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Isolated from Mine Tailings in the Presence of Various Electron Donors

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Microbial activity of iron-reducing bacteria and sulfate-reducing bacteria isolated from mine tailings in the presence of various electron donors.

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Abstract

Two abandoned mine tailings sites (Calumet near Ottawa, and Potter near Timmins, Ontario) have been shown to support active populations of iron- (IRB) and sulfate-reducing bacteria (SRB). The competition between IRB and SRB for similar electron donors was however never assessed. The present study looked into the potential competition between IRB and SRB isolated from those 2 sites since they represent different pH conditions and mineralogy. The Potter tailings are acidic to slightly acidic and contain large quantities of sulfides, whereas the Calumet tailings are alkaline and contain less pyrite and more carbonate minerals. Batch experiments were designed to test the competition between IRB and SRB for 3 electron donors (acetate, formate and lactate) and to determine the role of abiotic Fe(III) reduction. Results from abiotic systems indicated that Fe(II) was released overtime in the various systems due to the reduction of Fe(III)-rich minerals by the organic acids present in the medium and due to the chemical oxidation of pyrite by ferric iron. IRB could only grow in the Calumet systems containing inhibited SRB. In these systems, IRB growth was favored in the presence of acetate. In the systems containing active SRB, IRB growth was minimal in the presence of all electron donors, suggesting that they cannot compete with SRB under the conditions used in the systems. SRB present in all systems were capable of oxidizing all 3 electron donors, including acetate. Our results also showed that complete and incomplete lactate oxidizers were present in the Calumet and Potter systems.

Résumé

Il a été démontré que deux parcs abandonnés de résidus miniers (Calumet près d'Ottawa et Potter près de Timmins, Ontario) contiennent des populations actives de bactéries réductrices de fer (BRF) et de bactéries sulfato-réductrices (BSR). La compétition pour des donneurs d'électrons similaires entre ces deux groupes de bactéries n'a toutefois pas été étudiée. La présente étude porte donc sur la compétition pouvant exister entre ces deux groupes de bactéries provenant de deux sites miniers différents d'un point de vue chimique (pH) et minéralogique. Les résidus de la mine Potter sont acides à légèrement acides et contiennent de grandes quantités de sulfures de métaux, alors que ceux de Calumet sont alcalins et contiennent moins de pyrite, mais plus de minéraux carbonatés. Des systèmes fermés ont été utilisés pour tester la compétition entre les deux groupes de bactéries en présence de 3 donneurs d'électrons (acétate, lactate et formiate) et pour déterminer l'importance des processus abiotiques de réduction du Fe (III). Les résultats des systèmes abiotiques ont démontré que du Fe(II) était relâché en solution dans tous les systèmes suite a la réduction des minéraux riches en Fe(III) par les acides organiques et suite a l'oxydation chimique de la pyrite par le fer ferrique. Les bactéries réductrices de fer ont poussé en présence de populations de BSR inhibées, mais seulement dans les systèmes contenant les résidus de Calumet. Dans ces systèmes, les BRF ont préféré l'acétate. Dans les systèmes contenant des BSR actives, les populations de BRF n'ont pas été capables d'entrer en compétition avec les BSR pour les donneurs d'électrons disponibles. Nos résultats ont aussi indiqué que les BSR présentes dans tous les systèmes étaient capables d'utiliser les trois donneurs d'électrons, même l'acétate. Nos résultats ont aussi démontre que les BSR étaient capables d'oxyder partiellement et complètement le lactate.

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This project was part of a larger research project on the ecology of iron- and sulfate-reducing bacteria in mine tailings funded by NSERC (research grant to D. Fortin). I also want to thank the owners of the Potter and Calumet mines for giving us access to their sites.

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1.0 Introduction

Mining activity makes up a large portion of the Canadian economy (Environment Canada, 1996), but it also contributes to environmental pollution through the disposal of the wastes. In the case of base-metal mines, the tailings are composed of fine crushed minerals (i.e., originating from the host rock) and contain variable amounts of sulfide-bearing metals, such as pyrite and pyrrhotite, and smaller amounts of the ore itself (Cu, Zn, Ni, Cd, Pb, etc.). Residual by-products produced during mining operations are often disposed of in nearby open-air settings, such as natural or man-made tailings impoundments. Some mine tailings are buried and protected by a cover (such as vegetation or soil covers) which limits the penetration of oxygen and keeps the moisture in, thus limiting the oxidation of the mine tailings (Aube et al., 1995). However, several mining impoundments are left to oxidize and pose a serious environmental problem. The oxidation of mine tailings, more specifically the oxidation of the metal sulfide minerals left in the tailings, generates acid-mine drainage (AMD). The oxidation of reduced forms of iron and sulfur occurs in the tailings as a result of chemical and microbial processes (Nordstrom and Southam, 1997 and Emerson and Moyer, 1997). Several studies have looked at the factors influencing the chemical and microbial oxidation of mine tailings and at the populations of Fe- and S-oxidizing bacteria responsible for the generation of AMD (Baker and Banfield, 2003; Edwards et al., 2000; 2001; Bond et al., 2000; Schrenk et al., 1997, etc.), but very few studies have focused on the microbial ecology of anoxic mine tailings, i.e, where Fe- and S-oxidizing bacteria are no longer active because of the absence of oxygen. Recent studies (Koschorreck et al., 2003; Cummings et al., 2000, 2002; Küsel et al., 2001; Wielinga et al., 1999) have however indicated the presence of strictly anaerobic bacteria, such as sulfate and iron reducers, in environments impacted by mining activities. In mine tailings, the presence and potential activity of sulfate-reducing bacteria (SRB) have also been reported (Fortin et al., 2002; 2000a; Fortin and Beveridge; 1997), but competition between SRB and other anaerobic bacteria (such as iron-reducing bacteria (IRB)) was not discussed. Microbial iron reduction is an important biogeochemical process in fresh water sediments and aquifers (Nevin and Lovley, 2002), because it directly affects the mobility of iron (and associated metals) and generates alkalinity. The following sections discuss the general microbial ecology of Fe- and S-bacteria in mining environments, the iron cycle, the occurrence of SRB and IRB and the factors affecting their presence and activity and finally, the objectives and hypotheses of the present research.

1.1 The ecological diversity of microorganisms in mine tailings

The type of microorganisms found in mining environments is dependent on the *in situ* physical and chemical conditions. Nutrient availability, acidity, oxygen content, and the type and availability of organic substrates are all key elements in determining the presence and activity of microorganisms in such environments. Temperature might also play a role, but since most mine tailings are near ambient temperature, mesophilic bacteria are thought to be the most prominent bacterial populations; though some moderately thermophilic ones may also be present (Uberio and Bhattacharya, 1995).

1.1.1 Iron- and Sulfur-bacteria

Mesophilic microorganisms commonly found in mine tailings are either heterotrophic (i.e., they use organic carbon) or autotrophic (i.e., they use inorganic carbon) and are pH dependent, i.e. acidophilic to neutrophilic (Johnson, 1998). Most tailings share similar characteristics, i.e. they are oxic at the surface and become anoxic with increasing depth. The presence of a redox boundary is likely to impact the distribution of microorganisms that may inhabit such environments. Aerobic bacteria are generally abundant in oxic tailings, whereas anaerobic bacteria thrive under anoxic conditions (Fortin et al., 1995).

1.1.2 Oxic conditions

Oxic mining environments contain a wide range of microorganisms, including acidophilic to neutrophilic, and autothrophic and heterotrophic bacteria, archaebacteria and eukariotes (Baker and Banfield, 2003; Edwards, et al., 2000b). Within the bacteria domain, gamma-, beta-, alpha- and delta-proteobacteria have been detected, along with *Leptospirillum ferrooxidans*-group organisms, and organisms within the firmicute and acidobacteria divisions (Baker and Banfield, 2003). The most studied acidophilic autotrophic bacterium in acid-mine drainage environments, but not necessary the most relevant, is *Acidithiobacillus ferrooxidans* (Baker and Banfield, 2003; Johnson, 1998). This bacterium acquires its energy from the oxidation of reduced forms of Fe and S present in minerals and fixes inorganic carbon in order to sustain its metabolic activity and growth (Küsel and Dorsch, 2000). The microbial reactions mediated by Fe- and S oxidizing bacteria contribute to the generation of low pH conditions observed in most mine tailings, as a result of Fe-sulfide oxidation (Johnson, 1998).

1.1.3 Anoxic conditions

Strictly anaerobic bacteria have been recovered from environments impacted by mining acidity (Koschorreck et al., 2003; Cummings et al., 2000, 2002; Küsel et al., 2001; Wielinga et al., 1999) and from mine tailings (Fortin et al., 2002; 2000a; Fortin and Beveridge; 1997). The major groups identified in mining environments include sulfate-reducers and iron reducers (Peine et al., 2000; Fortin et al., 2000a; 2002; Cummings et al., 2000, Wielinga et al., 1999). SRB populations have also been detected in deep mine groundwaters (Nakagawa et al., 2002) and in AMD groundwater flow systems (Benner et al., 2000). Anaerobic IRB and SRB combine the oxidation of an organic carbon substrate to the reduction of inorganic material, such as ferric iron or sulfate (Küsel and Dorsch, 2000). Their occurrence and the factors affecting their growth are discussed in section 1.3.

1.2 The iron cycle

Several bacteria present in mining environments are involved in the cycling of iron. For instance, *A. ferrooxidans* is capable of oxidizing Fe(II) to Fe(III), which can lead to the formation of Fe(III)-rich minerals, whereas anaerobic iron-reducing bacteria can solubilize Fe(III)-oxides and release soluble Fe(II). Indirectly, anaerobic sulfate-reducers can also affect the solubility of Fe, because the production of sulfide (S^{2-}) can lead to the precipitation of Fe(II) sulfides, such as FeS and FeS_2. The following section is a summary of the iron cycle, as it applies to natural environments.

Iron is the fourth most abundant element in the Earth's crust and clearly an important component of sulfidic mine tailings. It is the reactivity of iron in natural environments that gives this particular element an important role in the regulation of many biogeochemical reactions and the distribution of heavy metals in soils and sediments (Wielinga et al., 1999). Iron possesses two oxidation states in the environment, i.e., ferric (Fe(III)) and ferrous iron (Fe(II)). Under oxic conditions, Fe(III) mainly exists as Fe-oxides over a pH range of 4 to 9, whereas under anoxic conditions, Fe(II) is either soluble or precipitated as Fe-carbonates and Fe-sulfides (Stumm and Morgan, 1981). Under aerobic conditions, the oxidation of Fe(II) can be chemical or catalyzed by microorganisms (Stumm and Morgan, 1981). At low pH, the oxidation of Fe(II) however becomes pH independent and the reaction is driven by the activity of specific bacteria (Singer and Stumm, 1970). In anaerobic environments, Fe-oxides have been shown to easily undergo reductive dissolution, as a result of chemical and microbial reactions (Sobolev and Roden, 2001). The reduction of iron bearing minerals is important from a geochemical point of view because Fe(III)-oxides are well known to act as scavengers for trace metals, most notably heavy metal cations, such As, Hg, Cu, Pb, etc. (Johnson, 1998). Heavy metals bound to Fe-oxides can then be released into solution upon the reduction of the oxides. The process of iron reduction may also limit the presence of nutrients in sediments (Roden and Edmonds, 1997). Phosphorous, an important nutrient for most organisms, can easily precipitate as a Fe(II) and Fe(III)-phosphate minerals in the environment, thus limiting the availability of phosphorous (Ehrlich, 1996). Phosphate can also be sorbed onto Fe-oxides, as shown by Châtellier et al. (2001) and Fortin et al. (1993).

1.2.1 Physico-chemical characteristics of iron-bearing minerals

The mineralogy of iron bearing minerals plays an important role in understanding the geochemical cycling of Fe in the environment. Many forms of iron-rich minerals exist in the environment, namely oxides, silicates, carbonates and sulfides. In the case of Fe-oxides (a general term used to describe oxides, hydroxides and oxyhydroxides), several studies (Fortin et al., 1993; Kosta et al., 1999a; Lovley and Phillips, 1987) have shown that they occur under a wide range of morphology and cristallinity, and that their physical, mineralogical and chemical characteristics will affect their reduction under anoxic conditions. The most reactive form is thought to be amorphous iron(III)-oxides or hydroxides which exist in natural sediments as discrete particles or as coatings on minerals (Fortin et al., 1993). Amorphous Fe-oxides are easily reducible and have been shown to be preferentially used up by iron-reducing bacteria (Urrutia et al., 1998). The mineral surface area has been shown to be more important than the crystalline nature of the Fe-oxides in controlling the rate of reduction (Kosta et al., 1999a). Recent studies have also shown that Fe(III)-silicates can be reduced by specific bacteria, such as iron reducers (Kostka et al., 1999b). When clay minerals, like smectite, are reduced, the clay layer collapses in response to the reduction of structural Fe(III), thus trapping cations (K and NH^{4+}) within the mineral structure (Blodau, et al., 1998).

1.2.2 Influence of microorganisms on the iron cycle: the importance of iron and sulfate reducers.

Select microorganisms utilize the redox properties of iron to gain available energy to sustain metabolic growth in both oxic and anoxic conditions (Kostka and Nealson, 1998). Because Fe(III) is second only to oxygen, as the most abundant electron acceptor, its use for microbial growth and activity is essential in anoxic sediments (Kostka et al., 1999b). The reduction of Fe(III) is coupled to the oxidation of simple organic substrates, as shown by the following equation:

$$CH_2O + 4FeOOH(s) + H_2O \rightarrow HCO_3^- + 4Fe^{+2} + 7OH^- \qquad (1)$$

The reduction of iron also generates alkalinity, as indicated by equation 1. In acidic environments, microbial iron reduction has been shown to increase the pH of the sediments (Peine, et al., 2000). Microbial iron reduction could then be an important process in acidic mine tailings because it could partially neutralize some of the acidity generated by the oxidation of metal sulfides.

Another important group of microorganisms that can impact the solubility of Fe are sulfate-reducing bacteria (SRB). They couple the oxidation of organic carbon to the reduction of sulfate to sulfide, as shown in equation 2:

$$2CH_2O + SO_4^{-2} \rightarrow 2HCO_3^- + H_2S \qquad (2)$$

Soluble Fe(II) can then easily react with sulfide to form new iron sulfide minerals, as shown in equation 3:

$$Fe^{2+} + H_2S \rightarrow FeS + 2\,H^+ \qquad (3)$$

Sulfate reduction also generates alkalinity (see equation 2), but the subsequent precipitation of Fe-sulfides produces acidity. If both IRB and SRB were present and active in mine tailings, they might play an important role in controlling the net accumulation of acidity. However, the acidity itself might be an important factor limiting their presence and activity, as discussed in section 1.3.

1.3 Factors affecting the growth of SRB and IRB and their occurrence in mining environments

1.3.1 Factors affecting the growth of SRB

Several factors can affect the presence and growth of SRB populations in natural environments. We discuss here the factors that are likely important in mining environments, i.e., pH, presence of oxygen, temperature, sulfide concentrations, availability of organic substrates and competition with other anaerobic bacteria.

The pH of the sediments can influence the presence and growth of SRB because they are considered neutrophilic (Postgate, 1984). Sulfate reducing bacteria have been recovered from slightly acidic (Küsel et al., 2001; Fortin et al., 2000a; 2002; Fortin and Beveridge, 1997) to acidic sediments (Gyure et al., 1990), but their activity has rarely been demonstrated under such conditions. A recent study by Koschorreck et al. (2003) however succeeded in demonstrating the activity of SRB in very acidic sediments ($pH < 3$) by measuring the *in situ* rate of sulfate reduction.

Oxygen is also considered an important inhibiting growth factor of SRB, because they are considered strictly anaerobic bacteria (Postgate, 1984). However, SRB populations have been recovered from oxic mine tailings (Fortin et al., 2000a; 2002), but their *in situ* activity was not measured. The recovery of SRB from oxic sediments could be explained by their ability to sporulate under harsh conditions, such as oxygen stress (Postgate, 1984). Recent work on cyanobacterial mats (Krekeler et al., 1998) has however indicated that SRB could remove oxygen by active respiration. On the other hand, studies by Sass et al. (1997) and Teske et al. (1996) have clearly indicated that the highest numbers of SRB and the highest rates of sulfate reduction were always measured below the oxic/anoxic interface in sediments, suggesting that SRB are only active in the absence of oxygen.

Temperature is also known to have an impact on microbial growth. In cold climate, it is often thought that microbial activity is as its lowest, but studies of cold Arctic sediments have indicated the opposite (Knoblauch et al., 1999, Jorgensen, 1977). In addition, Fortin et al. (2000a) showed that SRB populations in the sediments of a temperate wetland increased during the winter and that the apparent rate of sulfate reduction remained the same all year around.

Small concentrations of HS^- may also be toxic to SRB, thus increasing the abundance of other microorganisms in the sediments (Uberio and Bhattacharya, 1995). In sediments where DOC is found in excess, it can reduce the concentration of hydrogen sulfide by striping the toxins from the sediments via carbon dioxide production (Uberio and Bhattacharya, 1995). Heavy metals present in sediments could also affect the growth of SRB. Poulson et al. (1997) showed that high Zn concentrations inhibited SRB growth, however, it is still not known if the free ion species are the most toxic forms of metals.

Several low molecular weight compounds, such as acetate, formate, propionate, butyrate, pyruvate, lactate, can be utilized by SRB (Postgate, 1984). Some SRB can also use hydrogen (Postgate, 1984). SRB are capable of completely oxidizing most of them, but some electron donors are incompletely oxidized to acetate (Postgate, 1984). Even though acetate is not commonly used by SRB, with the exception of *Desulfotomaculum acetoxidans* (Postgate, 1984), it is an important carbon source for sulfate reduction in freshwater sediments (Hordijk and Cappenberg, 1983). Acetate, as an electron donor, has also been found to favor the growth of SRB in slightly acidic lake sediments impacted by coal mining (Küsel and Dorsch, 2000). It is important to mention here that the pH of the solution can affect the chemical form of the electron donor because most simple organic acids (like formate, etc.) exist as undissociated lipophylic acids at low pH (Johnson, 1998). Under such conditions, SRB cannot use the electron donors and have to rely on other compounds, such as glycerol and methanol (Johnson, 1998).

1.3.1.1 Occurrence of SRB in mining environments

Some studies have succeeded in isolating SRB from environments impacted by mining activity. SRB have been recovered from acidic lakes affected by coal strip-mining processes (Küsel et al., 2001; Küsel and Dorch, 2000) and radioactive waste dumps (Schippers, et al., 1995). These studies have shown that SRB do not seem to be limited to neutral-pH conditions and that they are sensitive to organic carbon availability (McCreadie et al., 2000). Large SRB populations have also been recovered in Cu-Zn and Au tailings (Fortin et al., 2000a, 2002).

1.3.2 Factors affecting the growth of IRB

The factors influencing the presence and activity of IRB in natural environments are somehow different from those affecting SRB. The major difference is the availability of reactive Fe(III) in the sediments (Sobolev and Roden, 2001). Iron-bearing minerals are common in natural sediments and possess different morphologies and cristallinities (Fortin et al., 1993). It has been shown that poorly ordered iron oxides (like ferrihydrite) are favored by IRB (Straub et al., 2001; Blodau et al., 1998). In a natural environments, the presence of sediments rich in reactive iron does not however imply that microbial iron reduction is occurring. A recent study by Cumming et al. (2000) showed a decrease of IRB populations with depth, even though Fe-rich minerals were present throughout the profile. This may support the hypothesis that iron-reducing bacteria are selective with respect to the morphology and cristallinity of the Fe(III)-rich minerals (like iron oxides) present in sediments and mine tailings. In addition, the rate at which Fe(III) is reduced depends on the direct contact between the organisms and iron oxides, the iron-oxide crystallinity, particle size and surface area, the sorption of ferrous iron onto the bacterial surface and the presence of electron shuttles, such as humic acids (Nevin and Lovely, 2002; Liu et al., 2001).

Acidic conditions will also affect the presence and activity of IRB, as shown by the study of Peine et al. (2000). This study indicated that elevated concentrations of Fe(III) and acidic sediments favored the activity of IRB over SRB. Küsel et al. (1999) isolated an acidophilic Fe-reducing bacterium from an acidic, Fe-rich sediment and demonstrated that *Acidiphilium cryptum* was capable of coupling the reduction of Fe(III) to the oxidation of several substrates, including glucose, hydrogen, but not lactate and acetate. The presence of oxygen can interfere in the dissimilatory reduction of Fe(III), but not necessary in the presence of IRB. In fact, some IRB, such as *Shewanella putrefaciens* and *Pseudomonas putida* are oxygen-tolerant, whereas *Geobacter* is strictly anaerobic (Kostka et al., 1999a). Küsel et al. (2002) showed that *Acidiphilium* species were capable of reducing Fe(III) in the presence of low levels of oxygen under acidic conditions and that Fe(II) was subsequently oxidized to Fe(III).

The presence of suitable electron donors for the dissimilatory reduction of Fe(III) is another important factor affecting the presence of IRB. Table 1 shows some electron donors that are coupled to the reduction of Fe(III) by IRB and used for their growth and energy production. However, the reduction of Fe(III) combined to the fermentation of sugars, such as glucose, might not lead to cell yields (Lloyd, 2003). In the case of hydrogen, an organic substrate (such as malate) must be added to the growth medium in order to sustain IRB growth under laboratory conditions (Roden and Urrutia, 1999). It is also well recognized that several IRB can use alternate electron acceptors, from oxygen to nitrate (Straub et al., 2001). In fact, Bridge and Johnson (1998) showed that some well known moderately thermophilic Fe- and S-oxidizing bacteria can couple the oxidation of simple organic compounds to the anaerobic reduction of Fe(III) (see Table 1).

Some heavy metals have been shown to affect the activity of IRB. Copper can inhibit the activity of iron-reducers at concentrations greater than 5 ppb (Markwiese et al., 2000). This may be very important in mine tailings because they often contain large concentrations of soluble toxic metals, such as Cu and Zn (Fortin et al., 2002). The presence of other microorganisms, such as fermenters, can also influence the extent to

Table 1.1: Type of electron donors coupled to dissimilatory Fe(III) reduction.

Electron donor	**IRB**	**Reference**
acetate	*Shewanella putrefaciens* MR-1	Kostka et al. (1999a)
acetate	*Shewanella putrefaciens* MR-4	Kostka et al. (1999b)
acetate	*Geobacter metallireducens*	Kostka et al. (1999b)
acetate	*Geobacter metallireducens* GS-15	Caccavo et al. (1992)
acetate	*Pseudomonas putida*	Kostka et al. (1999a)
acetate	*Geobacter sulfurreducens*	Straub et al. (2001)
Acetate	β Proteobacteria CdA-1	Cummings et al. (2000)
glycerol-yeast	*Sulfobacillus acidophilus* YTF1	Bridge and Johnson (1998)
glycerol-yeast	*Acidimicrobium ferooxidans* TH3	Bridge and Johnson (1998)
glucose	*Acidophilium cruptum*	Küsel et al. (1999)
tetrathionate	*Sulfobacillus acidophilus* ALV and THWX	Bridge and Johnson (1998)
tetrathionate	*Sulfobacillus thermosulfidooxidans* TH1	Bridge and Johnson (1998)
H_2	*Shewanella alga* BrY	Roden and Urrutia (1999)
lactate	*Shewanella alga* BrY	Roden and Urrutia (1999)
lactate	*Shewanella putrefaciens* CN32	Roden et al. (2000)
lactate	*Pseudomonas ferrireductans*	Arnold R.G (1986)

which copper can inhibit IRB. Fermentative microorganisms have been shown to reduce the overall concentration of Cu in sediments and soils, through sorption reactions (Markwiese et al., 2000).

1.3.2.1 Occurrence of IRB in mining environments

Several recent studies have focused on the activity of IRB in environments impacted by mining activity. Cummings et al. (2000) reported that the IRB populations in lake sediments impacted by AMD were in the order of 10^6 cells/g. sed. IRB populations were also isolated from fluvially deposited mine tailings (Wielinga et al., 1999). Wendt-

Pothoff et al. (2002) reported that IRB populations were abundant (>10^6 cells/g. sed.) in the sediments of a small acidic mining lake treated with lime. With respect to mine tailings, very little is known about the presence and activity of iron reducers. Fortin et al. (2002) showed strong evidence that IRB were active in Cu-Zn mine tailings, but they did not perform a specific IRB enumeration. They showed that soluble Fe was released in the tailings porewaters, just below the oxic-anoxic interface. The release of Fe was also paralleled by a decline of dissolved organic carbon (DOC) and an increase of inorganic carbon (DIC).

1.3.3 Potential competition between SRB and IRB and other anaerobic bacteria

In anaerobic environments, IRB and SRB often co-exist (Caccavo, et al., 1992), but the relative activity of each group depends on the *in situ* chemical conditions and on the type of organic substrates. The presence of common electron donors (such as acetate) for IRB, SRB and methanogens can lead to competition for the organic substrate. In acidic lake sediments where SRB and IRB were active, IRB were shown to out-compete SRB for acetate (Küsel and Dorsch, 2000). The addition of acetate to very acidic sediments can however inhibit certain acidophilic IRB, as shown by Küsel et al. (1999). Küsel and Dorsh (2000) also showed that iron reduction dominated over sulfate reduction in acidic environments. Roden and Wetzel (2003) showed that methanogens were able to out-compete iron-reducing bacteria in iron-rich sediments when acetate was the dominant electron donor. In environments poor in Fe(III) species, sulfate reducers and methanogens are likely to dominate over IRB (McCreadie et al., 2000). However, the addition of Fe(III) in a system of methanogenic activity can divert 90% of the electron flow towards iron reduction (Lovley and Phillips, 1986). In addition, the presence of various fermentable substrates obtained from fermentative bacteria can limit the electron flow in sediments (Peine, et al., 2000). Acetate, formate, hydrogen, pyruvate, and lactate are all by-products of fermentative microorganisms contributing to the electron flow. Pyruvate and lactate are not considered very important in the diversion of the electron flow because they are both minor composite of extra cellular intermediates in Fe(III) reduction (Roden et al., 2000). On the other hand, formate, acetate and to some extent hydrogen, all serve as dominant substrates regulating the electron flow in sediments, as shown in Figure (1.1).

The sorption of Fe to the surface of bacteria can also inhibit IRB in the environment. All microorganisms possess active binding sites on their cell wall that have the ability to sorb or immobilize nutrients and metals. The binding sites present on the cell wall on IRB may sorb soluble iron, thus inhibiting the reduction of Fe(III) in the sediments (Roden, et al., 1999).

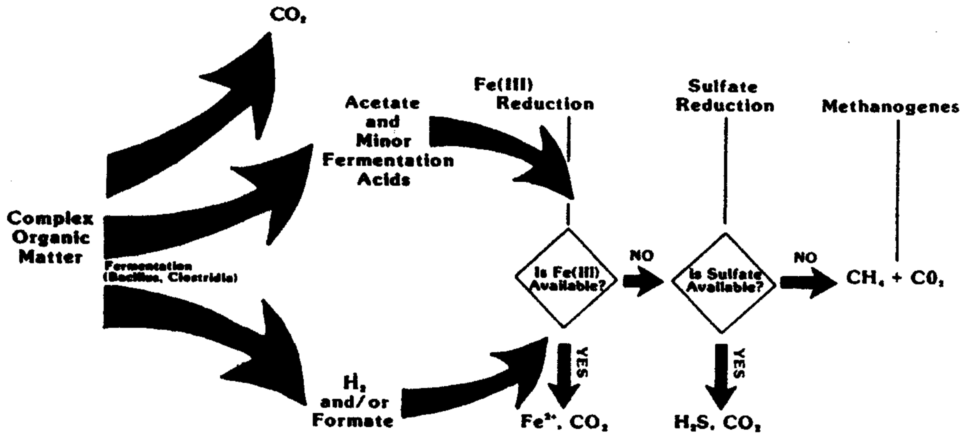

Figure 1.1 Model of carbon and electron flow in sediments with Fe(III) reduction, sulfate reduction, and methanogenesis as potential terminal-electron accepting processes (taken from Chapelle and Lovley, 1992).

1.4 Objectives and hypotheses of the present research

The main focus of this study was to look at the interactions between two groups of anaerobic bacteria, i.e., sulfate reducing bacteria (SRB) and iron reducing bacteria (IRB) isolated from two different mine tailings environments. Our objectives were

1. Assess the competitive nature between SRB and IRB for a given electron donor i.e., lactate, formate and acetate in systems containing 2 different types of mine tailings. These organic acids were chosen as important electron donors because they have been identified in the porewaters of acidic sulfide-rich mine tailings (Fortin et al., 1996)
2. Determine the effect of pH and tailings mineralogy on the competition for a given electron donor for each group of bacteria.
3. Assess the importance of abiotic iron reduction in systems containing different mine tailings.

The hypotheses of the study were:

1. Competition between SRB and IRB might occur for acetate, because it is an electron donor commonly used by both groups of bacteria.
2. Microbial iron reduction should dominate over sulfate reduction in the more acidic mine tailings systems (i.e., Potter tailings).
3. Abiotic iron reduction in the mine tailings systems should occur given the presence of strong reducing agents, such as soluble sulfide.

2. METHODOLOGY

2.1 Mine tailings sites

Two abandoned sulfidic mine tailings sites were selected for the present study. One site, the Potter mine, is located 100 km east of Timmins, Ontario, whereas the second site, Calumet Mine, is located 80 km northwest of Ottawa-Gatineau in the Province of Quebec. The Potter site (Cu-Zn) is highly oxidized and shows obvious signs of acid-mine drainage (Figure 2.1A). The tailings are sulfide-rich and display a net oxic-anoxic interface (Fortin et al., 2002). The Calumet tailings (Cu-Zn-Pb) are also oxidized (Figure 2.1B), but the tailings are pH neutral and contain less sulfide-bearing minerals (Fortin, 2003). The absence of acidic conditions within the tailings is caused by the presence of carbonates in the host rock, which offers a good buffering capacity.

2.2 Sampling of the mine tailings

All tailings samples were collected in the summer of 2002 at Potter and Calumet mines. Samples were collected near the oxic-anoxic interface, which was defined by a sharp redox change during the *in situ* Eh measurements. For Calumet, the samples were collected near the surface, i.e., between 0-5 cm, whereas for Potter, the tailings were taken at a depth of 10-15 cm. A total of nine samples were collected at each site and put in sterile 20 ml scintillation vials. Between each sampling, the spatula was washed with distilled water and sterilized with 70 % ethanol. The vials were completely filled with tailings in order to avoid oxygen contamination. All samples were kept on ice until their transport to the laboratory.

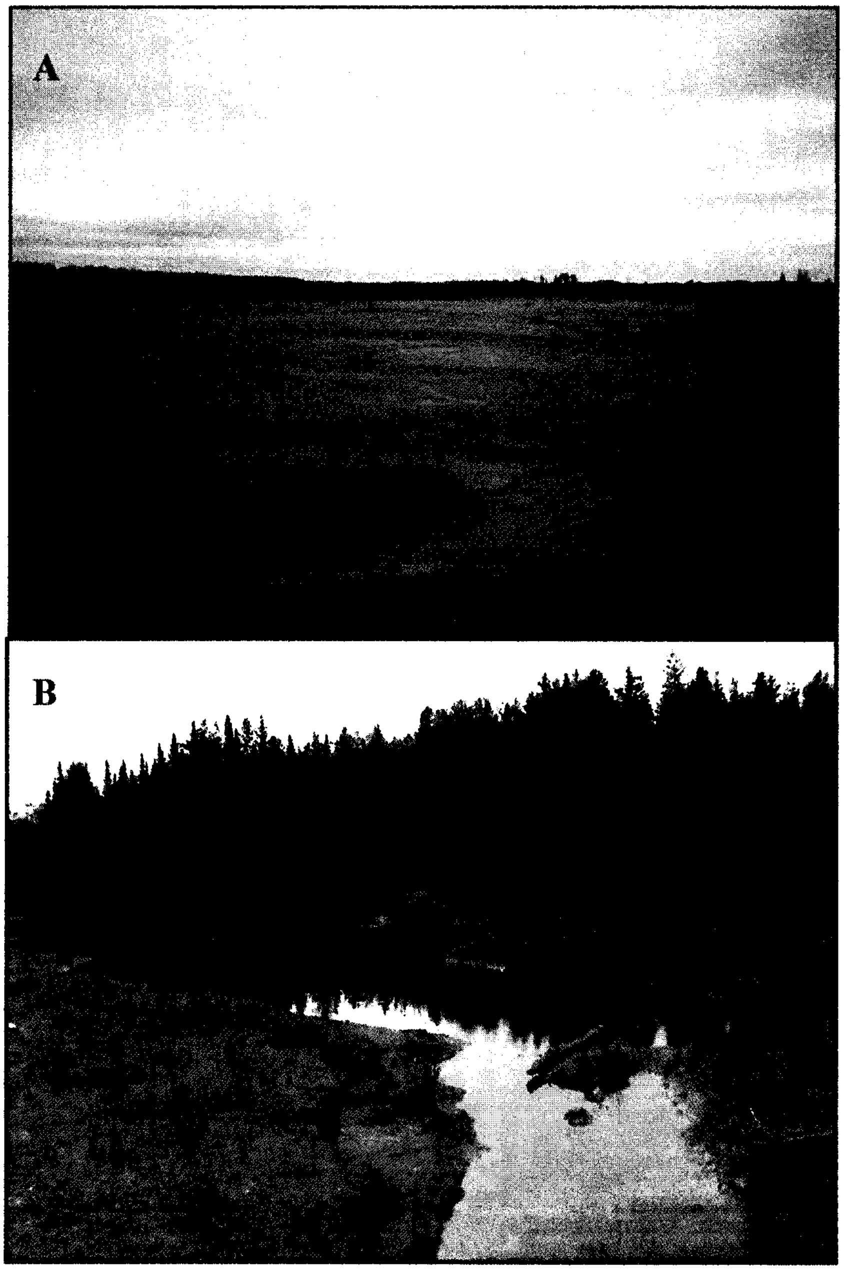

Figure 2.1: (A) Potter mine tailings near Timmins, Ontario. The Cu-Zn tailings are highly oxidized and show little vegetation, with the exception of a few cattail patches, (B) Calumet tailings near Ottawa. Notice the presence of dense vegetation on the tailings due to agricultural runoffs from the adjacent farm fields.

2.3 Batch systems

Nine different systems were setup for each mine tailings site (Figure 2.2). SRB and IRB were active in one set of systems, while the activity of SRB was inhibited in the second set of systems. A third set of systems was abiotic and used for comparison. For each set of systems, 3 different electron donors, including acetate, lactate and formate, were used and the pH of each system was adjusted to the *in situ* pH of the tailings sample. Each system was run in duplicate, with the exception of the control systems. All samples were left at room temperature in the dark and sub-sampling was carried out every 2 weeks over a period of 6 months. The weight of the tailings samples added to each system is indicated in Table A-1 (Appendix A).

Prior to sampling each individual system, a cotton swab moist with ethanol was used to sterilize the polyethylene septum. Injection of the anaerobic gas N_2:CO_2 (80:20) (30 mL) was performed with a sterile 60 mL syringe and 20-gauge needle. The addition of the gas eliminated the negative pressure build-up in the serum bottles, because the gas replaced the extracted liquid. From each system, a total of 23 mL was extracted using a sterile 60 mL syringe and a 20-gauge needle. A 15 mL aliquot sample was filtered with a 0.2 μm filter (Gelman Scientific) and dispensed into 20 mL glass scintillation vials sealed with polyethylene caps. The solutions were acidified immediately with omni-trace nitric acid (final concentration of 0.1%) and were stored in a cold room for future analysis. The remaining sample was used to measure pH, Eh, sulfide, and Fe(II) concentrations.

2.3.1 Growth medium

All systems contained a modified version of the growth medium of Roden et al. (2000). The medium contained the following reagents; Na_2SO_4 (3 g L^{-1}), NaCl (1.2 g L^{-1}), NH_4Cl (0.3g L^{-1}), $MgCl_2.6H_2O$ (0.4 g L^{-1}), KH_2PO_4 (0.2 g L^{-1}) and $CaCl_2.2H_20$ (0.15 g L^{-1}). A single electron donor (acetate (5 mM); formate (5 mM) and lactate (5 mM)) was added to the basal salt medium. Trace metals were added to all systems at a concentration of 1mL L^{-1}. The trace metal solution was composed of $MnCl_2.4H_20$ (2.5 mg L^{-1}), $FeSO_4.7H_2O$ (0.5 mg L^{-1}), $CoCl_2.6H_2O$ (0.5 mg L^{-1}), $ZnCl_2$ (0.5 mg L^{-1}), $NiCl_2.6H_2O$ (0.25 mg L^{-1}), H_3BO_3 (0.05 mg L^{-1}), and $NaMo_4.3H_20$ (0.05 mg L^{-1}). The growth medium was boiled on the counter and cooled by purging it with N_2:CO_2 (80:20) under anaerobic conditions. The solution for each site was adjusted to the *in situ* pH conditions of the tailings, i.e., 7.38 (for Calumet) and 5.26 for (Potter). 450 mL of the growth medium was dispensed into 500 mL serum bottles, capped with butyl rubber stoppers, sealed with aluminum crimps and sterilized at 121°C for 15 minutes. Once retrieved from the autoclave, the bottles were wrapped with aluminum foil and placed in the anaerobic chamber. Vitamins (1mL L^{-1}) were added to the warm growth medium by filter sterilization with 0.2 μm filters (Gelman Scientific,). The vitamin solution was prepared with the following ingredients; p-Amino benzoic acid (0.25 mg L^{-1}), Riboflavin (0.25 mg L^{-1}), Thiamine (0.25 mg L^{-1}), Cyanocobalamin (0.25 mg L^{-1}) and Biotin (0.1 mg L^{-1}).

2.3.2 SRB inhibition

Microbial sulfate reduction was inhibited in half of the prepared systems. 10 mM (final concentration) of sodium molybdate was added from a 1M stock solution via filter sterilization with a 0.2 μm filter (Smith and Klug 1981). Molybdate is frequently used to inhibit sulfate reduction because it is target specific. Molybdate is chemically similar to sulfate and is designed to inhibit adenosine triphosphate sulfurylase, the first enzyme in the sulfate-reducing pathway (Richard et al., 1981).

2.3.3 Inoculation

A tailings suspension was prepared for both acidic and alkaline tailings by mixing about 5 g of tailings to 50 mL of sterile anaerobic water in a 125 mL serum vial. For each system, a 10 mL aliquot was injected with a sterile needle and syringe. All control systems were inoculated by adding the same concentration of tailings, but autoclaved at 121°C for 20 minutes to render them sterile.

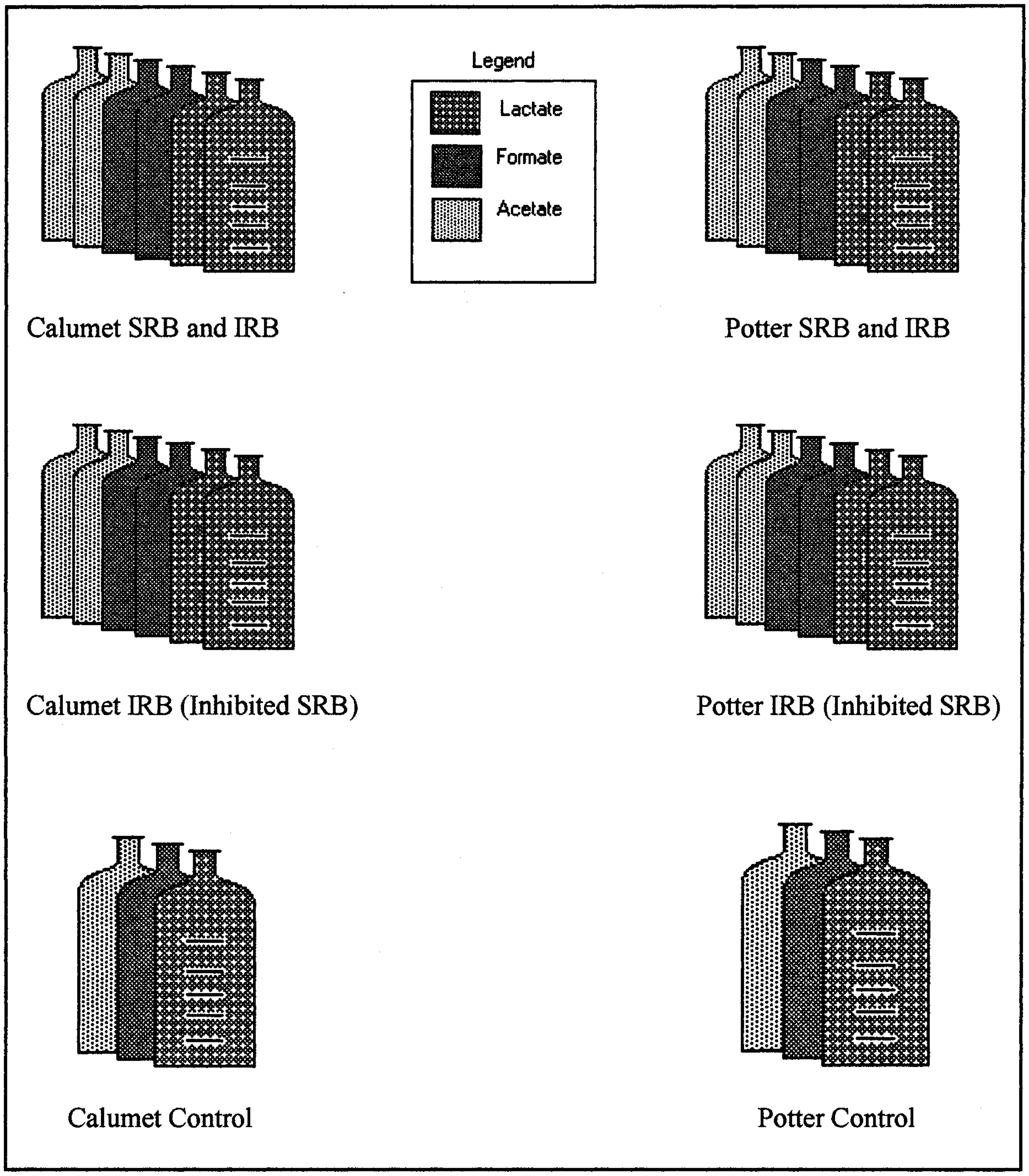

Figure 2.2 Diagram illustrating the different systems used in this study.

2.4 IRB enumeration

2.4.1 Growth medium for enumeration

The chemical composition of the medium used to enumerate IRB (with the MPN method, see section 2.4.4) was similar to the one previously described for the batch systems. The MPN medium was prepared as follows; pipes buffer (3.35 g L^{-1}), NH_4Cl (0.5349 g L^{-1}), KH_2PO_4 (0.1361 g L^{-1}), Na-Acetate (0.4101 g L^{-1}), Na-Lactate (0.3470 mL L^{-1}) (60% vol/vol) and Na-Formate (0.3405 g L^{-1}). Vitamin and trace metal solutions (1mL L^{-1}) were also added to the solution. All electron donors (i.e. lactate, formate, and lactate) were added simultaneously to the growth medium (final concentration of 5 mM each). The solution was boiled, cooled and purged with N_2:CO_2 (80:20) under anaerobic conditions. Once cooled, 30 mL of a 2.49 M $Fe(OH)_{3(s)}$ (iron oxide) stock suspension (see section 2.4.3) was added to 1 L of growth medium for a final concentration of 75 mM. The pH of the solution was adjusted to 6.8 using 2 M NaOH. 9 mL of the prepared growth medium was dispensed into 20-mL serum tubes. The tubes were crimp-sealed and autoclaved at 121°C for 15 minutes. 20 mM of $FeCl_2$ (final concentration) was injected via the filter sterilization technique into all sterile IRB MPN tubes from an anaerobic stock solution of $FeCl_2$ (200 mM).

2.4.2 Dilution water

Ultrapure distilled water was used in all dilutions for the enumeration of IRB using the MPN technique. The water was bubbled and agitated in the anaerobic chamber for 2 hours with N_2:CO_2 (80:20). 9 mL of distilled water was anaerobically dispensed into 20 mL serum tubes with the headspace being filled with the same N_2:CO_2 (80:20) gas. All the tubes were capped with butyl rubber stoppers, sealed and autoclaved at 121°C for 15 minutes.

2.4.3 Synthetic iron oxyhydroxide ($Fe(OH)_3$) preparation

Synthetic iron oxyhydroxides ($Fe(OH)_3$) were prepared by using the Schwertman method (Kostka and Nealson, 1998). In a 500 mL beaker, 24 g of $Fe(NO_3)_3.9H_2O$ was dissolved in 360 mL of distilled water. The pH of the solution was adjusted to 7.8 by adding 120 mL of 1M KOH. 100 mL of the base was added spontaneously, and the remaining 20 mL was added drop wise until the solution reached the required pH. The iron suspension was washed six times with ultra pure water and centrifuged at 6000 rpm for 10 minutes. The washed iron precipitate was re-suspended in 89.60 mL of ultra pure water for a final concentration of 2.49 M of $Fe(OH)_3$. The purity of the iron oxide precipitate was verified by X-ray diffraction (Appendix C, Fig. C-1).

2.4.4 MPN technique

Working on a bench, a sub-sample (1 mL) was taken with a sterile syringe and needle from each batch system and transferred into 9 mL of dilution water for the first 10-fold dilution. A sequence of five 10-fold serial dilutions was performed for all systems where bacterial population was active. Dilution tubes were then vortexed and 1 mL of the suspension was dispensed into the sealed MPN tubes (5 replicates per dilution series), using a sterile needle and syringe. All MPN tubes from the Calumet systems were stored in the dark for about one month. Because of the slow growth experienced with the acidic IRB populations, inoculated MPN tubes were incubated for six months. Microbial iron reduction was monitored visually by observing the formation of a black precipitate in the tubes. MPN values were calculated from statistical tables (Cochran, 1950) and expressed as colony forming units per milliliter (CFU mL^{-1}).

2.5 SRB enumeration

2.5.1 Growth medium for enumeration

The growth medium for SRB enumeration was prepared following a modified version of Postgate medium G (Postgate, 1984). The growth medium was prepared with the following ingredients; Bacto Tryptone (10 g L^{-1}), $MgSO_4.7H_2O$ (2 g L^{-1}), $FeSO_4.7H_2O$ (0.5g L^{-1}), Na_2SO_3 (0.5g L^{-1}), 60% Na-Lactate (0.347 (mL L^{-1}), Na-formate (0.34 g L^{-1}) and pyruvic acid (0.55 g L^{-1}). The growth medium was adjusted to a pH of 7.5 using 2M NaOH. 9ml of the prepared growth medium was dispensed into culture tubes, sealed with caps and autoclaved at 121°C for 15 minutes.

2.5.2 Reducing Agent Supplement (RAS)

Reducing agent supplement (RAS) was prepared on a daily basis prior to SRB inoculation. It was prepared with thioglycolic acid (7.5 mL L^{-1}) and L-ascorbic acid (7.5g L^{-1}). The pH of the solution was adjusted to 7.5 with 2M NaOH and the solution was transferred into 125 mL Erlenmeyer flask and autoclaved at 121°C for 15 minutes. RAS (1mL) was then aseptically added to each MPN tube containing the growth medium.

2.5.3 Dilution water

Dilution water was prepared with NaCl (2.1 g L^{-1}) and RAS 100 (mL L^{-1}). The solution pH was adjusted to 7.5 using 2 M NaOH, and 9 mL was transferred to culture tubes. Culture tubes were autoclaved at 121°C for 15 minutes and cooled prior to inoculation.

2.5.4 MPN technique

Serial dilution series (1:10) were prepared using the dilution water. A 1-mL sub-sample was taken from each system with a needle and syringe and transferred to 9 mL of sterile dilution water for the first 10-fold dilution using the flame-sterilization technique. A sequence of 10-fold serial dilutions was performed for all systems where bacterial population was active. Between each dilution, the suspension was vortexed to disperse the particles uniformly in the dilution tube. All MPN tubes were stored in the dark for a period of two weeks. SRB growth was assessed by the formation of a black Fe-sulfide precipitate in the tubes. MPN values were calculated from statistical tables (Cochran, 1950) and expressed as colony forming units per milliliter (CFU mL^{-1}).

2.6 Chemical analysis of the different systems

2.6.1 Eh and pH

All pH measurements were made with a VWR Scientific 8005 pH meter pH electrode. The electrode was calibrated at room temperature (25°C) with VWR pH buffer standards (4 and 7). A corning redox combo W/RJ Beckman electrode was used to measure the redox potential at room temperature. The redox probe was tested with the

Zobell solution (Nordstrom, 1997). A correction value of +199mV was applied to all Eh results obtained in the study. The same pH and Eh electrodes were used throughout the study to avoid instrumental variations in the readings.

2.6.2 Ferrous and ferric iron

Soluble Fe(II) filtered through a 0.2 μM filter (Gelman scientific, see Table B-1, Appendix B) were measured with the ferrozine method (Kostka and Nealson, 1998). For samples that exceeded the linear concentration range of the ferrozine method (i.e., 5.5 mg L^{-1}), a dilution was made with anaerobic distilled water. All samples were analyzed by measuring the absorbance at 562 nm with a Beckman Du-65 UV-Visible Spectrophotometer. The detection limit for the ferrozine method is 0.15 mg L^{-1}.

Soluble Fe(III) concentrations were estimated as being the difference between total dissolved iron and ferrous iron concentrations. Total Fe concentrations were measured using a spectrophotometer-analyzer HACH DR / 2010 with a detection limit of 3 mg L^{-1}. Prior to the total iron measurements, all samples were diluted 12.5 times with distilled water. However, the presence of sodium molybdate in the inhibited SRB systems interfered with the HACH reagents, and as a result, total dissolved iron concentrations were measured with an ICP-OES (Varian vista-pro). The ICP-OES was calibrated with iron standards prepared with a matrix similar to the growth medium used to enumerate the bacteria. For verification purposes, standard addition of iron was also practiced on random samples.

2.6.3 Sulfide (HS^-)

Hydrogen sulfide concentrations were measured with the Cline method, which has a detection limit of 0.01 μM with a concentration range between 0.05 μM to 2.50 μM (Cline, 1969). When the sulfide concentration of the samples exceeded the linear concentration range of the Cline's method (i.e., 0-2.5 μm), they were diluted with anaerobic distilled water. All diluted samples were immediately treated with the Cline reagent and the absorbance (670 nm) was read with a Beckman Du-65 spectrophotometer.

2.6.4 Sulfate (SO_4^{-2})

Dissolved sulfate concentrations were measured using the $BaCl_2$ turbidimetric method (Rodier, 1975). The initial sulfate concentrations in all batch systems exceeded the linear concentration range of the method (i.e., 0- 0.63 mM). Therefore, a dilution (1:20) was carried out with distilled water. The absorbance (650 nm) was read with a Beckman Du-65 plate Spectrophotometer.

2.6.5 Dissolved organic carbon (DOC)

DOC concentrations were determined with a 1010 model TOC-TIC analyzer calibrated with monopotasium salt standards. Because of the small volume recovered from each sampling, all samples were diluted four (4) times with distilled water. In order to limit inconsistencies in measurements between each day of analysis, all samples were measured at once. All samples were analyzed in Dr. David Lean's laboratory at the University of Ottawa.

2.6.6 Organic acids

The concentration of specific soluble organic acids was measured by High Performance Liquid Chromatography (HPLC). Filtered samples were analyzed with a Cecil Instruments ADEPT series HPLC sampler, with a UV/vis detector set at 230 nm. The eluent used to inject and analyze all samples was made with a solution of 0.0005 M phosphoric acid (pH -3.0) with a flow rate of 3.0 mL/min. The column used was a CSC-Tribute C-18 bonded phase column, 25 cm x 0.94 cm ("CSC-Tribute 120A / ODS 5um) with a 1.0 mL sample loop size. All samples were standardized with 20 mM lactic, formic and acetic acid. All samples were analyzed in the G.G. Hatch Isotope laboratory at the University of Ottawa.

2.7 Sequential chemical extraction

Residual precipitates that remained in all batch systems after 6 months were subjected to a sequential chemical extraction (Fortin et al., 2002). The tailings left in the batch systems were recovered by centrifugation and washed once with distilled water to remove any salts and impurities that could have precipitated while drying the mine tailings. Washed mine tailings were placed in the anaerobic chamber to dry in order to limit the extent of oxidation.

The sequential chemical extraction allowed to separate the tailings into 3 iron-rich fractions, i.e., Fe-reactive, Fe-silicates, and Fe-pyrite (Fortin et al., 2002). Tailings (0.025 g) were first digested with 1 mL of 1M HCl (omnitrace) for 16 hours. HCl dissolves reactive iron species, such as iron oxides and iron monosulfides. After centrifugation, the supernatant was collected and the residual sediment fraction was subjected to a second extraction with 10 N HF (3 mL) for 8 hours and with boric acid (0.05g) for another 8 hours. This second step extracts the Fe-silicate fraction. Finally, the supernatant was recovered and the residual sediment was treated with concentrated omnitrace HNO_3 for 2 hours in order to assess the pyritic fraction in the sample. After each step of the extraction, mine tailings were washed three times with cold distilled water, with the exception of the HF step, where hot water was used. Total iron ($Fe_{tot.}$) concentrations in each fraction were measured by flame atomic absorption spectroscopy (AAS, varian AA-1475) using VWR iron (Fe) standards acidified with 2-5% nitric acid.

2.8. Abiotic reduction of Fe(III)

Two different tests were performed to monitor the abiotic reducing potential of the anaerobic growth medium and the tailings themselves.

2.8.1 Abiotic reduction of Fe(III) in mine tailings

40 mL of growth medium identical to the one used for the batch experiments was dispensed into twelve 120 mL serum bottles. The electron donor (acetate, formate and

lactate) was added at a final concentration of 5 mM and the pH was adjusted to the *in situ* pH of each site, i.e., 5.26 for Potter and 7.38 for Calumet. Sodium molybdate was also added (10 mM final concentration) to a second set of systems. All serum bottles were capped with butyl rubber stoppers and autoclaved at 121°C for 15 minutes. Sterile mine tailings (~5 g), autoclaved at 121°C for 20 minutes, were used to inoculate all serum bottles. Each system was run in duplicate. Sub-sampling was performed every 2 weeks for a period of 10 weeks and Fe(II) concentrations were determined with the ferrozine method described earlier.

2.8.2 Abiotic reduction of synthetic Fe-hydroxide ($Fe(OH_3)$)

The reducing potential of the growth medium itself was tested on pure Fe-oxyhydroxides. The same growth medium, electron donors and SRB inhibitor (as described in section 2.8.1) were used, but a stock Fe-oxyhydroxide suspension (initial concentration of 2.49 M) was added to the systems, for a final concentration of 40 mM. All serum bottles were capped with butyl rubber stoppers and autoclaved at 121°C for 15 minutes. Sub-sampling was performed as described in section 2.8.1.

2.9 Mineralogy

X-ray diffraction was used to determine the purity of the Fe-hydroxide ($Fe(OH)_3$) prepared in the laboratory. Samples were analyzed with a Phillips x'pert diffractometer equipped with a Kevex (Si-Li) detector, using a Cu source, a voltage of 45 kV and a current of 40 mA. A step scan mode of 0.05° with a step time of 2.0 seconds was used between 4° and 90°.

3.0 RESULTS

3.1 Microbial populations

3.1.1 SRB populations

Bacterial counts reported in this study correspond to an average value (n=2) since duplicate systems were set up for each treatment. SRB growth was observed in the presence of all 3-electron donors in the non-inhibited systems containing the Calumet and Potter tailings (Figure 3.1). As expected, the inhibited (IRB systems) and abiotic control systems showed no growth. The initial number of cells in the Calumet tailings was higher than in the Potter systems. In the Calumet systems, SRB populations grew fast initially with lactate as the electron donor. In the presence of acetate or formate, growth was however slower, but the populations increased near the end of the 6 month period (weeks 16-20) and appeared to decrease shortly after. In the Potter systems, SRB growth was generally slow in the presence of lactate and acetate, but rapid in the presence of formate (Figure 3.1). In the latter system, SRB populations exceeded those present in the Calumet system. In all 3 systems, SRB populations decreased near the end of the experiment (i.e., 6 months).

3.1.2 IRB populations

In order to monitor the competitive nature between IRB and SRB in mine tailings, we inhibited the activity of SRB in some systems. It is important to mention here that IRB did not grow in the Potter systems containing inhibited SRB, even 6 months after the inoculation (Fig. 3.2). However, IRB populations grew well in the inhibited SRB Calumet systems.

In the Potter system containing non-inhibited SRB populations, IRB populations were generally low (10^1 -10^2 CFU/mL) in all systems (Figure 3.2). IRB populations did not appear to grow in the lactate and formate systems, whereas they died off shortly after inoculation in the acetate system. In the Calumet systems, IRB populations were also generally low ((10^1 -10^2 CFU/mL) and the trends were similar in the inhibited and non-inhibited systems containing lactate and formate and in the non-inhibited system containing acetate.

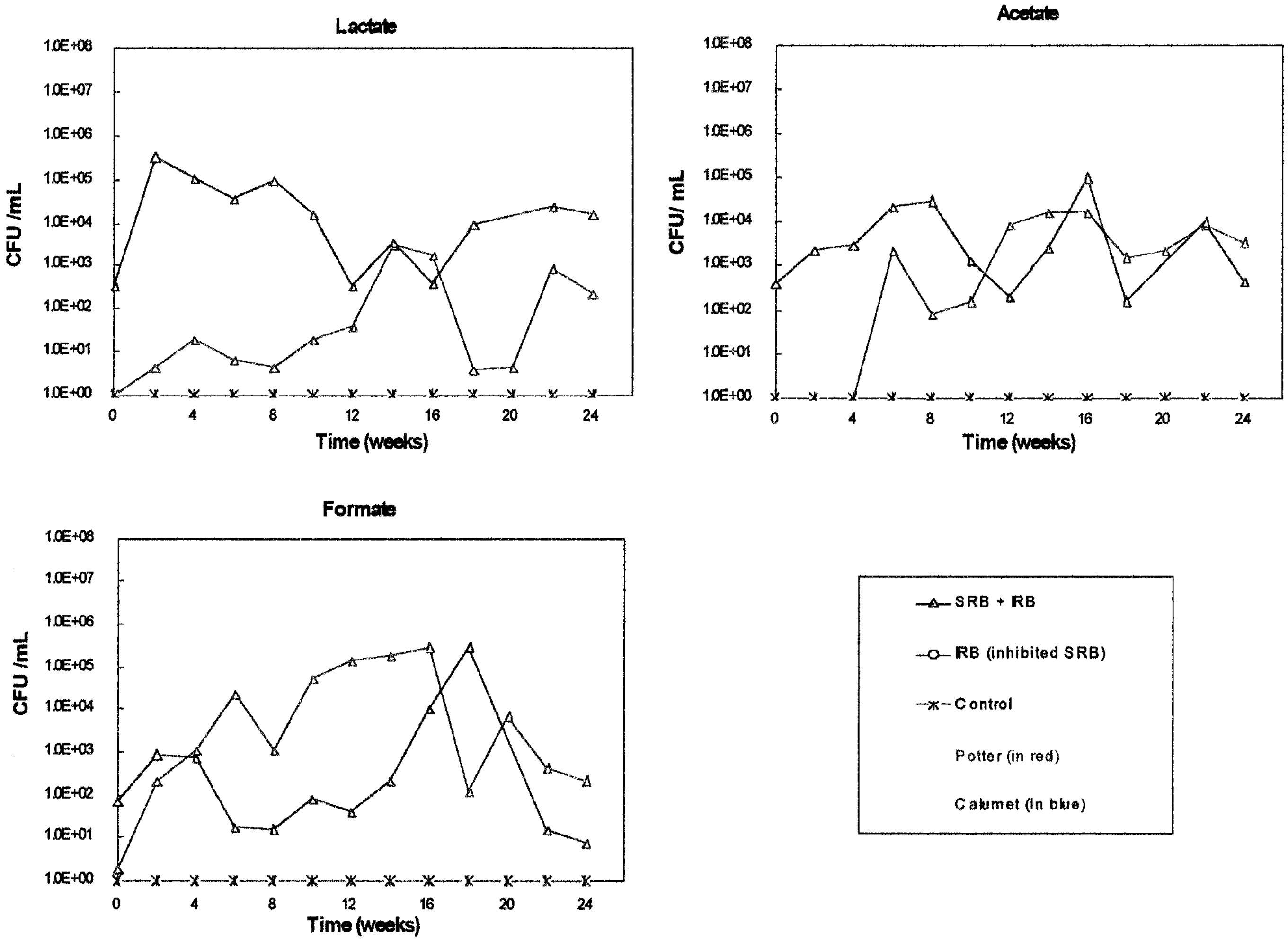

Figure 3.1 SRB populations in the various systems containing the Potter and Calumet mine tailings.

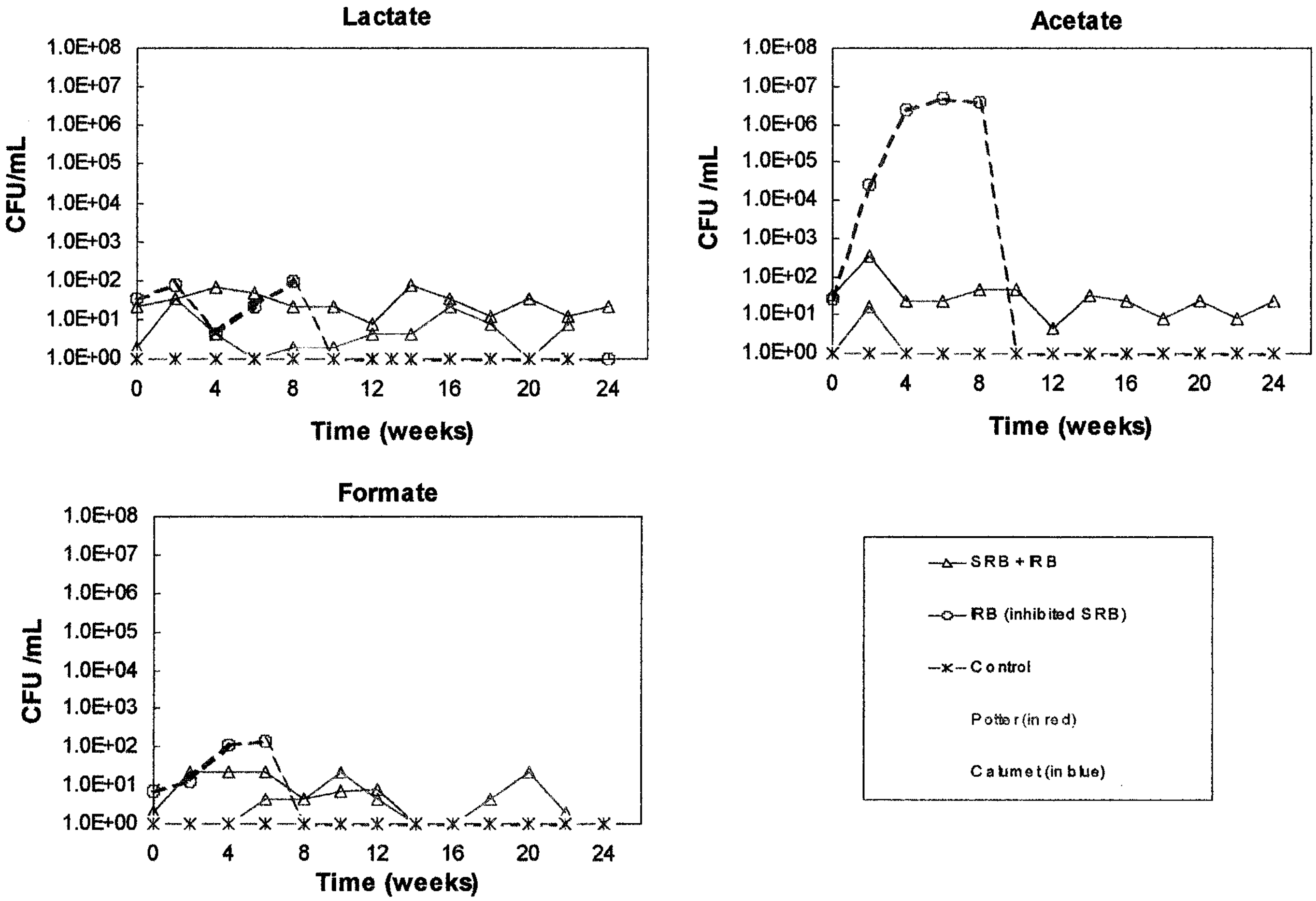

Figure 3.2 IRB populations in the various SRB and SRB-inhibited systems containing the Potter and Calumet mine tailings.

However, our results clearly indicate that IRB populations in the acetate system took advantage of the absence of SRB and showed a rapid growth (Figure 3.2), but they quickly died off after 8 weeks. Similar trends were observed in the Calumet systems containing lactate and formate.

3.2 Abiotic Fe(III) reduction

3.2.1 Abiotic reduction of Fe(III) in mine tailings

It is important to mention here that the concentration of tailings in the systems designed to test for abiotic Fe(III) reduction was greater than the concentration used in the systems containing bacteria and sterile tailings (i.e., control systems) (section 3.3). In addition, soluble iron concentrations were higher in the acidic tailings (Potter) than in the alkaline (Calumet) tailings due to the presence of large concentrations of soluble Fe in the Potter pore waters (D. Fortin, pers. com.). Ferrous iron was released into solution in the Potter sterile mine tailings in the presence of all three electron donors (Figure 3.3). The addition of sodium molybdate (the inhibitor for sulfate reducing bacteria) had little effect on the release of Fe(II) in both acidic and alkaline mine tailings (Figure 3.3). In the Calumet mine tailings, there was no apparent Fe(II) release. In fact, ferrous iron concentrations slightly declined overtime (Figure 3.3).

3.2.2 Abiotic reduction of synthetic Fe-hydroxide ($Fe(OH_3)$)

Pure synthetic Fe-hydroxides were reduced in abiotic systems (with and without Na-molybdate) adjusted to the *in situ* pH of the Calumet and Potter systems in the presence of lactate and acetate (Figure 3.4). However, the release of Fe(II) was generally greater in the systems containing no SRB inhibitor than in the systems containing Na-molybdate (Figure 3.4). In the systems containing formate, Fe(II) concentrations were relatively constant throughout the 10-week period (Figure 3.4).

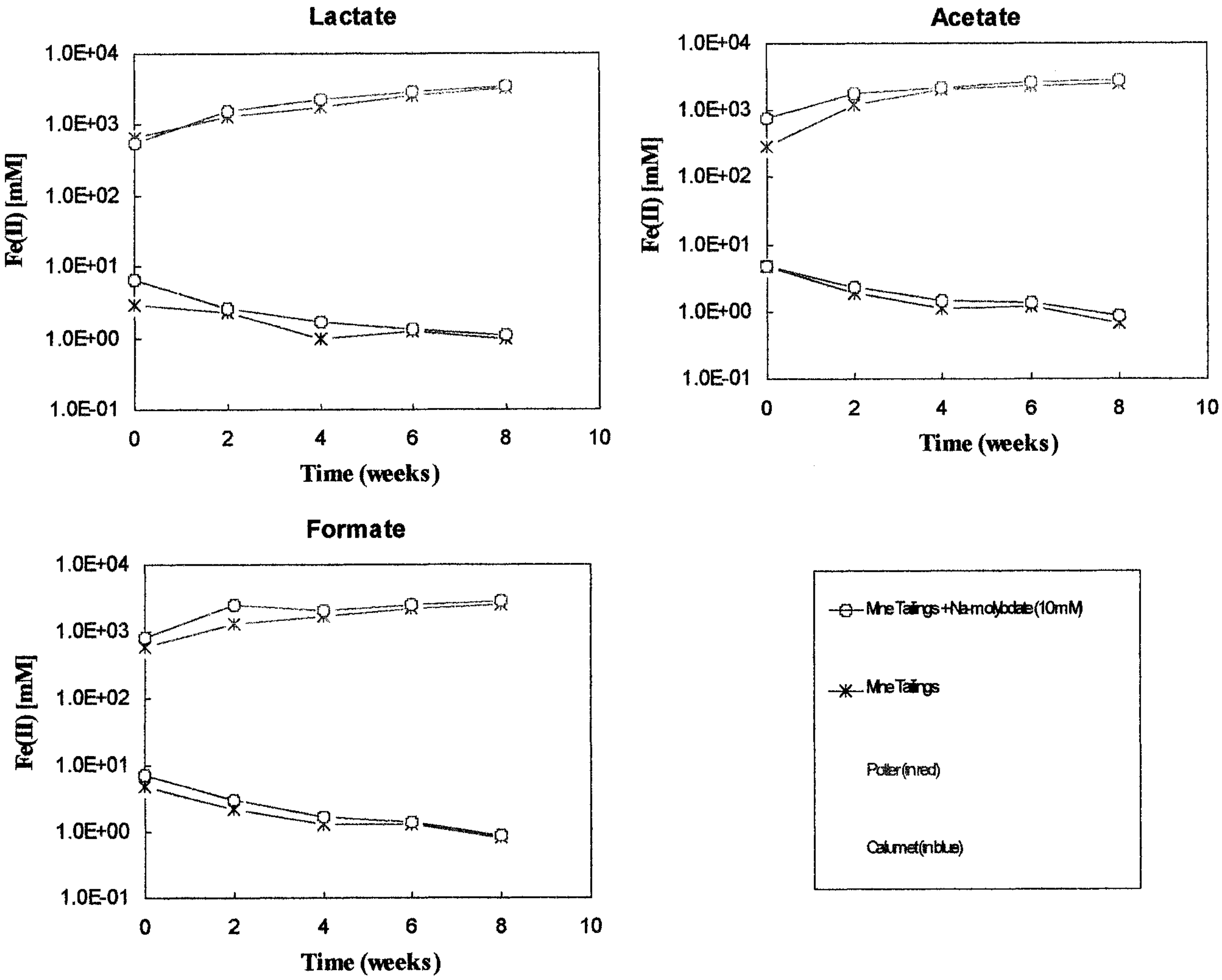

Figure 3.3 Abiotic iron reduction of sterile mine tailings with and without Na-molybdate from Calumet and Potter mine sites.

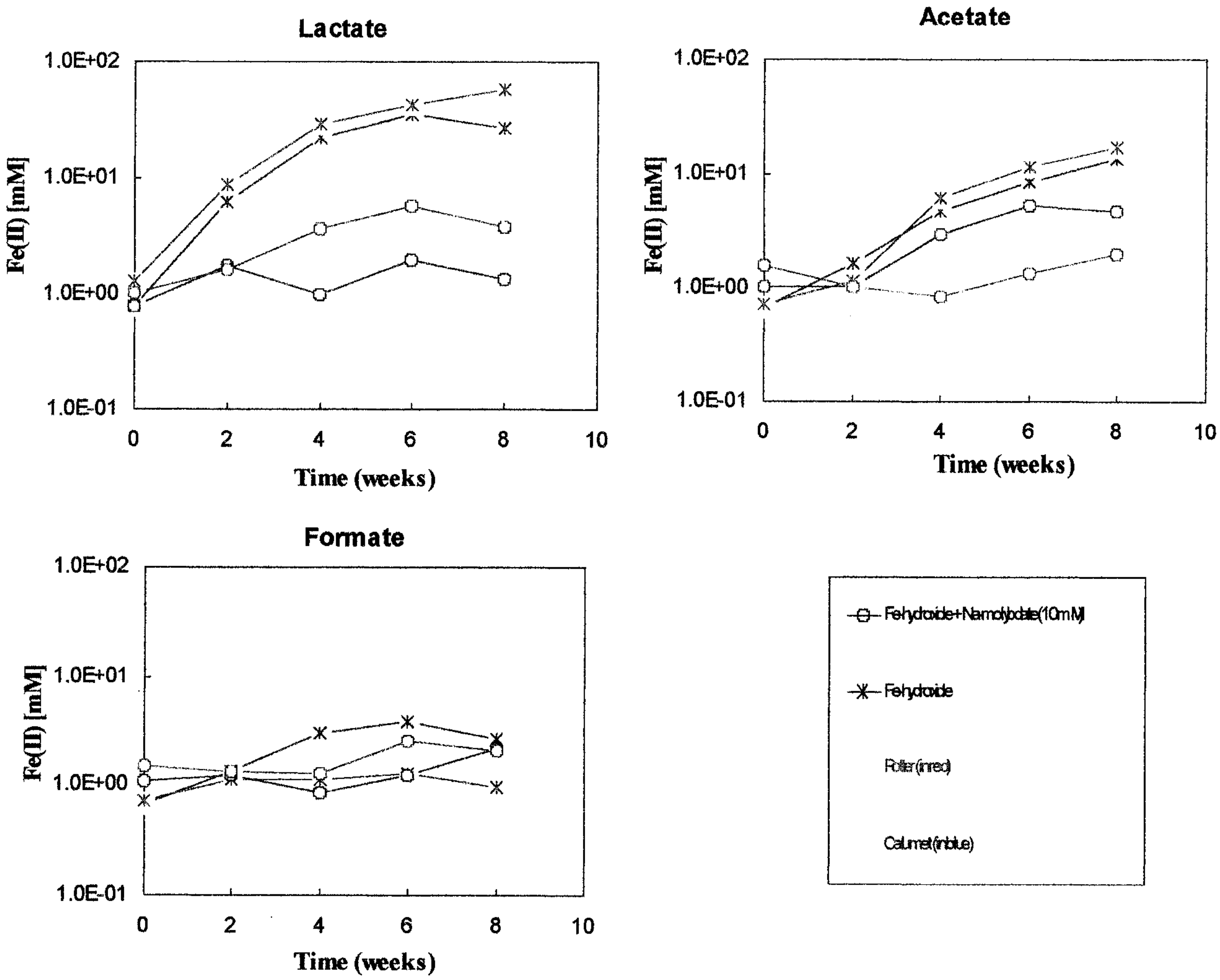

Figure 3.4 Abiotic reduction of synthetic iron hydroxide ($FeOH_3$) in the presence of various electron donors in the growth media adjusted to the *in situ* pH of the Potter and Calumet systems.

3.3 Chemical conditions of the systems

3.3.1 pH and Eh

All systems were analyzed bi-weekly to monitor the variations in Eh and pH. The trends are shown in Figures 3.5 and 3.6. First, even though the pH of the growth medium was adjusted to the *in situ* pH conditions of the tailings, i.e., 7.38 (for Calumet) and 5.26 for (Potter), the pH measured at T = 0 was slightly different (Figure 3.5). For the Potter systems, the initial pH at T = 0 varied between 5.00 and 5.70, whereas it ranged from 5.80 to 6.40 in the Calumet systems. The pH increased in the non-inhibited systems in the presence of all electron donors for both Potter and Calumet systems (Figure 3.5). On the other hand, the pH remained fairly stable over time for the abiotic control systems. Slight pH fluctuations were observed for the Calumet and Potter inhibited systems in the presence of all electron donors (Figure 3.5).

The general Eh trends for the Potter and Calumet non-inhibited systems indicated the development of reducing conditions in all systems during the course of the experiment, with the exception of the Calumet tailings in the presence of formate (Figure 3.6). For the inhibited and abiotic control systems in the presence of all 3 electron donors, the Eh measurements greatly fluctuated, but overall, the systems remained sub-oxic.

3.3.2 Ferrous iron (Fe(II))

First, the production of soluble Fe(II) was generally greater in the abiotic control systems than in the biotic ones, with the exception of the Calumet acetate system with inhibited SRB (Figure 3.7). In addition, the release of Fe(II) in the abiotic systems was larger in the Potter systems, than in the Calumet ones, with the exception of the lactate system. In the non-inhibited systems and in the presence of all 3 electron donors, there was no production of Fe(II), whereas in the inhibited systems (i.e., IRB), small quantities of Fe(II) were released during the course of the experiment in the presence of all electron donors (Figure 3.7).

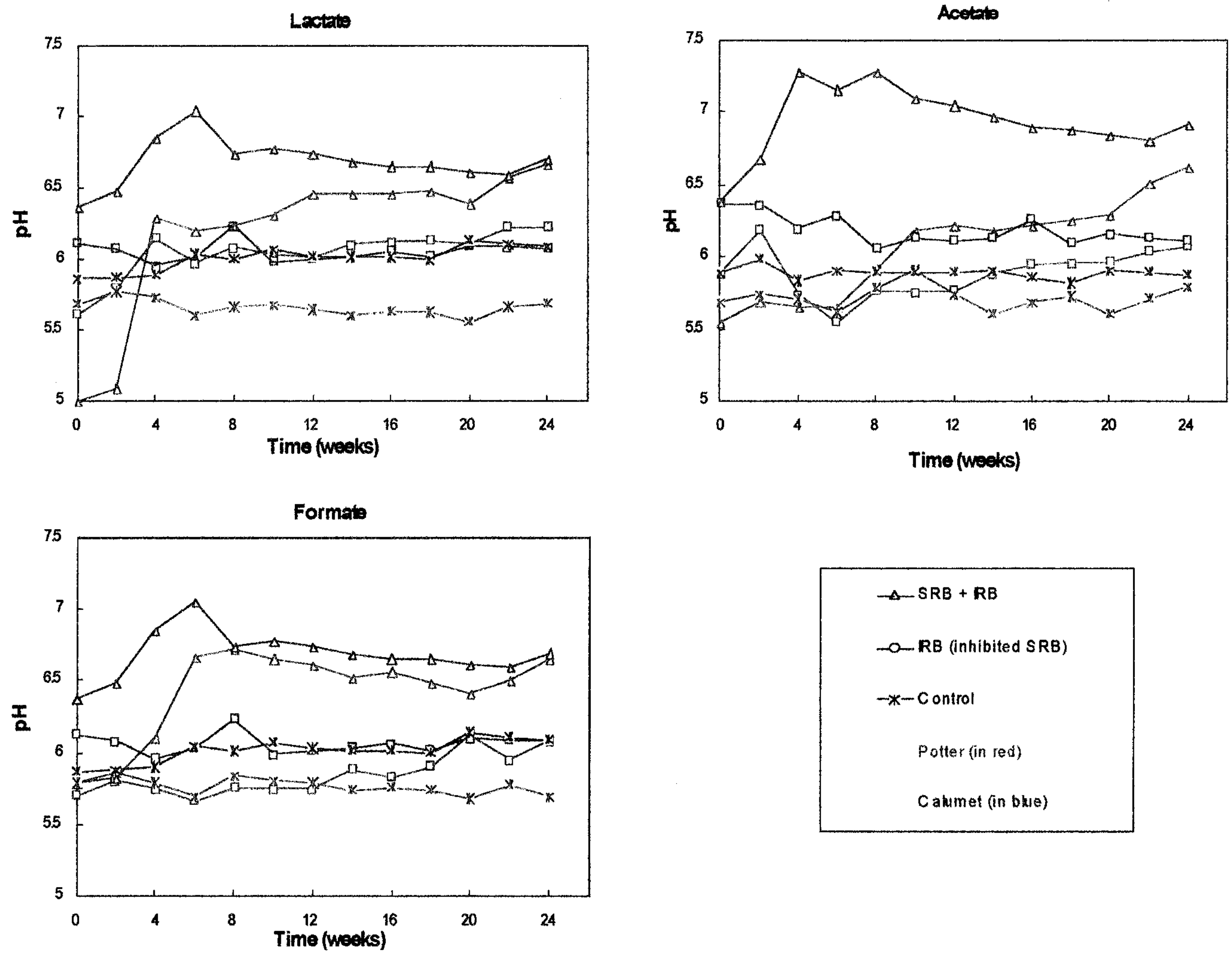

Figure 3.5 pH trends in the various systems containing the Potter and Calumet mine tailings.

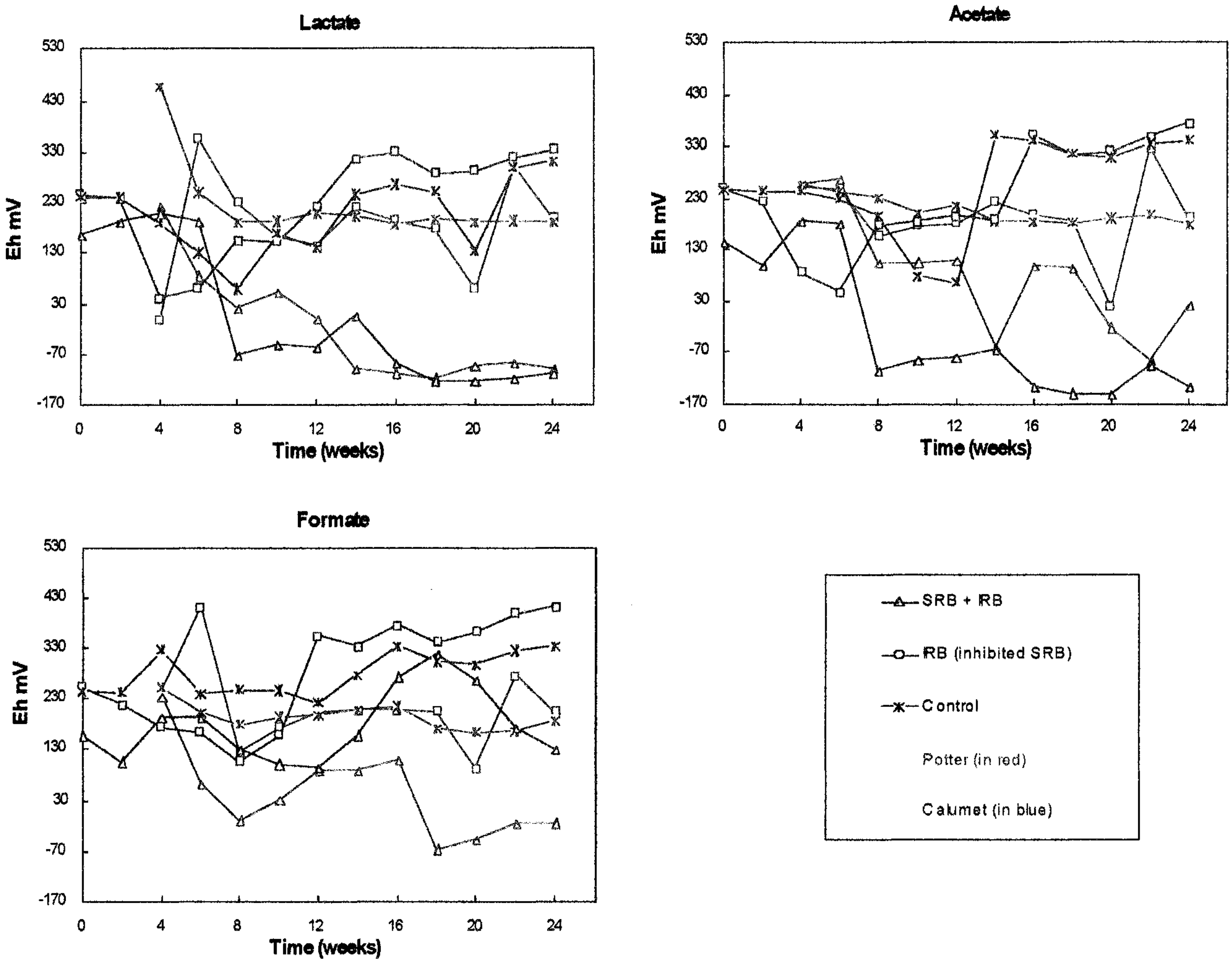

Figure 3.6 Eh trends in the various systems containing the Potter and Calumet mine tailings.

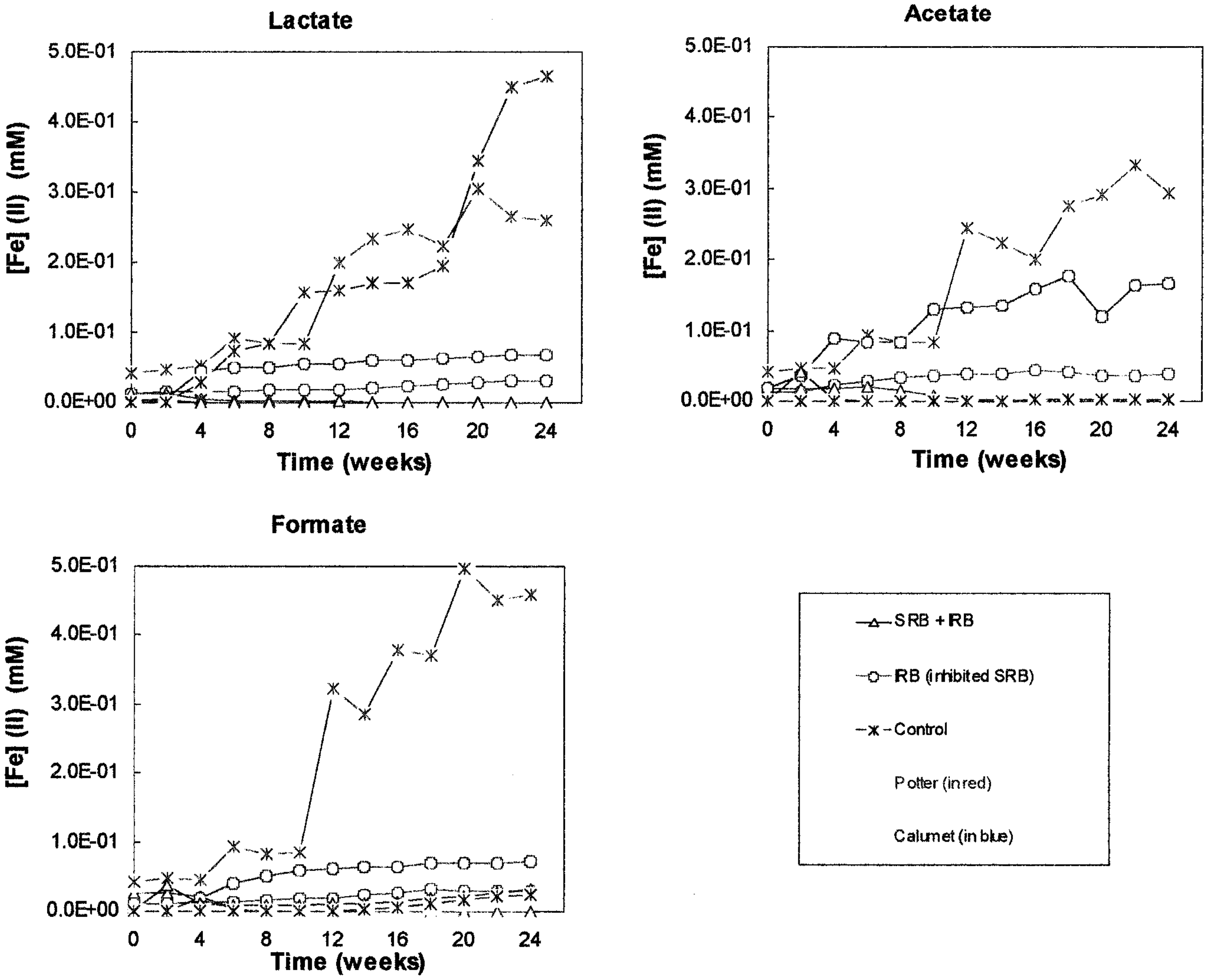

Figure 3.7 Concentration of soluble ferrous iron (Fe(II)) in the various systems containing the Potter and Calumet mine tailings.

3.3.3 Ferric iron (Fe(III))

Ferric iron concentrations remained fairly stable in all non-inhibited and inhibited systems (Figure 3.8), but increasing Fe(III) levels were observed in the inhibited SRB Potter system with acetate. On the other hand, Fe(III) concentrations slightly decreased over time in the control Potter systems, whereas they remained stable in the Calumet abiotic systems, with the exception of lactate system (Figure 3.8).

3.3.4 Hydrogen sulfide (HS^-)

Sulfide was not produced in the inhibited systems (i.e., containing only IRB) and in the abiotic control systems over the course of the experiment (Figure 3.9). However, it is important to mention here that sulfide concentrations were not measured in the inhibited systems after week 12 because of interference between the growth medium (containing the SRB inhibitor) and the Cline's reagents. Around week 12, all inhibited systems started to develop a blue color.

In the systems containing active SRB (i.e., non-inhibited), the production of sulfide was slow at first (0-6 weeks), but then increased over time (Figure 3.9). After 6 months, the production of sulfide was greater in the Potter systems containing acetate and formate than in the Calumet ones. The production of sulfide was however low in the Potter system containing lactate as an electron donor.

3.3.5 Sulfate (SO_4^{-2})

The initial sulfate concentration in the growth medium of all systems was around 18-20 mM. The initial sulfate concentration in some systems ($t = 0$) exceeded the concentration of sulfate in the growth medium because the tailings themselves (i.e., pore waters) contained variable amounts of sulfate (D. Fortin, pers. com.). In addition, due to the fact that all samples were diluted for sulfate analysis (section 2.6.4), a certain error is associated to each data point.

From the data presented in Figure 3.10, it appears that sulfate concentrations remained fairly stable in all inhibited systems (Potter and Calumet) in the presence of all 3 electron donors. Sulfate levels fluctuated over the course of the experiment in all abiotic systems, but no apparent trends were observed (Figure 3.10). On the other hand,

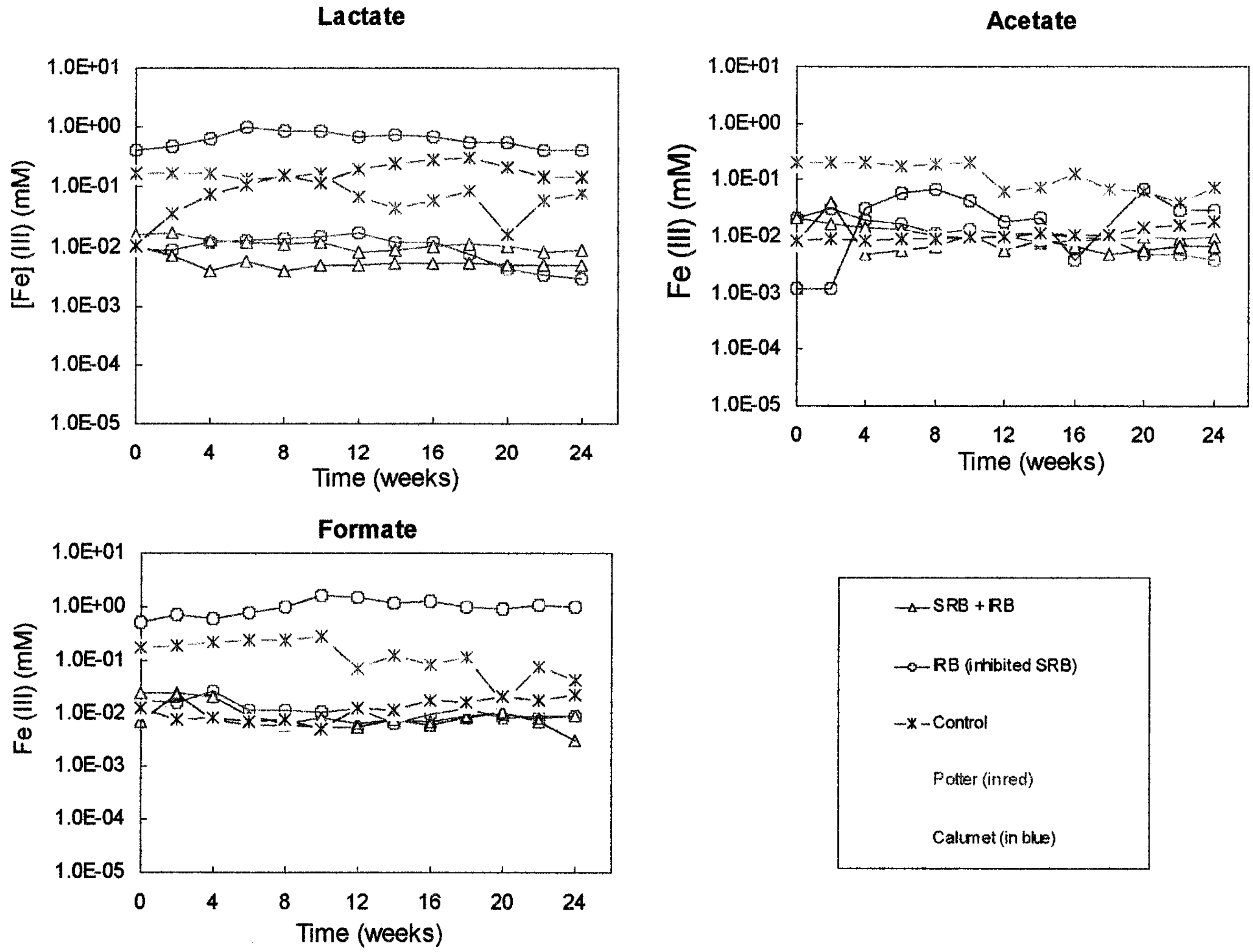

Figure 3.8 Concentration of soluble ferric iron (Fe(III)) in the various systems containing the Potter and Calumet mine tailings.

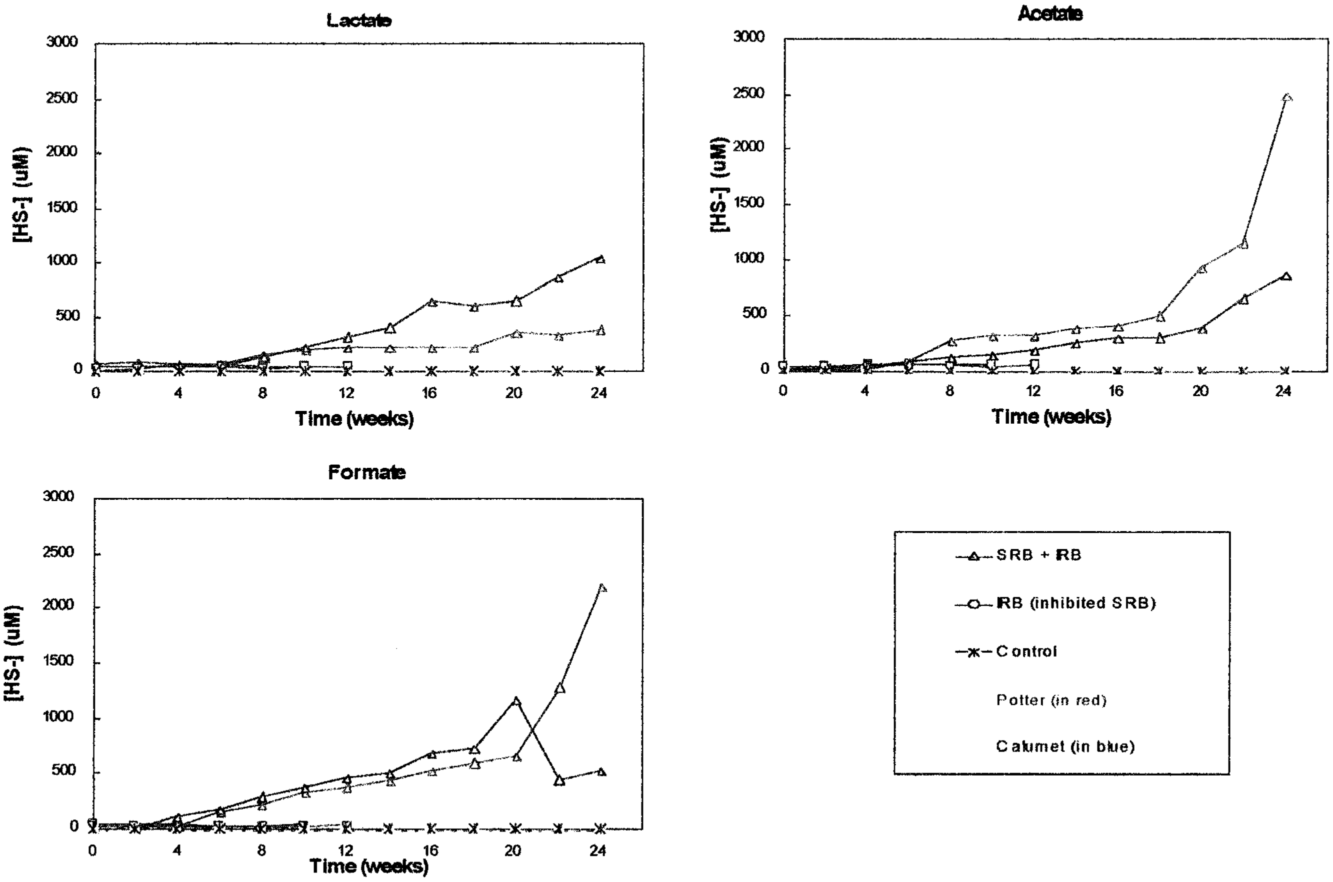

Figure 3.9 Concentration of hydrogen sulfide (HS$^-$) in the various systems containing the Potter and Calumet mine tailings.

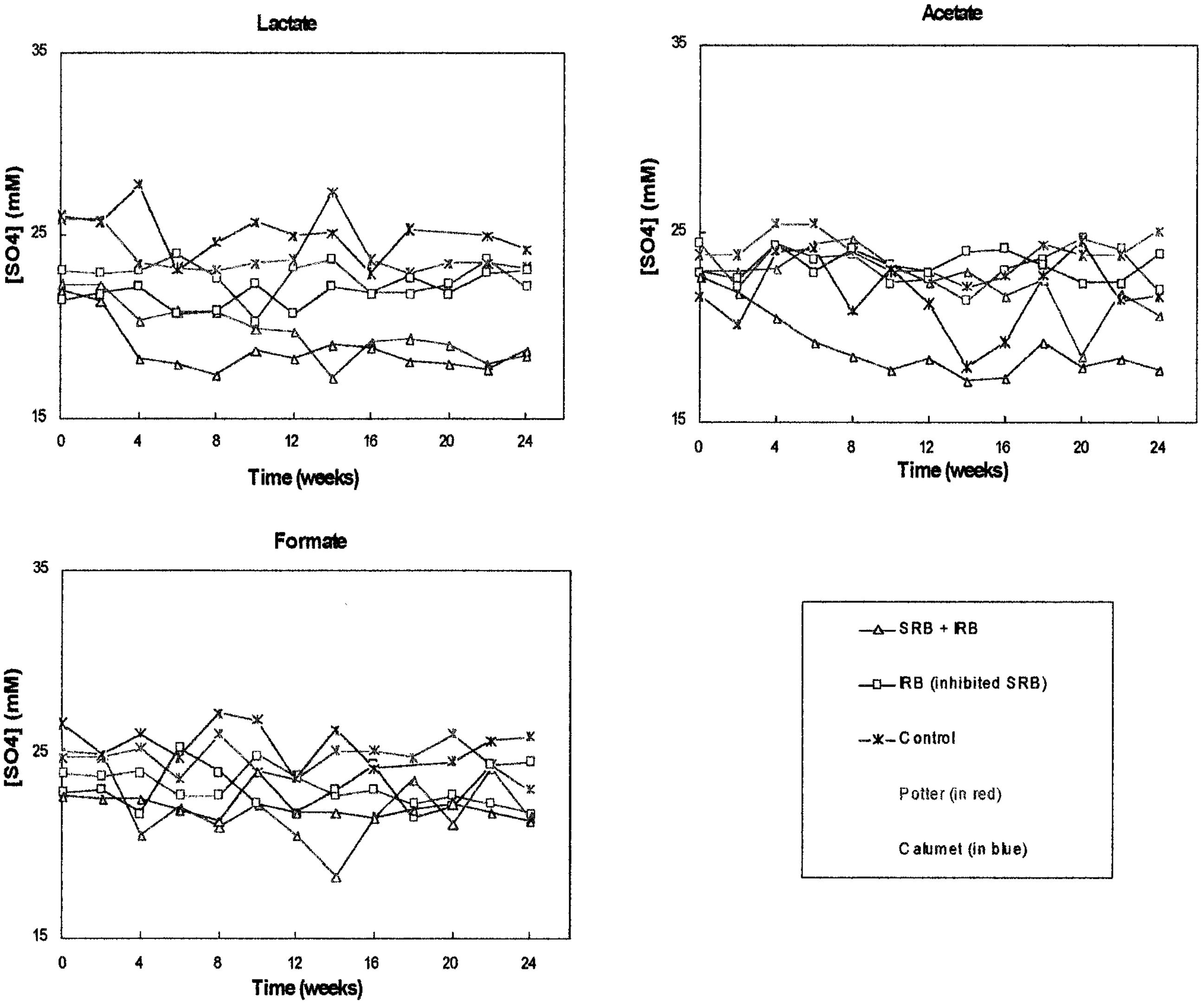

Figure 3.10 Concentration of sulfate (SO_4^{-2}) in the various systems containing the Potter and Calumet mine tailings.

sulfate concentrations slightly declined overtime in the systems containing active SRB (i.e., non inhibited systems), especially in the Calumet systems containing lactate and acetate as an electron donor, and in the Potter systems containing lactate and formate. In the presence of formate, the decline was only apparent from 0 to 16 weeks. Sulfate levels did remain stable in the non-inhibited Calumet system containing formate and in the non-inhibited Potter system with acetate (Figure 3.10).

3.3.6 Dissolved organic carbon (DOC)

The initial concentration of each electron donor was set at 5 mM (section 2. 3.1). This corresponded to 120 mg C/L for Na-acetate, 180 mg C/L for Na acetate and 60 mg C/L for Na-formate. Our results first indicate that DOC concentrations remained stable overtime for all abiotic systems containing the Potter and Calumet tailings (Figure 3.11). A sharp DOC decline (from 0 to 6 weeks) was observed in all Calumet non-inhibited systems (i.e., containing IRB and SRB) in the presence of all 3 electron donors (Figure 3.11). On the other hand, the non-inhibited Potter systems showed a slow decrease in DOC concentrations, but only in the systems containing lactate and acetate (Figure 3.11). In the Calumet inhibited systems, DOC levels remained stable in the lactate and acetate systems, but declined in the presence of formate. For the Potter systems, DOC concentrations remained constant in all inhibited systems (Figure 3.11).

3.3.7 Electron donors

3.3.7.1 Calumet systems

Due to a chemical interference with the HPLC column, formate was not detectable in the samples from both Calumet and Potter batch experiments. In addition, not all samples could be analyzed for acetate and lactate due to the lack of samples.

Lactate decreased rapidly within a few weeks in both biotic systems and in the control system containing lactate as the original electron donor (Figure 3.12). In the same systems, acetate was produced during lactate oxidation (Figure 3.12). In the systems originally containing acetate, the electron donor was rapidly consumed in the non-

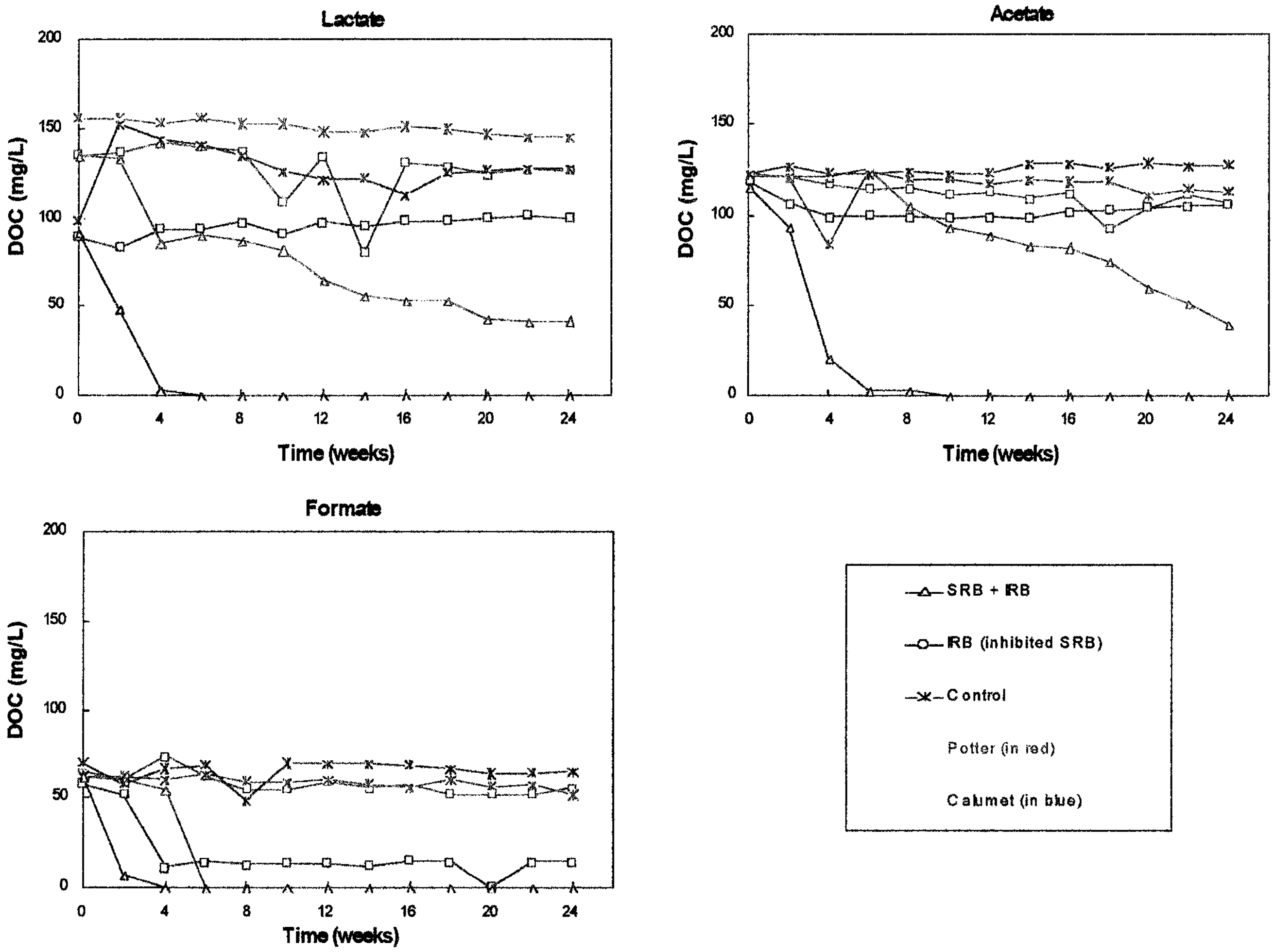

Figure 3.11 Concentration of dissolved organic carbon (DOC) in the various systems containing the Potter and Calumet mine tailings.

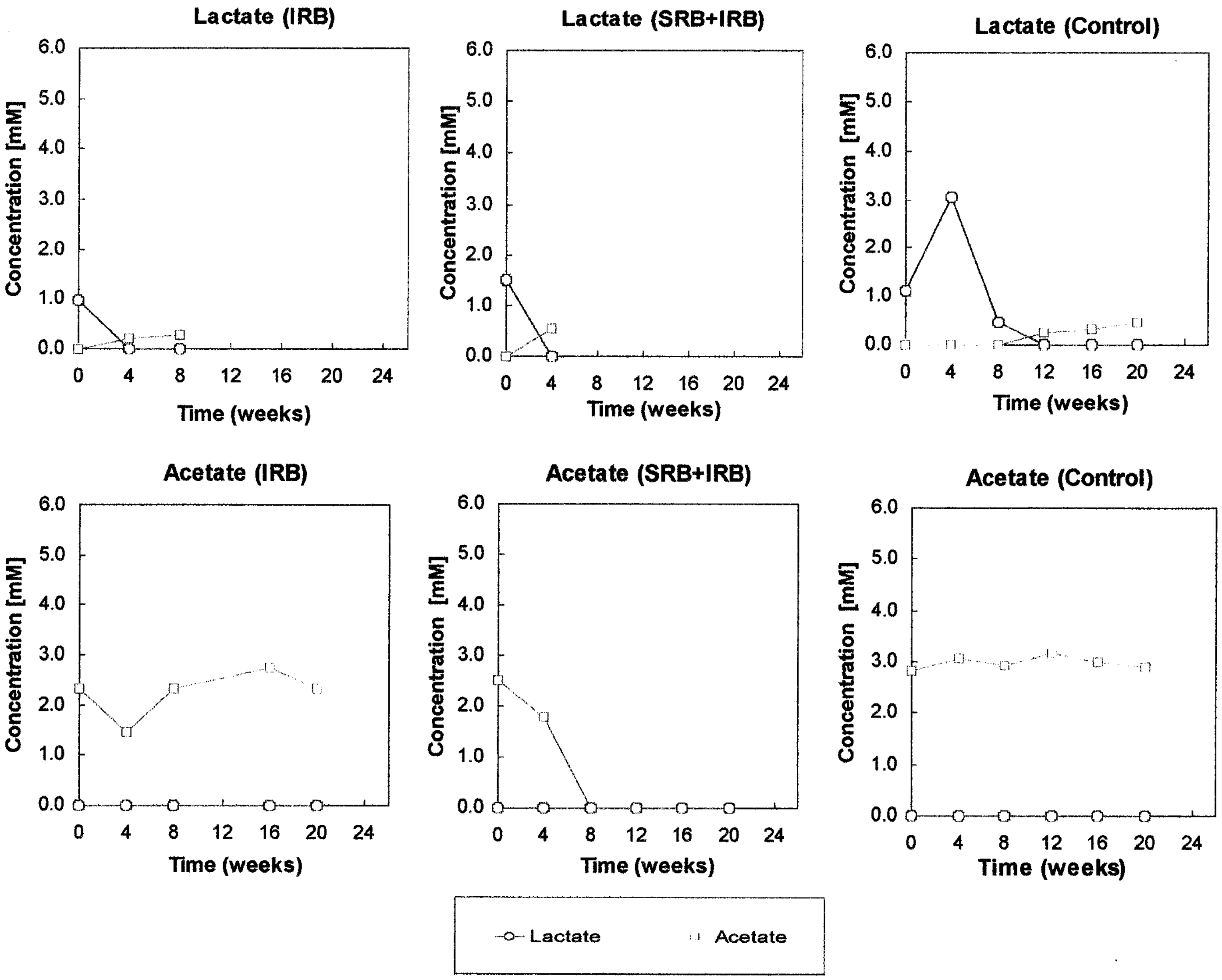

Figure 3.12 Concentration lactate and acetate in the various systems containing the Calumet mine tailings.

inhibited systems (i.e., active SRB), but remained fairly stable over time in the control and IRB systems (Figure 3.12).

3.3.7.2 Potter

Lactate, as an electron donor, was rapidly consumed in the non-inhibited systems (i.e., SRB), whereas it slightly decreased over time in the inhibited SRB systems (Figure 3.13). On the other hand, lactate concentrations remained stable in the control system. In the systems containing lactate as the original electron donor, acetate was however produced in the non-inhibited system (Figure 3.13).

Acetate levels declined over time in the non-inhibited systems containing acetate as the main electron donor, whereas it was not detected in the inhibited system after 8 weeks (no samples were available for the first 8 weeks). In the control system, acetate concentrations remained stable during the course of the experiment.

3.4 Solid phase geochemistry

Residual mine tailings collected from all systems (inhibited, non-inhibited and control) after 6 months were separated into 3 Fe-rich fractions using a sequential chemical extraction scheme (section 2.7). Because of the small quantity of tailings left in the systems after 6 months, the chemical extractions were performed without replicates. However, given the fact that all batch systems were prepared in duplicate, with the exception of the control systems, the results in Tables 3.1 and 3.2 represent an average of 2 analyses. In addition, only the result for the Fe-HCl (Fe-reactive) and Fe-HNO_3 (Fe-pyrite) fractions are presented because they correspond to the Fe-rich fractions most likely to change following the microbial activity of SRB and IRB.

3.4.1 Calumet mine tailings

For the lactate, formate and acetate systems, the Fe-HCl and Fe-HNO_3 fractions were generally higher in the SRB inhibited (i.e., IRB systems) and control systems than in the non-inhibited systems (Table 3.1). The Fe-HCl fractions of the various IRB systems (i.e., lactate, formate and acetate) were within the same range and did not appear to be significantly different (no statistical analyses were performed). The same trends were observed for the IRB+SRB systems and the control systems. With respect to the Fe-

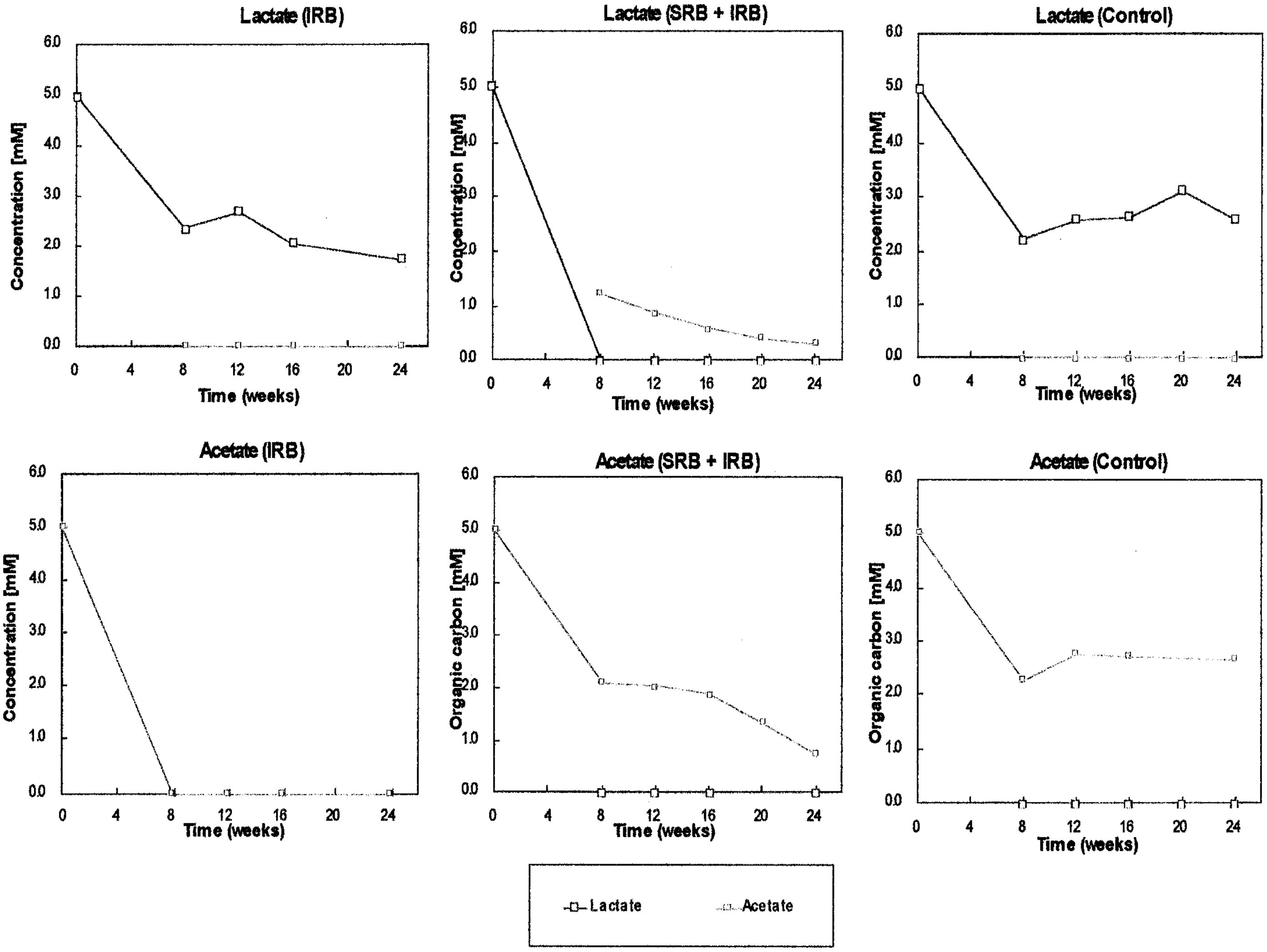

Figure 3.13 Concentration of acetate and lactate in the various systems containing the Potter mine tailings.

Table 3.1 Fe-rich fractions of the Calumet tailings left in the systems containing the various electron donors after 6 months.

System	**[Fe] HCl**	**[Fe] HNO_3**
	(mM/g. sed.)	(mM/g sed.)
CA-01 lactate IRB	1.231	1.387
CA-01 lactate IRB + SRB	0.223	0.515
CA-01 lactate Ctrl*	1.827	2.304
CA-01 acetate IRB	0.921	0.572
CA-01 acetate IRB + SRB	0.241	0.525
CA-01 acetate Ctrl*	1.088	4.637
CA-01 formate IRB	1.040	1.343
CA-01 formate IRB + SRB	0.322	0.521
CA-01 formate Ctrl*	1.569	2.898

*: n = 1 for the control systems.

HNO_3 fractions, similar values were obtained for the IRB+SRB systems for all electron donors, whereas the IRB system containing acetate contained less Fe-HNO_3 than the systems with lactate and formate (Table 3.1). The control system with acetate appeared to contain more Fe-HNO_3 than the lactate and formate systems (Table 3.1), but such trend might not be realistic because only one sample was analyzed.

3.4.2 Potter mine tailings

The Fe-HCl fractions were higher in the IRB system containing acetate than in the lactate and formate systems (Table 3.2). For the same fraction, but in the SRB+IRB systems, the Fe-HCl fraction was smaller in the lactate system than in the acetate and formate ones, whereas the control systems appeared similar (Table 3.2) with respect to the electron donors. No trends were observed for the Fe-HNO_3 fraction in the IRB+SRB and control systems with respect to the electron donors, whereas the IRB system containing acetate contained less Fe- HNO_3 than the other systems (i.e., lactate and formate) (Table 3.2).

Table 3.2 Fe-rich fractions of the Potter tailings left in the systems containing the various electron donors after 6 months.

System	**[Fe] HCl**	**[Fe] HNO_3**
	(mM/g. sed.)	(mM/g sed.)
PO-01 lactate IRB	0.796	6.687
PO-01 lactate IRB + SRB	0.829	7.691
PO-01 lactate Ctrl*	1.247	6.368
PO-01 acetate IRB	1.121	2.357
PO-01 acetate IRB + SRB	1.227	7.882
PO-01 acetate Ctrl*	1.430	5.541
PO-01 formate IRB	0.594	4.168
PO-01 formate IRB + SRB	1.508	5.293
PO-01 formate Ctrl*	1.035	6.512

*: n = 1 for the control systems.

4.0 Discussion

4.1 Importance of abiotic Fe(III) reduction

4.1.1 Iron oxide reduction by organic ligands.

One of the objectives (i.e., # 3) of the present study was to assess the importance of abiotic Fe(III)-reduction in order to fully understand the role of IRB in our systems. A survey of the literature on IRB growth has shown that abiotic reduction of Fe(III)-oxides by the components (the organic acids) of the growth medium is indeed a common problem (Bond and Lovley, 2002). According to these authors, when a growth medium containing 7 mM of acetate and 130 mM of Fe(III)-oxides is used to grow IRB, the production of 7.8 mM of Fe(II) can be initially ascribed to abiotic Fe(III) reduction alone. Complexing agents, such as NTA (nitrilotriacetic acid), added to certain growth media to prevent metal precipitation, have also been shown to reduce Fe(III)-oxides (Zachara et al., 1998). Such complexing agents were however not used in our growth medium. Experiments carried out with pure iron oxides (40 mM) and 5 mM of electron donors in the growth medium showed that Fe(II) was released into solution over time, especially in the presence of lactate and acetate (Figure 3.4). Concentrations of soluble Fe(II) within the first week of the experiment were similar to what was reported by Bond and Lovley (2002), but by the end of 8 weeks, most of the iron oxides originally present in the lactate systems (adjusted to the *in situ* pH of the Potter and Calumet tailings) were dissolved. These results indicate that lactate was a more powerful reducing agent for iron oxides than acetate and formate (Figure 3.4). To our knowledge, such findings have never been reported, even though several research teams are currently investigating the growth and isolation of IRB under laboratory conditions.

In addition, the presence of Na-molybdate in the iron oxide systems attenuated the reduction of Fe(III) in the presence of all 3 electron donors (Figure 3.4). Molybdate is known to sorbed onto mineral surfaces, including Fe-oxides (Zhang and Sparks, 1989) and it is possible that its presence on the surface of the iron oxides prevented their reduction by either stabilizing the minerals or blocking the binding sites for reductive dissolution. Urrutia et al. (1998; 1999) showed that the low reducibility of iron oxides was indeed caused by the sorption of metals, such as Fe(II), onto the surface of the iron oxides.

4.1.2 Redox reactions induced by sulfide minerals

In the control systems containing the tailings, it is more difficult to assess the effect of the electron donors on Fe(III) reduction because the systems were more complex than the Fe-oxide systems described in section 4.1.1, i.e., they contained tailings of various mineralogy and possibly other organic compounds originating from the ore extracting process. The results however clearly indicate that Fe(II) was released in these systems, as shown on Figure 3.7. Fe(II) concentrations reached about 0.5 mM in some of the systems during the course of the experiment. Additional experiments with higher tailings concentrations (Figure 3.3) produced similar results, but the levels of Fe(II) in solution (Figure 3.3) were higher than in the control systems containing less tailings (Figure 3.7). Interestingly, abiotic Fe(III) reduction occurred in the presence of all 3 electron donors in the systems containing the Potter tailings (Figures 3.3 and 3.7), but not in the Calumet systems, with the exception of the control lactate system (Figure 3.7). It is however not known why the Calumet control lactate system behaved differently. It was first thought that the slightly higher pH of the Calumet systems might have favored the precipitation of some Fe(II)-rich minerals, therefore limiting the Fe(II) concentrations in solution, but solubility calculations performed with the composition of the medium (at time zero) used for the control abiotic systems (Figure 3.7) and for the high tailings concentration systems (Figure 3.3) did not reveal any significant differences in terms of Fe(II)-rich mineral solubility between the Calumet and Potter systems (Table D-1, appendix).

It is more likely that that the production of soluble Fe(II) in the Potter abiotic control systems was caused by the oxidation of pyrite by soluble Fe(III) originally present in the medium (i.e., $FeS_2 + 14Fe^{3+} + 8\,H_2O = 15Fe^{2+} + 2SO_4 + 16H^{+}$). The source of soluble Fe(III) at time zero in the systems was likely from the tailings porewaters (Fortin et al., 2002). The oxidation of pyrite in the Potter tailings induced by the presence of soluble Fe(III) in the systems (Figure 3.8) is consistent with the decline of soluble

Fe(III) and the appearance of soluble Fe(II) overtime (Figure 3.7). The absence of Fe(II) production in the abiotic Calumet systems was likely caused by the lesser abundance of pyrite in these tailings, as shown by the HNO_3 extraction results (i.e., pyrite fraction) (Table 3.1 and 3.2). The abiotic oxidation of pyrite by ferric iron generates acidity, but such effect was not observed in the abiotic Potter control systems (Figure 3.5) because the growth medium was well buffered. It is also known that the reduction of Fe(III)-oxides by soluble sulfide is a mechanism responsible for the production of soluble Fe(II) (Peine et al., 2000), but it was not the case in the abiotic control systems since sulfide was not detected in the systems (Figure 3.9).

In summary, our results showed that organic acids can reduce iron oxides, but in the tailings systems containing pyrite, it appears that the production of soluble Fe(II) was likely caused by the abiotic oxidation of the iron sulfides by soluble ferric iron. The role of hydrogen sulfide as a reducing agent of Fe(III)-oxides could not be established in the abiotic systems because HS^- was not present.

4.2 Competition between IRB and SRB for similar electron donors

4.2.1 IRB activity in the absence of active SRB populations

The results first indicate that IRB populations did not grow well in the Calumet systems when SRB activity was inhibited and they did not grow at all in the Potter systems (Figure 3.2).

4.2.1.1 Potter systems

The absence of IRB growth in the SRB inhibited Potter systems in the presence of all 3 electron donors suggests that the electron donors (lactate, formate and acetate) were not suitable and/or that the *in situ* chemical conditions (mainly pH and Eh) inhibited their growth. However, IRB populations did grow in the Potter systems containing active SRB populations and the same electron donors, indicating that they were indeed capable of using a portion of the organic substrates. Despite the absence of IRB in the SRB inhibited systems (Figure 3.2), a slight decline of lactate and maybe acetate (not all samples were analyzed) concentrations was however observed, suggesting that a different process was responsible for the consumption of the electron donors (Figure 3.13). It is likely that abiotic Fe(III) reduction took place, as discussed in section 4.1, but other microorganisms (other than IRB and SRB) could have also been present.

Assuming that the electron donors were suitable for the IRB populations, it appears that the lack of IRB growth in the Potter systems was therefore related to the *in situ* chemical conditions, especially the redox potential and pH. Some IRB, such as *Shewanella and Acidiphilium*-like species, are facultative anaerobes, whereas *Geobacter* and *Geothrix* species are obligate anaerobes (Lovley, 2001; Coates et al., 2001; Küsel, 2003). Some IRB (i.e., *A. cryptum* and *A. ferrooxidans*) are also capable of only carrying Fe(III) reduction at low pH conditions (pH ~ 3), but not at pH 5 (Küsel, 2003). On the other hand, *Geobacter* tends to prefer pH neutral conditions (Coates et al., 1996). Based on the pH and Eh results of the inhibited SRB systems (Figures 3.5 and 3.6), the systems were sub-oxic and showed a constant pH around 5.70. Such conditions might have inhibited the *in situ* IRB populations in the Potter tailings if the latter were dominated by either acidophilic species incapable of carrying Fe(III) reduction at pH 5 or strictly anaerobic species, such as *Geobacter*. However, the growth of IRB in the non-inhibited SRB systems under the same pH conditions, but under reducing conditions, indicates that they were not acidophilic bacteria, but likely strictly anaerobes (Figures 3.2, 3.5 and 3.6). It is clear that additional molecular analyses are required to identify the various IRB species originally present in the tailings in order to better understand their growth behavior in the absence of active SRB.

4.2.1.2 Calumet systems

In these systems, IRB populations were relatively low and only present in the first 8-10 weeks of the experiment in the presence of lactate and formate, but very abundant in the acetate system (Figure 3.2). Their disappearance after 8-10 weeks also coincided to the appearance of sub-oxic to oxic conditions in the systems (Figure 3.6) whereas the pH remained fairly constant during the same period (Figure 3.5). These observations imply that the IRB populations present in the Calumet tailings were strict anaerobes and incapable of growth under sub-oxic conditions (Küsel, 2003). The complete consumption of the available electron donors (especially lactate; Figure 3.12) within the first 8-10 weeks might have also led them to their death or non-growth. The reason why oxic conditions developed in all 3 systems after 8-10 weeks is however unclear.

Our results also indicate that the IRB populations favored acetate (equation 4) over formate and lactate oxidation (equations 5 and 6) when SRB populations were not active (Figure 3.2). This is consistent with the fact that acetate oxidation is the most energetic reaction for IRB (Tor et al., 2001; Coates et al., 1996). In the presence of acetate, IRB populations grew rapidly within the first 8 weeks and reached ~ 10^7 CFU/mL (Figure 3.2).

Complete oxidation of acetate (Coates et al., 1996) (4)

$CH_3COO^- + 8Fe(III) + 4H_2O = 2HCO_3^- + 8\ Fe(II) + 9H^+$ $\Delta G°' = \sim 400$ kJ

The rapid growth of IRB in the presence of acetate also led to a net production of Fe (II) in the system (Figure 3.7). However, the consumption of 1 mM of acetate during the first weeks (Figure 3.12), should have produced 8 mM of Fe(II), according to equation 4, which is in excess of what was measured in the system (i.e., < 0.5 mM, Figure 3.7). This indicates that some Fe(II) likely precipitated as Fe(II)-rich minerals (see appendix D). The preferred oxidation of acetate is consistent with the presence of strict anaerobes, such as *Geobacter* species (Coates et al., 1996; 2001), which have been identified in mining-impacted lake sediments (Cummings et al., 2000). These authors showed that 2 isolates from the *Geobacter* cluster were strictly anerobic and oxidized acetate under slightly acidic pH conditions (pH 5.7 to 6.4).

Our results also show that IRB populations incompletely oxidized a portion of lactate to acetate (equation 5), as shown by the presence of acetate in the system (Figure 3.12). The low growth of IRB in such system consumed 1mM of lactate (Figure 3.12) and was accompanied by the release of some Fe(II) (Figure 3.7).

Incomplete oxidation of lactate (Lovley et al., 1989b) (5)

$C_3H_5O_3^- + 4\ Fe(III) + 2H_2O = CH_3COO^- + HCO_3^- + 4\ Fe(II) + 5H^+$ $\Delta G°' = -461$ kJ

However, it is clear that some of the acetate produced by the incomplete oxidation was further oxidized to CO_2 because the levels of acetate in the system (i.e., 10^{-4} mM; Figure 3.12) are not in agreement with the stoichiometric incomplete oxidation of 1mM of lactate (equation 5).

Growth in the presence of formate was low in the systems (Figures 3.2 and 3.7), because it is not an efficient energy yielding process (equation 7).

Complete oxidation of formate (Lovley et al., 1989b) (6)

$2HCOO^- + 2\,Fe(III) + H_2O = HCO_3^- + 2\,Fe(II) + 2H^+$ $\Delta G^{\circ\prime} = -227$ kJ

Similar results were obtained by Coates et al. (1996) when *Geobacter* was grown in the presence of formate as a sole electron donor. Formate was not analyzed by HPLC, but DOC levels rapidly declined in the first weeks of the experiment in the formate systems (Figure 3.11) suggesting that formate was completely oxidized.

Overall, IRB populations in the Calumet tailings were capable of using all 3 electron donors under anoxic and near pH-neutral conditions, but acetate appeared to be the favored electron donor. It is also clear that they were not capable of competing with the SRB populations, as described in the following section.

4.2.2 IRB activity in the presence of active SRB populations

Very low IRB populations (~ 10^2-10^3 CFU/mL) were detected in the Potter and Calumet systems containing active SRB populations in the presence of all 3 electron donors (Figure 3.2). IRB populations were generally present throughout the experiments, but they were by far less abundant than SRB (Figure 3.1)

The low IRB populations in the presence of active SRB indicate that iron reducers were not capable of competing with SRB for the various electron donors or that they were inhibited by the *in situ* chemical conditions (pH, Eh, etc.). Assuming that IRB populations in the Potter and Calumet tailings were likely neutrophilic and strictly anaerobes (based on the discussion in section 4.2.1), it is then unlikely that the *in situ* chemical conditions inhibited their growth since both the Potter and Calumet systems containing active SRB displayed near neutral pH conditions and anoxic conditions

(Figures 3.5 and 3.6). Our results therefore suggest that IRB were in fact incapable of competing with SRB for simple electron donors. This is best exemplified by the sharp decline of IRB populations in the Calumet acetate system in the presence of active SRB when compared to the system with inhibited SRB (Figure 3.2). Our results are in agreement with the findings of Küsel and Dorsch (2000), who demonstrated that the addition of acetate to slightly acidic lake sediments (with a pH similar to the Potter and Calumet tailing used in this experiment) was mainly coupled to sulfate reduction rather than iron reduction. The absence of Fe(II) release in the Potter and Calumet IRB systems containing active SRB (Figure 3.7) was the result of FeS precipitation following the microbial reduction of sulfate to sulfide (Fortin and Beveridge, 1997).

Results from our experiments do not support hypothesis #1, which stated that some competition might occur between IRB and SRB populations for acetate, since it is a common electron donor for both groups. The lack of competition could be related to the fact that the IRB populations in the Potter and Calumet tailings appear to be neutrophilic and strictly anaerobic IRB species. Such species have been shown to not be favored in the presence of SRB. Our results also refutes hypothesis # 2 which stated that IRB would out compete SRB in the more acidic tailings, i.e., Potter. The main explanation for the lack of competition is that the Potter tailings were not really acidic, at least not the samples collected for the present study. They were taken at the oxic-anoxic interface where the pH was around 5 (Fortin et al., 2002). Under such pH conditions, it appears that SRB can easily compete with IRB for the available electron donors.

4.2.3 Dominance of sulfate reducers

The discussion below is based on the assumption that IRB were not able to compete with SRB for the available electron donors. However, their presence, even in low numbers (Figure 3.2), indicates that they might have oxidized a very small fraction of the electron donors.

Sulfate reducers were abundant in the Potter and Calumet systems in the presence of various electron donors, but their growth was generally earlier in the Calumet systems than in the Potter ones (Figure 3.1). These observations are in agreement with the fact that SRB prefer neutral pH conditions (Widdel, 1988) and that the initial pH of the Calumet systems was slightly higher that the one in the Potter systems (Figure 3.5). However, the development of more alkaline conditions (as a result of sulfate reduction) in the Potter systems during the course of the experiment enhanced the growth of the SRB populations (Figure 3.1).

Our results indicate that SRB grew in the presence of all electron donors, despite the fact that some of them are less favorable from an energy point of view. The complete oxidation of lactate (equation 7) is more favorable than its incomplete oxidation (equation 8) and than the oxidation of formate and acetate (equations 9 and 10, respectively)

Complete (7) and incomplete (8) oxidation of lactate (Widdel, 1988)

$2C_3H_5O_3^- + 3SO_4^{2-} \rightarrow 6HCO_3^- + 3HS^- + H^+$ $\Delta G^{\circ\prime} = -255.3$ kJ (7)

$2\ C_3H_5O_3^- + SO_4^{2-} \rightarrow 2CH_3COO^- + 2HCO_3^- + HS^- + H^+$ $\Delta G^{\circ\prime} = -160.1$ kJ (8)

Oxidation of formate (Detmers et al., 2001)

$4HCOO^- + SO_4^{2-} + H^+ \rightarrow 4HCO_3^- + HS^-$ $\Delta G^{\circ\prime} = -146.9$ kJ (9)

Oxidation of acetate (Widdel, 1988

$CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^-$ $\Delta G^{\circ\prime} = -47.6$ kJ (10)

In the Potter systems, SRB growth in the presence of acetate and formate led to larger SRB populations that in the presence of lactate, even though lactate is the most favorable electron donor for energy requirement (equation 7). Such trend can be partially explained by the fact that the SRB in the Potter tailings appeared to incompletely oxidize lactate to acetate, as shown by the HPLC results (Figure 3.13). However, given the fact that the production of acetate in the lactate system (i.e., $< 2 \times 10^{-3}$ mM) was inferior than the expected concentration (i.e., 5×10^{-3} mM; calculated from the stoichiometric reaction in equation 8), it is likely that some acetate oxidizers were also present in the lactate system and that they further oxidized a portion of the acetate to CO_2. The appearance of acetate after week 8 (Figure 3.13) did in fact coincide with increasing SRB populations, suggesting that the original SRB populations in the Potter tailings were composed of incomplete oxidizers of lactate and acetate oxidizers. The overall lower SRB populations in the lactate systems also coincided to lower sulfide levels (Figure 3.9) when compared to the acetate and formate systems.

The large SRB populations in the acetate systems are intriguing since it is the least favored electron donor (equation 10). According to Figure 3.13, most of the acetate (5 mM) was consumed during the 6-month period, which led to a net production of sulfide of about 2.5 mM in the system (Figure 3.9). This is inferior to the expected 5 mM of sulfide (according to equation 10), and therefore indicates that Fe-sulfides likely precipitated as FeS or FeS_2. This is consistent with the appearance of black precipitates in the medium at the end of the experiment. The complete oxidation of 5 mM of acetate would have also consumed 5 mM of sulfate, which is in agreement with the data presented on Figure 3.10. The enhanced growth of SRB in the presence of acetate can only be explained by the fact that thea portion of the SRB consortium in the Potter tailings was composed of acetate oxidizers. According to Widdel (1988), acetate oxidation is a specific metabolic pathway carried out by only certain types of SRB (such as *Desulfobacter, Desulfobacterium, Desulfotomaculum acetoxidans*), but it appears to be ecologically relevant since acetate is commonly found in natural aquatic systems. Acetate oxidation was observed by Kusel and Dorsch (2000), who reported that in slightly acidic lake sediments impacted by mining activity, microbial sulfate reduction was indeed stimulated by the addition of acetate.

Our results indicate that the SRB present in the Potter tailings were also capable of growing in the presence of formate (Figure 3.1). Formate oxidation (equation 9) yields more energy than acetate oxidation (equation 10), which is consistent with the slightly higher SRB populations in the formate system than in the acetate one (Figure 3.1). Formate was not analyzed by HPLC, but the rapid DOC decline within the first 6 weeks of the experiment (Figure 3.11) indicates that formate was quickly oxidized. The complete oxidation of 5mM of formate should have produced 1.25 mM of HS^-, which is slightly greater than the net sulfide accumulation in the system up to week 20, i.e., ~ 0.75 mM. This indicates that Fe-sulfide precipitated, as suggested by the absence of soluble Fe(II) in the system (Figure 3.7) and the appearance of black precipitates at the end of the experiment. It is however not clear what caused the increased sulfide production in the last 4 weeks of the experiment (Figure 3.9). Sass et al. (1998) reported that formate, along with other electron donors, was completely oxidized by *Desulfovribrio* species isolated from lake sediments. In cyanobacterial mats, Teske et al. (1998) also reported that sulfate-reducing bacteria oxidized formate, but the same species were also capable of oxidizing acetate. These findings are in agreement with our results with respect to acetate and formate oxidation by the SRB populations in the Potter tailings.

In the Calumet systems, the presence of lactate and acetate led to similar SRB populations, whereas the formate systems contained less cells, at least in the first 12 weeks of the experiment (Figure 3.1). HPLC analyses indicated that acetate was produced in the lactate systems, indicating a partial oxidation of lactate (Figure 3.12). Since the quantity of acetate produced in the lactate system (i.e., ~ $5X10^{-4}$ mM) is slightly inferior to what should be expected from the incomplete oxidation of lactate (i.e., $1.5 X 10^{-3}$ mM), this suggests that SRB capable of oxidizing acetate were also present in the system and further oxidized acetate to CO_2. The incomplete oxidation of acetate (i.e., $1.5 X 10^{-3}$ mM) should have consumed 0.75 mM of sulfate and produced the same amount of sulfide (equation 8). From Figure 3.10, it appears that more sulfate was consumed during the course of the experiment (i.e., ~ 4mM) than what was expected from the stoichiometric oxidation of lactate to acetate. However, the levels of sulfide in the same systems (i.e., ~ 1 mM) are similar to what should be expected. The apparent larger consumption of sulfate could result from mineral (sulfate-rich) precipitation or from an experimental error on the sulfate measurement. However, solubility calculations showed that the growth medium was under-saturated with respect to several sulfate-rich minerals, such as gypsum, melanterite, epsomite, mirabilite, anhydrite and thenardite (data not shown) and that these minerals, if present, would have undergone dissolution and therefore produced more soluble sulfate in the systems. It then more likely that the an experimental error was associated to the sulfate measurement, as a result of several dilution steps in order to fit within the calibration curve of the sulfate assay. Despite the uncertainty associated with the sulfate measurements, it is clear that the SRB populations in the Calumet system containing lactate were incomplete oxidizers and that sulfate reduction was associated to sulfide production. These results are in agreement with the results obtained for the Potter tailings and with previous studies dealing with mining environments. Kusel (2003) showed that SRB capable of oxidizing acetate and lactate (complete and incomplete oxidation) have been identified in lake sediments impacted by mining activities.

Our results with the Calumet tailings also indicate that the SRB populations were capable of oxidizing acetate, as observed for the Potter tailings. The rapid consumption of acetate (Figure 3.12) was indeed accompanied by the fast growth of SRB within the first 8 weeks (Figure 3.1). The complete oxidation of 2.5 mM of acetate (equation 10) should have reduced 2.5 mM of sulfate and produced 2.5 mM of sulfide. These concentrations are somehow in agreement with the observed values, but the net production of sulfide in the acetate system (i.e., 1mM after 24 weeks; Figure 3.9) was inferior to the concentration expected from the stoichiometric oxidation. It is likely that the precipitation of Fe-sulfides in the systems (indicated by the formation of black precipitates) limited the concentrations of sulfide and soluble Fe(II) in the system (Figure 3.7).

In the formate system containing the Calumet tailings, SRB growth was slow at first and then increased near the end of the experiment (Figure 3.1). The slow growth was accompanied by the rapid consumption of formate as indicated by the declining DOC values (Figure 3.11), and by the production of sulfide in the system (Figure 3.9). For this system, the complete oxidation of formate (~ 5 mM) is in agreement with the production of sulfide (~ 1.25 mM; Figure 3.9) and the reduction of sulfate, i.e., ~ 1 mM (Figure 3.10).

Over all, SRB and IRB populations in the Calumet and Potter tailings appear to share the same energetic requirements with respect to the type of electron donors they can oxidize. Such lack of differences between these 2 distinct groups of bacteria could be related the fact that a certain number of sulfate reducers can also reduce Fe(III) (Lonergan et al., 1996). A more detailed molecular analysis of the SRB and IRB populations in both types of tailings would however be necessary in order to further investigate the similarities between these bacteria.

4.3 Effects of tailings mineralogy on the competition between SRB and IRB

Two different types of mine tailings were used in this work in order to see if the tailings mineralogy would have an impact on the activity of the microbial populations. The Calumet tailings are considered alkaline due to their high carbonate content and contain small amounts of pyrite (D. Fortin, pers. comm.). On the other hand, the Potter tailings are generally acidic and oxidized in the surface and contain large amounts of pyrite and other metal sulfides (Praharaj and Fortin, 2004a). Previous studies by Fortin et al. (2000; 2002 and Praharaj and Fortin 2004b) have shown that similar SRB populations (based on MPN enumeration) were present in the Potter and Calumet tailings and that they were involved in the reduction of sulfate. The rates of sulfate reduction were however higher in the Potter tailings than in the Calumet tailings (Praharaj and Fortin 2004b). More recent work on IRB in the same tailings (unpublished data) have also indicated that IRB were present, but their activity was more difficult to assess because no *in situ* rates of Fe reduction were performed. In the Potter tailings, the largest IRB populations were recovered from the acidic and oxic tailings, whereas the SRB populations were more abundant in the underlying anoxic tailings (unpublished data). On the other hand, both SRB and IRB populations occurred in the upper portion of the Calumet tailings where anoxic conditions prevailed (unpublished data).

The results obtained in this study showed that SRB were able to out compete the IRB under the conditions prevailing in the Potter and Calumet systems. It was also shown that SRB grew more quickly in the Calumet systems, likely as a result of the higher pH conditions (section 4.2.3). The enhanced growth of SRB in the Calumet tailings at higher pH conditions could however be linked to the presence of carbonate minerals in these tailings, which would have buffered the systems during the course of the experiment. Similar results were however obtained for the SRB and IRB populations in the Potter systems, but unlike the Calumet tailings, they do not contain much carbonate (unpublished data). This suggests that several factors, such as pH, Eh and presence or absence of certain minerals, affect the growth and activity of the bacteria and that the mineralogy of the tailings is not the only factor to consider.

5.0 Conclusion

The present study was undertaken to better understand the microbial ecology of sulfide-rich mine tailings, more specifically the behavior of iron- and sulfate-reducing bacteria. Laboratory experiments performed with 2 different types of mine tailings under slightly acidic to neutral pH conditions (pH 5 to 7) showed that SRB growth was favored over IRB in the presence of 3-different electron donors. The lack of competition was likely caused by the type of IRB living in both types of tailings, which appeared to be neutrophilic anaerobes incapable of competing with SRB at higher pH conditions. These results shed some light on the role of IRB and SRB in the Calumet and Potter mine tailings, but the results remain only applicable to the pH conditions used in the systems, and to the electron donors used, i.e., lactate, acetate and formate. In the Potter systems, pH conditions greatly vary within the first 50 cm of the tailings, ranging from 2 to 6 (Fortin et al., 2000), whereas in the Calumet tailings, the pH is more constant and neutral. It is clear that similar experiences should be carried out at lower pH with tailings samples collected from more acidic sections of the Potter tailings in order to fully assess the ecology of these bacteria in the natural settings. Unpublished data on the Potter site have also indicated that IRB populations were more abundant than SRB in the acidic and oxic portion of the tailings. Competition for electron donors under such conditions might be completely different that the results obtained in this study. Molecular analyses should also be performed in order to better understand the diversity of both SRB and IRB in the tailings.

The results from this study can also be helpful in the design of biological treatments (such as bioreactors) of acidic mine waters loaded with metals and sulfate. Some recent studies (Kaksonen et al., 2004; Christensen et al., 1996) have shown that bioreactors utilizing SRB were efficient in decreasing sulfate levels, generating alkalinity and favoring metal sulfide precipitation. Assuming that neutral pH conditions are maintained in the bioreactor, our results suggest that active SRB populations should be sustained and capable of competing with IRB populations.

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Appendices

Appendix A

Table A-1 Quantity of mine tailings used to inoculate each batch experiment system.

Site	Electron donor	System Classification	Initial weight (g) {bottle}+{50 mL of DI[a] water}	Final weight (g) {bottle}+{DI-water}+{tailings}	Weight of mine tailings added (g)
Calumet	Lactate	IRB	155.0739	160.0294	4.9555
		SRB +IRB	156.3586	160.8211	4.4625
		Control[b]	-	-	5.305
	Formate	IRB	155.2892	159.4994	4.2102
		SRB +IRB	154.7308	159.0569	4.3261
		Control	-	-	5.2159
	Acetate	IRB	152.2094	158.2924	6.083
		SRB +IRB	156.7048	161.5300	4.8252
		Control	-	-	5.2255
Potter	Lactate	IRB	153.6134	159.8143	6.2009
		SRB +IRB	153.6134	159.8143	6.2009
		Control	-	-	5.4298
	Formate	IRB	150.6314	159.8143	9.1829
		SRB +IRB	150.6314	159.8143	9.1829
		Control	-	-	5.9258
	Acetate	IRB	153.6155	158.1469	4.5314
		SRB +IRB	153.6155	158.1469	4.5314
		Control	-	-	5.9883

[a] DI: Distilled water.
[b] All control systems were inoculated with sterile mine tailings.

Appendix B

Table B-1 Effectiveness of Gelman filters used for Fe measurements

Systems	Volume filtered mL	[Fe] mg L^- with used filter[a]	[Fe] mg L^- with new filter	% Variation
1 Lac Ca	23.0	2.4370	2.3408	3.94
2 Lac Ca	23.0	0.0836	0.0836	0
3 Lac Ca	23.0	2.5190	2.4530	2.62
1 For Ca	23.0	2.2160	2.1500	1.85
2 For Ca	23.0	2.0510	2.0440	0.341
3 For Ca	23.0	0.4050	0.3800	4.93
1 Ace Ca	23.0	2.4460	2.4020	1.79
2 Ace Ca	23.0	2.2810	2.2750	0.26
3 Ace Ca	23.0	2.5890	2.5280	2.35

[a] used filter refers to the fact that the same filter was used to filter more than one sample from the same system.

Appendix C

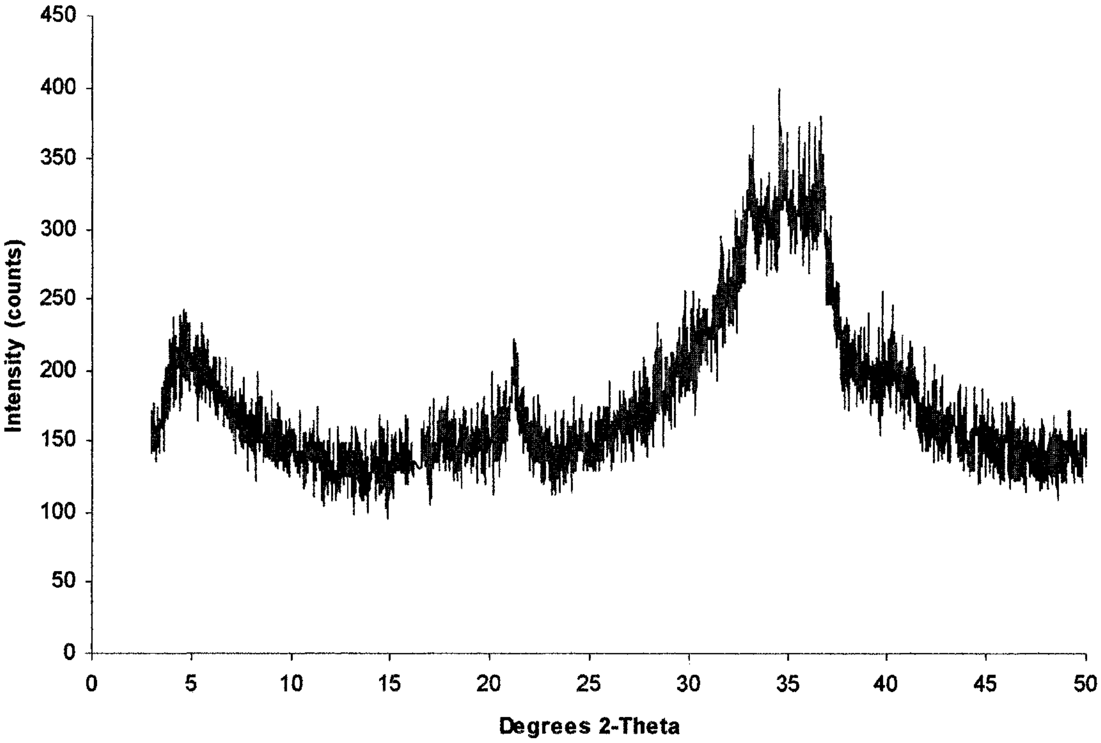

Figure C-1: XRD pattern of the hydrous ferric oxide (HFO) used in the growth medium for iron reducing bacteria

Appendix D

Table D-1 Saturations indices of some Fe(II)-rich minerals in the various abiotic systems* at time 0

System	$Fe_3(PO_4)_2$	Vivianite	Ca_2FePO_4
Acetate Potter high tailings concentration	5.42	5.42	13.10
Acetate Potter control	-4.29	-4.29	10.80
Acetate Potter Fe-oxides	0.22	0.22	12.30
Formate Potter high tailings concentration	5.43	5.43	13.60
Formate Potter control	-4.23	-4.23	10.80
Formate Potter Fe-oxides	0.28	0.28	12.30
Acetate Calumet high tailings concentration	2.50	2.50	13.50
Acetate Calumet control	-1.57	-1.57	12.20
Acetate Calumet Fe-oxides	2.95	2.95	13.60
Formate Calumet high tailings concentration	8.23	8.23	15.00
Formate Calumet control	-1.49	-1.49	12.20
Formate Calumet Fe-oxides	0.28	0.28	12.30

* Saturation indices were not calculated for the lactate systems because lactate was not in the MINEQL+ database