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ABSTRACT

Bacterial Biosorption of Heavy Metals From Liquid Wastestreams

by
Emilia Rus

In this study, immobilized activated sludge from a municipal wastewater aerobic treatment plant has been evaluated as a potential biosorbent for the removal of heavy metals from liquid waste streams. The method of immobilization consists of entrapment of the microorganisms in calcium alginate beads.

The affinity of immobilized activated sludge to cadmium, lead, chromium and copper has been evaluated under well defined laboratory conditions.

Initially, the time course of metallic ions uptake and the effect of pH and temperature on biosorption were studied for each metal. Then the biosorption pattern and the effect of biosorbent concentration on binding capacity were investigated. It was found that biosorption by the bacterial beads could be described by the Freundlich Isotherm.

A bench-scale system has been developed and tested to optimize the biosorption process. Promising results have been obtained for lead and chromium.

Results indicate that alginate-immobilized activated sludge has a capacity to adsorb a maximum of 240 mg Pb^{2+} /g dry biobeads; 36.9 mg Cr^{3+} /g dry biobeads; 18.9 mg Cu^{2+} /g dry biobeads, and 7.2 mg Cd/g dry biobeads. A residual Pb^{2+} concentration of 0.04 mg/l, which is below the drinking water limit, has been obtained with biobeads in a bench-scale biosorption treatment of lead from an initial concentration of 300 mg/l. This is a very encouraging result, because at a concentrations of Pb^{2+} as high as 300 mg/l the metabolic activity of the sludge is not inhibited. Therefore, biodegradation of organic contaminants, which might be found along with lead in some industrial effluents, can occur at the same time.

Other results suggest that the sludge biosorbent can also be used to selectively remove specific metals from solutions containing various metal ions. This may be done by adjusting the conditions of biosorption, especially the pH.

BACTERIAL BIOSORPTION OF HEAVY METALS
FROM LIQUID WASTESTREAMS

by
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TABLE OF CONTENT

	Page
1. INTRODUCTION.....	1
2. LITERATURE SURVEY.....	4
3. OBJECTIVE.....	9
4. MECHANISM OF BIOSORPTION BY FREE CELLS.....	10
4.1 EXPERIMENTAL METHODS.....	10
4.1.1 Effect of Nutrient Addition	10
4.1.2 Cadmium Toxicity.....	11
4.1.3 Effect of Metabolic Inhibition.....	14
4.2 RESULTS AND DISCUSSION.....	15
4.2.1 Effect of Nutrient Addition.....	15
4.2.2 Cadmium Toxicity.....	17
4.2.3 Effect of Metabolic Inhibition.....	19
5. BIOSORPTION OF METALS ON IMMOBILIZED ACTIVATED SLUDGE	21
5.1 MATERIALS AND METHODS.....	21
5.1.1 Chemicals.....	21
5.1.2 Microorganisms.....	21
5.1.3 Immobilization of Microorganisms.....	23
5.1.4 Metals Analysis.....	24
5.1.5 Preliminary Uptake Experiments.....	24
5.2 RESULTS AND DISCUSSION.....	27
5.2.1 Time Course.....	27
5.2.2 Effect of pH.....	27
5.2.3 Effect of Temperature.....	31

5.2.4 Biosorption Isotherm.....	35
5.2.5 Effect of Biosorbent Concentration.....	37
5.3 Experimental Set-up	37
5.3.1 Experimental Procedure.....	40
5.3.2 Bench-scale Study.....	45
5.3.3 Clean-up Level.....	45
6. ANAEROBIC CONCENTRATION	47
6.1 MATERIALS AND METHODS.....	48
6.1.1 Acquisition of the Anaerobic Inoculum.....	48
6.1.2 Preparation of Metal Biobeads.....	50
6.1.3 Analytical Methods.....	50
6.1.4 Anaerobic Digestion.....	50
6.2 RESULTS AND DISCUSSION.....	53
7. SUMMARY OF RESULTS.....	58

LIST OF TABLES

Table	Page
1. Effect of Nutrient Addition and Incubation Time on Cd Uptake and Extracellular Protein Production With and Without Cd.....	16
2. Cadmium Toxicity to Activated Sludge.....	18
3. Effect of Metabolic Inhibition on Cd Uptake and Extracellular Protein Production With and Without Cd...	20
4. Composition of Nutrient Medium # 1.....	49
5. Effect of Cd and Pb on Anaerobic Digestion of Calcium Alginate Biobeads.....	55
6. Metal Concentration in Solution at the End of Anaerobic Digestion.....	57

LIST OF FIGURES

Figure	Page
1. Experimental Set-Up of Microassay Reactor.....	13
2. Rime Course of Cadmium Uptake at 30+/-0.5°C and pH 6.6 +/-0.1 from Initial Cd Concentration of 25 and 10 mg/l.....	28
3. The Solubility of Cd Ions as a Function of pH in Experimental Conditions, Temperature=30+/-0.5°C, Initial Cd Concentration=10 mg/l.....	29
4. Effect of pH on Cd Uptake at 30+/-0.5°C and Initial Cd Concentration=10 mg/l, Cd-Biobeads Contact Time =24 Hours, Dry Cell Conc.=25 mg/g of Wet Biobeads....	30
5. Effect of pH on Cd Uptake at 30+/-0.5°C, Initial Cd Conc. 10 ppm, Cd-Biobeads Contact Time=24 Hours, Dry Cells Conc.=25 mg/g.....	32
6. Effect of Temperature on Cd Uptake at pH 6.6. Initial Cd Concentration=10 ppm, Cd-Beads Contact Time=24 Hours.....	33
7. Effect of Temperature on Cd Uptake at pH 6.6. Initial Cd Conc.=10 mg/l, Cd-Biobeads Contact Time=24 Hours, Dry Cells Concentration=25 mg/g of Wet Biobeads.....	34
8. Isotherm of Cd Biosorption by Immobilized Cells at Constant Biomass Conc., pH 6.6, Initial Cd Conc. 5 to 150 ppm.....	36
9. Experimental Set-Up for the Biosorption Experiments with Immobilized Activated Sludge.....	38
10. Isotherm of Pb(II) Biosorption by Biobeads in Column Reactor Operated at 25-30°C.....	41
11. Isotherm of Cd(II) Biosorption by Biobeads in Column Reactor Operated at 25-30°C.....	42
12. Isotherm of Cu(II) Biosorption by Biobeads in Column Reactor Operated at 25-30°C.....	43
13. Isotherm of Cr(III) Biosorption by Biobeads in Column Reactor Operated at 25-30°C.....	44

14. Anaerobic Digestion..... 54

CHAPTER 1
INTRODUCTION

Industrial development throughout the world has resulted in increased circulation of heavy metals in the environment. Heavy metals are present at high concentrations in some industrial effluents and also appear as side pollutants at low concentrations in many wastes. The contamination of the environment by heavy metals is a very serious public health problem due to the toxicity of most of these pollutants even at low concentrations.

The removal of toxic and heavy metals contaminants from aqueous waste is one of the most important environmental issues facing the world today. Although this issue has been addressed for many years, effective treatment technologies are limited. Physico-chemical methods such as hydroxide or sulfide precipitation, ion-exchange, reverse osmosis, solvent extraction and membrane microfiltration are the most commonly used procedures for removing metal ions from industrial effluents (1).

However, these procedures have significant disadvantages such as incomplete metal removal, relatively high costs and/or generation of large volumes of toxic sludges which must be disposed off at additional costs.

The research for new and innovative treatment methods has focused attention on the metal binding capacities of

various microorganisms. The literature is extensive, and a few patents have also been filed (2-4).

It has been demonstrated that microorganisms can concentrate heavy metals through passive or metabolic mediated mechanisms. These mechanisms depend on the metals, the types of microorganisms, and the composition of the medium (5).

Most of the research studies on heavy metals uptake by biological cells have involve a single microbial species (Horikoshi et al. 1981; Highman et al. 1986; Scott et al. 1986; Nakajima and Sakaguchi 1986.). However, the use of activated sludge appears to be more convenient for field application since it is used widely in sewage treatment processes and can be used as an inexpensive source of microorganisms. Fewer studies have been done on mixed cultures or activated sludges. The use of different species is based on the assumption that some species may behave differently from others with respect to metal biosorption.

Although biological cells are effective sorbents for toxic and heavy metals, available processing systems are cumbersome. The free cells often require maintenance of a healthy microbial population which can be difficult because of the toxicity of the wastewater processed. Also recovery of the metal-laden microorganisms from solution is troublesome due to liquid-solid separation problems. Researchers have recognized that immobilizing microorganisms in various inert matrices may offer the microorganisms in-

creased chemical resistance to environmental factors such as changes in pH and chemical concentrations.

The purpose of this study is to demonstrate the potential of the immobilized activated sludges for removing heavy metal contaminants from aqueous solutions and to indicate factors that can impact the sorption, and to optimize the biosorption of metals in bench-scale bioreactors. This potential biosorbent (activated sludge) is available at very low costs and may be readily used without additional facilities which would be required to grow pure species or obtain microbial biomass from other sources.

CHAPTER 2
LITERATURE SURVEY

Bowen (9) has stated that contamination is the release of substances into an environment at measurable concentrations, while pollution implies that these substances have a measurable effect on living organisms. A very serious form of pollution is the addition of toxic heavy metal compounds, frequently only in trace quantities, to the aqueous environment. The metallic ions have tremendous binding capacity for many types of naturally occurring organic ligands. These binding actions, therefore, constitute an accumulation step in certain aquatic compartments. Consequently, a characteristic feature of heavy metals pollution is its concentration and persistence in some aquatic sediments for years after the polluting operations have ceased.

Probably the most important feature which distinguishes the heavy metals from other toxic pollutants is that they are not biodegradable and having entered the environment their potential toxicity is controlled to a great extent by biological and geochemical factors (10).

Iverson and Brinckman (11) suggested that reaction between microorganisms and metals may be considered from two interrelated aspects, namely, the effect of the metal ion on the microorganism and, secondly, the effect of the

microorganism on the metal ion. The uptake of heavy metals by microorganisms is known under the general term of biosorption. This phenomenon includes both passive adsorption of metallic cations at binding sites on cells' envelopes and metabolically-mediated uptake. These mechanisms depends on the metal, the type of microorganisms, and the composition of the medium.

It has been reported (6,7,11) that microorganisms have the ability to synthesize extracellular metal binding proteins and polysaccharides, and to release molecules (such as phosphates) that can precipitate metal ions in the form of insoluble complexes. In addition, metabolic processes such as energy-dependent transport of metal cations into the cell or biosynthesis of metal-binding intracellular polymers that serve as traps for the removal of metals from the solution have also been documented (12).

Bacteria have the capability to develop tolerance to initially toxic concentrations of heavy metal ions. Organisms having or developing natural tolerance to one metal may also be tolerant to one or more other metals.

A relatively small number of studies have been done on mixed cultures or activated sludge (12-16). All these studies deal with the behavior of metals during biotreatment of municipal or industrial wastewaters, or investigate the toxic effects of heavy metals on the efficiency of biological treatments.

No work has been reported on the design of a specific process using activated sludge as a biosorbent to remove heavy metals from solutions, which is the objective of this study.

Extracellular polymers of the flocks in activated sludge provide many functional groups that may act as binding sites for metal cations (12). A combination of flocculation and settling is considered the major mechanism of metal removal in activated sludge treatment plants (13) but the role of the metabolic activity of the sludge in this process is not totally elucidated. It is also thought that metabolic metal uptake into the cells occurs in activated sludge biomass (14).

Cadmium is found in the effluents of many industries and is one of the most toxic heavy metals. The major sources of cadmium released into the environment are electroplating, smelting, alloy manufacturing, mining and refining processes (18). Cadmium is also found in the effluents from the industries of pigment, fertilizer, pesticide and rubber manufacturing. Cadmium accumulates in living organisms and is even more toxic to marine life than mercury (19). Toxicity to man results in renal and lung pathologies, hypertension, bone lesions (itai-itai disease), and cancer. Maximum cadmium concentration in potable water is set at 0.01 mg/l in USA.

Lead is a highly toxic and cumulative poison and has been recognized as an industrial hazard for many years. Its

long-term effects as an environmental hazard have now become more important. The natural concentration of lead is less than 10 ppm in the earth's crust and less than 0.03 ppb in sea water (20).

Lead poisoning can cause severe mental retardation or death. It is now known that lead interferes with the blood-forming process, vitamin D metabolism, kidney function and the neurological process. Lead has been associated with hypertension in adults, and low levels of lead have been shown to cause mental retardation in children.

Lead is released into the environment from natural causes as well from human industrial activity. Both natural and industrial lead are dispersed widely by atmospheric transport of aerosols and reentrained dusts and smokes. Settle and Patterson have estimated that a billion pounds of lead enters the earth's atmosphere each year (21). The chief source of this lead contamination is lead alkyls used in gasoline. Natural sources of lead such as volcanic dust account for less than 1% of the total. Other industrial activity that adds lead to the environment are : iron, lead, zinc and copper smelting, and coal burning. The land spreading of municipal sewage sludge as a fertilizer further adds to the environmental lead burden.

Chromium is commonly contained in a variety of industrial wastewaters, including those from the textile, leather tanning, electroplating, and metal finishing industry. Those effluents from the textile and leather tanning

industries may contain either hexavalent, Cr(VI), or trivalent, Cr(III), forms of chromium, depending upon the process used; however, electroplating and metal finishing effluents contain primarily Cr(VI) (22,23).

The toxicity of Cr(VI) is well documented and it is considered a health hazard to man and animals. Hexavalent chromium is corrosive to the flesh and is also carcinogenic(22). The presence of Cr(VI) in the aquatic environment at high concentration is lethal to marine species. The toxicity of Cr(III) is reportedly much less than that of Cr(VI); however, this is probably due to its relatively insolubility compared to Cr(VI) (24).

CHAPTER III

OBJECTIVES

The objectives of this research are:

1. to determine the biosorption mechanism of metal ions Cd (II), Pb(II), Cr(III) and Cu(II) by immobilized activated sludge,
2. to evaluate the biosorption parameters by varying pH, temperature, metal concentration and biomass concentration for a selected metal (Cd^{2+}),
3. to optimize the biosorption of a selected metal (Pb^{2+}) in a bench-scale bioreactor which exhibits the highest affinity to the biosorbent, and
4. to evaluate the feasibility of concentrating the metal ion in the biosorbent after the biosorption phase, by anaerobic digestion of the organic mass (biomass and alginate matrix).

CHAPTER 4

MECHANISM OF BIOSORPTION BY FREE CELLS

4.1 EXPERIMENTAL METHODS

4.1.1 Effect of Nutrient Addition

All runs were carried out at a dry biomass concentration of 200 mg/l and pH 6.6 in order to avoid chemical precipitation of insoluble cadmium compounds. Initial cadmium concentration was 10 ppm except when otherwise indicated. Each experiment was carried out in 250 ml flasks with reaction volume of 100 ml. The medium consisted of 0.5% (w/v) NaCl solution to which the biosorbent and the metal cations were added.

The 2g/l biomass suspension was used as the source of biosorbent. Ten-ml cell suspensions were used to get a biosorbent concentration of 200 ppm. The cell suspension concentration was checked each time by measuring the dry matter content of 20 ml of cell suspension.

One thousand ppm metallic solutions were used as the source of metal cations. The pH was adjusted with 0.01 N NaOH or 0.01 N HCl.

A series of blanks were initially used without biosorbent in order to determine the pH values above which chemical precipitation (mostly hydroxides) occurred for each metal. All experiments were done at pH values below these

limits. The temperature was set at $30 \pm 0.5^{\circ}\text{C}$. The biomass was equilibrated for 15 min at this temperature before the addition of metal ion at time zero.

When desired, nutrients were added as a solution of glucose 1,000 mg/l; $(\text{NH}_4)_2 \text{SO}_4$ 94.6 mg/l; KH_2PO_4 17.6 mg/l; yeast extract 1.0 mg/l; and NaCl 5000 mg/l. Final C, N, S and P concentration in the reaction mixture were respectively 4.00 mg/l, 0.20 mg/l, 0.23 mg/l and 0.04 mg/l.

The flasks were placed in a shaker where they were agitated at 130-150 rpm at $30 \pm 0.5^{\circ}\text{C}$. After 2 or 24 hours, pH was measured in each flask and the suspension filtered on syringe filters with 0.45- μm pore size cellulose acetate Millipore membranes. Cadmium and protein concentrations were immediately measured in the filtrates.

Protein concentration was measured by the Lowry procedure. The aim of these analyses was to provide information related to the physiological state and metabolic activity of the biomass and investigate a possible correlation between cadmium uptake and extra cellular protein production. The 24 hour incubation period was deemed long enough to allow any biological process to occur without significant cell autolysis.

4.1.2 Cadmium Toxicity

The toxicity of Cd^{2+} to the microorganisms in the sludge was studied by measuring inhibition of endogenous respiration and extracellular protein production due to exposure

to Cd^{2+} . The experimental set-up for the determination of respiratory activity is a microassay reactor, which is shown in Figure 1. The microassay reactor is a small jacketed vessel of 1.8 ml capacity, with provision for a Clark-type dissolved oxygen probe. Water at 30°C was circulated through the jacket and the reactor was mounted on a magnetic stirrer plate.

Before the start of each run, the reactor was sterilized in an autoclave at 120°C and then washed successively with methanol and distilled water. 1.18 ml of CdCl_2 solution (at the desired cadmium concentration between 0 and 500 ppm) in 0.5% w/v NaCl was added and saturated with oxygen by bubbling air through it. The saturation concentration of oxygen from air in water at 30°C is about 242 nmoles/ml.

When the saturation level was reached aeration was stopped and 20 μm of 0.01 NaOH was added to get a final pH of 6.6 ± 0.1 in the final volume of 1.8 ml. Then 0.6 ml of 2000 ppm cell suspension was added. The reactor was plugged in and the decrease in oxygen concentration due to endogenic respiration was recorded. A linear response was obtained between 90% and 60% of oxygen saturation and the rate of oxygen consumption could therefore be easily calculated in that range. Each assay was replicated 2 to 4 times.

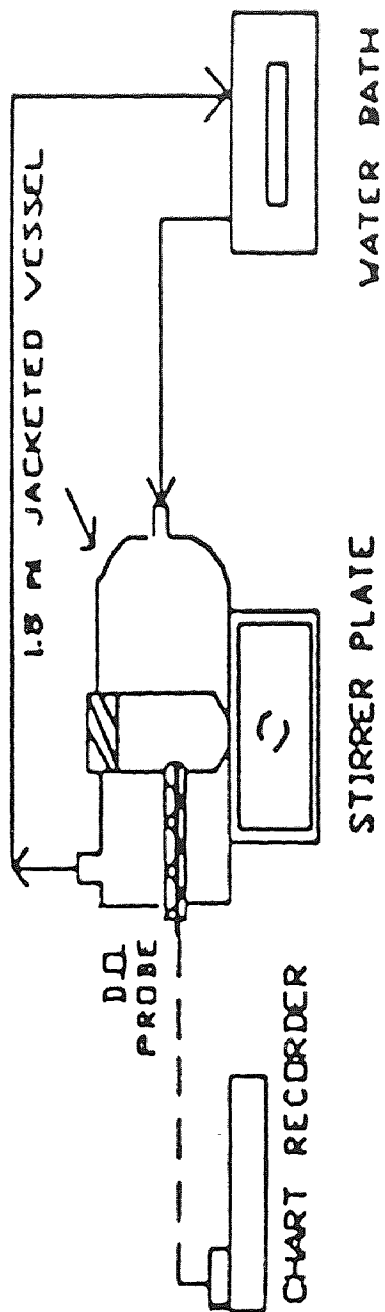


FIG. 1. EXPERIMENTAL SETUP OF MICROASSAY REACTOR

4.1.3 Effect of Metabolic Inhibition

Inhibition by UV rays was carried out by exposing the 2000 ppm biomass suspension to UV (240 nm and 20 W) for 20 minutes. The liquid layer of suspension was approximately 1 mm and the suspension was agitated periodically.

Inhibition by sodium azide (NaN_3) was performed by suspending the 2000 ppm biomass suspension in a 10^{-3} or 10^{-1} NaN_3 solution and mixing at room temperature on a magnetic stirrer plate for 30 or 45 minutes. Then the biomass was washed and resuspended in a volume of 0.5% NaCl solution to get a biomass concentration of 2000 ppm. The 2000 ppm suspension of inhibited cells was used to test cadmium uptake. The respiratory activity was measured at the same time.

4.2 RESULTS AND DISCUSSION

4.2.1 Effect of Nutrient Addition

Table 1. shows that the addition of nutrients had no effect on cadmium uptake during two hours of incubation, indicating that the lack of nutrients was not the cause of the low level of metabolic uptake.

After 24 hours, uptake remained unchanged when nutrients were not supplied, whereas a slight increase was observed in the presence of nutrients, suggesting that some low rate processes of metabolic uptake occurred.

These processes were probably not mediated by extra cellular protein production since the protein concentration in the medium after 24 hours of incubation with nutrients was less than that after 2 hours. Some other metabolic processes may therefore have occurred.

The results also show that the presence of nutrients in the medium induced a significant reduction of extracellular protein production. This suggests that a large part of these proteins might consist of hydrolytic enzymes such as cellulose or amylases that were released in the medium by chemo-organotrophic bacteria seeking carbon to support their metabolism. The presence of glucose may have partially inhibited the biosynthesis of those enzymes through a product-inhibition mechanism, resulting in the decrease in protein production actually observed.

Table 1. Effect of Nutrient Addition and Incubation Time on Cadmium Uptake and Extracellular Protein Production With and Without Cadmium

Initial Cd ²⁺ conc. (ppm)	Cadmium uptake*				Protein production**			
	no nutrients		nutrients		no nutrients		nutrients	
	2h	24h	2h	24h	2h	24h	2h	24h
0	-	-	-	-	125	0	75	0
10	13.5	13.5	13.5	14.5	110	135	65	25

* As ug cadmium per mg dry biomass in 2 hours at 40⁰C and pH 6.6+/-0.1

** As ug protein per mg dry biomass in 2 hours at 40⁰C and pH 6.0+/-0.1

Since biosorption in 2 hours was the same with and without nutrients, it was concluded that these proteins were not involved in cadmium uptake.

4.2.2 Cadmium Toxicity

Table 2. shows that both respiration and extracellular protein production were inhibited by cadmium even at the lowest concentration tested. The decrease in protein production could have reflected co-precipitation with corresponding amounts of cadmium but this assumption was invalidated by the other experimental data which showed no correlation between cadmium uptake and protein production. Respiratory activity was reduced by 65% when cadmium concentration was raised from 0 to 50 mg/l. When cadmium concentration was increased ten times from 50 to 500 mg/l, the inhibition level only slightly increased up to 75%, suggesting that some of the microorganisms in the sludge were resistant to this high concentration, or that the cadmium sensitive species were not totally inhibited.

These results suggest that cadmium uptake by the sludge occurred primarily through a passive mechanism. However, the occurrence of metabolically mediated uptake could not be totally rejected, for the inhibition by cadmium indicated that part of the metal entered the cell possibly through a mechanism of energy dependent transport.

Table 2. Cadmium Toxicity to Activated Sludge

Initial Cd ²⁺ concentration (ppm)	% Inhibition endogenous respiration*	% Inhibition of protein production**
0	0	0
10	20	11
25	nm	24
50	65	35
100	70	40
500	75	nm

* in the range of 60 to 90% oxygen saturation

** 2 hours incubation

Experimental Conditions: Temperature, 30+/-0.5⁰C; pH 6.6+/-0.1; no nutrients added. All assays were repeated at least three times. Data displayed in this table are average values. The intervals of confidence are indicated in brackets. nm = not measured.

It has been reported that metal-sensitive microorganisms have a better metal uptake capacity than metal resistant strains of the same species, suggesting that intracellular uptake of metal is the very cause of the toxicity (25).

4.2.3 Effect of Metabolic Inhibition

Table 3. shows that metabolic inhibition of the microorganisms either by sodium azide or by UV rays has a strong effect on respiratory activity and extracellular protein production.

However, the decrease in cadmium uptake after metabolic inhibition indicates that 5 to 10% of overall uptake is metabolically mediated.

It should be noted that inhibitors strongly decrease the protein production. This observation indicates that this production is mainly the result of metabolic activity. Inhibitors would not have decreased protein production if it had been due to autolysis of the biomass.

Table 3. Effect of Metabolic Inhibition on Cadmium Uptake and Extracellular Protein Production With and Without Cadmium

Treatment	% activity*	Cd uptake**	Protein production***	
			no Cd	10 ppm Cd
untreated	100	13.5	125	110
NaN ₃ 10 ⁻³ M, 45 min	65	12.5	45	25
NaN ₃ 10 ⁻¹ M, 30 min	55	12.5	65	20
NaN ₃ 10 ⁻¹ M, 45 min	20	12.5	30	30
UV 240 nm, 20W, 20 min	30	12.5	20	0

* as measured by the rate of endogenous oxygen consumption

** as ug cadmium per mg dry biomass in 2 hours.

at 40+/-0.5°C and pH 6.6+/- 0.1.

*** as protein per mg dry biomass in 2 hours at 40 +/- 0.5°C and pH 6.6+/-0.1.

No nutrients added.

CHAPTER 5

BIOSORPTION OF METALS ON IMMOBILIZED ACTIVATED SLUDGE

5.1 MATERIALS AND METHODS

5.1.1 Chemicals

All chemicals used were analytical grade reagents. Stock solutions of each metal at 1,000 ppm were prepared by dissolving respectively CdCl_2 , $\text{Pb}(\text{NO}_3)_2$, $\text{Cr}(\text{NO}_3)_3$, and CuCl_2 in NaCl 0.5% w/v. The pH was adjusted using a 0.01N HNO_3 or 0.1N NaOH in 0.5% w/v NaCl. These standard solutions were replaced every month. Bacterial beads were prepared by using sodium alginate of practical grade, type 1V, extracted from *Macrocystis pyrifera* (Kelp) and commercialized by Sigma Chemical Company.

5.1.2 Microorganisms

Activated sludge (a mixed microbial population) from the Parsippany-Troy Hills Water Pollution Control Plant, New Jersey, was used in this study. The sludge was sieved through a 500 μm opening screen to remove inert gross particles and kept for few hours at room temperature with good aeration. Then it was allowed to settle for 10-15 minutes and the supernatant was replaced by tap water. The sludge was then mixed, settled, and washed again with tap water. This step was repeated twice with distilled water. After

this pre-treatment the pellets were prepared by centrifugation of the sludge at 3,000 rpm at 4⁰C for 5 minutes (IEC model PR-2 Centrifuge).

Dry biomass content (dry weight of cells) of the pellets was determined by drying five sample of pellets in the oven at 105⁰C for 24 hours. The dry biomass concentration gave an average value of 58.2+/-0.2 mg/g wet pellets.

Pellets were frozen and stored at -20⁰C in 10 ml plastic tubes to prevent biological evolution over the duration of the study. Dried skim milk was used as a protective agent on the basis of 25 grams of wet pellets suspended in 100 ml of 12.5% (w/v) skim milk solution. Dry cell concentration in this suspension was measured by washing a known amount of suspension as detailed below and weighing the cells after drying at 105⁰C for 24 hours.

Before starting any biosorption experiment, the milk was washed from the suspension as follows:

The frozen cell suspension was quickly thawed by placing the tube(s) in water at room temperature, and an adequate amount of suspension was weighed to get the desired weight of the cell given the ratio dry cell weight/total weight of suspension which was previously determined.

The homogeneous suspension was then diluted approximately 15 times with 0.5% (w/v) NaCl solution at 4⁰C and centrifuged at 3,000 rpm for 5 minutes. Supernatant was removed and the pellets were resuspended in 50 ml of 0.5% NaCl solution at 4⁰C. Four successive centrifugations were

then carried out in the same way. The use of NaCl solution in preparation of cell suspension step as well as in the uptake experiments was meant to prevent cell damage due to osmotic pressure.

5.1.3 Immobilization of Microorganisms by Entrapment in Calcium Alginate Gel

For a given batch of 25 g pellets, a typical procedure for making beads was as follows. Wet bacterial pellets and 0.5% NaCl solution were taken in the ratio of 2:5 by weight in a blender. Sodium alginate (0.75% w/w) was then added slowly to the mixture with continuous stirring over a period of 2 to 3 minutes to obtain a homogeneous cell suspension. With the help of a syringe pump (Sage Instruments, Model 351) the homogeneous cell suspension was then extruded as discrete droplets in a slowly stirred solution of 0.1 M calcium chloride. On contact with calcium chloride, the droplets hardened to form beads about 3 to 3.5 mm in diameter. The beads (hereafter referred to as biobeads) were then cured in 0.1 calcium chloride solution for 24 hours at 4⁰C before use. In the present study, the average dry matter content of the biobeads was around 60 mg per gram of wet beads, corresponding to 25 to 30 mg of sludge solids and 30 to 35 mg of calcium alginate and sodium chloride per gram of wet beads.

Control beads were prepared with the same procedure by replacing the pellets by an equal volume water.

The average dry matter content of the control beads was 30 to 35 mg/ gram wet beads.

5.1.4 Metals Analysis

Metal concentrations were determined by atomic absorption with a Perkin-Elmer spectrophotometer, Model 305 B and Model 2380. The operating conditions were as recommended in the Perkin-Elmer operation manual. All analyses were done in the linear range of concentration.

5.1.5 Preliminary Uptake Experiments

Preliminary studies were done only for the biosorption of cadmium. Based on the results obtained earlier at the NJIT Biotechnology Laboratory with suspended sludges (26), it was assumed that the kinetics of biosorption observed for cadmium should be essentially representative of the kinetics of other metals.

Runs were carried out in 50 ml shaker flasks with 25 ml of medium, except those on the time course of cadmium uptake (see below). The medium consisted of a 0.5% w/v NaCl solution (pre-equilibrated at the desired temperature) in which the bacterial beads and, after 15 min for equilibration, Cd^{2+} ions were added. Bacterial beads were used on the basis of 200 mg (wet weight) in 25 ml medium, in order to get a 200 mg/L dry cells concentration. For each series of runs performed a series was carried out with control beads under the same conditions. The weight of the control

beads in the control flasks was the same as that of calcium alginate matrix contained in the bacterial beads of the corresponding test flasks. A series of blanks was also carried out without any beads. Bacterial and control beads were liberally washed with tap water and distilled water to remove all traces of calcium chloride and then left to drip on filter paper for a few minutes. Initial Cd^{2+} concentration was 10 mg/L in all runs except otherwise indicated. All the data presented were obtained at pH 6.6 \pm 0.1 in order to avoid chemical precipitation of cadmium hydroxide or carbonate. It was not easy to adjust the pH with good accuracy because no buffer solution was used. While it was found in preliminary runs that pH had a very high effect on cadmium biosorption, it was decided to bracket the desired pH by testing from 5 to 6 different pH levels between 6.4 and 7.0, and subsequently to determine cadmium uptake at pH 6.6 by interpolation. This procedure increased considerably the number of runs performed to achieve this study, but rendered the experimental results more reliable. The shaker flasks were placed in an incubator where they were permanently stirred at the desired temperature. The incubation times necessary to reach equilibrium were determined in a preliminary run and found to be 24 hours. After incubation, pH was measured in each flask. The liquid phase was then analyzed for Cd^{2+} concentration.

Time course of cadmium uptake was studied at 30 \pm 0.5 $^{\circ}$ C and pH 6.6 \pm 0.1 in 250 ml shaker flasks containing

100 ml of medium prepared as described above. The beads were equilibrated for 15 minutes at this temperature before cadmium addition at time 0. Then, 1 ml samples were taken after 0.5, 1, 2, 3, 5, 7, 24 and 48 hours of incubation and immediately analyzed for Cd^{2+} concentration. pH was measured at the end of the runs.

5.2 RESULTS AND DISCUSSION

5.2.1 Time Course

Figure 2. shows the course of cadmium uptake by bacterial beads. It can be seen that biosorption took approximately 15 hours to reach a plateau with a low slope. The observed rate of Cd^{++} uptake by alginate bacterial beads may also be limited by the diffusion of Cd^{++} ions through the gel matrix. It was reported by Tanaka et al. (27) that the diffusion coefficients of substrates with molecular weights less than 20,000 in calcium alginate beads were the same as the diffusion coefficients of the substrate in water. Therefore in the present case, the diffusion of Cd^{++} ions in the gel matrix should not limit the global rate of biosorption providing that Tanaka's results are also valid for ionic compounds.

5.2.2 Effect of pH

Experimental results are displayed in Figure 4. The dashed line in Figure 3. (blank) shows the solubility of Cd^{2+} ions as a function of pH in the experimental conditions mentioned in the legend. It can be seen that the chemical precipitation of Cd^{++} ions, most probably in the form of $\text{Cd}(\text{OH})_2$ or CdCO_3 , did not significantly occur below pH 7.0 in the present conditions. However, it was observed with higher Cd^{++} initial concentration or lower temperature that chemical precipitation occurs below pH 7.

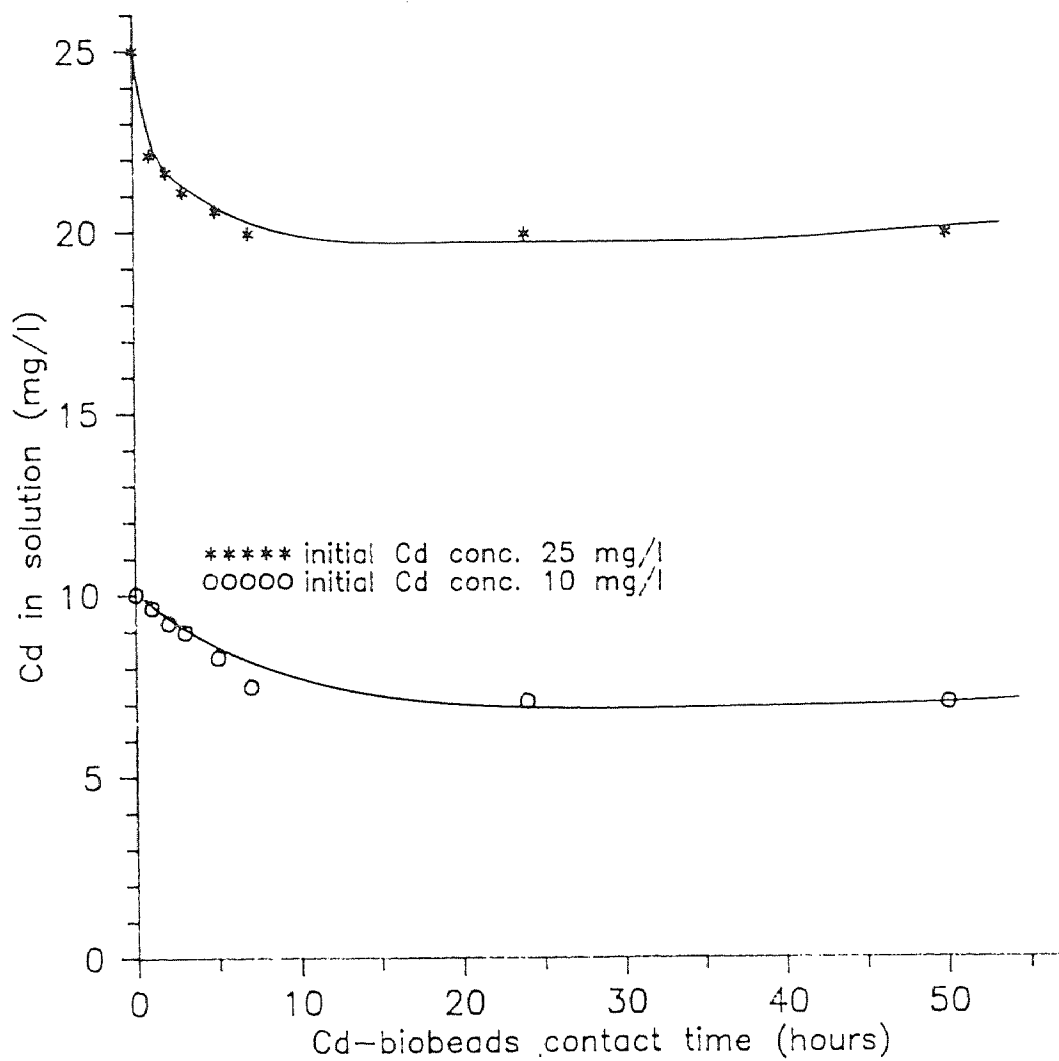


Figure 2. Time course of cadmium uptake at $30 \pm 0.5^{\circ}\text{C}$ and $\text{pH } 6.6 \pm 0.1$ from initial Cd concentration of 25 and 10 mg/l

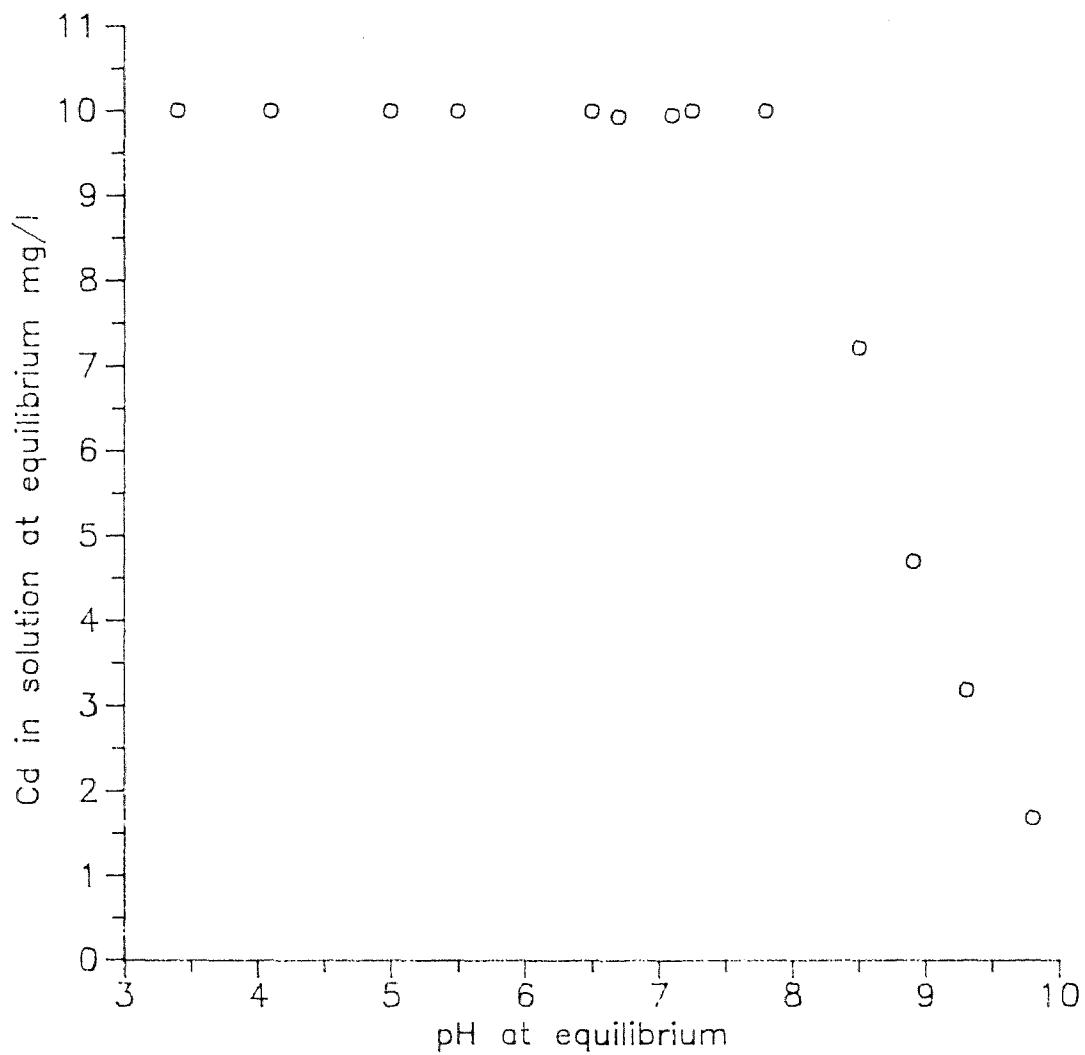


Fig.3. The solubility of Cd ions as a function of pH in experimental conditions, temperature = $30 \pm 0.5^\circ\text{C}$, initial Cd conc. = 10mg/l

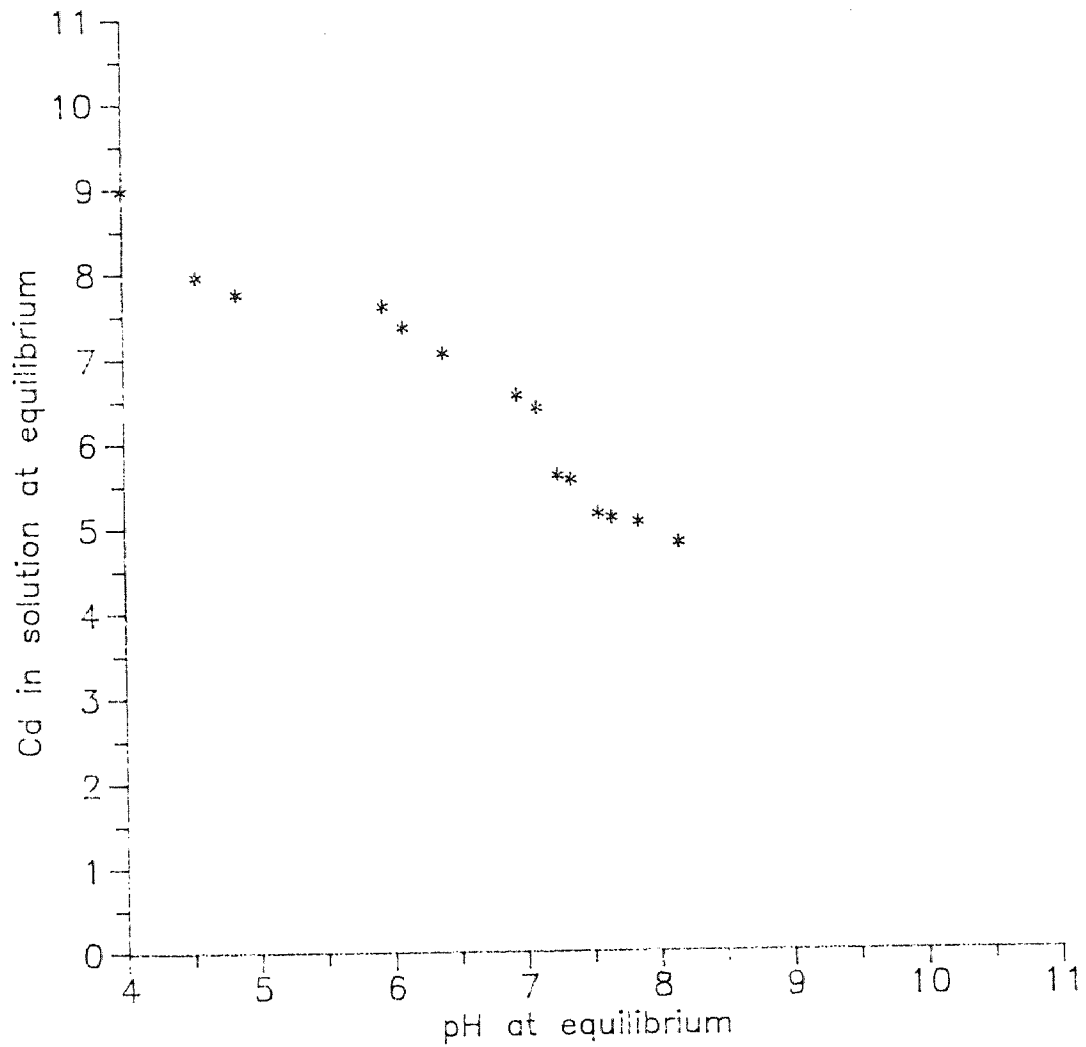


Fig.4. Effect of pH on Cd uptake at $30 \pm 1^\circ\text{C}$ and initial Cd concentration = 10 mg/l, Cd-biobeads contact time = 24 hours, dry cell conc. = 25 mg/g of wet biobeads

This is why a pH at 6.6 was taken throughout the study as the reference pH below which no chemical precipitation was ever observed. The curve in Figure 5., representing the amount of cadmium taken up per unit weight of beads vs. pH, was drawn from the difference between the ordinates of the solid line in Figure 4. (biosorption by bacterial beads) and those of the blank in Figure 3.

5.2.3 Effect of Temperature

The adsorption of solutes at liquid solid interfaces is generally an exothermic process (28) which therefore is favored by decreasing the temperature. This was actually observed when using control alginate beads (Fig.6) with which uptake was totally passive since no microorganisms were present. On the other hand, it was found that temperature had no significant effect on biosorption by bacterial beads, suggesting that metabolically-mediated biosorption increased with temperature to the same extent as passive adsorption decreased (Fig.6). The increase in metabolically-mediated uptake by bacterial beads is illustrated by the curve in Fig.7 which shows the amount of cadmium taken up by microorganisms in the beads as a function of temperature, expressed as ug cadmium per mg dry cells.

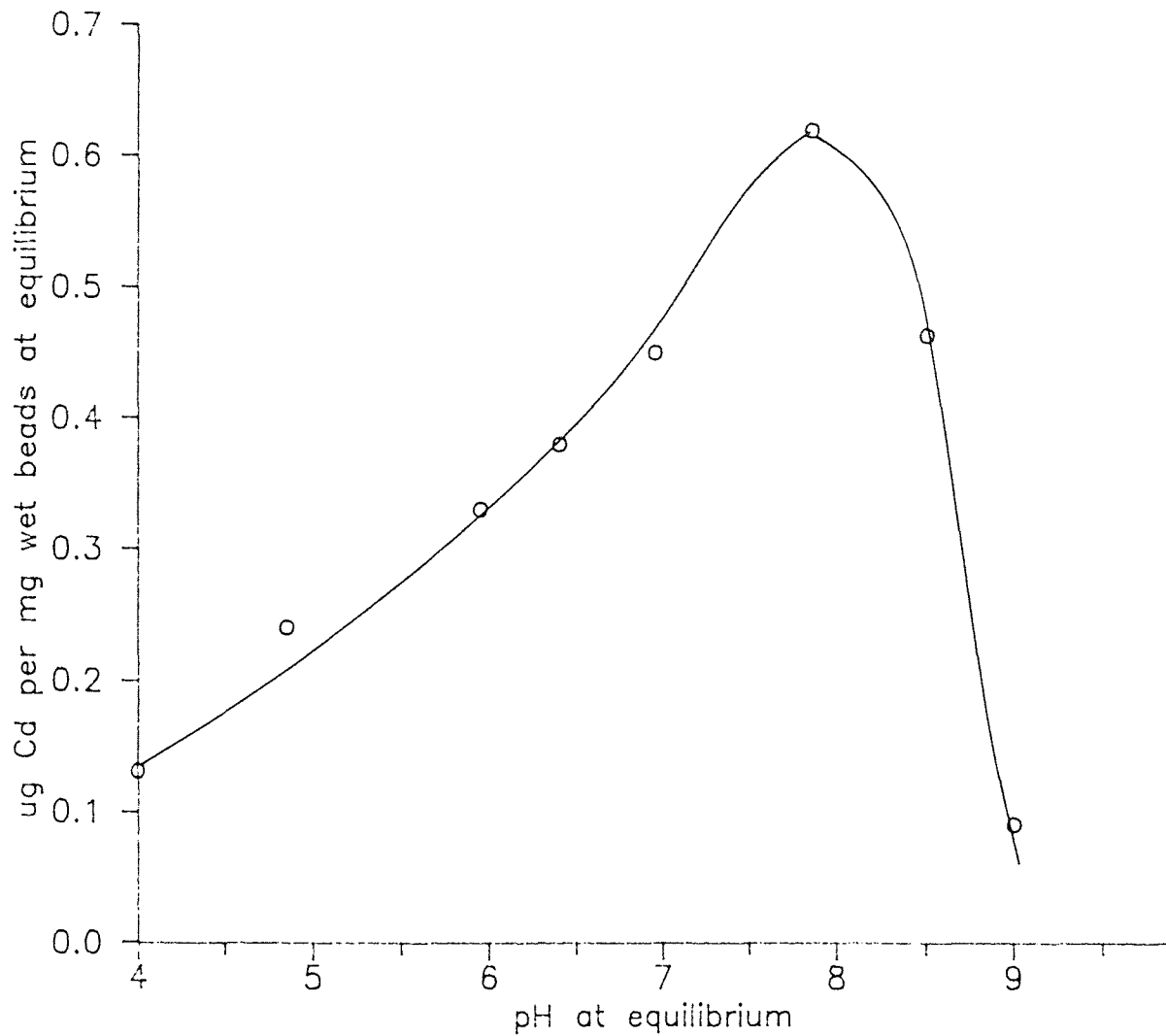


Fig.5. Effect of pH on Cd uptake at $30 \pm 0.5^\circ\text{C}$
 $30 \pm 0.5^\circ\text{C}$, initial Cd conc. 10 ppm, Cd-biobeads
contact time 24 hs., dry cells conc. = 25mg/g bead

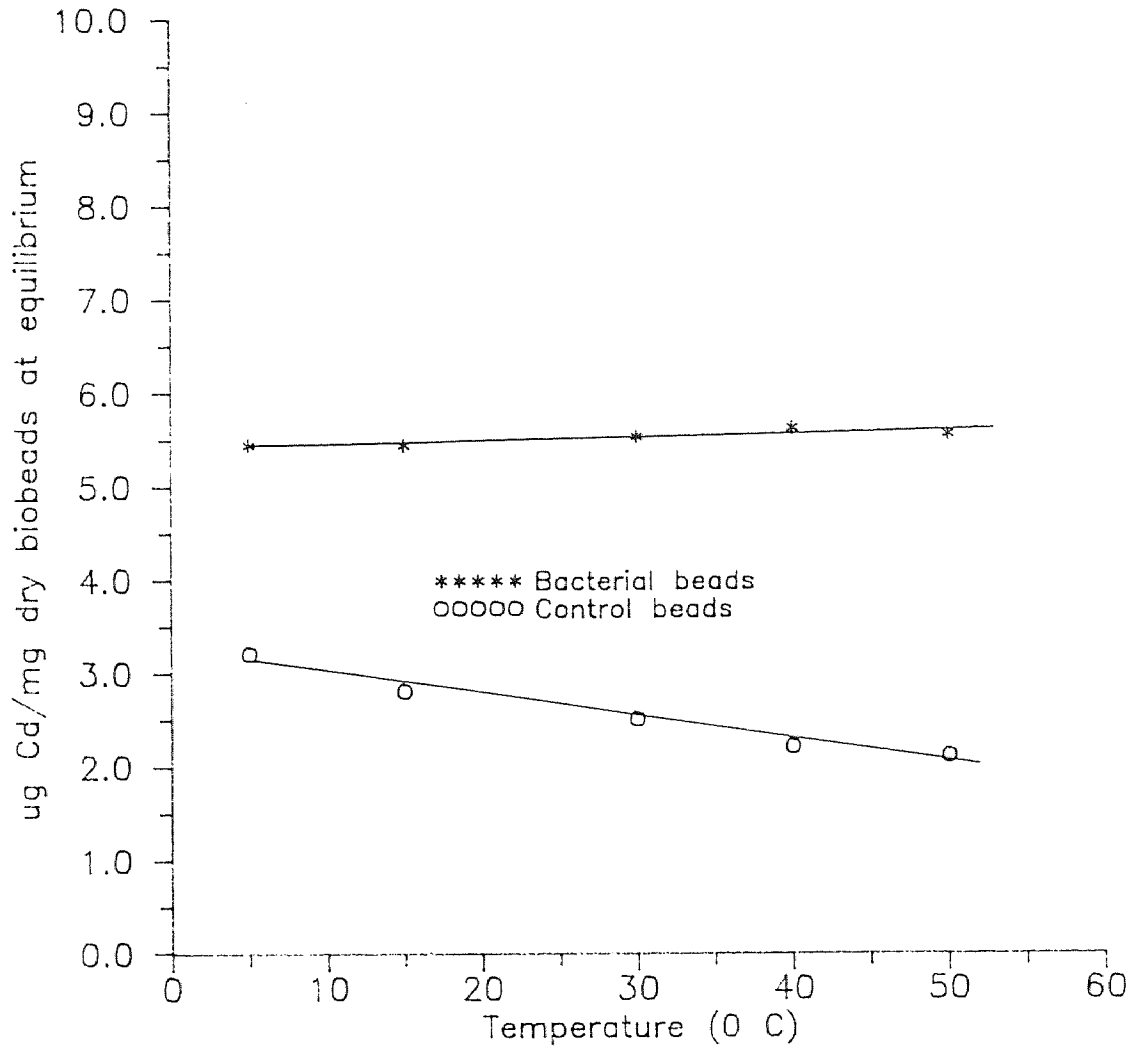


Figure 6. Effect of temperature on Cd uptake at pH 6.6. Initial Cd concentration = 10 mg/l, Cd-beads contact time = 24 hrs

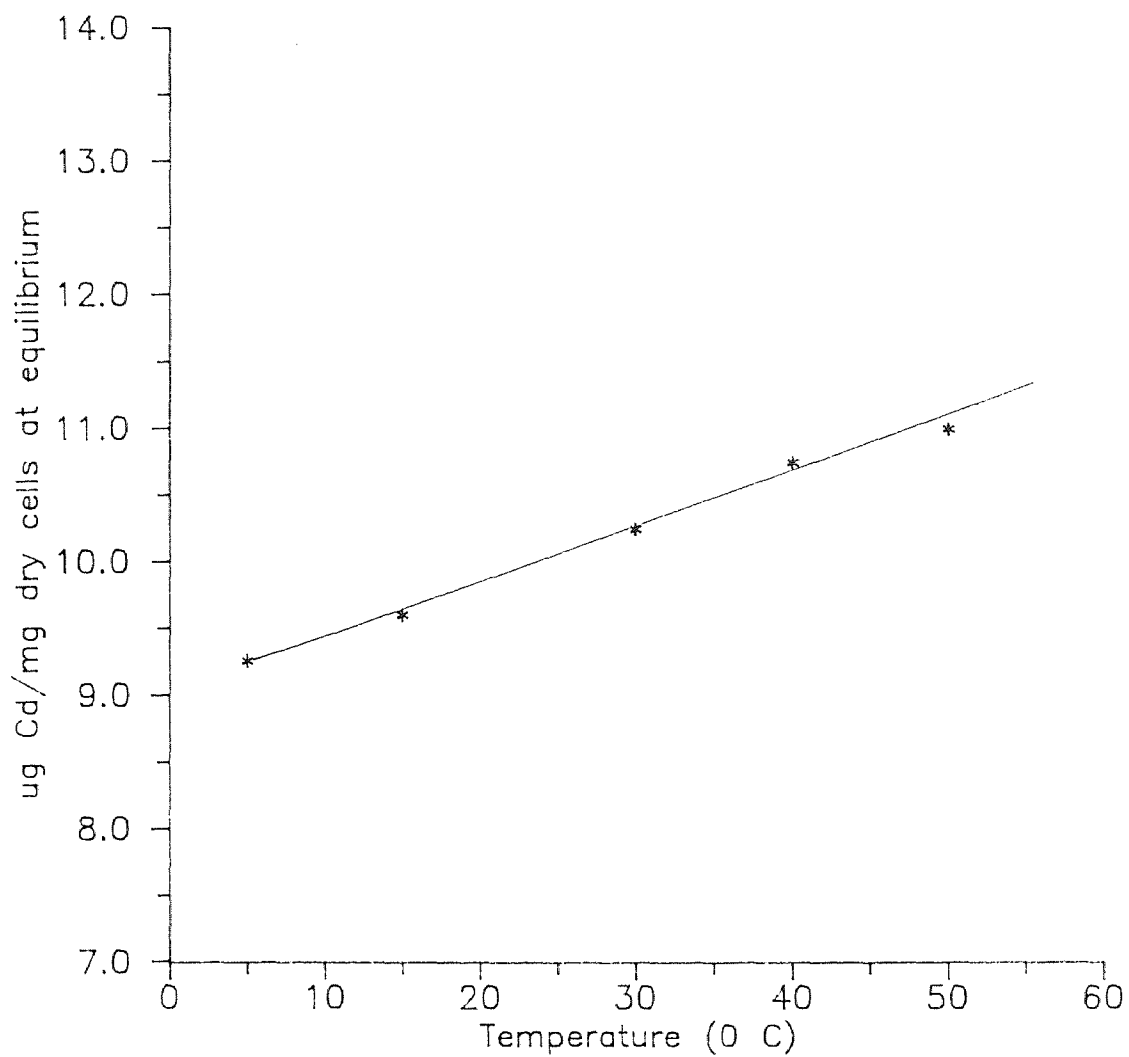


Fig.7. Effect of temperature on Cd uptake at pH 6.6. Initial Cd concentration = 10mg/l. Cd-biobeads contact time = 24 hours, dry cells concentration = 25 mg/g wet beads

5.2.4 Biosorption Isotherms

Biosorption isotherms were determined by investigating the effect of Cd^{2+} concentration in the solution on the amount of cadmium taken up by bacterial beads at equilibrium. To achieve this study, uptake experiments were carried out at various Cd^{2+} initial concentrations and constant temperature, pH, and bacterial beads dry concentration. The residual Cd^{2+} concentrations (C_e , in mg/l) were measured in the solution at equilibrium and the amounts of cadmium taken up per unit weight of dry beads (Q_e , in mg/g) were easily calculated by considering that cadmium was exclusively transferred from the liquid phase to the adsorbed solid phase.

This point was controlled by blank runs which showed that in the absence of bacterial beads, Cd^{2+} concentration in the solution remained unchanged in the present conditions ($30 \pm 0.5^\circ\text{C}$ and pH 6.6) whatever the initial cadmium concentration was.

Fig.8 shows that a linear relationship was observed between the logarithm of the amount of cadmium adsorbed per unit weight of biosorbent and the logarithm of the residual cadmium concentration. This result indicates that cadmium uptake followed the Freundlich Isotherm:

$$Q_e = kC_e^n, \text{ in which } k \text{ and } n \text{ are constants (with } n < 1).$$

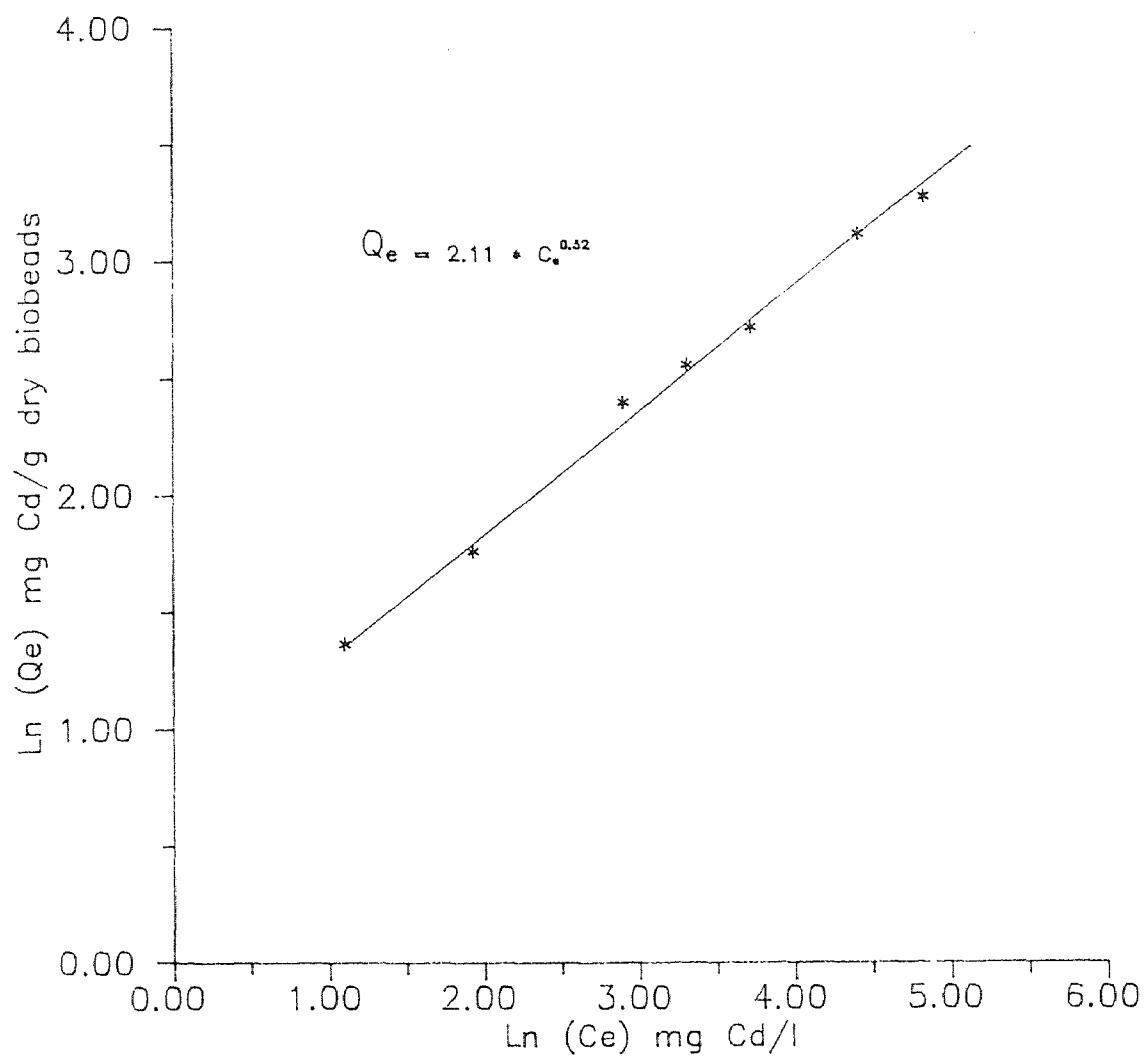


Fig.8. Isotherm of Cd biosorption by immobilized cells at constant biomass conc., pH=6.6, Initial Cd conc. 5 to 150 ppm

5.2.5 Effect of Biosorbent Concentration

The Freundlich parameters given in Fig.8 are exclusively valid for the experimental conditions of pH and temperature, and in the range of residual Cd^{++} concentrations investigated (namely 35-135 mg/l).

The interval of confidence on the calculated values may be estimated at

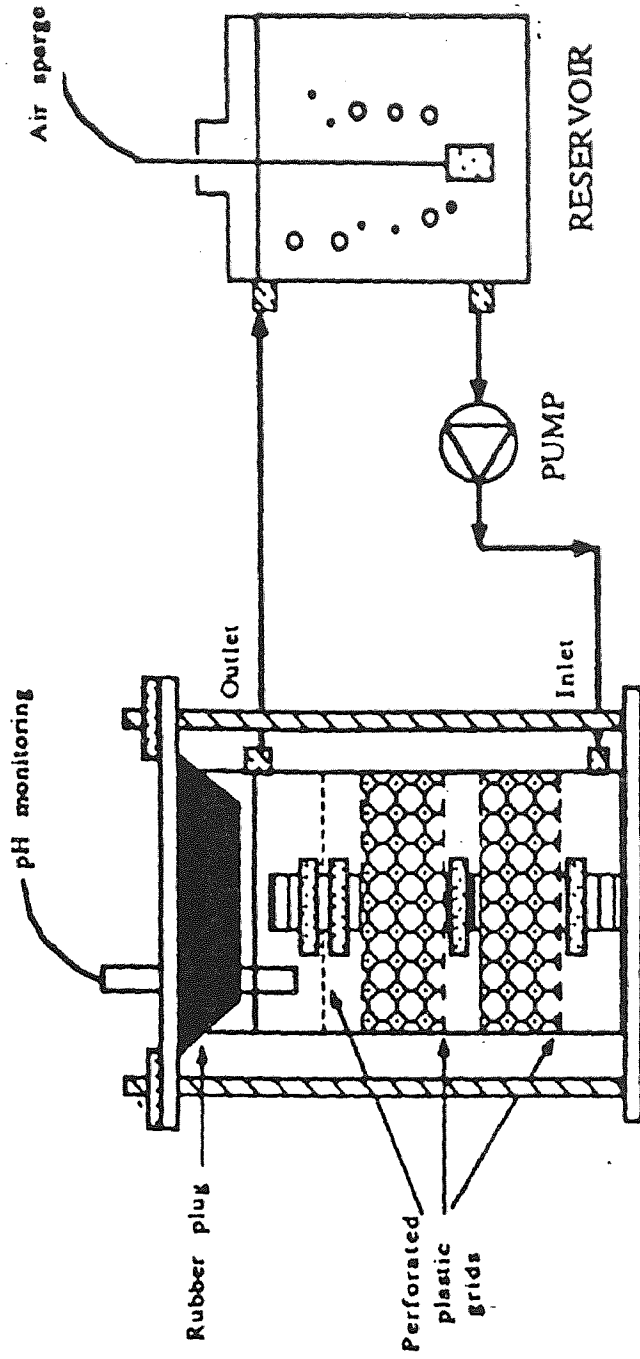
+/-10% by maximizing the experimental errors. It is clear that pH and temperature will affect the parameters of biosorption. However, beads concentration should not affect them. This latter point is critical if the parameters are to be used to design a bioreactor.

5.3 Experimental Set-up of the Calcium Alginate Biobead Reactor

The experimental set-up of the recirculation reactor (batch mode) is shown in Fig.9 Three identical bench-scale reactors were constructed. Each system consisted of a packed column, a reservoir in which air was continuously sparged, a pH monitoring device, and a pump which recirculated the solution through the column at a constant flow rate of 150 ml/min.

The reactor is 5 cm in diameter and 20 cm long. The reservoir is 6 cm in diameter and 10 cm long. The total reaction volume is 1.1 liters.

The biobeads or control beads were packed in the columns between three equally distant perforated plastic



**COLUMN REACTOR PACKED
WITH BIOBEADS OR CONTROL BEADS**

Fig. 9. Experimental set-up for the biosorption experiments with immobilized activated sludges

grids which maintained the beads in place. The volume of packed bed in each column was approximately 105 ml with a porosity of 0.28 to 0.35, corresponding to 70 grams of wet beads (i.e. about 4.2 g of dry biobeads or 2.2 g of dry control beads).

Those parameters were determined accurately for each experiment. The reaction medium was circulated between the reservoir and the reactor using a peristaltic pump. The linear velocity was maintained high enough to overcome the solid liquid film resistance to mass transfer by recirculating the stream at 150 ml/min. Also, a good recirculation rate was required to operate under a regime not limited by solid/liquid mass transfer resistance of the rate limiting compound, and also to maintain physical integrity of the beads.

In batch recirculation mode the parameter studied was concentration of metal cations. For each metal the pH was adjusted with 0.01N HNO₃ or 0.01 HCl and all experiments were done at pH values below limits determined in the blanks experiments. The pH ranges were as indicated below:

Cd:	pH 4.0 to 6.6
Pb:	pH 3.5 to 6.0
Cu:	pH 4.0 to 5.5
Cr:	pH 4.0 to 4.5

Temperature and pH were monitored inside the reactor. All experiments were done at room temperature (25-30⁰C).

5.3.1 Experimental Procedure

The biosorption of each metal cation on biobeads was studied at various pH levels below the limits of hydroxide precipitation determined earlier in the study.

The reactors were continuously operated over at least a week for each experiment, during which time pH was periodically adjusted to the desired value with 6N HCl or HNO₃ solutions.

The experimental reactor was initially filled (up to a reaction volume of 500 ml) with 0.5% w/v NaCl solution and the medium was recirculated for an hour. Then, the pH was adjusted. After 10 minutes, 5 or 12.5 ml of medium were removed from the reservoir and replaced at time zero by the same volumes of a 1000 ppm Cd⁺⁺, Pb⁺⁺, Cr³⁺ or Cu⁺⁺ solutions. Therefore initial metal concentrations were 10 or 25 ppm.

The solutions were spiked every 24 h with given amounts of metal cations. Samples were taken just before metal addition, and analyzed for metal concentration in solution.

At the end of each 24-h cycle, the mass Q_e of metal biosorbed per gram of dry biosorbent was calculated. Biosorption isotherms were obtained by plotting Q_e vs. C_e . (Fig.10, Fig.11, Fig.12, and Fig.13)

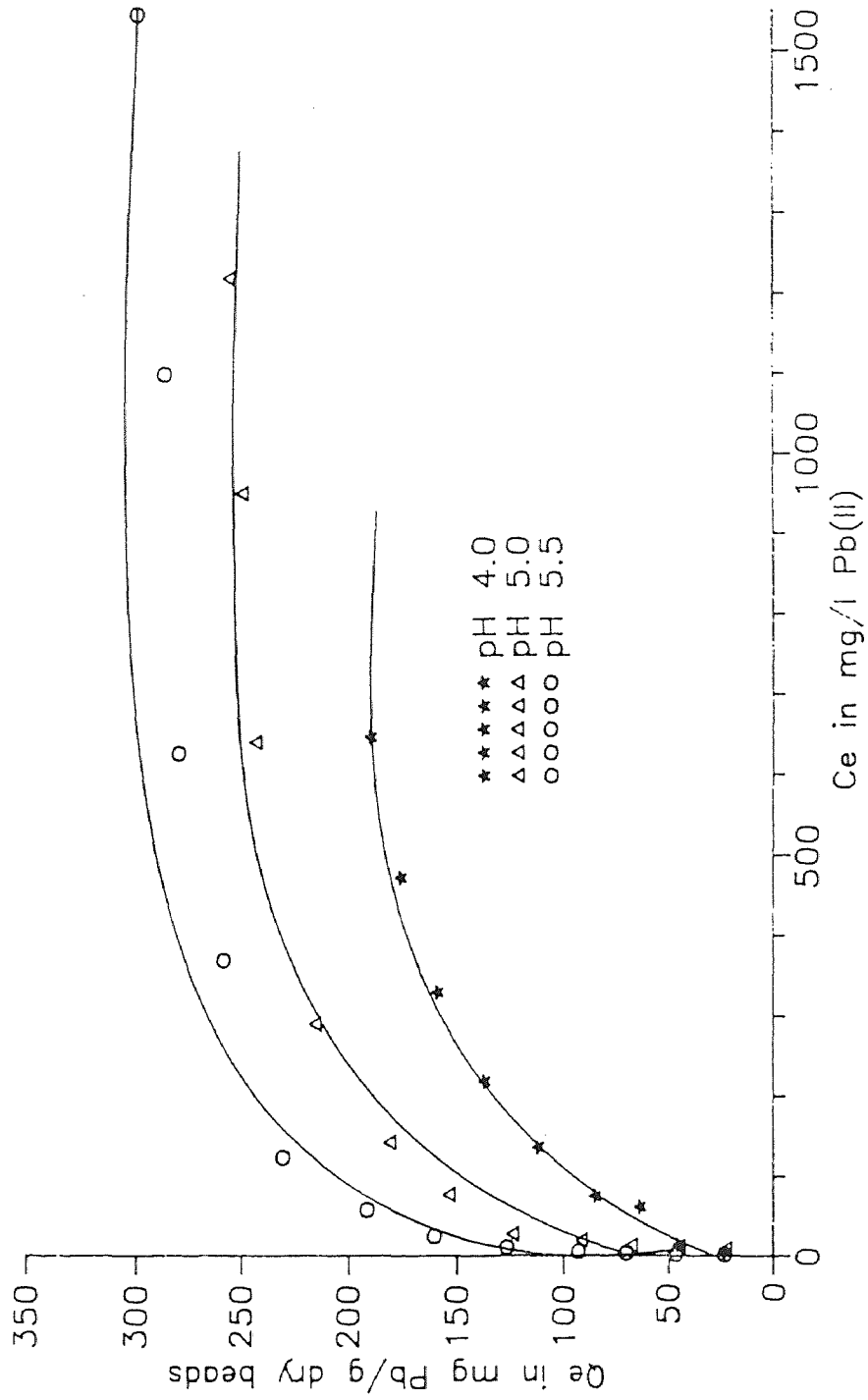


Fig. 10. Isotherm of Pb(II) biosorption₀ by biobeads in column reactor operated at 25--30°C

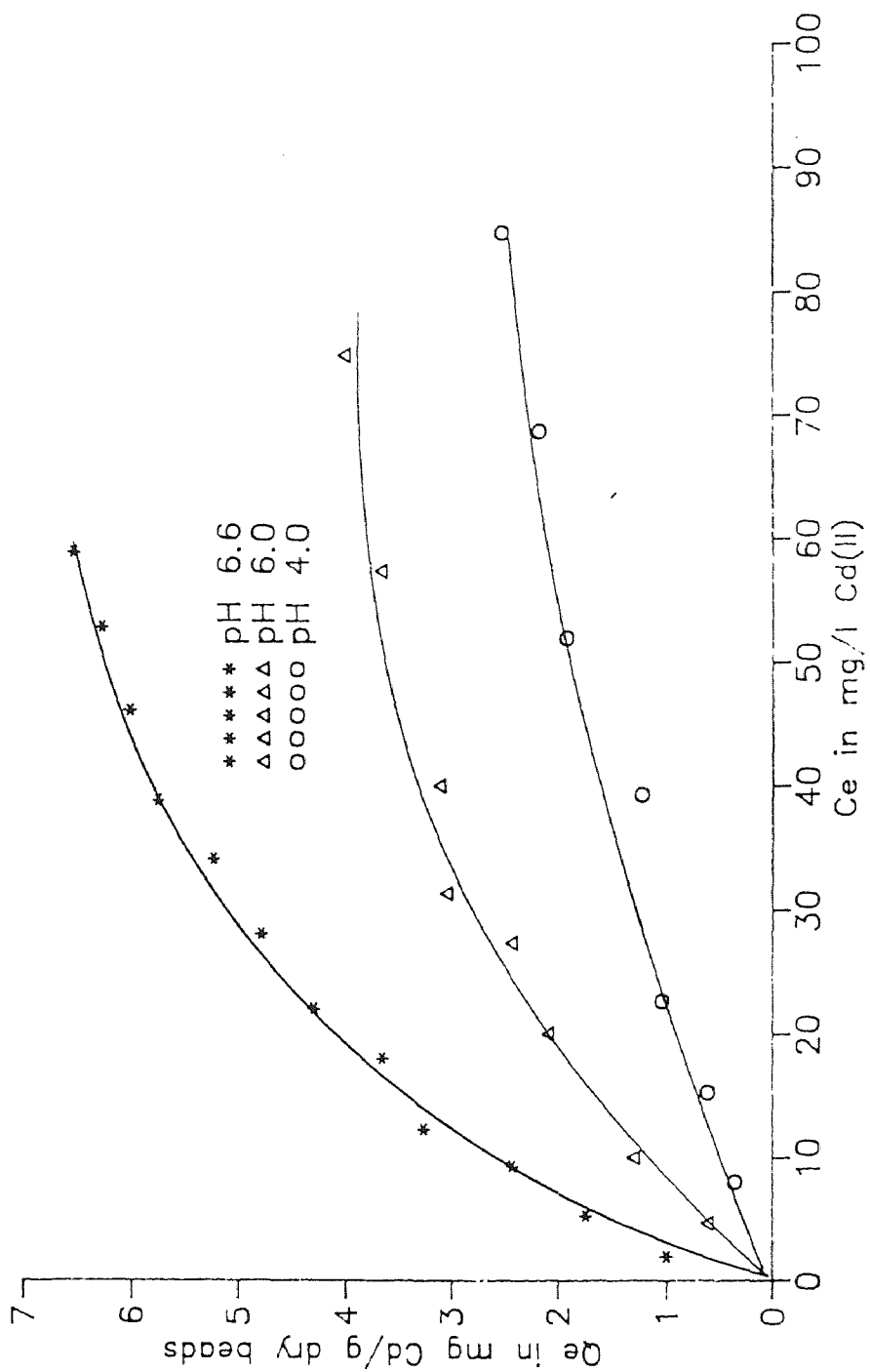


Fig.11. Isotherm of Cd(II) biosorption by biobeads in column reactor operated at 25-30°C

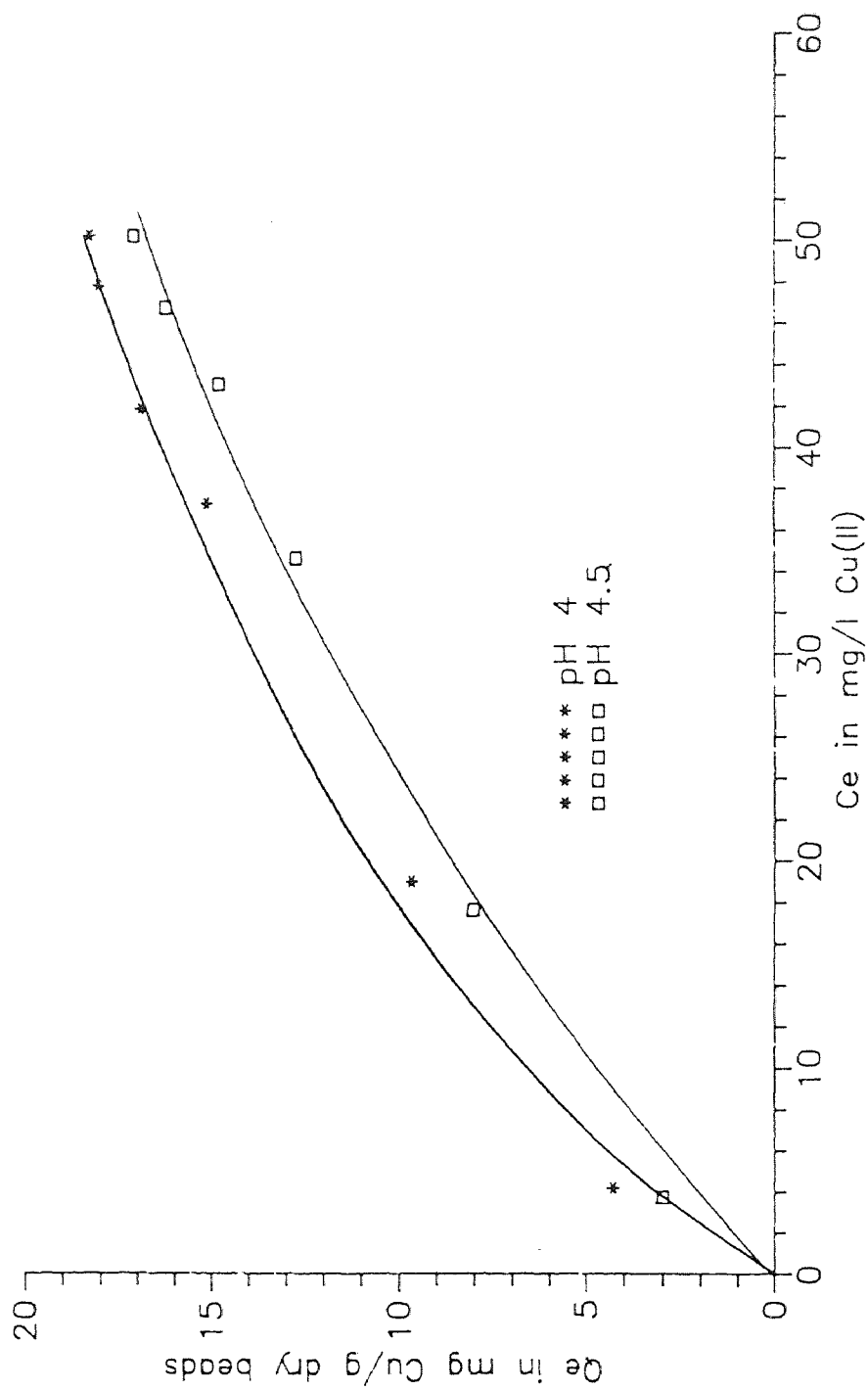


Figure 12. Isotherms of Cu (II) biosorption by biobeads in column reactors operated at 25--30°C

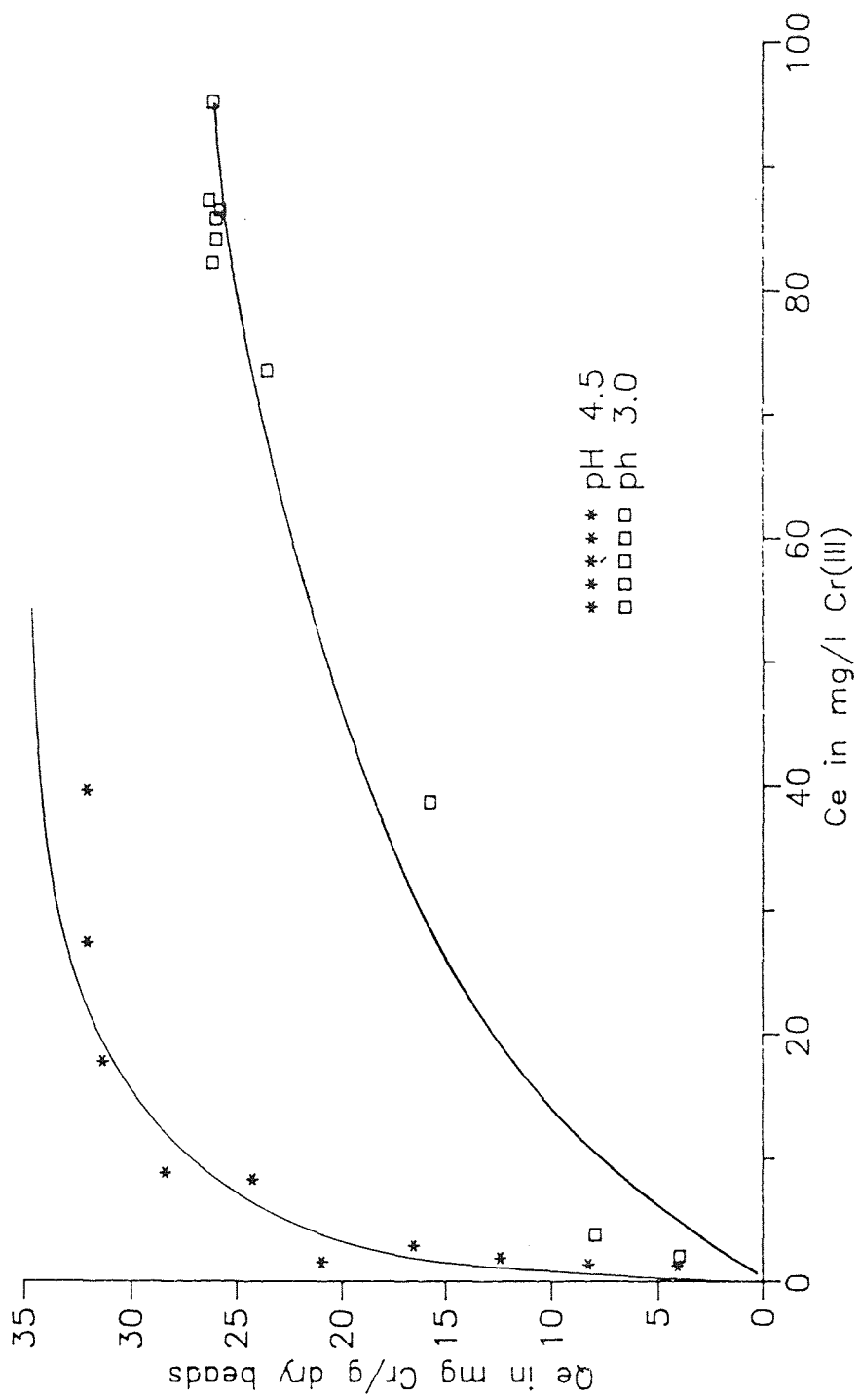


Figure 13. Isotherm of Cr (III) biosorption by biobeads in column reactor operated at 25–30°C

5.3.2 Bench-Scale Study

The experiment was done on a cascade of three reactors. The reactors were as shown in Fig.IV.9. Lead was selected as a model for this study.

The metal solution (input) treated in this experiment was 300 mg/l Pb^{2+} prepared by dissolving $\text{Pb}(\text{NO}_3)_2$ in a 0.5% w/v NaCl solution.

A total volume of 1300 ml of influent was treated. This volume corresponded to three times the volume of the liquid treated in each reactor at each cycle. Treatment was operated at pH 5.5 and room temperature. Each reactor was packed with 70 g of fresh biobeads, which contained approximately 4.2 g of dry biomass.

Based on the previous study the residence time of the solution in each reactor was set at 4 hours to optimize the process.

One-third of the volume of influent (430ml) was recirculated for 4 h in the first reactor, then transferred into the following reactor while the first reactor was filled again with a new batch of 430 ml of influent. The same operation was repeated until all the influent was treated (3 cycles per reactor).

5.3.3 Clean-Up Level

The effluent of the last reactor was analyzed for Pb^{2+} at the completion of the study. The concentration was below the analytical limit of detection, and therefore the ef-

fluent had to be concentrated by evaporation prior to the analysis. Pb^{2+} concentration in the effluent was 0.04 mg/l, which is below the drinking water limit for lead. This result shows that clean-up level can be achieved with the technique from an initial concentration of 300 mg/l.

The mass of lead retained in the biobeads was 0.39 g (i.e. 30.95 mg/g dry biomass).

This is a very satisfactory result, because at the low residual Pb^{2+} concentration attained, the metabolic activity of the sludge must not be inhibited by lead. Therefore, the biodegradation of organic contaminants, which might be found with lead in some industrial effluents, could have occurred at the same time. This is a significant potential advantage of this treatment process compared to the classical methods of metal precipitation or metal biosorption on dead biomass.

CHAPTER 6

ANAEROBIC CONCENTRATION

The concentration phase was planned as a possible intermediate stage between the biosorption and the recovery phases, during which the percentage of metal in the biosorbent would be increased with the objective of making metal recovery from the residual solids more feasible.

Our approach was to conduct a partial biodegradation of the organic biosorbent into methane and carbon dioxide under anaerobic conditions.

Although we were aware of the toxicity of metals to the anaerobic and aerobic microorganisms, we believed that it was worth trying due the following considerations:

1. The major part of the metal, we considered, would be bound to the solids and not in soluble form during anaerobic digestion. Therefore, the toxicity to the microorganisms would be very significantly reduced.

2. Metal cations are known to precipitate in the form of hydroxides or sulfides under anaerobic conditions, thereby reducing even more the metal concentration in the solution.

6.1 MATERIALS AND METHODS

6.1.1 Acquisition of the Anaerobic Inoculum

Anaerobic slurry from the Livingston Municipal Sewage Sludge Anaerobic Treatment Plant, New Jersey, was used in this study. The slurry contained approximately 10 % of suspended solids. It was taken to the laboratory and mixed with nutrient medium # 1 (Table 4.) on a volume to volume basis, under anaerobic conditions. Nitrogen was continuously bubbled through the medium during the dilution and 15 minutes thereafter in order to remove dissolved oxygen. Sodium alginate, an important constituent of nutrient medium was used as one of the carbon sources to acclimate the anaerobic microorganisms to the degradation of this substrate.

The slurry was digested in a 1 liter digester thermoregulated at $35 \pm 0.1^{\circ}\text{C}$. The digester was manually agitated twice a day. The pH was adjusted to 7.5 ± 0.1 and biogas production was continuously monitored.

Every 10-15 days, $1/5$ th of the total volume was removed from the reactor and replaced by the same volume of nutrient medium # 2 in which nutrient concentrations were 10 times as high as in nutrient medium #1. At the end of each 2 week cycle, dissolved organic carbon in the medium was below 500 mg/l. After 5 cycles of digestion, the medium was used as an inoculum for anaerobic digestion experiments.

TABLE 4. Composition of nutrient Medium # 1
(pH 7.4+/- 0.2)

Glucose3,000 mg
Yeast extract1,000 mg
Peptone	500 mg
Low viscosity sodium alginate1,000 mg
NH ₄ Cl	300 mg
K ₂ HPO ₄ .3 H ₂ O	270 mg
NaH ₂ PO ₄ .H ₂ O	40 mg
CaCl ₂ . 2H ₂ O	15 mg
MnSO ₄ .H ₂ O	5 mg
MgSO ₄ .7H ₂ O	10 mg
FeCl ₃ .6H ₂ O	5 mg
Tap water1,000 ml

6.1.2 Preparation of Metal Biobeads

Biobeads prepared as detailed in pg.25 were contaminated with lead and cadmium by contacting them with the respective metal solutions (CdCl_2 at pH 6.6 and $\text{Pb}(\text{NO}_3)_2$ at pH 5.5) for 24 hours in agitated shaker flasks. Then the beads were separated from the solution on a sieve, dripped for few minutes on filter paper, and subsequently tested for anaerobic digestion. Lead and cadmium concentrations in the biobeads were then:

7.2 mg Cd/ g dry biobeads

240.0 mg Pb/ g dry beads

6.1.3 Analytical Methods

Carbon dioxide and methane concentration for biogas obtained in anaerobic digestion were detected on a Shimadzu gas chromatograph (model GC8AIT) equipped with TCD. A stainless steel column (6' x 1/8') packed with Porapak, 100/200 mesh (Alltech) was used at an oven temperature of 50⁰C.

Dissolved organic carbon in the liquid medium was detected on a TOC Analyzer Model 700-O-I-Analytical. Metal concentrations were determined by atomic absorption with Perkin-Elmer spectrophotometer, Model 2380.

6.1.4 Anaerobic Digestion

The incubation medium was prepared by diluting the inoculum with a mineral nutrient medium on the basis of one volume

of inoculum for three volumes of mineral medium.

The mineral medium consisted of: NH_4Cl , 1.2g/l ; $\text{K}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$, 1.11g/l; $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ 0.17 g/l; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.06 g/l; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 0.02 g/l; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.04 g/l; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.02g/l; tap water, 1 liter. Traces of yeast extract and beef extract were also added to supply vitamins and growth factors.

Nitrogen was bubbled through the medium while the dilution was made and 15 minutes thereafter to remove dissolved oxygen. The pH of the incubation medium thus obtained was 7.4 +/-0.2.

The anaerobic degradation of the following substrates was investigated in this study:

- Biobeads (metal free)
- Plain alginate beads (metal free)
- Suspended activated sludge (metal free)
- Cadmium saturated biobeads (7.2 mg Cd/ g dry beads)
- Lead saturated biobeads (240 mg Pb/ g dry beads)

The organic substrates were introduced into a series of 100 ml glass serum bottles on the basis of 0.5 g dry solids per bottle. The substrates were tested in their wet form. Six bottles were used for each substrate.

The incubation medium was transferred into the bottles on the basis of 40 ml per bottle. Nitrogen was continuously bubbled through the bottles while filling them and 15 min thereafter. Then all bottles were air tight closed with rubber plugs, sealed with aluminum seals and incubated (Na-

tional Incubator) at $35 \pm 0.1^{\circ}\text{C}$. They were manually shaken twice a day.

At the same time, a series of blanks was carried out in the same way, in which no substrate was added. The activity of the anaerobic inoculum was also tested in a series of bottles in which the organic substrate was dextrose.

Biogas production was periodically measured by piercing the rubber plugs of the bottles with 10 ml, 20 ml or 50 ml glass syringes.

The digestion was conducted for a period of 70 days. The pH was controlled by opening one bottle of each series every 20 days approximately. Biogas was analyzed for methane and carbon dioxide at the completion of the 70 day period.

6.2 RESULTS AND DISCUSSION

The experimental results are presented in Fig.14., which shows the cumulative biogas productions for each substrate as a function of incubation time. The results are average data from all the bottles of each series. The very low biogas production from the blanks was due to the autodigestion of the inoculum because no carbon sources were available at significant concentration in the medium.

Biogas production from metal free biobeads was consistent with theoretical expectations. A maximum of 250 ml of biogas may be expected for 100 % degradation of 0.5 g of dry biobeads. Due to the solubilization of bicarbonates in the medium, the production of biogas was decreased and therefore a maximum of approximately 200 ml was actually expected. The cumulative volume of biogas obtained from biobeads was approximately 180 ml, indicating that the biobeads were degraded at 80%.

The production of biogas from metal saturated biobeads were drastically reduced as compared to metal free biobeads (83.1% and 70% reduction in the presence of cadmium and lead respectively) Table 5.

The results showed that the anaerobic digestion of biobeads was significantly inhibited by the presence of metals. One explanation might be that biodegradation of solids requires a contact between the degrading microorganisms or their enzymes and the substrate.

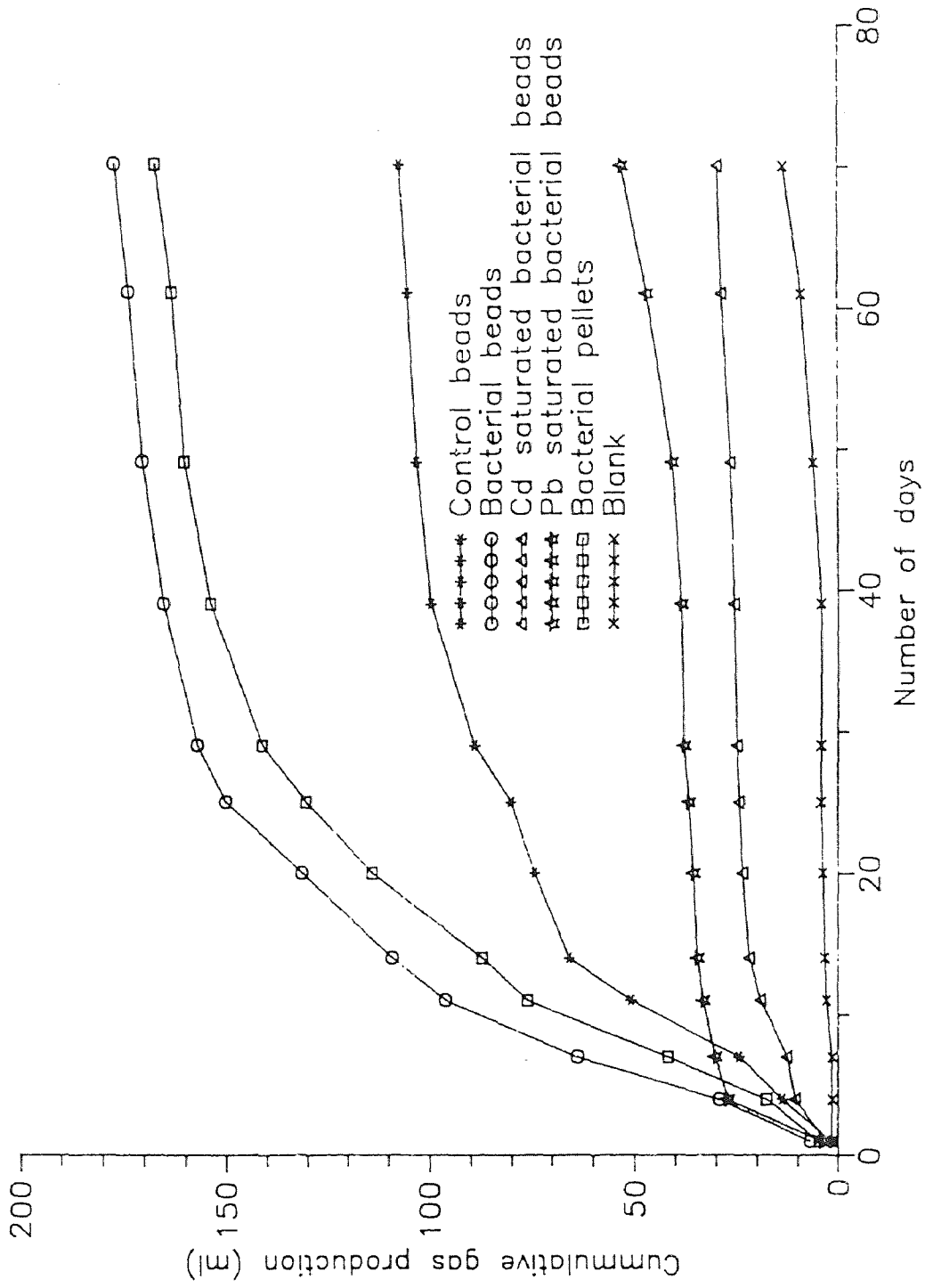


Fig.14. Anaerobic Digestion

Table 5. Effect of Cd and Pb on Anaerobic
Digestion of Ca Alginate Biobeads

Substrate	Cumulative biogas prod. (ml)	% CH ₄	% CO ₂	Final pH
Biobeads (metal free)	177	61	39	7.0
Alginate beads (metal free)	107	63	37	7.0
Suspended activated sludge	166	70	30	7.1
Cd saturated biobeads	30	62	38	6.3
Pb saturated biobeads	53	71	29	6.7

The initial phase of contact is often the adsorption of microorganisms or enzymes onto the solid surfaces. Due to the presence of sorbed metals, this contact may have been inhibited in the present study, or else the activity of the hydrolytic enzymes has been inhibited by the presence of the sorbed metals.

As expected, it was found that metal concentration in solution (determined after filtration at 0.45 μm) remained very low even at the end of anaerobic digestion, as shown in Table 6.

Table 6. Metal Concentration in Solution at
the end of Anaerobic Digestion

Substrate	Final Cd conc. in solution (mg/l)	Final Pb. conc. in solution (mg/l)
Biobeads (metal free)	0.0	0.0
Plain alginate beads	0.0	0.0
Suspended activated sludge	0.0	0.0
Cd saturated biobeads	0.1	0.0
Pb saturated biobeads	0.0	2.0

CHAPTER 7

SUMMARY OF RESULTS

Laboratory and bench-scale treatment studies performed at NJIT Biotechnology Laboratory have demonstrated the technical feasibility of utilizing immobilized activated sludge to concentrate heavy metals from aqueous streams via the mechanism of biosorption.

A residual metal concentration of 0.04 mg/l, which is below the drinking water limit, has been obtained with biobeads in a bench-scale biosorption treatment of lead contaminated water from an initial concentration of 300 mg/l. This result demonstrates the potential of the technique to treat lead-laden wastestreams. The process may be a viable alternative to existing techniques when both organics and metals have to be removed from wastestreams, because the classical methods do not have provision for the degradation of organics.

The immobilized cell reactor is more attractive than the free cell system because immobilization imparts greater operational flexibility due to the fact that it prevents biomass washout in continuous flow reactors, allows higher cell densities and facilitates the separation of the biomass from the treated effluent. In addition, the entrapment of the cells in calcium alginate beads is a good tech-

nique of immobilization, it is easy to implement and relatively inexpensive. Also the gel matrix has some adsorption properties which can be helpfull.

Biobeads can be stored for extended period of time prior to use, and have an operational lifetime of approximately 90 days.

The results also suggest that immobilized activated sludge may be used to selectively remove specific metals from solution containing various metal ions. This may be done by adjusting the conditions of biosorption, especially the pH.

Several additional areas of investigation remain to be completed before definitive conclusions can be made about the commercial application of the biobeads process (e.g. simultaneous treatment of several heavy metal contaminants, "non-ideal" waste characteristics, and synergistic/antagonistic effects of organics).

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