

MICROBIOLOGICAL TRANSFORMATION OF METAL CONTAMINATED EFFLUENTS

by

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EXECUTIVE SUMMARY

RESEARCH ON THE MICROBIOLOGICAL TRANSFORMATION OF METAL CONTAMINATED EFFLUENTS

Background and Motivation

Legislation governing protection of the environment is becoming progressively stricter. During this era of environmental protection, the environmental biotechnologist is faced with the special challenge to become increasingly innovative in the development of appropriate low-cost and efficient technologies for the treatment and/or reuse of waste products prior to discharge into the environment. The "heavy metals" have been the cause of particular environmental concern. Their toxic and carcinogenic potentials at low concentrations as well as the large quantities disposed to the environment have prioritised them as leading contaminants. Current technologies for the removal of metals from industrial waste solutions include chemical precipitation, chemical oxidation or reduction, filtration, electrochemical treatment, application of membrane technology and evaporative recovery. These methods are either ineffective or extremely expensive. There exists a need to develop cheaper, effective biosorbents to treat industrial wastes contaminated with metals.

Activated sludges have been shown to remove metal cations from water systems during the activated sludge process. This ability of sludges warranted further investigation into the potential for it to be developed into an attractive alternative to conventional systems for treatment of metal contaminated wastewaters. Hence, this study concentrated on

investigating the ability of ten activated sludges to biosorb metal-ions from wastewaters as well as to determine whether sludges could be recycled and metals recovered for possible reuse. Factors such as microbial constituents, mechanisms of biosorption and surface charge of activated sludge were also investigated for their contributions to the biosorptive capability of activated sludges.

Objectives

The objective of the project was to contribute to the development and optimisation of a bioreactor process which removes metals from effluents and concentrate them to complete biorecovery. To realize the above objective, three aims were:

- i) to survey several domestic and industrial activated sludges to;
 - a) quantify their microbiological populations,
 - b) analyse their metal contents, and,
 - c) develop appropriate sludge pretreatment methods, to facilitate metal chelation from sludges metals prior to their use as biosorbents.
- ii) adsorption/desorption studies in batch culture to ascertain;
 - a) their metal adsorption capacities, and,
 - b) appropriate methods for metal desorption and recovery from sludges.
- iii) bioreactor studies to optimise the parameters of the biorecovery processes developed.

Summary of Results

Activated sludge is comprised of bacteria, fungi and yeasts, algae and protozoa. Ten activated sludge plants in Natal (S1-S10) were surveyed for the microbial constituents listed above. Bacteria were enumerated, isolated and characterised. Filamentous bacteria, fungi and yeasts, algae and protozoa were identified. Gram positive eubacteria were predominant over their Gram negative counterparts and the activated sludge process favoured the survival of sporeformers compared with non-sporeformers. Type 0092 and *Microthrix parvicella* were the predominant filamentous bacteria in the ten sludges. Most of the fungi detected belonged to the common genera and yeasts were mostly common pathogens. Twenty six types of algae were detected in the ten sludges and sixteen types of protozoa were also detected. The microbiological survey facilitated greater understanding of the microbiology of activated sludge in the Natal region. The survey showed that there were no notable variations in microbial constituents of the sludges under investigation.

In a previous study, activated sludge biosorption was not enhanced by pretreatment with a metal chelator or inactivation by chemical or physical methods. Pretreatment was therefore not employed for biosorption studies. Ten sludges were exposed to six metal ions viz., Zn^{2+} , Ni^{2+} , Cu^{2+} , Cd^{2+} , Cr^{3+} and Cr^{6+} in individual solutions to determine their capacity for biosorption of these metals. Biosorptive capabilities of sludges were quantified to determine an affinity series of metal binding to sludges and to select the sludges which biosorbed metal ions most efficiently for further study. Batch experiments were also conducted to determine adsorption of metal ions from industrial wastewaters with mixed

metal ion content. All sludges exhibited metal biosorptive capability. An affinity series for metal/sludge biosorption was found to be, in descending order, $\text{Cu} > \text{Cd} > \text{Zn} > \text{Ni} > \text{Cr}^{3+} > \text{Cr}^{6+}$.

Mechanisms of sludge/metal interactions were investigated to determine how metal ions were being adsorbed onto sludge surfaces. The Langmuir Model was fitted to sludge/metal interaction data and adsorption isotherms were plotted. The shapes of these isotherms were indicative of the type of binding of metal ions to sludge surfaces. Transformation of the isotherms yielded the adsorption constants K_s , the sludge-metal bond strength and, X_m , the capacity of the sludge surface to adsorb metal ions. The latter constant was used to rank sludges according to their potential biosorptive capabilities. Ninety seven percent of the data fitted the Langmuir Model indicating that this Model can be used to predict binding mechanisms of metal ions to sludge surfaces. The predominant type of adsorption was of the type I or "L"-shaped adsorption. This is attributable primarily to charged carboxyl termini residues on sludge surfaces.

After equilibration with metal ions, sludges were exposed to desorbing agents in batch processes to determine whether metals biosorbed to sludge surfaces were recoverable. Two acids, namely, acetic and hydrochloric, were used to desorb metal ions from sludge surfaces. Both agents were found to be efficient at removing the metals adsorbed onto sludge surfaces. Desorption was also found to be agent-dependent rather than sludge dependent.

Desorbents were found to be selective with regard to the efficiency at which they removed metal ions from sludge surfaces.

Sludge surface charge was investigated with the aim of determining whether this was associated with its biosorptive capability. Three methods were employed to ascertain surface charge viz., streaming current, millivolt quantification and colloid titration. All sludges were found to demonstrate a net negative charge. There were differences in the magnitudes of these charges. Overall, S9 was found have the most electronegative surface. The millivolt quantification method was recommended because of the convenience and speed of analysis.

Adsorption and desorption of metal ions from synthetic and industrial wastewaters were further optimised in batch laboratory scale reactors. Activated sludge was found to adsorb metal ions optimally at the highest concentration of sludge tested viz., 25 000 mg ℓ^{-1} . The optimal time of adsorption was found to be 90 min., at 600 rpm in a 30 ℓ volume. Desorption by hydrochloric acid was found to be more efficient than desorption by acetic acid. The optimal time for desorption was found to be 15 min., at 300 rpm in a 10 ℓ volume. Multiple cycles of adsorption/desorption, however, revealed that sludge adsorptive capabilities decreased markedly after desorption. This led to the assumption that sludge adsorptive surfaces were disrupted by desorbing agents. Adsorption of metals from industrial wastewaters indicated that adsorption efficiency of metals is associated with pH. Zinc was shown to be adsorbed most efficiently at alkaline pH while Cu, Ni and Cr^{3+} were found to be most efficiently adsorbed at an acidic pH.

Sludges can be used as biosorbents of metal ions and these metals may be desorbed and recovered from their surfaces. Microbial analysis of sludges did not indicate a relationship between predominant populations and sludge adsorptive capabilities, hence, no comparisons could be made between the two. Sludge surface charge showed a greater correlation with biosorptive capability indicating, possibly, that sludge biosorption is more physicochemical rather than microbiological.

The findings of this study have shown that waste activated sludges may serve as metal biosorbents from industrial wastewaters. Furthermore, the metals biosorbed onto sludges may be recovered by desorption with appropriate agents in smaller, more manageable volumes. Metal concentrates may then be safely disposed of or metals may be recovered from them via chemical methods.

Technology transfer

A pilot-scale installation to confirm the potential of activated sludges as biosorbents for application for South African industry needs to be investigated. To this end, one or several industries with metal-bearing effluents need to be approached to set up pilot-plants with semi-skilled operators. These plants will then operate with recommendations based on findings of this report. If more than one local activated sludge is available for use, then the electronegativities may be assessed by methods described in this report and the most electronegative sludge should be used. Pilot-plant optimisation is envisaged with information from pertinent plants being compared with data from this report. The potential of activated

sludges to remediate metal contaminated wastewaters in an inexpensive and efficient manner is envisaged as a culmination of this project.

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LIST OF ABBREVIATIONS

A	-	Amanzimtoti
AAS	-	atomic adsorption spectrophotometry
AEV	-	A E Valves
ANOVA	-	Analysis of Variance
b	-	Constant related to the energy or net enthalpy (ΔH) of adsorption
B	-	constant expressive of energy of interaction with surface
B.E.T.-		Brunauer Emmett Teller
C	-	concentration of solute remaining in solution at equilibrium
CFU	-	Colony Forming Units
CGYA	-	Casitone Glycerol Yeast-Extract Agar
C'	-	saturation concentrating of solute in solution at given temperature
D	-	dielectric constant
DTPA	-	Diethylene triamine pentaacetic acid
E	-	Extend from Floc
(E)	-	Epiphytic growth
E2Cr	-	Kohler Flexible, Chromium rinse
E2FE	-	Kohler Flexible, Final effluent
E3Cd	-	Metal Protection Services, Cadmium rinse
E3FE	-	Metal Protection Services, Final effluent
E4FE	-	Glacier Bearings, Final effluent
E6FE	-	Fascor, Final effluent
EDTA	-	Disodium ethylenediaminetetraacetic acid
En	-	Effluent number
FC	-	Final concentration
FV	-	Final Volume
G	-	Granules
H	-	Hammarsdale
HAc	-	Acetic Acid
i	-	streaming current
I	-	Found mostly within Floc
IC	-	Initial concentration
IV	-	Initial volume
(K)	-	affinity constants
Ka	-	sludge-metal bond
KM	-	Kwa Mashu
(L)	-	uncomplexed ligand concentration
(M ⁺)	-	cationic metal ion
MGC	-	methyl glycolchitosan
MLSS	-	Mixed Liquor Suspended Solids
MPS	-	Metal Protection Services
N	-	viscosity of fluid
NDC	-	Natal Die Casting
NG	-	New Germany
NW	-	Northern Works
P	-	Phoenix
(P)	-	Peristomal disc

PHB	-	Polyhydroxybutyrate
PMB	-	Pietermaritzburg
PVSK	-	potassium polyvinyl alcohol sulphate
Q^0	-	number of moles of solute adsorbed per unit weight of adsorbent in forming
q^c	-	amount of solute adsorbed per unit weight solid adsorbent
RAS	-	Return activated sludge
RBCA	-	Rose Bengal Chloramphenicol Agar
RSD	-	relative standard deviation
(S ⁻)	-	anionic bacterial surface
SCV	-	streaming current values
SW	-	Southern Works
T	-	Tongaat
TB	-	toluidine blue
U	-	Umlaas
X _m	-	capacity of the sludge surface for metal-ions
YMEA	-	Yeast Malt Extract Agar
Z	-	Zeta potential

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1.0 GENERAL BACKGROUND, INTRODUCTION AND AIMS

1.1 BACKGROUND AND INTRODUCTION

As a result of the vigorous development of industries and increase in population since the middle of the nineteenth century and the introduction of piped water supplies to cities and municipal districts, the volume of sewage and other effluents has continued to increase. The water closet, the kitchen sink and the associated sewer network which transports the domestic sewage with all its constituents in a hygienically acceptable manner, thus eliminating a centuries-old source of infection, led to substantial improvement in sanitary and hygienic conditions of towns and cities. However, during the past century serious adverse effects on natural waters were already becoming apparent, such as unpleasant odours, fish kills and limitations on water re-use due to the introduction of sewage effluents (Hänel, 1988). During 1914, Ardern and Lockett introduced the activated sludge process as a biological method of organic matter reduction from sewage. This method is presently still employed, globally, for the treatment of waste water (Murray, 1987).

The principle of activated sludge is that, in a reactor, a community of microorganisms is constantly supplied with organic matter and oxygen. The microorganisms consume the organic matter and transform it by means of aerobic metabolism, partly into new microbial biomass and partly into carbon dioxide, water and minerals. The flow of water brings about constant wash-out of microorganisms from the reactor to the settler. Here, the microorganisms which grow in flocs are retained and then removed with the underflow. Part

of this sludge is then recycled to provide biomass to treat new influent. The surplus amount is discarded. In many respects, the aeration basin is comparable to a conventional fermentation reactor or chemostat. However, the purpose of the process is not to produce microbial biomass or a particular metabolite, but to mineralise incoming waste materials as much as possible. It is of paramount importance to minimize biomass production since the latter has to be removed and treated in a subsequent stage (Verstraete and van Vaerenbergh, 1986). The microorganisms present in activated sludge include eubacteria, filamentous bacteria, algae, fungi, protozoa and rotifers (Jenkins *et al*, 1986). These microorganisms contribute to transformation of organic matter by aerobic metabolism. Reactions occurring in the activated sludge process can be summarized as follows:

- Sorption of soluble, colloidal, and suspended organics in and on the sludge flocs,
- Biodegradation of organics materials resulting in end products (CO_2 , H_2O , minerals) and synthesis of new microbial biomass,
- Ingestion of bacteria and possibly other suspended matter by protozoa or other predators,
- Oxidation of ammonium to nitrite and further to nitrate by nitrifying bacteria, and,
- During insufficient supply of energy (waste) oxidation of cell reserves (internal and external) resulting in sludge mineralization and lysis. (Verstraete and van Vaerenbergh, 1986).

The removal of metal-ions from influents during the activated sludge treatment process has been reported (Brown *et al*, 1973, Lester and Sterritt, 1985). Waste activated sludges, a product of the activated sludge process, are being produced in increasingly larger volumes due to increases in population and industrial development. Current disposal methods for treated sludge include, landfill, land application, ocean disposal, incineration and lagoon disposal (Ekama, 1992). Industries utilising metals in their processes include, tanneries, metal plating, solid metal manufacturing, die-casting, and various other manufacturing processes (Nriagu and Pacyna, 1988). Conventional methods for removing metals from industrial waste solutions include, precipitation by chemical oxidation/reduction, filtration, electrochemical treatment, application of membrane technology and evaporative recovery (Volesky, 1987). Waste sludge biomass possesses metal-sorptive capabilities and may be used to adsorb metal-ions from solutions viz., metal-bearing effluents (Lester and Sterritt, 1985).

The use of activated sludge as the choice biosorbent for removal of heavy metals from industrial effluents would ultimately depend on, firstly, the potential for recovering metals that have been adsorbed by biomass and secondly, the potential for reusing the regenerated biomass in multiple adsorption - desorption cycles. The reuse capability would improve the economics of the process. Recovery of the adsorbed metal could be achieved by the use of an appropriate elution solution capable of effectively chelating adsorbed metal from the waste biomass. It is desirable that the elution be complete or that any irreversibly held metal be kept to a minimum. In addition, it is preferable that the least possible damage occurs to the adsorption properties of the biomass in order to facilitate the reuse of biomass in subsequent adsorption-desorption cycles. Previous research by Tsezos, 1984 showed that when

comparing six elution systems for desorption of Uranium from biomass, a sodium bicarbonate solution appeared to be the most promising. Mineral acids, although good elution agents, resulted in substantial damage to the biomass thus limiting potential for reuse (Tsezos, 1984).

1.2 AIMS

- i) Identification and characterization of microorganisms present in the activated sludge process.
- ii) Investigate the ability of waste activated sludge to perform as metal biosorbents and subsequently compare the biosorptive capacities of ten waste sludges by batch experimentation. Compare the differences in biosorption of metal ions from single solutions and industrial wastewaters.
- iii) Establish the mechanisms involved in the sludge metal interactions.
- iv) Determine a relationship, if any, between the microbiota of sludges under investigation and their comparative abilities to serve as metal biosorbents.
- v) Selection of suitable and efficient desorbing agents to remove adsorbed metal from biomass and, moreover, to render the biomass re-usable for multiple adsorption - desorption cycles.

- vi) Determine sludge surface charge and compare the magnitude of surface charge with the degree of biosorption among the ten sludges.
- vii) Investigate laboratory scale optimisation of a process for metal adsorption by and desorption from sludges.
- viii) Subsequent optimization of a process for metal adsorption from industrial wastewater by sludges and desorption of adsorbed metals from sludges.
- ix) Feasibility assessment.

2.0 MICROBIOLOGICAL SURVEY OF ACTIVATED SLUDGES

2.1 INTRODUCTION

Aerobic biological treatment systems are extensively used for stabilization of dilute organic wastes. Activated sludge, trickling filters, and oxidation ponds are the primary types of aerobic systems (McKinney, 1957). The most widely used of the above methods is the activated sludge process in which sludge flocs are maintained in suspension in flowing wastewater by air diffusion or mechanical agitation.

Activated sludge consists of yellow-brown to black coloured flocs, produced by growth of bacteria, fungi, algae and other micro-organisms in the presence of dissolved oxygen. Soluble and suspended matter are removed by the living mass of micro-organisms. After exhaustion of waste material, sludge flocs are allowed to settle, and separated from the carriage water which now contains 90-95 percent less of organic matter. The water is discharged from the settling tank as clear liquid (van Gils, 1962). Micro-organisms responsible for the transformation and removal of pollutants include eubacteria, filamentous bacteria, algae, fungi and protozoa (Jenkins *et al*, 1986). Rotifers, nematode worms and more rarely oligochaete worms and chironomid larvae may be found (Curds, 1982). Flocs are the basic ecological units of activated sludges. Heterotrophic bacteria form the basis of flocs by attaching to each other and to filamentous bacteria. The floc macrostructure is formed by filamentous bacteria which facilitate adhesion to floc-forming bacteria. Fungal hyphae are often associated with flocs, but rarely predominate under normal operating conditions. Protozoans contribute to the process by feeding on pathogenic bacteria and by

removing dispersed bacteria which results in larger flocs and improved sludge settleability (Curds and Cockburn, 1970).

Growth of specific micro-organisms in a given industrial waste disposal system will depend upon the chemical characteristics of the industrial waste, the environmental limitations of the particular waste system and the biochemical characteristics of the micro-organisms. All of the micro-organisms which proliferate in a given industrial waste disposal system contribute to its over-all characteristics, both good and bad. It is important to recognise contributions made by each type of organism to the overall stabilisation of the organic wastes (McKinney, 1957). Full understanding of the ecological, physiological and biochemical activities of the microflora is necessary for optimal control of the process (Adamse *et al*, 1984).

Previous researchers have postulated that many settling, compaction, and separation properties of activated sludge are related to the relative numbers of filamentous and floc-forming micro-organisms in the activated sludge floc (Eikelboom, 1975; Lau *et al.*, 1985). Studies on the bacterial flora of activated sludges have been the subject of a comparatively small number of publications. The results of the investigations are divergent, due to the use of different methods and examinations of different types of sludges.

Although activated sludge treatment systems in South Africa have been relatively widely studied, little quantitative information describing microbiological populations of sludges have been communicated. In particular, microbiological surveys of activated sludge plants in Natal have only received scant attention.

2.1.1 Aims

- i) Determination of the microbiological population of ten activated sludge plants in Natal treating influents of a domestic and industrial nature, by enumeration, isolation and subsequent characterization of the eubacteria, identification of filamentous bacteria, fungi, yeasts, algae and protozoa, and,
- ii) Comparison of the prevalence and predominance of eubacteria, filamentous bacteria, algae, protozoa, yeast and fungi.

2.2 METHODOLOGY

2.2.1 Sampling and Mixed Liquor Suspended Solids (MLSS) Determination

Grab samples of return activated sludge ($\pm 1\ 000\ \text{m}\ell$) were collected in sterile bottles from the following waste water works in Natal; Umlaas, Amanzimtoti, New Germany, Hammarsdale, Pietermaritzburg, Kwa Mashu, Tongaat, Northern Works, Southern Works and Phoenix (Table 2.1). Samples were stored at $4\ ^\circ\text{C}$ prior to use and processed within 24 h. Triplicate samples of $100\ \text{m}\ell$ liquid sludge were dried overnight in pre-weighed porcelain dishes in an oven at $105\ ^\circ\text{C}$ and re-weighed to determine the sludge MLSS.

2.2.2 Enumeration, Isolation, Characterisation and Identification of Eubacteria

A $50\ \text{m}\ell$ volume of each sludge sample was homogenised using a Sorvall Omni-Mixer 17106 (Du-Pont Instruments, Newton, USA) for 4 min., at $16\,000\ \text{r. min.}^{-1}$, to disperse flocs. Serial dilutions (10^{-1} to 10^{-6}) of samples were prepared. Triplicate, $0,1\ \text{m}\ell$, volumes from the 10^{-4} to the 10^{-6} dilutions were plated on Casitone Glycerol Yeast-Extract Agar (CGYA) (Appendix 1) using the spread plate technique. Plates were incubated at $30\ ^\circ\text{C}$ for 48 h and only those plates showing between 30 and 300 (above 300 too numerous to count) colonies were enumerated. Counts were expressed as CFU g^{-1} (Table 2.1). Bacterial colonies were differentiated on the basis of colonial morphology (configuration, margin and elevation) and pigmentation. Colonies were coded, subcultured on CGYA plates and re-incubated at $30\ ^\circ\text{C}$. Repeated subculturing was conducted to obtain pure cultures.

Purity was determined by Gram staining and microscopic observation. Eubacterial isolates were identified according to the procedure outlined in Figure 2.1. Staining procedures employed for characterisation included Gram stains (Pelczar and Chan, 1977) and spore stains (Pelczar and Chan, 1977) performed on 24 h cultures from CGYA plates. Determination of oxygen requirement was performed by using the anaerobic jar method. Motility was detected by examining wet-mount preparations of 24 h cultures (Pelczar and Chan, 1977). Measurement of bacterial cell diameters were conducted using light microscopy and an ocular micrometer (Pelczar and Chan, 1977). Biochemical tests conducted for the purpose of characterisation were performed at room temperature (25°C). Cytochrome oxidase was determined by testing for oxidation of tetramethyl-*p*-phenylene diamine dihydrochloride (Frobisher *et al*, 1974). The oxidase test was used to differentiate Enterobacteriaceae from other Gram-negative organisms (Frobisher *et al*, 1974). Presence of the enzyme catalase in isolates was assayed by employing a 3 % solution of hydrogen peroxide (Frobisher *et al*, 1974). The API 20E and 20NE identification systems (Montalieu-Vercieu, France) were used to identify Gram-negative enteric and non-enteric bacteria, respectively. Gram-positive isolates were identified using appropriate taxonomic techniques (Schleifer, 1986; Sneath, 1986; Kandler and Weiss, 1986; Jones and Collins, 1986). Bacilli were identified to generic level.

2.2.3 Characterisation and Identification of Filamentous Bacteria

Air-dried smears of freshly collected sludge were prepared on microscope slides. The morphological characteristics and staining reactions described by Jenkins *et al* (1986), were used to identify filamentous bacteria. Characteristics of filamentous bacteria determined using

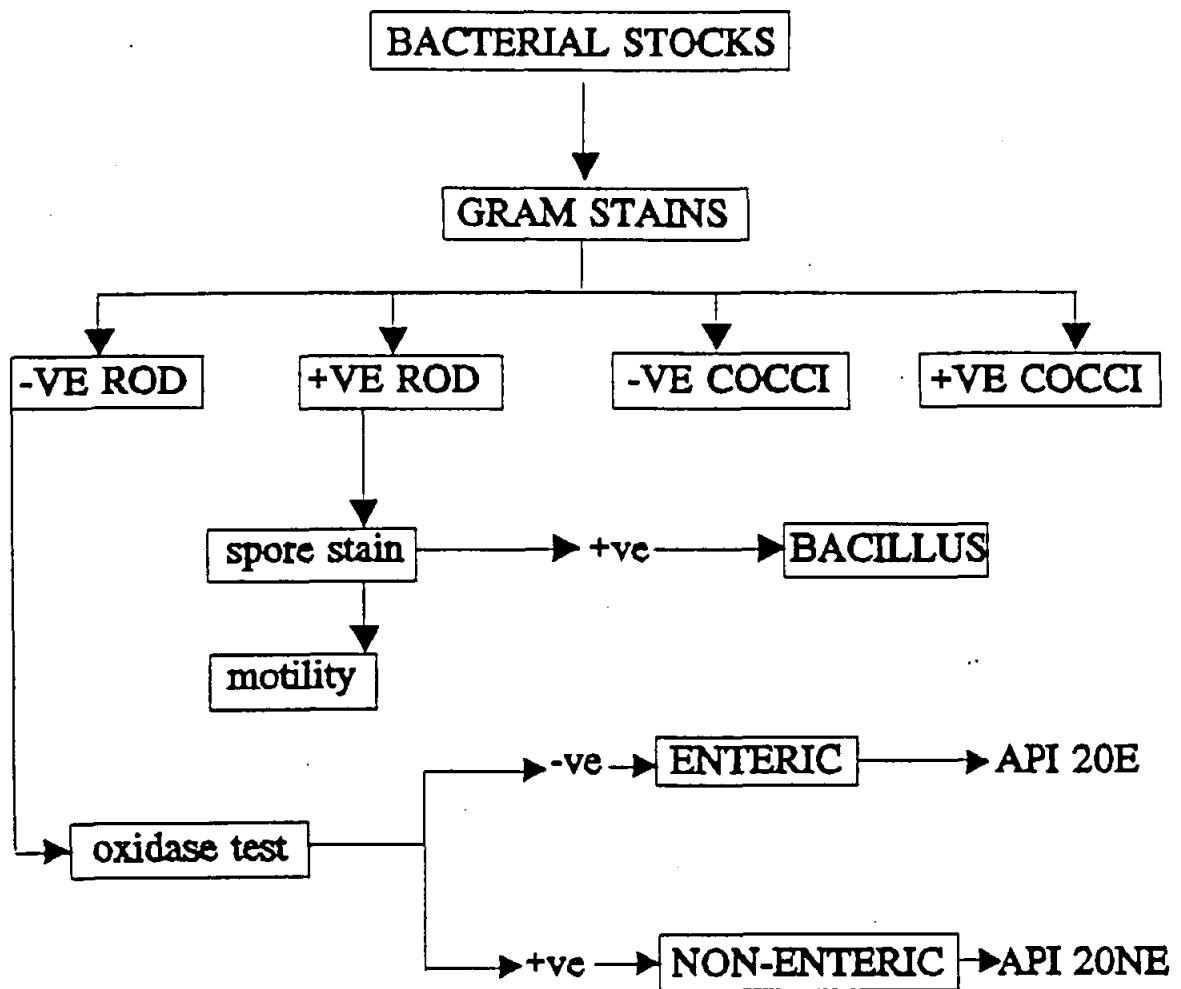


Figure 2.1. Schematic representation of procedure employed for identification of bacterial isolates.

light microscopy included: Gram reactions, Neisser reaction, presence of sulphur granules and polyhydroxybutyrate cell inclusions, trichome diameter, length, shape and location, presence of cell septa, indentations and sheaths, epiphytic growth, and cell shape and size (Jenkins *et al*, 1986). Identification was achieved using appropriate taxonomic keys and tables of Eikelboom and Van Buijsen (1983) and Jenkins *et al* (1986).

2.2.4 Isolation, Characterisation and Identification of Fungi

Aliquots, 0,1 ml, of homogenised sludge were plated on Rose Bengal Chloramphenicol Agar (RBCA) (Appendix 2). Plates were incubated at 30 °C for 7 d to facilitate development of pigment on colonies to expedite complete differentiation of fungal types. Repeated subculturing on RBCA was necessary to obtain pure cultures. Sporulation was induced by subjecting cultures to ultraviolet light (Alexopoulos and Mims, 1979). Isolates were characterised according to morphological features, cultural characteristics such as pigmentation of the mycelium and direction of growth of the hyphae, whether aerial or lateral, microscopic observation of structures involved in asexual reproduction eg., conidia or spores, and in sexual reproduction, and the presence of fruiting bodies. Identification was accomplished using appropriate taxonomic techniques (Gilman, 1959; Smith, 1967; Hazen *et al*, 1973; Webster, 1978; Alexopoulos and Mims, 1979).

2.2.5 Isolation, Characterisation and Identification of Yeasts

Aliquots of 0,1 ml homogenised sludge were plated on Yeast Malt Extract Agar (YMEA) (Clesceri *et al*, 1989). Plates were incubated at 30 °C for 72 h. Colonies were purified by

repeated subculturing on YMEA and purity confirmed microscopically. Microscopic observation of cellular morphology was conducted. Pure cultures were identified using ATB32C (Montalieu-Vercieu, France) identification kits in conjunction with the ATB32C data-base and appropriate literature (Barnett *et al*, 1983).

2.2.6 Characterisation and Identification of Algae and Protozoa

Wet mounts on microscope slides of freshly collected sludge samples were viewed and recorded under the photomicroscope. Algae were identified on the basis of pigmentation and gross cellular morphology (Palmer, 1980). Appropriate techniques were used to identify algae (Bellinger, 1980 and Clesceri *et al*, 1989). Protozoa were identified on the basis of cellular morphology eg., shape, size and inclusions, (Buchsbaum, 1976; Van Rensburg *et al* 1980; Eikelboom and Van Buijsen, 1983).

2.3 RESULTS AND DISCUSSION

2.3.1 Eubacteria

The choice of suitable culture media is essential in order to achieve the best possible representation and enumeration of microbial population in activated sludge. To isolate a large and representative eubacterial range, the medium employed must be nonselective. Previous work by Ishwarlall (1990) showed Casitone Glycerol Yeast Agar (CGYA), when compared, to several other media, to be the ideal medium supporting the growth of the greatest diversity of eubacterial types from activated sludge. Viable bacterial numbers in homogenised MLSS samples ranged from 1.38×10^9 to 3.28×10^{10} CFUg⁻¹ (Table 2.1). Analysis of variance (ANOVA) proved that viable counts of bacteria from all sludges except from Southern Works did not differ significantly ($p < 0,01$). The results produced relatively higher viable counts on CGYA, in all plants sampled, comparing favourably with similar work conducted by Pike and Carrington (1972) on domestic waste water treatment installations using CGYA. Some of the notable differences in viable numbers between the plants studied could be attributed to differences in operational parameters such as sludge age, plant design, environmental variables and waste water characteristics which determine the microbial population in activated sludges.

A diverse population of Eubacteria were isolated and identified from the ten sludges under investigation, namely twenty-two different genera. Initial differentiation of the eubacteria isolated, according to Gram reaction's presented larger numbers of Gram positive organisms, when compared to Gram negative isolates (Table 2.2 and 2.3). These were further

characterised according to presence or absence of spores. The non-sporeforming rods were further characterised and final identification presented a large variety of different genera (Table 2.2). The Gram-positive sporeforming rods (*Bacillus* spp.) represented a major portion of the overall eubacterial population (Table 2.4). Comparatively few cocci were detected during the present survey (Table 2.4).

Bacillus spp. were detected in all sludges investigated (Table 2.4, Figs. 2.2 - 2.11). The most predominant genera recorded for sludges under investigation were, *Bacillus* spp., *Cellulomonas* spp., *Pseudomonas* spp., *Listeria* spp., *Lactobacillus* spp., and *Microbacterium* spp. detected at 100%, 80%, 50%, 40% and 40% frequencies, respectively (Table 2.4). Sludges obtained from New Germany, Tongaat, Pietermaritzburg and Amanzimtoti works showed highest bacterial diversity (Table 2.4, Fig 2.2, 2.3, 2.4 and 2.7). Hammarsdale sludge showed lowest bacterial diversity (Table 2.4, Fig 2.11). Gram-positive *Bacillus* sp. was the predominant bacterial type detected in sludges from Tongaat (Fig 2.3), Pietermaritzburg (Fig 2.4), Kwa Mashu (Fig 2.5), Phoenix (Fig 2.6), Amanzimtoti (Fig 2.7), Southern Works (Fig 2.9), Northern Works (Fig 2.10) and Hammarsdale (Fig 2.11). Sludges from New Germany, (Fig 2.2) and Umlaas (Fig 2.8) were predominated by *Pseudomonas* spp. and *Alcaligenes* spp., respectively. Previous research by Allen (1940) on homogenised sludge of domestic origin and Pike (1975) on activated sludge showed that most of the eubacterial population were Gram-negative bacilli belonging to the genera *Pseudomonas*, *Flavobacterium*, and *Achromobacter*. In contrast, the results of the present study showed that except for the New Germany and Umlaas activated sludges, the remaining eight plants sampled exhibited a predominantly Gram-positive eubacterial population (Table 2.4). Gram-positive spore-forming bacteria predominated over Gram-positive non-spore

formers. This occurrence could be attributed to environmental stress such as a drastic change in the waste water being treated, agitation within the aeration basin, facilitating the adaptation of certain types of species which are more resistant to such environments. Several workers have reported that rod-shaped bacteria occur at higher frequencies than cocci (McKinney and Weichlein, 1953; Dias and Bhatt, 1964; Kasan and Baecker, 1989a). The results of the present study confirm these findings (Table 2.4).

The type of waste treated ie., domestic, industrial or mixtures, influences the eubacterial microflora present. Water treatment plants such as New Germany and Umlaas that treated primarily domestic waste contained larger numbers of Gram-negative rods such as *Pseudomonas* sp. and *Alcaligenes* sp. (Fig 2.8), which are potential pathogens of gastrointestinal and faecal origin. These results substantiated the findings of Dias and Bhatt (1964) and Hänel (1988). Plants that treated a mixed influent type ie., both domestic and industrial, displayed predominantly Gram-positive bacteria (Table 2.4). Therefore, the chemical nature of wastes treated and the type of substrate, determines bacterial diversity (McKinney and Weichlein, 1953; Hänel, 1988). Overall evaluation of all ten water treatment installations showed the activated sludge community to be specialised, and in agreement with previous research by Pike (1975).

2.3.2 Filamentous Bacteria

Filamentous bacteria have been classified by Eikelboom and van Buijsen (1983) into 29 categories. Classification is based on phase contrast microscopic observation of morphology,

relationship to other micro-organisms and floc particles, and staining characteristics. A diverse population of filamentous bacteria was identified in activated sludges from the ten waste water treatment plants under investigation. Eleven types were detected on the basis of morphology and staining characteristics from the ten sludges investigated (Table 2.5). All of these have previously been identified in domestic and industrial activated sludge plants (Cyrus and Sladaka, 1970; Farquhar and Boyle, 1971; Eikelboom, 1975; Strom and Jenkins, 1984). Some sludges contained a greater variety of filamentous types than others (Table 2.6). Predominant filament types identified were *Microthrix parvicella* (Fig 2.12), Type 0041 (Fig 2.13) followed by *Nocardia* sp. (Fig 2.14), *Sphaerotilus natans* (Fig 2.15) and Type 0675 (Fig 2.16) occurring at frequencies of 100%, 60%, 50%, 40% and 30%, respectively. The results of the present study confirmed the findings of Blackbeard *et al*, (1986) who showed Type 0092 and *M.parvicella* to be predominant filamentous types present in activated sludge plants in South Africa. The high frequency of occurrence of *M.parvicella*, Type 0041 and *Nocardia* sp. can be attributed to their ability to adapt and withstand environmental stress.

Some of the common problems experienced by waste water works are bulking and foaming. Studies have shown that such phenomena are related to increased presence of certain filamentous types such as Type 0041, *M.parvicella*, Type 0675 and *Nocardia* sp. (Eikelboom, 1977; Jenkins *et al*, 1986). The New Germany Waste Water Treatment Plant showed sludge bulking which could be related to an abundance of Type 0041, *M.parvicella* and Type 0675 whose presence was confirmed microscopically. In agreement with work done by Blackbeard and Ekama (1984), who related the presence of *Nocardia* sp. to foaming problems, the present work has demonstrated that overgrowth of *Nocardia* and *M.parvicella*

could be related to the foaming problems experienced at the Tongaat Waste Water Works. Pipes (1978) suggested that *Nocardia* growth (and foam formation) in activated sludge was a problem associated with warm temperatures ($> 18^{\circ}\text{C}$). The presence of high levels of emulsifiable fatty material (oil and grease) in the wastewater has been associated with *Nocardia* growth and foaming in activated sludge plants by several workers (Eikelboom, 1975; Pipes, 1978; Lewis, 1979). The present study also revealed no marked difference between filamentous bacterial populations of domestic and industrial sludges eg., New Germany treating primarily domestic waste, and Amanzimtoti treating industrial waste, displayed four out of five common filamentous types (Table 2.5). This was contrary to the findings of Eikelboom (1977) which showed that a clear difference exists between the population of filamentous bacteria in plants receiving domestic sewage and those treating industrial waste water.

2.3.3 Fungi

Fungi are not normally found as dominant organisms in the activated sludge treatment process (Tomlinson and Williams, 1975). Most isolates recovered from the ten sludges investigated belonged to common genera (Table 2.7) *Penicillium* sp., *Cladosporium* sp. and *Aspergillus* sp. were present in each of Tongaat, Phoenix and Southern Works (Table 2.6). Phoenix sludge contained the greatest diversity of fungal types (Table 2.6). Tomlinson and Williams (1975) reported *Geotrichum candidum* and *Trichosporon* sp. to be present in large numbers in activated sludge of industrial origin. In contrast, our results show that although fungi prevailed in all plants studied, there was no degree of predominance of specific genera

in any of the plants, although *Geotrichum* sp. did occur in a plant treating industrial waste only i.e., Amanzimtoti. The fungi are known to play an important role in the stabilization of organic wastes. Like the bacteria, the fungi can metabolize almost every type of organic compound found in industrial wastes. The fungi have the potential ability to predominate over the bacteria but they do not, except under unusual environmental conditions. The filamentous nature of most of the fungi found in industrial wastes makes them undesirable since they do not form a tight compact floc which settles easily, sometimes giving rise to bulking sludge (McKinney, 1957; Hänel, 1988). The effect of seasonal variations on fungal enumeration in activated sludge should be taken into account when conducting studies of this nature, since fungi are present in largest numbers during autumn, declining with summer and spring. Winter shows smallest number of colonies and fewest species (Cooke, 1969).

2.3.4 Yeasts

Although yeast are generally included with moulds and broadly termed fungi, for the purpose of the present study, they have been addressed separately. Studies by Kahn *et al* (1990) showed yeast to be present in insignificant numbers and sometimes absent from activated sludge plants. In contrast, the results of the present study have shown that yeasts were present in all plants although not predominating among the microflora. Eleven yeast types were isolated and identified from the ten sludges (Table 2.8). New Germany and Kwa Mashu sludges contained the greatest diversity of yeast types comparatively. *Candida* sp. was the most common type of yeast isolated. Water installations treating large domestic loads generally produce yeasts of gastrointestinal and faecal origin (Cooke, 1958). This has

been confirmed by the present research which showed most of the isolates to be common clinical pathogens (Table 2.8). Studies by Cooke, 1969 have shown that accuracy of yeast detection and enumeration in polluted waters could present problems, since not all yeasts respond to culture and agar media. A number of species commonly present in some sewage and polluted water samples respond well to agar culture, others propagate only in liquid culture, while others occur equally well in both. The YMEA medium selected for the present research accommodated a variety of yeast types (Table 2.8).

2.3.5 Algae and Protozoa

Algae are known to be common inhabitants in most water systems, and are known to play a role in the treatment of waste eg., sewage using stabilisation ponds (Palmer, 1980) but little is known about their contribution in the activated sludge process. The algae detected were of the common fresh and polluted water types (Table 2.9). Twenty-six types of algae were observed in the ten sludges under investigation. Northern Works, Phoenix, New Germany and Tongaat contained the highest diversity of algal types. *Chlorella* sp. (Fig 2.17) was detected in all plants except New Germany (Table 2.9). *Anabaena* sp. (Fig 2.18) and *Volvox* sp. (Fig 2.19) also occurred at relatively high frequencies (Table 2.9). The high frequency of occurrence of these types was similar to most fresh water systems. Other common algae detected in the plants studied were *Coelastrum* sp. (Fig 2.20), *Sphaerocystis* sp. (Fig 2.21), *Ulothrix* sp. (Fig 2.22), *Fragilaria* sp. (Fig 2.23), *Diatoma* sp. (Fig 2.24) and *Chlamydomonas* sp. (Fig 2.25) occurring at frequencies of 40%, 30%, 30%, 20%, 20%, 20%, respectively. These algae make a substantial impact in sewage stabilization ponds but little is known about their contribution in the activated sludge treatment process. The present

results displayed little comparative difference in the algal microflora of sludge of domestic and industrial origin.

In most activated sludges several higher organisms such as protozoans and rotifers may be found together with the bacteria that form flocs. These organisms feed on bacterial cells that are free in the liquid and at the edge of flocs, thus contributing to clarification and subsequently to improved effluent quality (Curds and Cockburn, 1970; Eikelboom and Van Buijsen, 1983). A variety of sixteen genera of protozoa were observed in the ten sludges under investigation, *Paramecium* sp. (Fig 2.26), *Aspidisca* sp. (Fig 2.27), *Euplotes* sp. (Fig 2.28) and *Amoeba* sp. (Fig 2.29) were the most predominant among the ten sludges, occurring at frequencies of 80%, 80%, 70% and 70% respectively (Table 2.10). Other genera detected in the plants were sedentary forms such as *Vorticella* sp. (Fig 2.30), *Potteriodendron* sp. (Fig 2.31) and *Opercularia* sp. (Fig 2.32). The predominant protozoans identified were mainly free swimming and crawling over the floc surface (Gray, 1990) and belonged to the class *Ciliata* although flagellates and rhizopods were also present when considered together as a single group. Contrary to the findings of Curds (1982) that the activated sludge process selects for sedentary forms, the present work has shown that the free swimming ciliates predominated. Previous research by McKinney (1957) showed that the free swimming *Ciliata* were motile and presented superior mechanisms for obtaining the bacteria and other food components. Therefore, they predominated over the *Zoo-Mastigophora* and *Phyto-Mastigophora*. In addition, this could be attributed to the high degree of agitation in the aeration basin, thus preventing surface attachment of the sedentary protozoans. This type of environment proved more suitable to free swimming ciliates. It was also noted that Northern Works sludge contained the greatest diversity of protozoan

types, when compared to other plants studied. The succession of protozoa offers a good index of stability of the biological waste treatment system (McKinney, 1957). Some of the positive effects of protozoans in the activated sludge treatment process are firstly, elimination of particulate sewage constituents such as viruses and pathogenic bacteria. Secondly, elimination of dissolved and colloiddally dispersed constituents and finally, the production of glutinous substances which assist in the formation of the sludge flocs by ciliates such as *Paramecium* sp. and *Vorticella* sp. (Hänel, 1988).

2.4 CONCLUSIONS

Gram positive eubacteria predominated when compared to Gram negative isolates. The activated sludge environment favoured sporeformers to non-sporeformers and rod shaped bacteria to cocci. In keeping with previous national findings, Type 0092 and *M. parvicella* were the most predominant filamentous types detected during the present investigation. Most of the fungi detected belonged to the common genera and the yeast were mostly common clinical pathogens. Twenty-six types of algae were observed, belonging to the fresh and polluted water genera. A variety of sixteen genera of protozoa were observed. The predominant types were mainly free swimming and crawling over the floc surface and belonged to the class *Ciliata*. The survey has facilitated greater understanding of the microbiology of activated sludge in the Natal region and facilitated global comparison of studies of a similar nature. The survey also showed that there was notable variations in the microbial constituents of the sludges under investigation and therefore, differences in the biosorptive capacities of sludges, if any, could be substantiated by referring to their

individual microbial content, which would determine the biosorption of heavy metals. Therefore, information obtained during the present survey provided the foundation for further investigations.

Table 2.1. Nature of wastes treated and eubacterial viable counts* for ten activated sludge plants.

PLANT NAME	WASTE TYPE	VIABLE COUNT (colony forming units g ⁻¹)
New Germany (NG)	15% Industrial 85% Domestic	$7.10 \times 10^9 \pm 4.70 \times 10^8$
Tongaas (T)	40% Industrial 60% Domestic	$5.51 \times 10^9 \pm 6.33 \times 10^8$
Pietermaritzburg (PMB)	5% Industrial 95% Domestic	$5.10 \times 10^9 \pm 3.86 \times 10^8$
Kwa Mashu (KM)	100% Domestic	$1.04 \times 10^{10} \pm 1.70 \times 10^9$
Phoenix (P)	100% Domestic	$4.99 \times 10^9 \pm 7.86 \times 10^8$
Amanzimtoti (A)	100% Industrial	$2.48 \times 10^9 \pm 2.81 \times 10^8$
Umlaas (U)	100% Domestic	$6.27 \times 10^9 \pm 3.19 \times 10^8$
Southern Works (SW)	16% Industrial 84% Domestic	$3.28 \times 10^{10} \pm 1.63 \times 10^{10}$
Northern Works (NW)	8% Industrial 92% Domestic	$3.79 \times 10^9 \pm 2.41 \times 10^8$
Hammarsdale (H)	95% Industrial	$1.38 \times 10^9 \pm 3.35 \times 10^8$

* Values are expressed as means and standard deviations of three determinations.

Table 2.2 Gram positive non-sporeforming rod-shaped bacteria.

ISOLATE CODE	FAC. ANAEROBE	CATALASE	MOTILITY	PIGMENT	DIAM. μ m	MORPHOLOGY	IDENTIFICATION
U1	-	+	+	-	1.14	short rods	<i>Arthrobacter</i>
U2	-	+	-	-	0.94	coccobacilli	<i>Renibacterium</i>
U19	+	+	+	-	0.92	coccobacilli	<i>Cellulomonas</i>
PMB1	+	+	+	+(blue)	0.94	short rods, filaments	<i>Cellulomonas</i>
PMB3	+	+-	+	+(brown)	0.83	short rods	<i>Listeria</i>
PMB8	+	+	+	-	0.89	coccobacilli	<i>Cellulomonas</i>
PMB10	-	+	-	-	1.50	long, thick rods in clumps	<i>Caseobacter</i>
PMB12	+	+	+	-	0.93	short rod	<i>Cellulomonas</i>
PMB13	-	+	-	-	1.85	long, thick rods in clumps	<i>Caseobacter</i>
PMB14	+	+	+	-(brown)	0.86	short, thin rods	<i>Cellulomonas</i>
PMB25	+	+	+	-	1.07	irregular rods	<i>Curtobacterium</i>
PMB26	+	+	+	+(pink)	1.19	irregular rods	<i>Curtobacterium</i>
NG9	+	+	+	+(yellow)	0.85	short rods	<i>Cellulomonas</i>
NG14	-	+-	+	+(yellow)	1.00	short rods	<i>Microbacterium</i>
NG15	-	+	+	+(yellow)	1.04	med. to long	<i>Microbacterium</i>
NG16	+	+	+	-	0.86	short rods	<i>Listeria</i>
NG17	-	+	+	+(cream)	1.10	short rods	<i>Arthrobacter</i>
P1	+	+	+	+(yellow)	0.84	coccobacilli	<i>Cellulomonas</i>
P11	-	+-	+	-	1.24	med. rods in chains	<i>Kurtzia</i>
P14	+	+	+	+(yellow)	0.97	short, fat rods	<i>Cellulomonas</i>
P17	-	+	+	-	0.97	med. rods	<i>Kurtzia</i>
P19	+	-	+	-	1.07	med. rods	<i>Lactobacillus</i>
P20	-	+	+	+(brown)	1.25	short, fat rods	<i>Arthrobacter</i>
P25	-	+-	+	-	1.14	med., fat rods	<i>Arthrobacter</i>
P27	+	+	-	-	1.28	short, fat coccobacilli	<i>Corynebacterium</i>
NW10	+	+	+	+(yellow)	1.12	short rods	<i>Cellulomonas</i>
NW16	+	+	+	+(orange)	1.42	short, fat rods	<i>Cellulomonas</i>
NW19	-	+	-	+(pink)	1.05	coccobacilli	<i>Caseobacter</i>
T1	+	-	-	-	1.86	short rods	<i>Erysipelothrix</i> sp.
T9	-	-	-	-	1.61	rod-cocci cycle	<i>Listeria</i> sp.
T18	+	+	-	-	1.92	short rods	<i>Listeria</i> sp.
T19	-	-	-	-	1.82	bent rods	<i>Lactobacillus</i> sp.
T25	-	-	-	-	1.43	slender, irreg. rods, rod-cocci	<i>Microbacterium laeviformans</i>
T33	-	-	-	-	1.48	slender, irreg. rods	<i>Microbacterium</i> sp.
A10	-	+	-	-	1.65	"	<i>Microbacterium</i> sp.
A25	+	+	+	-	1.48	rod-cocci cycle	<i>Listeria</i> sp.
S5	-	-	-	-	1.59	short rods	<i>Erysipelothrix</i> sp.
H4	+-	-	+	-	1.53	slender, irreg. rod, rod-cocci	<i>Microbacterium</i> sp.
H12	-	+	-	-	1.80	slender, irreg. rods	<i>Microbacterium</i> sp.

Table 2.3 Gram negative rod-shaped bacteria.

CODE	I.D. KIT	PROFILE	IDENTIFICATION
NG1	API 20NE	1004464	<i>Pseudomonas acidovorans</i>
NG2	API 20NE	1004464	<i>Pseudomonas acidovorans</i>
NG3	API 20NE	140124	<i>Pseudomonas sp</i>
NG18	API 20NE	7034644	<i>Vibrio sp</i>
NW6	API 20NE	0000014	<i>Pseudomonas diminuta</i>
NW11	API 20E	4246125	<i>Vibrio fluvialis</i>
NW21	API 20NE	0000156	<i>Alcaligenes spp</i>
KM5	API 20E	0004771	<i>Klebsiella rhinoscleromatis</i>
KM7	API 20E	0004151	<i>Klebsiella sp</i>
KM16	API 20E	3206127	<i>Aeromonas hydrophila</i>
A1	API 20NE	2450004	CDC Sr. II B
A5	API 20E	4244124	<i>Vibrio alginolyticus</i>
A13	API 20NE	2432004	<i>Flavobacterium spp</i>
S12	API 20NE	0400204	<i>Pseudomonas vesicularis</i>
S13	API 20NE	1467540	<i>Agrobacterium radiobacter</i>
PMB31	API 20NE	0461345	<i>Pseudomonas paucimobills</i>
U7	API 20NE	0000050	<i>Alcaligenes spp</i>
T37	API 20NE	1460004	<i>Pseudomonas vesicularis</i>
H1	API 20NE	2453204	CDC Sr. II B

Table 2.4 Eubacteria isolated and frequency of occurrence in ten activated sludge plants.

ORGANISM NAME	GRAM (±)	CELL MORPH- OLOGY	SPORES	NG	T	PMB	KM	P	A	U	SW	NW	H
<i>Bacillus</i> sp.	+	rod	+	28.14	49.15	54.28	61.74	66.27	65.34	37.04	63.61	65.30	61.34
<i>Cellulomonas</i> sp.	+	rod	-	3.88	10.18	11.42	6.17	8.43		0.93	13.64	2.61	
<i>Pseudomonas</i> sp.	-	rod	-	52.43	1.69	14.30					4.54	11.30	
<i>Listeria</i> sp.	+	rod	-	4.85	22.03	2.86			0.79				
<i>Lactobacillus</i> sp.	+	rod	-		1.69		14.81	7.83	8.67				
<i>Microbacterium</i> sp.	+	rod	-	1.94	6.78				4.72				19.33
<i>Arthrobacter</i> sp.	+	rod	-	1.94				4.22		4.63			
<i>Vibrio</i> sp.	-	rod	-	1.94					3.15			5.22	
<i>Staphylococcus</i> sp.	+	coccus	-	4.85		5.71							
<i>Eryipelothrix</i> sp.	+	rod	-		5.93						15.15		
<i>Propionibacterium</i>	+	rod	-		2.54		5.35						
<i>Alcaligenes</i> sp.	-	rod	-							53.70		1.74	
<i>Careobacter</i> sp.	+	rod	-			5.71						13.92	
<i>Curtobacterium</i> sp.	+	rod	-			5.71							
<i>Klebsiella</i> sp.	-	rod	-				11.93						
<i>Kurtzia</i> sp.	+	rod	-					7.23					
<i>Corynebacterium</i> sp.	+	rod	-					6.02					
<i>Flavobacterium</i> sp.	-	rod	-						1.57				
<i>Micrococcus</i> sp.	+	coccus	-						15.74				
<i>Rennibacterium</i> sp.	+	rod	-							3.70			
<i>Agrobacterium radiobacter</i>	-	rod	-								3.03		
CDC Ser 103	-	rod	-										19.33

KEY: NG = New Germany, T = Tongaat, PMB = Pietermaritzburg, KM = Kwa Mashu, P = Phoenix, A = Amanzimtoti, U = Umlaas, SW = Southern Works, NW = Northern Works, H = Hammarsdale.

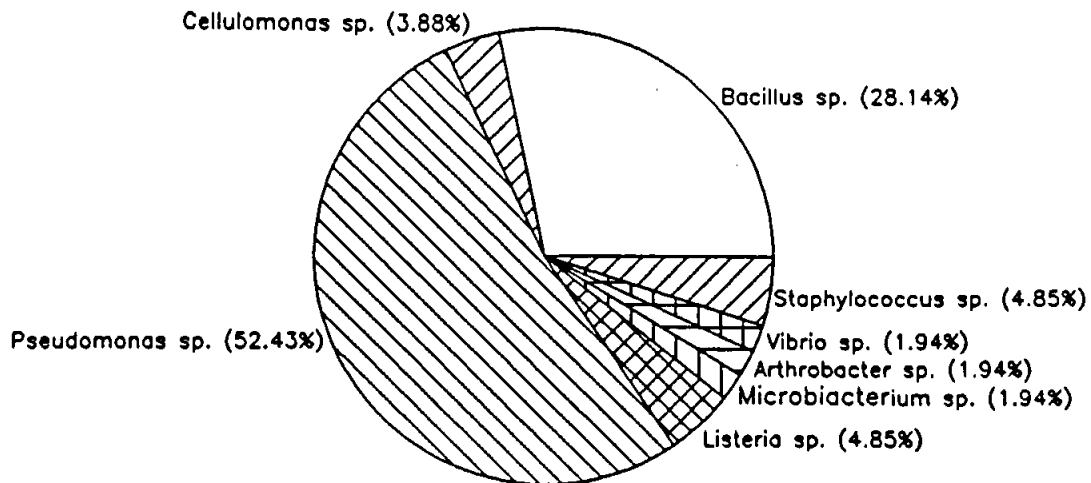


Fig 2.2 Percentage representation of Eubacteria isolated from the New Germany activated sludge plant.

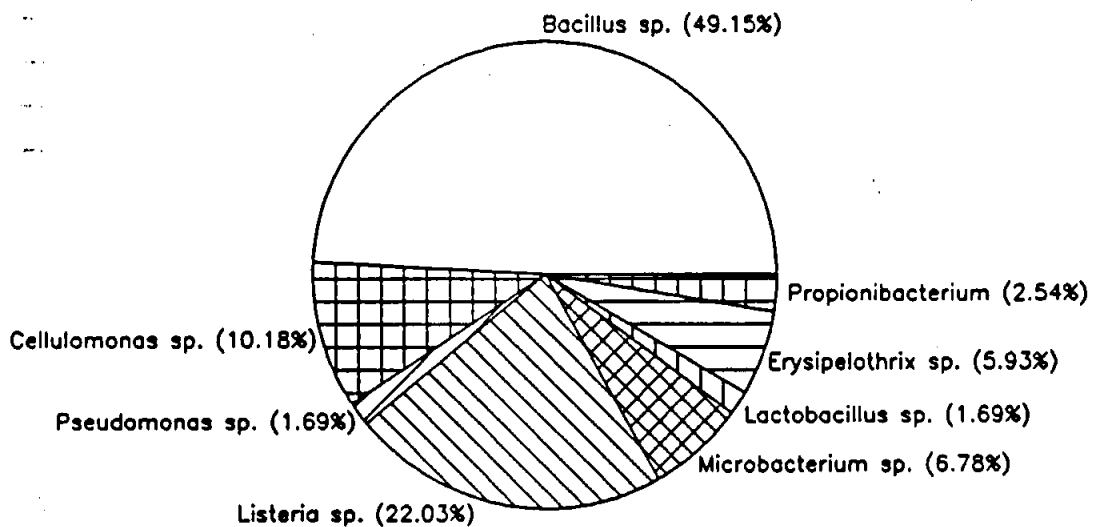


Fig 2.3 Percentage representation of Eubacteria isolated from the Tongaat activated sludge plant.

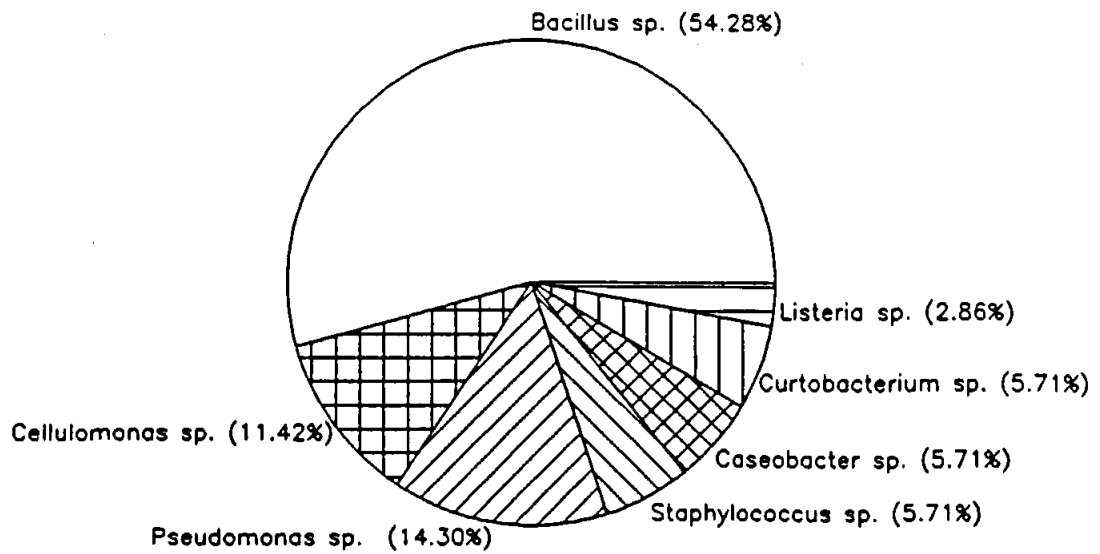


Fig 2.4 Percentage representation of Eubacteria isolated from the Pietermaritzburg activated sludge plant.

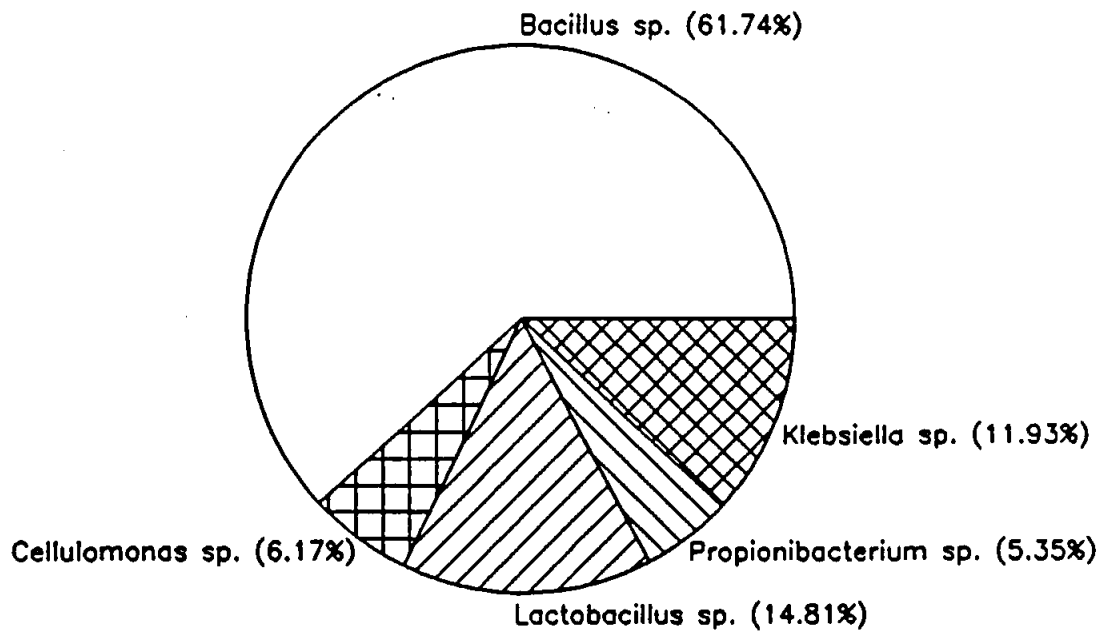


Fig 2.5 Percentage representation of Eubacteria isolated from the Kwa Mashu activated sludge plant.

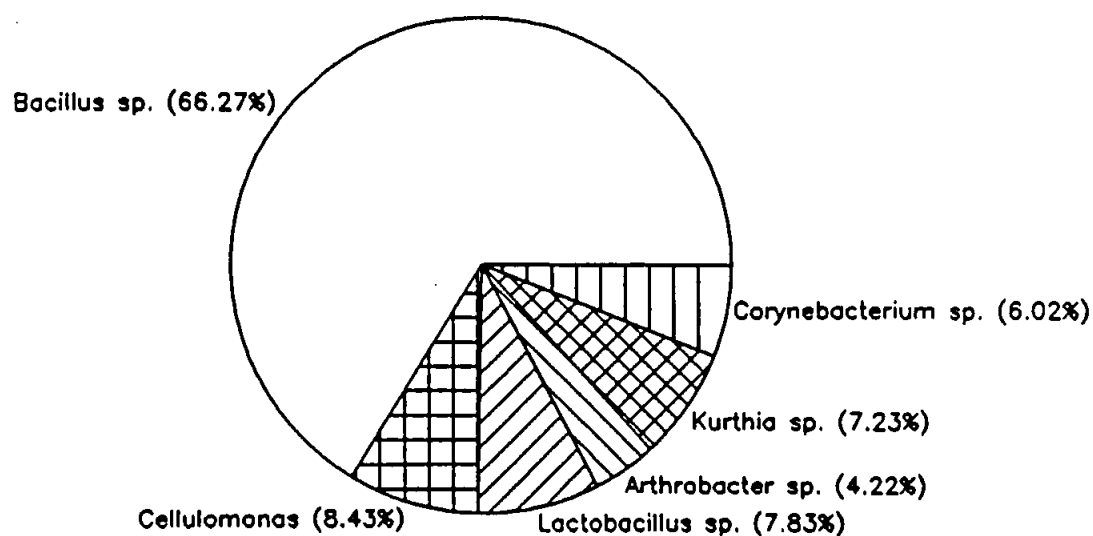


Fig 2.6 Percentage representation of Eubacteria isolated from the Phoenix activated sludge plant.

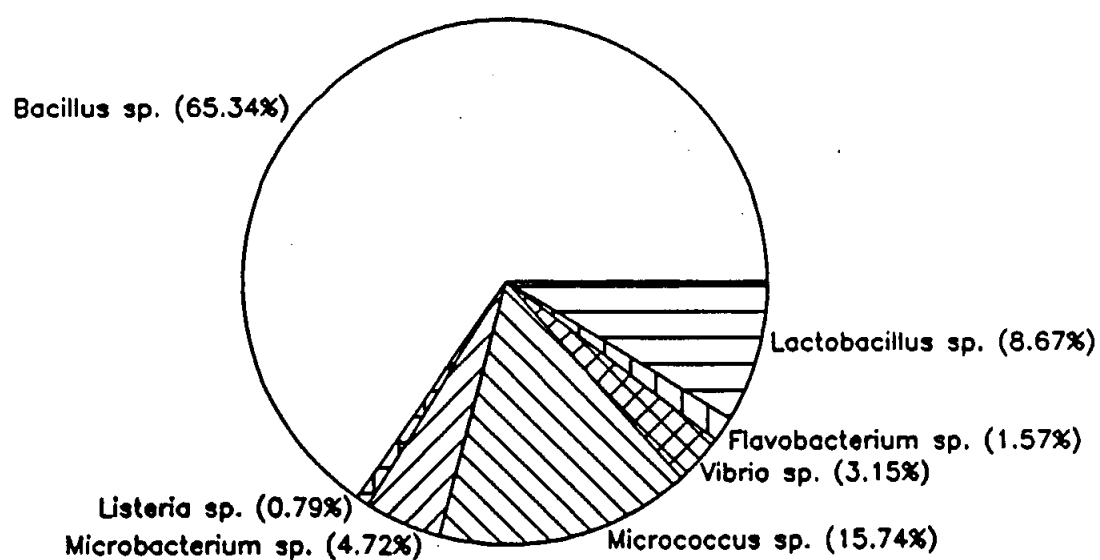


Fig 2.7 Percentage representation of Eubacteria isolated from the Amanzimtoti activated sludge plant.

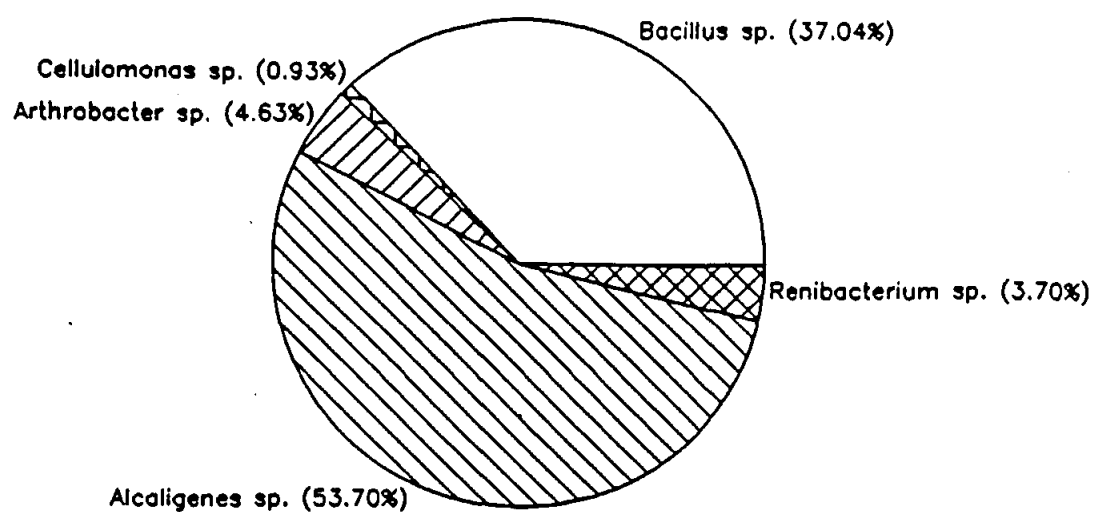


Fig 2.8 Percentage representation of Eubacteria isolated from the Umlaas activated sludge plant.

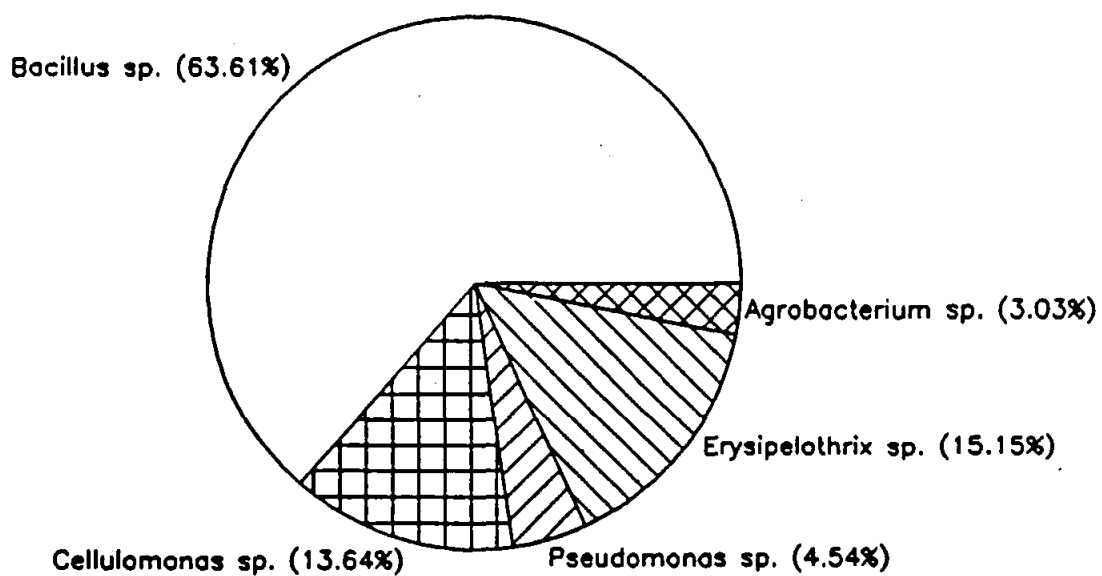


Fig 2.9 Percentage representation of Eubacteria isolated from the Southern Works activated sludge plant.

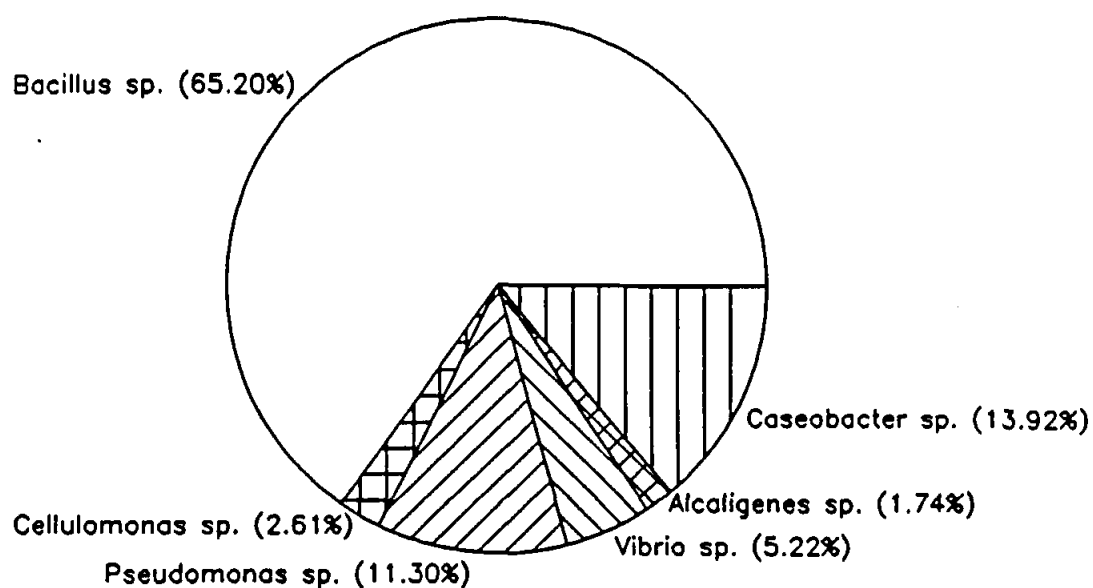


Fig 2.10 Percentage representation of Eubacteria isolated from the Northern Works activated sludge plant.

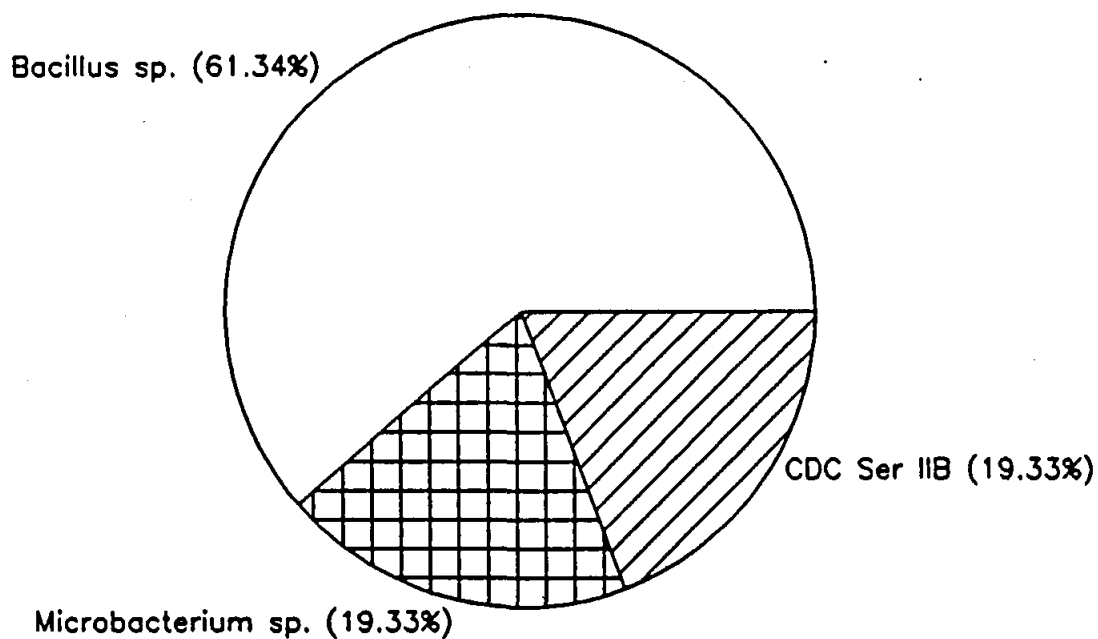


Fig 2.11 Percentage representation of Eubacteria isolated from the Hammarsdale activated sludge plant.

Table 2.5 Morphology, staining characteristics and frequency of occurrence of filamentous bacteria observed in ten activated sludge plants in Natal.

FILAMENT TYPE	GRAM	NEISSER	SULPHUR GRANULES	POLYHYDROXYBUTYRATE GRANULES	TRICHOME DIAMETER (μm)	TRICHOME LENGTH (μm)	TRICHOME LOCATION	CELL SEPTA	INDENTATION	SHEATH	ATTACHED GROWTH	SHAPE AND SIZE	PERCENTAGE FREQUENCY
<i>M. parvicella</i>	+	-	+	+	0.8	100	I	-	-	-	-	-	100
Type 0041	+	-	-	-	1.4	100	I	+	-	+	+	1.4x1.5 square	60
<i>Nocardia</i>	+	+	-	+	1.0	15	I	-	-	-	-	1.0x1.0 variable	50
<i>S. natans</i>	-	-	-	+	1.2	500	E	+	+	+	-	1.4x2.0 rod round end	40
Type 0675	+	-	-	-	1.0	100	I	+	-	+	+	1.0x1.0 square	30
<i>N. limicola II</i>	+	+	-	+	0.8	100	I	-	-	-	-	-	20
Type 0092	-	+	-	+	1.0	50	I	+	-	-	-	0.8x1.5 rectangles	20
Type 1701	-	-	-	+	0.7	70	I	+	+	+	+	0.8x1.2 rod round end	20
Type 021N	-	-	+	+	1.0	300	E	+	+	-	-	1.2x1.5 rectangles	20
<i>Thiothrix I</i>	+	+	+	+	1.7	300	E	+	-	+	-	2.0x3.5 rectangles	10
<i>Cyanophyceae</i>	+	-	-	-	2.7	250	E	+	+	?	-	-	10

Trichome location : E = extends from floc surface, I = found mostly within floc.

Table 2.6 Filamentous bacteria identified and frequency of occurrence in ten activated sludge plants.

NAME OF BACTERIA	NW	P	KM	SW	U	NG	H	PMB	T	A	FREQUENCY OF OCCURRENCE
<i>Microthrix parvicella</i>	*	*	*	*	*	*	*	*	*	*	100
Type 0041		*	*	*	*	*				*	60
<i>Nocardia</i> sp.						*	*	*	*	*	50
<i>Sphaerotilus natans</i>	*					*		*		*	40
Type 0675		*			*	*					30
<i>Nostocoida limicola II</i>									*	*	20
Type 0092	*				*						20
Type 1701				*			*				20
Type 021N				*					*		20
<i>Thiothrix l</i>			*								10
<i>Cyanophyceae</i>					*						10

KEY: NG = New Germany, T = Tongaat, PMB = Pietermaritzburg, KM = Kwa Mashu, P = Phoenix, A = Amanzimtoti, U = Umlaas, SW = Southern Works, NW = Northern Works, H = Hammarsdale, * = presence of organism.



Fig 2.12

M. parvicella showing intracellular granules (G) (X 1 000) phase contrast.

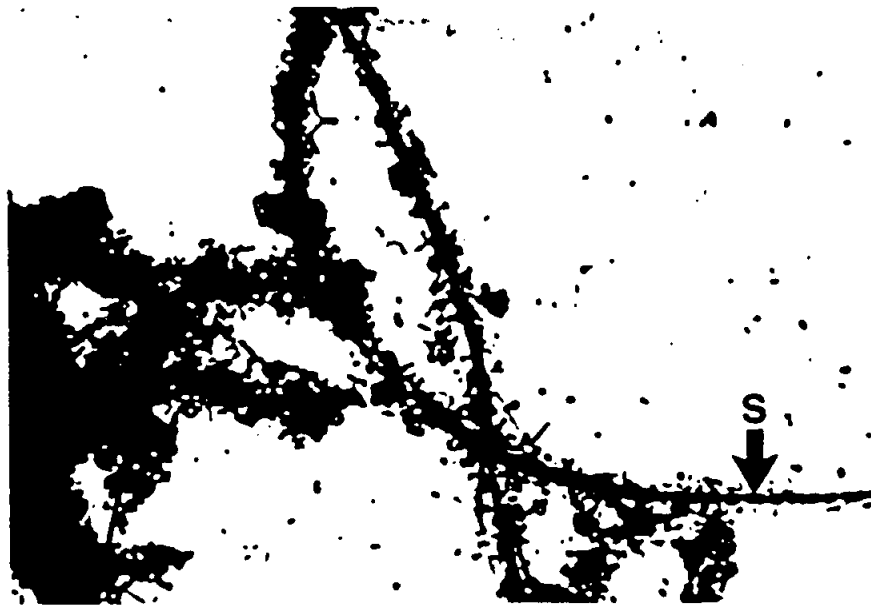


Fig 2.13

Smoothly curved filament Type 0041 exhibiting square-shaped cells (S) (X 1 000) phase contrast.



Fig. 2.14 True branching of *Norcardia* sp with irregularly bent short filaments (X 1 000) phase contrast.

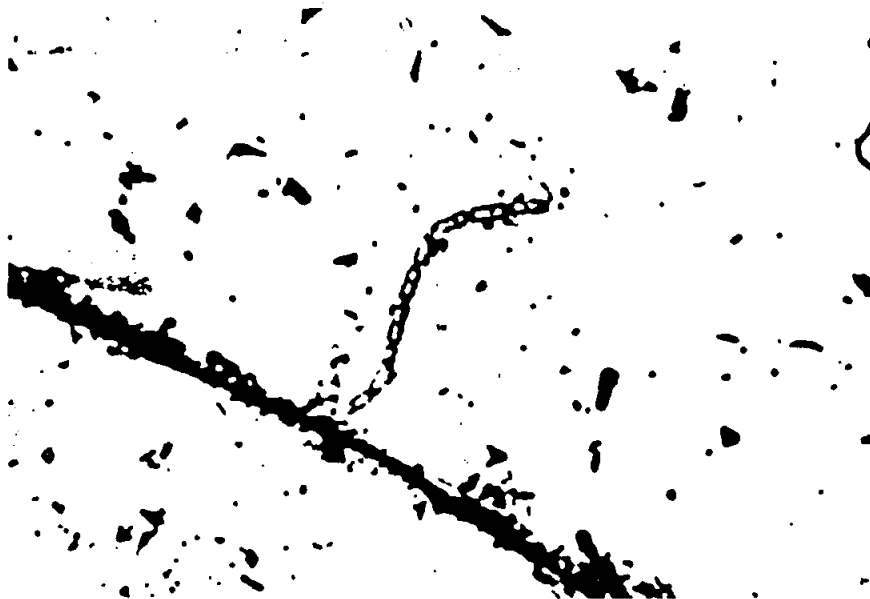


Fig. 2.15 *S. natans*, round-ended rod shaped cells with indentations at septa (X 1 000) phase contrast.

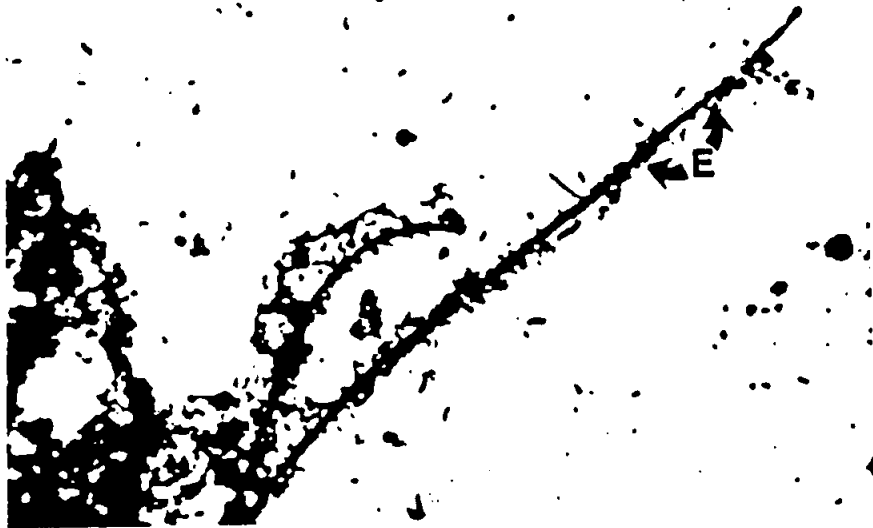


Fig 2.16

Epiphytic growth (E) covering Type 0675 (X 1 000) phase contrast.

Table 2.7 Fungi isolated and frequency of occurrence in ten activated sludge plants.

NAME OF FUNGUS	T	P M B	U	N W	A	P	S W	N G	K M	H	FREQ. OF OCCURR- ENCE
<i>Penicillium</i> sp.	*					*	*				30
<i>Cladosporium</i> sp.			*			*			*		30
<i>Aspergillus</i> sp.	*				*		*				30
<i>Harpella</i> sp.		*						*			20
<i>Fusarium</i> sp.			*				*				20
<i>Trichoderma</i> sp.	*		*								20
<i>Chrysosporium</i> sp.				*		*					20
<i>Helminthosporium</i> sp.					*	*					20
<i>Geotrichum</i> sp.		*			*						20
<i>Phoma</i> sp.				*							10
<i>Chaetomium</i> sp.					*						10
<i>Rhizopus</i> sp.	*										10
<i>Gonatobotrys</i> sp.			*								10
<i>Botrytis</i> sp.				*							10
<i>Epidermophyton</i> sp.					*						10
<i>Coriolus</i> sp.						*					10
<i>Zygorhynchus</i> sp.						*					10
<i>Enterobryus</i> sp.						*					10
<i>Pestalotia</i> sp.								*			10
<i>Colletotrichum</i> sp.								*			10
<i>Trichophyton</i> sp.									*		10
<i>Nematogonum humicola</i>										*	10
Deuteromycete		*									10
Myxomycete						*					10

KEY: NG = New Germany, T = Tongaat, PMB = Pietermaritzburg, KM = Kwa Mashu, P = Phoenix, A = Amanzimtoti, U = Umlaas, SW = Southern Works, NW = Northern Works, H = Hammarsdale, * = presence of organism.

Table 2.8 Yeasts isolated and frequency of occurrence in ten activated sludge plants.

NAME OF YEAST	T	P M B	U	N W	A	P	S W	N G	K M	H	FREQUENCY OF OCCURRENCE
<i>Candida</i> sp.		*			*	*			*	*	50
<i>Rhodotorula rubra</i>			*	*				*		*	40
<i>Tricho cutaneum</i>	*							*		*	30
<i>Rhodotorula</i> sp.			*								10
<i>Candida humicola</i>								*			10
<i>Candida inconspicua</i>									*		10
<i>Candida catenulata</i>									*		10
<i>Candida glabrata</i>	*										10
<i>Pichia</i> sp.					*						10
<i>Tricho capitatum</i>								*			10
<i>Crypto laurentii</i>									*		10

KEY: NG = New Germany, T = Tongaat, PMB = Pietermaritzburg, KM = Kwa Mashu, P = Phoenix, A = Amanzimtoti, U = Umlaas, SW = Southern Works, NW = Northern Works, H = Hammarsdale, * = presence of organism.

Table 2.9 Presence and frequency of occurrence of algae in ten activated sludge plants.

Name of alga	N W	P	K M	S W	U	N G	H	PM B	T	A	FREQUENCY OF OCCURRENCE
<i>Chlorella</i> sp.	*	*	*	*	*		*	*	*	*	90
<i>Anabaena</i> sp.	*	*	*		*				*	*	60
<i>Volvox</i> sp.	*			*		*	*			*	50
<i>Chlorococcum</i> sp.		*		*			*		*		40
<i>Coelastrum</i> sp.		*	*			*	*				40
<i>Anacystis</i> sp.			*	*		*	*				40
<i>Botryococcus</i> sp.	*							*		*	30
<i>Sphaerocystis</i> sp.		*	*			*					30
<i>Ulothrix</i> sp.		*		*				*			30
<i>Gomphosphaeria</i> sp.				*		*	*				30
<i>Fragilaria</i> sp.	*		*								20
<i>Hydrodictyon</i> sp.							*			*	20
<i>Diatoma</i> sp.			*			*					20
<i>Chlamydomonas</i> sp.	*								*		20
<i>Nodularia</i> sp.		*									10
<i>Lepocinclis</i>	*										10
<i>Nitzschia</i> sp.										*	10
<i>Navicula</i> sp.					*						10
<i>Chromulina</i> sp.									*		10
<i>Gonium</i> sp.									*		10
<i>Agmenellum</i> sp.									*		10
<i>Carteria</i> sp.									*		10
<i>Euglena</i> sp.	*										10
<i>Gomphonema</i> sp.		*									10
<i>Cyclotella</i> sp.						*					10
<i>Phytoconis</i> sp.						*					10

KEY: NG = New Germany, T = Tongaat, PMB = Pietermaritzburg, KM = Kwa Mashu, P = Phoenix, A = Amanzimtoti, U = Umlaas, SW = Southern Works, NW = Northern Works, H = Hammarsdale, * = presence of organism.

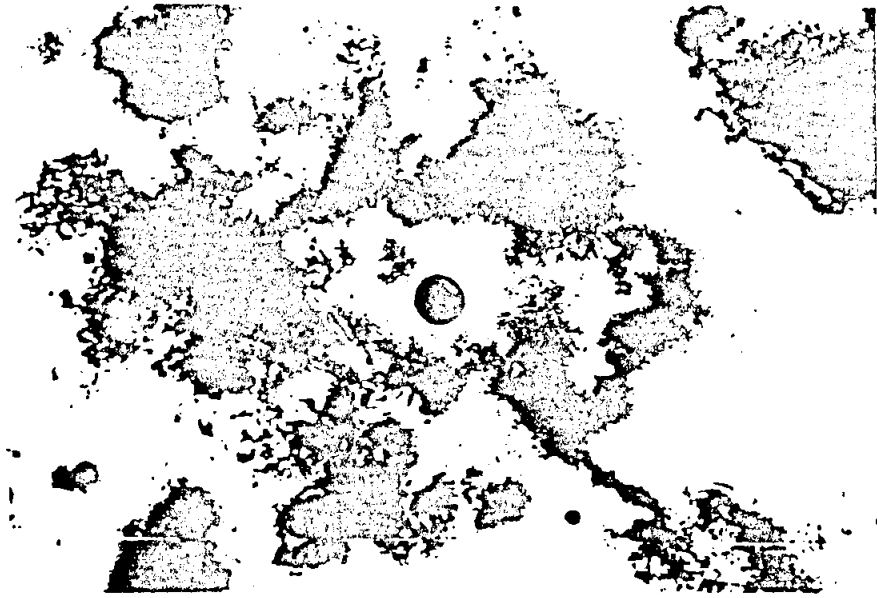


Fig. 2.17

Chlorella sp observed in Tongaat sludge sample (X 1 000) phase contrast.



Fig 2.18 *Anabaena* sp extending from within floc surface (X 1 000).

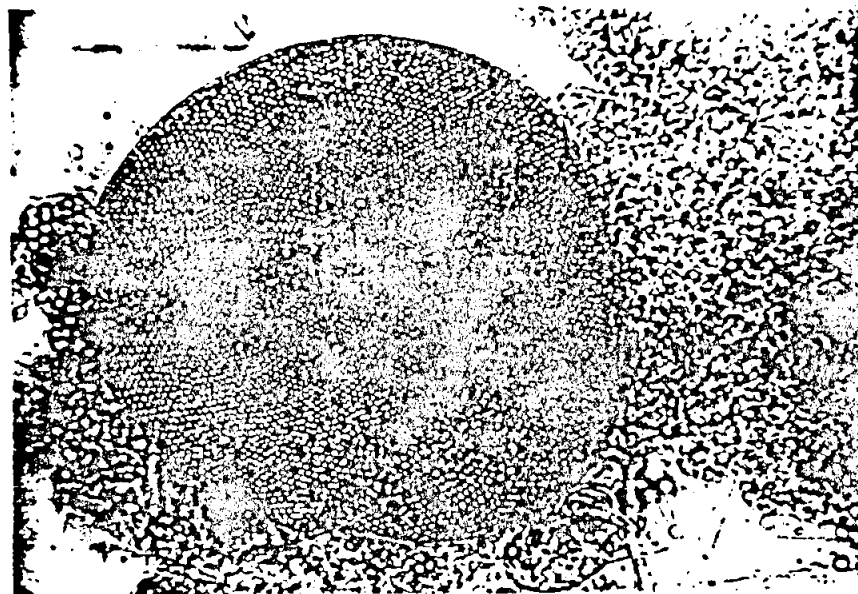


Fig 2.19 Typical *Volvox* sp showing more than 400 cells per colony (X 1 000).



Fig 2.20 *Coelastrum sp*, typical sphere shaped interconnecting cells (X 1 000).



Fig 2.21 *Sphaerocystis sp* associated with sludge floc (X 250).



Fig 2.22

Ulothrix sp, associated with floc material (X 400).

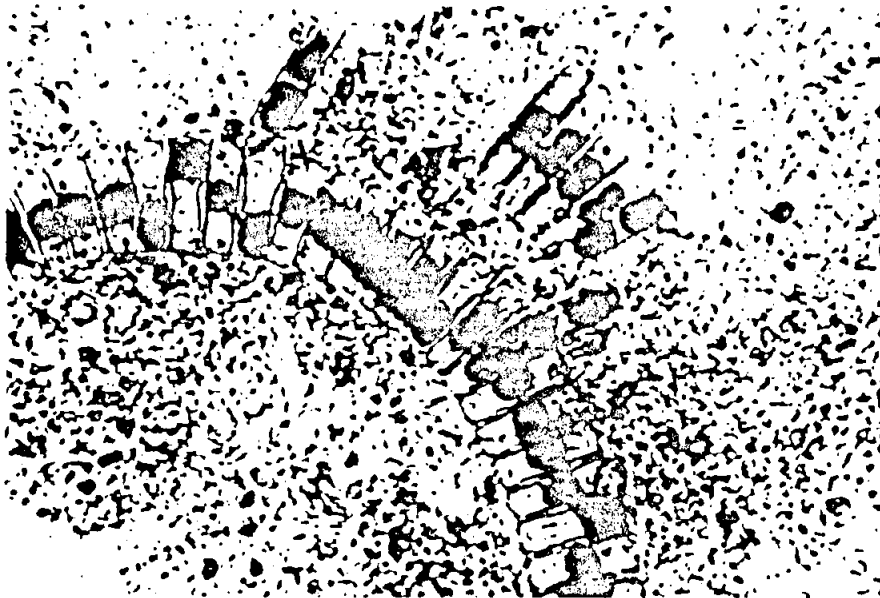


Fig 2.23

Fragilaria sp, showing cells attached side by side to form a ribbon (X 1 000).



Fig 2.24

Diatoma sp., showing characteristic transverse wall markings
(X 1 000).

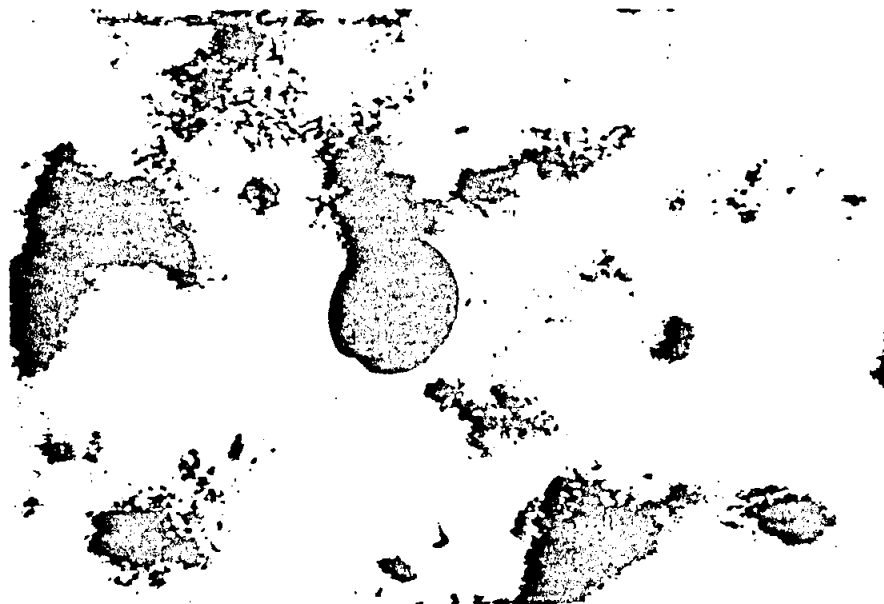


Fig 2.25

Chlamydomonas sp., solitary existence associated with sludge particles
(X 1 000).

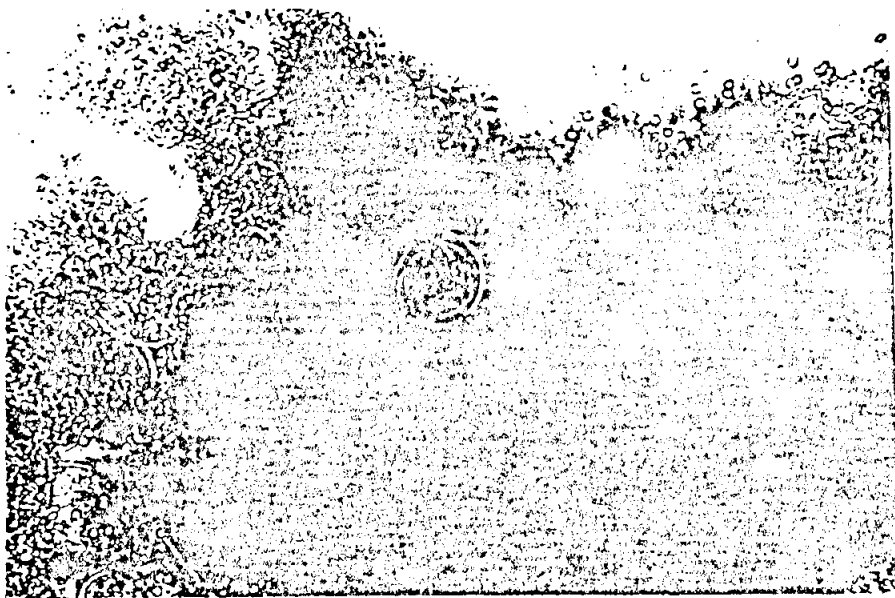


Fig 2.28

Euplotes sp., moving freely in the liquid between the flocs (X 400).

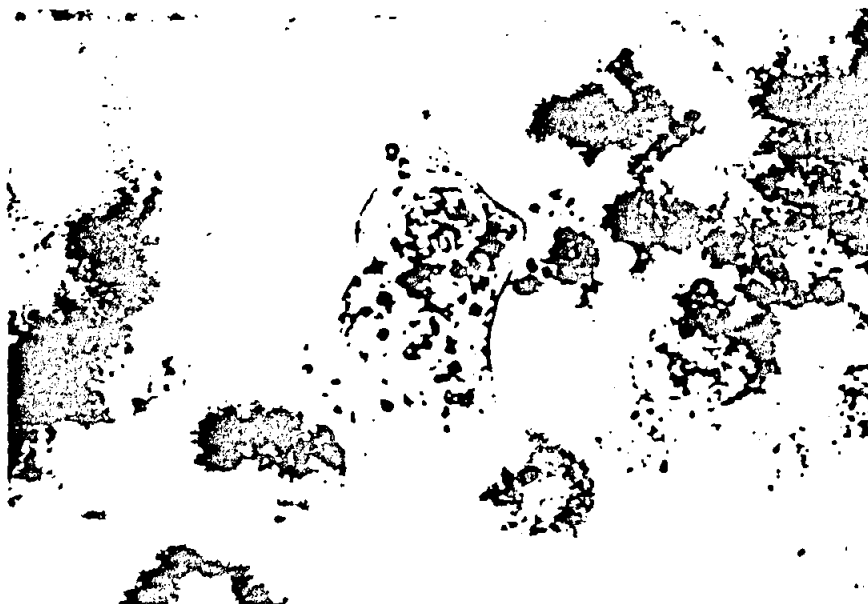


Fig 2.29

Amoeba sp., mobile between floc particles (X 1 000).

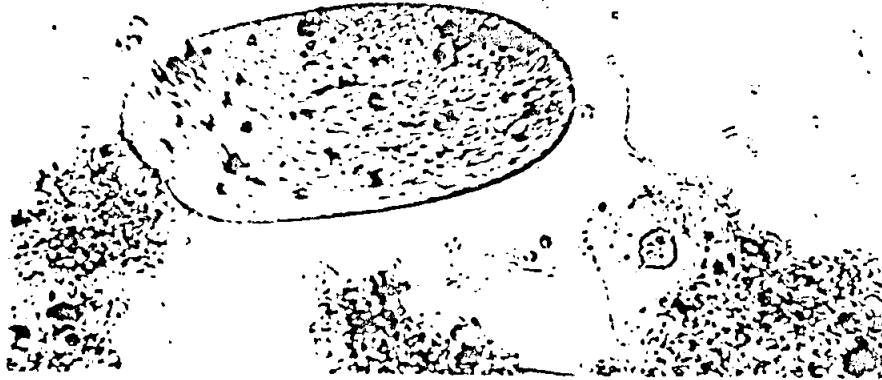


Fig 2.26 *Paramecium sp*, cigar shaped free-swimming ciliate (X 1 000).

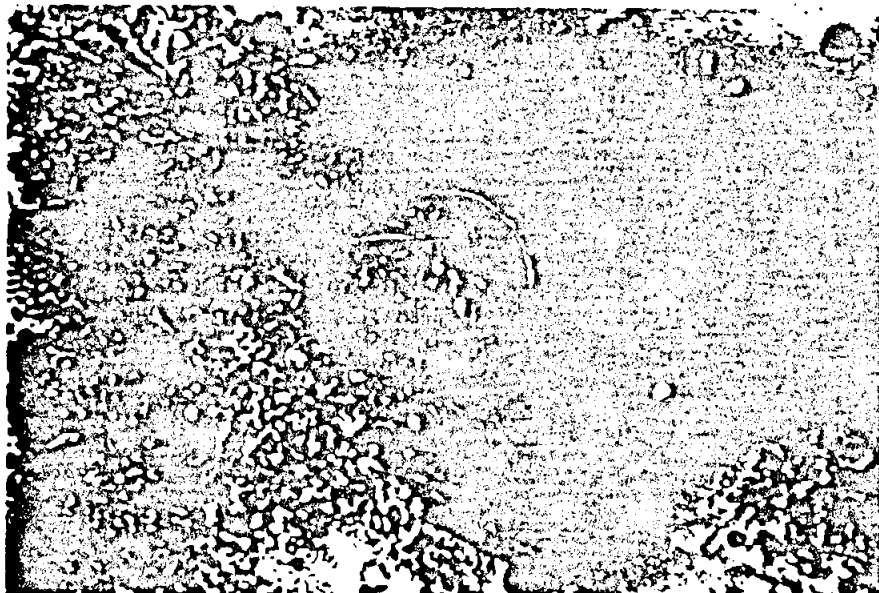


Fig 2.27 *Aspidisca sp*, creeping across the surface of the floc (X 1 000).

Table 2.10 Presence and frequency of occurrence of protozoa in ten activated sludge plants.

Name of protozoan	N W	P	K M	S W	U	N G	H	P M B	T	A	FREQ. OF OCCUR- RENCE
<i>Paramecium</i> sp.	*	*	*	*	*	*		*	*		80
<i>Aspidisca</i> sp.	*	*		*	*	*	*	*		*	80
<i>Euplotes</i> sp.	*	*		*	*		*	*		*	70
<i>Amoeba</i> sp.	*	*		*	*			*	*	*	70
<i>Vorticella</i> sp.	*	*			*			*			40
<i>Blepharisma</i> sp.			*	*		*			*		40
<i>Potteriodendron</i> sp.	*							*		*	30
<i>Colpidium colpodem</i>				*		*	*				30
<i>Epistylis</i> sp.	*							*			20
<i>Spirostomum</i> sp.	*					*					20
<i>Pleuromonas</i> sp.			*	*							20
<i>Opercularia</i> sp.				*	*						20
<i>Carchesium</i> sp.	*										10
<i>Bodo</i> sp.			*								10
<i>Trachelophylum pusillum</i>			*								10
<i>Chilodonella cuculatus</i>									*		10

KEY: NG = New Germany, T = Tongaat, PMB = Pietermaritzburg, KM = Kwa Mashu, P = Phoenix, A = Amanzimtoti, U = Umlaas, SW = Southern Works, NW = Northern Works, H = Hammarsdale, * = presence of organism.

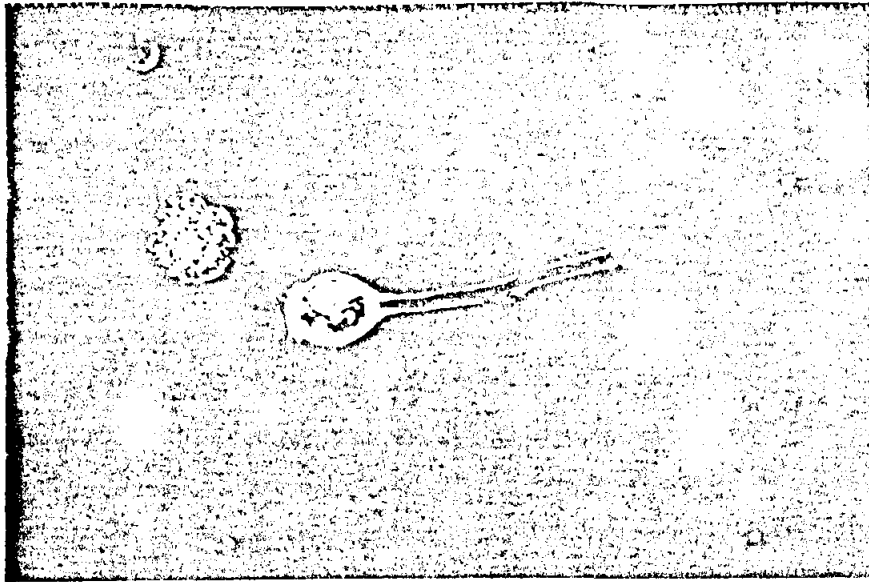


Fig 2.30 *Vorticella* sp, displaying its sessile, solitary nature (X 400).



Fig 2.31 *Poteriodendron* sp, cells are formed in a colony (X 1 000).



Fig 2.32

Opercularis sp., sessile ciliate displaying pronounce peristomal disc (P)
(X 1 000).

3.0 BIOSORPTION OF METAL-IONS BY WASTE ACTIVATED SLUDGES

3.1 INTRODUCTION

Legislation governing protection of the environment is becoming progressively stricter. The disposal of solid and liquid waste products resulting from industrial processes is receiving increasing attention on a global basis. During this era of environmental protection, the environmental biotechnologist is faced with the special challenge to become increasingly innovative in the development of appropriate low-cost and efficient technologies for the treatment and/or reuse of waste products prior to discharge into the environment (Kasan, 1993).

Previous research has shown that substantial quantities of metals present in wastewater may be removed in the activated sludge process of biological waste treatment (Brown *et al*, 1973; Oliver and Cosgrove, 1974; Stoveland *et al*, 1979). The removal of metals in the waste water treatment process is important in maintaining the quality of receiving waters, which is becoming increasingly important in areas where water re-use is essential to maintain potable water supplies. The average annual rainfall of about 497mm for South Africa as a whole is well below the world average of 860mm (Dept. of Water Affairs, 1986). Therefore the need to recycle and reuse water is a priority.

The "heavy metals" have been the cause of particular environmental concern. These are

generally accepted to be chromium, manganese, iron, cobalt, copper, zinc, molybdenum, silver, mercury, cadmium and nickel (Brown and Lester, 1979). Most wastewater treatment works employ primary sedimentation as preliminary treatment to remove the relatively coarse solids. Thus, particulate metals are removed at this stage, and this usually accounts for 40-60% of the total concentrations of metals in raw sewage, with the exception of nickel (15-35%) and manganese (30%) (Lester, 1983). The remainder pass into the biological treatment stage which is normally either activated sludge or trickling filters. A significant proportion of the heavy metals load to this stage is in a soluble form, and its removal depends on a modification of the matrix during the process to promote the formation of species which will settle out (Oliver and Cosgrove, 1974). Possible removal mechanisms have been outlined by Brown and Lester (1979) :-

- (i) physical trapping of precipitated metals in the sludge floc matrix;
- (ii) binding of soluble metal to extracellular polymers;
- (iii) accumulation of soluble metal by the cell; and,
- (iv) volatilisation of metal to the atmosphere.

Various relationships have been identified between metal removal and metal concentrations in activated sludge. Stoveland and Lester (1980) found that the concentrations of heavy metals in solution were directly proportional to the total heavy metal concentrations in samples from laboratory scale activated sludge simulations. Brown *et al*, (1973) observed that over the range of initial metal loading studied ($< 2 \text{ mg l}^{-1}$), the percentage heavy metal removal in full scale activated sludge plants was directly proportional to the initial metal loading. Several other workers have identified equilibrium relationships between metal

adsorbed by activated sludge flocs and free metal ions or soluble metal, which they have fitted to Langmuir and Freundlich isotherms (Cheng, 1973; Neufeld and Hermann, 1975; Sterritt *et al*, 1981). In some cases activated sludge flocs became saturated with metals once a certain concentration of metal had been adsorbed (Neufeld and Hermann, 1975).

The efficient removal of toxic metal ions from water is an important and widely studied research area. Conventional methods employed for the removal of metal ions from effluents include filtration, chemical oxidation/reduction, ion exchange, electrodialysis and evaporative processes. These methods are often only economically viable for large industrial units, due to high costs involved and skilled manpower required. Neither of these technologies, however, is capable of removing trace levels of metal ions. Therefore, a need exists for a low-cost, efficient, process for the removal of trace amounts of heavy metals.

3.1.1 Aims

- i) To determine whether activated sludge would serve as a biosorbent for the removal of heavy metals from pure solutions and industrial effluents.
- ii) To compare the biosorptive capacity of ten waste sludges.
- iii) Quantification of the removal of these heavy metals by the sludges investigated.
- iv) Determine an affinity series, i.e., if there is any preferential binding for metal ion species by the sludges.

- v) To assess any differences in biosorption of metals by sludges from synthetic wastewater containing single metal ions and industrial wastewaters containing mixtures of metal ions.

- vi) To select sludges exhibiting superior metal biosorptive capacities for further investigations.

3.2 METHODOLOGY

3.2.1 Pretreatment of Materials

Polypropylene tubes, vials and all glassware were soaked overnight at ambient temperature in 5% (v/v) Extran MA - 01 alkaline (Lasec, South Africa). Vials and centrifuge tubes were then rinsed three times in deionised water and subsequently dried. Glassware, including pipettes were subjected to a stringent acid wash procedure, i.e., rinsing in 50% (v/v) nitric acid (HNO_3), tap water followed by 50% (v/v) hydrochloric acid (HCl), tap water, and finally, rinsing in triple deionised water with subsequent drying.

3.2.2 Preparation of Solutions

Heavy metal species used for the batch experiments included Cu^{2+} , Cd^{2+} , Cr^{3+} , Cr^{6+} , Ni^{2+} , and Zn^{2+} . A-grade glassware was used throughout, for preparation of metal solutions. Aqueous metal stock solutions of $1\,000\text{ mg l}^{-1}$ were prepared in one litre volumes and stored in 1 l grade volumetric flasks for immediate use as required. Lower concentrations were prepared from stock solutions. Chemicals, $\text{K}_2\text{Cr}_2\text{O}_7$ (BDH, England), $\text{CrK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ (BDH, England), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (BDH, England), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (BDH, England), $(\text{CH}_3\text{COO})_2\text{Cd} \cdot 2\text{H}_2\text{O}$ (BDH, England) and $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (Hopkins and Williams, England) were Analar grade.

3.2.3 Sludge Collection and Preparation

Five litres of return activated sludge (RAS) samples were obtained from each of 10 wastewater treatment plants in Natal, namely, Umlaas, S1, Amanzimtoti, S2, New Germany, S3, Hammarsdale, S4, Pietermaritzburg, S5, Kwa Mashu, S6, Tongaat, S7, Northern Works, S8, Southern Works, S9 and Phoenix, S10. Samples were collected in glass bottles. Dry mass determinations were conducted by dispensing 50 ml of the sludge in to preweighed porcelain dishes. The sludge was subsequently dried overnight in an oven at 105°C. Dried sludge was then weighed. Initial volumes required to obtain final concentration (FC) of 25 000 mg ℓ^{-1} were calculated according to the formula-:

$$IV = \frac{FC \times FV}{IC}$$

IV = Initial volume

FC = Final concentration (25 000 mg ℓ^{-1})

FV = Final volume (100 ml)

IC = Initial concentration (MLSS)

Sludges were concentrated to 25 000 mg ℓ^{-1} by centrifugation using a J-6B Beckman centrifuge at 3 500 x g for 30 min., and pellets subsequently resuspended in 100ml deionised water and stored at 4°C for further use.

3.2.4 Biosorption Procedure

Each of the heavy metal species used in the experiments were prepared separately from 1 000 mg ℓ^{-1} stock solutions when required at final concentrations of 30, 60, 90, 120 and 150mg ℓ^{-1} . Experiments were performed per single metal solution in triplicate. Aliquots of 8 ml metal solutions were dispensed into polypropylene centrifuge tubes. This was followed by addition of 2 ml of 25 000 mg ℓ^{-1} sludge. The final concentration of sludge in each tube was 5 000 mg ℓ^{-1} . Tubes were capped, agitated using a vortex mixer and incubated in a shaking incubator at 25°C at 150 rpm for 3 h at a slant to ensure efficient mixing between sludge and metal solution. This was followed by centrifugation at 4 000 x g for 30 min using a J6B centrifuge. Supernatants were decanted into scintillation vials and analysed for metal-ions using a Varian Atomic Absorption Spectrophotometer.

3.2.5 Determination of Hexavalent and Trivalent Chromium

Total chromium content was determined using an atomic absorption spectrophotometer. Trivalent chromium was calculated by subtracting the hexavalent chromium (SABS method 206) from the total chromium.

3.2.6 Biosorption of Metal Ions from Effluents by Ten Sludges - Batch Experiment

Industrial wastewaters containing a mixture of metals at varying concentrations were substituted for pure metal solution. The procedure employed for the experiments was the same as 3.2.4. A range of industrial wastewaters were initially screened for their metal content (Table 3.1a & b). Six wastewaters were subsequently selected for experimental purposes on the basis of the variety of metal species and concentrations in the wastewater and different pH values (Table 3.1a & b) namely :- E2Cr (Kohler Flexible, Chromium rinse); E2FE (Kohler Flexible, Final effluent); E3Cd (Metal Protection Services, Cadmium rinse); E3FE (Metal Protection Services, Final effluent); E4FE (Glacier Bearings, Final effluent) and E6FE (Fascor, Final effluent).

3.3 RESULTS AND DISCUSSION

3.3.1 Biosorption of Metal ions from Single Solutions

Many workers have shown the ability of microorganisms to bioaccumulate metal-ions (Oliver and Cosgrove, 1974; Beveridge and Murray, 1976, 1980; Sterritt and Lester, 1981; Kasan and Stegmann, 1987a, 1987b; Kasan, 1988; Hughes and Poole, 1989). Bioaccumulation is essentially an active process and dependent on growth. Most studies on the concentration of metals by biomass have been conducted from a toxicological point of view, and have been concerned with accumulation due to the active metabolism of living cells, the effects of metal on the metabolic activities of the microbial cell and consequences of accumulation on the food chain. However, certain types of microbial biomass can passively bind and accumulate metals even when metabolically inactive or dead (Petrasek *et al*, 1983).

The results of the present research showed that all sludges tested were capable of metal biosorption (Figures 3.1 - 3.12). Stones (1958, 1959a, 1959b) reported that copper, nickel, iron, lead and zinc were removed by up to 90 % during the activated sludge process. The present research showed that except for Cr^{6+} , the percentage removal of all other metals tested, by many of the sludges at metal concentrations of 30, 60 and 90 mg ℓ^{-1} displayed efficiencies of approximately 90 %. It was also evident that there was a decrease in percentage removal with an increase in metal concentration in solution. (Figures 3.1 - 3.6). Although the latter occurred, it was evident that there was a distinct increase in biosorption of metals by sludges with increase in metal quantities (mg g^{-1}) in solution, with the exception of Cr^{6+} . This increased biosorption could be attributed to various physico-chemical factors,

eg., more binding sites were available on the sludge surface to facilitate free metal binding (Lawson *et al*, 1984, Alibhai *et al*, 1985). The physico-chemical properties which may be considered as being of significance to the binding of metals include pH, temperature, the oxidation-reduction potential and the presence of complexing agents (Alibhai, *et al*, 1985). When compared to other metals, Cr^{6+} biosorption by the sludges is relatively low (Figures 3.13 and 3.14). Previous results have attributed such a phenomenon to Cr^{6+} ions possessing the smallest ionic radii i.e., 0,35 Å when compared to Cr^{3+} and other heavy metals. In addition, more binding sites are available for larger ions than for smaller ones (Forster, 1985). Secondly, the hexavalent form of chromium is toxic to most biological systems such as bacteria, plants and animals (Benoit, 1976; Ross, 1981; Richard and Bourg, 1991), therefore, having a negative effect on the activated sludge micro-flora, resulting in reduced biosorption of the metal.

The present findings confirmed that when comparing the ten sludges, some of the sludges displayed superior metal biosorptive potential (Figure 3.13), although the differences in values were not very pronounced. Amanzimtoti sludge (S2) treating 100% industrial wastes appeared to be the most efficient biosorbent among the ten sludges (Figure 3.13). Industrial effluent particularly from electroplating industries could be responsible for the increased capability of S2 sludge to biosorb metal ions because the microbial populations might have acclimatised during exposure to heavy metals. An important biological factor that could account for this is the difference in the concentration of bacterial extracellular polymers present in individual sludges, which influence metal biosorption by sludges (Horan and Eccles, 1986). It was also observed that certain sludges displayed preferential behaviour. Sludges showed an affinity for certain metal species, e.g. S2 showed superior biosorption for

cadmium, copper, and zinc (Figure 3.13). This could be attributable to differences in the characteristics of the sludge which in turn, are a function of the physical and chemical properties imposed by the particular sludge treatment process (Alibhai *et al*, 1985). In addition, differences in the chemistry of binding sites on the individual sludges, determine the metal uptake capacity of the sludge (Lester and Sterritt, 1985). In considering the interactions of metal ions with biomass in the activated sludge system, it is necessary to determine whether the removal of metal from solution is simply the result of precipitation of the metal and subsequent entrapment of the precipitate in the biofloc or as a result of physical-chemical associations between biomass and soluble metal species (Cheng *et al*, 1975).

Efficiency of sludge biosorption of metal ions from pure solutions was found to follow the descending order of $\text{Cu} > \text{Cd} > \text{Zn} > \text{Ni} > \text{Cr}^{3+}$ and Cr^{6+} . (Figure 3.14). The affinity of the sludges for each of the metals followed the same pattern as reported by Lester and Sterritt, (1985). Alibhai *et al* (1985) confirmed that superior copper adsorption may be due to a combination of both chemical and physical adsorption. Copper binding was attributable to physical adsorption alone compared to Zn, Ni and Fe (Kasan and Baecker, 1989b). Similar affinity series of waste sludges for the metals studied was also shown by other researchers. Copper was the metal most strongly bound by activated sludge flocs, followed by Cd and Ni (Rudd *et al*, 1984).

3.3.2 Biosorption of Metal ions from Industrial wastewaters

When selecting industrial wastewaters for the present investigation every attempt was made

to ensure a variable pH range and in addition a wide representation of different metal species such as Cu^{2+} , Zn^{2+} , Cd^{2+} , Ni^{2+} , Cr^{3+} , Cr^{6+} and concentration in the effluent. The effluents used were E2Cr - pH 5.97, E2FE - pH 3.27, E3Cd - Ph 12.74, E3FE - pH 3.52, E4FE - pH 6.93 and E6FE - pH 6.88. Sludge-bound metal concentrations and percentage removal of metal ions were computed and the results expressed graphically (Figures 3.15a & b - 3.20a & b). All ten sludges investigated demonstrated an ability to remove metal ions from effluents. Biosorption of metal ions occurred at all wastewater pH's tested. The pH of a solution in which interaction between metal ions and biomass occurs is an important factor in determining the association of metal ions with the organic functional groups. Hydrogen ions will compete with other cations, including the metals, for binding sites on the sludge functional groups. As the pH of the solution is increased, resulting in an increase of free binding sites, the formation of metal-organic complexes may also increase. The hydroxyl ion concentration will also have an effect on the equilibrium of metal ion in solution, acting as a ligand with an affinity for the central metal ion and compete with other ligands for the metal ion. When the pH is increased to a level at which other ligands can no longer successfully compete with the hydroxyl ion, the metal will precipitate from solution as the hydroxide (Cheng *et al*, 1975). During the present study, although wastewaters of varying pH's were used, it was not possible to show how pH of solution determines biosorption by sludges because a variety of wastewaters were tested which comprised of different metal species and concentrations, and therefore the varying pH range would not be reflective of the biosorption potential. It is also known that in addition to pH, metal uptake by biomass is dependent on competition among the different metal ions present for binding sites on the sludge and concentrations of the biomass and metals present in the system (Cheng *et al*, 1975).

Differences in the biosorptive capacities of the ten sludges were found on exposure to wastewaters. Whereas removal efficiencies were up to 100% for some metal ions eg., Zn^{2+} removal by sludges 4,5,7 and 10 from wastewater E2Cr (Figure 3.15a) and Cu^{2+} removal by sludges 5,6 and 10 from wastewater E6FE (Figure 3.20a), in some cases metals present in effluent were not removed by certain sludges eg., Ni^{2+} was not removed by sludges 2,3 and 10 in E2FE (Figure 3.16a & b) and sludges 1,5,6,7,8,9 and 10 in E3FE (Figure 3.18a & b), Zn was not removed by any of the sludges except S2 in E3Cd (Figure 3.17a & b). This indicates that sludges possess metal-specific surface sites (Lester and Sterritt, 1985). Generally the metal present at the highest concentration in the wastewater was preferentially adsorbed by the sludges at higher removal efficiencies eg., chromium (Cr^{3+} and Cr^{6+}) present in E2Cr (Figure 3.15b), Cu^{2+} from wastewater E2FE (Figure 3.16b), Cr^{3+} from wastewater E3Cd (Figure 3.17b), Zn^{2+} from wastewater E3FE (Figure 3.18b), Ni^{2+} from wastewater E4FE (Figure 3.19b) and Zn^{2+} from wastewater E6FE (Figure 3.20b). These results confirmed the findings of Cheng *et al* (1975) who emphasized that concentration of metal was an important factor when comparing biosorption of metal in mixed solution by biomass. At this stage it was difficult to determine an affinity sequence for the metal species used since wastewaters used which were of different pH's and comprised mixtures of metal species at different concentrations. In addition, non-metallic ionic compounds present in the wastewaters could also interfere with the biosorption of metals by the ten sludges. The metal present in highest concentrations in the wastewater was preferentially adsorbed by the sludges at higher removal efficiencies (Figure 3.15b - 3.20b). This superior biosorption could be attributed to the advantage in concentration of a single metal ion species and thus competing more favourably for binding sites on sludge surface (Lester and Sterritt, 1985). There were pronounced differences in the results when comparing biosorption from wastewaters and pure

solutions. Sludges that presented superior biosorptive capacities for pure metal solutions eg. sludge 2,3,4 and 9 did not display the same results when challenged with industrial effluents of mixed metal content. Sludges exposed to pure metal solutions displayed superior biosorption than sludges exposed to mixed metal effluents (Figure 3.1 - 3.12, 3.15a & b - 3.20a & b).

3.3 CONCLUSIONS

All sludges investigated were capable of metal ion biosorption from pure metal solutions although variations were noted in their adsorptive capabilities. Results indicated increased quantities of metal biosorption with an increase in metal-ion concentration. Percentage biosorption decreased with increased metal concentration. An affinity series by sludges for specific metals, showing metal ion preference was determined viz., $\text{Cu} > \text{Cd} > \text{Zn} > \text{Ni} > \text{Cr}^{3+} >$ and Cr^{6+} . Sludges also showed the ability to remove metals from industrial wastewaters containing mixtures of metal ions, thus providing the foundation for further research focused on laboratory-scale bioreactor trials.

Table 3.1a Initial screening of Industrial effluents to determine their metal content and pH values.

EFF CODE	NICKEL	ZINC	COPPER	CHROMIUM	CADMIUM	pH
E4Ni	149.8	1.01	0.0	0.0	0.0	7.75
E4bCr	31.3	9.1	374.3	90730.1	1.0	0.91
E4Zn	0.1	9.7	0	0.0	0.0	10.51
E4FE	40.7	2.9	0	0.0	0.0	6.93
E3Cd	23.4	43.4	209	0.2	1125.6	12.74
E3Zn	0.1	46.6	1.5	0.4	0.0	12.13
E3FE	1.9	3832.5	8.0	1.4	4.4	3.52
E2Cr	0.1	0.9	1.1	45.6	0.0	5.97
E2FE	27.6	2.1	100.3	38.3	0.0	3.27
E1GL	0.0	29.3	0.1	24.2	0.0	6.03
E1Zn-P	0.1	30.3	0.0	0.0	0.0	5.77
E8S	83.7	2100.80	0.0	0.0	0.0	3.79
E7FR	0.0	23.2	29.8	0.0	0.0	3.05
E6Cu	27.6	1.3	101.6	48.2	0.0	10.98
E6Ni	475.2	1.5	0.0	0.0	0.0	6.99
E6Cr	0.1	413.6	0.3	44.3	0.0	3.93
E6Zn	0.1	309.4	0.4	0.0	0.0	12.60
E6FE	13.9	23.7	2.0	0.0	0.0	6.88
E5Cu	10.8	6.7	2977.7	2.5	0.0	11.14
E5Ni	1235.5	2.0	0.5	0.0	0.0	7.17
E5Cr	564.49	2367.3	654.9	15413.2	0.2	1.57

KEY: Ni = Nickel, Cr = Chromium, Zn = Zinc, Cd = Cadmium, Cu = Copper, FE = Final Effluent, GL = Galvanising Line, Zn-P = Zinc Phosphate line, S = Sludge, T = treated, FR = Final rinse.

Table 3.1b Industries from which effluents were obtained.

CODE	EFFLUENT
E1	XPANDA
E2	KOHLER FLEXIBLE
E3	METAL PROTECTION SERVICES
E4	GLACIER BEARINGS
E4 (b)	A E VALVES
E5	NATAL DIE CASTING
E6	FASCOR
E7	NON-FERROUS METAL EXTRUDERS
E8	TOYOTA

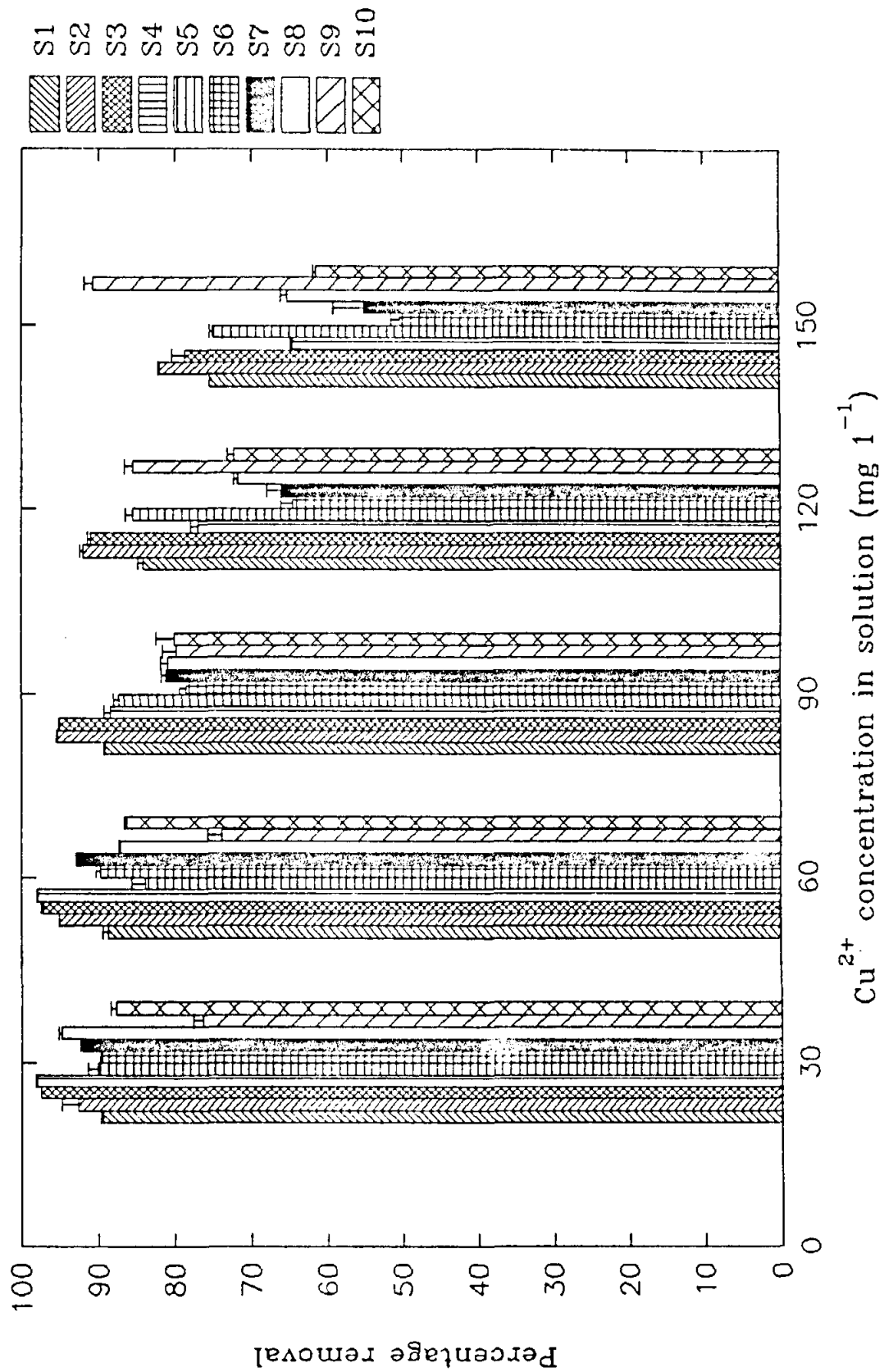


Fig. 3.1. Percentage copper biosorbed by ten activated sludges.

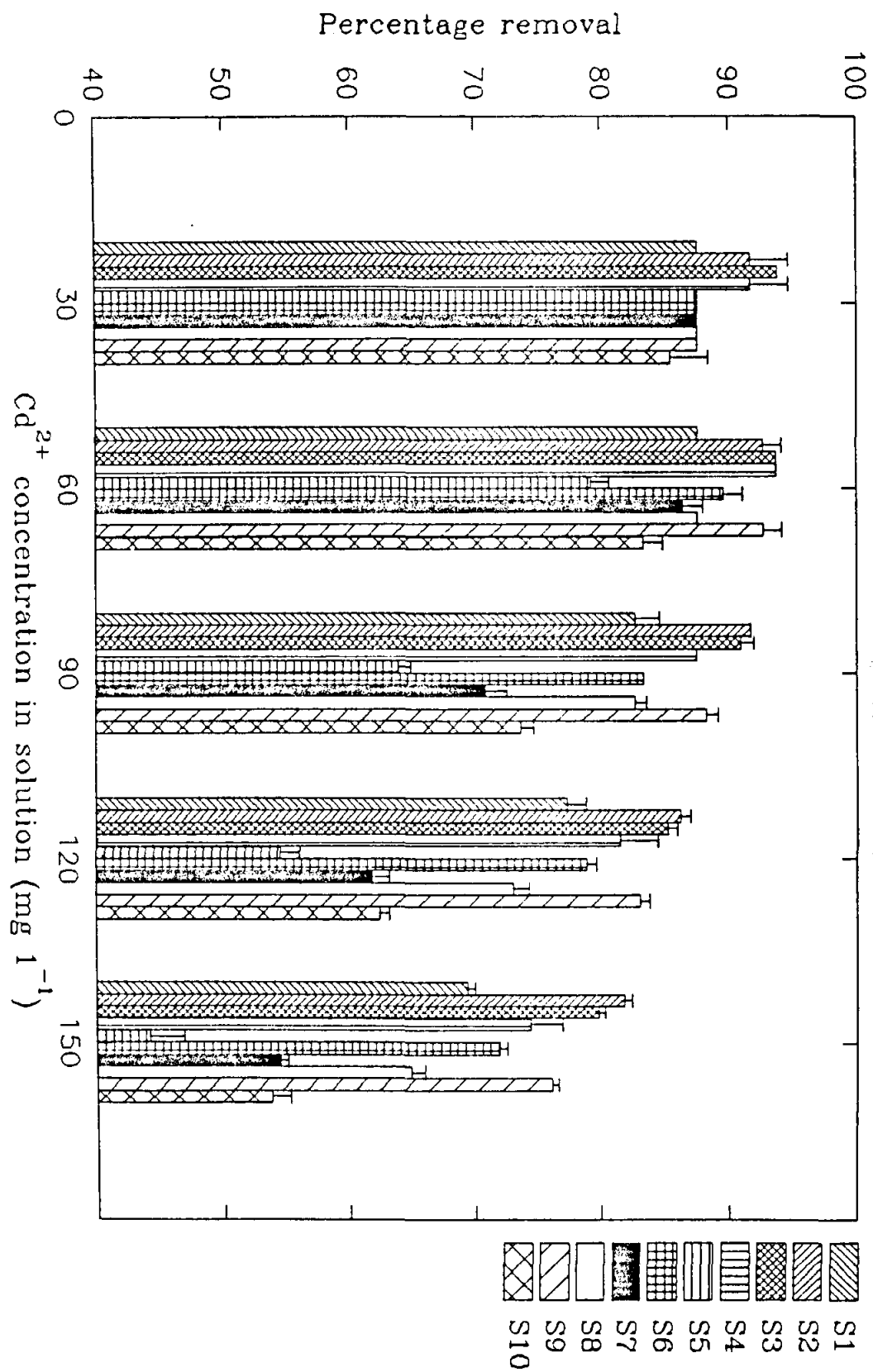


Fig. 3.2. Percentage cadmium biosorbed by ten activated sludges.

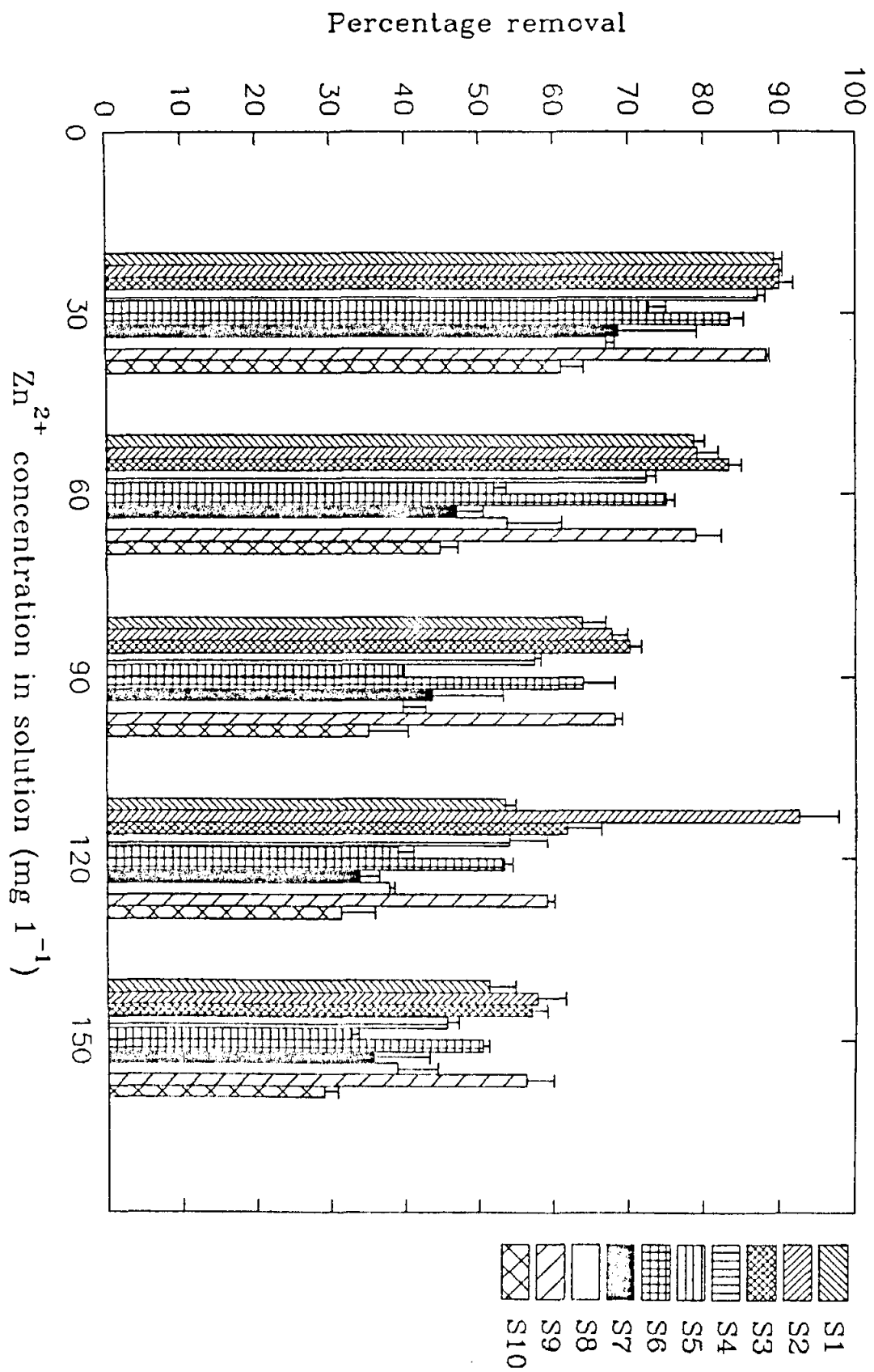


Fig. 3.3. Percentage zinc biosorbed by ten activated sludges.

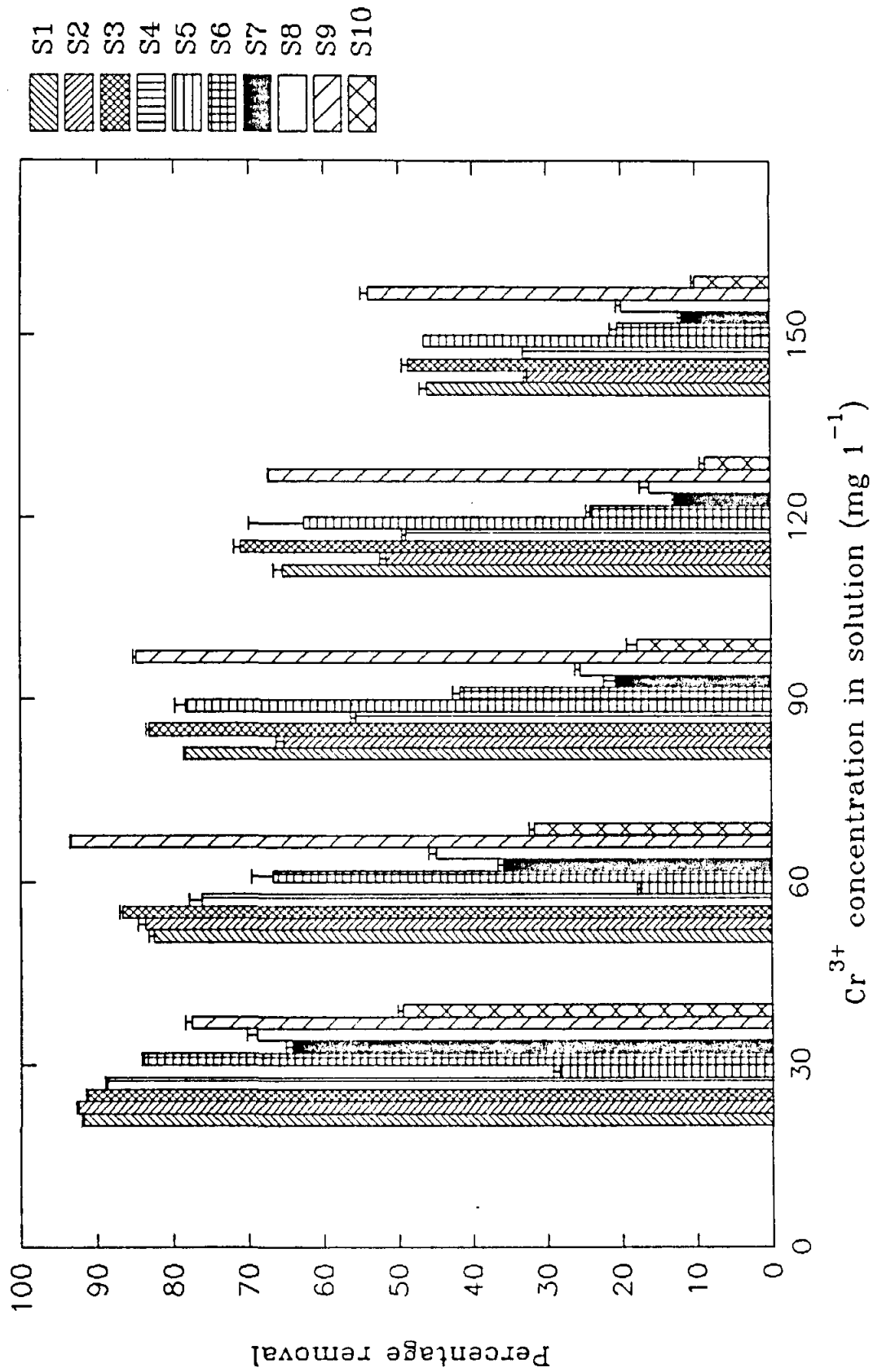
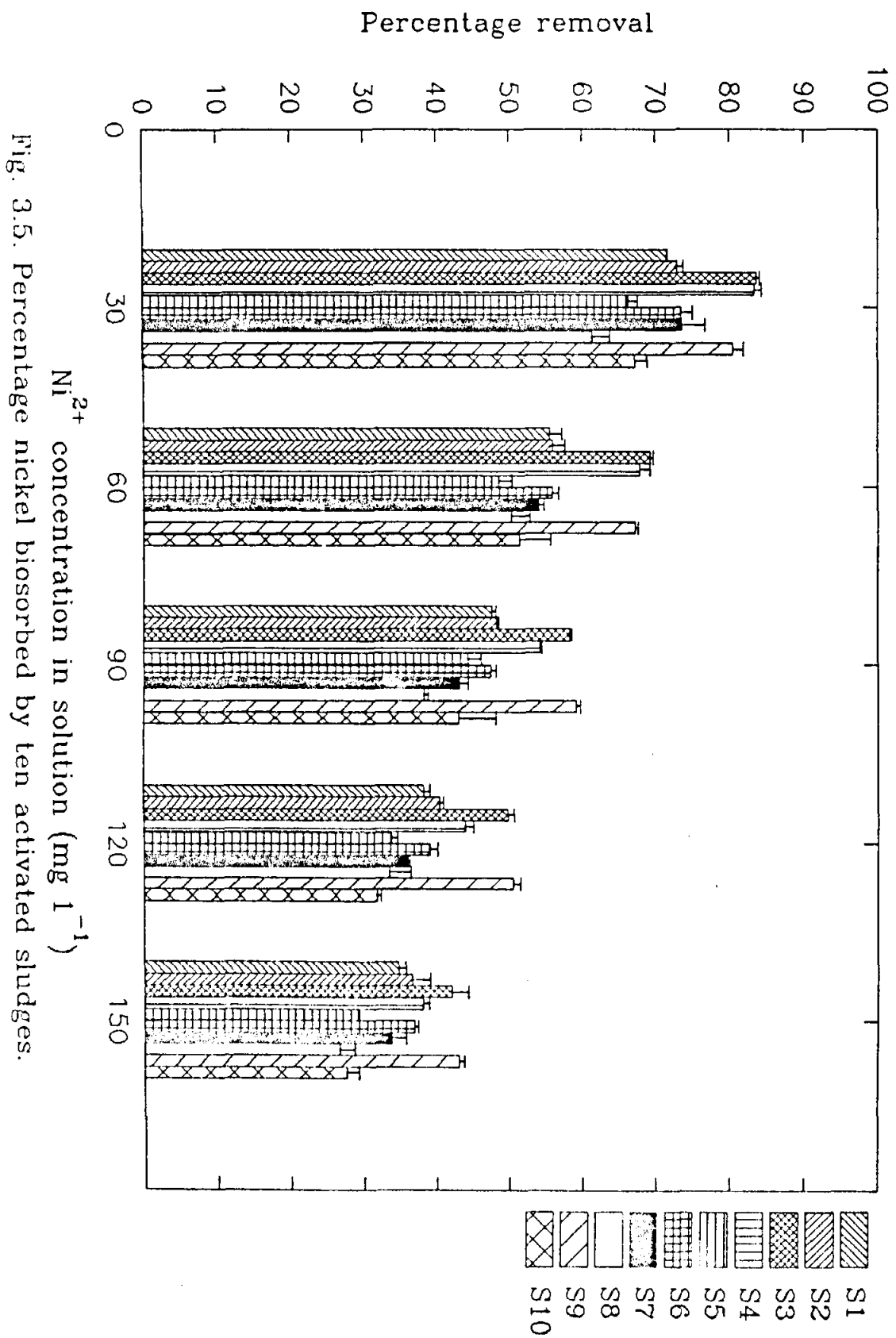


Fig. 3.4. Percentage chromium (III) biosorbed by ten activated sludges.



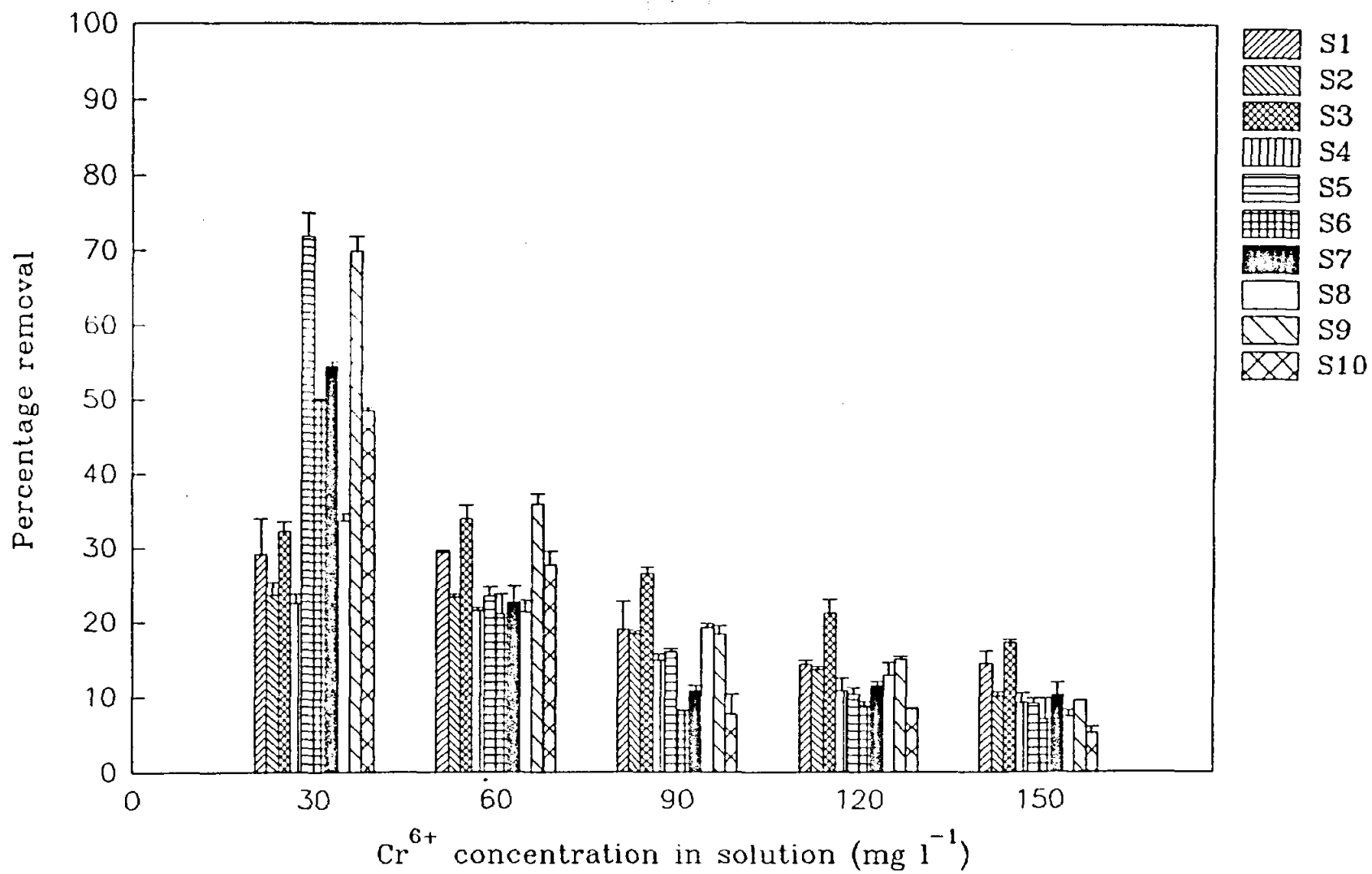


Fig. 3.6. Percentage chromium (vi) biosorbed by ten activated sludges.

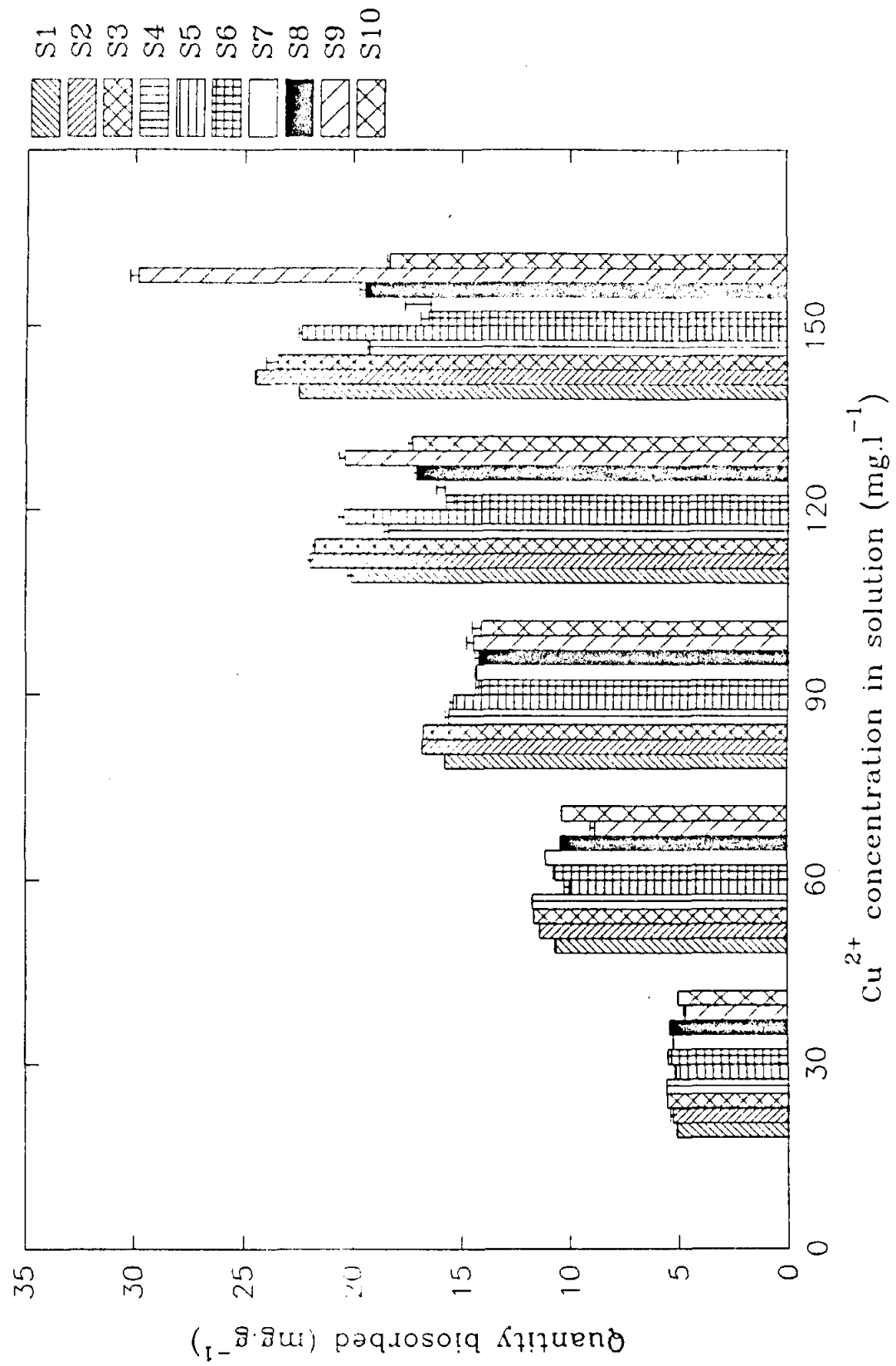


Fig. 3.7. Quantity of copper biosorbed by ten activated sludges.

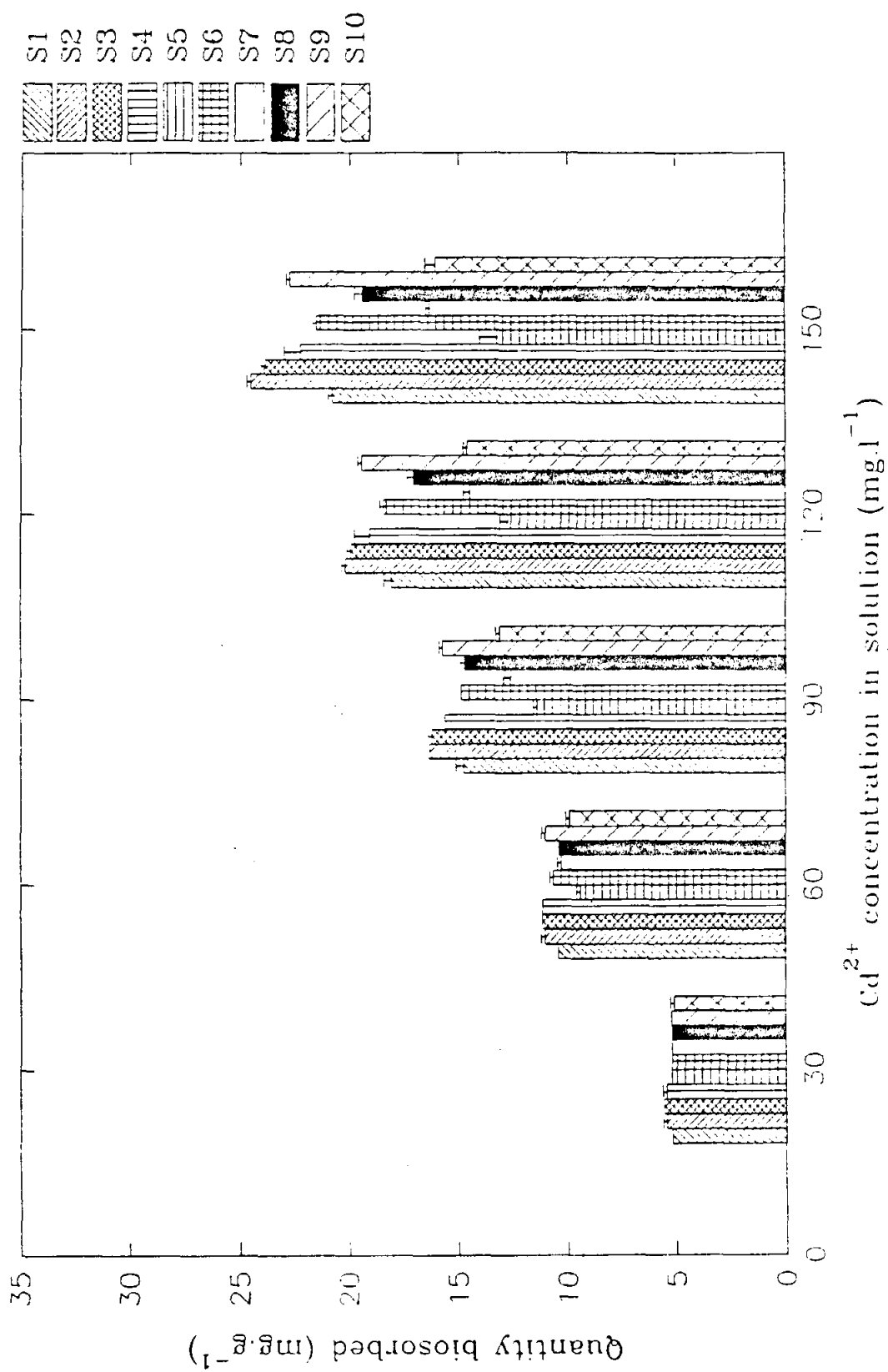


Fig. 38. Quantity of cadmium biosorbed by ten activated sludges.

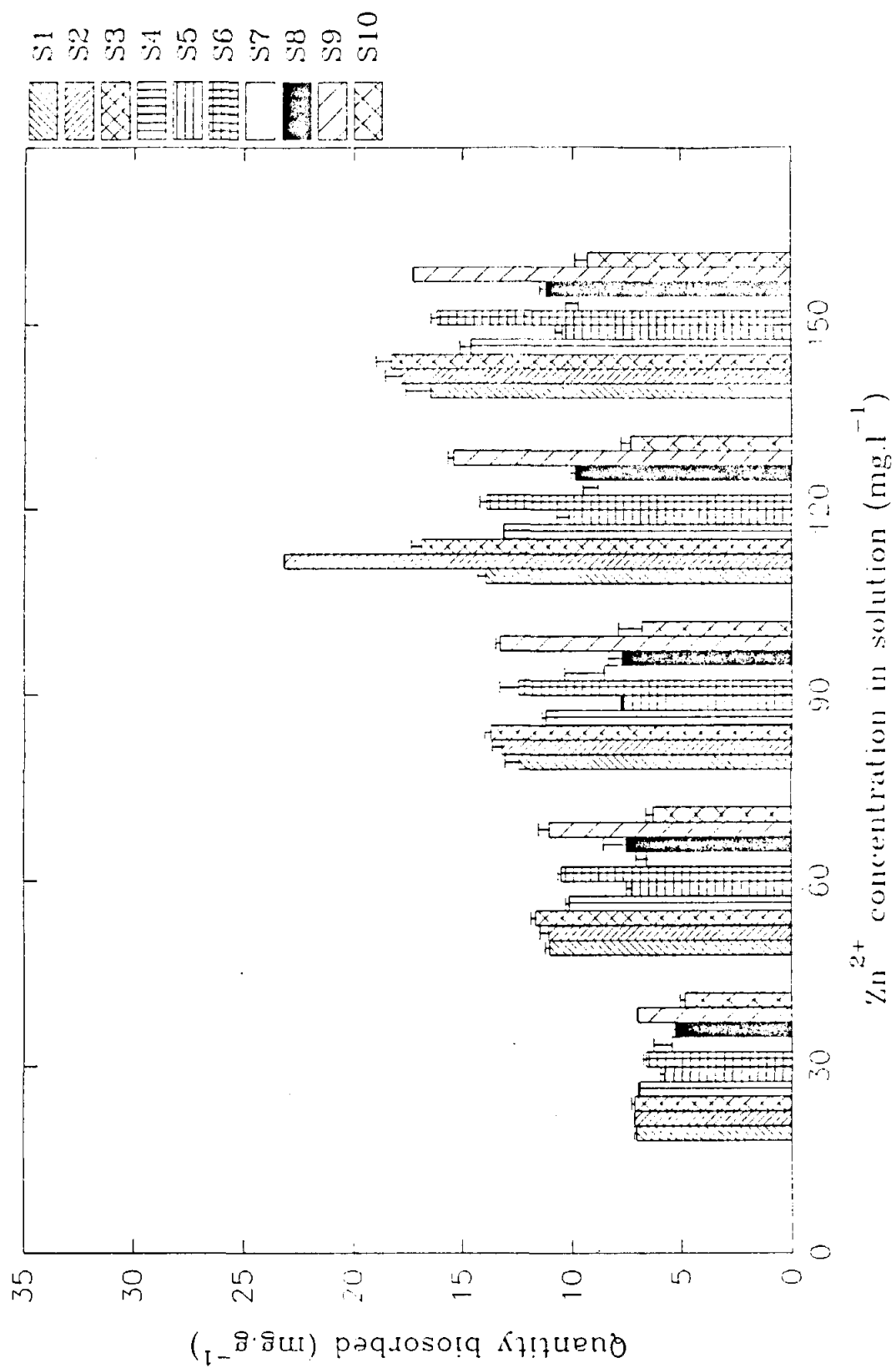


Fig. 3.9. Quantity of zinc biosorbed by ten activated sludges.

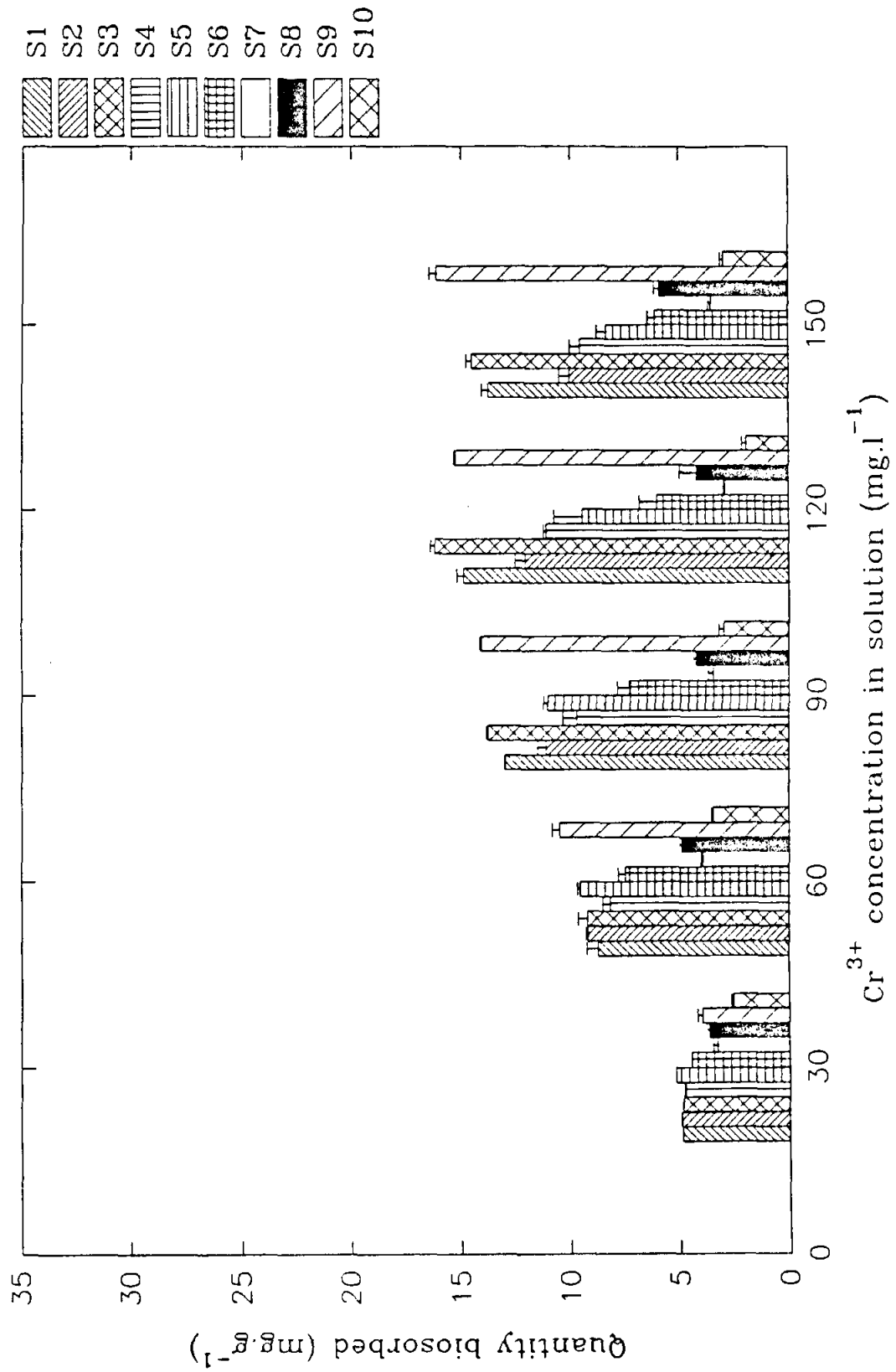


Fig. 3.10. Quantity of Chromium (III) biosorbed by ten activated sludges.

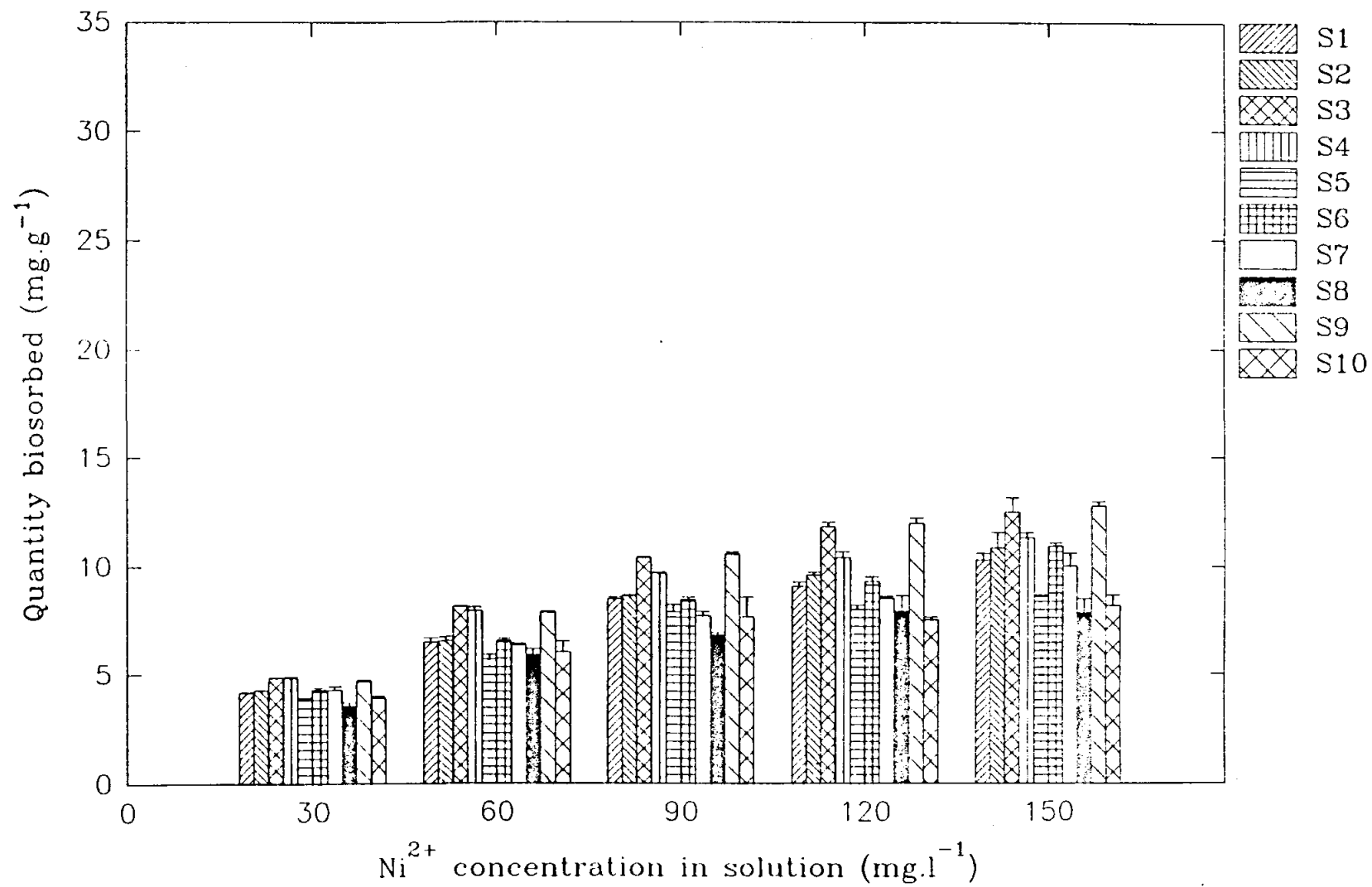


Fig. 3.11. Quantity of nickel biosorbed by ten activated sludges.

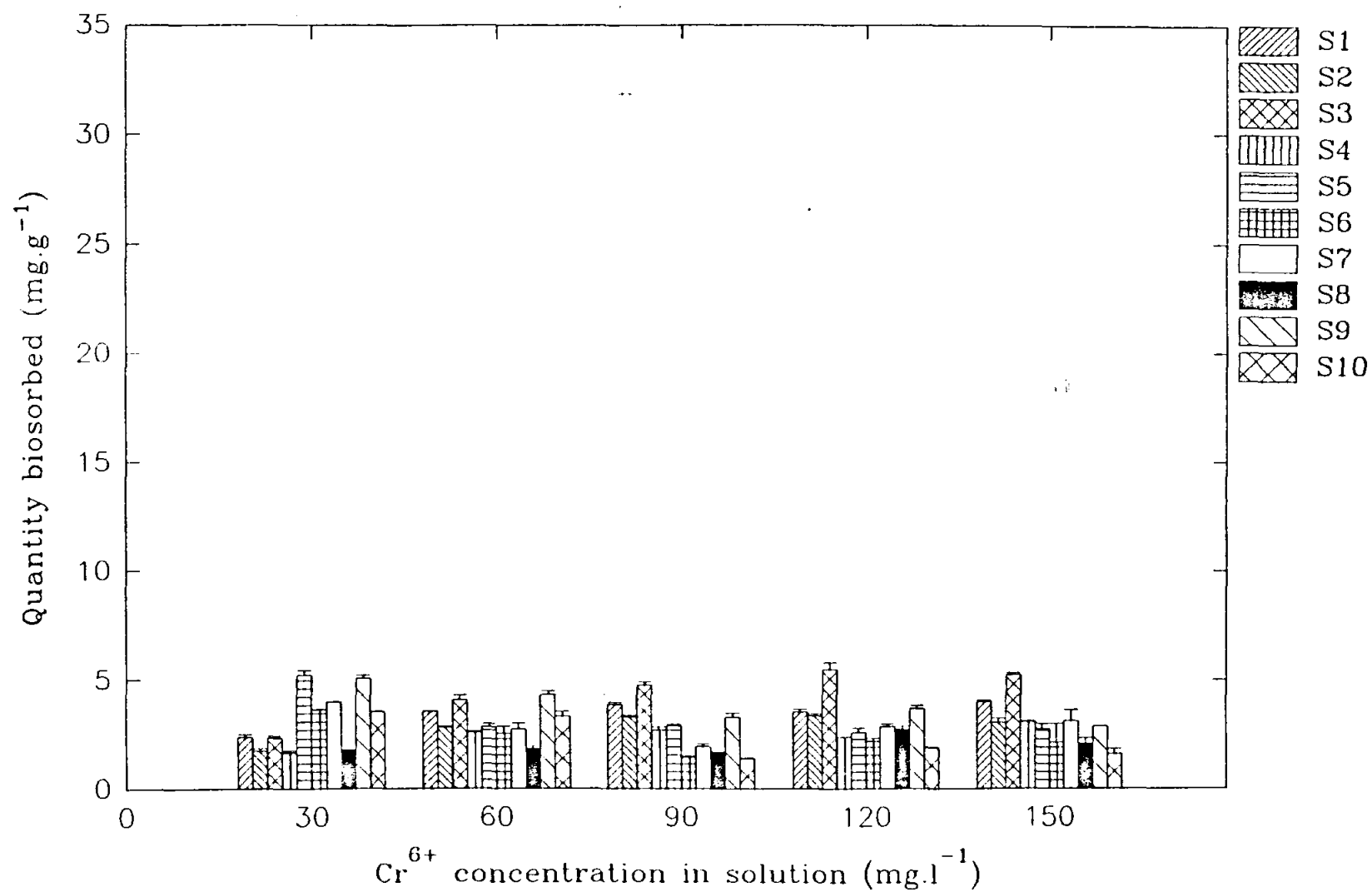


Fig. 3.12. Quantity of chromium (VI) biosorbed by ten activated sludges.

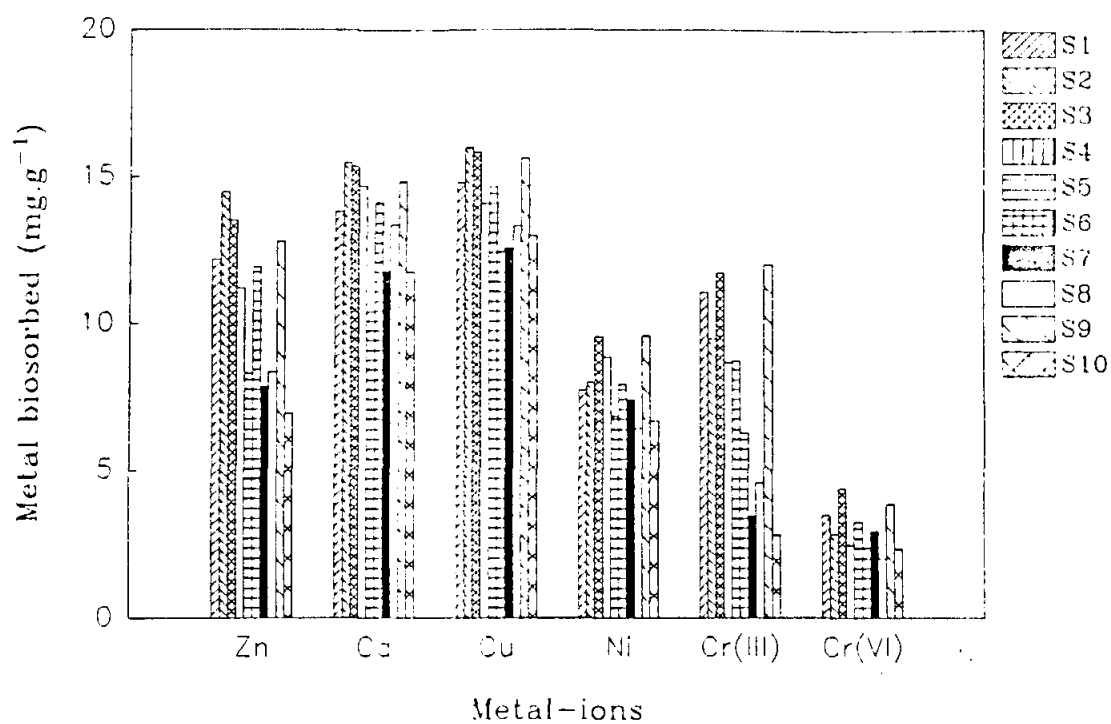


Fig. 3.13. Mean quantities of metal-ions biosorbed by 10 activated sludges.

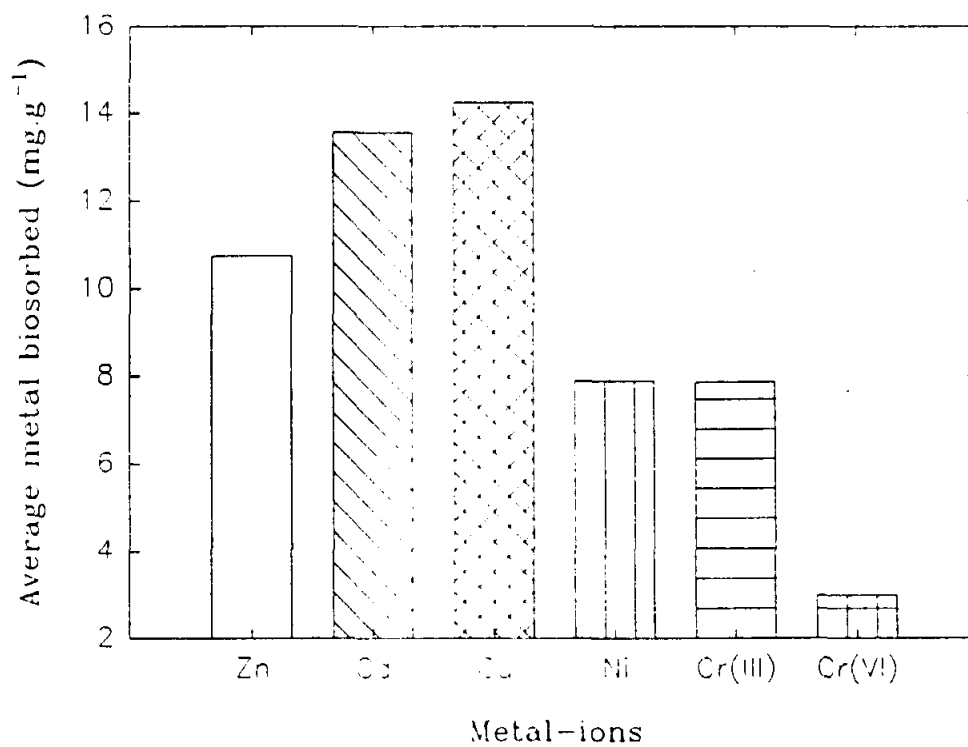


Fig. 3.14. Mean metal-ion concentrations biosorbed by 10 activated sludges.

Effluent code : E2Cr

Source : Kohler Flexible, Chrome rinse

pH : 5.97

Initial conc of metals : Ni = 0.08, Zn = 0.04, Cu = 0.34,
Cr = 28.30

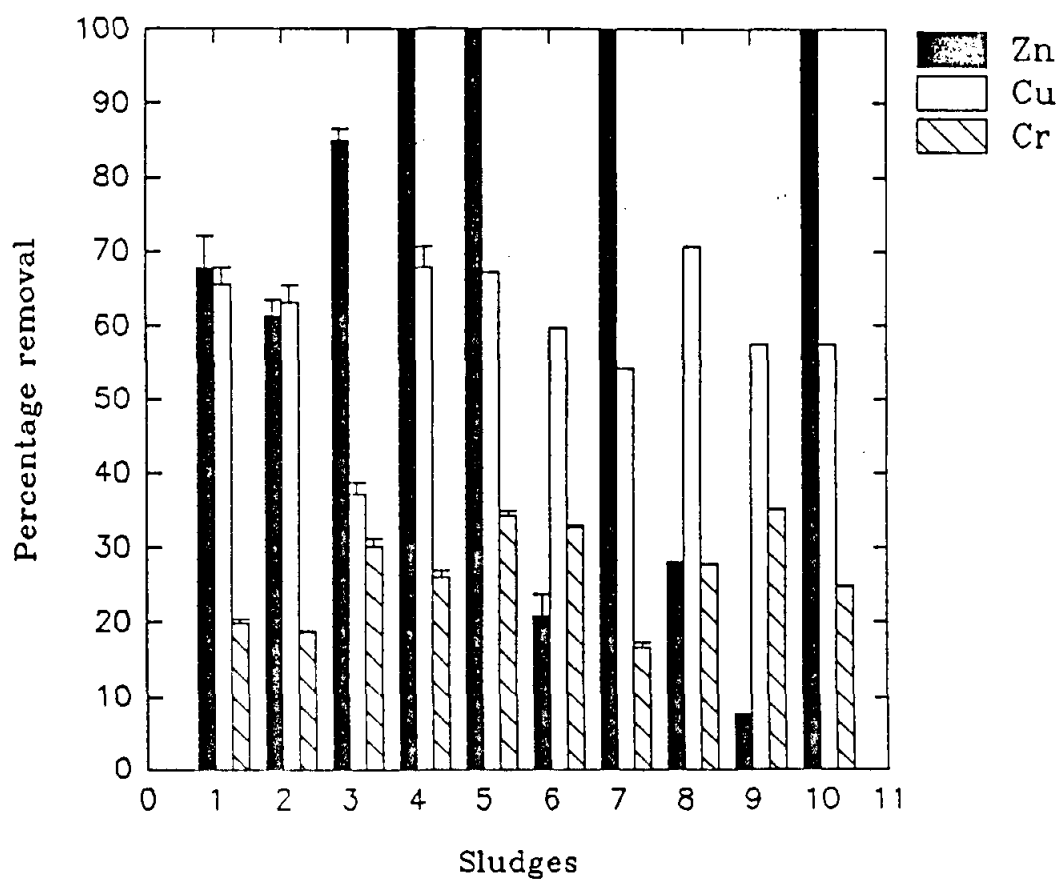


Figure 3.15a Percentage metal removal by ten waste activated sludges from effluent E2Cr.

Effluent code : E2Cr

Source : Kohler Flexible, Chrome rinse

pH : 5.97

Initial conc of metals : Ni = 0.08, Zn = 0.04, Cu = 0.34,
Cr = 28.30

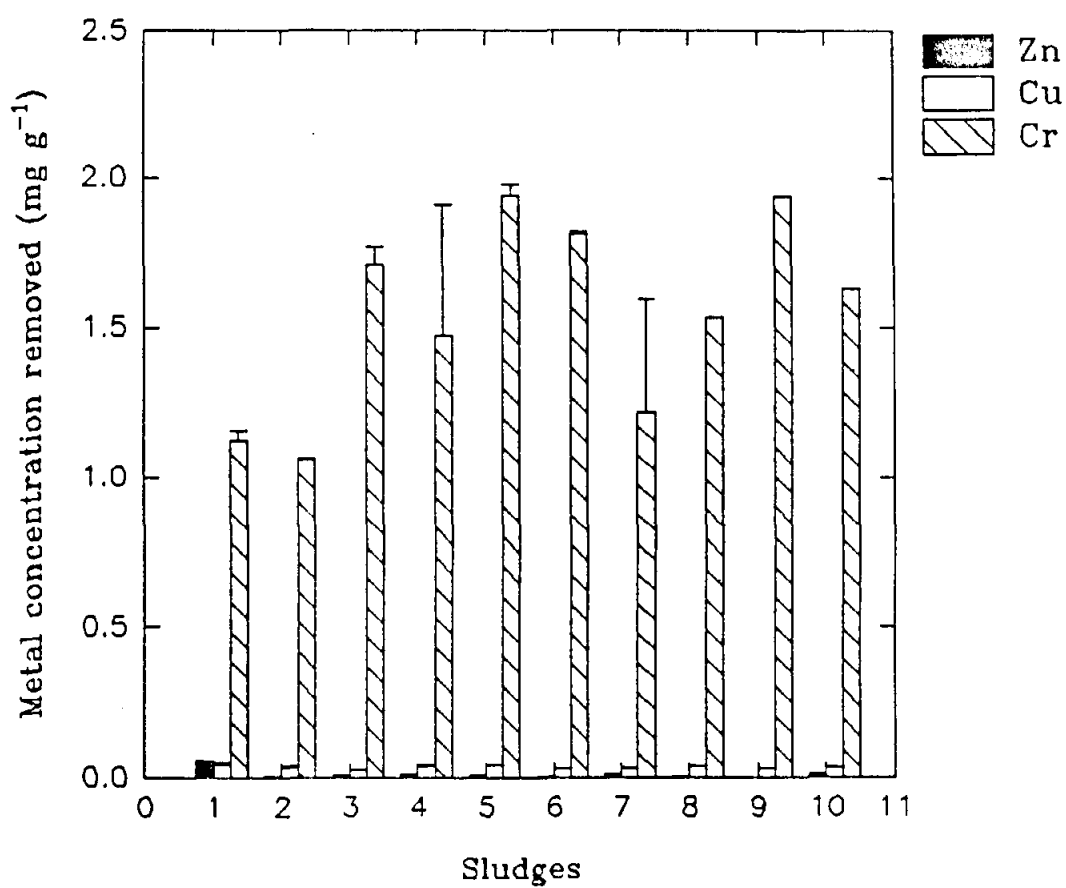


Figure 3.15b. Removal of metals from effluent E2Cr by ten sludges.

Effluent code : E2FE

Source : Kohler Flexible, Final Effluent

pH : 3.27

Initial conc of metals : Ni = 19.10, Zn = 0.53, Cu = 74.47,
Cr = 41.5

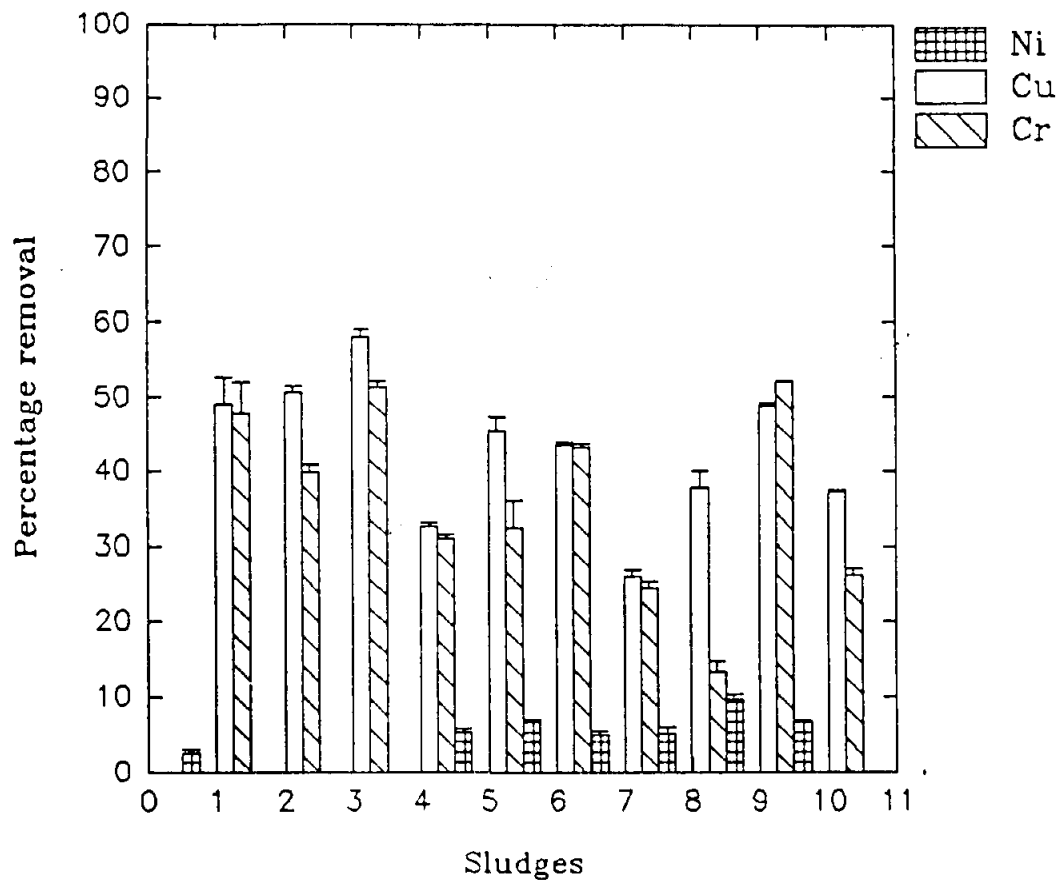


Figure 3.16a. Percentage metal removal by ten waste activated sludges from effluent E2FE.

Effluent code : E2FE

Source : Kohler Flexible, Final Effluent

pH : 3.27

Initial conc of metals : Ni = 19.10, Zn = 0.53, Cu = 74.47,
Cr = 41.5

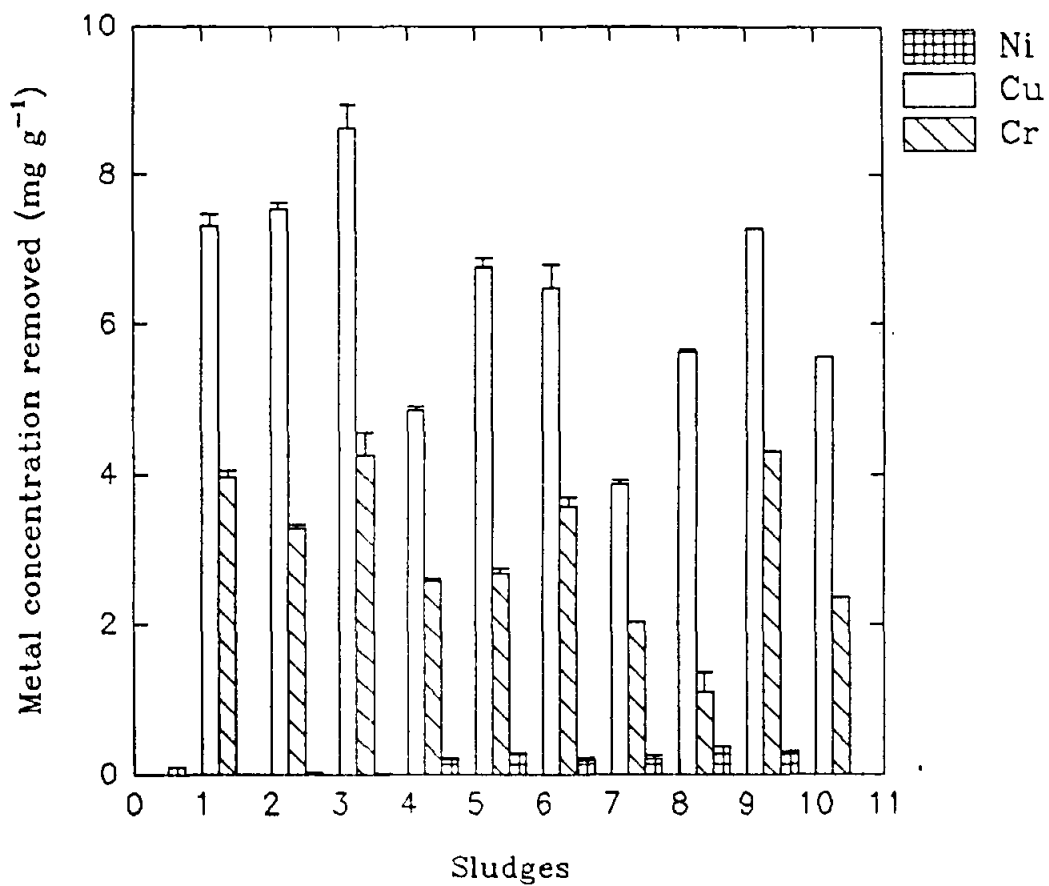


Figure 3.16b. Removal of metals from effluent E2FE by ten sludges.

Effluent code : E3Cd

Source : Metal Protection Services, Cadmium rinse

pH : 12.74

Initial conc of metals : Ni = 14.92, Zn = 41.16, Cu = 140.38

Cr = 0.16, Cd = 1384.53

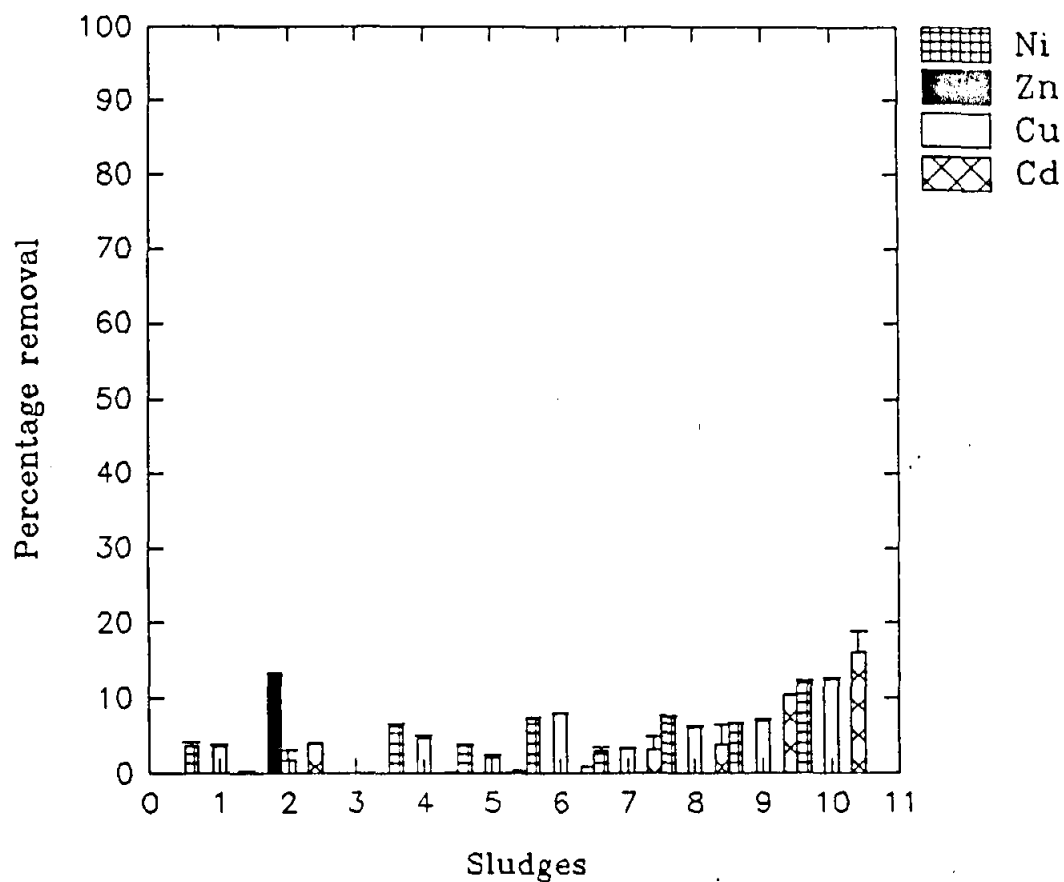


Figure 3.17a. Percentage metal removal by ten waste activated sludges from effluent E3Cd.

Effluent code : E3Cd

Source : Metal Protection Services, Cadmium rinse

pH : 12.74

Initial conc of metals : Ni = .14.92, Zn = 41.16, Cu = 140.38

Cr = 0.16, Cd = 1384.53

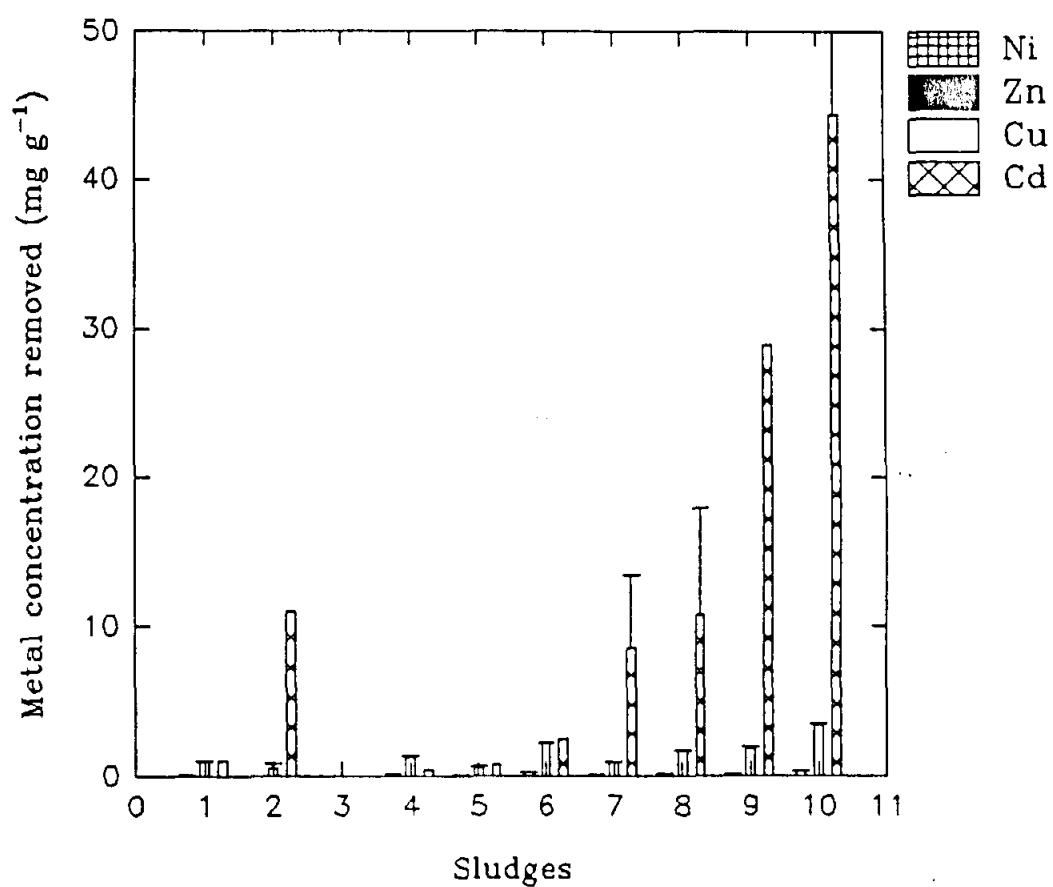


Figure 3.17b. Removal of metals from effluent E3Cd by ten sludges.

Effluent code : E3FE

Source : Metal Protection Services, Final Effluent

pH : 3.52

Initial conc of metals : Ni = 1.18 Zn = 3380, Cu = 6.47,

Cr = 14.5, Cd = 18.26

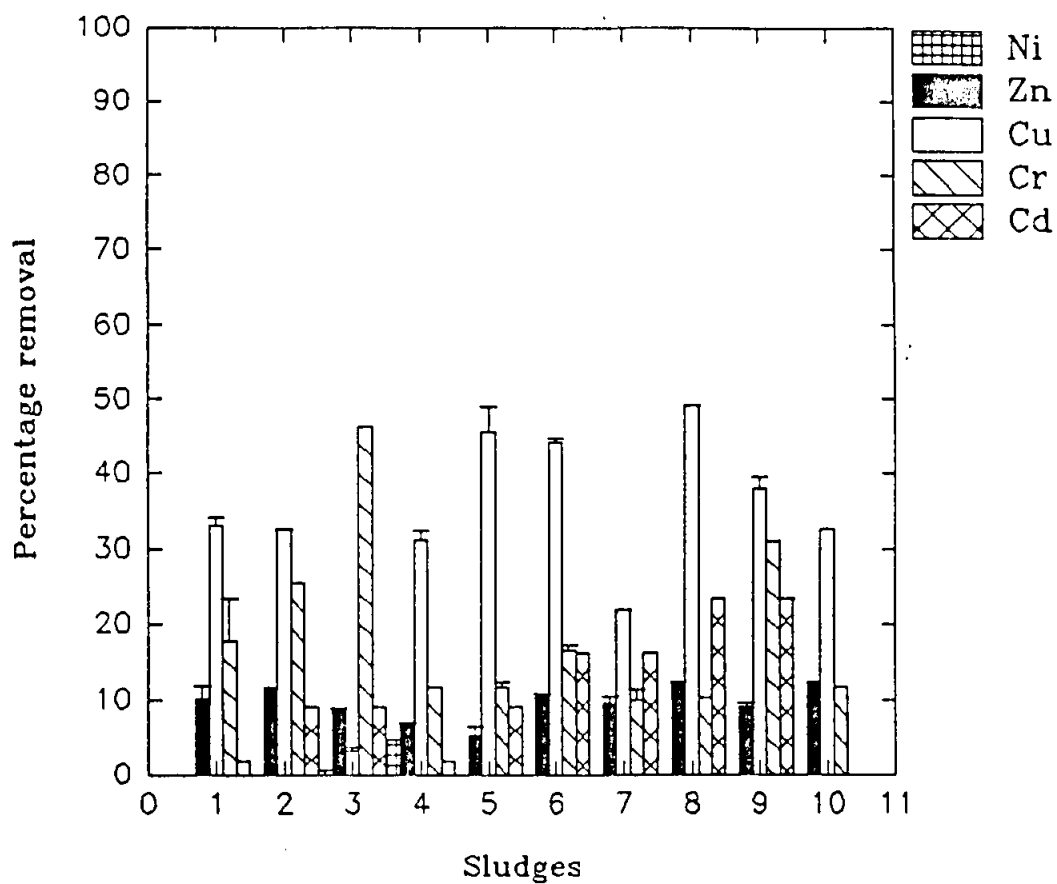


Figure 3.18a. Percentage metal removal by ten waste activated sludges from effluent E3FE.

Effluent code : E3FE

Source : Metal Protection Services, Final Effluent

pH : 3.52

Initial conc of metals : Ni = 1.18 Zn = 3380, Cu = 6.47,
Cr = 14.5, Cd = 18.26

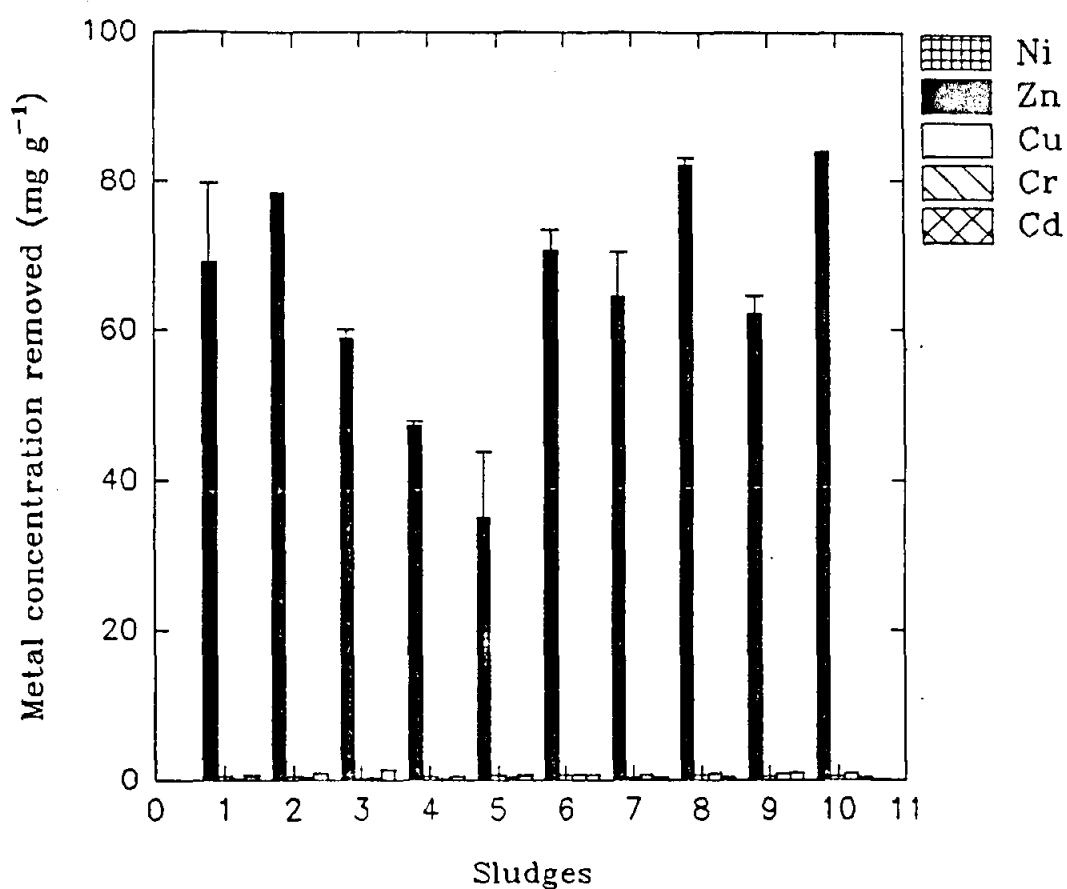


Figure 3.18b. Removal of metals from effluent E3FE by ten sludges.

Effluent code : E4FE
Source : Glacier Bearings, Final Effluent
pH : 6.93
Initial conc of metals : Ni = 30.82, Zn = 1.76

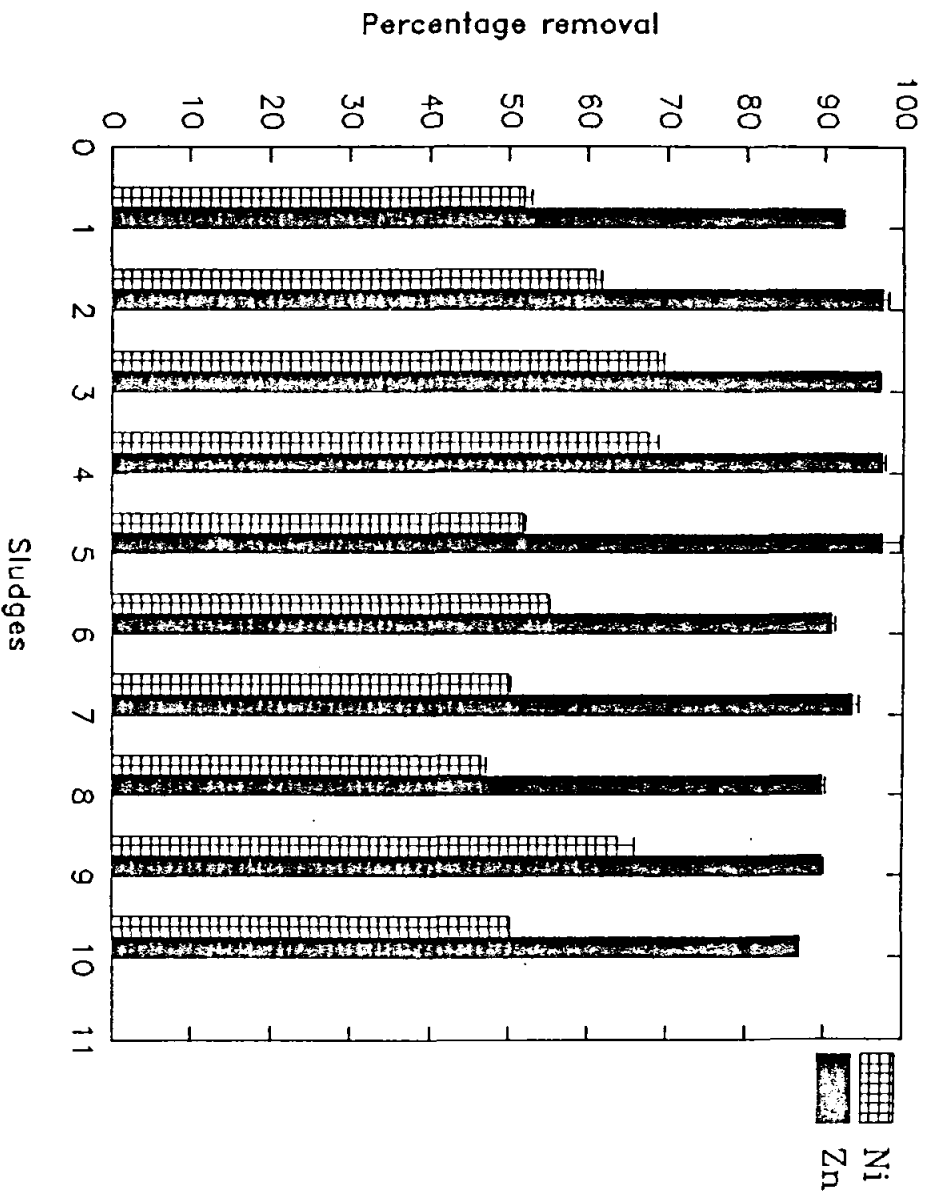


Figure 3.19a. Percentage metal removal by ten waste activated sludges from effluent E4FE.

Effluent code : E4FE

Source : Glacier Bearings, Final Effluent

pH : 6.93

Initial conc of metals : Ni = 30.82, Zn = 1.76

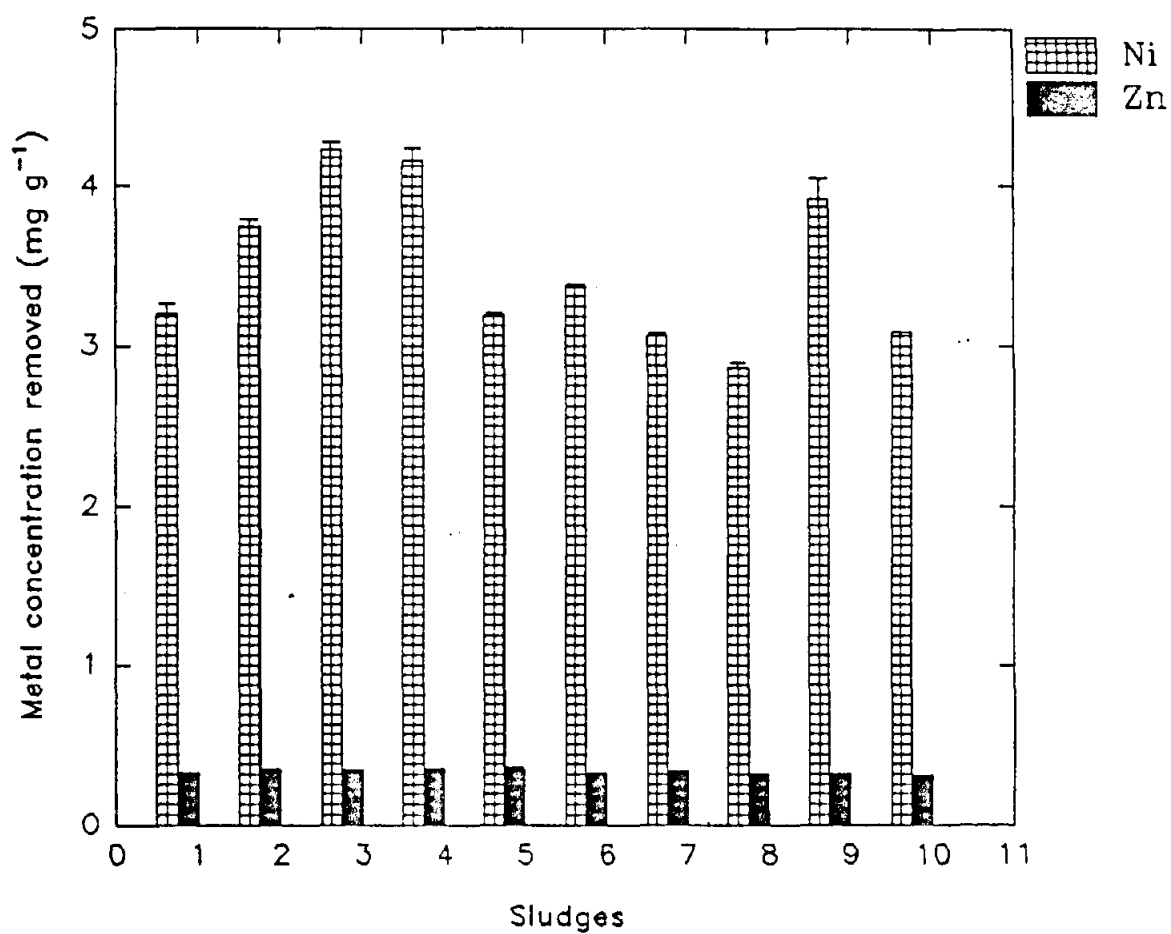


Figure 3.19b. Removal of metals from effluent E4FE by ten sludges.

Effluent code : E6FE

Source : Fascor, Final Effluent

pH : 6.88

Initial conc of metals : Ni = 9.0, Zn 12.21, Cu = 1.32

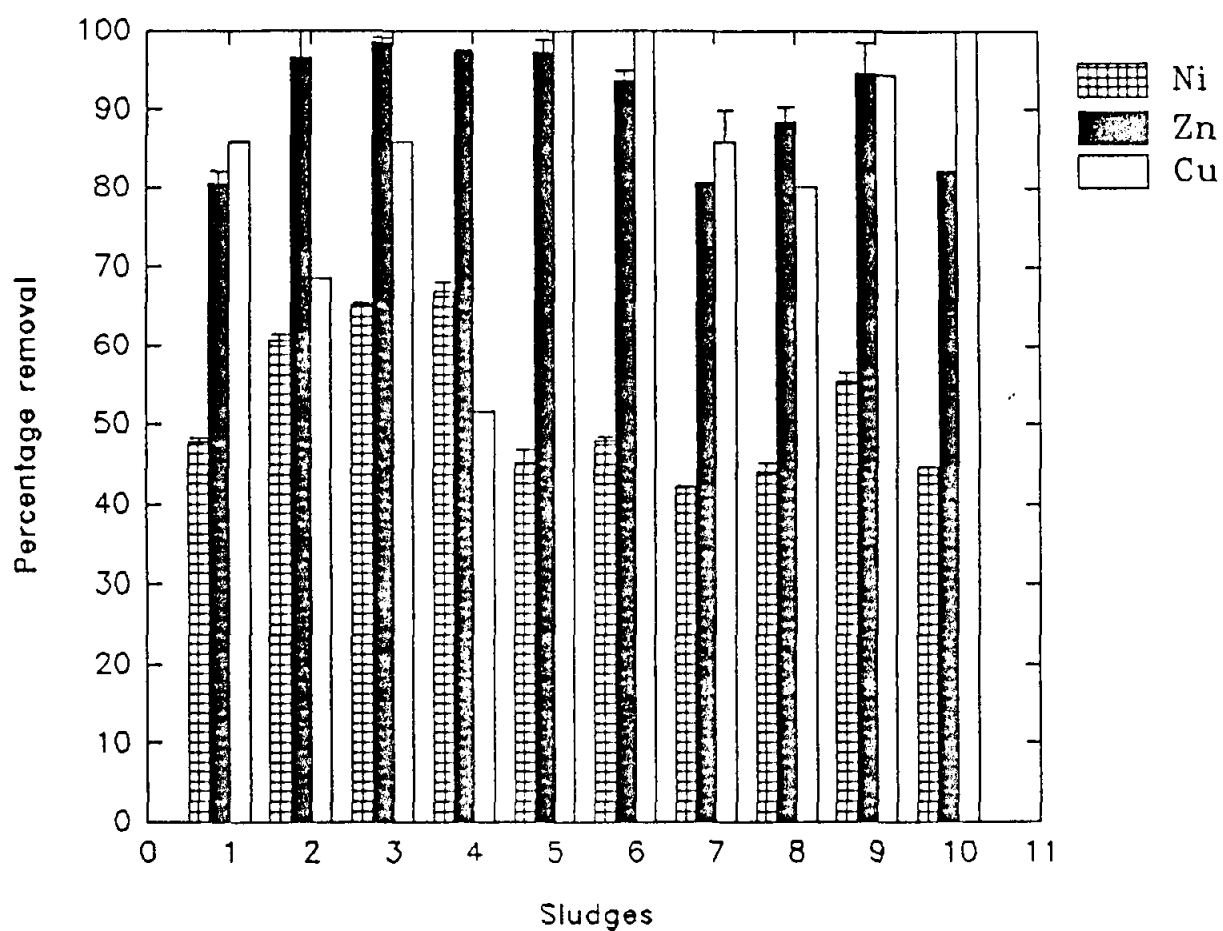


Figure 3.20a. Percentage metal removal by ten waste activated sludges from effluent E6FE.

Effluent code : E6FE

Source : Fascor, Final Effluent

pH : 6.88

Initial conc of metals : Ni = 9.0, Zn 12.21, Cu =1.32

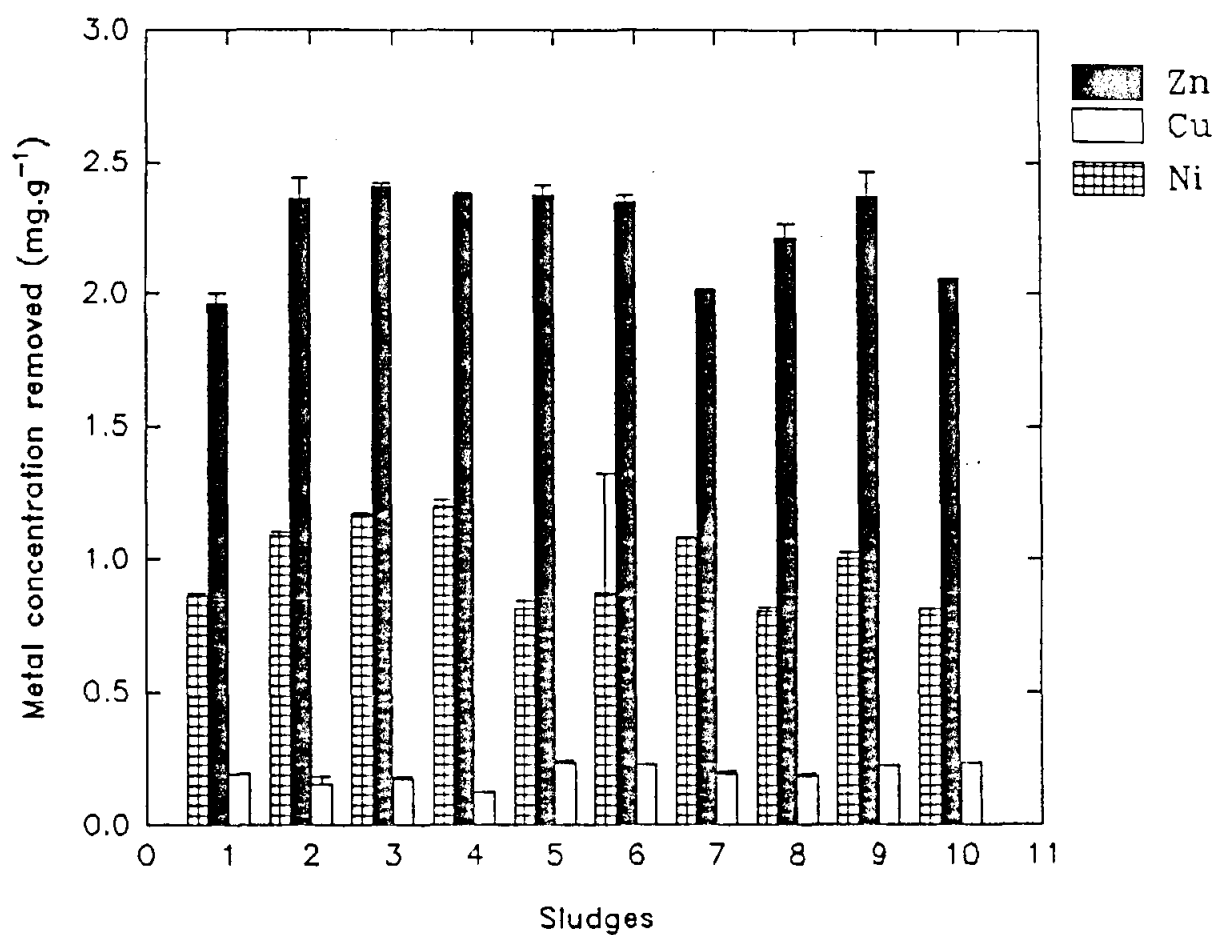


Figure 3.20b. Removal of metals from effluent E6FE by ten sludges.

4.0 MECHANISMS OF SLUDGE-METAL INTERACTIONS

4.1 INTRODUCTION

The removal of metal pollutants from watercourses is important if utilisable resources are to be recycled. Existing methods for removal of metal-ions from water include chemical precipitation by oxidation/reduction, filtration, electrochemical treatment, application of membrane technology and evaporation recovery which can be energy intensive and expensive (Volesky, 1987). Waste sludge, a by-product of the activated sludge treatment process, has demonstrated an ability to bind and remove metal-ions from various water sources by both adsorption and absorption processes (Cheng *et al.*, 1975). Evaluation of these mechanisms, determination of binding strengths and capacities of sludges can be ascertained by applying the Langmuir Adsorption Model to sludge-metal interactions. This information can be utilised to select sludges which demonstrate efficient metal removal capabilities and further to use them for specific metal removal processes. Waste sludge biomass acts as a biosorbent of metal cations from solution. The Langmuir Model of Adsorption provides the basis of deriving a fundamental interpretation of the nature of the sludge-metal interaction.

4.1.1 Adsorption Theory

Adsorption involves the interphase accumulation or concentration of substances at a surface or interface. This phenomenon can occur at an interface between any two phases, such as liquid-liquid, gas-liquid, gas-solid or liquid-solid interfaces. The material being concentrated

or adsorbed is the *adsorbate* and the adsorbing phase is termed the *adsorbent* (Weber, 1972). Sorption results from a variety of different types of attractive forces between solute molecules and molecules of the sorbent. Such forces usually act in concert but one type or another is generally more significant than the others in any particular situation. Three loosely defined categories of adsorption, viz., electrostatic, physical and chemical, are traditionally distinguished according to the class of active force which predominates (Weber *et al.*, 1991).

The primary driving force for adsorption may be a consequence of lyophobic (solvent disliking) character of the solute relative to the particular solvent or of a high affinity of the solute for the solid. For the majority of systems encountered in water and wastewater treatment practice, adsorption results from the combined action of both forces. The degree of solubility of a dissolved substance is by far the most significant factor in determining the intensity of the first of two driving forces. The second primary driving force for adsorption results from a specific affinity of solute for the solid. In this context, it is important to distinguish between three principal types of adsorption viz., electrical attraction, van der Waal's attraction, and chemical bonding between adsorbate and adsorbent. For electrical attraction, ions with similar ionic radii but higher ionic charge are more strongly attracted to sites of opposite charge. For ions of similar charge hydrated radius determines order of preference for adsorption with ions of smaller ionic radii being able to approach closer to adsorption sites. Van der Waal's interactions are normally termed "physical" or ideal adsorption where the adsorbed molecule is not affixed to a specific surface site but is allowed to undergo some translational movement. Where the adsorbed molecule undergoes chemical interaction with the adsorbent, molecules are considered to be restricted to one location on the surface and this type of adsorption is normally termed "chemisorption". Most adsorption

phenomena are combinations of the three different forms of adsorption, i.e., several forces which influence the different types of adsorption often interact to cause concentration of a particular solute at a surface. Thus it is generally difficult to distinguish between physical and chemical adsorption (Weber, 1972).

Brunauer *et al.*, (1940, cited by Ruthven, 1984) have classified the isotherms for physical adsorption into five classes (Fig. 1.1). The isotherms for true microporous adsorbents in which the pore size is not very much larger than the molecular diameter of the sorbate molecule, are normally of type I. This is because for such adsorbents there is a definite saturation limit corresponding to complete filling of the micropores. Occasionally if intermolecular attraction effects are large, an isotherm of type V is observed. An adsorption isotherm of type IV suggests the formation of two surface layers either on a plane surface or on the wall of a pore very much wider than the molecular diameter of the sorbate. Isotherms of types II and III are only observed in adsorbents containing a wide range of pore sizes. In each system there is a continuous progression with increasing loading from monolayer to multilayer adsorption and then to capillary condensation (Ruthven, 1984; Gasser, 1985).

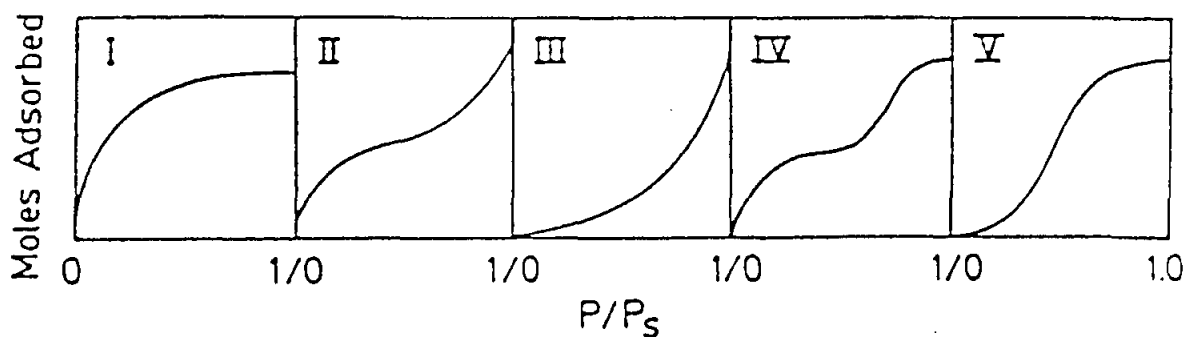


Fig. 4.1. Brunauer classification of adsorption isotherms (Hughes and Poole, 1989).

The preferred form for depicting this distribution is to express the quantity bound q_e as a function of C , at fixed temperature, the quantity q_e being the amount of solute adsorbed per unit weight of adsorbent and C the concentration of solute remaining in solution at equilibrium. Several types of isothermal adsorption relations may occur. The most common relationship between q_e and C is obtained for systems in which adsorption from solution leads to the deposition of an apparent single layer of solute molecules on the surface or within pores of the solid.

The simplest theoretical model for monolayer adsorption is due to Langmuir. The Langmuir model was originally developed to represent chemisorption on a set of distinct localised sites.

The assumptions on which the model is based are:

- a. molecules are adsorbed at a fixed number of well-defined localised sites;
- b. each site can hold one adsorbate molecule;
- c. all sites are energetically equivalent; and
- d. there is no interaction between molecules adsorbed on neighbouring sites.

The Langmuir isotherm is characterised by the equation:

$$q_e = \frac{Q^\circ bC}{1 + bC}$$

where:

- | | | |
|-----------|---|--|
| b | = | constant related to the energy or net enthalpy (ΔH) of adsorption |
| C | = | concentration of solute remaining in solution at equilibrium |
| q_e | = | amount of solute adsorbed per unit weight solid adsorbent |
| Q° | = | number of moles of solute adsorbed per unit weight of adsorbent in forming complete monolayers |

The Brunauer Emmet and Teller (BET) model represents isotherms reflecting apparent multilayer adsorption. Typical Langmuir and BET isotherms are represented in Fig. 1.2.

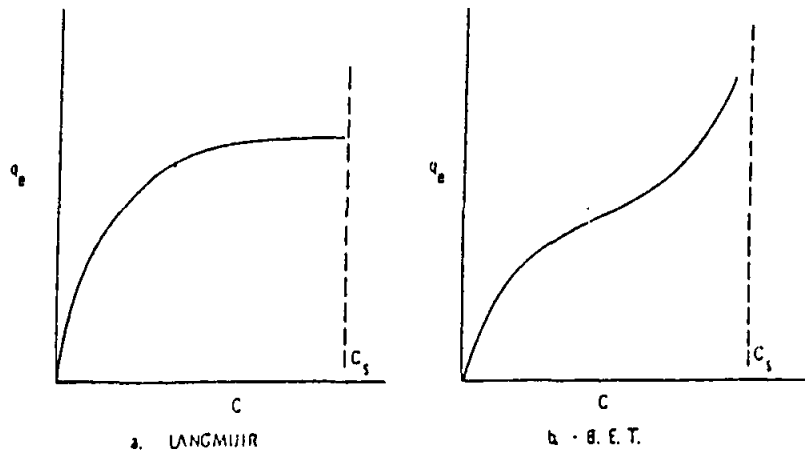


Fig. 4.2. Typical isotherms for Langmuir and BET adsorption patterns. C_s represents saturation concentration at a given temperature.

The BET isotherm is characterised by the equation:

$$q_e = \frac{BCQ^0}{(C_s - C) [1 + (B-1) (C/C_s)]}$$

where:

- B = constant expressive of energy of interaction with surface
- C_s = saturation concentrating of solute in solution at given temperature

This equation can be converted to the Langmuir form if:

- a) b is equal to B/C_s ;
- b) C is taken to be negligibly small compared to C_s ; and
- c) B is greater than 1 (Weber, 1972).

Two convenient linear forms of the Langmuir equation are (Kinnburgh, 1986):

$$\frac{C}{q_e} = \frac{1}{bQ^o} + \frac{C}{Q^o}$$

or

$$\frac{1}{q_e} = \frac{1}{Q^o} + \frac{1}{bQ^o} \left(\frac{1}{C} \right)$$

Linearisation of isotherms allows determination of whether a model will fit experimental data. Linearisation allows simple regression analysis with goodness of fit values indicating how well data fit models. However, linearisation formula should be carefully selected to protect against overoptimistic interpretation of goodness of fit by incorporating outliers. In this respect, the first equation tends to exaggerate deviations from the fitted equation and is ideal for application with the Langmuir Model (Kinnburgh, 1986)

4.1.2 Aims

- i) To determine the mechanisms of metal-ion adsorption by activated sludges by applying the Langmuir Model to data obtained during sludge-metal adsorption, and,
- ii) To quantitatively determine the strength of the sludge-metal bond (K_d) and the capacity of the sludge surface for metal-ions (X_m).

4.2 METHODOLOGY

Waste sludges from ten activated sludge plants in Natal were collected and concentrated to a mixed liquor suspended solids (MLSS) concentration of 25,000 mg ℓ^{-1} . Aliquots of 2 ml of each sludge were exposed to 8 ml of divalent nickel, copper, cadmium and zinc and trivalent and hexavalent chromium at final concentrations of 30, 60, 90, 120 and 150 mg ℓ^{-1} . Controls comprised deionised water substituted for sludges and all experiments were conducted in triplicate. Sludge-metal equilibration was allowed to proceed for 3h at 25°C and 150 rpm in polypropylene centrifuge tubes. Reactions were terminated by centrifugation at 4,000 x g in a Beckman J-6B centrifuge. Free metals present in supernatants were quantified by atomic absorption spectrophotometry. Bound and free metals were expressed as adsorption isotherms to determine adsorption mechanisms. First order regression coefficients, (>0.90) were calculated for linear reciprocal transformations of adsorption isotherms to determine whether the model was applicable to sludge metal interactions. Equations of these straight lines were determined from which sludge-metal bond strengths and capacities were calculated. Sludges were ranked according to the latter two criteria.

4.3 RESULTS AND DISCUSSION

All ten sludges investigated were capable of metal ion adsorption from solutions. Mechanisms of adsorption were ascertained from shapes of adsorption isotherms classified according to Brunauer (Ruthven 1984). The three most common types of adsorption isotherms obtained were of types I, II and IV, (Table 4.1). Type I isotherms are characteristic of monolayer adsorption while type II isotherms, also known as B.E.T., (Brunauer Emmett Teller), isotherms indicate monolayer to multilayer adsorption. Type IV isotherms are associated exclusively with multilayer adsorption. The most predominant type of the three adsorption isotherms was the Type I or "L" shaped isotherm, due predominantly to carboxyl termini present on amino and nucleic acid residues (Forster, 1976).

Ninety seven percent of the reciprocal plots showed a first order regression coefficient of greater than or equal to 90% indicating that the data conformed to the Langmuir Model. Zinc adsorption by sludges 1 to 10 (Figs 4.1 - 4.20) showed predominantly type II adsorption (Ruthven, 1984) with sludges 1, 6, 8, 9 and 10 all exhibiting this trend (Figs 4.1, 4.11, 4.15, 4.17 and 4.19). Type II adsorption indicates a monolayer to multilayer adsorption of Zn ions and was first characterised by Brunauer, Emmet and Teller. Hence, it is also known as the B.E.T., isotherm. The curve indicates a continuous progression with increasing load. As load increases further, though, multilayer adsorption occurs. This can arise by two means, i.e., a wide range of pore sizes on the sludge surfaces is available to accommodate Zn ions and the larger pores begin accumulating a second layer of ions or, planar sludge surfaces adsorbing metal-ions begin to accrue a second layer on their adsorbed surfaces.

Three type IV adsorption isotherms were observed, i.e., with zinc adsorption to sludges 4, 5 and 7 (Figs 4.7, 4.9 and 4.13). Type IV isotherms are indicative of multilayer adsorption, in particular, the formation of two surface layers either on a plane surface or on the wall of a pore with a diameter much larger than the diameter of sorbate.

Sludge 2 adsorption of Zn (Fig. 4.3) indicated an isotherm that was not classifiable but if the fourth point of the isotherm is discounted then the isotherm would closely resemble the type I isotherm which is commonly known as the 'L' shaped isotherm because it resembles the letter L whose shape describes a rectangular hyperbola.

Cadmium adsorption by the ten sludges indicated 'L' shaped isotherms. This is indicative that only one type of binding site on sludges adsorbed Cd from solution i.e., charged carboxyl groups which may exist on amino acid or protein moieties of the sludge flocs. These include cell walls, envelopes, membranes and various other organelle components of the cells which contain proteins and which constitute the sludge floc. Sludges 2, 3, 8 and 10 (Figs 4.23, 4.25, 4.35 and 4.39) indicated isotherms of sharp initial incline with a plateau at approximately 0.1 mM l^{-1} of Cd in solution. Sludges 4, 7 and 9 (Figs 4.27, 4.33 and 4.37) displayed sharp inclines with a plateau before 0.1 mM l^{-1} indicating that these sludge surfaces had a lower saturation capacity than those mentioned previously. Lester and Sterritt (1985) described biphasic or bilinear adsorption isotherms for copper adsorption to activated sludges which might be related to the isotherms obtained above. They postulated that this is indicative of two types of complexes being formed between the metal-ion and the activated sludge surface with individual affinity constants (k) and uncomplexed ligand concentration $[L]$ for each linear section.

They explained 'non-linear plots' as obtained for sludges 1, 5 and 6 (Figs 4.21, 4.29 and 4.31) to be due to a heterogeneous distribution of ligands each having different k and $[L]$ values. They also reported that k values obtained were an average rather than a constant for the metal for which it was obtained. Sludges 1, 5 and 6 exhibited gentle curves for adsorption isotherms more in keeping with the expected type I isotherm described by Brunauer (Ruthven, 1984).

Adsorption isotherms for copper adsorption to the ten sludges were predominantly of the 'L' type (Figs 4.41, 4.43, 4.45, 4.47, 4.51, 4.53, 4.55 and 4.59) with sludges 5 and 9 being the exceptions (Figs 4.49 and 4.57). The latter two indicated adsorption conforming most closely with that of the type IV adsorption isotherm described earlier. It is evident from these isotherms that two surface layers exist for the adsorption of copper onto the surfaces of sludges 5 and 9 (Figs 4.49 and 4.57). The type I adsorption isotherms show little difference in shape from each other with steep uptake curves. The exceptions to this trend are sludges 1, 8 and 10 (Figs 4.41, 4.55 and 4.59). These three sludges depict less steep uptake at low adsorbate pressures, reaching a plateau at higher pressures.

Nickel adsorption by sludges 1 to 10 indicated adsorption of two types viz., types I and II. Adsorption of six out of the ten sludges was due to type II adsorption. These included sludges 1, 2, 5, 6, 7 and 10 (Figs 4.61, 4.63, 4.69, 4.71, 4.73 and 4.79). Nickel adsorption therefore, occurs predominantly by a mono- to multi-layer mechanism. As load of Ni increases in solution, the multilayer mode of adsorption is preferred by sludge surfaces. Sludges 3, 4, 8 and 9 indicated 'L' shaped adsorption isotherms (Figs 4.65, 4.67, 4.75 and 4.77) with sludge 8 tending to be somewhat bilayered (Fig 4.75). However, all 'L' shaped

isotherms associated with Ni adsorption were gentle curves rather than steep uptake curves observed for Cu. This indicates the lower potential of sludges for adsorption of Ni compared to that of Cu.

Chromium (III) adsorption by the ten sludges was predominantly by the rectangular hyperbola mechanism which resembles closely the model for Langmuir adsorption. Six of the ten sludges adsorbed metal-ions by this mechanism (Figs 4.81, 4.83, 4.85, 4.87, 4.89 and 4.97). However the remaining four viz., sludges 6, 7, 8 and 10 showed isotherms approximating the 'L' shaped ones but decreasing and then increasing as adsorbate pressure increased (Figs 4.91, 4.96, 4.95 and 4.99). This could be due to a second layer of adsorption occurring which is not completely expressed as a type IV isotherm because sufficient adsorbate pressure is not applied to fully demonstrate the sludges' capabilities at adsorbing a further layer of Cr (III). Therefore, it seems that the potential of these four sludges to adsorb Cr (III) from solution is perhaps greater than that of the other six, evidenced by the tendency of these graphs to indicate an increase after approximately 2.0 mM ℓ^{-1} adsorbent pressure.

Chromium (VI) adsorption by sludges 2,3 and 4 displayed type I isotherms indicating a fixed demand for the metal by these sludges (Figs 4.103, 4.105 and 4.107). The majority of sludges showed a characteristic increase and then a decrease as adsorbate pressure increased. This was followed by a further increase in adsorptive capability, in most cases (sludges 4, 5, 6, 9 and 10) diminishing to reach saturation (Figs 4.107, 4.109, 4.111, 4.117 and 4.119). Sludge 1 displayed no apparent saturation point for adsorption indicating that it is capable of still further adsorption of Cr (VI) (Fig 4.101). The shapes observed for the seven sludges

adsorbing Cr (VI) indicate that there are possibly two types of adsorption occurring for Cr (VI) uptake or two layers of the metal-ions being formed on one adsorbent surface. However, the shape also indicates that there is a resistance to the second layer of adsorption because of the apparent decrease, in some cases, to below the saturation point before the second uptake cycle.

The highest capacity for nickel and trivalent chromium binding was shown by the Southern Work's sludge (Table 4.2). Maximum capacities for copper and hexavalent chromium were shown by Pietermaritzburg and New Germany Works' sludges, respectively. With the exception of Amanzimtoti Work's sludge which showed highest binding capacity as well as highest bond strength for zinc all other sludges showing highest X_m values for specific metals failed to portray corresponding high K_d values for the respective metal (Tables 4.2 and 4.3). This indicates that the surface binding capacity for metal-ions is not synonymous with the strength of binding.

Sludge biosorption ranking with respect to metal-ions was determined to be $S2 > S3 > S9 > S1 > S4 > S6 > S5$. This was determined by ascertaining the binding capacities, (Table 4.2), of sludges to metal-ions. Maximum binding capacities of sludges to metal-ions, with the exception of Zn adsorption to sludge 2, did not coincide with maximum bond strengths of sludges to metal-ions, (Tables 4.2 & 4.3). However, an ideal biosorbent should have a high metal binding capacity with a moderate to low bond strength to facilitate removal of large amounts of metal-ions and desorption. Sludges indicating highest binding capacities for Ni^{2+} , Cu^{2+} , Cr^{3+} and Cr^{6+} , ($S2$, $S5$, $S9$ & $S3$, Table 4.2), display correspondingly moderate bond strengths, (Table 4.3). Similarly, sludges can be chosen for adsorption of

Cd^{2+} and Zn^{2+} since the sludges with highest binding capacities for these metals also show corresponding high binding strengths.

4.4 CONCLUSIONS

Sludges have been shown to adsorb metal ions from solutions and these adsorptive capabilities were fitted to the Langmuir Model of adsorption which provides mathematical reductions to sludge-metal adsorption experiments to facilitate selection of the most efficient sludge for the adsorption of metal-ions. Sludges show a preference in capacity and affinity for certain metal-ions as determined by reciprocal linearisations of adsorption isotherms.

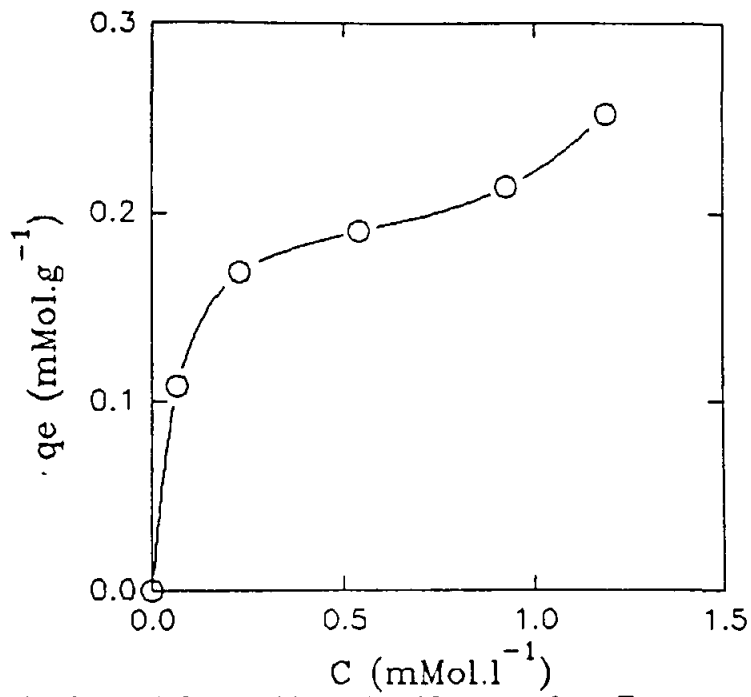


Fig. 4. 3. Adsorption isotherm for Zn uptake from solution by sludge 1.

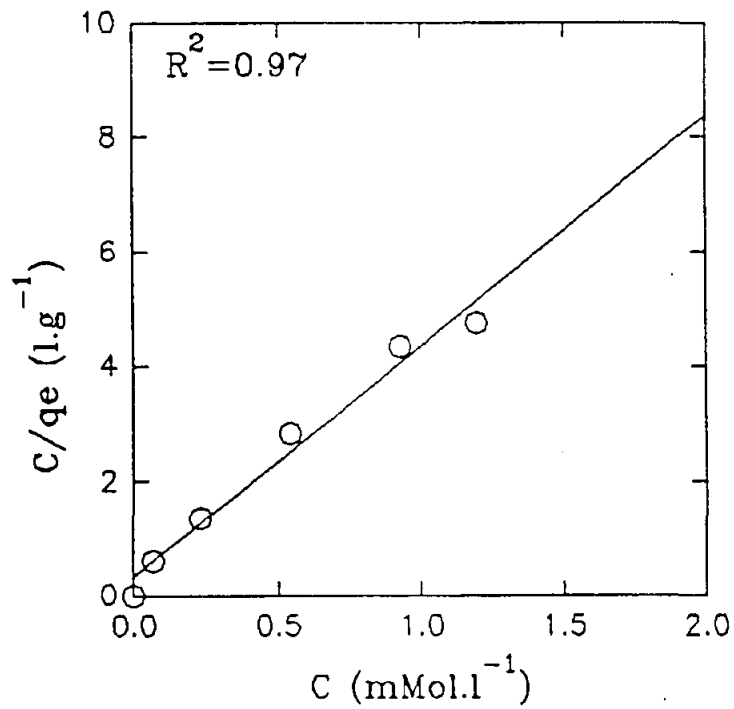


Fig. 4. 4. Reciprocal isotherm for Zn uptake from solution by sludge 1.

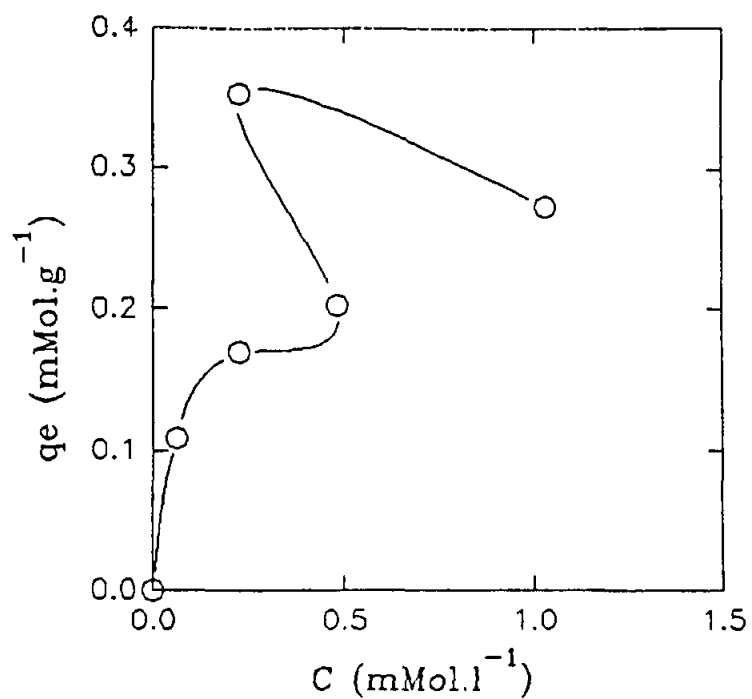


Fig. 4. 5. Adsorption isotherm for Zn uptake from solution by sludge 2.

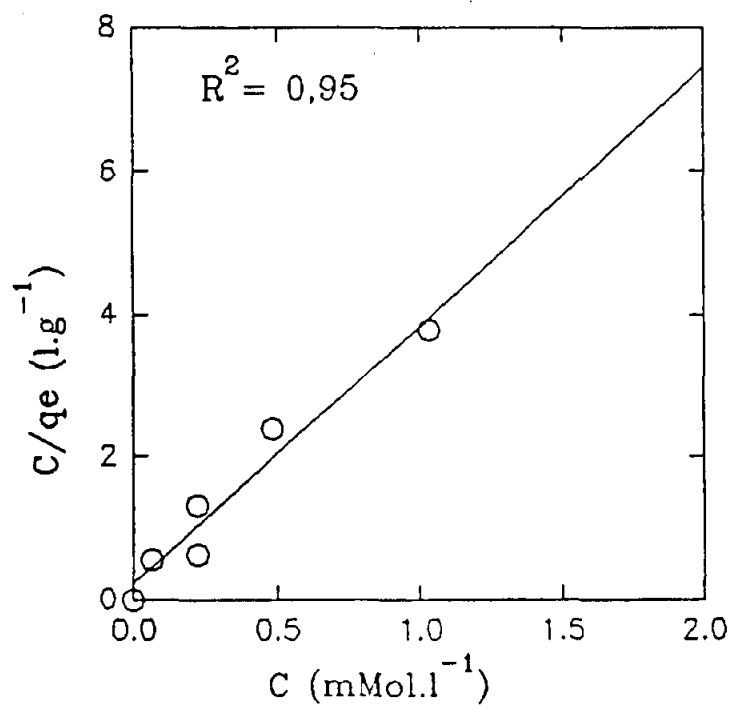


Fig. 4. 6. Reciprocal isotherm for Zn uptake from solution by sludge 2.

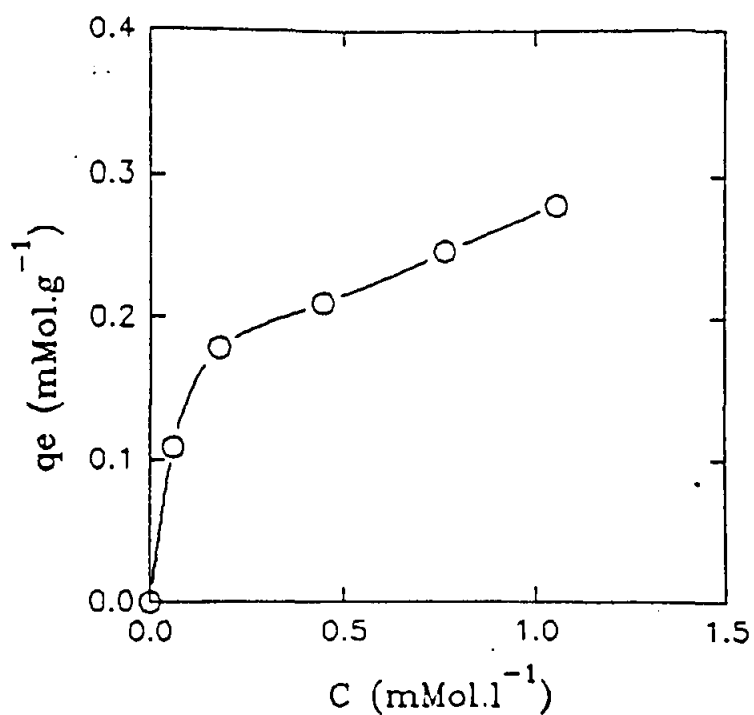


Fig. 4.7 Adsorption isotherm for Zn uptake from solution by sludge 3.

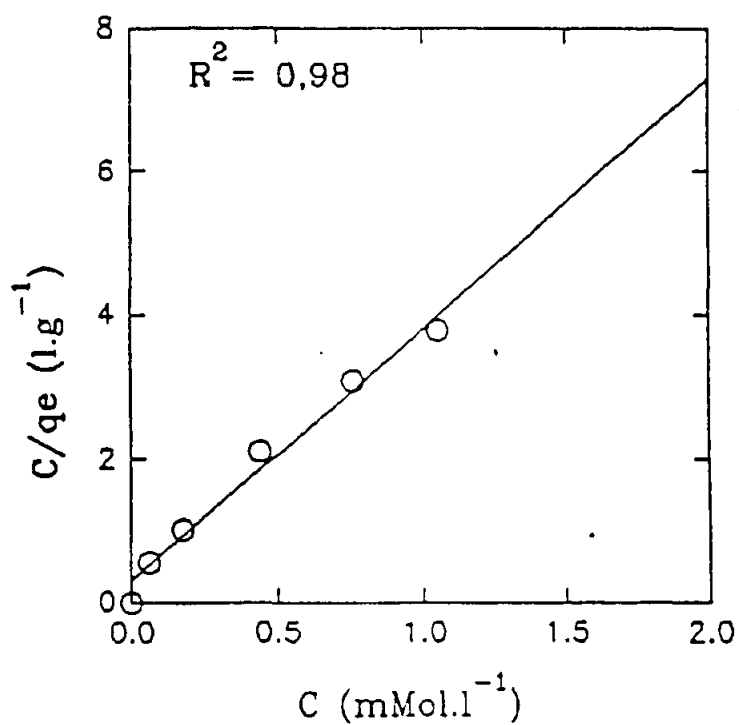


Fig. 4.8 Reciprocal isotherm for Zn uptake from solution by sludge 3.

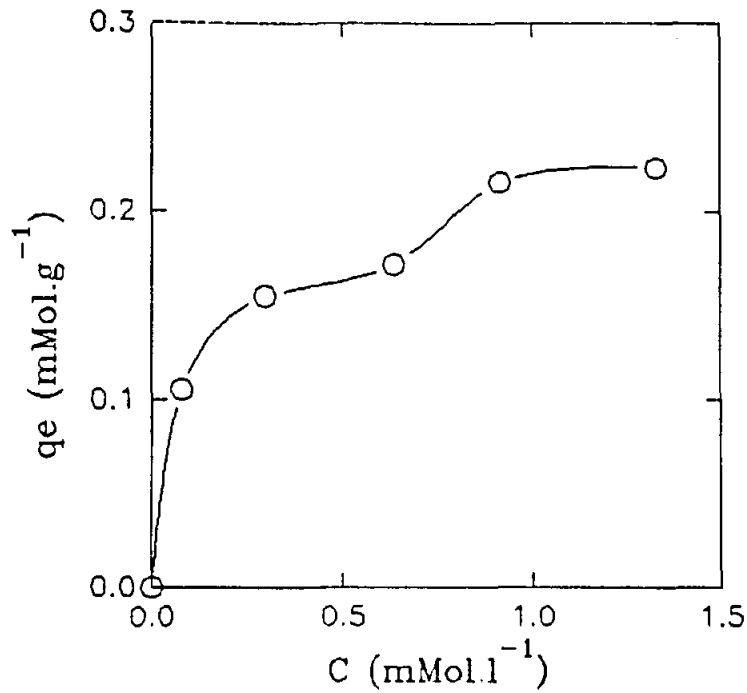


Fig. 4. 9. Adsorption isotherm for Zn uptake from solution by sludge 4.

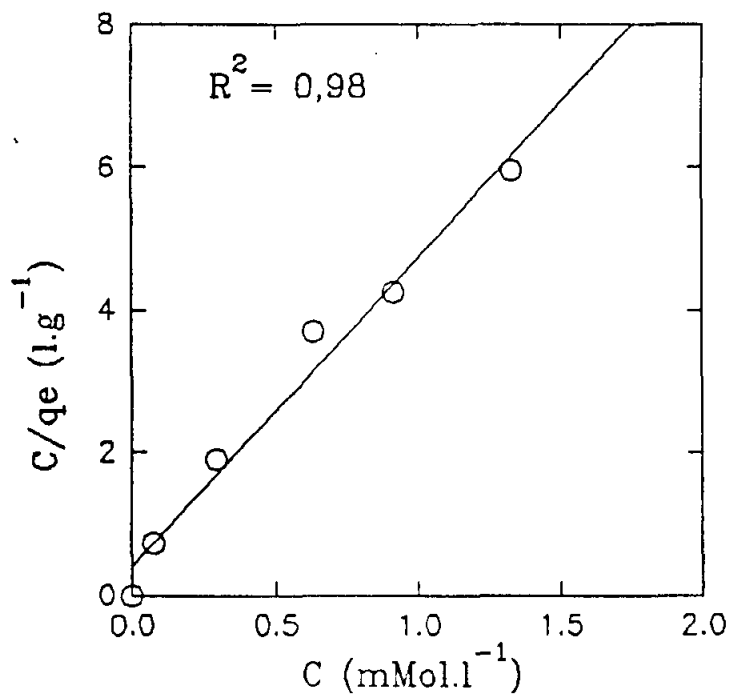


Fig. 4. 10. Reciprocal isotherm for Zn uptake from solution by sludge 4.

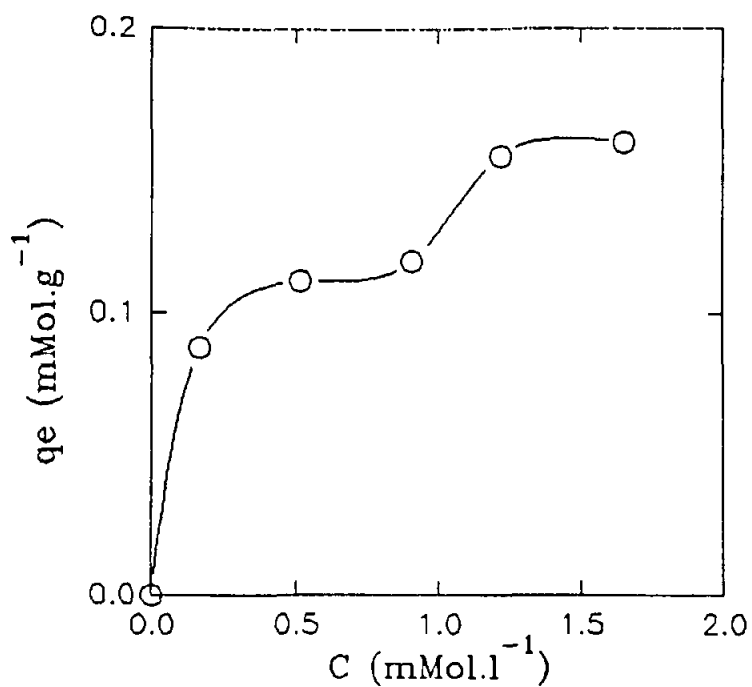


Fig. 4. 11. Adsorption isotherm for Zn uptake from solution by sludge 5.

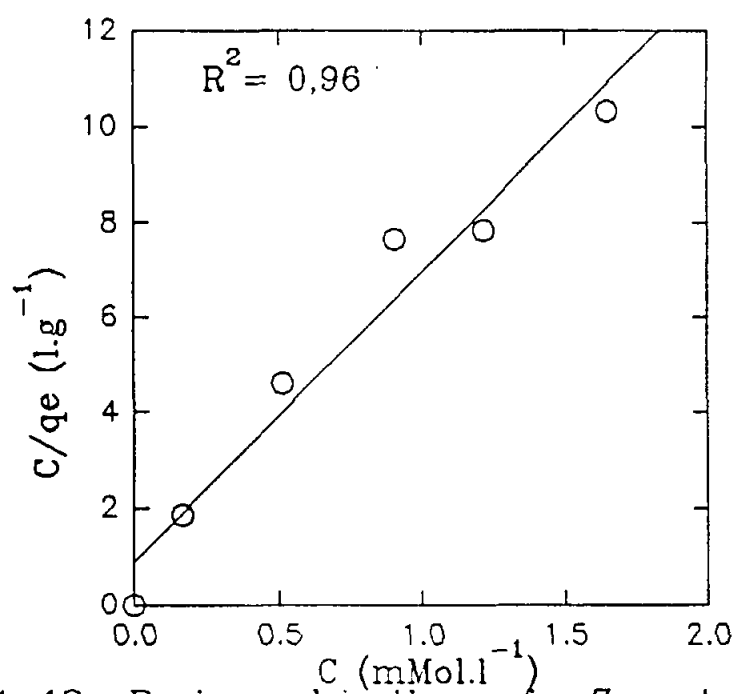


Fig. 4. 12. Reciprocal isotherm for Zn uptake from solution by sludge 5.

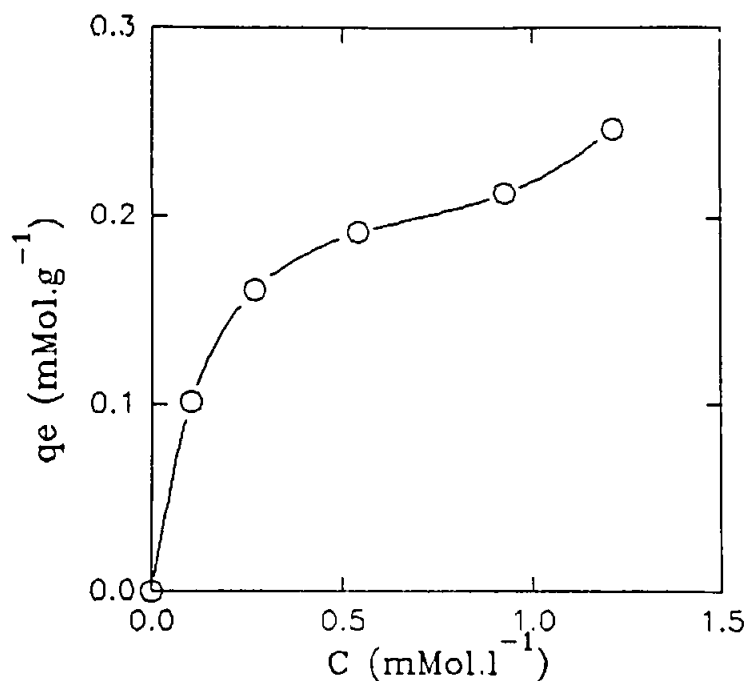


Fig. 4. 13. Adsorption isotherm for Zn uptake from solution by sludge 6.

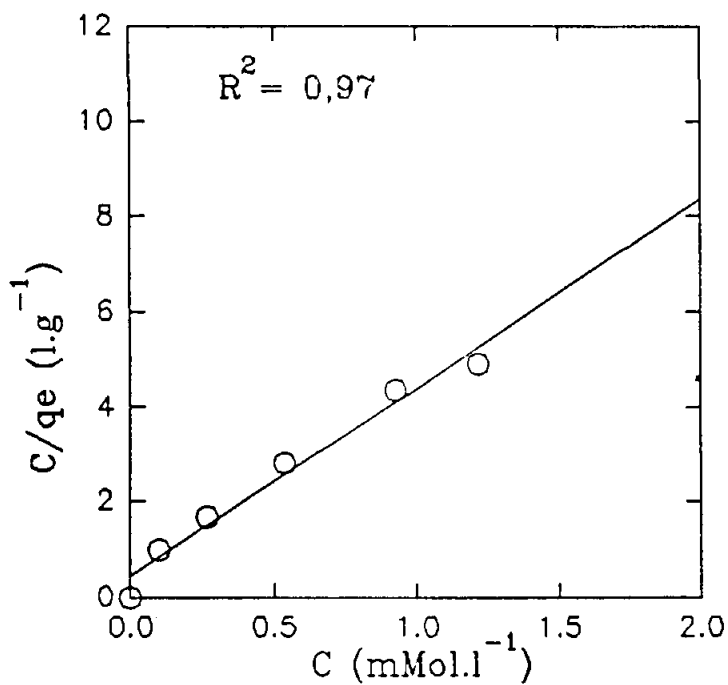


Fig. 4. 14. Reciprocal isotherm for Zn uptake from solution by sludge 6.

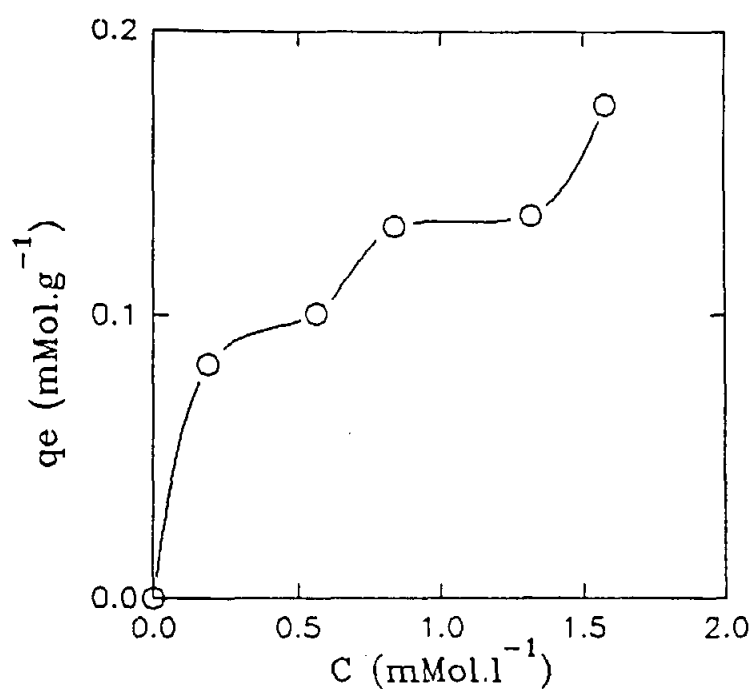


Fig. 4. 15. Adsorption isotherm for Zn uptake from solution by sludge 7.

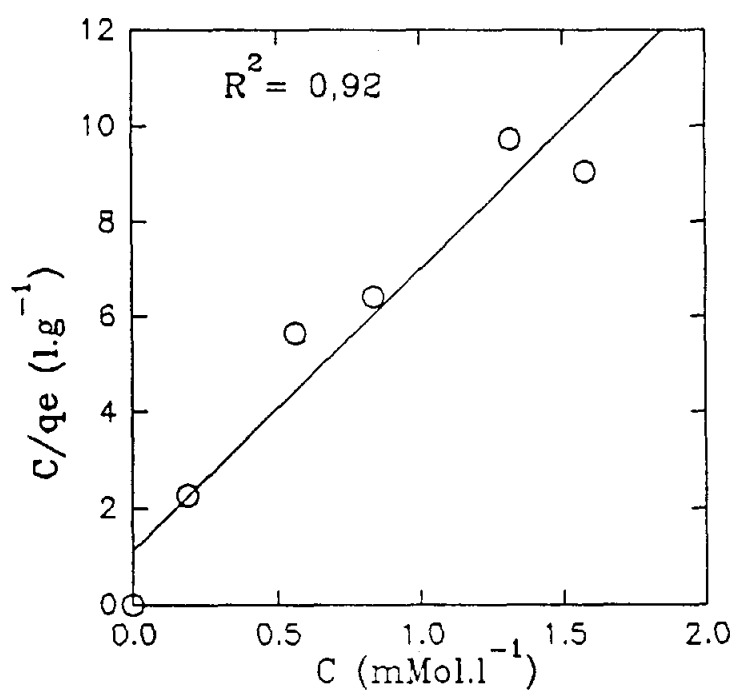


Fig. 4. 16. Reciprocal isotherm for Zn uptake from solution by sludge 7.

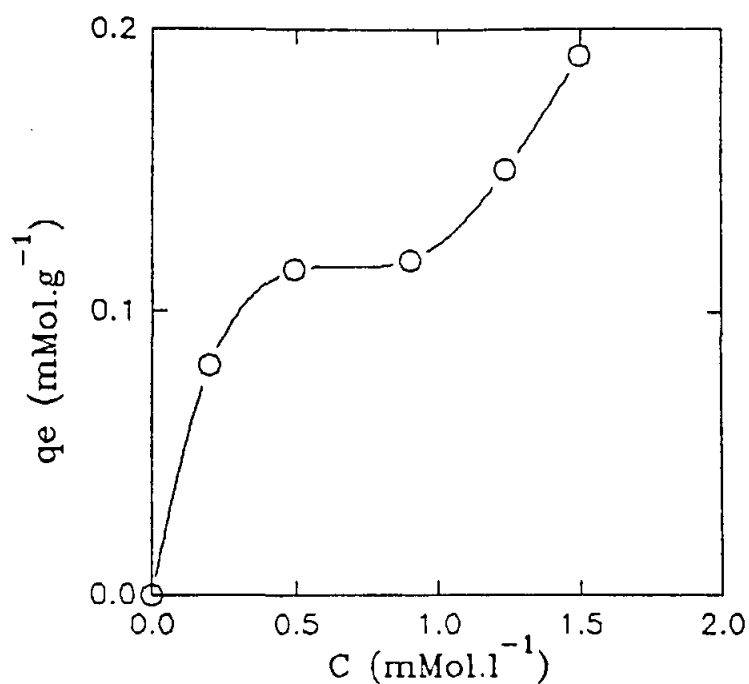


Fig. 4. 17. Adsorption isotherm for Zn uptake from solution by sludge 8.

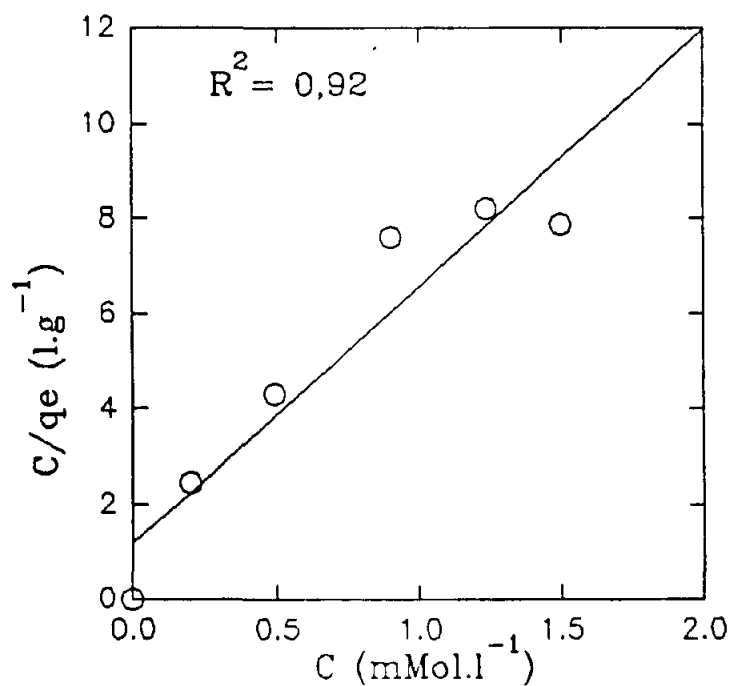


Fig. 4. 18. Reciprocal isotherm for Zn uptake from solution by sludge 8.

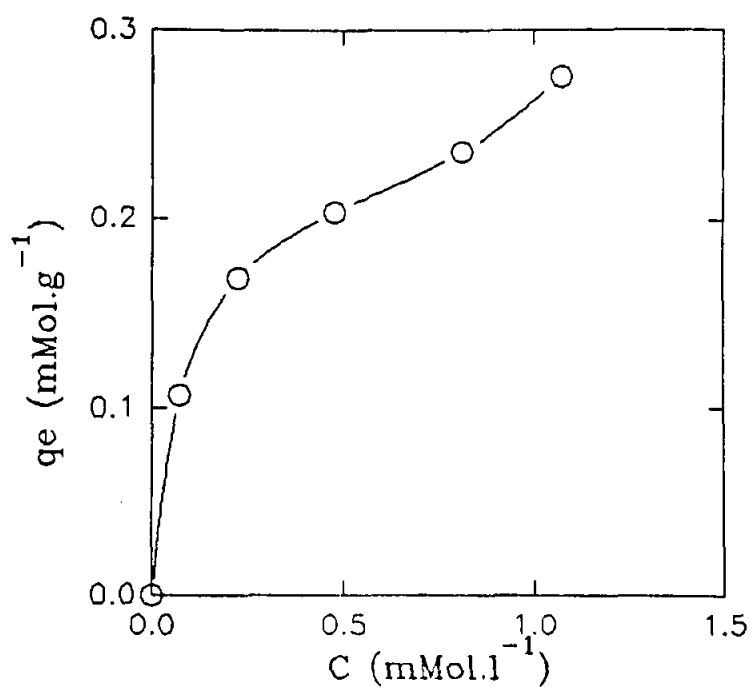


Fig. 4. 19. Adsorption isotherm for Zn uptake from solution by sludge 9.

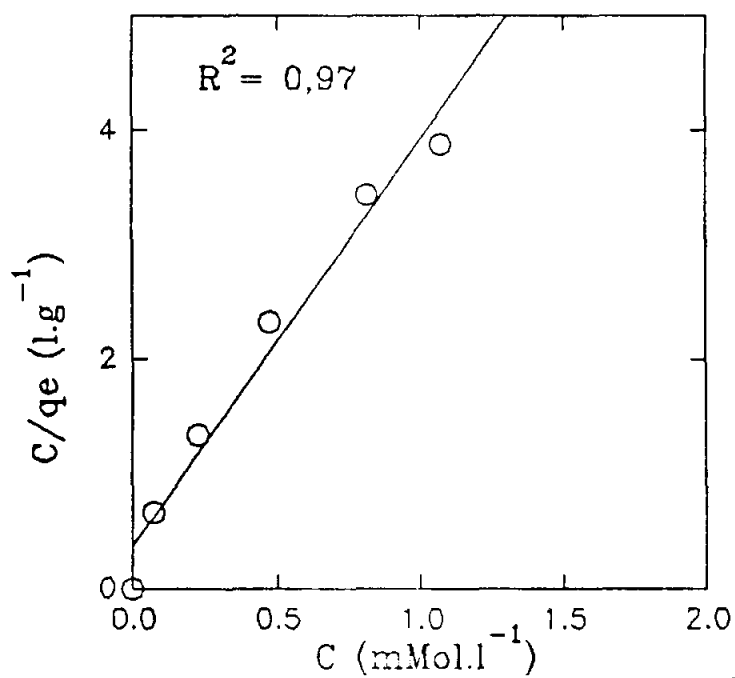


Fig. 4. 20. Reciprocal isotherm for Zn uptake from solution by sludge 9.

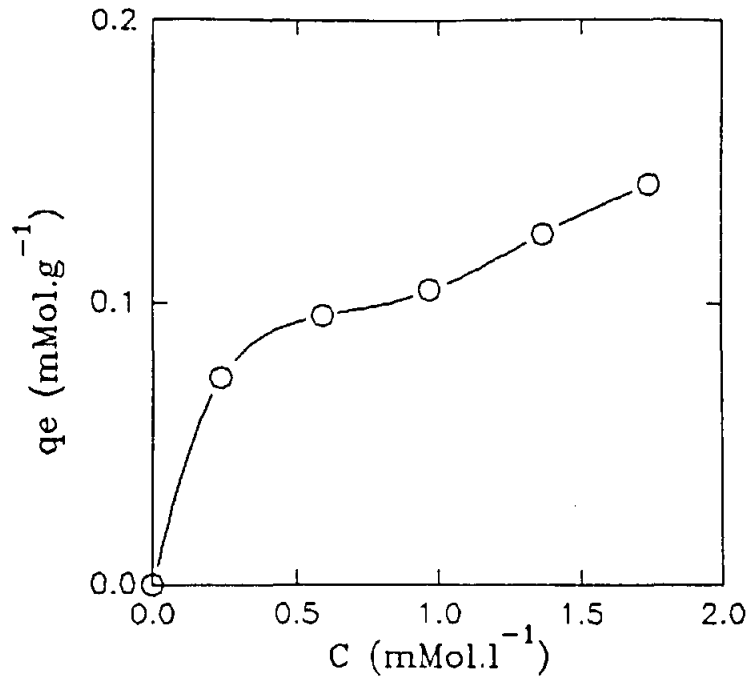


Fig. 4. 21. Adsorption isotherm for Zn uptake from solution by sludge 10.

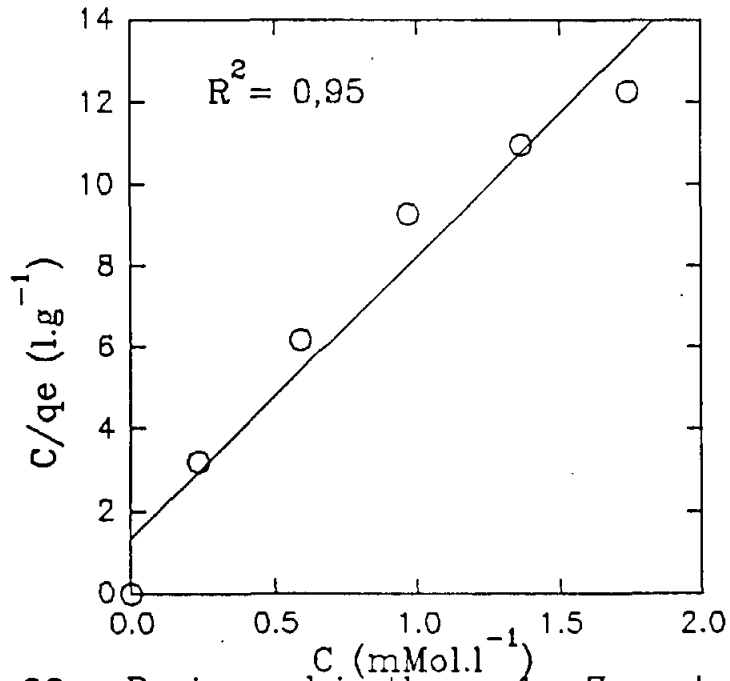


Fig. 4. 22. Reciprocal isotherm for Zn uptake from solution by sludge 10.

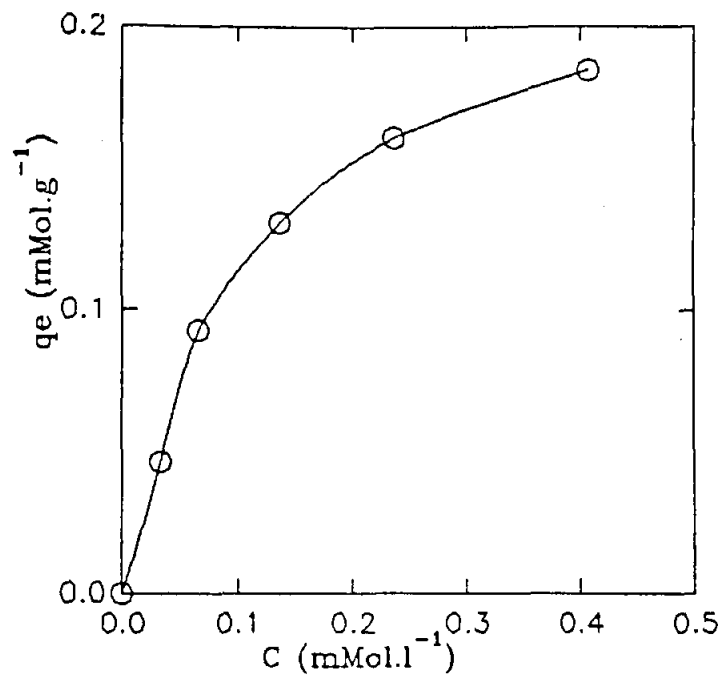


Fig. 4. 23. Adsorption isotherm for Cd uptake from solution by sludge 1.

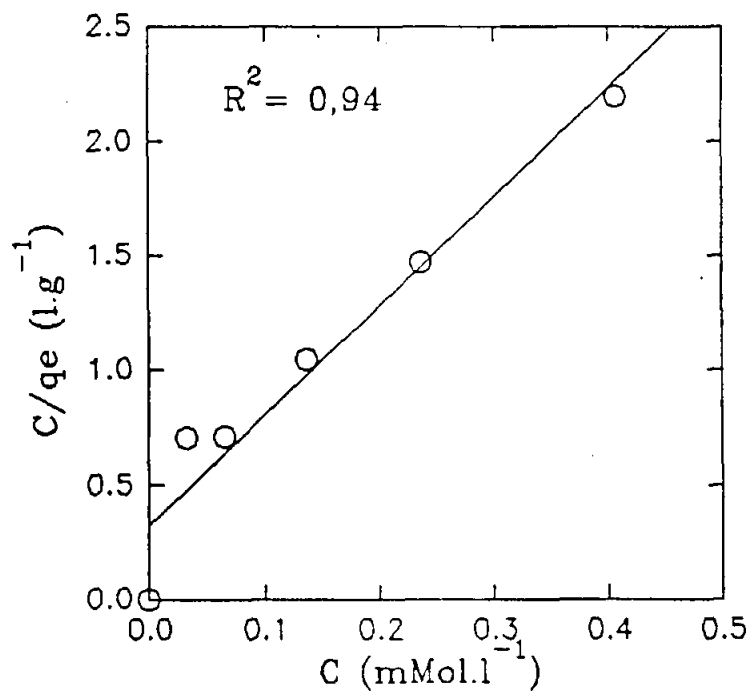


Fig. 4. 24. Reciprocal isotherm for Cd uptake from solution by sludge 1.

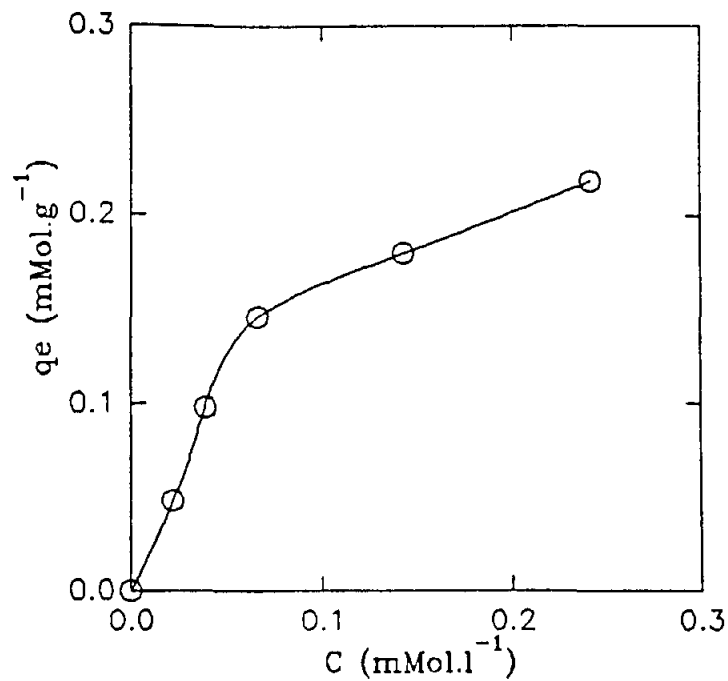


Fig. 4. 25. Adsorption isotherm for Cd in solution by sludge 2.

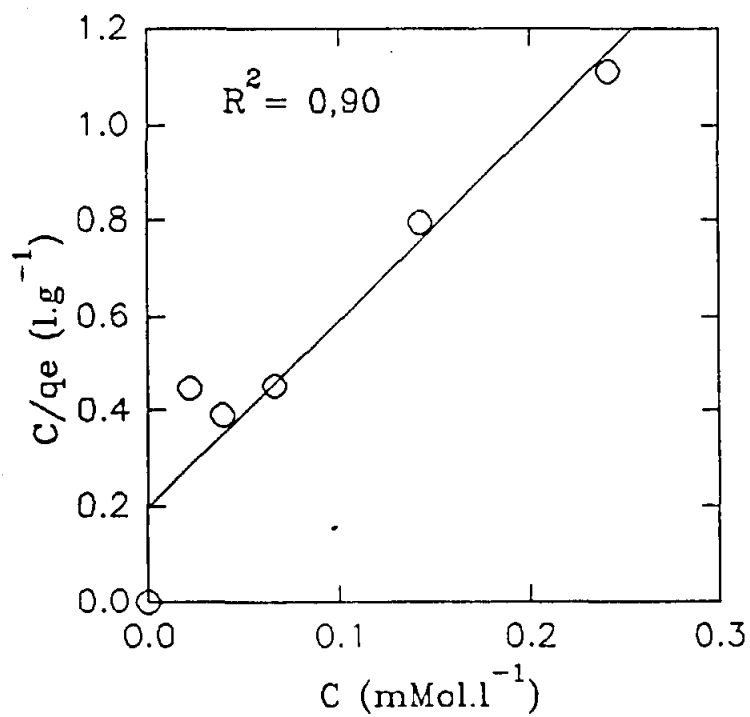


Fig. 4. 26. Reciprocal isotherm for Cd uptake from solution by sludge 2.

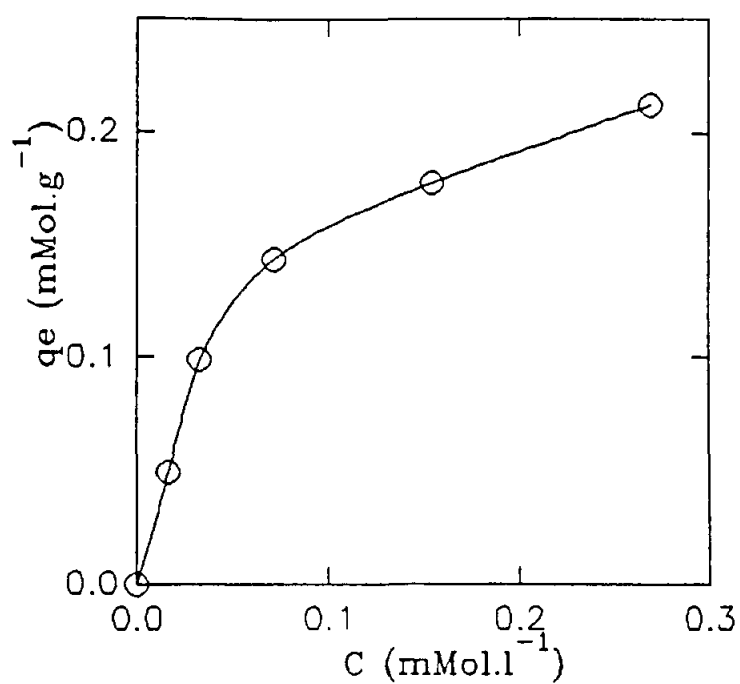


Fig. 4. 27. Adsorption isotherm for Cd in solution by sludge 3.

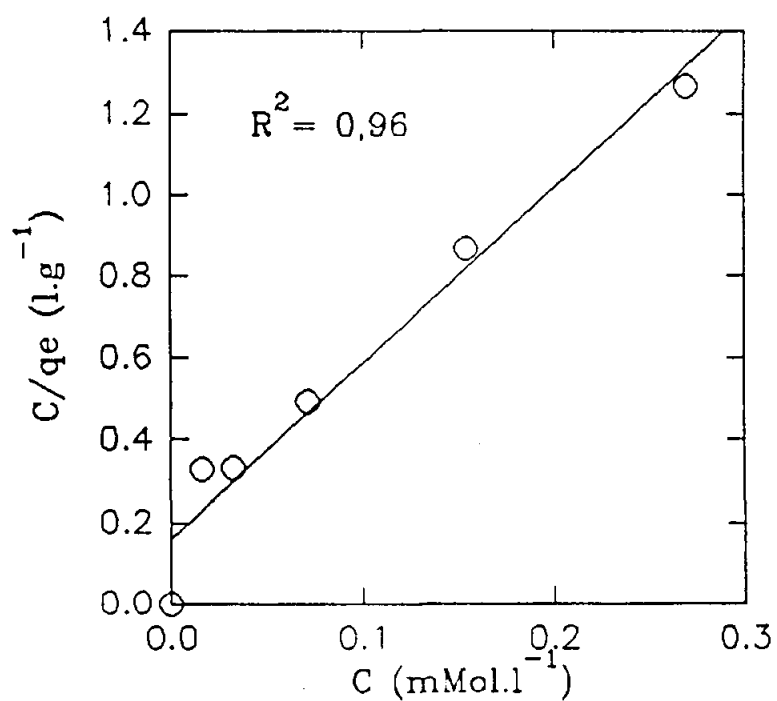


Fig. 4. 28. Reciprocal isotherm for Cd uptake from solution by sludge 3.

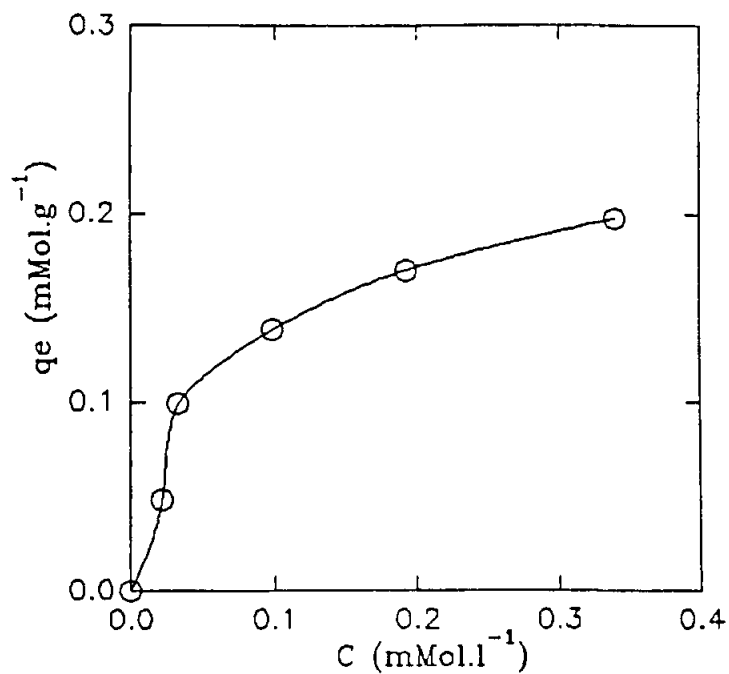


Fig. 4. 29. Adsorption isotherm for Cd uptake from solution by sludge 4.

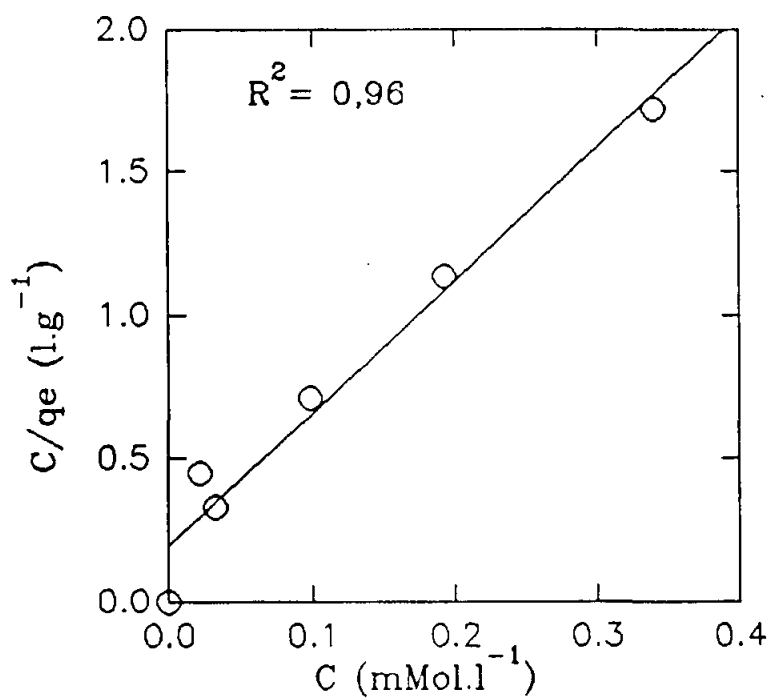


Fig. 4. 30. Reciprocal isotherm for Cd uptake from solution by sludge 4.

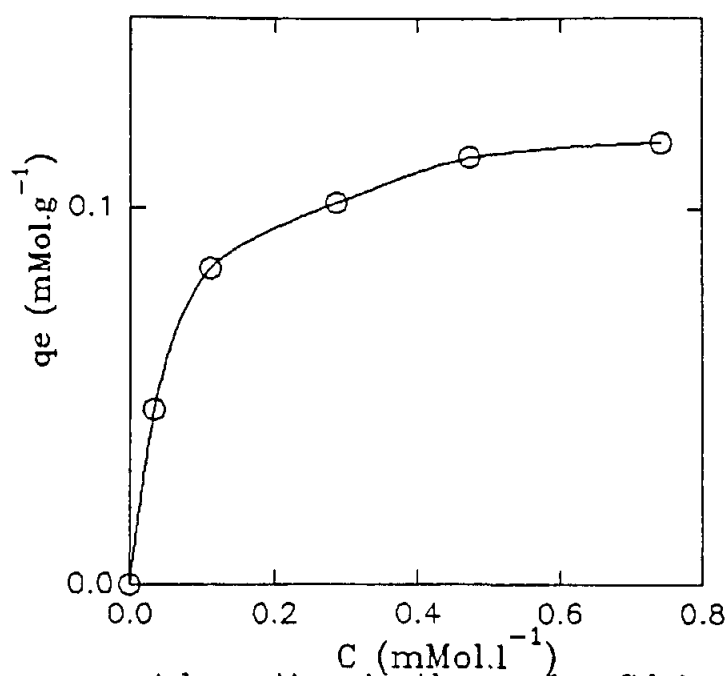


Fig. 4. 31. Adsorption isotherm for Cd in solution by sludge 5.

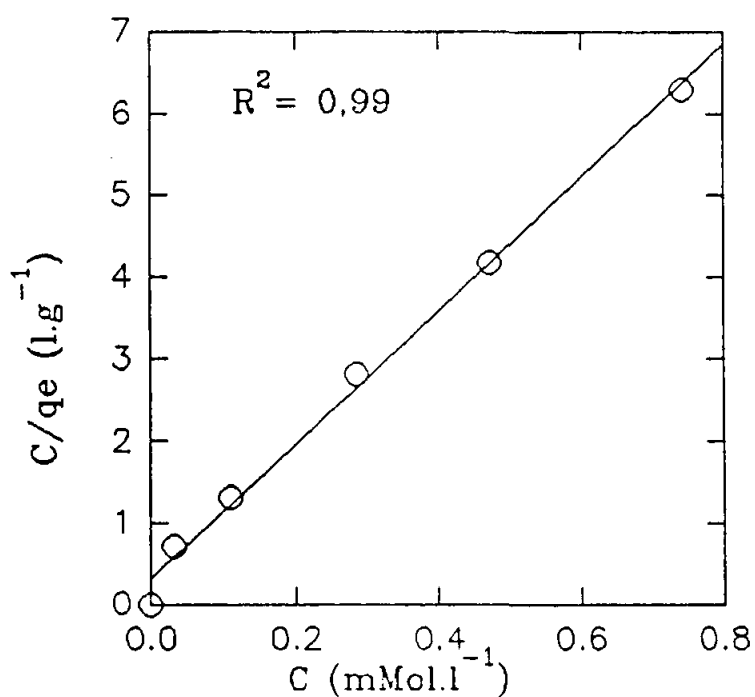


Fig. 4. 32. Reciprocal isotherm for Cd uptake from solution by sludge 5.

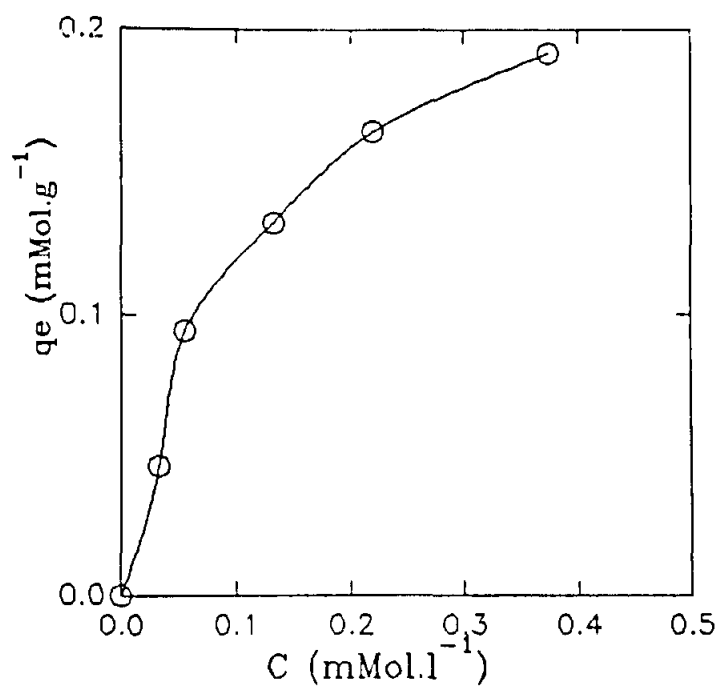


Fig. 4. 33. Adsorption isotherm for Cd uptake from solution by sludge 6.

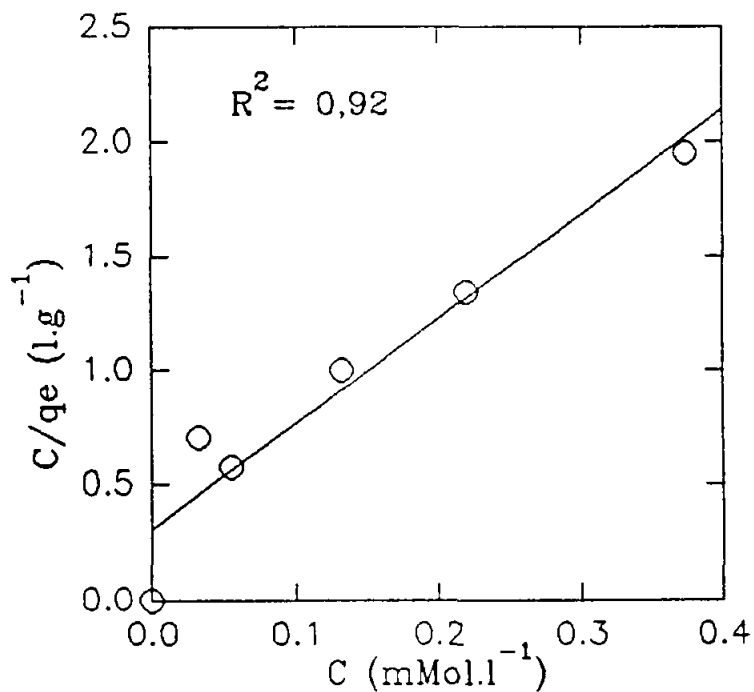


Fig. 4. 34. Reciprocal isotherm for Cd uptake from solution by sludge 6.

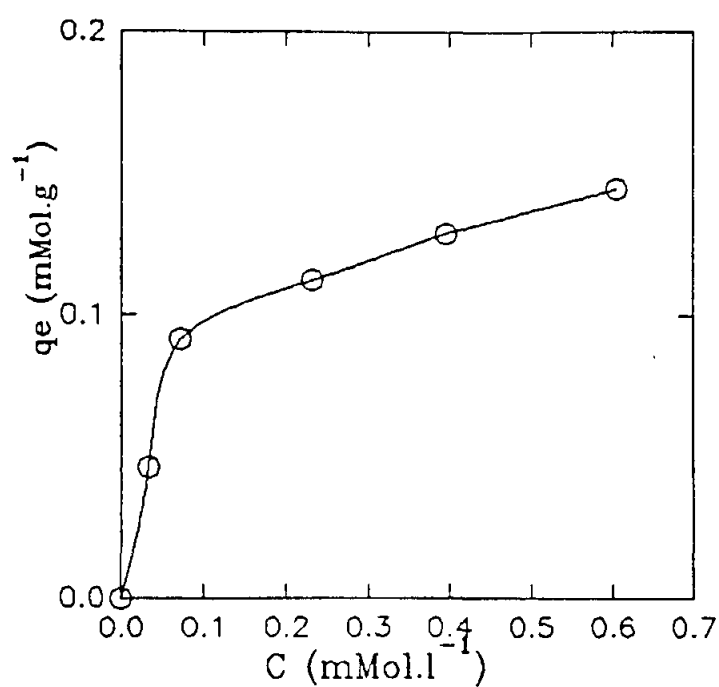


Fig. 4. 35. Adsorption isotherm for Cd uptake from solution by sludge 7.

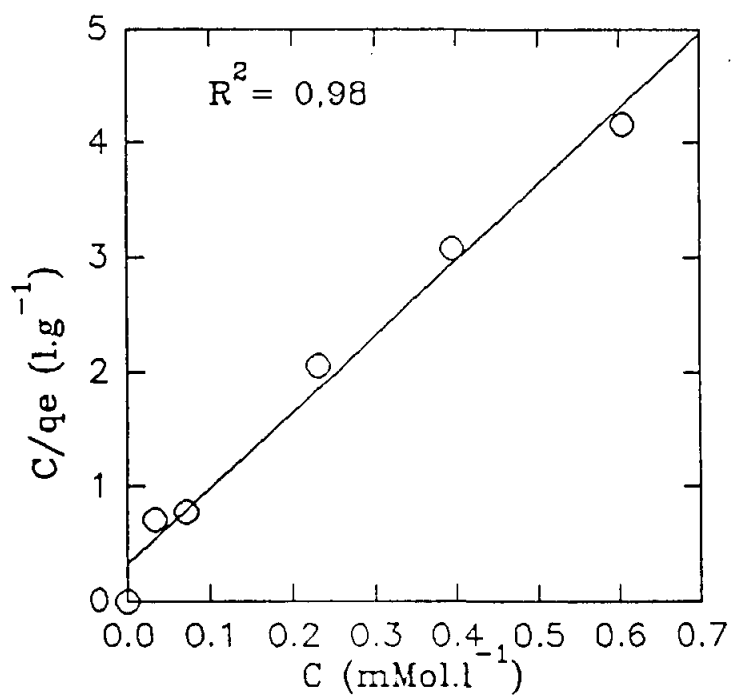


Fig. 4. 36. Reciprocal isotherm for Cd uptake from solution by sludge 7.

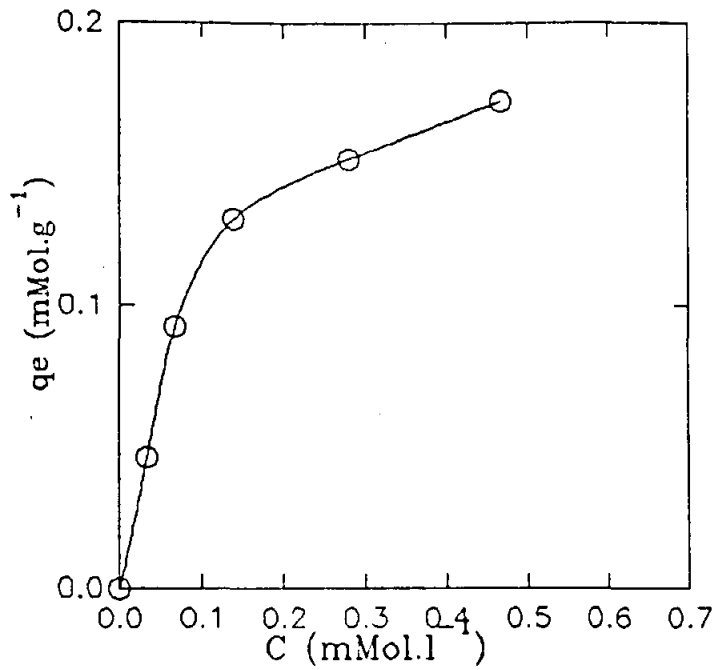


Fig. 4. 37. Adsorption isotherm for Cd uptake from solution by sludge 8.

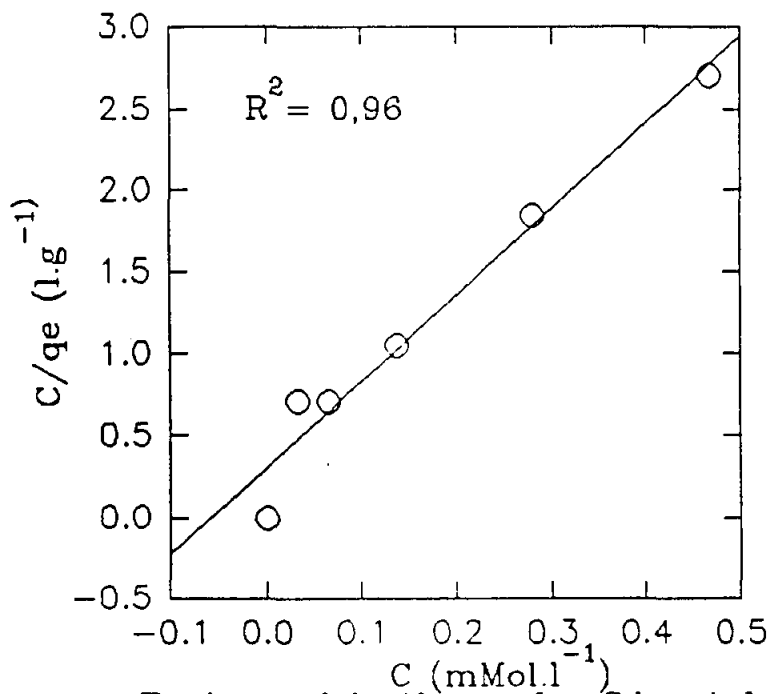


Fig. 4. 38. Reciprocal isotherm for Cd uptake from solution by sludge 8.

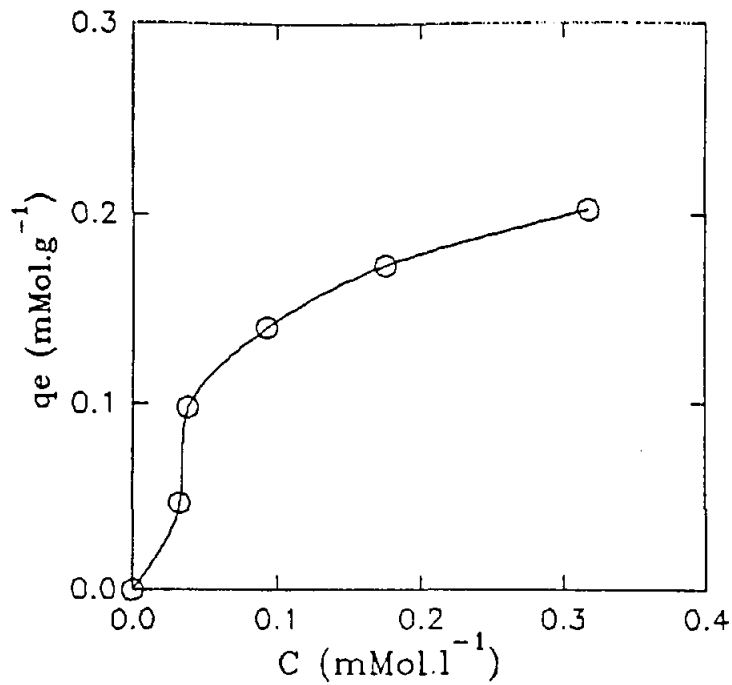


Fig. 4. 39. Adsorption isotherm for Cd uptake from solution by sludge 9.

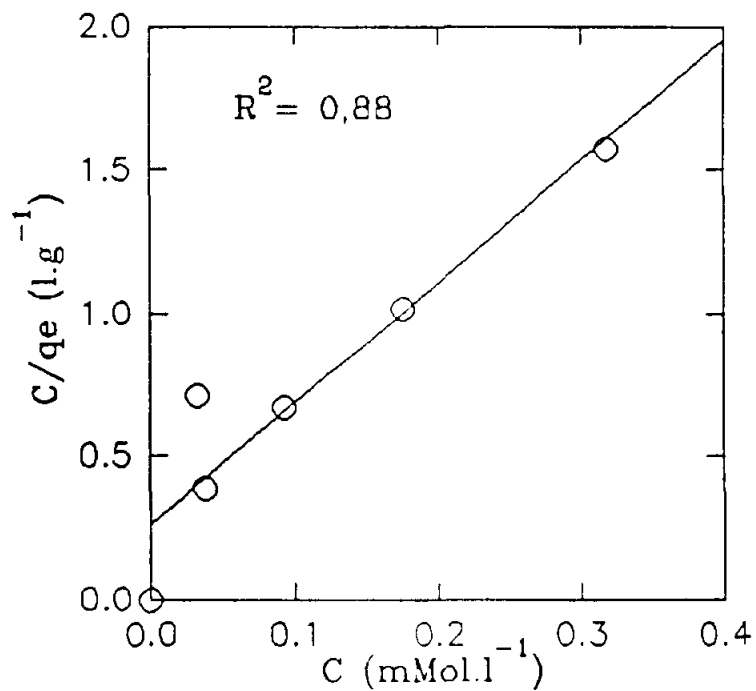


Fig. 4. 40. Reciprocal isotherm for Cd uptake from solution by sludge 9.

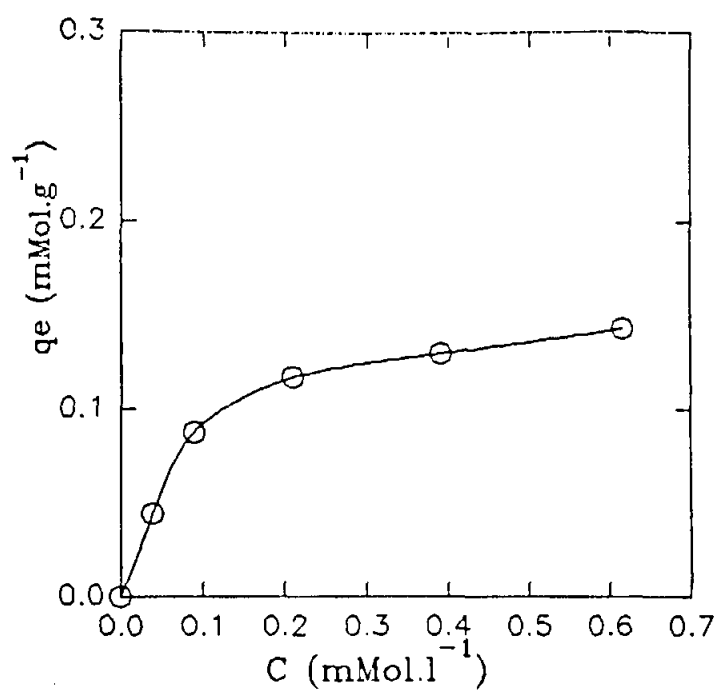


Fig. 4. 41. Adsorption isotherm for Cd uptake from solution by sludge 10.

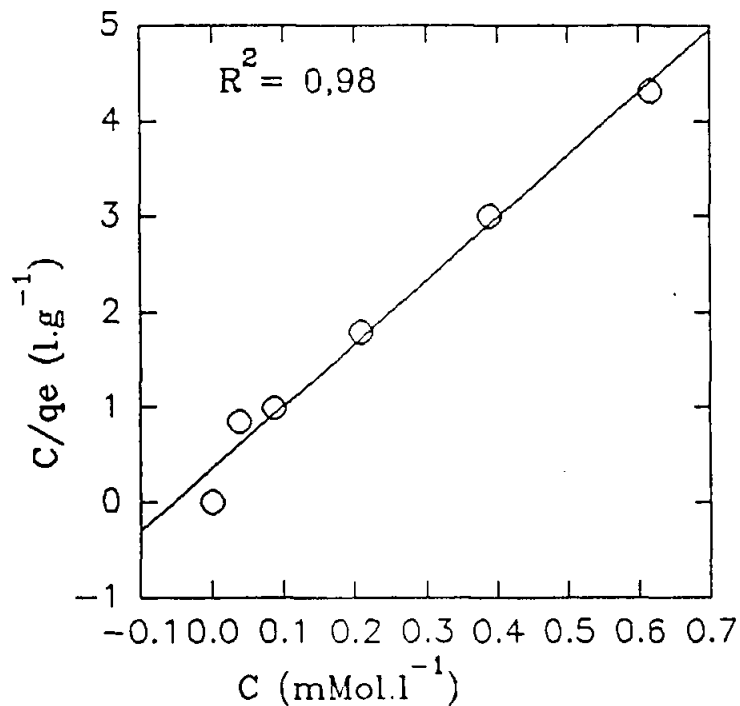


Fig. 4. 42. Reciprocal uptake for Cd from solution by sludge 10.

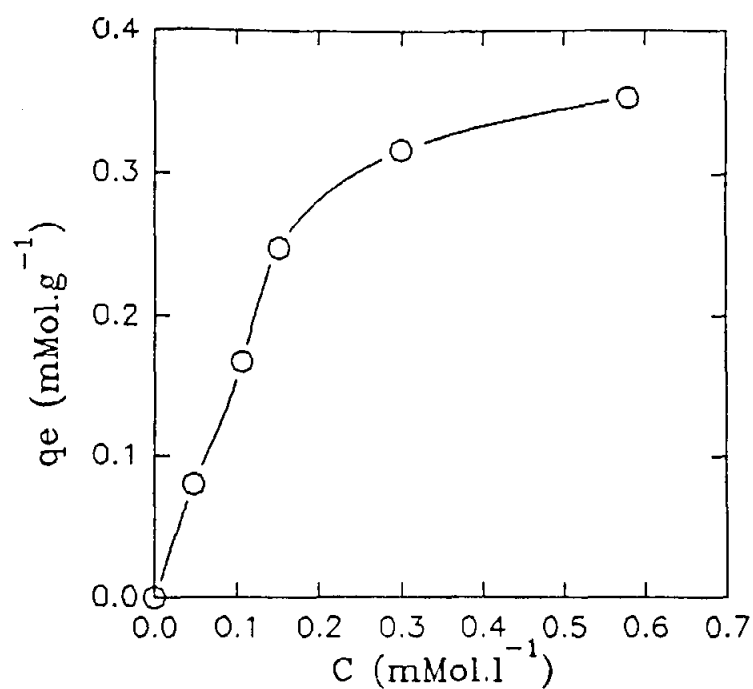


Fig. 4. 43. Adsorption isotherm for Cu uptake from solution by sludge 1.

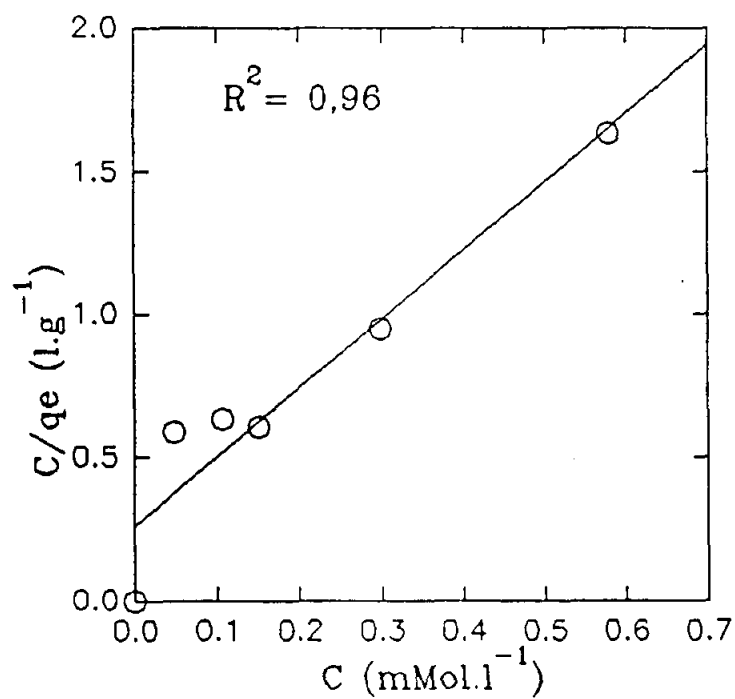


Fig. 4. 44. Reciprocal isotherm for Cu uptake from solution by sludge 1.

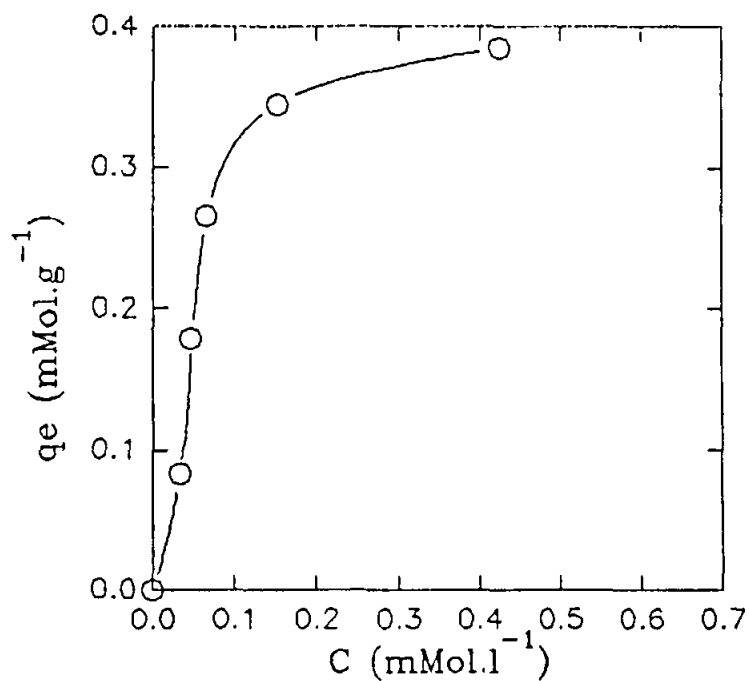


Fig. 4. 45. Adsorption isotherm for Cu uptake from solution by sludge 2.

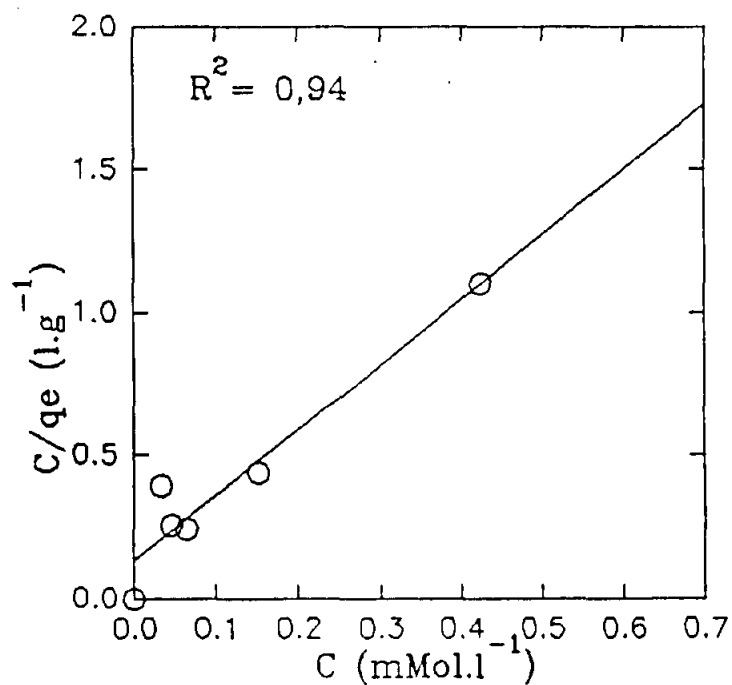


Fig. 4. 46. Reciprocal isotherm for Cu uptake from solution by sludge 2.

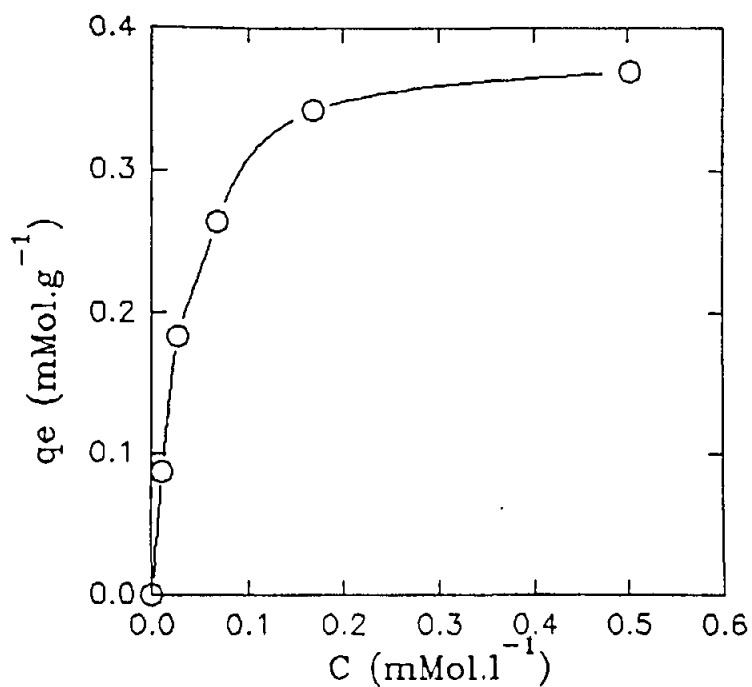


Fig. 4. 47. Adsorption isotherm for Cu uptake from solution by sludge 3.

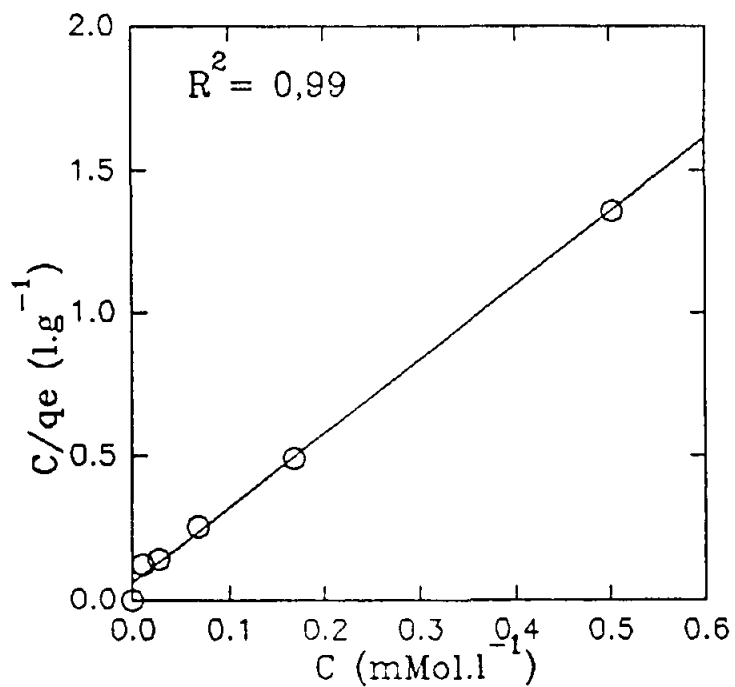


Fig. 4. 48. Reciprocal isotherm for Cu uptake from solution by sludge 3.

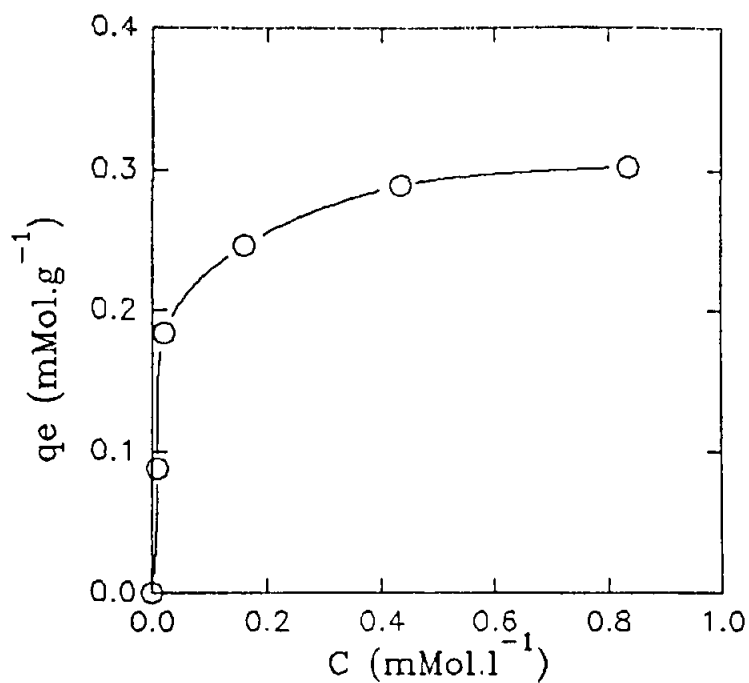


Fig. 4. 49. Adsorption isotherm for Cu uptake from solution by sludge 4.

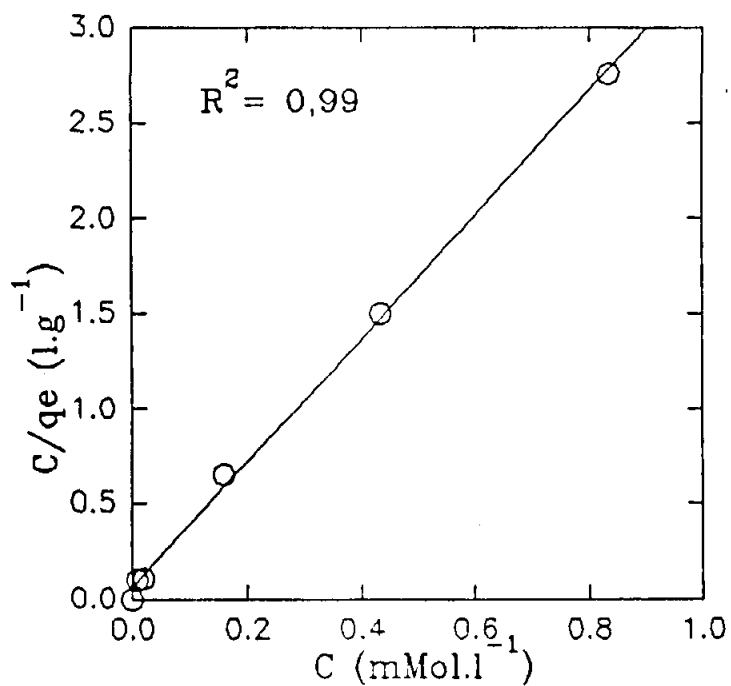


Fig. 4. 50. Reciprocal isotherm for Cu uptake from solution by sludge 4.

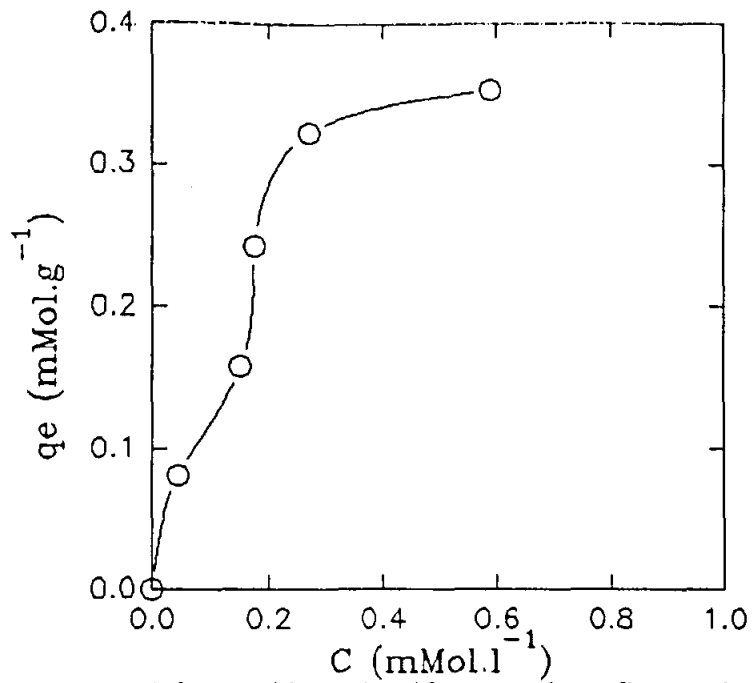


Fig. 4. 51. Adsorption isotherm for Cu uptake from solution by sludge 5.

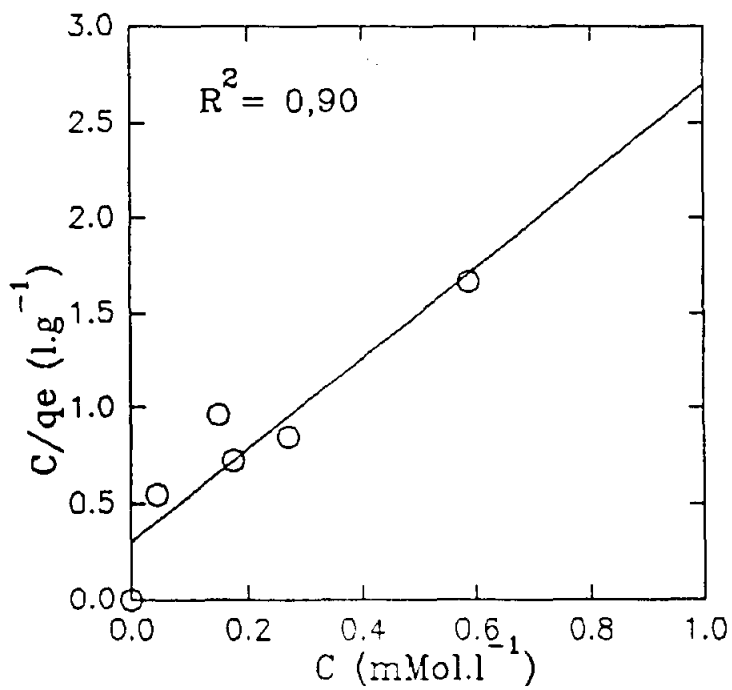


Fig. 4. 52. Reciprocal isotherm for Cu uptake from solution by sludge 5.

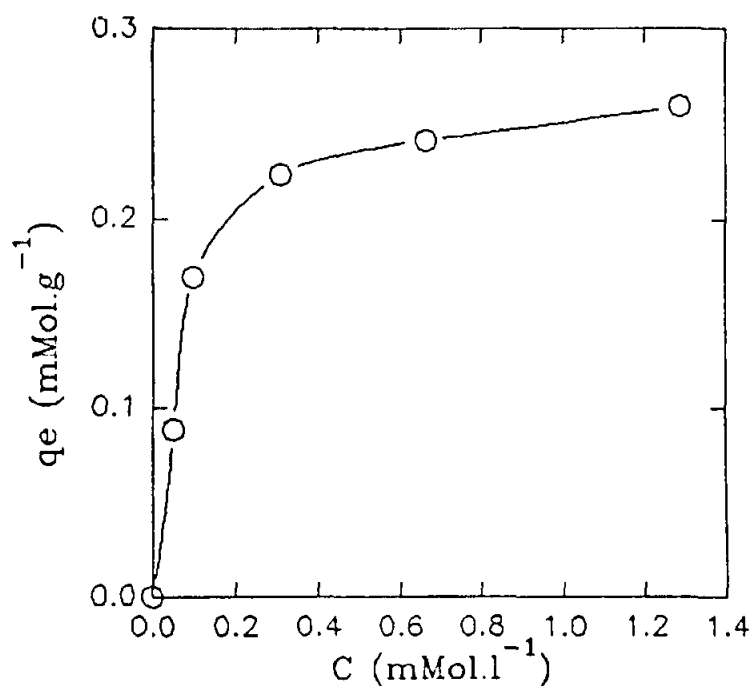


Fig. 4. 53. Adsorption isotherm for Cu uptake from solution by sludge 6.

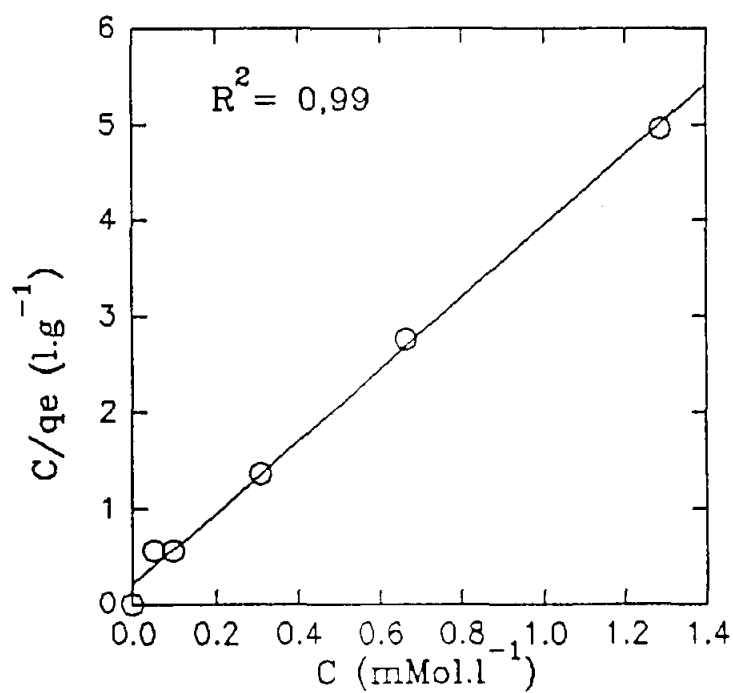


Fig. 4. 54. Reciprocal isotherm for Cu uptake from solution by sludge 6.

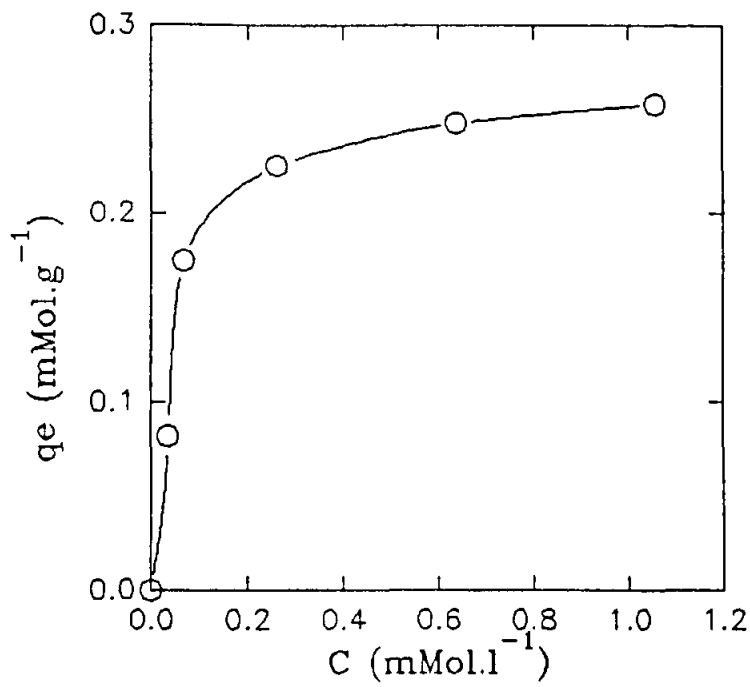


Fig. 4. 55. Adsorption isotherm for Cu uptake from solution by sludge 7.

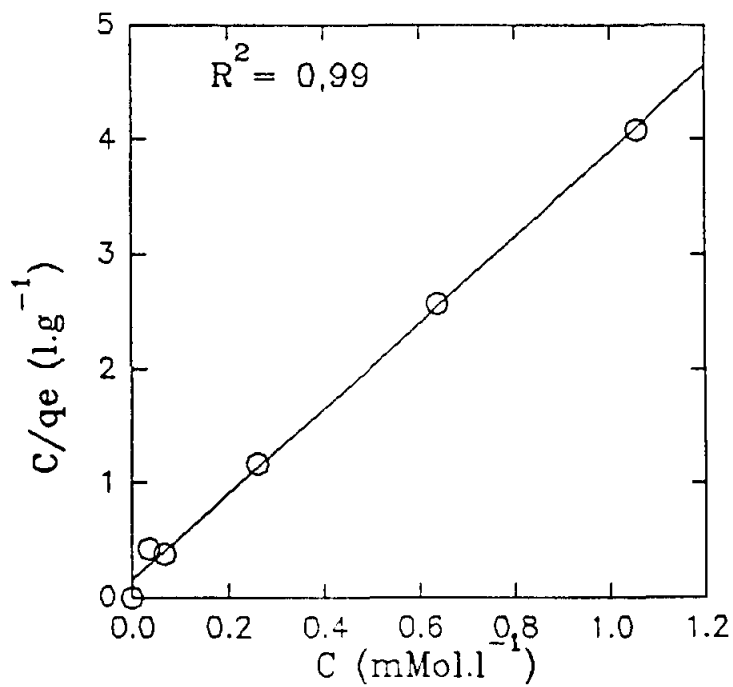


Fig. 4. 56. Reciprocal isotherm for Cu uptake from solution by sludge 7.

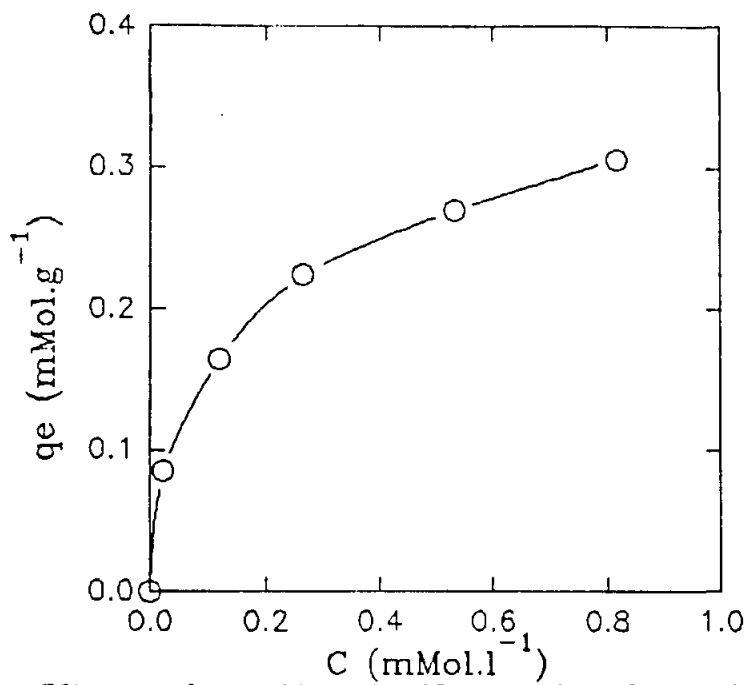


Fig. 4. 57. Adsorption isotherm for Cu uptake from solution by sludge 8.

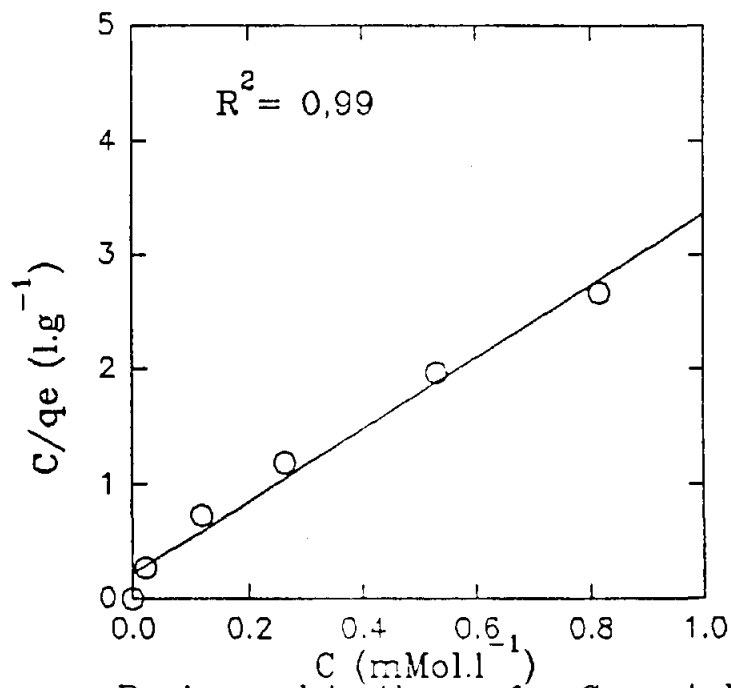


Fig. 4. 58. Reciprocal isotherm for Cu uptake from solution by sludge 8.

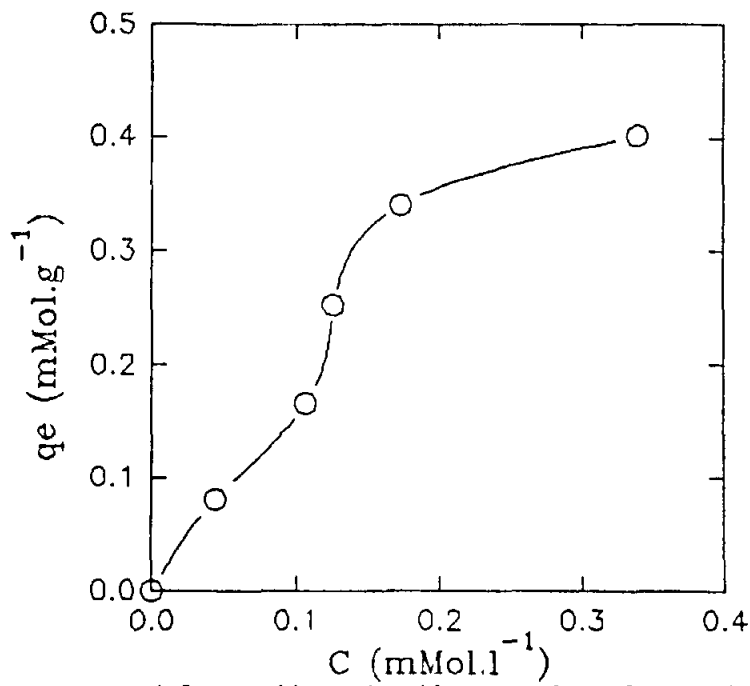


Fig. 4. 59. Adsorption isotherm for Cu uptake from solution by sludge 9.

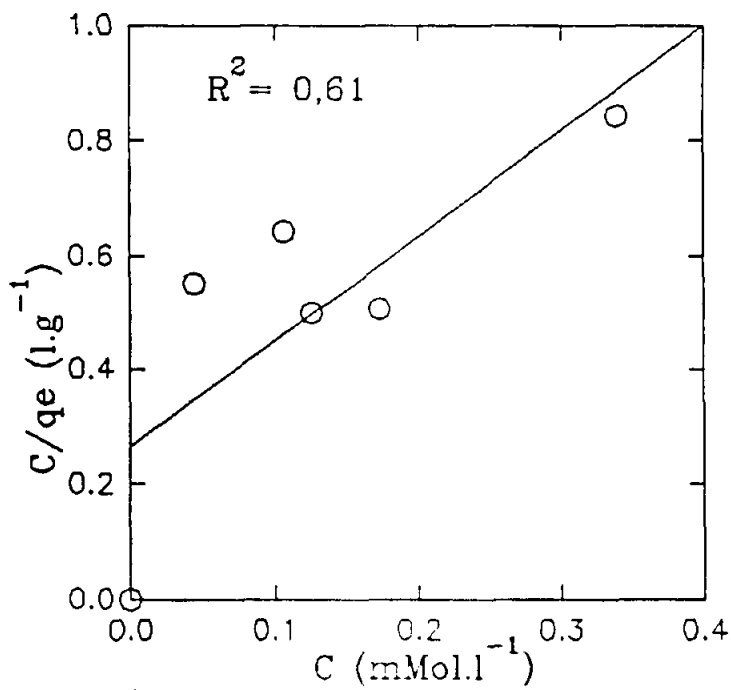


Fig. 4. 60. Reciprocal isotherm for Cu uptake from solution by sludge 9.

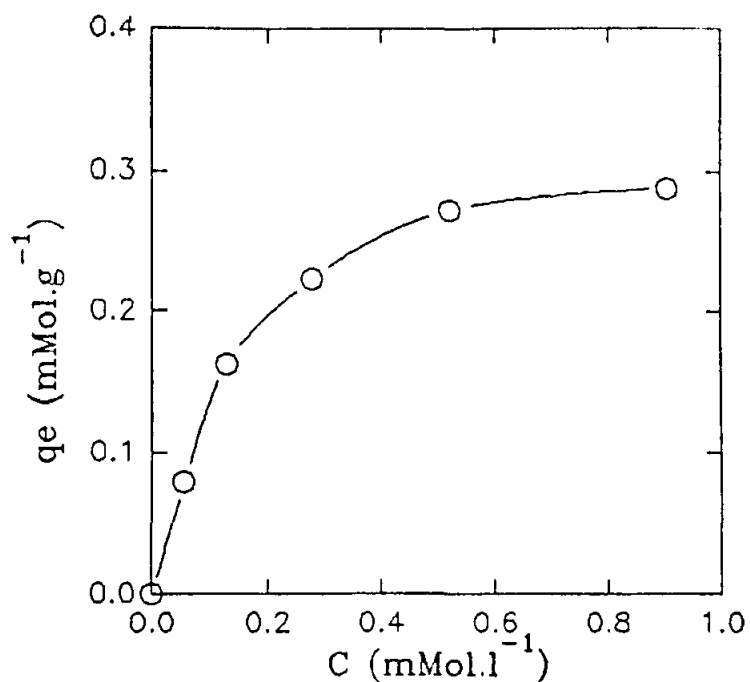


Fig. 4. 61. Adsorption isotherm for Cu uptake from solution by sludge 10.

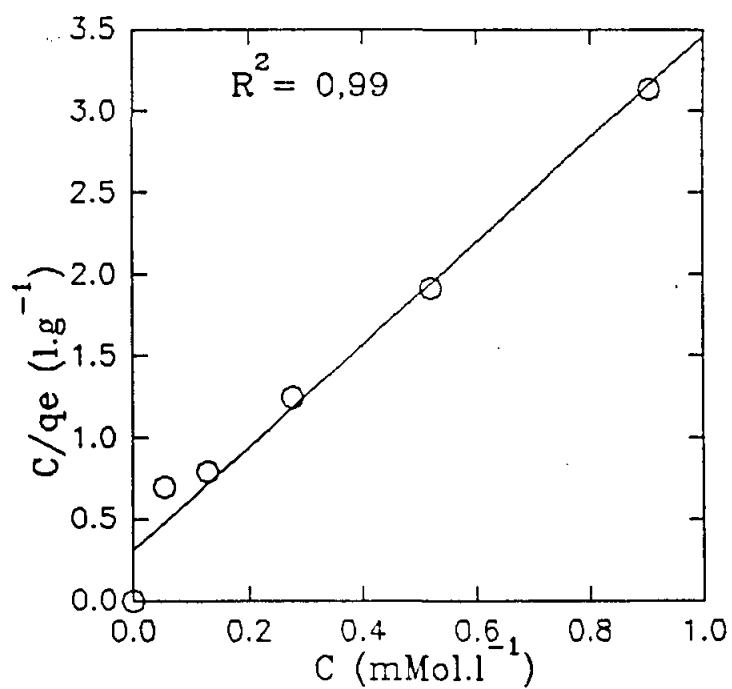


Fig. 4. 62. Reciprocal isotherm for Cu uptake from solution by sludge 10.

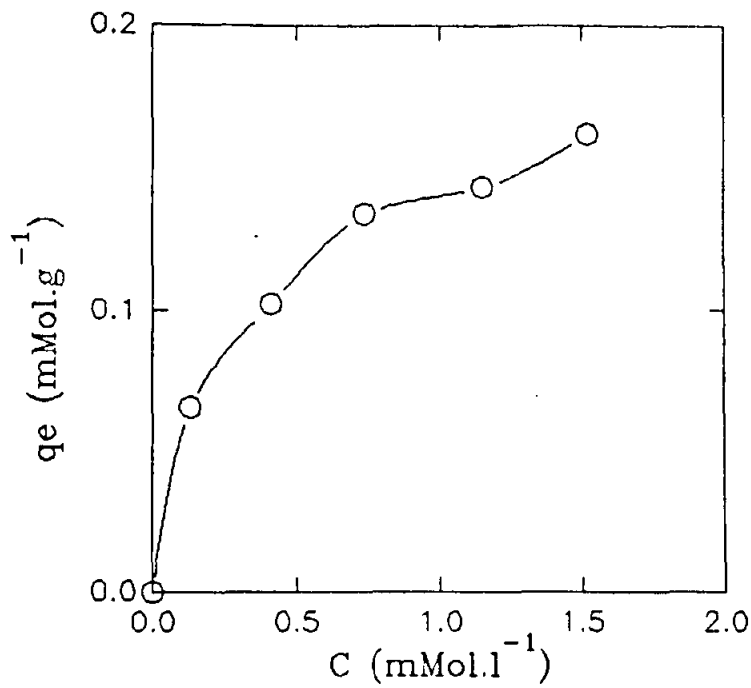


Fig. 4. 63. Adsorption isotherm for Ni uptake from solution by sludge 1.

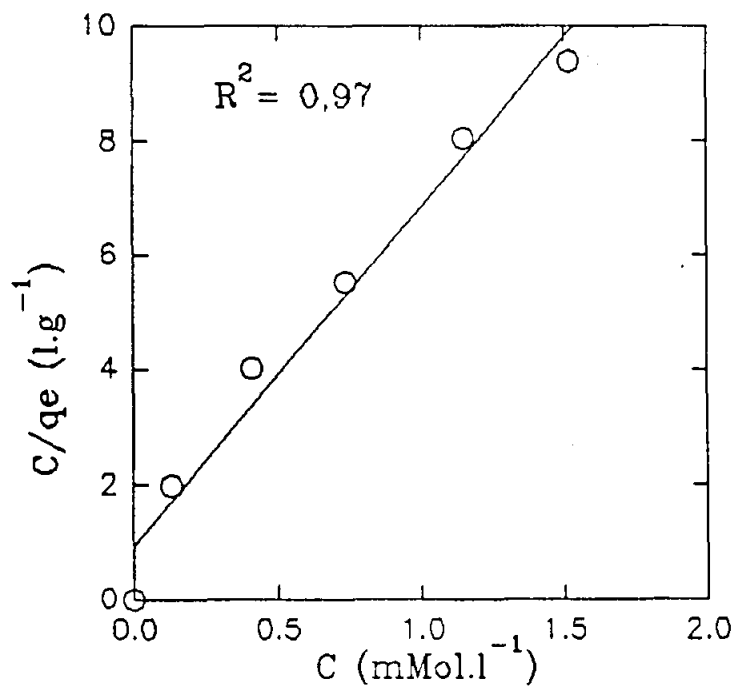


Fig. 4. 64. Reciprocal isotherm for Ni uptake from solution by sludge 1.

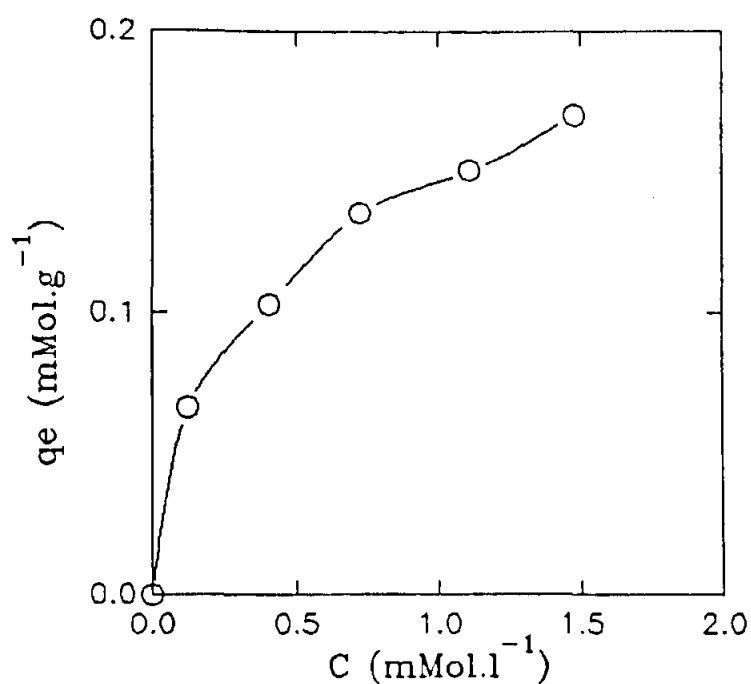


Fig. 4. 65. Adsorption isotherm for Ni uptake from solution by sludge 2.

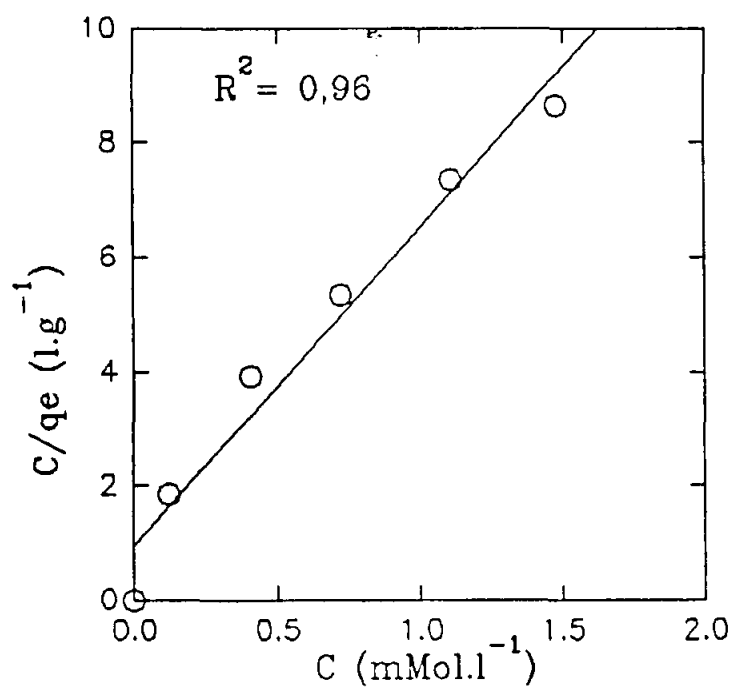


Fig. 4. 66. Reciprocal isotherm for Ni uptake from solution by sludge 2.

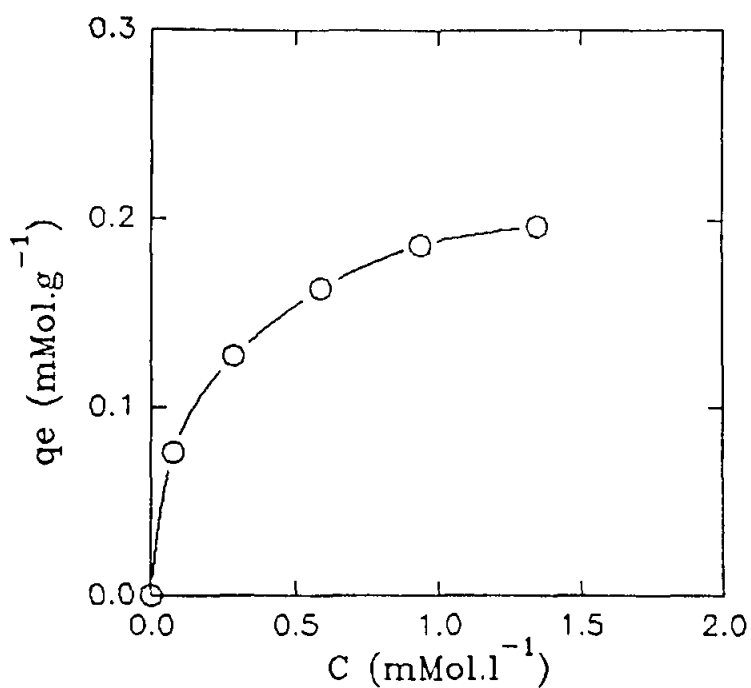


Fig. 4. 67. Adsorption isotherm for Ni uptake from solution by sludge 3.

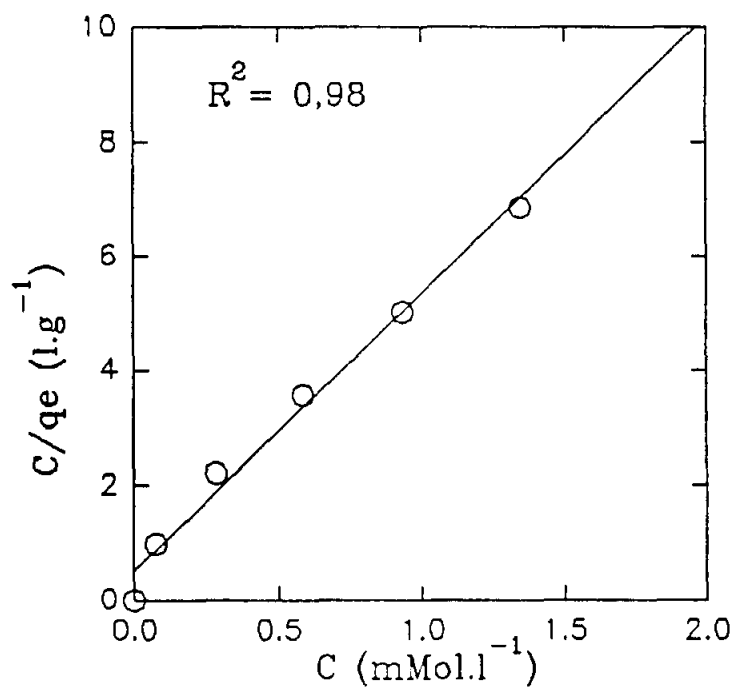


Fig. 4. 68. Reciprocal isotherm for Ni uptake from solution by sludge 3.

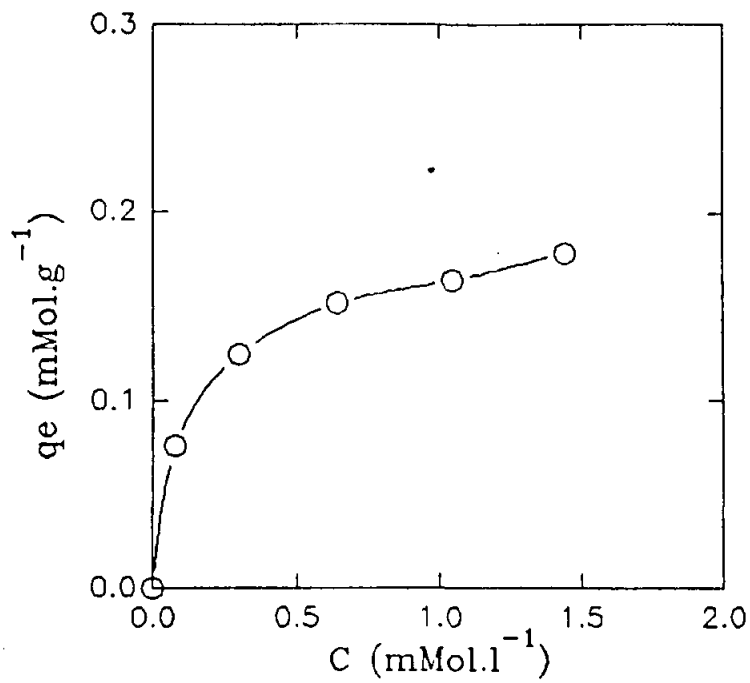


Fig. 4. 69. Adsorption isotherm for Ni uptake from solution by sludge 4.

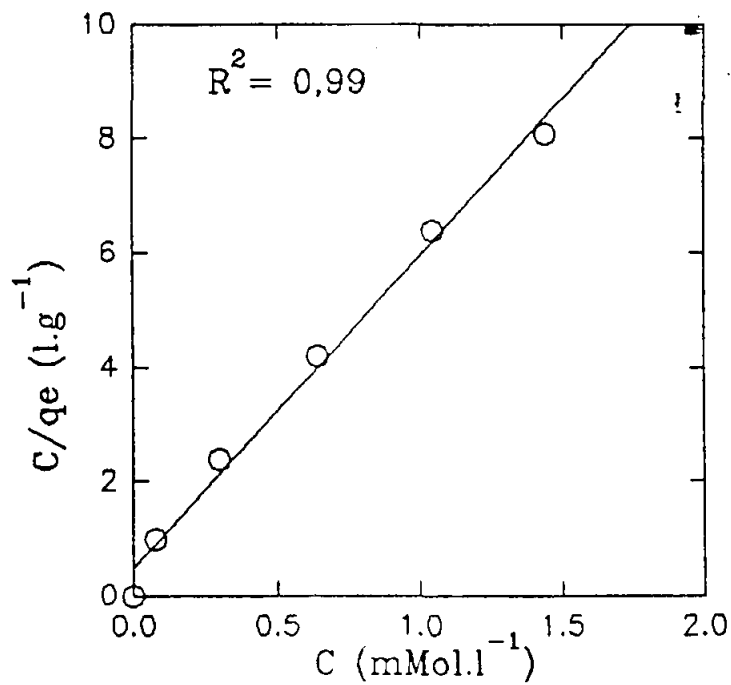


Fig. 4. 70. Reciprocal isotherm for Ni uptake from solution by sludge 4.

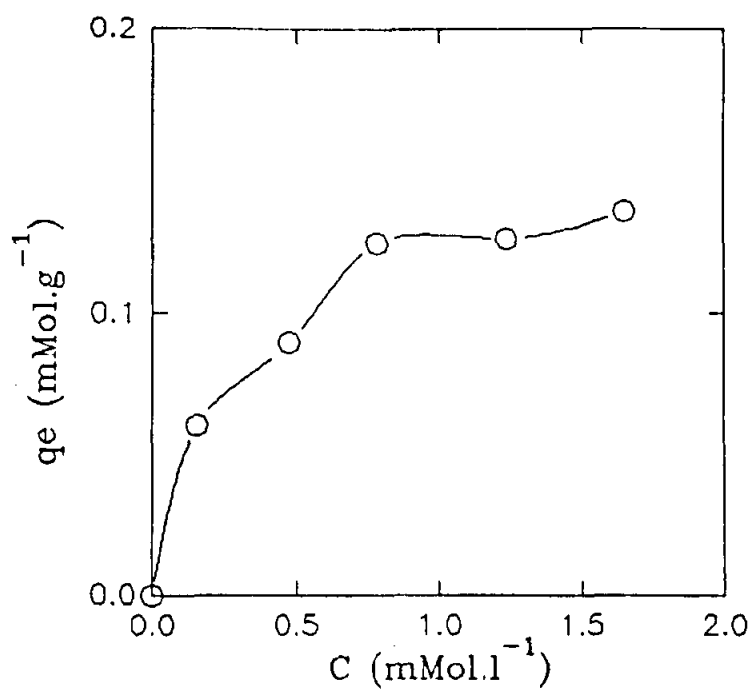


Fig. 4. 71. Adsorption isotherm for Ni uptake from solution by sludge 5.

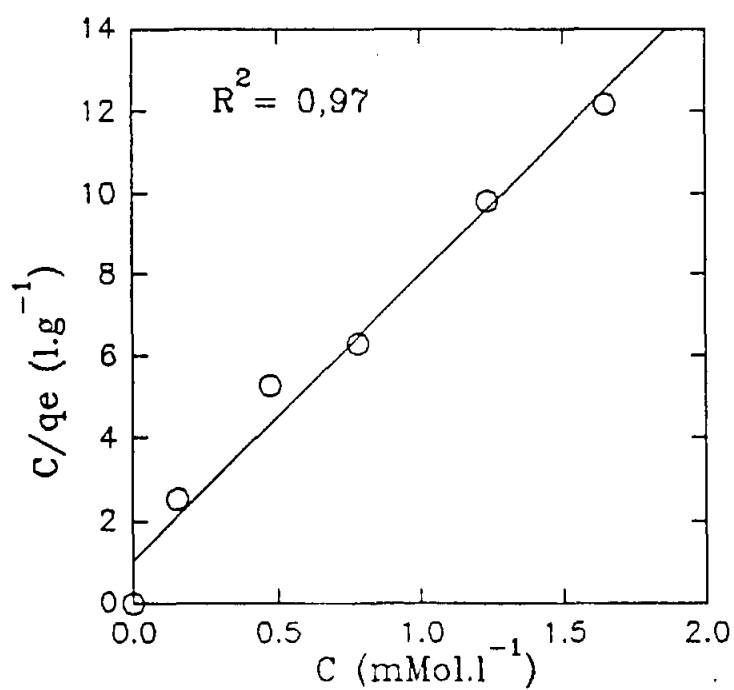


Fig. 4. 72. Reciprocal isotherm for Ni uptake from solution by sludge 5.

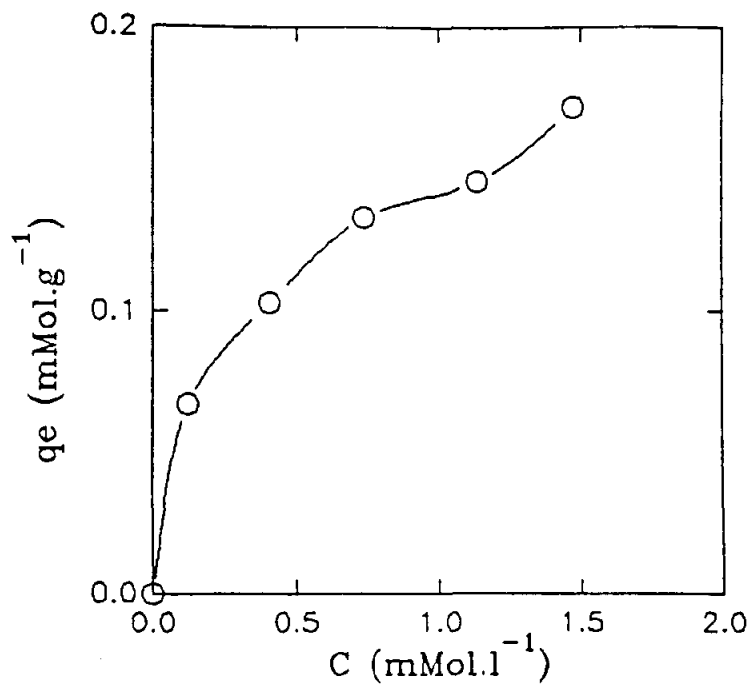


Fig. 4. 73. Adsorption isotherm for Ni uptake from solution by sludge 6.

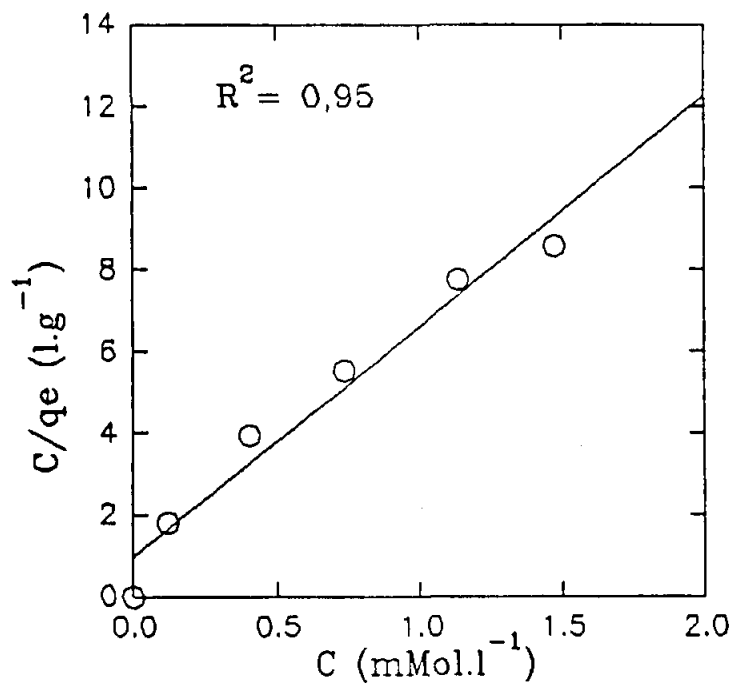


Fig. 4. 74. Reciprocal isotherm for Ni uptake from solution by sludge 6.

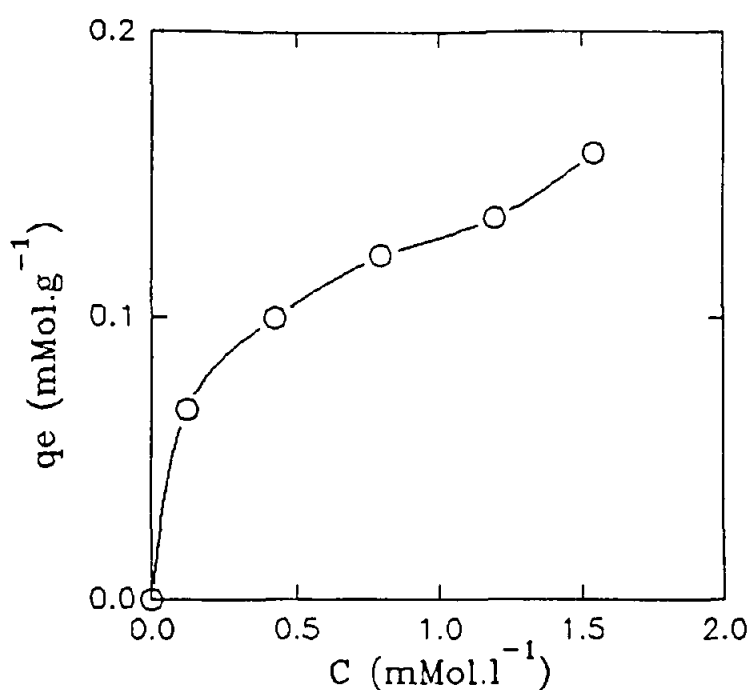


Fig. 4. 75. Adsorption isotherm for Ni uptake from solution by sludge 7.

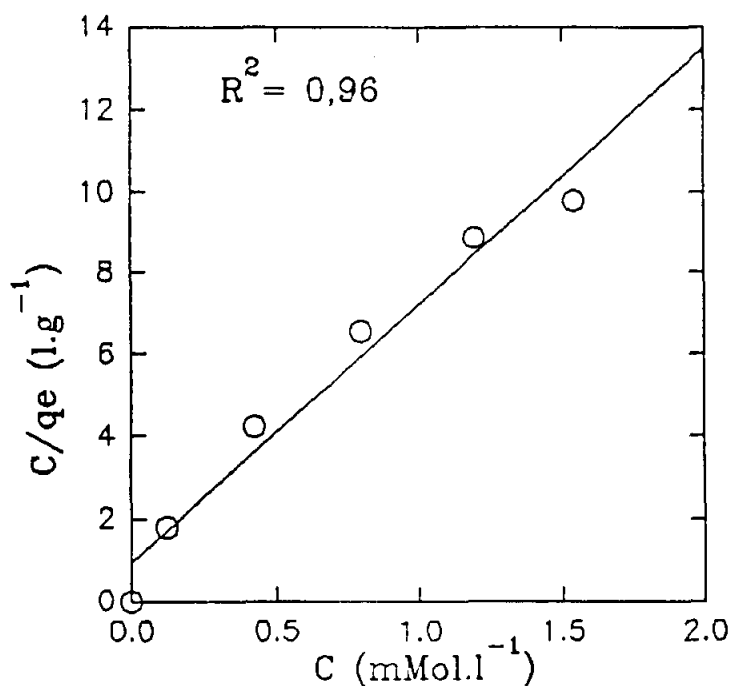


Fig. 4. 76. Reciprocal isotherm for Ni uptake from solution by sludge 7.

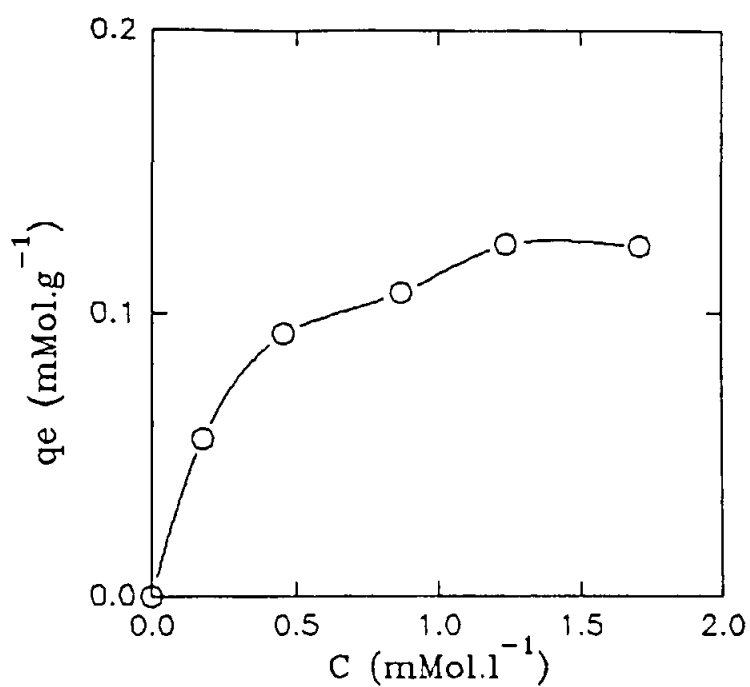


Fig. 4. 77. Adsorption isotherm for Ni uptake from solution by sludge 8.

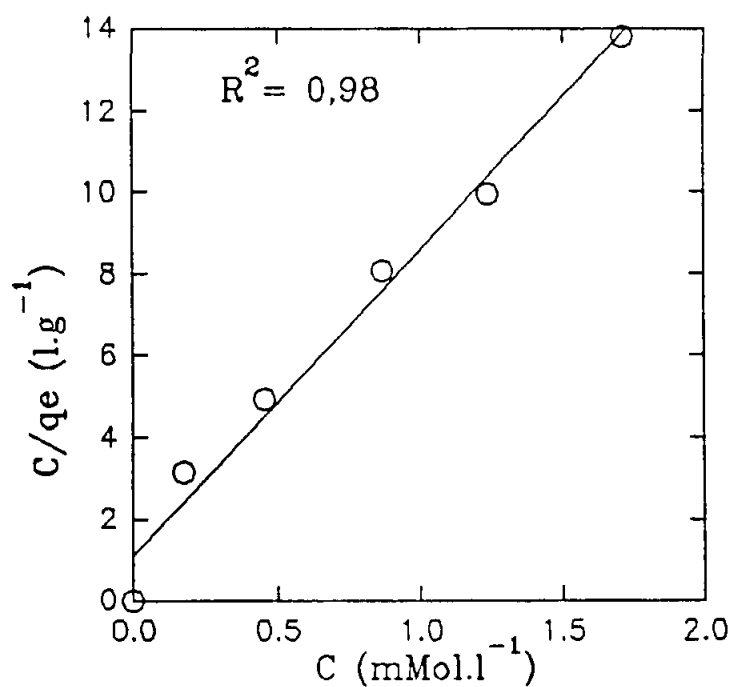


Fig. 4. 78. Reciprocal isotherm for Ni uptake from solution by sludge 8.

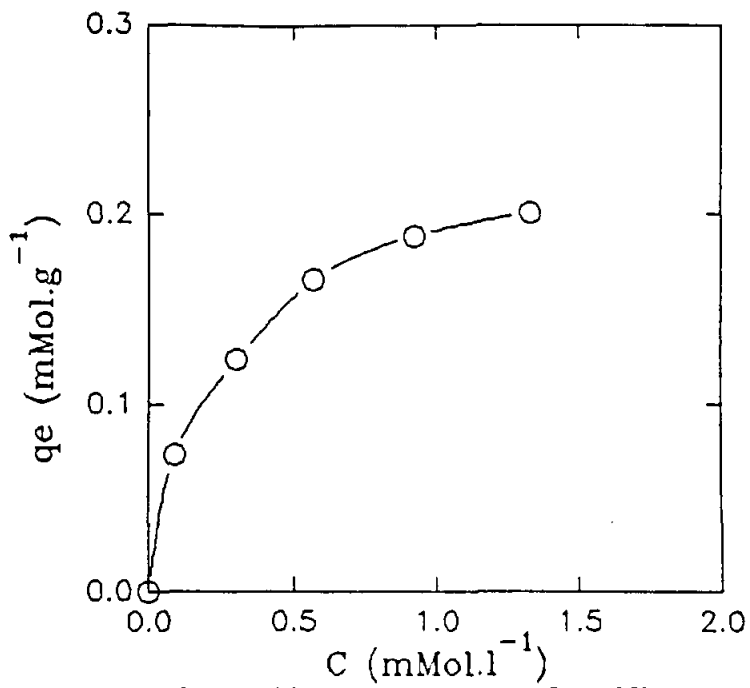


Fig. 4. 79. Adsorption isotherm for Ni uptake from solution by sludge 9.

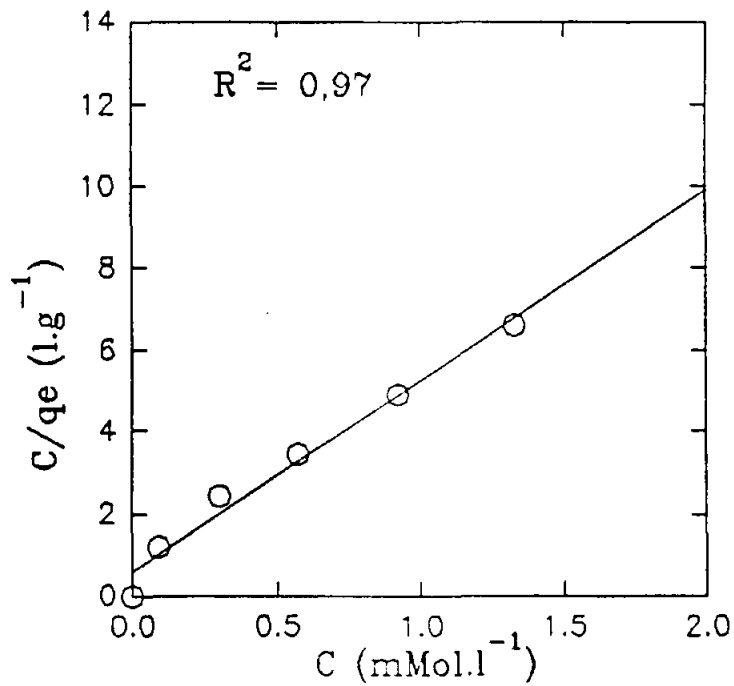


Fig. 4. 80. Reciprocal isotherm for Ni uptake from solution by sludge 9.

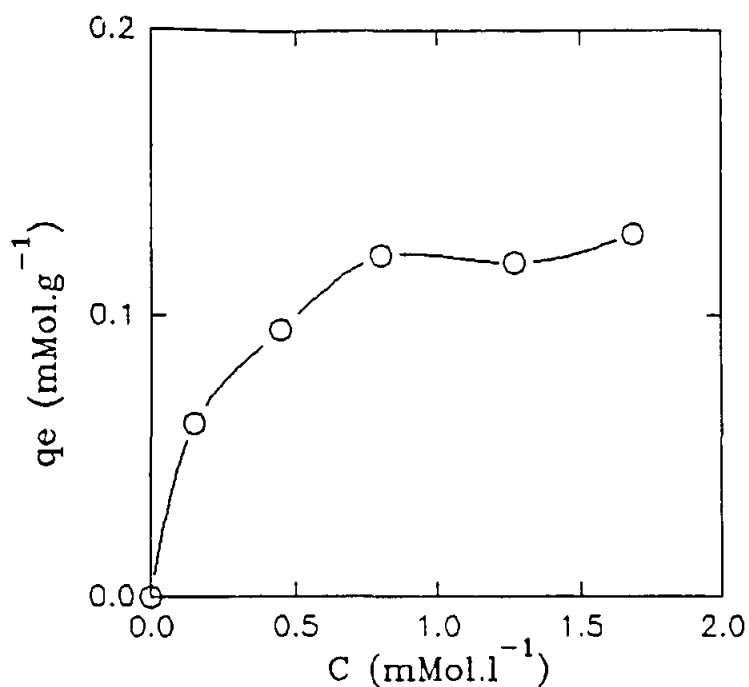


Fig. 4. 81. Adsorption isotherm for Ni uptake from solution by sludge 10.

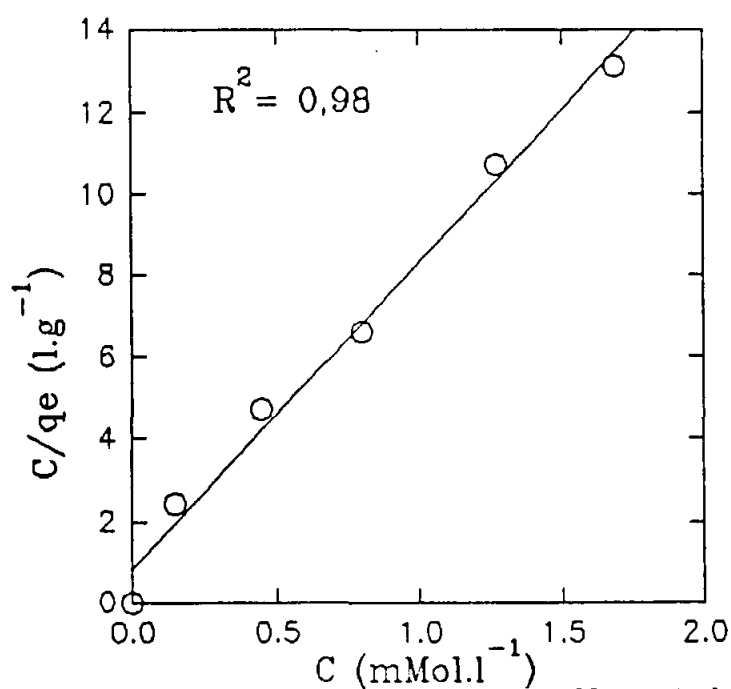


Fig. 4. 82. Reciprocal isotherm for Ni uptake from solution by sludge 10.

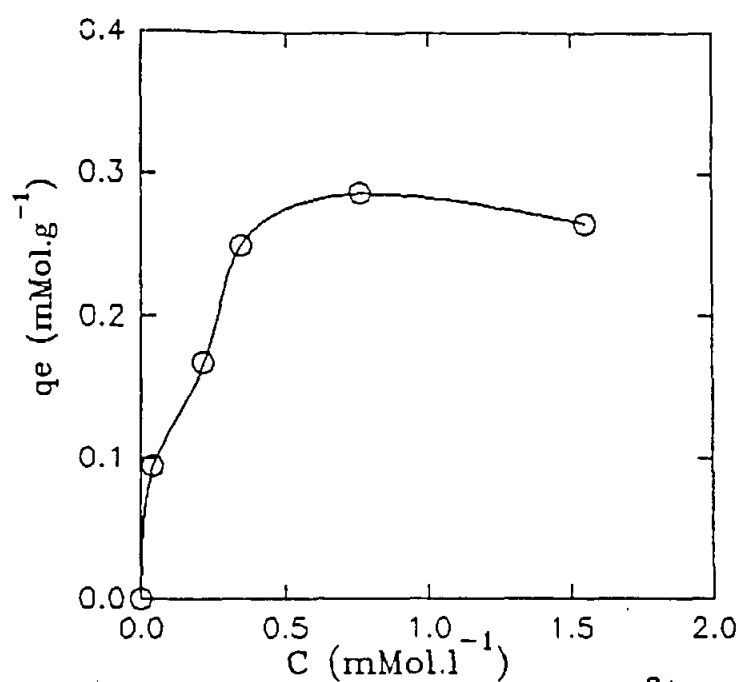


Fig. 4. 83. Adsorption isotherm for Cr^{3+} uptake from solution by sludge 1.

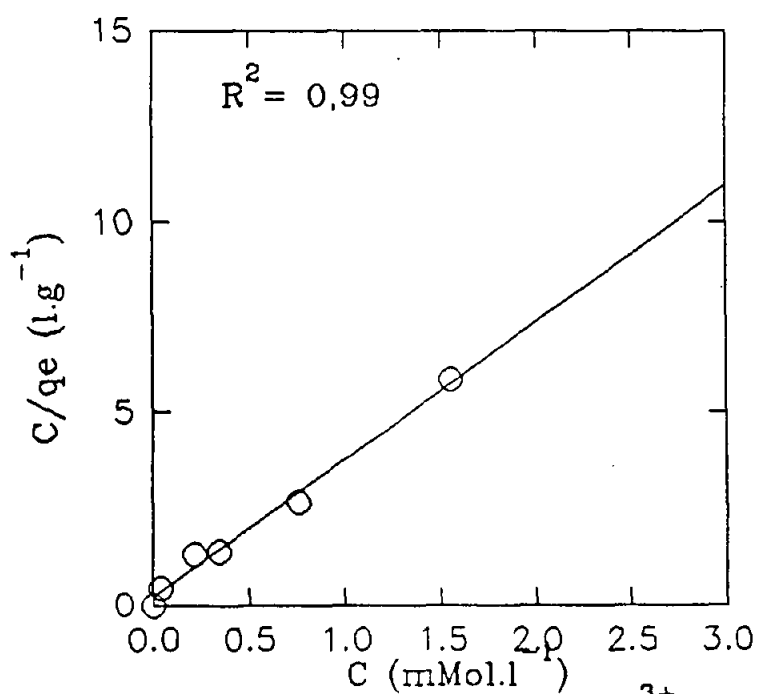


Fig. 4. 84. Reciprocal isotherm for Cr^{3+} uptake from solution by sludge 1.

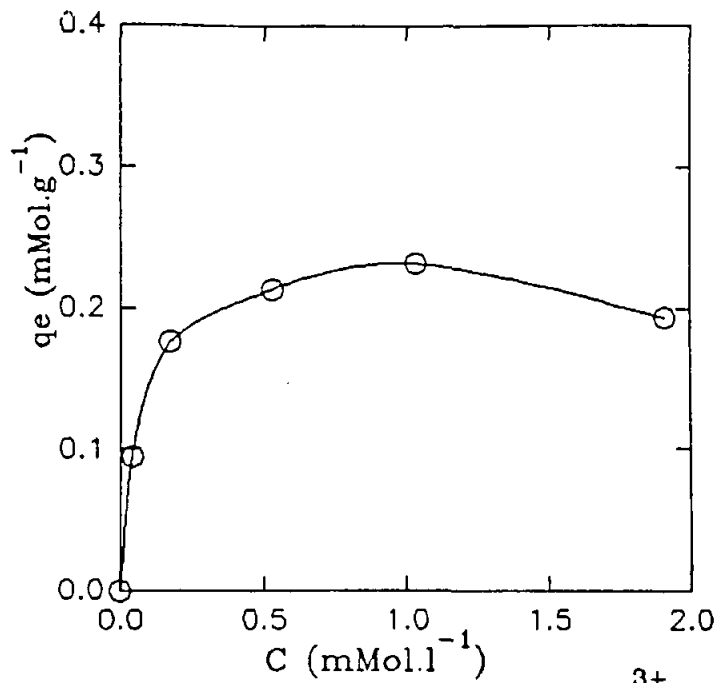


Fig. 4. 85. Adsorption isotherm for Cr^{3+} uptake from solution by sludge 2.

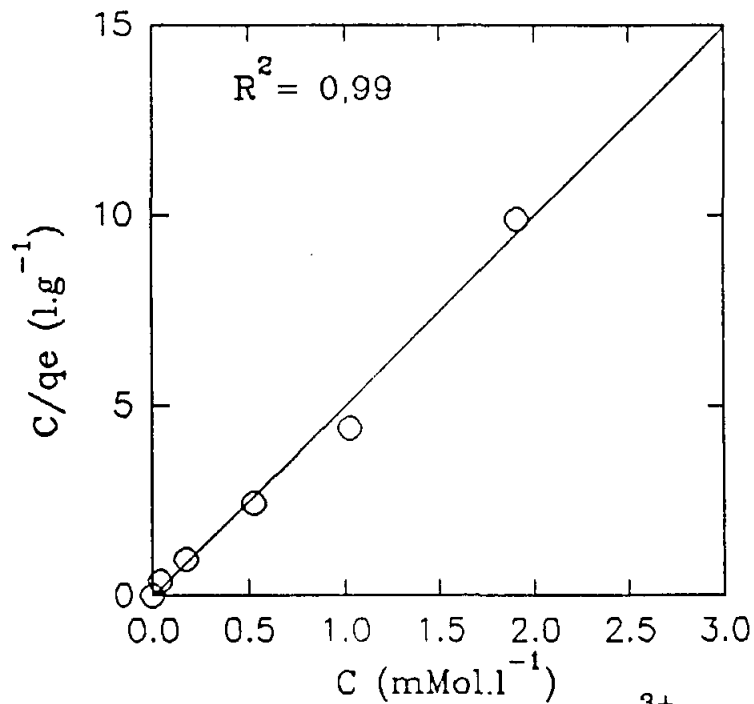


Fig. 4. 86. Reciprocal isotherm for Cr^{3+} uptake from solution by sludge 2.

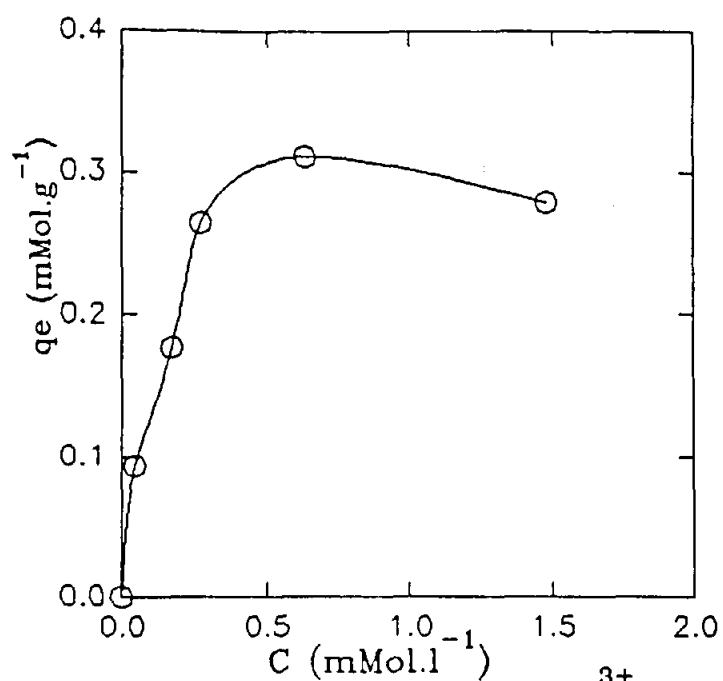


Fig. 4. 87. Adsorption isotherm for Cr^{3+} uptake from solution by sludge 3.

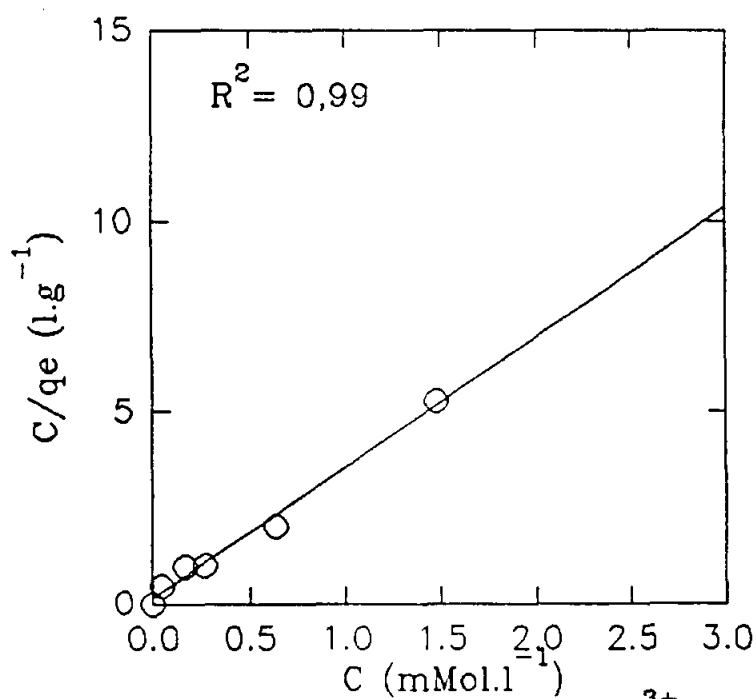


Fig. 4. 88. Reciprocal isotherm for Cr^{3+} uptake from solution by sludge 3.

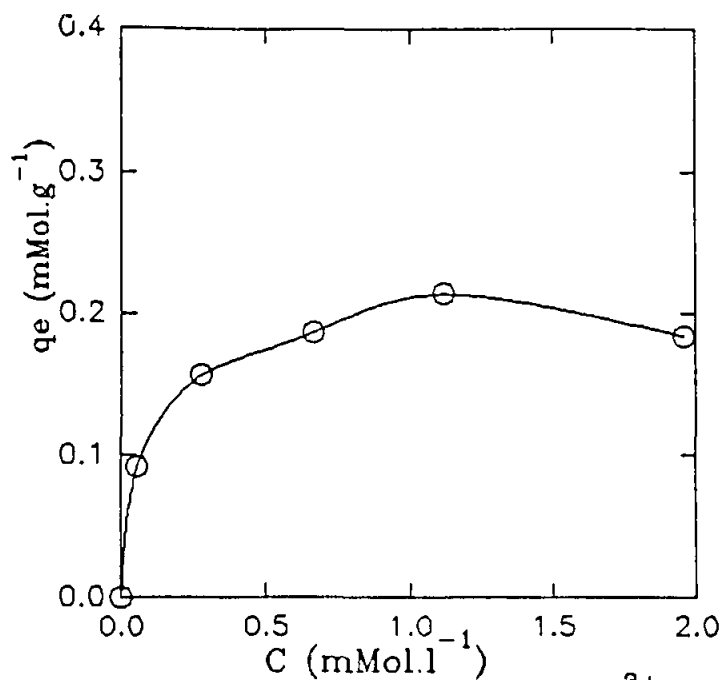


Fig. 4. 89. Adsorption isotherm for Cr^{3+} uptake from solution by sludge 4.

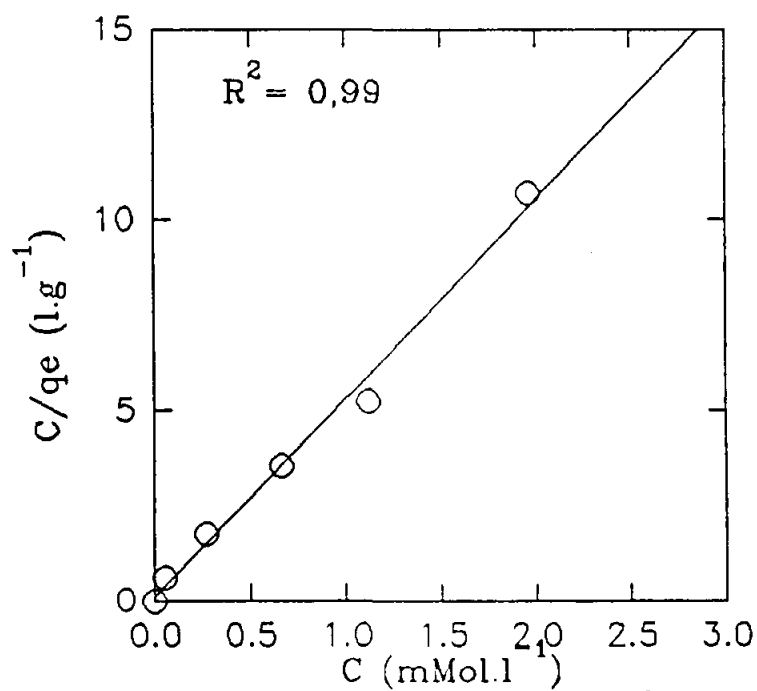


Fig. 4. 90. Reciprocal isotherm for Cr^{3+} uptake from solution by sludge 4.

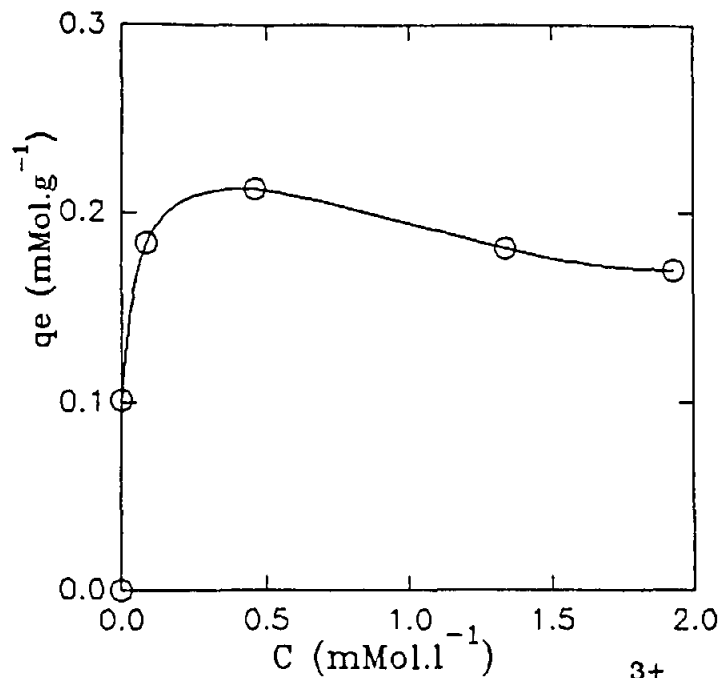


Fig. 4. 91. Adsorption isotherm for Cr^{3+} uptake from solution by sludge 5.

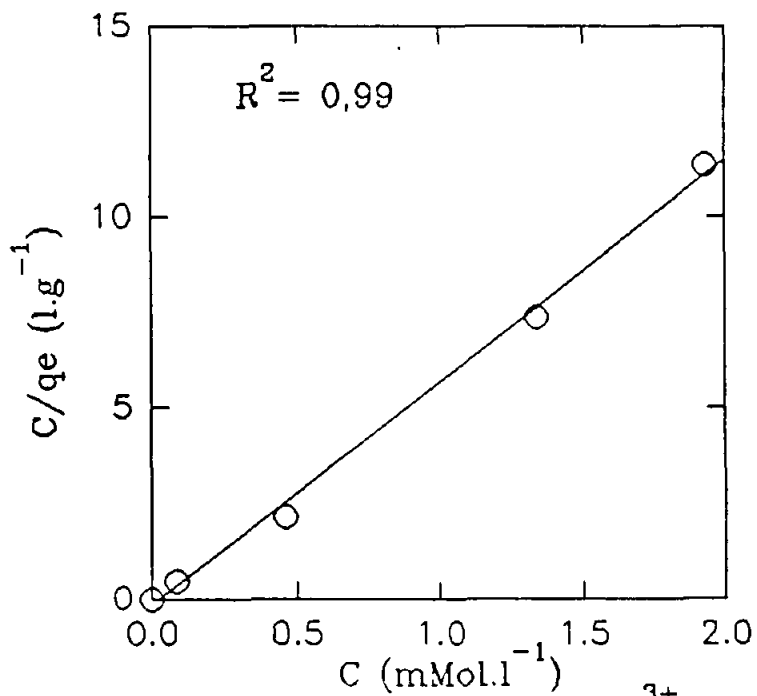


Fig. 4. 92. Reciprocal isotherm for Cr^{3+} uptake from solution by sludge 5.

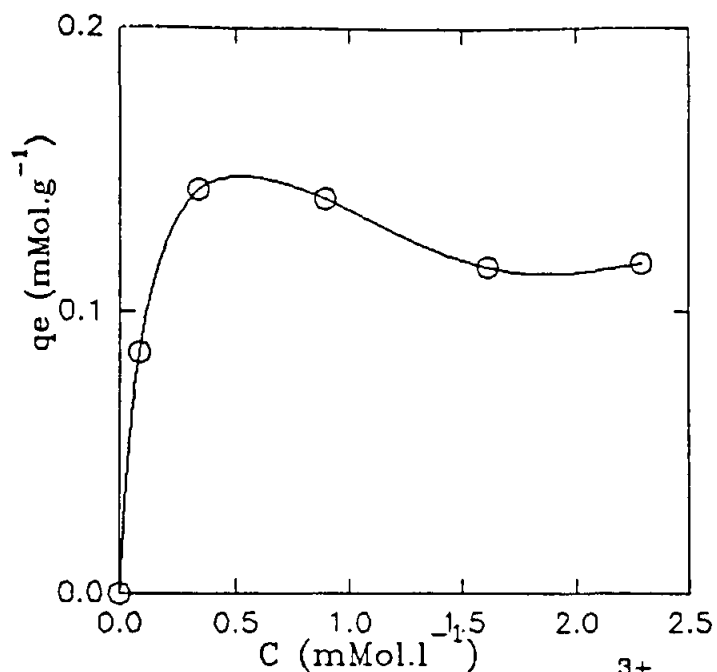


Fig. 4. 93. Adsorption isotherm for Cr^{3+} uptake from solution by sludge 6.

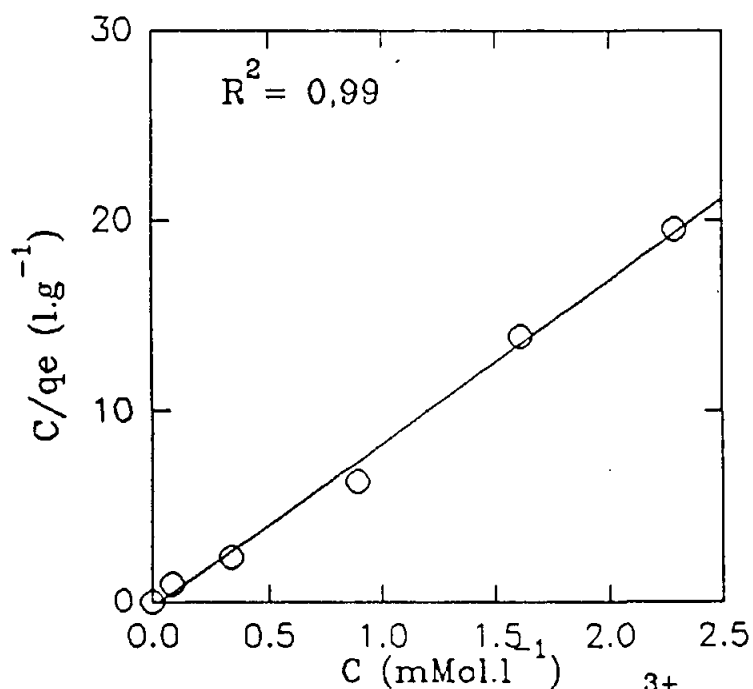


Fig. 4. 94. Reciprocal isotherm for Cr^{3+} uptake from solution by sludge 6.

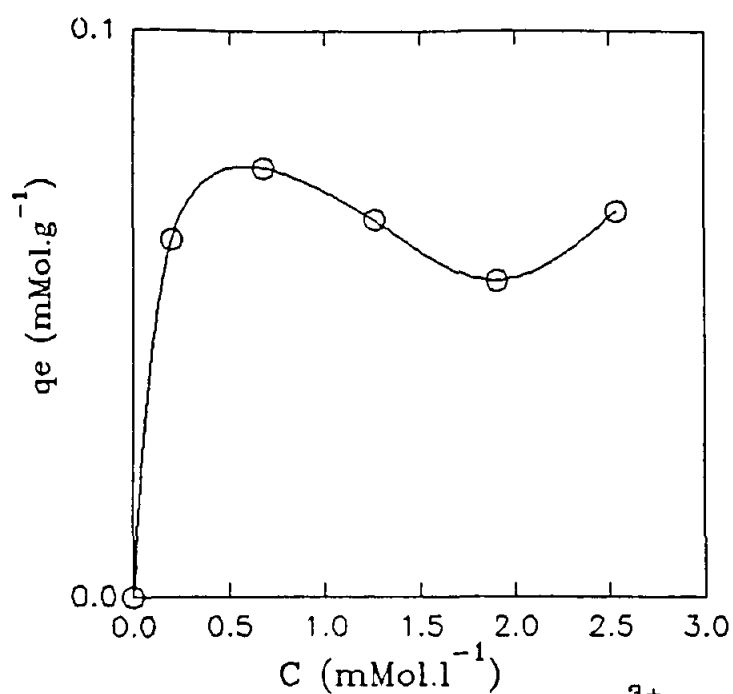


Fig. 4. 95. Adsorption isotherm for Cr^{3+} uptake from solution by sludge 7.

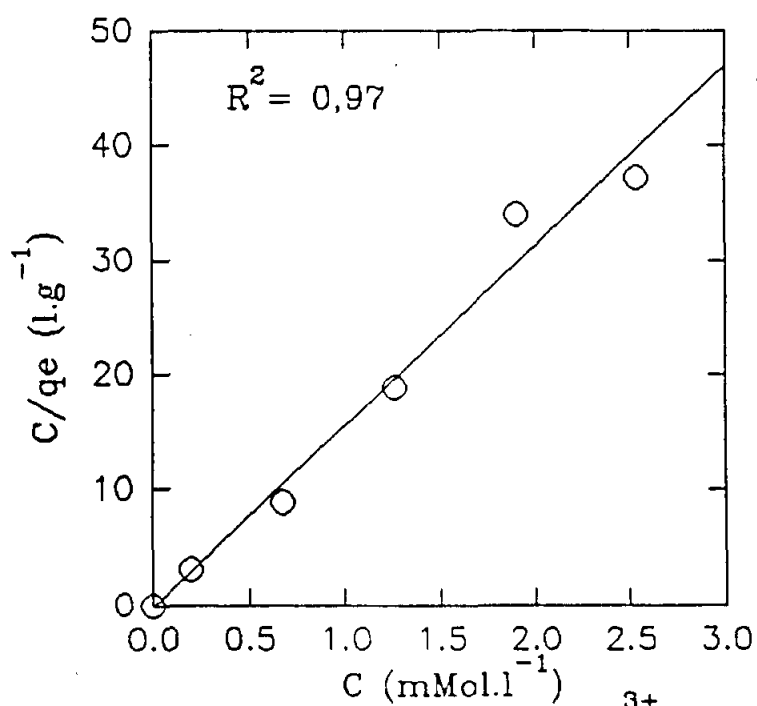


Fig. 4. 96. Reciprocal isotherm for Cr^{3+} uptake from solution by sludge 7.

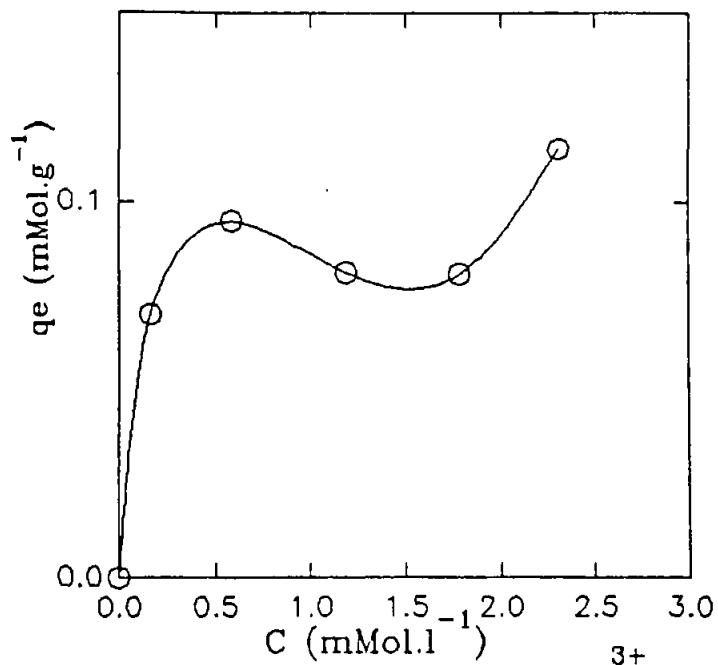


Fig. 4. 97. Adsorption isotherm for Cr^{3+} uptake from solution by sludge 8.

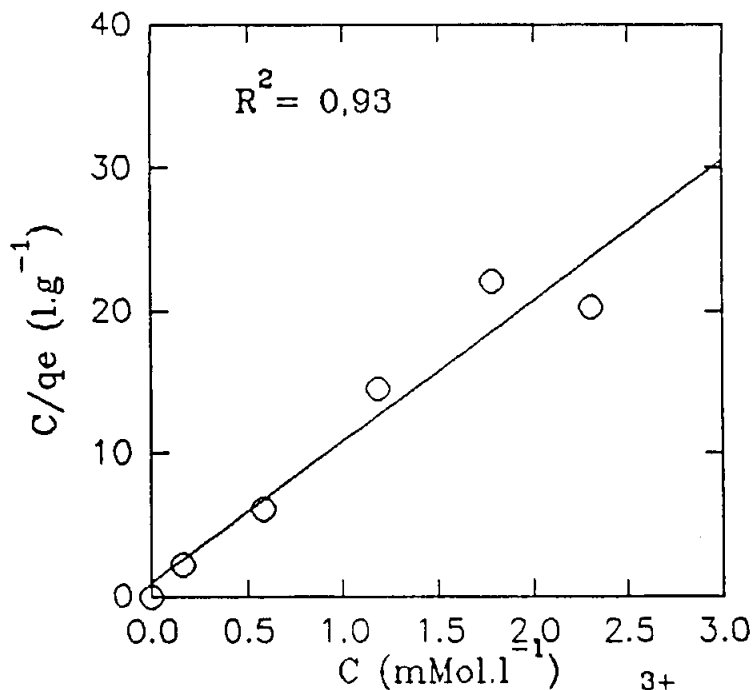


Fig. 4. 98. Reciprocal isotherm for Cr^{3+} uptake from solution by sludge 8.

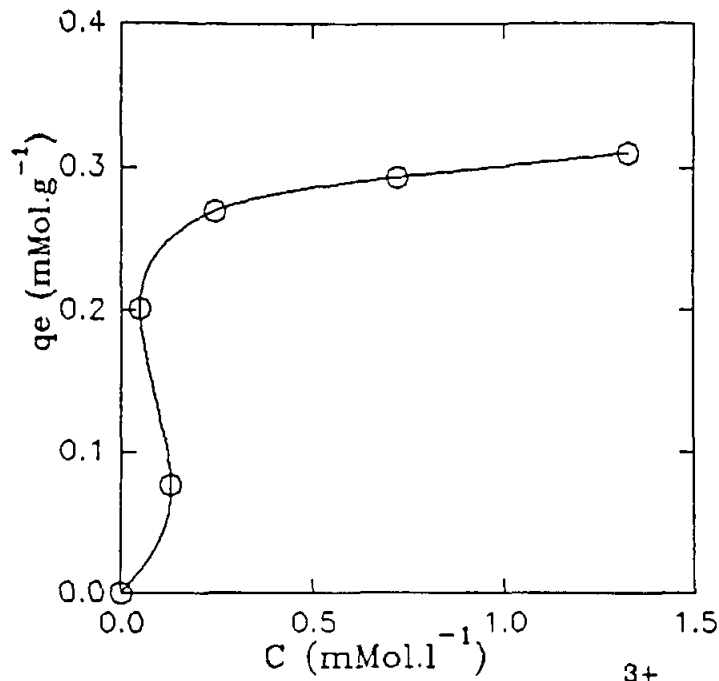


Fig. 4. 99. Adsorption isotherm for Cr^{3+} uptake from solution by sludge 9.

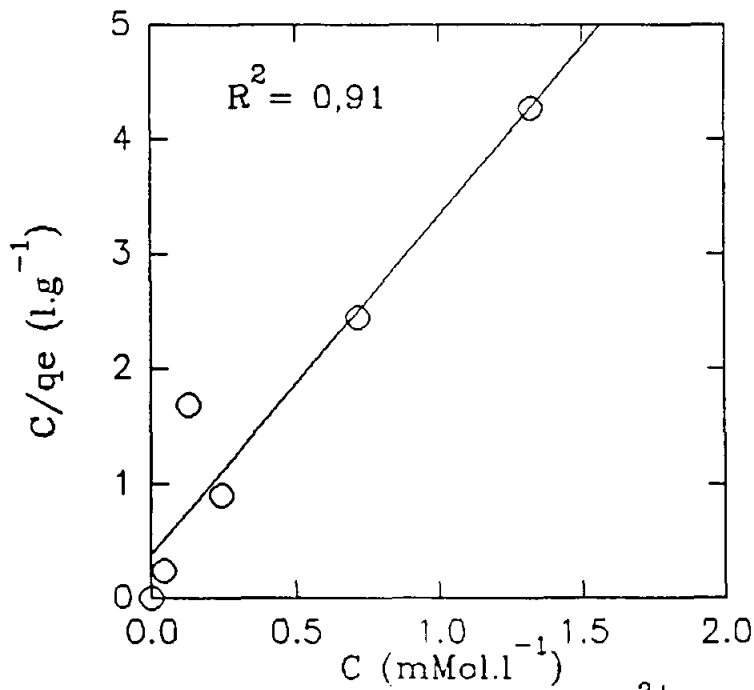


Fig. 4. 100. Reciprocal isotherm for Cr^{3+} uptake from solution by sludge 9.

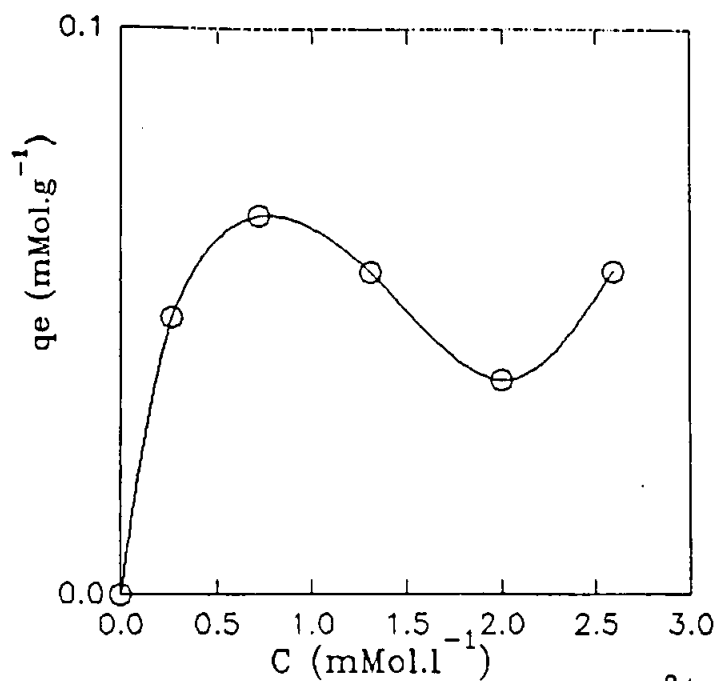


Fig. 4. 101. Adsorption isotherm for Cr^{3+} uptake from solution by sludge 10.

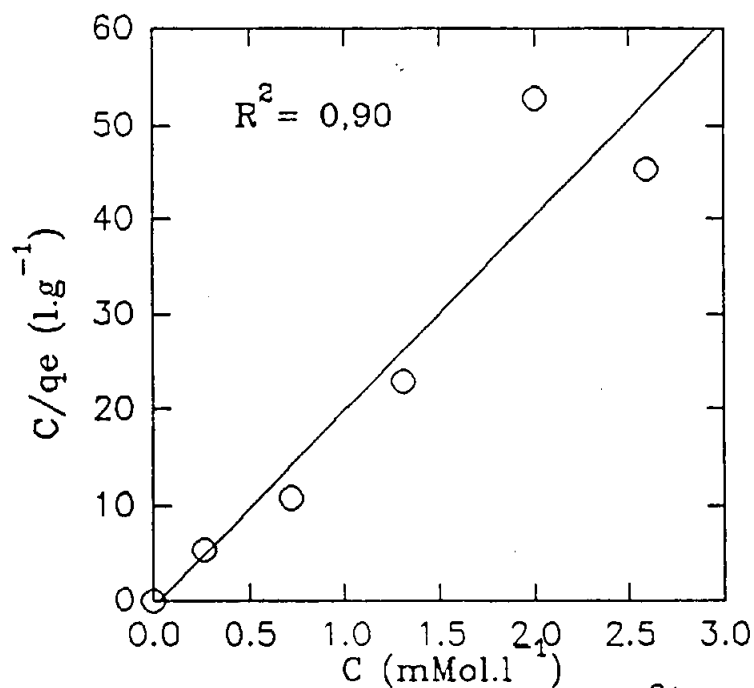


Fig. 4. 102. Reciprocal isotherm for Cr^{3+} uptake from solution by sludge 10.

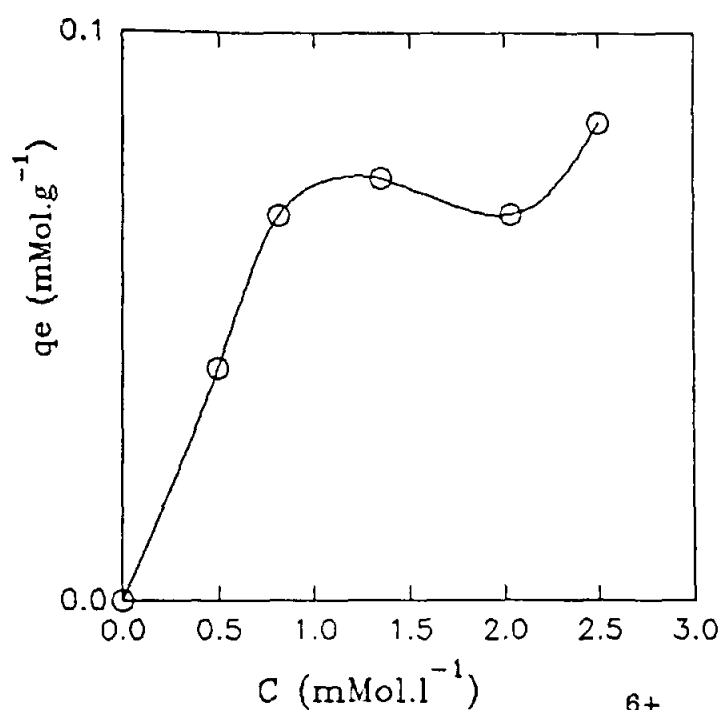


Fig. 4. 103. Adsorption isotherm for Cr^{6+} uptake from solution by sludge 1.

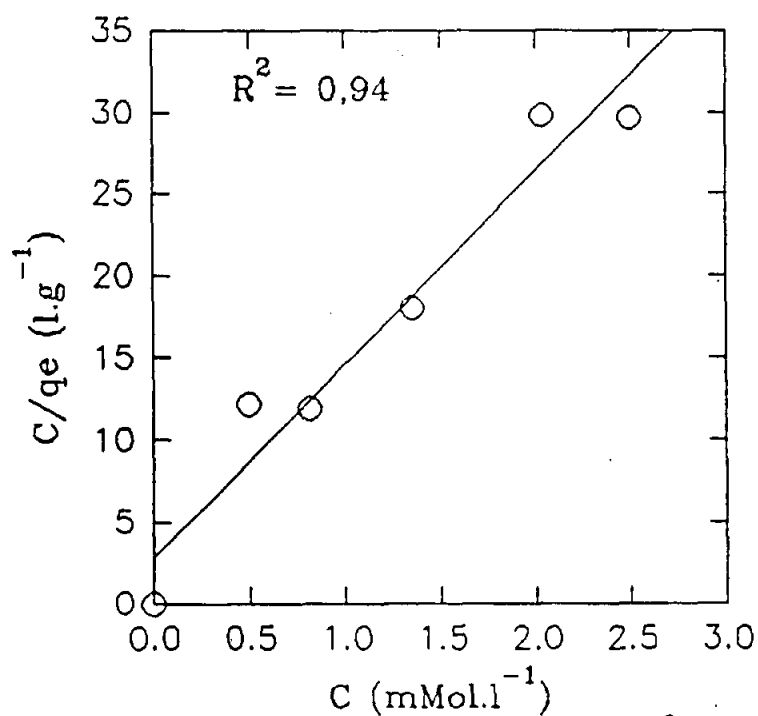


Fig. 4. 104. Reciprocal isotherm for Cr^{6+} uptake from solution by sludge 1.

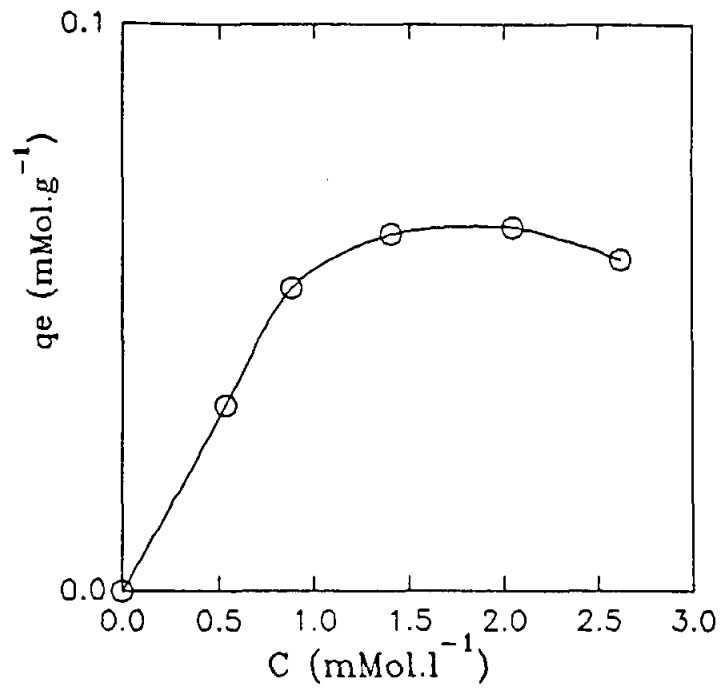


Fig. 4. 105. Adsorption isotherm for Cr^{6+} uptake from solution by sludge 2.

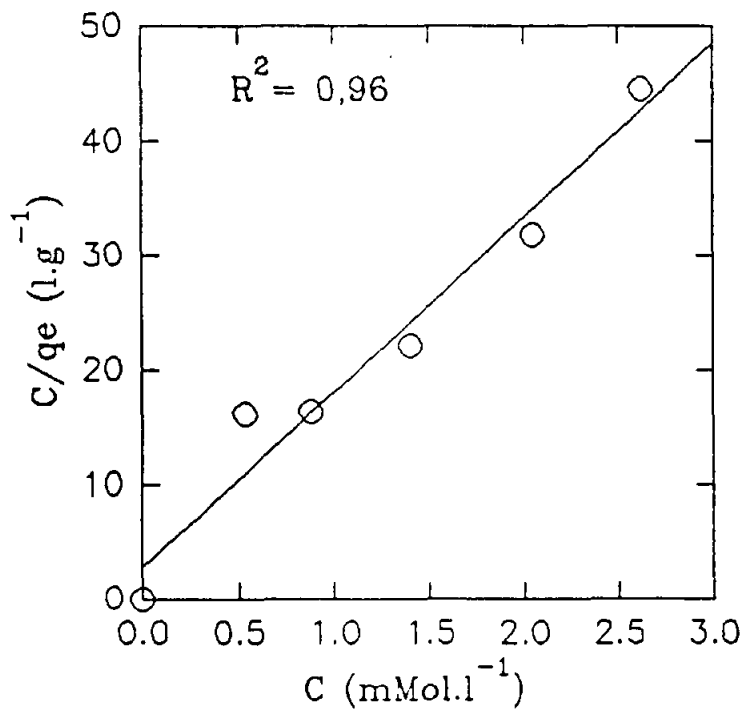


Fig. 4. 106. Reciprocal isotherm for Cr^{6+} uptake from solution by sludge 2.

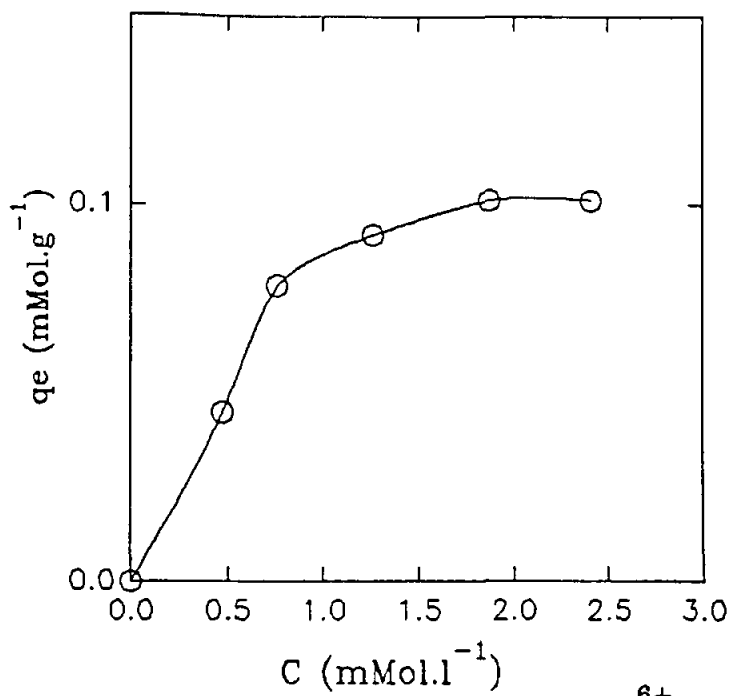


Fig. 4. 107. Adsorption isotherm for Cr^{6+} uptake from solution by sludge 3.

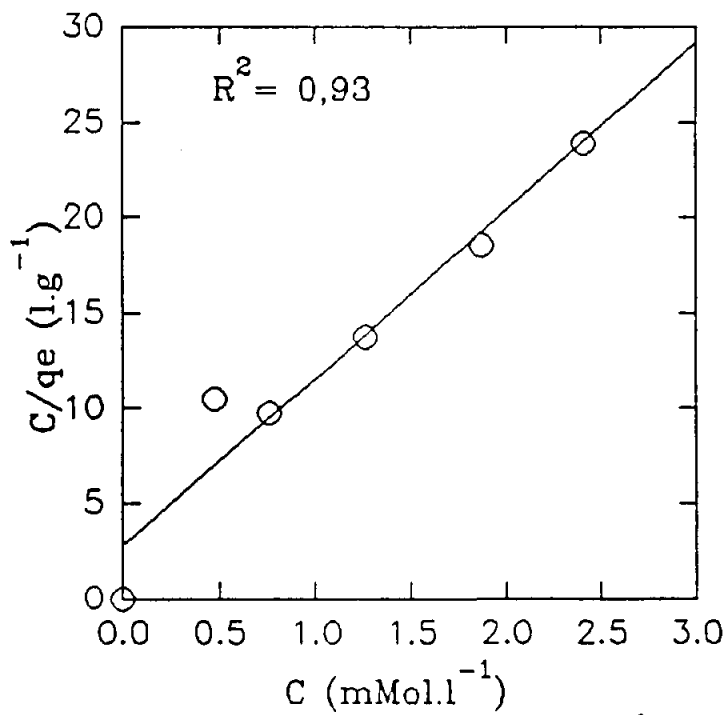


Fig. 4. 108. Reciprocal isotherm for Cr^{6+} uptake from solution by sludge 3.

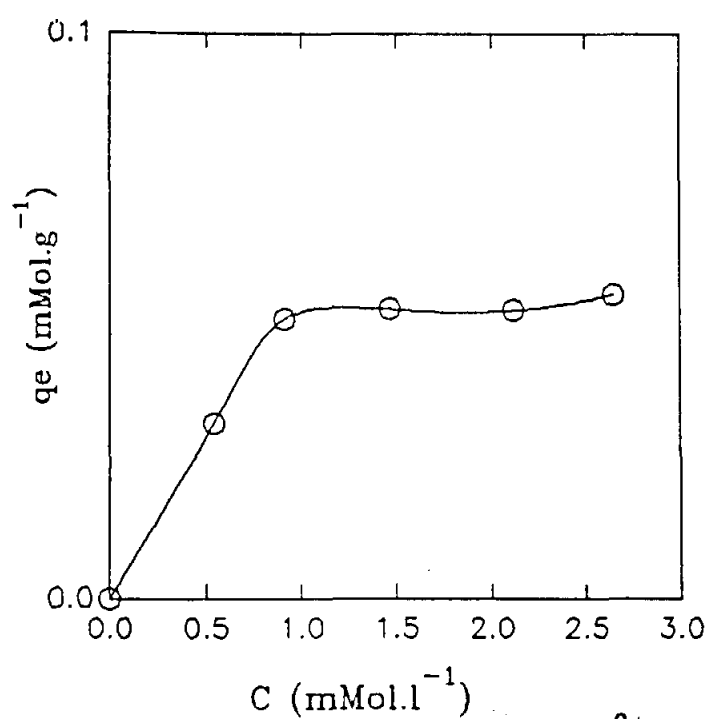


Fig. 4. 109. Adsorption isotherm for Cr^{6+} uptake from solution by sludge 4.

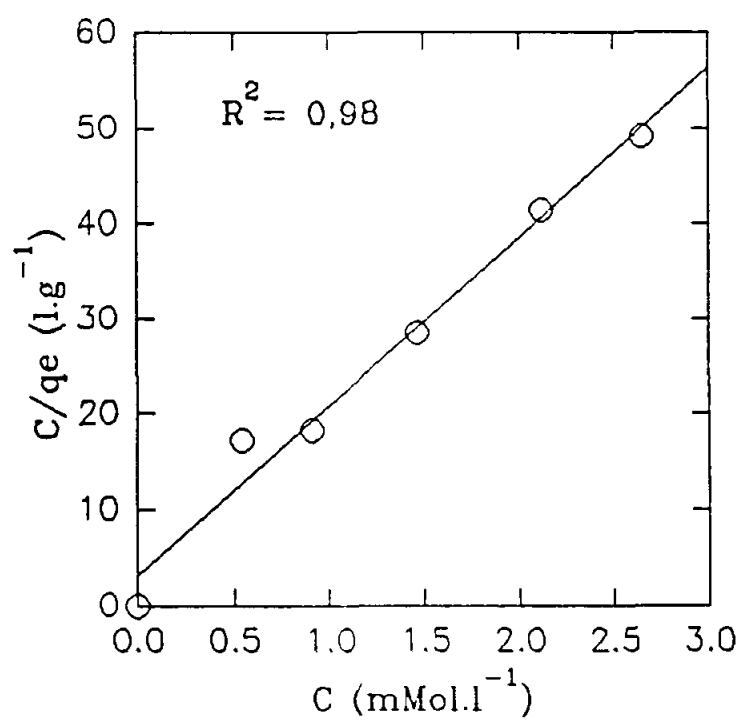


Fig. 4. 110. Reciprocal isotherm for Cr^{6+} uptake from solution by sludge 4.

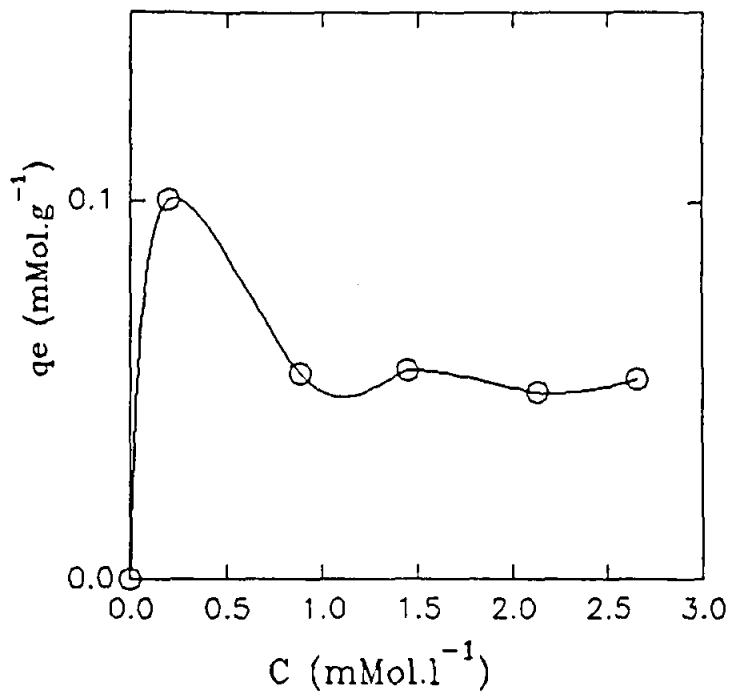


Fig. 4. 111. Adsorption isotherm for Cr^{6+} uptake from solution by sludge 5.

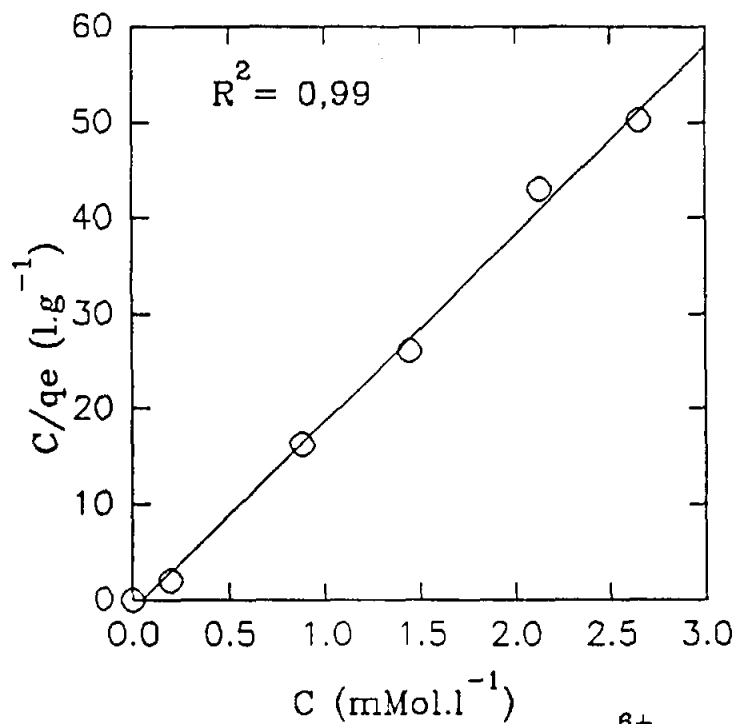


Fig. 4. 112. Reciprocal isotherm for Cr^{6+} uptake from solution by sludge 5.

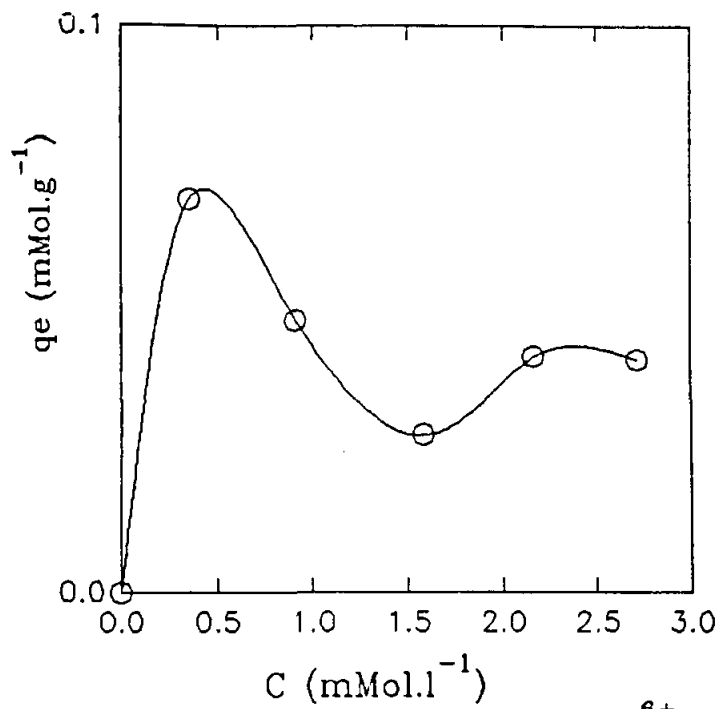


Fig. 4. 113. Adsorption isotherm for Cr^{6+} uptake from solution by sludge 6.

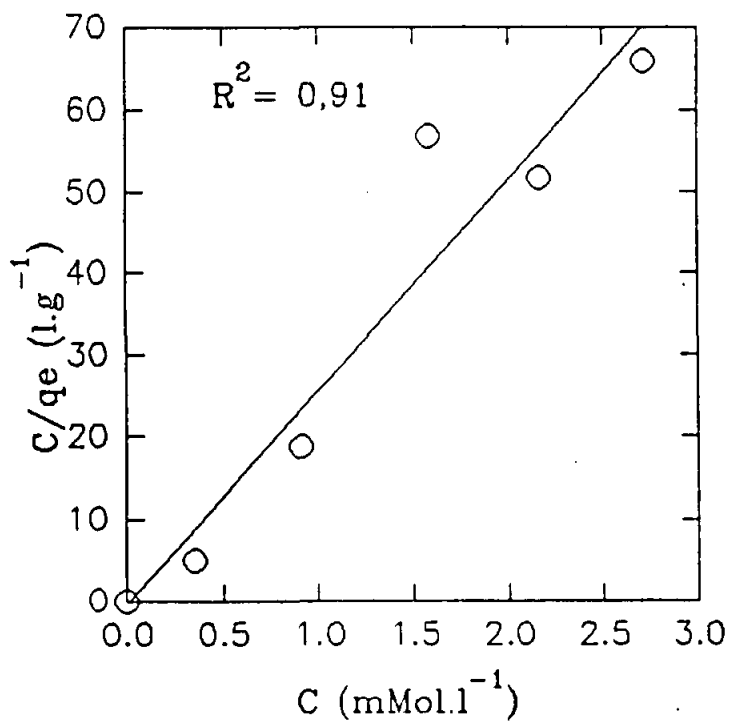


Fig. 4. 114. Reciprocal isotherm for Cr^{6+} uptake from solution by sludge 6.

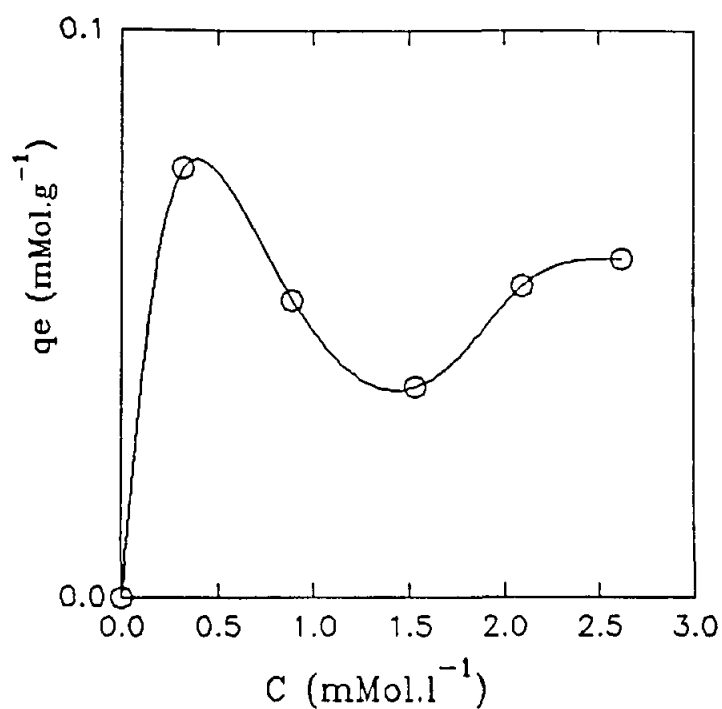


Fig. 4. 115. Adsorption isotherm for Cr^{6+} uptake from solution by sludge 7.

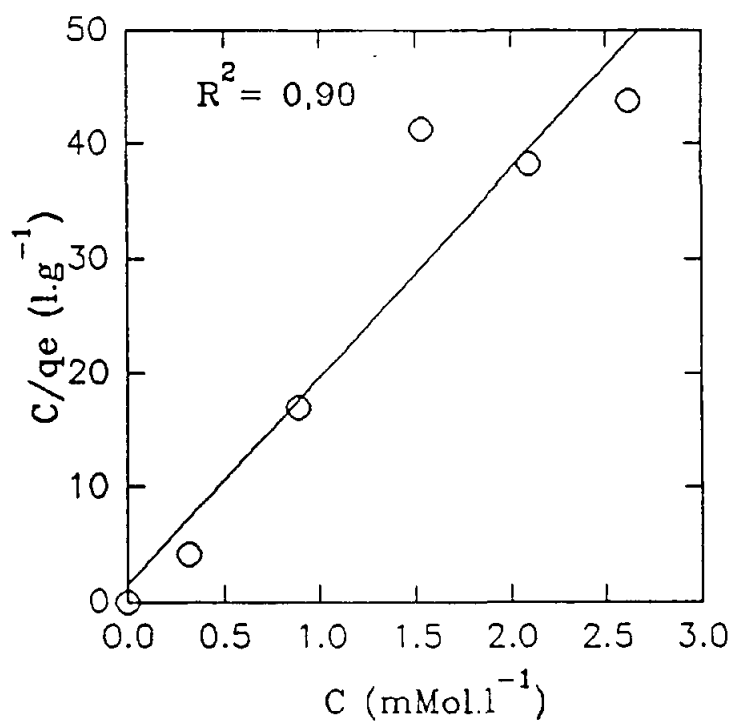


Fig. 4. 116. Reciprocal isotherm for Cr^{6+} uptake from solution by sludge 7.

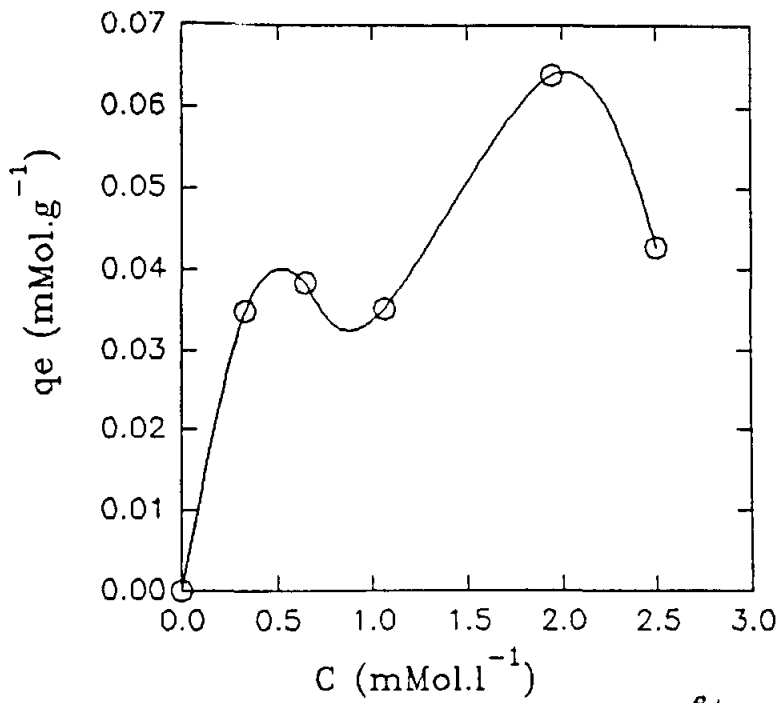


Fig. 4. 117. Adsorption isotherm for Cr^{6+} uptake from solution by sludge 8.

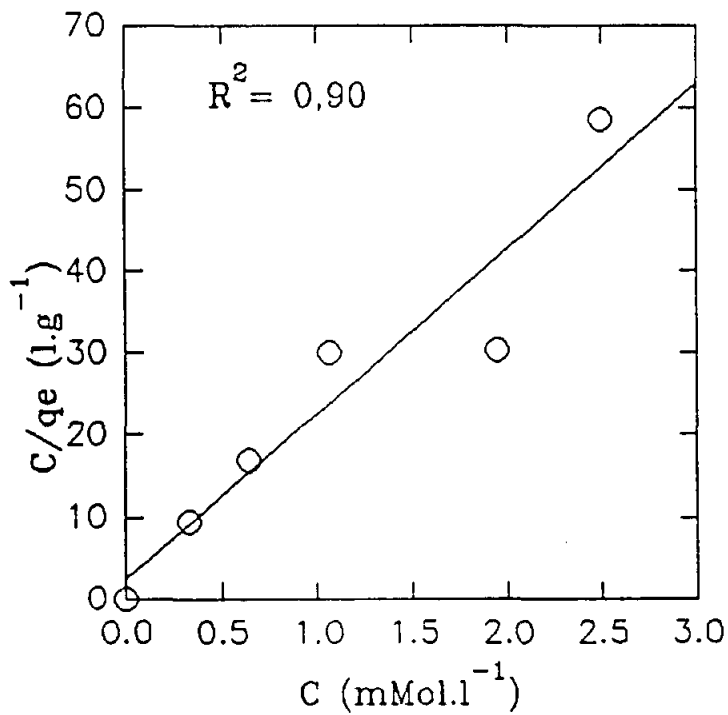


Fig. 4. 118. Reciprocal isotherm for Cr^{6+} uptake from solution by sludge 8.

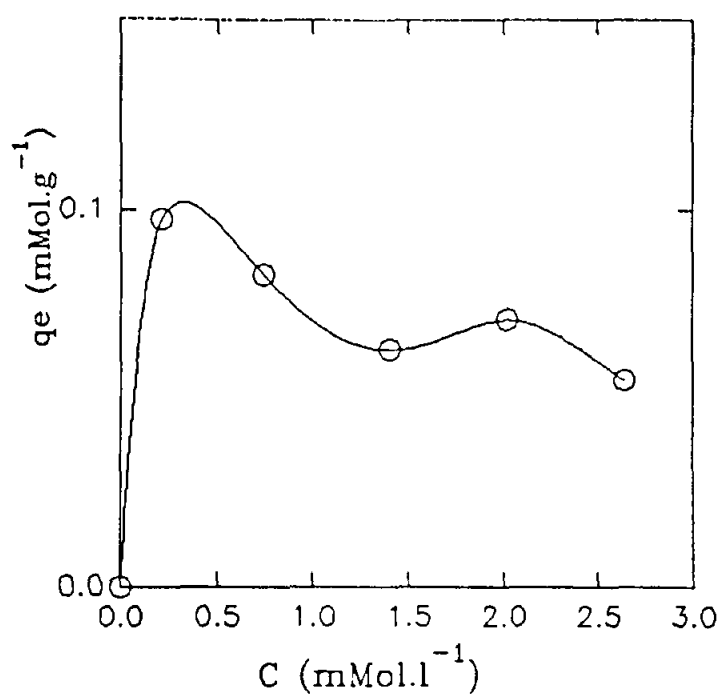


Fig. 4. 119. Adsorption isotherm for Cr^{6+} uptake from solution by sludge 9.

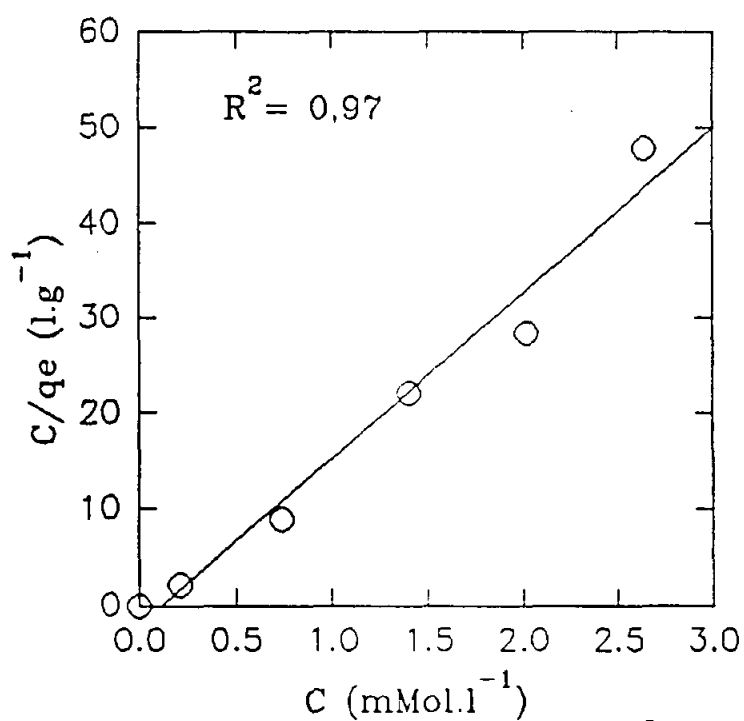


Fig. 4. 120. Reciprocal isotherm for Cr^{6+} uptake from solution by sludge 9.

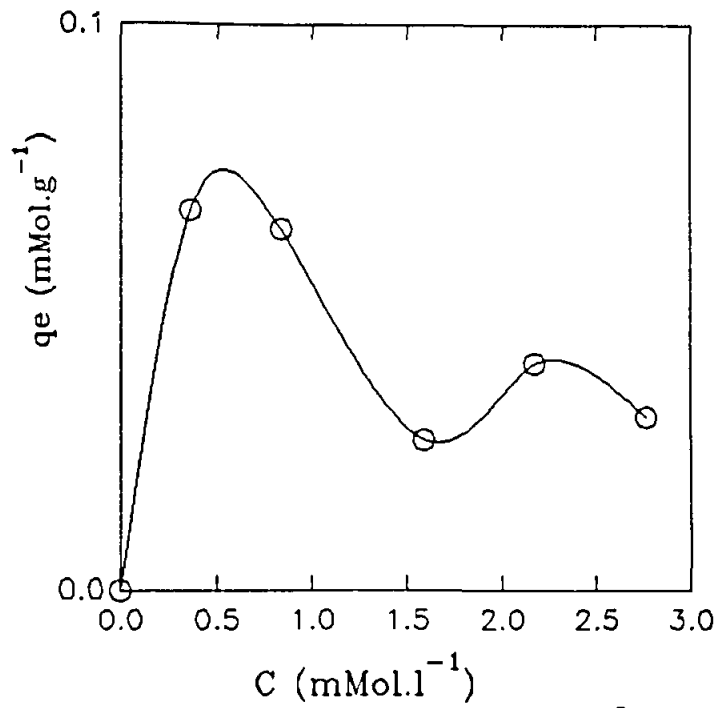


Fig. 4. 121. Adsorption isotherm for Cr^{6+} uptake from solution by sludge 10.

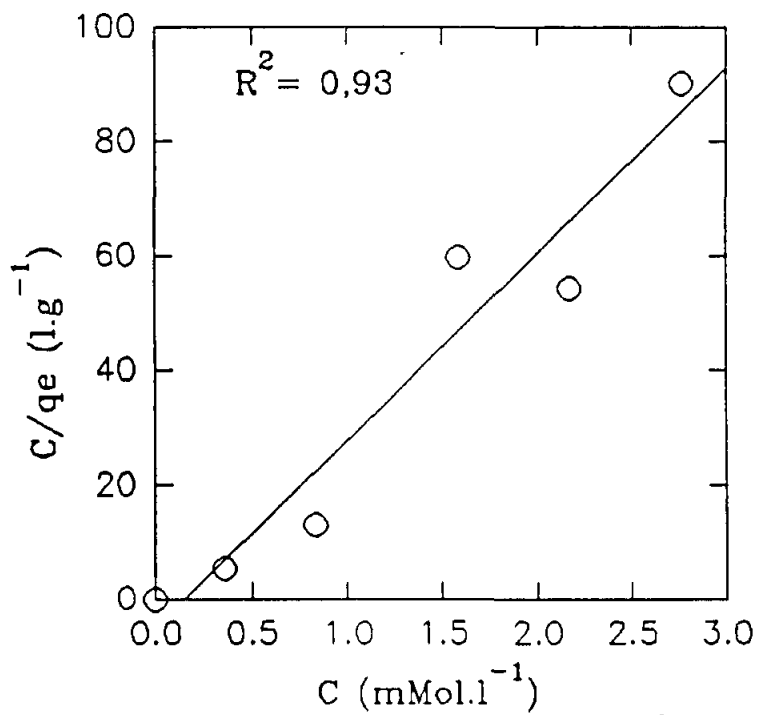


Fig. 4. 122. Reciprocal isotherm for Cr^{6+} uptake from solution by sludge 10.

Table 4.1. Summary of mechanisms of sludge-metal adsorption.

Sludge	Zn	Cd	Cu	Ni	Cr ³⁺	Cr ⁶⁺
1	II	I	I	II	I	*
2	?	I	I	II	I	I
3	I	I	I	I	I	I
4	IV	I	I	I	I	I
5	IV	I	IV	II	I	*
6	II	I	I	II	I	*
7	IV	I	I	II	*	*
8	II	I	I	I	*	II
9	II	I	IV	I	I	*
10	II	I	I	II	*	*

- I, II, IV - Types of adsorption
 ? - Unknown
 * - Unknown increase then decrease

Table 4.2. Sludge-surface capacity (X_m) for adsorption of metal-ions in single solutions.

X_m sludge (mol g ⁻¹)	Ni	Cu	Cd	Zn	Cr ³⁺	Cr ⁶⁺
S1	169.21	48428.49	209.58	248.63	278.71	84.54
S2	179.40	47648.54	253.51	288.35	200.27	65.58
S3	206.58	39491.35	233.54	287.94	293.51	113.68
S4	182.75	31030.84	215.67	230.94	191.73	56.29
S5	143.76	50671.39	122.20	164.88	171.99	50.85
S6	176.91	267.70	218.23	252.74	116.81	38.51
S7	159.30	27291.08	150.70	170.12	63.55	55.01
S8	133.37	33806.63	189.34	173.11	101.43	49.59
S9	214.59	543.05	235.95	280.91	338.90	57.78
S10	133.56	34281.79	152.05	144.37	48.79	30.64

Table 4.3. Sludge-metal binding strengths (K_a) for sludge adsorption of metal-ions in single solutions.

K_a sludge (l mol ⁻¹)	Ni	Cu	Cd	Zn	Cr ³⁺	Cr ⁶⁺
S1	62.76	16114.63	346.68	195.50	264.12	18.70
S2	61.73	34364.52	569.59	259.55	-1738.41	18.57
S3	116.10	76831.92	701.37	232.39	332.69	18.56
S4	122.24	75373.67	579.31	159.98	526.08	17.17
S5	58.71	13699.87	360.15	73.63	-383.39	-51.27
S6	61.50	304.17	364.11	145.72	-202.12	-155.68
S7	62.15	29430.29	350.94	58.37	-258.29	34.71
S8	54.08	19538.74	369.59	98.62	52.32	21.25
S9	97.43	239.85	430.89	178.16	137.55	-25.83
S10	70.54	13921.99	317.12	49.71	-84.25	-10.66

5.0 DESORPTION OF METAL-IONS FROM SLUDGES

5.1 INTRODUCTION

The need for development of biosorbents for the removal of metal-ions has already been outlined in Chapter 3. However, for these biosorbents to be cost effective and efficient, separation of metals from the sorbent (desorption) needs to be investigated. Volesky, (1987) in mapping requirements for a potential biosorbent process included desorption which has to be metal-selective and economically feasible with minimal loss of sorbent. From an economic standpoint, regeneration of biosorbent lowers waste treatment costs for users and can enhance profitability for biosorbent producers. Regeneration of biosorbent also affords an opportunity for reclamation of stripped metal into a form that is reusable by the waste generator, or conversion of metal into a form that can be reintroduced to commerce through further processing and refining (McLean and Beveridge, 1990). Adsorption and desorption are usually coupled processes. Desorption is also known as elution, particularly in processes involving adsorbents in column configurations.

Studies have been conducted on the feasibility of adsorption of uranium from dilute wastewaters generated during mining operations. Processes developed have suggested some desorbing agents for removal of this metal after its adsorption. Strandberg *et al.*, (1981) investigated uranium recovery by *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa*. They employed several desorbing agents to recover metals from these cells after biosorption. *S. cerevisiae* cells were exposed to 0.1M nitric acid, 0.1M disodium ethylenediaminetetraacetic acid (EDTA) and 0.1M ammonium carbonate for 16 h and

obtained only 59.3, 72.3 and 83.5% removal, respectively. They also attempted desorption with two other agents viz., Sodium citrate 0.1M and potassium oxalate 1.0M and obtained 57 and 14% removal, respectively. They did, however, report increased adsorption in biomass desorbed with ammonium carbonate, sodium citrate and potassium oxalate. Although nitric acid and EDTA decreased final adsorption of U, initial uptake rates were enhanced.

Tsezos (1984) adsorbed uranium to *Rhizopus arrhizus* biomass and desorbed the metal with six different eluants viz., sulphuric, nitric and hydrochloric acids, ammonium sulphate, sodium carbonate and sodium bicarbonate. All of the above were used at concentrations of 0.01, 0.1 and 1N at solids (biomass) to liquids (eluant) (S/L) ratios of 0-6. They found that ammonium sulphate was not an efficient eluant; even at S/L ratios of 1:1 there was only 10% uranium recovery. Sulphuric acid was reported to be an unsuccessful eluant. At low concentrations (0.1N) complete elution was achieved at a S/L ratio of 4, but this efficiency decreased to 90% at a concentration of 1N at the same S/L ratio. Hydrochloric acid was ineffective at 0.01N, increased to 94% at 0.1N and eluted all biosorbed uranium. The high solubility of uranyl chloride, which is the dominant form of uranium in solution at the low pH values during the elution with 1N HCl, and the increased competition towards uranium by hydrogen ions for the cell wall coordination sites were postulated to be the driving forces that reversed the equilibrium in favour of the liquid phase, thus returning the uranium held by the fungal wall to solution. Nitric acid also exhibited elution behaviour similar to that of hydrochloric acid. Sodium carbonate was shown to be very effective at eluant concentrations higher than 0.1N. However, this option does not seem very attractive, since the high pH values at equilibrium are between 11 and 12 and can be damaging to fungal cell walls.

Sodium bicarbonate emerged as the better uranium elution agent at concentrations above 0.1N since equilibrium pH ranged from 7.5-8.5 and this agent produced intact cells with no structural damage after elution. After further investigations, Tsezos *et al.*, (1989) developed a laboratory scale model for uranium recovery with sodium bicarbonate as desorbent and were capable of recovering all uranium from dilute ($300 \text{ mg } \ell^{-1}$) solutions concentrating it to $5\,000 \text{ mg } \ell^{-1}$ in the eluant. The biomass used was *R. arrhizus* immobilised by a proprietary process and removal decreased to 30% efficiency after 10 cycles. This was attributed to loss of approximately 49% of the biomass on elution (10 cycles). Clearly, their immobilisation process was not successful although they did obtain removal capacities of 50 mg g^{-1} , uranium to biomass.

Cadmium adsorption and desorption was investigated by Kurek *et al.*, (1982). They exposed Cd at a concentration of $10 \text{ mg } \ell^{-1}$ to soil, several bacteria and fungi and reported that bacteria adsorbed more metals than the soil constituents. They further extracted the adsorbed metal from each organism using 0.1N sodium hydroxide and 5mM diethylene triamine pentaacetic acid (DTPA). They reported that Cd sorbed to bacteria and fungi could be extracted with both agents. However, it was observed that with increasing biomass in a medium NaOH-extractable Cd increased while DTPA-extractable Cd decreased. The reason advanced for this was that different chemical forms of Cd were extracted by the different desorbing agents.

Huang *et al.*, (1990) used *Saccharomyces cerevisiae* to biosorb copper, lead and cadmium in batch and immobilised systems. They used strong acid (HClO_4) to strip immobilised biomass of bound metal after adsorption. Their results indicated that perchloric acid at a

concentration of 5mM was capable of completely stripping copper from immobilised columns. One volume of acid was used to strip columns after addition of 20 volumes of Cu at a concentration of $5 \times 10^{-5}\text{M}$ (approx 3.2 ppm).

Matheickal *et al.*, (1991) utilised a naturally occurring wood-rotting fungus, *Ganoderma lucidium* to biosorb copper and attempted desorption with 0.01M EDTA and 0.1M HCl. They reported that almost all the metal biosorbed was capable of being desorbed by the two agents after incubating the metal-laden biomass with the agents for three hours. They found HCl to be the more efficient desorbent since a small amount of Cu still remained bound to the biomass after treatment with EDTA. They postulated that the high concentrations of protons made available by HCl may dislodge Cu from active sites so as to make the bond between Cu and the biosorbent labile. The EDTA desorption was attributable to direct competition between ligands and EDTA for Cu ions since EDTA forms a strong complex with Cu with a strong stability constant ($\log K = 10.7$).

Wong *et al.*, (1993) investigated Cu removal and recovery from an industrial effluent using an immobilised *Pseudomonas putida* II-11 strain isolated from a metal plating effluent. They eluted biomass after adsorption with 0.1M HCl and were able to recover more than 90% of adsorbed Cu. They also eluted with 0.1M EDTA and obtained approximately 89% desorption and 94% desorption with HCl. They postulated that the unrecoverable Cu might be trapped intracellularly. They were capable of operating their immobilised column for five cycles before marked detrimental effects to adsorption were noted.

There are several advantages to using bacteria for adsorption of metals. The rapid growth of these organisms and their adaptability to an assortment of substrates and environments are some useful attributes. Following chemical treatment to desorb bound metals microbial biomass can often be reused for metal reclamation. In some cases biomass can be reused for metal reclamation.

5.1.1 Aim

To investigate the desorptive capabilities of deionised water, acetic and hydrochloric acids for sludge bound metal ions.

5.2 METHODOLOGY

Four of the ten sludges in chapter 3 were used in desorption studies. Sludges were chosen on their higher overall uptake of metal-ions from solutions than their counterparts. These sludges were 2, 3, 4 and 9.

Sludges were exposed to metal-ions in individual solutions as described in chapter 3.

After separation of sludges from solution by centrifugation at 4,000 x g in a Beckman, J6-B centrifuge, supernatants were analysed by atomic absorption spectrophotometry (AAS) to determine the quantities of metals adsorbed by sludges. Pellets were resuspended in 10 ml volumes of three desorbing agents viz., distilled water, 0.2 N hydrochloric acid and 2.5% acetic acid. The tubes were incubated at 25 °C for 3 h at 150 rpm on a Certomat U reciprocal shaker. Sludges were separated from desorbing agents by centrifugation at 4,000 x g. Supernatants were collected and analysed for metal-ions by AAS to determine efficiency of desorption. Efficiency of desorption was calculated by the following formula :

$$\text{Desorption efficiency} = \frac{\text{quantity adsorbed (mg } \ell^{-1})}{\text{quantity desorbed (mg } \ell^{-1})} \times 100\%$$

5.3 RESULTS AND DISCUSSION

Desorption of zinc and cadmium (Figs 5.1 - 5.8) from the four sludges to which it was adsorbed showed general removal trends which were similar among the sludges, i.e., the effect was desorbent-dependent rather than sludge-dependent. Cadmium desorption by both agents was more efficient than zinc. Both acetic and hydrochloric acids displayed high overall desorption of zinc and cadmium from sludges. The contribution of deionised water to desorption was low in comparison to these two agents. Comparing the two acids, hydrochloric acid was the better desorbing agent among all four sludges although acetic acid was present in greater concentration c.f., 0.2N to 2.5% (approx. 0.42N). This is most probably due to the fact that hydrochloric acid is a strong acid and liberates more protons to exchange with metal ions bound on the surface than acetic acid, which is a weak acid. Desorption of zinc by acids at all adsorption concentrations were high indicating that ions were effectively removed. In no instance, however, was all the metal removed from the sludge surfaces. There was, however, 97.43% removal of zinc from sludge 9 at $120 \text{ mg } \ell^{-1}$ (Fig. 5.4). Efficiencies of 96.76% and 97.21% zinc removal from sludges 2 and 3 at $150 \text{ mg } \ell^{-1}$ were also recorded (Figs 5.1 & 5.2). Cadmium desorption by hydrochloric acid ranged from 94.33% to 99.45%, the former being recorded for sludge 9 at $150 \text{ mg } \ell^{-1}$ (Fig. 5.8) and the latter, for sludge 4 at $120 \text{ mg } \ell^{-1}$ (Fig. 5.7).

Chromium (III) was also more efficiently desorbed by hydrochloric acid (Figs 5.9 - 5.12). However, the efficiency of desorption was not consistently high, increasing as concentration of metal-ions in solution increased. This could possibly be due to a multilayer adsorption of Cr^{3+} onto the surface of the sludge. Thus, as more layers are adsorbed onto sludge, it

becomes easier to desorb these surface layers, hence higher efficiencies at higher adsorption concentrations. Notably, in sludges 3 and 4 at higher concentration, desorption by HCl was almost complete. Acetic acid also progressively desorbed more Cr^{3+} from sludge surfaces as concentrations increased reaffirming the premise that adsorption of Cr^{3+} occurs in layers upon sludge surfaces.

Nickel and copper were desorbed at high efficiency by both acids (Figs 5.13 - 5.20). In contrast to the metals already discussed, Ni and Cu were more efficiently removed by acetic than hydrochloric acid. Near complete desorption of metal-ions was recorded at all concentrations. This could possibly be due to the metal cations being more amenable to form metal-acetate salts than metal-chloride salts. Therefore, since more metal ions form the acetate salt more exchange of metal ions will occur between the bound metal on sludge and hydrogen cations liberated by acetic acid.

Nickel in solution with acetate forms a tetrahydrate while nickel chloride forms a hexahydrate. The formation of tetrahydrate nickel salts is more favoured than the formation of hexahydrate salts in aqueous solutions. Therefore, the formation of nickel acetate would be favoured and this is possibly why acetic acid is more efficient at desorbing nickel ions from sludge surfaces. The same argument may be advanced for copper (II) acetate and chloride the former being known to exist in the monohydrate form while the latter can take on two water molecules. It is not certain what forms occur in solution, but these are most likely to occur when cationic and anionic species of the above types are present in aqueous solution.

Efficiency of removal from surfaces of Ni by acetic acid ranged from 92.27% for sludge 3 at 120 mg ℓ^{-1} (Fig. 5.14) to 99.48% for sludge 9 at 90 mg ℓ^{-1} . Desorption efficiency of copper ranged between 86.52% (Fig. 5.19) for sludge 4 at 120 mg ℓ^{-1} and 98.77% for sludge 9 at 30 mg ℓ^{-1} .

Chromium (VI) desorption was low compared to the other five metals. Also, definite trends were not observed across all sludges for the two acid desorbing agents. Acetic acid was more efficient at desorbing Cr^{6+} from sludges 2 and 3 than hydrochloric acid (Figs 5.21 & 5.22). This trend was not repeated in the other two sludges although it seemed that hydrochloric acid was the more efficient desorbent at lower concentrations and acetic acid, at the higher concentrations in sludges 4 and 9 (Figs 5.23 & 5.24). Deionised water displayed a clear trend among all sludges although it was not the most efficient desorbing agent. Increasing desorption was observed as concentrations in solution increased prompting the assumption that Cr^{6+} is bound in layers upon the sludge surface.

Desorption by the harsher agents viz., the acids was not capable of removing all the metals from the surfaces, in fact efficiencies were rarely over 50%. This could be due to the initial layers of Cr^{6+} being adsorbed strongly.

5.4 CONCLUSIONS

The two acids tested viz., acetic and hydrochloric acid, as desorbing agents were efficient at removing metal-ions from sludge surfaces. Desorption was agent-dependent rather than sludge-dependent. Desorbing agents were selective with regard to efficiency of removal of metal-ions from sludges.

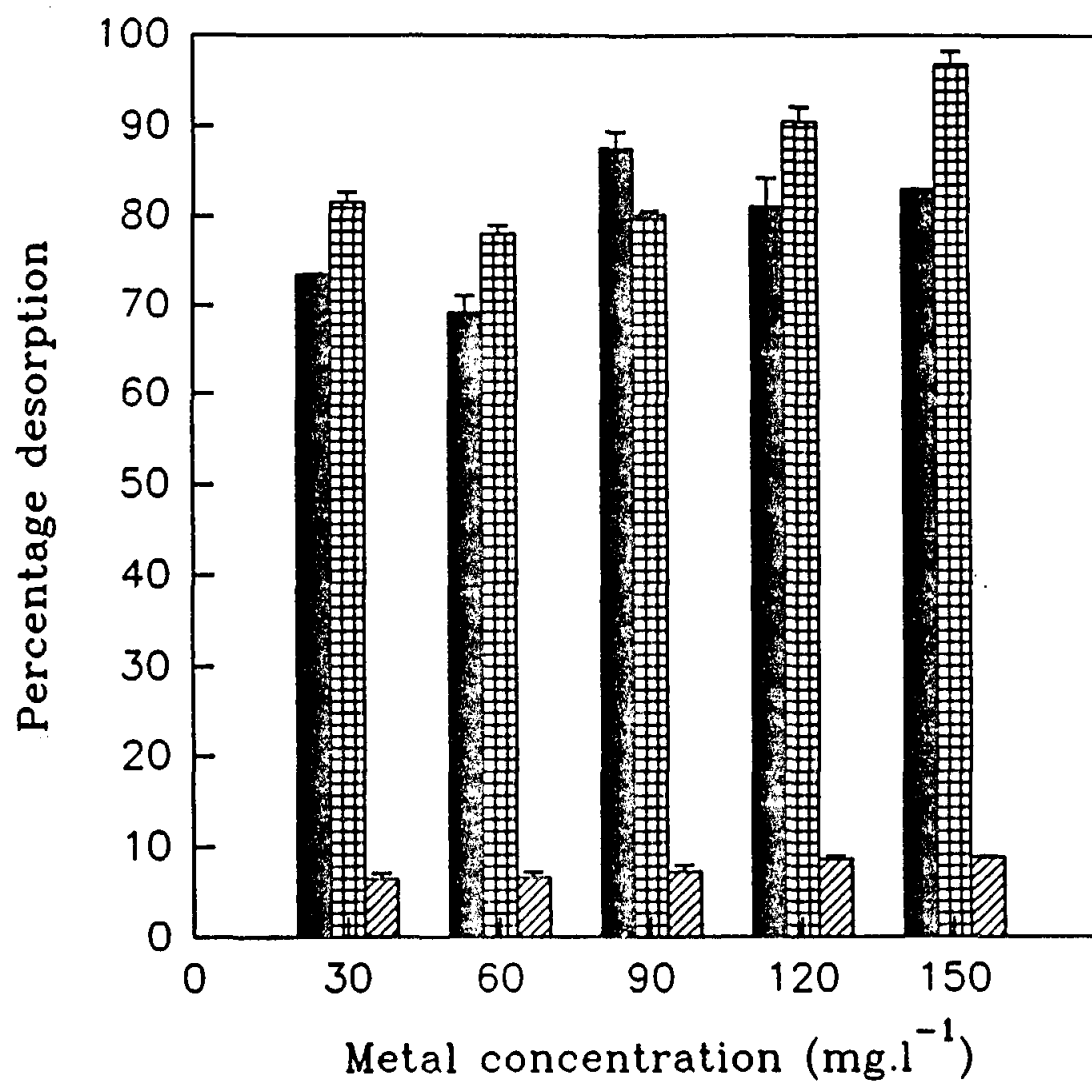


Fig. 5.1. Percentage desorption of Zn from sludge 2 by acetic acid (■), hydrochloric acid (▨) and deionised water (▧).

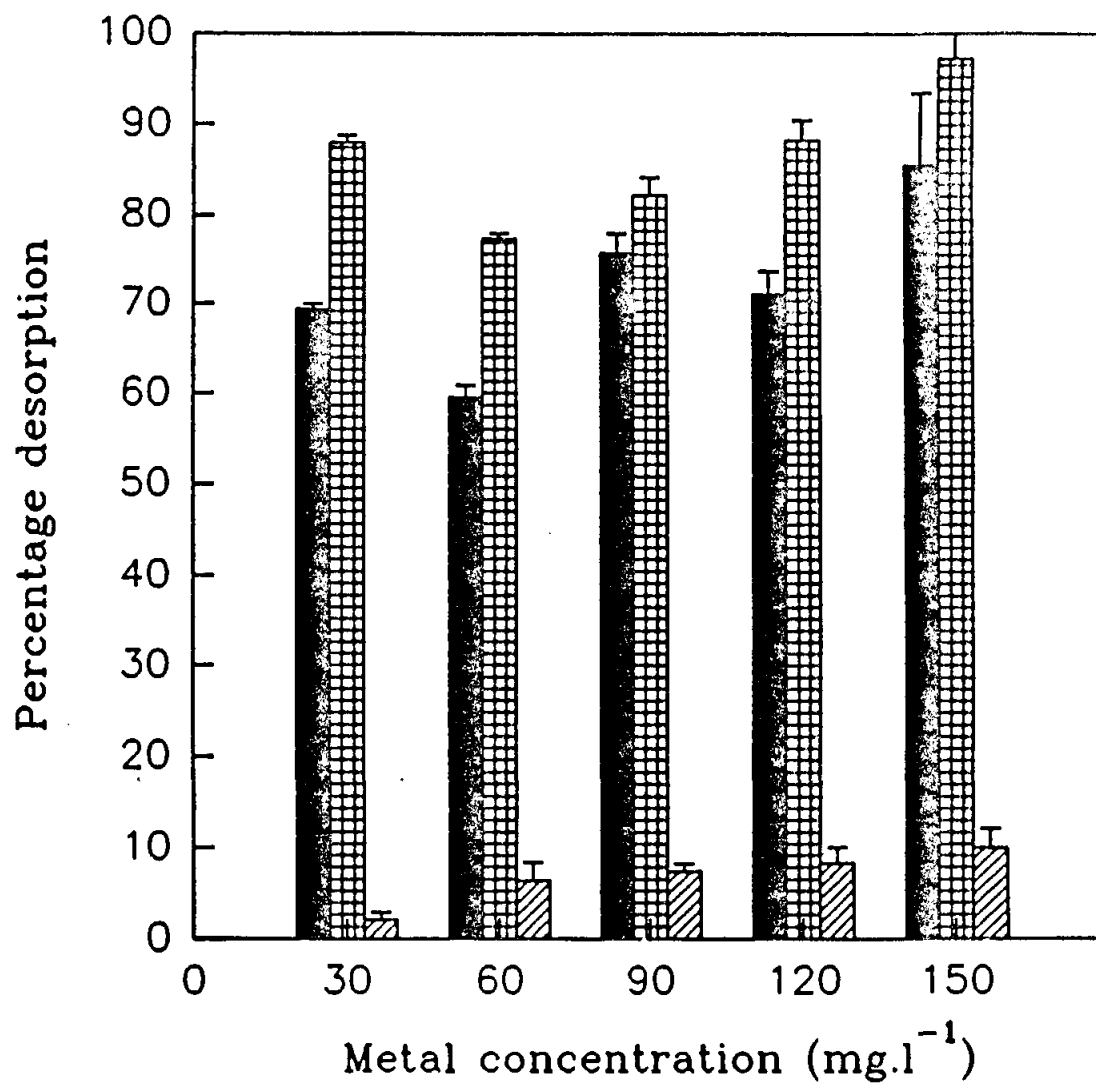


Fig. 5.2. Percentage desorption of Zn from sludge 3 by acetic acid (■), hydrochloric acid (▤) and deionised water (▨).

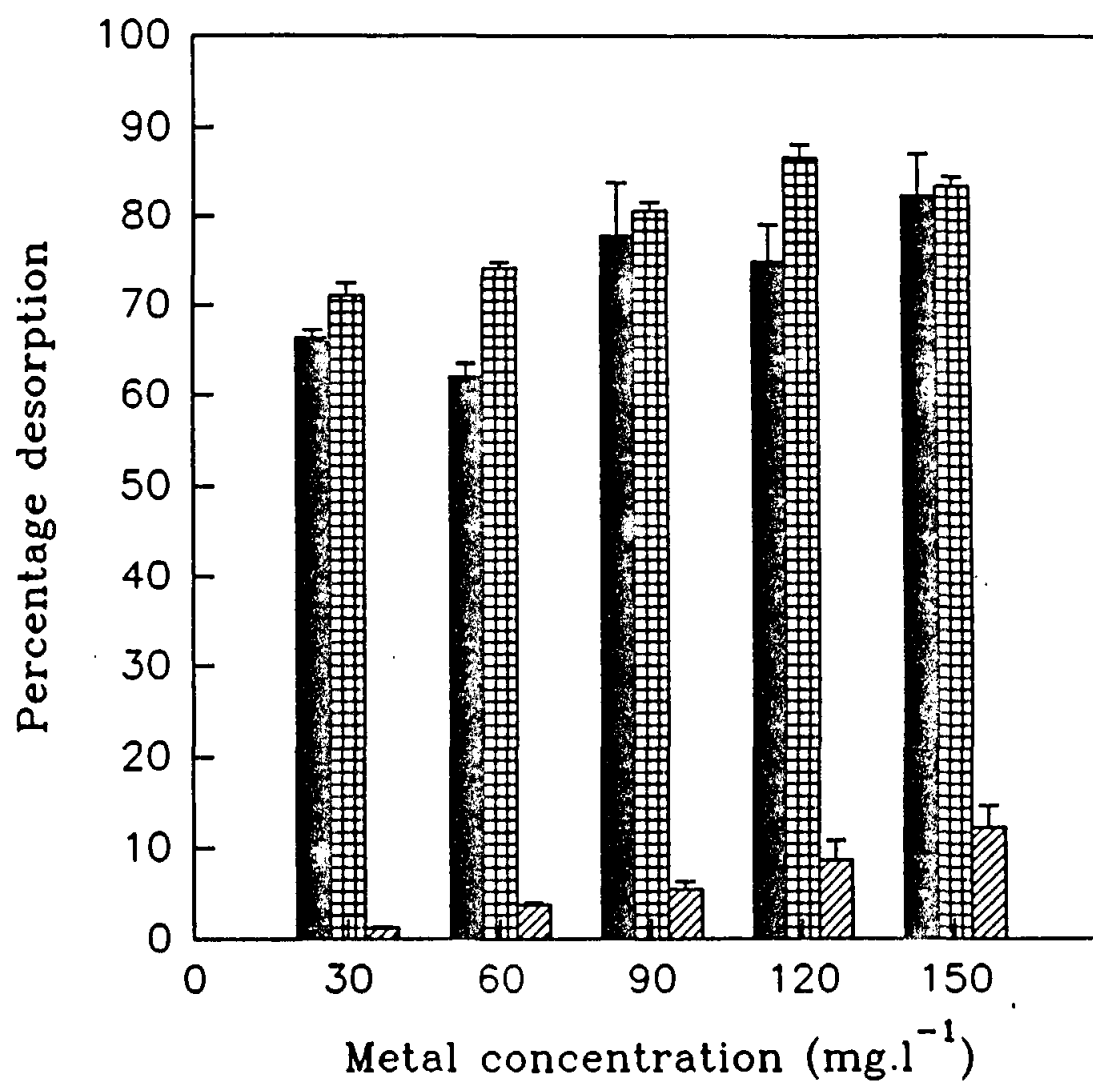


Fig. 5.3. Percentage desorption of Zn from sludge 4 by acetic acid (■), hydrochloric acid (▣) and deionised water (▨).

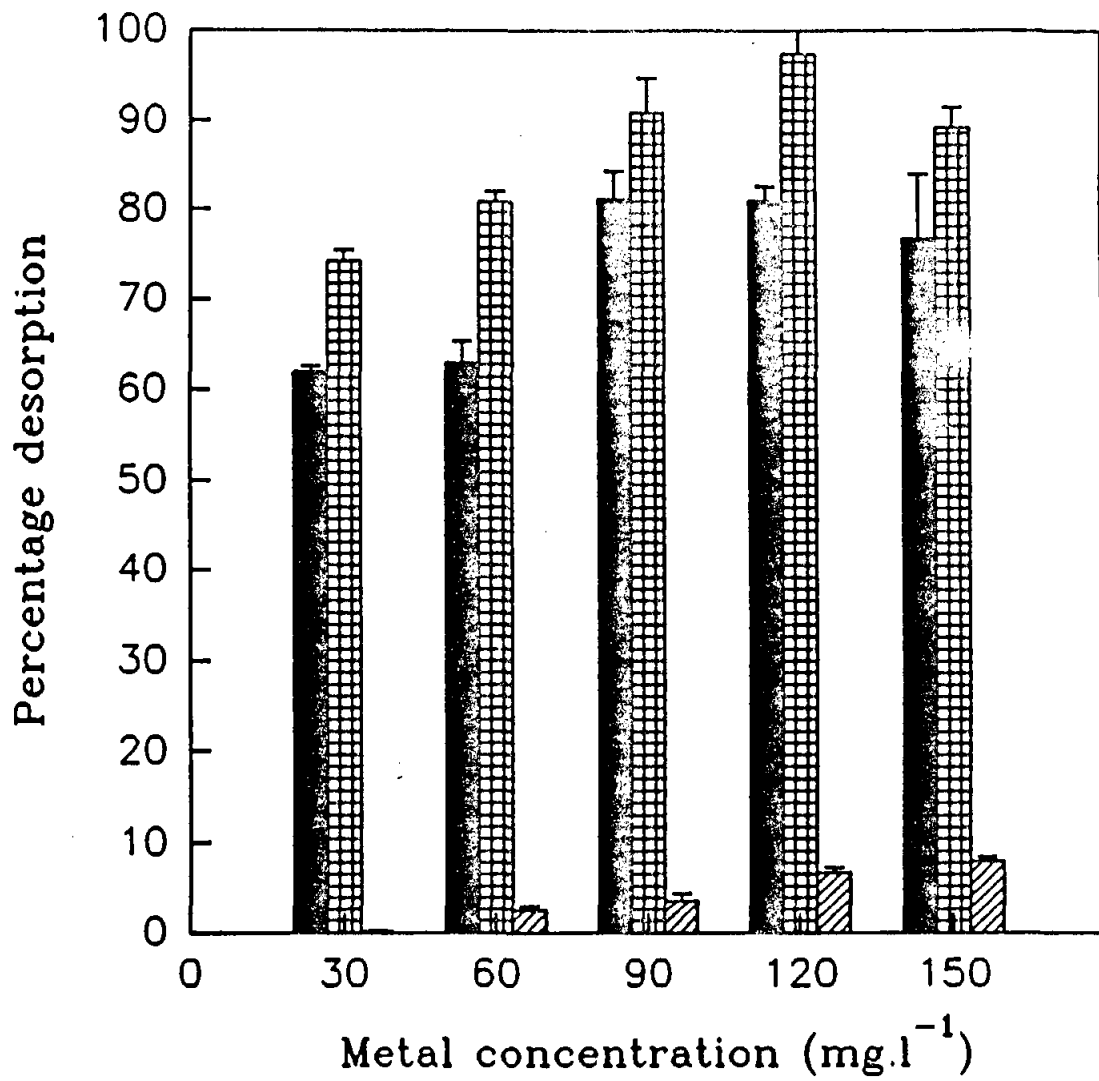


Fig. 5.4. Percentage desorption of Zn from sludge 9 by acetic acid (■), hydrochloric acid (▨) and deionised water (▧).

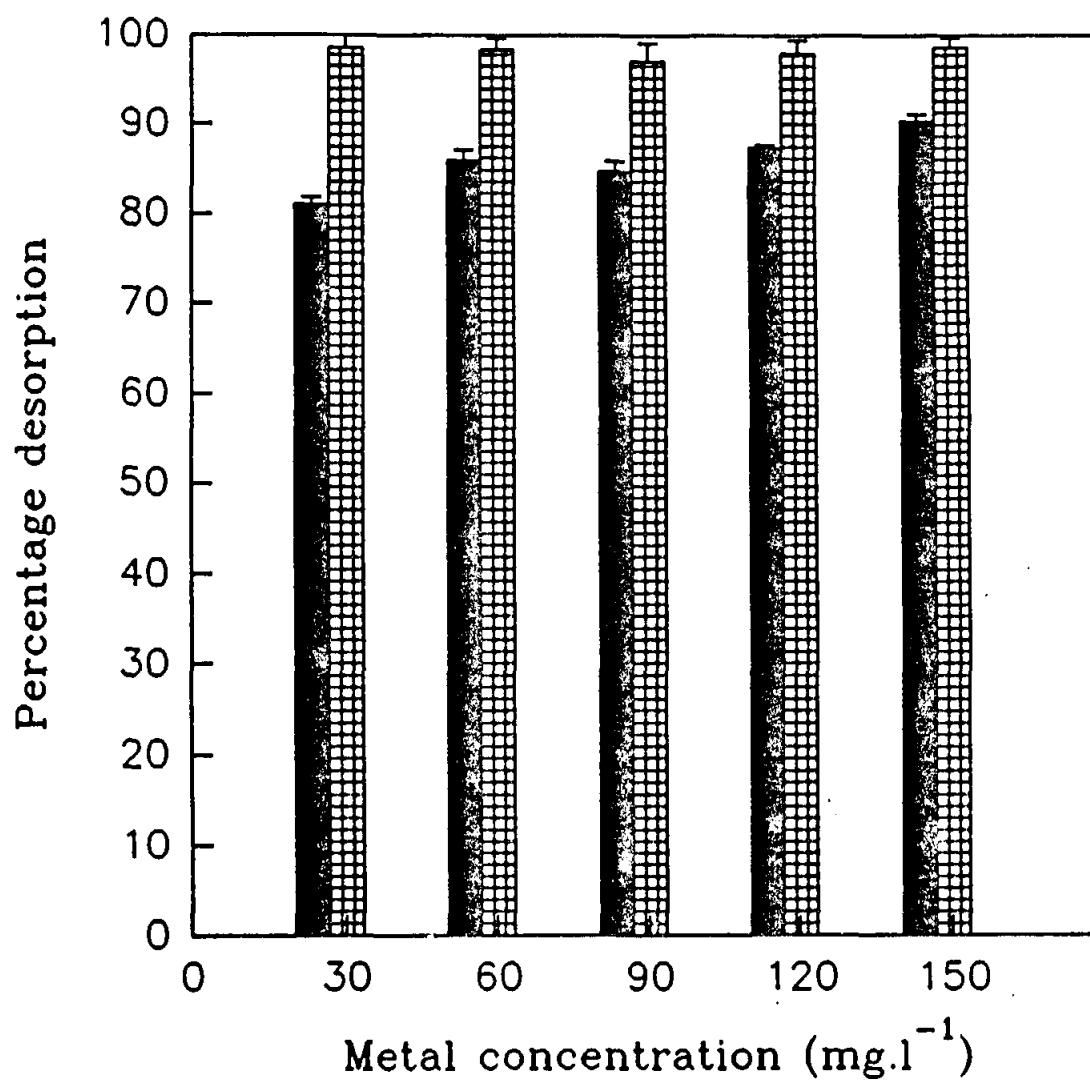


Fig. 5.5. Percentage desorption of Cd from sludge 2 by acetic acid (■), hydrochloric acid (▣) and deionised water (▤).

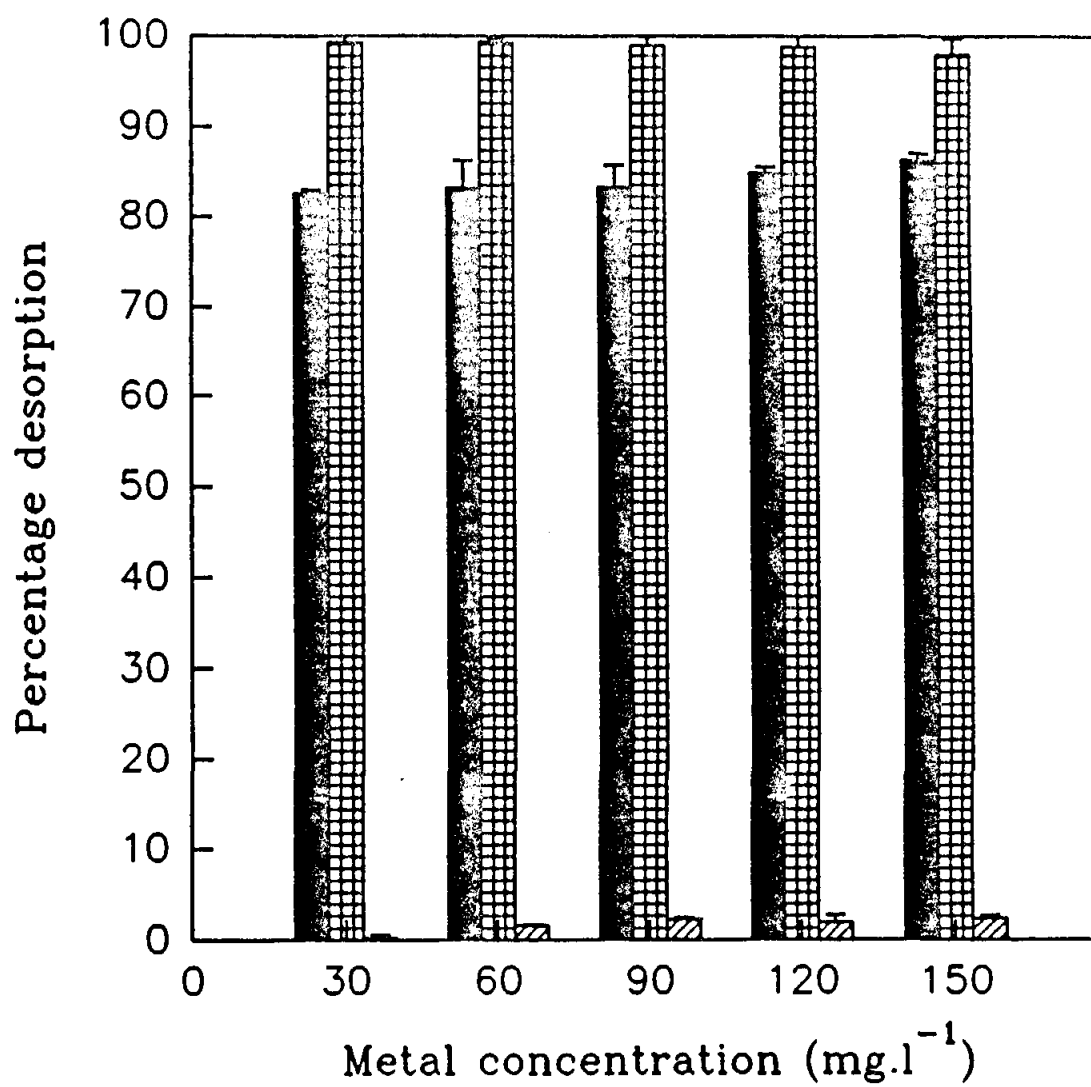


Fig. 5.6. Percentage desorption of Cd from sludge 3 by acetic acid (■), hydrochloric acid (▨) and deionised water (▧).

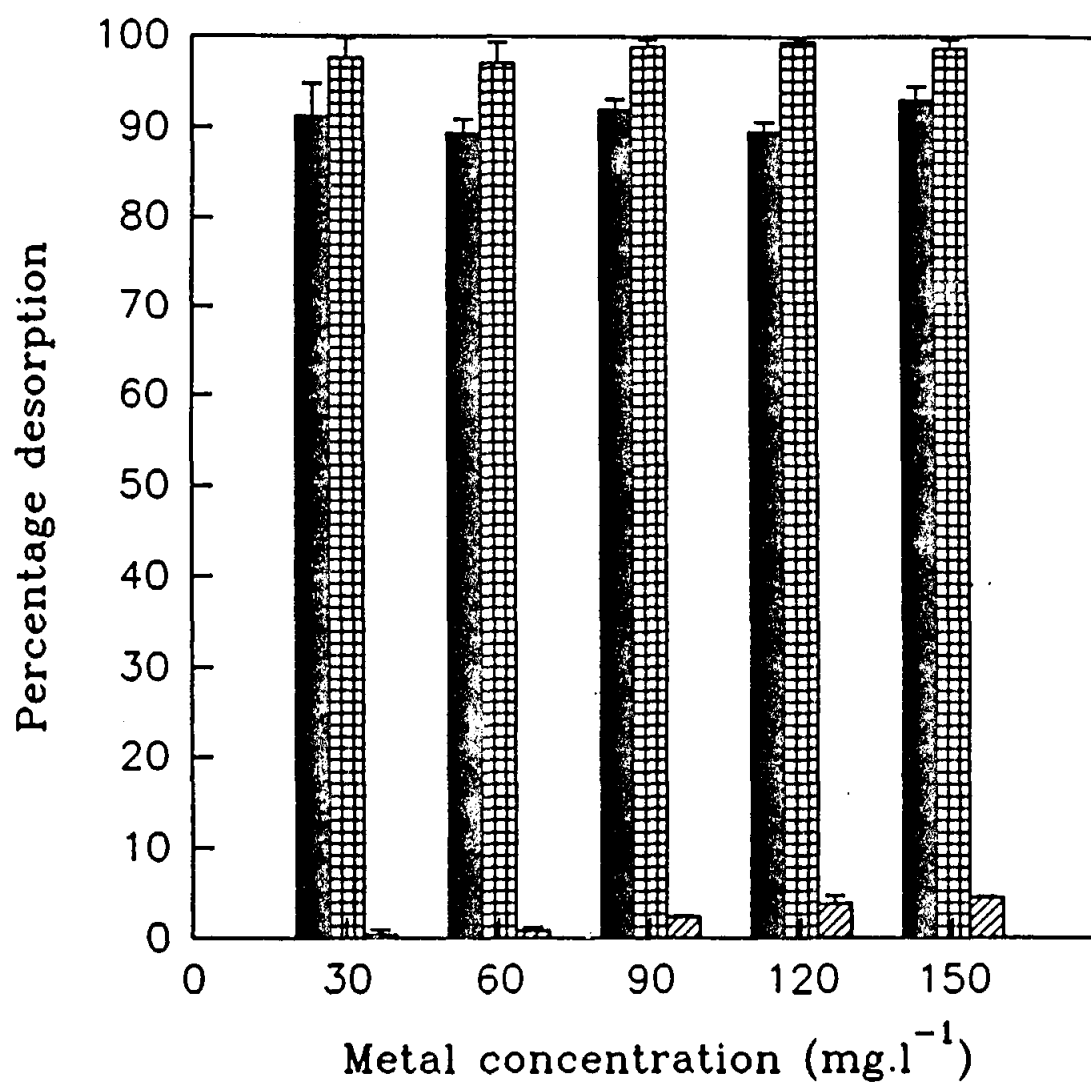


Fig. 5.7. Percentage desorption of Cd from sludge 4 by acetic acid (■), hydrochloric acid (▨) and deionised water (▧).

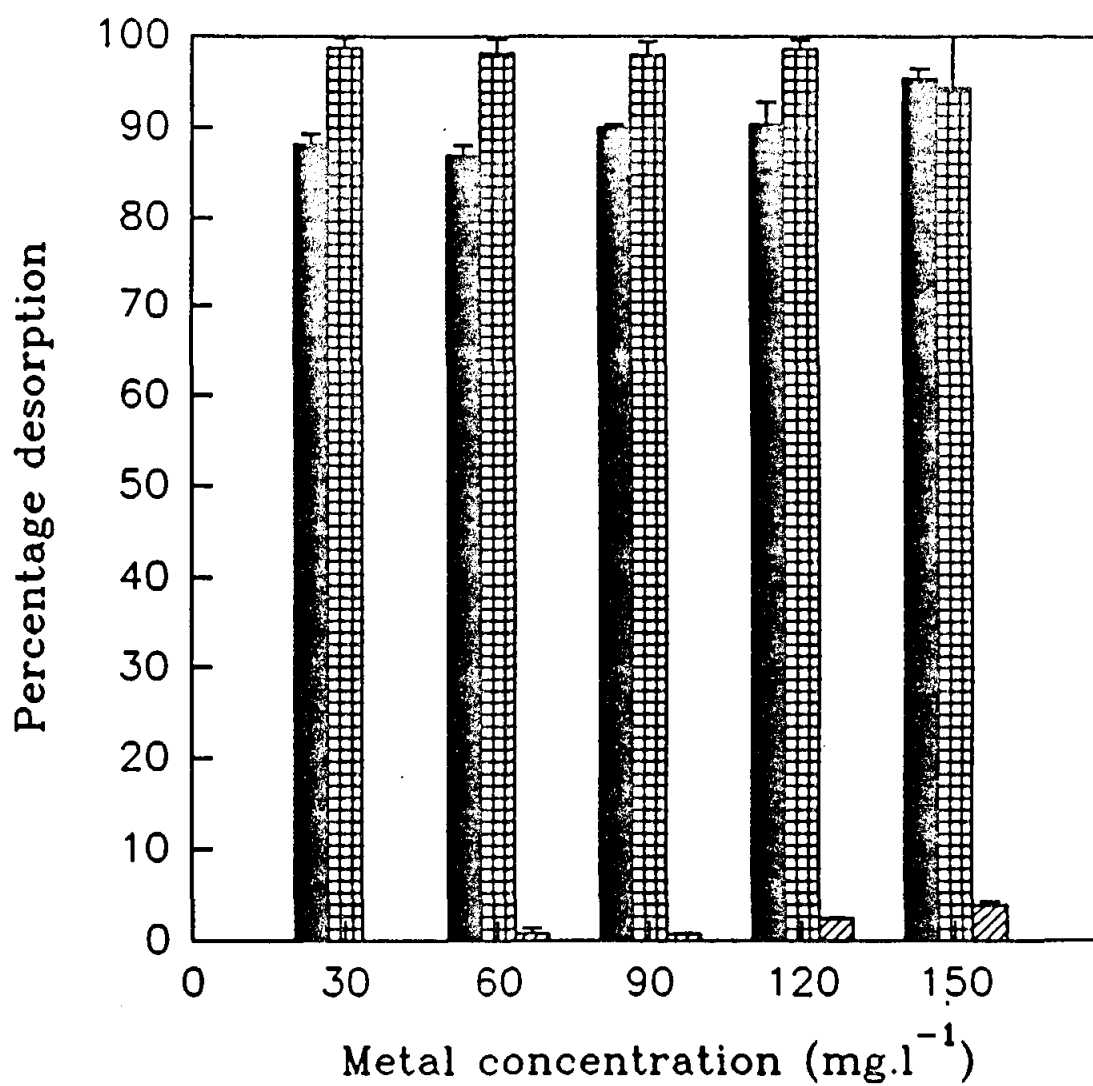


Fig. 5.8. Percentage desorption of Cd from sludge 9 by acetic acid (■), hydrochloric acid (▣) and deionised water (▨).

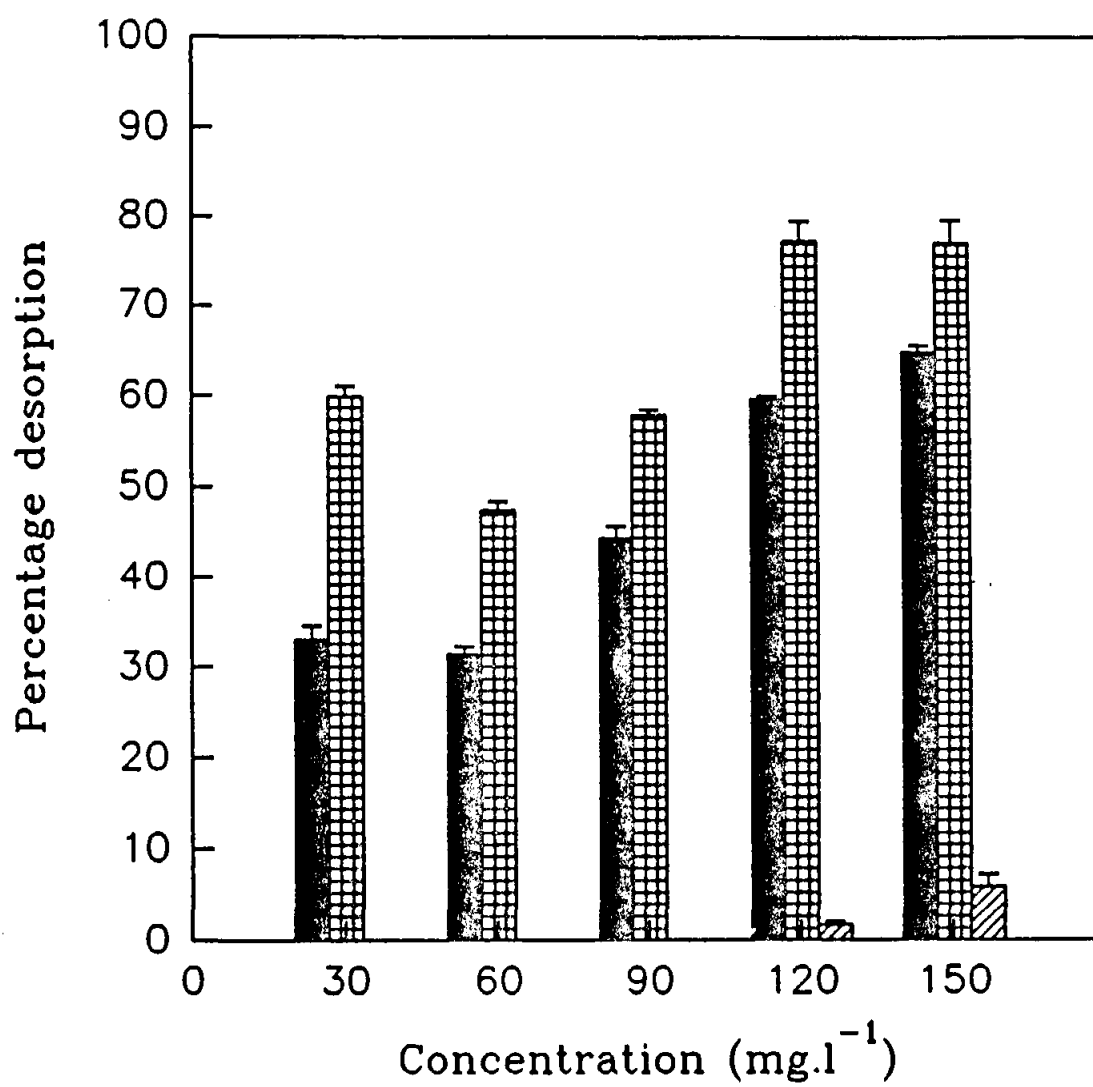


Fig. 5.9. Percentage desorption of Cr^{3+} from sludge 2 by acetic acid (■), hydrochloric acid (▤) and deionised water (▨).

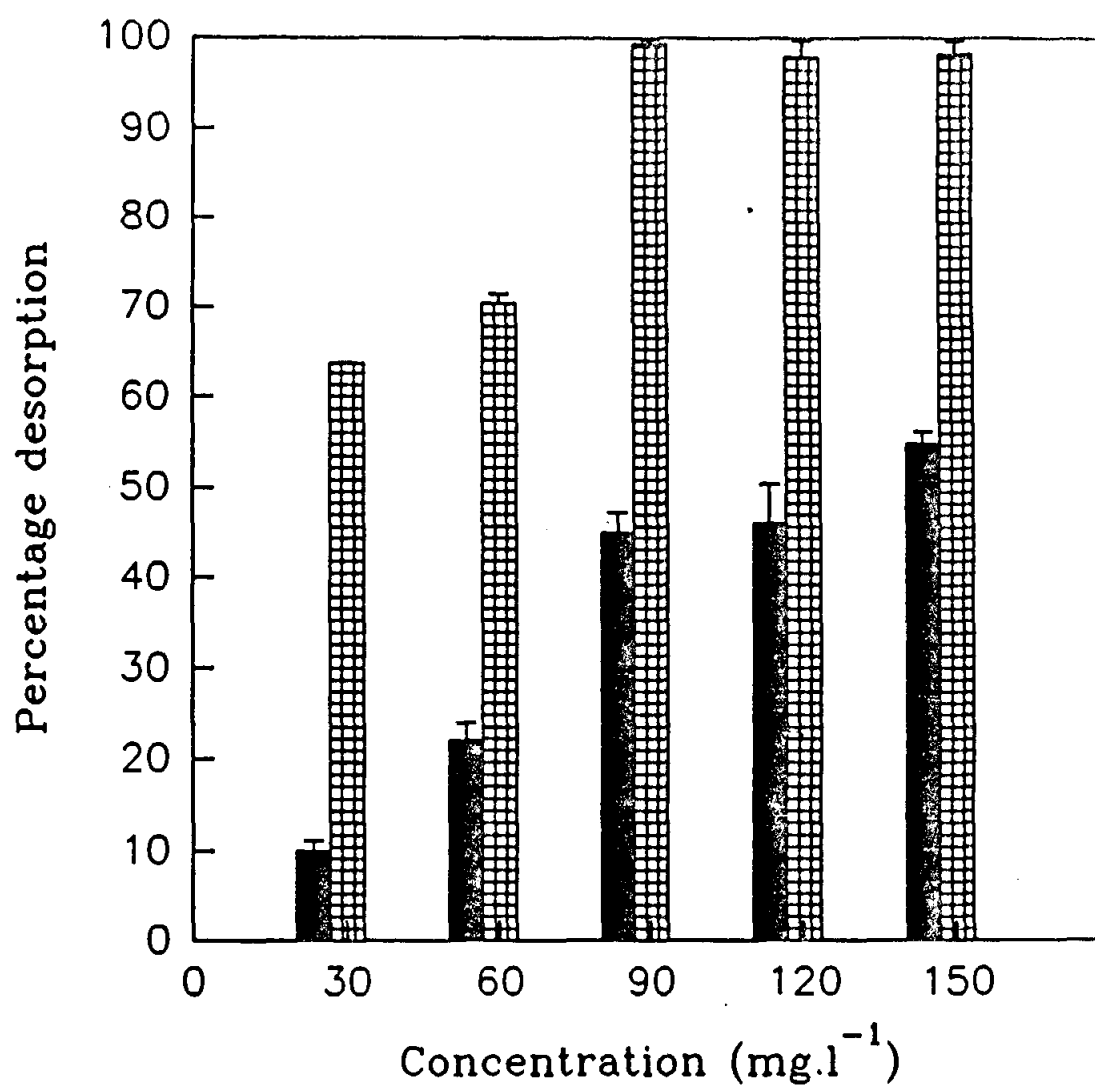


Fig. 5.10. Percentage desorption of Cr^{3+} from sludge 3 by acetic acid (■), hydrochloric acid (▨) and deionised water (▧).

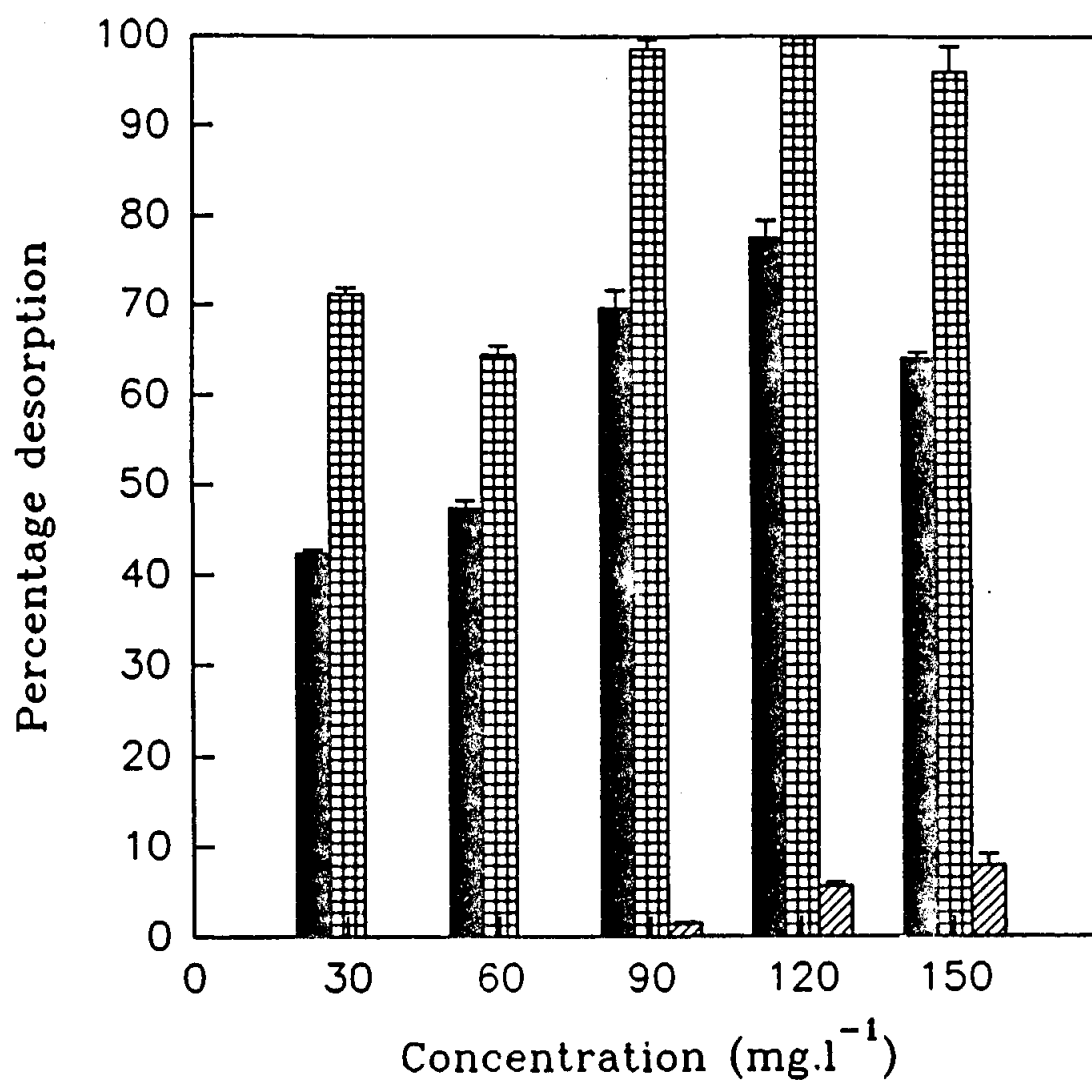


Fig. 5.11. Percentage desorption of Cr^{3+} from sludge 4 by acetic acid (■), hydrochloric acid (▤) and deionised water (▨).

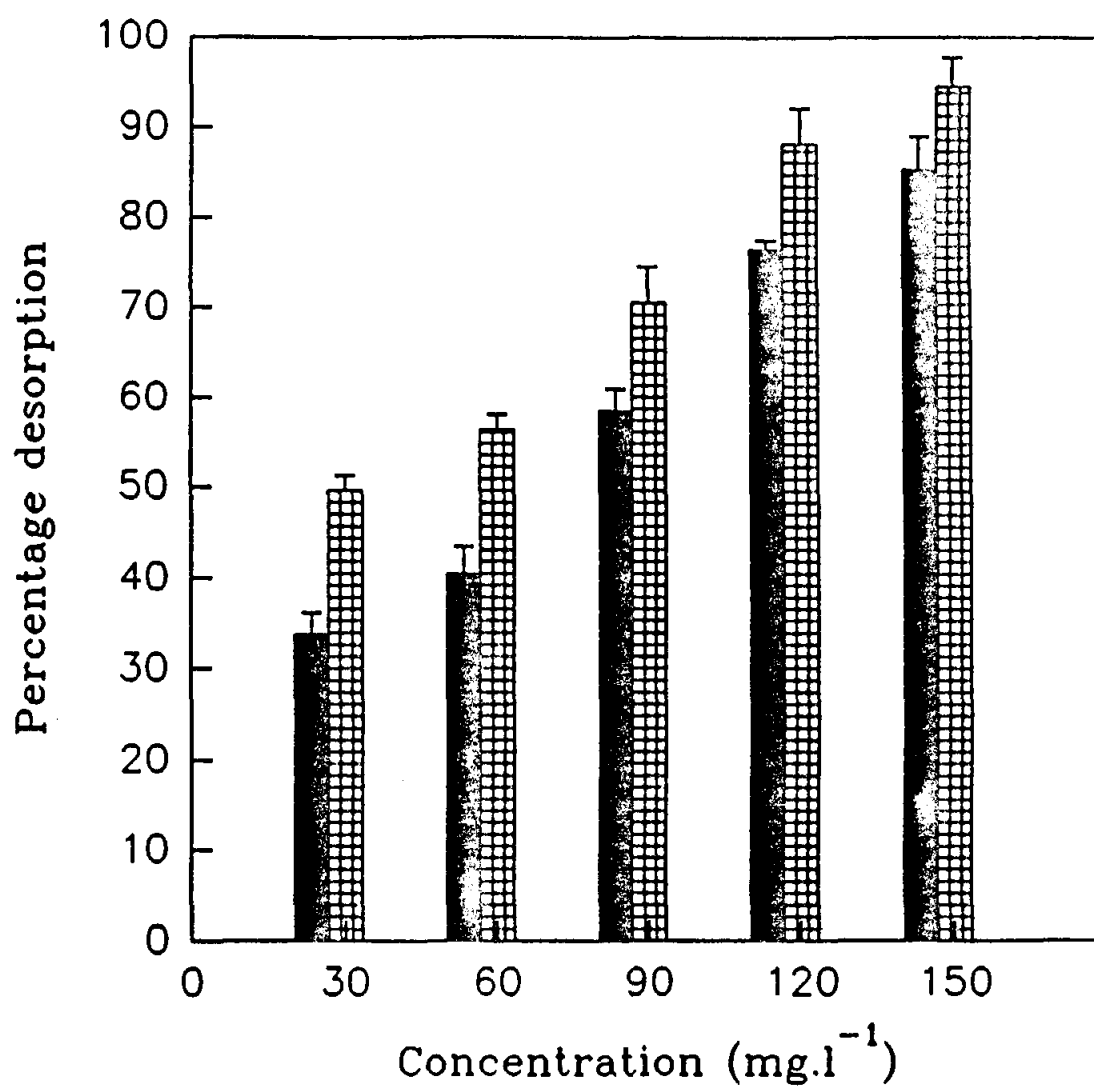


Fig. 5.12. Percentage desorption of Cr^{3+} from sludge 9 by acetic acid (■), hydrochloric acid (▤) and deionised water (▨).

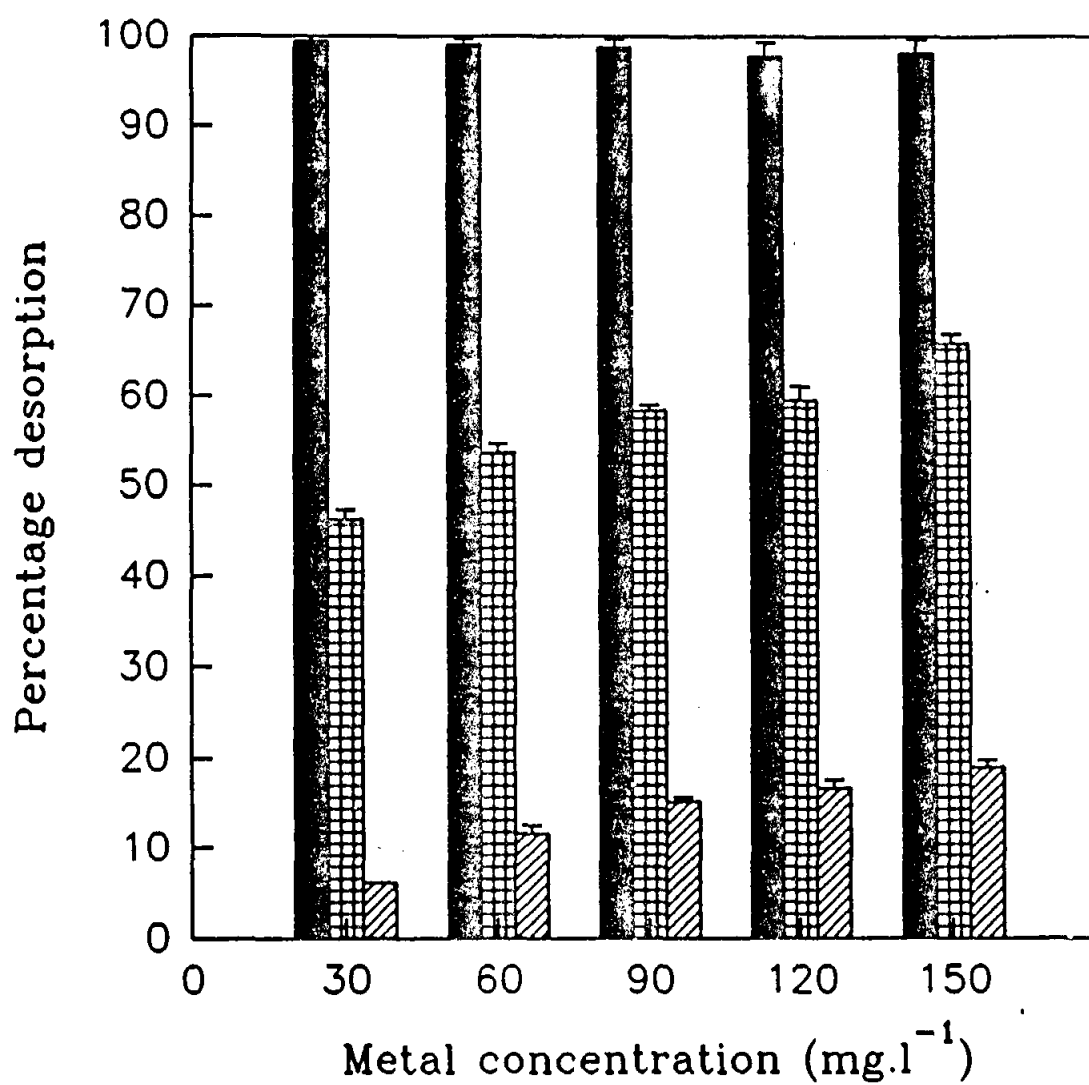


Fig. 5.13. Percentage desorption of Ni from sludge 2 by acetic acid (■), hydrochloric acid (▣) and deionised water (▨).

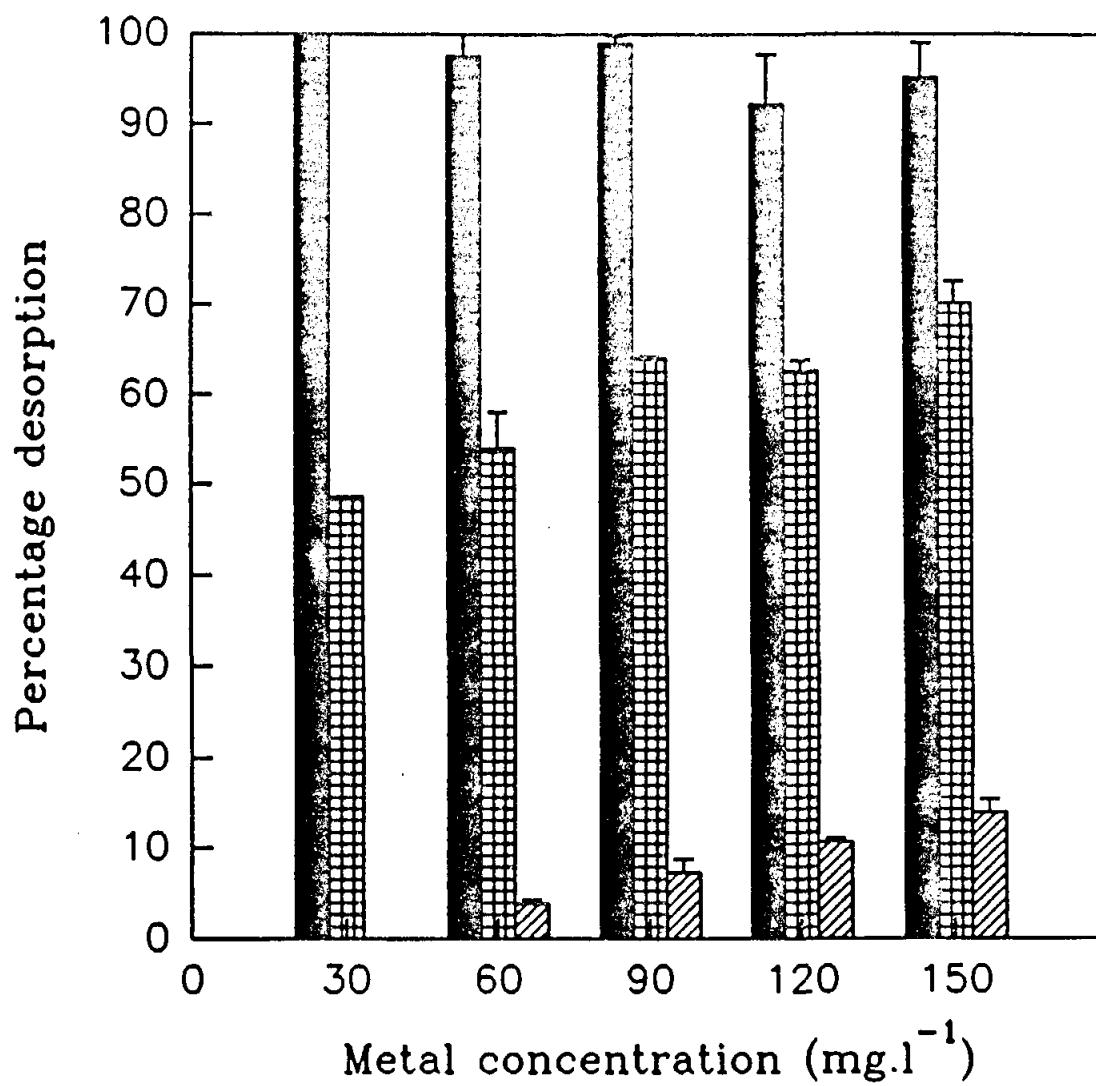


Fig. 5.14. Percentage desorption of Ni from sludge 3 by acetic acid (■), hydrochloric acid (▤) and deionised water (▨).

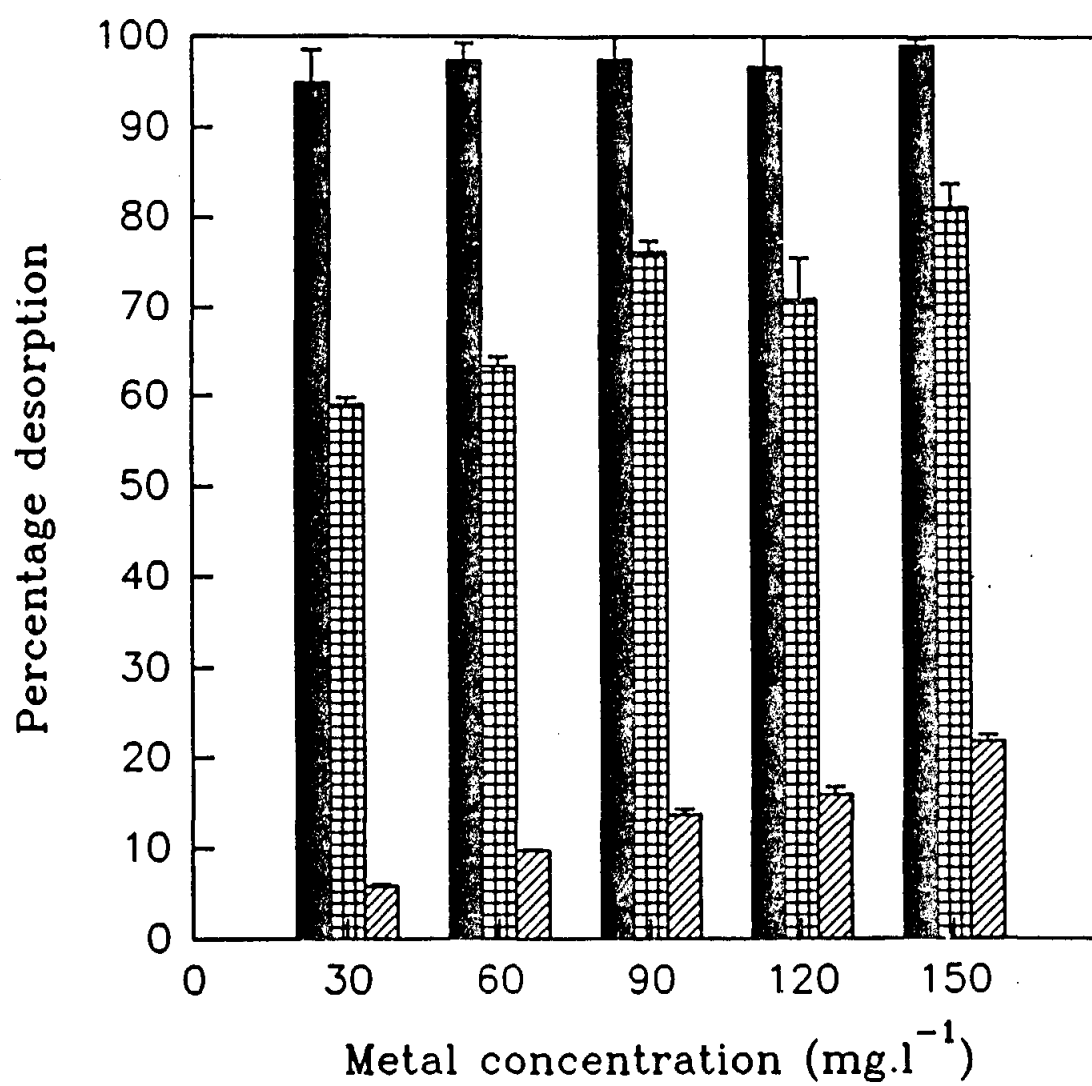


Fig. 5.15. Percentage desorption of Ni from sludge 4 by acetic acid (■), hydrochloric acid (▣) and deionised water (▨).

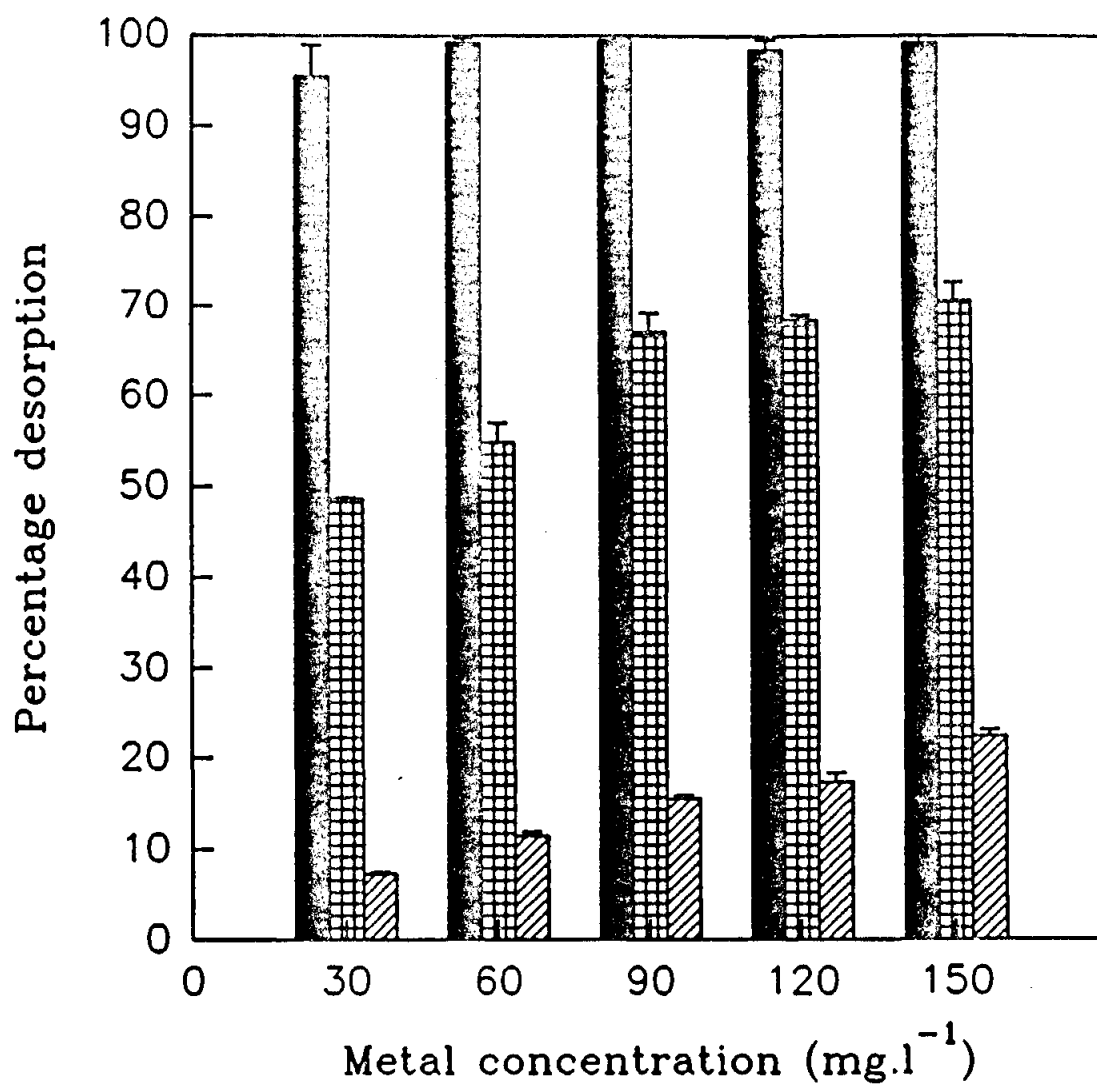


Fig. 5.16. Percentage desorption of Ni from sludge 9 by acetic acid (■), hydrochloric acid (▤) and deionised water (▨).

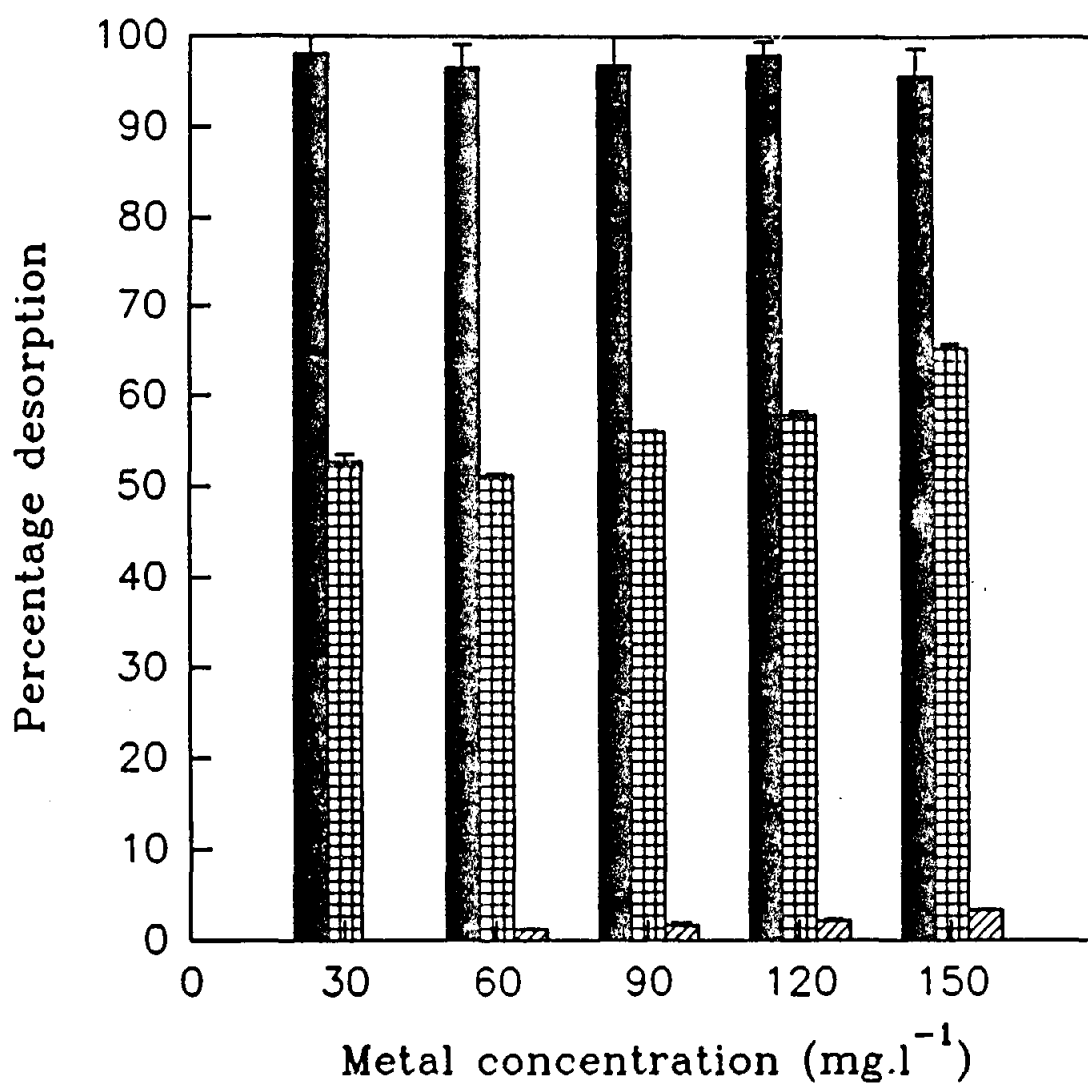


Fig. 5.17. Percentage desorption of Cu from sludge 2 by acetic acid (■), hydrochloric acid (▤) and deionised water (▨).

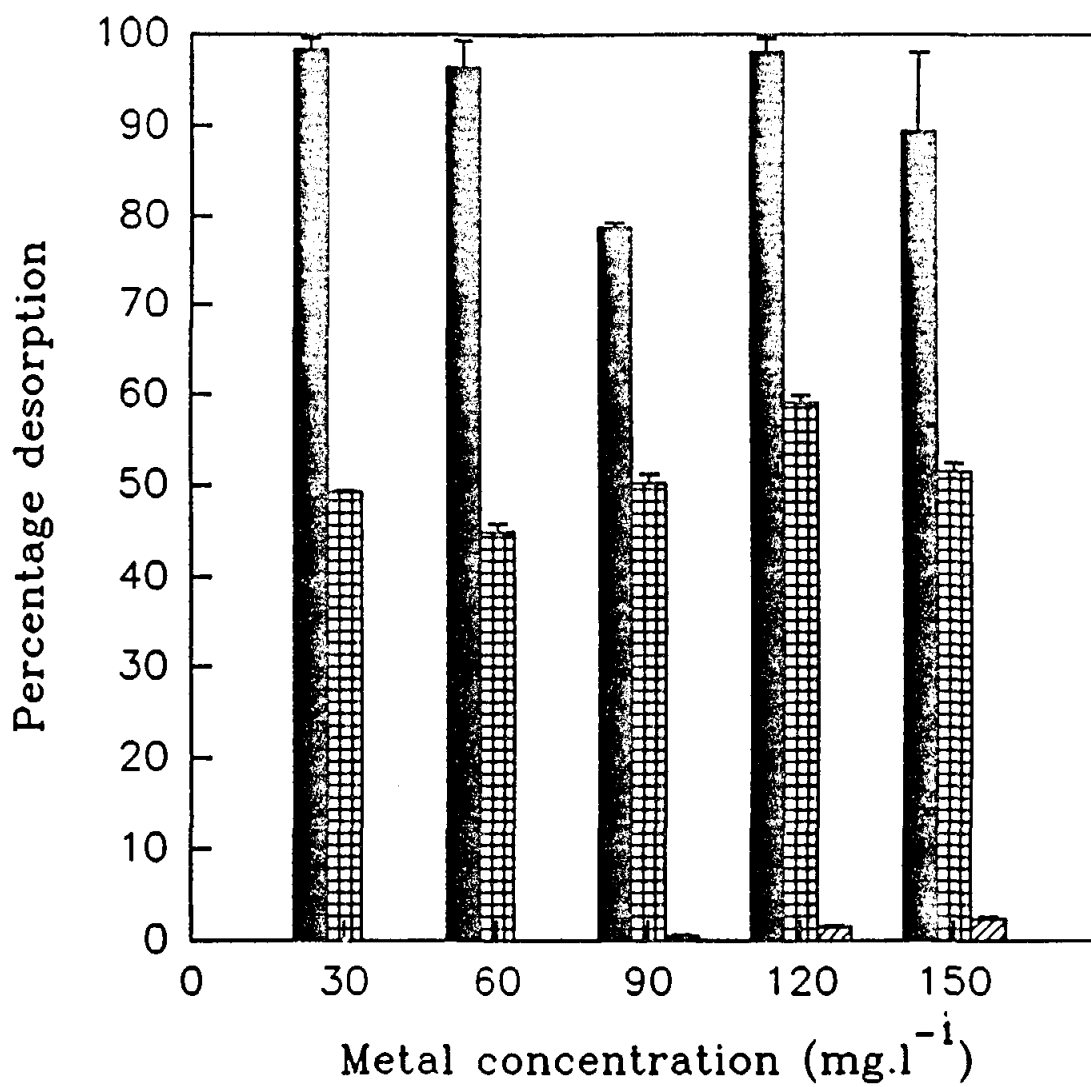


Fig. 5.18. Percentage desorption of Cu from sludge 3 by acetic acid (■), hydrochloric acid (▤) and deionised water (▨).

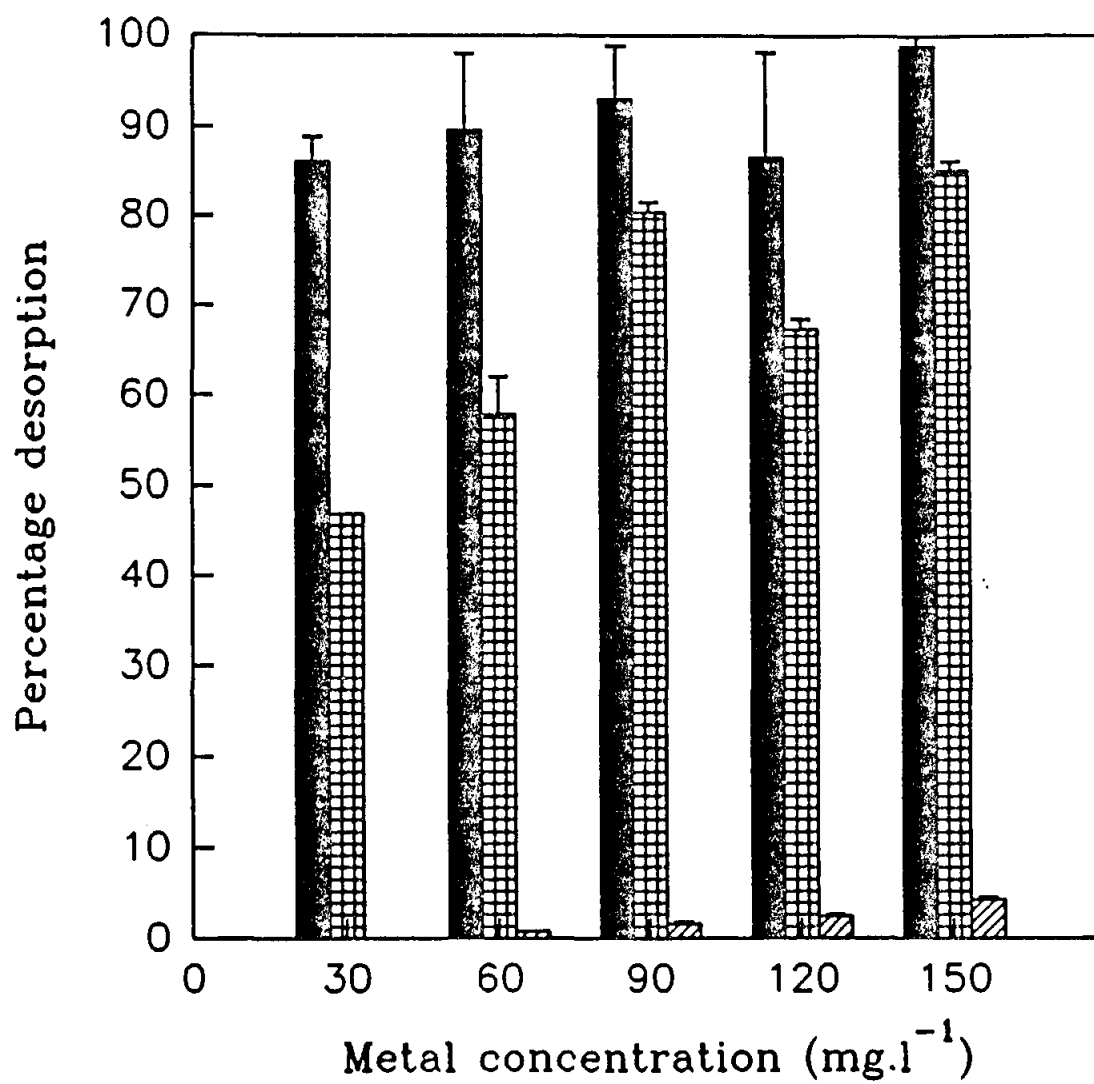


Fig. 5.19. Percentage desorption of Cu from sludge 4 by acetic acid (■), hydrochloric acid (▤) and deionised water (▨).

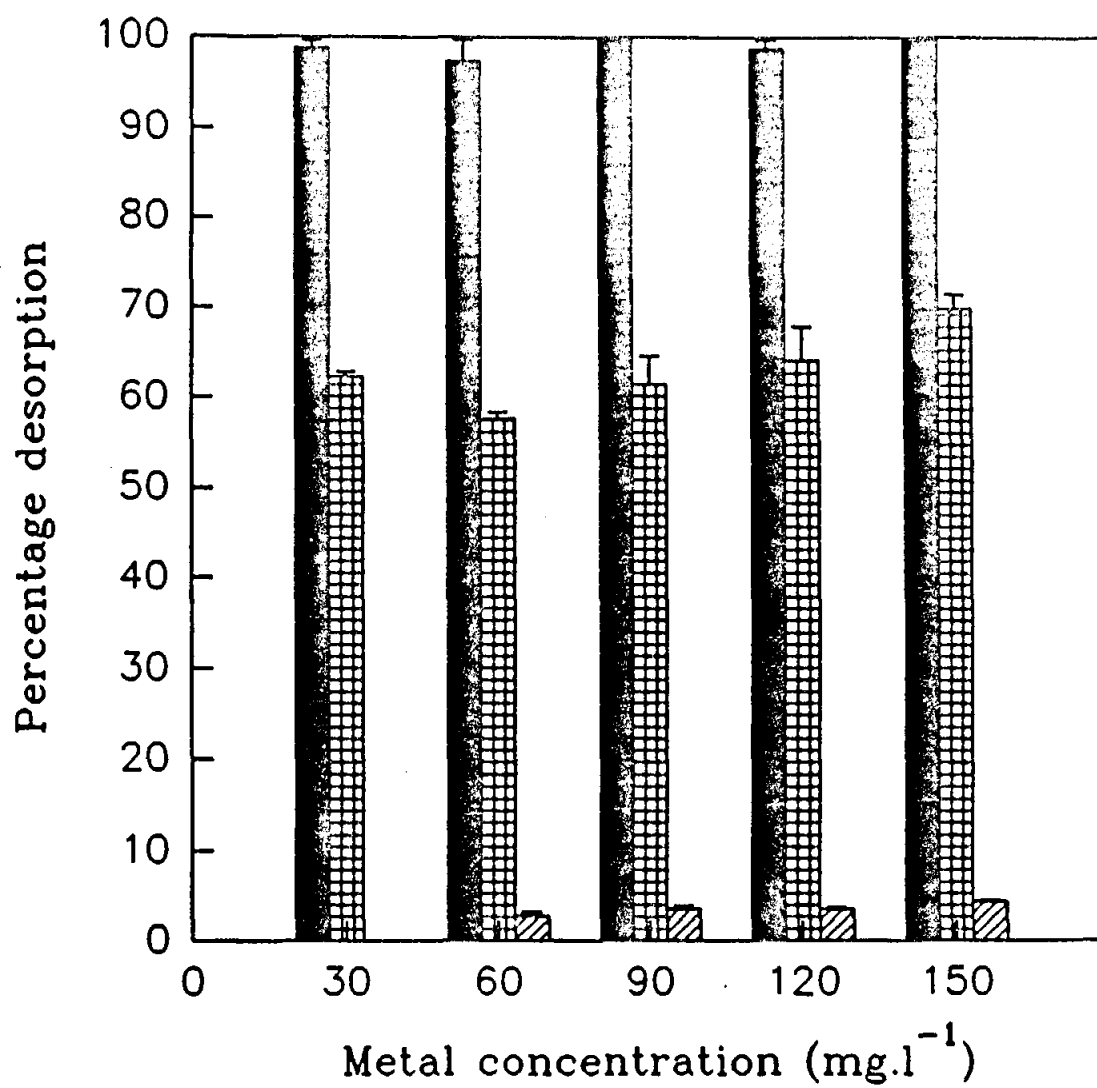


Fig. 5.20. Percentage desorption of Cu from sludge 9 by acetic acid (■), hydrochloric acid (▤) and deionised water (▨).

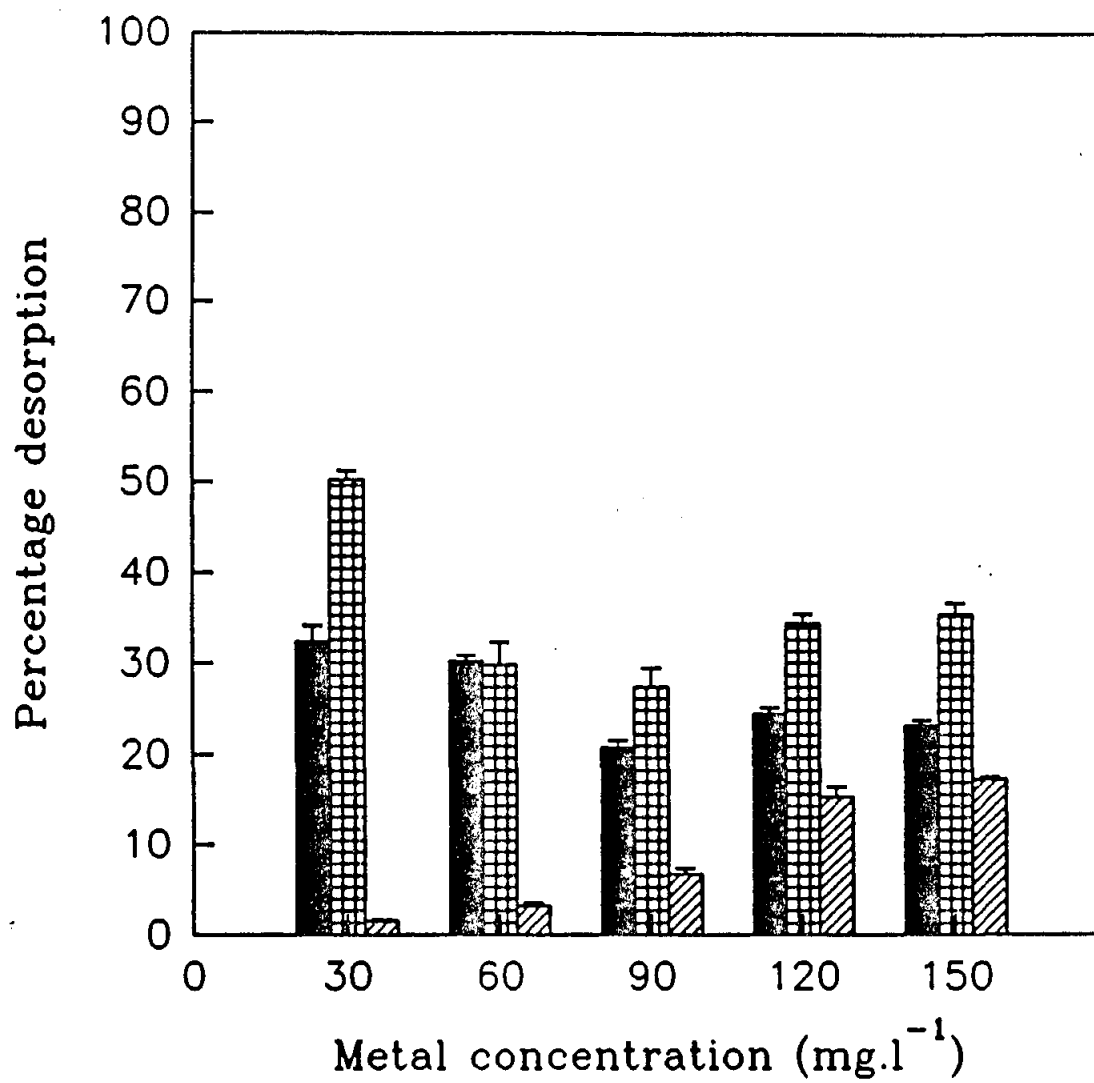


Fig. 5.21. Percentage desorption of Cr^{6+} from sludge 2 by acetic acid (■), hydrochloric acid (▤) and deionised water (▨).

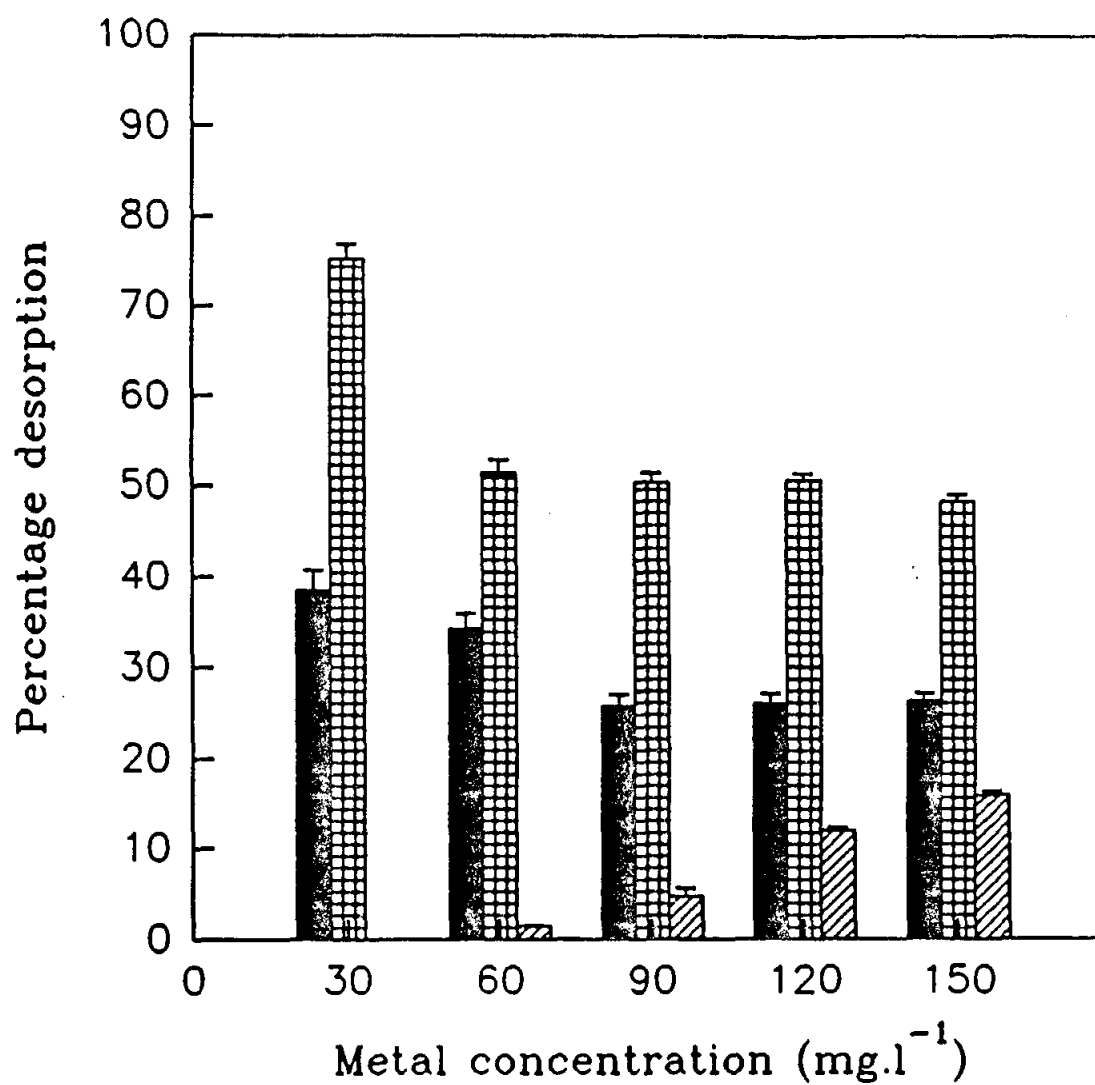


Fig. 5.22. Percentage desorption of Cr^{6+} from sludge 3 by acetic acid (■), hydrochloric acid (▨) and deionised water (▧).

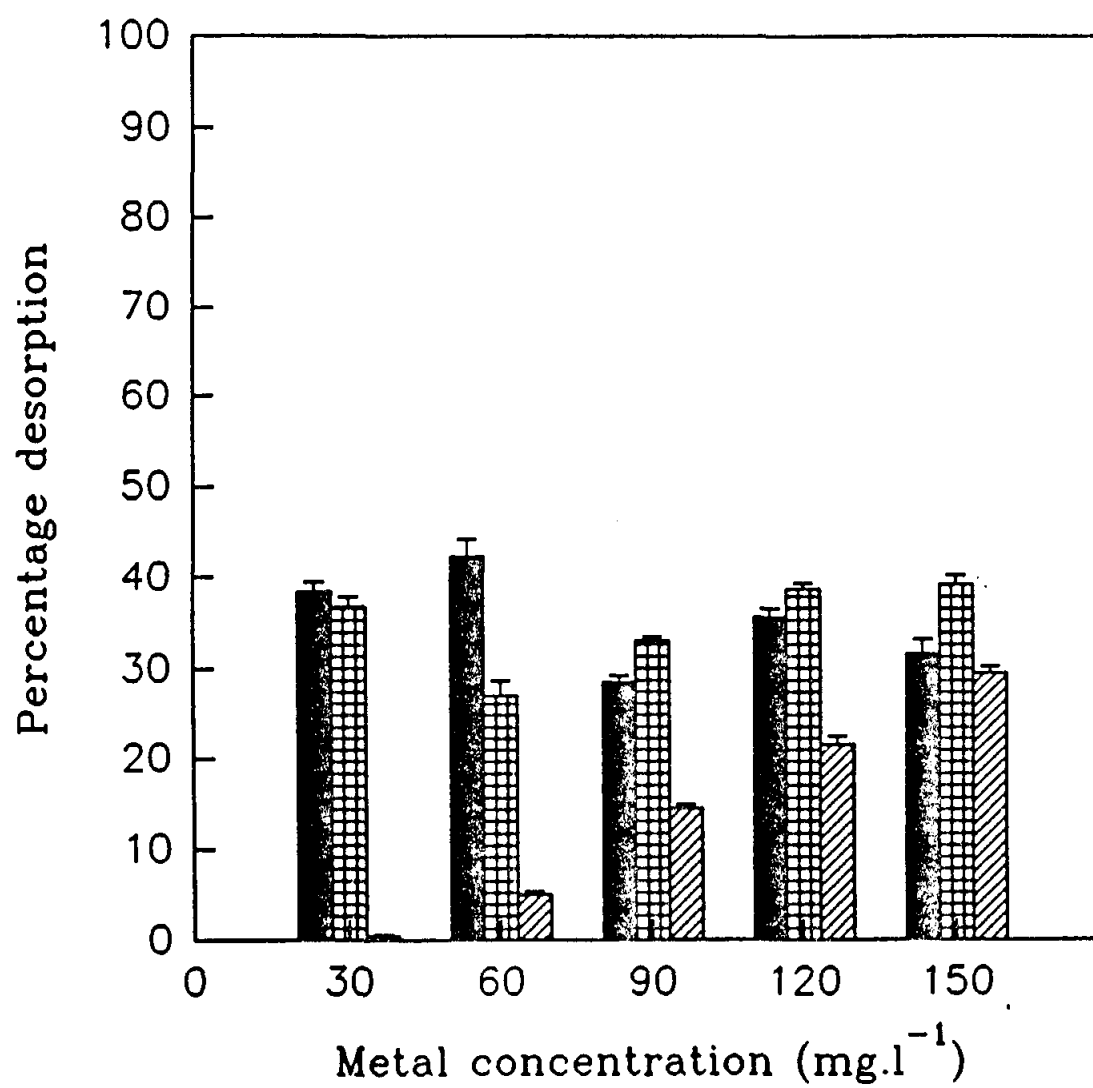


Fig. 5.23. Percentage desorption of Cr^{6+} from sludge 4 by acetic acid (■), hydrochloric acid (▤) and deionised water (▨).

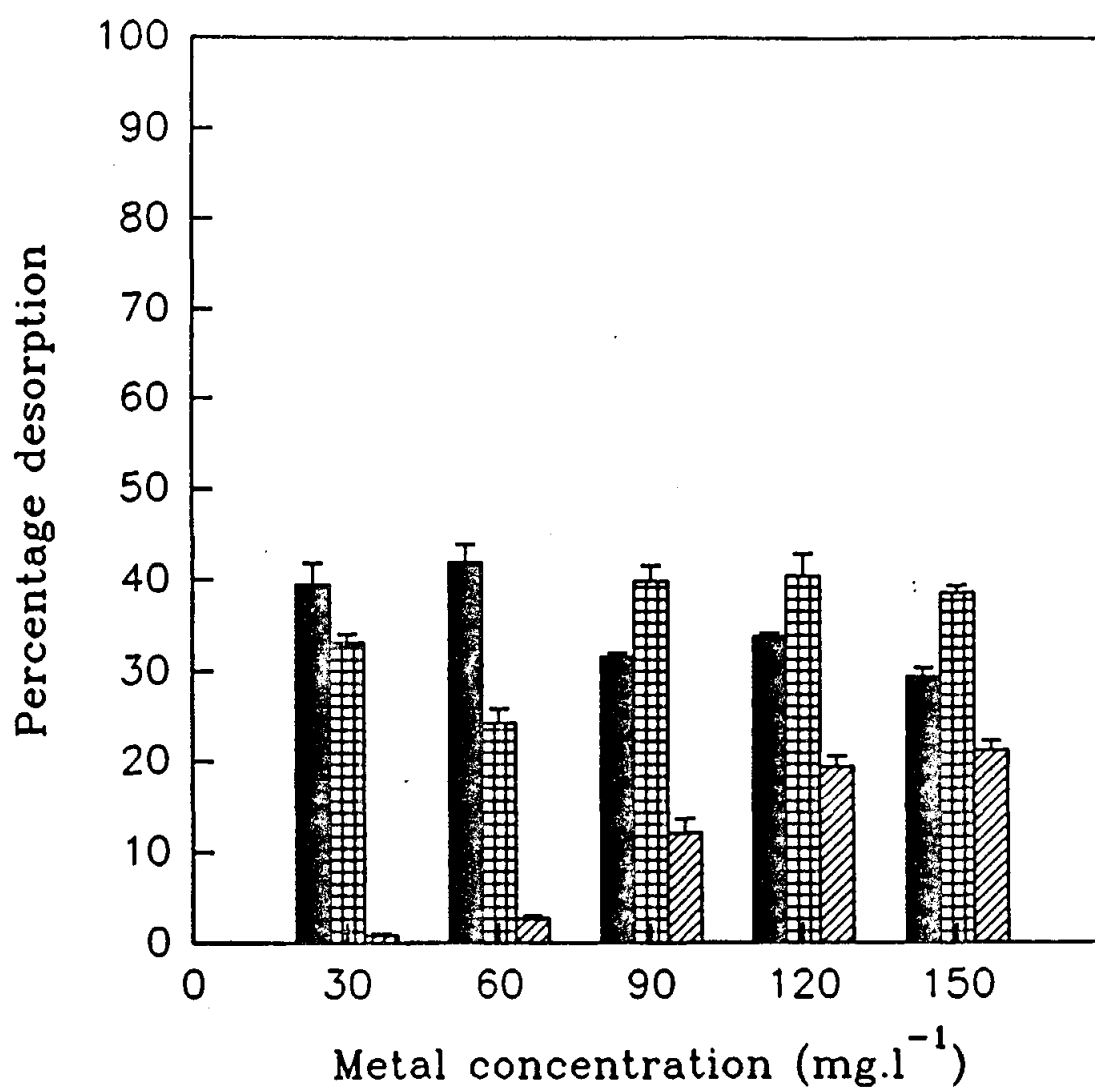


Fig. 5.24. Percentage desorption of Cr^{6+} from sludge 9 by acetic acid (■), hydrochloric acid (▤) and deionised water (▨).

6.0 DETERMINATION OF SLUDGE-SURFACE CHARGE

6.1 INTRODUCTION

Activated sludge biomass is composed of eubacteria, filamentous bacteria, fungi, yeast, algae and protozoa (Jenkins *et al.*, 1986; Bux *et al.*, 1992; 1993). Several studies have shown that the activated sludge treatment process is capable of reducing metal-ion concentrations from wastewater (Brown *et al.*, 1973; Chen *et al.*, 1974; Lester *et al.*, 1983; Fletcher and Beckett, 1987a). Many workers have attributed this finding to physico-chemical interactions between metal ions and sludge surfaces (Stoveland and Lester, 1980; Sterritt *et al.*, 1981; Fletcher and Beckett, 1987b; Lake *et al.*, 1989). Sludge surfaces are polymeric in nature comprising of protein, carbohydrate, nucleic acids and lipid (Goodwin and Forster, 1985).

Many of the extracellular polymers produced by microorganisms possess negatively charged inorganic groups such as carboxylic, aliphatic, aromatic, hydroxyl, sulphate and amino groups, which confer an overall negative charge to sludge floc surfaces. The quantity of bacterial extracellular polymers in activated sludge is controlled by the concentrations of various nutrients in the growth medium, sludge retention time and oxidation of polymers by other bacterial species present (Brown and Lester, 1979). The chemical reaction between an anionic bacterial surface (S^-) and a cationic metal ion (M^+) should ideally represent a simple electrostatic attraction: $M^+ + S^- \rightarrow S-M$. In reality, the chemistry of metal ions in aqueous solutions is much more complicated. In aqueous solution, metal ions are usually hydrated. In natural water systems, metal ions can be attracted to a number of dissolved, colloidal, or solid organic and inorganic substances. When the effects of pH, Eh interfacial

nature, and chemical attributes of the metal (heat of hydration, charge density, electronic shell, etc.), and presence of competing ions and other biological surfaces are also taken into account, we can begin to grasp the true complexity of bacterial metal binding (McLean and Beveridge, 1990). Tenneg and Verhoff (1973) reported that the electrophoretic mobility of activated sludge bacteria indicates an increase in surface charge negatively with increasing extracellular polymer concentration. Steiner, *et al.* (1976) have demonstrated that the ratio of glucuronic acid to carbohydrate in activated sludge has a more or less constant value of 1:6. Cellulase treatment of activated sludge was found to cause a steady release of sugars. Initially an increase in surface charge was monitored, but this began to decrease after 4h. The increase in charge was attributed to the production of hydroxyl groups by the cleavage of β -1,4-glucan linkages. The subsequent decrease was thought to be due to the release of 1,3- attached hexuronic acid molecules.

Much of the research conducted thus far on the magnitude of surface charge on biological matter such as sludge was restricted to its applications to sludge conditioning to increase particle size and facilitate solid/liquid separation, influence of the charge density of cationic polyelectrolytes on sludge conditioning and to determine the optimum coagulant dose in relation to the colloidal characteristics of the sludge (Tiravanti *et al.*, 1985). During the present investigation, the determination of sludge surface charge was conducted to assess differences in magnitude of surface charge in ten different sludges with the intention of substantiating their varying biosorptive potentials and thus using surface charge as an indicator of superior biosorbents. Recent findings have conclusively shown that waste activated sludges from a variety of sources are capable of biosorbing several metal ion species from solution (Swalaha and Kasan, 1992, Kasan 1993). Since the biosorption of

metal cations to sludges is also dependent on sludge surface charge.

6.1.1 Aims

- i) Establish whether the sludge surfaces possess a net negative charge and subsequently,
- ii) determine the magnitude of the charge and compare the relative surface charge of the ten sludges under investigation,
- iii) use three different methods, viz., streaming current, millivolt quantification and colloid titration technique in order to verify the results and moreover, to determine whether sludge electronegativity rankings are consistent by using all 3 methods, and
- iv) select a single, most applicable method to determine surface charge on sludge.

6.2 METHODOLOGY

Grab samples of return activated sludge were obtained from waste-water treatment plants in Natal, namely, Umlaas, S1, Amanzimtoti, S2, New Germany, S3, Hammarsdale, S4, Pietermaritzburg, S5, Kwa Mashu, S6, Tongaat, S7, Northern Works, S8, Southern Works, S9 and Phoenix, S10. Sludges were concentrated to 25 000 mg l^{-1} by centrifugation using a J6B Beckman centrifuge at 3 500 x g for 30 min., and pellets were resuspended in 100 ml deionised water in sealed bottles and stored at 4°C for further use. The electronegativity of the sludge particles was determined within 24 h of obtaining samples utilising different methods, i.e., streaming current method, use of a pH/millivolt meter and modification of a colloid titration method as described by Kawamura and Tanaka (1966).

6.2.1 Streaming Current Method

The movement of like charges is defined as an electrical current. This current is called the streaming current. The streaming current monitor utilises the principle of streaming current to obtain a measure of the colloidal charge and the values vary linearly with temperature. Streaming current is related to the zeta potential as follows:- (Smith and Somerset, 1971)

$$i \propto \frac{ZD}{N}$$

i = streaming current

Z = zeta potential

D = dielectric constant

N = viscosity of fluid

Sludge samples S1 - S10 were diluted to a final volume of 100 ml and standard concentration of 5 000 mg ℓ^{-1} using deionised water. The procedure involved using a Chemtrac Model 2 000 X R streaming current monitor (Floccotan, SA). The unit was initially flushed for approximately 15 min. with distilled water and subsequently standardised with deionised water. A 50 ml syringe was used to inject sludge samples into the boat of the monitor. Results were recorded when constant negative streaming current values were acquired. This procedure was conducted in triplicate. The instrument was rinsed with deionised water between each of the samples tested.

6.2.2 Millivolt Quantification Method

A Beckman 50 combination pH/millivolt meter was used. The meter was standardised using standard buffer solutions of pH 7 (Beckman), since the solution has no net charge. Sludges 1 - 10 were diluted to final volume of 100 ml with deionised water in conical flasks at a concentration of 5 000 mg ℓ^{-1} . The unit was operated at mV/Rel mV mode and tested with triple deionised water which produces no charge. Sludge samples were read in triplicate and the combination electrode rinsed with deionised water between each readings.

6.2.3 Modified Colloid Titration Method

Five ml of each of the sludge samples (S1 - S10) of 500 mg ℓ^{-1} concentrations were pipetted into 50 ml conical flask in triplicate. Three drops of 0,1 % of toluidine blue (TB) indicator was added to each of the flasks. Contents of the flasks were mixed using a magnetic stirrer. An aliquot of 3,5 ml of standard positive colloid i.e. 0,001N methyl glycolchitosan (MGC)

(Merck) was added and mixed for 10 s. This resulted in a blue colour. Excess positive colloid was then back - titrated with 0,01N potassium polyvinyl alcohol sulphate (PVSK) (Aldrich) i.e., standard negative colloid, using a 10 ml burette. The pH of PVSK was corrected to that of MGC with small additions of 0,1M NaOH. The colour change from blue to purple indicated by the addition of 2 to 3 drops of PVSK was the end point. If purple colouration remained for 30 s. to 2 min then end point was stably reached. In addition to the flask containing the sludge sample, a deionised water (5 ml) blank was treated in the same manner, with the rest of the additions to the blank being the same.

The colloidal charge of the sample was determined by the following calculation:-

(Kawamura and Tanaka, 1966)

$$\begin{aligned}\text{Colloidal Charge} &= [\text{sludge titration (ml)} - \text{blank titration (ml)}] \times 10^{-3} \\ &= \text{mEq } \ell^{-1}\end{aligned}$$

but for sludge samples it is preferable to express charge per gram of dried sludge solids :

$$\begin{aligned}\text{Charge of Sample} &= [\text{sludge titration (ml)} - \text{blank titration (ml)}] \times 2 \\ &= \text{mEq g}^{-1} \text{ suspended solids}\end{aligned}$$

since concentration of sludge used for colloid titration experiment was 500 mg ℓ^{-1}

6.3 RESULTS AND DISCUSSION

The charge present on the sludge surfaces under investigation is a function of the physico-chemical properties of the surfaces. The results showed that surface charge of the ten sludges using three different methods of detection were different. The significance of the different methods used during the present research was to compare the abilities of these techniques to assess surface charge and compare the results between ten sludges rather than to determine the absolute charge on individual sludge surfaces. The colloid titration technique is the conventional and most widely used method to detect surface charge of colloids (Kawamura and Tanaka, 1966; Tiravanti *et al*, 1985; Morgan, *et al.*, 1990). During the present study, this method was modified for application to sludges. The streaming current method for charge detection is utilised primarily by the flocculant manufacturing industry. Neither the latter method nor the millivolt technique have been reported to be used for detection of surface charge on sludges.

The surface charge determined by using the streaming current monitor were referred to as streaming current values (Table 6.1) (Smith and Somerset, 1971). Some sludges were found to be less electronegative than others, S9 was most electronegative and S7, least electronegative. The use of a pH/millivolt meter which was adapted to millivolt mode to detect surface charge (Table 6.2) showed S9 as most negative with S10 being the least negative sludge. It is important to note that when using this method, although conductivity is detected, it is reflective of the charge in the solution which is dictated by the charge on the sludge surface, since the suspension medium i.e., distilled water has no conductivity or net charge present. Therefore the pH meter in millivolt mode could be used to detect

differences in surface charge among sludges. The findings of the latter method partially substantiated the results of the conventional method of charge detection on sludge surface, i.e., colloid titration technique (Kawamura and Tanaka, 1966). The calculated surface charges of sludges as shown in Table 3 are expressed as milliequivalents per gram of suspended solids. Interestingly, S9 proved to be the most negatively charged sludge.

When comparing the three methods there was relative similarity in ranking of the high order sludges, viz., S9 and S6, whilst the middle and lower order sludges did not follow a similar pattern, displaying noticeable variations (Table 6.4). There was substantial difference in the magnitude of electronegativity of the high order sludge, i.e., S9 and the rest of the sludges (Table 6.1 - 6.3). This large difference in surface charge was detected by all three methods employed. The magnitude of difference in charges between the middle and lower order ranked sludges was small (Table 6.1 - 6.3). Therefore, the sensitivities of each of the techniques employed for charge detection influenced the ranking of these sludges. The three methods utilised to determine electronegativity of sludge biomass showed that all sludges tested produced an overall net negative charge. Several hypotheses have been postulated to explain this complex phenomenon (Forster, 1976) e.g., the chemical nature of the sludge surface influences the magnitude of the surface charge which subsequently affects the settlement properties of the sludge. The principle ionogenic component of sludge polysaccharide was shown to be glucuronic acid which, at neutral pH, contributes a strong negative charge, thus facilitating the polysaccharides to behave as a polyelectrolyte (Stumm and Morgan, 1962; Forster, 1971). Brown and Lester (1979) concluded that hexuronic acid is one of the main components contributing to the overall negative value of the zeta potential of activated sludge surfaces. Research conducted by Horan and Eccles (1986) showed that

the nature and concentration of the ionogenic materials present at sludge surfaces will determine the magnitude of the sludge surface charge. Morgan *et al* (1990) demonstrated that using the colloid titration technique, the surface charge for sludge solids from nine sources ranged from -0,110 to -0,907 m Eq g⁻¹ SS. In contrast to these findings, the results of the present study showed that using the same charge detection method, the charges on the sludges under investigation ranged from -12,52 to -29.86 m Eq g⁻¹ SS, thus displaying much greater electronegativity. This high degree of sludge electronegativity could be attributable to the nature of the individual sludge surfaces.

Overall, comparison of results showed that the titration and pH meter methods produced similar ranking patterns, indicating that these techniques share similar limits of detection. In addition, assessment of accuracy among the different methods used present the pH meter method to be superior, followed by the titration method due to minimal fluctuations in the percentage relative standard deviation (RSD) (Table 6.1 - 6.3). There are several other benefits associated with the use of the pH meter method, compared with the modified titration method viz., the technique is less time consuming, facilitates on-site field testing, can be more accurate and precise due to its simplicity and the absence of the need for colour indication. Both titration and pH meter method appear to be suitable to compare surface charge among sludges, although the latter method is more convenient.

6.4 CONCLUSIONS

All the sludges investigated demonstrated a net negative charge. There were noticeable differences in the magnitude of the surface charge of the ten sludges. An overview of the research using three different methods of charge detection presented the pH meter and titration methods showing similar ranking patterns. The method of choice for the present research application was the pH meter method.

Table 6.1. Electronegativity of ten waste activated sludges using the streaming current method.

Sludge	SCV	%RSD
1	-0.28	21.43
2	-0.36	5.56
3	-0.25	88.0
4	-0.17	29.41
5	-0.55	30.91
6	-0.42	11.90
7	-0.10	0.00
8	-0.22	18.18
9	-1.47	10.20
10	-0.43	0.00

SCV = Streaming Current Value
RSD = Relative Standard Deviation

Table 6.2. Electronegativity of ten waste activated sludges using the pH method.

Sludge	Millivolts	%RSD
1	-54.63	0.82
2	-64.70	0.54
3	-61.50	0.60
4	-63.90	1.71
5	-35.16	0.88
6	-68.80	0.71
7	-33.40	1.77
8	-29.87	0.13
9	-79.27	0.15
10	-19.37	0.46

RSD = Relative Standard Deviation

Table 6.3. Electronegativity of ten waste activated sludges using the titration method.

Sludge	PVSK (ml)	mEq g ⁻¹ SS	%RSD
1	10.56	-15.24	4.46194
2	08.80	-18.76	1.17271
3	09.18	-18.00	0.55556
4	09.67	-17.02	0.99882
5	11.92	-12.52	1.4377
6	07.19	-21.98	0.59145
7	11.34	-13.68	3.07018
8	07.26	-21.84	0.27473
9	03.25	-29.86	0.56932
10	10.61	-15.13	2.01586
B	18.18		

B = Blank

RSD = Relative Standard Deviation

Table 6.4. Ranking of ten waste activated sludges according to their electronegativities using three different methods.

RANKING	STREAMING CURRENT METHOD	pH METER METHOD	TITRATION METHOD
1	9	9	9
2	5	6	6
3	10	2	8
4	6	4	2
5	2	3	3
6	1	1	4
7	3	5	1
8	8	7	10
9	4	8	7
10	7	10	5

7.0 LABORATORY-SCALE OPTIMISATION OF A SLUDGE METAL ADSORPTION/DESORPTION PROCESS FROM SYNTHETIC WASTEWATER

7.1 INTRODUCTION

For adsorption of metal ions by biomass to be feasible, the biomass has to have a high uptake capacity and a high metal selectivity. Evaluation of biosorbents also has to consider desorption of metal ions (Volesky, 1987).

Several bioreactor configurations can be utilised to interact metal ions with biosorbent. The solid-liquid contact is very similar to those used in ion-exchange. Configurations used can range from batch, semi-continuous, to continuous-flow. These can be developed in any of the following process equipment:

Stirred tank contactors : The biosorbent is suspended in liquid containing metal and biosorbent laden with metal is recovered and metal eluted to regenerate the biosorbent for reuse.

Fixed packed bed contactors : The metal-bearing liquid percolates through a bed of active biosorbent. Equilibrium is not attained since the upper layers become saturated before the lower ones. Once all biomass is saturated, the bed becomes inactive and this is indicated by a sharp rise in metal concentration in the outlet, termed the 'breakthrough point'. The

saturated column has to be taken out of operation and the flow has to be stopped or re-routed to an active column. The saturated column has to be regenerated by eluting the bound metal from the surface of the biosorbent, usually by forcing low volumes of elutant through the column to keep the eluted metal in a concentrated state.

Packed bed contactors can be modified to operate as pulsating beds which are operated with upward flow of metal solutions. This allows periodic removal of biosorbent from the bottom and withdrawn biosorbent can be regenerated and inserted in a continuous manner. Fluidised beds are another modification where there is also an upward flow but at a higher rate to maintain the biosorbent in a fluidised state. Periodic or continuous removal of biosorbent along with metal solution allows this process to be operated continuously.

Some commercial processes have been developed utilising microbial biomass for recovery of metal ions. The most promising biosorbent has been developed by Brierley *et al.*, (1985) and is called AMT-BIOCLAIM. The authors claim that 99% of metals can be removed from 10 to 100 mg ℓ^{-1} metal solutions. In addition, approximately 86 mg silver, 213 mg cadmium, 152 mg copper, 600 mg lead and 137 mg zinc can be removed per gram of the biosorbent. The granules, can also be used in either fixed bed or fluidised bed reactor systems. These granules satisfy the requirements for high capacity and high selectivity.

Another process developed by Bennet and Jeffers (1990) utilises commercially available sphangum moss immobilised in polysulphone beads. This process carries the proprietary name, "BIO-BEADS" and is also called "Bio-Fix" which is an acronym for biomass-foam immobilised extractant. The beads were applied in a continuous three-column loading/elution

cycle to a zinc mining waste water containing 0.06 mg ℓ Cd, 6.0 mg ℓ Mn and 12.0 mg ℓ Zn. Over 99% of the metal was reported removed from the effluent. The subsequent eluate obtained by passaging dilute sulphuric acid through saturated columns allowed up to 100 times concentration of the metals compared to the initial effluent. Treated wastewater, further, complied with the U.S. National Drinking Water Standards. Ferguson and Jeffers (1991) applied the bio-fix beads to acidic mine waters and compared the recovery of metals to a conventional magnesium hydroxide precipitation. This was a large scale experiment with the beads packed in porous bags and lowered into tanks containing the mine wastewater. They obtained more than double the recovery efficiency with the process compared to the chemical precipitation of Al, Zn, Mn, Fe and Ca from the acidic mine wastewater.

7.1.1 Aims

The aims of this part of the study were to develop a batch scale adsorption-desorption process by:

- (i) Optimising adsorption parameters, and
- (ii) selecting a desorbing agent and optimising desorption parameters in a batch scale bioreactor.

7.2 METHODOLOGY

7.2.1 Batch Adsorption

The aim of this experiment was to determine optimum parameters for adsorption of metal-ions from a synthetic effluent in a batch tank reactor. Parameters optimised were, sludge (adsorbent) concentration, time of exposure and agitation rate.

Experiments were conducted in 40ℓ bioreactors. Thirty litre volumes of effluent composing 50 mg ℓ⁻¹ of each of the following metals : Zn, Cd, Cu, Ni, Cr(III) and Cr(VI), were decanted into each of five bioreactors. Sludge concentrations were 5 000, 10 000, 15 000, 20 000 and 25 000 mg ℓ⁻¹. Each bioreactor was exposed to one concentration of sludge. Immediately upon addition, triplicate 20 ml samples were extracted, as the 0 min., sample. Agitation was commenced and the rate optimised visually, optimum agitation being the presence of no non-agitated areas or 'dead spots' in the bioreactor. Optimum agitation was determined to be approximately 300±2% rpm. Samples were extracted at 15 min., intervals for the next one hour. Thereafter, samples were extracted at 30 min., intervals for the following three hours. The total time for the experiment was four hours. Samples were stored overnight at 4°C and metal content of each sample was determined by atomic absorption spectrophotometry.

7.2.2 Batch Desorption

Desorption was attempted on sludges which were already saturated with metal-ions for 90 min. from a synthetic effluent consisting of 50 mg ℓ^{-1} of the six metals used in this study. Desorbing agents were chosen by reference to previous research (Tsezos, 1984; Tsezos et al., 1989)

Two acids used as desorbing agents in vial experiments were again employed as desorbing agents. The two were, 2.5% acetic acid and 0.2N hydrochloric acid, individually. In addition, these two agents were used in combination at the concentrations listed and together at twice the individual concentrations i.e., 5% acetic acid and 0.4N hydrochloric acid. therefore, four desorbing solutions were used. Sludge at 25 000 mg ℓ^{-1} was exposed to 30 ℓ of synthetic effluent containing metal-ions in a bioreactor for 90 min. with agitation at 600 rpm. Effluent was drained after the exposure period and sludges were exposed to 10 ℓ of desorbing agent over a time period of 240 min. agitating at 300 rpm. Samples were withdrawn at 0 min., and 15 min., intervals for the first hour. Thereafter 30 min., samples were taken until 240 min. Controls comprised of desorbing agents exposed to unsaturated sludge with sampling times duplicated. All samples were taken in triplicate.

Optimum desorbing agent from the four above and desorption time were determined and applied to experiments to determine the effects of desorbing agents on sludges. Both acetic and hydrochloric acids were used in this experiment at the concentrations of 2.5% and 0.2N respectively. Sludges were saturated with metal-ions from a synthetic effluent for 90 min., and effluent was drained. Sludges were exposed to the two desorbing agents for 15 min. and

these were drained. Samples were taken in triplicate at 0 and 90 min. for adsorption and 0 and 15 min. for desorption. The adsorption-desorption process was repeated twice. Controls comprised of sludges desorbed by the agents after which two cycles of adsorption and desorption followed. These served to determine whether pre-stripping of the sludge with the desorbing agent enhanced adsorptive capability.

Other types of desorbing agents were also assessed for their desorptive capabilities. Calcium chloride at a concentration of 5mM and sodium bicarbonate at a 1M concentration were used as desorbing agents on pre-saturated sludges. Methodology was similar to that in the case of mineral acids (refer to section). Times for desorption were 480 min., for calcium chloride and 360 min., for sodium bicarbonate (Tsezos, 1984). Controls comprised unsaturated sludges exposed to desorbing agents.

7.3 RESULTS AND DISCUSSION

7.3.1 Adsorption

Exposure of all metals to all sludge concentrations showed that maximum adsorption occurred during a period of one hour and any further incubation time increased adsorption only minimally. This can be observed in the steep adsorption curves from 0 min., to 60 min., and the tapering-off of adsorption after this time (Figs 7.1 - 7.12).

Quantities of zinc adsorbed were erratic (Fig 7.1) showing no particular trend with respect to quantity of sludge and its adsorptive capability. However, efficiency of adsorption of Zn by concentrations of sludges showed that as concentration of sludge increased, there was a concomitant rise in efficiency of adsorption (Fig. 7.2). This indicates that there are finite number of sites on the surface of sludges for adsorption of zinc and an increase in the quantity of sludge leads to a proportional increase in the number of sites for adsorption.

Quantities and percentages of adsorption of cadmium showed clear trends of increasing adsorption with increasing sludge concentration (Fig. 7.3 and 7.4). The trend was repeated for copper and nickel (Figs 7.5 - 7.8). However, for Cu adsorption at 15 000 mg ℓ^{-1} of sludge, after 150 min., quantities adsorbed were less than those adsorbed by 10 000 mg ℓ^{-1} sludge (Fig. 7.5). This is possibly attributable to experimental error since no reason for this apparent decrease can be forwarded. Nickel adsorption by sludges at concentrations of 10 000 and 15 000 mg ℓ^{-1} increased till 60 min., after which they decreased (Figs 7.7 and 7.8). This can be attributable to the bond between Ni and sludge particles being weak, and

thus with extended agitation, some Ni is steadily dislodged. However, this trend is not observable in the two higher concentrations of sludge used. This could then lend credence to the theory that sludge particles were sheared at lower concentrations on extended agitation, releasing Ni from its bound form. However, since this trend is not clearly repeated for the other metals, this trend seems unique. Perhaps the outer layers of sludge particles are more actively involved in adsorption of Ni and it is these layers which are sloughed off in the reactor environment.

Adsorption of Cr^{3+} increased slightly, initially, but decreased to zero for all sludge concentrations (Figs 7.9 and 7.10) while adsorption of Cr^{6+} was higher compared to its Cr^{3+} counterpart (Figs 7.11 and 7.12). This indicates that in a fully mixed tank system, the higher valence chromium species is more efficiently adsorbed than the lower one. Also, for Cr^{6+} adsorption, sludge at 20 000 $\text{mg } \ell^{-1}$ adsorbed the metal more efficiently than sludge at 25 000 $\text{mg } \ell^{-1}$. This was contrary to the trends observed for the other sludges.

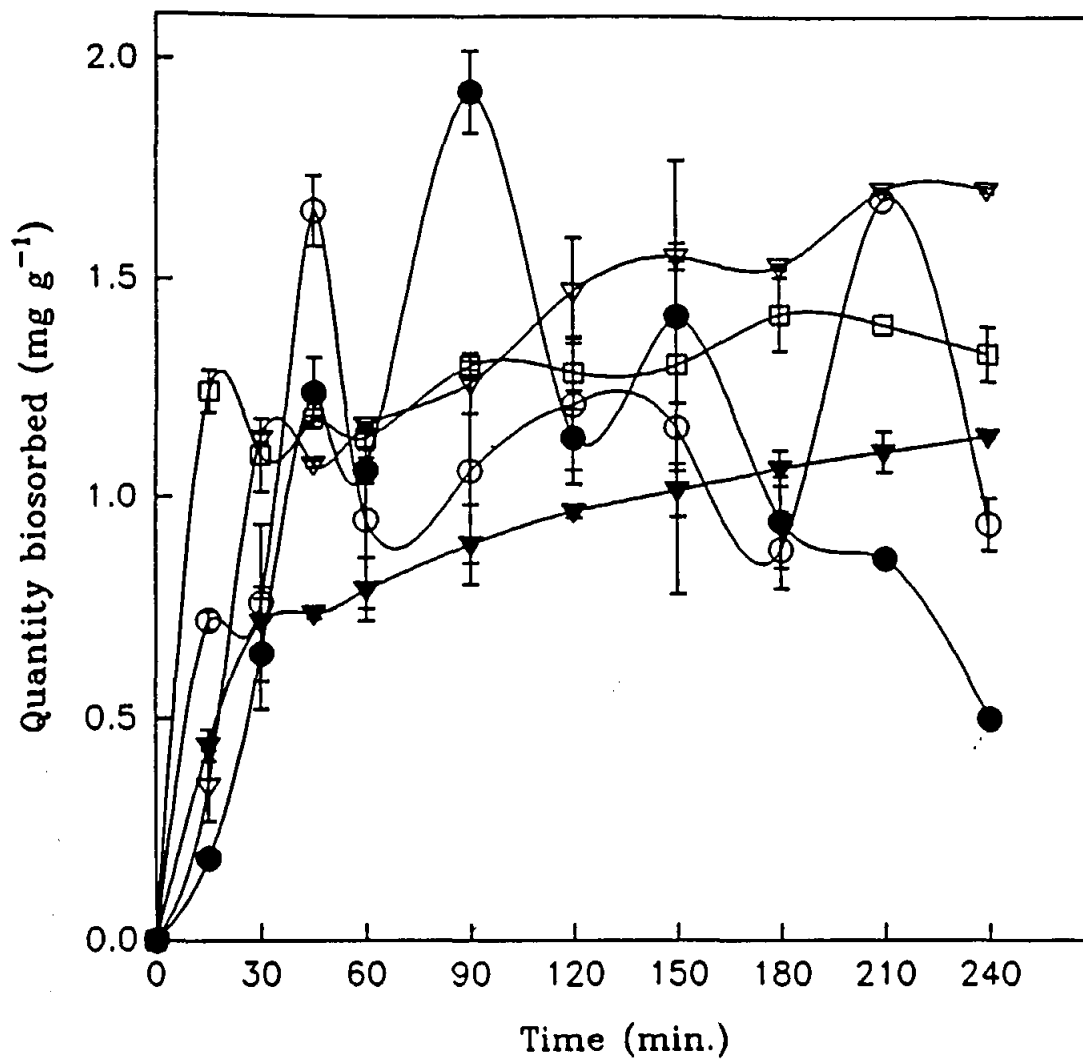


Fig. 7.1. Quantity of Zn biosorbed from synthetic effluent by sludges at concentrations of 5 000 (○), 10 000 (●), 15 000 (▽), 20 000 (▼) and 25 000 (□) mg l⁻¹.

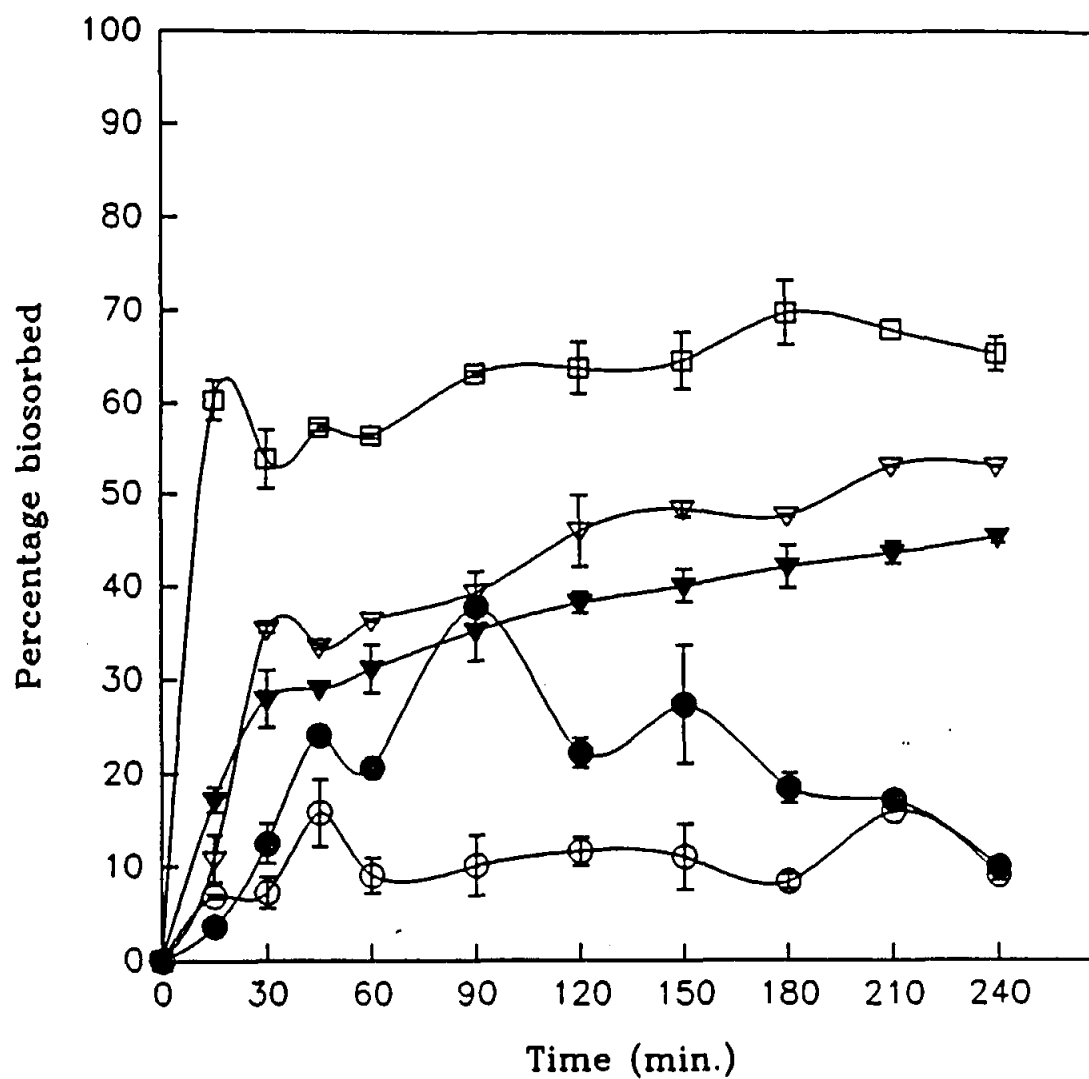


Fig. 7.2. Percentage Zn biosorbed from synthetic effluent by sludges at concentrations of 5 000 (○), 10 000 (●), 15 000 (▽), 20 000 (▼) and 25 000 (□) mg l^{-1} .

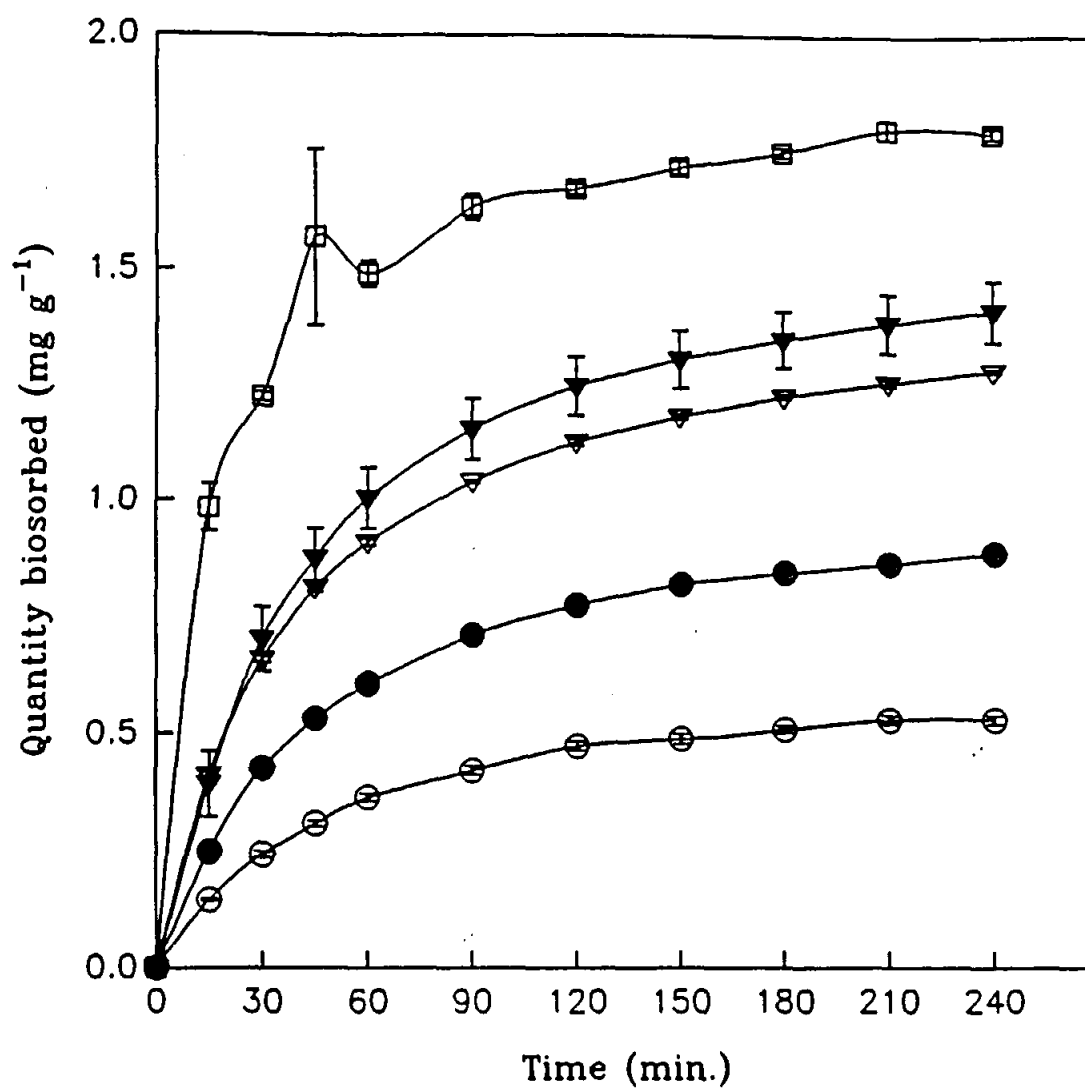


Fig. 7.3. Quantity of Cd biosorbed from synthetic effluent by sludges at concentrations of 5 000 (○), 10 000 (●), 15 000 (▽), 20 000 (▼) and 25 000 (□) mg l⁻¹.

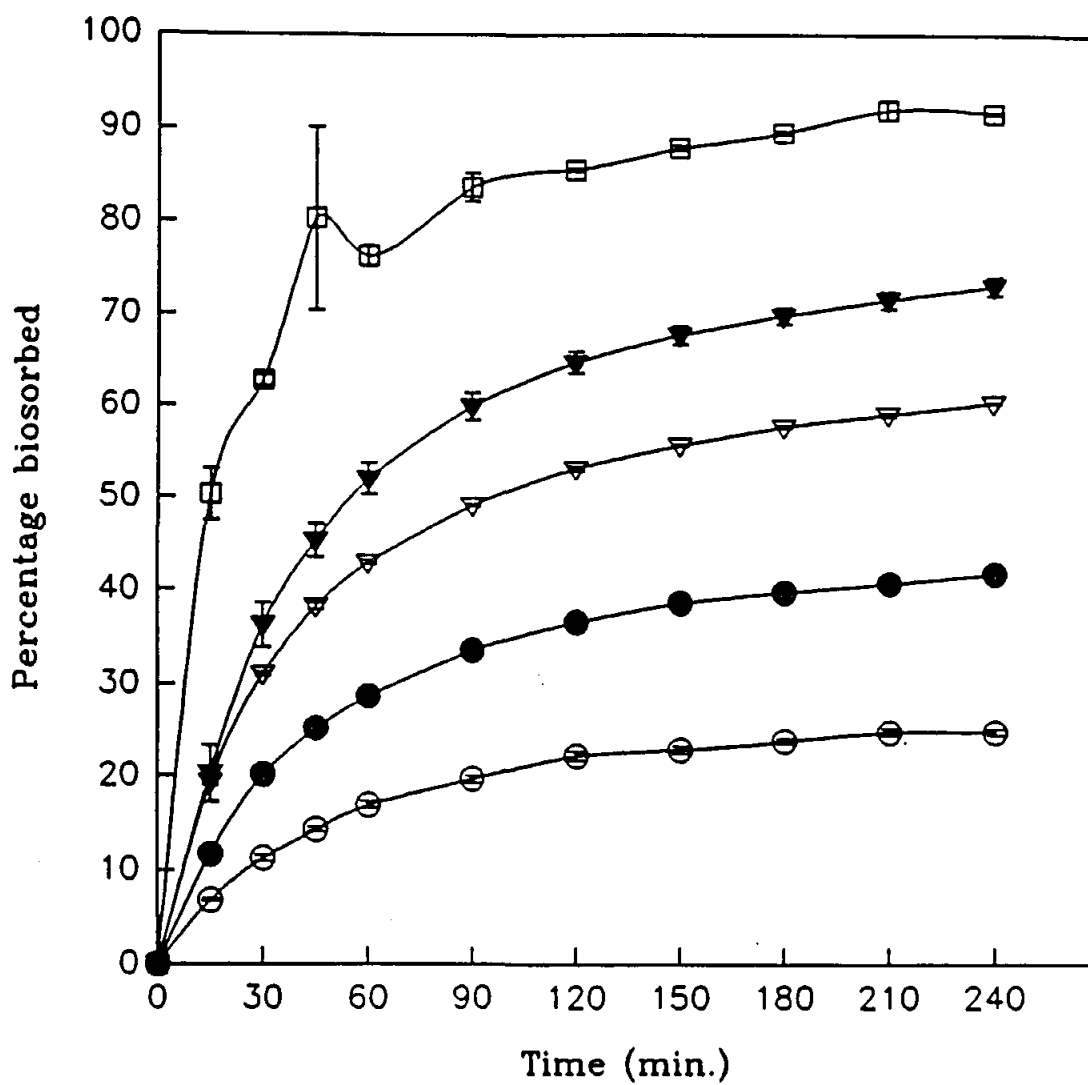


Fig. 7.4. Percentage Cd biosorbed from synthetic effluent by sludges at concentrations of 5 000 (○), 10 000 (●), 15 000 (▽), 20 000 (▼) and 25 000 (□) mg l⁻¹.

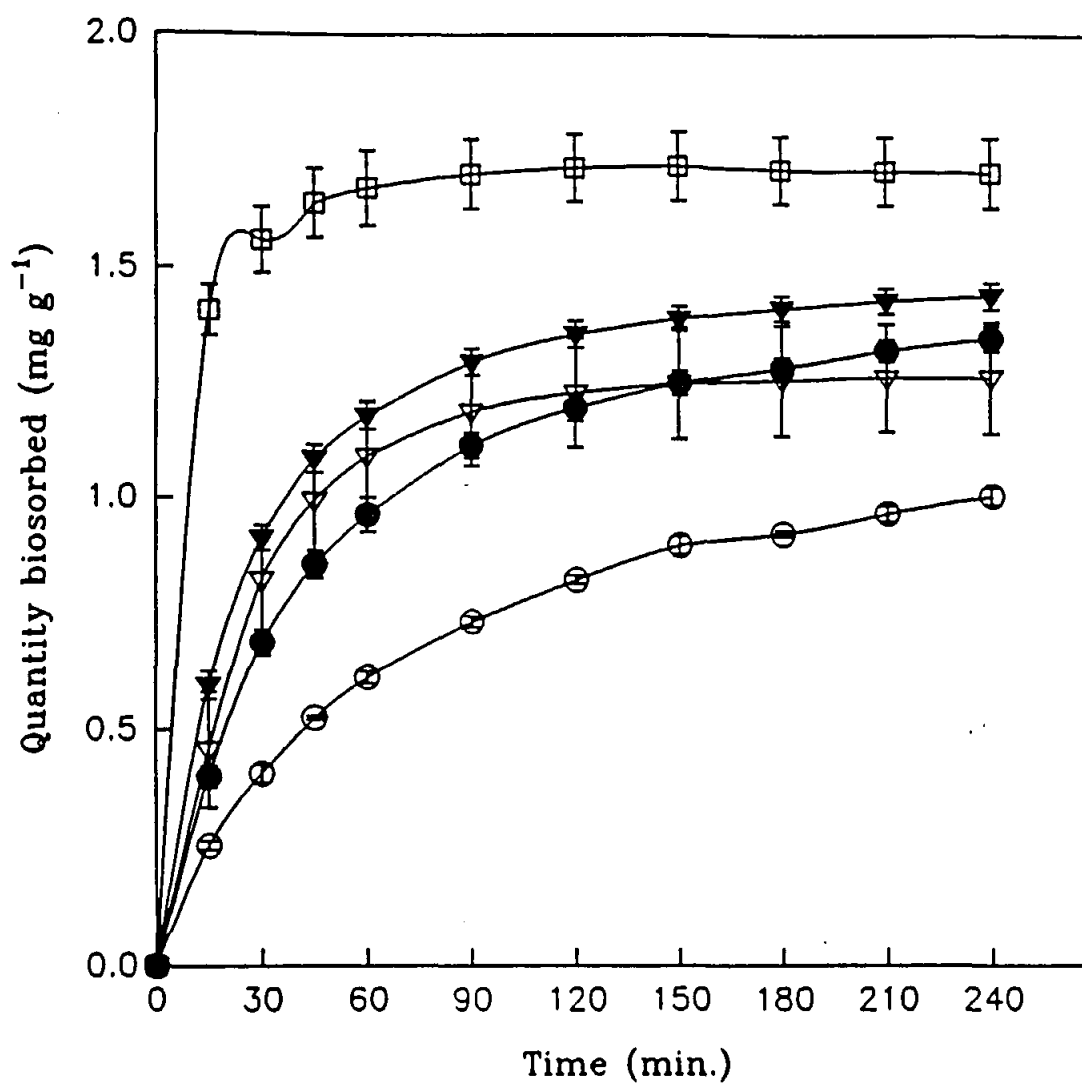


Fig. 7.5. Quantity of Cu biosorbed from synthetic effluent by sludges at concentrations of 5 000 (○), 10 000 (●), 15 000 (▽), 20 000 (▼) and 25 000 (□) mg l⁻¹.

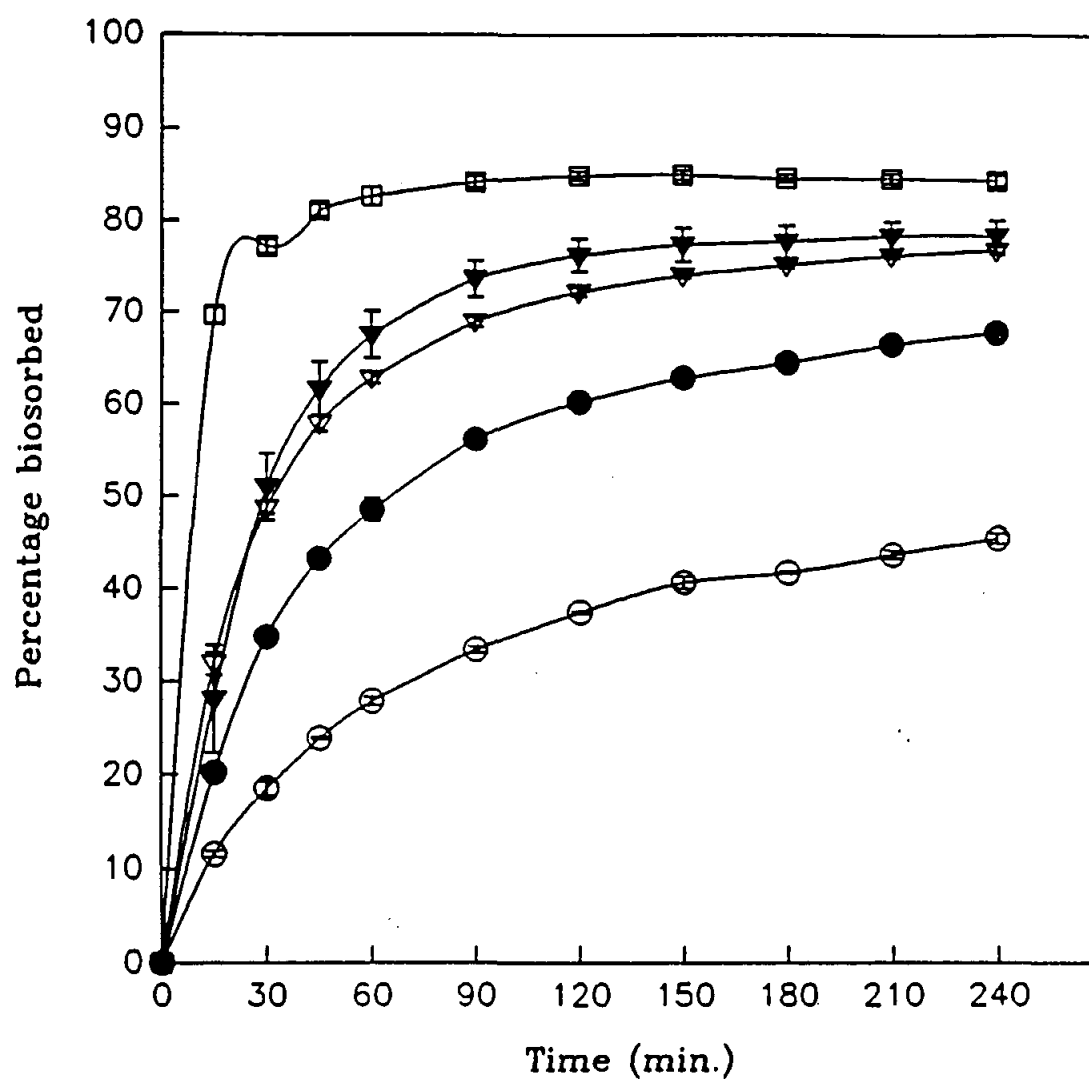


Fig. 7.6. Percentage Cu biosorbed from synthetic effluent by sludges at concentrations of 5 000 (○), 10 000 (●), 15 000 (▽), 20 000 (▼) and 25 000 (□) mg l^{-1} .

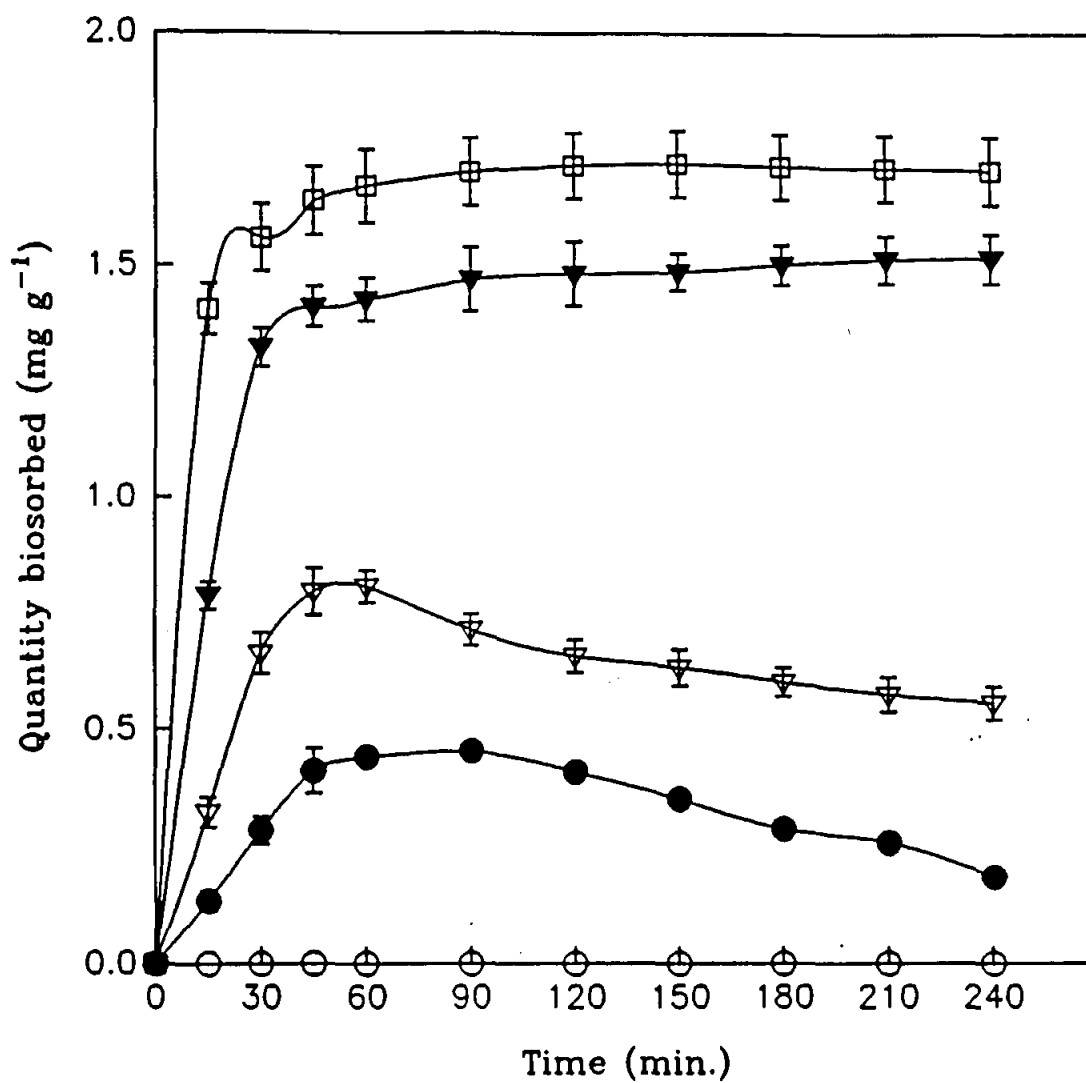


Fig. 7.7. Quantity of Ni biosorbed from synthetic effluent by sludges at concentrations of 5 000 (○), 10 000 (●), 15 000 (▽), 20 000 (▼) and 25 000 (□) mg l⁻¹.

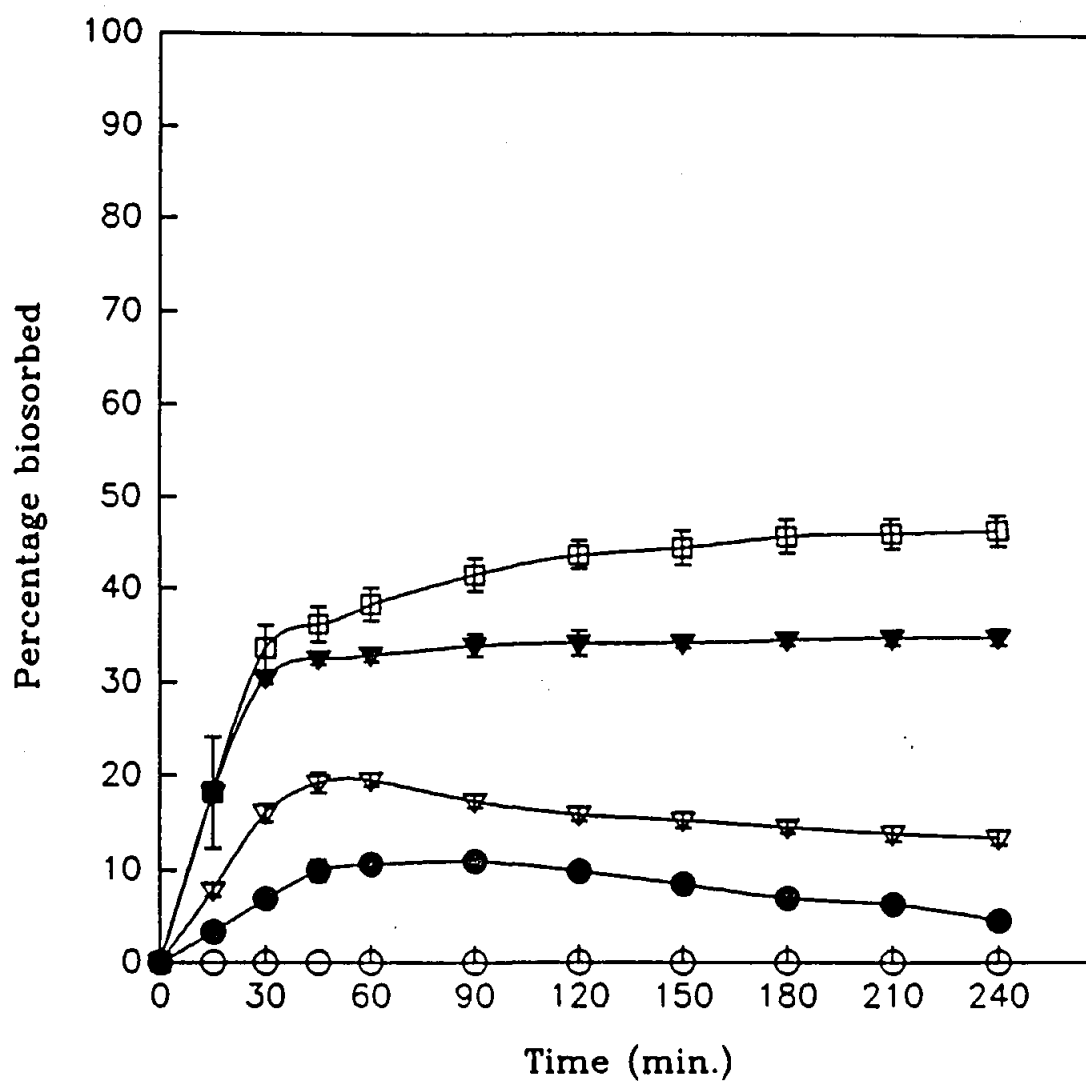


Fig. 7.8. Percentage Ni biosorbed from synthetic effluent by sludges at concentrations of 5 000 (○), 10 000 (●), 15 000 (▽), 20 000 (▼) and 25 000 (□) mg l⁻¹.

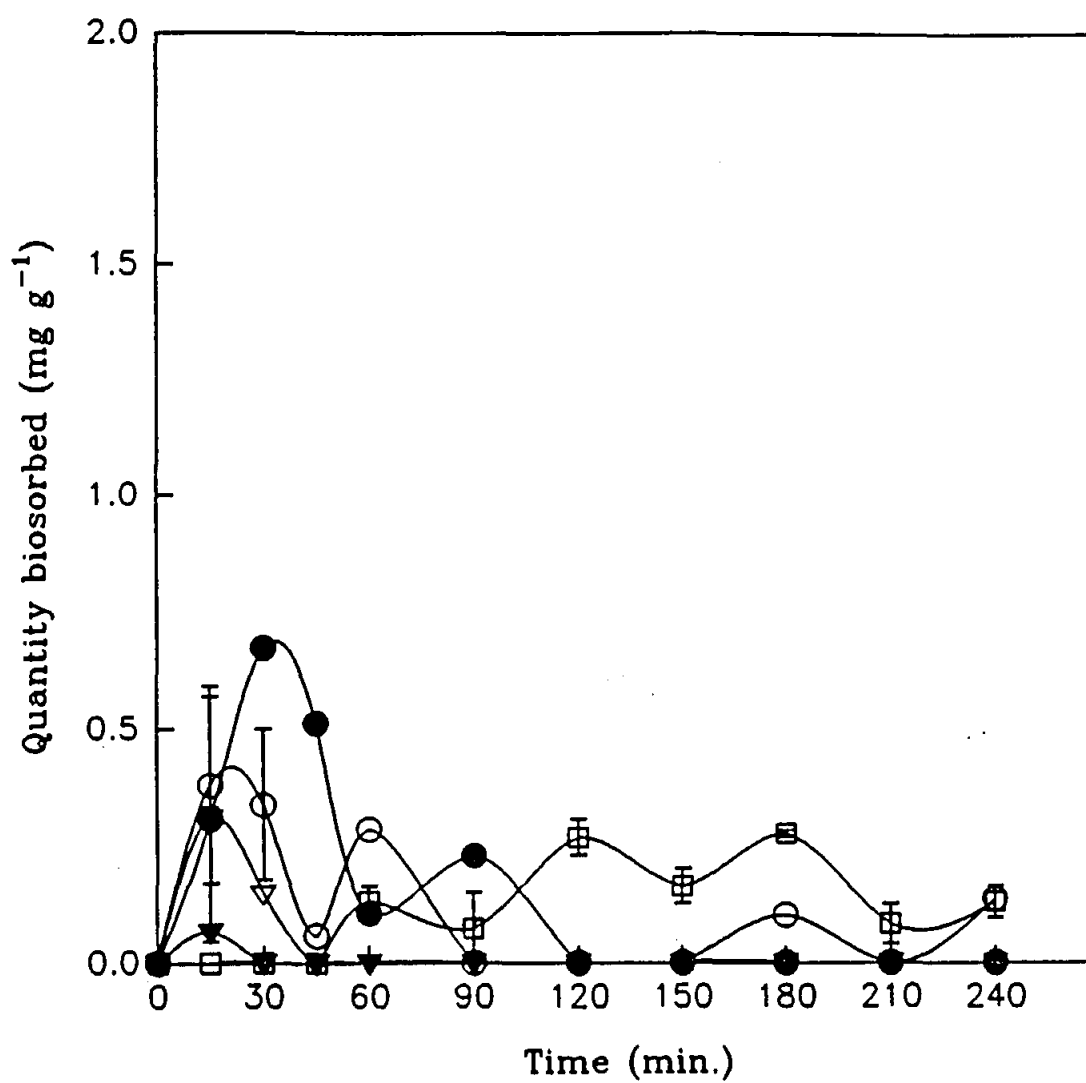


Fig. 7.9. Quantity of Cr^{3+} biosorbed from synthetic effluent by sludges at concentrations of 5 000 (\circ), 10 000 (\bullet), 15 000 (∇), 20 000 (\blacktriangledown) and 25 000 (\square) mg l^{-1} .

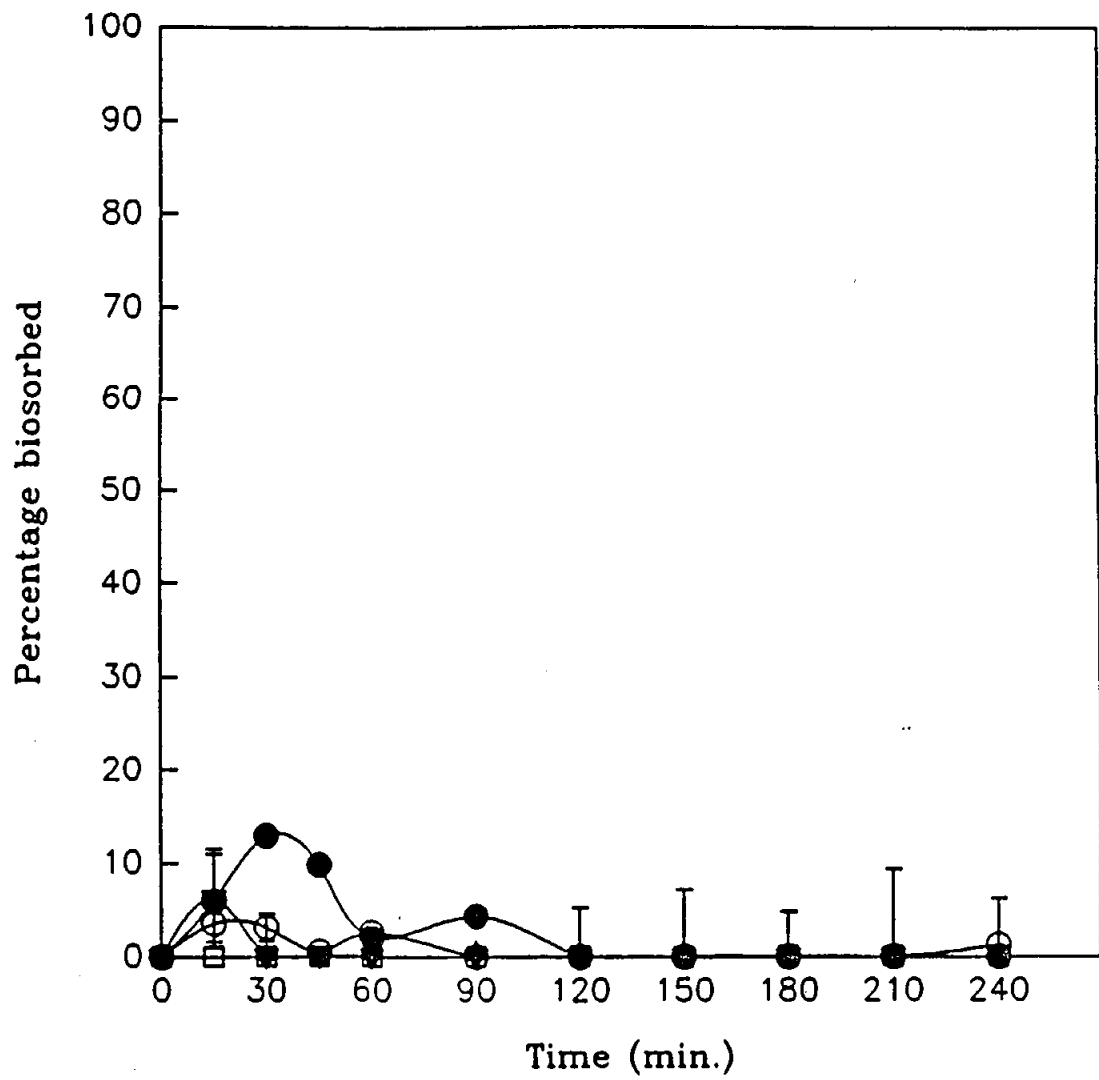


Fig. 7.10. Percentage Cr^{3+} biosorbed from synthetic effluent by sludges at concentrations of 5 000 (\circ), 10 000 (\bullet), 15 000 (∇), 20 000 (\blacktriangledown) and 25 000 (\square) mg l^{-1} .

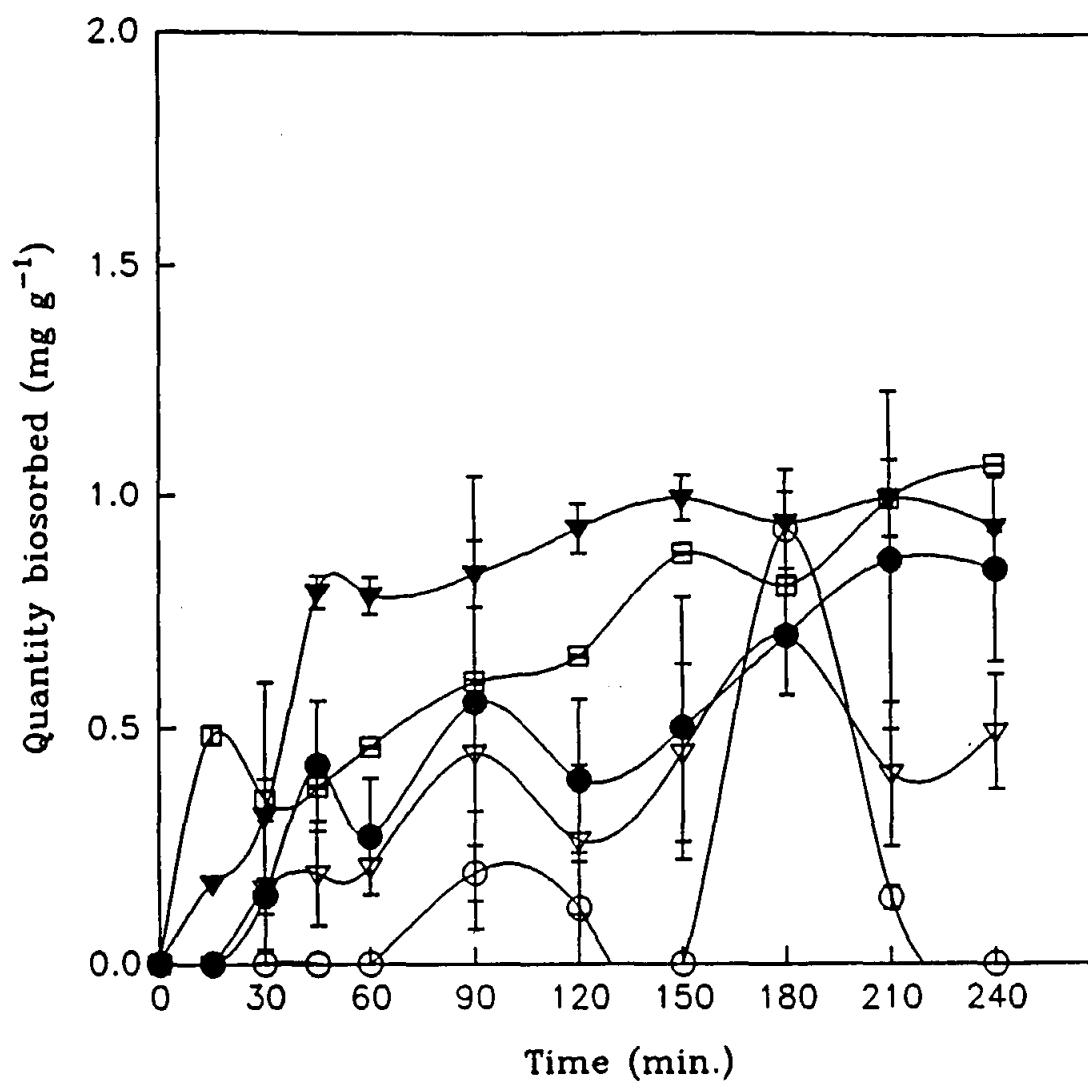


Fig. 7.11. Quantity of Cr^{6+} biosorbed from synthetic effluent by sludges at concentrations of 5 000 (\circ), 10 000 (\bullet), 15 000 (∇), 20 000 (\blacktriangledown) and 25 000 (\square) mg l^{-1} .

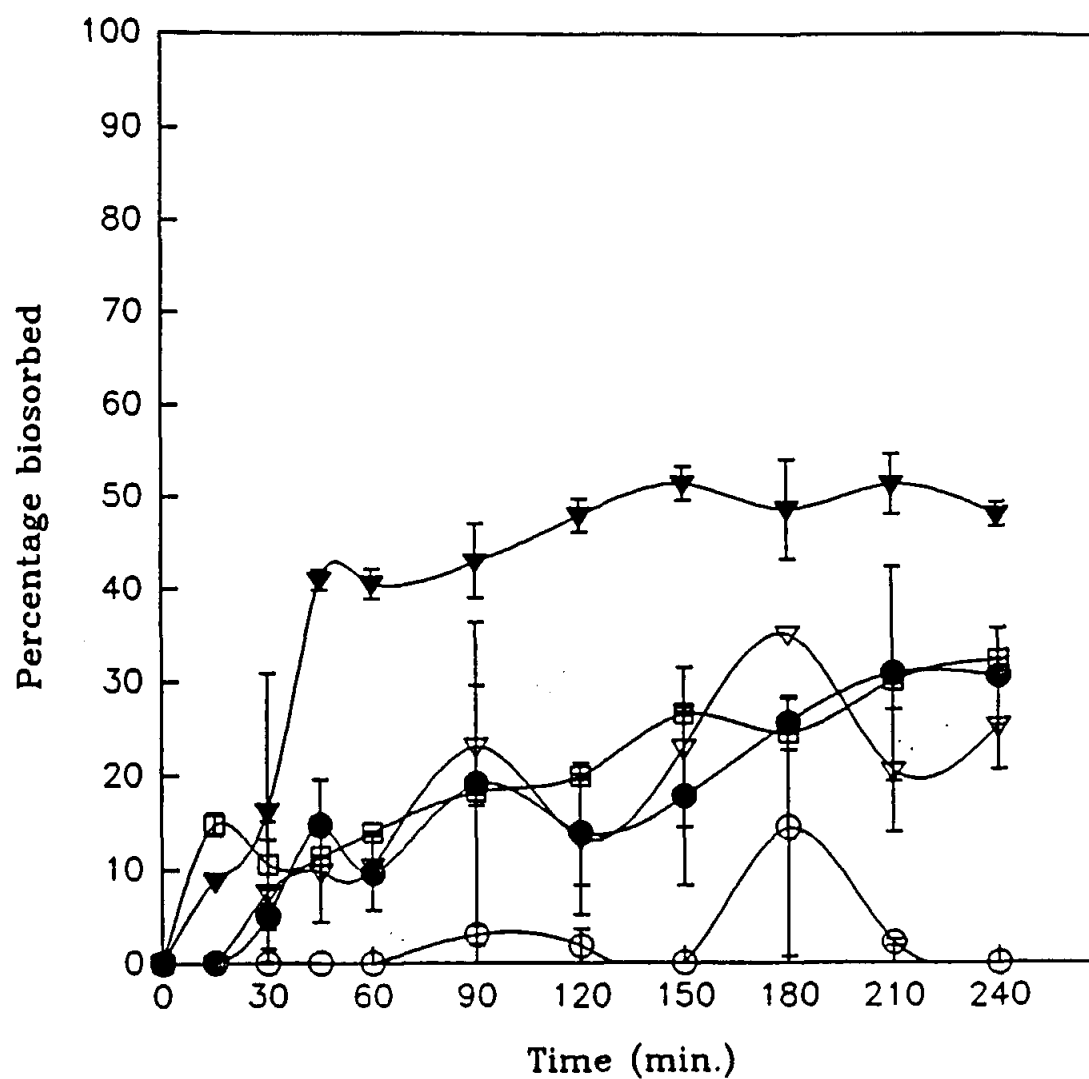


Fig. 7.12. Percentage Cr^{6+} biosorbed from synthetic effluent by sludges at concentrations of 5 000 (○), 10 000 (●), 15 000 (▽), 20 000 (▼) and 25 000 (□) mg l^{-1} .

7.3.2 Desorption

Results of the four desorbing agents exposed to sludges showed similar trends for desorption of zinc, cadmium and nickel (Figs 7.13, 7.14 and 7.16). All four agents were capable of completely desorbing the above three metals within 15 min. The graphs show profiles similar in nature for all four desorbing agents. Therefore, points are represented with the numeral 4 to denote that four points are at the same site. The mineral acids did not show a preference among these three metals for desorption whereas in batch experiments in vials it was shown that zinc, cadmium and chromium III were better desorbed by hydrochloric acids and copper and nickel were better desorbed by acetic acid. The turbulent environment of the bioreactor along with extended agitation could contribute to the non-specificity of the desorbing agents allowing complete contact of sludge with acid which was probably not possible in the vial experiments.

Complete copper desorption was obtained by all but the mixture of desorbing agents at 1x concentration (Fig. 7.15). In the latter case, desorption was rapid initially, tailing-off at approximately 60 min. and increasing linearly but slowly with time. However, 100% desorption was not attained in the 240 minute time period of the experiment. This slower rate of desorption clearly shows that a mixture of the two agents is not feasible although both individually are capable of complete desorption. Desorption by 2.5% acetic acid also decreased after approximately 60 min. desorption. This could be attributable to the surfaces of the sludges being sheared and damaged, releasing Cu into solution.

There was no chromium (III) desorption by all four desorbing agents which was contrary to

previous experiments conducted out with two of the four agents used viz., 2.5% acetic and 0.2N hydrochloric acids. Hydrochloric acid was previously capable of removing a substantial percentage of bound Cr^{3+} while acetic acid also desorbed a large amount of the bound metal. Chromium (VI) was also not desorbed by all four agents with acetic acid at 2.5% being the only successful desorbent (Fig. 7.17). Previous experiments indicated that both acetic and hydrochloric acids at the concentrations used were capable of removing bound chromium (VI) from sludges. In this instance it is not known why the mixtures of acids were incapable of desorbing Cr^{6+} with any efficiency.

Since complete desorption was obtained by all desorbing agents for all but the chromium species, the two individual desorbents viz., 2.5% acetic acid and 0.2N hydrochloric acid were selected to determine whether any damage was done to sludge surfaces when they were exposed to these agents. Metals were adsorbed onto sludge surfaces and repeatedly stripped off for three cycles (indicated by HAc and HCl). The efficiency of adsorption after stripping with desorbent indicated whether binding sites were removed along with the metals. Also, sludges were pretreated with the two desorbing agents (indicated by Pre HAc and Pre HCl) to determine whether stripping of the sludge surface detrimentally affected adsorptive capability.

Zinc desorption was detrimentally affected by exposure to both agents. There was a drastic drop in adsorption of Zn after the first cycle of adsorption-desorption by both agents (Fig. 7.18). Acetic acid desorption decreased adsorption from 76.60% to 3.93% while hydrochloric acid decreased adsorption from 76.51% to 0.00%. There was no adsorption after the second cycle of desorption indicating that the sludge surface was so damaged by the

desorbing agents that no metal was taken up, probably because all of the adsorption sites were stripped off in the two prior desorption cycles. Pretreatment with both agents did not enhance adsorption of Zn. After the first cycle only 37.25% of adsorption was recorded for acetic acid pretreatment while no adsorption was recorded on pretreatment with hydrochloric acid. Further treatment with acetic acid decreased adsorption to zero. This was also observed in the previous experiment.

Cadmium desorption was also detrimentally affected by both acetic and hydrochloric acid stripping (Fig. 7.19). Acetic acid desorption decreased adsorption from 86.44% to 32.27% for the first cycle and to 12.07% in the second cycle. This was also observed in the pre-treated sample where adsorption was 55.04% after pre-treatment and decreased to 26.27% after one cycle of acetic acid desorption. Hydrochloric acid also decreased adsorptive capability of sludges from approximately 86.05% to 0.00% and only 2.36% was adsorbed after the second cycle. Pre-treatment with hydrochloric acid drastically reduced the sludge's capability to adsorb cadmium to 3.19% and 3.57%, respectively, after two cycles.

Copper adsorption was similarly affected by both treatments with a sharp decrease in adsorption after each of the cycles of desorption by acetic acid and an even sharper decline in adsorptive potential after desorption by hydrochloric acid (Fig. 7.20). Pre-treatment with both acids showed similar trends of decreased adsorption.

Nickel adsorption was also adversely affected by both acid desorbents (Fig. 7.21). Desorption was decreased from 61.17% to 6.09 and 5.18%, respectively, by acetic acid after two cycles and from 65.72% to 0.00% for desorption by hydrochloric acid. This trend was

repeated for pre-treated sludge with the two agents. Clearly, hydrochloric acid desorption was the harsher of the two treatments in all of the above instances.

Chromium (III) desorption was low initially and decreased to zero percent after treatment with both desorbing agents (Fig. 7.22). Pre-treatment also decreased adsorptive capability.

Chromium (VI) adsorption was the only one to increase after treatment with both desorbing agents (Fig. 7.23). Adsorption increased from 26.11 to 42.46% after the three cycles of adsorption-desorption with acetic acid while adsorption increased from 21.17 to 100% after the three cycles of adsorption-desorption with hydrochloric acid. However, adsorptive capability decreased with pre-treatment with both acids. Chromium (VI) seems to bind to sites not located on the surface, hence, acids used as desorbents while removing the surface layers, did not remove the adsorptive sites for chromium, but rather exposed more of them. However, if this were true, then pre-treatment should have enhanced adsorption. Therefore, it is assumed that chromium (VI) bound to sludge surfaces previously, inhibits the action of the acids on the binding sites preventing their removal. Hence, if no chromium is available, i.e., on pre-treatment, the sites for chromium adsorption are stripped, leading to a decrease in the amount of chromium (VI) bound after pre-treatment.

Since the sludge was adversely affected by the acid desorbents, other agents were sought to determine whether they could be used in multiple adsorption-desorption cycles without adversely affecting sludge binding sites. The two agents selected were calcium chloride at a concentration of 5mM and sodium bicarbonate at a concentration of 1M. These two agents were previously used, among others, by Tsezos (1984). An adsorption experiment was

conducted and the saturated sludges were desorbed with the two agents.

Low zinc and cadmium quantities were desorbed by both agents over an extended time period (Figs 7.24 and 7.25). In addition, calcium chloride was the better desorbent of zinc while, sodium bicarbonate desorbed cadmium more efficiently. Zinc desorption by calcium chloride reached a maximum of 27.87% after a period of 480 min., while cadmium desorption by sodium bicarbonate only reached a maximum of 17.22% after 360 min (Fig. 7.24). Copper was efficiently desorbed by sodium bicarbonate, reaching 100% after 90 min., while calcium carbonate desorption of copper reached a maximum of 71.86% after 420 min (Fig. 7.26). Sodium bicarbonate also desorbed nickel more efficiently than calcium carbonate, but only after 90 min., being capable of completely removing nickel from sludge surfaces after 300 min (Fig. 7.27). Sodium bicarbonate also desorbed chromium (III) efficiently with 100% removal at 30 min. Calcium carbonate could only desorb a maximum of 7.96% of chromium (III) after 480 min (Fig. 7.28). Chromium (VI) desorption increased initially for both agents and decreased with time (Fig. 7.29). This was attributable to the sheared sludge particles exposing surfaces for adsorption which removed chromium from solution decreasing the amount desorbed with time. This is in keeping with the assumption that chromium (VI) binds to sites that are situated within sludge particles.

Although some of the metals were efficiently desorbed viz., bicarbonate desorption of copper and chromium (III) (Figs 7.26 and 7.28), the rest of the metals were either poorly desorbed or a prolonged time period was required to desorb all of the metal from the sludge surface. This latter phenomenon could be due partially to shearing of sludge particles in conjunction with the desorbing agents. Since the object was to reuse sludge, a prolonged time period

of desorption was an unfavourable criterion. In addition, the agents used did not demonstrate the efficiency for all metals as was observed by the acids. Therefore, these agents were deemed unsuitable for desorption and multiple adsorption-desorption cycles were not attempted.

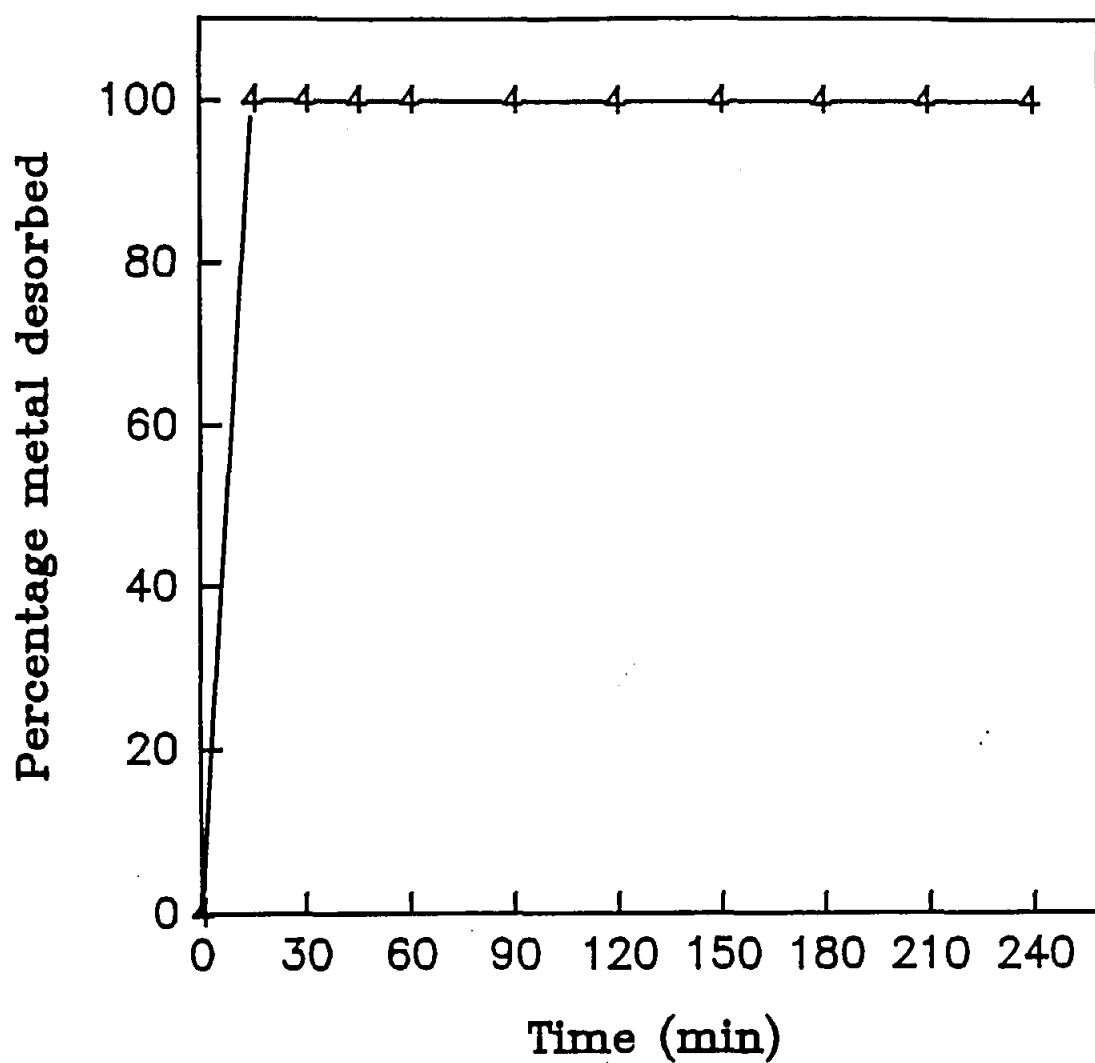


Fig. 7.13. Percentage desorption of zinc by 2.5% acetic (4), 0.2N hydrochloric (4), 2.5% acetic + 0.2N hydrochloric (4) and 5% acetic + 0.4N hydrochloric (4) acids. The numeral 4 indicates the number of points at each location.

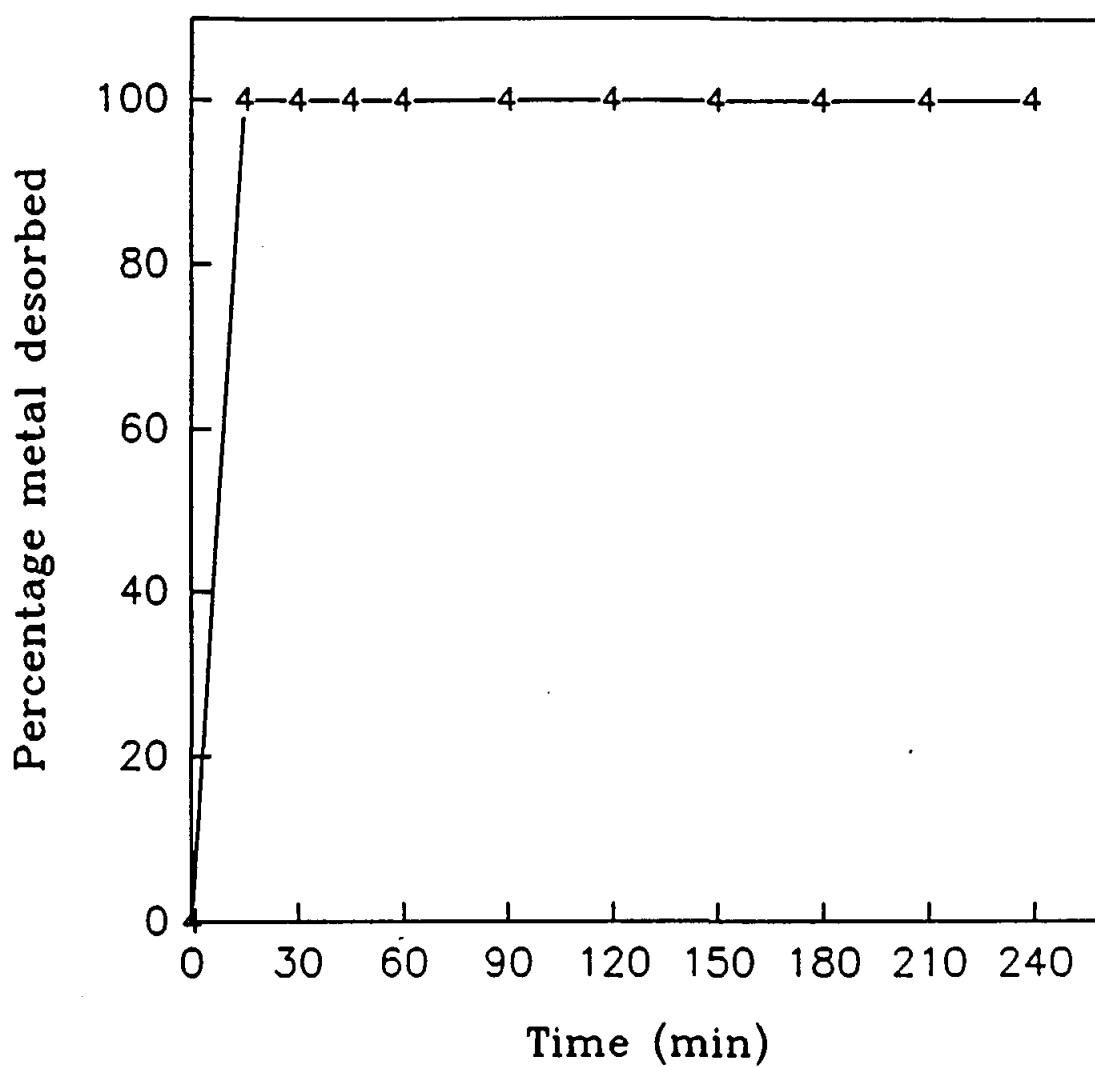


Fig. 7.14. Percentage desorption of cadmium by 2.5% acetic (4), 0.2N hydrochloric (4), 2.5% acetic + 0.2N hydrochloric (4) and 5% acetic + 0.4N hydrochloric (4) acids. The numeral 4 indicates the number of points at each location.

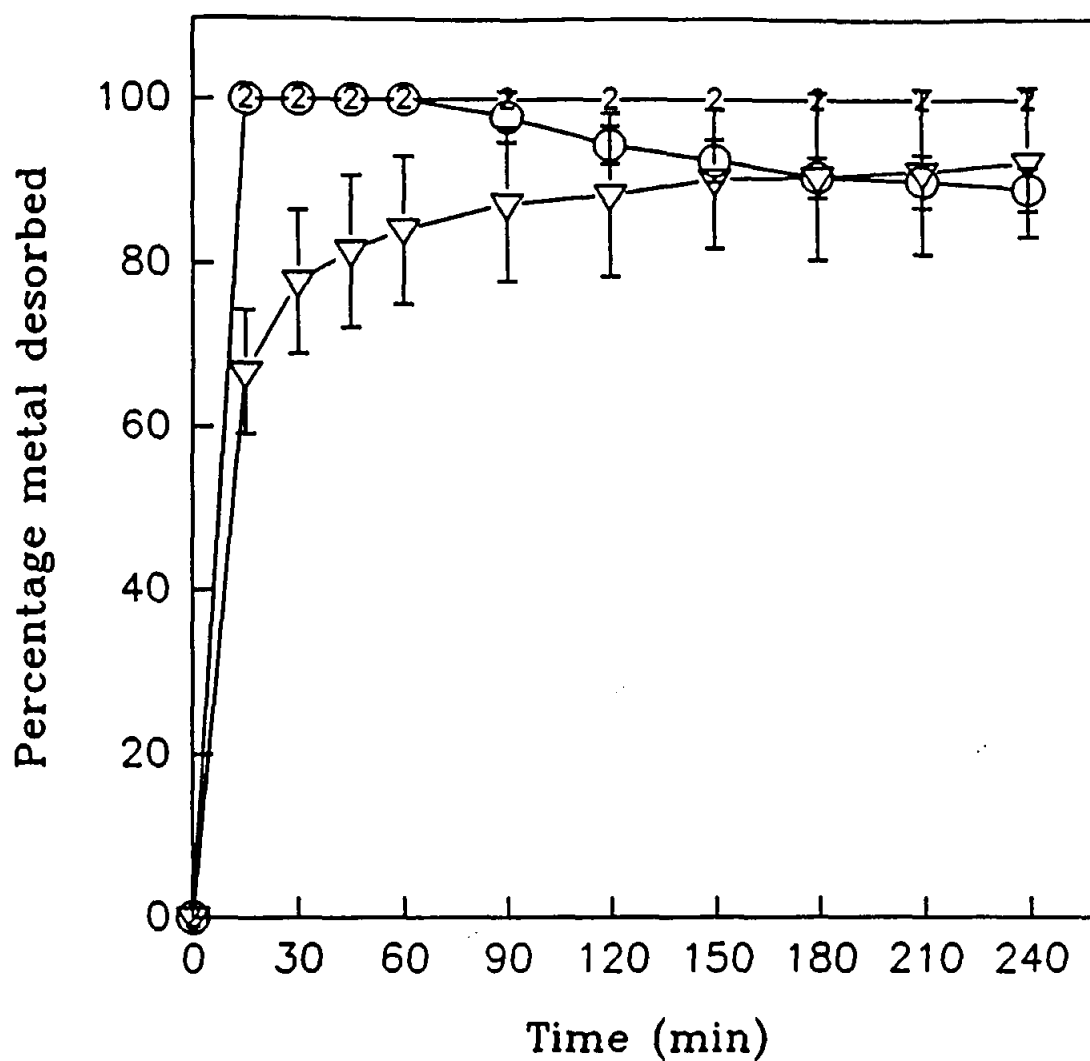


Fig. 7.15. Percentage desorption of copper by 2.5% acetic (○), 0.2N hydrochloric (2), 2.5% acetic + 0.2N hydrochloric (2) and 5% acetic + 0.4N hydrochloric (▽) acids. The numeral 2 indicates the number of points at each location.

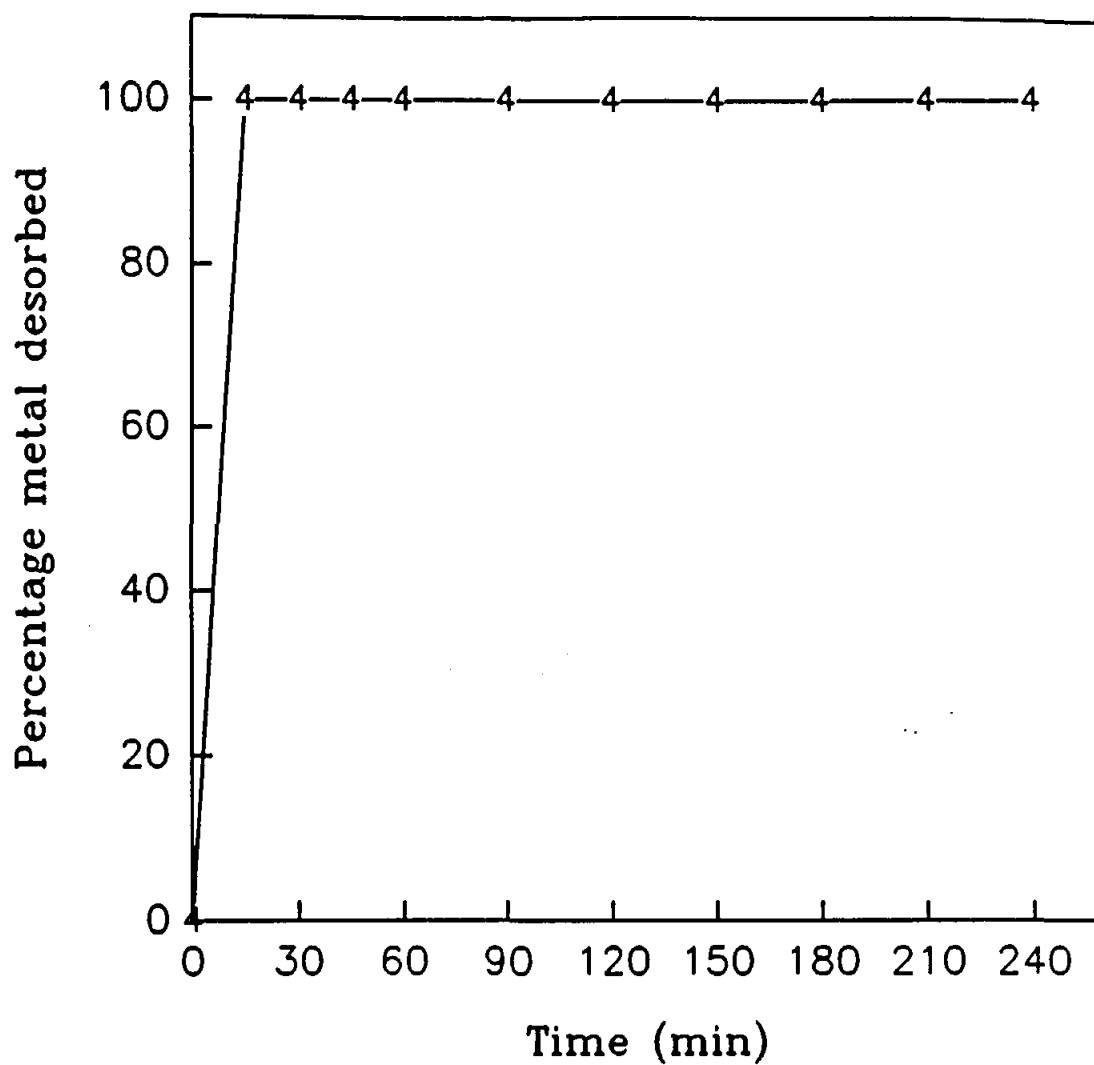


Fig. 7.16. Percentage desorption of nickel by 2.5% acetic (4), 0.2N hydrochloric (4), 2.5% acetic + 0.2N hydrochloric (4) and 5% acetic + 0.4N hydrochloric (4) acids. The numeral 4 indicates the number of points at each location.

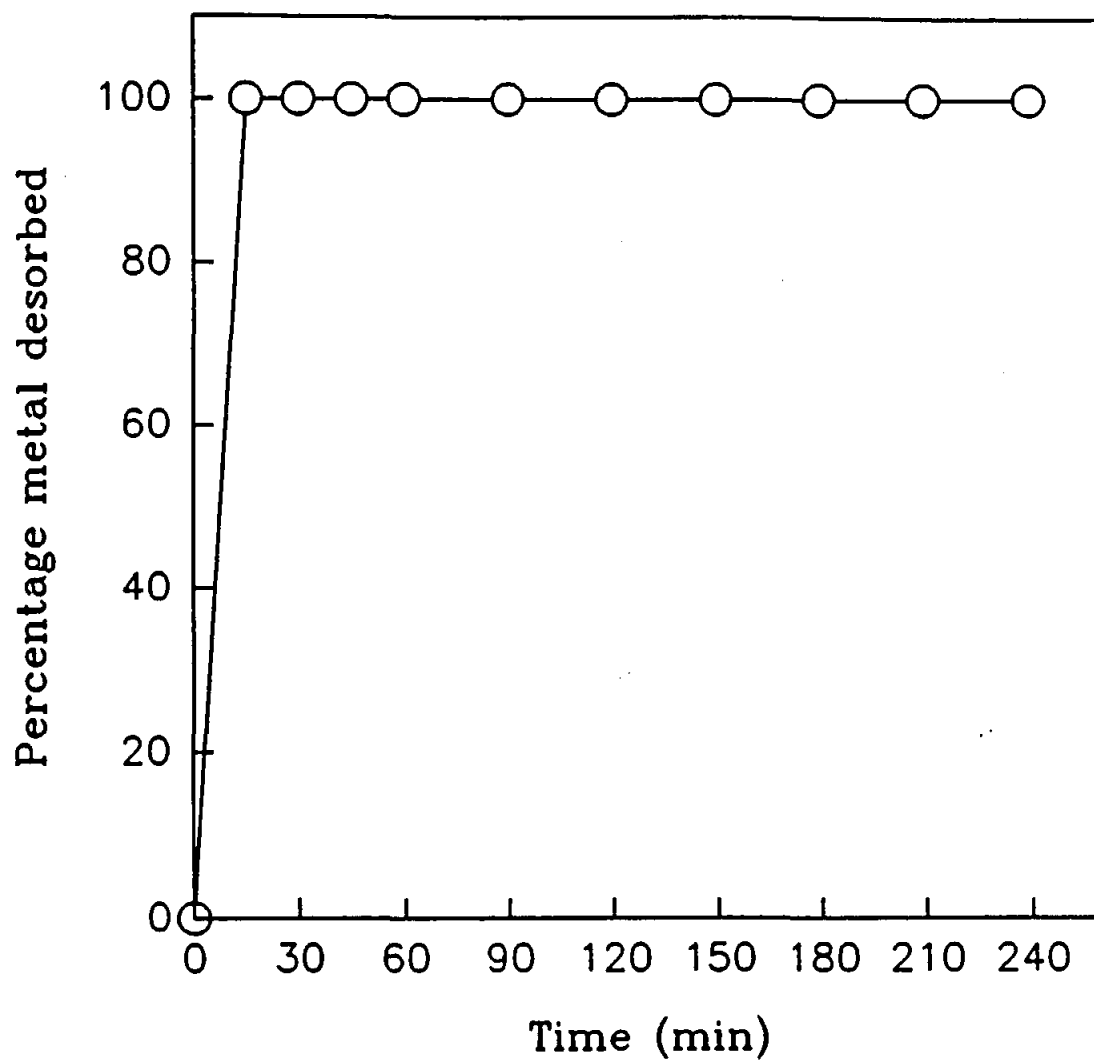


Fig. 7.17. Percentage desorption of chromium (VI) by 2.5% acetic acid (○).

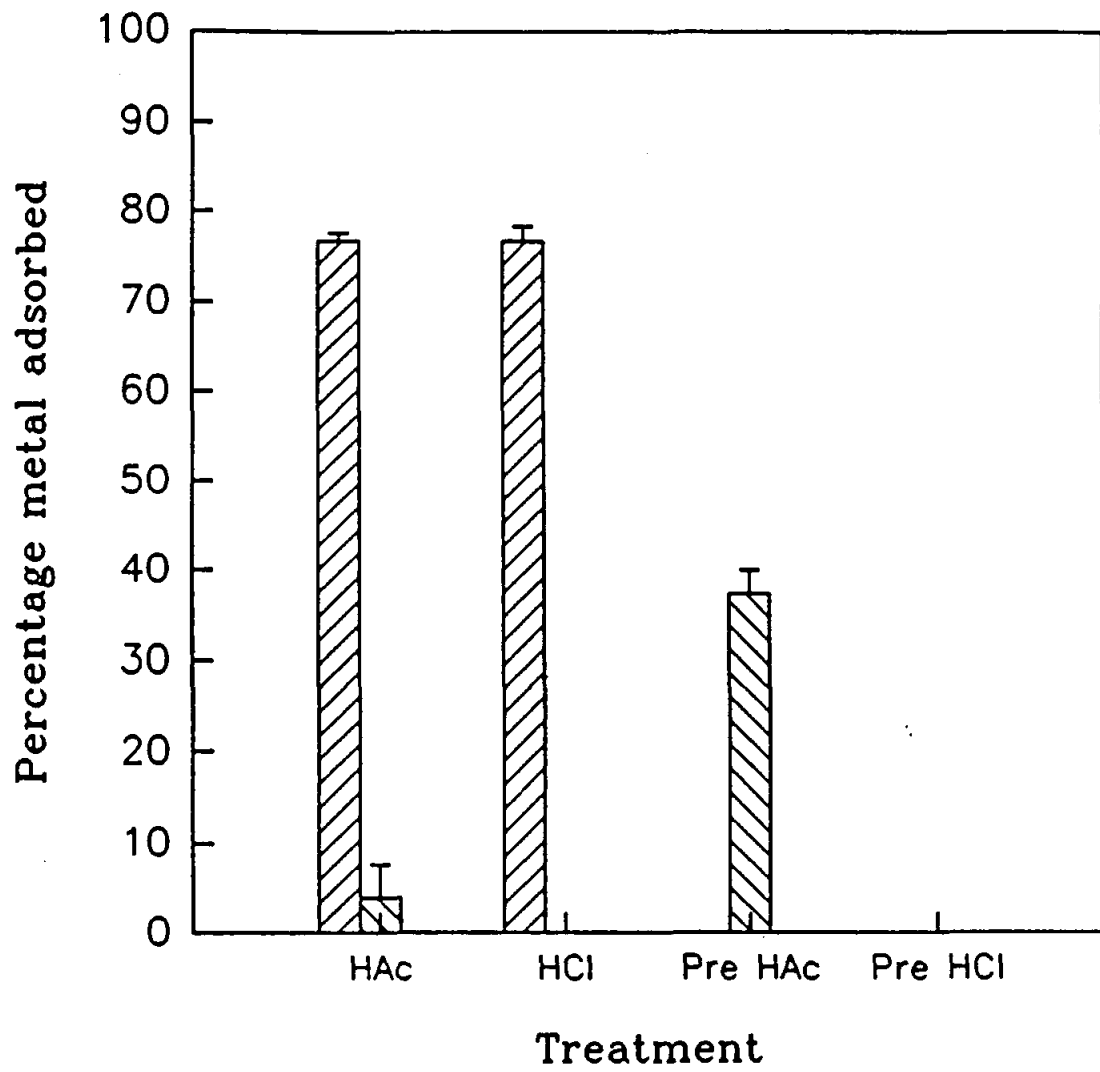


Fig. 7.18. Effect of desorbing agents on adsorption of zinc by sludges from synthetic effluent. Cycle 1 (▨), cycle 2 (▩) and cycle 3 (▤).

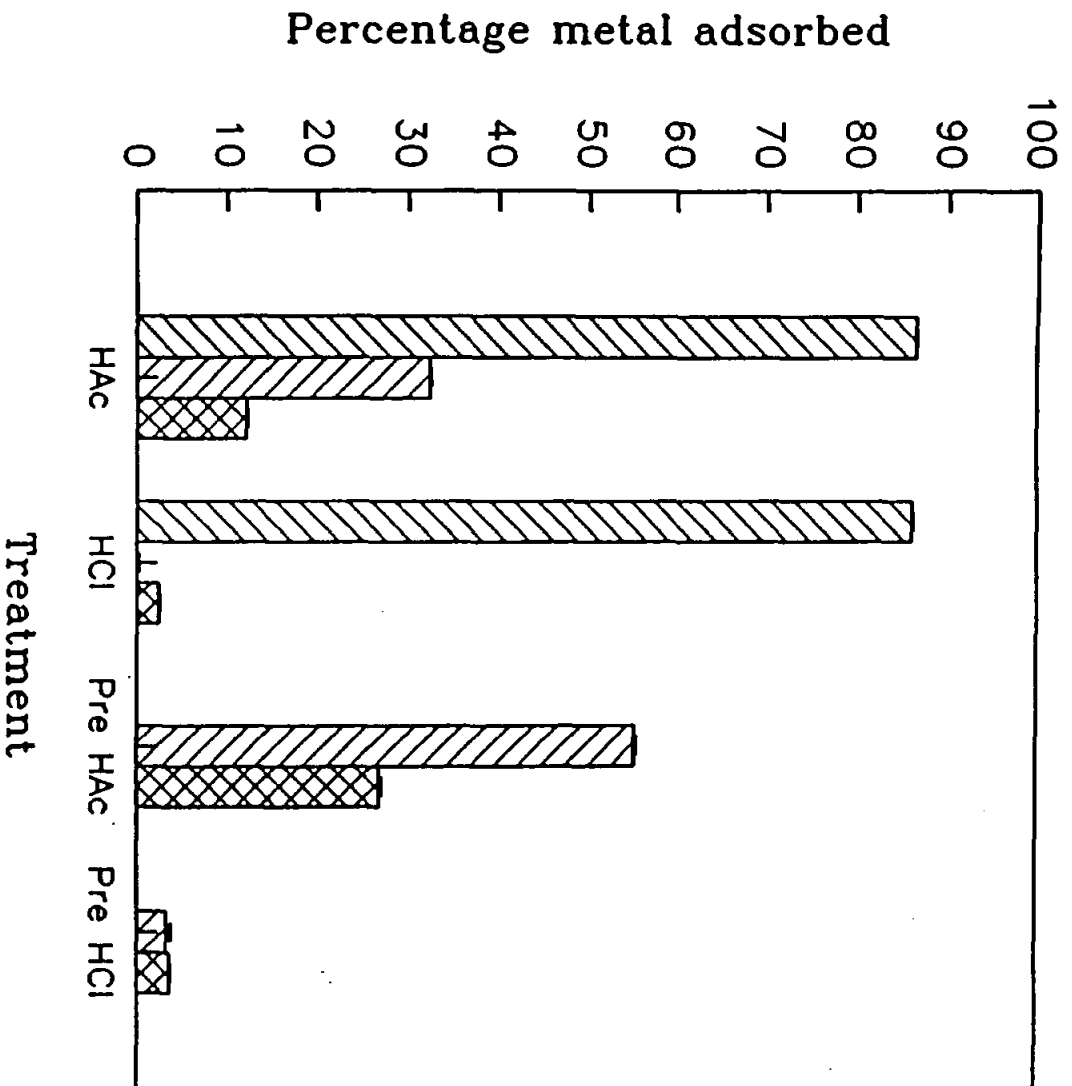


Fig. 7.19. Effect of desorbing agents on adsorption of cadmium by sludges from synthetic effluent. Cycle 1 (▨), cycle 2 (▩) and cycle 3 (▧).

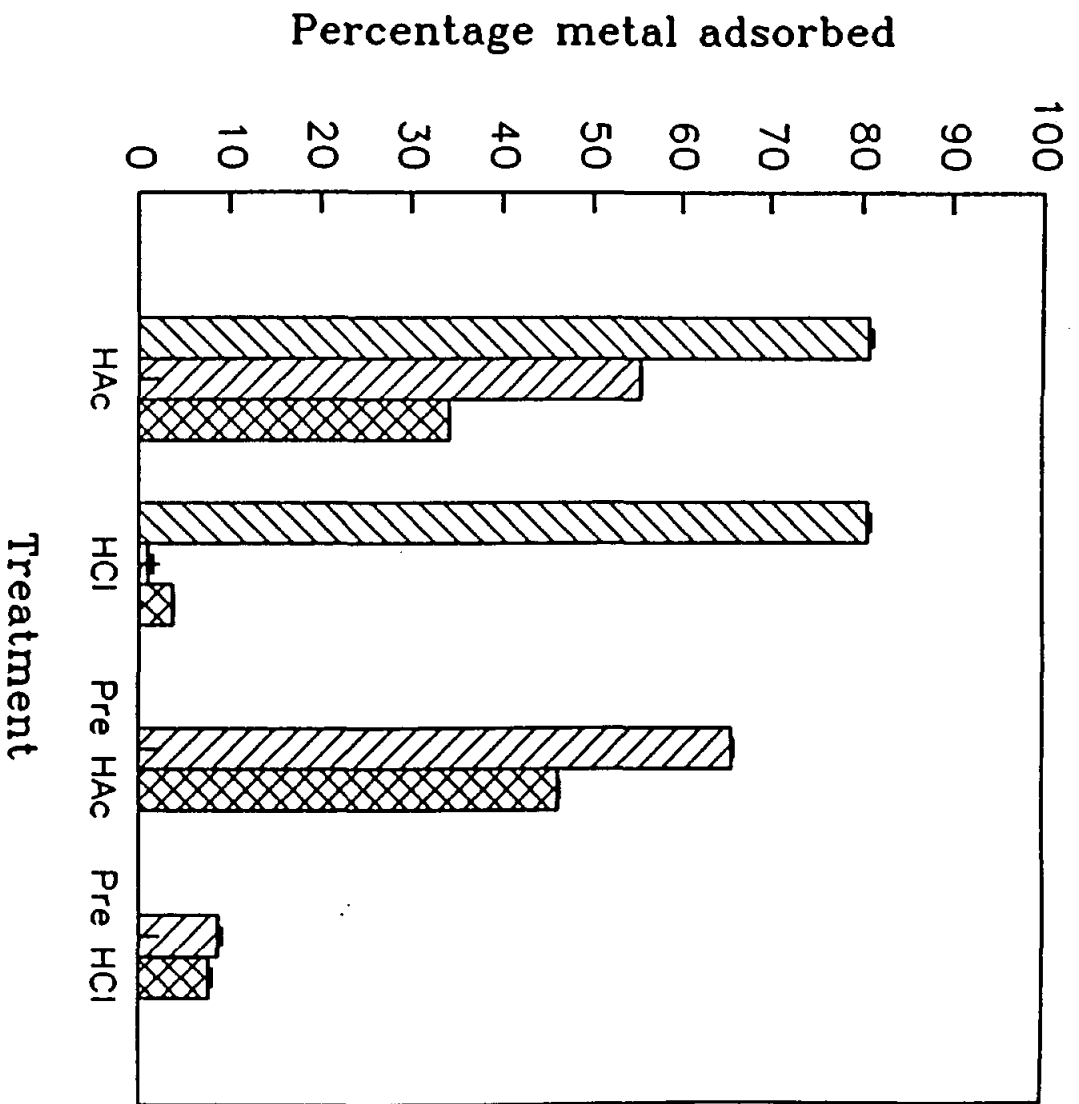


Fig. 7.20. Effect of desorbing agents on adsorption of copper by sludges from synthetic effluent. Cycle 1 (▨), cycle 2 (▩) and cycle 3 (▧).

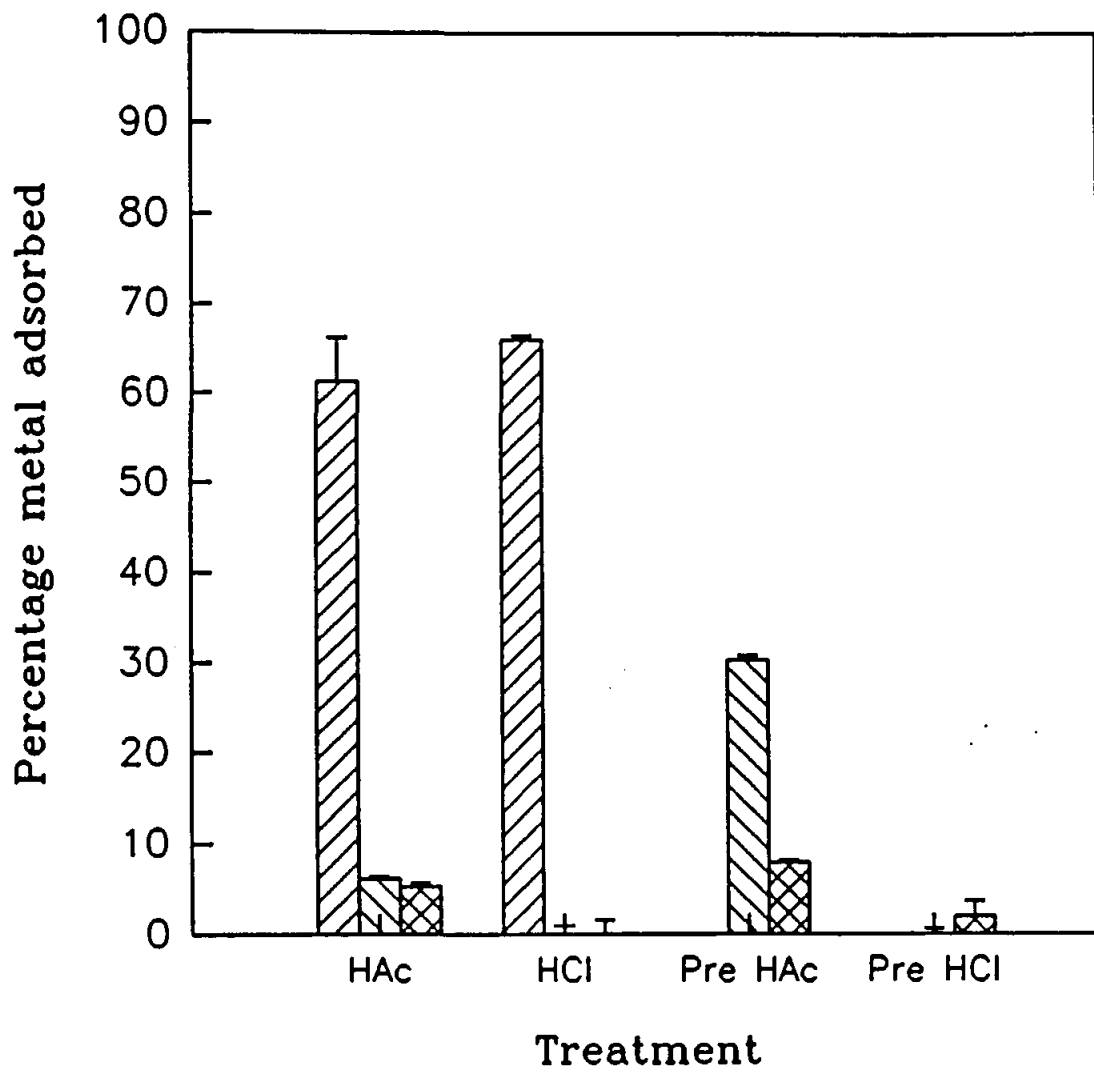


Fig. 7.21. Effect of desorbing agents on adsorption of nickel by sludges from synthetic effluent. Cycle 1 (▨), cycle 2 (▤) and cycle 3 (▥).

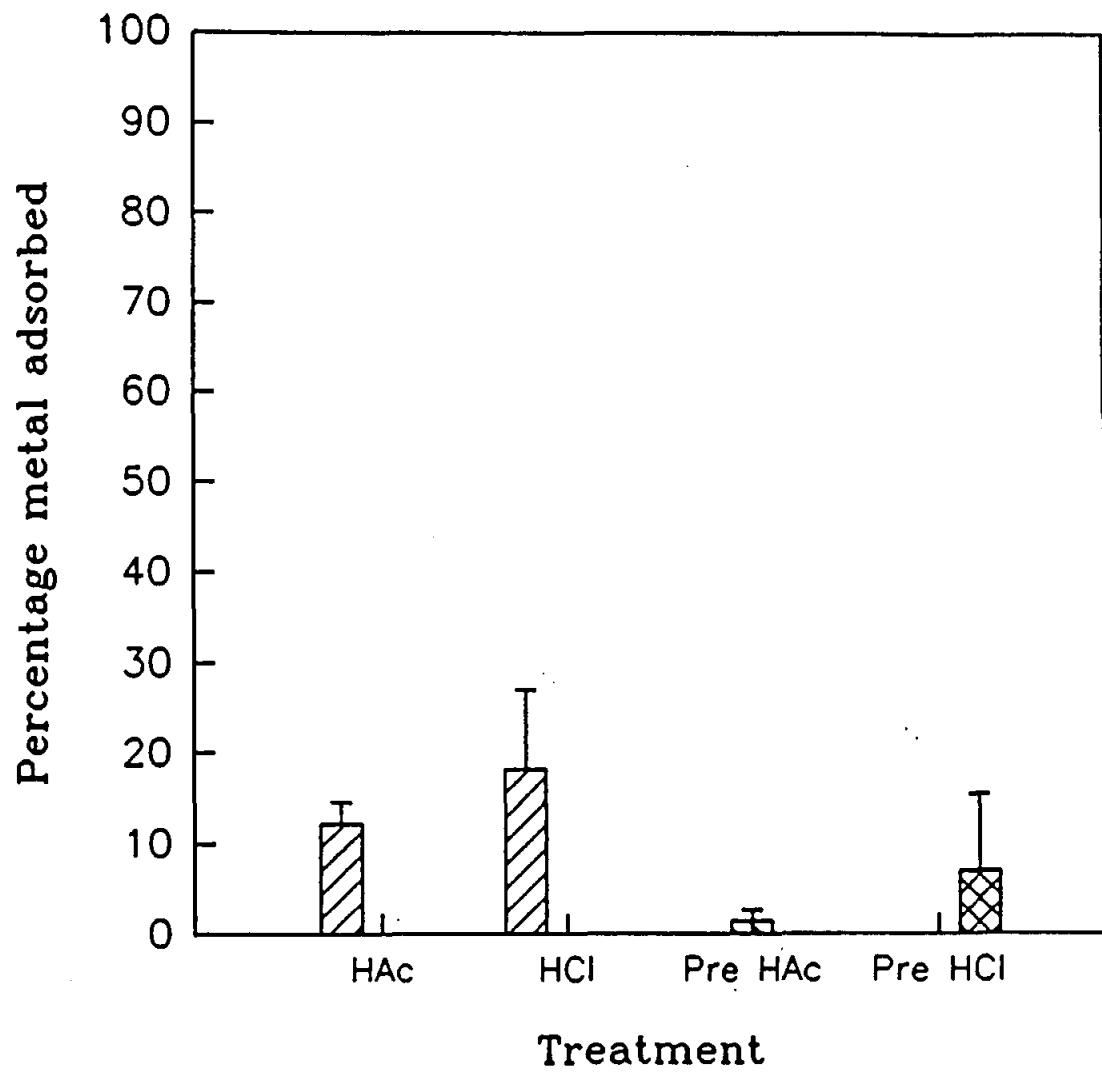


Fig. 7.22. Effect of desorbing agents on adsorption of chromium (III) by sludges from synthetic effluent. Cycle 1 (▨), cycle 2 (▩) and cycle 3 (▧).

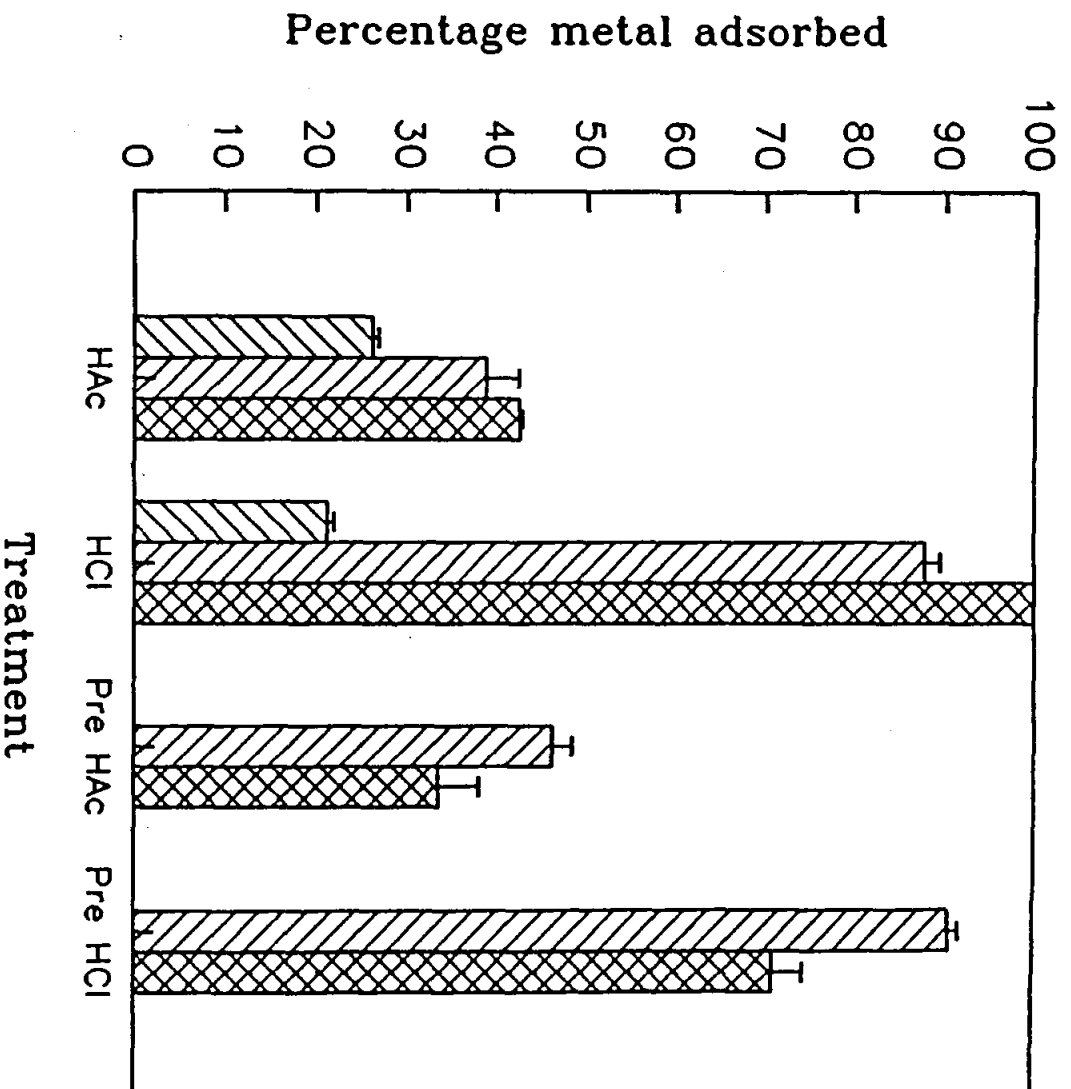


Fig. 7.23.

Effect of desorbing agents on adsorption of chromium (VI) by sludges from synthetic effluent. Cycle 1 (▨), cycle 2 (▩) and cycle 3 (▣).

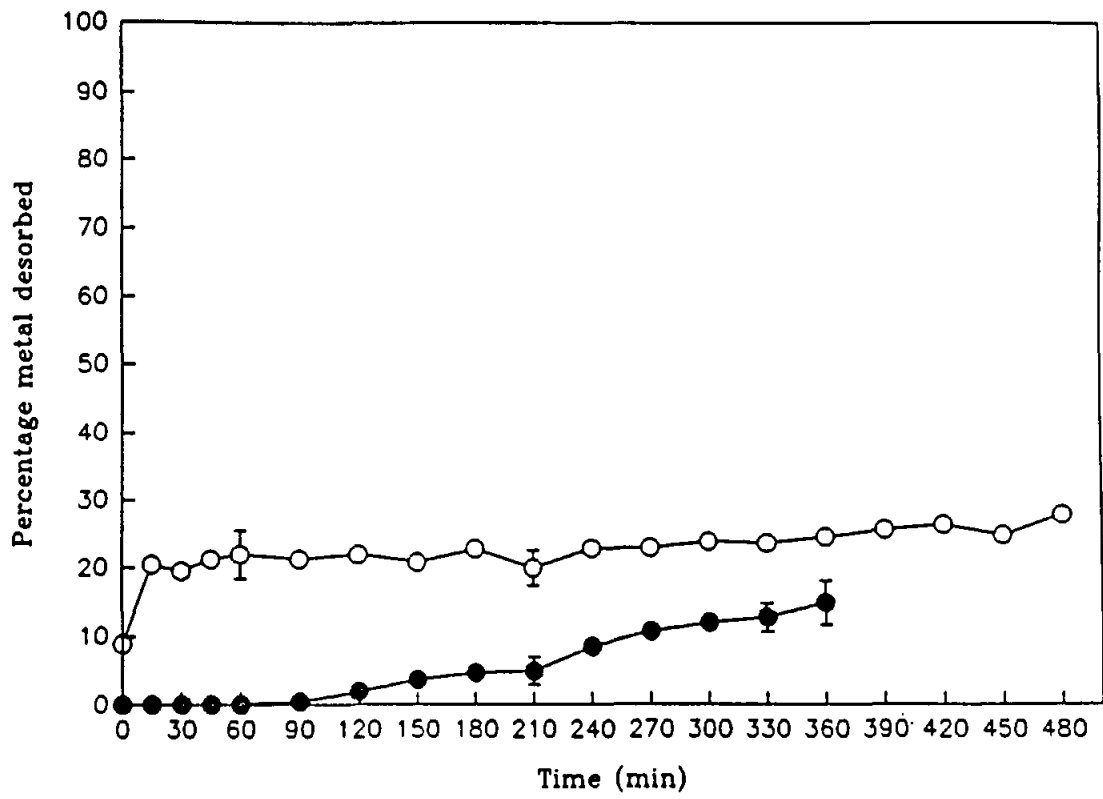


Fig. 7.24. Percentage desorption of zinc from sludge by 5mM calcium chloride (○) and 1N sodium bicarbonate (●).

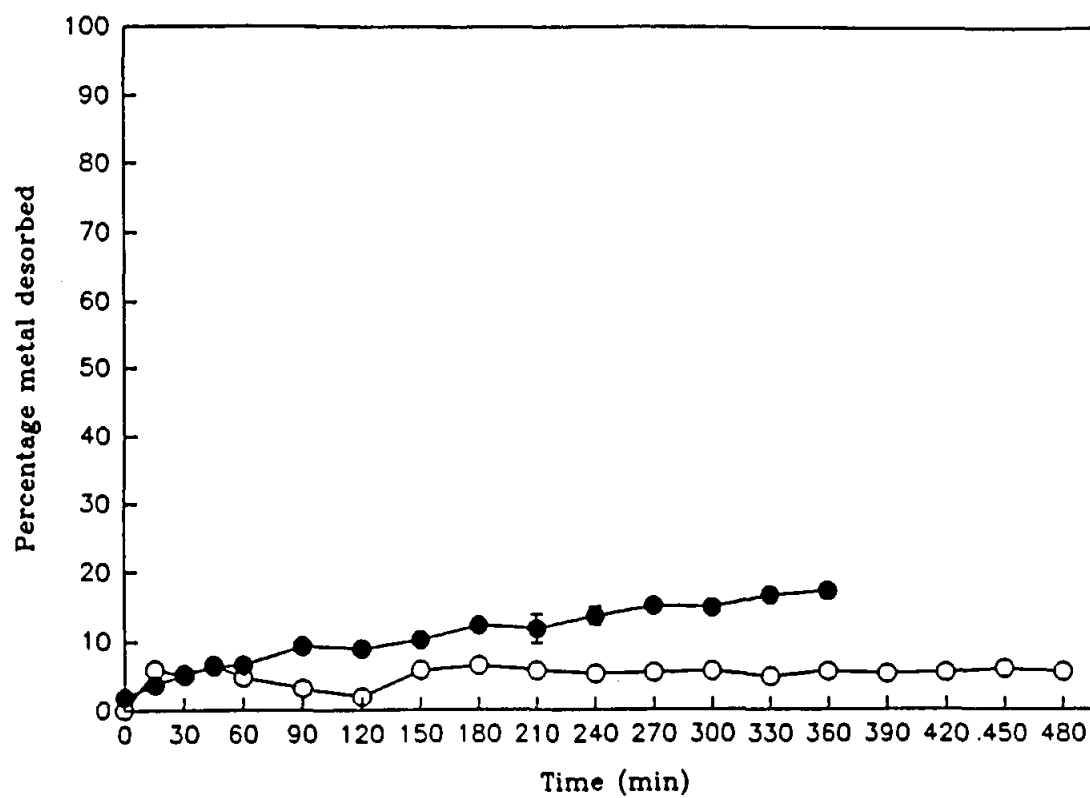


Fig. 7.25. Percentage desorption of cadmium from sludge by 5mM calcium chloride (○) and 1N sodium bicarbonate (●).

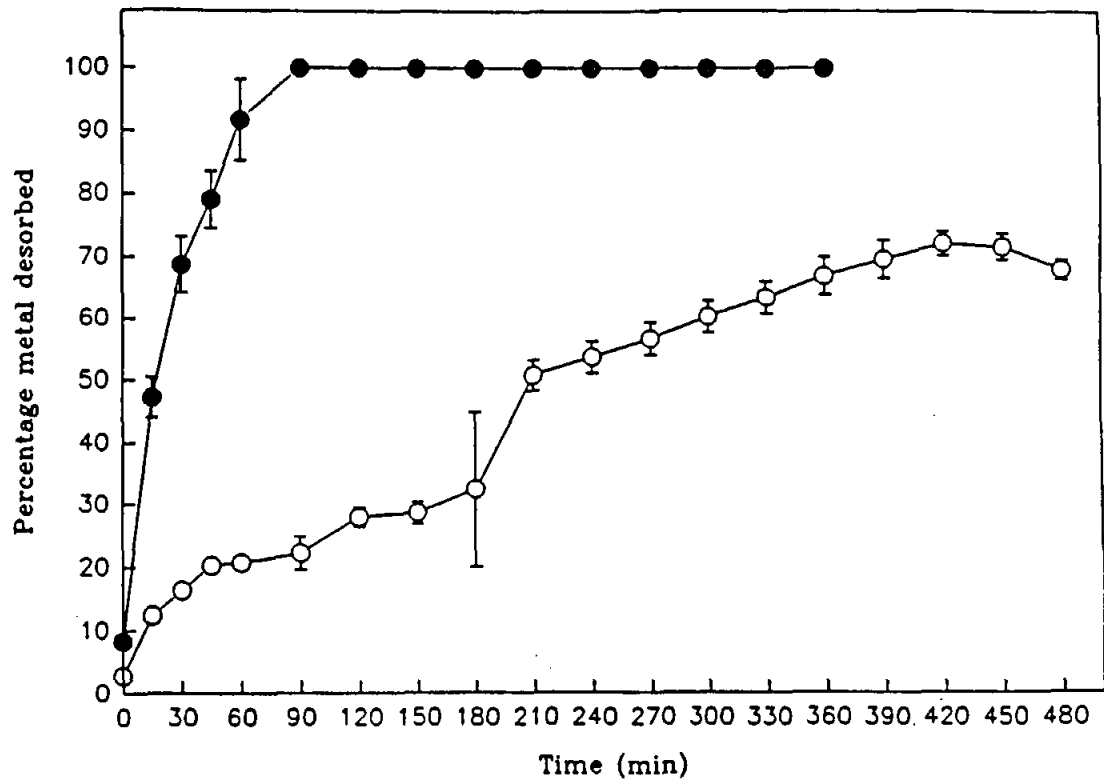


Fig. 7.26. Percentage desorption of copper from sludge by 5mM calcium chloride (○) and 1N sodium bicarbonate (●).

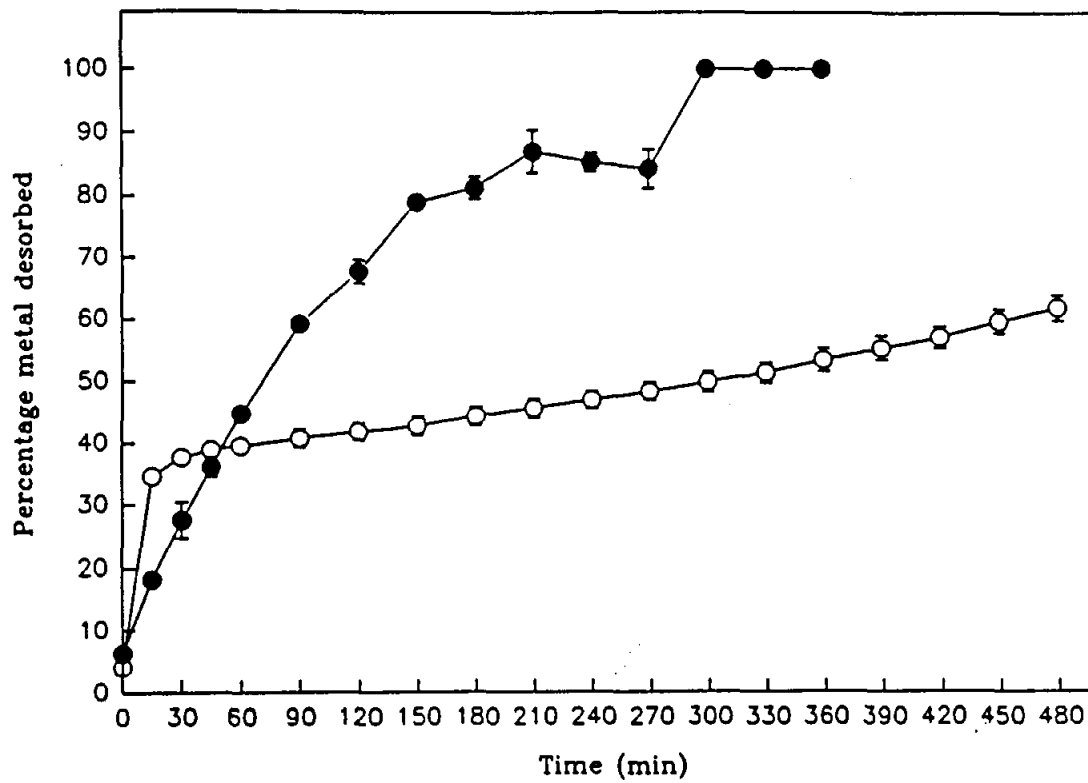


Fig. 7.27. Percentage desorption of nickel from sludge by 5mM calcium chloride (○) and 1N sodium bicarbonate (●).

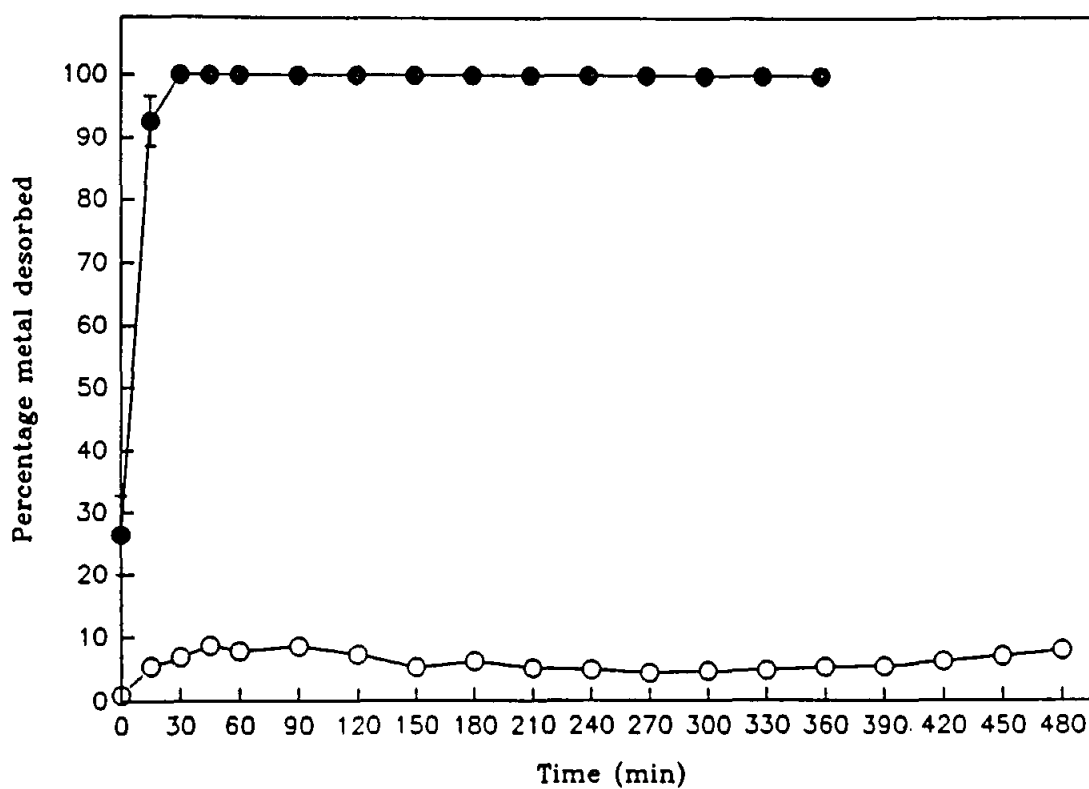


Fig. 7.28. Percentage desorption of chromium (III) from sludge by 5mM calcium chloride (○) and 1N sodium bicarbonate (●).

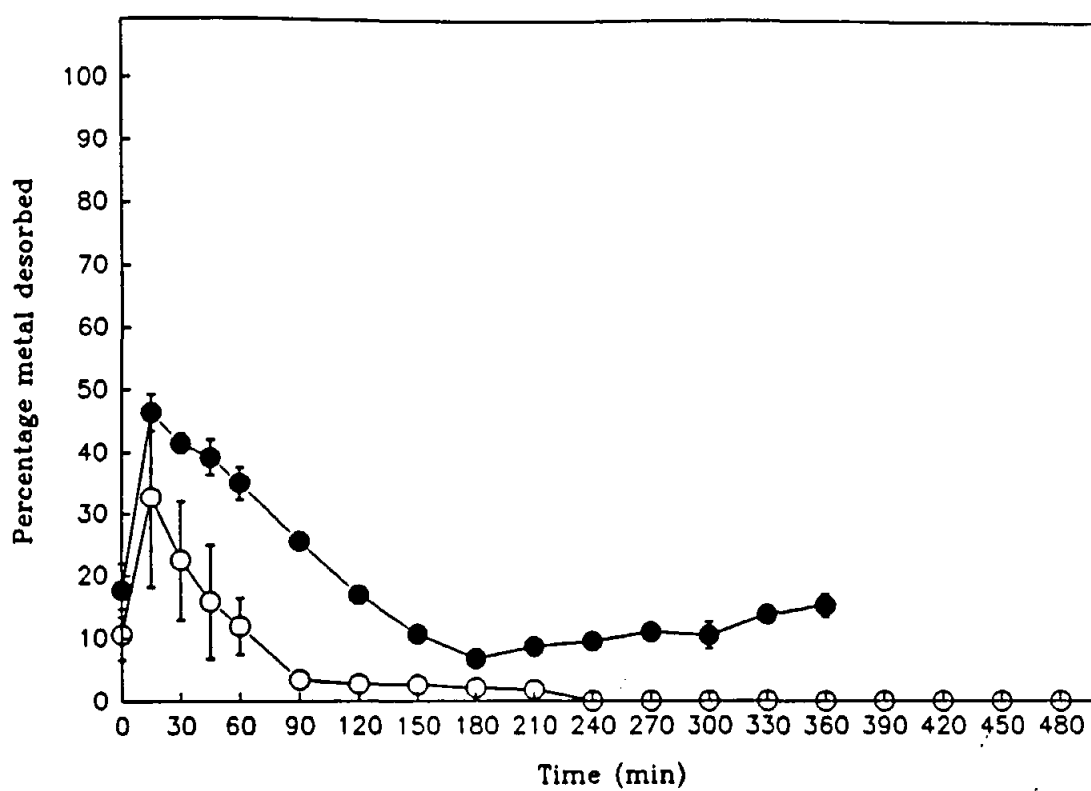


Fig. 7.29. Percentage desorption of chromium (VI) from sludge by 5mM calcium chloride (○) and 1N sodium bicarbonate (●).

7.4 CONCLUSIONS

The experiments with sludge concentrations show that the higher the sludge concentration, the greater the efficiency of biosorption, therefore the highest concentration used viz., 25 000 mg ℓ^{-1} was recommended for further experimentation.

Acetic and hydrochloric acids showed efficient desorption of metal ions either singly or in combination, however, these acids detrimentally affected sludge surfaces' ability to biosorb metal ions after one cycle. Calcium chloride and sodium bicarbonate were not efficient desorbents for some metals therefore, hydrochloric acid was used as the desorbent of choice.

8.0 LABORATORY-SCALE OPTIMISATION OF A SLUDGE METAL ADSORPTION/DESORPTION PROCESS FROM INDUSTRIAL WASTEWATERS

8.1 INTRODUCTION

Industrial wastes containing metal-ions originate from a number of sources and are disposed to, water and soils. Combustion of hard coal, lignites and brown coal in electric power plants and in industrial, commercial and residential burners is the major source of airborne Hg, Mo and Se and a significant source for Cr, Mn, Sb and Tl. Combustion of oil for the same purpose is the most important source of V and Ni and is an important contributor of Xn. The non-ferrous metal industry accounts for the largest fraction of Pb, Cd, Cu and Zn. The iron and steel industry are responsible primarily for the emission of Cr and Mn. The major sources of trace metal pollution in aquatic ecosystems including the ocean are domestic wastewater effluents (Cu, Mn and Ni), coal-burning power plants (Hg, Sn, Cu, Mn and Ni), non-ferrous metal smelters (Cd, Ni and Pb), iron and steel plants (Cr, Mo, Sb and Zn), and the dumping of sewage sludge (Mn and Pb). There are two principal sources of trace metals in soils, viz., disposal of ash residues from coal combustion and general wastage of commercial products on land. Urban refuse contributes significant quantities of Cu, Hg, Pb and Zn. Cadmium, Pb and V are contributed to soils from the atmosphere (Nriagu and Pacyna, 1988).

Effluents or wastewaters generated from industrial metal processing sources have to comply

with standards set by Government Notice No. R991 (1984). Some of the restrictions placed on metal-ion disposal are presented in Table 8.1.

Table 8.1. Quality standards for wastewater or effluent arising in the catchment area draining water to any river or tributary thereof.

Constituents	Maximum Concentration in mg ℓ^{-1}
Zinc (as Zn)	5.0
Cadmium (as Cd)	0.05
Hexavalent chromium (as Cr)	0.05
Total chromium (as Cr)	0.5
Copper (as Cu)	1.0
Lead (as Pb)	0.1
Manganese (as Mn)	0.4
Mercury (as Hg)	0.02
Selenium (as Se)	0.05

8.1.1 Aim

The aim of this aspect of the current study was to obtain wastewaters containing metal-ions and to subject them to activated sludges to determine whether these wastewaters could be treated by sludges to produce acceptable levels of metal-ions before disposal.

8.2 METHODOLOGY

The purpose of the current experiment was to determine whether industrial effluents containing metal-ions were treatable using activated sludges.

8.2.1 Wastewater Analysis

Wastewaters were obtained from three industrial sources with differing pHs. One was of a neutral pH while the other two were acidic and alkaline, respectively. Samples of effluents were diluted and analysed for their metal content by atomic absorption spectrophotometry. Metals analysed for included, zinc, cadmium, copper, nickel, chromium (III) and chromium (VI).

8.2.2 Metal Adsorption and Desorption from Wastewaters

Thirty litre volumes of each effluent were decanted into 40 ℓ bioreactors and these were exposed to sludge at a concentration of 25 000 mg ℓ⁻¹. A 0 min. sample was taken, and agitation commenced, at 600 rpm for 90 min., after which a sample was taken. The effluent was then decanted and replaced with 10 ℓ 0.2N hydrochloric acid. A 0 min., sample was taken and the sludge and desorbent was agitated for 15 min., before the next sample was taken. Controls comprised of sludge exposed to the desorbent only without them adsorbing any metals from the effluents before hand.

8.3 RESULTS AND DISCUSSION

Adsorption and desorption of metals from each of the three wastewaters is depicted in Figures 8.1 - 8.9. The three wastewaters are ascribed the acronyms AEV, MPS and NDC. Their pHs were 9.87, 3.24 and 7.18, respectively. The metal content of each wastewater accompanies the adsorption graph.

Adsorption graphs were presented in both quantity and percentage form since quantities of metals varied in the wastewaters and the forms presented allow comparative assumptions to be made. The adsorption efficiency of metals from wastewater AEV was $\text{Ni} > \text{Zn} > \text{Cu} > \text{Cr}^{3+}$ (Fig. 8.2). However, although zinc was less efficiently adsorbed by sludge than nickel, by far the largest quantity of metal uptake by sludge was zinc (Fig. 8.1). This large quantity of zinc uptake could be attributable to the high initial quantities present in wastewater AEV. Although quantities of uptake for other metals was low, their comparative efficiencies were higher. None of the metals were completely removed from the wastewater but a removal efficiency of 82.47% of zinc, from a high concentration wastewater of 121.67 mg l^{-1} is indicative of the great potential for sludge adsorption of this particular metal. The desorption of metals from the sludge was not as efficient with zinc being the only metal being desorbed in any significant quantity at 82.32% (Fig. 8.3). It seems that the small quantities adsorbed were so closely associated with sludges that they were not removable even by the harsh mineral acid.

Adsorption of metals from the acidic MPS wastewater indicated large quantities of zinc adsorbed (Fig. 8.4). However, again it was not the most efficiently adsorbed with adsorption

efficiency being in the order $\text{Cr}^{3+} > \text{Ni} > \text{Cu} > \text{Zn}$ (Fig. 8.5). This order is reversed in the previous figure for quantities adsorbed with the exception of zinc. Again this could be attributable to the large quantity of zinc present. It seems that adsorption is related, in this case to the amount of metal present with the quantities of metal adsorbed increasing (Fig. 8.4), albeit not in proportion (Fig. 8.5), to the quantity present. Desorption of zinc was again the highest from the sludge followed, this time, by copper and a small quantity of nickel (Fig. 8.6).

Metals adsorbed from the neutral ($\text{pH}=7.18$) NDC wastewater showed a similar trend for quantities adsorbed with the greatest initial concentration being adsorbed in greatest quantity (Fig. 8.7). However, the efficiency of adsorption of the predominant metal, zinc, was also the greatest, at 39.00%, followed by nickel and copper (Fig. 8.8). Desorption demonstrated that the largest quantity bound would be released most efficiently, while the smaller quantities were more difficult to remove (Fig. 8.9)

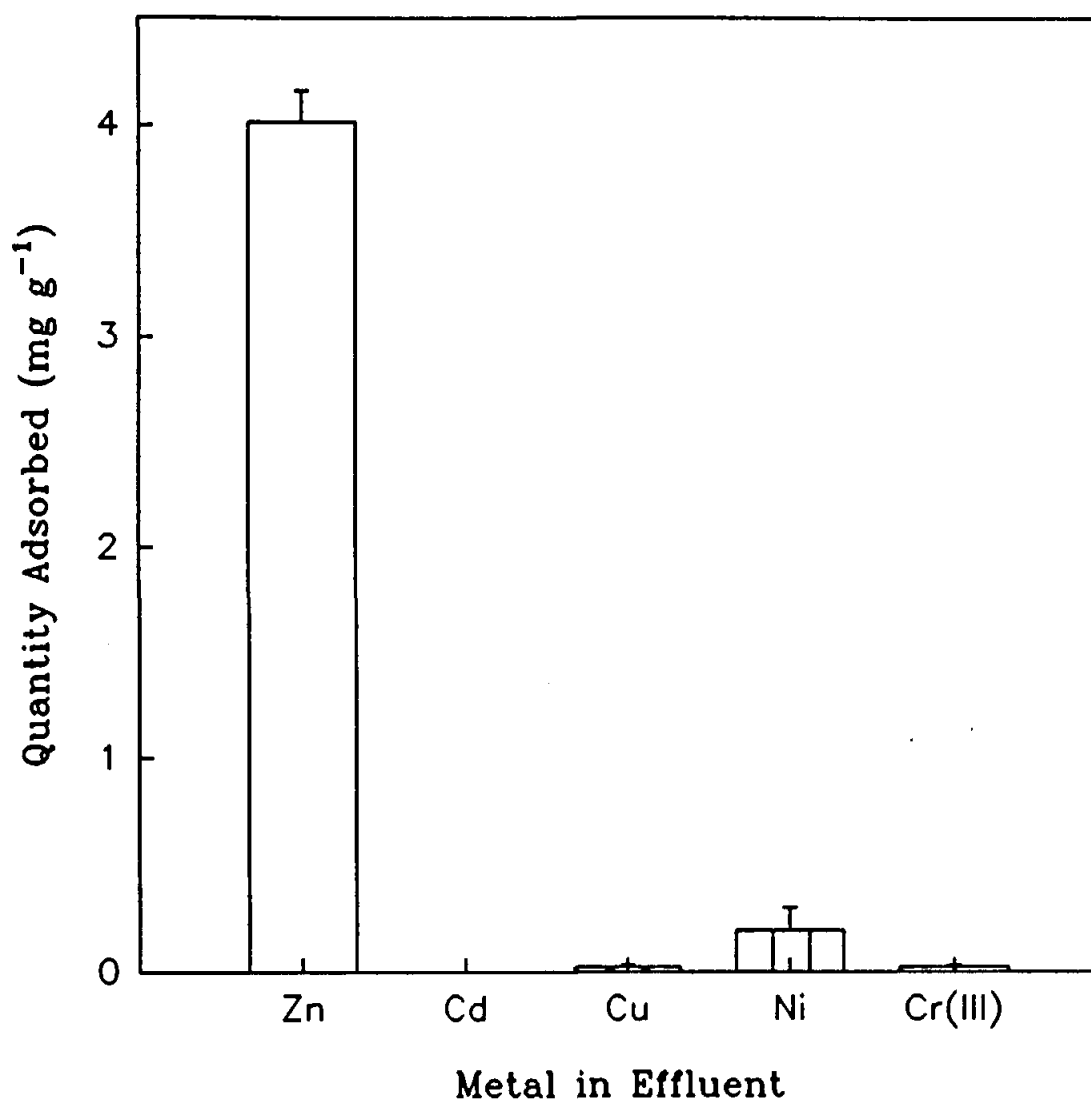


Fig. 8.1. Quantities of zinc (\square), cadmium (▨), copper (▩), nickel (▧) and chromium (III) (▨) adsorbed from wastewater AEV by 25 000 mg ℓ^{-1} activated sludge. Initial concentrations of Zn (121.67), Cd (0.00), Cu (1.76) Ni (5.48) and Cr^{3+} (3.02) in mg ℓ^{-1} .

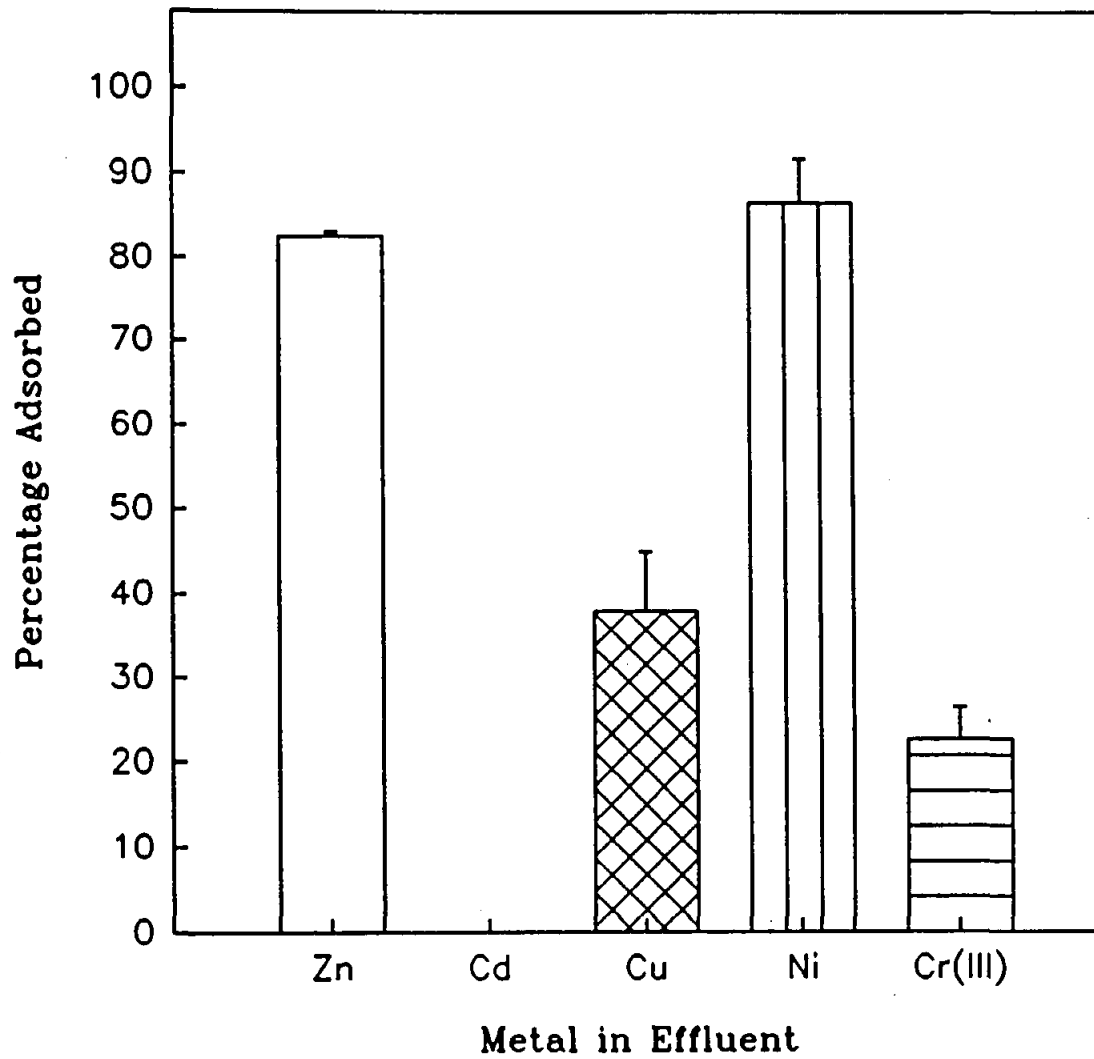


Fig. 8.2. Percentages of zinc (\square), cadmium (diagonal lines), copper (cross-hatch), nickel (vertical lines) and chromium (III) (horizontal lines) adsorbed from wastewater AEV by 25 000 mg l^{-1} activated sludge. Initial concentrations of Zn (121.67), Cd (0.00), Cu (1.76) Ni (5.48) and Cr^{3+} (3.02) in mg l^{-1} .

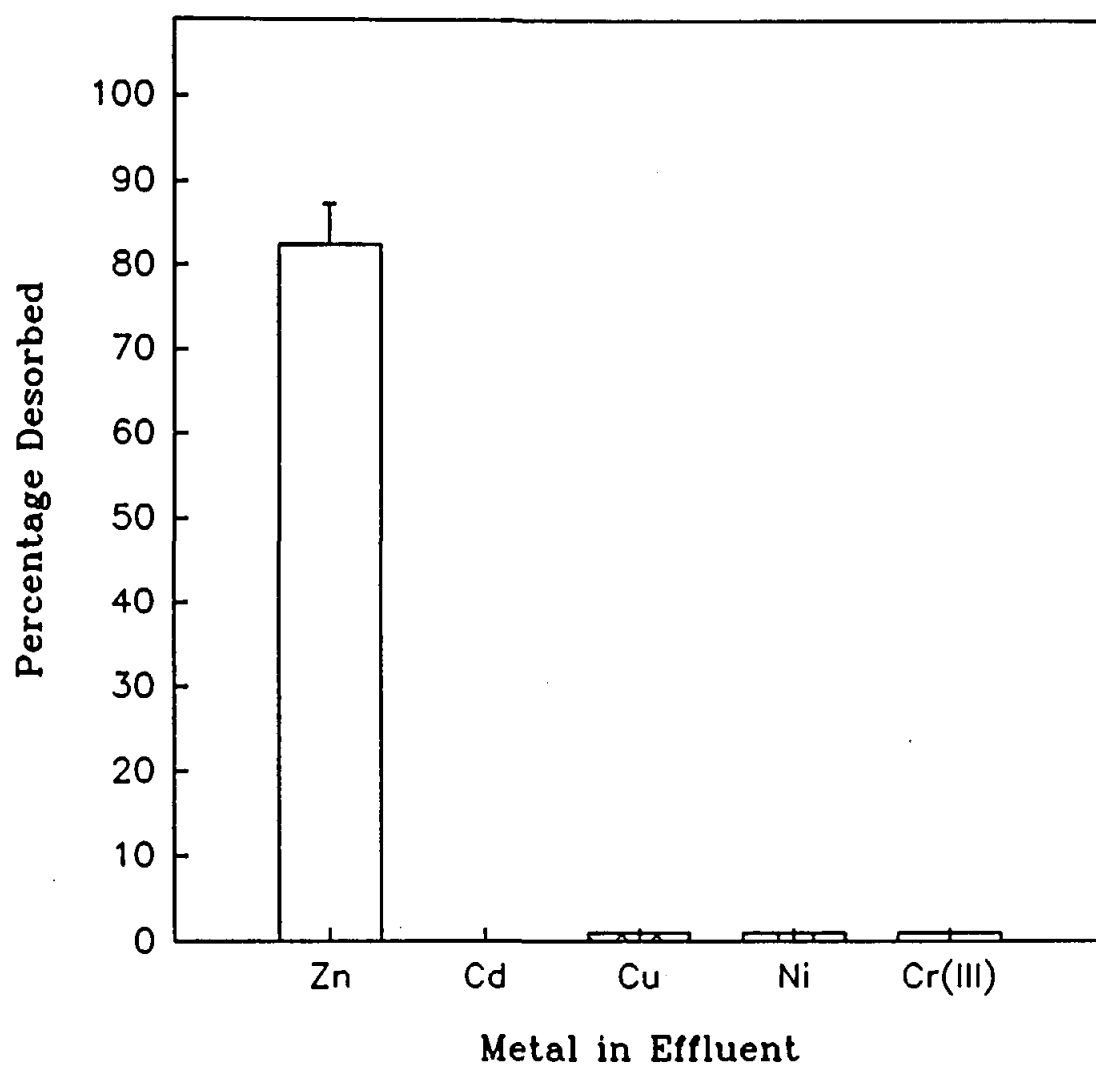


Fig. 8.3. Percentages of zinc (\square), cadmium (▨), copper (▩), nickel (▧) and chromium (III) (▥) desorbed from sludges by 0.2N HCl after adsorption from wastewater AEV.

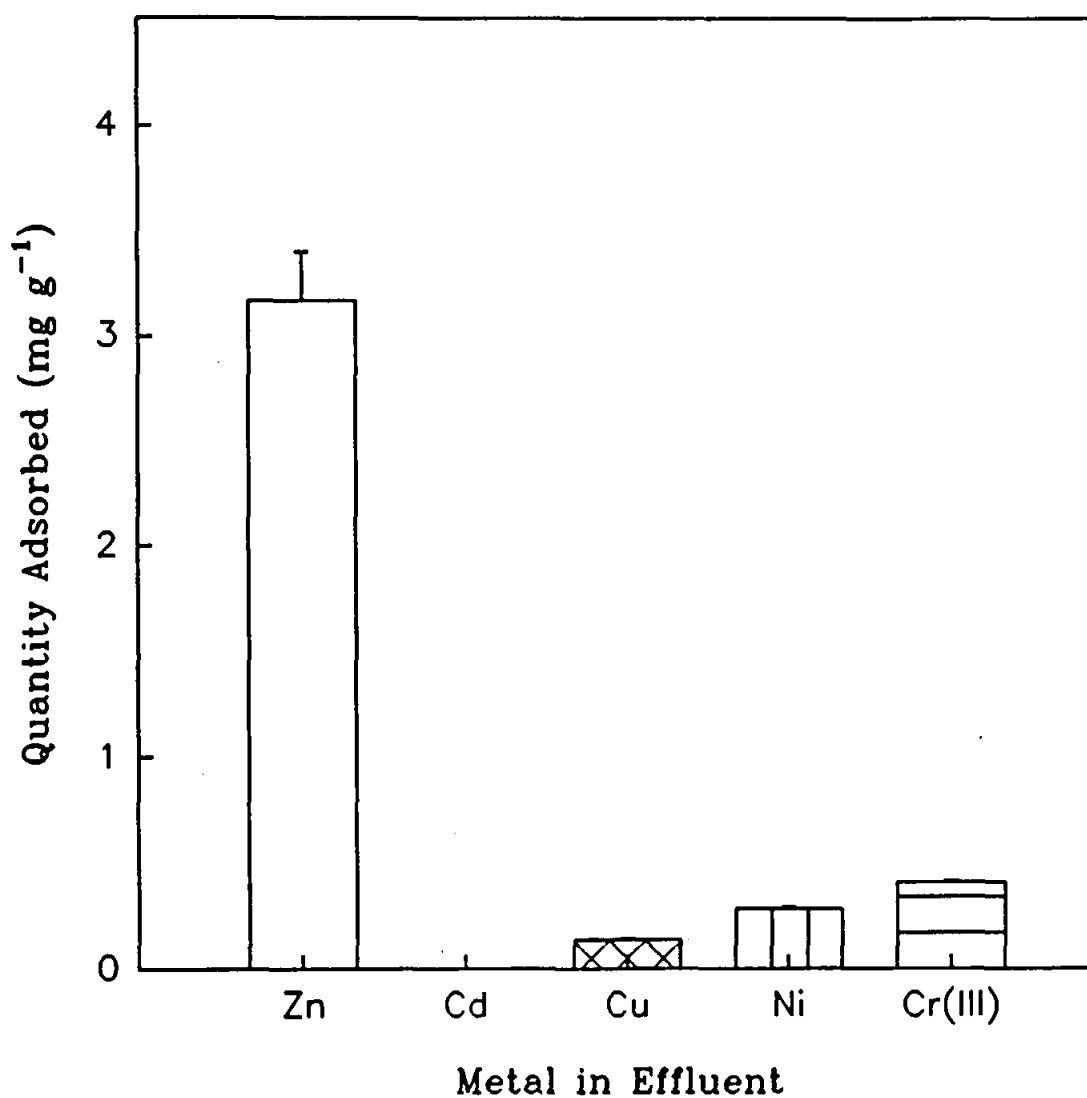


Fig. 8.4. Quantities of zinc (\square), cadmium (diagonal lines), copper (cross-hatch), nickel (vertical lines) and chromium (III) (horizontal lines) adsorbed from wastewater MPS by 25 000 mg ℓ^{-1} activated sludge. Initial concentrations of Zn (132.33), Cd (0.00), Cu (4.38) Ni (7.70) and Cr^{3+} (10.80) in mg ℓ^{-1} .

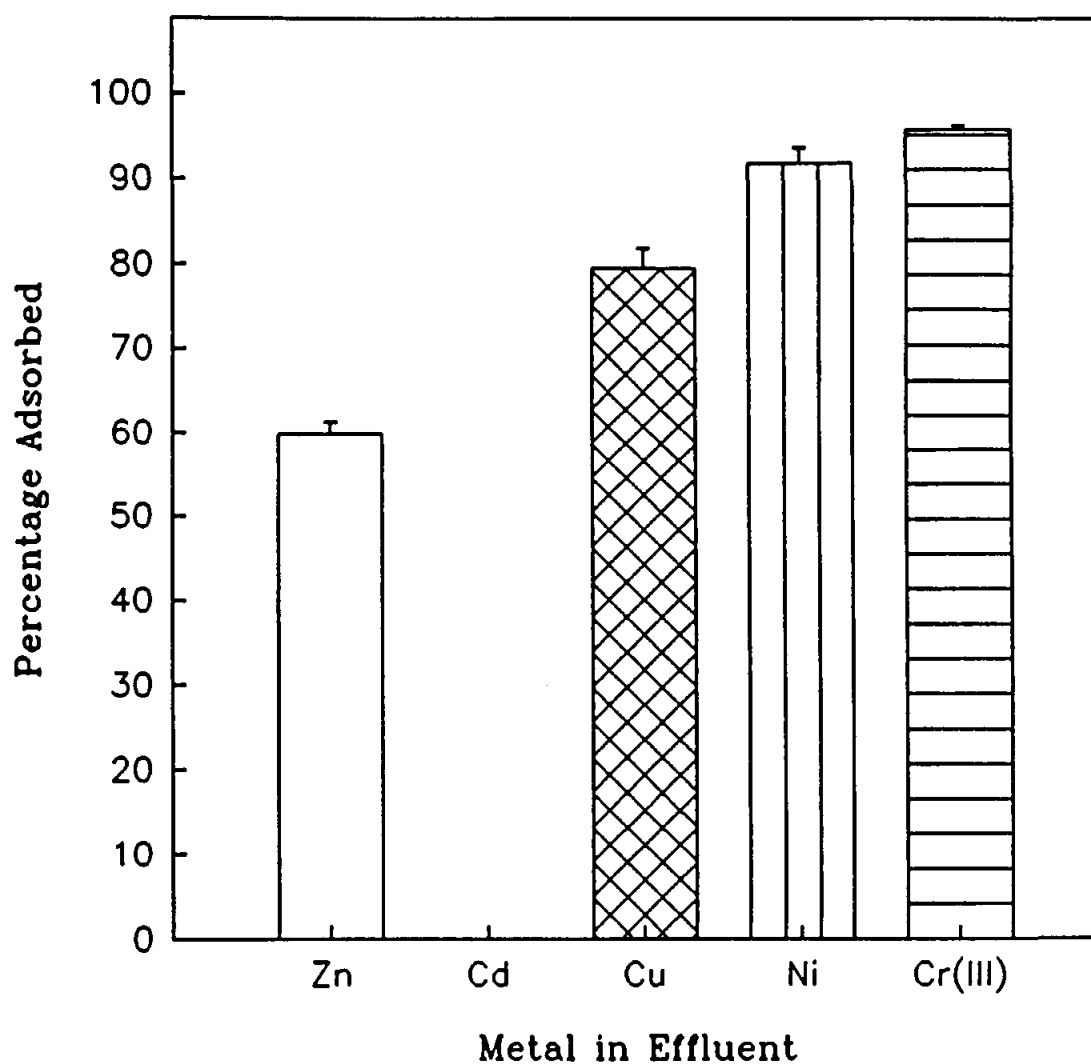


Fig. 8.5. Percentages of zinc (\square), cadmium (diagonal lines), copper (cross-hatch), nickel (vertical lines) and chromium (III) (horizontal lines) adsorbed from wastewater MPS by 25 000 mg ℓ^{-1} activated sludge. Initial concentrations of Zn (132.33), Cd (0.00), Cu (4.38) Ni (7.70) and Cr^{3+} (10.80) in mg ℓ^{-1} .

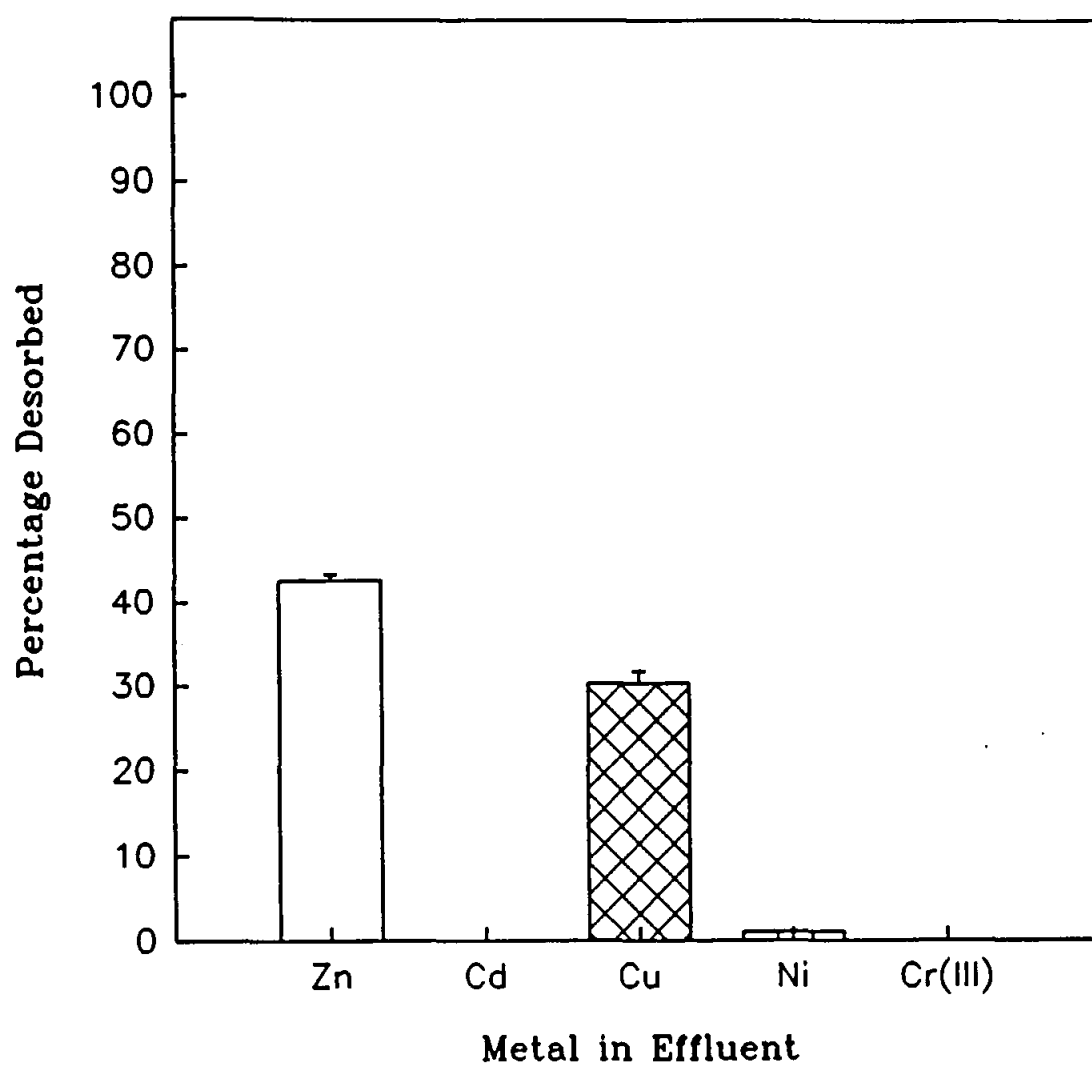


Fig. 8.6. Percentages of zinc (\square), cadmium (diagonal lines), copper (cross-hatch), nickel (vertical lines) and chromium (III) (horizontal lines) desorbed from sludges by 0.2N HCl after adsorption from wastewater MPS.

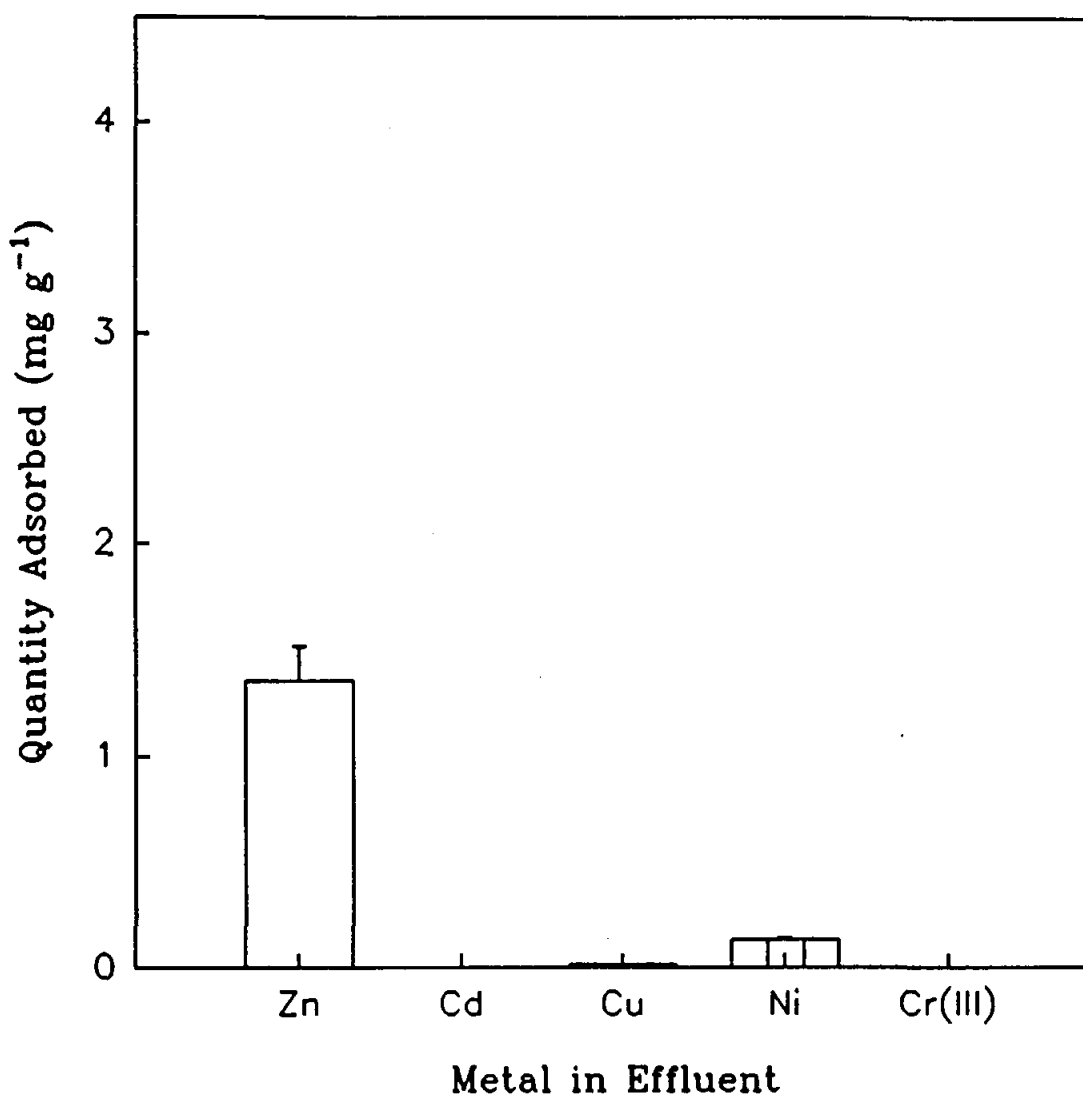


Fig. 8.7. Quantities of zinc (\square), cadmium (▨), copper (▩), nickel (▧) and chromium (III) (▦) adsorbed from wastewater NDC by 25 000 mg ℓ^{-1} activated sludge. Initial concentrations of Zn (86.47), Cd (0.00), Cu (1.40) Ni (10.25) and Cr^{3+} (0.00) in mg ℓ^{-1} .

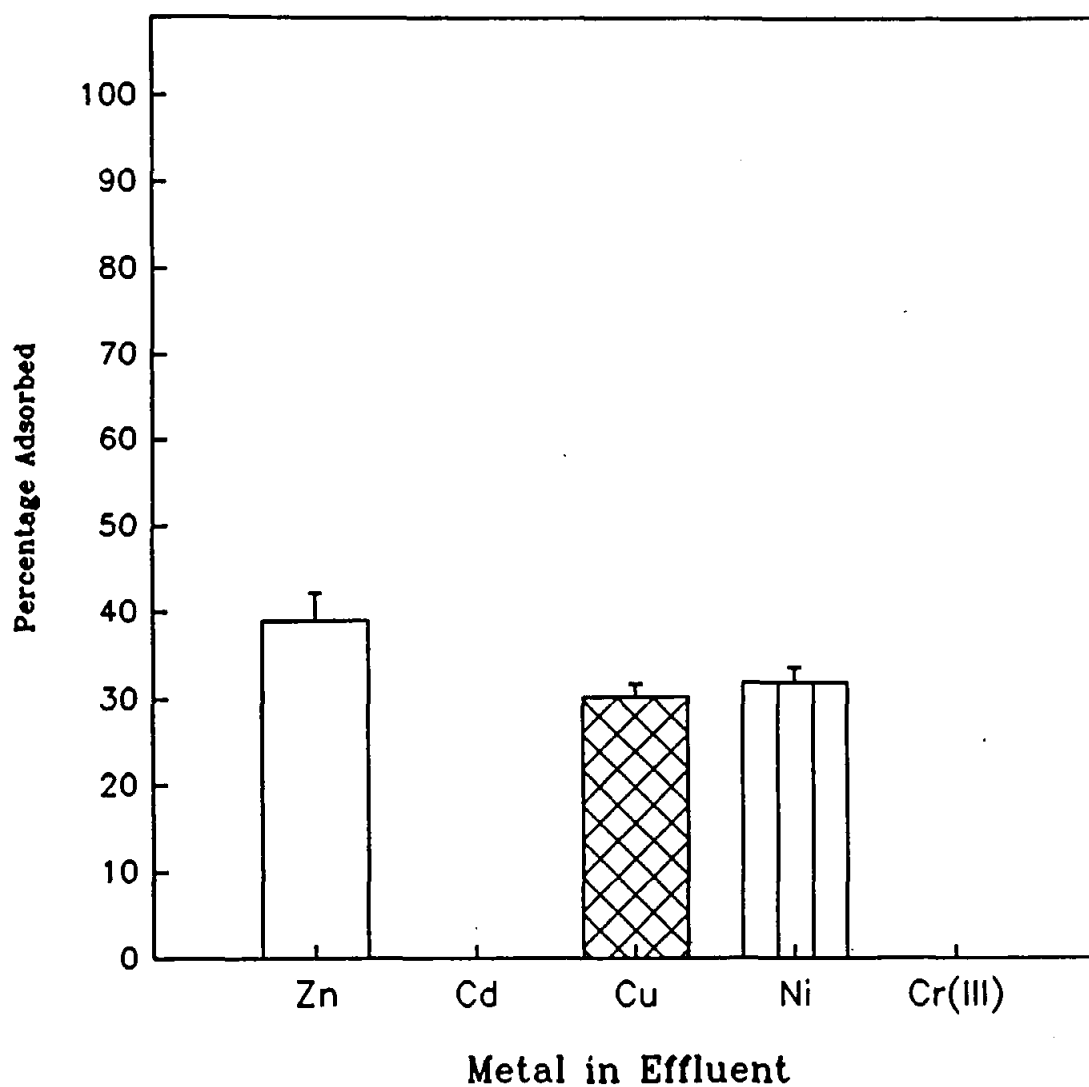


Fig. 8.8. Percentages of zinc (\square), cadmium (diagonal lines), copper (cross-hatch), nickel (vertical lines) and chromium (III) (horizontal lines) adsorbed from wastewater NDC by 25 000 mg l^{-1} activated sludge. Initial concentrations of Zn (86.47), Cd (0.00), Cu (1.40) Ni (10.25) and Cr^{3+} (0.00) in mg l^{-1} .

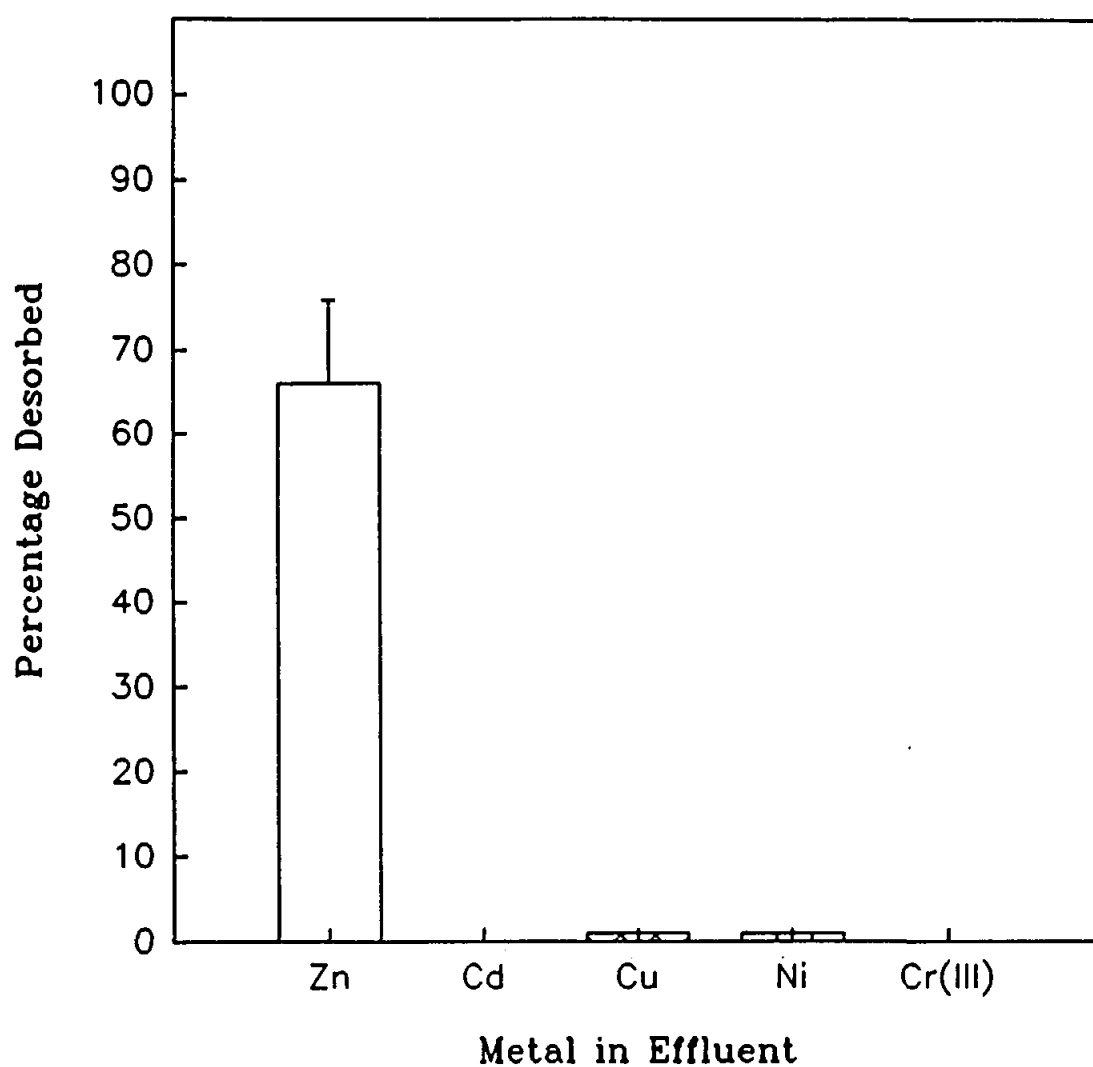


Fig. 8.9. Percentages of zinc (\square), cadmium (▨), copper (▩), nickel (▧) and chromium (III) (▨) desorbed from sludges by 0.2N HCl after adsorption from wastewater NDC.

Table 8.2 summarises quantities of metal-ions removed from sludges. This allows comparison with standards for wastewater discharge. The largest quantity of metal present in all three of the wastewaters is zinc. The greatest efficiency of removal occurred in the alkaline AEV wastewater at 82.46%. This, however, only reduced the quantity from 121.67 to 21.34 mg ℓ^{-1} . This is high considering the quantity of this metal needs to be reduced to approximately 0.3 mg ℓ^{-1} to be discharged legally to any river or tributary. This is only one cycle of adsorption of the metal from the wastewater by sludge. If it was assumed that the removal efficiency would remain at 82.46% then after two cycles the quantity would be reduced to 3.74 mg ℓ^{-1} , after three cycles, to 0.66 mg ℓ^{-1} and after four, 0.12 mg ℓ^{-1} . Four cycles of 90 min., each would last approximately 6 h. However, it has been demonstrated that lower concentrations of metal-ions are more efficiently adsorbed by sludges so 82.46% adsorption efficiency is a conservative estimate when considering further cycles of adsorption. The one drawback is that sludge cannot be reused and fresh adsorbent has to be used in successive cycles, but since the material is cheaply obtained, the cost factor is not a consideration. Adsorption efficiency of zinc seems to be less at the moderate and acidic pHs. One solution is to raise the pH of wastewaters, but the cost of this as opposed to the benefits of increased adsorptive efficiency have to be investigated.

In contrast, the acidic pH MPS wastewater containing high quantities of Cu Ni and Cr^{3+} was most efficiently treated by sludge with removal efficiencies of 79.45%, 91.81% and 95.56% respectively. Maximum quantity of copper disposable to the environment in wastewater may not exceed 1.0 mg ℓ^{-1} and this is achieved in two of the three wastewaters treated with AEV being 1.09 mg ℓ^{-1} after one cycle of treatment.

Total chromium levels disposed of are very stringent, and they need to be, considering the toxic and carcinogenic effects they have on plant and animal life. Their quantity is restricted to $0.05 \text{ mg } \ell^{-1}$ in discharged wastewaters. The two wastewaters containing Cr^{3+} did not have their levels reduced to this standard after one cycle. The projected total number of adsorption cycles required to reduce chromium in alkaline AEV wastewater to an acceptable level of $0.039 \text{ mg } \ell^{-1}$ is 18, while only two cycle is required to reduce acidic MPS chromium levels to acceptable discharge standards of $0.021 \text{ mg } \ell^{-1}$. Perhaps, then, it is feasible to lower the pH of wastewaters to remove chromium (III) species from wastewaters more efficiently.

Table 8.2. Initial, adsorbed and residual quantities ($\text{mg } \ell^{-1}$) of metal-ions in wastewaters treated with $25\,000 \text{ mg } \ell^{-1}$ activated sludge.

Wastewater		Zn	Cu	Ni	Cr^{3+}
AEV	Initial	121.67	1.76	5.48	3.02
	Adsorbed	100.33	0.67	4.87	0.68
	Residual	21.34	1.09	0.61	2.34
MPS	Initial	132.33	4.38	7.70	10.80
	Adsorbed	79.13	3.48	7.07	10.32
	Residual	53.20	0.90	0.63	0.48
NDC	Initial	86.47	1.40	10.25	0.00
	Adsorbed	33.67	0.42	3.27	0.00
	Residual	52.80	0.98	6.98	0.00

8.4 CONCLUSIONS

Sludges were capable of removing metal ions from effluents of different pHs but the efficiency of removal was related to the pH of the effluent. Highest adsorption of zinc occurred at a slightly alkaline pH while Cu, Ni Cr (III) were more efficiently removed at an acidic pH. For optimal biosorption of metal ions from effluents, the pH as well as the concentration of metal needs to be considered. The former may be optimised, and, if the latter is too high, the effluent may be diluted or the need for successive biosorption cycles must be considered.

9.0 GENERAL CONCLUSIONS, RECOMMENDATIONS AND FEASIBILITY STUDY

9.1 GENERAL CONCLUSIONS

The activated sludge environment contained a diverse population of microorganisms. The turbulent nature of activated sludge favoured the predominance of adaptable species of organisms.

All activated sludges were capable of biosorption of metals from single solutions and wastewaters containing mixtures of metal ions. Reduced biosorption was observed in mixtures, due predominantly to competitions among metals for binding sites on sludge surfaces.

Sludges demonstrated different uptake capacities for metals and were ranked accordingly. Mechanisms of metal uptake were inferred using an adsorption model. The predominant mechanism of sludge biosorption was by the Type I adsorption which indicates a fixed number of sites on sludge surfaces for metal biosorption. The model also allowed the calculation of sludge-metal biosorptive capacity as well as sludge-metal bond strength. The former was used to definitively and objectively rank sludge biosorptive capability. Although there were taxonomic differences as well as biosorptive differences among sludges, there was no correlation between these differences.

Two acids, acetic and hydrochloric acid, were assessed both singly and in mixtures as desorbing agents. Although these agents were capable of desorbing metal ions biosorbed to sludges, they detrimentally affected the sludges' biosorptive capabilities. Other agents tested were not as efficient at desorption as the acids.

Three methods were employed in an effort to determine sludge surface charge in an effort

to relate this to biosorptive capability. The method of choice was the pH/millivolt meter method since it was rapid and convenient. Sludge surfaces demonstrated a net negative quantifiable charge. Differences in magnitudes of these charges facilitated ranking of sludges according to their electronegativities. The sludge with highest electronegativity also displayed a high capacity for metal-ion adsorption.

Laboratory scale bioreactor trials established that sludges are capable of adsorbing and desorbing metal ions from synthetic and industrial wastewaters. Further, these metals may be recovered by desorption with appropriate agents.

9.2 RECOMMENDATIONS

1. Microbial analysis of sludges did not correlate with sludge metal-biosorptive capability. Rather measurement of sludge surface charge using a pH/millivolt meter would be more appropriate since it is less time consuming and less energy intensive and yields an objective indication of a good biosorbent.
2. Sludges may be used as metal biosorbents and metals may be recovered by desorption with mineral acids but the potential for sludge reuse is minimal since adsorptive surfaces are damaged after desorption. Thus, sludges may only be used once as biosorbents, desorbed and then discarded. Since reuse is a limitation, batch scale adsorption processes are envisaged.
3. The metal uptake capacities of sludges make them appropriate for treating concentrations of between 1 - 100 mg ℓ^{-1} of metal ions in wastewaters.

9.3 FEASIBILITY STUDY

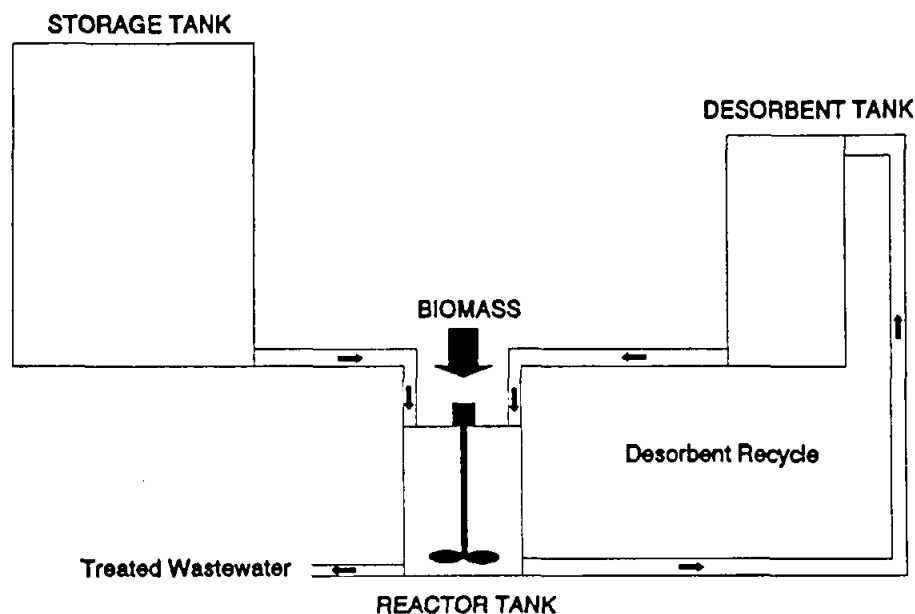


Fig. 9.1 Schematic representation of biological treatment process for the removal of metal ions from industrial wastewaters

During the current study it has been shown that activated sludges are capable of adsorbing and removing metal ions from solutions. Further, these bound metals may be removed by desorption, particularly with HCl.

An envisaged scale-up is depicted in Figure 9.1. Briefly, metal contaminated wastewaters can be stored in large volume tanks. Before treatment, wastewaters in storage tanks need to be assessed for their metal content. Wastewaters high in Cu, Ni and Cr may be pre-adjusted to pH 3 before treatment. Wastewaters high in Zn may be pre-adjusted to pH 9

before treatment. They can then be dispensed in appropriate volumes e.g., 1 000 ℓ volumes to reactor tanks where they may be treated with dried activated sludge. Since the working sludge concentration was found to be 25 000 $\text{mg } \ell^{-1}$ a quantity of 25 kg will be required for treatment of 1 000 ℓ of wastewater.

There is no cost other than transportation for acquisition of sludge since it is a waste product. Wastewater/sludge mixtures require agitation for a minimum of 90 min., for optimal removal of metal ions from wastewaters. Treated wastewaters may then be safely disposed of to municipal outlets. However, if levels of metal ions are not within acceptable limits, a further dose of 25 kg of sludge may be added to the reaction mixture and agitated for a further 90 min. After sludge has settled and wastewater is decanted, an appropriate volume of desorbent viz., 0,3 volumes hydrochloric acid of 0,2N concentration may be dispensed into the reactor tank to desorb metals adsorbed onto the sludge surface. This process normally takes approximately 15 min. After desorption, the desorbent can be recycled into the desorbent tank and reused till exhaustion. Sludge may then be safely discarded either to agricultural land, landfill sites or the sea. Concentrates containing high levels of metal ions may be disposed of in controlled landfill sites or, more economically, the metals may be recovered for reuse by chemical treatment.

The location of the process is an important factor. There are two possible locales in which the process may be established. Firstly, every metal industry discarding hazardous wastewater may need to install the process. Costs for start-up may be prohibitive, but maintenance of the process would be minimal since it is envisaged that only one part-time operator is required to dose sludge, wastewater and desorbing agent and to test levels of metals in untreated and treated wastewater. Also, most industries disposing metal

contaminated wastewaters have storage tanks, which would decrease the cost of start-up of a process of this nature. Secondly, the process can be established at waste sludge producing sites and industries can transport or pump their wastewater to the site for treatment. This will negate the cost of transporting sludge, although there will be cost implications for transport of wastewaters. Costs for start-up and maintenance of metal removal process are detailed in Table 9.1.

Table 9.1 Break-down of costs (approximate values) for establishment of metal removal process.

START-UP		
Planning and Drawing expenses	R10 000	
Labour to site	R10 000	
Storage Tank 3m x 3m x 2.5m	R 20 000	
Reactor Tank 1m x 1m x 1m	R 5 000	
Desorbent Tank 1m x 1m x 1m	R 5 000	
Agitator	R 2 000	
Pumps	R 5 000	
Valves	R 2 500	
		R59 000
MAINTENANCE PER ANNUAL		
Sludge 25 kg x 20 (Transport)	R 1 200	
Part-time Technician	R 24 000	
Desorbing Agent	R 18 000	
		R43 200
GRAND TOTAL		R102 200

10.0 REFERENCES

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APPENDIX 1

Casitone Glycerol Yeast Autolysate Agar

Bacto casitone	-	5g
Glycerol	-	5g
Yeast autolysate	-	1g
Agar	-	16g
Distilled water	-	1ℓ

Dissolve constituents by heating and adjust pH to 7.2 before autoclaving at 121°C for 15 min.1

APPENDIX 2

Rose - Bengal Chloramphenicol Agar

Mycological peptone	-	5g
Dextrose	-	10g
Dipotassium Phosphate	-	1g
Magnesium sulphate	-	0.5g
Rose - Bengal	-	0.05g
Agar	-	15.5g
Distilled water	-	1ℓ

Dissolve constituents in distilled water by heating. Add the contents of one vial of Chloramphenicol Selective Supplement SR 78 (reconstituted) and mix gently. Adjust pH to 7.2. Sterilize by autoclaving at 121°C for 15 min. Cool to 50°C, mix gently and pour into petri dishes.