Biofilm modified activated carbon surfaces for removal of heavy metals and organics

Karanjkar, A. M.

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Karanjkar, A.M.

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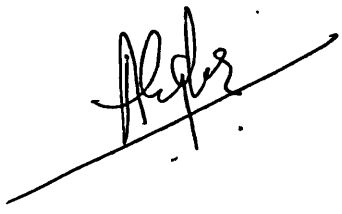

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SUMMARY

Presence of heavy metals in waste water is a major environmental threat. Micro-organisms are capable of concentrating heavy metal ions from an aqueous solution. This work reports removal of heavy metals from aqueous solution using microbial biofilms. Biofilms of polysaccharide producing *Pseudomonas* sp. were developed on fluidized granular activated carbon (GAC). These biofilms-GAC removed heavy metal in the preferential order of: silver, copper, zinc, cadmium, nickel and chromium from trace concentration. Rate constants and transfer parameters, *viz.* mass transfer coefficient and diffusion coefficient were obtained for metal biosorption. Biofilms developed under various physio-chemical conditions, such as pH, temperature, cell viability, age of cell, dissolved oxygen level and fluidization velocity were assessed for metal uptake. Effect of conditions in which the biofilm may encounter trace metal were also assessed.

Methods were developed for building up metal levels through biosorption onto the GAC. Metal build up of up to 8 mg/g of GAC was achieved by six exposure cycles. An integration of biosorption with toxic organic pollutants such as chloroform and atrazine (previously adsorbed onto GAC) was also approached. Biofilms were found to be preferential in take up of cadmium than the organic pollutants such as benzene, butanol and atrazine.

Influence of heat fixed dry biofilm on GAC properties, such as attrition, porosity, basicity and surface area were evaluated. The presence of biofilm on GAC reduced the rise in bed temperature during chloroform vapour adsorption. Zinc recovered through biofilm on the surface of GAC was successfully used to enhance chloroform vapour adsorption. A 15% increase in chloroform adsorption capacity over virgin GAC was achieved with heat fixed metal and biofilm over GAC.

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NOMENCLATURE

GAC	virgin granular activated carbon,
biofilm-GAC	wet biofilm attached to the GAC and used for metal biosorption,
GACBIO	biofilm only fixed to GAC by heat at 110°C
R-GAC	GAC exposed to nitrogen at 230°C for 3 hours
R-GACBIO	biofilm only on GAC exposed to nitrogen at 230°C for 3 hours
GACBIO-M	biofilm and metal (either cadmium, copper, zinc or nickel) fixed to GAC in nitrogen at 230°C for 3 hours

A,	cross sectional area of column, (cm^2)
C_a,	specific heat of adsorbate, (kcal/kg)
C_c,	specific heat of adsorbent, (kcal/kg)
C_v,	specific heat of carrier gas, ($kcal/m^3$)
C_e,	equilibrium metal concentration in solution, (mg/L)
C_o,	vapour inlet concentration, (g/cm^3)
C_x,	vapour exit concentration with time, (g/cm^3)
D and D_{M2+},	diffusion coefficient of metal ions, (cm^2/s)
G,	weight of adsorbed adsorbate, (kg)
H_b,	expanded bed height, (cm)
K,	atrazine adsorption rate constant, (min^{-1})
K_{ad},	metal adsorption rate constant, (min^{-1})
K_f,	measure of biosorption capacity (in Freundlich equation)
L,	height of adsorbent bed, (cm)
Mo,	initial mass of particles in attrition test (g)
M,	weight of adsorbate, (g)
M_f,	final mass of particles in attrition test (g)
$[M^{2+}]_1$,	final metal ions concentration in the solution, (mg/L)
$[M^{2+}]2$ and C_1,	initial metal ions concentration in the solution, (mg/L)
MW_{air},	molecular weight of air, (g)
$MW_{chloroform}$,	molecular weight of chloroform, (g)
P,	equilibrium pressure of chloroform, (mm Hg)
P_o ,	saturated vapour pressure of chloroform, (mm Hg).

Q_h, heat of adsorption, (kcal/kg)

Q, volumetric flow rate of air, (cm^3/min)

Q_o, Langmuir constant

R, gas constant, (cal/mole °K)

V, volume of carrier gas, (m^3)

V_L, superficial linear velocity of carrier gas air, (cm/s)

V_s, volume of GAC in bed, (cm^3)

W, adsorption space occupied by the condensed chloroform, (cm^3/g)

W_d, dry weight of the biomass sample, (mg)

W_e, adsorption capacity, (g/g)

W_o, maximum adsorption space available, (cm3/g)

Y, chloroform vapour concentration in air, (g/g)

a, area from which a biomass sample is collected, (cm^2)

b, Langmuir constant (dimensionless)

d_p, particle diameter of GAC, (cm)

k, constant related to the structure of adsorbent, $(cal/mole)^{-2}$

k_c, external mass transfer coefficient, (cm/s)

k_v, first order rate constant in Wheeler equation, (min^{-1})

m, weight of adsorbate (in Freundlich equation), (g)

m, weight of adsorbed adsorbate, (kg)

n, measure of biosorption intensity (in Freundlich equation)

r_1, radius of GAC, (cm)

r_2, radius of biofilm-GAC, (cm)

r^2, regression constant

t_b, breakthrough time, (min)

q, amounts of metal biosorbed by biofilm-GAC at any time t, (mg/g)

q_e, equilibrium metal level on biofilm-GAC, (mg/g)

v, metal flux velocity, (cm/s)

u, superficial upflow velocity of metal containing solution, (cm/s)

x, amount of adsorbent on adsorbate (in Freundlich equation), (mg/g),

β, dimensionless affinity coefficient

ρ, molecular parachor of the adsorbate

β_{ex}, experimental affinity coefficient

β_{th}, theoretical β values

Δt, rise in temperature, (°C)

ε, bed porosity

ρ_l, liquid density of metal containing solution, (kg/cm^3)

ρ_c, chloroform density, (kg/cm^3)

ρ_d, biofilm density based on dry weight of biomass, (mg/cm^3)

μ, liquid dynamic viscosity of metal containing solution, (kg.s/ cm)

ρ_b, bulk density of the packed bed, (g/cm^3)

ξ, adsorption potential, (cal/mole)

Chapter 1

INTRODUCTION

1.1 Heavy metals in the environment

The pollution of the environment by heavy metals is one of the most serious ecological threat in the present world due to their toxicity to living matter, even at very low concentrations. The main cause of pollution is rapid industrialisation in the last twenty years with increased use of heavy metals and their subsequent discharge into the environment. Table 1.1 summarizes total metal production in 1988 through anthropogenic sources and the percentage of these metals discharged into waste water. Heavy metals are introduced into the environment through the effluent of many industrial activities, such as metal finishing, surface coating, catalysis, mine tailings and petroleum refining. A summary of heavy metal discharges by various industrial activities is given in Table 1.2. Typically, the metals discharged are found in low concentrations and are usually accompanied with organic and inorganic chemicals. However, the appearance of metals in the environment is not limited due to industrial effluents alone. Domestic use of metals *e.g.* in batteries and pigments in paint, also leads to contaminating domestic water in similar way.

Heavy metals are of great concern as they become concentrated through the food chain to the extent of posing a serious health hazard to humans and other living organisms. In particular, the effects of acidic mine effluent on freshwater ecosystems are now well known. For example, the Itai-Itai catastrophe at the Jintsu River in Japan (Wilmonth *et al.*, 1991), has been connected with cadmium rich effluent. Other examples are, Cornwall (England), Silesia (Poland), Siberia (Russia), Colorado (USA), the Philippines and Tasmania (Australia) (Wilmonth *et al.*, 1991). Although different

metals were found in these sites, in all cases the levels of dissolved nickel, manganese and iron exceeded the normal surface water concentration by a factor of more than 10000; values of zinc and chromium increased 1000 fold; and lead and cadmium enrichments were higher than 100 fold. More recently, in 1992, 10 million gallons of water containing cadmium, zinc, arsenic and iron at different concentrations (4 to 100 mg/L) flowed down into the Carnon river in Cornwall (Williams, 1994).

Table 1.1 Toxic metals through anthropogenic sources and their discharges as solid waste and in sewage.

Metal	**Total source** tonnes/year	**% of Source metal in:**	
		Solid waste	**Sewage and Sludge**
Cadmium	38970	2.94	62.00
Chromium	172480	11.80	35.50
Copper	147370	18.97	27.75
Lead	346150	12.10	4.75
Nickel	380650	0.07	15.94
Zinc	1727880	3.76	6.00

Source: Nriagu and Pacyna (1988)

Standards for the level of metals in drinking water have been issued nationally by many countries, as well as internationally by the World Health Organisation (WHO) and the CEC (Council of European Communities). The level of treatment needed to meet these standards is indicated by comparison of heavy metal discharges levels

(Table 1.2) and acceptable levels (Table 1.3).

Table 1.2 Typical heavy metals concentrations in different discharges

Metal	mg/L of metals in different discharges					
	1	2	3	4	5	6
Cadmium	0.8	32	1.5 - 2.5	-	_	-
Chromium	1.73	-	20 - 200	-	6	-
Copper	2.11	223	6 - 140	1.0	68	65
Nickel	3.14	13	15 - 70	31.6	103	10
Zinc	42	61	100 -1000	1.5	-	-

1. Metal finishing waste (Cheremisinoff, 1992)
2. Boiler cleaning water (Holcombe *et al.*, 1987)
3. Sewage effluent (Sterritt and Lester, 1986)
4. & 5. Waste water from electronic industry (Sternberg, 1987)
6. Electroplating waste water (Low *et al.*, 1994)

Table 1.3 Standards and guidelines for metals in drinking water

Metal	WHO (mg/L)	CEC GL[1] (mg/L)	CEC MAC[2](mg/L)
Cadmium	0.005	-	0.005
Chromium	0.05	-	0.05
Copper	1.00	0.1	-
Lead	0.05	-	0.05
Nickel	-	-	0.05
Silver	-	-	0.01
Zinc	5	0.1	-

1. Guidelines 2. Maximum admissible concentration (WHO , 1984; Ewers, 1991)

1.2 Removal of heavy metals by biosorption

Removal of toxic heavy metal contaminants from aqueous waste streams has been tackled for many years, although effective treatment options are limited (Morper, 1986). Chemical precipitation, ion exchange, reverse osmosis and solvent extraction have all been used to remove metals from solutions. However, these methods can have disadvantages such as incomplete metal removal, high energy and reagent costs, and the generation of waste products or toxic sludge that would in turn require disposal. Table 1.4 gives an overview of the advantages and the disadvantages of present metal decontamination systems.

In the search for new and innovative treatment technologies, there has been a focus on the metal binding capacities of various micro-organisms. Micro-organisms and associated biomass are well known for their ability for sorbing toxic heavy metals (Volesky, 1990). Micro-organisms can interact with heavy metals, firstly, in order to use them in metabolic requirements (in very limited amounts), and secondly, to prevent excess heavy metals entering into the cell cytoplasm. The mechanism to prevent metal entry into the cell cytoplasm varies with the micro-organism. One mechanism is precipitation/chelation of metals on the cell wall. This accumulation of metals by microorganisms and their associated biomass is called Biosorption, and has been defined as the " ...sequestering of metal ions and organic molecules from aquatic solutions by microbial biomass" (Volesky 1990).

Biosorption offers a number of benefits over conventional metal removal techniques. These benefits include: a versatile process, non-generation of toxic wastes which would need further treatment, simplicity of in design and low process costs. However,

the mechanisms associated with biosorption are complex and involve both extracellular and intracellular binding of metals to the micro-organism.

Table 1.4 Comparison of treatment technologies to remove/recover metals (Brierley *et al.*, 1986)

Properties of Technique	**Technology**					
	1	**2**	**3**	**4**	**5**	**6**
Conc. dependence	Ok	No	Ok	Ok	No	Ok
Expected conc. of effluent (mg/L)	< 1	2-5	< 1	No*	1-5	1-5
pH adjustment	Ok*	No	Some	Some	Some	Ok
Selectivity	Ok*	No**	Ok*	No	No	No
Efficiency	Ok	No	Ok	No	No	No
Versatility	Ok	No	Ok	Ok	Ok	No
Organic tolerances	Ok	Ok	No	Ok	No	Ok
Regeneration	Ok	No	Ok	Ok**	No	No
Cost	Ok	Ok	Some	Some	No	No
Flow equalization	No	Ok	No	No	No	No

Technology:

1. Biosorption **2.** Precipitation **3.** Ion Exchange

4. Activated Carbon **5.** Membrane **6.** Evaporation

Key:

Ok: not required, good

No: required, not good

Ok*: sometimes required for specific application

Ok**: regeneration of GAC possible

No**: sulphide precipitation can be selective

No*: it is good in some special application *e.g.*, gold uptake

Some: average

Intracellular or active mechanisms involve metabolic metal uptake into the cell, which is often plasmid-linked (Doyle *et al.*, 1980; Volesky, 1987), whereas extracellular metal accumulation particularly excreted bacterial slim coatings, is a more rapid mechanism and probably plays the significant role in metal extraction from waste water (Scott and Palmer, 1988).

Although living microbial populations effectively sorb heavy metals, many biosorption systems presently available are cumbersome. In order to maintain a living biosorption system, it requires addition of nutrients and is even then difficult to maintain due to the toxicity of the waste water. However, with slime forming bacteria it has been found that the excreted polymers and not metabolic activity accounts for the majority of biosorption (Scott *et al.*, 1988). Similarly, with some organisms, dead biomass adsorbs metals as effectively as living organisms (Tsezos, 1985).

Researchers have recognised that by immobilising the micro-organisms and biomass into a granular or porous substrate, metal uptake performance improves. Immobilization also facilities subsequent separation of the biomass from the aqueous solutions. Immobilized viable micro-organisms can form comprehensive polymer based biofilms over the substrate. In addition, micro-organisms tend to produce more polymers as a result of being attached (Vandevivere and Kirchman, 1993), and have shown greater binding of heavy metal when immobilized (Lion *et al.*, 1988). A wide range of substrates can be used for immobilization; these include sand, wood, foams and activated carbon.

Granular activated carbon (GAC) is extensively used in waste water treatment

technologies (Cheremisinoff and Morresi, 1978). GAC has also shown a tendency to support the attachment of micro-organisms (Camper *et al.*, 1986). When the dissolved organic matter present in the waste is biodegradable and adsorbs onto GAC, bacterial growth can follow. The benefits offered by GAC as a support for the biofilm are, (i) large surface area, (ii) porous structure and (iii) an ability to adsorb a wide range of organic chemicals which can provide nutrients for the micro-organisms. Based on these useful properties, if the GAC can be used efficiently in heavy metal removal, its applicability and effectiveness in waste water treatment will extend. This consideration led to the process adopted during this research which comprised the development of a biofilm of exopolysaccharide producing bacteria over GAC, subsequently used for heavy metal(s) biosorption.

The use of GAC offers a number of advantages, other than simply the attachment of cells. These are as follows:

(i) GAC has an extensive adsorption capacity for organic materials, *e.g.*, chloroform, benzene, phenol and pesticides. The adsorbed toxic organic compounds could be utilized as a source of carbon for cell functions or biodegraded. (Bouwer and Zehnde, 1993). Therefore, an integrated biodegradation and biosorption approach could be developed.

(ii) As the presence of certain heavy metals enhance the surface activity of the GAC (Bansal *et al.*, 1988; Henning and Schäfer, 1993), the resulting material (*i.e.* the biosorbed metal over GAC) may be reused in other applications, such as catalysis and toxic organic compounds adsorption (Afzal *et al.*, 1993).

Over the last twenty years, biosorption of heavy metals has developed from a laboratory curiosity into a full scale industrial process. For example, a few biosorption processes have already been patented in North America (Volesky, 1990; US Patent, 1991; Gadd and White, 1993).

Major areas for the potential application of biosorption are:

(i) Decontamination of heavy metals from industrial waste waters (Barnes *et al.*, 1993).

(ii) Biological metal recovery in the treatment of nuclear waste. A wide range of micro-organisms have been investigated for radionuclides and in particular uranium recovery (Macaskie and Dean, 1985; Brierley, *et al.*, 1986; Ashely and Roach, 1990; Sakaguchi and Nakajima, 1991).

(iii) Commercialization in the recovery of precious metals, such as gold and silver, from mine effluent streams (Darnall, *et al.*, 1986; Finnegan *et al.*, 1991; Rusin and Sharp, 1991).

(iv) Mineral engineering is utilizing the metal accumulation ability of micro-organisms for recovering metals from lower grade, more finely disseminated and more refractory ores. There is a substantial research trend towards "mineral bioprocessing" (Ogunseitan and Olson, 1991).

1.3 Objectives

Micro-organisms which excrete extracellular polysaccharides can accumulate more metal than non-polysaccharide producing strains (Scott *et al.*, 1992). Polysaccharide producing micro-organisms are also known to have better resistance to metal toxicity (Hughes and Poole, 1989). Therefore, a bacterial strain, a *Pseudomonas* sp. (NCIMB

11592) was selected for this research on the basis of its proven polysaccharide producing ability. Although, micro-organisms can be immobilized on various support surfaces, GAC was chosen due to its key role in existing environmental technologies. In this research, the *Pseudomonas* sp., was immobilized over GAC surfaces in a fluidized bed and subsequently used for biosorption of heavy metals.

The overall objective of this research was to develop a microbial biofilm that would remove heavy metals from solution, and then see if a fixed biofilm-metal-GAC system could be advantageously applied in a vapour adsorption process.

In order to achieve the overall objective, the following studies were made in this research.

(1) Optimal microbial conditions for development of biofilm for biosorption, such as culture age and metabolic activity.

(2) The efficiency of metal removal (using optimal biofilm) from solution. Metals considered were cadmium, nickel, zinc, chromium, copper, and silver. These metals were looked at individually, as well as in combination.

(3) Metal uptakes in terms of rate kinetics and predictive adsorption capacity models, such as Langmuir and Freundlich. The process transfer parameters of biosorption system, such as mass transfer and diffusion coefficient were also assessed.

(4) Biofilms over GAC were developed under different physio-chemical conditions

and evaluated for their metal removal ability. Conditions considered were pH, temperature, fluidising velocity and the GAC (particle size, source and regeneration). The production of polysaccharide under different temperature culture conditions was assessed. Extracted bacterial polysaccharide was assessed for its ability to fix onto GAC and for subsequent metal removal capacity.

(5) The influence of the physio-chemical conditions of the aqueous metal containing solutions on metal removal using the biofilm-GAC system was evaluated.

(6) The simultaneous removal by the biofilm-GAC of metal and organic compounds such as a pesticide (atrazine), butanol and benzene, was analyzed. A potential integrated biosorption and biodegradation route for metal decontamination and organic compound degradation was assessed.

(7) The reuse of previously used GAC (*i.e.* in biosorption) for further metal removal, by repeated cycles of biofilm regeneration or metal fixation were assessed. The extent of metal loading was confirmed by X-ray diffraction techniques.

(8) Biofilms were developed over GAC which was previously saturated with toxic organic materials (chloroform and pesticide). These biofilms were subsequently assessed for their metal removal capacity.

(9) The GAC surface with fixed biofilm-metal was analyzed for its properties, such as surface area, porosity, particle size, basicity and attrition.

(10) The benefits and drawbacks of biofilm-metal laden GAC as a potential adsorbent of organic vapours, such as chloroform, was studied. Adsorption of vapours over virgin GAC and metal laden GAC was analysed in terms of adsorption capacity and rate constants using established mathematical models.

Chapter 2

LITERATURE REVIEW

In the course of this research, the ability of micro-organisms to interact with heavy metals was utilized through a biofilm formed by them over GAC surfaces. The first section of this chapter, therefore, deals with metal-micro-organism interactions, metal accumulation, and the conditions affecting the interactions. The second section is related to the formation of biofilm and factors contributing to it. Since GAC was chosen as a support for biofilm attachment, it is reviewed in the third section. Finally, in this research, the potential of metal attached to GAC to adsorb an organic vapour (*i.e.* chloroform) was examined. Therefore, GAC as an organic vapour adsorbent and applications of metal impregnated GAC are also reviewed in the third and final section.

2.1 Metal interaction with microbial biofilm

In a system consisting of metals, micro-organisms and a solid surface, several interactions are possible. Lion *et al.*, (1988) described metal binding in a system (metals, micro-organisms and a solid surface) as a result of binding to: (i) the original solid surface, (ii) the surface of free or attached cells and (iii) polymers produced by the cells. In this section, interactions of metal with micro-organisms and their extracellular polymer are reviewed.

2.1.1 Interaction between heavy metals and micro-organisms

Heavy metal-micro-organism interactions need to be considered, in order to understand metal accumulation. Micro-organisms are generally the first organisms to interact with

heavy metals discharged into the environment. The interaction between heavy metals and micro-organisms has been studied by researchers from two inter-related aspects, namely the effect of the metals on the micro-organism and secondly, the effect of the micro-organism on the metals.

(I) The nature of interaction

Physical, chemical and biological interactions have all been observed between metals and micro-organisms. With physical interactions, the cell wall captures the metal on its surface and stops the metal from penetrating into the cytoplasm (Ford and Mitchell, 1992). As a chemical response, cells release detoxifying chemicals or create a protective layer around the cell wall (Gordon *et al.*, 1993). For example, an excretion of extracellular polymer has been observed with marine bacteria when they are exposed to toxic metals (Jones, 1970). Biological interaction can result in a change in cell morphology and development of resistance against heavy metal toxicity (Novak *et al.*, 1992). An example of a biological response is that of the bacterium *Heterocystous cynobacteria,* which changes its growth and nitrogen fixing capacity (Rath *et al.*, 1986; Sahu *et al.*, 1987).

Volesky (1990), classified cell response on the basis of metal binding sites; metals either penetrate through the cell wall into the cytoplasm, or they are precipitated or chelated on the cell wall. Precipitation / chelation of metals on the cell wall is usually known as Biosorption, whereas, metal passage into the intracellular cytoplasm is called Bioaccumulation.

Ford and Mitchell (1992), went further and divided microbial-metal interactions into

six distinct categories. These are, (i) intracellular accumulation, (ii) cell wall adsorption of metals, (iii) metal-siderophore interaction (siderophore are low molecular weight organic compounds), (iv) extracellular mobilization by bacterial metabolites, (v) extracellular polymer-metal interactions and (vi) transformation and volatilization of metals.

A two stage metal-micro-organisms interactions, that is, active and passive, has been considered by many researchers (Norrsi and Kelly, 1977; Brown and Lester, 1979; Norberg and Persson, 1984; Ting *et al.*, 1989; Brieley, 1990; Grappelli *et al.*, 1992).

(i) Active interaction is carried out by living, metabolically active micro-organisms. Precipitation, oxidation, methylation and intracellular accumulation are the mechanisms taking part in an active process. These active interactions are slow and metal ions are gradually transported and accumulated within the cytoplasm.

(ii) An integrated physico-chemical action between metals and micro-organism is involved in passive interaction and follows instantaneous binding of metals to cellular surfaces. Metal chelation by extracellular cell products and physico-chemical binding to cell surfaces, are the typical mechanisms of passive interactions.

Most studies in the metal-micro-organism interaction have centred on the ability of micro-organisms to retain metals and only few have attempted to elucidate the metal binding mechanism (Brinckman and Olson, 1986; Simkiss and Taylor 1989).

Brinckman and Olson (1986) suggested that micro-organisms interact with molecules

containing heavy metals through one of several ways in metabolism or in resistance mechanism. In metabolism they interact as, (i) energy sources, (ii) electron acceptors, or (iii) enzyme cofactors. In mechanisms of toxic element resistance interaction result in, (i) oxidation, (ii) reduction or derivatization with subsequent precipitation or (iii) volatilization of the element. These reactions may occur intracellularly, pericellularly or extracellularly.

2.1.2 Sites for accumulation of heavy metals in the biomaterial

Interactions reviewed earlier, are essentially responses of micro-organisms to the metal. The removal or accumulation of metal by biomaterial (cell and associated biomass) that was used in this research can be classified into three categories:

(i) Cell wall adsorption, *e.g. Rhizopus arrhizus* (Tsezos and Volesky, 1981), *Saccharomyces cerevisiae* (Strandberg *et al.*, 1981), *Penicillium digitatum* (Galun *et al.,* 1984).

(ii) Intracellular-metal accumulation, *e.g. Escherichia coli* (Beveridge, 1986), *Citrobacter* sp. (Macaskie, 1990).

(iii) Binding of metal to extracellular material, *e.g. Pseudomonas aeruginosa* (Strandberg *et al.*, 1981), *Acinetobacter* (Zosim, 1983), *Arthrobacter, Pseudomonas* sp., *Enterobacter aerogenes* (Scott and Palmer, 1990). Some of these micro-organisms have been shown to be capable of accumulating metal intracellularly, *e.g.* cadmium accumulation by *Arthrobacter* and *Pseudomonas* sp. (Scott and Palmer, 1990), and zinc and lead accumulation by *Arthrobacter* sp. (Grappelli *et al.*, 1992).

(I) Cell wall accumulation

The cell wall forms the outermost limit of a cell, and is in immediate contact with the surrounding environment. Interaction between bacterial cell walls and metal ions results from different processes, such as adsorption, ion-exchange and covalent binding, redox reactions, entrapment of particles and precipitation (Gadd and White, 1993).

The cell wall is characterised as a negatively charged (anionic) surface with an affinity, therefore, for cations (Ehrlich, 1986; Hughes and Poole, 1989; Mann, 1990). These surface charges are due to functional groups and the spatial structure of the cell wall. It has been shown that the cell wall has a greater ion exchange capacity than that of commercial ion exchange resins (Tsezos and Volesky, 1981). However, these capacities are governed by functional groups present on the cell wall. Thus, both the chemical composition of the cell wall and its surface charges, can be responsible for metal interactions.

The cell wall is made up of two membrane bilayers, the outer layer contains lipopolysaccharides and is usually linked chemically to a peptidoglycan monolayer (Hughes and Poole, 1989). These lipopolysaccharides with closely associated reactive sites (*e.g.* phosphate group) bind them with metals. The exact chemical composition of the cell wall is strongly dependant on culture conditions which can subsequently affect metal-cell interactions (Tobin, *et al.*, 1994). Furthermore, some biomolecules function specifically to bind metals and are induced by their presence (Gadd and White, 1993). By this mechanism, metals are immobilized and their entry into the cell is prevented (Ford and Mitchell, 1992).

The specific influence of the cell wall composition on metal interactions has been the subject of several studies. Cho *et al.*, (1994), modified the surface functional carboxyl and amine groups of *Chlorella vulgaris* and subsequently observed that carboxylic groups provided the major site for adsorption of cadmium and zinc. An increase in the nickel binding ability of *Bacillus subtilis* was found with modified amine groups, but conversely a decrease in copper binding (McLean and Beveridge, 1990). The cell wall of *Bacillus subtilis* which contains peptidoglycan, has favoured Fe(III), Mn, Cu, Zn, Hg, Au, Ag, Ni and Pb metal ions (Beveridge and Murry, 1980). Teichoic and teichuronic acids in the cell wall of *Bacillus licheniforms* were found to attract Cu, Ni, Fe, Mn and Au (Beveridge *et al.*, 1982). In an investigation on effects of inorganic lead salts on *Azotobacter* sp., 37.6% of the lead accumulated was found in the cell wall (Sterritt and Lester, 1980).

Interactions of the cell wall are predominately of the ion-exchange type which can be modified. However, the contribution of the cell wall in total metal accumulation by biomaterials is not revealed in the literature.

(II) Intracellular accumulation

In intracellular accumulation, toxic metals flux across the cell wall membrane. These metals are transported by one, or a combination of, mechanisms. Suggested mechanisms are lipid permeation, complex permeation, carrier mediated, ion channel, ion pump and endocytosis (Simkiss and Taylor, 1989). Once through the cell wall, metals are released and incorporated into biochemical pathways, or trapped in an inactive form by complexation with high affinity ligands (Ford and Mitchell, 1992).

Metals and intracellular interactions were studied by Beveridge (1986) and Macaskie (1990). Research by Macaskie (1990), suggested that *Citrobacter* sp. accumulated cadmium through cell bound cadmium phosphate. Beveridge (1986), found that the hydrophillic faces of the outer membrane in *E. coli* provided the site of metal interactions. Outer membrane protein species form channels or pores. These pores (*e.g.* in *E. coli)* allow substances with a molecular weight of up to 600-1000 to freely pass through the membrane.

In general, interacellular metal accumulation is rather limited to specific species and only a few studies on it are reported.

(III) Extracellular accumulation

Extracellular interactions of metals with micro-organisms are predominant in the accumulation of metals. In particular, excreted polymers bind metals through entrapment or by forming metal-polymer complexes. A polymer excreting *Pseudomonas* sp. was used in this research. Therefore role of extracellular polymer in metal sorption is reviewed below.

(A) Role of extracellular polymers in metal accumulation

Metal and extracellular polymer interactions have been extensively studied. Attempts have been also made to use only the excreted polymers for the recovery of heavy metal from waste water (Onsoyan and Skaugrud, 1990; Grappelli *et al.*, 1992).

Extracellular polymers produced by micro-organisms act as a buffer between the cell wall and the external environment. These exopolymers may extend from 0.1 to 10 μm,

from the cell surface into the surrounding environment (Geesey and Jang, 1990).

Two types of interactions between extracellular polymers and metals have been suggested. Firstly, entrapment of metals by negatively charged functional groups on the exopolymers (Hughes and Poole, 1989; Ford and Mitchell, 1992). Secondly, formation of metal-polymer complexes (Rudd *et al.,* 1984), as metals have a greater tendency to associate with organic matter than the cell wall. Extracellular polymers excreted by strains of genus the *Zoogloea* form Ni-complexes (Mann, 1990), and copper complexation was observed with exopolymers produced by *Thermus* sp. (Black *et al.*, 1986). The ionic radius of the metal appears to determine whether polymer metal complexation will occur and the affinity usually decreases with increasing ionic radius of the metal (Geesey and Jang, 1990).

The metal binding capacities of extracellular polymer vary with chemical composition. Polysaccharides, proteins and nucleic acids have all been identified in samples of extracellular polymers. A wide range of metal-binding chemical groups have been found in polysaccharides produced by cells (Painter, 1983). These include, pyruvate, phosphate, hydroxyl, succinyl, and uronic acid. Many bacterial and algal polymers are composed of acidic polysaccharides, the properties of which are primarily due to free carboxyl groups of uronic acid subunits, or pyruvylated sugars (Geesey and Jang, 1990). Capsular extracellular polymers of *Klebsiella aerogenes* have been characterised as 50% glucose, 29% uronic acid, 10% fructose and 1% galactose (Brown and Lester, 1982b). These groups may render selectivity towards metals (Hughes and Poole, 1989). Ebube *et al.*(1992), isolated a polysaccharide from *Bacillus licheniformis* and identified it as a heteropolymer comprising D-Glucose, D-Mannose

and D-Xylose.

The presence of exopolymer around the cell surface has been shown to contribute significantly in metal biosorption. Grapepelli *et al.*, (1992) found that Cu and Cd were concentrated three to five times more by capsular polymers than cells. Enhanced cadmium uptake was found by polymers excreting *Arthrobacter viscosus*, as compared to non-polymer excreting *Arthrobacter globiformis* (Scott *et al.*, 1988). Extracellular components in *Actinomycetes* biomass have been found to play a profound role in lead and copper binding (Pradhan and Levine, 1992a). Cultures of *Azotobacter* sp. (Tornabene and Edward, 1972), *Sphaerotilus natans* (Hatch and Menawat, 1978), *Citrobacter* sp. (Macaskie and Dean, 1982), have all been examined for their metal interaction with exopolymers.

Extracellular polymers, when present, thus have a dominant role in metal accumulation through complex formation and physical entrapment. These polysaccharides offer great potential towards metal selectivity and accumulation through modification of functional groups. But the effects of pH, temperature and presence of the multi-cation along with metal on complex formation are not clear.

2.1.3 Action of heavy metals on micro-organisms

In this research biofilm-GAC system adopted was exposed to multi-metal system. Hence, in this section effects of the metal on micro-organisms and metal-metal interactions are reviewed.

(I) Metal toxicity and microbial tolerance

Several workers have studied the toxicity of heavy metals with pure bacterial cultures. Species have shown different responses, probably due to different biochemical and morphological characteristics (Sterritt and Lester, 1980). The major factor determining the toxicity of heavy metals is probably the extent to which they penetrate the cytoplasm and thus affecting enzymatic activity (Olson and Panigrahi, 1991).

Although different species exhibit different responses to heavy metal toxicity, some common trends are evident. Sub-lethal concentrations of metals affect bacteria in growth, viability, respiration (Sunda *et al.*, 1978; Hsieh *et al.*, 1994b), morphology and composition of the cell wall (Tobin, *et al.* 1994). For example, cadmium has the effect of extending the lag phase of *E. coli*, and during that phase up to 95% of cells may lose viability (Mitra *et al.*, 1975). Similar effects were observed with mercuric chloride on *Rhodobacter capsulata* (Vaituzis *et al.*, 1975) and copper on *Vibrio alginolyticus* (Gordon *et al.*, 1993). The toxicity of metal varies with the type of bacteria. For example, Gram negative bacteria generally exhibit greater metal tolerance than Gram positive bacteria (Hughes and Poole, 1989).

Resistance of micro-organism to toxic elements has been shown to occur through genetic modifications (Silver and Misra, 1983), or through production of extracellular material to protect the cell wall (Corpe, 1975). In the case of cadmium, resistance has been shown to be mediated by a plasmid, with two separate genes involved. These genes also prevent Zn^{+2} toxicity (Olson and Panigrahi, 1991).

(II) Effect of metal-metal interactions

The toxicity of a metal may be enhanced by the presence of another metals, a phenomenon referred to as synergism. Alternatively, one metal may protect an organism against the toxic effect of another, *i.e.* act antagonistically (Simkiss and Taylor, 1989). For example, zinc reduces the toxic effect of cadmium (Beyersmann, 1991). The presence of a non-toxic or less-toxic metal of similar ionic radius as a toxic metal, can decrease toxicity in competition for binding sites (Gadd and Griffiths, 1978). For example, Mg and Ca, reduces the toxicity of nickel to bacteria (Beyersmann, 1991).

2.1.4 Effect of environmental conditions on metal accumulation

Numerous industrial and mining activities discharge effluent containing recoverable metals. Any subsequent accumulation of these metals by micro-organisms varies, depending on the physico-chemical conditions of the environment, such as pH, temperature and presence of other competing ions.

(I) Effect of pH

Metal binding ability of micro-organisms is strongly affected by the presence of other anions and cations including, H^+ ions. Both bioaccumulation and biosorption of cations such as copper, cadmium, zinc and nickel are decreased by low pH (Holan *et al.*, 1993; Cho *et al.*, 1994; Tobin *et al.*, 1994). For example, in the case of cadmium uptake by the biomass of *Ascophyllum nodosum*, uptake declined by 90% at pH 2, compared to that recorded at pH 4.9.

As a consequence of low pH, the biomaterial alters through (i) the isoelectric point of

a protein, which subsequently alters surface charges (Dranell *et al.*, 1986); (ii) viability of cells (Tanner and James, 1992); (iii) extracellular polymers produced (Novak *et al.*, 1992). All these factors contribute to decline in metal uptake. The influence of pH on metal binding by biomaterial is similar to that of hydrous adsorbents (Park and Huang, 1987). That is, surface complexation between metal ions and the charged adsorption surface was employed to explain such pH dependent adsorption.

On the other hand, solubility is an important factor. For example, nickel, which is the most soluble of the metal sulphide at neutral pH, displays a rapid accumulation in cells as pH is lowered (Theis and Hayes, 1982). However, such an accumulation is specific to particular metals, *e.g.* lead compared to nickel is less accumulated by cells under identical condition (Theis and Hayes, 1982).

The affinity between metal and micro-organisms and their biomass is thus an important aspect. Any change in the affinity may alter metal binding in the biomaterial.

(II) Effect of temperature

With respect to metal interaction temperature can have both negative and positive influence. It enhances polysaccharide excretion, on the other hand, it can cross-link metal binding sites in cells or polysaccharides, thus affecting metal uptake.

Tsezos (1986) reported that an increase in temperature from 4°C to 25°C resulted in a 75% increase in copper and zinc biosorption capacity of viable cultures (*e.g.* *Penicillium* sp.). However, McKay et al., (1986), found 30% decline in mercury

uptake by chitosan at 60°C compared to that at 25°C.

2.1.5 Metals and uptake processes

The application of biosorption will depend on both the type of metal and the process employed to achieve a contact between biosorbent and metal. Different metals, in particular those studied during the course of this research and their uptake processes are reviewed in this section.

(I) Metals

Metals can be classified as class A, class B and borderline according to their interaction with cells (Simkiss and Taylor, 1989; Collins and Stotzky, 1992). Metals that are classified as A (*e.g.* Ca, K, Mg and Na) are essential for micro-organisms. Metals, such as copper, iron, manganese and zinc, which are required in trace concentrations are classified as borderline. Metals, such as cadmium, mercury and lead, which are not necessary for biological functions and are toxic, are class B type.

(A) Cadmium

Cadmium has no known metabolic role. High doses of cadmium can lead to pronounced toxic effects. Cadmium has been the most studied heavy metal in biosorption systems. A wealth of information is available on interaction of cadmium with the cell and associated biomass (Bruke *et al.*, 1991; Apel and Torma, 1993; Cunningham and Lundie, 1993; Holan *et al.*, 1993; Volesky *et al.*, 1993; Volesky and Prasetyo, 1994).

(B) Chromium

Over the past several decades, increased quantities of chromium compounds have been used by man and introduced into the environment. The danger of environmental contamination depends on the oxidation state of chromium. In its hexavelant form it is 100 to 1000 times more toxic than the most common trivalent compounds (Gauglhoffr and Bianchi, 1991). Due to toxicity of hexavalent chromium, in this research trivalent chromium was used. Interactions of micro-organisms with chromium has been reported by Torma and Apel, (1991); Gopalan and Veeramani (1994) and Nourbaksh *et al.*, (1994).

(C) Copper

Copper is involved in many metabolic processes in living organisms. However, excess concentration of copper is toxic to many bacteria. Copper has high affinity to, and forms complexes with, organic molecules (Geesey *et al.*, 1992). In its complex form, it is not toxic to cells *e.g.* copper-organic complex to *Vibrio alginolyticus* (Schreiber *et al.*, 1985). Copper has been studied in biosorption, both as a single metal, and along with other metals (Brown and Lester, 1982a, b; Clark and Ehrlich, 1992; Geesey *et al.*, 1992; Low *et al.*, 1994).

(D) Nickel

Nickel is a common toxic metal present in wastewater, particularly in metal-plating effluents. Studies focusing specifically on nickel biosorption are rare, *e.g.* Zouboulis and Kydros, (1993), and Holan and Volesky, (1994), as previous work mostly covered sorption of nickel in a range of other heavy metals (Brown and Lester, 1979; Collins and stotzky, 1992; Mattuschka and Straube, 1993). Nickel has low affinity for the

polymers (*e.g.* its binding to *Klebsiella aerogens* polymers, Brown and Lester, 1982b) and its binding is more dependant on the chemical nature of the cell wall (Mclean *et al.*, 1990).

(E) Silver

Silver is non-toxic to humans, but it is highly toxic to certain aquatic organisms. Silver impregnated filters are used in water purification. Only a few studies were found which consider the removal of silver from water by biosorption, *e.g.* Darnall *et al.*, (1988).

(F) Zinc

Zinc plays an important role (*e.g.* in growth) as an essential trace element in all living systems, from bacteria to humans. In effluents, zinc is often accompanied by impurities such as cadmium and lead, which are more toxic than zinc. Consequently, most of biosorption studies on cadmium also consider zinc removal (Scott *et al.*, 1986; Costa and Leite, 1991; US Patent, 1991; Apel and Torma, 1993, Mattuschka and Straube, 1993).

(II) Metal uptake processes

A critical review of the literature shows trends towards use of dead biomass or immobilization of live biomass for metal uptake (Volesky, 1990; Costa & Leite, 1991; Holan *et al.*, 1993; Niu *et al.*, 1993; Voleskey *et al.*, 1993). These biosorbents are analogous to ion exchange resins or carbon adsorbents, and the removal of metal ions from solution by biosorption is essentially a conventional solid-liquid contacting and separation process. A wide range of live cells and dead biomass are used in processes

to accumulate metals. Metal uptake by these processes usually takes places via two stages, *i.e.* rapid and slow uptake stages (Gadd, 1986).

Industrial potential of biosorption processes depends on factors such as loading capacities, efficiencies and selectivity. However, reported biosorption processes have been performed over a wide range of conditions and metal concentrations. Therefore, direct comparison of efficiencies and loading capacities is very difficult.

2.2 Biofilm

Some of the main processes that remove, immobilize or detoxify heavy metals in the natural environment result from microbial activity. Many of these micro-organisms have a natural tendency to attach to solid surfaces and form a biofilm. Such biofilms were utilized in this research for metal sorption. Therefore, the formation of extensive and profound biofilm was one of the fundamental aspect in this research. Mechanism and other aspects involved in biofilm formation are reviewed in this section.

Biofilm has a number of definitions, although a generally accepted version at the Dahlem Konferenze (Bryers, 1987) on microbial adhesion, was " a biofilm is a collection of micro-organisms and their extracellular product bound to a solid (living or inanimate) surface (termed, a substratum)".

Artificial immobilization and formation of biofilm has been shown for all types of cells -microbial, plant and mammalian (Hu and Dodge, 1985). Significant benefits are demonstrated by immobilized systems, which include facilation of biomass-liquid separation; provision of high concentrations of micro-organism; protection of biomass

from system hydraulics; an increase in overall productivity and reduced wash out effect (Webb, 1987; Tsezos and Deutschmann, 1992; Craik *et al.,* 1992; Durham *et al.*, 1994). These properties of immobilized systems make them suitable for use in a continuous process.

With respect to metal interaction, use of immobilised micro-organisms in a biofilm has four important benefits:

(i) Immobilised micro-organisms have shown an increase in resistance to toxicity to metals as well as organic compounds (Stewart *et al.*, 1990), *e.g.* bacteria attached to GAC were extremely resistances to chloroamines at 1.5 mg/L, even after 40 min of exposure.

(ii) Greater metabolic activity and growth (Stewart *et al.*, 1990; Volesky, 1990; Craik *et al.*, 1992; Konhauser *et al.*, 1994), *e.g.* glucose was assimilated 2 to 5 times faster by attached *Pseudomonas fluorescens* than by suspended cells.

(iii) An increase in exopolysaccharide excretion, *e.g.* micro-organisms attached to a solid surface gave a 2.5 times increase in polysaccharide excretion compared to the same micro-organisms in suspension (Vandevivere and Kirchman, 1993).

(iv) An increase in the metal binding ability of a cell, *e.g.* the maximum copper binding ability of attached *Thermus* sp. was 8 times greater than that of free cells (Ford and Mitchell, 1992).

2.2.1 Mechanism of biofilm formation

The formation and development of biofilm can be divided into three stages. The first stage is initial cell transfer from suspension to a solid surface. Micro-organisms in suspension are transported to the solid surface by a combination of motility and chemotaxis, gravity, molecular diffusion and/or eddy diffusion (Bryers, 1987). This first stage is followed by microbial adhesion and metabolic activity at the interface, and then finally growth of the biofilm will commence (Annachhatre and Bhamidimarri, 1992; Camper *et al.*, 1993). Cellular attachment to surface is 'reversible' at the initial stage, and becomes 'irreversible' over a period of time. Initial attachment is attributed to the physical interactions of mico-organisms with the surface, whereas irreversible attachment is associated with extracellular polymers.

Factors such as, substrate surface energies (Characklis, 1981), composition of the medium (Li and DiGiano, 1983) and initial preconditioning of the substrate by organic material (Dexter *et al.*, 1975), can all play an important role in the initial cell attachment. However, little is currently known concerning the quantitative aspect of cell transport and the exact role of each factor in initial cell adhesion to surface.

2.2.2 Factors affecting biofilm attachment and growth

Transport of micro-organisms, and biofilm formation is governed by; (i) cell type (size, surface hydrophobicity, (ii) infiltration rate and clogging efficiency, (iii) the nature of the support (size, type, heterogeneity); (iv) chemistry of growth medium (pH and ionic strength).

(I) Microbial culture

Certain micro-organisms are known to attach rapidly and firmly to inert or non-polar support surfaces. This attachment is contributed by specific cell characteristics, *e.g.* presence of capsular polymers, ability to aggregate, and large cell size (Rijnaarts *et al.*, 1993). The number of cells attached to a solid surface also depends on both the concentration and age of the cells. Fletcher (1977) showed that the log phase culture has the greatest facility for attachment, followed by the stationary and dead phases. Similar trends were also observed by Shabtai and Fleminger (1994) in the attachment of a *Rhodococcus* strain onto TiO_2 surfaces.

The influence of culture age on cell adhesion is reported to be through changes in cell motility, quality and quantity of excreted polymer, and cell hydrophobicity. Motile and non-motile cells, starved cells and hydrophobicity, have all been extensively studied for their influence on adsorption to surfaces. Starved cells have been found to have a higher adsorption rate than motile and non-motile cells, whereas, motile cells (*e.g. E. coli)* penetrated four times faster than non-motile cells through sand cores in static conditions (Camper *et al.*, 1993). On the other hand, the ability of cells to adhere decreases with decreasing hydrophobicity (Shabtai and Fleminger, 1994). Wan *et al.*, (1993) found that higher proportions of hydrophobic cells attached than hydrophillic, which was due to greater attractive forces between the cells and surfaces (Rijnaarts *et al.*, 1993). However, the effect and role of adhesion enhancing materials, *e.g.* lecitin macromolecules in *Chlorella* (Bryers, 1987), with such cells (starved, hydrophobic or motile) is not clear.

With regard to attached cells, researchers have reported different findings with respect

to their morphology and physiology. Craik *et al.*, (1992) reported an altered morphology and physiology of attached cells. Similarly, the bacterium *Vibrio alginolyticus* produces a single, polar flagellum in suspension, but this appears as unsheathed flagella when grown on a solid surface (Golten and Scheffers, 1975). However, Van Loosdrecht and Heijnen (1993), reported that generally, the physiology of immobilised or attached cells is not greatly different from that of free cells.

(II) Role of extracellular polymer

Extracellular polymers produced by micro-organisms act as:

(i) adhesion promoters, *e.g.* an increase in adhesion of microbial cells to a substrate has been observed in the presences of extracellular polymers (Imam *et al.*, 1984; Zaidi, 1984),

(ii) shelter to cells against shear, *e.g.* an extracellular polymer may adopt the form of gelatinous matrix and under shearing fluid conditions, this matrix shelters the micro-organisms (Bryers, 1987) and

(iii) growth promoters for entrapped cells, *e.g.* the attached cells adopt new survival strategy as they switch from production of new cells to production of excess polysaccharide (Andrews and Tien, 1981). Further, this excess, or secondary polysaccharide, is found to be responsible for strengthening the attachment of cells (Corpe, 1980).

Thus, extracellular polysaccharides are integral part of biofilm development along with cellular growth, replication, and substrate conversion. Biofilm development will continue until cells located inside it are deficient in nutrient.

Cells at the base of a biofilm can use polysaccharides for their existence. However, polysaccharide consumption by entrapped cells diminishes the biofilm density (Andrews and Tien, 1981). On the other hand, physiology of the cells has been shown to be altered due to the presence of excessive polysaccharides as a metabolic response for being attached (Bakke *et al.*, 1984; Craik *et al.*, 1992; Vandevivere and Kirchman, 1993).

(III) Support surface characteristics

Occurrence and biofilm development depend greatly on the characteristics of the support material. A variety of support media have been employed for biofilm development, such as sand, GAC, plastic, foam and various types of clays. These materials are summarised in Table 2.1

Table 2.1 Reported supports for biofilm attachment

Support	Diameter/ area	Organisms	Reference
Coal	0.59 to 0.7 mm	Bacteria*	Andrews and Tien (1981)
Glass Bead	0.9 to 1.23mm	Bacteria*	Chang and Rittman (1987 a, b)
Raschig Ring Reticulate Foam	85714 (m^2/m^3) 500 (m^2/m^3)	*Citro-bacter*	Macaskie (1990)
Sand	0.5mm	----	Pirbazari *et al.*(1990)
Sediments	0.149mm	Bacteria*	Burke *et al.*, (1991)
Z-Biocarrier	3.17mm	*Pseudo-monas* sp.	Durham *et al.*, (1994)
Metal mesh particle	2 to 6mm	Bacteria*	Kargi and Torpak (1994)

* Mixed or un defined culture

The properties of a support such as surface area, roughness, porosity, particle size and shape have been studied for biofilm development. These factors do not have significant negative effect on cellular attachment (Chang & Rittmann, 1988; Stewart *et al.*, 1990; Tsezos, 1990; Xing *et al.*, 1992). Similarly, ability of the surface to adsorb organic molecules enhances cell attachment (Characklis, 1981). On the other hand, dimension of the cell influences its attachment to the support (Messing and Oppermann, 1979).

Kida *et al.*, (1990) evaluated eight different support surfaces for attachment of micro-organisms. They concluded that good performance as a support medium was associated with a rougher surface, rather than large surface area. Micro-organisms also showed preference to attach to a positively charged surface (cristobalite *i.e.* inorganic silica), rather than to a negatively charged one (zeolite).

Ideally, the cell support should (i) be non-toxic, *e.g.* surface such as wood shavings, need washing in methanol in order to leach out antimicrobial compounds (Macaskie, 1990); (ii) give protection to the newly attached biomass from fluid shear, (iii) should not lead to blocked pores, *e.g.* Macaskie (1990), observed blockages of pores in Rasching ring and reticulate foam due to cell attachment and growth.

Although the attachment of a cell to the support is influenced by the support, it is also dependant on the micro-organism itself. There is a need for more information on the influence of microbial cell properties on attachment and biofilm development.

2.2.3 Reactors for biofilm development

The type of reactor can affect the quality (thickness and density) of biofilm, along with the rate of biofilm development. Therefore, a variety of reactor configurations have been adopted by researchers for cell attachment and biofilm development over solid support surfaces. The oldest forms of biofilm reactors are trickling filters, which are simple in operation and require low maintenance. Other configurations, such as stirred tank reactors, packed bed and fluidized bed, have all been used for cell immobilization and biofilm development. A comparison in the performance of reactors for biofilm development can be found in Rittman (1982) and Webb (1987).

Factors influencing choice of reactor include the immobilization method, particle characteristics (*e.g.* size, shape, density and robustness), nature of the substrate, inhibitory effects, and hydrodynamic and economic considerations (Webb, 1987). Similarly, bacterial adsorption was found to be a function of velocity, column cross-sectional area and porosity, along with bacterial concentration (Camper *et al.*, 1993).

Two reactor configurations, packed bed and fluidized bed are extensively used for biofilm development on a support. The packed bed reactor configuration accommodates the maximum number of support particles per unit volume and such a reactor has been regularly exploited for biofilm development (Paprowicz and Slodczyk, 1988; Annachhatre and Bhamidimarri, 1992; Porro *et al.*, 1993). This type of reactor, however, can suffer from poor liquid distribution, resulting in channelling and uneven biofilm growth, flooding and compressibility of the support material, all of which limit bed height (Webb, 1987). Additional problems associated with immobilised cells in this reactor are, (i) blockage of bed by growing cells, and (ii) an

initial slow development of biofilm due to low cell growth rate (Annachhatre and Bhamidimarri, 1992).

Fluidized beds are often the preferred choice for biofilm development (Andrews and Tien, 1981; Khan *et al.*, 1982; Hermanowicz and Ganczarczyk, 1983; Chang and Rittman, 1988; Chen *et al.*, 1988; Pfeffer and Suidan, 1989; Craik *et al.*, 1992; Scott and Karanjkar, 1992; Xing *et al.*, 1992). The performance of fluidized bed reactors under various condition is described by Shieh and Keenan (1986), Mulcahy and Shieh (1987) and Lin (1991). Fluidized bed bioreactor offers (i) maximum surface area available as a support for cell attachment, (ii) even distribution of substrate, and (iii) control of biofilm thickness through inter-particle and particle wall collision (Webb, 1987).

Table 2.2 Fluidized bed reactors for biofilm development

Temp. °C	Velocity cm/s	Bed Expansion (%)	Reference
21- 24	0.068	--	Andrews and Tien (1981)
--	0.0017	--	Khan *et al.*, (1982)
22	0.7	--	Mulcahy and Shieh (1987)
--	0.89	40	Chang and Rittman (1988)
35	0.38	--	Chen *et al.*, (1988)
30	--	25	Pfeffer and Suidan (1989)
20	0.9	30	Lin (1991)
25	0.8	--	Xing *et al.*, (1992)

Fluidized bed reactors limit fluid velocity so as to avoid particles to be carried over and/or loss of biofilm due to too high shear force. Shear loss rate of biofilm seems to be a function of factors such as biofilm density, extracellular polymers, support shape

and shear stress (Chang and Rittmann, 1988). Table 2.2 summarizes different conditions adopted in fluidized bed reactors for biofilm development.

(I) Hydrodynamics of process

The deposition of micrometer sized particles, such as bacteria, on solid surface and their ability to form a biofilm, is influenced by the system hydrodynamics (Stoodley *et al.*, 1994). Similarly, the adsorption of bacteria has been reported to be a function of velocity *e.g.* at higher velocities the "sloughing off" of bacteria from a surface was observed (Camper *et al.*, 1993). However, the transport of microbial particles from the liquid to the surface has been shown to be more efficient in dynamic, rather than in static, conditions (Rijnaarts *et al.*, 1993).

2.2.4 Environmental conditions

Regulation of cell attachment on solid surface can be effected by environmental conditions. These conditions include dissolved oxygen, pH, temperature and nutrient abundance.

(I) Oxygen

Biofilms are not flat and the distribution of cells in them is not uniform. Instead, they form highly complex structures containing "voids", "channels", "cavities", "pores" and "filaments", with cells arranged in clusters or layers (Beer *et al.*, 1994). Due to variable biofilm configurations, meeting oxygen demand can be an acute problem for cells in a biofilm, *e.g.* oxygen concentrations have been found to approach zero at less than 0.1mm from the film interface (Webb, 1987).

On the other hand, oxygen is one environmental constituent that interacts with the cell cytoplasm. It is also essential in the metabolic activity of aerobic micro-organisms and carbon fixation. Thus, deficiency in oxygen can hinder cell growth in a maturing biofilm (Marshall, 1978). Conversely, excess oxygen may vary the composition of cell walls due to oxygen reactions (Alberts *et al.*, 1989). Similarly, due to its role in carbon fixation, the excretion of extracellular polysaccharide is also altered. Therefore, achieving an optimal oxygen level with respect to polysaccharide excretion is essential.

(II) Nutrient availability

Polymer biosynthesis and growth of bacteria is influenced by nature of the carbon source and electrolyte composition of the fermentation medium. Medium constituents such as glucose and other sugars, form the building blocks for polysaccharide synthesis. Further, polysaccharide synthesis is also reported to be influenced by the type of sugar, *e.g.* maximum polysaccharide synthesis was observed with sucrose containing media, whereas xylose hindered excretion (Ebube *et al.*, 1992).

Availability of nitrogen, phosphorus and sulphur in the medium also affects polysaccharide synthesis (Characklis, 1973). The weight of biofilm has been reported to increase with an increase in the carbon to nitrogen ratio (Shieh and Keenan, 1986). An approximate optimal C/N ratio of 8 was reported by Brown (1979).

(III) Temperature

The system temperature can alter bacterial attachment and biofilm development. Temperature can affect:

(i) viscosity of the medium and/or polymer, *e.g.* cells motility at low temperatures decrease as the viscosity of the medium and/or polymer increases, which subsequently alters attachment efficiency (Camper *et al.*, 1993).

(ii) rate of adsorption, *e.g.* temperature influences physio-chemical adsorption of organic compounds. This adsorption of organic compounds increases initial cell attachment to the surface (Characklis, 1981).

Researchers have found biofilm development at a temperature, as low as 3°C (Fletcher, 1977) and as high as 60°C (Rogers *et al.*, 1994). At 3°C there was a noticeable decrease in the proportion of cells attached, compared to attachment at 20 °C (Fletcher, 1977). However, at low operating temperatures, specific microbial growth rates are low, and accordingly, biofilm development and growth is hindered. In addition, lower temperature results in decreased metabolic activity and can affect the physiology of the organisms.

Rogers *et al.*, (1994) demonstrated presence of abundant *Legionella pneumophila* in a biofilm on plastic surface at 40°C. However, a further temperature increase to 50°C resulted in a decline of cells on the surface (*e.g.* 6.84×10^4 declined to 6.00×10^1 CFU cm^{-2} on a PVC surface).

Another reported significant effect of temperature was on polysaccharide production. This was due to influence on the activity of enzymes responsible for excretion of polysaccharides. However, temperature for optimum polysaccharide production has been found to be usually less (by 5 to 6°C) than that for the cell growth (Brown, 1979;

Shieh and Keenan, 1986).

(IV) pH

The medium pH has a significant role in all three stages of biofilm formation, namely microbial adsorption to substrates, adhesion of adsorbed micro-organisms and biofilm development (Greenfield, 1987). These factors are related to cell growth and amount of polysaccharide produced.

Cell growth and rate of metabolism of the micro-organisms is affected by the pH of the medium. A low pH reduces growth (Tanner and James, 1992) and also denatures the enzymes responsible for polysaccharide excretion; as with most enzymes, they are stable only within a limited pH range (Brown, 1979). But, the overall effect of pH on polysaccharide synthesis is not well understood. Mild alkaline conditions (pH 7.5), however, do not affect cell growth and adhesion of polysaccharides to the support surface (Sheih and Keenan, 1986).

The majority of microbial systems reported for biofilm formation are operated near optimal neutral pH for maximum biofilm development (Sheih and Keenan, 1986; Pirbazari *et al.*, 1990; Annachhatre and Bhamidimarri, 1992; Ebube *et al.*, 1992).

2.3 Granular activated carbon (GAC)

GAC was selected in this research for attachment of a biofilm for subsequent use in metal sorption. An additional reason for using GAC was to explore the reuse of biosorbed metal without separating it from the surface of the GAC. Such GAC was assessed for organic vapour adsorption. In this section, use of GAC is reviewed from

two points of view. Firstly, as a support for biofilm and secondly, as an organic vapour adsorbent (with and without impregnated metal over its surface).

The term "activated carbon" covers a range of carbon based materials that possess adsorptive power. Activated carbon is defined by the European Commission (EC) as "a non-hazardous, processed, carbonaceous product, having a porous structure and a large internal surface area" (Henning and Schafer, 1993). It can adsorb a wide variety of substances and is able to attract molecules to internal surfaces. The volume of activated carbon is generally greater than 0.2 mL/g and the internal surface area, greater than 400 m^2/g. The width of pores range from 0.3 to several thousand nanometres.

Activated carbon in the form of carbonized wood has been known for its purifying properties for many centuries. The Egyptians used it around 1500 B.C. as an adsorbent (Bansal *et al.*, 1988). The basis for industrial production of activated carbon, however, was established at the beginning of the 20^{th} century.

Today, a number of commercial technologies for industrial effluent treatment are based on granular activated carbon (GAC). However, GAC has not been widely used for removal of heavy metals from water, which is due its low efficiency, which is variable depending upon the type of carbon (Huang, 1978; Taylor and Kunnen, 1994). However, a considerable amount of research has been conducted on this subject (Huang *et al.*, 1987; Tan and Teo, 1987; Petrov *et al.*, 1992; Reed and Matsumoto, 1993).

GAC is manufactured from different sources, including bones, coal, coconut shells, coffee grounds, fish fruit pits, kelp, molasses, nutshells, peat, petroleum coke, rice hulls, sawdust and all types of wood (Kohl, 1985; Bansal *et al.*, 1988). The principal operation in the manufacturing of GAC is heating of carbon containing material, so that volatile components, either originally present or formed during the heating operation, are distilled off, leaving a highly porous structure.

The large surface area of GAC is accompanied with a unique, intricate pore structure and a hydrophobic surface, which consists of partially inter-connected irregular shaped capillaries (Waller, 1975). An ideal GAC pore structure consists of micropores in the range 1 to 100 nm and macropores greater than 100 nm diameter.

The chemistry of GAC surfaces varies with the source of base material and its method of activation (Ishizaki *et al.*, 1983; Taylor and Kuennen, 1994). GAC develops a surface charge and exhibits amphoteric properties when immersed in water. Surface functional groups such as carboxyl, lactone type hydroxyl, phenolic hydroxyl and carbonyl are found on GAC. These groups strongly affect the polar properties of the GAC surface and its adsorption capacity (Gergova *et al.*, 1993, Reed and Matsumoto, 1993). GAC has the following general characteristics:

(i) Large surface area

(ii) Wide range of particle size

(iii) Good resistance to compression and abrasion

(iv) High porosity and good wetting ability

(v) Good resistance to chemicals

(vi) Extensive adsorption of organic and inorganic chemicals

2.3.1 Microbial interaction on GAC

Bacterial growth on GAC in drinking water treatment plants is well documented (AWWA Report, 1981; Camper *et al.*, 1986). A wide range of micro-organisms such as *Acinetobacter, Aeromonas, Alcaligenes, Bacillus, Chromobacterium, Flavobacterium* and *Pseudomonas* have been identified in GAC beds (Love and Symons, 1978; Cairo *et al.*, 1979; Stewart *et al.*, 1990). Several researchers have investigated and compared bacterial activity over GAC and other support surfaces (AWWA Report, 1981; , Khan *et al.*, 1982; Kida *et al.*, 1990; Pirbazari *et al.*, 1990; Olmstead and Weber, 1991).

In 1981, an American Water Works Association committee (AWWA) reported enhanced microbial activity on GAC due to several factors, such as enrichment of GAC by oxygen, promotion of growth due to abundance of substrate on the surfaces and ability of extracellular enzymes to enter into GAC pores (AWWA Report, 1981). However, contrasting results have been produced on the ability of extracellular enzymes to reach substrate within GAC micropores.

Khan *et al.*, (1982) found that micro-organisms on GAC have a higher rate of metabolic activity than on Anthracite. Pirbazari *et al.* (1990) compared growth of bacteria on GAC, glass beads, natural silica sand and adsorbent resin. Activated carbon provided a more favourable surface for cell attachment and growth than the other supports. They concluded that the higher growth was due to surface roughness, adsorptive capacity and the shelter provided by crevices against toxic effects and shear. Of the research groups studying bacterial adhesion on GAC, only Kida *et al.*, (1990) found cristobalite (inorganic silica) to be a better support surface than GAC.

Researchers are divided over the presence of bacteria inside pores of GAC. Den Blanken (1983) did show appearance of biological material inside the pores of GAC. However, the majority of research groups consider that bacteria remain on the surface of GAC on the basis of the relative dimensions of cells and pores. The outer pores of GAC have a diameter of $> 5 \times 10^{-8}$ m, which is smaller than the diameter of a typical cell (*e.g. Pseudomonas* sp. of about 5×10^{-7}m). Therefore, cells will attach on the external surface of GAC and will only enter some big macropores (Ehrhardt and Rehm, 1985).

A few researchers have attempted to identify the number of micro-organisms attached over GAC, *e.g.* Donlan and Yohe (1983) found 1.3×10^3 cells/mL in suspension as against 6.8×10^5 cells/g attached to GAC. However, researchers have been concerned as to whether the procedure of total plate count enumerates all the micro-organisms present on GAC. Other aspects of biofilm such as density, thickness or weight of biomass have been also measured and these are summarized in Table 2.3.

Table 2.3 Measured factors of biofilm on GAC

Diameter (mm)	**Factors**	**Reference**
0.6 to 0.7	Dry Density 35 mg/cm^3	Andrews and Tien(1981)
---	6.8 x 10^5 cells/g	Donlan and Yohe (1983)
1 to 4	4.2 X 10^9 cells/g	Ehrhardt and Rehm (1985)
0.7	Dry Density 100 mg/cm^3 Thickness 0.18 to 0.23 mm	Chen *et al.* (1988)
--	1.4 to 8.6x10^7 cells/g	Paprowicz and Slodczyk (1988)
0.42 to 0.84	0.091g biomass/g	Craik *et al.*, (1992)
--	1.5 to 1.8x10^6 cells/cm^3	Dalmacija *et al.*,(1992)

Thus, bacterial colonization can be considered to result from a GAC's (i) adsorptive properties and (ii) porous surface which provides protective environment from fluid shear force.

(I) SEM studies of biofilm on GAC

Several studies report use of Scanning Electron Microscopy (SEM) to observe the characteristics of biofilm on GAC (Schalekamp, 1979; LeChevallier *et al.*, 1984; Erhardt and Rehm, 1985; Kida *et al.*, 1990; Pirbazari *et al.*, 1990; Craik *et al.*, 1992). All these studies found a non-uniform and low density biofilm over the GAC surface.

Pirbazari *et al.*, (1990) observed that the total exterior surface coverage of GAC by biofilm was less than 25%. Craik *et al.*, (1992) observed an incomplete and non-uniform biofilm growth on GAC, even after 276 days. GAC with crevices, pores and surface irregularities provided the best attachment sites, as they protected the micro-organisms from shearing. All the SEM studies observed an abundant growth in craters and sheltered areas on the GAC surface.

(II) Bacterial growth on the GAC saturated with organic compounds

Almost all assessments of microbial activity on GAC have been conducted on virgin material. Many of these assessments have considered simultaneous adsorption of organic material from waste waters and formation of biofilm (Chen *et al.*, 1988; Pfeffer and Suidan, 1989; Kida *et al.*, 1990). Organic compounds from effluents have been also utilised as carbon sources for micro-organisms attached over GAC surfaces. For example, 0.013 mg of phenol per hour was degraded by 10^8 bacteria attached to 1 g of GAC (Den Blanken, 1983). Craik *et al.*, (1992) reported 0.15 to 0.22 mg

phenol degraded per mg of volatile solid per day.

Organic compounds adsorbed onto GAC during biofilm development can diffuse out of the GAC. For example, phenol was found to diffuse out and be subsequently metabolized by *Pseudomonas* sp. and *Candida* sp. (Ehrhardt and Rehm, 1985). Similar interactions between total organic carbon (TOC) and attached micro-organisms are reported by Maloney *et al.*, (1983). Thus, a possible ideal situation would be that GAC stores biodegradable materials that can be subsequently used by bacteria as the material is released back in to the aqueous phase.

(III) Interaction of biofilm on GAC with metals and organic compounds

The interactions of metals, organic and inorganic compounds with natural biofilms are well known, *e.g.* biofilms on submerged rocks in rivers (Characklis, 1981; Konhauser *et al.*, 1994). However, the use biofilm on GAC for removal of metal has not been reported extensively except by Macaskie, (1990) and Scott *et al.*, (1992).

With respect to organic molecule adsorption onto GAC with a biofilm presence, both beneficial and negative effects are reported. There are interferences to adsorption of organic molecules (Weber and Ying, 1978; Olmstead and Weber, 1991), but the degree of interference is varied. For example, a slow diffusion of organic molecules through biofilm into the surface of GAC has been observed (Fan *et al.*, 1990). On the other hand, there are specific cases in which biofilm enhances GAC adsorption capacity, *e.g.* 1.34 times more quinalphos (pesticide) was adsorbed due to the presence of biofilm over GAC (Dalmacija *et al.*, 1992). Also, 90% refractory organic pollutants and benzoic acid were degraded by micro-organisms attached to GAC (Iwami *et al.*,

1992; Nishijima *et al.*, 1992). Thus, the benefits of biofilm on GAC for organic pollutant treatment is dependant on the particular pollutant and specific micro-organisms. The interaction of organic compounds, which are neither biodegradable nor adsorbed by GAC, with a biofilm, was not found in the literature.

2.3.2 Adsorption of volatile organic compounds (VOCs) on GAC

In this research, GAC with attached metal through biofilm uptake, is to be exploited for adsorption of organic vapour, such as chloroform, from air. The use of virgin GAC and metal impregnated GAC to adsorb organic vapour is reviewed in this section.

The gas adsorptive properties of wood charcoal was recognised as early as 1773 by Scheele (Kohl, 1985). A major development in the use of GAC for gas adsorption occurred during the First World War, when it was used in gas masks. Today, use of GAC is at the forefront of VOC adsorption technology. Extensive literature is available on a wide range of GAC applications as a gas adsorbent (Rehrmann and Jonas, 1978; Bradley and Rand, 1993; Graham and Ramaratnam, 1993; Ruhl, 1993; You *et al.*, 1994).

VOC adsorption is particularly widely used in recovery of solvents. The adsorption of non-polar organic compounds, particularly VOCs, is mainly influenced by pore size distribution and surface chemistry (Rivera-Utrilla and Ferro-Garcia, 1986; Taylor and Kuennen, 1994). Commonly adsorbed solvents are toluene, heptane, hexane, acetone, carbon tetra chloride, ethyl acetate and chloroform.

GAC attracts VOC molecules and holds them on its surfaces and pores by physical

and exothermic processes (Ruhl, 1993). The exothermic interaction, however, is weak and liberates between 2 - 5 Kcal/ mole of heat (Graham and Ramaratnam, 1993). The adsorption capacity of GAC is decreased by this liberated heat (Kohl, 1985; Bansal *et al.*, 1988; Kovach, 1988; Graham and Ramaratnam, 1993) and it may restrict GAC use for high concentrations of volatiles, and low boiling point organic molecules. Due to this exothermic adsorption process, a fire suppression system is included in virtually all GAC solvent recovery systems (Ruhl, 1993).

(I) Applications of metal impregnated GAC

GAC is impregnated with a wide range of chemicals for a wide variety of applications, such as gas purification, civil and military gas protection and for catalysts. There are many applications which use metal impregnated GAC. A few are listed below:

(i) Activated carbon impregnated with metals, such as Zn/Cu, has been used for hydrogen cyanide adsorption (Rossin and Morrison, 1993).

(ii) Silver impregnated GAC, because of oligodynamic effects, has been successfully used in drinking water sterlization (Thomson, 1973).

(iii) Removal of sulphur from hydrocarbon feedstock is catalysed by GAC which has zinc impregnated on its surface (Catalyst Chemical Europe, 1983).

(iv) Noble metal (*e.g.* palladium, platinum) impregnated GAC has been used in organic synthesis and hydrogenation applications (Henning and Schafer, 1993).

(v) Copper impregnated GAC has enhanced removal of hydrogen cyanide and hydrogen sulphide gas (Capon *et al.*, 1981).

(vi) Increased cyclohexane dehydration activity was observed on a platinum/GAC catalyst (Youssef and Ahmed, 1981).

(vii) ASC Whetlerite carbon has been impregnated with copper, silver and chromium in the presence of ammonia for removal of phosgene, chloroform, hydrogen cyanide and cyanogen chloride from air (Chiou and Reucroft, 1977; US Patent, 1987).

(iix) GAC has been adsorbed with nickel, zinc, copper and cadmium for adsorption of methanol, acetone, acetaldehyde and methyl formate (Afzal *et al.*, 1993).

The amount of metal impregnated on GAC varied according to the application, *e.g.* 0.1% Zinc on GAC was used for sulphur removal from hydrocarbon feed stream (Catalyst Chemical Europe, 1983), whereas, 5% copper was impregnated onto the GAC in the case of hydrogen cyanide adsorption (Bansal *et al.*, 1988).

When impregnated metals are deposited on the surface of GAC, adsorption capacity can be enhanced, but the removal mechanisms change. For example, physical adsorption was found to be transformed into chemisorption (Barnir and Aharoni, 1975; Chiou and Reucroft, 1977; Afzal *et al.*, 1993; Henning and Schafer, 1994). Although metal impregnation on GAC is highly effective in applications such as those indicated above, GAC has been found to lose surface area and porosity, *e.g.* an 18% reduction in micropore volume was found due to metal impregnation (Barnir and Aharoni, 1975;

Bansal *et al.*, 1988; Afzal *et al.*, 1993).

(II) Adsorption of chloroform

Finally, chloroform adsorption is to be of specific interest in this work. The Commission of the European Communities (CEC, 1986) categorized chloroform among six chlorinated hydrocarbons that are considered to be particularly poisonous. The main uses of chloroform is in pharmaceutical and cosmetic products. It is used also as a solvent and raw material for dyes, pesticides and production of fluorocarbons. In 1986, chloroform production in the EEC was estimated to be 50,000 tonnes per annum (CEC, 1986).

Adsorption of chloroform on GAC is well documented (Jonas *et al.*, 1979; Kohl, 1985; Fang and Khor, 1989; Nirmalakhandan and Speece, 1993). Adsorption capacities for chloroform are summarised in Table 2.4.

Table 2.4 Chloroform adsorption capacity of GAC

g $CHCl_3$/ g of GAC	Reference
0.728	Jonas *et al.*, (1979)
0.490	Kohl (1985)
0.4 to 0.58	Nirmalakhandan and Speece (1993)

Chapter 3

MATERIALS AND METHODS

3.1 Culture and biofilm development

3.1.1 Culture of the bacteria

The bacteria used were the known polysaccharide producers; a *Pseudomonas* sp.(unclassified) NCIMB 11592 (National Collection of Industrial Bacteria), and *Enterobacter aerogenes* (University of Bath Culture), along with a non-polysaccharide producer, *Serratia marcescens* (NCIMB 8805).

Bacterial cultures were obtained from the National Collection of Industrial and Marine Bacteria (NCIMB), Aberdeen, Scotland.

Stock cultures of organisms were maintained by periodic sub-culture on nutrient agar (Code CM3, OXOID Ltd, Hampshire, U.K.) and stored at 4°C in a refrigerator. Unless otherwise stated in experiments, cultures of *Pseudomonas* sp. (unclassified) NCIMB 11592 were used.

(I) Culture medium and culture conditions

The standard culture medium used in this research, had composition as described in Table 3.1. It was obtained from OXOID Ltd., as Nutrient Broth (code CM1).

Medium pH was 7.4± 0.2 (except for studies on the influence of pH). Cultures were incubated as 100 mL aliquots of medium in 250 mL flasks in an incubated rotary shaker (25°C and 150 rpm). Standard incubation time was 24 hours after inoculation. Incubated cultures were used to seed fermenters containing sterile medium ($2dm^3$) of

the same concentration as in Table 3.1.

Table 3.1 Standard culture medium composition

Ingredient	Weight (g/L)
"Lab -Lemco" powder	1.0
Yeast extract	2.0
Peptone	5.0
Sodium chloride	5.0

(II) Cell growth

For cell growth studies, 0.1dm^3 of medium in a shaker flask was inoculated with the culture under study. The flask was then incubated at 25°C, and samples collected at desired time intervals for cell growth studies.

Growth of free cells was determined by measuring optical density using a UV spectrophotometer (CE 588 model, CECIL Instrument, Cambridge, England). Samples were examined at optical wavelength of 560 nm, with sterile nutrient medium as a reference.

3.1.2 Development of biofilm over GAC

(I) GAC preparation

The GAC used for all the experiments (except in the study of effects of GAC properties on biofilm metal sorption) was charcoal with a particle diameter within the range of 0.85 to 1.70 mm (obtained from BDH Ltd. Pool UK).

It has the following specifications:

Product code	33034
Source of carbon	Coal/Coke
Method of activation	Steam activated at 850-950°C
Particle size	0.85 - 1.70 mm (10-18 mesh)
Ash contain	≈ 8%

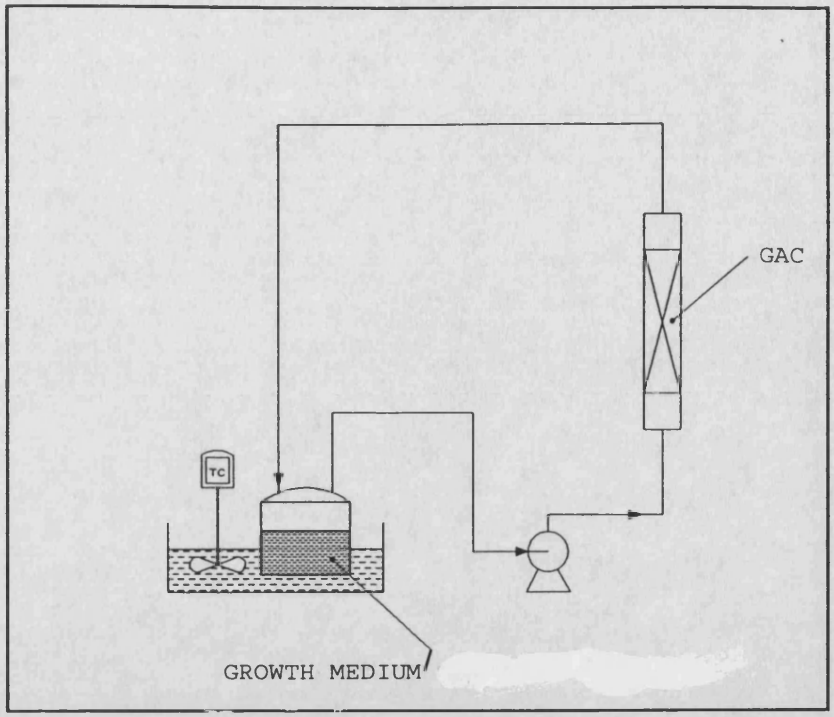
GAC was sieved to obtain a uniform size distribution of 0.85-1.70 mm. After sieving, the GAC was washed with distilled water to remove any carbon fines and dried overnight at 105°C. In all studies, before development of a biofilm, the GAC was autoclaved at 121°C for 15 min.

(II) Biofilm development

To grow and attach a biofilm, growth medium containing a bacterial culture was circulated by a peristaltic pump for 2 days (unless mentioned otherwise) through 15 mm diameter glass column, containing 10 g of fluidized GAC. Fermenter temperature was maintained at 25°C, except during temperature studies. The velocity of growth medium over the GAC in column was constant at 2.1 cm/sec (except in fluidization studies). The experimental set up was as shown in Figure 3.1.

(III) Determination of dry biomass weight attached to GAC

The biofilm development method adopted was as described in Section 3.1.2. To determine weight of biofilm attached, at regular time intervals the columns were disconnected and the GAC collected was washed with Ringer's solution (¼ strength, Lab M, Bury, U.K.). This material was dried at 105°C until a constant weight was obtained.



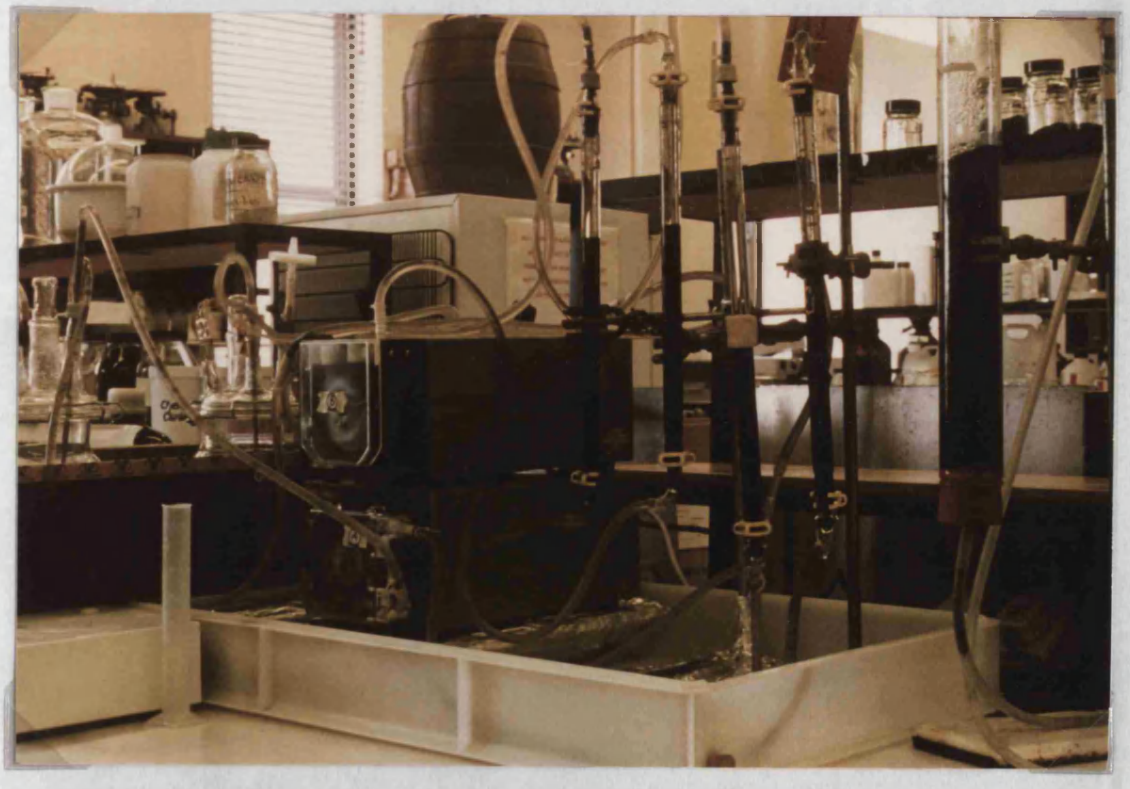


Figure 3.1 Biofilm development experimental set up

The attached biomass was then separated from GAC by the method described by Chen *et al.*, (1988). A weighed quantity of GAC was introduced into hot (50°C) 0.1N NaOH to extract the biomass attached to GAC. The total non-volatile solid in the extract was then measured by evaporating water and determining the % solid present.

After the extraction, the GAC was again dried at 105°C and stored in a desiccator. The GAC weight loss was measured and correlated to the extracted biomass. Control experiments were conducted as (i) GAC exposed to only sterile nutrient medium, and (ii) GAC without any biofilm. Approximately 3 to 4% of attached biomass was found to be due to solids from the medium, whereas with only GAC the weight loss was less than 1%.

3.1.3 Optimum age of biofilm for biosorption

To determine the optimum biofilm development period, biofilms were developed as described in Section 3.1.2. To obtain biofilms of different ages, the circulation of cultured medium was continued beyond 2 days.

Columns containing, 2, 4 and 6 days old biofilm-GAC system were exposed to solutions containing 25 mg/L of cadmium and its uptake rate and final loading were assessed.

3.1.4 Comparison of metal uptake between live and treated biofilm

For comparison of metal uptake by biofilms containing live and dead cells, biofilms were formed on GAC as described in Section 3.1.2. These biofilms were washed with Ringer's solution (¼ strength). Columns containing GAC and attached biomass were

exposed to different treatment conditions to kill the cells. One column was subjected to autoclaving at 121°C for 15 minutes; another exposed to 10% formaldehyde for 30 minutes; and a third column was left in a water bath maintained at 95°C for 45 minutes.

To check any viability after these treatments, samples of GAC along with biofilm were introduced into sterile nutrient medium and monitored for cell growth, as described in Section 3.1.

Columns were then exposed separately to solutions containing 25 mg/L of cadmium and its uptake was recorded.

3.2 Metal uptake and analysis

To study metal uptake by biofilms, solutions ($1dm^3$) were prepared at metal cadmium, copper, chromium, nickel, silver and zinc) concentrations between 5-100 mg/L. Columns containing GAC with attached biofilm were disconnected from the growth medium circulator and rinsed with Ringer's solution. Then they were reconnected to a metal solution circulator. Biofilm coated particles were re-fluidized at a flow rate of $0.1dm^3/min$ by a metal containing solution (25°C). Unless otherwise stated the metal uptake experiments were conducted in batch mode *i.e.* metal solution was circulated over GAC-biofilm until concentration of free metal had reached an equilibrium level.

Unless stated, the following conditions were maintained as a standard in all metal sorption studies:

Weight of GAC 10 g

Biofilm state	Live
Treatment	Washed through with sterile Ringer's solution (¼ strength)
Mode of contact	Batch
Metal solution volume	1 dm^3 (except continuous mode)
Metal concentration	25 mg/L
Solution temperature	18 ±2 °C
Solution flow rate	0.1dm^3/min.

Except for continuous mode, in all the other metal uptake studies, samples were taken at every 5 minutes during the first 20 minutes and then at intervals of 10 minutes until constant metal concentration was reached in the solution. In the continuous mode, samples were taken every 2 minutes.

3.2.1 Metal salts

In all the metal uptake experiments, unless stated, the following metal salts were used to make solutions. All the chemicals were obtained from BDH, unless indicated otherwise.

Cadmium	$CdCl_2$ 2½H_2O
Copper	$CuCl_2$ ½H_2O
Chromium	$CrCl_3$ 6H_2O (Fluka chemicals)
Zinc	$ZnCl_2$
Nickel	$NiCl_2$ 6H_2O
Silver	Ag $(NO)_3$

All salts used were of 'Analar' standard.

3.2.2 Metal Analysis

A Perkin Elmer Atomic Absorption Spectrometer (Model 3110) was used to measure metal concentrations in solution. All samples were analysed using an air-acetylene flame. The analysis was carried out at a wavelength (nm) of: 228.8 (cadmium), 359.4 (chromium), 324.8 (copper), 231.1 (nickel), 328.1 (silver) and 213.9 (zinc).

All samples of metal containing solutions were collected in plastic bottles and were analysed within 2 hours of exposure to the biofilm-GAC system. All glassware used were treated with Di-methyldichlorosilane (obtained from BDH Ltd; Poole, U.K) to make it non-adsorbent and 0.5 to 0.8 % of the initial metal level in the solution was found to be adsorbed onto the glass column.

The metal concentration of the initial solution was measured and the metal removed by biofilm-GAC determined by obtaining the difference in concentration between exposed and unexposed solutions.

3.2.3 Metal loading

Metal loading (q) was calculated from the initial concentration (C_i) and the analyzed equilibrium (final) concentration (C_f) of the metal in solution was determined according to the following formula:

$$q = V\frac{(C_i - C_f)}{W}$$

where: q is metal loading (mg/g); V is the volume of solution (L); and W is dry weight of GAC (g). The metal concentrations are expressed as mg/L.

3.2.4 Uptake from multi-metal system

To determine the uptake of metals from multi-metal systems, two combinations of solutions were made at a concentration of 25 mg/L for each metal. The first solution contained six metals (cadmium, copper, chromium, nickel, silver and zinc), whereas, the second solution contained four metals (cadmium, copper, zinc and nickel). Solutions were thoroughly mixed to obtain uniform composition. The pH of both solutions was observed to be 4.55, which was within the solubility range of all the metals used. These solutions were exposed to biofilm (developed as described in Section 3.1.2) and the uptake of each metal by biofilm-GAC was determined.

3.3 Biofilm development under different conditions

3.3.1 Variation in pH of the growth medium

The growth medium pH was observed to be 7.4± 0.2. To obtain the medium at a desired pH, additions of 0.1M NaOH or 0.1M HCl were used. Ebube *et al.*, (1992) reported that use of these chemicals had no influence on microbial cells. Once the desired pH of the medium was obtained, it was inoculated by the *Pseudomonas* sp. and subsequently used for biofilm development, as described in Section 3.1.2. The pH of the medium was checked periodically, but was not adjusted. Biofilms thus developed were exposed to 25 mg/L of cadmium aqueous solution and metal uptake was analysed.

3.3.2 Variation in temperature

To asses the effect of temperature on biofilm growth, cultures were initially incubated at 25°C for 24 hours. They were then acclimatised for 6 hours at a new temperature (*i.e.* 5, 15 or 30°C), before seeding them in a fermenter (at the same temperature)

containing sterile medium ($2dm^3$). This medium was used for the biofilm development, as described in Section 3.1.2. Biofilms thus developed were exposed to 25 mg/L of cadmium aqueous solution and metal uptake was analysed.

(I) Isolation and purification of polysaccharide

To recover polysaccharides, cultures were harvested after two days in a growth medium (25°C). The culture ($0.5dm^3$) was diluted by 1:4 volumes of distilled water to reduce the viscosity. A 50/50 mixture with ethanol was then made up and agitated for 30 minutes to detach any polysaccharide adhering to the bacterial cell walls. The mixture was then centrifuged at 25000g for 120 minutes to sediment out the cells.

The supernatant was decanted off and polysaccharide precipitated by addition of 1 g of KCl per 100 mL solution and 2.5 volumes of ethanol. The precipitate was redissolved in deionised water (500 mL) and re-centrifuged in a Microcentaure centrifuge at 11600g for 30 minutes and re-precipitated as previously. Finally, the precipitate was dehydrated in air at 105°C to determine the dry weight of polysaccharide produced. This method of isolation of polysaccharide is described by Cadmus *et al.*, (1963).

(II) Effect of temperature on polysaccharide production

To determine the amount of polysaccharide produced at different culture temperatures, $2dm^3$ of inoculated medium was harvested after two days at 5, 15, 25 or 30°C. Polysaccharide was isolated by the method described in Section 3.3.2. The viscosity of the medium was analysed at 25°C by using a Brook Field Viscometer (Model DV II).

(III) Metal uptake by extracted polysaccharides re-attached to GAC

To determine metal uptake capacity of extracted polysaccharide (2.68g obtained from a 1dm^3 growth medium inoculated with *Pseudomonas* sp.), it was redissolved in deionised water (0.2dm^3). This solution was mixed with GAC. Water from the mixture was evaporated at 105°C until a dry deposit of polysaccharide was obtained. The presence of the dry deposit was confirmed by using scanning electron microscopy (SEM). The GAC with attached deposit was further exposed to a 25 mg/L cadmium containing solution to determine metal uptake.

3.3.3 Biofilm development from two days old culture

Medium (2 dm^3) was inoculated with the *Pseudomonas* sp. and maintained at 25°C in an incubator for two days. This medium was then used to develop a biofilm by circulation through GAC column for 6, 8, 10, 18 or 24 hours. After the desired circulation period, the column was disconnected and rinsed with Ringer's (¼ strength) solution. The column containing GAC along with any attached biofilm was exposed to cadmium (25 mg/L) to assess metal uptake capacity.

3.3.4 Effect of dissolved oxygen on biofilm development

To assess the effect of oxygen, in this experiment air was continuously purged into the medium which was used for biofilm growth. Biofilm development was as described in Section 3.1.2. An air pump (Model 500 series II, LH Eng. Ltd., Bucks, England) fitted with a flow controller (1 to 10 cm^3/min) was used to purge air into the cultured medium. Biofilms thus developed, were exposed to a 25 mg/L solution of cadmium for metal uptake studies.

3.3.5 Variation in fluidization velocity

For biofilm development under different fluidization velocities, the method described in Section 3.1.2 was adopted, except that flow rate of the medium fluidizing the GAC was varied. The desired flow rate to give velocities of 1.4 to 4.8 cm/s, was obtained by using the flow controller of the peristaltic pump. The initial and expanded bed height (cm) was measured to obtain the expansion of the fluidized bed. Biofilms thus developed were exposed to 25 mg/L solution of cadmium for metal uptake studies.

3.4 Influence of environmental and other factors on metal biosorption

3.4.1 Effect of pH of aqueous metal solution

Solutions ($1dm^3$) were prepared at metal (cadmium, chromium, copper, nickel or silver) concentrations of 25 mg/L. Using buffer solution (*Tris (hydroxymethyl) methylamine*) and 0.1M HCl, a desired pH of the metal containing solution (in the range of 2 to 7) was obtained. These solutions were exposed to biofilm-GAC in a column, as described in Section 3.1, and free metal analysed periodically until an equilibrium was reached.

3.4.2 Effect of temperature of the metal containing solution

Biofilms were harvested on GAC as described in Section 3.1.2 and rinsed with sterile Ringer's solution (¼ strength). Different columns containing biofilm-GAC were exposed to cadmium containing solutions maintained at 5, 15, 20 or 25°C and free metal analysed periodically until equilibrium was reached.

3.4.3 Effect of the presence of organic and inorganic compounds

To analyze the effect of organic and/or inorganic contaminants, $1dm^3$ solutions

containing cadmium or zinc (25 mg/L) were prepared. To these solutions, 10 mg of atrazine and 10 mg of sodium nitrate were added to act as organic and inorganic contaminants. These solutions were thoroughly mixed and exposed to biofilm-GAC in a column. Cadmium or zinc uptake trends were then determined.

(I) Simultaneous metal and organic residue uptake

To asses the ability of biofilm-GAC system to remove metal and organic residue simultaneously, two combinations of solution were prepared. These solutions were each exposed to either a biofilm-GAC system (developed as per the method in Section 3.1.2) or to virgin GAC.

The first solution was a combination of six different metals (cadmium, chromium, copper, nickel, silver and zinc), each at 25 mg/L, together with either, butanol (100 mg/L), benzene (25 mg/L) or atrazine (10 mg/L). The second solution was either of butanol (100 mg/L), benzene (25 mg/L), or atrazine (10 mg/L) only.

Solvents (butanol, benzene and chloroform) used were of 'LR' grade, obtained from BDH (U.K.) or Fisons (U.K.). Atrazine was obtained from Ciba Geigy (U.K.), purified standards of atrazine were obtained from Promochem (U.K).

Samples were analysed for both metal and organic content before and after exposure to biofilm-GAC or virgin GAC. An analysis of benzene and butanol was carried out using gas chromatography, and atrazine by using HPLC (see below).

(II) Analysis of chloroform, benzene and butanol

All analyses were performed on a Hewlett Packard 5790 A series gas chromatograph (GC). The GC was equipped with a flame ionization detector and stainless steel column 300 cm in length and 0.325 cm outer diameter. The column, a PORAPACK-S, was held at 180°C for all the samples. Injector and detector temperatures were maintained at 200°C. Flow rates of N_2, H_2, and air were 40, 80, and 150 mL min^{-1}, respectively. The GC was standardised by injecting suitable standards before test runs and the calibration periodically checked.

(III) Analysis of atrazine

The analysis of atrazine was carried out by using a GILSON HPLC. All samples were pre-filtered through a 0.2 μm nylon filter. The HPLC analysis was carried out in SPHRISORB C8 column in mobile phase of acetone/water/ (50/50). Flow rate was 0.8 mL/min, with detection at 280 nm (0.5 sensitivity).

A calibration curve for atrazine was obtained before analysing experimental samples.

3.4.4 Metal uptake in continuous through flow mode

To assess metal uptake capacity of biofilm-GAC in a continuous through flow mode, solutions containing 25 mg/L concentration of metal (cadmium or chromium) were passed only once through columns containing biofilm-GAC. The metal concentration in the stream coming out of the column was measured every two minutes. The metal exposure to biofilm-GAC continued until the outlet concentration was identical to that of the inlet.

3.5 Effect of type and source of GAC on biofilm development and subsequent metal sorption

3.5.1 Adaptability of different GAC

In this study GAC obtained from BDH (U.K.) and Chemviron Carbon (Brussels, Belgium) (Filtrasorb, F200) were used. The properties of GAC supplied by BDH were given in Section 3.1.2. The F200 used had a 700-750 m^2/g surface area and 0.6 to 0.7 mm diameter. Biofilms developed on both the GACs were exposed to cadmium of 25 mg/L initial concentration and its uptake was analysed.

3.5.2 Effect of GAC particle size

GACs of three mean particle diameter (0.25, 0.5 and 1.42 mm) were obtained from BDH (U.K.). Biofilms were developed over these GACs (Section 3.1.2) and subsequently assessed for cadmium uptake from an initial 25 mg/L concentration.

3.5.3 Biofilm development on GAC regenerated at high temperature

GAC which was previously used in a biosorption study was pre-dried in air at 105°C for 3 hours. To regenerate it at high temperature, a crucible containing this GAC was kept for 5 or 10 minutes in a muffle furnace which was maintained at 900°C. The regenerated GAC was further used for biofilm development as described in section 3.1.2. Samples of this GAC were observed in SEM for any structural deformation (see Section 3.10).

3.6 Metal loading by repeated biosorption cycles

3.6.1 Regeneration of biofilm attached onto GAC

The biofilm was developed as described in Section 3.1.2. This biofilm was exposed

to an aqueous cadmium solution (at 25 mg/L) until a constant metal level was reached. The circulation of cadmium solution was stopped, the column disconnected and a sterile Ringer's solution (¼ strength) was passed through it. Then fresh sterile growth medium (without any fresh culture) was reconnected and circulated for two more days to develop the biofilm. Other conditions for biofilm development were similar to those described in Section 3.1.2. This cycle was repeated for up to six metal exposures.

3.6.2 Repeated use of GAC

Biofilms were developed as described in Section 3.1.2. These biofilms were exposed to aqueous metal solution until constant metal levels in solution were reached. The circulation of the metal solution was stopped and a sterile Ringers solution was passed through the column.

The GAC with attached biofilm and metal was dried at 230°C for 3 hours. The dried GAC was recharged into a column (without any further treatment) and freshly seeded sterile nutrient medium was circulated for two days to develop a fresh biofilm. These biofilms were then exposed to an aqueous metal (cadmium or zinc at 25 mg/L) solution. This procedure was repeated in a cyclic manner.

3.7 Use of organic compound adsorbed GAC for biosorption

3.7.1 Use of GAC (for biosorption) with previously adsorbed atrazine

Biofilms were developed over GAC which had been previously exposed to atrazine. The biofilm development method was described in Section 3.1.2.2. The adsorption of atrazine was carried out as described below.

Pretreated GAC (Section 3.1.2) was exposed in a column to an atrazine containing solution. Distilled water ($1dm^3$) containing either 10, 20, 50, 80 or 100 mg atrazine/L, was circulated through 10g of GAC until atrazine concentration in the circulating solution reached an equilibrium level. These columns were then drained and inoculated and growth medium was circulated through them for two days to develop a biofilm. The amount of atrazine in the water, as well as desorbed back into the medium during biofilm growth, was analyzed by HPLC (Section 3.4.3).

Biofilms developed on these GACs were exposed to aqueous cadmium solutions of 25 mg/L concentration.

3.7.2 Use of GAC with adsorbed chloroform for biosorption

Vapours of chloroform were adsorbed onto GAC by the method described in Section 3.9. The influent concentration of chloroform was maintained at 5% (± 0.3) in air. The GAC saturated with chloroform was then used for development of biofilm as described in Section 3.1.2. The biofilm developed on this GAC was exposed to an aqueous solution containing cadmium or zinc at 25 mg/L concentration.

(I) Cell growth with chloroform containing growth medium

Medium ($0.1dm^3$)in a shaker flask with a known amount of chloroform was inoculated by the *Pseudomonas* sp. under study. The shaker flask was then incubated at 25°C, and samples collected every 15 minutes during the initial three hours, and then every hour. Cell growth was measured as described in Section 3.1.

(II) Cell growth with chloroform adsorbed onto GAC

To 0.1 dm^3 of growth medium in a shaker flask, accurately weighed GAC (0.1 to 1 g) adsorbed with chloroform (0.6 g chloroform/g GAC) was added. The flask was inoculated with bacterial culture and incubated at 25°C in a rotary shaker. Samples were taken every 15 minutes during the initial three hours, and then every hour. Cell growth was measured as described in 3.1.

3.8 Testing of GAC properties

3.8.1 Regeneration of GAC with biosorbed metal over the surface in a nitrogen atmosphere

GAC with biosorbed metal (GACBIO-M) or only biofilm over the surface (GACBIO) was dried at 105°C for one hour to remove the excess fluid. This GAC was then kept in an airtight stainless steel column (10mm diameter and 140 mm length), which was maintained at a temperature of 230°C.

During regeneration nitrogen was passed through the column. A flow controller was used to monitor the nitrogen flow rate. Flow rate was maintained constant at 1 ±0.05 L/min. The temperature of nitrogen entering to the regeneration columns was 230°C, achieved by preheating the gas in an extension of copper tubing added to the column. After three hours of regeneration, GACs were cooled by passing cold nitrogen through the column.

Materials thus regenerated (GACBIO-M) were subsequently used for vapour adsorption studies. Chloroform adsorption capacity of GACBIO-M was compared with virgin GAC. Therefore, in order to compensate for any effects from regeneration, the

virgin GAC (without any biofilm) was also subjected to similar nitrogen regeneration conditions. At the end of a regeneration period, the amount of weight loss was determined. This weight loss ranged from 3 to 4% in the case of virgin GAC and 4 to 6% in other samples.

3.8.2 Analysis of particle size of GAC with attached biofilm and metal

Particle size analysis of virgin GAC, GACBIO and GACBIO-M was carried out by a sieve shaker. A quantified mass was used in a Test Sieve Shaker (EFL model, London), in which vibration cycles were maintained at 50 cycles/min for all the tests. The particle size distribution was analyzed as weight of particles in each sieve at the end of a 10 minute cycle. Sieves 8 (2 mm), 10 (1.70 mm), 14 (1.18 mm), 16 (1.0 mm), and 22 (0.710 mm) mesh were used in this test. They were classified according to BS 410:1962.

3.8.3 Surface area analysis

Surface area and pore size analysis were carried out via nitrogen adsorption in an Accelerated Surface Area and Porosimetry system (ASAP 2000) manufactured by Micromeritics Instrument, Norcross, USA. The surface area and porosity assessment was performed under the following specifications:

Pressure range	0 to 950 mm Hg
Accuracy of pressure	± 0.1%
Sample tube	¼ inch O.D. and 9 cm^3 bulb
Analysis gas	Nitrogen
Degas	Helium

Bath temperature	70 to 80°K
Sample Size	0.15 to 0.2 g

Samples of R-GAC, R-GACBIO and GACBIO-M were dried overnight at 120°C before using them in surface area analysis. The total surface area was calculated from measurement of adsorbed nitrogen as a function of relative pressure. Total pore volume and average pore radius were determined from the same nitrogen isotherms.

3.8.4 Testing for attrition resistances of GAC particles

The attrition rate of dry GAC was obtained by measuring the weight loss due to fluidization. The attrition rate of GACBIO and GACBIO-M was compared with virgin GAC. For comparison virgin GAC before the attrition test was subjected to fluidization for two days by using water or sterile growth medium.

All the particles before the attrition test were, (i) dried in air at 105°C for 3 hours, (ii) in equilibrium with air to avoid further oxygen adsorption (iii) sieved to ensure that they were above 0.85 mm in size, as breaking of particles below 0.85 mm was considered as loss of weight in this attrition test.

The columns were charged with 5 g of test particles and subjected to a fluidizing velocity of 1.66 cm/sec. This velocity gave approximately 60% bed expansion. At periodic intervals, columns were disconnected and particles sieved to determine their mass retained over on 18 mesh (0.85 mm) sieve. The loss of mass with time was compared with other test samples as an indication of degree of attrition. This procedure was continued until a stage when the weight of sample particles remained

constant.

3.8.5 Measurement of surface basicity of the adsorbent

The total surface basicity of the GAC, GACBIO and GACBIO-M was evaluated by mixing 0.5 g of adsorbent with 50 dm^3 of 0.1M HCl. This suspension was shaken for 48 hours at 25°C. After which, an aliquot of the supernant liquid was titrated with 0.1M NaOH. The basicity was calculated from the volume of 0.1M NaOH required to neutralize the aliquot. This method of basicity measurement of GAC was as suggested by Moreno-Castilla *et al.*, (1993).

3.9 Adsorption of chloroform vapour from an air stream onto GAC

For chloroform adsorption studies, the experimental set-up used was as shown in Figure 3.2. Similar method was used by Zilli *et al.*, (1993) for phenol vaporization. The set-up consisted of (i) chloroform vaporisation section, (ii) adsorption column (iii) condensation section.

(i) Vaporisation section

To obtain chloroform vapour, air was bubbled into a flask containing liquid chloroform (kept in water bath and maintained at 20 ±2°C). With the help of a flow controller the volume of air entering into the flask was controlled. This air conveyed chloroform to the adsorption column. During vaporisation, (a) humidity of the air entering into the flask was measured by a Hygrometer (found to be between 50 to 55% in all experiments) and (b) temperature of the air entering the flask was maintained at 20 ±2°C by pre-heating it.

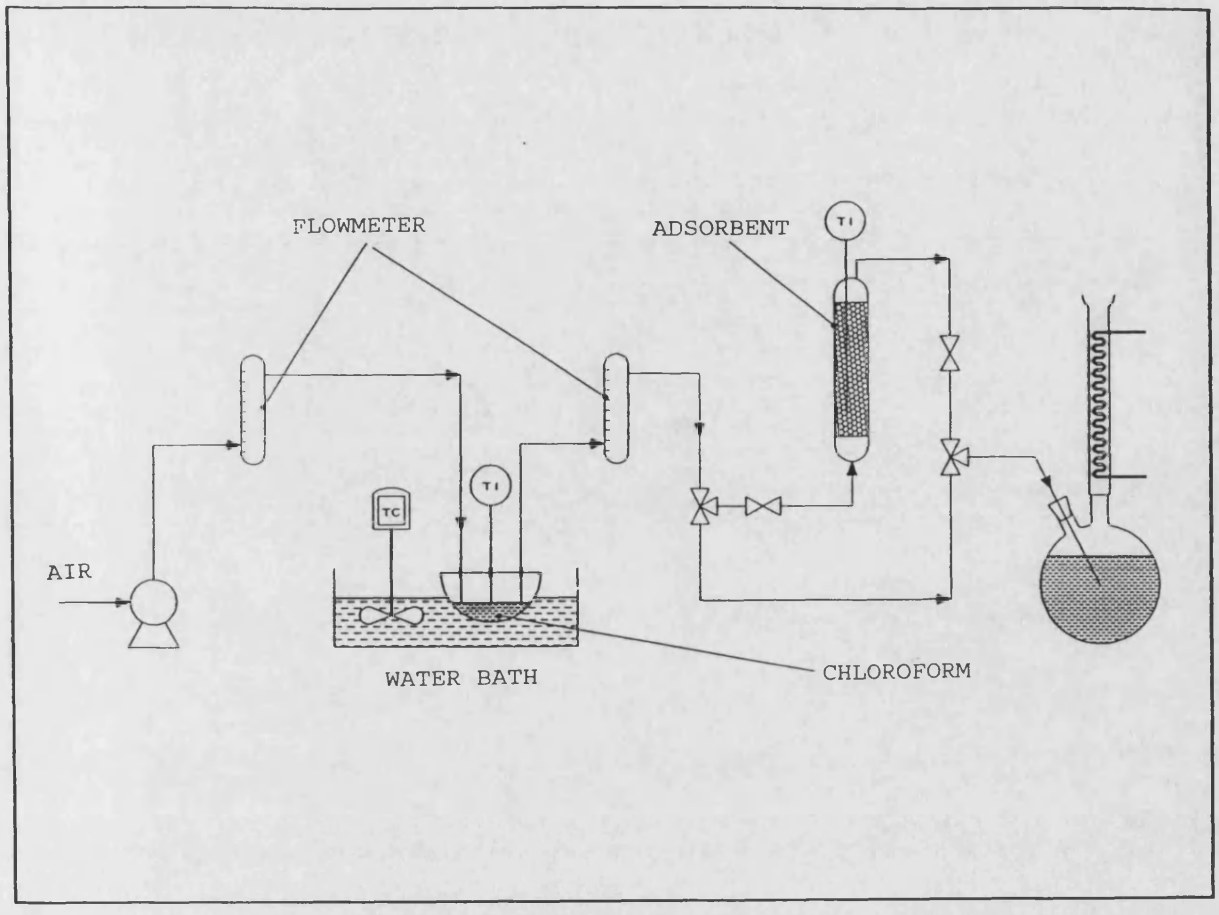


Figure 3.2 Chloroform adsorption experimental set up schematic diagram

(ii) Adsorption section

The adsorption section consists of a column and a flow meter (rotameter). The column had an inside diameter of 13 mm and was 60 mm long. The air containing chloroform passed up through a 1 g bed of accurately weighed adsorbent in the column. The adsorbent bed was fitted with a thermocouple (digital), located in the centre. The adsorption column and connected tubing was flushed with air between each experiment to remove any condensed chloroform. To determine chloroform concentrations in the inlet (C_i) and outlet (C_o) air of the column, samples were collected from sample ports. Further, air samples were collected every minute at the

column outlet and analysed for chloroform using GC. The adsorption was continued until chloroform concentration at the outlet (C_o) was equal to that of the inlet (C_i).

(iii) Condensation section

To calibrate the amount of chloroform in the air stream, chloroform was condensed in distilled water (0.5 dm3) and analysed by using GC (Section 3.4.3). A coolant maintained at -5 ±2°C was circulated through the condenser. A calibration curve was obtained for concentration of chloroform at different air velocities.

3.10 Electron microscopy

Scanning electron microscopy (SEM) was used to observe the surface of GAC and appearance of biofilm over a GAC surface. Energy dispersive X-ray microanalysis system (EDS) was used in conjunction with a scanning electron microscope to identify and map metals present over GAC surfaces. Samples of virgin GAC and biofilm-GAC exposed to metals (at 25 mg/L of cadmium, copper, nickel and zinc) were analysed by EDS. The SEM employed was a JOEL T330. The X-ray system was an EDS Link systems AN10000, attached to a JEOL 35c SEM.

Preparation of samples for microanalysis by the SEM required maintenance of specimen integrity. Sample preparation involved two stages.

(i) Biomass coated and/or metal exposed GAC particles were first freeze dried under vacuum between 10^{-4} to 10^{-6} Torr with the temperature maintained between -60 to -65°C, until all water was removed. Drying took 24 to 36 hours, depending on the sample. This reduced risk of sample contamination, loss of soluble substances and

chemical alterations.

(ii) Freeze dried samples were glued to uniform flat, polished aluminium or carbon stubs. Specimen stubs were then coated with a thin layer (approximately 5 nm) of conducting material such as gold or carbon. This coating protects the sample from electron bombardment during scanning.

3.11 Experimental errors and deviation

Unless otherwise mentioned following experimental variations were observed.

(i) Metal uptake values reported in this thesis are within the variation of ±3%. These values were obtained from results of 3 to 6 repeated experiments, their arithmetic mean, deviation, and standard deviation (Pantony, 1961; Jenson and Jeffreys, 1963).

$$\bar{x} = \sum \frac{x}{n}$$

where: x, is results of experiment; n, is number of experiments; and $\overline{x}$, is arithmetic mean. The deviation d and standard deviation s are given as:

$$d = x - \bar{x}$$

$$s = \sqrt{\frac{\sum d^2}{n-1}}$$

(ii) The comparative metal uptake values reported here, unless otherwise mentioned are within the experimental error.

(iii) Production of polysaccharides, unless otherwise mentioned was within 5 to 6%, experimental variation.

(iv) In chloroform adsorption capacity experiments, experimental errors were within a variation of 4 to 5%.

(v) The comparative chloroform adsorption capacities reported here, unless otherwise mentioned are within the experimental deviation.

Chapter 4

RESULTS AND DISCUSSION

This chapter is divided into ten sections which present the results of the studies initially set out in this research (Section 1.3). In the first section the appearance and optimal age of biofilm-GAC is discussed. The uptake of six different metals (as single and multi-metal systems) by biofilm is considered in the second section. Uptake kinetics and process design parameters, such as capacity, diffusion and mass transfer coefficients are discussed in section three. Section four deals with biofilm-GAC developed under different conditions (such as pH, temperature, oxygen *etc.*) for metal sorption. A discussion on environmental factors such as pH, temperature *etc.*, associated with metals, is presented in section five. Use of different GAC for biosorption (obtained from different source or regeneration), repeated metal biosorption cycles, and use of GAC saturated with organic compounds for biofilm development and subsequent biosorption, are discussed in section six, seven and eight, respectively. The influence of the heat fused biofilm on the properties of GAC (such as surface area, porosity, basicity *etc.*) is discussed in section nine. In the final section, the use of GAC-biofilm-metal for adsorption of an organic vapour (chloroform) is discussed.

4.1 Microbial culture for development of biofilm

The selection of suitable polysaccharide excreting bacteria for this research is considered in this section. Different Gram -ve rod species with common characteristics (particularly ease of culture) and similar growth rates, were selected for initial

screening. The species were a *Pseudomonas* sp. (NCIMB 11592), *Enterobacter aerogenes* (University of Bath Culture) and *Serratia marcescens* (NCIMB 8805). The first two have the capacity to excrete significant quantities of polysaccharides and it has been shown that this type of species has the ability to accumulate large quantities of heavy metals, such as cadmium (Scott and Palmer, 1990). *Serratia marcescens* was selected as it has a morphology similar to the other two, but is not capable of excreting polysaccharides.

Factors relating to the nature of the biofilm, such as metal sorption and physical appearance of the biofilm over GAC were compared. The physical appearance of the biofilm-GAC, was examined by Scanning Electron Microscopy (SEM). An optimum culture age and biofilm state (live or dead) was established, which was then used in further studies during this research.

4.1.1 Physical appearance of a biofilm

Formation of a biofilm over GAC was one of the basic aspects of this research. The physical appearance of the biofilm was examined by using SEM to determine the film structure and also degree of biomass attached to the GAC.

Virgin GAC particle surface (Figure 4.1) showed a rough, porous texture, capable of providing a favourable and protected environmental surface for the growth of micro-organisms. The crevices observed on the GAC surface were expected to provide initial cell attachment sites and protection for the cells from fluid shear. The majority (*i.e.* ≈75%) of the GAC particles were spherical in shape, although some irregular shapes were also present.

Figure 4.2, 4.3, and 4.4 show biofilm over GAC after 2 days of incubation. The biofilms developed by *Enterobacter aerogens* were scanty and thin, with excreted polysaccharides visible above the cracked surface (Figure 4.4). In contrast, biofilms of the *Pseudomonas* sp. were thick and highly dense (Figure 4.2 and 4.3) with polysaccharide forming an open net like matrix. However, biofilms did not have a uniform coverage over the entire surface of the GAC. It was observed that biofilm was localized over edges and irregular surfaces rather than the relatively flat surface of the GAC. Non-uniform coverage by biofilm over GAC has also been reported by Pirbazari *et al.*, (1990) and Craik *et al.*, (1992).

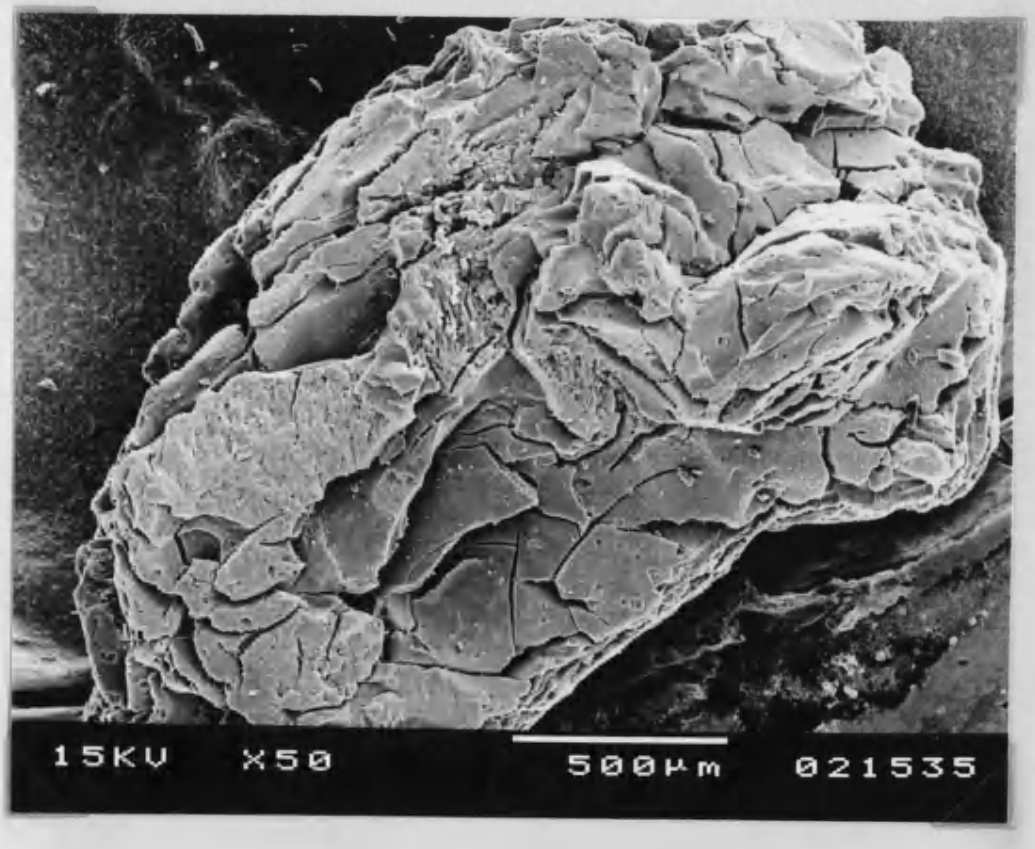


Figure 4.1 Virgin GAC (magnification X 50)

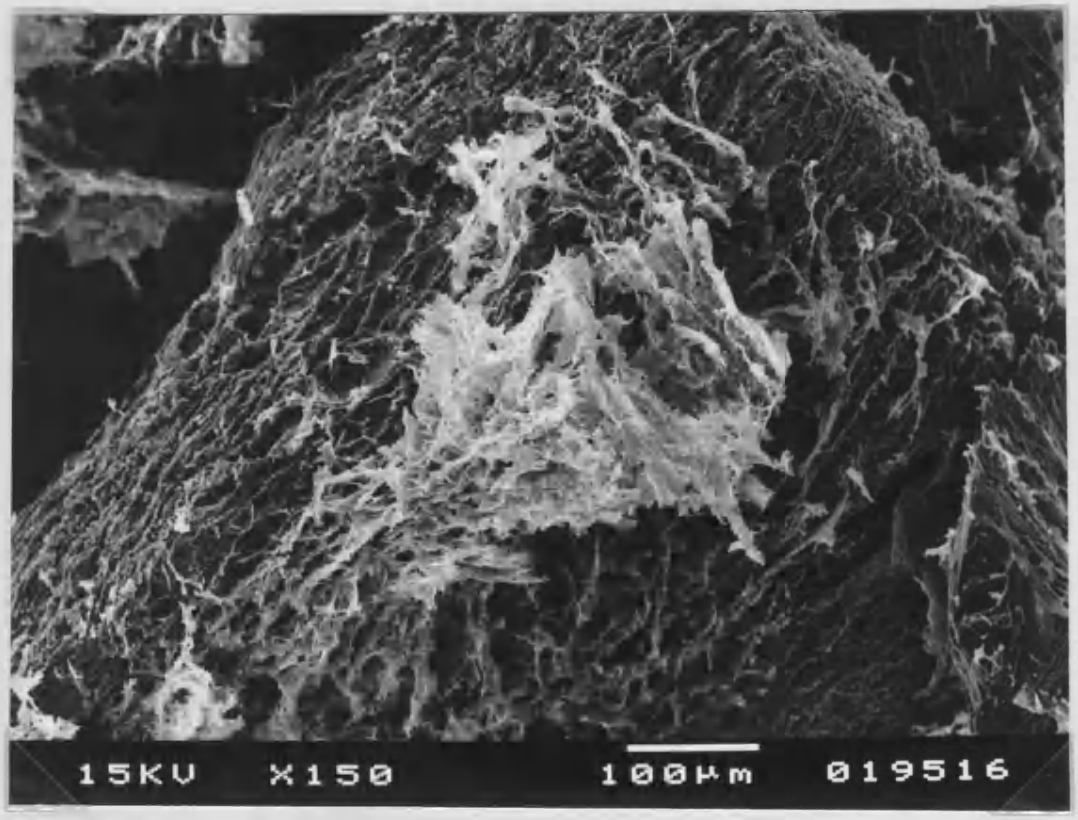


Figure 4.2 *Pseudomonas* sp. biofilm over GAC after 2 days, magnification X150

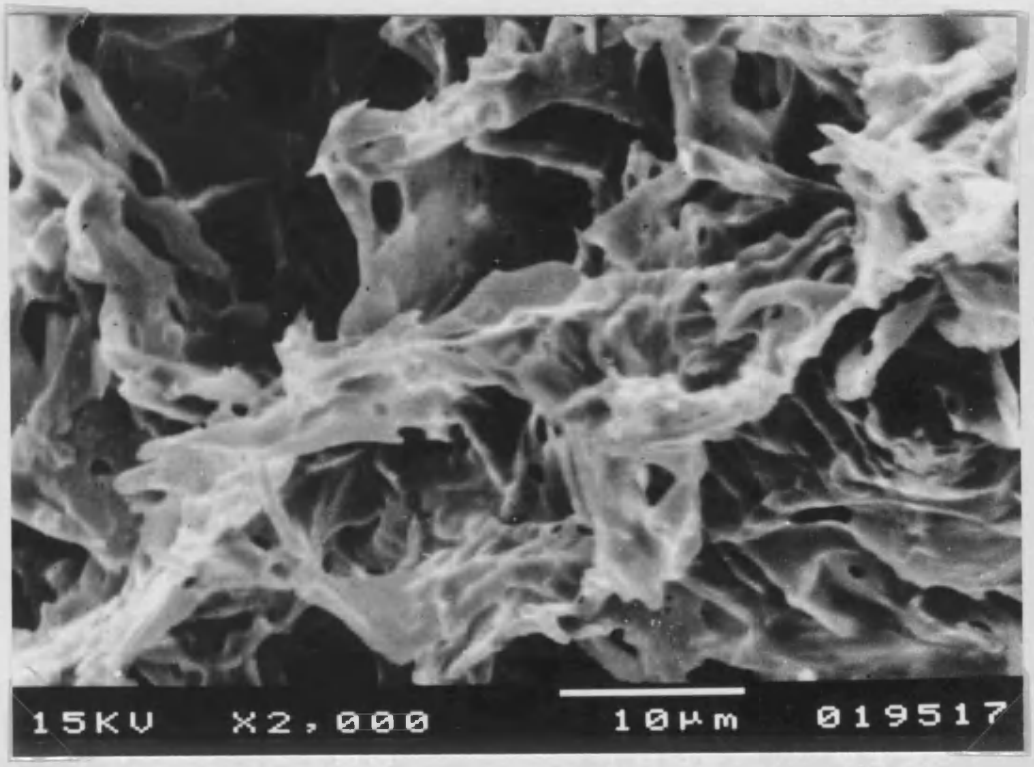


Figure 4.3 *Pseudomonas* sp. biofilm over GAC after 2 days, magnification X 2000

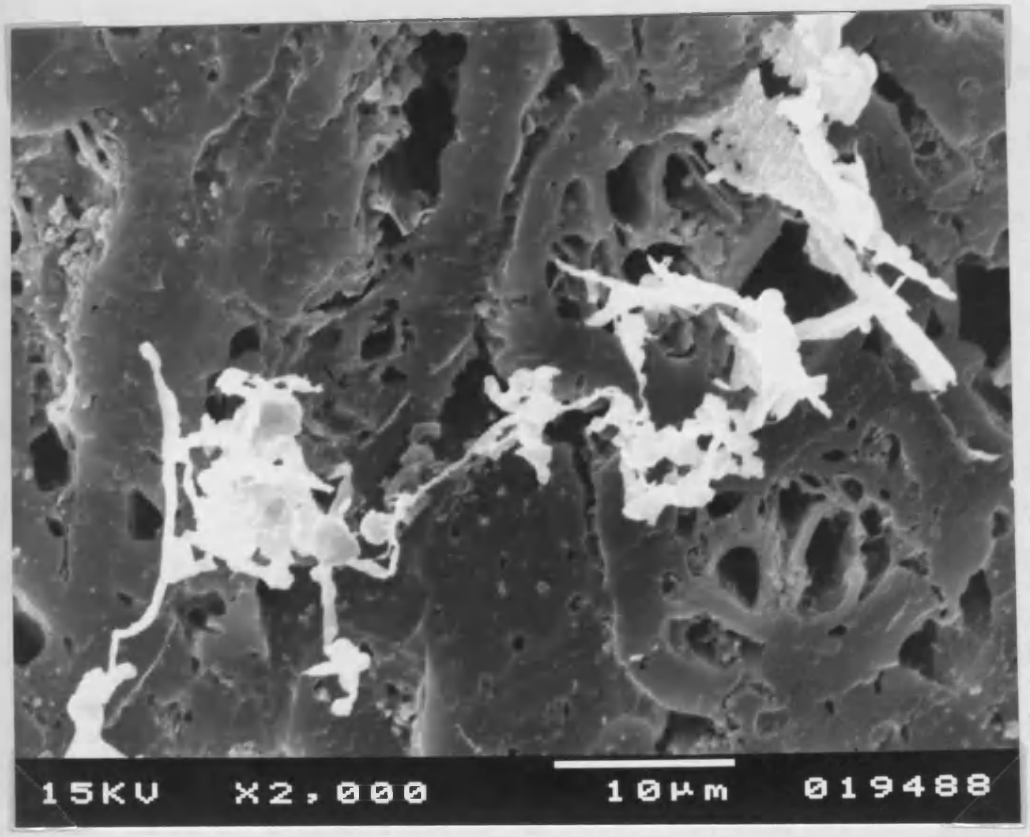


Figure 4.4 *Enterobacter aerogenes* biofilm over GAC after 2 days, magnification X 2000

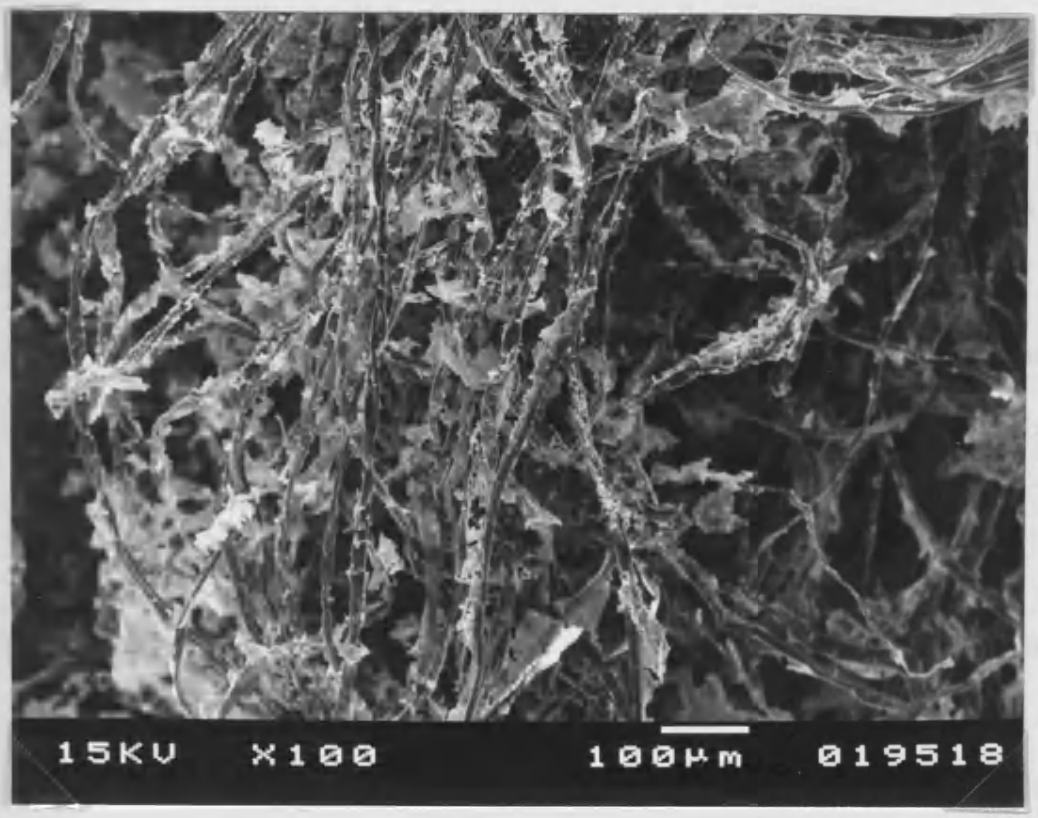


Figure 4.5 *Serratia marcescens* biofilm over GAC after 4 days, magnification X 100

The biofilm formed by the non-polysaccharide secreting species, *Serratia marcescens*, had a thin appearance, even after a 4 day growth (Figure 4.5). Further, the slime like biomass of *Serratia marcescens* biofilms, was observed in only small patches over the GAC.

4.1.2 Age of biofilm, cell growth and metal (cadmium) uptake

The optimum age of biofilms used in biosorption studies was determined by a combination of cell growth studies in suspension, and metal uptake trends. The age of biofilm with respect to metal uptake was assessed by using *Pseudomonas* sp. only, due to the good biofilm observed with the SEM. Biofilms (biomass and cells) attached to GAC were harvested between 1 to 6 days and exposed to metal containing solutions.

(I) Cell growth and biofilm dry weight

The three species (*Pseudomonas* sp., *Enterobacter aerogenes* and *Serratia marcescens*) showed similar cell growth trends in suspension (measured by optical density, Figure 4.6). They showed a 10 to 12 hour exponential growth phase followed by a 36 to 38 hour stationary phase. Cells in a one day old suspension were in their early to mid-stationary growth phase and by day 2, in the late stationary phase. Cell growth, thus appeared to be in a stationary phase at the end of two days.

In the case of the *Pseudomonas* sp., attachment of biofilm to the GAC surface and production of polysaccharides (in the growth medium) were also assessed (Table 4.1). The attachment of the biofilm to the GAC followed cell growth (Figure 4.8). In the 48 hours, 130 mg biofilm (dry weight/g of biofilm-GAC) was attached to the GAC.

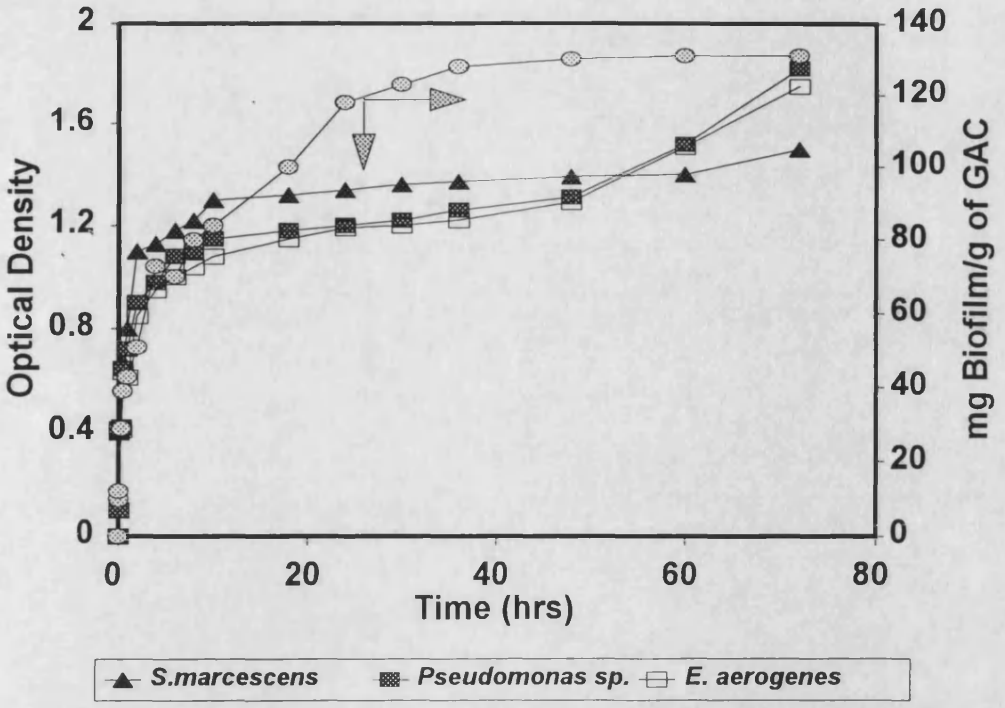


Figure 4.6 Cell growth trends of the three bacterial species at 25°C, and biofilm dry weight attached to GAC by the *Pseudomonas* sp.

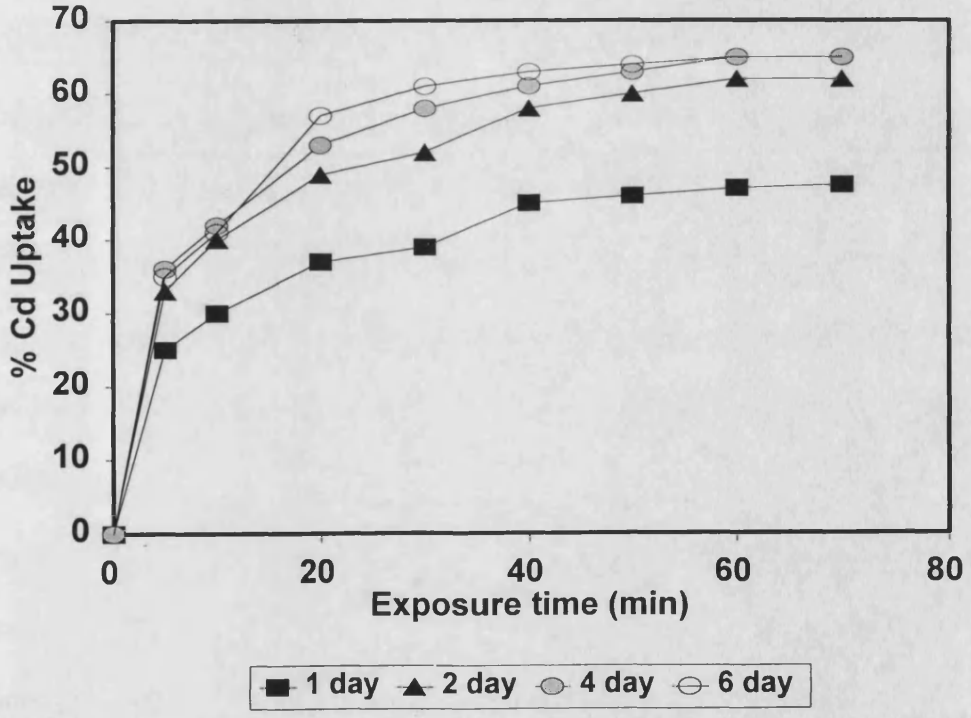


Figure 4.7 Cadmium biosorption (25 mg/L) by biofilm-GAC system using different age *Pseudomonas* sp. biofilms

Attachment of biofilm appeared to increase with cell growth, before reaching an equilibrium.

Table 4.1 Polysaccharide production and biofilm attachment to GAC with time

Time (hours)	Polysaccharide (mg/mL)	Biofilm (mg/g GAC)
18	1.30	100
24	2.34	112
36	2.42	120
48	2.68	130

(II) Age of biofilm and cadmium uptake

Figure 4.7 shows cadmium biosorptive trends of biofilms developed over different periods of time. With biofilms which were developed in one day, metal uptake was substantially less (by 22%) as compared to biofilms which were 2 to 6 days old. There was no major difference in metal uptake by biofilms which were harvested after two days.

The significantly lower metal sorption by one day old biofilm may be due to the reduced level of polysaccharide. Polysaccharide production was found to commence at mid to late exponential phase (Figure 4.8 and Table 4.1). Polysaccharide production continued until late stationary phase. Similar trends in polysaccharide production were reported by Piggot *et al.*, (1982). They reported that with *Pseudomonas aeruginosa*, polysaccharide production did not commence until late in the exponential phase, and that production was maximum during the stationary phase.

The observed metal uptake by one day old biofilms could be, to a greater extent, due to the attached cells over GAC. Such cell attachment in the early stages of growth was reported by Annachhatre and Bhamidimarri (1992), although, Roger *et al.*, (1990) observed that the adhesion of cells varied with species, *e.g. F. succinogenes* had the highest percentage of adherent cells at the end of exponential growth, whereas *R. flaverfaciens* adhered in the early stages of exponential growth.

The difference in the response of young and old cells to a toxic metal is not clear. The availability of energy source(s) may cause actively growing cultures to respond differently to resting cells. Energy dependant metal efflux and energy dependant metal uptake systems have both been reported (Bucheder and Broda, 1974; Nies and Silver, 1989), which may also be the underlying reasons for different levels of uptake.

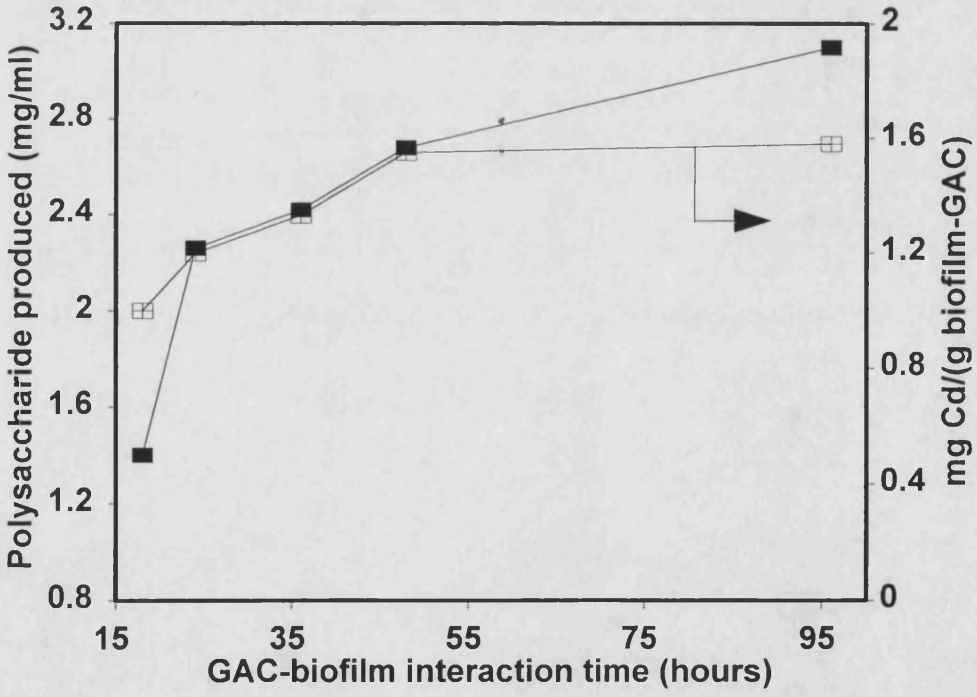


Figure 4.8 Polysaccharide production (at 25°C) with time and cadmium biosorption (25 mg/L) by biofilm-GAC system using *Pseudomonas* sp.

The micro-organisms within an old biofilm have undergone all phases of growth. In addition, micro-organisms when attached to solid surfaces can vary greatly in size, shape, and structure, suggesting a wide range of growth rates (Marshall, 1984; Vandevivere and Kirchman, 1993). Furthermore, Wiencek *et al.*, (1990) showed that the hydrophobicity of viable cells is dependant on culture age. Irrespective of all factors mentioned above, the metal biosorption performance of 2, 4 and 6 day old biofilm was not affected.

Table 4.2 shows the cadmium biosorption (at 25 mg/L) capacity of the three species. A two day old biofilm was used for this comparison. This result shows the superior metal biosorption capacity of the *Pseudomonas* sp. This also suggests that the biosorptive process was predominantly governed by interactions between extracellular polysaccharides and metal, rather than those between the viable cells and metal.

Table 4.2 Uptake capacity of two day old biofilm-GAC of three species after exposure to 25 mg/L cadmium

Species	**mg Cd/g biofilm-GAC**
E. aerogenes	1.10
S. marcescens	0.50
Pseudomonas sp.	1.55

These results were used to decide on the optimal biofilm age and species for subsequent metal biosorption studies. Since there was no major difference in metal uptake ability between 2 to 6 day old biofilms, it was decided that in all subsequent

studies, biofilms developed over 2 days would be used. Furthermore, due to good metal sorption and biofilm formation ability, the Pseudomonad was selected for this research project. That is, in all further experiments, the *Pseudomonas* sp. was used.

4.1.3 Metabolically inactive biomass for biosorption

The use of non-living (non-metabolizing) biomass may be supported by the fact that waste streams are often toxic to living systems, devoid of nutrients and can posses extremely variable conditions. Many biological metal-removal systems used in industry, or approaching commercialization, employ non-living or non-metabolizing systems (Brierley *et al.*, 1986; Tsezos, 1988; Mattuschka and Straube, 1993).

Experiments were conducted to ascertain whether any change in the biofilm cadmium uptake capacity occurs in the absence of viable cells. Cells in the biofilm were made non-viable by using three different methods: (i) autoclaving at 121°C; (ii) exposing to 10% formaldehyde; and (iii) heat treatment at 95°C (by keeping the columns of biofilm-GAC in a hot water bath for 45 minutes). However, each technique has a potential drawback. For example, biofilms may be disrupted by high temperature and pressure and may become compressed, such that the cell wall and polymers may deform. This can result in the unfolding of proteins and exposure of internal hydrophobic sites (Li Chan *et al.*, 1984). Formaldehyde treatment on the other hand may affect charges on the cell wall (Strandsberg *et al.*, 1981), and influence the metal biosorptive properties of the dead cells.

The biosorptive cadmium uptakes (from initial 25 mg/L) by active and non-active biofilms are shown in Figure 4.9. In all these studies, the live biofilms appeared

consistently better in their biosorptive capacity than the non-viable biofilms, despite of some destruction to the biofilm.

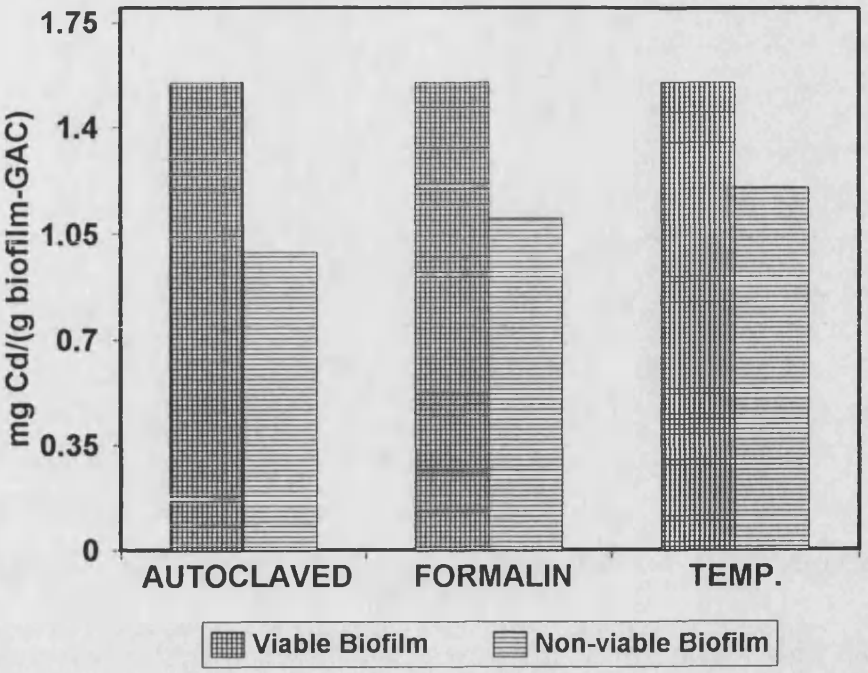


Figure 4.9 Cadmium uptake by active and non-active biofilms, at 25 mg/L initial cadmium concentration (biofilm exposed to autoclaveing, formalin or temperature)

The presence of viable cells enhanced cadmium accumulation from 1 to 1.55 mg Cd/(g of biofilm-GAC). It may therefore be inferred that metal accumulation by the biofilm is due to active (intracellular) and passive (surface adsorption) activities. Uptake of metals by non-viable biomass is predominantly due to the attached biomass (cell residue and polysaccharides). Even after formaldehyde treatment, it was found (in SEM studies during this research) that cells (debris) can still adhere onto GAC surface. Similarly, Gong and Forsberg (1989) reported that *F. succinogenes* species continued to adhere to a cellulose support after formaldehyde treatment. Furthermore, biomass surfaces can accumulate metal through an ion exchange type interaction

(Volesky, 1990).

Wiencek *et al.*, (1990) observed that heat treatment of adhered cells exposed internal hydrophobic moties within the cells. It was also found in SEM studies that the various treatment destroyed the part of the biofilm. However, such effects were not clear in SEM studies. Despite the possible alteration in cell configuration due to heat and formaldehyde treatment, the biofilms-GAC were still capable of sorbing significant metal.

Although the viability of cells in the biofilm greatly influence the cadmium uptake, this result indicates that cadmium uptake by the biofilm was mediated primarily by polysaccharides and cell biomass.

4.2 Metal uptake by biofilm-GAC

In the following sections, the biosorptive capacity of the biofilm for silver, cadmium, zinc, nickel, copper and chromium is described. These metals were exposed to the biofilm, either individually or in combination, over a concentration range of 5 to 100 mg/L. Metal uptake trends and loading capacities for various metals of biofilm-GAC system were compared with the result for cadmium.

4.2.1 Metal uptake from a single metal system

Figures 4.10 and 4.11 illustrates cadmium uptake trends by biofilm-GAC. Metal loading capacity, defined as the amount of metal that is loaded onto the biofilm-GAC, was observed to increase with increasing initial cadmium concentration in the solution. Cadmium uptake appears to be in two uptake stages, followed by an equilibrium stage.

However, appearance of these stages were not distinct. The first stage was an initial rapid uptake (passive uptake), which was followed by a second, much slower, uptake stage (active uptake). For example, with an initial concentration of 25 mg/L cadmium, uptake was observed to be 0.09 mg/(min. g of biofilm-GAC) during the initial ten minutes. This then decreased to 0.0016 mg/(min. g of biofilm-GAC) until an equilibrium was achieved. A combination of passive and active uptake is well documented (Brown and Lester, 1982 a, b; Ting *et al.*, 1989; Volesky 1990). The initial metal uptake is assumed to be due to attachment of metals to cell components and extracellular products. The second slower uptake is considered to be due to intracellular uptake by the cells (Scott and Palmer, 1990).

These uptake trends suggest the existence of a concentration gradient (driving force) between biofilm and bulk solution, which may influence the metal uptake capacity of the biofilm. The time to reach equilibrium metal levels was found to be inversely proportional to initial cadmium concentrations. For example, at 5 mg/L, the equilibrium metal loading was reached in 80 mins, which is 3 times slower than that for an initial concentration of 100 mg/L (25 minutes). The higher concentration gradient not only increased uptake rates, but also resulted in a rise in cadmium loading. Cadmium loading was increased by 8.5 times when the initial concentration was increased from 5 to 100 mg/L.

The contribution of the biofilm to the total accumulation of cadmium was significant (both by surface binding and intracellular accumulation) when compared to virgin GAC. The contribution of GAC, due to its small adsorption capacity for cadmium, was very small (20% of biofilm-GAC system at 25 mg/L initial concentration).

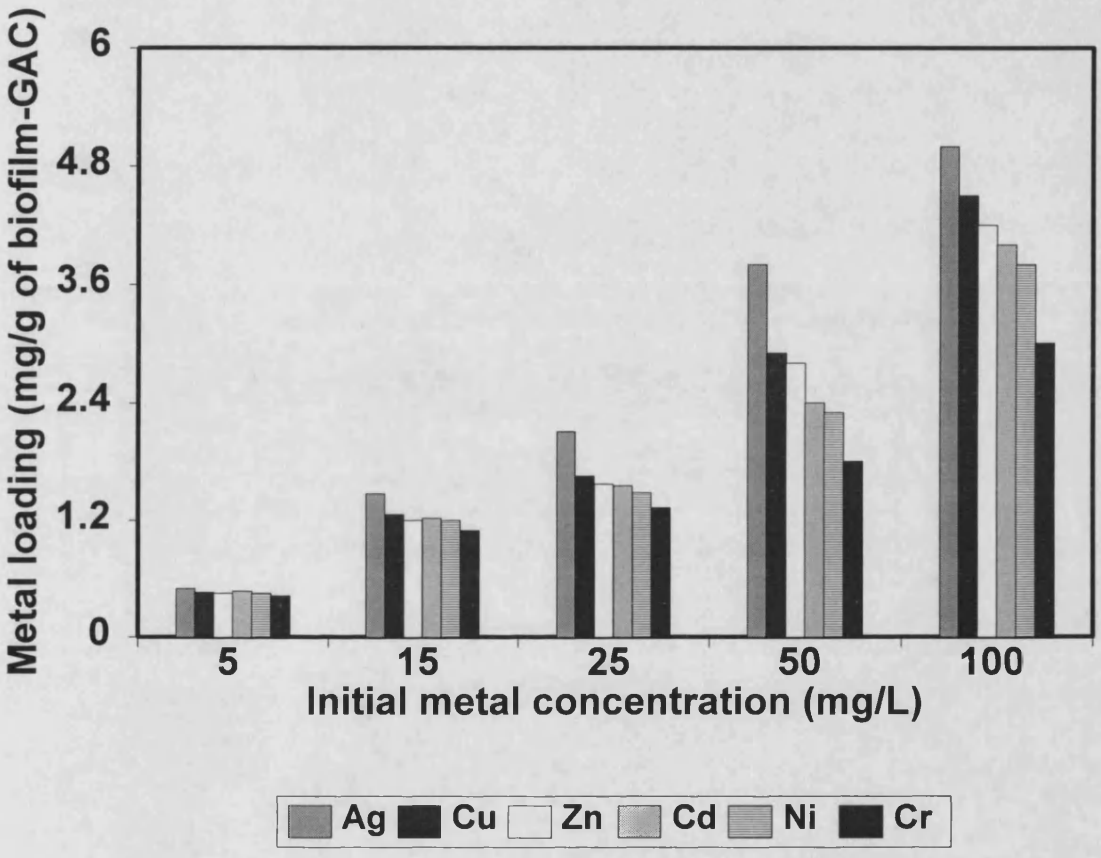


Figure 4.10 Metal loading capacity for Ag, Cu, Zn, Cd, Ni and Cr of biofilm-GAC system from a single metal solution: 5 to 100 mg/L initial metal concentration.

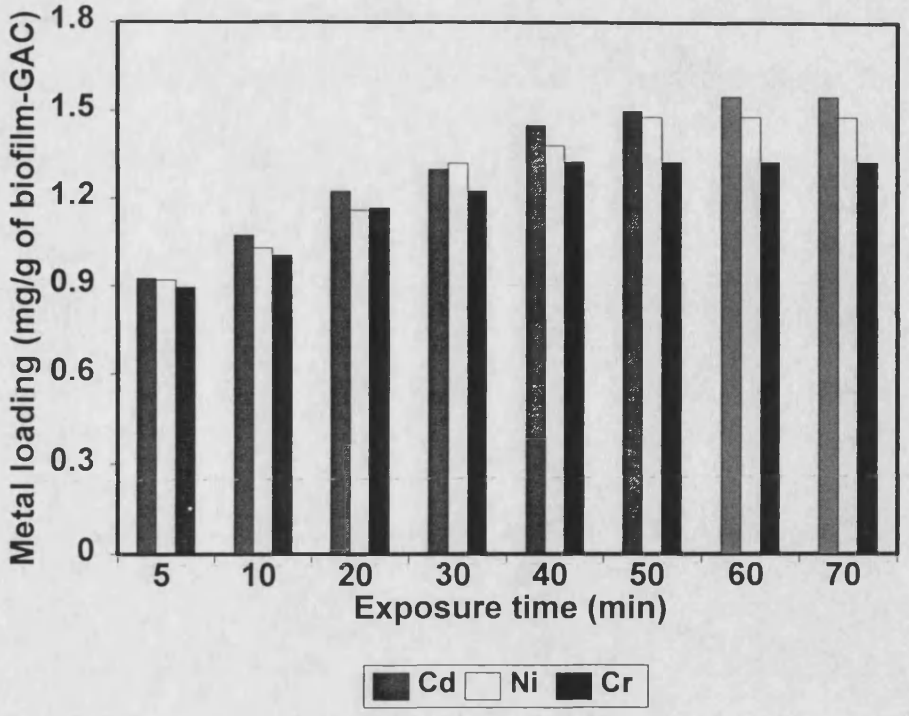


Figure 4.11 Metal uptake rate (Cd, Ni and Cr) by biofilm-GAC system from a single metal solution, at 25 mg/L initial metal concentration

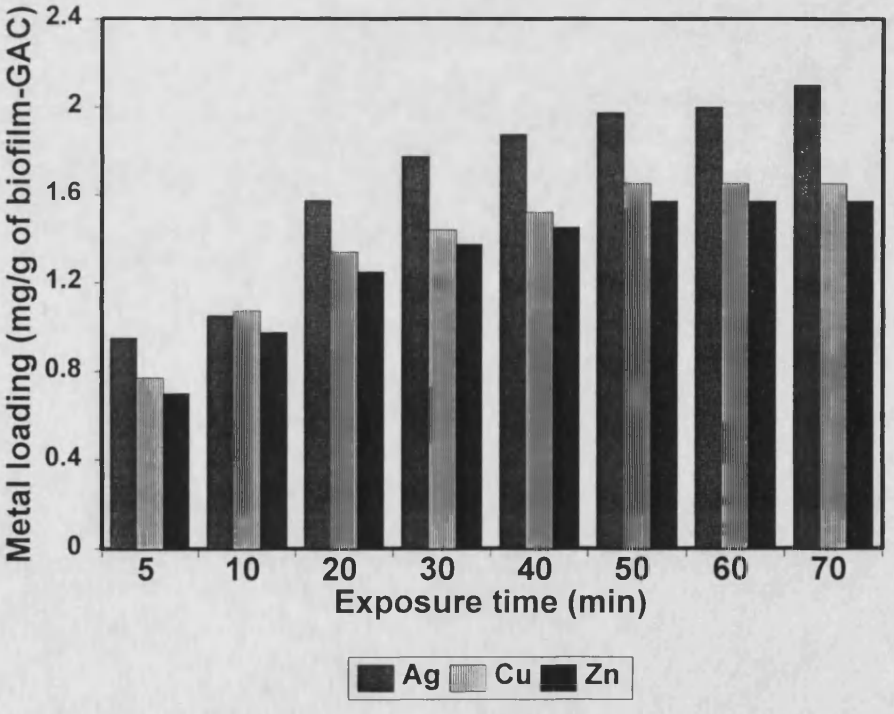


Figure 4.12 Metal uptake rate (Ag, Cu and Zn) by biofilm-GAC system from a single metal solution, at 25 mg/L initial metal concentration

Uptake trends by biofilm-GAC system for the other five metals (chromium, copper, nickel, silver and zinc) were similar to that for cadmium (Figures 4.10, 4.11 and 4.12). However, the final loading levels varied with the metal (Table 4.3). On the basis of metal loading capacity of biofilm-GAC, the following sequence was identified,

$$Ag^{+} \rangle Cu^{+2} \rangle Zn^{+2} \rangle Cd^{+2} \rangle Ni^{+2} \rangle Cr^{+3} \qquad [S\ 4.1]$$

Table 4.3 Metal loading capacities of biofilm-GAC system at different initial metal concentrations

Initial metal Conc. (mg/L)	Metal loading capacities (mg/g biofilm-GAC)					
	Ag	Cu	Zn	Cd	Ni	Cr
5	0.495	0.46	0.45	0.47	0.45	0.42
15	1.47	1.26	1.21	1.22	1.20	1.09
25	2.10	1.65	1.57	1.55	1.48	1.32
50	3.80	2.90	2.80	2.40	2.30	1.82
100	5.00	4.50	4.20	4.00	3.80	3.05

The following trends were seen in the uptake of these metals by the biofilm-GAC system in comparison with cadmium uptake.

(1) Chromium uptake capacity of biofilm-GAC was the lowest amongst metals tested in this research. This could be due to uptake mechanism of chromium and its interaction with polysaccharides. For example, Gaughofer and Bianchi (1991) reported that interactions of chromium (III) with micro-organisms take place by less efficient mechanisms. Its uptake has also been reported to be dependant on nature of the polysaccharides (Galli *et al.*, 1988).

(2) Copper loading was observed to be higher as compared to cadmium, *e.g.* it was 12% more at the initial metal concentration of 100 mg/L. This may be due to copper having higher interactions with exopolymers than cadmium has (Bitton and Freihofer, 1978).

(3) The loading capacity for nickel by biofilm-GAC was found to be lower than that for cadmium, zinc, copper and silver. In comparison with cadmium, nickel loading by biofilm was 5% lower at an initial concentration of 100 mg/L.

(4) Zinc uptake in comparison with cadmium was found to be similar at low concentrations, but increased by 5% at 100 mg/L. Although, zinc and cadmium are commonly found in association with each other, they have different influences on cell metabolism. Therefore, similar uptake capacities and kinetics of the two metals was rather unusual. Apel and Torma (1993) reported that zinc was biosorbed twice as fast as cadmium by calcium alginate beds. Ting *et al.*, (1989) reported a higher adsorption constant for zinc compared to cadmium as a result of a difference in metal-cell affinities.

(5) Silver loading capacity of biofilm-GAC system was found to be highest among all metals tested. In comparison to cadmium uptake, at 100 mg/L, 25% more loading of silver was observed. However, the equilibrium uptake stage in the case of silver was reached at a slower rate. That is, uptake of silver required 55% more time than cadmium at 25 mg/L.

Silver uptake was found to be a combination of both biofilm interaction and GAC

adsorption. This was supported by, (i) high silver adsorption capacity of GAC alone (1.7 mg/g GAC, from 25 mg/L), which was further increased in the presence of biofilm, (ii) considering the toxicity of silver and its oligodynamic nature at concentration as low as 200γg/L (Thomson, 1973), the possibility of high intracellular or metabolic accumulation was negligible, (iii) slower uptake rate of silver suggests that silver ions would migrate through the biofilm onto the GAC surface. Thus the biofilm hinders the migration of silver ions, affecting the sorption rate.

Interactions of silver with polysaccharides have been reported as highly favourable. Ghandour *et al.*, (1988) reported that Ag^+ cations bind strongly to the cell surface of *E. coli.* Similarly, Petering and McClain (1991) reported that silver ions have good affinity for amino and phosphate groups, and form complexes with them. Accordingly, silver loading onto biofilm-GAC was found to be more than its loading onto the GAC surface alone.

Metal uptake rates and capacities in the present study were varied with the type of metal. This could be due to the different nature of interaction between polysaccharides and metal. Further, metal uptake rates were independent of metals physical properties or its role in metabolic functions of the cell. This suggests that uptake was primarily due to surface attachment onto the biofilm.

4.2.7 Uptake from multi-metal solution system

Metal pollutants rarely exist singly. Effluent from industries such as petroleum refining, electroplating and electronics often contain multi-metal contaminants (Table 1.3). From single species solution, the biofilm-GAC sorbed the metal in sequence [S

4.1]. This metal sorption ability suggests the presence of specific as well as common binding sites over the biofilm. In this section, the metal removal efficiency and the metal loading capacities of biofilm are examined in multi-metal systems.

The uptake of each metal by biofilm from a mixture of cadmium, copper, chromium, nickel, silver and zinc (each at 25 mg/L) is illustrated in Figure 4.13. Total initial concentration of these metals was 150 mg/L, from which 32.6 mg of metal was removed. This gave a removal efficiency of 21%. In comparison to a single metal solution of cadmium (at 150 mg/L), total removal was 16% lower.

The fall in the total metal removal was due to a decline in uptake of each metal from the multi-metal solution. Table 4.4 shows the comparison of uptake of metals, from a single or a multi-metal solution (each at 25 mg/L). The uptake of silver was least affected by the presence of other metals.

Table 4.4 Metal uptake and general sites in biofilm-GAC occupied by each metal in a single metal and multi-metal solution, each metal at 25 mg/L

Metal	Metal level mg/g biofilm-GAC		% Uptake through total sites (B/ΣB) x100	% Uptake through common sites in single metal system {(A - B)/ΣB} x100
	Single A	Mixture B		
Ag^{+}	2.10	1.20	36.78	28
Cu^{+2}	1.65	0.575	17.62	32
Zn^{+2}	1.57	0.50	15.32	32
Cd^{+2}	1.55	0.39	11.87	35
Ni^{+2}	1.48	0.35	10.72	34
Cr^{+3}	1.325	0.25	7.66	32

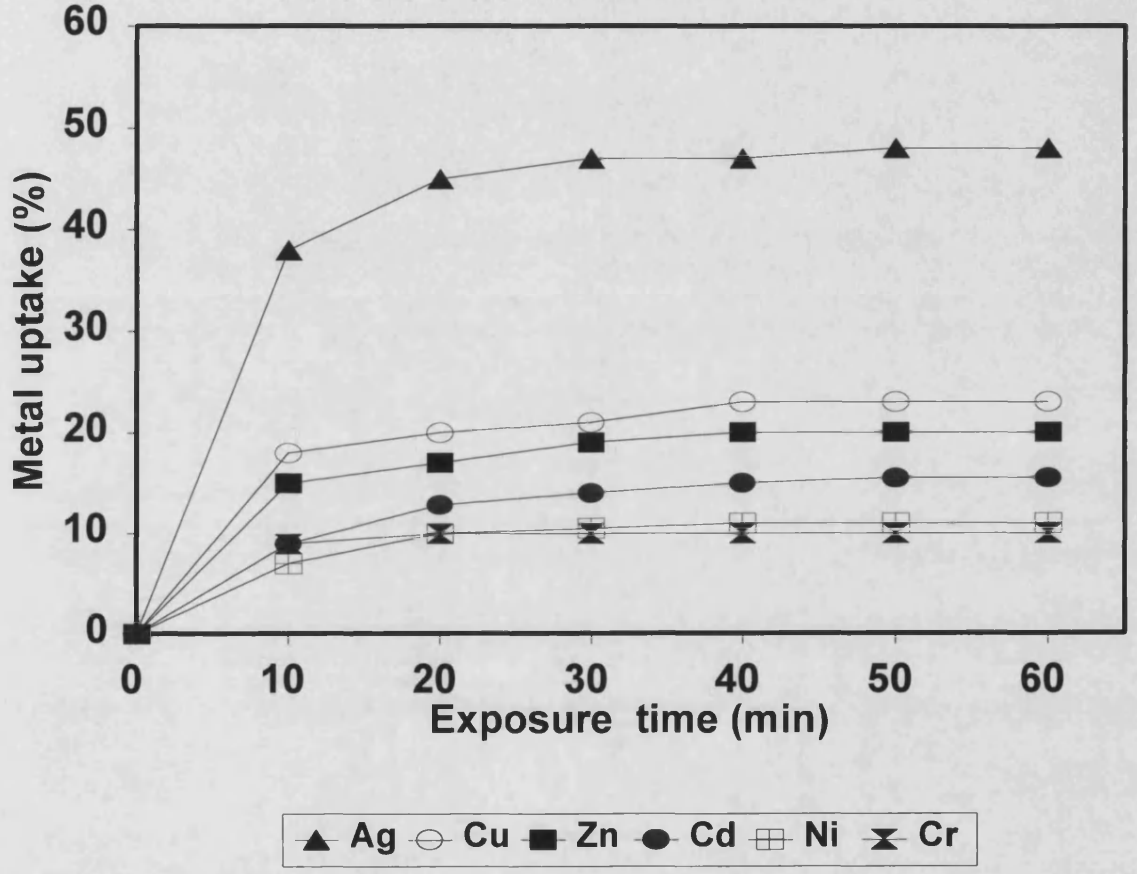


Figure 4.13 Metal uptake by biofilm-GAC system from six-metal solution each at 25 mg/L initial concentration

The metal uptake capacity sequence for the multi-metal solution was the same as that observed in the case of the single metal solution. Although preferential metal uptake remained unchanged, net metal accumulation declined. Factors such as competition for common or general binding sites, properties of metal ions and toxicity of metals may have contributed to the overall decline of metal uptake by the biofilm. These factors are discussed below.

An analysis of metal binding sites occupied by each metal is presented in Table 4.4. From the metal uptake data, possible binding through common sites in biofilm-GAC system were estimated. For the estimation of binding sites, two assumptions were made, (i) in multi-metal solution (each metal at 25 mg/L) the metal(s) binding is through specific site only, and (ii) total metal binding sites in the biofilm-GAC system are equal to the total amount of metal biosorbed from 150 mg/L.

The estimation shows that the majority of the metal uptake (as a single species at 25 mg/L) was at a specific type of site. Therefore, it can be presumed that metal uptake starts with the metal ions occupying specific sites. They then compete with other metals to occupy more general metal binding sites. Such competition for binding sites in a cell and accompanying cellular product has been reported previously. Shuttleworth and Unz (1993) reported competition between zinc and nickel ions to occupy binding sites on a *Thirothix* species. Collins and Stotzky (1992) reported existence of specific binding sites for copper and nickel on the cell wall of *S. cerevisiae*. The X-ray diffraction studies carried out during this research (see Section 4.2.8) also support the existence of both general and specific binding sites for the metal.

The existence of specific and common binding sites is also supported by the kinetic data. The uptake rate of a metal from a single metal solution was slower compared to its uptake from a multi-metal solution. For example, uptake of cadmium from a multi-metal solution reached an equilibrium in 30 min, compared to 40-45 min in a single metal solution. This suggests each metal rapidly occupies the specific sites and then competes for the common binding sites.

Table 4.5 Comparison with physical-chemical properties and the metal uptake sequence

[S 4.1]	Molecular Weight	Ionic Radius[1] ($\times 10^{-10}$m)	Cell-metal affinity Constant[2] K_B
Ag^{+}	110	0.89	----
Cu^{+2}	62	0.72	6.06×10^{17}
Zn^{+2}	65	0.83	1.26×10^{15}
Cd^{+2}	78	1.03	3.60×10^{17}
Ni^{+2}	59	0.78	5.10×10^{10}
Cr^{+3}	54	0.64	1.46×10^{10}

1. Emsley (1989), **2.** Theis and Hayes (1982)

The uptake of metals from the multi-metal solution was compared with metal ionic radius and molecular weight, and also with cell-metal affinity constants (Table 4.5). The metal uptake sequence (both in single and multi-metal solution) was found to be independent of the ionic radius. Apart from cadmium, the cell-metal affinity constants (those found for bacteria in activated sludge) and molecular weight of metals have a similar sequence as [S 4.1]. The uptake of metals by the biofilm declined with decrease in cell-metal affinity constant and molecular weight of metal. The uptake of cadmium was lower than that suggested from the cell-metal affinity constant and

molecular weights in Table 4.5. However, odd appearance of cadmium in molecular weight and ionic radius sequences is unclear and peculiar in this study.

Several examples are cited in the literature which suggest inhibition of metal uptake by biomass and cells in a multi-metal system. An interaction of copper with zinc for binding site has been reported by Scheinberg (1991). Stoeppler (1991) reported that zinc causes redistribution of cadmium and inhibits its cellular uptake. Polysaccharides through the functional amino groups acts as common binding sites for nickel and copper (Sunderman and Oskarsson, 1991). A competing effect between cadmium and zinc uptake by immobilized *Chlorella homosphaera* cells has been reported by Costa and Leite (1991). They also observed an increase in uptake of zinc with increasing Zn/Cd ratio in the initial solution.

Biofilm sorbed silver most, and chromium least, preferentially in the presence of other competing metals. Presence of silver and chromium may predominantly affect the uptake of the other four metals by the biofilm. That is, silver may occupy sites rapidly while chromium may interact with other metals as its uptake was least. Therefore, a mixture of metals consisting of cadmium, copper, zinc and chromium (each at 25 mg/L) was used to analyze metal uptake capacity of the biofilm. Figure 4.14 illustrates metal uptake trends. A 15 to 20 % increase in uptake of each metal from the mixture of four metals was found when compared to that of six metals. This suggests that silver and chromium, did not offer increased competition for binding sites and that the uptake increase may be due simply to the availability of more general metal binding sites in the biofilm.

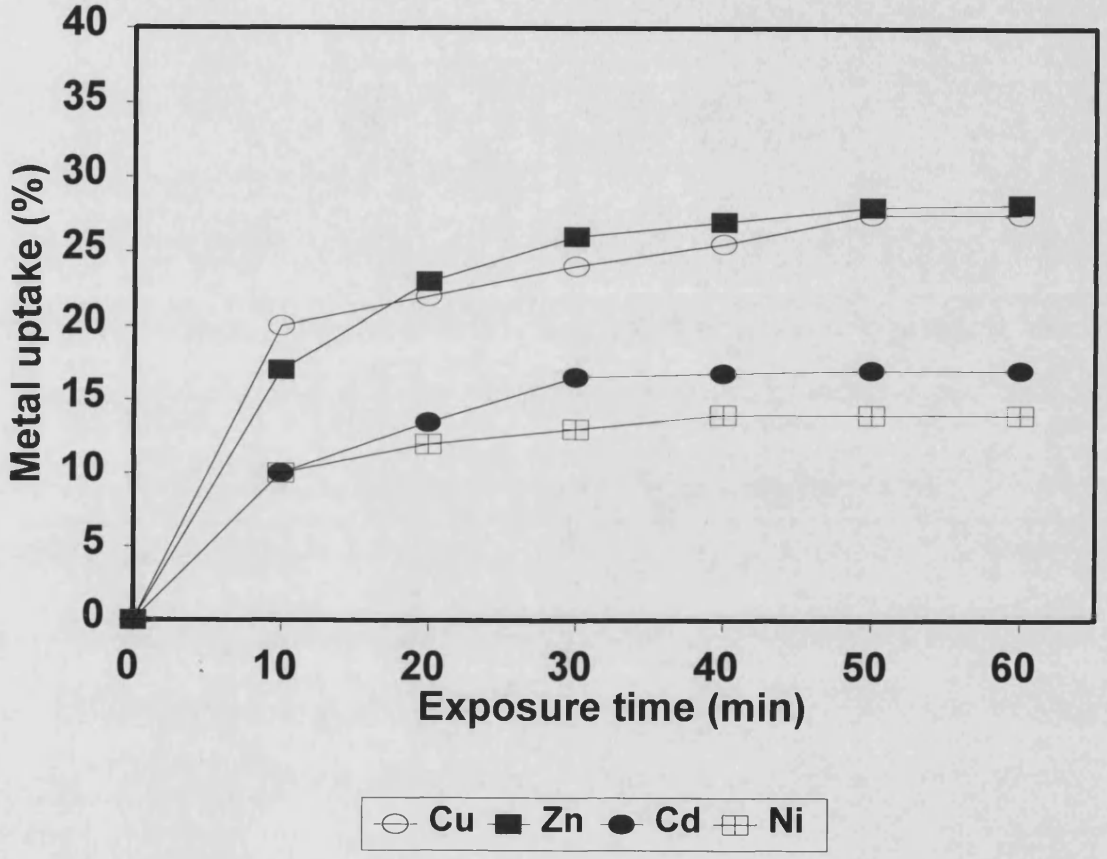


Figure 4.14 Metal uptake by biofilm-GAC system from four-metal solution each at 25 mg/L initial concentration

The contributing factors for the lower uptake from multi-metal system, other than those already discussed could be due to the toxicity of metal ions and metal-metal interactions. Metal uptakes were compared to their reported toxicity to bacteria (Sterritt and Lester, 1980). Their was no trend between the toxicity of metal ions to the cells and identified metal uptake Sequence [S 4.1]. This suggests that the majority of metal uptake was a function of attachment to sites in the biofilm-GAC, rather than intracellular accumulation.

4.2.8 Metal distribution and X-ray analysis studies

Energy Dispersive Spectrometry (EDS) analyses were carried out in order to: (i) confirm the presence of metal and (ii) determine whether the distribution of metal was directly associated with biomass attached to the GAC, or elsewhere on the GAC.

(I) Individual metal distribution

GAC particles, as well as GAC with attached biofilms and metal, were studied by SEM coupled with EDS. Both the biofilms-GAC and virgin GAC were exposed to metals with an initial concentration of 25 mg/L.

The spectrograph of the virgin GAC (Figure 4.15) shows the absence of cadmium, chromium, copper, nickel, silver and zinc metals. On the other hand, spectrograph in Figure 4.16 shows a cadmium energy spectrum, which confirms the presence of cadmium over biofilm-GAC. Similarly spectrographs in Figures 4.17 and 4.18 illustrate the energy spectrum of zinc and copper, respectively.

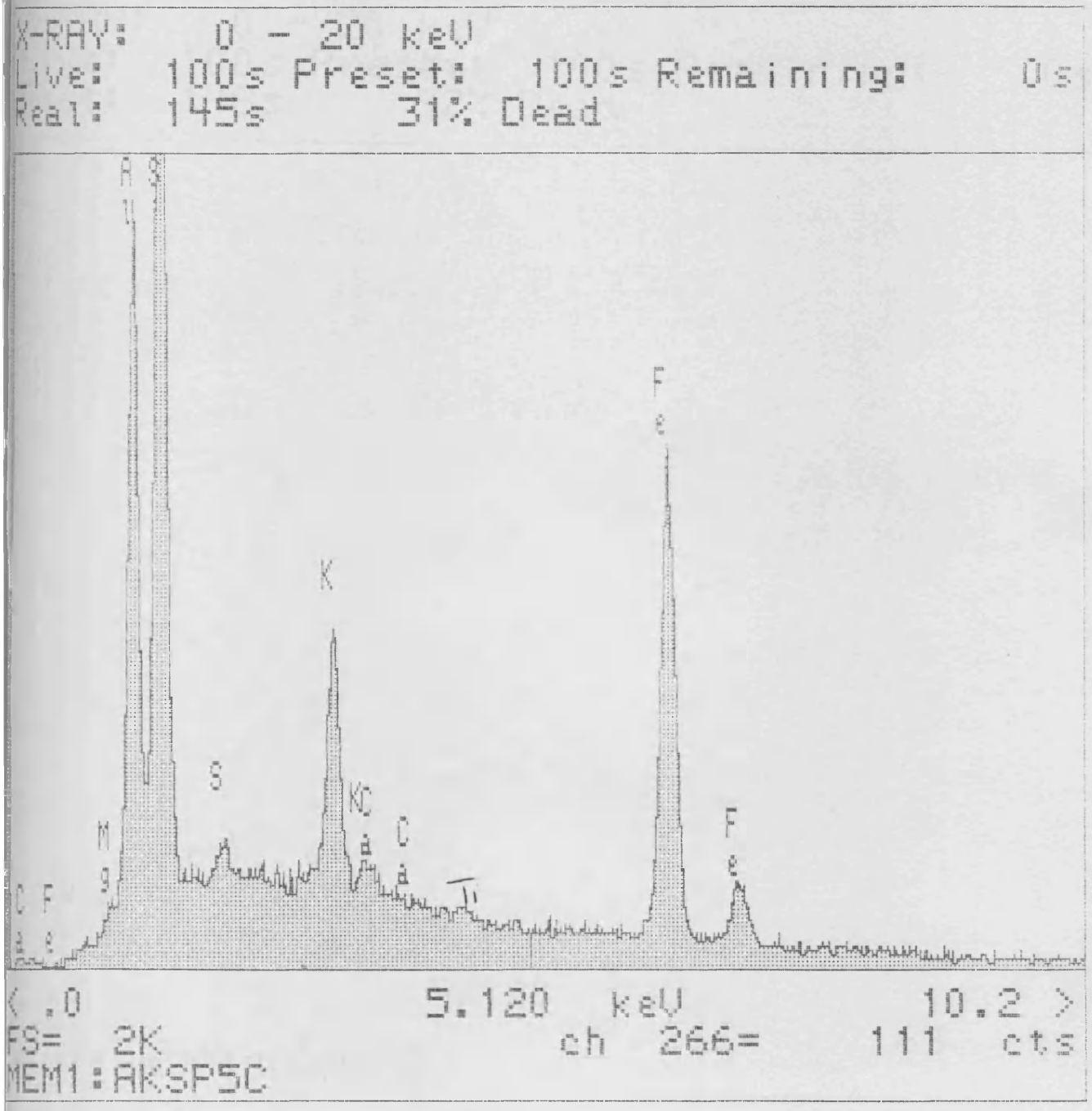


Figure 4.15 Spectrograph of virgin GAC

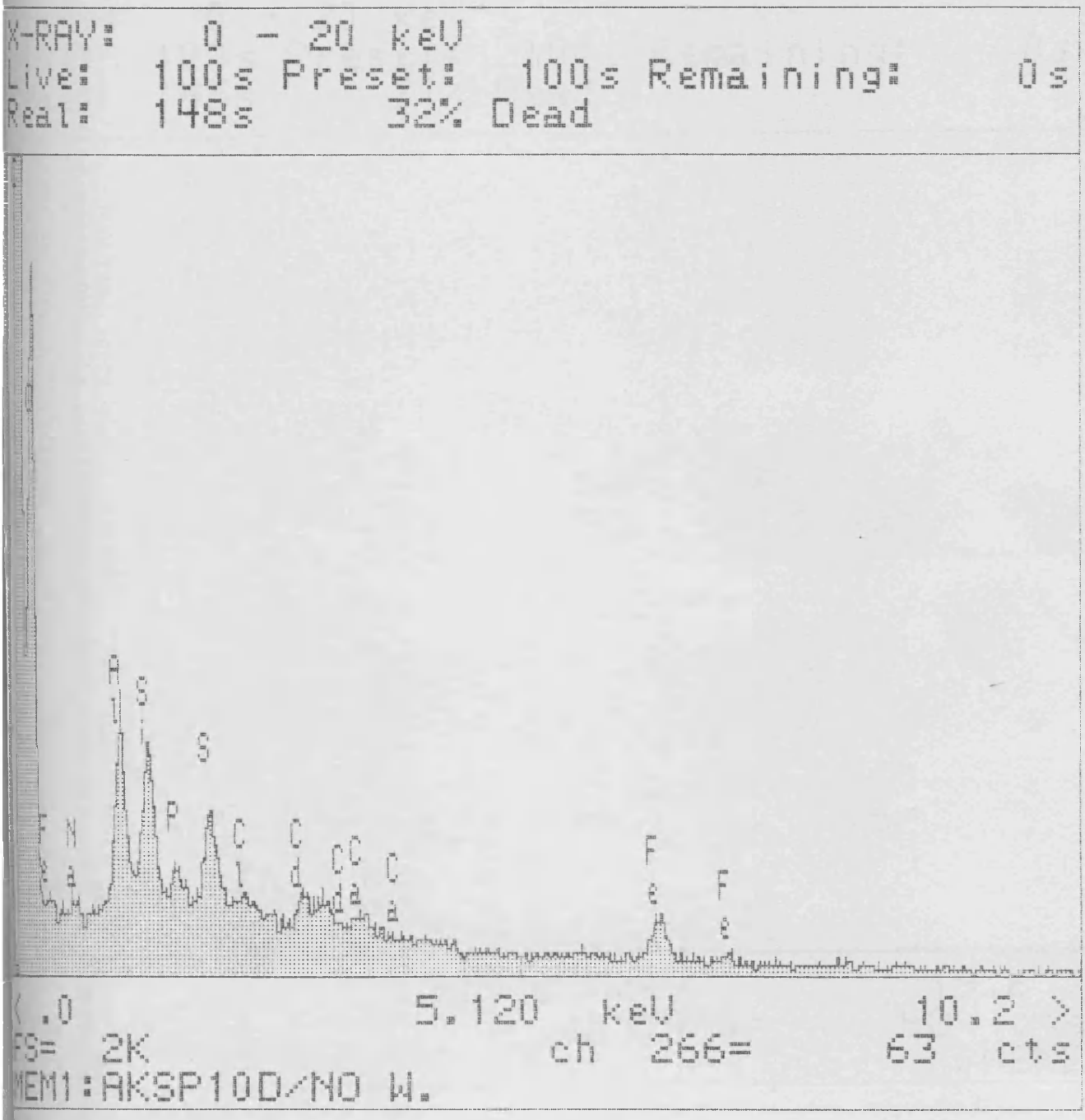


Figure 4.16 Spectrograph of biofilm-GAC exposed to cadmium (25 mg/L)

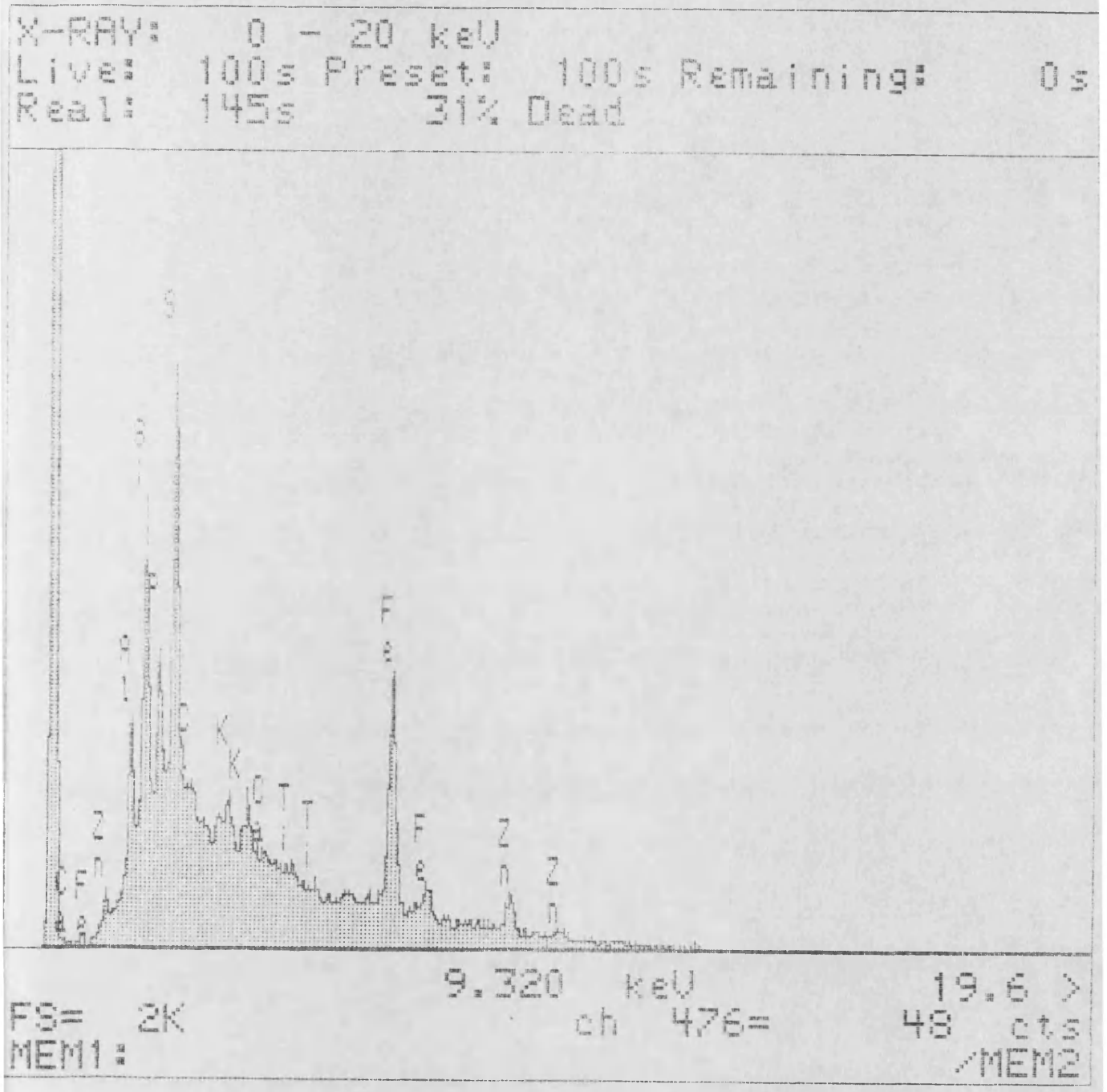


Figure 4.17 Spectrograph of biofilm-GAC exposed to zinc (25 mg/L)

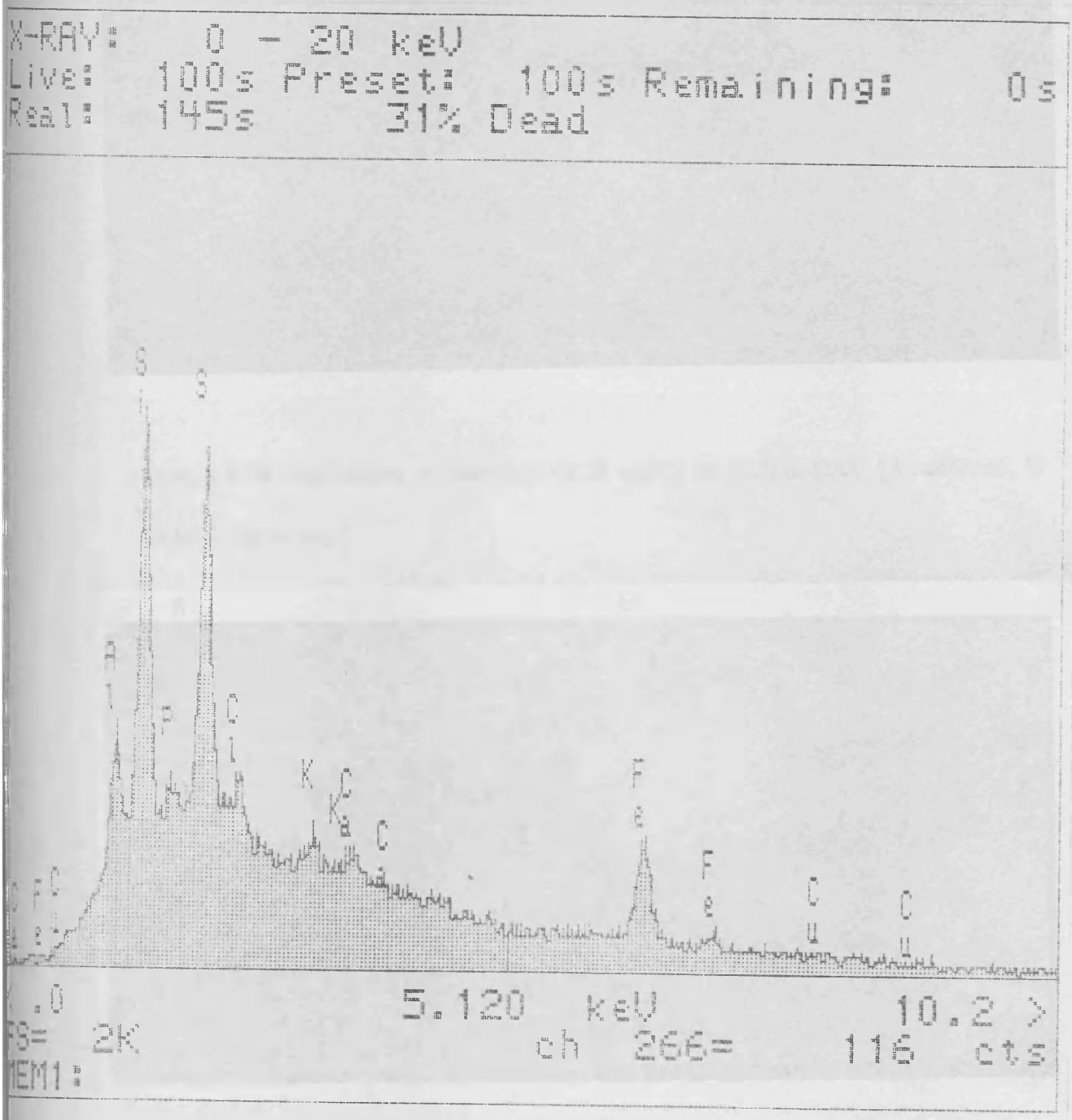


Figure 4.18 Spectrograph of biofilm-GAC exposed to copper (25 mg/L)

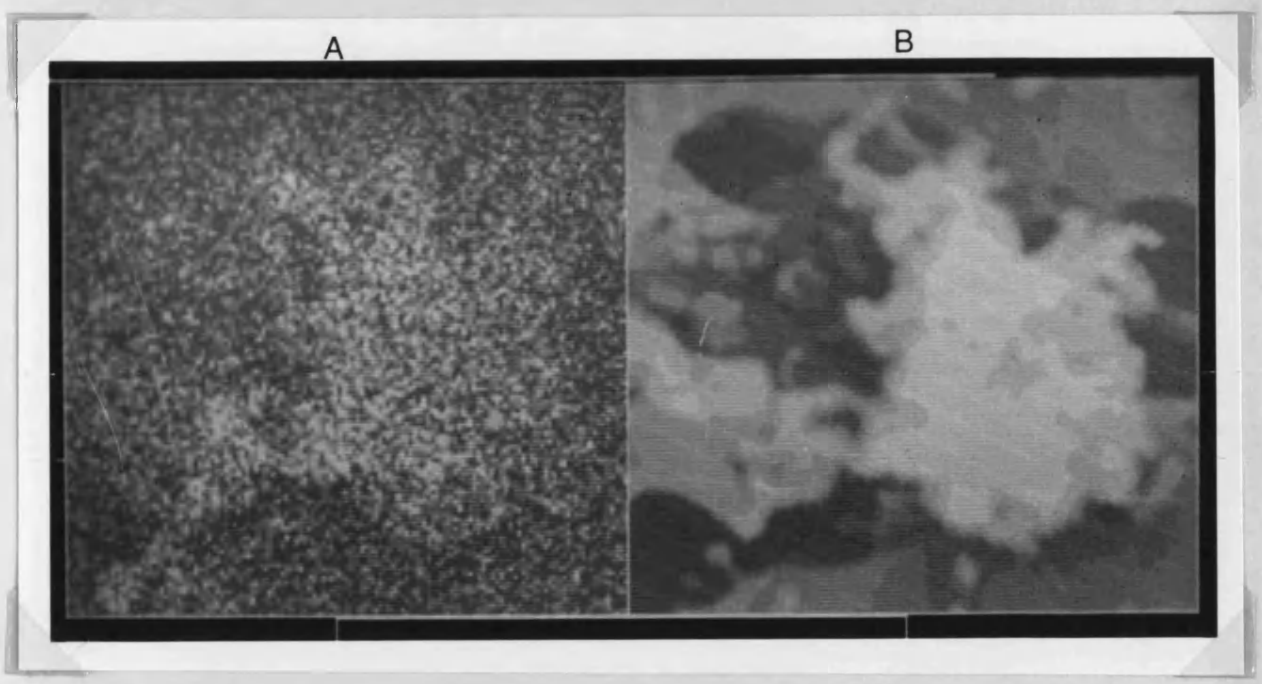


Figure 4.19 Distribution of cadmium (at 25 mg/L) on biofilm-GAC [A:cadmium, B: SEM of same area]

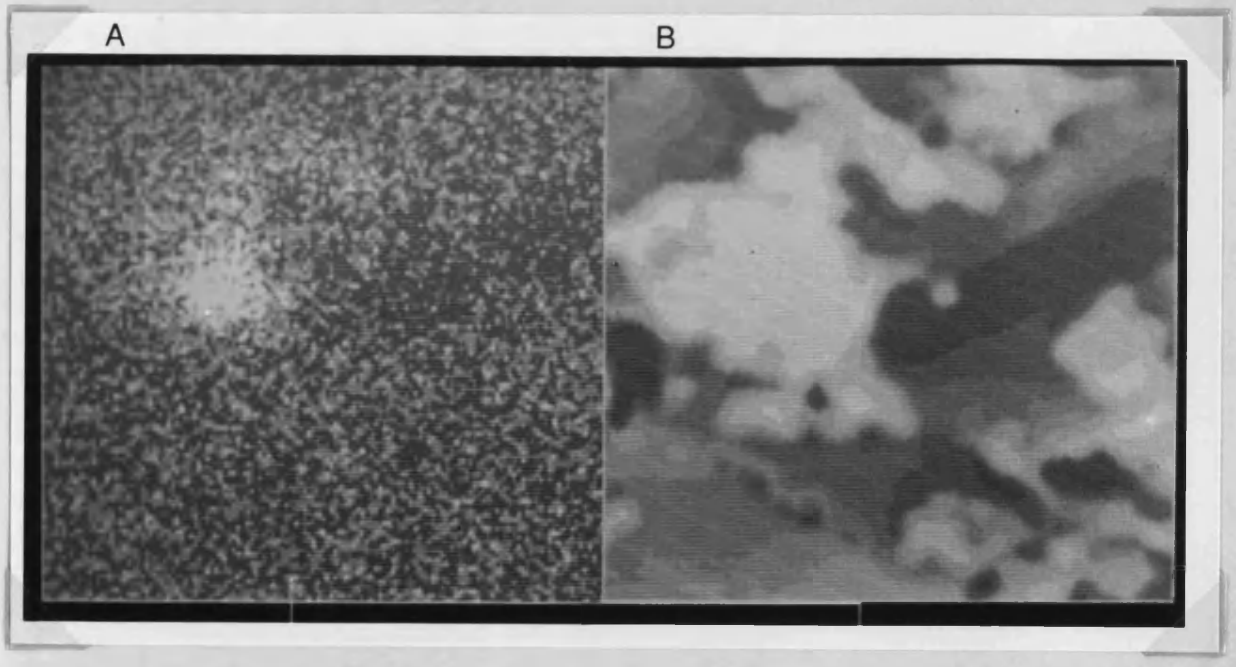


Figure 4.20 Distribution of silver (at 25 mg/L) on biofilm-GAC [A:silver, B: SEM of same area]

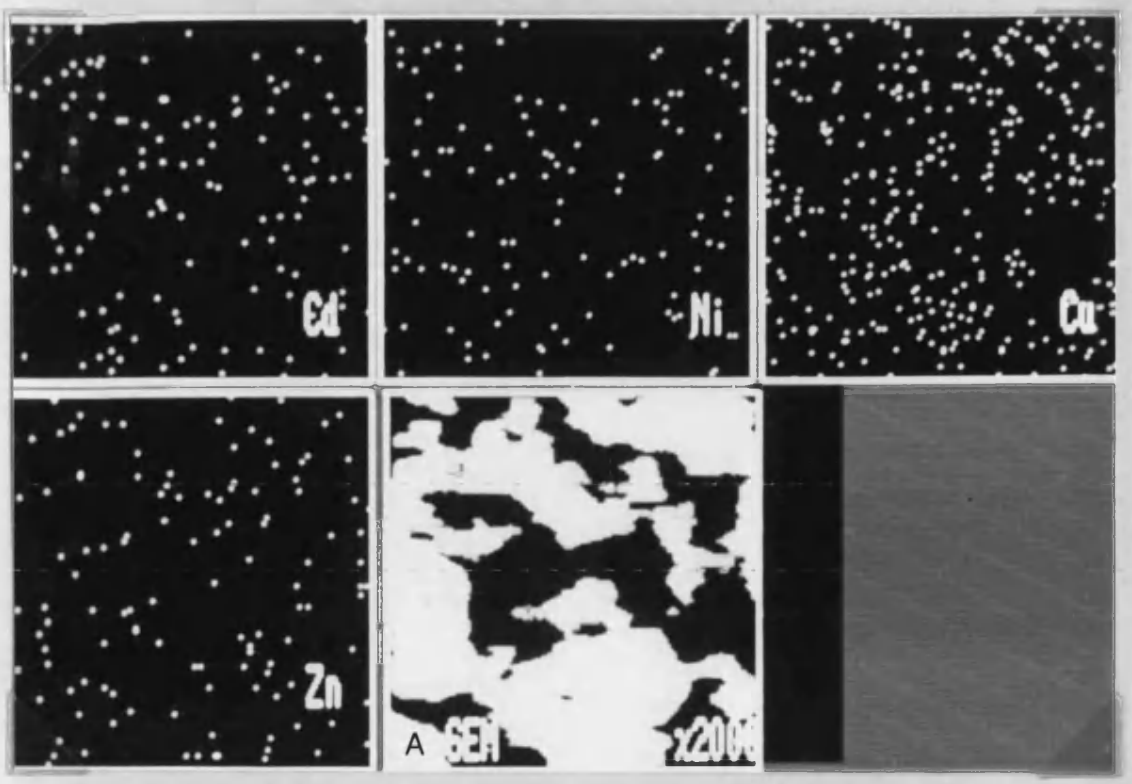


Figure 4.21 Distribution of cadmium, copper, zinc and nickel (each at 25 mg/L) on virgin GAC [A: SEM of same area]

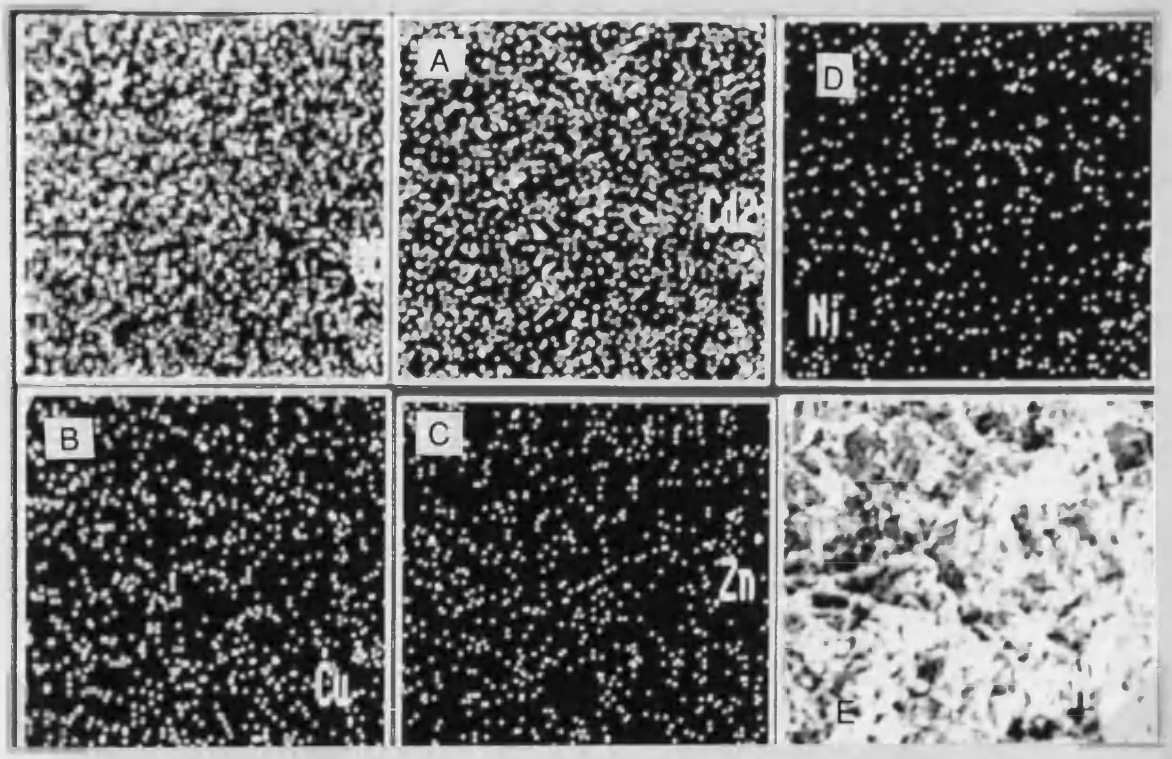


Figure 4.22 Distribution of cadmium [A], copper [B], zinc [C] and nickel [D] (each at 25 mg/L) on biofilm-GAC [E: SEM of same area]

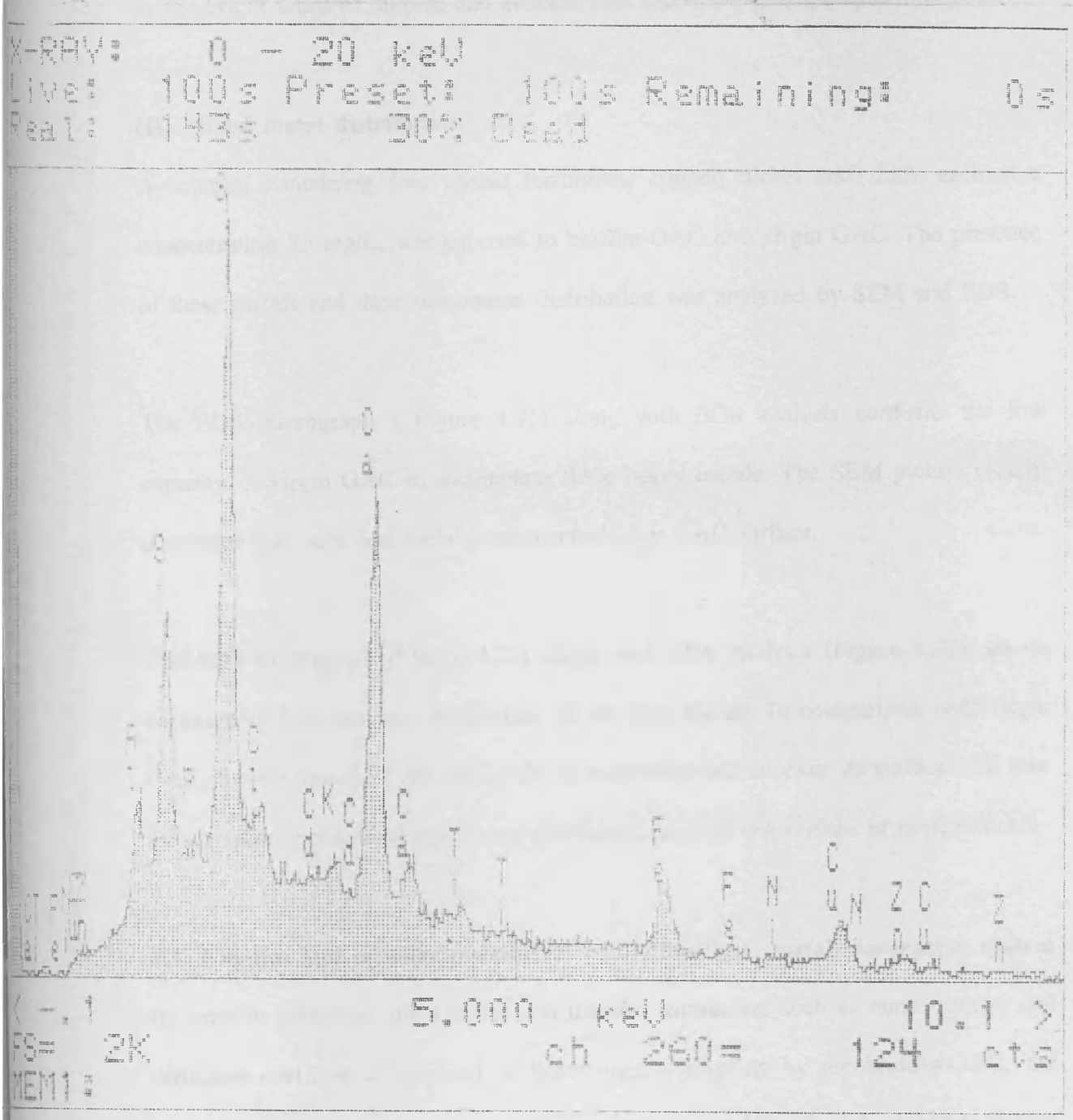


Figure 4.23 Spectrograph of biofilm-GAC exposed to four-metal solution (cadmium, copper, zinc and nickel) each at 25 mg/L

Same area as SEM picture (area) was analysed by X-ray EDS (micrographs in Figures 4.19 and 4.20). Metal distribution was found to be closely associated with the biofilm as the bright areas of biofilm and concentrated metal deposits coincide.

(II) Mixed metal distribution

A solution containing four metals (cadmium, copper, nickel and zinc), each at a concentration 25 mg/L, was exposed to biofilm-GAC and virgin GAC. The presence of these metals and their subsequent distribution was analyzed by SEM and EDS.

The EDS micrograph (Figure 4.21) along with EDS analysis confirms the low capacity of virgin GAC to accumulate these heavy metals. The SEM picture clearly illustrates that very few metal ions attached over GAC surface.

The EDS micrograph (Figure 4.22) along with EDS analysis (Figure 4.23), shows coverage of biomass and distribution of the four metals. In comparison with virgin GAC, the biofilm-GAC showed a dense metal distribution over its surface. All four metals were found to be bound and distributed all over the surface of biofilm-GAC.

4.3 Kinetics and transfer parameters of biofilm-GAC metal biosorption system

In order to determine the kinetics and transfer parameters such as mass transfer and diffusion coefficients involved in heavy metals sorption by the biofilm-GAC, the following studies were carried out. This information on kinetics and other data should help in the design of metal sorption process using bacterial biofilm attached to GAC.

4.3.1 Kinetics

The rate constants for adsorption, K_{ad}, for the uptake of metals by biofilm-GAC at metal concentrations of 25 mg/L, were determined using the first order Lagrergren expression. Singh *et al.*, (1989) and Periasamy and Namasivayam (1994), both reported use of the Lagergren expression for metal adsorption kinetics to virgin GAC. For metal sorption by the biofilm-GAC, the Lagergren expression used can be represented by Equation [4.1]:

$$\log(q_e - q) = \log q_e - \frac{K_{ad}}{2.303} t \qquad (4.1)$$

where: q_e and q are amounts of metal biosorbed (mg/g) by biofilm-GAC at equilibrium and any time, t (min), respectively. K_{ad} is the Lagergren metal adsorption rate constant, (min^{-1}).

A straight line plot of log (q_e - q) vs (t) (Figure 4.24 A & B) along with regression correlation coefficient (r^2) (Table 4.6) indicates the applicability of the above equation. The kinetics of metal adsorption onto biofilm over GAC was observed to follow the first order rate expression. The value of K_{ad} for each of the six different metals was calculated from the plots (Table 4.6).

These rate constants suggest that the biosorption of zinc, copper, cadmium was relatively rapid as compared to uptake rate of silver, nickel and chromium. The following metal uptake rate sequence was identified [S 4.2]:

$$Cu^{+2} > Zn^{+2} > Cd^{+2} > Ni^{+2} > Cr^{+3} > Ag^{+} \qquad [S\ 4.2]$$

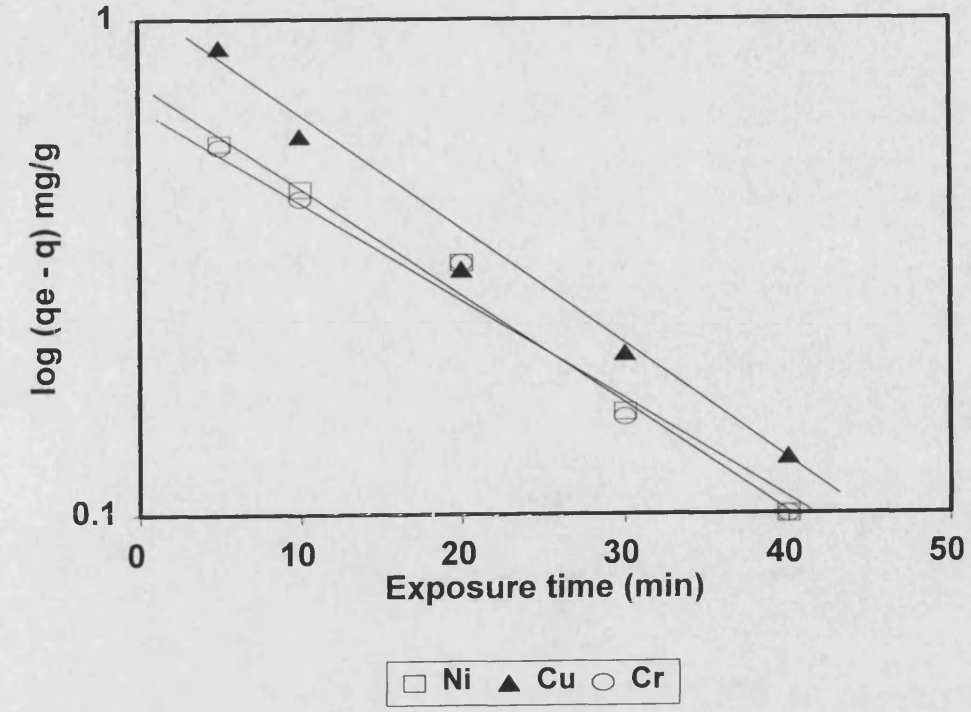


Figure 4.24A Lagergren plots for metal uptake (Ni, Cu and Cr) by biofilm-GAC, from a single metal solution, each: at 25 mg/L

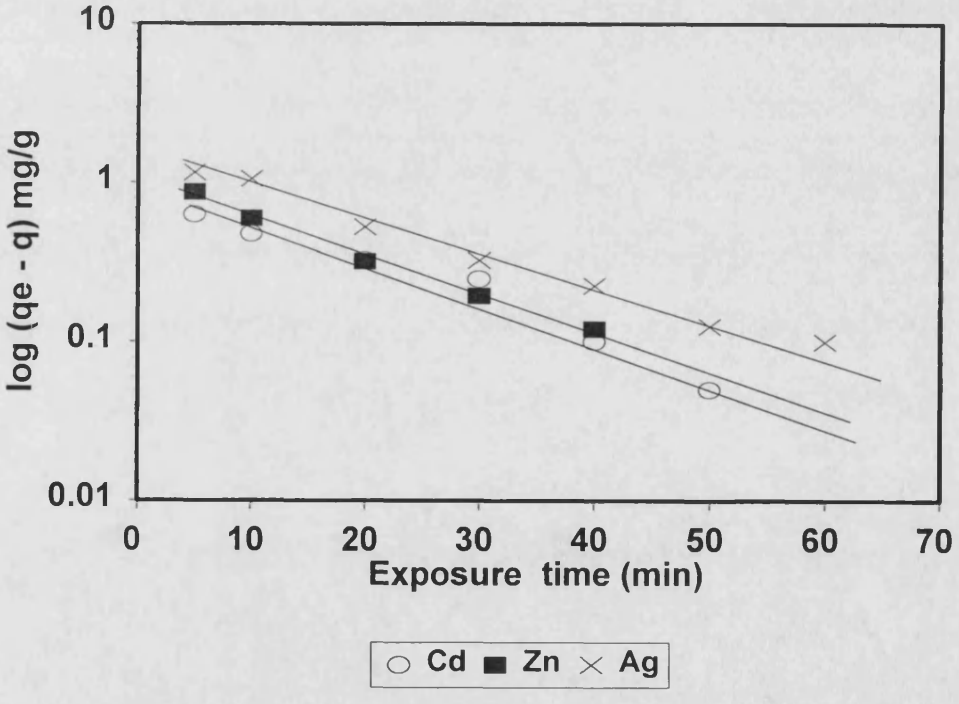


Figure 4.24B Lagergren plots for metal uptake (Cd, Zn and Ag) by biofilm-GAC, from a single metal solution, each: at 25 mg/L

Table 4.6 Biosorption rate constants

Metal ions	r^2	K_{ad} min^{-1} @25 mg/L
Cu^{+2}	0.98	0.062
Zn^{+2}	0.97	0.060
Cd^{+2}	0.96	0.058
Ni^{+2}	0.95	0.055
Cr^{+3}	0.98	0.053
Ag^{+}	0.98	0.049

The higher sorption rates for cadmium, zinc and copper could be due to their passive uptake, which saturates the binding sites rapidly. Although silver uptake capacity was highest compared to other metals, the uptake rate was the lowest, which support the earlier explanation of silver ions migrating through the biofilm onto the GAC. Therefore, presence of biofilm, although enhances silver uptake capacity, hinders adsorption rate.

In biosorption studies using Ca-alginate beds, Apel and Torma, (1993) reported the rate constants for cadmium and zinc to be 0.010 and 0.007 min^{-1} (50 mg/L initial concentration). The cadmium uptake rate obtained here was 5.8 times faster than that reported for Ca-alginate beds and 1.25 times faster than cadmium adsorption rate on to the virgin GAC (0.0448 min^{-1}, 20 mg/L initial concentration) reported by Periasamy and Namasivayam (1994). Thus, the biofilm-GAC system offers significantly better metal sorption rates.

4.3.2 Adsorption isotherms

The data on metal uptake by biofilm-GAC was used to assess the applicability of two basic isotherm models, Langmuir and Freundlich, to obtain the adsorption capacity of six different heavy metals at concentrations 5, 15, 25, 50 and 100 mg/L.

(I) Langmuir adsorption model

The Langmuir isotherm model is considered valid for monolayer adsorption on a surface containing a finite number of identical sites (Mckay *et al.*, 1982). This model assumes uniform energies of adsorption on the surface and no transmigration of adsorbate in the plane of the surface. The Langmuir expression is represented by Equation [4.2]:

$$\frac{C_e}{q_e} = \frac{1}{(Q_o)b} + \frac{C_e}{Q_o} \qquad (4.2)$$

where: C_e is the equilibrium concentration (mg/L); q_e is the amount adsorbed at equilibrium (mg/g); and Q_o and b are Langmuir constants related to adsorption capacity and energy of adsorption, respectively.

The Langmuir plot (C_e/q_e vs C_e) for the biosorption of heavy metals by biofilm-GAC is presented in Figure 4.25. From this plot constants Q_o and b for the six metals were determined (Table 4.7).

The Langmuir model agrees with the experimental data of all six metals up to an initial concentration of 50 mg/L (Figure 4.26 A, B, C and D) found during this research. However, for silver the model fitted well at all the tested concentrations.

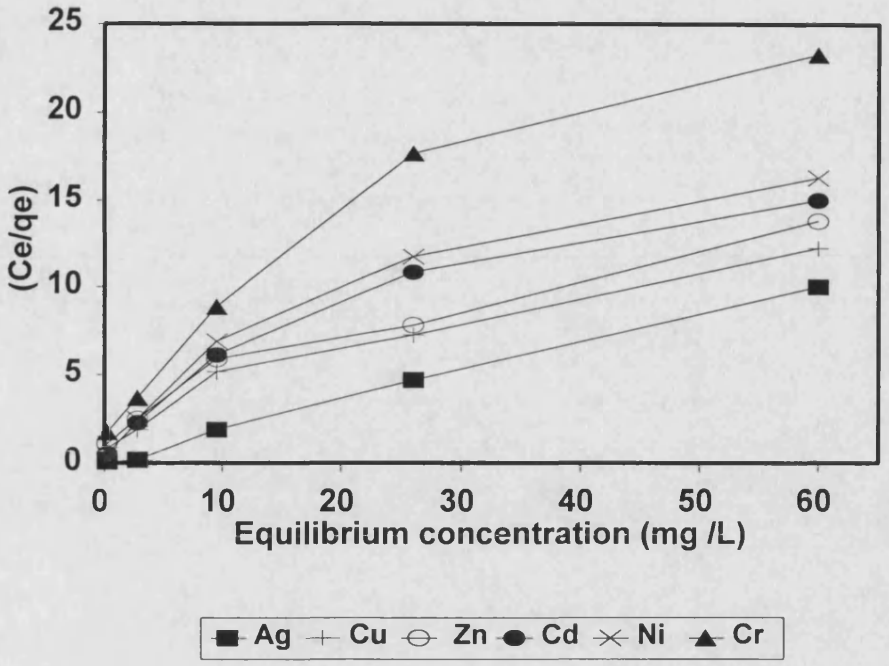


Figure 4.25 Langmuir plot for six different metals

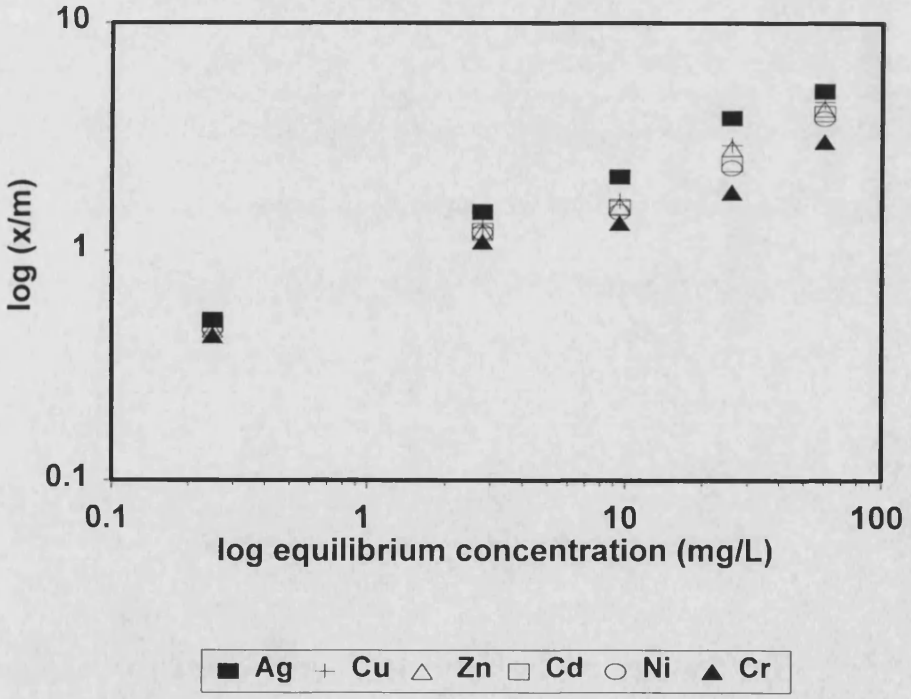


Figure 4.27 Freundlich plot for six different metals

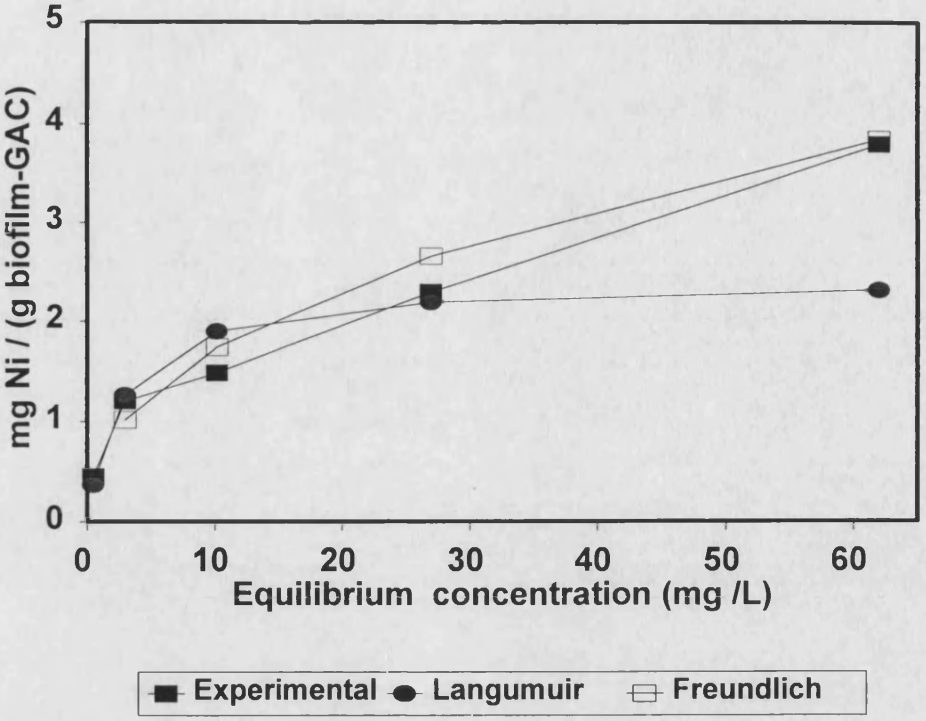


Figure 4.26A Predicted and experimental, uptake of nickel by biofilm-GAC

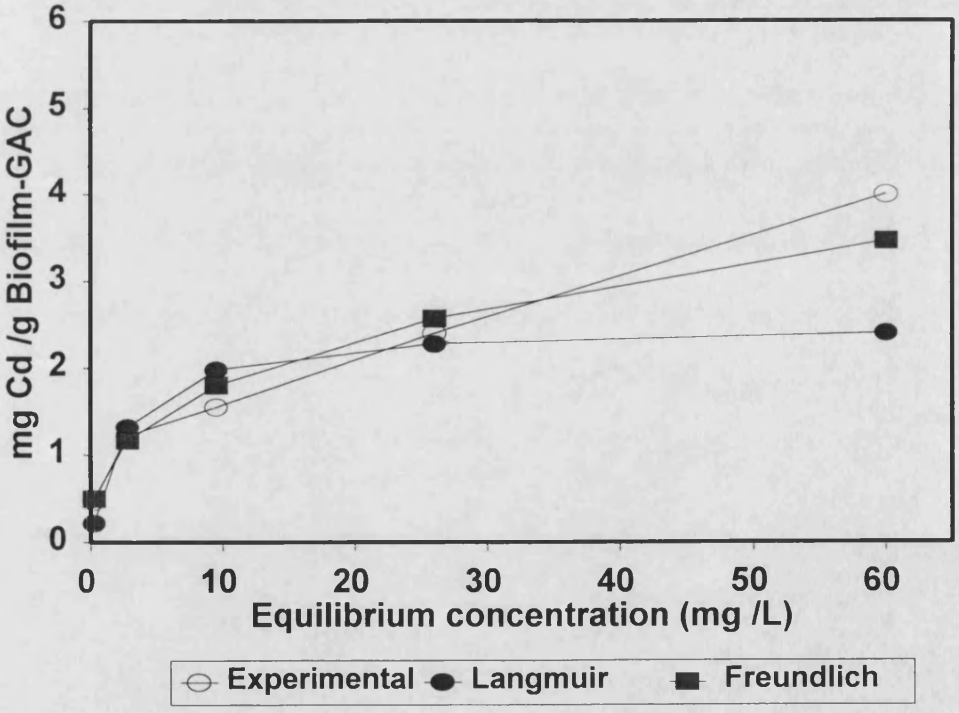


Figure 4.26B Predicted and experimental, uptake of cadmium by biofilm-GAC

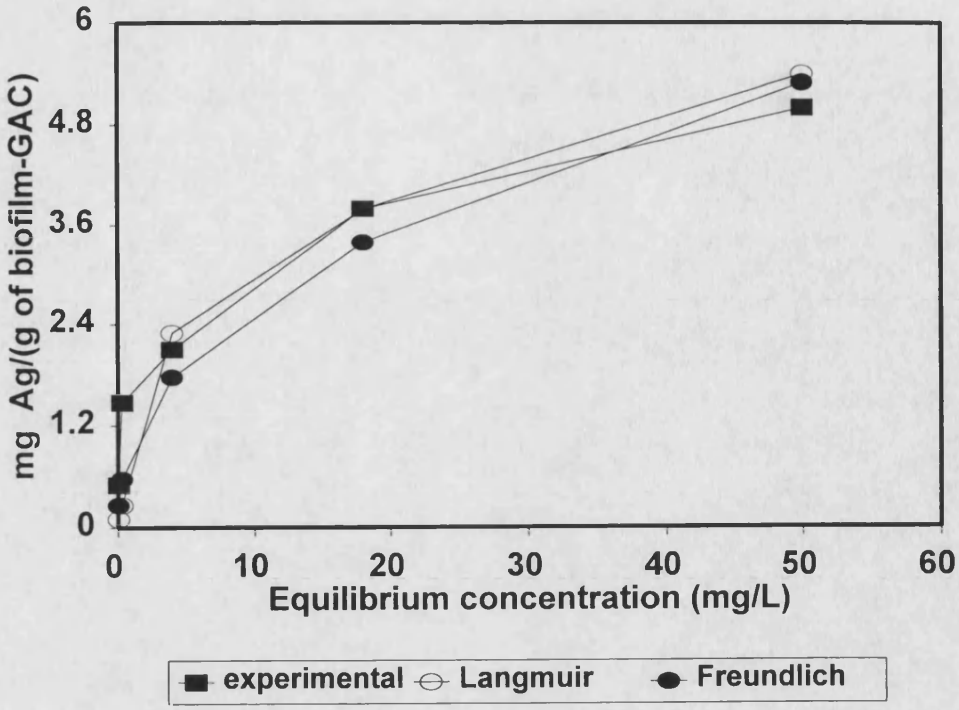


Figure 4.26C Predicted and experimental, uptake of silver by biofilm-GAC

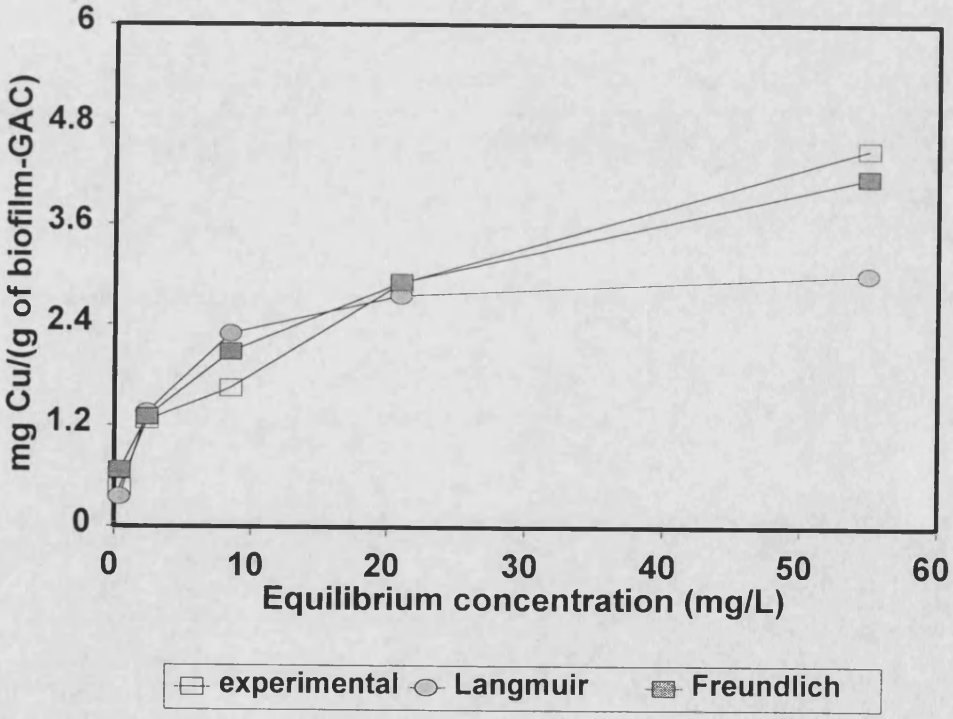


Figure 4.26D Predicted and experimental, uptake of copper by biofilm-GAC

The following factors could be responsible for the failure of the model to predict at higher concentrations than 50 mg/L:

(i) The mechanism of biosorption which consists of extracellular metal entrapment, followed by intracellular metal accumulation. This mechanism of metal uptake depends on the concentration and type of metal, the number of viable cells in the biofilm and the amount of extracellular polymer attached.

(ii) Biofilms attached to the GAC surface were of a non-uniform nature (Section 4.1.1). This could result in different surface energies in adsorption, particularly at high concentrations, whereas the model assumes uniform energies. In the case of silver, its uptake took place by combination of GAC and biofilm (Section 4.2).

(iii) Biofilms have different binding sites through surface functional groups. These groups may vary in electrostatic interactions (Cho *et al.*, 1994), which could result in non-uniform binding.

However, the model does provide a generalized prediction of metal loading for a range of metal concentration up to 50 mg/L. The preferential metal uptake sequence obtained from the model agrees well with the experimental sequence [S 4.1].

The essential characteristics of the Langmuir isotherm model can be expressed in terms of a dimensionless parameter, R_L (separation factor), which is defined by Equation [4.3]:

$$R_L = \frac{1}{(1+bC_1)} \quad (4.3)$$

where: b is the Langmuir constant and C_1 (mg/L) is initial concentration of metal (Mckay *et al.*, 1982). The value of R_L predicts the nature of adsorption, *i.e.* unfavourable ($R_L > 1$), favourable($0 < R_L < 1$), linear ($R_L = 1$), or irreversible ($R_L=0$). By this criteria, values of R_L shown in Table 4.7 suggest that the biofilm-GAC, favourably sorbs a wide range of metals, irrespective of the physical properties (ionic radii, molar volume, ionization potential and electron affinity).

Table 4.7 Langmuir constants for different metals sorption by biofilm-GAC

Metal ion	Q (mg/g)	b	R_L @25 mg/L
Ag^{+}	6.25	0.146	0.21
Cu^{+2}	3.18	0.314	0.11
Zn^{+2}	2.83	0.32	0.11
Cd^{+2}	2.50	0.398	0.09
Ni^{+2}	2.43	0.36	0.10
Cr^{+3}	2.08	0.309	0.11

(II) Freundlich adsorption models

The Freundlich isotherm is represented by Equation [4.4] (McKay *et al.*, 1982):

$$Y_e = K_f C_e^{\frac{1}{n}} \quad (4.4)$$

which can be linearised to:

$$\log(\frac{x}{m}) = \log K_f + (\frac{1}{n}) \log C_e \qquad (4.5)$$

where: C_e is the equilibrium concentration (mg/L), x is amount of adsorbent on adsorbate (mg/g), and m is the weight of adsorbate (g).

The constants K_f (a measure of biosorption capacity mg/g), and n (a measure of biosorption intensity) can be obtained from the plots of log (x/m) vs log (C_e) (Figure 4.27). The value of constants K_f and n for the six different metals are given in Table 4.8. The magnitude of the exponent n gives an indication of the favorability of the system, where values of n > 1 represent favourable adsorption conditions (Treybal, 1980). In all cases the calculated value of exponent n (1 < n < 10) indicate good adsorption. For none of the six metals were saturation effects observed over the range of metal concentrations studied, *i.e.* the isotherm showed a straight line relationship between free and adsorbed metal.

Table 4.8 Freundlich constants for different metals sorption by biofilm-GAC

Metal ion	**K_f (mg/g biofilm GAC)**	**r^2**	**n**
Ag^{+}	0.93	0.95	2.3
Cu^{+2}	0.856	0.93	2.7
Zn^{+2}	0.84	0.95	2.8
Cd^{+2}	0.81	0.95	2.83
Ni^{+2}	0.62	0.93	2.27
Cr^{+3}	0.59	0.94	2.23

The predicted and experimental metal loadings over biofilm-GAC are shown in Figure

4.26 (A, B, C and D). This figure illustrates that the Freundlich model is in good agreement with the experimental data again up to 50 mg/L initial metal concentration. However, for silver the model fitted well at all the tested concentrations. The sequence of preferential metal uptake by biofilm-GAC identified from Figure 4.27, agrees well with experimental Sequence [S 4.1]. The Freundlich model, in comparison with the Langmuir model, expresses a better fit for a biofilm-GAC biosorption system.

4.3.3 Biofilm-GAC metal sorption transfer phenomenon

(I) Biofilm-GAC metal biosorption diffusion coefficient

Biosorption of metal ions depends on several processes, such as diffusion from the bulk liquid (higher metal concentration) to the external biofilm surfaces (lower metal concentration), inward diffusion through the layer and chemical binding. In this section, inward diffusion coefficients of different metal ions at varying concentrations are calculated using measured metal uptakes.

Using Fick's second law of diffusion and assuming steady state conditions, Apel and Torma (1993) derived an expression for molecular diffusion flux of divalent metal ions into a biosorbent. The diffusion coefficients for various metal ions can be calculated by the following Equation [4.6]:

$$D_{M^{2+}} = \frac{[M^{2+}]_1 v r_1 (1 - \frac{r_1}{r_2})}{([M^{2+}]_2 - [M^{2+}]_1)} \qquad (4.6)$$

where: $D_{M\,2+}$ is the diffusion coefficient of metal ions; cm^2/s, $[M^{2+}]_1$ and $[M^{2+}]_2$ are respectively, final and initial metal ion concentrations (mg/L) in solution; v is metal

flux velocity (cm/s), r_1 and r_2 are respectively, radius of GAC and GAC with biofilm (cm). The thickness of biofilm over GAC was assumed to be 10μm, which was estimated from SEM studies (Section 4.1.1). The diffusion coefficients calculated for four different metals using Equation [4.6] are presented in Table 4.9.

Figure 4.28 suggests a dependence between diffusivity and concentration in the biosorption of metals. With all the metals tested, an increase in diffusion coefficient was found with increasing metal concentration. However, such apparent dependence of diffusion on concentration can in fact, be a dependence of diffusion on the thermodynamics (free energy, enthalpy and entropy, Birdi, 1984) of the process. Such a dependence was also observed in adsorption of organic molecules onto the zeolite by Ruthven (1984). (see Appendix II)

Influence of various biofilm thickness (in agreement with the thickness observed by, Shieh and Keenan (1986), Chen *et al.*, (1988), Yu and Pinder (1993)) on calculated cadmium diffusion coefficient is illustrated in Figure 4.29. It can be observed that as expected, an increase in biofilm thickness increases the diffusion coefficient (explanation in Appendix II).

The calculated diffusion coefficient for the four metals decreases in the following order:

$$Cu^{+2} < Zn^{+2} < Cd^{+2} < Ni^{+2} \qquad [S\ 4.3]$$

Apel and Torma (1993) found the diffusivity of zinc and cadmium (at 50 mg/L concentration) in biosorption by Ca-Alginate beds to be 1.02×10^{-7} and 1.34×10^{-7} cm^2/s, respectively. These values are in good agreement with the values obtained in this study (Table 4.9).

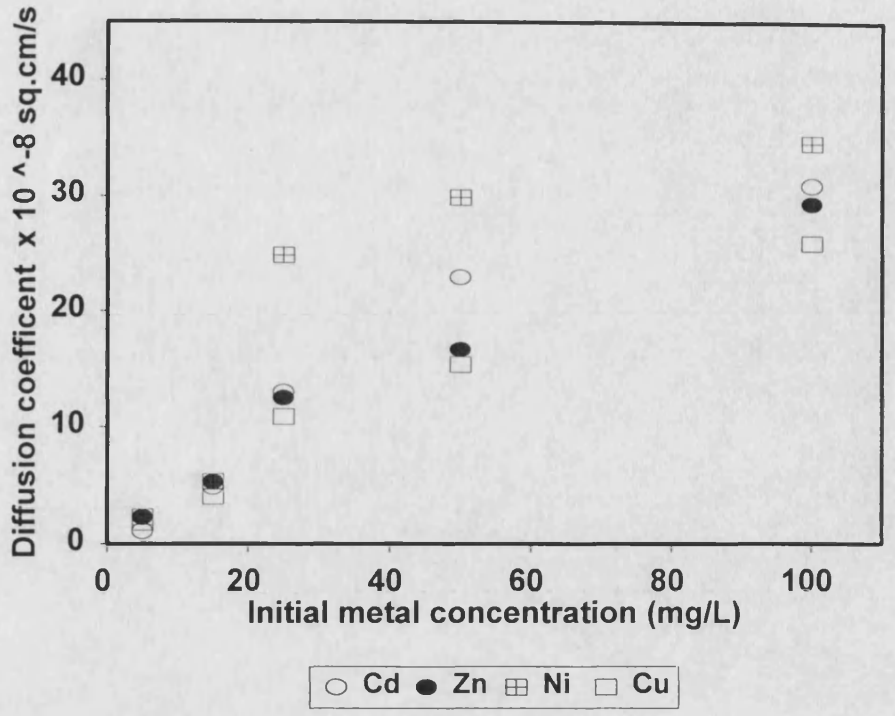


Figure 4.28 Effect of initial metal concentration on diffusion coefficient

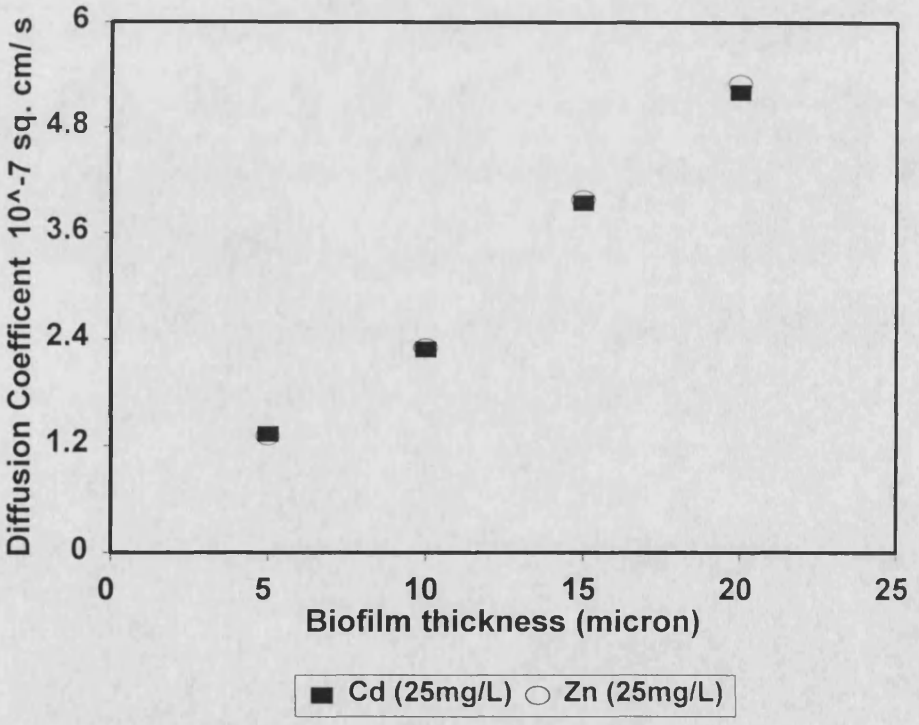


Figure 4.29 Effect of biofilm thickness on diffusion coefficient

Table 4.9 Diffusion coefficients for metal sorption by biofilm attached to GAC (50 mg/L initial concentration)

Metal ion	Diffusion coefficient (cm^2/s)
Cu^{+2}	1.54×10^{-7}
Zn^{+2}	1.67×10^{-7}
Cd^{+2}	2.3×10^{-7}
Ni^{+2}	2.49×10^{-7}

However, apart from diffusion, other processes are also responsible for the rate of metal uptake by biofilms. It is well documented that surface charges, metal-polymer complexion and intracellular accumulation are all involved in biosorption of metal (Volesky, 1990; Scott *et al.*, 1988). These processes are not considered in the calculation of diffusion coefficient.

(II) Biofilm-GAC biosorption system mass transfer coefficient

Metal uptake by the biofilm is a surface phenomenon. The surface mass transfer coefficient and diffusion resistance are the controlling elements in metal ion transport process from bulk solution to the metal sorbent biomass (Tsezos and Deutschmann, 1992). The metal uptake by biofilms in a fluidized GAC bed reactor is analogous to substrate utilization in a biofilm reactor and can be described in three steps:

(i) transport of metal from bulk liquid to the liquid-biofilm interface (external mass transfer);

(ii) transport of metal within the biofilm (internal mass transfer), and

(iii) intracellular interaction within the biofilm.

The last two steps are physio-chemical and are not fully understood in terms of their role in mass transfer. The significance of mass transfer coefficient in the fluidized bed reactor can be assessed using a correlation developed by Shieh and Keenan (1986):

$$k_c = \frac{0.81}{\epsilon}\left[\frac{D^{4/3} u\ \rho_1^{1/3}}{\mu^{1/3} d_p}\right]^{1/2} \qquad \textbf{(4.7)}$$

where: k_c is the external mass transfer coefficient (cm/s); ε is bed porosity; D is the diffusivity (cm^2/s); u is superficial upflow velocity (cm/s); ρ_1 is the liquid density (kg/cm^3); μ is the liquid dynamic viscosity (kg.s/ cm); and d_p is particle diameter (cm). Bed porosity is calculated from the following equation:

$$\epsilon = 1 - \frac{V_S}{H_b A} \qquad \textbf{(4.8)}$$

where: V_s is volume of GAC in bed (cm^3); A is cross sectional area (cm^2); and H_b is the expanded bed height (cm).

Table 4.10 gives the mass transfer coefficients obtained from Equation [4.7] and an estimated biofilm thickness of 10μm. Such mass transfer values would help in the design of biosorption system discussed in these thesis. For example, from known mass transfer coefficients and fluid properties, the optimum flow rate of a metal containing stream through the column can be estimated.

Table 4.10 Mass transfer coefficients for metal biosorption by biofilm-GAC

Metal ion	Mass transfer coefficient (cm/s)
Cu^{+2}	3.28×10^{-4}
Zn^{+2}	3.46×10^{-4}
Cd^{+2}	4.28×10^{-4}
Ni^{+2}	4.52×10^{-4}

The K_c value was reported by Mishra and Chaudhury (1994) to be dependant on the particle size of the adsorbate, the rate of energy dissipation per unit mass of fluid, and the kinematic viscosity of the fluid. The biofilm is likely to be affected by all these variables. Therefore, the biofilm properties will be the major controlling variables of the metal uptake process. The gelatinous structure of the biofilm can slow down the transport of metal through the biofilm. Therefore, metal concentration surrounding any micro-organism within the biofilm is expected to be less than that in the bulk solution. Thus, the mass transfer properties of the biofilm are of critical importance in assessing the overall performance of the system.

4.4 Biofilms development in various conditions and their influence on biosorption

Biofilms described in this section were developed under various influential conditions, such as pH, temperature, dissolved oxygen and fluid velocity. These biofilms were then assessed for metal biosorption capacity.

4.4.1 Growth medium pH

The biosorptive property of a biofilm can be influenced by pH. For example, when

cells were grown in different buffered media (pH range 4 to 9), they showed both variation in their extracellular polysaccharide production and composition of polysaccharides (Graham *et al.*, 1994).

Biofilms were developed over GAC using buffered growth medium (pH range 4 to 9) and subsequently assessed for their cadmium uptake capacity. Although the *Pseudomonas* sp. used in this research has shown viability in a wide pH range (3.2 to 9.5), the number of viable cells were expected to decrease with lowering the pH (*e.g.* with *P. aeruginosa* cell viability declined from 2×10^5 cells/mL at pH 7.6 to 2×10^0 cells/mL at pH 3.2, Tanner and James, 1992).

Irrespective of whether the growth medium pH of the non-proliferating cells was originally acidic or alkaline (within the tested pH range), during culturing the medium approached a pH in a range of 5.5 to 8.2 which was maintained. This was probably due to a combination of the buffering ability of the species, and acid or base adsorption onto the GAC. However, buffering capacity is not a recognised trend of *Pseudomonas* sp.; although similar trends in the case of *S. marcescens* were observed by Williams *et al.*, (1971). Table 4.11 gives the level of buffering of growth medium by combination of *Pseudomonas* sp. and GAC.

Cadmium or zinc uptake by biofilms developed at different growth medium pH is illustrated in Figure 4.30. Both metal solutions used in this study were at pH 4.6. Initial alkaline conditions (buffered close to neutrality) were found to have no negative influence on biosorptive capacity. However, biosorptive capacity of biofilms developed in mild acidic conditions (*i.e.* pH 5.6 and 6) declined by 40 to 55%.

Table 4.11 Buffering of growth medium by the *Pseudomonas* sp. after 24 hours.

Initial pH	pH after 24 hours
4.5	5.3
5.0	6.3
6.0	7.3
7.4	7.4
8.0	7.4
9.0	7.8
9.2	8.0

This decline in metal uptake in acidic conditions could be a combination of different factors.

(i) Changes in surface charge of binding sites. Geesey and Jang (1990) observed that as pH is lowered, overall surface charges on cells and polysaccharide become positive, which inhibits the binding of positively charged metal cations. Such changes in surface properties are reported to be due to the mechanism of self-protection shown by micro-organisms in an acidic environment (Leyer and Johnson, 1993).

(ii) With low pH, cell growth is inhibited, which in turn decreases extracellular polysaccharides (Novak *et al.*, 1992). Further, the loss in metal uptake capacity may be due to weak metal-polymer complexes which decompose under acidic condition. Urrutia-Mera *et al.*, (1992) observed similar trends in the metal binding ability of *Bacillus subtilis* cells in suspension. The observed metal uptake may be due to adsorption of metal ions to the cell wall and/or entrapment in the polymer matrix.

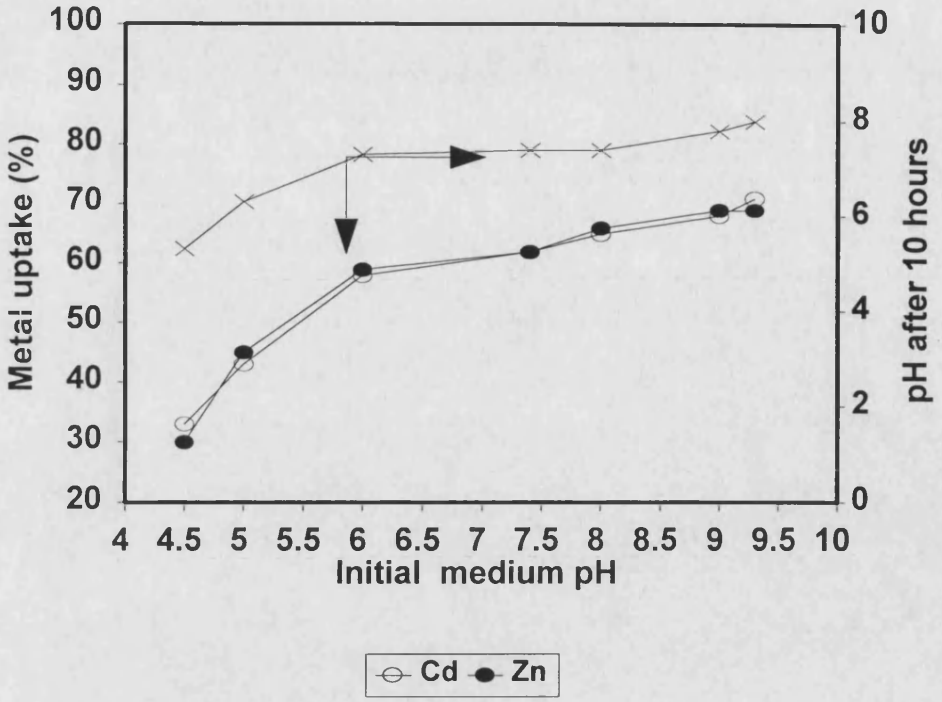


Figure 4.30 Buffering of medium and effect of growth medium pH on biosorption of zinc and cadmium (at 25 mg/L)

A biofilm developed in an alkaline growth medium was found to have a higher metal uptake capacity. A 10 to 12 % rise in biosorptive capacity was observed when initial pH was in the range of 8 to 9. Although, due to buffering of the medium the conditions were reduced close to neutrality. The significant metal uptake under these condition may be due to enhanced polysaccharide production by the cells, as in neutral or slightly alkaline conditions cells are known to yield the highest concentration of polysaccharides. For example, *A. viscosus* produces relatively copious polysaccharides at pH 8.0 (12 g/L) than at pH 5 (4 g/L) (Novak *et al.*, 1992). Furthermore, the increase in metal uptake may be due also to cross-linking of exopolysaccharides. Holan and Volesky (1994) observed higher metal sorption values with several cross-linked types of biomass. In addition, surface cell-metal affinity, surface charges and stability of metal-polymer complex contribute to metal accumulation by the biofilm.

Brown and Lester (1979) reported that anionic polysaccharides have stronger affinity for metal "counter-ions" and this cationic-anionic interaction is stronger in gelation. Similarly, Darimont and Frenay (1990) showed that at high pH, the complexes were more stable and longer lasting. Thus the alkaline growth medium conditions (up to pH 9.2) favour the biofilm-GAC biosorption system.

4.4.2 Growth medium temperature

Temperature is an environmental condition which can affect both, biofilm growth and metal-biofilm interaction. The effects of temperature on biofilm growth, attachment and on polysaccharide production were assessed through cadmium uptake.

(I) Cadmium uptake by biofilm developed at different temperatures

It is well known that within the growth-permissible temperature range, changes in temperature influence both the metabolism and biosorptive performance of bacteria (Heitzer *et al.*, 1991). In this study, biofilms were developed over GAC at four different growth medium temperatures (5, 15, 25 and 30°C) and their cadmium uptake capacity was subsequently assessed at 18°C (Figure 4.31).

A general trend of increasing cadmium uptake with increasing growth medium temperature was observed. An increase in temperature from 5 to 15°C resulted in a 10% increase in total metal loading. A further 10°C increase in temperature (*i.e* from 15 to 25°C) resulted in a further 25% increase in cadmium loading. However, between 25 to 30°C, there was little increase in metal loading. Increasing metal accumulation with increasing temperature has been also observed in the case of cells and immobilized biomass (Volskey, 1990).

The uptake data suggests that the increase in cadmium uptake with temperature can be related to an increase in metabolic activity, resulting in more cells and subsequent production of extracellular polysaccharides. Low temperature, low metal uptake, was observed also in case of bacteria and yeast by Gadd (1990), who suggested an inhibition of metabolism-dependent intracellular metal uptake. Fletcher (1977) reported a decrease in number of cells attached to a surface with decreasing temperature.

To understand the influence of temperature, a number of related factors, such as (i) viscosity of medium, (ii) cell growth, (iii) amount of polysaccharide produced and (iv) degree of biomass attached were determined and correlated to cadmium uptake.

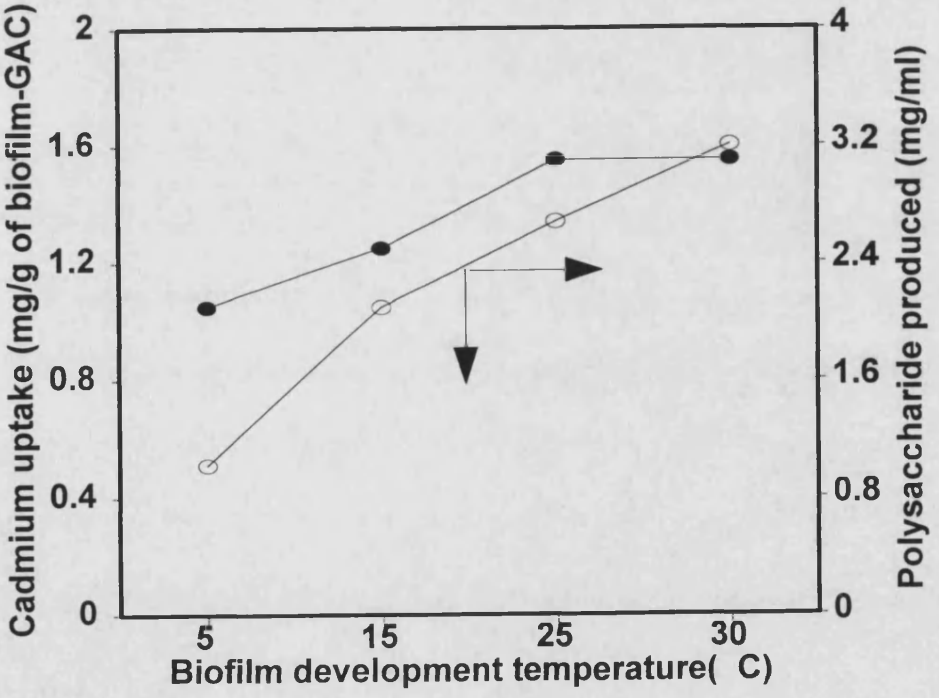


Figure 4.31 Effect of growth medium temperature on polysaccharide production and cadmium biosorption (at 25 mg/L and 18°C)

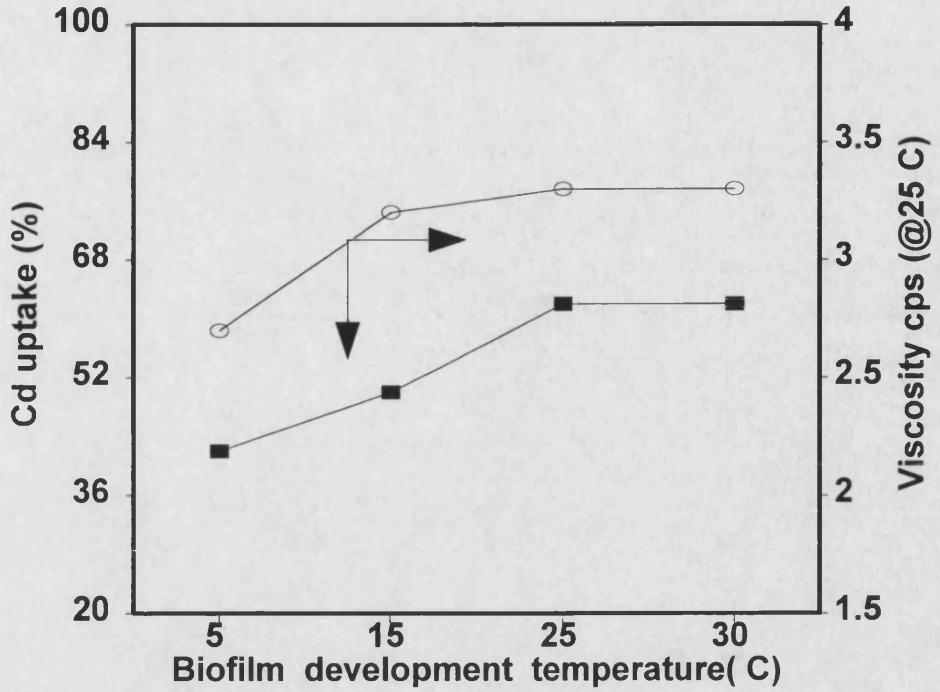


Figure 4.32 Effect of growth medium viscosity and temperature on polysaccharide production and cadmium biosorption (at 25 mg/L and 18°C)

As expected, cell growth (measured by optical density and cell dry weight) was observed to increase with increase in temperature. Similarly, the medium viscosity (tested after 48 hours at 25°C) was 2.2 cps at 5°C which increased to 3.3 cps at 25°C (Figure 4.32). The rise in medium viscosity with growth temperature could be due to a denser population of suspended cells and the degree of polysaccharide produced. Further, the nature of the polysaccharides may have been influenced by temperature, as they tend to weaken or get disordered (with respect to chemical structure) with increasing temperature (Bacon, 1979). Such disorder or polymerization often leads to higher viscosity. The increase in viscosity found here had no adverse effect on the metal uptake capacity of the biofilm. The effect of different growth medium temperatures on polysaccharide production, biofilm attachment and subsequently on cadmium uptake, is discussed next.

(II) Cell produced polysaccharides

In earlier studies (Section 4.1.1), it was shown that the *Pseudomonas* sp. used was much more effective for cadmium uptake than non-polysaccharide producing *S.marcencens*. In this study, an attempt was made to determine the amount of polysaccharide produced by the *Pseudomonas* sp. over time and at different temperatures.

Figure 4.31 illustrates the amount of polysaccharide produced at 5, 15, 25 and 30°C. An increase in temperature from 5 to 15°C, polysaccharide production increased from 1.0 to 2.68 mg/mL, which further enhanced to 3.2 mg/L at 30°C.

Reported polysaccharide production is found to vary with species and constituents of the growth medium. Grappelli *et al.*, (1992) reported 9% of the biomass to be polysaccharides produced by *Arthobacter* sp. Ebube *et al.*, (1992) recovered 3.9 to 17.6 mg/mL of polysaccharide from cultures of *Bacillus licheniformis*. Palmer (1988) reported 29 mg polysaccharide recovery from 100 mL culture fluid of *A. viscous*. Thus, the polysaccharides recovered in this research was within previously reported ranges.

The amount of polysaccharide produced was correlated to cadmium uptake. An increase of polysaccharide production from 1 to 2.68 mg/mL, increased cadmium uptake from 1 to 1.55 mg/g of biofilm-GAC. However, further increases in polysaccharide production did not result in any worthwhile increase in cadmium uptake. This small increase in cadmium uptake could be due to the biofilm (therefore polysaccharide) reaching an equilibrium level over GAC. This explanation is supported

by Figure 4.33, which shows the amount of biofilm attached to the GAC at four different temperatures.

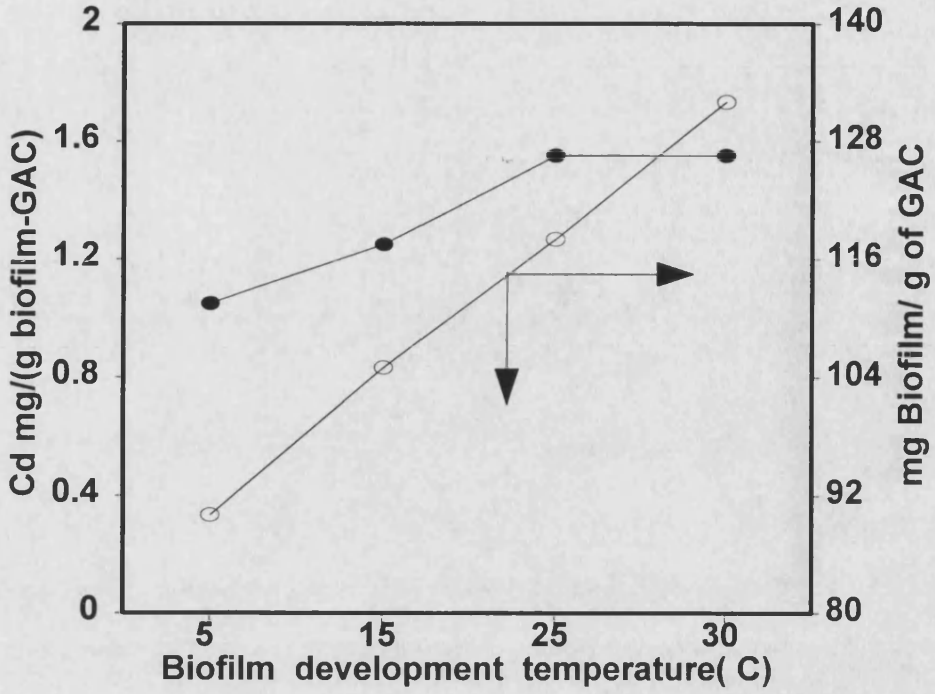


Figure 4.33 Effect of growth medium temperature on biofilm attachment and cadmium biosorption (at 25 mg/L and 18°C)

The amount of biomass attached to GAC increased by 11% (*i.e.* from 118 to 132 mg/g of GAC) with an increase in temperature of 5°C (from 25 to 30°C). Thus, biofilm attachment was approaching saturation level over the GAC.

Factors such as viscosity, cell growth and attachment, and polysaccharide production, were related to each other and influenced by temperature (Table 4.12). Consequently, these factors can play a significant role in the uptake of metal by the biofilm.

Table 4.12 Effect of growth medium temperature on polysaccharide production, biomass dry weight, medium viscosity and cadmium uptake.

Temp. °C	Viscosity of medium @25°C cps	Medium (Biomass) dry wt. mg/mL	Biomass attached (mg/g GAC)	Poly-saccharide mg/mL	Cd uptake[1] mg/g
5	2.7	10.50	90	1.00	1.05
15	3.2	11.24	105	2.32	1.22
25	3.3	12.08	118	2.68	1.55
30	3.4	12.80	130	3.20	1.58

1. Initial cadmium concentration 25 mg/L.
2. All results are average of 4 to 5 repeated runs with a variation of ±5% (except cadmium uptake, which was ±3%)

(III) Use of extracted polysaccharide for metal sorption

In this research it was found that polysaccharide excreting species exhibit very good metal uptake capacities (see Section 4.1). A similar role for polysaccharides in metal sorption, but with free cells, has been also reported (Brown and Lester, 1982 a; Scott *et al.*, 1986; Pradhan and Levine, 1992a, b). Thus, an efficient metal biosorbent could be GAC with extracted polysaccharides attached on its surface (*i.e.* using polysaccharides extracted from the biomass).

Attempts were made, therefore, to attach extracted polysaccharide (free of cells) to the surface of GAC, for subsequent cadmium uptake (Section 3.3). However, this attachment (deposit) was found to be non-uniform (observed in SEM studies), and

some of the deposit was loosely held.

When an aqueous metal solution was passed over this GAC, sloughing off of loosely attached polysaccharide was observed. This poor adhesion could be due to, (i) limitations in attachment technique *e.g.* heat drying at 105°C may have deformed the polysaccharides and (ii) absence of live cells. Adhesion of cells to a solid surface is reported to be mediated by excreted polysaccharide (Lion *et al.*, 1988), whereas this result suggests that the presence of polysaccharide producing cells is also essential for good adhesion. This observation is supported by the reported role of cell components and adhesion enhancing materials, (*e.g.* lectin) in cell attachment (Bryers 1987; Sorongon *et al.* 1991).

The attachment of extracted polysaccharide to GAC was assessed by subsequent cadmium uptake. The average cadmium uptake (from an initial 25 mg/L) was found to be 34% (±7%), which was still more than GAC on its own (12 to 15%). But uptake by attached polysaccharide (residual) was much lower than that for fresh biofilm-GAC (62%). This demonstrates the need for much more efficient means of polysaccharide attachment.

4.4.3 Metal uptake by biofilm formed from previously grown cells

One of the constraints of the biofilm-GAC metal biosorption system was the process time to obtain the desired biofilm. In all studies described so far, $2dm^3$ of growth medium inoculated with the *Pseudomonas* sp., was immediately circulated over GAC for the biofilm development. In this study, a new approach was adopted, in which a $2dm^3$ medium with the Pseudomonad was grown for 2 days before being circulated

over the GAC.

The biofilm formed by this approach was assessed for its biosorptive capacity. These biofilms may be different in biosorption performance due to, (i) adhesion of cells to the GAC, influenced by the state of the cells (Savage and Fletcher, 1985), (ii) cells grown before attachment would be free from alterations caused by fluid shear force, presence of excessive capsular polymer and any stress induced due to attachment to GAC, (iii) the majority of exposed cells to GAC were from early stationary growth phase, and were known to yield maximal polysaccharide (Section 4.1.2).

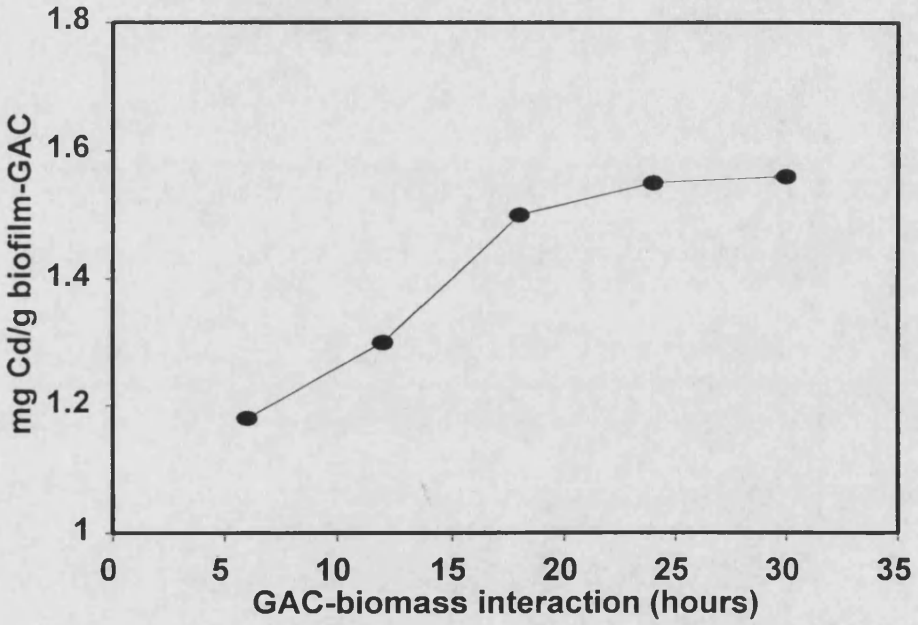


Figure 4.34 Use of two day old cultured medium for biofilm development and cadmium biosorption at 25 mg/L

Cadmium uptake capacity (from an initial 25 mg/L) of biofilms-GAC which were developed by cells grown prior to attachment was assessed after 6, 12, 18, 24 and 30 hours circulation (Figure 4.34). The biofilm formed after 6 hours circulation over the

GAC, sorbed 1.18 mg Cd/g of biofilm-GAC and increased to 1.3 and 1.5 mg Cd/g biofilm-GAC after 12 and 18 hours exposure, respectively. Further, increasing the interaction time between GAC and growth medium showed saturation in cadmium uptake (1.55 mg/g biofilm-GAC), which was similar to biofilms developed with growing cells. This result suggests that irrespective of the nature of the cells (*i.e.* young, fully grown and old), the biofilm sorbs metal. This use of previously grown biomass not only reduces the process time by 50%, it may also offer cost benefits. A typical process would be, growing the bulk biomass for two days and exposing it to the GAC for one day to obtain the desired biofilm for biosorption.

4.4.4 Growth medium with enhanced air supply for biofilm development

In this study, the reduction in biofilm development time was approached by enhancing cell growth by purging the air into the growth medium. This medium was circulated over GAC, and the metal uptake capacity determined at the end of 1 and 2 days of circulation.

Microbial cultures are often oxygen limited. When oxygen is limited, the carbon substrate is only partially oxidized, which leads to by-product secretion. Further, metabolic activity of cells is also affected by changes in oxygen level (Varma *et al.*, 1993).

Air was purged into a growth medium inoculated with the *Pseudomonas* sp. The air flow rate was maintained at 8 mL/min. Atkinson and Mavituna (1983) reported that at this level, dissolved oxygen (DO) would not usually be growth limiting for freely suspended cells. It was found that cell growth (measured as optical density) was

enhanced by air supply. However, changes in the sterile growth medium due to purged air contributed 5 to 8% in optical density measured.

The biofilm which was harvested after one day with purged air showed 2 to 4% more cadmium uptake, as compared to the one day biofilm developed without any air supply (Figure 4.35). On the other hand, with biofilms grown for two days with an air supply, uptake was 5% lower than that of two days old biofilm without any external air.

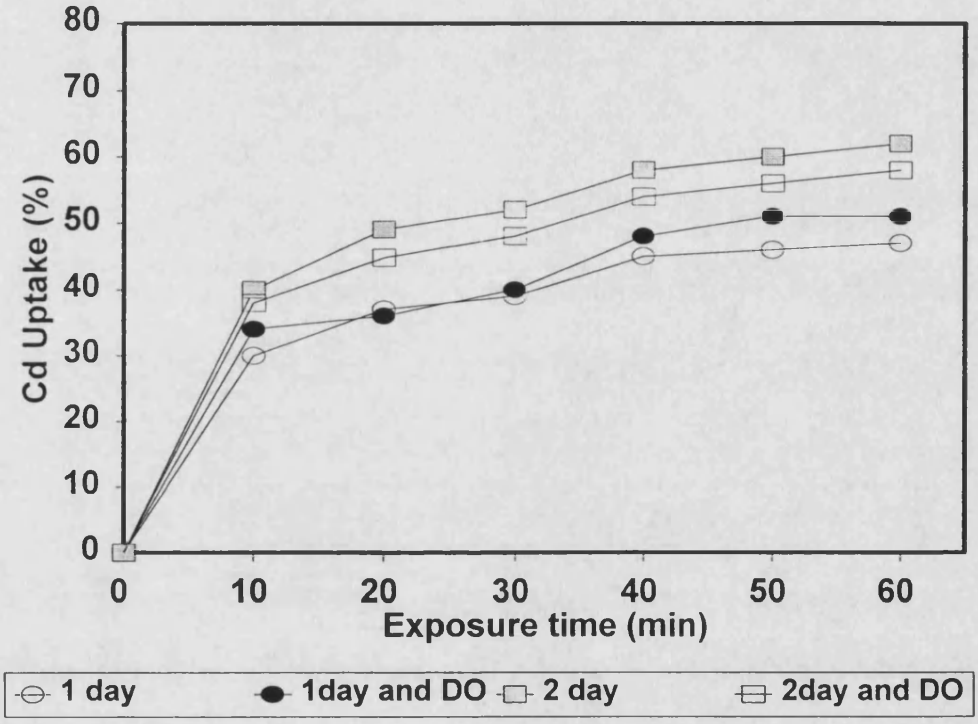


Figure 4.35 Effect of biofilm developed with dissolved oxygen (DO) in growth medium on cadmium biosorption at 25 mg/L

These results suggest that despite of a significant improvement in cell growth the biosorptive capacity of the biofilm-GAC system was not improved. One possible explanation for this decline could be the insufficient level of polysaccharides. For

example, Novak *et al.*, (1992) reported that the polysaccharide production is dependant upon cell growth, but it is also possible to obtain dense cell growth without reaching maximum polysaccharide levels. Another reason could be due to reaction of oxygen with metabolism products and by-products, as they could have damaged the biological (enzymatic activity) system (Cox *et al.*, 1990).

4.4.5 Influence of medium fluidization velocity on biofilm development and subsequent metal uptake.

The superficial velocity of the growth medium over fluidized GAC may affect cell immobilization and biomass attachment due to shear. Moreover, the fluidization velocity may alter the alignment of cells on GAC, causing elastic deformation and morphological changes. On the other hand, it is reported that cells increase their hydraulic resistance rapidly before any effect on metabolic activity occurs (Fowler and Robertson, 1991). This may help in attachment of cells to the GAC. In addition, the fluid velocity may influence biosorption by variations in cell hydrophobicity, *e.g.* Sorongon *et al.*, (1991) reported that a static culture of *F. maritimus* was less hydrophobic than one grown in shaker culture. Similarly, increasing fluidization velocities may provide more nutrients to growing cells, although higher velocities will reduce the contact time. Biofilms developed under different fluid velocities were examined for cadmium uptake (see Appendix II for minimum fluidization velocity).

Figure 4.36 shows trends of cadmium uptake by biofilm-GAC developed under different fluidization velocities. Superficial velocities were in the range of 1.7 to 4.8 cm/s. Cadmium uptake was not affected with increasing fluidization velocity up to 2.4 cm/s. However, any further increase in velocity resulted in significant decline in

cadmium uptake.

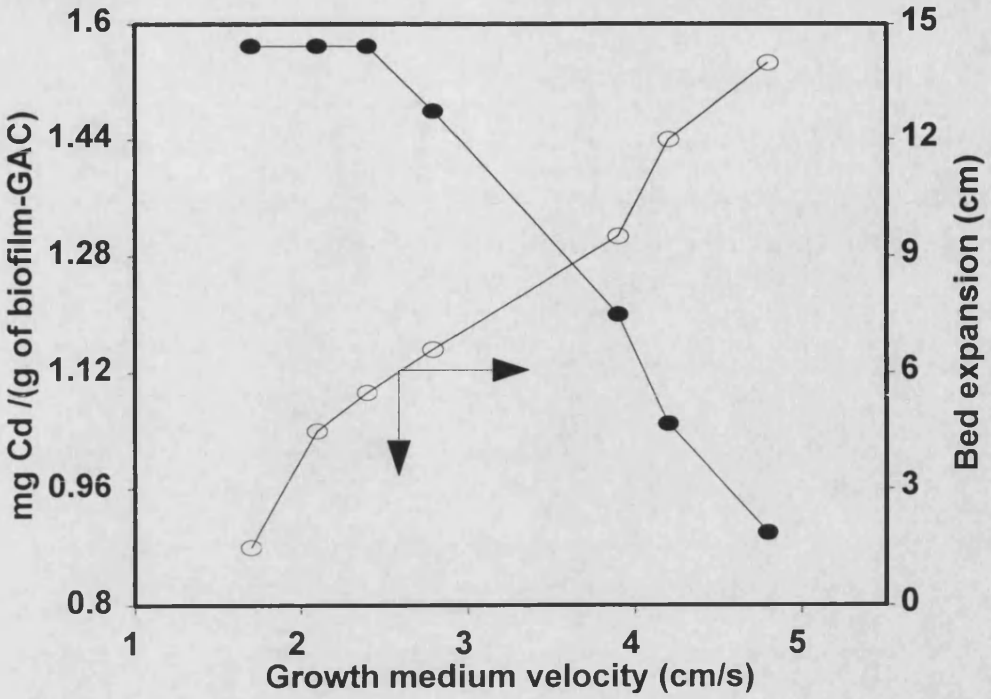


Figure 4.36 Effect of growth medium fluidization velocities during biofilm development on subsequent cadmium biosorption (25 mg/L)

This decline could be due to the weak initial cell attachment and/or sloughing off of the biofilm by fluid shear. Further, the metabolic behaviour of attached cells and amount of polysaccharide produced has been reported to be affected by fluid velocities (Fowler and Robertson, 1991). Thus, the optimal velocity for biofilm development was below 2.4 cm/s.

4.5 Influence of the metal solution environmental and other factors on biosorption

Effects on metal biosorption due to varying environmental conditions *e.g.* pH and temperature, and presence of inorganic and organic compounds along with metal are discussed in this section.

4.5.1 Effect of pH of aqueous metal solution on biosorption

Biofilms were developed at pH 7.4 to assess the effect of pH of the aqueous metal solution (at an initial metal concentration of 25 mg/L). Figures 4.37 A and B and Table 4.13, illustrate the effect of pH on biofilm biosorptive capacity. The exposed metal containing solutions were between pH 2 and 7.

Table 4.13 Specific metal uptake (mg metal/g biofilm-GAC) at different pH, from 25 mg/L

Metal @ 25 mg/L	Metal loading (mg/g biofilm-GAC) at different pH					
	2	3	3.5	4	5	7
Cd	0.25	0.90	1.47	1.55	1.56	1.56
Cr	0.37	0.48	1.32	1.32	1.33	LS
Cu	0.72	1.47	1.47	1.55	1.66	LS
Ni	0.24	0.53	1.27	1.48	1.48	LS
Ag	0.87	1.89	2.10	2.10	2.10	2.10
Zn	0.25	0.52	1.30	1.45	1.57	LS

LS: Limited metal solubility

Compared to pH 5, at pH 2 and below the overall reduction in uptake was: (i) 80% in the case of cadmium, nickel and zinc; (ii) 55% in copper; (iii) 60% in silver; (iv) 70% in chromium. This decline is likely to be due to a combination of more than one factor. Biosorption includes surface and internal accumulation by viable cells, as well as by the extracellular polymer. As pH falls, the surface charge of cell wall proteins becomes more positive (Bitton and Marshall, 1980), thereby inhibiting accumulation. Furthermore, in this work it was found that the cell lost complete viability around pH 3.2, which would cease any metabolically sponsored accumulation, making biosorption only a passive process. A further consideration effecting long term

exposures in low pH streams, is degradation and sloughing off of the extracellular polymer attached to the GAC.

Changes in protonation levels will also affect metal speciation. The cell wall carries surface functional groups such as -COOH and -OH, and as a result the surface should exhibit amphoteric behaviour depending upon the solution pH. That is, if metal accumulation is controlled by electrostatic interaction, then at a pH above 3-4 (isoelectric point), negatively charged surfaces would attract oppositely charged metal ions. Similar pH sensitivity has been reported for other micro-organism-metal interactions, *e.g. C. Vulgaris* (Darnall *et al.*, 1986; Cho *et al.*, 1994), *A. nodosum* (Holan *et al.*, 1993).

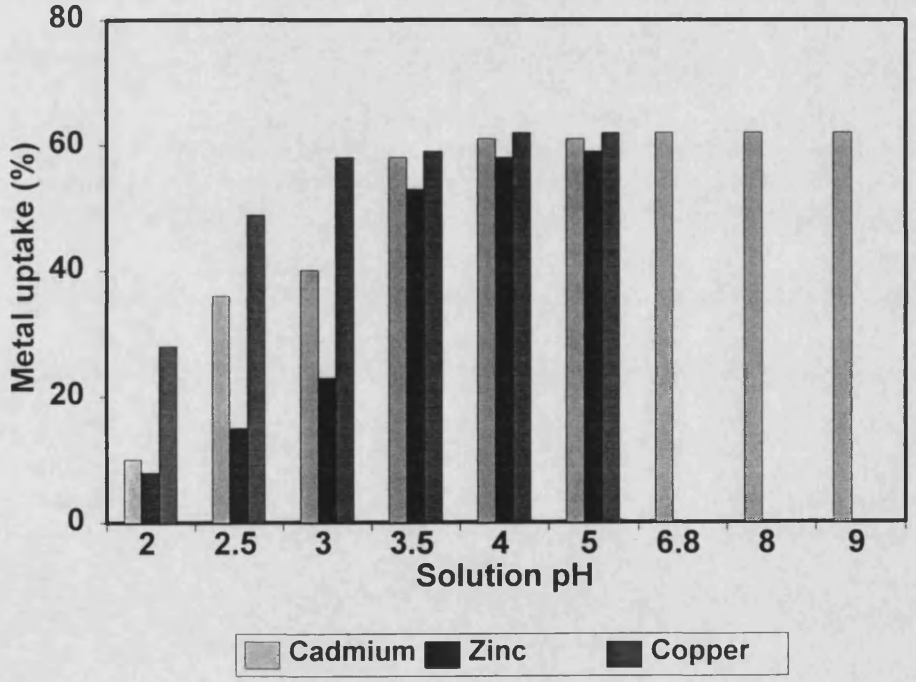


Figure 4.37A Effect of pH of metal containing solution on biosorption of Cd, Zn and Cu, in single metal solution (each at 25 mg/L)

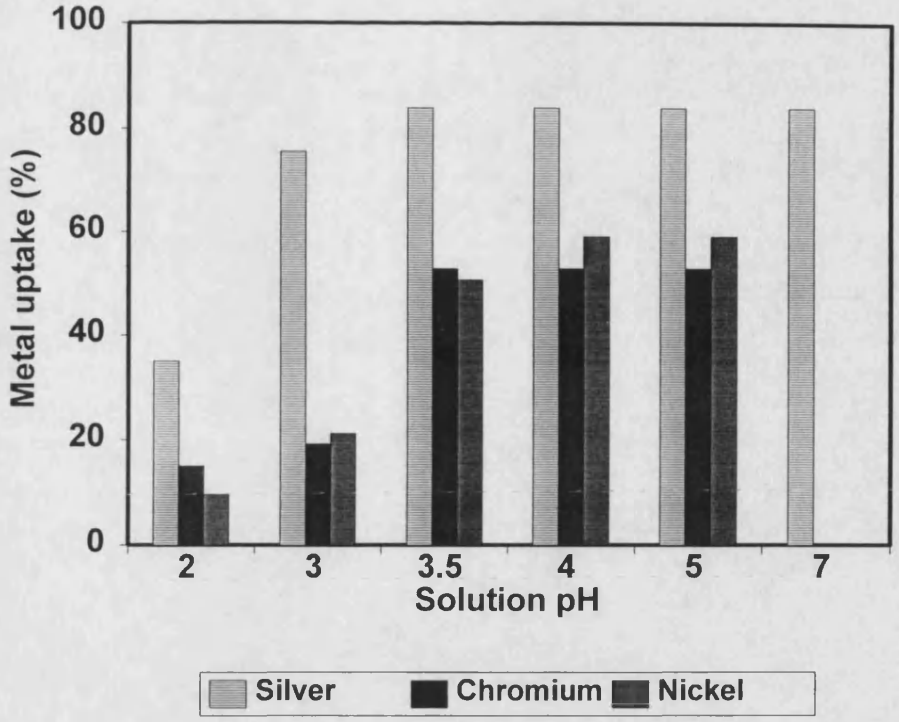


Figure 4.37B Effect of pH of metal containing solution on biosorption of Ag, Cr and Ni, in single metal solution (each at 25 mg/L)

4.5.2 Effect of aqueous metal solution temperature on biosorptive capacity of biofilm-GAC

Temperature of metal contaminated discharges will vary with the type of process and surrounding environmental conditions. Variation in temperature is likely to influence metal uptake for two reasons. Firstly, due to inorganic dissolution reactions, which are strongly endothermic; that is, metal become more soluble. Thus, at a higher temperature, more metal will be available for uptake by the biofilm. Secondly, the electrochemical potential of extracellular polymer in the biofilm can be modified due to changes in temperature (Bryers, 1987), which, in turn, may affect metal-polymer interaction.

The biosorptive capacity of biofilm was assessed at four different temperatures (5, 15,

20 and 25°C), which were within the viability range of the *Pseudomonas* sp. used in this research. Figure 4.38 demonstrates the direct relationship between cadmium solution temperature and its uptake by the biofilm. However, at 5°C, cadmium uptake was only 10% lower than at 25°C. These results show that within the range of most waste water stream temperatures, changes in temperature will not significantly affect the biofilm's metal sorptive capacity. Similar small influence of solution temperature was observed on the uptake of zinc. This low level of temperature dependent uptake suggests that the biofilm accumulates metal through surface attachment rather than metabolic interactions.

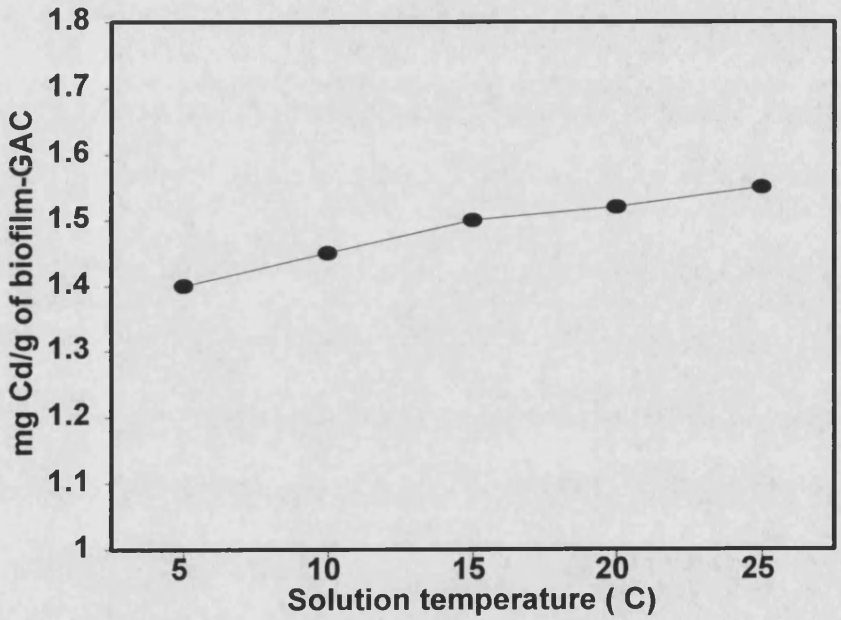


Figure 4.38 Effect of metal containing solution temperature on cadmium biosorption (25 mg/L)

Depending upon type of adsorbent, both increase and decrease of metal sorption with temperature of solution have been reported. Taylor and Kuennen, (1994) showed that

temperature decreased the physical adsorption of metal onto GAC. On the other hand, increase in temperature in the case of cell suspensions resulted in increases in metal uptake (Nourbakhsh *et al.*, 1994). Therefore, the metal uptake observed here is as expected, predominantly due to interaction of metal with the biofilm and not GAC.

4.5.3 Influence on metal sorption by biofilm-organic-inorganic interaction

(I) Metal uptake in the presence of organic and inorganic residues

A waste stream usually is a combination of metals, organic compounds and salts. The biosorption studies reported in this thesis so far, deal only with metal containing solutions. This is, however, often unrealistic in terms of actual conditions likely to be encountered. Although micro-organisms attached to GAC showed an increased resistance to the toxic effects of organic residue (Pfeffer and Suidan, 1989), the presence of other chemicals on the metal biosorptive performance of biofilm-GAC was not known.

In order to assess the effect of other compounds on biosorptive capacity, a solution containing organic and inorganic compounds (as well as metal), was exposed to the biofilm-GAC. The solution contained cadmium (25 mg/L), sodium nitrate (10 mg/L) and atrazine (8 mg/L). Cadmium uptake is shown in Figure 4.39. These preliminary results indicate that metal uptake capacity is not affected by the presence of other components. In fact presence of sodium in solution (as a competing metal ion for binding sites) did not affect cadmium biosorption. A literature survey did not reveal any similar study on biosorption.

A number of factors may be responsible for the relatively unaffected biosorptive

capacity of biofilm-GAC. They could be, (i) metal-polymer complexation; (ii) organic residues not competing for metal binding sites, but just diffuing through the biofilm to the GAC, *e.g.* as with diffusion of phenol through biofilm (Craik *et al.*, 1992).

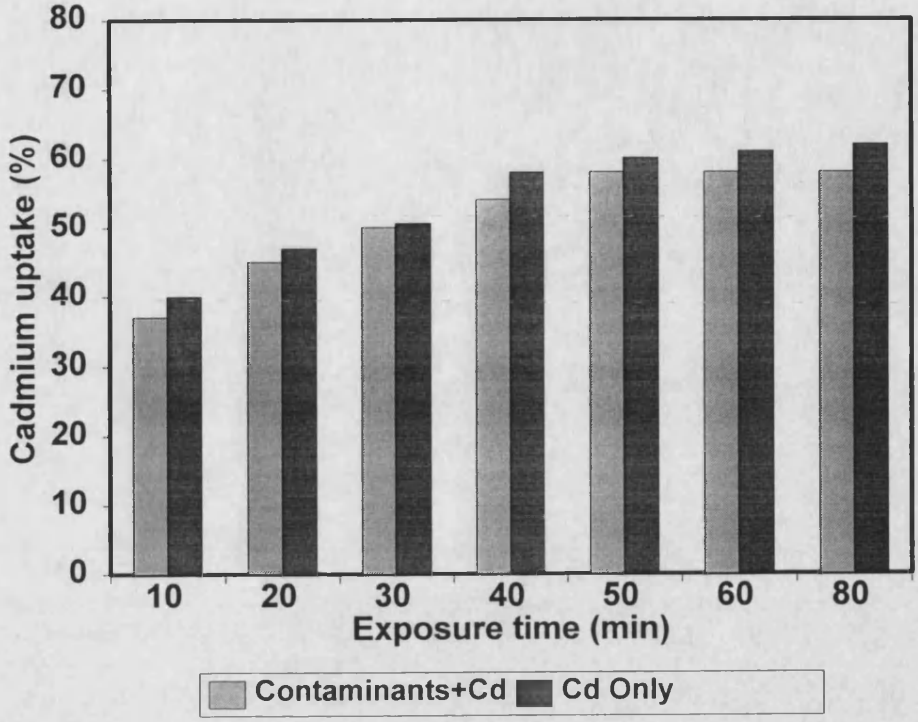


Figure 4.39 Effect of the presence of organic and inorganic pollutants on cadmium biosorption at 25 mg/L

(II) Combined organic residue and metal uptake

A similar study to the one described in the previous section was carried out in order to examine the selectivity of the biofilm for metal and an organic compound. Most organic contaminants found in effluents can be classed as either aliphatic or aromatic compounds (Bouwer and Zehnder, 1993). Therefore, the organic contaminants selected were butanol (aliphatic), benzene (aromatic) and atrazine (aromatic). During this study, in addition to the uptake of metals, uptake of organic contaminants by the biofilm-GAC was also considered.

(A) Atrazine

Atrazine is the most widely used herbicide in the control of broad-leaf weeds in corn, sorghum, and certain other crops (Mandelbaum *et al.*, 1993). Atrazine has a water solubility of 33 mg/L and is moderately persistent in the environment. Spillage of this compound at herbicide loading sites and subsequent run off can be a cause of ground water contamination.

The uptake of atrazine by biofilm covered GAC was compared to its uptake by virgin GAC, and also by biofilm in the absence of cadmium. Virgin GAC on its own in the absence, or presence, of cadmium was excellent atrazine adsorber. It adsorbed 95% of the atrazine (10 mg/L initial concentration) within 2 hours of exposure.

A mixture of atrazine (10 mg/L) and cadmium (25 mg/L) was then exposed to the biofilm. Subsequent atrazine and cadmium uptake trends are illustrated in Figure 4.40. Uptake of cadmium was found to be unaffected by the presence of atrazine. An equilibrium cadmium uptake level was reached in 40 to 50 minutes, whereas it took 24 hours in the case of atrazine. Interactions of cadmium with biomaterial (cell and associated biomass) are well known. However, interactions of atrazine with biofilm are not known, but a few studies on pesticide and micro-organisms have found that pesticides have low biodegradability (Dalmacija *et al.*, 1992).

These results clearly signify that the presence of the biofilm over GAC decreased the atrazine adsorption rate. The resulting slow uptake could be due to diffusional resistances to atrazine from the biofilm as its migrates from bulk solution to the GAC. Hindered diffusion of organic compounds through biofilm was also observed by

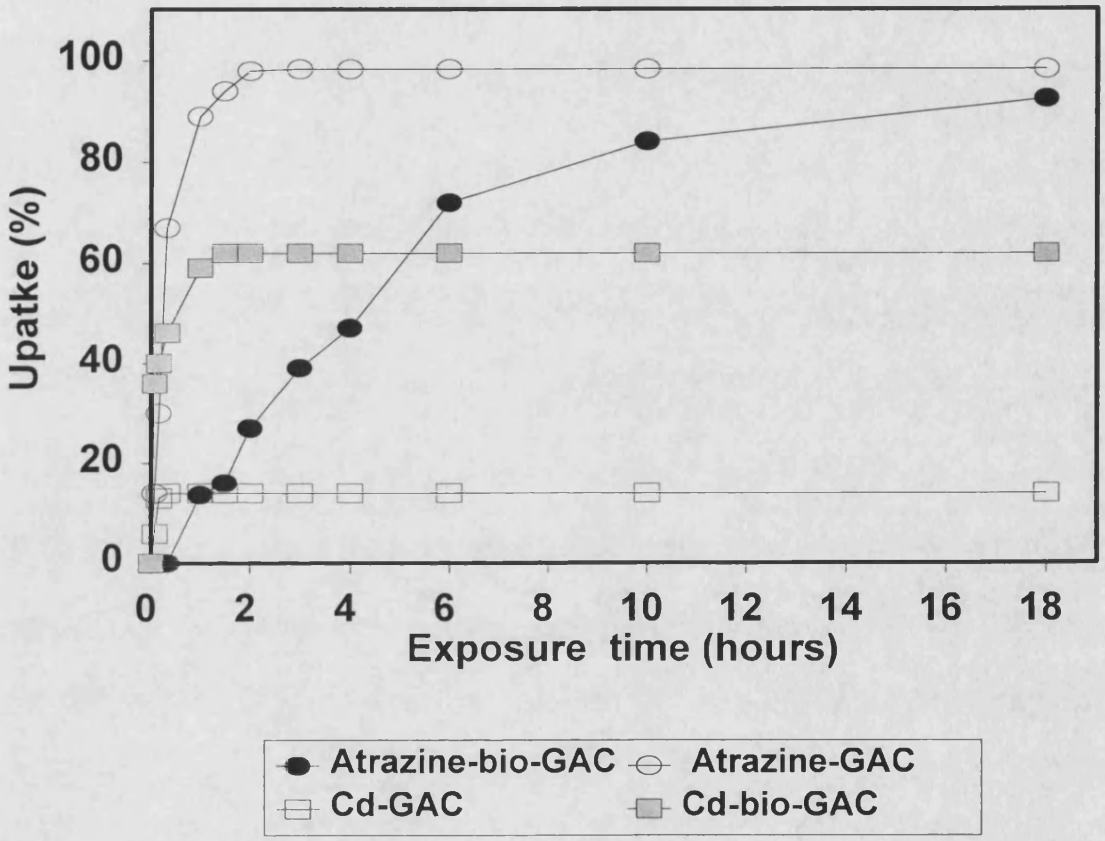


Figure 4.40 Simultaneous removal of atrazine (10 mg/L) and cadmium (25 mg/L) by virgin GAC and a biofilm-GAC system

Bouwer and Zehnder (1993). They reported that over a long contact time, sorbing pollutants diffuse into the organic matrix and also form bound residues.

The rate constant of atrazine uptake by virgin GAC and biofilm attached to the GAC are presented in Table 4.14. These constants are obtained by using the Lagergren Equation [4.1]. It can be seen that rate constant of atrazine uptake by biofilm-GAC was 10 times smaller than that of cadmium (Table 4.6).

Table 4.14 Kinetics of atrazine (10 mg/L) adsorption

Adsorbent	r^2	K (min^{-1})
GAC	0.65	0.035
biofilm-GAC	0.78	0.00554

The observed characteristics of the biofilm could be due to both the presence of capsular polymers and an inability of the *Pseudomonas* sp. to metabolize atrazine. Interactions of polysaccharides with atrazine are not clear, however, in the case of other organic compounds they have affected adsorption, *e.g.* dextran does not adsorb p-chloroaniline and cellulose does not adsorb toluene (Bengtsson *et al.*, 1986; Olmstead and Weber, 1991). Metabolized or degradation products of atrazine, such as des-isopropyl atrazine and des-ethyl atrazine, were not found by HPLC analysis, further suggesting an absence of biological activity during atrazine adsorption.

(B) Butanol

Butanol is relatively more miscible in water (9 g/ 100 g of water), and has a highly polar organic chain. It was, therefore, selected in this study to see if it competes with metal ions for binding to the biofilm. The miscibility of butanol with water should

help it to migrate into the biofilm. Further, it may help also in facilitating degradation of organic compounds by bacteria.

Figures 4.41 and 4.42, illustrate butanol uptake trends. Virgin GAC adsorbed 82% of butanol from an initial 100 mg/L, whereas, biofilm-GAC adsorbed 72%. Although the presence of biofilms over GAC decreased the butanol adsorption capacity when compared to virgin GAC, the uptake rate was not affected. Butanol uptake was also assessed in both the absence and presence of six heavy metals. In the presence of all the metals in solution, butanol adsorption on biofilm-GAC decreased from 80% to 10%. On the other hand the uptake of all six heavy metals (cadmium, copper, chromium, nickel, silver and zinc each at 25 mg/L) was not affected by the presence of butanol.

The interference to butanol adsorption may have been from only one of the six metals present in the solution. Therefore, all the metals were exposed individually to the biofilm along with butanol. The biofilm again showed more affinity to silver, zinc, cadmium, copper, nickel and chromium than butanol (uptake of butanol was 8 to 10%). This result signifies the selectivity of biofilm for metals, compared to butanol.

(C) Benzene

Benzene was selected for uptake studies along with metals due to its immiscibility with water and its potential as a carbon source for certain micro-organisms (Smith, 1990). Sayles and Suidan (1993) reported that benzene has a high octanol-water partition constant (91) and can be biodegraded easily. The selectivity of the biofilm-GAC between benzene and metals was assessed by comparison of uptake capacity.

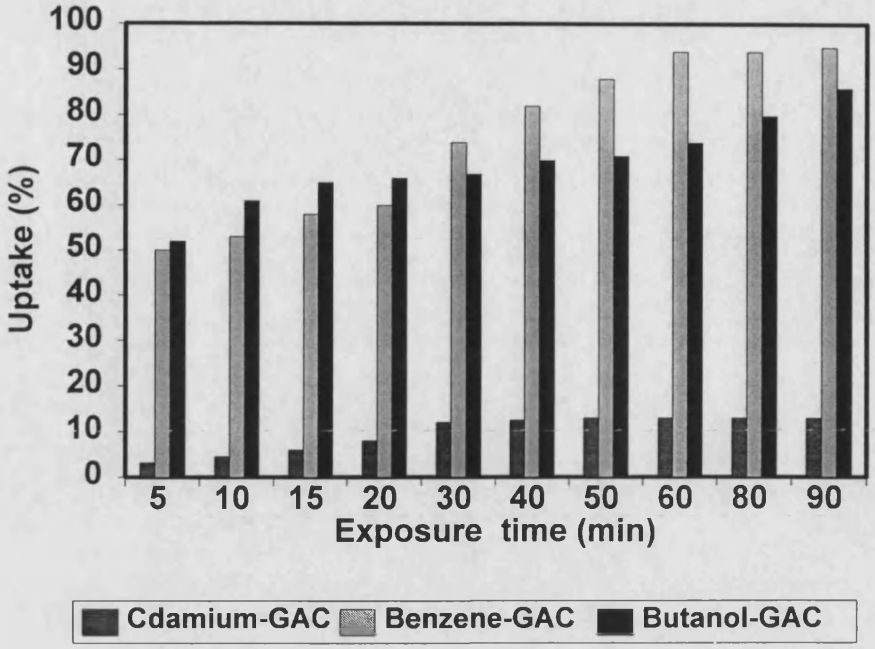


Figure 4.41 Adsorption of cadmium (25 mg/L), butanol (100 mg/L) and benzene (25 mg/L) from a single pollutant system by virgin GAC

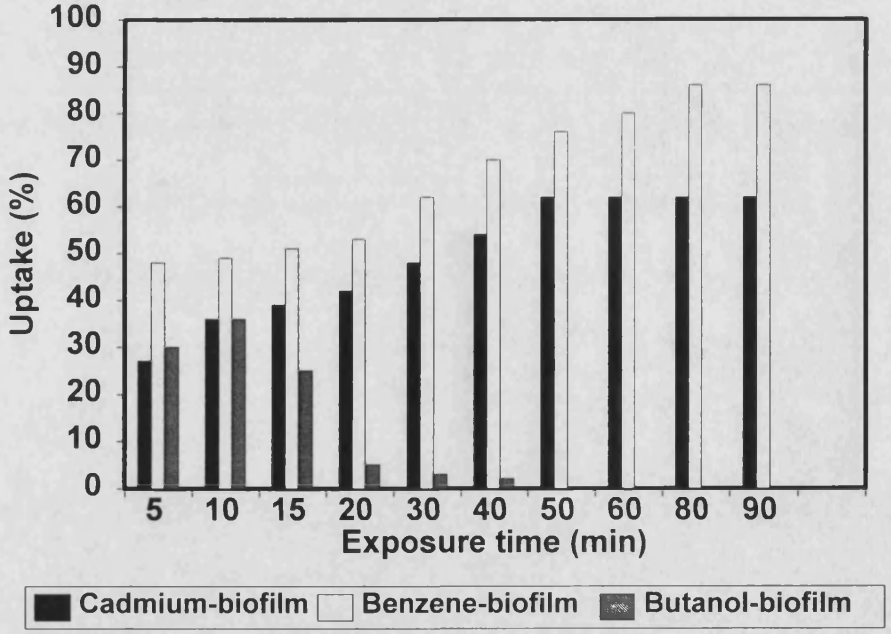


Figure 4.42 Adsorption of cadmium (25 mg/L), butanol (100 mg/L) and benzene (25 mg/L) from a cadmium-organic pollutant system by biofilm-GAC

Figures 4.41 and 4.42, illustrate benzene and metals uptake by the biofilm-GAC. Uptake trends of benzene on virgin GAC were also determined. Metal uptake capacity or rate was not affected by the presence of benzene (25 mg/L). Whereas, irrespective of presence or absence of metals, benzene uptake capacity of GAC declined by 12-14% due to the attached biofilm. However, the kinetics of benzene adsorption in the presence of biofilm over GAC were similar to virgin GAC.

This shows that the metal uptake capacity of the biofilm was not affected due to the presence of the three organic compounds tested. On the other hand, the biofilm-organic compound interaction varied significantly with the organic compound tested.

4.5.4 Continuous through flow biosorption

The work described so far was based upon batch processing, that is metal containing solutions were re-circulated until free metal reached a constant, reduced concentration level. This results in a diminishing concentration gradient between the bulk solution and the biofilm. In order to assess (i) the ability of a GAC-biofilm system to process metal containing effluent in a continuous mode and (ii) any effect of concentration gradient on metal loading capacity, biofilms were exposed to constant concentrations of metal by using a straight through system. Biofilms were exposed to a fresh metal containing solution until outlet metal concentration was similar to that of inlet.

Residence time (defined as average amount of time spent by the fluid in the reactor) was maintained constant at around 7 sec. The results of metal uptake from exposure to cadmium or chromium at a constant concentration of 25 mg/L, are given in Figure 4.43. The cadmium uptake rate was found to be 0.0079 mg/min. g of biofilm-GAC

over the initial 20 minutes as compared to 0.0065 mg/min. g of biofilm-GAC in batch operated biosorption. Metal equilibrium uptake was reached in 30 minutes as compared to 40-50 minutes in batch processing. Net equilibrium loading of cadmium was increased to 2.2 mg/g of biofilm-GAC, which is 40% more than the cadmium loading achieved in batch processing. With chromium equilibrium loading was increased by 35% when compared to batch processing.

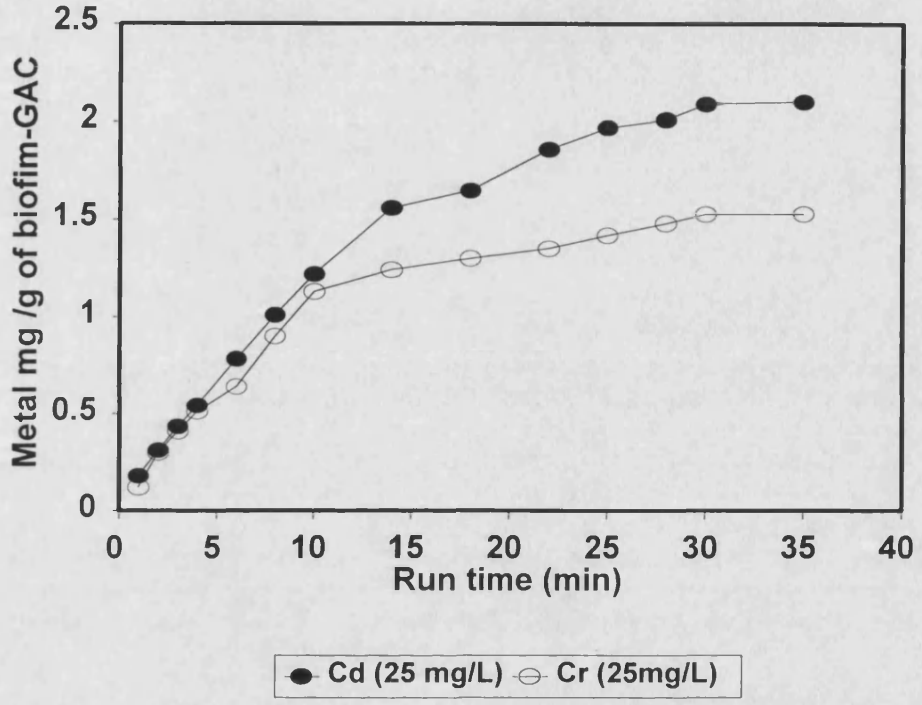


Figure 4.43 Biosorption of cadmium (at 25 mg/L) and chromium (25 mg/L) as single metal in a continuous through flow system.

Although a similar style of metal uptake (decreasing rate with time) was observed in both batch and continuous processes, these results confirm that biosorption process is influenced by concentration of metal. These results are support by the observed increase in diffusion coefficient due to increasing concentration gradients around the biofilm-GAC (Section 4.3.3). The results also show the ability of biofilm to sorb the

metal in a continuous mode.

4.6 Influences of GAC on the biosorptive properties of the biofilm

It is well known that the surface properties of GAC are variable, depending on both source of raw material and method of activation (Cheremisinoff and Ellerbusch, 1978; Rivera-Utrilla and Ferro-Garcia, 1986). This section presents the discussion on suitability of GAC, with different specifications, for biofilm development and subsequent biosorption. The metal uptakes by biofilms attached to GAC were compared with, (i) similar sources of GAC, but with known surface functional groups over the surface, (ii) different particle diameters and (iii) regenerated GAC.

4.6.1 Adaptability of biofilm metal sorption to different sources of GAC

The prime source of virgin GAC used in this research was from BDH (Section 3.1.2) and was of a coal/coke source. In order to consider the adaptability of biofilm metal sorption process, another, similar well characterised GAC, F200 (Chemviron Carbon, Filtrasorb 200) was used as a comparison with the GAC used primarily in this research. The GAC F200 and GAC (BDH) are of similar raw material source and produced by high temperature steam activation. Table 4.15 summarizes the physical and chemical properties of the two carbons.

Biofilms of the *Pseudomonas* sp. were developed over both types of GAC and subsequently exposed to an aqueous cadmium solution of 25 mg/L concentration. The performance of these biofilms showed identical cadmium uptake rates and equilibrium capacities. These results indicate that the cells have an ability to develop over surfaces with similar physical natures and to form a biofilm, despite 30% less surface area.

Table 4.15 Comparison of the properties of commercial GACs

Properties	GAC (BDH)	GAC (F200)[1,2]
Surface area (m^2/g)	900-1000	700-750
Particle Size (mm)	0.85 to 1.70	0.6 to 0.7
Surface groups	Unknown	phenolic: 65% f-Lactone: 26%

1. Ishizaki *et al.*, (1983); **2.** Huang (1978)

4.6.2 Effect of GAC particle size

Industrial water treatment technologies use GAC of various diameters. Incorporation of this biofilm-GAC metal sorption process in a water treatment process may be affected by the GAC particle diameter. In order to determine any effect of particle diameter on biosorptive capacity, particles of three different mean diameters (1.41, 0.50 and 0.250 mm) were selected.

Table 4.16 Effect of particle size on metal equilibrium sorption level from an initial 25 mg/L of cadmium

Mean particle diameter (mm)	Cadmium (mg/g biofilm-GAC)
0.250	1.60
0.50	1.70
1.41	1.55

Cadmium uptakes from an initial concentration of 25 mg/L by the biofilms attached to GAC of various particle diameters are presented in Table 4.16. There appeared to

be an optimal GAC diameter (within the tested range of diameters) for biofilm development with respect to cadmium uptake.

However, decreasing the particle size below 0.5 mm (mean diameter) resulted in operational difficulties, such as carry over of particles with the fluid. Such limitations resulted in reduced flow rates (by 20%), which in turn would affect the process.

4.6.3 Use of regenerated GAC for biofilm development and subsequent metal uptake

Regeneration of exhausted GAC by heat treatment is well known and very widely adopted in the industry (Chemviron, 1993). Thermal regeneration has small effects (depending on regeneration temperature) on the adsorptive capacity of GAC and its pore structure (Smithson, 1978). On the other hand, the effect of regeneration of GAC on biofilm attachment and subsequent metal uptake was not known. Consequently, previously used GAC for biosorption, was initially regenerated at 900°C for 5 or 10 min (in air), and used for further biofilm attachment and subsequent metal uptake. The regenerated GAC was also examined by SEM for any structural changes.

SEM photograph (Figure 4.44) of regenerated GAC shows a significant change in the surface appearance as compared to the surface of virgin GAC (SEM Photograph in Figure 4.1). A profound opening of pores in regenerated GAC was observed. The virgin GAC has few surface irregularities, whereas, the surface of regenerated GAC appears to be in a severely deformed state. These deformations and irregularities were expected to give enhanced cell attachment and biofilm growth, and thus improve metal uptake.

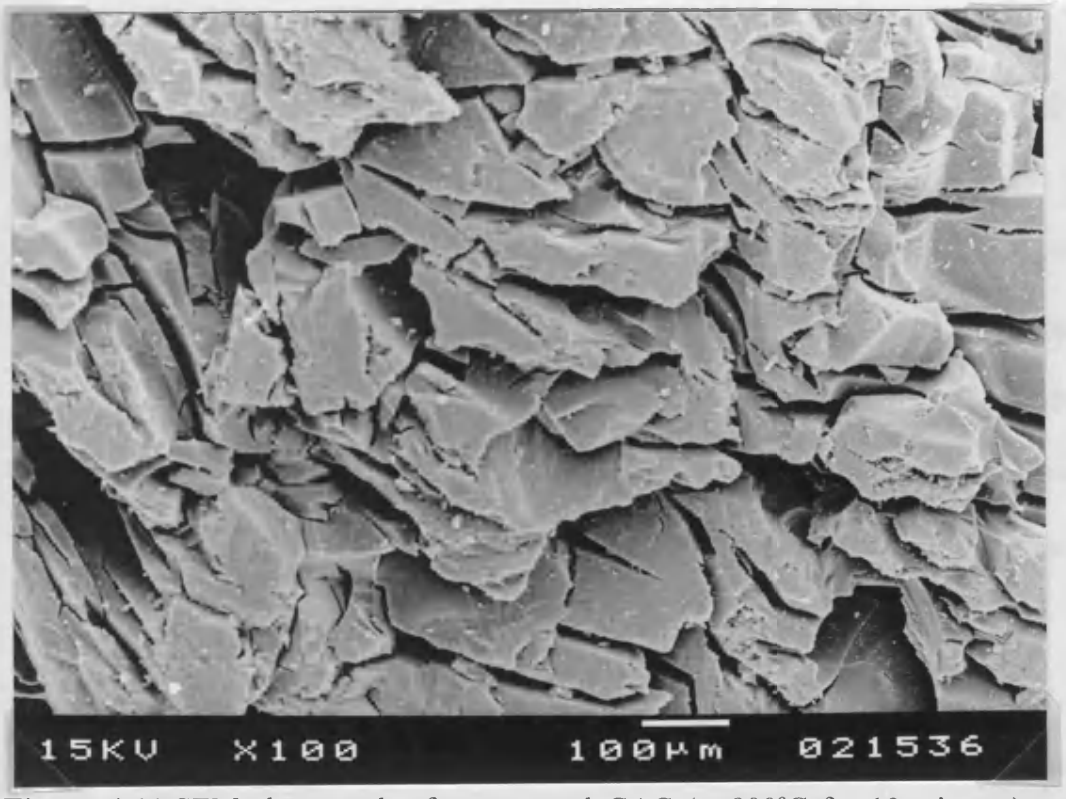


Figure 4.44 SEM photograph of regenerated GAC (at 900°C for 10 minutes)

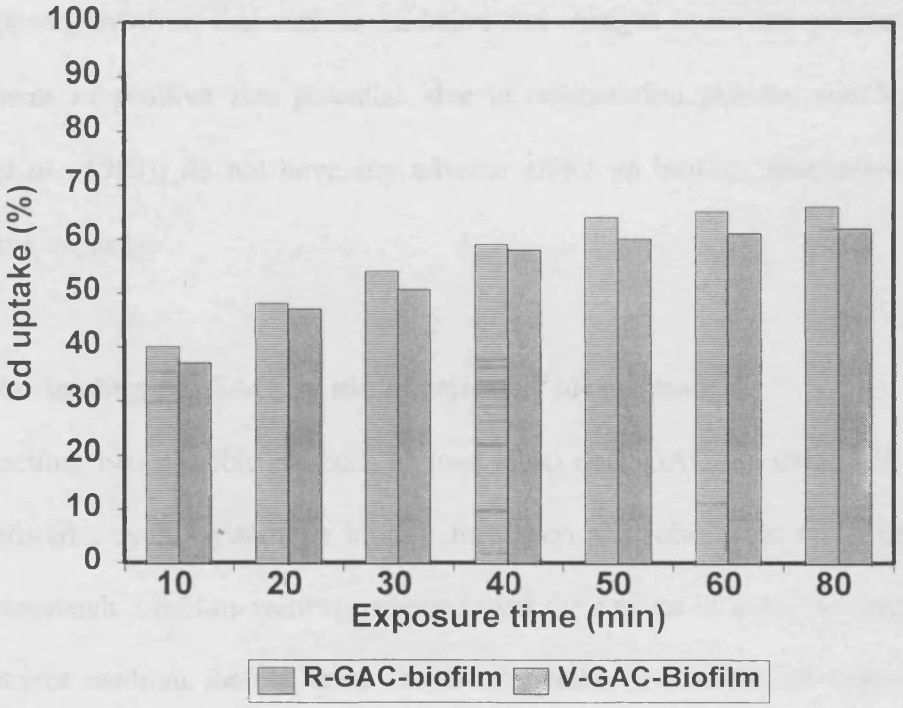


Figure 4.45 Use of regenerated GAC for biofilm development and subsequent cadmium (25 mg/L) biosorption [R- regenerated, V- Virgin]

Cadmium uptake by biofilm developed over virgin and regenerated GAC is illustrated in Figure 4.45. The uptake curves of biofilm attached to GAC regenerated for 5 and 10 minutes were similar to each other. However, with both the regenerated GACs, a consistent but small, increase in cadmium uptake was observed (4 to 5%), compared to biofilm attached to virgin GAC. The possible explanations for this small increase in cadmium uptake could be due to the increase in surface irregularities of the regenerated GAC, giving better sites for cell attachment, and/or an increase in adsorption of cadmium by the regenerated GAC itself.

Cadmium adsorption capacity of regenerated GAC (without biofilm) was subsequently found to increase by 6 to 8%. This suggests that any enhancement in cadmium uptake was primarily a factor of direct adsorption of cadmium onto the GAC surface. It would appear, however, that surface oxidation and changes in surface properties (*e.g.* development of positive zeta potential, due to regeneration (Martin and Ng, 1985; Bansal *et al.*, 1988)) do not have any adverse effect on biofilm attachment and its biosorptive capacity.

4.7 Metal loading on GAC by use of repeated biosorption

In this section, two possible methods to load metal onto GAC are discussed, both of which utilized a cyclic system for biofilm formation and subsequent metal uptake. In the first approach, biofilms were regenerated over the surface of GAC by recirculating fresh nutrient medium, before reuse for metal uptake. In the second approach, the biosorbed metal-GAC was heat treated to fix the metal. The GAC with metal fixed over it was then reused for biofilm development and subsequent metal accumulation.

4.7.1 Metal accumulation by regenerated biofilm cycle

Extracellular polymers offer protection to entrapped cells against the imposed toxicity by metals (Sterritt and Lester, 1980). In order to regenerate biofilm and re-initiate cell growth after the biofilm had been exposed to metals, it was flushed with sterile Ringer's solution followed by circulation of fresh sterile nutrient medium. This entire procedure was repeated several times in a cyclic manner.

The metal uptake efficiency, as net accumulation of metal on biofilm coated GAC, along with loss of metal to the surrounding medium, is shown in Figure 4.46. The quantity of metal taken up from fresh $1dm^3$ aqueous metal solutions during each exposure run was progressively reduced over the first four cycles, after which it did not diminish any further.

The net loading of cadmium over the biofilm-GAC increased by 3.5 times in six cycles (*i.e.* from 1.55 to 5.5 mg Cd/g of biofilm-GAC). The first exposure accounted for 29% (*i.e.* 1.55 mg/g of biofilm-GAC) of the total cadmium accumulation in six cycles. The contribution to total metal accumulation by each cycle after the fourth cycle was 0.77 mg/g of biofilm-GAC.

However, cadmium also desorbed into the circulating growth medium. During the first regeneration cycle, after two days, 16% of accumulated metal was found in the circulating medium. This implies areas of weak association between metals and the biofilm. A material balance illustrates that, during six cycles, 150 mg of cadmium is exposed to regenerated biofilm-GAC, of which 64 mg is accumulated and 15 mg is desorbed back into medium.

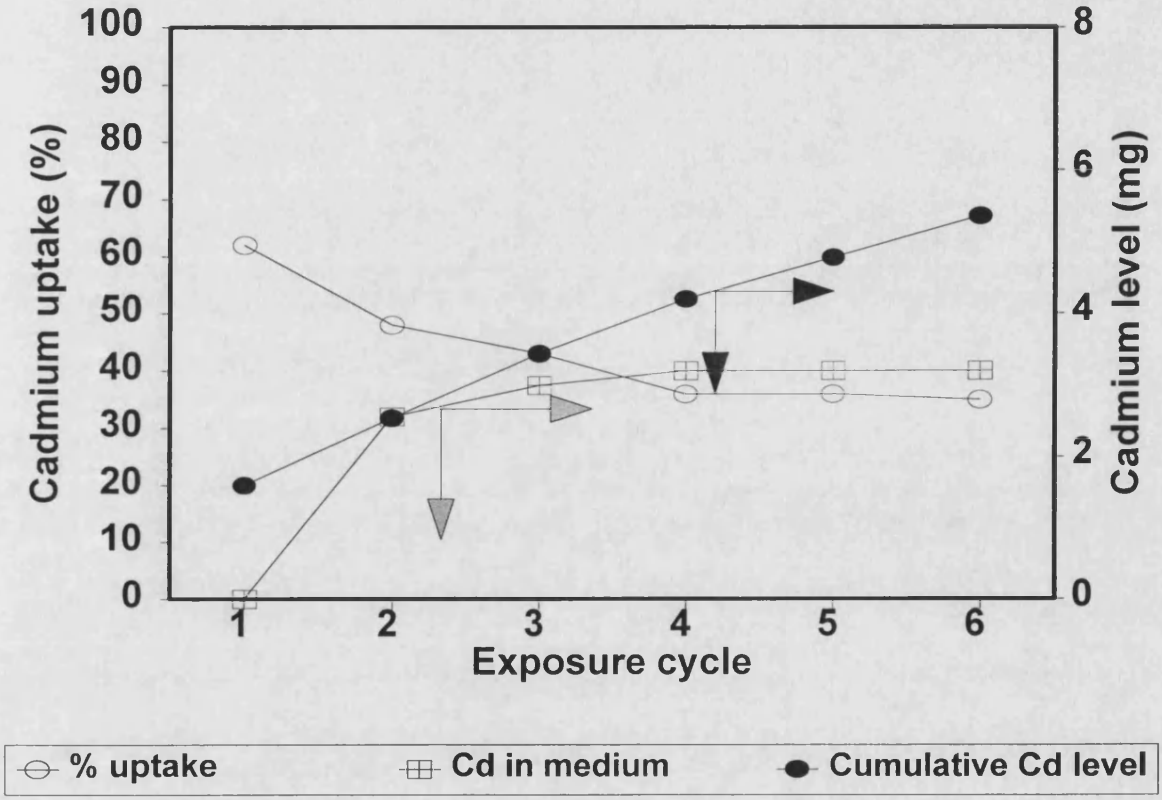


Figure 4.46 Cadmium loading by regenerated biofilm and cadmium(25 mg/L) biosorption in cyclic mode, and loss of weak bound cadmium into the medium

There are several reasons which may contribute to the decline of metal uptake efficiency in the subsequent cycles. These include availability of sites to hold excreted polymers, flushing off of polymers by shear forces (Chang and Rittmann, 1988), and morphological changes occurring in the cells (Craik *et al.*, 1992). Morphological changes may result in cells which are different from the parent cells and these cells may have different polysaccharide excretion level or metal interaction mechanisms.

Despite the drawback of weak binding, this approach shows that through the biosorption process, the GAC can be reused and metals can be obtained in a concentrated form.

4.7.2 Metal fixation-biosorption cycle

The regenerated biofilms demonstrated (Section 4.7.1) a potential to bind more metals over the surface of GAC (5.3 mg Cd/g biofilm- GAC after six cycles). However, after 3-4 cycles, up to 30% of the metal taken up during a biosorption cycle, desorbed back into the medium during biofilm regeneration.

A new approach was subsequently developed to load more metals onto the GAC through repeated use. The accumulated metal was fixed by heat treatment in air at 230°C for 3 hours after each biosorption cycle. A fresh inoculated nutrient medium was then provided for re-newed biofilm development. This approach was aimed at obtaining permanent attachment of accumulated metals over the GAC in order to avoid desorption during biofilm development. The fixation of metal over the surfaces of the GAC between each cycle was demonstrated by using EDS studies.

The observed cadmium uptake equilibrium after each repeated use of GAC with fresh biofilm is given in Figure 4.47. Cadmium uptake (from an initial 25 mg/L) by the biofilm-GAC after the first cycle was 62%, but it declined to 51% after six metal exposure-heat treatment-biofilm development cycles. Over six cycles, the net accumulation of cadmium reached 8.7 mg/g biofilm-GAC. This was four times higher than the cadmium loading achieved after a single exposure. In comparison to the biofilm regeneration approach discussed earlier, this method gave a 40% increase in cadmium accumulation. This method was also used to test zinc, which after a six exposure cycle had an accumulation of 9 mg Zn/ g biofilm-GAC. This zinc loading level was five times more than that obtained by a single use of biofilm-GAC.

Table 4.17 Heat treatment cadmium exposure cycle, the influence on GAC porosity and metal loading

Exposures	Relative porosity (%)	Cd (mg/g biofilm-GAC)
0	100	-
1	86	1.55
2	N	3.20
3	N	4.80
4	25	6.20

N: results not available

With both the metals tested, the % uptake from an initial 25 mg/L declined over the first four cycles, before reaching a constant uptake level. The decline in metal uptake observed during the first four cycles was considered to be due to various factors associated with the GAC surface with respect to biofilm attachment.

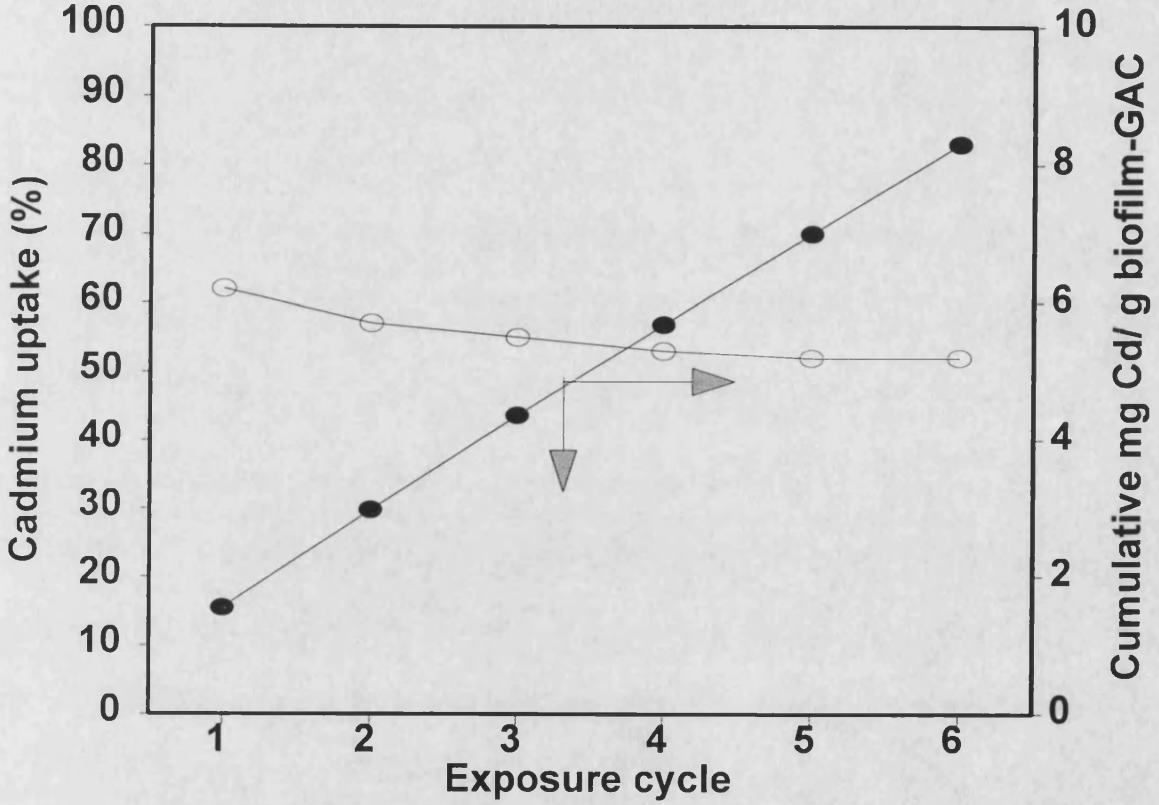


Figure 4.47 Cadmium loading by metal fixation cycle, biofilm-GAC exposed to cadmium (25 mg/L) in each exposure

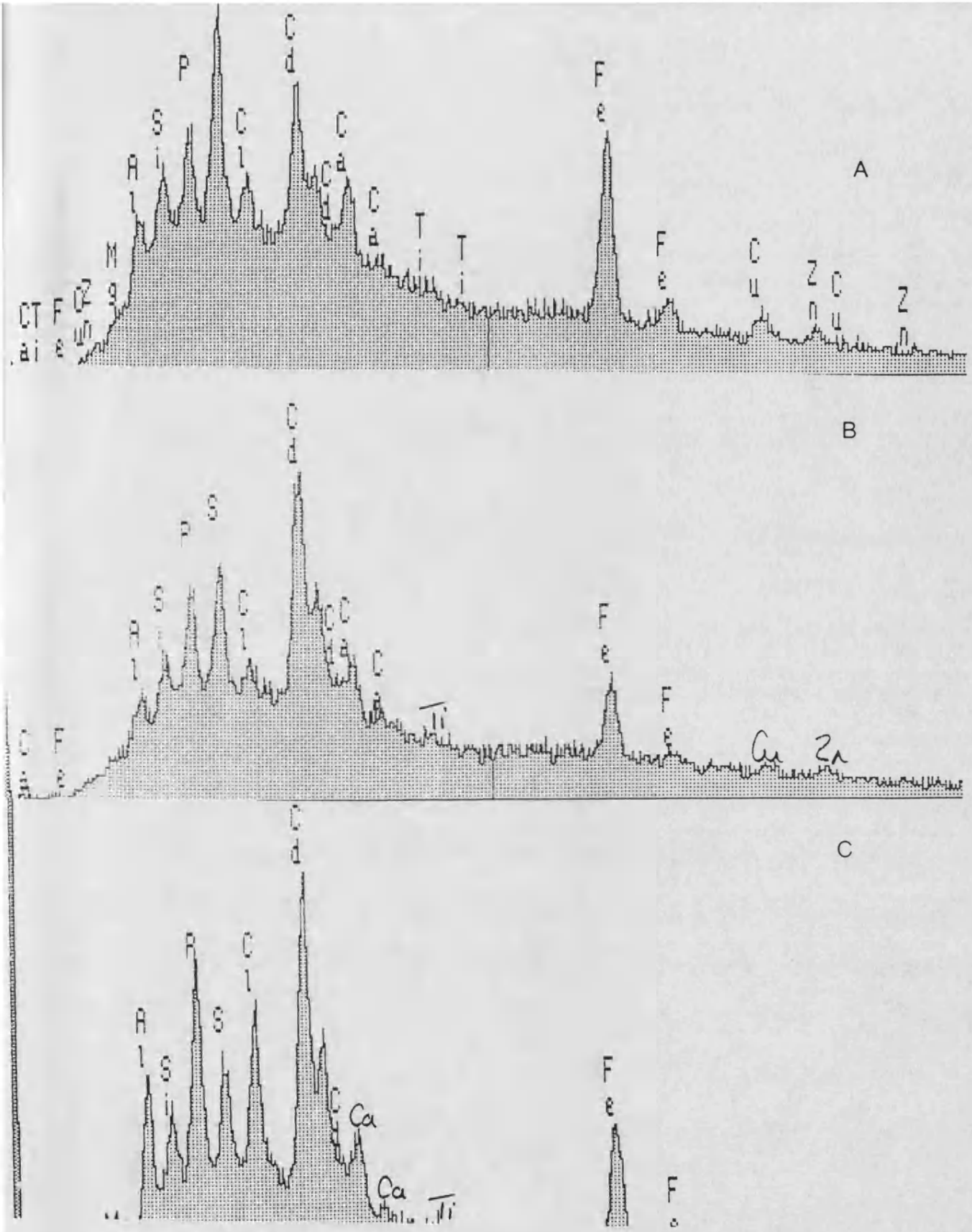


Figure 4.48 Spectrographs of biofilm-GAC in metal fixation cycle exposed to cadmium (25 mg/L) in each exposure (A: Exposure II, B: Exposure III; C: Exposure IV)

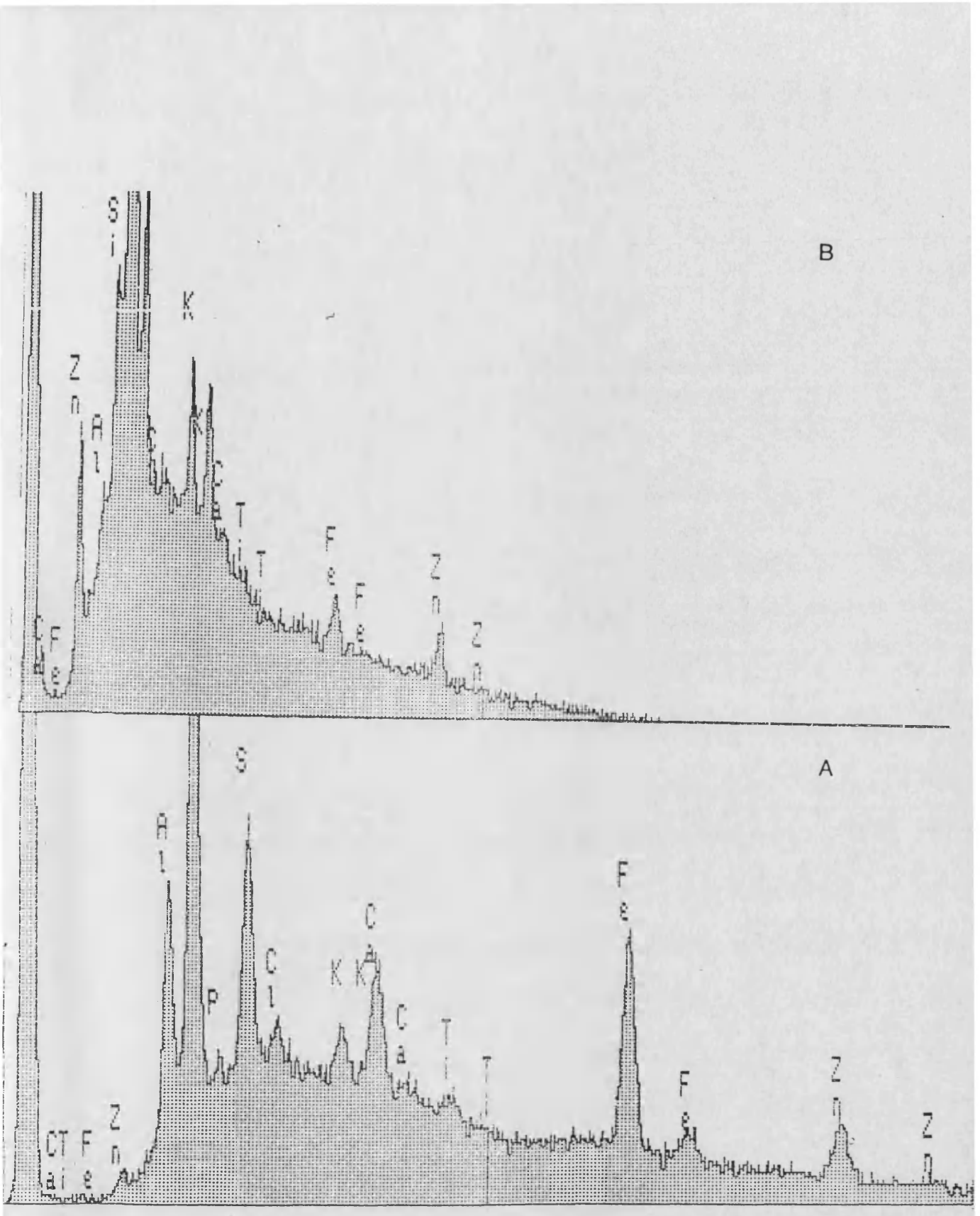


Figure 4.49 Spectrographs of biofilm-GAC in metal fixation cycle exposed to zinc (25 mg/L) in each exposure (A: Exposure II, B: Exposure IV)

The GAC pore volume was found to decline during each cycle. For example, the pore volume was fell by 14% and 75% that of virgin GAC after the first and fourth exposure cycles, respectively (Table 4.17).

It has been reported that the adsorption ability of a solid surface is influenced by the pore volume and structure (Tsutsumi *et al.*, 1993), which may also influence cell adhesion. The increase in coverage of heat fixed biofilm, including the penetration of medium into the pores during each cycle, will have affected the surface roughness of the GAC. In addition, the surface chemical properties such as acidity, were found to have changed due to the build up of heat fixed biofilm (Section 4.9.3). These surface chemical changes may weaken the attachment of polysaccharides, which may rely on interactions between acidic groups with basic molecules within the biofilm by acid-base interaction (Tsutsumi *et al.*, 1993).

A progressive build up of cadmium over the GAC surface was confirmed by EDS examination. This build up was well characterised by the magnitude of the cadmium peaks. EDS Spectrograph (Figure 4.48) clearly illustrates an increase in cadmium peak size after each exposure. Similarly, Spectrograph (Figure 4.49) illustrates the increasing peak size of zinc.

To assess the cadmium uptake capacity of the virgin GAC (without a biofilm), it was also used in a cyclic manner (*i.e.* metal exposure and heat fixing at 230°C for three hours). It was found that after the first cycle, uptake declined more than 30% and by the fourth cycle, the GAC did not adsorb any cadmium. Thus, from three cycles, from an initial 25 mg/L cadmium in each cycle, the final level of cadmium was 0.75 mg/g

of GAC. This level of cadmium was significantly lower than that from either of the repeated biosorption approaches.

4.8 Organic pollutants pre-adsorbed onto GAC used for biofilm development and subsequent metal sorption

GAC is capable of adsorbing a wide range of organic and inorganic chemicals. GAC with adsorbed pollutants can be reused after either chemical or thermal regeneration methods. In this study, spent GAC (saturated with toxic pollutants such as atrazine and chloroform) was assessed for biofilm development without any regeneration. The biofilm developed was subsequently used for uptake of metals.

The ability of micro-organisms to utilize organic pollutants is well documented (Galli and Leisinger, 1985; Walker and Kaplan, 1992). Ideally therefore, the attached cells within the biofilm during the process of biofilm formation would degrade or utilize adsorbed pollutants as nutrient sources. The two pollutants, atrazine and chloroform, were selected as examples of typical aqueous and a gaseous contaminants. Atrazine is a commonly used pesticide which is often found in ground water. Chloroform vapours are found in many industrial gaseous effluent streams.

4.8.1 GAC saturated with atrazine

Previous studies have shown that atrazine is biodegraded by mixed or pure cultures of micro-organisms (Mandelbaum *et al.*, 1993). In this study, three criteria were assessed. First of these was the ability of the *Pseudomonas* sp. to attach to a GAC surface previously contaminated with various concentrations of atrazine. Second was extent of any degradation of atrazine by the *Pseudomonas* sp. during the process of

biofilm development. Third criteria was the cadmium uptake capacities of biofilms developed over such GAC.

Prior to development of a biofilm, the GAC was exposed to various concentrations of atrazine (10-100 mg/L). An equilibrium level for adsorption of atrazine was achieved over 24 hours. After adsorption of atrazine, the GAC was exposed to a medium containing the *Pseudomonas* sp. to develop a biofilm (AE-GAC). Samples of the circulating medium were taken periodically in order to check for any desorption and/or breakdown of attached atrazine. After 48 hours, the biofilm-GAC was exposed to an aqueous solution containing 25 mg/g of cadmium.

Cadmium uptake was found to decrease with increasing levels of pre-adsorbed atrazine. In comparison to using virgin GAC, biofilms developed over AE-GAC that had previously been exposed to 30-100 mg/L of atrazine, showed a 25% fall in cadmium uptake (Figure 4.50). However, cadmium uptake was not affected when the virgin GAC was exposed to an initial concentration of 20 mg/L, or less, of atrazine. The fall in cadmium uptake may have been due to desorption of atrazine from GAC into the circulating medium as it affected cell growth and thus the biofilm development. It was found that in 24 hours, the maximum desorption level was reached. This level was 1 mg/L in the case of the GAC from a 30 mg/L atrazine exposure and increased to 8 mg/L with the GAC from a 100 mg/L atrazine exposure.

Increasing levels of atrazine in the medium was found to inhibit cell growth (tested using optical density) within the first 24 hours. After this initial period, cell growth was similar to that in a medium without any contamination. A dense polysaccharide

like precipitate was observed in the flask containing the *Pseudomonas* sp. and atrazine. This could be due to excess polysaccharide being produced to limit the toxic atrazine. But, this polysaccharide did not contribute to any significant enhancement in cadmium uptake rate, or capacity, of the biofilm-GAC. This could be due to saturation level of the biofilm attachment onto the GAC (Section 4.1.2).

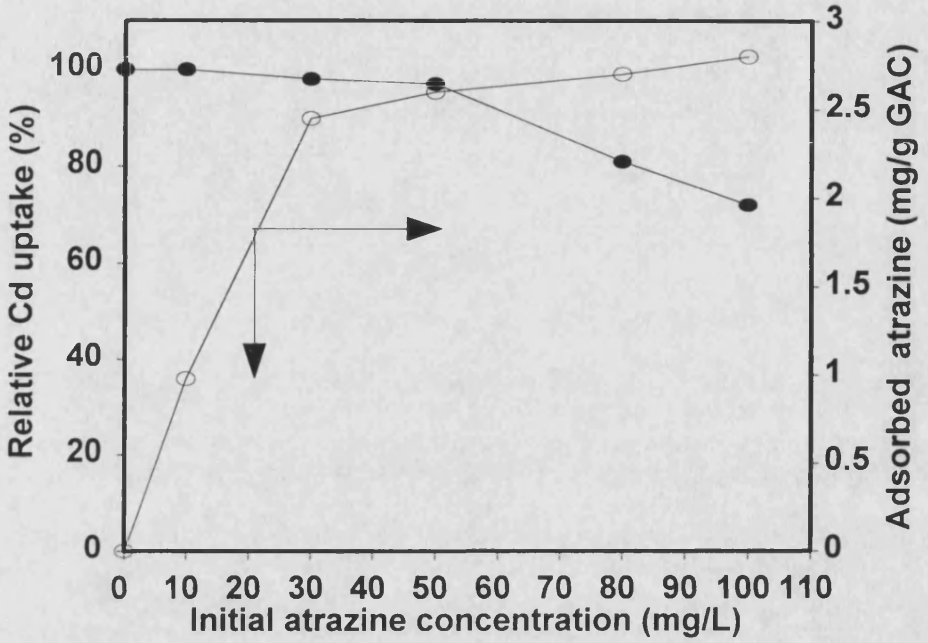


Figure 4.50 Use of GAC with adsorbed atrazine for development of biofilm and subsequent cadmium (25 mg/L) biosorption

Atrazine and its typical breakdown products, were analyzed in the circulating nutrient media. Breakdown products such as des-isopropyl atrazine and des-ethyl atrazine were not found in the medium. This suggests an inability of the *Pseudomonas* sp. to breakdown atrazine within the time of the experiment.

Thus, the presence of atrazine at reasonable levels (*i.e.* below 30 mg/L), appeared not to adversely influence the biosorptive capacity of the biofilm, which is within typical

atrazine levels (3 to 7.4 mg/L) found in ground water (Mandelbaum *et al.*, 1993). Hence, the development of biofilm over such AE-GAC leads to potential route of recycling the GAC without regeneration.

4.8.2 Use of GAC previously saturated with chloroform vapours

GAC which was previously saturated with chloroform, was also assessed for biosorption. In addition, any interaction occurring between the *Pseudomonas* sp. and chloroform during biofilm development was considered.

The GAC used for biofilm development carried 600 mg chloroform per g of GAC. If complete desorption of chloroform from the 10 g of GAC used is assumed, then the chloroform concentration in the 2 L of growth medium would be 3000 mg/L. However, the maximum observed concentration of chloroform in the medium (without a culture) was found to be 570 mg/L. This suggests that micro-organisms would face high levels of chloroform at both the GAC surface and in the nutrient medium.

During biofilm development, samples from the circulating medium were analyzed for the presence of chloroform. After 48 hours, this concentration declined by 15% (i.e. from 570 to 484 mg/L). To understand the chloroform uptake/degradation capacity of the *Pseudomonas* sp., the medium was further circulated for another 48 hours. It was found that a total of 23% (of 570 mg/L) chloroform was utilized. In control studies, that is with sterile medium, in 48 hours a 5 to 8% (of 570 mg/L) chloroform loss was found. This suggests that although the *Pseudomonas* sp. may have utilized some chloroform, the process may be more beneficial with a species better known for chloroform degradation (*e.g. Methylosinus trichosporium*, Alvarez-Cohen *et al.*, 1992).

In order to study the effect of pre-adsorbed chloroform on the biosorption of metals, columns containing GAC with chloroform and biofilm were exposed to 25 mg/L concentrations of either zinc or cadmium. Figure 4.51 demonstrates observed zinc and cadmium uptake. After 60 minutes, cadmium uptake, both in presence and absence of chloroform, was found to be 1.55 mg/(g of biofilm-GAC). In case of zinc uptake, the final metal level showed a small (2 to 3%) decline in uptake than that of the biofilm developed on the virgin GAC (*i.e.* without chloroform).

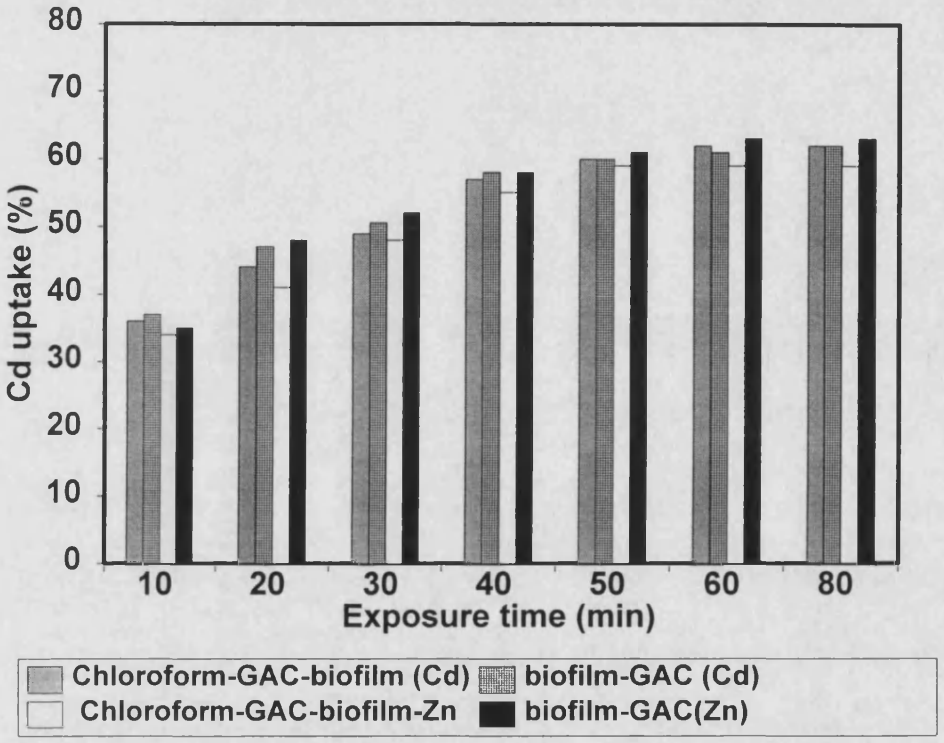


Figure 4.51 Use of chloroform adsorbed GAC for development of biofilm and subsequent cadmium biosorption

This small effect could be due to toxic effects of chloroform on the cell growth during biofilm formation. It was found that cell growth of the *Pseudomonas* sp., in presence of chloroform saturated GAC, was affected for the first 24 hours. However, after this initial phase growth was similar to cells which were inoculated in standard medium.

Similar cell growth trends have been reported for *Pseudomonas putida* in the presence of chloroform (Cruden *et al.*, 1992).

This data indicates that a species such as the Pseudomonad, which is known for its biosorption ability, also shows good adherence and subsequent metabolism and growth over a substrate contaminated with a chloroform. Further, the presence of chloroform do not significantly affect metal-biofilm interaction.

4.9 Properties of GAC with biosorbed metal over surface

In this section, changes in the chemical and physical properties of GAC due to the presence of a heat fused biofilm (with and without metal) are discussed.

To determine effects of heat fused biofilm on GAC properties and chloroform adsorption (Section 4.10) the following adsorbents were used:

(i) GAC (virgin), prepared as described in Section 3.1.2.1, and further dried a t 230°C for three hours.

(ii) GAC and only biofilm (without any metal), dried in air at 230°C for three hours to fix the biofilm (GACBIO).

(iii) virgin GAC, prepared as described in Section 3.1.2.1, and further dried at 230°C for three hours in a nitrogen atmosphere (R-GAC).

(iv) GAC and only biofilm (without any metal), dried /regenerated at 230°C for three hours to fuse/fix the biofilm over GAC in a nitrogen atmosphere (R-GACBIO).

(v) GAC with metal (cadmium, copper, nickel and zinc) and biofilm, dried/regenerated at 230°C for three hours to fuse/fix the biofilm-metal over

GAC in a nitrogen atmosphere (GACBIO-M).

4.9.1 Effect of dry biofilm and fixed metal on GAC

The thickness of dried biofilm over GAC was estimated based on biomass dry weight attached to the GAC, using the equation below (Yu and Pinder, 1993):

$$L = \frac{W_d}{\rho_d \, a} \tag{4.9}$$

where: a is the area from which a biomass sample is collected (cm^2), W_d is the dry weight of the biomass sample (mg), and ρ_d is the biofilm density based on dry weight of biomass (mg/cm^3). The density of biofilm, 100 mg/cm^3 reported by Shieh and Keenan, (1986) and Chen *et al.*, (1988) was used in this study. The dry weight of biomass over the GAC was determined by the method described in Section 3.1.2. The thickness of heat dried biofilm was then estimated to be 1μm.

(I) Particle size of biofilm fixed GAC

Analysis of particle size was carried out in order to see any significant effect of biofilm attachment on the particle size distribution of the GAC. Figure 4.52 illustrates the % particle size distributed in each unit diameter of: (i) virgin GAC (exposed to water circulating fluidized bed for two days and dried for 3 hours at 230°C), (ii) GACBIO and (iii) GACBIO-Cd used in four metal fixation cycles (Section 4.7). The presence of biofilm and metal (or the heat treatment) over GAC does not significantly affect the particle size distribution. This is not surprising, as the dried biofilm has a very small estimated thickness (*i.e.* ~ 0.1 % of the GAC particle diameter).

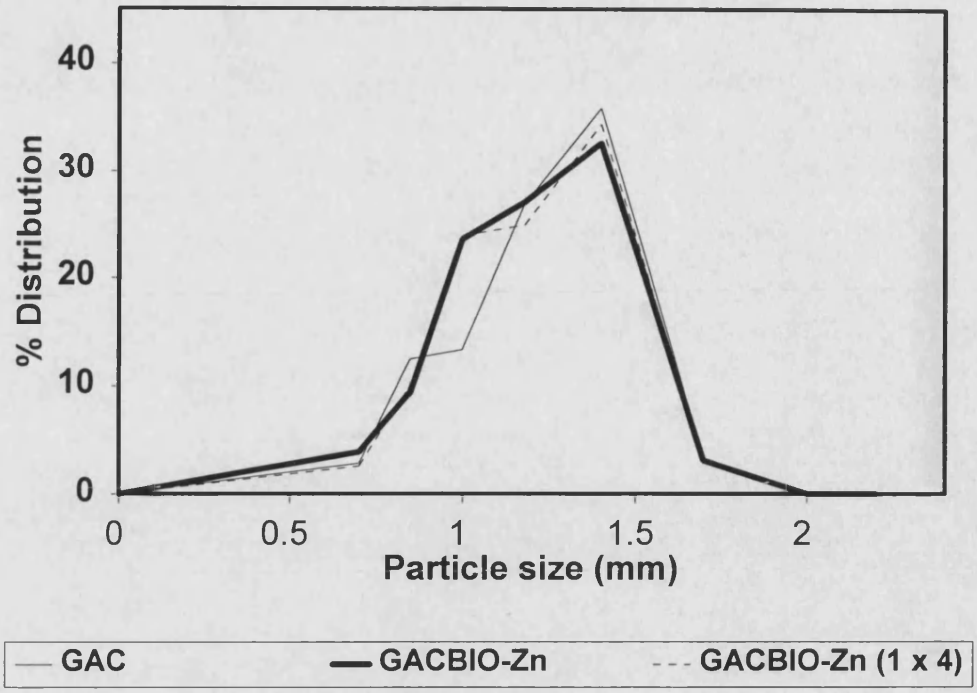


Figure 4.52 Particle size distribution of GAC with and without biofilm-metal (from I and IV exposure cycles, Section 4.7.2)

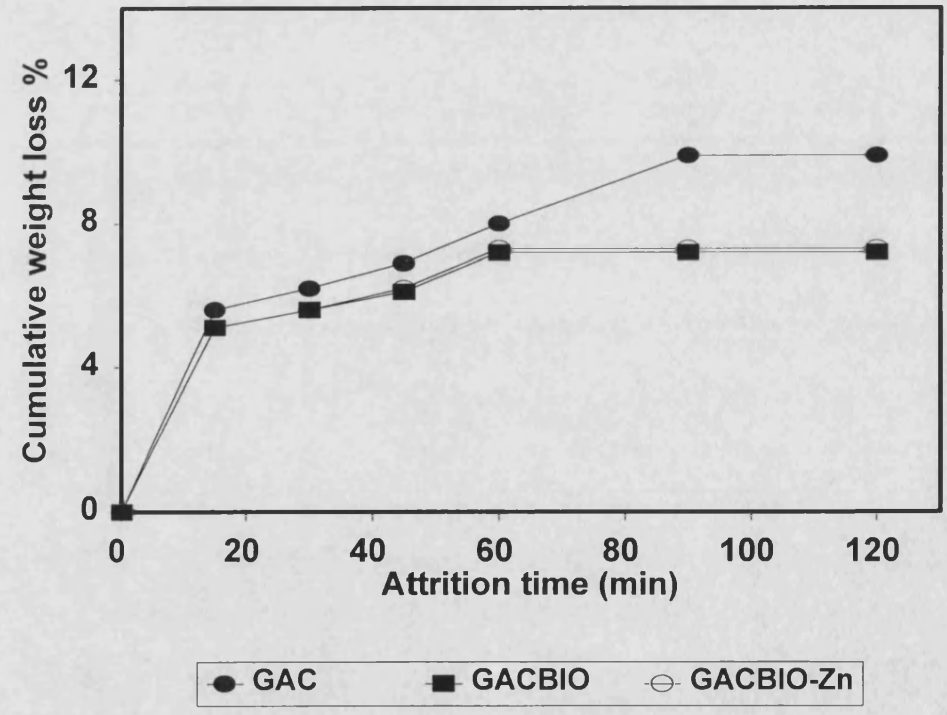


Figure 4.53 Effect of heat fixed biofilm on attrition resistance of GAC in fluidized bed containing 5 g of adsorbent and at a velocity of 1.66 cm/s

4.9.2 Effect of biofilm on attrition

Breaking of particles increases with increasing flow velocity in adsorption process (Kono, 1981). Therefore, in addition to loss of adsorbent due to such attrition, the flow rate of streams to be processed has to be limited.

The effect of the presence of biofilm and metal on the GAC was assessed in term of particle attrition. Particles of (i) virgin GAC (exposed to a water-fluidized bed for two days and then dried for 3 hours at 230°C), (ii) GAC-biofilm (dried for 3 hours at 230°C) and (iii) GAC-biofilm-metal (dried for 3 hours at 230°C), were all sieved to get particles of the size of 18 mesh or over for use in attrition tests. The exposure of the virgin GAC to the fluidization condition was essentially to knock off weak parts of the GAC in order to obtain GAC samples similar to those which underwent fluidization during biofilm growth.

These particles were then subjected to a fluidizing velocity of 1.66 cm/sec for 24 hours. The mass of fines (under 18 mesh) produced was periodically determined. Cumulative weight loss and Equation [4.10] (Pis et al., 1991) were used to determine extent of GAC attrition.

$$R = 100 \ln\left(\frac{M_o}{M_f}\right) \qquad \textbf{(4.10)}$$

where: Mo and M_f represent initial and final mass of particles (g), respectively. The extent of attrition with respect to time for virgin GAC and biofilm attached to GAC is illustrated in Figure 4.53. The results signify that GAC particles with the biofilm have a greater resistance to breakdown compared to the virgin GAC. This improved

mechanical strength may be due to the binding nature of the polysaccharides attached to the GAC and/or from the organic compounds in the medium.

4.9.3 Surface basicity

In order to assess surface chemical changes, the basicity of virgin GAC, GACBIO, GACBIO-Zn, and GACBIO-Cu were determined. The measured basicity of the GAC, as well as attached biofilm and metal is given in Table 4.18. In comparison to virgin GAC, the basicity of GACBIO was found to be increased by 0.08 mmole equivalent per g GAC. However, the presence of zinc reduced the basicity of GACBIO by 0.022 mmole equivalent per g. But, the basicity with the zinc and biofilm (GACBIO-Zn) together was still higher than that of the virgin GAC. This increase in basicity may be due to the different surface functional groups present in the biofilm *e.g.* hydroxyl and amino. It was also found that the samples of R-GAC and R-GACBIO exposed to inert regeneration conditions (Section 3.8.1) showed basicity similar to that measured before regeneration.

Table 4.18 Basicity of different GAC systems

Adsorbate	Basicity mmole eqv/g
GAC	0.029
GACBIO	0.110
GACBIO-Zn	0.088
GACBIO-Cu	0.103

This data shows that the presence of biofilm modifies the chemical nature of the GAC surface. This can be an advantage, (i) to obtain a consistent GAC surface, possibly

irrespective of the origin of GAC and (ii) to obtain biofilms with electrostatic affinities, which can favour the adsorption of positively charged compounds.

4.9.4 Surface area and porosity of GAC-biofilm

The surface area of the GAC with attached dry biofilm was measured by the nitrogen adsorption method (Section 3.8.3), using an ASAP (Accelerated Surface Area and Porosimetry) instrument. The surface area and porosity of R-GACBIO (regenerated in nitrogen) along with R-GAC (exposed to identical nitrogen conditions) are presented in Table 4.19. The nitrogen adsorption data obtained by ASAP, when analysed by different adsorption models (BET, Langmuir and Dubinin-Astakhov and M.P method) showed a small decrease (3%) in total R-GACBIO surface area, as compared to virgin GAC. Pore volume and radius were found to be more than 10% less compared to those of R-GAC. Thus, the biofilm may have partially blocked the pores of the GAC. The effect of heat on the GAC and other biofilm attached GACs can be negligible, as the deformation of GAC due to the heat was found to start above 250°C.

Bansal *et al.*, (1988) and Afzal *et al.*, (1993) reported that impregnation of GAC with different metals lowered the micropore volume, which was attributed to some pores being blocked or occupied by the impregnating species. Thus, the approach of metal attachment to the surface of GAC through a biofilm can offer a distinct advantage over other processes of metal impregnation. However, surface area of GAC which was used in four metal fixation/biosorption cycles (Section 4.7.2) was reduced by 50%. Porosity of this GAC also declined, by 75%.

Table 4.19 Surface area and porosity of GAC and biofilm-metal laden GAC

Method & parameter	GAC	GACBIO-Cd	
	Virgin	I[a]	IV[a]
Surface area (m2/g)			
BET	860	834	485
Langmuir	1144	1129	670
Dubinin- Astakhov	1128	1088	565
M.P. Method	1148	1100	715
Porosity			
Pore volume (cm^3/g)	1.00	0.86	0.25
Pore Hydraulic Radius (nm)	0.873	0.783	0.35

a: exposure of GAC in metal fixation biosorption cycle (Section 4.7.2)

4.10 Organic vapour adsorption onto GAC with heat fused biofilm and metal over the surface

The use of GAC for adsorption of toxic vapours is well known. Adsorption capacities can be improved by impregnation of the GAC by metals and other chemical compounds (Bansal *et al.* 1988). Impregnation, especially with metals, has been widely studied and adopted because of their importance in heterogeneous catalysis. In this study, GAC with metals deposited on it by the biofilm was assessed for adsorption capacity of chloroform vapour from air. In addition to its diverse use in various industries, chloroform was chosen as a test substance because it has critical properties for adsorption, such as, (i) low boiling point (60°C), (ii) low molecular weight and (iii) good diffusivity in air. It is also listed as a highly toxic pollutant by the European Commission (EC) and the Environmental Protection Agency (EPA) in the USA.

4.10.1 Adsorption of chloroform on virgin GAC and GAC-biofilm

Initial chloroform vapour adsorption experiments were conducted by using virgin GAC and GACBIO. The breakthrough curves and subsequent adsorption capacity of these adsorbents were compared with each other. The chloroform influent concentration, 110$\mu g/cm^3$ (± 5%), was maintained through a column containing 1 g of adsorbent and a bed height of 1.15 cm. The effluent breakthrough profiles for both adsorbents are shown in Figure 4.54.

For both adsorbents, the break through point (when effluent concentration was greater than 0.01% of influent concentration) was observed at 7 minutes. The adsorption capacities of both adsorbents at the breakthrough point were also the same. The breakthrough curve profiles show that the mass transfer zone proceed in a similar manner along the bed of both adsorbents. However, there was a small (3 to 4%) increase in the adsorption capacity of GACBIO compared to the virgin GAC.

As a result of adsorption, heat was liberated in the packed bed. The subsequent rise in bed temperature was measured by a thermocouple located at the centre of the bed (to give a real time signal). The exothermic temperature profile for both adsorbents is shown in Figure 4.55. An initial slow rise in temperature in both adsorbents was observed due to the time required for the mass transfer zone to travel through the bed to a zone where the thermocouple was located. After this initial slow rise in temperature, an exponential increase in the temperature was observed.

In the case of GACBIO, the thermal wave travelled rapidly in comparison with the virgin GAC. However, the degree and rate to which heat was liberated was different.

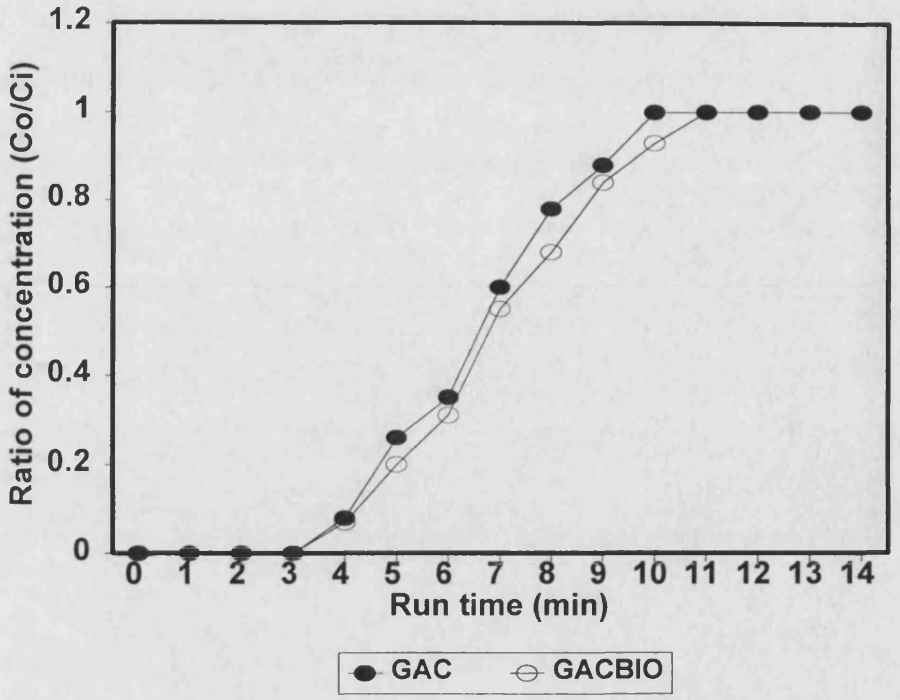


Figure 4.54 Chloroform adsorption breakthrough profile for GAC and GACBIO, with C_o outlet concentration of chloroform and C_i inlet concentration of chloroform

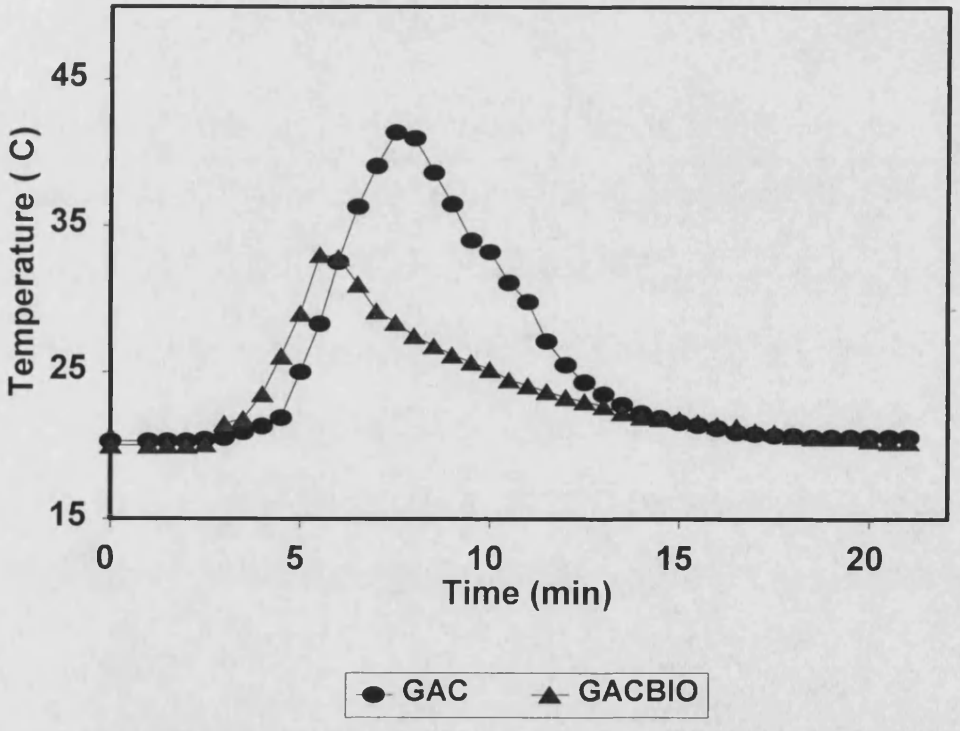


Figure 4.55 Chloroform adsorption exotherm temperature profile for GAC and GACBIO.

The peak temperature was reached in 7 to 9 minutes with GAC, and 4 to 5 minutes with GACBIO. This difference is likely to be due to differences in the surfaces and their interactions with chloroform. In the case of GAC, the overall rise in bed temperature was 18 to 20°C, whereas it was 10 to 12°C in case of GACBIO. This reduction in the exotherm could be attributed to the relatively less acidic surface of the GACBIO. For example, Tsutsumi *et al.*, (1993) reported a high heat of adsorption for GAC with an acidic surface.

In vapour adsorption, after an initial rapid surface adsorption phase, the adsorbate molecules start filling the pores of the adsorbents (Ruthven, 1984). Thus, the structural integrity of the adsorbent, as well as surface interactions, is an important aspect of adsorption. These surface interactions (molecular movement) are a function of temperature, as well as the GAC's surface properties (Bansal *et al.*, 1989; Tsutsumai, 1993). As a result of a high exotherm temperature, molecules of chloroform may be loosely held onto the adsorbent and they may flux back into the air. Considering the low boiling point of chloroform (60°C) and the heat of the exotherm, these molecular interactions were expected to be relatively vigorous in the virgin GAC.

The interaction of chloroform with adsorbents can be considered on the basis of molecular movements and temperature interaction.

(i) The virgin GAC had a higher pore volume than the GACBIO (Section 4.9.4), which should provide a greater volume for chloroform penetration. On the other hand, vapours inside pores may escape at a high pressure due to liberated heat. Such vapours escaping out from the pores of adsorbents would encounter the incoming fresh

molecules. These escaping vapours may therefore obstruct the adsorption of incoming chloroform molecules into the GAC.

(ii) As a VOC is adsorbed, the heat of adsorption can cause the GAC temperature to rise in the active adsorption zone. This heat is transferred to the surrounding air, which carries it further into the bed, rising the GAC temperature in advance of the adsorptive process. The hot zone thus proceeds through the adsorption bed, ultimately causing an appreciable increase in the temperature of the exit vapour and a corresponding decrease in the bed capacity.

However, both, hypothesised molecular activities and temperature effects discussed above were not reflected in any significant increase in adsorption capacity of chloroform over GACBIO. This is probably due to changes in the surface properties by the presence of heat fused and oxidized biofilm.

The biofilms used in adsorption were dried and fixed onto GAC in an oxidizing condition (air) at 230°C. Mattson and Mark (1971) reported the different surface properties (electrophoretic mobility and degree of hydrophobicity) of sugar based carbon activated at high temperatures to those activated at lower temperatures and in oxidizing conditions. Similarly, You *et al.*, (1994) also reported that presence of surface oxides reduce the adsorption capacity of GAC for n-hexane.

Nevertheless, in this work it was found that the biofilm on GAC can reduce the exotherm temperature, without affecting chloroform adsorption capacity. The reduction in the exotherm could be due to, (i) different surface properties of GACBIO (Section

4.9.3) resulting in reduced heat of adsorption, and/or (ii) different integral heat capacity of the GACBIO, which can be accounted for in the heat balance over the system, as indicated in Equation [4.11] (Kovach, 1988),

$$Q_h = (GC_a + MC_c + VC_v)\Delta t \qquad (4.11)$$

Where: Q_h is heat of adsorption, kcal/kg; G, weight of adsorbed adsorbate, kg; C_a, specific heat of adsorbate, kcal/kg; C_c, specific heat of adsorbent, kcal/kg; C_v, specific heat of carrier gas, kcal/m^3; M, weight of adsorbed adsorbate, kg; V, volume of carrier gas, m^3; and Δt is rise in temperature °C. In Equation [4.11], only two terms can differ between GAC and GACBIO, these are Q_h (heat of adsorption) and C_c (heat capacity of adsorbent).

4.10.2 Effect of metals on chloroform adsorption capacity of heat fused biofilm-GAC

The effect of metals (which were fixed onto the GAC by using biofilm) on chloroform adsorption was considered. In order to avoid an oxidation effect, the GAC-Biofilm-metal was regenerated at 230°C in an inert (N_2) atmosphere. Virgin GAC and GACBIO were also exposed to similar regeneration conditions. Cadmium, copper, nickel and zinc were fixed over the GAC by biofilm sorption, as described in Section 4.2. The metal levels per gram of GACBIO-M were 1.55, 1.65, 1.42 and 1.57 mg of cadmium, copper, nickel and zinc, respectively. The presence of metal on GACBIO-M was confirmed by EDS (Section 4.2.3). Chloroform adsorption tests were then carried out under the conditions described in Section 4.10.1.

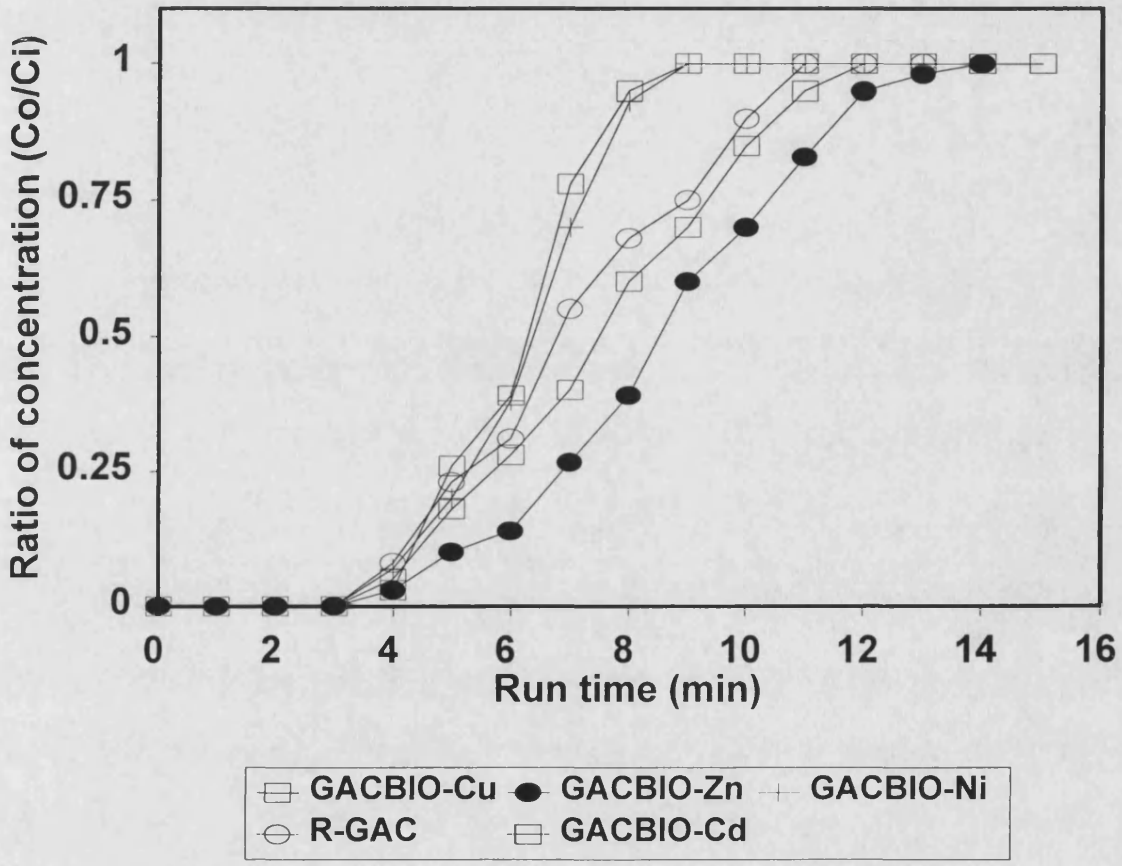


Figure 4.56 Breakthrough profile for chloroform adsorption with biosorbed Cd, Cu, and Zn over GAC

The adsorption capacity of GACBIO regenerated in inert condition was increased by 4 to 5 % compared to R-GAC (virgin GAC exposed to an inert regeneration conditions). The fall in adsorption temperature was again observed in case of R-GACBIO (regenerated in nitrogen). On the other hand the R-GAC showed a further 3°C increase in temperature and small (2 to 3%) increase in adsorption capacity, compared to virgin GAC. This rise in adsorption capacity could be due to the re-opening of "aged" pores of the GAC, (as GAC may oxidize during transport and with time, You *et al.*, 1994).

The presence of heavy metals (cadmium, copper, nickel and zinc) in GACBIO-M did not affect the exotherm reducing characteristics of GACBIO. The breakthrough curves of chloroform adsorption on GACBIO-M containing four different metals (as a single metal) are illustrated in Figure 4.56. The chloroform adsorption capacities in the presence of cadmium and nickel were decreased by more than 10%, when compared with R-GAC. In the presence of copper, capacity increased by 5 to 6% (including 4 to 5% due to the biofilm). The presence of zinc gave a 15 to 18 % increase in the adsorption capacity. These results signify that the metals fixed through biosorption process have much variable influence on the surface activity of GAC/biofilm system.

Use of impregnated GAC with copper is well known in adsorption of toxic vapours such as methanol, methyl formate, acetaldehyde and phosgene (Afzal *et al.*, 1993; Henning and Schäfer, 1993). A significant increase in adsorption capacity could therefore be expected with GACBIO-Cu. However, in this study, GACBIO-Cu did not display any significant advantage in terms of adsorption capacity for chloroform when compared to R-GAC. The copper-vapour interaction may have been affected by the

nature in which copper was available over the GACBIO surface. Copper has significant ability to form complex with polysaccharides (Brown and Lester, 1979). Geesey and Jang (1990) reported a preferential binding sequence: Cu > Ni, Co > Zn > Mn with polysaccharides. In this research, copper was also biosorbed preferentially over, cadmium, zinc and nickel. This complex formation may change the ionic activity of copper and its accessibility for interaction during chloroform adsorption. Similarly, the state of copper has been reported as an influential factor in interaction with vapours. For example, Brown *et al.*, (1989), found GAC impregnated with Cu^{++} was more effective in hydrogen cyanide adsorption as compared to Cu^{+}. In the case of cadmium and nickel, their is no reported beneficial interaction in vapour adsorption.

Zinc is also well known for its catalytic activity over GAC surfaces (Catalyst Chemical Europe, 1983; Afzal *et al.*, 1993; Henning and Schäfer, 1993). For example, GAC with 0.1% (w/w) Zn is used industrially for the removal of sulphur from hydrocarbon feed streams (Catalyst Chemical Europe, 1983). Similarly, the use of zinc and copper together, over the surface of GAC, has been reported for hydrogen cyanide adsorption (Rossin and Morrison, 1993).

The breakthrough profiles of chloroform appear to be different for each metal tested. In the case of zinc, after the breakthrough point, the curve profile was relatively dispersed rather than showing a sharp rise up to an equilibrium. Ruthven (1984) suggested that these types of dispersive breakthrough curves are due to a non-linear nature of adsorption. Thus, in both adsorbents (R-GAC and GACBIO-Zn), it appears that in addition to external mass transfer resistance, there may be two diffusional resistances from internal macropores and micropores. In the case of GACBIO-Zn,

resistances may be lower. This could be due to a decreased micropore volume. Thus, the interaction between chloroform and zinc can be increased as the chloroform would be held on the surface for a longer time.

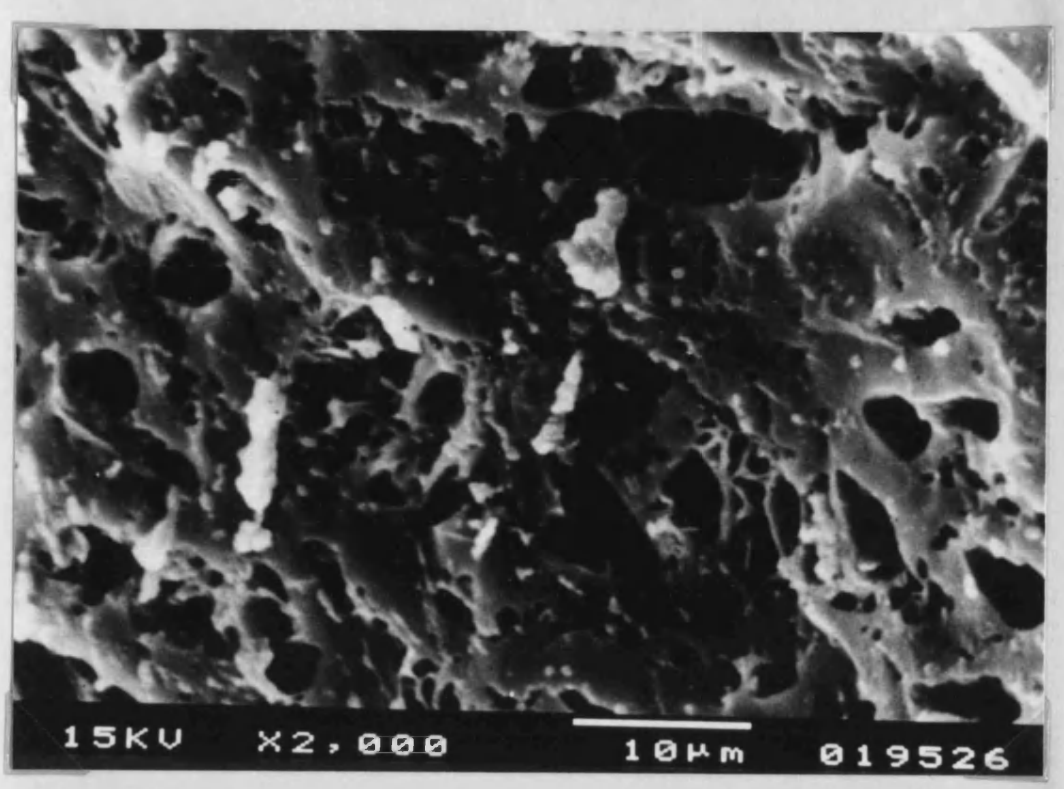


Figure 4.57 SEM photograph of heat fused biofilm over GAC (230°C for 3 hours in N_2)

The phenomenon of capillary condensation has been recognised as a significant factor in adsorption capacity. According to Bansal *et al.*, (1988), the adsorption first occurs at the most constricted portions of the adsorbents, where adsorption potential is likely to be the highest. Thus, the heat dried biomass distributed over the surface (SEM photograph in Figure 4.57) can increase the sites of adsorption. As the thickness of the adsorbed layer increases, this results in capillary condensation. Once liquid droplets of chloroform are formed over the surface of adsorbent, they would be revaporised

into the air stream depending on surrounding temperature. The capillary condensation of chloroform and lower surrounding temperature in the bed of GACBIO-Zn (rather than the R-GAC), can result in increased interaction between zinc and chloroform. This interaction may favour chemisorption.

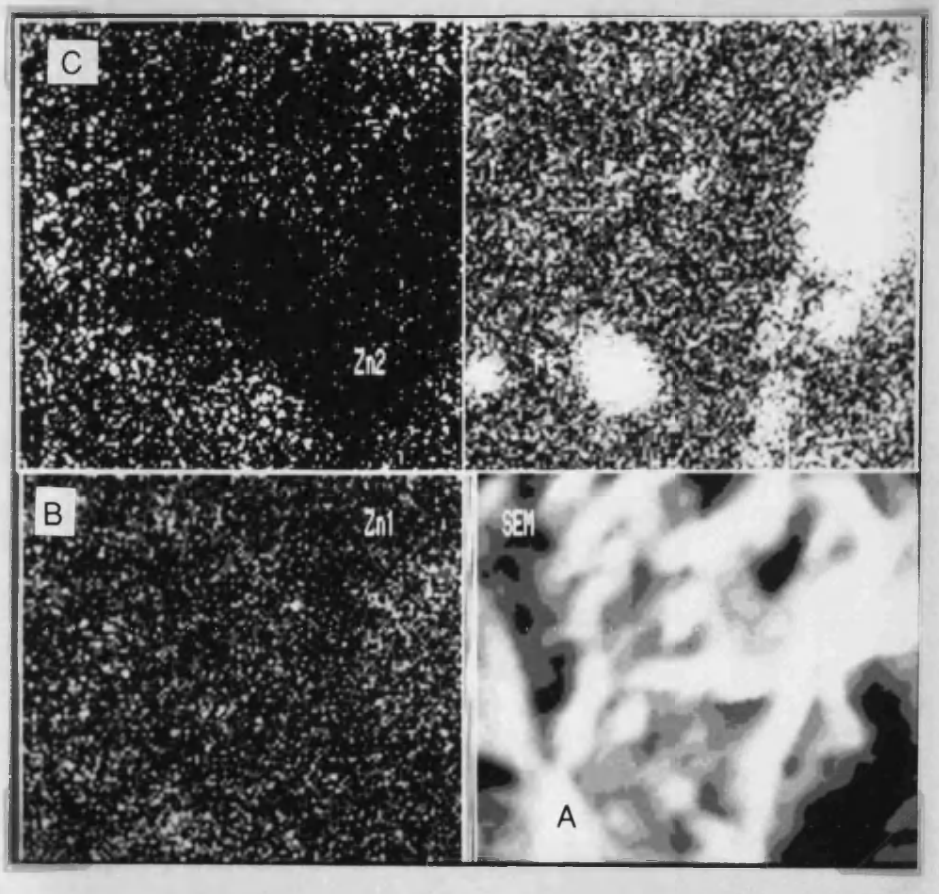


Figure 4.58 Distribution of zinc (from 25 mg/L) on biofilm-GAC. [A: SEM photo, B: zinc distribution on same area as A, C: zinc distribution on different area]

Bansal *et al.*, (1988), Rossin and Morrison (1993) and Brown *et al.*,(1989) all reported that when GAC is impregnated with metals or other chemicals, physical adsorption of vapours changes to chemisorption. In this research, therefore, the chloroform may have adsorbed through chemisorption on to the GACBIO-Zn, because zinc accumulation over the GAC could be simple attachment to the biofilm, rather than complex

formation. Further, it was found that zinc was well dispersed over the GAC (EDS Micrograph, Figure 4.58). Therefore, zinc may be easily accessible for interaction with chloroform promoting chemisorption, resulting in the observed increased adsorption.

Clearly, surface properties of the GAC are the important elements which influence adsorption capacity. Therefore, another contributing factor for the higher adsorption capacity of the GACBIO-Zn, could be increased surface basicity. As a result of a more basic surface, the electrostatic interaction between the adsorbate and adsorbent changes, which could be favourable for interaction between GACBIO-Zn and chloroform.

In the following sections, the increased adsorption capacity of GACBIO-Zn for chloroform is discussed. Various factors such as increasing zinc levels on the GAC, use of zinc on virgin GAC and different concentrations of chloroform and their effects on the kinetics of adsorption were assessed.

(I) Effect of the level of zinc over GAC on chloroform adsorption capacity

In Section 4.2, it was shown that zinc levels on GAC can be increased by exposing biofilm-GAC to a high initial concentration of zinc solution. From the six samples tested, three were R-GAC at 0.68, 1.2 and 1.35 mg of Zn /g of GAC, and the remaining three were of GACBIO-Zn at 1.57, 4.0 and 4.57 mg/g biofilm-GAC. All the samples were regenerated under an inert atmosphere at 230°C before using them for adsorption studies. The other adsorption conditions were maintained identical to those described in Section 4.9.1

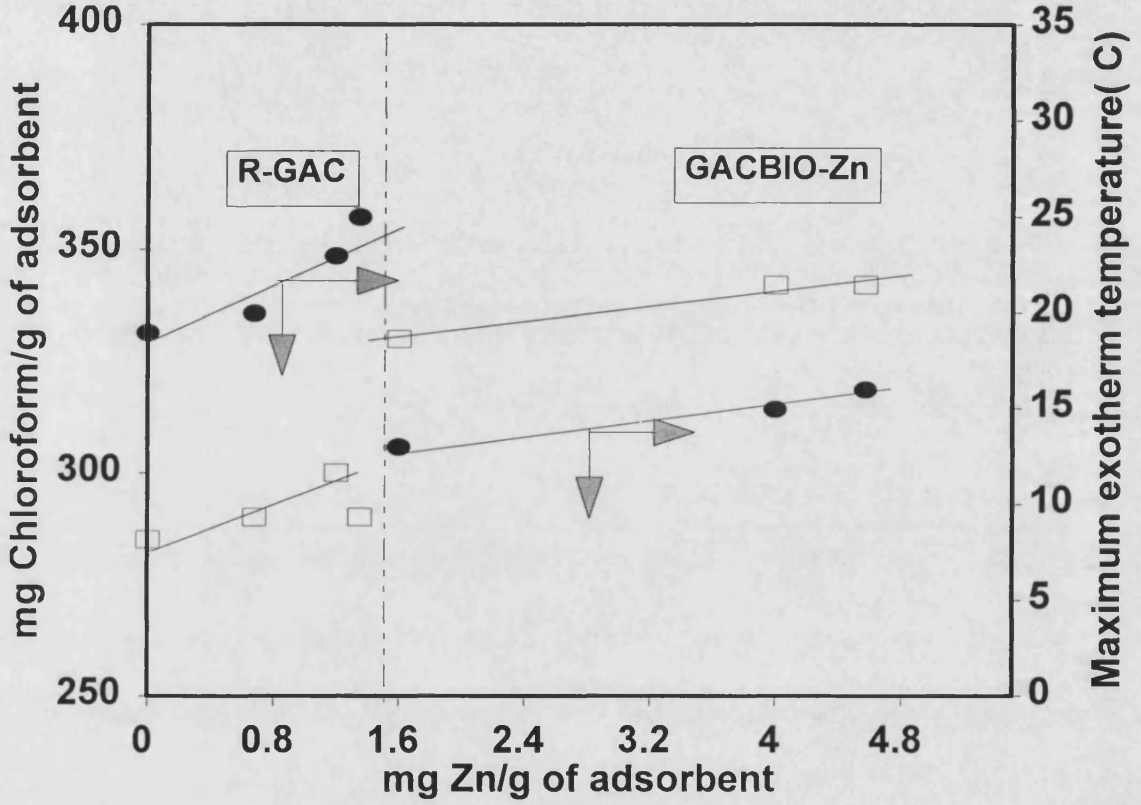


Figure 4.59 Effect of level of zinc on chlorofrom adsorption capacity of GACBIO-M

Figure 4.59 illustrates the effect of varying quantities of zinc on chloroform adsorption capacity and maximum rise in bed temperatures. The left hand part of Figure 4.59 shows R-GAC without any biofilm. A 7% rise in adsorption capacity was observed with 1.2 mg Zn/g of GAC. However, the bed temperature increased with increasing zinc content on adsorbent. A further increase of zinc from 1.2 to 1.35 mg/g on R-GAC resulted in a high heat of adsorption, such that the bed temperature was close to the boiling point of chloroform (60°C), and hence no significant increase in adsorption capacity was observed.

Table 4.20 Effect of zinc on chloroform adsorption capacity and temperature rise

Metal Content mg/g	Rise in Temp. °C	Adsorption Capacity g/g
Adsorbent: GAC		
0	19	0.285
0.68	20	0.290
1.20	23	0.300
1.35	25	0.290
Adsorbent: GACBIO-Zn		
0	13	0.286
1.57	13	0.330
4.00	15	0.342
4.52	16	0.342

The right hand part of Figure 4.59 shows the adsorption capacity and exotherm temperatures for GACBIO-Zn. An increase in the amount of biosorbed zinc from 1.57 mg/g to 4 mg/g resulted in a further 4% increase in adsorption capacity. However, the net increase in adsorption capacity of GACBIO-Zn was 18% more than R-GAC. Increasing biosorbed zinc to 4.57 mg/g did not increase adsorption capacity of

GACBIO-Zn any more, although, with increasing zinc content, rise in bed temperature was small amounting to 3 to 4°C beyond the bed temperature showed by GACBIO-Zn at 1.57 mg Zn/ g biofilm-GAC. Table 4.20 presents adsorption capacity and exotherm temperature of R-GAC and GACBIO-Zn at the different zinc levels.

These results confirm that the presence of zinc over GAC enhances chloroform adsorption capacity. The increase in zinc on both (R-GAC and GACBIO-Zn) adsorbents resulted in an increase in the bed temperature. The existence of heat fused biofilm reduced the exotherm temperature, possibly to allow better zinc interaction with the chloroform. Thus the biofilm and zinc were complimentary to each other.

The marked increase in adsorption capacity was strongly influenced by the diminishing exotherm. On the other hand, the degree of heat of adsorption is related to concentration of adsorbate. In the following section, the increase in adsorption capacity was evaluated, therefore, by varying the solute concentration.

4.10.3 Effect of chloroform concentration

The influence of concentration on adsorption capacity of GACBIO-Zn and consequently bed temperatures was assessed. Chloroform influent concentrations were 30, 73, 105 and 110 $\mu g/cm^3$ in air. The different concentrations were obtained by varying the air flow rate, as described in section 3.9. The superficial velocities of air varied from 1.11 to 2.78 cm/s. Rehermann and Jonas (1978) reported that within superficial velocities of 2 to 60 cm/s, adsorption capacity varied by only 3 to 4%). In all the following experiments, the GACBIO-Zn used was with 1.57 mg Zn/g of biofilm-GAC.

The chloroform adsorption capacities of R-GAC and GACBIO-Zn at various concentrations of chloroform are illustrated in Figure 4.60 and in Table 4.21. These data indicate that the presence of zinc in GACBIO-Zn has an influence on adsorption capacity. The adsorption capacities, irrespective of concentration, were consistently higher in the case of GACBIO-Zn.

Table 4.21 Effect of different chloroform vapour concentrations on adsorption capacity and exotherm temperature rise

Chloroform in Air μ/cm^3	Adsorption Capacity (mg/g)		ΔT°C	
	R-GAC	GACBIO-Zn	R-GAC	GACBIO-Zn
30	54	66	12	7
73	132	154	15	10
105	252	282	17	12
110	285	330	19	13

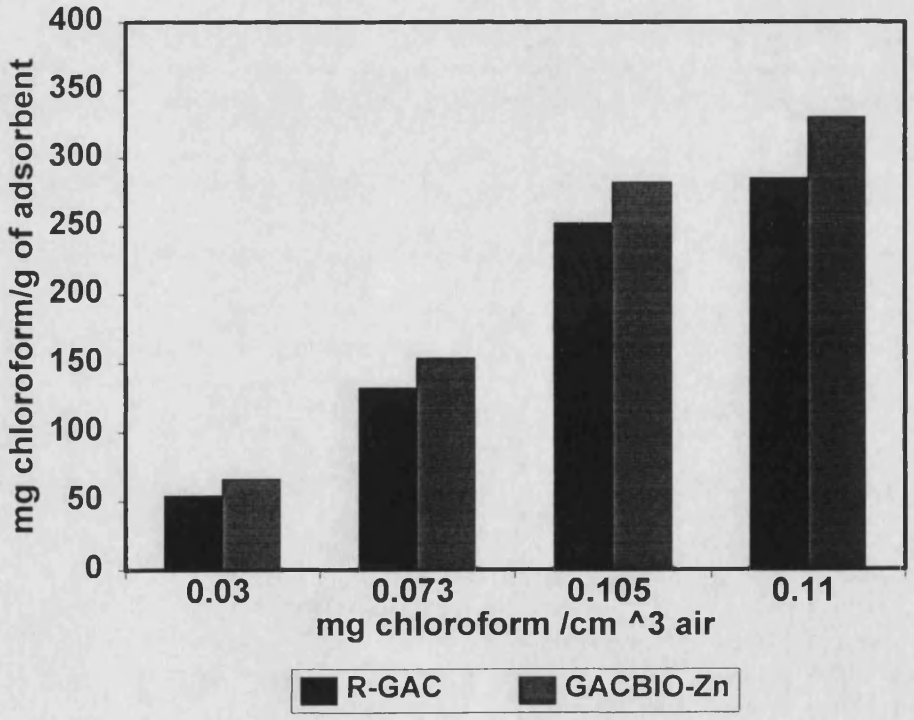


Figure 4.60 Chloroform adsorption capacities of R-GAC and GACBIO-Zn (1.56 mg Zn/g of GACBIO) at various concentration of chloroform in air (at 20°C)

4.10.4 Kinetics of chloroform adsorption over GAC with biosorbed metal

Adsorption of chloroform was found to be influenced by the presence of zinc over the GAC as well as GACBIO. To understand the effect of zinc on the nature of adsorption, the adsorption data on two adsorbents, R-GAC and GACBIO-Zn (1.57 mg Zn/g of biofilm-GAC), were evaluated by using well established models such as Wheeler and Dubinin-Polyani. The effect of zinc on adsorption rate kinetics and equilibrium capacity were assessed. From the outcome of these models, any changes in the nature of adsorption, *i.e.* from physical to chemisorption were considered.

(I) Adsorption kinetics and capacity

The dynamic adsorption behaviour of GAC and GACBIO-Zn was analysed in terms of capacity and the first order rate constant. This analysis of vapour breakthrough in adsorbent beds is often done in terms of the modified Wheeler equation (Jonas *et al.*, 1979; Busmundrud, 1993). That is, a kinetic adsorption equation was derived from the continuity equation to give:

$$t_b = \frac{W_e}{QC_o}\left[M - \frac{\rho_b Q}{k_v}\ln\left(\frac{C_o}{C_x}\right)\right] \qquad (4.12)$$

where: t_b, is the breakthrough time, min; C_x the exit concentration with time, g/cm^3; C_o the inlet concentration, g/cm^3; Q the volumetric flow rate, cm^3/min; M the weight of carbon, g; ρ_b the bulk density of the packed bed, g/cm^3; W_e the adsorption capacity, g/g; and k_v, the first order rate constant, min^{-1}. This equation is considered to be valid for only small exit concentrations, since the equation predicts exponentially increasing exit concentrations with time. Busmundrud (1993), modified the equation to involve superficial velocity and bed height to predict the whole breakthrough curve. The modified equation is represented by:

$$\ln\left(\frac{C_x}{C_o - C_x}\right) = \frac{k_v C_o}{\rho_b W_e} t - kv\frac{L}{V_L} \qquad \textbf{(4.13)}$$

where: L is height of adsorbent bed, cm; and V_L, is superficial linear velocity, cm/s.

Accordingly, ln(C_x / (C_o -C_X)) plotted against time, as predicted by Equation [4.13] gives a straight line for both adsorbents (GAC and GACBIO-Zn) (Figure 4.61). The adsorption capacity (W_e) and rate constant (k_v) for GAC and GACBIO-Zn were then obtained from the plot and are given in Table 4.22

The values of rate constant and adsorption capacity obtained here are lower than those reported by Jonas *et al.*, (1979). However, VOCs adsorption capacities and rate constants have been known to vary with GAC.

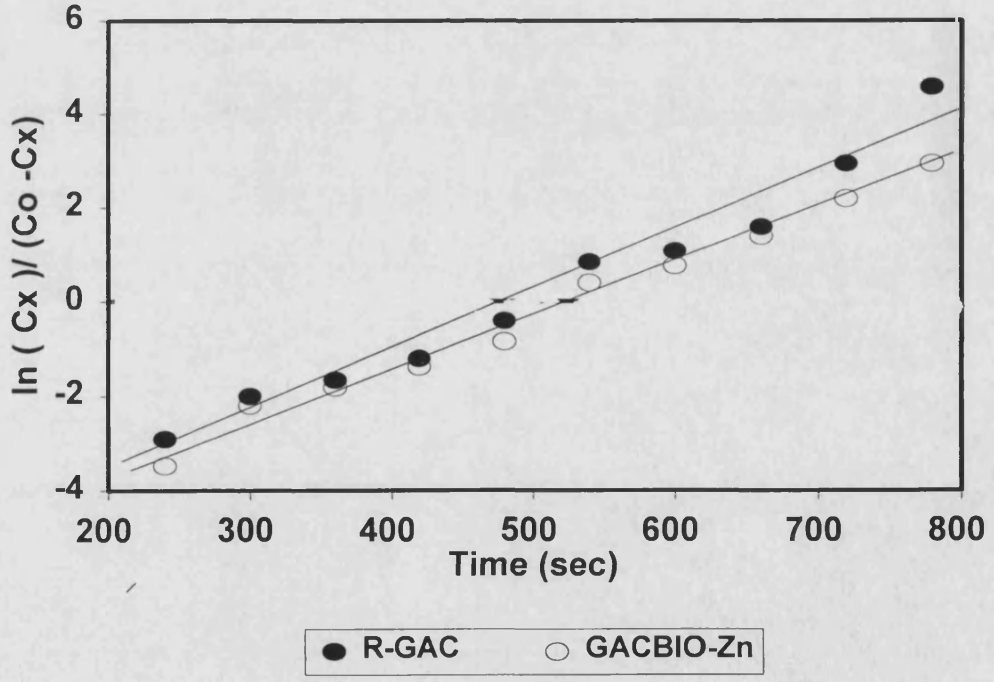


Figure 4.61 Chloroform adsorption kinetics, with R-GAC and GACBIO-Zn (1.56 mg Zn/g of GACBIO) at a chloroform concentration of 110 $\mu g/cm^3$ and 20°C

Table 4.22 Calculated adsorption capacities and rate constants for chloroform adsorption over GAC and GACBIO-Zn

Adsorbent	r^2	k_v (sec^{-1})	W_e (g/g)
R-GAC	0.96	2.56	0.53
GACBIO-Zn	0.98	2.46	0.58

Table 4.22 reveals that the rate constant was lower in the case of GACBIO-Zn than for R-GAC. Busmundrud, (1993) reported that the rate constant is reduced with increase in the contact time between adsorbate and adsorbent. Hence, the lower rate constant supports the observed increased interaction between chloroform and GACBIO-Zn. Similarly, the adsorption capacity of GACBIO-Zn was again found to be higher than that of R-GAC.

(II) Equilibrium adsorption isotherms

The key consideration in selecting and using GAC is the adsorption capacity. For preliminary evaluation, therefore, a predictive method (Dubinin-Polanyi) was used for estimation of adsorption capacity. The Dubinin-Polanyi model is based on adsorption potential theory. This model, in estimating the adsorption capacity, takes into account both pore volume of adsorbent and adsorbate properties, such as the molar volume and the surface tension (parachor).

The Dubinin-Polanyi equation for the GAC equilibrium VOC adsorption capacity X* was related to the vapour phase VOC concentration Y (Klobucar and Pilat, 1992; Nirmalakhandan and Speece, 1993). The Dubinin-Polanyi equation [4.14] described below, was subsequently used to relate the volume of chloroform adsorbed per unit

mass of adsorbent, to the vapour pressure of chloroform in the gas phase.

$$W = W_o \exp -k\xi^2 \tag{4.14}$$

where, the adsorption potential, ξ, is defined as:

$$\xi = RT \ln(\frac{P_o}{P}) \tag{4.15}$$

where: W is the adsorption space occupied by the condensed chloroform in (cm^3/g); W_0 is the maximum adsorption space available (cm^3/g); k is the constant related to the structure of adsorbent in $(cal/mole)^{-2}$; R, is the gas constant in (cal/mole °K); P is the equilibrium pressure of chloroform (mm Hg); and P_0 is the saturated vapour pressure of chloroform (mm Hg).

The term W can be defined as:

$$W\frac{(cm^3 chloroform)}{(g of GAC)} = \frac{(X^*)}{(\rho_c)} \tag{4.16}$$

where: X^*, g of chloroform/g of GAC; ρ_c, is density of chloroform (g/cm^3).

The value of the saturation pressure P_0 was calculated using the Antoine equation (Lange, 1988). The chloroform equilibrium partial pressure P can be related to the total pressure P_t by:

$$P = (\frac{MW_{air}}{MW_{chloroform}}) Y P_t \tag{4.17}$$

where: Y is the chloroform vapour concentration (g/g), MW_{air} and $MW_{chloroform}$ are molecular weights of air and chloroform (g), respectively.

Taking to natural log and substituting Equations [4.15], [4.16] and [4.17] into Equation [4.14] gives:

$$\ln\frac{X^*}{\rho} = \ln W_o - k[RT\ \ln\frac{(P_o MW_{chloroform})}{(YP_t MW_{air})}]^2 \qquad (4.18)$$

The experimental chloroform adsorption data was fitted into the Equation [4.18] and parameters k and W_o from the plot of W v/s [RT ln (P_o $MW_{chloroform}$)/ YP_t MW_{air}) (Figure 4.62) were obtained for R-GAC and GACBIO-Zn and are presented in Table 4.23.

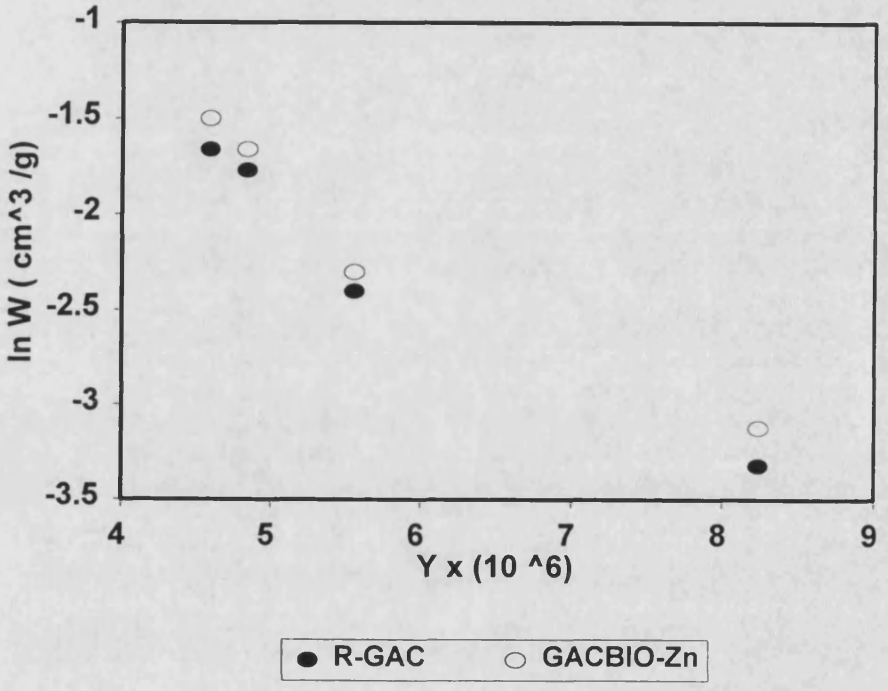


Figure 4.62 Plot derived from Dubinin-Polanyi model for chloroform adsorption on R-GAC and GACBIO-Zn (1.56 mg Zn/g of GACBIO)

The Dubinin-Polanyi model estimated 10% higher adsorption capacity on GACBIO-Zn. Thus, the model, which considers both adsorbent structure and chemical properties of adsorbate also confirmed the superior performance of GACBIO-Zn.

The outcome of this model can be used to estimate any changes in the chloroform-adsorbent interaction. That is, the affinity coefficient based on chemical properties of chloroform and k can indicate whether adsorption was changing towards chemisorption. Hence, any chemical effects present in addition to physical adsorption are also considered.

Table 4.23 Dubinin-Polanyi model prediction of chloroform adsorption over GAC and GACBIO-Zn

Adsorbent	r^2	k^a x10 $^{-7}$	W_o (g/g)
R-GAC	0.96	4.5	0.310
GACBIO-Zn	0.98	4.3	0.342

a: cal. $^{-2}$ mole $^{-2}$;

The dimensionless affinity coefficient (β_{th}) is a measure of the strength of the adsorptive interaction of the adsorbate vapour, relative to that of a reference adsorbate vapour. It has been defined as (Jonas *et al.*, 1979; Golden and Kumar, 1993; Bradley and Rand, 1993):

$$\beta_{th} = \left(\frac{\rho}{\rho_{ref}}\right)^{\frac{1}{2}} \qquad (4.19)$$

where: ρ is the molecular parachor of the adsorbate. The experimental affinity coefficient, β_{ex}, of a given adsorbate can be calculated with respect to that of a reference vapour, using the following equation:

$$\beta_{ex} = \left(\frac{k_{ref}}{k}\right)^{\frac{1}{2}} \qquad (4.20)$$

where: K and k_{ref} were parameters from Dubinin-Polanyi model,(cal. $^{-2}$ mole $^{-2}$). Chiou

and Reucroft (1977) reported that chemical interaction between adsorbate and adsorbent, (*i.e.* chemisorption), is generally expected to give rise to a higher β_{ex} than β_{th}. Table 4.24 presents both experimental and theoretical β values. These values of β were arrived at by choosing phosgene as a reference compound. Phosgene was selected as a reference vapour, because it showed chemisorption effects on adsorption onto GAC with impregnated metal (Chiou and Reucroft, 1977). The comparison of β_{th} and β_{ex} for both adsorbents indicates that the GACBIO-Zn has more affinity towards chloroform.

Table 4.24 Affinity coefficients

Adsorbent	β_{th}	β_{ex}
GAC	1.28	2.4
GACBIO-Zn	1.28	2.5

Reference vapour phosgene; $k_{ref} = 2.68 \times 10^{-8}$ (cal. $^{-2}$ mole $^{-2}$)

(Chiou and Reucroft, 1977)

Results discussed in the first seven sections of this chapter lead towards an optimized biofilm-GAC for sorption of different metals at a concentration range of 5 to 100 mg/L. Within tested concentration range the biofilm-GAC biosorbed metals preferentially to organic pollutants such as atrazine, benzene and butanol. Furthermore, it was shown that by repeated use of biofilm-GAC, either by biofilm regeneration or through a heat fixation cycle, the metal loading (cumulative) can be increased on the GAC. Conclusion drawn from this chapter are presented in chapter five.

It was shown that the metal recovered by biofilm-GAC system can possess catalytic activity. That is, biofilms not only bind metals, but also reduce the exothermic heat

in adsorption, modify surface properties and enhance the mechanical strength of GAC. The presence of zinc in particular, along with biofilm on GAC, enhanced the chloroform adsorption capacity of GAC by 15 to 18%.

Chapter 5

CONCLUSIONS

Biological means of heavy metal accumulation are now the focus of many research projects. Microbial systems such as fungi, yeast and bacteria have been successfully utilized in interaction with heavy metals. In general, such microbial systems were specific to the metals and micro-organisms employed. Initial research was dominated by use of live micro-organisms for biosorption, but there has been a shift towards use of immobilized biomass (dead or alive). However, very few industrial applications of biosorption systems have been reported.

Biosorption systems are a new concept for waste water treatment companies. A major drawback to exploit present biosorption systems has been the lack of properties *viz.*: (i) high metal accumulation capacities, (ii) reuse of the biosorbent, (iii) versatility to different environmental conditions and various metals and (iv) use as a continuous system. Furthermore, most existing systems only shift heavy metal contaminants (albeit concentrated) into another environmental phase, necessitating further treatment for true disposal of the recovered metal. Therefore, the search has continued for an efficient metal removal system, which could be easily incorporated into existing water treatment technologies.

This research has investigated a novel technique for heavy metal removal within the context of existing water treatment technologies. The objective was to develop a polysaccharide producing bacterial biofilm over granular activated carbon (GAC) to remove heavy metals from solution, and then to utilize the resulting GAC-biofilm-

metal in a vapour adsorption process. That is, making use of the metal(s) in an environmentally friendly way.

Current environmental clean up technologies handling organic effluent in gas and aqueous systems are dominated by GAC. Similarly, use of GAC as a support for catalytic metals is well established (Barnir and Aharoni, 1975; Chiou and Reucroft, 1977; Afzal *et al.*, 1993). GAC is well documented as one of the best available surfaces for the attachment of micro-organisms, due to high surface area and porosity, surface irregularities and mechanical strength. With this view GAC was selected in this research for attaching biofilm for metal sorption.

To address this objective, a number of studies were carried out which lead to the following findings.

From the three bacterial species (*Enterobacter aerogenes*, *Serratia marcescens* and *Pseudomonas* sp.) studied in the first part of this research, the Pseudomonad was found to have the best level of polysaccharide excretion. The biofilm of *Pseudomonas* sp. on the surface of GAC was extensive, but the coverage was non-uniform. The GAC surface appeared to reach a saturation state of biomass (cells and polysaccharide) attachment, even though the cells were growing and producing polysaccharides. This was as expected, as cells entrapped in the biofilm are known to utilize the polysaccharides for their survival (Annachhatre and Bhamidimarri, 1992).

The metal loading capacity of the biofilm-GAC was found to be in the following sequence:

$$Ag^{+} > Cu^{+2} > Zn^{+2} > Cd^{+2} > Ni^{+2} > Cr^{+3}$$

Although uptake efficiency of the biofilm-GAC decreased in the order of copper, zinc and cadmium, their final capacity (metal level) did not vary significantly. Most of the metal exposed to the biofilm was bound within 20 min, and almost no increase in bound metal occurred after 1 hour.

The biofilm-GAC systems developed sorbed different metals effectively from a multi-metal solution, although total metal removal and efficiency for each metal declined. Primarily, the sorption in multi-metal solution appears to be due to the existence of specific binding sites for each metal in the biofilm. However, in a multi-metal solution, all the metals sorbed were inhibited by other species as they also competed for common binding sites. The sorption efficiency of silver was the least affected by the presence of other cations. The preferential biofilm metal uptake sequence identified for multi-metal solution was the same as the uptake sequence of single metal solutions.

Metal sorption by the biofilm-GAC was found to be independent of the ionic properties of the metal, such as ionic radius, electron affinity, molar volume and ionization energy. However, with the exception of cadmium, the biofilm-GAC metal sorption in this research can be directly correlated to the cell-metal affinity constant and molecular weight of the metal.

Uptake rate followed first order kinetics for all metals tested. However, the identified rate kinetics sequence of metals,

$$Cu^{+2} > Zn^{+2} > Cd^{+2} > Ni^{+2} > Cr^{+3} > Ag^{+}$$

followed the metal loading capacity sequence except for silver. The metal sorption rates for cadmium, copper and zinc were very similar. The rates were found to be higher than those reported for GAC alone. High metal sorption rates observed in biofilm-GAC system can be beneficial in reduction of treatment time of metal containing streams.

The higher loading capacity of silver was through combination of uptake by the biofilm and GAC, which was dominated by GAC. However, biofilm acts as a barrier between the metal (silver) in solution and the GAC, making silver uptake rate relatively slow.

The adsorption models of Freundlich and Langmuir were found to have limitation in predicting the metal sorption ability of the biofilm-GAC. Except for silver, these models are only useful up to an initial metal concentration of 50 mg/L. Their inadequacy at higher concentrations is probably due, in part, to the basic assumption of monolayer adsorption. The biofilm used in this work consisted of a wide variety of surfaces and as such had a high metal sorbing potential in the form of multi-layer binding sites. In the case of silver, however, uptake is more a combination of GAC and attached biofilm. Hence, it showed a more linear relationship in terms of metal uptake over the tested concentration range. Prediction of these models (Langmuir and Freundlich) agree well with the preferential metal uptake capacity sequence obtained from experiments in this work.

Factors which influenced the metal sorption ability of this particular biofilm were:

(i) Biofilm related: age of the biofilm; growth temperature during biofilm attachment;

polysaccharide produced; fluidization velocity.

(ii) Metal related: type of metal; metal concentration; pH of aqueous metal solution; presence of competing metals; temperature of aqueous metal solution.

On the other hand, the metal sorption capacity was not affected by:

(i) presence of other toxic organic and inorganic chemicals (*e.g.* atrazine, benzene, butanol, nitrate and chloroform)

(ii) GAC surface properties

(iii) dissolved oxygen level (in growth medium) during biofilm attachment and growth.

Not surprisingly, metal sorption by the biofilm is influenced by the concentration gradient between the bulk solution and the biofilm. Higher metal sorption rates and loading capacities resulted from higher concentration gradients. Therefore, maintaining a constant metal concentration gradient in a continuous process was beneficial to achieve higher metal levels in the biofilm. Process transfer parameters, such as mass transfer coefficient and diffusion coefficient, were also influenced by concentration gradient and biofilm thickness.

The majority of previously reported metal sorption systems were sensitive to variations in pH of the metal containing solution. This sensitivity also existed in the biofilm-GAC metal sorption system. Both biofilm development over GAC and subsequent metal uptake were influenced by the pH of the culture and the metal containing solution. Despite the viability of cells in the pH range 3.2 to 9.5, lowering the pH of the metal containing solution to below 3.5, affected uptake. Hence, metal uptake by the biofilm appeared predominantly due to surface attachment, rather than cellular

interaction.

Increasing the pH of the culture medium, produced a more extensive biofilm over the GAC, which was in turn capable of sorbing more metal. The bacterial cultures also had a buffering effect, that is they would tend to neutralize either acid or base media. Furthermore, under alkaline conditions (pH 8), higher polysaccharide production has been reported (Ebube *et al.*, 1992), which may have contributed to increasing metal uptake by the biofilm-GAC in this work.

Irrespective of age, cells attached to the GAC surface and formed biofilm. But, culture age (up to 1 day) significantly affected metal sorption, as the excretion of polysaccharide by the *Pseudomonas* sp. did not become significant until after 24 hours. Biofilms developed from cultures containing cells in the stationary growth phase sorbed more metal than biofilm with cells in the logarithmic phase. Hence, there was little difference in metal uptake between biofilms which were 2 to 6 days old. Any initial precondition of the GAC surface, such as by adsorption of nutrients from the medium, was not essential as cells formed the biofilm in all viable stages of their life cycle. On the other hand, regardless of cell viability (live or dead), the biofilm-GAC system sorbed the metal, although their was a small decline in metal uptake after killing the cells. Enhanced cell growth due to increasing oxygen levels did not appear to increase polysaccharide production and hence metal sorption.

A successful biofilm for metal sorption was developed over a temperature range of 15 to 30°C with the *Pseudomonas* sp. It was observed that culture temperature had a significant effect on polysaccharide production and therefore on subsequent metal

uptake. The optimal temperature for biofilm development was found to be 25°C with respect to the biofilms ability to remove metal. Increasing the temperature from 25 to 30°C, a small increase in biofilm dry weight on GAC was found, resulting in a small rise in metal uptake. On the other hand, for temperature of metal containing solution in the range of 5 to 30°C, the biosorption efficiency (for zinc and cadmium) varied by 10%.

Extracellular polysaccharides excreted by the *Pseudomonas* sp. were the major contributors in the uptake of metals. Live cells in the biofilm played a minor part (25 to 30% of the total uptake) in the metal sorption, but they were more essential in the attachment and development of polysaccharides based biofilms over the GAC and maintaining the film integrity. The biofilm attachment and growth over the GAC withstood shear from the circulating growth medium. However, optimal biofilm development fluidization velocity with respect to metal uptake capacity was below 2.4 cm/s.

The biofilm adapted and functioned with equal effect on GAC (i) which had undergone thermal regeneration at 900°C, (ii) varied in size, or (iii) obtained from a different supplier. Because of this adaptability, the biofilm-GAC metal sorption process should be easily incorporated into most existing waste treatment technologies that are based on GAC.

The selectivity of the biofilm was very much towards metals as compared to organic compounds such as butanol, benzene and atrazine. The high metal decontamination efficiency of the biofilm persisted in the presence of organic compounds. However,

the biofilm interfered in the adsorption of atrazine and metals interfered in the adsorption of butanol.

Increasing net metal levels over GAC was achieved by repeating biofilm-GAC metal sorption system over same GAC. This was carried out by either regenerating the metal saturated biofilm, or by heat fixing the metal and biofilm over GAC, before exposing it to a fresh culture. The final metal loading (after six cycles) through regeneration of the biofilm was 40% less than those achieved by the initial metal fixation cycle. But, biofilm regeneration offers other advantages, such as lower down time in the plant, and lower energy cost. A metal fixation and GAC recycling system can be employed with removal of organic pollutants. For instance, such a cycle would involve adsorption of atrazine by virgin GAC, subsequent development of a biofilm, followed by metal uptake and metal fixation.

Integration of metal sorption by the biofilm with removal of other toxic pollutants by the GAC, is one of the most significant potential advantages offered by this process. In spite of a high concentrations of atrazine or chloroform, biofilm formation and metal uptake was sustained. Therefore, it may be possible to incorporate micro-organisms with the potential to interact and degrade adsorbed organic pollutants, during the biofilm formation.

This preliminary study on biofilm development on exhausted GAC, leads to biological regeneration of GAC. At the same time, through this process and subsequent use of fused biofilm-metal-GAC extends the potential of GAC as an adsorbent. Conventional methods of regeneration use either high temperature or chemical reagents, which

essentially transfer the pollutants into another phase of the environment *e.g.* in thermal regeneration of GAC, adsorbed organic pollutants are transferred into the air. Hence, the process adopted here could offer potential benefits over other regeneration methods.

Present drawbacks of the biofilm-GAC metal sorption system are (i) the time needed to obtain a suitable biofilm over the GAC surface, (ii) formation of biofilm in batch process, and (iii) its being non-selective with respect to metals (although at different efficiencies).

As an active metal recovery system, biofilm-GAC fulfils the following criteria endorsed by Volesky (1990).

(i) Uptake of the metal is efficient and rapid. The biofilm-GAC system can be used for different metals.

(ii) The active biosorbents can be produced at low cost and are reusable.

(iii) The biosorbent material has the desired particle size, shape and mechanical properties for use in a continuous system.

A novel approach adopted in this work was not only to recover heavy metals from the waste water but also to employ them in adsorption of volatile organic compounds (VOC).

GAC is a key element in a number of environmental control technologies designed to capture volatile organic compounds from air. This is due to its characteristics properties, which include very high surface area and porosity. Therefore effect on these properties due to the heat fixed biofilm was evaluated.

The biofilm system used in this work successfully attached and dispersed metals over the GAC surface. During metal fixation, the useful properties of GAC such as surface area and porosity were affected by 3 to 5% and 12 to 14%, respectively. This is one of the benefits offered by this method of metal fixation, compared to other techniques (adsorption) of metal ion impregnation. For example, when Afzal *et al.*, (1993) adsorbed cadmium, zinc, nickel and copper from solution on GAC, they observed a greater decrease in surface area (5 to 20%) and porosity (15 to 30%). With respect to metal level, up to 0.4 to 0.5% (w/w of GAC) can be fixed with a biofilm over GAC by varying the initial concentration of exposed metal.

The presence of biofilm over GAC improves its surface homogeneity and can offer a uniform surface for adsorption. The polysaccharides in the biofilm not only improved metal sorption, but may also have contributed to improving the attrition resistance of the GAC. This could be due to the adhesive properties of the polysaccharides. In addition, the basicity of the GAC surface increased due to the presence of a particular biofilm.

The most favourable effect of the biofilm on the GAC was in reduction of exothermic heat generated during organic vapour adsorption. This reduction was observed without any loss of chloroform adsorption capacity. Thus, the heat capacity of the GAC can be favourably modified by the biofilm.

Copper attached to the GAC surface through use of the biofilm was less effective in vapour adsorption than that reported. Thus, the polysaccharides appeared to affect the overall effectiveness of the metal, due to polysaccharide-copper interaction.

The adsorption of chloroform on GAC, however, was increased by the presence of bound in zinc, but the nature of the processes involved is not fully understood. It is generally believed that adsorption of VOCs on virgin GAC is essentially physical, whereas adsorption on metal laden GAC involves chemisorption. The adsorption of chloroform on GAC-biofilm-zinc was assessed by the Dubinin-Polanyi approach. An increased interaction (higher rate constants) and chemical affinity was indicated between chloroform and GAC with fused zinc and biofilm. Increased adsorption observed here could be due to the different adsorption thermodynamics. For example, the strong adsorbate-adsorbent interactions observed here, can result in lower free energy, enthalpy and entropy due to the presence of metal over the GAC (Afzal *et al.*, 1993; Chiou and Reucroft, 1977; Huber *et al.*, 1978).

Along with environmentally friendly metal recycling, the GAC-biofilm can offer a solution to the following drawbacks of virgin GAC as a vapour adsorbent:

(i) With increasing VOC concentration in the air stream, heat generated in the adsorption bed not only decreases the adsorption capacity of the GAC, but also can be a fire hazard. Therefore, GAC has limited use with low boiling point VOCs and with high concentration of VOCs in air.

(ii) Regeneration of GAC is carried out at elevated temperature, so the GAC has to be returned to the supplier for regeneration.

(iii) Attrition of GAC restricts gas flow.

Finally, research reported in thesis has successfully demonstrated the use of bacterial biofilm over GAC for the removal of heavy metals and recycling of these metal along with GAC in the existing process of VOC adsorption.

5.1 Further Work

As a result of work presented in this thesis, further investigation can be recommended both in terms of fundamental aspects (*e.g.* control of biofilm thickness or creating biofilm of known specific groups) and process development (*e.g.* rapid development of biofilm).

(I) Polysaccharides for biosorption

In biofilm-GAC systems, the role of polysaccharides was predominant in overall metal removal. The excretion of microbial polysaccharide varies with pH, temperature and dissolved oxygen. The majority of reported studies, on polysaccharides have focused on yield maximization, rather than the chemical composition. Hence, the following investigation should be carried out for enhancing polysaccharide role in metal biosorption:

(a) It was observed that the matrix-like capsular coating varied with different runs with the same bacterial species. The crystal structure and degree of polymerization, or chain length of polymer, would appear sensitive to small variations in parameters such as pH, temperature *etc.* The nature of produced polysaccharides could be analysed by using X-ray crystallographic techniques in order to identify the polysaccharide structure. Polysaccharides required for biosorption can be obtained by optimizing pH, temperature, flow rates and composition of growth medium.

(b) The chemical composition of polysaccharides under the influences of pH, temperature and growth medium composition should also be investigated (by HPLC analysis). Metal biosorption could then be correlated to chemical composition of the

polysaccharide. Revealing interactions of specific chemical groups from the polysaccharide with metals would enhance metal removal. Similarly, it would assist in selecting specific species, growth medium and pH and temperature conditions for a particular metal.

(II) Types of biofilm

The metal uptake data gathered in this research was apparently from a biofilm with both specific and general metal binding sites. To achieve high metal sorption, it may be possible to develop a metal specific biofilm. On the other hand, from economy point of view two types of biofilm should be investigated, (i) common biofilm capable of sorbing different metals (ii) natural biofilm, developed with activated sludge.

(a) Selective biofilm: Although the biofilm of the Pseudomonad studied interacted with six different metals, a more metal selective biofilm could be more effective when an effluent contains only one metal. These interactions can be made selective in a biofilm by incorporating species which are know to have a strong affinity to a specific metal. For example, *Rhizopus arrhizus* for uranium (Tsezos, 1983) or Penicillium for lead (Niu *et al.*, 1993).

(b) Common biofilm: This research has demonstrated that the metal sorption performance of biofilm-GAC was greatly enhanced by excreted polysaccharides. Investigated should be the best method by which polysaccharides along with cells can be concentrated and optimized for their attachment onto the GAC.

(c) Natural biofilm: Biofilms used in this research were more engineered than natural.

That is, they were obtained under conditions of well defined growth medium, controlled pH, temperature and flow conditions. Biological growth over GAC has been observed as a natural phenomenon in waste water treatment plants. These natural biofilms should be investigated for metal sorption ability. The metal sorption performance of these biofilms may be enhanced by exposing them to specific micro-organism. Such a biofilm development process would help introduction of biofilm-GAC biosorption systems into existing waste water treatment without major modifications.

(III) Mixed bacterial culture

In Section 4.8, it was shown that GAC previously used to adsorb pesticide or chloroform can be subsequently used for biofilm development. However, the *Pseudomonas* sp. used in this work failed to degrade atrazine and the uptake level of chloroform was low. Biofilms with mixed-cultures should be investigated for effective utilization of biofilm-GAC for combined metal biosorption and organic pollutant degradation. For example, the benzene utilising bacterium, *Acinectobacter calcoacetucus* (Smith, 1990) could be used along with the Pseudomonad for metal sorption. However, factors such as cell-cell interaction should be investigated before selecting the species. It is possible that one of the species will dominate over the other in attachment and cellular growth. Therefore, incompatibility of the species may adversely affect degradative or sorption process.

(IV) Pilot-plant studies

During this research, data was gathered on (i) efficient biofilm development, (ii) metal loading capacities and (iii) effect of environmental factors associated with a metal

containing stream. These laboratory data can be used to help scale up and evaluate the process under conditions more realistic of a water treatment plant. For example, it can be projected that a pilot plant column of 15 cm diameter, 1.3 m height and holding 10 kg GAC can be used to process around 100-150 L of a metal containing stream in 1 to 2 hours.

(V) Applications of GAC-biofilm-metal

In this research it was shown that heat treated metal-biofilm-GAC can be used for chloroform adsorption. This opens up a potential new research angle to metal-GAC catalyst systems. Such metal covering of GAC could be investigated for (i) adsorption of other organic vapours, such as benzene, phosgene, ethanol *etc.*, and (ii) catalysis of chemical reaction *e.g.* cyclohexane dehydrogenation over a carbon-platinum catalyst (Youssef and Ahmed, 1981).

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APPENDIX I

Publications

(i)"Heavy metal ion accumulation over activated carbon surface through biosorption"
Scott J.A., O'Reilly A., and Karanjkar A.M. (1992) In: *Waste Processes Recycling: Metal Waste Processing and Recycling,* (Ed. R.Rao) Pub.: CIM, Edmonton, Canada, pp 157-170

(ii)"Repeated cadmium biosorption by regenerated *Enterobacter aerogenes* biofilm attached to activated carbon"
Scott J.A., and Karanjkar A.M. (1992) *Biotechnol. Lett.*, **14**, pp 737-740.

(iii)"Combined metal biosorption and heat fixing for reclaiming metals and distributing them over granular activated carbon"
Karanjkar A.M. and Scott J.A. (1993) *Proc.I. Chem. E.Res. Event*, **2**,pp 399-401

(iv)"The use of biosorption to decontaminate streams and build up metal levels on the surface of granular activated carbon - influence of physical parameters"
Scott J.A., and Karanjkar A.M. (1994) *Progress in Biotechnol.*, **9**, pp 1231-1234

(v)"Organic vapour adsorption activity onto granular activated carbon (GAC) with biosorbed metal over the surface"
Karanjkar A.M., and Scott J.A. (1994) *Proc.I. Chem. E.Res. Event*, **1**, pp 377-380

(vi)"Exploitation of biofilm covered granular activated carbon for use in removal of metals from waste stream"
Scott J.A., Karanjkar A.M. and Rowe D.L. (1994) *Resources Conservation and Environmental Technologies in Metallurgical Industries*, (Eds. Mahant P., Pickles, C., and Lu, W.K.) Pub.: CIM, Montreal, Canada, pp 345-356

(vii)"Biofilm covered granular activated carbon for decontamination of streams containing heavy metals and organic chemicals"
Scott J.A., Karanjkar A.M. and Rowe D.L. (1994) *Minerals Engineering* (In press)

APPENDIX II

(A) Diffusion coefficient

The relationship between diffusion coefficient and the amount of adsorbate over GAC has been described by Suzuki(1990) as:

$$D_s = D_o(a_o q)^n$$

where, D_s is effective diffusion coefficient (cm^2/sec); D_0 is diffusion coefficient (cm^2/sec); a_0 is a constant (g/mole); q is equilibrium amount adsorbed, (mg/g or mmole/g) and n is reciprocal exponent of Freundlich isotherm. That research, however, was based on organic compounds adsorption onto the GAC and did not involve studies with biofilm.

In the case of adsorption of propionic acid onto GAC, Suzuki (1990) reported that the diffusion coefficient increased from 4 X 10^{-10} to 4 X 10^{-9} (m^2/sec) by increase in equilibrium adsorption (q) from 0.4 to 0.8 mmole/g. Similar increase in diffusion coefficient with increasing amount adsorbed has been reported by Sudo *et al.*, (1978) achieved again with organic compounds onto the GAC (chlorophenol-GAC, benzoic acid-GAC and d-chlorophenol-GAC). However, literature does not reveal similar studies involving heavy metals and GAC (with or without biofilm). Therefore, the effect of metal concentration and biofilm thickness on diffusion coefficient observed here are particular to this research.

Figures 4.28 and 4.29 show the effect of metal concentration and biofilm thickness on diffusion coefficient. Such a dependency of diffusion coefficient can still be explained on the basis of activation energy and amount of metal adsorbed as shown in equation described above. Amount of metal sorbed by biofilm-GAC increased with an increase

in initial metal concentration as shown in Figure 4.10. Hence, the result achieved with biofilm-GAC agree with the trends in diffusion coefficient reported earlier for organic compounds over GAC.

(B) Fluidization velocity

Several solid-liquid fluidization correlations have been proposed for determining minimum fluidization velocity (Kunni and Levenspile, 1969; Perry and Green, 1984; Coulson and Richardson, 1991). Most of these correlations are based on voidage at minimum fluidization condition, the voidage being a function of particle shape and size.

Accordingly, Kunni and Levenspiel (1969) developed the following correlation for u_{mf} (minimum fluidization velocity, cm/s) and u_t (terminal velocity, cm/s) based on the first principle:

$$u^2_{mf} = \frac{d_p(\rho_l - \rho_s)\, g}{24.5\rho_l}$$

and

$$u_t = \left(\frac{0.017(\rho_s - \rho_l)^2 g^2}{\rho_l \mu}\right)^{0.33} d_p$$

where: d_p is mean particle diameter (cm); ρ_l and ρ_s are liquid and solid density, respectively, (g/cc); g is gravitational acceleration constant (cm/sec^2) and μ is fluid viscosity, (g/cm sec). The constant terms (24.5 and 0.017) in the above correlations correspond to spherical particles of mean diameter greater than 0.5 mm under uniform fluidization conditions.

In this research, following assumptions were made in the calculation of minimum and terminal fluidization velocities: (i) spherical shaped particles; (ii) mean diameter of GAC as 1.2 mm and (iii) uniform fluidization condition (regular and even expansion of bed). Therefore, above correlations for u_{mf} and u_t were used in the calculations.

The minimum fluidization velocity was found to be 1.46 cm/sec and terminal velocity as 8.23 cm/sec. These velocities (u_t and u_{mf}) signifies the operational range in which fluidized bed can be operated. However, these calculations did not take into account the effect of the biofilm growth over GAC and the breaking of particles.

(C) Non-uniform biofilm over GAC

The ability of a solid material to support biological growth on its surface depends, in part, on the roughness of surface. Under the conditions used in this study, biofilm attachment and growth tended to be non-uniform (Section 4.1). The biofilm growth was found in the form of clusters in some areas and with part of the surface uncovered in other areas. The clusters occurred, in most cases, where the surface was rough or had cervices. On the other hand, the biofilm did not grow where the surface was relatively smooth. The variation in surface texture could have contributed to non-uniform attachment and growth of biofilm.

The ability of solid material to support biological growth is also partially dependent upon its adsorptive characteristics. GAC with distributed surface functional groups (see Section 4.6) could result in non-uniform adsorption of organic compounds from the growth medium. As a result, cell attachment tends to be confined to nutrient reach sites over the surface of GAC.

These were the two major factors considered to be responsible for the non-uniform growth of the biofilm over GAC surface.