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EXTRACTION AND REMOVAL OF HEAVY METALS FROM CONTAMINATED SLUDGE USING RAW AND FERMENTED LIQUID FROM PINEAPPLE WASTES

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A Thesis Presented

By

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Doctor of Philosophy
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Abstract

One of the main concerns in the land disposal of sludge is the presence of toxic heavy metals which concentrate during various physico-chemical and biological interactions occurring in sludge treatment. Other factors that heighten the concern over the presence of these heavy metals in the environment are their nonbiodegradability and consequent persistence. This study investigated the potential of utilizing pineapple wastes as a source of citric acid in the extraction of cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni) and zinc (Zn) from anaerobically digested sewage sludge for land application. Leaching experiments were done to determine optimum conditions of metals removal using citric acid, unfermented, naturally fermented, and *A. niger* fermented raw liquid from pineapple wastes. Chemical speciation studies using sequential chemical extraction procedure (SCE) were also done to understand how the metals are bound to the sludge and their effect on extraction efficiency as well as their mobility in the soil after land application.

Results of lab-scale studies revealed the high potential of raw liquid from pineapple wastes as extractant in the removal of heavy metals from sewage sludge, with the best removal of Cd (64.6%), Cr (83.4%), Ni (82.4%) and Zn (100%) achieved by leaching with *A. niger* fermented liquid at optimum conditions, though effectivity of removal seemed to be less apparent for Cu and Pb. Removal of heavy metals seemed to be affected by pH, contact time as well as the nature of the sludge sample and the forms of metals in the sludge. Moreover, the treated sludge being low in faecal coliform (almost pathogen free for *A. niger* treated sludge), low in heavy metals, high in nutrient content (2% for N, 3.5% P), high in organic matter content and dominant in residual fractions for most metals, has also a high potential for land application. Furthermore, this study demonstrated a novel and sustainable way of managing contaminated sewage sludge by utilizing one form of wastes (pineapple wastes) to treat another form of wastes (sewage sludge), with practically no wastes produced.

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List of Symbols and Abbreviations

AAS	Atomic Absorption Spectrophotometer
AIT	Asian Institute of Technology
A. niger	*Aspergillus niger*
ABS	Absorbance
Al	Aluminum
AOAC	Association of Official Agricultural Chemists
As	Arsenic
BMA	Bangkok Metropolitan Administration
Cd	Cadmium
CEC	Cat-ion Exchange Capacity
COD	Chemical Oxygen Demand
Cr	Chromium
Cu	Copper
CWTP	Central Wastewater Treatment Facility
DDT	Dichloro-diphenyl-trichloroethane
DI	Deionized water
DPS	Digested primary sludge
DS	Dry solids
DTPA	Diethylenetriaminepentaacetic acid
DWAS	Digested waste activated sludges
EDTA	Ethylenediaminetetraacetic acid
EEC	European Economic Community
EU	European Union
Fe	Iron
ha	Hectare
H_3BO_3	Boric acid
HCl	Hydrochloric acid
HF	Hydrofluoric acid
Hg	Mercury
HNO_3	Nitric acid
H_2O_2	Hydrogen peroxide
HPLC	High Performance Liquid Chromatography
ICP/MS	Inductively Coupled Plasma Mass Spectrometry
IR	Infrared
$MgCl_2$	Magnesium Chloride
Mn	Manganese
Mo	Molybdenum
NaAc	Sodium Acetate
Na_2EDTA	EDTA disodium salt
Ni	Nickel

List of Symbols and Abbreviations (cont.)

NTA	Nitrilotriacetic acid
NTU	Nephelometric Turbidity Units
$NH_2OH.HCl$	Hydroxylamine hydrochloride
ORP	Oxidation reduction potential
Pb	Lead
PCBs	Polychlorinated biphenyls
PDA	Potato dextrose agar
ppm	Parts per million
PS	Primary sludge
rpm	Revolutions per minute
Sb	Antimony
SCE	Sequential Chemical Extraction
Se	Selenium
tds	Tonnes of dry solids
TOC	Total Organic Carbon
TS	Total Solids
TVS	Total Volatile Solids
Tween 80	Polyoxyethylene-sorbiton monooleate
µm	Micron
WAS	Waste activated sludge
YPG	Yeast, Peptone, Glucose
Zn	Zinc

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Chapter 1

Introduction

1.1 General Background

Wastewater treatment plants usually generate millions of tons of residual sludges worldwide every year. The management of this sludge is a major part of waste treatment, involving substantial cost and effort. For instance, estimates for 1999 sludge production in the U.K. were close to 1.2 million tons of dry solids or tds (Twigg et al., 2002) and were predicted to double to 2.18 x 10^6 tds by the year 2006 (Wilson, 1998). Sludge production in other countries is similarly excessive. In the United States, about 6.9 million tds per year of municipal sewage was produced in 1998 and is expected to increase to 8.2 million tds in 2010. Sludge production in the European Union (EU) in 1997 was about 5.9 x 10^6 tds per year (Marmo, 2005). It was predicted that this figure will rise to 10 million tons by the year 2005 (Lue-Hing et al., 1998). In Taiwan, the volume of sludge originating from municipal and industrial wastewater treatment plants was estimated to be 2.9 x 10^7 and 7.3 x 10^7 m^3/year, respectively (Lo and Chen, 1990). In Thailand, sludge production from 14 small wastewater treatment plants and six central wastewater treatment plants, including septic tank sludge, in the Bangkok Metropolitan area (BMA), is expected to reach up to 63,000 tds/year in 2010 (Stoll, 1995; Eckhardt and Khatiwada, 1998). In Sao Paulo State, Brazil, it was predicted that there would be an estimated sludge production of 615 dry metric tons per day in the year 2005 (225,000 tds per year). In Europe, the progressive implementation of the Urban Wastewater Treatment Directive 91/271/EEC in Member States of the European Economic Community (EEC) is resulting in an increase in the quantities of sewage sludge requiring disposal. This increase is mainly due to the practical implementation of the Directive and the slow but constant rise in the number of households connected to sewers thus increasing quantity of wastewater treated (Europa, 2005). In the developing countries, so far, almost all of the sludge is disposed of in open fields due to the shortage of appropriate disposal facilities, resulting in serious problems and leaching of heavy metals to groundwater, surface water and soil (Marchioretto et al., 2002)

The basic disposal methods for such large quantities of sludge are land application, landfilling, incineration, ocean dumping and lagooning. (Metcalf and Eddy, 2003). The digested or stabilized sludge from municipal wastewater treatment plants (known as sewage sludge) contains a high quantity of nutrients required for plant growth. When spread onto soil, sludge can increase soil fertility and improve soil ventilation and moisture retention, and therefore, is a good soil conditioner (Lo and Chen, 1990). However, for land application of sludge, two types of environmental risks (temporary and persistent) have been identified. Temporary risks disappear within one year or at most a few years following sludge application and include malodor, pathogens, groundwater contamination with nitrate-nitrogen, and phytotoxicity due to soluble salts or toxic biodegradation products from inadequately stabilized sludge. Persistent risks on the other hand, remain long after the temporary ones have disappeared, and include increased concentrations of industrially produced organic compounds such as polychlorinated biphenyls (PCBs) and toxic heavy metals in soil (Lue-Hing et al., 1998).

The presence of metallic contaminants in sewage sludges applied to agricultural land were suspected to be the cause of crop failure as early as 1977 (Lester, 1987a). One study led to the conclusion that "foreign" metals can be retained in soil for several years at least, which emphasizes the danger of accumulation when manures containing metal – contaminated organic material are applied repeatedly (Lester, 1987a). These findings have lead to substantial research programs on the effect of heavy metal contaminants in plant growth, particularly in the US, Canada and U.K. Ultimately, guidelines for the disposal of sludge were formulated in both the UK in 1977 and the US in 1979, which have been updated since then (Lester, 1987a). These guidelines, which are generally based on phytotoxic effects and limited plant uptake studies, normally specify the maximum allowable total metal concentration and exhibit considerable variation. For instance, the maximum annual loading for cadmium (Cd) on agricultural land in Denmark and Sweden, is 15g/ha, Finland and the Netherlands allows up to 20 g/ha, and Norway, up to 30 g/ha. These requirements may be compared with 167 g/ha for the UK, 280 g/ha for Canada (Ontario) and 1250 g/ha for the United States (Lake et al., 1984). In Asia, guidelines for the land application of sludge have been formulated by Japan (Lue-Hing et al., 1998) and Thailand (AIT, 1998).

1.2 Rationale of the Study

Until a few years ago, sewage sludge could be re-used directly in agriculture as fertilizer. Recently, however, there has been an increased concern because of the legal criteria for the heavy metal concentration in sewage sludge (Lue-Hing et al., 1998; Scheltinga, 1987). Concern for heavy metals is due to its nonbiodegradability, toxicity and consequent persistence (Dutta, 2002). Reduction of heavy metals in sewage sludge can be achieved either by source control of industrial and domestic discharges to the sewer systems or by extractive removal of metals from the sludge. Source control includes control of the processes and materials used in production at the industries; removal and controlled disposal of hazardous constituents before they reach the wastestream; separation of highly contaminated industrial wastewater from the domestic wastewater; and pretreatment of the wastes before discharging to the municipal collection system. The major difficulty in source control arises in identifying the sources, which leaves metal removal from the sludge as the only practical solution (Sreekrishnan et al., 1993). It was reported that the total heavy metal content of sewage sludges is about 0.5 to 2.0% on a dry weight basis and in some cases may rise up to 4% on a wet weight basis, especially for metals such as cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn) (Jain and Tyagi, 1992). These heavy metals can come from industrial and domestic discharges, stream runoff and groundwater infiltration from soil to the sewage system (Koch et al., 1982; Wozniak and Huang, 1982). In the United States, for instance, sludges from 50 to 60% of the municipal wastewater treatment plants cannot be applied on agricultural land because the Cd content of the sludge exceeds the standard (Couillard and Mercier, 1990). Recent developments in waste management have also led to a decree which forbids the landfilling of solid wastes with organic matter content higher than 5% (Veeken and Hamelers, 1999). Incineration, on the other hand, causes air pollution and hence requires expensive off–gas treatment (White et al., 1995). Compared to landfilling and incineration, utilization of sludge for agricultural use is the best alternative for sludge disposal because it recycles both nutrients and organic matter, once the heavy metals in sludge are reduced (Levlin, 2004; Pogrzeva et al., 2004; Veeken and Hamelers, 1999; Sreekrishnan et al., 1993). The extraction of heavy metals in sludge before land application is therefore a vital step to achieve a more sustainable form of sludge management.

In recent years, effective methods for heavy metal removal from sludge have been extensively studied in order to minimize the prospective health risks during land application (Wong and Henry, 1988). Various technologies used for the extraction of heavy metals in sludges, such as chemical extraction, bioleaching, electrokinetics, supercritical fluid extraction (SFE), etc., have previously been used in the removal of metals from soils. Chemical extraction uses extracting chemicals such as inorganic acids (sulfuric, hydrochloric and nitric), organic acids (citric and oxalic acid), chelating agents (ethylenediaminetetraacetic acid or EDTA and Nitrilotriacetic acid or NTA) and inorganic chemicals such as ferric chloride, to reduce the volume of the hazardous wastes from soils, sludges or sediments that must be treated. Experimental studies revealed a broad range in metal extraction efficiencies of the different extracting agents. It was also found that inorganic acids and complexing agents are not applicable on a practical scale due to the costs of the process and the negative environmental impacts of the discharged solid and liquid wastes streams. Organic acids especially citric acid can be more promising because the extraction can be performed at mildly acidic condition (pH 3-4) and the acids are readily degradable under aerobic and anaerobic conditions. This implies that the decontaminated sludge does not have to be conditioned resulting to a substantial reduction of wastewater. Citric and oxalic acid both qualify for the extraction process and form relatively strong complexes with heavy metal ions. At mildly acidic pH, both oxalic acid and citric acid seemed to be highly effective in heavy metal extraction. However, citric acid has better prospects because oxalic acid is removed from solution by precipitation as calcium oxalate. Hence, interest is directed toward utilizing citric acid over oxalic acid as extracting agent for the removal of heavy metals.

So far, limited studies have been done on the use of citric acid as extracting agent in the removal of heavy metals from sludge. However, the laboratory scale studies done on this organic acid, proved to be promising. Marchioretto et al. (2002), while assessing the chemical extraction efficiency in heavy metals removal from anaerobically digested sludge, confirmed that at higher pH values (3-4) citric acid achieved the highest extraction efficiency as compared to the inorganic acids used.

Citric acid [$C_3H_5O(COOH)_3$], is a 6-carbon containing tricarboxylic acid and exists as an intermediate in the citric acid cycle when carbohydrates are oxidized to carbon dioxide. Citric acid is produced either by direct extraction from fruits; fermentation of sucrose by fungi; and by chemical synthesis (Alben and Erkmen, 2004; Yigitoglu, 1992; Sun, 1984). At present, citric acid is produced commercially by fermentation of sucrose using mutant strains of *Aspergillus niger (A. niger),* and chemical synthesis. Carbohydrates and wastes that have been considered experimentally to produce citric acid by *A. niger* includes, date fruit syrup, soya whey, cheese whey, pineapple wastes, corncobs, and cane molasses (El-Holi and Al-Delaimy, 2003; Ali et al., 2002; Hang and Woodams, 1998; Sun, 1984). Among those wastes which can be potential sources of citric acid, pineapple solid wastes is of interest, especially in

Thailand where pineapple is one of the major food products in the country. It was reported that in 2002, fresh pineapple production in Thailand reached up to 2.0 million metric tons (Food Market Exchange, 2006). Since 70-80% of the pineapple fruit is discarded as solid waste (Sun, 1984), an equivalent of 1.4 - 1.6 million tons of pineapple solid wastes is also produced. Although some research have been conducted on the utilization of these wastes (such as for animal feed, alcohol, vinegar and wine production), this enormous quantity of discarded material has not been utilized efficiently in practice for a long time. At present, the pineapple solid wastes is still disposed off into the environment at a considerable cost for transportation and causing environmental degradation. Therefore, research on the alternatives for utilization of these wastes is still of interest.

The measurement of the total metal content of sewage sludges and related matrices using analytical methods is well established. However, while total concentrations of metals indicate the extent of contamination, they provide less information into the forms in which the metals are present in sludge or their potential for mobility and bioavailability once released to the environment (Lake et al., 1984; Lester et al., 1983). Hence, a detailed knowledge of the speciation of metals in the sludge itself and the changes in speciation likely to occur following disposal is also necessary to determine the suitability of decontaminated sludge for land application.

1.3 Objectives of the Study

The main objective of the study is to investigate the potential of utilizing pineapple wastes as a source of citric acid in the extraction of heavy metals from contaminated sewage sludge, using commercial citric acid as a reference. Chemical speciation studies were also done to determine various forms of metals in sludge, and to understand how the metals are bound to the sludge and their effect on extraction efficiency as well as their mobility in the soil after land application. The following are the specific objectives of the study:

- To determine the various forms of heavy metals present in sewage sludge.
- To investigate the efficiency of using commercial citric acid ($C_6H_8O_7$) for heavy metal extraction from sewage sludge.
- To investigate the efficiency of using unfermented, naturally fermented, and *A. niger* fermented raw liquid from pineapple waste as a source of citric acid for use in the extraction of heavy metals from sewage sludge.
- To compare the efficiencies of commercial citric acid, unfermented, naturally fermented and *A. niger* fermented raw liquid from pineapple wastes, in extracting heavy metals from sludge.
- To assess the removal of metal forms by the extractants.
- To assess the suitability of treated sludge for land application.

1.4 Scope of the Study

This study included batch scale laboratory experiments to extract heavy metals mainly from anaerobically digested sludge from Nong Khaem sludge treatment facility under the Bangkok Metropolitan Administration (BMA), using acid extraction technology with the following extractants: commercial citric acid, *A. niger* fermented raw liquid from pineapple wastes, naturally fermented raw liquid from pineapple wastes, and unfermented raw liquid from pineapple wastes. The choice of heavy metals was based on the toxicity of the metal and compliance with the proposed local standards for land application of sludge. Based primarily on the recommended standards for heavy metals in sludge for agricultural application under the BMA, the following metals were analyzed: cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni) and and zinc (Zn). The amount of iron (Fe) and manganese (Mn) in the sludge were also determined as these metals are known to affect citric acid production.

Specific description of the various activities covered in the study are as follows:

- Forms of heavy metals in sludge were determined by Sequential Chemical Extraction (SCE) procedure.
- Enhanced citric acid production from pineapple wastes was done by fermentation of raw liquid with *A. niger* and without *A. niger* (natural fermentation).
- Presence of citric acid and other carboxylic acids in raw and fermented liquid from pineapple wastes was confirmed by total acidity, IR spectroscopy and HPLC studies.
- During the extraction process, the following parameters were analyzed to determine conditions that would give the best metal removal efficiency (optimum conditions):
    - pH (2-7)
    - Leaching time (1 hour, 2 hours, 6 hours, 1 day, 5 days, 8 days and 11 days)
    - Metal removal efficiency by measuring metal concentration before and after leaching
- The suitability of sludge for land application was assessed by evaluating the effects on the quality of sludge after treatment with different extractants at optimum conditions.

Chapter 2

Literature Review

2.1 Sludge Production and its Impact on the Environment

Conventional wastewater treatment system particularly the activated sludge system, is a worldwide technology used to treat society's sewage. Such a system, although produces a high quality effluent, also produces excessive amounts of sludge (Marchioretto et al., 2002). Sewage sludge has been defined as a combination of the organic and inorganic solids removed at different stages of wastewater treatment (Stoll, 1995). Municipal sewage sludge processing, utilization, and disposal are some of the most difficult and expensive operations conducted by municipalities today. In the United States, about 16,900 dry tons per day of municipal sewage is produced, which must be managed in an environmentally acceptable way (Lue-Hing et al., 1998). In the UK, 30 million wet tones (1.2×10^6 tds) of sewage sludge were produced from 7,800 publicly owned sewage works serving approximately 82% of its population. The treatment and disposal of the sludge cost the UK government 200 million British pounds in 1987 (Davis, 1987). The 1991 sludge production in the UK was about 1.11×10^6 tds per year. Table 2.1 shows the 1991-92 data on sludge production in the European Community. As shown, the total sludge produced in the European Union (EU) in 1991- 92 was about 6.5×10^6 tds per year. It was predicted that sludge production in the EU will rise to 10 million tonnes by the year 2005 (Lue-Hing et al., 1998).

Table 2.1 Populations Served and Sludge Production in the European Community in 1991-92

Country	Total[a] Population (millions)	Population Connected to Sewer (%)	Sludge Production (tds[b] /yr)
Belgium	9.9	70	59,200
Denmark	5.1	93	170,300
France	56.9	65	852,000
Germany	79.7	89	2,681,200
Former West	62	92	2,449,200
Former East	17	77	232,000
Greece	10.2	45	48,200[c]
Ireland	3.5	67	36,700
Italy	57.7	75	816,000
Luxembourg	0.4	97	7,900
Netherlands	15.0	97	322,900
Portugal	9.9	52	25,000[c]
Spain	39.0	70	350,000
United Kingdom	57.5	96	1,107,000

Total (mean)[d]	344.8	(79)	6,476,400

[a]Population data for 1991
[b]tds = tons of dry solids
[c]Upper estimate
[d]Weighted means in parentheses
Source: Lue-Hing et al. (1998)

Sludge production in other countries are similarly excessive. In New Zealand, it is estimated that 40,000 dry tonnes/ year of domestic source sludge is produced which is likely to increase to around 55,000 dry tonnes/year within the next decade (Lue-Hing et al., 1998). In Taiwan, the volumes of sludge originating from municipal and industrial wastewater treatment plants were estimated to be 2.9 x 10^7 and 7.3 x 10^7 m^3/year, respectively (Lo and Chen, 1990). In Japan, 1.95 x 10^6 m^3 of sludge was disposed in 1988 (Lue-Hing et al., 1998). In Thailand, sludge production from six central wastewater treatment plants in the Bangkok Metropolitan Area (BMA), was estimated to be 457 m^3/day (166,800 m^3/yr) serving a population of 2.4 million (Stoll, 1995). In Malaysia, 38 x 10^6 m^3 of sludge is produced annually costing the Malaysian government about US $ 0.26 billion. Sludge production is expected to double by the year 2020 (Mannan et al., 2005). In Sao Paulo State, Brazil, it was predicted that there would be an estimated sludge production of 615 tds per day (7,380 tds per year) in the year 2005. So far almost all of this sludge is disposed of in open fields resulting, more and more, in serious problems due to the shortage of disposal facility and leaching of heavy metals to underground water, surface water and soil (Marchioretto et al., 2002).

Clearly, the management of the large amounts of sludge produced from treatment facilities is a major part of waste treatment involving substantial cost and effort. Levlin (2004) introduced the concept of sustainable sludge handling in which he defined the concept as a method that meets the requirements of efficient recycling of resources without supply of harmful substances to the humans or the environment. Since wastewater sludge may be regarded both as a resource (nutrient source) which should be recycled properly, and a threat to the environment (due to the presence of heavy metals, organics and pathogens), sludge handling should be performed in an energy and resource efficient way, and should consider most of the sludge components as resources suitable for manufacturing of products, the pollutants being separated into a small stream or be destroyed.

2.2 Sludge Sources, Characteristics and Composition

2.2.1 General

Sludge produced from a treatment plant vary according to the type of plant and its method of operation. Typically, sludge from a conventional wastewater treatment plants come from screening, grit removal, pre-aeration treatment, primary sedimentation, biological treatment, secondary sedimentation and solids-processing facilities (Metcalf and Eddy, 2003; Torrey, 1979). The characteristics of sludge produced from these treatment processes vary depending upon the origin of the solids and sludge, the amount of aging that has taken place, and the type of processing to which they have been subjected. In general, the majority of sludge applied to land will be either anaerobically or aerobically digested sewage sludge. After digestion, sewage sludge may be further processed to reduce the water content by vacuum filtration or centrifugation, resulting in a sludge "cake" containing 30 – 40% solids. In many cases, sewage sludge from digester settling tank, will be applied to land as a suspension containing from 1 to 10% solids. The dewatering of sludge not only influences the economics of sludge disposal but it also alters the chemical composition of the sludge and thus the rate of application on agricultural land (Torrey, 1979).

The composition of municipal wastewaters is highly dependent on the proportion and nature of the community's industrial base. Extremely variable loadings of industrial wastes to municipal sewage systems will increasingly affect treatment plant operations and ultimately the characteristics of the sludge. Some industrial discharges such as various food and agricultural industry wastes may increase usable landspreading constituents. However, many discharges associated with chemical process, metallurgical, mineral products, petroleum, and wood processing industries may contribute undesirable constituents and as a result may affect sludge disposal practices. Sludge characteristics are also markedly influenced by the affluency of the municipal residential area served by the wastewater treatment plant. The increasing household use of marketed chemical compounds (cleaning complexes, drugs, etc.) affect sewage treatment operations and, finally, the sludges produced. The design and integrity of the wastewater collection system can also alter the sludge characteristics. Treatment plant operations are affected by increased hydraulic and solid loadings due to captured urban storm water runoff (combined sewers and/ or groundwater infiltration). Storm water runoff reaching the sewage treatment plant often transports various street refuse such as litter, dirt, bird and animal droppings, air pollution fallout particles, oils, chemical compounds, etc. (Torrey, 1979).

Many of the chemical constituents of sludge, including nutrients, are important in considering the ultimate disposal of the processed sludge and the liquid removed from the sludge during processing. The content of heavy metals, pesticides, and hydrocarbons has to be determined when incineration and land application methods are considered. The typical data on the chemical composition of untreated and digested sludges is shown in Table 2.2.

Table 2.2 Typical Chemical Composition and Properties of Untreated and Digested Sludge

Item	Untreated primary sludge		Digested primary sludge		Activated sludge, range
	Range	Typical	Range	Typical	
Total dry solids (TS) , %	2.0 – 8.0	5.0	6.0 – 12.0	10.0	0.83 – 1.16
Volatile solids (% of TS)	60 – 80	65	30 – 60	40	59 - 88
Grease and fats (% of TS)					
Ether soluble	6-30	-	5-20	18	-
Ether extract	7-35	-	-	-	5-12
Protein (% of TS)	20 – 30	25	15-20	18	32-41
Nitrogen (N, % of TS)	1.5 – 4	2.5	1.6 – 6.0	3.0	2.4 – 5.0
Phosphorous(P_2O_5, % of TS)	0.8 – 2.8	1.6	1.5 – 4.0	2.5	2.8 – 11.0
Potash (K_2O, % of TS)	0 – 1	0.4	0.0 – 3.0	1.0	0.5 – 0.7
Cellulose (% of TS)	8.0 – 15.0	10.0	8.0 – 15.0	10.0	-
Iron (not as sulfide)	2.0 – 4.0	2.5	3.0 – 8.0	4.0	-
Silica(SiO_2, % of TS)	15.0 – 20.0	-	10.0 – 20.0	-	-
pH	5.0 – 8.0	6.0	6.5 – 7.5	7.0	6.5 – 8.0
Alkalinity (mg/L as $CaCO_3$)	500 – 1,500	600	2,500 – 3,500	3,000	580 – 1,100
Organic acids (mg/L as HAc)	200 – 2,000	500	100 - 600	200	1,100 – 1,700
Energy Content, Btu/lb	10,000 – 12,500	11,000	4,000 – 6,000	5,000	8,000 – 10,000

Source: Metcalf and Eddy (2003)

The characteristics of sludge that affect its suitability for land application and beneficial uses include organic content (usually measured as volatile solids), nutrients, pathogens, metals and toxic organics. The fertilizer value of sludge, which should be evaluated where the sludge is to be used as a soil conditioner is based primarily on the content of nitrogen, phosphorous, and potassium (potash). Typical nutrient values of sludge as compared to fertilizers are shown in Table 2.3.

Table 2.3 Comparison of Nutrient Levels in Commercial Fertilizers and Wastewater Sludge

	Nutrients, %		
	Nitrogen	**Phosphorus**	**Potassium**
Fertilizers for typical agricultural use[a]	5	10	10
Typical values for stabilized wastewater sludge	3.3	2.3	0.3

[a] The concentration of nutrients may vary widely depending upon the soil and crop needs.
Source: Metcalf and Eddy (2003)

In most land application systems, sludge provides sufficient nutrients for good plant growth. In some applications, the phosphorus and potassium content of wastewater sludge may be too low to satisfy specific plant uptake requirements.

Trace elements in sludge are those inorganic chemical elements that, in very small quantities, can be essential or detrimental to plants and animals. The term “heavy metals” is used to denote several of the trace elements present in sludge and biosolids (wastewater sludge). The concentrations of heavy metals may vary widely as indicated in Table 2.4.

Table 2.4 Typical Metal Content in Wastewater Sludge

Metal	*Dry Sludge, mg/kg*	
	Range	**Median**
Arsenic (As)	1.1 – 230	10
Cadmium (Cd)	1-3,410	10
Chromium (Cr)	10 – 99,000	500
Cobalt (Co)	11.3 – 2,490	30
Copper (Cu)	84 – 17,000	800
Iron (Fe)	1,000 – 154,000	17,000
Lead (Pb)	13 – 26,000	500
Manganese (Mn)	32 – 9,870	260
Mercury (Hg)	0.6 – 56	6
Molybdenum (Mo)	0.1 – 214	4
Nickel (Ni)	2 – 5,300	80
Selenium (Se)	1.7 – 17.2	5
Tin (Sn)	2.6 – 329	14
Zinc (Zn)	101 – 49,000	1,700

Source: Metcalf and Eddy (2003)

2.2.2 Characteristics of Bangkok Sludge

The Bangkok Metropolitan Administration (BMA) includes Bangkok, the capital of Thailand, and five other provinces of Samutprakan, Pathumthani, Samutsakorn, Nonthaburi and Nakorn Pathom (National Statistical Office, 2004). The BMA has a total of 14 small wastewater treatment plants with a combined capacity of 25,700 m^3/day serving a population of 133,415. The treatment plants produce about 7.3 tds/day (36 m^3/day at 20% dry solids or DS) of sludge. In addition, BMA has also constructed six central wastewater treatment plants (CWTP) with a combined treatment capacity of 1.018 million m^3/day serving a population of about three million. The CWTPs produce about 91.4 tds/day (457.0 m^3/day at 20% DS) of sludge. These central treatment plants treat mostly domestic wastewater and a small amount of industrial wastewater. Sludge produced from these plants is characterized as semi-solid, brown-black in color with unpleasant odor. The properties of the sludge varies depending on the characteristics of the raw wastewater entering the treatment plants (AIT, 1998). Table 2.5 shows the characteristics of sludges obtained from three wastewater treatment plants in the BMA. The Huay Kwang plant employs the conventional activated sludge process while Si Phraya plant employs modified contact stabilization in treating sewage sludge. The plant in Nongkhaem has two treatment facilities. One plant treats nightsoil sludge and employs activated sludge/extended aeration for treatment. The other plant is a central sludge treatment facility which treats sludges (mixed) from five (5) central wastewater treatment facilities and employs anaerobic digestion process.

Table 2.5 Characteristics of Sludge Obtained from Three Central Wastewater Treatment Plants of BMA (Adapted from Sae-Tang, 2004)

Parameter	**Sewage Sludge**					**Nightsoil Sludge/Mixed Sludge**		
	Huay Kwang		**Si Praya**			**Nongkhaem**		
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)*
pH (sludge:water ratio = 1.5)	6.6	7.6	7.2	7.4	6.94	6.72	7.8	7.4
Moisture Content (%)	-	86	86	84	85	-	80	96
Total Kjeldahl Nitrogen (%)	3.15	3.71	3.87	4.2	3.6	5.72	6.1	2.74
Total Phosphorus (%)	1.38	-	4.7	1.32	1.35	-	-	3.23
Organic Matter (%)	58.51	11.17	-	45	46	-	-	
Total Volatile Solids (%)	-	-	-	-	-	-	-	39.6
Organic Carbon (%)	34.42	3.01	-	26.47	27.06	-	-	32.8
C/N ratio	10.93	-	-	6.3	7.52	-	-	12
Heavy metals (mg/Kg dry basis)								
Cd	2.9	2.61	0.77	2.2	1.89	3	2.5	2.5
Cr	49	21	654	208	208	27	19	385
Cu	322	135	1,449	1,269	1,437	395	328	4,673
Pb	125	109	178	146	28	21	1	139
Fe	1.62	16,652	-	23,852	23,852	-	-	-
Mn	358	106	1,638	2,197	1841	353	354	-
Hg	7.57	-	-	4.3	4.3	-	-	0.19
Ni	-	13	426	460	107	41	20	156
Zn	2,000	848	781	1,776	1,607	2,390	1,101	2,387

(a) Gupta (1999)
(b) Sreesai and Parkpian (2001)
(c) JICA (1999)
(d) Poonpolwatanaporn (2000)
(e) Klankrong (2002)
(f) Amerasinghe (1997)
(g) Chanya (1998)
(h) *Sae-Tang (2004) (for mixed sludge)

2.3 Land Application of Sludge

Land application of sludge has been practiced successfully for decades. Metcalf and Eddy (1991) has defined land application of stabilized municipal wastewater sludge, as the spreading of sludge on or just below the soil surface. Sludge may be applied to agricultural land; forest land; disturbed land; and dedicated land disposal sites. Experience in western countries has shown that of these four cases, disposal of sewage sludge on agricultural or forest lands has been shown to be an economical means for final sludge disposal (Stoll, 1995; Davis, 1987).

2.3.1 Sludge Characteristics Affecting Land Application

The characteristics of sludge that affect its suitability for land application or affect the design of land application systems include organic content (usually measured as volatile solids), nutrients, pathogens, metals and toxic organics.

Organic Content and Pathogens

Degradable organic material in unstabilized sludges can lead to odor problems and attract vectors (flies, mosquitoes, and rodents) in a land application setting. Pathogens (bacteria, viruses, protozoa, and eggs of parasitic worms) are concentrated in sludges and can spread diseases if there is human exposure to the sludge. To meet prescribed limits, organic content and pathogens must be reduced significantly prior to land application by means of preapplication treatment processes (Metcalf and Eddy, 1991).

Nutrients

Nitrogen (N), phosphorus (P), and potassium (K), which are major plant nutrients, are not removed substantially during sludge processing but are taken up by vegetation after sludge has been applied to land. Nitrogen is normally the nutrient of concern in land application because of the potential for nitrate contamination of groundwaters. The nitrogen uptake rate of vegetation, therefore, is a key design parameter in determining sludge-loading rates. When nutrient content of wastewater sludge is compared to commercial fertilizers as shown in Table 2.3, sludge can meet only a portion of the complete nutrient needs of plants in most cases, with N, P, K contents of 3.3%, 2.3%, and 0.3% respectively for sludge, as compared to 5,10 and 10 respectively for typical fertilizers (Metcalf and Eddy, 2003).

Organics

Wastewater sludges contain organic compounds that are retained in the soil and pose potential toxic risks to plants, animals, and humans. These organic compounds such as EDCs (Endocrine Disrupting Chemicals) are substances that are persistent, hard to be decomposed naturally, accumulative in environment and food chain and likely affective or deprivable to the normal function of human or animal's metabolite, growth and reproduction hormones. EDCs include the well known Persistent Organic Pollutants (POPs), including DDT, Dieldrin, chlordane, heptachlor, polychlorinated biphenyls (PCBs) and dioxins (Europa, 2007). It is estimated that the half-life of persistent organics in soil is about 10 years (Lue-Hing et al., 1998). The principal concern with such organics is not with plant uptake, which does not occur, but with the direct ingestion of compounds by animals, particularly dairy cattle grazing on sludge treated grasses. There is also evidence that organics can be adsorbed onto the surface of root crops such as carrots. Consequently, loading limits for specific organic compounds are of concern when designing land application systems for sludge (Metcalf and Eddy, 1991).

Other emerging pollutants of concern are the PPCPs (Pharmaceuticals and Personal Care Products) which are bioactive chemicals i.e. substances that have an effect on living tissue. PPCPs include prescription and over - the counter therapeutic drugs, veterinary drugs, cosmetics, fragrances, nutraceuticals (e.g., vitamins), etc. which can come from human activity, residues from pharmaceutical manufacturing, hospitals, and other sources. Although some PPCPs are easily broken down and processed by the human body or degrade quickly in the environment, others are not easily broken down and processed and so enter domestic sewers (Europa, 2007).

Heavy Metals

The typical heavy metals content of sewage sludge is about 0.5 % - 2% on dry weight basis but can rise as high as 4% w/w (wet weight basis) for some metals (Sreekrishnan et al., 1993; Wong and Henry, 1984). Because of the heavy metals content of the sludge, more than 50% of municipal sewage sludge can not be used on agricultural land (Couillard and Mercier, 1990). Unlike the hazardous organic constituents, metals can not be degraded or readily detoxified. The presence of metals in the waste can pose a long-term environmental hazard. The fate of the metal depends on its physical and chemical properties, the associated waste matrix, and the soil. Significant downward transportation of metals from the soil surface occurs when the metal retention capacity of the soil is overloaded or when metals are solubilized (e.g. by low pH). As the concentration of metals exceeds the ability of the soil to retain them, the metals will travel downward with the leaching waters (Dutta, 2002). It has been estimated that approximately 8,000 tons/year of the six heavy metals Cd, Cr, Cu, Ni, Pb and Zn are incorporated into sludges in the UK (Lester et al., 1983).

The heavy metals and other potentially toxic elements present in sludges can be divided into two categories, based on whether or not they present a potentially serious hazard to plants (phytotoxicity), animals (zootoxicity) or humans (Jain and Tyagi, 1992; Couillard and Mercier, 1990; Davis, 1987; Lester, 1987a; Lester et al., 1983; Torrey, 1979). This classification applies to sludge-borne metals that enter plants through the roots and not to metals that may be ingested directly by grazing animals from sludge present on plant foliage or on the soil surface. This subdivision assumes that correct management practices are implemented at the application site.

Manganese (Mn), Fe, aluminum (Al), Cr, As, Se, antimony (Sb), Pb, and Hg pose relatively little hazard to crop production and plant accumulation when sludge is applied to soil because all either have low solubility in slightly acid or neutral, well-aerated soils, or as with selenium, are present in such small amounts that the concentration is low in soils. As a result, the availability of these elements to plants is relatively low, and little uptake by plants occurs. Although many sludges, particularly those from tertiary treatment plants, contain considerable quantities of iron and/or aluminum, these elements will not pose a problem provided that the application is well managed.

For Cr and Pb, in addition to low solubility in soil, these metals are not readily taken up by plants, and this also limits their entry into the food supply. Although Pb can cause central and peripheral nervous system damage, kidney effects, and it is highly toxic to infants and pregnant woman. This metal appears to be largely retained in the roots of the plants (Ratanachoo, 1995; Lester et al., 1983).

Addition of sludge to soil seldom increases the Cr concentration in plant tissue. However, because there is evidence that Cr may be deficient in the diets of animals and humans, such small increase in concentration of this metal in plants as might result from application of sewage sludge are not to be viewed with alarm. Chromium (Cr) can exist in soil in two forms: the trivalent Cr (III) form, Cr^{+3}, and the hexavalent Cr (VI) forms, $(Cr_2O_7)^{-2}$ and $(CrO_4)^{-2}$. The dichromate ions present a greater health hazard than chromate ions, and both Cr (VI) ions are more toxic than Cr (III) ions (Dutta, 2002).

Most sludges are relatively low in mercury (Hg), and very little increase in mercury concentration in plants has resulted from sludge application. In soils, mercury exists primarily in the mercuric and mercurous forms as a number of complexes with varying water solubilities (Dutta, 2002; Torrey, 1979).

Considerable quantities of arsenic (As) can be added to soil in the form of sludge, but because most plants tend to exclude arsenic from their aerial tissues, little hazard arises from this element. Arsenic (As) exists in the soil environment as arsenate, As (V), or as arsenite, As (III). Both are toxic. However, arsenite is the more toxic form, and arsenate the most common form (Dutta, 2002; Torrey, 1979).

In the case of selenium (Se), very few reports are available to indicate the quantities likely to be applied to land in sludge. Data now available indicate that selenium does not present a hazard. In some cases, it is deficient in animal diets, and so somewhat elevated levels in plant tissue could be an advantage.

Research is lacking on antimony (Sb), but on the basis of present evidence, antimony is unlikely to be a potential hazard to plants or animals.

The remaining heavy metals, Cd, Cu, Mo, Ni and Zn can accumulate in plants and may pose a hazard to plants, animals, or humans under certain circumstances.

Cadmium (Cd) is a nonessential element which can be a serious hazard to animals and humans if dietary levels are increased substantially. Increased Cd intake by humans can cause adverse chronic effects such as kidney disease (renal tubular dysfunction) and a concentration of approximately 200 ppm in the kidney appears to be the threshold value for kidney dysfunction. Median concentrations of cadmium in sludge are low, but some sludges contain appreciable quantities of cadmium. Cadmium uptake by plants from sludges applied to land is a very complicated phenomenon. Lester et al. (1983) observed that Cd is easily absorbed by roots of plants and subsequently translocated to the aerial parts of the plant. Many crops may contain undesirable concentrations of cadmium in their vegetative tissues without showing symptoms of cadmium toxicity (Torrey, 1979).

Copper (Cu), although essential to plants, can become toxic to them at high concentrations. Sludges often contain appreciable levels of copper, but application of sludge to soil results in only slight to moderate increases in the copper content of plants.

Copper is an essential element used in processes of blood formation and iron utilization, with a human daily requirement of 0.03 mg/kg (Lester, 1987a). In general, animal diets are deficient in copper; hence, slightly elevated concentrations in animal feeding could be advantageous. Under good management practices, copper in sludges will seldom be toxic to plants and should not present a hazard to the food supply. Copper toxicity in animals would be expected to occur only when copper toxicity is severe in the plants used as feed (Torrey, 1979).

Molybdenum (Mo) is not particularly toxic to plants, even when applied at relatively high levels. As a result, molybdenum may accumulate in plants at concentrations sufficient to cause molybdenosis in ruminant animals without prior warning from plant behavior. The recommended practice of maintaining the soil pH at 6.5 or higher at sludge application sites results in greater solubility and availability of Mo than would occur at lower pH values. However, since sludges are usually very low in Mo, it is doubtful that it would present a serious hazard to the health of grazing animals except for the unusual circumstances in which forages from sites receiving high-molybdenum sludge form the major part of the animal diet (Torrey, 1979).

Nickel (Ni) is not essential to plant growth but seems to be required for poultry. Sludges often contain substantial amounts of nickel, which appears to be more readily available from sludges than from inorganic sources. Nevertheless, toxicity of nickel to plants occurs only on acid soils. If the soil pH is maintained at 6.5 or above, nickel should not cause toxicity to plants or pose a threat to the food supply (Lester et al., 1983; Torrey, 1979).

Zinc (Zn), an essential element for both plants and animals, is often found in sludge at relatively high concentrations. The greatest percentage of total zinc in polluted soil and sediment is associated with iron (Fe) and manganese (Mn) oxides (Dutta, 2002). Addition of sludge to soil may cause substantial increases in the zinc content of plants, but toxicity seldom occurs. Zinc is easily absorbed by roots of plants and subsequently translocated to the aerial parts of the plant (Lester et al., 1983). Many animal diets are deficient in zinc, and a wide margin of safety usually exists between normal dietary intakes of zinc and those that produce toxicity in birds and animals. Slightly elevated levels of zinc in plants may, therefore, be regarded as beneficial. In general, if the pH of sludge-treated soils is maintained at 6.5 or greater, Zn should not be a hazard to plants or to the food supply unless exceptionally high amounts are added in the sludge (Torrey, 1979).

Davis et al. (1985) investigated the interactive effects of Cu, Ni and Zn in relation to their concentrations in soil. They found that the toxic effect of a mixture of Cu, Ni and Zn in soil is a function of the element present in highest concentration relative to its upper critical soil concentration. In the pot trial, the critical soil concentrations were 105 mg/kg Cu, 221 mg/kg Ni, and 319 mg/kg Zn.

2.3.2 Heavy Metal Requirements for Land Application of Sludge

Due to the often high concentrations of heavy metals in sludge, many countries have developed legislation or recommendations which specify the maximum concentration of metals in sludge which may be applied to agricultural land. In general, total concentrations are specified, and the metal speciation is not taken into account, with few exceptions (Lester et al., 1983). National guidelines for the safe utilization of sludge in agriculture have been formulated in countries like UK since 1977, and the US since 1979, and have been updated since (Lester, 1987a). Similarly, there have been guidelines and statistics in many other European countries, such as France and Germany since 1982, the Netherlands since 1980, Switzerland since 1980, and Japan since 1998. The US, German and Japan guidelines are shown in Table 2.6.

For the European countries, the guidelines for the individual countries have been modified and combined into the EU directive formulated in 1986, and is summarized in Table 2.7. As shown, specified metals are controlled by setting maximum concentrations in the soils (as mg/kg dry solids) and maximum concentrations in sludge (as mg/kg dry solids) or maximum rates of addition expressed as an annual average over a ten-year period. Limits are set for total Zn, Cu, Ni, Cd, Hg and Pb, and consideration is still being given to Cr.

Table 2.6 Standards for Heavy Metals in Sludges Used in Agriculture

Pollutant	Germany, 1992 (mg/kg DS [a])	US EPA, 1992 (mg/kg DS)	EU, 1986 (mg/kg DS)	Japan, 1998
Arsenic (As)	-	41	-	50 mg/kg DS
Cadmium (Cd)	10/5[b]	39	20-40	5 mg/kg DS
Chromium (Cr)	900	1200	-	1.5 mg/L
Copper (Cu)	800	1500	1,000-1,750	-
Lead (Pb)	900	300	750-1,200	3 mg/L
Mercury (Hg)	8	17	16-25	2 mg/kg DS
Molybdenum (Mo)	-	18	-	-
Nickel (Ni)	200	420	300-400	-
Selenium (Se)	-	36	-	-
Zinc (Zn)	2,500/2,000 [b]	2,800	2,500-4,000	120 mg/kg DS

[a] Dry solids
[b] Low standards for sludges in soils with 5<pH<6. For pH <5, no application allowed.
Source: Stoll (1995); Lue-Hing et al. (1998)

Table 2.7 EU Directive Restrictions on Metals in Sludges/Soils During Agricultural Use

	Soil (mg/kg DS) [a,b]	Sludge (mg/kg DS)	Rate of Application (kg/ha/yr)[c]
Cadmium (Cd)	1-3	20-40	0.15
Copper (Cu)	50-140	1,000-1,750	12.0
Nickel (Ni)	30-75	300-400	3.0
Lead (Pb)	50-300	750-1,200	15.0
Zinc (Zn)	150-300	2,500-4,000	30.0
Mercury (Hg)	1-1.5	16-25	0.1

[a] pH 6-7, but Cu, Ni, Zn limits can be increased by 50% in soils of pH >7.
[b] Where dedicated land used for farming and sludge disposal exceeded these values and it can be demonstrated that there is no hazard, and commercial crops are grown and used only for animal consumption, the practice may continue.
[c]10 – year average, can be applied in one go.
DS – Dry solids
Source: Lue-Hing et al.(1998)

In Thailand, the recommended standards for heavy metals in sludge for application in agriculture is shown in Table 2.8.

Table 2.8 Recommended Standards for Heavy Metals in Sludge for Agricultural Application in Bangkok and Surrounding Areas[a]

Heavy Metals	Limiting Sludge Concentration (mg/kg DS)
Cadmium (Cd)	20
Chromium (Cr)	1,000
Copper (Cu)	900
Lead (Pb)	1,000
Mercury (Hg)	10
Nickel (Ni)	400
Zinc (Zn)	3,000

[a]Sludge should not be applied to soils having pH value<5; if applied, extensive monitoring should be done.
Source: AIT (1998)

Monitoring of soil in terms of pH, cation exchange capacity (CEC), N, P, K and heavy metals content of concern must be done every after 5 years of application according to the regulations. Moreover, the sludge should be free of pathogen if it is to be used for raising edible crops and must first be applied to the soil before agricultural use.

2.3.3 Summary of Benefits and Limitations of Land Application

Due to its high nutrient content, experience in western countries has shown, that one of the most promising options for sludge disposal is to economically reuse sludge as fertilizer in agriculture. The term “agricultural use” incorporates all activities in the application of wastewater sludges related to agriculture, gardening, and forestry. In general, the utilization of sludge in agriculture has the following advantages (Levlin, 2004; Pogrzeva et al., 2004; Veeken and Hamelers, 1999; Stoll, 1995; Sreekrishnan et al., 1993):

- It encourages recycling and reuse of waste materials to agricultural advantage.
- The sludge contains a high nutrient value which can be utilized as a means of fertilizing crops.
- The sludge increases the pH of highly acidic soils.
- Utilization for agricultural purposes is more economical in comparison to incineration and landfill since municipalities do not have to buy land for sludge disposal.
- The sludge improves poor soil condition for germination and growth of crops.

However, land application of sludge is not without its disadvantages which has resulted to low acceptance of this method in other countries. In Germany, for instance, only 20% of municipal sewage sludges were used in 1995. The disadvantages identified in the utilization of sludge for land application include the following (Stoll, 1995):

- The main concern is the potential hazards of sludge to plants, animals and humans associated with the presence of high concentration of heavy metals.

- The sludge also enriches other pollutants (pathogens, and organics) in the soil.
- The sludge can be offensive to senses (mainly odor) especially if it is not properly digested.
- The applied sludge can be a means of transmission of toxins (pathogens or chemicals) into water supplies through leaching and through crops to animals and humans.
- The method requires large land areas, since sludge application rates (dry weight/unit land area/time) for agricultural purposes are usually relatively low.

Concerns expressed by those countries who have low acceptance of land disposal of sludge for agricultural purposes, such as Germany, include the following:

- Unbalanced nutrient content and uncertain availability of nitrogen compounds for plant uptake;
- The standards becoming even more stringent in the future and having a limited knowledge on the impact of pollutants on soil, crops, animals and humans;
- Recently detected pollutants (such as dioxins);
- Risk of soil contamination leading to depreciation of land prices;
- Competition between manure and organic composts and municipal sludge; and
- Discrimination of products produced on sludge applied land.

However, despite these limitations, utilization of sludge for agricultural use is still considered to be the best alternative for sludge disposal especially when the heavy metals in sludge are reduced (Levlin, 2004; Pogrzeva et al., 2004; Sreekrishnan et al., 1993; Veeken and Hamelers, 1999).The extraction of heavy metals in sludge before land application is therefore a vital step to achieve a more sustainable form of sludge management.

2.4 Extraction of Heavy Metals from Sludge

The extraction of heavy metals from sewage sludge has been extensively studied (Wong and Henry, 1988). Various technologies such as chemical extraction, bioleaching process, electrokinetic process, supercritical extraction, etc., used for the extraction of heavy metals in sludges, have previously been used in the removal of metals from soils. Several studies done on these technologies revealed the broad range in metal extraction efficiencies which were found to be due to differences in sludge composition, pretreatment of sludge and extraction conditions (Veeken and Hamelers, 1999).

The measurement of the total metal content of sewage sludges and related matrices using analytical methods are well established. However, while total concentrations of metals indicate the extent of contamination, they give little information into the forms in which the metals are present in sludge or their potential for mobility and bioavailability once released to the environment. This can only be attained from a detailed knowledge of the speciation of metals in the sludge itself and the changes in speciation likely to occur following disposal (Lester, 1987a). Hence, a study of the different forms of metals in the sludge would be helpful in evaluating the removal efficiencies of heavy metal extraction technologies.

2.4.1 Chemical Speciation of Heavy Metals in Sewage Sludge

Lester (1987b) identified the different forms in which metals may occur in sewage sludges based from several studies, to be (1) soluble, (2) adsorbed, (3) organically complexed, (4) precipitated, (5) coprecipitated in metal oxide, or (6) residual forms. The definitions for these terms are vague with the definition of one often overlapping that of another.

Soluble metals may exist in solution as simple ionic forms, or complexed to soluble organics or inorganics. A metal complex is formed by association between an electron-deficient metal atom or ion and an electron-rich species called a ligand, which is either an anion (e.g., Cl^-, OH^-, HCO_3^-, SO_4^{2-}) or a polar molecule (e.g., NH_3). Complexation is principally by coordinate covalent bonding which is established through donation of a pair of electrons from the ligand to the metal–ion acceptor. However, ionic bonding by electrostatic attraction also plays a major role in many metal-ligand complexes. If two or more electron-donating atoms are present within a ligand, they may coordinate to the same metal ion to form a heterocyclic ring termed a chelate**.** Complexes with multidentate ligands (i.e., more than one donor atom) are generally more stable than those with monodentate ligands. Humic acids and fulvic acids are examples of multidentate ligands found in sludges and therefore form stable complexes (Lester, 1987a; Sawyer et al., 1994).

Adsorption based on ion exchange is a chemical process in which a negative or positive charge on a particle surface is equalized by ions possessing opposite charges. Most particle surfaces have a negative electrical charge; thus in solution, an equivalent number of cations will gather around the particle, where an electric double layer occurs. By definition, cation held on negatively charged sites on the surface of colloids or particles are easily exchangeable with the soil solution and are frequently referred to as “exchangeable” metals. Clay minerals and organic matter, in addition to hydrous oxide materials, all constitute adsorption sites (Lester, 1987a).

Organically complexed metals incorporate those forms which are bound to insoluble organic matter, in addition to components of living cells, their exudates, and degradation products, by simple complexation or chelation. Research relating to the mechanisms involved in complexation and chelation between metal ions and solid-phase organic compounds of sewage sludges is limited, but the principles involved are likely to be similar to those described previously for solution-phase organometallic interactions. The metals in soil that occur in insoluble combinations with organic matter are largely those that are bound to components of the humic fractions, particularly humic acids. Since a major proportion of the sludge organic fraction consists of humic compounds, these may also play an important role in the formation of insoluble organometallic complexes in this matrix (Lester, 1987a).

Precipitated metal forms are defined as insoluble substances formed in solution as the result of a chemical reaction, and include metal hydroxides, carbonates, phosphates, and sulfides. Metals may also be coprecipitated, under aerobic conditions, with manganese and iron oxides which may occur as concretions, stains or coatings on the surfaces of sludge particles.

Finally, residual metals may occur as ions inertly bound in crystal lattices of highly stable primary and secondary minerals.

The distribution of metals between the specific forms varies widely according to the chemical properties of the individual metal and the characteristics of the sludge, which are a function of the physical and chemical properties imposed by the particular sludge-treatment processes. These include such parameters as pH, temperature, oxidation-reduction (ORP) potential, the presence of complexing agents, and the concentration of precipitant ligands (Lester et al., 1983; Lester, 1987b). ORP, for instance, can determine the geochemical mobility of pollutants and nutrients (especially, N, P and heavy metals) in the environment and consequently, their influence on ecosystems, and is expected to vary with depth in soils since they contain microorganisms which consume oxygen. Well-aerated surface layers may have high ORP values, indicating oxidizing conditions due to the availability of oxygen. Deeper layers may be completely devoid of oxygen, giving rise to highly reducing conditions and consequently low ORP values (Radojevic and Bashkin, 1999).

Segregation of heavy metals in sewage sludges into all-specific physicochemical forms is not possible, however, with current analytical techniques. This is due not only to the limitation of analytical techniques available as regards constraints imposed by interferences, selectivity, and sensitivity, but also to the complex nature of sewage sludge. Valuable information on the partitioning of heavy metals in such a complex matrix into several component fractions has been obtained, however, using chemical-extraction techniques based on selective chemical reagents. Such techniques may either be of discrete nature, employing a single selective reagent to release a specific metal fraction from within the sludge sample or, alternatively, may incorporate several reagents of increasing extraction strength to release a number of different metal forms in sequence called sequential chemical extraction or SCE (Lester, 1987a).

2.4.1.1 Sequential Chemical Extraction (SCE)

SCE techniques are widely used to determine the geochemical partitioning of heavy metals in sludge and the potential of heavy metals removal from sludge. It includes the utilization of a series of chemical extractants in a sequence of reagents of increasing strength (Marchioretto et al., 2002). Although more time consuming, they provide additional information on the origin, mode of occurrence, biological and physicochemical availability, mobilization, and transport of heavy metals. Such techniques initially involve extraction of a dried sludge sample by shaking over a predetermined time period with an appropriate volume of a relatively weak reagent followed by centrifugation. The resultant supernatant is subsequently decanted, and possibly filtered in preparation for metal analysis, while the remaining sludge pellet is then resuspended and washed in water prior to extraction by another reagent. This procedure is followed for a sequence of reagents of progressibly increasing strength. Finally, the “residual” metal is extracted from the sludge pellet using mineral acids, at ambient or increased temperatures, or homogenization techniques.

The major sequential extraction schemes applied to wastewater sludges are presented in Table 2. 9. Reagents utilized were chosen on the basis of their selectivity and specificity towards particular physicochemical forms of heavy metals, although variations in reagent strength, volume and extraction time between schemes are apparent.

Table 2.9 Reagents Utilized in the Sequential Extraction of Heavy Metals from Sewage Sludges

Designated Chemical Form of Metals	Reagents for Specific Sludge Types								
	Digested (Cd, Cu, Pb, Zn)	**Digested (Cd, Cu, Ni, Pb, Zn)**	**Raw Activated Digested (Cd, Cr, Cu,Ni, Pb, Zn)**	**Raw Digested (Cd, Cu, Ni, Pb, Zn)**	**Digested (Cd, Cu, Ni, Zn)**	**Digested (Zn)**	**Activated (Cd, Cu, Pb, Zn)**	**Digested Ash (Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn)**	**Digested (Cd, Cu, Ni, Pb, Zn)**
Soluble	H_2O				(Footnote[a])				(Footnote[a])
Exchangeable	KNO_3	KNO_3	KNO_3	KNO_3	KNO_3 Ion-exchange	NH_4OAc	NH_4OAc (Footnote[b])	$MgCl_2$ (Footnote[b])	$BaCl_2$
Adsorbed		KF	KF	KF	H_2O	EDTA			
Organically bound		$Na_4P_2O_7$	$Na_4P_2O_7$	$Na_4P_2O_7$	NaOH	NaOCl NH_4OAc	H_2O_2- HNO_3 and NH_4OAc (Footnote[c])	H_2O_2- HNO_3 and NH_4OAc (Footnote[c])	H_2O_2- HNO_3 and NH_4OAc (Footnote[c])
"Available"	DTPA								
Carbonate		EDTA	EDTA	EDTA	EDTA		$NH_2OH.HCl$- HNO_3	NaOAc-HOAc	NaOAc-HOAc
Sulfide		HNO_3	HNO_3	HNO_3	HNO_3		H_2O_2- HNO_3 and NH_4OAc (Footnote[c])	H_2O_2- HNO_3 and NH_4OAc (Footnote[c])	H_2O_2- HNO_3 and NH_4OAc (Footnote[c])
Oxide bound[d]						Oxalate mixture (dark and UV)	$NH_2OH.HCl$- HNO_3, and oxalate[e]	$NH_2OH.HCl$- and HOAc	$NH_2OH.HCl$- -HOAc and NH_4OAc
Residual	HNO_3			Homogenization	HNO_3		C. HNO_3	H_2O_2-HF-HCl	HCl-HF

[a]Soluble phase separated from solid phase.
[b]Correct order of extraction scheme is exchangeable, carbonate, oxide-bound, organic/sulfide, residual.
[c]Organic and sulfide forms classed as a single "oxidizable" phase.
[d]Coprecipitated with or occluded in iron and manganese oxides; also designated as the "reducible" phase.
[e]Consists of oxalic acid $(CO_2H)_2$ and oxalate $(CO_2NH_4)_2$.

Source: Lester (1987a)

Marchioretto et al. (2002), however, cited the disadvantages of SCE scheme such as lack of specificity; absence of selectivity; readsorption; dependence on many factors such as type of sample; size of particulates; pH; temperature; contact time; concentration of extractant; and solid-liquid ratio. These limitations have been confirmed by Nirel and Morel (1990). Despite these drawbacks however, the SCE has continued to be recognized as a valuable tool for metal removal studies (Wasay et al., 1998).

2.4.1.2 Application of SCE in the Determination of Forms of Metals in Sludges

Early studies on speciation were done by Stover et al. (1976), who used the SCE scheme incorporating potassium nitrate (KNO_3), potassium fluoride (KF), sodium pyrophosphate ($Na_4P_2O_7$) in anaerobically digested sewage sludge from wastewater treatment plants in 12 Indiana cities in the US. Oake et al. (1984), using the modified scheme devised by Stover et al. (1976), also conducted speciation studies on selected types of sewage sludge (primary, activated sludge and co-settled sludge before and after mesophilic anaerobic digestion) from major UK treatment plants. Using the same extraction procedure, Ali (1994) and Ratanachoo (1995), determined the different forms of metals in anaerobically digested sludges from two treatment plants in Thailand. Marchioretto et al. (2002) also employed SCE to determine the distribution of heavy metals in the sludge matrix while assessing the chemical extraction process efficiency in the removal of heavy metals from anaerobically digested sludge from a wastewater treatment plant in the Netherlands. The different SCE methods developed by Tessier et al. (1979), Veeken (1998) and Sims and Kline (1991), were used to identify the forms of heavy metals in the sludge. The SCE schemes are presented in Table 2.10. The results of all studies on SCE are summarized in Table 2.11

As shown, there is wide variation in the forms of metals present in sewage sludges studied. Based on the selection of work presented in Table 2.11, the following forms of metals seem to predominate: Cd in carbonate and residual forms; Cr in organic and residual forms; Cu in residual and organic forms; Pb in carbonate and organic forms; Mn mostly in organically bound form; Ni in carbonate and residual forms; and Zn mostly in organically bound form. Oake et al. (1984) found that each individual metal had a characteristic fractionation profile independent of the type of sludge, with metals in the exchangeable, adsorbed and organically-bound fraction likely to be comparatively mobile once disposed to land. Moreover, mobilization of metals could result from dissolution of the carbonate fractions of Cd, Pb, and Ni or oxidation of the sulfide fraction of Cu. Furthermore, as organic matter decays to low levels, the nature of the major inorganic constituents in sludge (Fe, Al, Ca, or P) as well as the properties of the soil receiving the sludge will ultimately affect the mobility of heavy metals disposed to land (McBride, 1995). Ali (1994) found that residual, organic and carbonate fractions contained more than 95% of metals studied, whereas metals in exchangeable and adsorbed forms were negligible (less than 5%). The results of the study done by Ratanachoo (1995), on the other hand, revealed that, although most of the heavy metals studied were associated in residual form, the metals in adsorbed and exchangeable forms contained more than 10% of the total metals studied. Marchioretto et al. (2002) compared the heavy metals behavior in all the fractions among the three SCE schemes used, and found that the distribution in each fraction is almost the same for both Veeken, and Sims and Kline schemes, although it was difficult to distinguish between what is bound to organic and what is bound to inorganic

Table 2.10 Summary of Different Sequential Chemical Extraction (SCE) Schemes

SCE Scheme	Step No.	Chemical Form of Metals	Reagent	Time and Temperature
Tessier	1	Exchangeable	$MgCl_2$ 1 mol/l, pH = 7, 8(v/w)[1]	1 h at 20°C
	2	Bound to carbonates	NaAc[2] 1 mol/l, pH = 5, 8(v/w)	5 h at 20 °C
	3	Bound to Fe-Mn Oxides	$NH_2OH.HCl$ 0.04 mol/l, pH = 2, 20(v/w)	6 h at 96 °C
	4	Bound to organic matter	HNO_3 0.02 mol/l, 3(v/w), 30% H_2O_2, 5(v/w) pH = 2 30% H_2O_2, 5(v/w) NH_4Ac 3.2 mol/l, 5(v/w)	2 h at 85 °C 3 h at 85 °C 30 min at 20 °C
	5	Residual	5 ml H_2O, 4 ml HNO_3 70%, 1 ml HCl 35%, 2 ml HF 48%	26 min-microwave oven
Veeken Method	1	Exchangeable	NH_4Ac 0.5 mol/l, pH =7, 10(v/w)	3 h at 20 °C
	2	Bound to carbonates	NaAc/HAc 0.1 mol/l, pH = 5.5, 10(v/w)	5 h at 20 °C
	3	Bound to Fe-Mn Oxides	$NH_2OH.HCl$ 0.1 mol/l, pH = 4, 20(v/w)	6 h at 20 °C
	4	Bound to organic- inorganic matter	EDTA 0.1 mol/l, pH = 4.5, 20(v/w)	16 h at 20 °C
	5	Incorporated in organic matter and organic-mineral aggregates	HNO_3 0.02 mol/l, 3(v/w), 30% H_2O_2, 5(v/w) pH = 2, 30% H_2O_2, 5(v/w) NH_4Ac 3.2 mol/l, 5(v/w)	20 h at 85 °C 30 min at 20 °C 4 h – microwave oven
	6	Residual	Aqua regia – $HCl:HNO_3$-3:1	30 min-microwave oven
Sims and Kline	1	Exchangeable	KNO_3 0.5 mol/l, 12.5(w/w)[3], pH = 6.2	16 h at 20 °C
	2	Bound to organic matter	NaOH 0.5 mol/l, 12.5(w/w), pH = 12.6	16 h at 20 °C
	3	Inorganic precipitate	Na_2EDTA 0.05 mol/l, 12.5(w/w), pH = 4.5	6 h at 20 °C
	4	Residual	HNO_3 4 mol/l, 12.5 (w/w), pH = 0.6	16 h at 80 °C

[1]Liquid-to-solid ratio (v/m); v is the volume of the extractant (ml); m is the mass of the sample (g)

[2]Ac: acetate

[3]Weight-to-weight (w/w): w is the mass of the extractant (g); w is the mass of the sample (g)

Source: Marchioretto et al. (2002)

Table 2.11 Chemical Speciation of Heavy Metals in Anaerobically Digested Sludges

Metal	**Metal Forms**	**Reference**
Cd	Carbonates>Sulfides>Organic bound>Adsorbed=Exchangeable	Stover et al., 1976
	Mainly carbonate	Oake et al., 1984
	Residual (67.23%)> Carbonate (32.77%)	Ali, 1994
	Mainly bound to Fe/Mn (Tessier); Bound to organic and inorganic matter (Veeken); Organically bound and present as inorganic precipitate (Sims and Kline)	Marchioretto et al., 2002
Cr	38-62% in organic fraction	Oake et al., 1984
	Residual (73.91%)>> Organic (17.39%) > Carbonate (8.7%)	Ali, 1994
	Organic (59%) >> Residual (20%) >Carbonate (16%) >> Adsorbed (4%) >Exchangeable (1%)	Ratanachoo, 1995
	Mostly bound to organic matter (Tessier); Bound to organic and inorganic matter (Sims and Kline)	Marchioretto et al., 2002
Cu	Sulfides>Carbonates>Organic bound=Adsorbed>Exchangeable	Stover et al., 1976
	43-70% in sulphide fraction	Oake et al., 1984
	Residual (62.58%)>> Carbonate (20.55%)> Organic (13.5%)>> Adsorbed (3.07%)>Exchangeable (0.31%)	Ali, 1994
	Organic (45%) >> Residual (19%)> Adsorbed (16%)>Carbonate (12%)>Exchangeable (8%)	Ratanachoo, 1995
	Mainly bound to organic matter (Tessier); Essentially incorporated in organic matter and organic-mineral aggregates (Veeken); Mainly bound to organic matter (Sims and Kline)	Marchioretto et al., 2002
Pb	Carbonates> Organic bound > Sulfides > Adsorbed > Exchangeable	Stover et al., 1976
	Predominantly in the organic and carbonate fraction	Oake et al., 1984
	Carbonate (51.57%) > Organic (33.96%) >> Residual (11.32%) >> Exchangeable (2.52%) > Adsorbed (0.63%)	Ali, 1994
	Organic (57%) >> Carbonate (21%) > > Adsorbed/ Exchangeable (9/8%)> Residual (6%)	Ratanachoo, 1995
	Almost 80% in residual portion (Tessier); Mostly bound to organic and inorganic matter (Veeken); Present as inorganic precipitate (Sims and Kline)	Marchioretto et al., 2002
Mn	Organic (46.96%) > Carbonate (38.44%) >> Residual (12.90%) >> Exchangeable (1.46%)> Adsorbed (0.24%)	Ali, 1994
	Organic (80%) >> Carbonate (11%) > Adsorbed/ Exchangeable (3/3%)> Residual (2%)	Ratanachoo, 1995
Ni	Carbonates>Organic bound>Exchangeable>Adsorbed>Sulfides	Stover et al., 1976
	>40% in exchangeable/sorbed fractions	Oake et al., 1984
	Residual (63.04%) >> Carbonate (28.26%)> Organic (8.70%)	Ali, 1994
	Residual (40%) >>Organic (29%) > Carbonate (15%) > Exchangeable (10%)> Adsorbed (7%)	Ratanachoo, 1995
	Especially bound to Fe/Mn (Tessier); Bound to organic and inorganic matter (Veeken); In the residual and bound to organic matter (Sims and Kline)	Marchioretto et al., 2002
Zn	Organic bound >Carbonates >Sulfides > Adsorbed > Exchangeable	Stover et al., 1976
	Predominantly in organic fraction	Oake et al., 1984
	Organic (65.84%) >> Carbonate (22.06%) > Residual (11.32%)>>Adsorbed (0.41%) > Exchangeable (0.37%)	Ali, 1994
	Organic (58%) >> Exchangeable (19%) > Residual (9%) >Carbonate /Adsorbed (7/7%)	Ratanachoo, 1995
	Prevalent in the fraction bound to Fe/Mn oxides (Tessier); Bound to organic and inorganic matter (Veeken); Present as inorganic precipitate (Sims and Kline)	Marchioretto et al., 2002

matter in the Veeken scheme. On the other hand, the Tessier and Veeken schemes were different in several aspects.

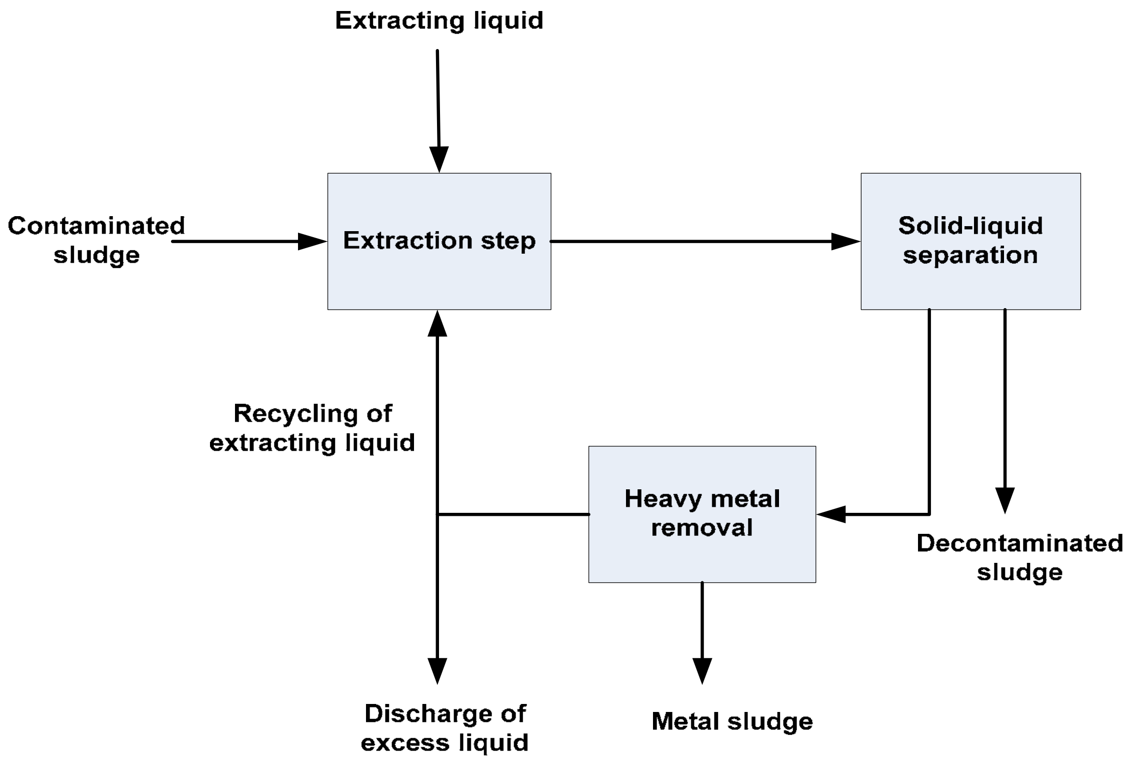
In general, the forms of heavy metals present in sewage sludges vary widely according to the nature of the individual metal, the characteristics of the wastewater treated and the sludge treatment employed (Lester et al., 1983; Lake, 1987b).

2.4.2 The Chemical Extraction Technology

Chemical extraction is a means of separating hazardous contaminants from soils, sludges, and sediments by using extracting chemicals such as acids, chelating agents and inorganic chemicals, to reduce the volume of the hazardous wastes that must be treated. The process has been used in the remediation of soils contaminated with heavy metals (Paff et al., 2004). According to Wong and Henry (1988), the process has also been extensively used in the extraction of heavy metals from sewage sludge.

2.4.2.1 Process Scheme

The chemical extraction process consists of three steps namely, (1) the actual extraction process; (2) separation of solids and liquid; and (3) cleaning and recycling of extracting fluid. The process scheme is shown in Figure 2.1.



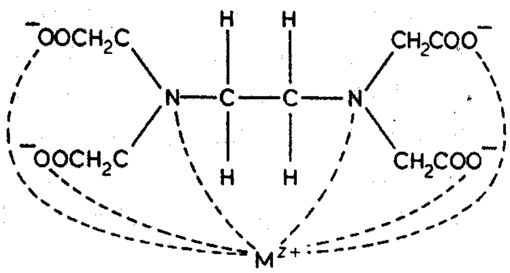
Source: Veeken and Hamelers (1999)

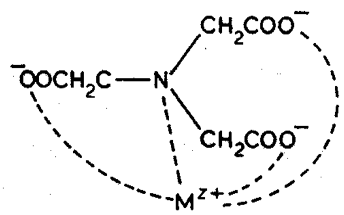
Figure 2.1 Process Scheme for Extraction of Heavy Metals from Sewage Sludge

During the extraction process, the heavy metals are transferred from the solid phase to the aqueous phase. This requires good contact between solids and the extracting liquid which is brought about by intensive mixing. According to Waidmann et al. (1984) one of the most influential parameters controlling the transfer of metals from immobile solid-phase forms to a more mobile and possibly more bioavailable form is pH. Aside from pH, ORP was also observed to influence the solubilization of metals in sludge (Marchioretto, 2003). A decrease in the pH of sludge (specifically anaerobically digested sludge) with a previous increase in the ORP of the sludge, using aeration or addition of oxidizing agent, such as H_2O_2, seemed to promote the solubilization of heavy metals (Marchioretto, 2003; Yoshizaki and Tomida, 2000). In the single extraction process of heavy metals from sludge, the inorganic acids such as nitric, hydrochloric and sulfuric, and organic acids such as citric and oxalic have been widely used. For heavy metals adsorbed to the solid phase, the extraction process is brought about by the exchange of the protons by solubilization of heavy metal precipitates. The protons can be supplied by addition of acid solution. If acid is added to the sludge, the heavy metals present in the sludge can be dissolved and then exist in solution. The whole reaction can be expressed by Equation 2.1 as follows:

$$\text{Sludge-M} + \text{Acid} \longrightarrow \text{Sludge} + M^{z+} + \text{Acid} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(2.1)}$$

Strong chelating agents such as EDTA and NTA can also be used. These extractants function by complexing heavy metals to form EDTA-metal (or NTA-metal) complexes. Theoretically, one mole EDTA or NTA can remove one mole of heavy metals. Since EDTA is a hexadentate ligand and NTA a tetra-dentate ligand, the two reactions can be expressed by Equations 2.2 and 2.3 as follows (Lo and Chen, 1990):

Sludge–M + EDTA → Sludge +  … (2.2)

Sludge–M + NTA → Sludge +  …………… (2.3)

The next step involves the separation of the aqueous phase from the 'cleaned' sludge by a solid-liquid separation process, e.g. decanting centrifuge. In the final step, the heavy metals are removed from the extracting solution to recover the economic value of the extracting agent and prevent environmental impact associated with the discharge of extracting fluid. Removal of the solubilized heavy metals from the extracting solution can be accomplished by precipitation process followed by a separation step. Common precipitating reagents include alkalis such as CaO, NaOH, $NaHCO_3$, and sulfides such as

NaS, H_2S, or FeS (Brooks, 1991). The most appropriate and economically feasible technologies for removal of heavy metals from solutions are chemical sulphide precipitation and selective ion-exchange (Veeken and Hamelers, 1999).

2.4.2.2 Stability Constants of Various Metal Chelates for some Extracting Agents

First stability constants are well known tools to help determine the properties of metal-ligand reactions in water and biological systems. It describes how tightly or loosely the chelating agent (ligand) holds the metal ion. In general terms, the stability constant of a metal complex can be calculated as follows: K = [ML]/[M][L], where K is the stability constant (expressed as a logarithm); M is the amount of metal ion, and L is the amount of a ligand.

The first stability constants of various metal chelates for citric acid, oxalic acid, EDTA and NTA are shown in Table 2.12. Very low stability constant numeric values (between negative values and 1) mean that the metal-ligand is not only soluble in water but readily dissociates into the metal ionic form and the ligand, yielding essentially all metal in ionic form at pH as low as 2 -3, to as high as 7.4. Generally, metal-ligands with stability constants of 3 and lower are soluble and substantially (~ over 5%) ionized at pH 7.4. Between 3 and perhaps as high as 6, metal ligands are likely to disassociate in very low pH, but not greatly at pH 7.4. For stability constants above 6, there is less and less metal release regardless of how low the pH may be. All metal-ligand compositions release increasingly more metal ions as pH is lowered towards increased acidity, and more metal hydroxides are released as pH is raised into the basic end of the pH scale.

Table 2.12 First Stability Constants (log K_1) of Various Metal Chelates

Metals	First Stability Constants of Metal Chelates			
	Citric Acid	EDTA	NTA	Oxalic Acid
Al	-	16.13	>10	7.26
Ba	2.3	7.78	4.82	2.31
Ca	3.5	10.70	6.41	3.0
Co (II)	4.4	16.21	10.6	4.7
Cu	6.1	18.8	12.68	6.3
Fe(II)	3.2	14.3	8.84	>4.7
Fe(III)	11.85	25.7	15.87	9.4
Mg	2.8	8.69	5.41	2.55
Mn	3.2	13.56	7.44	3.9
Ni	4.8	18.56	11.26	5.16
Sr	2.8	8.63	4.98	2.54
Zn	4.5	16.5	10.45	4.9

Source: Furia (1972)

2.4.2.3 Heavy Metal Removal Kinetics

According to Lo and Chen (1990), when the heavy metals in the sludge contact and react with the extracting agent, the removal ratio increases with increasing time. Hence, the removal rate is proportional to the concentration of remaining metal and can be expressed by Equation 2.4 as follows:

$$\frac{dC}{dt} = - KC \qquad (2.4)$$

Where: K is the reaction rate constant (1/t)

The K values of various heavy metals under different conditions can be obtained by regression analysis. The required reaction time for the desired percentage removal of various heavy metals can then be calculated.

2.4.2.4 Application to Heavy Metals Removal in Contaminated Sludge

Heavy Metal Extraction with Inorganic and Organic acids

Using inorganic acids, Jenkins et al. (1981), assessed the removal of metals from sludges obtained from a central wastewater treatment plant in California, USA. The types of sludge used in the experiment were primary sludge (PS) containing 2.92% solids; digested primary sludge (DPS) containing 4.66% solids; waste activated sludge (WAS) containing 0.68% solids; and digested waste activated sludges (DWAS) containing 1.54% solids. The sludges were dosed with sulfuric acid (1N H_2SO_4) to effect metal solubilization. The results revealed that the most difficult metal to solubilize for any of the sludges was Cu (only about 1% removal), followed by Cd and Pb. This suggested that part or all of the Cu in the sludges exists as an organic complex and that the organic-metal bond strength is greater than Cd and Pb (Wozniak and Huang, 1982). In general, the experiment showed that if the pH is decreased to about 2 for contact time of 24 hours, the maximum removal efficiencies for Cd, Cr, Fe, Ni and Zn were found to be about 70% or greater. Removal efficiency for Pb was less at only 13%. Preliminary estimates indicated that about 0.5 metric ton of acid would be required for each dry metric ton of sludge solids for metal removal of more than 50% of the Cd. Based on US$52.27/metric ton H_2SO_4, the total cost for acid treatment of PS and DPS at maximum metal removals was determined to be US$ 25.88 /dry metric ton and US$37.08 /dry metric ton, respectively.

Using hydrochloric acid (HCl) as an extracting agent to remove metals from a waste activated sludge sample obtained from a treatment plant in Wisconsin, USA, Wozniak and Huang (1982), determined the variables affecting the metal removal efficiency. They observed variations in metal removal with pH, sludge solids concentration, individual metals, and time of extraction. They found that solubilization increased with increasing time, up to approximately 12 hours, and with lower values of pH and sludge solids. The rate of metal extraction was also found to be related to the possible metal species present. Up to 15% of the nickel and chromium extracted was solubilized almost immediately after acid addition, indicating dissolution of inorganic precipitates. The initial removals of other metals such as Cd, Cu, Pb and Zn, which were less than 10%, indicated the presence of these metals in organometallic complexes. At approximately pH 2, as much as 100% Zn removal was attained. The removal efficiencies for other metals were 92.5 % for Cd, 88% for Ni, 73% for Cu, 65% for Pb and only 24% for Cr.

Logan and Feltz (1985) also used HCl to observe the effect of aeration, cadmium concentration and solids content on acid extraction especially of Cd from an anaerobically digested sludge obtained from a municipal wastewater treatment plant in Ohio, USA. The average solid content of the sludges used was 3.3%.They found that extended aeration for at least 14 days was necessary for the oxidation of organic and inorganic forms of Cd prior to acid extraction, and that aeration during acid extraction was sufficient to maintain the metal in the extractable form. At pH 2 and leaching time of 18 hrs, an average of 76% of Cd was removed. Other metals removed were Zn (77%), Mn (75%), Ni (70%), and Cu (26%). Not much removal was attained for Fe (15%), Pb (4%), Cr (2%), and Al (1%).

Lo and Chen (1990) explored the removal efficiencies of heavy metals from urban and industrial sludges in Taiwan, using sulfuric acid (H_2SO_4) at 4 hours contact time and twice the stoichiometric dose (one stoichiometric dose equals one equivalent acid per one equivalent of metals). The thickened aerobic digested sludge was taken from a municipal treatment plant in Taipei City, while the anaerobic digested sludge was taken from an industrial district wastewater plant in north Taiwan. Results of acid extraction indicated that better removal efficiencies were obtained at pH 1.5 or 2. For the municipal sludge, Cd and Zn were the most readily solubilized metals with removal > 95%. For the industrial sludge, the removal percentages of Cd, Cr and Ni were > 97%. The remaining amount of most metals in the decontaminated sludge was within the USEPA 1993 standards for sludges used in agriculture.

Blais et al. (1992) also studied the removal of heavy metals from municipal sewage sludge (1.69 – 31.44 g/L solids) by acid treatment. The sludge samples were collected from 11 wastewater treatment plants in Canada and the US. A total of 23 sludges of the following sludge types were used: secondary activated sludge; aerobically digested sludge; sludge from an oxidation pond; anaerobically-digested primary sludge; anaerobically digested secondary sludge; primary sludge; and anaerobically digested sludge. The sludges were dosed with H_2SO_4 at pH of 1.5 and a temperature of 21^oC for 24 hours. It was found that Mn gave the highest yield of metal solubilization of 83%, followed by Ni (68%), Zn (66%) and Cd (59%). It was also observed that the sludge types and solids contents used in the study did not considerably influence the leaching capacity of the acid extraction.

Using both organic and inorganic acids, Veeken and Hamelers (1999) conducted acid leaching study on the removal of heavy metals from anaerobically digested sewage sludge with 20% dry matter (DM) collected from a municipal sewage treatment plant in the Netherlands. The metal extraction was performed at pH range of 2-6 for 0.1 M oxalic acid; 0.1 M citric acid; and nitric acid (HNO_3) at room temperature. Only two heavy metals (Cu and Zn) and competing metals (Ca and Fe) were measured during extraction. The results revealed that both citric and oxalic acids had increased heavy metal extraction (70% and 60% respectively for Cu; 90% and 70% respectively for Zn) at mildly acidic pH of 3-4 as compared to HNO_3 (38% and 65% respectively for Cu and Zn) at pH of 1.5 to 2. The extraction efficiencies for the two metals were high enough to remove metals from sewage sludge originating from agro–industries to levels below the Dutch legal standards of 75 mg/kg DM and 300 mg/kg DM, respectively. Citric acid, being tri-carboxylic, was found to be better than oxalic acid, which is di-carboxylic, because oxalic acid is removed from solution by precipitation as calcium oxalate. Moreover, oxalic acid is a strong reducing agent that might become oxidized in an organic matter matrix. This causes the decrease of oxalate ions available for leaching, resulting to a lower extraction for the metals compared to citric acid.

Marchioretto et al. (2002), assessed the chemical extraction efficiency of both organic and inorganic acids in the removal of heavy metals from anaerobically digested sludge (25g/L DM) from a wastewater treatment plant in the Netherlands. The plant received both industrial and municipal wastewater as influent. Nitric, hydrochloric, oxalic and citric acids were used in the experiment. The results indicated that oxalic acid did not show good results compared to the other acids. It is less soluble than citric acid at the same pH value. At higher pH values (3 and 4), citric acid achieved the highest extraction efficiency of 85% for Cd, Cr and Zn. This was probably due to its chelating properties. It was also observed that at pH values lower than 3, the extraction efficiency of HNO_3 or hydrochloric acid (HCl) is improved, achieving 100% removal for Cd and Pb. Except for Cu (for organic acids) and Cr, Cu and Zn (for inorganic acids), the heavy metals in decontaminated sludge were well within the Dutch legal standards.

Heavy Metal Extraction with Chelating Agents

Jenkins et al. (1981), using the four sludges from a central wastewater treatment plant in California (the same sludges that were treated with inorganic acids), studied the extraction of heavy metals using EDTA, at 1 hour contact time and twice the stoichiometric dose. Based on metals solubilization, results revealed the maximum removal efficiency of 37% for Pb, and 8% for Cu. The corresponding maximum removal efficiencies for Fe, Zn, Ni and Cr were 89%, 45%, 53%, and 33%, respectively. The solubilized metals were recovered by precipitation with lime, after pH adjustment to 7.0 with $Ca(OH)_2$.

Lo and Chen (1990) used EDTA and NTA to solubilize heavy metals from municipal and industrial sludges in Taiwan after 4 hours contact time and twice the stoichiometric dose (two moles of EDTA/NTA per mole of metal). EDTA extraction results showed that the removal efficiency was dependent on the nature of the sludge. For municipal sludges, the order of solubilization was Pb > Cd > Zn > Ni ≈ Cu > Cr. For the industrial sludges, the order was Cd > Ni > Pb > Cr > Zn > Cu. Compared with acid extraction, the EDTA removal for Pb was higher, while removal for Cr, Ni and Zn was lower. Cd and Cu removals were, however, nearly equal. For NTA extraction, the order of metal solubilization from the municipal and industrial sludges was Cd ≈ Ni > Pb > Zn ≈ Cu ≈ Cr. These differences in the resulting order of solubilization reflected the effects of the nature or forms of metals in the sludges as discussed earlier in section 2.4.1. The remaining amount of heavy metals in decontaminated sludges was well within the USEPA standards.

Heavy Metal Extraction with Inorganic Chemicals

Zhiping (1995) investigated the effect of leaching by ferric chloride ($FeCl_3$) of heavy metals from anaerobically digested sludge (11.29% total solids) and undigested sludge cake (20.82% total solids) that were collected from a sewage treatment facility in Bangkok, Thailand. Using $FeCl_3.6H_2O$, leaching was done using a laboratory shaker at solution pH of 3 for 4 to 7 days. Results revealed Zn solubilization was the largest at 92% and 65%, respectively, for the anaerobically digested sludge and undigested sludge cake. The decontaminated anaerobically digested sludge and undigested sludge contained about 412.4 mg/kg dry solids (DS) and 881 mg/kg dry solids (DS), respectively of Zn metal, well below the proposed Thai standard of 3,000 mg/kg dry solids for Zn. Removals attained for other metals were 30% for Cu, 59% for Mn and 39% for Ni, for anaerobically digested sludge, while for undigested sludge cake, metal removals were 46% for Cu, 51% for Mn and 51% for Ni. In general, the experiment revealed that for anaerobically digested sludge, lower pH, lower solid content and higher ferric ion dosage favored metal solubilization, although the strong buffer capacity of the sludge seems to make $FeCl_3$ less effective. For undigested sludge, it was assumed that its weak buffer capacity resulted in a drastic drop of sludge pH when ferric salt was added, favoring the dissolution of acid soluble heavy metals and oxidation of oxidizable heavy metals.

2.4.2.5 Comparison of Extracting Agents

Table 2.13 shows a summary of the efficiencies of the different extracting agents used to solubilize heavy metals from sludge. Initial metal concentration in mg/kg of dry solids, are provided wherever available in order to show the wide variation in the metal content of various types of sludges. As shown, for inorganic acids, as much as 100% removal was attained for Cd, Pb and Zn using HCl and H_2SO_4 as extracting agents. For Cr, the maximum removal of 97% was attained using H_2SO_4 at pH 1.5 while maximum removal of 99% was attained for Ni, using the same reagent. Cu, on the other hand, attained only as much as 80% removal using HCl and HNO_3.

The use of chelating agents resulted to lesser removal efficiencies for most of the metals except for Cd and Ni which attained as much as 98% and 95% respectively, using NTA.

For organic acids, the removal efficiency attained was relatively good at higher pH conditions compared to inorganic acids and chelating agents. In comparison with oxalic acid, citric acid attained higher removal efficiency for all metals studied.

Compared to other extracting agents, the use of inorganic chemicals such as ferric chloride, resulted to lower removal efficiencies for most metals, except for Zn, which is comparable to that attained using organic acids.

In summary, it seems that inorganic acids are superior over the other extracting agents in removing heavy metals from sludge at a lower pH of 1.5 to 2. However, like chelating agents and other inorganic chemicals, the use of inorganic acids can produce solid and liquid wastestreams which can have negative impacts in the environment. Organic acids can be more promising because the extraction can be performed at mildly acidic conditions (pH 3-4). Moreover, organic acids are biodegradable which implies that the decontaminated sludge does not have to be conditioned, resulting in a substantial reduction of wastewater required for washing the sludge.

2.5 Citric Acid and its Sources

2.5.1 General Properties

Citric acid is a weak organic acid which is a natural component of many citrus fruits. It is a good natural preservative and is also used to add an acidic (sour) taste to foods and softdrinks. In biochemistry, it is important as an intermediate in the citric acid cycle and therefore occurs in the metabolism of almost all living things. It also serves as an environmentally friendly cleaning agent and acts as an antioxidant. Citric acid exists in a variety of fruits and vegetables, but it is most concentrated in lemons and limes, where it can comprise as much as 8% of the dry weight of the fruit. The formula of citric acid is $C_6H_8O_7$ (2-Hydroxy-1,2,3-propanetricarboxylic acid) (Science Daily, 2004).

Table 2.13 Comparison of Metal Removal Efficiencies for Different Chemical Extracting Agents

Extracting Agent/pH	Maximum Metal Removal Efficiency (%)								Reference
	Cd	**Cr**	**Cu**	**Fe**	**Pb**	**Mn**	**Ni**	**Zn**	
1. Inorganic acids									
H_2SO_4 pH ~2	74 (68-331)[a]	76 (1,147-1,377)	1 (882-1195)	96 (11,029-41,558)	13 (377-1,009)	- -	74 (248-665)	72 (2,574 - 3,948)	Jenkins et al., 1981
H_2SO_4 pH= 1.5	97[b] (17)[b] 95[c] (5)[c]	69 (69) 97 (360)	10 (243) 10 (1,518)	- -	66 (133) 36 (161)	- -	70 (62) 99 (449)	100 (1,856) 50 (6,533)	Lo and Chen, 1990
H_2SO_4 pH = 1.5	59 (10-31)	27 (26-1,719)	41 (147-3,689)	- -	22 (15 - 646)	83 (48-5,696)	68 (13-274)	66 (151-1,926)	Blais et al., 1992
HCl pH=2	76	2	26	15	4	75	70	77	Logan and Feltz, 1985
HCl pH <3	100 (3)	80 (300-500)	80 (700-900)	- -	100 (150)	- -	60 (20-40)	80 (1,500-2,000)	Marchioretto et al., 2002
HNO_3 pH<3	-	80	80	-	100	-	60	80	Marchioretto et al., 2002
HNO_3 pH = 3	-	-	38 (472)	15 (7.9)	-	-	-	65 (425)	Veeken and Hamelers, 1999
2. Chelating agents									
EDTA pH ~2	23	33	8	89	37	-	53	45	Jenkins et al., 1981
EDTA pH = 1.5	65 95	7 32	16 4	-	71 61	-	17 80	26 23	Lo and Chen, 1990
NTA pH = 1.5	98 96	8 30	5 8		24 44		95 87	15 50	Lo and Chen, 1990
3. Organic acids									
Citric Acid pH= 3	85	85	25	-	60	-	60	85	Marchioretto et al., 2002
Citric acid pH = 3	-	-	70	90	-	-	-	90	Veeken and Hamelers, 1999
Oxalic acid pH = 3	-	-	60	58	-	-	-	70	Veeken and Hamelers, 1999
4. Inorganic chemicals									
Ferric Chloride pH = 3	-	14[d] (149)[d] 14[e] (429)[e]	30 (1,061) 46 (2,574)	-	15 (272) 15 (205)	59 (1,572) 51 (4,119)	39 (181) 51 (196)	65 (5,155) 92 (2,517)	Zhiping, 1995

[a] (   ) – Initial metal concentration in mg/kg dry solids
[b] Sludge from municipal wastewater treatment plant
[c] Sludge from industrial wastewater treatment plant
[d] Anaerobically digested sludge
[e] Undigested sludge cake

The acidic nature of citric acid results from the three carboxy groups COOH which can lose a proton in solution forming the citrate ion. Citrates make excellent buffers for controlling the pH of acidic solutions. Citrate ions form salts with many ions. Citrates can chelate metal ions, and thus can be used as preservatives, water softeners, chelating and sequestering agents. At room temperature, citric acid is a white crystalline powder. Chemically, citric acid shares the properties of other carboxylic acids. When heated above 175°C, it decomposes through the loss of carbon dioxide and water. Citric acid is recognized as safe for use in food by all major national and international food regulatory agencies. It is naturally present in almost all forms of life, and excess citric acid is readily metabolized and eliminated from the body (Alben, and Erkmen, 2004).

Production of citric acid involves the feeding of cultures of filamentous fungus *Aspergillus niger* (*A. niger*) on sucrose to produce citric acid. After the mold is filtered out of the resulting solution, citric acid is isolated by precipitating it with lime (calcium hydroxide) to yield calcium citrate salt, from which citric acid is regenerated by treatment with sulfuric acid. Alternatively, citric acid is sometimes isolated from the fermentation broth by extraction with a hydrocarbon solution of the organic base trilaurylamine, followed by re-extraction from the organic solution by water (Yigitoglu, 1992).

2.5.2 Sources of Citric Acid

Citric acid is produced either by direct extraction from fruits; fermentation of sucrose by fungi; and by chemical synthesis (Sun, 1984). Until about 1920, all commercial citric acid was produced from lemon and lime juices. At present, citric acid is produced commercially by fermentation of sucrose using mutant strains of *A. niger*; and chemical synthesis. Carbohydrates and wastes that have been considered experimentally to produce citric acid by *A. niger* included, date fruit syrup, soya whey, and cheese whey (El-Holi and Al-Delaimy, 2003). The following describes studies done on other wastes used to produce citric acid:

Sun (1984) conducted an experimental study on the use of raw liquid obtained from pineapple solid waste (peel, crushed core, top and bottom cuts and damaged parts of the fruit) as raw material for the production of citric acid. The study proved the suitability of the liquid for fermentation with *A. niger*. The yield of citric acid obtained varied from 5.0 - 13.6 g/liter of the liquid depending on the sugar content in the samples.

Hang and Woodams (1998) investigated the use of corncobs (solid waste produced from sweet corn processing) as raw material for the production of citric acid. Fermentation of corncobs with *A. niger* produced up to 400 g of citric acid per kg dry matter of corncobs .

Ali et al. (2002) worked on the use of cane molasses as raw material for the production of citric acid. Fermentation of molasses with *A. niger* produced up to 99.56 g of citric acid per liter of molasses.

Alben and Erkmen (2004) explored the use of wheat wastes (such as undersized semolina) for citric acid production. Fermentation of undersized semolina with *A. niger* produced about 0.04-0.0506 gram citric acid per liter of wheat waste.

Of those wastes which can be potential sources of citric acid, pineapple solid wastes is of interest. It was reported that in 2002, the fresh pineapple production in the world was about 14 million metric tons (Food Market Exchange, 2006). Among those countries producing fresh pineapple, Thailand has the highest market share which produced about two million metric tons in 2002. So far, Thailand has been considered as the world's largest producer of both fresh and canned pineapple. This also implies that the largest solid wastes arising from pineapple processing come from Thailand. Utilization of these wastes to produce citric acid for the removal of heavy metals from contaminated sludge would indeed be a sustainable way of sludge management.

2.6 Citric Acid Fermentation Process

There are basically three different batch fermentation used in industry: the Japanese koji process; the liquid surface culture; and the submerged fermentation process (Yigitoglu, 1992). Nearly all commercial citric acid is currently produced by submerged culture fermentation.

2.6.1 Microorganism

The main fungus used in citric acid fermentation is *A. niger*, although other strains of fungi and various kinds of yeast and some bacteria are known to accumulate citric acid in the medium. *A. niger* has the advantage of producing high consistent yields of citric acid using cheap raw materials such as molasses (Ali et al., 2002; Alben and Erkmen, 2004; Yigitoglu, 1992).

A. niger Inoculum and Growth Form

Earlier studies recommended that between 120 x 10^3 and 280 x 10^3 pellets per liter (obtained from spore inoculated shake flasks) is a suitable inoculum level. Yigitoglu (1992), employed a less complex method of inoculum where spores were harvested from potato dextrose agar (PDA) plates and incubated in an orbital shaker at 30^{o}C and 250 rpm for 48 hrs. At the end of this period, the small pellets which had formed in the flasks were used as inocula for fermenter experiments, using an inoculum level of 2%.

There is general agreement in the literature that the pelleted form is favorable for acid production because pellet cultures have low culture viscosity which improves bulk mixing and aeration condition, by lowering oxygen consumption as compared to cultures composed mainly of filamentous (dispersed forms). Furthermore, problems of wall growth and pipe blockage are reduced and separation of biomass from culture liquid by filtration is considerably enhanced by pelleted growth form. An ideal pellet configuration of 1.2 to 2.5 mm diameter after five days was described in early studies (Yigitoglu, 1992).

2.6.2 Factors Affecting the Fermentation Process

Based on other studies, Yigitoglou (1992) has identified the various factors that affect the yields (grams acid per gram sugar) of citric aid fermentation by *Aspergillus niger*.

2.6.2.1 Medium Constituents

Trace Elements: The levels of Mn, Fe, Cu and Zn are particularly critical in citric acid fermentation. If the levels of these trace elements are carefully controlled, high citric acid production can be attained, and other factors have less pronounced effects. Manganese ions (Mn^{2+}) in the nutrient medium plays a key role in the accumulation of citrate by *A. niger*. When the Mn^{2+} concentration is maintained below 0.02 mM (which does not affect growth rate or biomass yield) large amounts of citric acid are produced. Adding more or less would result to reduced yield. Up to 1 mg iron per liter medium is essential for high yields of citric acid by *A. niger*, but amounts in excess of this interferes with citric acid accumulation. Copper ions play an important role in reducing the deleterious effect of iron on citric acid production and has also been reported to successfully counteract addition of Mn to citric acid fermentation media and are inhibitors of cellular Mn uptake. The optimum concentration for Cu^{2+} to obtain high citric acid yield is 40 ppm. Low concentration (below 0.2 μM) of Zn in the fermentation medium are generally favored in most citric acid production media.

Sugars: For high yield of citric acid, the usual carbon sources are glucose, fructose, or sucrose. For strain *A. niger* B60, maltose and sucrose, two disaccharides were found to be better carbon sources for production of citric acid than the monosaccharides glucose and fructose. Not only the type but also the concentration of the carbon source is important in citric acid production. High yields of citric acid were obtained at 14 to 22% of sugar in the medium.

Nitrogen Source: Usually ammonium sulfate or ammonium nitrate has been used as a nitrogen source. Physiologically, acid ammonium compounds are preferred since their consumption lowers the pH of the medium to below 2 which is an additional prerequisite of citric acid fermentation. The optimal concentration of ammonium sulfate can be between 3-5 kg/m^3.

Phosphate: Although the effect of phosphate is not very pronounced, the balance between Mn, Zn and phosphate is critical. Requirement of phosphate for fungal growth is 0.1 to 0.2%.

Magnesium: Mg is essential for growth and citric acid production due to its role as a cofactor in a number of enzyme reactions in the cell. It has been reported that the optimum concentration of magnesium sulphate to produce maximum citric acid varies from 0.02 to 0.025%.

2.6.2.2 Environmental Conditions

Aeration: Aeration has been shown to have a critical effect on the submerged citric acid production process. Aeration rate of 0.9 to 1.3 vvm (liter air per liter medium per minute) is favorable for citric acid production. Interruption in aeration during the fermentation process has found to adversely affect the citric acid yield.

Agitation: Agitation in stirred tank fermenter is critical. Higher yields of citric acid is obtained by increasing agitator speed because it breaks up the inoculum pellets leading to dispersion of more than 95% of pellets. Maximum citric acid yields can be obtained at agitator speeds between 400 and 700 rpm.

pH: There is no agreement in literature about the optimal initial pH, although it was reported that culture pH increased yield has been attained at 1.8 to 6.5.

Incubation Temperature: The incubation temperature can be in the range of 28 to 32^{o}C with 30^{o}C being found to be the optimum.

Duration of Fermentation: It has been reported that the citric acid fermentation is completed from 6 to 9 days. Extension of fermentation period does not increase the citric acid yield.

2.6.2.3 Other Factors

Alcohols: It was found that addition of lower alcohols, methanol, ethanol, n-propanol, to crude carbohydrate raw materials could increase the yield of citric acid. Optimal concentration of methanol, which was said to be more effective than ethanol varied from 1 to 4% by volume. The exact mechanism of the alcohol effect however is unexplained, though it is postulated that addition of methanol increases the tolerance of the fungi to Fe^{2+}, Zn^{2+} and Mn^{2+}.

Lipids: Addition of natural oils with a high content of unsaturated fatty acids and oleic acid at 2% (v/v) to fermentation media led to increases in the yield by 20% without affecting dry weight of mycelium. The unsaturated fatty acids act as an alternative hydrogen acceptor to oxygen during the fermentation, since only high levels of unsaturated acids were effective in improving the yield of citric acid. A concentration of fatty acid of 0.05 to 0.3% has been suggested to be maintained during fermentation.

Vitamins: It has been observed that ascorbic and p-amino benzoic acid inhibited both growth of fungi (*A. niger* AL 29) and citric acid production. On the other hand, the presence of thiamine and riboflavin (at concentrations of 3×10^{-5} M and 4×10^{-5}M respectively) stimulated the citric acid formation by 59% and 50% respectively. Biotin (3×10^{-5} M) produced the greatest enhancement stimulating growth and increasing the production of acid by 66.4%.

Amino Acids: The presence of glutamic acid (4×10^{-3}) and aspartic acid (3×10^{-3} M) stimulated citric acid production by 79 and 76.7% respectively. Presence of lysine (5×10^{-3}) and serine (4×10^{-3} M) also could influence the formation of citric acid by 62.3 and 50.4% respectively. Cystein on the other hand was found to have negative effect in all concentrations.

Toxic Chemicals: Results of a study done on the effect of addition of chemicals (in the range of 0 to 60 ppm) on spore germination, mycelial dry weight and citric acid production in a based glucose medium using *A. niger* EU-1 are described as follows: Phenol (at 20 ppm) and b-naphthol (at 20 ppm) caused a slight increase in citric acid production while hydroquinone (at 30 ppm) and o-cresol (at 15 ppm) increased the acid production by 85 and 80 kg/m^3 respectively. Acid formation in the presence of resorcinol (at 50 ppm) was 78 kg/m^3. The increase in acid production may be due to either the direct effect of these phenols on the growth process i.e. metabolism of *A. niger* or to the inhibition of enzymes involved in further metabolism of citric acid.

Ammonium Sulphate and Citrate Supplementation: Studies found that the optimum addition time of N was in the range of 40 to 75 h. It was found that final citric acid concentration achieved was increased when the amount of supplemental N source added was between 0.25 and 0.5 kg/m^3. The addition of 60 kg/m^3 of citric acid at time 90 h, led to a 40.3% increase in the final citric acid concentration (80kg/m^3) compared to the standard run in which 57 kg/m^3 citric acid was produced.

2.7 Extraction of Citric Acid from Pineapple Wastes

The most common preservation method of pineapple is canning. During canning, the peel and core of the fruit are automatically removed by machine, yielding pineapple flesh which only make up 20-30% of the fruit. The flesh is cut into slices, chunks, and tidbits. About 70-80% of the fruit is discarded as solid wastes, which consists of shell, core, top, and bottom cuts and damaged fruit or parts of fruit (Sun, 1984). Although some research have been conducted on the utilization of these wastes (such as animal feed; and for production of alcohol, vinegar and wine), its utilization has not been done in practice for a long time. At present, most pineapple solid wastes are still disposed off into the environment at a considerable cost for transportation and causing environmental pollution. Hence, research on the alternatives for utilization of these wastes are still of interest.

Citric acid extraction from pineapple wastes has been done by Sun (1984) using laboratory and pilot scale studies. The waste which was collected from a local canning factory, consisted of the skin (shell), crushed core, top and bottom cuts as well as damaged parts of the fruit. The raw liquid obtained from the pineapple solid wastes was analyzed to determine the suitability for citric acid fermentation. The physical and chemical characteristics of the raw liquid are presented in Table 2.14.

Table 2.14 Physical and Chemical Characteristics of Raw Liquid from Pineapple Wastes

Parameters	Values
pH	3.5
Turbidity (NTU)	170
Colour (dilute 20 times)	70
Volatile solid (mg/L)	58,200
Total solids (mg/l)	62,400
Ash (mg/L)	416
Reducing Sugar(mg/L)	42,800
Sucrose (mg/l)	8,700
Total Sugar(mg/l)	51,500
Protein (mg/L)	1,880
Kjeldahl Nitrogen (mg/L)	301
Phosphorus (mg/L)	45.6
COD (mg/L)	164,020

Source: Sun (1984)

In the study, raw liquid obtained from the pineapple solid waste, was used as a fermentation medium for the production of citric acid. The chemical analysis showed that the raw liquid contained about 5-7% sugar which increased to 7-8% after autoclaving. The study proved the suitability of raw liquid for fermentation. It was also found that the sugar content in raw liquid fluctuated according to seasons, obtaining low sugar content during rainy season, and high sugar content during dry season. The inoculum used which was identified as *Aspergillus niger* MAT-1, is a wild type of strain from tropical countries. Shake flask studies revealed that the optimum conditions for fermentation were as follows: inoculum size of 10% (v/v); spore suspension of 50% transmittance; fermentation duration of 144 hours (6 days); pH of 3.0-4.0; and added phosphate (KH_2PO_4) of 0.5 – 1.0 g/liter. The yields of the citric acid varied from 5.0-13.6 g/liter of the liquid, depending on sugar content in the samples. Pilot plant studies in a fermenter resulted to citric acid yield of 5.65 g/liter of fermentation liquid, and 36.8% on the basis of the sugar consumed. The yield of mycelial dry weight was 14.6 g/L and 65.5% based on the sugar consumed. The mycelium which contained 27.3% protein, is a good source of single-cell protein for feed supplement. Addition of nitrogen compounds not only reduced the amount of citric acid formed but also reduced fungus growth. The addition of methanol at a concentration of 2-4% proportionately increased the formation of citric acid but suddenly dropped at 5% methanol, while the mycelial growth increased at first then slowly reduced.

Citric acid produced from the fermentation process was analyzed using the microcolorimetric method (Taussky's Method) which is known to have high sensitivity. Within the concentration range of 10 ppm to 60 ppm of citric acid, a straight line correlation between the colorimetric reading and the citric acid concentration is obtained. The method is based on the conversion of citric acid to pentabromoacetone and alcoholic sodium iodide with the development of yellow color complex.

Chapter 3

Methodology

In this study, batch laboratory scale experiments were carried out to remove heavy metals from sludge using acid extraction technology. The study comprised of three major parts namely: sludge characterization, chemical speciation studies, and metal extraction studies. An overview of the methodology is shown in Figure 3.1. Sludge characterization was done before and after metal extraction. Characterization of sludge after metal extraction was done only for optimum conditions where the maximum metal removal efficiency was observed. Metal extraction studies were conducted using the following treatments:

1. Leaching with commercial citric acid,
2. Leaching with *A. niger* fermented raw liquid from pineapple wastes,
3. Leaching with naturally fermented raw liquid from pineapple waste; and
4. Leaching with unfermented raw liquid from pineapple waste

Results of each metal extraction studies were analyzed and compared in terms of heavy metal removal efficiencies.

3.1 Reagent Preparation

Before the start of analysis, all glasswares were cleaned with 5N HCl and thoroughly rinsed several times with tap water and once with distilled water. Reagents for the analysis of parameters such as nitrogen, phosphorous and heavy metals were prepared according to the Standard Methods for the Examination of Water and Wastewater (APHA et al., 1998).

3.2 Sewage Sludge Sample Collection

The sludge samples were collected from the sludge treatment facility at Nongkhaem on February 3, 2005 (5 days old); October 25, 2005 (fresh sludge) and June 15, 2006 (fresh sludge). The plant with a capacity of 500 m^3/day, receives dewatered sludges from five (5) central wastewater treatment facilities under the Bangkok Metropolitan Administration (BMA), and Siriraj hospital, including fresh sludge from Nongkhaem nightsoil treatment facility. The five (5) central wastewater treatment plants include Si Phraya, Chon Nongsri, Ratburana, Rattanakosin and Din Daeng. The sludge treatment facility in Nongkhaem employs anaerobic digestion at 19 days retention time. The anaerobically digested sludge is dewatered using belt filter press before being disposed of to a landfill, and applied on land after composting. The process flowchart of the treatment facility is attached in Appendix A.

Three types of sludge were collected for preliminary analysis namely: raw sludge from the nightsoil treatment facility at Nongkhaem, and wet and dewatered anaerobically digested sludge from the sludge treatment facility. For commercial citric acid leaching, both wet and dewatered anaerobically digested sludge were used. For leaching studies other than using commercial citric acid, only dewatered anaerobically digested sludge was used. After collection, the sludge samples were stored at 4^{o}C when not directly used.

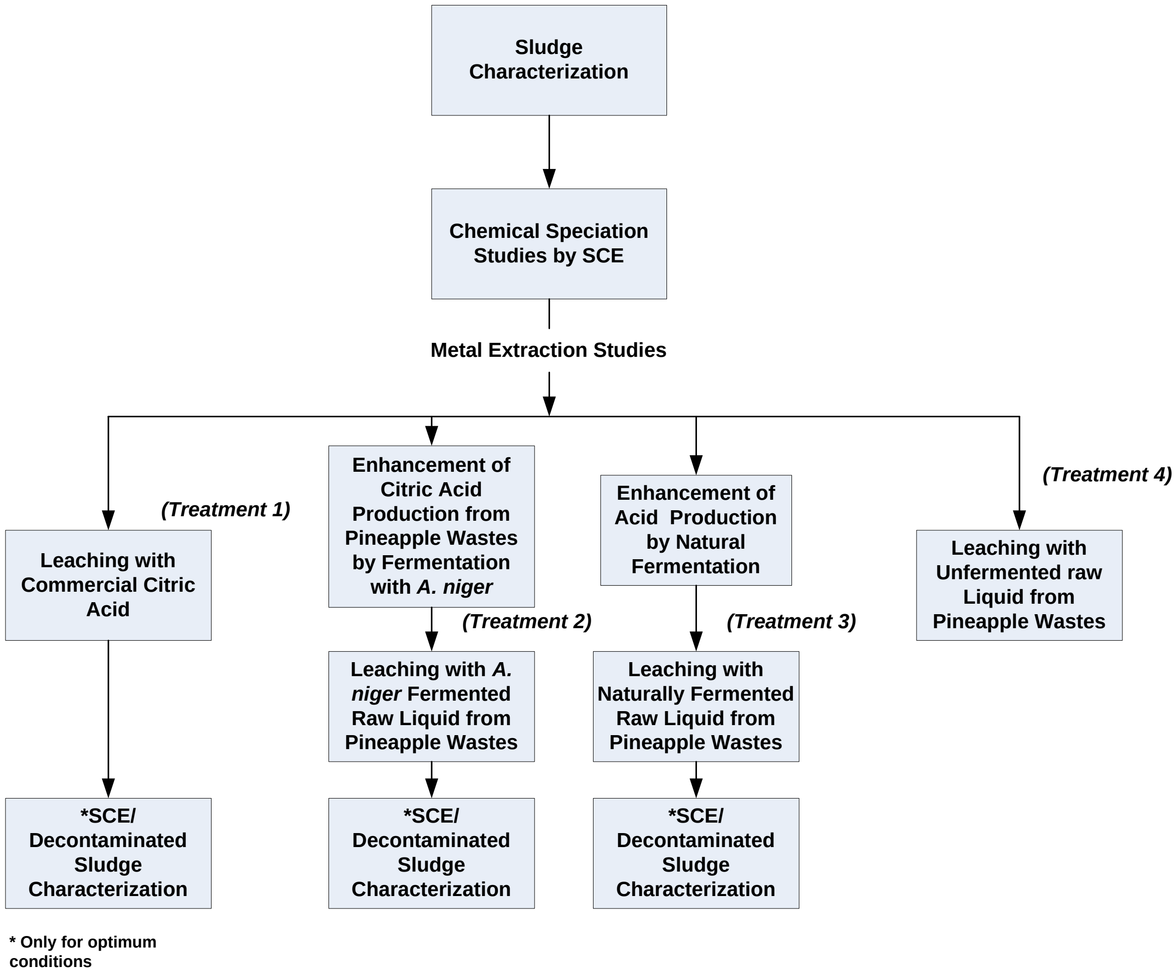


Figure 3.1 Overview of Methods used in the Study

3.3 Sludge Characterization

The sludge samples were analyzed in terms of their physical and chemical characteristics including the presence of heavy metals. Analyses were done in duplicates. Table 3.1 shows the instruments and methods used for analysis.

3.4 Heavy Metal Analysis

The analysis of heavy metals was done according to the Standard Methods for the Examination of Water and Wastewater using flame atomic absorption spectrophotometer (AAS). Before metal analysis, the sludge samples were digested with acids (HCl, HF, HNO_3 and H_3BO_3) using the Microwave digestion procedure for sludges. The procedure employed in the digestion of sludge samples before heavy metal analysis is attached in Appendix B. The heavy metal concentration was expressed in mg/kg dry solids (DS) and was calculated as follows:

$$\text{Metal concentration (mg/kg DS)} = \frac{A \times B}{\text{Wt. of sample (g)}} \times \frac{100}{D} \quad \ldots\ldots\ldots\ldots\ldots (3.1)$$

Where:

A = Concentration of metal in digested solution, mg/L
B = Final volume of digested solution, mL, and
D = Total solids, %

Table 3.1 Analytical Methods Employed in Sludge Characterization

Parameters	Analytical Methods	Instrument
1.pH	Electrometric Method (Radojevic and Bashkin, 1999)	pH Meter Oaklon Model No. 35614-70
2. Total Solids (%)	Standard Methods Part 2540 B	-
3. Total Volatile Solids (%)	Standard Methods Part 2540 E	-
4.Total Nitrogen (%)	Titrimetric Method with Digestion (Standard Methods, 1998; Radojevic and Bashkin, 1999; Ryan et al., 2001)	TKN Apparatus (Gerhardt Turbotherm and Vapodest 20)
5. Total Phosphorous (%)	Spectrophotometry (Standard Methods, 1998; Radojevic and Bashkin, 1999)	Spectrophotometer Cecil (CE 1021) model 1000 series
6. Heavy Metals (mg/Kg dry basis)	Atomic Absorption Spectrophotometry (AAS) with Microwave Digestion Technique – Standard Methods Part 3111	- Microwave Digester with WinWave Application Software - AAS Hitachi Z-8230
7. Faecal Coliform	Standard Methods Part 9221 E	-

3.5 Chemical Speciation Studies

Chemical speciation studies were done to determine the different forms of metals in the sludge. For untreated sludge, two types of samples were used, namely: dewatered anaerobically digested sludge (oven-dried at 105 ^oC and air dried) and wet anaerobically digested sludge (oven-dried at 105 ^oC). For treated sludge, only oven-dried samples were used. Sequential chemical extraction (SCE) procedure employed by Tessier et al. (1979) and Sims and Kline (1991), and that modified by Marchioretto (2003), was used in the speciation studies. The procedure is summarized in Table 3.2 and presented in Figure 3.2.

The sequential extraction was carried out in two grams of air dried sludge samples in 250 mL erlenmeyer flasks and shaken at 125 rpm. The sludge samples were first ground using mortar and pestle, to pass through a 2 mm sieve. Between each of the successive extractions, separation was done by centrifuging at 4,000 rpm for 30 minutes where the supernatant was removed and analyzed for trace metals by flame AAS and the residue washed with 40 mL deionized water.

Table 3.2 Modified Tessier/Sims and Kline Scheme for Determination of Metal Forms in Sludge

Chemical Form of Metals	**Extraction Conditions**	
	Reagent	**Time and Temperature**
Exchangeable (*Tessier*)	$MgCl_2$ 1 mol/l, pH = 7, 8(v/w)[1]	1 h at 20°C
Bound to carbonates or acid extractable phase (*Tessier*)	NaAc[2] 1 mol/l, pH = 5, 8(v/w)	5 h at 20 °C
Bound to Fe-Mn Oxides or reducible phase (*Tessier*)	$NH_2OH.HCl$ 0.04 mol/l, pH = 2, 20(v/w)	6 h at 96 °C (occasional shaking)
Bound to organic and inorganic matter or oxidisable phase (*modified Sims and Kline, Marchioretto*)	Na_2EDTA 0.05 mol/l, pH 4.5, 20 (v/w)	6 h at 20 °C
Residual (*modified Tessier and Sims and Kline*)	5 ml H_2O, 4 ml HNO_3 70%, 1 ml HCl 35%, 2 ml HF 48% HNO_3 4 mol/L,pH = 0.6, 12.5 (v/w)	24 h at room temperature 16 h at 80°C (occasional shaking)

[1]Liquid-to-solid ratio (v/w); v is the volume of the extractant (ml); w is the mass of the sample (g)

[2]Ac: acetate

3.6 Metal Extraction Studies to Determine Optimum Conditions

3.6.1 Leaching with Commercial Citric Acid: Determination of Optimum pH (by varying dose) and Leaching Time

The citric acid used for leaching the sludge sample under this treatment was of commercial grade and obtained from the local market. The following acid leaching procedure modified from Ali (1994), Marchioretto (2003) and Veeken and Hamelers (1999), was used:

A. Determination of Optimum pH

1. For each sludge type, duplicate samples containing about two gram dry solids were transferred to reactors (erlenmeyer flasks) and filled with 40 mL deionized water.
2. Varying amounts of citric acid were added to the sludge to obtain the desired pH of 2-6 for dewatered sludge, and 2-7 for wet sludge.
3. A rotary shaker was used to mix the reactors continuously at 125-150 rpm at room

temperature for two hours, and the pH monitored.

4. From each reactor, two samples of 10 ml were collected and centrifuged at 4000 rpm for 30 minutes and then filtered using 0.45 µm membrane filters before analyzing the filtrate for heavy metals.
5. Further experimentation on the effect of leaching time were then carried out at three pH values (2.33, 3.04 and 3.90 for dewatered sludge; and 2.55, 3.02 and 4.0 for wet sludge) corresponding to the optimum acid dosages.

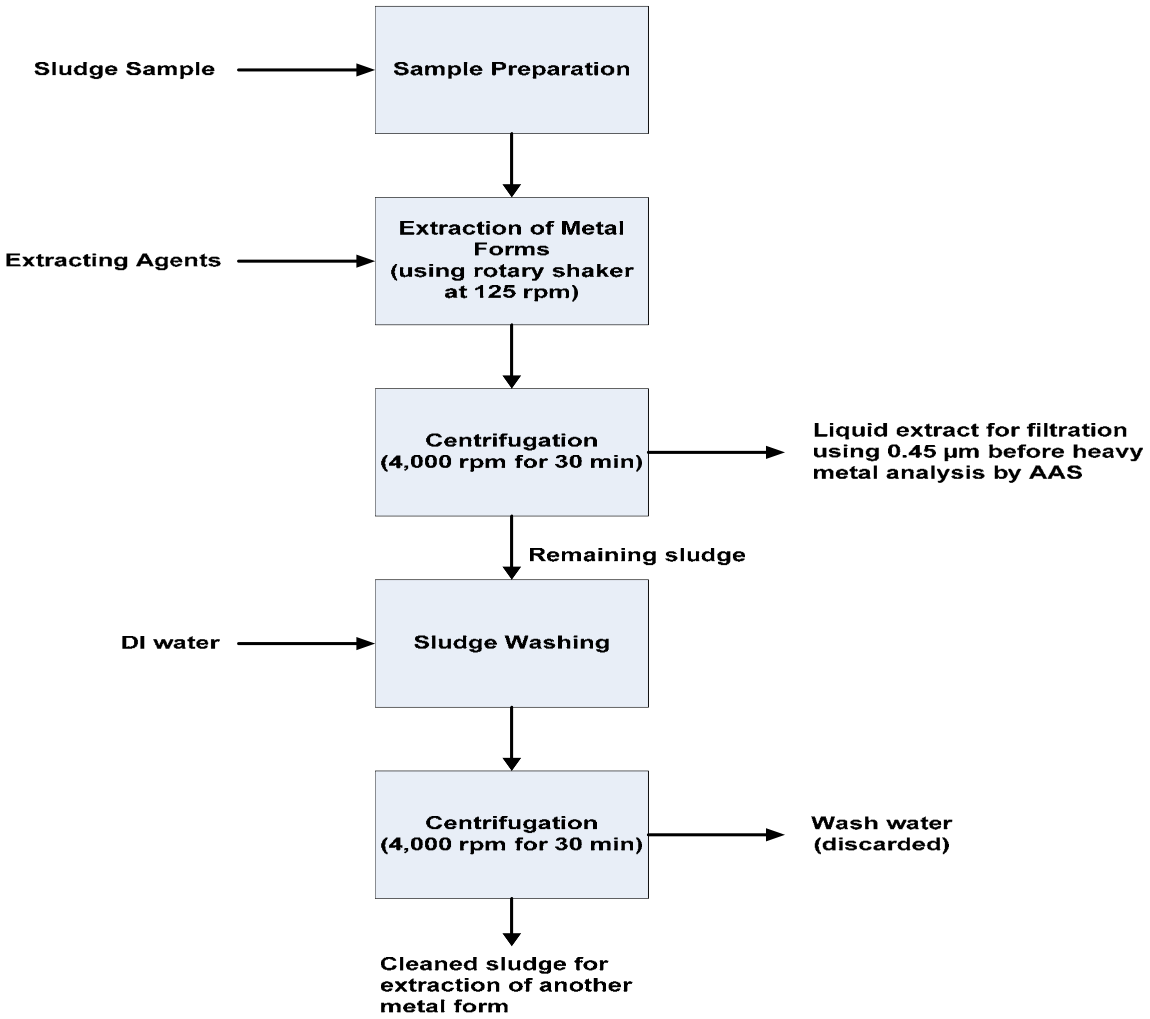


Figure 3.2 Overview of Sequential Chemical Extraction Procedure (SCE)

B. Determination of Optimum Leaching Time

1. For each sludge type, duplicate samples containing about 7 grams air-dried samples were transferred to reactors (erlenmeyer flasks) and filled with 140 mL deionized water.
2. Varying amounts of citric acid were added to the sludge to obtain the desired optimum pH obtained from part A.
6. A rotary shaker was used to mix the reactors continuously at 125-150 rpm at room

temperature.

7. Samples of appropriate amount were then collected from the reactor at certain time intervals (1 hour, 2 hours, 6 hours, 1 day, 5 days, 8 days and 11 days) for centrifugation at 4,000 rpm for 30 minutes and then filtered using 0.45 μm membrane filters, before analyzing the filtrate for heavy metal analysis.
8. Leaching was done at room temperature for up to 11 days.

An overview of the leaching procedure is presented in Figure 3.3.

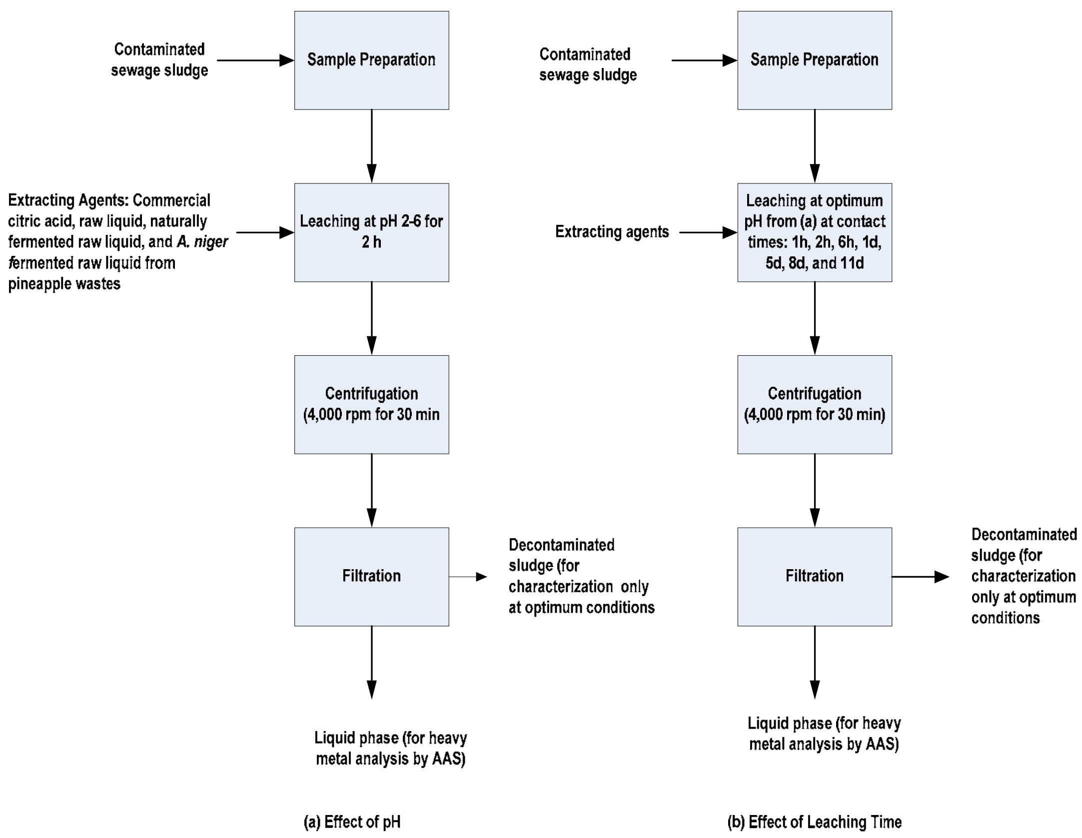


Figure 3.3 Overview of Acid Leaching Procedure

3.6.2 Leaching with Unfermented Raw Liquid from Pineapple Waste: Determination of Optimum pH

3.6.2.1 Source of Pineapple Waste and Sample Preparation

Pineapple waste samples were collected from fruit vendors at Thammasat University main canteen and AIT Cafeteria. The pineapple wastes collected consisted of the core, peel (shell) and top and bottom cuts (Figure 3.4). The wastes were then crushed and ground for extraction of raw liquid. The raw liquid was manually squeezed from the pulp with a fine cloth. The extracted liquid (Figure 3.5) was then used to extract the metals from sludge sample. The pulp (Figure 3.6) which is rich in protein (Sun, 1984) can still be used as animal feed.

Figure 3.4 Pineapple Wastes

Figure 3.5 Unfermented Raw Liquid

Figure 3.6 Pulp from Pineapple Wastes

Figure 3.7 Naturally Fermented Raw Liquid

3.6.2.2 Raw Liquid Characterization

The raw liquid from pineapple wastes was analyzed for physico-chemical parameters including heavy metals using AAS, total sugar using high performance liquid chromatography (HPLC-Agilent Technologies 1100) and total acidity as citric acid using the Glass electrode method following AOAC Official Methods of Analysis (AOAC, 2000). Infrared Spectroscopy (IR) studies using FT-IR Spectrometer (Perkin Elmer System 2000R) was also done to confirm the presence of citric acid as carboxylic acid in the raw liquid.

3.6.2.3 Leaching Procedure

Extraction of metals from sample sludge was done using the procedure described in section 3.6.1. The sludge sample was dosed with raw liquid from pineapple waste to obtain the desired pH of 3.94, 4.0, 5.07 and 6.02. Further experimentation was also conducted to determine the optimum pH at two hours leaching time, for the removal of heavy metals.

3.6.3 Leaching with Naturally Fermented Raw Liquid from Pineapple Waste: Determination of Optimum pH and Leaching Time

3.6.3.1 Source of Pineapple Waste and Sample Preparation

Pineapple waste samples were collected from fruit vendors at Thammasat University main canteen and AIT Cafeteria. Raw liquid preparation was done as described in section 3.6.2.1.

3.6.3.2 Natural Fermentation of Raw Liquid by Shake Flask Studies

Based on optimum conditions on citric acid fermentation by Sun (1984), the raw liquid obtained after manually squeezing the pulp, was allowed to undergo natural fermentation (i.e. without adding *A. niger*) for 144 hours (6 days) at room temperature. The naturally fermented liquid (Figure 3.7) was then used to extract the metals from sample sludge.

3.6.3.3 Naturally Fermented Liquid Characterization

The naturally fermented raw liquid from pineapple wastes was analyzed for physico-chemical parameters as described in section 3.6.2.2

3.6.3.4 Leaching Procedure

Extraction of metals from sample sludge was also done using the procedure described in section 3.6.1. The sludge sample was dosed with the naturally fermented raw liquid from pineapple waste to obtain the desired pH of 3.67, 3.94, 4.21, 4.36. Further experimentation was conducted to determine the optimum pH and leaching time for the removal of heavy metals from sludge.

3.6.4 Leaching with *A. niger* Fermented Raw Liquid from Pineapple Wastes

The extractant used for removing metals from the sample sludge under this treatment was the raw liquid extracted from pineapple wastes after fermentation with the fungus *A. niger*, as described below.

3.6.4.1 Enhancement of Citric Acid Production from Pineapple Wastes

The method employed by Sun (1984) and Mcintyre et al. (2001), with some modifications, was used to enhance citric acid production from pineapple wastes. The following optimum conditions for acid fermentation using *A. niger* was applied:

- Spore size of 10^9-10^{10} spores/L
- Fermentation duration of 144 hours (6 days)
- pH of 4.0; and
- Phosphate (KH_2PO_4) addition of 0.5-1.0 g/liter.

An overview of the extraction procedure is presented in Figure 3.8.

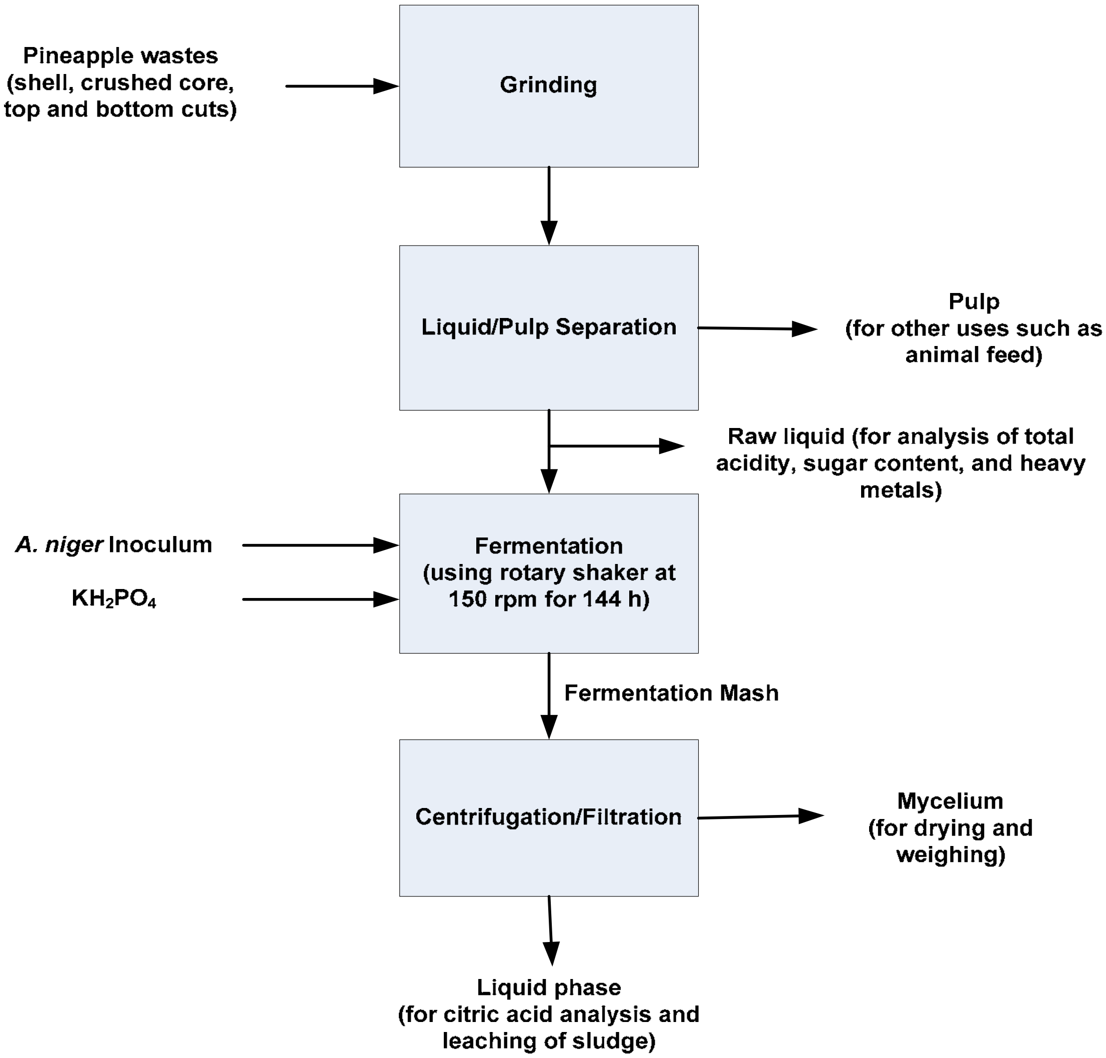


Figure 3. 8 Overview of Raw Liquid Fermentation with *A. niger* to Enhance Citric Acid Content

3.6.4.2 Source of Pineapple Waste and Sample Preparation

Pineapple waste samples were collected from fruit vendors at Thammasat University main canteen and AIT Cafeteria. Sample preparation was done as described in section 3.6.2.1. The raw liquid which was manually squeezed from the pulp was allowed to undergo aerobic fermentation with *A. niger* for 144 hours (6 days) at room temperature. The fermented liquid was then used to extract the metals from sample sludge.

3.6.4.3 *A. niger* Fermented Liquid Characterization

The *A. niger* fermented liquid from pineapple wastes was analyzed for physico-chemical parameters as described in section 3.6.2.2. Citric acid analysis was done as will be described in section 3.6.4.6.

3.6.4.4 *Aspergillus niger* and Inoculum Preparation

The individual strain of *Aspergillus niger* obtained from BIOTEC Central Research Unit in Pathumthani, Thailand, was inoculated on potato dextrose agar slant (PDA) at 30 ^oC for 7 days. After incubation, some were maintained on the same slant in the refrigerator and some were used immediately. Whenever inoculum is needed, spore suspension was prepared by first cultivating the fungus strain in flasks containing 29-30 g rice grain (Figures 3.9 and 3.10), pre-autoclaved with 6 ml medium (YPG) for 7 days at 30^{o}C. The YPG contained the following : yeast extract, 5 g/L; peptone, 10 g/L and glucose, 20 g/L. The resulting spores were then harvested in a 0.1% Tween 80 (Polyoxyethylene-sorbiton monooleate) solution and used to inoculate the raw liquid at $10^9 – 10^{10}$ spores per liter (Mcintyre et al., 2001). The *A. niger* strain was also cultivated using mycelium inoculum prepared using YPG incubated at 25 – 30^{o}C at 200 rpm for 36-48 hrs. An overview of the procedure for the preparation of spore suspension and mycelium inoculum are presented in Figures 3.11 and 3.12. The prepared *A. niger* inoculum, if properly stored, can be used several times for fermentation of the raw liquid.

Figure 3.9 *A. niger* Spore Formation in Rice Culture after Incubation

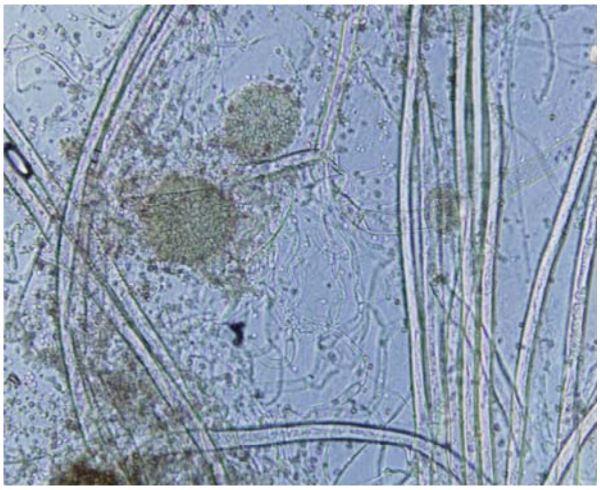


Figure 3.10 Microscopic View of *A. niger* showing Spores

3.6.4.5 Citric Acid Fermentation by Shake Flask studies

Optimum conditions for fermentation of raw liquid from pineapple wastes using *A. niger* by Sun (1984) were adapted in this study. Prior to the fermentation, raw liquid from pineapple wastes (250 mL) was dispensed into 1000 mL erlenmeyer flasks. Since the pH of the raw liquid was within the optimum range of 3.0-4.0, no pH adjustment was made for citric acid fermentation. Phosphorus in the form of potassium dihydrogen phosphate (KH_2PO_4) was added at the rate of 0.5-1.0 g/liter. The flasks were plugged with cotton and autoclaved at 103.42 kPa (15 lb_f/in^2) at 121^{o}C for 15 minutes prior to use. Each flask was inoculated with 2 mL spore suspension and was shaken at 150 rpm for 144 hours (6 days) at 30^{o}C (Figure 3.13). Mycelium inoculum was also used by transferring 25 mL of the inoculum in 225 ml of raw liquid in flasks (Figure 3.14). However, the mycelium inoculum was discarded because it was found to be contaminated.

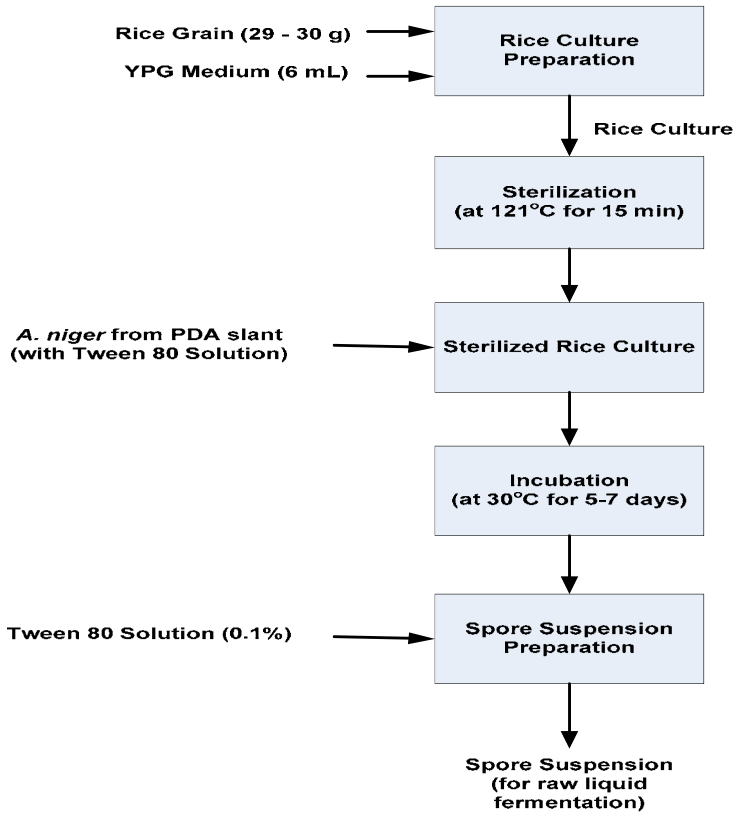


Figure 3.11 Overview of Procedure for *A. niger* Spore Suspension Preparation (Aseptic Technique)

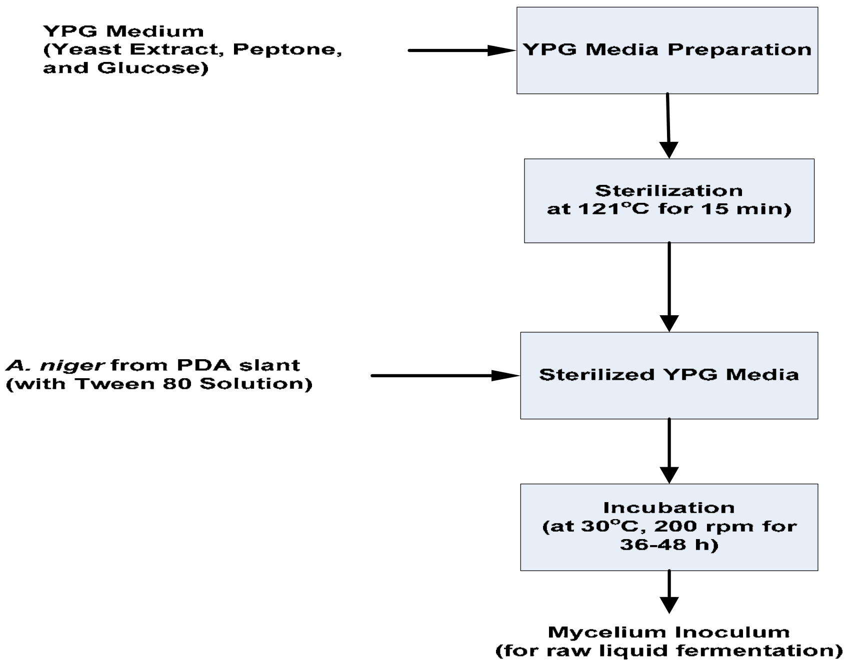


Figure 3.12 Overview of Procedure for *A. niger* Mycelium Inoculum Preparation (Aseptic Technique)

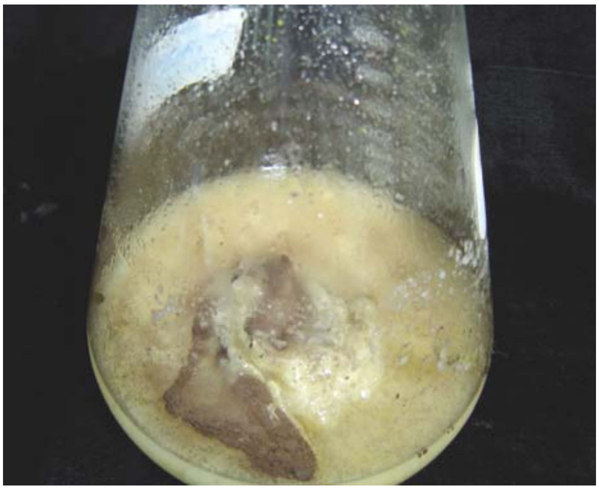


Figure 3.13 Fermentation with *A. niger* Spore Suspension

Figure 3.14 Fermentation with *A. niger* Mycelium Inoculum

3.6.4.6 Citric Acid Analysis

Samples taken from fermentation (fermented mash) were filtered to separate the mycelium (Figure 3.15) from the liquid phase. The mycelium obtained was dried in the oven at 103°C for 24 hours, then weighed. The filtrate (Figure 3.16) was sent for citric acid analysis immediately in order to avoid decomposition. Citric acid was analyzed as total acidity using the Glass electrode method (AOAC Official Method 942.15). The amount of citric acid extracted was also analyzed using high performance liquid chromatography (HPLC) with an Aminex HPX-87H column (Bio-Rad, Hercules, Calif., USA) at 60 °C, using 5 mM H_2SO_4 as a mobile phase at a flow rate of 0.6 ml/min. Citrate was detected refractometrically (Waters 410 Differential Refractometer Detector, Millipore Corp., Milford, MA, USA).

Figure 3.15 Mycelium from *A. niger* Fermented Liquid

Figure 3.16 *A. niger* Fermented Raw Liquid after Filtration

3.6.4.7 *A. niger* Fermented Raw Liquid Leaching: Determination of Optimum pH and Leaching Time

Extraction of metals from the sludge sample was also done using the procedure described in section 3.6.1. The sludge sample was dosed with the *A. niger* fermented raw liquid from pineapple waste to obtain the desired pH of 3.73, 3.88, 3.98, 4.47, and 4.51. Further experimentation was conducted to determine the optimum pH and leaching time for the removal of heavy metals.

3.7 Metal Extraction Studies at Optimum Conditions

A separate metal extraction study was done using treatment options at optimum conditions. Optimum conditions refer to the sludge pH and contact time with various extractants that give the best metal removal efficiencies. Metal extraction studies were carried out at the following optimum conditions only for three types of extractants:

1. Leaching with commercial citric acid: pH = 3.04 at 1 day leaching time
2. Leaching with *A. niger* fermented raw liquid from pineapple wastes: pH = 3.73 and 3.88 at 5 days leaching time.
3. Leaching with naturally fermented raw liquid from pineapple waste: pH = 3.67 at 1 day leaching time

Sludge characterization and chemical speciation studies were also done both before and after treatment with the extractants.

The overview of metal extraction studies at optimum conditions is presented in Figure 3.17.

3.8 Characterization of Decontaminated Sludge for Land Application

The sludge samples after undergoing metal removal treatment at optimum conditions, were analyzed in terms of its physico-chemical characteristics including remaining heavy metals, pathogens, as well as nutrients such as nitrogen and phosphorus, and compared with local standards to determine its suitability for land application. Comparison of the characteristics of the treated sludge with the standards for composted sludge was also made. Analysis was done using the methods described in section 3.3, according to soil and sludge analysis manuals (Ryan et al., 2001; Radojevic and Bashkin, 1999) and the Standard Methods for the Examination of Water and Wastewater (APHA et al., 1998), where appropriate.

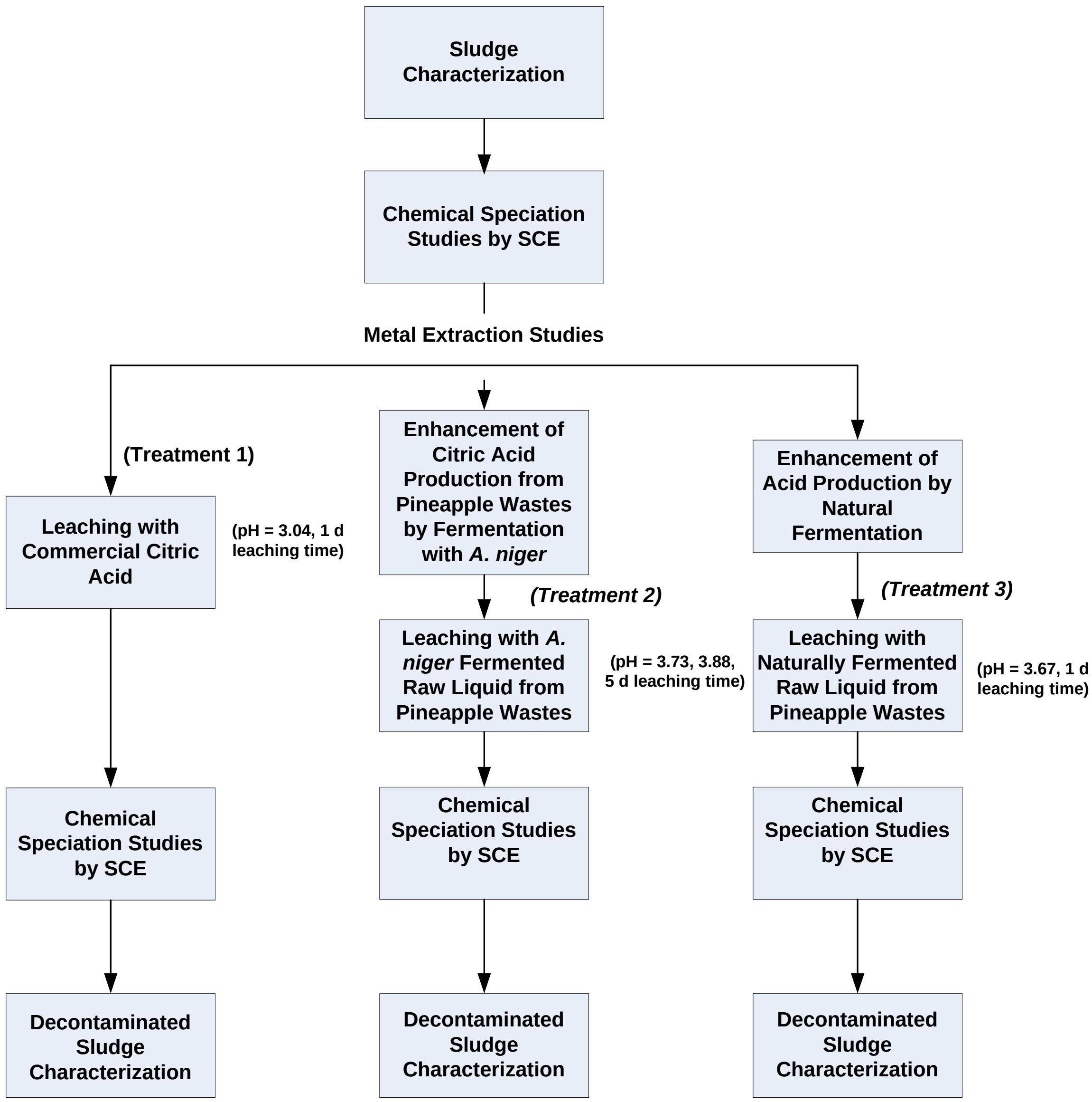


Figure 3.17 Overview of Methods for Experimental Studies at Optimum Conditions

Chapter 4
Results and Discussions

4.1 Sludge Characterization

For preliminary analysis, three types of sludge samples were used namely: raw sludge from Nongkhaem nightsoil treatment plant, wet and dewatered anaerobically digested sludges from Nongkhaem sludge treatment facility.

Table 4.1 summarizes the results of the general characterization of sludge samples. For leaching experiments, only anaerobically digested sludge samples were used. Wet anaerobically digested sludge sample was used only for leaching with commercial citric acid; while dewatered anaerobically digested sludge was used for leaching (to determine optimum conditions) with commercial citric acid, raw liquid from pineapple wastes, naturally fermented liquid and *A. niger* fermented liquid from pineapple wastes. The range of values shown in the table for dewatered anaerobically digested sludge was the result of analysis of sludge collected on February 3 and October 25, 2005. The sludge used for leaching at optimum conditions was analyzed separately and is discussed in section 4.5.1.

Table 4.1 General Characteristics of BMA Sewage Sludge

Parameter	Raw Sludge (Nongkhaem Nightsoil Treatment Plant)	Mixed Sewage Sludge (Nongkhaem Sludge Treatment Plant)		Previous Analysis (Nightsoil Sludge/Mixed))		
		Wet Sludge	Dewatered Sludge	(a)	(b)	(c)
pH (sludge:water ratio = 1:2)	6.77	6.99	6.33 - 6.5	6.72	7.80	7.40
Total Solids (%)	0.33	2.13	23.1 - 27.82	-	20.0	4.00
Moisture Content (%)	99.67	97.87	76.9 - 72.18	-	80.0	96.0
Total Volatile Solids (TVS)	41.25	38.16	38.30 - 44.7	-	-	39.6
Total Nitrogen (%)	3.97	2.94	1.53 - 3.6	5.72	6.1	2.74
Total Phosphorus (%)	5.20	1.95	2.26 – 4.63	-	-	3.23
Organic Matter (%)	41.25	38.16	38.30 - 44.7	-	-	39.6

(a) Amerasinghe (1997) – nightsoil sludge
(b) Chanya (1998) – nightsoil sludge
(c) Sae–Tang (2004)– mixed

4.1.1 Sludge pH

The pH of sludges is one of the most frequently measured parameters and gives an indication of the acidity or alkalinity of the sludge samples and the availability of the different nutrients. For example, sludges of around 7 have a higher availability of Mg, Ca, K, N and S, while Fe, Zn and Cu are less available at high pH (Radojevic and Bashkin, 1999). From the experiment, it was observed that the pH of both the raw sludge from Nongkhaem night soil treatment facility and the anaerobically digested sludge from the sludge treatment facility have almost the same values, but compared to that obtained by Amerasinghe (1997) and Sae-Tang (2004), the values were much lower. This must be due to the quality of sludges that come from a variety of sources that were treated at that time. The dewatered sludge, having a pH value of only 6.33 seem to indicate a slightly acidic character of the raw sludges treated in the facility. To increase pH of sludge before disposal, lime, kiln dust and other alkaline products may be added.

4.1.2 Total Solids (TS)

The solids content of sludge or biosolids affects the method of land application. Liquid or low – solids biosolids will generally be injected into the soil to prevent vectors and increase the moisture content of the soil. Dewatered or semisolid biosolids are usually spread on the surface and subsequently plowed to the soil. The concentration of solids adds organic matter to the soil which improves the physical properties of the soil, especially soil structure, soil moisture retention, soil moisture content and cation exchange capacity (Epstein, 2003). The results showed a variation in the total solids of the various sludge samples. The dewatered sludge studied had the highest total solids content since the sludge after dewatering has been exposed to open air for 5 days, thus also dried naturally.

4.1.3 Total Volatile Solids (TVS)

The total volatile solids can serve as a rough indicator of the amount of organic matter present in the sample. Organic matter is an important constituent of sludge the content of which varies depending on the solids content and extent of treatment. Use of sludge for land application enhances the organic content of soils which increase its water-holding capacity, aggregation, and cation exchange capacity (Epstein, 2003). Decomposition of organic matter releases nitrogen which is a very important plant nutrient (Radojevic and Bashkin, 1999). The results showed that the raw sludge from the nightsoil treatment plant in Nongkhaem seems to have the highest TVS indicating high organic matter content. The organic matter content of both the digested and dewatered sludge from the central treatment facility is comparable to that analyzed by Sae -Tang (2004). Most of the organic matter in the sludge might have come from domestic sources.

4.1.4 Plant Nutrients

Plant nutrients are among the most important chemical characteristics of sludge as discussed in section 2.3.1. Sludge for agricultural application is normally valued for the nitrogen and phosphorous content. The high nitrogen and phosphorous content of the sludges studied, especially the raw sludge from the nightsoil treatment facility, seemed to indicate the fertilizer value of the sludges.

4.1.5 Heavy Metals

Table 4.2 shows the results of the heavy metal analysis for the three sludge samples. As shown, variation on heavy metals content of the sludges analyzed seemed to be apparent which may be due to the sludge sources and sampling time. Moreover, almost all of the heavy metals seem to comply with the recommended BMA standards for agricultural application, except Cu which is way beyond the standard of 900 mg/Kg dry solids. However, the presence of heavy metals which can still pose a long-term environmental hazard due to bioaccumulation, and a more stringent regulation in the future, still call for further reduction or elimination of heavy metals in the sludge before land application. Iron (Fe) and manganese (Mn) are not included in the regulated heavy metals, but were analyzed to determine the effect of their concentration on citric acid production.

Table 4.2 Results of Heavy Metal Analysis of BMA Sludge

Heavy Metals (mg/Kg Dry Solids)	Raw Sludge (Nongkhaem Sewage Treatment Plant)	Mixed Sewage Sludge (Nongkhaem Sludge Treatment Plant)		Previous Analysis (Nightsoil Sludge/Mixed Sludge)			Proposed BMA Heavy Metal Standards for Agricultural Application (mg/Kg Dry Solids)
		Wet Sludge	Dewatered Sludge	(a)	(b)	(c)	
Cd	0.98	1.02	2.7 - 25.6	3.0	2.5	2.5	20
Cr	636.07	430.06	264 - 404.4	27	19	385	1000
Cu	5,708.30	5,727.12	1,500 – 4,610	395	328	4,673	900
Pb	89.96	165.30	100 – 318.2	21	1	139	1000
Fe	53,821.15	55,401.12	34,190 – 56,290	-	-	-	-
Mn	573.16	1,316.07	682.6 – 2,011	353	354	-	-
Ni	172.41	170.64	78.9 – 220	41	20	156	400
Zn	-	-	1,430 – 2,290	2,390	1,101	2,387	3000

(a) Amerasinghe (1997) – nightsoil sludge
(b) Chanya (1998) – nightsoil sludge
(c) Sae-Tang (2004) – mixed

4.2 Chemical Speciation Studies

4.2.1 Sequential Chemical Extraction I-SCE I (Dewatered and Wet Anaerobically Digested Sludge)

The sludge samples analyzed under the first SCE studies (SCE I) were collected on February 3, 2005 and were treated with commercial citric acid and unfermented raw liquid from pineapple wastes, to determine optimum conditions.

Results of the SCE I studies are summarized in Figures 4.1 and 4.2, while the actual amounts of metal forms extracted are presented in Appendix C-1.

As shown, there was a wide variation in the forms of metals present in both types of sludge, which could be due to the nature of the individual metal, the characteristics of the wastewater treated and the sludge treatment employed (Lake, 1987; Lester et al., 1983). For both the dewatered and wet digested sludge samples, Cr, Cu and Ni seem to predominate in residual fractions, while Pb and Zn were found mostly in the bound to organic and inorganic matter forms i.e., in the oxidizable phase. Cadmium was also found to predominate in the bound to organic and inorganic matter form for dewatered digested sludge, however, for wet sludge, Cd was not detected in any form. This is because the concentrations found in all fractions of the metal were lower than their respective detection limit. Overall, the results obtained are comparable to that from previous studies (Table 2.11). For Cd which was found to be mainly bound to organic and inorganic matter is comparable to that found by Marchioretto et al. (2002) using the Veeken Scheme and Sims and Kline schemes. The forms of Cr and Cu which are mainly residual is comparable to that found by Ali (1994) who studied the anaerobically digested sludge from Hwai Khwang treatment plant. Lead, which was found to be mainly in the bound to organic and inorganic matter form, is comparable to that found by Marchioretto et al. (2002) using the Veeken and Sims and Kline schemes. The form of Ni which was mainly residual for both types of sludge is comparable to that found by Ali (1994) and Ratanachoo (1995). Ratanachoo (1995) studied the sludge cake from Sri Praya treatment plant. Finally for Zn, which was found to be mainly bound to organic and inorganic matter for both types of sludges, is comparable to that found by Stover (1976), Oake et al. (1984), Ali (1994) and Marchioretto et al. (2002) using the Veeken scheme.

Although dewatered sludge seems to offer the advantages of relative homogeneity, stability and ease of handling, previous studies (Oake et al., 1984) have shown that forms of metals in sludge may be influenced by the drying process. Liquid (wet) sludges on the other hand are not stable materials, and chemical, biological and physical changes are continuously taking place. Such changes may have profound effects on the metals present. The results in this study suggest that the distribution of the forms of metals in sludge is almost qualitatively the same for both wet and dewatered sludges, but different quantitatively.

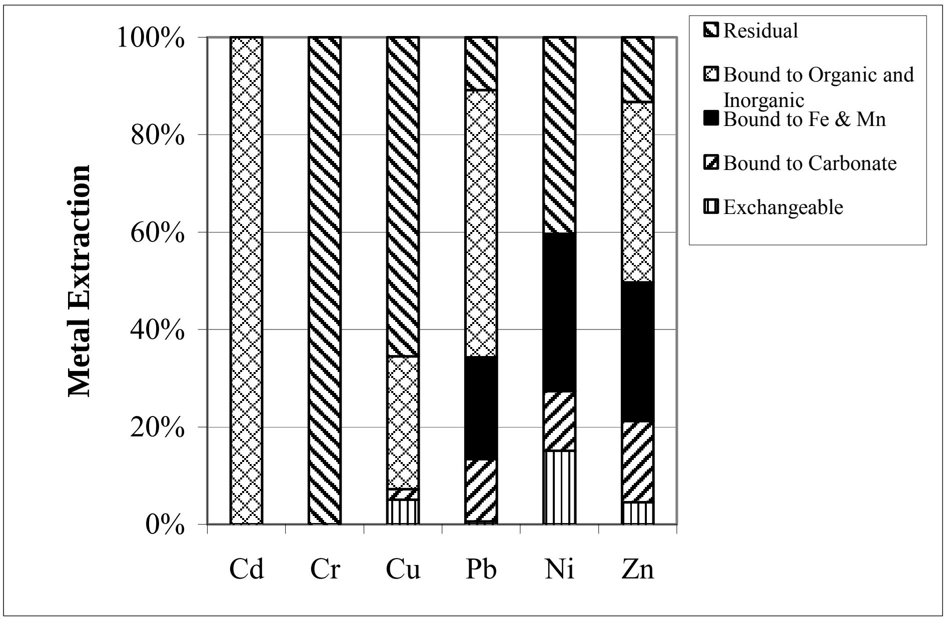


Figure 4.1 Metal Fractionation Profile for Dewatered Digested Sludge (SCE 1)

For dewatered anaerobically digested sludge, the forms of metals are summarized in the following rankings:

Cd: bound to organic and inorganic matter form (~ 100%)
Cr: the residual form (~100%)
Cu: Residual (65.5%) >> Bound to organic and inorganic matter (27%) > exchangeable (5%) > bound to carbonate (2%)
Pb: Bound to organic and inorganic matter (54.9%) >> bound to iron and manganese (20.9%) > bound to carbonate (12.8%) > residual (10.8%) > exchangeable (0.6%)
Ni : Residual (40.3%) >> bound to iron and manganese (32.4%) > exchangeable (15.1%)> bound to carbonate (12.2%)
Zn: Bound to organic and inorganic matter (37.1%) >> bound to iron and manganese (28.5%) > bound to carbonate (16.6%) > residual (13.3%) > exchangeable (4.6%)

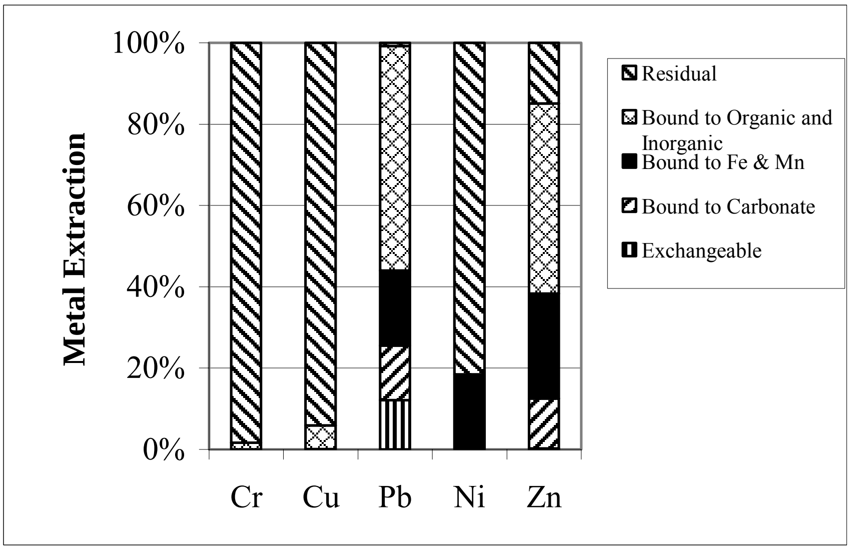


Figure 4.2 Metal Fractionation Profile for Wet Digested Sludge (SCE 1)

The forms of metals for wet digested sludge are summarized in the following rankings:

Cd: No form detected.
Cr: Residual form (~100%)
Cu: Residual (94.1%)>> Bound to organic and inorganic matter (5.9%) > exchangeable (0.05%)
Pb: Bound to organic and inorganic matter (55.2%) >> bound to iron and manganese (18.5%) > bound to carbonate (13.4%) > exchangeable (12.1%) > Residual (0.8%)
Ni: Residual (81.5%) >> bound to iron and manganese (18.5%)
Zn: Bound to organic and inorganic matter (46.8%) >> bound to iron and manganese (25.8%) > residual (14.9%) > bound to carbonate (12.3%) > exchangeable (0.2%)

Since for both the dewatered and wet digested sludge samples, the metals studied seem to predominate in bound to organic and inorganic matter and residual fractions, the mobility of these metals once disposed to land is less compared to other forms. In terms of metal removal from the sludge, metals in these forms tend to exhibit less leachability.

Metals in the exchangeable phase are easily exchangeable with the soil solution, and metals in the acid extractable phase (or carbonate bound) are very sensitive to pH changes and are readily leached when the pH of the environment is decreased, and hence both forms are comparatively mobile (Oake et al., 1984; Staelens et al., 2000). In terms of metal removal from the sludge, these forms exhibit ease of leachablity.

4.2.2 Sequential Chemical Extraction II-SCE II (Oven-Dried Sludge and Air-Dried Sludge)

The sludge sample analyzed under SCE II was collected on October 25, 2005, and was treated with naturally fermented and *A. niger* fermented raw liquid from pineapple wastes, to determine optimum conditions.

Results of the SCE II studies are summarized in Figures 4.3 and 4.4 , while the actual amounts of metals extracted are shown in Appendix C-2. As shown, there was also a wide variation in the forms of metals present in the sample sludge. Moreover, the forms of some metals in the sludge seem to vary significantly for oven-dried and air-dried sludge. For Cd metal, only bound to carbonate form (62%) predominates in the oven-dried sludge with a small amount of exchangeable form present, while in the air-dried sludge, exchangeable and bound to carbonate form equally predominate. Lead (Pb) metal seems to exhibit a significant change in metal forms as the sludge is oven-dried. High percentage of bound to organic and inorganic matter (59.3%) is found in oven-dried sludge as compared to air – dried sludge (39%), while less percentage of residual form (38%) is in oven-dried sludge as compared to air-dried sludge (53%). For Ni, in oven-dried sludge, all forms of metals are almost equally distributed, while in the air-dried sample, only residual, bound to carbonate and bound to iron and manganese forms are present, with the bound to iron and manganese being predominant. For Zn, the significant difference is evident in bound to organic and inorganic matter form which is relatively high in oven-dried sludge (44%) as compared to air-dried sludge (3%), although for other forms, the percentage seem to be almost similarly distributed. Chromium (Cr) and Cu metals on the other hand, do not seem to vary much in the forms of metals for oven and air-dried sludge samples. For Cr, in both forms of sludge, the residual form seem to be the only form found, although in oven-dried sludge, 5% of the total forms of metal is bound to organic and inorganic matter. For Cu, the predominant form which is residual, has almost the same percentage for both forms of metals.

As discussed earlier, although dry sludge seems to offer the advantages of relative homogeneity, stability and ease of handling, previous studies (Oake et al., 1984) have shown that forms of metals in sludge may be influenced by the drying process. The results in this study suggest that the distribution of the forms of metals in sludge is almost qualitatively the same for corresponding oven-dried and air-dried sludges, but different quantitatively. According to McLaren and Clucas (2001), drying of sludge clearly influences the distribution of metals between fractions as determined by sequential extraction, but the significance of this observation is difficult to predict. They speculated that the drying process may have affected the breakdown (oxidative) of organic components which could either (i) release associated (bound) metals from the solid phase organic matter and/or (ii) lead to greater complexing of metals by the increased concentrations of water-soluble organic components.

For oven-dried dewatered digested sludge, the forms of metals are summarized in the following rankings:

Cd: Bound to carbonate (62%) > bound to Fe and Mn = bound to organic and inorganic matter (16%) > exchangeable (6%).
Cr: residual (95%) > bound to organic and inorganic matter (5%)
Cu: Residual (81%) >> bound to organic and inorganic matter (14%) > bound to carbonate (3%) > exchangeable (2%)
Pb: Bound to organic and inorganic matter (59.3%) > residual (37.7%) > bound to iron and manganese= bound to carbonate (1.4%)
Ni: Residual (29.7%) > bound to carbonate (21.5%) > bound to organic and inorganic matter (20.1%) > bound to iron and manganese (18.7%) > exchangeable (10%)
Zn: Bound to organic and inorganic matter (43.4%) >> bound to carbonate = bound to iron and manganese (19.7%) > residual (13.9%) > exchangeable (3.3%)

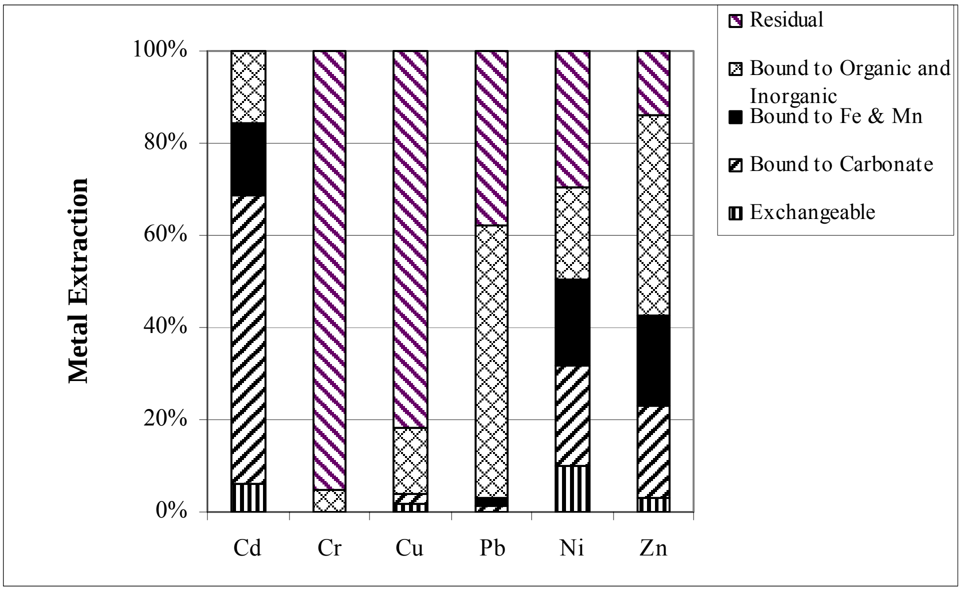


Figure 4.3 Metal Fractionation Profile for Oven-Dried Dewatered Digested Sludge (SCE II)

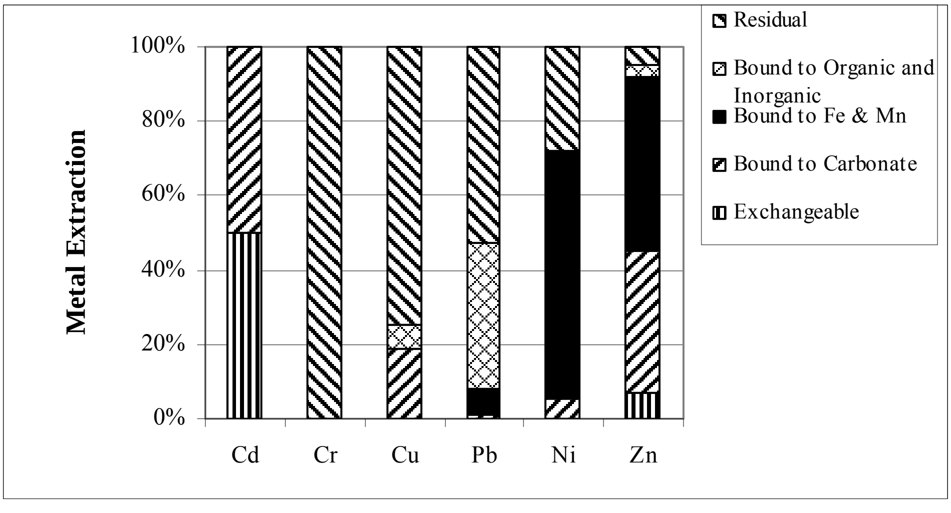


Figure 4.4 Metal Fractionation Profile for Air-Dried Dewatered Digested Sludge (SCE II)

The forms of metals for air – dried dewatered sludge are summarized in the following rankings:

Cd: Bound to carbonate = exchangeable (50%)
Cr: Residual form (~100%)
Cu: Residual (74.5%) >> bound carbonate (18.8%) > bound to organic and inorganic matter (6.5%) > exchangeable (0.15%)
Pb: Residual (52.5%) > bound to organic and inorganic matter (39.3%) >> bound to iron and manganese (7.2%) > bound to carbonate (1%)
Ni: Bound to iron and manganese (66.7%) > residual (28.1%) > bound to carbonate (5.1%)
Zn: Bound to iron and manganese (46.5%) > bound to carbonate (38.3%) > exchangeable (6.9%) > residual (4.9%) > bound to organic and inorganic matter (3.4%).

Moreover, it was observed that the sum of five metal fractions obtained from sequential extraction procedure for some metals, was higher than the total metal contents in the sludge following acid digestion. This may be due to the slow but increasingly stronger attack by the various reagents used in the sequential extraction procedure which removed specific forms of metals in the process. Whereas after acid digestion, not all forms of metals may have been removed. Similar observation was recorded by Paré et al. (1999) and Staelens et al. (2000).

4.3 Metal Extraction Studies

4.3.1 Leaching with Commercial Citric Acid

For leaching with commercial citric acid, two forms of sewage sludge samples were used namely: dewatered and wet anaerobically digested sludge.

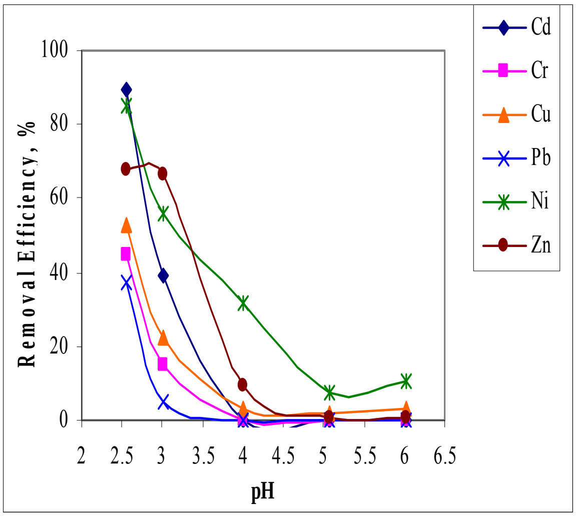
4.3.1.1 Determination of Optimum pH

Table 4.3 summarizes the citric acid dosages used in the leaching process with the corresponding pH. As shown, since the average initial pH of the dewatered digested sludge sample was about 6.09, only the pH conditions shown on the table were used for leaching in this type of sludge. For wet digested sludge, only the pH conditions shown in the table were selected since the average initial pH of the sludge sample was about 8.7. Results of leaching study at 2 hours leaching time are shown in Figure 4.5 and discussed as follows:

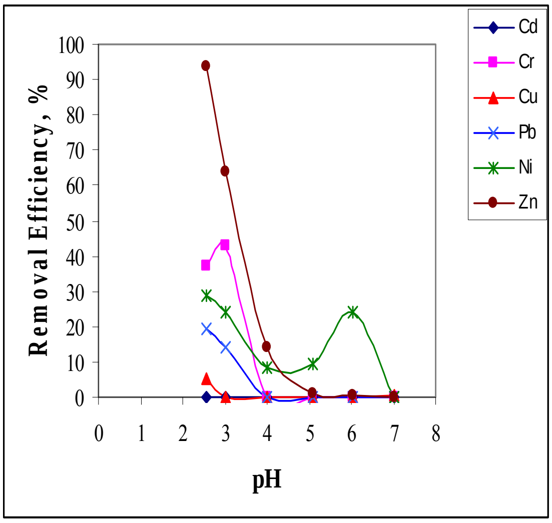
For Cd, the highest removal obtained for dewatered digested sludge was 89.2% at pH of about 2.33, followed by 39.4% at pH of about 3. Removal for other pH conditions were undetectable. For wet digested sludge, no removal was detected for all pH conditions. The highest removal attained for Cr was about 45 % for dewatered digested sludge at a pH of about 2.38, followed by only 14.9% at a pH of about 3. No removals were detected for other pH conditions. For wet digested sludge, the highest removal of 43.02% was attained at pH 3, followed by 37.43% at pH 2.38. No removal was detected for other pH conditions.

Table 4.3 Citric Acid Dosages Used in the Experiment

pH	Citric Acid (0.1 M) Dosage (mL/g)	Equivalent wt of Citric Acid (g citric acid/g sludge)
Dewatered Digested Sludge		
6.09 (initial)	0	0
2.33	100	2.1
3.04	7.9	1.4×10^{-1}
3.9	1.4	3.0×10^{-2}
5.01	0.2	4.2×10^{-3}
6.0	0.03	5.3×10^{-4}
Wet Digested Sludge		
8.67 (initial)	0	0
2.55	125	2.63
3.02	30	6.3×10^{-1}
4.0	5	1.1×10^{-1}
5.08	1.3	2.3×10^{-2}
6.03	0.65	1.4×10^{-2}
7.02	0.1	2.1×10^{-3}



(a) Dewatered sludge



(b) Wet Sludge

Figure 4.5 Metal Removal Efficiencies for Cd, Cr, Cu, Pb, Ni and Zn during Leaching with Commercial Citric Acid at various pH Conditions and 2 hours Leaching Time

For Cu, the highest removal attained was 52.6% at pH 2.33, followed by 22.4% at pH 3. At pH of about 4, the removal was only about 3%, and at pH 5, 1.94% removal was attained. A slight increase in metal removal (2.96%) was obtained at pH 6. For wet digested sludge, only a small percentage of removal (5.14%) was attained at pH of 2.38. No removal was detected at pH 3, 4 and 5. However, at pH 6, 0.16% removal was detected, and at pH 7, a slightly higher removal of 0.42% was detected.

The highest removal attained for Pb was 37.43% at pH 2.33, followed by only 5.01% at pH 3. No removal was detected for other pH conditions. The same is true with wet digested sludge where metal removal was detected only at pH 2.38 (19.3%) and pH 3 (13.91%).

For Ni, metal removal was detected at all pH conditions for dewatered digested sludge sample. The highest removal of 85.38% was attained at pH 2.33 , followed by 56.15% at pH 3. At pH 4, a removal of 31.58 % was attained while 7.67% was attained at pH 6. A slightly higher increase in metal removal was attained at pH 7. For wet digested sludge, metal removal was also detected at almost all pH conditions, with 28.87 % removal obtained at pH 2.38, followed by 24.03% at pH 3. A decrease in metal removal to 8.2% was observed at pH 4, while a slight increase was observed at pH 5 with 9.58% removal. The removal was observed to increase further at pH 6 with removal of 24.2%. However, at pH 7, no removal was detected .

For dewatered digested sludge, Zn metal removal was detected at all pH conditions with the highest removal of 67.91 % attained at pH 2.38 followed by a slight decrease to 66.23% at pH 3. The removal was decreased further down to 9.57% at pH 4, and further down to 0.84% and 0.57% at pH 5 and 6 respectively. The results observed using wet digested sludge seemed to be promising with as much as 93.52% removal attained at pH 2.38. At pH 3, the result (63.87% removal) is almost the same as in the dewatered digested sludge. Metal removals for other pH conditions are 14.03%, 1.13%, 0.69% and 0.25% at pH 4, 5, 6, and 7 respectively.

Results of commercial citric acid leaching study in terms of the actual amount of metals removed are shown in Appendix D-1. The overall results revealed that there was a wide variation of removal efficiencies for all metals at various pH conditions. It was observed though that high metal removal efficiencies (as much as 93.5% removal for Zn on wet sludge) were attained at pH of about 2 to 3 for all metals for both types of sludge. At pH 4, a much lower efficiency was attained for Ni and Zn, but still higher than that obtained at pH 5, 6, and 7 although for Ni, a higher efficiency was attained at pH 6 for wet sludge. Compared to that obtained by Veeken and Hamelers (1999) and Marchioretto et al (2002) using citric acid at pH 3-4, the results obtained in this study is comparatively less for most metals. For Cd, only a maximum of 39.4% was attained at pH 3 compared to 85% in the previous studies. For Cr, a much lower result was obtained attaining only 15% for dewatered sludge and 43% for wet sludge as compared to 85% in the previous studies. The results obtained for Cu is comparable to the lower range of 25 % from previous studies attaining about 22% in this study. Lead removal was the lowest, with only 5% and about 14% for dewatered and wet sludge respectively, compared to 60% from previous studies. The results obtained in Ni removal was comparable to the previous studies, attaining about 56% for dewatered sludge and 24% for wet sludge respectively, compared to 60% from previous studies. For Zn, about 66% and 64% removal were obtained for both dewatered and wet sludges respectively as compared to 80-90% from previous studies. A summary of the comparison of metal removal efficiencies at pH 3-4 is shown in Table 4.4.

Table 4.4 Comparison of Heavy Metal Removal Efficiencies at pH 3-4

Metals	Veeken and Hamelers (1999)		Marchioretto, et al (2002)		This Study			
	Initial Metal Concentration (mg/Kg DM)	Metal Removal (%)	Initial Metal Concentration (mg/Kg DM)	Metal Removal (%)	Initial Metal Concentration (mg/Kg DM)		Metal Removal (%)	
					[1]Dew S	[2]Wet S	Dew S	Wet S
Cd	1.99	-	2.6	85	2.69	1.02	39.4	-
Cr	21.4	-	300 –500	85	370	430	14.9	43
Cu	472	60 –70	700-900	25	4,129	5,726	3.04-22.4	-
Pb	38	-	150	60	106	165	5.01	14
Ni	17	-	20-40	60	126	171	32-56	8.2-24
Zn	425	90-100	1,500-2000	85	1,933	1,907	9.6-66.2	14-64

[1]Dew S – Dewatered anaerobically digested sludge
[2]Wet S – wet anaerobically digested sludge

The variation in metal removal efficiencies could be attributed to several factors such as the nature of the sludge sample, pretreatment of sludge, extraction conditions, the forms of metals present, and the concentration of metals in the sludge (Lo and Chen, 1990; Wong and Henry, 1988)

From the results of the study, pH conditions of about 2, 3 and 4 were selected for further experimentation on the effect of leaching time.

4.3.1.2 Determination of Optimum Leaching Time

Table 4.5 summarizes the citric acid dosages used in the leaching process with the corresponding pH selected.

Table 4.5 Citric Acid Dosages Used in the Experiment (Determination of Optimum Leaching Time)

pH	Citric Acid (0.1 M) Dosage (mL/g)	Equivalent wt of Citric Acid (g citric acid/g sludge)
Dewatered Digested Sludge		
6.09 (initial)	0	0
2.33	100	2.1
3.04	7.86	0.17
3.9	1.43	0.03
Wet Digested Sludge		
8.67 (initial)	0	0
2.55	125	2.63
3.02	30	0.63
4.0	5	0.11

Using these pH conditions corresponding to the citric acid dosages, acid leaching was done at leaching times of 0.042 day (1h), 0.083 day (2h), 0.25 day (6h), 1 day, 5 days, 8 days and 11 days. The optimum leaching time was determined based on the contact time between the sludge and the citric acid (extractant) which gives the best removal efficiency.

Metal removals achieved at different leaching times are shown in Figures 4.6 and 4.7 for dewatered and wet sludges respectively, and described as follows:

Dewatered Anaerobically Digested Sludge

As shown in Figure 4.6 (a), for Cd, all the metal seemed to be removed after leaching for 1 hour (0.042 day) at pH 2, while at pH 3, 100% removal was attained after 2 hours (0.083 day), and at pH of 4, 100% removal was attained after 5 days of leaching.

For Cr metal, about 100 % removal was attained after 5 days of leaching at pH 2. At pH 3, the maximum removal attained was 45% after 1 day of leaching. At pH 4, no removal was detected at any leaching time, as shown in Figure 4.6 (b).

For Cu, the highest removal attained was 88% at pH 2 after 1 day of leaching. At pH 3, only 29% maximum removal was attained after 1 day leaching time. Not much removal was attained at pH 4 with only a maximum of 8% after 1 h of leaching, as shown in Figure 4.6 (c).

The highest removal attained for Pb metal at pH 2 was 95% after 11 days of leaching. At pH 3, 100% removal was attained after 1 day of leaching. No removal was detected at pH 4 as shown in Figure 4.6 (d).

For Ni, metal removal was detected at all pH conditions. The highest removal of 98% was attained at pH 2 after 5 days of leaching. At pH 3, the maximum metal removal of 70% was attained after 2 h of leaching. At pH 4, a maximum removal of 47% was attained after only 1 hour of leaching, as shown in Figure 4.6 (e).

All Zn was removed from the sludge sample only after 2 hours of leaching at pH 2. At pH 3, a maximum removal of 80% was attained also after 2 hours of leaching. Metal removal at pH 4 was less with only a maximum of 10% at leaching time of 1 hour, as shown in Figure 4.6 (f).

Wet Anaerobically Digested Sludge

For Cd, 100 % metal removal was attained at all pH conditions only after 1 h leaching time, although at leaching time of 5 days at pH 3, no removal was detected and at 11 days leaching time, removal was reduced to 72%. Similarly, at pH 4, no removal was detected at 5 days of leaching, as shown in Figure 4.7 (a).

The highest removal attained for Cr metal at pH 2 was about 100% after 5 days of leaching . At pH 3, the highest removal of 88% was also attained after 5 days of leaching time. Metal removal at pH 4 was very low attaining only a maximum of 2% after 2 hours leaching time, as shown in Figure 4.7 (b).

For Cu, the highest removal attained at pH 2 was only 63% after 5 days of leaching. At pH 3, very low removal was attained with only a maximum of 9% after 1 day of leaching. Metal removal at pH 4 was almost undetectable with only 1% attained after 11 days of leaching as shown in Figure 4.7 (c).

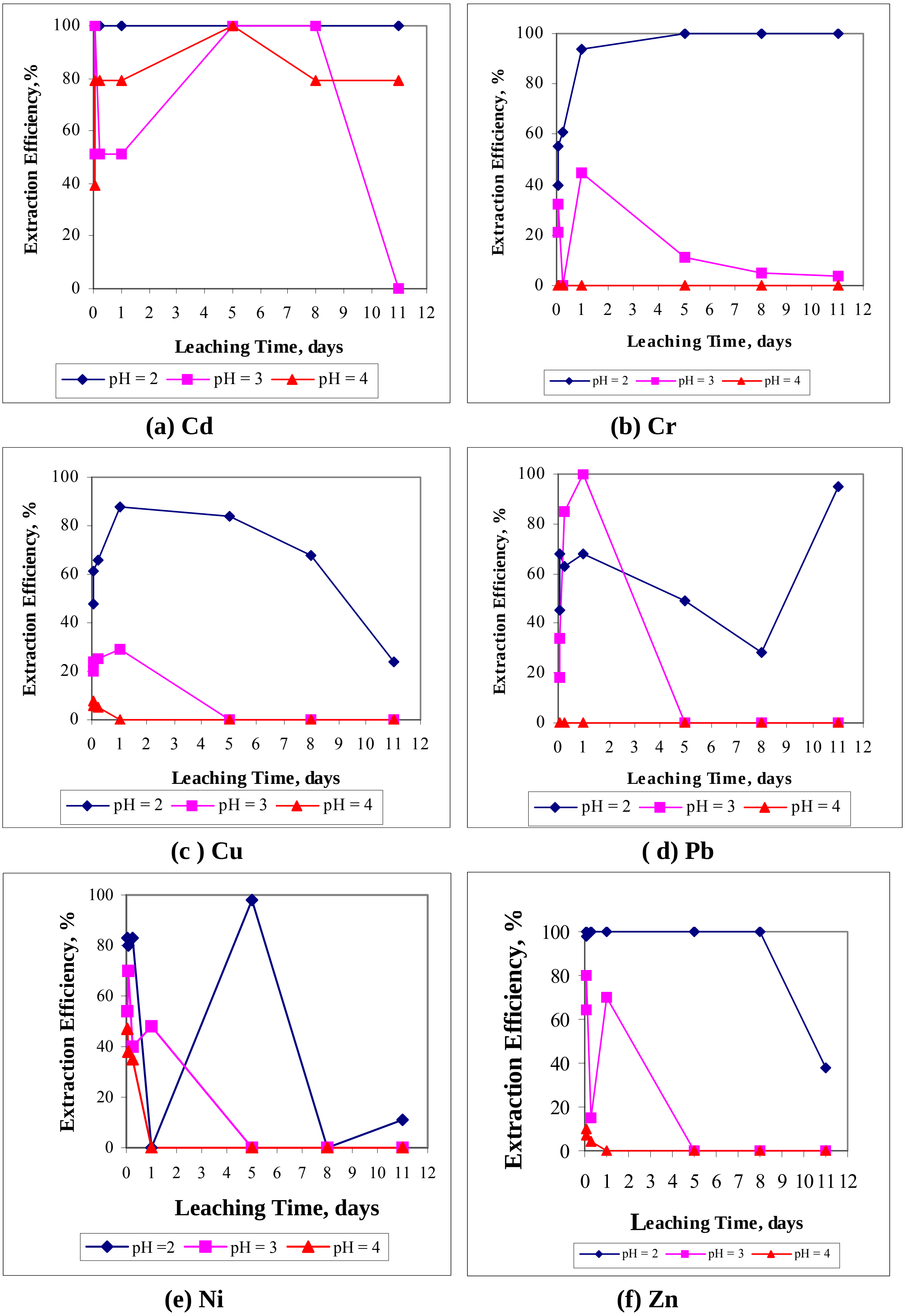
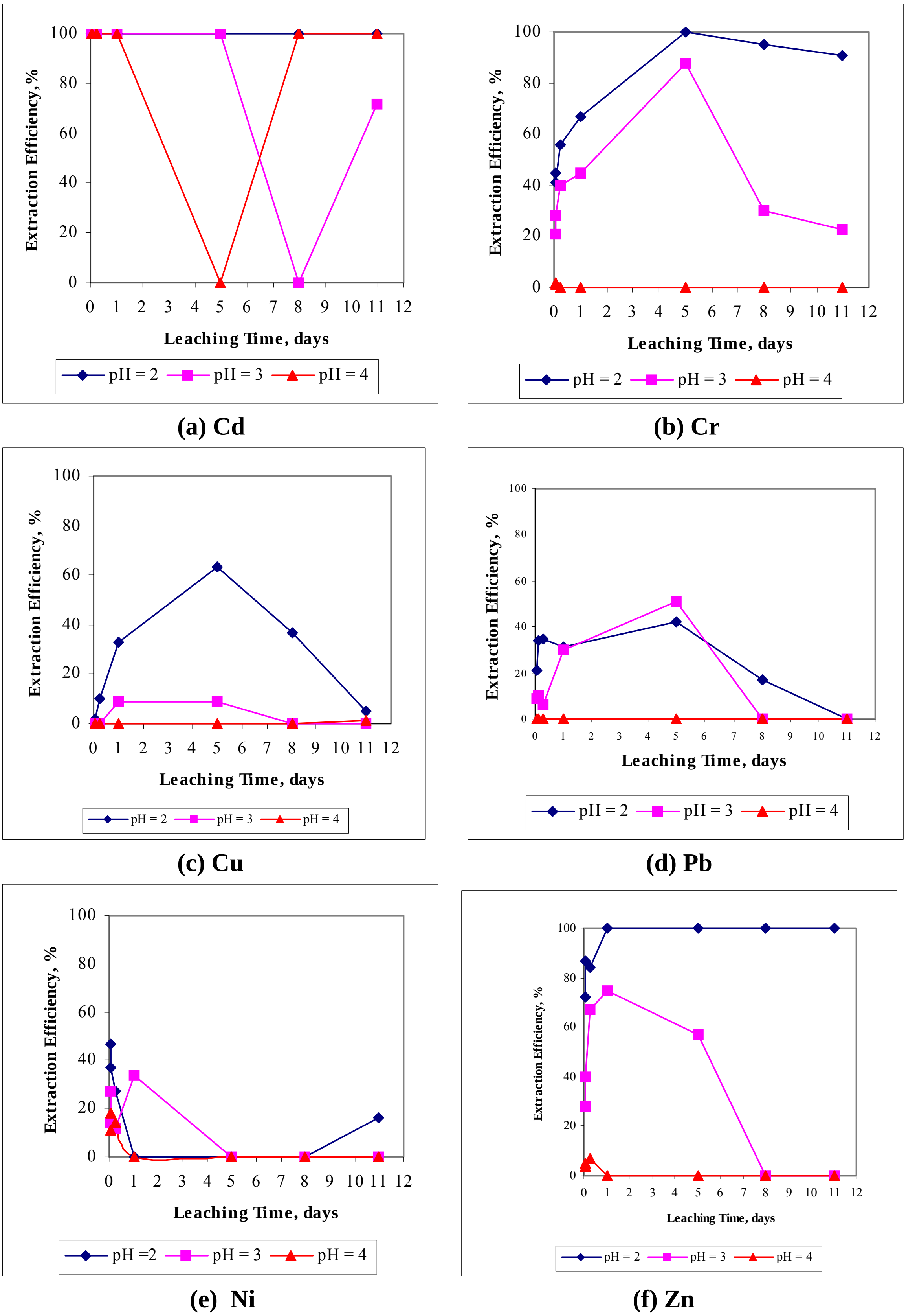


Figure 4.6 Metal Removal Efficiencies for Cd, Cr, Cu, Pb, Ni and Zn at pH 2, 3 and 4 and Various Leaching times (Dewatered Sludge)



(a) Cd
(b) Cr
(c) Cu
(d) Pb
(e) Ni
(f) Zn

Figure 4.7 Metal Removal Efficiencies for Cd, Cr, Cu, Pb, Ni and Zn at pH 2, 3 and 4 and Various Leaching times (Wet Sludge)

The highest removal attained for Pb metal at pH 2 was 42% after 5 days of leaching. At pH 3, 52% metal removal was attained after 5 days of leaching. No removal was attained at pH 4 at any leaching time as shown in Figure 4.7 (d).

For Ni, at pH 2, the highest metal removal attained was 47% after only 1 hour of leaching. At pH 3, the highest removal was 34% at 1 day leaching time. A metal removal of 18% was the highest attained at pH 4 after only 1 hour of leaching, as shown in Figure 4.7 (e).

Zinc removal at pH 2 from wet digested sludge was 100% at 1 day leaching time. At pH 3, the highest removal was attained after 1 day of leaching was 75%. Removal at pH 4 was very low with only as much as 7% after 6 hours of leaching, as shown in Figure 4.7 (f).

The overall results in terms of the actual amount of metals removed are presented in Appendix D-2. As shown, there was a wide variation of removal efficiencies for all metals at various leaching times.

For Cd, it was observed that for both the dewatered and wet sludges, metal removal was unusually high reaching more than 100% especially for wet sludge. The results obtained were much higher compared to the previous results obtained during the determination of optimum pH of the same samples. This might be due to the difference in preservation technique used where HNO_3 was used to preserve the samples for this experiment. Another reason might be the change in metal forms and concentration of the sludge as the sludge aged. According to Forstner (1993) and Rapin et al. (1986), there is possibility of metal phase changes resulting from sludge storage and pretreatment. The same reasons could be attributed to the unusually high removals for other metals. For dewatered sludge, optimum Cd metal removal seemed to be at 1 h leaching time at pH 2 (100%), 2 hours leaching time at pH 3 (100%), and 5 days leaching time at pH 4 (100%). For wet sludge, the optimum metal removals seemed to be attained at 1 h leaching time for all pH conditions.

For Cr, optimum metal removal seemed to be attained at 5 days leaching time at pH 2 (100%), and 1 day at pH 3 (45%) for dewatered sludge. For wet sludge, the optimum leaching time seemed to be 5 days at both pH 2 (100%) and 3 (88%).

Copper removal for dewatered sludge seemed to be optimum at 1 day leaching time for both pH 2 (88%) and 3 (29%), although removal at pH 3 is just about one third that at pH 2. Removal for pH 4 was almost negligible at 1 h leaching time. For wet sludge, removal seemed to be optimum at 5 days leaching time at pH 2 (63%) and 1 day leaching time at pH 3, although removal at this leaching time was very much less at only 9%.

For Pb, removal seemed to be optimum at 11 days leaching time (at 95%) at pH 2, although at 2 hours leaching time, a relatively higher removal of 68% was also attained, while at pH 3, removal seemed to be optimum at 1 day leaching time, for dewatered sludge. For wet sludge, optimum leaching time seemed to be at 5 days for both pH 2 and 3. No removal was attained at pH 4.

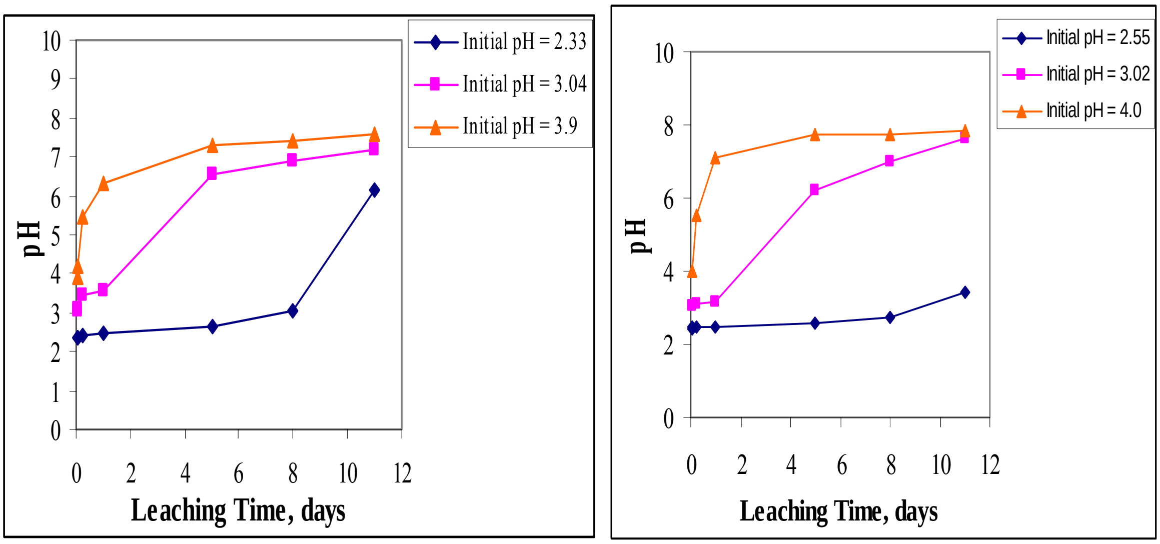
For dewatered sludge, Ni removal seemed to be optimum at 5 days leaching time (at 98%), although at 1 hour leaching time, a relatively higher removal of 83% was also attained. At pH 3, the optimum leaching time seemed to be at 2 hours, while at pH 4, it seemed to be only at 1 hour. For wet sludge, a relatively lower metal removal efficiency was attained with the optimum leaching time being at 1 hour at both pH 2 and 4, and 1 day at pH 3.

For Zn, optimum removal seemed to be attained at 2 hours leaching time at pH 2 (100%) and 3 (80%), while at pH 4 almost no removal was attained even at 1 hour leaching time. For wet sludge, optimum metal removal seemed to be attained at 1 day leaching time at both pH 2 (100%) and 3 (75%), while at pH 4, removal was almost negligible.

Since the extraction pH seemed to be proportional to the citric acid concentration, the results also confirmed the direct relationship between acid concentration and extraction rate. This is attributed to the fact that since the extraction efficiency at equilibrium solely depends on competition between citric acid and the solid phase for metal ions, the rate of extraction, also depends on the spatial distribution of the metals (Wong and Henry, 1988; Lo and Chen, 1990; Veeken and Hamelers, 1999). Veeken and Hamelers (1999) cited the fact that since heavy metals are predominantly incorporated in sludge flocs, the extraction takes time because the heavy metals have to diffuse from the sludge matrix to the bulk solution and the rate of extraction depends on the accessibility of the metals for the extracting agent. Protons which are small ions can penetrate the sludge flocs to exchange with metal ions while the larger sized citrate anion is not able to enter the sludge flocs and in that case metal ions can only reach the bulk solution by diffusion. This process takes a considerable amount of time since the driving force is the very small concentration gradient which is established between the metal concentration in the aqueous phase of the sludge flocs and the bulk solution. This explains also why some of the heavy metals required more contact time while using citric acid for solubilization.

The forms of the metals present in the sludge sample may have also affected the extraction efficiency of citric acid as observed in the acid leaching study. Cr and Ni which are predominantly in residual form tend to be solubilized most at longer acid contact times at pH 2, although for Cu which is also predominantly residual requires a shorter time of 1 day to solubilize. This might be due to the presence of acid extractable and exchangeable forms of Cu. At a higher pH of 3, solubility is less. Metals in residual form tend to be bound strongly to the sludge matrix. Lead and Zn which were found mostly in the bound to organic and inorganic matter forms i.e., in the oxidizable phase, also exhibit almost similar properties although for Zn the presence of other forms especially acid extractable and exchangeable forms may have increased its solubility at a shorter contact time. About 60% of sludge are organic fraction containing mainly humic acid. According to Wild (1993) humic acid reacts with metal cations to form complexes and the stability of these complexes varies with pH.

Variation of metal removal efficiencies can also be attributed to the change of pH of the sample as the leaching time is increased, as shown in Figure 4.8.



(a) Dewatered Sludge **(b) Wet Sludge**

Figure 4.8 Change of pH over Time for Leaching with Commercial Citric Acid

As shown, for dewatered digested sludge samples, drastic increase in pH was observed starting from 8 days leaching time at pH 2, while at pH 3, at 5 days leaching time. At pH 4, a rapid increase in pH took place over the entire leaching period of 11 days. For wet digested sludge samples, there seemed to be no drastic increase in pH over time at pH 2, but at pH 3 a rapid increase was observed starting from 5 days leaching time. At pH 4, a rapid increase in pH was also observed throughout the leaching time of 11 days. The increase in pH may have been caused by the consumption of acid in the hydrolysis of polysaccharides making up the cellular material of the biomass within the sludge (Wozniak and Huang, 1982), or in other possible reactions. In general, it was observed that the solubilization of metals decreased as pH is increased.

The variation in metal removal efficiencies could also be attributed to pretreatment of sludge and extraction conditions (Lo and Chen, 1990; Wong and Henry, 1988).

4.3.2 Characteristics of Raw and Fermented Liquid from Pineapple Wastes

The amount of raw liquid extracted per kilogram of pineapple waste was 530 grams. The physical characteristics and metal content of the unfermented and fermented raw liquid are summarized in Table 4.6. As shown, some heavy metals are contained in the raw liquid which may come from the peeling operations or cutting and grinding of pineapple peels. According to Yigitoglu (1992), the levels of Mn, Fe, Cu and Zn are particularly critical in citric acid fermentation. If the levels of these trace elements are carefully controlled, high citric acid production can be attained, and other factors have less pronounced effects as discussed in section 2.6.2.

Table 4.6 Characteristics of Raw and Fermented Liquid from Pineapple Wastes

Parameters	Raw Liquid	Naturally Fermented	*A. niger* Fermented
Density, Kg/L	1.05 - 1.25	1.05 - 1.25	1.05 - 1.25
pH	3.9 - 4.09	3.4 - 3.64	3.67 – 3.70
Total Sugar (%):	5.17 - 12.42	4.43	2.43 – 5.59
Fructose	0.7 - 2.2	2.0	2.49
Glucose	2.79	2.43	2.43 – 3.10
Sucrose	4.47 - 7.43	ND	ND
Maltose	ND	ND	ND
Lactose	ND	ND	ND
Acidity as Citric Acid (g/100g)	0.5 – 0.97	0.62	4.54
Citric Acid Content (g/L)	NA	NA	5.34 - 6.36
Heavy Metals (mg/L):			
Cd	0.01	0.01	ND
Cr	2.5	ND	ND
Cu	0.06	ND	ND
Pb	0.3	0.3	ND
Ni	0.18	0.06	0.2
Zn	2.3 - 3.3	2.3 - 2.9	1.9
Fe	3.2	0.85	1.65
Mn	14.7	29.7	12.3

ND= Not detected;
NA = Not Analyzed

As observed, only three types of sugar (fructose, glucose and sucrose) seemed to be present in the raw liquid which is favorable in the production of citric acid. According to Yigitoglu (1992), the usual carbon sources for fermentation by the fungus *A. niger* to produce high yield of citric acid are glucose, fructose, or sucrose. Yigitoglu (1992) also quoted that not only the type but also the concentration of the carbon source is important in the citric acid fermentation, with maximal citric acid production rate achieved at 14 to 22% of sugar in the medium. The absence of sucrose in both the naturally fermented raw liquid and the *A. niger* fermented raw liquid indicated that sucrose fermentation has taken place, although the process may have not been completed as indicated by the increased production of fructose and glucose in the *A. niger* fermented liquid. According to Batista, et al. (2004), it is generally accepted that sucrose fermentation proceeds through extracellular hydrolysis of the sugar producing glucose and fructose. The total acidity of a solution is a measure of all the hydrogen ions of both the fixed and volatile acids present. The acidity then indicates the presence of citric acid and all other acids such as malic and ascorbic acids (Chan et al., 1973) in the raw liquid. An increase in total acidity was observed after fermentation of raw liquid indicating the increase in the amount of citric acid and other acids present. Results of HPLC measurements of duplicate samples of *A. niger* fermented liquid revealed a citric acid content of 5.34 g/L- 6.36 g/L. The result seemed to be consistent with the findings of Sun (1984) who was able to obtain from 5.0 – 13.6 g/L of citric acid from *A. niger* fermented liquid from pineapple wastes, depending on the sugar content of the raw liquid. However, since the sugar content in the raw liquid was found to vary according to seasons as pointed out in section 2.7, citric acid content of the liquid would be expected to be low during rainy season due to low sugar content of the liquid.

Infrared (IR) spectroscopy studies were also done in the raw liquid, naturally fermented liquid and *A. niger* fermented liquid to determine the functional groups present in the samples. The results of the analysis are shown in Figures 4.9, 4.10 and 4.11, respectively. As shown, the presence of the carboxy groups (COOH) indicates the presence of citric acid, and other carboxylic acids. Carboxylic acid dimers display very broad, intense O-H stretching absorption in the region of 3300-2500 cm^{-1} (Silverstein and Webster, 1998).

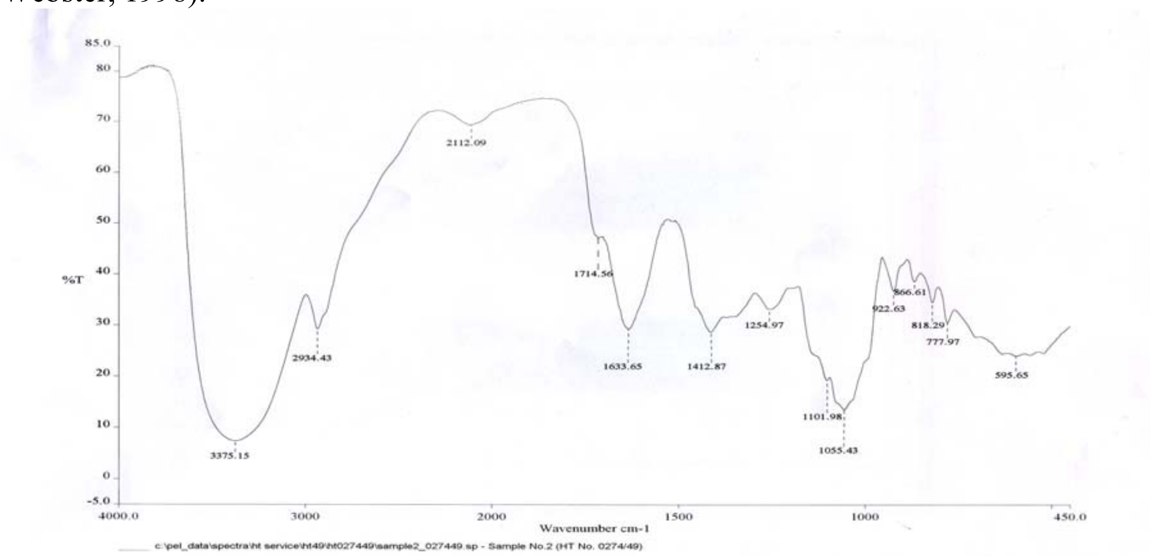


Figure 4.9 IR Spectrogram of Raw Liquid from Pineapple Wastes

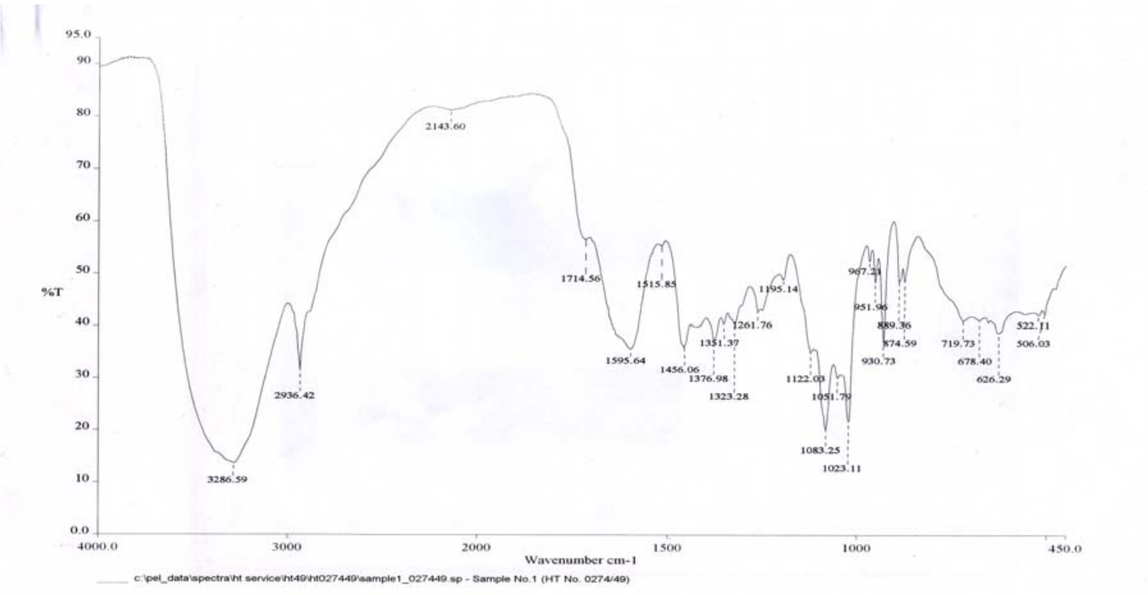


Figure 4.10 IR Spectrogram of Naturally Fermented Raw Liquid from Pineapple Wastes

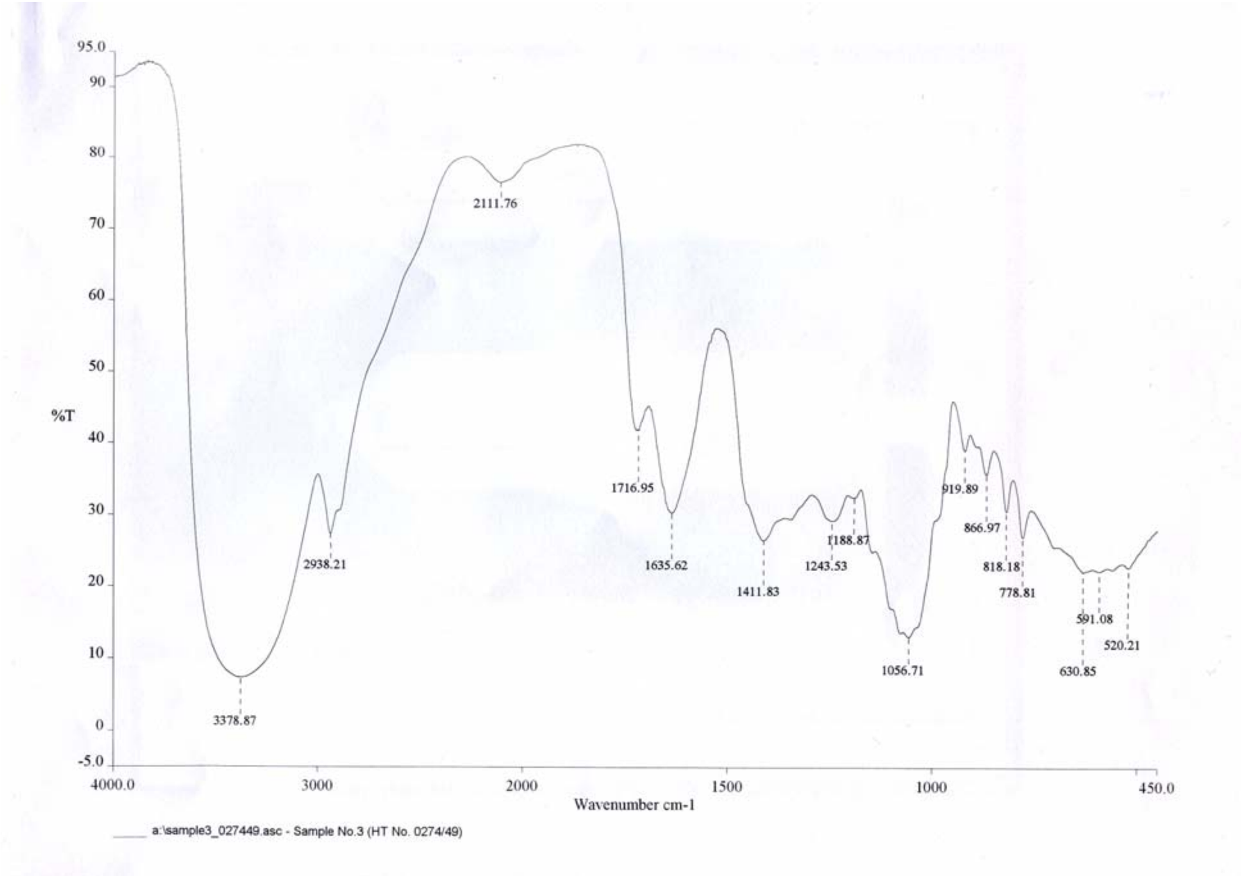


Figure 4.11 IR Spectrogram of *A. niger* Fermented Raw Liquid from Pineapple Wastes

4.3.3 Leaching with Unfermented Raw Liquid from Pineapple Waste: Determination of Optimum pH

For this leaching experiment, unfermented raw liquid from pineapple wastes (pH=3.9) was used to extract heavy metals from air-dried dewatered sludge samples at 2 hours leaching time. Table 4.7 summarizes the dosages used in the leaching experiment with the corresponding pH.

Table 4.7 Unfermented Raw Liquid Dosages Used in the Experiment

pH	Raw Liquid Dosage (mL/g)	Equivalent wt of Raw Liquid (g raw liquid/g sludge)
6.09 (initial pH of sludge)	0	0
3.94	125	127.5
4.00	12.5	12.8
5.07	2.5	2.6
6.02	0.5	0.51

Since the initial pH of the raw liquid was only 3.9, to reduce the pH of sludge to 2 would require a considerable amount of raw liquid. As observed, the sludge pH was reduced to 3.94 after addition of 125 mL of raw liquid. Further addition of large amount of raw liquid resulted to a very slight reduction in sludge pH. Hence, the pH of 3.94 was selected as the minimum for the leaching experiment. Moreover, since the initial pH of the dewatered digested sludge sample was about 6.09, the pH condition used for leaching was only until 6.0. Using these pH conditions corresponding to the raw liquid dosages, the following metal extraction removals for dewatered sludge samples were obtained. Figure 4.12 shows the metal removal efficiencies of all metals analyzed.

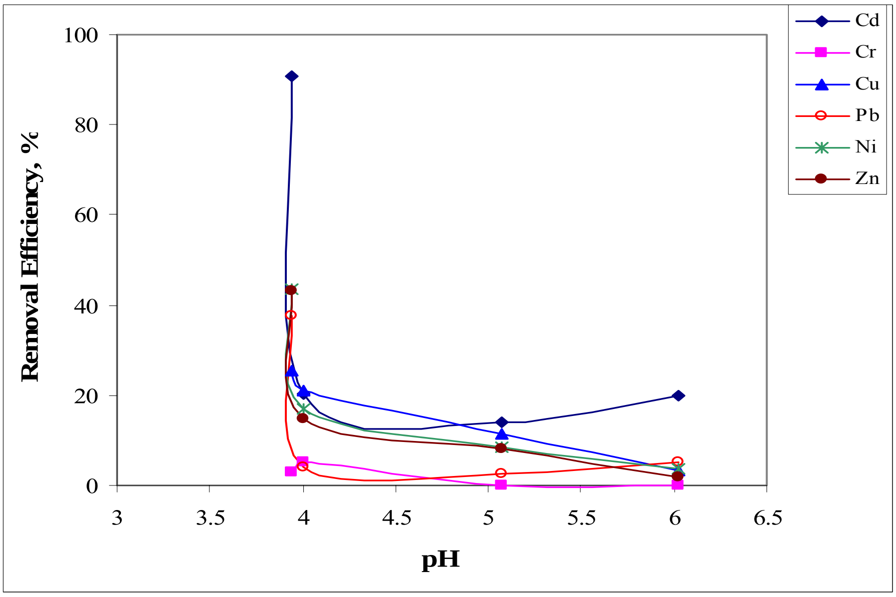


Figure 4.12 Metal Removal Efficiency for Cd, Cr, Cu, Pb, Ni and Zn at Two Hours Leaching Time and Various pH Conditions during Leaching with Unfermented Raw Liquid

For Cd, the highest removal obtained was 90.63 % at pH of about 3.94. Removals for other pH conditions were very low at 20.3%, 14.1 % and 19.8% for pH 4, 5.07 and 6.02, respectively.

The highest removal attained for Cr metal was only 5.3 % at a pH of about 4.0 followed by only 2.87% at pH of 3.94. No removals were detected for other pH conditions.

For Cu, the highest removal attained was 25.5 % at pH 3.94, followed by 20.9% at pH 4. At pH of about 5, the removal was only about 11.6%, and at pH 6.02, 3.3 % removal was attained.

The highest removal attained for Pb metal was 37.7% at pH 3.94, followed by only 3.9 % at pH 4. At pH 6.02, the removal was surprisingly higher at 5.1 % compared to 2.7% attained at pH 5.07.

For Ni, metal removal was detected at all pH conditions. The highest removal of only 43.5% was attained at pH 3.94 followed by 17.1 % at pH 4. At pH 5.07, a removal of 8.6% was attained while 3.8% removal was attained at pH 6.02.

For Zn, metal removal was detected at all pH conditions with the highest removal of 43.3 % attained at pH 3.94 followed by only 14.7% at pH 4. The removal was decreased further down to 8% at pH 5.07, and further down to 1.68% at pH 6.02.

The actual amount of metals removed from dewatered sludge after leaching with the unfermented raw liquid is presented in Appendix E. For the calculation of metal removals, the initial metal concentration used was that obtained from the analysis of the dewatered sludge after 6 months of storage. As observed, the highest removal of 90.6 % was attained at pH 3.94 for Cd, followed by Ni at 43.5%, then by Zn at 43.3%, Pb at 37.7% and Cu at 25.5% at the same pH condition of 3.94. Removal for Cr are almost negligible at this pH and other pH conditions. The results seemed to confirm that wide variation in metal removals can be attributed to the change in the properties of sludge over time. This seemed to agree with the findings of Forstner (1993) and Rapin et al. (1986) who suggested that there is a possibility of metal phase changes resulting from sludge sample storage. Moreover, the metal concentration of the aged sludge seemed to increase for Cd, Cr and Ni, while for Cu, Pb and Zn, a decrease in concentration was observed. Overall, the results seemed to reveal that minimal metal removal was observed for unfermented raw liquid at pH of 3.94 corresponding to a relatively high raw liquid dosage of 125 (mL/g). thus, no further experimentation was done on the determination of optimum leaching time, since prolonging the contact time would result in the natural fermentation of the raw liquid.

4.3.4 Leaching with Naturally Fermented Raw Liquid from Pineapple Waste

4.3.4.1 Determination of Optimum pH

The raw liquid after fermentation for 144 hours (6 days) was filtered using a very fine cloth to remove solid particles. This fermented liquid (pH= 3.55) was used in leaching experiment to determine the optimum pH for removal of metals from air – dried dewatered sludge. Table 4.8 summarizes the dosages used in the leaching experiment with the corresponding pH.

Table 4.8 Naturally Fermented Raw Liquid Dosages Used in the Experiment

pH	Naturally Fermented Liquid Dosage (v/w)	Equivalent wt of Naturally Fermented Liquid (g liquid/g sludge)
6.83 (initial pH of sludge)	0	0
3.67	45	46.6
3.82	2.9	3.0
4.02	1.4	1.5
4.24	0.7	0.74
4.36	0.5	0.53

Since the initial pH of the fermented raw liquid is now 3.55, the pH of the sludge was reduced down to 3.67. To reduce the pH of sludge further down would require a considerable amount of the liquid. As observed, further addition of large amount of raw liquid resulted to a very slight reduction in sludge pH. Hence, the pH of 3.67 was selected as the minimum for the leaching experiment. The highest pH selected was 4.36 since increasing the pH further would only require a very minimal dosage of the liquid which would result in a negligible metal removal. Using these pH conditions corresponding to the raw liquid dosages, the following metal removals were obtained. Figure 4.13 shows the metal removal efficiencies of all metals analyzed.

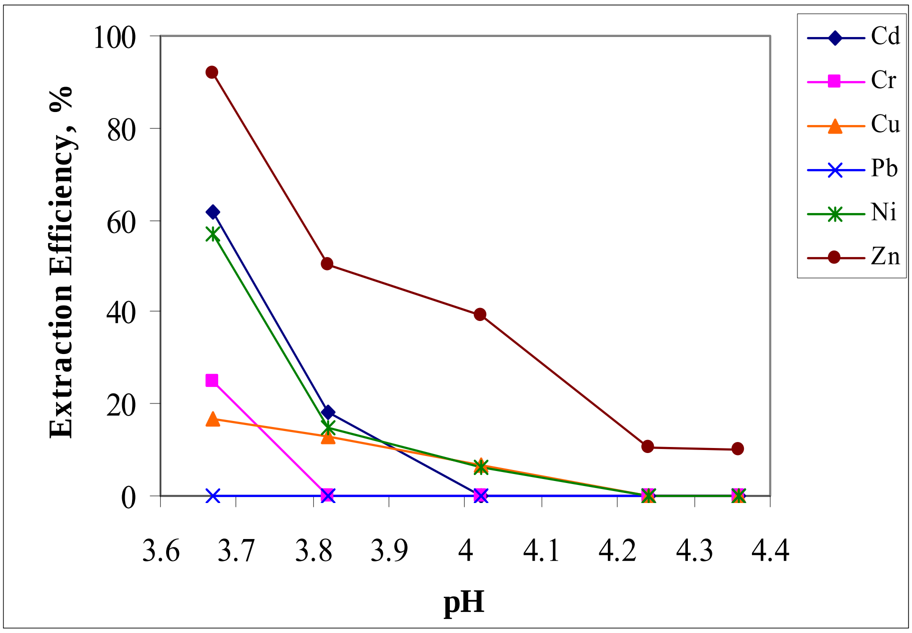


Figure 4.13 Metal Removal Efficiency for All Metals at Two Hours Leaching Time and Various pH Conditions during Leaching with Naturally Fermented Raw Liquid

For Cd, the highest removal obtained was about 34% at pH of about 3.67. Metal removals for other pH conditions were almost the same at about 3%.

The highest removal attained for Cr metal was only 25 % at a pH of about 3.67. No removals were detected for other pH conditions.

For Cu, about 16.6 % removal was attained at pH 3.67, followed by about 13% at pH 3.82. At other pH conditions, removal was relatively lower at 6.9% at pH 4.02 and no removals at other pH conditions.

Removal for Pb metal was undetected for all pH conditions.

For Ni, the highest removal attained which was 57 % was at pH 3.67. Removals for other pH conditions was very minimal at 15% at pH 3.82 and 6% at pH 4.02. No removals were detected at other pH conditions.

For Zn, metal removal was detected at all pH conditions with about 92% removal at pH 3.67 followed by 50% at pH 3.82. Removal for other pH conditions was relatively low at 39.4%, 10.4% and 10.01% at pH 4.02, 4.24 and 4.36 respectively.

Removals in terms of actual amount of metals removed are presented in Appendix F-1. For the calculation of metal removals, the initial metal concentration used was that obtained from the analysis of the dewatered sludge after 6 months of storage. From the foregoing results, the pH 3.67, 3.82 and 4.02 were selected for further experimentation on the determination of optimum leaching time.

4.3.4.2 Determination of Optimum Leaching Time

Further leaching experiment was done on the determination of optimum leaching time after adjusting the pH of the sludge to the desired conditions of 3.67, 3.82 and 4.02 using the naturally fermented liquid from pineapple wastes (pH = 3.64). Table 4.9 summarizes the dosages used in the leaching process with the corresponding pH selected.

Table 4.9 Naturally Fermented Liquid Dosages Used in the Experiment

pH	**Naturally Fermented Raw Liquid Dosage (mL/g)**	**Equivalent wt of fermented liquid (g liquid /g sludge)**
6.76 (initial)	0	-
3.67	45	46.6
3.82	2.9	3.0
4.02	1.4	1.5

Using these pH conditions corresponding to the fermented liquid dosages, leaching was done at duration of 0.083 day (2h), 0.25 day (6h), 1 day, 5 days, 8 days and 11 days. Metal removals achieved at different leaching times are shown in Figure 4.14 and described as follows:

For Cd, all the metal seemed to be removed after leaching for 8 days at pH 3.67. At pH 3.82, highest removal of 38% was also attained at 8 days leaching time. No removal was observed at pH 4.02 as shown in Figure 4.14 (a).

The highest removal for Cr metal was 74% and seemed to be attained at 11 days leaching time for pH 3.67. No removal was observed at other pH conditions as shown in Figure 4.14 (b).

For Cu, the highest removal attained was 33% at pH 3.67 at 1 day leaching time. No removal was observed at 5, 8 and 11 days leaching time at the same pH condition. Removals for other pH conditions was comparatively less at 2h and 6h leaching times as shown in Figure 4.14 (c).

Removal for Pb at pH of 3.67 was attained at longer leaching times of 8 and 11 days at 24.04% and 37.4% respectively. No removal was observed in other pH conditions as shown in Figure 4.14 (d).

For Ni, metal removal was detected at all pH conditions with the highest attained at 1 day leaching time (58%) at pH 3.67. At pH of 3.82, the highest removal attained (33.6%) was at 6 hours leaching time. Minimal removal of 6% was attained at pH 4.02, as shown in Figure 4.14 (e).

Most of Zn metal was removed at various leaching times for pH 3.67 with the highest of 93% attained at 1 day leaching time, while at pH 3.82 only a maximum removal of 55% was attained at 6 hours leaching time. Removal was also attained at pH 4.02 with the highest (39.5%) at 2 hours leaching time, as shown in Figure 4.14 (f).

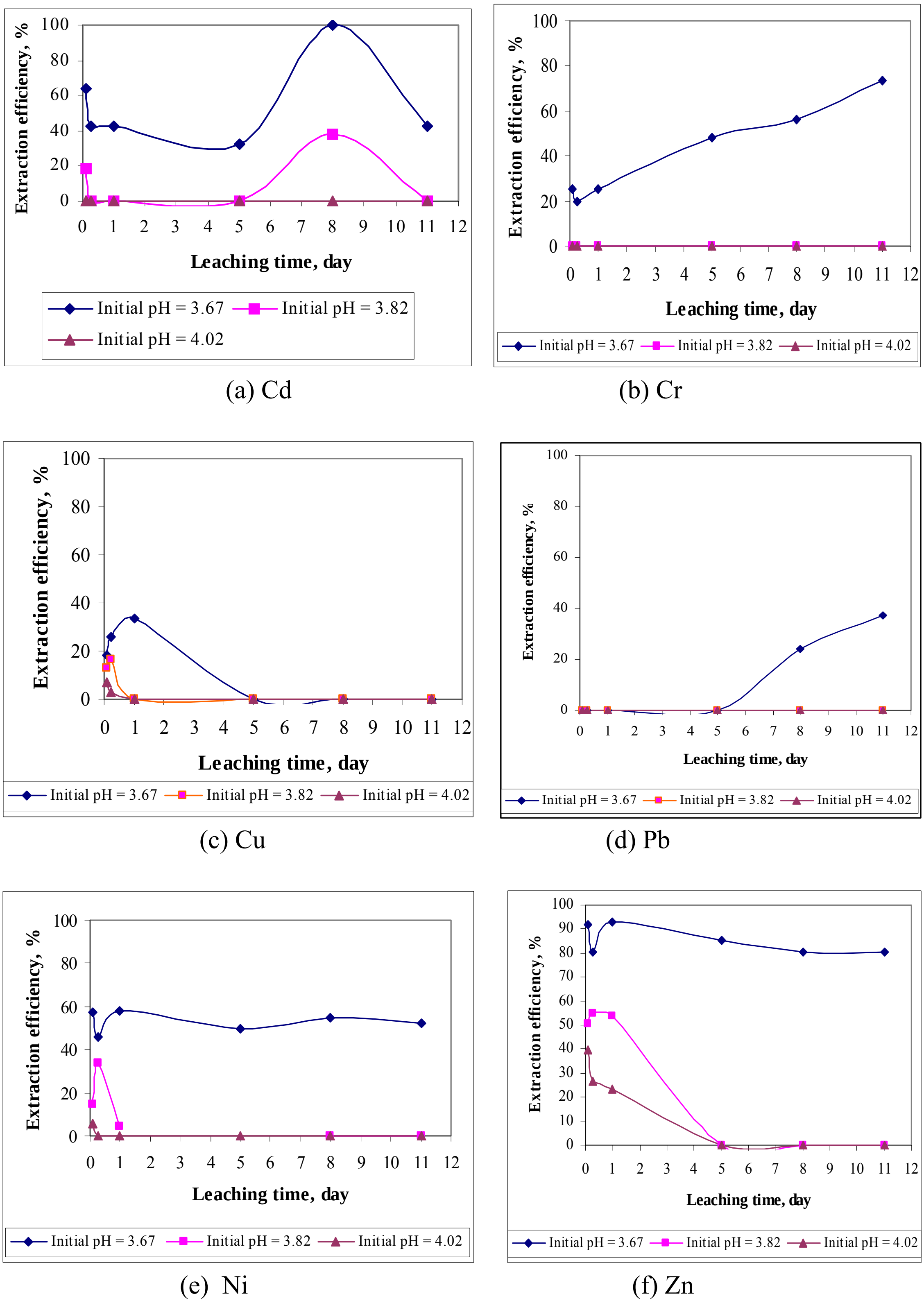


Figure 4.14 Metal Removal Efficiencies by Naturally Fermented Raw Liquid for Cd, Cr, Cu, Pb, Ni and Zn at 3<pH<4.5 at Various Leaching Times

The actual amount of metals removed during leaching with naturally fermented liquid is presented in Appendix F-2. As shown, there was a wide variation of removal efficiencies for all metals at various leaching times.

Results of the leaching study on the effect of leaching time are summarized as follows: For Cd, the optimum leaching time seemed to be 8 days at pH 3.67 (at 100% removal). For Cr, 73.5% of the metal was removed at the optimum leaching time of 11 days for pH 3.67. No removals were observed at other pH conditions. It was observed that at pH 3.73, 50% of Cr was removed from the sludge immediately after acid addition. This behavior can be due to the presence of some portion of inorganic precipitate or weakly chelated form of the Cr within the sludge as revealed in chemical speciation studies. Such precipitates or weakly-held chelates are thought to react and therefore attain solubilization equilibrium much more rapidly than strongly complexed organic metal chelates. Copper removal was relatively lower for leaching at pH 3.67 with 33.2% optimum removal at 1 day leaching time. At other pH conditions, a very minimal removal was observed. This could be due to the predominance of residual portion of the metal in the sludge. For Pb, it was observed that very minimal removal was attained even at the optimum leaching time of 11 days for all pH conditions. The highest removal of 37.4% was attained at pH 3.67, followed by 24.04% at the same pH. No removals were observed at other pH conditions. This might be due to the significant amount of residual portion of Pb present in the sludge.

Nickel removal was observed to be optimum at 1 day leaching time (at 58 %) for pH 3.67, although removals for other leaching time at the same pH condition did not vary much. At pH 3.82, 6 hours seem to be the optimum at removal of 33.7%. No removals were observed at a higher pH of 4.02. Ni is characterized by about 50% solubilization immediately after acid addition. This may be due to the predominance of Ni as an inorganic precipitate or weakly complexed species, and the presence of exchangeable form of the metal. For Zn, the optimum leaching time was observed at 1 day (at 93% removal) for pH 3.67, although removals at other leaching time for the same pH condition did not vary much. Zinc was the most readily solubilized metal of those investigated which may be due to the predominance of bound to organic and inorganic form of the metal and the low amount of residual fraction. It was also observed that the amount of Zn removed from the sludge seemed to exceed the total amount of the metal in the sludge. This might be due to the presence of the metal in the raw liquid used for leaching which was measured to be 2.3 mg/L.

A comparison of removal efficiencies for all metals show that orders of metal removal vary depending upon the pH. At pH 3.67 for instance and 2 hours leaching time (Figure 4.13), the following order of metal removal can be deduced:

Zn >Ni > Cd> Cr > Cu > Pb

This order follows the general pattern of the Irving-Williams series, which describes the relative orders of metal-organic chelate bond strengths (Wozniak and Huang, 1982). These bond strengths are thought to be dependent only upon the metal involved in the organic complex, and not the organic structure alone, and that the greater the organic-metal bond strength, the less soluble the complex.

Variation of metal removal efficiencies can also be attributed to the change of pH of the sample as the leaching time is increased, as shown in Figure 4.15.

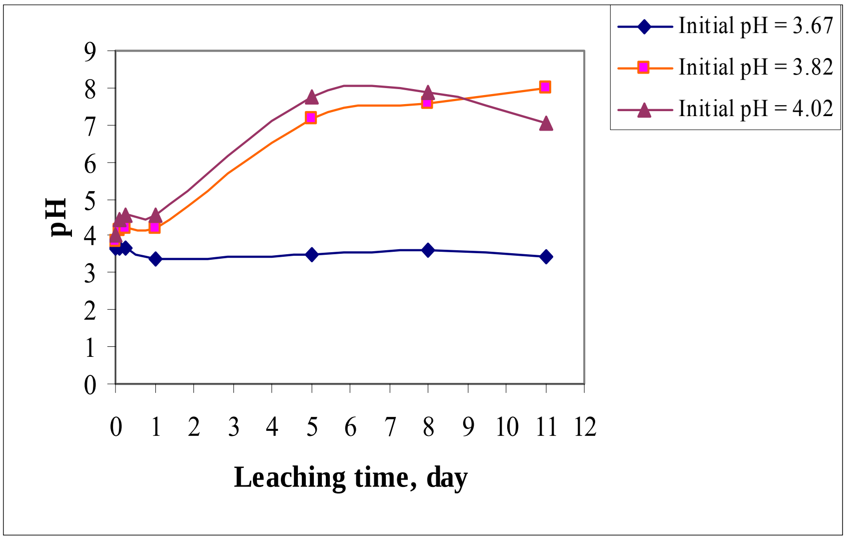


Figure 4.15 Change of pH Over Time for Leaching with Naturally Fermented Raw Liquid from Pineapple Wastes

As shown, a slight decrease in pH seemed to be observed for leaching at initial pH of 3.67, as compared to the other pH conditions where a continuous increase in pH occurred until the 11^{th} day of leaching, although a slight decrease in pH was observed for leaching at initial pH of 4.02. At a lower initial pH of 3.67, continued fermentation might have occurred during leaching up to 11 days. While for other pH conditions, no further fermentation has taken place. This observed change in pH reflects better removal efficiencies for most metals at pH 3.67 (Figure 4.14).

One other factor which might have affected the removal efficiency of metals from sludge is the nature and quality of the raw liquid from pineapple wastes. The presence of Zn in the raw liquid, for example, might have affected the results of the study.

4.3.5 Leaching with *A. niger* Fermented Raw Liquid from Pineapple Waste

4.3.5.1 Determination of Optimum pH

The raw liquid after fermentation with *A. niger* spore suspension for 144 hours (6 days) was filtered using a very fine cloth to separate the mycelium. The yield of mycelial dry weight was 28.18 g/L. The *A. niger* fermented liquid (pH=3.7) was used in the leaching experiments to determine the optimum pH or dosage for removal of metals from dewatered sludge. Table 4.10 summarizes the dosages used in the leaching experiment with the corresponding pH. The minimum pH selected was 3.73 because reducing the pH of the sludge sample further down from pH 6.5 using the *A. niger* fermented liquid (pH = 3.7), would require a considerable amount of liquid while achieving only a very minimal sludge pH reduction. As observed, further addition of large amount of raw liquid resulted to a very slight reduction in sludge pH. Hence, the pH of 3.73 was selected as the minimum for the leaching experiment. The highest pH selected was 4.51 since increasing the pH further would only require a very minimal dosage of the liquid which would result in a negligible metal removal. Using these pH conditions corresponding to the raw liquid dosages, the following metal removals for leaching at 2 hours leaching time, were obtained.

Table 4.10 *A. niger* Fermented Raw Liquid Dosages Used in the Experiment

pH	Raw Liquid Dosage (mL/g)	Equivalent wt of Fermented Liquid (g liquid/g sludge)
6.5 (initial pH of sludge)	0	0
3.73	40.0	42.0
3.88	8.57	9.0
3.98	2.86	3.0
4.47	1.0	1.1
4.51	0.5	0.53

Figure 4.16 shows the metal removal efficiencies of all metals analyzed and described as follows:

For Cd, the highest removal obtained was only 19% at pH of about 3.73. Metal removals for other pH conditions were undetected.

The highest removal attained for Cr metal was high at 50.24 % at a pH of about 3.73. A lesser removal of 14.1% was attained at pH 3.88. No removal was detected for other pH conditions.

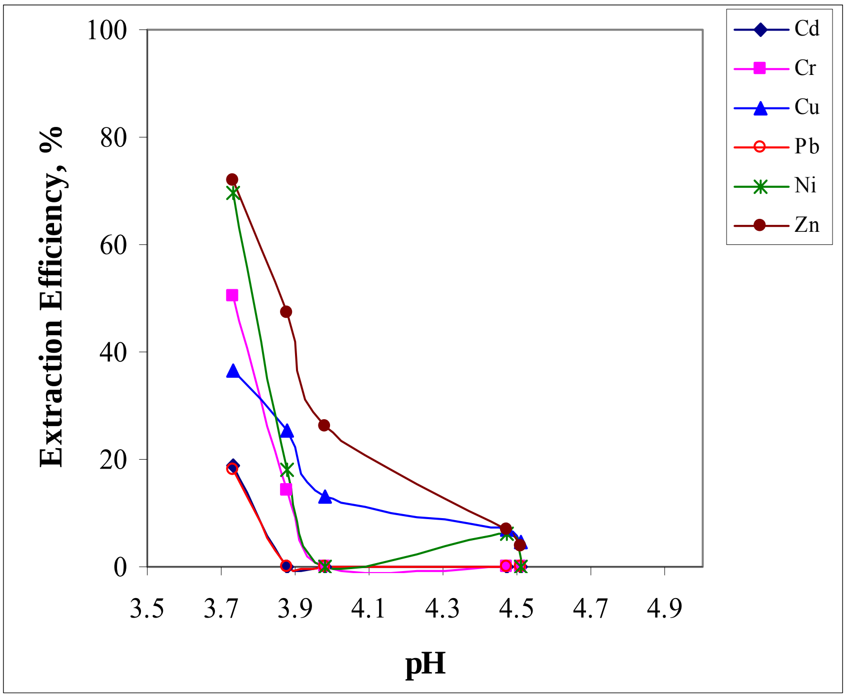


Figure 4.16 Metal Removal Efficiency for All Metals at Various pH Conditions during Leaching with Raw Liquid Fermented with *A. niger* at Two Hours Leaching Time

For Cu, about 36.5% removal was attained at pH 3.73, followed by about 25.3% at pH 3.88. At other pH conditions, removal was relatively low with only about 13% removal at pH 3.98, 7 % at pH 4.47 and 5% at pH 4.51.

The highest removal attained for Pb was 12% at pH 3.73. No removal was detected for other pH conditions.

For Ni, the highest removal attained which was 69.5% was at pH 3.73. Removals for other pH conditions were low at 18 % at pH 3.88 and 6 % at pH 4.47. No removal was detected at other pH conditions.

For Zn, metal removal was detected at all pH conditions with about 72% removal at pH 3.73, 47% at pH 3.88 and 26.2% at pH 3.98. Removal for other pH conditions was low at 7% and 4% at pH 4.47 and 4.51 respectively.

Removals in terms of amount of metals removed in mg/kg DM are presented in Appendix G-1. As observed, the highest removal of about 72% was attained at pH 3.73 for Zn followed by Ni at 69.5%, Cr at 50.2% and Cu at 36.5%. Removals for Cd and Pb were almost negligible at this pH and other pH conditions. Good results were also obtained at pH 3.88 and 3.98 for Cu and Zn. However, for pH 4.47 and 4.51, metal removals were negligible and for some metals undetectable. Hence, the optimum pH conditions were then selected to be 3.73, 3.88 and 3.98.

4.3.5.2 Determination of Optimum Leaching Time

Further experimentation was done on the use of *A. niger* fermented liquid for the removal of metals from air – dried dewatered sludge sample after adjusting to the selected pH conditions of 3.73, 3.88 and 3.98. Table 4.11 summarizes the dosages used in the leaching process with the corresponding pH selected.

Table 4.11 *A. niger* Fermented Liquid Dosages Used in the Experiment

pH	***A. niger* Fermented Liquid Dosage (v/w)**	**Equivalent wt of fermented liquid (g liquid /g sludge)**
6.5 (initial pH of sludge)	0	0
3.73	40.0	42.0
3.88	8.57	9.0
3.98	2.86	3.0

Using these pH conditions corresponding to the *A. niger* fermented liquid dosages, leaching was done at duration of 0.083 day (2h), 0.25 day (6h), 1 day, 5 days, 8 days and 11 days. Metal removals achieved at different leaching times are shown in Figure 4.17 and described as follows:

For Cd, the highest removal was attained at 5 days leaching time with all the metals removed at pH 3.98. At pH 3.88, 88.8% removal was attained, with the least removal at pH 3.73 at the same leaching time. Figure 4.17 (a) shows the metal removals attained for Cd at different leaching times.

For Cr metal, the highest removal of 100% seemed to be attained at 11 days leaching time for pH 3.73 although a high removal of about 91% was attained only at 5 days leaching time for the same pH. At pH 3.88, the highest removal attained was 84.4% at 11 days leaching time, while at pH 3.98 all metals seemed to be removed at 11 days leaching time, as shown in Figure 4.17 (b).

For Cu, the highest removal of 65 % was attained at 5 days leaching time at a higher pH of 3.98 and 8 days leaching time at pH 3.88. At pH 3.73, a lower removal of 46% was attained, as shown in Figure 4.17 (c).

The highest removal for Pb at all pH conditions seemed to be attained at 5 days leaching time, i.e., 34 %, 14%, and 26% at pH 3.73, 3.88 and 3.98 respectively, as shown in Figure 4.17 (d).

For Ni, metal removal was detected at almost all pH conditions. The highest removal of about 93% was attained at 11 days leaching time at pH 3.73. At pH of 3.88, the highest removal attained (64.6%) was at 8 days leaching time. Minimal removal of about 25% was attained at pH 3.98, as shown in Figure 4.17 (e).

All Zn metal were removed at 11 days leaching time for pH 3.73, although a high removal of 99% was obtained at 5 days leaching time. For pH 3.88, the highest removal seemed to be attained also at 11 days leaching time at 94%, while for pH 3.98, the highest removal of 81% seemed to be attained at only 5 days leaching time. For this metal, removal was detected for all pH conditions at various leaching times as shown in Figure 4.17 (f).

The actual amount of metals removed in terms of mg/Kg DM is presented in Appendix G-2. As shown, there was a wide variation of removal efficiencies for all metals at various leaching times.

For Cd, it was observed that for all pH conditions, the optimum leaching time seemed to be 5 days with removals of 56%, 88.8% and 100% attained at pH 3.73, 3.88 and 3.98 respectively. This high degree of solubilization is may be due to the presence of exchangeable form of the metal.

Chromium removal was excellent (about 100%) at the optimum leaching time of 11 days for pH 3.73 and 3.98, although at 5 days leaching time, removal of about 92% was attained at pH 3.73, while at 8 days leaching time, 95% removal was attained at pH 3.98. At pH 3.88, optimum removal of 84% was attained at 11 days leaching time. It was observed that at pH 3.73, significant amount of Cr (at 50%) was removed from the sludge immediately after acid addition, as shown in Figure 4.17 (b). This behavior is may be due to the presence of some portion of inorganic precipitate or weakly chelated form of the Cr within the sludge as revealed in chemical speciation studies. Such precipitates or weakly-held chelates are thought to react and therefore attain solubilization equilibrium much more rapidly than strongly complexed organic metal chelates

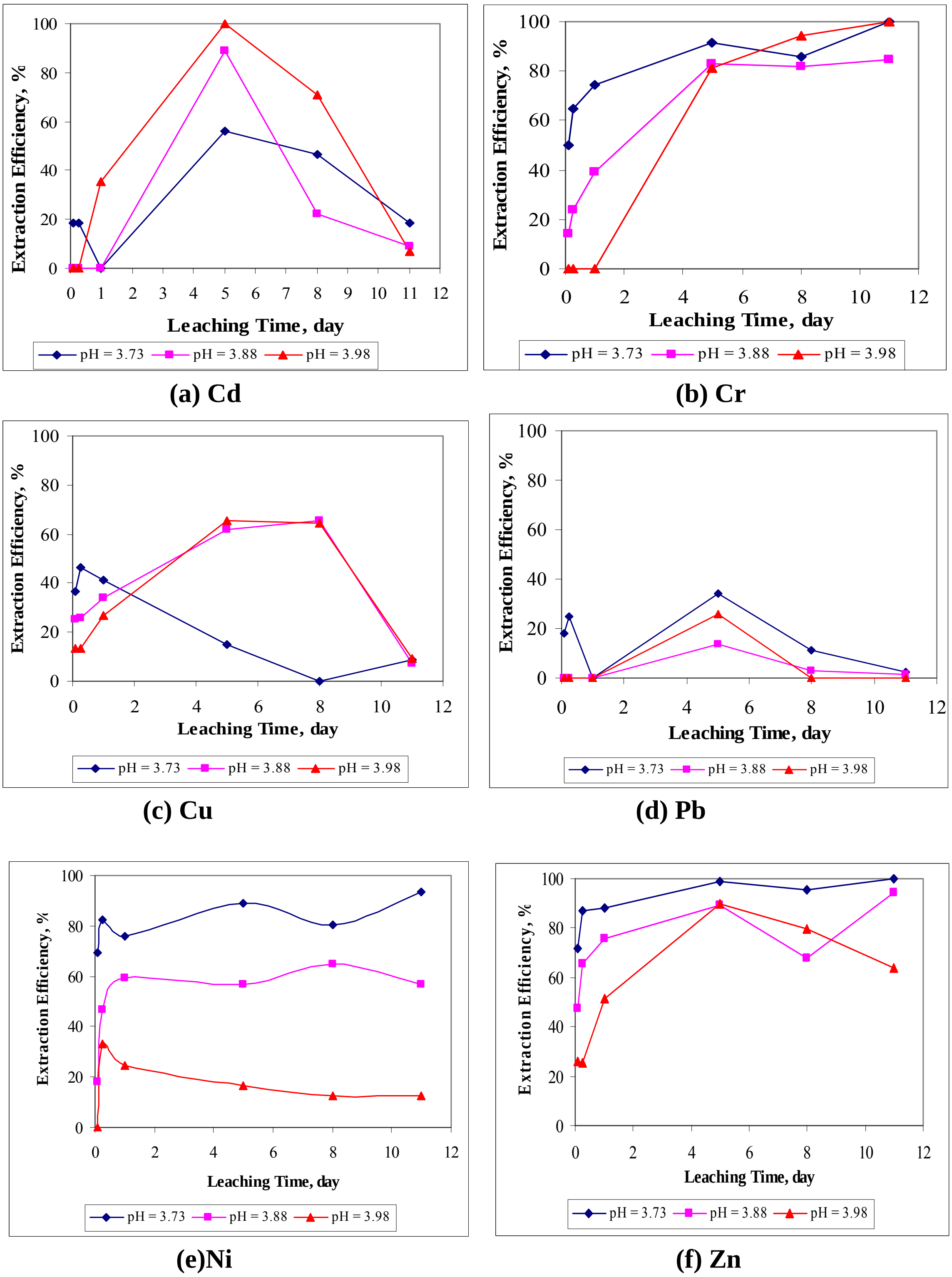


Figure 4.17 Metal Removal Efficiencies by *A. niger* Fermented Raw Liquid for Cd, Cr, Cu, Pb, Ni and Zn at 3<pH<4 at Various Leaching Times

Copper removal was relatively lower for leaching at pH 3.73 with 6 hours leaching time (46% removal). Optimum leaching times at pH 3.88 and 3.98 were observed to be 8 days and 5 days respectively, with 65% removal efficiency. The presence of the carbonate bound and exchangeable forms in Cu in the sludge sample seemed to improve its metal removal efficiency, although the residual fraction predominates in the metal.

For Pb, it was observed that a minimal removal was attained even at the optimum leaching time of 5 days for all pH conditions. The highest removal of 34% was attained at pH 3.73, followed by 26% at pH 3.98 and 14% at pH 3.88.This might be due to the significant amount of residual portion present in Pb.

Nickel removal was observed to be optimum at 11 days leaching time (at 94%) for pH 3.73. At other pH conditions of 3.88 and 3.98, it was observed to be at 8 days (65%) and 6 hours (35%) respectively. At pH 3.73, Ni is characterized by a large amount of solubilization (70%) immediately after acid addition. This may be due to the predominance of Ni as an inorganic precipitate or weakly complexed species, and the presence of exchangeable form of the metal.

For Zn, the optimum leaching time was observed at 11 days with 100% removal for pH 3.73 although at 5 days leaching time, a high removal of 99% was also attained. For pH 3.88 and 3.98, the optimum leaching time seemed to be at 11 days (at 94%) and 5 days (at 81%) respectively. Zinc was the most readily solubilized metals of those investigated which may be due to the predominance of bound to organic and inorganic form of the metal and the low amount of residual fraction.

A comparison of removal efficiencies for all metals show that orders of metal removal vary depending upon the pH. From Figures 4.16 and 4.17, the various pH values at 2 hours leaching time for instance, resulted in the following orders of metal removal:

pH 3.73: Zn > Ni > Cr > Cu > Cd >Pb
pH 3.88: Zn > Cu > Ni > Cr > Cd = Pb
pH 3.98 : Zn > Cu > Cr = Cd =Pb = Ni

At pH 3.73, the order of removal follows the general pattern of the Irving-Williams series, which describes the relative orders of metal-organic chelate bond strengths (Wozniak and Huang, 1982). These bond strengths are thought to be dependent only upon the metal involved in the organic complex, and not the organic structure alone, and that the greater the organic-metal bond strength, the less soluble the complex.

Variation of metal removal efficiencies can also be attributed to the change of pH of the sample as the leaching time is increased, as shown in Figure 4.18. As shown, for all pH conditions, a decrease of pH occurred after 1 day of leaching with the minimum reached at 5 days leaching time. It was observed that the sample with the initial pH of 3.88 surprisingly dropped to as low as 2 after 5 days leaching time which was the lowest among the samples. This might be due to the continued fermentation process occurring during leaching since the process is favored by pH 3-4 (Sun, 1984). Moreover, this might also explain the higher removal especially for Pb at 5 days leaching time. The observed decrease of pH at 11 days leaching especially for samples with initial pH of 3.73 might also explain the highest removal attained for some metals (e.g. Ni and Zn) at this leaching time.

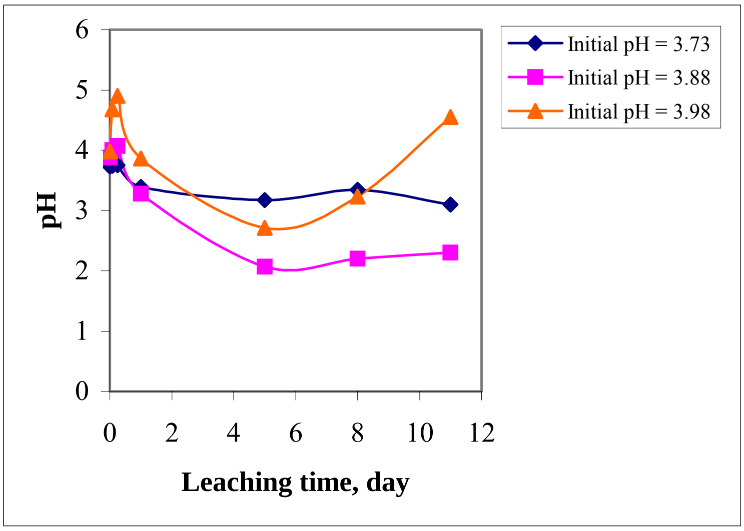


Figure 4.18 Change of pH over Time for Leaching with *A. niger* Fermented Raw Liquid from Pineapple Wastes

Several workers have reported increases in organometallic complex stability with increasing pH, and solubilization of metallic ions with decreasing pH. Such an effect may result from the competition that exists between the metal and hydrogen ions for the weakly acidic complexation sites (Lester et al., 1983).

4.4 Summary of Optimum Conditions for Heavy Metal removal using Various Extractants

Table 4.12 shows the summary of optimum conditions for heavy metal removal from sludge using commercial citric acid, naturally fermented raw liquid and *A. niger* fermented raw liquid from pineapple wastes. The unfermented liquid was not included since leaching studies were done only at two hours contact time. As shown, the optimum leaching time for commercial citric acid for pH around 3 ranged from one hour to 5 days. For leaching with naturally fermented raw liquid, the optimum leaching time varied from two hours to 11 days, while for *A. niger* fermented raw liquid, the time varied from 6 hours to 11 days. In terms of the amount of metal removed at optimum conditions, leaching with *A. niger* fermented raw liquid seemed to give the best results for most metals except for Pb and Cd. Based on the foregoing results, further experimentation on the following optimum conditions for various extractants were conducted:

Commercial citric acid: pH = 3.04, leaching time = 1 day
Naturally Fermented Raw liquid: pH = 3.67, leaching time = 1 day
A. niger Fermented Raw Liquid: pH = 3.73, leaching time = 5 days
A. niger Fermented Raw Liquid: pH = 3.88, leaching time = 5 days

Since for *A. niger* fermented liquid, better removals were also attained at pH 3.88, metal extraction at this pH condition and 5 days leaching time, was also done.

4.5 Leaching with Various Extractants at Optimum Conditions to Determine Suitability for Land Application

For leaching with various extractants at optimum conditions, only one sludge sample was used i.e., air-dried dewatered anaerobically digested sludge sample collected on June 15, 2006. Characterization before and after treatment of the sludge sample with various extractants at optimum conditions, is discussed separately in this section.

4.5.1 Sludge Characterization

Table 4.13 shows the characteristics of the sludge sample before and after treatment at optimum conditions with the following extractants: commercial citric acid (CA), naturally fermented raw liquid from pineapple wastes (NF), *A. niger* fermented raw liquid at pH 3.73 (AF1) and *A. niger* fermented raw liquid at pH 3.88 (AF2). As shown, in terms of the physical characteristics, the total solids of the treated sludges are lesser compared to the untreated sludge since the treated sludge were not subjected to any dewatering process prior to analysis. There is also an observed increase in the organic matter content of treated sludges compared to the untreated sludge which may be due to the organic nature of the extractants added to the sludge during treatment. For total nitrogen, there seemed to be an observed decrease for sludge treated with *A. niger* fermented liquid which might be attributed to the use of nitrogen by *A. niger* as nutrient in the fermentation process (Yigitoglu, 1992). The phosphate content of the *A. niger* treated sludge seemed to be unaffected since a phosphate source in the form of potassium dihydrogen phosphate (KH_2PO_4) has been added as nutrient in the fermentation process. It was observed that the phosphorous content of *A. niger* treated sludge seemed to be the highest. In terms of coliform removal, an almost complete removal of faecal coliform was observed for the sludge treated with *A. niger* fermented raw liquid as compared to the sludge treated with citric acid and naturally fermented raw liquid from pineapple wastes. In terms of remaining metals, the sludge treated with *A. niger* fermented liquid seemed to contain the least amount of most metals indicating a higher metal removal attained for the extractant. However, the remaining copper metal for sludge treated with all four extractants is still high and so cannot comply with the proposed BMA standard. It can also be noticed that a high amount of Fe (removal at 71.4% for naturally fermented; while 70.6% and 69.7% for *A. niger* fermented liquid at pH 3.73 and 3.78, respectively) and Mn (removal at 57.2% for naturally fermented; while 81.4% and 85.1% for *A. niger* fermented liquid at pH 3.73 and 3.78, respectively) was also removed in the process, indicating that Fe and Mn may have been utilized in the fermentation process and may have also acted as competing metals in the extraction process. The removal of Fe and Mn may have also affected the forms of metals in the sludge as will be discussed in the succeeding sections. With the exception of Cu, all the other parameters including the nitrogen and phosphorous content of the sludge seem to indicate the potential of the treated sludge for land application. However, further treatment may be necessary to increase the total solids and decrease the organic matter content of the treated sludge which do not meet the compost standards. The pH of the treated sludges which is also below the compost standard would not pose a problem since increase in pH would occur over time as all the extractants, being biodegradable, will decompose with time.

Table 4.12 Optimum Conditions for Heavy Metal Removal using Various Extractants at pH 3-4.5

Extractants	pH	Dosage (v/w)	Heavy Metals											
			Cd		Cr		Cu		Pb		Ni		Zn	
			Leaching Time	Removal (mg/Kg)	Leaching Time	Removal (mg/Kg)	Leaching Time	Removal (mg/Kg)	Leaching Time	Removal (mg/Kg)	Leaching Time	Removal (mg/Kg)	Leaching Time	Removal (mg/Kg)
CA	3.04	7.86	2h	2.8 (~100%)	1d	168.91 (45%)	1d	1,230.82 (29%)	1d	138.45 (~100%)	2h	88.61 (70%)	2h	1,552.86 (80%)
	3.90	1.43	5d	4.26 (~100%)	ND	ND	1h	350.39 (~0%)	ND	ND	1h	60.71 (47%)	1h	193.83 (10%)
NF	3.67	45	8d	42.74 (~100%)	11d	293.87 (73%)	1d	488.88 (10.6%)	11d	37.4 (37.4%)	1d	125.56 (58%)	1d	1,650 (93%)
	3.82	2.9	8d	9.39 (37.58%)	ND	ND	6h	244.26 (5.3%)	ND	ND	2h	32.88 (15%)	6h	977.03 (43%)
	4.02	1.4	ND	ND	ND	ND	2h	101.28 (2.19%)	ND	ND	2h	13.21 (6%)	2h	700.18 (39.5%)
AF	3.73	40	5d	14.33 (55.97%)	11d	410.74 (~100%)	6h	2,134.87 (46.3%)	5d	71.64 (34.1%)	11d	205.37 (93.95%)	11d 5d	2,471.58 (~100%) 2,268.6 (99%)
	3.88	8.57	5d	22.74 (88.84%)	11d	341.14 (84.4%)	8d	3,007.7 (65.24%)	5d	28.43 (13.54%)	8d	142.14 (64.61%)	11d	2,160.57 (94.35%)
	3.98	2.86	5d	36.4 (~100%)	11d	427.57 (~100%)	5d	3,011.15 (65.32%)	5d	54.58 (25.99%)	1d	54.48 (24.81%)	5d	2,055.95 (89.78%)

CA = Citric Acid; NF = Naturally Fermented Raw Liquid from Pineapple Wastes; AF = *A. niger* Fermented Raw Liquid from Pineapple wastes
ND= not detected; v/w = volume of extractant in mL / weight of sludge in grams

Table 4.13 General Characteristics of Untreated and Treated Sewage Sludge at Optimum Conditions

Parameters	Untreated Anaerobic Sludge	[1]CA Treated Sludge	[2]NF Treated Sludge	[3]AF1 Treated Sludge	[4]AF2 Treated Sludge	Proposed BMA Standards/ [8]USEPA Standards	Compost Standards
pH (sludge:water ratio = 1:2)	6.5	3.04	3.67	3.73	3.88	-	[6]5.5 - 8.5
Total Solids (%)	23.1	17.04	17.55	10.72	13.79	-	≥ 65
Total Volatile Solids (TVS)	44.74	96.4	85.72	81.6	95.68	-	-
Total Nitrogen (%)	2.5	2.1	3.0	1.7	1.5	-	0.2 – 1
Total Phosphate (%)	3.35	3.2	3.4	3.7	3.6	-	0.2 – 1
Organic Matter (%)	44.74	96.4	85.72	81.6	95.68	-	15 - 60
Faecal Coliform (MPN/g)	1.6×10^4	9.2×10^3	490	<2	<2	- /1×10^3 – 2×10^6	-
Heavy Metals (mg/Kg)							
Cd	4.8	2.4	4.3	2.4	0.6	20/ 39	[7]19
Cr	264	187.2	175.2	43.2	57.6	1000/ 1,200	-
Cu	3,043.2	2,937.6	2,764.8	2,025.6	2,587.2	900/ 1,500	725
Pb	175.2	156	175	165.9	175	1000/300	565
Fe	34,190	[5]NA	9,776.4	10,053.6	10,360.8	-	-
Mn	2,011.2	NA	860.4	374.4	300	-	-
Ni	297.6	228	194.4	57.6	129.6	400/420	-
Zn	1,908	1,166.4	984	432	484.8	3000/2800	1000

[1]CA – Citric acid
[2]NF – Naturally fermented raw liquid from pineapple wastes
[3]AF1 – *A. niger* fermented raw liquid from pineapple wastes (pH 3.73)
[4]AF2 - *A. niger* fermented raw liquid from pineapple wastes (pH 3.88)
[5]NA– Not analyzed
[6] Thai compost standard, source: Suksawad (2002)
[7] US compost standard, source: Epstein (1997)
[8]Source: USEPA (2002)

4.5.2 Chemical Speciation Studies (SCE III)

4.5.2.1 Untreated Sludge

Results of the chemical speciation studies for the sludge sample collected are shown in Figure 4.19. The actual amount of metals extracted for each form of metals analyzed is presented in Appendix H-1.

As shown, Cd seemed to predominate in the bound to Fe and Mn form (36.08%) and least predominant in residual form. Chromium and Cu seem to be predominantly in the residual form (92% and 89.6% respectively), although for Cu, a very minimal exchangeable form (0.51%) was detected which is not present in Cr. Lead predominates in bound to organic and inorganic matter (53.4%) with a relatively high residual portion at 26.5%. Nickel contains an almost equal amount of residual (at 32.4%) and bound to Fe and Mn form (at 31.1%) and a relatively high exchangeable form (7.45%). Zinc on the other hand has a lesser amount of residual form (at 9.6%) and predominantly in the bound to Fe and Mn form (at 43.8%). All forms of Zn metal were detected.

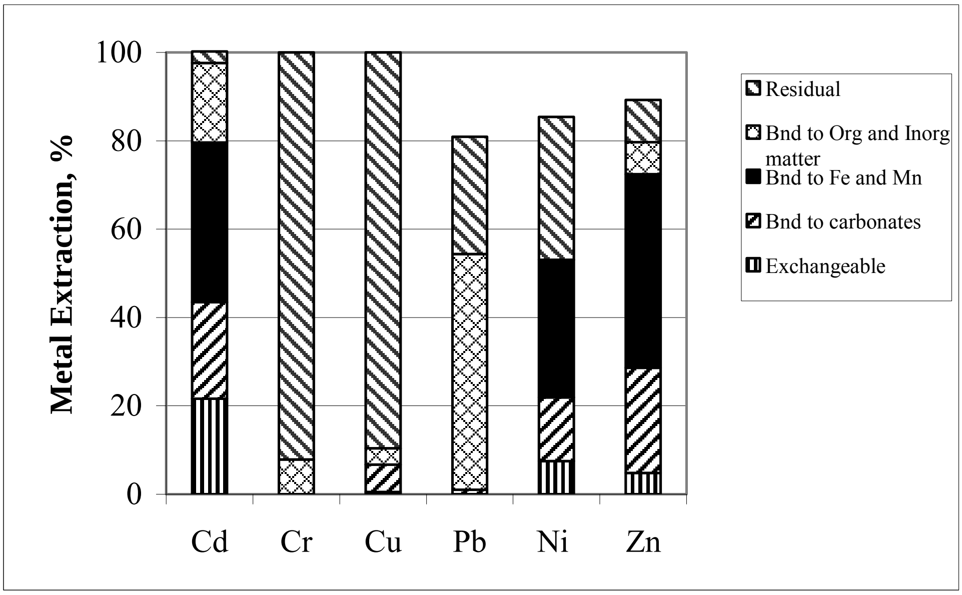


Fig 4.19 Metal Fractionation Profile of Untreated Sewage Sludge

The rankings of the different forms of all metals studied is shown as follows:

Cd: Bound to Fe and Mn (36.1%) > Exchangeable = Bound to carbonate (22%) > Bound to organic and inorganic matter (18%) > Residual (2.6%)
Cr: Residual (92.1%) >>> Bound to organic and inorganic matter (7.9%)
Cu: Residual (89.6%) >> Bound to carbonate (6.2%) > Bound to organic and inorganic matter (3.7%) > Exchangeable (0.51%)
Pb: Bound to organic and inorganic matter (53.4%) >Residual (26.6%) > Bound to carbonate (1%)
Ni: Residual (32.4%) > Bound to Fe and Mn (31.1%) > bound to carbonate (14.4%) > Exchangeable (7.5%)
Zn: Bound to Fe and Mn (43.8%) >Bound to carbonate (23.9%) > Residual (9.6%) > Bound to organic and inorganic matter (7.3%) > Exchangeable (4.8%)

Moreover, it was observed that the sum of five metal fractions obtained from sequential extraction procedure was higher than the total metal contents in the sludge following acid digestion. This may be due to the slow but increasingly stronger attack by the various reagents used in the sequential extraction procedure which removed specific forms of metals in the process. Whereas after acid digestion, not all forms of metals may have been removed. This difference was particularly pronounced for Cd, Cr and Cu. Similar observation was recorded by Paré et al. (1999) and Staelens et al. (2000).

4.5.2.2 Sludge Treated with Commercial Citric Acid (CA Treated)

Results of the SCE studies for sludge treated with commercial citric acid are shown in Figure 4.20. The actual amount of metals extracted for each form of metals analyzed is presented in Appendix H-2. As shown, for Cd, compared with the untreated sludge, the portion which is bound to Fe and Mn and the residual forms seemed to increase to 41.3% and 7.9% respectively, while the exchangeable form of the metal seemed to be unchanged. For Cr, the residual form of the metal seemed to predominate completely while it is less in the untreated sludge. Copper seemed to predominate in residual form at 89.6%, with most metal forms at a lesser proportion and observed absence of bound to Fe and Mn forms. For Pb, the presence of bound to Fe and Mn form (at 24%) seemed to be noticeable where it is absent in the untreated sludge, as well as the decrease in residual form to 12.5% in the CA treated sludge. The forms of Ni were almost the same although there is an observed decrease in proportion, except for the exchangeable form where an increase in proportion to 12.1% was observed. For Zn, all forms of the metal were detected although at a lesser proportion. The increase in the proportion of exchangeable form to 17.1% was also noticeable.

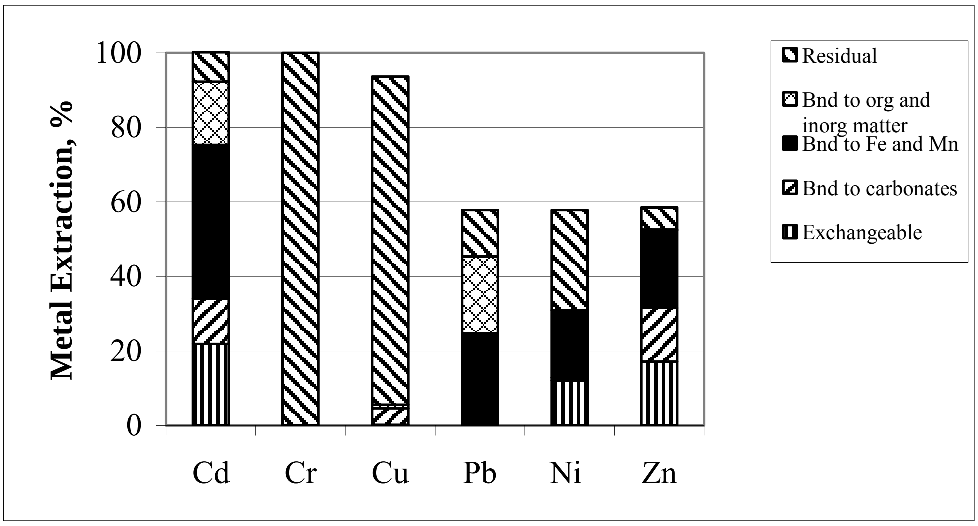


Figure 4.20 Metal Fractionation Profile of CA Treated Sewage Sludge

The rankings for the different forms of all metals for CA treated sludge are summarized as follows:

Cd**:** Bound to Fe and Mn (41.3%) > Exchangeable (21.9%) > Bound to organic and inorganic matter (17%) > Bound to carbonate (12.1%) > > Residual (7.9%)
Cr: Residual (100%)
Cu: Residual (88.1%) >> Bound to carbonate (4.4%) > Bound to organic and inorganic matter (0.96%) > Exchangeable (0.15%)
Pb: Bound to Fe and Mn (23.96%) > Bound to organic and inorganic matter (20.6%) > Residual (12.5%) > Bound to carbonate (0.8%)
Ni**:** Residual (26.9%) > Bound to Fe and Mn (18%) > Exchangeable (12.1%) > Bound to carbonate (0.8%)
Zn**:** Bound to Fe and Mn (20.8%) > Exchangeable (17.1%) > Bound to carbonate (14.4%) > Residual (5.9%) > Bound to organic and inorganic matter (0.3%)

The results revealed the potential of the CA treated sludge for land application since most metals contain residual fractions especially Cr and Cu. However, the high percentage of exchangeable forms of metals such as in Cd, Ni and Zn, could affect the mobility of these metals in the environment. Metals in the exchangeable phase are easily exchangeable with the soil solution and hence comparatively mobile (Oake et al., 1984).

While residual fractions are tightly bound to the sludge and therefore less mobile once the treated sludge is disposed to land.

4.5.2.3 Sludge Treated with Naturally Fermented Raw Liquid (NF Treated)

Results of the SCE studies for sludge treated with naturally fermented raw liquid from pineapple wastes is shown in Figure 4.21. The actual amount of metals extracted for each form of metals analyzed is presented in Appendix H-3.

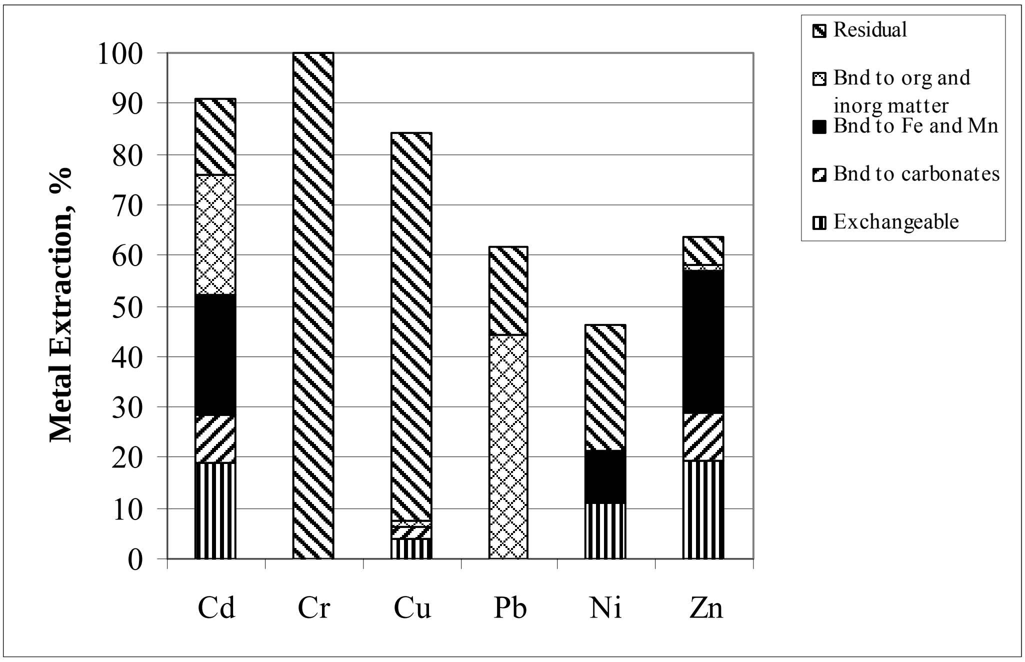


Fig 4.21 Metal Fractionation Profile of NF Treated Sewage Sludge

As shown, for Cd, compared to the untreated sludge, it seemed that the portion which is bound to Fe and Mn have decreased to 23.8% while the residual form has increased to 14.8%. Moreover, a slight decrease in the exchangeable form of the metal to 19% has been observed, as well as in bound to carbonate form. For Cr, the residual form of the metal seemed to predominate completely while it is less in the untreated sludge. Copper still predominates in residual form at a lesser proportion of 76.4% but with an observed increase in the exchangeable form to 4.1%. For Pb, the absence of bound to carbonate form is noticeable where it is present in the untreated sludge, as well as the decrease in bound to organic and inorganic matter form and residual form to 44.3% and 17.5%, respectively in the CA treated sludge. The forms of Ni metal observed were of lesser proportion although an increase of exchangeable form to 11% was observed in the CA treated sludge. For Zn, all forms of the metal were also detected although at a lesser proportion. The increase in the proportion of exchangeable form to 19.5% was also noticeable. The rankings for the different forms of all metals are summarized as follows:

Cd**:** Bound to Fe and Mn = Bound to organic and inorganic matter (23.8%) > Exchangeable (19%) > Residual (14.8%) > Bound to carbonate (9.5%)
Cr**:** Residual (100%)

Cu**:** Residual (76.4%) > Exchangeable (4.1%) > Bound to carbonate (2.5%) > Bound to organic and inorganic matter (1.12%)
Pb: Bound to organic and inorganic matter (44.3%) > Residual (17.5%)
Ni: Residual (24.9%) > Exchangeable (11.03%) > Bound to Fe and Mn (10.3%)
Zn: Bound to Fe and Mn (28.1%) > Exchangeable (19.5%) > Bound to carbonate (9.2%) > Residual (5.6%) > Bound to organic and inorganic matter (1.19%)

The results revealed that the naturally fermented treated sludge contains metals mostly high in residual fractions, hence its potential also for land application. However, the high percentage of exchangeable forms of metals such as in Cd, Ni and Zn, could affect the mobility of these metals in the environment. As discussed in previous sections, metals in the exchangeable phase are easily exchangeable with the soil solution and hence comparatively mobile. While residual fractions are tightly bound to the sludge and therefore less mobile once the treated sludge is disposed to land.

4.5.2.4 Sludge Treated with *A. niger* Fermented Raw Liquid at pH 3.73 (AF1 Treated)

Results of the SCE studies for sludge treated with *A. niger* fermented raw liquid at pH 3.73 is shown in Figure 4.22. The actual amount of metals extracted for each form of metals analyzed is presented in Appendix H-4.

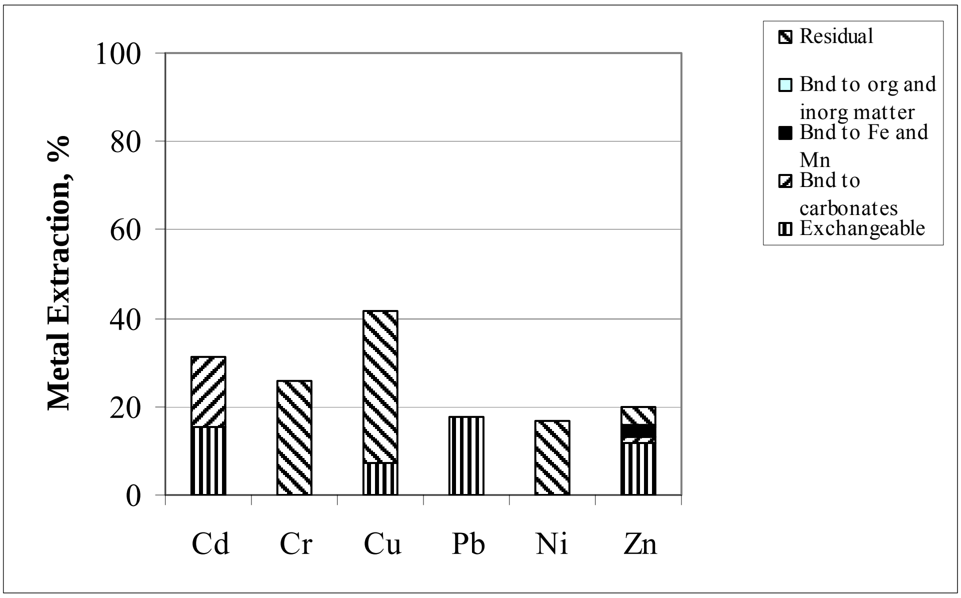


Fig 4.22 Metal Fractionation Profile of Sewage Sludge Treated with *A. niger* Fermented Raw Liquid at pH 3.73 (AF1 Treated)

As shown, for Cd, compared with the untreated sludge, equal amount of only two forms of the metal (exchangeable and bound to carbonate forms) were detected at lesser proportion. For Cr, the residual form of the metal seemed to predominate completely at a much lesser proportion (26%). Copper still predominates in residual form at a lesser proportion of 34.7% but with an observed increase in the exchangeable form to 7.1%. Lead metal seemed to be present only in exchangeable form in contrast with the untreated sludge where it is present in three forms (bound to organic and inorganic matter, residual and bound to carbonate) and not in exchangeable form. Only one form (residual) of Ni at a lesser proportion (16.8%) was observed in the sludge treated with *A. niger* fermented liquid at pH 3.73, in contrast to the untreated sludge where almost all forms of metals were present. For Zn, all forms of the metal were also detected with the predominance of the exchangeable form at 11.7% in contrast to the untreated sludge where the predominant form is bound to Fe and Mn. Other forms of metals were observed to be at a much lesser proportion.

The rankings for the different forms of all metals are summarized as follows:

Cd**:** Exchangeable = Bound to carbonate (15.5%)
Cr: Residual (26%)
Cu**:** Residual (34.7%) > Exchangeable (7.1%)
Pb**:** Exchangeable (17.9%)
Ni**:** Residual (16.8%)
Zn**:** Exchangeable (11.7%) > Residual (4.3%) > Bound to Fe and Mn (2.0 %)
Bound to carbonate (1.6%) > Bound to organic and inorganic matter (0.5%)

Results revealed that for this treated sludge, Cr, Cu , Ni and Pb do not seem to pose a problem of mobility once disposed to land because of their relatively high proportion of residual forms. However, Cd and Pb which seem to predominate in exchangeable and carbonate forms may affect the mobility of these metals to the environment. As discussed in previous sections, metals in the exchangeable and carbonate bound are comparatively mobile, while residual fractions which are tightly bound to the sludge are less mobile once the treated sludge is disposed to land.

4.5.2.5 Sludge Treated with *A. niger* Fermented Raw Liquid at pH 3.88 (AF2 Treated)

Results of the SCE studies for sludge treated with *A. niger* fermented raw liquid at pH 3.88 is shown in Figure 4.23. The actual amount of metals extracted for each form of metals analyzed is presented in Appendix H-5.

As shown, for Cd, compared with the untreated sludge, only the exchangeable form of the metal was detected at lesser proportion. For Cr, the residual form of the metal seemed to predominate completely at a much lesser proportion (19.9%). Copper still predominates in residual form at a lesser proportion of 26.4% but with an observed increase in the exchangeable form to 3.7%. Lead metal seemed to be present only in exchangeable form in contrast with the untreated sludge where it is present in three forms (bound to organic and inorganic matter, residual and bound to carbonate) and not in exchangeable form. Only two forms (residual and exchangeable) of Ni at a lesser proportion (18.9% and 1% respectively) were observed in the sludge treated with *A. niger* fermented liquid at pH 3.88, in contrast to the untreated sludge where almost all forms of metals were present. For Zn, not all forms of the metal were detected in contrast to the untreated sludge. The exchangeable form of the metal (at 7.14%) predominates while all the other forms are of much lesser proportion.

The rankings for the different forms of all metals are summarized as follows:

Cd**:** Exchangeable (12.1%)
Cr: Residual (19.9%)
Cu**:** Residual (26.4%) > Exchangeable (3.7%)

Pb: Exchangeable (12.6%)
Ni**:** Residual (18.9%) > Exchangeable (0.97%)
Zn**:** Exchangeable (7.14%) > Residual (2.6%) > Bound to carbonate (1.5%) > Bound to organic and inorganic matter (1.9%)

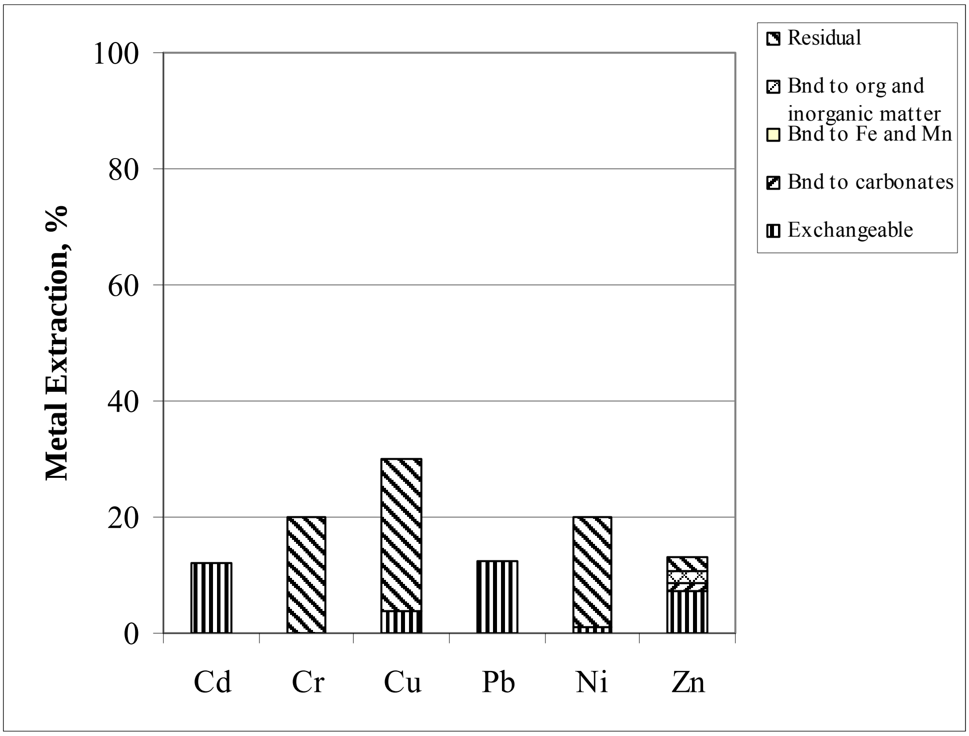


Fig 4.23 Metal Fractionation Profile of Sewage Sludge Treated with *A. niger* Fermented Raw Liquid at pH 3.88 (AF2 Treated)

For sludge treated with *A. niger* fermented liquid at pH 3.88, similar observation is made at pH 3.73, as to the mobility of the metals present in the treated sludge once disposed to land. Chromium, Cu , Ni and Pb do not seem to pose a problem of mobility because of their relatively high proportion of residual forms. However, Cd and Pb which seem to predominate in exchangeable form may affect the mobility of these metals to the environment.

4.5.3 Leaching with Various Extractants

Figure 4.24 and Table 4.14 present the results of leaching at optimum conditions with the following extractants: commercial citric acid or CA (1.57 mg/L), naturally fermented raw liquid or NF (1.7 mg/L), *A. niger* fermented raw liquid at pH 3.73 or AF1 (13.3 mg/L), and *A. niger* fermented raw liquid at pH 3.88 or AF2 (6 mg/L).

For Cd, the highest removal of 64.6 % was attained using *A niger* fermented liquid at pH 3.73 at 5 days leaching time, followed by citric acid at 52.5% and naturally fermented liquid at 51.6%. The least removal of 39.3% was obtained using *A. niger* fermented liquid at pH 3.88 and optimum leaching time of 5 days.

The highest removal attained for Cr metal was 83.4 % using *A. niger* fermented raw liquid at pH 3.73, followed by the same extractant at pH 3.88 (59.3% removal), both at 5 days leaching time; and citric acid at 23.4% at optimum conditions. No removal was detected using naturally fermented raw liquid at optimum conditions.

Copper removal was very minimal using all extractants at optimum conditions with citric acid attaining the highest removal of only 10%. No removal was detected using *A. niger* fermented liquid at optimum conditions.

Lead removal was highest at 30.2% using citric acid as extractant at optimum conditions. Using *A. niger* fermented liquid at pH 3.73, a very minimal removal of 5.3% was attained. No removal was detected for other extractants.

For Ni, the highest removal of 82.4% was attained using *A. niger* fermented liquid at pH 3.73 as extractant followed by the same extractant at pH 3.88, attaining about 48.1% removal, and naturally fermented raw liquid at 34.9% removal. The least removal of 18.6% was attained using citric acid as extractant.

For Zn, metal removal was attained using all extractants at optimum conditions, with all metals being removed using *A. niger* fermented liquid at pH 3.73 and 3.88. A removal of 51.6 % was attained using naturally fermented raw liquid at optimum conditions, and the least removal of 44.6% was attained using commercial citric acid.

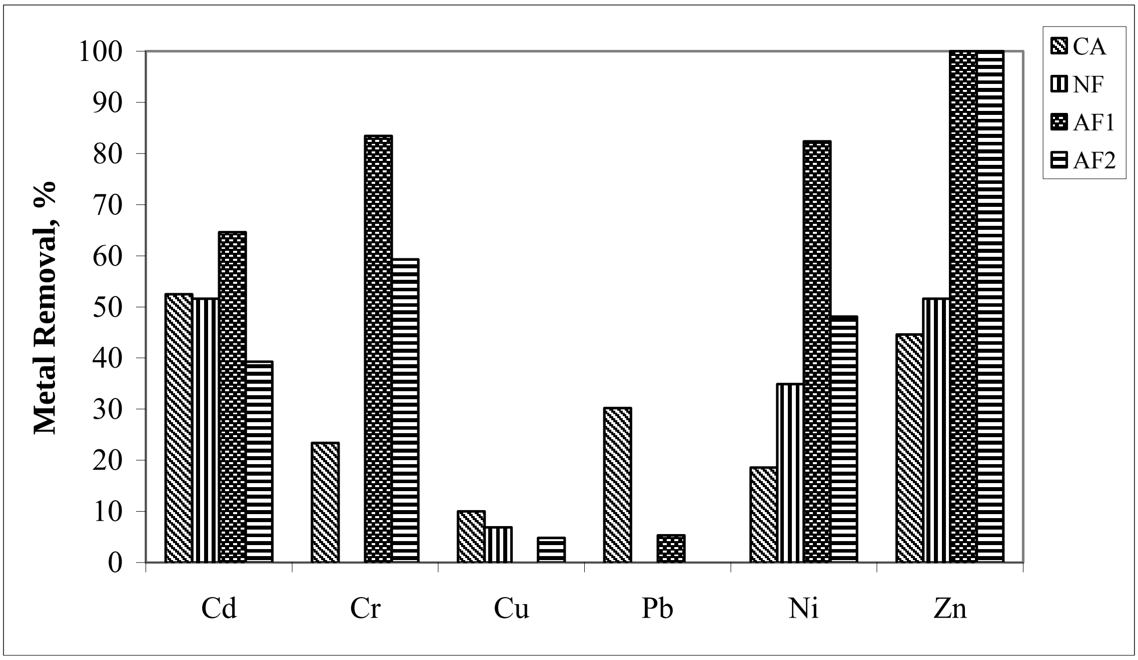


Figure 4.24 Metal Removal at Optimum Conditions

A comparison of performance of various extractants at optimum conditions with earlier studies (summarized in section 4.4) as shown in Table 4.14, revealed wide variation in terms of metal removal efficiencies. The use of commercial citric acid seemed to give better removal efficiencies in earlier study, especially for Pb, which may be due to a very minimal change in pH after optimum leaching time of 1 day, and higher acid dosage, compared to this study. For leaching with naturally fermented raw liquid, the same trend was observed, and removal of more metals seemed to be attained in earlier study. For leaching with *A. niger* fermented liquid both at pH 3.73 and 3.88, the trend in metal removal did not seem to vary much, although a higher extractant dosage and lower pH in earlier studies, may have resulted in the removal of some Cu and Pb, which were not detected in this study. Moreover, it was generally observed that leaching with lesser amount (two grams) of sludge required a higher dosage of extractants to adjust the pH to the desired value, as compared with seven grams sludge. Although this seems to imply less extractant usage for leaching with large quantity of sludge, unfavorable change of pH would decrease removal efficiency over time. It should be noted however, that comparison of removal efficiencies between studies may not be practical since the sludges used in both studies were collected at different times and therefore vary in physico-chemical characteristics.

Table 4.14 Comparison of Heavy Metal Removals at Optimum Conditions

Extractants	Dosage (v/w)	Leaching Time (day)	pH at Leaching	pH after Leaching	*Metal Removal (mg/Kg)*					
					Cd	**Cr**	**Cu**	**Pb**	**Ni**	**Zn**
[1]CA [5](A)	1.57	1	3.04	5.7	2.5 (52.5%)	61.7 (23.4%)	303.7 (10%)	52.9 (30.2%)	55.4 (18.6%)	913.6 (44.6%)
[6](B)	7.86	”	’’	3.6	1.4 (52%)	168.91 (45.7%)	1,230.82 (29.8%)	138.5 (~100%)	60.92 (48%)	1,359 (70%)
[2]NF (A)	1.7	1	3.67	4.1	2.5 (51.6%)	[7]ND	210.4 (7%)	ND	104 (35%)	984 (51.6%)
(B)	45	”	’’	3.4	10.7 (41.8%)	101.5 (25.1%)	488.9 (33%)	ND	117.6 (~100%)	1,517.4 (~100%)
[3]AF1 (A)	13.3	5	3.73	3.5	3.1 (64.6%)	220.3 (83.4%)	ND	9.3 (5.3%)	245.1 (82.4%)	2,016.4 (~100%)
(B)	40	’’	’’	3.2	14.3 (56%)	370.1 (91.6%)	687.7 (14.9%)	71.6 (34.1%)	195.8 (89%)	2,268.6 (99%)
[4]AF2 (A)	6	5	3.88	3.1	1.9 (39.3%)	156.5 (59.3%)	145.1 (4.8%)	ND	143.3 (48.1%)	2,064.1 (~100%)
(B)	8.57	”	’’	2.1	22.7 (84.8%)	335.5 (83%)	2,848.5 (62%)	28.4 (13.5%)	125.1 (56.9%)	2,046.9 (89.4%)

[1]CA – Citric acid
[2]NF – Naturally fermented raw liquid from pineapple wastes
[3]AF1 – *A. niger* fermented raw liquid from pineapple wastes (pH 3.73)
[4]AF2 - *A. niger* fermented raw liquid from pineapple wastes (pH 3.88)
[5] (A) – this study
[6] (B) – previous study
[7]ND – Not detected

Chapter 5
Conclusion and Recommendations

5.1 Conclusion

The following conclusions are drawn from the study:

Results of the sludge characterization study showed wide variation in the sludge characteristics especially on the quantities of heavy metals in sludge, which could be mainly due to the nature of the wastewater treated. The high organic matter and nutrient content (in terms of nitrogen and phosphorous) of the sludge indicated its appropriateness for agricultural applications, although the presence of heavy metals can pose a long term environmental hazard once disposed to land.

Sequential chemical extraction (SCE) studies showed a wide variation in metal forms present in the sample sludge which could be due to the nature of the individual metal, the characteristics of the wastewater treated and the sludge treatment employed. For both the dewatered and wet digested sludge samples, Cr, Cu and Ni seem to predominate in residual fractions, although for Ni the presence of other forms such as bound to Fe and Mn, bound to carbonate and exchangeable forms, equally predominate. Cadmium, Pb and Zn on the other hand, were found mostly in the bound to organic and inorganic matter forms. Metal forms other than residual seemed to exhibit ease of leachability from sludge. In terms of mobility after land application of sludge, exchangeable and carbonate bound metal forms are readily leached especially at low pH soil and therefore the most mobile.

Results of all leaching studies with various extractants (commercial citric acid, unfermented, naturally fermented, and *A. niger* fermented raw liquid from pineapple wastes) showed a wide variation of removal efficiencies for all metals which could be attributed to the nature of the sludge sample, pretreatment of sludge, extraction conditions (pH, contact time) and forms of metals present. In general, lower pH and longer contact times favor metal removal. Most metals which are predominantly in the residual form exhibited low leachability, although for some metals, enhanced metal removal from sludge was observed due to the chelating properties of citric acid in the extractants.

Leaching with citric acid at pH 3-4 and various contact times, and unfermented raw liquid from pineapple wastes at pH 3-4 and two hours contact time, seemed to result in a relatively high removal for Cd, Pb, Ni and Zn. For naturally fermented liquid, a relatively high removal was attained for Cd, Cr, Ni and Zn, while *A. niger* fermented liquid seemed to be effective in the removal of Cd, Cr, Cu, Ni and Zn at pH 3 - 4.5 and various contact times. The ability of the unfermented and fermented liquid to extract heavy metals from sludge may be due to the presence of citric acid and other carboxylic acids, as confirmed by the total acidity, IR spectroscopy and HPLC studies of the liquid.

Leaching studies for all extractants revealed that the most readily solubilized metal is Zn. This might be due to the predominance of the more soluble bound to carbonates and Fe and Mn forms of the metal. Overall, effective metal removal seemed to be in the following order: Zn>Ni>Cd>Cr>Cu>Pb, which in general, also describes the increasing order of the organic-metal bond strength.

At selected optimum conditions, *A. niger* fermented raw liquid from pineapple wastes seemed to be the best extractant for the removal of Cd (64.6%), Cr (83.4%), Ni (82.4%) and Zn (100%) at pH 3.73, while commercial citric acid seemed to be best for the removal of Cu and Pb at pH 3.04. Moreover, *A. niger* fermented liquid seemed to exhibit effectiveness in the removal of bound to Fe and Mn form of Cd, residual and bound to organic and inorganic matter form of Cr, and most metal forms including residual form of Ni and Zn. The superiority of the *A. niger* fermented liquid over the other extractants for metal removal, seemed to be due to the presence of relatively high amount of citric acid as confirmed by the infrared (IR) spectroscopy and HPLC analyses.

The sludge treated with the different extractants seemed to exhibit a high potential for land application due to the following: (i) improvement in treated sludge characteristics especially in terms of decrease in pathogen and heavy metals content which were low enough to comply with regulatory standards for land application; and (ii) dominance of residual fractions in most metals (Cr, Cu and Ni) present in the treated sludge. However, the high organic matter content of the treated sludge might pose odor problem once disposed to land and hence may need to undergo further treatment or stabilization such as air drying, for several days before final disposal. Furthermore, the treated sludge may also be applied by subsurface injection to minimize odor problem.

The main drawback of the process seemed to be its inefficiency in the extraction of the Cu metal. It was observed that even after treatment with *A. niger* fermented liquid at optimum conditions, Cu concentration (2,025.6 mg/Kg DM) in the sludge still exceeded the recommended BMA standard (900 mg/Kg DM). However, this may not seem to pose a serious problem since after treatment, Cu metal still predominates in residual form and hence comparatively immobile.

The effectivity of the extraction process in the removal of heavy metals from sewage sludge as revealed in this study, seemed to indicate its potential for use in industrial wastewater sludges.

Finally, it can be concluded that this study demonstrated a novel process which provides a sustainable way of managing contaminated sludge since one form of wastes (pineapple wastes) is utilized to treat another form of wastes (sludge). Moreover, besides the extracted metals, practically no wastes is produced in the process since the by-products such as pineapple pulp and mycelium, can be used as animal feed.

5.2 Recommendations for Future Research

Based on the results of the study, the following recommendations for future research are presented.

The present study assessed the effectiveness of the metal removal of various extractants using only anaerobically digested sludge. It is suggested that additional samples of sludges from different treatment processes such as raw and aerobically digested sludges be used to assess the applicability of the extractants in these sludge types. Moreover, variability of results should be observed as the sludge type varies, including effect of sludge age and length of sludge storage.

Studies have shown (Marchioretto, 2002), that in the case of anaerobically digested sludges, the highest heavy metals solubilization, especially Cu, can be obtained if the oxidation reduction potential (ORP) of the sludge is increased before low pH conditions are applied for acidification. This will favor the formation of soluble metal complexes, oxidizing insoluble reduced metal forms to soluble forms. It is suggested therefore, that the ORP of the sludge be determined and increased either by biological or chemical oxidation, and observe its effect in heavy metal removal by the extractants.

Since the buffer capacity gives an indication of the sludge resistance to a pH variation (Zagury et al., 1999), determination of this parameter, would be useful in assessing the effectiveness of the extractants in the removal of metals from the sludge. Buffer capacity is determined experimentally by measuring the pH change when a strong acid is added to a sludge suspension.

Although various parameters have already been analyzed in sludge characterization, a more detailed analysis would be useful to assist in determining the metal removal mechanisms from sludge. This may include determination of organic nitrogen, amines, carboxyl and sulfidal groups.

The present study used only sewage sludge sample from wastewater treatment facilities receiving mostly domestic wastewater. It is suggested that leaching experiments with the three extractants (citric acid, naturally fermented liquid and *A. niger* fermented liquid), be also done on mostly industrial wastewater sludges, to determine the applicability of the study to other types of sludge.

Since the present study was only done in the laboratory, a pilot scale study is recommended to better assess the feasibility of the leaching process. Studies can be done for both the naturally fermented and *A. niger* fermented raw liquid from pineapple wastes.

It is also recommended that pot trial or field application be done after pilot scale studies, to determine the suitability of the treated sludge for growing crops.

Since the present study dealt only with the extraction of heavy metals from the sludge using the different extractants, it is recommended that separation of the metals from the leachate be also done to determine potential for metal recovery. This may be accomplished by chemical sulfide precipitation and selective ion exchange.

Economic evaluation of the metal extraction process may be done based on pilot scale studies, to asses the economic feasibility of the implementation of the process.

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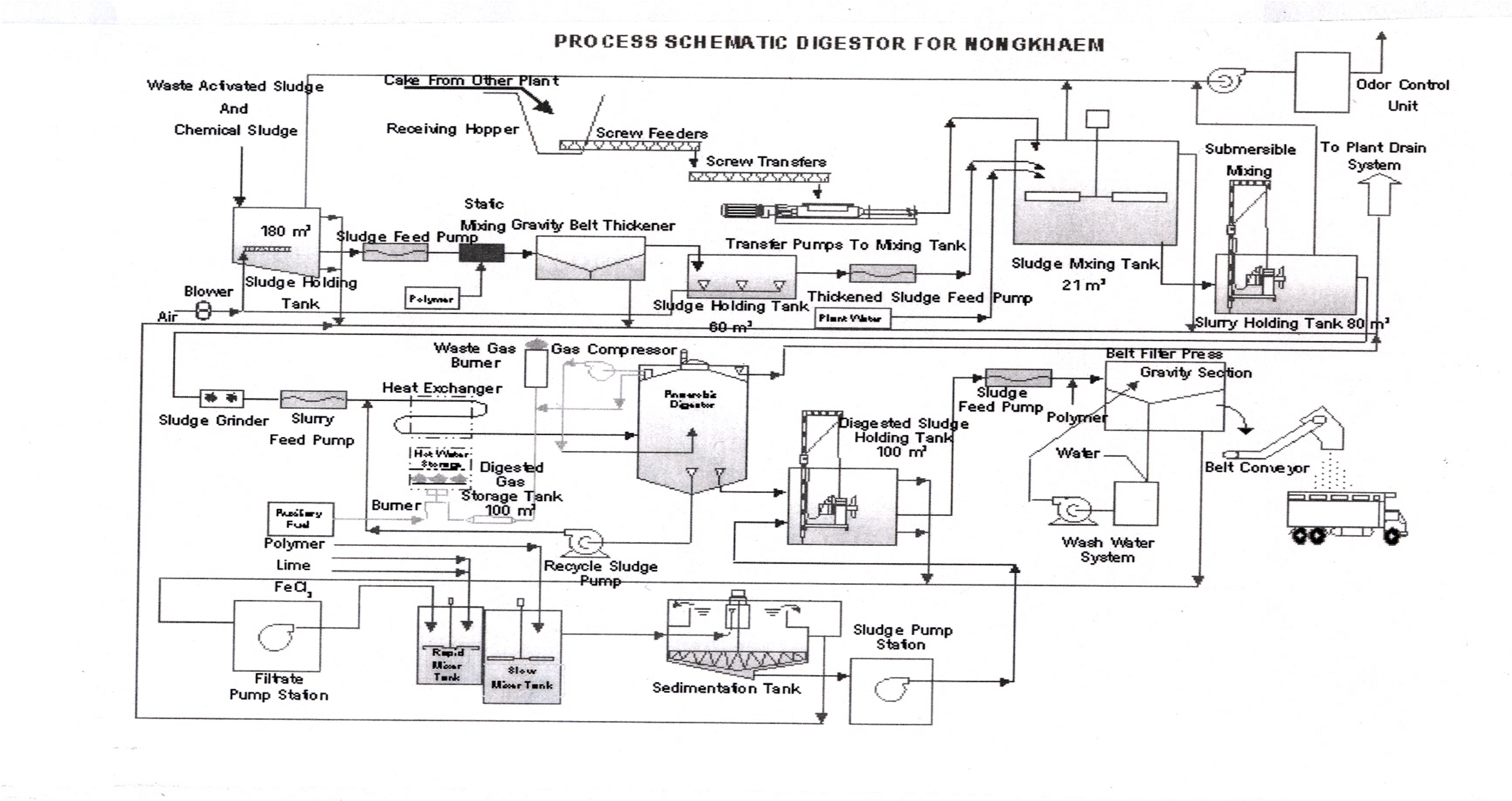
APPENDICES

Appendix A



Process Flowchart for Nongkhaem Central Sludge Treatment Facility

Appendix B

Digestion Procedure for Heavy Metals in Sludge

The microwave used in the digestion procedure was **Analytical Microwave System (AMS)** operated by the **WinWave** application software. Digestion of heavy metals in sludge was done with HCl (37%), HF (8%), HNO_3 (70%), and H_3BO_3 (5%) using the following procedure:

1. Weigh 0.2 g sample and place it in a perfluoroalkoxy polymer resin (PFA) Teflon vessel. The sample must be fine enough to pass through 100 mesh sieve. Add 2 mL of HNO_3 and 3 mL of HCl, washing down any sample that may cling to the vessel wall. After the reaction stops (wait at least 10 minutes), add 3 mL of HF and swirl the acid mixture. Place the safety disk on the vessel, ensuring that a single disk is in place, and cap the vessel.
2. Repeat this procedure until the digestion turntable contains 6 vessels. Verify that the vessels are evenly spaced within the vessel rack.
3. Ensure that the pressure control vessel is connected to the pressure control unit. The pressure control vessel should be placed in the slot nearest the oven door when connecting the pressure control transducer line.
4. The preparation blank must contain the same amount of reagent (2 mL of HNO_3, 3 mL of HCl, and 3 mL of HF) and 0.2 mL of water.
5. Select **Pressure Control Mode** from the **METHOD PANEL** in **WinWave**, and program as follows:

Stage	Power (%)	Pressure (psi)	Dwell Time (min)	Max Time
1	30	20	2:00	5:00
2	30	40	5:00	6:00
3	30	60	2:00	3:00
4	40	80	2:00	3:00
5	50	100	15:00	16:00

6. Allow vessels to cool to room temperature. Open vessels in a fume hood. Lift each vessel cap and add 40 mL H_3BO_3 solution to each of the 6 vessels. Close and recap the vessels, then return them to the microwave.
7. Select **Power Control mode** from the **METHOD PANEL** in **WinWave**, and program as follows:

Stage	Power (%)	Max Time
1	60	3:00

8. Allow the vessels to cool to room temperature. Open vessels and transfer contents to a plastic volumetric flask by rinsing the safety disk and the PFA Teflon vessel with distilled water. Filter the solution.

Appendix C-1

Results of Sequential Chemical Extraction Study for Dewatered and Wet Anaerobically Digested Sludges

Metals	Metal forms (mg/kg DM)										Total Extracted in SCE (mg/Kg)		Metal Concentration in Sludge (mg/Kg)		Percent Recovery (SCE Study)	
	Exchangeable		Bound to Carbonates		Bound to Fe and Mn		Bound to Organic and Inorganic Matter		Residual							
	Dew S	Wet S	Dew S	Wet S	Dew S	Wet S	Dew S	Wet S	Dew S	Wet S	Dew S	Wet S	Dew S	Wet S	Dew S	Wet S
Cd	ND	ND	ND	ND	ND	ND	0.8	ND	-	-	-	-	2.69	1.02	-	-
Cr	ND	ND	ND	ND	ND	ND	ND	7.2	331.2	420	331.2	427.20	369.92	430.06	89.5	99.3
Cu	166.0	1.6	71.6	ND	ND	ND	896	200	2,155.3	3,195.3	3,288.85	3396.94	4,128.8	5,727.1	79.7	59.3
Pb	1.6	38.4	33.2	42.4	54.5	58.4	142.8	174.8	28.2	2.4	260.2	316.4	105.81	165.3	246	191.4
Ni	27.6	ND	22.4	ND	59.2	29.6	ND	ND	73.8	130.8	183.00	160.40	126.49	170.64	144.7	94.0
Zn	88.0	4.0	321.6	234.4	550	492	716	892	256.55	284.63	1,932.15	1,907.03	-	-	-	-

ND = Not Detected

Note:1. For Cd, the total metals extracted in SCE could not be determined since residual form was not measured
2. For Zn, percent recovery could not be calculated since the amount of metal in the sample sludge was not determined

Appendix C-2

Results of Sequential Chemical Extraction Study for Oven-Dried and Air-Dried Anaerobically Digested Sludge Samples

Metals	Metal forms (mg/kg DM)										Total Extracted in SCE (mg/Kg)		Metal Concentration in Sludge (mg/Kg)		Percent Recovery (SCE Study)	
	Exchangeable		Bound to Carbonates		Bound to Fe and Mn		Bound to Organic and Inorganic Matter		Residual							
	Oven - Dried	Air - Dried	Oven - Dried	Air - Dried	Oven - Dried	Air - Dried	Oven - Dried	Air - Dried	Oven - Dried	Air - Dried	Oven - Dried	Air - Dried	Oven - Dried	Air - Dried	Oven - Dried	Air - Dried
Cd	0.08	3.29	0.8	3.29	0.2	ND	0.2	ND	ND	ND	1.28	6.58	NA	25.6	NA	25.8
Cr	ND	ND	ND	ND	ND	ND	14.4	ND	281.88	228.62	296.28	228.62	NA	404.2	NA	56.5
Cu	35.2	3.29	56.8	417.58	ND	ND	320.8	144.67	1,842.5	1,651.7	2,246.50	2,217.24	NA	1,479.6	NA	149.9
Pb	ND	ND	2.4	3.29	2.4	23.02	99.2	124.94	63.13	166.97	167.13	318.22	NA	318.22	NA	100
Ni	11.2	ND	24	3.29	20.8	42.74	22.4	ND	33.13	17.98	111.53	64.01	NA	78.91	NA	81.2
Zn	54.4	108.5	329.6	598.42	329.6	726.65	724	52.61	231.88	77.1	1,669.48	1,563.24	NA	1,430.3	NA	109.3

ND = Not Detected
NA = Not Analyzed

Appendix D-1

Amount of Metals Removed during Citric Acid Leaching at various pH Conditions

pH	Metal Removal (mg/Kg DM)											
	Cd		Cr		Cu		Pb		Ni		*Zn	
	Dew S	Wet S	Dew S	Wet S	Dew S	Wet S	Dew S	Wet S	Dew S	Wet S	Dew S	Wet S
2	2.4 (89.2%)	-	165.6 (44.8%)	160.95 (37.4%)	2,170.8 (52.6%)	294.35 (5.14%)	39.6 (34.4%)	31.9 (19.3%)	108 (85.38%)	49.3 (28.9%	1,312.3 (67.9%)	1,783.5 (93.5%)
3	1.06 (39.4%)	ND	55.12 (14.9%)	185 (43.0%)	914.85 (22.4%)	ND	5.3 (5.01%)	23 (13.91%)	71.02 (56.15%)	41 (24%)	1,279.95 (66.2%)	1,218 (63.9%)
4	ND	ND	ND	ND	117.3 (2.8%)	ND	ND	ND	39.95 (31.58%)	14 (8.2%)	184.9 (9.6%)	267.5 (14%)
5	ND	ND	ND	ND	78 1.94%)	ND	ND	ND	9.7 (7.67%)	16.3 (9.6%)	16.2 (0.84%)	21.5 (1.13%)
6	ND	ND	ND	ND	122.21 (2.96%)	9.09 (0.16%)	ND	ND	13.1 (10.38%)	41.3 (24.2%)	11.1 (0.57%)	13.2 (0.69%)
7	-	ND	-	ND	1070.4 (25.93%)	24.12 (0.42%)	-	ND	-	ND	-	4.8 (0.25%)
Total Metal in Sludge (mg/Kg)	2.69	1.02	369.92	430.06	4,128.75	5,726.12	105.82	165.30	126.49	170.64	1,932.55	1,907

ND = Not Detected

* The total Zn metal concentration used in the calculation of percent metal removal was the total metal extracted in SCE (Sequential Chemical Extraction Procedure) since Zn metal in the original sludge was not analyzed.

Appendix D-2A

Amount of Metals Removed during Citric Acid Leaching at Different Leaching Times (pH 2)

Leaching Time	Metal Removal (mg/Kg DM)											
	Cd		**Cr**		**Cu**		**Pb**		**Ni**		***Zn**	
	Dew S	**Wet S**	**Dew S**	**Wet S**	**Dew S**	**Wet S**	**Dew S**	**Wet S**	**Dew S**	**Wet S**	**Dew S**	**Wet S**
1h	4.8	5.8	148.8	197.2	2011.2	87	48	34.8	105.6	81.2	1,896.0	1,374.6
2h	6	5.8	204	179.8	2,532.0	133.4	72	40.6	102	63.8	1,972.8	1,661.7
6h	4.8	5.8	225.6	173.52	2,764.8	609	67.2	58.0	105.6	46.4	2,121.6	1,609.5
1 d	6.0	5.8	348	290.0	3,654.0	1,925.6	72.0	52.2	ND	ND	2,320.8	1,445.7
5 d	4.8	5.8	470.4	440.8	3,508.8	3,636.6	52.8	69.6	124.8	ND	2,107.2	2,668
8 d	6.0	5.8	396	411.8	2,832.0	2,169.2	30	29	ND	ND	2,335.2	2,372.2
11 d	4.8	5.8	379.2	394.4	1,017.6	319	100.8	ND	14.4	29	739.2	2,148.9
Total Metal in Sludge (mg/Kg)	2.69	1.02	369.92	430.06	4,128.75	5,726.12	105.82	165.30	126.49	170.64	1,932.55	1,907

ND=Not Detected

* The total Zn metal concentration used in the calculation of percent metal removal was the total metal extracted in SCE since Zn metal in the original sludge was not analyzed.

Appendix D-2B

Amount of Metals Removed during Citric Acid Leaching at Different Leaching Times (pH 3)

Leaching Time	Metal Removal (mg/Kg DM)											
	Cd		Cr		Cu		Pb		Ni		*Zn	
	Dew S	**Wet S**	**Dew S**	**Wet S**	**Dew S**	**Wet S**	**Dew S**	**Wet S**	**Dew S**	**Wet S**	**Dew S**	**Wet S**
1h	1.4	1.5	80.301	93.04	843.16	ND	19.38	16.24	69.23	47.26	1,255.47	552.32
2h	2.8	1.9	121.84	121.84	1,024.53	ND	72.0	16.61	88.61	24.00	1,552.86	764.98
6h	1.4	3.7	ND	173.52	1,032.84	ND	89.99	11.08	51..23	22.15	303.48	1,284.82
1 d	1.4	1.5	168.91	196.41	1,230.82	524.26	138.45	50.21	60.92	59.07	1,359.03	1,445.79
5 d	2.8	3.7	41.54	380.28	ND	535.34	ND	84.92	ND	ND	ND	1,101.69
8 d	2.8	ND	19.38	131.07	ND	ND	ND	ND	ND	ND	ND	ND
11 d	ND	0.74	16.61	101.90	ND	5.17	ND	ND	ND	ND	ND	ND
Total Metal in Sludge (mg/Kg)	2.69	1.02	369.92	430.06	4,128.75	5,726.12	105.82	165.30	126.49	170.64	1,932.55	1,907

ND= Not Detected

* The total Zn metal concentration used in the calculation of percent metal removal was the total metal extracted in SCE (Sequential Chemical Extraction Procedure) since Zn metal in the original sludge was not analyzed.

Appendix D-2C

Amount of Metals Removed during Citric Acid Leaching at Different Leaching Times (pH4)

Leaching Time	Metal Removal (mg/Kg DM)											
	Cd		Cr		Cu		Pb		Ni		*Zn	
	Dew S	Wet S	Dew S	Wet S	Dew S	Wet S	Dew S	Wet S	Dew S	Wet S	Dew S	Wet S
1h	1.1	1.14	2.13	7.95	350.39	ND	ND	ND	60.71	31.81	193.83	102.01
2h	2.1	2.3	ND	9.01	264.12	ND	ND	ND	49.0	20.45	149.74	91.33
6h	2.13	0.23	ND	ND	210.87	ND	ND	ND	44.73	25.0	90.53	144.27
1 d	2.13	2.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5 d	4.26	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8 d	2.13	2.3	ND	ND	ND	4.54	ND	ND	ND	ND	ND	ND
11 d	4.8	1.14	ND	ND	ND	60.21	ND	ND	ND	ND	ND	1.59
Total Metal in Sludge (mg/Kg)	2.69	1.02	369.92	430.06	4,128.75	5,726.12	105.82	165.30	126.49	170.64	1,932.55	1,907

ND = Not Detected

* The total Zn metal concentration used in the calculation of percent metal removal was the total metal extracted in SCE (Sequential Chemical Extraction Procedure) since Zn metal in the original sludge was not analyzed.

Appendix E

Amount of Metals Removed from Dewatered sludge during Unfermented Raw Liquid Leaching at Various pH Conditions

pH	Metal Removal (mg/Kg DM)					
	Cd	Cr	Cu	Pb	Ni	Zn
3.94	23.2	11.6	377	37.7	95.7	768.5
4.0	5.2	21.45	309.4	3.9	37.7	261.3
5.07	3.6	ND	171.45	2.7	18.9	142.2
6.02	5.06	ND	48.3	5.06	8.28	29.9
*Total Metal in Sludge (mg/Kg)	26	404.42	1,479.6	100	220	1,775

* After six (6) months of sludge storage
ND = Not Detected

Appendix F-1

Amount of Metals Removed from Dewatered sludge during Naturally Fermented Raw Liquid Leaching at Various pH Conditions

pH	Metal Removal (mg/Kg DM)					
	Cd	**Cr**	**Cu**	**Pb**	**Ni**	**Zn**
3.67	16.03	101.52	245.78	ND	125.56	1,634.96
3.82	4.7	ND	192.59	ND	32.88	892.48
4.02	ND	ND	101.28		13.21	700.18
4.24	ND	ND	ND	ND	ND	184.95
4.36	ND	ND	ND	ND	ND	177.76
*Total Metal in Sludge (mg/Kg)	26	404.42	1,479.6	100	220	1,775

*After six (6) months of sludge storage
ND = Not Detected

Appendix F-2A

Amount of Metals Removed during Aerobically Fermented Raw Liquid Leaching at Different Leaching Times (pH =3.67)

Leaching Time	Metal Removal(mg/Kg DM)					
	Cd	**Cr**	**Cu**	**Pb**	**Ni**	**Zn**
2h	16.03	101.52	245.78	ND	125.56	1,634.96
6h	10.69	80.15	376.68	ND	101.52	1,431.92
1d	10.69	101.52	488.88	ND	127.6	1,650
5d	8.01	192.35	ND	ND	109.53	1,512.10
8d	42.74	224.41	ND	24.04	120.22	1,426.58
11d	10.69	293.87	ND	37.40	114.87	1,549.47
*Total Metal in Sludge(mg/Kg)	26	404.42	1,479.6	100	220	1,775

*After six (6) months of sludge storage
ND = Not detected

Appendix F-2B

Amount of Metals Removed during Aerobically Fermented Raw Liquid Leaching at Different Leaching Times (pH =3.82)

Leaching Time	Metal Removal(mg/Kg DM)					
	Cd	**Cr**	**Cu**	**Pb**	**Ni**	**Zn**
2h	4.70	ND	192.59	ND	32.88	892.48
6h	ND	ND	244.26	ND	74.02	977.03
1d	ND	ND	ND	ND	9.39	958.24
5d	ND	ND	ND	ND	ND	ND
8d	9.39	ND	ND	ND	ND	ND
11d	ND	ND	ND	ND	ND	ND
*Total Metal in Sludge(mg/Kg)	26	404.42	1,479.6	100	220	1,775

*After six (6) months of sludge storage
ND = Not detected

Appendix F-2C

Amount of Metals Removed during Aerobically Fermented Raw Liquid Leaching at Different Leaching Times (pH =4.02)

Leaching Time	**Metal Removal(mg/Kg DM)**					
	Cd	**Cr**	**Cu**	**Pb**	**Ni**	**Zn**
2h	ND	ND	101.28	ND	13.21	700.18
6h	ND	ND	44.04	ND	ND	471.20
1d	ND	ND	ND	ND	ND	413.94
5d	ND	ND	ND	ND	ND	ND
8d	ND	ND	ND	ND	ND	ND
11d	ND	ND	ND	ND	ND	ND
*Total Metal in Sludge(mg/Kg)	26	404.42	1,479.6	100	220	1,775

*After six (6) months of sludge storage
ND = Not detected

Appendix G-1

Amount of Metals Removed from Dewatered sludge during Fermented Raw Liquid Leaching at Various pH Conditions

pH	Metal Removal (mg/Kg DM)					
	Cd	**Cr**	**Cu**	**Pb**	**Ni**	**Zn**
3.73	4.78	202.98	1,781.15	38.208	152.83	1,647.72
3.88	ND	56.86	1,165.57	ND	39.8	1085.97
3.98	ND	ND	609.51	ND	ND	600.414
4.47	ND	ND	324.29	ND	13.37	160.47
4.51	ND	ND	215.39	ND	ND	88.11
Total Metal in Sludge (mg/Kg)	25.6	404.42	4,610	210	220	2,290

ND = Not Detected

Appendix G-2A

Amount of Metals Removed during A. niger Fermented Raw Liquid Leaching at Different Leaching Times (pH =3.73)

Leaching Time	Metal Removal (mg/Kg DM)					
	Cd	**Cr**	**Cu**	**Pb**	**Ni**	**Zn**
2h	4.78	202.98	1,681.15	38.21	152.83	1,647.72
6h	4.78	262.68	2,134.87	52.54	181.49	1,993.98
1d	ND	300.89	1,905.62	ND	167.16	2,017.86
5d	14.33	370.14	687.74	71.64	195.82	2,268.6
8d	11.94	346.26	9.55	23.88	176.71	2,185.02
11d	4.78	410.74	410.74	4.78	205.37	2,471.58
Total Metal in Sludge (mg/Kg)	25.6	404.42	4,610	210	220	2,290

ND = Not detected

Appendix G-2B

Amount of Metals Removed during A. niger Fermented Raw Liquid Leaching at Different Leaching Times (pH =3.88)

Leaching Time	Metal Removal (mg/Kg DM)					
	Cd	**Cr**	**Cu**	**Pb**	**Ni**	**Zn**
2h	ND	56.86	1,165.57	ND	39.8	1,085.97
6h	ND	96.66	1,188.31	ND	102.34	1,501.03
1d	ND	159.2	1,563.57	ND	180.77	1,728.46
5d	22.74	335.46	2,848.54	28.43	125.09	2,046.86
8d	5.69	329.77	3,007.74	5.69	142.14	1,552.2
11d	2.27	341.14	341.14	3.41	125.09	2,160.57
Total Metal in Sludge (mg/Kg)	25.6	404.42	4,610	210	220	2,290

Appendix G-2C

Amount of Metals Removed during A. niger Fermented Raw Liquid Leaching at Different Leaching Times (pH =3.98)

Leaching Time	Metal Removal (mg/Kg DM)					
	Cd	**Cr**	**Cu**	**Pb**	**Ni**	**Zn**
2h	ND	ND	609.51	ND	ND	600.41
6h	ND	ND	618.61	ND	72.78	582.22
1d	9.1	ND	1,237.2	ND	54.58	1,182.63
5d	36.4	327.49	3,011.15	54.58	36.39	2,055.95
8d	18.19	382.08	2,974.77	ND	27.29	1,819.43
11d	1.82	427.57	427.56	ND	27.29	1,455.54
Total Metal in Sludge (mg/Kg)	25.6	404.42	4,610	210	220	2,290

Appendix H-1

Results of SCE study for Untreated Sludge

Metals	Metal Forms (mg/Kg)					Total Extracted in SCE (mg/Kg)	Metal Concentration in Sludge (mg/Kg)	Percent Recovery (SCE Study)
	Exchangeable	Bound to Carbonate	Bound to Fe and Mn	Bound to Organic and Inorganic Matter	Residual			
Cd	1	1	1.7	0.9	1.1	5.7	4.8	118.75
Cr	ND	ND	ND	20.8	384.3	405.1	264	153.45
Cu	15.6	187.1	ND	112.6	2914.6	3229.9	3,043.20	106.14
Pb	ND	1.7	ND	95.5	46.5	143.7	175.2	82.02
Ni	22.2	43	92.7	ND	96.3	254.2	297.6	85.42
Zn	91.8	453.8	835.7	138.6	184	1703.9	1908	89.30

Appendix H-2

Results of SCE study for CA Treated Sludge

Metals	Metal Forms (mg/Kg)					Total Extracted in SCE (mg/Kg)	Metal Concentration in Sludge (mg/Kg)	Percent Recovery (SCE Study)
	Exchangeable	Bound to Carbonate	Bound to Fe and Mn	Bound to Organic and Inorganic Matter	Residual			
Cd	1.4	0.9	2.3	1.2	0.7	6.5	2.4	270.83
Cr	ND	ND	ND	ND	307.5	307.5	187.2	164.26
Cu	4.7	135.3	ND	29.2	2681.8	2851	2,937.60	97.05
Pb	ND	1.4	42	36.1	21.7	101.2	156	64.87
Ni	35.9	2.3	53.6	ND	80.2	172	228	75.44
Zn	326.5	275.2	396.4	5.8	113	1116.9	1166.4	95.76

ND = Not Detected

Appendix H-3

Results of SCE study for Sludge Treated with Naturally Fermented Raw Liquid from Pineapple Wastes

Metals	Metal Forms (mg/Kg)					Total Extracted in SCE (mg/Kg)	Metal Concentration in Sludge (mg/Kg)	Percent Recovery (SCE Study)
	Exchangeable	Bound to Carbonate	Bound to Fe and Mn	Bound to Organic and Inorganic Matter	Residual			
Cd	0.9	0.5	1.1	1.1	0.7	4.3	4.3	100
Cr	ND	ND	ND	ND	282.9	282.9	175.2	161.47
Cu	123.1	75.2	ND	34.2	2326.3	2558.8	2,764.20	92.57
Pb	ND	ND	ND	77.5	30.6	108.1	175	61.77
Ni	32.8	ND	30.8	ND	74.1	137.7	194.4	70.83
Zn	371.6	175.6	535.8	22.8	106.9	1212.7	984	123.24

Appendix H-4

Results of SCE study for Sludge Treated with *A. niger* Fermented Raw Liquid from Pineapple Wastes at pH 3.73

Metals	Metal Forms (mg/Kg)					Total Extracted in SCE (mg/Kg)	Metal Concentration in Sludge (mg/Kg)	Percent Recovery (SCE Study)
	Exchangeable	Bound to Carbonate	Bound to Fe and Mn	Bound to Organic and Inorganic Matter	Residual			
Cd	0.7	0.7	ND	ND	ND	1.4	2.4	58.33
Cr	ND	ND	ND	ND	67.6	67.6	43.2	156.48
Cu	216.2	ND	ND	ND	1054.3	1270.5	2,025.60	62.72
Pb	31.3	ND	ND	ND	ND	31.3	165.9	18.87
Ni	ND	ND	ND	ND	50.1	50.1	57.6	86.98
Zn	223.7	29.8	37.3	9.3	81.6	381.7	432	88.36

Appendix H-5

Results of SCE study for Sludge Treated with *A. niger* Fermented Raw Liquid from Pineapple Wastes at pH 3.88

Metals	Metal Forms (mg/Kg)					Total Extracted in SCE (mg/Kg)	Metal Concentration in Sludge (mg/Kg)	Percent Recovery (SCE Study)
	Exchangeable	Bound to Carbonate	Bound to Fe and Mn	Bound to Organic and Inorganic Matter	Residual			
Cd	0.6	ND	ND	ND	ND	0.6	0.6	100
Cr	ND	ND	ND	ND	52.6	52.6	57.6	91.32
Cu	113.1	ND	ND	ND	802	915.1	2,587.20	35.37
Pb	22	ND	ND	ND	ND	22	175	12.57
Ni	2.9	ND	ND	ND	56.2	59.1	129.6	45.60
Zn	136.3	29	ND	36.3	49.8	251.4	484.8	51.86

Appendix I

Analysis of Raw and Fermented Liquid using IR Spectroscopy

Instrument : FT-IR Spectrometer (Perkin Elmer System 2000R)
Test method : FT-IR technique
Conditions : Light source: Middle range infrared (4000-450 cm^{-1})
Resolution : 4 cm^{-1}
Detector : TGS
Sample preparation : 1. The liquid samples were evaporated with solvent or water at oven temperature of 65 °C for 12 hours.
2. The liquid sample was then placed in a NaCl cell, and then examined using FT-IR Spectrometer.

Appendix J

(a) Wet (b) Dry (17.04% TS)

Figure J-1 Anaerobically Digested Sludge Treated with Commercial Citric Acid

(a) Wet (b) Dry (17.6% TS)

Figure J-2 Sludge Treated with Naturally Fermented Liquid at Optimum Conditions

(a) Wet (b) Dry (10.7% TS)

Figure J-3 Sludge Treated with *A. niger* Fermented Liquid (pH 3.73) at Optimum Conditions

(a) Wet (b) Dry (13.8 % TS)

Figure J-4 Sludge Treated with *A. niger* Fermented Liquid (pH 3.88) at Optimum Conditions