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Irreversible immobilization of heavy metal

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ABSTRACT
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Heavy metals are contaminants of both solid and liquid wastes. For example, the ash produced from the incineration of domestic or industrial wastes often contains substantial concentration of lead, mercury, cadmium and other heavy metals that make it a hazardous waste. These heavy metals in wastes are hazardous because they are mobile. Immobilization of the metals is an effective method of rendering the metals harmless, provided that immobilization is irreversible. This study focused on the heavy metal immobilization potential associated with the activities of anaerobic bacteria known as the dissimilatory sulfate reducers (Postgate, 1984). The sulfate-reducing bacteria produce sulfide and form highly insoluble precipitates of the metals. Cupric sulfide, cadmium sulfide, mercuric sulfide and lead sulfide are examples of these insoluble salts. Our research will have an additional advantage because it means that the biological process of immobilization of the heavy metals can be made to continue underground for many years after the treated ash has been buried thus providing extra protecting against remobilization of the metals.

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by
Hyoung-Seog Ko

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This thesis is dedicated to
Jesus Christ

ACKNOWLEDGMENT

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CHAPTER 1. INTRODUCTION

It is estimated that 197 million tons of municipal solid waste (MSW) were generated in 1990 (1). Approximately 144 million tons of this waste were disposed of in municipal landfills (1). However, the rapid disappearance of landfill space, coupled with rising costs of disposal and increasing awareness of both environmental and health dangers posed by MSW have led to the exploration of alternate methods of waste processing and disposal, particularly incineration.

Thermal destruction, i.e., incineration of solid wastes as a mean of waste management, is becoming increasingly more popular as a method of disposal for many industrial and municipal wastes. The method involves the destruction of solid wastes through oxidation, i.e., the exposure of the waste to high temperatures in the presence of oxygen. The products include exhaust gases, solid residuals (termed ash) and contaminated quench and scrubbing waters (2). A simplified flow diagram of a municipal solid waste incinerator is shown in Figure 1.1 (3).

1.1. Physical and Chemical Properties of Ash

The incineration ash produced from MSW can be further subdivided into two specific types of ash: fly ash and bottom ash. Fly ash is the gas-borne particulate residue collected from incinerator flue gases using a state-of-the-art air pollution control device.

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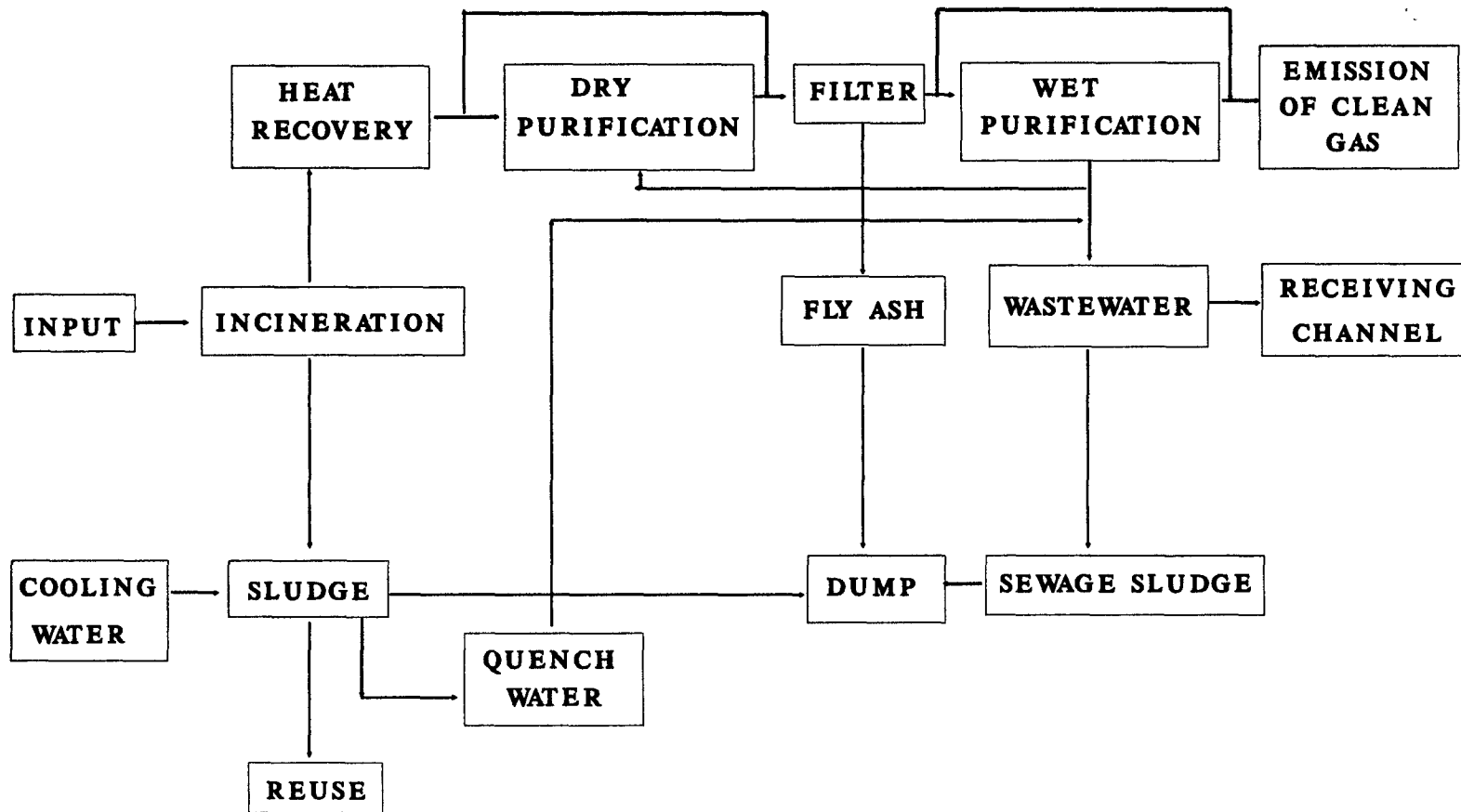
Figure 1. Flow diagram of MSW incineration

Fly ash is composed of the inorganic by-products of paper, wood, plastic, rubber and food wastes (4). Bottom ash, on the other hand, is the heavier preliminary ash residual collected from the primary combustor, quench water, and scrub water. It is composed of bottles, cans, autoparts, broken appliances and a number of other materials that are not easily burned, and accounts for 85 to 90 percent of the total ash (4). Table 1.1 lists the elemental composition of both bottom and fly ash as well as that of MSW for some selected elements (3). The physical properties of the ash are tabulated as follows (4):

Density : one to two tons per cubic yard

Specific Gravity : approximately one to three

Moisture : 15% to 25%

Grain Size : 10% similar to clay

                 40% similar to sand

                 30% similar to gravel

                 20% larger than gravel

Permeability : 10^{-3} to 10^{-4} cm/sec as landfilled

Texture : wet concrete like or wet sand and gravel-like

1.2. Ash Variation

While the reported chemical and physical properties of the ash represent the most common, or average value, it is important to realize that ash composition is quite variable and is dependent on such factors as the economic status of the area collected, and time of year. As an example, homes of moderate and affluent stature frequently

Table 1.1 Lists the Elemental Composition of both Bottom and Fly Ash

Some reported concentration ranges of elements in MSW and bottom ash, fly ash and suspended particulates from MSW incineration

Element	Concentration (μg g^{-1} unless % indicated)				Element enrichment in fine ash[e]	Possible carcinogens[f]
	MSW (combustible fraction)[a]	Bottom ash[b]	Fly ash[c]	Suspended particulates[d]		
Ag	<3–7		52–220	84–2000	x	
Al(%)	0.54–1.17	2.6–14.2	9.0–14.2	0.58–4.8		
As			9.4–74	81–510	x	x
Ba	47–447	80–9000	1600–3600	40–1700	x	
Be	<2					x
Bi	<15–30					
C(%)		1.0–28.7	1.7–7.4	1.8–2.2		
Ca(%)	0.59–1.65	3.6–11.2	3.3–8.6	—0.66–5.3	x	
Cd	4–22	3.8–442	<1–477	520–2100	x	x
Cl(%)		0.2–1.0	0.12–1.12	9.29	x	
Co	<3–5		25–54	3.8–28	x	
Cr	22–96		730–1900	122–1800	x	x
Cu	79–877	630–4281	69–2000	3000	x	
F	140–200	130–250	1500–3100	990–6800		
Fe(%)	0.10–0.35	2.1–32	2.4–8.7	0.17–1.8		
Hg	1–4.4	0.03–3.5	0.09–25	20–2000	x	
K(%)	0.09–0.19	0.42–2.41			x	
Mg(%)	0.09–0.21	0.04–0.86	0.5–2.1	0.31–2.8	x	
Mn(%)	0.005–0.02	0.08–39	0.20–0.85	0.03–0.57	x	
Mo					x	
N(%)		0–0.35	0			
Na(%)	0.18–0.74	2.3–14.2	1.12–1.94	5.1–9.8	x	
Ni	9–90	110–210	38.6–960	65–440	x	x
P(%)		0.04–0.83				
Pb(%)	0.01–0.15	0.04–0.80	0.06–0.54	2.5–15.5	x	x
S(%)		0.27–1.0	1.9–3.6	0.001–0.01		
Sb	20		139–760	610–12000	x	
Se			1.4–13	7.0–122	x	
Si(%)		4.7–9.4				
Sn(%)	<0.002–0.004	0.01–0.1	0.12–0.26	0.4–1.51	x	
Sr	11–35		110–220		x	
Ti(%)	0.14–0.31	0.04–0.90	2.5–4.2	0.13–1.29	x	
Tl				150	x	
V			110–166	6–60	x	
Zn(%)	0.02–0.25	0.35–3.61	0.08–2.6	4.7–24	x	

[a] From Haynes et al., 1977; Law and Gordon 1979, Reimann, 1986; Baccini et al., 1987.
[b] From Hrudey et al., 1974; Hocking, 1975; Brunner and Mönch, 1986; Carlsson, 1986; Lisk, 1988.
[c] From Greenberg et al., 1978a; Brunner and Mönch, 1986; Carlsson, 1986; Lisk, 1988
[d] From Greenberg, 1978b; Brunner and Mönch, 1986; Carlsson, 1986; Vogg, 1987.
[e] From Davison et al., 1974; Kaakinen et al., 1975; Klein et al., 1975; Campbell et al., 1978; Greenberg et al., 1978a,b; Smith et al., 1979; Henry and Knapp, 1980; Gounon and Milhau, 1986; Brunner and Mönch, 1986.
[f] From Goyer, 1986.

have garbage disposals, and their refuse will contain fewer foodstuffs as compared to homes without disposals. Similarly, the quantity of yard wastes incinerated will vary from season to season, with the summer and fall months producing the more copious amounts (3).

The variation does not end there, however. Other, less obvious factors may also affect the content of the ash. Polyvinyl chloride (PVC) acts as a major source of chloride in the residue. Furthermore, chlorides of such elements as As, Cd, Ni, Pb, Sb and Zn may also be formed during incineration of MSW and become part of ash. Magazines and paper products provide a source of Fe, Zn, Pb, Ba, Cr, Cu, Mg and Mo. Perhaps the most toxic element found in MSW is mercury. The element usually exists in oxidized forms such as halide and is commonly derived from the incineration of mercury batteries (3).

1.3. Advantages of Solid Waste Incineration

There are several advantages to combusting solid waste. First and foremost, raw, unprocessed municipal solid waste can be reduced in volume if incinerated to ash. In fact, ash comprises only one-tenth to one-twentieth the volume of MSW. The obvious beneficial result is long term land preservation, since ash requires less landfill space for disposal (4). But it is much more hazardous.

Raw municipal solid waste, when disposed of by conventional landfilling is subjected to biological decomposition. The by-products of this degradation include

noxious odors, methane gas, and many toxic and reactive gases. Odors, of course, are more often an annoyance than a health hazard. However, methane gas poses a substantial health risk. Its migration from poorly designed and/or operated landfills results in millions of dollars worth of damage and loss of life. In sharp contrast, incineration ash is biologically inert and products none of the odors or gases common to landfills (4).

Groundwater contamination by landfill material also poses a severe threat. Moisture contained in the waste, as well as infiltration by rain and surface water can cause leaching of toxic contaminates. Since raw municipal waste is predominately organic in nature, and acidic due to biological activity within the landfill, there is much greater potential for toxic compounds and elements to be carried by its leachate into groundwater, than from leachate from an ash landfill. In fact, ash leachate forms in only small amounts since water infiltration through dense ash is very slight. Moreover, the leachate contains only inorganic compounds (salts, metals) which move very slowly through soil. Therefore, proper hydrogeological location of the landfill, coupled with proper liners and leachate collection system greatly reduces the potential for ground water contamination over raw municipal solid waste landfills (4).

Another problems indigenous to landfills which may be reduced by waste combustion is dust. MSW, by its nature, is a dusty material. If not adequately controlled, small pieces of it can blow around. The situation is further compounded when large bulldozers are driven over the waste to compact it. In contrast, the semi-wet ash, when placed in a landfill, releases very few dust particles. Also, since the wet ash

is quite dense, it requires very little compacting and even less airborne material is released into the environment (4).

Finally, disposal of raw waste in landfills requires continuous maintenance for many years. Unlike ash residues, raw waste undergoes gradual biological decomposition resulting in a slow volume reduction over time. This continuous shrinkage causes the surface of the landfill to subside and extensive reworking of the soil is required. Ash landfills, however, produce no subsidence of the upper soil layer, and only the simplest maintenance of the final cover is required (4).

1.4. Ash Disposal

As discussed earlier, the major advantage in combusting solid waste is the reduction of the material to a much smaller volume. This allows landfill space to be used much more efficiently. However, special precautions must be taken to assure the stability of the ash in the landfill so that no hazardous leachate from the ash contaminates the environment or poses a risk to humans. To this end, the Environmental Defense Fund recommends the following disposal procedure (5).

The ash is to be disposed of in a monofill, constructed with two liners. The upper most liner, termed the primary liner, must be composed of a minimum 60-mil thickness of high density polyethylene or equivalent synthetic material. The lower liner, or secondary liner, is to be a composite liner composed of a synthetic liner identical to the primary liner. It is to rest immediately above liner constructed of compacted clay, with a minimum thickness of three feet and compacted to allow a hydraulic

conductivity of 10^{-7} cm/sec or less. Any leachate is to be collected by systems installed above and between the primary and secondary liners. The collection systems are to be tested monthly and the surrounding ground water checked for contamination. Once in the fill, the ash is to be covered with at least six inches of clay daily.

CHAPTER 2. BIOLOGICAL TREATMENT TO SOLVE THE HEAVY METAL PROBLEM IN THE ASH

The toxicity of heavy metals depends on their concentration in the soluble or ionic form and not on the total concentration. Thus, effective removal of metal ions from the soluble phase and, hence, from the biological environment should be effective in preventing toxicity to the organisms responsible for the anaerobic process. The role that sulfide can play in such a scheme of detoxification is illustrated by a consideration of the solubility of metal sulfide.

The presence or addition of sulfide to a digester containing heavy metals results in the formation of the corresponding metal sulfides which are extremely insoluble salts. This is illustrated by the solubility and solubility products given in Table 2.1.

2.1. The Sulfide Sources and Interactions under Anaerobic Condition

At present, four different microbial process are known in which hydrogen sulfide is formed as an endproduct under anaerobic conditions: 1. Ecologically the most significant process is the dissimilatory sulfate reduction of the strictly anaerobic *Desulfovibrio* and *Desulfotomaculum* species which sulfate, sulfite, thiosulfate and tetrathionate are excellent electron acceptor for sulfate-reducing bacteria, elemental sulfur cannot be reduced (Postgate, 1965). 2. Hydrogen Sulfide is liberated in the course of the anaerobic microbial degradation of sulfhydryl group containing organic compounds. 3. Hydrogen Sulfide is produced very slowly from extracellular and

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intracellular elemental sulfur by phototrophic green and purple sulfur bacteria in the course of their anaerobic fermentative dark metabolism (Lasen, 1953, Van Gemerden, 1968). 4. H_2S may be produced slowly by an incidental reduction of elemental sulfur in the course of the anaerobic fermentative metabolism of different microorganisms. It is a non-specific process in which thiosulfate, methylene blue and aldehydes may also be reduced; it is questionable whether it plays any significant role in the normal metabolism of the cell (Roy and Trudinger, 1970). Considering these results it is apparent that no chemoorganotrophic bacterium is known in which the reduction of elemental sulfur to H_2S is a regular and essential dissimilatory metabolic process for growth.

Table 2.1 Solubility of heavy metals sulfide at 18° C(6)

Heavy Metal	Sulfide Salt	Solubility Product	Solubility (mg/l)
Copper	Cu_2S	2×10^{-47}	3×10^{-11}
Copper	CuS	8.5×10^{-45}	9×10^{-18}
Lead	PbS	3.4×10^{-28}	4×10^{-9}
Cobalt	CoS	3.0×10^{-26}	2×10^{-8}
Nickel	NiS	1.4×10^{-24}	1×10^{-7}
Zinc	ZnS	1.2×10^{-23}	3×10^{-7}
Iron	FeS	3.7×10^{-19}	5×10^{-5}

H₂S gas

Soluble Sulfide

| Sulfate Reduction |
| Protein Degradtion |
| Sulfide Addition |

H₂S aq. + HS⁻
+
Heavy Metals

Metal Sulfides

Insoluble Metal
Metal Sulfide
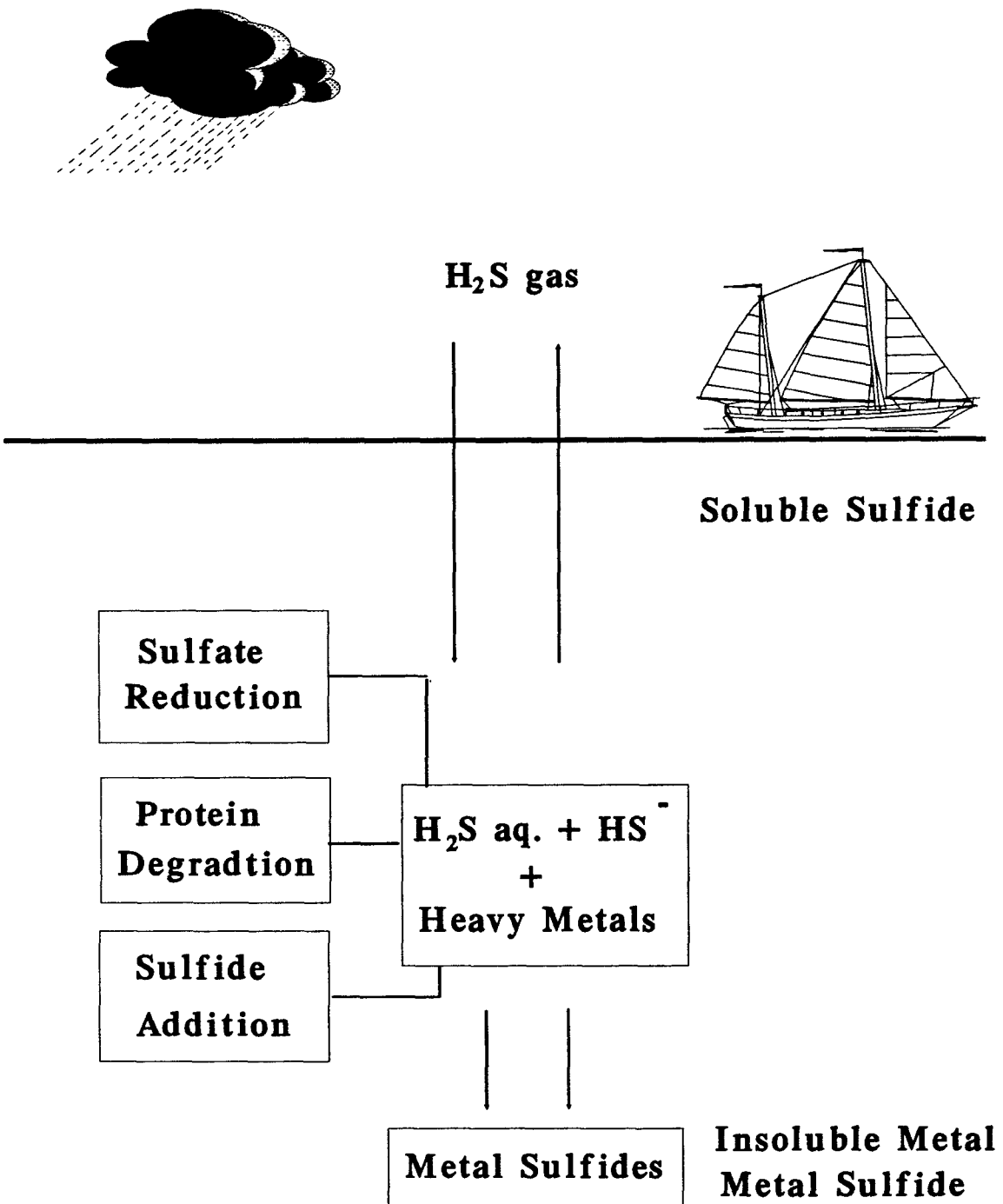
Figure 2.1 The Sulfide Source and Interactions

2.2. The Role of Sulfide in Preventing Heavy Metal Toxicity

The successful stabilization of organic wastes by the anaerobic waste treatment process has been shown to depend on the maintenance of an environment favorable to the organisms involved. The control or elimination of toxic materials is of major importance in maintaining such a favorable environment. In the realm of toxicity-producing materials, "heavy metals" long have been known to cause retardation or complete cessation of the anaerobic process.

Table 2.2 Maximum concentration of contaminants for characteristic of EP toxicity (*)

Contaminant	Regulatory Level(mg/l)
Arsenic	5.0
Barium	100.0
Cadmium	1.0
Chromium	5.0
Lead	5.0
Mercury	0.2
Silver	5.0

* [55FR 11862, Mar. 29, 1990, as amended at 55 FR 22684, June 1, 1990; 55FR 26987, June 29, 1990]
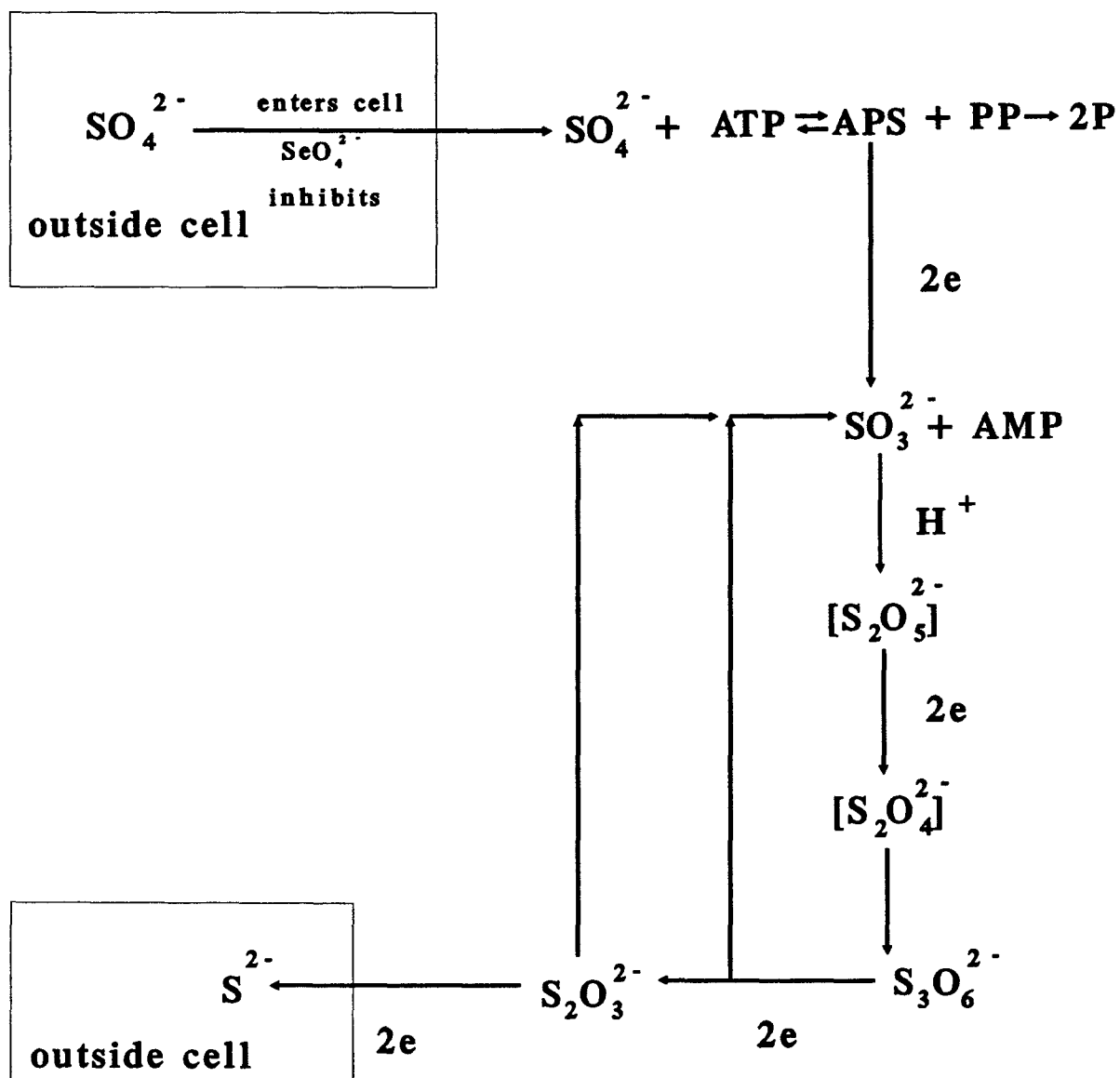
Figue 3.1 Cyclic Pathwasy for DSRs

CHAPTER 3. LITERATURE SURVEY

3.1. The History of Research on Metal Sulfide Use by Microorganisms

The effect of copper, zinc, nickel, and iron on aerobic waste treatment has received considerable attention in the past. Masselli *et al.*, (7) presented an excellent literature review of the occurrence of copper and zinc in domestic sewage and sewage sludge. In several investigations, copper was reported to exert toxic effects on anaerobic waste treatment processes (8,9,10,11); another report showed high concentrations to be nontoxic (12). Previous studies indicated that high concentrations of heavy metals can cause rapid toxicity and cessation of digestion. However, for the toxic concentrations reported, the variations may be related to the different quantities of sulfide as copper salts present in digesters as proposed for copper by Masselli *et al.* (7).

The principle that emerges from this study is that a sulfate-reducing organisms are protected from heavy metals toxicity by the sulfide they produce. This principle would apply to sulfate-reducing agents for any metal that formed an insoluble metal sulfide.

3.2. Classification of Sulfate-Reducing Bacteria

Classification of the presently known types of sulfate-reducing bacteria is based primarily on nutritional and morphological characteristics; this classification is supported by some chemical criteria, such as the guanine plus cytosine content of the DNA and the presence of special pigments. Detailed data from nucleic acid studies such as DNA/rRNA hybridization or rRNA oligonucleotide cataloging were insufficient or not available when the species and genera of sulfate-reducing bacteria were established.

The classification is complicated by the fact that morphologically similar types many differ by their nutrition, whereas nutritionally similar types may have different morphology. Also, the presence of pigments does not always correlate with nutritional and morphological groups. This complexity of features made compromises necessary in classification of the sulfate-reducing bacteria. The species and genera were established for determinative purposes and do not necessarily indicate phylogenetic relationships (13).

As in the case of many other bacteria, the genus names of sulfate reducers, apart from the prefix "Desulfo", have been derived from features observed by microscopy. However, cell shape and motility are not always constant features of strains and may vary with growth conditions (14). Typical *Desulfovibrio* species grow well on lactate that is incompletely oxidized to acetate; fatty acid are not used. For growth with hydrogen or formate, these species require acetate as a carbon source for cell synthesis (15,16,17,18).

(a) *Desulfuromonas acetoxidans*
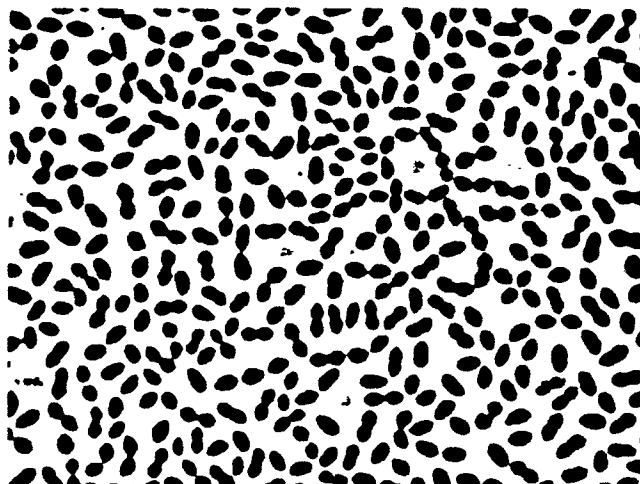
(b) *Desulfobulbus*
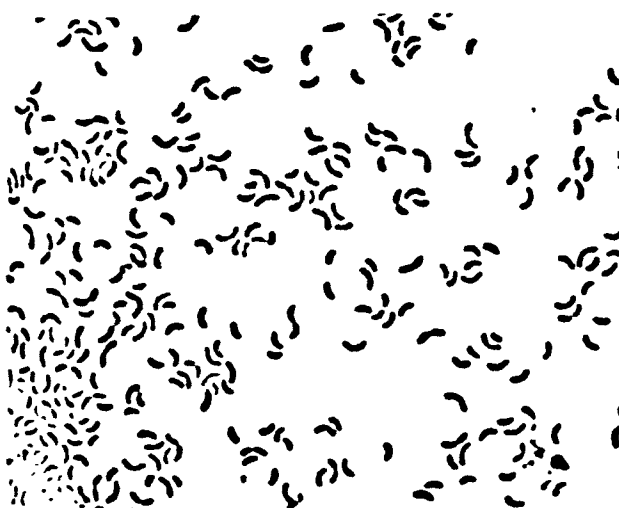
(c) *Desulfovibrio vulgaris*
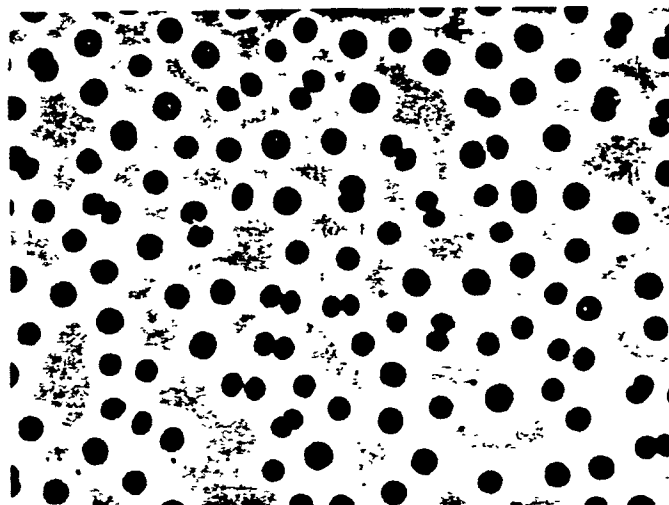
(d) *Desulfococcus multivorans*
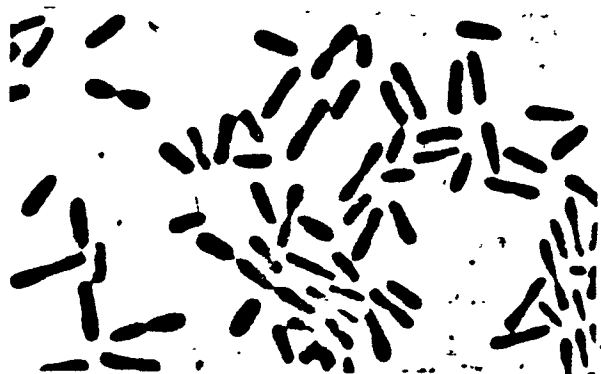
(e) *Desulfobacter sp*

(f) *Desulfosarcina variabilis.*

Figure 3.2 Representative Sulfur-Reducing Bacteria

3.3. The Leaching and Toxicity of Ash

Mass burning of Municipal Solid Waste(MSW) is practiced to reduce waste volume, preserve landfill capacity, and in some instances, to recover energy. While incineration is a feasible alternative for managing solid waste, it is essential for incinerator designers to realize that incinerator ash is not necessarily a chemically inert material. The ash may contain leachable metals and salts that, if improperly disposed, may adversely impact groundwater quality (7).

Among U.S. plants, ash residue has generally been about one-third of the weight of the waste stream. About 10 percent of the ash residue is fly ash. Thus, for each 100 tons of waste that is burned, about 3 tons of fly ash and 27 tons of bottom ash will remain (19).

As incineration has grown in popularity, so has concern over the management of increasing volumes of ash. Ashes from Municipal Waste Combustion(MWC) facilities have, on occasion, exhibited a hazardous waste characteristic as determined by the EP Toxicity Test. The debate regarding the regulatory status of ash and the representativeness and validity of EP test continues. Congress is considering several legislative initiatives that would give EPA clear authority to develop special management standards for ash under subtitle D of RCRA (20).

Acid rainfall can mobilize trace metals, although extensive data on leaching by rain water are scanty. In laboratory experiments on leaching potentials, it has been observed that 5-30% of the toxic elements initially contained in the ash, especially Cd, Cu, and Pb are leachable. The leachability is dependent on the pH of the make-up

waters, bonding of element to fly ash, and diffusivity of each species. With a lowering of pH, the release of soluble trace metals increases and causes concern to the biotic ecosystem. Solubility plays an important role in trace element concentration levels in the aquatic environment. Toxic elements in fly ash are preferentially enriched in a thin layer at the particle's surface, and may be more readily leached (5-40%) in water than the bulk ash constituents (21).

Boyle *et al* (22), studied foundry wastes and concluded that the extraction of Cd and Pb were highly pH dependent. Wastes containing smaller particles contain more Cd or Pb because of their increased surface area. They also found that there was basically no relationship between the percentage of Cd or Pb released in the EP test and concentration of metal in the waste, i.e., the metal content of a waste did not relate directly to the level or amount of metal in the EP test.

CHAPTER 4. MATERIALS AND METHODOLOGY

4.1. Source of the Microorganisms Used in This Study

Soil sediment samples were taken from a wetland area in the Hackensack Meadowland (NJ), adjacent to the Bergen County Landfill. They were collected in plastic screw top containers at a depth of one foot. Care was taken to ensure that there was no headspace in the collecting vessel, in order to prevent the sediment from being exposed to the atmosphere. Since at the time of collection, weather conditions were cold, the sample was maintained under refrigeration until the time of inoculating the medium. Medium was inoculated as soon as possible with a maximum lag time of 48 hours.

Culture Medium

The first experimental step in this work was to find a suitable energy source (carbon source) for *Desulfovibrio* dealing with the ash. To do this, a live inoculum of *Desulfovibrio* (from the Hackensack Meadowlands (NJ)) was placed into a series of glass bottles (160 ml) containing either ash or no ash each with different carbon sources. These carbon sources were the organic compound that fed the bacteria. The compound can either feed the Dissimilatory Sulfate Reducers (DSRs) directly or can feed other bacteria (fermentative bacteria) that produce fermentation products that feed the DSRs carbon source used; sodium lactate, sodium succinate, cellulose and, no carbon sources. 2 bottles (160 ml) were prepared for each of the 4 carbon source conditions.These bottles were separated into two sets; one set with no ash, the other set

Table 4.1 DSRs Enrichment Cultures Experimental Design

Culture #	Ash (gm)	Inoculum (ml)	water (ml)	Medium(ml)
1	10	3	125	32(with Na lactate)
2	10	3	125	32(with Na succinate)
3	10	3	125	32(with cellulose)
4	10	3	125	32(no carbon source)
5	-	3	125	32(with Na lactate)
6	-	3	125	32(with Na succinate)
7	-	3	125	32(with cellulose)
8	-	3	125	32(no carbon source)
9	-	-	128	Medium B

Note; Bottle 9 was set up as a control 8 days after the other culture bottles were set up.

Note: Volume of liquid in each bottle is 160 ml.

* Medium; Table 4.2

with ash. Therefore, this experimental design used 8 bottles (Table 4.1). In this work, a modified Medium B (x10 fine concentration) was used in which the reducing agents, ascorbic acid and thioglycollic acid originally present in medium B were substituted with sodium sulfide (Table 4.2).

Table. 4.2 Medium B for sulfate-reducing bacteria (14)

Salts	Concentration(g/l)
KH_2PO_4	0.5
NH_4Cl	1
$CaSO_4$	1
$MgSO_4.7H2O$	2
Yeast extract	1
Ascorbic Acid	0.1
Thioglycollic Acid	0.1
$FeSO_4.7H2O$	0.5

Note; 2 liters of distilled water, was added, the reaction was adjusted to between pH 7.0 and 7.5 with 1N HCl.

Note; Sodium Lactate(60% of con.), Cellulose(filter paper), Sodium Succinate(0.03M) were used for carbon sources.

Medium B contained a precipitate that is supposed to aid the growth of tactophilic strains. It was also ideal for long term storage of the cultures. All the

ingredients except the reducing agent were added together and the pH adjusted to between 7.0 and 7.5 with 1 N HCl.
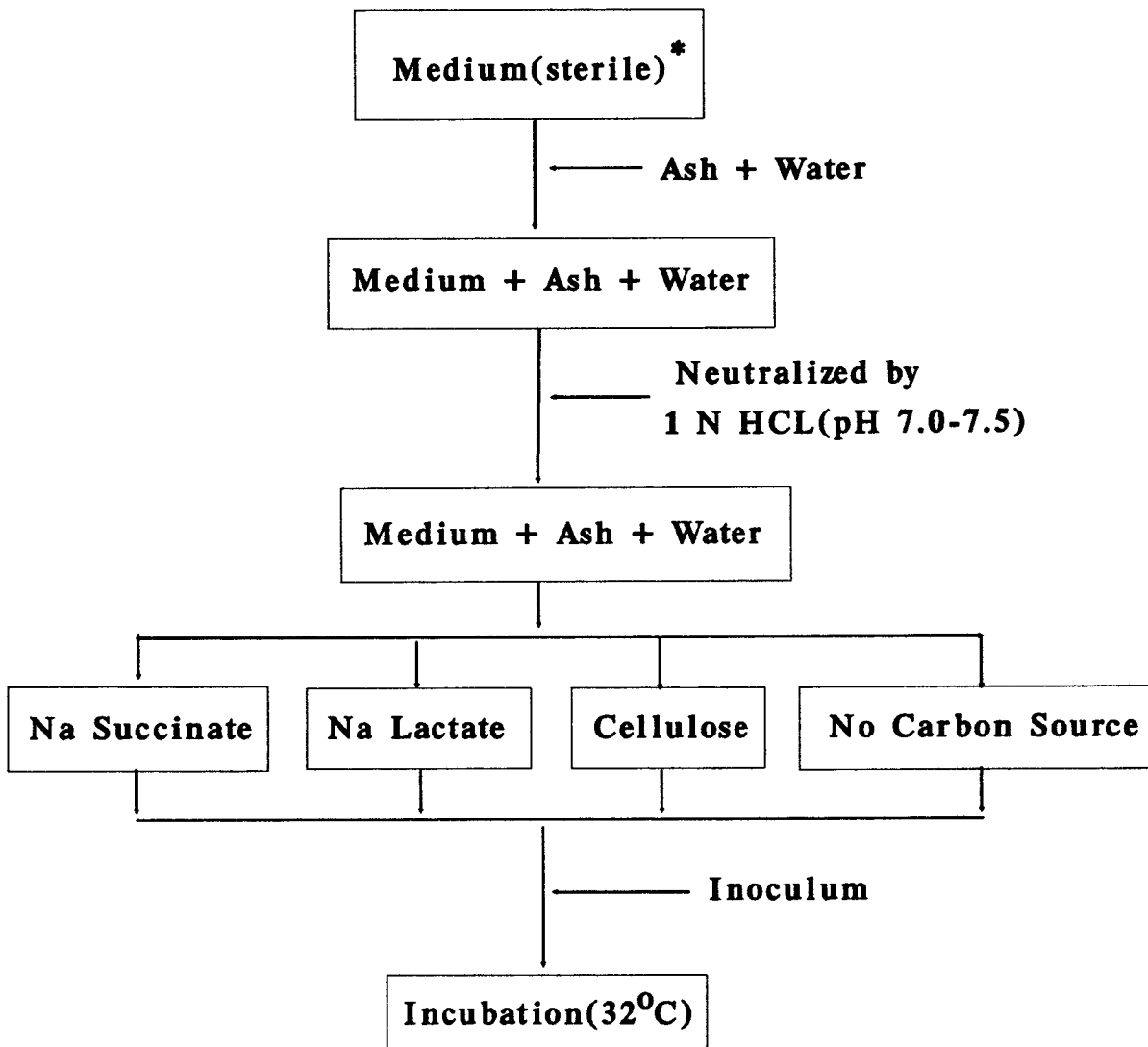
Because DSRs are obligate anaerobes, all traces of oxygen were removed by passing nitrogen gas through the Medium. The nitrogen gas was first passed through copper filings heated in a Sargent-Welch furnace (Sargent-Welch Scientific Co., Skokie, Ill.), prior to being channeled through a gas manifold which contained the mixture, the mixture then dispensed into serum bottles (figure 4.1). The following apparatus was used in the experimentation process;

- Glass serum incubation bottles (160 ml volume) Wheaton Glass Company, Millville, NJ
- Butyl rubber stoppers; Bellco Biotechnology, Vineland, NJ, catalog number 2048-118000
- Aluminum seals (20 ml); Bellco Biotechnology, Vineland, NJ, catalog number 2048-1120
- Nitrogen gas; Methane Gas Products, Inc., East Rutherford, NJ
- Incubator (32°C); Environmental Growth Chamber, Chagrin Fells, Ohio
- Harvard/Lte series 300 lab autoclave; catalog number 302/0662/00, 121°C for 20 minutes
- Corex glass centrifuge tubes (50 ml); Corning Incorporated, Corning, NY
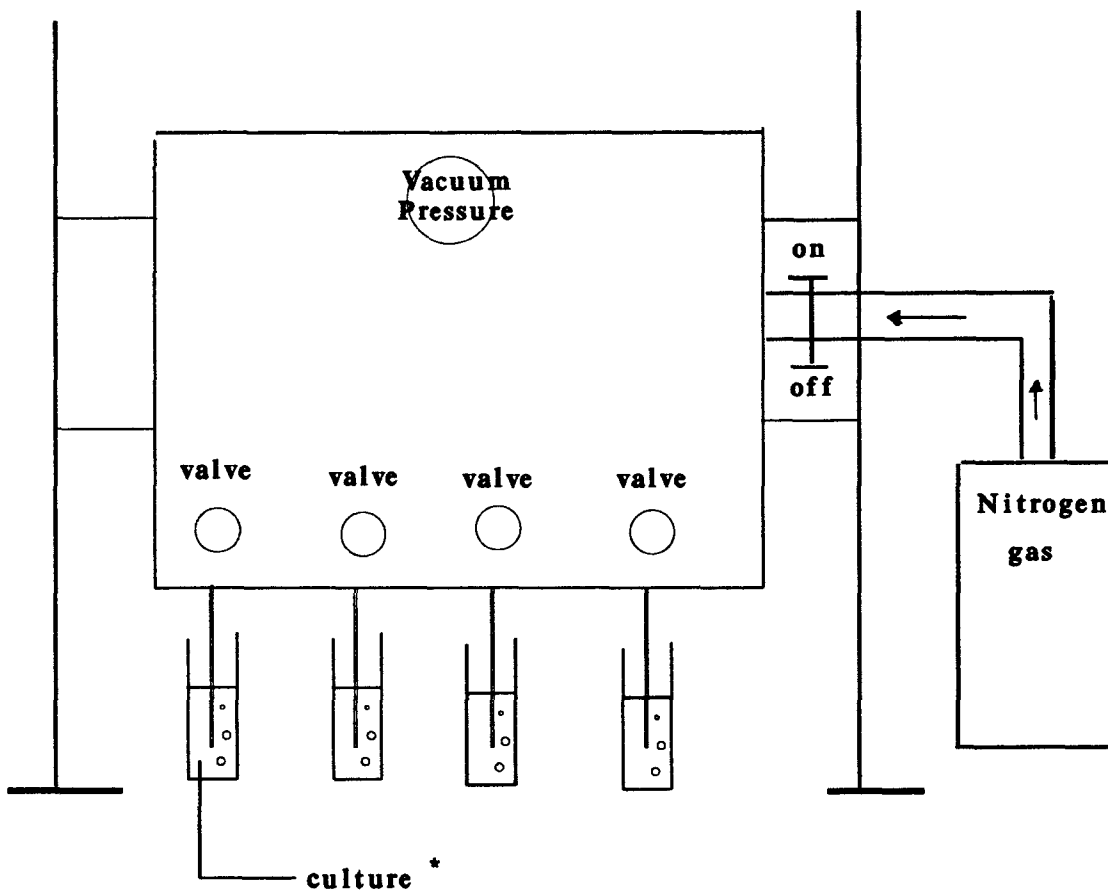
* Culture : media + ash + inoculum

Figure 4.1 Anaerobic Instrumentation

```
        ┌─────────────────────────┐
        │  Medium(sterile)*       │
        └─────────────────────────┘
                    │
                    │ ──── Ash + Water
                    │
        ┌─────────────────────────┐
        │ Medium + Ash + Water    │
        └─────────────────────────┘
                    │
                    │ ──── Neutralized by
                    │       1 N HCL(pH 7.0-7.5)
        ┌─────────────────────────┐
        │ Medium + Ash + Water    │
        └─────────────────────────┘
                    │
```

| Na Succinate | Na Lactate | Cellulose | No Carbon Source |

Inoculum

Incubation(32°C)

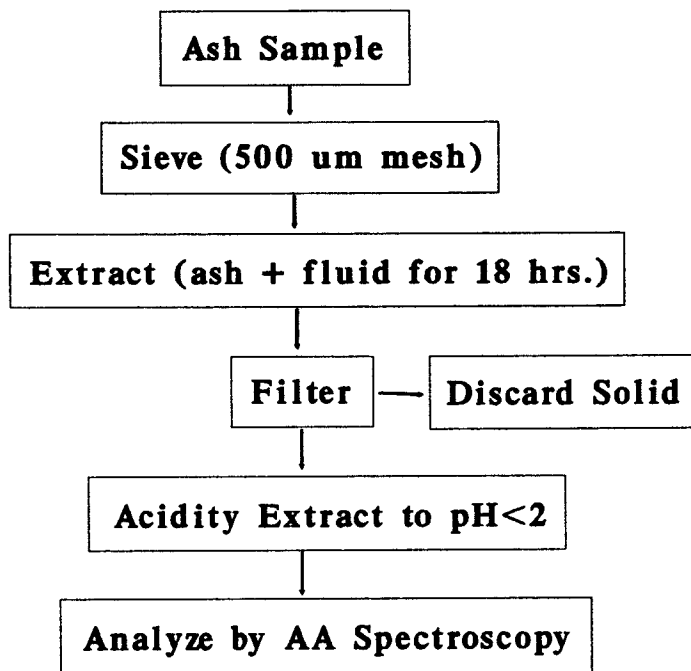* Modified Medium B(Postgate, 1984)

Figure 4.2 Experimental Design to Find Optional Carbon
Source for DSR

4.2. Analysis of Heavy Metals in Incinerator Ash

The content of the heavy metals lead, cadmium, chromium, and copper in incinerator ash was determined by conducting a "digestion" of the ash with a strong acid (23).This method involved mixing 10 grams of incinerator ash (obtained from American Ref-Fuel, Newark, New Jersey) with 200 ml of 1 N nitric acid. The combined ash (bottom ash and fly ash) was oven dried at 105°C for 24 hours. The ash was sieved through 500 uM mesh without additional grinding. After grinding and sieving, samples were dried again. The mixture (ash, HNO3, and water) was stirred vigorously at room temperature for 72 hours, and the supernatant was removed and filtered for analysis at intervals of 10 minutes, 60 minutes, 24 hours, and 72 hours.

Analysis of heavy metals was performed on a Smith Hietje Flame Atomic Absorption (AA) Spectrometer (model number 12) manufactured by Thermo Jarrel Ash Corporation. Standards were prepared from 1000 ppm stock solutions of lead oxide (PbO), cadmium nitrate, ammonium dichromate and copper (the metal salts were purchased from J.T. Baker Chemical Corporation). Standards were prepared by successive dilutions to ,the following concentrations: 2.0, 4.0, and 8.0 ppm of copper; 2.0, 4.0, and 8.0 ppm of chromium; 0.5, 1.0, and 2.0 ppm of cadmium; and 1.0, 5.0 and 10.0 ppm of lead.

TCLP Procedure

Ash Sample

Sieve (500 um mesh)

Extract (ash + fluid for 18 hrs.)

Filter — Discard Solid

Acidity Extract to pH<2

Analyze by AA Spectroscopy

pH 5 Method Procedure

Ash Sample

Sieve (500 um mesh)

Extract (ash + fluid for 18 hrs.) — pH=5

Filter — Discard Solid

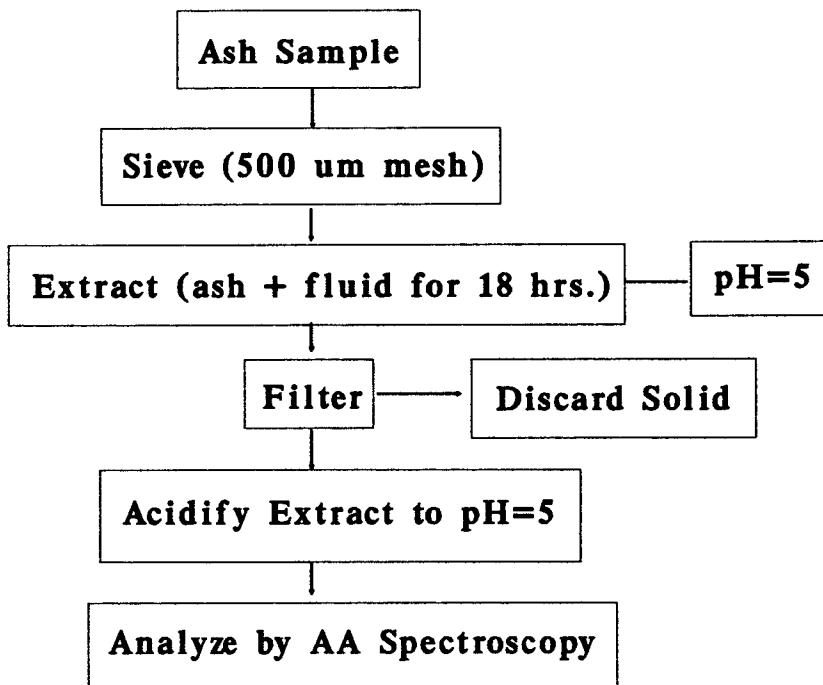Acidify Extract to pH=5

Analyze by AA Spectroscopy

Figure 4.3 TCLP & pH 5 Method

Leaching Methods

In order to determine the amount of lead, cadmium, copper, and chromium that would leach from the fresh ash and treated ash.

The pH 5 method involved leaching the ash for the same time period, but at a pH at or below 5.0. The pH was tested every 15 minutes for the first two hours, and every two to three hours after that. In this method 1 N nitric acid was added to bring the pH to 5 or below during the extraction (Figure 4.3).

The Toxic Characteristic Leaching Procedure (TCLP) (40 CER Chapter I; 7-1-88 edition) involves leaching the ash over an 18 hours period with a slightly acidic solution, then acidifying the extract prior to AA spectroscopic analysis. Both methods were used due to the inherent alkalinity of the ash; the TCLP is a federally mandated test, but it does not maintain an environment acidic enough for the leaching of metals, and was therefore of limited use for the purpose of this study. The pH 5 method is more stringent, and provided useful data the ash had already passed the TCLP test.

4.3. Analysis of Sulfate and Sulfide in the *Desulfovibrio* cultures

In order to determine, if the *Desulfovibrio* cultures were active, the rate of sulfate disappearance and excess soluble sulfide appearance in the cultures was determined. The preferred method for this monitoring is Ion Chromatography (IC) manufactured by Millipore Corporation. A sulfate and sulfide determination method devised by

Millipore Coporation was used (Method number A-111) on Waters Instrumentation (Figure 4.4).

The cultures were sampled at 3-5 day intervals by inserting a needle attached to a syringe through the rubber stopper under anaerobic conditions (nitrogen gas was blown in through the stopper to displace any oxygen in the atmosphere). Prior to sampling, the culture bottle was not shaken. This was done to avoid to sample the thick precipitate originally present in the medium, and the black ferrous sulfide formed once the *Desulfovibrio* was introduced to the medium. Hence, sample may not have been homogenous in their salt concentration. The 1 ml samples were stored in plastic centrifuge vials (eppendrof) in the freezer (below 0^oC) until the time of analysis. The sample was diluted 1:50 with a 25 mM sodium phosphate 10 mM mannitol solution. The mannitol was used as a reducing agent to protect the sulfide in solution from being oxidized. The dilutions were then loaded in 100 ul amounts into the IC for analysis.

Table 4.3 Ion Chromatography Instrumentation

1. Pump system(water 600E system)

2. Sample process, housing the injection system(water 715, ultra wisp)

3. Column IC-PAK A HC, 150 x 4.6 mm, 10 uM

4. Tunable absorbance detector(water 484)

5. Conductivity detector(water 431)

6. PC minichrome 1990 VG date system Ltd., software version 1.5

Ion Chromatography Instrumentation for
Sulfate/Sulfide Ion[Millipore Corp., Milford, Mas.]
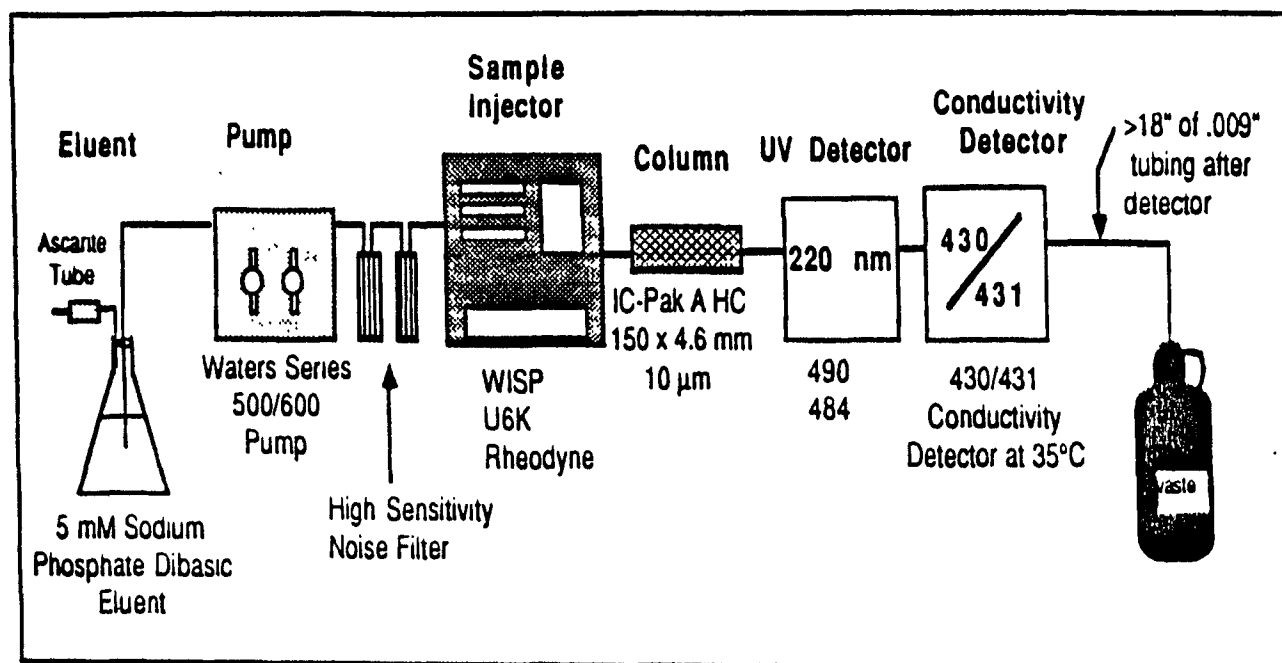

Figure 4.4 Ion Chromatography Instrumentation

Instrument Conditions

1. Eluent; 5.0 mM sodium phosphate dibasic

2. Pump; 510 solvent delivery module

3. Injector; U6K

4. Column; IC-Pak AHC

5. Data; 840 data system

6. Flow rate; 2.0 ml/min

7. Injection; 100 ul of working standard

8. Detector; 430 conductivity

9. Range; 1000 uS

10. Temperature ; on

11. Background; 960 uS

Table 4.4 Components of culture tubes of inoculated incinerator ash

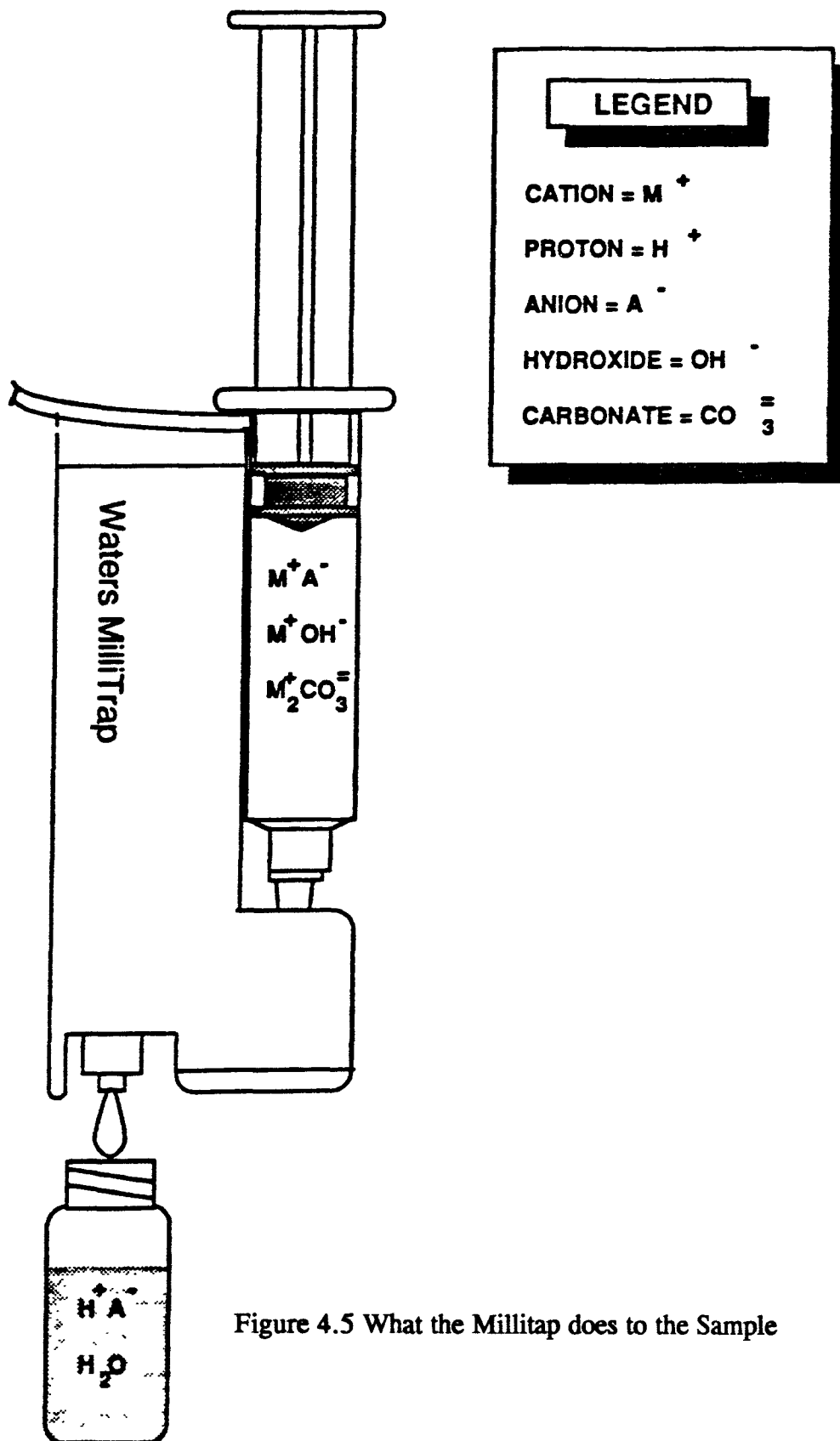| Culture #	Ash (gm)	Inoculum (ml)	Water (ml)	Medium(ml)
1	1.0	2.0	18.0	5.0
2	1.0	-	20.0	5.0
3	-	2.0	18.0	5.0

Figure 4.5 What the Millitap does to the Sample

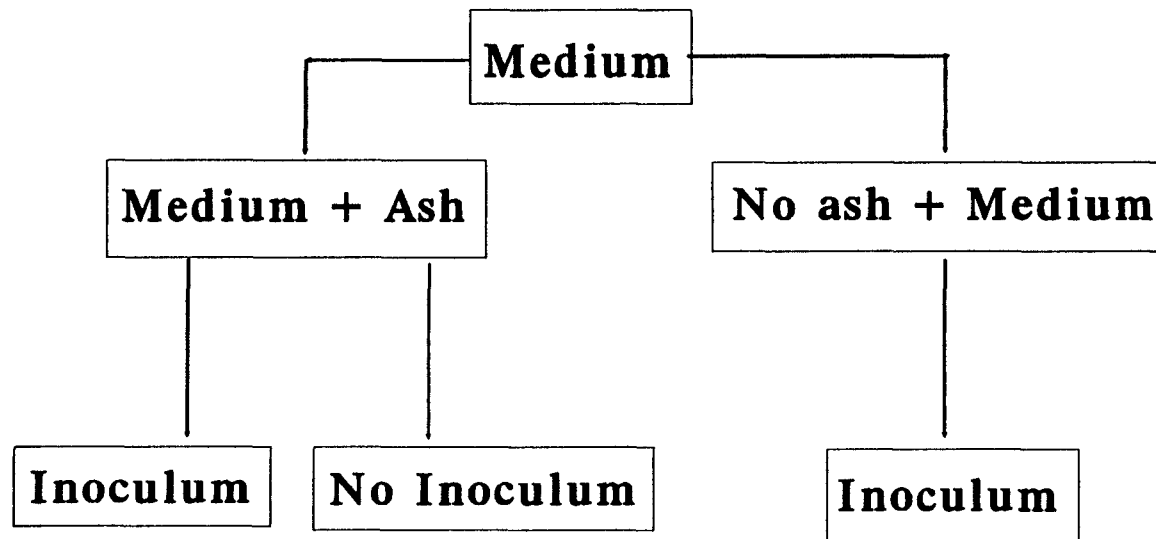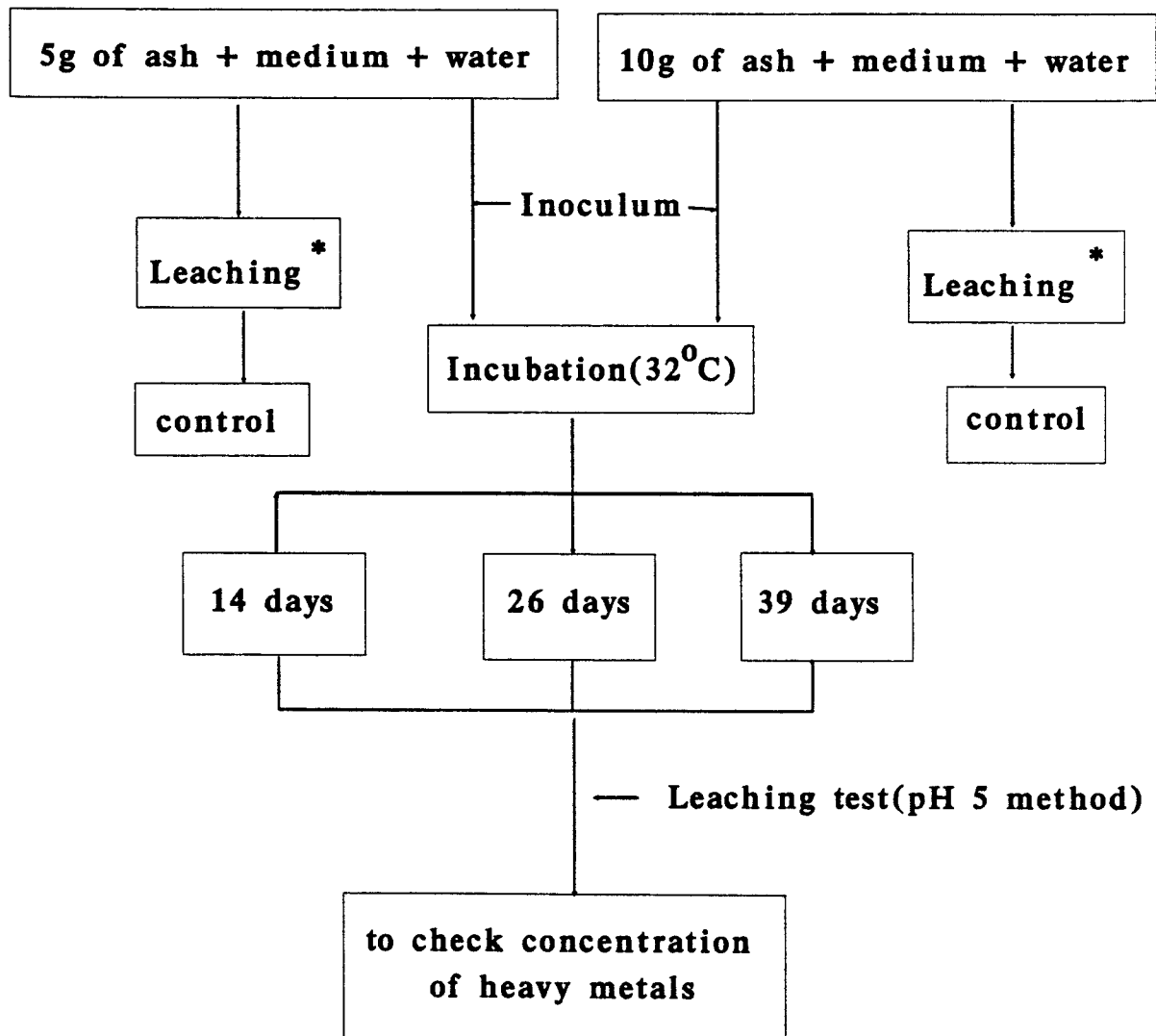Figure 4.6 Experimental Design of Inoculated Incinerator Ash

Figure 4.7 Design for Treated Ash

Table 4.5 Components of culture tubes of treated ash

Culture (No.)	Ash (gm)	Inoculum (ml)	Water (ml)	Medium (ml)
1	5	3	125	32
2	5	3	125	32
3	5	3	125	32
4	10	3	125	32
5	10	3	125	32
6	10	3	125	32

Sodium sulfate standards of 75.00, 37.50, and 18.75 ppm were prepared fresh daily in sodium phosphate and mannitol solution. Sodium sulfide standards were similarly prepared at 20.00, 10.00, and 5.00 ppm concentrations.

CHAPTER 5 RESULTS AND DISCUSSION

5.1. Determination of an appropriate Carbon Source for *Desulfovibrio*

It was necessary to find a suitable energy source (carbon source) for *Desulfovibrio* acting on ash. The Dissimilatory Sulfate Reducers (DSRs) in these experiments were cultured with sodium lactate, sodium succinate, cellulose and in control cultures with no carbon source (Section 4.1). The growth of DSRs without ash was faster than the growth of bacteria with the ash (elapsed time: 8 days). The DSRs grew well in the four different sources without ash. No growth of DSRs with ash occurred with sodium succinate or cellulose. However, DSRs with ash grew in sodium lactate and in the control compounds without carbon source. The DSRs can be inhibited by phenolics, quaternary, antibiotics, and heavy metals (14). The inhibition by metals is largely due to the organism's precipitation of Hg^{+2}, Cu^{+2}, Cd^{+2}, etc., as sulfides, in absence of H_2S, these metals are fairly toxic. Chemical composition of incinerator ash includes Al, Ca, Fe, P, Na, Mg, Cd, Cu, Hg, Pb, Zn, etc.,(3). It is possible that one of main reasons for the poor growth of DSRs with is the toxic effect of heavy metals.

5.2. Determination of the Concentration of Heavy Metals in Incinerator Ash

A strong acid digestion was performed with an ash sample (section 4.2). Ten grams of incinerator ash were added to 200 ml of 1 N nitric acid and mixed for 72 hours at room temperature. The analysis of the supernatant was done by at intervals of 10 minutes, 60

minutes, 24 hours, and 72 hours. Figure 5-1 shows that the amounts of lead, copper, chromium, and cadmium found in the incinerator ash.

Table 5.1 Amounts of Heavy Metals Released from Incinerator Ash after

72 Hours of Digestion

Metals	Amounts (mg/1 gm ash)
lead	72.0
cadmium	18.4
copper	60.1
chromium	1.3

5.3. Variation of Sulfate and Sulfide in the Cultures

Analysis of sulfate and sulfide in the ash was performed with ion chromatography. Three cultures are shown in Table 4.5.. These data indicate a gradual decreasing of sulfate in active culture tubes. In Figures 5.2 and 5.3, the amount of sulfate is decreasing, however, the amount of sulfide shows increasing in the Figure 5.4. and 5.5.
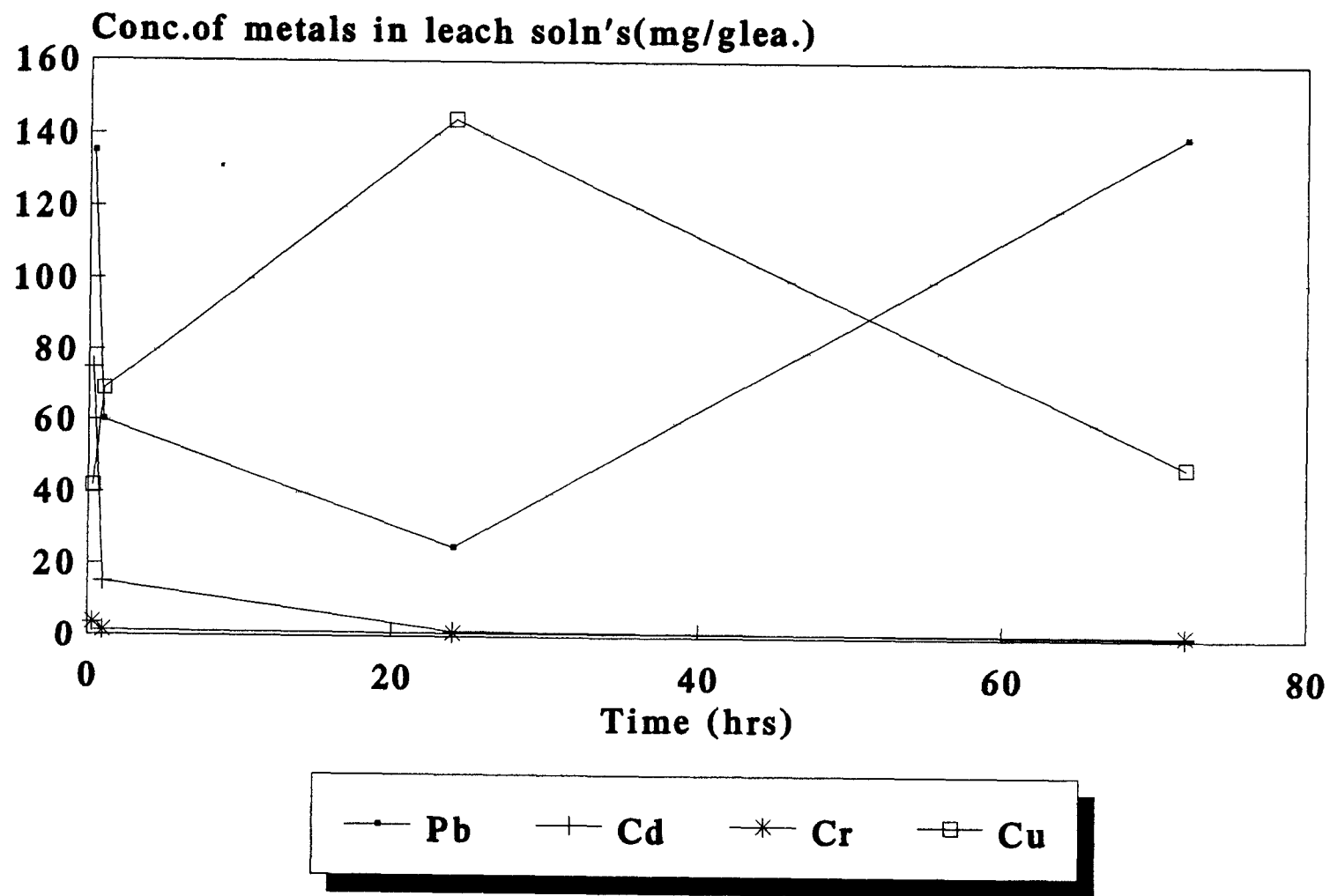
Figure 5.1 The Leaching of Heavy Metals from Ash
(10 g of ash with 200 ml of 1 N HNO₃)

All the samples initially contained 850 micromoles of sulfate (Table 4.5). After a 21 day growth period all the culture series inoculated with DSRs showed almost complete consumption of the sulfate. No soluble sulfide was detected in the control Medium B because this medium did not contain any sulfide (Table 4.2).

Culture tubes 1 and 3 (Table 4.5) were inoculated media with ash and no ash, respectively. In both of these series, no initial sulfide was present. According to Figure 5.5, the sulfide concentration increased from 0 micromole to around 850 micromoles in Culture tube 3 (Table 4.5). Culture tube 1 also increased at a lesser rate compared to culture tube 3 because of the ash added to the culture.

These results clearly show that the DSRs consumed the sulfate for their growth and produced sulfide in the culture. Sulfide detection was difficult due to the rapid oxidation of sulfide into sulfate and sulfite. Some amount also precipitated as FeS; a black precipitate was very noticeable in the cultures. These processes caused the large difference between the cultures, in the amount of sulfate and sulfide. For example, the amount of sulfate in culture tube 3 was 850 micromoles but the amount of sulfide detected in culture tube 1 was quite smaller (Table 4.5). Some of the sulfide loss could not be accounted for.

5.4. Activity of DSRs in Incinerator Ash

To determine the activity of DSRs in incinerator ash, the culture tubes (Table 4.6) were designed as shown in Figure 4.6. The analysis of heavy metals in the cultures was
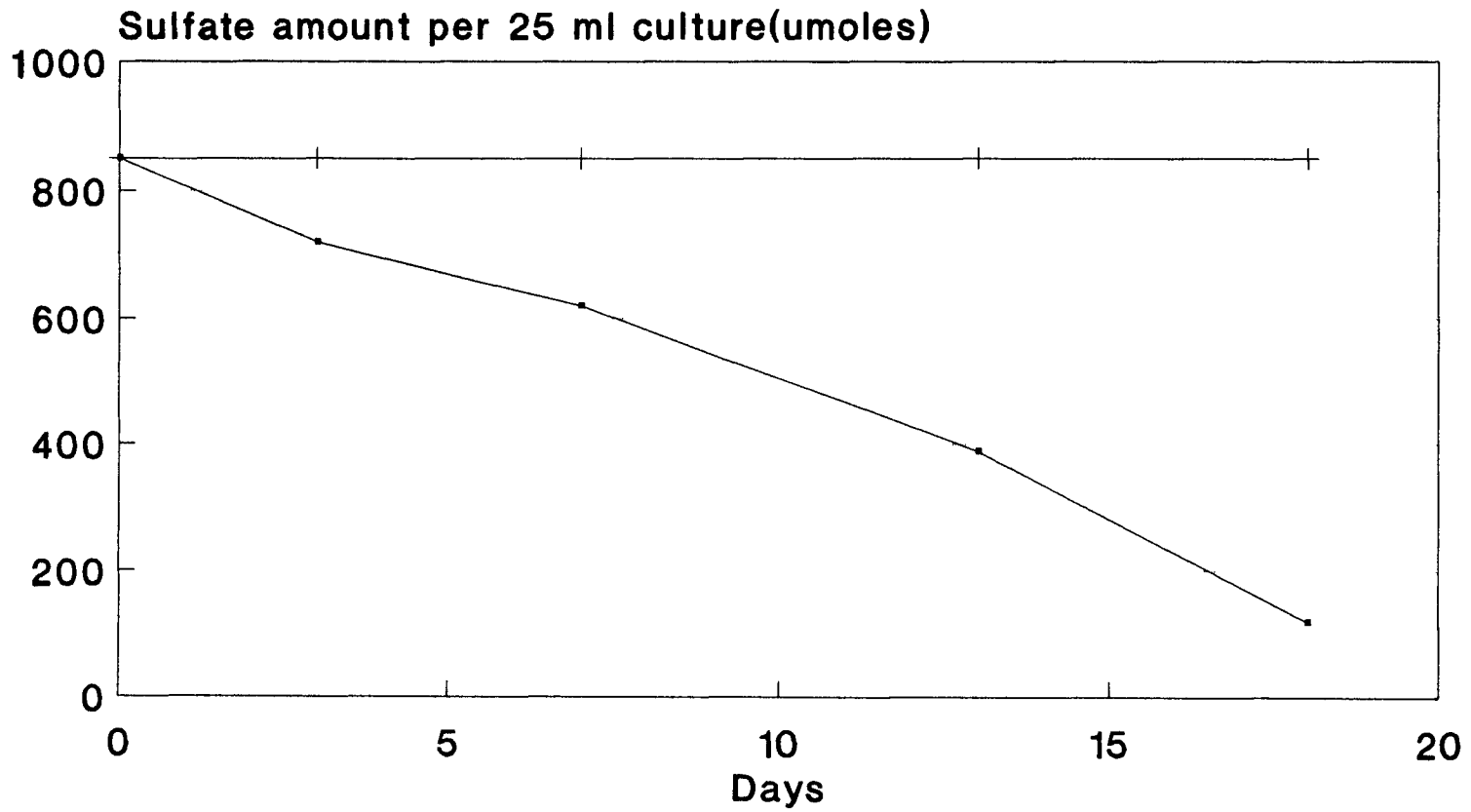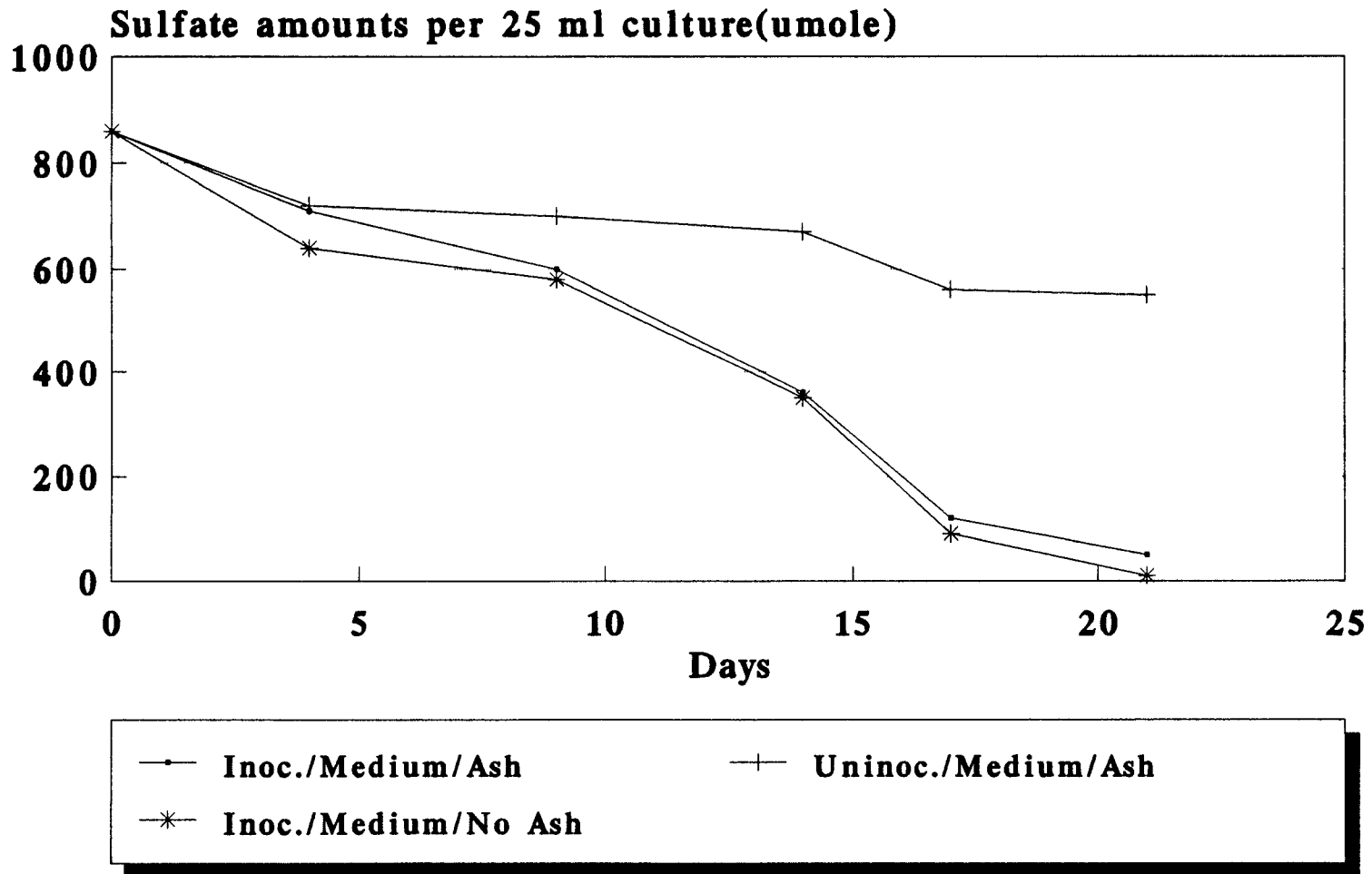
Figure 5.2 Sulfate in Culture without Ash

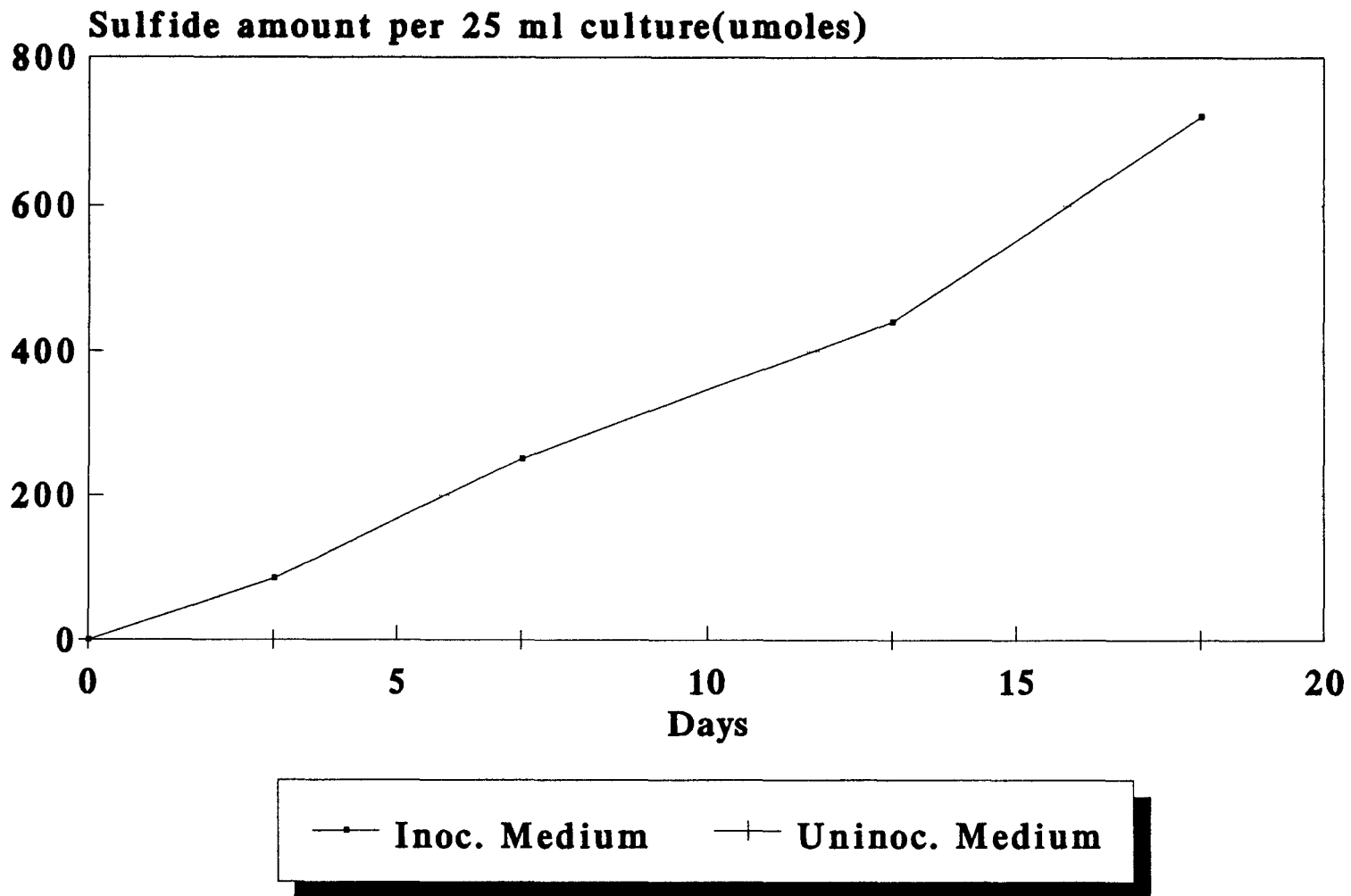**Figure 5.3 Sulfate in Culture with Ash**

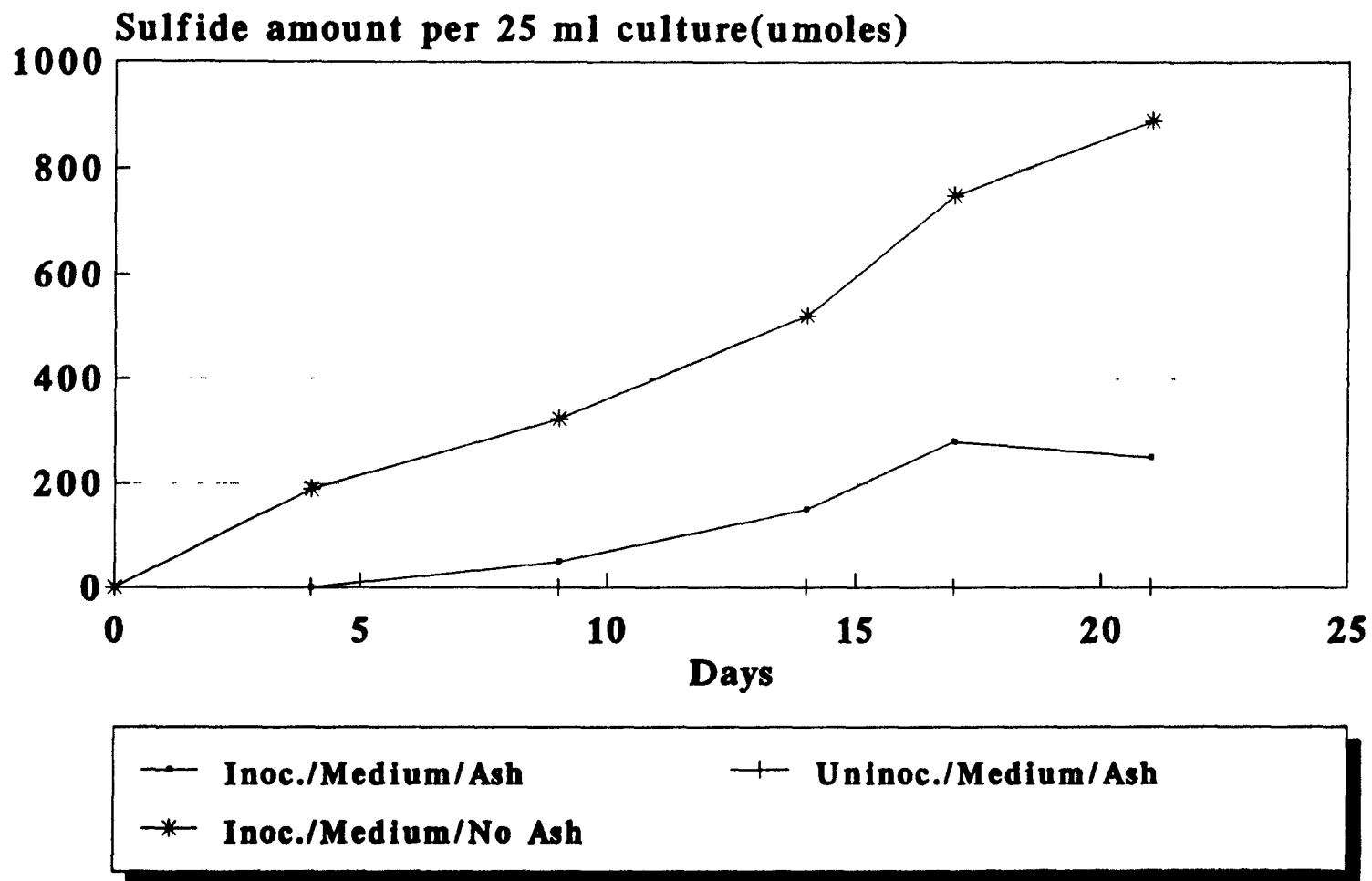**Figure 5.4 Sulfide in Culture without Ash**

Figure 5.5 Sulfide in Culture with Ash

done using A.A. spectroscopy. The activity of DSRs did not entirely depend on the different amounts of ash. DSRs activity was analyzed at level of 5 g of ash and 10 g of ash. After 40 day growth period, the activity did not seem to differ with different amounts of ash (Figure 5.6, 5.7, 5.8, 5.9). This means they might be acted with different amount of ash by same immobilizing ability.

The concentration of heavy metals such as copper, cadmium, and chromium was dropping under the limit of EP Toxicity; lead remained about the limit (Figure 5.6, 5.7, 5.8, 5.9). The effectiveness of anaerobic treatment of incinerator ash is shown in Table 5.2. The amount of lead also decreased but never blow the limit of EP Toxicity (5.0(mg/l)) during 40 days. In the case of lead leaching, a longer time period than other metals, will be required. However, it is not clear why DSRs were acting relatively ineffectively.
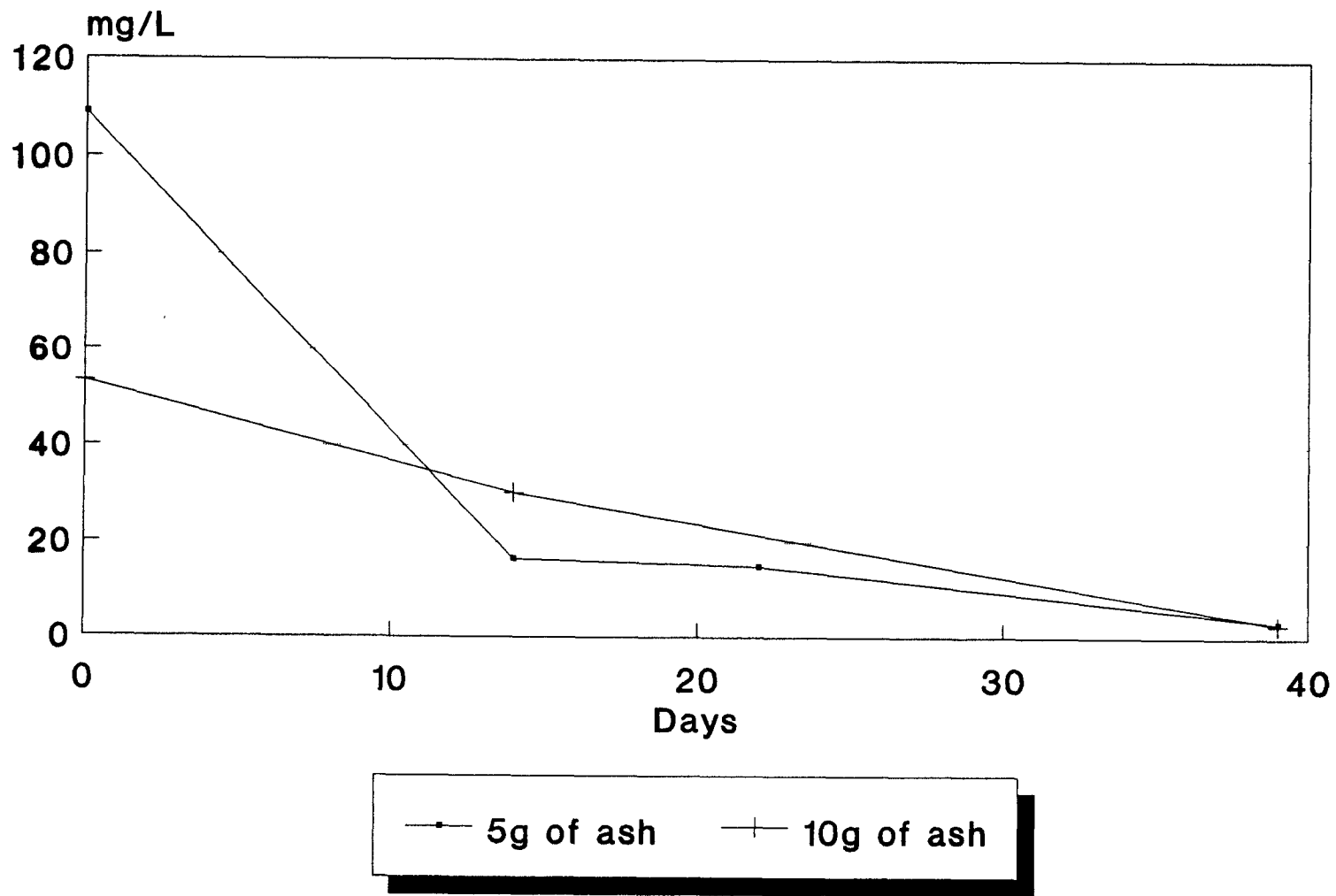
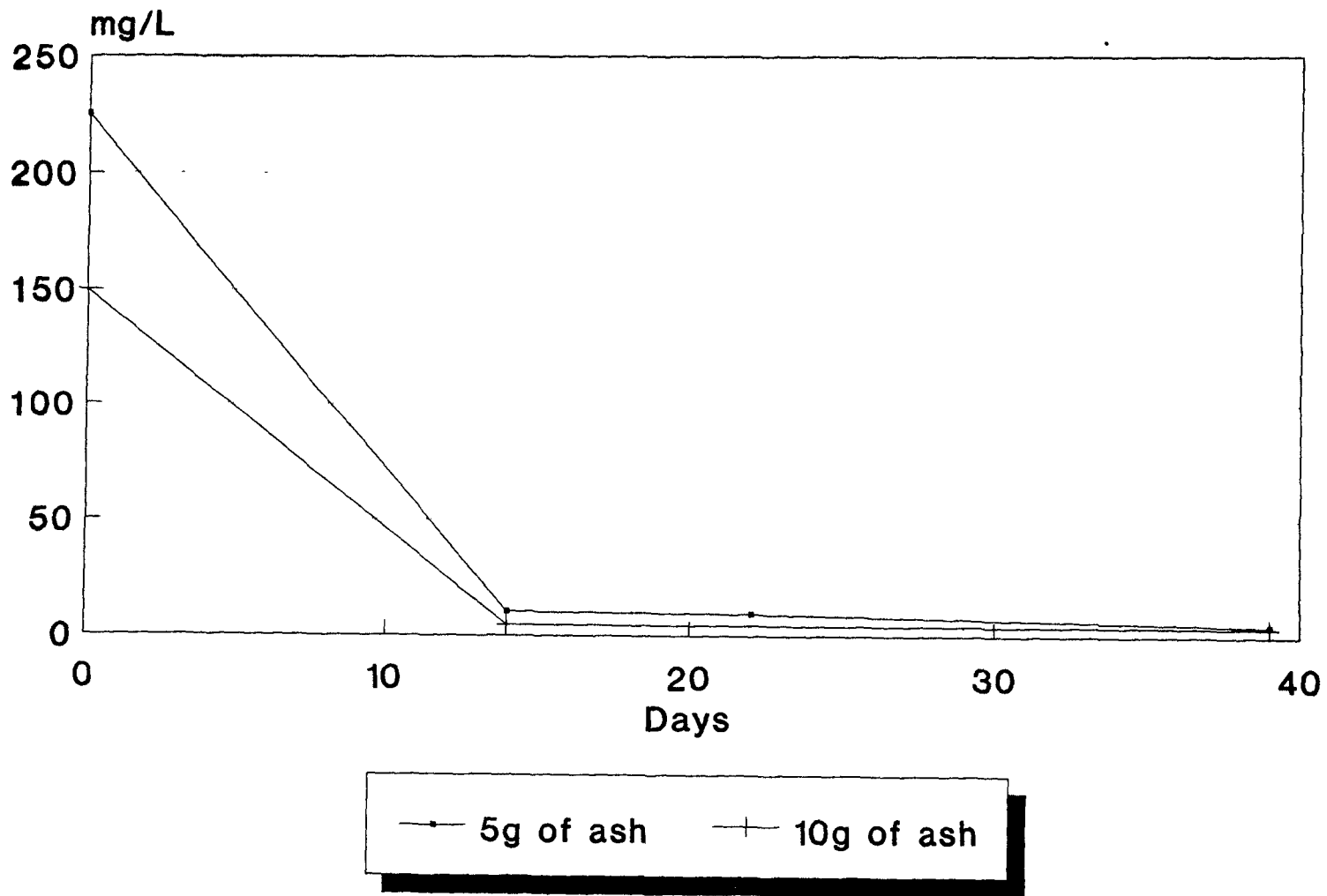**Figure 5.6 Concentration of Cu(pH 5 Method)**

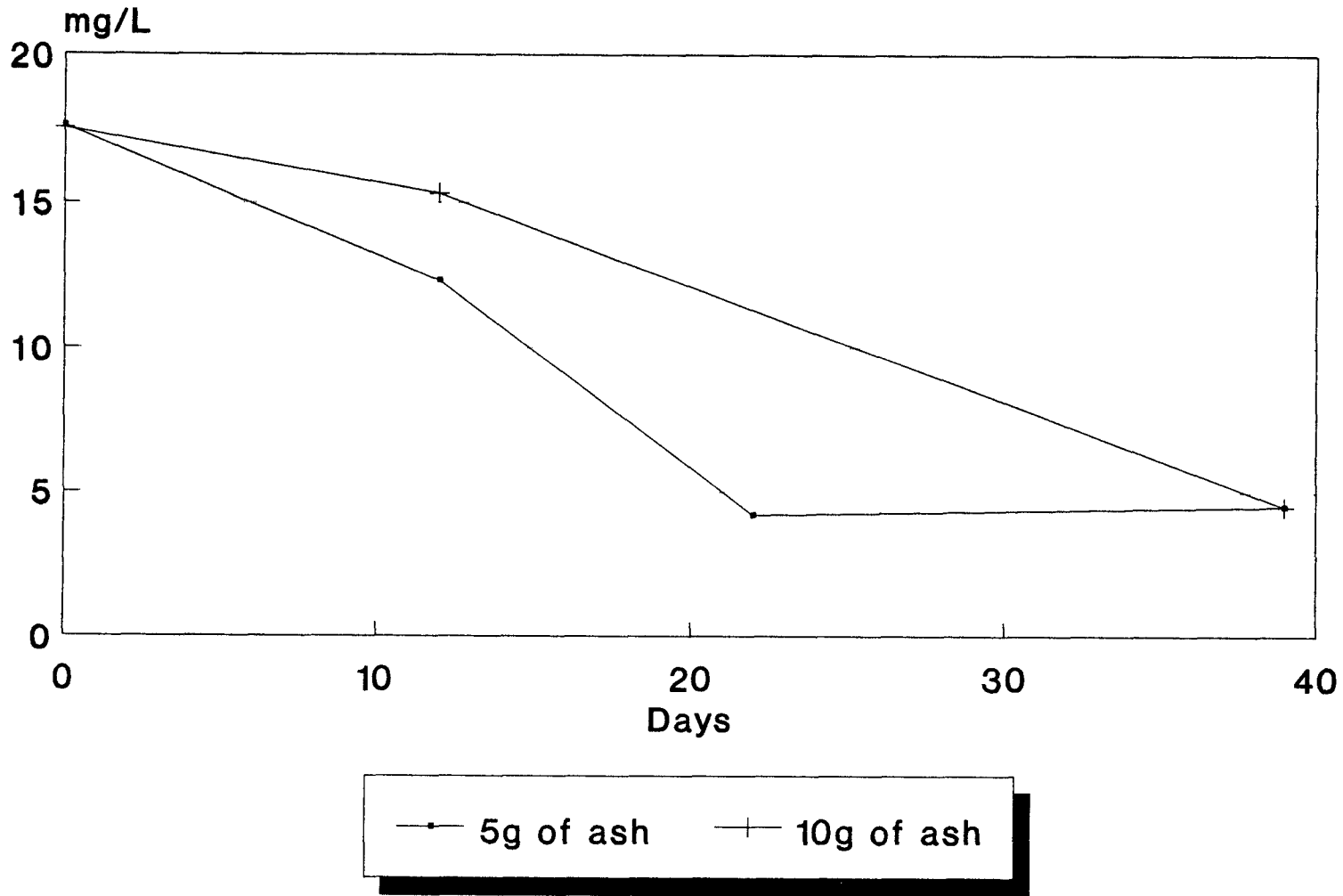Figure 5.7 Concentration of Cd(pH 5 Method)

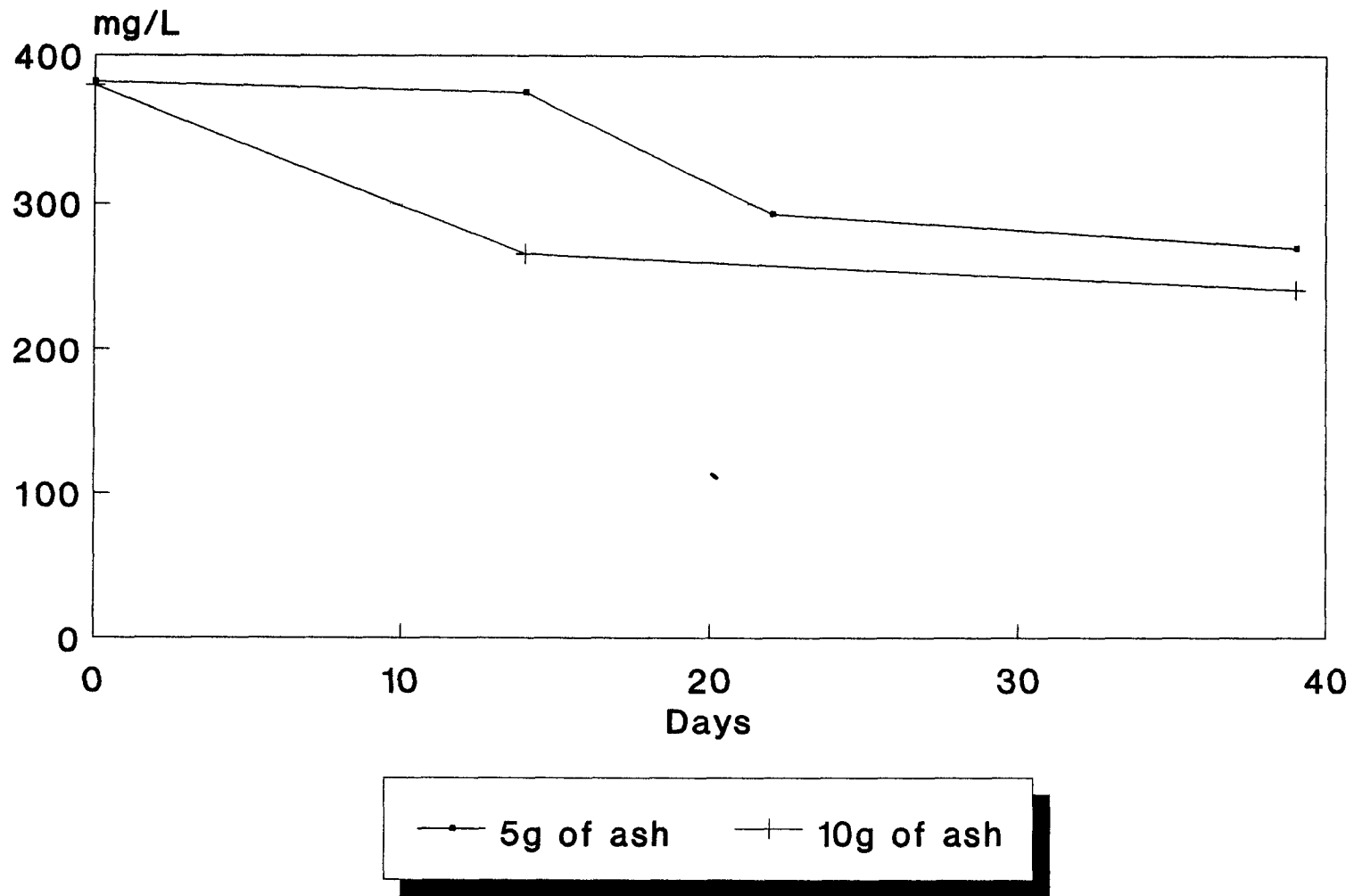**Figure 5.8 Concentration of Cr(pH 5 Method)**
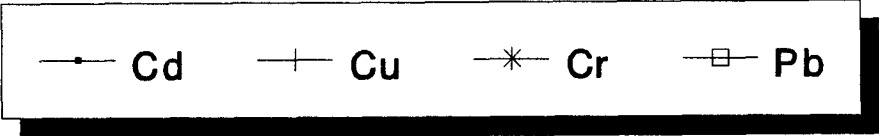
Figure 5.9 Concentration of Pb(pH 5 Method)
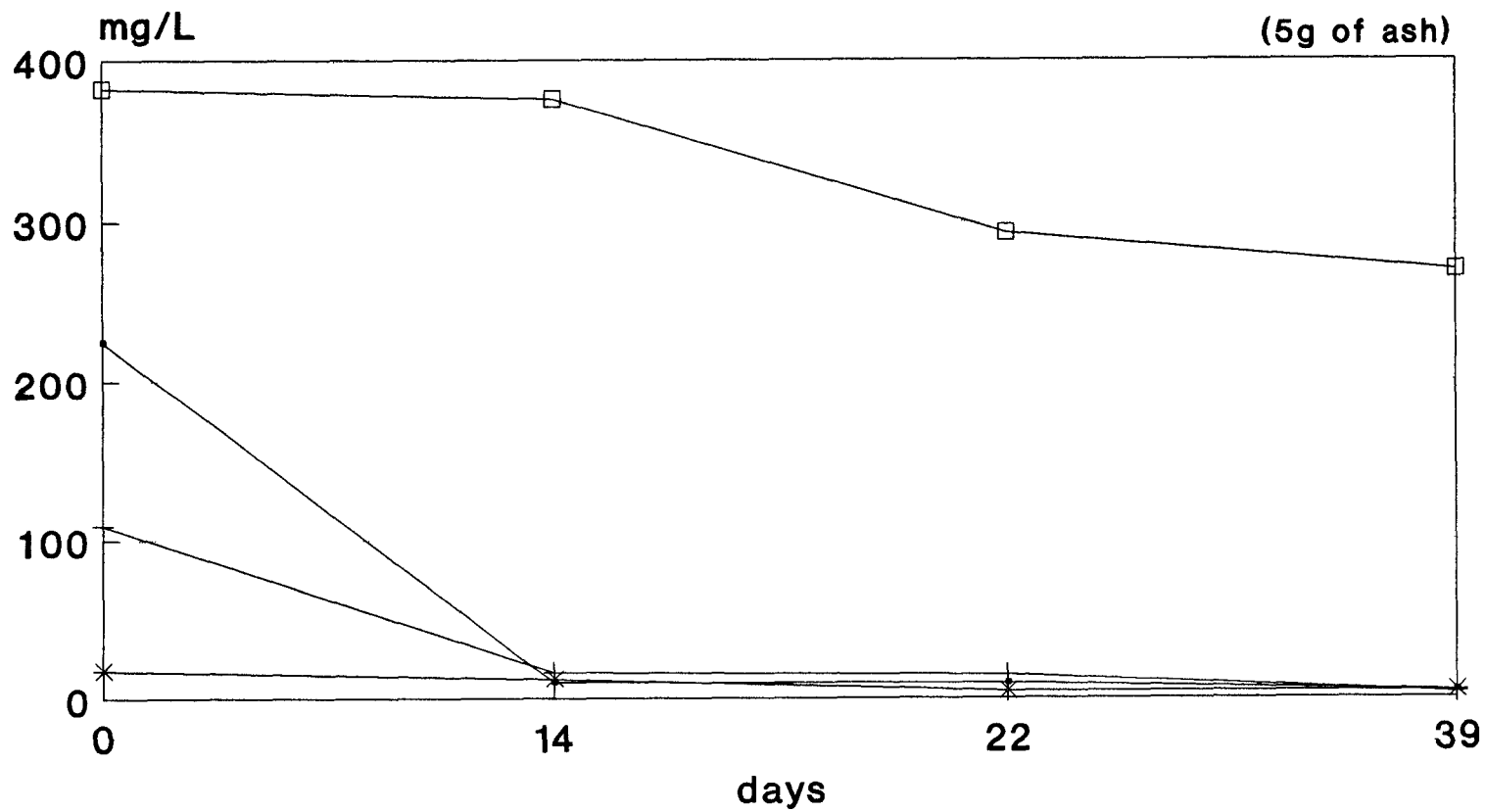
Figure 5.10 Heavy Metals In The Ash(pH 5 Method)

ppm

(10g of ash)
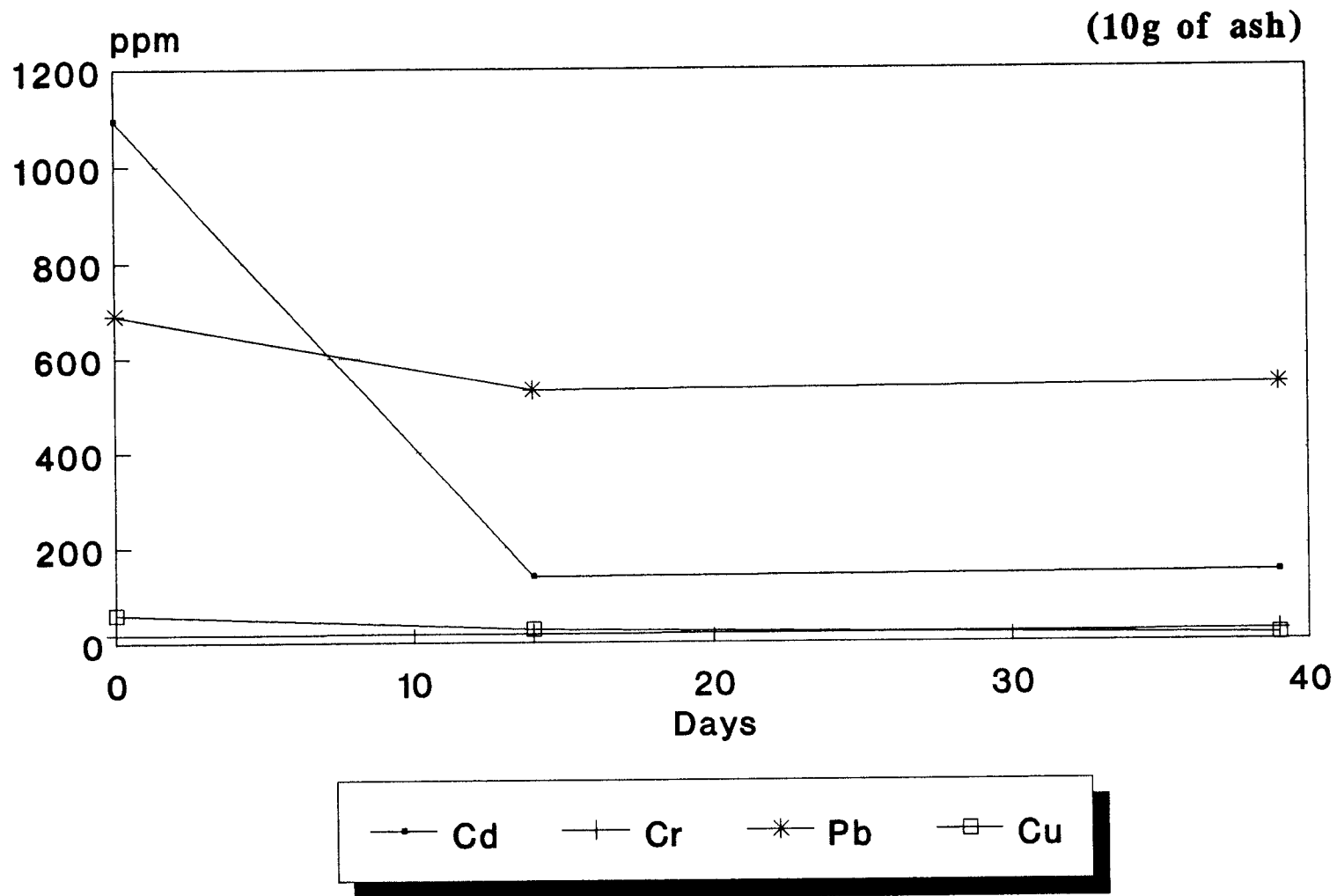
Figure 5.11 Heavy Metals In The Treated Ash

Table 5.2 Effectiveness of Anaerobic Treatment

of Incineration Ash

Concentration of Contaminants in Leachate(mg/L)				
System	Pb	Cd	Cr	Cu
Untreated Ash	382	225	17.6	109
Ash + Anaerobic Inoculum(40days)	270	0.8	4.2	3.08
EPA Standard	5.0	1.0	5.0	5.0

* Method of Extraction: leaching with acid solution at pH <5

* Ash; American Ref-Fuel of Essex County, Newark, NJ

CHAPTER 6 CONCLUSIONS

The results of these experiments show that sodium lactate is a suitable carbon source. This will be very useful for enriching cultures of DSRs in batch reactors in future work. As mentioned in chapter 5, the amount of sulfate and the amount of sulfide did not fit the theoretical mass balance. Our work can supply data to apply in the design for immobilizing heavy metals to acceptable limits of EP Toxicity; lead is the exception. However, it would be of value to demonstrate for future work, the amount of sulfide that disappeared. Also, the proper treatment of remaining heavy metals after treatment by DSRs should be developed such as by landfill or some other method. This research could develop an immobilization process by which heavy metals contaminants of liquid and solid wastes are precipitated The metals are immobilized as insoluble sulfides by the biological action of DSR anaerobic consortia.

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APPENDIX

- Heavy Metals in The Ash (pH 5 Method)
- Application of Metal Immobilization

Heavy Metals In The Ash(pH 5 Method)
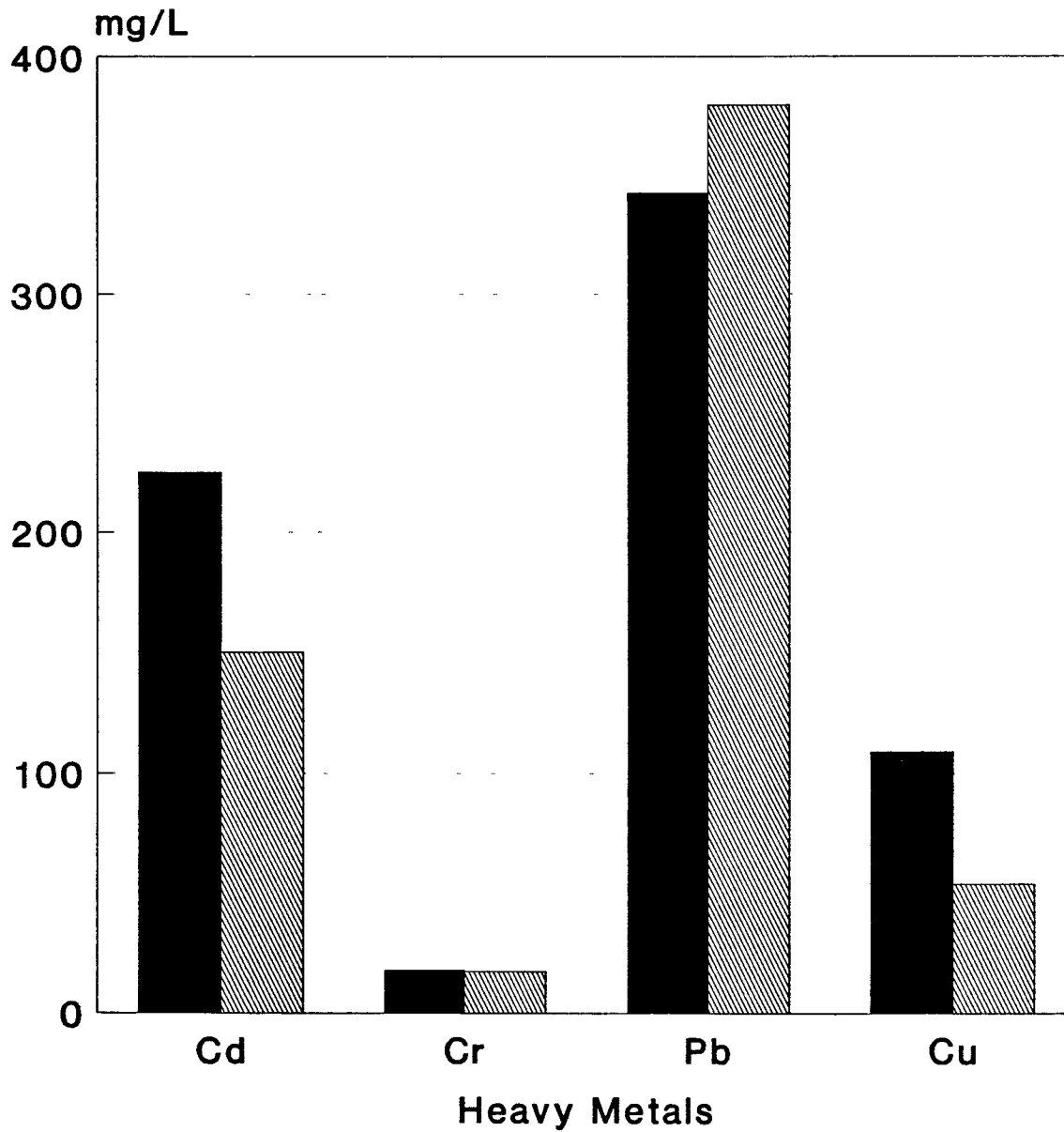
* The ash is came from American Ref-Fuel of Essex County, Newark, NJ 07105

Metal Immobilization

sludge   sludge

ash

reactor
with DSRs

water

solid waste treatment

(landfill)