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Cu-Zu and Au Mine Tailings from Timmins, Ontario

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**The Effects of Electron Donors
on the Growth of Sulfate-Reducing Bacteria in
Cu-Zn and Au Mine Tailings
from Timmins, Ontario**

by
Michael Roy

A thesis submitted to the School of Graduate Studies and Research
in partial fulfillment of the requirements
for the degree of M.Sc. in Earth Sciences

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Abstract

Previous studies have shown that sulfate-reducing bacteria (SRB) are present and possibly active in gold and copper-zinc mine tailings. Sulfate-reducing bacteria can play an important role in the geochemistry of the mine tailings as they are responsible for the precipitation of diagenetic iron monosulfides and pyrite, a potential source for the generation of acid mine drainage. On the other hand, the formation of iron monosulfides can also serve to immobilize trace metals, and therefore has potential benefits to the tailing water systems. In addition, microbial sulfate reduction generates alkalinity which can be used to neutralize some of the acidity generated by the oxidation of metal sulfides.

To better understand the role that sulfate-reducing bacteria play on the geochemistry of mine tailings, this present study was designed to identify some of the factors controlling the growth of sulfate-reducing bacteria in the tailings. The main goal was to determine the influence of organic electron donors (specifically lactate, acetate, formate and pyruvate) on microbial sulfate reduction in closed batch systems possessing physico-chemical conditions (pH, redox potential) matching the *in situ* conditions of the tailings in order to identify the preferred electron donor.

Fresh tailings samples were taken from four mine tailings in Timmins, Ontario. The Potter and Broulan tailings are from Cu-Zn mines, whereas Delnite and Hollinger tailings are from Au mines. From each site, one sample was taken from an area without vegetation, whereas a second sample was taken in a zone with vegetation. Each sample (for a total of 8) was used to inoculate four systems, i.e., a lactate system, an acetate system, a formate system and a pyruvate system. Sub-samples were taken from each system for SRB enumeration, dissolved Fe and SO_4^{2-} measurements and electron donor

(indirectly through DOC) concentrations over time. Each biotic system was compared to an abiotic (control) system.

In general, the results indicate that sulfate-reducing bacteria only appear to be active in circa-neutral pH. However, they can apparently survive in acidic conditions, as once the pH increases above 6, their population levels start to rise again. SO_4^{2-} measurements generally show a decrease with time, however, there does not appear to be any correlation between the rate of sulfate decrease and the rate of sulfate-reducing bacterial population increase. Despite this lack of correlation, the decrease in SO_4^{2-} in the systems does appear to be related to bacterial processes rather than simple chemical precipitation, i.e., sulfate-rich mineral precipitation. Soluble Fe concentrations generally decrease over time, likely as a result of iron monosulfides or pyrite precipitation. DOC concentrations show a steady decline over time, which supports bacterial presence and activity in the systems.

Of all electron donors, pyruvate appears to be the favored electron donor in all eight sites, because it led to the highest SRB population growth. Lactate was the second most favored electron donor with respect to sulfate-reducing bacteria population growth. Acetate and formate were the least favored, each having the lowest SRB populations.

Finally, the SRB population increase varies even between individual Cu-Zn tailings as well as between the two Au tailings, suggesting that the *in-situ* geochemical conditions control the growth of SRB populations.

Sommaire

Des études ont démontré que les bactéries sulfato-réductrices (BSR) sont présentes et possiblement actives dans les résidus miniers de mines d'or et de cuivre-zinc. Les bactéries sulfato-réductrices peuvent jouer un rôle important dans la géochimie des résidus miniers car elles sont responsables de la précipitation de monosulfures de fer et de pyrite, lesquels peuvent générer du drainage minier acide. D'un autre côté, la formation de monosulfures de fer peut immobiliser les métaux traces, ce qui diminue leur toxicité et leur mobilité. De plus, la réduction microbienne du sulfate génère de l'alkalinité qui peut être utilisée pour neutraliser l'acidité générée par l'oxydation des sulfures de métaux.

Afin de mieux comprendre le rôle des bactéries sulfato-réductrices dans la géochimie des résidus miniers, cette étude a pour but d'identifier certains facteurs contrôlant la croissance des bactéries sulfato-réductrices dans les résidus miniers. L'objectif principal de cette recherche est de déterminer l'influence des donneurs d'électron organiques (spécifiquement le lactate, l'acétate, le formate, et le pyruvate) sur la réduction microbienne du sulfate dans des systèmes fermés contenant des résidus miniers frais et possédant des caractéristiques physico-chimiques (Eh, pH) similaires à celles observées dans les résidus miniers afin d'identifier le donneur d'électron préféré.

Des échantillons frais ont été prélevés dans quatre parcs à résidus miniers dans la région de Timmins, Ontario. Les sites Potter et Broulan sont des résidus de mine de Cu-Zn, tandis que les résidus miniers de Delnite et Hollinger proviennent de mine d'or. Pour chaque site, un échantillon a été pris dans des résidus sans végétation, alors qu'un second échantillon a été prélevé d'une section avec végétation. Chaque échantillon (pour un total de 8) a servi à inoculer quatre systèmes fermés, soit un système de lactate, un système

d'acétate, un système de formate, et un système de pyruvate. Des sous-échantillons ont été prélevés dans chaque système afin d'énumérer les BSR et de mesurer la concentration de Fe et SO_4^{2-} dissous et la concentration de donneurs d'électron (indirectement par mesure de COD) en fonction du temps. Chaque système a été comparé à un système abiotique (contrôle).

De façon générale, les résultats indiquent que les bactéries sulfato-réductrices sont seulement actives sous des conditions de pH neutre. Par contre, les BSR semblent être capables de survivre sous des conditions acides, car lorsque le pH augmente au dessus 6, les populations augmentent en nombre. Les concentrations de SO_4^{2-} décroissent le temps, mais il ne semble pas y avoir une corrélation entre la diminution de sulfate et la croissance des bactéries sulfato-réductrices. Par contre, la baisse de concentration de SO_4^{2-} dans les systèmes n'est pas liée à des réactions de précipitation de minéraux riches en sulfate, ce qui suggère que le sulfate est en fait réduit par les bactéries. Les concentrations de Fe diminuent aussi dans le temps, possiblement suite à la précipitation de monosulfures de fer. La baisse de concentration de COD supporte aussi l'hypothèse que les BSR sont actives dans les divers systèmes.

Parmi les 4 donneurs d'électrons étudiés, le pyruvate semble être le donneur d'électron préféré, et ce pour tous les systèmes. Le lactate est le deuxième donneur d'électrons préféré, suivi de l'acétate et du formate. Ces conclusions sont basées sur le nombre de BSR dans chaque système.

Finalement, l'augmentation des populations de BSR varie entre les 2 sites de résidus miniers dev Cu-Zn, ainsi qu'entre les résidus miniers d'or, ce qui suggère que les conditions géochimiques *in-situ* contrôlent la croissance des populations de BSR.

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1 INTRODUCTION

1.1 Mining Activities, Mine Tailings and Environmental Problems

Mining and milling have always been a major activity of the human race. They have supplied humans with metals, non-metals and minerals that have, throughout the ages, advanced their technology and standard of living. Europeans mined for flint as early as 4000 B.C. In 2500 B.C., Egyptians mined for copper while the ancient Greeks mined silver and lead. By 100 A.D., the Romans operated mines for iron, gold, tin and coal (James and Thorpe, 1994). In fact, the metals mined throughout the ages were so important that historians named various human epochs, such as the Bronze Age and Iron Age, after them.

Mining is still just as important in the modern day. However, one of the growing changes in modern mining is the incorporation of environmental maintenance and reclamation in the economics and engineering of the mine site.

There are numerous environmental concerns that relate to mining. Quarries, open pits and strip mining can devastate a large area, destroying the local vegetation and environment. The milling and smelting processes can release harmful gases into the atmosphere. However, although harmful, these activities are usually temporary. Stripped areas can be re-vegetated after the mine is gone, while holes can be plugged or filled. The smelting, meanwhile, lasts only as long as there is ore remaining.

But some environmental impacts can have effects long after the mine has been abandoned. Mine tailings, the unprofitable portion of the mined rocks which is dumped by the mining company, often create numerous problems. Tailings require a large amount of space, and can impact vast areas in the most productive of mines. The most notorious

environmental effect related to tailings is acid rock drainage which results from the oxidation of the newly exposed metal sulfides that can be found within the tailings.

1.1.1 Acid Rock Drainage (ARD)

Two aspects of ARD are so common that they are often used in the terminology surrounding ARD. First of all, ARD is so often associated with mines and mining activity that it is often referred to as acid mine drainage (AMD) in the literature. Second, although any metal sulfide can be the source of ARD, it is so often associated with pyrite (FeS_2) that many geologists refer to pyrite as the source of ARD instead of sulfides in general. The process of acid rock drainage can be simplified into the following equation (Montgomery, 1995):

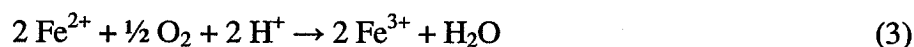


The resulting product of reaction (1) is an orange-red tainted water caused by the release of Fe(III) and the formation of iron oxides. The water is also highly acidic due to the presence of sulfuric acid (H_2SO_4) generated by the oxidation of reduced sulfur compounds. Of course, the above equation is a simplification of the exact processes which are occurring. No one is sure what reaction is actually taking place, and there may well be numerous intermediate steps and half-reactions involving the formation of peroxide, various sulfur compounds, elemental sulfur, and thiosalts (Knapp, 1987). Still, most geochemists agree that there are obviously three main steps in the reaction (Knapp, 1987).

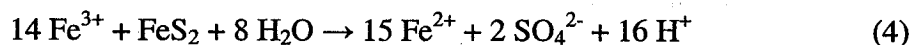
First, there is a chemical oxidation of the pyrite resulting in a relatively small quantity of acid. The formula for this reaction is as follows:



As indicated, both water and oxygen are needed to accomplish this oxidation. The source of oxygen is the atmosphere, while water tends to come from ground runoff. The end result is slightly acidified water as only a relatively small amount of H^+ is created. Once the pH of the water starts to reach 4, the second step begins. At that point, acidophilic bacteria, such as *Acidithiobacillus*, start a biological oxidation of the Fe^{2+} produced in equation (2). The lower the pH gets, the more bacteria play an important role in the oxidation, because the oxidation of Fe(II) is pH independent under very acidic conditions (Kim et al., 1982). The second phase of the overall ARD reaction is as follows:



The Fe^{3+} generated by the microbial oxidation acts as another powerful oxidant and can oxidize more pyrite according to:



Equation (4) is the final step which creates the very acidic waters. Ferrous iron generated in reaction 4 can then be re-oxidized to ferric iron, as shown in reaction 3. This can potentially lead to an endless loop between equations (3) and (4), assuming there is sufficient pyrite left to react.

As previously mentioned, pyrite is not the only source for ARD. Any metal sulfide can be oxidized, resulting in the release of sulfate and sulfuric acid as well as the release of the metal into solution. Common metallic ions that can be released as part of the ARD process include Cu, Ni, Pb, Zn, and As (Veldhuizen et al., 1987). This process of removing metals from the rock is often referred to as metal leaching.

1.2 Iron and Sulfur Cycles

ARD, taken to its most simplistic definition, is the oxidation of a metal sulfide. An understanding of the sulfur cycle is therefore a key element to understanding ARD. Also associated with ARD is the iron cycle. The most common sulfide involved with ARD is pyrite, which is an iron sulfide. Therefore, knowledge of the iron cycle is also important for a complete understanding of ARD, its impacts on the environment, as well as of possible methods of mitigating its effects.

1.2.1 Iron Cycle

Iron is the fourth most abundant element in the crust, after oxygen, silicon, and aluminum. Iron can be found in numerous igneous rocks such as pyroxenes, amphiboles, olivines, and silicates (particularly micas). Iron is even more common in sedimentary rocks (Lundgren and Dean, 1979), with iron carbonates, such as siderite (FeCO_3), iron sulfides, such as pyrite (FeS_2), iron oxides (Fe_2O_3), iron hydroxides ($\text{Fe}(\text{OH})_3$), iron oxyhydroxides, such as goethite ($\alpha\text{-FeOOH}$) and lepidocrocite ($\gamma\text{-FeOOH}$), and many more.

Iron also plays an important role in the biosphere and is frequently used by plants and animals in significant concentrations (Kadlec and Knight, 1996). In animals, iron is a key component of hemoglobin, which allows the transportation of oxygen through the bloodstream. For plants, the production of chlorophyll and cytochromes is also heavily dependent on iron.

Iron has three oxidation states: 0, +2 (ferrous iron, or $\text{Fe}(\text{II})$), and +3 (ferric iron, or $\text{Fe}(\text{III})$). Taken down to its most simplistic form, the iron cycle is essentially the

transformation of iron between ferric and ferrous states. It is because ferrous and ferric iron readily oxidize and reduce, respectively, that iron is so mobile in our environment and can perform all of the important roles attached to it.

In aerobic environments, Fe(III) is the most stable ionic form. Ferrous iron will oxidize into ferric iron by a reaction similar to the one in the following equation:



Ferric iron can only remain soluble in extremely acidic conditions (pH of 2 or less). In less acidic and more neutral conditions (pH 4 to 9), ferric iron will quickly react with water forming various $\text{Fe}_x(\text{OH})_y$ species. This hydrolysis of ferric iron usually leads to the precipitation of reddish-brown iron oxides and hydroxides such as $\text{Fe}(\text{OH})_3$ and FeOOH . (Stumm and Morgan, 1996). When the pH is greater than 5, the oxidation of ferrous iron is generally a purely chemical process. In more acidic environments, the process requires the presence of iron-oxidizing bacteria, such as *Acidithiobacillus ferrooxidans* and *Leptospirillum* (Ehrlich, 1995).

In anaerobic environments, Fe(II) is the dominant form of iron. When an iron (hydr)oxide (with ferric iron) enters an anaerobic environment, it is reduced to soluble ferrous iron, as shown in equation 6:



This reaction shows the dissimilation of organic material (referred to as $\langle \text{CH}_2\text{O} \rangle$) by bacteria. With a lack of oxygen for aerobic respiration, the iron (hydr)oxide serves as the final electron acceptor in the reaction. Iron oxidation defines one of the redox zones, which will be explained in further details in section 1.2.3.

The formation of soluble ferrous iron may be controlled somewhat by the presence of sulfides. Ferrous iron can react with sulfides to form relatively insoluble iron monosulfides (FeS) or pyrite (FeS₂) according to the following equations:



In fact, it is during the reactions described in equations (7) and (8) that metals can become trapped with the iron monosulfides. These are the reactions that can limit the effects of metal leaching that occurs with ARD.

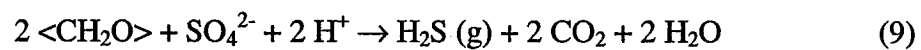
1.2.2 Sulfur Cycle

Sulfur moves throughout the biosphere by a complex interconnection of biological and chemical reactions. The burning of fuels liberates sulfur dioxides (SO₂) into the atmosphere. It returns to the ground below as sulfuric acid rain. Wetlands can function as sulfur sinks through their internal production and release of hydrogen sulfide as a gas, release of elemental sulfur (S⁰) or methyl sulfide gas, precipitation of elemental sulfur, and precipitation and burial of insoluble metallic sulfides (Kadlec and Knight, 1996). Within shallow waters and aerated soils, sulfur will generally be found in the form of a sulfate (SO₄²⁻). In deeper anaerobic waters and soils, sulfur is generally in the form of a sulfide (S²⁻). Sulfides are often bound to metals, such as iron sulfide (FeS or FeS₂).

Sulfur is also an essential nutrient to living organisms because its reduced sulfhydryl (-SH) form is used in the formation of amino acids (Kadlec and Knight, 1996). No known animal can reduce sulfur, and must rely on plants to acquire its organic sulfur requirements.

Plants can reduce sulfur through a process known as assimilatory sulfate reduction. It is referred to as assimilatory because the sulfur is assimilated into the plant's amino acids.

Sulfates can also be reduced by certain bacteria, such as *Desulfovibrio desulphuricans* or *Desulfotomaculum*, through a process called dissimilatory sulfate reduction (Postgate, 1984). Those bacteria which conduct dissimilatory sulfate reduction are commonly referred to as sulfate-reducing bacteria. During this process, sulfate is the final electron acceptor during the dissimilation (oxidation) of organic material. Most of the sulfur is released back into the environment as gaseous H₂S. One possible pathway for dissimilatory sulfate reduction can be defined as follows:



Again, <CH₂O> is the representation of generic organic material. As can be seen, the process ends with the formation of sulfide. The exact form of sulfide (H₂S, HS⁻ or S²⁻) will depend on the physico-chemical conditions of the solution and on the pH (Morse et al., 1987). However, since ferrous sulfide (FeS) is highly insoluble, sulfide does not tend to accumulate until all reduced iron is removed from the system.

1.2.3 Redox sequence

In the carbon cycle, plants absorb CO₂ from the atmosphere during photosynthesis. Photosynthesis incorporates the carbon into generic organic carbon material (i.e. <CH₂O>), which is used for the growth of the plant. When the plant is consumed by an herbivore, the carbon is then used by the animal to build its own tissues. The carbon proceeds in this manner up the food chain to all the animals. During the animal's respiration, the organic

carbon material is dissimilated, and the transfer of electrons to an acceptor produces energy for the animal. The respiration process releases the carbon back into the atmosphere as CO_2 .

Alternatively, when an organism decomposes, the carbon returns to the soil through the process of decomposition. The carbon in the soil is taken in by plants through their root system, and thus returns to the carbon cycle.

Both iron and sulfur have a part in the carbon cycle in which they are reduced by the process of accepting an electron from the dissimilation of organic material. These processes are essentially identical to the aerobic respiration, in which oxygen acts as the electron acceptor, except that in these instances, ferric iron and sulfate act as the electron acceptors. As can be seen in equations (6) and (9), CO_2 is also released from the reduction of iron oxides and sulfate. This is why the processes have sometimes been called "iron respiration" or "sulfate respiration" respectively.

There are several "respiration" pathways possible in the carbon cycle. Generally, the most energetically favorable pathway will be used by the organisms whenever possible. Table 1 presents the general respiration pathways in order of most favorable to least favorable. However, the real situation is far more complicated because there are more species present and more redox routes than only those presented in Table 1.

As Table 1 illustrates, oxygen respiration is the most energetically favored. Because of this, whenever free oxygen is present, aerobic respiration is the dominant respiration pathway. In areas where there is no rapid mixing of oxygen, such as within soils, free oxygen will deplete. Once the oxygen is depleted, denitrification begins as it is the next most favored pathway. With the depletion of nitrates, manganese (Mn (IV)) reduction proceeds, followed

by iron (III) reduction and sulfate reduction. Finally, once all possible electron acceptors are depleted, fermentation begins. This sequence of favored respiration pathways is often referred to as the redox sequence.

The decrease in free oxygen can be measured as an increasingly negative redox potential. Redox potential (Eh) is the electric potential between a standard platinum electrode and the concentration of oxygen, measured in mV. An environment with freely available oxygen will have an Eh greater than 300 mV. Such environments are considered aerobic. If the Eh is less than -100 mV, the local conditions are anaerobic as there is no free oxygen present. The zone between aerobic and anaerobic is referred to as anoxic by some authors (Kadlec and Knight, 1996).

Table 1: Typical Respiration Redox Sequence in the Carbon Cycle

<u>Process</u>	<u>Equation</u>	<u>Free Energy change*</u> (kJ/mol)
Aerobic respiration	$\langle \text{CH}_2\text{O} \rangle + \text{O}_2 \rightarrow \text{CO}_2 + \text{H}_2\text{O}$	-119
Denitrification	$5\langle \text{CH}_2\text{O} \rangle + 4\text{NO}_3^- + 4\text{H}^+ \rightarrow 2\text{N}_2 + 5\text{CO}_2 + 7\text{H}_2\text{O}$	-113
Manganese reduction	$\langle \text{CH}_2\text{O} \rangle + 2\text{MnO}_2 + 4\text{H}^+ \rightarrow \text{CO}_2 + 2\text{Mn}^{2+} + 3\text{H}_2\text{O}$	-97
Iron Reduction	$\langle \text{CH}_2\text{O} \rangle + 4\text{Fe}(\text{OH})_3 + 8\text{H}^+ \rightarrow 4\text{Fe}^{2+} + \text{CO}_2 + 11\text{H}_2\text{O}$	-47
Sulfate Reduction	$2\langle \text{CH}_2\text{O} \rangle + \text{SO}_4^{2-} + \text{H}^+ \rightarrow \text{HS}^- + 2\text{CO}_2 + 2\text{H}_2\text{O}$	-21
Fermentation	$2\langle \text{CH}_2\text{O} \rangle \rightarrow \text{CO}_2 + \text{CH}_4$	-18

*: Morel and Hering, 1993.

1.3 Microbial Ecology of Mine Tailings and Mining Environment

The microbial ecology in soils, including mine tailings, generally correlates with the redox sequence. In the first oxic zone, aerobic oxygen utilizing bacteria will be present.

After, in the anoxic zone, the general profile of bacteria is, in theory, denitrifiers, followed by manganese- and iron-reducing bacteria, sulfate-reducing bacteria and fermentative bacteria.

As mentioned, this is merely a generalization, as actual *in-situ* conditions will favor some bacteria, and not others. For example, in areas without any iron oxides, iron-reducing bacteria will not be active due to the lack of suitable final electron acceptors. However, if conditions are equally favorable for all bacteria, they will follow a profile matching the order of redox sequences.

1.3.1 Microbial activity of Fe- and S-oxidizers

Iron-oxidizing bacteria and sulfide-oxidizing bacteria can be found in the oxic zone of mine tailings. These bacteria play important roles in the iron and sulfur cycles respectively. However, the focus of this study is on anaerobic bacteria found within the anoxic zone, specifically sulfate-reducing bacteria.

1.3.2 Sulfate-Reducing Bacteria

The discovery of sulfate-reducing bacteria was the ultimate conclusion to experiments that attempted to discover the mechanism behind the production of hydrogen sulfide from sulfate in natural waters. Meyer (1864), who had recognized the process as biological in origin, first believed that the reaction was caused by certain algae. It was only a few years later that Cohn managed to link the sulfide formation to filamentous microorganisms which he classified as *Beggiatoae* (Cohn, 1867). From there, it took 30 years for Beijerinck to

actually demonstrate that a unique kind of bacteria were responsible for the entire process (Beijerinck, 1895). He had managed to isolate curved motile cells which he named *Vibrio desulfuricans*, but they eventually received the name *Desulfovibrio desulfuricans*.

The genus designation of *Desulfovibrio* was used for any non-sporing sulfate reducers usually having curved motile cells. The genus *Desulfotomaculum* was used to identify similar bacteria which can create spores. Other types of sulfate-reducing species which differed from either genus physiologically or morphologically were also discovered (such as *Desulfobacter*, *Desulfococcus*, *Desulfosarcina* and *Desulfonema*), indicating that sulfate-reducing bacteria are a rather heterogeneous assemblage of microorganisms with only a dissimilatory sulfate metabolism in common (Widdel, 1988). However, such discoveries only came much more recently, and many previously discovered sulfate-reducing bacteria have been mislabeled as one of two above genus. That is why the generic term “sulfate-reducing bacteria” is so commonly used.

1.3.2.1 Gram-Staining

As expected, gram-staining tests revealed that the non-sporing sulfate-reducing bacteria have gram-negative cell walls. Spore-forming bacteria should have gram-positive cell walls, but only small traces of gram-positive cells appeared in the various *Desulfotomaculum* cultures (Postgate, 1984). It took electron microscopy and catalogs of RNA to show that *Desulfotomaculum* indeed had a gram-positive cell wall structure (Nazina and Pivovarova, 1979). These studies also lead to the discovery that normal gram-staining tests are inconclusive when it comes to sulfate-reducing bacteria.

1.3.2.2 Electron Donors

As with all heterotrophic bacteria, sulfate reducers obtain their energy by the degradation of organic material in the presence of a suitable electron acceptor (i.e. sulfate).

The equation for dissimilatory sulfate reduction is:



Since sulfate reducers are anaerobes, the amount of dry cell mass formed per amount of energy substrate used is relatively low (Widdel and Pfennig, 1981). This means that only a small amount of the hydrogen sulfide formed is used for cell synthesis of sulfate-reducing bacteria. The remainder is therefore released into the surrounding environment.

The electron donor, represented in equation 10 by $\langle\text{CH}_2\text{O}\rangle$, is always a low-molecular-weight compound. Most are fermentation products from the anaerobic degradation of carbohydrates, proteins, and other components of dead biomass. In the nutritiously poor setting of mine tailings, the most likely source of biomass is thought to be iron and sulfur oxidizers that arrive with pore water percolating down from the oxic layer (Fortin and Beveridge, 1997) and organic carbon present in surface runoffs. The oxidation of the organic substrate allows the creation of ATP by electron transport phosphorylation. In this case, sulfate plays the role of oxygen in aerobic organism, which is why the process is also known as sulfate respiration.

There are two main groups of sulfate-reducing bacteria, at least metabolically speaking. The first group includes all the species that incompletely oxidize their substrate; they end up with acetate and cannot oxidize any further. The second group consists of those

capable of complete oxidation of their organic substrates, releasing carbon dioxide as their end product.

The incomplete oxidizers include species of the genera *Desulfotomaculum*, *Thermodesulfobacterium*, *Desulfomonas*, and *Desulfovibrio*. What unites these species is the fact that they lack an operation enzymatic mechanism, like the citric acid cycle, that allows the oxidation of acetyl-CoA (Brandis-Heep et al., 1983). The end product excrete is therefore acetate. Substrates that are already more oxidized than acetate, such as oxalate and glycine, can actually be completely oxidized by incomplete oxidizers. On the whole, the incompletely oxidizing sulfate reducers are nutritionally less versatile than the completely oxidizing species. The most common organic substrates for this group are hydrogen, lactate, pyruvate, malate, fumarate and ethanol. However, their specialization in substrates grants them a significantly faster growth than the more versatile complete oxidizers. Growth rates for *Desulfovibrio* and *Desulfotomaculum* species using pyruvate or lactate can achieve doubling times of 3 to 4 hours (Stams et al., 1985). Because of this fast growth rate, when a substrate can be used by both complete and incomplete oxidizers, it is usually the latter that dominate the growth medium.

The complete oxidizers include species of the genera *Desulfobacter*, *Desulfococcus*, *Desulfosarcina*, *Desulfonema* and *Desulfobacterium*. Also, although *Desulfotomaculum* and *Desulfovibrio* are considered genera of incomplete oxidizer, the species of *Desulfotomaculum acetoxidans*, *Desulfotomaculum sapomandens*, and *Desulfovibrio baarsii* brake the mold and fall into the complete oxidizers category. In theory, the complete oxidizers are all capable of oxidizing any substrate completely to carbon dioxide. In reality,

however, several are specialized and can only use a specified number of substrates. Those that use only acetate (*Desulfobacter*) have relatively faster growth rates, reaching doubling times as low as 20 hours (Widdel, 1988). Those who truly are more versatile tend to prefer other substrates. They can still use acetate, but very slowly and have a much lower cell yield. Also, despite being able to oxidize acetate, some complete oxidizers have been known to excrete acetate. It is believed that in these specific instances, oxidation to acetate was more energy effective than completely oxidizing the substrate to carbon dioxide.

Regardless if a species is a complete or incomplete oxidizer, one cannot ignore the significance of the electron donor.

1.3.2.2.1 Acetate

Acetate is an electron donor that is used best by species such as *Desulfobacter* and other complete oxidizers. Interestingly enough, although having the unique ability among sulfate reducers to use acetate (which is what makes them complete oxidizers), they also have the most specialized diet. Some strains use no further substrate besides acetate, while others can also use ethanol (Laanbroeck et al., 1984).

In the laboratory, *Desulfobacter* species have an optimum growth rate in a saline media with sodium and magnesium concentrations that mimic the concentrations found in their habitat (Widdel and Pfennig, 1981).

The oxidation reaction of acetate is (Widdel, 1988):



The low energy yield of such a reaction explains why growth with acetate as the organic substrate is relatively slow when compared to other substrates.

1.3.2.2.2 Hydrogen and Formate

With the exception of *Desulfovibrio sapovorans*, sulfate reducers that incompletely oxidize lactate are also able to grow using hydrogen as their electron donor (Brandis and Thauer, 1981). Accordingly, species isolated from natural sources with hydrogen were all able to grow on lactate. The utilization of hydrogen was the first hint that sulfate reducers can conserve energy solely by electron transport phosphorylation.

The reaction representing the use of hydrogen as electron donor is (Widdel, 1988):

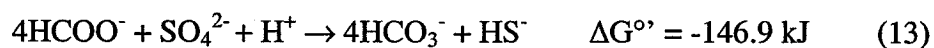


Although the high energy yield would seem to indicate that sulfate reducers will have a relatively fast growth rate with hydrogen as the electron donor, this is not necessarily the case. When hydrogen is used as the electron donor, there are no useful end products that can be used for the creation of cell walls. For continuous growth, the bacteria therefore need to assimilate other substances found in the medium to provide building materials. Usually, carbon dioxide and acetate serve as building materials in a 2/3:1/3 ratio respectively (Badziong et al., 1979).

Hydrogen can also be used by completely oxidizing sulfate-reducing bacteria. However, such growth is relatively slow, suggesting that the bacteria grow autotrophically. Most sulfate reducers that use hydrogen can also use formate as their electron donor,

however a few species can use only hydrogen or formate (such as *Desulfobulbus propionicus* and *Desulfovibrio baarsii* respectively).

The use of formate as electron donor is shown in the following equation (Detmers et al., 2001):



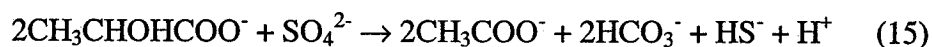
1.3.2.2.3 Lactate

Lactate is considered the substrate of choice to isolate and cultivate *Desulfovibrio* and *Desulfotomaculum* species (Postgate, 1984). Lactate can be degraded by both complete and incomplete oxidizers. *Desulfovibrio* can use both L-lactate and D-lactate. Several of the complete oxidizers, however, are incapable of oxidizing lactate. These include various *Desulfobacter* and *Desulfobacterium* species, *Desulfotomaculum acetoxidans*, *Desulfovibrio baarsii*, *Desulfococcus niacini*, and *Desulfonema magnum*.

The equations for both complete (14) and incomplete (15) oxidations of lactate are, respectively:



$$\Delta G^{\circ'} = -255.3 \text{ kJ}$$



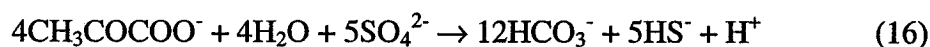
$$\Delta G^{\circ'} = -160.1 \text{ kJ}$$

With such a relatively high energy yield, it is easy to see why lactate is the favored organic substrate of both completely and incompletely oxidizing sulfate-reducing bacteria.

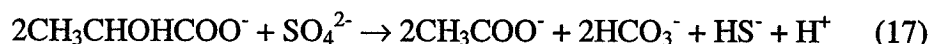
1.3.2.2.4 Pyruvate

Pyruvate appears to be the least favored substrate, and generally results in a very slow growth rate (Tinoco et al., 1995). Widdel (1988), on the other hand, seems to rank pyruvate as similar to lactate, anticipating similar results with both electron donors. The main reasoning behind this is that the degradation pathway of lactate by bacteria into acetate is believed to create pyruvate as an intermediate step (Postgate 1984, Widdel 1988, Oude Elferink et al., 2001).

The equations for both complete (16) and incomplete (17) oxidations of pyruvate are, respectively (Detmers et al., 2001):



$$\Delta G^{\circ\prime} = -106.3 \text{ kJ}$$



$$\Delta G^{\circ\prime} = -340.9 \text{ kJ}$$

According to the $\Delta G^{\circ\prime}$, incomplete oxidation of pyruvate appears to be the most energetically favorable of all of the reactions.

1.3.3 Factors Affecting the Presence and Growth of SRB

1.3.3.1 Temperature

Temperature plays an important role in the rate of the reactions taking place as all bacteria are poikilotherms, meaning that their internal temperature is the same as the ambient temperature. For sulfate reducers, the optimum temperature for growth is usually found within 30 to 40°C (Widdel, 1988).

Growth rate below the optimum temperature can be calculated using the Arrhenius equation. The Q_{10} values obtained for most sulfate-reducing bacteria in marine sediments range between 2.0 and 3.9. This means that for every change in temperature by 10°C , the growth rate will change by a factor between 2.0 and 3.9.

Above the optimum temperature, the growth rate declines much more rapidly. In fact, most sulfate-reducing bacteria have a maximum temperature just slightly higher than their optimal temperature (Widdel, 1988). Of course, once the maximum temperature is reached for a given species, the bacteria will die as their enzymes deteriorate by the added energy. Although sulfur-reducing archaeobacteria are still known to thrive in temperatures of 100°C and higher, the highest known maximum temperature for sulfate reducers is 85°C (specifically for *Desulfovibrio thermophilis*).

Not much is known about the minimum temperatures that sulfate reducers can survive in. Several species have been isolated from Antarctica with temperatures as low as -51°C and were still able to grow at room temperature. However, the optimum growth-rate temperature for these cold-surviving bacteria was found to be around 20°C , which is significantly higher than the in situ temperature. This indicates that some sulfate reducing bacteria species are psychotrophic. True psychophilic sulfate reducing species have yet to be discovered. The optimum sulfate reduction rate temperature for psychotrophic sulfate reducers appears to be even higher than their optimum growth-rate temperature, generally around 30°C (Isaksen and Jørgensen, 1996; Sageman et al, 1998). The implications are that in below optimum reduction temperatures, although the sulfate reduction rate will decrease, the growth of the sulfate reducers will still be relatively high in comparison. Recent work by Fortin, Goulet,

and Roy (2000) showed that sulfate-reducing bacteria populations were indeed higher in the winter than in the summer in wetland sediments, suggesting that the cells were active all year around.

Other studies have suggested that cold temperatures might not inhibit bacterial life but would instead diminish the affinity of the bacteria for the substrates (Wiebe et al., 1992; Nedwell and Rutter, 1994). This diminished affinity for the substrate supports the decrease in sulfate reduction rates as well as the fact that there is not a similar decrease in bacterial growth rate.

1.3.3.2 Oxygen

One trait that all sulfate-reducing bacteria share in common (beside the fact that they reduce sulfate) is that they are all obligate anaerobes (Postgate, 1984). However, contrarily to what this statement implies, sulfate reducers have survived temporary exposure to oxygen and again become active once anaerobic conditions are restored (Widdel, 1988). The actual sensitivity to oxygen depends greatly on the specific species of sulfate reducers. Regardless of species, if the habitat receives continuous exposure to oxygen, eventually only *Desulfomaculum* will survive by creating spores.

So how do they survive for a limited oxygen exposure? *Desulfovibrio desulfuricans* were studied to understand this better (Abdollahi and Wimpenny, 1990). In that study, oxygen prevented the reduction of sulfate and the use of lactate (the electron donor for these experiments) also declined. However NADH oxidase production increased significantly to counter the effects of oxygen. This allowed the conversion of oxygen to H₂O₂. The activity

of superoxide dismutase also increased, although for what reasons still remains unclear. Although the NADH oxidase prevented the bacteria from dying immediately, the bacteria were no longer reducing sulfate, and no longer receiving any energy. Death of the cultures occurred after 48 hours.

However, even if exposed to oxygen, microniches of sulfate-reducing bacteria can continue to thrive. Hydrogen sulfide, the main end product of sulfate reduction, can itself be oxidized back to sulfate. This can create a mini sulfide-oxygen cycle that would allow a colony to thrive even though sulfate is absent (Cypionka et al., 1985). And since the oxygen is reacting with the hydrogen sulfide, it will not affect the bacterial population at all. Of course, this requires an enormous amount of hydrogen sulfide to react with all the oxygen. Usually, this is only possible in areas with only a small amount of oxygen present, such as in lakes with a large quantity of dead organic matter.

1.3.3.3 pH

Although sulfate-reducing bacteria generally prefer pH neutral conditions (Postgate, 1984), recent studies have indicated that the bacteria can survive in more acidic environments (Tuttle et al., 1968; Gyure et al., 1990). The rate of sulfate reduction, however, is a function of pH. Studies by Peine et al. (2000) have shown that the rate increases significantly above a pH of 5, but recent work by Koschorreck et al. (2003) has shown very high rates of sulfate reduction in acidic lake sediments (pH 3) in Argentina.

1.3.3.4 Presence of iron-reducing bacteria

According to the redox sequence (Table 1), iron reduction takes place prior to sulfate reduction. However, the situation is much more complex in the natural environment. Many factors can prevent iron-reducing bacteria from reducing iron and allow sulfate reduction to occur first. Recent studies have even found sulfate reducing bacteria coexisting with iron reducing bacteria in mine tailings (Fortin et al., 2002).

Although the present study does not focus on iron-reducing bacteria, it is important to note that iron-reducing bacteria do use the same electron donors as sulfate-reducing bacteria. The two types of bacteria will therefore compete for the same substrates. Because iron reduction is more energetically favorable, iron reducing bacteria will generally out-compete sulfate-reducing bacteria, and their presence will generally result in the decrease of the growth rate of sulfate-reducing bacteria, particularly in acidic environments (Peine et al., 2000; Küsel and Dorsch, 2000)

2 OBJECTIVES AND HYPOTHESES

Numerous recent studies (Blodau et al., 1998; Friese et al., 1998; Benner et al., 2000; Fortin et al., 2000; Peine et al., 2000, etc.) have found that sulfate-reducing bacteria are present in mine tailings and in the sediments of mining lakes, even under oxic and acidic conditions. Additionally, it appears that sulfate-reducing bacteria are active and participate in sulfur cycling, thus affecting the geochemistry of the tailings (Fortin et al., 2002). However, mine tailings are poor in organic substrates, and it has long been a mystery as to the source of electron donors used by the bacteria. In fact, that very lack of organic substrates led to the long-held, but now known to be inaccurate belief, that sulfate-reducing bacteria were not present or active within tailings (Fortin et al., 1995).

The main goal of this study was to increase our understanding as to which organic substrates are favored by sulfate-reducing bacteria living in mine tailings. By attempting to grow sulfate-reducing bacteria obtained from mine tailings in cultures containing only a single specific electron donor, it should be possible to determine what electron donors the sulfate-reducing bacteria are most adapted to using. Future studies can then focus on determining possible *in situ* sources of those specific electron donors.

The second goal was to assess if sulfate-reducing bacteria from vegetated tailings behaved differently from sulfate-reducing bacteria from non-vegetated tailings in terms of preferred organic substrates.

The third objective was to determine if sulfate-reducing bacteria from Cu-Zn tailings differed from sulfate-reducing bacteria from Au tailings in terms of preferred organic substrates.

The following hypotheses have been made prior to beginning the experiments:

1. Pyruvate systems will be the most favorable to sulfate-reducing bacterial growth as it is the most energetically favorable. The next most favorable electron donors will be lactate, formate, then finally acetate, as per the order of most energetically favorable to the least energetically favorable.
2. Vegetated areas will have greater overall populations of sulfate-reducing bacteria as there is likely to be greater organic carbon sources within the vegetated tailings, which would originate from decomposing vegetation. There is also likely more different types of available electron donors, and thus the sulfate-reducing bacteria are likely to, as a whole, be adapted to various sources.
3. There should not be any inherent difference in sulfate-reducing bacteria populations between those taken from Cu-Zn tailings and those taken from Au tailings. The type of tailings is unlikely to have any influence on the source of electron donors.

3 METHODOLOGY

3.1 Site Location

Samples were collected during the month of June, 2000, from four mine tailings sites in the vicinity of the Municipality of Timmins, Ontario, Canada (43°07'N, 80°20' W). Two Au tailings and two Cu-Zn tailings impoundments were chosen. The gold sites were the Hollinger (HO) and Delnite (DE) tailings, while the Cu-Zn sites were the Broulan (BR) and Potter (PO) tailings. All sites are old tailings deposits no longer in use by their respective mines.

The Hollinger tailings site is located just south of downtown Timmins. A light and sporadic vegetation cover exists on top of the tailings pile, and a large pond of water has accumulated in the center. The Delnite mine tailings site is located a few kilometers south-east of Timmins and is partially covered by vegetation. The Broulan tailings are located near Timmins, approximately 1 kilometer east of South Porcupine. The Broulan tailings are composed of old Au-tailings covered by a layer of Cu-Zn tailings (~ 30 cm thick). There was no apparent recent vegetation on those tailings, however grasses and small bushes were creeping in from the edges of the tailings mound. Near the center of the tailings, close to the remnants of an old tailing pump house, were small ponds of accumulated rainwater. The Potter tailings site is located 100 km east of Timmins on highway 101. The tailings were bare of vegetation, but there was on site abundant growth of naturally occurring (i.e. non-planted) cattails.

3.2 Sampling

At the Hollinger tailings, the samples were collected near the pond on top of the tailings. Sample HO-01 was collected about a dozen meters from the edge of the pond in an area with the least amount of vegetation, whereas HO-02 was collected near the edge of the pond with a definite presence of vegetation. At the Broulan site, sample BR-01 was taken near the ponds where the tailings were water-saturated, whereas sample BR-02 was taken closer to the edge of the tailings, in a grassy area. The DE-01 sample was taken in a grassy section of the tailings while DE-02 was taken in an area of the tailings without vegetation. Sample PO-01 was taken at the edge of the cattail region at the Potter site, whereas sample PO-02 was taken approximately 5 m away from both the cattail zone and on the edge of the mine tailings in a non-vegetated area. The general physical, chemical and microbial characteristics of the tailings samples are given in Table 2, whereas the chemical characteristics of the porewaters of each sample are shown in Table 3.

Table 2: Physical, chemical and microbial characteristics of the tailings samples (from the 1999 sampling season)

Sample	Depth (cm)	Vegetation	pH	Eh (mV)	Water content (%)	*organics (%)	SRB** (CFU/g dry wt. sed.)
BR-01	20	No	7.09	253	18.45	0	3.43×10^5
BR-02	5	Yes	3.83	251	25.29	2.05	9.24×10^3
DE-01	30	No	6.08	312	10.59	0	1.03×10^4
DE-02	30	Yes	6.51	354	5.02	0	1.47×10^4
HO-01	20	No	7.26	180	25.62	0	2.09×10^3
HO-02	30	Yes	7.05	197	26.78	0	9.93×10^4
PO-01	15	Yes	4.13	100	28.11	0	2.53×10^4
PO-02	25	No	5.29	178	23.01	0	2.60×10^3

*: based on loss on ignition (Fortin, 2001, personal communication)

** : based on bacterial enumeration (Fortin, 2001, personal communication)

Table 3: Chemical characteristics of the porewaters of the mine tailings samples* (from the 2000 sampling season)

Sample	[Fe] mM	[SO ₄ ²⁻] mM	DIC mM	DOC mM	Cu mM	Zn mM
BR-01-20 cm	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BR-02-5 cm	1.68 X 10 ⁻²	5.14	0.02	0.8	0	0
DE-01-30 cm	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DE-02-30 cm	0	21.10	1.43	1.19	0	0
HO-01-20 cm	0	1.64	2.81	3.96	0	0
HO-02-30 cm	3.67 X 10 ⁻²	27.40	n.d.	8.41	0	0
PO-01-15 cm	7.55	22.20	0.34	1.53	0.032	0.55
PO-02-25 cm	9.43	36.10	1.69	6.27	0	0

n.d. = not determined because of the lack of porewaters

*D. Fortin, 2001 (personal communication)

At each of the sampling sites, a small pit was dug down to a depth of approximately 50 cm. One of the edges of the pit was brushed clean of any debris created by digging the pit to present a clear and smooth facing. This enabled visible identification of color changes in the tailings, from bright orange and yellow near the surface to darker gray tailings at the bottom. The change in color roughly corresponded to the oxic/anoxic interface, as indicated by the *in situ* redox measurements (Fortin, 1999; Fortin et al., 2000; Fortin et al., 2002). The samples were collected just below the oxic/anoxic interface, where previous studies had indicated the largest populations of sulfate reducing bacteria (Fortin, 1999; Fortin et al., 2000; Fortin et al., 2002).

Using a spatula sterilized with ethanol just prior to sampling, two autoclaved vials were completely filled with the tailings. The first vial was immediately sealed to minimize oxygen diffusion and was used for the inoculation of the various systems. The second vial was used for *in situ* pH and Eh measurements. Both vials were placed in a cooler for

transportation back to the laboratory at the University of Ottawa.

3.3 Experimental Setup

Each tailings sample was used to inoculate 4 different growth media. A total of 32 biotic systems were prepared (see Table 3). Each system contained a different electron donor in the growth medium. A modified version of Postgate's Growth Medium G (Postgate, 1984) was used:

Distilled water	970 mL
Na ₂ SO ₄	3 g
NaCl	1.2 g
KCl	0.3 g
NH ₄ Cl	0.3 g
MgCl ₂ •6H ₂ O	0.4 g
KH ₂ •PO ₄	0.2 g
CaCl ₂ •2H ₂ O	0.15 g
electron donor	varied (see below)
trace metal solution	1 mL (see below)

The electron donors were pyruvate (pyruvic acid, 0.440 g/L), formate (Na-formate, 0.272 g/L), acetate (Na-acetate, 0.320 g/L) and lactate (60% Na-lactate, 0.59 mL/L). These four particular electron donors were chosen for this experiment because previous studies have shown that they are present within the mine tailings pore waters of the sample sites (Fortin et al., 2000). The final concentration of each electron donor was 0.4 mM in each system.

The trace metal solution contained: 6.5 mL/L HCl (25%), 1.5 g/L FeCl₂•4H₂O, 0.06 g/L H₃BO₃, 0.1 g/L MnCl₂•4H₂O, 0.12 g/L CoCl₂•6H₂O, 0.07 g/L ZnCl₂, 0.025 g/L NiCl₂•6H₂O, 0.015 g/L CuCl₂•2H₂O and 0.025 g/L Na₂MoO₄•2H₂O. The pH of the medium was then adjusted with NaOH and/or HCl to match the *in situ* pH of the mine tailings. The

various growth media were poured into erlenmeyer flasks for a final volume of 225 mL and autoclaved for 20 minutes. This resulted in a loss of 5 to 10 mL of growth medium in most of the flasks. The filter-sterilized vitamin solution (0.1 mL/L) was then added to each system shortly after the sterilization. The vitamin solutions contained 0.1 mg/L biotin, 0.5 mg/L p-Aminobenzoic Acid, 0.5 mg/L vitamin B₁₂, and finally a solution of 1mg/L of thiamine. A sterile solution (filtered through 0.22 um) of Fe (II) (as FeCl₂•4H₂O) was also added to the medium for a final concentration of 5 mg/L. Each system was then quickly inoculated with approximately 1 g of wet tailings on the bench and put back in the anaerobic chamber under N₂ atmosphere. Each flask was sealed with a rubber lid. The systems were taken out of the chamber and stored at 20°C in the dark for a period of 3-4 months.

Each biotic system was compared to a control system containing no tailings (Table 4). Their chemical composition was identical to the one of the biotic counterparts. Sub-samples were taken on a regular basis for chemical analysis. Control systems were only created to match the pH and electron donor of the actual systems used in this experiment; if a specific electron donor was not found at a certain pH in the actual systems of the experiment, then no control system was created for that electron donor at that particular pH level. Likewise, if more than one system of a specific electron donor had the same pH, only a single control system of that pH was required for the two (or more) systems.

3.3.1 Sub-Sampling

Shortly after inoculation, 10 mL of sample was taken out with a sterile syringe and needle (t = 0). Additional 10 mL samples were taken at progressively larger intervals during

the next three to four months. Those samples were used for sulfate-reducing bacteria enumeration (section 3.4) and for the analysis of pH, Eh, dissolved Fe, SO_4^{2-} , DOC, and HS^- (sections 3.5.1 and 3.5.2). The same sub-sampling protocol was used for the control systems.

Table 4: Physico-chemical characteristics of the various systems

System	Electron donor	Weight of wet tailings (g)	Initial pH	Initial Eh (mV)	Initial pH of control system*
BR-01	Acetate	1.1	6.63	-21	6.5
	Formate	1	6.74	-11	7
	Lactate	1	6.66	-27	6.5
	Pyruvate	1	6.74	-2	6.5
BR-02	Acetate	1.4	3.88	134	4
	Formate	1.5	4.26	47	4
	Lactate	1.3	4.22	-88	4
	Pyruvate	1	4.28	45	4
DE-01	Acetate	1.1	6.02	163	5.5
	Formate	1	6.46	42	6
	Lactate	1	5.95	184	6
	Pyruvate	1	6.02	177	6
DE-02	Acetate	1	5.41	297	5.5
	Formate	1.1	5.51	184	5.5
	Lactate	1	5.93	26	6
	Pyruvate	1	5.58	171	5.5
HO-01	Acetate	1.4	6.71	73	6.5
	Formate	1.2	6.68	164	7
	Lactate	1.1	6.81	174	7
	Pyruvate	1.2	7.22	-4	7
HO-02	Acetate	1.2	6.24	174	6.5
	Formate	1	6.33	164	6
	Lactate	1.2	6.22	162	6
	Pyruvate	1	6.26	160	6
PO-01	Acetate	1	4.13	-94	4
	Formate	1	4.16	47	4
	Lactate	1	4.16	52	4
	Pyruvate	1	4.27	64	4
PO-02	Acetate	1	5.98	-7	5.5
	Formate	1	5.47	189	5.5
	Lactate	1.1	5.85	5	6
	Pyruvate	1.5	6.16	1	6

*: control systems contained no tailings

3.4 SRB Enumeration (MPN)

The sub-samples (1 mL out of 10 mL) were used for bacterial enumeration. The sulfate-reducing bacteria populations were estimated with the Most Probable Number (MPN) dilution in liquid medium technique (American Public Health Association, 1965). This method involves probabilities and gives a relative magnitude of population, not an exact count. The liquid medium used for the MPN technique was a modified Postgate's Medium E (Postage, 1984). It contained:

Tryptone	10 g/l
MgSO ₄ •7H ₂ O	2 g/l
FeSO ₄ •7H ₂ O	0.5 g/l
Na ₂ SO ₃	0.5 g/l
Na-Lactate (60%)	5.9 mL/l
Pyruvic Acid	0.44 g/l
Na-Acetate	0.32 g/l
Na-Formate	0.27 g/l
adjusted to pH 7.5 using 2 M NaOH	

For the MPN, all 4 electron donors were used in the growth medium in order to not favor any given type of sulfate-reducing bacteria. Tryptone was also used in the growth medium to facilitate sulfate-reducing bacteria growth. The pH was adjusted to 7.5 even though the initial pH of some systems was acidic. This was done to maximize sulfate-reducing bacteria growth. 9 mL of medium was put into culture tubes and autoclaved for 20 minutes. The growth medium was used in conjunction with a reducing agent supplement (RAS) containing 7.5 g/L of ascorbic acid and 7.5 mL/L of thioglycolic acid. The pH was adjusted to 7.5 and autoclaved for 20 minutes. 1 mL of RAS was added to each tube prior to the inoculation.

The sub-samples were submitted to dilution series, starting with the 1 mL of the

sample in 9 mL of reduced water (100 mL/l RAS, 2.1 g/l NaCl and adjusted to pH 7.5 using 2 M NaOH). Between each dilution, the tubes were vortexed to assure a proper dispersion of the bacteria. Each dilution set was used to inoculate 5 tubes containing 9 mL of growth medium and 1 mL RAS. Once inoculated, the MPN tubes were placed in the dark at room temperature (20°C). After two weeks, the MPN tubes were counted to determine how many tubes were positive at each dilution. Tubes showing a dark gray to black precipitate at the bottom were considered positive. The black precipitate was an iron sulfide resulting from the reduction of sulfate. The number of positive tubes in each dilution after two weeks was then referred to Mendel's probability table to determine the Most Probable Number (MPN) of sulfate-reducing bacteria present.

3.5 Chemical Analyses

3.5.1 pH and Eh Measurements

The sub-samples (2 mL out of 10 mL) were analyzed for pH and Eh with a VWR meter. The pH measurements were taken with a VWR electrode calibrated at pH 4 and 7, whereas the redox measurements were performed with an Orion redox electrode. The redox probe was tested with ZoBell's solution (Nordstrom, 1977) and the measurement was corrected with respect to the hydrogen reference electrode (+ 199 mV).

3.5.2 Iron, Sulfate, and DOC Measurements

The remaining sub-samples (7 mL) were filtered through a 0.22 μm filter (Gelman) and acidified with 7 μL of 10% HNO_3 . Samples were refrigerated at 4°C until analysis. Iron

concentrations were measured by flame atomic absorption spectroscopy (Varian Spectra AA-250 plus) using appropriate standards. Sulfate concentrations were determined with the barium turbidity method (see paper Fortin et al., 1996). The absorbance at 650 nm was read with a spectrophotometer (Beckman Du-65plate Spectrophotometer). Dissolved organic carbon (DOC) was analyzed with an O-I Analytical model 1010. All analyses were performed in duplicate.

3.5.3 Thermodynamics Calculations

The geochemistry of the abiotic systems was interpreted with the aid of the geochemical code MINEQL+ 3.1 (Schecher and McAvoy, 1994). We used the corrected thermodynamics database of Fortin and Gauthier (1995). Additional solubility data were added to the database for schwertmannite, according to the values reported by Bigham *et al.* (1996).

3.6 X-ray Diffraction

The original fresh tailings and the tailings present in the systems after the end of the experiments (only from the Delnite and Potter systems) were freeze-dried and then suspended in isopropanol, ground with a mortar and dropped on a XRD spinning holder. Once the isopropanol had evaporated, XRD measurements were performed for 1 hour with a Philips X'Pert diffractometer, using a Cu source operating at 45 kV and 40 mA, and a Kevex Si(Li) solid-state detector. All samples were run in a step-scan mode for the angle 2θ , at $0.02^\circ/1s$ from 4° to 90° .

3.7 Transmission Electron Microscopy (TEM)

Tailings from each system were collected at the end of the experiment and analyzed by TEM, along with the original fresh tailings. The samples were gelled in agar, dehydrated for 15min in a series of ethanol solutions (25 %, 50%, 75%, 95% and 99% three times), infiltrated with a 1:1 ratio of 100% ethanol/TAAB solution for 1 hour and twice with a 100% TAAB solution. The samples were finally embedded with a TAAB resin (Marivac, Halifax NS, Canada) and polymerized at 60°C overnight. Ultra-thin-sections (60-80 nm) were obtained with an ultramicrotome (Reichert Ultracut E.) and some were stained with uranyl acetate and lead citrate. Ultra-thin sections were observed with a JEOL JEM 1200 EX TEMSCAN scanning transmission electron microscope (STEM) operated at 100 kV in the transmission mode at McMaster University. Some unstained sections were analyzed X-Ray Energy Dispersive Spectroscopy (EDS), using the same STEM instrument equipped with a Tracor Northern X-ray detector and EDS2000 microanalysis software (IXRF Systems Inc., Houston, TX).

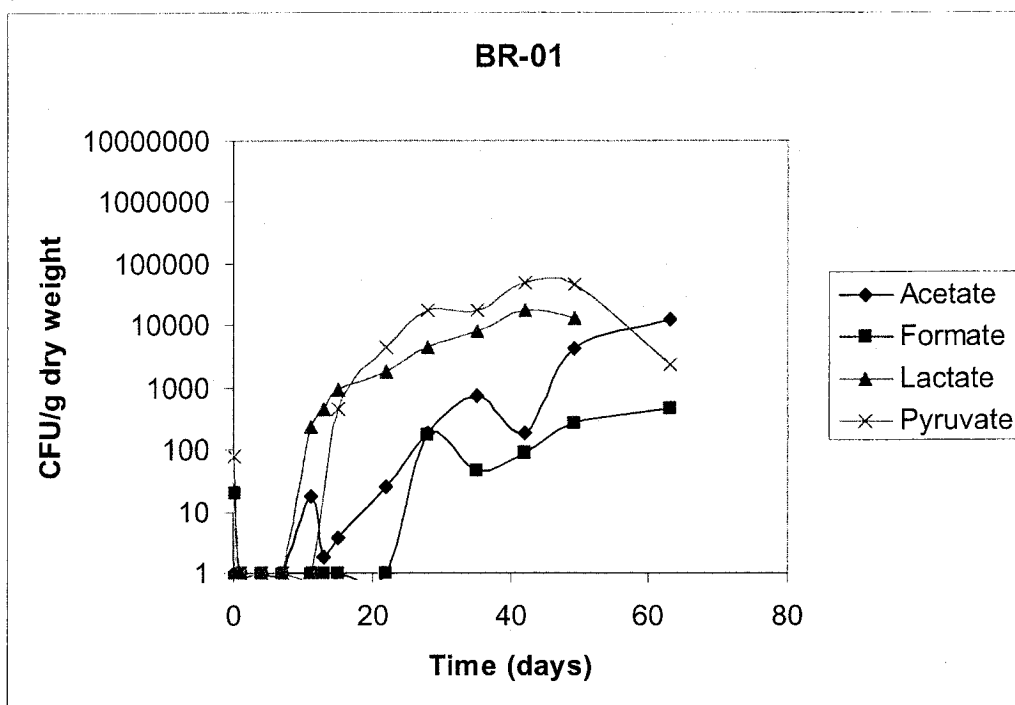
4 RESULTS

4.1 SRB Growth Over Time

4.1.1 Broulan Tailings

The growth of sulfate-reducing bacteria populations from the non-vegetated and vegetated Broulan mine tailings (BR-01 and BR-02) in the presence of various electron donors is shown in Figure 4-1. In the non-vegetated BR-01 site (Fig. 4-1a), the initial populations in all the systems showed a sharp decline shortly after inoculation, but they all increased in number after 10 days. In the acetate system, the initial lag period was followed by a slow-but-steady growth over time, whereas in the lactate and pyruvate systems, sulfate-reducing bacteria populations increased rapidly after the first week and leveled off after 24 days. Populations started to decline in those systems, especially in the pyruvate system, at the end of the experiments, i.e., at 60 days. In the formate system, sulfate-reducing bacteria populations remained lower than in the other systems and grew slowly after a 3-week lag period. In the vegetated BR-02 site (Fig. 4-1 b), the initial sulfate-reducing bacteria populations were extremely low at time 0, with the exception of the pyruvate system. In the acetate system, sulfate-reducing bacteria populations remained almost non-detectable over the entire course of the experiment, whereas the populations in the lactate and formate systems increased after 60 days. A population increase was also observed in the lactate system after 10 days. Sulfate-reducing bacteria growth was only apparent in the pyruvate system where populations increased rapidly after 2 weeks and then slightly declined over time.

a)



b)

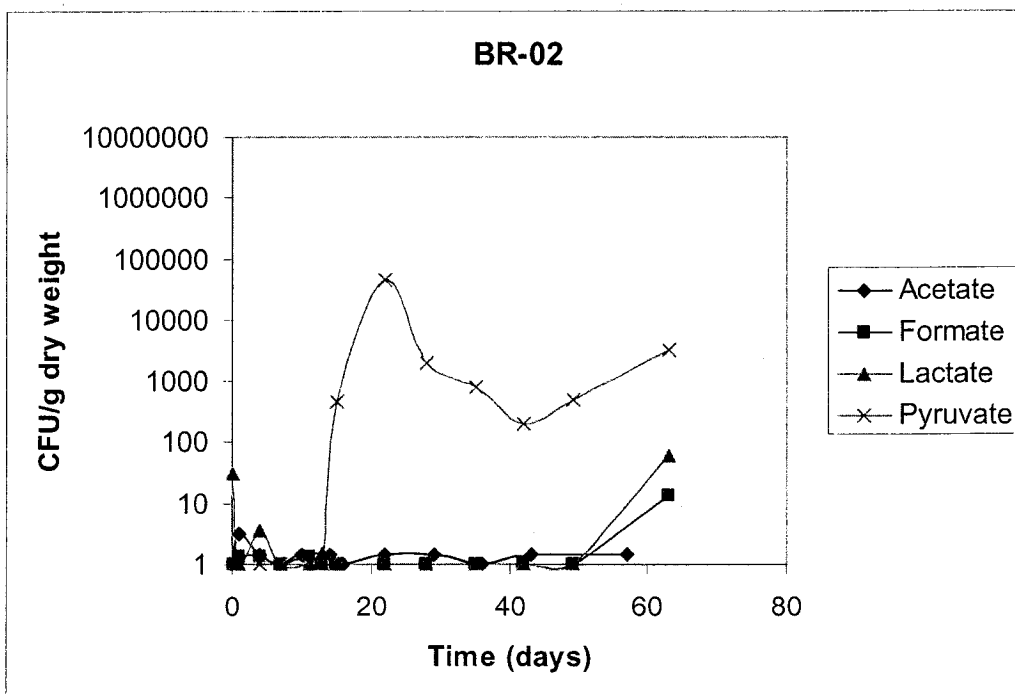


Figure 4-1 SRB populations as a function of time in the various systems for the Broulan tailings.

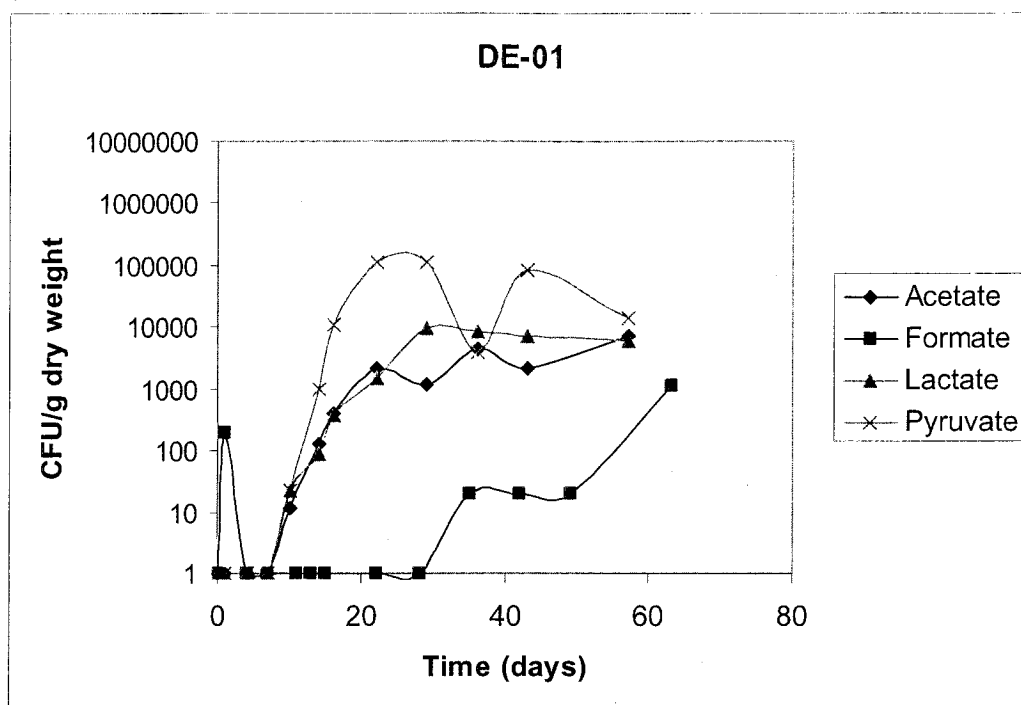
4.1.2. Delnite Tailings

The results for the sulfate-reducing bacteria populations present in the Delnite tailings are found in Figure 4-2 a and b. In the non-vegetated site (DE-01), the initial sulfate-reducing bacteria populations in the formate system dropped in the first few days and remained at near-zero levels until about day 10, where there was a slow increase (Fig. 4-2 a). In the acetate, pyruvate and lactate systems, sulfate-reducing bacteria populations were extremely low for the first week, but then increased rapidly around 20 days and leveled off over time. In the systems containing the vegetated DE-02 tailings (Fig. 4-2 b), sulfate-reducing bacteria populations remained very low in all systems in the first 20 to 30 days of the experiments. In the acetate, lactate and pyruvate systems, populations increased rapidly after 20 to 30 days and slightly fluctuated overtime, whereas in the formate system, sulfate-reducing bacteria slightly increased near the end of the experiment, but then appeared to die off.

4.1.3 Hollinger Tailings

The growth of sulfate-reducing bacteria in the various systems for the vegetated and non-vegetated Hollinger tailings are shown in Figure 4-3 a and b. For the non-vegetated HO-01 tailings (Fig. 4-3 a), the initial sulfate-reducing bacteria populations were very low, with the exception of the pyruvate system. Sulfate-reducing bacteria growth occurred rapidly in all systems and the populations leveled off after 10 days in the pyruvate, acetate and lactate systems and after 20 days, in the formate system. In that system, sulfate-reducing bacteria populations were lower than in all the other system. Sulfate-reducing bacteria growth for the vegetated HO-02 tailings is shown on Figure 4-3 b. In all systems, sulfate-reducing bacteria

a)



b)

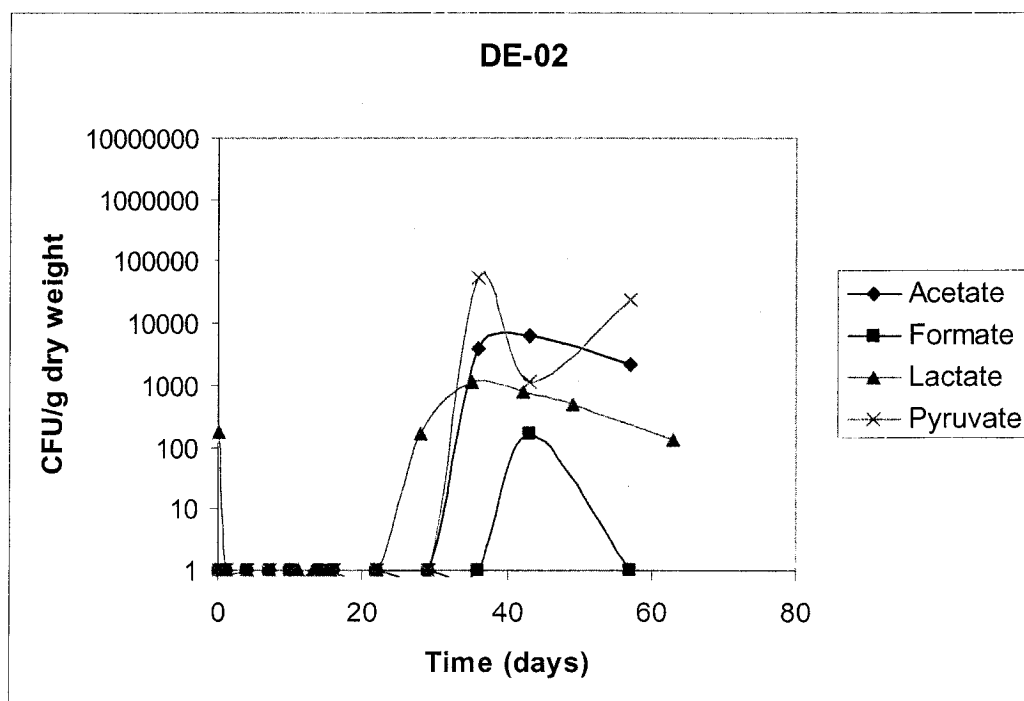
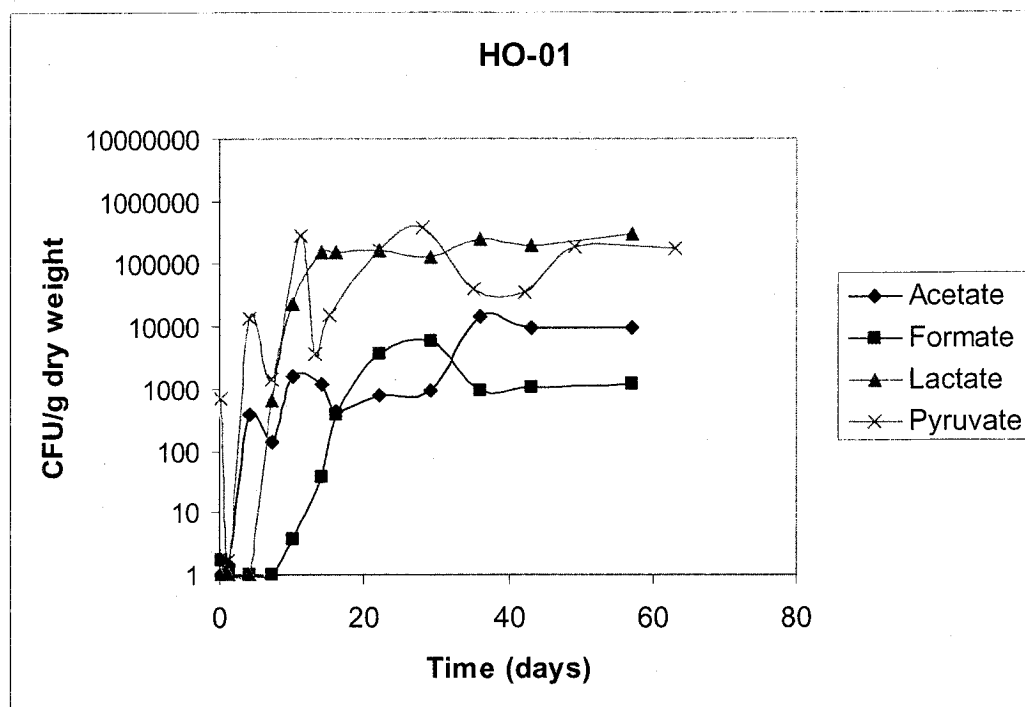


Figure 4-2 SRB populations as a function of time in the various systems for the Delnrite tailings.

a)



b)

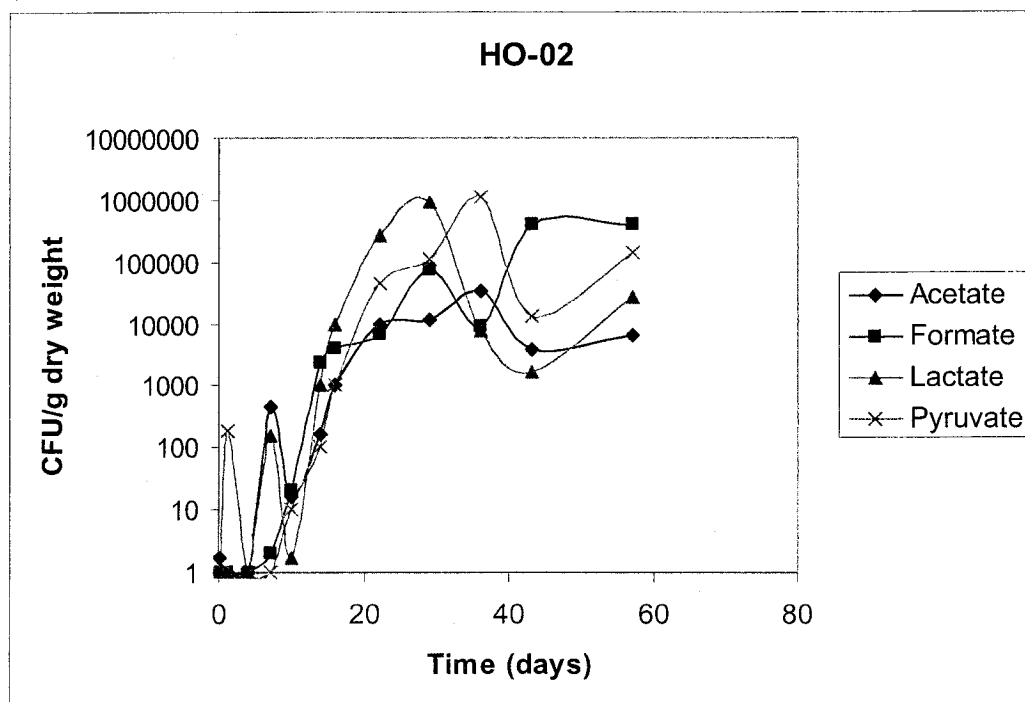


Figure 4-3 SRB populations as a function of time in the various systems for the Hollinger tailings.

populations remained low for the first 10 days, but then increased rapidly over a short period of time. In all systems, the populations slightly fluctuated over time, but the cells did not die off, with the exception of the lactate system, where sulfate-reducing bacteria decreased in number near the end of the experiment.

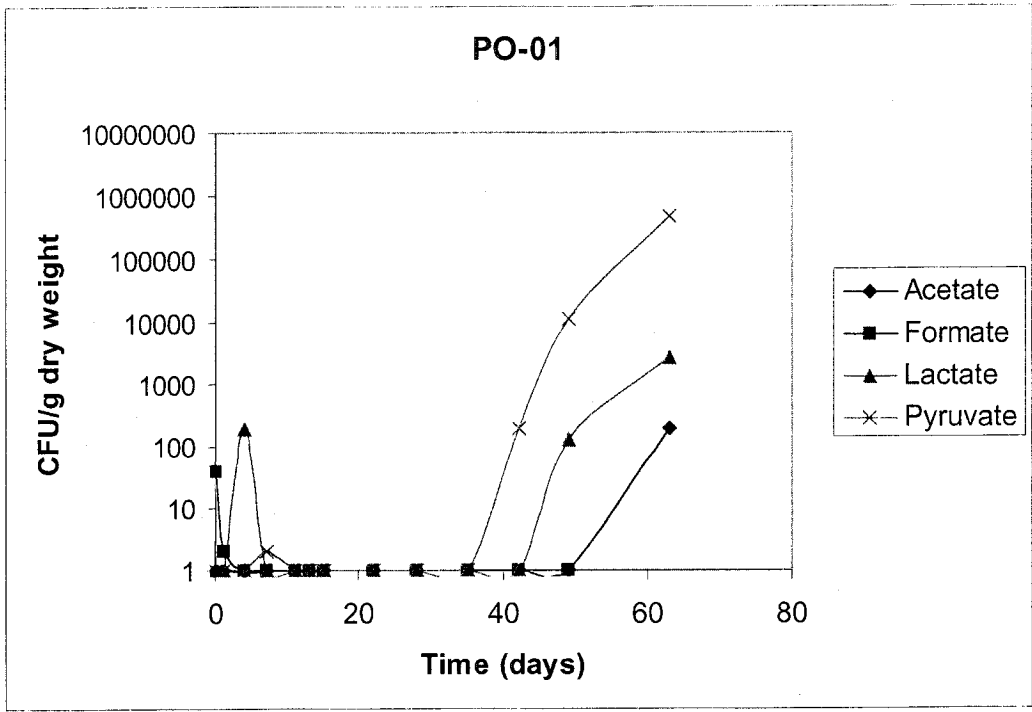
4.1.4 Potter Tailings

The results for the sulfate-reducing bacteria populations from the Potter tailings are shown on Figure 4-4 a and b. Sulfate-reducing bacteria populations were almost not detectable in the first 40 days of the experiment, with the exception of the formate and lactate systems where sulfate-reducing bacteria were present at the beginning (Fig. 4-4 a). In the formate system, sulfate-reducing bacteria did not grow at all, whereas populations slightly increased after 60 days in the acetate system. In the lactate and pyruvate systems, sulfate-reducing bacteria populations increased rapidly around 40 days. For the vegetated PO-02 tailings, sulfate-reducing bacteria populations increased slowly but steadily over time in all systems (Fig. 4-4 b). Populations leveled off near the end of the experiment in the acetate and pyruvate systems, whereas they slightly decreased in the formate and lactate systems.

4.1.5 Abiotic Control Systems

The abiotic control systems were not inoculated, and thus no bacterial enumeration were performed. Unlike all the systems inoculated with tailings, there were never any visible traces of black iron monosulfide precipitates in any of abiotic control systems throughout

a)



b)

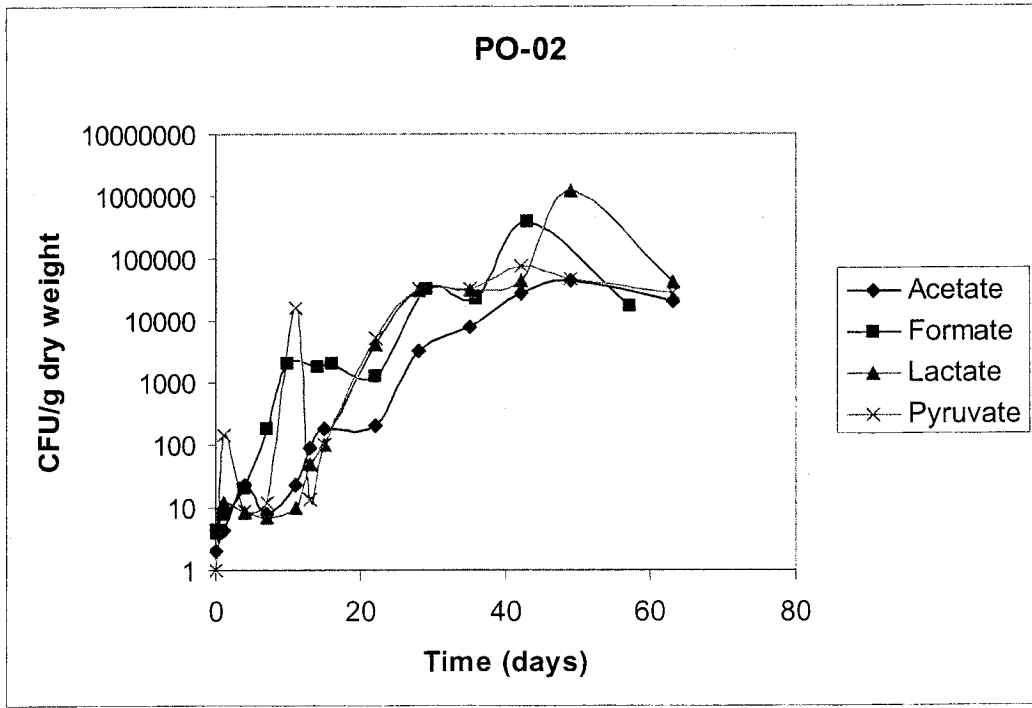


Figure 4-4 SRB populations as a function of time in the various systems for the Potter tailings.

the entire experiment.

It should be noted that a moss-like biofilm was observed in the pH 4 lactate control system on day 102.

4.2 pH and Eh trends

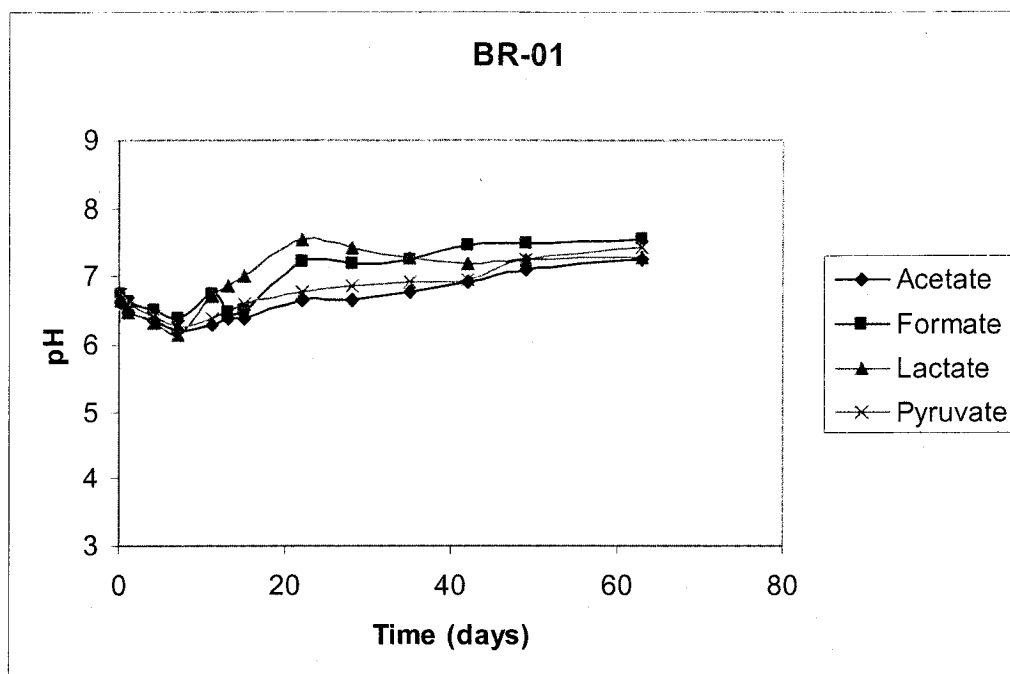
4.2.1 pH

The pH of all systems was measured at regular intervals over a period of 60 days. The initial pH of each system corresponded to the *in situ* pH of the tailings.

For the Broulan tailings, the initial pH was set near neutrality for the BR-01 site and near 4.0, for the BR-02 site (Fig. 4-5). In BR-01, the pH slowly increased over time for all the systems (Fig. 4-5 a), whereas for the more acidic BR-02 system, a sharp pH increase was observed in all systems, especially in the pyruvate system (Fig. 4-5 b). In that system, the pH went from 4 at day 10 to 7 by day 20. In the acetate, formate and lactate systems, the pH increased from 4 to 5, 5.5 and 6.5 (respectively) by the end of the experiment.

For the Delnrite tailings, the pH of the DE-01 systems remained near neutrality but slightly increased over time (Fig. 4-6 a). The pH of the formate and pyruvate systems was slightly higher at the end of the experiment than the pH of the lactate and acetate systems. In the DE-02 systems (Fig. 4-6 b), the initial pH was set 5.5 and it increased to 7 after 2 weeks in all systems. The pH leveled off for the remainder of the experiment.

a)



b)

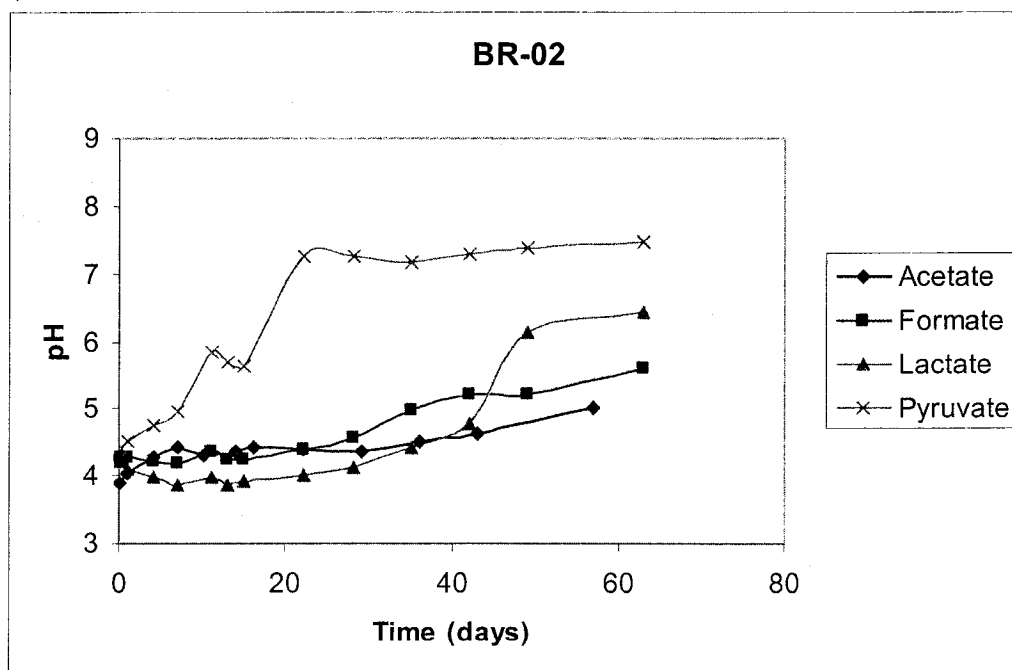
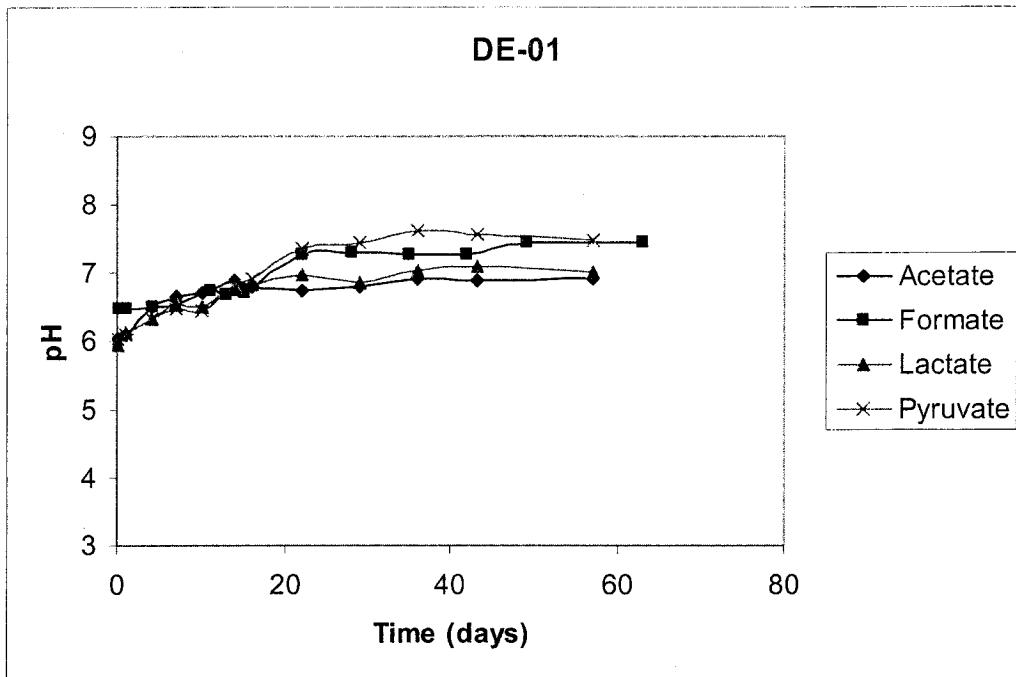


Figure 4-5 pH evolution of the various systems containing the Broulan tailings.

a)



b)

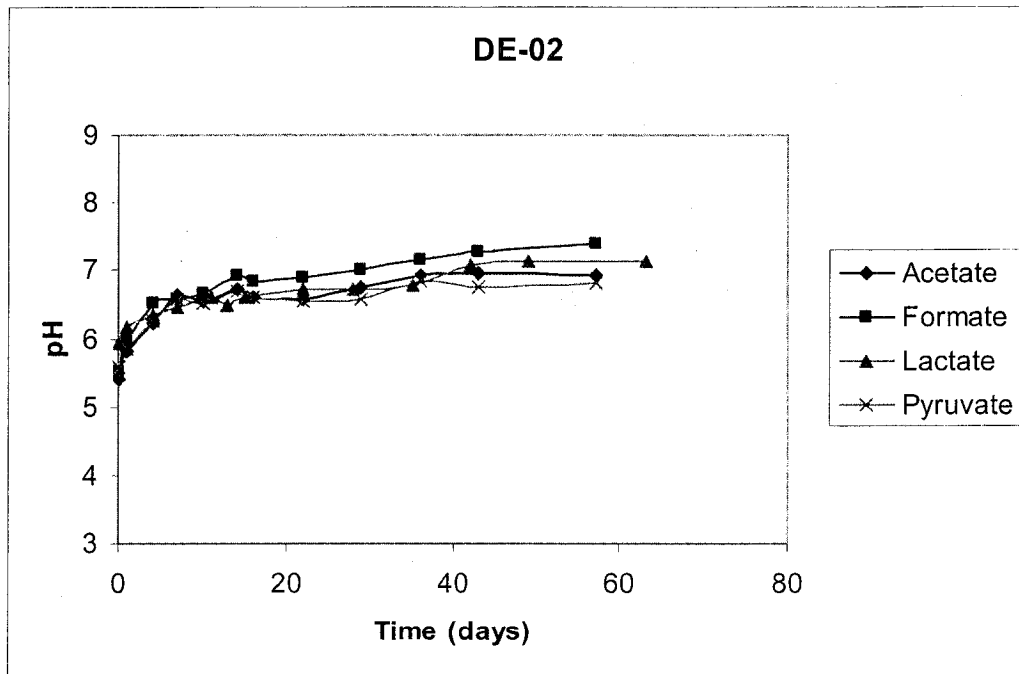


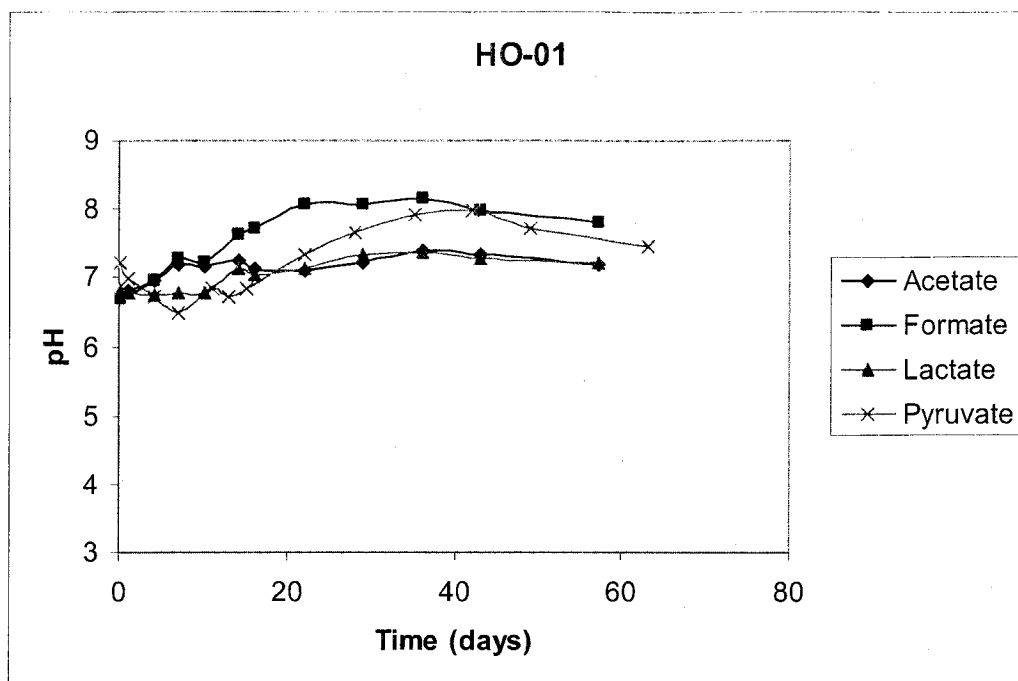
Figure 4-6 pH evolution of the various systems containing the Delnrite tailings.

In the HO-01 systems (Fig. 4-7 a), the pH of the acetate and lactate systems remained constant around 7 throughout the entire 60 days. The pyruvate and formate systems both increased from pH 7 to pH 8 by day 40, then slowly dropped back down to pH 7.5 by day 60. In the HO-02 systems, the pH increased from 6 to 7 and 7.5 for the acetate and formate systems, respectively, whereas the pH increased to 8 by day 20 for the acetate and lactate systems.

In PO-01 systems, the pyruvate system showed a slow but constant pH increase from 4 to 7 over 40 days (Fig. 4-8 a). It slightly dropped off by the end of the experiment. In the formate system, the pH remained at 4 until about day 20, and then started to slowly increase, attaining pH 5 by day 30. The pH of the lactate system started increasing at day 20, rising from pH 4 to pH 6.5 by day 40, where it then stabilized (Fig. 4-8 a). In the acetate system, the pH remained stable for the first 20 days, and then slowly increased to pH 6 by the end of the experiment. In the PO-02 systems (Fig. 4-8 b), the initial pH was set near 5.5 and slowly increased to pH 7 by day 40. The pH then remained constant in all the systems until day 60.

The pH in almost all of the abiotic control systems remained relatively constant throughout the entire experiment (Figures 4-9 to 4-11), showing only a very slight increase for the initial pH 4 and pH 5.5 systems, or a very slight decrease for the initial pH 6, pH 6.5, and pH 7 systems. The pH 4 pyruvate system (Figure 4-9 a) is the only exception; it started with a pH slightly over 4 and increased to just over pH 6 by day 60, at which point it stabilized.

a)



b)

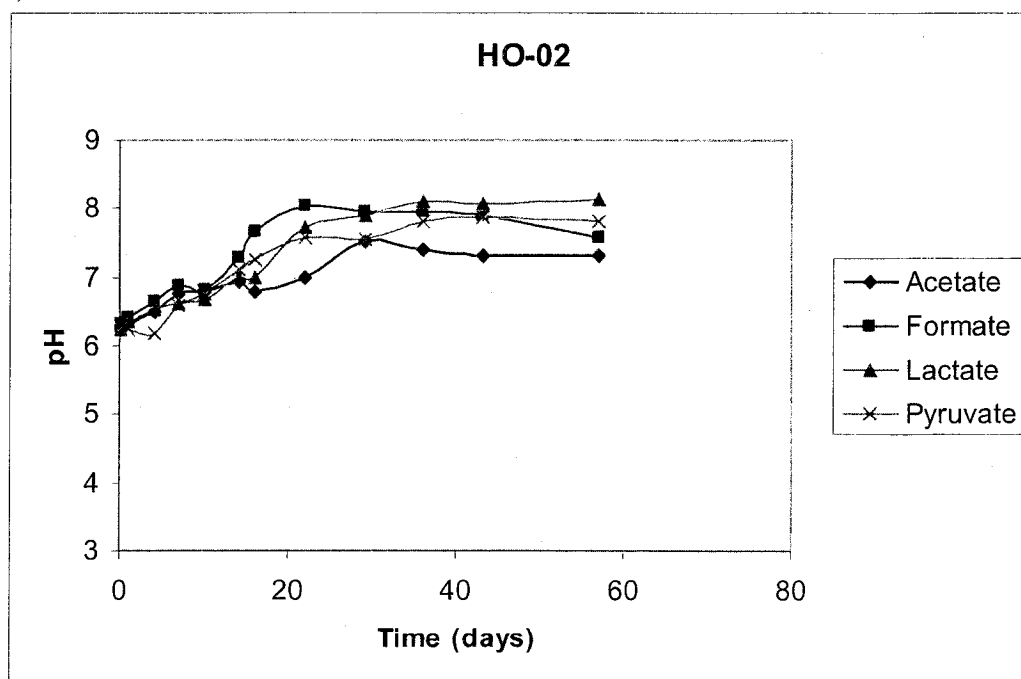
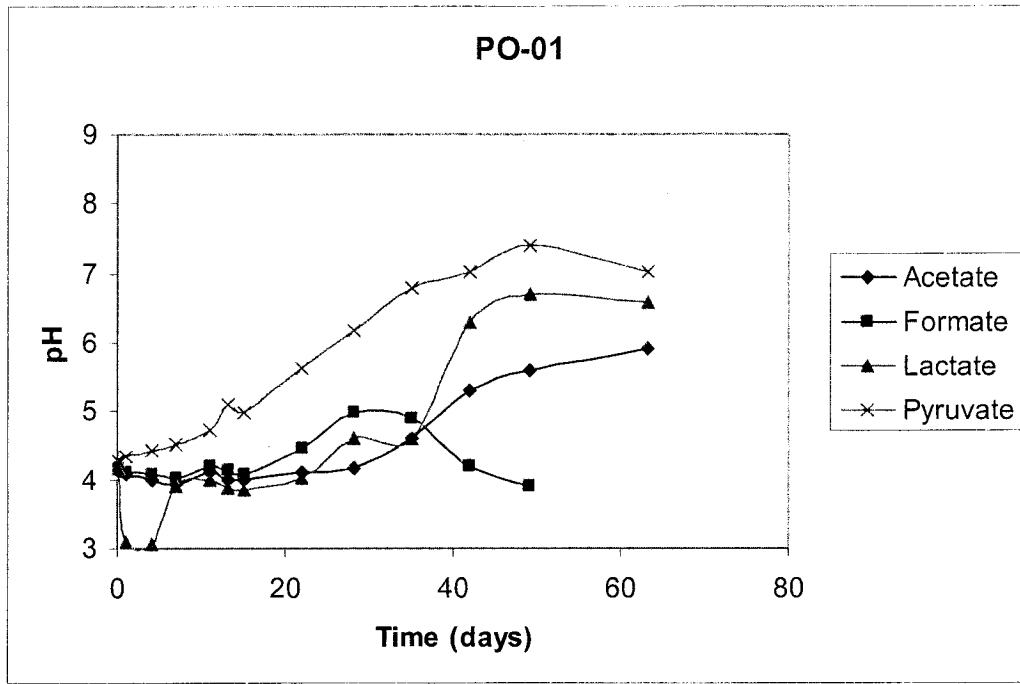


Figure 4-7 pH evolution of the various systems containing the Hollinger tailings.

a)



b)

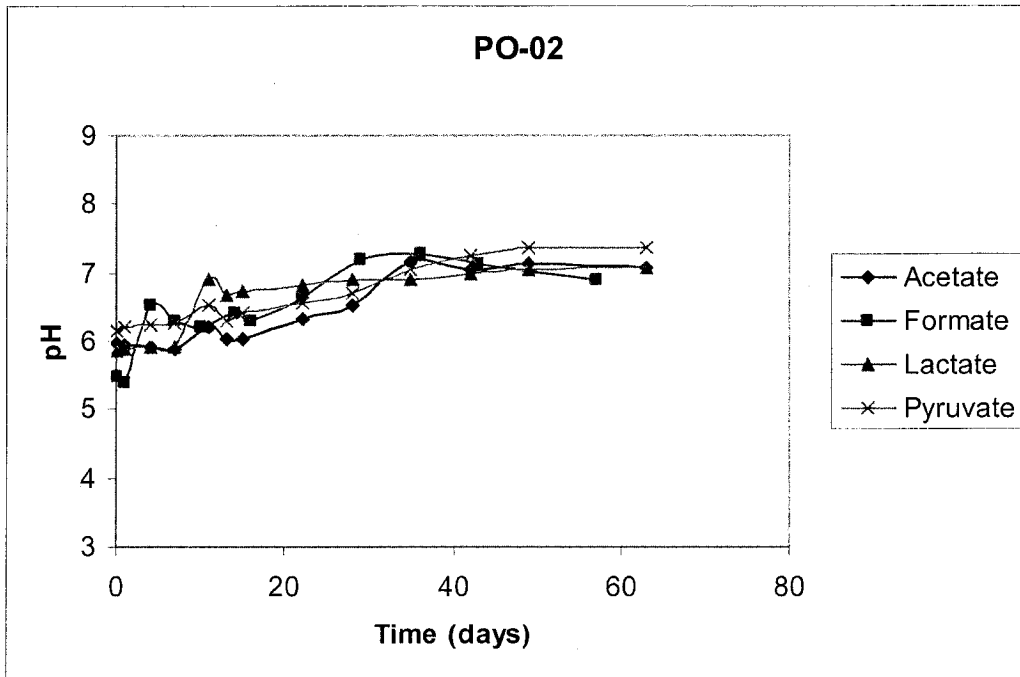
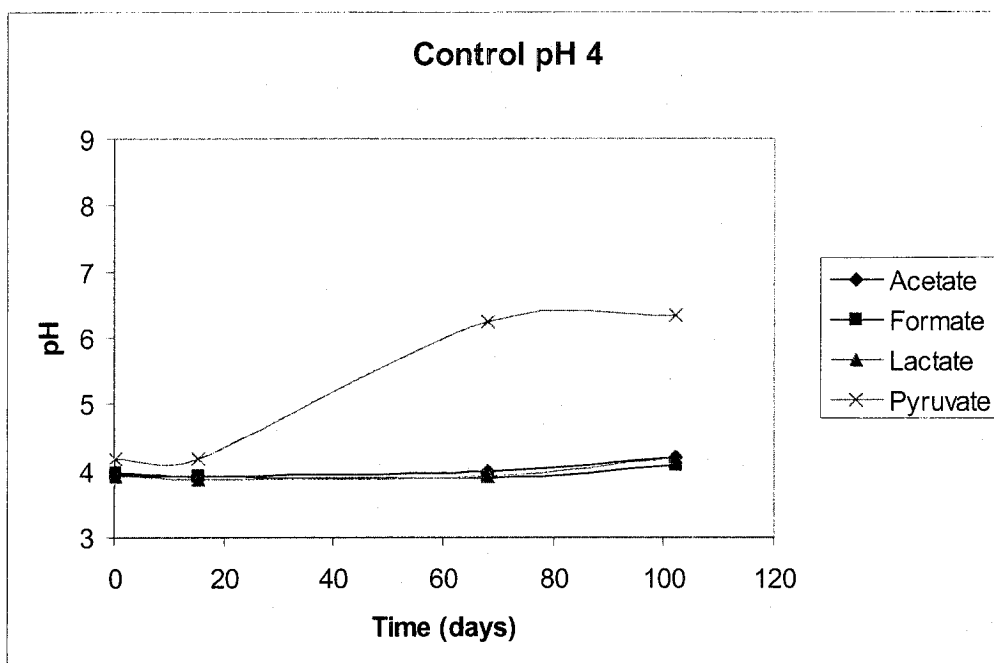


Figure 4-8 pH evolution of the various systems containing the Potter tailings.

a)



b)

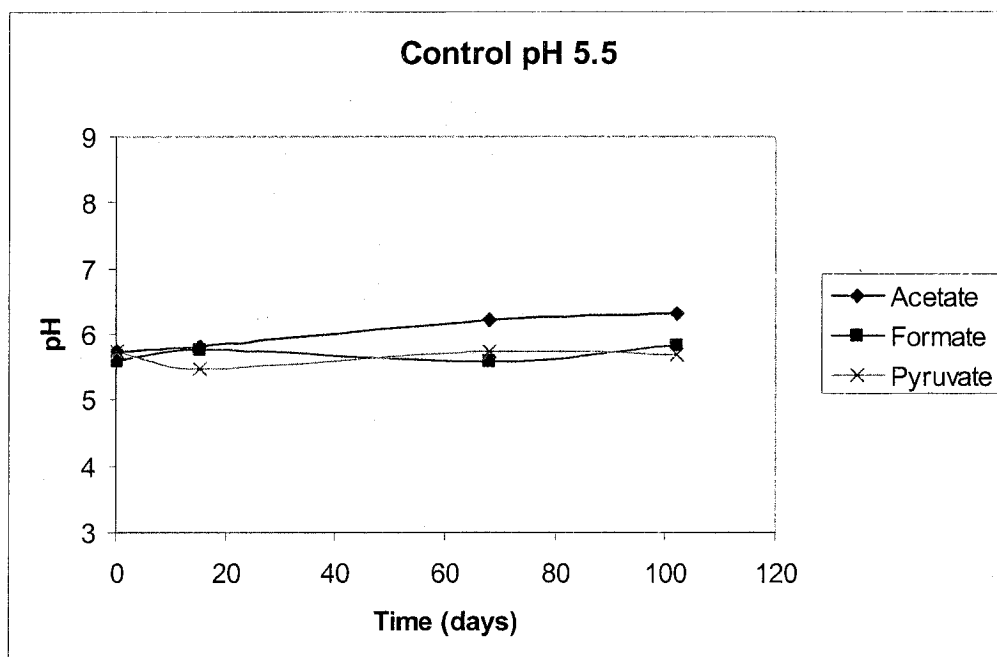
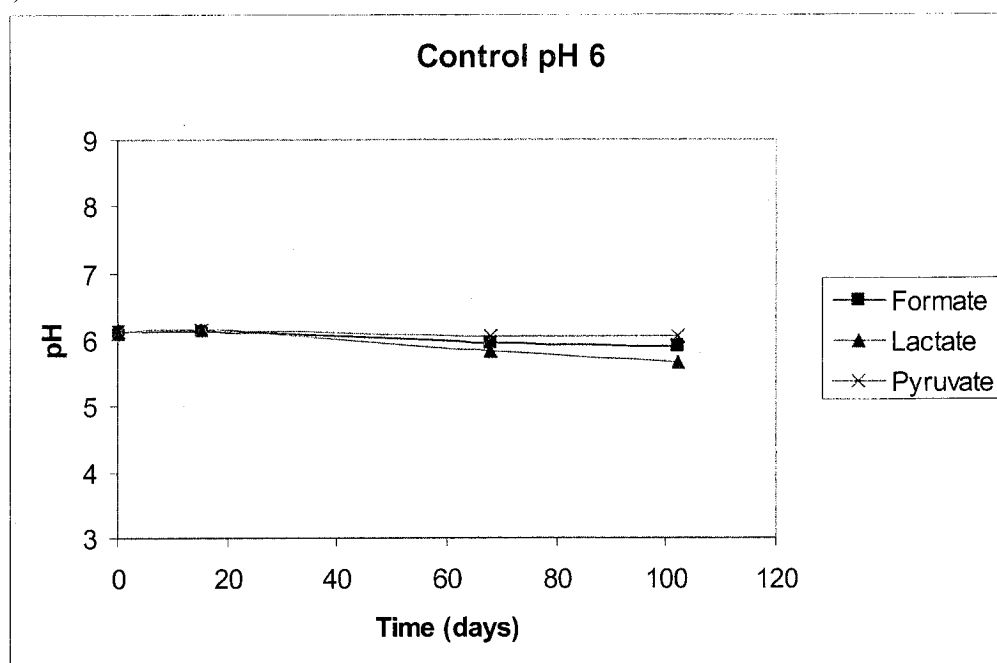


Figure 4-9 pH evolution of the abiotic control systems with an initial pH of a) 4 and b) 5.5.

a)



b)

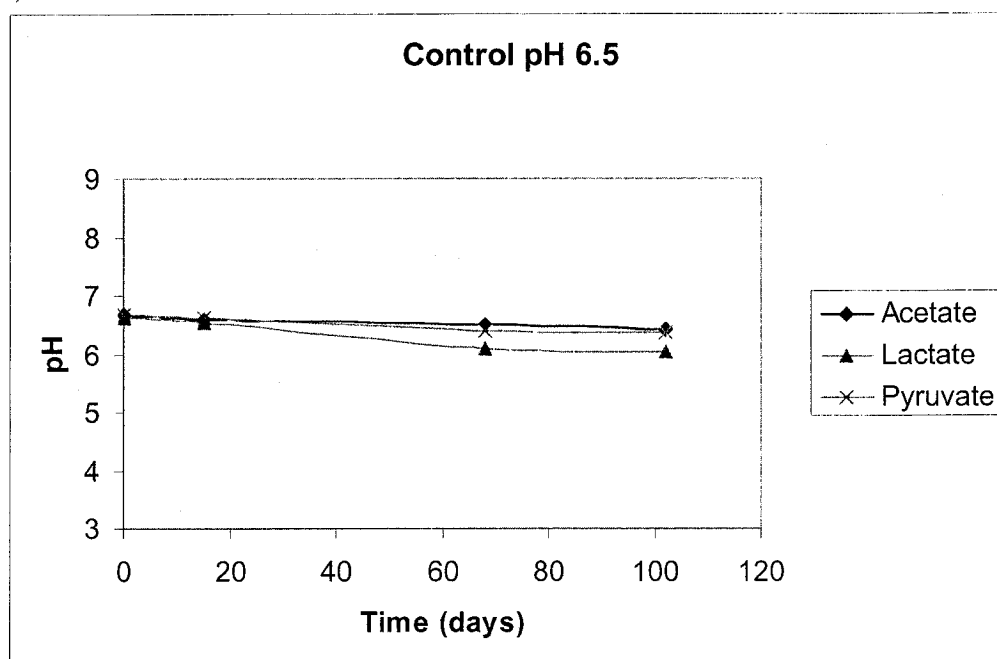


Figure 4-10 pH evolution of the abiotic control systems with an initial pH of a) 6 and b) 6.5.

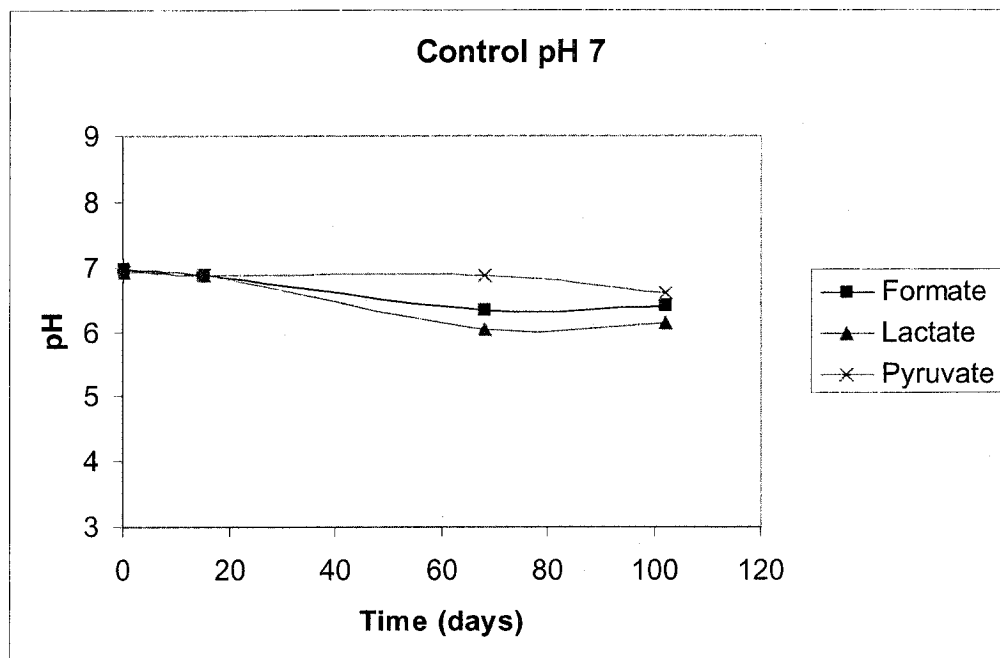


Figure 4-11 pH evolution of the abiotic control system with an initial pH of 7.

4.2.2 Eh

The Eh measurements were made at regular intervals throughout the course of the experiment with 2 different probes, because the first one started to show an erratic behavior after 20 days. As a result, it is suspected that the Eh trends are not reliable, and thus they are not presented here in the results. The data collected from the Eh measurements are presented in Appendix A. We can only report that all the systems remained anoxic throughout the course of the experiment.

4.3 Dissolved Sulfate

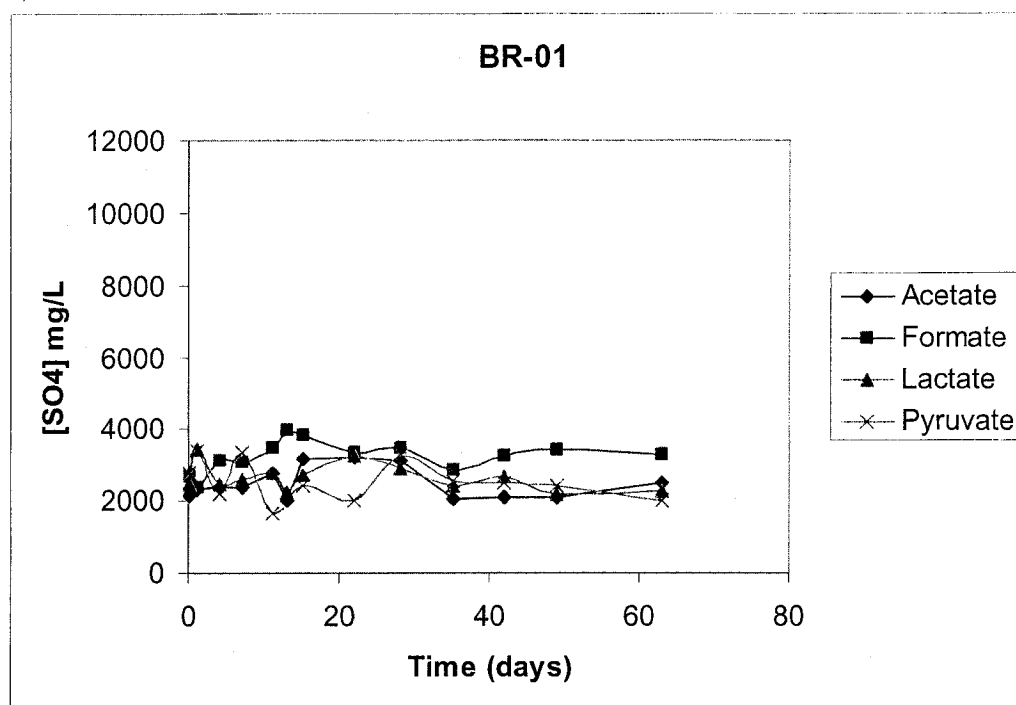
4.3.1 Broulan Tailings

The concentration of sulfate in the systems containing the Broulan tailings is shown in Figure 4-12. In the BR-01 and BR-02 systems, the sulfate levels remained relatively stable, fluctuating slightly between 2000 and 3000 mg/L throughout the course of the experiment.

4.3.2 Delnite Tailings

The SO_4 concentrations of the Delnite systems are shown in Figure 4-13. It is important to mention that the initial concentration of sulfate in all systems is greater than the concentration of the growth medium, which was around 3000 mg/L. This indicates that the wet tailings were a source of dissolved sulfate. In all the DE-01 systems, SO_4 concentrations slowly declined over time (Fig. 4-13 a). In the acetate system, SO_4 levels dropped from 10,000 to 8000 mg/L over 60 days. In the formate and pyruvate systems, the initial

a)



b)

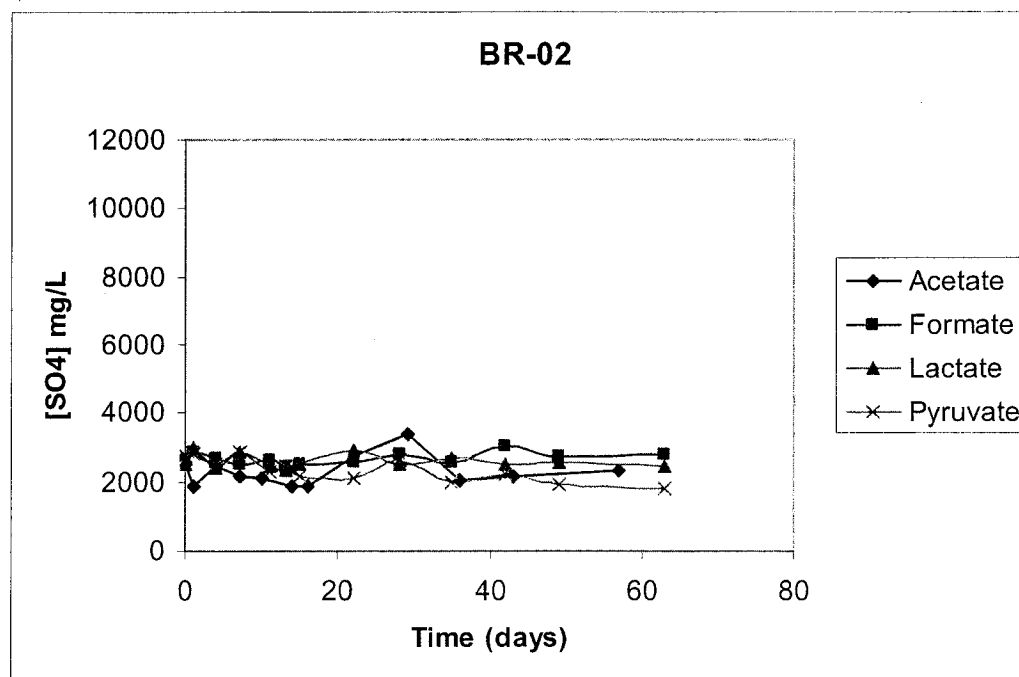
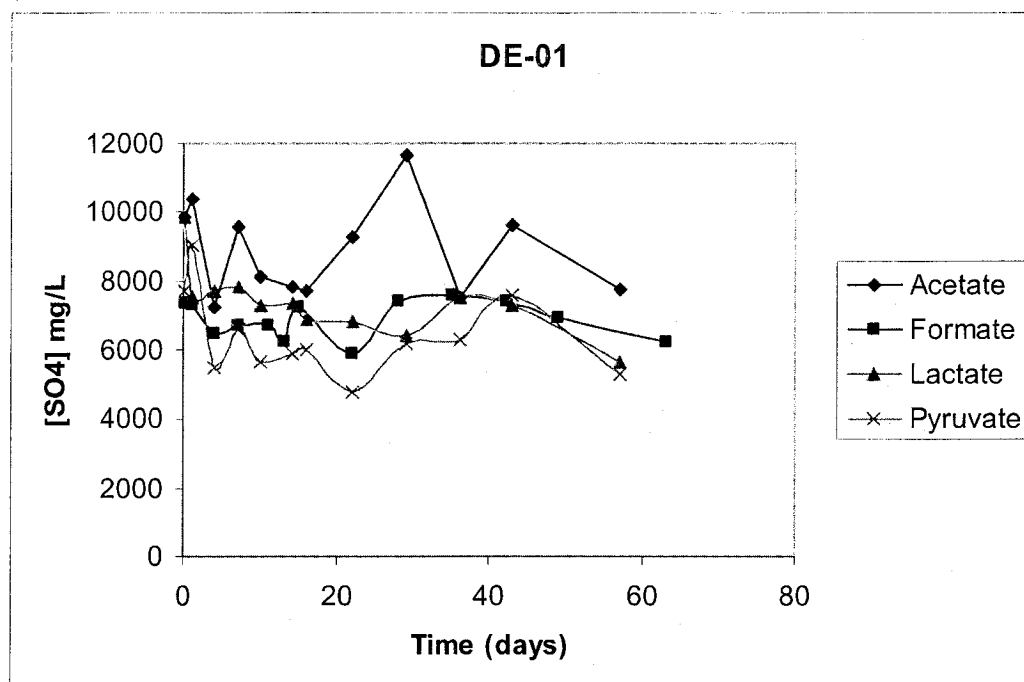


Figure 4-12 Dissolved SO₄ (mg/L) concentrations in the various systems as a function of time for the Broulan tailings.

a)



b)

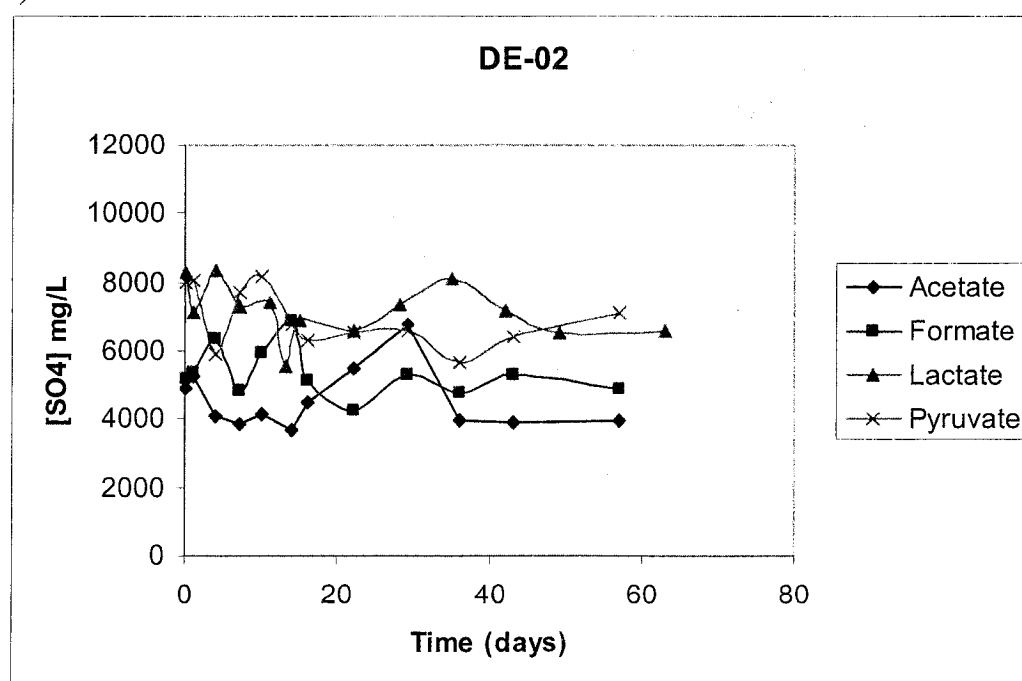


Figure 4-13 Dissolved SO₄ (mg/L) concentrations in the various systems as a function of time for the Delnite tailings.

concentration was around 8000 mg/L, but it decreased to 6000 mg/L by day 60. The lactate system showed a sharper decrease, dropping from 10,000 to 6000 mg/L over the course of the experiment. In the DE-02 systems, a decline in SO_4 was also observed, but at a much lower rate than in the DE-01 systems (Fig. 4-13 b). In the acetate, formate and pyruvate systems, the sulfate concentrations declined by about 1000 mg/L over 60 days, whereas in the lactate system, the concentration dropped by 2000 mg/L by day 60.

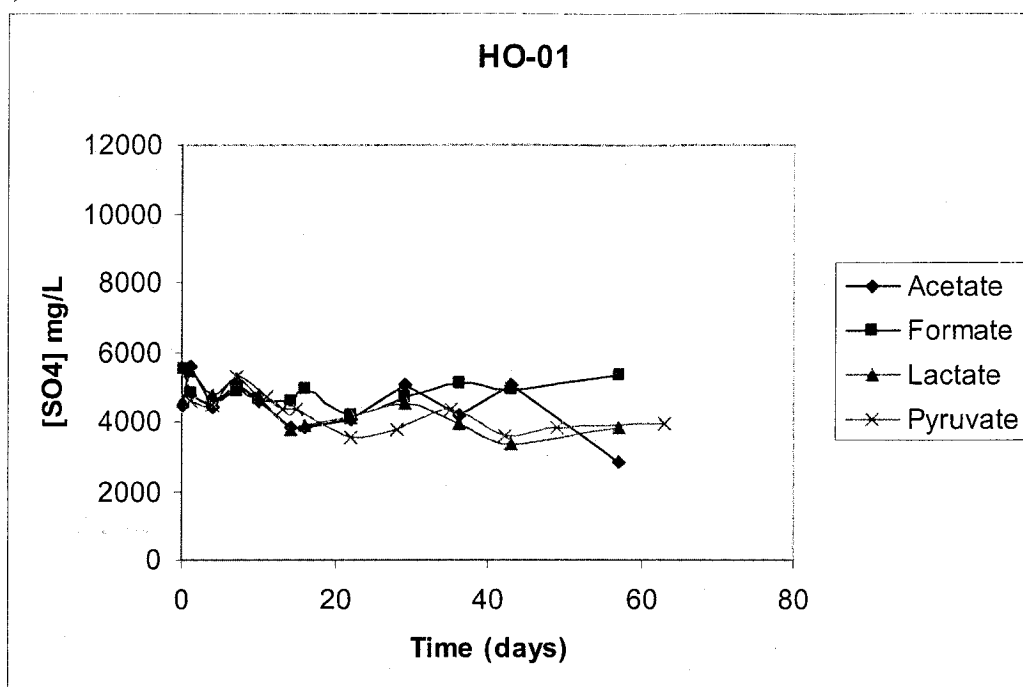
4.3.3 Hollinger Tailings

In the systems containing the Hollinger tailings, the initial sulfate concentration was also higher than the one of the growth medium. All the Hollinger systems showed a decline in SO_4 over time (Fig. 4-14). In the HO-01 systems, SO_4 decreased from 5000 to 4000 mg/L by day 60 (Fig. 4-14 a), whereas in the HO-02 systems, sulfate levels dropped from 5000 to 3000 mg/L over the 60 days, with the exception of the acetate system. In that system, the initial sulfate concentration was lower than in the other system. It fluctuated slightly throughout the course of the experiment (Fig. 4-14 b).

4.3.4 Potter Tailings

The SO_4 concentrations of the systems containing the Potter tailings are shown in Figure 4-15. In the PO-01 systems (Fig. 4-15 a), the formate, lactate and pyruvate systems all showed a sharp decline in sulfate concentration, even though the initial concentration was different in each system. The initial concentration was also generally greater than the one in

a)



b)

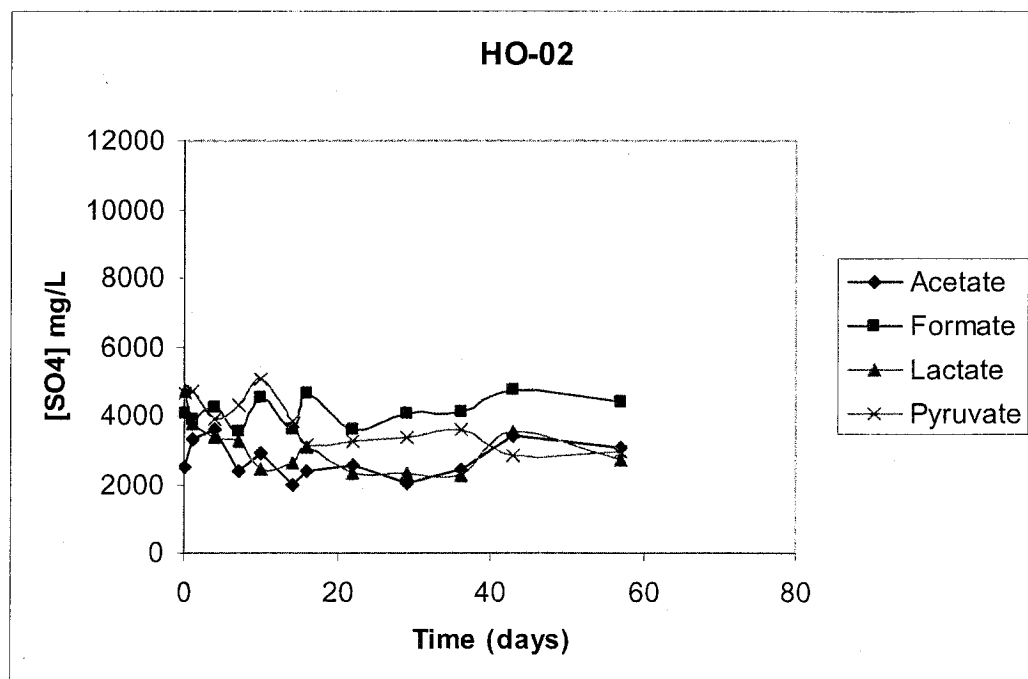
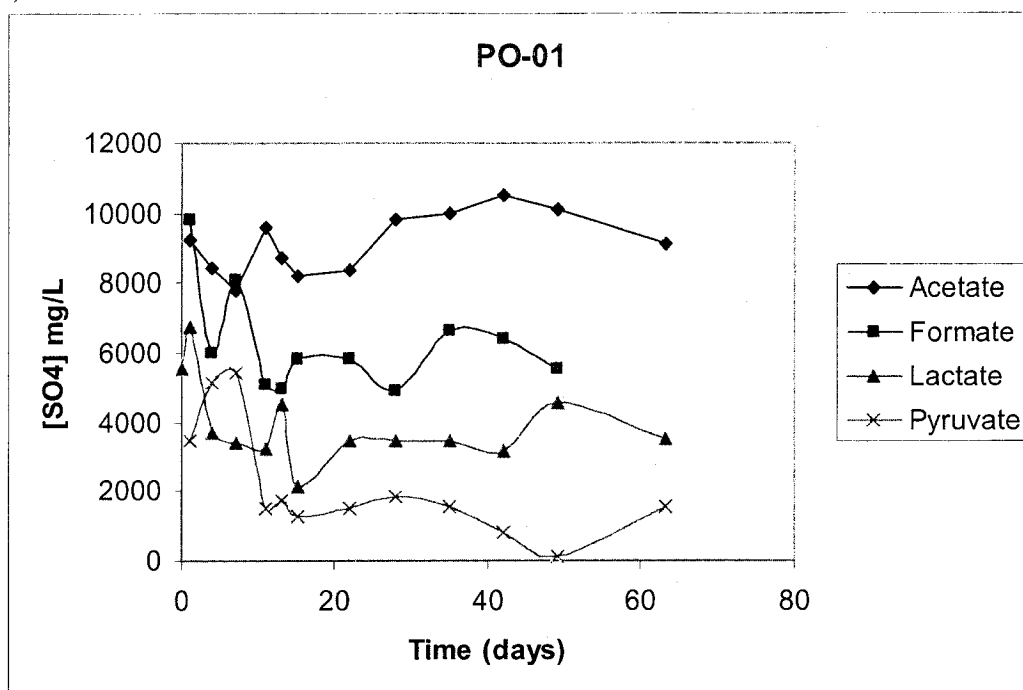


Figure 4-14 Dissolved SO₄ (mg/L) concentrations in the various systems as a function of time for the Hollinger tailings.

a)



b)

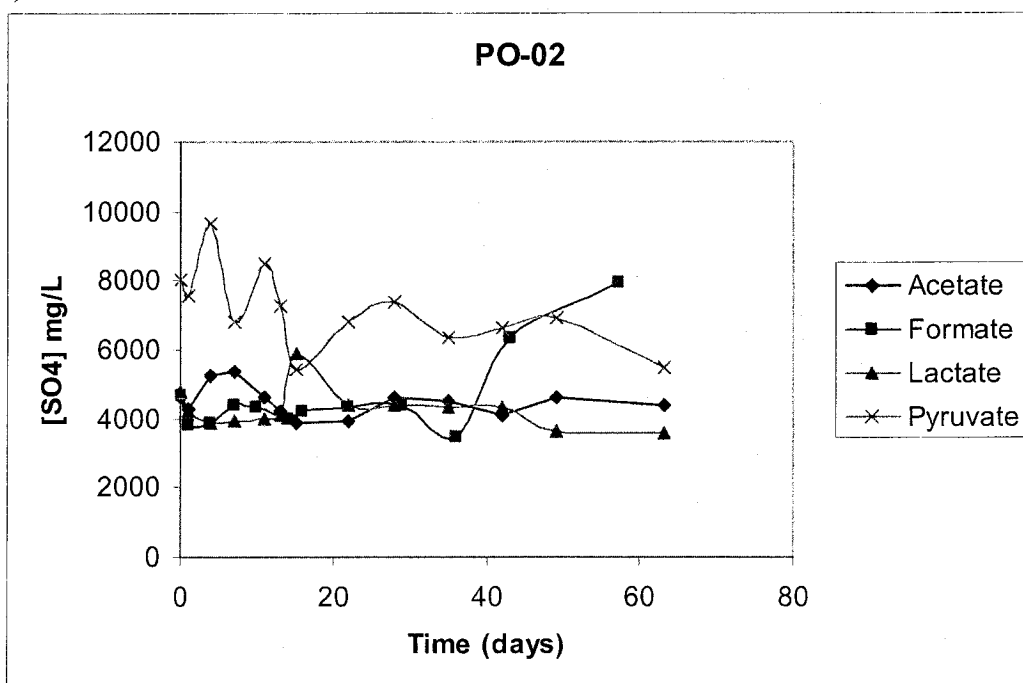


Figure 4-15 Dissolved SO₄ (mg/L) concentrations in the various systems as a function of time for the Potter tailings.

the growth medium. In the acetate system, the sulfate concentration remained fairly stable, with only minor fluctuations over time. In the PO-02 systems (Fig. 4-15 b), the pyruvate system showed the highest initial sulfate concentration and it decreased at a constant rate, dropping from 8000 mg/L to under 6000 mg/L by day 60. The formate, acetate and lactate systems showed a small increase in sulfate concentrations in the first week, but the concentrations slightly declined afterwards.

4.3.5 Abiotic Control Systems

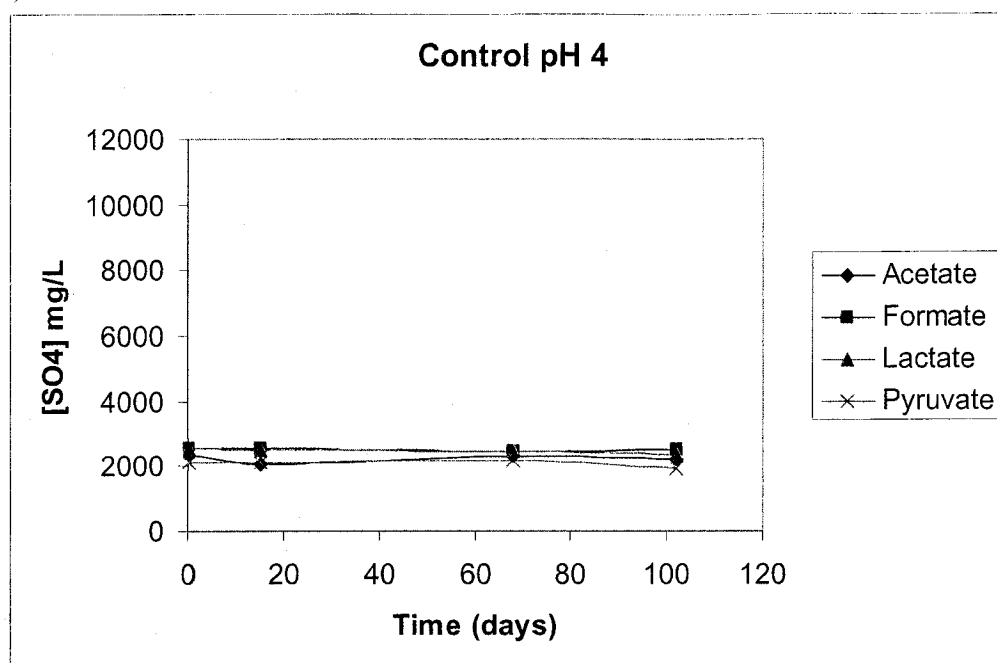
The SO_4 concentrations of the abiotic controls systems remained constant, without any real fluctuations, throughout the entire length of the experiment, as shown in Figures 4-16 to 4-18. The concentration levels ranged between 2000 mg/L to 3000 mg/L among the various systems.

4.4 Dissolved Iron

4.4.1 Broulan Tailings

The dissolved Fe concentrations of the Broulan tailing systems are shown in Figure 4-19. In the BR-01 systems (Fig. 4-19 a), the initial Fe concentration was lower than the concentration in the growth medium. In the lactate system, a small release of Fe was observed at the beginning of the experiment but the levels went down afterwards. In the acetate and formate systems, Fe levels went up around 10 and 40 days, but slightly declined

a)



b)

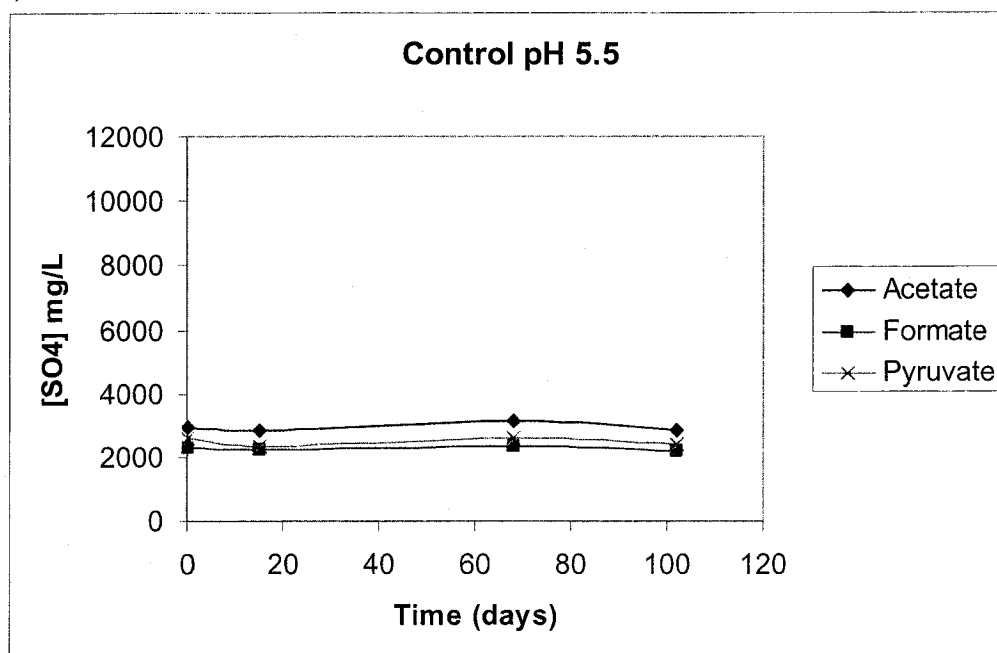
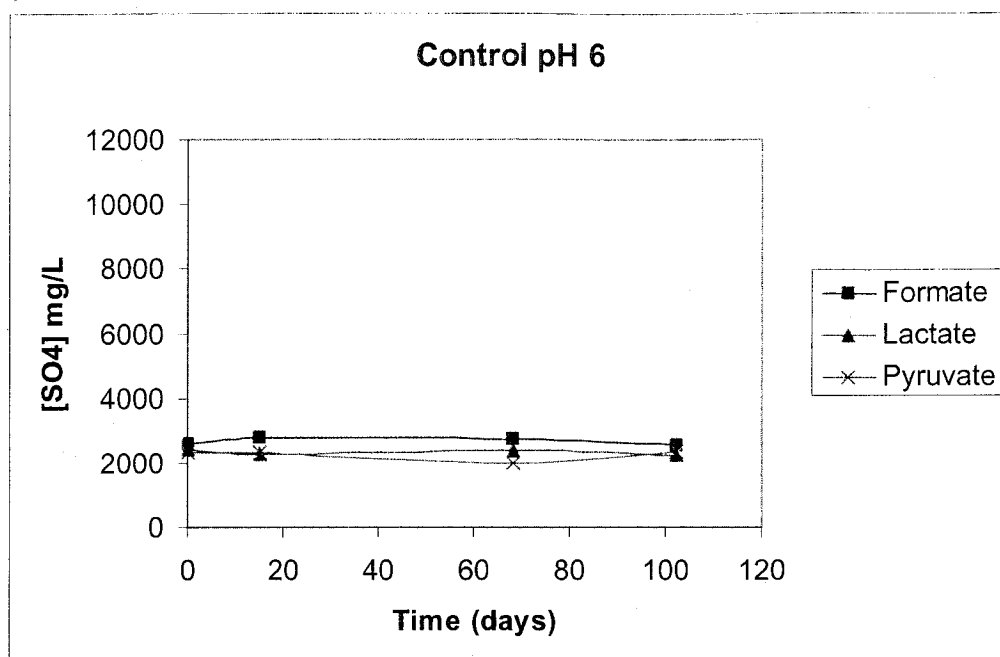


Figure 4-16 Dissolved SO₄ (mg/L) concentrations in the abiotic control systems with an initial pH of a) 4 and b) 5.5.

a)



b)

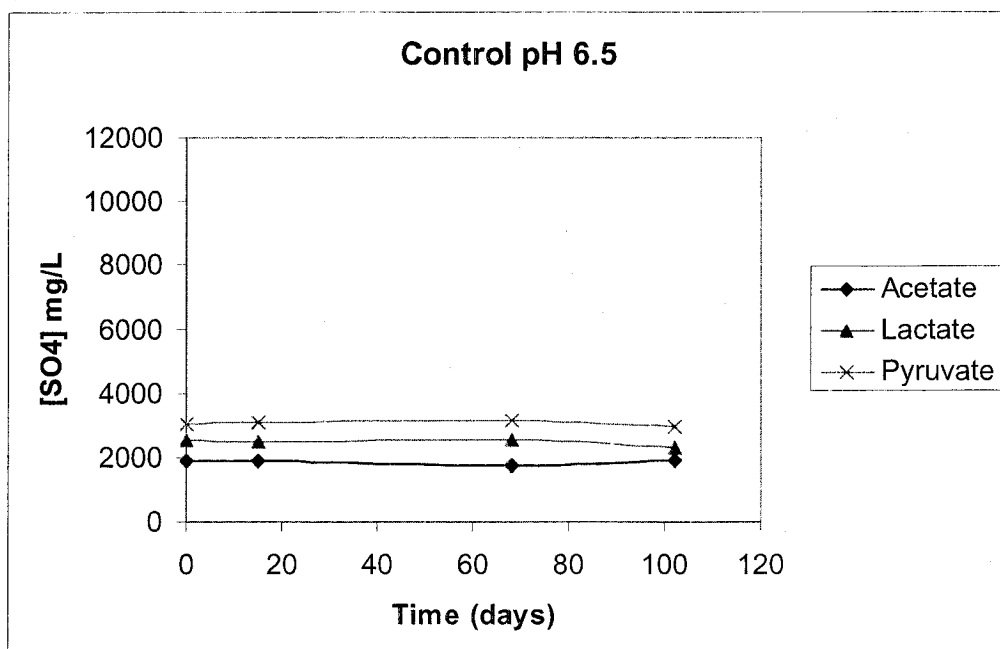


Figure 4-17 Dissolved SO₄ (mg/L) concentrations in the abiotic control systems with an initial pH of a) 6 and b) 6.5.

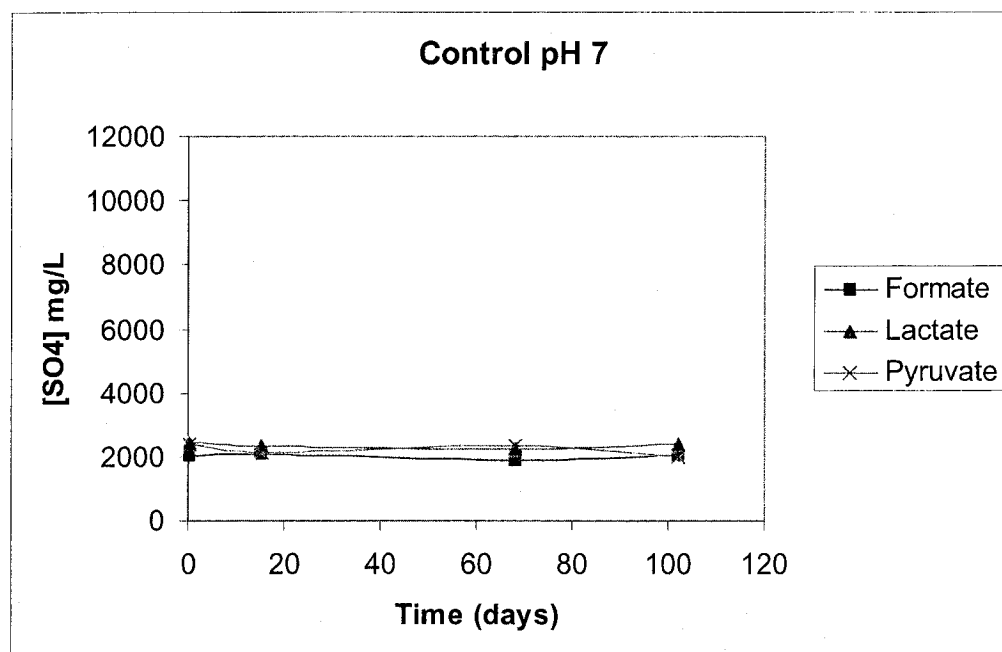
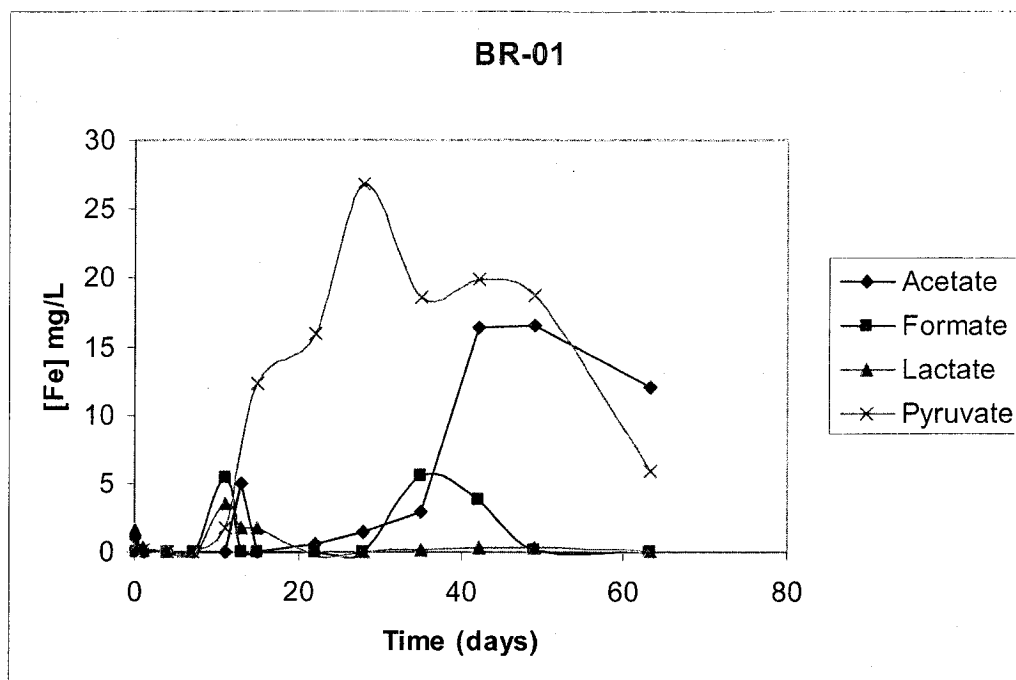


Figure 4-18 Dissolved SO_4 (mg/L) concentrations in the abiotic control systems with an initial pH of 7.

a)



b)

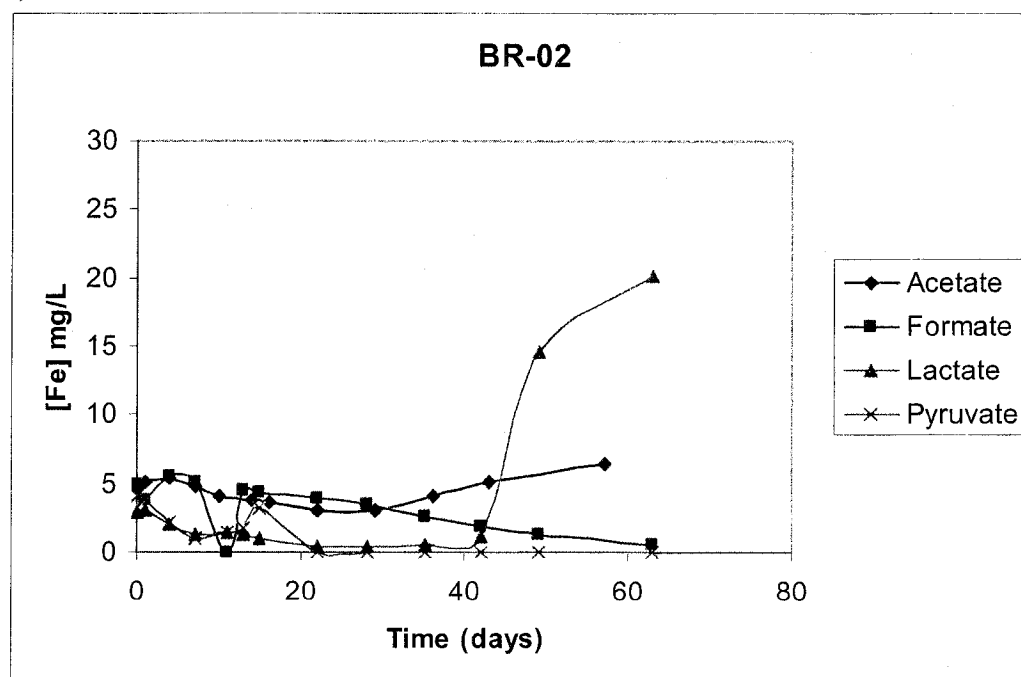


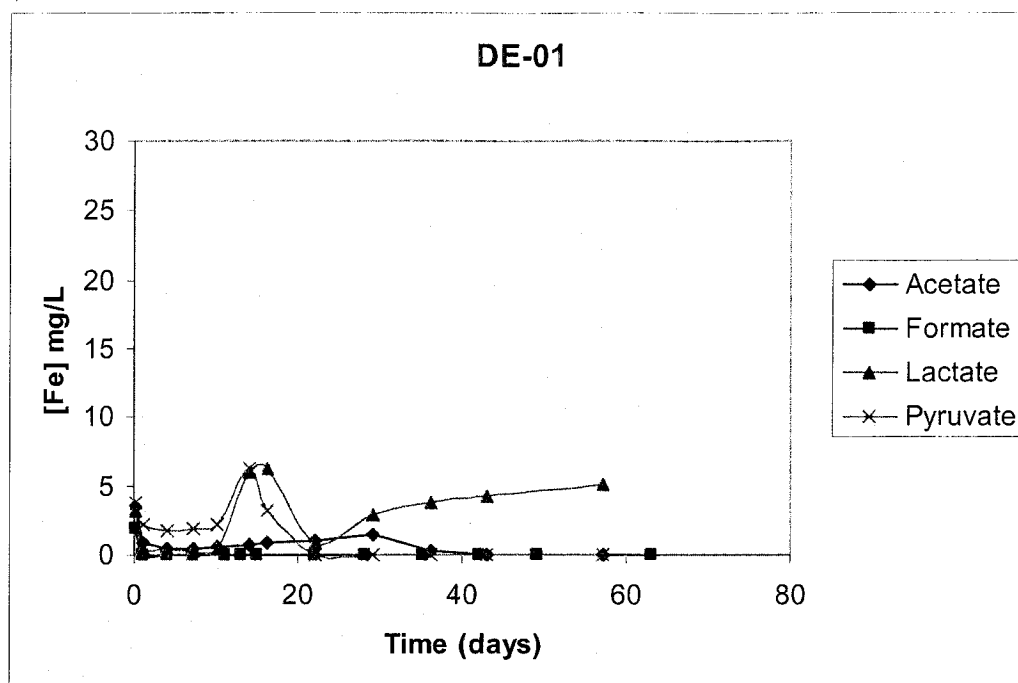
Figure 4-19 Dissolved Fe (mg/L) concentrations in the various systems as a function of time for the Broulan tailings.

by day 60. In the pyruvate system, a large release of Fe was observed between 10 and 50 days. In that system, the concentration of soluble Fe reached 25 mg/L and then slightly declined by the end of the experiment. In the BR-02 systems (Fig. 4.19 b), the initial Fe concentration was identical to the one of the growth medium. Soluble Fe concentrations in the pyruvate and formate systems slowly decreased over time from 5 mg/L to near 0. The acetate system maintained a constant Fe concentration of 5 mg/L over the 60 days. In the lactate system, Fe levels also decreased initially, but after day 40, they rapidly increased to 20 mg/L by day 60.

4.4.2 Delnrite Tailings

The concentrations of soluble Fe of the systems containing the Delnrite tailings are shown in Figure 4-20. In the DE-01 systems, the initial Fe concentration of 5 mg/L rapidly declined to non-detectable levels in the acetate and formate systems over the first few days of the experiment (Fig. 4-20 a). In the pyruvate system, a small Fe increase was observed after day 20, but the concentration dropped to zero by the end of the experiment. In the lactate system, a small release of Fe was observed at day 20, but unlike the other systems, Fe remained in solution for the rest of the experiments. In the DE-02 systems, a rapid decrease of soluble Fe was observed in all systems. Soluble Fe was not detected in the acetate and formate systems for the remainder of the experiment, whereas a release of Fe was observed at

a)



b)

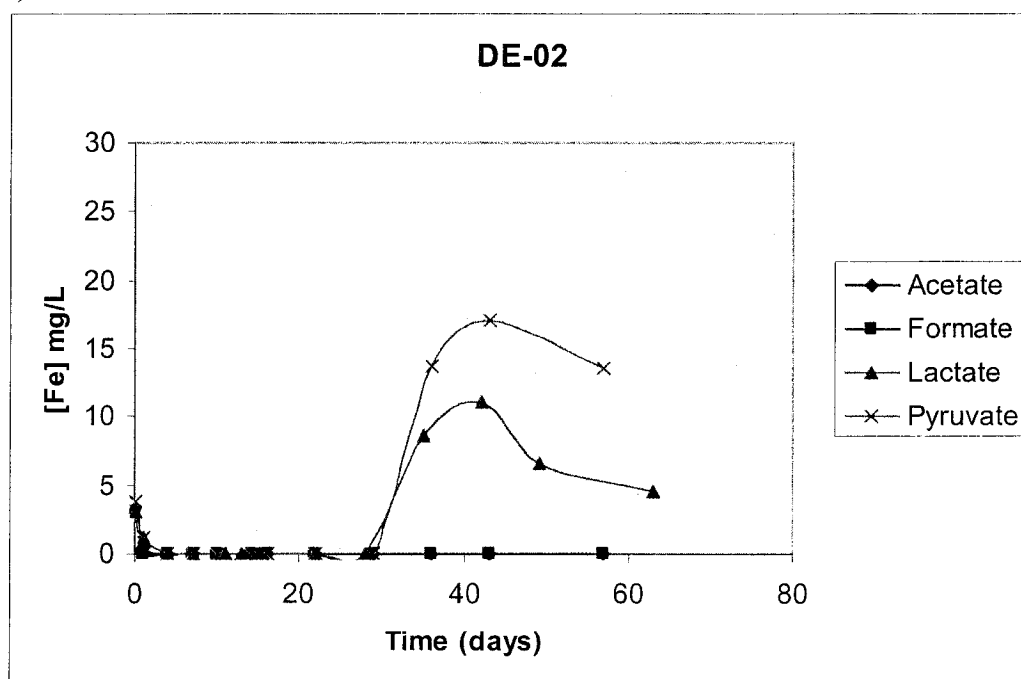


Figure 4-20 Dissolved Fe (mg/L) concentrations in the various systems as a function of time for the Delnite tailings.

day 30 in the pyruvate and lactate systems (Fig. 4-20 b). Both systems then showed a small decline of soluble Fe after day 40.

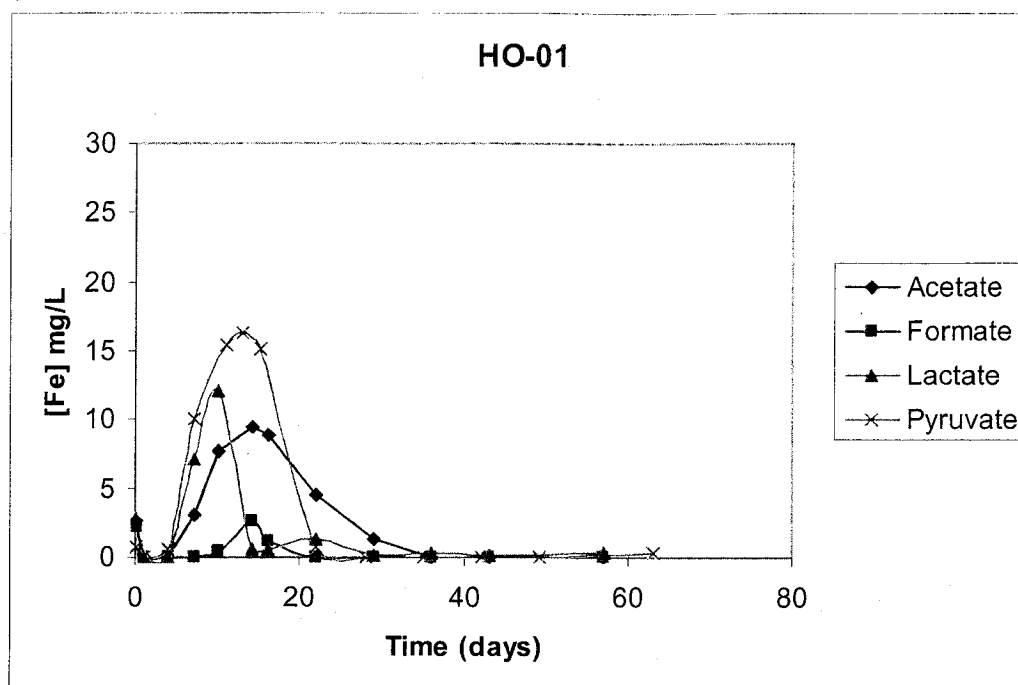
4.4.3 Hollinger Tailings

The soluble Fe concentrations of the systems containing the Hollinger tailings are shown in Figure 4-21. In all the HO-01 systems, an initial decline of Fe was followed by a release of iron by day 10 (Fig. 4-21 a). Fe concentrations reached 5 mg/L in the formate system, whereas the lactate and acetate systems showed Fe concentrations as high as 10 mg/L. The pyruvate system also released soluble Fe by day 10 and the concentrations reached 15 mg/L. By day 15, all systems showed a decrease of soluble Fe over the remainder of the experiment. In the HO-02 systems (Fig. 4-21 b), the formate system showed a constant concentration of soluble Fe at the beginning of the experiment but the levels went below detection limit by day 15. In the other systems, the small decrease of soluble Fe at the beginning of the experiment was followed by a release of Fe by day 10-15. Fe concentrations in those systems then dropped to near-zero levels by day 20.

4.4.4 Potter Tailings

The soluble Fe concentrations of the systems containing the Potter tailings are shown in Figure 4-22. In the PO-01 tailing systems, the initial Fe concentration of the 4 systems was greater than the one of the growth medium, indicating that the wet tailings were a source of soluble Fe (Fig. 4-22 a). The acetate system showed a continuous release of Fe over the course of the experiment. Fe concentrations were greater than 30 mg/L by the end of the

a)



b)

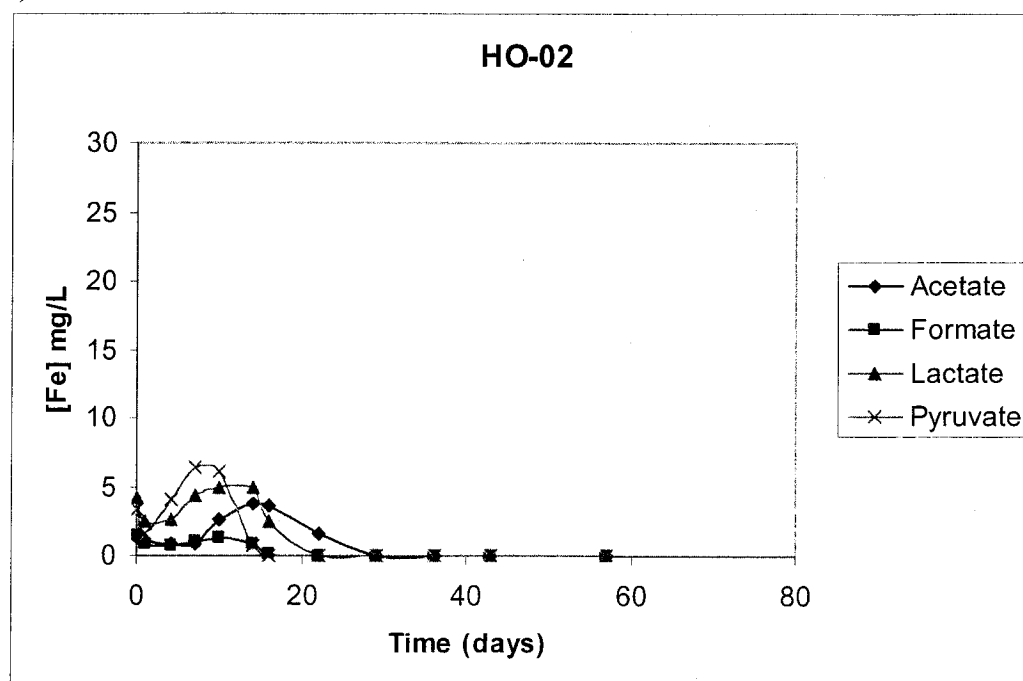
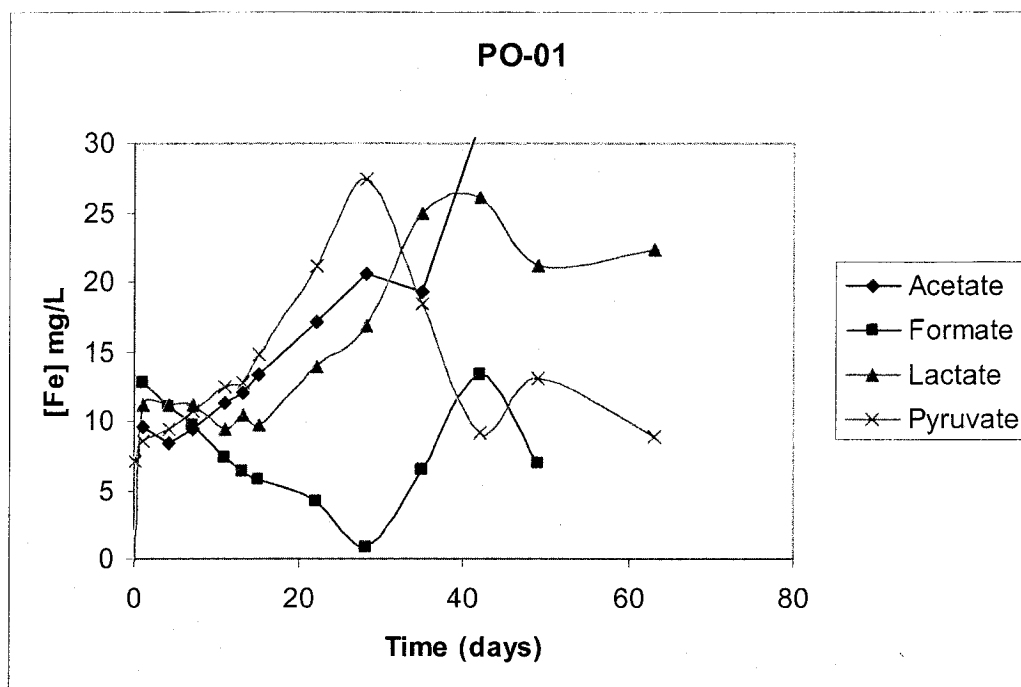


Figure 4-21 Dissolved Fe (mg/L) concentrations in the various systems as a function of time for the Hollinger tailings.

a)



b)

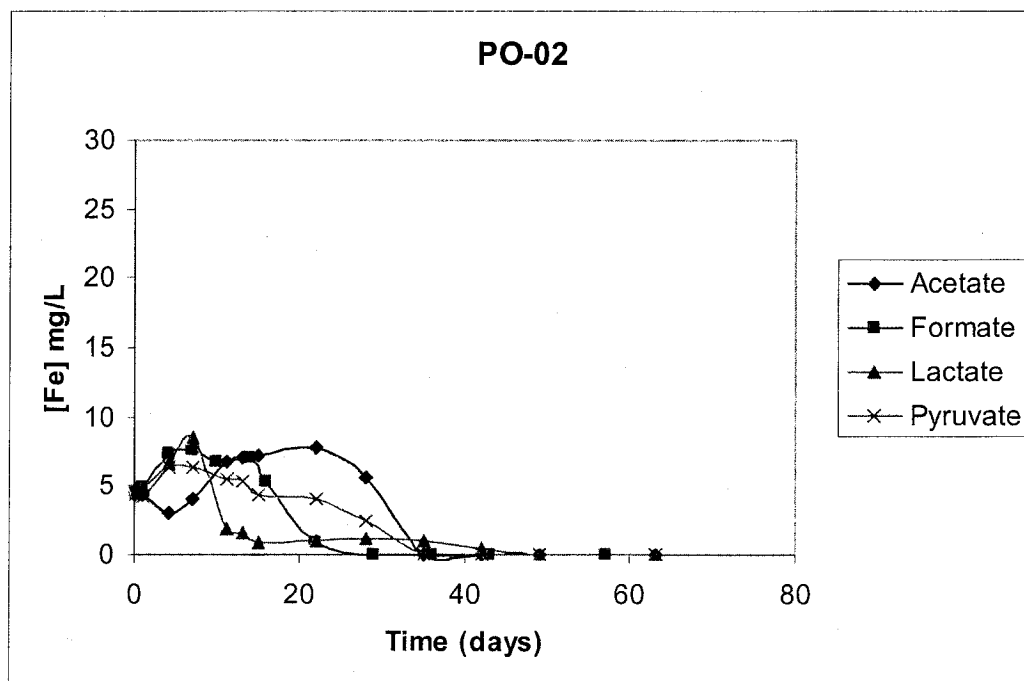


Figure 4-22 Dissolved Fe (mg/L) concentrations in the various systems as a function of time for the Potter tailings.

experiment. The pyruvate and lactate systems also showed an increase of soluble Fe for the first 25 days, but the concentrations declined by day 60.

Unlike the other system, the soluble Fe concentrations in the formate system decreased in the first 25 days, then slowly rose back by day 40. In the PO-02 systems, the concentration of soluble Fe slightly increased for the first 15 days, and then dropped below detection levels by day 40 (Fig. 4-22 b).

4.4.5 Abiotic Control Systems

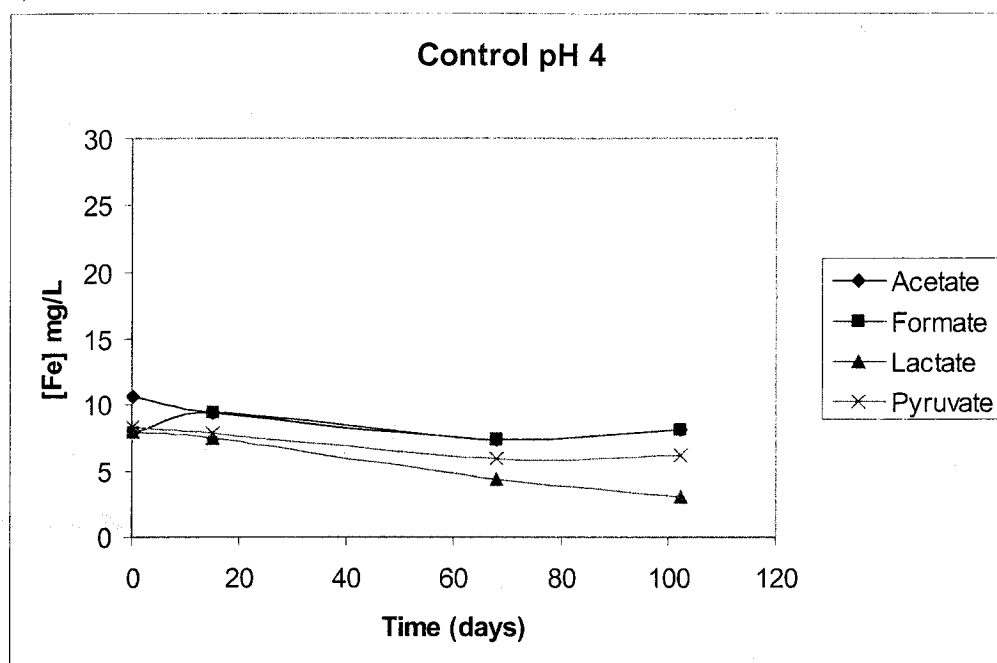
The soluble Fe concentrations of the abiotic control systems all decreased over time, as indicated in Figures 4-23 to 4-24. Specifically, the initial pH 4 abiotic control systems started with approximately 10 mg/L of Fe(II) and slowly decreased, reaching approximately 5 mg/L by day 100. The initial pH 5.5 abiotic control systems started with approximately 5 mg/L and dropped below detection limit by day 20. All the other more circa-neutral systems started with levels below detection limit.

4.5 Dissolved Organic Carbon (DOC)

4.5.1 Broulan Tailings

The DOC concentrations of the various systems containing the Broulan tailings are shown in Figure 4-26. In all BR-01 systems, there was a decrease in DOC over time, especially after the first week of the experiment (Fig. 4-26 a). In the BR-02 systems (Fig. 4-26 b), the pyruvate and lactate systems showed the largest decrease in DOC over the entire 60 days (from 250 mg/L to 50 mg/L), whereas the acetate system showed a slight DOC

a)



b)

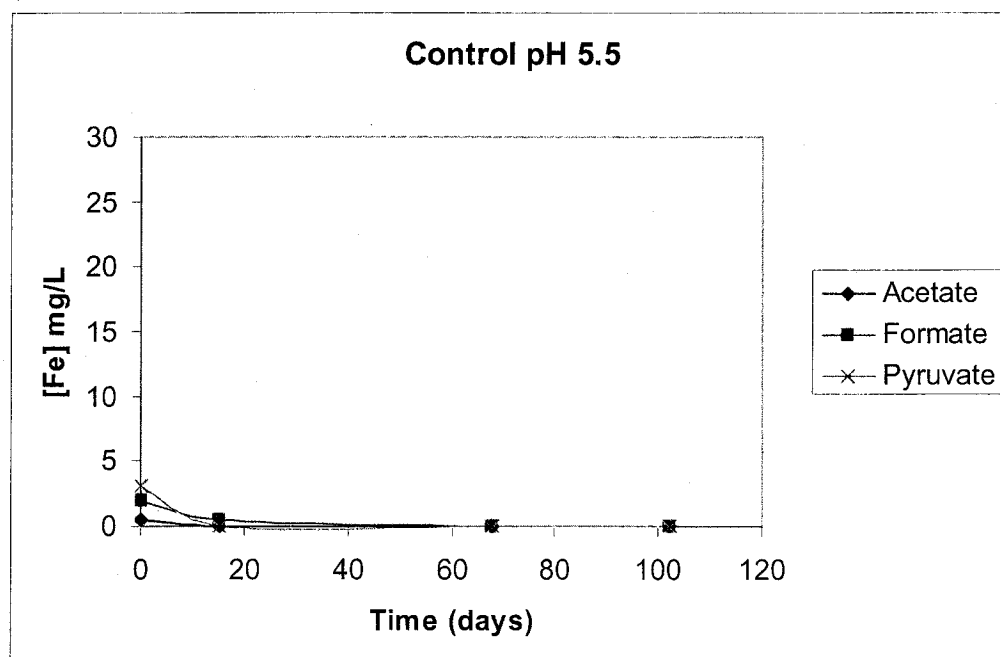


Figure 4-23 Dissolved Fe (mg/L) concentrations in the abiotic control systems with an initial pH of a) 4 and b) 5.5.

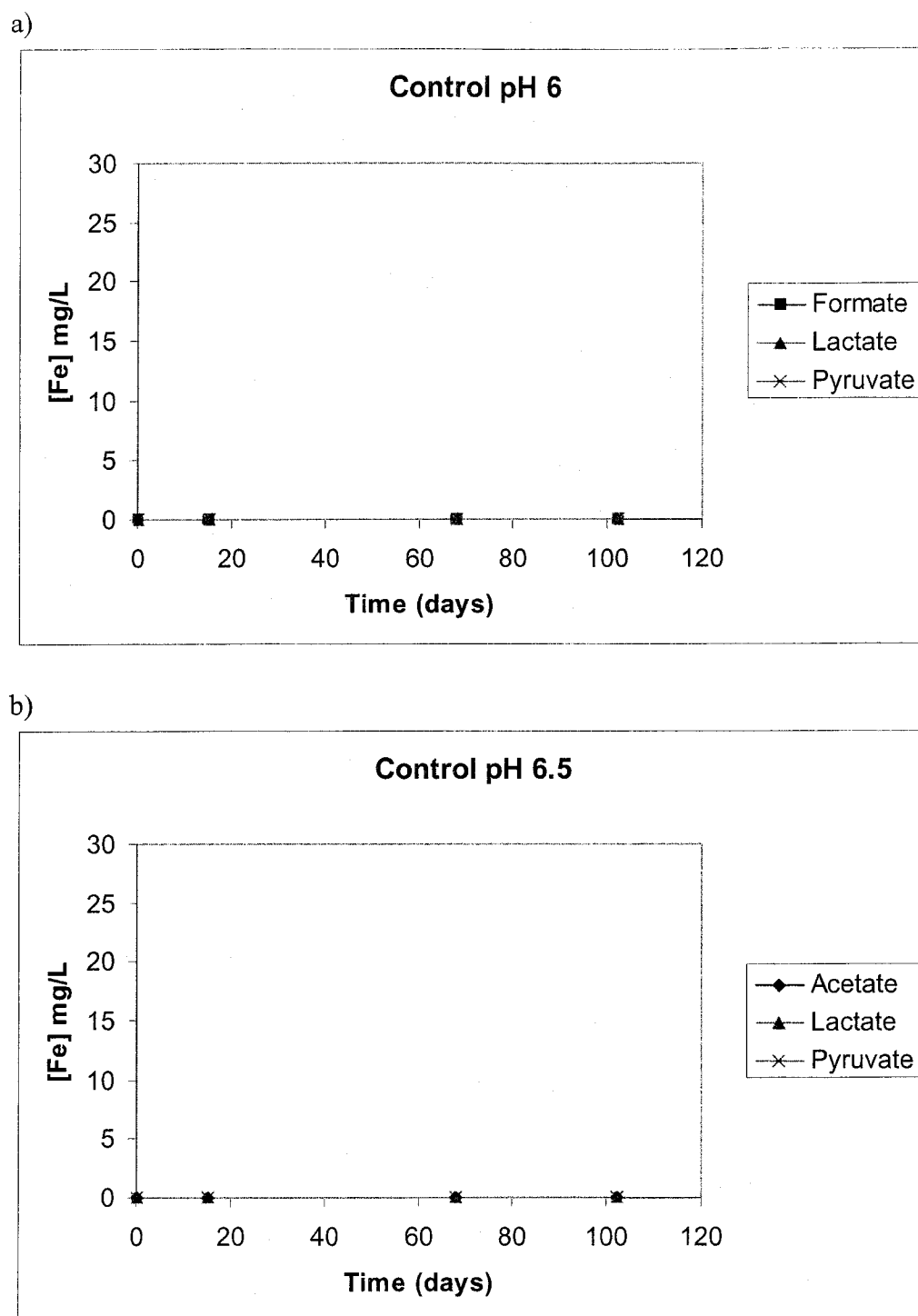


Figure 4-24 Dissolved Fe (mg/L) concentrations in the abiotic control systems with an initial pH of a) 6 and b) 6.5.

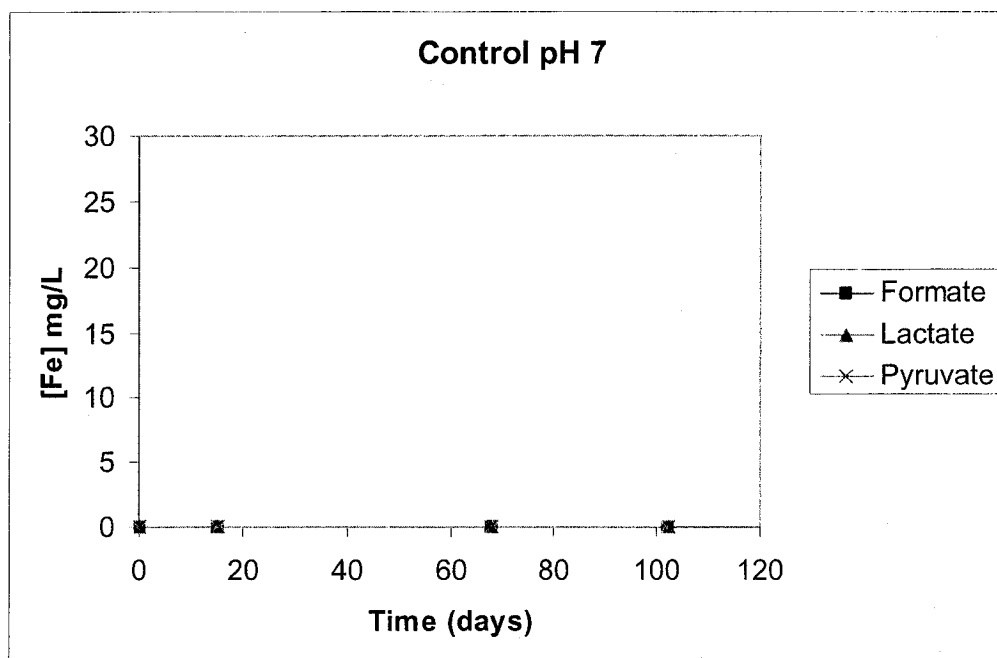
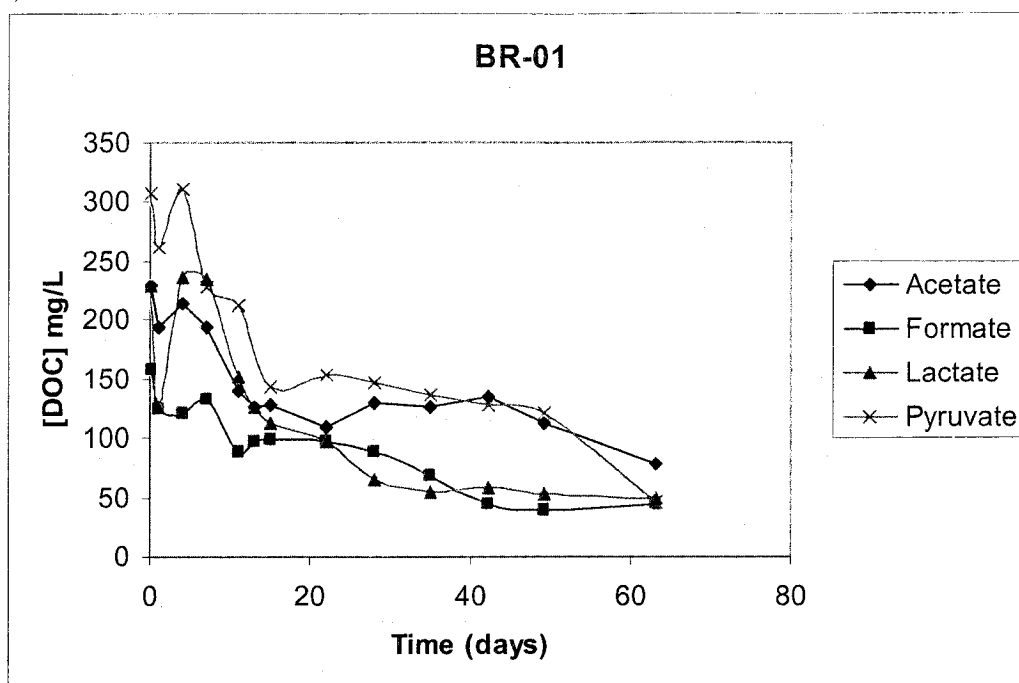


Figure 4-25 Dissolved Fe (mg/L) concentrations in the abiotic control systems with an initial pH of 7.

a)



b)

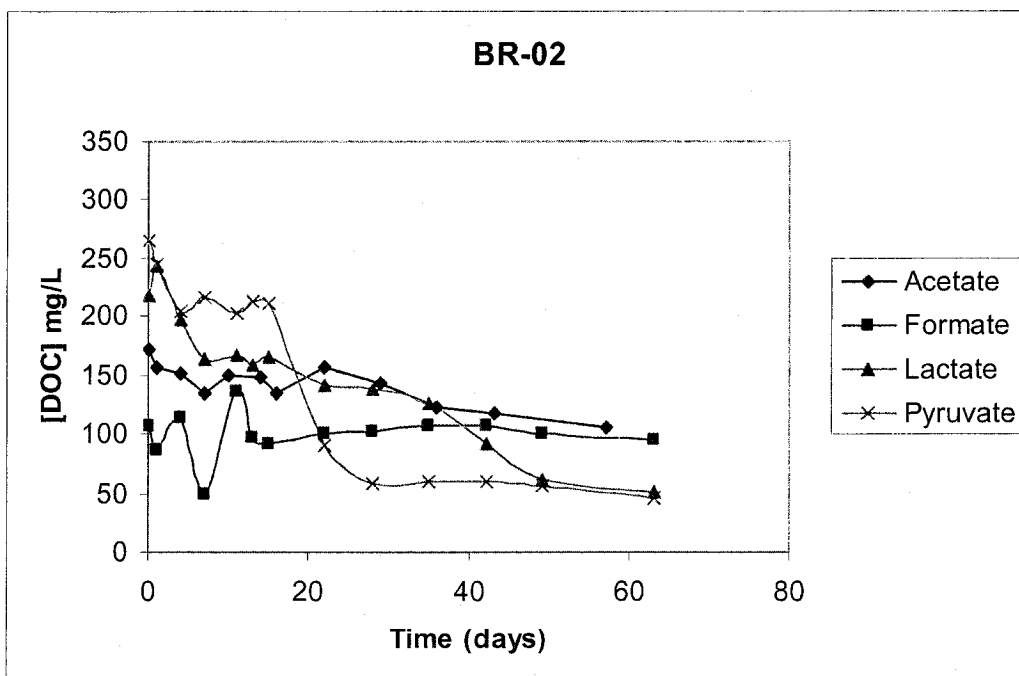


Figure 4-26 DOC concentrations (mg/L) in the various systems as a function of time for the Broulan tailings.

decline (from 150 mg/L to 100 mg/L). In the formate system, the DOC concentrations remained fairly constant over time.

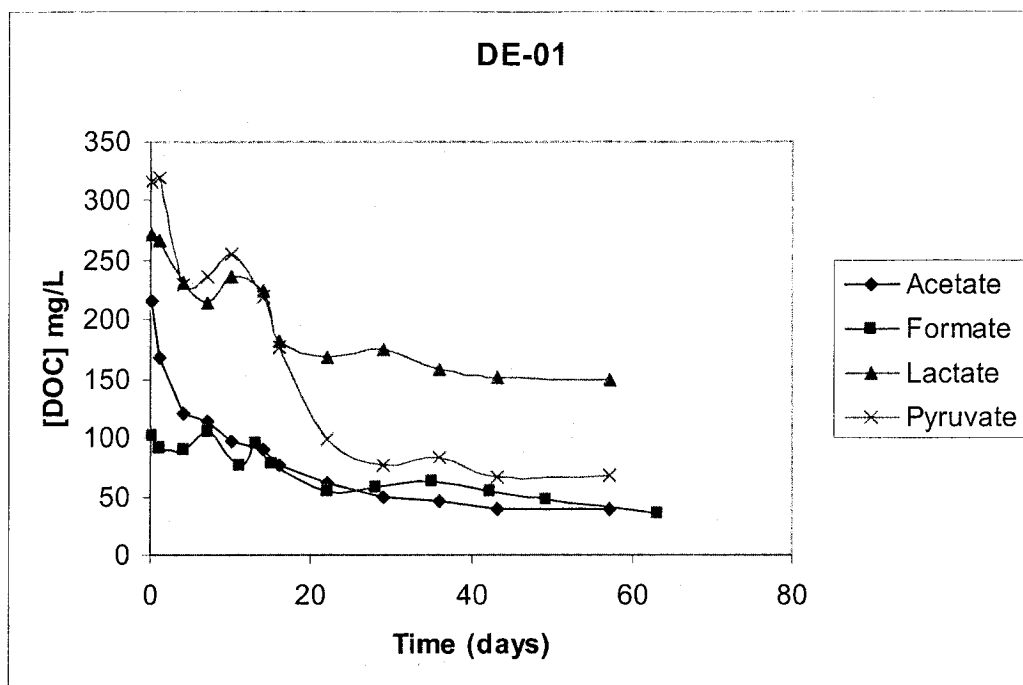
4.5.2 Delnite Tailings

The DOC concentrations of the systems containing the Delnite tailings are shown in Figure 4-27. In the DE-01 systems, the acetate and pyruvate systems showed an initial rapid decrease in DOC concentrations (Fig. 4-27 a). The DOC levels in those systems leveled off and eventually stabilized near day 20. The lactate and formate systems also showed a small decline in DOC concentrations over time, and the levels stabilized around day 20 (Fig. 4-27 b). In the DE-02 systems, the DOC levels declined over time and the trends for each system were similar to the ones observed for the DE-01 systems (Fig. 4-27 b).

4.5.3 Hollinger Tailings

The DOC concentrations of the systems containing the Hollinger tailings are shown in Figure 4-28. All four HO-01 systems showed an initial rapid decrease in DOC concentrations between 0 and 30 days (Fig. 4-28 a). The concentration then leveled off by the end of the experiment and reached 50-75 mg/L. In the HO-02 systems, the DOC concentrations in acetate, lactate and pyruvate systems rapidly declined between 0 and 30 days. The concentrations remained stable for the remainder of the experiment but they were higher in the acetate system than in the two other systems (Fig. 4-28 b). In the formate system, the DOC concentrations slightly decreased in the first 20 days and then remained stable over time.

a)



b)

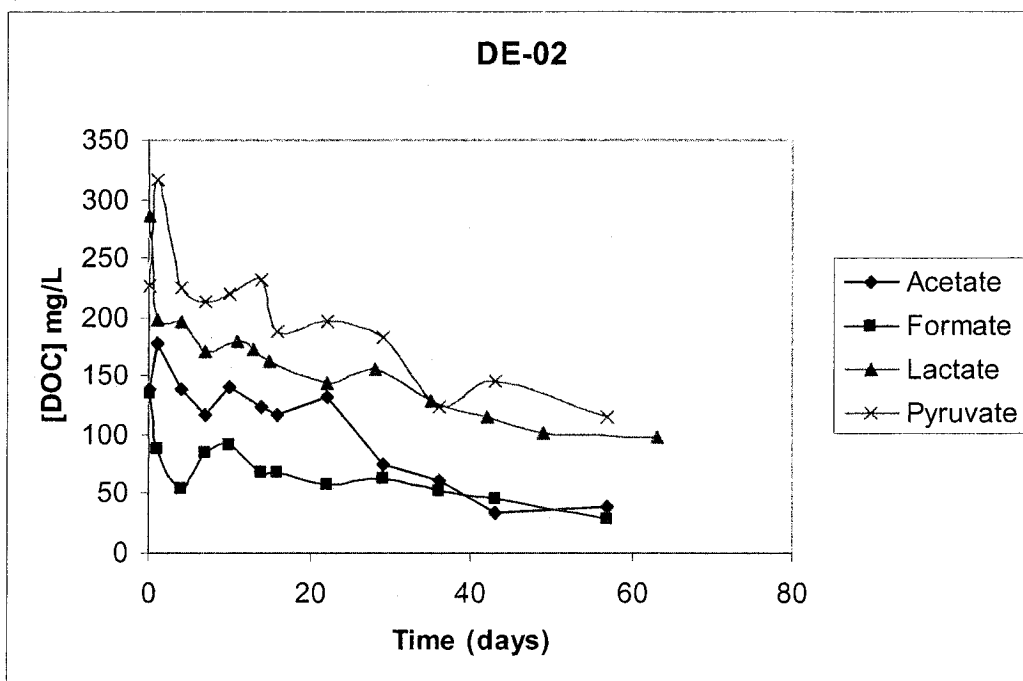
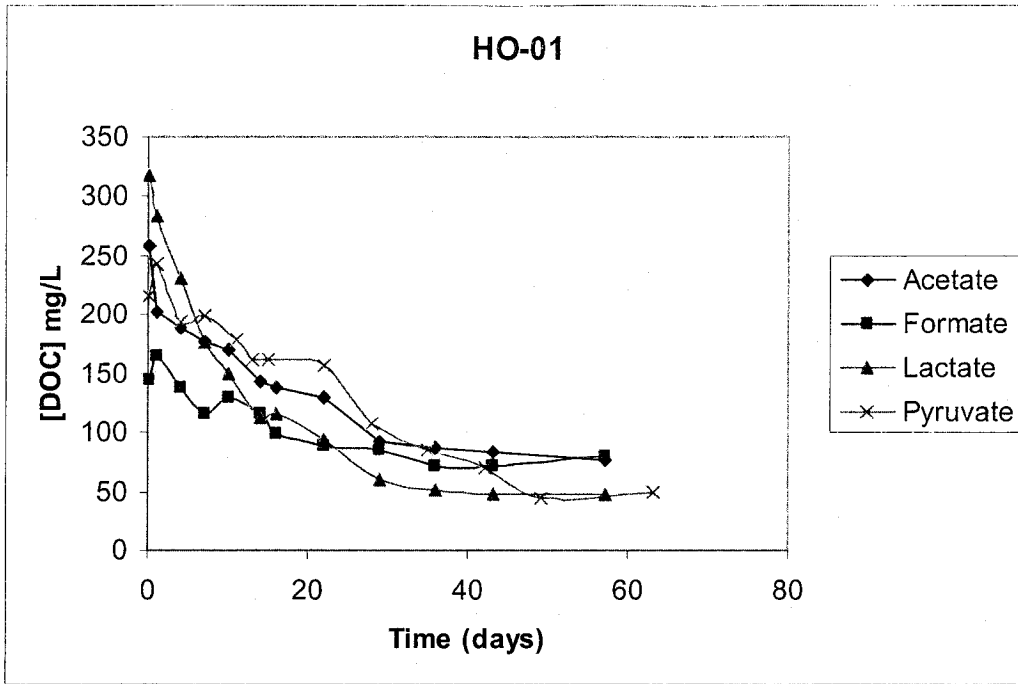


Figure 4-27 DOC concentrations (mg/L) in the various systems as a function of time for the Delnite tailings.

a)



b)

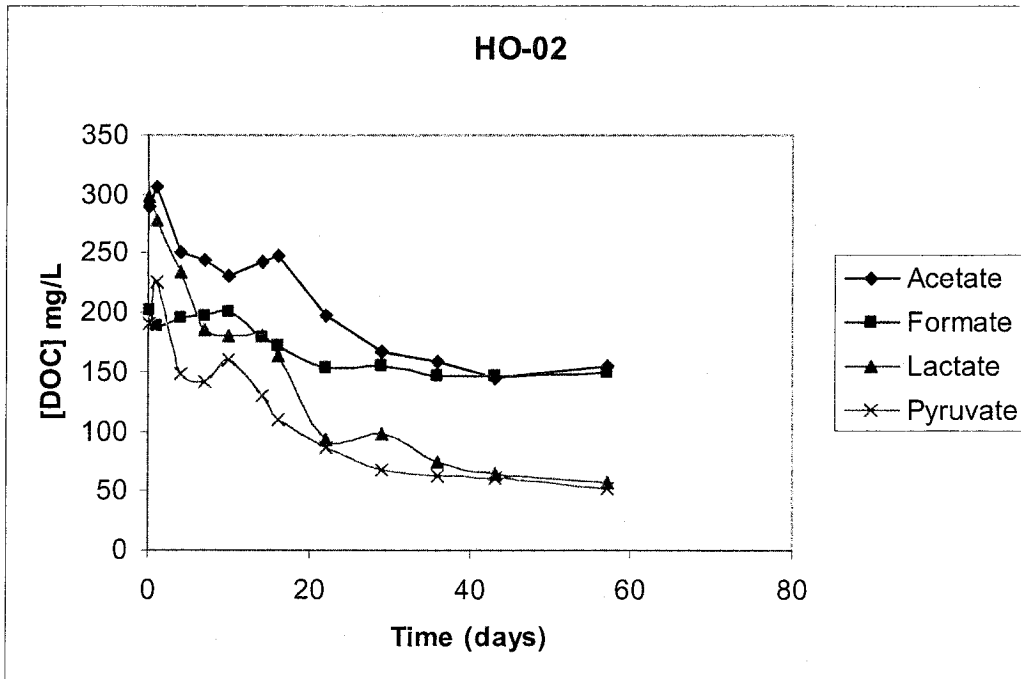


Figure 4-28 DOC concentrations (mg/L) in the various systems as a function of time for the Hollinger tailings.

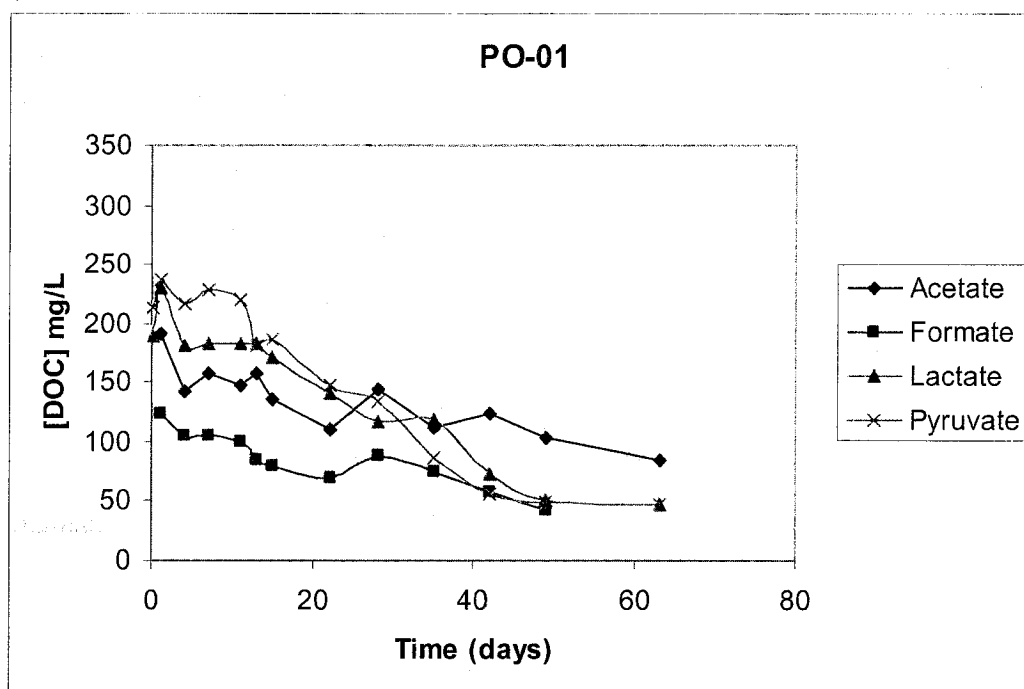
4.5.4 Potter Tailings

The DOC concentrations of the systems containing Potter tailings are shown in Figure 4-29. In the PO-01 systems, the lactate and pyruvate systems showed a constant DOC decline between 0 and 50 days, whereas in the acetate and pyruvate systems, the DOC concentrations slightly decreased over time (Fig. 4-29 a). In the PO-02 systems, the lactate system showed a constant DOC decline over time, whereas in the pyruvate system, the DOC concentration dropped twice during the course of the experiment, i.e., shortly after the inoculation and near day 20 (Fig. 4-29 b). In the formate and acetate systems, the DOC levels remained stable over the entire course of the experiment.

4.5.5 Abiotic Control Systems

Overall, the DOC concentrations of the abiotic control systems mostly remained constant throughout the experiment, as seen in Figures 4-30 to 4-32. They did not seem to fluctuate by more than 50 mg/L from their initial concentrations. A few exceptions, however, have a more significant decrease in DOC concentration levels. These include the initial pH 4 pyruvate system which decreased from 275 mg/L to 125 mg/L (Fig 4-30 a), the pH 4 lactate system which decreased from 250 mg/L to 125 mg/L (Fig 4-30 a), and the pH 6 formate system which started at 240 mg/L, dropped to 125 mg/L by day 20, then increased again back to 200 mg/L by day 100 (Fig 4-31 a).

a)



b)

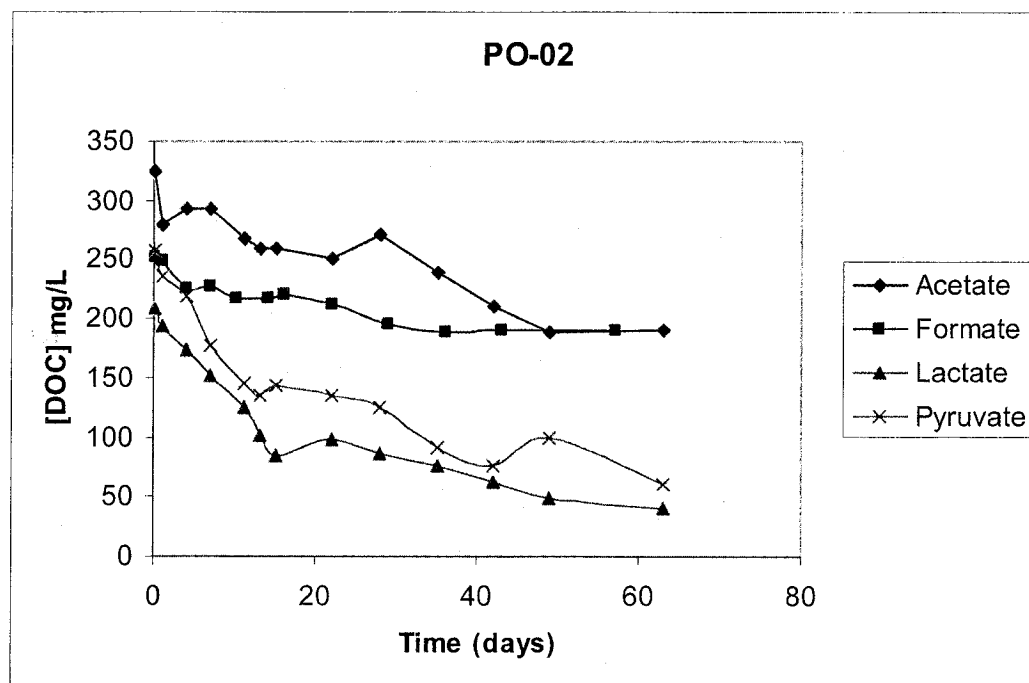
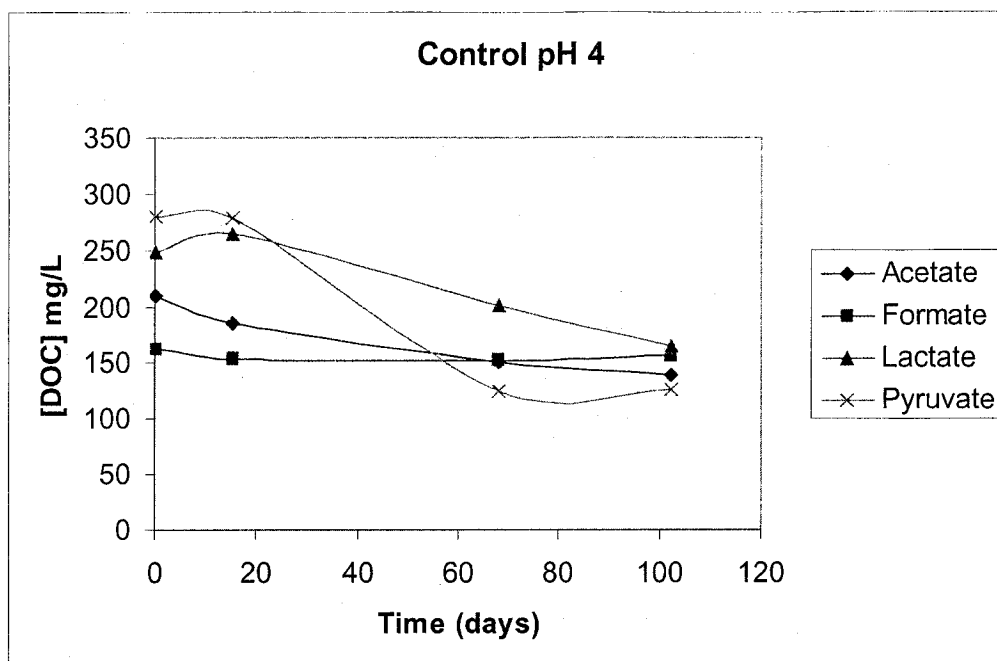


Figure 4-29 DOC concentrations (mg/L) in the various systems as a function of time for the Potter tailings.

a)



b)

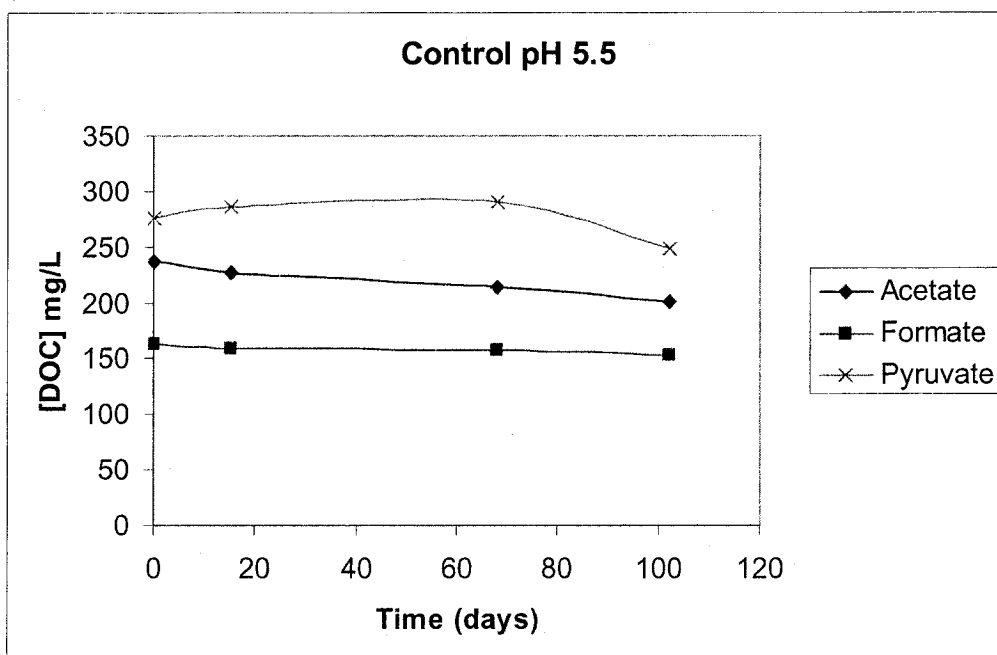
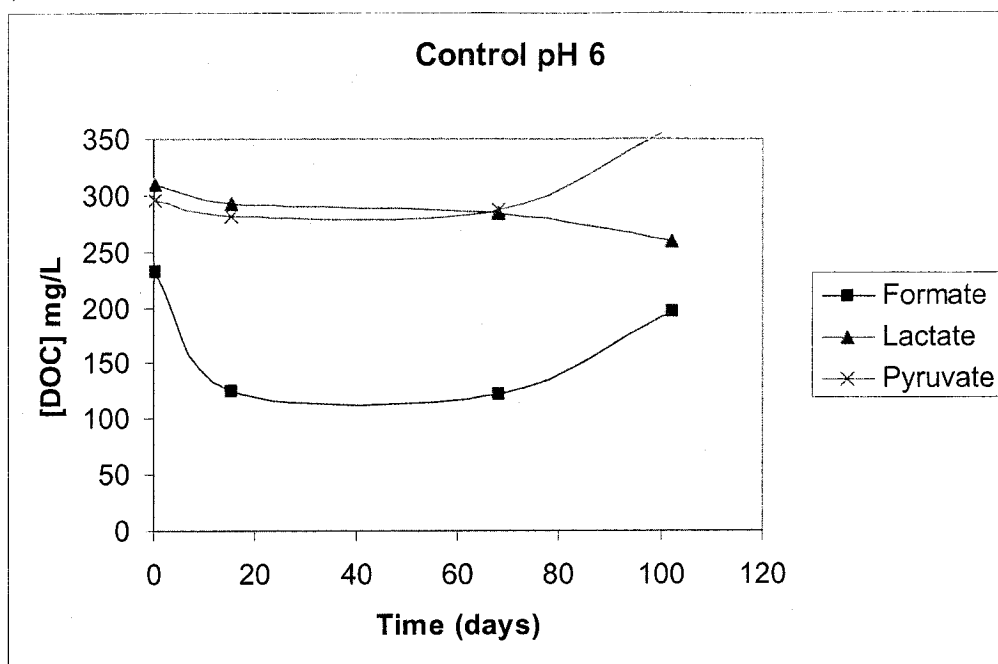


Figure 4-30 DOC concentrations (mg/L) in the abiotic control systems with an initial pH of a) 4 and b) 5.5.

a)



b)

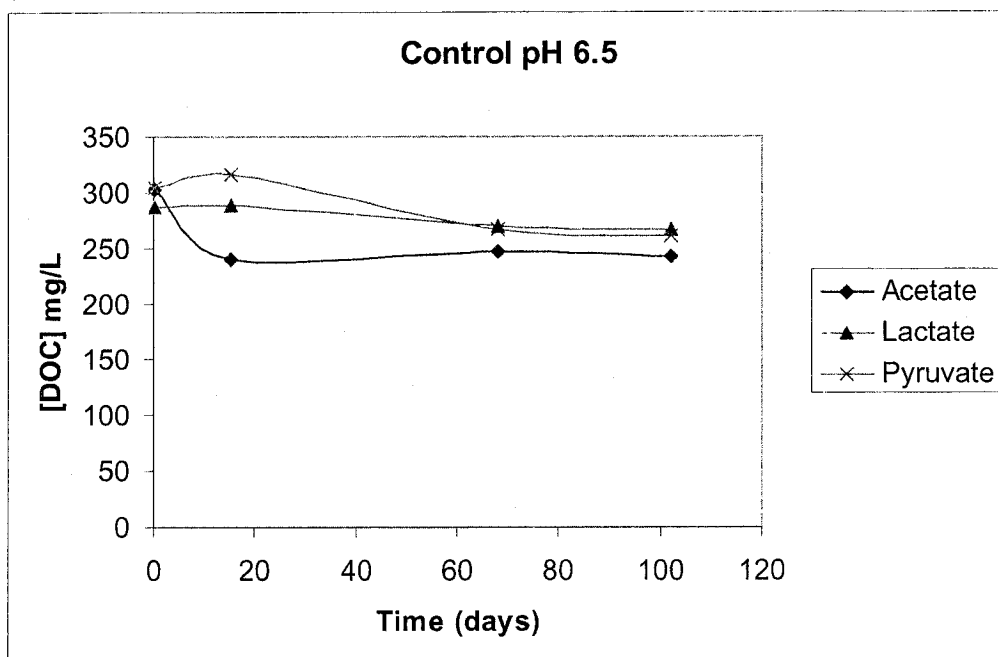


Figure 4-31 DOC concentrations (mg/L) in the abiotic control systems with an initial pH of a) 6 and b) 6.5.

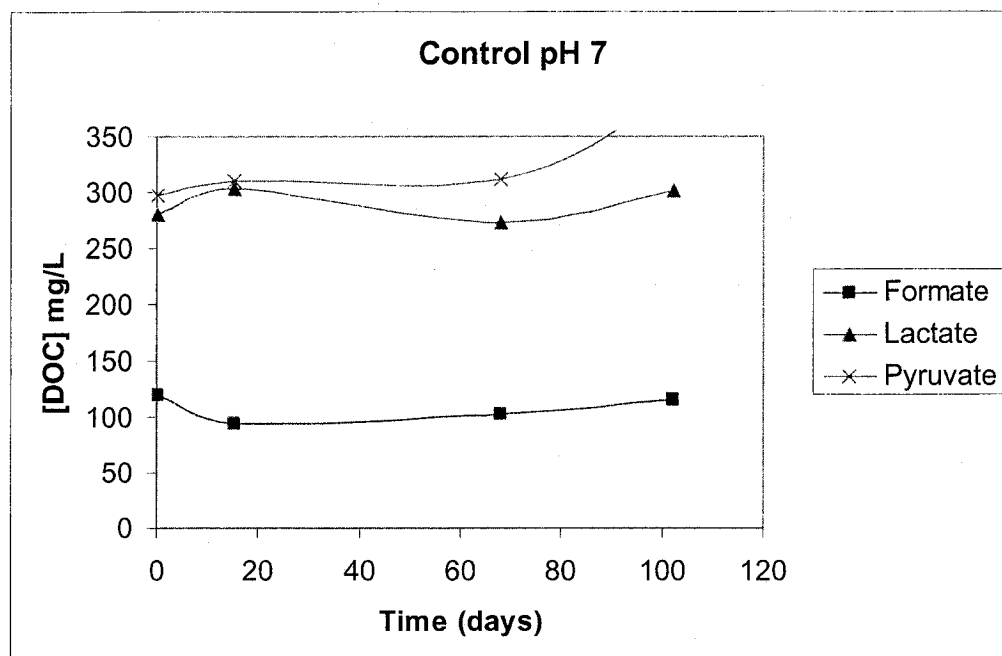


Figure 4-32 DOC concentrations (mg/L) in the abiotic control systems with an initial pH of 7.

4.6 Saturation Index Calculations

The full results of the MINEQL+ 3.1 thermodynamic saturation index calculations are presented in Appendix B. As such, the results indicated that all systems were under-saturated with respect to sulfate-rich minerals. The saturation index (SI) values ranged from circa -1 for gypsum to circa -30 for $Zn_3O(SO_4)_2$.

Most systems were under-saturated with respect to iron-rich minerals, with the exception of $Ca_2(PO_4)_2Fe$ (see appendix A). Most systems were also close to saturation with respect to vivianite and $Fe_3(PO_4)_2$. The acidic systems were generally under-saturated, whereas the more neutral systems were over-saturated with respect to these minerals.

4.7 X-Ray Diffraction

The XRD results are displayed in Figures 4-33 and 4-34. The interpretation of these results is explained in more detail in Appendix C.

The XRD analysis of the DE-01 sample (Figure 4-33) indicated the possible presence of jarosite, muscovite, quartz, and vermiculate both in the before and after results. Goethite and diaspore also appeared to be present, with a slight decrease in quantity in the after graph when compared to the before graph. Finally, gypsum appeared to be present in the before graph, but there were no traces of gypsum in the sample at the end of the experiment.

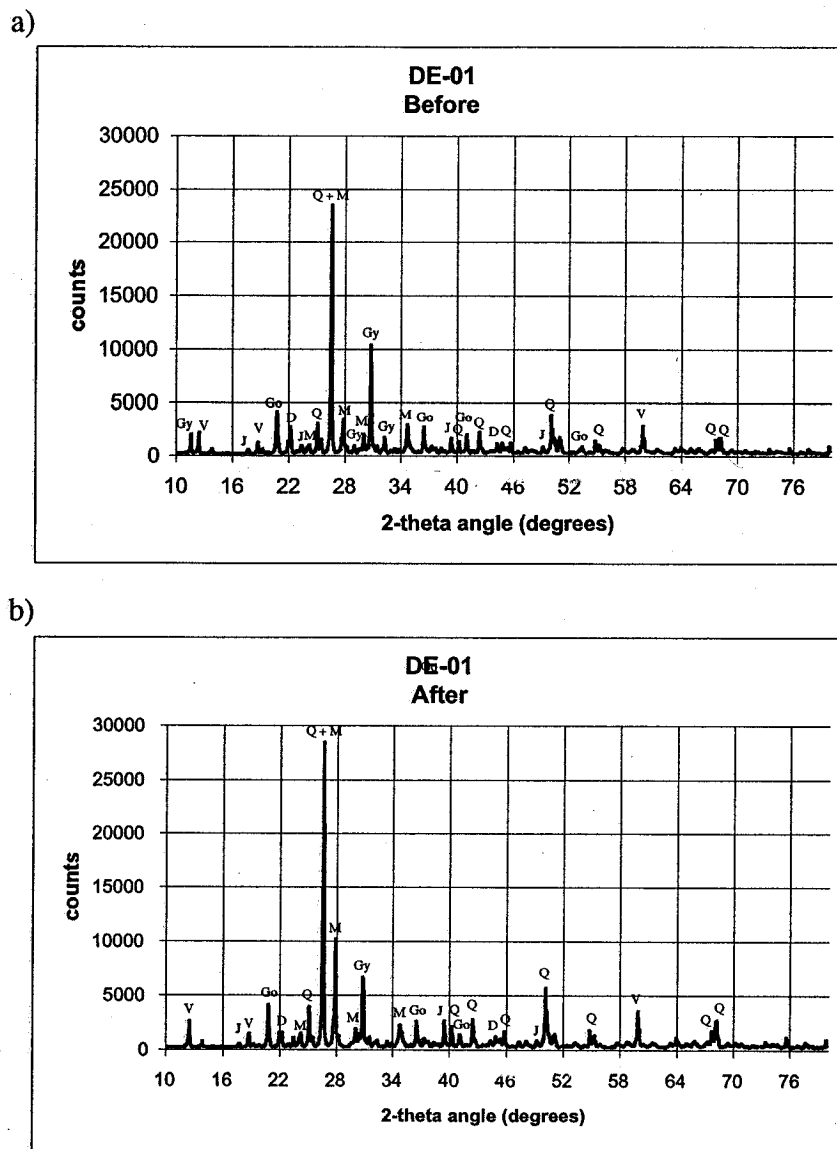
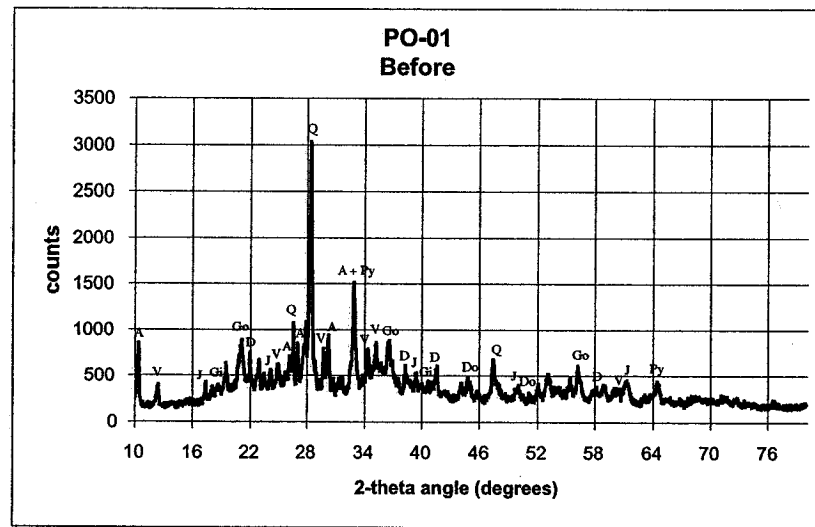


Figure 4-33 XRD patterns of the initial DE-01 tailings (a) and the tailings collected at the end of the experiment (b). D = Diaspore, Go = Goethite, Gy = Gypsum, J = Jarosite, M = Muscovite, Q = Quartz, V = Vermiculite

a)



b)

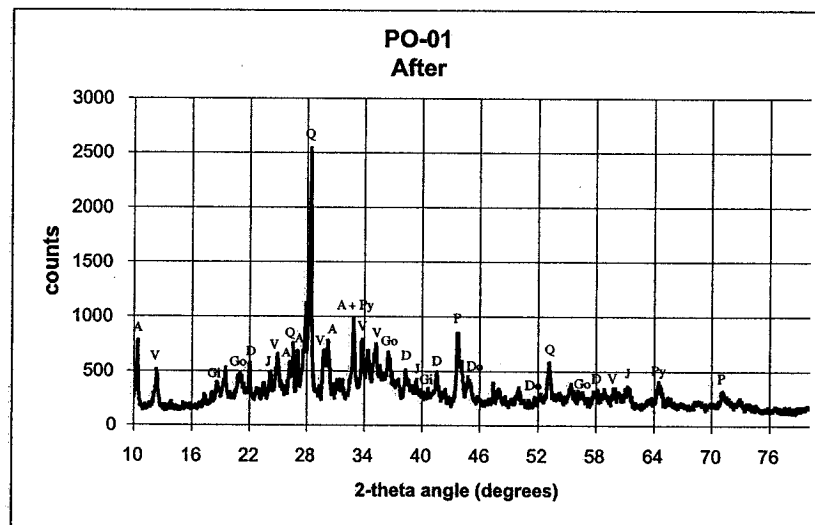


Figure 4-34 XRD patterns of the initial PO-01 tailings (a) and the tailings collected at the end of the experiment (b). A = Actinolite, D = Diaspore, Do = Dolomite, Go = Goethite, Gi = Gibbsite, J = Jarosite, Py = Pyrite, P = Pyrrhotite, Q = Quartz, V = Vermiculite

The XRD results for the PO-01 sample (Figure 4-34) indicate the possible presence of actinolite, diaspore, dolomite, goethite, gibbsite, jarosite, pyrite, pyrrhotite, and vermiculite. Of these, goethite appears to have possibly decreased by the end of the experiment. Pyrrhotite, on the other hand, did not appear to be present before the experiment, but did appear to be present after the experiment (see Appendix C).

4.8 TEM/EDS

Of all samples embedded, only 2 sets of samples were observed by TEM/EDS, i.e., the Potter (PO-01 and PO-02) and the Delnite (DE-01 and DE-02) systems. TEM observations of all 16 systems (i.e., 4 systems X 4 electron donors) indicated that they were very similar with respect to their particulate content. Various mineral fragments of variable size (10 nm to 5 μm) (Fig. 4-35 a and b) were identified as Ca- and Fe-silicates, Fe-oxides and Fe-, Cu- and Zn-sulfides based on EDS analysis. Bacterial remains and entire cells were also observed within the mineral matrix. Cells occurred as rods and vibrio (Fig. 4-35 c and d). Several bacterial cells were also partially covered with a Fe-rich precipitate occurring as amorphous to fibrous coatings (Fig. 4-35 e).

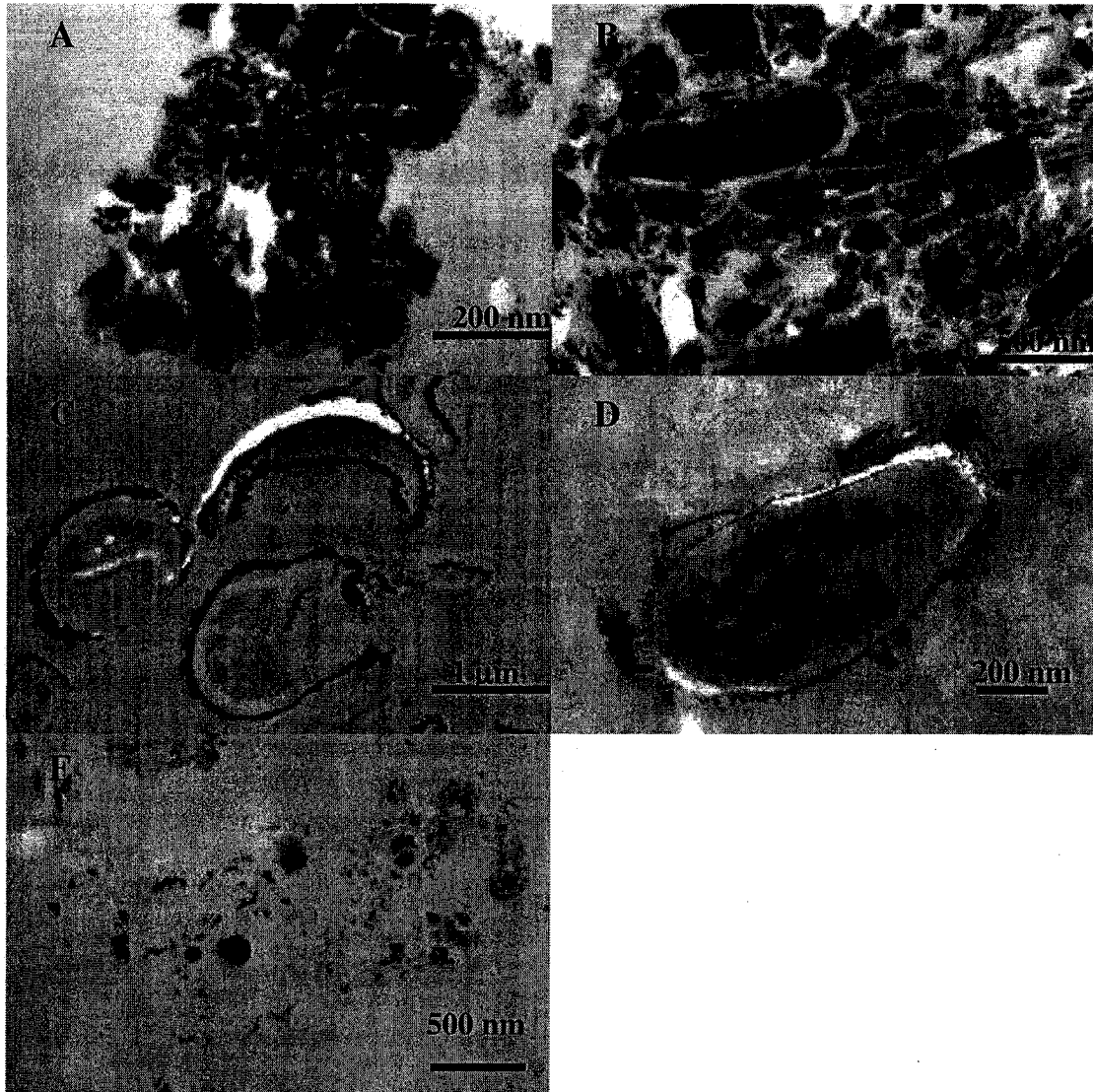


Figure 4-35: TEM micrographs of the tailings particles (from various sites) at the end of the experiment. Tailings were composed of granular material such as pyrite (A) and silicates (B) and bacteria (C, D and E). Bacteria were often encrusted with Fe-rich minerals which occurred as precipitates on their cell wall.

5 DISCUSSION

5.1 Trends

5.1.1 pH and Sulfate-Reducing Bacteria

When comparing the pH of various systems with respect to sulfate-reducing bacteria populations, the circa-neutral systems generally showed immediate signs of growth. On the other hand, the more acidic systems (BR-02 and PO-01) had a long delay before the first significant signs of sulfate-reducing bacteria growth. As the acidic systems became more pH neutral, the sulfate-reducing bacteria population levels began to increase. This is most obvious with the BR-02 systems. The pH in the BR-02 pyruvate system increased more rapidly than in the other BR-02 systems. Likewise, sulfate-reducing bacteria populations increased faster in the BR-02 pyruvate system than in the other BR-02 systems (see Figures 4-1 b and 4-5 b). These results are in agreement with previous studies that indicated that sulfate-reducing bacteria have an optimum growth in circa-neutral pH. (Postgate, 1984; Peine et al., 2000).

Since the pH of the control systems remained stable over time in almost all systems (Figures 4-9 to 4-11), it is likely that bacterial activity was responsible for the change of pH in most biotic systems. Several studies have shown that microbial processes generate alkalinity and neutralize acidity (Capone and Kiene, 1998; Lazzaretti-Ulmer and Hanselmann, 1999). In our systems, alkalinity generated during microbial sulfate reduction (equation 9) should be responsible for the pH increase since sulfate-reducing bacteria populations were present. However, with no apparent growth of sulfate-reducing bacteria at low pH (i.e. BR-02 (Fig. 4-1) and PO-01 (Fig. 4-4)), it is unlikely that sulfate reduction was

the cause of the pH increase. Although it is possible that there may have been circa-neutral microenvironments which allowed a small quantity of sulfate-reducing bacteria to eventually produce enough alkalinity to increase the pH of the entire system, it is more likely that other microbial processes, such as iron reduction, took place in the acidic systems. Microbial iron reduction, as shown in equation 6, generates alkalinity and has been shown to occur in low pH mining environments (Peine et al., 2000; Fortin et al., 2002). The release of soluble Fe in the acidic systems (Figures 4-19 and 4-22), along with the consumption of organic substrates (Figures 4-26 and 4-29) tend to support the fact that iron-reducers were active in the acidic systems, especially in the Potter site (PO-01). This would then support the findings of Peine et al (2000) that the microbial reduction of iron is the main cause of pH increase in acidic mining environments.

One control system did show a noticeable increase in pH. This was the initial pH 4 pyruvate system (Figure 4-9 a). It is likely that this system was contaminated, as it is unlikely that the pyruvate system of only the pH 4 system would show such an increase, while none of the other initial pH pyruvate systems showed any sign of increase. The fact that the initial pH 4 pyruvate system's dissolved organic carbon concentration levels also decreased (Figure 4-30 a) provides additional support that microbial contamination was the likely cause of this pH change.

5.1.2 SO_4^{2-} and Sulfate-Reducing Bacteria

Most of the systems showed a slight decrease in sulfate (SO_4^{2-}) over time. There are two possible explanations for this. The first is that sulfate precipitated as a mineral in the

systems. The second is that sulfate-reducing bacteria were active and reducing the sulfate.

With respect to mineral precipitation, the thermodynamic calculations of the saturation indices (SI) performed by MINEQL+ 3.1 indicated that all systems were undersaturated with respect to sulfate-bearing minerals (see Appendix B). Likewise, none of the various abiotic control systems showed any significant decrease in sulfate concentration (Figure 4-16 to 4-18). These results suggest that there was no precipitation of sulfate minerals in the systems.

Since most systems showed the growth of sulfate-reducing bacteria populations, the latter explanation of sulfate being reduced by bacterial activities is more probable. However, there is no clear relationship between the rate of disappearance of sulfate in the systems and the sulfate-reducing bacteria population numbers. Additionally, some systems (such as the various Broulan systems) have a growth in sulfate-reducing bacteria populations without any significant decrease in sulfate concentrations.

One possible explanation is that the strains of sulfate-reducing bacteria found in the Broulan tailings are those that do not require sulfate to grow. Although the primary characteristic of sulfate-reducing bacteria is that they grow by reducing sulfate to sulfide, they can also grow with sulfite, thiosulfate, and tetrathionate as an electron acceptor (Postgate, 1984). Additionally, most species of *Desulfotomaculum* and some *Desulfovibrio* are known to have facultative "non-sulfate" growth provided an appropriate carbon source is available. It is believed that the carbon source is incompletely oxidized into "incomplete" substrates (such as fumarate) which can then also serve as the electron acceptor (Postgate, 1984). Appropriate carbon sources include pyruvate (Postgate, 1984), choline (Hayward and

Stadtman, 1960), malate and fumarate (Miller and Wakerley, 1966). Similarly, there are numerous reports of *Desulfovibrio* and *Desulfobulbus* species being able to reduce nitrate as well as sulfate (Widdel, 1980; Widdel and Pfennig, 1982; Postgate, 1984). In some cases, nitrate reduction into ammonia only occurred in conjunction with sulfate reduction if the concentration of nitrate was relatively low. In other cases, the sulfate-reducing bacteria could only reduce nitrate if there was no sulfate present in the system (McCready et al., 1983).

Since the exact species of sulfate-reducing bacteria were never identified in this study, it is impossible to determine if the species present are non-sulfate growth strains of sulfate-reducing bacteria. However, one fact that makes this possibility less than a certainty is that the above-mentioned studies generally indicated the use of non-sulfate alternatives when there was a clear lack of sulfate, or in conjunction with sulfate reduction. None of the studies indicated that the sulfate-reducing bacteria would ignore available sulfate to use the alternative non-sulfate growth options. This suggests that non-sulfate growth options are likely less energetically favorable than normal sulfate reduction. If the sulfate-reducing bacteria of these systems were indeed non-sulfate growth strains, there must be a reason why the non-sulfate growth option was preferred over the sulfate-reduction since the systems were not lacking in sulfate. Perhaps some element found within the tailings somehow impeded or prevented sulfate reduction, thus encouraging non-sulfate growth.

One commonality of all the systems that did not have any significant decrease in sulfate levels is that they were all Cu-Zn tailings (specifically, the BR-01 systems, the BR-02 systems, and a few PO-02 systems). Perhaps the concentration levels of Cu or Zn within the system prevented the reduction of sulfate, which stimulated non-sulfate growth in the

bacteria. Perhaps other elements found within Cu-Zn tailings but not Au tailings are responsible for this. Since a complete breakdown of the tailings and elements found within them was not done as part of this study, any implication that the type of tailings is responsible for this non-sulfate growth is purely speculative.

It was also noted that there was black iron monosulfide precipitation visible in all the systems, suggesting that there must have been some hydrogen sulfide in the systems, and thus at least some sulfate reduction taking place. This possibility is not negated despite the lack of any measurable decrease in sulfate within the systems. It takes 1 mol of sulfate in sulfate reduction to produce 1 mol of hydrogen sulfide (as indicated in equation 9), which reacts with 1 mol of ferrous iron to precipitate as iron monosulfide (as indicated in equation 7). The amount of ferrous iron in the systems was, at most, 15 mg/L, which is equal to 0.57 mol/L of ferrous iron. This means that the ferrous iron will react with 0.57 mol/L of sulfate, which is the equivalent of 27.7 mg/L of sulfate, to precipitate as iron monosulfides or pyrite. With the concentration of sulfate remaining near the 2000 mg/L, 27.7 mg/L easily falls within the margin of error of the sulfate measurements. It is therefore possible that the sulfate concentration did drop by a sufficient amount to account for the iron monosulfide precipitation, yet to still remain insignificant when compared to the overall sulfate concentration measurements found in Figure 4-12. Following a similar logic, it is possible that all of the sulfate reducing bacteria are indeed sulfate-growth strains, and that their rate of sulfate reduction was merely much lower than in the other systems, making the reduction in sulfate much less apparent.

A second possible explanation lies in the fact that the original concentration level of

sulfates in the Broulan and Potter systems, that are without any significant decrease in sulfate concentrations, were already relatively low. They mostly seem to start at and hover around 2000 mg/L, compared to the other systems that do have a decrease in sulfate. These systems instead have starting concentrations of 4000 mg/L, 8000 mg/L, or even 10,000 mg/L. Perhaps a sulfate concentration below 2000 mg/L encourages the dissolution of sulfides found within the tailings placed within the system. As the sulfate-reducing bacteria transform the sulfate into hydrogen sulfide, additional sulfate is added back into the system to keep the levels balanced. Verification of this hypothesis would have required an analysis of the sulfur/sulfide concentrations within the mine tailing sediments prior to the experiment and comparing it with the sulfur/sulfide concentrations that remained within the tailing sediments after the experiment. Such measurements, however, were unfortunately not performed. Future studies should therefore pay closer attention to the sulfur geochemistry of the tailings in addition to aqueous sulfate levels within the system.

The thermodynamic MINEQL+ 3.1 saturation index (SI) calculations (as presented in Appendix B) do indicate that the systems were under-saturated with respect to sulfate minerals, thereby suggesting that the dissolution of sulfate bearing minerals is a possibility. On a particular note, the sulfate-bearing minerals with the lowest saturation index (SI) values were usually from the zinc-sulfate species, such as $Zn_3O(SO_4)_2$, $Zn_4(OH)_6SO_4$, and $Zn_2(OH)_2SO_4$. Since all of the systems without any significant decrease in sulfate concentrations are from Cu-Zn tailings, it is likely that there was a significant source of zinc-sulfate compounds provided from the tailings added during inoculation, which could easily have served as the source of additional sulfate to replenish the diminishing supply.

In addition to the overall decline in sulfate noticed in most systems, there were also small fluctuations in the concentration over time. That is, the concentration would sometimes increase slightly from one measurement to the next, even though overall there was a steady decline in total concentration from the start to the end of the experiment. This suggests that sulfur was potentially cycled throughout the duration of the experiment in the systems, with the decrease in sulfate outpacing its production. Since none of the abiotic control systems show any fluctuations in sulfate concentration (Figures 4-16, 4-17, and 4-18), it is likely that this potential cycling is a result of bacterial activity. If there is indeed sulfate/sulfide cycling occurring in the systems, perhaps these two reactions reach an equilibrium at approximately 2000 mg/L, which could also help explain why the sulfate concentrations never decreased below that level.

5.1.3 Fe and Sulfate-Reducing Bacteria

In several systems, notably BR-02, DE-01, HO-01, HO-02, and PO-02, the growth of sulfate-reducing bacteria was matched with a decrease in soluble iron concentrations. As all these systems had a pH of 4 or greater at any given point in time, all soluble Fe in the systems should have existed as ferrous iron (Fe(II)). Since the growth of sulfate-reducing bacteria populations generate hydrogen sulfide (H₂S), as shown in equations 7 and 8, Fe(II) likely precipitated as iron monosulfides or pyrite, therefore explaining the disappearance of soluble Fe in the systems. Visible black iron monosulfides within all the systems support this hypothesis.

Some systems, however, showed a release of soluble iron over time. The most

obvious example is in the PO-01 systems (see Figure 4-22 a). As stated earlier, this is a likely indication of the presence of iron-reducing bacteria in the systems. Supporting this theory is the fact that the sulfate-reducing bacteria populations were practically non-existent during the first several weeks (as indicated in Figure 4-4 a), yet there was a steady decline in dissolved organic carbon during the same period (as indicated in Figure 4-29 a). It is therefore likely that the iron-reducing bacteria used the electron donors for their own growth, and thus out-competed the sulfate-reducing bacteria. The ferrous iron levels only started to decline once the sulfate-reducing bacteria populations started to increase, which resulted in the production of HS^- , and thus the precipitation of the ferrous iron as iron monosulfides and pyrite.

All the abiotic control systems indicated a decrease in soluble iron over time, most to levels below detection limit (Figures 4-30 to 4-32). However, as previously mentioned, there were never any traces of black iron monosulfide precipitates in the control systems. In this case, the likely explanation is the precipitation of iron oxides, but since our Eh results are not reliable, we cannot ascertain this hypothesis.

5.1.4 DOC and Sulfate-Reducing Bacteria

As seen in Figures 4-26 to 4-29, dissolved organic carbon (DOC) decreased with time in all the systems. This can be interpreted as a sign of bacterial activity within the systems. However, the amounts and rates of reduction in DOC were not easily correlated with an increase in sulfate-reducing bacteria populations. Some systems, notably BR-02 and PO-01, had no initial growth in sulfate-reducing bacteria populations, yet there was an initial decline

in the DOC concentration. This indicates that other bacteria likely oxidized the organic substrates for their own growth, and thus competed with sulfate-reducing bacteria for the available substrates. These results support the *in situ* observations of the Potter site, whereby iron reducers were shown to be active (Fortin et al., 2002). The BR-02 systems also had a low pH, which again suggests that iron-reducing bacteria might have been present and oxidized the dissolved organic carbon in the systems.

The abiotic control systems generally did not show any decrease in DOC levels, as indicated in Figures 4-30 to 4-32. Two exceptions exist, however, notably the initial pH 4 systems for pyruvate and lactate. With the lactate system, it is known to have been contaminated with some sort of fungal biofilm which was visible in the system at day 102, thus explaining the decrease in DOC. With respect to the pyruvate system, it can only be speculated that the system was also contaminated. In this case, the possibility of contamination is supported by a concurrent increase in pH, which can be interpreted as the generation of alkalinity from microbial processes (Capone and Kiene, 1998).

5.2 General Overview by System

Using the interpretations and discussion presented above about the trends noticed in all the systems, the following is a quick overview of the various systems.

5.2.1 Broulan Systems

In the BR-01 systems, the initial pH was near-neutral, and thus optimal for sulfate-reducing bacteria growth. Correspondingly, all four systems showed the growth of sulfate-

reducing bacteria. All systems also showed a decrease in DOC, which would seem to indicate that bacteria were active and using the electron donors. However, contrarily to what would be expected with active sulfate-reducing bacteria, SO_4 remained fairly constant. This suggests that perhaps the Broulan tailings had non-sulfate growth strains of sulfate-reducing bacteria, or that perhaps the rate of sulfate reduction from the involved species was significantly slower.

Another aspect of the BR-01 systems is the increase in soluble iron over time in all the systems, except for lactate, followed by a decrease in ferrous iron. This is especially prevalent in the pyruvate and acetate systems. This increase in Fe(II) is a good indication of the presence of iron-reducing bacteria. Since the sulfate-reducing bacteria showed an early and relatively rapid growth, it is likely that they were able to out-compete the iron-reducing bacteria for the acetate at the beginning of the experiment. However, near day 40, there was a sudden drop in the population of sulfate-reducing bacteria. This drop corresponded to an increase in Fe(II) in the system, suggesting that the iron-reducing bacteria must have started to dominate in the competition for acetate. The amount of ferrous iron in the system then started to drop shortly thereafter, and the sulfate-reducing bacteria population once more began to increase, which means that the sulfate-reducing bacteria had likely once more become dominant in the system. A similar event occurred in the BR-01 formate system near day 35. It is, however, unclear why the iron-reducing bacteria became dominant for a short period of time.

The BR-01 pyruvate system started with a large increase in ferrous iron early during the experiment, suggesting the presence of iron-reducing bacteria. The increase in ferrous

iron corresponded to a large increase in sulfate-reducing bacteria in the system. A possible explanation for this co-existence is that the sulfate-reducing bacteria were incomplete oxidizers, oxidizing pyruvate into acetate, which was then used by the iron-reducing bacteria. This explanation is supported by the fact that incomplete oxidation of pyruvate appears to be much more energetically favored than complete oxidation of pyruvate (see equations 16 and 17). If, further to being incomplete oxidizers of pyruvate, the strains were incapable of oxidizing acetate, this would have removed the need to compete with the iron-reducing bacteria, which generally favor acetate as their electron donor, allowing for the coexistence of the two types of bacteria.

Additionally, the relatively large increase in ferrous iron in the BR-01 pyruvate system also supports the hypothesis of non-sulfate growth bacteria, as the reduction of sulfate would have produced hydrogen sulfide, which would have reacted with the ferrous iron and precipitated as iron monosulfides or pyrite.

In the BR-02 systems, the initial pH was acidic. Correspondingly, there was no growth in sulfate-reducing bacteria population until the pH reached circa-neutral levels. This was particularly obvious in the pyruvate system, where the increase in pH and in bacterial population occurred rather rapidly. As with the BR-01 systems, there was no reduction of the concentration of sulfate within the BR-02 systems, once more suggesting that the bacteria in the Broulan tailings might have been facultative non-sulfate growth bacteria.

Fe(II) concentrations in the BR-02 system generally showed a decrease over time. The pyruvate system showed the most rapid decrease, and this correlated well with the large increase in sulfate-reducing bacteria population. This is, therefore, likely the result of the

production of hydrogen sulfide and the precipitation of the iron as iron monosulfides and pyrite. The sulfate concentration in the pyruvate system also showed a slow but steady decline, which substantiates the above conclusion.

Additionally, the acetate and lactate systems showed an increase in ferrous iron near the end of the experiment. In the case of the BR-02 acetate system, there was no significant growth of sulfate-reducing bacteria, and the pH remained below 5. Such conditions are ideal for iron-reducing bacteria, which are known to out-compete sulfate-reducing bacteria in low pH, and are known to favor acetate conditions (Küsel and Dorsch, 2000). The initial lag in the appearance of ferrous iron, and thus the likely activity of iron-reducing bacteria, cannot be explained with certainty but is likely a result of the bacteria adjusting to their new environment.

In the case of the BR-02 lactate system, the increase in ferrous iron by day 40 was rather significant. This corresponded with a simultaneous increase in sulfate-reducing bacteria populations, indicating that the iron-reducers were not out-competing the sulfate-reducers. Since iron-reducing bacteria generally do not prefer lactate, this suggests that the sulfate-reducing bacteria were probably incomplete oxidizers, which oxidized lactate into acetate, which was then used by the iron reducing bacteria. These facts remain circumstantial since iron-reducing bacteria were never enumerated in the systems.

5.2.2 Delnite Systems

Overall, all DE-01 systems had a circa-neutral pH, which was optimal for sulfate-reducing bacteria growth. The increase in bacteria population produced hydrogen sulfide, as

suggested by the decrease in sulfate concentrations, and allowed FeS precipitation, as indicated by the presence of black precipitates in the systems and low levels of soluble Fe. Bacterial activity was also confirmed by the decrease in dissolved organic carbon levels in the systems.

The only unexpected result was the increase in iron concentration in the DE-01 lactate system. This suggests that iron-reducing bacteria might have been active or that abiotic Fe(III) reduction occurred. However, iron-reducing bacteria generally favor acetate over lactate, thus their presence is difficult to explain. It is possible that the incomplete oxidizers of lactate (which would produce acetate as an intermediate compound) dominated over the complete oxidizers, even though the complete oxidation is more energetically favorable (see equations 14 and 15). The production of acetate could have then favored the iron-reducing bacteria. As indicated in equations 14 and 15, complete oxidation of lactate requires significantly more sulfate than incomplete oxidation. The switch between the dominance of complete oxidizers and incomplete oxidizers may have been due to the decrease in available sulfate. On the other hand, recent work has indicated that abiotic reduction of Fe(III) by lactate is also possible (Pers. Comm., J-P. Rioux). It is clear that more work is needed to fully assess the production of soluble Fe in some systems.

In the DE-02 systems, despite the circa-neutral pH, there was a delay before the first significant signs of growth of sulfate-reducing bacteria. This corresponded with a slow decline of sulfate in the DE-02 systems. Contrary to this, however, there was a rapid decrease in dissolved organic carbon during the first few days of the experiment, particularly in the acetate and lactate systems. This is a strong indication that other heterotrophic bacteria were

likely active within the systems. Since there was generally no corresponding increase in ferrous iron concentration levels over time, the presence of iron-reducing bacteria was unlikely. There was, however, some indication of iron reducing bacteria with the increase of dissolved iron in the lactate and pyruvate systems near day 30. Since no analysis of the species of bacteria present was done during this experiment, it is impossible to say what other heterotrophic bacteria were actually present.

5.2.3 Hollinger Systems

As with DE-01, the Hollinger systems (both the HO-01 systems and the HO-02 systems) had a circa-neutral pH which favored the growth of sulfate-reducing bacteria. Consequently, the bacteria populations began to increase almost immediately after inoculation. The sulfate-reducing bacteria were active, as indicated by the reduction in sulfate concentration. There was an initial increase in ferrous iron in the systems, likely the result of the reduction of Fe(III)-minerals, but the concentrations soon dropped to levels below the detection limit, probably by precipitating as iron monosulfides and pyrite. There was also a continuous decrease in dissolved organic carbon concentrations, which confirms that there were active bacteria within the system.

5.2.4 Potter Systems

The PO-01 systems started with an acidic pH, which inhibited the initial growth of sulfate-reducing bacteria. The acidic environment likely favored the iron-reducing bacteria, as suggested by the increase in soluble iron concentration in the systems. The activity of the

iron-reducing bacteria is also supported by the initial decrease of dissolved organic carbon despite the lack of sulfate-reducing bacteria populations. However, as indicated in equation 6, the reduction of iron resulted in the neutralization of acidity, and thereby increased the acidic pH to circa-neutral levels. Once the pH of a system reached 6 or higher, this permitted the growth of sulfate-reducing bacteria. Correspondingly, once sulfate-reducing bacteria appeared, they seemed to out compete the iron-reducing bacteria. The production of hydrogen sulfide likely caused the ferrous iron to precipitate as iron monosulfides and pyrite, as indicated by the decrease in soluble iron concentration.

The PO-02 systems started with a more neutral pH, and thus were optimal for the growth of sulfate-reducing bacteria. There was an initial increase in ferrous iron in the systems, likely the result of iron-reducing bacteria, but the concentrations soon dropped to levels below the detection limit, probably due to iron monosulfides and pyrite formation, as indicated by the formation of black precipitates in the systems. This indicates that the sulfate-reducing bacteria quickly became dominant over the iron-reducing bacteria, which is to be expected in circa-neutral conditions (Küsel and Dorsch, 2000). There was a continuous decrease in dissolved organic carbon concentrations, which confirms that there were active bacteria within the system.

With the exception of the PO-02 pyruvate system, the sulfate concentrations appeared to remain relatively stable throughout the experiment, with a slight increase in the beginning and slight decrease near the end. As mentioned before, it is possible that the bacteria in the acetate, lactate, and formate PO-02 systems were non-sulfate growth bacteria, which would explain the lack of significant decrease in sulfate concentrations. Alternatively, perhaps the

rate of sulfate reduction was simply too low that the decrease was insignificant compared to the total levels of sulfate present in the systems.

5.3 Effects of Type of Tailings on Sulfate-Reducing Bacteria

When comparing the Cu-Zn tailings (Broulan and Potter) with the Au tailings (Delnate and Hollinger), superficially there does not appear to be any significant difference between the two with respect to their effects on the growth of sulfate-reducing bacteria. Other factors, most notably the pH, have a much more significant effect on their growth rate.

However, overall, the Cu-Zn tailings had a much less significant decrease in sulfate concentration than in the Au tailings. This leads to the speculation that perhaps the sulfate-reducing bacteria within those tailings are non-sulfate growth strains. Alternatively, it is possible that the Cu-Zn tailings are rich in sulfur/sulfides, which could provide a significant source of sulfides for the cycling of sulfates. This could therefore allow the systems to remain at equilibrium with respect to sulfate reduction and the dissolution of sulfate from the sulfides in the tailings, thereby maintaining a relatively constant sulfate concentration throughout the duration of the experiment.

This indicates that hypothesis number 3, which suggested that the tailings would not have any effect on the population levels of sulfate reducing bacteria, has therefore not been refuted.

5.4 Effects of Vegetation on Sulfate-Reducing Bacteria

When comparing tailings with vegetation to the tailings without vegetation with

respect to the growth of sulfate reducing bacteria, there does not appear to be any significant difference. This is most obvious when comparing the two Hollinger systems (HO-01 and HO-02), which have nearly identical results in sulfate-reducing bacteria growth, as well as pH, soluble iron concentrations, and sulfate concentration.

Hypothesis number 2, which suspected that the vegetated tailings would have overall larger sulfate reducing bacteria populations, is therefore inaccurate.

5.5 Effects of Electron Donor on Sulfate-Reducing Bacteria

When comparing the acetate, formate, lactate, and pyruvate systems to each other, there are a few trends that are readily visible.

Pyruvate systems always seem to have the greatest and fastest increase in sulfate-reducing bacteria population levels. Since the incomplete oxidation of pyruvate is the most energetically favorable reaction, this comes as no surprise. Lactate systems always follow closely behind pyruvate with respect to growth rates and population levels. Again, since the oxidation of lactate, both complete and incomplete, is the next most favorable reaction, it is only logical that lactate is the next most favorable electron donor.

Acetate and formate systems alternated between the third and fourth most effective electron donor, with acetate having more significant growth and populations slightly more often than formate. Although formate is much more energetically favored than acetate, the oxidation of formate does not produce any material that is useful for cell wall development and growth (Badziong et al., 1979). To the contrary, acetate is useful as building material for sulfate-reducing bacteria, which would explain why acetate is generally more favored than

formate. In the systems in which the rate of growth with formate dominated over the growth with acetate, it is likely that other elements within the systems provided the formate-using species other sources of material for the construction of their cell walls.

Finally, it should be noted that despite the observations from this experiment, several considerations are required before drawing specific conclusions with respect to the exact rate or exact population levels according to electron.

With both pyruvate and lactate, it is likely that acetate is generated from incomplete oxidation, which in turn can allow acetate using sulfate-reducing bacteria to also grow within the systems. Since the MPN solutions used all electron donors equally, acetate using bacteria could easily have been included in the population count, thereby creating a false reading that may be higher than the actual population levels of only pyruvate or only lactate using populations. Likewise, some bacteria, which only use specific electron donors not present in any given system, may have adapted alternative non-sulfate growth pathways, such as nitrate reduction, thereby allowing them to grow within the system even without their normal electron donor. Overall, such considerations would likely affect the results of all systems equally, and thus not affect overall conclusions, just specific values and rates. Still, such possibilities should always be taken into consideration.

Future studies should aim to specifically identify the species of sulfate-reducing bacteria present and determine what metabolic pathways are available to those species. This would help clarify specific effects of any given electron donor, or other element, found within the system. Likewise, future studies should look into separate measurements for the various electron donors rather than overall dissolved organic carbon, thereby accounting for

the possibility of the production of acetate during the incomplete oxidation of lactate or pyruvate.

Hypothesis number 1, which suspected that the favored electron donor would be pyruvate, followed by lactate, formate, and acetate (in that order) remains inconclusive. Although pyruvate and lactate were indeed the favored electron donor according to the growth rate of the bacteria, the methodology and results of this experiment cannot account for the possibility that bacteria using other substrates within the system may have been present, or if acetate was generated by the incomplete oxidation of pyruvate or lactate, thus artificially increasing their numbers. In either case, formate does not seem to be preferred over acetate.

6 CONCLUSION

The results of this study indicate that sulfate-reducing bacteria are present and likely active in mine tailings. The bacteria in the tailings seem capable of using multiple sources of carbon as electron donor, including acetate, pyruvate, lactate, and formate. Whether this is simply strains of bacteria that can use more than one electron donor, or multiple strains specialized in one electron donor, or a combination thereof, cannot be determined with the results of this experiment.

pH is likely one of the most important *in situ* factors affecting the growth of sulfate reducing bacteria. It is clear that sulfate-reducing bacteria do not seem to grow or be active in acidic conditions. The bacteria do not die out at low pH, however, as they quickly become active again once the pH reaches circa-neutral conditions. In the more acidic conditions, iron-reducing bacteria appear to dominate over the sulfate-reducing bacteria.

The presence of vegetation over the tailings does not appear to have any effect on the growth of the sulfate-reducing bacteria found below them.

The type of tailings – whether Cu-Zn or Au – does not seem to have any real direct effect on the growth of sulfate-reducing bacteria. It is possible, however, that the type of tailings has an indirect effect on the growth of sulfate reducing bacteria. For one, the type of tailings will generally determine local geochemical factors such as pH, which has a much more direct influence on sulfate-reducing bacteria populations. Likewise, the type of tailings may also play a role in determining the availability of sulfate (perhaps through its sulfide concentration and sulfur cycling), or iron (which is necessary to precipitate the toxic HS⁻ product of sulfate reduction). Therefore, the overall geochemistry of the tailings is more

important than the specific type of tailings (i.e., whether it is Cu-Zn or Au tailings)

Finally, the most favored electron donor of the sulfate-reducing bacteria taken from the tailings appears to be pyruvate, followed closely by lactate. Acetate and formate seem more or less equally acceptable to the bacteria, but not nearly as favored as pyruvate or lactate.

Ultimately, this study reinforces the fact that sulfate-reducing bacteria, and iron-reducing bacteria, likely play a major role in the geochemistry of mine tailings. Modeling of geochemical cycling cannot be based solely on chemical reactions and must take bacterial processes into consideration. Likewise, it suggests that bioremediation of mine tailings and acid rock drainage may indeed be a feasible approach to environmental management of mine sites. Although sulfate reducing bacteria will likely play a major role in this process due to their ability to precipitate metals, they are clearly not the only bacteria required as they do not appear to be active in acidic conditions. However, if combined with bacteria that can generate alkalinity, such as perhaps iron-reducing bacteria, such a complex biogeochemical system may indeed provide a workable solution to current mining issues.

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APPENDIX A: Eh Results

The Eh measurements were made at regular intervals throughout the course of the experiment. The measurements of the various inoculated systems are presented in Figures A-1 to A-4, while the Eh measurements of the abiotic control systems are presented in Figures A-5 to A-7. However, it became apparent during the course of the experiment that the Eh probe was not functioning correctly. A second probe was therefore used to measure the final Eh values of the systems.

Because the Eh values of all the inoculated systems have a significant drop at approximately the same time (circa day 20 for most systems), followed by a significant increase near the time of the change of probes (circa day 40 for most systems), this leads to the speculation that perhaps the readings of the first non-functioning probe were inaccurately lower than the actual Eh values in the systems between days 20 to 40.

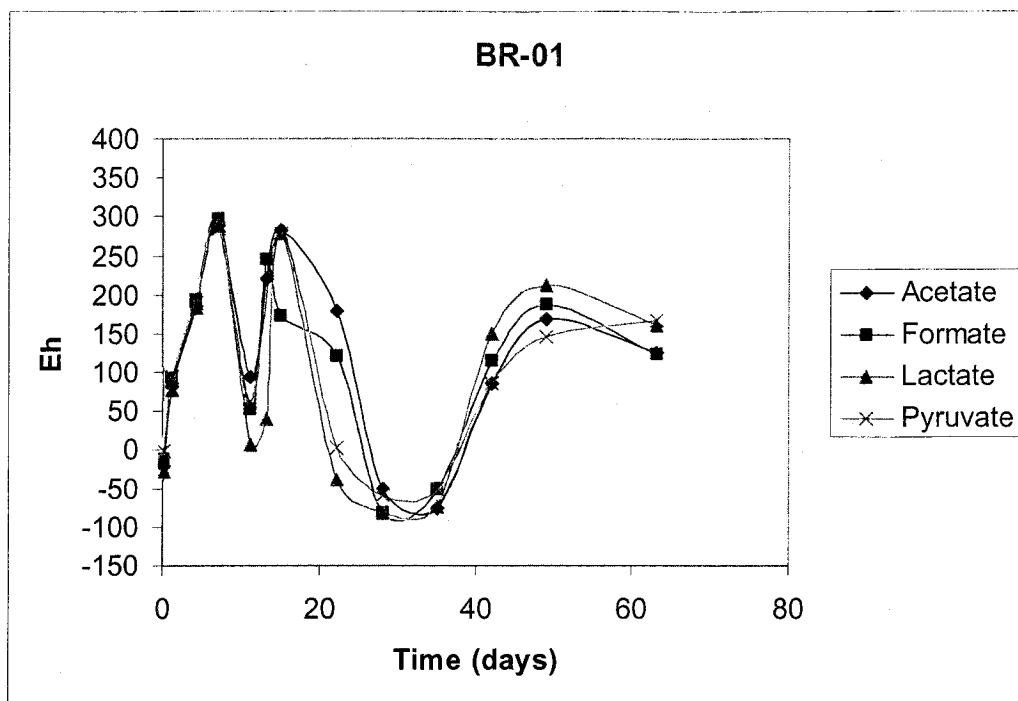
The Eh measurements in the abiotic control systems are likely more accurate, because they were performed after changing the Eh probe. In all the control systems, the Eh started near 0 mV, increased rapidly in the first few days to approximately 400 mV, where it leveled off for the remainder of the experiment (Figures A-5 to A-7). The only exception is the initial pH 4 pyruvate abiotic control system which started off like the other systems, but quickly decreased at day 20, dropping to 150 mV by the end of the experiment.

The control systems would indicate that the Eh values should not have dropped and gone back up between days 20 to 40, thereby supporting the speculation that the measurements between days 20 to 40 are erroneous. However, it is also acknowledge that

microbiological activity could easily have been the cause for the decrease and subsequent rise of the Eh level.

Ultimately, it is impossible to conclude if the obtained results are truly a representation of what is occurring in the systems, or merely erroneous readings from a faulty probe. The Eh results were therefore separated from the other results of this study and placed in this Appendix to clearly indicate that the values are unreliable.

a)



b)

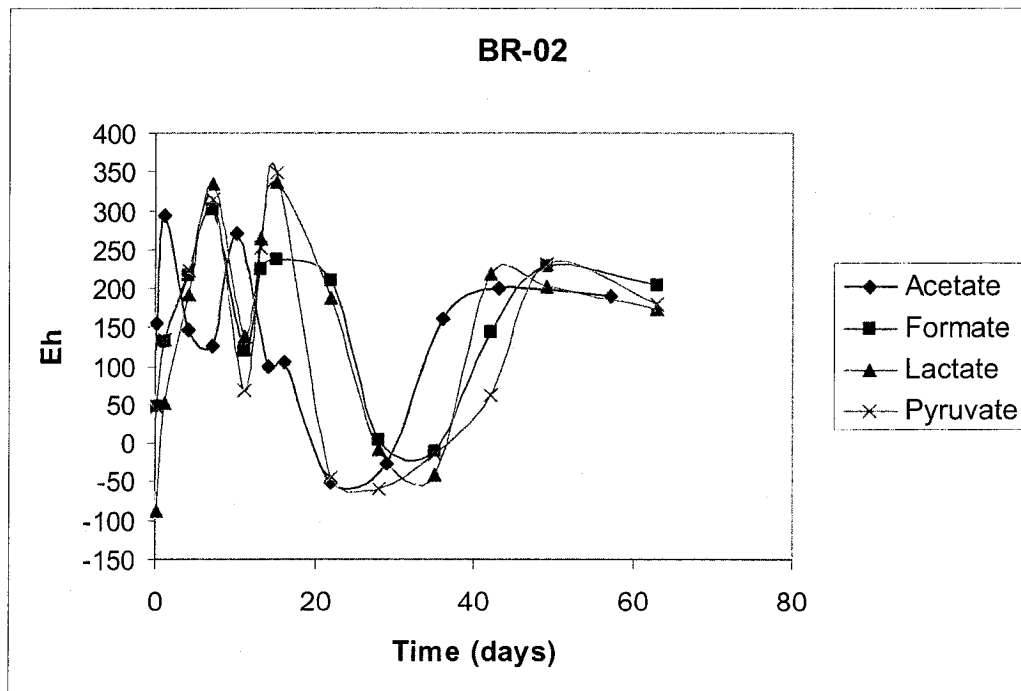
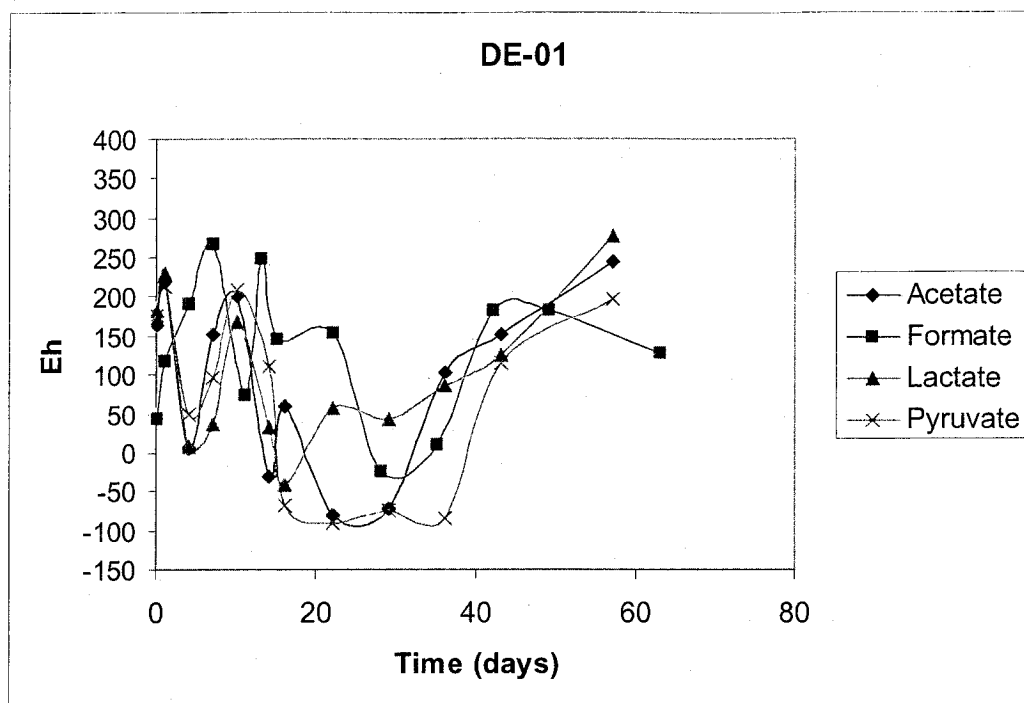


Figure A-1 Eh (mV) evolution of the various systems containing the Broulan tailings. **Note:** These values are unreliable.

a)



b)

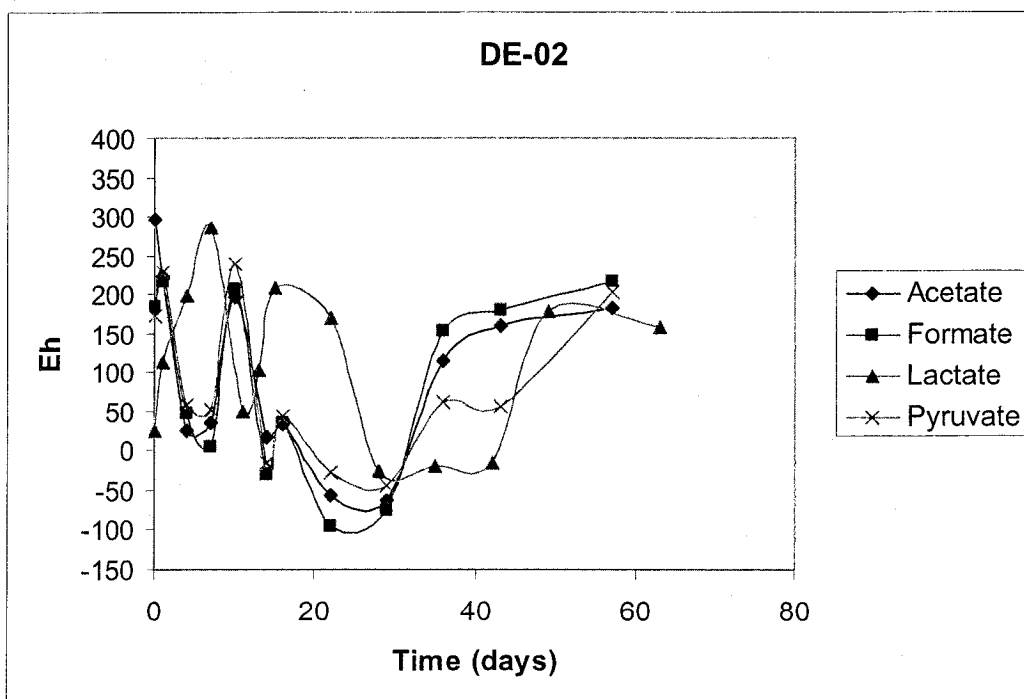
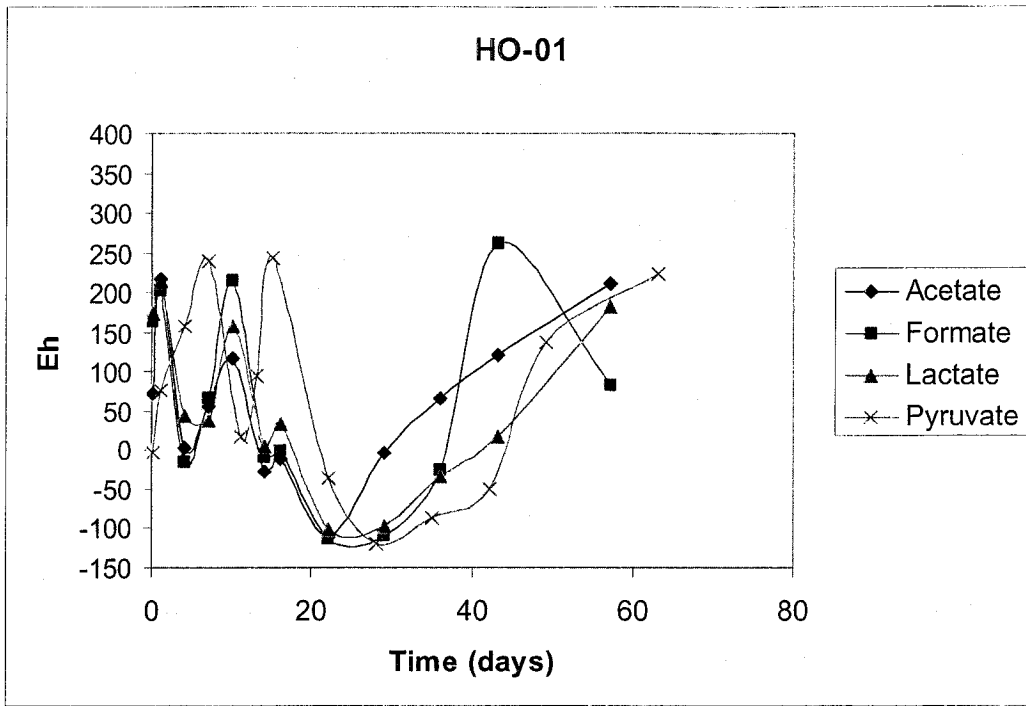


Figure A-2 Eh (mV) evolution of the various systems containing the Delnrite tailings. **Note:** These values are unreliable.

a)



b)

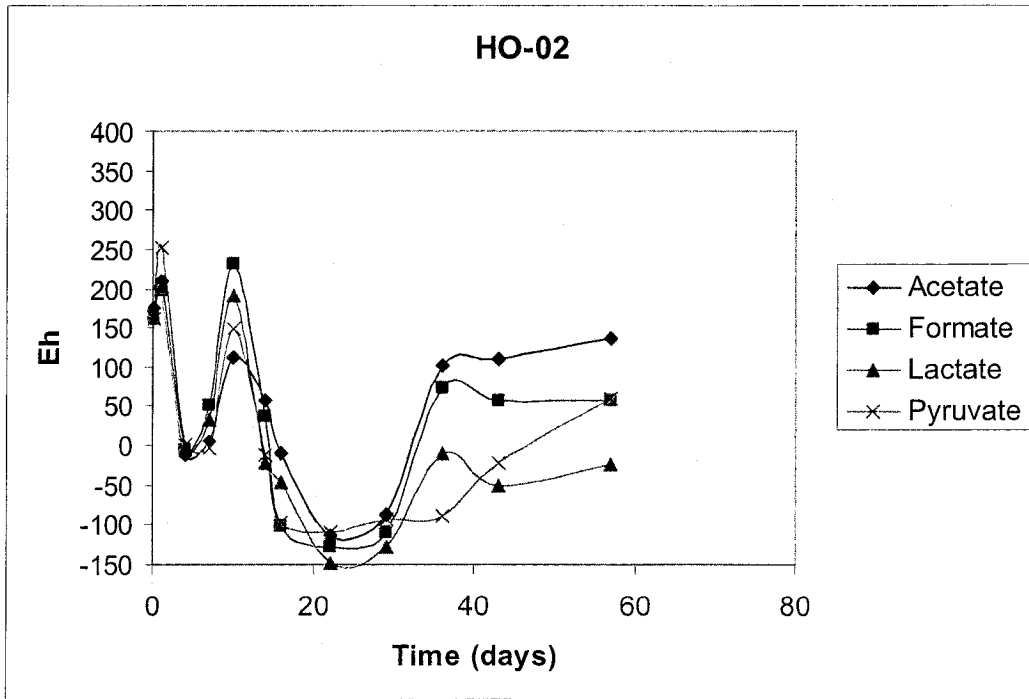
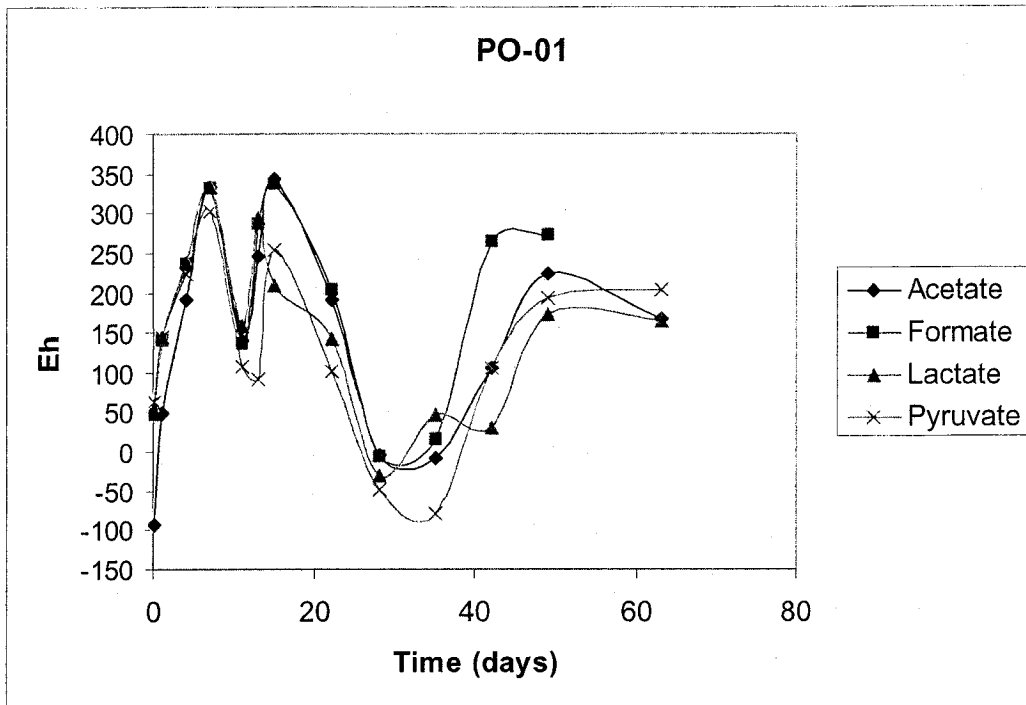


Figure A-3 Eh (mV) evolution of the various systems containing the Hollinger tailings.
Note: These values are unreliable.

a)



b)

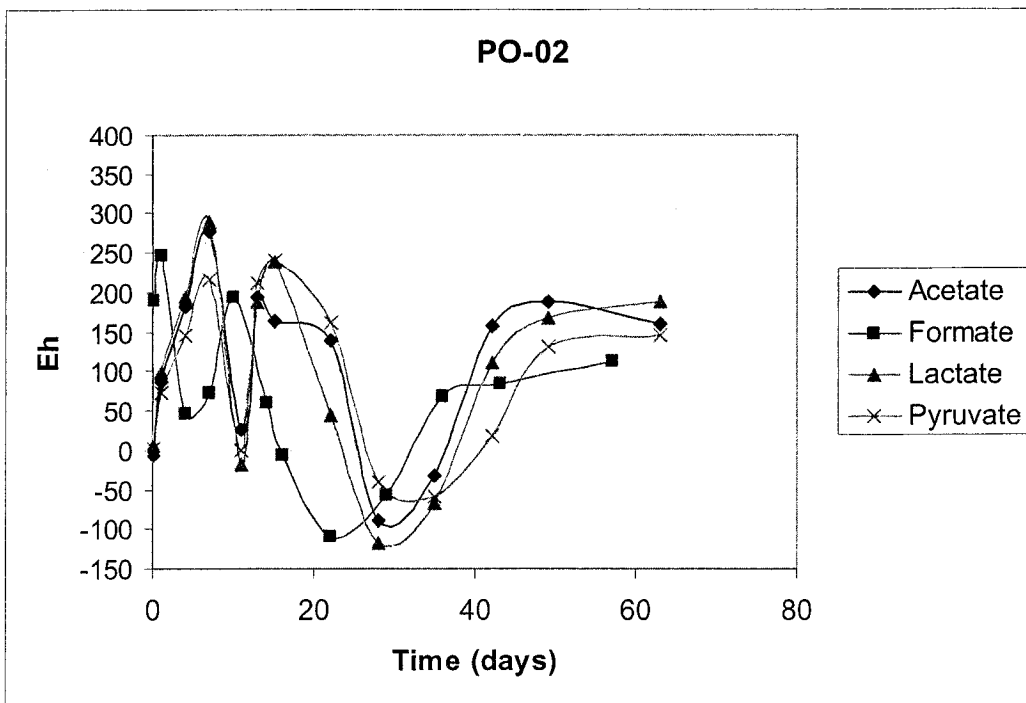
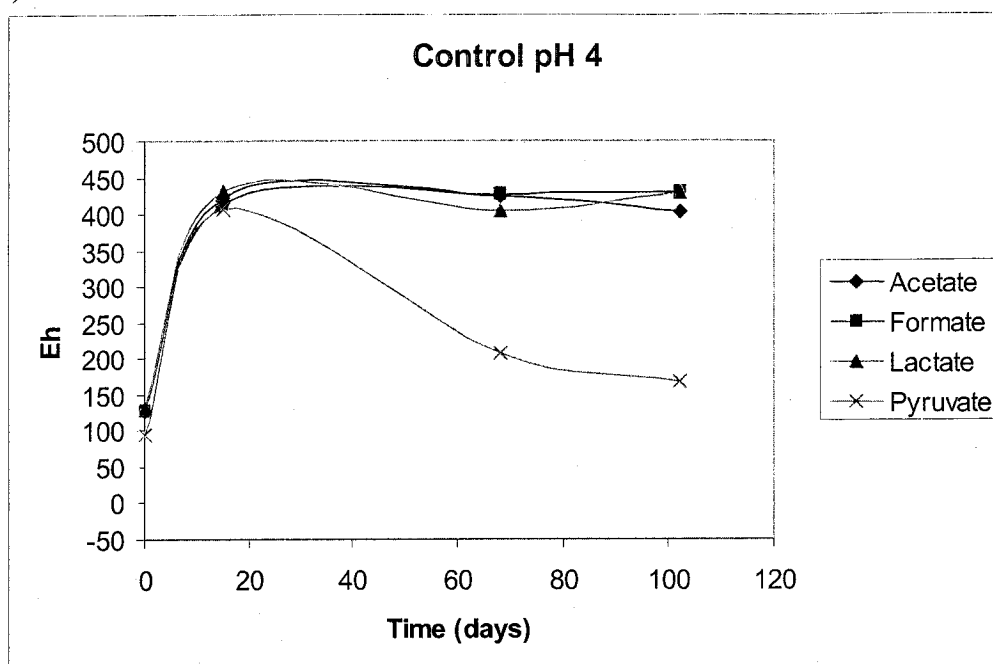


Figure A-4 Eh (mV) evolution of the various systems containing the Potter tailings. **Note:** These values are unreliable.

a)



b)

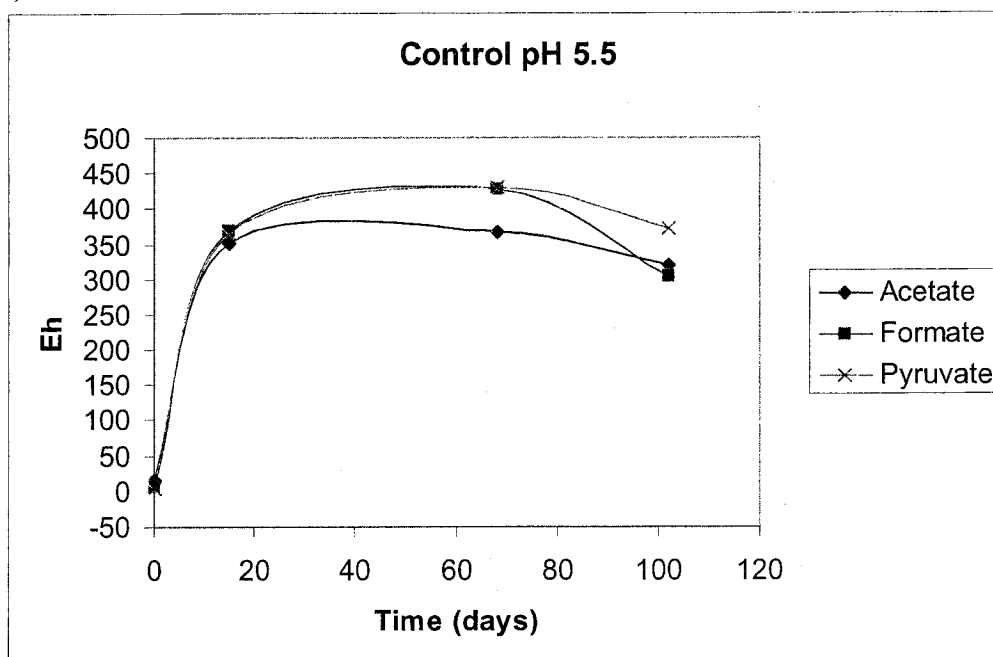
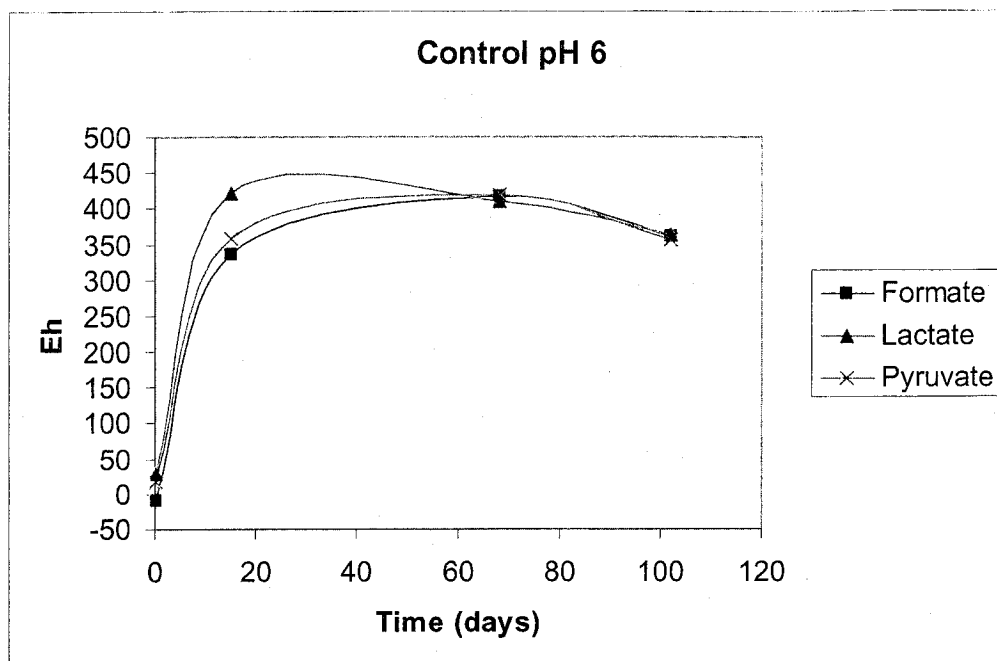


Figure A-5 Eh (mV) evolution of the abiotic control systems with an initial pH of a) 4 and b) 5.5.

a)



b)

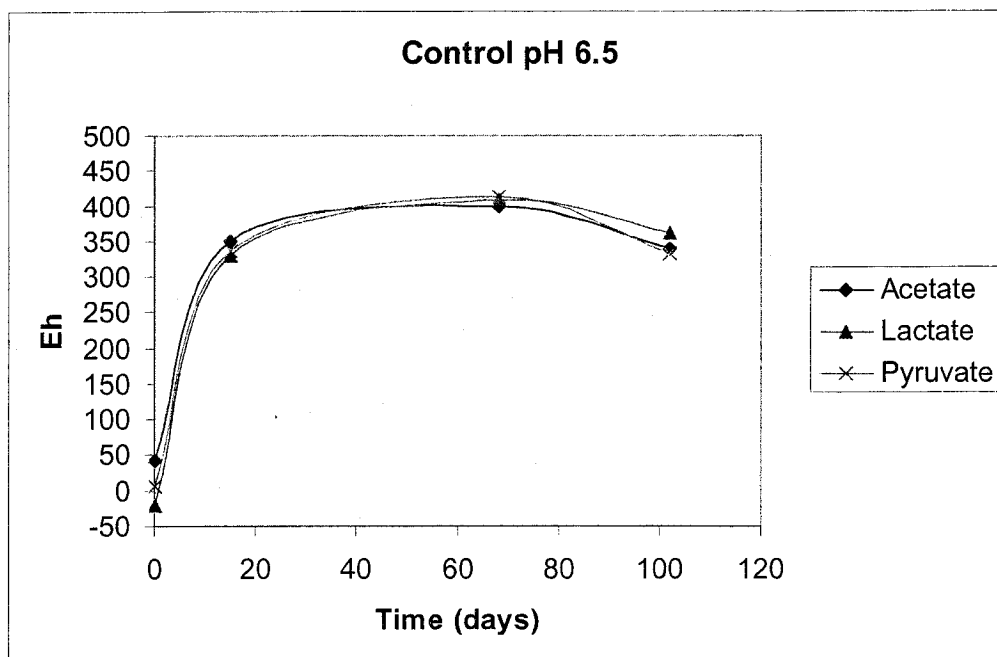


Figure A-6 Eh (mV) evolution of the abiotic control systems with an initial pH of a) 6 and b) 6.5.

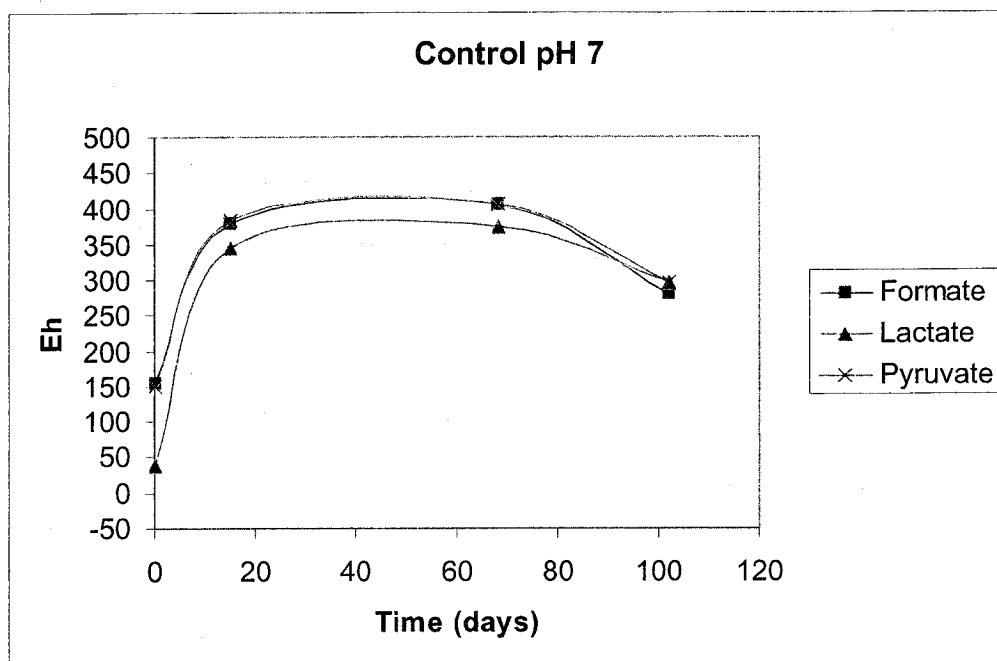


Figure A-7 Eh (mV) evolution of the abiotic control systems with an initial pH of 7.

APPENDIX B: MINEQL+ 3.1 Results (Saturation Indices)

Table B-1: Saturation Indices for BR-01 and BR-02 systems

	BR-01 A	BR-01 F	BR-01 L	BR-01 P		BR-02 A	BR-02 F	BR-02 L	BR-02 P
Sulfate minerals	pH 6.63	pH 6.74	pH 6.66	pH 6.75		pH 3.88	pH 4.26	pH 4.22	pH 4.28
ANTLERITE	-6.776	-6.283	-6.536	-6.278		-17	-15.444	-15.605	-15.362
CuOCuSO4	-16.239	-15.95	-16.079	-15.943		-21.2	-20.408	-20.494	-20.36
BROCHANTITE	-8.047	-7.35	-7.729	-7.347		-23.535	-21.214	-21.45	-21.098
LANGITE	-9.992	-9.295	-9.674	-9.292		-25.48	-23.16	-23.395	-23.043
Ni4(OH)6SO4	-24.251	-23.572	-24.048	-23.573		-40.732	-38.451	-38.688	-38.333
Zn3O(SO4)2	-28.856	-28.529	-28.717	-28.52		-34.197	-33.416	-33.508	-33.359
Zn4(OH)6SO4	-14.112	-13.477	-13.93	-13.483		-30.511	-28.24	-28.469	-28.133
Zn2(OH)2SO4	-8.267	-8.009	-8.175	-8.007		-12.683	-12.917	-12.999	-12.872
GYPSUM	-1.064	-0.986	-1.012	-0.978		-1.003	-0.987	-0.998	-0.972
CHALCANTHITE	-7.385	-7.3	-7.304	-7.291		-7.082	-7.055	-7.066	-7.04
MELANTERITE	-5.21	-6.863	-4.979	-5.373		-4.554	-4.501	-4.749	-4.568
EPSOMITE	-2.443	-2.363	-2.39	-2.356		-2.378	-2.362	-2.373	-2.347
MIRABILITE	-3.637	-3.541	-3.577	-3.531		-3.573	-3.552	-3.567	-3.532
RETGERSITE	-7.875	-7.795	-7.823	-7.787		-7.82	-7.804	-7.815	-7.788
MORENOSITE	-7.532	-7.452	-7.48	-7.444		-7.477	-7.461	-7.472	-7.445
ZnSO4, 1H2O	-7.857	-7.788	-7.81	-7.781		-7.782	-7.768	-7.777	-7.755
GOSLARITE	-6.293	-6.224	-6.246	-6.217		-6.217	-6.203	-6.213	-6.191
BIANCHITE	-6.531	-6.462	-6.484	-6.455		-6.456	-6.442	-6.451	-6.429
ANHYDRITE	-1.325	-1.247	-1.273	-1.24		-1.264	-1.248	-1.259	-1.234
CuSO4	-13.279	-13.195	-13.198	-13.186		-12.977	-12.95	-12.961	-12.935
MnSO4	-12.193	-12.113	-12.138	-12.105		-12.056	-12.039	-12.051	-12.023
THENARDITE	-4.816	-4.72	-4.756	-4.71		-4.752	-4.731	-4.746	-4.712
ZINCOSITE	-11.544	-11.475	-11.497	-11.468		-11.469	-11.455	-11.464	-11.442
Iron minerals									
FeHPO4	-5.709	-7.421	-5.527	-5.941		-7.446	-7.036	-7.305	-7.104
FeH2PO4	-6.039	-7.861	-5.887	-6.381		-5.026	-4.996	-5.225	-5.084
Ca2(PO4)2Fe	13.224	11.588	13.418	13.062		8.73	9.513	9.21	9.455
WUSTITE	-3.621	-5.066	-3.401	-3.661		-8.561	-7.771	-8.071	-7.814
Fe(OH)2	-3.995	-5.527	-3.766	-4.049		-8.905	-8.114	-8.426	-8.162
VIVIANITE	0.787	-4.169	1.379	0.269		-7.596	-5.985	-6.835	-6.169
MELANTERITE	-5.21	-6.863	-4.979	-5.373		-4.554	-4.501	-4.749	-4.568
Fe3(PO4)2	0.787	-4.169	1.379	0.269		-7.596	-5.985	-6.835	-6.169

Table B-2: Saturation Indices for DE-01 and DE-02 systems

	DE-01 A	DE-01 F	DE-01 L	DE-01 P		DE-02 A	DE-02 F	DE-02 L	DE-02 P
Sulfate minerals	pH 6.02	pH 6.46	pH 5.95	pH 6.02		pH 5.41	pH 5.51	pH 5.93	pH 5.58
ANTLERITE	-8.802	-7.096	-8.972	-8.674		-11.047	-10.544	-9.017	-10.333
CuOCuSO ₄	-16.975	-16.169	-17.041	-16.921		-18.156	-17.88	-17.08	-17.729
BROCHANTITE	-11.364	-8.759	-11.638	-11.161		-14.672	-13.942	-11.688	-13.671
LANGITE	-13.31	-10.704	-13.583	-13.106		-16.617	-15.887	-13.634	-15.616
Ni ₄ (OH) ₆ SO ₄	-28.24	-25.465	-28.649	-28.123		-31.651	-31.052	-28.693	-30.777
Zn ₃ O(SO ₄) ₂	-29.939	-28.972	-30.069	-29.847		-31.009	-30.8	-30.044	-30.726
Zn ₄ (OH) ₆ SO ₄	-18.633	-15.686	-19.04	-18.35		-21.632	-21.06	-18.962	-21.012
Zn ₂ (OH) ₂ SO ₄	-9.604	-8.628	-9.737	-9.511		-10.632	-10.434	-9.712	-10.395
GYPSUM	-0.688	-0.739	-0.685	-0.729		-0.825	-0.808	-0.714	-0.721
CHALCANTHITE	-6.829	-6.924	-6.793	-6.851		-6.949	-6.899	-6.825	-6.808
MELANTERITE	-4.282	-4.596	-4.321	-4.294		-4.486	-4.498	-4.38	-4.289
EPSOMITE	-2.048	-2.104	-2.045	-2.092		-2.192	-2.175	-2.076	-2.083
MIRABILITE	-3.079	-3.172	-3.078	-3.156		-3.313	-3.291	-3.132	-3.145
RETGERSITE	-7.488	-7.54	-7.485	-7.531		-7.633	-7.616	-7.516	-7.524
MORENOSITE	-7.145	-7.197	-7.142	-7.188		-7.29	-7.273	-7.173	-7.181
ZnSO ₄ , 1H ₂ O	-7.603	-7.612	-7.599	-7.604		-7.645	-7.634	-7.6	-7.599
GOSLARITE	-6.038	-6.048	-6.035	-6.04		-6.081	-6.07	-6.036	-6.035
BIANCHITE	-6.277	-6.286	-6.273	-6.278		-6.319	-6.308	-6.274	-6.273
ANHYDRITE	-0.95	-1.001	-0.947	-0.99		-1.086	-1.069	-0.976	-0.982
CuSO ₄	-12.724	-12.819	-12.688	-12.745		-12.843	-12.794	-12.72	-12.703
MnSO ₄	-11.732	-11.812	-11.725	-11.779		-11.872	-11.855	-11.759	-11.757
THENARDITE	-4.259	-4.351	-4.258	-4.336		-4.492	-4.471	-4.311	-4.324
ZINCOSITE	-11.29	-11.299	-11.286	-11.291		-11.332	-11.321	-11.287	-11.286
Iron minerals									
FeHPO ₄	-5.749	-5.67	-5.841	-5.661		-6.176	-6.122	-5.846	-6.031
FeH ₂ PO ₄	-5.469	-5.83	-5.491	-5.381		-5.286	-5.332	-5.476	-5.311
Ca ₂ (PO ₄) ₂ Fe	12.175	12.796	12.019	12.353		11.359	11.505	12.053	11.534
WUSTITE	-4.552	-3.862	-4.729	-4.472		-5.704	-5.537	-4.762	-5.361
Fe(OH) ₂	-4.912	-4.232	-5.091	-4.828		-6.06	-5.895	-5.124	-5.717
VIVIANITE	-0.211	0.629	-0.574	0.049		-2.212	-1.939	-0.615	-1.579
MELANTERITE	-4.282	-4.596	-4.321	-4.294		-4.486	-4.498	-4.38	-4.289
Fe ₃ (PO ₄) ₂	-0.211	0.629	-0.574	0.049		-2.212	-1.939	-0.615	-1.579

Table B-3: Saturation Indices for HO-01 and HO-02 systems

	HO-01 A	HO-01 F	HO-01 L	HO-01 P	HO-02 A	HO-02 F	HO-02 L	HO-02 P
Sulfate minerals	pH 6.71	pH 6.68	pH 6.81	pH 7.22	pH 6.24	pH 6.33	pH 6.22	pH 6.26
ANTLERITE	-6.386	-6.369	-6	-5.264	-8.05	-7.55	-7.926	-7.786
CuOCuSO ₄	-15.928	-15.869	-15.734	-15.526	-16.806	-16.465	-16.621	-16.557
BROCHANTITE	-7.579	-7.604	-7	-5.735	-10.03	-9.369	-9.965	-9.75
LANGITE	-9.525	-9.549	-8.946	-7.681	-11.975	-11.314	-11.91	-11.695
Ni ₄ (OH) ₆ SO ₄	-23.833	-24.048	-23.221	-20.748	-26.588	-26.077	-26.767	-26.524
Zn ₃ O(SO ₄) ₂	-28.472	-28.51	-28.262	-27.476	-29.537	-29.212	-29.407	-29.332
Zn ₄ (OH) ₆ SO ₄	-13.858	-14.143	-13.26	-10.796	-16.435	-16.038	-16.771	-16.524
Zn ₂ (OH) ₂ SO ₄	-8.062	-8.134	-7.859	-7.052	-9.004	-8.794	-9.019	-8.94
GYPSUM	-0.851	-0.8	-0.841	-0.861	-1.01	-0.871	-0.832	-0.837
CHALCANTHITE	-7.152	-7.052	-7.15	-7.472	-7.244	-7.062	-6.998	-7.011
MELANTERITE	-4.587	-4.599	-4.547	-5.168	-5.122	-4.862	-4.369	-4.468
EPSOMITE	-2.222	-2.169	-2.212	-2.234	-2.387	-2.243	-2.201	-2.207
MIRABILITE	-3.344	-3.271	-3.335	-3.362	-3.571	-3.383	-3.325	-3.333
RETGERSITE	-7.654	-7.603	-7.645	-7.664	-7.822	-7.678	-7.638	-7.644
MORENOSITE	-7.311	-7.26	-7.302	-7.321	-7.479	-7.335	-7.295	-7.301
ZnSO ₄ , 1H ₂ O	-7.677	-7.643	-7.671	-7.693	-7.801	-7.686	-7.656	-7.66
GOSLARITE	-6.113	-6.079	-6.107	-6.128	-6.237	-6.121	-6.091	-6.096
BIANCHITE	-6.351	-6.317	-6.345	-6.367	-6.475	-6.36	-6.33	-6.334
ANHYDRITE	-1.112	-1.061	-1.103	-1.122	-1.272	-1.132	-1.093	-1.099
CuSO ₄	-13.046	-12.947	-13.045	-13.366	-13.138	-12.957	-12.893	-12.906
MnSO ₄	-11.958	-11.896	-11.951	-11.988	-12.111	-11.961	-11.909	-11.918
THENARDITE	-4.524	-4.451	-4.515	-4.542	-4.751	-4.563	-4.505	-4.512
ZINCOSITE	-11.364	-11.33	-11.358	-11.38	-11.488	-11.373	-11.343	-11.347
Iron minerals								
FeHPO ₄	-5.358	-5.462	-5.295	-5.801	-5.876	-5.764	-5.4	-5.467
FeH ₂ PO ₄	-5.768	-5.842	-5.805	-6.721	-5.816	-5.794	-5.32	-5.427
Ca ₂ (PO ₄) ₂ Fe	13.489	13.31	13.653	13.575	12.642	12.745	12.957	12.937
WUSTITE	-3.167	-3.315	-2.937	-2.679	-4.382	-4.147	-3.959	-3.965
Fe(OH) ₂	-3.526	-3.679	-3.295	-3.066	-4.755	-4.517	-4.308	-4.319
VIVIANITE	1.957	1.597	2.315	1.532	-0.306	0.155	1.092	0.948
MELANTERITE	-4.587	-4.599	-4.547	-5.168	-5.122	-4.862	-4.369	-4.468
Fe ₃ (PO ₄) ₂	1.957	1.597	2.315	1.532	-0.306	0.155	1.092	0.948

Table B-4: Saturation Indices for PO-01 and PO-02 systems

	PO-01 A	PO-01 F	PO-01 L	PO-01 P		PO-02 A	PO-02 F	PO-02 L	PO-02 P
Sulfate minerals	pH 4.13	pH 4.16	pH 4.16	pH 4.27		pH 5.98	pH 5.47	pH 5.85	pH 6.16
ANTLERITE	-16.122	-15.983	-15.867	-15.413		-8.908	-10.689	-9.261	-8.167
CuOCuSO ₄	-20.603	-20.523	-20.52	-20.31		-17.118	-17.965	-17.262	-16.671
BROCHANTITE	-22.376	-22.179	-21.949	-21.25		-11.433	-14.149	-11.994	-10.398
LANGITE	-24.321	-24.124	-23.894	-23.195		-13.378	-16.094	-13.94	-12.343
Ni ₄ (OH) ₆ SO ₄	-39.543	-39.387	-39.17	-38.472		-28.22	-31.267	-28.991	-27.299
Zn ₃ O(SO ₄) ₂	-33.671	-33.635	-33.492	-33.272		-29.889	-30.882	-30.129	-29.582
Zn ₄ (OH) ₆ SO ₄	-29.868	-29.758	-29.195	-28.435		-18.203	-21.231	-18.979	-17.556
Zn ₂ (OH) ₂ SO ₄	-13.344	-13.308	-13.138	-12.898		-9.498	-10.501	-9.749	-9.246
GYPSUM	-0.695	-0.685	-0.791	-0.825		-0.839	-0.832	-0.826	-0.722
CHALCANTHITE	-6.766	-6.745	-6.855	-6.89		-7.01	-6.922	-6.945	-6.858
MELANTERITE	-3.867	-3.724	-18.218	-4.126		-4.381	-4.362	-4.354	-4.22
EPSOMITE	-2.055	-2.044	-2.157	-2.193		-2.208	-2.2	-2.195	-2.085
MIRABILITE	-3.098	-3.08	-3.267	-3.32		-3.332	-3.329	-3.319	-3.143
RETGERSITE	-7.497	-7.486	-7.599	-7.635		-7.646	-7.641	-7.634	-7.523
MORENOSITE	-7.154	-7.143	-7.256	-7.292		-7.303	-7.298	-7.291	-7.18
ZnSO ₄ , 1H ₂ O	-7.595	-7.596	-7.622	-7.642		-7.659	-7.649	-7.648	-7.604
GOSLARITE	-6.031	-6.031	-6.058	-6.078		-6.095	-6.085	-6.083	-6.04
BIANCHITE	-6.269	-6.269	-6.296	-6.316		-6.333	-6.323	-6.322	-6.278
ANHYDRITE	-0.957	-0.946	-1.053	-1.087		-1.101	-1.093	-1.087	-0.983
CuSO ₄	-12.661	-12.639	-12.75	-12.785		-12.905	-12.817	-12.84	-12.753
MnSO ₄	-11.719	-11.706	-11.826	-11.864		-11.907	-11.881	-11.886	-11.777
THENARDITE	-4.278	-4.259	-4.446	-4.499		-4.512	-4.508	-4.498	-4.323
ZINCOSITE	-11.282	-11.283	-11.309	-11.329		-11.346	-11.336	-11.335	-11.291
Iron minerals									
FeHPO ₄	-7.074	-6.927	-21.176	-6.915		-5.569	-5.979	-5.656	-5.505
FeH ₂ PO ₄	-4.904	-4.787	-19.036	-4.885		-5.249	-5.149	-5.206	-5.365
Ca ₂ (PO ₄) ₂ Fe	8.991	9.149	-4.918	9.497		12.547	11.638	12.329	12.635
WUSTITE	-7.914	-7.74	-21.298	-7.634		-4.443	-5.448	-4.691	-4.137
Fe(OH) ₂	-8.252	-8.073	-22.343	-7.971		-4.793	-5.797	-5.04	-4.49
VIVIANITE	-6.199	-5.727	-48.495	-5.6		0.269	-1.554	-0.152	0.7
MELANTERITE	-3.867	-3.724	-18.218	-4.126		-4.381	-4.362	-4.354	-4.22
Fe ₃ (PO ₄) ₂	-6.199	-5.727	-48.495	-5.6		0.269	-1.554	-0.152	0.7

APPENDIX C: XRD Results and Interpretation

The graphs presented in Figures 4-33 and 4-34 are the X-ray diffraction results of the tailings used in this experiment. The main purpose of the XRD analysis was to compare the mineralogy of the tailings before and after the experiment to aid in the determination of possible geochemical changes that have occurred in the tailings as the likely results of bacterial activity.

In the XRD graph, each peak, referred to as a Bragg Peak, corresponds to one or more minerals. Each mineral will have multiple identifying Bragg Peaks, each one representing a cleavage plane, preferential orientation, or other unique feature of the mineral that is detectable by X-ray diffraction. If all of the major peaks of a mineral are present, then it is likely that the mineral in question is present in the sample.

However, there are a few limitations to be aware of with respect to XRD analysis. Although in theory the Bragg Peaks for a specific mineral should have a specific 2-theta angle, the actual value will vary slightly. This variance can be caused by impurities or non-homogeneity of the sample and/or minerals, or by incorrectly placing the holder in the diffractometer. Such variances, however, will generally not be more than a few hundredths of a degree. Still, this may result in minerals being incorrectly identified. This is most likely with structurally similar minerals which have very similar 2-theta angle values for their peaks.

Additionally, the variance in peak position can cause multiple nearby peaks to effectively overlap. This will mean that a single Bragg Peak may represent more than one mineral. This can result in the failure to identify minerals that are present in the sample.

The minerals identified on the XRD graphs of Figures 4-33 and 4-34 therefore only represent the most probable minerals found in the samples.

Second, XRD can be used for a comparison of the quantity of minerals present, thereby determining if there was an increase or decrease in the quantity of minerals between the before and after samples. However, the counts of a peak do not determine the quantity of the mineral in the sample. Instead, the amount of the mineral is represented by the height of the peak (i.e., its counts) relative to the counts of the background level. A peak of 500 counts over a background of 50 counts therefore has the same quantity of minerals as a peak of 5000 counts over a background of 500 counts. In this case, they both represent a factor of 10x background, and thus both have the same quantity of that particular mineral present. The comparison of the quantities of minerals is therefore, by default, relative.

For this study, the graphs were resized to obtain identical background levels, thereby allowing a direct visual comparison of the peaks to determine relative mineral concentrations between the two samples. This was achieved by assuming that the quartz in the tailings was likely to have remained unchanged by chemical or microbial processes. Comparing the counts value of the quartz peaks, the counts values of the graph with the lower peaks was increased (by multiplication) until they matched the counts value of the second graph. The same multiplication factor was then applied to all count values of the line. The “before” and “after” graphs were then merged into single graphs, as presented below. The matching of the background levels seems to support the assumption that the quantity of quartz did not change in the samples. The resized and merged graphs are presented in Figure C-1 and C-2.

As can be seen in Figure C-1, representing the DE-01 sample, there appears to be clear presence of gypsum in the before sample, and a lack of its presence in the after sample, suggesting that gypsum was depleted or otherwise dissolved during the experience. There also appears to be a possible slight decrease in goethite and diaspore. Otherwise, possible minerals identified in the samples include jarosite, muscovite, quartz, and vermiculite. None of the latter minerals appear to change in quantity during the experiment.

The PO-01 minerals, as presented in Figure C-2, appear to include actinolite, diaspore, dolomite, goethite, gibbsite, jarosite, pyrite, pyrrhotite, quartz, and vermiculite. Of these, goethite appears to decrease by the end of the experiment. Pyrrhotite, on the other hand, does not appear to be present before the experiment, suggesting that it precipitated during the experiment.

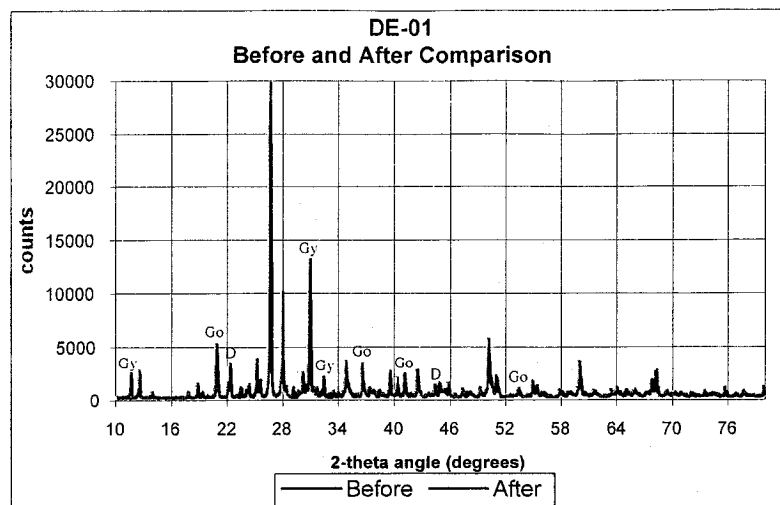


Figure C-1 Comparison of XRD patterns of the DE-01 tailings before and after expirement. Minerals with noticeable changes include: D = Diaspore, Go = Goethite, Gy = Gypsum.

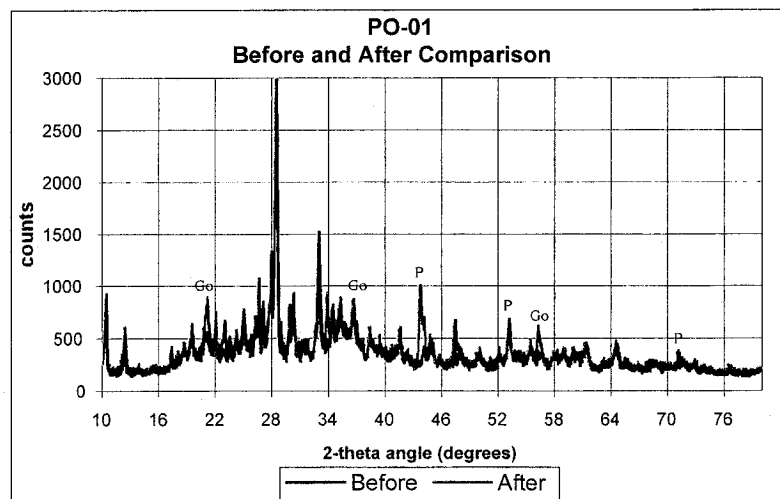


Figure C-2 Comparison of XRD patterns of the PO-01 tailings before and after expirement. Minerals with noticeable changes include: Go = Goethite, P = Pyrrhotite.