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Anaerobic biological treatment of incinerator ash to immobilize toxic metal residues

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ABSTRACT

ANAEROBIC BIOLOGICAL TREATMENT OF INCINERATOR ASH

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In this study, a reliable and inexpensive method for biologically immobilizing toxic heavy metal residues present in very high concentrations in incinerator ash was developed. *Desulfovibrio*, a *genus* of sulfate-reducing bacteria was cultured and treated with combined ash under anaerobic conditions. The carbon source, lactate, and the electron acceptor, sulfate, were provided to the cultures for their normal growth. Since the sulfate reducers utilize organic matter using sulfate as the oxidizing agent, sulfides are the corresponding end products of the reaction. Ash was added to well established cultures of *desufovibrio.* Immobilization of the heavy metals in the ash matrix occured as the metals precipitated as sulfides (PbS, CdS). The culture medium was analysed periodically for sulfate decrease until a steady state value was reached indicating the completion of bacterial activity. Next, the biologically treated ash was subjected to a number of leaching tests. The results showed a high degree of immobilization especially for the ash treated with cultures grown at room temperatures. The treated ash passed all existing and previous EPA leaching tests and met the required standards for Pb, Cd, and Cr, the metals of interest in this project. The proposed approach appears to have significant potential in the treatment of heavy metal contaminated incinerator ash prior to land burial, since it minimizes the problems associated with metal leaching and groundwater contamination.

ANAEROBIC BIOLOGICAL TREATMENT OF INCINERATOR ASH TO IMMOBILIZE TOXIC METAL RESIDUES

by

UMA PARASAR

Submitted to the Department of Chemical Engineering, Chemistry and Environmental Science of
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Chapter 1

INTRODUCTION

The shortage of available land and the increase in the generation of Solid Waste has created a Solid Waste crisis in the state of New Jersey. Incineration of refuse is considered the most convenient method of reducing waste volume and recovering some of the energy bound in waste. Ash is the non-combustible portion of the residue of municipal waste incineration and consists of $80 - 90\%$ (by weight) bottom ash from the furnace bottom, and $10 - 20\%$ of fly ash from the dust collectors system.

Many communities in the Northeast and other parts of the USA have chosen municipal waste incinerators, as their primary waste disposal alternative. In 1987, 111 municipal solid waste incinerators (MSWI) were operating in the USA, and EPA predicts that the number will increase to 300 facilities by the year 2000. Thus, environmentally sound disposal options for ash have received great attention both in Congress and in the general public. Landburial presents the danger of polluting bordering ecosystems due to leachability of non-combustible substances present in the ash, such as heavy metals.

The volume reduction accomplished by incineration inevitably results in an increase in the concentration in the ash of the inorganic elements that were present in the materials that were burned. In particular, heavy metals are ef-

ficiently concentrated by incineration and even domestic garbage produces ash with significant levels of a number of heavy metals. After burial in a landfill the metals can be leached from the ash by rain and enter the groundwater over a period of time [Repa, 1987], leading to contamination. In fresh water systems, trace metal levels must be limited to the water quality standards since they affect human consumption. In seawater systems, the levels are important due to their toxicity to marine biota. At the present time metal leaching is one of the major obstacles to the use of incineration as an environmentally acceptable waste disposal method.

Fly ash released into the atmosphere or disposed of in landfills or water bodies is eventually incorporated in the biogeochemical cycles. Toxic elements move through the environment under natural conditions and are available to biological processes. The metabolic activities of micro-organisms plays a significant role in the mobility of toxic elements in the environment. Micro-organisms are exceedingly versatile in the way they metabolize natural substances, and it is said that if they do not degrade a particular compound then it is unlikely that higher organisms will have the capacity to do so.

1.1 Microbiological approach to metal immobilization

Microbes which conduct *dissimilatory sulfate reduction* are called the *sulfate-reducing bacteria.* Here the sulfate ion plays the same role as does oxygen in conventional respiration, i.e., it acts as an oxidizing agent, or in other words the terminal eletron acceptor, for the utilization of organic matter. Virtually all the reduced sulfur is released into the environment as the sulfide ion, usually liberated as H_2S. This process is called dissimilatory sulfate reduction since the sulfur is

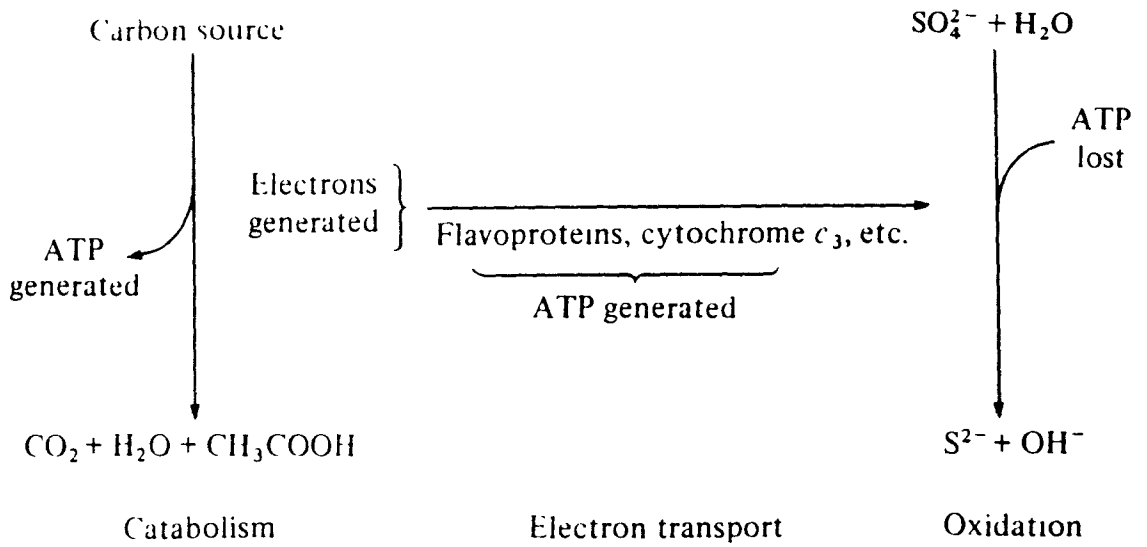


Fig 1.1 - General Process of dissimilatory sulfate reduction (Postgate 84)

not incorporated into the organism's protein, as opposed to assimilatory sulfate reduction, a common-on going reaction in nature, by which green plants, as well as several bacteria, and fungi are capable of utilizing the sulfate from the soil.

The sulfate reducing bacteria grow by coupling the oxidation of simple compounds such as lactate, acetate, hydrogen gas, to the reduction of sulfate ions. Sulfide ions are their metabolic endproduct.

$$4H_2 + SO_4^{-2} \text{ -> } 4H_2 + 2O_2 + S^{-2}$$

A formalized scheme for dissimilatory sulfate reduction is shown in Fig. 1.1 (Postgate, 1984). Catabolism ceases at the acetate level of oxidation and the oxidative step involves removal of oxygen atoms from sulfate and its reduction to sulfide. ATP is lost at this stage. The two longest established genera of sulfate-reducing bacteria are *Desulfovibrio* and *Desulfotomaculum*. Morphologically *desulfovibrios*, as the name indicates are vibrios or coma shaped although pleomorphism, which is a change in the morphological appearance due to incompletely satisfactory environment, is very frequent (shape also varies with age, environment, carbon source, temperature, and salinity). As a general rule, whenever sulfate, organic matter, and anaerobiosis coexist in a non acid locality there *desulfovibrio*, and sulfide biogenesis will be discovered. *Desulfovibrio* is thus a common inhabitant of marine, brackish, estuarine and regularly polluted environments.

1.2 Objective of this work

The objective of this thesis is to develop a method to immobilize the heavy metal content of incinerator ash as metal sulfides and keep them immobile indefinitely by using sulfate-reducig microorganisms. Hence, a prime objection to incineration, the problem of ash disposal can be overcome.

Heavy metals of concern to the environment such as Cadmium and Lead, form insoluble sulfides [Anon, 1982]. Hence the rationale behind this thesis, is the ability of the bacteria to convert the metals into their sulfide salts, thereby rendering them fixed, and the ash safe for land disposal.

Chapter 2

OVERVIEW OF INCINERATION

Incineration is the process of thermally reducing the volume of solid waste while producing *inoffensive gases and sterilized residue*, by the application of the combustion process [National Center for Resource Recovery, Inc.].

Large incinerators were first developed in England in 1874. After World War II and the growth in chemical wastes there was widespread installation of incinerators in the USA.

2.1 Advantages and disadvantages of incineration

Incineration of wastes offers both benefits as well as drawbacks to the community.

The potential **advantages** of incineration are:

(1) Volume Reduction: This is especially suitable for bulky solids with a high combustible content. High temperature incineration can reduce the volume of waste collected by as much as 90-95%.

(2) Detoxification: Mainly for combustible carcinogens, pathogenically contaminated material, toxic organic compounds or biologically active materials finally producing an inert ash.

(3) Energy Recovery: Energy production offsets incinerator costs and can also be used as community energy source.

(4) Byproducts: The high free lime content (the source being salts of Ca and Mg) of incinerator residue, creates the potential for several reutilization processes. Some of the feasible ones are now briefly discussed.

(i) Waste minimization strategy through use of fly ash [University of N. Dakota, Energy Research Center, 1988]

Coal burning utility plants, municipal solid waste incinerators, and resouce recovery facilities produce large quantities of fly ash that must be either used in economical applications or disposed in an environmentally acceptable manner. All new landfills are required to have liner systems to protect groundwater from leachate contamination.

Effective liner systems are a pre-requisite to waste disposal, and the material used to develop liners have to meet numerous criteria. Fly ash has been found to be a cost effective liner material meeting state regulatory requirements for liner permeability. Liner development criteria include a permeability of less than 10^{-7} cm/s, and a durability comparable to that of conventional clay liners. A representative sample from six companies located in five states when mixed with $3-9.5\%$ by weight of cement or lime yielded respective liners with permeabilities significantly less than 10^{-7} cm/s, and compression strength generally exceeding 400 psi. Liner construction costs ranged from \$ 0.05-0.44 per foot , with the cost depending on the quantities of fly ash and the additives used.

(ii)Road base ad-mixtures [Turgeon, 1988]

With the amendment of the Solid Waste Management Act in 1987 by the Pennsylvania legislature, coal ash is now a natural resource and no longer a solid waste. The theory that fly ash produced by electric power companies

should be put to good use rather than buried in landfills was finally put into practice by Penndot. 400,000 tons of fly ash were used as fill material along route I-279, near Pittsburg. This geotextile blanket spans the entire width of the valley for the 1,200 feet length of the fill which will serve the eight lane East Street Valley Expressway. Incentives for using fly ash as a structural fill came from the Federal Highway Act, passed in December 1987, which increased Federal funds for projects where significant amounts of material from coal ash was used on any construction project with a view to promote reuse of power plant ash and reduce disposal costs for utilities that generate ash.

The **Disadvantages** of incineration are:

(1) Cost: Both initial investment and operation costs are high because of the new designs and pollution control equipments mandated by EPA.

(2) Staffing: Skilled labor adds to the cost.

(3) Public reaction: Hostility from the public and/or regulatory agencies, NIMBY(not in my backyard) syndrome contributes to the hurdles incinerators have to face.

(4) Secondary Impact Problems: Emissions of SO_2, NO_x, CO and other noxious materials pollute the air. The ash routinely contains dangerous levels of toxic metals such as Pb, Cd, and can also carry excessive amounts of carcinogenic dioxin [Star Ledger, 1989]. Hence residue disposal can present a variety of problems.

2.2 Regulatory aspects of ash disposal

The growth of the resource recovery industry has ash disposal as its main obstacle. It is today a complex and politically volatile issue for several reasons. First, no standards exist outlining what constitutes hazardous or non-hazardous

ash. Second, the quality of ash and its toxicity vary with incinerator feed material and operation characteristics, leading to variable test results. Finally, no standards exist to determine how ash should be managed, reused, or disposed of. Regulations are often developed out of emotional community reaction to the issue.

Confusion reigns as to whether incinerator ash is classified as an hazardous waste or not. The regulatory difference between household waste ash and municipal solid waste ash was clarified by Congress when it enacted the Hazardous Solid Waste Ammendments (HSWA) in 1984. The law states that if a facility takes only household and non-hazardous commercial and industrial waste, and has a program to keep subtitle C hazardous waste out, then it is not deemed to be generating, treating or otherwise managing hazardous waste. When EPA placed the law into the Code of Federal Regulation, they did not interpret the law in the same way as the Congress intended. EPA stated that if the ash was tested and exhibited the characteristics of a hazardous waste, it had to be managed as such.

Since the success of incinerators depends on increasing amounts of trash brought to the facility, officials are determined to maintain low disposal costs which can be achieved only if the ash remains as a non-hazardous material [Star ledger, 1989]. Present disposal costs are $100-150 per ton for non hazardous and $300-350 per ton for hazardous ash.

Possible solutions of the burial issue of ash are new reutilization techniques, use of ash monofills where all the *bad eggs* are buried in one basket or treating the ash to render the toxics stable.

2.3 Composition of fly ash

Fly ash is the net result of coal burning in thermal power stations. Fly ash varies in its chemical composition depending on the parent coal and the operating conditions of the furnace. In general, approximately $95 - 99\%$ of fly ash consists of oxides of Si, Al, Fe, and Ca and about 0.5-3.5 % consists of Na, P, K, and S. The remainder of the ash is composed of trace elements. As indicated in Table 2.1 it is composed of all the elements found in most soils.

Thus, fly ash can be called an amorphous ferro-alumino silicate mineral with the major matrix elements being Si, Al, and Fe, together with significant percentages of Ca, K, Na, and Ti (Table 2.2). An emperical formula for fly ash has also been worked out to be [Fisher *et al.*, 1976]:

$$Si_{1.00}Al_{0.45}Ca_{0.051}Na_{0.047}Fe_{0.039}Mg_{0.020}K_{0.017}Ti_{0.011}$$

The particle size in fly ash varies from less than 0.002 mm to as high as 2.00 mm. The particle size distribution of a typical fly ash from a U.S. plant is given in Table 2.3. The size of the particles is directly related to the elemental concentration. Davison *et al.*, 1974, and Natusch *et al.*, 1975, have observed that As, Cd, Cu, Ga, Mo, Pb, S, Sb, Se, Ti, and Zn tend to increase in concentration with decreasing fly ash particle size. While the exact mechanism of such a behavior is not known, it is believed that after combustion there is a tendency of condensation due to fall in ambient temperature.

In the U.S. fly ash production has increased from a mere 24.5 million tons/year in 1971 to 44 million tons/year in 1977, and it has been estimated that the ash production reached about 100 million tons/year in 1985. Essentially the environmental impact study of fly ash is a two-step activity: (1) the physicochemical changes due to fly ash discharge in soil and water systems need to be carefully monitored from the crop production and human health viewpoints and (2) a short

Table 2.1 - Chemical composition of fly ash and soil (Sharma 1989)

Element	Range (%)	
	Fly Ash	Soil
Aluminum (Al)	0 1—17 3	1—30
Barium (Ba)	0.011—0.5	0 01—0 3
Calcium (Ca)	0.11—12.6	0 7—50
Iron (Fe)	1—26	0.7—55
Magnesium (Mg)	0 01—6 02	0 06—0 6
Manganese (Mn)	0 01—0 3	0 01—0·4
Phosphorus (P)	0 01—0 8	0 005—0·1
Potassium (K)	0 19—3 0	0 04—3·0
Silicon (Si)	19 1—28 6	25—33
Sodium (Na)	0 01—0 66	0 01—3 0
Strontium (Sr)	0 03—0 3	0 05—0 4
Sulfur (S)	0.1—1 5	0 01—0 2
Titanium (Ti)	0.16—0 7	—

	Range (ppm)	
	Fly ash	Soil
Antimony (Sb)	1 6—202	0 6—10
Arsenic (As)	2.8—6300	0 1—40
Beryllium (Be)	3—7	0.1—100
Boron (B)	48—618	2—100
Bromine (Br)	0.7—5.3	—
Cadmium (Cd)	0.7—130	0.01—7
Cerium (Ce)	22—300	50
Cesium (Cs)	3.1—10	—
Chlorine (Cl)	—	10—1000
Chromium (Cr)	10—690	5—300
Cobalt (Co)	7—49	1—40
Copper (Cu)	14—1000	2—100
Fluorine (F)	100—610	30—300
Gadolinium (Gd)	—	—
Gallium (Ga)	15—93	15—70
Germanium (Ge)	—	—
Lanthanum (La)	12—99	30
Lead (Pb)	7—279	2—100
Lithium (Li)	50—1064	1—2000
Molybdenum (Mo)	7—117	0.2—5 0
Nickel (Ni)	10—4300	10—1000
Niobium (Nb)	—	—
Rubidium (Rb)	49—220	30—600
Scandium (Sc)	3.7—141	10—25
Selenium (Se)	0.2—134	0.1—2.0
Silver (Ag)	0.04—5	0.1—8.0
Tellurium (Te)	0.02—0.2	—
Thallium (Th)	0.5—1.7	—
Tin (Sn)	3.3—63	0.1—300
Vanadium (V)	50—1000	20—250
Zinc (Zn)	36—1333	10—300
Zirconium (Zr)	50—1286	60—2000

Table 2.2 - Chemical composition of fly ash (Sharma 1989)

Name	Formula	Percentage
Silica	SiO_2	62
Iron oxide	Fe_2O_3	63
Alumina	Al_2O_3	26
Titanium dioxide	TiO_2	1.80
Potassium dioxide	K_2O	1.28
Calcium oxide	CaO	1.13
Magnesium oxide	MgO	0.49
Phosphorus pentoxide	P_2O_5	0.40
Sulfate	SO_4	0.36
Disodium oxide	Na_2O	0.28

Table 2.3 - Size distribution in U.S. fly ash (Sharma 1989)

Category based on grain size	Size (mm)	U.S. fly ash (%)
Very coarse sand	2.00—1.00	0 2
Coarse sand	1 00—0 50	0 9
Medium sand	0 50—0 25	3 4
Fine sand	0.25—0.10	14.8
Very fine sand	0 10—0.05	13.1
Silt	0 05—0 002	63·2
Clay	<0.002	4.3

term goal should be to approach the 40-50% utilization of fly ash occuring in some European countries [Adriano *et al.*, 1980]. Feasible reutilization techniques being evaluated in the U.S. have been mentioned earlier in this chapter.

Due to the strict guidelines issued by many countries, several types of pollution control equipment like scrubbers and electroststic precipitators are mandatory in incinerators and power plants. The use of these devices produces a 98-99.5% removal of fly ash from the flue gas stream. As a result millions of tons of ash are produced each year. Since fly ash reutilization is still in its infant stage there is an immediate need to solve the issue of ash disposal.

Chapter 3

LITERATURE SURVEY

3.1 The Sulfate-Reducing Bacteria

John R. Postgate, a pioneer who has done extensive work with the sulfate-reducing bacteria, believes that *"studies on the bacteriology and biochemistry of the sulfate-reducing bacteria have been obscured by the inconsistency and capriciousness of these organisms often not described explicitly in the literature, but a common experience among workers in the field"* [Postgate, 1953].

However, the *Desulfovibrios* have been found especially interesting due to their involvement in the formation of metallic sulfides and elemental S, the corrosion of iron and steel pipes, and the genesis of a potent toxin, H_2S.

Barghoorn and Nichols (1961), found that without enzymatic intervention, the H_2S formed by *desulfovibrio* will react with heavy metals in solution to yield insoluble metal sulfides. Thus, much of the H_2S that is generated in oxygen-free muds, soils, and water containing ferrous iron, combines immediately with the iron, the final product often being a black preciptate of FeS_2 (pyrite).

In vitro studies by Bass Becking and Moore (1961), showed that the bacterial reduction of sulfate in an artificial marine ecosystem is a prelude to the formation of Cu, Ag, Pb, and Zn sulfides. It is plausible to suggest that many of the commercially mined sulfides, were deposited originally when soluble ions of

the element reacted with and were precipitated by H_2S [Alexander, 1971].

In addition, the reducing conditions engendered by the activity of *Desulfovibrio* in sulfate-rich waters have been implicated in the reduction of the soluble uranyl ion and precipitation of UO_2 in natural uranium deposit [Jensen, 1958]. Reduction of uranium under anaerobic conditions by sulfate reducing bacteria has been reported by Evardsson (1984) and West *et al.* (1988) as:

$$UO_2^{2+} + H_2S \text{ —> } UO_2 + 2H^+ + S^o$$

Francis *et al.* (1989) investigated anaerobic microbial transformations of uranium and toxic metals presented in depleted uranium wates. Though the wastes were found to contain very high concentrations of (3000 ppm) of uranium, little uranium was detected in the solution. This was attributed to reduction and precipitation of uranium under reducing conditions brought about by microbial action.

Miller (1950), grew these bacteria in the presence of carbonates or oxides of Pb, Zn, Sb, Bi, Cd, Co, and Ni and considered that they might be responsible for the deposition of several types of metal sulfide ores in a sulfuretum, i.e., a zone of sulfate reduction in an ecosystem.

Trudinger (1976, 1982), reviewed the role of these bacteria in ore formation and suggested that sulfide deposits of Cu, Ag, and U could also be biogenic, in addition to those mentioned earlier.

Douglas J. Cork and Michael A. Cusanovich (1978), demonstrated that a mutualism may be established between *desulfovibrio* and the green and purple sulfur bacteria *Chlorobrium* and *Chromatium* respectively. By combining the activities of these organisms sulfate, a component of solvent extractions posing disposal problems, is quantitatively converted to elemental S.

Using anaerobic marine sediments, Jorgensen and Fenchel (1974), and Jor-

gensen (1977), showed that more than half the added organic matter was completely degraded to CO_2 in the course of sulfate reduction. In experiments with anaerobic sediment, Winfrey and Zeikus (1977), demonstrated that the carbon and electron flow was altered from methanogenesis to the formation of CO_2 and sulfide, when sulfate was added to the samples.

When sulfate becomes exhausted in deep layers or microniches of such habitats, the terminal degradation is taken over by the methanogenic bacteria. Even under these conditions, hydrogenase-containing sulfate reducing bacteria may maintain metabolic activity by cleaving certain reduced fermentation products to acetate and hydrogen, thus providing substrates for methanogenic bacteria [Bryant *et al.* (1977); McInerney and Bryant, (1981)].

Peck (1959), and Ishimoto (1959), established that ATP was consumed in the process of sulfate reduction; thereafter, a cyclic mechanism leads to sulfide formation. Though details of the sulfide-generating system are still uncertain, work by Postgate (1984), Lee and Peck (1971), Kobayashi *et al.* (1972), Skyring and Jones (1977), Vainshtein *et al.* (1980), and Akagi (1981), concluded that a cyclic pathway for dissimilatory sulfate reduction exists.

In Figure 3.1 (Postgate 1984), the selenate ion competetively inhibits the process by which the sulfate ion outside the cell is accumulated inside the cell. The sulfate which enters the cell reacts with ATP to form adenosine phosphosulfate (APS) and pyrophosphate (PP), the latter being removed as inorganic phosphate. APS is reduced to sulfite and AMP. Sulfite, via intermediates (not mentioned due to dissension among authors) gives trithionate ($S_3O_6^{2-}$). This splits reductively to give thiosulfate, which is then reduced to give sulfide.

Postgate, (1984) presented the following reasons why yield studies with sulfate-reducing bacteria on batch cultures can have variable data.

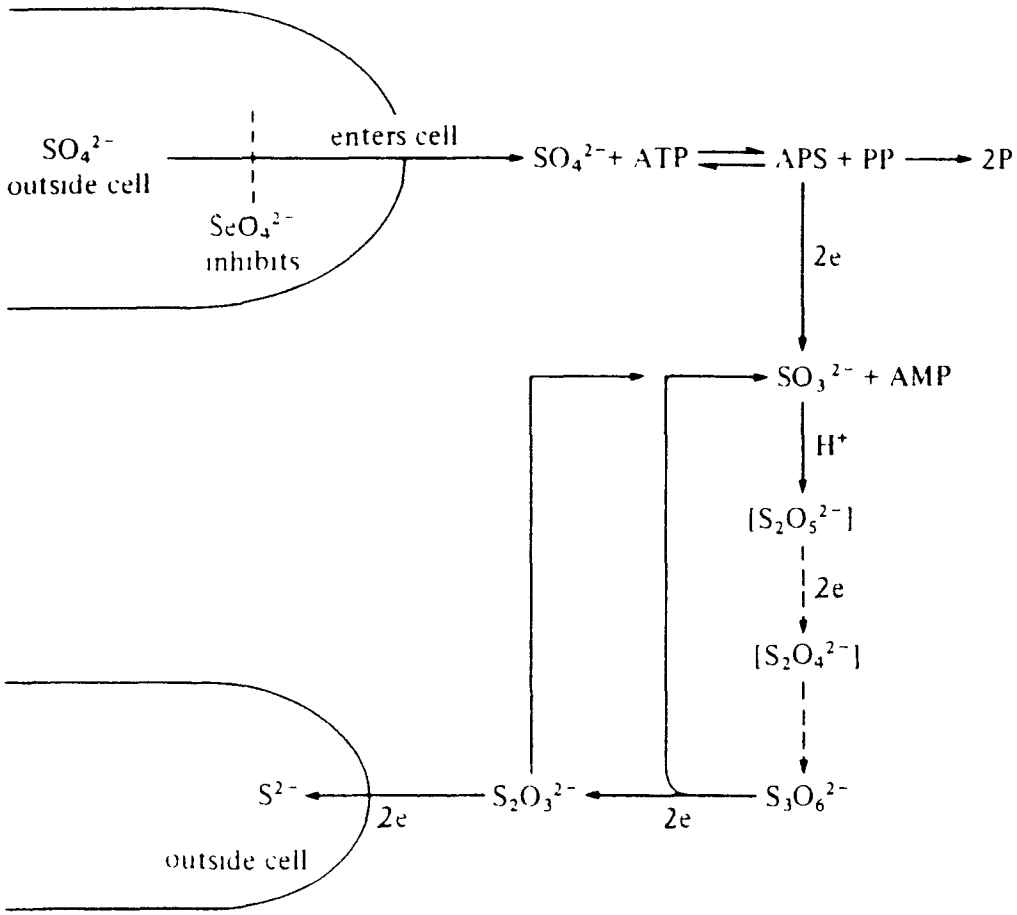


Fig 3.1 - Cyclic pathway for dissimilatory sulfate reduction (Postgate 84)

(i) Growth of sulfate-reducing bacteria is usually linear, not exponential. This phenomenon occurs because sulfide, although a product of metabolism, is also an inhibitor of growth: the higher the sulfide concentration, the longer the doubling time of the population. Thus, energy is being expended in countering the inhibitory effect of sulfide and, in a batch culture, this energy loss interferes with the ATP calculation since the *maintenance coefficient* increases as the culture grows.

(ii) If the above problem is avoided by removal of sulfide, the pH shifts to an alkaline value, imposing another stress on the population which also requires ATP *maintenance.*

(iii) Sulfide precipitates iron, so that iron, and not the carbon/energy source, may become the limiting substrate.

Domka, Gasiowek, and Klemm (1977), surveyed a variety of municipal wastes (sewage, yeast, sugar, and dairy wastes) for purification by way of bacterial sulfate-reduction. Here sulfate is the terminal oxidant for anaerobic biological waste treatment unlike the more common activated sludge process.

Tuttle et al. (1969), described a system in which acid mine water flowed through a porous dam of wood dust, within which a consortium of sulfate reducing bacteria reduced sulfate and generated sufficient alkalinity to render the effluent acceptable. Iron, sulfate and acidity were thus removed in one treatment.

3.2 Previous studies on the leaching and toxicity of ash

Presently, fly ash is exempt from subtitle C of the Resource Conservation and Recovery Act [USEPA, 40 CFR. 270], and is therefore not classified as an hazardous waste. However, since some of the criteria that define wastes as hazardous

(corrosiveness, radioactivity, and toxicity) are potentially applicable to fly ash, attention has focused on leachates generated by fly ash deposits [Roy *et al.*, 1981]. 56 million tons of ash were produced in the USA in 1980 of which only 15% was recycled. Approximately 36% of the fly ash was buried in landfills [National Research Couoncil, 1980].

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 [Natusch, 1975]. The leachability is dependent on the pH of the make-up waters, bonding of element to fly ash, and diffusivity of each species [Phung *et al.*, 1979]. With a lowering of pH, the release of soluble trace metals increases and causes concern to the biotic ecosystem [Theis and Wirth, 1977]. Solubility plays an important role in trace element concentrations levels in the aquatic environment. Toxic elements in fly ash are preferentially enriched in a thin layer at the particle surface and may be more readily leached $(5 - 40\%)$ in water than the bulk ash constituents [Linton *et al.*, 1975].

The ability of a wide variety of aquatic organisms to concentrate trace elements is well documented [Patric, 1978; Allen *et al.*, 1980]. Goldberg (1963), demonstrated that trace element uptake increases with their ability to be complexed by ligands. Studies suggest that free metal ions are the toxicant chemical species since the stronger the metal complex, the lower the toxicity of the metal under a given concentration. Certain microbes concentrate trace metals in their systems and pass them on to other organisms in the food chain [Patrick and Loutit, 1976]. One of the principal routes of some of the trace elements through the environment is from waste fly ash streams into receiving waters, where ac-

cumulation of trace elements from the water leads to *ecological magnificaton* in higher species. Bioaccumulation factors for trace elements are several orders of magnitude higher than factors for biomagnification along food chains. Hazardous chemical substances consistently found in fly ash have been documented in literature to reach concentrations harmful to man and other consumers [Gutenmann, *et al.*, 1976].

Lopat Enterprises, Inc. a publicly held corporation, has developed a site-specific formulation used in control and remediation of hazardous leachable toxic metals (Pb, Hg, Cd, Zn, Ba, Cr, Cu, Ar, Ni, and B) in bag hose dust, incinerator ash, furnace slag, shredder waste, sludge, soil, and particulate material. Marketed under the brand name K-20 Lead-in-Soil-System (K20/LSC), toxicity reductions are achieved by both physical encapsulation in a cementitious matrix and chemical complexing with the contaminant to form insoluble metal metasilicates. EPA recognition as to reliability, stability and longevity of treatment have been corroborated by Toxic Characteristic Leaching Procedure (TCLP), and Extraction Procedure (EP) toxicity tests [Lopat Enterprises, Inc.].

A number of procedures have been proposed and evaluated for the leaching of solids. All of them seek to assess the worst case scenario of waste material that has been buried, leaching out with time under the forces of nature and thus polluting nearby ecosystems. Cote and Constable (1983), used distilled water, an acidic solution and a synthetic leaching solution (composed of acetic acid, sodium acetate, glycine and salicylic acid, having higher available acid than the other two solutions) as the three leaching media on a number of wastes from various locations across Canada. They found that distilled water had little effect on the leachate pH. Acid solution shifted the leachate pH towards the pH of the leaching medium. The synthetic leaching solution appeared to complex some contaminants

and increase their release above that to be expected from pH considerations. The contaminant release from the same waste was found to vary over several orders of magnitude within a short pH range. A linear relationship was observed between the leachate pH and the logarithm of release for some wastes and contaminants.

Boyle *et al.* (1983), 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 availability of metal in the EP test.

Langley *et al.* (1989), incorporated high volumes of ASTM (American Society for Testing and Materials Method) class F ash in structural concrete. Walter D. Munn, 1989 states that the use of lime/fly ash cuts cost of Highway construction. With the copious amounts of ash being produced, reutilization processes are extremely necessary, as landfill space is scarce. Adding ash as an aggregate for concrete and in road-base mixtures are typical uses of fly ash (described in detail in Chapter 2 of this thesis).

Chapter 4

MATERIALS AND METHODS

4.1 Growth and culture of *Desulfovibrio.*

The methods used to grow these bacteria were based on Postgate's work on these organisms [Postgate, 1984]. Though the media and procedures described were straightforward, it took several months to establish the desired cultures.

Soil sediment samples were procured from a waterbody in the Hackensack Meadowlands(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 till the time of inoculating the medium. Medium was inoculated as soon as possible with a maximum lag time of 48 hours.

Initially, media differing in carbon sources (lactate, cellulose, glycerol) and reducing agents were used. Baars medium (1930) Starkey's medium(1938) (Pankhurst, 1971), and Postgate's medium (1984), were the three experimented with, of which Postgate's Medium B was found to be most suited. Growth was quicker in Medium B as indicated by the formation of a black precipitate of sulfide. Moreover, production of hydrogen gas as an indication of growth of methanogens

Table 4.1 - Basic Salts medium, modified Postgate's medium B

Salt	*Concentration(g/l)*
KH_2Po_4	0.5
NH_4Cl	1
$CaSO_4$	1
$MgSO_4.7H_2O$	2
Sodium lactate(60% solution)	4.08
Yeast extract	1
$Na_2S.9H_2O$	0.4
$FeSO_4$ $.7H_2O$	0.5
Sodium chloride	25

was noticed in other media.

In this work a modified medium B was used in which the reducing agents, ascorbic acid and thyoglycollic acid originally present in medium B were substituted with sodium sulfide (Table 4.1).

The reducing agent, $Na_2S.9H_2O$, was added to the culture as a 0.04% solution (1 ml/100 ml medium). The medium was made to a liter with distilled water.

This medium contained a precipitate that aided 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 7-7.5. The mixture was then brought to a boil to drive out oxygen. Resazurin, a redox indicator, was added to the medium as a 0.1% solution, to help monitor the reduced environment needed for the growth of the sulfate reducers. While the medium was still hot, as indicated by the steam effusing out, the container was gassed with nitrogen, to prevent re-entry of air. Since the *desulfovibrios* are obligate anaerobes, to remove all traces of oxygen, the nitrogen gas used was passed through copper filings heated in a Sargent- Welch furnace (Sargent-Welch Scientific Co., Skokie, 111.), prior to being channelled through a gas manifold

through which the mixtures were dispensed into serum bottles.

The following apparatus was used in the experimentation process:

Serum bottles(200 ml)

Manifold system

Rubber tubing

Syringe and needles

Rubber stoppers

Metal crimps

Magnetic stirrer

Assorted glassware.

The Hungate technique was used to transfer medium to the serum bottles (Skerman 1967). The serum bottles were also subjected to 2 minutes of gassing (N_2), after which they were stoppered and clamped. At the time of inoculation of the sample 0.04% $Na_2S.9H_2O$ was added to the bottles at a dose of 1 ml per 100 ml, along with a 1 ml inoculum.

The bottles were incubated at $30°C$ for 3 days (the normal growth period of *desulfovibrios*). Wet mounts of the culture, when observed under the phase contrast microscope, showed a mixed population of microrganisms. Hence serial dilutions of the culture, in a series of test tubes, with medium B were made. On re-examination of the cultures, from 10^{-3}, and 10^{-4} dilutions, under the microscope, a culture predominant in rapidly moving vibrios was observed. These were used as inoculum for the experimental process.

4.2 Composition of ash sample

The ash sample used in this project was obtained from Dr. David Kosson, Department of Chemical and Biochemical Eng., College of Eng., Bush Campus,

Rutgers University, New Brunswick. It was a combined ash sample (bottom ash and fly ash), generated at an incinerator (origin undisclosed). Studies had been conducted on this ash at Dr. Kosson's laboratory. Acid digestion was performed on the ash to indicate total metal content of the sample. Concentrations of Pb, Cd, and Cr, were of significance to us since these were the metals of interest, and were found to be:

Lead 1660 mg/kg of ash

Cadmium 37.5 mg/kg of ash

Chromium 72.8 mg/kg of ash

The values given above were those received from the acid digestion performed at Dr. Kosson's laboratory.

4.3 Experimental set up of *desulfovibrio* cultures

700 ml glass bottles with screw caps were used as the anaerobic culture vessels. All the glassware and media were autoclaved at 125°C for 25 minutes. Two culture types were used as inoculum in the experiment. The first, referred to as MB, was obtained from Mitch Berman, Department of Microbiology, New York University and Medical Center, New York, who had been working with the *desulfovibrios* for several years. The second, referred to as U, was cultured as described in section 4.1 of this Chapter. Cultures were set up as shown in Table 4.2. Cultures A, B, C, and E were control culture vessels. Culture A contained no lactate, the carbon source of *desulfovibros*. Culture B contained no sulfate, the electron acceptor of the bacteria. Culture C contained no inoculum, and Culture E contained a sterile inoculum. Triplicate cultures of inoculum MB (F, G, H), and U (J, K, L) were set up at 30°C. Culture I represented a culture of inoculum MB at 21°C.

After inoculating the medium, the cultures were given a three day period

Table 4.2 - Set up of *Desulfovibrio* cultures

Culture	*Temp.(°C)*	*Medium*	*Inoculum(1ml)*	*Ash*
A	30	no lactate	MB	10 g
B	30	SO_4^{2-} substituted by Cl^-	MB	10 g
C	30	Med. B	-	10 g
D	30	Med. B	MB	-
E	30	Med. B	Sterile(MB)	10 g
F	30	Med. B	MB	10 g
G	30	Med. B	MB	10 g
H	30	Med. B	MB	10 g
I	21	Med. B	MB	10 g
J	30	Med. B	U	10 g
K	30	Med. B	U	10 g
L	30	Med. B	U	10 g

Note: MB- Mitch Berman's Culture
U-inoculum cultured as described in 4.1
21 °C- Room Temperature

to acclimatize themselves before the addition of ash. 10 grams of ash was added to each culture except D, which contained no ash.

After analyzing the cultures, Culture I was replicated as I_a, I_b, and I_c under exactly the same conditions to confirm results obtained with I.

4.4 Sulfate and Sulfide analysis by Ion Chromatography (IC)

4.4.1 Sulfate analysis

Waters ion chromatography method No. A-102 was used to measure the amount of sulfate (ppm) present in each culture bottle.

Cultures were sampled at 3-4 day intervals over a 14 day period. Prior to sampling, the culture bottle was not shaken. This was because of not wanting to sample the thick precipitate originally present in the medium, and the black ferrous sulfide formed once the desulfovibrios were introduced to the medium.

Hence, samples may not have been homogenous in their salt concentration. The 1 ml samples were stored in plastic centrifuge vials (eppendorf) in the freezer (below 0° C) until the time of analysis.

Preparation of Samples and Standards

Prior to the analysis each sample was diluted (1:50 times) with Milli Q^R water. To prevent chromatographic interference from carbonates, metals such as magnesium, and calcium, the samples were pretreated with a Millitrap^{TM} H^+ Membrane Cartridge. The Millitrap^{TM} H^+ cartridge is a hand held, multiple use, disposable device that removes interferences from the culture medium [Fig. 4.1]. Furthermore, because of the high chloride (NaCl) content in Medium B, the samples were also passed through a Maxi-$Clean^{TM}$ IC- Ag cartridge, a solid phase extraction device used to eliminate chloride interferences prior to analysis by ion chromatography [Fig. 4.2]. In the Ag^+4 cartridge Ag contained in the packing reacts with the chloride from the sample to form an insoluble precipitate of AgCl. This has no significant effect on the sulfate in solution.

Standards of concentration 10 ppm, 25 ppm, and 50 ppm were prepared from a stock concentrate of 1000 ppm sulfate (J.T. Baker Chemical Co.), immediately prior to the analysis, and were run in duplicate.

Instrumentation [Fig.4.3]

The IC apparatus consists of the following:

(i) Pump system (Waters 600E- system controller)

(ii) Sample processor, housing the injection system (Waters 715, Ultra Wisp)

(iii) Column IC-PAK A HC, 150 x 4.6 mm, 10μm

(iv) Tunable absorbance detector (Waters 484)

(v) Conductivity detector (Waters 431)

(vi) PC Minichrom, a chromatography data handling system,

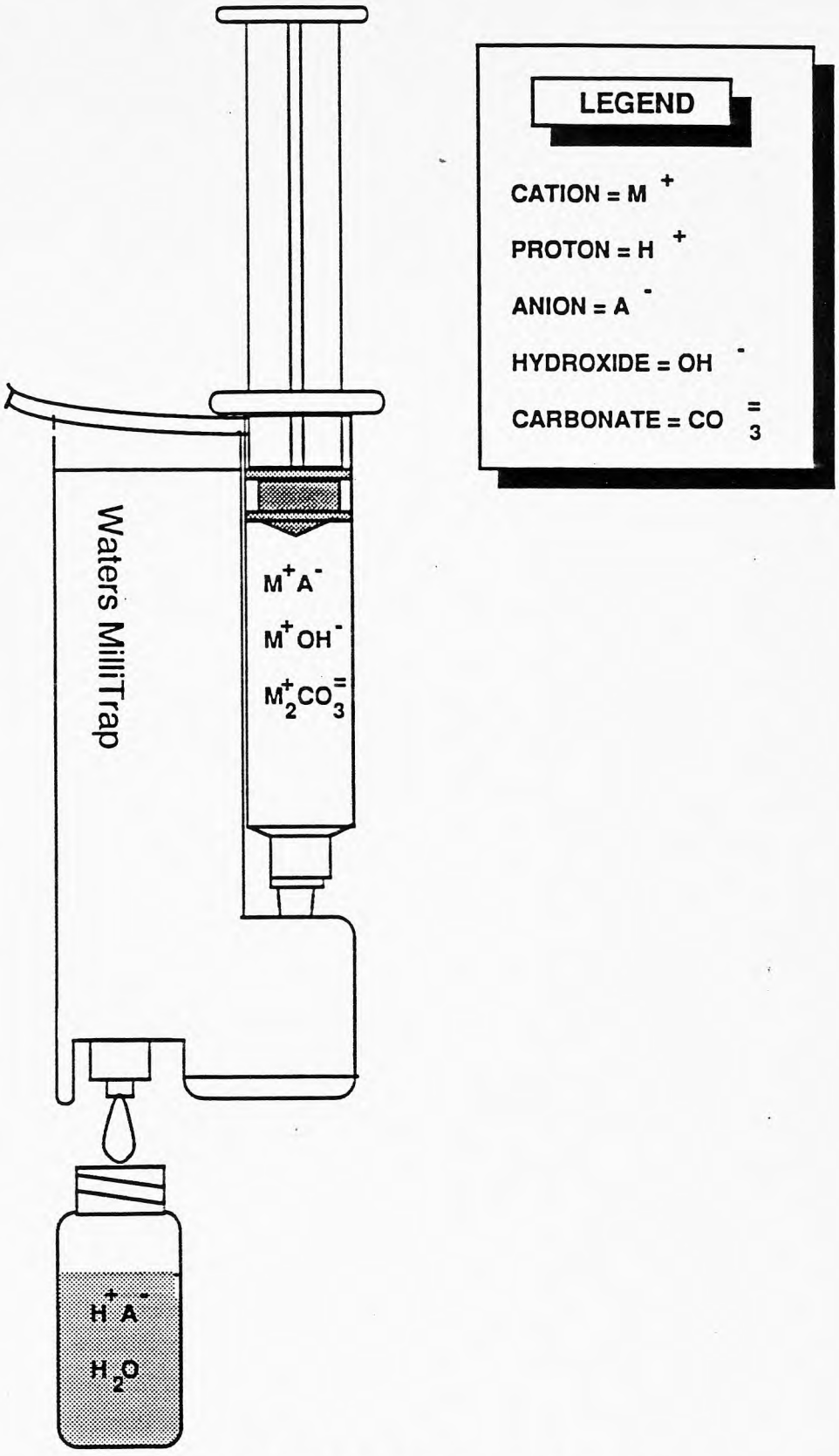


Fig 4.1 - *Millitrap*^{TM} H^+ Membrane Cartridge

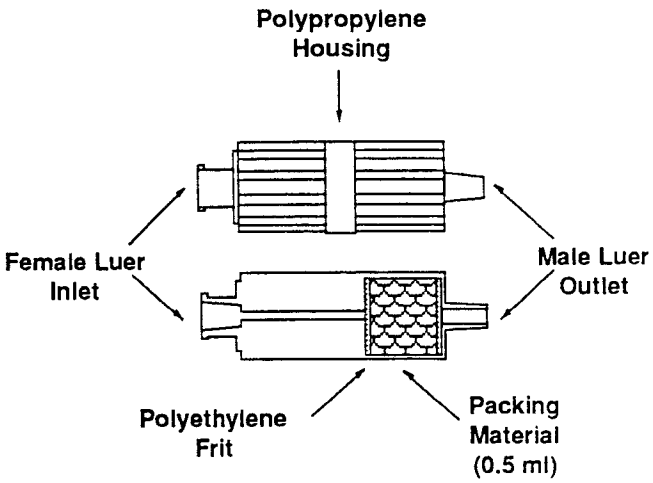


Fig 4.2 - $Maxi - Clean^{TM}$-IC-AG Cartridge

Software version 1.5, 1990 VG Data System Ltd.

Instrument conditions

Eluent: Borate/Gluconate

Flow rate: 2.0 ml/min

Injection: 100μl of sample

Detection: 431 Conductivity

Range: 500μS

Conductivity detector temperature: 35°C

Polarity: +ve

Background: 220-230μS

Sulfate standards and samples were placed in the wisp, for auto sampling, each sample requiring an approximate run time of 30 minutes. The calibration curve for sulfate standard is shown in Fig. 4.4.

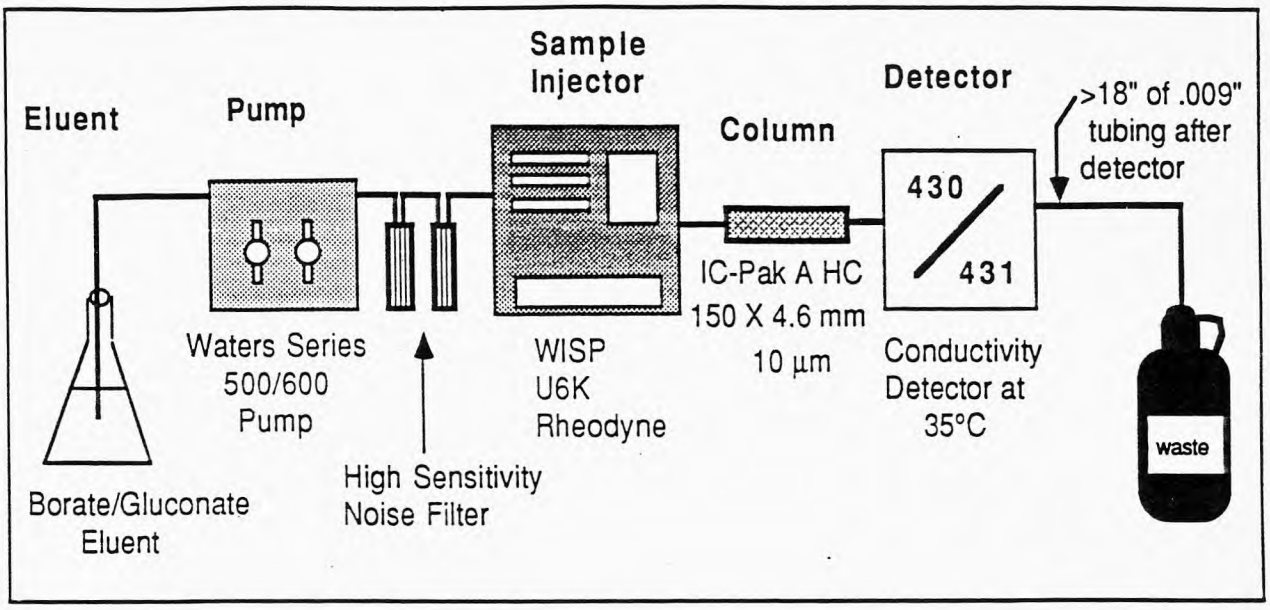


Fig 4.3 - Instrumentation of IC

STANDARD CALIBRATION CURVE FOR SULFATE

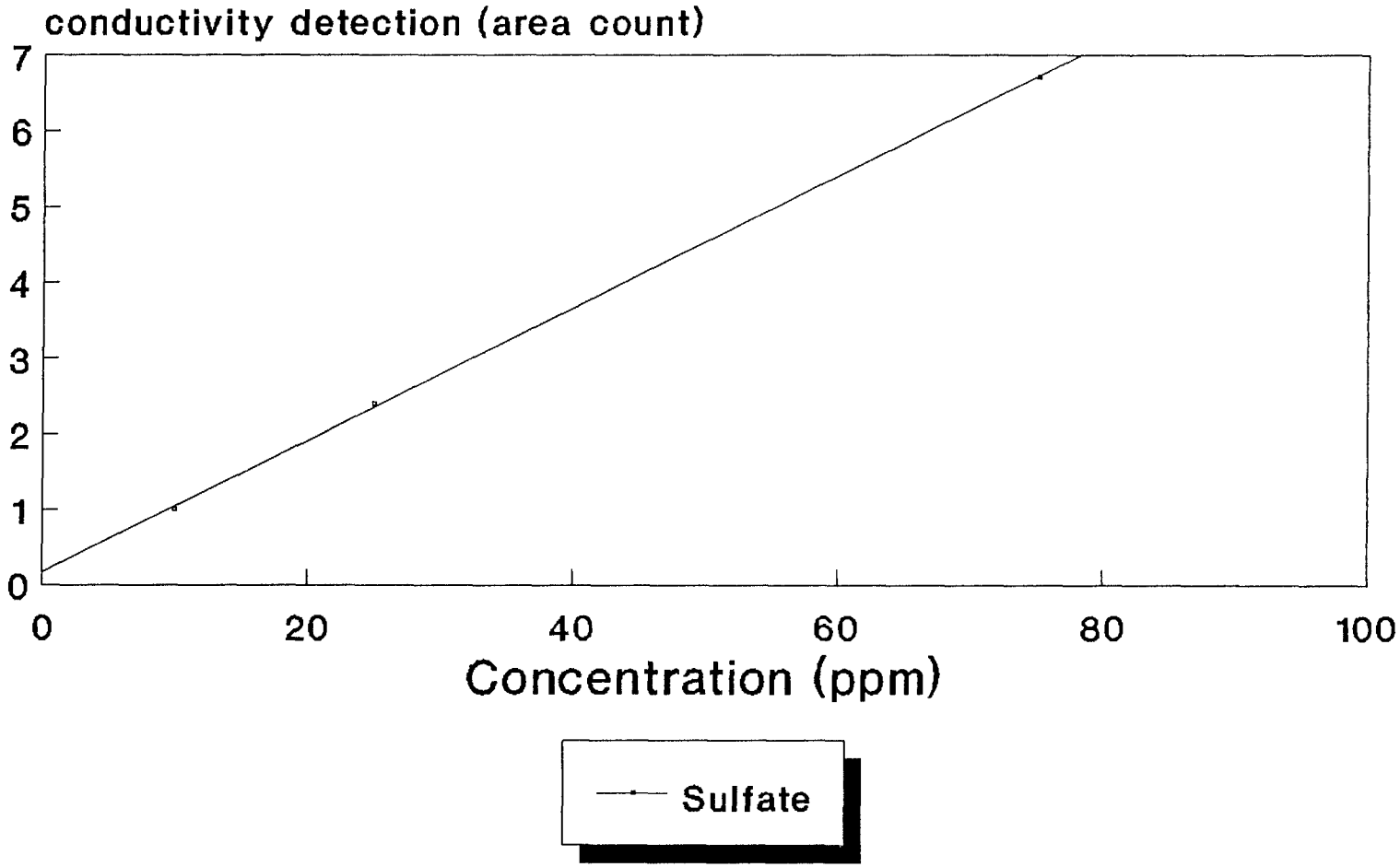


Fig 4.4 - Standard calibration curve for sulfate

4.4.2 Sulfide analysis

Waters ion chromatography method No. A-111 was used, to measure the amount of sulfide and sulfate simultaneously, as a means of confirming previous analysis described earlier in this section. Sulfide concentrations also helped assess the degree of immobilization, since the basis of bacterial activity is the formation of sulfide, which would fix the toxic metals.

Sulfide ion is highly unstable in solution. Therefore, it was necessary to sample and analyze the cultures immediately. Each sample was diluted (1:50 times) with a diluent consisting of 250 ml of 0.1 M Na_2HPO_4, which is the eluent for the method, and 1.8218 g of mannitol, the solution being made to a liter. Mannitol is the preservative used to prevent oxidation of sulfide for 3-4 hours so as to enable sulfide detection. Neither the $Millitrap^{TM}$ $H+$, nor the AG cartidge was used since they might remove the sulfide. Instead $SEP - PAK^R$ $C - 18$ Cartridge was used so as not to overload the column.

Four standards, each of them containing sulfate, sulfide, and sulfite were run with sulfate concentrations ranging from 10, 25, 50 and 75 ppm, and the corresponding sulfide and sulfite standards being 15, 10, 5, and 1 ppm. The standard curves for the above used in calculating sample values are shown in Figures 4.4, 4.5, and 4.6 respectively.

Instrumentation Same as method A-102.

Instrument conditions

Eluent: 5.0 mM Sodium Phosphate Dibasic

Flow rate: 2.0 ml/min

Injection: 100μ l of sample

Detection: 430 Conductivity

Range: 1000μ s

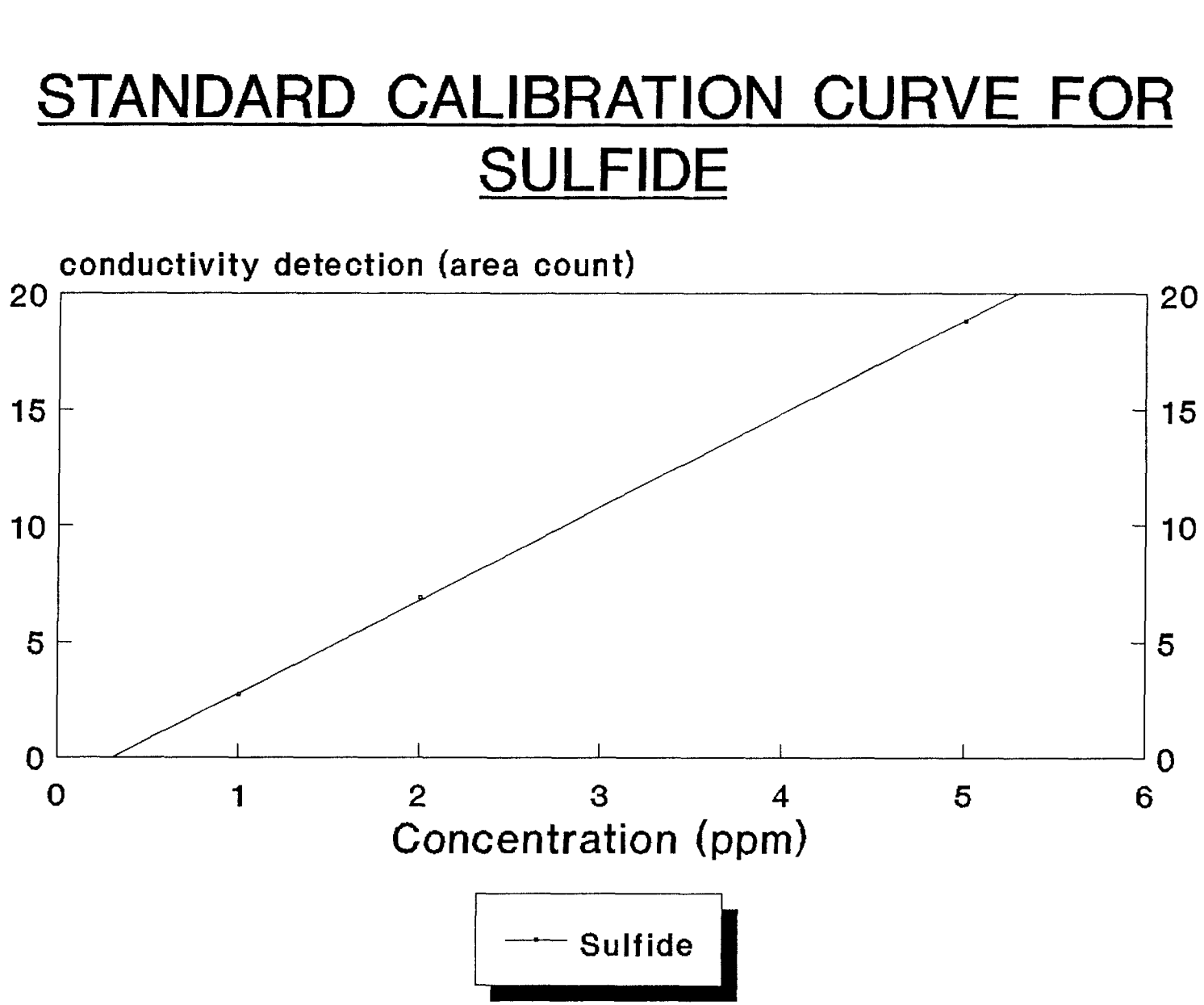


Fig 4.5 - Standard calibration curve for sulfide

STANDARD CALIBRATION CURVE FOR SULFITE

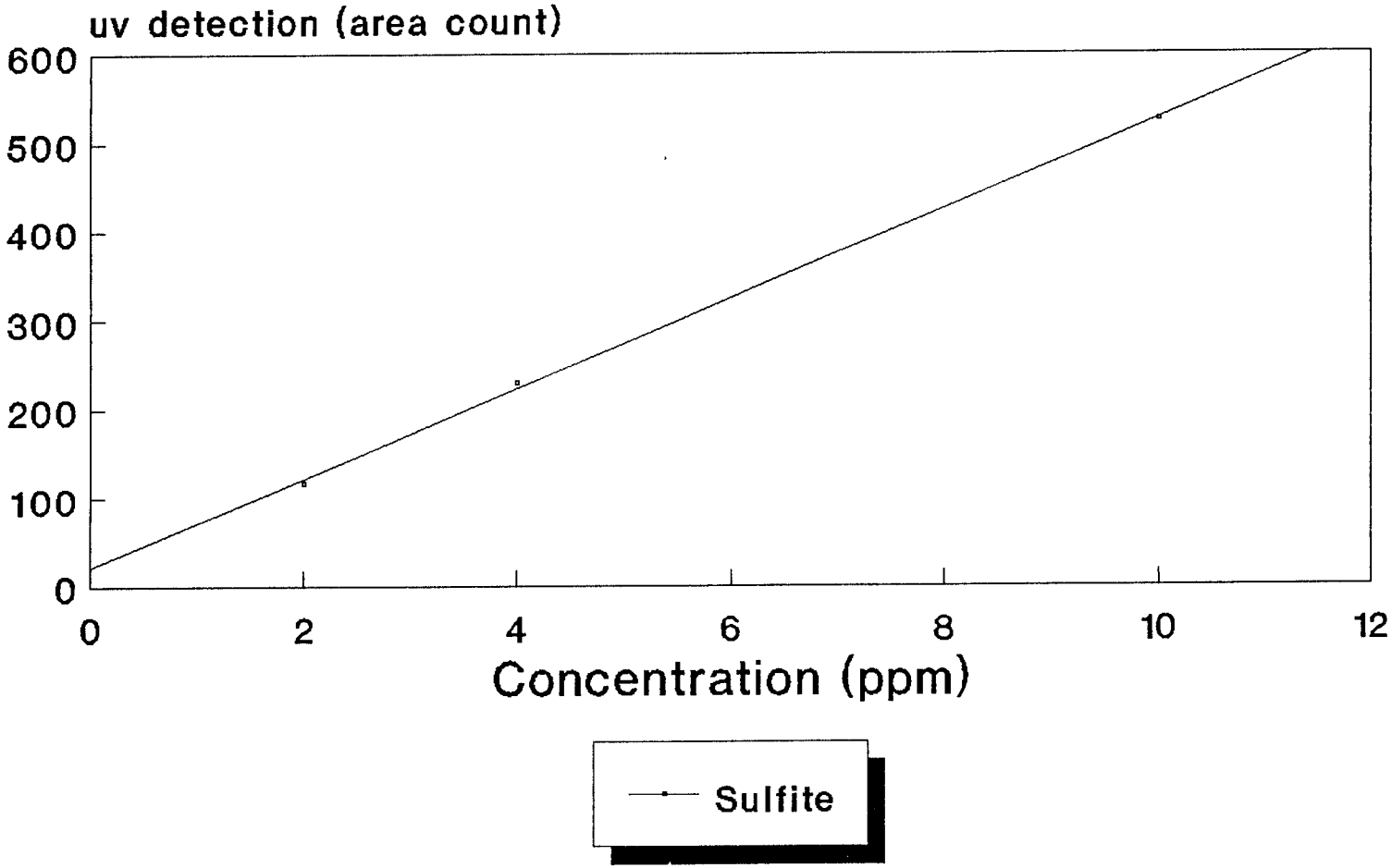


Fig 4.6 - Standard calibration curve for sulfite

Conductivity detector temperature: $35°C$

Polarity: -ve

Background: 960μ s

4.5 Leaching tests

Toxic Characteristic Leaching Procedure (TCLP)

The TCLP, [40 CFR Ch I (7-1-88 Edition)] is the EPA recommended method designed to determine mobility of both organic and inorganic contaminants present in liquid, solid and multiphasic wastes. It is designed to simulate conditions in nature, and for our purpose, metal leaching from fly ash, buried in a landfill. TCLP procedures are outlined in the flow charts; Table 4.3 for ash analysis, and Table 4.4 for the multiphasic culture. It is important to note that acid digestion as required in the TCLP procedure for metal analysis was performed using two methods: (i) Nitric acid digestion (Standard Methods, 1985); (ii) Hydrochloric acid (37%) HNO_3 (70%) microwave digestion using microwave energy (CEM Corp. Method EW-2).

Both digestion methods yielded the same results irrespective of the fact whether the sample was digested or not, indicating that digestion is not necessary and it was therefore not performed on further samples. The TCLP extract was then subjected to Atomic Absorbance (AA) analysis for determining concentration of Cadmium, Lead, and Chromium.

Extraction Procedure (EP) Toxicity Test(Method 1310, EPA, Sept 1986.)

This is the older method employed by EPA to determine whether a waste is toxic. Since the leaching of metals is highly pH dependent, and the TCLP did not call for stringent pH monitoring, the EP TOX was also performed on the culture and ash. The EP Tox procedure is outlined im Table 4.5. It is important

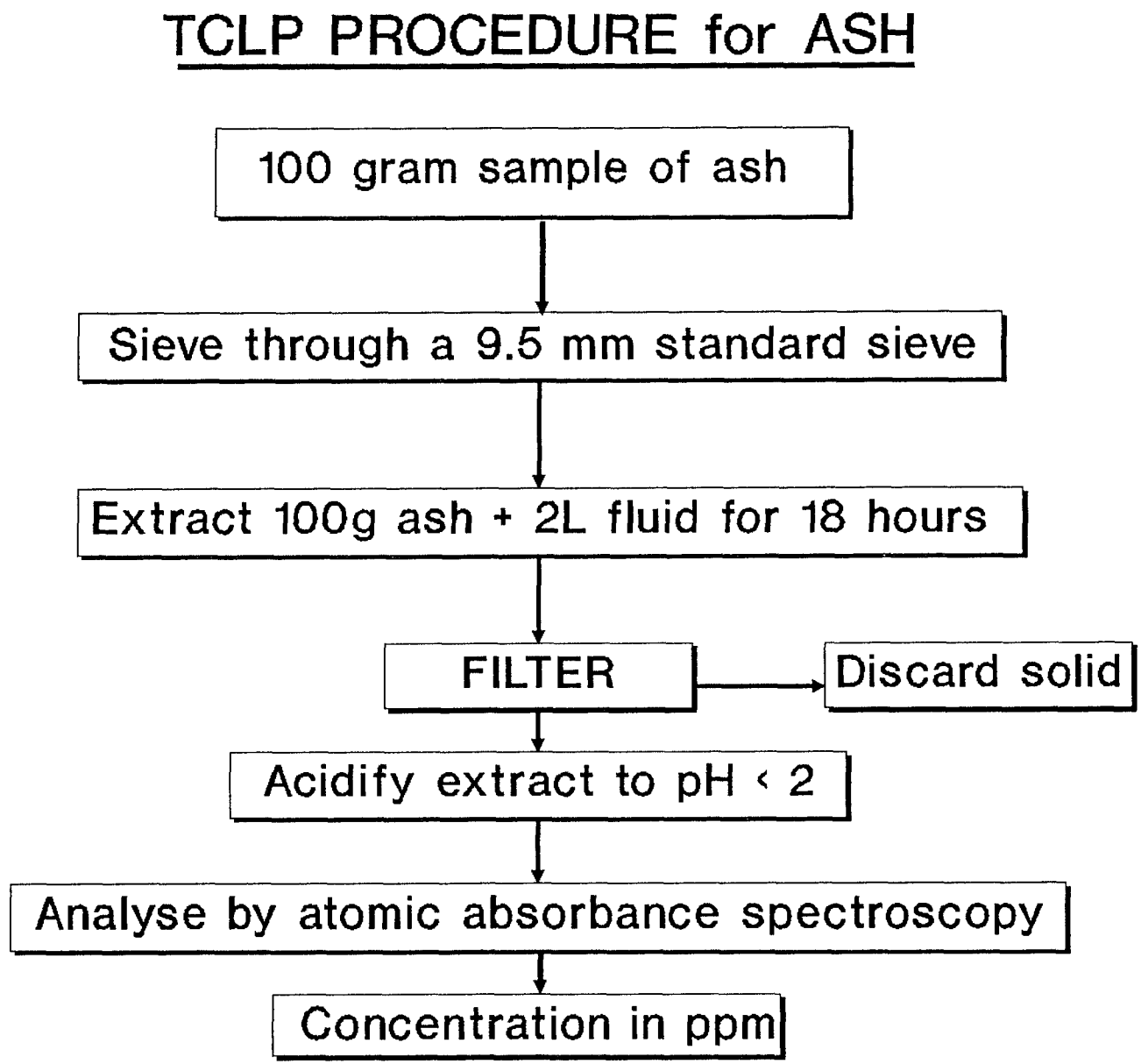


Table 4.3 - TCLP procedure for ash

TCLP PROCEDURE for CULTURE

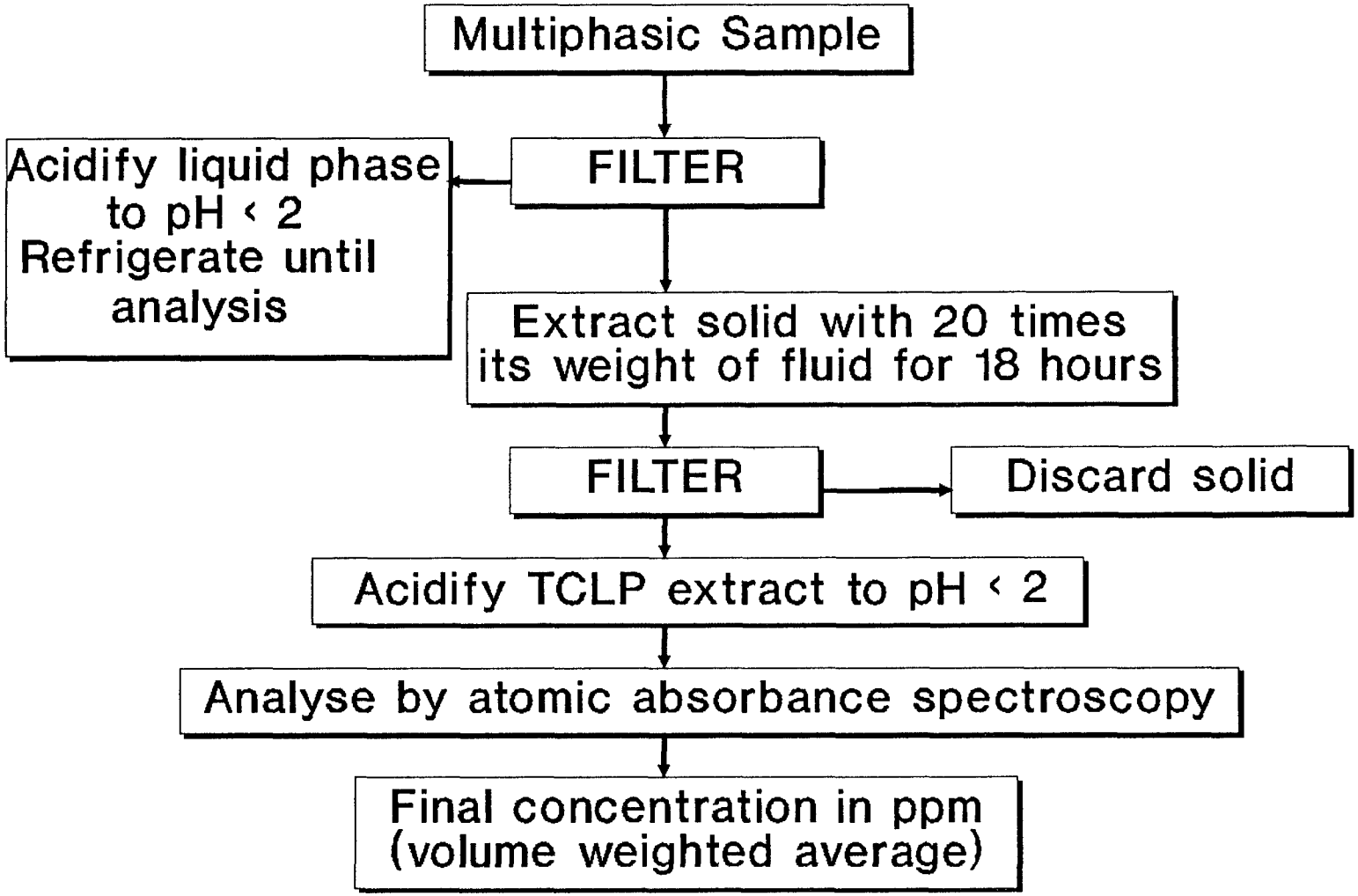


Table 4.4 - TCLP procedure for culture

to note that the EP TOX regulates the maximum total amount of acid which can be added during extraction (4 ml of acid /gram of waste).

pH 5 Method

The ash used in the experiment was highly alkaline. Since Pb leaches at pH 5 there was a need to lower the pH further, below the EP TOX pH of 6. Hence, a new method was designed which was called the pH 5 Method. In this method concentrated Acetic acid was added to bring the pH to 5 or below during the extraction.

Acid Digestion

In an effort to test the immobilization of the bacteria under highly acidic conditions (pH 2), acid digestion was performed with a few cultures with a soultion of 0.5 N acetic acid followed by addition of concentrated acetic acid to maintain pH at 2.5. Flow charts have not been added to depict pH 5 method or Acid digestion since they are modifications of the EP TOX.

4.6 Atomic absorbance (AA) spectroscopic analysis

An AA Smith Hieftje, 12 manufactured by Thermo Jarrell Ash Corp. was used in the analysis of the extracts from the leaching tests.

TCLP, EP TOX, pH 5 Method, and Acid Digestion extracts were analyzed within an hour and a half after filtration in order to minimize metals plating on to the glassware.

Matrix match was performed in each analysis, i.e., all standards were prepared with the same solution as the solution used in the extraction. Hence standards were made using the extraction fluid of the method being analysed.

Pb standards used were 1, 5, and 10 ppm. Cr standards were 1, 2, and

EP TOX PROCEDURE

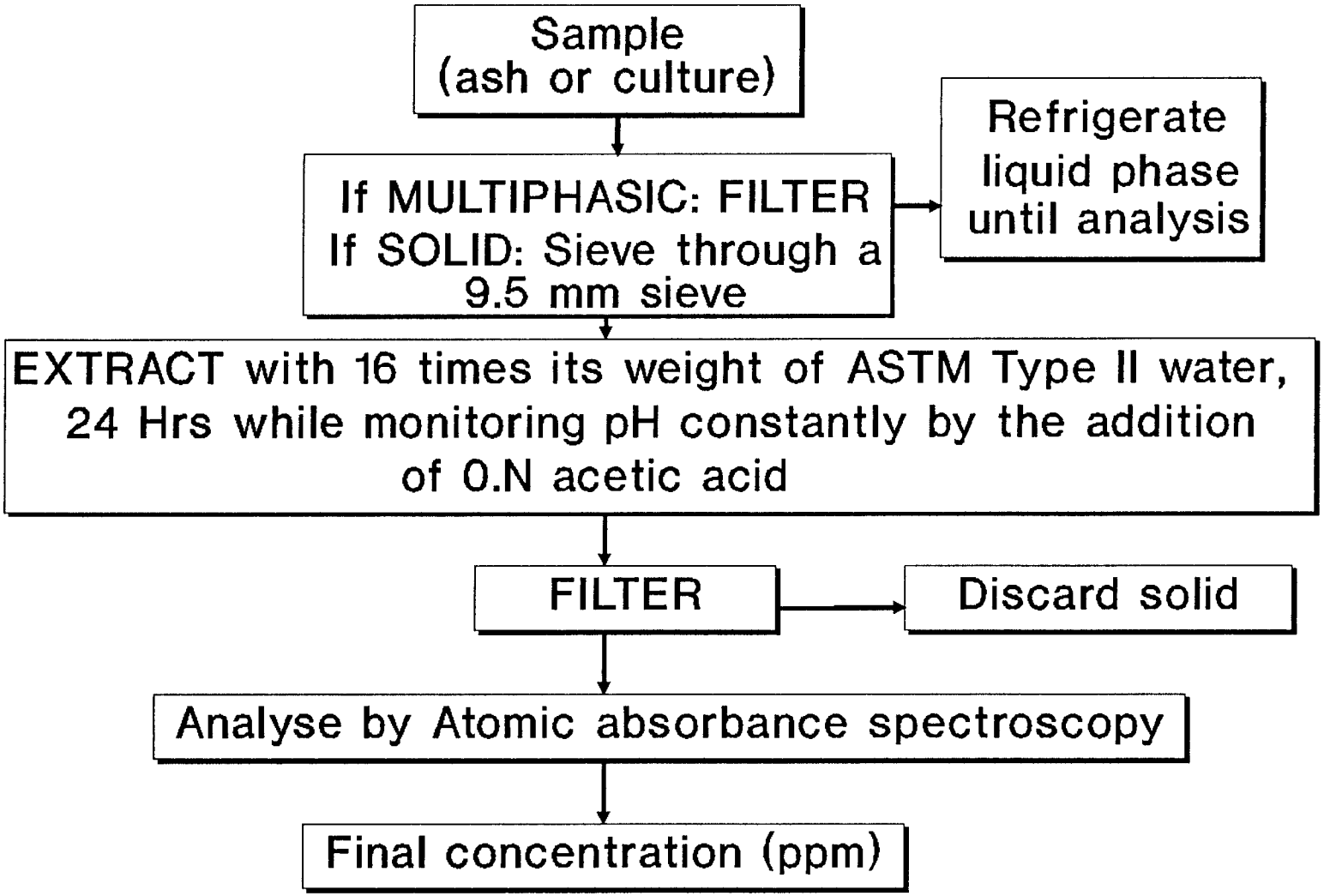


Table 4.5 - EP TOX procedure

4 ppm. Cd standards were 0.25 , 0.5, and 1 ppm. Acid blanks (10% HNO_3) and water blank (extraction fluid) were also used at each analysis. Custom stock standard solutions of 1000 ppm Pb, Cd, Cr (J.T. Baker Chemical Co.) were used from which working standards of 100 ppm containing 1% HNO_3 were prepared. Standards of 100 ppm and lower were freshly made at each analysis.

The value read on the AA is reported as the concentration in ppm of the respective metal present in the ash. This is irrespective of whether 100 g ash is used or 10 g since an equivalent dilution is performed for both at the time of extraction. Standard curves for the anlaysis are shown in Fig. 4.7, 4.8, 4.9, 4.10, and 4.11.

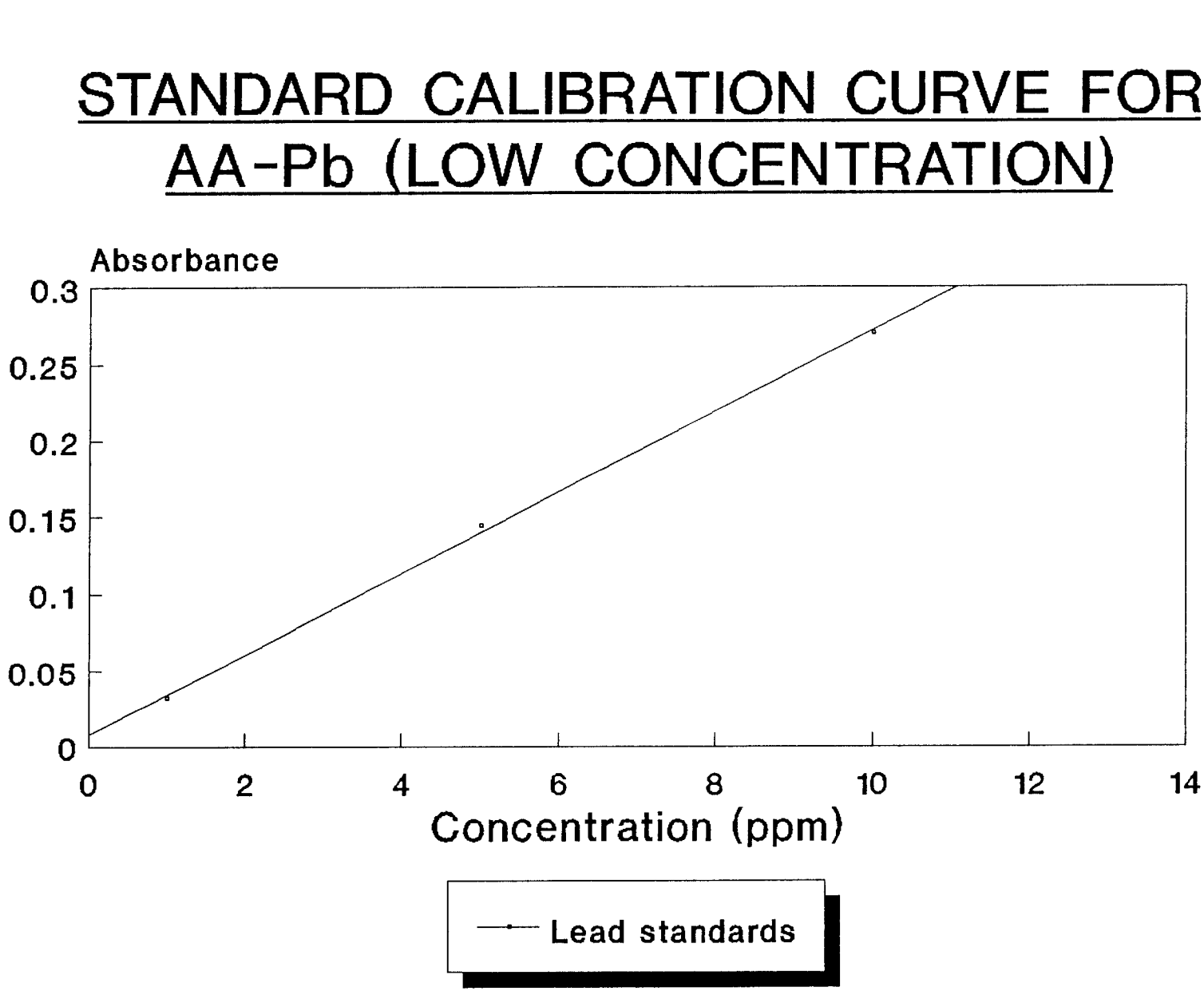


Fig 4.7 - Standard calibration curve for AA (PB, low conc.)

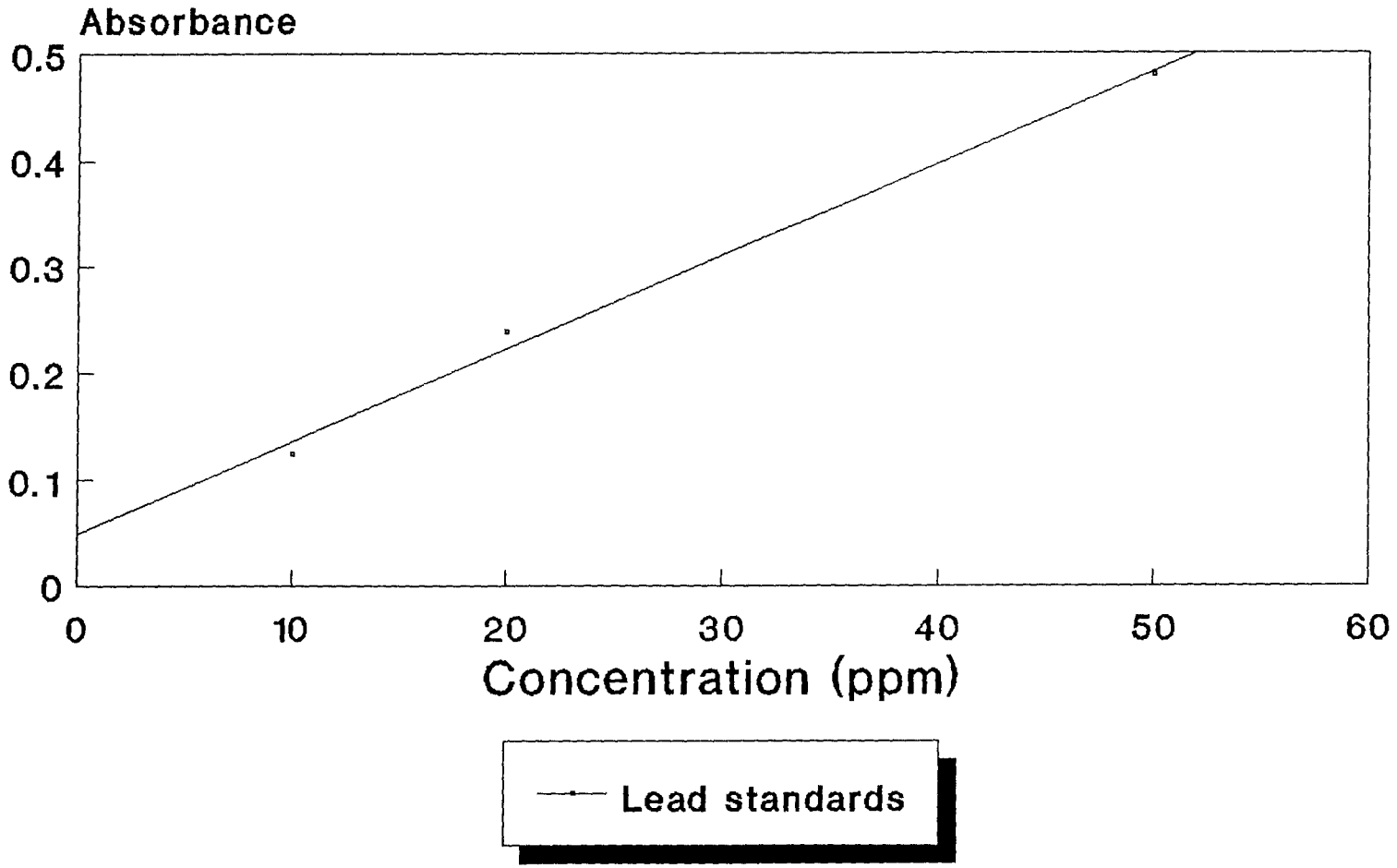


Fig 4.8 - Standard calibration curve for AA (Pb, high conc.)

STANDARD CALIBRATION CURVE FOR AA-Cd (LOW CONCENTRATION)

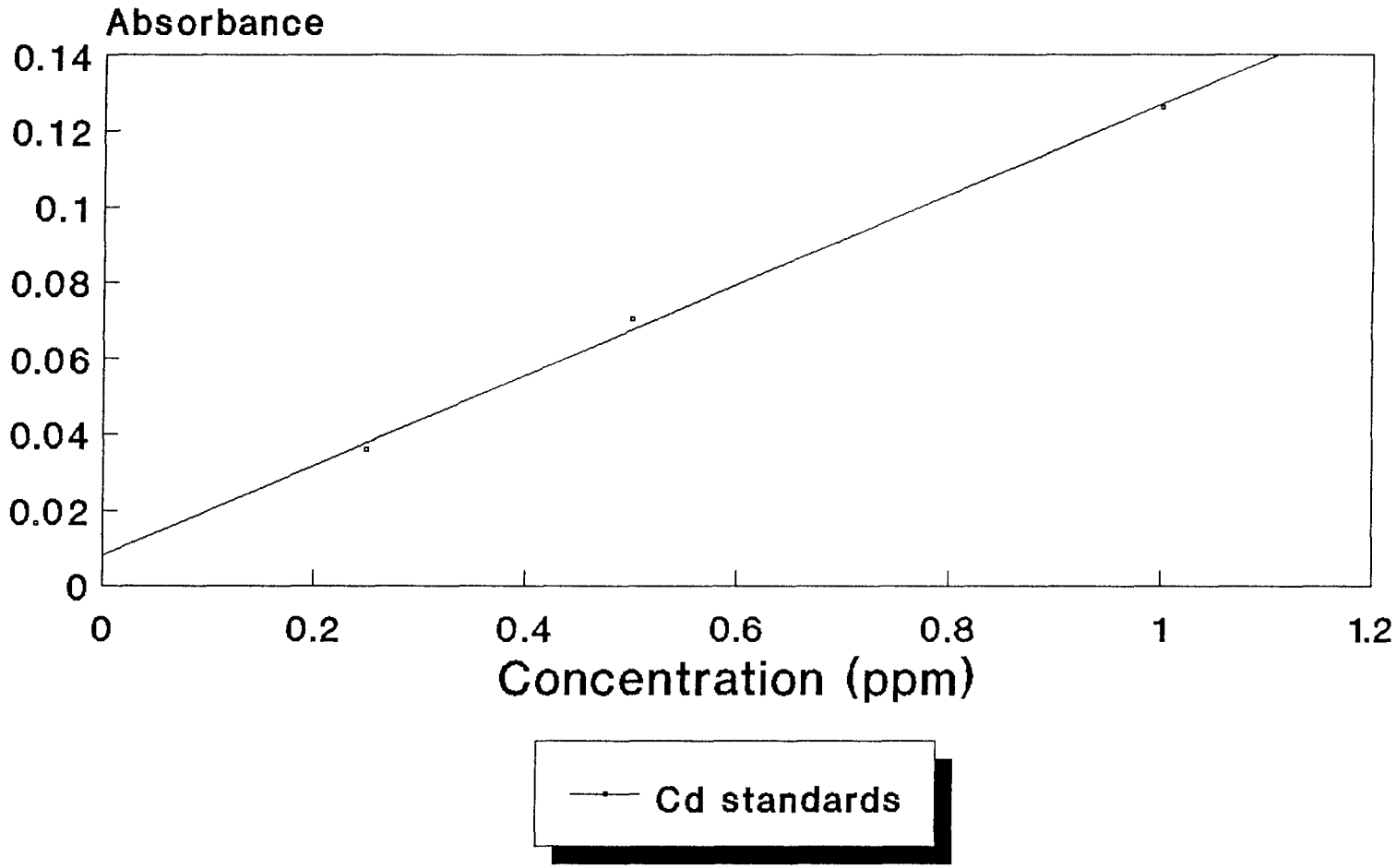


Fig 4.9 - Standard calibration curve for AA (Cd, low conc.)

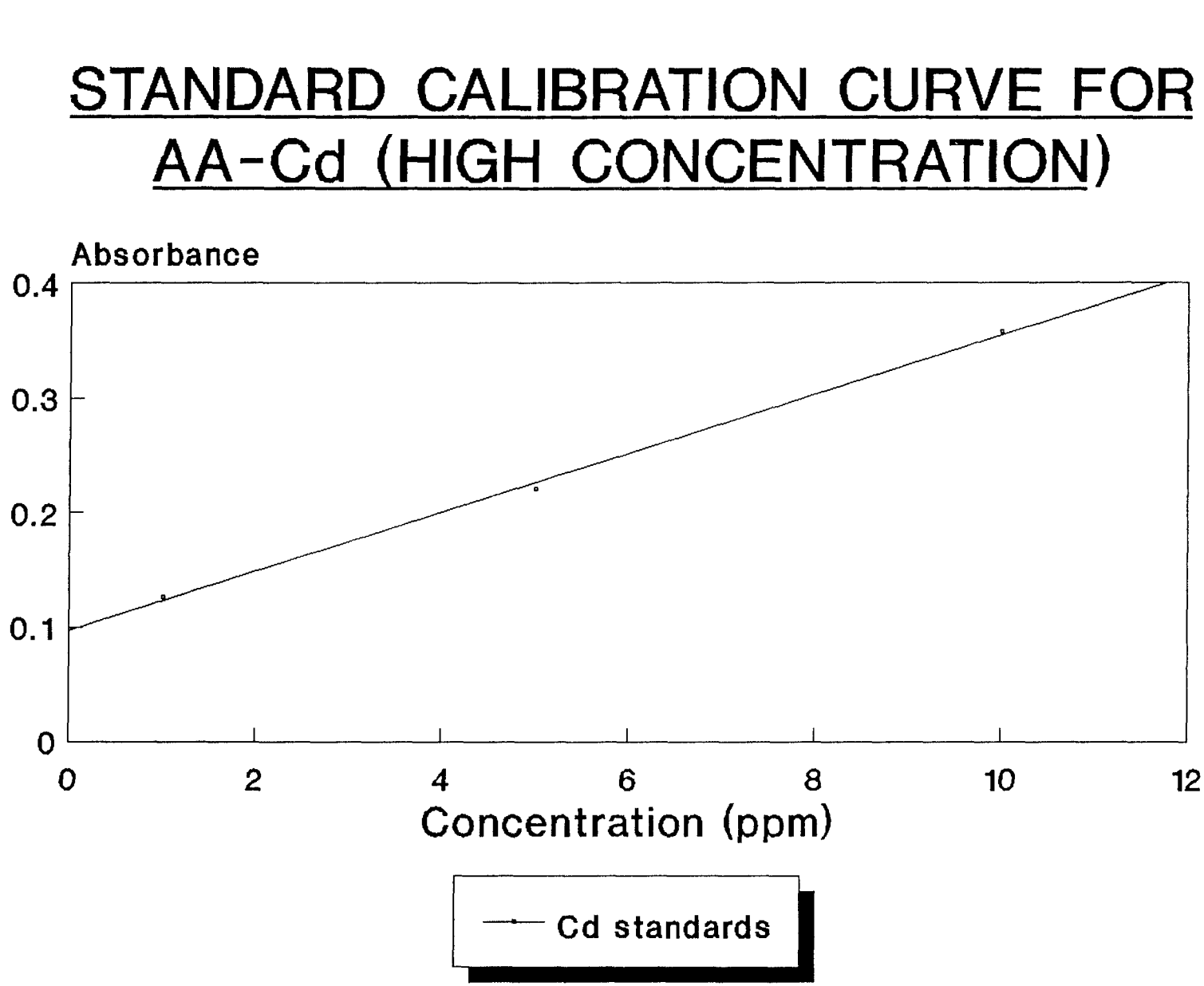


Fig 4.10 - Standard calibration curve for AA (Cd, high conc.)

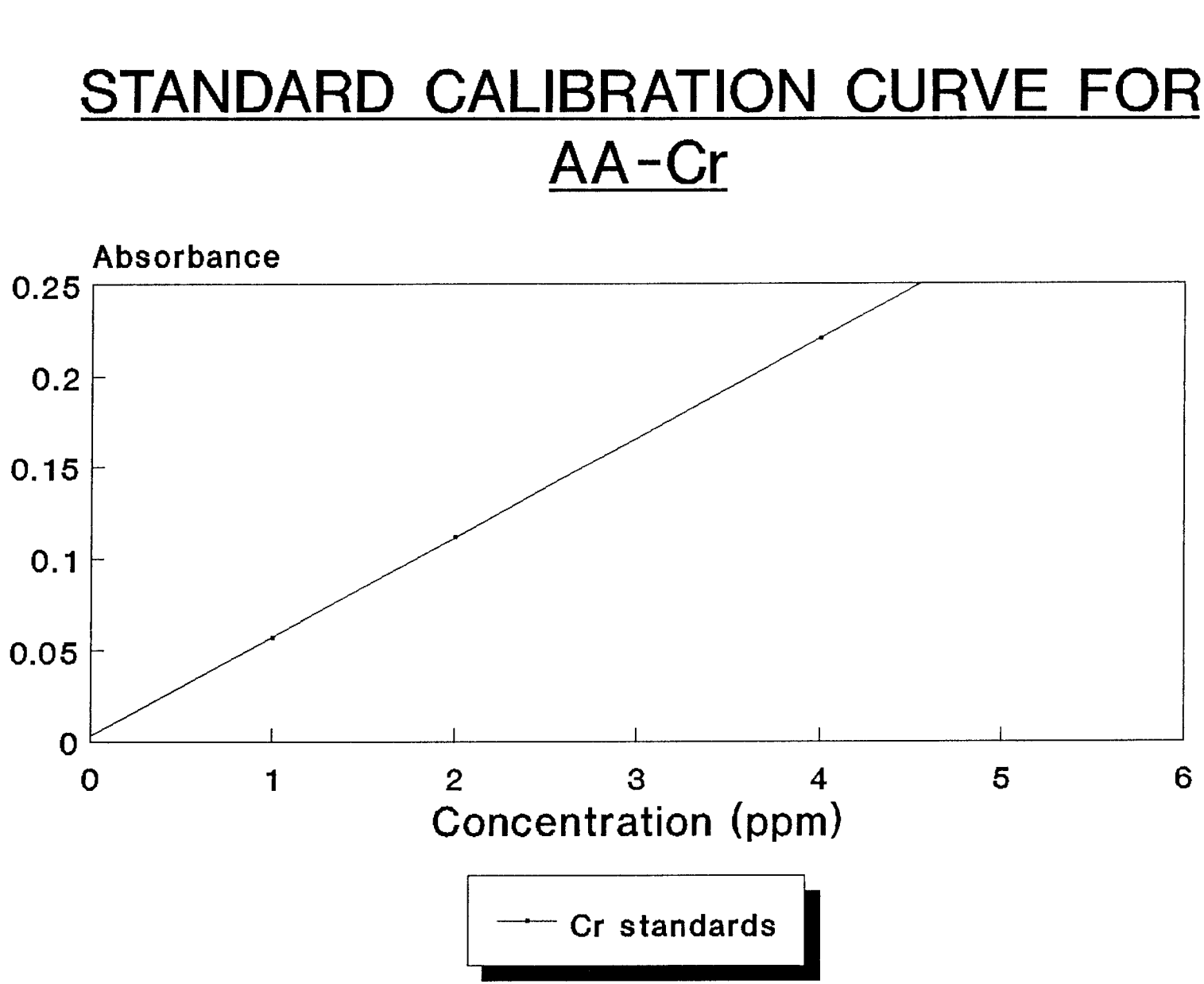


Fig 4.11 - Standard calibration curve for AA (Cr)

Chapter 5

RESULTS AND DISCUSSION

5.1 Sulfate concentration in culture medium as determined by IC analysis

The sulfate analysis performed on all cultures indicates a gradual decrease of sulfate in active cultures. Control cultures containing no lactate, no sulfate, no inoculum, and sterile inoculum show no decrease at all, indicating no growth.

Fig. 5.1 represents the sulfate consumption related to the growth of *desulfovibrio*. Regular sampling over a period of 25 days shows sulfate levels decreasing from an initial 50 ppm to 23 ppm in Culture I, and to 13.6 ppm in Culture D (all numbers represented are 1:50 dilution values, since a dilution of original medium was performed before the IC analysis, (see Chapter 4 section 4.3). There is a difference in the decrease of sulfate concentration in the two cultures. This is expected since Culture D contains no ash, and hence there is no initial lag as seen in Culture I. As a contrast a control culture with no inoculum shows no decrease and in fact a gradual increase in sulfate. This is attributed to the sulfate leaching into the medium from the ash.

Fig. 5.2 represents the sulfate concentration in control cultures, A (no lactate), C (no inoculum), and E (sterile inoculum). As expected there is no decrease in sulfate concentrations and hence no growth. Fig. 5.3 shows sulfate

Sulfate Consumption Related to the Growth of *Desulfovibrio*

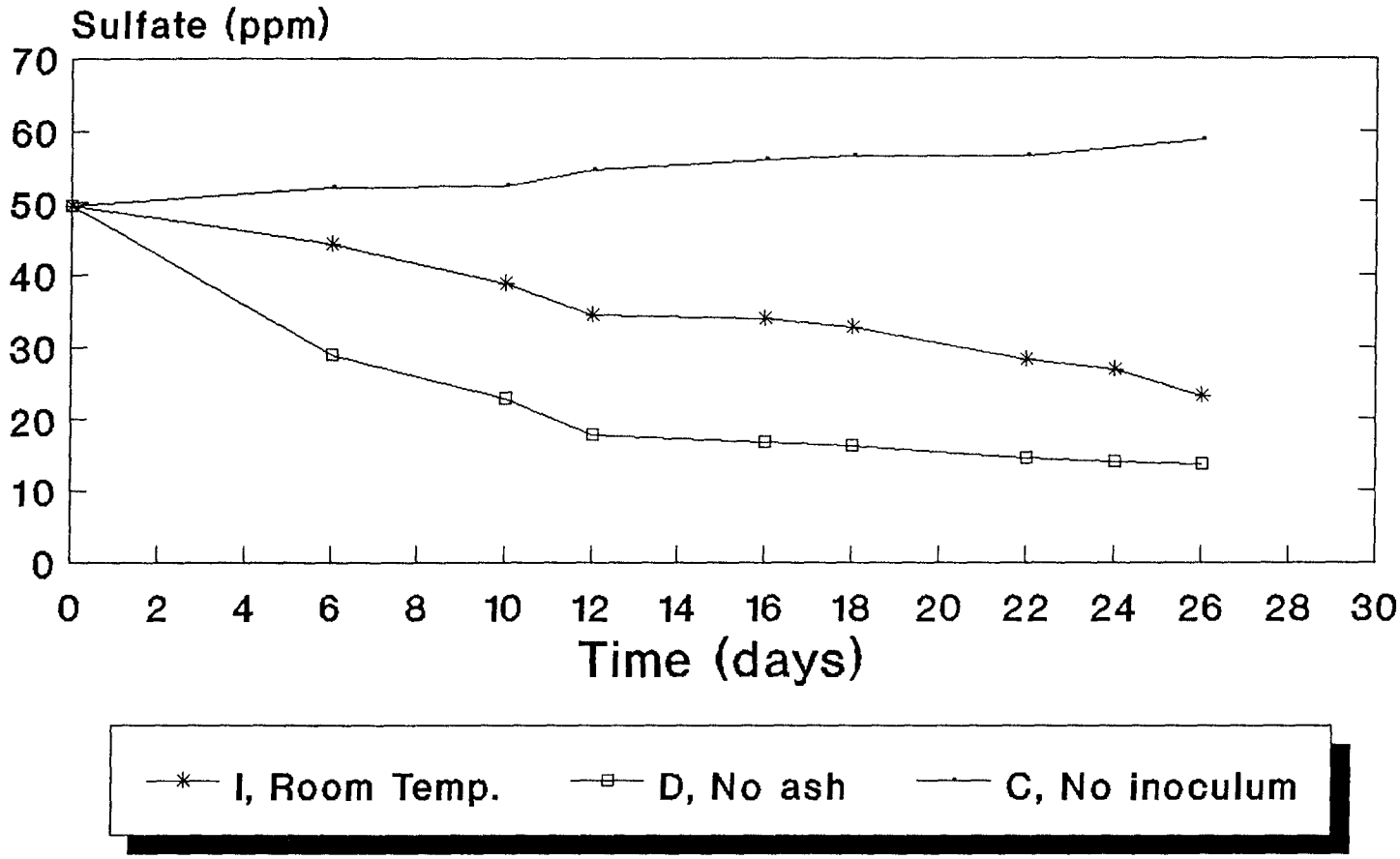
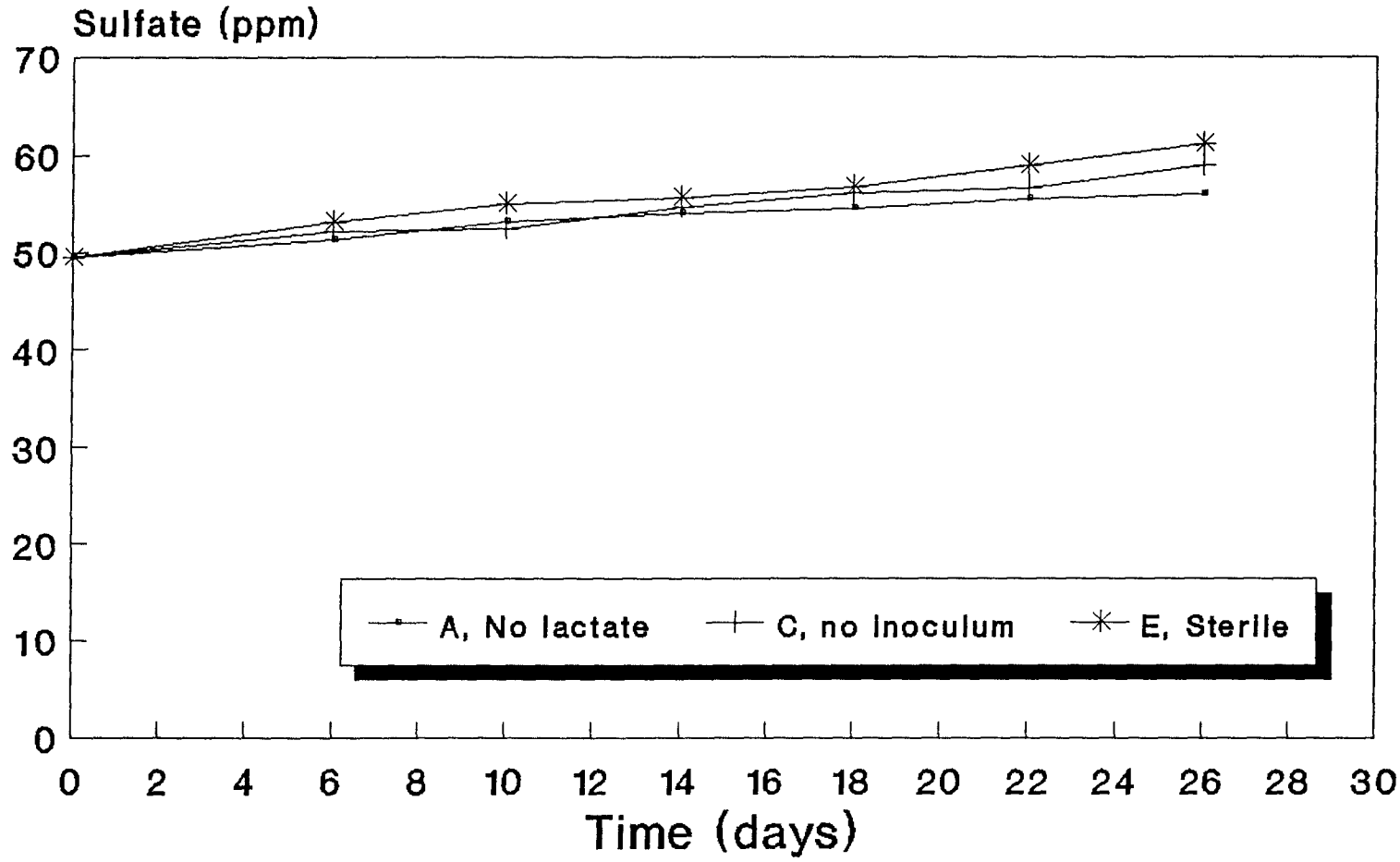


Fig 5.1 - Sulfate consumption related to the growth of *desulfovibrio*

Control Parameters
(No Growth of *Desulfovibrio*)



B, No sulfate not depicted

Fig 5.2 - Control parameters (no growth of *desulfovibrio*)

leaching into the medium from the ash. From figure 5.3 we can see that over a period of two weeks the sulfate concentration in a culture initially containing no sulfate (B), increased to 6 ppm of sulfate. This accounts for the upward trend in Fig. 5.2, where sulfate leaching from the ash over a period of 25 days has lead to a 12 ppm increase in sulfate in culture E (sterile), 9 ppm increase in C (no inoculum), and a 6.5 ppm increase in A (no lactate). These results can be compared to a study conducted by Spencer and Drake (1987), on the hydrogeology of an alkaline fly ash landfill in Eastern Iowa. Leachate studies showed that sulfate concentrations in the leachate plume was very high and exceeded EPA drinking water standards.

Sulfide formation with growth of *desulfovibrio* is shown in Fig. 5.4. Here Culture D, with no initial ash showed higher sulfide formation of 5.3 ppm while Culture I at room temperature showed 1.1 ppm. Sulfide detection is difficult due to the rapid oxidation of sulfide into sulfate and sulfite. Hence, the number 5.3 ppm does not truly represent all detectable sulfide. Some amount was also precipitated as FeS, the black precipitate very noticeable in all cultures. In Culture I, detectable sulfide was low since most of the sulfide formed had reacted with the metals in the ash forming PbS, CdS etc. Culture C, with no inoculum, showed that minimum amount of sulfide formed which is due to the addition of the reducing agent sodium sulfide and not due to bacterial activity.

Fig. 5.1 and Fig. 5.4 were combined to produce Fig. 5.5. The Δ Sulfate curve represents the difference between the two lines I and D in Fig. 5.1. Similarly, Δ sulfide is the difference between the two lines I and C in Fig. 5.4.

Fig. 5.6 shows sulfate decrease with time in duplicate cultures of I: I_a and I_c. These cultures were set up and analysed in order to duplicate and confirm the results obtained with Culture I. Results for Culture I_b were not shown in Fig. 5.6

Sulfate Leaching into Medium from the Ash

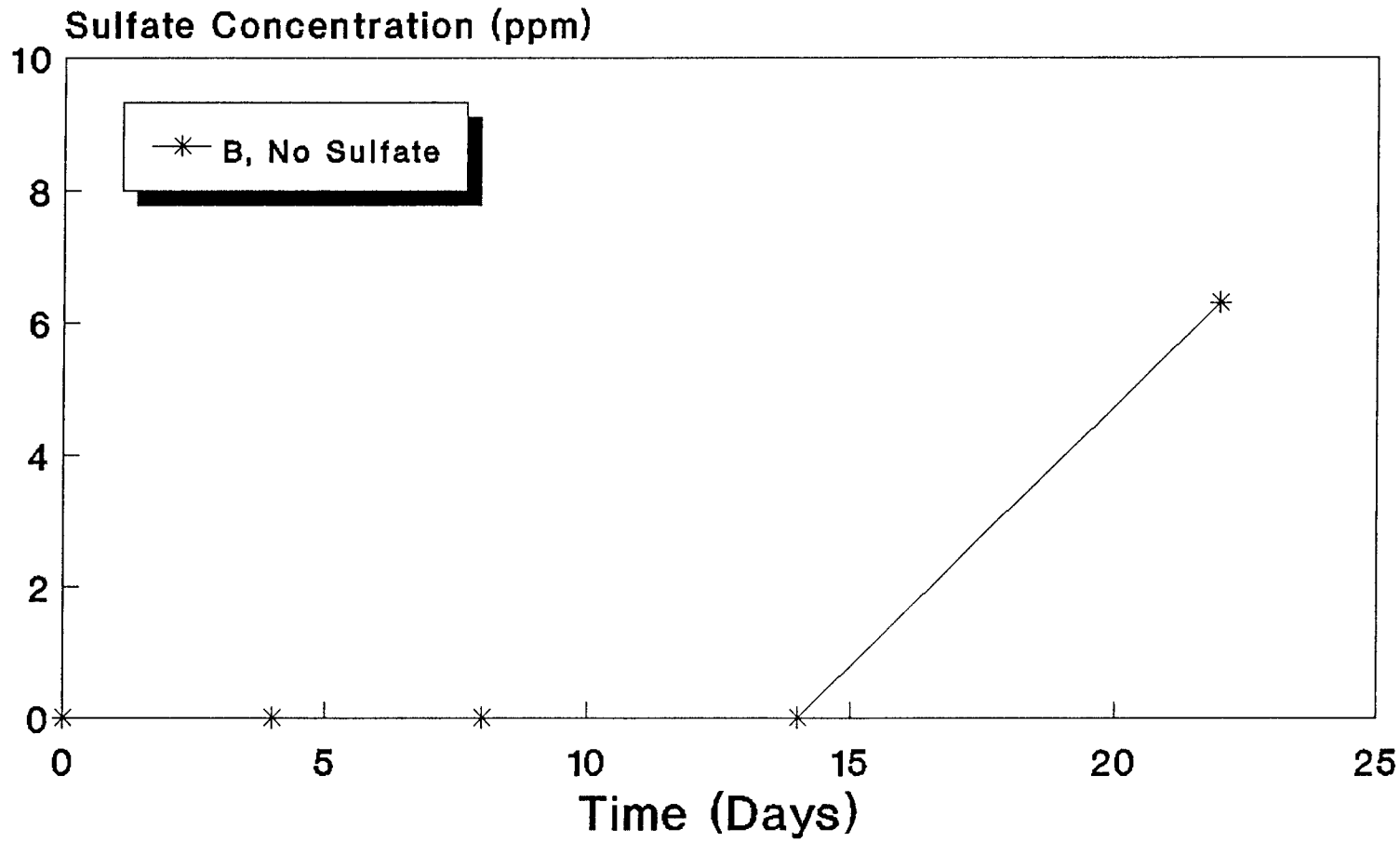


Fig 5.3 - Sulfate leaching into the medium from the ash

Sulfide Formation with Growth of *Desulfovibrio*

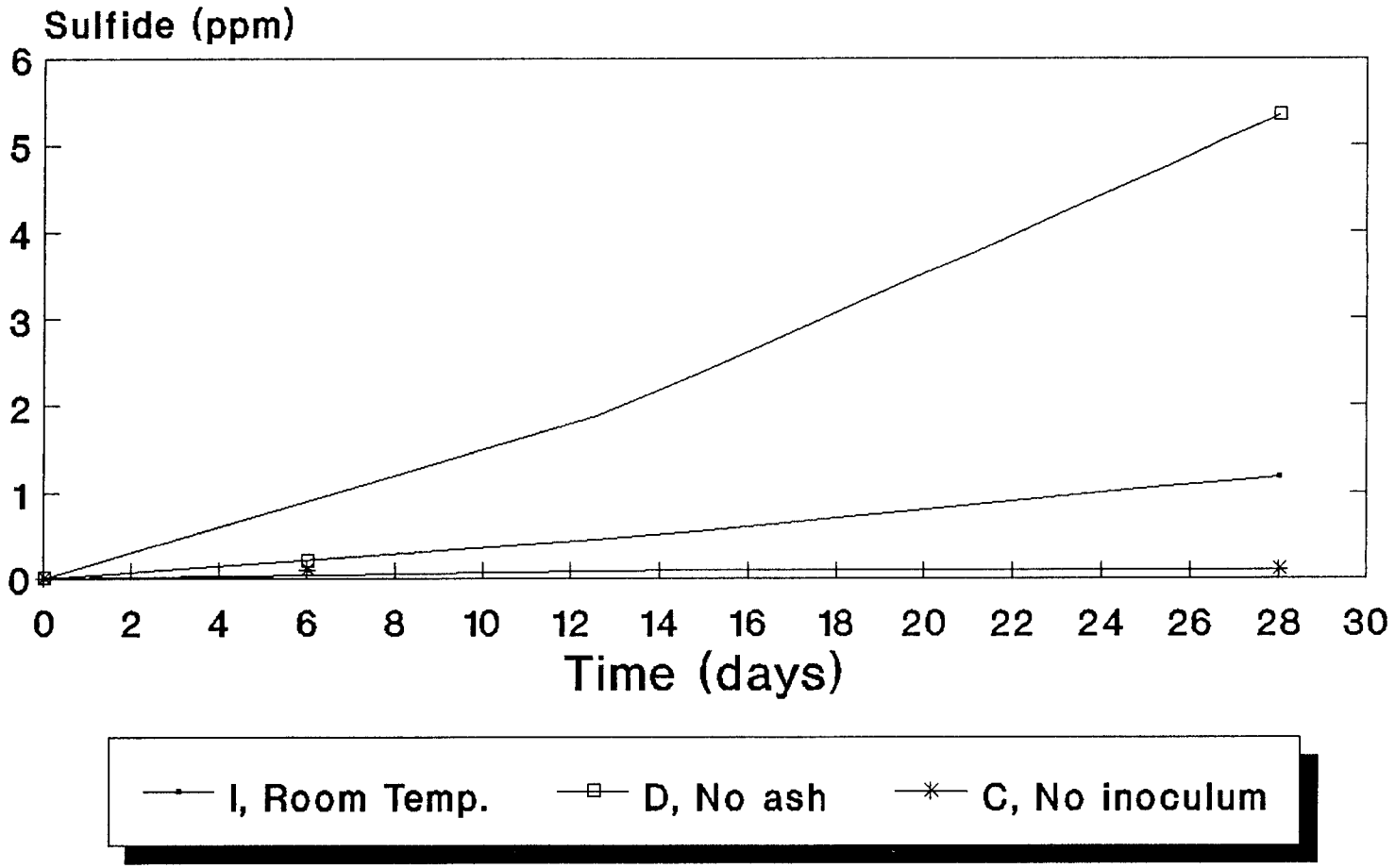
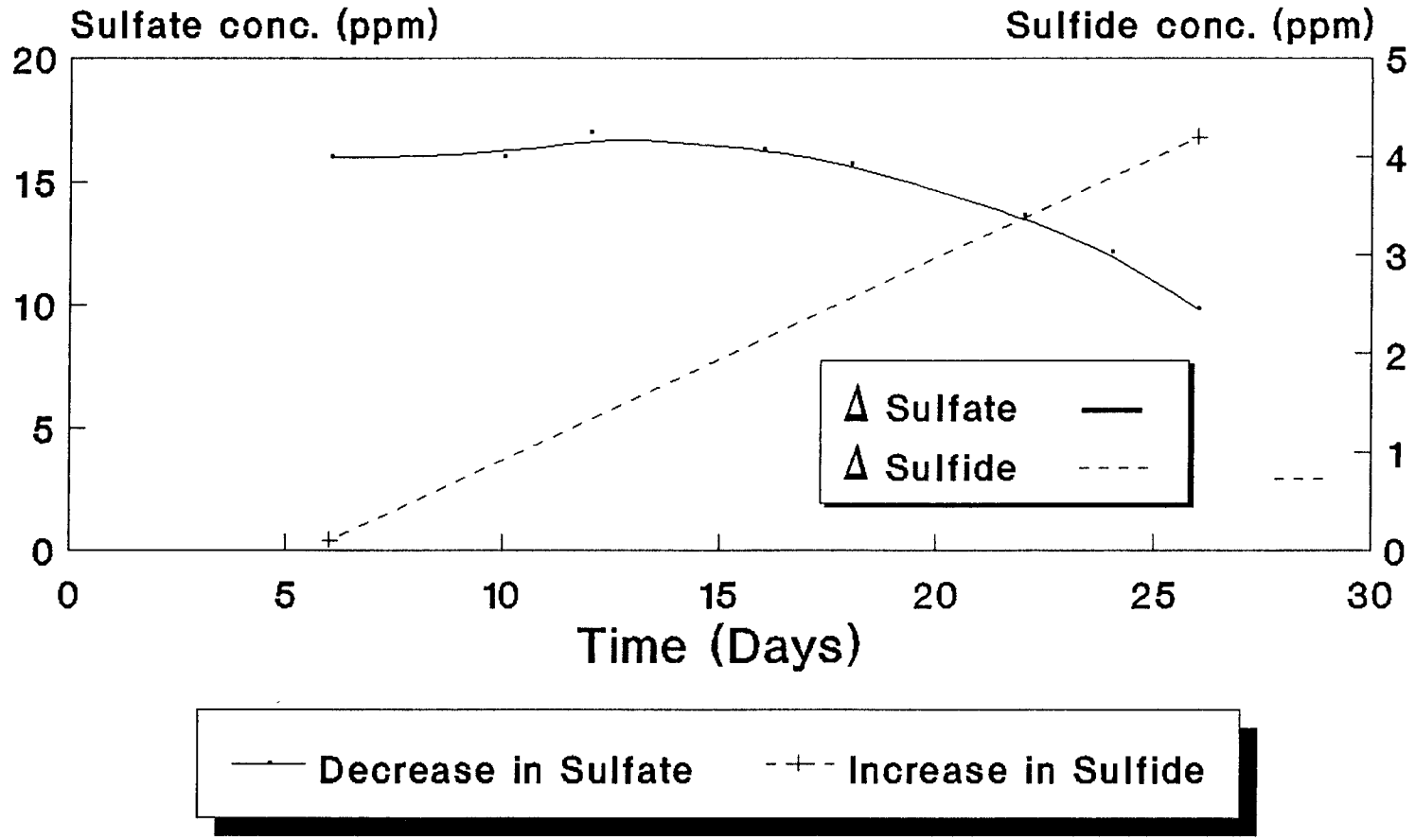


Fig 5.4 - Sulfide formation with growth of *desulfovibrio*

Sulfate Conversion to Sulfide by *Desulfovibrio*



2 ppm sulfate = 1 ppm sulfide

Fig 5.5 - Sulfate conversion to sulfide by *desulfovibrio*

Sulfate Consumption Related to the Growth of *Desulfovibrio*

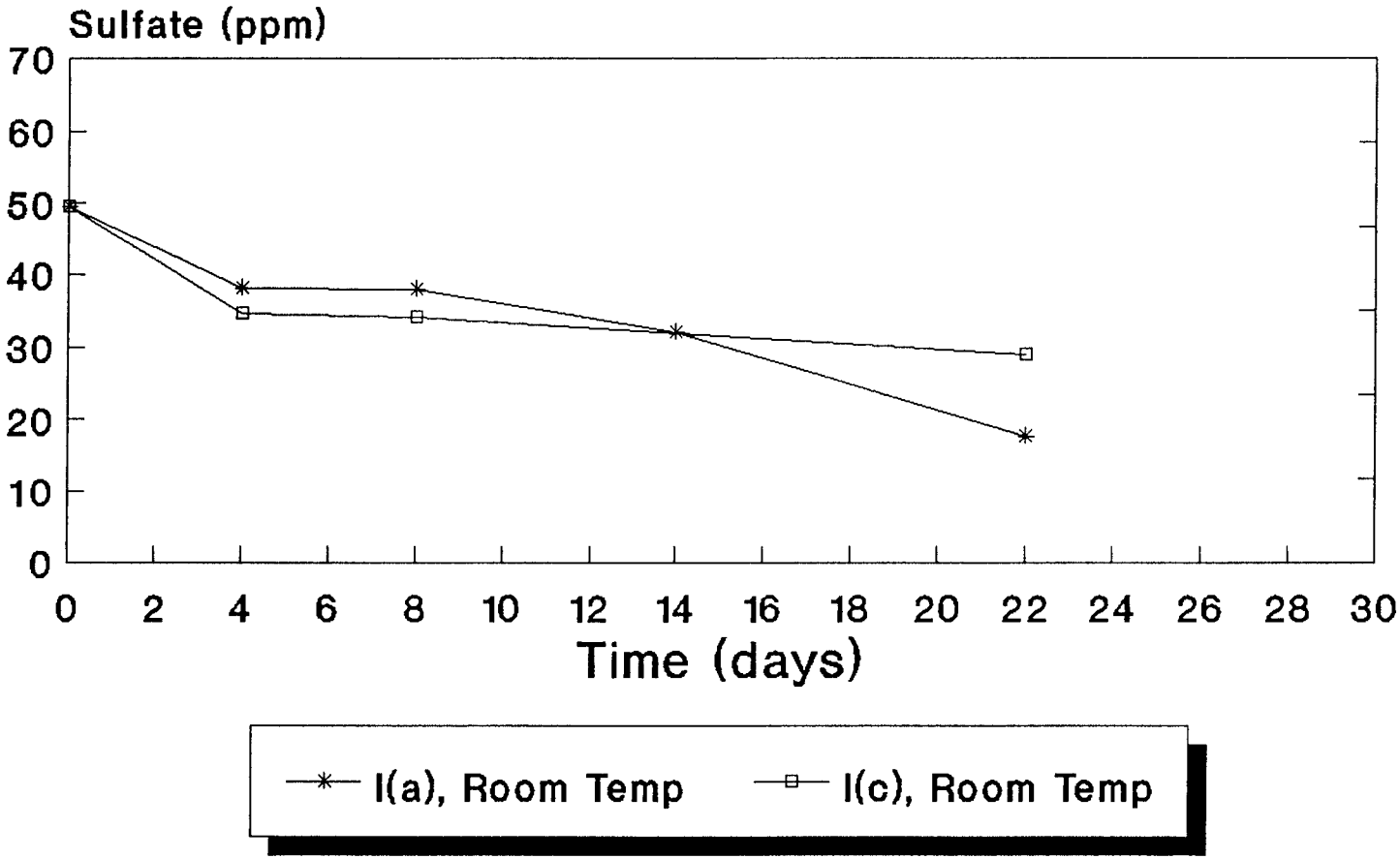


Fig 5.6 - Sulfate consumption related to growth of *desulfovibrio*

due to inconsistent data.

5.2 Analysis of Leachate Composition of Untreated Ash

A heavy metal analysis was done on the untreated ash sample alone using TCLP, EP TOX and pH 5 Methods.

The results are represented in Table 5.1. EPA standards for Pd, Cd, and Cr were obtained from 40 CFR Part 26.24.

The ash sample was reported to contain 1660 mg of Pb/kg of ash, 37.5 mg of Cd/kg of ash, and 72.8 mg of Cr/kg of ash (Acid digestion values received from previous study on the same ash at Rutgers University, New Brunswick). The TCLP procedure was found to be too mild a test since it was capable of detecting only 0.4 ppm of Pb. The EP TOX was no better with a value of 0.9 ppm. Cd failed the EP TOX with a value of 1 ppm. Controlling the pH was seen to be the key in the leaching of metals. At about pH 5; 39.2 ppm of Pb was detected and 1.7 ppm of Cd. Cr was a low 0.9 ppm.

The pH dependency in the leaching of metals is depicted in Figs. 5.7, 5.8 and 5.9 for Pb, Cd, and Cr respectively. As seen the pH 5 method is the most significant of the three.

5.3 Analysis of Leachate Composition of Treated Ash

Leachate analysis was performed on all cultures using TCLP, EP TOX and pH 5 Method. Cultures I, I_a, I_b, and I_c showed a high degree of immobilization. The results obtained are shown in Table 5.2.

Inoculum MB was more successful than inoculum U for metal immobilization.

CONCENTRATION OF METALS IN THE LEACHATE FROM UNTREATED ASH (ppm)

Method / 100g ash	TCLP	EP TOX	pH 5 Method	EPA Standard (ppm)
Pb	0.4	0.9	39.2 *	5
Cd	0.7	1.0 *	1.7 *	1
Cr	0.0	0.1	0.9	5

* FAILS EPA STANDARDS

Table 5.1 - Concentration of metals in the ash (ppm)

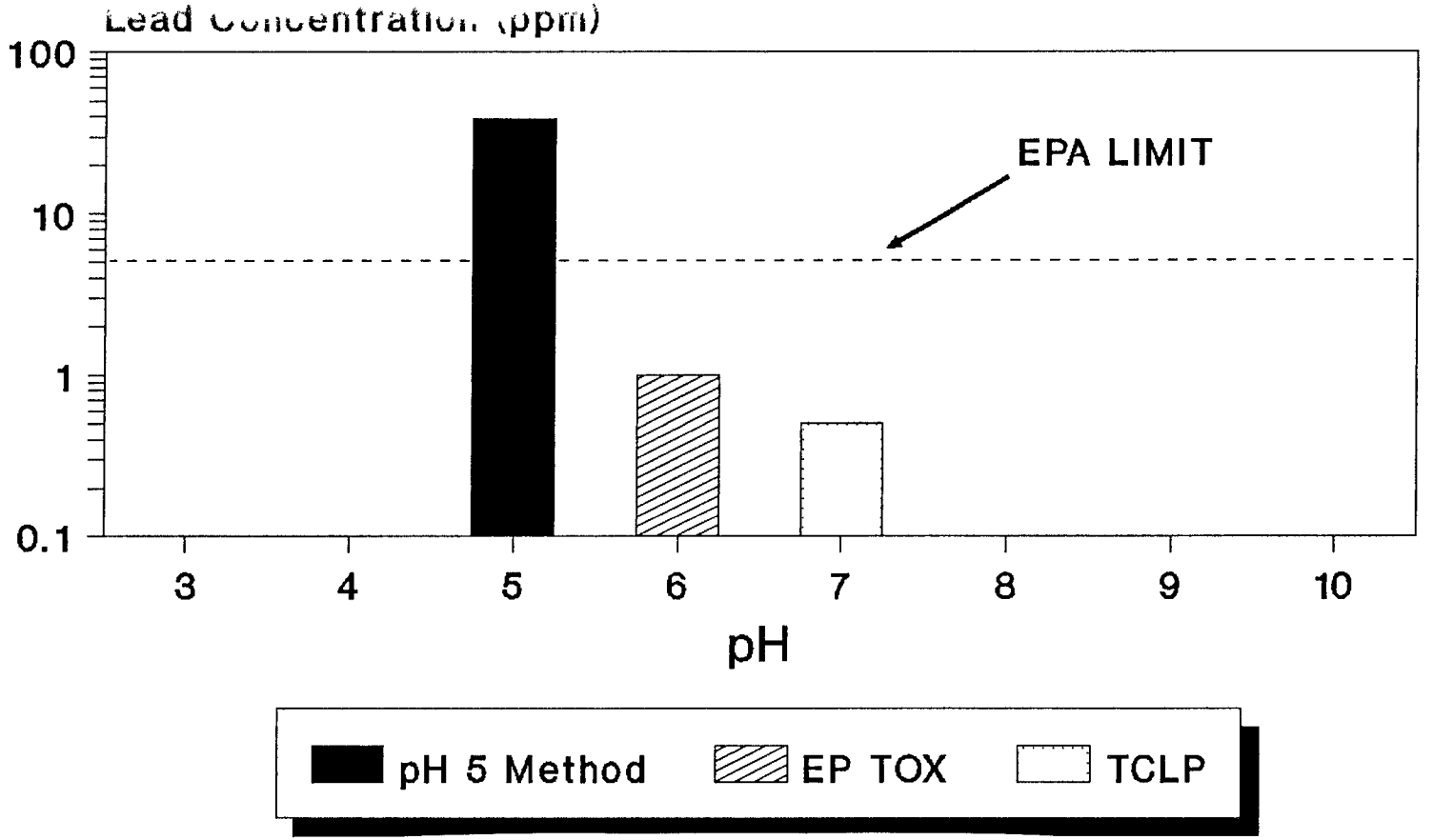


Fig 5.7 - pH dependency in the leaching of metals (Pb)

pH Dependency in the Leaching of Metals (Cd)

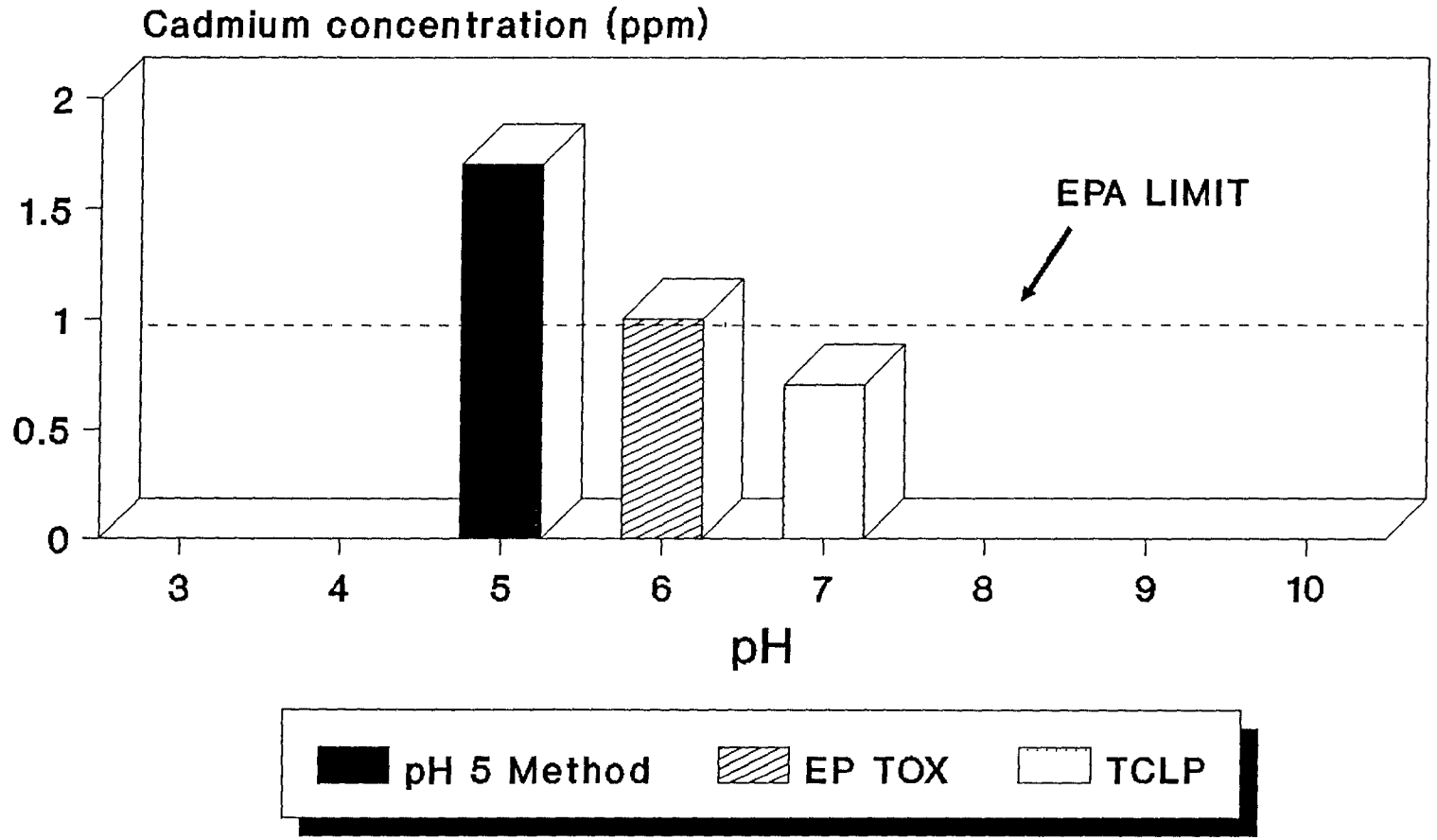


Fig 5.8 - pH dependency in the leaching of metals (Cd)

pH Dependency in the Leaching of Metals (Cr)

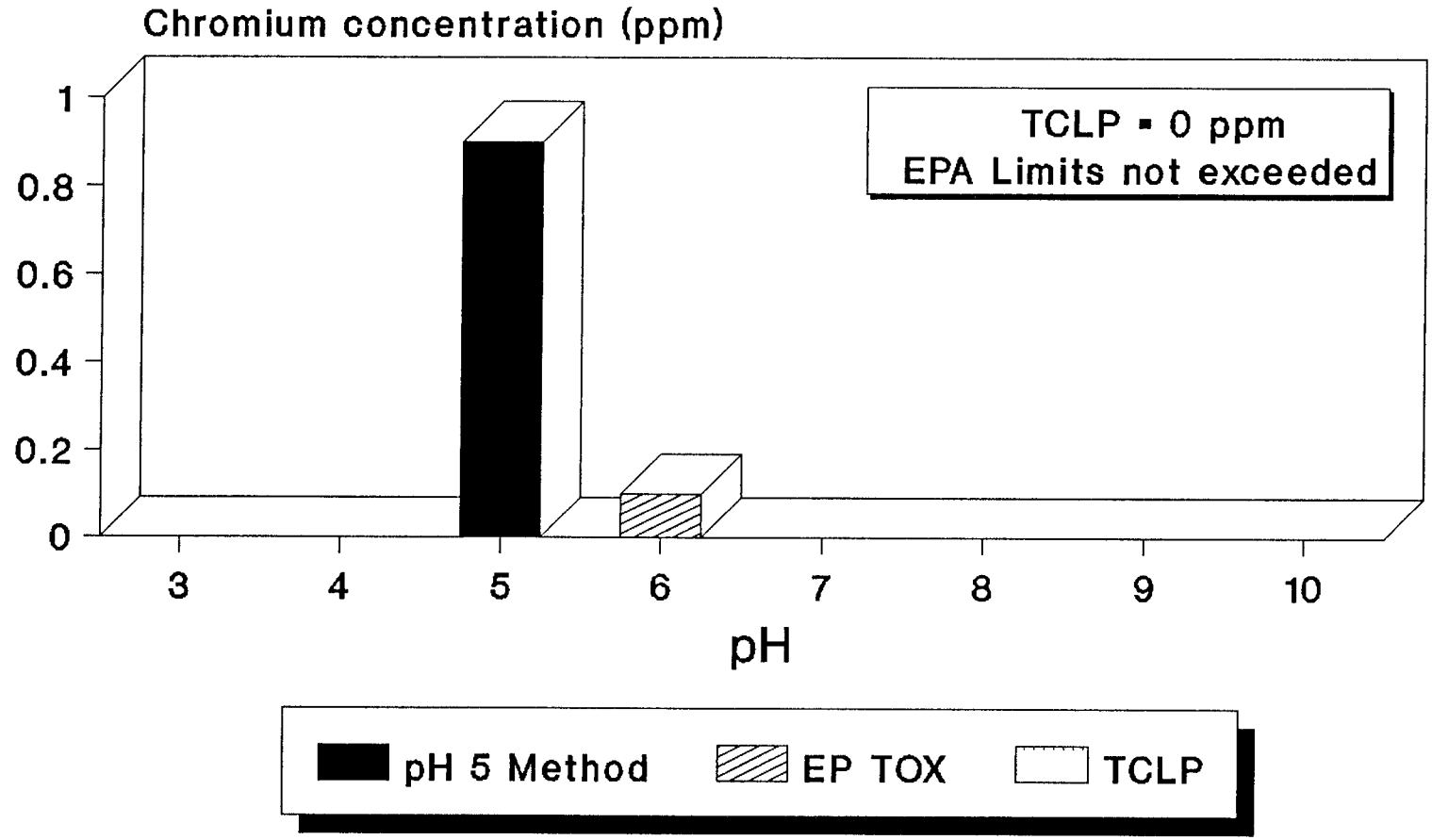


Fig 5.9 - pH dependency in the leaching of metals (Cr)

Table 5.2 - Leachate analysis of cultures

Culture	*Initial pH*	*Final pH*	*Pb*	*Cd*	*Cr*	*Leach Test*
Untreated ash	10.8	> 5	39.2	1.7	0.9	pH 5 Method
A-no lactate	8.4	5.25	2.0	0.15	0.1	EP TOX
B-no sulfate	6.7	4.1	19.8	0.1	0.6	pH 5 Method.
C-no inocul.	8.7	6.0	1.5	-	0.2	EP TOX
D-no ash	-	5	0.0	0.0	0.0	TCLP
E-sterile	7.74	5.5	0.4	0.0	0.0	TCLP
G-MB	6.85	5	0.3	0.0	0.0	TCLP
H-MB	8.0	>5	0.3	0.0	0.3	EP TOX
I-MB@21°C	-	5	0.0	0.0	0.0	TCLP + EP TOX
J-U	8.2	4.7	1.0	0.1	0.07	pH 5 Method
K-U	8.2	<5	1.1	0.1	0.06	pH 5 Method
L-U	7.5	>5	0.4	0.07	0.0	EP TOX
I_a@21°C	6.6	4.2	3.3	0.66	0.2	pH 5 Method
I_b@21°C	-	4.4	2.6	0.09	0.1	pH 5 Method
I_c@21°C	-	4.3	3.9	0.3	0.1	pH 5 Method
1-U@21°C	7.5	4.3	15.0	0.25	0.2	pH 5 Method
2-U@21°C	7.3	4.1	27.2	0.45	0.3	Acid Dig.

MB- Mitch Berman's Culture
U- My Culture
Initial pH- pH before extraction
Final pH- pH after extraction
Metal concentrations are in ppm

Culture I showed 0 ppm for all three metals by TCLP and EP TOX, the current and previous EPA recommended leaching tests. To conclusively prove the extent of immobilization, triplicate cultures of I, I_a, I_b, and I_c, were set up and analyzed by the more stringent pH 5 Method. This method detected 39.2 ppm of Pb in the untreated ash but a corresponding 3.3, 2.6, and 3.9 ppm in cultures I_a, I_b, and I_c respectively. Cultures J and K containing inoculum U show low values of metals by the pH 5 Method. Cultures 1 and 2 which also contained inoculum U, however show a high concentration of 15 ppm of Pb by the same method indicating no immobilization. The reason for no growth and subsequently no immobilization in the duplicate cultures is not definite. Hence it is concluded that culture MB was more sucessful since the results were reproducable.

TCLP and EP TOX tests on control cultures E and C, show results comparable to those obtained on untreated ash with the respective methods. The TCLP on Culture E, gives a value of 0.4 ppm for Pb, comparable to 0.4 ppm obtained on the ash, indicating no immobilization ocurred. EP TOX values on controls A and C are 2.0 and 1.5 for Pb comparable to the corresponding 0.9 ppm for untreated ash indicating no immobilization again. Culture B, no sulfate, a control for the pH 5 Method, showed a value of 19.8 ppm for Pb. All the leachable Pb was not detected in Culture B. Some amount adheres stongly to the filter paper at the time of filtration, which is required for the multiphasic culture as described in Table 4.5 (EP TOX procedure).

Acid digestion was performed on Culture 2 which contained inoculum U, to see the extent of metal extractable. The pH for this was maintained at 2.5. Metal recovery was 25 ppm for Pb comparable to the 39.2 ppm in the untreated ash. Some amount of immobilization seems to have occured and some Pb is not detected because of adhering to the filter paper.

Normal final pH values for *Desulfovibrio* cultures is 8.5 (Postgate, 1984). Under these conditions it is unlikely that any metal leaching can occur as demonstrated by Table 5.1 and Figs. 5.7, 5.8, and 5.9. pH values of most cultures prior to extraction are listed in Table 5.2. Thus even a mixed culture of *desulfovibrios* in screw top bottles, maintains an alkaline pH of above 8 in most cases.

Contaminant release from the same waste can vary over several orders of magnitude within a short pH range. This is to be expected since the leachate pH has a major influence on contaminant solubility. Leachate pH in turn, is a function of the alkalinity of the waste and of the amount of acid available in the leaching medium to react with the waste. This accounts for the higher value of metal concentration obtained by the pH 5 method.

Studies by Cote and Constable (1983) on Cd and Pb showed that a strong linear correlation exists between the metal release and the final pH leachate. However, they found that this linear trend is not observed with all contaminants. No readily discernable pattern was apparent for the release pH-curves for Cr. They also demonstrated that the use of a synthetic municipal landfill leachate appeared to complex Cr and increase its release above that to be expected from pH solubility considerations alone. In our work Chromium values were found to be low when more acid was added as in the pH 5 method. This could also be a consequence of Cr forming complexes with the leachate.

Though the initial metal concentration in the ash sample was very high, especially for Pb, the amount of metal released from the tests was low. Boyle *et al.* (1983) in their study of leach testing of foundry process wastes reported that the percentage of metals released in the EP TOX test ranged from 0-39% for Cd, <1-12% for Pb, and 0-0.5% for Cr. The metals released by the pH 5 Method were 2.3% for Pb, 4.5% for Cd, and 1.2% for Cr. Boyle's study on 21 foundries found

9 of them toxic with respect to Pb/Cd or both; but none were Cr toxic. The ash sample used in this project was also non toxic with respect to Cr.

5.4 Analysis of the Effectiveness of Current EPA Leaching Tests

The EPA recommended tests, both the TCLP and the EP TOX are not representative tests for the leaching potential of ash, since an initial addition of base to the ash makes the ash resistant to such moderate acid leaching tests.

TCLP

The TCLP which has come into effect from September 1990, does not call for pH monitoring, which, as demonstrated by the results as well as various literature cited in this manuscript, is crucial in the detecting of metals. The ash sample used in the analysis, as mentioned earlier, had a total Pb content of 1660 mg/Kg of ash. However, the TCLP could detect only 0.4 ppm of the same. This is because it is believed that the incinerator from which the ash originates had good management practice. By the addition of excess amounts of alkali (Ca, Mg or other source) they suceeded in increasing the alkali content of the ash, to such a high degree that it passed both existing and previous EPA tests. Hence, from the results obtained, and according to present environmental regulations, this ash is safe for burial in a landfill. The pH 5 Method, however, demonstrates the possible contamination of the ecosystem as a result of burial.

EP TOX

Though the EP TOX is definitely better than the TCLP since it mandates pH control at 5 very strongly, it is also not stringent enough for clever waste generators. According to this method addition of acid should not exceed 4 ml per gram of waste. Hence the acid is not sufficient to bring the pH to 5 in a waste as

alkaline as the ash sample used in this analysis.

Hence there is a need to establish a *real test* to continue further work on these same lines.

Chapter 6

Conclusions

A novel method for the immobilization of the heavy metal content of incinerator ash was developed. The method is based on the use of sulfate-reducing anaerobic microorganisms (*desulfovibrio*) capable of producing sulfides and in the process precipitate the heavy metals as metal sulfides.

Desulfovibrio cultures grown at 21° C were found to be more effective in immobilization than cultures grown at 30° C, though literature (Postgate, 1984) cites 30° C as the preferred temperature of *desulfovibrios.*

The bacteria were not found to be adversely affected by the toxicity of the ash, which was an initial consideration at the begining of this work.

The TCLP and the EP TOX leaching tests were found to be limited in their leaching capability on the ash samples used in this work. Therefore, a new method called the pH 5 Method was introduced to overcome the shortcomings of the EPA recommended tests, and the high alkalinity of the ash sample. In this method, concentrated acetic acid was added as required to maintain the pH below 5 and the pH monitored constantly during extraction. When this leaching test was applied to the untreated ash samples, the concentration of heavy metals in the leachate was found to be much higher than the EPA recommended standards. However, when the same test was conducted on biologically treated samples of

the same ash, the heavy metal concentration of the leachate was below the EPA standards.

The proposed approach to ash pretreatment prior to burial appears to have a significant potential for solving the problems associated with heavy metal leaching in landfills.

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Appendix: Raw Data

Table A.1 - Sulfate concentration of duplicate cultures with time

Date	9/13	*9/20*	*9/24*	*9/26*	*9/30*	*10/2*	*10/8*	*10/10*	*10/12*
A	49.5	51.1	51.3	53.3	54	54.5	55.9	-	-
C	49.5	52.1	52.4	54.6	56	56.4	56.48	-	58.8
D	49.5	28.9	22.7	17.7	16.7	16.3	14.4	13.9	13.6
E	49.5	53.0	54.9	55.6	56.6	58.9	61.1	-	-
I	49.5	44.1	38.8	34.4	33.8	32.6	28.2	26.8	23.1

Sulfate concentrations are in ppm (1:50 dilution)
49.5 = initial sulfate concentration in medium B

Table A.2 - Sulfate concentrations of cultures with time

Date/Culture	*11/9*	*11/13*	*11/19*	*11/27*
1	34.1	32.6	29.9	27.0
3	32.8	36	32.5	23.7
I_a	38.1	38.0	32.0	17.6
I_b	31.6	43.2	39.8	39.4
I_c	34.7	34.1	-	28.9
B-no sulfate	0.0	0.0	0.0	6.3

sulfate conc. are in ppm (1:50 dilution)
49.5 = initial sulfate conc. in medium B