-edge spectra of inoculated solid phase samples over time shows a slight shift (ca. 1 eV) to the lower energy As(III) edge (eV) at 168 and 336 hours and the emergence of an energy shoulder not seen in control samples (Figures 3.2 and SI Figure S3.1). While S reduction was not expected, inoculated sample spectra were compared to that of realgar (AsS) and orpiment (As₂S₃) to rule out the possibility of S reduction and precipitation. The energy shift and emergence of a lower energy shoulder in inoculated samples corresponded to the sodium meta-arsenite spectrum rather than those of the sulfides (SI Figure S3.1). The proportion of As(III) to As(V) in the solid phase samples as the microbial experiments progressed was determined by linear least squares combination fitting of XANES spectra using the spectra of sodium meta-arsenite, orpiment, realgar and the pure un-reacted Pb-As jarosite. Least-squares

fitting of earlier samples showed 100% As(V) (as in the unreacted Pb-As jarosite, Section S3.6), and fitting of the inoculated sample at 168 and 336 hours showed 14.7% As(III) (as sodium meta-arsenite)/ 85.3% As(V) (R factor: 0.0082) and 20.4% As(III) (as sodium meta-arsenite)/ 79.6% As(V) (R-factor: 0.0096), respectively.

In contrast to the As(V) reduction lag time, Fe(III) reduction was observed in the aqueous phase within the first sampling period and likely occurred in the time between inoculation and ferrozine analysis (ca. 1 hour) (Figure 1). Fe(II) concentrations increased throughout the experiment at a rate of $7.03 \pm 0.46 \mu\text{M} \cdot \text{h}^{-1}$ ($r^2 = 0.997$) to a maximum of $2166.68 \pm 323.18 \mu\text{M}$ at the termination of the experiment. Based on this, and on the As-Kedge XANES spectra and the arsenic speciation data, both Fe(III) and As(V) reduction occurred simultaneously from at least 168 hours onward (Figures 3.1, 3.2 and SI Figure S3.1). The ratio of $\text{Fe(II)}_{(\text{aq})}:\text{As(V)}_{(\text{aq})}$ remained between 1.0 - 1.7 for the first 72 hours at which point it increased to 4.6 and 11.3 by 168 and 336 hours, respectively. Therefore, based on the increase in $\text{Fe(II)}_{(\text{aq})}:\text{As(V)}_{(\text{aq})}$, coupled with the low analytical sensitivity of the As speciation method and the detection limits of XAFS ($> 5 \text{ wt.}\%$), it is likely As reduction began once As(V) concentrations decreased at 72 hours. The normalized first derivative Fe-K edge XANES spectra of the reacted solid phase at selected time intervals does not show a shift to the lower Fe(II) energy edge at the end of the experiment, suggesting the majority of Fe(II) (at least 95%) is associated with the aqueous phase (Figure 3.2). By combining aqueous concentrations with XANES fitting at 336 hours, As(III) and Fe(II) represented 23.2 % and 12.4%, respectively, of the total As and Fe available from the original Pb-As jarosite.

The initial ~ 1:1 release of Fe(II):As(V) for the first 72 hours of the experiment is not surprising because while Pb-As jarosite has a Fe:As molar ratio of 9.2:1, structurally the Fe octahedra is sterically remote (SI Section S3.6, SI Figure S3.11) and less susceptible to dissolution than the sulfate and arsenate tetrahedral sites.¹² Yet despite the structural constraints imposed on Fe reduction and the presence of aqueous As(V), Fe reduction by *S. putrefaciens* occurred first. Therefore, to elucidate the potential underlying mechanisms behind the simultaneous reduction of Fe and As, the thermodynamic constraints of each redox couple in the aqueous and solid phase was considered.

3.3.3 Standard versus Reaction state Thermodynamic Favorability. Pb-As jarosite presents an interesting opportunity for metal and metalloid reducing bacteria because it contains three potential terminal electron acceptors (TEA) to derive energy from: Fe, As and S. Therefore, to determine the relative thermodynamic favorability of TEAs in the Pb-As jarosite, the constraints of both aqueous and structural Fe, As and S on standard state (ΔG°_f) versus reaction state (ΔG) Gibbs Free energy were considered. Calculations of ΔG°_f under standard state conditions predicted aqueous $\text{Fe}(\text{OH})_2^+$ reduction as the most favorable reaction on a per mole of lactate basis followed by structural Fe, aqueous HAsO_4^{2-} , structural S and aqueous SO_4^{2-} (Table 3.1). Moreover, with a $\Delta G^\circ_{\text{rxn}} = +181.92$, structural As(V) reduction is not favorable, which may partly account for the aqueous As(V) reduction observed in this study. The contribution of the concentration gradients established by the reactants and products over time under non-standard conditions (Q) were considered and the ΔG under non-standard state was calculated.²⁸ The reaction quotient Q (Equation 3) at each time interval was calculated

and incorporated into the Lewis equation for ΔG (Equation 1) for the 3 most likely reduction reactions predicted to occur: $\text{Fe}(\text{OH})_2^+$ (Equation 4), structural Fe (Equation 5) and aqueous HAsO_4^{2-} (Equation 6):

$$\Delta G_r = \Delta G_r^\circ + 2.3RT \log \left(\frac{\{\text{CH}_3\text{COO}^-\}\{\text{HCO}_3^-\}\{\text{Fe}^{2+}\}^4}{\{\text{Fe}(\text{OH})_2^+\}^4\{\text{C}_3\text{H}_5\text{O}_3^-\}\{\text{H}^+\}^3} \right) \quad (\text{Equation 4})$$

$$\Delta G_r = \Delta G_r^\circ + 2.3RT \log \left(\frac{\{\text{CH}_3\text{COO}^-\}\{\text{HCO}_3^-\}\{\text{Pb}^{2+}\}^{0.45}\{\text{Fe}^{2+}\}^4\{\text{HAsO}_4^{2-}\}^{0.43}\{\text{SO}_4^{2-}\}^{2.37}}{\{\text{C}_3\text{H}_5\text{O}_3^-\}\{\text{H}^+\}^{2.31}} \right) \quad (\text{Equation 5})$$

$$\Delta G_r = \Delta G_r^\circ + 2.3RT \log \left(\frac{\{\text{CH}_3\text{COO}^-\}\{\text{HCO}_3^-\}\{\text{H}_3\text{AsO}_3\}^2}{\{\text{HAsO}_4^{2-}\}^2\{\text{C}_3\text{H}_5\text{O}_3^-\}\{\text{H}^+\}^3} \right) \quad (\text{Equation 6})$$

Calculations of ΔG at each time interval for the redox couples (SI Figure S3.5) show that the concentration gradient established by the accumulation of Fe^{2+} , SO_4^{2-} , HAsO_4^{2-} and Pb^{2+} during the reduction of structural Fe (Equation 5) serves to potentially drive the reaction forward (ΔG) by increasing the initial ΔG to below the $\Delta G_{\text{rxn}}^\circ$ (i.e. more negative) and decreasing thereafter (Figure 3.3). On the other hand, the accumulation of Fe^{2+} coupled with the expected low concentrations of Fe(III) at circumneutral conditions drive the ΔG for aqueous Fe(III) reduction (Equation 3.4) lower (i.e. more positive) than the ΔG for structural Fe reduction. Similarly, low aqueous As(III) coupled with higher As(V) concentrations also decreased the ΔG for aqueous As(V) reduction lower (i.e. more positive) than the ΔG for structural Fe reduction. Thermodynamically, structural Fe reduction is predicted as the dominant reaction yet does not consider the structural constraints imposed on the reaction. In this study, we did not experimentally determine if *S. putrefaciens* reduced structural or

aqueous Fe. However, in order for aqueous Fe(III) reduction to proceed, rapid abiotic dissolution would need to occur to continuously provide aqueous Fe(III) activities sufficient for continued energy gain by *S. putrefaciens*.²⁸ Based on the minimal dissolution of the control samples (i.e. no bacteria) and the structural constraints placed on Fe octahedra, it is unlikely that abiotic dissolution would provide enough aqueous Fe(III) for continued reduction. Moreover, if aqueous Fe(III) reduction was the governing reaction, then the ΔG of aqueous Fe and As would be similar and simultaneous aqueous As(V) and Fe(III) reduction would have occurred earlier (Figure 3.3). The thermodynamic favorability of structural Fe reduction coupled with the higher Fe:As (~9:1) in the structure leads us to hypothesize that additional factors are driving As (V) reduction.

3.3.4 Arsenate reduction mechanism. The increased concentrations of As(V) over time may be a potential trigger for the As detoxification mechanism (*arr* system). Saltikov et al. (2005) demonstrated a correlation between As(V) concentrations and *ars/arr* expression in *Shewanella* sp. ANA-3 whereby respiration occurred at low concentrations (100 nM) earlier in cell growth and detoxification at higher concentrations (~100 μ M) during stationary phase.² Jiang et al. (2009) showed 93.7 and 100 % protein sequence similarities in *S. putrefaciens* CN32 to the proteins encoded by the *arr-ars* operon from *Shewanella* sp. strain ANA-3 thereby providing evidence for both As reduction pathways in *S. putrefaciens* CN32.⁵⁰ Therefore, As(V) reduction in inoculated Pb-As jarosite samples may be the result of detoxification (*ars*) induced by high aqueous As(V) concentrations (328 μ M) (Figure 3.1).

An ATP-based cell viability assay was used to evaluate ATP concentrations over time to examine the potential toxicity of As and Pb on *S. putrefaciens* CN32 (Figure 3.1). Statistical analyses (t-test) of the variation in initial and ATP concentrations at each time interval between inoculated minimal media with or without Pb-As jarosite showed significantly higher ATP in samples containing Pb-As jarosite at 48, 72 and 168 hours where $p = 0.0039$, 0.0033 and 0.0007 , respectively. Increased ATP in the inoculated Pb-As jarosite samples may be attributed to the ATP generated during normal Fe reduction, however, in our experience when *S. putrefaciens* CN32 is exposed to potentially toxic metals such as Pb, the ATP concentrations in bacterial control samples (i.e. no jarosite) are consistently higher over time.²⁵ As proposed by Chow et al. (2008), the higher ATP concentrations in the Pb-As jarosite samples may be the result of increased Fe reduction triggered by *S. putrefaciens* to compensate for the ATP lost during hydrolysis to maintain active transport As(III) out of the cell.⁵¹

In a previous study, the reductive dissolution of Pb-jarosite by *S. putrefaciens* under similar conditions showed minimal Fe(II) reduction with a maximum of $\sim 200 \mu\text{M}$ Fe(II) versus the $2166 \mu\text{M}$ observed in the current study.²⁵ Therefore, the enhanced Fe(III) reduction ($10 \times$) observed in this study may be a response by *S. putrefaciens* to high As(V) concentrations to maintain the ATP required for As(III) efflux during the detoxification mechanism. The time lag in this study is consistent with previous studies which suggest rates of As(V) reduction via the detoxification pathway (*ars*) may be slower and occur during the stationary growth phase than the respiratory pathway (*arr*) which occurs earlier during exponential growth.^{2,3,52} However, in the absence of gene

expression data the process cannot be confirmed and therefore the As(V) reduction mechanism still requires elucidation.

Alternatively, the accumulation of precipitates or metabolites may have diminished the thermodynamic favorability of Fe(III) reduction and triggered simultaneous respiratory As reduction (*arr*). However, bulk X-ray diffraction patterns collected on inoculated samples over time were similar to the original unreacted jarosite and did not show any new peaks (SI Figure S3.2). Moreover, SEM images do not show extensive secondary precipitation over time (SI Figure S3.6), which is also corroborated by the lack of a shift in the solid phase Fe-K edge and the high concentrations of aqueous Fe(II) (Figures 3.1 and 3.2). High resolution SEM images of inoculated Pb-As jarosite at 336 hours reveal a surface coating of secondary precipitates (Figure 3.3A). Areas of isolated secondary precipitates could not be located, so SEM-EDS spectra were collected at randomly selected areas on the bulk jarosite at 0, 168 and 336 hours and demonstrated enrichment of Pb (from 16.55 to 20.56 %). By contrast, As increases from 1.44 wt.% at time 0 to 4.73 wt.% at 168 hours, followed by a decrease to 1.61 wt.% by 336 hours (SI Table S3.6), the latter of which is also coincident with the increase of aqueous As(III) (Figure 3.1). While secondary precipitation was not extensive, TEM images of a *S. putrefaciens* cell at 72 hours (Figure 3.3B) show precipitates in close proximity to the cell. An elemental map of precipitates shows relative enrichment of Fe, P and O (SI Figure S3.7). Furthermore, TEM-EDS analysis of several precipitates shows enrichment of Fe (24 wt.%), P (3.38 wt.%), O (63.90 wt.%) and sometimes As (8.56 wt.%) (SI Figure S3.8), while HR-TEM images reveal an amorphous structure (Figure 3.3C and D). ICP-OES analysis of the minimal media at 0 and 336 hours shows a

background P concentration of $\sim 8 \mu\text{M}$. Inoculated minimal media without Pb-As jarosite contributed to an increase in P over background concentrations likely related to cell lysis (Figure 3.1). Release of P in these samples showed an increase in P from $10.86 \mu\text{M}$ at Time 0 to $16.59 \mu\text{M}$ followed by a decrease to $12.45 \mu\text{M}$ by 336 hours. Inoculated Pb-As samples showed a gradual P decline from 6.3 to $3.4 \mu\text{M}$ at the end of the experiment. Mineral saturation indices ($\log Q/K$) predict the formation of vivianite ($\text{Fe}_3(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$) and siderite (FeCO_3) and the under saturation of $\text{Fe}(\text{OH})_2$ and cerussite (PbCO_3) (SI Table S3.3). Potential sorption of As(III) onto secondary precipitates was not modeled due to the lack of As(III) surface complexation constants for vivianite and siderite.

3.3.5 Fate of As and Pb. While vivianite has been proposed as an As sink in previous studies, the low concentrations of P ($\sim 12 \mu\text{M}$) in our samples make it unlikely that vivianite is the primary sink for As sorption and retention in this experiment. Therefore, it is more plausible that arsenite and P was sorbing to the siderite or to Fe(II)/(III) oxyhydroxides predicted to form. The identification of siderite could not be determined using TEM-EDS due to the background contribution of carbon from the carbon-coated formvar sample grid. The observed release of aqueous As(III) at 336 hours may be due to increasing As(III) reduction and the lack of sorption sites due to minimal secondary precipitation. Tufano et al. (2008) showed As(III) retention during early Fe reduction from ferrihydrite-coated sands presorbed with As(III) using *S. putrefaciens* CN32, but over time the cessation of phase transformations resulted in the release of aqueous As(III).²⁹

Interestingly, intracellular Pb accumulation was not observed in this study, yet was previously demonstrated during the reductive dissolution of Pb-jarosite by the same freezer stock of *S. putrefaciens* CN32.²⁵ Therefore, it is possible that the energy derived from ATP efflux was sufficient to remove Pb from the cell. Elemental maps of cross sections of *S. putrefaciens* cells show relative increased localization of As and Pb at the cell wall from Time 0 to 336 hours (SI Figures S3.9 and S3.10). The enrichment of As(V) at the cell wall likely represents As(V) sorption prior to uptake, reduction and efflux of As(III) from the cell. While, the localization of Pb at the cell wall is likely due to the formation of adsorption complexes with carboxyl and phosphoryl functional groups commonly reported at circumneutral conditions.^{25,53,54}

3.3.6 Environmental Implications. This study demonstrates the simultaneous reduction of Fe(III) and As(V) from synthetic Pb-As jarosite. Additionally, we did not observe aqueous Pb release in both the control and inoculated samples. Despite the low molar content of As in the original Pb-As jarosite, high concentrations of aqueous As(V) were observed as compared to the abiotic control. Our results suggest that structural Fe(III) reduction was thermodynamically driven while aqueous As(V) reduction was triggered by a detoxification mechanism (*ars*) induced to offset high As(V) concentrations. Therefore, depending on the microbial communities present, Pb-As jarosite does not represent a suitable candidate for arsenical waste storage under anaerobic circumneutral conditions. However, the effects of temperature, flow, competing microbial communities, natural organic matter, suspension density or removal of As onto potentially co-existing Fe-oxides on the reduction kinetics and fate of As were not considered, leaving opportunity for future refinement.¹² Finally, future

studies should assess the reductive dissolution of Pb-As jarosite using arsenate and sulfate reducing bacteria and microbial consortia collected from mine waste repositories to improve our understanding of Pb-As jarosite solubility.

Table 3.1. Standard State Gibbs Free Energy for Pertinent Redox Reactions

Reactions considered		$\Delta G^{\circ}_{\text{rxn}}$ (kJ/mol)
Iron Reduction		
1	$1.40(\text{H}_3\text{O})_{0.68}\text{Pb}_{0.32}\text{Fe}_{2.86}(\text{SO}_4)_{1.69}(\text{AsO}_4)_{0.31}(\text{OH})_{5.59}(\text{H}_2\text{O})_{0.41} + \text{C}_3\text{H}_5\text{O}_3^- + 2.31\text{H}^+ \rightarrow \text{CH}_3\text{COO}^- + \text{HCO}_3^- + 0.45\text{Pb}^{2+} + 4.00\text{Fe}^{2+} + 0.43\text{HAsO}_4^{2-} + 2.37\text{SO}_4^{2-} + 7.35\text{H}_2\text{O}$	-377.04
2	$4.00\text{Fe}(\text{OH})_2^+ + \text{C}_3\text{H}_5\text{O}_3^- + 3\text{H}^+ \rightarrow 4.00\text{Fe}^{2+} + \text{CH}_3\text{COO}^- + \text{HCO}_3^- + 6.00\text{H}_2\text{O}$	-480.68
Arsenic Reduction		
3	$2\text{HAsO}_4^{2-} + \text{C}_3\text{H}_5\text{O}_3^- + 3\text{H}^+ \rightarrow 2\text{H}_3\text{AsO}_3 + \text{CH}_3\text{COO}^- + \text{HCO}_3^-$	-296.20
4	$6.45(\text{H}_3\text{O})_{0.68}\text{Pb}_{0.32}\text{Fe}_{2.86}(\text{SO}_4)_{1.69}(\text{AsO}_4)_{0.31}(\text{OH})_{5.59}(\text{H}_2\text{O})_{0.41} + \text{C}_3\text{H}_5\text{O}_3^- \rightarrow \text{CH}_3\text{COO}^- + \text{HCO}_3^- + 0.23\text{H}^+ + 2.065\text{Pb}^{2+} + 18.45\text{Fe}(\text{OH})_2^+ + 2.00\text{H}_3\text{AsO}_3 + 10.90\text{SO}_4^{2-} + 6.19\text{H}_2\text{O}$	+181.92
Sulfate Reduction		
5	$0.30(\text{H}_3\text{O})_{0.68}\text{Pb}_{0.32}\text{Fe}_{2.86}(\text{SO}_4)_{1.69}(\text{AsO}_4)_{0.31}(\text{OH})_{5.59}(\text{H}_2\text{O})_{0.41} + \text{C}_3\text{H}_5\text{O}_3^- \rightarrow \text{CH}_3\text{COO}^- + \text{HCO}_3^- + 0.41\text{H}^+ + 0.09\text{Pb}^{2+} + 0.85\text{Fe}(\text{OH})_2^+ + 0.09\text{HAsO}_4^{2-} + 0.5\text{HS}^- + 0.28\text{H}_2\text{O}$	-96.66
6	$0.5\text{SO}_4^{2-} + \text{C}_3\text{H}_5\text{O}_3^- \rightarrow 0.5\text{HS}^- + \text{CH}_3\text{COO}^- + \text{HCO}_3^- + 0.5\text{H}^+$	-65.39

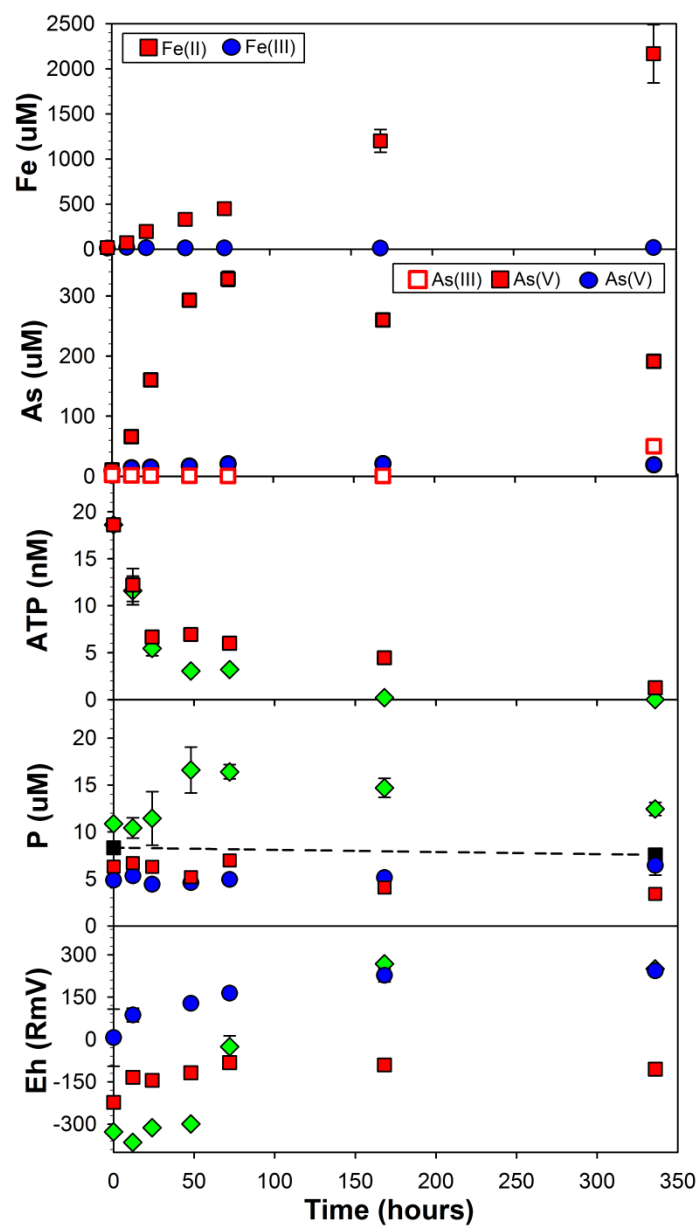


Figure 3.1. Redox potential, ATP concentrations and release of aqueous Fe (II/III), As(III/V) and P in minimal media control (■), inoculated minimal media control (◆), Pb-As jarosite control (●), and inoculated Pb-As jarosite (■ or □) over time. Error bars represent standard error (n=3).

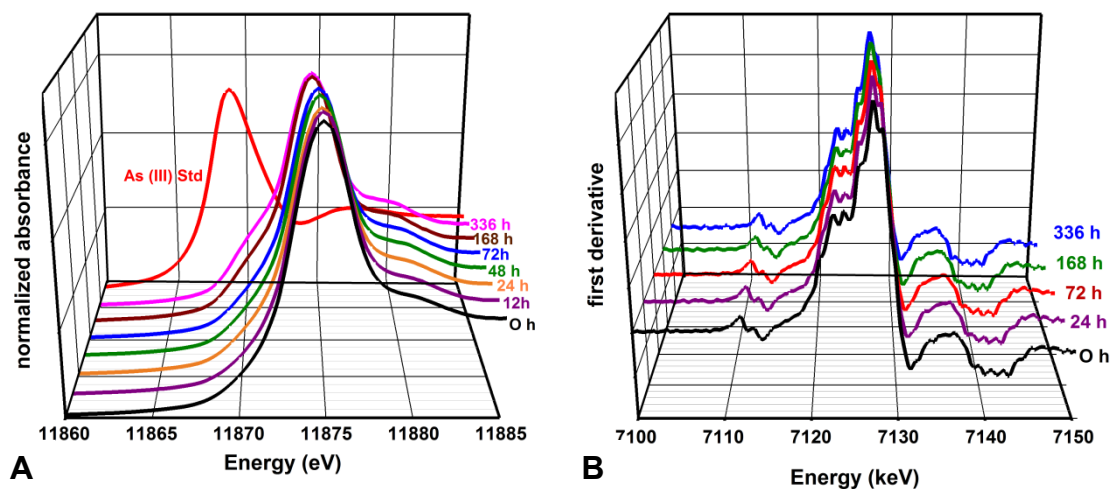


Figure 3.2. Normalized A) absorbance As K-edge and B) first derivative Fe K-edge XANES spectra for inoculated Pb-As jarosite samples over time.

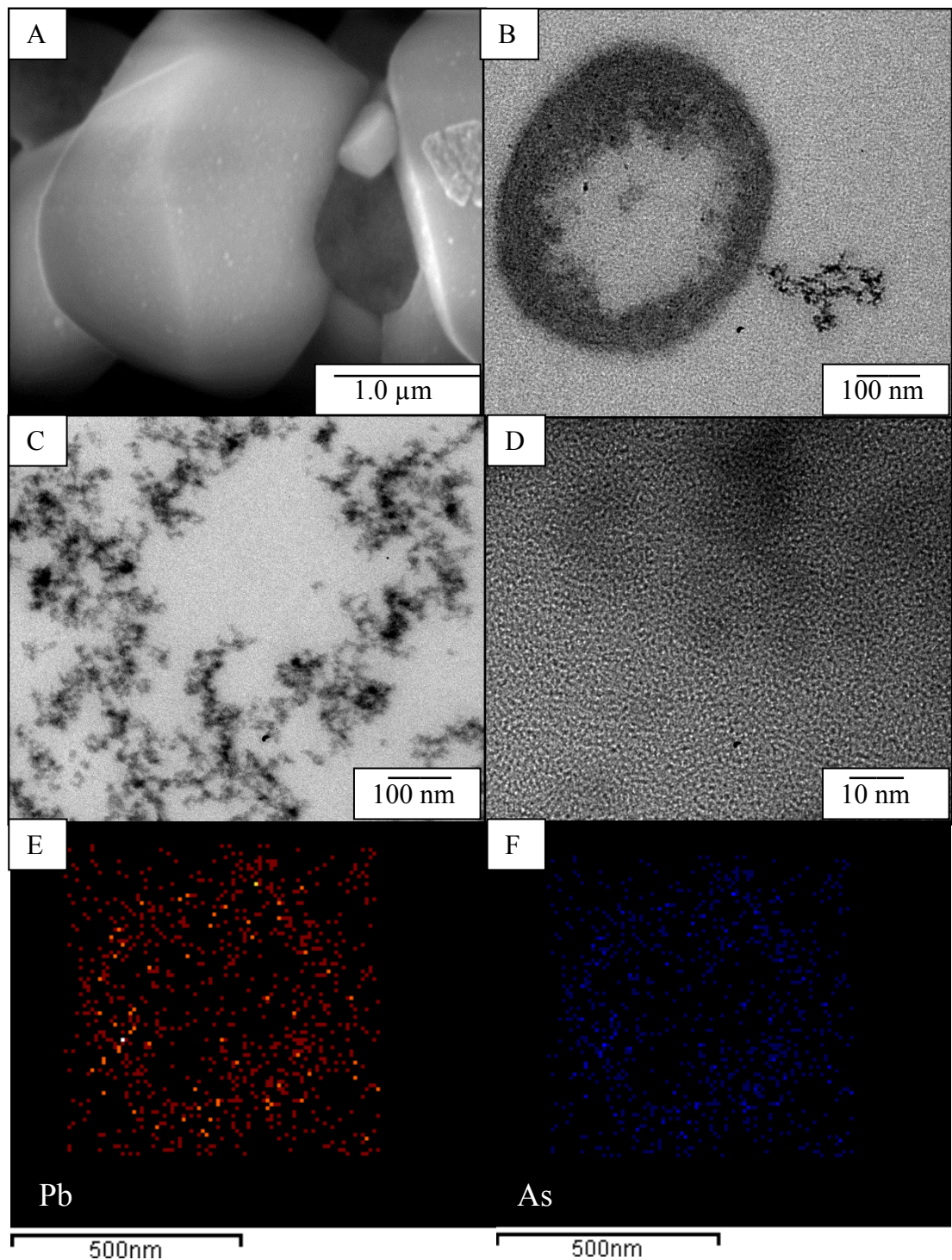


Figure 3.3 A) SE-SEM image of secondary mineral precipitates associated with inoculated Pb-As jarosite at 336 hours, and TEM images of B) *S. putrefaciens* associated with secondary minerals at 72 hours; C) secondary precipitates at 336 hours and the ; D) corresponding HR-TEM image of the precipitate; E) and F) TEM-EDS Pb and As maps of a cross sectioned *S. putrefaciens* cell at 336 hours. Maps of P, Fe and O of the same cell are found in SI Figure S10.

3.4 SUPPORTING INFORMATION

S3.1. Bacterial cultivation method

Pure cultures of *Shewanella putrefaciens* were prepared from 1.5 mL frozen glycerol stocks maintained at -80 °C and transferred to Trypticase Soy Agar (TSA) plates and grown aerobically for 24 hours. Single colonies were inoculated into 25 mL of sterile Trypticase Soy Broth (TSB) and incubated aerobically at 32 °C for 16 hours. Seed cultures (10 mL) were used to inoculate 4 x 100 mL aliquots of sterile Luria-Bertrani (LB) media and incubated aerobically at 32 °C until the culture reached late log phase (16 hours). Bacteria were harvested by centrifugation at 3000 rpm for 20 minutes and washed twice using sterile 0.01M NaNO₃, decanting the supernatant and centrifuging at each step. The wet biomass was washed once using 100 mL of N₂ purged minimal media containing: 1.34 mM potassium chloride (KCl), 28 mM ammonium chloride (NH₄Cl), 0.68 mM calcium chloride (CaCl₂), 50 mM sodium perchlorate (NaClO₄), 24mM Na-lactate (60% syrup), 20 mM PIPES (1,4-piperazine diethanesulfonic acid) buffer at pH 7.4. The minimal media was decanted and the cells were suspended in 1.0 L of media with a final wet mass of 1.08 g/1 L of media. All solutions were prepared from ACS grade reagents and were either filter sterilized (0.2 µm) or autoclaved.

S3.2. ATP Analysis

Briefly, 150 µL of the BacTiter-Glo™ reagent was added to 150 µL sample, shaken for 5 minutes and luminescence was measured using a GloMax® 20/20 Single Tube Luminometer (λ 350-650 nm) at an integration time of 2 seconds and the output

was reported in relative luciferase units (RLU). Control samples were also analyzed to determine the intensity of the luminescent signal produced by the minimal media and the Pb-As jarosite in the absence of *S. putrefaciens*. The RLU values were converted to ATP concentrations using the average of three calibration curves prepared with an ATP standard in minimal media (10 mM; Promega Corporation).

S3.3. TEM preparation and analysis:

The primary fixative used for TEM samples preparation was 0.2M glutaraldehyde (2% v/v) in 0.1M phosphate buffer pH 6.8. The samples were rinsed 2X in the buffer solution, then post-fixed in 1% osmium tetroxide in 0.1M phosphate buffer for 1 hour. Samples were dehydrated through a graded ethanol series (50%, 70%, 70%, 95%, 95%, 100%, 100%) and final dehydration was done in 100% propylene oxide (PO). Samples were infiltrated with Spurr's resin in a series (2:1 PO:Spurr's, 1:1 PO:Spurr's, 1:2 PO:Spurr's, 100% Spurr's, 100% Spurr's, 100% Spurr's) with rotation of the samples in between solution changes. The samples were transferred to embedding moulds and were filled with fresh 100% Spurr's resin and polymerized overnight at 60°C. Thin sections were cut on a Leica UCT Ultramicrotome and placed onto both uncoated grids and some Formvar-coated grids. Samples were lightly C coated (10-15 Å). Specimens were examined using field emission- transmission electron microscopy (FE-TEM) (FEI Titan 80-300). Bright field (BF) images and high angle annular dark field (HAADF) scanning transmission electron microscopy (STEM) images were collected at 200 kV. Energy dispersive x-ray spectra (EDX) were collected in STEM mode with a probe size of 1 nm or less.

S3.4. X-ray Absorption Spectroscopy (XAS):

Absorption spectra were collected at room temperature in transmission mode using a 13 element solid state Ge-detector from -200 (pre-edge) to +1000 eV (post-edge) of the As (11 867 eV) and Fe (7110.75 eV) K-edges. The energy of the Si(111) double crystal monochromator was calibrated using an Au foil and each standard/ sample was scanned 3-4 times. Proportions of As(III)/As(V) and Fe(II)/Fe(III) were determined by linear least squares combination fitting of the XANES spectra using the spectra of As(III) as arsenic (III) trioxide (As_2O_3), As(V) as the pure Pb-As-jarosite, Fe(II) as wustite (FeO) and Fe(III) as the pure Pb-As-jarosite as standards.

Extended X-ray Absorption Fine Structure (EXAFS) data for the original Pb-As jarosite were collected at the CLRC Synchrotron Radiation Source at Daresbury Laboratory, UK, which operates at 2 GeV with a typical current of 150 to 250 mA. X-ray absorption spectra were collected at the Fe and As K-edges (7.12054 and 11.8695 keV) on station 9.2 which was equipped with a Si(111) double crystal monochromator, and ion chambers for measuring incident and transmitted beam intensities. 10 μm Fe and As foils were used to calibrate the monochromator position. In order to reduce the presence of higher harmonics the focused beam was detuned by approximately 50 %. The sample was mounted as a pressed pellet (diluted with diffused silica, $\varnothing 15\text{mm}$) on to a liquid nitrogen cryostat (80 K) and between 7-10 quick (Q)EXAFS data scans were collected for each sample at both edges. XAS data were processed using EXCALIB, EXBSPLINE and EXCURV98.¹

S3.5. Arsenic Speciation Method

For As(V), 100 μ L of a color reagent was added to 900 μ L of the acidified sample and analyzed following a 30 minute reaction period. The color reagent contained 0.613 M L-ascorbic acid ($C_6H_8O_6$), 24 mM ammonium molybdate ($[NH_4]_6Mo_7O_{24}$), 8 mM potassium antimonyl tartrate ($C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$) and 2.5 M sulphuric acid (H_2SO_4). The aforementioned color reagent solutions were prepared separately and mixed in a 2:2:1:5 ratio (by volume) in the order listed. Special care was taken to prevent turbidity in the coloring reagent by adding the H_2SO_4 immediately following the addition of the first 3 reagents. Absorbance was measured using a GENESYS 20 UV-VIS Spectrophotometer at 873 nm. Total arsenic was determined by adding 100 μ L of an oxidizing reagent to 900 μ L of the sample. The oxidizing reagent contained 2 mM potassium iodate (KIO_3) in 2% v/v HCl. Samples were heated at 95°C for 10 minutes in a weller hot-plate and cooled on ice for 5 minutes. Following cooling, 100 μ L of the color reagent was added to the sample and the absorbance was measured. Arsenite and arsenate concentrations were determined using calibration curves of sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$) and sodium *m*-arsenite ($NaAsO_2$) stock solutions and were matrix matched with experimental samples and contained minimal media, 1% v/v HCl and 1 μ M KH_2PO_4 . Arsenite and arsenate detection limits were 6.25 and 0.625 μ M, respectively. As(III) concentrations were determined by subtracting As(V) from total arsenic. Total As concentrations determined in this method were compared to ICP-OES As totals and were within the standard errors of each measurement at each time interval.

S3.6. EXAFS analysis of the original Pb-As jarosite

Fitting of the EXAFS data for the Pb-As jarosite was performed in k-space using curved wave theory and in accordance with previous work both single and multiple scattering events (using Rehr-Albers small atom theory) were considered.²⁻⁴ Specifically for the multiple scattering calculations a cluster was constructed with C1 symmetry that was based on the local structure around a sulfur site in jarosite, derived from the crystal structure.⁵ In order to ensure that the number of refineable parameters did not exceed the number of experimental observations, coordination numbers were constrained to a value of 1 and the Debye-Waller factors of similar types of scattering species were grouped together.⁶ Bond lengths and bond angles around the cluster were refined in order to obtain detailed information on the local structure around the arsenic site and the refinement that yielded the best fit of the data whilst retaining chemically sensible bond distances between the nearest neighbours.

The fitted Fe K-edge EXAFS spectra and associated FT for the Pb-As-jarosite sample are shown in SI Figure S11 and the derived parameters are listed in Table S7. The results from a first shell analysis suggested that the Fe species in the sample existed as Fe(III) in octahedral coordination. It is known from crystallographic studies of jarosite that the Fe site is distorted and therefore contains four equatorial oxygens at a distance of 1.97 Å and two axial oxygens at 2.06 Å.⁷ The EXAFS technique is unable to resolve these two different contributions but a bond distance of 2.01 Å can be rationalised as being an average of 4×1.97 Å and 2×2.06 Å. In the structure two of the equatorial oxygens corner share with other FeO₆ octahedra whilst the remaining two

bind to hydrogen. The axial ligands coordinate to the interconnecting sulfur species which we observe at a distance of 3.25 Å away. The As that is substituting for some of the S sites is located at a slightly longer distance from the Fe of 3.31 Å.⁸ The identification of a shell of four Fe atoms at a distance of 3.67 Å is also typical of the jarosite structure.

A simple first shell analysis of the As data yielded four oxygens at an average distance of 1.69 Å (Table S3.7) and is consistent with the presence of tetrahedral As(V) species in the structure. Further proof for the substitution of As for S within the structure and a more detailed picture of the local coordination around the As substituent, was obtained by fitting the higher shells and by considering multiple scattering events. A best fit of the higher shell data was obtained in which three Fe species were observed at distances of 2.87, 3.34 and 3.38 Å, respectively (Table S3.7). The contribution of oxygen atoms at distances around 3.40 Å were found to be negligibly small owing to a large static disorder (large Debye–Waller factors) and therefore excluded from the model.

Table S3.1. Gibbs Free Energy of Formation for Reaction Constituents

Constituent	ΔG°_f (kJ/mol)
Pb ²⁺	-24.2 ⁹
Fe ³⁺	-16.28 ¹⁰
Fe(OH) ₂ ⁺	-438 ¹¹
Fe ²⁺	-91.5 ¹⁰
SO ₄ ²⁻	-744.46 ¹²
HS ⁻	11.97 ¹³
C ₃ H ₅ O ₃ ⁻	-512.67 ¹³
C ₂ H ₃ O ₂ ⁻	-369.33 ¹³
HAsO ₄ ²⁻	-713.73 ¹⁴
H ₃ AsO ₃	-640.03 ¹⁴
HCO ₃ ⁻	-586.94 ¹³
H ₂ O	-237.18 ⁹
Pb-As jarosite	-3041.64*

*calculated in this study.

Table S3.2. Measured chemical parameters and log activities used in React (GWB 7.01) for the control and inoculated Pb-As jarosite samples at 336 hours.

Component	Treatment			
	Control	Log activity	Biotic	Log activity
Fe ³⁺ (as Fe(OH) ₃)	20 µM	-4.75	---	---
Fe ²⁺	---	---	2166 µM	-2.99
Pb ²⁺ (as PbCO ₃)	5 µM	-5.82	5 µM	-5.31
SO ₄ ²⁻	109 µM	-4.25	1040 µM	-3.31
Cl ⁻	30 mM	-1.55	30 mM	-1.56
CH ₃ CH(OH)COO ⁻	24 mM	-1.69	19 mM	-1.80
CH ₃ COO ⁻	---	---	566 µM	-3.41
HCO ₃ ⁻	---	---	566 µM	-3.36
HAsO ₄ ²⁻	20 µM	-5.05	191 µM	-4.07
H ₃ AsO ₃	---	---	50 µM	-4.31
pH	7.40	-7.40	7.42	-7.42
Eh	+243 mV	---	-104.9 mV	---

Table S3.3. Predicted saturation indices (log Q/K) of potential secondary precipitates using calculated activities of control and inoculated Pb-As jarosite samples at 336 hours.

Mineral	Treatment	
	Control	Inoculated
Hematite	14.50	---
Schwertmannite	9.68	---
Goethite	6.77	---
Fe(OH) ₃	2.38	---
Pb ₃ (AsO ₄) ₂	-0.55	---
Anglesite	-2.25	---
Vivianite	---	5.29
Siderite	---	1.28
Fe(OH) ₂	---	-1.00
Cerrusite	---	-1.75

Table S3.4. GWB predicted moles of Pb and As aqueous and surface species.

Pb aqueous and surface species	moles	% Pb _{TOT(aq)}	As aqueous and surface species	moles	% As _{TOT(aq)}
>(w)FeOPb ⁺	2.89 x 10 ⁻⁶	58.40	HAsO ₄ ²⁻	1.12 x 10 ⁻⁵	56.49
Pb ²⁺	1.13 x 10 ⁻⁶	22.85	H ₂ AsO ₄ ⁻	7.61 x 10 ⁻⁶	38.44
PbCl ⁺	7.97 x 10 ⁻⁷	16.10	>(w)FeOHAsO ₄ ³⁻	9.60 x 10 ⁻⁷	4.85
PbCl ₂	2.92 x 10 ⁻⁸	0.59	>(w)FeHAsO ₄ ⁻	4.99 x 10 ⁻⁸	0.25
>(s)FeOPb ⁺	9.90 x 10 ⁻⁸	2.00	>(w)FeH ₂ AsO ₄	2.46 x 10 ⁻⁹	0.01
>(w)FeOHPbSO ₄	2.21 x 10 ⁻⁹	0.04			
>(s)FeOHPbSO ₄	1.99 x 10 ⁻¹²	0.00			
Total Pb _(aq)	1.96 x 10 ⁻⁶	39.54	Total As _(aq)	1.88 x 10 ⁻⁵	94.93
Total >(w,s) Pb	2.99 x 10 ⁻⁶	60.44	Total >(w,s) As	1.01 x 10 ⁻⁶	5.11

Percentages were determined by comparing predicted Pb and As species to original aqueous concentrations. The >(w) and >(s) correspond to weak(low-affinity) and strong (high-affinity) surface complexation sites, respectively.

Table S3.5. Locations of spectra collected during TEM-EDX analysis and corresponding relative elemental concentrations reported in weight %

Element	Spectra (wt%)			
	1	2	3	4
O K	37.50	39.33	37.33	36.10
Fe K	32.24	25.15	31.89	38.19
As K	---	19.64	30.79	---
Pb L	22.83	15.88	---	17.74
S K	7.44	---	---	7.96

Table S3.6. SEM-EDS Analysis of relative elemental weight percent and standard deviation (n=3) of bulk Pb-As jarosite at randomly selected areas over time

Element	Time 0		168 hours		336 hrs	
	Wt %	S.D	Wt %	S.D	Wt %	S.D
OK	41.46	2.19	36.60	1.22	43.47	1.80
SK	12.65	0.82	10.13	1.51	9.16	0.39
FeK	27.90	2.35	32.02	0.65	25.19	0.96
AsK	1.44	0.19	4.73	0.52	1.61	0.78
PbL	16.55	0.47	15.50	3.01	20.56	1.25

Table S3.7. Parameters derived from the EXAFS fitting of the Fe and As K-edges shown for the Pb-As-jarosite sample.

Edge	Shells	N	R (Å)	$2\sigma^2(\text{Å}^2)$	Angle As-O-Fe	R (%)
Fe-K	O	6	2.01	0.016		22.89
	S	1.61	3.25	0.012		
	As	0.4	3.31	0.005		
	Fe	4	3.67	0.021		
As-K	O	2	1.69	0.005		26.67
	Fe	1	2.87	0.018	101	
	Fe	1	3.34	0.010	127	
	Fe	1	3.38	0.010	130	

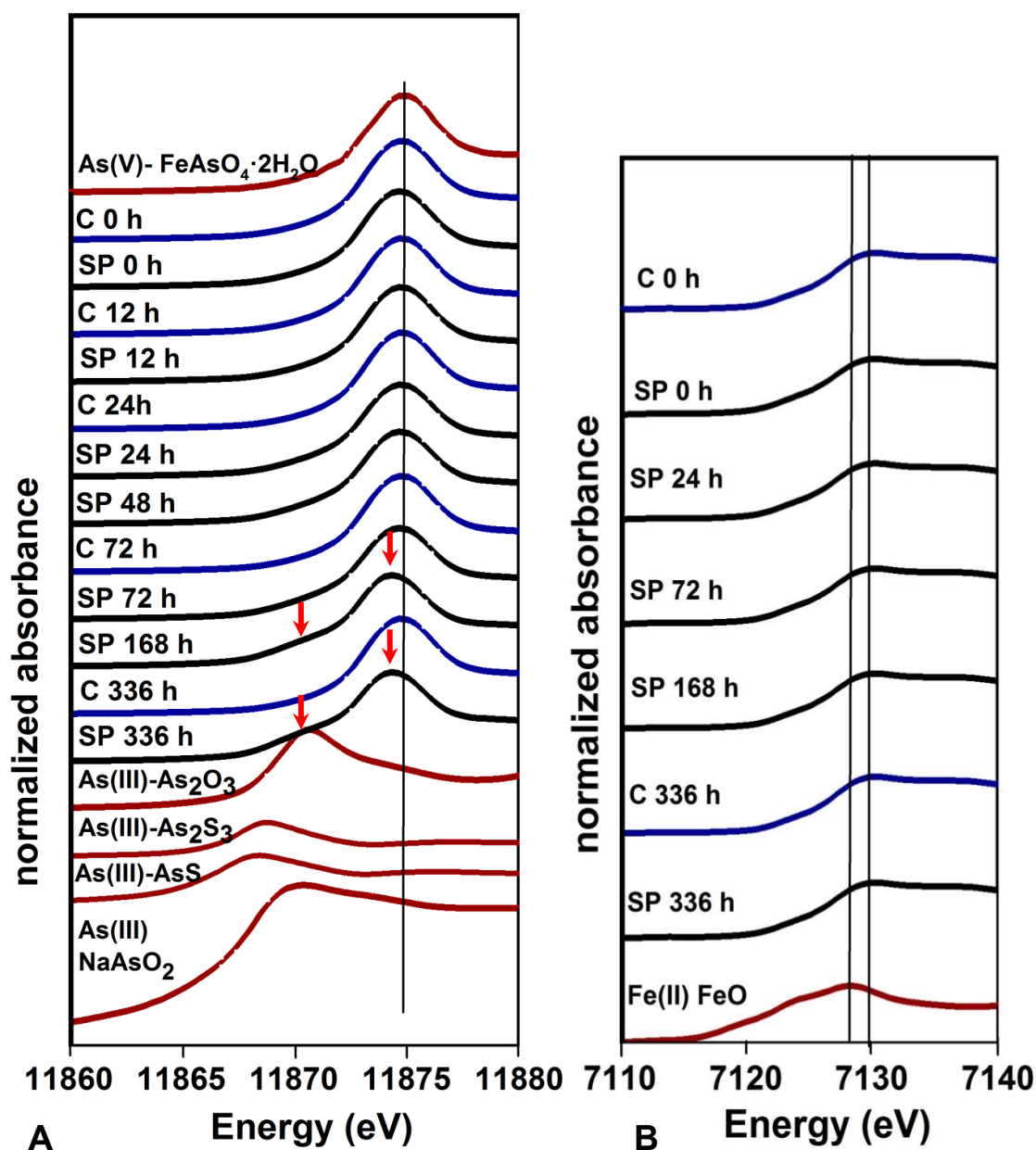


Figure S3.1. Comparison of A) As-K and B) Fe K-edge XANES spectra on standards (red line), inoculated (black line) and control (blue line) Pb-As jarosite samples over time. Red arrows in (A) mark the shift in the As-K edge in inoculated samples and the emergence of an As(III) shoulder at 168 and 336 hours. Note: the inoculated data corresponds to the same spectra presented in Figure 3.2 of the main document.

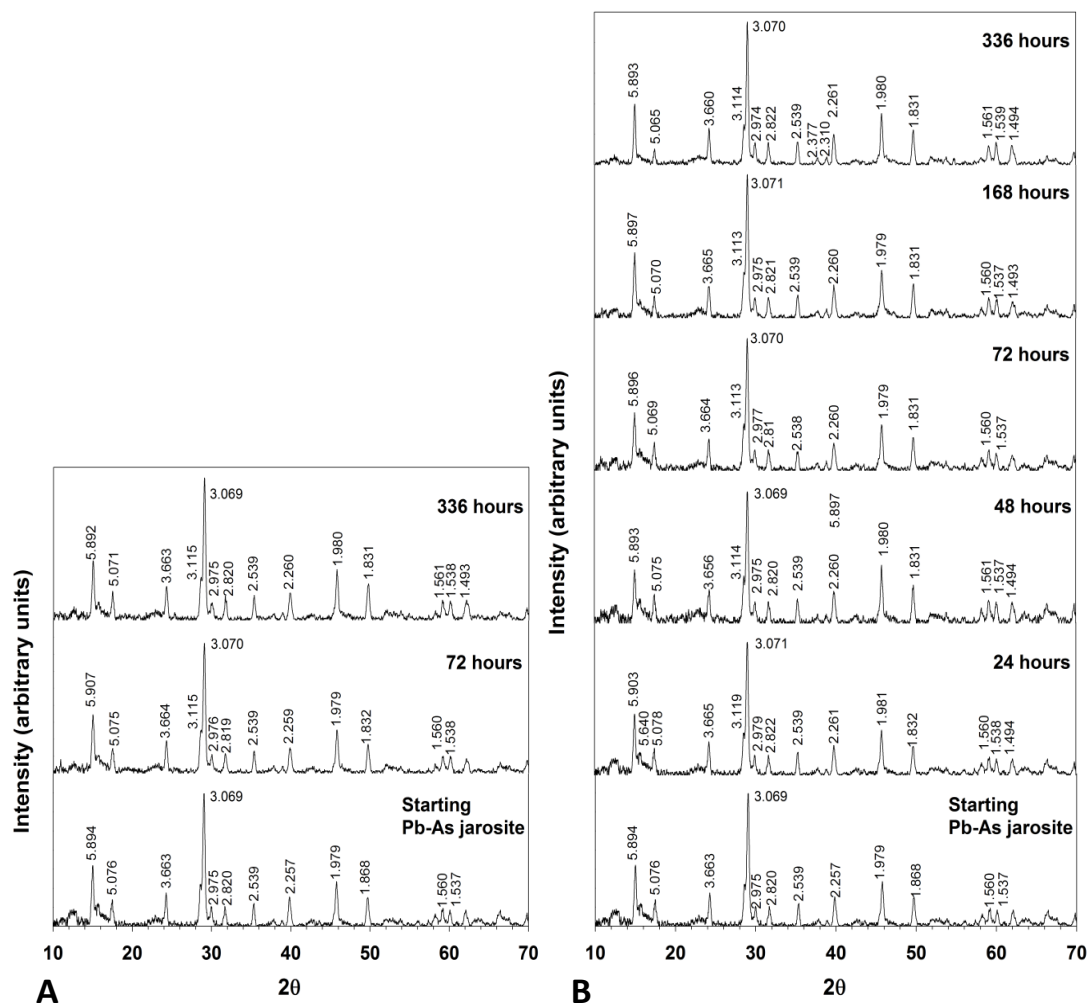


Figure S3.2. Powder X-ray diffraction patterns of A) control, and B) inoculated Pb-As jarosite samples taken at selected time intervals.

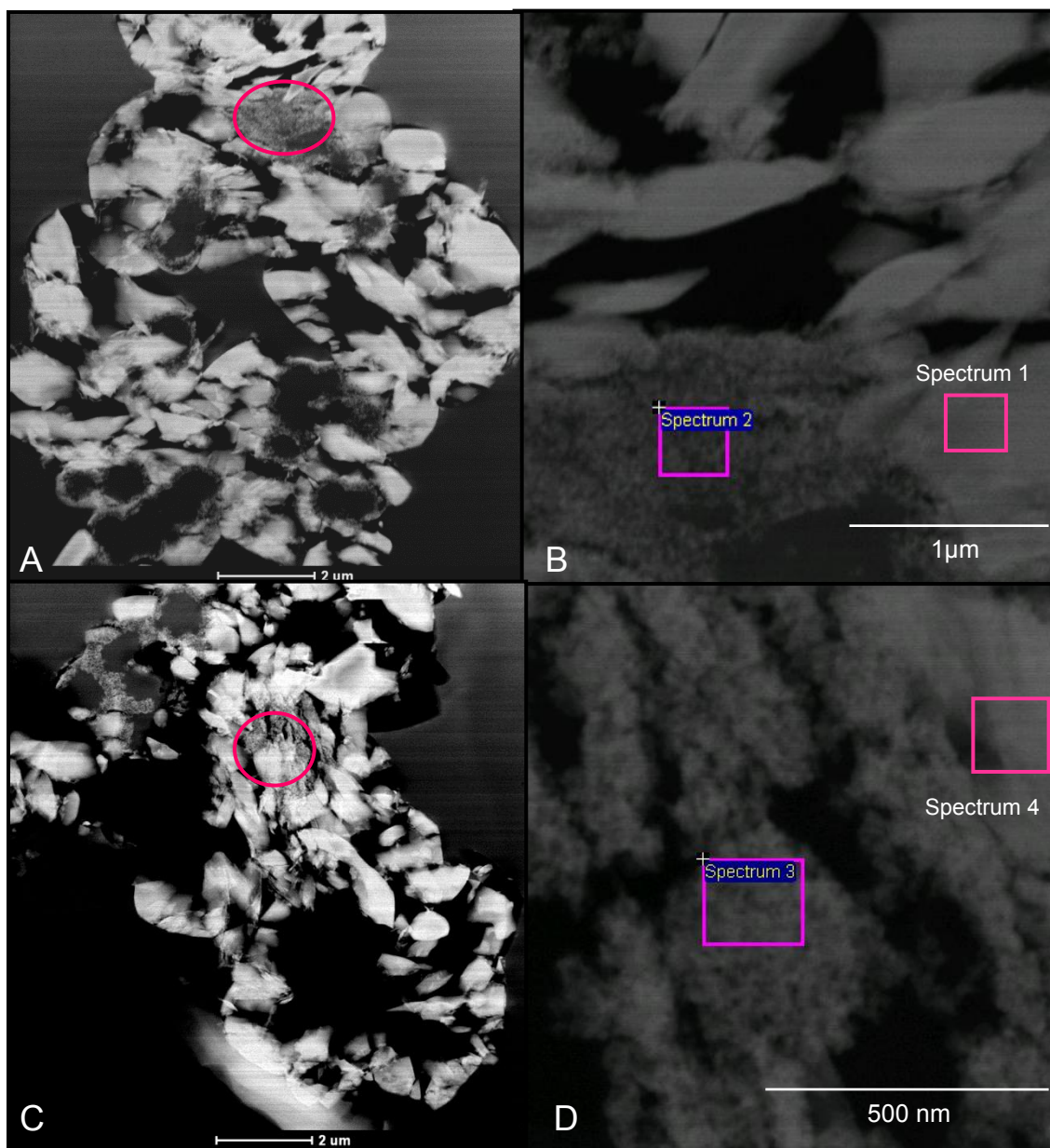


Figure S3.3. TEM images of control Pb-As jarosite samples at 336 hours. Ovals correspond to the area of secondary precipitates magnified in images B and D. Spectrum numbers in B and D denote areas of EDS analysis corresponding to Table S3.5 and EDS spectra in Figure S3.3.

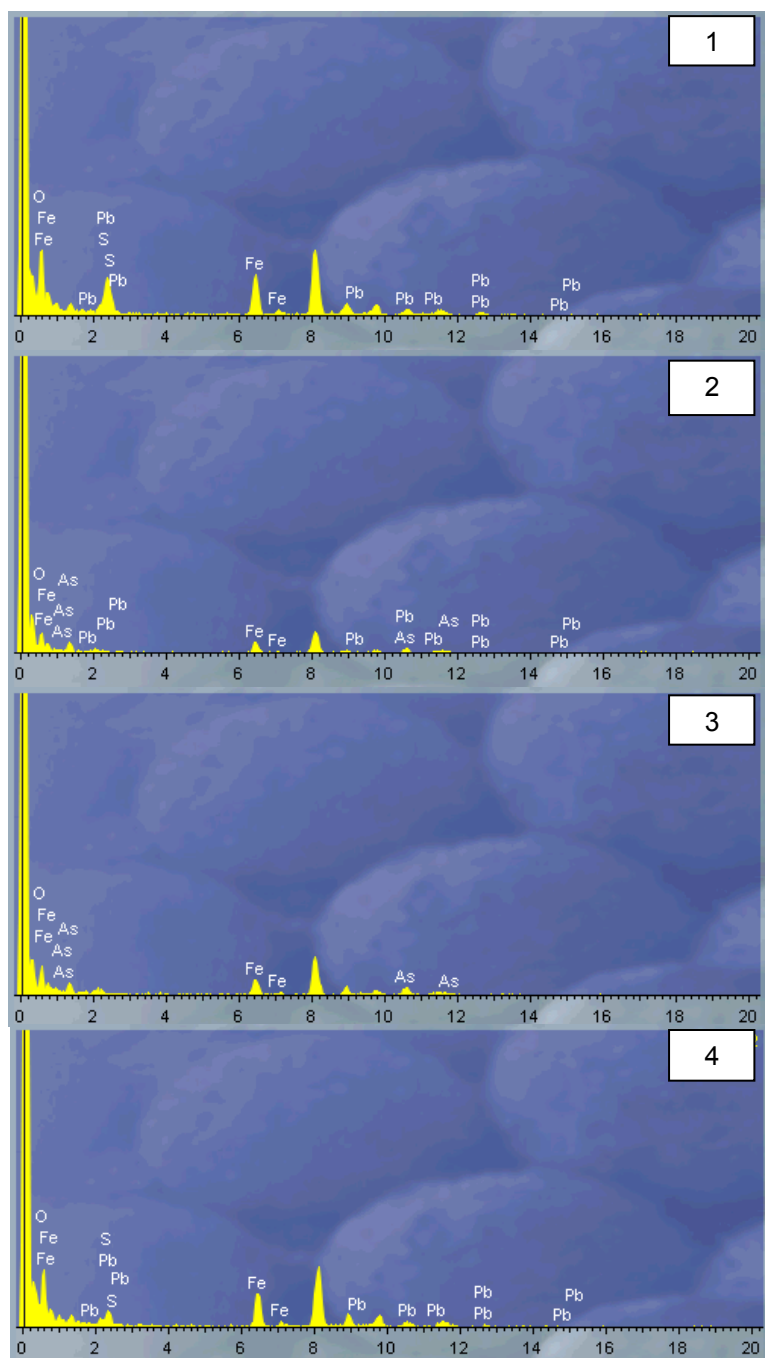


Figure S3.4. Corresponding EDS spectra of areas denoted as Spectrum 1, 2, 3 and 4.

Note: C and Cu peaks at 1 and 8.01 keV were not labeled due to contribution from formvar grids and carbon coating, respectively.

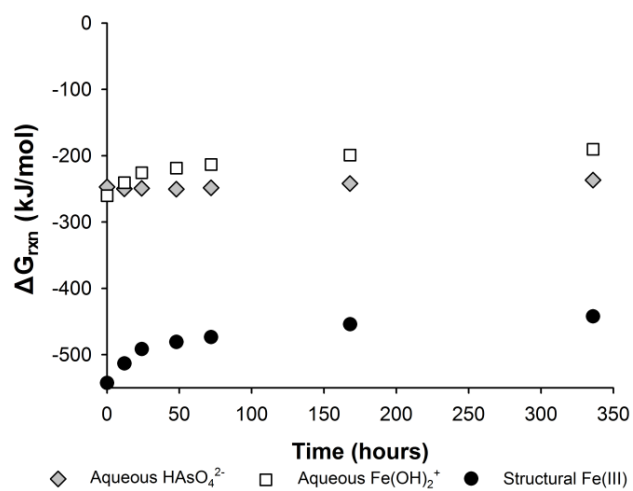


Figure S3.5. ΔG_{rxn} calculated for aqueous As(V), structural and aqueous Fe(III) coupled to lactate oxidation based on experimental conditions over time.

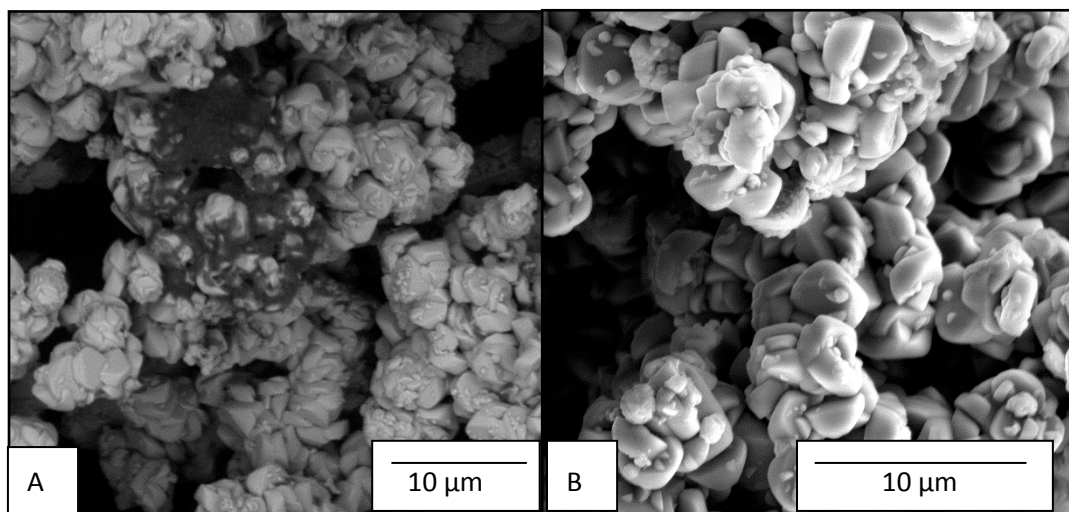


Figure S3.6. A) BSE-SEM image of inoculated Pb-As jarosite at 0 h and B) SE-SEM image of inoculated Pb-As jarosite at 336 hours.

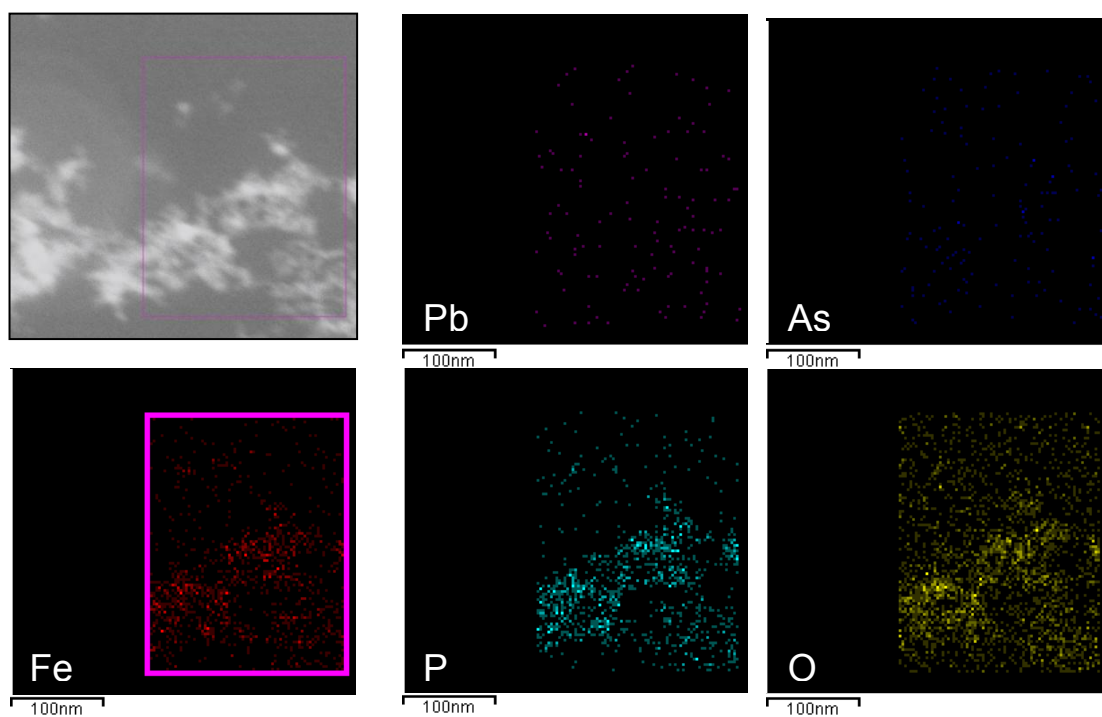


Figure S3.7. TEM-EDS elemental maps of secondary precipitates in the inoculated Pb-As jarosite sample at 336 hours.

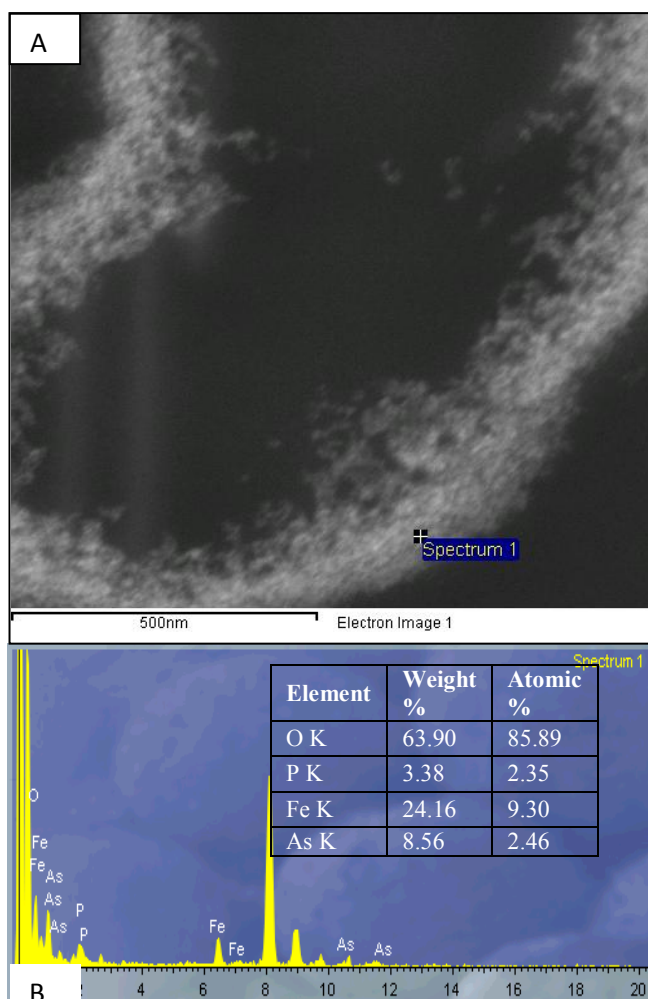


Figure S3.8. A) STEM image of secondary precipitate taken at 336 hours, and B) EDS spectra of area labeled Spectrum 1 and the inlaid table is the relative wt% and at% elemental concentrations of O, P, Fe and As. Note: C and Cu peaks at 1 and 8.01 keV were not labeled due to contribution from formvar grids and carbon coating

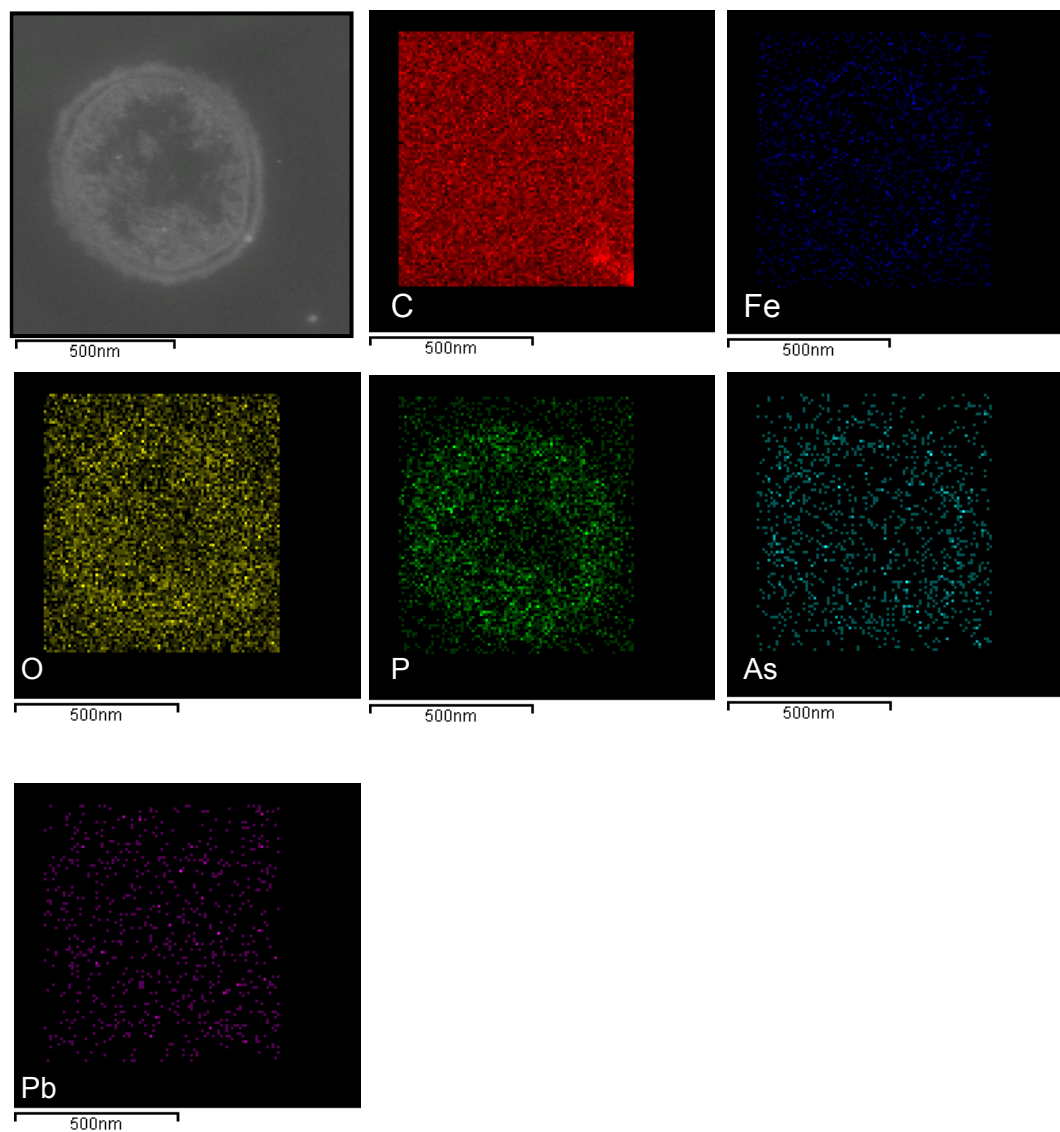


Figure S3.9. TEM-EDS elemental maps of a cross sectioned *S. putrefaciens* cell at 0 hours.

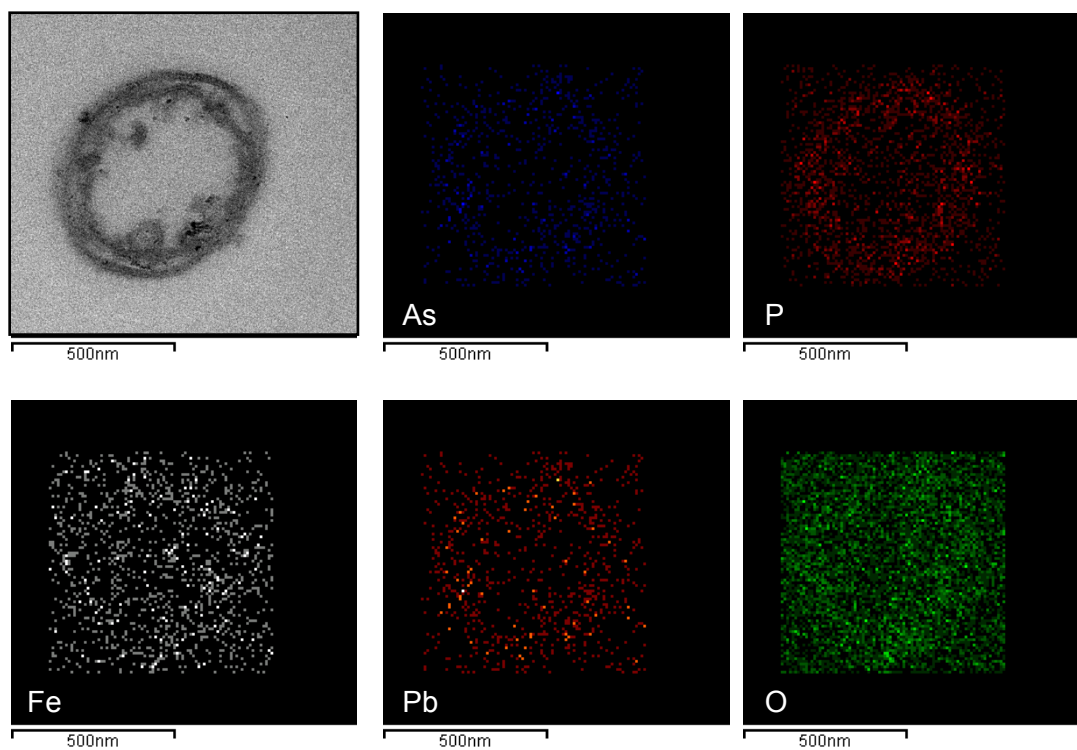


Figure S3.10. TEM-EDS elemental maps of a cross sectioned *S. putrefaciens* cell at 336 hours.

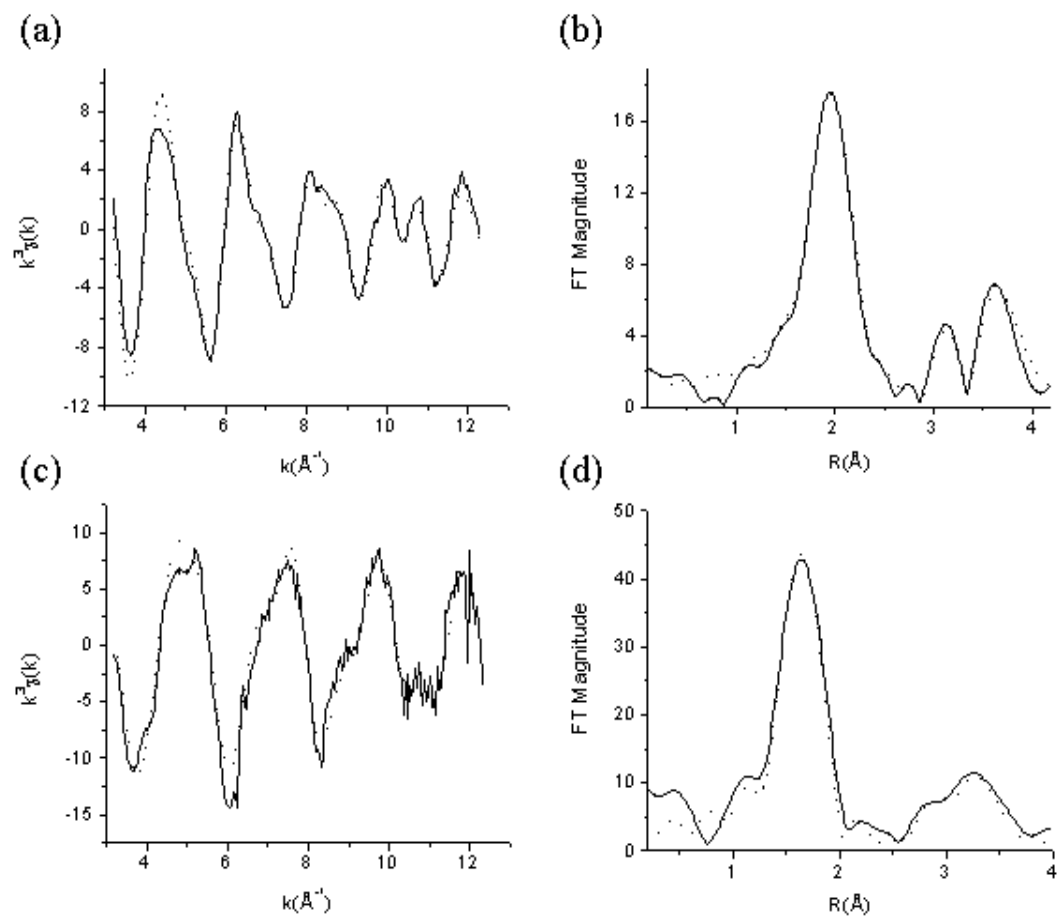


Figure S3.11. (a) Fitted Fe K-edge EXAFS and (b) associated FT. In (c) and (d) are shown the fitted As K-edge EXAFS and FT.

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CHAPTER 4

Reductive dissolution of Tl(I)-jarosite by *Shewanella putrefaciens*:

Providing new insights into Tl biogeochemistry

4.1 INTRODUCTION

An estimated 2 to 5 thousand tonnes of thallium (Tl) per year is mobilized worldwide, primarily through the burning of fossil fuels and the smelting of ferrous and non-ferrous ores.¹ Tl is a priority pollutant and is of particular importance because it is highly toxic to biota and studied to a lesser degree than other prominent metals such as arsenic (As), lead (Pb), cadmium (Cd) and mercury (Hg).^{1,2} Average Tl concentrations range from 0.17-2.8 µg/g in uncontaminated soils, 0.001-0.25 µg/L in groundwater, 0.001-0.036 µg/L in lake water and 0.012-0.016 µg/L in sea water.^{2,3} Elevated Tl concentrations are reported in soils (125 mg/kg), surface waters (534 µg/L), groundwater (1100 µg/L), mine tailings (43 µg/L) and in plants (65 µg/L) at several historical and current mining sites.^{1,4-11} As a chalcophile, Tl is a trace constituent of most zinc (Zn) sulfides (~ 2.2 µg /g), consequently, US electrolytic Zn and Cd plants generate an estimated 3.1 tonnes of Tl per year in solid and liquid wastes.^{12,13} Zn concentrates contain 20-100 mg/L Tl which co-deposits with Zn during electrolysis and is removed during processing to less than 5 mg/L Tl to avoid contamination.¹³ The Zn industry uses the Jarosite process to eliminate iron (Fe) and other impurities such as Tl and Pb through the precipitation of a jarosite compound, $(\text{AFe}_3(\text{SO}_4)_2(\text{OH})_6)$, where A sites are occupied by monovalent (e.g. K^{+1} , Na^{+1} and Tl^{+1}) and divalent cations (e.g. Ca^{+2} , Pb^{+2} and Ag^{+2}).¹⁴ Jarosite precipitation is used in 80% of world Zn production (8 Mt/year) and is advantageous because it is easily filtered, economical and gives low losses of Zn metal.¹⁵ K-jarosite is the most thermodynamically stable jarosite compound, yet Tl is extensively incorporated into K-jarosite when present in synthesis solutions and will preferentially precipitate relative to ammonium or sodium jarosite.¹⁶

While naturally occurring Tl-jarosites are rarely reported, a Tl-jarosite analogue, dorallcharite ($\text{Tl}_{0.8}\text{K}_{0.2}\text{Fe}_3(\text{SO}_4)_2(\text{OH})_6$), was identified on the surface of Tl-rich sulfide ores in Macedonia.^{17,18} The low natural concentrations and historically poor analytical sensitivity of Tl coupled with the use of conventional bulk analytical methods such as x-ray diffraction (XRD) may preclude the identification of Tl-jarosite in the environment. Nevertheless, Tl-jarosite is expected to form under oxidizing, ferric rich and acidic (pH < 3) environments.^{13,17,19} The stability of jarosite is limited by conversion above pH 3.5 to goethite or to meta-stable phases such as schwertmannite or ferrihydrite. Despite the importance of jarosite precipitation for Tl control in the Zn industry and the potential for Tl-jarosite formation under acid mine drainage conditions, very little is known about the mobilization of Tl from these phases. This issue is further exacerbated by the large quantities of jarosites produced in the Zn industry; for example, a plant annually producing 150,000 tonnes of metallic Zn will generate 125,000 tonnes of jarosite.¹⁴

Currently, the most widely used disposal method is in lined ponds under circumneutral conditions. Under these conditions, aqueous Tl(I) is predicted to dominate over Tl(III) due to the high reduction potential ($E^\circ = +1.28\text{V}$) for the Tl(III)/Tl(I) redox couple.^{11,20} However, thermodynamic predictions of Tl speciation versus field measurements has yielded contradictory results.²⁰ As expected, aqueous Tl(I) dominated (> 98%) river waters downstream from an abandoned Pb-Zn mine in Southern France.¹¹ On the other hand, aqueous Tl(III) complexes dominated (68%) water samples taken from the Great Lakes due to Tl(I) oxidation by planktonic bacteria.^{21,22} Tl(I) is also unique compared to many aqueous metals such as Pb or As

because it does not sorb significantly to iron oxides yet may be immobilized as a Tl_2O_3 precipitate via oxidation of Tl(I) to Tl(III) on the surface of Mn-oxides.^{11,23,24}

Determining the biogeochemical stability of jarosites is important to evaluate the potential mobility of metals such as Tl from jarosite disposal sites. Due to the increasing awareness of Tl contamination in countries such as China, Tl is quickly emerging as a pollutant of concern, yet the potential influence of bacteria on Tl mobility remains to be elucidated.^{3,25,26} Tl binds to the sulfhydryl groups of proteins and inhibits a variety of enzymatic reactions and due to the non-discriminatory uptake of Tl(I) over K(I) , Tl(I) will also interfere with a range of K-dependant processes such as $(\text{Na/K})/\text{ATPase}$ synthesis.²⁵ Accordingly, Tl(I) inhibited growth in *Saccharomyces cerevisiae*, *Escherichia coli* and *Bacillus megaterium* while increased aqueous K concentrations alleviated Tl toxicity in *S. cerevisiae* and *B. megaterium*.²⁷ Similarly, increased intracellular Tl accumulation decreased intracellular K accumulation in the cyanobacterium *Synechocystis* PCC 6803.²⁸ The toxicity of Tl is also dependant on oxidation state; for example, Tl(III) was 50,000-fold more toxic than Tl(I) to the unicellular green algae *Chlorella*.²⁹ Biomethylation of Tl was also shown in a mixed bacterial culture isolated from sewage sludge in lake sediment under anaerobic conditions.³⁰

Despite the environmental relevance of Tl and jarosites, few studies have focused on the abiotic and biotic dissolution of jarosites under anaerobic circumneutral conditions with even fewer studies focused on general Tl biogeochemistry.³⁰⁻⁴¹ Therefore, the objectives of this study were to: 1) assess the effects of the model metal reducing bacterium, *Shewanella putrefaciens* CN32, on the reductive dissolution of

synthetic Tl-jarosite under circum-neutral anaerobic conditions, and 2) evaluate the fate and toxicity of Tl on *S. putrefaciens* CN32.

4.2 MATERIALS AND METHODS

4.2.1 Preparation of Tl-jarosite/Cell Suspensions and Sampling Protocol.

Tl(I) jarosite was synthesized using ACS grade reagents according to the method outlined by Dutrizac (1997) with the resulting chemical formula:

$(\text{H}_3\text{O})_{0.29}\text{Tl}_{0.71}\text{Fe}_{2.74}(\text{SO}_4)_2(\text{OH})_{5.22}(\text{H}_2\text{O})_{0.78}$.^{13,42} (See Supporting Information (SI)

Section 4.1 for more details). *Shewanella putrefaciens* CN 32 cultures (ATCC# BAA-453) were prepared from 1.5 mL frozen glycerol stocks maintained at -80°C and were grown, harvested and transferred into a modified M1 minimal media (SI Section S4.2). The inoculated media was divided and one portion was autoclaved to serve as a heat killed experimental control. All solutions were prepared from ACS grade reagents and were either filter sterilized (0.2 µm) or autoclaved.

Minimal media was transferred to an anaerobic chamber (95% N₂/5% H₂) and 15 mL aliquots were dispensed into 20 mL polypropylene test tubes containing 0.0561 ± 0.0097 g of Tl-jarosite. Sample treatments varied based on the presence and/or viability of *S. putrefaciens* and contained either a) no cells (48 samples), b) viable cells (48 samples), or c) autoclaved cells (18 samples). Additional sets of controls containing only minimal media were prepared to examine background pH, Eh, ATP and elemental concentrations over time in the absence of Tl-jarosite and contained either a) no cells (8 samples), or b) viable cells (8 samples). All samples were capped, sealed with parafilm, covered with aluminum (Al) foil and rotated end-over-end at 20 rpm in the anaerobic chamber using a bench top rotator (Glas-Col®) at 28 °C and were sampled at selected

time intervals. Samples containing Tl-jarosite were sampled in triplicate while minimal media controls (i.e. no Tl-jarosite) were sampled in duplicate.

All samples were monitored for pH (Thermo Ross Sure-flow semi micro pH probe) and Eh (Thermo Ross Sure Flow combo redox/ORP) at each sampling interval. Slurries from each sample were collected at selected time intervals (0, 14, 24, 84, 132, 325, 493 and 893 hours) and imaged immediately (i.e., usually within 2 hours) using field emission-environmental scanning electron microscopy (FE-ESEM, FEI-Quanta 200F) (SI Section S4.3). Microbial metabolism was monitored using the Promega BacTiter-Glo™ Microbial Cell Viability Assay, a rapid and sensitive ATP-based luminescent technique. A slurry (1.0 mL) from each sample was collected and divided into two aliquots. To avoid an overestimation of intracellular ATP, extracellular and intracellular (i.e. microbial) ATP were separated by filtration through a 0.1 µm syringe filter (Millex®-VV, Millipore) and analyzed separately using the same analytical protocol (SI Section S4.4).⁴³ Intracellular ATP (referred to as ATP_{INT}) was calculated by subtracting extracellular ATP from total ATP. Starting ATP concentrations were converted to cell counts using an average ATP-per-biovolume concentration of 1.75×10^{-10} nmol/cell ATP and the starting cell concentration were 1.3×10^8 cells/mL.⁴³

The remaining sample was syringe filtered (0.20 µm) and a subsample (100 µL) of the filtrate was analyzed immediately for Fe(II) and total Fe concentrations via the Ferrozine method as described by Viollier et al. (2000).^{44,45} The remaining filtrate was diluted and acidified with sub-boiling doubly distilled 0.016 M HNO₃ and stored at 4°C until analysis. Aqueous concentrations of Tl, Fe, S and all minimal media components

(i.e. K, Mg etc.) of the stored samples were determined using inductively-coupled plasma optical emission spectroscopy (ICP-OES).

4.2.2 Tl sorption and toxicity experiment. To evaluate Tl toxicity and the potential for Tl sorption onto *S. putrefaciens* cells, a solution of reagent grade Tl_2SO_4 (80 mM) was added to suspensions of living and autoclaved bacteria dispersed in 0.1M NaCl to give a final concentration of 35 μM Tl and sampled over time (0, 24, 72 hours). Additional cell-free controls were prepared to examine the potential for abiotic Tl removal via precipitation. The pH was adjusted to pH 5 and 6.2 using 0.1M HCl. The pHs were chosen to reflect the final pH of the biotic experiment (pH 6.30) and to examine changes in Tl sorption at a lower pH (pH 5). Peter et al., (2007) showed optimal Tl adsorption onto *Aspergillus niger* between pH 4 and 5.⁴⁶ The initial and final pH were measured and ATP_{INT} concentrations were determined (SI Section S4.4). Samples were filtered through a 0.1 μm syringe filter (Millex[®]-VV, Millipore) and analyzed for aqueous Tl concentrations using ICP-OES. Initial cell concentrations were $0.265 \text{ mg}_{\text{dw}} \text{ mL}^{-1}$ (1.3×10^8 cells/mL) and cell growth was also monitored by measuring the cultures' optical density at 600 nm.⁴³

4.3 RESULTS AND DISCUSSION

4.3.1 Tl sorption experiment. Batch sorption tests were conducted to compare Tl removal from solution in autoclaved cells, viable cells and Tl spiked media control samples in the absence of Tl-jarosite over 72 hours at pH 5 and 6.3. Differences between initial and final aqueous Tl concentrations were compared between the three treatments and showed no significant statistical difference after 72 hours at pH 5

(ANOVA, $F=0.05$, $p = 0.948$) and 6 (ANOVA, $F=1.33$, $p= 0.333$) (SI Table S4.1). Tl sorption onto *S. putrefaciens* biomass may be undetectable due to the low mass of *S. putrefaciens* (dry weight: 0.265 mg/mL, 4.04×10^8 cells/mL) chosen to correspond with bacterial concentrations used in the Tl-jarosite experiment. Despite low cell concentrations, Norris et. al (1976) showed Tl uptake in similar cell concentrations of *E. coli* (dry weight: 0.40 mg/ mL) at higher Tl concentrations (100 μ M). Aside from differences in bacterial species, the lack of detectable Tl sorption onto *S. putrefaciens* over *E. coli*. may also be due to redox conditions. Haas et al. (2004), showed decreased molar site concentrations of functional groups in *S. putrefaciens* 200R under anaerobic conditions and suggested *S. putrefaciens* may be a poorer adsorptive agent under anaerobic conditions.⁴⁷ Sorption is also not expected to be a significant mechanism for Tl immobilization because Tl(I) does not form strong complexes with ligands due to anti-bonding electrons in the outer *s* orbital; particularly with the phosphoryl functional groups expected to dominate at the cell wall at pH 5 and 6.⁴⁸

The toxicity of Tl to *S. putrefaciens* was investigated as a potential obstacle to Tl sorption. Internal ATP concentrations provide a useful indirect metric for microbial biomass estimates in both uncontaminated and metal-contaminated soils; even at high metal concentrations.^{49,50} A particular advantage of determining ATP concentrations is the short 1/2 life of extracellular ATP released from cells during cell lysis (0.5 h, 16°C).^{51,52} Intracellular ATP (referred to as ATP_{INT} hereafter) concentrations in both treatments at pH 5 decreased to below detection limits within 24 hours and showed no significant differences in the decline of ATP_{INT} between control (i.e. no Tl) and Tl-spiked samples (t-test, $p= 0.588$) (Figure 4.1A). By 48 hours, samples at pH 6

containing Tl had significantly higher ATP_{INT} concentrations than control samples (t-test, $p = 0.011$). Irrespective of Tl treatment, differences in pH showed significant differences in ATP_{INT} concentrations within the control (t-test, $p=0.030$) and Tl-spiked (t-test, $p=0.0003$) samples. At 72 hours, ATP_{INT} concentrations in pH 6 samples remained detectable (~ 0.2 nM) and undetectable in pH 5 samples with no significant differences (t-test, $p > 0.05$) between ATP_{INT} decline between 0 and 72 hours between pH regimes or treatments. ATP_{INT} concentrations complemented the OD_{600nm} measurements which also showed decreased turbidity over time in all samples (SI Figure S4.1). Cell death at pH 5 was attributed to stress associated with the acidic pH rather than Tl toxicity and corroborates previous studies that show low viability of *S. putrefaciens* under low pH (< 4) conditions. At pH 6, viability was not related to Tl toxicity and may be due to the lack of nutrients in the NaCl medium. Interestingly, there was greater ATP_{INT} in pH 6 samples containing Tl rather than in the control. The slightly lower pH of the control (6.15) versus the Tl-spiked (6.28) samples may have contributed to decreased viability.

Previous studies demonstrated rapid and active Tl uptake/efflux in fungal and bacterial biomass related to the substitution of Tl(I) over K(I) in cellular Na/K-ATPase transport systems due to the greater electronegativity of Tl (1.62) versus K (0.82).²⁷ Accordingly, Tl toxicity may be reduced by increasing the K:Tl ratio to suppress Tl uptake. Hassler et al., (2007) showed decreased Tl toxicity in the microalga *Chlorella* sp. by increasing K concentrations to an excess of 40 -160 times that of Tl which suppressed intracellular Tl accumulation.⁵³ Inhibition of Tl uptake by K could be particularly important for Tl-jarosite disposal because it is expected to co-precipitate

with K-jarosite and the simultaneous release of K and Tl during dissolution may serve to decrease toxicity in biota. Alternatively, depending on the sorptive capacity of the microbial community, high concentrations of K may also serve to reduce uptake in native bacteria and potentially increase Tl mobility.

The high background concentrations of the typical major cellular cations, K, Mg and Ca contributed by the minimal media in the Tl-jarosite experiment precluded the measurement of their potential flux from *S. putrefaciens*. The simple, unbuffered media (0.1M NaCl) used in the sorption tests afforded the measurement of aqueous K, Mg and Ca release over 72 hours (See SI Figure 4.2). Student T-tests were used to determine the statistical significance of the variation in initial and final aqueous concentrations (normalized to OD_{600nm}) of the major cellular cations (i.e. K¹⁺, Ca²⁺ and Mg²⁺) in samples containing viable cells between different treatments (i.e. no Tl versus Tl) and pH values (pH ~ 5 and 6) to determine if cation cellular efflux was driven by either: a) the presence of Tl, b) the pH of the samples, or C) both pH and Tl. Within 24 hours, aqueous K concentrations increased in samples containing viable cells (SI Figure 4.2). However, it was the pH rather than Tl which contributed to a significant statistical difference in K release in the control (i.e. no Tl, p = 0.005) and spiked Tl (p = 0.004) samples and confirms previous observations which showed release of aqueous K from *S. putrefaciens* 200R during acid-base titrations.⁵⁴

In the viable Tl-free and Tl-spiked samples at pH 5, Mg concentrations increased between 0 and 24 hours to autoclaved concentrations followed by a plateau for the rest of the experiment. In contrast, in the corresponding pH 6.3 samples there is a linear increase (i.e. no plateau) over time in the control and Tl-containing samples.

Interestingly, in samples containing Tl, initial Mg concentrations were higher and similar to autoclaved cell concentrations (3.4 μM Mg) and increased linearly to 5.5 μM at 72 hours while in Tl-free control samples, initial Mg was lower (0 μM) and increased to autoclaved cell concentrations (2.87 μM Mg) by the end of the experiment. The only significant difference in the variation of aqueous Mg release over time was between samples under different pH conditions which contained Tl ($p = 0.024$). The difference is likely attributed to cell death in the lower pH samples (pH 5) as a result of the low pH conditions which likely inhibited an active Mg efflux transport process.

Despite the higher overall concentrations ($\sim 12 \mu\text{M}$) of Ca in viable samples containing Tl at pH 6.3 compared to all other treatments ($\sim 6 \mu\text{M}$), there was no significant difference in the variation of aqueous Ca release in the presence of Tl or across different pH values (SI Figure 4.2). Given that Tl uptake is a rapid and active process, the higher initial concentrations of Ca may be an immediate, metabolic response by *S. putrefaciens* to control Tl influx and efflux. Previous studies have shown that Tl(I) acts as an analogue for K^+ in Na, K ATPase, and is actively transported across the cell membranes. However, it is likely that if Tl substitutes for K, there would be a significant difference in aqueous K release between Tl-free and Tl-spiked samples at the same pH which was not observed in this experiment. An alternative Tl uptake mechanism may be due to the higher Ca concentrations observed in the samples containing viable cells and Tl at pH 6.3 to maintain uptake/efflux. In fact, Ca was shown to compete with Tl during uptake in the aquatic plant *Lemna minor* where Tl uptake was inhibited in the presence of high Ca concentrations. This may be due to Tl movement through $\text{Ca}^{2+}/\text{K}^+$ ATPase which are not as well studied or understood in bacteria as

Na⁺/K⁺-ATPase facilitated transport. Ca²⁺-ATPase channels are not documented in *S. putrefaciens*, however, they have been reported in close association with K⁺ channels in *Streptomyces lividans* and were shown to regulate Ca efflux in *E. Coli*.^{55,56} Therefore, it is possible that the higher aqueous Ca concentration baseline established at the beginning of the experiment occurred very quickly and prior to sampling (< 2 hours) as a result of constant Tl uptake and Ca efflux. However, in the absence of ATPase measurements and experiments conducted at varying Tl concentrations and cell concentrations, the Tl detoxification mechanism in *S. putrefaciens* remains to be elucidated and provides a framework for future experimental refinement.

4.3.2 Tl-jarosite Control Samples. Aqueous Fe(II), Fe(III) and Tl concentrations were below analytical detection limits in both sterile and inoculated minimal media samples (i.e., in the absence of Tl-jarosite) and remained buffered at pH 7.2 throughout the experiment. Sulfate release over time could not be used to evaluate Tl-jarosite dissolution due to the background contribution of S (34 mM) by the PIPES buffer which destabilizes upon acidification of samples during ICP-OES preparation and contributes to higher sulfate concentrations during analysis.⁵⁷ In cell-free and autoclaved cell Tl-jarosite samples, total Fe_{aq} concentrations remained below the detection limits of the Ferrozine method (i.e., 0.3 uM in undiluted samples) and were between 3-5 uM (ICP-OES) for the duration of the experiment (Figure 4.1D).⁵⁸ Low Fe concentrations in the control samples were consistent with minimal Fe concentrations observed during K, Pb, As and Pb-As jarosite dissolution and is attributed to the low solubility of Fe(III) at circumneutral pH.^{32,33,59,60}

Aqueous Tl release from Tl-jarosite was linear in both control treatments and was 3.25 times slower in samples containing autoclaved cells ($0.20 \pm 0.18 \mu\text{M} \cdot \text{h}^{-1}$, $r^2 = 0.955$) than in cell-free samples ($0.65 \pm 0.29 \mu\text{M} \cdot \text{h}^{-1}$, $r^2 = 0.966$) (Figure 4.1D). At the termination of the experiment, final $\text{Tl}_{(\text{aq})}$ concentrations were $514.81 \pm 74.25 \mu\text{M}$ ($n=3$) and $2124.89 \pm 65.13 \mu\text{M}$ ($n=3$) in autoclaved cell and cell-free samples, respectively. Sorption onto autoclaved *S. putrefaciens* was discounted as a possible sink for Tl because *S. putrefaciens* did not show significant Tl uptake in the sorption experiment (SI Table S4.1). Attraction between the cell and jarosite surface was not considered in this study, yet previous electrophoretic mobility measurements of K-jarosite in 0.01M NaCl revealed two isoelectric points (pH_{IEP}): 3.9 and 5.7 with a zeta potential of ~ 10 mV at pH 7.⁶¹ Between the isoelectric points, jarosite was electronegative which was attributed to iron(III) hydroxide formation. However, the jarosite surface was electropositive below pH 3.9 and above pH 5.7. Chubar et al. (2008), measured a $\text{pH}_{\text{IEP}} \sim 4$ in both autoclaved and viable *S. putrefaciens* CN32 cells in 0.1M NaCl with a zeta potential of ~ 18 mV at pH 7.⁶² Therefore, decreased dissolution of samples containing autoclaved cells may be related to the electrostatic surface attraction between the negatively charged cell surface of dead *S. putrefaciens* cells with the positively charged Tl-jarosite surface. This attraction may serve to passivate the mineral surface by reducing the Tl-jarosite surface area available for dissolution. However, in the absence of electrophoretic measurements in our study, the mechanism remains to be elucidated and the comparison to other studies should be interpreted cautiously due to the differences in jarosite phases and experimental conditions. Aqueous Tl concentrations of cell-free samples were higher than mass normalized K concentrations ($\sim 1500 \mu\text{M}$) reported by Smith et al.

(2006) during the unbuffered abiotic dissolution of K-jarosite and is likely a reflection of the structural strain imposed by the larger Tl(I) ion in the jarosite structure expected for alkali site (A-site) substitutions.¹³ The substitution of Tl (1.49 Å) versus K (1.33 Å) expands the structural *c* parameter of the crystal lattice resulting in the largest known inter-layer spacing in the alunite-jarosite family thereby decreasing the stability of Tl-jarosite over K-jarosite.^{13,17,63}

A distinctive colour change typically associated with iron hydroxide formation was observed in cell-free control samples, whereby the original yellow Tl-jarosite changed to a reddish hue at 132 hours and darkened to a deep red by the termination of the experiment (SI Figure S4.3).⁶⁴ BSE-SEM images of the cell-free Tl-jarosite surface showed extensive pitting after 168 hours (SI Figure S4.4 A-C) and channelling (SI Figure S4.3 B) at 893 hours similar to surface textures observed during K-jarosite dissolution.⁶⁵ Over time, SEM images show a gradual comminution of cell-free samples and extensive secondary mineralization characterized as spherical precipitates similar to those identified as goethite during the dissolution of Pb and K-jarosite (Figure 4.2B and D).^{32,63} Mineral saturation indices were calculated by the React program within the Geochemist's Workbench® (GWB, version 7.01) using solution chemistry at 893 hours (SI Table S4.2) and predicted the precipitation of hematite > schwertmannite > goethite > Fe(OH)₃ (SI Table S4.3). Hematite was discounted because it is expected to form over longer durations at circumneutral pH.⁶⁶ Energy dispersive X-ray (EDX) spectra of a representative secondary precipitate at 893 hours taken away from the original Tl-jarosite shows the presence of Fe (25.24 At %) and O (74.76 At %) potentially confirming the formation of an Fe oxide or hydroxide (SI Figure S4.5).

The predicted formation of goethite or $\text{Fe}(\text{OH})_3$ is important because iron oxides are common minerals in soils and colloids in aquatic environments and exert significant control over the concentrations and speciation of trace elements such as Pb, As and Cd.²³ For example, during the abiotic dissolution of Ag and Pb-jarosite, Ag and Pb concentrations were below detection limits and were either sorbed or co-precipitated with Fe-oxides.^{31,67,68} In contrast, Tl(I) is highly soluble and has a low affinity for hydrous ferric oxides. Jacobsen et. al (2005), reported 1.5% Tl versus 100% Ag sorption onto ferrihydrite at pH 7.^{11,67,69} Based upon atomic radii and oxidation state, one may expect similar iron hydroxide surface complexation mechanisms for monovalent Ag (1.31 Å) and Tl (1.49 Å). However, given the electron configuration of Tl(I): ($[\text{Xe}] 4f^{14}5d^{10}6s^2$), the 6s orbital electrons are σ -antibonding whereas the two electrons in $4d_{z^2}$ orbital of Ag(I):($[\text{Kr}]4d^{10}$) move to the empty 5s orbital to form relatively strong bonds with surface complexes.⁶⁹ Liu et al. (2011), showed 35.4-78.5% Tl sorption onto goethite and suggested that increased Tl sorption may also be related to the differences of surface structure and crystallinity between ferrihydrite and goethite.⁷⁰

To account for the high concentrations of Tl observed in this experiment, the React program (GWB ®) was used to predict the speciation of aqueous and sorbed Tl. The Dzombak and Morel (1990) diffuse layer complexation model (DLM) was incorporated into the reaction model and the database was amended to include surface complexation constants for weak and strong $>\text{FeOTl}$ sites.¹¹ Using solution data collected at 893 hours, React predicted that 8.0% and 0.2% of Tl was sorbed to the weak and strong sites of the iron hydroxide, respectively. A small percentage (0.6 %) would remain as aqueous $\text{TlCl}(\text{aq})$ and 91.2 % would dominate as aqueous Tl(I) . While

thermodynamic modelling was useful to evaluate Tl-jarosite dissolution, the predicted Tl speciation should be interpreted qualitatively rather than quantitatively because the predictions were not confirmed experimentally. Moreover, at 863 hours experimental samples did not reach equilibrium. Nonetheless, the predicted high concentrations of free $\text{Tl}^+_{(\text{aq})}$ are consistent with field observations and theoretical predictions where the aqueous Tl(I) species dominates in near neutral pH and low Eh environments.^{11,20} Based on aqueous Tl data and thermodynamic modelling, Tl did not sorb significantly to the iron oxides predicted to form in this study which even under abiotic conditions may ultimately increase Tl mobility and bioavailability in the environment.^{31,32,34}

4.3.4 Microbial Reduction of Tl-jarosite. Aqueous Fe(III) concentrations remained below detection limits in all Tl-jarosite samples containing viable cells. However, Fe reduction, $\text{Fe(II)} = 31.97 \pm 4.41 \mu\text{M}$ ($n=3$), was observed within the first sampling period and likely occurred in the time between initial inoculation and ferrozine analysis (approx. 1 hour) (Figure 4.1D). Early onset of Fe reduction coincided with increased Eh as compared to inoculated minimal media controls where Fe reduction was absent and is presumably due to electron transfer to Fe(III) (Figure 4.1B). Aqueous Fe(II) concentrations increased at a rate of $7.3 \pm 2.22 \mu\text{M} \cdot \text{h}^{-1}$ ($r^2 = 0.866$) until steady state was reached at 493 hours. Subsequently, aqueous Fe(II) increased minimally from $4266.72 \pm 85.60 \mu\text{M}$ to $4569.49 \pm 150.01 \mu\text{M}$ between 493 and 893 hours (Figure 4.1D). Maximum Fe(II) concentrations ($4569.49 \pm 150.01 \mu\text{M}$) observed in this study were higher than those reported in a similar study using Ag-jarosite ($1701 \mu\text{M}$) with similar cell densities of *S. putrefaciens* under comparable conditions.³⁴ Differences are likely explained by structural differences between Tl-jarosite and Ag-jarosite whereby Tl(I)

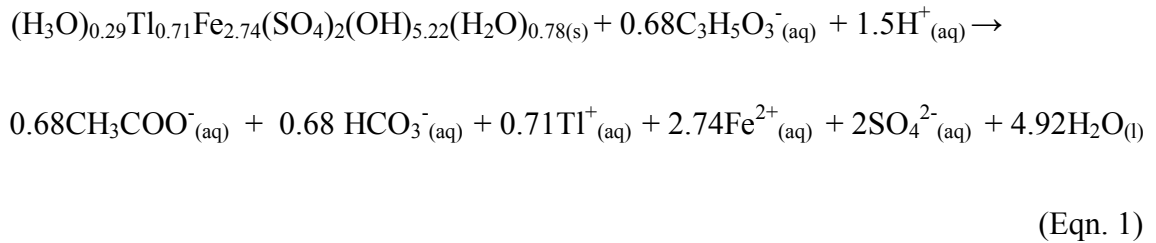
(1.49 Å) is larger than Ag(I) (1.31 Å) resulting in larger inter-layer spacing thereby increasing solubility. Additionally, *S. putrefaciens* demonstrated Ag(I) reduction to elemental Ag(0) from Ag-jarosite and may have preferentially used Ag as a terminal electron acceptor over Fe(II) thus reducing total Fe reduction.³⁴

Initial Tl_(aq) release from Tl-jarosite was rapid in samples containing viable *S. putrefaciens* cells ($19.15 \pm 3.69 \mu\text{M} \cdot \text{h}^{-1}$, $r^2 = 0.938$) until 156.5 hours when concentrations began to plateau and increased gradually from $2613.30 \pm 29.34 \mu\text{M}$ to $3169.25 \pm 70.41 \mu\text{M}$ between 156.5 and 893 hours, respectively (Figure 4.1D). BSE-SEM images from 893 hours show an electron dense cubic secondary precipitate in close proximity to Tl-jarosite and EDS analysis of the precipitate confirmed the presence of Tl (20.56 At%), S (46.94 At%) and O (32.51 At %)(Figure 4.3D and SI Figure S4.6). The phase is likely Tl(I) sulfate (Tl₂SO₄), and the non-ideal stoichiometry of the precipitate is attributed to the elemental signature from the underlying Tl-jarosite. Based upon the lack of Tl uptake by *S. putrefaciens* in the sorption experiment, it is unlikely that Tl sorption onto *S. putrefaciens* is a significant Tl sink during the reductive dissolution of Tl-jarosite.^{27,46}

The largest variation of ATP_{INT} (10.11 ± 9.7287) in the Tl-jarosite samples was at 38.5 hours and coincided with the largest variations in aqueous Tl (860.16 ± 417.06) and Fe(II) (516.89 ± 267.83) concentrations. At this time interval, the greatest decrease in ATP concentrations occurred in replicates containing the highest Tl and Fe concentrations (Figure 4.1C). During the Tl sorption experiment, a subsample of viable cells were exposed to a similarly high concentration of Tl (2,455 μM) encountered in

this experiment at pH 6.65 and the ATP concentrations were measured immediately in order to track the ratio of extracellular ATP to total ATP. The ratio of filtered to total ATP was 0.69 ± 0.05 (data not shown) at time 0 which corresponds to only 31% ATP_{INT} associated with intracellular (viable) ATP as compared to > 99% intracellular ATP (data not shown) associated with *S. putrefaciens* at the low Tl concentration (35 μ M) in the sorption experiment. Therefore, high Tl concentrations likely resulted in substantial decreased cell viability. Despite the potentially low viability of *S. putrefaciens*, Fe reduction continued until sometime between 325 and 493 hours.

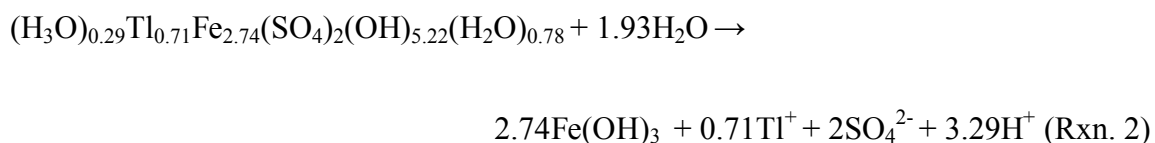
Based on aqueous Fe(II) and Tl concentrations, samples reached equilibrium between 493 and 893 hours. Equilibrium concentrations of Tl, Fe(II), pH and Eh at 893 hours were used to predict saturation indices (log Q/K). Ratios of Fe to acetate, sulfate and bicarbonate were used to estimate equilibrium concentrations according to the following reaction (SI Table S4.2):



The React program (GWB) predicted the formation of hematite > magnetite > goethite (SI Table S4.4). Hematite was discounted because it is expected that a Fe(II) phase will form. While the particle morphology is characteristic of the "pin cushion" morphology associated with schwertmannite (Figure 2A), EDX analysis of 2 of these locations show low concentrations of sulfur (1.85 - 2.66%) and enrichments in Fe and O (SI Figure S4.7). Given the dark brown colour change (SI Figure S4.3), pH and redox conditions

(Figure 4.1B) the phase is likely magnetite or a precursor such as green rust. Due to the lack of equilibrium constants for Tl adsorption onto Fe(II) minerals, sorption could not be modelled. However, based on SEM observations, it is expected that secondary precipitation of a Tl(I) precipitate such as the Tl(I) sulfate observed in this study and is likely the most important sink for Tl during the dissolution of Tl-jarosite.

4.3.5 Influence of pH. Due to the instability of jarosite minerals at circum-neutral pH and the production of H^+ observed during dissolution in previous studies, decreases in pH may also be used as a proxy for jarosite dissolution.⁷¹⁻⁷³ The pH of the cell-free and inoculated minimal media control samples (i.e., no Tl-jarosite) in this study remained buffered at pH 7.3 throughout the experiment (Figure 4.1B). Between 156.5 and 325 hours, the buffering capacity of the cell-free Tl-jarosite samples was exceeded and the pH decreased from 7.12 ± 0.06 to 6.66 ± 0.04 and to 5.88 ± 0.05 at 893 hours. The decrease in pH is reflective of the increased acidity associated with jarosite dissolution and is consistent with previous abiotic dissolution studies of buffered (Ag)⁷⁴ and unbuffered (K, Pb-As) jarosites and is described by the following reaction:^{32,33}



The pH of Tl-jarosite samples containing autoclaved cells did not exceed the buffering capacity of the media and decreased minimally from 7.25 ± 0.01 at 0 hours to 6.96 ± 0.06 by 893 hours. The buffering capacity of the minimal media was exceeded earlier in Tl-jarosite samples containing viable cells as compared to cell-free samples. Between 59 and 84 hours, the pH decreased from 7.06 ± 0.15 to 6.36 ± 0.10 and reached the lowest

pH value of 6.02 ± 0.04 until 325 hours followed by an increase to 6.40 ± 0.07 at 893 hours (Figure 4.1B). The increase in pH observed later in the inoculated samples was consistent with previous magnetite formation by *S. putrefaciens*. The pH buffering effect by *S. putrefaciens* CN 32 the result of HCO_3^- production and H^+ during the Fe reduction (Eqn. 1) According to Eqns. 1 and 2, the abiotic dissolution of jarosite may have a greater impact on the acidity released during Tl-jarosite dissolution and may be buffered by microbial processes via Fe reduction.

4.3.6 Environmental Implications. The decrease in pH and the increase in aqueous Tl concentrations over time in the cell-free Tl-jarosite dissolution studies demonstrate that Tl-jarosite is susceptible to significant incongruent dissolution under abiotic conditions. However, Tl-jarosite dissolution may be enhanced through the microbial reduction of structural Fe. The major difference between each regime is related to the secondary Fe mineralization (i.e. Fe(II) versus Fe(III)) associated with dissolution and the role secondary precipitates may play to sequester Tl remains to be elucidated. Given the conservative chemical nature of Tl demonstrated in both experiments, Tl may remain soluble under circum-neutral to acidic anaerobic conditions.

The results from this study provide important yet preliminary insights into the fundamental mechanisms governing the abiotic and biotic dissolution of Tl-jarosite and Tl biogeochemistry. The effects of temperature, aqueous phase mixing rate, competing microbial communities, natural organic matter, suspension density or removal of reaction products on dissolution kinetics were not considered and leaves opportunity for future refinement.³² The Tl/bacteria sorption experiment was useful to provide a Tl mass

balance for results observed in the Tl-jarosite dissolution experiment yet did not provide a comprehensive assessment of Tl sorption onto *S. putrefaciens*. Future studies should consider Tl sorption across a pH range, microbial concentrations, nutritional conditions and Tl concentrations to elucidate the Tl-detoxification mechanism. Moreover, while *Shewanella putrefaciens* CN32 is a well characterized, model metal reducing bacterium, future studies should assess the reductive dissolution of Tl-jarosite using a suite of other environmentally relevant bacteria such as *Geobacter sp* or microbial consortia collected from mine waste repositories to improve our understanding of thallium jarosite solubility.

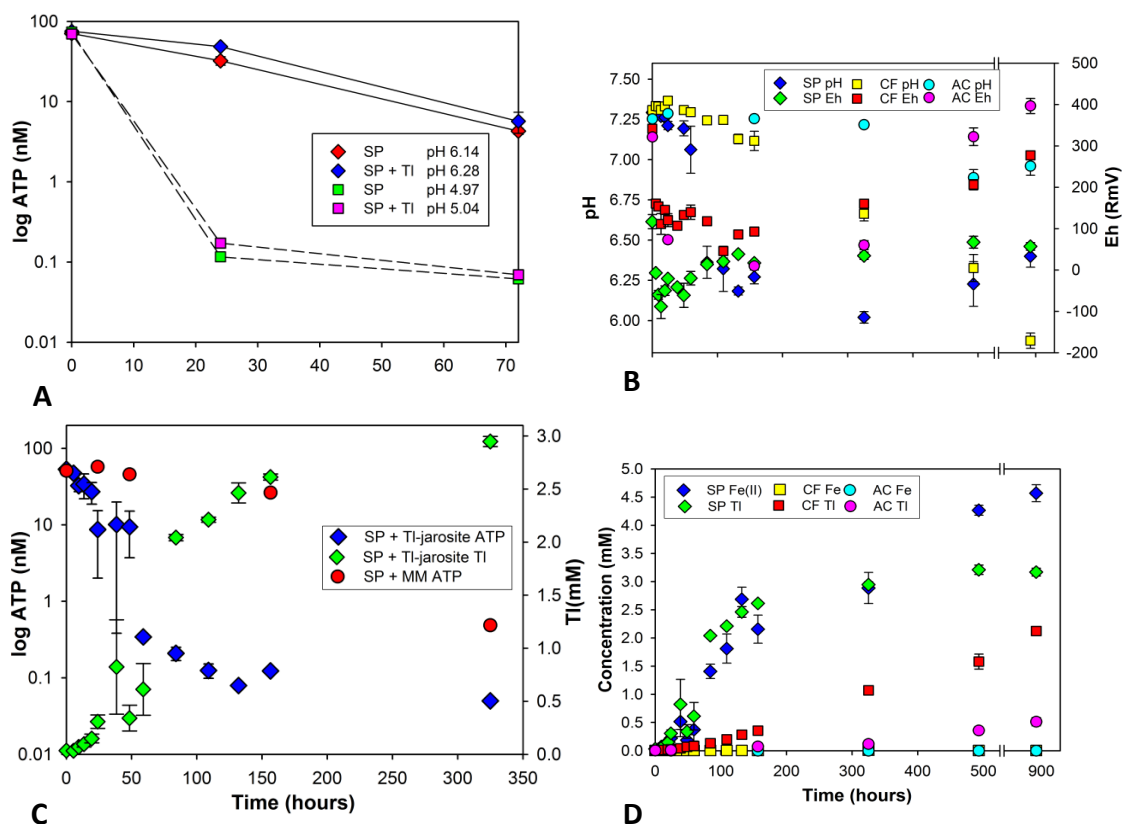


Figure 4.1. SP, CF and AC denotes viable *S. putrefaciens* cells, cell-free and autoclaved cell samples, respectively. A) Intracellular ATP in viable *S. putrefaciens* + minimal media TI-free and TI-spiked sorption samples at pH ~ 5 and 6 as a function of time; B) Redox potential (Eh) and pH in viable *S. putrefaciens*, cell-free and autoclaved *S. putrefaciens* TI-jarosite samples over time; C) Aqueous TI concentrations in TI-jarosite samples containing viable *S. putrefaciens* and intracellular ATP concentrations in viable *S. putrefaciens* + minimal media (MM) and viable *S. putrefaciens* + TI-jarosite samples over time; and D) Aqueous TI and Fe(II) concentrations in viable *S. putrefaciens*, cell-free and autoclaved *S. putrefaciens* TI-jarosite samples over time. Error bars represent standard error (n=3).

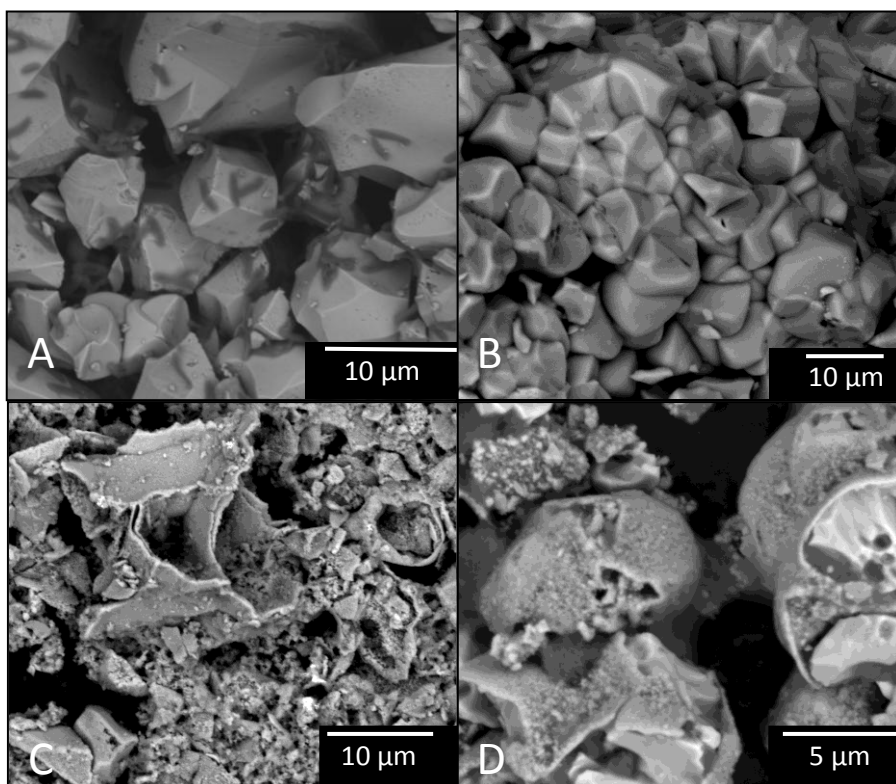


Figure 4.2. Backscattered-electron (BSE) image of the inoculated (viable) Tl-jarosite samples at (A) 0 and (C) 893 hours; and the control (cell-free) Tl-jarosite samples at (B) 0 and (D) 893 hours.

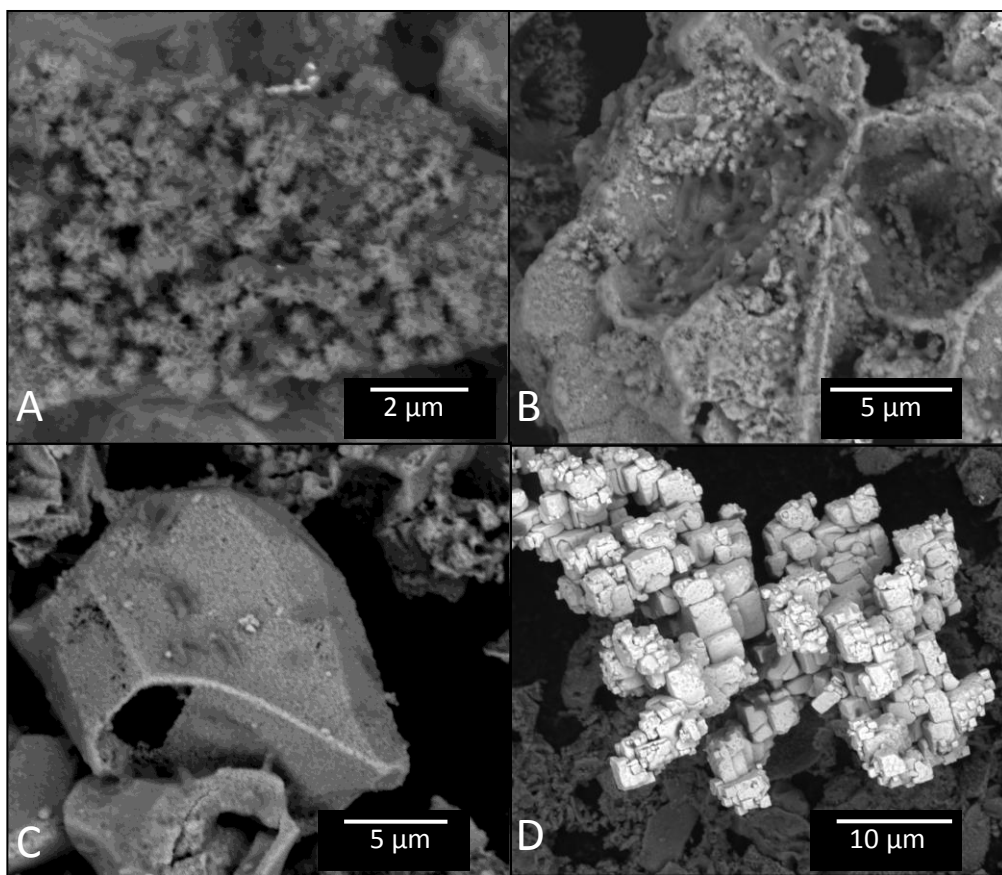


Figure 4.3. Backscattered-electron (BSE) images of *S. putrefaciens* associated with the surface of Tl-jarosite samples and secondary mineral precipitates at (A) 493 and (B),(C) 893 hours; and (D) a secondary Tl precipitate at 893 hours identified as a thallium sulfate.

4.4 SUPPORTING INFORMATION

S4.1. Synthesis of Tl-jarosite

Due to the toxicity of Tl, special safety precautions were taken at all times during the synthesis and experiment. When the samples were not in the anaerobic chamber, handling was conducted in a fume hood, while wearing protective goggles, clothing, a mask and gloves. Tl-jarosite synthesis and in the Briefly, Tl(I) jarosite was synthesized using ACS grade reagents according to the method outlined by Dutrizac (1997). and the pH of a 0.30 L solution containing 0.05 M Tl_2SO_4 and 0.3 M $\text{Fe}_2(\text{SO}_4)_3 \cdot x\text{H}_2\text{O}$ was adjusted from 0.087 to 1.059 using 5.0 M sodium hydroxide (NaOH).¹ The resulting solution was transferred to a 1.0 L round bottom flask connected to a condenser to minimize evaporation. The apparatus was submersed in a closed thermostatic water bath maintained at 98 ± 0.5 °C for 24 hours after which point a golden yellow precipitate characteristic of Tl-jarosite formed.² After 24 hours, 4000 mL of ultrapure Milli-Q water ($18 \text{ M}\Omega \text{ cm}^{-1}$, 98 °C) was applied in 100 mL aliquots to eliminate any dissolved salts and the precipitate was dried at 110 °C for 24 hours. For quantitative total elemental analysis, approximately 2 mg of the Tl-jarosite was dissolved in polypropylene test tubes by adding concentrated HCl drop wise until no solid remained. The resulting solution was diluted with 0.016 M HNO_3 and analysed for Tl, Fe, and S by inductively coupled plasma emission spectrometry (ICP-OES). The resulting chemical formula, $(\text{H}_3\text{O})_{0.29}\text{Tl}_{0.71}\text{Fe}_{2.74}(\text{SO}_4)_2(\text{OH})_6$, was calculated using total elemental concentrations and the modified formula of Kubisz (1970).³

S4.2. Bacterial Growth Procedures

S. putrefaciens CN 32 cultures (ATC# BAA-453) were prepared from a 1.5 mL frozen glycerol stock maintained at -80 °C and were transferred to Trypticase Soy Agar (TSA) plates and grown aerobically for 24 hours. Single colonies were inoculated into 25 mL of sterile Trypticase Soy Broth (TSB) and incubated aerobically at 32 °C for 18 hours. The 25 mL seed cultures were used to inoculate 4 x 100 mL aliquots of sterile Luria-Bertrani (LB) media and incubated aerobically at 32 °C until the culture reached late log phase (18 hours). Bacteria were harvested by centrifugation at 3000 rpm for 20 minutes and washed twice using sterile 0.01M NaNO₃, decanting the supernatant and centrifuging at each step. The wet biomass was washed once using 100 mL of N₂ purged minimal media containing: 1.34 mM potassium chloride (KCl), 28 mM ammonium chloride (NH₄Cl), 0.68 mM calcium chloride (CaCl₂), 50 mM sodium perchlorate (NaClO₄), 24mM Na-lactate (60% syrup), 20 mM PIPES (1,4-piperazine diethanesulfonic acid) buffer at pH 7.3 using 0.01M NaOH. The minimal media was decanted and the final wet mass was 1.65 g bacteria/ 2.0 L of minimal media.

S4.3 SEM Analysis.

The abundance and spatial distribution of bacteria with respect to the Tl-jarosite was examined using a backscattered electron (BSE) detector and enhanced surface detail was investigated using a secondary electron (SE) detector. Energy dispersive x-ray spectroscopy (EDX) spectra were collected to examine changes in elemental composition of the Tl-jarosite and any secondary precipitates formed over time.

S4.4 Cell Viability Analysis (ATP_{INT})

Prior to analysis, all BacTiter-Glo™ reagents were stored at -80°C to prevent degradation and thawed immediately before use. To prepare the BacTiter-Glo™ reagent, lyophilized BacTiter-Glo™ substrate was reconstituted using the BacTiter-Glo™ buffer and equilibrated at room temperature for 10 minutes. Samples from the TI-jarosite experiment were stored at 4°C for up to 48 hours prior to analysis and samples from the TI/bacteria sorption experiments were analyzed immediately upon sampling. The sample slurries were divided into 2 x 1.0 mL aliquots. A 1.0 mL aliquot was filtered through a 0.1 µm syringe filter (Millex®-VV, Millipore) and 150 µL of the filtrate was transferred to a sterile 1.5 mL microcentrifuge tube and represented the extracellular fraction of ATP. An additional 150 µL of the slurry was transferred to a sterile 1.5 mL microcentrifuge tube and represented total ATP.

An equal volume (150 µL) of the BacTiter-Glo™ reagent was added to each sample and slowly shaken for 5 minutes. Luminescence was measured using a GloMax® 20/20 Single Tube Luminometer (λ 350-650 nm) at an integration time of 2 seconds and the output was reported in relative luciferase units (RLU). Control samples were also analyzed to determine the intensity of the luminescent signal produced by the minimal media and the TI-jarosite in the absence of bacteria. The RLU values were converted to ATP concentrations using the average of three calibration curves prepared with an ATP standard (10 mM; Promega Corporation).

Treatment	Final pH	Initial Tl Concentration ($\mu\text{M} \pm \text{S.D.}$)	Final Tl Concentration ($\mu\text{M} \pm \text{S.D.}$)	Tl Difference (μM)
Control	4.57 $\pm$ 0.04	35.97 $\pm$ 0.37	34.31 $\pm$ 0.43	1.66
Control	6.57 $\pm$ 0.24	35.02 $\pm$ 0.24	34.52 $\pm$ 0.21	0.50
Autoclaved	4.95 $\pm$ 0.03	35.38 $\pm$ 0.04	34.93 $\pm$ 0.16	0.45
Autoclaved	6.70 $\pm$ 0.10	36.26 $\pm$ 0.06	35.94 $\pm$ 0.07	0.33
Viable cells	5.04 $\pm$ 0.01	33.89 $\pm$ 0.21	32.50 $\pm$ 0.14	1.39
Viable cells	6.28 $\pm$ 0.03	35.84 $\pm$ 0.04	35.57 $\pm$ 0.12	0.27

Table S4.1. Initial, final and differences in concentrations of Tl in the control (i.e., no cells), autoclaved cells and viable cells at pH $\sim$ 5 and 6. The variation of differences among the treatments were analyzed were not significant (ANOVA, $p < 0.05$) pH 7, $p = 0.333$ and pH 6, $p = 0.948$.

Component	Treatment			
	Control	Log activity	Biotic	Log activity
Fe ³⁺ (as Fe(OH) ₂ ⁺)	5 μM	-5.57	---	---
Fe ²⁺	---	---	4569 μM	-2.74
Tl ⁺	2112 μM	-3.52	3169 μM	-2.69
SO ₄ ²⁻	5949 μM	-2.57	3336 μM	-2.87
Cl ⁻	34 μM	-1.56	34 μM	-1.56
CH ₃ CH(OH)COO ⁻	24 mM	-1.87	22.86 mM	-1.73
CH ₃ COO ⁻	---	---	1142 μM	-3.18
HCO ₃ ⁻	---	---	1142 μM	-7.04
pH	5.8	-5.80	6.40	-6.40
Eh	277 mV	---	57 mV	---

Table S4.2. Measured chemical parameters and log activities used in React (GWB 7.01) for the control and inoculated experiments at 893 hours.

Mineral	log Q/K
Hematite	12.49
Schwertmannite	8.60
Goethite	5.76
Fe(OH) ₃	1.40
Thallium jarosite	-5.42

Table S4.3. Predicted saturation indices (log Q/K) of hematite, schwertmannite, goethite, Fe(OH)₃ and thallium jarosite during the abiotic dissolution Tl-jarosite at 893 hours.

Mineral	log Q/K
Hematite	9.06
Magnetite	9.02
Goethite	4.05
Fe(OH) ₃	-0.32
FeO(c)	-1.14
Tl ₂ CO ₃ (s)	-2.17
Siderite	-3.09

Table S4.4. Predicted saturation indices (log Q/K) of hematite, magnetite, goethite, FeO, Tl₂CO₃ and Fe(OH)₃ and siderite at 893 hours during the biotic dissolution Tl-jarosite.

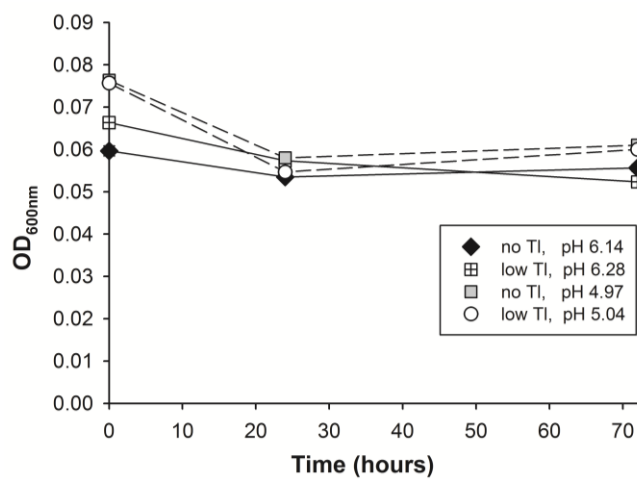


Figure S4.1. Optical density (OD₆₀₀) in the inoculated TI-free control and TI-spiked sorption samples at pH ~ 5 and 6 as a function of time. Error bars represent standard error (n=3).

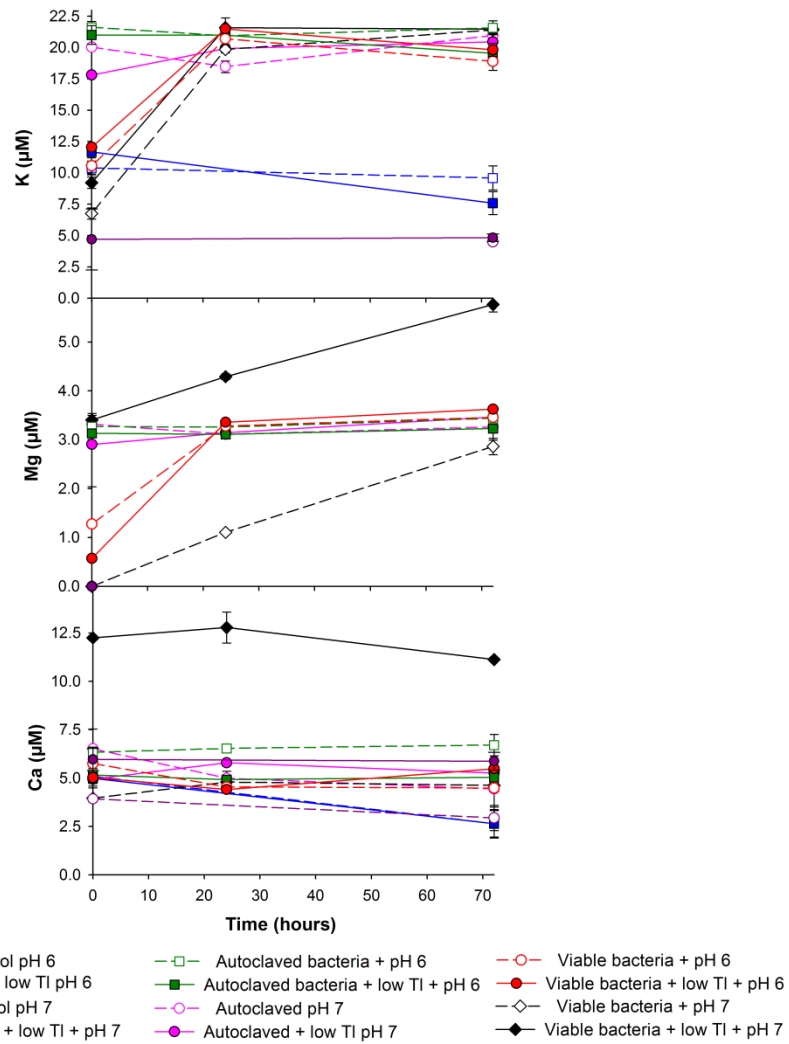


Figure S4.2. Release of K, Mg and Ca over time.



Figure S4.3. Digital photographs of: A) autoclaved cells and Tl-jarosite, B) Tl-jarosite and *S. putrefaciens* and C) cell-free Tl-jarosite at 893 hours

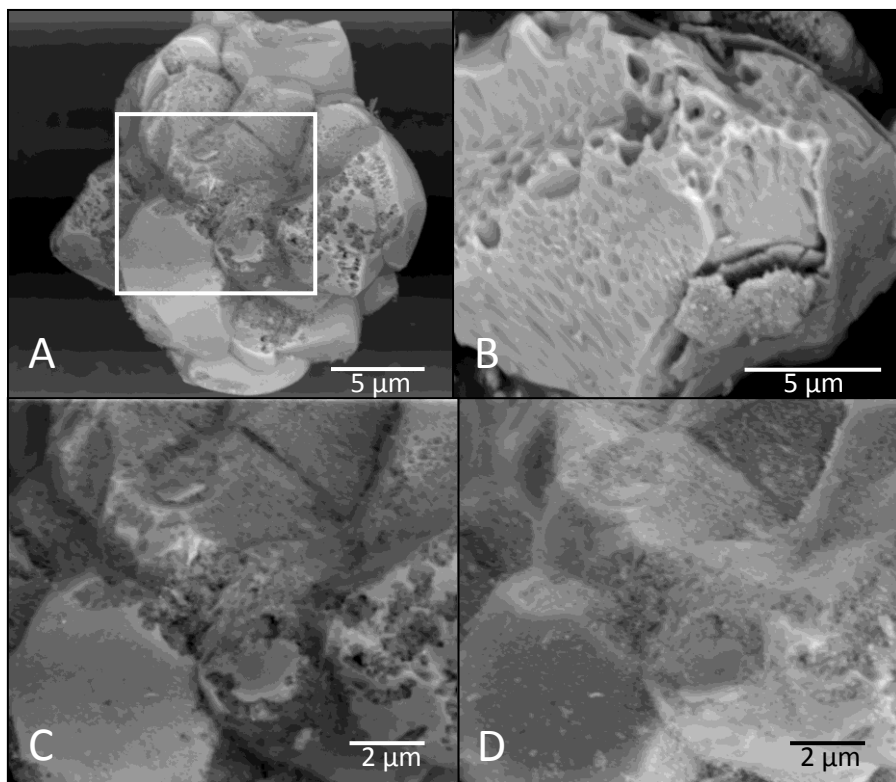


Figure S4.4. A) Backscattered-electron (BSE) image of the cell-free Tl-jarosite control samples taken at 10 kV at 168 hours and; B) 893 hours C) Magnified BSE image of area denoted as a white square in image A; and D) Corresponding secondary-electron (SE) image of C.

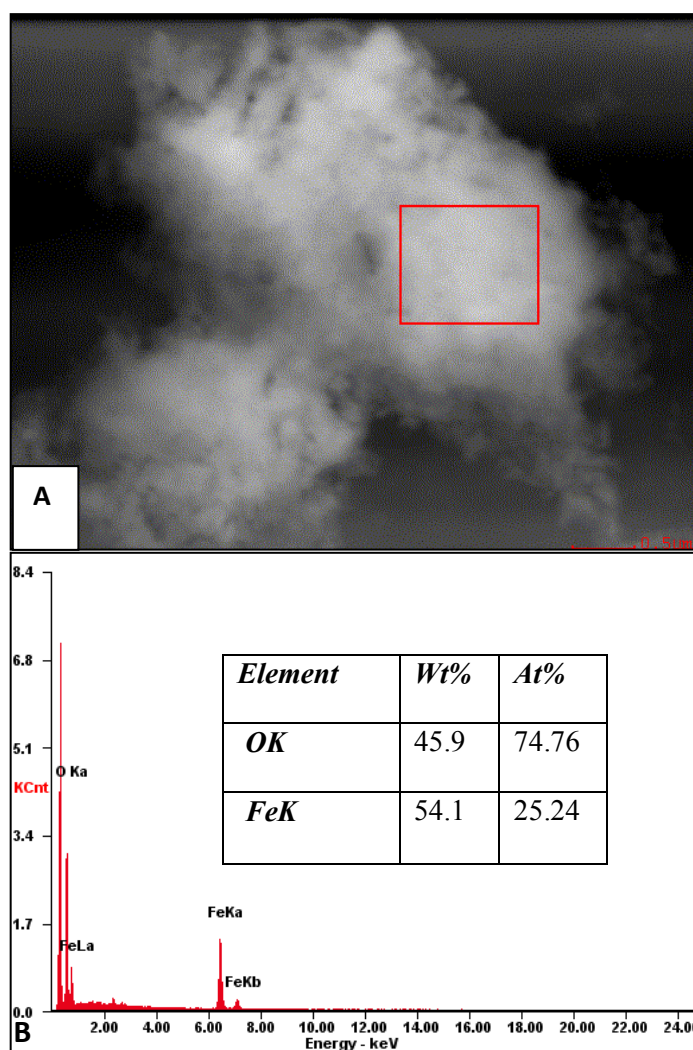


Figure S4.5. (A) BSE-SEM image of a secondary precipitate in the cell-free inoculated Tl-jarosite at 893 h taken at 25 kV, red square denotes location of EDX analysis and (B) EDX spectra of chosen area (red square) in A, inlaid table is the relative wt% and at% elemental concentrations of O and Fe.

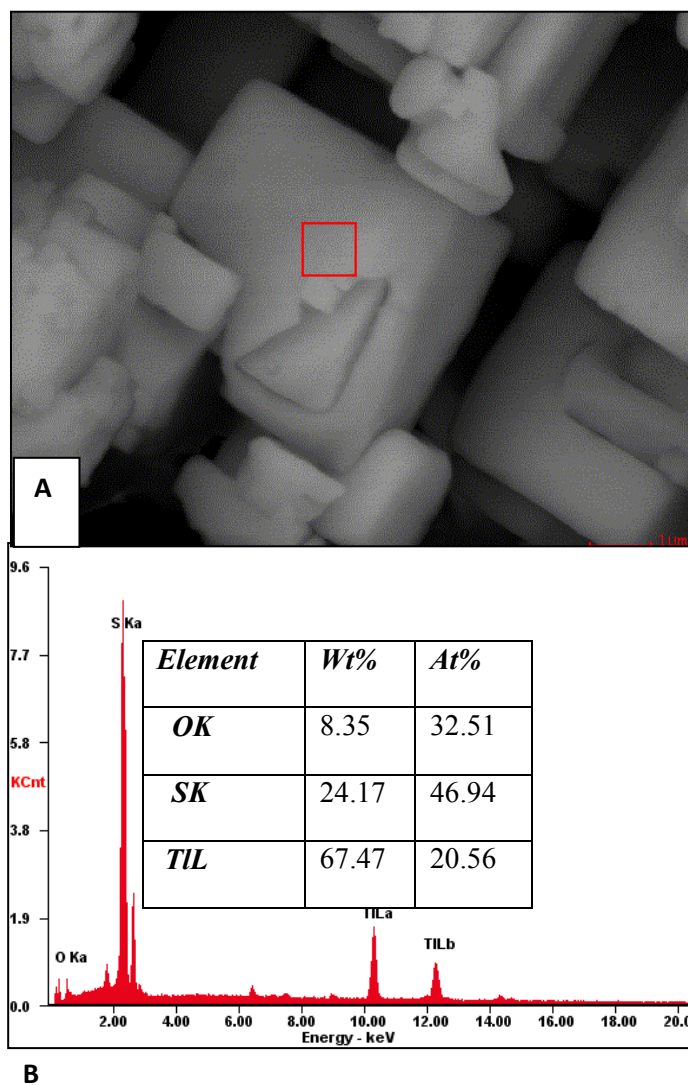
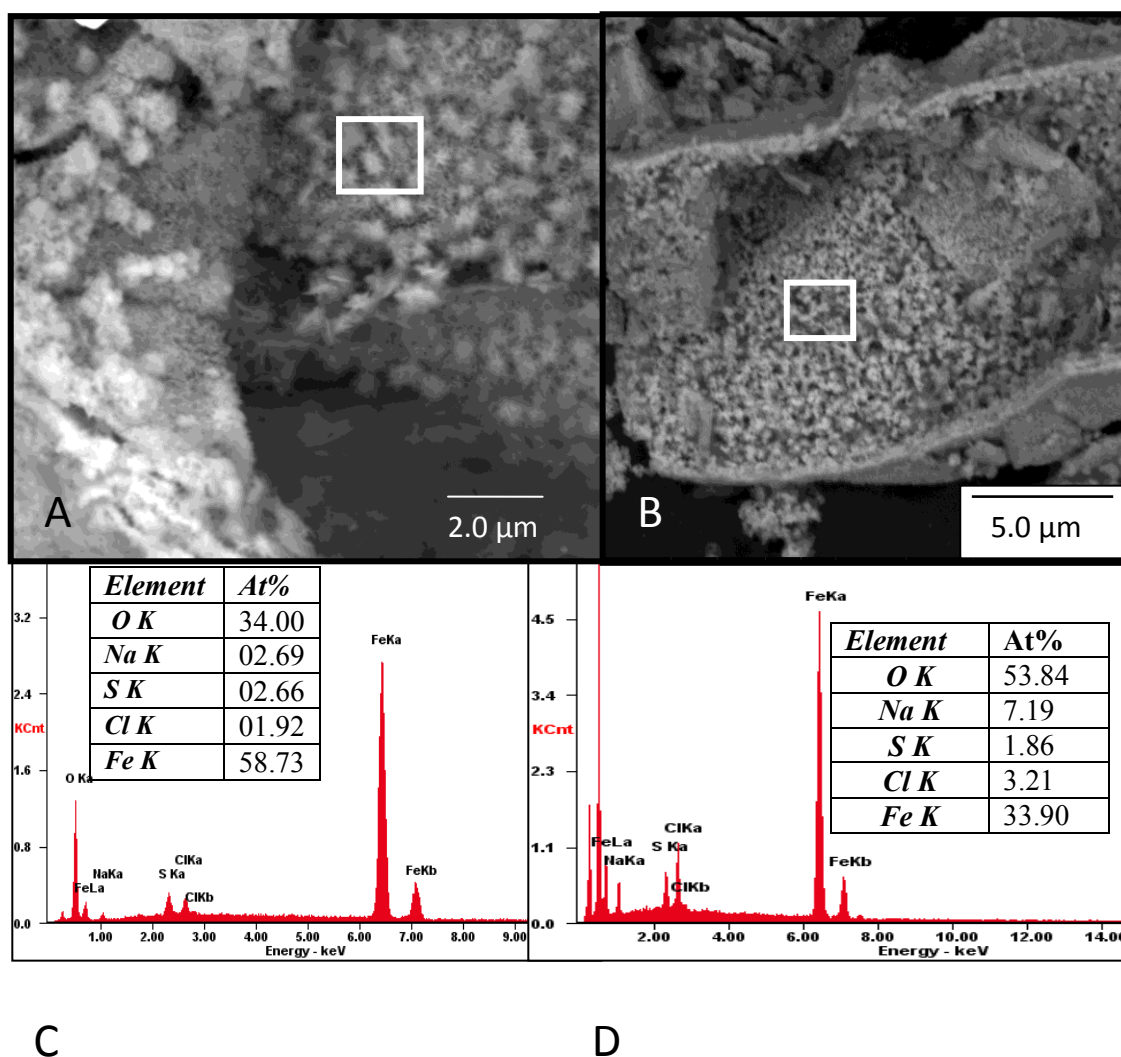


Figure S4.6. (A) BSE-SEM image of a secondary precipitate in the inoculated (viable) Tl-jarosite at 893 h taken at 25 kV, red square denotes location of EDX analysis and (B) EDX spectra of chosen area (red square) in A, inlaid table is the relative weight % and atomic % elemental concentrations of O and Fe.



SI Figure S4.7. BSE-SEM image of secondary mineralization associated with the inoculated (viable cells) Tl-jarosite at 10 kV taken at : (A) 493 hours and (B) 893 hours. White square denoted location of EDX analysis ; and (C) EDX spectra taken at 25 kV of chosen area (white square) from image (A), inlaid table is the relative atomic % elemental concentrations; (D) EDX spectra taken at 25 kV of chosen area (white square) in B, inlaid table is the relative atomic% (at%) elemental concentrations. The Na and Cl peaks are likely contributed by the background minimal media.

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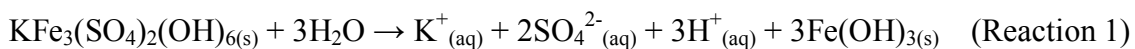
CHAPTER 5

Comparing the susceptibility of natural and synthetic jarosite-group minerals to reductive dissolution by *Shewanella putrefaciens* CN32

5.1 INTRODUCTION

Jarosite-group minerals are members of the alunite mineral supergroup and have the general formula: $AB_3(TO_4)_2(OH)_6$, where the A site is occupied by monovalent or divalent cations (e.g. K^+ or Pb^{2+}), the B site is occupied by cations with octahedral (O) coordination (e.g. Fe^{3+} or Al^{3+} and the T sites correspond to tetrahedral coordination (T) (e.g. SO_4^{2-} and AsO_4^{3-}).^{1,2} Natural jarosites are typically formed through either hydrothermal ($> 100\text{ }^\circ\text{C}$) or low-temperature processes ($< 100\text{ }^\circ\text{C}$). Low-temperature are commonly referred to as "young" jarosites and typically form under oxidizing, ferric and sulfate rich and acidic ($pH < 3.5$) conditions such as in acid sulfate soils or in mine wastes formed during the oxidation of sulfide minerals.^{3,4} At pH values above 3.5, jarosites dissolve to form goethite or meta stable phases such as schwertmannite or ferrihydrite.^{5,6} Jarosite precipitation is widely used in the Zn industry to remove unwanted impurities such as Fe, sulfate, alkalis and other heavy metals (e.g. Pb, Tl and As) from processing solutions prior to Zn electrolysis.⁷ The amount of iron waste produced during this process generally ranges between 1/3 and 1/6 of the total Zn tonnage.⁸ Consequently, large volumes of jarositic wastes are produced yearly and typically stored in disposal ponds under circum-neutral conditions, thereby rendering the jarosite unstable and resulting in dissolution/re-precipitation reactions that may potentially release toxic metals into disposal environments.^{9,10}

Jarosite dissolution is characterized as an incongruent reaction with selective dissolution at the A and T-sites and is written as:¹¹

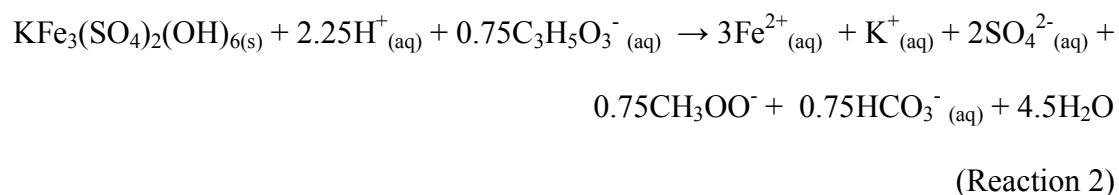


The production of H^+ during dissolution contributes to the total acidity of mine wastes. Accordingly, a survey of 130 metal-mine-waste piles in Colorado conducted by the U.S Geological Survey showed that the presence of jarosite minerals in mine wastes, particularly hydronium-jarosites, was the best indicator of acid-generation potential.¹² In fact, several studies have established that hydronium bearing jarosites are significantly more soluble than K-jarosite.¹³⁻¹⁶ Natural jarosites are difficult to physically separate because they are very fine-grained ($< 5 \mu m$) and are commonly associated with other minerals such as quartz, clays and iron oxides.^{12,17} Therefore, synthetic jarosites are used as analogs for natural jarosites in order to evaluate solubility and dissolution properties.¹⁷ Based on chemical composition and cell dimensions, synthetic jarosites serve as a relatively good proxy for low-temperature natural jarosites typically formed through sulfide oxidation.¹⁷ They are similar because both are non-stoichiometric, formed at low-temperatures and meta-stable due to substitution of H_3O^+ or Fe^{3+} and B site deficiencies in the mineral structure. However, the widespread practice of heating synthetic jarosites to $110^\circ C$ post synthesis drives off structural water thereby increasing stability and may lead to underestimations of dissolution rates.¹⁷ In contrast, hydrothermal jarosites are older, stoichiometric and do not have extensive B site or Fe^{3+} site deficiencies or significant H_3O^+ substitutions in the mineral structure and are thereby more stable. Therefore, it is important to identify the origin of natural jarosite samples when comparing synthetic versus natural dissolution rates because the origin will dictate the reactivity of the jarosite phase.

Few studies have compared the abiotic dissolution rates of synthetic versus natural jarosite samples. Welch et al. (2008) demonstrated that abiotic dissolution of

synthetic samples was at least order of magnitude faster than the rates determined for natural jarosite samples from acid sulfate soils.¹⁸ Dissolution was reduced in the natural samples due to pre-existing Fe hydroxide surface coatings formed thereby limiting the reactivity of the mineral phase. The stability and reactivity of jarosites is related to the degree of substitution in the A and B structural sites. The general relationship between A site cations and dissolution rates in synthetic jarosites increases in the order K(Fe, Cr)- > K- > Na- > H₃O-jarosite.¹⁶ Atomic force microscopy (AFM) demonstrated that abiotic dissolution in synthetic jarosite [(K, H₃O)Fe₃(SO₄)₂(OH)₆] at pH 5.5 is governed by surface reactions involving the formation and expansion of monolayer-deep etch pits on surface terraces.¹⁶ As dissolution proceeds, newly formed surface layers of Fe(III) oxyhydroxides passivate the jarosite surface and decelerates further dissolution. However, if aqueous Fe undergoes complexation (i.e. removed from the surface) congruent dissolution will proceed unhindered by the formation and growth of etch pits.

Dissimilatory Fe reducing bacteria (DIRB) acquire energy for growth by coupling the oxidation of an organic carbon source such as lactate to the reduction of structural Fe from jarosites. The reduction of insoluble Fe³⁺ from the surface of jarosite into soluble Fe²⁺ enhances dissolution through the following reaction:



In contrast to abiotic dissolution processes, bacterial Fe(III) reduction from jarosites is an acid consuming reaction. Moreover, the reductive dissolution of biogenic, synthetic and natural jarosite-group phases is well-documented under low pH (< 2.5) and circum-

neutral anaerobic conditions via both Fe and SO_4^{2-} reducing bacteria such as *Shewanella putrefaciens*, *Geobacter sp.*, *Acidiphilium sp.* and *Desulfovibrio desulfuricans*.¹⁹⁻²⁵ To date, there is a lack of studies comparing bacterial Fe reduction in natural and synthetic jarosite minerals. Yet, given the potential for enhanced dissolution and metal release from jarosites, comparing the biogeochemical stability of synthetic versus natural jarosites is paramount for evaluating the use of synthetic samples as proxies for natural samples. In contrast to jarosites, the ability of DIRB to reduce iron oxides is well documented and is dependent on several factors including particle size, surface area, and crystallinity. Generally, the rate of Fe reduction increases with increasing surface area, but is inversely related to particle size and crystallinity.^{26,27}

Therefore, the primary objectives of this study are to: 1) evaluate the susceptibility of two synthetic and two natural jarosites to reductive dissolution using the model Fe reducing bacterium *Shewanella putrefaciens* CN32; 2) examine the influence of particle size and surface area on reductive dissolution; and 3) examine the influence of *S. putrefaciens* on the release of trace metals from the natural jarosite phases.

5.2 MATERIALS AND METHODS

5.2.1 Jarosite Synthesis and Natural Jarosite Information. The first synthetic jarosite (hereinafter referred to as Syn1) was synthesized following the method of Baron and Palmer (1996) whereby a 200 mL solution of 1.0 M KOH and 0.299 M $\text{Fe}_2(\text{SO}_4)_3 \cdot 5\text{H}_2\text{O}$ was covered and heated to 98°C at constant stirring for 4 hours.²⁸ The yellow precipitate was vacuum filtered and washed with 4000 mL of ultrapure Milli-Q water (18 $\text{M}\Omega \text{ cm}^{-1}$) and dried at 110°C for 24 h. The second synthetic jarosite

(hereinafter referred to as Syn2) was synthesized according to the method of Dutrizac (2008).²⁹ A round bottom flask containing 400 mL of 0.301 M K₂SO₄ and 0.425 M Fe₂(SO₄)₃·5H₂O was adjusted to pH 1.6 using 0.1M H₂SO₄ and connected to a condenser coil to minimize evaporation. The apparatus was submersed in a close thermostatic water bath maintained at 98 ± 0.5 °C (to minimize evaporation) for 12 h. The resulting yellow precipitate was vacuum filtered and washed with 4000 mL of ultrapure Milli-Q water (18 MΩ cm⁻¹) and dried at 110 °C for 24 h.

Two natural jarosite-group mineral specimens were acquired from the Smithsonian National Museum of Natural History (NMNH) Mineral Sciences Collection. Beyond results presented in this study, the only information available for the samples was collection locations. Sample 1 (NMNH 95924-1) (hereinafter referred to as Nat1) was collected from Velardino, Durango Province, Mexico. Sample 2 (NMNH R7598-1) (hereinafter referred to as Nat2) was collected from Sabina, Chihuahua Province, Mexico. All samples were ground into fine powders using a mortar and pestle.

5.2.2 Characterization of Jarosites All samples were identified as their respective jarosite-group minerals with powder X-ray diffraction (XRD) analysis with a Rigaku D/MAX 2500 rotating anode powder diffractometer with monochromatic CuKα radiation. For quantitative total elemental analysis, 50 mg of each synthetic jarosite was dissolved in polypropylene test tubes by adding concentrated HCl dropwise until all solid dissolved. However, natural jarosites did not dissolve using this method and required a two step acid digestion procedure. In step 1, samples (50 mg) were transferred to 30 mL Teflon digestion bombs containing 1 mL of concentrated HNO₃ and 1 mL of concentrated HF, capped and heated at 100 °C for 6 days. Following HF/ HNO₃

digestion, the Teflon bombs were uncapped and the HF was evaporated to dryness at 60 °C. In step 2, samples were re-suspended in aqua regia (1 mL of concentrated HCl/ 0.5 mL of concentrated HNO₃), capped and heated at 60 °C for 3 days at which point no solid remained. Natural and synthetic samples were both diluted with 1% HNO₃ and analyzed for elemental concentrations by inductively coupled plasma optical emission spectrometry (ICP-OES). Particle morphology of each sample was determined using a FEI-200F Field-Emission Environmental Scanning Electron Microscope (ESEM) with an accelerating voltage of 5-10 kV. Surface area was determined by nitrogen gas multipoint Brunauer-Emmett-Teller (BET) analysis with a Quantachrome NOVA 1200e surface area analyzer.

5.2.3 Preparation of Jarosite/Cell suspensions. Pure cultures of *Shewanella putrefaciens* CN32 (ATC# BAA-453) were grown to late log phase (17 hours), harvested, and inoculated into a modified M1 minimal media. All minimal media were transferred to an anaerobic chamber (95% N₂/5% H₂) and 15 mL of the cell suspension was dispensed into 20 mL polypropylene test tubes containing 0.0580 ± 0.0059 g of either A) Syn1, B) Syn2, C) Nat1 or D) Nat2. Special care was taken to vigorously shake the bottle containing bacteria after every 10th inoculation in order to ensure similar cell numbers. Experimental controls contained 15 mL of sterile minimal media and 0.0580 ± 0.0059 g of either A) Syn1, B) Syn2, C) Nat1 or D) Nat2 in 20 mL polypropylene tubes. In total, there were 18 samples per treatment giving a total of 144 jarosite samples. Additional sets of controls containing only minimal media were prepared to examine background pH, Eh, ATP and elemental concentrations over time in the absence of jarosite and contained either a) no cells (6 samples), or b) viable cells

(18 samples). All samples were capped, sealed with parafilm, covered with aluminum (Al) foil and rotated end-over-end at 20 rpm in the anaerobic chamber using 2 bench top rotators (Glas Col) at 28° C and were sampled in triplicate at 0, 24, 48, 65, 192 and 720 hours.

5.2.4 Sampling Protocol. At each sampling period, pH (Thermo Ross Sure-flow semi micro pH probe) and Eh (Thermo Ross Sure Flow combo redox/ORP) was measured in each sample. *S. putrefaciens* metabolism was monitored using the Promega BacTiter-Glo™ Microbial Cell Viability Assay, an ATP-based luminescent technique. All bacteria originated from the same minimal media jar and starting ATP concentrations were converted to cell counts using an average ATP-per-biovolume concentration of 2.95×10^{-9} nmol/cell ATP and the starting cell concentration were 1.2×10^8 cells/mL.³⁰

Sample slurries were collected and stored at 4°C and imaged under low vacuum at a low accelerating voltage (5-10 kv) using field emission-environmental scanning electron microscopy (FE-ESEM, FEI-Quanta 200F). Samples were filtered through a 0.2 um nylon syringe filter and subsamples (100μL) were analyzed immediately for Fe(II) and total Fe concentrations via the Ferrozine method.³¹ The remaining filtrate was diluted, acidified and aqueous elemental concentrations were determined using inductively-coupled plasma optical emission spectroscopy (ICP-OES).

5.3 RESULTS

5.3.1 Characterization of Synthetic and Natural Jarosites

5.3.1.1 Synthetic Jarosites. Syn1 and Syn2 were identified as hydronium substituted jarosite, $(K,H_3O)Fe_3(SO_4)_2(OH)_6$, by XRD analysis (Figure 5.1).

Concentrations obtained from acid digestion of the synthetic jarosites were converted to molar concentrations and the chemical formulas were determined based upon the ideal chemical formula for jarosites of Kubisz (1970): $A_{1-x}(H_3O)_x Fe_{3-y}(OH)_{6-3y}(H_2O)_{3y}(SO_4)_2$ whereby K and Fe are normalized to 2 S.³² Accordingly, the molecular composition of Syn1 and Syn2 is $(H_3O)_{0.04}K_{0.96}Fe_{2.92}(SO_4)_2(OH)_{5.76}(H_2O)_{0.24}$ and $(H_3O)_{0.19}K_{0.81}Fe_{3.02}(SO_4)_2(OH)_6$, respectively (Table 5.1). Potential percent mole occupancies at each site in the synthetic jarosite structure were calculated and compiled in Table 2 whereby the lower K^+ occupancy at the A site in Syn1 and Syn2 is charge balanced by incorporation of hydronium $(H_3O)^+$ ions as confirmed by the XRD patterns (Table 5.2, Figure 5.1). Acid digestions of samples also revealed minor concentrations of Pb, Zn, Ca, Bi, Ba, Ti and Cd that likely originated from the synthesis reagents (Table 5.3). While chemical and XRD analysis of the samples are similar, back scattered electron (BSE) scanning electron microscope (SEM) images taken of both samples show strikingly different morphologies (Figure 5.2). Syn1 is characterized by irregular globular precipitates typically ranging in size from 0.5 - 2 μm . In contrast, Syn2 crystals are intergrown with a pseudo-rhombohedral to globular morphology and a wider range of grain sizes from 0.5 - 8 μm . This is not surprising since different synthesis procedures were chosen to yield different particle morphologies and sizes. The constant stirring and short synthesis time in Syn1 was chosen to yield small spherical precipitates while the longer synthesis time and reflux procedure for Syn2 was expected to yield larger crystals. Despite different particle sizes, BET surface area measurements of Syn1 and Syn2 were the same at $1.68 \pm 0.19 \text{ m}^2/\text{g}$ and $1.67 \pm 0.22 \text{ m}^2/\text{g}$, respectively and are similar to surface areas of synthetic jarosites reported elsewhere (Table 5.4).¹¹

5.3.1.2 Natural Jarosites.

Natural Sample 1 (Nat1). Nat1 was identified as jarosite, $\text{KFe}_3(\text{SO}_4)_2(\text{OH})_6$ by XRD analysis (Figure 5.1). Based on ICP-OES acid digest concentrations, mol % Fe at the B site was in excess by 25.2 % and may be the result of iron hydroxide surface coatings formed during weathering as demonstrated in other natural jarosites (Table 5.2).³³ While XRD identified K-jarosite as the primary phase, acid digestion of Nat1 revealed relatively high concentrations of Pb, As and Zn (Figure 5.1, Table 5.3). Total ICP-OES concentrations in Nat1 showed potential 98.4 mol% K occupancy of the A-site with minor potential mol% occupancies of Pb (3.49 %) in the A-site, Zn (7.00 %) in the B-site and As (2.84 %) in the T-site (Table 5.2). Arsenate (AsO_4^{3-}) commonly substitutes for SO_4^{2-} in the T-site in jarosite group minerals to charge balance Pb^{2+} substitution in the A-site while substitution of Zn^{2+} for Fe^{3+} in the B site is also known to occur.^{2,34}

Due to the excess occupancy (i.e. > 100%) of (Fe + Zn) and (K + Pb) in the A and B- sites, respectively, the chemical formula of Nat1 could not be determined. However, SEM-EDS analysis of randomly selected Nat1 jarosite crystals demonstrated minor relative concentrations of Pb (3.61 ± 0.93 wt %), As (0.39 ± 0.07 wt %) and Zn (0.52 ± 0.15 wt %) (Table 5.5). The low Zn wt % does not corroborate with the higher B-site occupancy (7 %) expected based on ICP-OES concentrations. Therefore, it is likely that an additional Zn phase is associated with Syn1. Indeed, SEM images of Nat1 show a mineral phase exhibiting the "hairy" or fibrous morphology typical of clays such as illite $(\text{K},\text{H}_3\text{O})(\text{Al},\text{Mg},\text{Fe})_2(\text{Si},\text{Al})_4\text{O}_{10}[(\text{OH})_2,(\text{H}_2\text{O})]$ (Figure 5.3A).³⁵ EDS analyses of the phase showed the presence of Al (1.00 - 1.22 wt %), Si (4.57 - 5.80 wt %) and Zn

(6.12 - 7.64 wt %) which was not observed in the jarosite mineral phase (Figure 5.3B,C,D). Accordingly, the secondary phase is likely a clay which has retained Zn either through ion exchange or adsorption processes.³⁶ Based on ICP-OES concentrations coupled with SEM observations, Nat1 is likely a Pb and As substituted K-jarosite. SEM images taken of Nat1 show no resemblance in morphology to the synthetic samples (i.e. Syn1 and Syn2) (Figure 2 and 3). Nat1 crystals exhibit thin euhedral hexagonal crystal habits typically ranging in size from 0.5 - 6 μm wide and 0.5 μm thick with a larger surface area ($4.66 \pm 1.01 \text{ m}^2/\text{g}$) than the synthetic jarosites (Table 5.4).

Natural Sample 2 (Nat 2). XRD analysis identified several potential jarosite phases in Nat2 in the order: hydronium jarosite $(\text{H}_3\text{O})\text{Fe}_3(\text{SO}_4)_2(\text{OH})_6 >$ natrojarosite, $\text{NaFe}_3(\text{SO}_4)_2(\text{OH})_6 >$ synthetic jarosite $\text{KFe}_3(\text{SO}_4)_2(\text{OH})_6$ (Figure 5.1). Similarly to Nat1 and based upon total acid digest concentrations, Nat2 also showed 25.2% excess Fe mol occupancy of the B-site (Table 5.2). BSE-SEM images show spherical mineral phases associated with the jarosite and EDS analyses reveal that the spherical precipitates are enriched in Fe, O and Al (Figure 5.4). Therefore, the excess Fe is likely contributed by iron oxides enriched in Al. In contrast to Nat1, the occupancy of the A-site did not exceed 100 % and showed 33.8 and 48.2 % potential occupancy by K and Na, respectively (Table 5.2). Minor concentrations of Sr and As corresponded to 0.97 and 0.99 % substitution in the A and T sites, respectively (Table 5.2, 5.3). In contrast to Nat1, the chemical formula of Nat2 was determined because the potential % occupancy at the A-site did not exceed 100 % and the sample was already identified as $(\text{H}_3\text{O}, \text{Na}, \text{K})$ jarosite during XRD analysis (Figure 5.1, Table 5.2). Similarly to the Pb/As

substitution in Nat1, As also substitutes at the T site to charge balance Sr^{2+} substitution in the A-site. Therefore, Sr and As were also incorporated into the Nat2 chemical formula because their respective potential occupancies are similar at 0.97 and 0.99 %, respectively, with the formula: $(\text{H}_3\text{O})_{0.17}\text{Na}_{0.48}\text{K}_{0.34}\text{Sr}_{0.01}\text{Fe}_3(\text{AsO}_4)_{0.02}(\text{SO}_4)_{1.98}(\text{OH})_6$ where the % Fe occupancy was assumed at 100 % (Table 5.1). SEM images of Nat2 show similar euhedral hexagonal morphology to Nat1 but also show broken and fragmented crystals which likely occurred from weathering processes in the field or mechanical abrasion during sample processing (Figure 5.2). Crystal size was larger than Nat1 and typically ranged from 0.5 - 12 μm with a surface area of $1.81 \pm 0.32 \text{ m}^2/\text{g}$ (Table 5.4).

5.3.2 Abiotic and Reductive Dissolution of Synthetic and Natural Jarosites

5.3.2.1 Eh and pH. The pH in the minimal media (MM) control and natural control samples (Nat1 and Nat2) remained buffered at pH 7.5 throughout the experiment (Figure 5.5A). Despite using PIPES buffer in the minimal media, by the end of the experiment (720 hours) the pH of the control synthetic samples, Syn1 and Syn2, decreased to pH values of 4.98 ± 0.04 and 5.46 ± 0.22 , respectively thus reflecting the acid generation potential (net H^+) of jarosite as described in Reaction 1 (Figure 5.5A). Overall, the greatest pH decrease in control samples was observed in the decreasing order: Syn2 > Syn1 > Nat1 = Nat2 = MM. In the inoculated minimal media (i.e. no jarosite), the pH remained buffered at 7.5 over the entire experiment. In contrast, the pH of the inoculated synthetic samples, Syn1 and Syn2, decreased earlier compared to controls with the lowest pH values at 5.32 ± 0.46 at 24 h and 4.87 ± 0.13 at 120 h,

respectively (Figure 5.5B). The pH decrease in inoculated Syn1 and Syn2 samples was subsequently followed by an increase after 65 hours to pH 6.34 ± 0.56 and 6.21 ± 0.34 , respectively, by 720 hours. The increase in pH reflects the increase in Fe reduction, an acid consuming reaction (Reaction 2), observed after 24 hours (Figure 5.6E). The enhanced decrease in Syn2 is likely due to the higher mol % of H_3O^+ that is expected to decrease the stability of the jarosite phase.¹³⁻¹⁶ The pH decrease in inoculated Nat1 and Nat2 samples was slower than synthetic samples and reached values of 6.52 ± 0.03 and 6.24 ± 0.27 by 720 hours and may be an indicator of the relative stability of the phases. Therefore, the greatest decrease in pH in inoculated jarosite samples was observed in $\text{Syn2} > \text{Syn1} > \text{Nat2} > \text{Nat1} > \text{MM}$ (Figure 5.5B).

The redox potential (Eh) of the control minimal media samples and jarosite samples decreased from moderately oxidizing ($\sim +187$ RmV) to moderately reducing conditions by the end of the experiment (~ -51 RmV) (Figure 5.5C). In contrast, in inoculated MM, Nat1 and Nat2 samples, redox potential was initially lower and increased over time (Figure 5.5D). More specifically, Eh in inoculated MM samples originally increased from -360.3 ± 1.5 at Time 0 to -204.0 ± 1.3 RmV at 720 hours. In the inoculated natural samples, Nat1 and Nat2, Eh values increased from -324.23 ± 1.9 and -268.6 ± 4.0 RmV to -159.4 ± 4.0 and -113.8 ± 0.7 RmV, respectively, by the end of the experiment. Inoculated synthetic samples had higher Eh values at the beginning of the experiment which decreased in Syn1 and Syn2 from -15.53 ± 4.18 and -113.9 ± 4.18 RmV to -153.85 ± 4.37 and -144.20 ± 5.0 RmV by 720 hours.

5.3.2.2 Release of Major Elements. All elements exceeding concentrations of 10,000 ppm in the original jarosite structure as determined by bulk ICP-OES digestions

were classified as major elements and these included Fe, S, K, Na and Pb (Table 5.3). The contribution of jarosite dissolution to Na concentrations could not be evaluated due to the high background concentration of Na contributed by reagents in the minimal media

5. 3.2.3 Sulfur. PIPES buffer dissociates upon acidification during ICP-OES sample preparation and contributed to a background minimal media S concentration of $1830 \pm 22 \mu\text{M}$ ($n=24$). Therefore, all aqueous S concentrations were determined by subtracting the background concentration. S concentrations were consistently higher than all other measured elements in control and inoculated synthetic and natural samples over time (Figure 5.6A and B). Aqueous S release from control Syn1 and Syn2 jarosite samples was initially rapid but subsequently declined after 65 hours reaching maximum concentrations at the end of the experiment of $9678 \pm 95 \mu\text{M}$ and $8672 \pm 58 \mu\text{M}$, respectively (Figure 5.6A). Aqueous S release from control Nat1 and Nat 2 was slower and reached maximum concentrations of $833 \pm 29 \mu\text{M}$ and $1105 \pm 356 \mu\text{M}$ at 720 hours. The percent S release was calculated by comparing the original solid phase concentrations to aqueous concentrations and decreased in the order: Syn1 (79.4 %) > Syn2 (65.3%) > Nat 2 (8.7%) > Nat 1 (6.9 %). Ideally, comparison of aqueous S to the corresponding reacted solid would provide a better mass balance of dissolution on a per sample basis.³³ Therefore, the calculated values are only estimates and may vary based on sample heterogeneity.

Sulfur concentrations were higher in inoculated synthetic and natural jarosite samples as compared to control samples. The majority of aqueous S released from Syn1 samples occurred within the first 24 hours and reached a maximum concentration of

11,962 $\pm$ 348 μ M and corresponded to 98 % S release from the original sample (Figure 5.6B). S release from inoculated Syn2 and Nat2 samples also occurred rapidly with the majority of dissolution occurring within the first 65 hours of the experiment with rates declining over time and reaching maximums of 12,049 $\pm$ 585 μ M and 10,372 $\pm$ 479 μ M, respectively. S release from inoculated Nat 1 samples was slower and reached a maximum of 2032 $\pm$ 462 μ M at 720 hours. Therefore, S release decreased in the order: Syn1 (98.2 %) > Syn2 (90.8 %) > Nat 2 (81.5 %) > Nat 1 (16.7 %). The difference in initial and final S concentrations between control and inoculated jarosite samples were compared using Student t-tests and was statistically significant in Syn 2 (p= 0.038), Nat 1 (p=0.041) and Nat2 (p= 0.0001) and not significant in Syn1 (p= 0.117). The non-significance between control and inoculated Syn1 S dissolution profiles is likely due to the higher surface area of Syn1 thus rendering it more susceptible to abiotic dissolution.

5.3.2.4 Iron Reduction. Aqueous Fe(II) release was not observed in any control samples during ferrozine analysis and total Fe concentrations (ICP-OES) remained below 1 μ M throughout the experiment (data not shown). The minimal aqueous Fe concentrations in control jarosite samples is attributed to the poor solubility of Fe(III) (10^{-9} μ M) under circum-neutral conditions whereby any Fe(III) released during the dissolution of jarosites will form ferrihydrite or goethite.^{11,37} Fe(III) reduction as indicated by aqueous Fe(II) production was highest in inoculated synthetic samples in the decreasing order: Syn1 (8761.03 $\pm$ 9.05 μ M) $\approx$ Syn2 (8628.11 $\pm$ 592.58 μ M) > Nat2 (5123.36 $\pm$ 63.52 μ M) > Nat1 (592.58 $\pm$ 0.24 μ M) (Figure 6E). To account for variations in Fe concentrations within the original jarosites, total solid phase Fe reduction was determined by comparing aqueous Fe(II) concentrations at 720 hours to

the initial solid phase concentrations and is reported based on decreasing concentrations of released Fe(II): Syn1 ($52.83 \pm 0.02 \%$) > Syn2 ($39.07 \pm 0.10 \%$) > Nat2 ($23.63 \pm 3.19 \%$) > Nat1 ($2.37 \pm 0.12 \%$). Fe(III) reduction rates in inoculated jarosite samples were generated by linear least-squares regression analysis of the linear portion of the aqueous Fe(II) data which corresponded to the first 65 hours. Rates were normalized to starting mass and the original surface areas of each jarosite-group phase and showed similar reduction in rates in synthetic samples in the descending order: Syn1 ($1122 \pm 311 \mu\text{M} \cdot \text{h}^{-1} \cdot \text{m}^{-2}$) $\approx$ Syn2 ($802 \pm 155 \mu\text{M} \cdot \text{h}^{-1} \cdot \text{m}^{-2}$) > Nat2 ($415 \pm 73 \mu\text{M} \cdot \text{h}^{-1} \cdot \text{m}^{-2}$) > Nat1 ($15 \pm 3.5 \mu\text{M} \cdot \text{h}^{-1} \cdot \text{m}^{-2}$) (Table 5.5).

5.3.2.5 Potassium. Initial aqueous K release for control samples (Syn1 and Syn2) occurred rapidly reaching a steady state at 48 hours with maximum concentrations of $4672 \pm 62 \mu\text{M}$ and $3600 \pm 6 \mu\text{M}$, respectively at the end of the experiment (Figure 5.6C). In contrast, aqueous release from control Nat1 and Nat 2 was slower and reached maximum concentrations of $142 \pm 9.3 \mu\text{M}$ and $196 \pm 24 \mu\text{M}$ at 720 hours. The percent solid phase K release was determined by comparing aqueous K concentrations at 720 hours to initial solid phase concentration. Aqueous K release in control samples was greatest in the order: Syn1 (79.75%) > Syn2 (67.28 %) > Nat2 (8.79 %) > Nat1 (2.38 %).

Aqueous K release from inoculated jarosite samples was elevated relative to control samples and final concentrations were higher (Figure 5.6D). In inoculated Syn1 samples, aqueous concentrations reached steady state after 24 hours with a maximum concentration of $6033 \pm 215 \mu\text{M}$ by 720 hours. In inoculated Syn2 samples, aqueous K release was slower and began to plateau after 65 hours with a maximum concentration of

5547 $\pm$ 368 μ M by 720 hours. K release was slower from inoculated Nat1 and Nat2 whereby concentrations gradually increased overtime and reached maximums of 779 $\pm$ 87 μ M and 2126 $\pm$ 292 μ M, respectively, at 720 hours. The variation in K concentrations over time between control and inoculated jarosite samples was compared and was statistically significant in all samples where Syn 1 (p= 0.035), Syn 2 (p= 0.006), Nat 1 (p =0.001) and Nat2 (p= 0.001). Moreover, aqueous K release from all inoculated jarosites samples decreased in the order: Syn1 (102.98 %) > Syn2 (103.66 %) > Nat2 (95.39 %) > Nat1 (13.05 %). The excess aqueous release of K versus that of the solid phase (> 100 %) in Syn1 and Syn2 is likely related to heterogeneity of the original samples and reflects the need to use reacted solids in future experiments. Despite the relatively high Pb concentrations (21,565. ppm) in the Nat1 starting material, aqueous Pb release from all control and inoculated samples including Nat 1 was minimal and remained below 0.3 μ M throughout the experiment (data not shown).

Therefore, based on aqueous S, Fe(II) and K concentrations, dissolution was enhanced in inoculated synthetic and natural samples over control samples in decreasing order: Syn1> Syn2 > Nat2 > Nat 1 with the majority of dissolution generally occurring within the first 65 hours of the experiment with rates declining rapidly over time.

5.3.2.6 Release of Trace Elements. The reductive dissolution of the natural jarosite-group minerals by *S. putrefaciens* also served to enhance the release of trace elements from the solid phase in the following order of decreasing concentrations: Sr (Nat 2) > As (Nat 1, Nat 2) > Zn (Nat 1) > P (Nat 2) > Ba (Nat 2) (Figure 5.7). Sr release from inoculated Nat2 samples contributed to the largest concentration of trace elements observed in this study and reached 93 $\pm$ 1.1 μ M by 720 hours representing 76

% of total Sr (Figure 5.7A and Table 5.3). By comparison, abiotic dissolution of control samples released $2.97 \pm 0.24 \mu\text{M}$ Sr by 720 hours and accounted for 2% Sr release from the solid phase. The difference between Sr release in inoculated and control Nat 2 was significant ($p < 0.0001$). In addition to Sr, the reductive dissolution of Nat2 also increased trace aqueous As concentrations to $4.42 \pm 0.17 \mu\text{M}$ (4 % of Total As) at 190 hours at which point concentrations decreased to below detection limits by 720 hours (Figure 5.7B). In contrast, aqueous As in control Nat2 samples remained below detection limits throughout the experiment. Total acid digest of the original Nat 2 also revealed minor amounts of P ($532.13 \mu\text{M}$) (Table 5.3). Accordingly, trace aqueous P concentrations in inoculated Nat2 samples increased over time to $5.76 \pm 0.36 \mu\text{M}$ by 720 hours while P concentrations remained below $0.05 \mu\text{M}$ in the other control and inoculated jarosite-group samples (i.e. Syn1, Syn2 and Nat1) (Figure 5.7C). Trace concentrations of aqueous Ba also increased overtime in inoculated Nat2 samples to $1.30 \pm 0.08 \mu\text{M}$ by 720 hours while all other control and inoculated samples remained below $0.1 \mu\text{M}$ (Figure 5.7D).

As release from inoculated Nat1 samples contributed to the second largest concentration of trace elements observed in this study and reached $16.87 \pm 1.27 \mu\text{M}$ (5% total As) by 720 hours (Figure 5.7B, Table 5.3). In contrast, aqueous As in control Nat1 samples remained below $1 \mu\text{M}$ throughout the experiment. Previously, *S. putrefaciens* demonstrated As(V) reduction during the reductive dissolution of Pb-As jarosite when As(V) concentrations exceed $\sim 320 \mu\text{M}$ (Chapter 5.3). Unfortunately, As oxidation state was not examined in this experiment and given that As concentrations were minimal ($< 100 \mu\text{M}$), it is unlikely that As(V) reduction occurred.

Zn was released from both inoculated and control Nat1 samples and reached $4.79 \pm 0.64 \mu\text{M}$ (1% Tot Zn) and $3.94 \pm 0.12 \mu\text{M}$ (0.9% Tot Zn), respectively, and the variation in initial and final concentrations between control and inoculated samples was not statistically significant ($p = 0.329$) (Figure 5.7E). The similarities in concentrations are likely attributed to exchange or dissolution of the clay phase. It should also be noted that Zn ($< 1 \mu\text{M}$) was also released from the other jarosite-group phases and was contributed by synthesis reagents (Table 5.3).

5.3.2.7 ATP Concentrations. Using the Bactiter Cell viability assay, ATP concentrations were measured at each time interval. ATP concentrations provide a useful indirect metric for microbial biomass estimates in both uncontaminated and metal-contaminated soils; even at high metal concentrations.^{38,39} The toxicity of substituted elements to *S. putrefaciens* was investigated as a potential contributor to cell death and obstacle to Fe reduction. By the end of the experiment, ATP in all inoculated samples decreased to below 1 nM (Figure 5.7F). However, in inoculated synthetic samples ATP concentrations increased in both Syn1 and Syn2 samples which is likely due to the energy gained during the intense Fe reduction observed in those samples, while concentrations decreased in minimal media, Nat1 and Nat2. Interestingly, ATP decreased quickly in Nat1 more rapidly than the other samples and may be linked to the high concentrations of Pb within the mineral phase (Table 5.3). Overall, ATP concentrations decreased the fastest in the order Nat1 > Nat2 > MM > Syn2 > Syn1.

5.3.2.8 SEM Characterization. SEM images of inoculated Syn1, Syn2 and Nat 2 samples taken at Time 0 and 720 hours shows extensive dissolution and secondary precipitation. SEM images of Nat1 also corroborate solution data whereby there was less

release of elements and shows minimal dissolution. More specifically, BSE-SEM images of Syn1 showed extensive dissolution and pitting of the solid phase (Figures 5.8A, B and 5.9A). Images of Syn2 show secondary precipitates even at Time 0 which likely occurred between sampling and sample storage (~ 2 hours) (Figure 5.8C). By 720 hours, there is extensive dissolution along Syn2 grain boundaries revealing hollow centers in larger intergrown crystals (Figure 5.8D). Extensive pitting on the surface of Syn2 was also observed in association with *S. putrefaciens* (Figure 5.9B). However, pitting may also be due to abiotic dissolution and has been previously demonstrated using atomic force microscopy (AFM).¹⁶ Secondary precipitation occurred on the surface of some Nat 1 crystals, although to a lesser degree than the other jarosite samples (Figures 5.8E, F and 5.9C). Interestingly, electron dense particles associated with *S. putrefaciens* in Nat 1 were observed in samples at 720 hours (Figure 5.10). These particles closely resembled those identified as intracellular Pb phosphates formed by *S. putrefaciens* in response to Pb toxicity during the reductive dissolution of Pb-jarosite (Chapter 2).²² Given the high Pb concentrations (21,565 ppm) of the original Nat1 and the sharp decrease in ATP concentrations at the beginning of the experiment, it is possible that the same mechanism may be occurring in Nat1 samples. However, in the absence of TEM data, it is not certain if the particles are indeed intracellular Pb phosphates.

Similarly to Syn1 and Syn2, Nat2 also underwent extensive dissolution as demonstrated by the extensive secondary mineralization at the surface of the jarosite (Figures 5.8G, H and 5.9D). SEM images of Nat2 at 720 hours shows acicular habits of the precipitated phases associated with the original jarosite surface (Figure 5.9D). The

acicular precipitate is likely the result of the abiotic dissolution of jarosite with the formation of goethite or ferrihydrite. The larger crystal size of Nat 2 at 720 hours allows us to see surface transformation of the original jarosite mineral where surface precipitates and voids have replaced the original structure. In a previous study, Smith et al. (2006) demonstrated selective dissolution of the SO_4^{2-} tetrahedra and K^+ sites relative to the Fe octahedra sites.¹¹ Therefore, depending on microbial concentrations, a portion of the Fe(III) will be reduced to Fe(II) and potentially precipitated as an Fe(II) phase while the remainder of available Fe(III) will precipitate as goethite $\text{FeO}(\text{OH})$ or ferrihydrite $(\text{Fe}(\text{OH})_3)$.

5.4 DISCUSSION

By comparing S, K and Fe(II) concentrations over time in control and inoculated samples we demonstrated that *S. putrefaciens* enhances dissolution of jarosites and the degree of dissolution is greater in synthetic over natural samples. It is expected that the major factors influencing the susceptibility of synthetic and natural jarosite group minerals to dissimilatory Fe reduction will be: 1) surface area; 2) structure; and the 3) potential toxicity of the constituent elements to bacteria.

5.4.1 Surface area. The role of surface area on jarosite dissolution has never been elucidated. However, a previous long term study (27.5 months) on the influence of particle size on K-jarosite dissolution showed enhanced abiotic dissolution in smaller particles.¹⁷ Interestingly, BET surface area measurements of Nat1 ($4.66 \pm 1.01 \text{ m}^2/\text{g}$) were higher than the other jarosite samples used in this study (Table 5.4). This is surprising due to the smaller crystal size of Syn1 (0.5 - 2 μm) as compared to Nat1 (0.5 -

12 μm). However, as compared to geometric surface area, the BET surface area analysis method used in this study includes all of the surface area generated by surface roughness such as etch pits, pores, steps, and kinks.^{40,41} As demonstrated in silicate minerals, the surface roughness and pitting of the naturally weathered jarosites along with any minor amounts of clays or unidentified minerals will contribute to increased surface area.⁴² Furthermore, particle aggregation of the fine-grained synthetic jarosites during BET analysis will change the total surface area available for N_2 adsorption and potentially underestimate surface area in synthetic samples.²⁶ The latter case, may be an explanation for the similar surface areas of Syn1 ($1.68 \pm 0.19 \text{ m}^2/\text{g}$) and Syn2 ($1.67 \pm 0.22 \text{ m}^2/\text{g}$) whereby Syn1 was expected to have a higher surface area over Syn2 because it had smaller crystals (0.5 - 2 μm) versus those of Syn2 (0.5 - 8 μm). The aggregation of Syn1 can be seen in the Figure 5.2 which may have reduced the total surface area available for N_2 adsorption. Therefore, particle size is potentially a better indicator of reactivity over surface area. Higher surface area is expected to enhance reductive dissolution because smaller crystallites exhibit higher solubility due to greater structural disorder and surface tension effects and therefore will have greater surface Fe(III) sites available for reduction.^{26,27}

Production of Fe(II) over time in inoculated samples showed that total % Fe reduction of the jarosite samples was greatest in Syn1 by the end of the experiment in the following order of decreasing reduction: Syn1 ($52.83 \pm 0.02 \%$) > Syn2 ($39.07 \pm 0.10 \%$) > Nat2 ($23.63 \pm 3.19 \%$) > Nat1 ($2.37 \pm 0.12 \%$) (Table 5.4). However, surface area normalized Fe(II) production rates were higher in Syn1 but within experimental error of Syn2 where Syn1 = $1122 \pm 311 \mu\text{M}\cdot\text{h}^{-1}\text{m}^{-2}$ and Syn2 = $802 \pm 155 \mu\text{M}\cdot\text{h}^{-1}\text{m}^{-2}$.

Similarly to Fe oxides, surface area is expected to exert an influence on Fe reduction from jarosites in synthetic samples. However, this was not demonstrated in natural samples where Fe(II) production was slowest in the highest surface area jarosite, Nat1. Therefore, the difference between Fe(III) production rates is likely related to the structural constraints imposed on the reductive dissolution pathway.

5.4.2 Structural Constraints on Fe reduction. Aside from surface area, the enhanced reductive dissolution of synthetic jarosites over natural jarosites is likely to be complicated by structural factors such as: 1) H_3O^+ ion substitution; 2) additional A-site substitutions; and 3) surface coatings or accessory minerals. Based on comparison of samples used in this study, Nat1 was the least susceptible to both abiotic dissolution and Fe(III) reduction by *S. putrefaciens*. According to XRD patterns collected on the jarosites used in this study, all jarosites were identified as containing H_3O^+ with the exception of Nat1 (Figure 5.1). H_3O^+ substitution is well documented to increase solubility in jarosite minerals by increasing the *c*-parameter of the crystallographic structure which decreases its stability while increasing reactivity.^{12,17} Therefore, the lack of H_3O^+ in the structure of Nat1 may contribute to its overall relative stability compared to the other jarosites used in this study. The total acid digestion and SEM-EDS of the original Nat1 sample also revealed relatively high Pb concentrations corresponding to a potential structural occupancy of 3.49 mol % (Table 5.2). The substitution of Pb^{2+} into the mineral lattice will increase stability because it has twice the charge and is a smaller cation with an atomic radius of 1.20 Å while K^+ is larger at 1.31 Å. Indeed, our previous study with *S. putrefaciens* under similar conditions using

synthetic Pb-jarosite showed comparably low Fe (II) production of $197.1 \pm 2.29 \mu\text{M}$ versus the $592.58 \pm 0.24 \mu\text{M}$ as observed in Nat1 (Chapter 2).²² The lower Fe(II) concentrations in the Pb-jarosite is likely due to the higher structural Pb content of the pure Pb-jarosite which is toxic to bacteria. Therefore, the lower reductive dissolution of Nat1 in this study as compared to Syn1, Syn2 and Nat2 is not necessarily a reflection of synthetic versus natural differences but rather a reflection of the overall structural constraints hindering Fe reduction by *S. putrefaciens*.

The initial difficulty dissolving the natural samples during total acid digestion suggested the presence of a refractory phase not identified by XRD analysis. The refractory phase such as the clay seen in Nat1 or the Al-Fe oxide in Nat2 or another unidentified phase may have hindered dissolution as a surface coating or may have physically aggregated with the natural jarosites to prevent physical access to the surface. XRD and total acid digests indicated that Nat 2 contained H_3O^+ , Na and K of which the 2 former cations are expected to enhance solubility and reactivity of the jarosite (Table 5.3, Figure 5.1).¹⁶ However, Fe(II) production from Nat2 was lower than the two synthetic K-jarosites (Figure 5.6E). Therefore, the decreased reductive dissolution for both natural jarosites may be due to interference by unidentified refractory phases.^{12,33}

5.4.3 Toxicological Constraints on Fe reduction. The exact role that metal toxicity plays in inhibiting Fe(II) reduction in the natural samples is difficult to discern. ATP concentrations of Nat1 samples (i.e. contains Pb) show decreased viability after 24 hours as compared to the other treatments, which may be the result of Pb toxicity on *S. putrefaciens* (Figure 5.7F). Moreover, SEM images taken at 720 hours show potential evidence of intracellular accumulation of Pb as observed in a previous study in response

to Pb toxicity by *S. putrefaciens* from Pb-jarosite (Figure 5.10) (Chapter 2).²² However, based on the minimal release of K and S over time from control Nat1 samples, it is clear that Nat1 is less susceptible to dissolution due to structural constraints which in addition to toxicity, may pose the greatest barrier to reductive dissolution (Figure 6). The release of $94 \pm 1.1 \mu\text{M}$ Sr from Nat2 inoculated samples may also pose a toxicological constraint on reductive dissolution (Figure 5.7A). However, Brown et al. (2006) showed that *Shewanella oneidensis* was able to withstand Sr concentrations less than 180 mM suggesting that it is unlikely Sr posed a large obstacle to reductive dissolution.⁴³

5.4.4 Additional Factors. The role of pH on cell viability in synthetic samples may have influenced the decrease in cell viability and Fe(II) production after 65 hours whereby the pH decreased to 5.62 and 4.9 in Syn1 and Syn2 samples, respectively (Figure 5.5). The initial decrease in pH may be due to the overriding abiotic dissolution occurring simultaneously with biotic Fe(III) reduction thus creating external stress on *S. putrefaciens* and leading to cell lysis. Our previous study with synthetic Tl-jarosite and *S. putrefaciens* showed cell lysis at pH 5 and corroborates previous studies with *S. putrefaciens* which showed the bacterium's sensitivity to low pH environments (Chapter 4).^{44,45} Additionally, the build up and sorption of Fe^{2+} onto the jarosite surface may have served to passivate the surface and decrease Fe(III) reduction rates.²⁷ More importantly, SEM images revealed extensive secondary precipitation in Syn1, Syn2 and Nat2 which is known to decrease jarosite dissolution rates (Figure 5.9).¹⁶ While secondary mineralization characterization was beyond the scope of this study, our previous experiments have documented extensive mineralization and characterization of amorphous Fe hydroxide precipitates which are expected to serve as sinks and sorb

elemental constituents such as Pb from samples such as Nat1 during jarosite dissolution (Chapters 2-4).^{22,23} Unfortunately, due to the lack of sample collection information on the natural samples, the origin of the samples could not be used as a potential indicator of reactivity. Future studies should investigate the potential role of sample origin (i.e low-temperature versus hydrothermal) on their susceptibility to Fe reduction to better elucidate if synthetic samples are indeed representative of low-temperature natural jarosites.

5.5 CONCLUSIONS

In this experiment we showed that the synthetic and natural jarosite used were both susceptible to reductive dissolution by *S. putrefaciens* CN32. However, the extent of Fe(III) reduction was greater in the synthetic H₃O,K-jarosite samples (Syn1 and Syn2) than in natural K,Pb,As-jarosite (Nat1) and H₃O,Na,K,Sr-jarosite (Nat2) samples in the following order of decreasing susceptibility: Syn1 > Syn2 > Nat2 > Nat1. We also demonstrated that particle size rather than surface area was a better predictor of susceptibility to Fe(III) reduction in synthetic samples but was not a significant factor in natural samples. Moreover, we also showed that the susceptibility of natural jarosites to Fe(III) reduction is likely to be affected by substitutions into the mineral lattice by cations such as H₃O⁺, Na, Pb²⁺ or accessory minerals and/or surface coatings. Lastly, despite decreased Fe(III) reduction in the natural samples, trace metal release of Sr, As, Zn, P still occurred and the reductive dissolution of jarosites may serve as a source of such elements in disposal environments.

ID	Jarosite phases (identified by XRD)	Chemical Formula	ICD PDF
Syn1	(K, H ₃ O)Fe ₃ (SO ₄) ₂ (OH) ₆	(H ₃ O) _{0.04} K _{0.96} Fe _{2.91} (SO ₄) ₂ (OH) _{5.76} (H ₂ O) _{0.24}	00-036- 0427
Syn2	(K, H ₃ O)Fe ₃ (SO ₄) ₂ (OH) ₆	(H ₃ O) _{0.19} K _{0.81} Fe _{3.02} (SO ₄) ₂ (OH) ₆	00-036- 0427
Nat1	KFe ₃ (SO ₄) ₂ (OH) ₆	Could not be determined, potential substitution of Pb and As	00-022- 0827
Nat2	(H ₃ O)Fe ₃ (SO ₄) ₂ (OH) ₆ NaFe ₃ (SO ₄) ₂ (OH) ₆ KFe ₃ (SO ₄) ₂ (OH) ₆	(H ₃ O) _{0.17} Na _{0.48} K _{0.34} Sr _{0.01} Fe ₃ (AsO ₄) _{0.02} (SO ₄) _{1.98} (OH) ₆	00-021- 0932 00-051- 1567 97-001- 2107

Table 5.1. Identity of jarosite samples determined by X-ray powder diffraction analysis and calculated chemical formulas of jarosites where ICDD PDF corresponds to the International Centre for Diffraction Data powder diffraction file identified as a match during analysis.

Sample Code	Potential Occupancy (%)								
	A-site					B-site		T-site	
	K	Na	Sr	Pb	H ₃ O ⁺	Fe	Zn	As	S
Syn1	96.4	---	---	---	3.6	97.3	---	---	100
Syn2	80.8	---	---	---	19.2	100.7	---	---	100
Nat1	98.4	---	---	3.49	---	125.2	7.00	2.84	97.16
Nat2	33.8	48.2	0.97	---	17.0	124.0	---	0.99	99.01

Table 5.2. Potential % occupancy of the A, B and T sites within the jarosite-group minerals based on the formula: A_{1-x}(H₃O)_xB_{3-y}(OH)_{6-3y}(H₂O)_{3y}(TO₄)₂ whereby S is assumed at 100 % occupancy in Syn1 and Syn2 and molar A and B site potential constituent concentrations are normalized to molar S concentrations as determined by ICP-OES.

Element	Syn1 (ppm)	Syn2 (ppm)	Nat1 (ppm)	Nat2 (ppm)
Fe	239,592.69	319,260.88	312,367.48	293,618.95
S	94,317.04	121,371.68	92,756.57	89,649.52
K	55,475.13	59,835.28	57,623.28	18,659.65
Na	---	---	209.63	29,416.76
Pb	20.99	30.33	21,565.58	71.19
Zn	61.07	84.59	6,822.52	60.91
As	---	---	6,285.84	2,086.24
Ca	88.80	135.70	3,085.50	264.86
Sr	---	1.57	52.17	2,412.06
P	---	---	532.13	---
Se	---	---	242.07	---
Bi	64.54	78.32	134.37	76.42
Ba	5.32	7.17	48.39	92.27
Ti	20.69	40.93	48.07	6.19
Mn	---	---	44.60	10.98
Cd	21.08	26.10	39.94	32.04
Mo	---	---	13.44	25.18

Table 5.3. Elemental Concentrations (ppm) of 50 mg of acid digested jarosite minerals as determined by ICP-OES.

Sample Code	Surface area (m ² /g)	Fe(II) _{aq} release rate (μM·h ⁻¹ m ⁻²)	% Total Fe reduced
Syn1	1.68 ± 0.19	1122 ± 311	52.83 ± 0.02
Syn2	1.67 ± 0.22	801 ± 155	39.07 ± 0.10
Nat1	4.66 ± 1.01	15 ± 3.5	2.37 ± 0.12
Nat2	1.81 ± 0.32	416 ± 73	25.30 ± 0.02

Table 5.4. BET surface area, Fe(II)_{aq} release rate and % Total Fe reduction of Syn1, Syn2, Nat1 and Nat2 samples. Rates were normalized to starting jarosite mass and surface area.

Element	Syn 1 (wt %)	Syn 2 (wt %)	Nat1 (wt %)	Nat2 (wt %)
O	46.41 ± 2.56	49.28 ± 1.93	42.65 ± 4.03	48.09 ± 4.98
S	14.44 ± 0.21	14.51 ± 0.27	13.35 ± 0.61	14.04 ± 0.85
K	7.68 ± 0.30	7.91 ± 0.72	7.15 ± 0.45	2.13 ± 0.31
Fe	31.30 ± 2.22	28.30 ± 1.68	32.33 ± 2.73	31.22 ± 4.17
Zn	---	---	0.52 ± 0.15	---
As	---	---	0.39 ± 0.07	0.44 ± 0.10
Pb	---	---	3.61 ± 0.93	---
Na	---	---	---	4.08 ± 0.28

Table 5.5. Relative elemental concentrations (wt%) from EDS analysis of randomly selected Syn1 (n=5), Syn2 (n=6), Nat1(n=4) and Nat2 (n=4) crystals.

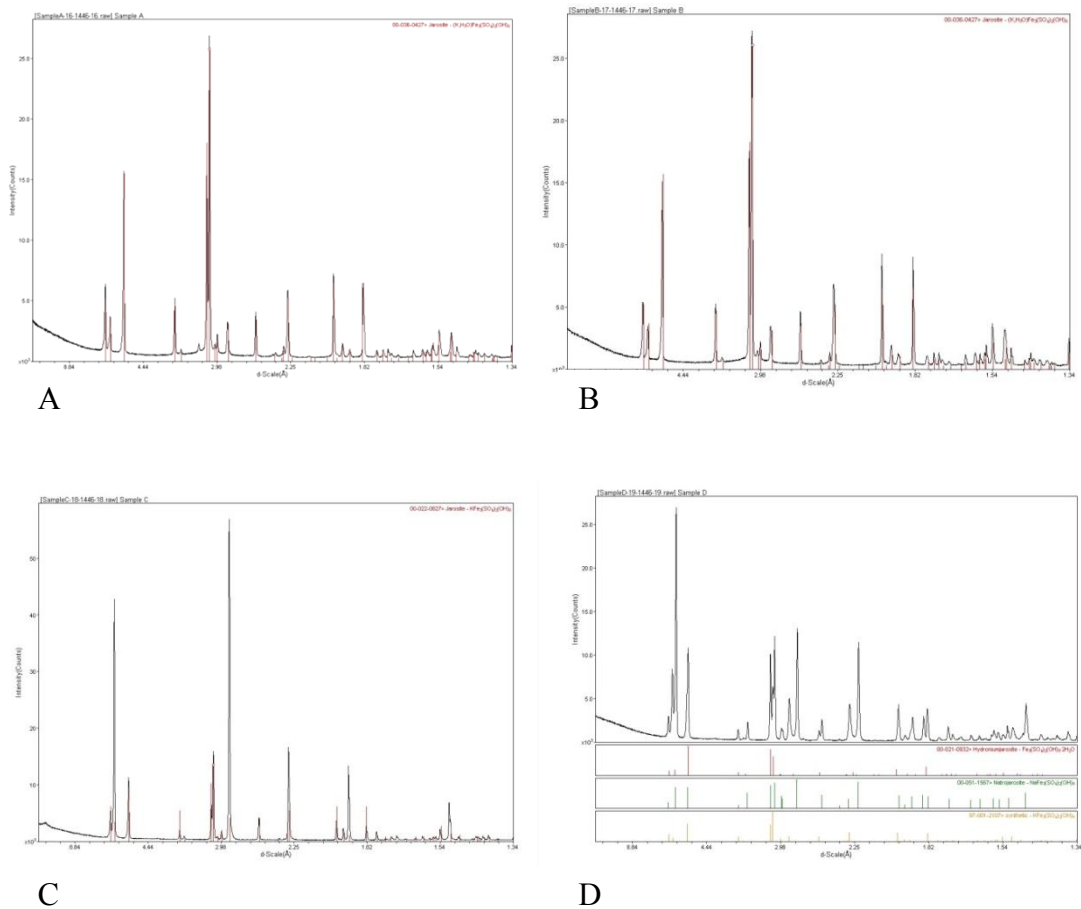


Figure 5.1. Powder X-ray diffraction spectrum of A) synthetic jarosite (Syn1); B) synthetic K- jarosite (Syn2); C) natural jarosite (Nat1); and D) natural jarosite (Nat 2)

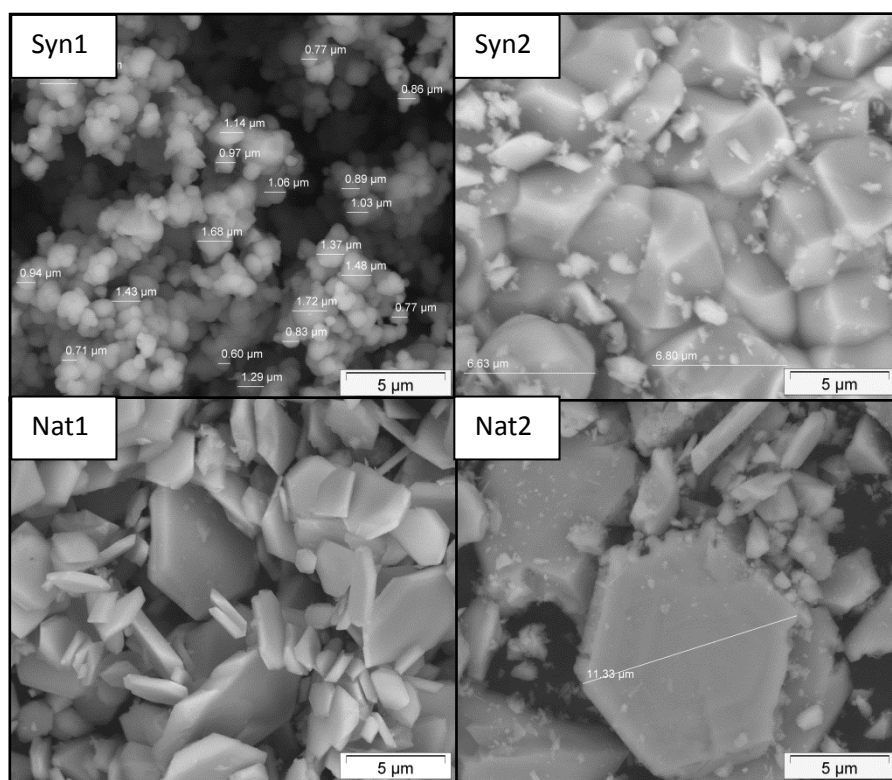


Figure 5.2. Back-scattered scanning electron (BS-SEM) images of the jarosite-group phases used in the experiment. Sample identity is noted on each image.

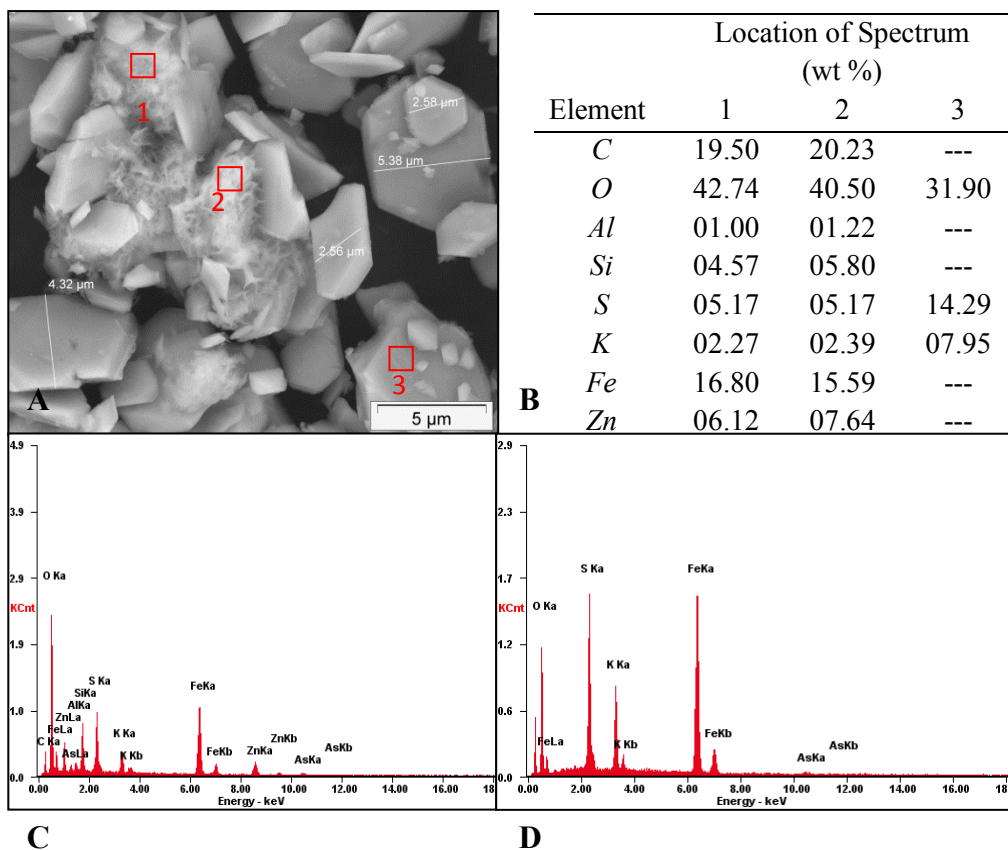


Figure 5.3. A) BSE-SEM image of a secondary mineral phase associated with natural jarosite, Nat1 taken at 30 kV; B) Relative elemental concentrations (wt %) of spectra taken from the locations denoted as 1, 2 and 3 in A; (C) EDS spectra taken at 30 kV of area denoted as 1 from image A; and (D) EDS spectra taken at 30 kV of area denoted as 3 in A.

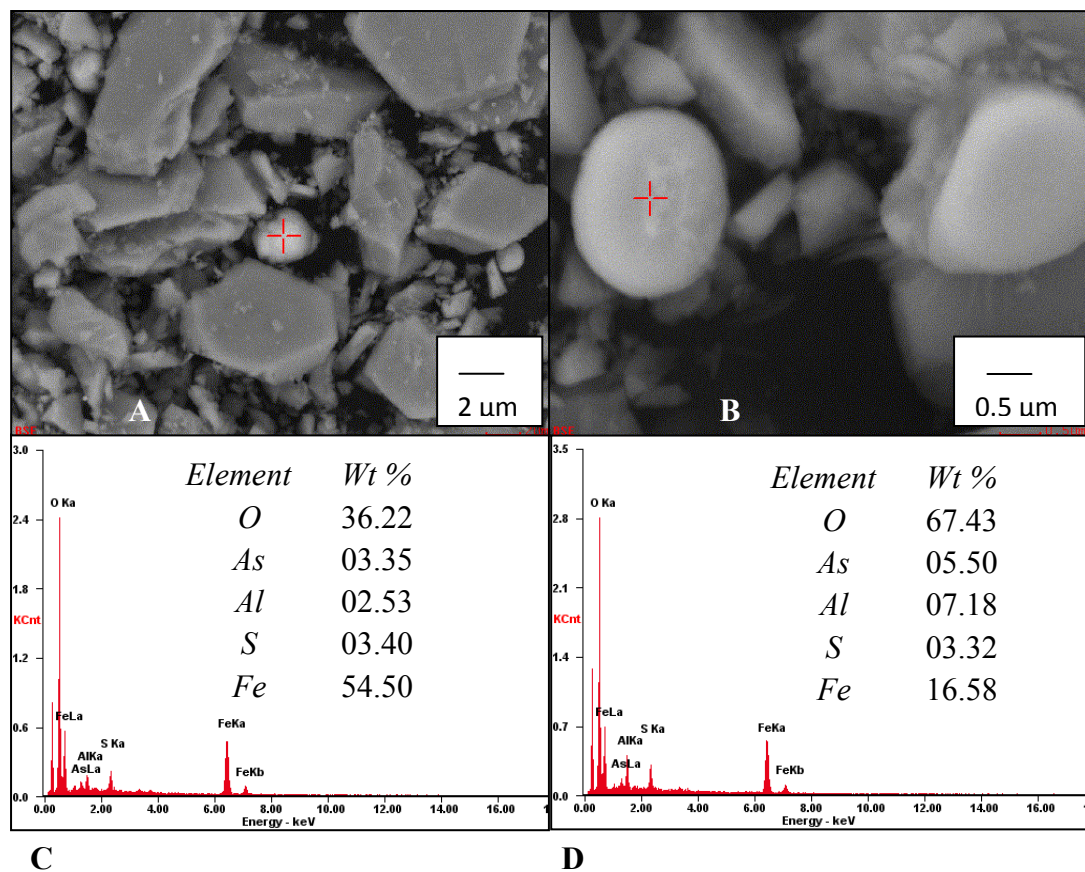


Figure 5.4. A and B) BSE-SEM image of accessory minerals associated with natural sample, Nat2. Red cross-hair denotes the accessory mineral and the area of location of EDS analysis; and (C) EDX spectra taken at 30 kV from the location denoted by red cross-hair in image A, inlaid table is the relative elemental concentrations (wt %); (D) EDX spectra taken at 30 kV from the location denoted by the red cross-hair in image B, inlaid table is the relative elemental concentrations (wt %).

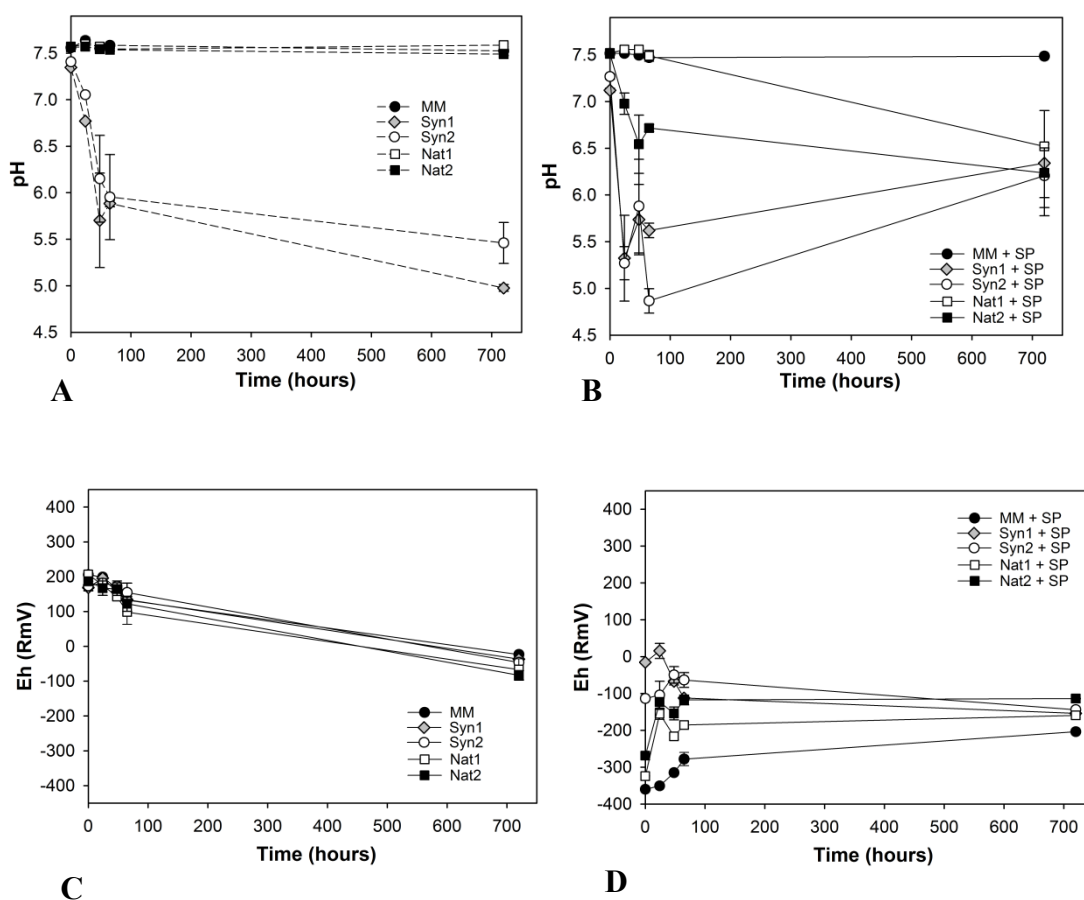


Figure 5.5. SP denotes samples inoculated with *S. putrefaciens*. pH in A) control; and B) inoculated jarosite samples over time. Redox potential (Eh) in C) control; and D) inoculated jarosite samples over time. Error bars represent standard error (n=3).

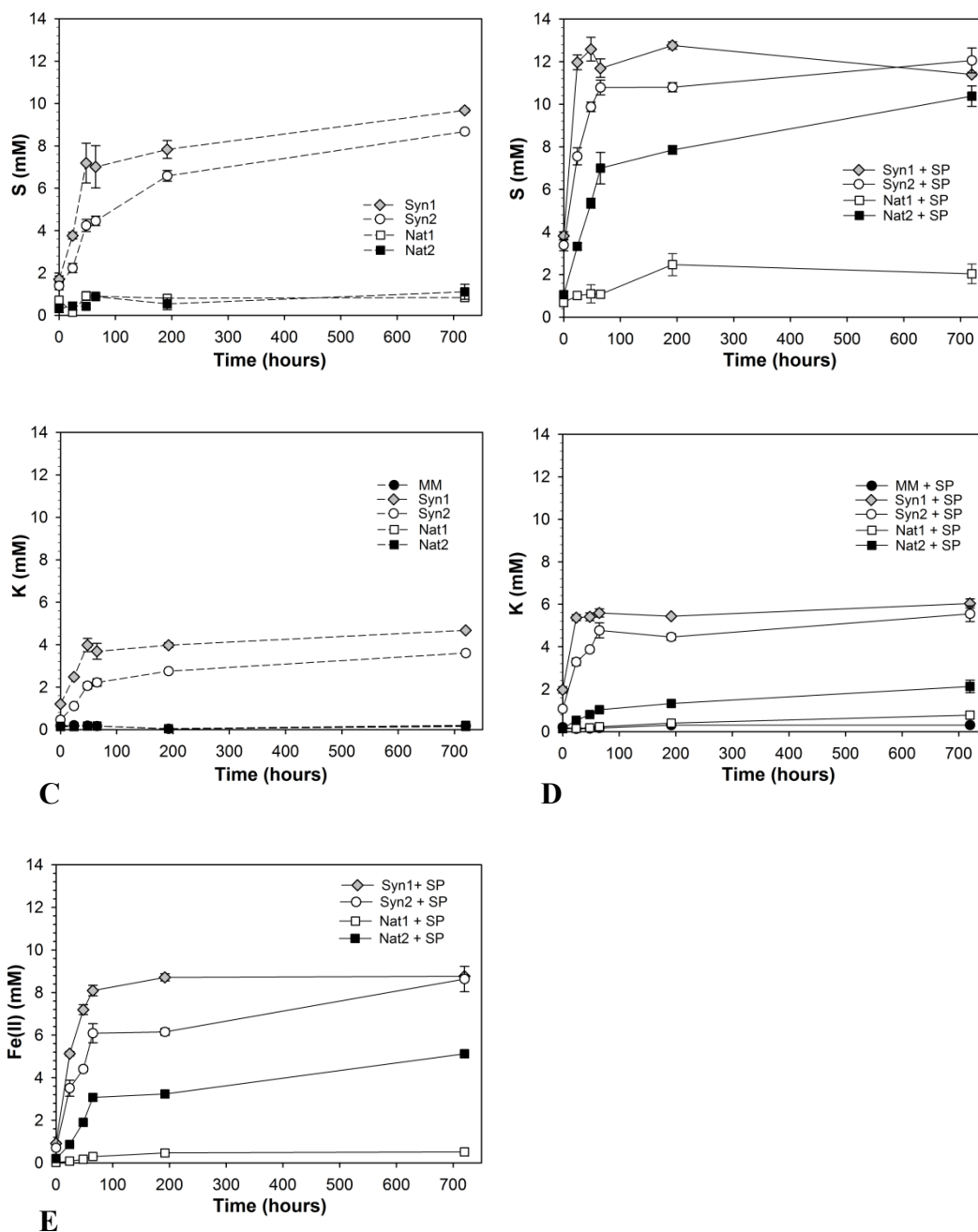


Figure 5.6. SP denotes samples inoculated with *S. putrefaciens*. Aqueous S concentrations in A) control; and B) inoculated samples over time. Aqueous K concentrations in C) control; and D) inoculated jarosite samples over time, E) Aqueous Fe(II) production in inoculated samples over time. Error bars represent standard error (n=3).

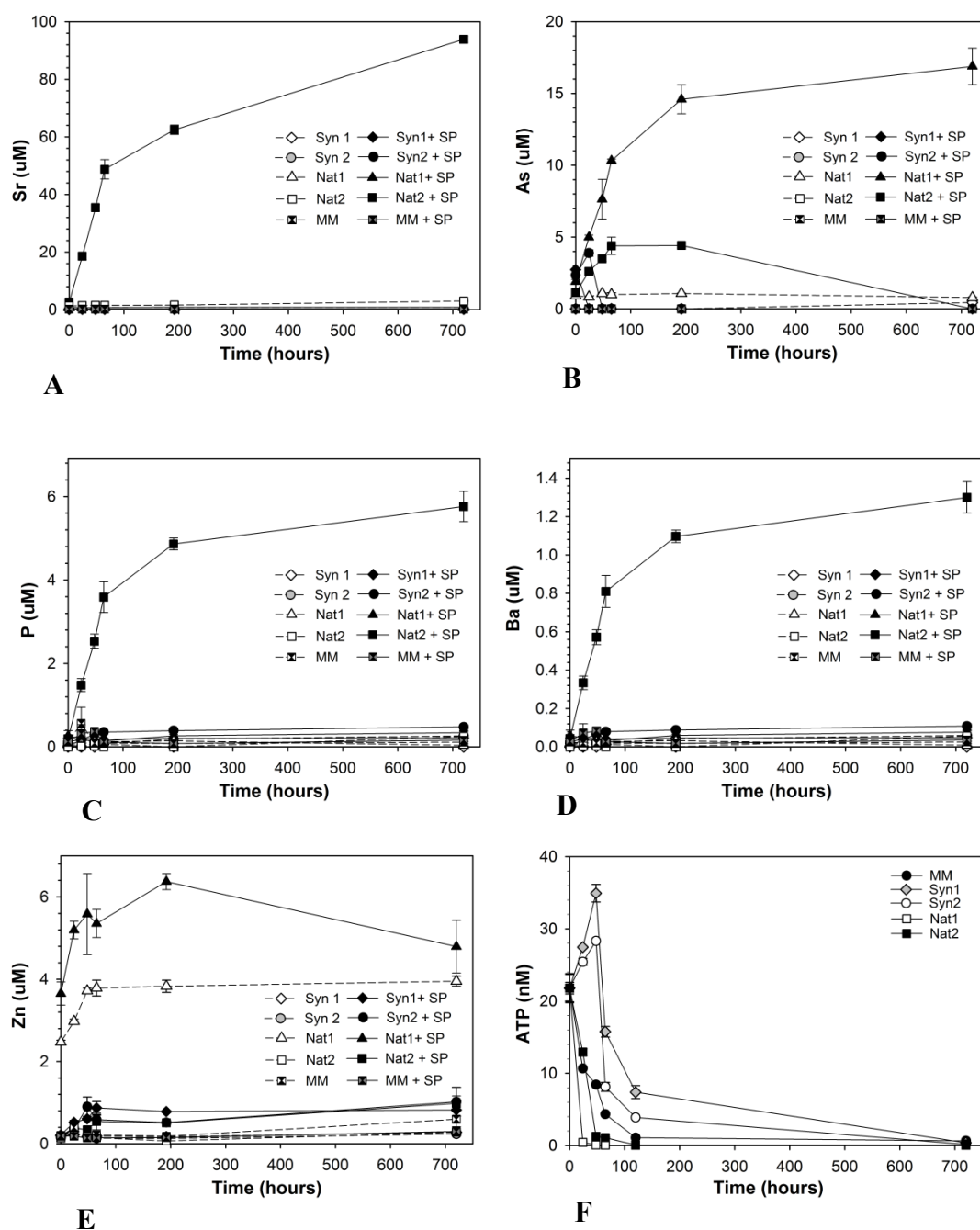


Figure 5.7. SP denotes samples inoculated with *S. putrefaciens*. Aqueous A) Sr; B) As; C) P; D) Ba; and E) Zn concentrations in control and inoculated samples over time. Note the different scales; F) ATP concentrations in inoculated samples over time. Error bars represent standard error (n=3).

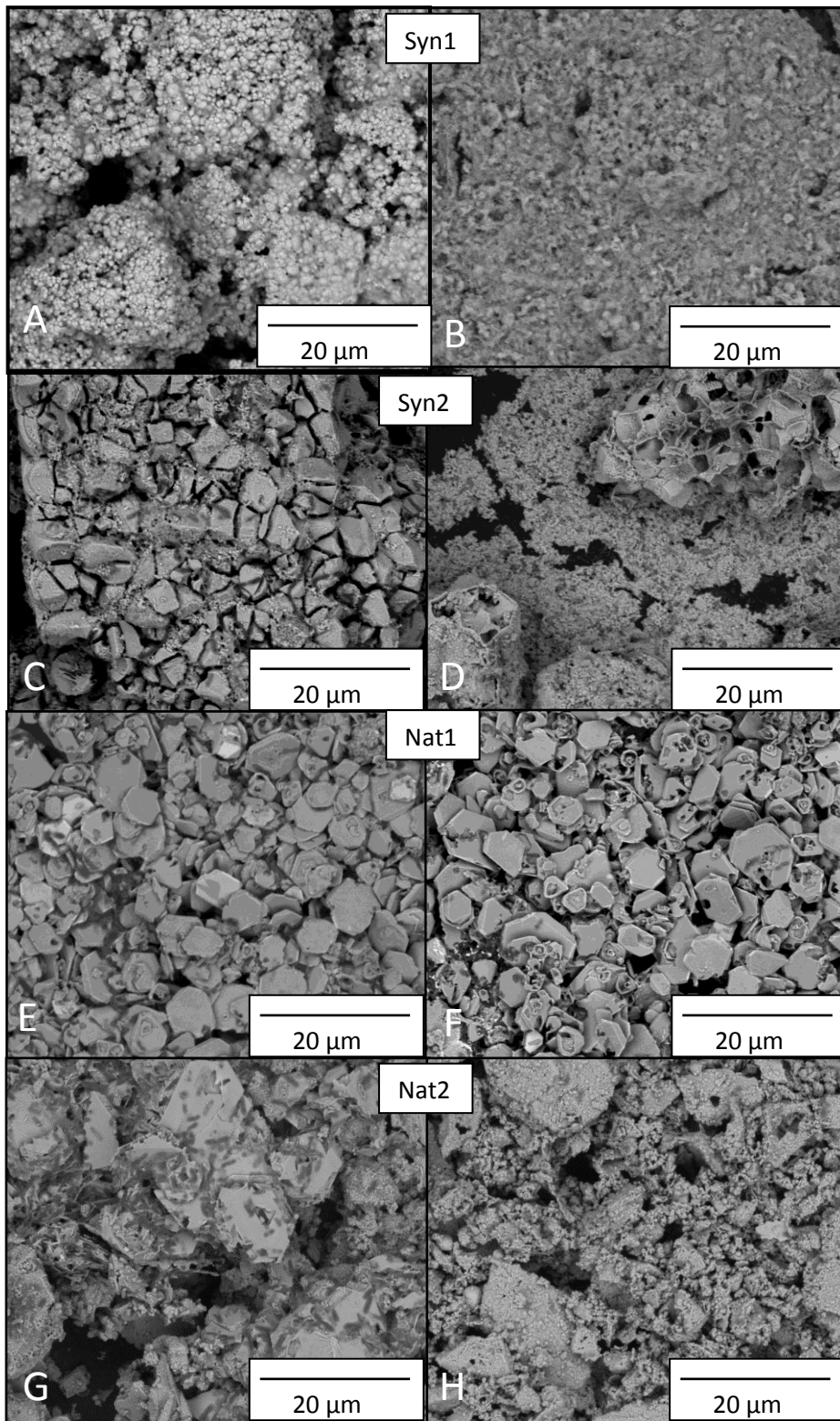


Figure 5.8. BS-SEM images of inoculated jarosites at A,C,E,G) Time 0 and; B, D, F, H) 720 hours. Note: sample code is indicated on the image.

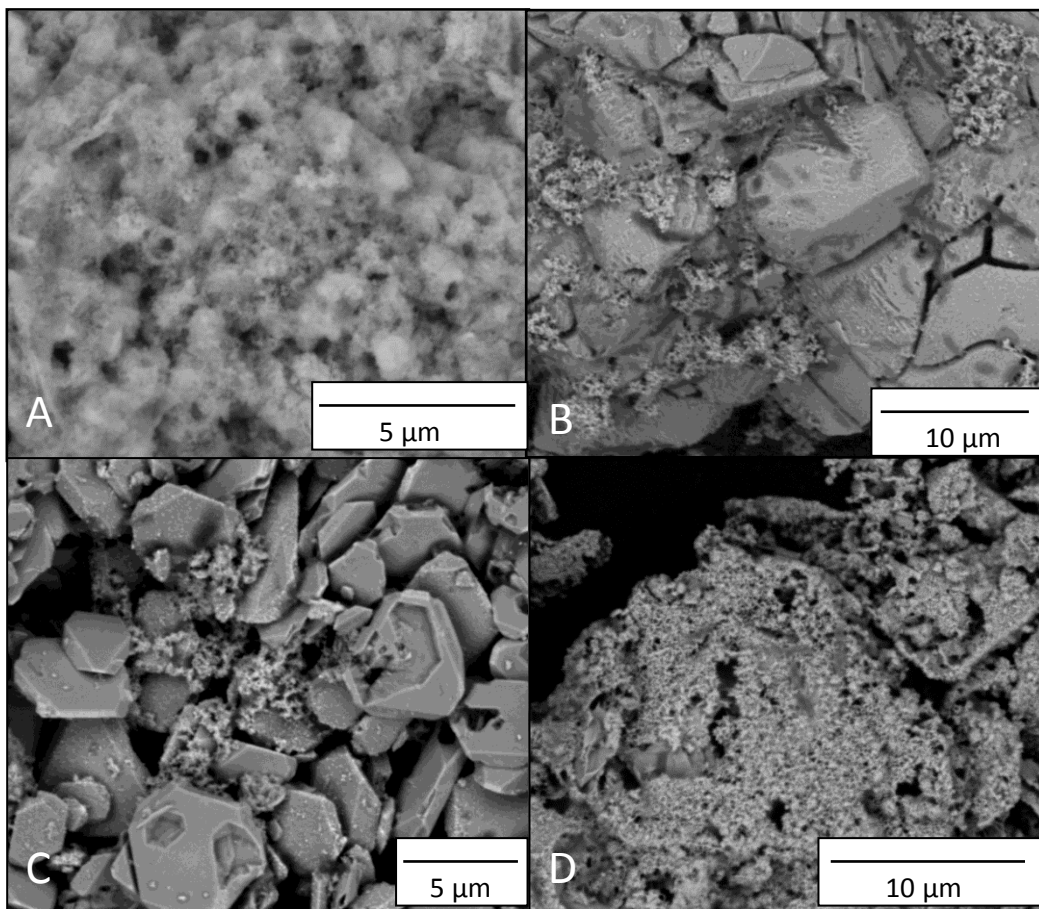


Figure 5.9. BS-SEM images of inoculated jarosites of A) Syn1; B) Syn2; C) Nat1; and D) Nat2 at 720 hours.

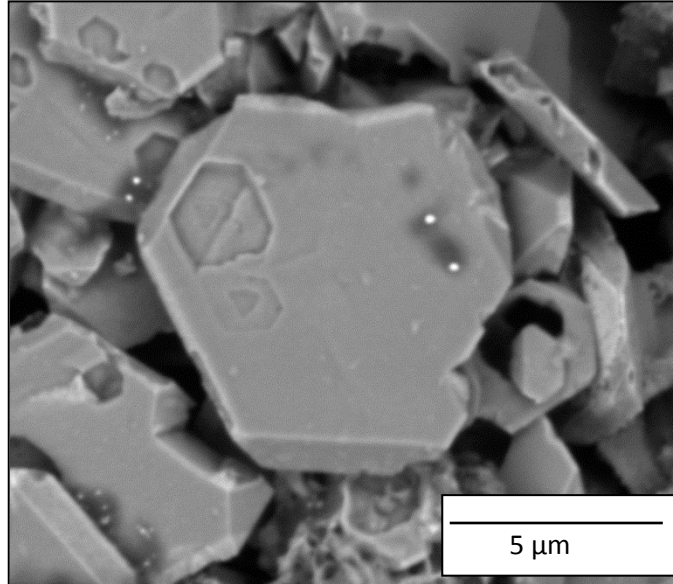


Figure 5.10. BS-SEM images of inoculated Nat1 at 720 hours. Note the electron dense precipitate associated with the cell.

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CHAPTER 6

Discussion and Conclusions

6.1 SUMMARY

The primary goal of this dissertation was to examine the influence of jarosite structure on dissimilatory Fe(III) reduction. A secondary goal was to examine the potential toxicity and fate of metals released during dissolution of the jarosite phases on *S. putrefaciens* CN32. This was accomplished by incubating a variety of synthetic and natural jarosites with the facultative anaerobe, *Shewanella putrefaciens* CN32 using lactate as the sole electron donor. The experiments were conducted in a simplified minimal medium, containing no phosphate or added trace elements at $\sim 25^{\circ}\text{C}$ under anaerobic conditions and buffered at $\text{pH} \sim 7$. The electron donor, lactate, was present in excess so its availability would not affect the rate of Fe reduction across jarosites. Aqueous Fe(II) production was used as a proxy for Fe(III) reduction. Solution chemistry, pH, Eh and cell viability were monitored over time while the solid phase was characterized using SEM-EDS, TEM-EDS, X-ray Absorption Spectroscopy (XAS) and XRD.

In Chapter 2, I examined the reductive dissolution of synthetic Pb-jarosite, $\text{PbFe}_6(\text{SO}_4)_4(\text{OH})_{12}$ whereby divalent Pb^{2+} is in the A-site of the jarosite structure. *S. putrefaciens* reductively dissolved Pb-jarosite via the reduction of structural Fe(III) with minimal release of aqueous Pb ($< 1.5 \mu\text{M}$) over time in both inoculated and control samples. Moreover, I demonstrated for the first time the intracellular precipitation of Pb by *S. putrefaciens* CN 32 thus providing new insights into the biogeochemical cycling of Pb in reducing environments.

Building upon the previous chapter, Chapter 3 examined the reductive dissolution of synthetic Pb-As jarosite,

$(\text{H}_3\text{O})_{0.68}\text{Pb}_{0.32}\text{Fe}_{2.86}(\text{SO}_4)_{1.69}(\text{AsO}_4)_{0.31}(\text{OH})_{5.59}(\text{H}_2\text{O})_{0.41}$, whereby AsO_4^{3-} was incorporated into the T-site of the jarosite structure. This experiment demonstrated enhanced Fe(III) reduction in synthetic Pb-As jarosite relative to Pb-jarosite in Chapter 2 (Table 6.1, Figure 6.1). Simultaneous structural Fe(III) and aqueous As(V) reduction was also observed in inoculated samples. By considering the thermodynamic constraints on structural Fe, As and S reduction from Pb-As-jarosite and calculating the ΔG for each redox reaction, I showed that aqueous As(V) reduction was likely due to detoxification while Fe(III) reduction was driven by respiration. Similarly to Chapter 2, aqueous Pb concentrations were below detection limits in both control and inoculated samples. Interestingly, in contrast to Chapter 2 intracellular Pb precipitation was not observed in *S. putrefaciens* during SEM or TEM analyses. The lack of intracellular Pb precipitation may have been due to increased Fe reduction triggered by *S. putrefaciens* to compensate for ATP lost during hydrolysis to maintain active transport of As(III) out of the cell.¹

In Chapter 4, I examined the reductive dissolution of a monovalent A-site cation jarosite, Tl-jarosite $(\text{H}_3\text{O})_{0.29}\text{Tl}_{0.71}\text{Fe}_{2.74}(\text{SO}_4)_2(\text{OH})_{5.22}(\text{H}_2\text{O})_{0.78}$ and showed extensive dissolution over time. While Fe(II) concentrations at ~ 330 hours in both Pb-As jarosite and Tl-jarosite were similar on a $\mu\text{M} \cdot \text{nM ATP}^{-1} \cdot \text{g}^{-1}$ basis (Figure 6.1), initial Fe(III) reduction was higher in Tl-jarosite samples and there was extensive secondary Fe mineralization not seen in Pb-As jarosite (Table 6.1, Figure 6.1 A). In contrast to Chapters 2 and 3, high aqueous concentrations of the A-site cation (Tl^{+1}) were observed over time due to its high solubility under anaerobic circumneutral conditions (Figure 6.1 B). In contrast to the preceding studies, H^{+} production exceeded the buffering capacity of the PIPES buffer in the minimal media in both the inoculated and control samples

which decreased to $\text{pH } 6.40 \pm 0.07$ and $\text{pH } 5.88 \pm 0.05$ by 893 hours, respectively (Figure 6.2A). To elucidate the potential role of *S. putrefaciens* in Tl sequestration under the experimental conditions, batch sorption tests were conducted at $\text{pH} \sim 5$ and 6 and showed a lack of Tl uptake by *S. putrefaciens*. To the best of our knowledge, this was the first study to examine the fate of Tl under anaerobic circumneutral conditions and is therefore not only expected to contribute to jarosite research but also to the Tl biogeochemistry literature.

In Chapter 5, I expanded the reductive dissolution of monovalent A-site jarosites to include the non-toxic and more commonly found K-jarosite. In this experiment, I also examined the differences between the reductive dissolution of synthetic and natural jarosites. Based on linear regression analysis of initial Fe reduction (< 65 hours), I showed that Fe(III) reduction was greater in synthetic $\text{H}_3\text{O}, \text{K}$ -jarosite samples (Syn1 and Syn2) than in natural $\text{K}, \text{Pb}, \text{As}$ -jarosite (Nat1) and $\text{H}_3\text{O}, \text{Na}, \text{K}, \text{Sr}$ -jarosite (Nat2) samples in the following order of decreasing susceptibility: $\text{Syn1} > \text{Syn2} > \text{Nat2} > \text{Nat1}$. Similarly to Chapter 4, the pH decreased in all inoculated samples to $\text{pH} \sim 6.2$ -6.5 (Figure 6.2A). Additionally, by using two different synthesis methods in order to yield different particle sizes in synthetic K-jarosite, I also demonstrated that while surface area influenced Fe(III) reduction in synthetic samples it was not a significant factor in natural samples. The lower susceptibility of natural jarosites to Fe(III) reduction was more likely affected by substitutions into the mineral lattice by cations such as H_3O^+ , Na, AsO_4^{3-} , Pb^{2+} or potentially accessory minerals or surface coatings. Lastly, despite decreased Fe(III) reduction in the natural samples, trace metal release of Sr, As and Zn occurred and may present a potential pathway for metal mobility in the environment.

6.2 DISCUSSION

While it is tempting to compare the reductive dissolution of jarosites to Fe-oxides it is important to consider the stability of both mineral phases. Fe-oxides are stable under circumneutral conditions, are more prevalent and with metals typically complexed to Fe(III)-oxide surfaces rather than structurally incorporated as in jarosites. In contrast to Fe(III) oxides, the instability of jarosites at circumneutral pH also renders them susceptible to abiotic dissolution. Therefore, when examining the results of a biotic jarosite dissolution study it is also critical to examine the dissolution of control samples which may in turn provide indicators of the stability of the jarosite phase. Interestingly, the decrease in pH in the control samples (Figure 6.2) may potentially be used as an indicator for dissolution. Across all jarosites studied, the buffering capacity of the minimal media in the control synthetic monovalent A-site jarosites (i.e. K and Tl) was exceeded in the order Syn 1 > Syn 2 > Syn. Tl-jarosite while the natural K, Pb, As-jarosite (Nat1) and H₃O, Na, K, Sr-jarosite (Nat2) jarosites along with the synthetic Pb-jarosite and Pb-As jarosite remained buffered throughout the experiment.

By comparison, the pH buffering capacity was exceeded in inoculated samples more rapidly in the synthetic K and Tl-jarosites and also in Nat1 and Nat 2 in the following order where Syn2 > Syn1 > Syn. Tl-Jarosite > Nat 2 > Nat 1 while synthetic Pb- and Pb-As jarosite samples remained buffered (Figure 6.3A). The decrease in pH may be used as an indicator for enhanced dissolution through Fe(III) reduction. Moreover, the subsequent increase in pH over time due to consumption of H⁺ during reduction demonstrates a potential buffering effect by dissimilatory reduction. However the decrease in pH is not necessarily a direct metric to predict the order of jarosite

stability with respect to Fe(III) reduction because the rate of Fe(II) production decreased in the order of Syn1 > Syn2 > Nat2 > Syn. Tl > Syn Pb-As > Nat1 > Syn. Pb (Table 6.1, Figure 6.1).

Based on Fe(II) production, the three jarosites least susceptible to Fe(III) reduction all included Pb as a divalent A-site cation and susceptibility decreased in the order synthetic Syn. Pb-As jarosite > Nat1 > Syn. Pb-jarosite (Figure 6.1). It is expected that substitution of Pb^{2+} into the mineral lattice increases stability because it has twice the charge and is a smaller cation with an atomic radius of 1.20 Å while Tl^{+1} and K^{+} are monovalent and have larger atomic radii at 1.31 Å and 1.49 Å, respectively. However, while Nat1 was the jarosite least susceptible to Fe reduction, it also had the least amount of Pb as it was only partially substituted into the A-site at 3.46 %. The decrease in Fe(III) reduction is more likely related to additional structural or physical parameters such as less H_3O^{+} substitution, physical aggregation or potential surface coatings as discussed in Chapter 5. ATP concentrations also decreased quickly in these samples so perhaps the decreased Fe(III) reduction may be due to Pb or As toxicity on the bacteria. A potential response by *S. putrefaciens* was observed in SEM images of Nat1 taken at 720 hours whereby electron dense particles were associated with cells which were previously identified as intracellular Pb precipitates in Chapter 2.

The potential role of substituent toxicity may have also played a role in the decreased reductive dissolution observed in Tl-jarosite over its non-toxic monovalent A-site analogue, K-jarosite. However, it is difficult to discern the exact role of Tl toxicity on the reductive dissolution of Tl-jarosite by *S. putrefaciens* due to potential structural constraints which is evident in the lower H^{+} production observed during abiotic

dissolution (Figure 6.2B). Moreover, H_3O^+ is expected to increase solubility and is higher in Tl-jarosite than the 2 synthetic jarosites but did not enhance reductive dissolution relative K-jarosite samples.²⁻⁵ Rather it is more likely that particle size affected dissolution as was demonstrated in Chapter 5 between the synthetic K-jarosites.⁶ The surface area was not measured in Tl-jarosite, however the particles sizes were much larger ($>10\text{ }\mu\text{m}$) as opposed to the smaller Syn 1 ($0.5 - 2\text{ }\mu\text{m}$) and Syn2 ($0.5 - 8\text{ }\mu\text{m}$) samples.

However, Bonneville et al. (2009) demonstrated that mineral solubility provided a better predictor of relative iron reduction rates in Fe(III) oxyhydroxide minerals by *S. putrefaciens* 200R over surface area.⁷ They showed that Fe(III) reduction rates and measured solubility products defined a single linear free energy relationship (LFER) whereby the least soluble Fe(III) oxyhydroxides had the lowest reduction rates. This is not surprising as solubility products are related to the standard Gibbs free energies of dissolution reactions and reflect the thermodynamic stability of the mineral lattice structure. In terms of the jarosites examined in this dissertation, previous empirical measurements of solubilities of pure synthetic jarosite phases at 298K showed that solubility decreased in the order Na-jarosite ($\log K_{\text{sp}} = -8.56$) $>$ K-jarosite ($\log K_{\text{sp}} = -12.50$) $>$ plumbojarosite ($\log K = -25.5$).^{8,9} Therefore, the low susceptibility of Pb-containing jarosite phases to microbial Fe reduction may be attributed to the general low solubility expected for the mineral phase. However, in the absence of experimentally derived solubilities of the jarosites used in this study, a linear free relationship of Fe(III) reduction to solubility remains to be elucidated and should be considered in future research.

In comparison to the complex biogeochemical situations encountered in nature, the experimental results derived from this dissertation were conducted in a highly simplified system whereby a well studied model iron-reducing microorganism, *Shewanella putrefaciens* was added to well-characterized synthetic and natural jarosites. Therefore, these batch experiments provide important, preliminary insights into both the abiotic and reductive dissolution of jarosites. Future studies should examine the effects of temperature, mixing rate, redox gradients, flow, solubility, suspension and microbial densities and the removal of reaction products on reductive dissolution kinetics. Moreover, this dissertation did not consider the effects of differing microbial communities such as sulfate reducing microorganisms or population dynamics.

6.3 CONCLUSIONS

My original hypothesis states: "*Shewanella putrefaciens* CN32 will reductively dissolve jarosites and the degree of dissolution will be influenced by jarosite structure". Based upon the results from this dissertation, jarosite structure does play a significant role in the degree of reductive dissolution of jarosites. However, Fe(III) reduction is also complicated by the accompanying metal toxicity to *S. putrefaciens*, abiotic dissolution and physical factors such as surface coatings, particle size and surface area. Overall, the reductive dissolution of jarosites resulted in enhanced release of soluble A-site cations such as Ti^+ and K^+ as compared to control samples and resulted in a pH buffering effect not seen in control samples. Moreover, the reduction of arsenate (AsO_4^{3-}), to arsenite (H_3AsO_3^0) from the T-site also presents a potential mechanism for enhanced As(III) mobility in human mediated and natural environments. In addition to improving our understanding of the susceptibility of jarosites to reductive dissolution, these

experiments also provided additional insights into bacteria + Pb /As /Tl interactions in reducing environments and may help to better predict the impact of microbes on metal mobility in the environment.

Jarosite Phase	Rate of Fe(II) production ($\mu\text{M} \cdot \text{nM ATP}^{-1} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$)
Syn1 (K-jarosite)	86.3 ± 9.8
Syn2 (K-jarosite)	61.3 ± 5.9
Nat 2 (Na, K-jarosite)	34.3 ± 3.1
Syn. Tl-jarosite	10.9 ± 1.8
Syn. Pb-As jarosite	5.6 ± 0.2
Nat 1 (K, Pb-jarosite)	3.2 ± 0.4
Syn. Pb-jarosite	2.1 ± 0.4

Table 6.1. Fe(II) production rates based on linear regression analysis of measured Fe(II) concentrations in the first 72 hours normalized to initial ATP concentrations and mass of jarosite samples.

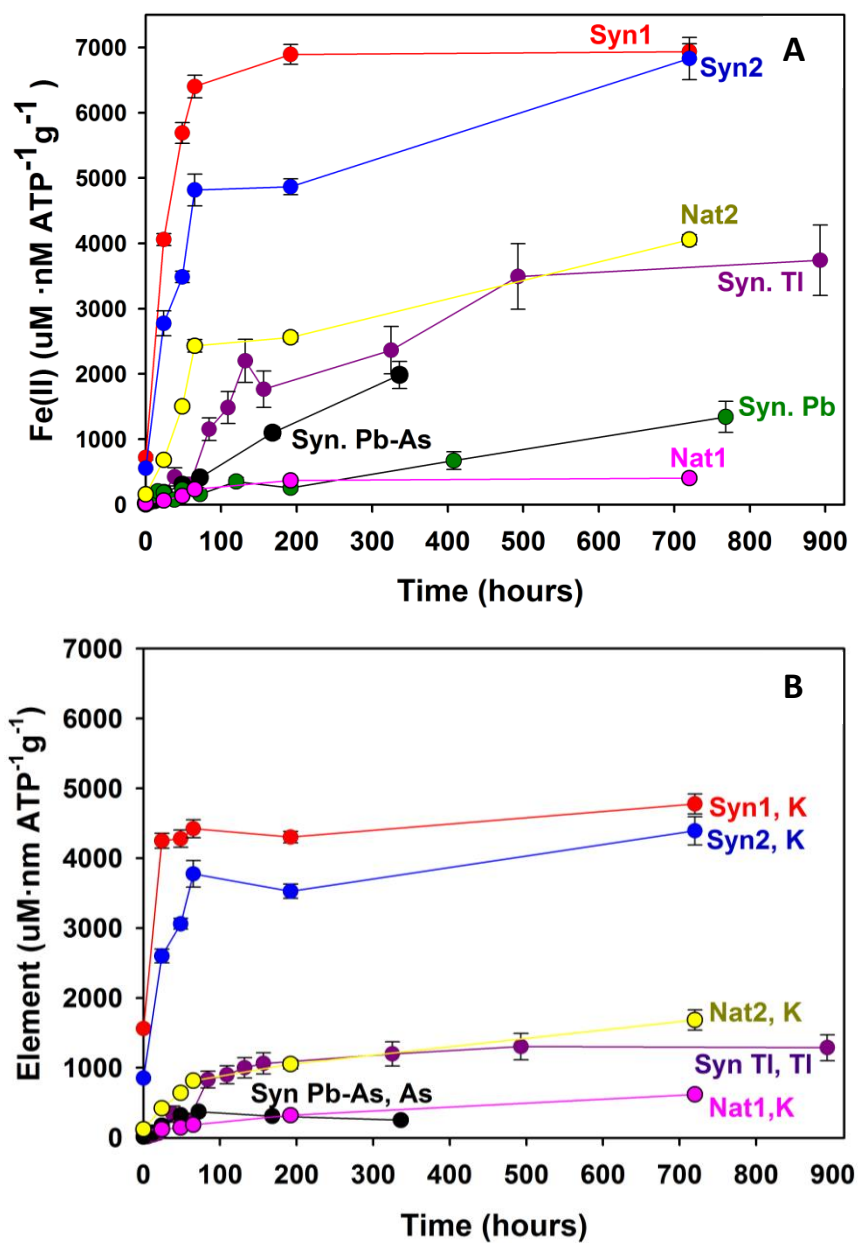


Figure 6.1. Aqueous A) Fe(II) concentrations; and B) Elemental substituents released over time across the jarosites studied. Values were normalized to initial ATP concentrations and mass of jarosite samples and error bars represent standard error ($n = 3$) and take into account variation from initial ATP concentrations, sample mass and ICP-OES measurements.

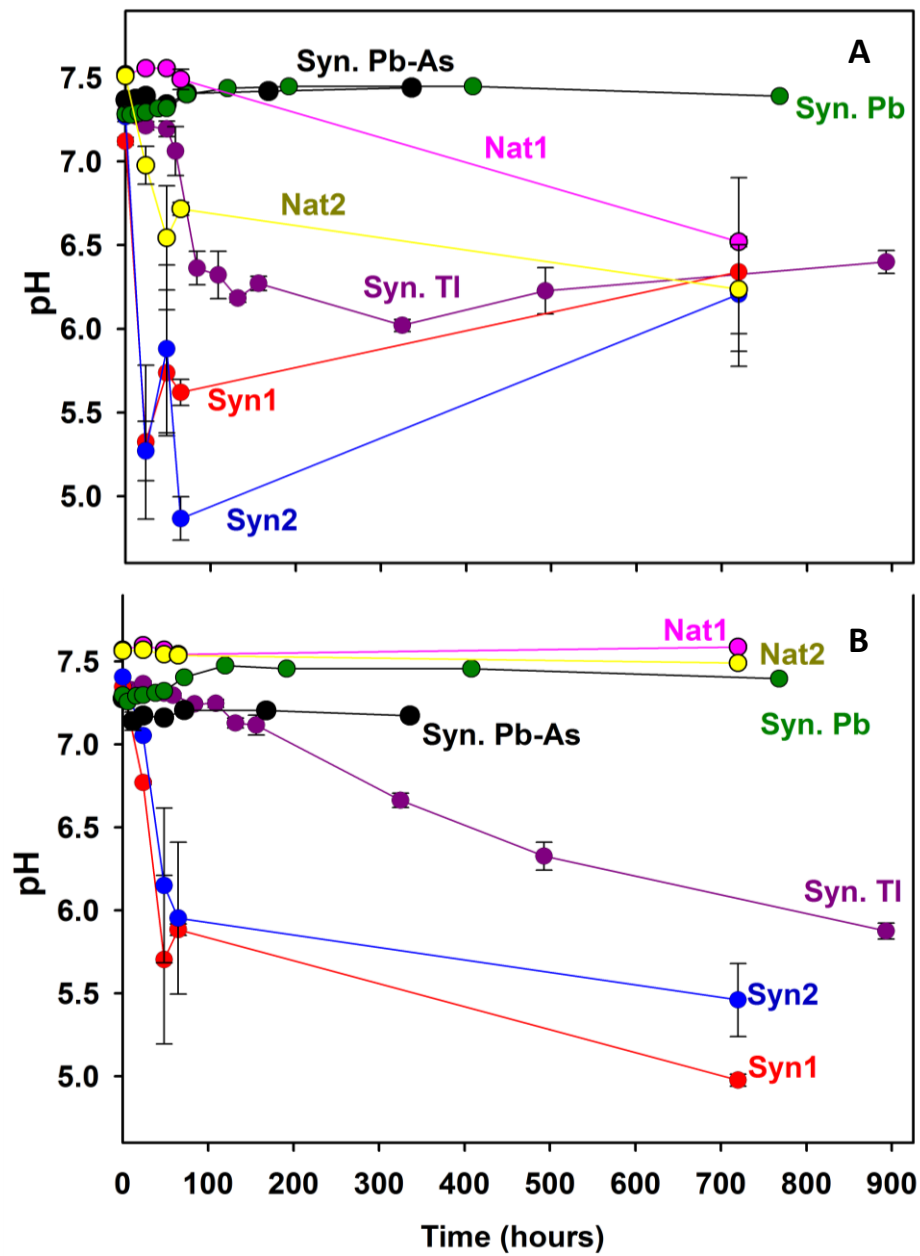


Figure 6.2. pH of A) inoculated jarosites; and B) control jarosites over time across the suite of jarosite samples studied. Error bars represent standard error (n =3).

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APPENDIX A

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Christina M. Smeaton was born in 1978 in Placentia, Newfoundland, Canada. She holds a B.Sc. in Environmental Science (Chemistry) from Grenfell Campus, Memorial University of Newfoundland. She also holds an M.Sc. in Environmental Science from the Great Lakes Institute for Environmental Research at the University of Windsor.