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Evaluation of bioleaching process for the simultaneous reduction of metals and total coliform from sewage sludge

Safika Akter
Ryerson University

EVALUATION OF BIOLEACHING PROCESS FOR THE SIMULTANEOUS REDUCTION OF METALS AND TOTAL COLIFORM FROM SEWAGE SLUDGE

by

Safika Akter

(MSc. in Sanitary Engineering, UNESCO-IHE Institute for Water Education, the Netherlands, 2004)

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EVALUATION OF BIOLEACHING PROCESS FOR THE SIMULTANEOUS REDUCTION OF METALS AND TOTAL COLIFORM FROM SEWAGE SLUDGE

M. Eng., Civil Engineering, 2009

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ABSTRACT

Bioleaching has been proven to be a promising technology for removing heavy metals from the sewage sludge over many years. The main objective of this research is to evaluate the bioleaching process for the simultaneous reduction of three heavy metals – copper(Cu), cadmium(Cd), zinc(Zn) and total coliform from the sewage sludge of the Ashbridges Bay Treatment Plant (ABTP). Bioleaching was carried out with adapted activated sludge containing high concentration of iron oxidizing bacteria T. ferrooxidans using ferrous sulphate as a substrate without adjusting the sludge initial pH to about 4 with acid. The results demonstrated that simultaneous metal removal efficiencies of Cu, Zn were 70% and 74% for Cd respectively after 10 days of bioleaching. The final pH and ORP were found 2.44 and 533 respectively. After this research, it was also observed that the process of bioleaching by T. ferrooxidans is very efficient for the reduction of total coliform from the sludge. This process allows a considerable reduction in total coliform (3 to 4 log removal) for the activated sludge examined over a 10 day period.

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List of Abbreviations

ABTP	Ashbridges Bay Treatment Plant
MOE	Ministry of Environment
OMAFRA	Ontario Ministry of Agriculture, Food, and Rural Affairs
ORP	Oxidation/Reduction Potential
TS	Total Solid
CSTR	Continuously stirred tank reactor
AA	Atomic-Absorption Spectroscopy

1. Introduction

1.1 General Review

Sewage sludge is defined by Harper-Collins Dictionary of Environmental Science as "a semi-solid mixture of bacteria, virus-laden organic matter, toxic metals, synthetic organic chemicals, and settled solids removed from domestic and industrial waste at sewage treatment plants."(www.sewagesludge.com). Sludge originates from the process of treatment of waste water. Due to the physical-chemical processes involved in the treatment, the sludge tends to concentrate heavy metals and poorly biodegradable trace organic compounds as well as potentially pathogenic organisms (viruses, bacteria etc) present in waste waters. During sewage treatment, about 50–80% of the total quantity of heavy metals present in sewage gets fixed into the sludge by various physicochemical and biological interactions (Lester et al., 1983b). Sludge is, however, rich in nutrients such as nitrogen and phosphorous and contains valuable organic matter that is useful when soils are depleted or subject to erosion.

Wastewater treatment plants generate a huge amount of sewage sludge every year. In recent years, the quantity of the total sludge generated all over the world has increased dramatically, and this trend is expected to increase in future years. Now the disposal of sludge is a serious environmental concern. Traditionally sludge has been disposed of by land filling, incinerating, composting, or, prior to 1988, dumping into oceans. An alternative method of sludge disposal was introduced in the mid 1970's known as the land application of sludge (Crittenden, 2002). Land application is the most commonly used method around the world and is being considered as one of the most economical methods of sludge disposal (Metcalf and Eddy, 2003). The land application of sludge is more economical than incineration, while preventing air pollution and at the same time promoting the recycling of valuable resources. But the presence of pathogens and high concentrations of heavy metals in sewage sludge constitutes a major constraint to the use of sludge as agricultural fertilizer. Epidemiological studies conducted to sludge as fertilizer have shown that risk of infection is associated with the presence of pathogenic bacteria and helminthic worms (Alderslade, 1981; Hays, 1977; Yeager, 1980). As well, trace metal assimilation by plants and subsequent bioaccumulation in the food chain can occur through the agricultural use of sludge (Lester et al., 1983; Tyler et al., 1989). A safe use of sewage sludge as a fertilizer requires sludge with low heavy metal content. Several chemical processes for metal removal have been proposed over the years. But the practical application of the chemical processes is still limited due to the requirement of large amount of chemicals, the high operating cost, the operational difficulties and the secondary pollution problems associated with them (Blais et al., 2005). Therefore, the research interest was shifted towards bioleaching process that has been reported to be an efficient and economical method for removal of heavy metals from the sludge (Wong et al., 2004). The bioleaching process aims at especially the solubilization of heavy metals but it also causes a reduction of pathogenic organisms. Thus, ideally the sludge coming out from a bioleaching system would present reduced amounts of both toxic metals and pathogenic organisms (Lombardi and Garcia, 1999). A joint process which concurrently removes metals and destroys pathogens to levels compatible with agricultural use would be desirable. Over the years, bioleaching process using iron-oxidizing microorganisms and sulphur-oxidizing microorganisms has been reported to be an efficient and economical method for removal of heavy metals from the sludge. In iron-oxidizing process ferrous sulphate is used as an energy source that has less risk of soil re-acidification.

Every year about 120,000 tonnes of sewage biosolids (one third of provincial production) are spread on 5,000 to 6,000 acres of farmland in Ontario. Toronto plans to spread half of the sludge from the city's largest plant at Ashbridge's Bay - about 25,000 dry tonnes per year. The intent of the Ashbridges Bay Treatment Plant (ABTP) biosolids management program is to use approximately half of the biosolids generated for agricultural land application. An average of 66,400 wet tonnes per year was applied to agricultural land during the 1998 to 2001 period. In the year 2008, 21582 wet tonnes of biosolids sent to agricultural land application.

According to the previous research (Nie,2003), the concentration of three metals Cu, Cd and Zn of ABTP exceeded the recommended limit for agricultural use most frequently and the average amount of metals found in digested sludge at ABTP, compared to Ontario Ministry of Agriculture, Food, and Rural Affairs (OMAFRA) guidelines is shown in Appendix A in Table A1. As the half of the sludge produced in ABTP applied to agricultural land, previous research was to remove heavy metals from the sludge by bioleaching process which involved the incubation of adapted sludge using fresh raw digested sludge and activated sludge of ABTP and was tested in a continuously stirred tank reactor (CSTR) in combination with a series of jar tests. The presence of pathogens or indicator bacteria (total coliforms, fecal coliforms, etc.) in sewage sludge is also a concern as heavy metals as they are constraints for the land application of sludge. In the present study bioleaching was carried out with Activated sludge of ABTP using adapted iron-oxidizing microorganisms by using ferrous sulphate (FS) as an energy source without adjusting the initial pH with acid to examine the heavy metal removal as well as reduction of total coliform. According to the data provided by city of Toronto (shown in Tabl2, Appendix 1) and considering the historical record, the three heavy metals Cu, Cd and Zn were used as a monitored element in this research.

1.2 Objectives

The main objective of this research is to evaluate the bioleaching process for the simultaneous reduction of heavy metals and total coliforms from the sewage sludge of ABTP. To achieve this objective first the sludge adaptation was carried out. After that with the adapted sludge, the bioleaching experiment was performed with iron oxidizing bacteria T. ferrooxidans in shake flasks using ferrous sulphate as a substrate. To investigate the simultaneous reduction of heavy metals and total coliform from the sludge during bioleaching process heavy metal measurements for three problematic metals Cu, Cd, Zn and total coliform tests were done. Furthermore comprehensive literature review of bioleaching process, microorganisms, recent research and studies regarding bioleaching process was done in this research.

2. Literature Review

2.1 Bioleaching

Bioleaching is defined as "the solubilization of metals from solid substrates either directly by the metabolism of leaching bacteria or indirectly by the products of metabolism" (Rulkens et al., 1995). Now-a-days bioleaching is gaining importance as a low cost environment friendly process for the treatment of the contaminated sewage sludge, solid waste and other industrial wastes (Krebs et al., 1997).

2.2 Bioleaching Mechanisms

The process of Bioleaching, also known as bacterial leaching, is used to extract specific metals from their ores through the use of bacteria. It was found that the metal sulphides present in coal, in a series of oxidation reaction, could be oxidized and solubilized to the corresponding metal sulphates and sulphuric acid by bacteria. The dissolution of metal sulphides occurs by two mechanisms (Jensen and Webb, 1995): direct and indirect mechanisms. In the direct mechanism, the T. ferrooxidans adhere to the sulphide surface and solubilize the metal according to following reactions, using CuS, ZnS and CdS as examples:

$$CuS + 2O_2 \rightarrow Cu^{2+} + SO_4^{2} \quad (1)$$

$$ZnS + 2O_2 \rightarrow Zn^{2+} + SO_4^{2-} \quad (2)$$

$$CdS + 2O_2 \rightarrow Cd^{2+} + SO_4^{2--} \quad (3)$$

In the indirect mechanism, T. ferrooxidans have a very active oxidation function. They make a high contribution to oxidize the ferrous ion (Fe^{++}) to become ferric ion (Fe^{+++}) in a microbial reaction. The ferric ion then solubilizes the metal sulphides into soluble Cu^{++}, Zn^{++}, and Cd^{++} chemical reactions according to the following principle formulas:

$$2Fe^{3+} + CuS \rightarrow Cu^{2+} + S + 2Fe^{2+} \quad (4)$$

$$2Fe^{3+} + ZnS \rightarrow Zn^{2+} + S + 2Fe^{2+-} \quad (5)$$

$$2Fe^{3+} + CdS \rightarrow Cd^{2+} + S + 2Fe^{2+} \quad (6)$$

The relative contribution of the two leaching mechanisms depends on the type of sulphide mineral, the condition of ferric iron and on the operating conditions.

In sulphur based bioleaching process, the dissolution of metal sulphides takes place by direct and indirect mechanisms (Suzuki, 2001). In direct bacterial leaching, the bacteria are in direct contact with the metallic sulphide in the sludge. Metal sulphide is directly oxidized by the T. thiooxidans into soluble metal sulphate according to the following reaction:

$$MeS + 2O_2 \rightarrow MeSO_4 \quad (7)$$

where Me is the metal.

Generally, the metallic sulphides such as NiS, CuS, ZnS etc. present in the sewage sludge can be solubilized by the above mechanism. In indirect bacterial leaching, the elemental sulphur or reduced sulphur compounds in the sludge are oxidized by sulphur-oxidizing bacteria into sulphuric acid which reduces the pH of the sludge medium, thereby enhancing the solubilization of the metal.

$$S° + H_2O + 1.5O_2 \rightarrow H_2SO_4 \qquad (8)$$

$$H_2SO_4 + sludge - Me \rightarrow sludge - 2H + MeSO_4 \qquad (9)$$

where Me is a bivalent metal.

In reaction (8) thiobacillus take active part, whereas reaction (9) takes place chemically without any involvement of bacteria. In indirect leaching, bacteria accelerate the oxidation of elemental sulphur, which otherwise takes place very slowly in the absence of bacteria. Bioleaching mechanisms are shown in Figure 2.1:

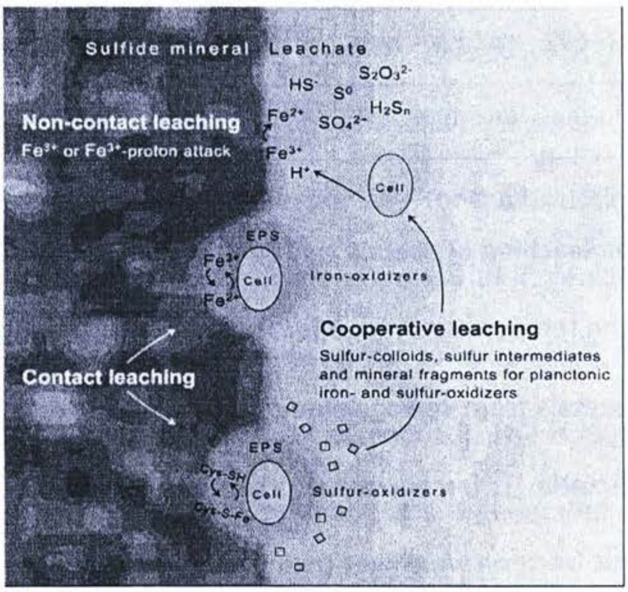


Figure 2.1: bioleaching mechanisms

(Source: http://wiki.biomine.skelleftea.se/wiki/index.php/Bioleaching_reactions)

2.3 Microorganisms

Different types of microorganisms play an important role in the bioleaching process. The maximum leaching occurs when conditions are optimum for bacterial growth and metal solubilisation. The Gram-negative thiobacilli are a group of organisms with physiological and morphological similarity which are used in bioleaching process and grow by oxidizing reduced sulphur compounds (Laskin and Lechevalier, 1973). Table 2.1 shows the characteristics of selected Thiobacillus (Prescott et al., 1999):

Table 2.1 Characteristics of selected Thiobacillus

Species	Inorganic Substrates	Terminal Electron Acceptor	Cell Type
T. ferrooxidans	$S_2O_3^{=}$, Fe^{++}	O_2	Gram-negative rods, polar flagella
T. thiooxidans	S^0, $S_2O_3^{=}$	O_2	Gram-negative rods, polar flagella
T. thioparus	$S_2O_3^{=}$	O_2	Gram-negative rods, polar flagella

Source: (Nie,2003)

Generally, the microorganisms exploited for bioleaching of metals can be classified broadly as mesophiles and thermophiles on the basis of the temperature range for their growth. The most dominant mesophiles used in bioleaching of metals from sewage sludge are sulphur-oxidizing bacteria (T. thiooxidans) and iron-oxidizing bacteria (T. ferrooxidans) (Villar and Garcia, 2003; Wong et al., 2004). These are chemolithotrophic bacteria which get their energy by oxidation of ferrous iron or reduced sulphur compounds. The optimum temperature for growth of T. ferrooxidans is around 33°C, although it can grow at any temperature in the range 20–40°C. The growth occurs at a pH in the range 1.0–4.5 with an optimum value between 2.0 and 2.3

(Ruamsap , et al., 2003; Mousavi et al., 2006). At a higher temperature, Archeans are the dominant species in the bioleaching environments. Sulfobacillus thermosulfidoxidans and other closely related species are moderate thermophiles which permit the use of higher temperature for faster bioleaching rate. The extreme thermophiles which grow at 70° C and use sulphur or thiosulfate as an energy source mainly include genus Sulfolobous viz. S. ambivalens (Kletzin, 2006), S. brierleyi (Konishi et al., 1998) and Thiobacter subterraneus(Hirayama et al., 2005). Microorganisms used in bioleaching of metal from sewage sludge are summarized in Table 2.2.

2.3.1 Iron Oxidizing Bacteria

One species of thiobacillus, T.ferrooxidans are gram-negative bacteria. They are rod shaped and move using polar flagella. Cells are 0.5~0.6 μm wide and 1.0~2.0 μm long in size and is non-spore forming (Jensen and Webb, 1995). Main Properties of T. ferrooxidans are: chemolithotropic, autotrophic, obligate aerobic, acidophilic and mesophilic. T. ferrooxidans are recognized as being responsible for the oxidation of iron and inorganic sulphur compounds, the optimal conditions for the growth of T. ferrooxidans are high temperature, low pH, and an abundance of reduced sulphur compounds. T. ferrooxidans has an optimum pH range of 1.5–4 and grows best at a pH of about 2. The ability to resist low pH is an important physiological characteristic since sulphuric acid is generated when reduced inorganic sulphur compounds are being oxidized (Blais et al., 1992a,b).The optimum temperature is 30-35°C.

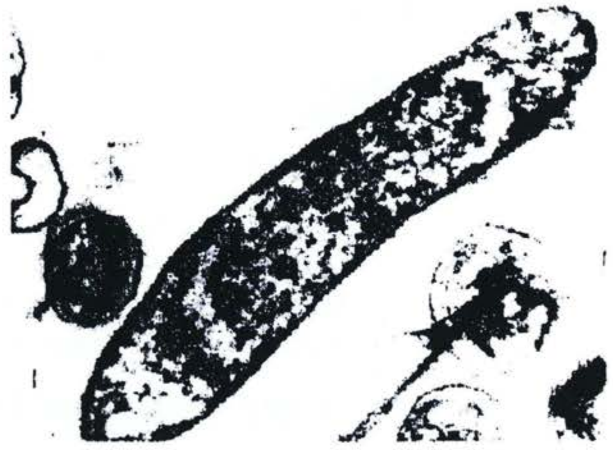


Figure 2.2: T. Ferrooxidans cell suspension magnified 30,000 times.

(Source: http://www.mines.edu/fs_home/jhoran/ch126/microbia.htm)

2.3.2 Sulphur Oxidizing Bacteria

The other microorganism involved in microbial leaching is Thiobacillus thiooxidans which is morphologically and physiologically similar to T. ferrooxidans. The fundamental difference between the two species is generally recognized by the inability of T. thiooxidans to oxidize ferrous iron and insoluble metal sulphides (Torma, 1986). The presence of sulphur-oxidizing microorganisms has been verified in 23 different sewage sludges procured from plants (Tyagi et al., 1994).

Table2.2 Microorganisms used in bioleaching of metal from sewage sludge

Microorganisms	Initial pH	Reference
T.ferrooxidans+T.thioxidans	4	Tyagi et al.(1988)
T.ferrooxidans(ATCC19859)	4	Couilliard and Marcier, 1990
T.ferrooxidans(ATCC23270)+Mixed culture of T.ferrooxidans(ATCC19859) and T.thioxidans(ATCC19377)	4	Couilliard and Zhu, 1992
Indigenous sulfur-oxidizing microorganisms	6.04-6.95	Blais et al.(1993)
T.ferrooxidans	4	Couilliard et al.
Indigenous sulfur-oxidizing bacteria	6.6	Tyagi et al.(1994)
Indigenous sulfur-oxidizing bacteria	7	Meknassi et al.(2000)
Indigenous iron-oxidizing bacteria	3	Xiang et al.(2000)
T.ferrooxidans	2.5	Cho et al.(2002)
T.ferrooxidans	4	Nie,2003
Sulfur-oxidizing bacteria	7	Villar and Garcia(2002)
Iron and sulfur-oxidizing bacteria	7.19	Chan et al.(2003)
Indigenous iron-oxidizing bacteria	3	Wong et al.(2004)
Indigenous sulfur-oxidizing bacteria	6.8	Chen et al.(2004)
Indigenous sulfur-oxidizing bacteria	7	Villar and Garcia(2006)

2.4 Recent Research and Study on Bioleaching

Over the last two decades bioleaching of heavy metals from sewage sludge has been widely studied (e.g., Lombardi and Garcia Jr., 2002; Filali-Meknassi et al., 2000; Sreekrishnan and Tyagi, 1996; Couillard and Mercier, 1994; Hayes et al., 1980). Tyagi and Blais from the University of Quebec have done a great deal of research on sludge metal bioleaching since 1990.

2.4.1 Governing Factors

pH, temperature and solid concentration of sludge are the three main governing factor in metal bioleaching process. The performance of the bioleaching process is influenced by various parameters such as sludge solids concentration, pH, temperature etc. Effect of pH, temperature and solid concentration are described in this report based on the results of different researchers.

pH

An initial sludge pH of 4.0 produced the maximum sulphate production rate, but the initial sludge pH had little effect on the final metal solubilization levels achieved. For a given sludge, the amount of metal solubilised at a particular time will depend on the system pH, regardless of whether the acidification is partially or entirely microbial in origin. The optimum pH for T.ferrooxidans is summarized in Table 2.3.

Table 2.3 Optimum pH for T. ferrooxidans

Optimum pH	References
2.5~5.8	Buchanan and Gibbons(1974)
2.5~3.5	MacDonald and Clark(1970)
2.0	Karamanev and Nikolov (1988); Ingeldew(1986)
2.3	Torma(1977)

(Source: Nie, 2003)

Temperature

The effect of temperature on simultaneous sludge digestion and metal bioleaching process was studied by Blais and Tyagi, 1996. From their result it is observed that the process can be employed efficiently for metal solubilisation, elimination of indicator microorganisms and sewage sludge stabilization at temperature between 10°c and 30°C. The rates of pH reduction, sulphur oxidation, growth of thiobacilli, elimination of indicator microorganisms and solid degradation were found to decrease with temperature. Low metal solubilisation efficiency was observed at 10°C. The results of another study by Blais et al., 1993 suggested that the metal removal from sludge by bioleaching methods was strongly influenced by the temperature of the bioreaction. Optimum temperature for bioleaching process studied by different researchers is shown in Table 2.4.

Table 2.4 Optimum Temperature for T. ferrooxidans

Optimum Temperature(°C)	References
15-20	Buchanan and Gibbons(1974)
29-33	MacDonald and Clark(1970)
31	Lacey and Lawson (1970)
30	Karamanev and Nikolov (1988)

(Source: Nie, 2003)

Solid Contents

The sludge solids concentration plays a major role in regulating the acid production indirectly, by influencing the nature of the pH drop in the system; the sludge solids concentration does not directly affect the metal solubilization process (Sreekrishnan et al., 1993). The study by Blais and Tyagi, 1996 demonstrated that an increase in sludge solids concentration increased the sulphuric acid production rates in the range of 8 to 30 g/l of solids. From the result it was found that the rate of ORP rise decreases as the solid content increases, similarly, the rate of pH reduction decreases with increasing solid content (Chen and Lin, 2000). From the result of the research by Nie, 2003, it has been observed that an increase in sludge solids concentration in the range of solid 4 to 16 g/l (25% of TS to 100% TS, TS=16g/l) does not affect the removal efficiency for Cu and Zn very much, but the Cd removal efficiency increases with decreasing TS during the bioleaching process.

2.4.2 Modes of Operation

Most studies regarding bioleaching of metal removal from the sludge have been conducted in batch and continuous processes.

Bioleaching in batch mode

The most of the bioleaching studies for sludge decontamination have been reported using laboratory scale batch reactors which are easy to operate and are conventionally used to generate data required for development of the process for large-scale applications. Wong and Henry (1983, 1984a) reported, batch bioleaching with anaerobic digested sludge using T. ferrooxidans and $FeSO_4$ as an energy source. A batch bioleaching study (Blais et al., 1992) conducted with 23 municipal sewage sludges showed that sulphur-oxidizing bacteria were more efficient in bioleaching (62.5% mean metal solubilisation) as compared to iron-oxidizing bacteria (49.5% mean metal solubilisation). On the other hand, T. ferrooxidans was reported to be highly effective in reducing the sludge pH from 7.86 to 1.80 and within 6 days of batch operation, complete removal of Cu and Zn was observed (Zhou et al., 2003). The results of the batch study reported by Wong et al. (2002) using T. ferrooxidans showed that the bioleaching process was efficient at different initial sludge pH ranging from 3 to 7. Similar results were obtained using sulphur oxidizing bacteria at initial sludge pH of 4 and 7 for 16 days of batch operation by Villar and Garcia, 2006 (Pathak et al.,2009).

Bioleaching in continuous mode

Batch bioleaching process takes longer time even up to 16 days when pH of the medium reaches the desired level, i.e., pH <2 for solubilization of metals. On the other hand, a continuous process can treat larger volume of sludge in shorter time period and hence appears to be more suitable for applying on a larger scale. However, very few studies have been carried out in continuous flow system for bioleaching of metals. The most of the studies on bioleaching in continuous mode have been carried out in continuous stir tank reactor (CSTR) having provision for aeration and agitation to mix up the reactor content. Couillard and Mercier, 1990 reported, bioleaching with T. Ferrooxidans using CSTR and CSTR with sludge recycling (CSTRWR). Tyagi et al. (1991) studied the effect of HRT and recycling rate in a continuous bioleaching process using pure culture of T. ferrooxidans with 4 g/ L of $FeSO_4.7H_2O$. Bioleaching process in continuous mode studied by different researchers is shown in Table 2.5.

Simultaneous sewage sludge digestion and metal leaching (SSDML) process

An integrated technique termed as 'simultaneous sewage sludge digestion and metal leaching (SSDML) involves the coupling of bioleaching process with sludge digestion process in a single stage reactor, which results in simultaneous removal of metals, sludge volatile solids and pathogens (Benmoussa et al., 1997). The SSDML process consists of aerobic sludge digestion followed by solubilisation of heavy metals due to the acid production by the oxidation of sulphur. Generally, sulphur based bioleaching process starts at neutral pH of the sludge and hence can be combined with the aerobic sludge digestion process. SSDML process was studied by Tyagi et al. (1997), MeKnassi et al. (2000), Blais et al. (2001), (Blais et al., 2004) and the percentage removal of heavy metals are shown in Table 2.5.

2.4.3 Applications

The main application of bioleaching process is the heavy metal removal. Bioleaching process has been proven to be a very efficient and economical method to remove heavy metal from sewage sludge compared with traditional methods of metal removal. Percentage removal of heavy metals in different modes of bioleaching operation studied by different researchers is summarized in Table 2.5. Another application of bioleaching is the elimination or reduction of indicator bacteria from sewage sludge. The several research and studies showed that this process of microbial leaching destroys the indicator bacteria present in the sludge to a level normally found in agricultural soil and contributes substantially toward the reduction of volatile suspended solids (15-70%). The reduction of volatile solids in the sludge is an indication of stabilization of the sludge and reduction of its odor. Shooner and Tyagi (1996) have shown that thermophilic bioleaching process was efficient in the reduction of volatile solids (VS) and volatile suspended solids (VSS) in the sludge with simultaneous removal of odor in less than 4 days of bioleaching. So, odor control is also an application of bioleaching process.

Table 2.5 Heavy metals removal in different bioleaching processes.

Mode of operation	Heavy metals removal efficiencies (%)							References
	Cu	Ni	Zn	Cr	Cd	Mn	Pb	
Batch	66–80	70–78	84–90	–	80–85	–	6–9	Wong and Henry (1984)
Batch	75	–	97	–	50	–	55	Tyagi et al. (1988)
Batch	47–80	42–60	–	–	–	81–89	–	Couillard and Chartier, 1991
Batch	46.7–95.8	–	–	–	49.5–88.2	58.1–95.9	–	Blais et al. (1992)
Batch	69–92	77–88	88–97	19–41	83–90	88–99	10–54	Blais et al. (1993)
Batch	16–90	86–97	18–91	26–71	7–60	–	48–98	Shanableh and Ginige (2000)
Batch	80	100	100	60	–	–	–	Villar and Garcia (2002)
Batch	63.7–74.1	15.5–38.6	74.9–88.2	50.2–78.4	–	–	–	Wong et al. (2002)
Batch	100	–	100	80	–	–	–	Zhou et al. (2003)
Batch	–	–	–	65–69	–	–	–	Ryu et al (2003)
Batch	79	87	91	42	–	–	52	Villar and Garcia (2003)
Batch	87.86	–	92.14	–	32.72	–	–	Chen et al. (2004)
Batch	74	84	99	65	–	–	58	Wong et al. (2004)
Batch	34	–	38	18	–	–	–	Kim et al. (2005)
Batch	80	80	80	50	–	–	50	Villar and Garcia (2006)
SSDML	61–100	57–84	83–96	34–46	83–100	91–100	17–44	Benmoussa et al. (1997)
SSDML	69–86	19–21	79–100	6–26	33–63	80–100	5–69	Blais et al. (1997)
SSDML	41.53–80.72	26.37–56.75	48.01–92.98	9.12–18	17.32–47.03	–	9.12–17.60	Meknassi et al. (2000)
SSDML	48–100	15–33	66–100	9–32	25–78	77–99	12–47	Couillard and Mercier (1993)
Continuous	50–62	93–94.8	64–77	–	64.6–66.75	–	–	Couillard and Mercier, 1990
Continuous	91	67	94	8	93	7		Tyagi et al. (1991)
Continuous	91	67	94	–	67	93	–	Couillard and Mercier, 1991
Continuous	91	–	–	–	–	–	–	Couillard and Zhu, 1992
Continuous	52	–	62	–	–	78	–	Couillard and Mercier (1993)
Continuous	73.78	52.72	86.49	6.7	–	66.49	2.89	Ratanchco (1995)
Continuous	33	48	74	–	50	–	–	Seth et al. (2006)

Source: (Pathak et al., 2009)

3 Materials and Methods

In this research, the bioleaching experiment was performed in 500 ml Erlenmeyer flasks with incubated and adapted sewage sludge of the Ashbridges Bay Wastewater Treatment Plant (ABTP) to study metal removal efficiency of three problematic heavy metals such as Cu, Cd and Zn. In addition in this research, the bioleaching process with iron oxidizing bacteria (T. ferroxidans) was evaluated whether this process is potential for the elimination of indicator bacteria such as total coliforms.

3.1 Sample Collection

The sewage sludge sample was collected from ABTP in temperature-controlled PVC containers, shipped cold and kept covered at a constant temperature of 4°C in the refrigerator before use. Two types of sludge were collected from ABTP. The first type is taken from the aeration tank of ABTP, called activated sludge, and the other from the outlet of sludge digester, called digested sludge. This research was first started with two types of sludge.

3.2 Sludge Adaptation

Bacterial leaching involving T. ferrooxidans requires sludge to be pre-acidified so that bacteria are in an adapted environment for optimal growth. Bacterial growth generally proceeds through a series of phases, including Lag grown phase, Log growth phase, Stationary phase and Endogenous phase. To get a high concentration of T. ferrooxians, that is to reach the log growth phase for bacteria, an optimum living condition must be maintained. As the growth of T. ferrooxidans are quite sensitive to pH, the measurement of pH plays an important role in identifying and controlling the activity of T. ferrooxidans in the bioleaching experiment. This is because the metal solubilization efficiency was found to increase with decreasing pH during the bioleaching process (Chen and Lin, 2000). Another important monitoring factor is Oxidation/Reduction Potential (ORP), which empirically reflects the oxidation status from Fe^{++} to Fe^{+++} by T. ferrooxidans. An ORP in the positive range indicates oxidation. For example, a reading of 450-500mV in bioleaching process would often indicate a strong oxidation. So during the adapted sludge incubation stage, the ORP value increases steadily over time. When the ORP value reaches 450mV, the population of T. ferrooxidans is assumed to be fully adapted at that time. In this research, the incubation and adaptation procedure was adopted from Blais and Tyagi (1992) with some modification. The sludge adaptation was done in three stages: Stage I, Stage II and Stage III to get an enriched sludge. A small portion of the collected sludge is firstly incubated and used for adapting the remaining sludge for the experiments.

Stage I

Two 150 ml of sludge samples (one from the Aeration Tank and the other one from Sludge Digester) were transferred to each of four 250 ml Erlenmeyer flasks. The mouths of the flasks were covered with tin foil to maintain aerobic process. The pH of the sludge was adjusted to 4.0±0.5 with 2N H_2SO_4. Then 0.5% of $FeSO_4 \bullet 7H_2O$ (equivalent to 0.75g) was added to every flask containing sludge. In order to adapt the iron oxidizing bacteria, the flasks with sludge for the test runs were incubated at 28°C (because some research suggested under 25°C, some suggested under 30°C, the average incubation temperature was adopted as 28°C) in a gyratory shaking incubator (Model C-25, New Brunswick Scientific Co.) at 125 revolutions per minute (rpm). Samples was drawn at regular intervals (every 24 hr) during the initial period of adaptation to measure the pH and oxidation-reduction potential (ORP).The first stage was considered complete after the initial adaptation, when the pH reaches a value around 2.5 or the ORP was increased to around 450 mV. To compare the activity of adapted sludge incubated from sampled sludge of the aeration tank, the sludge of the digester was also incubated at the same time. After Stage I adaptation, it has been observed that the digested sludge needs more time to reach pH 2.5 and ORP value 450 mV. After that, the rest of the research was done with only activated sludge.

Stage II

In this adaptation stage, 10% of adapted activated sludge incubated during stage I was mixed with 150 ml of fresh activated sludge containing 0.5% of $FeSO_4 \bullet 7H_2O$ in 250 ml Erlenmeyer flask for re-incubation (28°C). In this stage the pH was not adjusted to 4.0±0.5 with acid. The pH and ORP was measured at 24 hr intervals until pH was about 2.5 or the ORP increased to around 450mV. In this research to get a better adapted iron-oxidizing thiobacilli this step was repeated in Stage III.

Stage III

This is the final stage of adaptation in this research. In this adaptation stage, 10% of adapted activated sludge incubated during stage I was mixed with 150 ml of fresh activated sludge containing 0.5% of $FeSO_4 \bullet 7H_2O$ in 250 ml Erlenmeyer flask for re-incubation (28°C). In this stage the pH was also not adjusted to 4.0±0.5 with acid. The pH and ORP was measured at 24 hr intervals until pH was about 2.5 or the ORP increased to around 450mV. During this stage the iron oxidizing bacteria was assumed to be fully adapted and this adapted sludge was used for the bioleaching experiment to study heavy metal removal and reduction of total coliform.

3.3 Bioleaching experiment

Bioleaching experiment was performed in 500 ml Erlenmeyer flasks with 250 ml of fresh activated sludge. 1% of $FeSO_4 \cdot 7H_2O$ (equivalent to 2.5g) and 10% adapted sludge (equivalent to 25 ml) was added to the flask containing activated sludge. Although the bioleaching experiments were done by iron oxidizing bacteria, the pH was not adjusted to 4.0±0.5 with acid. The flasks were incubated at 28°C and 180 rpm in shaker incubator. Samples were drawn from the flasks at every 48 hr for analysis. The changes in pH, oxidation reduction potential (ORP) and concentration of indicator bacteria will be measured at every 48 hr. To determine the metal removal efficiency, initial and final concentration of Cu, Cd and Zn in the sludge was measured during bioleaching experiment. The duration of bioleaching experiment was 10 days. Experiments were done duplicates.

Figure 3.1: Bioleaching experiment

3.4 Analytical Methods

3.4.1 pH and ORP measurements

During adaptation of the sludge and the bioleaching experiments, some samples were drawn from the flasks for pH and oxidation-reduction potential (ORP) measurements. The pH of the sludge was measured by an Accumet Basic pH meter, model AB15 and the ORP was measured by portable Horiba pH/Ion meter, model D-53 as shown in Figure 3.2.

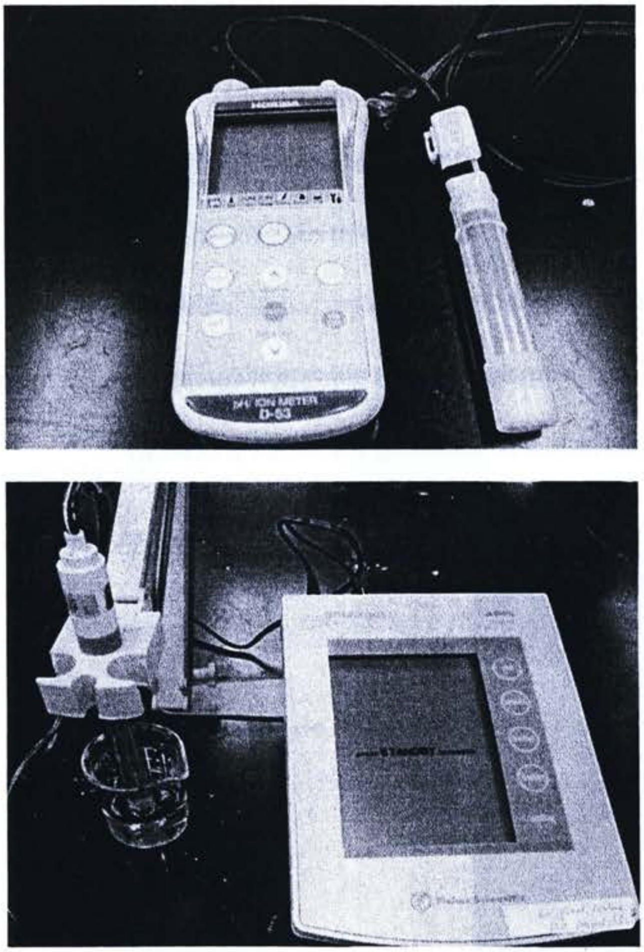


Figure 3.2: pH and ORP meters

3.4.2 Heavy Metal Measurements

A heavy metal concentration in the raw sludge and bioleached sludge were measured by Atomic-Absorption Spectroscopy (AA), Model PE–800 and is shown in figure. Sample to be analyzed by AA must be vaporized or atomized, typically by using a flame or graphite. For this research the flame furnace was utilized to measure metal concentrations. Before AA analysis, the bioleached samples were centrifuged at 16500 rpm for 15 minutes to separate the solids from the liquid fraction. The supernatant was filtered by filtration apparatus and the liquid portion was prepared for AA analysis of heavy metal concentration. Some samples were prepared by acidified the filtered solution with HNO_3 to about pH 1 and then stored at 4°C prior to determination of heavy metal. To determine the metal concentration in raw sludge, the sludge was digested with acid according to the procedure EPA 3050B. Duplicate samples were also prepared according to the procedure followed by standard methods for the examination of water and wastewater APHA(1989).After that the heavy metal in the digested liquid were determined by using atomic adsorption spectroscopy (Perkin Elmer AAnalyst 800). Calibration curves for each metal were determined using standardized metal solutions prepared following the APHA procedure.

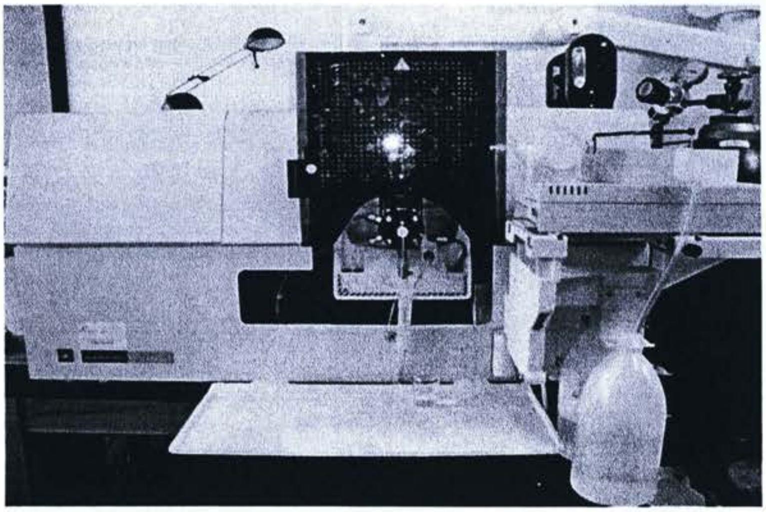


Figure 3.3 Atomic-Absorption Spectroscopy (AA)

3.4.3 Total Coliform Test

In this research, the member filter technique was used for enumeration of total coliform. This technique involves filtering a known volume of sample through a sterile 0.45 µm membrane filter. When the sample is filtered, bacteria are trapped on the surface. The filter is then placed on media in a petri dish to allow the bacteria to grow. During test, first samples were diluted with sterile phosphate buffer solution. After dilution of samples media plates were prepared in petri dishes. M-Endo b broth was used in absorbent pad. Samples were filtered with suction in 0.45 µm membrane filters and after filtering sample the filter was placed on the prepared absorbent pad. The procedure is demonstrated in Figure 3.4. The petri dishes were then incubated for 24h at 35°C for total coliform. After 24h all colonies which are pink to dark red with a golden or greenish metallic sheen were counted between 20-200.A colony count of 20-200 is statistically correct for enumeration. If colonies overlap one another, coliform counts were recorded as TMTC (too many to count). If there are less than 20 colonies coliform counts were recorded as TFTC (too few to count).

Calculation:

$$\frac{\textit{Total Coliform}}{100\ mL} = \frac{\textit{number of coliform colonies x D.F.}}{\textit{mL Sample filtered}} \times 100 \qquad (10)$$

D.F. = Dilution Factor (ie. If dilution is 10^{-1} then D.F. = 10^{1})

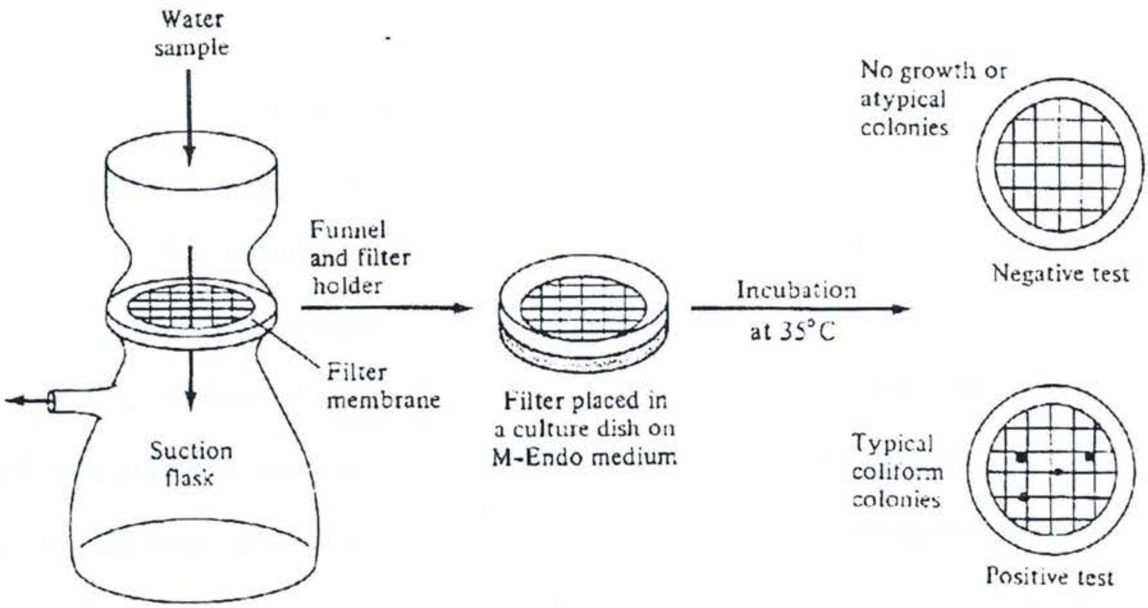
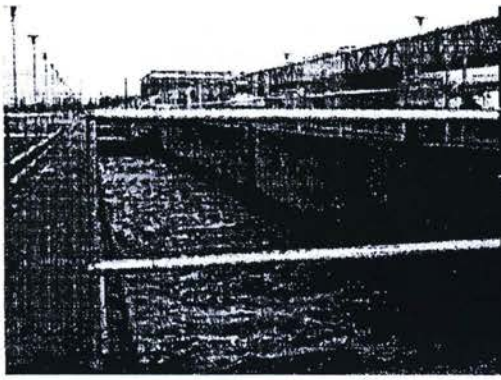


Figure3.4 Diagram of membrane filter technique for coliform testing

4. Results and Discussion

4.1 Initial Characteristics of Sludge

Sludge samples were collected from ABTP. For comparison during adaptation of sludge, two types of sludge were studied. The first was taken from the Aeration Tank and the other from the Sludge Digester as shown in Figure 4.1 and Figure 4.2. Some initial characteristics of activated sludge and digested sludge such as pH, oxidation–reduction potential (ORP), total solids content are shown in Table 4.1.



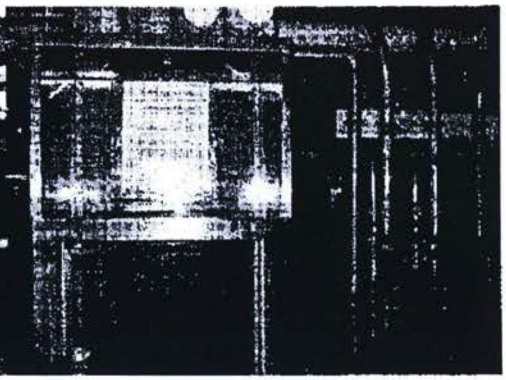


Figure 4.1 Aeration Tank of ABTP

Figure 4.2 Digester's Sludge Feed

(Source: Nie,2003)

Table4.1 Initial Characteristics of Sludge Samples

Parameters	Activated Sludge	Digested Sludge
pH	7.01	7.22
ORP	-113 mV	-253 mV
Temperature	23.5°C	22.6°C
ORP(mV) after preacidification(pH=4)	151	-133
Total Solid content(TS)	N.A	2.02%

4.2 Results of Sludge Adaptation

4.2.1 Stage I Result

In this research sludge adaptation was performed in three stages. In stage I, the activated sludge is initially adapted for around 7 days. During this stage pH changes from 3.99 to 2.14 in 7 days and oxidation/reduction potential (ORP) increased from initial value 151 mv to 450mv. In stage I adaptation of sludge, first the pH was adjusted to around 4 with acid. The pH and ORP variation during stage I for activated sludge are shown in Table in Appendix A and Figure 4.3. For digested sludge, pH changed from 4.05 to 2.70 and ORP increased from initial value -133 mV to 504 mV are shown in Table in Appendix A and Figure 4.4. During adaptation, ORP value increased due to the increase of Fe^{3+}/Fe^{2+} ratio because ferrous ion in solution was oxidized by microorganisms. The formation of ferric hydroxide also contributes to the reduction of sludge pH. The incubation period for activated sludge is 168 hours whereas for digested sludge is 288 hours. After Stage I adaptation, it has been observed that the digested sludge needs more time to reach pH 2.5 and ORP value 450 mV. After that, the rest of the research was done with only activated sludge.

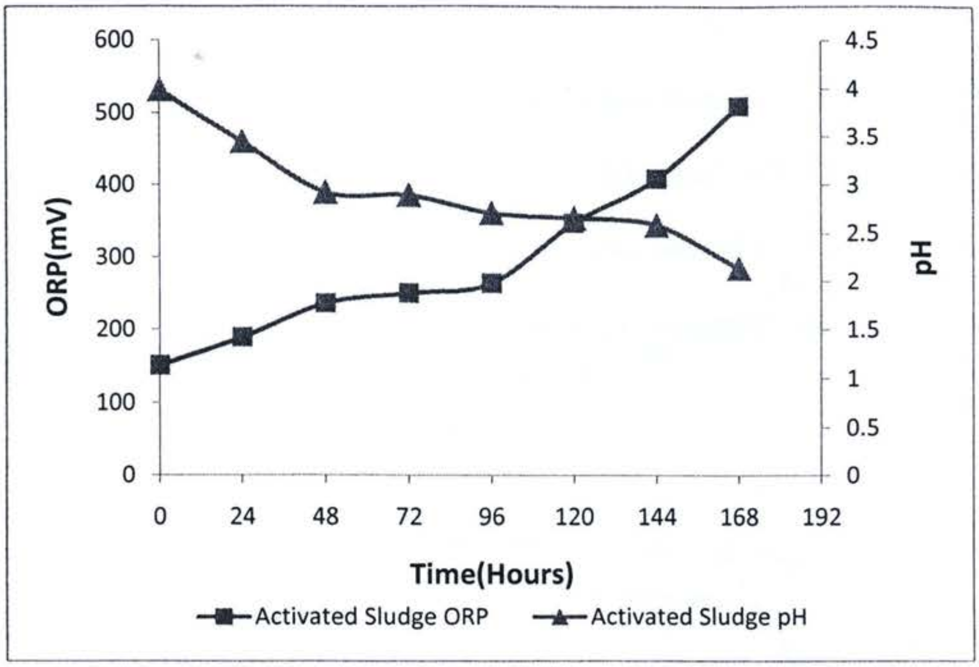


Figure 4.3 pH and ORP variation during adaptation of activated sludge (Stage I)

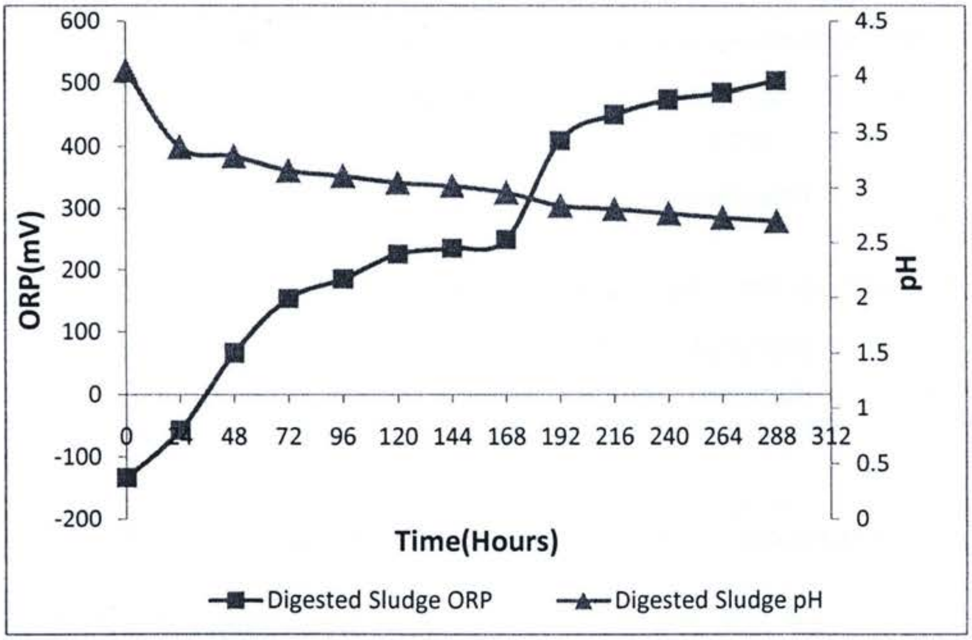


Figure 4.4 pH and ORP variation during adaptation of digested sludge (Stage I)

4.2.2 Stage II Result

The change in pH with time and changes in oxidation/reduction potential for activated sludge are shown in Table in Appendix A and Figure 4.5. This is the 2nd stage of adaptation and at this stage, pH changed from 6.05 to 2.78 and ORP increased from -115 mV to 518 mV. The incubation period for stage II was 192 hours. For the sludge to be fully adapted, Stage III was also required in this research.

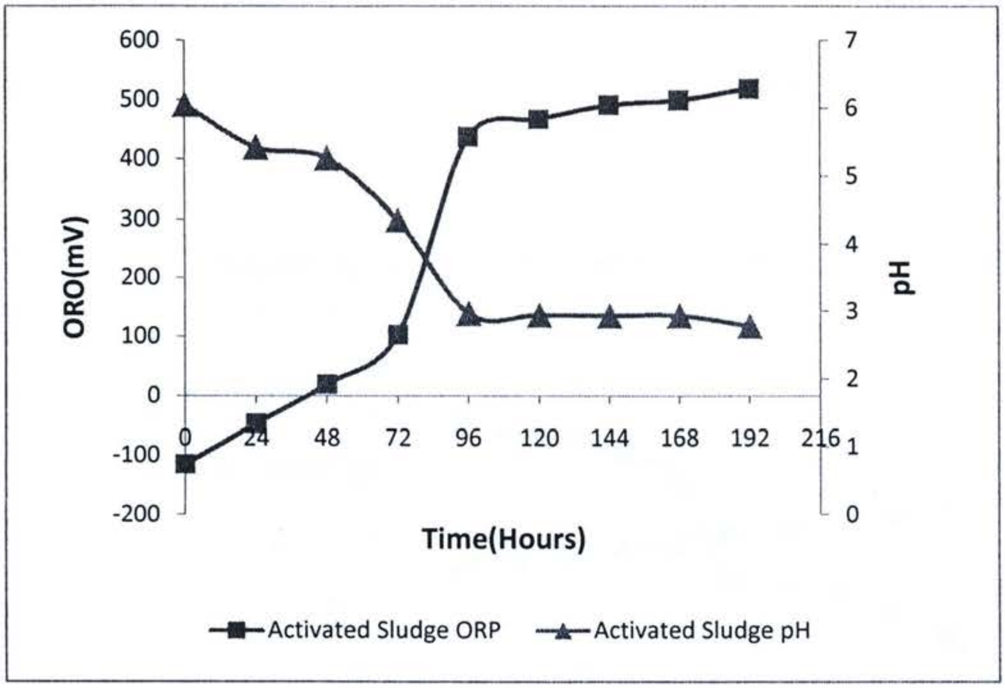


Figure 4.5 pH and ORP variation during adaptation of activated sludge (Stage II)

4.2.3 Stage III Result

This is the final stage of adaptation. At this stage, pH changed from 6.22 to 2.75 and ORP increased from -141mV to 445 mV and iron-oxidizing bacteria T. ferrooxidans is assumed to be fully adapted. This adapted sludge (or adapted iron-oxidizing bacteria in the adapted sludge) was used in the subsequent bioleaching experiments. The incubation period for stage III was

216 hours. The pH varying trend and changes in oxidation/reduction potential with time for activated sludge are shown in Table in Appendix A and Figure 4.6.

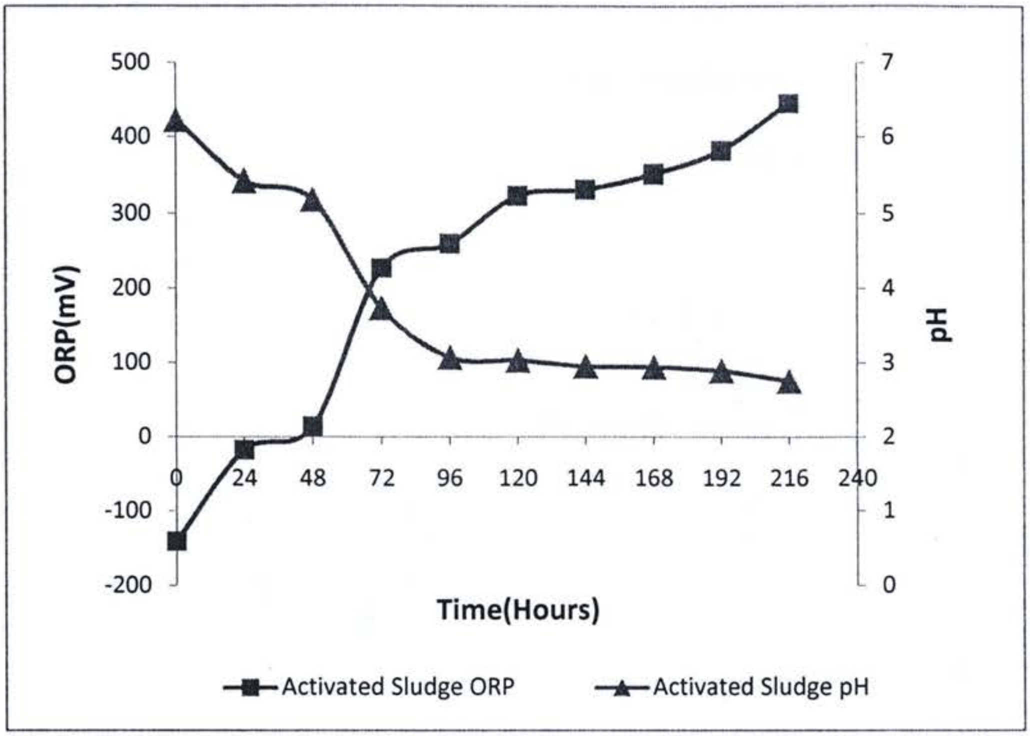


Figure 4.6 pH and ORP variation during adaptation of activated sludge (Stage III)

4.3 Results of Bioleaching Experiment

4.3.1 pH & ORP variation during bioleaching

The change in pH and ORP with time during bioleaching using adapted iron-oxidizing microorganisms and 1%ferrous sulphate as a substrate is shown in Fig 4.7. From the figure, it has been observed that there was a rapid decrease in pH from 6.18 to 2.6 in only 2 days, then slowly to 2.44 in 10 days. Again there was also rapid increase of ORP from -107 mV to 449 mV in only 2 days and finally to 533 mV on the 10th day. The increase in ORP coupled with a low pH value during bioleaching is an indicator of substantial growth of microorganisms. The increase in ORP is due to the oxidation of Fe^{2+} to Fe^{3+}, which occurs naturally in the presence of air and also through biological oxidation. Without adjusting the pH to about 4, the bioleaching experiment was carried out with iron oxidizing bacteria at initial pH of the sludge. The results of the present study show that adapted iron oxidizing microorganisms were efficient at initial pH of the sludge. The similar results indicating decrease in pH were also obtained in the previous study carried out at initial neutral pH of the sludge using iron oxidizing bacteria (Wong et al., 2001).

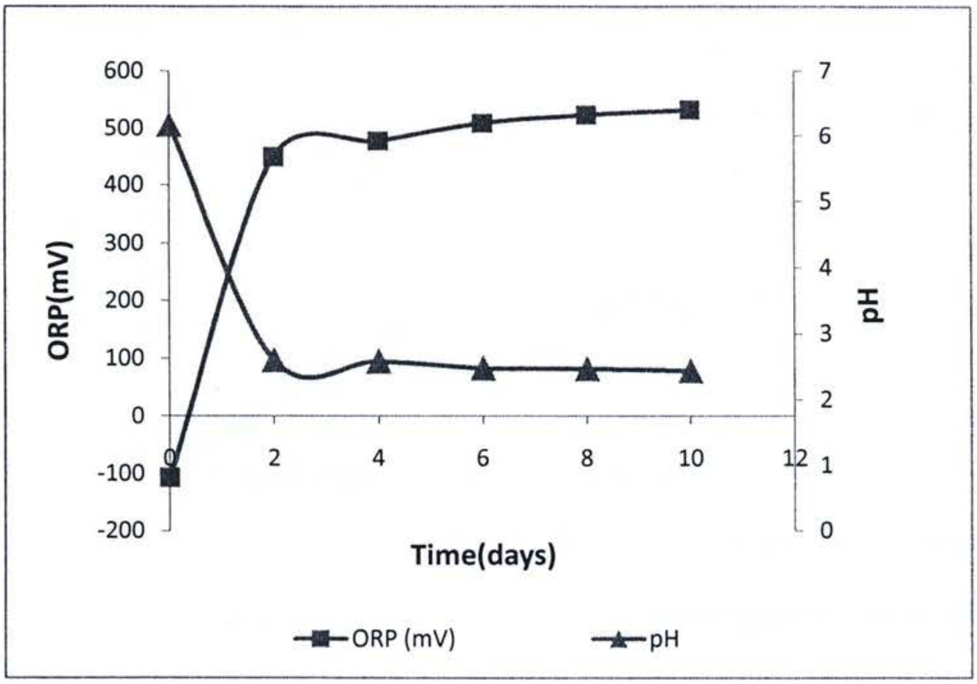


Figure 4.7 pH and ORP variation during bioleaching period.

4. 3.2 Heavy Metal Removal after bioleaching

In this research, concentrations of three heavy metals: Cu, Cd , Zn in the original sludge and the sludge after 10 days of bioleaching treatment were measured. Concentrations of heavy metals in the raw and bioleached sludge, percent removal of heavy metal and data statistics are summarized in Table 4.2. The concentration of Cu, Zn and Cd in raw sludge and bioleached sludge is shown in Figure 4.9. In the present study, after 10 days of bioleaching 70% Cu, 70% Zn and 74% Cd were removed from the sludge. Figure 4.8 presents the percent removals of the three metals after 10 days of bioleaching. The removal efficiencies of heavy metal achieved in the present study appear to be very close to the values of previous studies.

Table 4.2 Heavy metal concentrations, percent removal of heavy metal and data statistics

	Cu		Zn		Cd	
	Conc(ppm)	%removal	Conc(ppm)	%removal	Conc(ppm)	%removal
bioleach	3.02	64	1.65	74	0.0067	77
bioleach	2.58	69	2.00	69	0.0090	70
bioleach	1.96	76	2.20	66	0.0078	74
Average	2.52	70	1.95	70	0.0078	74
STDEV	0.53	6.03	0.28	4.04	0.0011	3.51
95% CI	0.60	6.82	0.32	4.57	0.0013	3.97
Raw	8.28		6.38		0.0296	

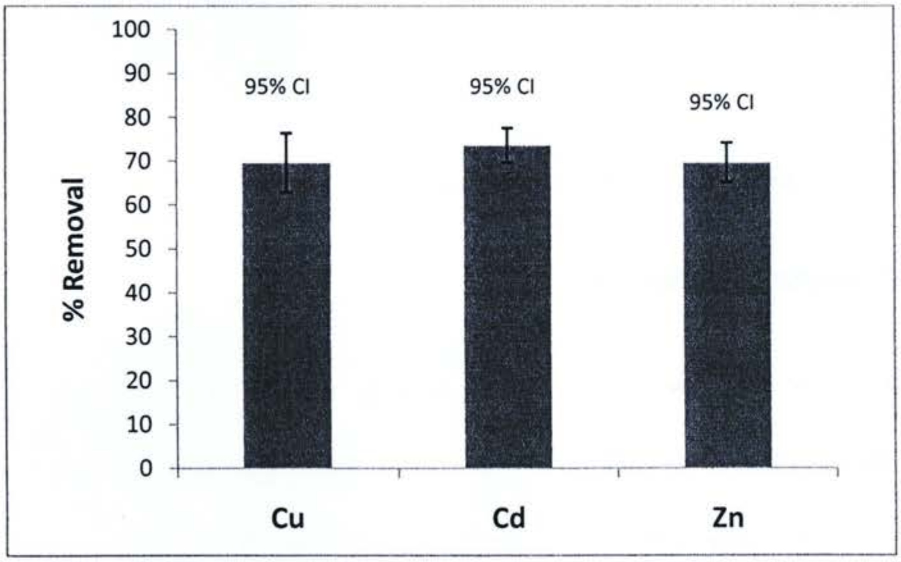


Figure 4.8 Metal percent removals after 10 days of bioleaching

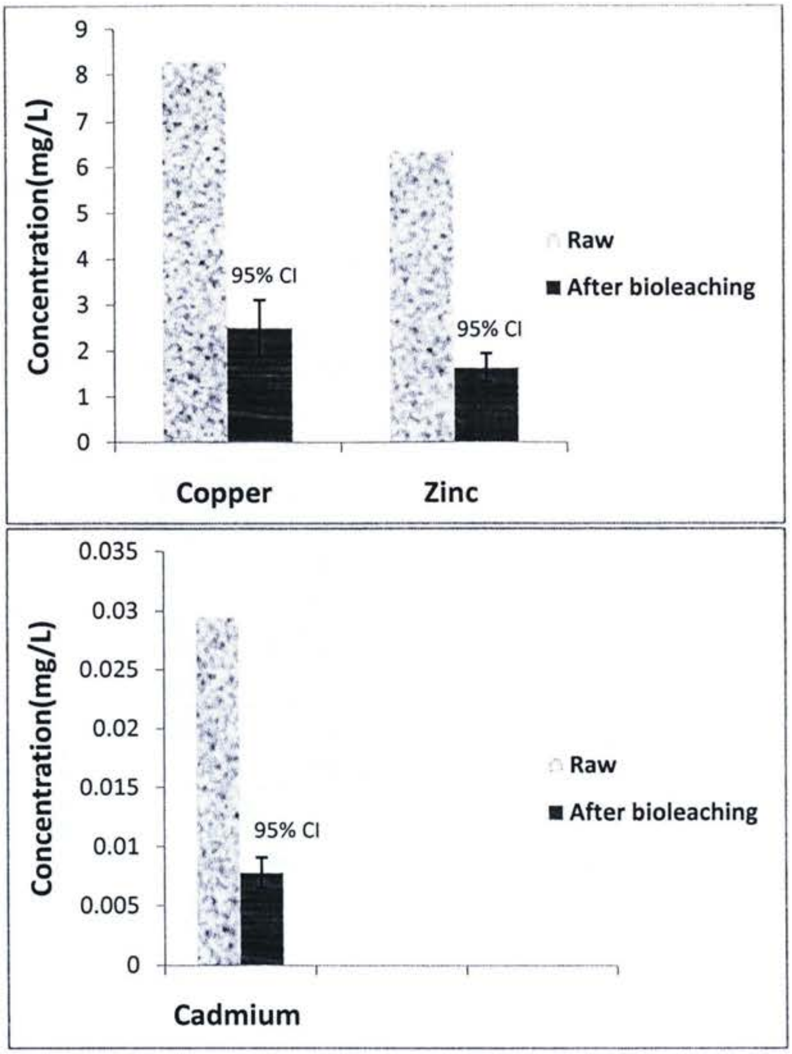


Figure 4.9 Comparison of metal concentration in the raw and bioleached sludge

4.3.3 Total Coliform reduction during bioleaching

Total coliform test was done for the raw sludge. During bioleaching in every 48 hour total coliform tests were done by member filter technique. Coliform colonies grow on the petridish CC plate. By counting the colonies, after 24h incubation, total coliform/100 ml was calculated for raw sludge and bioleached sludge. Test results are shown in Tables in Appendix C. The typical total coliform colony has a pink to dark red colour with a metallic surface sheen.

Coliforms that lack sheen may be pink, red, white or colourless are considered to be non coliforms (APHA, 1989). After the tests it has been observed that for raw sludge, coliform colonies were dark red colour with a metallic surface sheen as shown in Figure 4.10. After bioleaching colonies that found were white coloured as shown in Figure 4.11. The reduction of total coliform occurred after two days of bioleaching due to rapid decrease of pH below 2.7 within two days. At the end of 10 days bioleaching period, the elimination of the total coliforms occurred with a large morality (3 log removal). Reduction of total coliforms in the sludge during bioleaching period is shown in Figure 4.12.

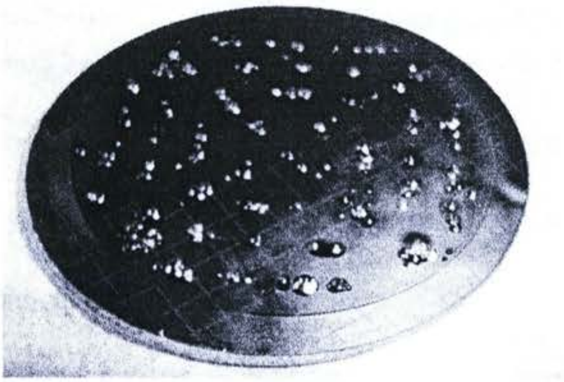


Figure 4.10 Coliform colonies in raw sludge

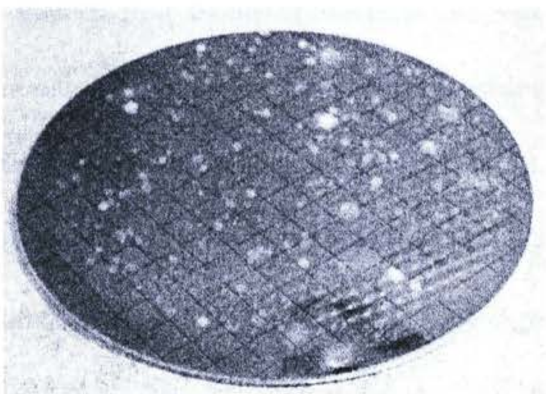


Figure 4.11 Coliform colonies after bioleaching

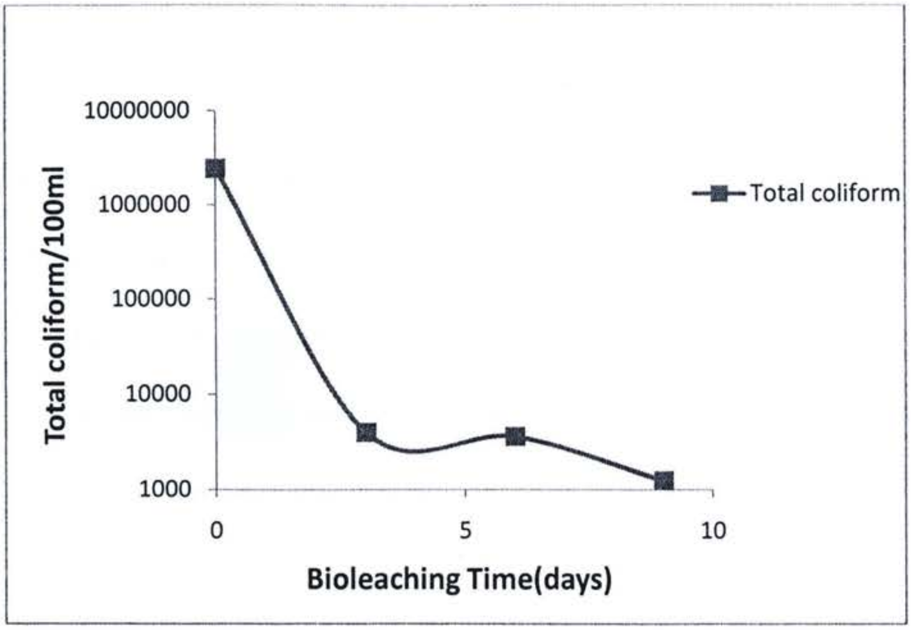


Figure 4.12 Reduction of total coliforms in the sludge during bioleaching period

5. Conclusions and Recommendations

5.1 Conclusions

The results of the present study show that bioleaching of sewage sludge with adapted iron oxidizing bacteria T. ferrooxidan using ferrous sulphate as an energy source T. ferrooxidan was successfully carried out for simultaneous reduction of three problematic heavy metals and total coliform. The results demonstrated that simultaneous metal removal efficiencies of Cu, Zn and Cd were 70%, 70% and 74% respectively after 10 days of bioleaching. The final pH and ORP were found 2.44 and 533 respectively. It is observed that iron oxidizing bacteria are naturally present in the sewage sludge, including sludge in the aeration tank and digester of ABTP. It is very easy and convenient to prepare adapted sludge using raw sludge (either activated sludge or digested sludge) in the plant. But adaptation of bacteria in digested sludge needs more time than activated sludge. In this research, during bioleaching experiment acid is not required to adjust the initial sludge pH as is required in previous research. Therefore, pre-acidification of sludge for the bioleaching can be avoided or reduced in order to further reduce the acid consumption and the cost of operation for the bioleaching process. It can be concluded that the adapted iron oxidizing bacteria did not require a low starting pH of less than 4 but still maintained a comparable metal leaching efficiency. After this research, it also observed that the process of bioleaching by T.ferrooxidans is very efficient for the reduction of total coliform in the sludge. This process allows a considerable reduction in total coliform (3 log or under the limit detection of 10^3 CFU/100ml) for the activated sludge examined over a 10 day period.

5.2 Recommendations

Bioleaching process has been investigating by the researchers over many years. Based on the findings gained from the research, the following recommendations could be proposed:

1. This research evaluated the bioleaching process for the simultaneous reduction of heavy metal as well as total coliform from the sewage sludge of ABTP. After this research it can be recommended that lowering of initial pH to as low as 4.0 prior to bioleaching process with T. ferrooxidans with acid is not absolutely essential. Therefore, pre-acidification of sludge for the bioleaching can be avoided or reduced by increasing the substrate amount in order to further reduce the acid consumption and the cost of operation for the bioleaching process.

2. After sludge adaptation, it is observed that iron oxidizing bacteria are naturally present in the sewage sludge. It is very easy and convenient to prepare adapted sludge using raw sludge (either activated sludge or digested sludge) in the plant. But adaptation of bacteria in digested sludge needs more time than activated sludge. Metals which exceed recommended levels can be removed by using adapted sludge with high concentration of T. ferrooxidan bacteria, standard T. ferrooxidans strains are not required which are costly.

3. In every year, half of the ABTP sludge is sent to agricultural land as a fertilizer. The potential leaching loss of Nitrogen (N) and Phosphorus (P), which in turn will reduce its value as a fertilizer is also a concern for the sludge used for land application. Besides, bioleaching at a high initial pH would add an additional benefit of reducing the leaching

loss of N and P (Wong et al., 2001). In this research, bioleaching with iron oxidizing bacteria was also carried out with high initial pH. So, sludge nutrient level (N, and P) after bioleaching should be examined in future study.

4. The advantages of the bioleaching method are numerous and clear. Moreover, research for the bioleaching process to remove metal from sewage sludge has been carried out for many years, but still now it is at the experimental stage. So, special attention should be taken to develop this process on a larger scale to treat large volume of sludge.

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Appendices

Appendix A: Sludge Adaptation and Bioleaching Experiment

Table A1 Annual average heavy metal concentration in digested sludge at ABTP in 2001

Sampled Metals	Conc. in Wet Sludge (mg/L)	Conc. on a Dry weight Basis(a) (mg/kg)	Maximum Allowable Dry wt conc. (mg/kg)	Recommended levels Dry wt conc. (b) (mg/kg)	Percent of **Guideline (a/b)**
As	0.10	5.1	170	170	3.0%
Cd	**<0.2**	**3.5**	**34**	**15**	**23.3%**
Co	<0.1	2.6	340	340	0.8%
Cr	2.7	134	2800	1000	13.4%
Cu	**21.8**	**1083**	**1700**	**1000**	**108.3%**
Hg	0.045	2.3	11	11	20.9%
Mo	0.22	11.1	94	94	11.8%
Ni	0.7	37.8	420	180	21.0%
Pb	1.4	70	1100	500	14.0%
Se	0.06	3.0	34	34	8.8%
Zn	**17.2**	**856**	**4200**	**2500**	**34.2%**

Source: Nie,2003.

Table A2 Annual average concentration of three heavy metals in digested sludge at ABTP in 2008

Date	Cadmium (mg/L)	Copper (mg/L)	Zinc (mg/L)
02/10/2008	0.0134	17.4	12.5
27/10/2008	0.0121	18.5	13.8
11/11/2008	0.0059	14.8	11.4
24/11/2008	0.0072	8.28	6.38
01/12/2008	0.0174	19.3	14.2
15/12/2008	0.0141	18.6	13.2
28/04/2008	0.0178	15.2	10.5
21/05/2008	0.0251	25.5	16.6
05/05/2008	0.0295	27.2	18.7
23/06/2008	0.0277	26.3	17.4
09/06/2008	0.0025	1.67	1.29
21/07/2008	0.02	24.5	17.9
07/07/2008	0.0299	26	20.2
11/08/2008	0.0162	18.3	13.1
18/08/2008	0.0148	17.4	13.2
22/09/2008	0.0132	18.5	13.8
08/09/2008	0.0141	17.8	13.8
	0.2809	315.25	227.97
Annual Average	0.0165235	18.5441176	13.41

Source: city of Toronto

TableA3 pH & ORP variation of Activated sludge during sludge Adaptation (Stage I)

Hours	Activated Sludge	
	pH	ORP
0	3.99	151
24	3.45	189
48	2.92	236
72	2.89	250
96	2.71	264
120	2.66	348
144	2.59	409
168	2.14	510

Table A4 pH & ORP variation of Digested Sludge during sludge Adaptation (Stage I)

Hours	Digested Sludge	
	pH	ORP
0	4.05	-133
24	3.37	-57
48	3.29	67
72	3.16	154
96	3.11	186
120	3.05	226
144	3.02	236
168	2.96	250
192	2.84	410
216	2.81	451
240	2.77	474
264	2.73	485
288	2.7	504

Table A5 pH & ORP variation of Activated sludge during sludge Adaptation (Stage II):

Hours	Activated Sludge	
	pH	ORP
0	6.05	-115
24	5.42	-47
48	5.26	20
72	4.36	103
96	2.96	437
120	2.94	467
144	2.93	491
168	2.93	499
192	2.78	518

Table A6 pH & ORP variation of Activated sludge during sludge Adaptation (StageIII):

Hours	Activated Sludge	
	pH	ORP
0	6.22	-141
24	5.43	-18
48	5.18	14
72	3.73	227
96	3.07	260
120	3.03	324
144	2.95	332
168	2.94	352
192	2.89	382
216	2.75	445

Bioleaching experiment:

$FeSO_4$ = 10 g/L, 1%

Inoculums = 10%

Table A7 pH & ORP variation during bioleaching experiment

Day	pH	ORP (mV)
0	6.18	-107
2	2.6	449
4	2.58	477
6	2.48	509
8	2.47	524
10	2.44	533

Appendix B Calibration and Heavy Metal Measurements

Calibration for Cu:

signal	conc.
0.053	1
0.162	3
0.253	5
0.498	10
0.729	15

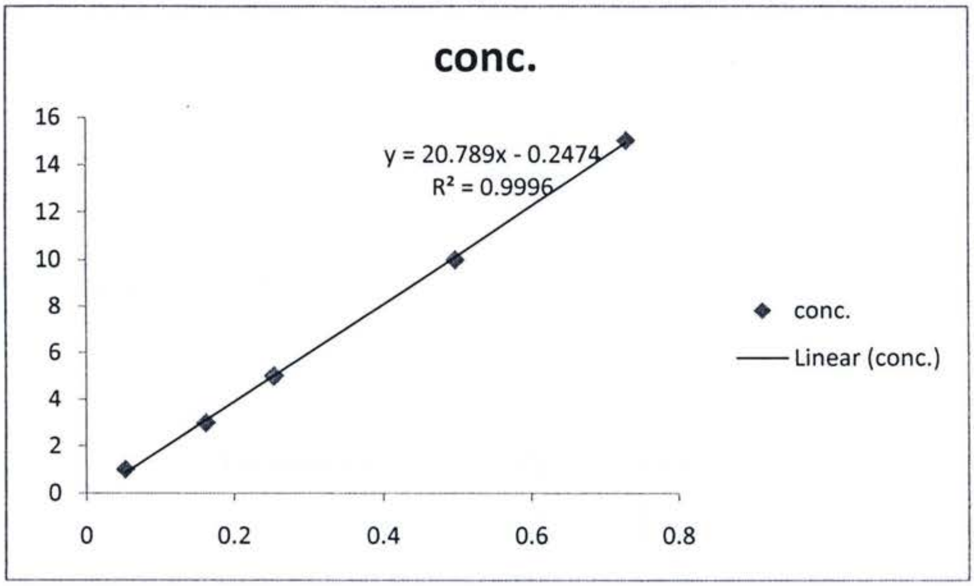

Figure B1 Calibration curve for Cu

	signal	con(ppm)	Removal
bioleach	0.157	3.016473	64%
bioleach	0.136	2.579904	69%
bioleach	0.106	1.956234	76%
raw	0.41	8.28	

Calibration for Zn:

signal	conc
0.273	1
0.771	3
1.148	5
1.291	6
1.473	8

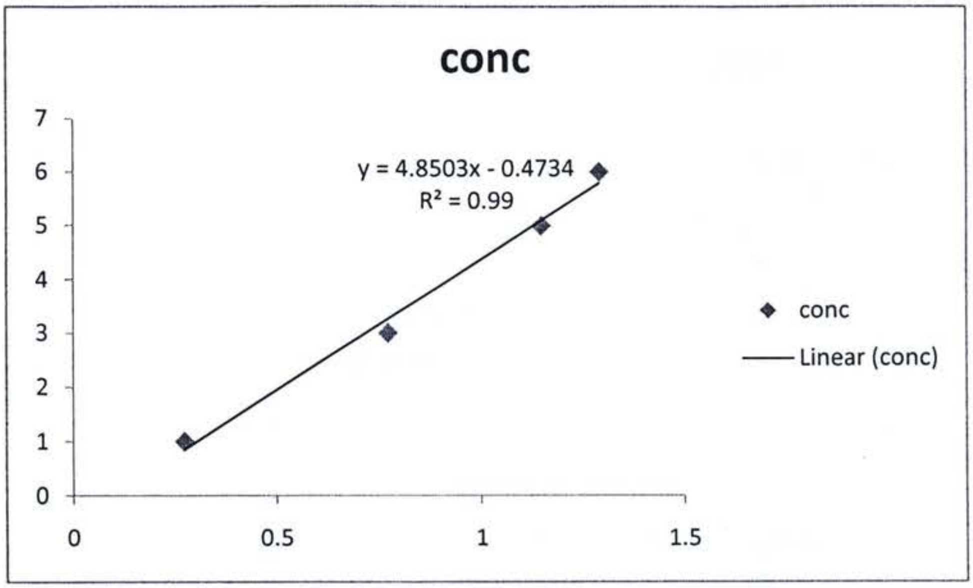

Figure B2 Calibration curve for Zn

Zn

	signal	conc
bioleach	0.437	1.646181
bioleach	0.509	1.995403
bioleach	0.551	2.199115
raw	1.412	6.38

	Signal	Conc(ppm)
bioleach	0	0.0067
bioleach	0.0002	0.0089856
bioleach	0.0001	0.0078428
raw	0.002	0.029556

Calibration for Cd:

signal	conc
0.009	0.1
0.016	0.2
0.087	1

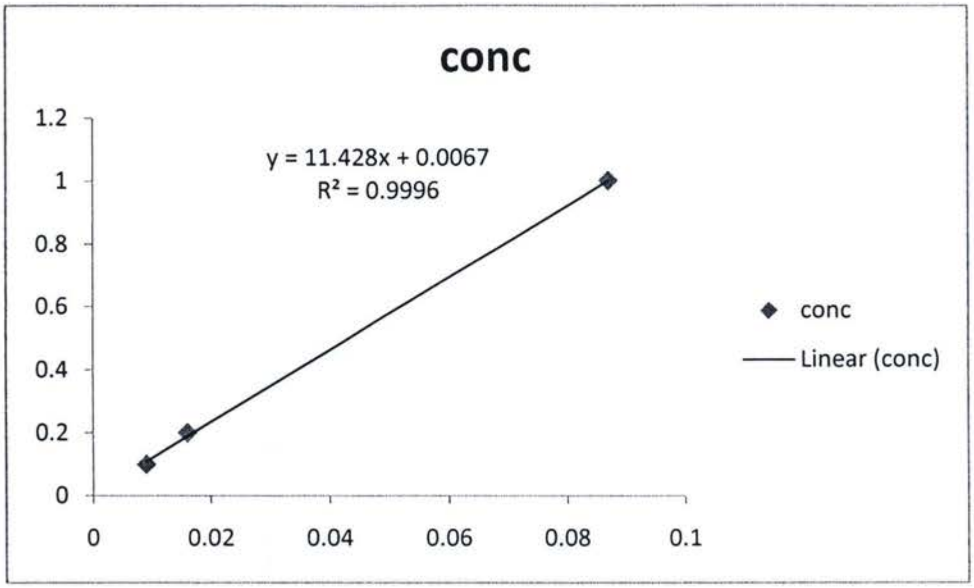

Figure B3 Calibration curve for Cd

	Signal	Conc(ppm)
bioleach	0	0.0067
bioleach	0.0002	0.0089856
bioleach	0.0001	0.0078428
raw	0.002	0.029556

Appendix C Results of Total Coliform Test

Table C1 Enumeration of Total Coliform (Raw Sludge), Sample No.1

Original sludge sample diluted (dilution Factor, 10^{-2})

Bottle	1	2	3	4	5
Sample Volume	50 ml	45 ml	45 ml	50 ml	50 ml
Dilution		10^{-1}	10^{-2}	10^{-3}	
Dilution Factor		10	100	1000	
Number of colonies	TMTC	TMTC	105	12	no
Total coliform/100 ml			23333.33*100	24000*100	

Table C2 Enumeration of Total Coliform (Raw Sludge), Sample No. 2

Original sludge sample diluted (dilution Factor, 10^{-1})

Bottle	1	2	3	4	5
Sample Volume	50 ml	45 ml	45 ml	50 ml	50 ml
Dilution		10^{-1}	10^{-2}	10^{-3}	
Dilution Factor		10	100	1000	
Number of colonies	TMTC	TMTC	TMTC	112	no
Total coliform/100 ml				224000*10	

Table C3 Enumeration of Total Coliform (Bioleached Sludge), Sample No.1

Original sludge sample diluted (dilution Factor, 10^{-1})

After 3 days of bioleaching period

Bottle	1	2	3	4	5
Sample Volume	50 ml	45 ml	45 ml	50 ml	50 ml
Dilution		10^{-1}	10^{-2}	10^{-3}	
Dilution Factor		10	100	1000	
Number of colonies	199	TFTC	TFTC	TFTC	no
Total coliform/100 ml	398*10				

Total coliform/100 ml=3980

Table C4 Enumeration of Total Coliform (Bioleached Sludge), Sample No.2

After 3 days of bioleaching period, without dilution

Bottle	1	2	3	4	5
Sample Volume	50 ml	45 ml	45 ml	50 ml	50 ml
Dilution		10^{-1}	10^{-2}	10^{-3}	
Dilution Factor		10	100	1000	
Number of colonies	TMTC	179	19	TFTC	no
Total coliform/100 ml		3978	4222		

Total coliform/100 ml=4100

Table C5 Enumeration of Total Coliform(Bioleached Sludge), Sample No.1

Original sludge sample diluted (dilution Factor, 10^{-1})

After6 days of bioleaching period

Bottle	1	2	3	4	5
Sample Volume	50 ml	45 ml	45 ml	50 ml	50 ml
Dilution		10^{-1}	10^{-2}	10^{-3}	
Dilution Factor		10	100	1000	
Number of colonies	155	19	TFTC	TFTC	
Total coliform/100 ml	3100 (310*10)	4220 (422*10)			

Total coliform/100 ml=3660

Table C6 Enumeration of Total Coliform(Bioleached Sludge), Sample No.2

After6 days of bioleaching period, without dilution

Bottle	1	2	3	4	5
Sample Volume	50 ml	45 ml	45 ml	50 ml	50 ml
Dilution		10^{-1}	10^{-2}	10^{-3}	
Dilution Factor		10	100	1000	
Number of colonies	TMTC	139	21	TFTC	
Total coliform/100 ml		3089	4667		

Total coliform/100 ml=3878

Table C7 Enumeration of Total Coliform (Bioleached Sludge), Sample No.1

After9 days of bioleaching period, 10^{-1} dilution

Bottle	1	2	3	4	5
Sample Volume	50 ml	45 ml	45 ml	50 ml	50 ml
Dilution		10^{-1}	10^{-2}	10^{-3}	
Dilution Factor		10	100	1000	
Number of colonies	62	62	TFTC	TFTC	no
Total coliform/100 ml	1240 (124*10)				

Total coliform/100 ml=1240

Table C8 Enumeration of Total Coliform(Bioleached Sludge), Sample No.2

After9 days of bioleaching period, without dilution

Bottle	1	2	3	4	5
Sample Volume	50 ml	45 ml	45 ml	50 ml	50 ml
Dilution		10^{-1}	10^{-2}	10^{-3}	
Dilution Factor		10	100	1000	
Number of colonies	TMTC	65	TFTC	TFTC	no
Total coliform/100 ml		1300			

Total coliform/100 ml=1300