ENHANCED BIOWEATHERING OF COAL FOR RARE EARTH ELEMENT

EXTRACTION AND CONCENTRATION

By

Ankur Sachan

A Thesis Submitted in Partial Fulfillment of the Requirements

for the degree of

Master of Science

in

Mining Engineering

University of Alaska Fairbanks

May 2019

APPROVED:

Tathagata Ghosh, Committee Co-chair
Brandon Briggs, Committee Co-chair
Rajive Ganguli, Committee Member
Srijan Aggarwal, Committee Member
Margaret Darrow, Chair
Department of Mining and Geological Engineering
William Schnabel, Dean
College of Engineering and Mines
Michael Castellini,
Dean of the Graduate School

Abstract

Rare earth elements (REEs) are a group of seventeen elements that include scandium, yttrium, and fifteen of the lanthanide series elements, which are used in a variety of consumer goods and for defense purposes. Acquiring a domestic profitable source of REEs is a critical national need as most of the global supply comes from one country, China. To counter this problem, the US is actively looking at alternative sources of REEs by implementing unconventional methods of extraction. Coal is one of the alternative sources of REEs. Alaskan coal from Wishbone Hill and Healy are known to contain REEs up to 286 ppm and 524 ppm, respectively, while having concentrations as high as 950 ppm on ash basis in some density fractions.

Microbial leaching or bioleaching is a novel method that can be used for extraction of REEs from coal as microbes are known to affect earth's surface over geologic time by playing critical roles in weathering of minerals. A certain species of bacteria, *Shewanella oneidensis* MR-1, was used to separate the REEs from Wishbone Hill and Healy coal samples. The experiments were performed for various density fractions of both coals by varying solids percentage, temperature, size of coal, and bacterial concentration, and recovery of REEs for these conditions was recorded. Highest individual recovery of neodymium, 75.3%, was obtained for Wishbone Hill 1.3 floats, while a maximum of 98.4% total REE recovery was obtained for Healy 1.3 sinks. Healy coal has the higher total recovery of REEs in comparison to Wishbone Hill coal.

Bioleaching process was also compared to the acid leaching process. Healy coal responded better to bioleaching than the acid leaching process. The Wishbone Hill coal had comparable recoveries of bioleaching with acid leaching, although they were always less than acid leaching.

Dedicated to

My Beloved Parents, Sister, Brother-in-law, Friends

And

The Advisors for their support and inspiration.

Table of Contents **Page**

List of Figures   Page

Acknowledgements

CHAPTER 1 INTRODUCTION

1.1 Introduction

The modern world economy is exclusively dependent on technology ranging from electronics, defense and medical equipment, and other industries like ceramics, electrical, chemical, nuclear, optical, catalytic, and metallurgical applications (Gupta and Krishnamurthy 2004). Most of these industries require a certain group of elements known as the Rare Earth Elements (REEs). The REEs are a group of seventeen elements including scandium (Sc) and yttrium (Y), and fifteen lanthanide series elements, namely, lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), promethium (Pm), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb), and lutetium (Lu). As these elements are used in critical industries, to secure their supply is of utmost importance for every country.

Unfortunately, China controls the global market by contributing more than 85% of the total world REE production. For a prolonged period of time, China produced the majority of REEs and fluctuated their prices. This has provided China the monopoly to regulate REE prices. In the recent past, the US was self-sustainable in REE production but due to the Chinese monopoly in the international market, the US has become dependent mainly on China for its imports of REEs. As of 2015, the US imported 77% of its REE demand from China, 7% from Estonia, 4% from France, 4% from Japan, and the remaining 8% from other countries (Gambogi 2018). Also, as China is the main supplier of REEs in the world, they reduce their prices whenever there is a new competitor in the market. The US had one such company, Molycorp, which had to cease its operations in 2015 owing to low prices of REEs for a lengthened period and it could not compete with Chinese suppliers. Since 2015, the US has not produced any REEs, which has led to the dependence on China for the majority of its

REE imports. The value of these imports is estimated to be USD $150 million in 2017, a steep increase from USD $118 million in 2016 (Gambogi 2018).

China has also implemented export quota on REEs since 2009, which has limited the global supply of REEs in the market. Due to high demands coupled with the decreasing REE output from China, this resulted in the significant increase of REE prices. Also, China has 42% of the world's known REE reserves and by restricting the export of REEs in international market, it is ensuring that it remains a force in the market (Gambogi 2018).

The REEs in the US are used mainly as catalysts (55%), ceramics and glass (15%), metallurgical applications and alloys (10%), polishing (5%), and others (15%). Due to over-dependence on China for REEs, the Department of Energy (DoE) is trying to find alternative sources of REEs and their extraction, mostly to meet domestic requirements. As the world is getting more conscious of the ill-effects of mining and refining of metals, more emphasis is given to developing eco-friendly methods for REE extraction. The extraction of REEs from traditional sources (ores) and methods consumes a lot of energy and uses a lot of harmful chemicals, which, if released, could be hazardous for the environment. Due to these problems, there is a need for developing an alternative method that can prove to be economical and environment-friendly for REE extraction, preferably from unconventional sources.

One such unconventional source of REEs is coal. Considering the amount of coal mined throughout a year in the world, it can prove to be a long-lasting source of REEs. The US has large reserves of coal that on an average contains 66 ppm of REEs in it (Zhang et al. 2015). Moreover, Alaska contains 40% more coal than the whole of the contiguous US and previous research has shown that some Alaskan coal contains up to 525 ppm REEs which makes it a perfect candidate for research on extraction using novel methods that are environment-friendly (Gupta 2016).

1.2 Objective of the Work

Coal and coal ash have been known to contain REEs, as high as 1000 ppm, as it has been demonstrated by previous research (Seredin and Dai 2012). Wishbone Hill and Healy coal, both from Alaska, contain up to 857 ppm REEs on basis of ash in certain density fractions and 286 ppm and 524 ppm, respectively, on whole coal basis (Gupta 2016). The amount of REEs in Alaskan coal is significant and it can serve as an alternate source of REEs. The main objective of this research project is to find an alternate method of REE extraction that is environmentally friendly and cost-effective. If either of the objectives are met, the project can proceed further with optimizing the process in the future.

The alternative method that is used in this research is called enhanced microbial weathering or bioleaching, in which the microbes can "leach" out the elements from coal and ash by including them in their metabolic processes. This is a novel approach and if proven to be successful, can be applied for REE extraction process and can change the coal mining industry, especially in Alaska, significantly.

The main objectives of the research are:

1) To demonstrate that microbes can be successful in leaching out the REEs trapped within coal.
2) To conduct experiments on coal in different conditions and find out the optimum temperature, size, and pulp density for extraction and concentration of REEs.
3) To analyze the samples after performing experiments for REE content in the solution using ICP-MS (Inductively Coupled Plasma-Mass Spectrometry).

1.3 Thesis Outline

The first chapter of this thesis provides the general information about the demand and supply of REEs, and current scenarios in the international market and the need

for research in this area are discussed. Also, in this chapter, the new method used for REE extraction and concentration is introduced and the outline for the research objectives are stated. The second chapter provides the background information about the REEs including their abundance, chemical and physical properties, applications, current extraction techniques, and global demand and supply. This chapter also discusses the problems associated with conventional extraction techniques of REEs, which leads to a dialogue on alternative methods of extraction. Chapter 3 contains elaborate details on how the microbes perform different kinds of biohydrometallurgical processes. The alternative method, i.e., bioleaching that is studied for this particular research has its details about the different mechanisms involved in bioleaching in this chapter. Chapters 2 and 3 contain the literature review of the research, which paves the way for determining the methodology for the experiments. Chapter 4 contains the information about the methodology including how the experiments were performed, the chemicals and instruments used, and the analysis technique used in the research. Chapter 5 contains the detailed discussion about the results obtained and their interpretations. The conclusions and further recommendations of this research are stated in Chapter 6.

CHAPTER 2 BACKGROUND

2.1 What are REEs?

Rare earth elements (REEs) are a set of seventeen elements that occur together in nature. The REEs consists of scandium (Sc), yttrium (Y) and fifteen elements within the chemical group known as lanthanide series, from lanthanum (La) to lutetium (Lu). Scandium and Yttrium are classified as REEs because they have chemically corresponding characteristics to the lanthanide series and because of their general availability in REE-bearing ores. The REEs are studied together because they have similar chemical properties that result in their occurrence in similar mineral deposits. These elements were not isolated from each other until the 20th century. The REEs have metallic properties and sometimes are also called rare earth metals (Binnemans et al. 2013; LePain et al. 2011).

They are referred to as "rare" because they are found in only a handful of places on Earth. The most common ores of REEs are bastnaesite, xenotime, monazite and ion-adsorption clays. Bastnaesite is the most abundant of all followed by monazite, while xenotime and ion-adsorption clays contribute to a smaller part of the total production (Gambogi 2018).

Contrary to their name, most of the REEs are not as uncommon in nature. Table 1 shows the abundance (in ppm) of every REE along with their atomic number and symbol. Most of the REEs are relatively plentiful in the Earth's crust with cerium being the most abundant of all the REEs, even more than copper, gold, and silver. Most of the REEs are more common than tin, molybdenum, and mercury except for promethium (Taylor and McLennan 1985). Promethium does not occur naturally on Earth's crust because it has no stable or long-living isotopes. Due to its isotopes being radioactive, there are only 500-600 grams of promethium in Earth's crust (Yardley 1986).

Table 1. REE's along with their symbol, atomic number, crustal abundance (in ppm) on Earth and electronic ground state configurations.

	Element	Symbol	Atomic Number	Crustal Abundance (ppm)	Configuration
Light Rare Earth Elements (LREE's)	Scandium	Sc	21	22	$[Ar]4s^2 3d^1$
	Lanthanum	La	57	39	$[Xe]6s^2 5d^1$
	Cerium	Ce	58	66.5	$[Xe]4f^1 6s^2 5d^1$
	Praseodymium	Pr	59	9.2	$[Xe]4f^3 6s^2$
	Neodymium	Nd	60	41.5	$[Xe]4f^4 6s^2$
	Promethium	Pm	61	N/A	$[Xe]4f^5 6s^2$
	Samarium	Sm	62	7.05	$[Xe]4f^6 6s^2$
	Europium	Eu	63	2.0	$[Xe]4f^7 6s^2$
	Gadolinium	Gd	64	6.2	$[Xe]4f^7 6s^2 5d^1$
Heavy Rare Earth Elements (HREE's)	Terbium	Tb	65	1.2	$[Xe]4f^9 6s^2$
	Dysprosium	Dy	66	5.2	$[Xe]4f^{10} 6s^2$
	Holmium	Ho	67	1.3	$[Xe]4f^{11} 6s^2$
	Erbium	Er	68	3.5	$[Xe]4f^{12} 6s^2$
	Thulium	Tm	69	0.5	$[Xe]4f^{13} 6s^2$
	Ytterbium	Yb	70	3.2	$[Xe]4f^{14} 6s^2$
	Lutetium	Lu	71	0.8	$[Xe]4f^{14} 6s^2 5d^1$
	Yttrium	Y	39	33	$[Kr]5s^2 4d^1$

2.2 Classification of REEs

The REEs are generally classified as "light rare earth elements (LREEs)" and "heavy rare earth elements (HREE's)" based on their respective atomic numbers. Elements from lanthanum to gadolinium, and scandium are classified as LREEs, while elements from terbium to lutetium, and yttrium are classified as HREEs. The only anomaly in this classification is yttrium which is placed under HREEs, despite it

being lighter than LREEs. This is because yttrium has a chemical and physical association with HREEs in natural deposits (Dai et al. 2012). There are many ways for classifying REEs, for example, based on specific gravity and supply and demand. In all the classifications, the elements may change groups, and this can lead to confusion, so for all the purposes in this thesis, LREEs are elements from lanthanum to gadolinium, and scandium while HREEs are elements from terbium to lutetium, and yttrium. LREEs are generally more abundant than HREEs, as this is the case with most of the lighter elements. As the atomic number increases, the natural occurrence of the element decreases. Also, REEs with even atomic numbers are more abundant than odd atomic numbers because of the Oddo-Harkins rule.

The REEs are enriched in Earth's crust and occur together naturally because most of them are trivalent except for cerium (occurs as Ce^{+4}) and europium (occurs as Eu^{+2}) and have similar ionic radii. As the minor increase in ionic radii in the lanthanide series is not accompanied by the change in charge, the lanthanide elements occupy the same cell in the periodic table. Due to similar radii and oxidation states of REEs, they can substitute each other in various crystal lattices and hence they have similar chemical and physical properties.

2.3 Properties

Atoms consist of electrons, protons, and neutrons, with the electrons revolving around the nucleus, which is made up of neutrons and protons. The electrons revolve around the nucleus and spins on their own axes which produces a magnetic field. The pairing of electrons in an orbital cancels out their magnetism as the electrons in the same orbital have opposite spin. So, if the electron is unpaired in an orbital, the atom can be magnetic in nature and as the number of unpaired electrons increases, the magnetism and paramagnetic behavior of the element increases. In the case of REEs, except for scandium, yttrium, lanthanum, ytterbium, and lutetium, the rest of the elements are strongly paramagnetic. The change in temperature affects the electrons present in the outermost shell of the atoms and leads to the electrons skipping from

one orbital to another depending on the change. If the temperature decreases, they tend to pair up, while, if the temperature increases, they become unpaired. On decreasing the temperature, many REEs become antiferromagnetic, while decreasing the temperature further, they become ferromagnetic. REEs also have low ionization potential, which is due to the outermost shell being far away from the nuclei and the electrons can get to an excited state and leave the atom with very little energy input. Due to this fact, the REEs primarily occur as ionic compounds (Gupta and Krishnamurthy 2004).

The REEs, being metals, have relatively higher melting and boiling points as compared to non-metals. The melting point of REEs varies from 798°C for cerium to 1,663°C for lutetium. Similarly, the boiling point ranges from 1,194°C for ytterbium to 3,512°C for praseodymium (Cordier and Hedrick 2008). REEs are soft, malleable, and ductile and have iron-gray to a silvery luster. They also have low ionization potential due to which they occur as ionic compounds (Gupta and Krishnamurthy 2004).

Lanthanide contraction is another phenomenon that is exhibited by the lanthanide series elements. This process is explained as the significant difference in the size of atoms and ions as the atomic number of elements increases, which leads to a similarity in chemical properties between REEs and their compounds. Lanthanide contraction occurs due to poor shielding of nuclear charge by 4f electrons, which results in these electrons being drawn towards the nucleus and thus resulting in a smaller atomic radius (Hughes et al. 2007). This also explains the chemically similar properties between HREEs and yttrium because of similarity in the outer electronic configuration and ionic size. As for scandium, it is smaller than the LREEs both in atomic and ionic radius, thus its chemistry is different from other REEs (Gupta and Krishnamurthy 1992). Due to the small difference in ionic radii of REEs, there is a slight difference in chemical and physical properties and results in separation of REEs into deposits rich in heavy and light REEs. Also, the REEs are readily soluble

in acids, a property that is extensively used in their extraction process (Gupta and Krishnamurthy 2004).

2.4 Application of REEs

The REEs are vital to every modern economy as they are used in a host of consumer products such as computers, cell phones, rechargeable batteries, magnets, etc. Furthermore, they are also used in military equipment like night-vision goggles, precision-guided weapons, GPS equipment, guidance systems and "white noise" production in stealth technology. Although the REEs are more expensive as compared to their heavy metal counterparts, they pose relatively fewer environmental problems during their disposal and recycling (Haxel et al. 2002).

The REEs and their alloys are used in a wide variety of products ranging from day-to-day use electronics like smartphones, laptops, lighter flints, and fluorescent lighting to high-end products like magnetic resonance imaging (MRI) and computerized axial tomography (CAT) scans, electric cars, catalysts, magnets, and lasers. They are also used in aerospace technology as well as in the manufacture of defense equipment. The REEs are used in these products because they have special electronic, catalytic, magnetic and optical properties. For example, lanthanum is exploited for its catalytic property and is used in the manufacture of low-octane fuel from heavy crude oil in the petroleum industry. Praseodymium, when doped with zirconia, produces a bright yellow pigment that is highly valued for ceramics. Samarium is used in laser application and when it is alloyed with cobalt, it is used in the production of permanent magnets. The use of REEs in the US and in the world are shown in Figure 1.
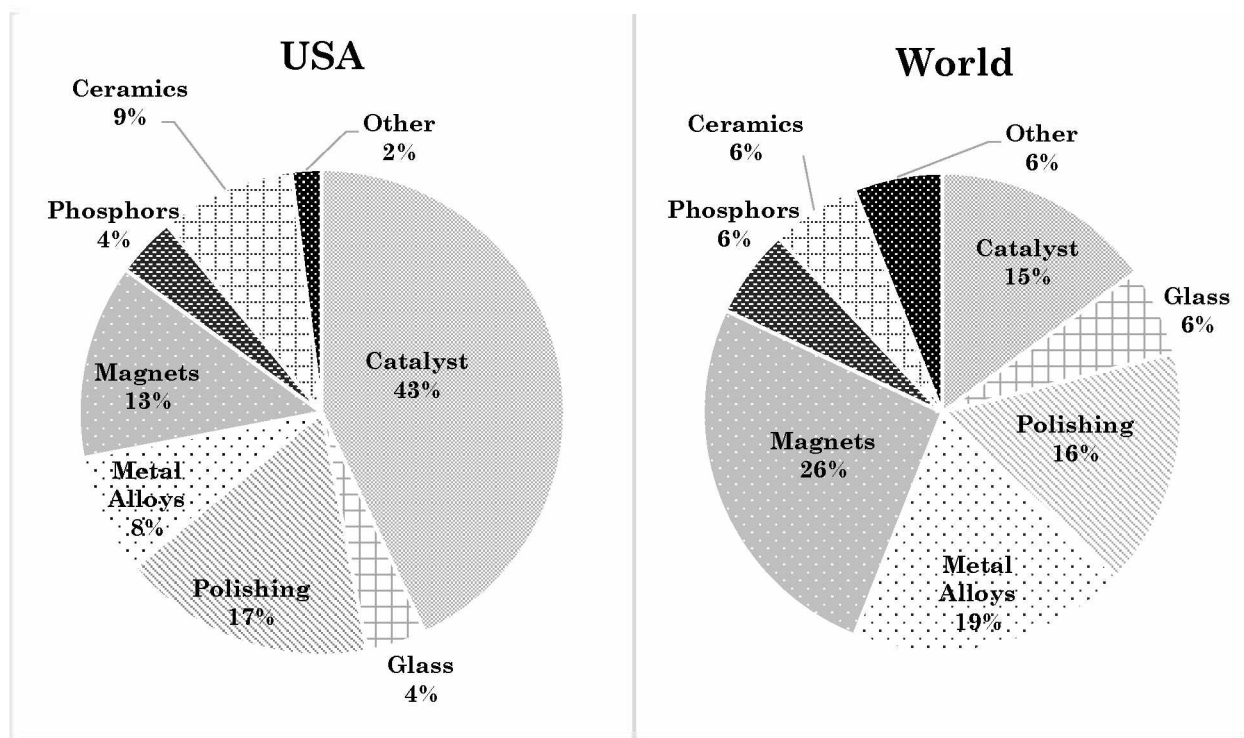
Figure 1. REE usage in the US and world in 2015 (Humphries 2011).

When gadolinium is mixed with EDTA (ethylenediaminetetraacetic acid) it is injected as a contrast agent into patients undergoing magnetic resonance imaging. Ytterbium metal has a rather unique property of increasing its electrical resistance when it is subjected to very high stresses and is used in stress gauges for monitoring ground deformation after earthquakes. Thulium is mainly used in making crystals and lasers but one of its most important uses is in the production of portable X-ray sources. Lutetium has the smallest atomic radius of all the lanthanide series elements and produces the densest white material, lutetium tantalite ($LuTaO_4$), which is an ideal host for X-ray phosphors. The REEs are also used as additives to ductile iron and steel to make them harder, which then are used in ordinance and research (Gupta and Krishnamurthy 2004). The major uses of REEs are summarized in Table 2.

REEs are indispensable and irreplaceable in their applications such as electronic devices, defense, medical purposes, and optical quality glasses. As the world is getting

more technology driven by the day, the demand for these products is increasing, thereby increasing the demand for REEs.

Table 2. Application of REEs (Haque et al. 2014; Gupta and Krishnamurthy 2004; Humphries 2011).

Element	Applications
Scandium	Aluminum-scandium alloys for aerospace industry, ceramics, lasers
Lanthanum	Hydrogen storage batteries, manufacture of low-octane fuel from crude oil, carbon arc lamps, and in night vision goggles
Cerium	Catalyst for auto industry, for making glass that can block UV light, and computer chips
Praseodymium	Magnets, coloring ceramics, optical fibers, and scintillator for CAT scans
Neodymium	Lasers, glass coloring and tinting, magnets for automotive and parts and computer disks, CRT displays and dielectrics
Promethium	Nuclear battery, pacemakers, and compact fluorescent bulbs
Samarium	Samarium-cobalt magnets, lasers, dielectrics, aerospace equipment and servomotors
Europium	Luminescence valuable for medical, surgical and biochemical applications, red color for TV and computer screens, and energy-efficient fluorescent lighting
Gadolinium	Magnets, Nuclear MRI, and host for X-ray cassettes and scintillated materials for computer tomography
Terbium	Fluorescent lamps, magnets, X-ray phosphor and defense technologies
Dysprosium	Nuclear reactors, computer hard disks, hybrid engines, and special ceramic compositions

11

Table 2. Continued

Holmium	Glass coloring agent, magnets, lasers, and nuclear control rods
Erbium	Glass coloring agent, amplifier for fiber optics, lasers, eyewear and decorative glassware
Thulium	Medical X-ray units, crystals, magnetic and ceramic materials for microwave technologies
Ytterbium	Ground control monitoring systems, lasers, steel alloys and fiber optics
Lutetium	Catalyst for petroleum refining, and positron emission tomography
Yttrium	Fluorescent lamps, lasers, computer displays, increasing strength of metallic alloys, and ceramics

China produces approximately 85% of the total REE output of the world, but due to environmental concerns regarding mining and processing of REEs, there are fears that they will reduce the production significantly in the coming years (Wübbeke 2013). Due to this and the fact that REEs not widely available (although abundant in nature), the US is actively looking for developing novel methods for REE extraction from secondary sources.

2.5 Global Demand and Supply

The conventional methods for extraction of REEs from their ores require grinding huge volumes of rocks and then digesting it in acids to leach the REEs. This process produces enormous quantities of toxic waste that is harmful to the environment and is cost-intensive. Due to the above-mentioned reasons, China, the largest supplier of REEs in global markets is decreasing its REE production. Countries like the US are dependent on REE imports for technological advancements and consumer products. To reduce the imports from foreign countries, researchers are trying to search for cheaper alternative substitutes or secondary sources for REEs. The problem with alternatives for REEs is they can only be substituted in applications like metallurgy and magnets, but not in applications that rely on optical and chemical properties of

REEs. Also, the substitutes are rarely available and expensive, which has ensured the demand for REEs will be long-lasting, especially in applications such as optics, pigments, and x-ray intensifiers. Recently, however, a technological breakthrough has been achieved in the production of large wind turbines (10 MW or more) that, except for yttrium, does not require the other REEs. The demand for dysprosium can be reduced by achieving better material efficiency. But, as the demand for wind turbines is increasing due to more offshore installations, the REE requirement is set to increase in coming years (Pavel et al. 2017).

Similarly, Tesla and Renault have developed an electric induction motor and a wound motor, respectively, that do not require any REEs, and BMW is lowering the use of REEs in its hybrid motors. Also, organic light emitting devices can replace the lamps that contain REEs and substitutes for red phosphor in fluorescent lighting are currently being tested (Ganguli and Cook 2018).

Bastnaesite deposits of China and the US contain the largest percentage of the world's REE in terms of economic resource. The initial demand of REEs was mainly for use as catalysts, but with technological advancements, the demand shifted towards electronics and high-value applications. The US was the major producer of the REEs until the 1980's and most of its production came from Mountain Pass Mine in California. With the discovery of REEs in Bayan Obo region of China, cheap labor and lax environmental regulations, China started producing huge quantities of REEs, which drove the prices of REEs down. Due to this factor, the US mines could not compete with the prices and it did not produce any REEs from the year 2003 until 2011 (Figure 2). The production restarted with 800 tons in 2012 rising up to 5,900 tonnes in 2015, but in 2016 all the production ceased (USGS 1994-2008).

The world is moving towards "green energy" due to rising global pollution and climate change concerns. As the REEs are used in producing products that harness renewable sources of energy like wind turbines, electric cars, and solar panels, their demand is increasing by the day. It is projected that the demand for electronics, MRI contrast
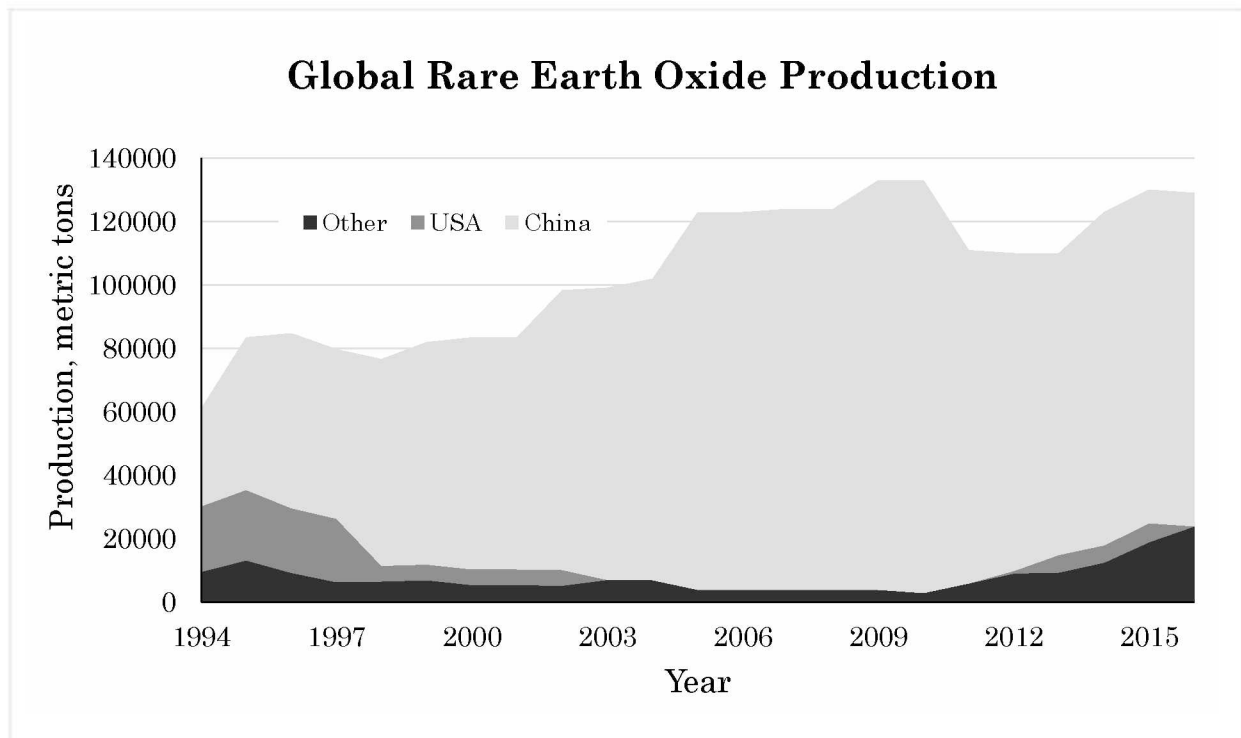
Figure 2. Worldwide REE-oxides production from 1994 to 2016 (U.S. Geological Survey, 1994-2018).

agents, medical isotopes, lasers, and magnets, which use various types of REEs, is not going to slow down in future, hence fueling the demand for REEs in the market (Hedrick 1998).

The demand for REEs in 2010 was estimated to be 134,000 tonnes, but the actual production was 133,600 tonnes, meaning that there was a negative difference between demand and supply and this difference was covered by the previously mined materials and industrial stockpiles (Humphries 2011). Historically, China has monopolized the global market and until the year 2009, it produced more than 95% of the total REEs in the world (Figure 2). After consolidating their domination in the world market, China controlled the prices of REEs, which led to shutting down of mines around the world as the REE producers outside China could not compete with the low REE prices. After the year 2009, China implemented export quota on REEs, which limited the global supply. Due to high demands coupled with decreasing REE output from China, this resulted in the significant increase of REE prices.

2.6 Sources of REEs

The world has about 120 million tonnes of REEs and about 126,000 tonnes were mined in 2016 (Ober 2017). Around 85% of the total production of REEs came from China alone with Australia in second place, producing 11% of the total world production. The REEs are spread across the earth and are known to be found in fourteen countries in Asia, six countries in Europe, the US, Canada, Australia, Brazil and ten countries in Africa. In total there are thirty-four countries with REE deposits in the world.

The sources of REEs can be classified as primary and secondary sources. Primary sources are defined as REE-bearing minerals like bastnaesite and monazite that occur in nature, while secondary sources are mainly limited to metal scavenging or recycling through the use of electronic waste, wastewater, incinerator ash, red mud, phosphogypsum, metallurgical slags, mine tailings, and coal. Due to advancement in technology in recent times, metal recycling mainly through electronic waste has gathered interest and is performed significantly for precious metals, but can be used for REEs also (Du and Graedel 2011).

2.6.1 Primary Sources

More than 200 minerals have been identified that contain an essential or significant amount of REEs. Bastnaesite [(Ce, La) CO_3 (F, OH)], monazite [(Ce, La, Nd, Th) PO_4], and xenotime [YPO_4] are the three most economically significant minerals from which REEs are extracted commercially at present and they account for almost 95% of the REEs currently used in the world. Bastnaesite and monazite contain LREE's while xenotime contains HREEs. Bastnaesite is a fluorocarbonate mineral and contains approximately 70% rare earth oxides. Large deposits of bastnaesite are located in Bayan Obo in China, and at Mountain Pass, California in the U.S. Monazite is a phosphate mineral that contains up to 30% thorium, and a considerable amount of

uranium, both having radioactive properties (Gupta and Krishnamurthy 2004). Table 3 shows the list of minerals that contain REEs (Kanazawa and Kamitani 2006).

Table 3. Minerals containing REE along with the weight% rare earth oxide (REO) (Ln=Lanthanide series element) (Kanazawa and Kamitani 2006).

Mineral	Chemical Formula	Weight% REO
Aeschynite	$(Ln,Ca,Fe,Th)(Ti,Nb)_2(O,OH)_6$	36
Allanite (orthite)	$(Ca,Ln)_2(Al,Fe)3(SiO4)_3(OH)$	30
Anatase	TiO_2	3
Ancylite	$SrLn(CO_3)_2(OH).H_2O$	46
Apatite	$Ca_5(PO_4)_3(F,Cl,OH)$	19
Bastnaesite	$LnCO_3F$	76
Brannerite	$(U,Ca,Ln)(Ti,Fe)_2O_6$	6
Britholite	$(Ln,Ca)_5(SiO_4,PO_4)_3(OH,F)$	62
Cerianite	$(Ce,Th)O_2$	81
Cheralite	$(Ln,Ca,Th)(P,Si)O_4$	5
Churchite	$YPO_4 \cdot 2H_2O$	44
Eudialyte	$Na_{15}Ca_6(Fe,Mn)_3Zr_3(Si,Nb)Si_{25}O_{73}(OH,Cl, H_2O)_5$	10
Euxenite	$(Ln,Ca,U,Th)(Nb,Ta,Ti)_2O_6$	<40
Fergusonite	$Ln(Nb,Ti)O_4$	47
Florencite	$LnAl_3(PO_4)_2(OH)_6$	32
Gadolinite	$LnFeBe_2Si_2O_{10}$	52
Huanghoite	$BaLn(CO_3)_2F$	38
Hydroxylbastnaesite	$LnCO_3(OH,F)$	75
Kainosite	$Ca_2(Y,Ln)_2Si_4O_{12}CO_3 \cdot H_2O$	38
Loparite	$(Ln,Na,Ca)(Ti,Nb)O_3$	36
Monazite	$(Ln,Th)PO_4$	71

Table 3. Continued

Mosandrite	$(Ca,Na,Ln)_{12}(Ti,Zr)_2Si_7O_{31}H_6F_4$	<65
Parisite	$CaLn_2(CO_3)_3F_2$	64
Samarskite	$(Ln,U,Fe)_3(Nb,Ta,Ti)_5O_{16}$	12
Synchisite	$CaLn(CO_3)_2F$	51
Thalenite	$Y_3Si_3O_{10}(OH)$	63
Xenotime	YPO_4	61
Yttrotantalite	$(Y,U,Fe)(Ta,Nb)O_4$	<24

HREEs are contained in minerals like apatite, cheralite, eudialyte, loparite, ion adsorption clays and xenotime (Dutta et al. 2016). Xenotime is an yttrium phosphate that contains approximately 67% rare earth oxide (REO), mostly heavier elements.

Recently, the deep-sea mud along the western Pacific coast of Japan was found to contain more than 8,000 ppm of total REE content. It is estimated to contain 1.2 million tonnes of REEs spread over an area of 105 km², this deep-sea mud can provide yttrium, europium, terbium and dysprosium for 62, 47, 32, and 56 years, respectively (Takaya et al. 2018).

2.6.2 Secondary Sources

The world has limited natural sources of REEs, which has led towards the search for alternate sources of REEs. Moreover, even after implementing export quotas, China still produces almost 85% of the total REE in the world, which means it has continued its monopoly over the international market as it can manipulate the prices of REEs in the global market. The combined effect of these two problems has led to research for better understanding and developing the processes for extraction of REEs from unconventional sources. These unconventional sources are also known as secondary sources of REEs.

Although there were known occurrences of REEs in bauxite residue, phosphogypsum, incinerator ash, mine tailings, metallurgical slags, wastewater, and electronic waste, it is only in recent times that processes are being developed for extraction of REEs from them. Wastes such as end-of-life (EoL) fluorescent lamps, permanent magnets, and nickel metal hydride batteries have a relatively low volume, but they have high REE content. REEs present as residue in different industrial processes, mine tailings, wastewater streams, and coal have a low concentration of REEs but are available in huge quantities and hence they are classified as a secondary source. Most common secondary sources of REEs are discussed below.

2.6.2.1 Bauxite Residue

Bauxite residue or red mud is a mixture of impure hydrated aluminum oxides that is formed when aluminum is extracted from bauxite using the Bayer process. A large quantity of bauxite is mined and processed every year to get valuable aluminum metal. The process of aluminum extraction from bauxite starts with its digestion in concentrated NaOH under high temperature and pressure (Bayer process), resulting in the formation of pure Al_2O_3 from which aluminum metal is produced. In this process, aluminum minerals are converted to sodium aluminate that gets dissolved in NaOH solution, while the impurities do not dissolve and are left as residue, which is known as bauxite residue or red mud. The REEs end up in red mud because they are associated with iron and titanium minerals that do not undergo any change during the Bayer process.

The concentration of REEs in red mud is generally high, except in Russia, where it contains 135 ppm of scandium oxide (Sc_2O_3), which is still six times more than the concentration of scandium in earth's crust (Fomin et al. 2004). In other places, the red mud contains 1040 ppm of REEs in Greece (Ochsenkühn-Petropoulou et al. 2002), 1500-2000 ppm in Jamaica (Wagh and Pinnock 1987), and 1700 ppm in Suriname (Logomerac 1971). There are some problems regarding the extraction of REEs from red mud as there is not enough information about their mineralogy phase. In 2018,

according to a report by USGS, about 300 million tonnes of bauxite was mined. Considering these high quantities of bauxite being mined every year, it can be safely assumed that red mud can serve as a critical source of REEs in the coming years, given that technological advancements are made in the same direction.

2.6.2.2 Phosphogypsum

Phosphogypsum is the main by-product when phosphoric acid (H_3PO_4) is manufactured by pulverizing the phosphate ores and digesting them in sulfuric acid. These phosphoric ores contain thorium and uranium 0.01% by weight and 0.01-0.1% REEs by weight (Binnemans et al. 2015). The concentration of individual REEs varies from a maximum of 0.015% by weight for cerium to a minimum of 0.0003% by weight for scandium (Binnemans et al. 2015). Historically, huge quantities of phosphate rocks have been mined and processed in the US. According to an estimate, about 6 million tonnes of phosphate rock was processed in 1964, which could have yielded about 3,500 tonnes of rare earth oxides (REO) (Binnemans et al. 2013).

During the processing of phosphate ores, the REEs become concentrated in phosphogypsum and about 70-85% of REEs originally present in the rock end up in phosphogypsum, while the rest of them remain in the solution with sulfuric acid (Binnemans et al. 2015). The REEs present in phosphogypsum can be recovered with 50% efficiency at ambient temperature by dissolving it in 0.1-0.5 M H_2SO_4 with a liquid to solid ratio of 10:1, but this process produces some solid residue (Habashi 1985), while when leaching is done by using HNO_3 or HCl, it does not have the same problem.

The phosphoric acid produced during leaching of phosphogypsum from sulfuric acid contains 1gm/L of REEs in it, which constitutes about 15-30% of the REEs present in the phosphate rock (Binnemans et al. 2015). The phosphoric acid contains most of the uranium present in the original rock, so this can also serve as an alternate source of

uranium. Recovery of the REEs from phosphoric acid leaching solutions can be done by solvent extraction or ion exchange (Kumar et al. 2011).

2.6.2.3 Incinerator Ash

The ashes obtained from incineration plants contain a wide variety of metals and sometimes REEs. Studies have been performed in various countries around the world that yielded similar results of low REE concentration in incinerator slag. Studies have been performed on incinerator ash in Switzerland (Morf et al. 2013) and Denmark (Allegrini et al. 2014), and on medical waste incinerator ash in China (Zhao, Zhang, and Zhang 2008) with a similar conclusion that they have low REE content and extraction of these elements would not be economically feasible. The research done on the ashes obtained from incineration plants burning municipal waste stored in landfills are unlikely to become a secondary source of REEs but they have high concentrations of base metals (Jones 2012; Jones et al. 2013), which can be explained by the fact that REE containing consumer goods are a relatively new phenomenon and they would require dedicated landfills for it to become a source of REEs (Binnemans et al. 2015).

2.6.2.4 Mine Tailings

During the initial years of REE beneficiation from ores, the methods that were used were not efficient, which resulted in huge losses during flotation process and led to the generation of REE-containing solid waste (Jordens, Cheng, and Waters 2013). The concentration of REEs in these solid wastes is high enough for them to be considered as rare-earth deposits. For example, according to an estimate, the tailings obtained from Mountain Pass mine in California consists of 3-5% of rare earth oxide (Binnemans et al. 2015). Similarly, in Bayan Obo (China) the efficiency of REE extraction process is low and only about 10% of REEs are recovered (Zhang et al. 2014). Near most of the Chinese REE processing plants, large volumes of REE tailings are present that can serve as an alternate untapped source or deposit of

REEs. Apart from tailings obtained from processing of REE ores, some REEs are present in apatite associated with iron ore (Long et al. 2010).

2.6.2.5 Metallurgical Slags

Recovery of valuable metals from lithium-ion and nickel metal hydride batteries using pyrometallurgy produces a slag that contains oxides of calcium, aluminum, silicon, and iron, and is also rich in REEs. Firstly, the plastic casing of the battery is removed and then the electrodes and polymers are heated at 600°C to remove organics. When the organics are removed, the residue is added to a calcium slag where nickel and cobalt were recovered as an alloy of iron-copper-cobalt and nickel while the REEs reported to the slag phase (Tang et al. 2012). Some companies like Solvay and Umicore have developed techniques and plants that recycle these batteries for REEs by feeding them in a vertical shaft furnace along with coke and a slag former, which results in the formation of slag at the bottom of the furnace when some oxygen is injected. The slag is then used to produce rare-earth concentrates that are then used as a feed to the REE separation plant (Binnemans et al. 2015). Around 50-60% by weight of REEs were collected from the slag phase in both laboratory and pilot scale settings (Müller and Friedrich 2006).

2.6.2.6 Wastewater

Wastewater, in most cases, contains lower concentration of rare elements (1-100 mg/L) despite the high total annual wastewater generation of more than 7.2 million tons (Li et al. 2013). Yessoufou et al. (2017) considered the amount of REEs in the urban sewage sludge in the Republic of Benin. Results showed Ce, La, and Nd were present in both sediments and sewage sludge at concentrations ranging from 5.80–41.30 mg/kg dry matter (DM), 3.23–15.60 mg/kg DM, and 2.74–19.26 mg/kg DM, respectively (Yessoufou et al. 2017). The low contents of REEs in the wastewaters and the solid suggests that if the recovery of rare earth elements was possible, there would only be small potentials for the economic and environmental benefit (Li et al.

2013). The levels of REEs in wastewaters and sewage sludge are usually quite low, hence the recovery of REEs by conventional methods is not economical. Therefore, there is a strong requirement to develop and apply a low-cost and environmentally friendly method like microbial leaching to recover them. However, to date, there is no evidence for recovering them from wastewater by bioleaching methods (Binnemans et al. 2015).

2.6.2.7 Electronic Waste

Among all the EoL products containing REEs, electronic waste (e-waste) has attracted the most attention in recent years throughout the world (Song et al. 2013). E-waste is the fastest-growing waste stream, increasing at a rate of 20-50 million tonnes per year and accounting for about 5% of the total municipal waste (Cui and Forssberg 2003). REEs are used mainly in fluorescent material, battery alloys and permanent magnetic material in electrical and electronic equipment. The fluorescent material is used mainly in the production of lighting such as fluorescent lamps (FLs), the backlight of flat panel displays, and in cathode-ray tube (CRT) monitors and display devices. The REEs are also present in circuit boards of electronics, but in very small quantities.

Before extraction of REEs from e-waste, they have to be shredded to small pieces ranging in size from 5-10 mm, which adds to the cost of extraction considerably as they have to be sorted before shredding, mostly manually, before processing (Cui and Forssberg 2003). There are several other challenges in extracting REEs from e-waste. With a very low recycling rate, as low as 1% (Reck and Graedel 2012) but with huge quantities of e-waste generated every year, more research is required in developing safe processes for extraction of REEs. Recycling REE resources from e-waste could be an effective measure for compensating for the gap between supply and demand and promoting the sustainable use of natural resources. As a potential source of REEs from e-waste recycling, REE recovery from waste phosphors in the EoL FLs (or waste

FLs) has attracted attention from researchers all over the world, although mostly at the laboratory scale (Binnemans et al. 2013).

2.6.2.8 REEs in Coal

Coal has been mined in huge quantities worldwide and it has been known to contain REEs around the world. High concentrations of REEs were found in the Pavlovka and Rakovka coal deposits of Russia (300-1000 ppm), and in the Appalachian deposits in the US (500-4000 ppm). Lower concentrations of REEs were found in Chinese (101 ppm) and Turkish coal (116 ppm), while the average world concentration of REEs is 72 ppm (Seredin and Dai 2012). Even though the concentration of REEs in the coal is less, because of the huge volumes of coal being mined, it can prove to be an important alternative source of REEs, especially in current unstable market conditions. Studies have shown that a concentration of 800-900 ppm of REOs in the combustion ash has the potential to be recovered economically (Dai et al. 2012; Hower et al. 1999; Seredin and Dai 2012; Ketris and Yudovich 2009).

Alaskan coal, namely from Wishbone Hill and Healy, contains 286 ppm and 524 ppm of REEs on ash basis, respectively. On basis of whole coal, Wishbone Hill had more REEs compared to Healy coal and both had more LREEs as compared to HREEs. The concentration of REEs reached as high as 857 ppm and 504 ppm on ash basis for lighter specific gravity fractions (Gupta 2016). It is hypothesized that the REEs present in the mineral grains are only a few microns in size and they can easily pass through the openings in the coal and enrich the lighter density fractions, usually less than 1.4 specific gravity, and thus this fraction has 70-80% of the total REEs present in coal (Gupta 2016; Seredin 1996).

The REE analysis based on size of coal revealed that in the size fraction between 1/4 inch (6 mm) and 100M (Tyler Mesh) (0.149 mm), Healy had more REE concentration, while Wishbone Hill had more REEs in size greater than 30M (0.54 mm). The distribution of REEs in Healy and Wishbone Hill coal follow the same pattern as seen

in coal from around the world. They have a high percentage of scandium, lanthanum, cerium, neodymium, and yttrium while having low concentrations of other REEs. The percentage distribution of individual REEs in Healy and Wishbone Hill coal is shown in Figure 3. It can be clearly seen that both coals have very high LREE content as compared to HREEs. Table 4 shows the comparison between the concentration of each rare earth element in the U.S., Chinese, Turkish and DPR Korean coal.

Table 4 .REE concentration (in ppm) in coal worldwide (Zhang et al. 2015).

REE	U.S. Coal	Chinese Coal	Turkish Coal	DPR Korea coal	World coal
La	12	18	21.12	14.5	11
Ce	21	35	39.24	27.2	23
Pr	2.4	3.8	4.71	2.9	3.5
Nd	9.5	15	16.85	11.1	12
Sm	1.7	3	3.18	2.3	2
Eu	0.4	0.65	0.76	0.5	0.47
Gd	1.8	3.4	3.00	1.4	2.7
Tb	0.3	0.52	0.45	0.3	0.32
Dy	1.9	3.1	2.42	2	2.1
Ho	0.35	0.73	0.47	0.4	0.54
Er	1.0	2.1	1.37	1.1	0.93
Tm	0.15	0.34	0.21	0.3	0.31
Yb	0.95	2	1.35	1	1.0
Y	8.5	9	12.76	7.2	8.4
Sc	4.2	4	7.92	4.9	3.9
Lu	0.14	0.32	0.21	-	0.20
LREE	48.8	78.85	88.86	59.9	54.67
HREE	17.49	22.11	27.16	17.4	17.7
Total	66.29	100.96	116.02	77.3	72.37

On the basis of specific gravity, generally for lower specific gravity, the Alaskan coal contains more REEs on ash basis. The only exception to this pattern is Healy 1.3-1.5 specific gravity, which has a slight increase in REE concentration as compared to Healy 1.3 floats. For Wishbone Hill, the highest concentration of REE is 857 ppm for 1.3 floats, which is attributed to the fact that it has a very low ash content for that specific gravity range (6.09% ash). The comparison of REE content between Healy and Wishbone Hill for 1.3 floats, 1.3 to 1.5, and 1.5 sink specific gravity range is shown in Figure 4 (Gupta 2016).
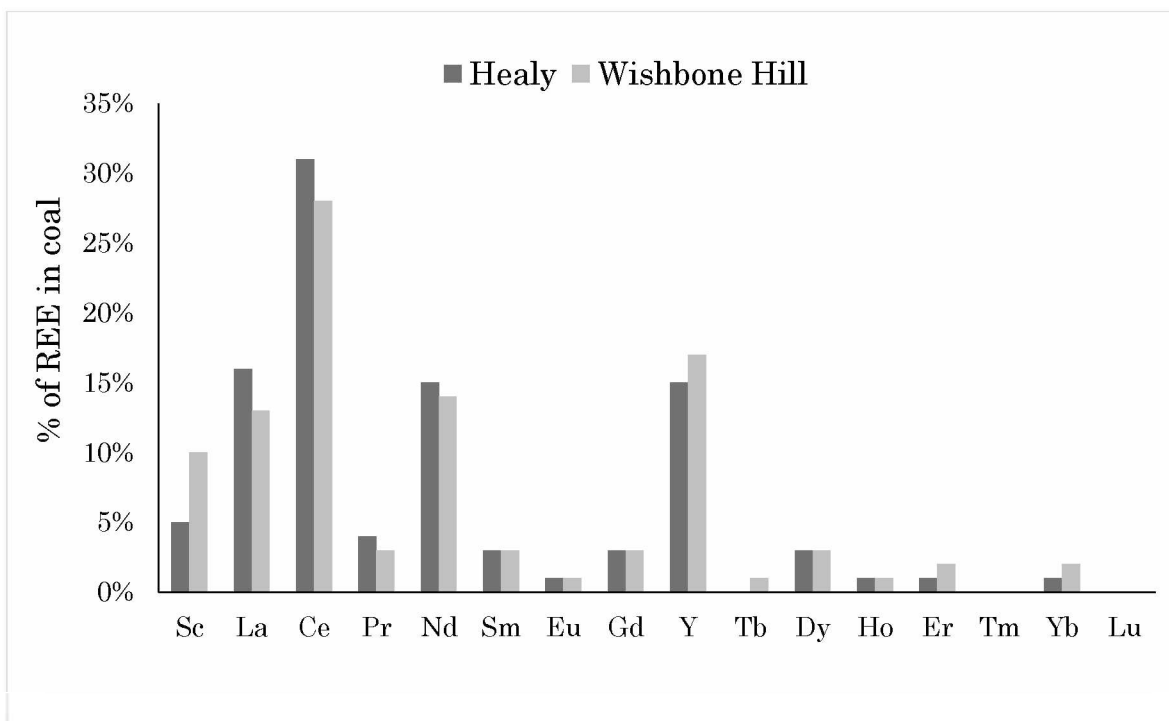
Figure 3. Comparison of total REEs in Healy and Wishbone Hill coal.
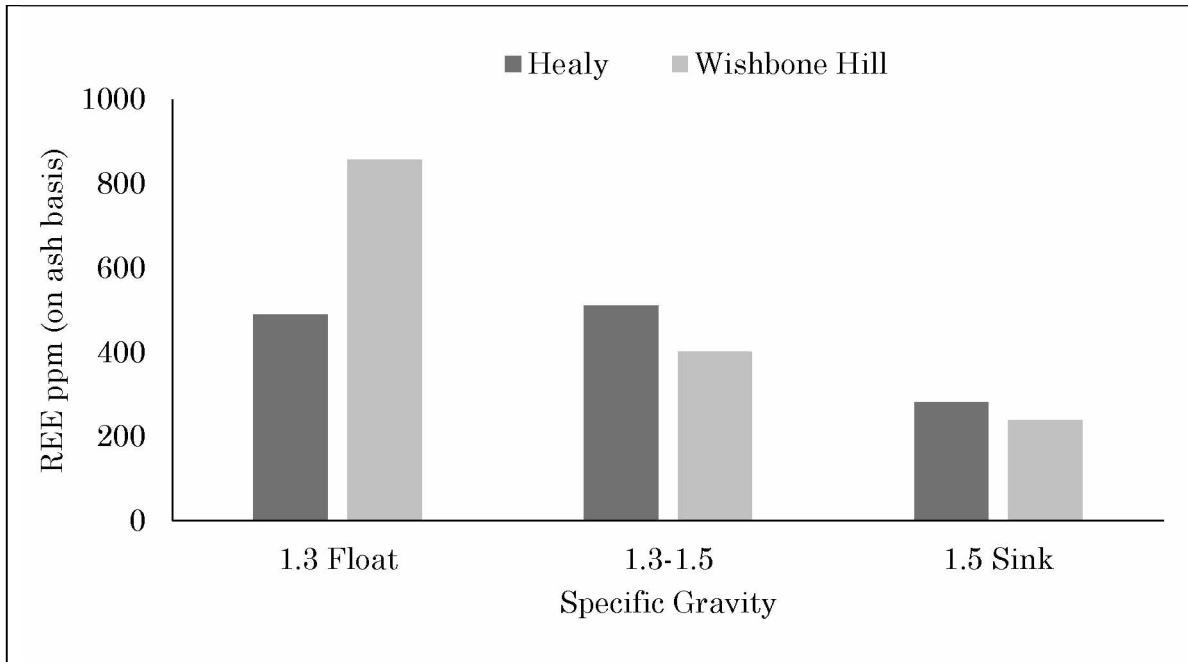
Figure 4. Comparison of total REEs in Healy and Wishbone Hill coal on ash basis (<1/4 inch to 30 Mesh size range).

2.7 Extraction Techniques for REEs

The primary sources of REEs, bastnaesite, monazite, and xenotime, usually have a cut-off grade about 1.5% to 2.0%. As the cut-off grade for REEs is very low, any deposit having a grade less than that will not be viable for economical extraction irrespective of the size of the reserve. HREEs are mostly found in ores that are harder to process, hence they have a brief history of processing and extraction while no such problem exists for LREEs (Zhang et al. 2015). The beneficiation of REEs is done by two methods, namely, physical and chemical, each of which is discussed further.

2.7.1 Physical Beneficiation

As is done for all the ores of other elements, the difference in physical properties of the mineral and gangue are exploited during initial beneficiation process. After the crushing and grinding of the ore, it undergoes various physical processes including gravity separation, magnetic separation, electrostatic separation, and froth flotation.

All these processes are widely employed for beneficiation of REE ores. Bastnaesite, monazite, and xenotime are paramagnetic and have a density range of 3.9-5.43 g/cm³. As they are paramagnetic, mostly magnetic separation is used for REE mineral beneficiation (Zhang et al. 2015). The run-of-mine ores are subjected to a series of physical mineral processing steps like crushing, screening, grinding, and flotation which finally produces a pre-concentrate having products with different rare REOs.

Monazite is a heavy mineral sand and it is obtained from placer deposits. It is usually mined by dredging or scraping. After mining the sand, it is then screened and concentrated using spiral concentrators. This mineral concentrate is then washed, dried, and sent to a magnetic separator, which removes ilmenite and other magnetic minerals. In the following step, an electrostatic plate separator is used to separate the electrically conducting and non-conducting heavy minerals. This step is then followed by using magnetic induction roll separators that help in distinguishing the induced magnetic from non-magnetic minerals, like xenotime and monazite from zircon. Xenotime and monazite can be separated by using wet-table or air table. This process takes advantage of their slightly different specific gravities and helps in easier separation.

Flotation is also used for beneficiation of REE-bearing minerals. The most common method for REE processing using flotation is to add soda ash, sodium fluorosilicate, and steam to the powdered ore slurry followed by steam conditioning. After this stage, ammonium lignin sulfonate is added while further steam conditioning is performed. The last stage of processing is the addition of steam-distilled tall oil and further bubbling of steam through the slurry and this is pumped to rougher flotation circuit, usually having 30-35% solids in it. The flotation concentrate obtained from flotation process is sent to a thickener and the resulting pulp is dewatered using a pressure filter. The concentrate obtained from rougher circuit usually has 30% REO while after dewatering and drying, it contains 60% REO (Fuerstenau 2013).

2.7.2 Chemical Beneficiation

The concentrate obtained from the flotation of REE ores is often subjected to the hydrometallurgical process, although pyrometallurgy is also sometimes used. As discussed previously, REEs are readily soluble in acids, which also allows for easy precipitation from acid solutions. As the technology for metal extraction is advancing for methods like complex reagents, solvent extraction, and ion-exchange, it is getting easier for separation of REEs, even though they only have minor differences in chemical properties (Gupta 2016).

Leaching is a form of hydrometallurgy that is mainly used for recovery of low-grade ores, which is generally the case with REEs. The leachates are usually roasted, resulting in formation of extractable REOs, an important process in making commercial REE products, then used by the general population as well as the industry (Gupta and Krishnamurthy 2004; Fleming 1992).

Bastnaesite is a fluorocarbonate mineral while monazite is a phosphate and contains predominantly either cerium, lanthanum or yttrium. These REO contents can be further separated using thermal and chemical processing steps. The REE concentrate is then mixed with hydrochloric acid, which dissolves and separates the REO contents from gangue minerals. The resulting REE-containing liquid is passed through a sequence of solvent extraction process that contains several mixer-settler steps. The products of this element-selective extraction process are separated REO. Finally, the extracted liquids are treated with sodium carbonate (Na_2CO_3) and neutralized with magnesium oxide (MgO) and precipitated using Na_2CO_3 solution or oxalic acid. The precipitated products are filtered and are further treated to obtain REO or carbonate products.

The monazite concentrate obtained from physical beneficiation is typically processed in hot sulfuric acid and then partially neutralized. Sodium sulfate (Na_2SO_4) is then added to separate the LREEs from HREEs as LREEs can be precipitated from the

sulfuric solution while it holds the remaining HREEs. The highly valuable HREEs are currently largely obtained either from xenotime or from ion adsorption clays.

Processing of ion adsorption clays is a fairly simple and low-cost operation that typically involves ion exchange by washing with ammonium sulfate ($(NH_4)_2SO_4$), followed by removal of uranium and thorium from the pregnant solution. After this step, the rare earth carbonates are precipitated and filtered and finally heated to high temperature in presence of air to obtain a mixed REO concentrate. This concentrate is further separated and purified into individual REO. Recovery of REEs from these operations typically exceeds 90%.

After the concentration of REE-bearing minerals, REEs have to be extracted from the concentrate. There are several methods for decomposition of REE-bearing minerals. The most important part of decomposition includes thermal treatment of the ore in the presence of acidic or caustic reagents. The composition of the ore concentrate is an important factor in deciding the method to be applied for decomposition. Acid baking with sulfuric acid is the most common process. The powdered ore obtained from previous processes is mixed with concentrated sulfuric acid and baked at temperatures between 200 and 400°C for several hours. The REE sulfates are soluble in water so the resulting cake is leached with it to dissolve REEs as sulfates. This process is very sensitive and requires optimal reaction conditions, and reagent use have to be matched specifically with each tested ore. There are different factors that influence this reaction, like the presence of iron oxide leads to an increase in the consumption of acid. When the roasting temperatures are above 300°C, the recovery of REEs decrease in most cases and also leads to reduced thorium leaching. Generally, thorium is an undesired leaching product, which means that the roasting temperature will be a trade-off between REE recovery and thorium leaching. Acid baking is the standard process since it is applicable to most of the common rare earth minerals like monazite, bastnaesite, xenotime, apatite or aeschynite.

The carbonate minerals like bastnaesite, parisite, synchisite or similar minerals are decomposed in HCl, but it can also be used to decompose allanite, cerite or gadolinite. The ore is mixed with concentrated HCl at temperatures >90°C and if the ore contains fluorine (e.g., bastnaesite), some part of the REEs forms insoluble REE-fluorides that are left behind in the solid residue. The solid residue has to be converted into hydroxides and soluble sodium fluoride to recover those REEs. For this process, the solid residue is further decomposed with sodium hydroxide. Due to the addition of sodium hydroxide (NaOH), the fluorides are washed away and REE hydroxides are dissolved by excess HCl in the leaching liquid from the HCl decomposition step. If calcite or similar carbonate phases are present in the ore, then it has to be purified with diluted HCl at room temperature prior to its decomposition as these reaction conditions will dissolve the unwanted carbonate phases without attacking bastnaesite. Eudialyte is a special ore as it is easily soluble in any mineral acid, which makes its decomposition rather simple. The problem with eudialyte is that it is often accompanied by zeolites, which tend to form silica gels when dissolved in acid. To minimize the formation of silica gels, precautions have to be taken depending on the zeolite content. Alternatively, caustic decomposition can be applied to specific ores. The most common process is decomposition with NaOH, which is applicable for monazite and bastnaesite. The ore is mixed with 50-60 weight% NaOH and is decomposed at temperatures greater than 140°C. This process transforms the REEs to hydroxides, while the phosphates present in monazite or carbonates and fluorides present in bastnaesite are transformed into soluble sodium salts, which can be easily washed off and the resulting solids are leached in diluted HCl. If it is necessary to remove residual thorium, it can be done so by dissolving it in nitric acid and extraction of the resulting solution with organophosphates (Xie et al. 2014; Kim et al. 2016; Gupta and Krishnamurthy 2004).

One of the challenges of REE extraction is the use of industrial grade acids in the leaching process that poses environmental hazards, as well as an unsafe work environment. There is always a risk of acid leaks, which can cause serious

environmental contamination. To prevent these risks, novel approaches and sources are needed for REE extraction and one of the methods that is gaining the attention of the researchers is microbial weathering or bio-leaching of coal, as it contains sizeable amount of REEs in it, to release the REEs entrapped in it.

CHAPTER 3 MICROBIAL LEACHING

3.1 Introduction to Microbial Leaching

Microbes have profoundly affected earth's surface over geologic time by playing critical roles in weathering of minerals and rocks, like carbonates, silicates, basalts, etc. Microbial weathering rates are very high compared to abiotic reactions and the most intense weathering occurs when the microbes are attached to the surface of particles. Several mechanisms of microbial weathering have been identified such as acid production (organic and strong acids), physical disruption, siderophore or chelator production, and electron transfer. This is done by using microbes that oxidize sulfide minerals mainly in the form of pyrite, which produces acid that causes metal dissolution. But coals that have high concentrations of REEs typically have low sulfur content. Wishbone Hill has 0.26% sulfur while Healy has 0.44% sulfur, which means conventional microbial leaching methods will not be suitable (Gupta 2016). To alleviate the environmental problems related to conventional methods of REE extraction and concentration, microbes that perform bio-weathering processes employing physical distribution, chelator, acid production, and electron transfer as their mechanisms can be used. One bacterium with the potential to aid in this process is *Shewanella oneidensis* MR-1.

3.2 What is Bioleaching?

Bioleaching is the process in which living organisms are used to solubilize metals from sulfidic ores or solid wastes into aqueous solutions. Metal recovery from sulfide minerals is performed by chemolithotrophic bacteria that convert insoluble metal sulfides into soluble metal sulfates while non-sulfide ores can be treated by heterotrophic bacteria or fungi. *S. oneidensis* MR-1 can reduce iron without coming into contact with it under anaerobic conditions. This microorganism can adapt to different conditions fairly easily as it is demonstrated by the fact that when the cells are pre-grown anaerobically prior to conducting the experiment, they can reduce iron

from a distance faster than compared to the cells which are grown aerobically (Lies et al. 2005b). If given enough time to adapt to the anaerobic conditions, the cells that are grown aerobically can also reduce iron from a distance. Lies et al. (2005a) performed experiments on iron beads and suggested that the iron present was more reactive as it was exposed to the water interface and therefore accessible for reduction. The iron present in the beads was inside the pores and therefore away from the cells, which suggest that the cells used nanowires (a hair-like appendage on the surface of bacteria) or electron shuttles to transfer electrons to the metal and reduce it. The process of bioleaching is applied at a commercial scale to extract base metals (e.g., Au, Cu, Co, and Ni) and can also be used to extract REEs from coal, wastewater, industrial wastes and mine tailings (Sethurajan et al., 2018).

The bioleaching mechanism falls under the biohydrometallurgy subdivision in which microorganisms are used to produce the leaching agents (oxidants and/or acids) needed for extraction of metals from low-grade ores, tailings, or end-of-use wastes. This method is economically beneficial because of its lower operating costs, reduced environmental impact, and use of low-grade ores or wastes.

The process of bioleaching can be classified into direct and indirect leaching based on the contacts between microbes and ores. The direct leaching can be categorized as biomineralization, bioprecipitation, biosorption, bioaccumulation, and bioreduction. The indirect leaching can be sub-grouped into three type such as redoxolysis, acidolysis, and complexolysis. Figure 5 shows the classification of bioleaching based on different mechanism and contact while Table 5 shows the comparison among the three different bioleaching mechanisms.
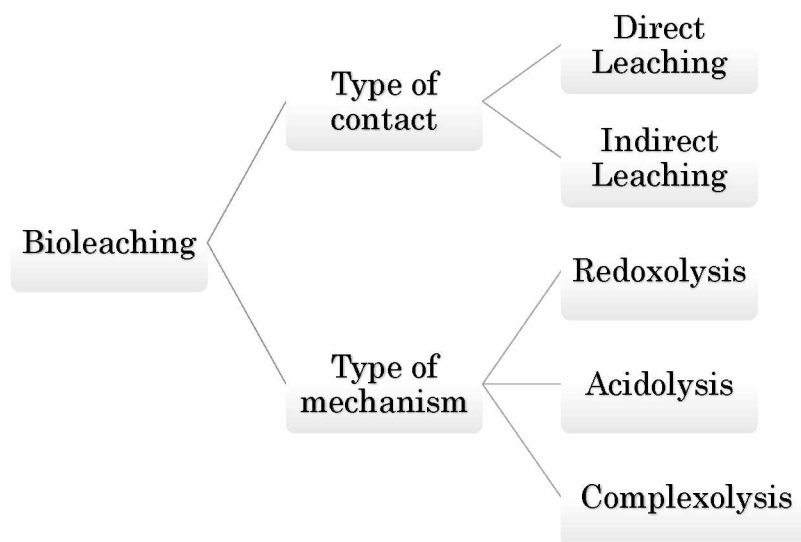
Figure 5: Different types of bioleaching based on mechanism and type of contact.

Table 5: Comparison among three indirect bioleaching mechanisms

Redoxolysis	Acidolysis	Complexolysis
Metal solubilization is achieved by electron transfer from mineral to microbe (direct leaching) or oxidation of $Fe^{2+} \rightarrow Fe^{3+}$ (indirect leaching).	Proton mediated dissolution of insoluble metal species is performed by microbes that acidify the environment by producing acids while consuming nutrients.	Soluble metal species are formed by chelation of microbial metabolites with metal ions to form stable metal-chelating compound.
A. ferrooxidans initially catalyzes the oxidation of Fe^{+2} to Fe^{+3} ($4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O$) and then Fe^{+3} oxidizes the chalcopyrite to Cu^{+2} ($CuFeS_2 + 4Fe^{3+} \rightarrow 5Fe^{2+} Cu^{2+} + 2S^0$).	*A. thiooxidans* uses elemental sulfur and produce biogenic sulfuric acid (that can bioleach Zn from Zn-metallurgical wastes).	*P. putida* produce extra-cellular cyanide that helps in solubilizing gold, hence improving recovery from electronic waste.
Ex: *Acidithiobacillus ferrooxidans*	Ex: *Acidithiobacillus thiooxidans*	Ex: *Pseudomonas putida, Pseudomonas aeruginosa*

3.2.1 Biomineralization

The biomineralization process results in the formation of biominerals within or outside the microbial cells. A large variety of chemical composition and structures of biominerals like aragonite, calcite (both carbonates), opal (silicate) or iron/manganese oxides are induced or controlled by a huge diversity of eukaryotes, bacteria, or archaea. *Saccharomyces cerevisiae, Pseudomonas flourescens,* and *Bacillus subtilis* are recorded to have nano-mineralized Sm from solutions into their cell bodies (Jiang et al., 2018) while *S. cerevisiae* is also recorded to have nano-mineralized cerium phosphate (Jiang et al. 2010). Recently, *Serratia* OT II 7 has been found to biomineralize uranium under acidic and alkaline conditions. Selenium, a non-metal, has been found to be used as a respiratory substrate by *Thauera selenatis,* which results in the formation of a red allotrope of Se° in the form of nano-spheres (Butler et al. 2012). These allotropes of selenium are different from each other depending on what kind of bacteria synthesized it and are yet to be reproduced by chemical synthesis. The bioaccumulation of selenium starts by reduction of selenate (SeO_4^{2-}) to selenite (SeO_3^{2-}) in the periplasm of *T. selenatis* by molybdoenzyme selenite reductase. The electrons required for this reduction are drawn from the quinone pool by the c-type cytochrome (Butler et al. 2012). The selenite is then transported across the membrane to the cytoplasm where it is reduced again, which leads to its accumulation as selenium nano-spheres that are ultimately secreted into the surrounding medium.

Biomineralization has also been used in treating underground water for uranium contaminants. This process has applications in nanotechnology, wastewater treatment, bioremediation, and metal recovery (Chandwadkar et al., 2018). *S. cerevisiae* is also recorded to have nano-mineralized cerium phosphate (Jiang et al. 2010). It was hypothesized that the cerium reacted with phosphorous released by *S. cerevisiae* to form monazite nanocrystallites on the cell surface. The mineralization on cell surface happens after the adsorption of cerium, hence, this is of great importance for durable migration of LREEs (Jiang et al. 2010).

3.2.2 Bioprecipitation

When living organisms form mineral phases like bioprecipitates or biominerals, the ensuing process is known as bioprecipitation wherein the chemical composition of the fluid is changed and the supersaturation of the solution with a mineral phase is raised and/or the cell or the associated extracellular polymers act as a nucleation surface. Recovery and removal of metals from the metal solutions like acid mine drainage or industrial wastewater are done through precipitation in the form of sulfides, hydroxides or in some rare cases, carbonates. There are three critical stages in precipitation process namely: a) nucleation, b) growth of nucleus, and c) aggregation and precipitation. Certain chemicals/agents are added to the metal solution to cause precipitation, but this process has a disadvantage as it forms sludge and has high requirement of chemicals to adjust pH. To overcome this problem, biogenic sulfide precipitation has received special interest for application in the industrial setting.

Sulfate-reducing bacteria reduce the oxidized sulfur compounds while oxidizing organic compounds for energy and producing sulfides. Eq.1 and Eq.2 show that sulfide (biogenic sulfide) produced during sulfate reduction can potentially precipitate metals (Sethurajan et al., 2018).

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^- \qquad \text{Eq. 1}$$

$$M^{2+} + HS^- \rightarrow MS + H^+ \qquad \text{Eq. 2}$$
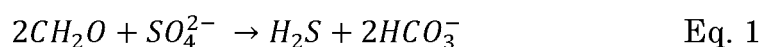
The biogenic sulfidic precipitation follows two different mechanisms such as assimilatory and dissimilatory sulfate reduction (Sethurajan et.al, 2018).

Uranium recovery from waste streams through bioprecipitation by biogenic phosphate has been of special interest in recent times. In this process, the microorganisms with phosphate activity are used to release inorganic phosphate for precipitation of uranium. Several microorganisms like *Bacillus sp., Rahnella sp., Citrobacter sp., Pseudomonas sp., Serratia sp.,* and *Deinococcus radiodurans* are

capable of reducing uranyl nitrate and uranyl carbonate, which are soluble, to hydrogen uranyl phosphate, which is insoluble (Nancharaiah et al., 2016). Bioprecipitation has a major advantage over other processes as it gives the possibility of selective recovery of metals from multiple metallic leachates with minimal generation of toxic sludge and no requirement of external chemicals/agent to maintain pH levels.

Bioprecipitation has a major advantage over other processes as it gives the possibility of selective recovery of metals from multiple metallic leachates with minimal generation of toxic sludge and no requirement of external chemicals/agent to maintain pH levels.

3.2.3 Biosorption

When certain types of inactive or dead microbial biomass like algae, bacteria or fungi bind and concentrate heavy metals on their cell walls either by a property or as defense mechanism even from dilute solutions, the process is called biosorption. The difference between biosorption and bioaccumulation is that in the case of bioaccumulation, the process is performed by metabolically active living cells while in the case of biosorption it is performed by either live (active) or dead (inactive) microorganisms. Bacteria or fungi can sorb metals by two processes, i.e., active process (by releasing energy) or inactive process (by electrostatic attachment to the cell wall). Biosorption has served as an alternate approach for removing metals from groundwater contaminated with heavy metals like As, Cd, and Cr because it is economical and works well with low metal ion concentration (Nancharaiah et al., 2016). Past studies have shown that yeast cells, fungus, and algae can concentrate REEs from dilute solution but the bioconcentration requires a nearly neutral or mildly acidic environment, which makes it difficult to extract REEs from fly ash as the solutions are acidic (Sethurajan et al., 2018). Biosorption can also be applied to REE leachates obtained as waste from conventional leaching of REEs during beneficiation. Some strains of *C. crescentus* and *E. coli* are used to produce lanthanide

binding tags (LBTs) on these leachates, which are anchored to the cell surface and has specificity towards REEs (Jin et al. 2017). *Candida utilitis* was reported to have adsorbed La, Sm and Y on its surface when the pH of the solution containing them was above 7.0, while at pH of 5.0, there was no adsorption (Korenevsky et al., 1998).

This process does not produce any chemical sludge, and it is easy to operate and efficient in the removal of pollutants from low concentrations. Most of the biosorption application studies are limited to laboratory scale; pilot and field scale application is necessary to identify its feasibility towards REE extraction from industrial wastes.

3.2.4 Bioreduction

Bioreduction is a process in which the soluble metal species are converted to insoluble species. This process is most commonly applied to Au, Ag, and Pd but can also be applied to toxic metal species like U^{+6}, Cr^{+6}, Se^{+4}, Se^{+6}, Co^{+3}, and REEs like Eu. *Shewanella oneidensis*, *Geobacter uraniireducens* and *Geobacter daltonii* are the three prominent uranium reducing bacteria. U^{+6} is toxic to most of the bacteria and to overcome the toxicity, high concentrations of bicarbonate are added to U^{+6} contaminated water. Vanengelen et al. (2010) demonstrated that when high concentrations of bicarbonate are used, the toxicity of U^{+6} significantly reduces due to the formation of the negatively charged uranyl-carbonate aqueous complex $((UO_2)_x(CO_3)_y(OH)_z^{2x-2y-2z})$. Due to the formation of these complexes, U^{+6} becomes less bioavailable to the bacteria. The rate of U^{+6} reduction by *S. oneidensis* was measured by Sheng et al., (2011) in the presence of dissolved calcium and ethylene diamine tetraacetic acid (EDTA). They found that there was a strong correlation between the concentration of new complexes of U^{+6} and the reduction rate of U^{+6} on the cell envelope. The bicarbonate forms the uranyl-carbonate aqueous complex, which is then adsorbed on the cell envelope of *S. oneidensis*. After the adsorption, the electrons are transferred by the bacteria to the complex and U^{+6} is reduced to U^{+4}. The Eu-Au nanoparticles were most efficiently recovered at pH of 8 with 100% of particles being smaller than 20 nm and 63% particles greater than 10 nm (Ascencio et al. 2003).

Bioreduction also has a potential for biorecovery of precious metals from the leachates; however, rigorous lab-scale investigations are necessary prior to implementing it at large-scale applications.

3.2.5 Bioaccumulation

Bioaccumulation is another process in which the heavy metals can be removed from wastewater by accumulation in living biomass. While in biosorption both living and non-living biomass can act as sorbents, in bioaccumulation only living biomass can be used. Bioaccumulation is an active process and is highly dependent on structural properties, bioavailability of heavy metals and genetic properties of bacteria. The fungal strain *Penidiella* sp. T9 obtained from an abandoned mine rich in REEs and other metals at high concentrations show high selectivity towards REEs and accumulation of dysprosium (Dy) from low pH (~2.5) solutions. The same amount of removal of Dy and Nd was reported when both of them were present in equal concentrations in a solution. Parallels were drawn between the bioaccumulation of Yb via *S. cerevisiae* and Dy by T9 and it was proposed that the bioaccumulation of all Yb consisted of two processes: (i) the cell created a large amount of phosphorous that led to the formation of phosphates that combined with the ytterbium to form precipitates of large-sized ytterbium phosphates, and (ii) the nano-sized Yb phosphates accumulated via Yb uptake by cytoplasm and a biological reaction with the functional group of a cell surface. This also suggested phosphorous has an important role in bioaccumulation of Yb and proposed that the Dy bioaccumulation with P is same as proposed for Yb/P bioaccumulation by *S. cerevisiae* mentioned in the process Table 7 (Horiike and Yamashita 2015).

All the biohydrometallurgical processes discussed above can be applied for treating wastewater, acid mine drainage or ores to recover a wide variety of REEs like Eu, Dy, Nd, Yb, Y, La, and Sm. Industrial wastewater can be treated by microorganisms; this has two benefits as it clears the water of these elements but also serves as an alternate source of REEs. Dysprosium and neodymium, both REEs, can be

bioaccumulated by a fungal strain, *Penidiella* sp. T9 by combining them with phosphates and then accumulating it in their cytoplasm in the form of nano-particles. Europium can be bioreduced to nanocrystals while lanthanum and yttrium can be bioreduced by *C. utilitis*. *P. flourescens* and *B. subtilis* are reported to bioprecipitate samarium. Rigorous and intensive laboratory tests are required for the microbial application in industries as they can serve as an alternate source of REEs in the future.

3.3 Factors Influencing Bioleaching of REE

The effectiveness of bioleaching depends on a number of factors ranging from the activity of microorganism to the mineralogical extraction and chemical composition of the leachate. Some of the factors affecting bioleaching include characteristics of microorganisms, nutrients, pH, temperature, and the mineral substrate. The maximum yield of extracted metals occurs only when the leaching conditions correspond to the optimum growth conditions of the bacteria.

3.3.1 Characteristics of Microorganisms and Nutrients

Heterotrophic leaching can be performed by a diverse set of microorganisms and is affected by the microbial population, its metal tolerance and the adaptability to the mineral environment. Also, for bioleaching process, selection of appropriate microorganism is the first step. Several indigenous thermophilic bacteria obtained from acid mine drainage and tailings pond like *Acidothiobacillus, Leptospirllium spp., Sulfobacillus spp.* have been tested for bioleaching of REEs (Sethurajan et al., 2018). These bacteria are useful because they live and perform their metabolic processes in the presence of REE containing minerals and have evolved to incorporate them in their energy cycle. Actinobacteria, a phylum of Gram-positive bacteria, are highly resistant to metal toxicity and radiation. Due to these physiological traits, they might be able to leach REEs from minerals containing it (Zhang et al. 2018). The study performed by Zhang et al. (2018) found that *Streptomyces sp*. FXJ1.172 was

able to grow in nutrient-deficient conditions in the presence of bastnaesite and hence it can be used for leaching REEs from e-waste, mine tailings, and other solid wastes. The study also found that bastnaesite provides some nutrients for the growth of bacteria, but the biomass was low and no growth was observed when bastnaesite was not present.

3.3.2 pH of Solution

The pH of the solution is an important factor that controls the biosorption process and affects the speciation of metals in solution through hydrolysis, complexation and redox reactions during metal recovery. Lanthanum biosorption using *Sargassum fluitans* at a pH 5.0 was 10 times more than at pH 2.0 although at pH higher than 5.0 results in the formation of insoluble hydroxide which inhibits biosorption (Palmieri et al., 2002). Similarly, biosorption of cerium by *Platanus orientalis* yields the maximum result at a pH of 4.0; however, in the case of *Agrobacterium sp.* HN1, La and Ce adsorption is increased by 188% and 606%, respectively, when pH is changed from 2.0 to 6.8 (Das and Das 2013). *Penicillium tricolor* RM-10 is known to reduce the pH of red mud solution from 10.0 to 3.0 in 200 hours and this was demonstrated during bioreduction of red mud (Qu and Lian 2013). For bacterial oxidation of ferrous iron and sulfide, a pH of 2.0-2.5 is optimum. *Thiobacillus ferrooxidans* undergoes severe inhibition at a pH less than 2.0 but it can be adapted to lower pH values by increasing acid in the solution through laboratory evolution of the species (Bosecker 1997).

3.3.3 Temperature

Optimum temperature is required for maintaining the correct growth and activity of any bacteria. For ferrous iron and sulfide oxidation by *T. ferrooxidans,* a temperature range of 28-30° C is required. At lower temperatures, the bacteria will be alive, but the activity decreases and it does not function properly. The bacteria are divided into three categories depending on the temperatures in which they can survive and grow.

Thermophilic bacteria can survive in a high-temperature range of 41° C to 122° C, like *Thiobacillus*, which has been used for leaching purposes at temperatures ranging 5°-80° C (Bosecker 1997). Mesophilic bacteria grow best in temperatures between 20° C and 45° C, as demonstrated by *Agrobacterium sp.* HN1, which is reported to have responded positively to a high-temperature range of 35-40° C for adsorption of lanthanum and cerium (Aksu et al., 1992). Psychrophilic (cryophilic) bacteria grow best at a temperature below 15° C; for example, a strain of bacteria B0665 was found to have increased dissolution of feldspar by 20 times when the temperature was kept at 5° C (Welch and Ullman 1999). Microbes are found in each of the three divisions that can perform bioleaching, which provides us the opportunity to use bioleaching at various temperature ranges.

3.3.4 Mineral Substrate

The mineralogical composition of leaching substrate is of foremost importance. If the leaching substrate has high carbonate content, the pH of the leaching liquid will increase and it will inhibit the bacterial activity or even completely suppress it (Bosecker 1997). The leaching process also depends on the particle size. Previous research has shown that smaller particle size fractions (42 µm) give better results compared to large size fraction (Torma 1977). Pulp density of the substrate plays an important role in bioleaching of REEs. *P. tricolor* RM-10, used for leaching REEs from red mud, showed best results when the pulp density was 2% (w/v) for one step bioleaching, while for a two-step process, maximum yield was obtained at 10% pulp density (w/v) (Qu and Lian 2013); however, if the pulp density is increased too much it can lead to the dissolution of inhibitory compounds that may prove to be toxic for leaching bacteria.

3.4 *Shewanella oneidensis*

3.4.1 Introduction

S. oneidensis can survive and grow under both oxic and anoxic conditions and it can reduce metal ions under anoxic conditions. This species has a single polar flagellum that helps in swimming in marine environments. The ability of *Shewanella* to respire on insoluble substances is a true biological feat that has recently stimulated a deep interest by researchers. They are usually found in marine sediments and experiments have proven that this bacterium can reduce ionic mercury and ionic silver to elemental mercury and silver and Fe^{+3} and U^{+6} to Fe^{+2} and U^{+6}, respectively (Lies et al. 2005a; Sheng and Fein 2014). Due to this metal-reducing capacity of *S. oneidensis* and its use in reducing iron present in clay, recent interest has developed in its use for extraction of REEs from coal. This thesis will focus primarily on the metabolism of *S. oneidensis* and its potential use in the extraction of REEs from coal. Table 6 shows the taxonomic classification of *S. oneidensis* MR-1.

Table 6. Taxonomic classification of *S. oneidensis* MR-1

Taxonomic Rank	
Kingdom	Bacteria
Phylum	Proteobacteria
Class	Gamma Proteobacteria
Order	Alteromonadales
Family	Shewanellaceae
Genus	Shewanella

3.4.2 History and Taxonomy

Shewanella is the only genus included in the Shewanellaceae family of marine bacteria, is found in extreme aquatic habitats with low temperature and high pressure and consists of facultative anaerobic gram-negative rods. It was originally

44

identified in 1931 as one of the numerous species of bacteria growing on decaying butter and was first classified as part of the genus *Achromobacter* (Derby and Hammer 1931). Due to its status as a non-fermentative marine bacterium, and the guanine/cysteine content of its DNA, it was reclassified numerous times and finally in 1985, the species was given a new name on the basis of 16S rRNA sequencing as a tribute to Dr. James Shewan for his outstanding work in fisheries microbiology. As the *Shewanella* species can live in both oxic and anoxic environments, it has developed diverse metabolic capabilities that provides it with a competitive edge in a range of environments especially where the type and concentration of electron acceptors fluctuate. Figure 6 shows *S. oneidensis* MR-1 growing on hematite.
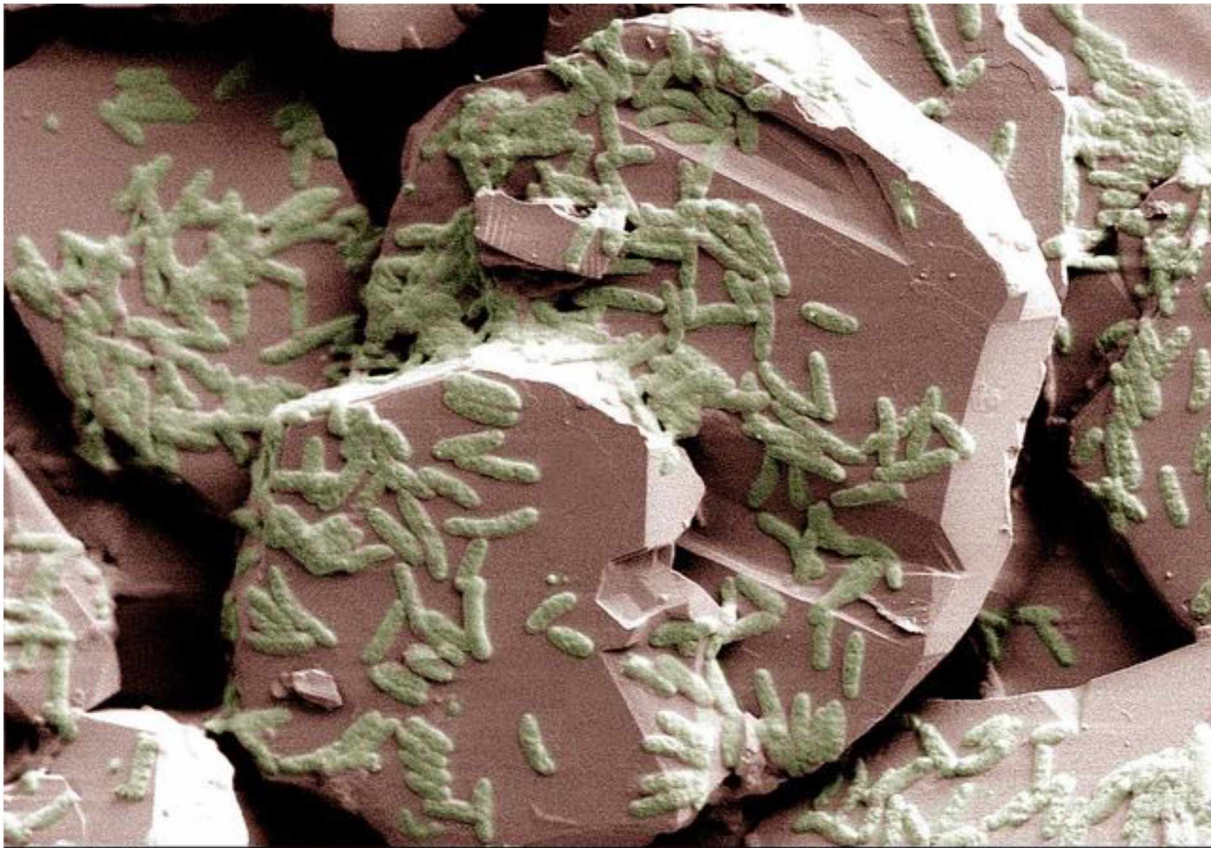
Figure 6: *S. oneidensis* **MR-1 growing on hematite (Wigginton 2014)**

S. oneidensis was first isolated from Lake Oneida, New York in 1988, where the species gets its name from and is sometimes referred to as *S. oneidensis* MR-1. MR-1

indicates the "manganese reducing" attribute that is generally misunderstood to be "metal-reducing." This bacterium has the ability to couple metal reduction with its metabolism and belongs to a group called "dissimilatory metal-reducing bacteria (DMRB)." DMRB is a group of microorganisms that perform anaerobic respiration and use metal as a terminal electron acceptor instead of using molecular oxygen (O_2). They reduce metals from a higher oxidation state to a lower oxidation state while other microbes produce water by reducing molecular oxygen. Due to this metal-reducing capacity, *S. oneidensis* has the potential to be used for extraction of rare earth elements present inside the coal.

3.4.3 Metabolism

Shewanella is present in both marine and freshwater environments, is typically found in places with high quantities of organic matter with favorable redox conditions, and is well adapted to the environments where electron donors are abundant, but electron acceptors are limiting and vary spatially. To put it simply, it is well adapted to the redox surface environments and most of the members of this genus can utilize a wide range of electron acceptors like oxygen (O_2), nitrate (NO_3^-), nitrite (NO_2^-), fumarate ($C_4H_2O_4^{2-}$), sulfite (SO_3^{2-}), Fe^{+3}, and Mn^{+4}.

As *Shewanella* can reduce metals and radionuclides, it has been used for investigating redox transformations of environmental contaminants such as uranium, plutonium, and technetium (Park et al. 2007). Due to the tendency of *Shewanella* to transfer electrons to extracellular substrates, recent interest has developed for its application in microbial fuel cells. The *Shewanella* species can perform a complete tricarboxylic acid (TCA) cycle which helps it in oxidizing acetate to CO_2 under aerobic conditions. Earlier, it was understood that oxidative phosphorylation was the primary pathway for ATP synthesis in *Shewanella* (Park et al. 2007; Reed et al. 2003) but it was demonstrated that *S. oneidensis* MR-1 could ferment pyruvate to gain energy for survival (Price et al., 2004). Although the physiological significance of this process in *S. oneidensis* MR-1 is yet to be

understood, fermentative metabolism in obligately respiratory bacteria may represent an important mechanism of survival in the absence of available electron acceptors. It is very important to have a clear knowledge of the underlying mechanism and other metabolic traits to have a better understanding of the function of *Shewanella* in various important environmental processes and also in its potential applicability in biotechnological applications.

S. oneidensis MR-1 utilizes pyruvate to produce formaldehyde, which then reacts with glycine to produce serine and enters into the TCA cycle as oxaloacetate, thereby consuming one $NADH_2$ and one ATP in the process. This is the central anaerobic carbon metabolic pathway of *S. oneidensis* MR-1 and is known as "serine isocitrate pathway." *S. oneidensis* MR-1 can grow on limited organic compounds such as lactate, pyruvate, DNA, and *N*-acetylglucosamine during anaerobic respiration whereas during aerobic respiration it can use a wider range of organic compounds. Unlike *Geobacter* (Esteve-Nunez et al. 2005) and *Anaeromxyobacter* (He and Sanford 2003), *Shewanella* is unable to grow by coupling acetate oxidation with reduction of electron acceptors other than O_2. This happens because when the electrons are transferred to protons to produce hydrogen, the production of hydrogen is limited due to the chemical kinetics and requires the removal of hydrogen. Moreover, during anaerobic respiration, the growth of *Shewanella* on lactate is accompanied by acetate accumulation (Lovley et al., 1989).

3.4.4 Mechanism for Metal Reduction

Biofilm formation helps but is not required in the metal reduction process. Nonetheless, *Shewanella* uses biofilm for maintaining adjacent contact between the bacteria and the oxidized metal (Thormann et al. 2004). After the formation of biofilm, *Shewanella* transfers electrons to the metal for respiration where special types of molecules, called cytochromes, help in metal reduction which facilitates the transfer of electrons in the electron transport chain. The cytochromes are electron transport proteins and facilitate in small, reversible energy transitions with electron

heme cofactor consists of an iron atom surrounded by a ring of conjugated double bonds. These double bonds and iron atoms have low redox potentials, which means that they can facilitate the transfer of electrons that have low energy requirement. This outstanding ability of cytochromes to acquire and transfer electrons gives them an advantage over other microbes in the metal reduction process. This mechanism of transfer of electrons is shown in Figure 7. The main advantage of the low redox potential is that it prevents the loss of energy in the form of heat, and instead uses that energy to reduce metals or pump protons across a membrane. *S. oneidensis* MR-1 contains at least 42 putative cytochrome c molecules and has CymA protein in the inner membrane, MtrA protein in the periplasm and MtrC and OmcA protein in the outer membrane, which is fixed in the outer membrane by type II protein secretion pathway (Meyer et al. 2004).

Figure 7: Transfer of electrons from the inner membrane using MQH$_2$ to the metal oxide (Fe^{+3} via periplasm and outer membrane (left) and the conventional method of transfer of electrons when the metal oxide is in contact with the cell (Fredrickson et al. 2008).

After the formation of biofilm, *S. oneidensis* has three options to transfer electrons to the metal oxides and these methods depend on the surrounding environmental conditions. These conditions are: a) the biofilm is in contact with the metal oxide, b) low metal oxide concentration leading *S. oneidensis* MR1- to form nanowires, and c) using electron shuttles instead of nanowires. Condition (a) is classified as direct metal reduction while conditions (b) and (c) are classified as indirect leaching as they lead to the transfer of electrons without direct contact of metal oxide and biofilm, enabling the transfer of electrons "at a distance." The conventional method of electron transfer occurs when the metal oxide is in contact with the cell. MtrC and OmcA are lipoproteins associated with the outer membrane protein MtrB (Figure 7) and facilitate the reduction process of Fe^{+3} during anaerobic respiration. These cytochromes couple oxidation of organic carbon to electron acceptors such as Fe^{+3}, oxygen, nitrate, and other metals. The position of MtrC and OmcA are fixed in the outer membrane, hence they are exposed to the extracellular environment. It is at this place where they can contact metals and reduce them by transferring electrons. The *Shewanella putrefaciens* species does not have MtrC and OmcA proteins in its cytochromes, which makes it 45% and 75% slower, respectively, at reducing MnO_2 than the other non-mutated strains, and highlights the importance of MtrC and OmcA proteins in the metal reduction process (Myers and Myers 2001).

When the environment surrounding *S. oneidensis* is low in metal oxide concentration, they have a special ability to grow a pilus-like structure that helps them to locate and reduce metal oxides (Marsili et al. 2008). Menaquinol (MQH_2) is a membrane-soluble quinone and transfers electrons to CymA, present in the periplasm. The electrons then move from the periplasm to the outer membrane proteins (MtrA and MtrB) finally ending up at the extracellular surface (MtrC and OmcA) (Marsili et al. 2008). Here these electrons come in contact with the insoluble metal oxides and transfer electrons to them directly or with the aid of nanowires or electron shuttles (at a distance) depending on the surrounding environment. Hence, *S. oneidensis* has the ability to reduce metal oxides "at a distance" by transferring electrons via nanowires

or electron shuttles to the metal oxides. Figure 7 shows the complete mechanism of electron transfer using electron shuttle (riboflavin) (left) and the conventional method (right) from the inner membrane to the metal oxide.

The difference between using riboflavins and conventional method for electron transfer is that riboflavin needs a media surrounding the biofilm, whereas the conventional method does not require media. Riboflavin (vitamin B2) has a polar tail that increases its solubility in the media and helps in shuttling electrons from the cell surface to external metals. So when the media surrounding the biofilm is removed, the electron transfer rate drops by more than 70% (Marsili et al. 2008). Conversely, the organisms which only use outer membrane cytochromes for electron transfer, like Geobacter, the rate of electron transfer is not affected significantly (less than 5% drop) by the removal of media surrounding the biofilm (Marsili et al. 2008).

3.4.5 Examples of Metal Reduction by *S. oneidensis* MR-1

3.4.5.1 Iron Reduction

As previously mentioned, *S. oneidensis* MR-1 can reduce iron without coming into contact with it under anaerobic conditions. One important observation is that the cells that were pre-grown anaerobically (e.g., with fumarate or ferric citrate as the electron acceptor) prior to conducting the experiment reduced iron at a distance faster as compared to the cells grown aerobically. If given time to adapt to the anaerobic conditions, the cells that are grown aerobically can also reduce iron at a distance (Lies et al. 2005a). Experiments done by Lies et al. (2005a) on iron beads suggested that the iron present was more reactive as it was exposed to the water interface and therefore accessible for reduction. The iron present in the beads was inside the pores and therefore away from the cells, suggesting that the cells used nanowires or electron shuttles to transfer electrons to the metal and reduce it. Similar to the iron present in the pores of bead, the REEs are also trapped within the coal matrix. Hence,

S. oneidensis can be used to reach them and leach out in the solution containing microbes along with substrates.

3.4.5.2 Uranium Reduction

U^{+6} is toxic to most of the bacteria and to overcome the toxicity, high concentrations of bicarbonate are added to U^{+6}-contaminated water. Van Engelen et al. (2010) demonstrated that when high concentrations of bicarbonate are used, the toxicity of U^{+6} significantly reduces due to the formation of the negatively charged uranyl-carbonate aqueous complex $((UO_2)_x(CO_3)_y(OH)_z^{2x-2y-2z})$ as shown in Figure 8. Due to the formation of these complexes, U^{+6} becomes less bioavailable to the bacteria. The rate of U^{+6} reduction by *S. oneidensis* was measured in the presence of dissolved calcium and ethylenediaminetetraacetic acid (EDTA) and it was found that there was a strong correlation between the concentration of new complexes of U^{+6} and the reduction rate of U^{+6} on the cell envelope (Sheng and Fein 2014). Additional studies suggest that metal bioavailability can be linked to the extent and speciation of metal adsorption onto bacterial cell envelopes. *E. coli* is recorded to have moved away from nickel present in the aqueous solution and this was strongly correlated to the extent of nickel adsorbed onto functional groups on the cell envelope (Borrok et al. 2005). Similarly, the toxicity of uranium and copper to bacteria is positively correlated with the concentration of the metals that bind to bacterial cells (Franklin et al. 2000). Figure 8 shows the U^{+6} reduction process. The bicarbonate forms the uranyl-carbonate aqueous complex which is then adsorbed on the cell envelope of *S. oneidensis*. After the adsorption, the electrons are transferred by the bacteria to the complex and U^{+6} is reduced to U^{+4}.
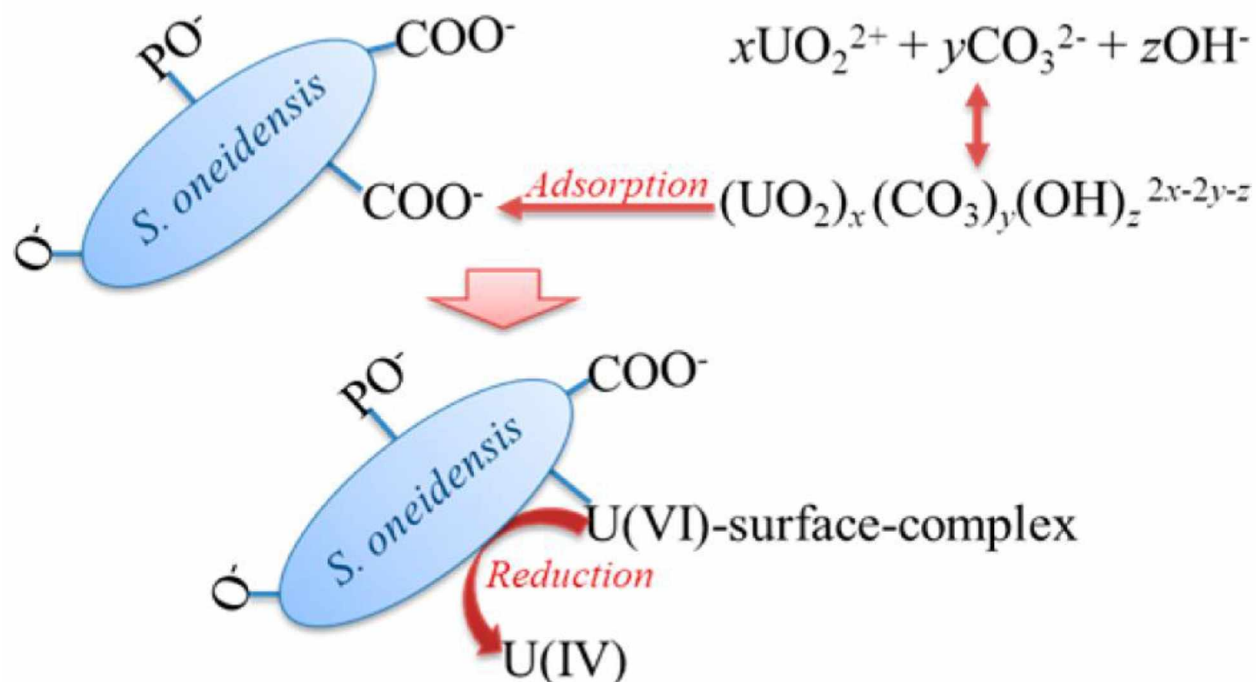
Figure 8. Mechanism of Uranium reduction (Sheng and Fein 2014).

$$xUO_2^{2+} + yCO_3^{2-} + zOH^-$$

$$(UO_2)_x(CO_3)_y(OH)_z^{2x-2y-z}$$

3.4.6 Potential for Microbial REE Extraction from Coal

S. oneidensis has demonstrated the capability to "reach out" to the metal oxides and reduce them, which makes it a potential candidate for extraction of REEs because the REEs present in coal are trapped within its structure. The cost of mineral processing mainly consists of milling and grinding. Of the total mineral processing cost, 40% is accounted for by these two-unit processes. Similarly, to extract the REEs, the coal needs to be ground to very small size fractions (less than 50 microns). Generally, this is the size range of REEs that are present in the coal in the form of oxides. So, if there is a process that can by-pass milling and grinding, it would make mineral processing cheaper. This can be achieved by performing redox reactions on iron present in the coal by using *S. oneidensis* MR-1. When the redox reactions occur, the structure of the iron present in the mineral constantly changes, which causes the weathering to occur faster resulting in the dissolution of REEs present inside the iron mineral to come out into the solution.

The extraction of REEs after milling and grinding also involves the use of sulfuric acid and hydrochloric acid, which poses an environmental risk and also workplace hazards. Instead of using acids for this process, if microorganisms can be used to achieve the results, it could prove to be a novel and improved method for REE concentration and extraction. As the concentration and extraction of REEs from coal by using *S. oneidensis* MR-1 does not occur due to acid generation but because of redox cycling of iron present in it, this method is environmentally safer than the conventional methods of REE extraction. If it is implemented, bio-leaching of REEs from coal can turn out to be a relatively environment-friendly method for their extraction and also pose less risk to the workers.

CHAPTER 4 METHODS AND MATERIALS

4.1 Coal Samples

The coal samples for this research were obtained from two Alaskan mines, Wishbone Hill and Usibelli Coal Mine (also known as Healy mines). Usibelli Coal Mine (UCM) has long been the only operating mine in Alaska. Alaskan coal has low sulfur content, ranging from 0.2% to 0.4%, as compared to the coal in the contiguous United States. Wishbone Hill has 0.26% sulfur, while Healy has 0.44% sulfur in it. The main differences between the two coals are the ash percentage and moisture content. Healy has low ash content, 23.72% ash, while Wishbone Hill has a very high ash content at 45.81%. On the other hand, Healy has a relatively higher moisture content of 9.46% while Wishbone Hill coal moisture content is 2.72%

The coal samples were originally obtained for previous research that characterized the REEs in coal. The samples were obtained in 5-gallon buckets and were mass reduced to 200 kg. These samples were classified into four different size ranges and different physical tests were performed on them. Float-sink tests, ash analysis, sulfur content, and moisture content was calculated for each size and specific gravity range. Also, the coal samples were analyzed using a Scanning Electron Microscope for determining particle size distribution and mineralogical composition (Gupta 2016).

Most REEs in their primary ore are present at the -200M (0.074 mm) particle size. Additionally, bioleaching has been found to be more effective in smaller size ranges. Therefore, coal was sized down to -200M (0.074 mm) in this research (using tumbling mill) (Gupta and Krishnamurthy 2004; Bosecker 1997).

Table 7 and 8 summarize the float-sink data for both coals in different specific gravity ranges.

Table 7. Float-sink data of Wishbone Hill coal sample (1/4" to 30M (0.54 mm)) (Gupta 2016)

Sink	Float	% wt.	% ash	Total Sulfur	Cumulative ash
Float	1.3	34.65	6.09	0.5	6.09
1.3	1.5	9.36	28.81	0.38	10.92
1.5	1.6	5.05	42.23	0.29	14.15
1.6	1.7	4.40	51.08	0.23	17.19
1.7	1.8	2.59	62.51	0.15	19.28
1.8	2	7.57	74.73	0.08	25.88
2	2.2	8.12	78.38	0.06	31.82
2.2	Sink	28.26	81.33	0.04	45.81
Total		100.00	45.81	0.26	

Table 8. Float-sink data of Healy coal sample (1/4" to 30M (0.54 mm)) (Gupta 2016)

Sink	Float	% wt.	% ash	Total Sulfur	Cumulative ash
Float	1.3	38.26	11.89	0.43	11.89
1.3	1.5	44.17	21.09	0.56	16.82
1.5	1.6	3.81	28.47	0.39	17.33
1.6	1.7	1.91	38.24	0.32	17.79
1.7	1.8	0.64	52.70	0.15	18.04
1.8	2	6.79	62.00	0.10	21.16
2	2.2	3.73	77.47	0.06	23.28
2.2	Sink	0.69	88.19	0.03	23.72
Total		100.00	23.72	0.44	

4.2 Ash Samples

The ash samples were prepared by burning the coal under standard conditions in Model 490 Coal Analyzer oven (Figure 9). The coal samples were placed in crucibles

that were then inserted into the oven under the "Fisher Ash" settings. The coal was combusted completely and the ash samples obtained were put in Ziplock™ bags to prevent oxidation.

Figure 9. Model 490 coal analyzer used for the preparation of ash.

The previous research has shown that when the coal is burnt, the silica present in it forms a coating on the REEs that makes it difficult for analysis. The size of these beads is usually less than 10 microns, so for bioleaching purposes, this glassy bead

has to be removed. To do the same, the ash samples were ground in a tumbling mill to be sized down to 10 microns and less size range.

4.3 Experiment Preparations

4.3.1 *S. oneidensis* MR-1 Growth

The first step in the preparation for performing experiments is to grow the *S. oneidensis* samples obtained from the University of Alaska Anchorage (UAA). *S. oneidensis* MR-1 samples were transported from the microbiology laboratory at UAA to the University of Alaska Fairbanks (UAF). The samples were originally stored at -80° C for long-term potency and were grown in normal conditions for the transportation purpose. To store the samples in -80° C and not damage the cell structure of the bacteria, glycerol is added to the bacteria, so that the cells do not rupture when they freeze.

For their transportation and growth at UAF, they were grown in Luria-Bertani (LB) broth (Figure 10). The sterilization of vials, beakers and their contents was performed in the Consolidated SSR series of sterilizers (Model SSR-3A-PB) (Figure 11).

Figure 10. *Shewanella oneidensis* **MR-1 growing in LB broth.**

Figure 11. Consolidated SSR series autoclave.

This process eliminated most of the microbes present in water, broth, and the vessels containing them, in turn, sterilizing them. An autoclave tape, having white strips on it, was used to confirm if the temperature inside the autoclave reached $121°$ C. All the vessels going inside the autoclave were marked with an autoclave tape, which turned black from white. After confirming the sterilization, all the items were taken out of the autoclave and allowed to cool before inoculation of *S. oneidensis* MR-1 in the LB broth solution. This was done because if the solution is hot when the bacteria are introduced, they will perish because of excessive heat.

When the broth solution cooled, the bacteria samples were taken out of the refrigerator and kept on the countertop. The countertop was sterilized by spraying 70% ethanol (%v/v) and wiping it. Pure ethanol (100% v/v) is not used for sterilization purpose because the microbes were only dehydrated through it and not eliminated. The Bunsen burner was lighted and everything was kept close so that it was easier and faster to complete the process. Firstly, the broth was transferred to the test tubes using the 10mL glass pipettes (sterilized when packed) and a 10 mL sample was sucked into the vial. When the flask was opened by removing the foil covering it, the mouth of the flask was heated by the Bunsen burner, which ensures that no bacteria entered the flask and contaminated the solution. This technique of sterilization is called aseptic technique and was followed while opening and closing any test tube or flask. When the broth was transferred in the pipette, the test tube was opened carefully by removing the foil placed on it and the same aseptic technique was followed to transfer the broth without any contamination. The desired amount of test tubes were filled with broth as needed for experiment purposes. When the test tubes were filled, the bacteria samples taken out from the refrigerator are opened and the pipette tips (100 µL) were used to transfer the bacteria samples in the test tube. Whenever any vial was opened or closed, the aseptic technique was strictly observed as contamination can ruin the experiment. After the inoculation of bacteria, the test tubes were closed, but not tightly (so that oxygen supply remained intact), and placed in the incubator at $30°C$ for their proper growth. The bacteria samples needed 24

hours to grow their colonies within the test tubes, which were later used for experiments.

4.3.2 Sterilization of Coal and Ash

The most important aspect of this research is the maintenance of sterility of the samples and storage of bacteria. For transferring coal/ash from the test tubes to the vials, the aseptic technique was used, while for sterilizing coal/ash a different process, known as pasteurization, was used. Pasteurization process is based on the principle of killing bacteria by exposing it to extreme temperature for prolonged period of times.

For pasteurizing coal/ash, it was first filled in centrifuge tubes and then placed in a hot water bath. The temperature of this water bath was set at $80°$ C and the samples were kept in this bath for one hour. After one hour is complete, the tubes were taken out and stored in a refrigerated room at $4°$ C for an hour. This process was repeated three times, which ensured that any microbes present in coal/ash were eliminated and the samples were now ready to be inoculated by *S. oneidensis* MR-1. Alternatively, coal/ash can also be sterilized in the autoclave by adding it to the vials along with the media.

4.3.3 Experimental Setup

The experiments performed for this research tested the effect of different factors like size of coal/ash, temperature of incubation, initial bacterial volume, and pulp density. Three different sizes of coal, namely, -200 M (<0.074 mm), -48 M to +200 M (0.074 mm-0.295 mm), and -14 M to 48 M (0.295 mm-1.651 mm) were used for experiments while the temperature of incubation was also kept at three different ranges, $30°$ C, $32°$ C, and $34°$ C. The initial bacterial volumes that was used in the experiments were 100 µL, 200 µL and 500 µL. The volume of the media used for the experiments was kept at a constant of 35 mL. So, to change the solids percentage (pulp density), the amount of the solids (coal or ash) added to the media was changed. The amount of

solids added to the media was 2 gm, 3.5 gm, and 5 gm to achieve a solids percentage of 5.7%, 10%, and 14.3%, respectively.

The bacteria samples were grown at least one day prior to making samples for inoculation so that they had a healthier population by the time experiments were conducted. The next step after bacterial growth and sterilization of coal was to inoculate bacteria in the vials along with coal and media. The media used for experiments was a mixture of chemicals that are known to promote redox cycling of iron when another species of *Shewanella, Shewanella putrefaciens*, is used. This research also tested if the *S. oneidensis* MR-1 reacts the same way towards this media as *S. putrefaciens* does.

For the purpose of these experiments, a shaking incubator and a non-shaking incubator were used to test the different conditions. Both the incubators were capable of maintaining temperatures that were required for this research. The vials in which the samples were prepared had a volume of 50 mL, which leaves enough space for air to be present inside them after they were filled with coal and the media. Syringes, needles and syringe filters were used to inject the vials with air when the experiment proceeded further.

4.3.4 Media Preparation

For media preparation, 18-ohm deionized water was placed in a flask. The constituents of the media were 20 mM of sodium L-lactate ($C_3H_5NaO_3$, 98+%), 10 mM of sodium bicarbonate ($NaHCO_3$), 29.76 mM of 1, 4 piperazinediethanesulfonic (PIPES, ≥99% titration), 1.34 mM of potassium chloride (KCl, BioXtra, ≥99%) and 0.2 mM of anthraquinone-2, 6-disulfonate (AQDS). The sodium lactate acts as an electron donor, AQDS acts as an electron acceptor, while $NaHCO_3$ and PIPES act as buffers (Zhao et al. 2015).

The required amount of each component, except AQDS, was weighed and mixed thoroughly in the water. After the mixing was complete, the mouth of the flask was sealed with the aluminum foil. The vials that were used for storing the samples were covered with aluminum foil and those along with the beaker were autoclaved for sterilization purpose. The mouths of the vials and the beakers were covered with aluminum foil during autoclave process because when the sterilization was complete and they wear taken out of the autoclave, they did not get infected through air or contact. The sterility was maintained on the insides of the vials, flasks, and their contents.

4.3.5 Sample Preparation

After the sterilization of media, coal, and vials was complete and the bacteria were allowed to grow for 24 hours, sample preparation was started. To prepare the samples, the first step was to sterilize the countertop by spraying 70% ethanol on top of it and wiping it off completely. Then, the burner was lighted and the vials were placed on the countertop. The latex gloves were also sprayed with the ethanol and wiped off to sterilize them.

The samples were prepared so that required datasets were obtained when the experiments were completed. Firstly, the coal samples were divided into three specific gravity ranges, namely, 1.3 floats, 1.3 to 1.5, and 1.5 sinks. Composite samples of coal were also taken to test for REE leachability from coal. So for the experiment purposes, the samples were named as Wishbone Hill composite, Wishbone Hill 1.3 floats, Wishbone Hill 1.3 sinks, Wishbone Hill 1.5 sinks, Healy composite, Healy 1.3 floats, Healy 1.3 sinks, and Healy 1.5 sinks. Five samples were made for each specific gravity fraction along with the composite sample, out of which three were inoculated with *Shewanella* while the other two were not. This was done to test the theory that the bacteria was leaching the REEs and not the chemicals present in the media. So, in total, there were 40 samples (vials) for one set of experiment.

The first step in the sample preparation was to fill the vials with the media by following the aseptic technique. 40 mL of media was added to the vials using pipettes while being careful about contamination. When all the vials were filled, coal was added to them using an appropriate amount to maintain the required pulp density, by observing aseptic technique. To maintain 0.2 mM of AQDS concentration in each vial, the required amount of AQDS was weighed and dissolved in 18-ohm deionized water and using a syringe fitted with an air filter (0.22 μm opening). Usually, for maintaining the required concentration of AQDS in the vials, 1 mL of solution was added to them.

After the addition of AQDS in the vials, resazurin, a color indicator was added to the vials. Resazurin is a blue dye that is used as a color indicator. The color of resazurin remains blue in the presence of oxygen but turns clear in its absence. This was used to detect whether the reduction-oxidation (redox) cycles were completed or not. When the blue color changed to clear, it was an indication that the vials lost the oxygen, meaning that oxygen needed to be added to the sealed vials for the next redox cycle to start. As previously mentioned in the thesis, while *S. oneidensis* MR-1 can live in both oxic and anoxic conditions, it still needs oxygen to restart the redox cycle, which stops in the absence of oxygen.

The samples that now contained media, coal, AQDS, and resazurin were sealed and placed in the incubator. These samples (16 in total) were sealed for the whole duration of the experiment and were opened only for analysis (Figure 12). The remaining vials (24 in total) then were inoculated with *S. oneidensis* MR-1 by following the aseptic technique as shown in Figure 13. For different tests, the initial quantity of the bacteria used were changed to determine its effect on the bioleaching process. The samples were sealed and stored in the incubator at 32°C (different for different tests, ranging from 30°C to 34°C) along with the vials that did not have the bacteria in them.

Figure 12. Sealed samples ready for incubation.
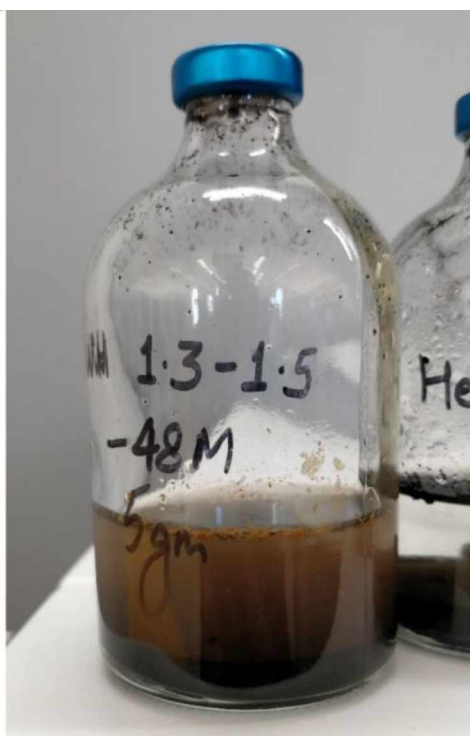
Figure 13. A sealed vial consisting of Wishbone Hill 1.3-1.5 (SG) coal (5 gm, -48 M size) with the growth media and *S. oneidensis* MR-1 ready for incubation at 32ºC.

4.3.6 Air Injection

The air (oxygen) was added to the vials so that the *S. oneidensis* MR-1 did not discontinue their redox cycling. This process was done every 5 days (120 hours) and repeated for 4 times. So, one experiment took 20 days to complete. To add air in the vials, first they were taken out of the incubator and placed on the sterile countertop. The caps of the vials were wetted using 70% ethanol and then put on the flame. This process destroyed any microbes that were present on the caps. When the fire burned out, syringes fitted with air filters (0.22 µm opening) and needles were used to inject the air in the vials. The capacity of the syringes was 12 mL and each vial was injected three times resulting in the injection of 36 mL of air. After all the vials were injected with air, the caps again were wetted with 70% ethanol and put on flame to burn off any microbes and then placed in the incubator. This process was repeated every 5 days until the experiments were completed.

4.3.7 Extraction of Solution

After the completion of 4 redox cycles, the vials were taken out of the incubator and opened carefully. Here it is worth noting that at this stage there was no need to follow the aseptic technique because the samples were sent out for analysis and it can be safely assumed that after the end of the redox cycles, the bacteria would remain inactive. Also, when the samples were filtered, there was no more coal present and the bacteria could not leach anything else from the solution.

The seals of the vials were unclamped and the liquid was separated from the solids by vacuum filtration. The liquids were stored in centrifuge tubes (Figure 14) and labelled accordingly. If the samples, for any reason, were not analyzed soon after the extraction from the vials, they were stored in a refrigerator at 4°C.

Figure 14. Solution collected from vials after vacuum filtration.

4.3.8 Analysis by ALS

After the extraction of liquid from the vials into the centrifuge tubes, they were sent to ALS Geochemistry lab situated in Fairbanks. The correct method for REE analysis was found and the samples were analyzed. As the concentration of the REEs is low in coal, a correct method was necessary for the analysis to give correct results. For analysis, a method named "ME-MS 14L + ME-MS 14L-REE" was preferred as this method is used for super trace analysis of water, or a solution in this case, for measuring the concentration of REEs present in it. The method is discussed in detail in the following paragraphs.

4.3.8.1 Analytical Method

The analytical method used for the analysis is Inductively Coupled Plasma Atomic Spectroscopy (ICP-AES) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The trace metal analysis in ground and surface waters can reveal mineralization-related anomalies in areas where traditional soil sampling is difficult or impossible, such as swampy terrain, desert, and agricultural disturbance. Water

samples are analyzed directly as submitted using the latest generation of collision-cell equipped ICP-MS instruments to provide the lowest detection limits available (ALS 2016). Table 9 displays the lower and upper detection limit for various REEs.

This method was selected for analysis of the solution generated in the research because it is advantageous in detecting the infinitesimal amount of elements (as low as 0.005 µg/L or 5 ppb), which is perfect for this research as the original concentration of REEs in coal is very little. Also, as the initial weight of coal/ash used in the experiments was very low, the amount of REEs present was also low. Hence, a method was needed that can detect the low concentration of REEs in the solution, which can be achieved by ICP-MS.

Table 9. List of reportable REE analytes from ME-MS 14L and ME-MS 14L REE method (ALS 2016).

Analyte	Symbol	Units	Lower Limit	Upper Limit
Cerium	Ce	µg/L	0.005	400,000
Lanthanum	La	µg/L	0.005	400,000
Scandium	Sc	µg/L	0.01	400,000
Yttrium	Y	µg/L	0.005	400,000
Dysprosium	Dy	µg/L	0.005	10,000
Erbium	Er	µg/L	0.005	10,000
Europium	Eu	µg/L	0.005	10,000
Gadolinium	Gd	µg/L	0.005	10,000
Holmium	Ho	µg/L	0.005	10,000
Lutetium	Lu	µg/L	0.005	10,000
Neodymium	Nd	µg/L	0.005	10,000
Praseodymium	Pr	µg/L	0.005	10,000
Samarium	Sm	µg/L	0.005	10,000
Terbium	Tb	µg/L	0.005	10,000
Thulium	Tm	µg/L	0.005	10,000

| Ytterbium | Yb | µg/L | 0.005 | 10,000 |

CHAPTER 5 RESULTS AND DISCUSSION

5.1 Testing on Different Conditions

The experiments related to this research were performed on various specific gravity fractions of coal under different conditions. The specific gravity fractions used were 1.3 floats, 1.3 sinks, and 1.5 sinks and a composite sample of coal. Ash samples were prepared for the above-mentioned specific density fractions of coal. The composite sample was taken to test for REE recovery from a homogenous sample of coal. These density fractions were selected for both Wishbone Hill and Healy coal. The different conditions for which solids (coal and ash) were tested are as follows:

1) Testing for the evidence of bioleaching,
2) Different weight of solids (to maintain different pulp density or solids percent),
3) Different initial bacterial concentration,
4) Different incubation temperature, and
5) Different size of solids.

The experiments were conducted on these conditions and are discussed in the following paragraphs.

5.1.1 Evidence of Bioleaching

The first experiments performed for this research were to test if *S. oneidensis* MR-1 is able to leach out the REEs or not. To test this, duplicates were prepared with the exact same solids %, namely 5.4%, 10%, and 14.3% solids, and the media volume of 35mL, but the microbes were not added to them and the incubation settings were also kept the same when the samples were prepared.

In Table 10, "S" denotes the presence of *S. oneidensis* MR-1 in the samples while "NS" denotes its absence. From Table 10, it can be deduced that the presence of *S. oneidensis* MR-1 facilitates the leaching of REEs from coal. For all the elements in all

the specific gravity fractions, the REE recovery is more in the microbe inoculated samples. The same tests were performed for Healy coal and ash from both Wishbone and Healy coals, the results for which are shown in Appendix A . It can be deduced from these tables that the leaching of REEs was occurring primarily due to the microbes which allow us to test further for different variable affecting bioleaching process. As the recovery from ash was minuscule, the research focused more on recovery of REEs from coal and subsequent experiments were performed on it.

5.1.2 Different Solids Percentage

To test the recovery of REEs from coal using different pulp density, the weight of coal was changed from 2 gm to 3.5 gm and 5 gm, while keeping the volume of media constant at 35 mL. The corresponding solids percentage for these weights are 5.7%, 10%, and 14.3%, respectively. The variables for the experiments were 2 gram coal, 100µL *S. oneidensis* MR-1, -48M coal size, 30°C incubation temperature; 3.5 gram coal, 500µL *S. oneidensis* MR-1, -14M size coal, 32°C incubation temperature; and 5 gram coal, 100µL *S. oneidensis* MR-1, -200M size coal, 30°C incubation temperature. The results of these tests for different specific gravity of both coals are discussed below.

5.1.2.1 Wishbone Hill Coal

The recovery of REEs from Wishbone Hill coal for 2 gm, 3.5 gm, and 5 gm of coal are shown in Table 11. In the table, 5, 3.5, and 2 stands for the weight of that particular coal sample (in grams) and it can be concluded that 10% solids (3.5 gm coal) for Wishbone Hill gives the best results in terms of REE recovery, followed by 5.7% (2 gm coal) and 14.3% (5 gm coal). Also, the best recoveries are obtained at 1.3 floats with a decrease in recovery as the specific gravity of coal increases. One of the critical REE, neodymium, had the best recovery of 75.3% in Wishbone Hill 1.3 float coal sample, while scandium had a recovery of 67.2% in the same sample. When a composite sample of Wishbone Hill is taken for the experiment, the recoveries of all

the REEs are very low with a maximum of 7.5% recovery of scandium at 10% solids at 3.5 gm coal. With a higher solids percentage, the recoveries fall further to minuscule. For 1.3 floats of Wishbone Hill, their recoveries are higher than all the other samples with maximum recoveries at 3.5 gm coal. The trend of recoveries shows that the optimum solids percentage for 1.3 floats is 10%, while at 5.7%, the recoveries are better than at 14.3%. This can be attributed to the fact that when more solids are present, there is not much space for the microbes to feed on coal, which leads to lesser recovery. The similar trend is followed in 1.3 sinks and 1.5 sinks where the maximum recovery is obtained at 10% solids.

Another important indicator in the effectiveness of bioleaching process is the total REE recovery. As the original concentration of REEs in coal varies, individual recovery is not of much importance as in some cases it is possible that the recovery of some REEs is very high, but they have less original concentration in coal. For example, for Wishbone Hill 1.3 floats, the concentration of neodymium is 5.7 ppm (Gupta 2016) and its recovery is 75.3% (Table 10), but the overall recovery for 1.3 floats is 43.2%. As our primary aim in this research is to concentrate the REEs, total recovery is a better indicator. The trend for total REE recovery from various specific gravity fractions of Wishbone Hill coal is shown in Figure 15.

The highest recovery of 43.2% REEs is obtained for 1.3 floats at 10% solids (3.5 gm coal), which is the only respectable result for Wishbone Hill. The optimum total recovery for all specific gravity fractions is obtained at 10% solids.

Table 10. Recovery of REEs for Wishbone Hill Coal with (S) and without (NS) *S. oneidensis* MR-1.

	Composite			1.3 floats			1.3 sinks			1.5 sinks		
	ppm	S	NS	ppm	S	NS	ppm	S	NS	ppm	S	NS
Sc	13.1	4.6%	1.6%	7.1	51.0%	29.0%	11.9	14.2%	11.4%	17.0	0.7%	0.6%
Y	21.1	1.5%	0.8%	13.3	9.8%	3.1%	19.4	1.8%	1.3%	26.4	0.0%	0.0%
La	16.4	1.2%	0.4%	4.8	11.2%	5.1%	15.3	0.9%	0.6%	23.7	0.0%	0.0%
Ce	35.3	1.3%	0.4%	10.6	13.1%	2.6%	31.9	1.0%	0.7%	51.1	0.0%	0.0%
Pr	4.2	0.3%	0.0%	1.3	6.0%	1.3%	3.8	0.7%	0.3%	6.1	0.0%	0.0%
Nd	17.5	0.2%	0.0%	5.7	6.9%	0.8%	15.8	0.9%	0.3%	25.1	0.0%	0.0%
Sm	4.1	0.2%	0.0%	1.4	4.9%	1.8%	3.7	0.8%	0.5%	5.8	0.0%	0.0%
Eu	0.9	0.5%	0.0%	0.3	17.5%	13.9%	0.8	2.0%	3.7%	1.2	0.0%	0.0%
Gd	4.0	0.4%	0.0%	1.7	7.0%	2.3%	3.6	2.0%	0.0%	5.4	0.0%	0.0%
Tb	0.6	0.2%	0.0%	0.3	5.5%	3.2%	0.6	2.0%	0.0%	0.9	0.1%	0.0%
Dy	3.8	0.2%	0.0%	2.1	5.9%	3.3%	3.4	0.9%	0.2%	4.9	0.0%	0.0%
Ho	0.8	0.3%	0.0%	0.5	6.3%	1.2%	0.7	2.6%	0.6%	1.0	0.0%	0.0%
Er	2.2	0.1%	0.0%	1.4	5.1%	1.7%	2.1	2.1%	0.4%	2.7	0.0%	0.0%
Tm	0.4	0.1%	0.3%	0.2	0.0%	0.0%	0.4	2.8%	0.0%	0.4	0.0%	0.0%
Yb	2.1	0.1%	0.0%	1.5	3.6%	1.4%	2.2	1.0%	0.0%	2.5	0.0%	0.0%
Lu	0.3	0.1%	0.0%	0.2	4.1%	0.0%	0.3	4.2%	0.0%	0.4	0.1%	0.0%

Table 11. Recovery of REEs from Wishbone Hill coal at different solids percentage.

	Composite			1.3 floats				1.3 sinks				1.5 sinks			
	ppm	5	3.5	ppm	5	3.5	2	ppm	5	3.5	2	ppm	5	3.5	2
Sc	13.1	2.9%	7.5%	7.1	27.3%	67.2%	13.4%	11.9	12.4%	34.7%	16.6%	17.0	0.4%	4.8%	0.9%
Y	21.1	0.8%	3.1%	13	9.6%	22.4%	19.5%	19.4	8.2%	17.7%	18.9%	26.4	0.1%	1.7%	1.6%
La	16.4	0.7%	2.6%	4.8	13.7%	30.1%	32.2%	15.3	1.5%	14.2%	10.5%	23.7	0.0%	1.5%	1.7%
Ce	35.3	0.7%	2.8%	10.6	5.9%	49.8%	9.6%	31.9	1.8%	23.4%	5.1%	51.1	0.0%	1.4%	0.4%
Pr	4.2	0.5%	3.1%	1.3	5.1%	34.6%	10.1%	3.8	2.1%	23.9%	6.1%	6.1	0.0%	1.4%	0.2%
Nd	17.5	0.9%	5.6%	5.7	7.1%	75.3%	9.3%	15.8	2.7%	24.5%	6.5%	25.1	0.0%	1.5%	0.3%
Sm	4.1	0.6%	2.4%	1.4	10.0%	38.8%	17.4%	3.7	4.0%	23.8%	7.9%	5.8	0.1%	2.0%	0.5%
Eu	0.9	0.9%	2.6%	0.3	7.1%	36.1%	10.2%	0.8	6.4%	26.0%	10.9%	1.2	0.0%	1.8%	0.8%
Gd	4.0	0.6%	2.8%	1.7	11.7%	33.6%	12.1%	3.6	6.8%	17.8%	12.2%	5.4	0.1%	1.6%	0.9%
Tb	0.6	0.7%	2.4%	0.3	8.6%	20.8%	20.5%	0.6	7.5%	17.6%	11.9%	0.9	0.1%	1.9%	0.9%
Dy	3.8	0.4%	2.6%	2.1	9.5%	21.6%	20.3%	3.4	7.4%	16.7%	12.6%	4.9	0.1%	1.9%	0.8%
Ho	0.8	0.7%	2.6%	0.5	8.8%	19.9%	13.2%	0.7	8.2%	14.7%	9.7%	1.0	0.1%	1.6%	0.9%
Er	2.2	0.6%	2.3%	1.4	10.1%	21.1%	16.7%	2.1	8.4%	12.0%	14.5%	2.7	0.1%	2.0%	1.2%
Tm	0.4	1.3%	2.4%	0.2	6.6%	9.4%	18.4%	0.4	4.9%	13.5%	15.5%	0.4	0.1%	1.5%	1.1%
Yb	2.2	0.8%	3.9%	1.5	5.7%	14.4%	22.9%	2.2	6.1%	11.0%	18.0%	2.5	0.1%	2.2%	1.7%
Lu	0.3	1.7%	2.9%	0.2	6.9%	38.5%	24.0%	0.3	5.5%	13.7%	22.8%	0.4	0.1%	2.3%	2.3%
Total	126.8	0.6%	3.6%	52.2	11.2%	43.2%	16.1%	115.9	4.8%	21.2%	10.7%	174.8	0.1%	1.8%	0.9%
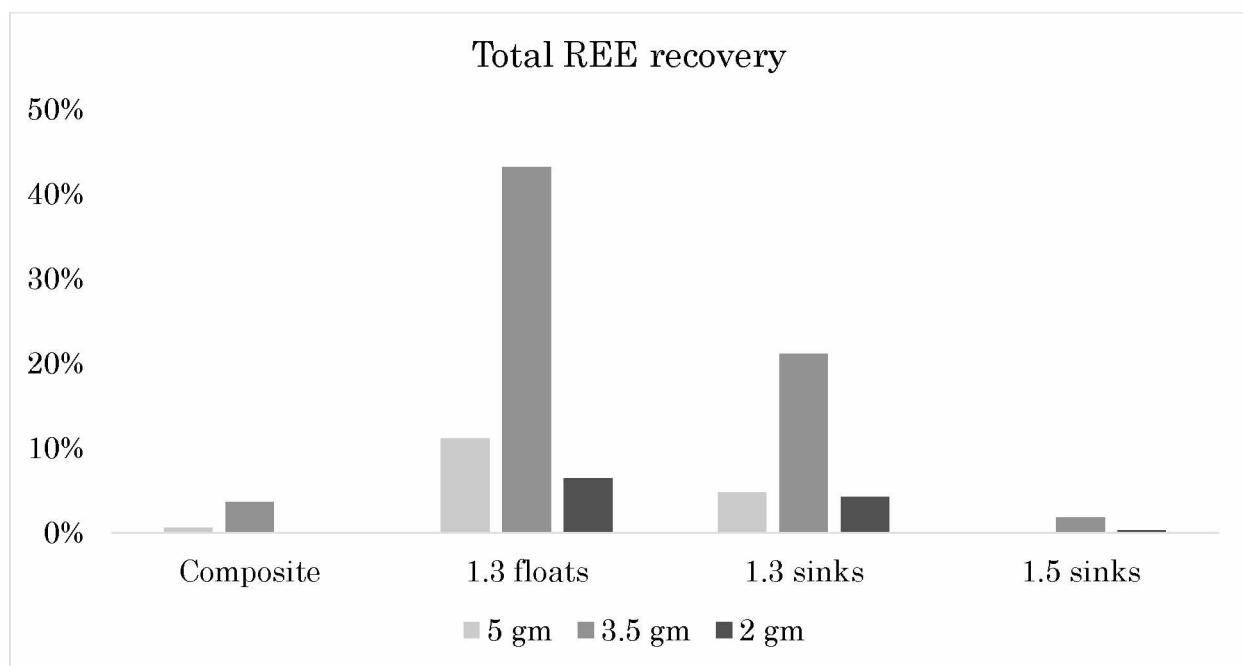
Figure 15. Total REE recovery from Wishbone Hill Coal at various solids percentage for different specific gravities of coal.

5.1.2.2 Healy Coal

The recovery of REEs from Healy coal for different solids percentage does not follow the same pattern as Wishbone Hill coal. For Healy coal, the best results are achieved at 2 gm coal (5.7% solids) for every density fraction. It is worth noting here that the recovery of REEs is more for heavier density fractions as compared to 1.3 floats, unlike Wishbone Hill coal where maximum recoveries were achieved at 1.3 floats fraction. The recoveries are much better for Healy when compared to Wishbone Hill, which can be attributed to the fact that Healy has more REEs compared to Wishbone Hill. For 1.3 floats in Healy, there is no significant difference in the recovery of REEs for different solids percentage, but for heavier fractions, there is a considerable difference when solids percentage increase from 10% to 14.3%, which leads to a steep fall in recovery. For solids percentage between 5.7% and 10%, the recovery is better for all specific gravity fractions. As the specific gravity of Healy coal increases, the recovery for lesser solids percentage increases drastically. Table 12 shows the recovery of REEs for Healy coal at various solids percentage.

76

Table 12. Recovery of REEs from Healy coal at different solids percentage.

	Composite			1.3 floats				1.3 sinks				1.5 sinks			
	ppm	5	3.5	ppm	5	3.5	2	ppm	5	3.5	2	ppm	5	3.5	2
Sc	4.9	4.9%	81.4%	3.4	5.9%	14.6%	0.0%	4.6	3.0%	5.0%	0.0%	9.0	6.8%	8.2%	14.4%
Y	14.6	0.5%	62.7%	9.7	35.5%	56.4%	28.8%	16.6	14.9%	25.0%	90.1%	19.9	14.8%	39.5%	83.4%
La	15.6	0.1%	70.1%	8.6	80.2%	77.1%	96.6%	16.8	12.5%	20.5%	96.4%	28.1	7.1%	19.0%	68.8%
Ce	30.7	0.1%	69.7%	17.3	42.5%	67.8%	62.4%	33.8	7.3%	12.4%	87.6%	52.1	4.3%	11.5%	51.2%
Pr	3.6	0.1%	71.7%	2.0	35.1%	57.5%	53.2%	4.0	6.4%	11.4%	78.2%	6.2	3.5%	10.3%	46.3%
Nd	14.5	0.2%	64.1%	8.6	30.3%	51.3%	46.6%	16.2	6.4%	12.0%	69.1%	23.4	4.0%	11.7%	46.9%
Sm	3.3	0.3%	58.6%	2.1	22.9%	35.5%	41.4%	3.7	5.5%	7.7%	73.2%	4.8	3.5%	10.6%	51.2%
Eu	0.7	0.1%	65.8%	0.4	26.0%	31.9%	45.2%	0.8	7.3%	10.9%	89.4%	0.9	4.9%	14.2%	68.1%
Gd	3.0	0.2%	56.1%	2.0	23.6%	39.5%	36.8%	3.4	7.4%	12.3%	78.2%	4.0	7.4%	18.2%	69.0%
Tb	0.5	0.2%	58.2%	0.3	20.0%	34.2%	30.1%	0.5	7.8%	11.6%	73.5%	0.6	5.9%	16.6%	61.9%
Dy	2.6	0.4%	66.2%	1.6	19.3%	28.5%	24.3%	3.0	8.1%	15.5%	70.7%	3.7	7.4%	19.6%	64.1%
Ho	0.5	0.4%	64.5%	0.3	18.6%	35.5%	19.3%	0.6	8.7%	13.9%	62.4%	0.8	7.5%	20.6%	56.9%
Er	1.4	0.6%	67.4%	0.9	20.8%	33.9%	19.7%	1.6	8.9%	13.8%	62.2%	2.1	7.1%	22.4%	62.0%
Tm	0.2	0.4%	67.3%	0.1	12.6%	21.6%	16.0%	0.3	6.1%	11.9%	59.2%	0.3	6.6%	19.9%	53.8%
Yb	1.4	0.4%	71.3%	0.8	14.9%	25.2%	19.3%	1.6	6.7%	14.3%	80.4%	2.0	6.0%	20.0%	68.6%
Lu	0.2	0.2%	73.8%	0.1	16.8%	22.0%	23.1%	0.2	6.9%	11.4%	93.3%	0.3	7.5%	24.8%	85.6%
Total	97.7	0.5%	67.1%	58.2	33.5%	53.5%	50.5%	107.8	8.7%	14.3%	98.4%	158.3	6.4%	16.4%	73.1%

From Table 12, it can be deduced that the lanthanum recovery is best at 2 gm coal for all the density fractions, while scandium recovery, other than from the composite sample, is negligible. The best recoveries of all the REEs, except scandium, is obtained at 5.7% solids for 1.3 sinks.

The trend for total REE recovery from various specific gravity fractions of Healy coal is shown in Figure 16. The total recovery of REEs from Healy coal is much better than Wishbone Hill coal, with the highest recovery of 98.4% in 1.3 sinks at 5.7% solids. The total recovery remains high even for higher density fractions at 5.7% solids. The 1.3 sinks fraction of Healy coal contains 107.8 ppm of total REEs (Gupta 2016), while having a total recovery of 98.1% for 5.7%. Similarly, 1.5 sinks contain 158.3 ppm of REEs while having a 73.1% total recovery for 5.7% solids.
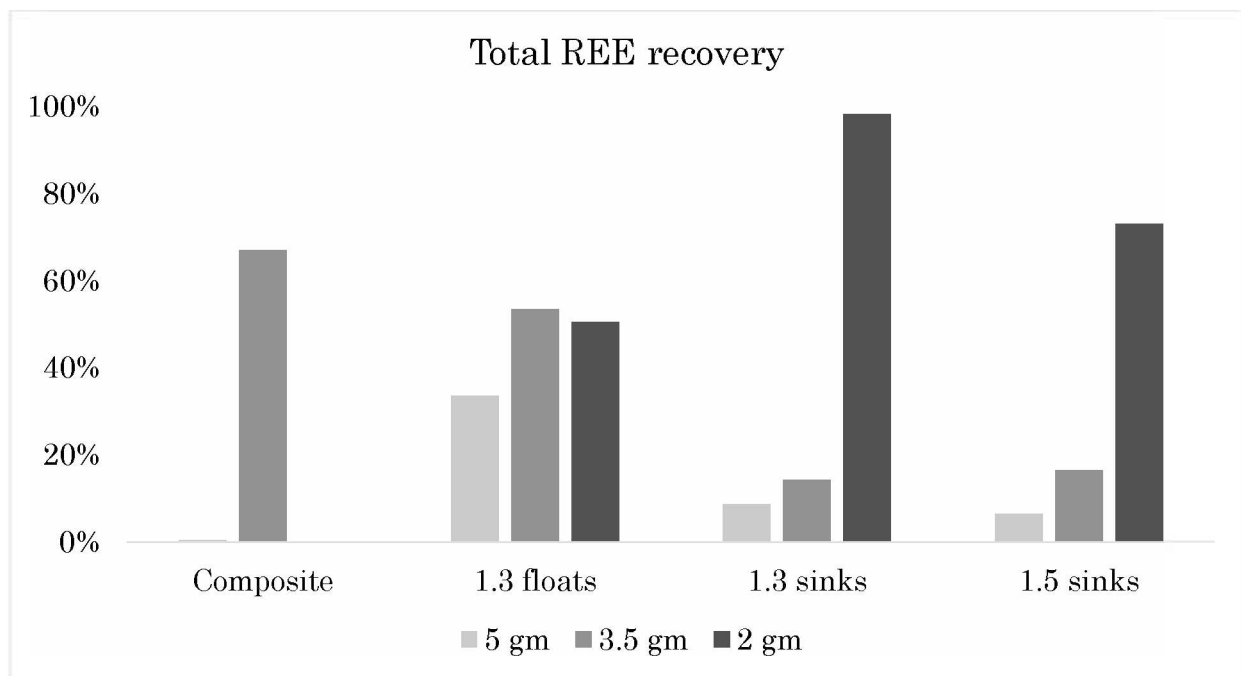
Figure 16. Total REE recovery from Healy Coal at various solids percentage for different specific gravities of coal.

78

5.1.3 Different Initial Bacterial Concentration

One of the variables that could affect the REE recovery is the initial bacterial concentration in the vial. It can happen due to the fact that when more microbes are present, they can perform the redox cycles better and faster. To test this, experiments were performed with an initial bacterial concentration of 100 µL, 200 µL and 500 µL in the media volume of 35 mL, which are depicted as 100, 200, and 500 in the data tables. The other variables for the experiments were 100µL *S. oneidensis* MR-1, 5 gram coal, -200M size coal, 30°C incubation temperature; 200µL *S. oneidensis* MR-1, 5 gram coal, -48M size coal, 30°C incubation temperature; and 500µL *S. oneidensis* MR-1, 3.5 gram coal, -200M size coal, 32°C incubation temperature. The experiments were performed on all the specific gravity fractions of both coals, the results of which are discussed in the following pages.

5.1.3.1 Wishbone Hill Coal

The recovery of REEs for all the fractions of Wishbone Hill coal generally increases with an increase in initial bacterial volume while following the same trend of decreasing recovery for heavier specific gravity fractions. Again, 1.3 floats of Wishbone Hill coal provide better recovery for all the REEs when compared to other specific gravity fractions.

Scandium shows the most robust recovery in all three conditions with a minimum of 27.3% recovery at 100 µL and a maximum of 67.2% at 500 µL for 1.3 floats. Tellurium has the minimum recovery of all the elements with a maximum of 9.4% recovery at 500 µL bacterial volume for 1.3 floats. Cerium shows the highest jump in recovery when microbial volume increases. The recovery of cerium increases from 10.8% at 200 µL to 49.8% at 500 µL. One interesting thing of note here is that the recovery of elements mostly decreased when the bacterial volume increased from 100 µL to 200 µL, the only exceptions being europium, cerium, lanthanum, scandium, and yttrium for the composite sample europium, cerium, and scandium for 1.3 floats, and

scandium for 1.3 sinks and 1.5 sinks. From Table 13, it can also be deduced that higher bacterial volume (500 µL) increases the recovery, even for 1.5 sinks, significantly, from almost 0% at 100 µL for all the REEs up to 5% at 500 µL. The total recovery of REEs from Wishbone Hill coal is shown in Figure 17. From this figure it can be clearly seen that the recovery of REEs mostly follows a positive relationship with the volume of bacteria, the only exception being the 1.3 sinks, where the recovery decreases slightly when volume is increased from 100 to 200 µL although it rises significantly when the volume is further increased. The maximum total recovery of REEs is achieved at 1.3 floats and 500 µL of bacteria, while for composite sample and 1.5 sinks, the total recovery is very low. For 1.3 sinks, a maximum recovery of 21.2% is achieved at 500 µL of bacteria. From these observations, it can be safely stated that recovery of REEs from Wishbone Hill coal has a positive relationship with the initial bacterial volume.

5.1.3.2 Healy Coal

For Healy coal, the recovery of REEs for a composite sample increases drastically when more bacterial volume is used. The recovery increases from almost negligible amount to more than 60% for all the REEs, with the highest recovery of scandium at 81.4%. Although, the recovery increases for all density fraction with higher bacterial volume, the jump in percentage is not as dramatic as in the composite sample. Here, unlike the case of solids percentage, the recovery of REEs does not increase with an increase in specific gravity of coal and remains mostly comparable or increases by a fraction. The recoveries of all the REEs for Healy coal for different bacterial volumes are mentioned in Table 14. The total recovery of REEs from Healy coal is significantly better than Wishbone Hill coal. A maximum of 67.1% of total REEs were recovered in the composite sample of Healy coal at 500 µL of *S. oneidensis* MR-1. The total recovery falls as the specific gravity of coal increases, although, it has a positive relationship with the bacterial volume. Figure 18 shows the recovery of REEs from Healy coal at various volumes of *S. oneidensis* MR-1.

80

Table 13. Recovery of REEs from Wishbone Hill coal for 100, 200, and 500 µL of *S. oneidensis* MR-1.

	Composite				1.3 floats				1.3 sinks				1.5 sinks			
	ppm	100	200	500	ppm	100	200	500	ppm	100	200	500	ppm	100	200	500
Sc	13.1	2.9%	8.4%	7.5%	7.1	27.3%	43.0%	67.2%	11.9	12.4%	14.2%	34.7%	17.0	0.4%	0.7%	4.8%
Y	21.1	0.8%	1.7%	3.1%	13.0	9.6%	7.9%	22.4%	19.4	8.2%	1.8%	17.7%	26.4	0.1%	0.0%	1.7%
La	16.4	0.7%	1.2%	2.6%	4.8	13.7%	11.2%	30.1%	15.3	1.5%	0.9%	14.2%	23.7	0.0%	0.0%	1.5%
Ce	35.3	0.7%	1.3%	2.8%	10.6	5.9%	10.8%	49.8%	31.9	1.8%	1.0%	23.4%	51.1	0.0%	0.0%	1.4%
Pr	4.2	0.5%	0.3%	3.1%	1.3	5.1%	3.8%	34.6%	3.8	2.1%	0.7%	23.9%	6.1	0.0%	0.0%	1.4%
Nd	17.5	0.9%	0.2%	5.6%	5.7	7.1%	4.1%	75.3%	15.8	2.7%	0.9%	24.5%	25.1	0.0%	0.0%	1.5%
Sm	4.1	0.6%	0.3%	2.4%	1.4	10.0%	2.7%	38.8%	3.7	4.0%	0.8%	23.8%	5.8	0.1%	0.0%	2.0%
Eu	0.9	0.9%	0.6%	2.6%	0.3	7.1%	19.0%	36.1%	0.8	6.4%	2.0%	26.0%	1.2	0.0%	0.0%	1.8%
Gd	4.0	0.6%	0.4%	2.8%	1.7	11.7%	4.4%	33.6%	3.6	6.8%	2.0%	17.8%	5.4	0.1%	0.0%	1.6%
Tb	0.6	0.7%	0.2%	2.4%	0.3	8.6%	3.2%	20.8%	0.6	7.5%	2.0%	17.6%	0.9	0.1%	0.1%	1.9%
Dy	3.8	0.4%	0.2%	2.6%	2.1	9.5%	4.2%	21.6%	3.4	7.4%	0.9%	16.7%	4.9	0.1%	0.0%	1.9%
Ho	0.8	0.7%	0.3%	2.6%	0.5	8.8%	2.4%	19.9%	0.7	8.2%	2.6%	14.7%	1.0	0.1%	0.0%	1.6%
Er	2.2	0.6%	0.1%	2.3%	1.4	10.1%	4.0%	21.1%	2.1	8.4%	2.1%	12.0%	2.7	0.1%	0.0%	2.0%
Tm	0.4	1.3%	0.1%	2.4%	0.2	6.6%	0.0%	9.4%	0.4	4.9%	2.8%	13.5%	0.4	0.1%	0.0%	1.5%
Yb	2.2	0.8%	0.1%	3.9%	1.5	5.7%	2.6%	14.4%	2.2	6.1%	1.0%	11.0%	2.5	0.1%	0.0%	2.2%
Lu	0.3	1.7%	0.1%	2.9%	0.2	6.9%	4.1%	38.5%	0.3	5.5%	4.2%	13.7%	0.4	0.1%	0.1%	2.3%
Total	126.8	0.6%	1.3%	3.6%	52.2	11.2%	15.0%	43.2%	115.9	4.8%	2.5%	21.2%	174.8	0.1%	0.1%	1.8%

Figure 17. Total recovery of REEs in Wishbone Hill coal for 100, 200, and 500 μL of *S. oneidensis* MR-1.
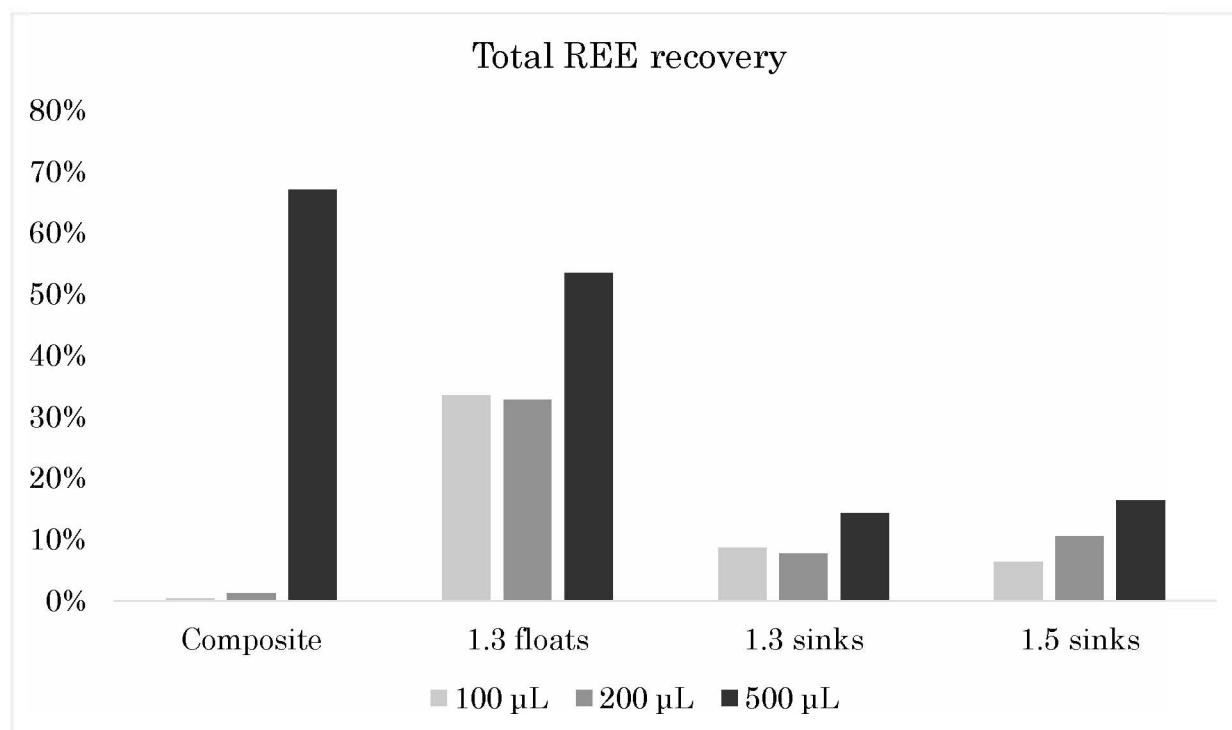
Figure 18. Total recovery of REEs in Healy coal for 100, 200, and 500 μL of *S. oneidensis* MR-1.

Table 14. Recovery of REEs from Healy coal for 100, 200, and 500 µL of *S. oneidensis* MR-1.

	Composite				1.3 floats				1.3 sinks				1.5 sinks			
	ppm	100	200	500	ppm	100	200	500	ppm	100	200	500	ppm	100	200	500
Sc	4.9	4.9%	6.8%	81.4%	3.4	5.9%	16.3%	14.6%	4.6	3.0%	2.7%	5.0%	9.0	6.8%	3.5%	8.2%
Y	14.6	0.5%	1.8%	62.7%	9.7	35.5%	33.6%	56.4%	16.6	14.9%	13.3%	25.0%	19.9	14.8%	21.8%	39.5%
La	15.6	0.1%	0.6%	70.1%	8.6	80.2%	72.1%	77.1%	16.8	12.5%	11.6%	20.5%	28.1	7.1%	13.5%	19.0%
Ce	30.7	0.1%	0.6%	69.7%	17.3	42.5%	35.9%	67.8%	33.8	7.3%	6.2%	12.4%	52.1	4.3%	7.6%	11.5%
Pr	3.6	0.1%	0.5%	71.7%	2.0	35.1%	34.7%	57.5%	4.0	6.4%	5.5%	11.4%	6.2	3.5%	6.3%	10.3%
Nd	14.5	0.2%	0.8%	64.1%	8.6	30.3%	32.5%	51.3%	16.2	6.4%	5.9%	12.0%	23.4	4.0%	7.4%	11.7%
Sm	3.3	0.3%	0.9%	58.6%	2.1	22.9%	20.8%	35.5%	3.0	5.5%	5.1%	7.7%	4.8	3.5%	7.3%	10.6%
Eu	0.7	0.1%	0.9%	65.8%	0.4	26.0%	25.6%	31.9%	0.8	7.3%	7.1%	10.9%	0.9	4.9%	10.4%	14.2%
Gd	3.0	0.2%	0.9%	56.1%	2.0	23.6%	28.0%	39.5%	3.4	7.4%	6.6%	12.3%	4.0	7.4%	11.6%	18.2%
Tb	0.5	0.2%	1.0%	58.2%	0.3	20.0%	21.2%	34.2%	0.5	7.8%	6.3%	11.6%	0.6	5.9%	11.1%	16.6%
Dy	2.6	0.4%	1.1%	66.2%	1.6	19.3%	17.7%	28.5%	3.0	8.1%	7.8%	15.5%	3.7	7.4%	11.6%	19.6%
Ho	0.5	0.4%	1.4%	64.5%	0.3	18.6%	20.9%	35.5%	0.6	8.7%	7.8%	13.9%	0.8	7.5%	13.1%	20.6%
Er	1.4	0.6%	1.6%	67.4%	0.9	20.8%	21.8%	33.9%	1.6	8.9%	7.8%	13.8%	2.1	7.1%	13.7%	22.4%
Tm	0.2	0.4%	1.3%	67.3%	0.1	12.6%	16.9%	21.6%	0.3	6.1%	6.8%	11.9%	0.3	6.6%	11.3%	19.9%
Yb	1.4	0.4%	1.9%	71.3%	0.8	14.9%	15.8%	25.2%	0.6	6.7%	6.6%	14.3%	2.0	6.0%	12.5%	20.0%
Lu	0.2	0.2%	2.3%	73.8%	0.1	16.8%	15.0%	22.0%	0.2	6.9%	8.8%	11.4%	0.3	7.5%	11.1%	24.8%
Total	97.7	0.5%	1.2%	67.1%	58.2	33.5%	32.8%	53.5%	107.8	8.7%	7.8%	14.3%	158.3	6.4%	10.5%	16.4%

5.1.4 Different Incubation Temperatures

The growth of microbes is affected by the changes in temperature, so the incubation temperature of the samples is an important factor in testing the bioleaching of coal. For the experiments performed for this research, the incubation temperature was changes from 30°C to 32°C to 34°C, and are depicted as 30, 32, and 34 in the data tables. The other variables for the experiments were 30°C incubation temperature, 5 gram coal, 100μL *S. oneidensis* MR-1, -200M size coal; 32°C incubation temperature, 3.5 gram coal, 500μL *S. oneidensis* MR-1, -14M size coal; and 34°C incubation temperature, 2 gram coal, 500μL *S. oneidensis* MR-1, -14M size coal. The experiments were performed for all the specific gravity fractions of both coals and are discussed in detail in the following pages.

5.1.4.1 Wishbone Hill Coal

The recovery of REEs from the composite sample of Wishbone Hill coal is not hugely affected by the change in temperature although it can be noticed that, in general, the recovery increases on increasing the temperature for most elements. The trend for recovery can be seen from 1.3 floats of the coal where the recovery increases sharply when temperature increases from 30°C to 32°C. The sharpest increase is in the recovery of neodymium, where it increases from 7.1% at 30°C to 75.3% at 32°C. For most of the REEs, the recovery maximizes at 34°C for 1.3 floats, the only exceptions being neodymium, samarium, cerium, lanthanum, and scandium where the maximum recoveries are obtained at 32°C.

The situation is reversed for 1.3 sinks where the maximum recoveries are mostly obtained at 32°C, the only exception being gadolinium, which has maximum recovery at 34°C. For 1.5 sinks, the maximum recovery is obtained at 32°C except for neodymium, which achieves a maximum recovery at 34°C. The individual recoveries of elements for various specific gravity at varying temperature does not give a clear

picture, but the total recovery follows a pattern. The maximum total recoveries, in all the cases, are obtained at 32°C, which can be clearly seen from Table 15 and Figure 19. Figure 19 shows the trend for the total recoveries at various specific gravities and temperatures for Wishbone Hill coal. The maximum total recoveries for all the cases are obtained at 32°C, varying with a minimum highest recovery for the composite sample at 3.6% (32°C) to a maximum of 43.2% (32°C) for 1.3 floats. The 1.5 sinks and the composite samples do not have a significant total recovery that can be attributed to the fact that Wishbone Hill has an overall high ash percentage (45.81%) with the ash percentage even higher for heavier density fractions. This is observed in almost all the samples where higher the ash percentage, the lower is the recovery and vice versa. The 1.3 floats have 6% ash and have a higher recovery of all elements compared to higher ash content coal.

Figure 19. Total recovery of REEs in Wishbone Hill coal at 30, 32, and 34°C incubation temperature.

Table 15. Recovery of REEs from Wishbone Hill coal at 30, 32, and 34ºC incubation temperature.

	Composite				1.3 floats				1.3 sinks				1.5 sinks			
	ppm	30	32	34	ppm	30	32	34	ppm	30	32	34	ppm	30	32	34
Sc	13.1	2.9%	7.5%	4.6%	7.1	27.3%	67.2%	65.9%	11.9	12.4%	34.7%	20.4%	17.0	0.4%	4.8%	2.3%
Y	21.1	0.8%	3.1%	2.7%	13.0	9.6%	22.4%	28.8%	19.4	8.2%	17.7%	10.1%	26.4	0.1%	1.7%	1.0%
La	16.4	0.7%	2.6%	2.7%	4.8	13.7%	30.1%	26.1%	15.3	1.5%	14.2%	7.6%	23.7	0.0%	1.5%	1.3%
Ce	35.3	0.7%	2.8%	2.7%	10.6	5.9%	49.8%	26.2%	31.9	1.8%	23.4%	8.3%	51.1	0.0%	1.4%	1.1%
Pr	4.2	0.5%	3.1%	3.4%	1.3	5.1%	34.6%	56.1%	3.8	2.1%	23.9%	12.0%	6.1	0.0%	1.4%	1.2%
Nd	17.5	0.9%	5.6%	3.8%	5.7	7.1%	75.3%	40.8%	15.8	2.7%	24.5%	11.7%	25.1	0.0%	1.5%	2.0%
Sm	4.1	0.6%	2.4%	2.0%	1.4	10.0%	38.8%	31.9%	3.7	4.0%	23.8%	6.7%	5.8	0.1%	2.0%	0.8%
Eu	0.9	0.9%	2.6%	2.1%	0.3	7.1%	36.1%	41.0%	0.8	6.4%	26.0%	13.3%	1.2	0.0%	1.8%	1.0%
Gd	4.0	0.6%	2.8%	4.3%	1.7	11.7%	33.6%	40.8%	3.6	6.8%	17.8%	19.2%	5.4	0.1%	1.6%	0.9%
Tb	0.6	0.7%	2.4%	2.7%	0.3	8.6%	20.8%	38.8%	0.6	7.5%	17.6%	11.5%	0.9	0.1%	1.9%	1.0%
Dy	3.8	0.4%	2.6%	4.2%	2.1	9.5%	21.6%	34.3%	3.4	7.4%	16.7%	9.9%	4.9	0.1%	1.9%	1.5%
Ho	0.8	0.7%	2.6%	2.9%	0.5	8.8%	19.9%	25.4%	0.7	8.2%	14.7%	11.7%	1.0	0.1%	1.6%	1.2%
Er	2.2	0.6%	2.3%	3.4%	1.4	10.1%	21.1%	26.8%	2.1	8.4%	12.0%	9.0%	2.7	0.1%	2.0%	1.5%
Tm	0.4	1.3%	2.4%	1.9%	0.2	6.6%	9.4%	26.5%	0.4	4.9%	13.5%	6.4%	0.4	0.1%	1.5%	1.4%
Yb	2.2	0.8%	3.9%	2.5%	1.5	5.7%	14.4%	25.5%	2.2	6.1%	11.0%	9.6%	2.5	0.1%	2.2%	1.9%
Lu	0.3	1.7%	2.9%	2.5%	0.2	6.9%	38.5%	85.7%	0.3	5.5%	13.7%	6.1%	0.4	0.1%	2.3%	2.1%
Total	126.8	0.6%	3.6%	3.1%	52.2	11.2%	43.2%	35.9%	115.9	4.8%	21.2%	10.8%	174.8	0.1%	1.8%	1.4%

5.1.4.2 Healy Coal

The composite sample of Healy coal shows a drastic improvement in recovery of all the REEs when the temperature increases from 30°C to 32°C. The recovery is maximum for all the composite samples at 32°C with a sharp fall at 34°C. This trend can be clearly established from Table 16. For all the other specific gravity fractions, the change in recovery between various temperatures is not drastic as the composite samples, although the maximum total recoveries are obtained at 32°C for all the samples (Figure 20). For 1.3 floats, the maximum recoveries for all the elements are obtained at 32°C with the exception of lanthanum, which has a maximum recovery at 30°C. For all the other samples, there is no other exception and the recoveries are maximum at 32°C.

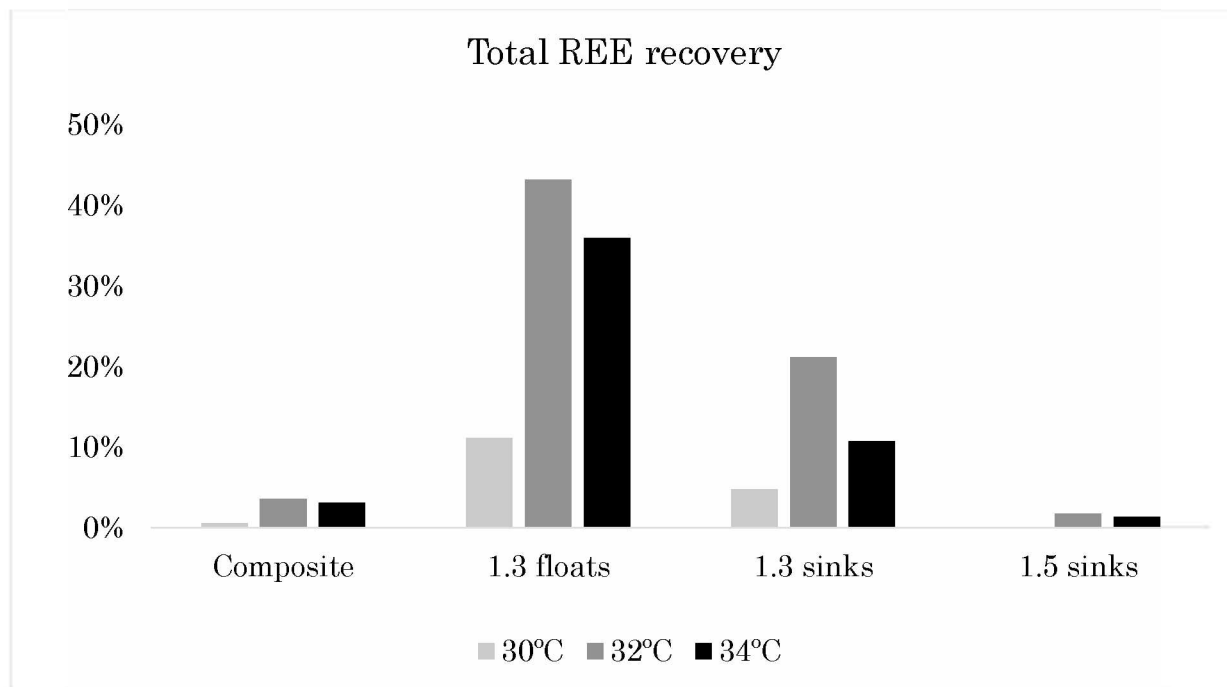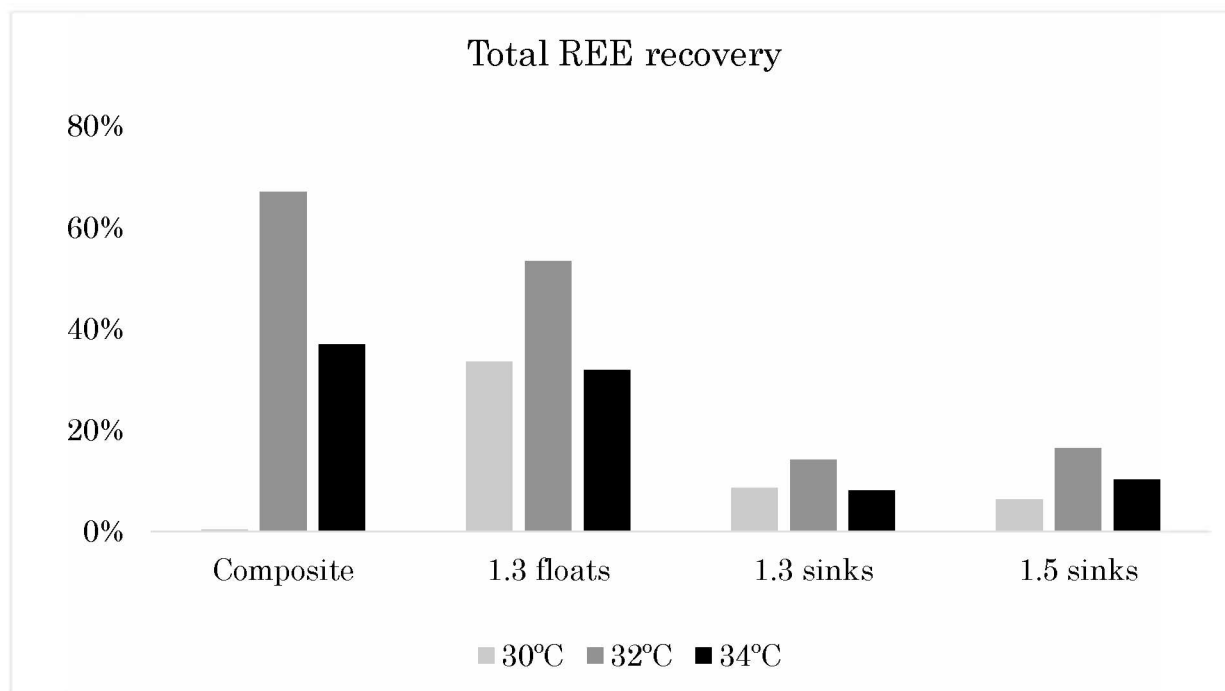**Figure 20. Total recovery of REEs in Healy coal at 30, 32, and 34°C incubation temperature.**

Table 16. Recovery of REEs from Healy coal at 30, 32, and 34°C incubation temperature.

	Composite				1.3 floats				1.3 sinks				1.5 sinks			
	ppm	30	32	34	ppm	30	32	34	ppm	30	32	34	ppm	30	32	34
Sc	4.9	4.9%	81.4%	45.1%	3.4	5.9%	14.6%	8.7%	4.6	3.0%	5.0%	2.5%	9.0	6.8%	8.2%	3.6%
Y	14.6	0.5%	62.7%	31.3%	9.7	35.5%	56.4%	38.3%	16.6	14.9%	25.0%	15.8%	19.9	14.8%	39.5%	24.6%
La	15.6	0.1%	70.1%	36.4%	8.6	80.2%	77.1%	40.9%	16.8	12.5%	20.5%	9.7%	28.1	7.1%	19.0%	11.3%
Ce	30.7	0.1%	69.7%	38.2%	17.3	42.5%	67.8%	37.7%	33.8	7.3%	12.4%	6.1%	52.1	4.3%	11.5%	6.8%
Pr	3.6	0.1%	71.7%	43.2%	2.0	35.1%	57.5%	35.9%	4.0	6.4%	11.4%	6.0%	6.2	3.5%	10.3%	7.2%
Nd	14.5	0.2%	64.1%	38.7%	8.6	30.3%	51.3%	32.6%	16.2	6.4%	12.0%	6.4%	23.4	4.0%	11.7%	6.9%
Sm	3.3	0.3%	58.6%	29.7%	2.1	22.9%	35.5%	22.4%	3.0	5.5%	7.7%	4.4%	4.8	3.5%	10.6%	6.6%
Eu	0.7	0.1%	65.8%	34.0%	0.4	26.0%	31.9%	26.8%	0.8	7.3%	10.9%	7.9%	0.9	4.9%	14.2%	10.5%
Gd	3.0	0.2%	56.1%	32.2%	2.0	23.6%	39.5%	29.8%	3.4	7.4%	12.3%	9.2%	4.0	7.4%	18.2%	14.3%
Tb	0.5	0.2%	58.2%	31.7%	0.3	20.0%	34.2%	24.3%	0.5	7.8%	11.6%	6.1%	0.6	5.9%	16.6%	13.0%
Dy	2.6	0.4%	66.2%	34.2%	1.6	19.3%	28.5%	27.5%	3.0	8.1%	15.5%	8.1%	3.7	7.4%	19.6%	13.3%
Ho	0.5	0.4%	64.5%	33.0%	0.3	18.6%	35.5%	23.3%	0.6	8.7%	13.9%	10.2%	0.8	7.5%	20.6%	16.1%
Er	1.4	0.6%	67.4%	40.0%	0.9	20.8%	33.9%	28.4%	1.6	8.9%	13.8%	9.1%	2.1	7.1%	22.4%	16.6%
Tm	0.2	0.4%	67.3%	34.7%	0.1	12.6%	21.6%	22.2%	0.3	6.1%	11.9%	8.0%	0.3	6.6%	19.9%	14.0%
Yb	1.4	0.4%	71.3%	39.1%	0.8	14.9%	25.2%	21.3%	0.6	6.7%	14.3%	8.1%	2.0	6.0%	20.0%	12.7%
Lu	0.2	0.2%	73.8%	48.3%	0.1	16.8%	22.0%	19.9%	0.2	6.9%	11.4%	9.1%	0.3	7.5%	24.8%	14.4%
Total	97.7	0.5%	67.1%	37.0%	58.2	33.5%	53.5%	31.9%	107.8	8.7%	14.3%	8.1%	158.3	6.4%	16.4%	10.3%

Unlike the Wishbone Hill samples, the maximum total recovery of 67.1% is obtained for the composite sample at 32°C. The total recovery decreases as the specific gravity of coal increases, while the maximum recovery for all the individual cases is obtained at 32°C.

5.1.5 Different Size of Coal

The last variable that was tested for the research was to understand the effect of the size of the coal to be leached on the recovery of the REEs. For this purpose, the coal samples were crushed and sieved, and divided into three size ranges, namely, -200M, -48M to +200M, and -14M to +48M which are depicted as -200, -48, and -14 in Table 17. The other variables for these experiments were -14M size coal, 32°C incubation temperature, 3.5 gram coal, 500µL *S. oneidensis* MR-1; -48M size coal, 30°C incubation temperature, 5 gram coal, 200µL *S. oneidensis* MR-1; and -200M size coal, 30°C incubation temperature, 5 gram coal, 100µL *S. oneidensis* MR-1. The experiments were performed for both types of coal and all the different specific gravities and their results are discussed in the following pages.

5.1.5.1 Wishbone Hill Coal

The composite samples of Wishbone Hill coal have a very low recovery for all the three size ranges, although, the maximum recoveries are obtained for -14M+48M size range. For 1.3 floats, the recoveries are minimum for -48M+200M size range, while the maximum recoveries are obtained at -14M+48M. The recoveries of individual REEs for different size ranges and specific gravities of coal are given in Table 17.

For -48M+200M, the recoveries are lowest for all the specific gravities of Wishbone Hill coal, while for -14M+48M, the recoveries are highest. The recoveries for all the elements decrease as the specific gravity increases with lowest recoveries obtained for 1.5 sinks.

Table 17. Recovery of REEs from Wishbone Hill coal for -200M, -48M+200M, and -14M+48M coal size.

	Composite				1.3 floats				1.3 sinks				1.5 sinks			
	ppm	-200	-48	-14	ppm	-200	-48	-14	ppm	-200	-48	-14	ppm	-200	-48	-14
Sc	13.1	2.9%	8.4%	7.5%	7.1	27.3%	43.0%	67.2%	11.9	12.4%	14.2%	34.7%	17.0	0.4%	0.7%	4.8%
Y	21.1	0.8%	1.7%	3.1%	13.0	9.6%	7.9%	22.4%	19.4	8.2%	1.8%	17.7%	26.4	0.1%	0.0%	1.7%
La	16.4	0.7%	1.2%	2.6%	4.8	13.7%	11.2%	30.1%	15.3	1.5%	0.9%	14.2%	23.7	0.0%	0.0%	1.5%
Ce	35.3	0.7%	1.3%	2.8%	10.6	5.9%	10.8%	49.8%	31.9	1.8%	1.0%	23.4%	51.1	0.0%	0.0%	1.4%
Pr	4.2	0.5%	0.3%	3.1%	1.3	5.1%	3.8%	34.6%	3.8	2.1%	0.7%	23.9%	6.1	0.0%	0.0%	1.4%
Nd	17.5	0.9%	0.2%	5.6%	5.7	7.1%	4.1%	75.3%	15.8	2.7%	0.9%	24.5%	25.1	0.0%	0.0%	1.5%
Sm	4.1	0.6%	0.3%	2.4%	1.4	10.0%	2.7%	38.8%	3.7	4.0%	0.8%	23.8%	5.8	0.1%	0.0%	2.0%
Eu	0.9	0.9%	0.6%	2.6%	0.3	7.1%	19.0%	36.1%	0.8	6.4%	2.0%	26.0%	1.2	0.0%	0.0%	1.8%
Gd	4.0	0.6%	0.4%	2.8%	1.7	11.7%	4.4%	33.6%	3.6	6.8%	2.0%	17.8%	5.4	0.1%	0.0%	1.6%
Tb	0.6	0.7%	0.2%	2.4%	0.3	8.6%	3.2%	20.8%	0.6	7.5%	2.0%	17.6%	0.9	0.1%	0.1%	1.9%
Dy	3.8	0.4%	0.2%	2.6%	2.1	9.5%	4.2%	21.6%	3.4	7.4%	0.9%	16.7%	4.9	0.1%	0.0%	1.9%
Ho	0.8	0.7%	0.3%	2.6%	0.5	8.8%	2.4%	19.9%	0.7	8.2%	2.6%	14.7%	1.0	0.1%	0.0%	1.6%
Er	2.2	0.6%	0.1%	2.3%	1.4	10.1%	4.0%	21.1%	2.1	8.4%	2.1%	12.0%	2.7	0.1%	0.0%	2.0%
Tm	0.4	1.3%	0.1%	2.4%	0.2	6.6%	0.0%	9.4%	0.4	4.9%	2.8%	13.5%	0.4	0.1%	0.0%	1.5%
Yb	2.2	0.8%	0.1%	3.9%	1.5	5.7%	2.6%	14.4%	2.2	6.1%	1.0%	11.0%	2.5	0.1%	0.0%	2.2%
Lu	0.3	1.7%	0.1%	2.9%	0.2	6.9%	4.1%	38.5%	0.3	5.5%	4.2%	13.7%	0.4	0.1%	0.1%	2.3%
Total	126.8	0.6%	1.3%	3.6%	52.2	11.2%	15.0%	43.2%	115.9	4.8%	2.5%	21.2%	174.8	0.1%	0.1%	1.8%

The total recoveries of REEs for various specific gravities are depicted in Figure 21. It can be seen that the total recovery is the highest for 1.3 floats specific gravity, -14M+48M size range. Within a sample, the total recoveries increase as the particle size increases, while the total recoveries decrease as the specific gravity of coal increases. The total recoveries of REEs from 1.5 sinks and composite samples are negligible, while from 1.3 sinks is respectable with the highest of 21.2% recovery from -14M+48M size range.
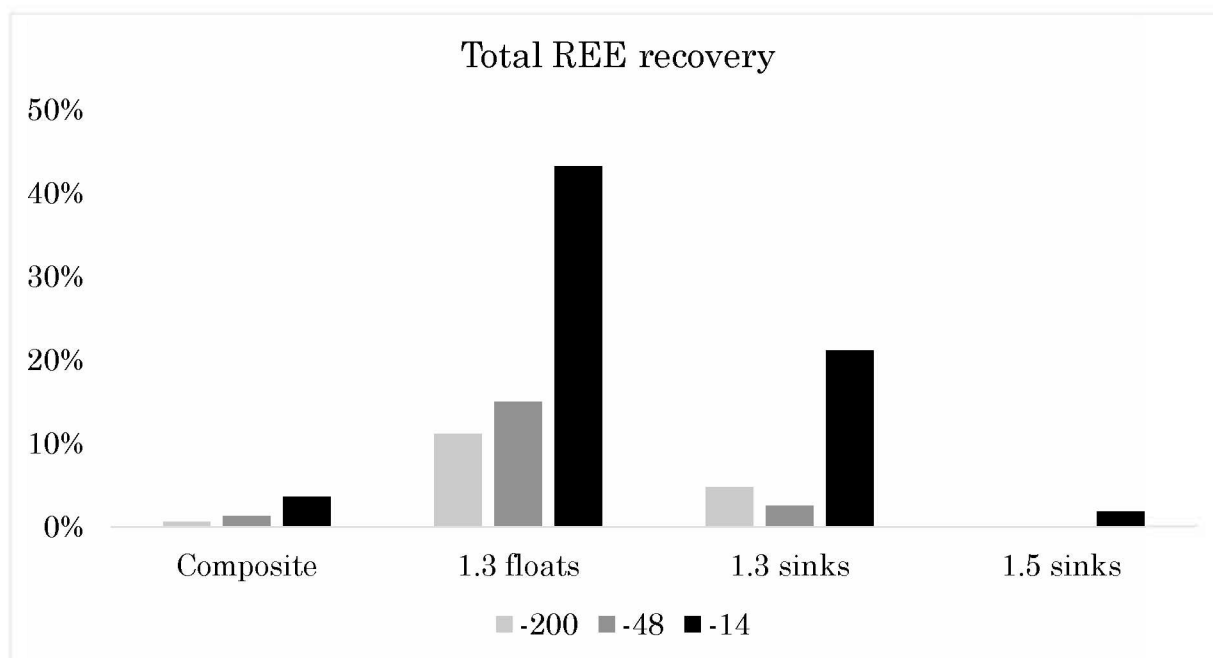
Figure 21. Total recovery of REEs in Wishbone Hill coal for -200M, -48M+200M, and -14M+48M coal size.

5.1.5.2 Healy Coal

For the composite samples, the recovery of all the REEs increases as the size of coal particles increases, with an exponential increase in recovery when the size increases from -48M+200M to -14M+48M. For 1.3 floats and 1.3 sinks, the recovery decreases as the size increases from -200M to -48M+200M, while it again increases from -48M+200M to -14M+48M achieving the maximum recovery at -14M+48M.

The 1.5 sinks fraction of Healy coal follows the composite samples in terms of the pattern of individual REE recovery as it increases when the size of coal increases. The only exception to this rule is the recovery of scandium where it decreases as the size increases but then increases as the size increases further. The recoveries of individual REEs for different size ranges and specific gravities are given in Table 18, while the pattern for the total recovery with the change in specific gravity of Healy coal in depicted in Figure 22.

The total recoveries of REEs from Healy coal for different specific gravities decreases as the coal gets heavier. The maximum total recovery is obtained for the composite sample while the minimum recovery is obtained for 1.3 sinks, although there is not much difference between the recoveries from 1.3 sinks and 1.5 sinks.
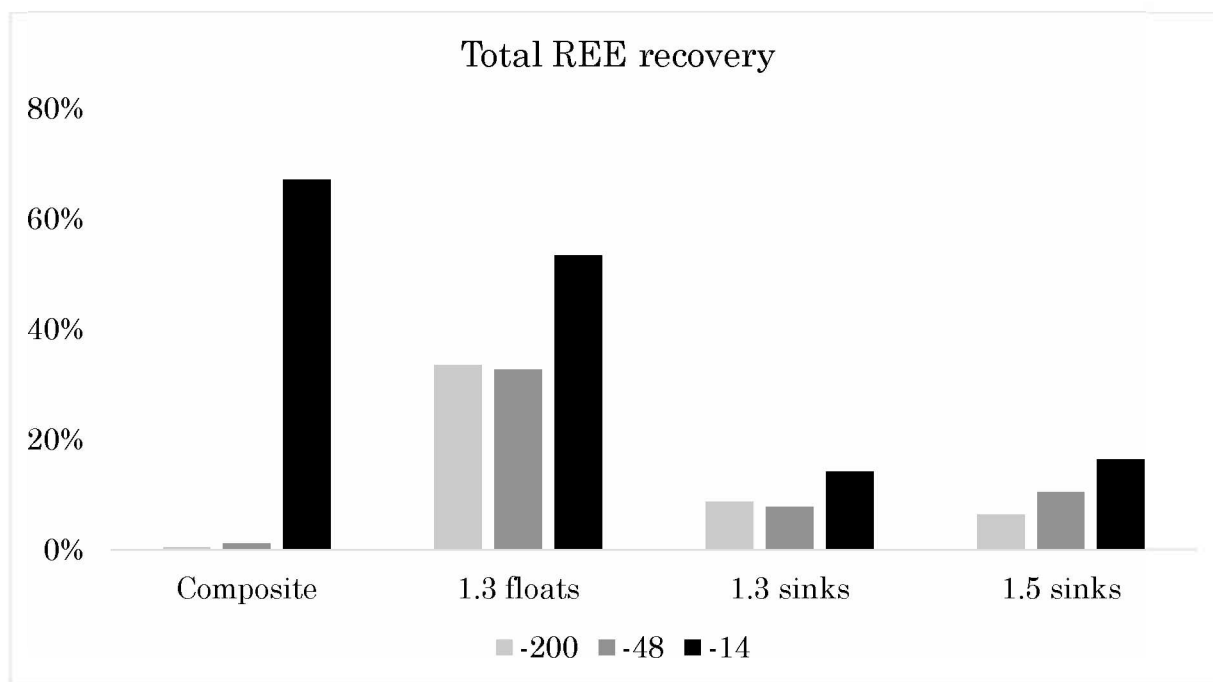

Figure 22. Total recovery of REEs in Healy coal for -200M, -48M+200M, and -14M+48M coal size.

Table 18. Recovery of REEs from Healy coal for -200M, -48M+200M, and -14M+48M coal size.

	Composite				1.3 floats				1.3 sinks				1.5 sinks			
	ppm	-200	-48	-14	ppm	-200	-48	-14	ppm	-200	-48	-14	ppm	-200	-48	-14
Sc	4.9	4.9%	6.8%	81.4%	3.4	5.9%	16.3%	14.6%	4.6	3.0%	2.7%	5.0%	9.0	6.8%	3.5%	8.2%
Y	14.6	0.5%	1.8%	62.7%	9.7	35.5%	33.6%	56.4%	16.6	14.9%	13.3%	25.0%	19.9	14.8%	21.8%	39.5%
La	15.6	0.1%	0.6%	70.1%	8.6	80.2%	72.1%	77.1%	16.8	12.5%	11.6%	20.5%	28.1	7.1%	13.5%	19.0%
Ce	30.7	0.1%	0.6%	69.7%	17.3	42.5%	35.9%	67.8%	33.8	7.3%	6.2%	12.4%	52.1	4.3%	7.6%	11.5%
Pr	3.6	0.1%	0.5%	71.7%	2.0	35.1%	34.7%	57.5%	4.0	6.4%	5.5%	11.4%	6.2	3.5%	6.3%	10.3%
Nd	14.5	0.2%	0.8%	64.1%	8.6	30.3%	32.5%	51.3%	16.2	6.4%	5.9%	12.0%	23.4	4.0%	7.4%	11.7%
Sm	3.3	0.3%	0.9%	58.6%	2.1	22.9%	20.8%	35.5%	3.0	5.5%	5.1%	7.7%	4.8	3.5%	7.3%	10.6%
Eu	0.7	0.1%	0.9%	65.8%	0.4	26.0%	25.6%	31.9%	0.8	7.3%	7.1%	10.9%	0.9	4.9%	10.4%	14.2%
Gd	3.0	0.2%	0.9%	56.1%	2.0	23.6%	28.0%	39.5%	3.4	7.4%	6.6%	12.3%	4.0	7.4%	11.6%	18.2%
Tb	0.5	0.2%	1.0%	58.2%	0.3	20.0%	21.2%	34.2%	0.5	7.8%	6.3%	11.6%	0.6	5.9%	11.1%	16.6%
Dy	2.6	0.4%	1.1%	66.2%	1.6	19.3%	17.7%	28.5%	3.0	8.1%	7.8%	15.5%	3.7	7.4%	11.6%	19.6%
Ho	0.5	0.4%	1.4%	64.5%	0.3	18.6%	20.9%	35.5%	0.6	8.7%	7.8%	13.9%	0.8	7.5%	13.1%	20.6%
Er	1.4	0.6%	1.6%	67.4%	0.9	20.8%	21.8%	33.9%	1.6	8.9%	7.8%	13.8%	2.1	7.1%	13.7%	22.4%
Tm	0.2	0.4%	1.3%	67.3%	0.1	12.6%	16.9%	21.6%	0.3	6.1%	6.8%	11.9%	0.3	6.6%	11.3%	19.9%
Yb	1.4	0.4%	1.9%	71.3%	0.8	14.9%	15.8%	25.2%	0.6	6.7%	6.6%	14.3%	2.0	6.0%	12.5%	20.0%
Lu	0.2	0.2%	2.3%	73.8%	0.1	16.8%	15.0%	22.0%	0.2	6.9%	8.8%	11.4%	0.3	7.5%	11.1%	24.8%
Total	97.7	0.5%	1.2%	67.1%	58.2	33.5%	32.8%	53.5%	107.8	8.7%	7.8%	14.3%	158.3	6.4%	10.5%	16.4%

5.2 Comparison Between Wishbone Hill and Healy Coal

After testing of all the specific gravities of coal on varying conditions, the results were then compared for Wishbone Hill and Healy coal. The comparison of the recoveries for both coals will give an insight on their leachability capability. The discussion on the comparison is provided in the following pages.

5.2.1 Comparison on Pulp Density

The total recovery of REEs from Healy coal is higher than from Wishbone Hill coal for all the cases except for 1.3 sinks and 3.5 gm (10% solids) where the total recovery is higher for Wishbone Hill coal. The lower recovery from Wishbone Hill can be attributed to the higher ash content in it. For 1.5 sinks, Wishbone Hill has 73.23% ash, while Healy has 56.09% ash for the same specific gravity of coal. For the 1.3 floats, although the ash content in Wishbone Hill coal is only 6.09%, the amount of total REEs present in it is less compared to Healy coal (Appendix B). The other factor that affect the low leachability in 1.5 sinks and composite samples of Wishbone Hill is the relatively higher concentration of arsenic, cadmium, and lead in these fractions as compared to 1.3 floats and 1.3 sinks (Appendix C). These elements can be inhibitory for the growth of *S. oneidensis* MR-1, thereby reducing the bioleaching capability resulting in lower recoveries. The concentration of these elements in Healy coal is lesser when compared to Wishbone Hill and hence Healy has better total recovery in almost every case. The total recoveries of REEs from both the coals at varying solids percentage is given in Figure 23.

5.2.2 Comparison on Initial Bacterial Concentration

The total recovery of REEs from Healy coal is higher in all the cases except in 1.3 sinks, 500 µL bacterial concentration. For every other case, the recoveries are significantly higher in Healy coal and follow almost the same pattern as for pulp density discussed previously. Also, there is no significant effect of the increase in microbial concentration for Wishbone Hill coal containing higher arsenic, cadmium,

and lead content, hence proving that these three elements are toxic to *S. oneidensis* MR-1, thereby reducing the recovery of REEs. The total recoveries of REEs for different initial bacterial concentration are given in Figure 24.

5.2.3 Comparison on Incubation Temperature

The effect of temperature on total recovery is more pronounced as compared to other factors as the total recovery is relatively higher for all the cases. The maximum recoveries are obtained at 32°C for every sample. *S. oneidensis* MR-1 is known to survive in a temperature range of 28°C to 38°C. By the results obtained from the research it can be concluded that the optimum temperature for achieving maximum total recovery of REEs is 32°C. The recoveries at 30°C were the lowest in every case except for Healy coal, where the total recoveries were lowest at 34°C for Healy 1.3 floats and 1.3 sinks. The total recoveries of REEs for various temperatures are mentioned in Figure 25.

5.2.4 Comparison on Coal Size

The total recovery of REEs in the composite samples for Healy coal is less in -200M and -48M+200M size range as compared to Wishbone Hill, although the difference is minor. The total recovery in -14M+48M size range is higher for Healy coal at 67.1% as compared to 3.6% for Wishbone Hill coal. For 1.3 floats and 1.5 sinks, the recoveries for all the size ranges is higher in Healy coal. For 1.3 sinks, the recovery from Healy coal is higher in the finer size ranges and lower for -14M+48M. From the above discussion, it can be concluded that the optimum size range for bioleaching is -14M+48M. The total recovery of REEs from Wishbone Hill and Healy coal for different size ranges is depicted in Figure 26.

Figure 23. Comparison of total recovery between Wishbone Hill and Healy coal for different pulp densities.
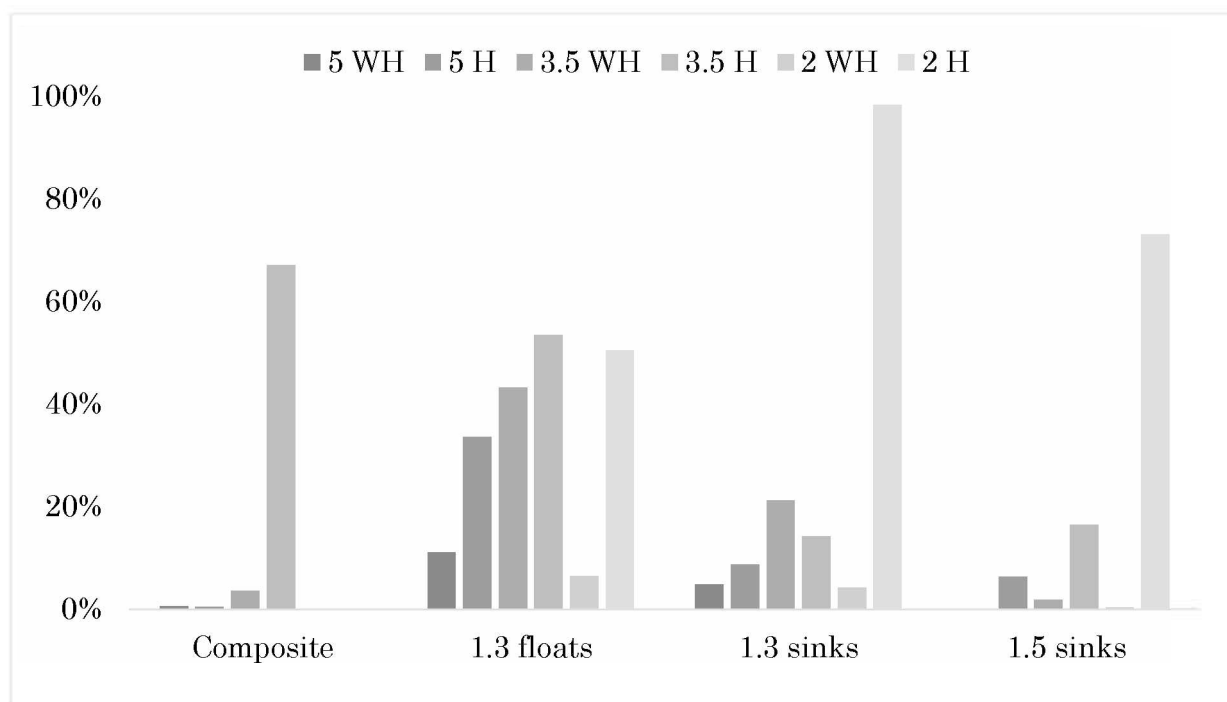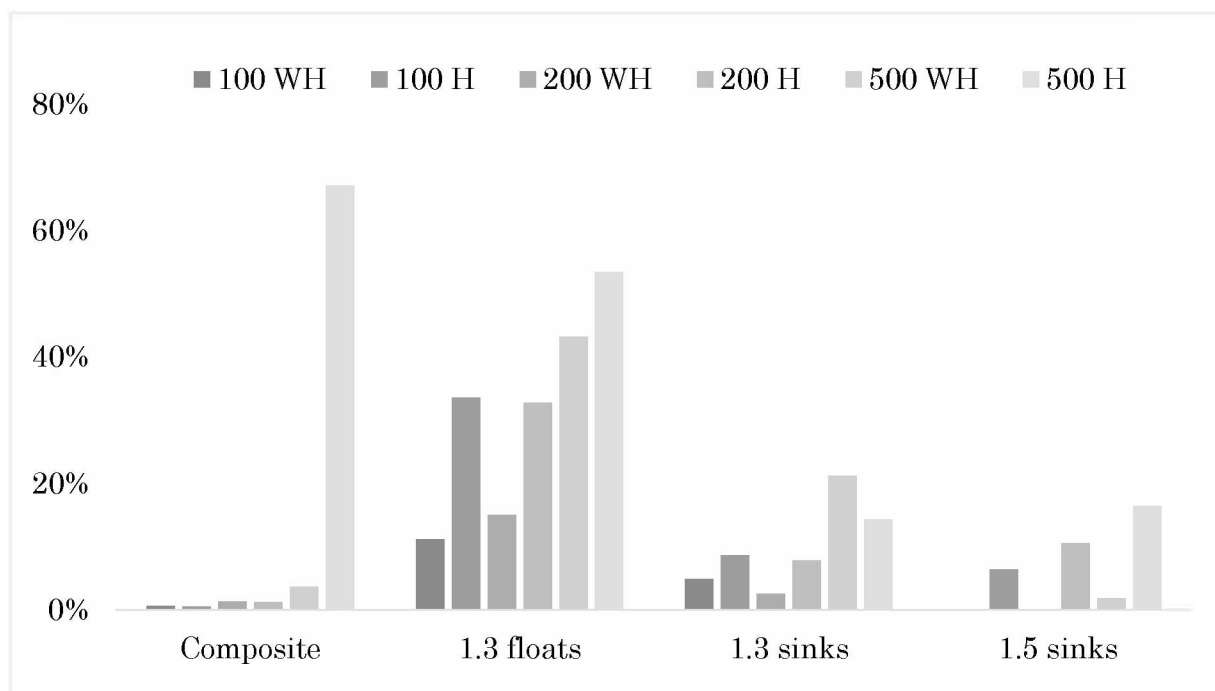

Figure 24. Comparison of total recovery between Wishbone Hill and Healy coal for different bacterial concentrations.
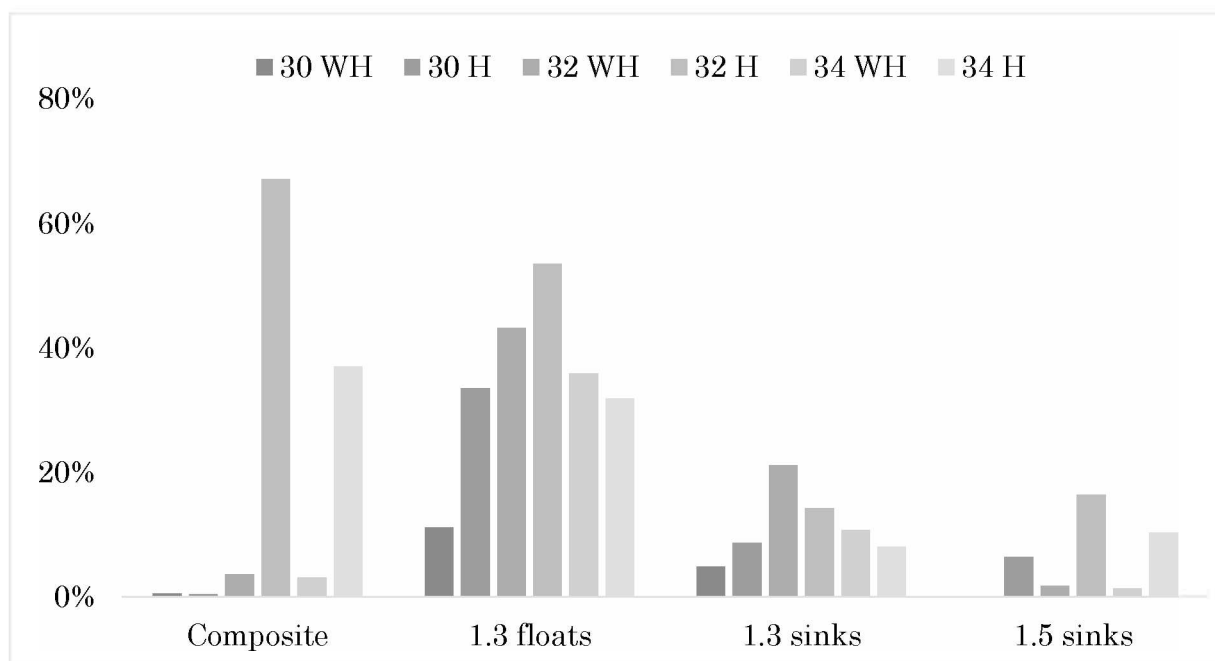
Figure 25. Comparison of total recovery between Wishbone Hill and Healy coal for different incubation temperatures.
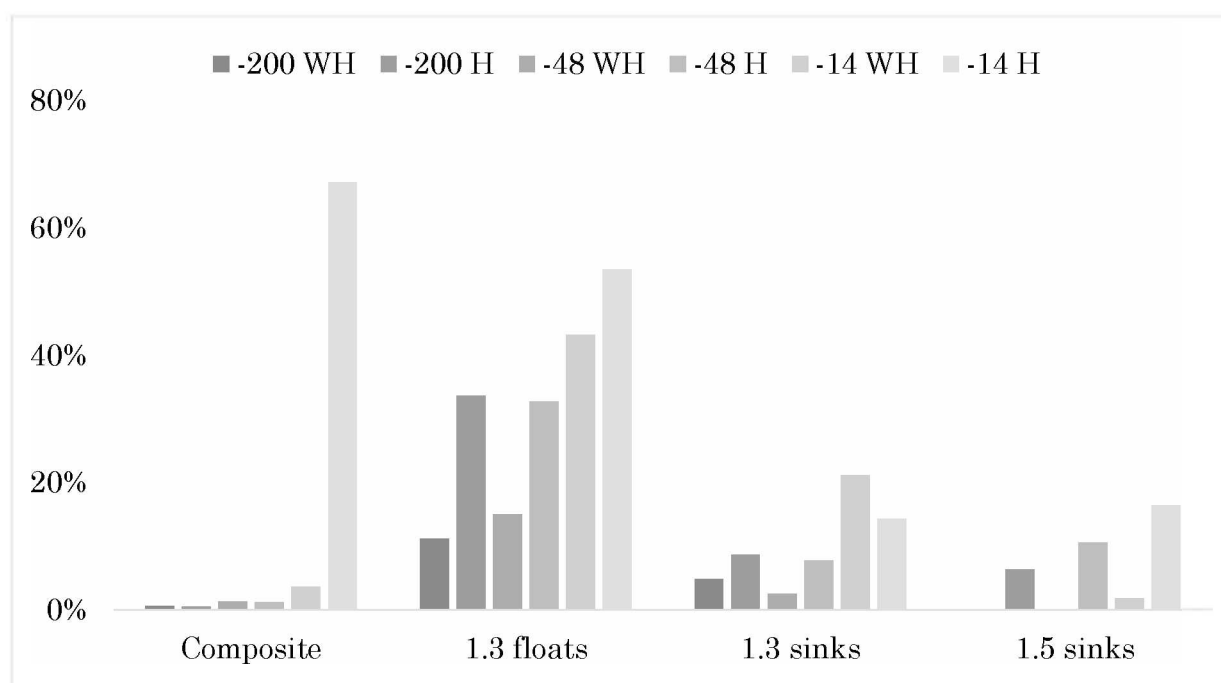

Figure 26. Comparison of total recovery between Wishbone Hill and Healy coal for different size ranges.

5.3 Yield of the Bioleaching Process

The yield of the process was determined by weighing the coal after the completion of bioleaching cycles. The difference in weight of coal was calculated and subsequently the yield was computed. The yield was calculated for three sets of experiments with the following conditions, (i) -200M, 2 gm, 30°C, 500μL, (ii) -200M, 3.5 gm, 32°C, 500μL, and (iii) -48M, 5 gm, 30°C, 100μL. For tabular and graphical purposes, the three conditions are named A, B, and C, respectively. For other conditions the coal samples could not be filtered out correctly so no data for those experiments could be obtained. Table 19 summarizes the yield of the process for both types of coal and all the specific gravities. The yield is less than 2% for every condition and specific gravity of both the coals. The highest yield (1.73%) is obtained for Healy 1.3 floats when the experiments are performed with -48M size 5 gram coal with an initial bacterial concentration of 100μL at 30°C. For conditions A and B, the maximum yields were obtained for Healy coal in all the specific gravity fractions, while for condition C in the composite samples the maximum yield was obtained for Wishbone Hill coal instead of Healy coal. There is no definite pattern between yield percentages in relation to the different conditions, although the yields for Wishbone Hill are less than 1% for all the conditions. Figure 27 depicts the yield percentage of bioleaching process for Wishbone Hill and Healy coal for conditions A, B, and C. In the graph, C stands for composite sample, F for 1.3 floats, M for 1.3 sinks, and S for 1.5 sinks.

5.4 Comparison of Bioleaching with Acid Leaching

One of the last objectives of the research was to compare the results from bioleaching with the traditional acid leaching operations. This comparison can provide the evidence for the selective leaching of REEs from coal through bioleaching. For the acid leaching, -14M+48M coal was used for each specific gravity fraction. A 1.2 molar sulfuric acid (H_2SO_4) at a temperature of 75°C along with the coal samples was placed in vials for 48 hours. The individual recovery of REEs from both the coals at different specific gravities are shown in Table 20.

98

Table 19. Comparison of yield of bioleaching process for Wishbone Hill and Healy coal at different conditions.

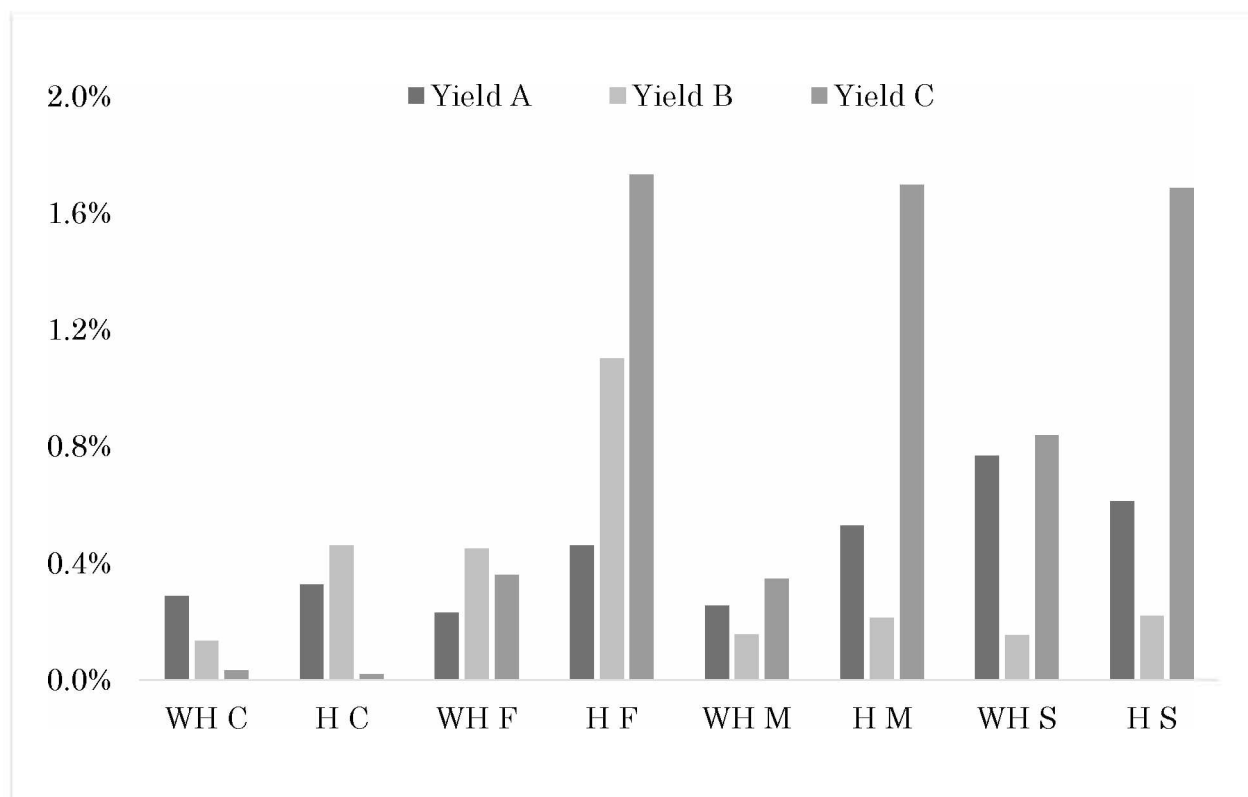|	Yield (%)		
	A	B	C
WH Composite	0.29%	0.13%	0.03%
H Composite	0.33%	0.46%	0.02%
WH 1.3 Floats	0.23%	0.45%	0.36%
H 1.3 Floats	0.46%	1.10%	1.73%
WH 1.3 Sinks	0.25%	0.16%	0.35%
H 1.3 Sinks	0.53%	0.21%	1.70%
WH 1.5 Sinks	0.77%	0.15%	0.84%
H S 1.5 Sinks	0.61%	0.22%	1.69%

Figure 27. Yield (%) of the bioleaching process for conditions A, B, and C for Wishbone Hill and Healy Coal.

Table 20. Total and individual recoveries of REEs by 1.2 M Sulfuric Acid for Wishbone Hill and Healy coal.

	Wishbone Hill				Healy			
	Composite	1.3 floats	1.3 sinks	1.5 sinks	Composite	1.3 floats	1.3 sinks	1.5 sinks
Sc	7.2%	15.7%	12.3%	3.4%	17.7%	18.9%	14.9%	18.6%
Y	6.8%	23.0%	16.3%	2.6%	9.8%	48.2%	15.8%	14.6%
La	3.1%	97.9%	26.3%	1.4%	9.1%	79.4%	20.0%	10.3%
Ce	4.1%	97.3%	33.2%	1.4%	4.7%	59.0%	9.6%	5.6%
Pr	4.1%	99.6%	30.7%	1.9%	30.3%	91.8%	57.4%	37.5%
Nd	4.7%	98.2%	32.8%	2.4%	9.8%	98.6%	19.4%	12.4%
Sm	6.1%	98.8%	34.0%	3.4%	28.0%	86.2%	46.0%	35.9%
Eu	6.5%	77.7%	32.6%	3.7%	30.0%	97.3%	46.6%	36.8%
Gd	7.3%	67.3%	30.3%	4.4%	42.2%	94.2%	45.5%	39.1%
Tb	7.2%	52.1%	26.8%	4.5%	34.3%	80.4%	42.8%	37.5%
Dy	7.3%	37.9%	23.7%	4.8%	46.1%	68.8%	42.0%	36.8%
Ho	6.4%	25.9%	17.9%	4.4%	34.9%	58.3%	36.6%	32.5%
Er	6.3%	20.5%	14.7%	4.5%	35.3%	56.2%	34.7%	31.9%
Tm	4.8%	13.5%	10.7%	3.7%	30.2%	42.0%	27.4%	26.4%
Yb	4.9%	11.9%	10.0%	3.8%	29.8%	41.2%	27.6%	26.0%
Lu	4.7%	10.2%	8.7%	3.6%	28.9%	39.6%	26.2%	23.8%
Total	5.2%	58.3%	25.8%	2.3%	13.0%	60.9%	20.2%	14.2%

From Table 20 it can be concluded that Healy coal has better recovery of REEs in comparison to Wishbone Hill coal. Also, Wishbone Hill has a better recovery of cerium and lanthanum for 1.3 floats and 1.3 sinks in comparison to Healy coal of the same specific gravities. The comparison of the recoveries between acid leaching and bioleaching for Wishbone Hill are shown in Figure 28 and for Healy coal are shown in Figure 29, where WH denotes Wishbone Hill, H denotes Healy, while A and B denote acid leaching and bioleaching, respectively.
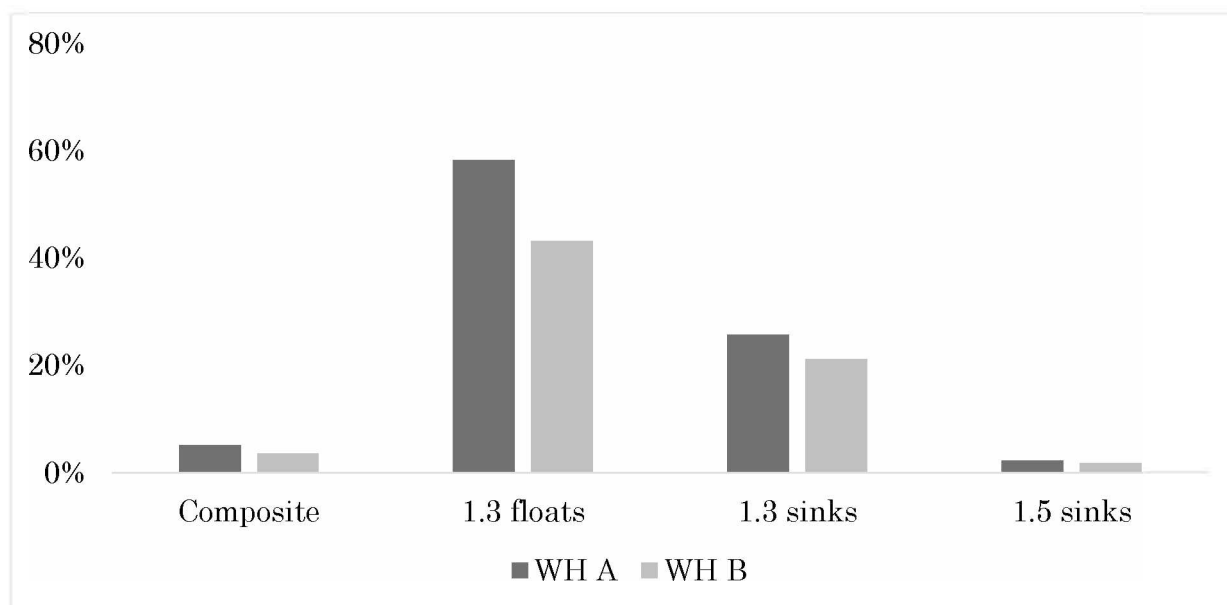
Figure 28. Comparison of recovery percentage between acid leaching and bioleaching for Wishbone Hill coal.
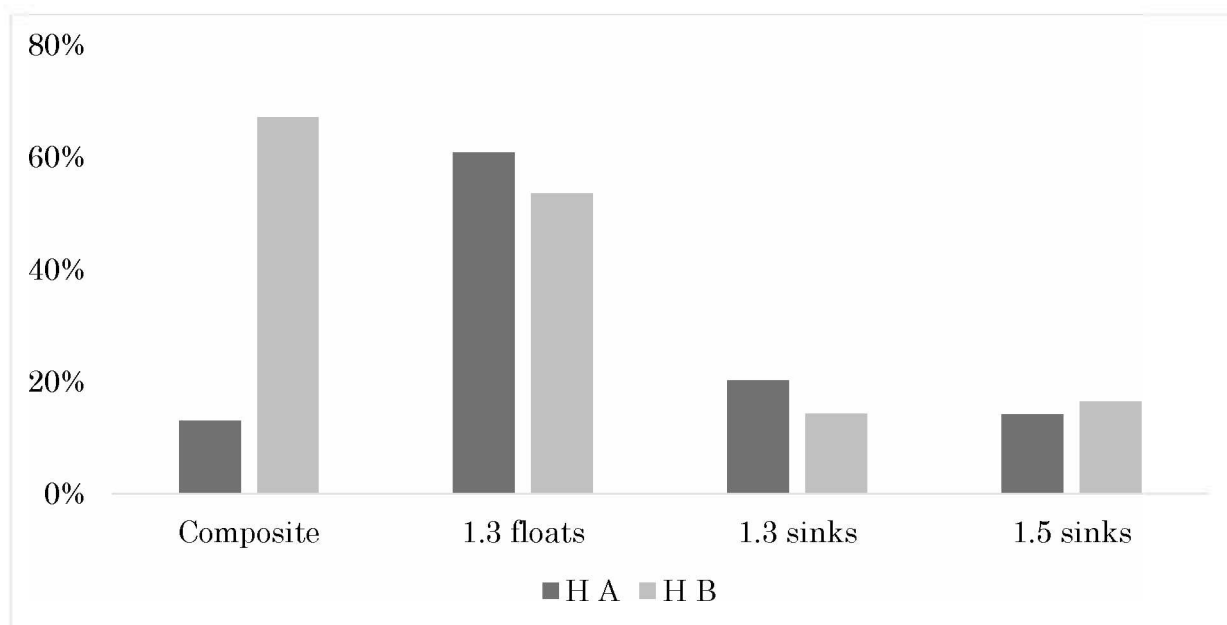
Figure 29. Comparison of recovery percentage between acid leaching and bioleaching for Healy coal.

5.4.1 Wishbone Hill Coal

For Wishbone Hill coal, the total recovery of REEs is lower for bioleaching in comparison to acid leaching for all the samples. Through acid leaching, a maximum total recovery of 58.3% is achieved for Wishbone Hill 1.3 floats. Also, similar to the bioleaching process, a trend can be observed in the pattern of total recoveries as they decrease when the specific gravity of coal increases. Although, the total recovery of REEs through bioleaching is less when compared to acid leaching for every case, the results are still close to each other. When the individual recoveries are compared for both processes, the evidence for selective leaching can be obtained. For Wishbone Hill composite samples, the recovery of neodymium and scandium is higher in bioleaching as compared to acid leaching, indicating a preference towards these elements (Figure 30).
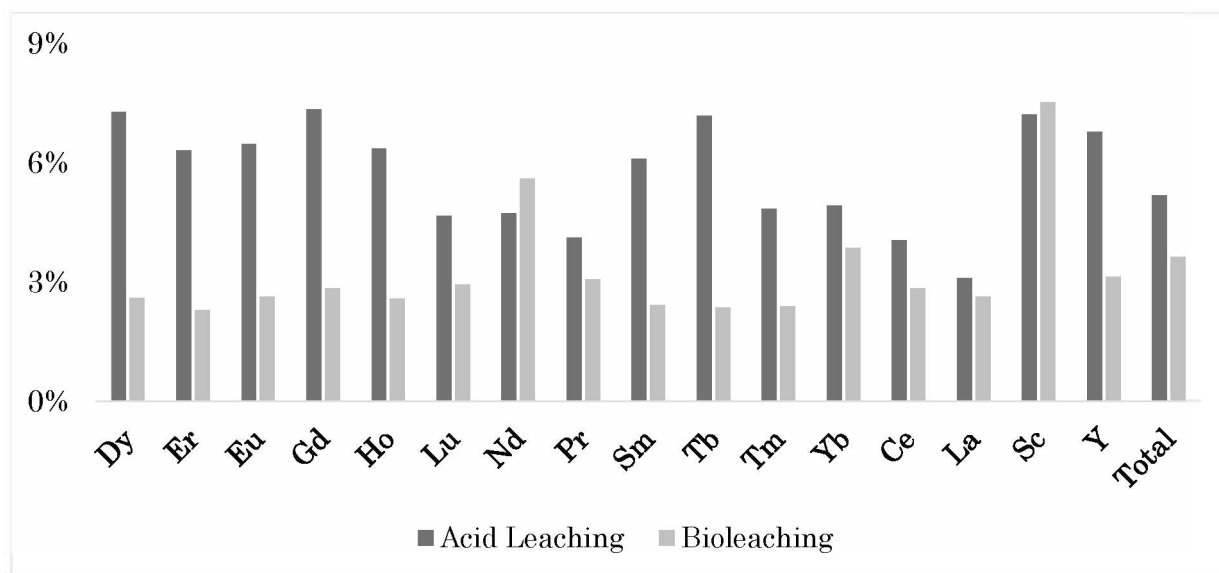
Figure 30. Comparison between recoveries of acid leaching and bioleaching for Wishbone Hill composite sample.

The recoveries from the composite sample of Wishbone Hill are less for both processes. For 1.3 floats, erbium, lutetium, neodymium, and scandium have higher recoveries in bioleaching process (Figure 31), while for 1.3 sinks, lutetium, tellurium,

ytterbium, and scandium have higher recoveries (Figure 32). For 1.5 sinks of Wishbone Hill coal, cerium, lanthanum, and scandium have a higher recovery in bioleaching process (Figure 33). From the above discussion, it can be concluded that scandium is selectively leached out in all the specific densities of Wishbone Hill coal in the bioleaching process, followed by neodymium and lutetium.

Figure 31. Comparison between recoveries of acid leaching and bioleaching for Wishbone Hill 1.3 floats.

Figure 32. Comparison between recoveries of acid leaching and bioleaching for Wishbone Hill 1.3 sinks.

Figure 33. Comparison between recoveries of acid leaching and bioleaching for Wishbone Hill 1.5 sinks.

5.4.2 Healy Coal

Healy coal responds to bioleaching better than Wishbone Hill coal which is evident from the fact that it has more total recovery from Healy composite and 1.5 sinks samples. The highest total recovery is obtained from Healy composite sample through bioleaching. For acid leaching, the recoveries fall as the specific gravity of coal increases. From Figure 29, it can also be concluded that bioleaching is a better option for recovering REEs from coal as the composite samples offer better recovery than from acid leaching.

For the composite sample of Healy, the bioleaching process achieves the better recovery of all the REEs in comparison to the acid leaching process (Figure 34). This fact alone is an indication that the Healy coal is more suited for bioleaching for the recovery of REEs from it. For 1.3 floats, only cerium and yttrium have better recoveries in bioleaching, although, for lanthanum and scandium, the difference is not considerable (Figure 35). For 1.3 sinks, cerium, lanthanum, and yttrium have better recoveries in the bioleaching process (Figure 36), while for 1.5 sinks of Healy coal, better recoveries are obtained for lutetium, cerium, lanthanum, and yttrium,

104

along with a higher total recovery of the REEs (Figure 37). From the discussion above, it can be concluded that cerium and yttrium are selectively leached out in bioleaching process more efficiently than any other REE in Healy coal. Lutetium and lanthanum also have better recovery through bioleaching in comparison to acid leaching.
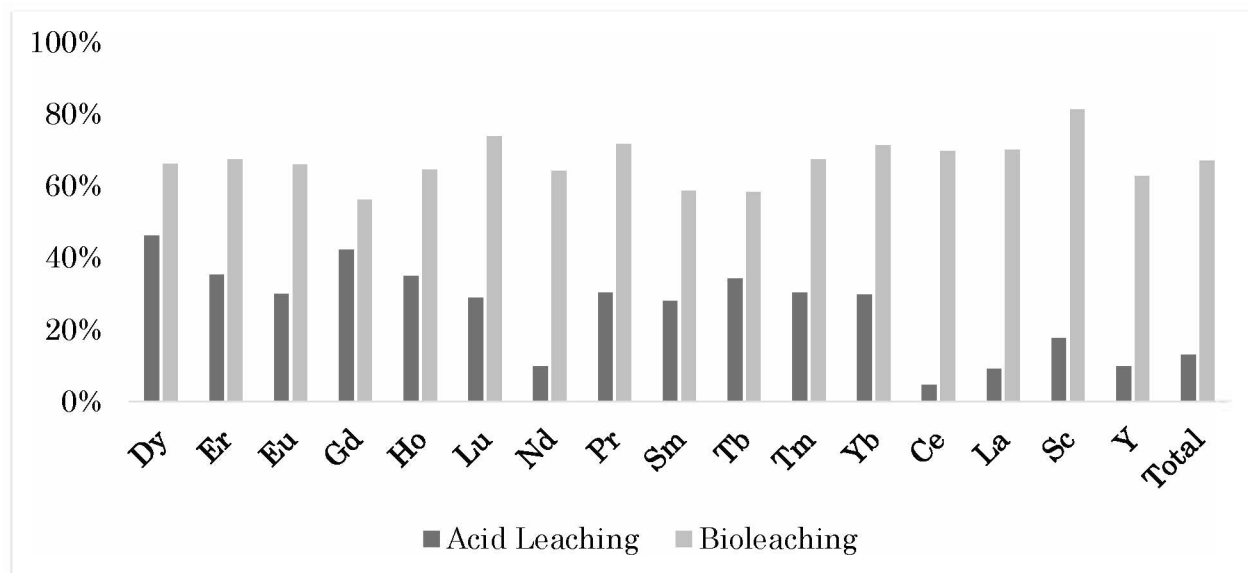
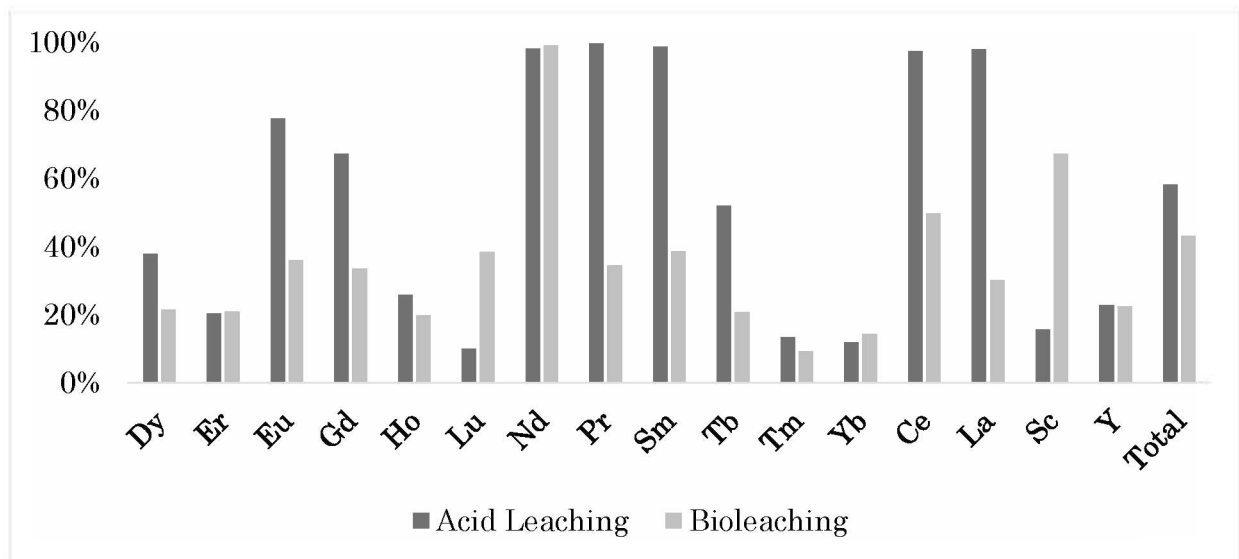**Figure 34. Comparison between recoveries of acid leaching and bioleaching for Healy composite sample.**
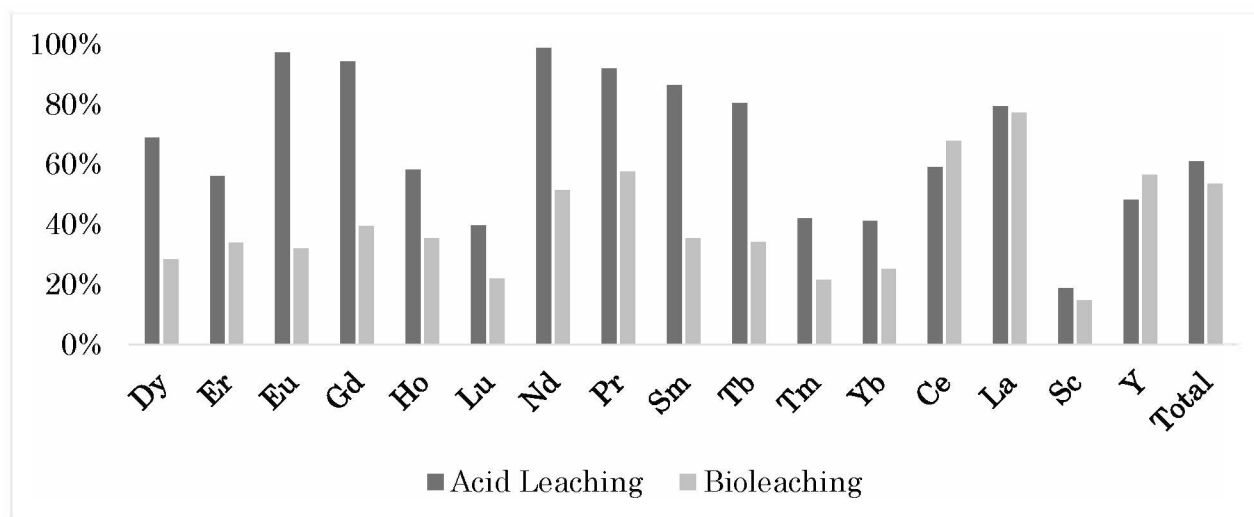
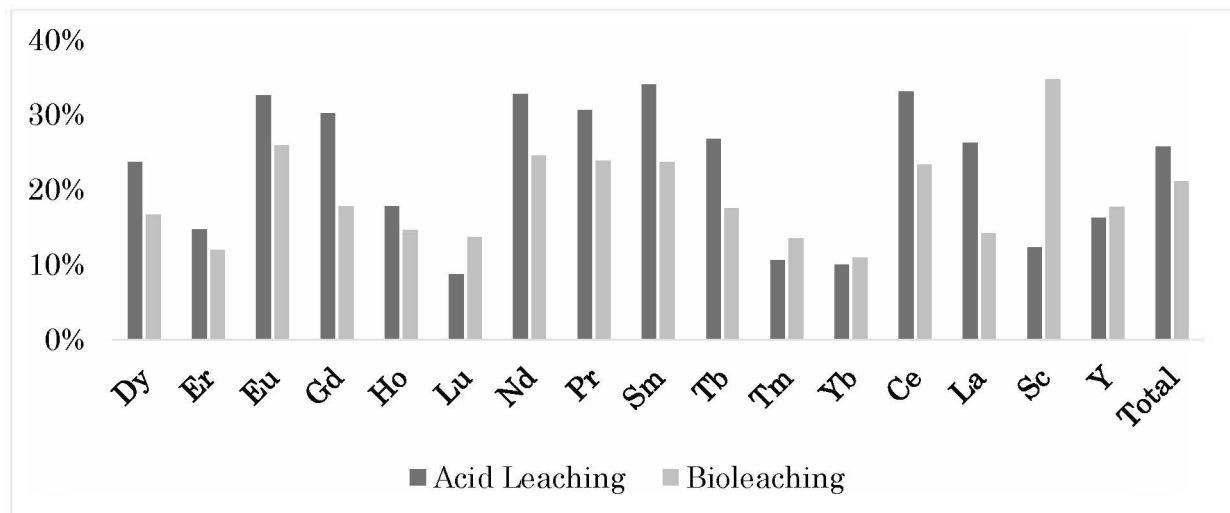**Figure 35. Comparison between recoveries of acid leaching and bioleaching for Healy 1.3 floats.**
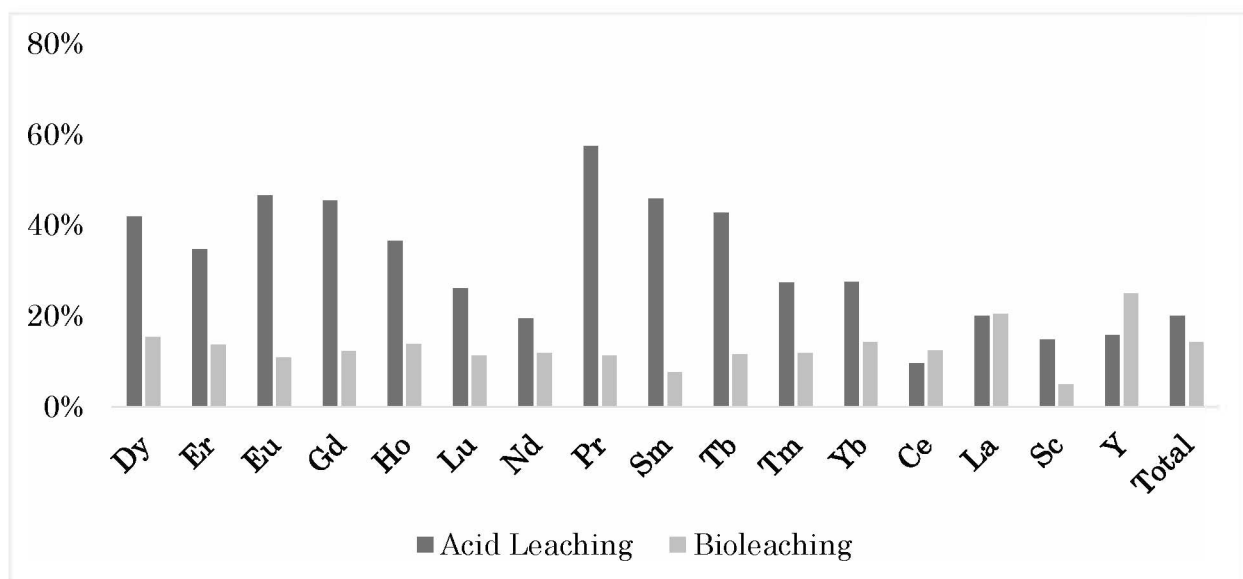
Figure 36. Comparison between recoveries of acid leaching and bioleaching for Healy 1.3 sinks.

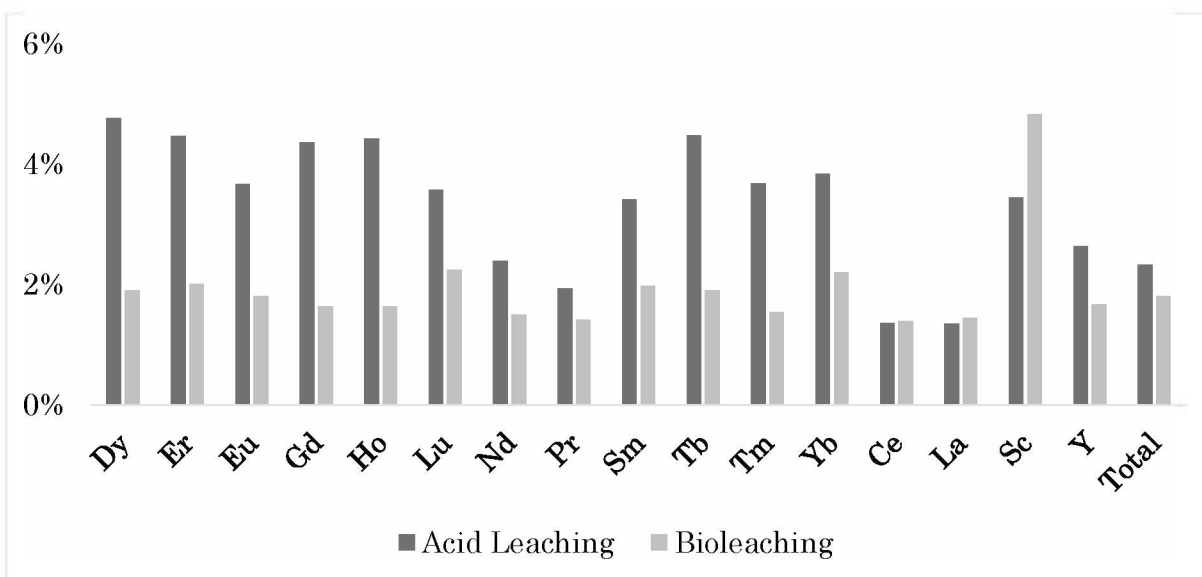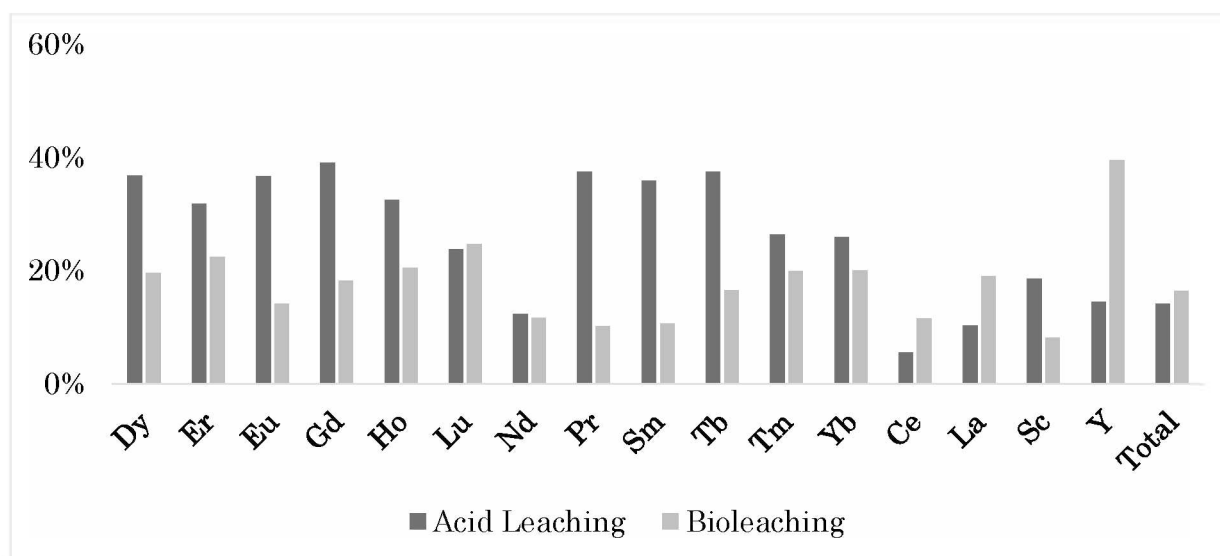**Figure 37. Comparison between recoveries of acid leaching and bioleaching for Healy 1.5 sinks.**

Bioleaching has proved to be an alternative method for the extraction of REEs from coal. Some REEs like scandium, yttrium, neodymium, and lutetium have better leachability through bioleaching in comparison to acid leaching. Also, Healy coal has a better response to bioleaching in comparison to Wishbone Hill coal.

CHAPTER 6 CONCLUSIONS

6.1 Conclusions

As the world moves towards green energy, the demand for REEs is going to increase because of their applications in these technologies. The world has a limited supply of REEs through conventional sources, with the Chinese monopoly in the international market. This domination leads to volatility in the international market, forcing other countries to look for alternative sources of REEs. The United States is the leader for the technological innovations and front runner for development of environmental-friendly technologies in the world. Due to its commitment to green energy use and to reduce its dependence on China for the supply of REEs, the US is actively trying to develop new methods for the extraction of REEs from unconventional sources, one of these sources being coal.

Coal from the two mines in Alaska, Wishbone Hill and Healy, was used to perform the experiments using a novel approach of bioleaching. Previous studies had shown that these coals contain as high as 524 ppm of REEs, which is higher than the world average content of REEs in coal. This makes them a perfect candidate for performing further research involving bioleaching.

S. oneidensis MR-1, a microbe, was used for conducting the redox cycles that are able to leach out the REEs from coal. This is performed by the microbe by including the electrons present in the iron-content of coal in its metabolism process, thereby leading to the reduction of the iron compounds in coal. These iron compounds contain the REEs trapped within them, and hence when it is reduced it leads to the dissolution of the REEs in the solution. The sample of *S. oneidensis* MR-1 was obtained from University of Alaska Anchorage and a growth media was determined, which maximizes the recovery of REEs. The experiments were performed in various conditions including different sizes of coal, incubation temperatures, initial bacterial

concentration, and solids percentage. The bioleaching was also compared to the conventional acid leaching process as well.

Some of the salient conclusions that can be drawn from the analysis of these experiments are:

1) Significant difference in the recovery of the REEs was observed from the samples that were inoculated with *S. oneidensis* MR-1 in comparison to the ones that were not inoculated, indicating that the leaching of elements was due to the microbe. There was some leaching of REEs in the samples that did not have any bacteria, but that can be due to the prolonged exposure (25 days) in the media containing various organic and inorganic chemicals.

2) The experiments were also performed for ash to recover the REEs from it, but the recoveries were poor. Healy 1.3 floats had a recovery of 96.6% lanthanum, but under the same conditions for Healy 1.3 floats ash, the recovery merely was 2.5% (Appendix A). Similarly, for Wishbone Hill coal, scandium had a recovery of 51% for 1.3 floats, while for 1.3 floats ash, the recovery was down to 3.3%. Subsequent experiments were not performed on ash as the recoveries for all the REEs was miniscule and more preference was given to coal while changing the conditions.

3) For different solids percentage, the maximum total recovery of REEs for Wishbone Hill was obtained for 1.3 floats at 10% solids, while for Healy coal, it was obtained for 1.3 sinks at 5.4% solids. The optimum solids percentage (pulp density) for bioleaching of Wishbone Hill was determined to be at 10%, while for Healy it was 5.4%.

4) The bacterial volume was found to be directly linked to the recovery of REEs for both coals. The total recovery was the maximum for the highest bacterial volume which, from the experiments, was determined to be 500µL of bacteria.

5) The growth of the bacteria was linked to the incubation temperature and hence the temperature was changed within different set of experiments to determine the optimum temperature. For both coals, the maximum total recovery was obtained at 32°C, with the recovery falling when the temperature decreased to 30°C or increased to 34°C.

6) The maximum total recoveries for both coals were obtained at -14M+48M size range with the recoveries falling for finer particles. This proves that less grinding of coal is required to prepare it for bioleaching, hence reducing the preparation costs.

7) The comparison of bioleaching with acid leaching was one of the main aspect of this research. The comparison between them indicated that for Wishbone Hill coal, the recovery of neodymium, scandium, erbium, lutetium, tellurium, and ytterbium was more in certain conditions than in acid leaching. The same comparison for Healy coal indicated more recovery of all the REEs in the composite sample than in acid leaching while for other specific gravity fractions, more recovery was obtained for cerium, yttrium, lanthanum, and lutetium.

8) It can be concluded that the best condition for the recovery of REEs from Wishbone Hill coal is at 10% solids, 500µL of bacteria, 32°C incubation temperature, and -14M+48M size provided five redox cycles of five days each are run.

9) For Healy coal, the optimum condition for total recovery of REEs was determined to be 5.4% solids, 500µL of bacteria, 32°C incubation temperature, and -14M+48M as long as five redox cycles are completed.

6.2 Recommendations

From the experience of conducting experiments for this research, the following recommendations are advised for further research:

1) Although in most cases the recoveries from bioleaching are comparable to the recoveries obtained from acid leaching, bioleaching is a time-consuming process with one experiment taking 25 days for completion. Further research can be performed for reducing the time consumed in experiments. This would also make bioleaching more acceptable in the mineral processing and coal industry.

2) An in-depth economic analysis of the process is required for its application in the industry. The main concern with bioleaching is maintaining the sterility of the samples as in this research pure bacteria samples were used that can be easily contaminated by other microbes. Further research can be performed to develop a similar method using another microbe that is more resistant to other species.

3) Only a few of the factors that can affect bioleaching were tested for this research. More size ranges, temperature ranges, bacterial concentration, and solids percentage as well as the use of another growth media can also be tested in the future research.

REFERENCES

Aksu, Z., Yesim S., and Tulin, K. 1992. 'The biosorpnon of copperod by C. vulgaris and Z. ramigera', *Environmental Technology*, 13: 579-86.

Allegrini, E., Maresca, A., Olsson, M.E., Holtze, M.S., Boldrin, A., and Astrup, F.A. 2014. 'Quantification of the resource recovery potential of municipal solid waste incineration bottom ashes', *Waste Management*, 34: 1627-36.

ALS. 2016. "ME-MS 14L and ME-MS 14L REE super trace analysis of natural waters." In, edited by ALS Geochemistry, 1-2.

Ascencio, J.A., Mejia,Y., Liu, H.B., Angeles, C., and Canizal, G. 2003. 'Bioreduction Synthesis of Eu−Au Nanoparticles', *Langmuir*, 19: 5882-86.

Binnemans, K., Peter, TJ., Bart, B., Tom, V.G., and Yiannis, P. 2015. 'Towards zero-waste valorisation of rare-earth-containing industrial process residues: a critical review', *Journal of Cleaner Production*, 99: 17-38.

Binnemans, K., Peter, T.J., Bart, B., Tom, V.G., Yongxiang, Y., Allan, W., and Matthias, B. 2013. 'Recycling of rare earths: a critical review', *Journal of Cleaner Production*, 51: 1-22.

Borrok, D., Borrok, M.J., Fein, J.B., and Kiessling, L.L.. 2005. 'Link between chemotactic response to Ni2+ and its adsorption onto the Escherichia coli cell surface', *Environ Sci Technol*, 39: 5227-33.

Bosecker, K. 1997. 'Bioleaching: metal solubilization by microorganisms', *FEMS Microbiology Reviews*, 20: 591-604.

Butler, C.S., Debieux, C.M., Dridge, E.J., Splatt, P. and Wright, M. 2012. 'Biomineralization of selenium by the selenate-respiring bacterium Thauera selenatis', *Biochem Soc Trans*, 40: 1239-43.

Chandwadkar, P., Misra, H.S., and Acharya, C. 2018. 'Uranium biomineralization induced by a metal tolerant Serratia strain under acid, alkaline and irradiated conditions', *Metallomics*, 10: 1078-88.

Cordier, D., and Hedrick, J.B. 2008. "Rare Earths: U.S. Geological Survey Minerals Yearbook." In, 1-60.

Cui, J., and Forssberg, E. 2003. 'Mechanical recycling of waste electric and electronic equipment: a review', *Journal of Hazardous Materials*, 99: 243-63.

Dai, S., Jiang, Y., Ward, C.R., Gu, L., Seredin, V.V., Liu, H., Zhou, D., Wang, X., Sun, Y., Zou, J., and Ren, D. 2012. 'Mineralogical and geochemical compositions of the coal in the Guanbanwusu Mine, Inner Mongolia, China: Further evidence for the existence of an Al (Ga and REE) ore deposit in the Jungar Coalfield', *International Journal of Coal Geology*, 98: 10-40.

Das, N., and Das, D. 2013. 'Recovery of rare earth metals through biosorption: An overview', *Journal of Rare Earths*, 31: 933-43.

Derby, H. A., and Hammer, B.W. 1931. *Bacteriology of butter. IV, Bacteriological studies on surface taint butter* (Agricultural Experiment Station, Iowa State College of Agriculture and Mechanic Arts: Ames, Iowa).

Du, X., and Graedel, T. E.. 2011. 'Uncovering the Global Life Cycles of the Rare Earth Elements', *Scientific Reports*, 1: 145.

Dutta, T., Kim, K.H., Uchimiya, M., Kwon, E.E., Jeon, B.H., Deep, A., and Yun, S.T. 2016. 'Global demand for rare earth resources and strategies for green mining', *Environmental Research*, 150: 182-90.

Esteve-Nunez, A., Rothermich, M., Sharma, M., and Lovley, D. 2005. 'Growth of Geobacter sulfurreducens under nutrient-limiting conditions in continuous culture', *Environ Microbiol*, 7: 641-8.

Fleming, C. A. 1992. 'Hydrometallurgy of precious metals recovery', *Hydrometallurgy*, 30: 127-62.

Fomin, E. S., Sabirzyanov, N. A., Anashkin, V. S., Yatsenko, S. P. and Diev, V.N. 2004. 'New approaches to processing of the muds of alumina production', *Trav. ICSOBA*, 31: 66-71.

Franklin, N. M., Stauber, J. L., Markich, S. J., and Lim, R. P.. 2000. 'pH-dependent toxicity of copper and uranium to a tropical freshwater alga (Chlorella sp.)', *Aquat Toxicol*, 48: 275-89.

Fredrickson, J. K., Romine, M. F., Beliaev, A. S., Auchtung, J. M., Driscoll, M. E., Gardner, T. S., Nealson, K. H., Osterman, A. L., Pinchuk, G., Reed, J. L., Rodionov, D. A., Rodrigues, J. L., Saffarini, D. A., Serres, M. H., Spormann, A. M., Zhulin, I. B., and Tiedje, J. M. 2008. 'Towards environmental systems biology of Shewanella', *Nat Rev Microbiol*, 6: 592-603.

Fuerstenau, D. W. 2013. 'Design and development of novel flotation reagents for the beneficiation of Mountain Pass rare-earth ore', *Minerals & Metallurgical Processing*, 30: 1-9.

Gambogi, J. 2018. "Mineral Commodity Summaries." In, 132-33. Reston, VA.

Ganguli, R., and Cook, D. R. 2018. 'Rare earths: A review of the landscape', *MRS Energy & Sustainability*, 5: E9.

Gupta, C. K., and Krishnamurthy, N. 1992. 'Extractive metallurgy of rare earths', *International Materials Reviews*, 37: 197-248.

Gupta, C. K., and Krishnamurthy, N. 2004. "Extractive Metallurgy of Rare Earths." In. London: CRC Press.

Gupta, T. 2016. 'Recovery of Rare Earth Elements from Alaskan Coal and Coal Combustion Products', University of Alaska, Fairbanks.

Habashi, F. 1985. 'The recovery of the lanthanides from phosphate rock', *Journal of Chemical Technology and Biotechnology. Chemical Technology*, 35: 5-14.

Haque, N., Hughes, A., Lim, S., and Vernon, C. 2014. 'Rare Earth Elements: Overview of Mining, Mineralogy, Uses, Sustainability and Environmental Impact', *Resources*, 3: 614.

Haxel, G.B., Hedrick, J.B., Orris, G.J., Stauffer, P.H., and Ii, J.W.H. 2002. "Rare earth elements: critical resources for high technology." In *Fact Sheet*.

He, Q., and Sanford, R.A. 2003. 'Characterization of Fe(III) Reduction by Chlororespiring Anaeromxyobacter dehalogenans', *Applied and Environmental Microbiology*, 69: 2712-18.

Hedrick, J.B. 1998. "Rare-earth metals." In *USGS Mineral Information, 2001*.

Horiike, T., and Yamashita, M. 2015. 'A new fungal isolate, Penidiella sp. strain T9, accumulates the rare earth element dysprosium', *Appl Environ Microbiol*, 81: 3062-8.

Hower, J.C., Ruppert, L.F., and Eble, C.F. 1999. 'Lanthanide, yttrium, and zirconium anomalies in the Fire Clay coal bed, Eastern Kentucky', *International Journal of Coal Geology*, 39: 141-53.

Hughes, I. D., Däne, M., Ernst, A., Hergert, W., Lüders, M., Poulter, J., Staunton, J. B., Svane, A., Szotek, Z., and Temmerman, W. M. 2007. 'Lanthanide contraction and magnetism in the heavy rare earth elements', *Nature*, 446: 650.

Humphries, M. 2011. "Rare earth elements: The global supply chain." In *Congressional Research Service*, 2-7.

Jiang, M., Ohnuki, T., Kozai, N., Tanaka, K., Suzuki, Y., Sakamoto, F., Kamiishi, E., and Utsunomiya, S. 2010. 'Biological nano-mineralization of Ce phosphate by Saccharomyces cerevisiae', *Chemical Geology*, 277: 61-69.

Jiang, M., Ohnuki, T., and Utsunomiya, S. 2018. 'Biomineralization of Middle Rare Earth Element Samarium in Yeast and Bacteria Systems', *Geomicrobiology Journal*, 35: 375-84.

Jin, H., Park, D.M., Gupta, M., Brewer, A.W., Ho,L., Singer, S.L., Bourcier, W.L., Woods, S., Reed, D.W., Lammers, L.N., Sutherland, J.W., and Jiao, Y. 2017. 'Techno-economic Assessment for Integrating Biosorption into Rare Earth Recovery Process', *ACS Sustainable Chemistry & Engineering*, 5: 10148-55.

Jones, P.T. 2012. 'Closing Material Loops', *JOM*, 64: 743-44.

Jones, P.T., Geysen, D., Tielemans, Y., Passel, S.V., Pontikes, Y., Blanpain, B., Quaghebeur, M., and Hoekstra, N. 2013. 'Enhanced Landfill Mining in view of multiple resource recovery: a critical review', *Journal of Cleaner Production*, 55: 45-55.

Jordens, A., Cheng, Y.P., and Waters, K.E. 2013. 'A review of the beneficiation of rare earth element bearing minerals', *Minerals Engineering*, 41: 97-114.

Kanazawa, Y., and Kamitani, M. 2006. 'Rare earth minerals and resources in the world', *Journal of Alloys and Compounds*, 408-412: 1339-43.

Ketris, M. P., and Yudovich, Y.E. 2009. 'Estimations of Clarkes for Carbonaceous biolithes: World averages for trace element contents in black shales and coals', *International Journal of Coal Geology*, 78: 135-48.

Kim, R., Cho, H., Han, K., Kim, K., and Mun, M. 2016. 'Optimization of Acid Leaching of Rare-Earth Elements from Mongolian Apatite-Based Ore', *Minerals*, 6: 63.

Korenevsky, A.A., Sorokin, V.V., and Karavaiko, G. 1998. 'Biosorption of Rare Earth Elements', *Mineral Processing and Extractive Metallurgy Review*, 19: 341-53.

Kumar, B.N., Surampally, R., Mannepalli L. K., and Reddy, B.R. 2011. 'Solid–liquid extraction of terbium from phosphoric acid solutions using solvent-impregnated resin containing TOPS 99', *Journal of Chemical Technology & Biotechnology*, 86: 562-69.

LePain, D.L., Clough, J.G., Gillis, R.J., Herriott, T.M., and Wartes, M.A. 2011. "Energy Resources FY11 project descriptions, in DGGS Staff, Alaska Division of Geological & Geophysical Surveys Annual Report: Alaska Division of Geological & Geophysical Surveys Annual Report 2010-A." In, 34-41.

Li, C., Zhuang, Z., Huang, F., Wu, Z,. Hong, Y., and Lin, Z. 2013. 'Recycling Rare Earth Elements from Industrial Wastewater with Flowerlike Nano-Mg(OH)2', *ACS Applied Materials & Interfaces*, 5: 9719-25.

Lies, D.P., Hernandez, M.E., Kappler, A., Mielke, R.E., Gralnick, J.A., and Newman, D.K. 2005a. '*Shewanella oneidensis* MR-1 Uses Overlapping Pathways for Iron Reduction at a Distance and by Direct Contact under Conditions Relevant for Biofilms', *Applied and Environmental Microbiology*, 71: 4414-26.

Lies, D.P., Hernandez, M.E., Kappler, A., Mielke, R.E., Gralnick, J.A., and Newman, D.K. 2005b. '*Shewanella oneidensis* MR-1 Uses Overlapping Pathways for Iron Reduction at a Distance and by Direct Contact under Conditions Relevant for Biofilms', *Applied and Environmental Microbiology*, 71: 4414.

Logomerac, V. G. 1971. 'Distribution of rare-earth and minor elements in some bauxite and red mud produced', *Proceedings of the Second International Symposium of ICSOBA*, 3: 383-93.

Long, K.R., Van Gosen, B.S., Foley, N.K., and Cordier, D. 2010. "The principal rare earth elements deposits of the United States-A summary of domestic deposits and a global perspective." In *Scientific Investigations Report*. Reston, VA.

Lovley, D. R., Phillips, E. J., and Lonergan, D. J. 1989. 'Hydrogen and Formate Oxidation Coupled to Dissimilatory Reduction of Iron or Manganese by Alteromonas putrefaciens', *Appl Environ Microbiol*, 55: 700-6.

Marsili, E., Baron, D.B., Shikhare, I.D., Coursolle, D., Gralnick, J.A., and Bond, D.R. 2008. 'Shewanella secretes flavins that mediate extracellular electron transfer', *Proceedings of the National Academy of Sciences*, 105: 3968-73.

Meyer, T. E., Tsapin, A. I., Vandenberghe, I., de Smet, L., Frishman, D., Nealson, K. H., Cusanovich, M. A. and van Beeumen, J. J. 2004. 'Identification of 42 possible cytochrome C genes in the *Shewanella oneidensis* genome and characterization of six soluble cytochromes', *Omics*, 8: 57-77.

Morf, L.S., Gloor, R., Haag, O., Haupt, M., Skutan, S., Di Lorenzo, F., and Böni, D. 2013. 'Precious metals and rare earth elements in municipal solid waste – Sources and fate in a Swiss incineration plant', *Waste Management*, 33: 634-44.

Müller, T., and Friedrich, B. 2006. 'Development of a recycling process for nickel-metal hydride batteries', *Journal of Power Sources*, 158: 1498-509.

Myers, J. M., and Myers, C. R.. 2001. 'Role for outer membrane cytochromes OmcA and OmcB of Shewanella putrefaciens MR-1 in reduction of manganese dioxide', *Appl Environ Microbiol*, 67: 260-9.

Nancharaiah, Y. V., Mohan, S. V., and Lens, P. N. L.. 2016. 'Biological and Bioelectrochemical Recovery of Critical and Scarce Metals', *Trends Biotechnol*, 34: 137-55.

Ober, J.A. 2017. "Mineral commodity summaries 2017." In *Mineral Commodity Summaries*, 202. Reston, VA.

Ochsenkühn-Petropoulou, M.T., Konstantinos, S.H., L.N. Mendrinos, and C.E. Salmas. 2002. 'Pilot-Plant Investigation of the Leaching Process for the Recovery of Scandium from Red Mud', *Industrial & Engineering Chemistry Research*, 41: 5794-801.

Palmieri, M. C., B. Volesky, and O. Garcia Jr. 2002. 'Biosorption of lanthanum using Sargassum fluitans in batch system', *Hydrometallurgy*, 67: 31-36.

Park, J.H, K.H. Lee, T.Y. Kim, and S.Y. Lee. 2007. 'Metabolic engineering of Escherichia coli for the production of l-valine based on transcriptome analysis and in silico gene knockout simulation', *Proceedings of the National Academy of Sciences*, 104: 7797-802.

Pavel, C.C., R. Lacal-Arántegui, A. Marmier, D. Schüler, E. Tzimas, M. Buchert, W. Jenseit, and D. Blagoeva. 2017. 'Substitution strategies for reducing the use of rare earths in wind turbines', *Resources Policy*, 52: 349-57.

Price, N. D., J. L. Reed, and B. O. Palsson. 2004. 'Genome-scale models of microbial cells: evaluating the consequences of constraints', *Nat Rev Microbiol*, 2: 886-97.

Qu, Y., and B. Lian. 2013. 'Bioleaching of rare earth and radioactive elements from red mud using Penicillium tricolor RM-10', *Bioresource Technology*, 136: 16-23.

Reck, B.K., and T. E. Graedel. 2012. 'Challenges in Metal Recycling', *Science*, 337: 690-95.

Reed, J. L., T. D. Vo, C. H. Schilling, and B. O. Palsson. 2003. 'An expanded genome-scale model of Escherichia coli K-12 (iJR904 GSM/GPR)', *Genome Biol*, 4: R54.

Seredin, V. V. 1996. 'Rare earth element-bearing coals from the Russian Far East deposits', *International Journal of Coal Geology*, 30: 101-29.

Seredin, V.V., and S. Dai. 2012. 'Coal deposits as potential alternative sources for lanthanides and yttrium', *International Journal of Coal Geology*, 94: 67-93.

Sethurajan, M., E. D. van Hullebusch, and Y. V. Nancharaiah. 2018. 'Biotechnology in the management and resource recovery from metal bearing solid wastes: Recent advances', *J Environ Manage*, 211: 138-53.

Sheng, L., and J. B. Fein. 2014. 'Uranium Reduction by *Shewanella oneidensis* MR-1 as a Function of NaHCO3 Concentration: Surface Complexation Control of Reduction Kinetics', *Environmental Science & Technology*, 48: 3768-75.

Sheng, L., J. Szymanowski, and J.B. Fein. 2011. 'The effects of uranium speciation on the rate of U(VI) reduction by *Shewanella oneidensis* MR-1', *Geochimica et Cosmochimica Acta*, 75: 3558-67.

Song, Q., Z. Wang, and J. Li. 2013. 'Sustainability evaluation of e-waste treatment based on emergy analysis and the LCA method: A case study of a trial project in Macau', *Ecological Indicators*, 30: 138-47.

Takaya, Y., K. Yasukawa, T. Kawasaki, K. Fujinaga, J. Ohta, Y. Usui, K. Nakamura, J. Kimura, Q. Chang, M. Hamada, G. Dodbiba, T. Nozaki, K. Iijima, T. Morisawa, T. Kuwahara, Y. Ishida, T. Ichimura, M. Kitazume, T. Fujita, and Y. Kato. 2018. 'The tremendous potential of deep-sea mud as a source of rare-earth elements', *Scientific Reports*, 8: 5763.

Tang, K., A. Ciftja, C. Van der Eijk, S. Wilson, and G. Tranell. 2012. *Recycling of the Rare Earth Oxides from Spent Rechargeable Batteries Using Waste Metallurgical Slags*.

Taylor, S.R., and S.M. McLennan. 1985. 'review-article', *Geological Magazine*, 122: 673-74.

Thormann, K.M., R.M. Saville, S. Shukla, D.A. Pelletier, and A.M. Spormann. 2004. 'Initial Phases of biofilm formation in *Shewanella oneidensis* MR-1', *Journal of Bacteriology*, 186: 8096-104.

Torma, A.E. 1977. "The role of Thiobacillus ferrooxidans in hydrometallurgical processes." In, 1-37. Berlin, Heidelberg: Springer Berlin Heidelberg.

U.S. Geological Survey, Mineral Commodity Summaries, Rare Earth, 1994-2018.

Vanengelen, M. R., E. Field, R. Gerlach, B. D. Lee, W. A. Apel, and B. M. Peyton. 2010. 'UO(2) 2+ speciation determines uranium toxicity and bioaccumulation in an environmental Pseudomonas sp. isolate', *Environ Toxicol Chem*, 29: 763-9.

Wagh, A.S., and W.R. Pinnock. 1987. 'Occurrence of scandium and rare earth elements in Jamaican bauxite waste', *Economic Geology*, 82: 757-61.

Welch, S. A., and W. J. Ullman. 1999. 'The effect of microbial glucose metabolism on bytownite feldspar dissolution rates between 5° and 35°C', *Geochimica et Cosmochimica Acta*, 63: 3247-59.

Wigginton, N.S. 2014. 'How bacteria manage to breathe on rust', *Science*, 344: 984-84.

Wübbeke, J., 2013. 'Rare earth elements in China: Policies and narratives of reinventing an industry', *Resources Policy*, 38: 384-94.

Xie, F., T. A. Zhang, D. Dreisinger, and F. Doyle. 2014. 'A critical review on solvent extraction of rare earths from aqueous solutions', *Minerals Engineering*, 56: 10-28.

Yardley, B. W. D. 1986. 'The continental crust: Its composition and evolution by S. R. Taylor and S. M. McClennan, Blackwell Scientific Publications, Oxford, 1985. No. of pages: 312 + XV pp. Price: £16.80 (soft covers)', *Geological Journal*, 21: 85-86.

Yessoufou, A., B.E. Ifon, F. Suanon, B. Dimon, Q. Sun, C. A. Dedjiho, D. Mama, and C.P. Yu. 2017. 'Rare earth and precious elements in the urban sewage sludge and lake surface sediments under anthropogenic influence in the Republic of Benin', *Environmental Monitoring and Assessment*, 189: 625.

Zhang, B., C. Liu, C. Li, and M. Jiang. 2014. 'A novel approach for recovery of rare earths and niobium from Bayan Obo tailings', *Minerals Engineering*, 65: 17-23.

Zhang, L., H. Dong, Y. Liu, L. Bian, X. Wang, Z. Zhou, and Y. Huang. 2018. 'Bioleaching of rare earth elements from bastnaesite-bearing rock by actinobacteria', *Chemical Geology*, 483: 544-57.

Zhang, W., M. Rezaee, A. Bhagavatula, Y. Li, J. Groppo, and R. Honaker. 2015. 'A Review of the Occurrence and Promising Recovery Methods of Rare Earth Elements from Coal and Coal By-Products', *International Journal of Coal Preparation and Utilization*, 35: 295-330.

Zhao, L., F. S. Zhang, and J. Zhang. 2008. 'Chemical properties of rare earth elements in typical medical waste incinerator ashes in China', *Journal of Hazardous Materials*, 158: 465-70.

Zhao, L., H. Dong, R. K. Kukkadapu, Q. Zeng, R. E. Edelmann, M.Pentrák, and A. Agrawal. 2015. 'Biological Redox Cycling of Iron in Nontronite and Its Potential Application in Nitrate Removal', *Environmental Science & Technology*, 49: 5493-501.

APPENDICES

Appendix A. Recovery of REEs from Healy coal, Wishbone Hill ash, and Healy ash.

	Healy Coal								
	1.3 floats			1.3 sinks			1.5 sinks		
	ppm	S	NS	ppm	S	NS	ppm	S	NS
Sc	3.4	0.0%	0.0%	4.6	0.0%	0.0%	9.0	14.4%	0.0%
Y	9.7	28.8%	10.1%	16.6	90.1%	18.3%	19.9	83.4%	22.5%
La	8.6	96.6%	35.3%	16.8	96.4%	23.6%	28.1	68.8%	17.3%
Ce	17.3	62.4%	22.5%	33.8	87.6%	15.6%	52.1	51.2%	12.3%
Pr	2.0	53.2%	18.9%	4.0	78.2%	13.4%	6.2	46.3%	10.7%
Nd	8.6	46.6%	15.8%	16.2	69.1%	11.6%	23.4	46.9%	10.8%
Sm	2.1	41.4%	15.7%	3.0	73.2%	12.2%	4.8	51.2%	10.5%
Eu	0.4	45.2%	15.6%	0.8	89.4%	14.4%	0.9	68.1%	16.2%
Gd	2.0	36.8%	11.4%	3.4	78.2%	13.0%	4.0	69.0%	18.1%
Tb	0.3	30.1%	10.6%	0.5	73.5%	13.5%	0.6	61.9%	16.5%
Dy	1.6	24.3%	8.6%	3.0	70.7%	13.8%	3.7	64.1%	16.9%
Ho	0.3	19.3%	6.3%	0.6	62.4%	12.6%	0.8	56.9%	15.4%
Er	0.9	19.7%	6.3%	1.6	62.2%	12.9%	2.1	62.0%	15.9%
Tm	0.1	16.0%	5.1%	0.3	59.2%	12.8%	0.3	53.8%	15.2%
Yb	0.8	19.3%	6.1%	0.6	80.4%	17.9%	2.0	68.6%	13.0%
Lu	0.1	23.1%	7.8%	0.2	93.3%	23.7%	0.3	85.6%	19.2%

	Wishbone Hill Ash											
	Composite			1.3 floats			1.3 sinks			1.5 sinks		
	ppm	S	NS	ppm	S	NS	ppm	S	NS	ppm	S	NS
Sc	13.1	0.7%	0.8%	7.1	3.3%	1.8%	11.9	5.7%	4.4%	17.0	1.0%	0.5%
Y	21.1	0.3%	0.0%	13.0	1.8%	0.5%	19.4	1.7%	0.4%	26.4	0.4%	0.1%
La	16.4	0.1%	0.0%	4.8	0.2%	0.1%	15.3	0.2%	0.0%	23.7	0.1%	0.1%
Ce	35.3	0.1%	0.0%	10.6	0.1%	0.0%	31.9	0.8%	0.0%	51.1	0.3%	0.1%
Pr	4.2	0.3%	0.0%	1.3	0.1%	0.0%	3.8	0.2%	0.0%	6.1	0.2%	0.1%
Nd	17.5	0.1%	0.1%	5.7	0.2%	0.1%	15.8	0.4%	0.0%	25.1	0.1%	0.1%
Sm	4.1	0.1%	0.0%	1.4	0.4%	0.1%	3.7	0.7%	0.0%	5.8	0.1%	0.1%
Eu	0.9	0.1%	0.0%	0.3	0.4%	0.2%	0.8	0.7%	0.0%	1.2	0.2%	0.1%
Gd	4.0	0.1%	0.0%	1.7	0.5%	0.2%	3.6	1.4%	0.0%	5.4	0.2%	0.1%
Tb	0.6	0.1%	0.0%	0.3	0.5%	0.0%	0.6	1.1%	0.0%	0.9	0.3%	0.2%
Dy	3.8	0.1%	0.0%	2.1	0.8%	0.2%	3.4	1.0%	0.1%	4.9	0.3%	0.1%
Ho	0.8	0.2%	0.0%	0.5	1.0%	0.2%	0.7	1.2%	0.0%	1.0	0.4%	0.1%
Er	2.2	0.1%	0.0%	1.4	1.4%	0.2%	2.1	1.0%	0.1%	2.7	0.4%	0.1%
Tm	0.4	0.1%	0.0%	0.2	1.3%	0.0%	0.4	1.3%	0.0%	0.4	0.4%	0.1%
Yb	2.2	0.3%	0.0%	1.5	1.4%	0.2%	2.2	2.0%	0.2%	2.5	0.5%	0.2%
Lu	0.3	0.5%	0.0%	0.2	1.5%	0.2%	0.3	2.0%	0.0%	0.4	0.6%	0.1%

	Healy Ash											
	Composite			1.3 floats			1.3 sinks			1.5 sinks		
	ppm	S	NS	ppm	S	NS	ppm	S	NS	ppm	S	NS
Sc	4.9	3.4%	2.8%	3.4	13.4%	10.1%	4.6	6.3%	3.5%	9.0	9.1%	9.1%
Y	14.6	0.1%	0.0%	9.7	3.8%	0.1%	16.6	3.0%	1.1%	19.9	4.0%	1.7%
La	15.6	0.0%	0.0%	8.6	2.5%	0.1%	16.8	0.8%	0.9%	28.1	0.8%	0.2%
Ce	30.7	0.0%	0.0%	17.3	1.5%	0.1%	33.8	0.5%	0.3%	52.1	0.7%	0.2%
Pr	3.6	0.0%	0.0%	2.0	1.2%	0.0%	4.0	0.5%	0.3%	6.2	0.7%	0.1%
Nd	14.5	0.0%	0.0%	8.6	1.4%	0.0%	16.2	0.5%	0.5%	23.4	0.9%	0.2%
Sm	3.3	0.0%	0.0%	2.1	0.9%	0.0%	3.0	0.4%	0.2%	4.8	0.7%	0.3%
Eu	0.7	0.0%	0.0%	0.4	1.3%	0.0%	0.8	0.6%	1.0%	0.9	1.2%	0.5%
Gd	3.0	0.1%	0.0%	2.0	1.8%	0.0%	3.4	0.9%	0.4%	4.0	2.0%	0.6%
Tb	0.5	0.0%	0.0%	0.3	0.0%	0.0%	0.5	0.4%	0.3%	0.6	1.6%	0.3%
Dy	2.6	0.2%	0.0%	1.6	1.5%	0.0%	3.0	0.9%	0.4%	3.7	2.0%	0.5%
Ho	0.5	0.0%	0.0%	0.3	1.7%	0.0%	0.6	1.1%	0.4%	0.8	2.7%	0.7%
Er	1.4	0.2%	0.0%	0.9	2.3%	0.0%	1.6	1.5%	0.6%	2.1	3.4%	1.4%
Tm	0.2	0.0%	0.0%	0.1	2.7%	0.0%	0.3	1.0%	0.0%	0.3	2.8%	1.8%
Yb	1.4	0.3%	0.0%	0.8	2.0%	0.0%	0.6	1.5%	0.6%	2.0	3.9%	2.1%
Lu	0.2	0.4%	0.0%	0.1	1.0%	0.0%	0.2	2.5%	0.0%	0.3	4.9%	3.5%

Appendix B. Comparison between the total REE content and ash percentage for Wishbone Hill and Healy coal.

Specific Gravity		Wishbone Hill		Healy	
Sink	Float	% ash	Total REE	% ash	Total REE
1.2	1.3	6.09%	52.2	11.89%	58.2
1.3	1.5	28.81%	115.9	21.09%	107.7
1.5	2.5	73.23%	174.8	56.09%	158.2

Appendix C. Concentrations of arsenic, cadmium, and lead in Wishbone Hill and Healy coal

	As	Cd	Pb	Total
	ppm	ppm	ppm	ppm
WH Composite	4.62	0.364	12.05	17.03
WH 1.3F	0.96	0.123	5.97	7.05
WH 1.3S	0.87	0.25	8.45	9.57
WH 1.5S	6.37	0.706	24.7	31.78
H Composite	0.94	0.526	2	3.47
H 1.3F	0.28	0.217	1.38	1.88
H 1.3S	0.67	0.481	2.16	3.31
H 1.5S	1.45	0.434	0.78	2.26