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Anaerobic Reductive Bioleaching of Manganese Ores

Neha Sharma
Michigan Technological University, nehashar@mtu.edu

ANAEROBIC REDUCTIVE BIOLEACHING OF MANGANESE ORES

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Neha Sharma

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Department of Chemical Engineering

Thesis Advisor:	*Timothy C. Eisele*
Committee Member:	*Rebecca G. Ong*
Committee Member:	*Lei Pan*
Department Chair:	*Pradeep K. Agrawal*

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Abstract

The increasing demand of manganese in the industries and various hindrances in its production from low grade ores by conventional method has made it imperative for researchers around the world to develop a method of manganese extraction from low grade ores that is both environment friendly and economical. Bioleaching has shown significant potential in manganese extraction and efficiencies of extraction have been found to be 70-98% with the help of various bacteria and fungi.

This study focuses on extraction of manganese with the help of mixed bacterial strains that have been collected from their natural anaerobic environment. The extraction of manganese from reagent grade manganese dioxide and high grade manganese ore has been studied over 130 days at room temperature and pH around 5. Highest concentrations of dissolved manganese have been found to be 866.7 mg Mn/L for reagent grade manganese dioxide and 545.7 mg Mn/L for ore grade manganese.

1 INTRODUCTION

Global manganese ore deposits are abundant but are selectively scattered around the world. United states, as one of the industrialized countries, uses 441,000 metric tons of manganese ores annually (as of 2015) and imports all the manganese that it uses [1, 45]. Large deposits of manganese are present in various districts, but the deposits are low grade and production of manganese from these deposits via conventional methods is uneconomical [1]. Among other uses, manganese is used as an alloying agent in the steel industry [2] and is an important constituent in battery making [3] (Figure 1). The conventional methods of manganese production being a major environment concern [4, 5, 6] along with the lack of high grade manganese ore deposits, require us to look towards alternative sustainable methods of manganese production such as bioleaching.

Scores of researchers have investigated the extraction of manganese from its ores with the help of bacteria and have found various microorganisms that can be employed in such processes [7, 8, 9, 10]. Recent research has also shown the potential with which bioleaching can be applied to extract manganese either from low grade ores or mine wastes [11, 12, 13], but the mechanism involved in these studies varies vastly. The process of extraction of manganese from ores using bacteria can be aerobic or anaerobic. Some organisms might reduce manganese directly as a part of their life process, while others might produce acids which dissolve the manganese

into solution [26, 29]. Convincing evidence for MnO_2 reduction by microbes which will perform this process anaerobically has so far been demonstrated only in enrichment cultures with lactate, succinate, and acetate as electron donors [8].

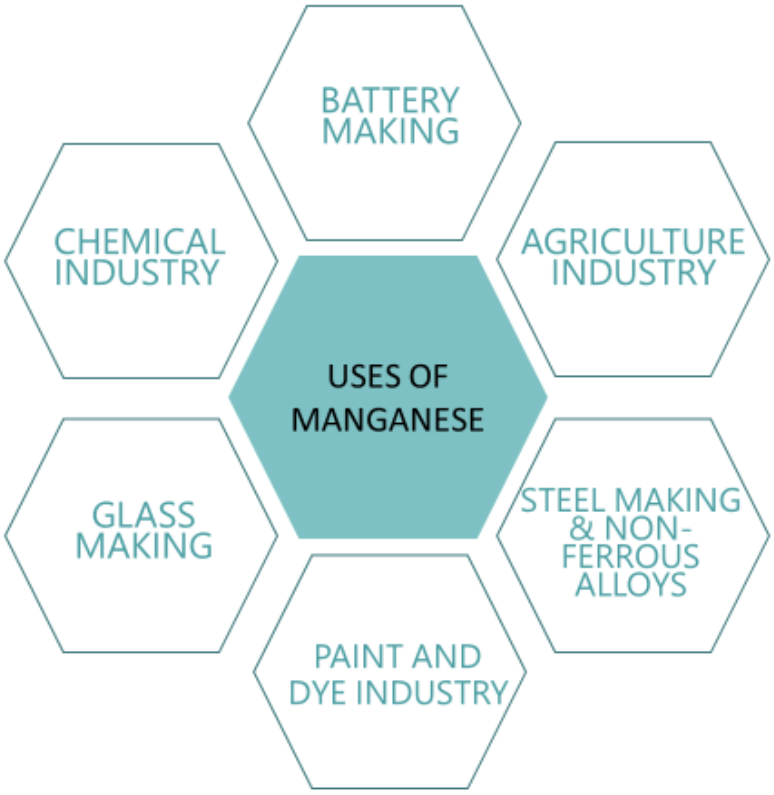

Figure 1. Uses of Manganese. After Ghosh et al., 2016.

Figure 1 shows the common uses of manganese in different forms. More than 90% of manganese in the US and globally is used in the steel industry. Significant amount is used in battery making and other industries. A total of 336,000 metric tons of ferromanganese was used in the steel industry in the US in 2015 according to the

US geological survey. 20,600 metric tons of manganese metal was also consumed by the US in 2015. A total of 441,000 metric tons of manganese ores of all grades were imported by the US of which about 7,000 metric tons was manganese dioxide mainly used in battery making. [45]

In-situ mining of manganese using bacteria and fungi has also been discussed in some studies [12, 31, 32]. The lack of oxygen might decrease the efficiency of manganese extraction as seen in some cases [12], but this problem could be solved using anaerobic bacteria since there will be no need to supply oxygen into the in-situ mine in order to obtain a high efficiency of bioleaching. Sulphur dioxide has extensively been investigated as a lixiviant for manganese dissolution in case of in-situ manganese extraction [40] but the use of anaerobic bacteria could make the process more economical and environment friendly.

1.1 Current Manganese Extraction Technologies

Manganese ores are treated using the common methods of washing, gravity separation, magnetic separation, flotation, and chemical processing. Washing is usually done under hydraulic force which removes most of the mud and surface gangue thereby enriching the ore. Most carbonate ores require washing which can be done with a shaker water spray. [41]

Gravity separation is an important step in manganese ore beneficiation as it separates the ores from gangue particle like silicates which decrease the efficiency of

extraction of manganese in later steps [41, 42]. Gravity separation serves as an efficient method for separating manganese oxide ores and silicates which have a considerable difference in their specific gravity. This process, however, is not very efficient for other manganese ores, in which case magnetic separation is the preferred process. Manganese oxide and carbonate ores are weakly magnetic to paramagnetic [41] which make them a suitable candidate for magnetic separation. High intensity magnetic separation has shown to increase the grade of a manganese ore containing 44% manganese to 51% manganese at 95% recovery [43].

Froth flotation is used for the beneficiation of low to medium grade ores because of their floatability characteristics. Pyrolusite (MnO_2) and Psilomelane ($(Ba,H_2O)_2Mn_5O_{10}$) are commonly enriched using froth flotation. Oleic acid as a collector and pine oil as a frother are commonly used in the flotation of manganese ores. Various other factors such as particle size, gangue minerals, and pH along with the dosage of collector and frother can play important role in the recovery of manganese by froth flotation. [44]

These methods of beneficiation and extraction consume a large amount of energy and are not always suitable for low grade manganese ores. These processes are only economical if the starting grade of ore is good. Bioleaching is a possible solution to the problems faced by the conventional manganese extraction technologies.

1.2 Aerobic and Anaerobic Reduction of Manganese Dioxide

MnO_2 serves as a terminal electron acceptor in respiration for various microorganisms. This is one of the several possible electron acceptors, with the most common electron accepting reactions shown here:

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \qquad E^0 = 1.272 \text{ Volts} \qquad (1)$$

$$2NO_3^- + 12H^+ + 10e^- \rightarrow N_2 + 6H_2O \qquad E^0 = 1.225 \text{ Volts} \qquad (2)$$

$$MnO_2 + 4H^+ + 2e^- \rightarrow Mn^{2+} + 2H_2O \qquad E^0 = 1.224 \text{ Volts} \qquad (3)$$

$$Fe^{3+} + e^- \rightarrow Fe^{2+} \qquad E^0 = 0.770 \text{ Volts} \qquad (4)$$

A higher E^0 value for the oxygen reaction tells us that, everything else being equal, oxygen is more favored as an electron acceptor than MnO_2. However, these reactions ignore the relative availabilities of O_2 and MnO_2 in the environment. In aerobic environment oxygen is present as an electron acceptor but in anaerobic environment, due to the lack of free oxygen, any MnO_2 in soil or rock becomes preferable. Why some bacteria can reduce MnO_2 in aerobic environments lies in the fact that the concentration of dissolved O_2 in water that bacteria encounter is far less than the concentration of Mn(IV) in MnO_2, which is insoluble, and as a consequence, bacteria must be in physical contact with it to reduce it [14].

In anaerobic environments, owing to the lack of oxygen, and nitrate ion not being readily available in the environment, MnO_2 becomes a promising candidate as an electron acceptor. Bacterial reduction of Mn(IV) oxide has been reported by many studies [14, 15]. It can also be seen from the above equations that iron can act as an electron acceptor in the absence of other favorable species. This can be seen in the environment and has been demonstrated successfully in previous studies [16, 17, 18]. The reduction of iron and manganese oxides in the environment can, to some extent, be attributed to the fact that they are insoluble solids and are contained in the mineral component of solids and sediments, where they remain until anoxic conditions allow their utilization as electron acceptors [19]. In environments where manganese and iron oxides coexist, manganese oxides may be reduced preferentially as has been observed in the bacterial attack of ferromanganese nodules [20], but whether this is because of the lower midpoint potential for the Fe(III)/Fe(II) couple relative to Mn(IV)/Mn(II) couple or some other reason remains to be determined [14].

As the anaerobic reductive leaching of manganese requires physical contact with bacteria in most cases [14], particle size plays an important role. Lower particle size offers higher surface area as a result of which high yields can be expected. A previous study has proposed a particle size of 42 micron to be optimum [25, 30]. Bacteria have also been known to attach themselves to particles using nanowires which are used for electron transfer [39].

1.3 Mechanism of Manganese Dioxide Reduction

1.3.1 Direct reduction

The mechanism of manganese oxide reduction depends on the life processes of bacteria being used, which vary with one bacterium species to another. In the case of reductive bioleaching in anaerobic environment, two pathways of manganese reduction can be discussed [14, 22]:

$$MnO_2 + 4H^+ + 2e^- \rightarrow Mn^{2+} + 2H_2O \quad (5)$$

The above process is the direct reduction of Mn(IV) to Mn(II) in a single step with 2 electron change. Below is the reduction involving two successive steps with one electron change in each step. The process below was reported to form an unidentified Mn(III) complex as an intermediate [14, 22], which was reported to diffuse and then be reduced to Mn(II) by bacteria.

$$MnO_2 + 4H^+ + e^- \rightarrow Mn^{3+}_{complex} + 2H_2O \quad (6)$$

$$Mn^{3+}_{complex} + e^- \rightarrow Mn^{2+}\ (aq) \quad (7)$$

Considering the standard equilibrium potentials for the above half cell reactions at pH 7.0, which are +0.45V, -0.55V, +1.54V, for reactions 5, 6, and 7 respectively, the half cell reaction 5 would seem more favorable. However, enzymes specific to the reactions 6 and 7 could make this sequence of reactions possible. [14]

There can be numerous electron pathways involved in the reduction of manganese [9, 23], but the above reactions 5, 6, and 7 summarize the main mechanism of manganese reduction. This mechanism is usually encountered during the direct bioleaching manganese ores which involves a direct contact between the bacteria and the surface of the ore [25]. Manganese plays an important role in microorganism life functions and takes part in various redox reactions. MnO_2 is used as a final electron acceptor during respiration in some manganese reducing bacteria in the absence of oxygen during the direct reduction process [26, 27]. Figure 2 [26] explains the process of manganese reduction when the bacteria are in contact with the surface of ores.

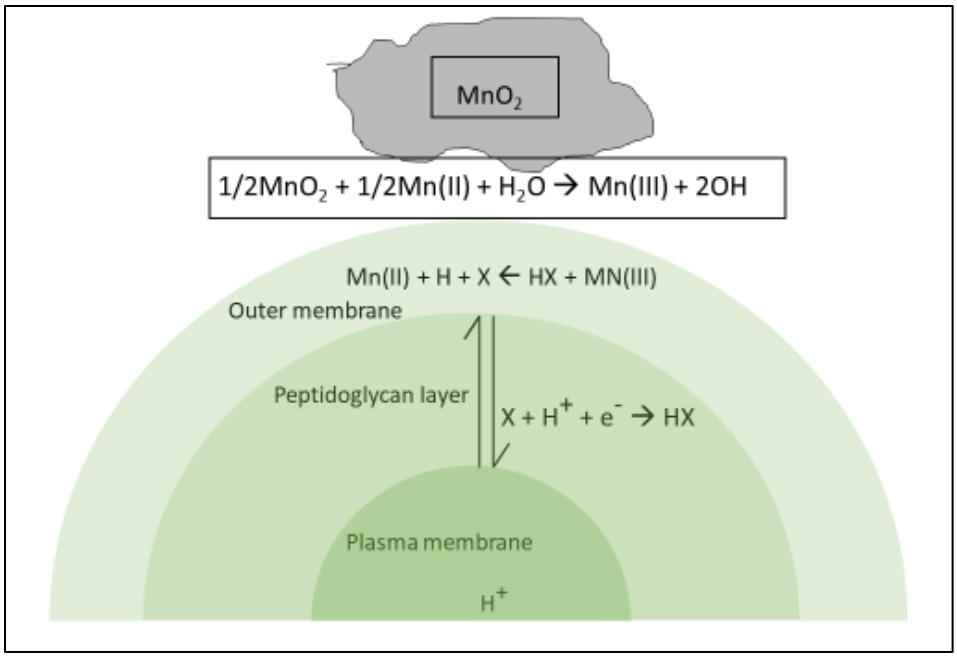

Figure 2. A model explaining the transfer of electrons across the interface between a bacterial cell surface and MnO2. Source: https://doi.org/10.1016/j.chemosphere.2016.04.028 after [26].

Since bacteria can take up manganese in their cell membranes [28], there is usually a maximum tolerable concentration of manganese in which bacteria can survive. A previous study done on manganese reducing abilities of bacterial strain *Acinetobacter* sp. show a maximum tolerable concentration of manganese for these bacteria to be 1000 mM [11] or 55 g/L. This limit can vary from one bacterium species to another.

Figure 3. shows the pourbaix diagram for manganese and it can be seen that pH plays an important role in the dissolution of manganese. Adjustment to the

correct pH is also a necessary condition for the growth of leaching bacteria. pH values in the range of 2.0–2.5 are optimum for the bacterial oxidation of ferrous iron and sulfide, below which considerable inhibition of bacteria will occur [25]. A previous study has found a pH of 3.5 to be optimum for high efficiency reductive bioleaching of manganese in anaerobic conditions using *Microbacterium trichothecenolyticum* Y1 [24].

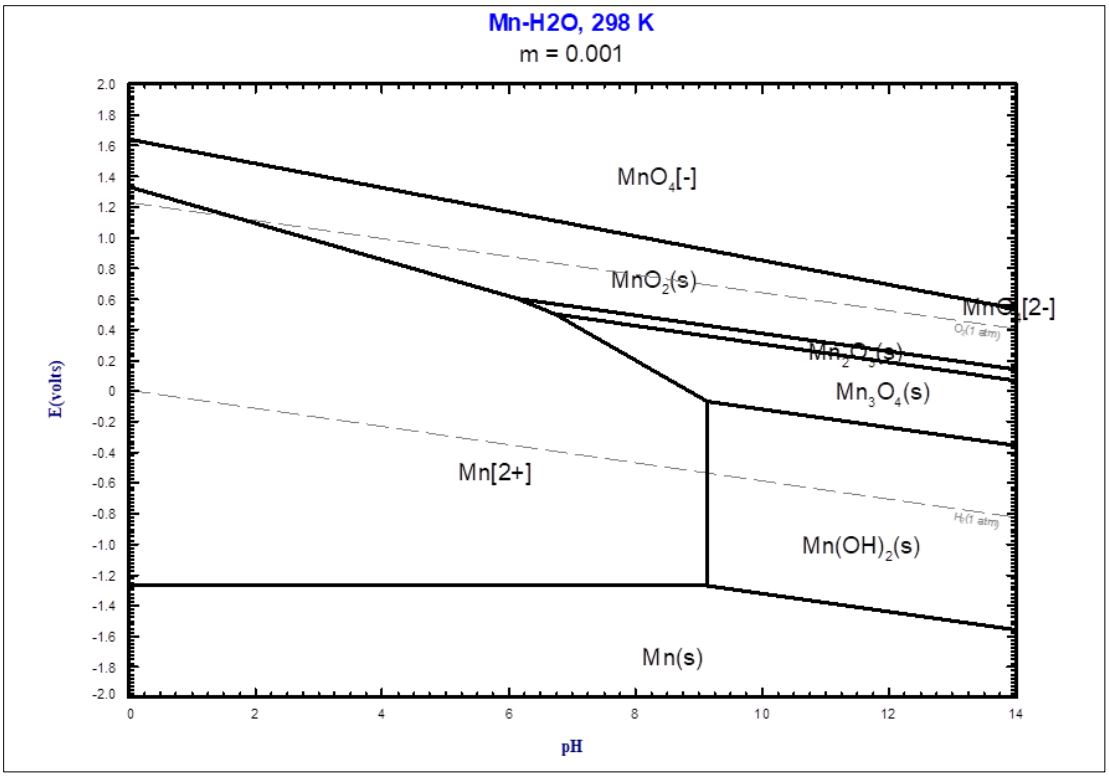

Figure 3. Manganese pourbaix diagram

Table below shows a list of some bacteria that have been used in manganese extraction and the corresponding efficiencies with which the process occurred. The first two studies in Table 1 do not specify the environment conditions in which bioleaching was conducted. *Acinetobacter sp.* MSB 5 is a strictly aerobic species of

bacteria which were used to solubilize manganese from mining waste, hence it is possible that the reduction was aerobic. *Bacillus cereus* is an aerobe or a facultative anaerobe, hence the reduction process in the study could have been aerobic or anaerobic.

Table 1. Manganese reducing bacteria and their Mn recovery.

Microorganism	%Mn recovery	Reference
Acinetobacter sp. MSB 5	76	Ghosh and Das (2017)
Bacillus cereus	76	Das and Ghosh (2018)
Microbacterium trichothecenolyticum	98	Lan et al., (2020)

1.3.2 Indirect Reduction

Indirect reduction of manganese oxides usually involves the production of organic acids by microorganisms, which in turn digest the ores to make Mn^{2+} ions available in the solution [26, 27, 29]. The microorganisms involved here have been found to be various types of fungi along with bacteria [9, 11, 12, 26, 31, 32]. These microorganisms have been known to produce organic acids like oxalic acid, citric acid, and gluconic acid [33, 34] which play an important role in the reduction of manganese [35].

A recent research on the fungal bioleaching of manganese ores by *Aspergillus niger* [36] has detailed out the mechanism with which the fungus reduce manganese by the production of various organic acids. Previous literature has shown that oxalic acid can leach manganese more effectively than other organic acids produced by *A. niger* [29], in which case the nutrient media plays an important role since *A. niger* produces more oxalic acid in a glucose medium compared to a sucrose medium where it produces more citric acid [36]. Two mechanism of manganese reduction, an autocatalytic and a noncatalytic pathways were suggested [37] and discussed [36].

The autocatalytic pathway involves the dissolution of a little amount of manganese as Mn(II) which is complexed with oxalic acid on the surface of MnO_2. The manganese complex formed (manganese oxalate, MnC_2O_4) attaches to the surface of MnO_2 and catalyzes the reduction of Mn(IV) to Mn(II). As an end product, manganese is reduced and mobilized, some oxalic acid is degraded, and some oxalate ions are reproduced. A system of equations for the process is given below. [36, 37]

$$Mn^{2+} + C_2O_4^{2-} \rightarrow MnC_2O_4\ (aq) \quad (8)$$

$$H_2C_2O_4 + (MnO_2)_m + MnC_2O_4 \rightarrow (MnO_2)_{m-1} + 2[Mn(C_2O_4)]^+ + 2OH^- \quad (9)$$

$$[Mn(C_2O_4)]^+ \rightarrow Mn^{2+} + CO_2 + C_2O_4^{2-} \quad (10)$$

The noncatalytic pathway includes the adhesion of oxalic acid on the surface of MnO_2 which is reduced to MnO accompanied by the formation of CO_2 and H_2O.

MnO is then reduced and leached out as Mn(II) by other organic acids produced by the fungus in the medium. [36, 37, 38]

Table below shows a list of some fungi that have been used in manganese extraction and the corresponding efficiencies with which the process occurred.

Table 2. Manganese reducing fungi and their Mn recovery.

Fungal species	% Mn recovery	Reference
Aspergillus sp.	79	Mohanty et al., 2017
Penicillium citrinum	64.58	Acharya et al., 2002
Aspergillus niger	80	Keshavarz et al., 2021

1.4 Project Hypothesis

The current project aims to develop a method of manganese extraction, and future iron extraction, by a completely natural process in a natural habitat from low grade manganese and iron ore sites. The experiments involve the use of mixed bacterial cultures which are provided with a nutrient media (*typha latifolia*, also called cattails) obtained at the site from which bacteria cultures were taken. These organisms were selected for their ability to reduce manganese to the highly soluble Mn^{2+} state, without the need for mineral acids. The aim of the project is to study the

manganese extraction by these bacteria and modifying the environment to an extent which is sustainable as well as efficient and economical.

The study focuses on direct reduction of manganese ores by bacteria and developing a method by which manganese can be digested into the solution to an extent where electrolytic manganese dioxide can be produced efficiently and economically.

2 MATERIALS AND METHODS

2.1 Manganese Leaching Flask Setup

Experiments were performed with bacteria that were freshly obtained from a fen at location 47°06'57.1"N 88°36'46.3"W where metal-reducing activity was evident in the Keweenaw peninsula of Upper Michigan, US, and bacteria that had been taken from the same fen and cultivated in the laboratory for 6 months in a manganese leach bucket setup explained in section 2.3, so that they had been adapted to a manganese rich environment. Six leaching flasks were set up with 200 ml of the following base nutrient media: 0.5g ammonium acetate ($C_2H_7NO_2$); 0.6g sodium phosphate ($NaH_2PO_4.H_2O$); 0.1g potassium chloride (KCl); 10g sodium acetate ($NaC_2H_3O_2$); 5g Sucrose, adjusted to 1L with distilled water. Each flask contained 100g of reagent grade MnO_2 or MnO_2 ore with 200 ml base nutrient media and 120 ml of inoculant. The Manganese ores used in the experiments were obtained from Manganese mine, Copper Harbor, Keweenaw Co., MI, USA. The location of the mine is 47° 27' 17" North, 87° 51' 54" West. Figure 6 and 7 show the manganese ore obtained from the mine. The ore was crushed to pass through 6 mesh using a jaw crusher, gyratory crusher and a short head crusher. The particle size distribution of the crushed ore is shown in figure below. The XRF analysis of the ore resulted in an 80% MnO_2 content (±1%).

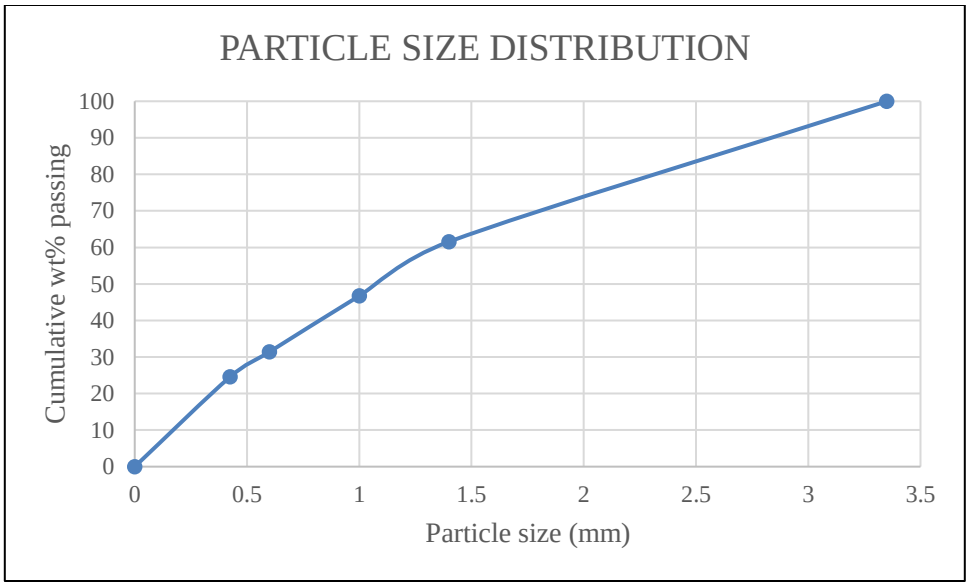

Figure 4. Particle size distribution for manganese ore.

Figure 5. Manganese mine, Copper Harbor, Keweenaw Co., MI, USA

Figure 6. Manganese ore from Manganese mine

Figure 7. Anaerobic bioleaching flask setup

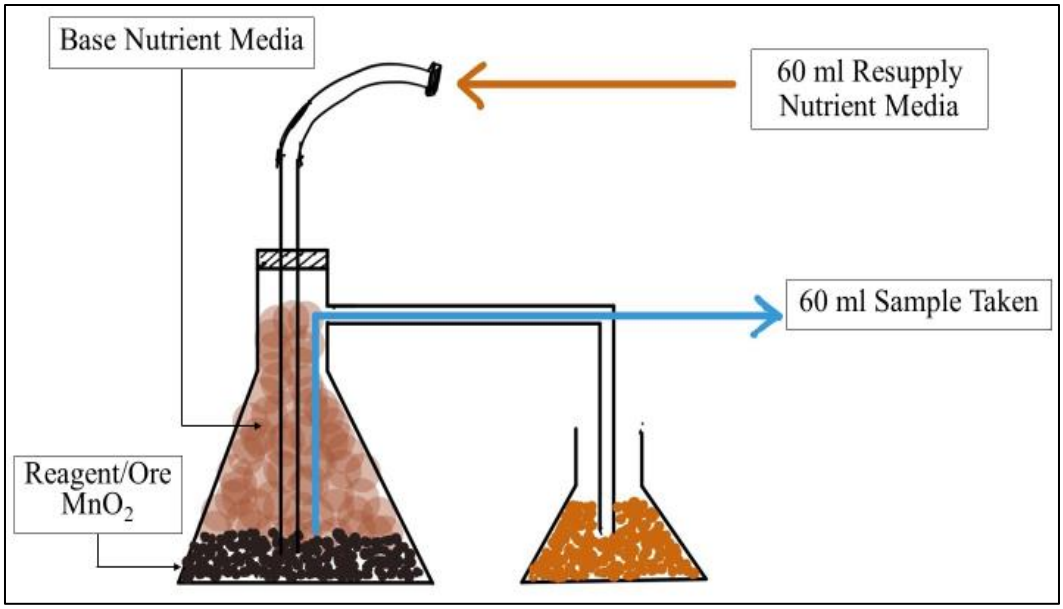

Figure 8. Anaerobic bioleaching process diagram

The cattails decant was produced by putting the cattails in a 5 gallon container and flooding it with water so that it fermented over time. The pH of cat tails decant was found to be 6.1. The leaching flasks were resupplied with 60ml of nutrient media which was produced by adding 500ml of cattails decant and 2.5g granulated sugar (Figure 8). Leaching flasks were maintained at anaerobic conditions by installing pipes that remained submerged in another flask containing cattails decant. Manganese leaching was performed at room temperature. The pH of resupply nutrient media was found to be 6.9. The pH of leaching flasks is given in the table below (Table 3).

Table 3. pH of bioleaching flasks.

Flask	pH
BR1: Fresh fen bacteria; Reagent grade MnO2	5.2
BO2: Fresh fen bacteria; MnO2 Ore	4.9
BO3: Duplicate of BO2	4.9
MR1: Cultivated bacteria; Reagent grade MnO2	5.5
MO2: Cultivated bacteria; MnO2 Ore	5.1
MO3: Duplicate of MO2	5.2

2.2 Qualitative and Quantitative Manganese Analysis

2.2.1 Qualitative analysis

The initial samples were qualitatively tested for manganese by adding the sample solution to 0.01ml (2 drops) of 5M $CuSO_4$ solution, 0.5ml (10 drops) of saturated $NaHCO_3$ solution and 0.5ml (10 drops) of bleach. The solutions turned pink or purple to indicate the presence of manganese. Figure below shows the color of solution if manganese was present in it.

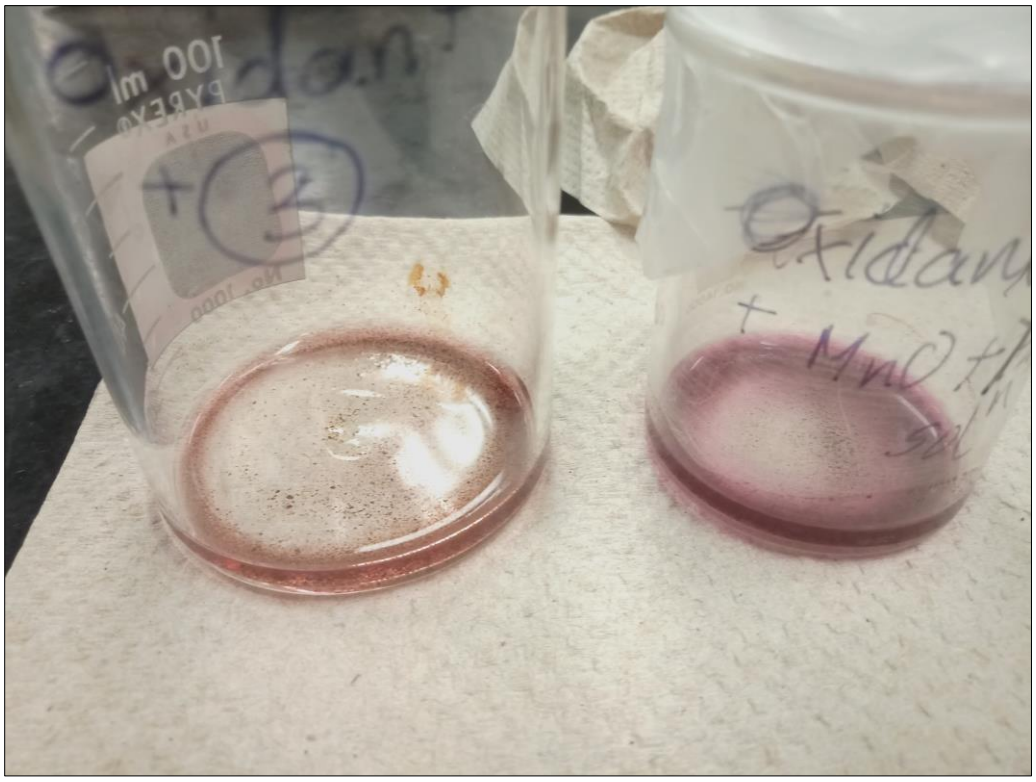

Figure 9. Qualitative manganese analysis showing pink colored solutions indicating the presence of manganese.

The above method also detected iron in the solution, and hence a more reliable method, the periodate method was used to detect and quantify manganese in the solution with the help of a Hach test kit as detailed below.

2.2.2 Quantitative Analysis

Leaching flasks were supplied with 60 ml of resupply nutrient media once a week and 60 ml of samples from the leaching flasks were taken out (at the same time)

to test for manganese concentration using a Hach Test kit. The Hach test kit, model MN-5, product number 146700, supplied by Hach was used for analysis.

The samples obtained from leaching flasks were high in concentration and the test kit used detected manganese up to a concentration of 3mg/L (±0.14 mg Mn/L). The samples were hence diluted by adding 5 to 10 drops (0.25ml to 0.5ml) of the sample solution to 80ml of distilled water. The diluted sample was then used to fill a flat bottomed test tube which was inserted into the manganese color disc setup. A citrate buffer pillow was then added to the diluted sample in another test tube and mixed. A periodate pillow was added next and mixed and was let to sit for 2 minutes by which time the solution turned pink to indicate the presence of manganese. This pink solution test tube was inserted into the manganese color disc setup and the reading was taken by matching the color on the disc. The above described method is the periodate method for determining the amount of manganese in a solution.

2.3 Manganese Leach Bucket setup

A leaching bucket setup was made with soil from the bog, which contained bacteria, and a small hole to collect the percolating water from the bottom of the bucket. The bottom of the bucket was fitted with a nylon mesh topped with crushed ore and gravel. The bucket was supplied with cattails decant once a week to supply organic nutrients to the bacteria. The obtained percolated water was allowed to settle, the soluble manganese was allowed to reoxidise in the presence of air and precipitate,

and the precipitate was separated and dried. 0.6g of the dried precipitate was digested in 30ml of 2M H_2SO_4 and analyzed for manganese content. Figure below shows the leach bucket setup.

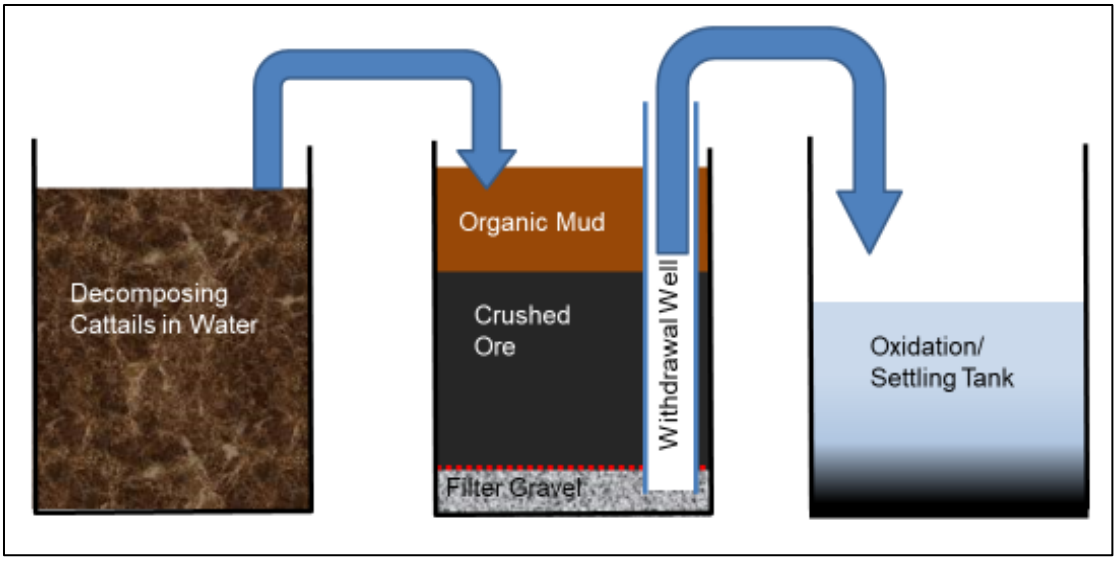

Figure 10. Manganese leach bucket setup.

2.4 MR1 Fungus Manganese Analysis

The bioleaching flask MR1 developed a layer of pink fungus (Figure 13). The same fungus developed on the sample taken from that flask after it was allowed to sit for a few days (Figure 14). The fungus from two samples dated 10/28/20 and 11/30/20 were isolated and dried at 100 degrees Celsius. 0.07g and 0.06g of the respective samples were digested with 10ml 2M H_2SO_4. The solution was then analyzed for manganese using the Hach test.

3 RESULTS

The samples obtained were tested for the concentration of Manganese in mg Mn/L. The first result was taken 20 days after the installation of leaching flasks and inoculating the solution. The results are shown below:

Table 4. Concentration of manganese obtained for each leaching flask over interval of days.

	Concentration (±0.14 mg Mn/L)					
Day\Sample	**BR1**	**BO2**	**BO3**	**MR1**	**MO2**	**MO3**
0	0	32.2	16.6	0	0	8.02
14	483	123	64.8	0	0	64.4
21	577.8	192.6	160.5	0	0	0
33	578	385.2	224.7	642	160.5	321
42	770.4	353.1	160.5	642	160.5	192.6
86	866.7	353.1	321	674.1	192.6	256.8
94	449.4	224.7	192.6	385.2	160.5	256.8
101	385.2	192.6	224.7	385.2	192.6	128.4
108	513.6	192.6	256.8	513.6	256.8	321
113	385.2	160.5	192.6	706.2	288.9	449.3
120	449.4	128.4	256.8	449.4	192.6	256.8
129	577.8	256.8	449.4	642	449.4	545.7
134	449.4	128.4	321	385.2	128.4	192.6

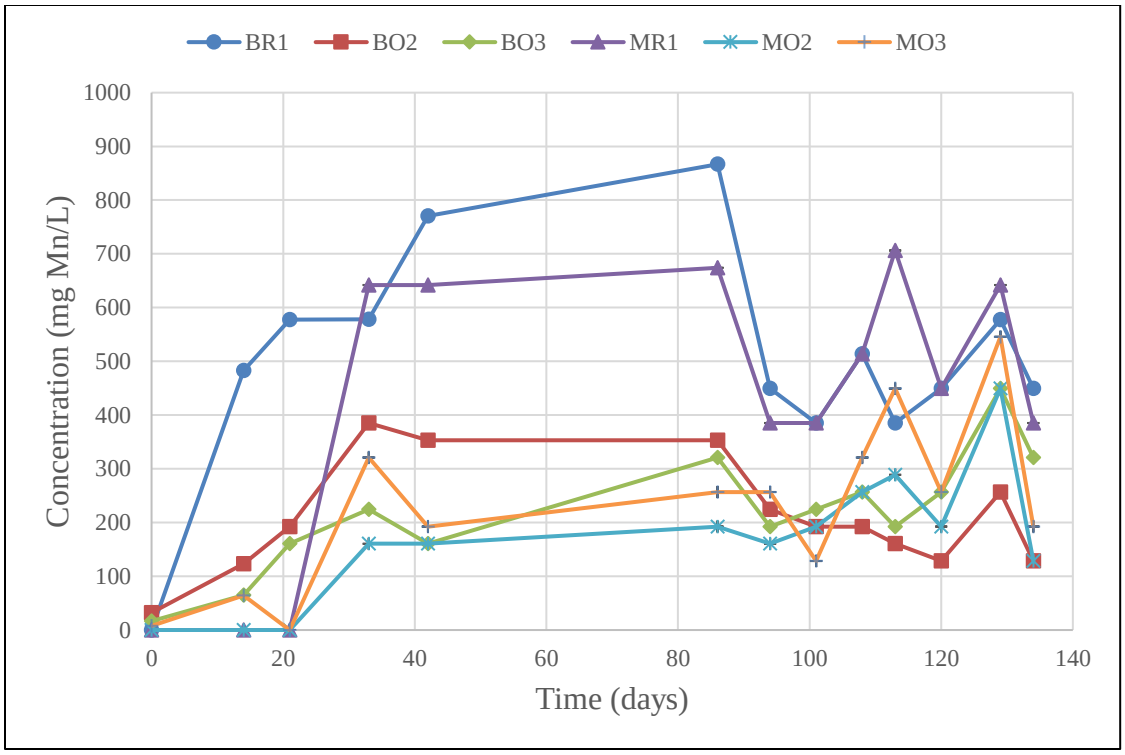

Figure 11. Graph showing the variation of concentration of dissolved manganese (mg Mn/L) over time (days) for data Table 4.

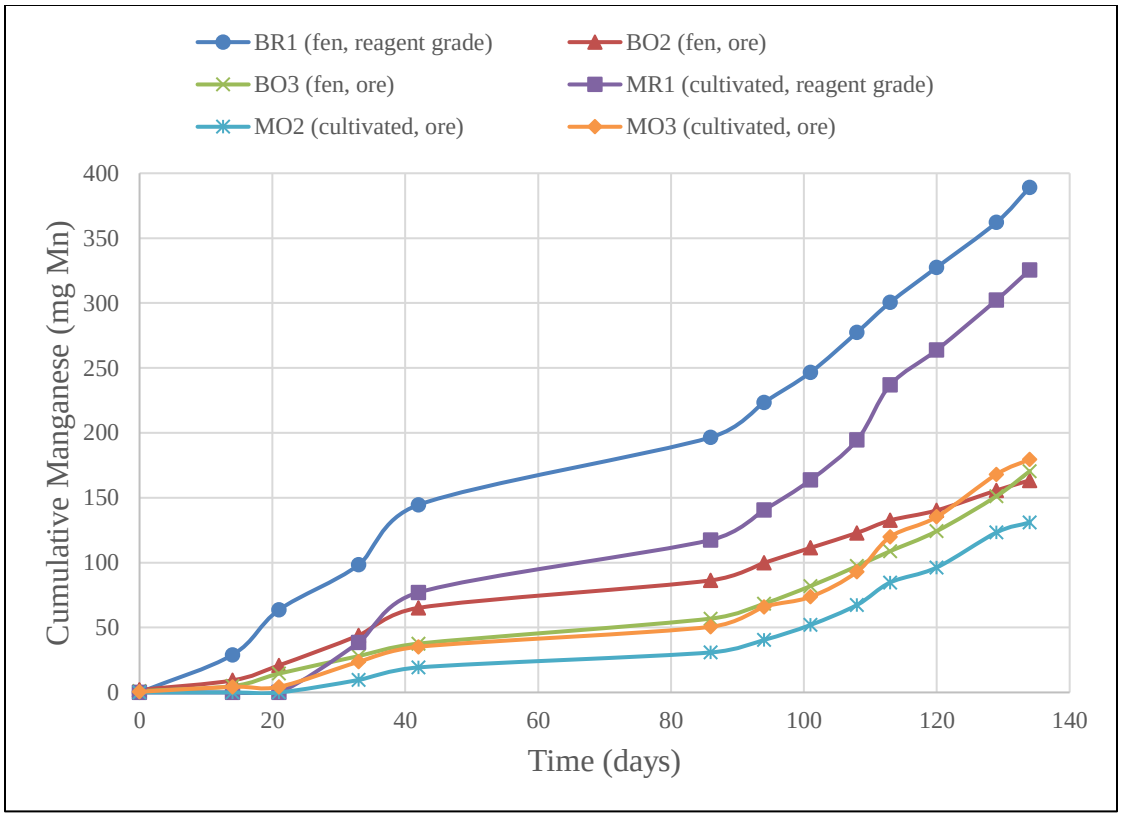

Figure 12. Graph showing Cumulative manganese recovered in mg manganese vs time.

We also observed that a layer of pink fungus was forming at the surface of the solution in the flask MR1. The flask used to keep the system anaerobic also had a layer of the pink fungus developed after a few days (Figure 13). The samples collected from this flask, when allowed to sit for a few days, developed the same pink fungus (Figure 14). The procedure for analysis of manganese in this fungus is described in section 2.4.

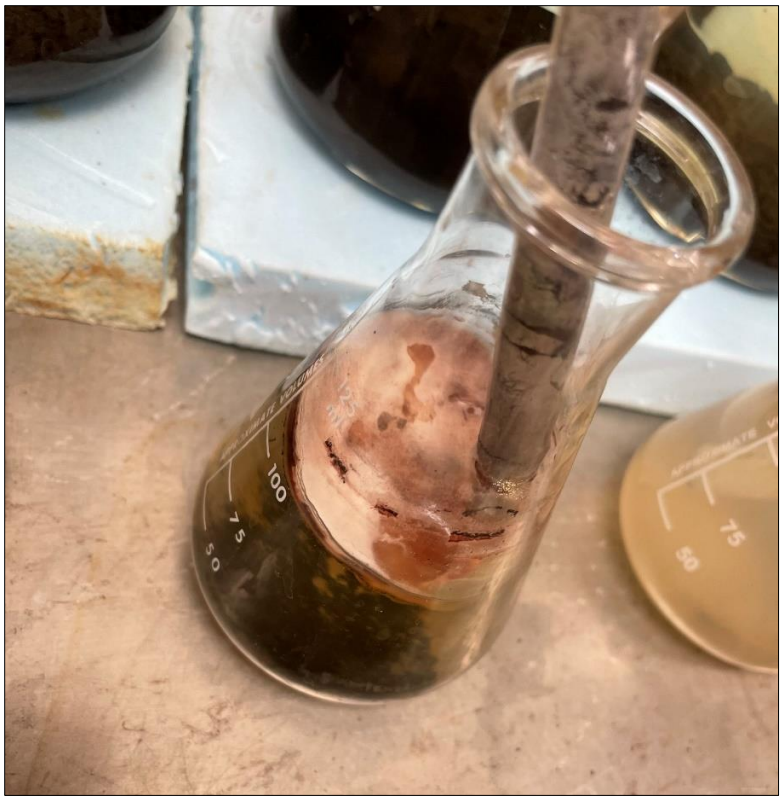

Figure 13. A layer of pink fungus developed in the leaching flask MR1.

Figure 14. Pink fungus on the sample collected from leach flask MR1 after being allowed to sit for a few days.

The analysis of Mn in fungus and precipitate from leach bucket, which was obtained by allowing the dissolved Mn^{2+} in percolated water to reoxidise in presence of air in the settling tank, as described in sections 2.4 and 2.3 respectively, are given in Table 5. The leaching bucket sample was tested for manganese on two occasions. The first test was conducted after adding 2M H_2SO_4 and letting it sit for a day. The second test was conducted on the same solution after it had been sitting for a month and shaken occasionally. The tests are labeled in table 5 as "leaching bucket (2/09)" and "leaching bucket (3/20)" respectively.

Table 5. Manganese analysis results for leaching bucket precipitate and fungus from sample collected from leach flask MR1.

	Leaching bucket (2/09)	**Leaching bucket (3/20)**	**10/28 fungus**	**11/30 fungus**
% Mn in dried sample	1.4	12.81	6.42	11.01

4 DISCUSSION

Figure 11 shows data collected over 130 days for the concentration of dissolved manganese in the solution. The concentrations of dissolved manganese were not significant during the initial days but started increasing as the bacteria was allowed to grow. The highest concentrations were observed for the leaching flasks containing reagent grade Manganese dioxide as opposed to ore grade Manganese dioxide. This might be due to the greater surface area of MnO_2 available for the bacteria to digest. Reagent grade MnO_2 contained 98% MnO_2 and had a particle size of 200 mesh. It is also important to note that the flask BR1 had the highest concentration of dissolved manganese among the two reagent grade manganese dioxide flasks. This might be due to the different bacterial strains used in the two leaching flasks. Figure 12 shows the cumulative mass of manganese recovered from each flask. The recovered amount of manganese is significant and suggests that bioleaching could be considered as a potential method for manganese extraction.

Dissolved manganese in the ore grade manganese dioxide leaching flasks was lower than the reagent grade leaching flasks, it was, nevertheless, a significant amount. There is also some difference in the concentration of dissolved manganese for flasks using the bacterial strain "B" as compared to the ones using "M". The nutrient media supplied to the leaching flasks was the same, but a change in nutrient media might affect the dissolution rates.

It can also be seen in the figure that the concentration of dissolved manganese decreases after attaining a certain peak. The study focused on manganese bioleaching kinetics of *Thiobacillus thiooxidans* [21] concluded an inverse relationship between manganese concentration and manganese leaching efficiency and attributed it to the inhibitory effect of manganese at higher concentration. The same reasoning could be applied here. Alternatively, this could also be due to bacteria not being able to access the manganese dioxide below the surface since the leach flasks were not being stirred.

The graph shows a gap of 25 days where the leaching flasks were not supplied with nutrient media and samples were not taken out for analysis. A decrease in the dissolved manganese concentration follows, although the concentrations did not drop to near zero, and started increasing after two weeks. It can be concluded that the bacterial communities in the leaching flask are quite sturdy, can live through a spell of low nutrient supply, and build up again quickly when nutrients are provided again.

pH of the leaching flasks was around 5 (table 1) which was lower than the pH of nutrient media which observed to be 6.9. This might indicate the production of acids by the bacteria in the leaching flasks. Alternatively, this could also be due the decomposition of sugar in the flasks which was added in the nutrient media, by the bacteria. A significant difference in pH was not observed for the flasks containing bacterial strain "B" and those containing bacterial strain "M" which might suggest a similar mechanism of manganese reduction being carried out by the two strains. It

might be interesting to note the change in dissolved manganese concentration at a lower pH (say a pH of 3.5 as suggested by a previous study [24]).

The content of manganese obtained from the manganese leach bucket precipitate was initially found to be 1.4% when it was digested with 2M H_2SO_4. The same solution was analyzed for manganese content after about a month, and it was found to be 12.81%. Since manganese is difficult to dissolve with sulphuric acid, the true content of manganese in the precipitate could be higher than what was calculated here.

We have talked about fungal species that can dissolve manganese from their ores to an appreciable extent [13, 31, 36]. These fungal species, however, reduce manganese indirectly by the production of acids. The fungus found in this study seemed to take up manganese in its structure, which was indicated by its pink color and became evident after tests were conducted which showed a manganese content of about 11% which was comparable to the manganese content obtained from leach bucket precipitate. The manganese could have been present in a complex form since it was easy to digest with 2M H_2SO_4. We unfortunately lost the fungus after the 25 day period of no resupply nutrient media and hence further analysis on the fungus could not be done.

5 CONCLUSION AND FUTURE WORK

The concentration of dissolved manganese in the experiments conducted was significant keeping in mind that the tests were run in a natural setting with little to no alterations of external factors. Alterations of some parameters, for example carbon source or pH, could lead to better dissolution of manganese. The manganese dissolution rates could also increase with time as has been seen in the case of iron [16]. Future work in this project will include the variation of parameters affecting the dissolution efficiency of bacteria. Figure below shows an example of a mine site setup that could be used for manganese extraction on a large scale. This method could also be applied for the extraction of iron after manganese has been preferentially leached out from the system.

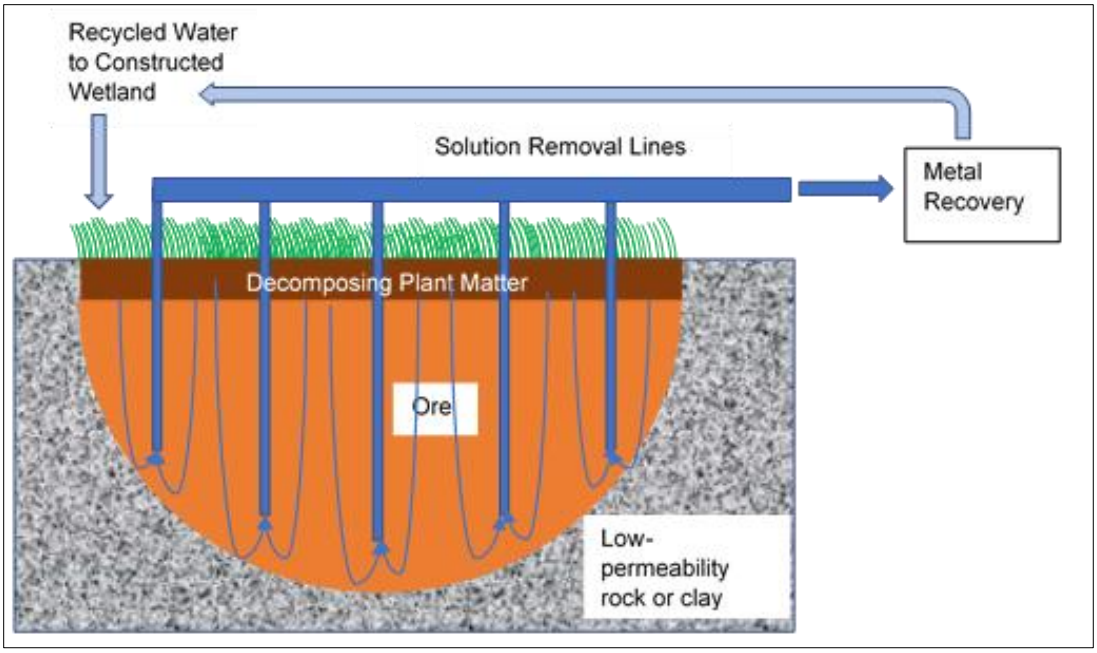

Figure 15. Large scale manganese production.

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