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Using bioleaching to remove metals from sewage sludge intended for land application

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NOTE TO USERS

USING BIOLEACHING TO REMOVE METALS FROM SEWAGE SLUDGE INTENDED FOR LAND APPLICATION

by

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Using Bioleaching to Remove Metals from Sewage Sludge intended for Land Application

Master of Applied Science, 2003, Jun Nie

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Abstract

Removal of heavy metal contaminants from sewage sludge is a necessity before it is used as an agricultural fertilizer (biosolid), due to environmental concerns and municipal, provincial and federal regulations. The bioleaching method is recommended as an economical and effective process for the removal of heavy metals from the Ashbridges Bay Treatment Plant (ABTP) sludge, some of them with concentrations exceeding the recommended level by the Ontario Ministry of Agriculture, Food, and Rural Affairs guidelines.

The Gram-negative thiobacilli is a group of organisms with physiological and morphological similarity and grows by oxidizing ferrous ion and reduced sulphur compounds. One species of thiobacillus, *T.ferrooxidan*, was recommended as an effective bacterium for the heavy metal removal from sewage sludge.

This research involved the incubation of adapted sludge using fresh raw digested sludge and activated sludge of ABTP. Using adapted sludge for the bioleaching process, the method was tested in a continuously stirred tank reactor (CSTR) in combination with a series of jar tests. Results showed that the metal removal efficiency increases with decreasing pH, and the solids content does not affect the removal efficiency of cupper

and zinc very much during short term jar test. The results from the long-term (20-day) CSTR test demonstrated that the high *T. ferrooxians*-contained adapted sludge could remove copper from the sewage sludge of ABTP very effectively, by as much as 79.2%. In comparison, the simultaneous removal efficiency of zinc and cadmium were also studied for the same process and, they are 82.0% and 83.9% respectively. The TSS degradation constant rate during the 20 days' bioleaching was found to be 0.0522 day^{-1}.

It is concluded that Ontario should continue to apply sludge to agricultural land, as sludge is an economic alternative, promotes recycling of resources, and is a valuable fertilizer. However, the toxic metals in sludge should be removed from sewage sludge using the bioleaching process to recommended level before it is disposed as a fertilizer for land application.

Acknowledgements

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List of Abbreviations and Symbols

ABTP: Toronto Ashbridges Bay Treatment Plant

CSTR: Continuously Stirred Tank Reactor

DDW: Distilled Deionized Water

DO: Dissolved Oxygen

G+C: Genomic Guanine Plus Cytosine Content

GFAA: the Graphite Furnace Atomic Absorption

MOE: the Ministry of the Environment

OMAFRA: Ontario Ministry of Agriculture, Food, and Rural Affairs

ORP: Oxidation/Reduction Potential

RCO: the Recycling Council of Ontario

SCIA: The Soil and Crop Improvement Association

SSDML: Simultaneous sewage sludge digestion and metal leaching

TS: Total Solid

TSS: Total Suspended Solids

VS: Volatile Solids

VSS: Volatile Suspended Solids

1. Introduction

Wastewater treatment plants generate a waste by-product known as sludge, which may be collected from the grit channels and sedimentation basins. 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). Sludge contains nutrients for plant growth and is a source of organic matter for soil conditioning, making it an excellent candidate as a valuable fertilizer (Viessman and Hammer, 1998). 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. However, the possibility exists for contamination of surface water, groundwater, soil, crops, livestock, and ultimately humans as sludge contains harmful contaminants such as dioxins, pathogens, polychlorinated biphenyls, pesticides, and especially heavy metals, including cadmium, chromium, cobalt, copper, iron, manganese, mercury, molybdenum, nickel, silver and zinc.

As a result, the land application of sludge is still a controversial issue. There are scientists, farmers, and public who support the land application of sludge, claiming that sludge is a valuable resource, and land application provides a more economical and an environmentally preferable disposal alternative. On the other hand, there are many others who strongly oppose the land application of sludge, arguing that the possibility of water, soil, and crop contamination is too high and ultimately the health of livestock and humans may be at serious risk (Crittenden, 2002).

In the year of 2000, Ontario spreads approximately 200,000 tonnes of sludge on 25,000 acres of agricultural land (Sidhwa, 2001). In 1997, the City of Toronto assigned about half the sludge generated, 53,000 tonnes, from their Ashbridges Bay wastewater treatment plant (ABTP) to land application. This initiative not only saved the City money, but also helped the City to phase out the operation of their sludge incinerator, as community members had been lobbying the City to stop the operation of the sludge incinerator owing to air pollution problems.

However, according to the sampled heavy metal concentration (provided in Appendix A) in the digested sludge of ABTP in the year 2001, the concentration of copper exceeded the recommended limit for agricultural use most frequently. Table 1-1 shows the average amount of metals found in digested sludge at ABTP, compared to Ontario Ministry of Agriculture, Food, and Rural Affairs (OMAFRA) guidelines (Appendix D). As indicated in the table, there are three sludge metals concentrations that either exceed or are relatively close to recommended levels (Blais et al., 1992), and they are Cadmium (Cd), Copper (Cu) and Zinc (Zn) (presented in bold). Figure 1-1 is a summary of the most important findings from Table 1-1.

Table 1-1 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%**

□ Conc. In ABTP

■ OMAFRA Guideline

□ Recommended Conc.
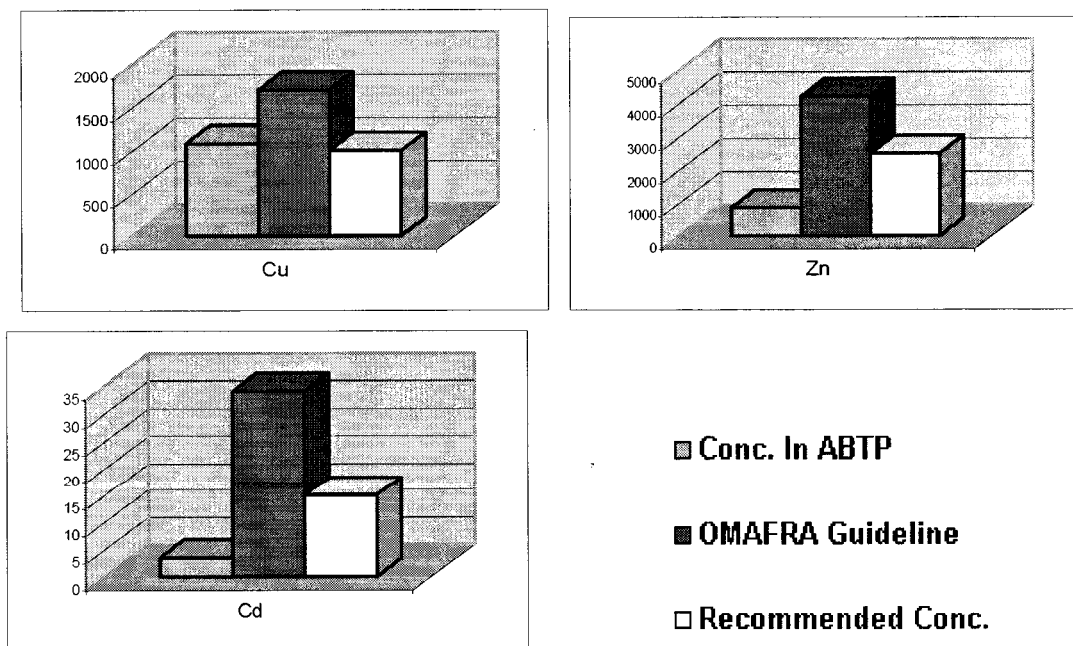
Figure 1-1 Heavy Metal Concentration Comparison

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The higher is the sludge concentration of heavy metals, such as copper, mercury or zinc, allowed for land application, the greater is the possibility of contamination. The transport and fate of metals from the land application of sludge has been extensively studied, and most studies concluded that problems of a low annual flux of metals from soil, a build-up of heavy metals in soil, and possible emissions from soil are more significant if the concentrations of contaminants are high. Furthermore, metals can be absorbed up by vegetation, and this poses an added potential to contaminate crops, water, livestock, and eventually humans through the food chain.

The municipality has already considered ending sludge incineration at the ABTP for long time. Incineration has now been reduced by 50%; and full closure is expected to happen in the very near future. The firm USF has been licensed for marketing the City's biosolids pellet product (a fertilizer suitable for agricultural and lawn use).

Considering the current situation of sludge treatment and utilization, the objectives of this thesis are to investigate the effect of pH, solid concentrations and other factors including bioleaching time, temperature and dissolved oxygen (DO) on the bioleaching process, to monitor the variation of solid concentration during bioleaching process. This is done by inoculating a high concentration of Thiobacillus bacteria (e.g. *T. ferrooxidans*) into the sewage sludge of ABTP and uses them in a bioleaching process to remove copper before the sludge is produced as a fertilizer for agricultural application. Another objective of this thesis is to review the existing legislation, the transport and fate of contaminants, current issues in Ontario regarding land application of sludge, and finally make recommendations based on the information gathered.

The research involves the inoculation of adapted sludge containing high concentration of useful thiobacillus bacteria into the sewage sludge of ABTP, and demonstrates the effectiveness of Cu removal from sewage sludge by the bioleaching method. A series of long- and short-term experiments covering the governing factors for bioleaching, such as solid contents, pH and reaction time will be carried out. Simultaneously, to verify the removal efficiency by bioleaching method for other metal removal, Cd and Zn are also tested and monitored. The reasons are: (a) Zn and Cd have the second (34.2%) and third (23.3%) highest concentration rates following Cu (108.3%), the rate is the concentration of metal in digested sludge against maximum allowable recommended metal concentration in sludge of OMAFRA guideline; (b) According to previous research (Agostinelli, 1995), as shown in Table 1-2, which contains the average amount of metals found in digested sludge at the Main Treatment Plant (MTP, now called Ashbridges Bay Treatment Plant) in 1995, the most problematic metals are Cd and Cu. In fact, Cd is the only metal that exceeds ministry guidelines in this table. Although the concentration of Cd is currently less than required level of guideline, considering the historical record, it is also used as a monitored element in this thesis.

Table 1-2 Annual average heavy metal concentration in digested sludge at MTP in 1995
(Unit: mg/kg of solids)

Metals	MTP	OMAFRA guideline	Recommended level
As	4.9	170	170
Cd	**39.8**	**34**	**15**
Co	8.4	340	340
Cr	504.7	2800	1000
Cu	**1371.8**	**1700**	**1000**
Hg	6.6	11	11
Mo	21.4	94	94
Ni	67.8	420	180
Pb	411.0	1100	500
Se	4.5	34	34
Zn	1446.0	4200	2500

2. Background Information

2.1 Land Application of Sewage Sludge

2.1.1 Risks of Land Application

There are three main risks associated with the agricultural land application of sludge:

1. The possibility for contamination of surface water, ground water, soils, crops, and air.

2. A health risk to humans, livestock, and natural habitat from consuming contaminated food, water, or breathing contaminated air.

3. The production of greenhouse gases like methane and nitrous oxide.

The possibility for mobility of contaminants through the various media is dependent on four major factors: the site, weather conditions, management practices, and heavy metal content of sludge.

The site is an important factor when addressing the transport of contaminants. It involves the topography, the presence and nature of buffer zones (such as natural vegetation between the sludge application site and any surrounding habitat, water bodies, or residential areas) and the soil characteristics.

Weather conditions may influence the mobility of contaminants. Alloway and Jackson (1991) investigated a number of studies where they found a potential correlation between downward movement of metals in soil and climate. Richards et al., (1998) found that the rate of metal percolation through soils is related to the amount of precipitation. Increased percolation rates typically occur during the wet seasons such as spring and fall. Management of farmland influences the level of contaminant mobility.

There are two application methods, surface spreading and direct injection, that influence mobility (Payne, 2001). Surface spreading involves spraying sludge directly onto the soil surface. This method increases the potential for odour, contaminant run-off, and nitrogen loss. Direct injection, on the other hand, involves injecting sludge directly into the soil. This method reduces the chances of odour, contaminant run-off, and nitrogen loss. Finally farmers must be aware of the contaminant uptake rates of different crops and different livestock, and manage the land to minimize the contaminant build-up in crops and livestock. For example any root and leaf vegetables grown on sludge treated soil tend to have much higher concentrations of heavy metals and harmful organic matter than non-leafy and non-root vegetables (Davis and Coker, 1980), therefore farmers should plant non-leafy and non-root vegetables.

Finally the content of sludge applied will definitely influence the mobility of contaminants. If sludge with a higher concentration of metals is applied then the possibility of mobility and contamination will be higher. So, it is strongly recommended the sludge be prohibited from being applied in agricultural soils without any further treatment (metal removal).

2.1.2 Transport and Fate of Contaminants in Sewage Sludge

Contaminants found in sludge can be classified into two main categories, metals and organic matter (nutrients). The transport and fate of metals from the land application of sludge has been extensively studied and most studies illustrate a low annual flux of metals from soil, often a build-up of heavy metal in soil, and possible emissions from soil or leaches to the groundwater. The possible fates of sludge contaminants including heavy metals and organic pollutants are shown in Figure 2-1(Lowe and Min 1996).

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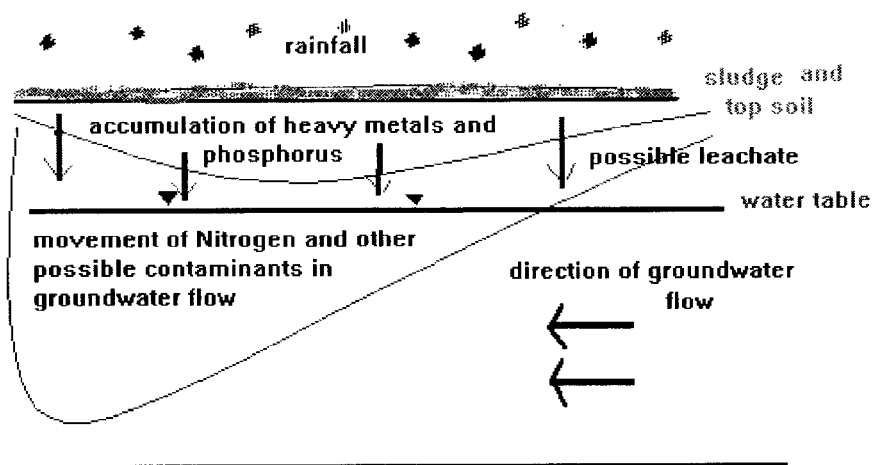
Figure 2-1 Possible fates of sludge contaminants

There is the possibility that land treated with sludge may leach heavy metals and harmful organic matter and ultimately contaminate groundwater, surface water, and well water. Joshua et al., (1998) found that soils treated with sludge had considerably less surface runoff than non-treated soils, as sludge increases the water bearing capacity of most soils. Therefore less runoff reduces the possibility for contaminant runoff and surface water contamination. Furthermore, heavy metal and nutrient concentrations in surface water runoff are rarely significant enough to result in any surface water contamination (Joshua et al., 1998). Joshua et al., (1998) found the potential for surface water contamination is minimal from the land application of sludge, as long as the proper guidelines and management practices are followed.

The foregoing studies illustrate the potential for surface water contamination is minimal from both metals and organic matter; however the potential for groundwater contamination by metals is more significant than organic matter. These studies also reveal that metals and organic matter often had low annual flux rates from soils, but these

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low flux rates may influence long-term water quality standards (Richards et al., 1998 and Wilson et al., 1996). Ultimately it suggests as long as management practices are followed, little threat exists for the contamination of groundwater.

Applying sludge to agricultural land may result in a concentration of contaminants in surface soil. Richards et al., (1998) found concentrations of metals (calcium, cadmium, chromium, copper, magnesium, nickel, phosphorous, lead, zinc) in surface soils (0-25cm) were much greater in sites treated with sludge than non-treated sites after a 30 year time period. Below a depth of 25cm metal concentrations were fairly similar between sites treated with sludge and without sludge (Richards et al., 1998).

Minimal leaching of metals and organic matter from soils is often viewed as a favourable situation for sludge application, as the possibility for contamination of ground water is minimized. However if little leaching occurs, then metals and organic matter may concentrate in the surface soil, resulting in contaminated soil. Contaminated soil may lead to the contamination of crops from adsorption, the contamination of livestock from direct ingestion, and the contamination of surface water from run-off. Some experts also believe that sludge application over time will build up concentrations of metals and organic matter in surface soils, resulting in a reduction in soil productivity (Renner, 2000).

Crops that are grown on sludge treated soil may adsorb and concentrate metals from the sludge. In general the level of metal adsorption is dependent on the type of crop, contaminant characteristics, and environmental conditions such as temperature (Duarte-Davidson and Jones, 1996). Metals tend to concentrate in the roots and leaves of most

crops, resulting in leaf and root crops being high risk crops for land treated with sludge (Frost and Ketchum, 2000; Duarte-Davidson and Jones, 1996; Davis and Coker, 1980).

Davis and Coker (1980) investigated the relationship between cadmium and the land application of sludge and found cadmium levels are dependent on plant species. In general the highest amount of cadmium adsorption occurs in the roots of any plant, followed by the leaves. Therefore high-risk crops for humans and livestock are leafy vegetables or root vegetables like lettuce, spinach, and carrots. The lowest concentrations of cadmium are found in fruits, seeds and storage organs. Therefore low risk crops would be cereal grains and fodder crops like oats and wheat.

Richards et al., (1998) found no difference in metal concentrations of apple trees grown on sludge treated soil and non-sludge treated soil. However grasses treated with sludge had elevated levels of cadmium, nickel, copper and zinc as compared to non-sludge treated grass. Therefore, livestock that are allowed to graze on sludge treated grasses may tend to accumulate contaminants.

Ultimately, metals in sewage sludge are often organically bound and generally less available for plant uptake (Frost and Ketchum, 2000). However after repeated application of sludge, the metal concentration in soils will accumulate and increase the possibility for crop contamination.

The potential contamination of livestock from sludge is a function of many factors including the livestock species, the crop, the season, their diet, and management practices.

Crittenden (2002) reported that cabbages grown on cadmium-contaminated sludge have resulted in lesions of the liver and thyroid gland when eaten by sheep and elevated

levels of cadmium when eaten by pigs. However cabbages are a high-risk crop, as they are leafy and most likely to concentrate metals and organic contaminants. Smith et al., (2001) has found that bioconcentration of metals is likely to occur in livestock that graze on sludge treated land.

To address this problem many countries have introduced bans or "no grazing" waiting periods. The United States has outright banned sludge application for grazing lands (Smith, 2001). The United Kingdom has a 3-week waiting period before animals are allowed to graze. Ontario has a waiting period of 2 months for horses and cattle and 6 months for pigs, sheep, and goats (Payne, 2001). However concentrations of metals in grasses were found to remain elevated one year after application (Molina et at., 2000). Therefore the use of a waiting period may not be effective in reducing the risk of livestock contamination.

Finally, the possibility also exists for harmful air emissions to be released from sludge treated land, like ammonia, amines, and nitric oxide that may be harmful to human health or methane and nitrous oxide that are known greenhouse gases.

2.1.3 Current Initiatives in Ontario

The land application of sludge started out as a great option for sludge disposal, but has become a very controversial program. Communities are questioning the health risks of sludge. Others are questioning the legislation with doubts about how well the Ministry of the Environment (MOE) can actually control and monitor the land application of sludge (Crittenden, 2002). Some people believe that the land application of sludge is simply moving pollutants from the city to the country. Recently there was a drinking water catastrophe in Walkerton, Ontario where 7 people died and hundreds were

hospitalized as a result of *E.coli* bacteria found in the drinking water. This catastrophe has had a direct impact on the land application of sludge, and three major initiatives have been undertaken as a result (Crittenden, 2002).

(1) Recycling Council of Ontario:

The first initiative, undertaken by the MOE, is a large consultation project undertaken by the Recycling Council of Ontario (RCO) to explore public and professional opinions resulting in the potential modification of the *biosolid guidelines*. The council intends to visit each municipality, review all complaints that have been submitted and review new scientific evidence relating to the land application of sludge in an attempt to improve the existing legislation.

(2) The Nutrient Management Bill:

The second initiative also by the MOE, is the introduction of Bill 81, the Nutrient Management Bill, which will attempt to update the legislation that governs the management of manure, sewage sludge, and paper mill sludge. This Bill will address how sludge is processed, how it is stored, how it is transported, and ultimately how it is disposed. The land application of sludge will play a minor role in Bill 81, as it is only a sub-component. According to Guy Crittenden, the editor of *Solid Waste and Recycling* magazine, Bill 81 can either have a profound effect rewriting the *biosolid guidelines* completely or a minor affect with minor changes, depending on the course of action the Ministry chooses.

(3) Soil and Crop Improvement Association:

The Soil and Crop Improvement Association (SCIA) based in Southern Ontario is currently involved in a three-year study to measure the impact that land application of

sludge has on agriculture and the environment. This study will address the possibility for surface water and groundwater contamination by monitoring metal and nutrient movement through the soil profile (Sidwha, 2001). This study is partly sponsored by one of North America's largest private sludge haulers and spreaders, Azurix North America.

2.2 A Brief History of Bioleaching

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 *T.ferrooxidans*. Reaction mechanisms are given by the following equations, using pyrite (FeS_2) as an example (Agostinelli, 1995):

(2.1) $$2FeS_2 + 7O_2 + 2H_2O \rightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+$$

(2.2) $$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O$$

(2.3) $$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+$$
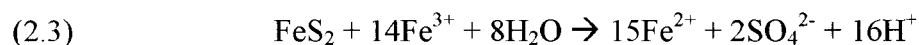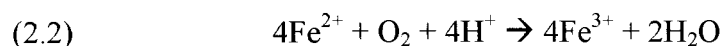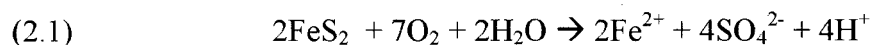
Some ferric ions hydrolyse in water to form ferric hydroxide as shown in this reaction:

(2.4) $$4Fe^{3+} + 12H_2O \rightarrow 4Fe(OH)_3 + 12H^+$$
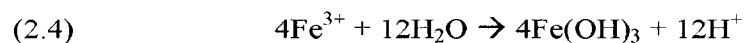
This process releases even more hydrogen ions into aquatic environment and continues to reduce pH.

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The above reaction can be applied to other mineral sulphides, such as galena, millerite and others. Regarding the copper, zinc and cadmium removals from sewage sludge by bioleaching method, as the major forms of those metals in sewage sludge are sulphides (Ito et al., 1998). The dissolution of metal sulphides occurs by two mechanisms (Jensen and Webb, 1994): 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:

(2.5) $$CuS + 2O_2 \rightarrow Cu^{2+} + SO_4^{2-}$$

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

(2.7) $$CdS + 2O_2 \rightarrow Cd^{2+} + SO_4^{2-}$$
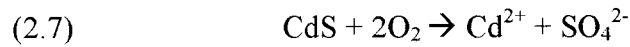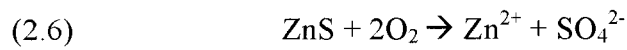
In the indirect mechanism, *T. ferrooxidans* have a very active oxidation function. They make a high contribution to oxidize the ferrous ion (Fe^{++}) to become ferric ion (Fe^{+++}) in a microbial reaction. The ferric ion then solubilizes the metal sulphides into soluble Cu^{++}, Zn^{++}, and Cd^{++} in chemical reactions according to the following principle formulas:

(2.8) $$2Fe^{3+} + CuS \rightarrow Cu^{2+} + S + 2Fe^{2+}$$

(2.9) $$2Fe^{3+} + ZnS \rightarrow Zn^{2+} + S + 2Fe^{2+}$$

(2.10) $$2Fe^{3+} + CdS \rightarrow Cd^{2+} + S + 2Fe^{2+}$$
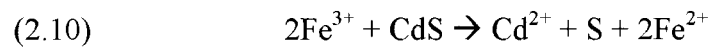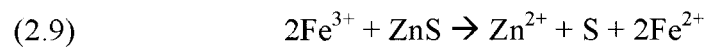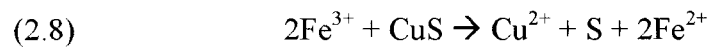
The relative contribution of the two leaching mechanisms depends on the type of sulphide mineral, the condition of ferric iron and on the operating conditions.

Bioleaching has been used in mining for many years and the technology is not new. However, the process of applying the bioleaching method to sludge treatment is quite innovative, and still remains at the experimental stage.

2.3 Bioleaching Bacteria - Thiobacilli

The Gram-negative thiobacilli are a group of organisms with physiological and morphological similarity and grow by oxidizing reduced sulphur compounds (Laskin and Lechevalier, 1973). The following table 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

One species of thiobacillus, T.ferrooxidans, is shown in Figure 2-2. They are Gram-negative bacteria (they lose the violet colour after decolourisation during Gram staining process). 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 are non-spore forming (Jensen and Webb, 1994).

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Figure 2-2 *T. ferrooxidans* cell suspension magnified 30,000 times

Source-online: http://www.mines.edu/fs_home/jhoran/ch126/microbia.htm

Table 2-2 shows the five main properties of this species:

Table 2-2 Main Properties of *T. ferrooxidans*

Properties	Description
1. Chemolithotropic	Energy for growth and maintenance is derived from the oxidation of ferrous iron or reduced sulphur compound (Bacon and Ingledew, 1989; Drobner and Stetter, 1990)
2. Autotrophic	CO_2 is the cellular carbon source with N and P also required as nutrients for cellular growth and synthesis along with trace mineral nutrients of K^+, Ca^{++}, Na^+, etc.,
3. Obligate Aerobic	Strictly need oxygen
4. Mesophilic	A temperature between 20 and 40 °C promotes growth and iron oxidation with an optimal temperature near 33 °C and with the growth and oxidation rate decreasing rapidly above the optimal temperature (MacDonald and Clark, 1970)
5. Acidophilic	Growth occurs at a low pH, typically ranges between 1.3 and 4.5

T. ferrooxidans are recognized as being responsible for the oxidation of iron and inorganic sulphur compounds. The following table shows their summarized physiological characteristics. As observed from the table, the optimal conditions for the growth of *T.*

ferrooxidans are high temperature, low pH, and an abundance of reduced sulphur compounds.

Table 2-3 Physiological characteristics of *T. ferrooxidans*

Condition	Characteristic
Optimum growth pH	1.3-4.5
Temperature range	10-37°C
Optimum temperature	30-35°C
Motility	0 to several polar or peritrichous flagella
Mol% G+C	56-59
Gram staining	Gram-negative
Spore formation	none
Shape	rod, 0.5-1 micrometers
Trophy	obligate chemolithoautotroph
Energy pathway	oxidation of Fe^{2+} and reduced sulphur
Oxygen requirements	obligate aerobe
Electron acceptor	oxygen
Nitrogen source	Ammonium salts, nitrate, fix dinitrogen

Source-online: http://www.mines.edu/fs_home/jhoran/ch126/microbia.htm

According to the literature (Sreekrishnan and Tyagi, 1996), heavy metals accumulated in sewage sludge can be leached out by (1) acidification process, (2) iron oxidation using *T. ferrooxidan*, or (3) sulphur oxidation using *T. thioparus* and *T. thiooxidans*. In this research, the combination of (1) and (2) will be utilized for the bioleaching experiments.

2.4 Recent Research and Study on Bioleaching

Tyagi and Blais from the University of Quebec have done a great deal of research on sludge metal bioleaching since 1990. The following excerpt contains their main findings, in addition to some of the most important findings of other investigators (Buchanan and Gibbons, 1974; Karamanev and Nikolov, 1988; Chen and Lin, 2000, etc.) regarding metal leaching from sewage sludge. All the results were obtained from experiments performed on simultaneous sewage sludge digestion and metal leaching (SSDML) process.

Effect of temperature

The results obtained show that the process can be employed efficiently for metal solubilization, 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 (the most frequently used is the coliform bacterium *E. coli.*) and solid degradation were found to decrease with temperature. A low metal solubilization efficiency was observed at 10°C. The solubilization of organic matter and nutritive elements (N, P and K) was not significantly affected by the variation in temperature. The fertilizer value (nutrient content, such as N, P and K) of sludge after leaching and digestion did not change significantly (Tyagi and Blais, 1996). Temperature optima for *T. ferrooxidans*, as studied by other investigators are listed 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)

The different optimum values in Tables 2-4 and 2-5 result from the diverse strains of bacteria used in the studies. In each case, specific bacteria resulted in different optimum temperature levels and different optimum pH levels.

Effect of pH

The results obtained on microorganism growth, sulphur oxidation, solids reduction and metal solubilisation suggest that the SSDML process can be conducted within a constant range of pH 2.0-2.5. The rate of sludge solids reduction under highly acidic conditions is similar to that of non-acidified sludge. Sludge digestion under acidic conditions reduces the indicator microorganisms more efficiently than in conventional mesophic aerobic or anaerobic sludge digestion. The partial solubilization of nutrients during the operation of the SSDML process does not reduce the fertilizer value of the sludge (Blais and Tyagi, 1992-3). The pH optima for *T. ferrooxidans*, are summarized in Table 2-5:

Table 2-5 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)

Effect of sludge solids concentration

The study 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. The metal solubilization efficiency (Cu, Mn, Ni and Zn) during the SSDML process in the bioreactor was not affected by sludge solids concentration to a large extent, except for Cd, Cr and Pb removal. The sludge solids content, in the range of 16-30g/l, did not affect the sludge solids reduction during the SSDML process operation at a bioreactor scale. The fertilizer value of the processed sludge remained intact. The process was more efficient than aerobic sludge digestion for the destruction of total coliforms in the range of solids concentration 10 to 50 g/l (Tyagi et al., 1997). Hence, it was found that 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).

One of the objectives of sludge treatment is to extract water from the solids and dispose of the dewatered residue. Since the volume reduction obtained by sludge concentration is beneficial to the overall sludge management, the solids degradation becomes important. According to the research (Blais et at., 1995), the solids including Total Solids (TS), Volatile Solids (VS), Total Suspended Solids (TSS), Volatile Suspended Solids (VSS) degradation rate constant(k, day^{-1}) during bioreactor tests at constant pH were summarized in the following table. As observed, a decreased degradation of various solids is observed with decreased pH from 7.0 to 4.0, however the reduction of various solids at pH 2.5 was observed to be similar to those at pH 5.5 and 7.0.

Table 2-6 Solids degradation rate constant (k, day^{-1})

Solids	pH			
	2.5	4.0	5.5	7.0
TS	0.0522	0.0398	0.0552	0.0626
VS	0.1000	0.0870	0.1035	0.1033
TSS	0.0818	0.0671	0.0937	0.0983
VSS	0.1126	0.0804	0.1032	0.0989
R^2(TS)	0.914	0.917	0.917	0.955
R^2(VS)	0.907	0.944	0.972	0.996
R^2(TSS)	0.974	0.960	0.944	0.996
R^2(VSS)	0.972	0.964	0.930	0.989

Other Growing Factors

- Metals removal from sewage sludge by indigenous iron-oxidizing bacteria

Indigenous sewage sludge iron-oxidizing bacteria were acclimated in twenty-three different sludge samples. The acclimation was carried out at pH 4.0 and 21±1°C. The required microflora were adapted in two to three successive transfers. The results showed that the oxidation of added Fe^{2+} in the form of $FeSO_4 \cdot 7H_2O$ decreased the sludge pH to 2.5. This pH reduction solubilized toxic metals to a level compatible with the recommended norms for agricultural use of the sludge. The adaptation of the microflora reduced the metal leaching period from 11-28 days to 2-10 days depending upon the sludge used (Blais et al., 1993).

- Comparison of acid and microbial leaching for metal removal from municipal sludge

Bioleaching process with sulphur as substrate and sulphur-oxidizing bacteria (bioreaction time of 5 days) was found to be better than the acid treatment process and microbial leaching with ferrous sulphate and iron-oxidizing bacteria (bioreaction time of 10 days) for solubilization of all heavy metals. Microbial leaching process with ferrous sulphate as substrate permits a better simultaneous solubilization of Cd, Cu, Mn and Zn than the acid treatment with sulphuric acid for one day, at a pH level of 1.5. However, the solubilization of aluminium, chromium, nickel and lead was less than acid leaching (Blais et al., 1992).

Other researcher also have done some work and produced the following interesting findings:

(1) Anaerobic leaching of covellite by *T. ferrooxidans*: *T. ferrooxidans* were found to be able to grow under anaerobic conditions on copper sulphide with ferric ion as the electron acceptor. The dissolution of covellite under these conditions (68% after 35 days) was higher than values observed aerobically in cultures with similar media composition and almost as high as under aerobic conditions without iron (Donati et al., 1997).

(2) Biosorption of Zn (II) by *T. ferrooxidans*: Variables like pH and biomass chemical pretreatment have been studied for its effect on the capacity for zinc biosorption by *T. ferrooxidans*. Also, studies to determinate the time for zinc adsorption were carried out. Results indicate that a capacity as high as 82.61 mg of Zn(II)/g of dry biomass can be obtained at a temperature of 25 °C and that the biosorption process occurs in a short time of 30 minutes (Celaya et al., 2000).

(3) Growth and Maintenance of *T. ferrooxidans* cells were studied and investigated, and a detailed description of the procedure is given in Barron and Lueking (1990).

(4) Removal and recovery of heavy metals by bacteria isolated from activated sludge treating industrial effluents and municipal wastewater were investigated (Leung et al., 2000), a total of nineteen metal-resistant and non-resistant bacteria from activated sludge were isolated and identified. The study of pH effect on metal removal for both species indicated that the metal biosorption increased with increasing pH from 2 to 6. Over 90% of copper absorbed on the cells could be recovered by washing with sulphuric acid within five minutes.

(5) Heavy metals bioleaching efficiency by Thiobacillus bacteria genera in activated sludge; Biological and chemical characterization of the sludge before and after the bioleaching process, and isolation of native microorganisms that was capable of leaching heavy metals were investigated (Blais and Tyagi, 1992), the metal solubilization efficiencies were Cd: 55-98%, Cu: 39-94%, Mn: 71-98%, Ni: 37-98%, Pb: 0-31% and Zn: 66-98%.

3. Materials and Methods

Very few studies have been performed on the removal of copper and other metals from sewage sludge by the bioleaching method. In addition, no reports have been found on any heavy metal removal issues by bioleaching for the biggest wastewater treatment plant in Toronto, the ABTP. Therefore, as shown in Figure 3-1, my research will involve a short-term experiment with the standard jar test, and a long-term experiment with continuously stirred tank reactor (CSTR), to study the metals removal efficiency under different control conditions. Incubation of adapted sludge was carried out before these tests, and the inoculation by 9K-medium was prepared afterwards. Normally, a control experiment should be carried out without incubated microorganism. However, it was observed from literatures (Blais et al, 1993; Tyagi et al., 1996) that toxic metal removal rates. are extremely low and negligible under normal circumstances. Therefore, the control experiment was omitted.
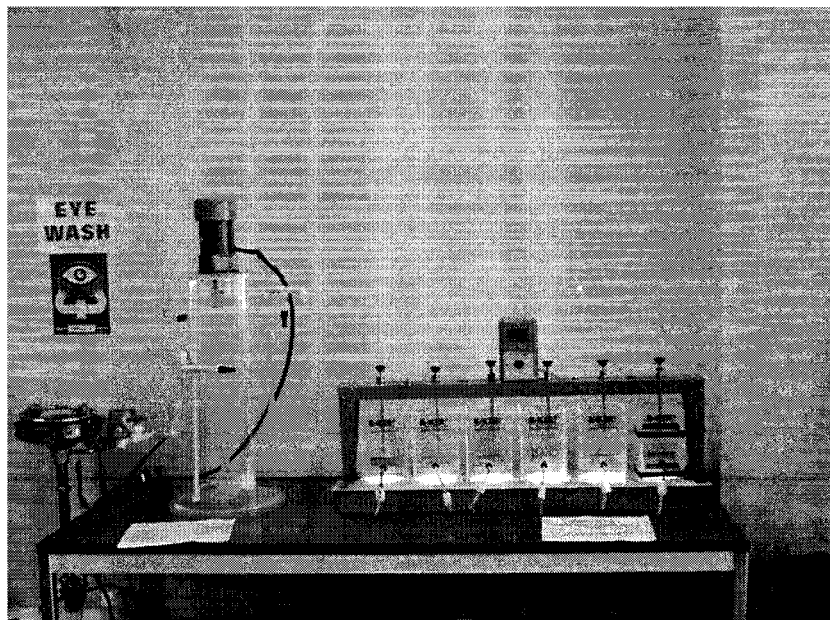
Figure 3-1 CSTR and Jar test devices

3.1 Incubation & Inoculation of Thiobacillus

Bacterial growth generally proceeds through a series of phases, as described in the following:

- Lag phase: time for microorganisms to become accustomed to their new environment. There is little or no growth during this phase.

- Log growth phase: bacteria logarithmic, or exponential, growth begins; the rate of multiplication is the most rapid and consistent.

- Stationary phase: the rate of multiplication slows down due to lack of nutrients and build-up of toxins. At the same time, some bacteria are dying so the numbers remain more or less constant.

- Endogenous phase: cell numbers decrease as growth stops and existing cells die off.

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). Bacterial leaching involving *T. ferrooxidans* requires sludge to be pre-acidified so that bacteria are in an adapted environment for optimal growth. As indicated in Table 2-3, the optimum growth pH for *T. ferrooxidans* is 1.3-4.5.

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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. From an initial value of around 150mV, the ORP eventually reaches 450mV. At that point, the population of *T. ferrooxidans* is assumed to be fully adapted. In the bioleaching process of *T. ferrooxidans* in sewage sludge, the increase in ORP can be attributed to the increase in the Fe^{+++}/Fe^{++} ratio.

Sampling sludge: In the study performed by Tyagi and Blais (1992), nineteen sewage sludge samples were obtained from nine wastewater treatment plants in the provinces of Quebec and Ontario, and in the state of Maryland (USA). The sampled sludge samples were from different units which include: secondary activated sludge, aerobically digested sludge, sludge from an aeration tank, primary sludge digested anaerobically, secondary sludge digested anaerobically, primary sludge and anaerobically digested sludge. The following table is the copper removal efficiency comparison among sampled sludges from different units.

Table 3-1 Copper removal efficiency comparison among sampled sludges
from different treatment unit (after Tyagi and Blais, 1992)

Sludges from different locations	Removal achieved (%) of Cu
Secondary activated sludge[a]	88
Aerobically digested sludge[b]	81
Sludge from aeration tank[c]	94
Primary sludge digested anaerobically[d]	52
Sludge digested anaerobically[e]	45
Primary sludge[f]	40

a: The activated sludge collected from secondary sedimentation tank, which

returns to aeration tank, also called "returned activated sludge".

b: Collected sludge after aerobic digestion.

c: Activated sludge collected from aeration tank.

d: Collected sludge after anaerobic digestion, the sludge for digestion is only

from primary sedimentation tank.

e: Collected sludge after anaerobic digestion.

f: Sludge collected from primary sedimentation tank

The copper removal efficiency is the highest for the sludge from aeration tank, the

reason for that is because of high DO in this unit, which is the basic living requirement

for *T. ferrooxidans*.

For this study, two types of sludge will be studied for comparison. 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. Sampled sludge will be collected and

shipped in temperature-controlled PVC containers, and kept at a constant temperature of 4° C in the refrigerator before use. 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 incubation, the adapted sludge is used for CSTR Phase I. 2mL of adapted sludge is also incubated on 9K medium to get inoculums which are used for CSTR Phase II.

A small portion of the collected sludge is firstly incubated and used for adapting the remaining sludge for the experiments. A summary of the samples used in the short-term jar tests and long-term CSTR (Phase I & II) experiments is provided in Table 3-2. It should be noted that while incubated & adapted sludge is used in the jar and CSTR Phase I tests, a pure culture incoculum has been applied to the CSTR Phase II test.

Table 3-2 Sludge samples used in experiments

Experiments	Sludge Sample
Jar Test	1. Mixture of incubated & adapted sludge (activated + digested) 2. Fresh raw digested sludge
CSTR Phase I	1. Mixture of incubated & adapted sludge (activated + digested) 2. Fresh raw digested sludge
CSTR Phase II	1. Pure culture inoculums 2. Fresh raw digested sludge

Incubation of adapted sludge: The following is the detailed incubation procedure (Tyagi and Blais, 1992); which is divided into two stages: Stage I - initial sludge adaptation and Stage II - final sludge adaptation.

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Stage I:

- A total of four 150 ml of sludge samples (two from the Aeration Tank and the other two 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 and 2N NaOH. Then 0.5% of $FeSO_4 \cdot 7H_2O$ (equivalent to 0.75g) was added to every flask containing sludge.

- 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) in a gyratory shaking incubator (Model C-25, New Brunswick Scientific Co.) at 125 revolutions per minute (rpm) as shown in Figure 3-2.
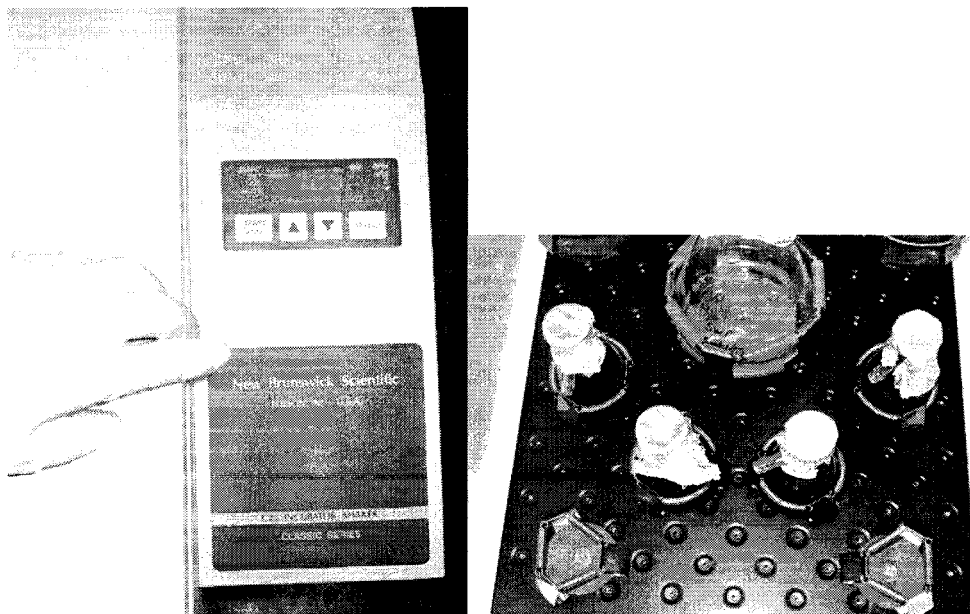
Figure 3-2 RPM adjustment for shaker incubator

- Samples were drawn at regular intervals (every 8 hr) during the initial period of adaptation to measure the pH and oxidation-reduction potential (ORP) by an Accumet Basic pH Meter, Model AB15. To measure pH, the glass pH electrode was used, while a platinum indicator electrode combined with a reference electrode was used to measure ORP.

- The first stage was considered complete after the initial adaptation, when the pH reaches a value around 2.5 or the ORP increased to around 450 mV.

Stage II:

- Based on the design of the incubator, a total of 175 ml adapted activated sludge incubated during stage I was transferred and mixed with 1750 ml of fresh activated sludge containing 0.5% of $FeSO_4 \cdot 7H_2O$(5 flasks) for re-incubation (28°C). The same transferring and mixing was arranged for the digested sludge. The detailed arrangement is shown in Table 3-3.

- The mixtures in the 10 flasks (5 for sludge of aeration tank and 5 for sludge of digester) are then re-incubated in the incubator (28°C). The pH and ORP are measured at 8 hr intervals until pH is about 2.5 or the ORP increased to around 450mV.

- When the rate at which the ORP increased to around 450mV was constant, the iron-oxidizing thiobacillis were assumed to be fully adapted. Considering that the pH and ORP activities of adapted sludge of activated sludge and digested sludge are quite similar, a mixture of them was used for the series of jar test and CSTR test.

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Table 3-3 Transferring & mixing arrangement for adapted sludge

Location of sampled sludge	Volume of adapted sludge (ml)	Volume of raw sludge (ml)	Volume of Flask (ml)	Added amount of $FeSO_4 \cdot 7H_2O$ (g)
Aeration Tank	80	800	1800	4
Aeration Tank	50	500	1000	2.5
Aeration Tank	15	150	250	0.75
Aeration Tank	15	150	250	0.75
Aeration Tank	15	150	250	0.75
Sub-total	175	1750		8.75
Sludge Digester	80	800	1800	4
Sludge Digester	50	500	1000	2.5
Sludge Digester	15	150	250	0.75
Sludge Digester	15	150	250	0.75
Sludge Digester	15	150	250	0.75
Sub-total	175	1750		8.75
Grand Total	350	3500		17.5

Inoculation

In numerous studies of *T. ferrooxidans* the 9K medium, developed by Silverman and Lundgren (Jensen and Webb, 1994), has been applied. The composition of the 9K-medium (containing 9000 ppm ferrous iron) is given in Table 3-4. The basal salts and iron solution were autoclaved separately and combined when cool.

The presence of iron-oxidizing bacteria in the adapted sludge was tested by mixing 2 ml of the sludge in 200 ml of 9K medium mentioned above. The samples were incubated for 2- 4 weeks at 30 °C. According to the foregoing research and study of Tyagi and Blais

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(1992), if dark reddish brown colour and/or precipitate appears, the sample can be considered positive for the presence of iron-oxidizing thiobacillus bacteria. Other methods of positive identification of thiobacillus bacteria include: Gram-staining, culturing and bacterial measurements (Blais, et al., 1996). Gram-staining method can be used to differentiate bacteria by using the purple dye. This dye combines with each bacterium turning it a purple colour. Bacteria that retain this colour after decolourising alcohol is added are classified as Gram-positive. Bacteria that lose the dark violet or purple colour after decolourisation are classified as Gram-negative. Thiobacillus bacteria are gram-negative. To culture bacteria, the growth media should be used to prepare agar plates. The thiobacillus bacterial population can be enumerated by this kind of direct plating. However, the colour and precipitation formation are adopted as indications of the thiobacillus bacteria for this study.

Table 3-4 Composition of 9K-medium (after Silverman and Lundgren, 1959)

Components	Content
Basal salts	
$(NH_4)_2SO_4$	3.0g
$MgSO_4.7H_2O$	0.50g
K_2HPO_4	0.50g
KCl	0.01g
$Ca(NO_3)_2$	0.01g
Distilled H_2O	700ml
5M H_2SO_4	Adjust pH to 2.3
Energy source	
$Fe_2SO_4.7H_2O$	44.25g
Distilled H_2O	300ml

3.2 Jar Test

Six Paddle Stirrer Model 7790 Jar (PHIPPS & BIRD) was used when adapted sludge was prepared. This equipment is designed to operate on nominal 120 volt, 60Hz AC current. The rotary speed was set as 66rpm. During every 48-hour Jar experiment, 6 different pHs and 3 different solid contents were set up.
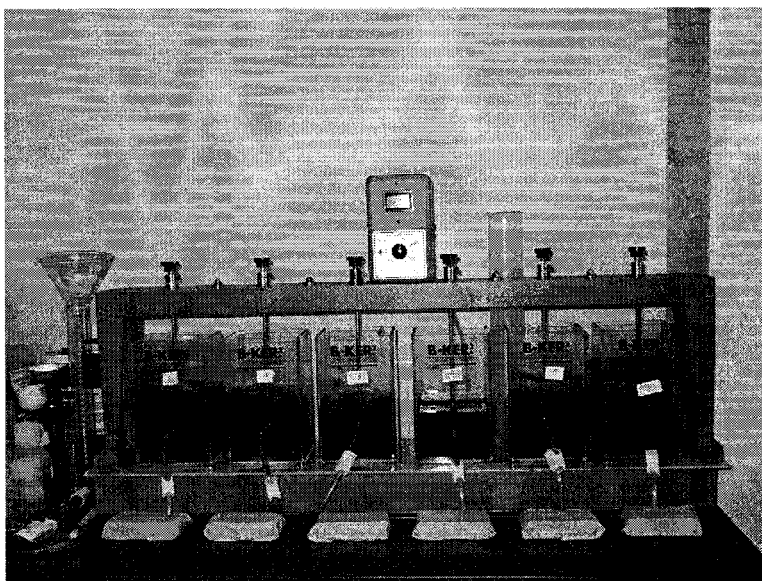
Figure 3-3 Jar Test

100 ml of adapted sludge was mixed with 900 ml of raw sludge sampled from digester as one unit jar test, thus 10% of adapted sludge was utilized for leaching experiment during jar test. The reason for this high content of adapted sludge (the standard is 5% adapted sludge) is that the jar test period is very short and a high concentration of *T. ferrooxidans* is required to get expected optimal pH and solid content. In addition, 0.5% (weight/volume) of $FeSO_4 \cdot 7H_2O$ (4.5g) was added to the raw sludge in every jar.

There are 3 runs and each run involves 6 jars, as shown in Figure 3-4:
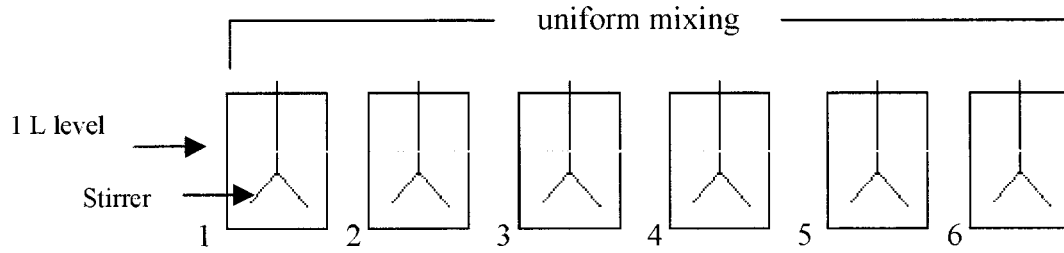
Figure 3-4 Schematic diagram of Jar Test

The volume of sample to be added to each jar is 1 litre. The objectives of the experiments are to determine the optimal performance of Cu removal under these conditions:

(1) Run 1: low pH range (4~6) and different solids (50% and 100%) contents;

(2) Run 2: high pH range (7~9) and different solids (50% and 100%) contents;

(3) Run 3: low and high pH range (4~9) with constant solids contents (25%).

The following table shows the arrangement for each run and different experimental parameters.

Table 3-5 Jar Test Arrangements

Jar # Runs	Jar 1	Jar 2	Jar 3	Jar 4	Jar 5	Jar 6
Run 1	50% TS pH 4	50% TS pH 5	50% TS pH 6	100% TS pH 4	100% TS pH 5	100% TS pH 6
Run 2	50% TS pH 7	50% TS pH 8	50% TS pH 9	100% TS pH 7	100% TS pH 8	100% TS pH 9
Run 3	25% TS pH 4	25% TS pH 5	25% TS pH 6	25% TS pH 7	25% TS pH 8	25% TS pH 9

In this table, 100% Total Solid (TS) here means the fresh digested sludge without any dilution; 50% TS is 0.5L fresh sludge + 0.5L DDW; 25% TS is 0.25L fresh sludge + 0.75L DDW.

3.3 CSTR Test

Figure 3-5 shows the set-up of the Continuously Stirred Tank Reactor (CSTR) system. The reason for adopting the CSTR for this research is because of high oxygen transfer efficiency, good mixing and simple operation. The CSTR could also be regarded as an aerobic sludge bioleaching reactor, which could be developed in future studies for treatment plant applications.

The CSTR is a cylindrical tank, with a continuous speed stirrer; complete mixing within the reactor is achieved by a 152 rpm, 115 volt, 2.8 amp electrical motor. A pure air cylinder is connected with reactor by a regulator, to adjust the air pressure introduced into the reactor. The pure air cylinder was used because of high content of oxygen, dust-free, and easy control. The sludge in the reactor is aerated by compressed air through the holes (air diffusers) placed at the bottom side of reactor. This compressed air can be recirculated through the reactor and this promotes mixing. This is demonstrated in Figures 3-5 and 3-6.
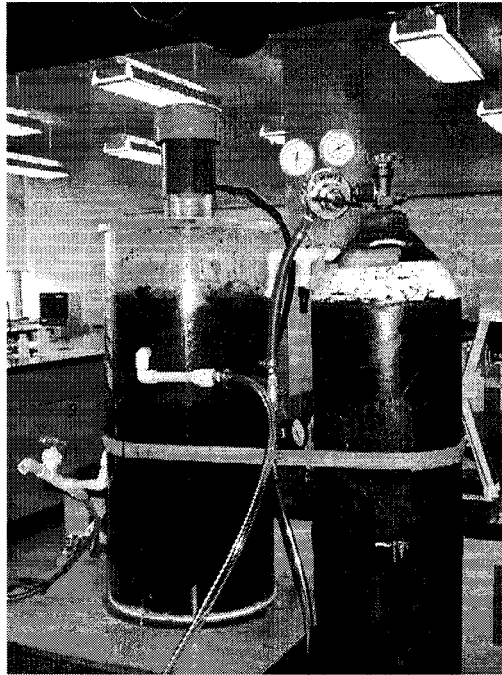
Figure 3-5 CSTR
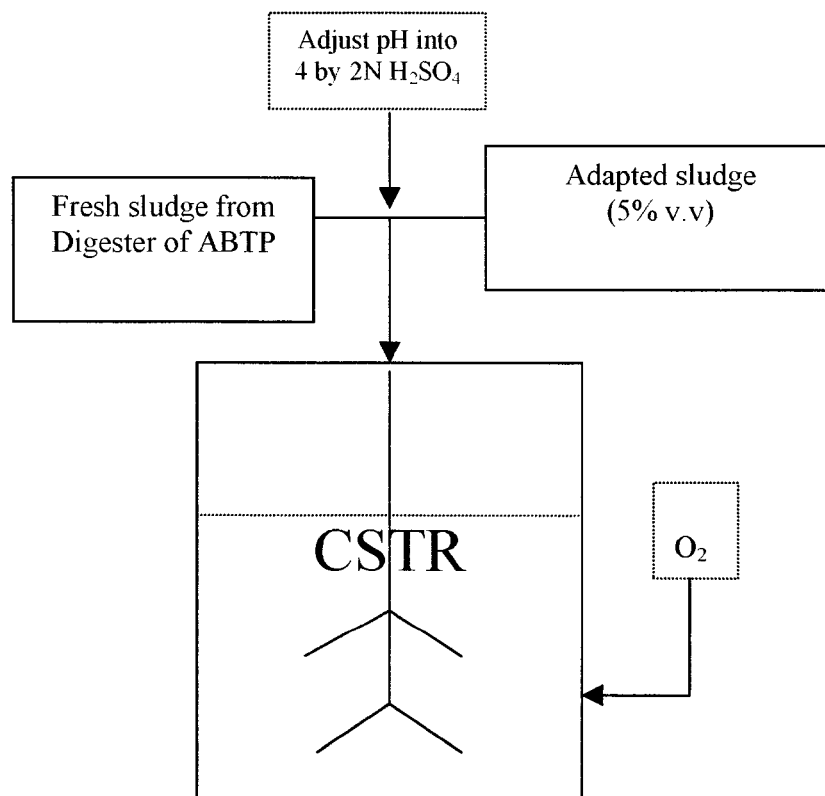
Figure 3-6 Schematic diagram of CSTR

According to information from previous research, the bioleaching period were found to vary between 2 day to 28 days, so this long term CSTR experiment was selected to be 3 weeks, with the sampling time for AA analysis conducted every 48 hours. In order to test the metal solubilization efficiency of adapted indigenous iron-oxidizing bacteria, a volume of 20L raw sampled sludge from the digester at ABTP is adjusted to pH 4.0. This is followed by the addition of $0.5\%(10g)$ $FeSO_4.7H_2O$ and 5% (1L) of adapted sludge incubated previously.

To compare the bioleaching efficiency of adding adapted sludge as opposed to adding inoculums (pure medium), when the 3-week period of the CSTR test for digested sludge is finished (Phase I), the fully inoculated sludge in 9K-medium was added into fresh digested sludge for another 10 days' CSTR operation (Phase II). The portion of inoculums with 9K-medium for the Phase II test is $5\%(0.8L)$, with the volume of fresh sludge as 16L. The pH was also adjusted to 4 initially. The sampling interval was once every 24 hours.

3.4 Analytical Methods

<u>**Atomic-Absorption Spectroscopy (AA) analysis:**</u>

Samples to be analyzed by AA must be vaporized or atomized, typically by using a flame or graphite furnace, for this research only the flame furnace was utilized. Atomic-absorption (AA) spectroscopy uses the absorption of light to measure the concentration of gas-phase atoms. Since samples are usually liquids, the analyte atoms or ions must be vaporized in a flame furnace. The atoms absorb ultraviolet or visible light and make transitions to higher electronic energy levels. The analyte concentration is determined

from the amount of absorption. Concentration measurements are usually determined from

a working curve after calibrating the instrument with standards of known concentration.

The Atomic-absorption equipment used was a market model PE-800, as shown in Figure

3-7.
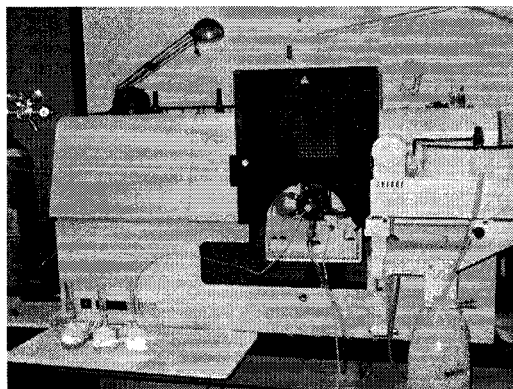
Figure 3-7 Atomic-absorption Spectroscopy

The concept of the AA equipment for the measurement of metal concentration in

liquid sample is described in Figure 3-8.
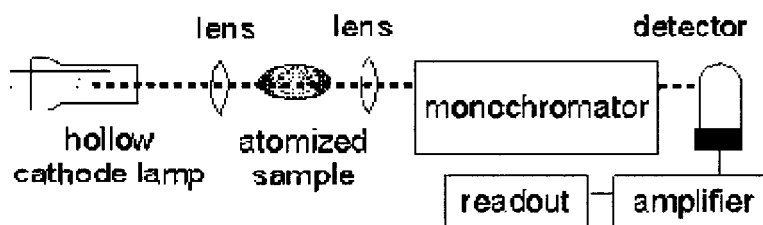
Figure 3-8 Schematic of an atomic-absorption experiment

Source-online: http://www.tu-bs.de/institute/pci/aggericke/PC4/Kap_I/aa.htm

The standard solution for Cu was set up as 5, 15 and 30 ppm, Cd at 2, 6 and 12 ppm,

and Zn at 1, 3 and 6 ppm, which are all within the detection range of the flame detection.

According to historic monitoring record provided in Table 2-5 and Appendix B of these

three metal concentrations of ABTP sludge, the concentration of Zn is out of the detection limit of the flame detection, which is from 1 to 6 ppm, so the sample must be diluted before feeding into AA equipment. For this experiment, all the samples for measuring Zn was diluted by 3 times, so that the value of Zn concentration of 14 mg/l from historical record can be converted to a third ($\approx$ 4.7), to be kept within the detection range.

Table 3-6 Historic monitoring record (sampled date: September 26, 2002)

Metals	Concentration in Wet Sludge (mg/L)
As	0.071
Cd	0.051
Co	0.08
Cr	2.07
Cu	21.4
Hg	0.02
Mo	0.33
Ni	0.48
Pb	1.34
Se	0.075
Zn	14

AA analytical procedure:

Setting up the software:

1. Switch on the power after opening the gas (acetylene) cylinder and air compressor.

2. Open the operating program AAnalyst, displayed on the computer screen, and select "Technique" and open 'Flame". The flame is ignited, exhaust fan is turned on; the source lamps are turned on; the optics/electronics have the proper settings; and the computer screen displays the operating menu.

3. From the File menu, select "Creating Methods". The "Create New Method" dialog appears.

4. In this dialog, select the element (Cu, Zn or Cd) and select one of the sets of parameter values to use as starting values; the system puts the selected recommended parameter values for the element into the "Method Editor".

5. In the "Method Editor", select parameter values that are suitable for the analysis of Cu, Zn or Cd.

6. Save the method for future use.

Setting up normal calibration and sample detection with flame techniques:

1. In the column of "Conc", type "5, 15, 30" for "Standard 1, 2, 3 of Cu, "2, 6, 12" for Cd, and "1, 3, 6" for Zn.

2. Bring the previously prepared solutions required for the AA analysis, as well as the 3 standard solutions for each metal. Also have a beaker of deionized water available as the "blank".

3. When ready to begin the analysis procedure, place the capillary in the blank solution and wait about 5 seconds. This requires about 20 sec and will automatically zero the output absorbance reading.

4. The computer will ask for [Standard 1], [Standard 2], [Standard 3]. These must be aspirated in order of ascending concentration. For improved precision, the AA

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procedure performs three repeated measurements on each solution. Continue
aspirating each solution until all replicate determinations are completed.

5. The computer will then request aspiration of [Sample]. Just put the capillary into
   the samples.

6. In the File menu, select Print > result.

7. When "Analysis is Complete", select the [OK] button. Remove the capillary from
   the solution; wipe it off; Switch off the Power and Gas cylinder; finally exit
   AAnalyst.

Test Procedure for Solid Content of Sludge:

According to the historic Total Solid (TS) record of digested sludge from ABTP

between October and December 2002, the variation in TS is very minor, between 1.6-

1.7%. It was therefore decided that no test for TS would be required for the jar test.

However, to study the variation of solid content caused by solid degradation during long

term CSTR test, the monitoring of total suspended solid (TSS) was performed. The

detailed sampling procedure is as following. Samples were taken every 48 hours:

1. Pre-heat clean aluminium dishes for 1 hr in an oven at 103-105 °C.

2. Remove a pre-heated aluminium dish from the oven with tongs and store in a
   dessicator.

3. Set up a filtration apparatus using the side-arm filtration flask, filter holder, filter
   funnel and tubing as shown in Figure 3-9.
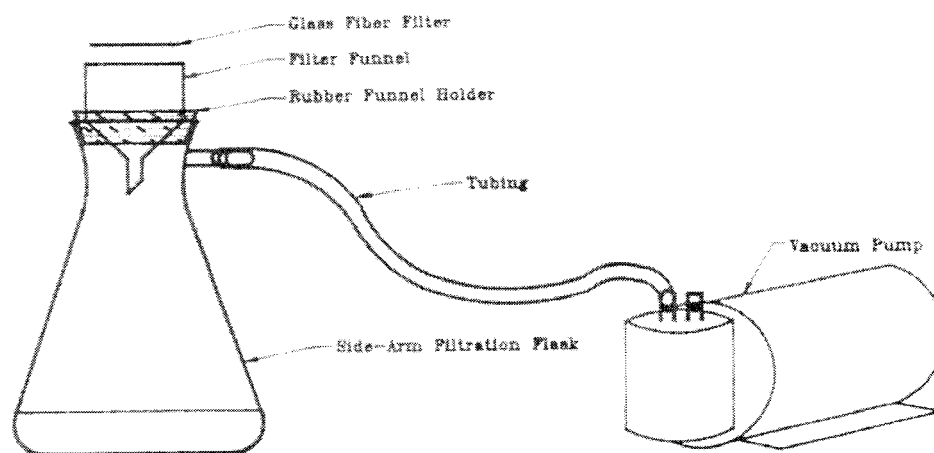
Figure 3-9 Filtration Apparatus

4. Weigh and record the mass of the aluminium dish, put a glass-fibre on it and weigh.

5. Carefully place preweighed filter with wrinkled side up into the funnel of the filtration apparatus with forceps.

6. Start the vacuum pump.

7. With a pipette, filter 20ml of a thoroughly mixed sample through the filter a little a time making sure the sample remains on the filter. After the sample is completely drained, rinse the filter with 10 ml volume of DDW, allowing complete drainage after rinsing, and continue suction for about 3 min after filtration is complete.

8. Using forceps, carefully remove the filter with residue from the filtration apparatus and transfer to the weighed aluminium dish.

9. Dry for 30 min in an oven at 103-105 °C.

10. After drying, cool the dish in a desiccator to balance temperature, and weigh. Record the weight.

<u>Calculations</u>

(3.1) $$\frac{MgTSS}{L} = \frac{(A - B)*1000}{sample\,volume, L}$$

A= weight of filter and solids (g)

B= weight of filter (g)

L= volume of sample (the actual volume of mixed liquor sample excluding DDW)

<u>Test Procedure for Heavy Metals Content of Raw Sludge</u>

1. Considering the AA test bottom limit, take 100ml of raw sludge from Digester of ABTP using volumetric cylinder and put into a 250ml beaker.

2. Put into beaker with 10ml HNO_3 and 5ml H_2SO_4 (to solubilize all metal from organic material in sludge).

3. Put the beaker on the heating block (300 °C) and mix up with glass stirring rod thoroughly for about 60 min so that metals in the sludge could be thoroughly acidified and solubilized.

4. Set up a filtration apparatus using the side-arm filtration flask, filter holder, and filter funnel and tubing as shown in Figure 3-9.

5. Carefully place preweighed filter with wrinkled side up into the funnel of the filtration apparatus with forceps.

6. Start up the pump

7. Slowly pour all acidified sludge sample through the filter. After the sample is completely drained, rinse the filter with 10 ml volume of DDW, allowing

complete drainage after rinsing, test the pH of the filtered solution, if the pH is less than 6, rinse the filter with DDW until the pH reaches 6~7, which means metals in the sample on the filter have been mostly rinsed into the flask

8. Continue suction for about 3 min after filtration is complete.

9. Transfer the filtered solution into a 250 ml quantitive flask and dilute it with DDW until it reaches 250 ml, this solution was prepared for AA analysis for metals Cd, Cu and Zn.

10. Using forceps, carefully remove the filter with residue from the filtration apparatus and transfer to the weighed aluminium dish.

11. Dry for 30 min in an oven at 103-105 °C.

12. After drying, cool the dish in a desiccator to balance temperature, and weigh. Record the weight.

13. Calculate the metal content per wet sludge (mg/L) and per dry sludge (mg/g)

Test Procedure for Heavy Metals Content of Bioleached Sludge

1. Samples of 100 ml were drawn from the reactors for both the jar test and the CSTR test.

2. The samples were centrifuged at 1650 rpm by Centrifuge for 15min.

3. The supernatant was filtered by filtration apparatus (Fig3-9) following filtration procedure in the previous section; the liquid portion was prepared for AA analysis of metals Cd, Cu and Zn.

4. Results and Discussion

4.1 Sludge Samples

As mentioned previously, two types of sludge will be studied for comparison during incubation of adapted sludge. The first was taken from the Aeration Tank, and the other was from the Sludge Digester (shown in Figure 4-2). Before the Incubation Stage for adapted sludge started, colour photographs were taken of the sludge samples, as shown in Figure 4-3. The darker coloured sludge in the figure is from the digester.
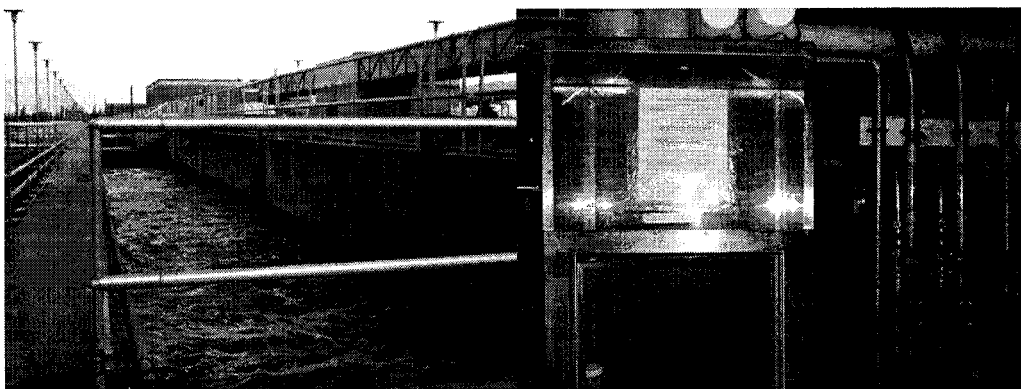
Figure 4-1 Aeration tank of ABTP   Figure 4-2 Digester's sludge feed

before incubation   after incubation
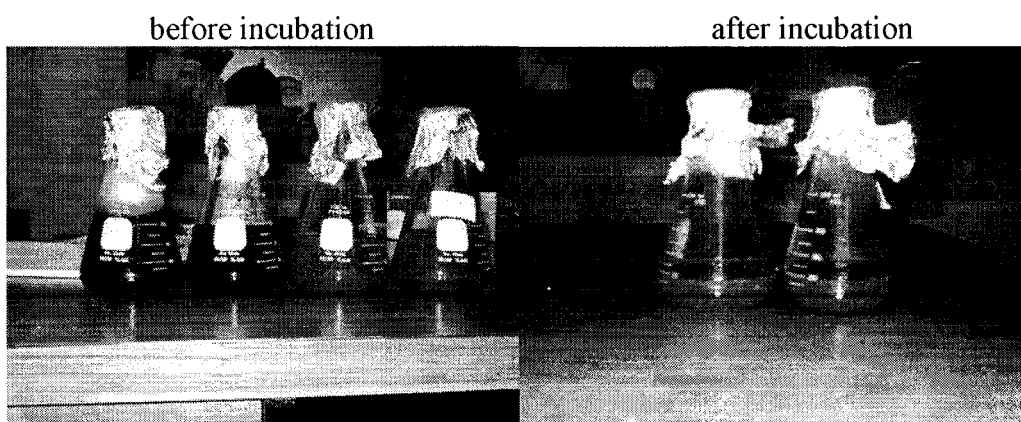
Figure 4-3 Colours of sludge from digester and aeration tank before & after incubation

The initial characteristics of the sludge used are presented in Table 4-1.

Table 4-1 Initial characteristics of the sludge

Parameters	Activated sludge	Digested sludge
pH	6.9	7.1
ORP (mV) after pre-acidification (pH=4)	161	138
Total Solid Content (TS)	N.A	1.6%
Total Suspended Solid (mg/L)	2000	8290
Temperature ($^\circ$C)	23.6	30.8

4.2 Sludge Incubation

4.2.1 Stage I Results

Stage I is the initial sludge adaptation period, the objective of this stage is to pre-adapt the sludge for around 7 days during which the pH of the sludge decreases steadily from around pH 7.0 to around pH 2.5, while the ORP increases from an initial value of around 150 mV to 450 mV.

Figures 4-4 & 4-5 present the sludge acidification pattern and changes in oxidation/reduction potential of two tested sludge.
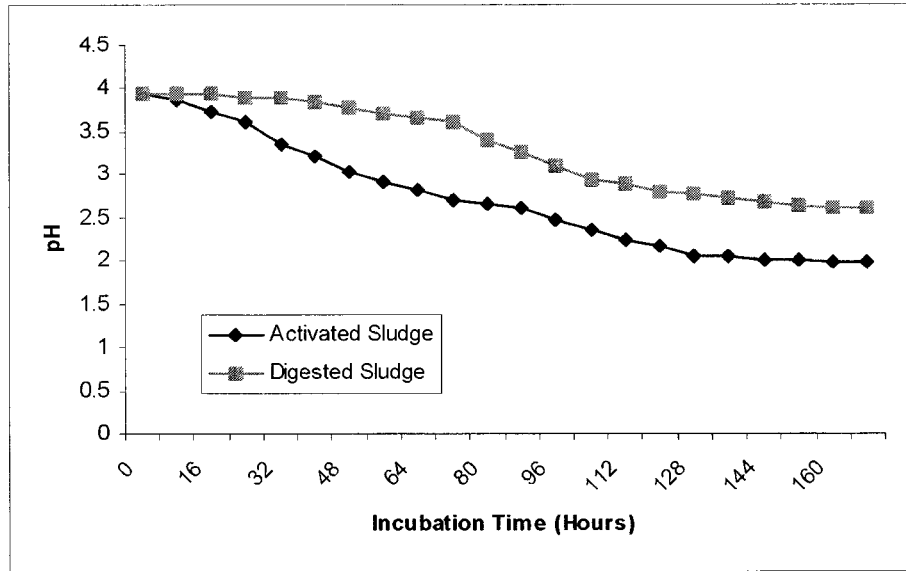
Figure 4-4 pH variation during incubation of activated and digested sludge(Stage I)
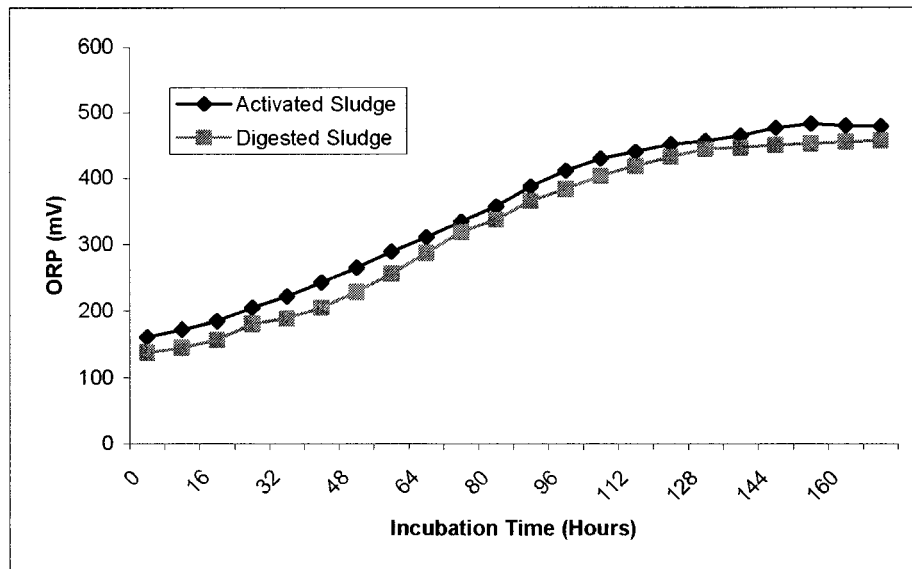
Figure 4-5 ORP variation during incubation of activated and digested sludge(Stage I)

The incubation period of Stage I is summarized in Table 4-2. The incubation period was set by 168 hr, because at this time, both pH and ORP for either activated sludge or digested sludge could reach the required range for adapting sludge.

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Table 4-2 Stage I Incubation Period

Condition	Digested Sludge	Activated Sludge
pH=2.5	168 hr	96 hr
ORP = 450 mV	120 hr	144 hr
Required incubation period	168 hr	144 hr
Actual incubation period	168 hr	168 hr

4.2.2 Stage II Results

Stage II is the period of final sludge adaptation. After this stage, the rate at which the

ORP increased to around 450~500 mV was constant, the pH also was at constant acidic

level to around 2~2.5, the iron-oxidizing thiobacillis is assumed to be fully adapted.

Figures 4-6 and 4-7 present the pH varying trend and changes in oxidation/reduction

potential for activated sludge and digested sludge.
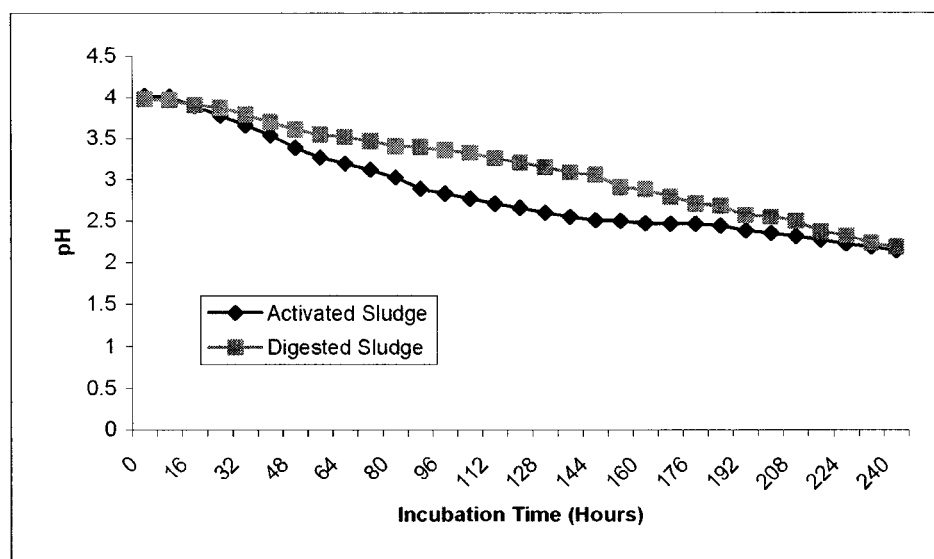
Figure 4-6 pH variation during incubation of activated and digested sludge(Stage II)

49
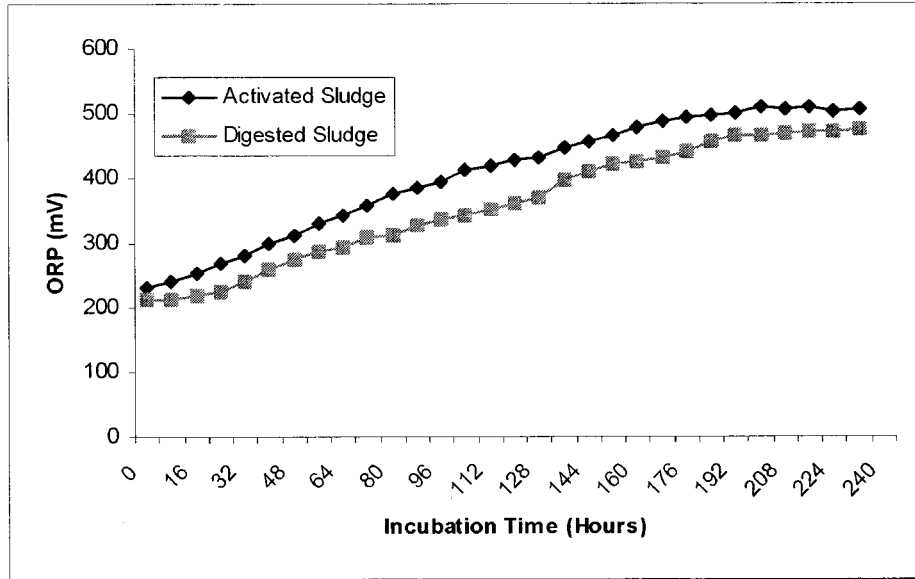
Figure 4-7 ORP variation during incubation of activated and digested sludge(Stage II)

Table 4-3 Stage II Incubation Period

Condition	Digested Sludge	Activated Sludge
pH=2.5	192 hr	128 hr
ORP = 450 mV	144 hr	184 hr
Required incubation period	192 hr	184 hr
Actual incubation period	232 hr	232 hr

The incubation period for stage II is summarized in Table 4-3, same as stage I, the longer period was selected to let both kinds of sludge be adapted. The incubation term for stage II is 11 days, while the stage I is 8 days. To get constant pH and ORP, and considering the large amount of the input raw sludge which is needed for incubation, and also to keep the required ORP (450mV) and pH (2.5) constant for a while, a longer incubation period was required for stage II.

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4.2.3 9K-medium Inoculation Results

Different from the study of Silverman and Lundgren (1959), the incubator used for the 9K-medium inoculation was not shaker incubator, but a stationary incubator that maintained a temperature at 30 °C. Also, this incubation did not involve any forced aeration, but instead used interval mixing by manual and surface aeration every 12 hours.

Initial colour   Colour after one week
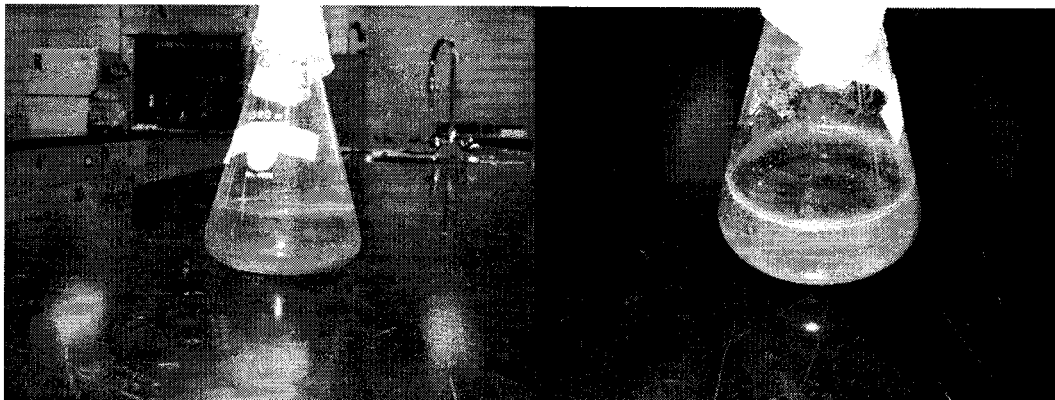
Colour after 2 weeks   Colour and precipitation after 3 weeks

Figure 4-8 The colour changes of adapted sludge mixed with 9K-medium

As shown in Figure 4-8, a reddish brown colour and some precipitate appeared after 21 days. At this point, the samples were considered positive for the presence of iron-oxidizing bacteria, and were used for Phase II of CSTR bioleaching process.

The development of a brown colour and precipitation took between 2-10 days in the study of other researchers (Tyagi and Blais, 1992). The reason for a longer development time is this study can be explained by the lack of shaking and forced aeration. The inoculation of adapted sludge in 9K synthetic medium showed that the autotrophic growth of *T. ferrooxidans* in the liquid medium depends upon temperature, mixing and proper aeration. Based on previous studies, the brown colour as well as precipitation indicated the presence of bacteria similar in morphology to *T. ferrooxidans*.

4.3 Jar Test Results

4.3.1 Solid Content (Low pH Range)

As mentioned previously, the total solid (TS) of digested sludge in ABTP is quite stable, as monitored by the ABTP. The TS of digested sludge on sampling days October 31, November 20 and December 10 of 2002 were 1.6%, 1.6% and 1.7%, respectively. To check the relation between metal removal efficiency and solid content of sludge, three solid contents were set up by adding DDW, as follows:

- 100%TS: fresh raw sludge without adding any DDW

- 50%TS: 50% fresh sludge + 50% DDW, and

- 25%TS: 25% fresh sludge + 75% DDW.

The results of the experiment are tabulated in Appendix E. In each trial, 3 different levels of TS were prepared and the metal removal was recorded. First, initial metal concentration readings were taken and recorded. The remaining readings were taken at 3 different TS levels.

For the analysis of the results, the following curves (Figures 4-9, 4-10 and 4-11) were produced from the data obtained experimentally, same as the data tabulated in Appendix E.

Metal percent removal (or removal efficiency) is expressed by the following equations:

$$(4.1) \qquad R = \frac{C - C_0}{C_{raw}} *100\%$$

Here R is the metal percent removal, C is the metal concentration in filtered bioleached solution (mg/L), C_0 is the initial metal concentration before bioleaching starts (mg/L), C_{raw} is the metal concentration in filtered solution after thoroughly acidified and solubilized on raw sludge by strong acid (HNO_3 and H_2SO_4). Based on the monitored metal concentration in sludge under pH=7, it is assumed that the concentration C_0 of metal is negligible in the liquid phase of raw sludge. So, the equation (4.1) can be arranged as:

$$(4.2) \qquad R = \frac{C}{C_{raw}} *100\%$$
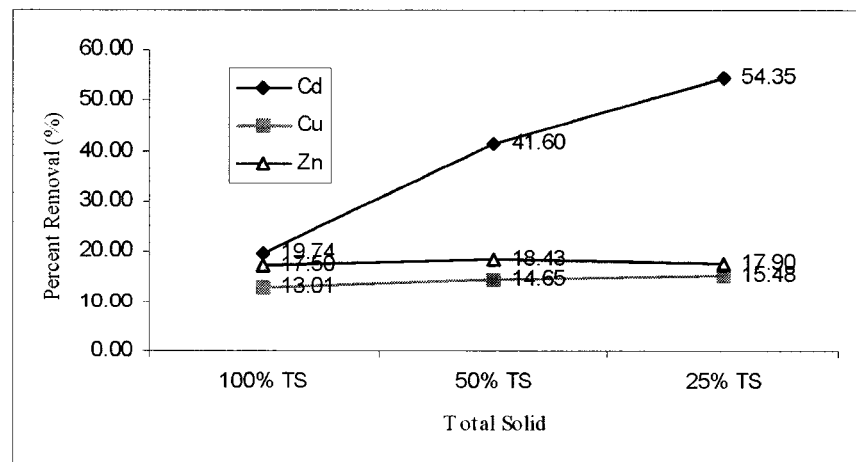
Figure 4-9 Metal percent removals under different TS (pH=4)

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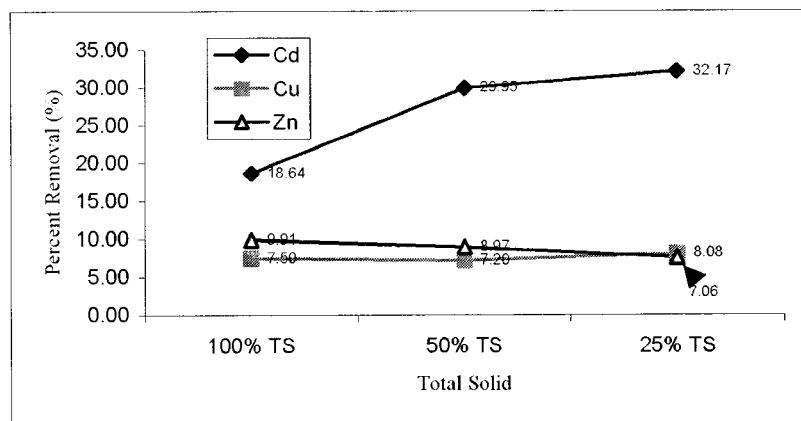
Figure 4-10 Metal percent removals under different TS (pH=5)
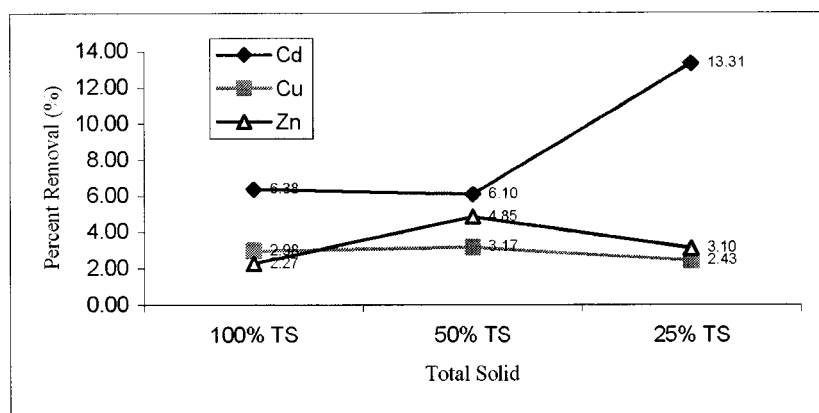
Figure 4-11 Metal percent removals under different TS (pH=6)

It may be observed from the curves that the solubilization rates for Cu and Zn were not affected significantly by the solid contents. However, the removal of Cd did change significantly at different solid contents, with a higher TS resulting in lower metal removal efficiency. The reason for this may be that the increase in TS resulted in increased sludge buffering capacity (Tyagi et al., 1996) for Cd, which has a relative low concentration in sludge and hence is very sensitive to the variation of solid content. Buffering capacity is

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the ability of sludge to withstand rapid pH fluctuations. Sludge with a high buffering capacity requires incorporation of a greater quantity of acid to alter the pH than sludge with a low buffering capacity. So increased sludge buffering capacity finally generates a lower sludge acidification. Based on the routine monitoring record of ABTP, the results obtained by this research show 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.

So, under low pH range, the metal bioleaching efficiency for Cu and Zn was not affected by sludge solids concentration, except for Cd, which has less bioleaching efficiency at increased solids contents.

4.3.2 Solid Content (High pH Range)

Tables 4-4 to 4-6 present the bioleaching efficiency under the condition of high pH range (7~9) and different solids (25%~100% TS) for the three monitored metals.

Table 4-4 Copper percent removals under different TS (pH7~9)

TS	pH (±0.1)	Mean Conc. (mg/L)	Percent Removal (%)
100% TS	7	0.015	0.09
50% TS	7	0.017	0.10
25% TS	7	0.020	0.46
100% TS	8	0.034	0.20
50% TS	8	0.027	0.16
25% TS	8	0.011	0.06
100% TS	9	0.012	0.07
50% TS	9	0.017	0.10
25% TS	9	0.016	0.09

Note: Copper concentration of raw sludge is 17.860 mg/L

Table 4-5 Zinc percent removals under different TS (pH7~9)

TS	pH (±0.1)	Mean Conc. (mg/L)	Percent Removal (%)
100% TS	7	0.045	0.30
50% TS	7	0.285	1.25
25% TS	7	0.264	2.31
100% TS	8	0.102	0.67
50% TS	8	0.081	0.36
25% TS	8	0.039	0.33
100% TS	9	0.036	0.24
50% TS	9	0.054	0.23
25% TS	9	0.060	0.52

Note: Zinc concentration of raw sludge is 15.285 mg/L

Table 4-6 Cadmium percent removals under different TS (pH7~9)

TS	pH (±0.1)	Mean Conc. (mg/L)	Percent Removal (%)
100% TS	7	0.002	1.94
50% TS	7	0.003	2.55
25% TS	7	0.002	1.66
100% TS	8	0.002	1.94
50% TS	8	0.003	2.22
25% TS	8	0.003	2.22
100% TS	9	0.001	1.11
50% TS	9	0.002	1.66
25% TS	9	0.002	1.39

Note: Cadmium concentration of raw sludge is 0.122 mg/L.

The results demonstrated that almost all the percent removals for trials above a pH of 7 with varied solids contents are very low and insignificant.

4.3.3 Effect of pH

The correlations between pH and metal removal efficiency in the bioleaching process are shown in Figures 4-12 to 4-14. It was observed that the metal removal efficiency was increased when pH is decreased for all three metals in the low pH range. This pattern, however, is a lot less noticeable when the pH value is above 7.
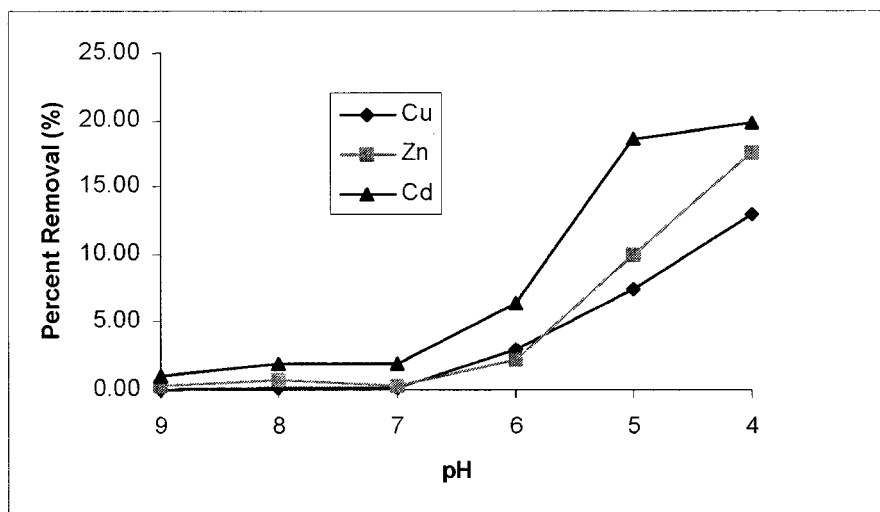
Figure 4-12 Metal percent removals under different pH (100% TS)
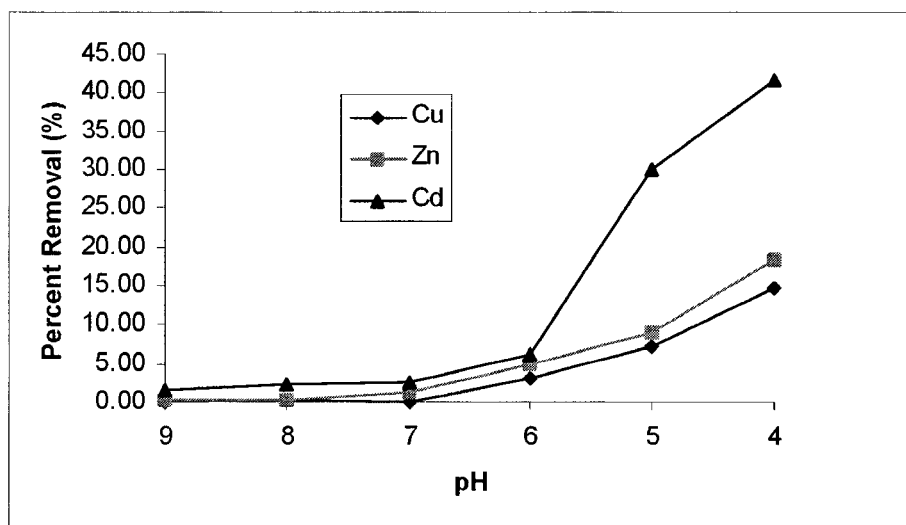
Figure 4-13 Metal percent removals under different pH (50% TS)

57
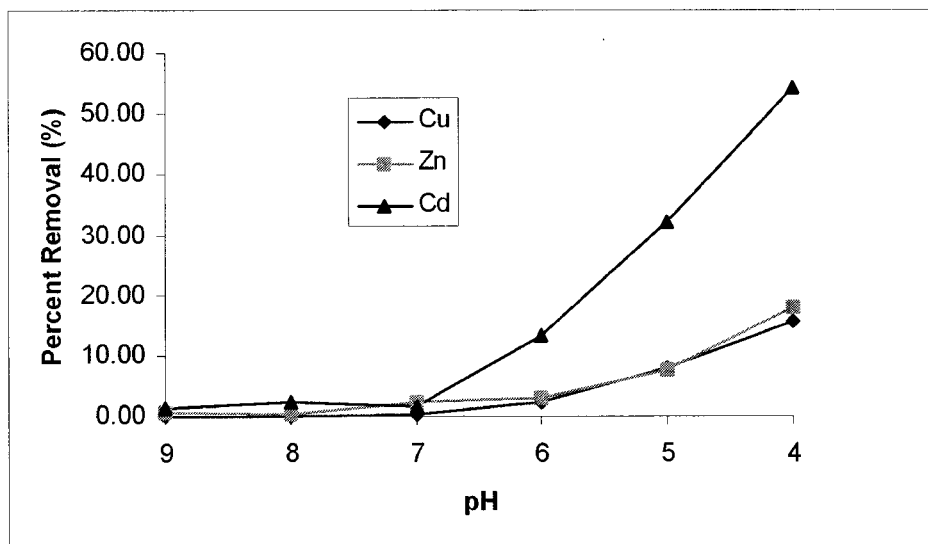
Figure 4-14 Metal percent removals under different pH (25% TS)

From Figures 4-12 to 4-14, it is clearly indicated that the metal removal efficiency is basically affected by the pH conditions. Metal solubilisation by *T.ferrooxdians* was much more efficient in the strong acid environment than neutral or alkaline conditions. It was also observed that the metal percent removals of the three metals in the low pH range follow the order of: Cd>Zn>Cu. However when actual concentration is considered as shown in Table 4-7, then bioleaching process in more efficient for Zn and Cu. The reason for the high percent removal of Cd is due primarily to the very low initial concentration, of 0.122 mg/L.

Table 4-7 Metal removal comparisons

Metal	Conc. in raw sludge (mg/L)	Conc. after 2 days bioleaching (mg/L)	Percent Removal (%)	Conc. decrease (mg/L)
Cd	0.122	0.098	19.7	0.024
Zn	15.285	12.620	17.5	2.665
Cu	17.860	15.540	13.1	2.320

The results show that iron oxidation bacteria rely on the acid condition to solubilise the metals. They also demonstrated that *T.ferrooxdians* are effective bioleaching microorganisms, it can assume that no growth happened at neutral or in high pH condition for acidophic *T.ferrooxdians*.

4.4 CSTR Results

4.4.1 Phase I Results

As mentioned previously, the long-term CSTR test was divided into 2 phases. During Phase I, the adapted sludge was added into the fresh digested sludge to initiate the bioleaching process. Samples to be processed by the AA analysis were taken every 48 hrs from CSTR reactor. The test results are shown in Fig.4-15.
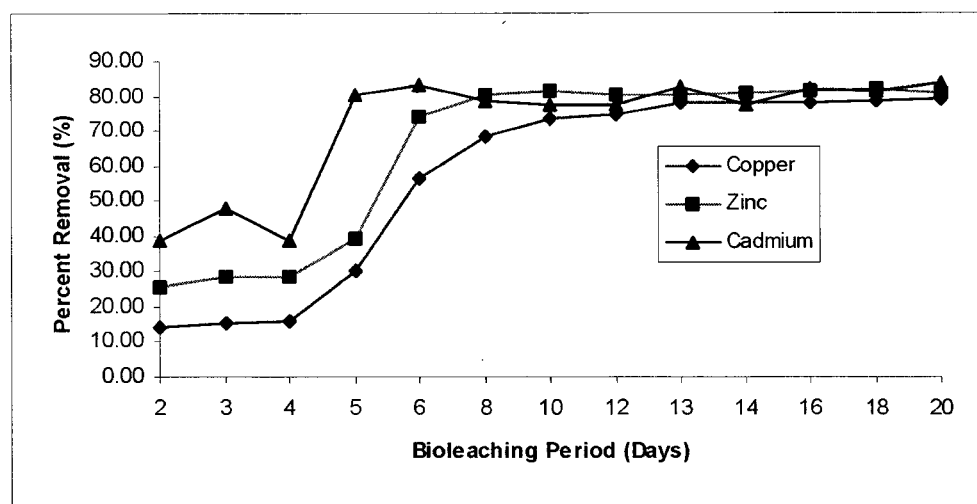
Figure 4-15 Metal Percent removals under different bioleaching period with adapted sludge

To achieve different percent removal targets for the later management, the following table is created based on 20-day bioleaching process for the three metals.

Table 4-8 Treatment periods to achieve different removal efficiencies

Metals	Targeting	Removal		
	25% [a]	50%	75%	100%
Cu	5 days	6 days	8 days	20 days
Zn	2 days	5 days	6 days	18 days
Cd	2 days	3 days	5 days	16 days

(a): the percent of the maximum removal efficiency during 20-day bioleaching

Each trend line is described by the equations below for each removal efficiency during the 20-day bioleaching for the 3 metals:

(4.3)   For Cu: $R = (1 - e^{-0.1443T}) *100\%$

(4.4)   For Zn: $R = (1 - e^{-0.2316T}) *100\%$

(4.5)   For Cd: $R = (1 - e^{-0.3370T}) *100\%$

Among which R is the percentage removal, T is the bioleaching time. It is clearly demonstrated that Cd has the highest removal constant (0.3370), while Cu has the lowest removal constant (0.1443). That is reason why it only took 5 days for Cd to reach the peak removal efficiency, while Zn needed 14 days.

4.4.2 Phase II Results

During Phase II of CSTR, the 9K-medium inoculated adapted sludge (inoculums) was used. Samples to be processed by the AA analysis were taken every 24hrs from CSTR reactor. The test results are shown in Fig.4-16.
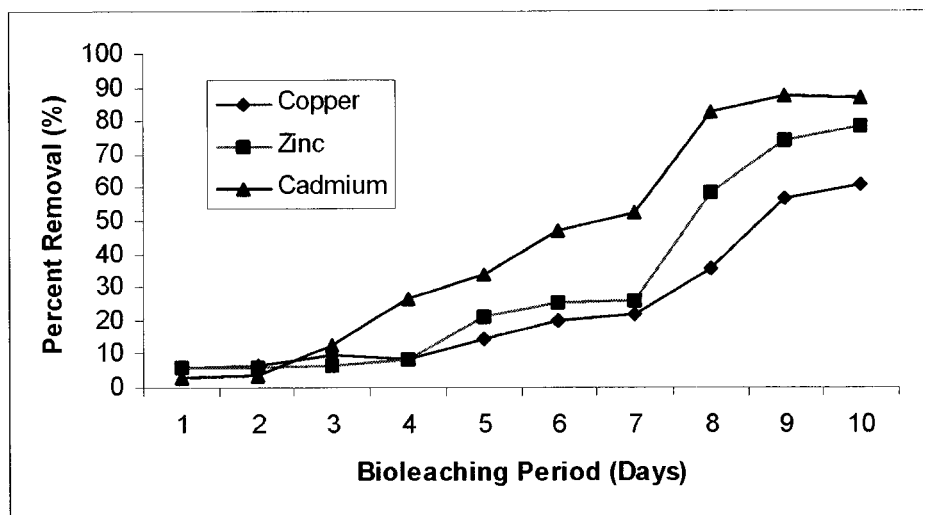
Figure 4-16 Metal percent removals under different bioleaching period with inoculums

4.4.3 Adapted Sludge Vs. Inoculums

For comparison between the bioleaching process using adapted sludge and inoculums, Figures 4-17 to 4-19 are prepared for the bioleaching efficiency comparison between the two phases. Both results are shown for a 10-day bioleaching period for the three metals, because Phase II only involved 10 days bioleaching period.
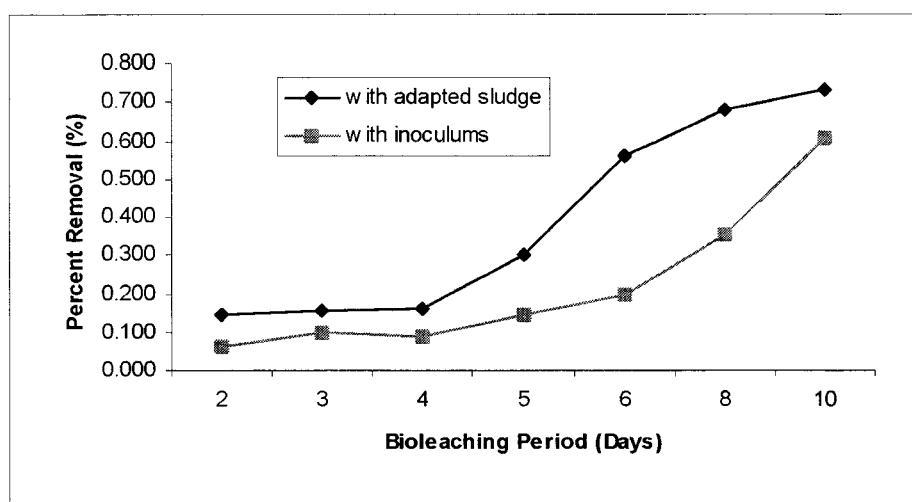
Figure 4-17 Cu removal comparison between using adapted sludge and inoculums

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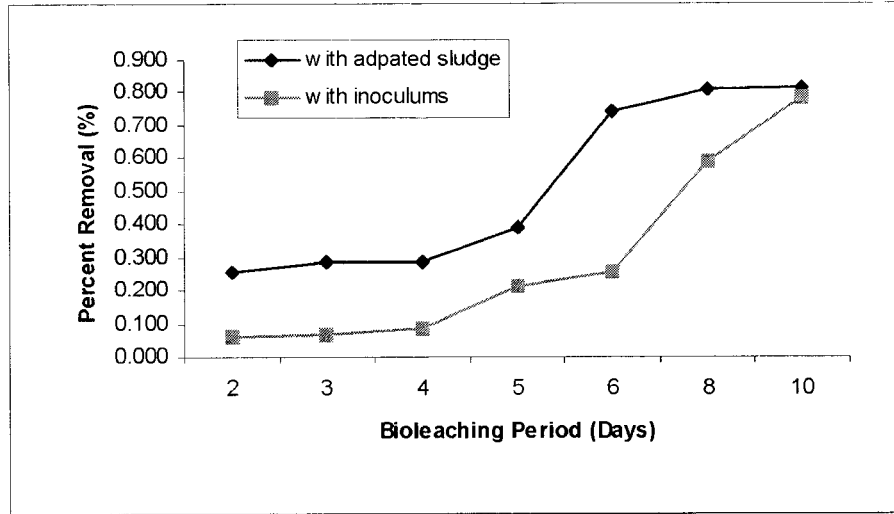
Figure 4-18 Zn removal comparison between using adapted sludge and inoculums
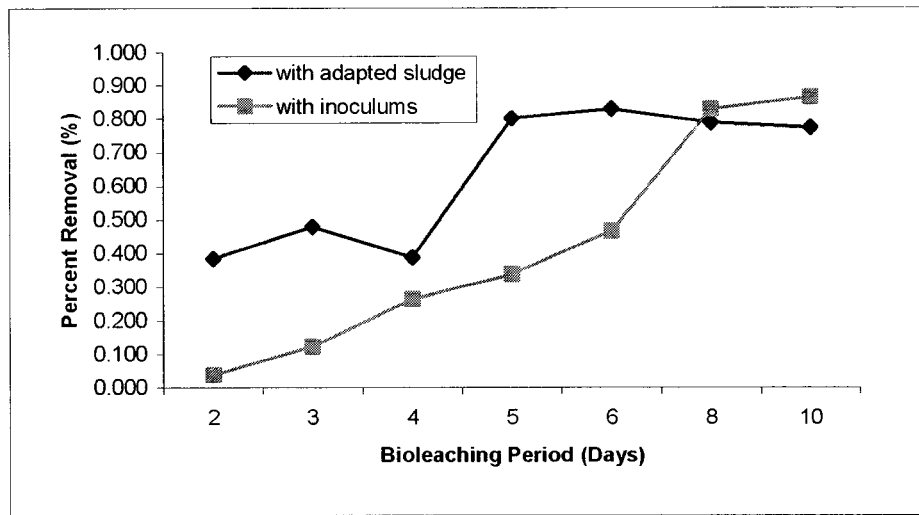
Figure 4-19 Cd removal comparison between using adapted sludge and inoculums

After 2 weeks of stage I, the Cu percent removal was near the peak value (around 80%). For Zn and Cd, the times to reach the peak removal rate were 8 days and 5 days respectively. It is observed that the bioleaching process of digested sludge with added inoculums, takes even longer to reach the peak removal rate. Figures 4-17 to 4-19 clearly

demonstrate, after 10 days, the percent removal of copper was 60.7% when adding inoculums while the rate is 73.2% for adding adapted sludge. For Zn the percent removals are 78.2% vs. 81.6 % for adding inoculums against adapted sludge. However, the metal percent removals comparison curves show that using inoculums have much more increasing room of percent removal than that of using adapted sludge.

Those results indicate (1) ABTP could use a 2 weeks bioleaching period to get the highest percent removal for Cu, saving operational cost. (2) The bioleaching time was longer when inoculums are added into digested sludge compared with adding adapted sludge. As the three curves of Cu, Zn and Cd (with added inoculums) show, all the percent removal begun to increase dramatically after the 7th day, indicating that the inoculums had started to fully adapt to the surrounding condition. This result demonstrates that even though more active iron oxidizing bacteria are present in the inoculums, those inoculums need more time to adapt to the digested sludge inside the CSTR than adapted sludge, which is quite "familiar" and suitable to the living conditions of the system.

4.4.4 Solid Degradation

According to the study results of other researchers (Tyagi, et al, 1997), when pH decreases to 3.0-2.5, metal solubilisation increases, but the bacterial sludge digestion activity decreases. Sludge solids destruction is about 3 times slower at this pH level than normal sludge digestion at neutral pH. It would be difficult to operate a "simultaneous sludge digestion and metal leaching" process in the digester because of this. According to the technical design of digesters of ABTP, the sludge detaining time inside the digester averages about 17-19 days. A separate bioleaching stage is recommended after sludge

digestion and before sludge dewatering, which is not only beneficial for metal removal, but also for solids reduction.

Total Suspended Solid (TSS) was tested during the 3 week CSTR test with adapted sludge (Phase I). The variation of TSS was monitored, and the results in Figure 4-20 showed that the TSS was degraded during the bioleaching process.

According to the study of Blais (1995), solids degradation is based on the following first order kinetic equation:

$$(4.6) \qquad \frac{dC(t)}{dt} = -kC(t)$$

Here k is the degrading constant (day^{-1}), C is the TSS concentration (mg/L), t is the bioleaching time of CSTR. Then:

$$(4.7) \qquad C = C_0 * e^{-kt}$$

The calculated degrading constant k is 0.0522day^{-1}. When compared with the data in Table 2-6, in which the TSS degradation rate ranges from 0.0670 day^{-1} (pH=4) to 0.0983 day^{-1} (pH=7), the result from this experiment is very consistent with previous works.
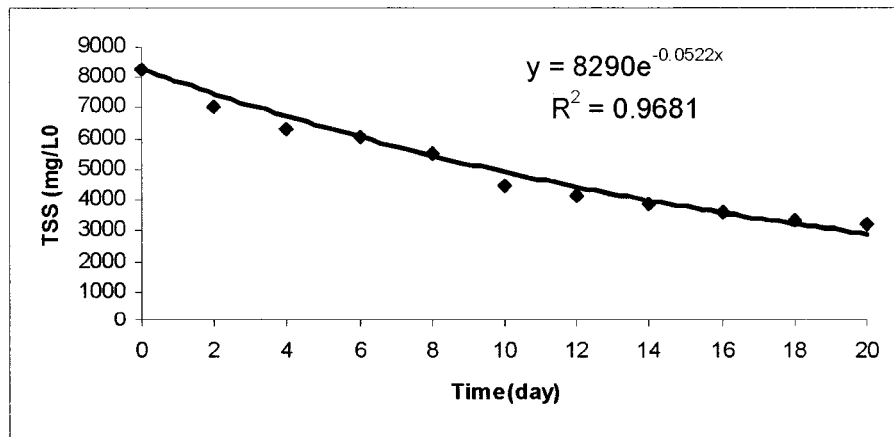
Figure 4-20 TSS degradation during bioleaching process of CSTR with adapted sludge

These results demonstrate that the reduction of sludge solids happened through the bioleaching process, which is quite beneficial for sludge stabilisation. A major objective of aerobic or anaerobic sludge digestion is to reduce the mass of the solids for disposal. The efficiency of sludge digestion is generally expressed in terms of reduction of Volatile Suspended Solids (VSS). A 40% reduction of VSS has been suggested by U.S.EPA (Blais et al., 1995). Because the sludge digestion for ABTP is an anaerobic process that only degrades the solids very little. According to the information collected from ABTP, the VSS of sludge before and after digestion are about 70% and 60% in the year 2002. So only 10% of VSS can be reduced after sludge anaerobic digestion. Adding an aerobic sludge bioleaching process would resolve the issue of excess solids. According to the study of other researchers (Tyagi et al., 1996), the possible reasons could be the production of sulphuric acid during bioleaching process and the solubilization of a small part of the sludge organic matter of sludge under the strong acid condition (pH <2.5).

4.4.5 Other Factors

The results of other factors that could affect bioleaching process are presented in Tables 4-9 and 4-10.

Table 4-9 Other monitored factors during bioleaching process (Phase I)

Days	pH	DO(mg/L)	Temp(°C)
1	4.0	0.5	19.1
2	3.4	2.3	19.2
3	3.1	3.1	19.1
4	3.0	4.5	19.3
5	2.9	5.8	19.1
6	2.9	5.8	19.2
8	2.8	6.2	16
10	2.7	6	16.1
12	2.7	2.6	13.3
13	2.6	1.3	13.4
14	2.6	5.8	21.7
16	2.5	7.1	21.8
18	2.5	7.1	21.9
20	2.5	7.2	21.8

Table 4-10 Other monitored factors during bioleaching process (Phase II)

Days	pH	DO(mg/L)	Temp(°C)
1	4.0	0.9	20.8
2	3.9	5.6	20.5
3	3.8	5.7	20.5
4	3.6	5.8	20.7
5	3.4	5.5	20.6
6	3.3	5.8	20.5
7	3.3	5.8	20.5
8	2.6	6	20.6
9	2.6	5.8	20.4
10	2.6	5.6	20.5

During Phase I and II of CSTR, it was observed when pH decreased to 3.0-2.5, metal solubilisation increased dramatically. This suggests that from an initial value around 4.0 to around 2.5, a quantity of sulphuric acid was generated by sulphuric oxidizing bacteria, thus promoting the solubilization of the metals. Figure 4-21 presents the sludge acidification pattern and comparison of Phase I and II.
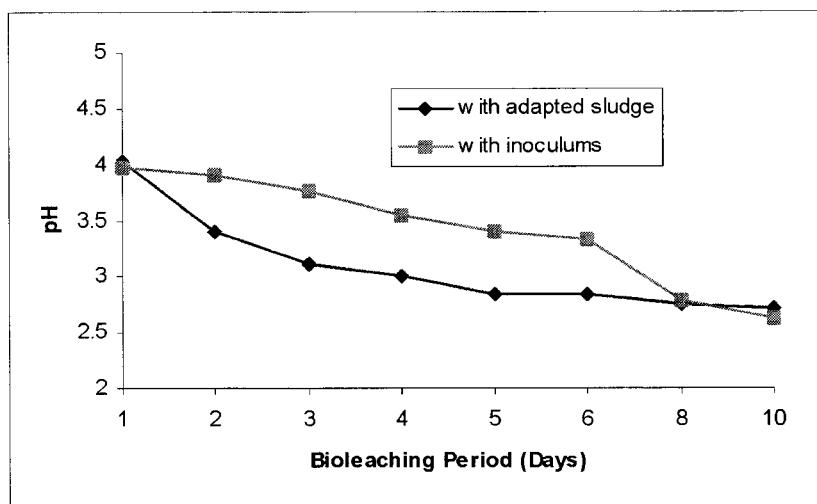
Figure 4-21 Comparison of pH variation during long term CSTR

During stage I, there were two incidents (between day 1-2 and 12-13) where the air supply was shut down to change the air cylinder. For day 1-2, the pH kept decreasing but with a very slow rate, the percent removal was also slow, demonstrating that the *T. ferrooxidans* in adapted sludge were trying to adapt to the new environment of a sudden accident. For day 12-13, because the percent removals for all three metals have already reached the peak level, this sudden air supply accident does not affect system performance for the process. This chance incident also demonstrated that short-term anaerobic condition does not affect bioleaching process significantly, the *T. ferrooxidans* can survive under anaerobic condition and recover the bioleaching function quickly when the condition switches back to aerobic condition. Of course this anaerobic stage must be

quite short. The percent removal jumped when air supply was resumed, showing that dissolved oxygen (DO) is a very important factor for aerobic metabolism of *T.ferrooxidans*. Aeration is one of the key parameters to be considered for efficient removal of metal during bioleaching process.

Another factor that varied during the CSTR test was temperature (Phase I), ranging from 13-22°C because the weather changed (windows of Lab kept open during the test). The metal removal trend did not appear to be affected significantly by temperature, thus showing that the bioleaching process can be employed efficiently for metal removal within this temperature range. This agrees with the study result of other researchers (Tyagi, et al., 1995) regarding the effect of temperature for metal leaching.

5. Recommendations

5.1 Recommendations on metal bioleaching

For many years researchers have been investigating bioleaching, as an environmentally friendly means to remove heavy metals from sewage sludge before they are applied as fertilizers. The following recommendations are based on the findings of this thesis, specifically of the beneficial effect of *T. ferrooxidans* on Cu, Cd and Zn removal in sludge.

Effective and economical method: The presence of metals in domestic sewage comes from the use of soaps, detergents, cosmetics, medicine as well as the waste from hospitals, warehouses, car washes, automobile garage, etc. Furthermore, considering industrial uses of metals such as metal plating, tanneries, and metal catalysts, these sources also contribute to high levels of heavy metals in sewage sludge. At present, there is no standard treatment process for such metals in sewage sludge before they are either landfilled or applied as the fertilizer. For the time being, some industries treat metal-polluted industrial effluents by chemical methods, such as chemical precipitation, electrochemical treatment and ion exchange (Leung et al., 2000). These methods are costly and only partially effective compared to the bioleaching method. The main materials used for bioleaching are (1) industrial-grade ferrous sulphate to be added as energy substrate for *T. ferrooxidans*, (2) sulphuric acid, and (3) lime (for sludge neutralization after bioleaching). As described in Chapter 3 of this thesis, it is very easy and convenient to prepare adapted sludge using raw sludge (either activated sludge or digested sludge) in the plant. The removal efficiency of 79% for Cu, 88% for Cd and 82% for Zn obtained from the conducted experiments, indicate that the concentration of

these metals in the digested sludge may be decreased enough to meet the guideline before land application.

Continued Research: According to the previous research by Sreekrishnan and Tyagi(1994), the leaching methods of metal removal from sewage sludge include (1) addition of inorganic acid followed by thorough mixing; (2) using *T. ferrooxidans* (iron oxidation), and (3) using sulphur oxidizing bacteria(*T. thioparus* or/and *T. thiooxidans*). Research from this thesis has demonstrated an approach that combined method (1) and (2). Although the results of study are acceptable, future studies in this field should include the "internal" combination among acidification process, iron oxidation process and sulphur oxidation process; and the "external" combination between bioleaching methods and chemical methods. The goal of these studies is to find the optimal metal removal method for sewage sludge.

For example, more research is needed to find out which pH level maximizes the solubilisation yield of different types of metals. It is apparent that different metals require different pH levels for sludge acidification, so some compromise among the maximization points are necessary in order to solubilise most metals efficiently. Also, considering the sludge digestion at pH 2.0-2.5 is much slower than normal sludge digestion at neutral pH, it is strongly recommended that the bioleaching process be set up between sludge digestion and sludge dewatering. This is demonstrated in the recommended diagram in Figure 5-1.

Put bioleaching method into application: Finally, research for the bioleaching process to remove metal from sewage sludge has been carried out for over 20 years, and yet it is still at the experimental stage. The reasons for this are: (1) a lack of research

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funding from the three levels of government, (2) a lack of interest among the municipalities to apply new technology, and (3) lack of public demonstration and education. So public education, in terms of large-scale awareness campaigns on environmental issues associated with conventional methods of land application by biosolid without any treatment, and the importance of using bioleaching to remove the metals in the sewage sludge as the most economical and effective method towards environmental protection for our land and food, is much needed. Regulated requirements, as well as subsidies for municipal sewage plants that are willing to apply the bioleaching technology during sludge treatment, are also recommended.
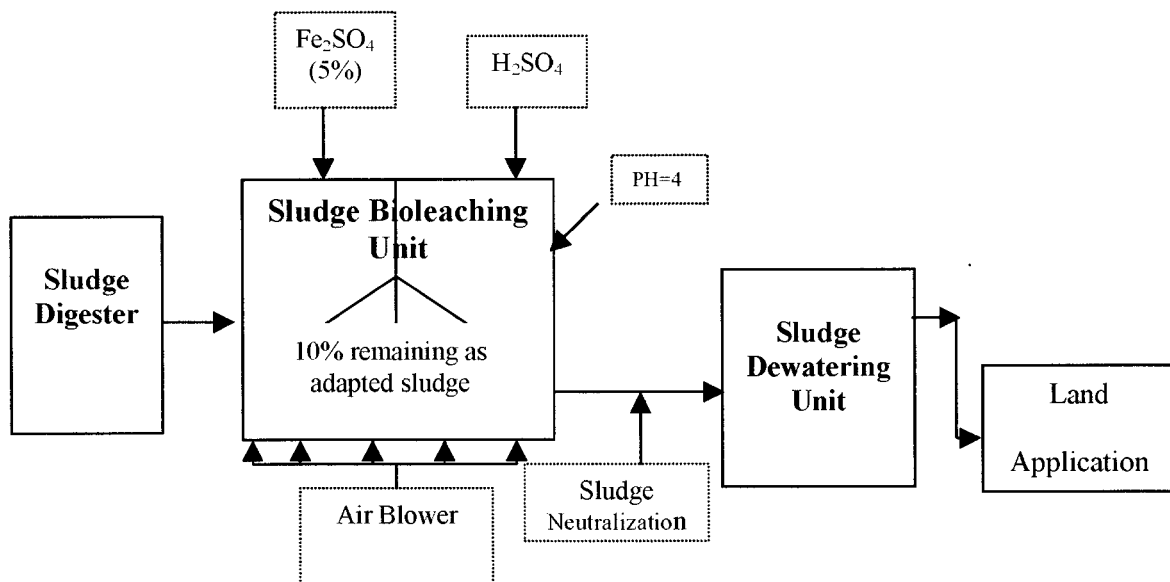
Figure 5-1   Diagram of recommended sludge bioleaching unit

5.2 Recommendations for land application

After reviewing the agricultural land application of sludge in Ontario, the following recommendations can be made: (1) the land application of sludge should be administered at the municipal level, (2) amendments to the existing guidelines are necessary, and (3) research must continue to address environmental risks from sludge spreading.

Municipal Government Control: Many experts have criticized the ability of the Ministry of the Environment to properly administer the land application of sludge. These critics feel that land application is simply beyond the Ministry's control due to staff and resource constraints. Many papers have proposed municipal government control of land application of sludge (Harrison and Eaton, 2001). Municipalities do not currently have the power to regulate the land application of sludge. For example, the Simcoe County recently objected to the agricultural land application of sludge within their municipality. However, Ontario's Ministry of the Environment overruled their objection on the grounds that "the county's waste management mandate does not extend to agricultural utilization" and the municipality has no authority to regulate a "normal farm practice" (Crittenden, 2002). American municipalities are having a similar problem trying to control the land application of sludge. The Spencer Township in Michigan recently tried to impose ordinances to control the land application of sludge but these ordinances were denied by State officials who said that the "ordinances duplicate the state provisions and cause undue burden on local farmers" (Essenburg, 2002). The Township feels that municipal government should be included in these decisions and could administer land application, as municipalities understand the land and properties much better than the state (Essenburg, 2002). The Desoto County in Florida has also tried to restrict the amount of

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sludge spread in their municipality. However a federal judge intervened and stopped the implementation of these restrictions, stating that restrictions are the responsibility of the State, not the municipality (Carroll and Manfuso, 2002).

There is clearly an interest to control the land application of sludge at the municipal level. Municipal governments are much more familiar with the community interest and the land than the provincial or state government. Municipal governments are also knowledgeable in the area of sludge as many wastewater treatment plants are operated at the municipal level.

A municipal government could control a land application of sludge program much more efficiently and easily than provincial or state governments. Municipalities are dealing with the public on a daily basis, and already have the structure in place to successfully implement land application of sludge programs. Municipal governments could hold public meetings about potential land application sites. The community could be directly involved in the process.

Improvements to Existing Guidelines: There are a few areas where the existing *Biosolid Guidelines* could be improved to ensure a safer land application of sludge process. First, soil samples should be taken by an independent party. Second, standards should be set for organic contents. Third, a ban should be implemented on grazing lands. Finally, sludge should be classed as containing pathogens or pathogen-free.

Soils samples are currently the responsibility of the private hauler. However the private hauler is running a business and maximizing profit is in their best interest. As a result, the hauler may submit biased or inaccurate soil samples to the Ministry. The responsibility of soil samples should be moved to an independent party like the provincial

government or the municipality (in the case of municipal control). This will allow for accurate soil samples and ensure maximum concentrations of metals are not exceeded. Another criticism of the legislation has been the lack of soil organic matter standards. The guidelines do not specify an organic matter standard, because data on organic matter was not available when the *Biosolid Guidelines* were being written. In the last 4 years many studies have addressed the transport and fate of organic matter from sludge treated soil. Now that data is available, there should be a review of the guidelines with the intent to develop maximum concentrations of organic matter in soil.

Research on adsorption by crops and ingestion by livestock suggest metals and organic matter tend to bioconcentrate in livestock grazing on sludge treated soil. Elevated levels of both metals and organic matter were noted in grasses after sludge application. Therefore, instead of imposing waiting periods, there should be an outright ban on sludge application to grazing land. This has already been done in American legislation has done. A ban will ensure the safety of livestock, and also reduce the potential for harming public health. Without a ban, there should be continued research into the area of contaminant adsorption by crops and ingestion by livestock, to identify risk levels for specific crops and livestock.

In 1992 the American Environmental Protection Act modified its agricultural land application of sludge standards with Part 503, which designated two different types of sludge: Class A (a pathogen-free sludge) and Class B (a pathogen containing sludge). Class A sludge can be spread on all agricultural land, while Class B sludge can only be applied on restricted land away from residential areas.

The Ontario *Biosolid Guidelines* do not differentiate between pathogen sludge and non-pathogen sludge. In Ontario, all sludge is regulated simply by the nitrogen, phosphorous and heavy metal concentrations. Ontario may want to consider distinguishing between pathogen and non-pathogen containing sludge in an attempt to reduce the possible health impacts from the land application of sludge.

Future Research: Finally there should be continued research into the health impacts from agricultural land application of sludge. No studies on transport and fate of metals and organic matter have provided certainty about groundwater contamination, surface water contamination, crop contamination, or livestock contamination. Many of the studies make general conclusions about the possibility of contamination. Therefore research in the area of contamination from metals and organic matter should continue.

The Environmental Protection Act of the United States acknowledged that data on the health risks from land applied sewage sludge have not been addressed (Crittenden, 2002). Very few studies have looked at health risks from land-applied sludge. Most studies have examined pathways for exposure but failed to look at a dose/response relationship. Therefore more research into the area of health risks from land application of sludge must be undertaken.

6. Conclusions

6.1 Conclusion on Sewage Sludge Bioleaching

After experimental research and study on the bioleaching process to remove the three selected metals-Cu, Cd and Zn for the sewage sludge of ABTP, it has been found that bioleaching is an effective bacterial process to remove these metals from digested sludge at ABTP to meet the guideline for land application.

Control studies on the effects of solid content, pH as well as other factors such as bioleaching time, DO, and temperature on the metal leaching process for sewage sludge show that the bioleaching process can be employed efficiently for metal solubilization. The results obtained on bioleaching efficiency under different pH, TS and bioleaching period suggest that the bioleaching process can be conducted efficiently under aerobic acidic conditions, and that the solids content has only a minor effect on removal of Cu and Zn, but affects the Cd significantly. It only took 2 weeks to remove 80% of copper; and even a shorter period for Zn and Cd. According to literature (Blais et al., 1993), the use of a bioleaching process, with ferrous sulphate as substrate to remove toxic metals to recommended level for land application from sewage sludge, normally takes about 3-4 weeks. However, we found that 2 weeks is sufficient from this research. Considering the typical period of a sludge digestion is around 1-2 months, the incorporation of a bioleaching process will not affect the total treatment time very much. Therefore, the bioleaching process may be incorporated into the sludge treatment process for an extra 2 weeks to provide metal removal.

Overall, considering the practical operational condition of ABTP, the suggested bioleaching method should include the addition of industrial-grade ferrous sulphate as

energy substrate for *T. ferrooxidans.* Other operational recommendations include the installation of an air compressor or blower for aeration into the bioleaching tank, adjustment of pH to 4 initially, and the use of a bioleaching period equal to the digestion time for a consistent control of the sludge management process rates.

The incubation and inoculation of adapted sludge show that iron-oxidizing bacteria are naturally present in sewage sludge, including sludge in the aeration tank and digester of ABTP. The metals which exceed their recommended levels can frequently be bioleached and separated using adapted sludge containing high concentration of *T. ferrooxidans.* Purchase of the standard *T. ferrooxidans* strains is not required to remove the metals in sewage sludge before it is disposed as a biosoild for land application, which means the treatment cost can be reduced dramatically. However, as the storage period of adapted sludge in the lab is very short before it loses its bioleaching activity, a new research challenge is how to store it long term. It may possible that the *T. ferrooxidans* bacteria found in activated sludge of the aeration tank could be adapted to remove toxic metals earlier in the activated sludge process, for example in the aeration process of wastewater treatment.

The bioleaching process is an affordable, clean and sustainable method for sewage sludge treatment before it is disposed as a fertilizer. It puts us in a strong position to recycle waste in the future, and it enhances agricultural development without causing pollution, generating hazardous wastes, or depleting natural resources. However, there should be continued research into the combined methods of bioleaching and chemical treatment so that the most cost-effective strategies could be adapted to municipal sewage treatment plants.

The advantages of bioleaching method are numerous and clear, and the technology itself has taken a leap forward in recent years. We must make special efforts to encourage this development today to ensure that this biological technology becomes a significant part of our environmental protection strategies in the coming years.

6.2 Conclusion on Land Application of Sewage Sludge

There have been many criticisms of the guidelines that govern the land application of sludge. The Recycling Council of Ontario is currently reviewing the *Biosolid Guidelines* and hopefully will address some of the criticisms that have been made. The review should address the existing maximum metal concentrations to ensure they do not represent a health risk, introduce maximum organic matter concentrations, and distinguish between pathogen and non-pathogen sludge. Ultimately the Ontario Ministry of the Environment may want to consider downloading the power to administer the land application of sludge to the municipal level. Municipal governments have shown a vested interest in the land application of sludge and feel they can better administer the program.

In conclusion, Ontario should continue to apply sludge to agricultural land, as this provides an economical alternative to sludge disposal by landfilling, and promotes the recycling of resources. Sludge is also a valuable fertilizer. The possibility for contamination is always present; therefore municipalities, haulers, and farmers must apply sludge in a responsible manner and follow the management practices outlined in the *Biosolid Guidelines*. Ongoing research is necessary to identify the health risks associated with the land application of sludge, as there is still a lack of knowledge about the long term effects of sludge treated land.

References:

Agostinelli, M., (1995) "Bioleaching of Heavy Metals from Sewage Sludge: Experimental Investigation of Sulphur-Oxidizing Bacteria". *Undergraduate Thesis, Civil Engineering Department, Ryerson University.*

Alloway, B. and Jackson, A., (1991) "The behaviour of heavy metals in sewage sludge amended soils". *Science of the Total Environment,* Vol. 100, pp. 151-176.

Bacon, M. and Ingledew, W.J., (1989) "The reductive reaction of *Thiobacillus ferrooxidans* on sulphur and selenium". *FEMS Microbiology Letters,*Vol. 58(2-3), pp.184-94.

Barron, J. L., and Lueking, D., (1990) "Growth and Maintenance of *Thiobacillus ferrooxidans* Cells", *Applied and Environmental Microbiology,* Vol.56, No.9, pp.2801-2806.

Blais, J.F., Tyagi, R.D. and Auclair, J.C., (1993) "Metals removal from sewage sludge by indigenous iron-oxidizing bacteria". *Journal of Environmental Science and Health,* Vol. A28 (2), pp.443-467.

Blais, J.F., Auclair. J. C. and Tyagi, R.D., (1992). "Cooperation between two Thiobacillus strains for heavy metals removal from municipal sludge". *Canadian Journal of Microbiology,* Vol. 38, pp.181-187.

Blais, J.F., Tyagi,R.D, Huang, C.P. and Auclair, J.C., (1992). "Comparison of acid and microbial leaching for metal removal from municipal sludge". *Water Science and Technology,* Vol. 26(1/2), pp.197-206.

Buchanan, R. E. and Gibbons, N. E., (1974), "Bergey's Manual of Determinative Bacteriology", Vol. 8. The Williams & Wikins Company, Baltimore.

Carroll, Scott and Manfuso, J., (2002). "DeSoto fights judges sludge ban order". *Sarasota Herald Tribune*. Sarasota: Florida, pp. BM1.

Celaya,R. J., Noriega, J. A., Yeomans, J. H., Ortega, L. J., (2000) "Biosorption of Zn(II) by *Thiobacillus ferrooxidans*", *Bioprocess Engineering*, Vol.22, pp. 539 - 542.

Chen, SY and Lin, JG., (2000) "Factors affecting bioleaching of metal contaminated sediment with sulphur-oxidizing bacteria", *Water Science and Technology*, Vol. 41 No.12, pp. 263-270.

Corwin, D., and David, A., and Goldberg, S., (1999) "Mobility of arsenic in soil from the Rocky Mountain Arsenal area", *Journal of Contaminant Hydrology*, Vol. 39, pp. 35-58.

Crittenden, G., (2002) "Sludge Fight", *Solid Waste and Recycling*, Vol. 6, No. 6, pp. 8-15.

Davis, R.D. and Coker, E.G., (1980) "Cadmium in agriculture, with special reference to the utilisation of sewage sludge on land", Medmenham, United Kingdom, Water Research Centre (Technical Report TR/139).

Donati, E., Pogliani, C., Boiardi, J.L., (1997), "Anaerobic leaching of covellite by *Thiobacillus ferrooxidans*". *Applied Microbiological Biotechnology*, Vol. 47, pp.636-639.

Drobner, E., Huber, H. & Stetter, K. O., (1990) "*Thibacillus ferrooxidans*, a facultative hydrogen oxidizer", *Applied Environment Microbiology*, Vol. 56(9), pp.2922-2923.

Duarte-Davidson, R. and Jones, K., (1996) "Screening the environmental fate of organic contaminants in sewage sludge applied to agricultural soils: 2. The potential for transfers to plants and grazing animals", *The Science of the Total Environment*, Vol. 185, pp. 59-70.

Essenburg, K., (2002) "Township gains ammunition in its fight to control sludge", *The Grand Rapids Press*. Grand Rapids: Michigan, pp. 2.

Frost, H., and Ketchum. L., (2000) "Trace metal concentration in durum wheat from the application of sewage sludge and commercial fertilizer", *Advances in Environmental Research*, Vol. 4, pp. 347-355.

Harrison, E., and Eaton, M., (2001) "The role of municipalities in regulating the land application of sewage sludge and septage", *Natural Resources Journal*, Vol. 41, pp. 1-47.

Ingeldew, W.J., (1986) "Ferrous iron oxidation by *Thiobacillus ferrooxidans*", *Biotechnology and Bioengineering Symposium*, Vol. 16, pp.23-33.

Ito, A., Umita,T., Aizawa, J., and Kitada, K., (1998) "Effect of inoculation of iron oxidizing bacteria on elution of copper from anaerobically digested sewage sludge". *Water Science and Technology*, Vol.38, No.2, pp.63-70.

Jensen, A., and Webb, C., (1995) "Ferrous Sulphate Oxidation Using *Thiobacillus ferrooxidans*", *Process Biochemistry*, Vol. 30, No.3, pp.225-236.

Joshua, W., Michalk, D., Curtis, I., Salt, M., and Osborne, G., (1998) "The potential for contamination of soil and surface water from sewage sludge in a sheep grazing study, Australia", *Geoderma*, Vol. 84, pp.135-156.

Karamanev, D.G. and Nikolov, L.N., (1988) "Influence of some physicochemical parameters on bacterial activity of biofilm: ferrous iron oxidation by *Thiobacillus ferrooxdians*", *Biotechnology and Bioengineering*, Vol. 31, pp. 295-9.

Laskin, A.I., and Lechevalier, H.A., (1973) "Handbook of Microbiology" *CRC Press*, Vol. I, pp. 7-9.

Leung, WC., Wong, M-F., Chua, H., Lo, W., Yu, PH., and Leung, CK., (2000) "Removal and recovery of heavy metals by bacteria isolated from activated sludge treating industrial effluents and municipal wastewater", *Water Science and Technology*, Vol. 41, No12, pp. 233-240.

Lowe, S., Min J., (1996) "Land Application of Sewage Sludge"- Online: http://www.cee.vt.edu/program_areas/environmental/teach/gwprimer/landappl/sewer.htm

MacDonald, D.G. and Clark, R.H., (1970) "The oxidation of aqueous ferrous sulphate by *Thiobacillus ferrooxidans*", *Canadian journal of chemical engineering*, Vol.48, pp. 669-76.

Molina, L., Diaz-Ferrero, J., Coll, M., Marti, R., Broto-Puig, F., Comellas, L., Rodriguez-Larena, M., (2000) "Study of evolution of PCDD/F in sewage sludge-amended soils for land restoration", *Chemosphere*, Vol. 40, pp. 1173-1178.

Payne, M., (2001). "Land application of sewage biosolids for crop production", *Environmental Science and Engineering*.

Online: http://www.gov.on.ca/OMAFRA/english/crops/facts/00-023.htm

Prescott, L.M., Harley, J. P., Klein, D. A., (1999) "Microbiology- Fourth Edition", *McGreen-Hil*, pp. 180-185; 955, 957, 469-470, 837-838.

Renner, R., (2000) "Scientists debate fertilizing soils with sewage sludge". *Environmental Science and Technology*, Vol. 34, Is. 11, pp. 242-243A.

Richards, B., Steehuis, T., Peverly, J., and McBride, M., (1998) "Metal mobility at an old, heavily loaded sludge application site", *Environmental Pollution*, Vol. 99, pp. 365-377.

Sidhwa, P., (2001) "Biosolids research and demonstration field trials in Southern Ontario", *Environmental Science and Engineering*.

Online: www.esemag.com/1101/biosolids.html

Silverman, M.P. & Lundgren, D.G., (1959) "Studies on the chemoautotrophic iron bacterium *Thiobacillus ferrooxidans*. I. An improved medium and a havesting procedure for securing high cell yields", *Journal of Microbiology*, Vol. 77, pp. 642-7.

Smith, K., Green, M., Thomas, G., and Jones, K., (2001) "Behaviour of sewage sludge-derived PAH's on pasture", *Environmental Science and Technology*, Vol. 35, pp. 2141-2150.

Sreekrishnan, T. R., Tyagi, R.D., (1996) "A Comparative Study of the Cost of Leaching Out Heavy Metals from Sewage Sludges", *Process Biochemistry*, Vol. 31, No. 1, pp. 31-41.

Torma, A. E., (1972) "The effect of carbon dioxide and particle surface area on the microbiological leaching of a zinc sulphide concentrate", *Biotechnology and Bioengineering*, Vol.14, pp. 777-786.

Tyagi, R.D., Blais, J.F., (1992) "Bacterial leaching of metals from sewage sludge by sulphur-oxidizing bacteria", *Journal of environmental engineering*, Vol. 118(5), pp. 690-707.

Tyagi, R.D., Sreekrishnan, T.R., Campbel, 1 P.G.C. and Blais, J.F., (1993) "Kinetics of heavy metals bioleaching from sewage sludge-II: Mathematical model", *Water Resources*, Vol. 27(11), pp.1653-1661.

Tyagi, R.D., Blais, J.F., (1994) "Comparison of microbial sulphuric acid production in sewage sludge from added sulphur and thiosulfate", *Journal of Environmental Quality*, Vol. 23, pp. 1065-1070.

Tyagi, R. D., Miller, N., and Blais, J. F., (1996) "Simultaneous sewage sludge digestion and metal leaching - Effect of temperature", *Applied Microbiological Biotechnology*, Vol. 46, pp. 422-431.

Tyagi, R. D., Blais, J.F., Miller, N., and Benmoussa, H., (1997) "Simultaneous sewage sludge digestion and metal leaching - Effect of sludge solids concentration", *Toilets LMBO*, Vol. 31 (1), pp. 105-118.

U.S. Environmental Protection Agency: Environmental Regulation and Technology. *Use and Disposal of Municipal Wastewater Sludge*, EPA625/10-84-003, September 1984.

Vasseur, L., Cloutier, C., and Ansseau, C., (2000) "Effects of repeated sewage sludge application on plant community diversity and structure under agricultural field conditions on Podzolic soils in eastern Quebec", *Agriculture, Ecosystems, and Environment*, Vol. 81, pp. 209-216.

Viessman, W., and Hammer, M., (1998), *Water Supply and Pollution Control*, 6th Edition. Addison Wesley Longman Inc: California.

Wastewater Engineering, Treatment, Disposal and Reuse. Third Edition. Metcalf & Eddy, 1991.

Wilson, S., Duarte-Davidson, R., and Jones, K., (1996) "Screening the environmental fate of organic contaminants in sewage sludges applied to agricultural soils: 1. The potential for downward movement toward groundwaters", *The Science of the Total Environment*, Vol. 185, pp. 45-57.

Source On-line:

- CELLS Alive - http://www.cellsalive.com

- University of Maryland University College - http://www.chem.vt.edu/chem-ed/spec/atomic/graphics

- Colorado School of Mine, Chemistry Department (1999) - http://www.mines.edu/fs_home/jhoran/ch126/microbia.htm

Appendix A: LABORATORY REPORT METALS ANALYSIS: Year 2001

TORONTO WORKS & EMERGENCY SERVICES DEPARTMENT: WASTEWATER QUALITY LABORATORY SERVICES

Sampled 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.10	5.1	170	8359	(100)
Cd	<0.2	3.5	34	6827	(500)
Co	<0.1	2.6	340	8126	(50)
Cr	2.7	134	2800	301	(6)
Cu	21.8	1083	1700	37	(10)
Hg	0.045	2.3	11	19043	(1500)
Mo	0.22	11.1	94	6342	(180)
Ni	0.7	37.8	420	1202	(40)
Pb	1.4	70	1100	567	(15)
Se	0.06	3.0	34	19138	(500)
Zn	17.2	856	4200	46	(4)

Correction:

Total solids in sludge =	2.0%	
Volatile Total Solids =	43.0%	45.1
Ammonia nitrogen (as N) in sludge =	777 mg/L	
Nitrate nitrogen (as N) in sludge =	0.1 mg/L	
Total solids in cake =	13016.4%	28.4
Volatile Total Solids =	36.2%	47.3
Total phosphorus (as P) in cake =	15600 mg/L	31200
TKN (as N) in cake =	39600 mg/L	47900

TORONTO WORKS & EMERGENCY SERVICES DEPARTMENT : WASTEWATER QUALITY LABORATORY SERVICES

ASHBRIDGE'S BAY TREATMENT PLANT BIO-SOLIDS DEMONSTRATION PROJECT : LABORATORY REPORT

Sampled 15 Jan 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.13	6.6	170	6461	(100)
Cd	<0.2	<10.53	34	>4100	(500)
Co	<0.1	<5.26	340	>8100	(50)
Cr	4.79	252.1	2800	170	(6)
Cu	20.8	1094.7	1700	39	(10)
Hg	0.040	2.1	11	20403	(1500)
Mo	0.10	5.3	94	8141	(180)
Ni	0.74	38.9	420	1100	(40)
Pb	1.20	63.2	1100	678	(15)
Se	0.050	2.6	34	16282	(500)
Zn	13.9	731.6	4200	59	(4)

Sampled 28 Jan 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.09	4.7	170	10437	(100)
Cd	<0.2	<11.11	34	>4400	(500)
Co	<0.1	<5.56	340	>8900	(50)
Cr	3.12	173.3	2800	284	(6)
Cu	20.3	1127.8	1700	44	(10)
Hg	0.049	2.7	11	18031	(1500)
Mo	0.20	11.1	94	4436	(180)
Ni	0.59	32.8	420	1504	(40)
Pb	1.35	75.0	1100	657	(15)
Se	0.029	1.6	34	30590	(500)
Zn	13.4	744.4	4200	66	(4)

Total solids in sludge = 1.9 %
Volatile total solids in sludge = 60 %
Ammonia nitrogen in sludge = 814 mg/L
Nitrate nitrogen in sludge = 0.09 mg/L

Centrifuge Cake   (Sampled   14-Jan)
Total solids in cake = 30.1 %
Volatile total solids in cake = 59.5 %
Total phosphorus (as P) in cake = 28500 mg/Kg dry weight
TKN in cake = 46700 mg/Kg dry weight

Total solids in sludge = 1.8 %
Volatile total solids in sludge = ** %
Ammonia nitrogen in sludge = 887 mg/L
Nitrate nitrogen in sludge = 0.12 mg/L

Centrifuge Cake   (Sampled   28-Jan)
Total solids in cake = 26.7 %
Volatile total solids in cake = ** %
Total phosphorus (as P) in cake = 27400 mg/Kg dry weight
TKN in cake = 43100 mg/Kg dry weight

* As per MOEE regulations for sludge utilisation on agricultural lands.
** No result : Equipment Failure

N = Sum of ammonia nitrogen and nitrate nitrogen
M = Metal concentration in wet sludge

ASHBRIDGE'S BAY TREATMENT PLANT BIO-SOLIDS DEMONSTRATION PROJECT : LABORATORY REPORT

Sampled 14-Feb 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.09	4.3	170	9789	(100)
Cd	<0.2	<9.09	34	>4600	(500)
Co	<0.1	<4.55	340	>9200	(50)
Cr	2.94	133.6	2800	313	(6)
Cu	24.2	1100.0	1700	38	(10)
Hg	0.040	1.8	11	23121	(1500)
Mo	0.01	0.6	94	65729	(180)
Ni	0.63	28.6	420	1461	(40)
Pb	1.47	66.8	1100	626	(15)
Se	0.068	3.1	34	13532	(500)
Zn	19.0	863.6	4200	46	(4)

Sampled 27-Feb 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.16	7.4	170	4909	(100)
Cd	<0.2	<9.09	34	>4000	(500)
Co	<0.1	<4.55	340	>8000	(50)
Cr	3.24	147.3	2800	245	(6)
Cu	23.9	1086.4	1700	33	(10)
Hg	0.038	1.7	11	20708	(1500)
Mo	0.19	8.6	94	4185	(180)
Ni	0.58	26.4	420	1371	(40)
Pb	1.64	74.5	1100	485	(15)
Se	0.057	2.6	34	13951	(500)
Zn	18.1	822.7	4200	44	(4)

Total solids in sludge =	2.2 %
Volatile total solids in sludge =	58 %
Ammonia nitrogen in sludge =	920 mg/L
Nitrate nitrogen in sludge =	0.20 mg/L

Centrifuge Cake (Sampled	15-Feb)
Total solids in cake =	31.2 %
Volatile total solids in cake =	55.4 %
Total phosphorus (as P) in cake =	32300 mg/Kg dry weight
TKN in cake =	40800 mg/Kg dry weight

Total solids in sludge =	2.2 %
Volatile total solids in sludge =	59 %
Ammonia nitrogen in sludge =	795 mg/L
Nitrate nitrogen in sludge =	0.20 mg/L

Centrifuge Cake (Sampled	27-Feb)
Total solids in cake =	28.9 %
Volatile total solids in cake =	59.1 %
Total phosphorus (as P) in cake =	29500 mg/Kg dry weight
TKN in cake =	42600 mg/Kg dry weight

* As per MOEE regulations for sludge utilisation on agricultural lands.

N = Sum of ammonia nitrogen and nitrate nitrogen
M = Metal concentration in wet sludge

ASHBRIDGE'S BAY TREATMENT PLANT BIO-SOLIDS DEMONSTRATION PROJECT : LABORATORY REPORT

Sampled 13-Mar 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.12	5.8	170	6977	(100)
Cd	<0.2	<9.52	34	>4300	(500)
Co	<0.1	<4.76	340	>8500	(50)
Cr	3.28	156.2	2800	260	(6)
Cu	22.0	1047.6	1700	39	(10)
Hg	0.044	2.1	11	19302	(1500)
Mo	0.21	10.0	94	4053	(180)
Ni	0.56	26.7	420	1520	(40)
Pb	1.29	61.4	1100	660	(15)
Se	0.044	2.1	34	19346	(500)
Zn	17.2	819.0	4200	49	(4)

Sampled 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.14	6.5	170	5800	(100)
Cd	<0.2	<9.09	34	>4200	(500)
Co	<0.1	<4.55	340	>8400	(50)
Cr	2.58	117.3	2800	324	(6)
Cu	23.7	1077.3	1700	35	(10)
Hg	0.032	1.5	11	26102	(1500)
Mo	0.23	10.5	94	3632	(180)
Ni	0.56	25.5	420	1492	(40)
Pb	1.32	60.0	1100	633	(15)
Se	0.026	1.2	34	32126	(500)
Zn	19.1	868.2	4200	44	(4)

Total solids in sludge = 2.1 %
Volatile total solids in sludge = 60 %
Ammonia nitrogen in sludge = 851 mg/L
Nitrate nitrogen in sludge = 0.21 mg/L

Centrifuge Cake   [Sampled 14-Mar)
Total solids in cake = 27.0 %
Volatile total solids in cake = 60.6 %
Total phosphorus (as P) in cake = 46900 mg/Kg dry weight
TKN in cake = 66500 mg/Kg dry weight

Total solids in sludge = 2.2 %
Volatile total solids in sludge = 62 %
Ammonia nitrogen in sludge = 835 mg/L
Nitrate nitrogen in sludge = 0.27 mg/L

Centrifuge Cake   [Sampled 27-Mar)
Total solids in cake = 27.6 %
Volatile total solids in cake = 59.2 %
Total phosphorus (as P) in cake = 32600 mg/Kg dry weight
TKN in cake = 47500 mg/Kg dry weight

* As per MOEE regulations for sludge utilisation on agricultural lands.

N = Sum of ammonia nitrogen and nitrate nitrogen
M = Metal concentration in wet sludge

ASHBRIDGE'S BAY TREATMENT PLANT BIO-SOLIDS DEMONSTRATION PROJECT : LABORATORY REPORT

Sampled 17 Apr 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.13	6.0	170	6609	(100)
Cd	<0.2	<9.09	34	>4400	(500)
Co	<0.1	<4.55	340	>8800	(50)
Cr	3.32	150.9	2800	265	(6)
Cu	21.9	995.5	1700	40	(10)
Hg	0.048	2.2	11	18237	(1500)
Mo	0.14	6.4	94	6279	(180)
Ni	0.65	29.5	420	1352	(40)
Pb	1.57	71.4	1100	560	(15)
Se	0.060	2.7	34	14651	(500)
Zn	16.8	763.6	4200	52	(4)

Sampled 25 Apr 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.12	5.7	170	7067	(100)
Cd	<0.2	<9.52	34	>4200	(500)
Co	<0.1	<4.76	340	>8400	(50)
Cr	2.88	137.1	2800	292	(6)
Cu	22.1	1052.4	1700	38	(10)
Hg	0.034	1.6	11	25031	(1500)
Mo	0.11	5.2	94	7646	(180)
Ni	0.67	31.9	420	1255	(40)
Pb	1.57	74.8	1100	536	(15)
Se	0.048	2.3	34	17632	(500)
Zn	17.2	819.0	4200	49	(4)

Total solids in sludge =	2.2	%
Volatile total solids in sludge =	q	%
Ammonia nitrogen in sludge =	879	mg/L
Nitrate nitrogen in sludge =	0.03	mg/L

Total solids in sludge =	2.1	%
Volatile total solids in sludge =	q	%
Ammonia nitrogen in sludge =	841	mg/L
Nitrate nitrogen in sludge =	0.03	mg/L

Centrifuge Cake (Sampled 17-Apr)

Total solids in cake =	30.1	%
Volatile total solids in cake =	q	%
Total phosphorus (as P) in cake =	33000	mg/Kg dry weight
TKN in cake =	48000	mg/Kg dry weight

Centrifuge Cake (Sampled 29-Apr)

Total solids in cake =	28.5	%
Volatile total solids in cake =	63.1	%
Total phosphorus (as P) in cake =	28400	mg/Kg dry weight
TKN in cake =	50800	mg/Kg dry weight

* As per MOEE regulations for sludge utilisation on agricultural lands.
q Equipment failure : No result

N = Sum of ammonia nitrogen and nitrate nitrogen
M = Metal concentration in wet sludge

ASHBRIDGE'S BAY TREATMENT PLANT BIO-SOLIDS DEMONSTRATION PROJECT : LABORATORY REPORT

Sampled 15-May 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.15	6.1	170	4530	(100)
Cd	<0.2	<8.00	34	>3400	(500)
Co	<0.1	<4.00	340	>6900	(50)
Cr	2.58	103.2	2800	267	(6)
Cu	25.7	1028.0	1700	27	(10)
Hg	0.036	1.4	11	19341	(1500)
Mo	0.27	10.8	94	2550	(180)
Ni	0.57	22.8	420	1208	(40)
Pb	1.22	48.8	1100	564	(15)
Se	0.058	2.3	34	11871	(500)
Zn	17.3	692.0	4200	40	(4)

Sampled 27-May 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.18	8.8	170	3602	(100)
Cd	<0.2	<10.00	34	>3200	(500)
Co	<0.1	<5.00	340	>6300	(50)
Cr	2.66	133.0	2800	237	(6)
Cu	25.7	1285.0	1700	25	(10)
Hg	0.049	2.5	11	12863	(1500)
Mo	0.19	9.5	94	3317	(180)
Ni	0.54	27.0	420	1167	(40)
Pb	1.65	82.5	1100	382	(15)
Se	0.045	2.3	34	14006	(500)
Zn	18.2	910.0	4200	35	(4)

Total solids in sludge =	2.5 %		Total solids in sludge =	2.0 %
Volatile total solids in sludge =	60 %		Volatile total solids in sludge =	63.0 %
Ammonia nitrogen in sludge =	688 mg/L		Ammonia nitrogen in sludge =	630 mg/L
Nitrate nitrogen in sludge =	0.54 mg/L		Nitrate nitrogen in sludge =	0.27 mg/L

Centrifuge Cake (Sampled 15-May)

Total solids in cake =	27.2 %
Volatile total solids in cake =	61.2 %
Total phosphorus (as P) in cake =	39700 mg/Kg dry weight
TKN in cake =	41300 mg/Kg dry weight

Centrifuge Cake (Sampled 28-May)

Total solids in cake =	29.4 %
Volatile total solids in cake =	56.7 %
Total phosphorus (as P) in cake =	28100 mg/Kg dry weight
TKN in cake =	42500 mg/Kg dry weight

* As per MOEE regulations for sludge utilisation on agricultural lands.

N = Sum of ammonia nitrogen and nitrate nitrogen
M = Metal concentration in wet sludge

ASHBRIDGE'S BAY TREATMENT PLANT BIO-SOLIDS DEMONSTRATION PROJECT : LABORATORY REPORT

Sampled 10-Jun 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.15	6.1	170	5619	(100)
Cd	<0.1	<4.00	34	>8500	(500)
Co	<0.1	<4.00	340	>8500	(50)
Cr	2.56	102.4	2800	334	(6)
Cu	25.4	1016.0	1700	34	(10)
Hg	0.047	1.9	11	18251	(1500)
Mo	0.37	14.8	94	2308	(180)
Ni	0.57	22.8	420	1498	(40)
Pb	1.75	70.0	1100	488	(15)
Se	0.119	4.8	34	7178	(500)
Zn	19.1	764.0	4200	45	(4)

Sampled 26-Jun 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.11	5.9	170	6965	(100)
Cd	<0.1	<5.26	34	>7800	(500)
Co	<0.1	<5.26	340	>7800	(50)
Cr	3.21	168.9	2800	243	(6)
Cu	19.8	1042.1	1700	39	(10)
Hg	0.040	2.1	11	19308	(1500)
Mo	0.28	14.7	94	2786	(180)
Ni	0.59	31.1	420	1322	(40)
Pb	1.31	68.9	1100	595	(15)
Se	0.086	4.5	34	9070	(500)
Zn	16.2	852.6	4200	48	(4)

Total solids in sludge =	2.5 %
Volatile total solids in sludge =	60 %
Ammonia nitrogen in sludge =	854 mg/L
Nitrate nitrogen in sludge =	0.13 mg/L

Total solids in sludge =	1.9 %
Volatile total solids in sludge =	63.0 %
Ammonia nitrogen in sludge = .	780 mg/L
Nitrate nitrogen in sludge =	0.03 mg/L

Centrifuge Cake (Sampled   15-Jun)

Total solids in cake =	27.3 %
Volatile total solids in cake =	60.0 %
Total phosphorus (as P) in cake =	27500 mg/Kg dry weight
TKN in cake =	43500 mg/Kg dry weight

Centrifuge Cake (Sampled   26-Jun)

Total solids in cake =	26.4 %
Volatile total solids in cake =	62.0 %
Total phosphorus (as P) in cake =	36300 mg/Kg dry weight
TKN in cake =	45900 mg/Kg dry weight

* As per MOEE regulations for sludge utilisation on agricultural lands.

N = Sum of ammonia nitrogen and nitrate nitrogen
M = Metal concentration in wet sludge

ASHBRIDGE'S BAY TREATMENT PLANT BIO SOLIDS DEMONSTRATION PROJECT : LABORATORY REPORT

Sampled 15-Jul 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.11	5.1	170	9402	(100)
Cd	<0.1	<4.76	34	>10000	(500)
Co	<0.1	<4.76	340	>10000	(50)
Cr	3.01	143.3	2800	334	(6)
Cu	17.9	852.4	1700	56	(10)
Hg	0.045	2.2	11	22258	(1500)
Mo	0.25	11.9	94	4024	(100)
Ni	0.61	29.0	420	1649	(40)
Pb	1.55	73.8	1100	649	(15)
Se	0.013	0.6	34	79217	(500)
Zn	20.4	971.4	4200	49	(4)

Sampled 29-Jul 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.11	5.3	170	8896	(100)
Cd	<0.1	<5.00	34	>9300	(500)
Co	<0.1	<5.00	340	>9300	(50)
Cr	2.41	120.5	2800	388	(6)
Cu	17.4	870.0	1700	54	(10)
Hg	0.044	2.2	11	21277	(1500)
Mo	0.24	12.0	94	3892	(100)
Ni	0.49	24.5	420	1906	(40)
Pb	1.43	71.5	1100	653	(15)
Se	0.012	0.6	34	76561	(500)
Zn	19.4	970.0	4200	48	(4)

Total solids in sludge = 2.1 %

Volatile total solids in sludge = 56 %

Ammonia nitrogen in sludge = 1006 mg/L

Nitrate nitrogen in sludge = <0.1 mg/L

Total solids in sludge = 2.0 %

Volatile total solids in sludge = 61.0 %

Ammonia nitrogen in sludge = 934 mg/L

Nitrate nitrogen in sludge = <0.1 mg/L

Centrifuge Cake   (Sampled 15-Jul   )

Total solids in cake = 27.4 %

Volatile total solids in cake = 59.8 %

Total phosphorus (as P) in cake = 31000 mg/Kg dry weight

TKN in cake = 46800 mg/Kg dry weight

Centrifuge Cake   (Sampled 29-Jul   )

Total solids in cake = 26.5 %

Volatile total solids in cake = 61.6 %

Total phosphorus (as P) in cake = 29000 mg/Kg dry weight

TKN in cake = 52700 mg/Kg dry weight

* As per MOEE regulations for sludge utilisation on agricultural lands.

N = Sum of ammonia nitrogen and nitrate nitrogen

M = Metal concentration in wet sludge

ASHBRIDGE'S BAY TREATMENT PLANT BIO-SOLIDS DEMONSTRATION PROJECT : LABORATORY REPORT

Sampled 12 Aug 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.10	5.7	170	7347	(100)
Cd	<0.1	<5.88	34	>7100	(500)
Co	<0.1	<5.88	340	>7100	(50)
Cr	2.71	159.4	2800	262	(8)
Cu	25.7	1511.8	1700	28	(10)
Hg	0.036	2.1	11	19899	(1500)
Mo	0.35	20.6	94	2030	(180)
Ni	0.55	32.4	420	1292	(40)
Pb	1.56	91.8	1100	455	(15)
Se	0.093	5.5	34	7606	(500)
Zn	21.0	1235.3	4200	34	(4)

Sampled 26 Aug 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.06	3.6	170	6451	(100)
Cd	<0.1	<4.76	34	>4900	(500)
Co	<0.1	<4.76	340	>4900	(50)
Cr	1.92	91.4	2800	255	(8)
Cu	22.4	1066.7	1700	22	(10)
Hg	0.104	5.0	11	4714	(1500)
Mo	0.31	14.8	94	1581	(180)
Ni	0.56	26.7	420	875	(40)
Pb	1.59	75.7	1100	308	(15)
Se	0.105	5.0	34	4669	(500)
Zn	16.9	804.8	4200	29	(4)

Total solids in sludge = 1.7 %

Volatile total solids in sludge = q %

Ammonia nitrogen in sludge = 710 mg/L

Nitrate nitrogen in sludge = 0.41 mg/L

Centrifuge Cake (Sampled 15-Aug)

Total solids in cake = 29.3 %

Volatile total solids in cake = q %

Total phosphorus (as P) in cake = 31300 mg/Kg dry weight
TKN in cake = 51300 mg/Kg dry weight

Total solids in sludge = 2.1 %

Volatile total solids in sludge = q %

Ammonia nitrogen in sludge = 490 mg/L

Nitrate nitrogen in sludge = 0.24 mg/L

Centrifuge Cake (Sampled 31-Aug)

Total solids in cake = 28.5 %

Volatile total solids in cake = q %

Total phosphorus (as P) in cake = 32300 mg/Kg dry weight
TKN in cake = 52400 mg/Kg dry weight

* As per MOEE regulations for sludge utilisation on agricultural lands.

q Equipment failure : No results

N = Sum of ammonia nitrogen and nitrate nitrogen

M = Metal concentration in wet sludge

Italicised results : QC data indicates there may be reason to doubt the accuracy of the ammonia nitrogen results. This, in turn, places doubt on the N : M ratios. The samples were discarded in error before the test could re-run and the results verified.

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Sampled 16 Sep 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.05	3.5	170	12928	(100)
Cd	<0.1	<7.14	34	>6400	(500)
Co	<0.1	<7.14	340	>6400	(50)
Cr	1.33	95.0	2800	476	(6)
Cu	16.1	1150.0	1700	40	(10)
Hg	0.055	3.9	11	11649	(1500)
Mo	0.16	11.4	94	3975	(180)
Ni	1.56	111.4	420	408	(40)
Pb	0.68	48.6	1100	935	(15)
Se	0.049	3.5	34	12928	(500)
Zn	12.3	878.6	4200	52	(4)

Sampled 30 Sep 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.06	3.6	170	10220	(100)
Cd	<0.1	<5.56	34	>6500	(500)
Co	<0.1	<5.56	340	>6500	(50)
Cr	1.78	98.9	2800	367	(6)
Cu	21.3	1183.3	1700	31	(10)
Hg	0.037	2.1	11	17488	(1500)
Mo	0.34	18.9	94	1924	(180)
Ni	1.28	71.1	420	511	(40)
Pb	1.41	78.3	1100	464	(15)
Se	0.073	4.1	34	8960	(500)
Zn	16.1	894.4	4200	41	(4)

Total solids in sludge =	1.4 %
Volatile total solids in sludge =	q %
Ammonia nitrogen in sludge =	636 mg/L
Nitrate nitrogen in sludge =	<0.1 mg/L

Total solids in sludge =	1.8 %
Volatile total solids in sludge =	63.0 %
Ammonia nitrogen in sludge =	654 mg/L
Nitrate nitrogen in sludge =	<0.1 mg/L

Centrifuge Cake   (Sampled 15-Sep   )

Total solids in cake =	28.5 %
Volatile total solids in cake =	q %
Total phosphorus (as P) in cake =	29000 mg/Kg dry weight
TKN in cake =	42000 mg/Kg dry weight

Centrifuge Cake   (Sampled 29-Sep   )

Total solids in cake =	27.6 %
Volatile total solids in cake =	61.4 %
Total phosphorus (as P) in cake =	23000 mg/Kg dry weight
TKN in cake =	43400 mg/Kg dry weight

* As per MOEE regulations for sludge utilisation on agricultural lands.
q Equipment failure : No result

N = Sum of ammonia nitrogen and nitrate nitrogen
M = Metal concentration in wet sludge

ASHBRIDGE'S BAY TREATMENT PLANT BIO-SOLIDS DEMONSTRATION PROJECT : LABORATORY REPORT

Sampled 11-Oct 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.05	2.2	170	13301	(100)
Cd	<0.1	<4.76	34	>6300	(500)
Co	<0.1	<4.76	340	>6300	(50)
Cr	2.35	111.9	2800	266	(6)
Cu	22.7	1081.0	1700	28	(10)
Hg	0.029	1.4	11	21336	(1500)
Mo	0.22	10.5	94	2842	(100)
Ni	1.04	49.5	420	601	(40)
Pb	1.54	73.3	1100	406	(15)
Se	0.041	2.0	34	15248	(500)
Zn	18.6	883.3	4200	34	(4)

Sampled 24-Oct 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.09	4.9	170	8266	(100)
Cd	<0.1	<5.26	34	>7800	(500)
Co	<0.1	<5.26	340	>7800	(50)
Cr	2.14	112.6	2800	363	(6)
Cu	21.7	1142.1	1700	36	(10)
Hg	0.096	5.1	11	8077	(1500)
Mo	0.21	11.1	94	3700	(100)
Ni	0.79	41.6	420	984	(40)
Pb	1.58	83.2	1100	492	(15)
Se	0.064	3.4	34	12104	(500)
Zn	16.8	884.2	4200	46	(4)

Total solids in sludge = 2.1 %
Volatile total solids in sludge = 58 %
Ammonia nitrogen in sludge = 625 mg/L
Nitrate nitrogen in sludge = 0.15 mg/L

Total solids in sludge = 1.9 %
Volatile total solids in sludge = 60.0 %
Ammonia nitrogen in sludge = 777 mg/L
Nitrate nitrogen in sludge = <0.1 mg/L

Centrifuge Cake (Sampled 19-Oct)

Total solids in cake = 30.0 %
Volatile total solids in cake = 59.6 %
Total phosphorus (as P) in cake = 33100 mg/Kg dry weight
TKN in cake = 57000 mg/Kg dry weight

Centrifuge Cake (Sampled 28-Oct)

Total solids in cake = 30.4 %
Volatile total solids in cake = 61.7 %
Total phosphorus (as P) in cake = 23900 mg/Kg dry weight
TKN in cake = 44500 mg/Kg dry weight

* As per MOEE regulations for sludge utilisation on agricultural lands.

N = Sum of ammonia nitrogen and nitrate nitrogen
M = Metal concentration in wet sludge

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Sampled 14 Nov 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.06	3.2	170	12095	(100)
Cd	<0.1	<5.00	34	>7700	(500)
Co	<0.1	<5.00	340	>7700	(50)
Cr	2.14	107.0	2800	362	(8)
Cu	21.2	1060.0	1700	37	(10)
Hg	0.029	1.5	11	26329	(1500)
Mo	0.22	11.0	94	3519	(180)
Ni	1.53	76.5	420	506	(40)
Pb	1.43	71.5	1100	541	(15)
Se	0.077	3.9	34	10053	(500)
Zn	17.0	850.0	4200	46	(4)

Sampled 28 Nov 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.07	3.0	170	10732	(100)
Cd	<0.1	<4.55	34	>7200	(500)
Co	<0.1	<4.55	340	>7200	(50)
Cr	2.93	133.2	2800	245	(8)
Cu	23.1	1050.0	1700	31	(10)
Hg	0.034	1.6	11	20903	(1500)
Mo	0.27	12.3	94	2663	(180)
Ni	1.00	45.5	420	719	(40)
Pb	1.56	70.9	1100	461	(15)
Se	0.086	3.9	34	8361	(500)
Zn	18.1	822.7	4200	40	(4)

Total solids in sludge =   2.0 %
Volatile total solids in sludge =   57 %
Ammonia nitrogen in sludge =   774 mg/L
Nitrate nitrogen in sludge =   0.07 mg/L

Centrifuge Cake   (Sampled   16-Nov)
Total solids in cake =   26.6 %
Volatile total solids in cake =   57.8 %
Total phosphorus (as P) in cake =   35602 mg/Kg dry weight
TKN in cake =   49547 mg/Kg dry weight

Total solids in sludge =   2.2 %
Volatile total solids in sludge =   58.0 %
Ammonia nitrogen in sludge =   719 mg/L
Nitrate nitrogen in sludge =   0.07 mg/L

Centrifuge Cake   (Sampled   28-Nov)
Total solids in cake =   29.0 %
Volatile total solids in cake =   59.1 %
Total phosphorus (as P) in cake =   26433 mg/Kg dry weight
TKN in cake =   47393 mg/Kg dry weight

* As per MOEE regulations for sludge utilisation on agricultural lands

N = Sum of ammonia nitrogen and nitrate nitrogen
M = Metal concentration in wet sludge

ASHBRIDGE'S BAY TREATMENT PLANT BIO-SOLIDS DEMONSTRATION PROJECT : LABORATORY REPORT

Sampled 17-Dec 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.07	3.9	170	11265	(100)
Cd	0.048	2.8	34	15418	(500)
Co	0.06	3.8	340	11564	(50)
Cr	1.75	102.9	2800	423	(6)
Cu	18.3	1076.5	1700	40	(10)
Hg	0.035	2.0	11	21452	(1500)
Mo	0.21	12.4	94	3524	(180)
Ni	0.50	29.4	420	1480	(40)
Pb	1.15	67.6	1100	644	(15)
Se	0.060	3.5	34	12438	(500)
Zn	15.0	882.4	4200	49	(4)

Sampled 27-Dec 2001	Conc in Wet Sludge mg/L	Conc on a Dry weight Basis mg/Kg	Maximum * Allowable Dry wt conc mg/Kg	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.07	3.8	170	10948	(100)
Cd	0.044	2.3	34	18140	(500)
Co	0.06	3.4	340	12471	(50)
Cr	3.20	168.4	2800	249	(6)
Cu	18.9	994.7	1700	42	(10)
Hg	0.038	2.0	11	20949	(1500)
Mo	0.23	12.1	94	3470	(180)
Ni	0.51	26.8	420	1565	(40)
Pb	1.09	57.4	1100	732	(15)
Se	0.073	3.8	34	10948	(500)
Zn	15.4	810.5	4200	52	(4)

Total solids in sludge =	1.7 %
Volatile total solids in sludge =	64 %
Ammonia nitrogen in sludge =	740 mg/L
Nitrate nitrogen in sludge =	0.08 mg/L

Total solids in sludge =	1.9 %
Volatile total solids in sludge =	60.0 %
Ammonia nitrogen in sludge =	798 mg/L
Nitrate nitrogen in sludge =	0.14 mg/L

Centrifuge Cake   (Sampled   13-Dec)

Total solids in cake =	27.4 %
Volatile total solids in cake =	59.6 %
Total phosphorus (as P) in cake =	28840 mg/Kg dry weight
TKN in cake =	44658 mg/Kg dry weight

Centrifuge Cake   (Sampled   19-Dec)

Total solids in cake =	28.2 %
Volatile total solids in cake =	58.3 %
Total phosphorus (as P) in cake =	35736 mg/Kg dry weight
TKN in cake =	59561 mg/Kg dry weight

*   As per MOEE regulations for sludge utilisation on agricultural lands.

N = Sum of ammonia nitrogen and nitrate nitrogen
M = Metal concentration in wet sludge

Appendix B: LABORATORY REPORT METALS ANALYSIS-Date: Sep-26-2002

TORONTO WORKS & EMERGENCY SERVICES DEPARTMENT
WASTEWATER QUALITY LABORATORY SERVICES

Sampled 26-Sep 2002	Conc in Wet Sludge (mg/L)	Conc on a Dry weight Basis (mg/Kg)	Maximum * Allowable Dry wt conc (mg/Kg)	Nitrogen to Metal Ratio in Wet Sludge	Minimum * Allowable N/M Ratio
As	0.071	3.7	170	10199	100
Cd	0.051	2.7	34	14198	500
Co	0.08	4.2	340	9051	50
Cr	2.07	108.9	2800	350	6
Cu	21.4	1126.3	1700	34	10
Hg	0.02	1	11	36756	1500
Mo	0.33	17.1	94	2228	180
Ni	0.48	25.4	420	1499	40
Pb	1.34	70.5	1100	540	15
Se	0.075	3.9	34	9655	500
Zn	14	736.8	4200	52	4

Total solids in sludge =   1.9   %
Volatile total solids in sludge =   62   %
Ammonia nitrogen in sludge =   724   mg/L
Nitrate nitrogen in sludge   =   0.1   mg/L

Appendix C: Plant Influents: Metals Analysis

TORONTO WORKS & EMERGENCY SERVICES DEPARTMENT

WASTEWATER QUALITY LABORATORY SERVICES
LABORATORY REPORT

PLANT INFLUENTS : METALS ANALYSIS

2001 AVERAGE VALUES

2001	HIGHLAND CREEK	NORTH TORONTO	HUMBER	ASHBRIDGES BAY
As	0.001	0.001	0.001	0.002
Cd	<0.01	<0.01	<0.01	<0.01
Cr	0.01	0.02	0.02	0.03
Cu	0.45	0.73	1.39	1.85
Fe	0.46	0.79	1.41	1.88
Hg(ug/L)	0.11	0.15	0.12	0.16
Ni	<0.01	<0.01	<0.01	<0.01
Pb	<0.06	<0.06	<0.06	<0.06
Zn	0.14	0.11	0.20	0.23

Results in mg/L unless stated otherwise

For each metal reported there is a minimum detection limit (MDL), below which accurate analysis by the method employed is not possible. Such results are reported as <MDL.

For those numbers reported as <MDL in the monthly reports, a value of MDL/2 has been used in calculating the yearly average. Any calculated average which turns out to be below the MDL is reported as <MDL

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Appendix D: Provincial Guidelines for Sewage Sludge Utilization on Agricultural Lands

PROVINCIAL GUIDELINES
FOR SEWAGE SLUDGE UTILIZATION
ON AGRICULTURAL LANDS

METAL CRITERIA FOR FLUID[a] ANAEROBICALLY DIGESTED SEWAGE SLUDGES

1 Metal	2 Mean Metal Content of Uncontaminated Ontario Soils ug/g[b]	3 Maximum Permissible Metal Content in Soil (ug/g)[b]	4 Maximum Permissible Metal Addition to Soil kg/ha[d]	5 Minimum Ammonium plus Nitrate Nitrogen (NH$_4^+$-N plus NO$_3^-$-N) to metal Ratios Required in Sewage Sludge	6 Number of Years To Reach Maximum Recommended Metal Content in Soil (col. 3), Based on cols. 2, 3 and 5[c],[d]	7 Minimum ammonium plus Nitrate Nitrogen (NH$_4^+$-N plus NO$_3^-$-N) to Metals Ratios Required To Give Maximum Permissible Metal Content in Soil (col. 3) in 250 years[d]
ARSENIC	7	14	14	100	50	480
CADMIUM	0.8	1.6	1.6	500	30	4200
COBALT	5	20	30	50	55	220
CHROMIUM	15	120	210	8	45	32
COPPER	25	100	150	10	55	45
MERCURY	0.1	0.5	0.8	1500	45	8400
MOLYBDENUM	2	4	4	100	25	1700
NICKEL	16	32	32	40	45	210
LEAD	15	60	90	15	50	75
SELENIUM	0.4	1.6	2.4	500	45	2800
ZINC	55	220	330	4	50	20

a   Dewatered and dried sludges are to meet the Column 5 criteria prior to dewatering and drying.

b   Based on dry weight at 100°C. The terms ug/g and mg/kg are interchangeable.

c   Based on 135 kg. of ammonium plus nitrate nitrogen per hectare per five years and sewage sludge having minimum ratios. Number of years is rounded off to the nearest five years.

d   Columns 4, 6 and 7 apply to soils of mean metal content (column 2) and require adjustment for soils lower or higher in metal content.

PROVINCIAL GUIDELINES
FOR SEWAGE SLUDGE UTILIZATION
ON AGRICULTURAL LANDS

METAL CRITERIA[a]
FOR ALL AEROBIC SEWAGE SLUDGES AND
FOR ALL DRIED AND DEWATERED ANAEROBIC SEWAGE SLUDGES

Metal	Maximum Permissible Metal Concentration (mg/kg of solids)[b]
Arsenic	170
Cadmium	34
Cobalt	340
Chromium	2800
Copper	1700
Mercury	11
Molybdenum	94
Nickel	420
Lead	1100
Selenium	34
Zinc	4200

a   For permissible number of years to reach maximum metal concentrations in soils, refer to Table 2, column 6.

b   Acceptability will be judged on the basis of the ratio of the average metal concentration during the preceding 12 months to the average concentration of solids during the same period or, at the discretion of local MOE staff and when solids and metals analyses are conducted once a month, on the basis of the last 3 results.

CALCULATIONS FOR AEROBIC SLUDGE USE ON LAND

1. To determine sludge acceptability, calculate 'Actual Metal Concentrations' and compare with the above Permissible Values

i.e. $\dfrac{\text{Metal Concentration (mg/l)} \times 10^6}{\text{Sludge Solids concentration (mg/l)}} = \dfrac{\text{mg of metal}}{\text{Kg of solids}}$

2. Calculate maximum application rate per 5 year period.

i.e. $\dfrac{8 \times 10^6}{\text{Sludge Solids (mg/l)}} = \dfrac{\text{Cubic Metres of Sludge}}{\text{Land Area in Hectares}}$

Appendix E   Experimental Data

E1: pH & ORP variation of activated & digested sludge during incubation (Stage I)

	Digested	Sludge	Activated	Sludge
Hours	pH	ORP(mV)	pH	ORP(mV)
0	3.9	161	4.0	138
8	3.9	172	3.9	145
16	3.9	185	3.7	157
24	3.9	205	3.6	181
32	3.8	222	3.4	189
40	3.8	243	3.2	205
48	3.8	265	3.0	229
56	3.7	289	2.9	256
64	3.6	311	2.8	287
72	3.6	335	2.7	319
80	3.4	358	2.7	338
88	3.3	388	2.6	366
96	3.1	412	2.5	384
104	2.9	430	2.4	404
112	2.9	441	2.3	419
120	2.8	452	2.2	433
128	2.7	457	2.1	445
136	2.7	465	2.1	447
144	2.6	477	2.1	451
152	2.6	483	2.1	453
160	2.6	480	2.0	456
168	2.5	479	2.0	458

E2: pH & ORP variation of activated & digested sludge during incubation (Stage II)

Hours	Digested	Sludge	Activated	Sludge
	pH	ORP(mV)	pH	ORP(mV)
0	4.0	231	4.0	210
8	4.0	240	4.0	211
16	3.9	251	3.9	219
24	3.9	268	3.8	223
32	3.8	281	3.7	240
40	3.7	297	3.5	258
48	3.6	312	3.4	273
56	3.6	330	3.3	287
64	3.5	341	3.2	291
72	3.5	359	3.1	308
80	3.4	377	3.0	312
88	3.4	385	2.9	327
96	3.4	395	2.8	337
104	3.3	412	2.8	343
112	3.3	419	2.7	351
120	3.2	428	2.6	360
128	3.2	433	2.5	371
136	3.1	449	2.6	397
144	3.1	456	2.5	411
152	2.9	467	2.5	422
160	2.9	479	2.5	426
168	2.8	489	2.5	433
176	2.7	493	2.5	440
184	2.6	498	2.4	457
192	2.5	502	2.4	465
200	2.6	509	2.4	467
208	2.5	508	2.3	468
216	2.4	509	2.3	471
224	2.3	504	2.2	471
232	2.2	506	2.2	475

E3: Copper removal efficiency under different TS (pH4~6)

TS	pH (±0.1)	Mean Conc. (mg/L)	Percent Removal (%)
100% TS	4	2.324	13.01
50% TS	4	2.474	14.65
25% TS	4	1.308	15.48
100% TS	5	1.267	7.50
50% TS	5	0.608	7.20
25% TS	5	0.171	8.08
100% TS	6	0.504	2.98
50% TS	6	0.268	3.17
25% TS	6	0.051	2.43

Note: Copper concentration of raw sludge is 17.86 mg/L

E4: Zinc removal efficiency under different TS (pH4~6)

TS	pH (±0.1)	Mean Conc. (mg/L)	Percent Removal (%)
100% TS	4	0.892	17.50
50% TS	4	2.816	18.43
25% TS	4	2.732	17.90
100% TS	5	0.505	9.91
50% TS	5	0.686	8.97
25% TS	5	0.289	7.60
100% TS	6	0.116	2.27
50% TS	6	0.371	4.85
25% TS	6	0.117	3.10

Note: Zinc concentration of raw sludge is 15.285 mg/L

E5: Cadmium removal efficiency under different TS (pH4~6)

TS	pH (±0.1)	Mean Conc. (mg/L)	Percent Removal (%)
100% TS	4	0.024	17.50
50% TS	4	0.050	18.43
25% TS	4	0.064	17.90
100% TS	5	0.023	18.64
50% TS	5	0.018	29.95
25% TS	5	0.01	32.17
100% TS	6	0.007	6.38
50% TS	6	0.004	6.10
25% TS	6	0.004	13.31

Note: Cadmium concentration of raw sludge is 0.122 mg/L.

E6: Metal removal efficiency under different pH (25~100% TS)

TS	Cu		Zn		Cd		pH
	Mean Conc.	Removal (%)	Mean Conc.	Removal (%)	Mean Conc.	Removal (%)	pH(±0.1)
	(mg/L)		(mg/L)		(mg/L)		
100% TS	2.324	13.01	0.892	17.50	0.024	19.74	4
100% TS	1.267	7.50	0.505	9.91	0.023	18.64	5
100% TS	0.504	2.98	0.116	2.27	0.007	6.38	6
100% TS	0.015	0.09	0.015	0.30	0.002	1.94	7
100% TS	0.034	0.20	0.034	0.67	0.002	1.94	8
100% TS	0.012	0.07	0.012	0.24	0.001	1.11	9
50% TS	1.237	14.65	1.408	18.43	1.237	14.65	4
50% TS	0.608	7.20	0.686	8.97	0.608	7.20	5
50% TS	0.268	3.17	0.371	4.85	0.268	3.17	6
50% TS	0.017	0.10	0.095	1.25	0.017	0.10	7
50% TS	0.027	0.16	0.027	0.36	0.027	0.16	8
50% TS	0.017	0.10	0.018	0.23	0.017	0.10	9
25% TS	0.327	15.48	0.683	17.90	0.327	15.48	4
25% TS	0.171	8.08	0.289	7.60	0.171	8.08	5
25% TS	0.051	2.43	0.117	3.10	0.051	2.43	6
25% TS	0.020	0.46	0.088	2.31	0.020	0.46	7
25% TS	0.011	0.06	0.013	0.33	0.011	0.06	8
25% TS	0.016	0.09	0.020	0.52	0.016	0.09	9

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E7: Metal removals under different reaction period with adapted sludge
(CSTR Phase I)

Reaction	Metal Conc. after bioleaching		(mg/L)	Percent	Removal	(%)
days	Cu	Zn	Cd	Cu	Zn	Cd
2	2.440	3.963	0.046	14.4	25.5	38.6
3	2.594	4.353	0.058	15.4	28.4	48.0
4	2.734	4.359	0.047	16.2	28.5	38.8
5	5.078	5.973	0.096	30.1	39.2	80.1
6	9.492	11.406	0.100	56.2	74.2	82.9
8	11.530	12.333	0.095	68.3	80.6	78.8
10	12.370	12.693	0.093	73.2	81.6	77.4
12	12.600	12.525	0.093	74.6	80.5	77.5
13	13.220	12.261	0.099	78.2	80.5	82.6
14	13.180	12.294	0.093	78.0	80.8	77.7
16	13.170	12.633	0.099	77.9	81.3	82.1
18	13.310	12.966	0.098	78.8	82.0	81.5
20	13.380	12.657	0.101	79.2	80.8	83.9

Note: Cu, Zn and Cd concentrations of raw sludge are 17.860, 15.285 and 0.122 mg/L, respectively.

E8: Metal removals under different reaction period with inoculums
(CSTR Phase II)

Reaction	Metal Conc. after bioleaching (mg/L)			Percent	Removal	(%)
days	Cu	Zn	Cd	Cu	Zn	Cd
1	0.973	0.888	0.005	5.8	5.8	3.3
2	1.079	0.957	0.005	6.4	6.3	3.8
3	1.65	1.032	0.018	9.8	6.8	12.4
4	1.446	1.302	0.038	8.6	8.5	26.5
5	2.409	3.261	0.048	14.3	21.3	34.0
6	3.326	3.894	0.066	19.7	25.5	46.7
7	3.609	4.002	0.074	21.4	26.2	52.3
8	5.998	8.970	0.118	35.5	58.7	82.8
9	9.611	11.340	0.124	56.9	74.2	87.1
10	10.25	11.955	0.123	60.7	78.2	86.5

Note: Cu, Zn and Cd concentrations of raw sludge are 15.401, 12.959 and 0.142 mg/L,

respectively.

E9: TSS degradation during bioleaching process of CSTR with adapted sludge

Days	TSS (mg/l)
Initial	8290
2	7060
4	6280
6	6065
8	5500
10	4480
12	4110
14	3870
16	3595
18	3375
20	3195