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ABSTRACT

IMMOBILIZATION OF METALS IN INCINERATOR ASH USING A MICROBIAL SYSTEM

(December 1991)

Gordon Hinshalwood, M.S.Ev.Sc., NJIT

Thesis Advisor: Dr. Piero M. Armenante

The heavy metals contained in incinerator ash constitute an environmental hazard because they can be leached out of the ash matrix by rain water after the ash is landfilled. This study focused on a novel biological treatment process in which immobilization of the heavy metal content of incinerator ash is achieved using naturally occurring microorganisms. Specifically, immobilization was obtained by the use of a sulfide producing bacteriological system. The genus *Desulfovibrio* was cultured under anaerobic conditions, providing a source of sulfide from the reduction of sulfate as a natural metabolic function. The sulfide produced then formed highly insoluble precipitates with the metals present after incinerator ash was introduced into the system. Untreated ash was tested for lead, cadmium and chromium content using a new leaching test known as the "pH 5 method". The ash failed EPA limits for both lead and cadmium. Following treatment, the ash passed the EPA leaching test (TCLP) and the more stringent pH 5 method for all three metals, suggesting that this treatment has potential as an ash treatment option prior to disposal.

IMMOBILIZATION OF HEAVY METALS IN INCINERATOR ASH USING A MICROBIOLOGICAL SYSTEM

by

GORDON HINSHALWOOD

Submitted to the Department of Chemical Engineering, Chemistry and Environmental Science of the New Jersey Institute of Technology in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE IN ENVIRONMENTAL SCIENCE

December 1991

APPROVAL SHEET

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1 INTRODUCTION

1.1 Overview

Of the numerous environmental problems currently facing the United States, perhaps the most challenging is the solid waste disposal crisis. In 1990 293 million tons of solid waste were disposed of in municipal facilities*, and it is estimated that within the next decade more than half of all American cities will run out of landfill space (Glen & Riggle, 1991). While other environmental issues such as acid rain or global warming seem more abstract to most Americans, the waste disposal issue affects all citizens in their daily existence. It is therefore essential that this issue be addressed before time runs out.

Using the technology currently available, three basic disposal options exist; landfilling, recycling/reduction, and incineration. Of these three, landfilling has

* The figure used to represent the amount of waste disposed of in municipal facilities (Glen & Riggle, 1991) differs from the EPA estimate of 180 million tons annually due to a lack of agreement concerning the definition of municipal waste between the Glen & Riggle and the EPA.

historically been the favored approach to waste disposal in the United States. Even today as much as 77% of the total municipal waste stream is still landfilled in spite of recent increases in recycling and reuse nationwide (Glen & Riggle, 1991).

Within the past fifteen years, however, municipalities have been searching for other disposal alternatives to replace landfills. Significant difficulties with landfilling are an increasing shortage of space, and opposition to siting new facilities in many municipalities. Additionally, odor, debris, fugitive dust and, most critically, ground water contamination concerns make landfilling an undesirable disposal option (Boynton, 1988).

Recycling and reuse, combined with waste stream reduction, provide the most environmentally sound options. Within the past year, curbside recycling programs have increased by an extraordinary 80% across the United States. Nevertheless, within the same period of time, one estimate suggests that the total municipal solid waste produced increased by 23 million tons (Glen & Riggle, 1991). Even if implementation of new recycling programs can continue at last year's extraordinary rate, recycling alone cannot solve the growing waste disposal crisis.

A successful waste disposal scenario for the future should be a combination of vigorous recycling/reduction programs and advanced technology incineration.

Incineration has traditionally met with extreme resistance from the public due to concern for the air quality in communities surrounding these facilities. In spite of these concerns, incineration has increasingly become a favored option among state and municipal legislators as landfilling costs increase (Perkins, 1989). In 1987, 6% of the nation's municipal waste stream was incinerated, while it has been predicted that by the turn of the century over 30% will be disposed of in this manner (Davis, 1987).

While it appears that incineration will continue to be considered as a waste disposal option, the public continues to perpetuate the Not In My Backyard (NIMBY) syndrome due to concerns over dioxin production and release during the combustion process along with SOx, NOx, CO and heavy metal stack emissions (Denison, 1987). Beyond stack emissions, however, lie additional concerns relating to the ash produced during incineration. Heavy metals bind to the ash during incineration, creating a potentially hazardous product that must be disposed (Sen & De, 1985). The disposal method most commonly used for incinerator ash is landfilling, providing an opportunity for the landfill leachate to be contaminated by the heavy metals. If incineration is to be used as an option for municipal waste disposal in the future, some means of detoxifying metals in the ash must be developed so that ash can be disposed of in

an environmentally responsible manner.

The purpose of this work is to detoxify the metals in incinerator ash by immobilizing them. To accomplish this goal, a microbiological system is used. Details concerning this system are introduced in sections 2.0 and 2.1. The remainder of chapter 1 is devoted to background information concerning ash, heavy metals and regulations.

1.2 Heavy Metals in Ash

A growing concern about incineration in recent years involves the ash produced from the combustion process. Ash residue is collected from both the kiln (bottom ash) and the stack (fly ash) following combustion. It is then generally combined and disposed of in a municipal waste landfill. By the year 2000, projected figures for municipal incinerator ash are 7 million tons per year, of which up to 80% will likely be landfilled, if current trends continue (Fisher & Gustin, 1989).

The ash produced varies a great deal in composition, depending on the type of waste incinerated, the actual combustion process, and the pollution control equipment used at each facility. In general, however, all types of ash produced share a characteristic of environmental concern:

they all contain heavy metals.

Incineration removes the physical matrix surrounding metals in combustible refuse, effectively concentrating the metals in a smaller, more available volume (Bagchi & Sopcich, 1989). During combustion, the metals are vaporized. They then adsorb to the surface of the ash particles as they cool. In general, the finer the ash particles, the greater the surface area for adsorption and, consequently, the greater the concentration of metals in the ash. Additionally, chlorine, prevalent in many plastic polymers, reacts during incineration to form metal chlorides, which readily solubilize the metals in water (Denison, 1987).

Once heavy metals are in the ash, there is an increased opportunity of exposure to the public prior to landfilling through a variety of pathways. These pathways include inhalation, ingestion, and dermal absorption of airborne ash. Further opportunity for exposure occurs after landfilling from groundwater leaching and surface water runoff contamination. Since the vast majority of ash is landfilled, groundwater contamination is the exposure route of greatest concern to the EPA (Denison, 1987).

Heavy metals are an environmental concern because of their proven effect on human health. Some of them, such as As, Cd, Be and Pb, are carcinogens and are known to cause neurological, hepatic and renal disorders (Kirchner &

Reilly, 1983). Metals tend to accumulate in adipose tissue. Even exposure to small amounts over a long period of time can be detrimental. Marine life and other biotia are also extremely sensitive to metals in the environment.

1.3 Regulations Concerning Ash

In municipal landfills, acidic conditions are produced by waste degradating bacteria. Under these conditions, metals in incinerator ash will tend to leach out of the ash. The Extraction Procedure (EP) toxicity test is an EPA approved protocol for testing the leachability of metals. The test is designed to mimic the bacterially induced acidic conditions found in municipal solid waste landfill facilities by exposing the waste to a mildly acidic solution for an 18 hour period, then testing the supernatant solution for metals. EP toxicity tests were performed on ash samples from 45 different incinerators across the United States by the Environmental Defense Fund (Denison, 1987). Their results showed that most ash samples, especially fly ash, failed the federal EP toxicity test for leachability of lead and cadmium, and should therefore be classified as a hazardous waste based on Subtitle C of the Resource Conservation and Recovery Act (RCRA) of 1976 and the

Hazardous and Solid Waste Amendments (HSWA) to RCRA of 1984.

Incinerator ash has not, however, been classified as a hazardous waste by the EPA, and more stringent disposal methods mandated by RCRA Subtitle C do not apply. Federal regulations concerning hazardous waste landfills currently require cover monitoring and leachate collection for a 30 year period following closure, while municipal landfills, where ash is typically disposed of, do not have even these

TABLE I

SUMMARY OF EDF EP TOXICITY TESTS FOR 45 INCINERATORS (DENISON, 1987)

	Lead	Cadmium	Either
Fly Ash			
No. Samples Analyzed	185	97	185
No. Over EP Limit	168	94	173
% Over Limit	91%	97%	94%
Bottom Ash			
No. Samples Analyzed	773	271	773
No. Over EP LImit	276	5	278
% Over Limit	36%	2%	36%
Combined Ash			
No. Samples Analyzed	933	806	933
No. Over EP LImit	373	115	390
% Over Limit	40%	14%	42%

requirements (Santoleri, 1989). Of particular concern are heavy metals, which survive indefinitely in the environment and are not monitored for extended periods once they are landfilled.

The proven adverse health effects of heavy metals in the environment combined with the questionable disposal practices for ash federally mandated by the EPA clearly show that innovations in the area of metal treatment in ash are necessary to ensure the environmentally sound disposal of ash in the future.

CHAPTER 2 DESULFOVIBRIO AND SLUDGE

2.1 Microbiological Approach to Metal Immobilization and Objectives of this Work

Although various methods of chemical treatment have been devised for metal detoxification in ash, using microbiological activity to accomplish this goal is a novel approach. A species of sulfate reducing bacteria, *Desulfovibrio*, can be used under reducing conditions to produce sulfide from its oxidized form (sulfate). The free sulfide anion in solution then binds to the solubilized heavy metals, forming an insoluble precipitate. As indicated by Table II, metal-sulfide precipitates are extremely insoluble, with solubility products ranging up to 2.0E-47 for copper (II) sulfide (Lawrence & McCarty, 1965). Formation of metal sulfide precipitates within the ash prior to disposal would effectively prevent mobility of the metals after the ash is landfilled.

The genus *Desulfovibrio* carries out dissimilatory reduction using a common carbon source such as lactate or acetate as a substrate, and sulfate as a terminal electron

acceptor. Figure I illustrates the general biochemistry of the dissimilatory sulfate reduction process (Postgate, 1984).

TABLE II

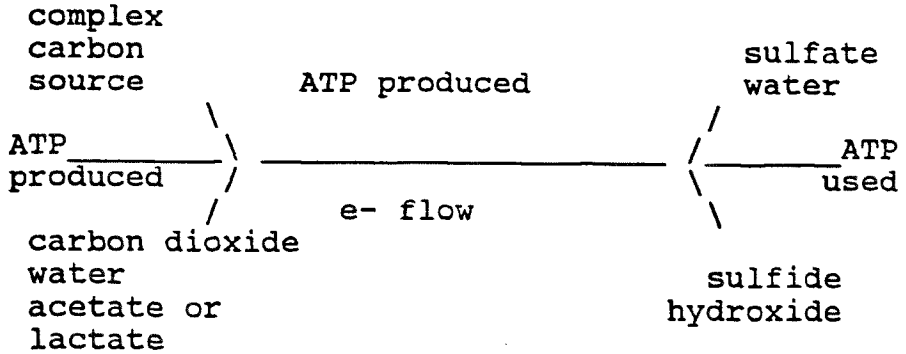
SOLUBILITY OF HEAVY METAL SULFIDES AT 18 DEGREES CELCIUS

Heavy Metal	Sulfide Salt	Solubility Product	Solubility (mg/L)
Copper	Cu2S	2.0E-47	3E-11
Copper	CuS	8.5E-45	9E-18
Lead	PbS	3.4E-25	4E-9
Cobalt	CoS	3E-26	2E-8
Nickel	NiS	1.4E-24	1E-7
Zinc	ZnS	1.2E-23	3E-7
Iron	FeS	3.7E-19	5E-5

FIGURE I

BIOCHEMISTRY OF DISSIMILATORY SULFATE REDUCTION IN

DESULFOVIBRIO (Postgate, 1984)



Complex carbon sources are commonly broken down to acetate by catabolic bacterial species, at which point the dissimilatory sulfate reduction process begins. *Desulfovibrio* then oxidizes acetate (or another carbon source) by reducing sulfate. This step is driven by ATP consumption. *Desulfovibrio* is commonly found in a variety of anaerobic mediums, ranging from acid mine waters to marine sediments and wastewater sludge (Tuttle, 1968).

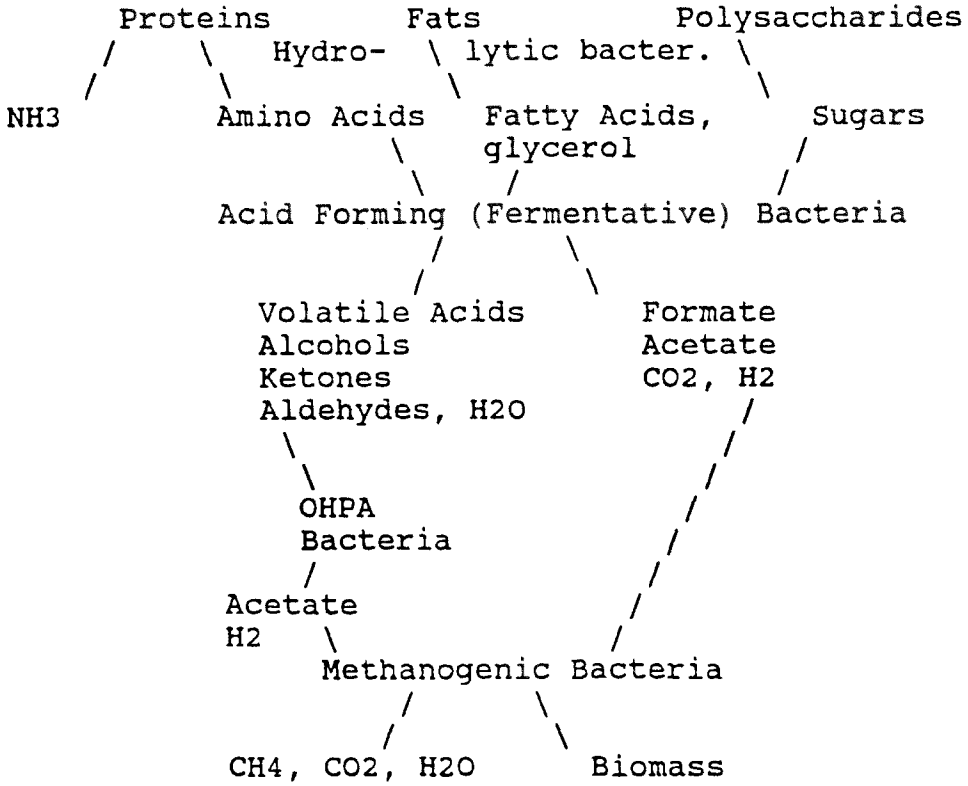
Therefore, the objective of this work is to immobilize the heavy metals found in municipal incinerator ash through the use of *Desulfovibrio*. Once a productive culture of *Desulfovibrio* is developed in wastewater sludge, the sulfide produced should be able to bind and immobilize the metals present in the ash.

2.2 Waste Water Sludge as a Source of Desulfovibrio

In order for *Desulfovibrio* to survive, an anaerobic environment must be established. An ideal choice for such an environment is the anaerobic sludge taken from a wastewater treatment process.

FIGURE II

BIOCHEMISTRY OF ANAEROBIC DIGESTION (Sterritt & Lester, 1988)



Traditionally, municipal wastewater in most communities has been treated using aerobic methods, primarily because of the limitations inherent in the anaerobic process. Anaerobic treatment requires a much lower loading capacity, longer retention times in the reactor, and is generally thought to be more sensitive to metal contamination from plumbing lines (Giller, 1989). The advantage of using an anaerobic process is its production of methane as an end

product. Since methane is a gas, it diffuses out of solution, lowering the Biochemical Oxygen Demand (BOD) of the waste water without the costly necessity of aeration (Sterritt & Lester, 1988). For this reason anaerobic treatment, or a combination of anaerobic and aerobic treatment procedures have gained popularity in recent years.

Anaerobic treatment involves three basic steps (see Figure II for details on the biochemistry involved); hydrolysis, fermentation, and conversion to methane (Sterritt & Lester, 1988). This process is often carried out in a double reactor system, where the primary tank is used for microbiological activity and the secondary tank is used for settling organic products.

The bacterial species prevalent in most anaerobic sludges correspond to the three metabolic steps listed above. The facultative anaerobes, including *Bacteroides*, *Pseudomonas* and *Bacillus* are the most common, while fermentative species such as *Clostridium* and *Butyribacterium* also occur frequently (Kucnerowicz, 1983).

Desulfovibrio, the species of most concern for the purpose of this project, is also commonly found in anaerobic sludge (Lawrence & McCarty, 1965). A small amount of the sulfide in anaerobic sludge comes from the degradation of sulfur containing amino acids, but the majority comes from sulfate reduction in the wastewater by *Desulfovibrio* (Lawrence & McCarty, 1965).

If a substrate such as acetate or lactate and a sulfate source were added to anaerobic waste water treatment sludge, conditions selectively favorable to *Desulfovibrio* should result.

CHAPTER 3 LITERATURE REVIEW

With the advent of incineration as a prevalent waste disposal solution, new technology for improving the process and eliminating environmental impacts has been developing at an unprecedented pace (Fisher & Gustin, 1989). A variety of new technologies have been developed to address concerns over the disposal of incinerator ash. Some of the more common or innovative solutions to ash disposal currently being pursued are listed below.

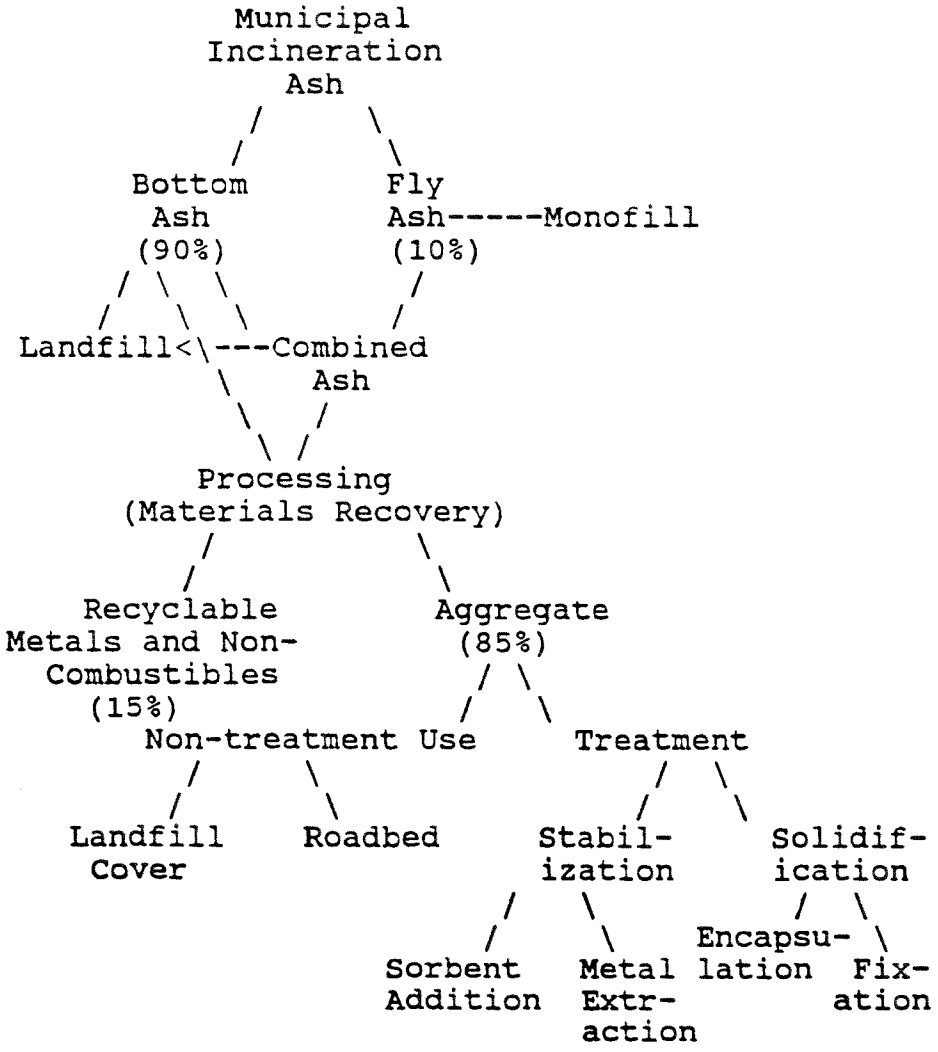
Material Recovery System (MRS) is a method of separating ash by size. Of the total municipal incinerator ash produced today, about fifteen percent is removed in the form of noncombustibles by MRS and recycled (Kellermeyer and Stewart, 1989). Incinerator ash can also be used as a fill for various construction materials such as cement and asphalt (Fisher and Gustin, 1989). Some environmentalists object to this use since studies proving the immobilization of heavy metals within the cement or asphalt matrices have not been done.

Several chemical treatment solutions have also been explored. Sorbent addition involves the addition of clay or vermiculite to the ash. The metals adsorbed to the ash surfaces will then react and stabilize with the sorbent material (Behel, 1986). Metal extraction uses an

FIGURE III

MUNICIPAL WASTE COMBUSTION ASH DISPOSAL ALTERNATIVES

(KELLERMEYER & STEWART, 1989)



acid bath to strip metals from the ash followed by treatment of the extract by ion exchange, precipitation, or adsorption (Berry, 1988).

Other methods suggested for ash disposal management include separation of fly and bottom ash due to differences in their metal content, and disposal in separate "monofills" to avoid the acid effects of municipal landfills (Denison, 1987).

The approach for heavy metal management in ash used in this study is dependent on the sulfide reducing capabilities of the *Desulfovibrio* bacteria. These bacteria have been used in research for close to half a century. Bass Becking and Moore (1961) linked the reduction of sulfate to the production of metal sulfides, while Sorokin (1966) discovered carbon dioxide and acetate as carbon sources and hydrogen as an electron donor in the sulfate reduction process. While Miller determined that *Desulfovibrios* were responsible for metal sulfide ore deposits as early as 1950, a significant amount of the research was carried out by Postgate (between 1953 and 1984) including numerous studies on classification and biochemical activity. Badziong and Thauer (1978) conducted an experiment which quantified ATP formation using hydrogen and sulfate as sole energy sources in *Desulfovibrio vulgaris*. This was later confirmed in *D. desulfuricans* by Brandis and Thauer (1981).

Lawrence and McCarty (1964) used *Desulfovibrio* to

control heavy metal toxicity in activated wastewater sludge through the formation of metal sulfide precipitates. Parasar (1990) precipitated metals from incinerator ash using *Desufovibrio* cultured in a media developed by Postgate. This study is an extension of the work begun by Parasar.

CHAPTER 4 MATERIALS AND METHODS

4.1 Determination of Metal Content in Ash

The content of the heavy metals lead, cadmium and chromium in incinerator ash was determined by conducting a "digestion" of the ash with a strong acid (Boyle, 1983). This method involved mixing 10 grams of incinerator ash (obtained from American Refuel, Newark, New Jersey) with 200 mL glacial sulfuric acid. The mixture 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. After 72 hours the pH of the mixture was below 0.5. The aliquots collected were then diluted 1:50 with a 2% solution of nitric acid.

Analysis of the aliquots was performed on a Smith Hieftje flame atomic absorption (AA) spectrometer (model number 12) manufactured by Thermo Jarrell Ash Corporation. Standards were prepared from 1000 ppm stock solutions of lead oxide (PbO), cadmium nitrate, and ammonium dichromate (the metal salts were purchased from J.T. Baker Chemical

Corporation). Standards were prepared by successive dilutions to the following concentrations: 1.0, 5.0, 10.0 and 15.0 ppm of lead; 0.5, 1.0, 1.5 and 2.0 ppm of cadmium; and 1.0, 4.0, 8.0 and 12.0 ppm standards of chromium.

A 1:50 dilution of fresh anaerobic wastewater sludge (obtained from the Township of Livingston Water Pollution Control Facility, Livingston, New Jersey) was filtered (0.45 um) and analyzed for lead, cadmium and chromium content using flame AA spectroscopy.

4.2 Determination of Desulfovibrio Activity in Wastewater Sludge

The first experimental step in this work was to establish live *Desulfovibrio* cultures in wastewater sludge. To do this, a live inoculum of *Desulfovibrio* (from a culture stored by Parasar (1990) and maintained in Postgate B media) was introduced into a series of culture tubes (25 mL) containing either media (Postgate B) or whole sludge. Table IV details the content of each tube, while Figure IV illustrates the experimental design. The tubes were set up under anaerobic conditions, using 100% nitrogen gas to displace the air above the liquid.

TABLE III

CONTENTS OF POSTGATE B MEDIUM (Postgate, 1957)

Compound	Amount Added (gms)
KH_2PO_4	0.5
NH_4Cl	1.0
$CaSO_4$	1.0
$MgSO_4-7H_2O$	2.0
Sodium Lactate	3.5
Yeast Extract	1.0
Ascorbic Acid	0.1
Thioglycollic Acid	0.1
$FeSO_4-7H_2O$	0.5

Adjust final volume to 1.0 L with tap water and final pH to between 7.0 and 7.5 with 1.0 N HCl.

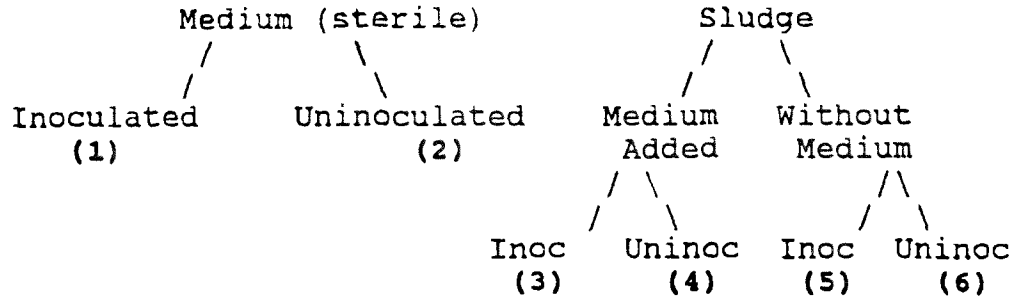
TABLE IV

CONTENTS OF DESULFOVIBRIO CULTURES IN WASTEWATER SLUDGE

Tube Number	Medium (mL)	Water (mL)	Inoculum (mL)	Sludge (mL)
1	5	18	2	-
2	5	20	-	-
3	5	-	2	18
4	5	-	-	20
5	-	-	2	23
6	-	-	-	25

FIGURE IV

EXPERIMENTAL DESIGN TO DETERMINE DESULFOVIBRIO ACTIVITY IN WASTEWATER SLUDGE



All of the tubes were sealed with rubber stoppers and incubated at 30 degrees Celsius.

In order to determine if the *Desulfovibrio* cultures were active, a method for determining sulfate and sulfide concentrations in solution was used. The preferred method for this determination is Ion Chromatography (IC). A sulfate/sulfide determination method devised by Waters Corporation was used (method number A-102) on Waters instrumentation (see Table V for details on the instrumentation used).

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 was blown in through the stopper to displace any oxygen in the

atmosphere). The samples (0.5 mL) were then placed in eppendorf tubes and centrifuged for 10 minutes at 10,000 RPM to remove the large organic molecules inherent in wastewater sludge. Following centrifugation, the samples were passed through a SEP-PAC filtering system to further remove any organics in solution, then 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 1:50 dilutions were then loaded in 100 uL amounts into the IC for analysis.

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

4.3 Determination of Desulfovibrio Activity in the Presence of Incinerator Ash

Once a culture of *Desulfovibrio* was established in wastewater sludge, a new series of tubes containing the cultures with incinerator ash added were prepared. A time stop assay of *Desulfovibrio* inoculated sludge with ash was developed in which a series of seven identical tubes for each experimental culture and control was set up according to Table VI. A total of 63 tubes were used.

TABLE V

ION CHROMATOGRAPHY INSTRUMENTATION USED FOR SULFATE/SULFIDE DETERMINATION

A) Pump System (Waters 600E system)

B) Sample Processor/Injection System (Waters 715, Ultra Wisp)

C) IC-PAK A HC Column, 10 um

D) Ultraviolet Absorbance Detector (Waters 484)

E) Conductivity Detector (Waters 431)

F) PC Minichrom 1990 VG Data System Ltd., Softwate version 1.5

Instrumental Conditions:

5 mM sodium phosphate eluent

2.0 ml/minute flow rate

100 uL automated injection volume

1000 uS conductivity detection range with a background of 960 uS

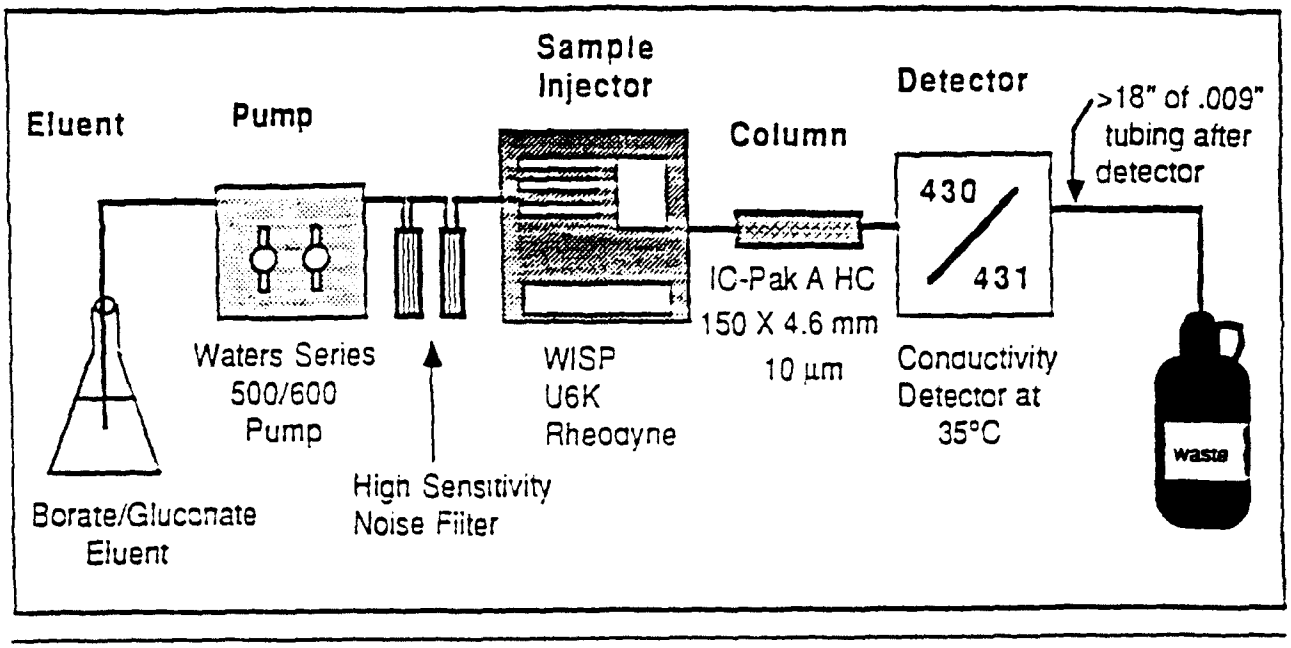


FIGURE V

SCHEMATIC DIAGRAM OF WATER'S ION CHROMATOGRAPHY SYSTEM

(Water's IC Method Number A-111, Millipore Corp., 1989)

TABLE VI

COMPONENTS OF CULTURE TUBES IN TIME STOP ASSAY OF INOCULATED WASTEWATER SLUDGE WITH INCINERATOR ASH

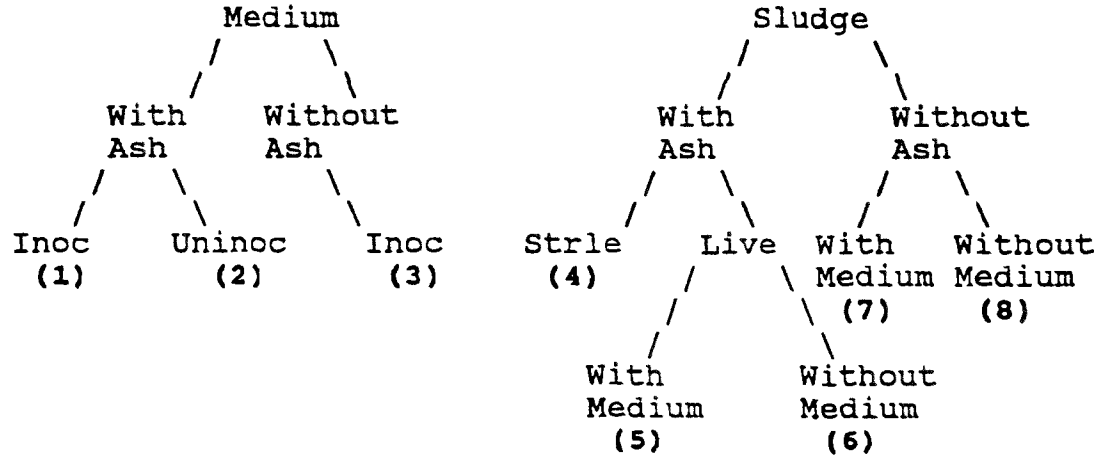
Culture Series	Ash (gm)	Sludge (mL)	Inoculum (mL)	Water (mL)	Medium (mL)
1	1.0	-	2.0	18.0	5.0
2	1.0	-	-	20.0	5.0
3	-	-	2.0	18.0	5.0
4	1.0	23.0*	-	-	-
5	1.0	18.0	2.0	-	5.0
6	1.0	23.0	2.0	-	-
7	-	18.0	2.0	-	5.0
8	-	23.0	2.0	-	-
9**	1.0	18.0*	2.0	-	5.0

* Sterile sludge

** Tube 9 was set up as a control nine days after the other culture tubes were set up.

FIGURE VI

EXPERIMENTAL DESIGN OF TIME STOP ASSAY OF INOCULATED WASTEWATER SLUDGE WITH INCINERATOR ASH



Seven identical tubes were set up for each of the culture tube series 1 - 8 above. Before the various components of the cultures were added to the tubes, one gram aliquots of ash were dispensed to tube series 1, 2, 4, 5 and 6. The ash was then suspended in 5.0 mL of water or sludge, depending on the culture series. Each tube was then tested with a pH meter and brought to a pH between 7.0 and 7.5 with 1 N hydrochloric acid. The tubes were then stored over night at room temperature. The following day each tube was pH tested again and adjusted to pH 7.3 with hydrochloric acid. This step was completed in order to minimize the effect of wide pH variances on the *Desulfovibrio* cultures.

Following pH adjustment the sludge, inoculum, media and water components were added under anaerobic conditions. All of the culture tubes were sealed and incubated at thirty degrees Celsius.

Sampling was conducted every 3 - 4 days, and involved "sacrificing" (opening) the culture tubes in order to remove and analyze the ash inside. Prior to opening the tubes, a portion of the supernatant in each was removed and analyzed for sulfate/sufide content on the IC using the methodology previously described.

4.4 Determination of Metals Leached from Incinerator Ash

In order to determine the amount of lead, cadmium and chromium that would leach from the treated ash, a three step process was used; (1) removal of the ash from the culture tubes, (2) testing for leachability using the Toxic Characteristic Leachate Procedure, and (3) testing for leachability using the "pH 5 Method". The Toxic Characteristic Leaching Procedure (TCLP) (40 CFR Chapter I: 7-1-88 Edition) involves leaching the ash over an 18 hour period with a slightly acidic solution, then acidifying the extract prior to AA spectroscopic analysis.

The pH 5 method (Parasar, 1990) involves leaching the ash for the same time period, but at a constant pH at or below 5.0. The pH is tested every 15 minutes for the first two hours, and every two to three hours after that. 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 after the ash had already passed the TCLP test. A pictorial description of the TCLP and pH 5 methods is given in Figures VII and VIII.

FIGURE VII

TCLP PROCEDURE FOR ASH

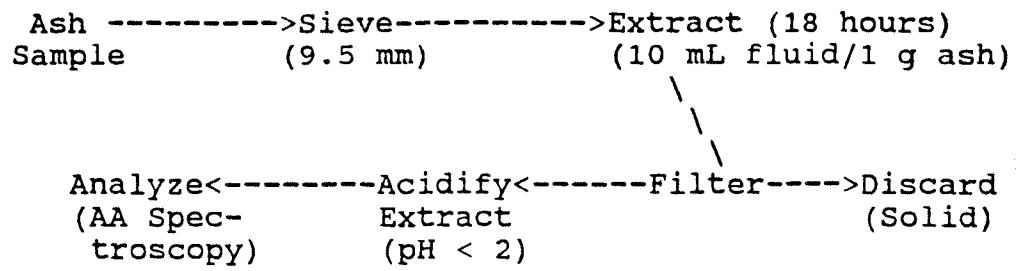
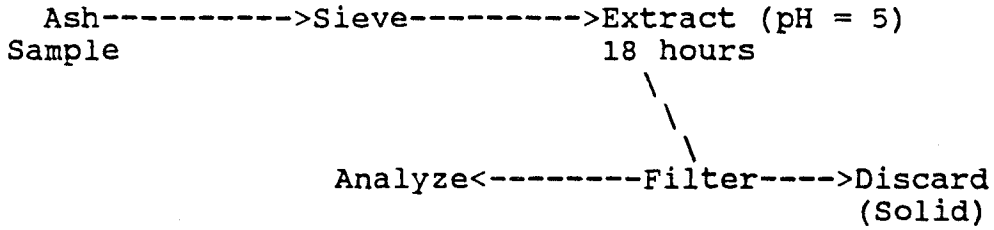


FIGURE VIII

pH 5 METHOD LEACHATE PROCEDURE



The detailed procedure for the above-mentioned three-step process is now outlined. First, the tubes of Culture Series 1,2,4,5,6 and 9 were opened by removing the rubber stoppers. The tubes were then centrifuged at 5,000 RPM to concentrate the ash at the bottom. The supernatant was decanted, leaving the ash and a layer of large organic particles at the bottom of the tube. The ash was then washed

three times with deionized water, and centrifuged after each wash. Finally, the ash was filtered through nitrocellulose filter paper and dried over night at room temperature in preparation for the leachate tests.

The TCLP test was the first leachate test used on the ash. The dry ash was mixed with 20 mL of Deionized water for an 18 hour period. The mixture was then filtered (0.45 um), and the ash dried for further analysis. The liquid phase from the filtration step was acidified below pH 2 with glacial acetic acid, diluted 1:50, and analyzed for lead, cadmium and chromium content using flame AA spectroscopy.

The "pH 5 Method" leachate test was conducted on the ash after the TCLP was completed. The dry ash was mixed with 10 mL of deionized water, and 1N nitric acid was added dropwise until the pH was between 4 and 5. The volume was then brought to 20 mL with deionized water. The pH of the mixtures was tested every 15 minutes for a 2 hour period, and nitric acid was added dropwise as needed to maintain the pH at or below 5. After 2 hours, the pH was tested every 2 to 3 hours for the remainder of the 18 hour period. The mixture was then filtered, and the liquid phase was diluted 1:50 for flame AA spectroscopic analysis.

CHAPTER 5 RESULTS AND DISCUSSION

5.1 Determination of Metal Content in Incinerator Ash

An acid digestion was performed on an ash sample as described in Section 4.1. A 10 g aliquot of ash was mixed with 200 mL of 1N nitric acid. At intervals of 10 minutes, 1 hour, 24 hours, and 72 hours the leachate was removed by filtration and analyzed for lead, cadmium and chromium content. Each time the leachate was removed, the ash was mixed with 200 mL of fresh 1N nitric acid. As Figure IX shows, large amounts of lead, cadmium and chromium were initially released from the ash, with significant releases continuing for the following 72 hours.

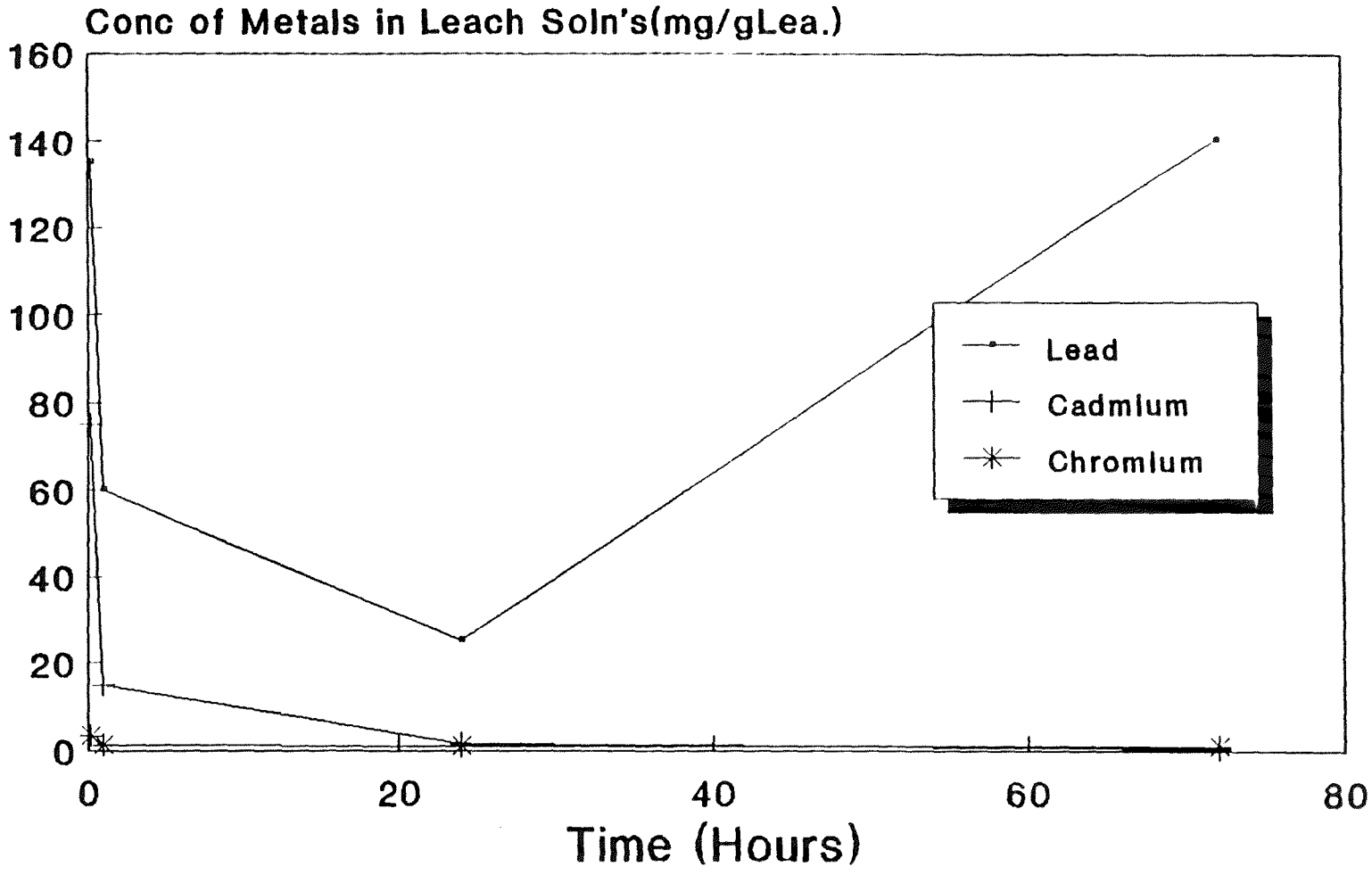
TABLE VII

TOTAL AMOUNT OF METALS RELEASED FROM INCINERATOR ASH AFTER STRONG ACID DIGESTION

Metal	Amount Digested in a 72 Hour Period (mg metal per 1 gm Ash)
lead	72.0
cadmium	18.4
chromium	1.3

FIGURE IX

CONCENTRATION OF METALS IN 200ml 1N HNO3 LEACHATE SOLUTION EXPOSED TO 10g ASH



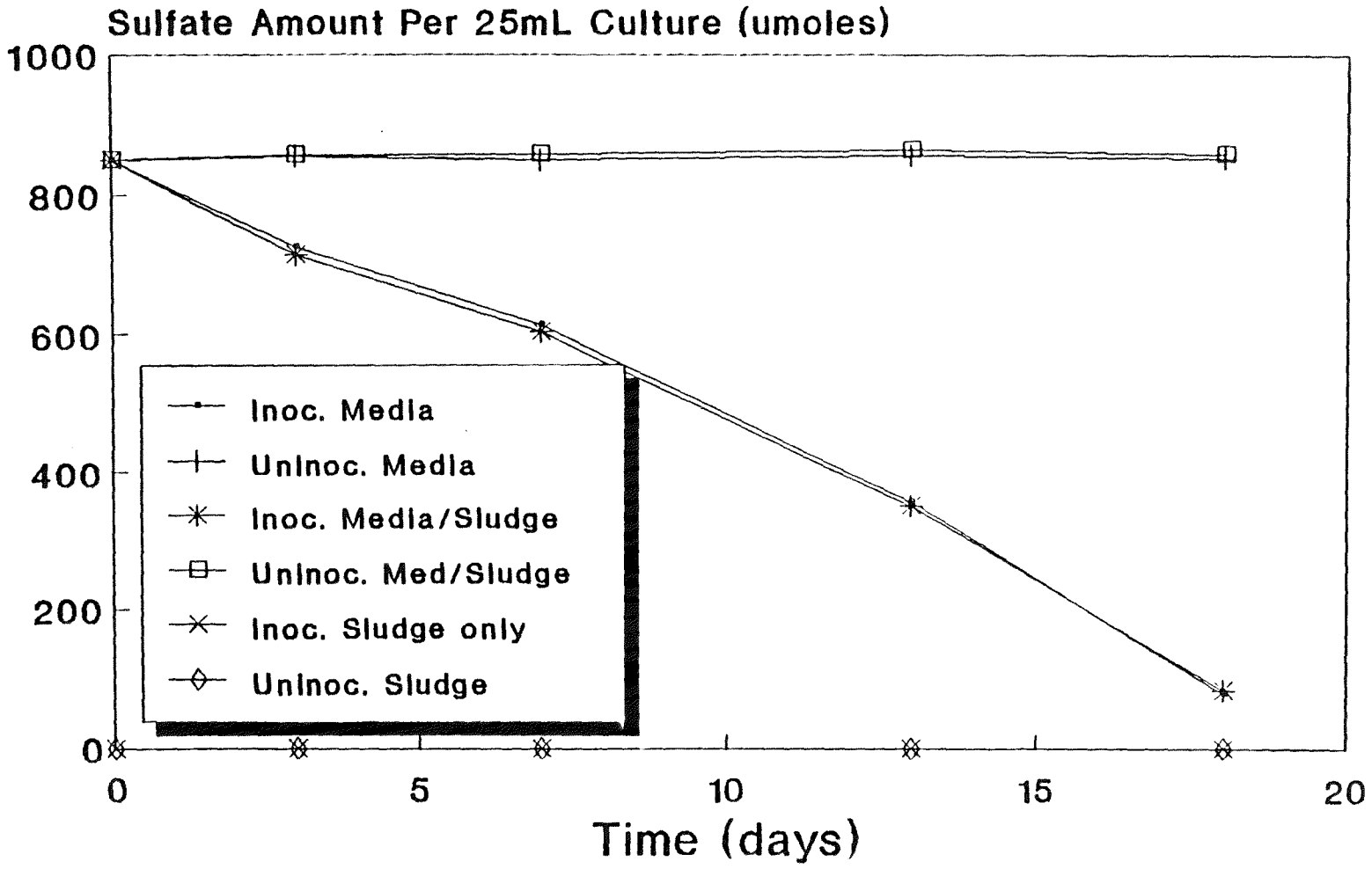
Leachate was changed at 0.25,1,24,&72 hr

5.2 Determination of Desulfovibrio Activity in Wastewater Sludge

The experiment described in Figure IV, Section 4.2 was conducted using ion chromatography to determine the sulfate and sulfide content of each sample. As demonstrated in Figure X, the inoculated culture containing media (culture tube 1) showed complete conversion from sulfate to sulfide over a three week period, as did the inoculated culture containing wastewater sludge and media (culture tube 3). The uninoculated cultures of media and sludge showed no conversion to sulfide. These results suggest that uninoculated sludge does not contain *Desulfovibrio* in amounts able to convert micromolar quantities of sulfate. Additionally, since Postgate B medium contains 850 micromoles of sulfate (see Table III, Section 4.1) and 835 micromoles of sulfide were produced in culture tube number 1, (842 micromoles in culture tube number 3) mass balance for these cultures was satisfied.

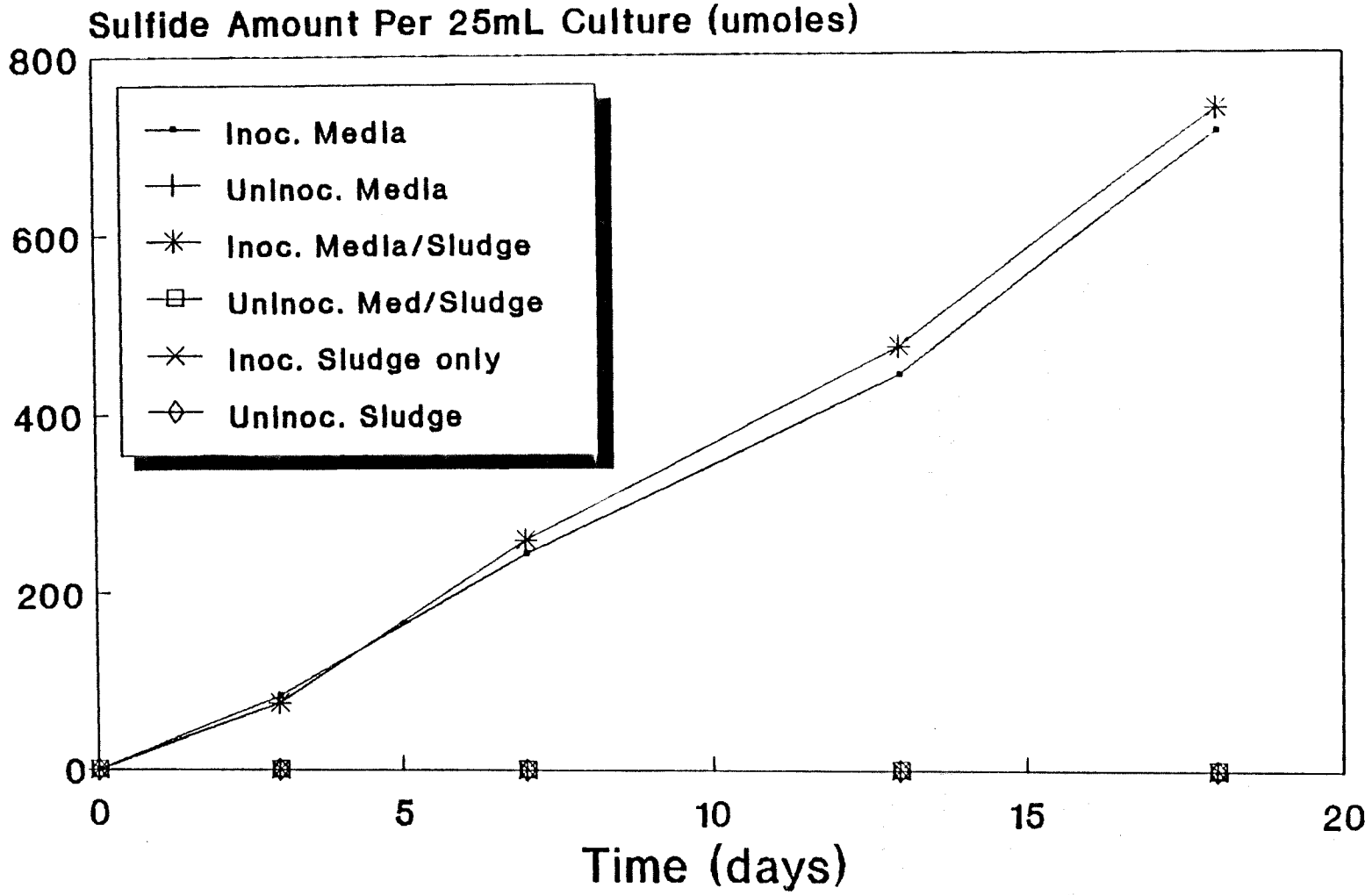
These results demonstrate that *Desulfovibrio* is active in wastewater sludge as long as sulfate and a source of carbon substrate are present.

FIGURE X
SULFATE IN DESULFOVIBRIO CULTURE TUBES



Culture Tubes 1-6 (Table IV,Section 4.2)

FIGURE XI
SULFIDE IN DESULFOVIBRIO CULTURE TUBES



Culture Tubes 1-6 (Table IV,Section 4.2)

5.3 Determination of Desulfovibrio Activity in the Presence of Incinerator Ash

Nine culture series (each one containing 7 tubes) were prepared as described in Section 4.3. The contents of these tubes were "sacrificed" at three to four day intervals over a 21 day period in order to analyze the leachability of the metal content in the ash. An analysis of sulfate and sulfide content was also conducted using ion chromatography. The results of the IC analysis are shown in Figures XII through XV, while the AA spectroscopic results on the ash from each sample are shown in Figures XVI through XXI.

All of the samples containing Postgate B media initially contained 850 micromoles of sulfate. None of the other samples contained any sulfate. Over the 21 day experimental period all of the culture series containing inoculated media showed almost complete disappearance of sulfate, including those tubes containing ash, sludge, or both. Soluble sulfide was detected only in tubes containing inoculated media or an inoculated media/sludge mixture (not in those tubes which showed no initial levels of sulfate).

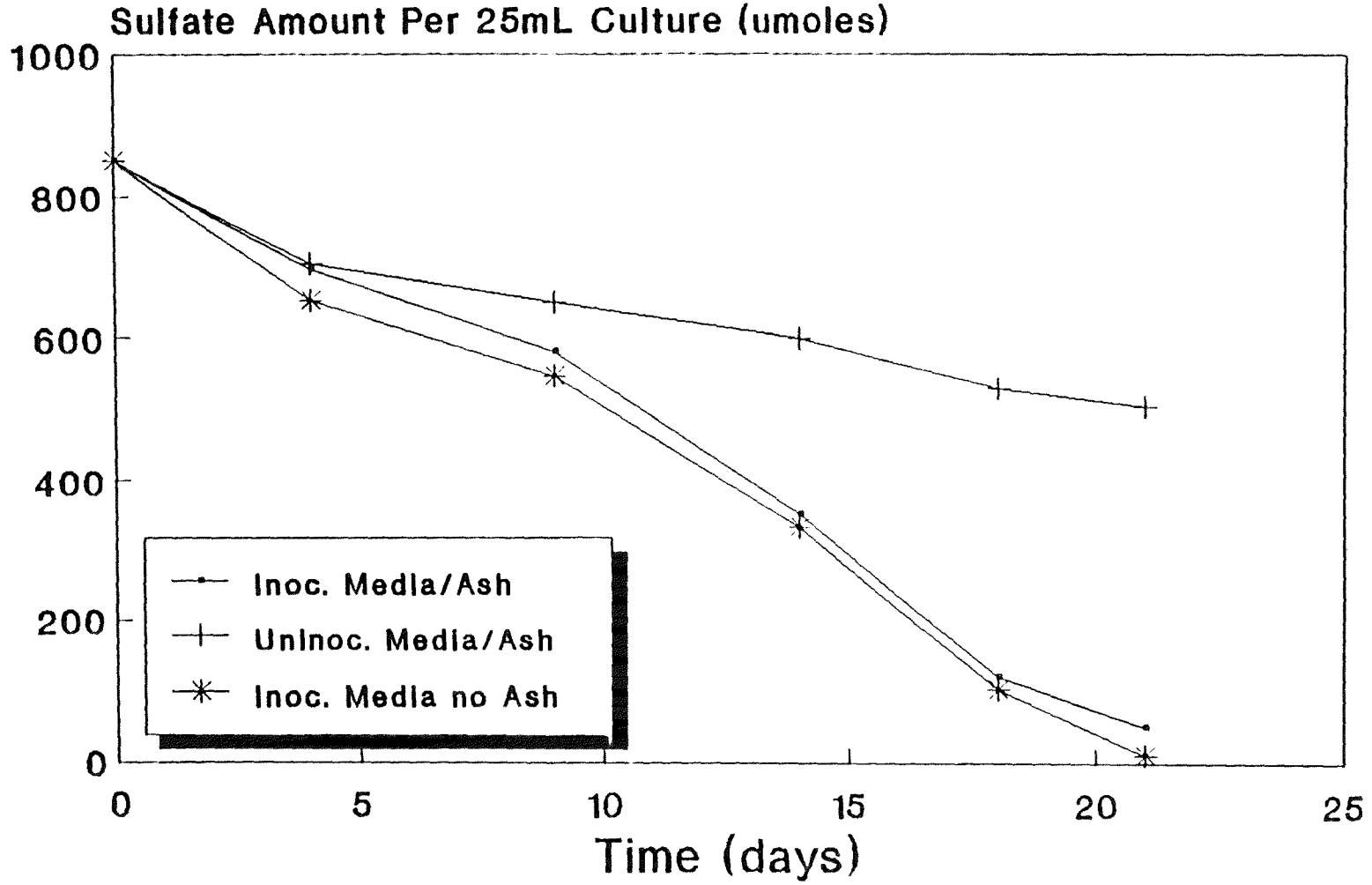
Culture series 1 and 5 (Table VI, Section 4.3) contained inoculated media with ash, and inoculated media mixed with sludge and ash, respectively. In both of these

series, the initial amount of sulfate present almost entirely disappeared during the experimental period. Corresponding sulfide production, however, was not stoichiometric, indicating that some of the sulfide produced was consumed in the formation of other products. Levels of sulfide production amounted to 250 micromoles in series 1, and 100 micromoles in series 5; substantially less than the 850 micromoles of sulfate initially present in both series. Culture series 3 (inoculated media without ash) and 7 (inoculated media/sludge mixture without ash), by contrast, exhibited stoichiometric conversions of sulfate to sulfide. Clearly, then, the sulfide missing in series 1 and 5 must be used by the ash present in those tubes.

Culture series 2 and 9 contained uninoculated media (series 2) or an uninoculated media/sludge mixture (series 9) along with ash. Both series exhibited a gradual decrease in sulfate content over the 21 day period without any corresponding sulfide production. This suggests that the ash present somehow binds some of the sulfate, removing it from solution. This effect accounts for about 250 micromoles of sulfate (see the IC analyses of tubes 2 and 9 in Figures XII and XIV).

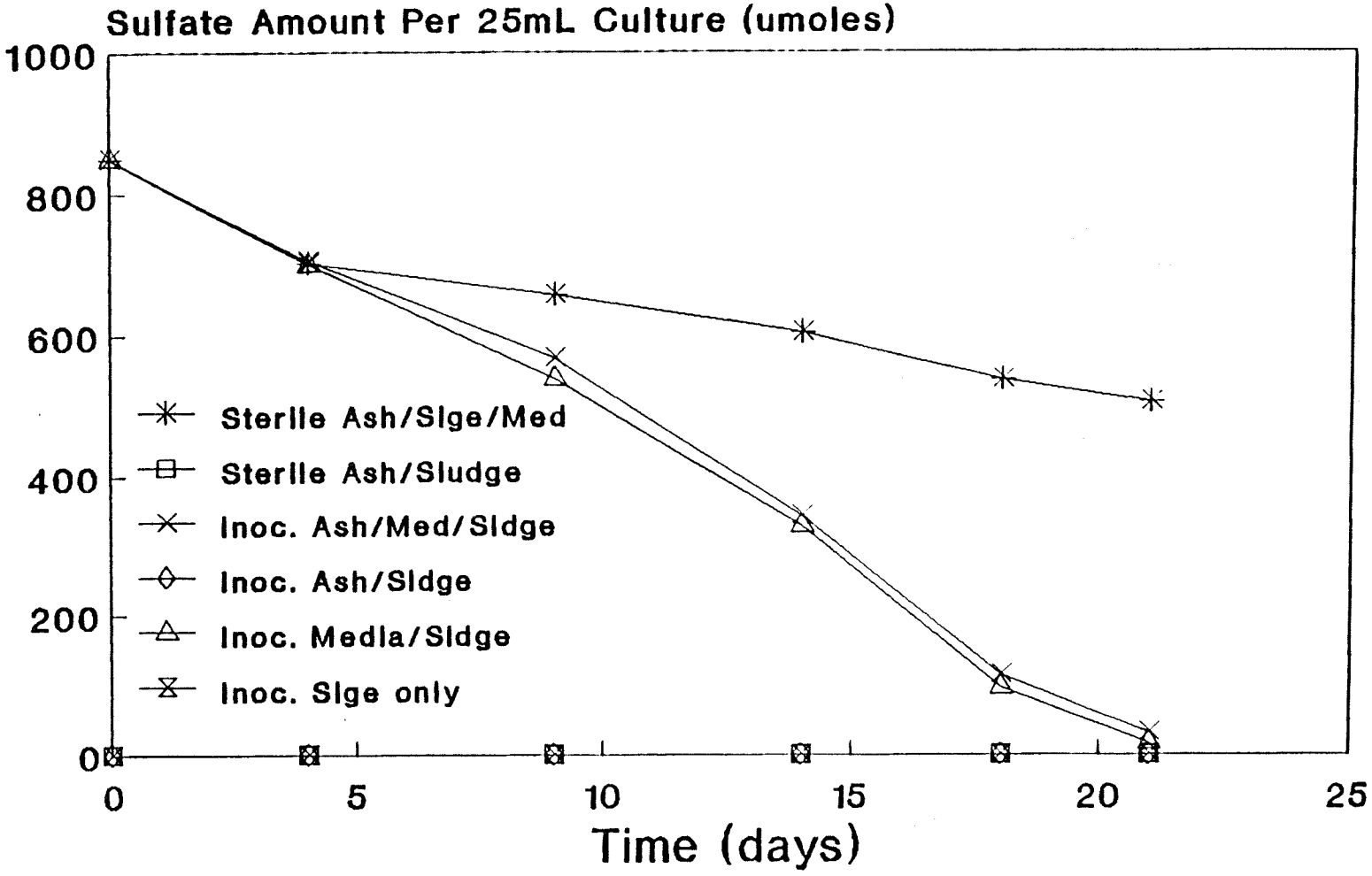
As already noted in this section, culture series 1 (inoculated media with ash) showed an initial sulfate level of 850 micromoles. At the end of the experimental period,

FIGURE XII
SULFATE IN CULTURE SERIES CONTAINING ASH



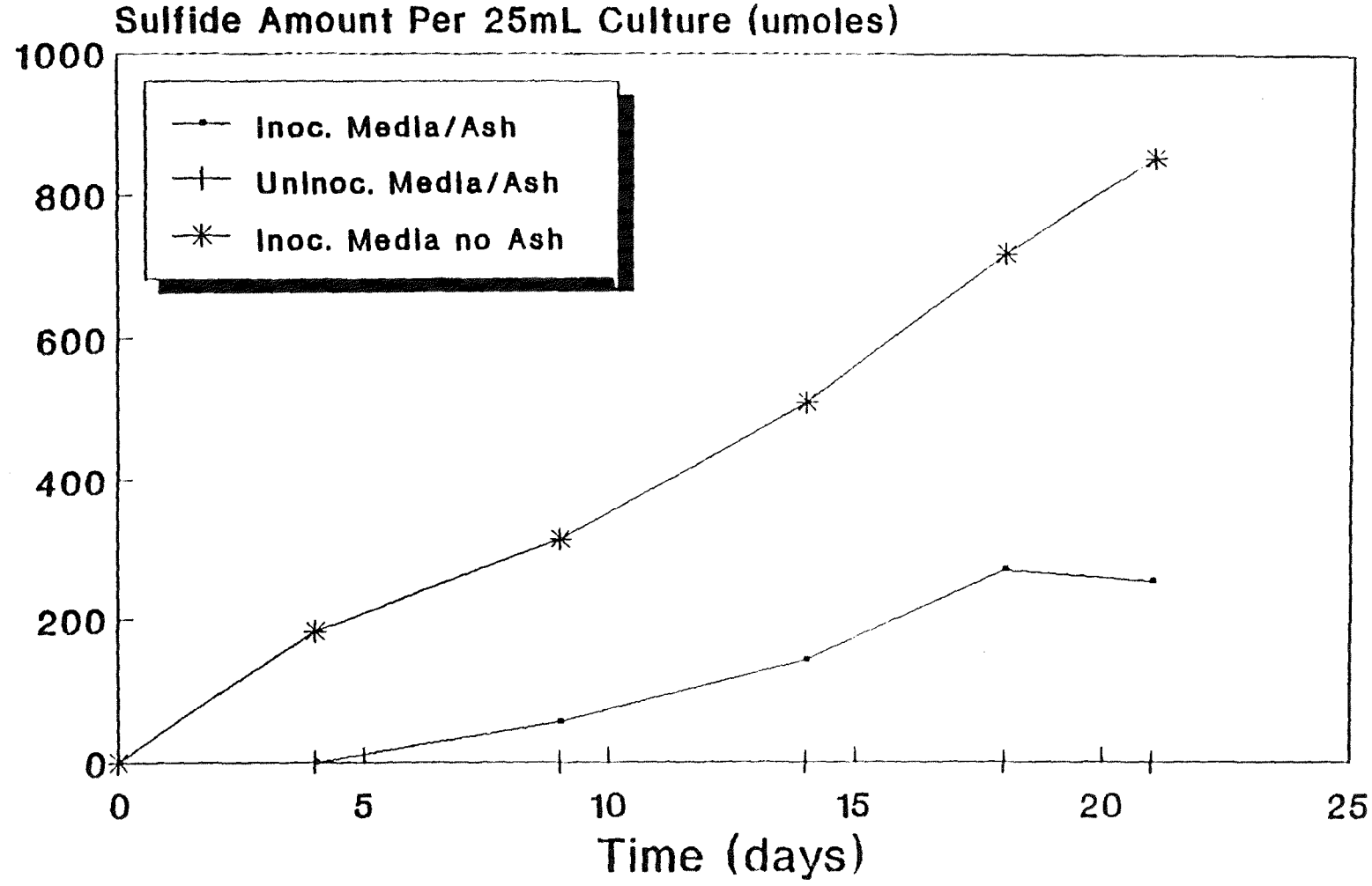
Series 1-3 (Table VI, Section 4.3)

FIGURE XIII
SULFATE IN CULTURE SERIES CONTAINING ASH



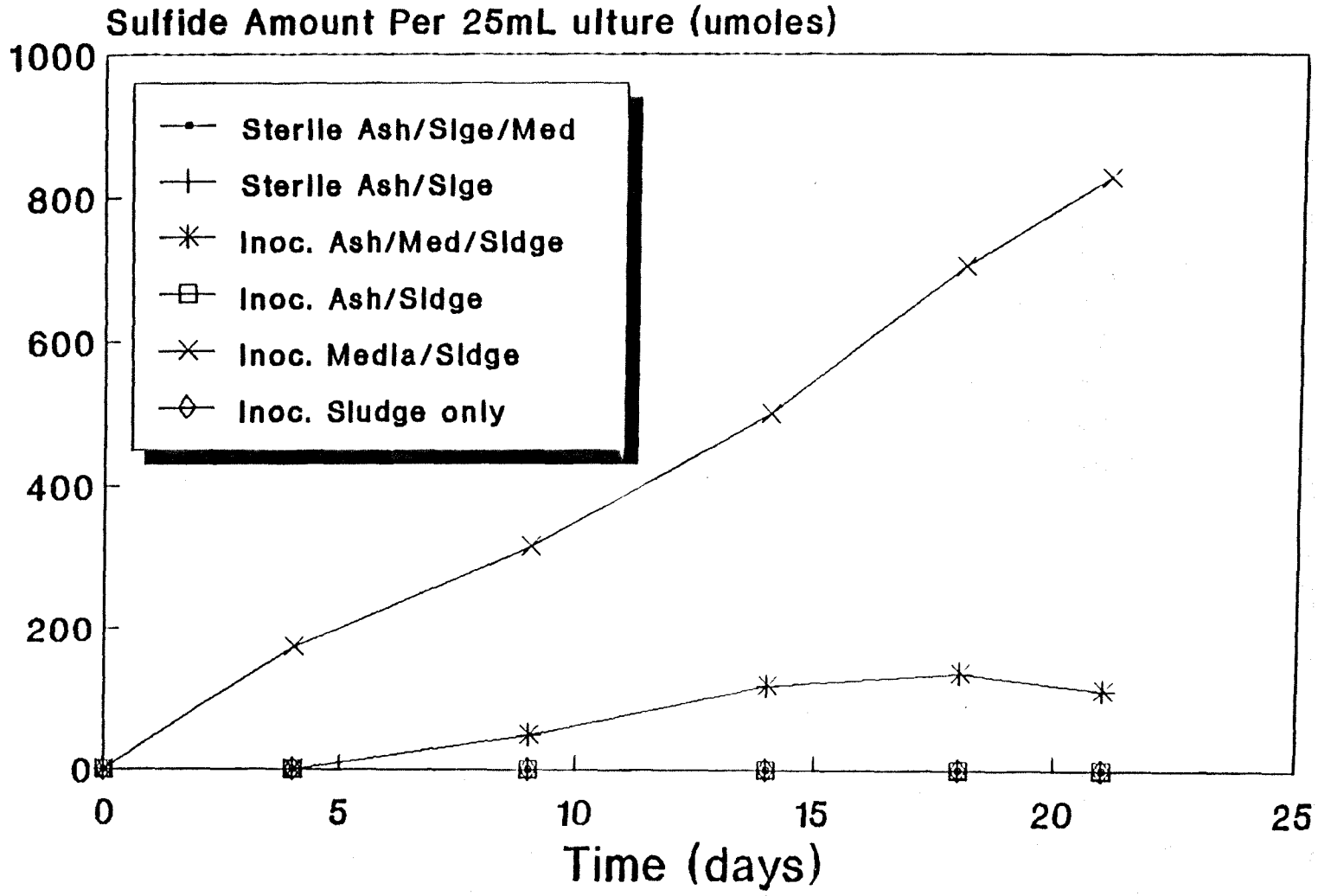
Series 4-9 (Table VI, Section 4.3)

FIGURE XIV
SULFIDE IN CULTURE SERIES CONTAINING ASH



Series 1-3 (Table VI, Section 4.3)

FIGURE XV
SULFIDE IN CULTURE SERIES CONTAINING ASH



Series 4-9 (Table VI, Section 4.3)

there were 50 micromoles left, representing a net disappearance of 800 micromoles of sulfate from the solution. Of the 800 micromoles, 250 can be accounted for by sulfide production measured (Figure XIII), and another 250 can be accounted for by the binding effect ash has on sulfate discussed above. This leaves 300 micromoles of sulfate unaccounted for; presumably precipitated as metal-sulfide. In order to establish this, an AA spectroscopic analysis was conducted on the ash to determine changes in metal content.

5.4 Analysis of Metals Leached from Incinerator Ash

As discussed in Section 4.4, the ash from culture series 1, 2, 4, 5, 6 and 9 (Table VI, Section 4.3) was removed and subjected to two leaching tests and subsequent spectroscopic analysis. The first leaching test was the EPA mandated TCLP test. As expected, both untreated ash and ash treated by the method described in this work passed minimal EPA standards for lead, cadmium and chromium concentrations. This method was therefore determined to be ineffective, and the results are not represented here (see Appendix for the TCLP test data).

The second leaching method used was Parasar's pH 5 method (1990). Figures XVI, XVIII and XX show gradual immobilization of the three metals tested from culture series 1 and 5 throughout the experimental period. These results suggest that metal immobilization is dependent upon microbiological activity in this system. Table VIII lists the amount of each metal immobilized during the experimental period.

TABLE VIII

ANALYSIS OF METAL IMMOBILIZATION IN INCINERATOR ASH

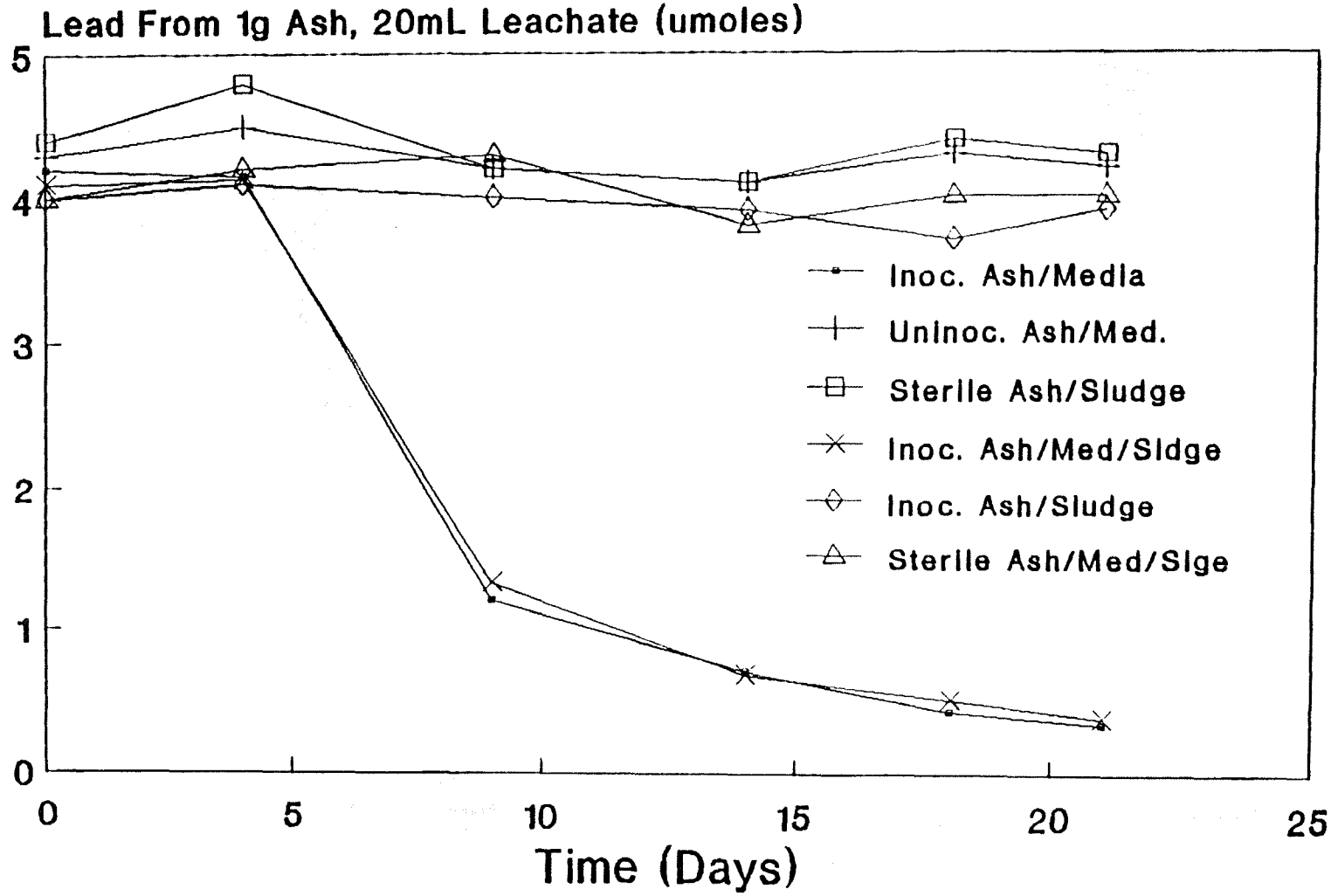
Metal	Initial Amount (mg/g Ash)	Amount After 21 Days (mg/g Ash)	Total Amount Immobilized (mg/g Ash)	Total Amount Immobilized (umol/g Ash)
Pb	2.30	0.18	2.12	10.2
Cd	0.16	0.03	0.13	1.2
Cr	0.09	0.02	0.07	1.3

Total micromoles of metal immobilized: 12.7 per gm Ash

As discussed in Section 5.3, 300 micromoles of sulfate remain unaccounted for in culture series 1 (inoculated media with ash), presumably having precipitated from solution as

metal sulfide. In order to close mass balance, the micromolar amount of total metals immobilized in the ash should equal the micromolar amount of sulfate not already accounted for. Since the amount of measured total metals immobilized is 4.88 micromoles, mass balance was not closed in this study. It should be noted, however, that a number of other metals are likely to be present in the ash in addition to the three analyzed here. Without a complete analysis of every sulfide precipitating metal present, (a task beyond the scope of this work) it is not possible to close mass balance. This situation does not, however, undermine the validity of the results overall.

FIGURE XVI
LEAD CONTENT IN LEACHATE OF TREATED ASH



Series 1-9 (Table VI, Section 4.3)

FIGURE XVII
LEAD IN ASH LEACHATE (pH 5 Method)

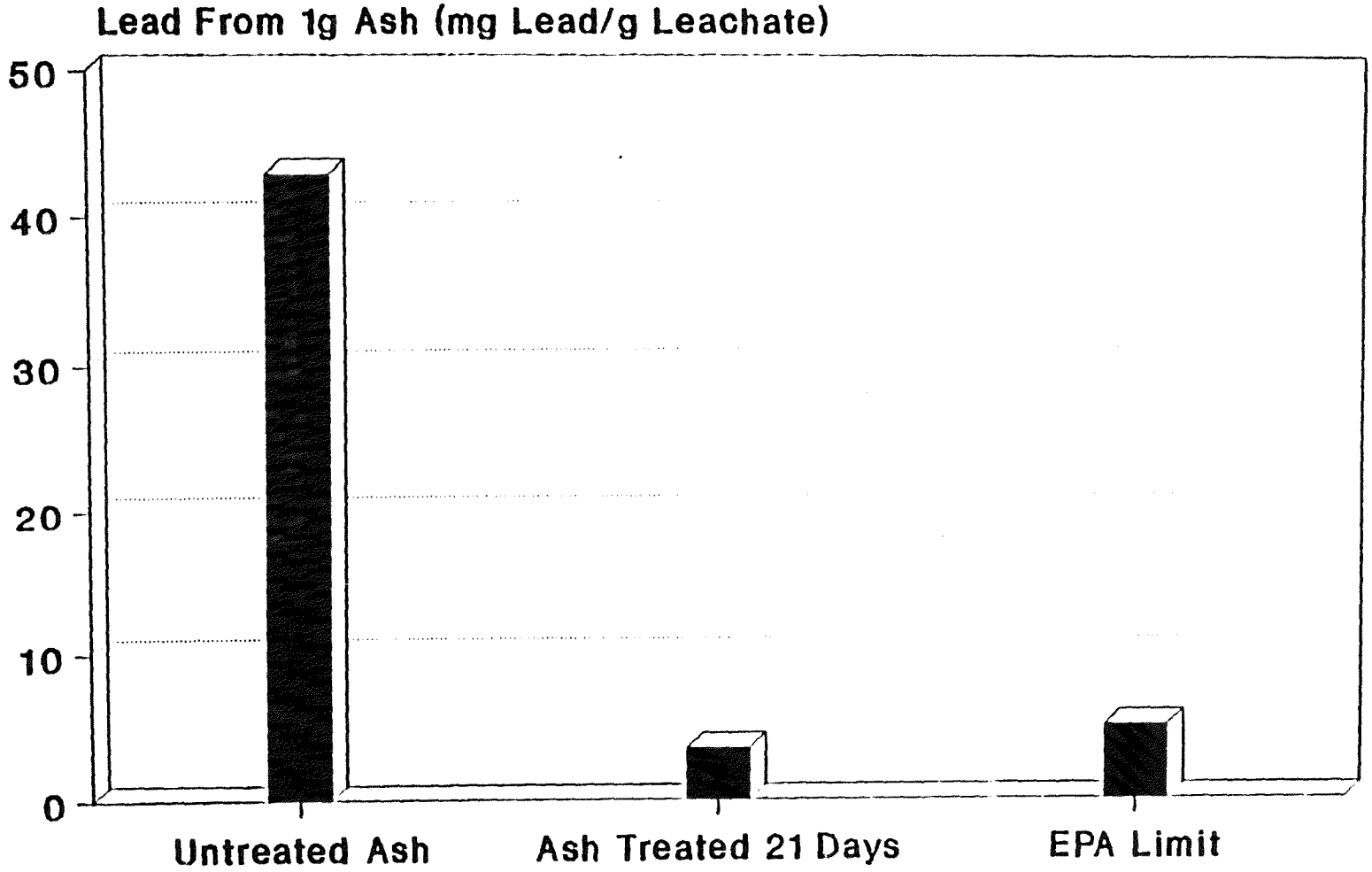
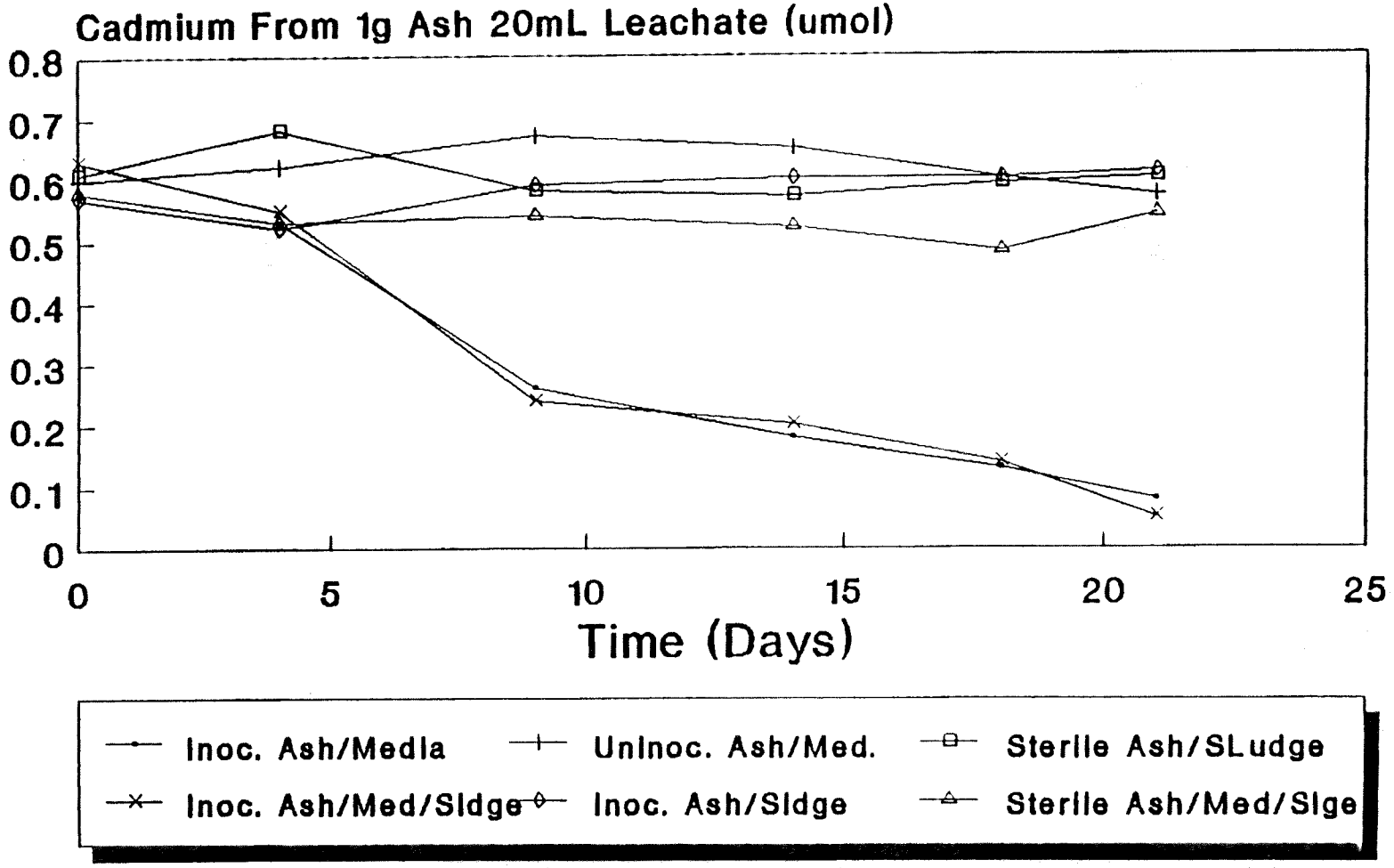


FIGURE XVIII
CADMIUM IN LEACHATE OF TREATED ASH



Series 1-9 (Table VI, Section 4.3)

FIGURE XIX
CADMIUM IN ASH LEACHATE (pH 5 Method)

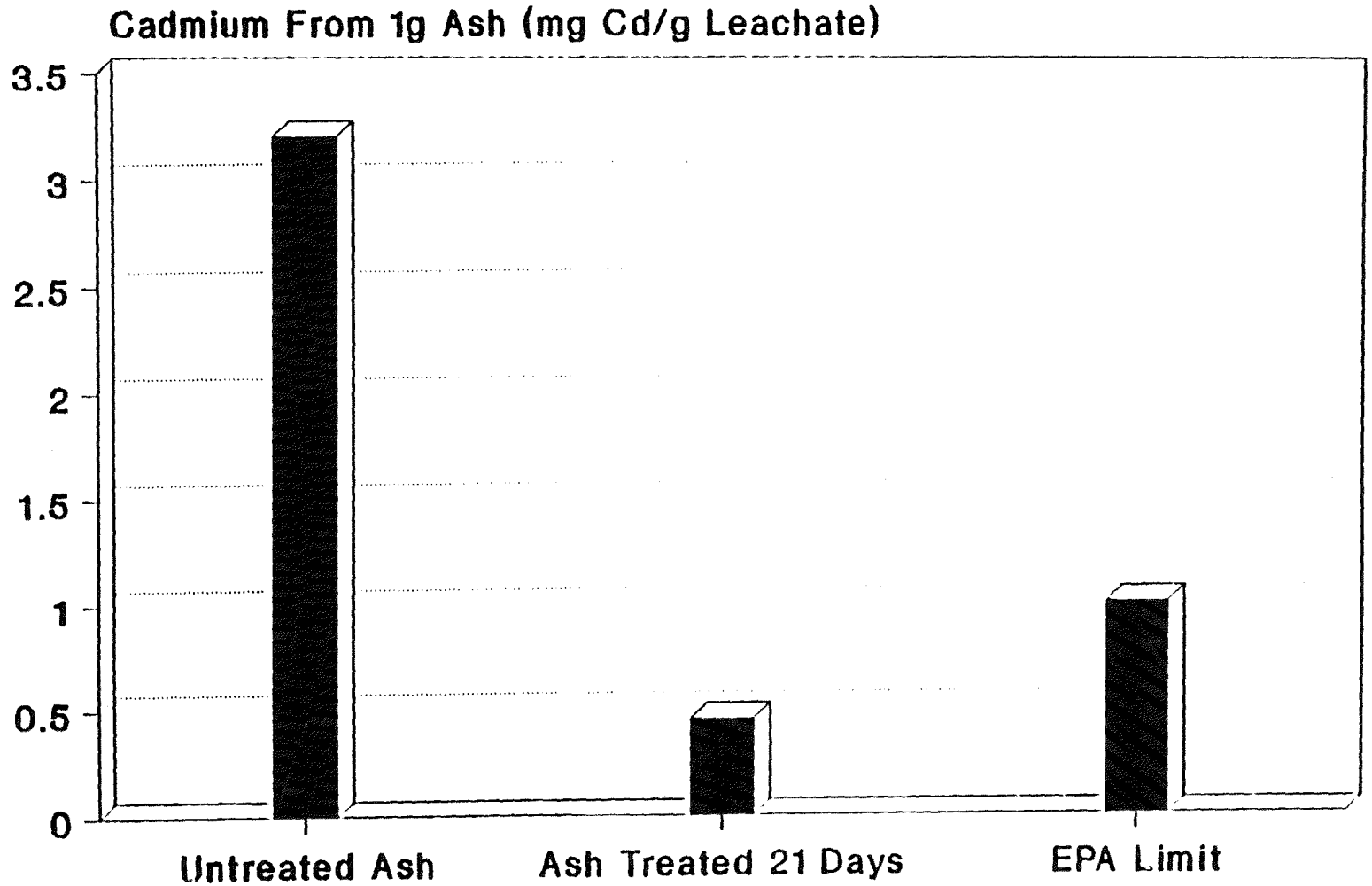
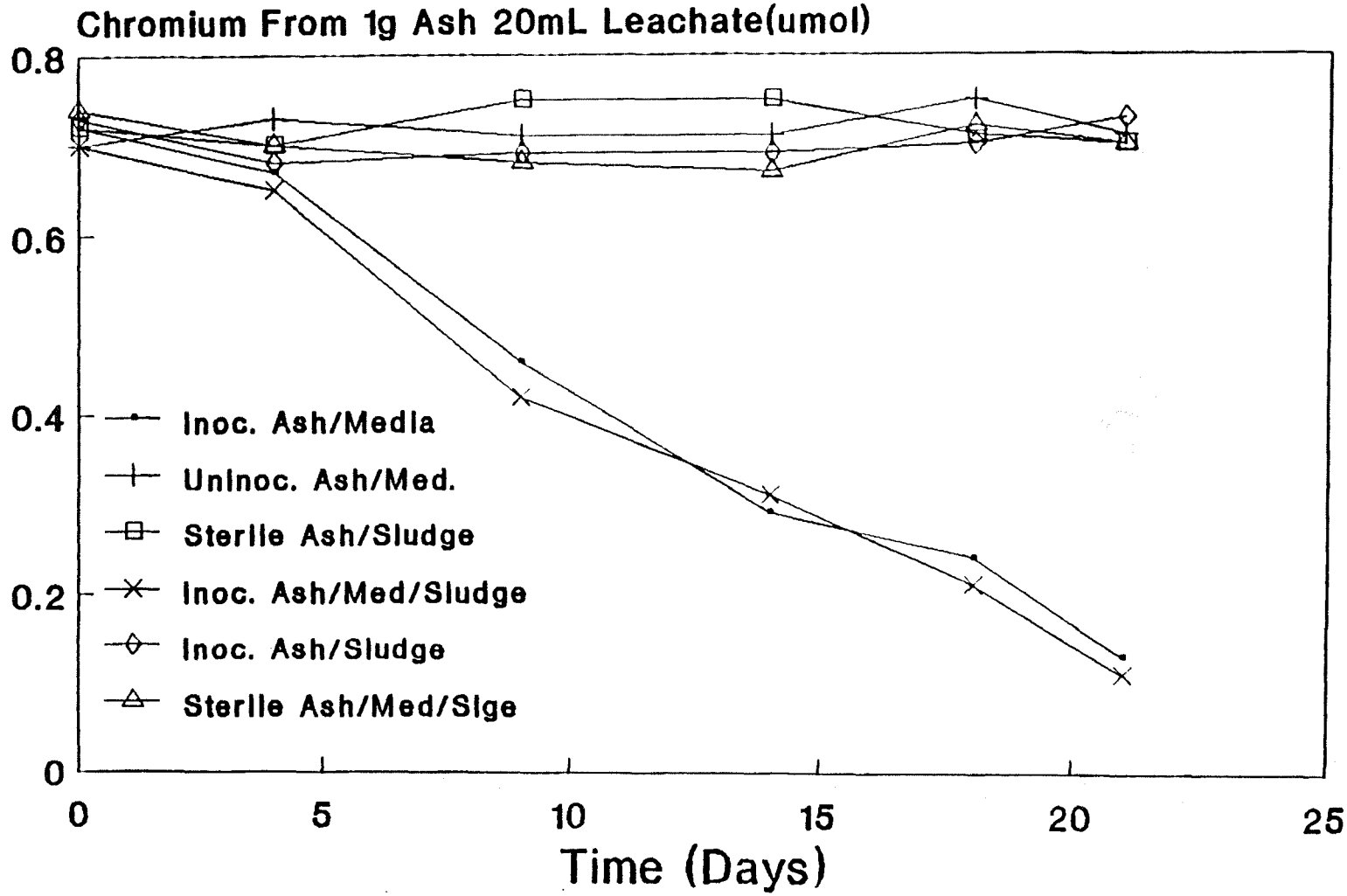
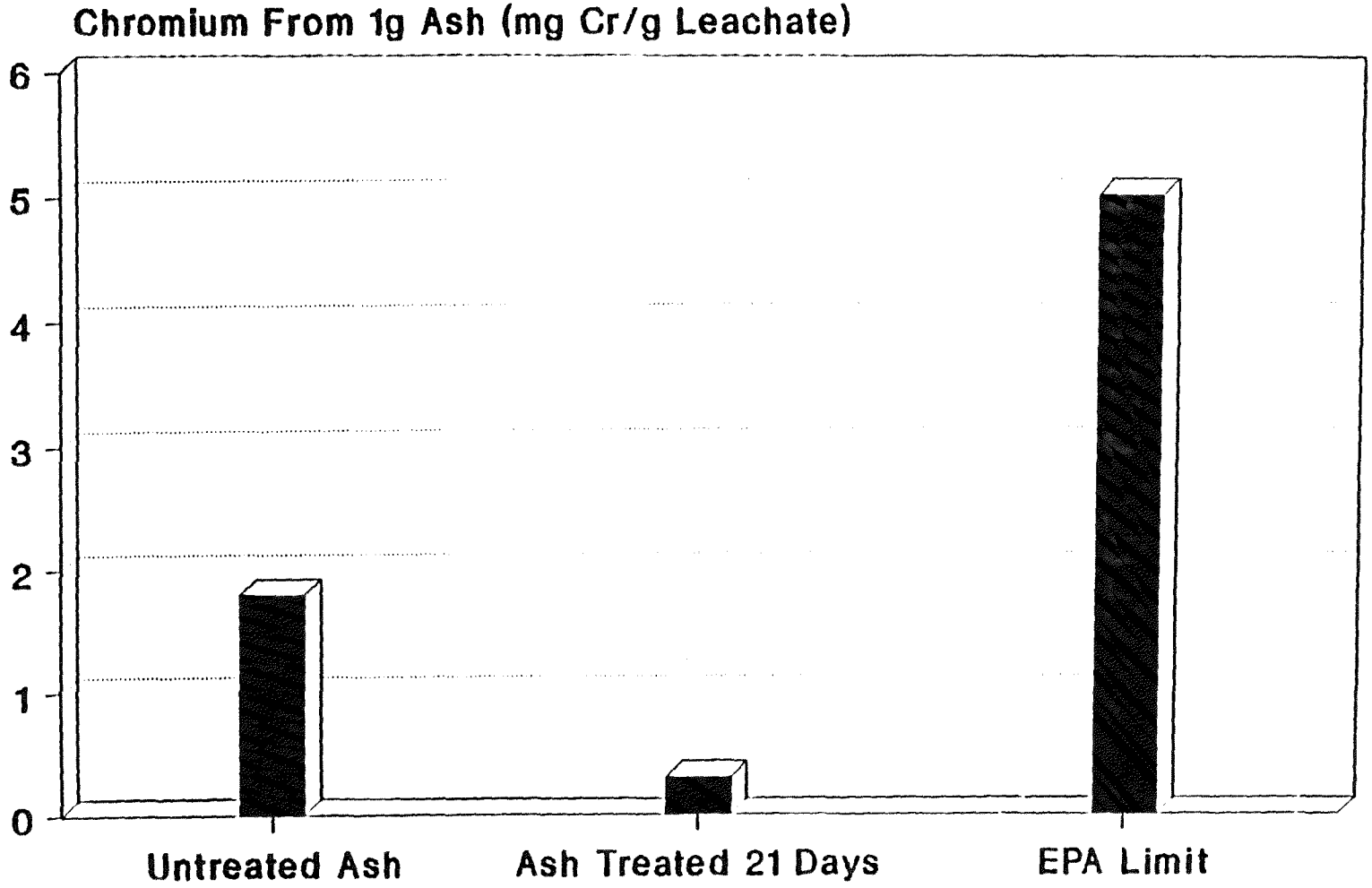


FIGURE XX
CHROMIUM IN LEACHATE OF TREATED ASH



Series 1-9 (Table VI, Section 4.3)

FIGURE XXI
CHROMIUM IN ASH LEACHATE (pH 5 Method)



CHAPTER 6 CONCLUSIONS

The results of this work show that heavy metals present in incinerator ash are immobilized in the presence of the bacterial species *Desulfovibrio*. As illustrated in Figures XVII, XIX and XXI, the incinerator ash treated with this method passed EPA leaching standards for all three metals, even though a more stringent test than the EPA mandated TCLP was used. Clearly, the *Desulfovibrio* treatment is an effective method of metal immobilization. Further work should be conducted in the future in order to establish the working mechanism by which the system functions. Mass balance should be established for the system as a whole, and the treated ash should be subjected to even more stringent leachate tests to determine the total amounts of metals left in the ash after treatment. Further efforts on this subject should also concentrate on ash to sludge ratios and initial sulfate levels in anticipation of scaling up the system.

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APPENDIX: EXPERIMENTAL DATA

TABLE 1

ASH DIGESTION (SECTION 4.1)

Metal	Hours			
	0.3	1	24	72
Pb	135	60	25	140
Cd	75	15	1.5	0.5
Cr	3.5	1.2	1.0	1.0

The amounts represented above are in ppm per 200 mL leachate, 10g ash

TABLE 2

TCLP AND pH 5 METHOD TEST DATA FOR ASH (SECTION 4.4)

	Concentration Before Treatment (mg/g leachate)		Concentration After Treatment (mg/g leachate)	
Metal	TCLP	pH 5	TCLP	pH 5
Pb	3.0	43	2.8	3.5
Cd	0.2	3.2	0.0	0.45
Cr	0.3	1.8	0.3	0.3

The above tests were conducted with 1g of ash, 20ml leachate

TABLE 3

SULFATE DISAPPEARANCE (SECTION 4.3)

Series#	Dy 0	Dy 4	Dy 9	Dy 14	Dy 18	Dy 21
1	850	695	580	359	123	53
2	850	708	655	608	532	500
3	850	653	545	332	103	12
4	-	-	-	-	-	-
5	850	701	568	342	114	33
6	-	-	-	-	-	-
7	850	698	538	327	96	18
8	-	-	-	-	-	-
9	850	700	654	612	552	518

Values in Table 3 are in micromoles

TABLE 4

APPEARANCE OF SULFIDE (SECTION 4.3)

Series#	Dy 0	Dy 4	Dy 9	Dy 14	Dy 18	Dy 21
1	-	-	56	142	268	253
2	-	-	-	-	-	-
3	-	182	312	508	713	850
4	-	-	-	-	-	-
5	-	-	48	117	136	112
6	-	-	-	-	-	-
7	-	173	314	498	702	850
8	-	-	-	-	-	-
9	-	-	-	-	-	-

Values in Table 4 are in micromoles