EFFECT OF EXTRACELLULAR POLYMER COMPOSITION OF ACTIVATED SLUDGE ON THE REMOVAL OF HEAVY METALS BY BIOSORPTION

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ABSTRACT

EFFECT OF EXTRACELLULAR POLYMER COMPOSITION OF ACTIVATED SLUDGE ON THE REMOVAL OF HEAVY METALS BY BIOSORPTION

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Activated sludge microorganisms can remove many hazardous substances from wastewater by adsorbing and concentrating them on their surfaces. Biosorption of these substances onto activated sludge surfaces are influenced by the chemical properties of the substance in question as well as the surface properties of the microorganisms. The purpose of this study is to identify the biosorption mechanisms of heavy metals and the effect of extracellular polymer (ECP) composition of activated sludge on the biosorption of Pb(II), Cd(II), Cu(II), Zn(II) and Ni(II). Microorganisms cultured under different growth conditions are expected to have different compositions of ECPs and hence, different biosorption capacities. For this purpose, three sets of reactors with C/N ratios of 9, representing a carbon-limited case; 21, representing conventional municipal wastewater treatment plant activated sludge and 43, representing nitrogen-limited condition, were set up. The semi continuous reactors were fed synthetically and operated at a sludge age of 8 days.

Isotherm and kinetic experiments that were held with three different C/N ratios was indicated that the biosorptive capacity of activated sludge was highly dependent on metal species and the C/N ratio. Although, the dependence of biosorptive capacity on C/N ratio was different for each metal, biosorption properties of activated sludge were found to be directly related with ECP composition. Among the heavy metals tested, Pb(II) was the one that was adsorbed at the highest capacity at all C/N ratios.

Also, with the purpose of understanding the mechanism of the process, Ca(II) and Mg(II) ions and carbohydrates released into the solution were also monitored and it was indicated that an ion exchange process is involved in the biosorption of heavy metals especially at high metal concentrations but the whole metal removal can not be explained by ion exchange.

Key words: Activated sludge; biosorption mechanism; C/N ratio; extracellular polymers; heavy metal removal.

ÖZ

AKTİF ÇAMUR BİYOPOLİMER KOMPOZİSYONUNUN AĞIR METALLERİN BİYOSORBSİYONLA GİDERİMİNE OLAN ETKİLERİ

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Aktif çamur mikroorganizmaları tehlikeli maddeleri adsorblayıp yüzeylerinde konsantre ederek atık sudan uzaklaştırabilmektedir. Bu maddelerin aktif çamur yüzeyinde biyosorplanması, incelenen maddenin kimyasal özelliklerinin yanı sıra kullanılan mikroorganizmanın yüzey özelliklerine de bağlıdır. Bu çalışmanın amacı ağır metal biyosorpsiyon mekanizmalarını araştırmak ve aktif çamur mikroorganizmalarının hücre dışı polimer kompozisyonunun Pb(II), Cd(II), Cu(II), Zn(II) ve Ni(II) biyosorpsiyonu üzerindeki etkilerini ortaya koymaktır. Farklı besi koşullarında üretilen mikroorganizmaların hücre dışı polimer kompozisyonu farklı olacağı için biyosorpsiyon kapasitelerinin de farklı olması beklenmektedir Bu amaçla C/N oranı 9 olup azot yükü fazla sistemi; C/N oranı 21 olup konvansiyonel aktif çamur sistemini ve C/N oranı 43 olup karbon yükü fazla olan sistemi temsil eden üç farklı karbon/azot oranında (C/N) işletilen üç set reaktör kurulmuştur. Bütün reaktörler 8 günlük bir çamur yaşı ile kesikli olarak çalıştırılmış ve sentetik bir besi ortamıyla beslenmiştir.

Üç değişik C/N oranına sahip reaktörlerle yapılan kinetik ve denge deneyleri sonucunda, incelenen metal ile çalışılan C/N oranının aktif çamurun biyosorpsiyon kapasitesi üzerinde önemli bir etkiye sahip olduğu ortaya konmuştur. Maksimum biyosorpsiyon kapasitesinin C/N oranına göre artması ya da azalması metalden metale değişiklik göstermesine rağmen, hücre dışı polimer kompozisyonunun aktif çamurun biyosorpsiyon özellikleriyle doğrudan ilgili olduğu saptanmıştır. Çalışılan bütün metaller içinde, Pb(II) metal iyonunun bütün C/N oranlarında en yüksek kapasitede adsorblandığı gözlemlenmiştir

Ayrıca biyosorbsion mekanizmasını açıklamaya yönelik olarak sistemde açığa çıkan Ca(II)), Mg(II) ve karbonhidratlı polimer miktarları da ölçülmüş, ve özellikle yüksek metal konsantrasyonlarında sistemde iyon değiştirme mekanizması yer aldığı görülmüş fakat tüm biyosorbsiyonu sadece iyon değişimi ile açıklamak mümkün olmamıştır.

Anahtar kelimeler: Aktif çamur; biyosorpsiyon mekanizması; karbon/azot oranı; hücre dışı polimerler; ağır metal giderimi.

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ABBREVIATIONS

AAS : Atomic Absorption Spectrophotometer
BOD : Biochemical Oxygen Demand
C/N : Carbon / Nitrogen
ECP : Extracellular Polymer
MLSS : Mixed Liquor Suspended Solids
MLVSS : Mixed Liquor Volatile Suspended Solids
TKN : Total Kjeldahl Nitrogen

CHAPTER 1

INTRODUCTION

Heavy metals discharged into the environment from various industries and municipal sewers constitute one of the major causes of water and soil pollution. The heavy metal impact on the biosphere is estimated to amount to approximately 5 million tons worldwide every year. The potential toxicity, partly in low concentrations, to humans, animals, plants, and microorganisms is the common ecological feature of approximately 40 heavy metals of the periodic table (Sandau *et al.*, 1996; Volesky, 1994). For humans poisoning by most of these metals causes severe dysfunction of the kidney, reproductive system, liver, brain and central nervous system (Manahan, 1994).

In recent years, the research for heavy metal removal has focused on new technologies rather than traditional methods of metal removal such as ion exchange, chemical precipitation, electrochemical processes and/or membrane processes and solvent extraction, which are expensive and inefficient, especially for low strength wastewaters.

These drawbacks of traditional metal removal techniques have led researchers to the investigation of the use of microbial biomass as biosorbents for heavy metal removal.

The sequestering of metal ions by solid materials of biological origin is known under the general term "biosorption". It is well known that pure and mixed cultures of bacteria, algae, yeast, and other microorganisms can remove heavy metal ions from solution in significant quantities (Chang *et al.*, 1995). This biosorption process offers the advantages of low operating costs, minimization of the volume of chemical and/or biological sludge to be disposed of and high efficiency in detoxifying very dilute effluents (Kratochvil, 1998).

Shumate and Strandberg (1985) reported that pure cultures of microorganisms exhibited significant heavy metal uptakes ranging from 8% to 35% of the dry cell weight. They also suggested that mixed microbial cultures were more efficient than pure cultures in removing heavy metals. This means that biological pollution control processes could be effective in removing heavy metals from polluted waters.

Factors which may influence the biosorption process include pH, the concentration of metal present in the wastewater and the composition and characteristics of wastewater and the biosorbent. Heavy metal removal or uptake by sludge is a consequence of interaction between metals in the aqueous solution phase and the bacterial cell surface (Artola *et al.*, 1997). Biosorption of metals is not based on only one mechanism. It consists of several mechanisms that quantitatively and qualitatively differ according to the species used, the origin of the biomass, and its processing.

Metal sequestration follows complex mechanisms, mainly ion exchange, chelation, adsorption by physical forces; and ion entrapment in inter- and intrafibrillar capillaries and spaces of the structural polysaccaharide network as a result of the concentration gradient and diffusion through the cell walls and membranes (Volesky and Holan, 1995).

Binding of heavy metals on the sludge surface can be attributed to the formation of surface complexes between metals and surface functional groups such as carboxyl, hydroxyl, and phenolic groups of the extracellular polymer (ECP). Many different species of bacteria isolated from the activated sludge have been shown to be able to produce ECP as capsules or a gelatinous matrix and such polymers have been shown to be involved in the adsorption of exogenous metal ions from solution (Rudd *et al.*, 1984; Tien and Huang, 1987). Heavy metals are complexed by anionic legend found in polymers (e.g., proteins, lipids, polysaccharides, nucleic acids, lipoproteins, glycocalyxes, and others) of the cell membrane and cell wall (Fukushi *et al.*, 1996).

It has been suggested that the composition of the growth medium is important in the production of metal-complexing proteins and exopolymers (Chang *et al.*, 1995). Feeding microorganisms with substrates at various carbon to nitrogen (C/N) ratios was shown to affect the composition of extracellular polymers in terms of protein and polysaccharide content (Durmaz and Sanin, 2001). Microorganisms cultured under different growth conditions have different composition of ECP and hence it is expected that they will have different biosorption capacities.

The main objective of this study is to identify the effect of ECP composition of activated sludge on biosorption process and in connection the mechanisms of heavy metal biosorption. There are many studies in literature about the biosorption of heavy metals by activated sludge; nevertheless there is not much specific information on the bisorption mechanism(s) involved and especially the role of ECP in biosorption.

CHAPTER 2

THEORETICAL BACKGROUND

2.1 Heavy Metal Biosorption

The removal of heavy metals from wastewater is important in the protection of the environment and human health. Chemical precipitation is the most commonly used method for the removal of dissolved metals from wastewaters. Alternative processes include reverse osmosis, electrodialysis, ion exchange, and passive treatment using engineered wetlands (Noyes, 1991; Brierley *et al.*, 1989). These methods primarily result in the transformation of the dissolved metals into a more concentrated and manageable form prior to final disposal, usually to landfill. However, the metals can continue to represent a further long-term environmental hazard. As a result, the disposal of such wastes is becoming increasingly expensive as costly environmental protection measures are imposed (Butter *et al.*, 1998). Also, these conventional treatment methods are either ineffective or expensive when heavy metals are present in the wastewater in low concentrations, or when very low concentrations of heavy metals in the treated water are required (Kuyucak and Volesky, 1988).

Biosorption is an alternative technology in which an increased amount of study is being focused. Biosorption utilizes the ability of various biological materials to accumulate heavy metals from aqueous solutions by either metabolically mediated or purely physico-chemical pathways of uptake (Fourest and Roux, 1992). Recently, research efforts have been directed towards the use of industrial wastes and non-living microorganisms, as adsorbent materials, for heavy metal biosorption in an attempt to minimize process costs (Zhang *et al.*, 1998; Fourest *et al.*, 1994; Lopez-Delgado *et al.*, 1998; Brady *et al.*, 1994). In fact, biosorption process offers the advantages of low operating costs, minimization of the volume of chemical and/or biological sludge to be disposed of and high efficiency in detoxifying very dilute effluents (Kratochvil and Volesky, 1998).

It is well known fact that various types of algae, bacteria, fungi and yeasts can readily adsorb or accumulate metal ions (Volesky, 1986; Shumate and Strandberg, 1985; Tsezos, 1985; Gadd, 1988; Volesky and Holan, 1995).There are many studies reported in literature worked with different types of algae, bacteria, fungi and yeasts for different heavy metals. Some of these studies are summarized in Table 2.1.

Table 2.1. Summary of literature on biosorption of heavy metals

Metal	Biosorbent Material	Study	Capacity (mg/g)
Cadmium	*Chlorella vulgaris* (microalgae)	Sandau *et al.*., 1996	0.02-12.45
Cadmium	*Fusarium flocciferum (fungus)*	Delgado et al.., 1998	192
Cadmium	*Phanerochaete chrysosporium* (white-rot fungi)	Say *et al.*., 2001	27.8
Cadmium	*Pseudomonas aeruginosa* (bacteria)	Chang *et al.*., 1997.	4.4-57.4
Cadmium	*Sargassum fluitans* (marine algae)	Schiewer and Volesky, 1995	34-292

Table 2.1. Summary of literature on biosorption of heavy metals (*continued*)

Metal	**Biosorbent Material**	**Study**	**Capacity (mg/g)**
Cadmium	*Sphaerotilus natans* (bacteria)	Lodi *et al*.., 1998	114.2
Cadmium	*Streptomyces clavuligerus* (bacteria)	Butter *et al*.., 1998	30.3-66.7
Copper	*Chlorella vulgaris* (microalgae)	Sandau *et al*.., 1996	0.77-10.9
Copper	*Fucus vesiculosus* (marine algae)	Bakkaloglu *et al*.., 1998	15.7-17.3
Copper	*Fusarium flocciferum* (fungus)	Delgado *et al*.., 1998	40-60
Copper	*Padina sp.* (marine algae)	Kaewsarn, 2002	51
Copper	*Penicillum chrysogenum* (fungi)	Bakkaloglu *et al*.., 1998	19.2
Copper	*Phanerochaete chrysosporium* (white-rot fungi)	Say *et al*.., 2001	26.6
Copper	*Pseudomonas aeruginosa* (bacteria)	Chang *et al*.., 1997.	23.7
Copper	*Saccharomyces cerevisae* (yeast)	Bakkaloglu *et al*.., 1998	2-3.5
Copper	*Sargassum fluitans* (marine algae)	Schiewer and Volesky, 1995	19-172
Copper	*Sphaerotilus natans* (bacteria)	Lodi *et al*.., 1998	130.6
Copper	*Streptomyces rimosus* (bacteria)	Bakkaloglu *et al*.., 1998	6.7
Lead	*Chlorella vulgaris* (microalgae)	Sandau *et al*.., 1996	0.8-17.1
Lead	*Ecklonia radiata* (marine algae)	Matheickal and Yu, 1996	282
Lead	*Phanerochaete chrysosporium* (white-rot fungi)	Say *et al*.., 2001	85.9
Lead	*Phanerochaete chrysosporium* (white-rot fungi)	Yetis *et al*.., 2000	9-80
Lead	*Pseudomonas aeruginosa* (bacteria)	Chang *et al*.., 1997	70-110
Lead	*Saccharomyces uvarum* (yeast)	Ashkenazy *et al*.., 1997	48.9
Nickel	*Chlorella vulgaris* (algae)	Wong *et al*.., 2000	1.3
Nickel	*Fucus vesiculosus* (marine algae)	Bakkaloglu *et al*.., 1998	0.69-2.9
Nickel	*Fusarium flocciferum* (fungus)	Delgado *et al*.., 1998	52
Nickel	*Penicillum chrysogenum* (fungi)	Bakkaloglu *et al*.., 1998	1.25
Nickel	*Saccharomyces cerevisae* (yeast)	Bakkaloglu *et al*.., 1998	1.47
Nickel	*Streptomyces rimosus* (bacteria)	Bakkaloglu *et al*.., 1998	1.63
Silver	*Sphaerotilus natans* (bacteria)	Lodi *et al*.., 1998	477

Table 2.1. Summary of literature on biosorption of heavy metals (*continued*)

Metal	Biosorbent Material	Study	Capacity (mg/g)
Uranium	*Mucor miehei* (fungus)	Guibal *et al.*, 1992	7-240
Zinc	*Chlorella vulgaris* (microalgae)	Sandau *et al.*., 1996	0.88-6.42
Zinc	*Fucus vesiculosus* (marine algae)	Bakkaloglu *et al.*., 1998	15.7-17.3
Zinc	*Penicillum chrysogenum* (fungi)	Bakkaloglu *et al.*., 1998	19.2
Zinc	*Saccharomyces cerevisae* (yeast)	Bakkaloglu *et al.*., 1998	1.95-3.45
Zinc	*Sargassum fluitans* (marine algae)	Schiewer and Volesky, 1995	13-157
Zinc	*Sphaerotilus natans* (bacteria)	Lodi *et al.*., 1998	741.4
Zinc	*Streptomyces rimosus* (bacteria)	Bakkaloglu *et al.*., 1998	6.63
Zinc	*Streptomyces rimosus* (bacteria)	Mameri *et al.*., 1999	80

Also, there are numerous approaches for the development of cheaper and effective bisorbents by using some industrial waste material such as fly ash (Ricou *et al.*, 2001), peat (Brown *et al.*, 2000; Ho and McKay, 2000), and agricultural byproducts (Laszlo and Dintsiz, 1994; Marshall *et al.*, 2000). Some of these byproducts include soybean hulls, peanut hulls, almond hulls, cottonseed hulls, and corncobs (Wartelle and Marshall, 2000). In a recent study, Reddad *et al.* (2002) have used sugar beet pulp generated by sugar-refining factories for the removal of several metal cations, namely Pb(II), Cu(II), Zn(II), Cd(II) and Ni(II). Senthilkumaar *et al.* (2000) used waste residues obtained from the juice processing industry as biosorbent for the removal of mercury, lead, cadmium, copper, zinc and nickel. Marques *et al.* (2000) used waste brewery biomass for biosorption of copper, cadmium and lead. Some seaweeds and seaweed derivatives were also used for the biosorption of heavy metals (Gonzalez *et al.*, 2001; Williams and Edyvean, 1997).

2.1.1 Heavy Metal Removal by Activated Sludge

Many studies have shown that mixed cultures can also remove considerable amount of heavy metal ions from aqueous solutions. Microbial metal uptake varies from a few micrograms per gram to several percent of the dry cell weight. Shumate and Strandberg (1985) suggested that mixed microbial cultures were more efficient than pure cultures in removing heavy metals. This observation suggests that biological pollution control processes could be effective in removing heavy metals from polluted waters (Chang *et al.*, 1995; Oliver and Cosgrove, 1974; Kasan and Stegmann, 1987; Volesky, 1987; Bux *et al.*, 1994a, b).

In an early report, Ruchoft (1949) indicated the capability of activated sludge cultures to remove heavy metals. Considerable work has been done since the report of Ruchoft confirming the abilities of microorganisms in activated sludge and also in anaerobic digester to remove a variety of heavy metals (Brown *et al.*, 1973; Gould and Genetelli, 1978; Stoveland *et al.*, 1979; Lester *et al.*, 1979, Brown and Lester, 1982; Rosin *et al.*, 1982; Tien and Huang, 1987; Aksu *et al.*, 1991; Kennedy *et al.*, 1992; Kodukula *et al.*, 1994; Atkinson *et al.*, 1996; Fukushi *et al.*, 1996; Aksu and Yener, 1998; Bux *et al.*, 1999; Aksu *et al.*, 1999, Utgikar *et al.*, 2000).

Bux *et al.* (1999) indicated the high biosorptive capacity of dried biomass of waste activated and digested sludge in the removal of zinc. They showed that activated sludge is more effective than digested sludge possibly due to the higher microbial content and the diverse nature of the activated sludge biomass in terms of its microbial consortium, which result in higher concentrations of cellular polysaccharides and other active materials that are thought to be responsible for metal biosorption. On the other side, several investigations reported that anaerobically digested sludge is an effective biosorbent for the removal of heavy metals (Solari *et al.*, 1996; Artola *et al.*, 1997; Haytoglu *et al.*, 2001).

2.2 Mechanism of Biosorption

In order to progress the understanding and application of these biosorbent systems, it is important that the mechanisms involved in metal ion binding are elucidated and optimized. The sorption of metal ions onto activated sludge is considered to be rather complicated and usually consists of more than one mechanism such as ion exchange, complexation, electrostatic attraction, and microprecipitation (as metal or metal salt) (Guibal *et al.*, 1992; Schiewer and Volesky, 1995; Kodukula *et al.*, 1994)

Biosorption mechanisms are various and in some cases they are still not very well understood. They may be classified by the following different criteria (Veglio and Beolchini, 1997):

According to the dependence on the cells' metabolism, biosorption mechanisms can be divided into:

1. metabolism dependent,
2. metabolism independent.

According to the location where the metal removed from the solution is found, biosorption may be classified as:

1. extracellular accumulation/precipitation,
2. cell surface sorption/ precipitation,
3. intracellular accumulation.

Transport of the metal across the cell membrane yields intracellular accumulation, which is dependent on the cells' metabolism. This implies that this kind of biosorption may take place only with viable cells (Veglio and Beolchini, 1997).

In the case of physicochemical interaction between the metal and functional groups of the surface, based on physical adsorption, ion exchange and complexation, we have cell surface adsorption, which is not dependent on the metabolism. This physicochemical phenomenon of metal biosoption, non-metabolism dependent, is relatively rapid and can be reversible (Kuyucak and Volesky, 1988).

In the case of precipitation, the classification is not unique. In fact the precipitation of the metal takes place both in solution and on the cell surface. Furthermore, it may be dependent on the cells' metabolism if, in the presence of toxic metals, the microorganism produces compounds, which favour the precipitation process. On the other hand, precipitation may not be dependent on the cells' metabolism, occurring after a chemical interaction between the metal and the cell surface (Veglio and Beolchini, 1997).

According to Tsezos (1980) various steps in the sorption mechanism are proposed which deal with metal transfer through layers constituting or bordering the cell wall:

1. Transport from the bulk solution to the boundary film present around the cell wall
2. Transport from the boundary film to the cell surface
3. Transfer from the cell surface to active sites of uptake
4. Uptake phases: complexation, adsorption and intramembranar precipitation.

Although, it is known that biosorption consists of several mechanisms that quantitatively and qualitatively differ according to the species used, the origin of the biomass, and its processing; many researchers regarded that generally, ion exchange is the predominant biosorption mechanism (Tau *et al.*, 1971; Kuyucak and Volesky, 1982; Xue *et al.*, 1988; Crist *et al.*, 1990; Fourest *et al.*, 1994; Utgikar *et al.*, 2000; Gonzalez *et al.*, 2001). Bupp and Ghosh (1991) indicated that less than 10% of the removed metals were adsorbed and/or precipitated, and that organic binding together with ion exchange accounted for 90% to 95% of the total metal removal.

Kuyucak and Volesky (1989) reported that cobalt biosorption by a nonliving biomass of *Ascophyllum nodosum* is predominantly an ion exchange process. They suggested that the carboxyl groups of the cell wall alginates play an important role in cobalt binding.

An ion exchange adsorption mechanism was also suggested by Fourest *et al.* (1994). Schneider and Rubio (1999), found that the sorption mechanism by the dried biomass of *Potamogeton lucens*, *Salvina herzogii*, and *Eichhornia crassipes* proceeded mainly by ion exchange reactions between the metal ions and the cationic weak exchanger groups present on the plant surface.

Crist *et al.* (1988) suggested that the biosorption of heavy metals consists of two phases: a fast phase (less than 4 s) is attributed to surface adsorption, mainly based on ion exchange with the participation of the carboxyl groups of uronic acids; and much slower metal uptake (2 h) phase represents the diffusion of ions into the cell structures.

Treen-Sears *et al.* (1984) have shown that uranium biosorption on *Rhizopus arrhizus* resulted in exchange of hydrogen ions from biomass for uranyl ions, showing that ion-exchange is the principal mechanism for metal biosorption. Muraleedharan *et al.* (1994) have shown that calcium and hydrogen ions were released on biosorption of copper by *G. lucidium*. Recent studies have shown that magnesium ions, in addition to calcium and hydrogen ions, were also released from biomass as a result of biosorption (Akthar *et al.*, 1996, Avery and Tobin, 1993; Brady and Tobin, 1994). These studies also demonstrate that ion exchange plays an important role in biosorption. Kapoor and Viraraghavan (1997) showed that biosorption of metal ions on dried biomass of *Aspergilus niger* released potassium ions, in addition to calcium and magnesium ions.

As opposed to the studies presented above, some researchers reported that some mechanisms other than ion exchange could be dominant in biosorption process. For example, complexation was indicated to be the only mechanism responsible for calcium, magnesium, cadmium, zinc, copper and mercury accumulation by *Pseudomonas syringae* (Cabral, 1992). Aksu *et al.* (1992) hypothesized that biosorption of copper by non-living cells of *Chlorella Vulgaris* and *Zoogloea Ramigera* takes place through both adsorption and coordination bonds between metals and amino and carboxyl groups of cell wall polysaccharides. Brown and Lester (1982) have suggested that both precipitation and adsorption of metals have taken place in their batch cultures of *K.aerogenes*, an organism commonly found in activated sludge.

To explore the biosorption mechanisms, it is necessary to identify the functional groups involved in the biosorption process. Tien and Huang (1987) have suggested that binding of heavy metals on the sludge surface can be attributed to the formation of surface complexes between metals and surface functional groups. Where several different types of metal-binding functional group are present, different metals may associate preferentially with different specific types of functional group and that the number of effective binding sites may depend to some extent on the relative distributions of these groups (Rudd *et al.*, 1984). There are many studies in literature present different results about the main active sites, which take place in biosorption.

There are several chemical groups that could attract and sequester the metals in biomass and where coordination complexes with metals can be formed: acetamido groups, amino and phosphate groups in nucleic acids, amino, amido, sulfhydryl, and carboxyl groups in proteins, and hydroxyl, carboxyl and sulfate groups in the polysaccharides (Tobin *et al.*, 1984; Volesky and Holan, 1995). Some researchers suggested that the main functional group involved in biosorption is the carboxyl group. For example, Beveridge and Murrey (1979) suggested that the carboxyl groups of peptidoglycan were primarily responsible for the interactions between cell walls and cations. Also, Marquis *et al.* (1973) have reported that carboxyl groups are involved in the binding of metal ions to the cell wall of *Streptococci*.

Doyle *et al.* (1980) who selectively modified the free carboxyl and amino groups of *Bacillus Subtilis*, showed that when amino groups were replaced by neutral, bulky, or negatively charged groups, the number of sites available for cation complexing generally increased and introduction of positive charges onto the cell wall decreased the numbers of metal binding sites. They suggested that the ionic interaction of lead cations with anionic groups, especially carboxyl groups, is the major biosorption mechanism responsible for the acetone-washed biomass metal uptake.

Gardea *et al.* (1990) observed that after the blocking of carboxyl groups of algal species by esterification the binding capacity of copper decreased. Fourest *et al.* (1996) used the same technique to demonstrate that the Zn binding by fungi was at least 30-70 % achieved by carboxyl groups. Tobin *et al.* (1990), through a series of chemical treatments to *R. arhizus*, indicated that phosphate and carboxyl groups were important in biosorption, and amine groups did not play any significant role.
Also, Akthar *et al.* (1996) and Gonzalez *et al.* (2001) indicated that carboxyl groups are largely responsible for biosorption.

Although carboxyl groups have been identified as the main-sequestering sites, they are not likely to be the only strongly active sites. In addition to the carboxyl groups of amino acids, the amino groups may also be ionized in solution and may contribute to the metal binding capacity (Hunt, 1986; Volesky and Holan, 1995). Amino acid groups, present in the cell wall are suggested as the most active sites in the sludge-metal binding process (Tien and Huang, 1991). Artola *et al.* (1997) found that the main binding groups for metal (especially for copper) in the anaerobically digested sludge are of amino acid type.

Kapoor and Viraraghavan (1997) showed that, in dried fungal biomass of *Aspergillus niger*, amine and carboxyl groups were important functional groups involved in lead, cadmium and copper biosorption and they reported that phosphate groups and the lipids fraction of the biomass did not play a significant role in biosorption of the metal ions studied. Also, Mameri *et al.* (1999) suggested that the carboxylic and amino groups are mainly involved in Zn(II) biosorption of a dried biomass *of Streptomyces rimosus*. The infrared analysis of the biomass performed before and after the Zn (II) biosorption showed that the amino and carboxylic bands disappear after saturation of active sites of the biomass.

The composition of the medium may also have a direct effect on both passive adsorption and metabolic uptake. Fukushi *et al.* (1996) explained the higher uptake of copper than uptake of cadmium in their study by the presence of peptone in the growth medium of the test culture of dead and viable biomass. They also suggested that metal uptake was related to unit mass of protein because it is believed that proteinaceous biopolymers play an important role in complexing and removing heavy metals from solution. Beveridge and Murray (1980) also observed that modification of amine and carboxyl groups resulted in a significant drop in copper biosorption by *Bacillus subtilis*.

Schneider and Rubio (1999) also showed importance of protein in biosorption in a different way with the nonliving biomass of freshwater macrophytes. They found that main functional group in the ion exchange reactions at neutral pH values is the carboxyl present on the plant tissues, but they related the carboxyl groups to the protein content in the tissues since *P.lucens*, which showed the highest protein content, also presented the highest concentration of carboxyl groups.

Many activated sludge bacteria produce ECPs as capsules or a gelatinous matrix. These polymers appear to play an important role in flocculation (Brown and Lester, 1979). A typical floc is formed by different species of bacteria as well as other organisms like protozoa, fungi, filamentous microorganisms and viruses along with some abiotic suspended material all of which are held together in a polymeric network called ECP.

The main components of ECPs are known to be polysaccharides, proteins, nucleic acids and lipids. Brown and Lester (1979) reported that the extraction products from pure bacterial cultures consisted of hexose sugars, pentose sugars, hexuronic acids and hexosamines.

As it is mentioned above, many species of bacteria isolated from activated sludge have been shown to produce ECP, which provide cation exchange and chelation sites for adsorbing and complexing heavy metals. Increased production of extracellular polymers may enhance metal binding (Bitton and Freihofer, 1978; Corpe, 1975; Brown and Lester, 1982).

Several factors have been identified which effect concentrations of ECPs in bacterial cultures and activated sludge: the ratios in the growth medium of carbon to nitrogen, carbon to phosphorus, and carbon to sulphur (Duguid and Wilkinson, 1953; Corpe, 1964; Wu, 1978); oxidation of ECPs (Obayashi and Gaudy, 1973); dissolved oxygen concentration (Novak *et al.*, 1977); biomass loading rates (Kiff, 1978); biochemical oxygen demand (BOD) (Coackley, 1969). Duguid and Wilkinson (1953), studying the influence of cultural condition on polysaccharide production by *Aerobacter aerogenes*, found that a deficiency in nitrogen or phosphate increases polysaccharide production. Wilkinson (1958) discovered that potassium, calcium and magnesium ions all stimulated ECP synthesis; a deficiency of any of these ions caused a decrease in ECP production.

The study of Tien and Huang (1987) indicated that the higher the C/N ratio resulted the higher the concentration of polysaccharide and the lower the concentration of protein content in ECP. This can be explained by the fact that at higher C/N ratios, there is an abundance of carbon to synthesize the polysaccharide portion of the ECP; a relatively low supply of nitrogen causes the protein portion of the ECP to decrease. Also, Durmaz and Sanin (2001) reported that feeding microorganisms with substrates at various carbon to nitrogen (C/N) ratios affected the composition of extracellular polymers in terms of protein and polysaccharide content.

2.3 Factors Affecting Heavy Metal Biosorption

Bioremoval of a pollutant using microorganisms is affected by several factors. These factors include the specific surface properties of the microorganism and the physicochemical parameters of the solution such as temperature, pH, metal ion concentration, metal solubility, metal valency, concentration of complexing agents and particle size. The main biological factor is, as discussed in detail in Section 2.2, the concentration of bacterial ECPs present (Brown and Lester, 1979).

2.3.1 Temperature

Butter *et al.* (1998) showed temperature variations from 15-35^{o}C did not affect the cadmium uptake by dead *Streptomyces* biomass. Also, Kasan (1993) found that the complexation/removal of the toxic metals Cr, Pb, and Zn, by the use of living activated sludge was independent of the temperature. On the other hand, Mameri *et al.* (1999) indicated that the increase in temperature led to a reduction of the biosorption capacity.

2.3.2 pH

It is known that pH has a very significant effect on the metal removal from solutions. Taking into account this importance of the pH on metal ion biosorption several studies are reported in literature, which have investigated the effect of pH on biosorption.

Most investigators have reported negligible sorption in their studies at pH values lower than 4.0 (Tien and Huang, 1987; Delgado *et al.*, 1998; Wang *et al.*, 1999). Mameri *et al.* (1999) found that adsorption capacity of dried biomass *of Streptomyces rimosus* for Zn(II) to be negligible at pH 3.0. Also, Leung *et al.* (2001) stated that metal uptake was negligible at pH 2.0 and then increased rapidly with increasing pH. Schiewer and Volesky (1995) also investigated the influence of pH on sorption. A trend of increasing metal ion binding with increasing pH could by nonliving biomass of the marine alga *Sargassum fluitans* be observed for three metal ions (Cd(II), Cu(II) and Zn(II)) examined in this work. The same trend was noted by other researchers e.g. for the sorption of cobalt on *Ascophyllum* (Kuyucak and Volesky, 1989) and sorption of copper, cadmium and nickel on nonliving biomass of *Fusarium flocciferum* (Delgado *et al.*, 1998).

These results could be explained by the competition between hydrogen ions and metal ions for the sorption sites of cells. At very low pH values, metal cations and protons compete for binding sites on the cell walls, which results in lower metal uptake. As pH levels are increased, more ligands with negative charge would be exposed with a subsequent increase in attraction for positively charged metal ions. It has been recognized by Crist *et al.* (1994) that the main effect of pH on metal ion binding consists of a reduction in the number of binding sites available with decreasing pH.

In addition to this competition between protons and metal ions for binding sites, there are also some other ways in which pH influences sorption. Since adsorption depends not only on the attraction of the sorbate to the solid surface but also on its lyophobic behavior (sorption increases with decreasing solubility), for most metals that means adsorption increases with increasing pH (Schiewer and Volesky, 1995).

Also, the solution pH affects the surface charge of the adsorbent, the degree of ionization, and the speciation of the surface functional groups like carboxylate, phosphate and amino groups of the cell wall. Moreover, pH variations can modify the speciation and the availability of the metal cations in solution as well as the chemical state of the biomass functional groups responsible for metal binding (Fourest *et al.*, 1994; Aksu *et al.*, 2000; Reddad *et al.*, 2002).

Gonzalez *et al.* (2001) stated that the ionization constants for different carboxyl groups were around 3.0-4.0. This means at pH values less than 3, the carboxyl groups become protonated and thus no longer available to attract metal ions from solution. On the contrary, when the pH is higher than 4, the carboxyl groups are deprotonated and therefore negatively charged and able to bind positively charged metal ions.

On the other hand, too high pH values, which cause precipitation of metal complexes where distinguishing between sorption and precipitation metal removal becomes difficult, should be avoided during sorption experiments. Holan and Volesky (1994) found that the danger of microprecipitation starts at pH above 5.0

for lead and at pH 6.0-7.0 for nickel. Similarly, Stumm and Morgan (1996) reported that hydroxyl species are formed above pH 8 for cadmium.

Marques *et al.* (2000) have undertaken some tests with different initial pH values between 4.5 and 8.0 of unbuffered Cu(II), Cd(II) and Pb(II) solutions in water without any addition of biosorbent. Without biosorbent, it was verified that an initial solution pH increase led to an immediate ion concentration decrease, for all metals, due to precipitation phenomena. This effect was observed in different pH zones and in different degrees for each cation, but mostly in the pH range above 6.0.

The effect of pH depends strongly on the metal species used; so, various investigators reported different pH values as optimum pH for different metal ions. Matheickal and Yu (1996) reported the optimum pH value as 5.0 for biosorption of lead by dried biomass of marine algae, *Ecklonia Radiata*. Also, Ashkenazy *et al.* (1997) found that the optimum pH for lead adsorption by acotene-washed yeast biomass was 5.5. Fourest and Roux, (1992) stated that the optimal pH for adsorption of lead by mycelial, by-products of *Rhizopus arrhizus,* was 5.0 and the optimal pH for adsorption of the same metal ion was around 4.5 for biomass of *Penicilluium chrysogenum* (Niu *et al.*, 1993).

Tien and Huang (1987) reported that the optimum pH values for biosorption of copper by activated sludge are between 5.5 and 6.0. Also, an optimal pH of 6.5 for the adsorption of copper was found using *Saccharomyces cerevisiae* as the biosorbent (Brady and Duncan, 1994) and Gould and Genetelli (1978) have concluded that an increase in pH above 7.0 did not increase the degree of copper adsorption on anaerobically digested sludge. Schiewer and Volesky (1995) stated that for copper pH 4.5 can be maintained as optimal when the nonliving biomass of marine alga was used as biosorbent.

Gonzalez *et al.* (2001) studied the mechanism of cadmium biosorption by dealginated seaweed waste and found that the optimal cadmium removal occurred at a solution pH of 6. The least cadmium was removed when the solution pH was maintained at 2.0. Butter *et al.* (1998) showed that cadmium uptake by dead biomass of *Streptomyces* strain varied only slightly over the pH range 5.0-9.0 (optimum at pH 5.5), and the optimum pH for Cd(II) uptake was 6.0 for immobilized *Zoogloe ramigera* cells (Park *et al.*, 1999). Also, Schiewer and Volesky (1995) found the optimum pH as 6.0 for the sorption of Cd(II) and Zn(II) by the nonliving biomass of marine alga.

Regarding these results reported above, it seems that influence of pH on biosorption varies with the type of adsorbates and also the type of adsorbent.

2.3.3 Adsorbent and Adsorbate Properties

Physico-chemical properties (e.g. metal ion concentration, metal solubility, metal valency) of adsorbate are important factors for biosorption. Holan and Volesky (1994) stated that metal sorption increases with increasing valence and atomic number. Also, Brown and Lester (1982) found that the sequence of solubilities of cadmium, manganese, cobalt and nickel is the reverse of the sequence of metal affinities for adsorption onto cells of *K aerogenes* indicating that the more soluble metals displayed the lowest removals.

The study of Irving and Williams (1948) has suggested a series of stabilities of complexes of bivalent metal ions, formed regardless of the nature of the complexing agent:

Pb > Cu > Ni > Co > Zn > Cd >Fe> Mn > Mg

Metal ions show different biosorption affinities for different types of biomass. It is stated in various studies that in general lead shows high affinities for different types of biomass where as removal of nickel is poor (Brown and Lester, 1979; Chan *et al.*, 1991; Tsezos *et al.*, 1996; Holan and Volesky, 1994; Bakkaloglu *et al.*, 1998; Wong *et al.*, 2000; Leung *et al.*, 2001). Rosin *et al.* (1982), pointed out that average heavy metal removal of as low as 1 % for nickel and as high as 92% for lead were reported for activated sludge. It has been shown by other researchers that lead, copper, and zinc are removed relatively more efficiently by activated sludge organisms, whereas cadmium, nickel, and manganese are removed poorly (Rudd *et al.*, 1984; Kodukula *et al.*, 1994; Chang *et al.*, 1995; Matheickal and Yu, 1996; Solari *et al.*, 1996).

The biosorption mechanism depends also on the nature of the metal in question. Tsezos and Volesky (1982a, b) showed that there are different biosorption mechanisms for uranium and thorium. Also, Holan and Volesky (1994) stated a conspicuous difference between the uptake of lead and nickel by dead biomass of Marine Algae. This varying metal behavior is a common observation in biosorption reports. Reddad *et al.* (2002) classified the metal ions according to their adsorption mechanism (Table 2.2).

Table 2.2. Adsorption mechanisms for different metal ions (Reddad *et al.*, 2002)

Metal	**Adsorption Mechanisms**
Pb^{2+}	58 % ion exchange + 42 % complexation
Cu^{2+}	60 % ion exchange + 40 % complexation
Zn^{2+}	78 % ion exchange + 22 % complexation
Cd^{2+}	90 % ion exchange + 10 % complexation
Ni^{2+}	100 % ion exchange

The results of the study presented by Marques *et al.* (2000) suggested that Pb(II)' Cu(II) and Cd(II) were removed through different mechanisms, in the standard biosorption runs which with had no pH control or adjustment. Also, data of Kapoor and Viraraghavan (1997) concerning the same cations showed that, in dried fungal biomass of *Aspergillus niger*, amine and carboxyl groups were important functional groups involved in lead, cadmium and copper biosorption. However, the biosorption of copper by binding to the amino groups was much more significant than by binding to the carboxyls.

In the study of Brown and Lester (1982), it was shown that cadmium was complexed to much greater extent by ECPs from activated sludge than nickel at the lower metal concentrations added. The results suggest that the binding of nickel to activated sludge was less specific than that of cadmium. The role of ECPs appeared to be much more significant in the removal of cadmium by activated sludge than that of nickel.

Ashkenazy *et al.* (1997) suggested that the ionic interaction of lead cations with anionic groups, especially carboxyl groups, is the major biosorption mechanism responsible for the acetone-washed biomass metal uptake. Also, Kapoor and Viraraghavan (1997) observed that lead biosorption was more sensitive to modifications of the carboxyl group than of the amine group.

These phenomena stated in above examples suggested that in cases where several different types of metal-binding functional groups are present, different metals may associate preferentially with different specific types of functional group and that the number of effective binding sites may depend to some extent on the relative distributions of these groups. Differences in biosorption can be attributable to variations in the chemistry of binding sites on individual sludges, which ultimately determine the metal uptake capacity of biomass.

State of the biomass also is an important factor determining the metal biosorption efficiency. Yetis *et al.* (2000) studied the removal of Pb(II) by live, resting and dead cells of white-rot fungus *Phanerochaete chrysosporium*. At all the initial Pb(II) concentrations tested, the highest removal rates were observed with resting cells, and the lowest with live cells. Surprisingly, the capacity of dead cells in sorbing Pb(II) was lower although heat treatment is thought to erode microbial cell surface integrity causing the walls to become leaky with a marked increase in the passive diffusion of metal to the interior. Having the least capacity of the live cells was attributed to the toxic effect of Pb(II) on *Phanerochaete chrysosporium*, resulting in lower biomass concentration. On the other hand, the sorptive capacity of resting cells was relatively high indicating the occurrence of both surface biosorption and bioaccumulation mediated by enzymes which may be active in complexing and binding the metal and also transporting and eventually depositing the metal into the vacuoles. The difference observed between the resting and dead cells' Pb(II) removal capacities could only be due to metabolism-dependent biosorption, which is not involved in metal removal by dead cells. Metabolism-dependent biosorption involves transport of the metal across the cell membrane that yields intracellular accumulation and precipitation/extracellular accumulation associated with the growth associated products (Chang *et al.*, 1995; Churchill *et al.*, 1995; Yetis *et al.*, 2000).

Cheng *et al.* (1975) carried out experiments to compare the metal uptake in active and non-active sludge. Sterilization at 121 ^{o}C and blending for 10 min were used to inactive the sludge. The uptake efficiency of non-activated sludge was found to be lower than that of the original viable sludge. Same phenomena was also observed by Chang *et al.* (1997) who worked with *Pseudomonas aeruginosa*, both with resting cells and inactivated cells. These results were attributed the techniques used to inactivate the sludge, which were very harsh treatments, that may have altered the structure of the sludge surface and destroy or lose some of metal binding sites in a way which interfered with metal adsorption and resulting in the decrease in metal uptake capacity for the inactivated cells.

2.4 Adsorption Isotherms

Adsorption isotherms are often employed to characterize metal uptake by the biomass and typically yield S- or L- shaped curves as shown in Figure 2.1. The S-shaped curve is indicative of competitive adsorption with moderate intermolecular attraction between the adsorbed species; and it shows that adsorption probably met strong competition from another component of the system. The L-shaped curve, on the other hand, is indicative of reduction in the number of available binding sites as free or soluble metal concentration increases (Lawson *et al.*, 1984)

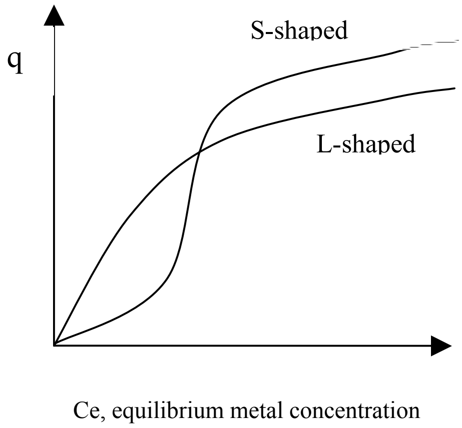


Figure 2.1. S- and L- shaped adsorption isotherms
(q: mass of metal adsorbed/mass of biomass)

Langmuir or Freundlich isotherm models are the most widely used models for biosorption. The Langmuir equation is valid for monolayer sorption onto a finite number of identical sites at surface and given by Equation 2.1 and the Freundlich expression is an empirical equation based on sorption at heterogenous surface suggesting that binding sites are not equivalent and/or independent. The mono-component Freundlich equation is given by equation 2.2.

$$\frac{C_e}{q_e} = \frac{Q^0 b C_e}{1 + b C_e} \qquad (2.1)$$

where q_e is the equilibrium capacity as mg metal/g dry biomass; C_e is the equilibrium metal concentration as mg/L; Q^0 is the maximum possible amount of substance adsorbed per unit weight of adsorbent to form a complete monolayer on the surface as mg metal/g dry biomass and b is an equilibrium constant related to the affinity of the binding sites as L/g.

$$q_e = K C_e^{1/n} \qquad (2.2)$$

where K and n are the Freundlich constants of the system which are indicators of adsorption capacity as $(mg/g)(L/mg)^{1/n}$ and adsorption affinity respectively.

Many researchers used these models in order to describe the biosorption process and they showed good correlations with the process (Lopez-Delgado *et al.*, 1998; Delgado *et al.*, 1998; Wong *et al.*, 2000; Leung *et al.*, 2001; Say *et al.*, 2001; Haytoglu *et al.*, 2001; Reddad *et al.*, 2002).

Langmuir or Freundlich sorption models are the most frequently used models for biosorption but they do not take into account electrostatic interactions or the fact that metal ion biosorption is largely an ion exchange phenomenon. They do not allow the prediction of the remaining binding of those ions (e.g. protons or sodium) that were initially loaded onto the biosorbent, and neither do they incorporate the concentration of the exchanged species (e.g. protons) as a parameter. As a result the conventional and tedious determination of the effects of these parameters has been necessary, normally not allowing any calculated predictions of the biosorbent performance (Schiewer and Volesky, 1995).

Schiewer and Volesky (1995) modeled the metal ion and proton binding at equilibrium as a function of pH and metal ion concentration using a modified multi-component Langmuir sorption model. It is recommended to use this two-site model instead of Langmuir or Freundlich isotherm models in order to accommodate the significance of ion exchange in biosorption. Also, Tien and Huang (1987) suggested a modified Langmuir adsorption isotherm, incorporating the proton effect to describe the adsorption behavior of Cu(II) onto sludge particulates.

CHAPTER 3

MATERIALS AND METHODS

3.1 Reactor Operation

Mixed culture bacteria grown in semi-continuous reactors were used during the experiments. The microbial seed was obtained from the primary settling tank effluent of Ankara Wastewater Treatment Plant. Reactors had a working volume of 2L and were operated at a mean cell residence time (MCRT) of 8 days. The reactors were placed into a water bath where temperature of the system was kept constant at 20^{o}C. The pH was adjusted to 7.0 ± 0.2. Oxygen was supplied by air pumps which were also providing completely mixed conditions in the reactors. Dissolved oxygen concentration in the reactors was kept at a minimum of 3 mg/L.

Three sets of replica reactors were operated under three different C/N ratios. Each reactor set had two replica reactors. The first C/N ratio was selected as 21 (in terms of the ratio of COD to TKN as mg/L per mg/L). This set of reactors was operated to represent the typical operational conditions in activated sludge systems treating municipal wastewaters. These reactors were fed with the synthetic medium given in Table 3.1. The second set of reactors was operated at a C/N ratio of 9 to represent a carbon-limited situation. To adjust the amount of carbon, the synthetic medium given in Table 3.1 was modified by decreasing the glucose amount in the feed. The third set of reactors was operated at a C/N ratio of 43, represented nitrogen limited situation. The carbon content of these reactors was again adjusted by modifying the glucose amount in the feed given in Table 3.1.

Table 3.1. Synthetic feed medium composition for C/N of 21

Constituent	Concentration (mg/L)
Glucose	935
Peptone	200
K_2HPO_4	600
KH_2PO_4	300
NH_4Cl	225
$MgSO_4.7H_2O$	112.5
$FeSO_4.7H_2O$	3.75
$ZnSO_4.7H_2O$	3.75
$MnSO_4.7H_2O$	3.75
$CaCl_2$	15
$NaHCO_3$	180

Reactors operated under the above listed conditions were brought to steady state which was demonstrated by measuring mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) concentrations daily as described in Section 3.4.

Once the steady state was reached, biosorption kinetic and equilibrium (isotherm) tests were conducted using a total of 500 mL of sludge by combining the 250 mL of daily wasted sludge from each replica reactor. The wasted sludge was first centrifuged for 15 min, the supernatant was discarded and remained biomass was resuspended in deionized water. This centrifugation-resuspension procedure was repeated once more; and finally, the MLSS concentration of biomass suspension to be used in biosorption tests was measured. Hence, the biomass used in soption tests was at resting state. Five different heavy metals, which are Pb(II), Ni(II), Zn(II), Cu(II) and Cd(II) were used during the biosorption tests.

3.2 Biosorption Tests

Biosorption studies that are mainly composed of sorption kinetic and sorption isotherm tests, were performed as batch experiments using biomass suspension with a predetermined biomass concentration. Tests were held for each C/N ratio and five different heavy metals. Since the sorption performance may vary with temperature and pH, in all experiments the temperature was kept constant at 25°C and, the initial pH of metal solutions was set to 4.0 using 0.01 M HNO_3 and NaOH. The initial pH of the biomass was around 7 and no initial pH adjustment was carried out for biomass suspension. Lead, nickel, zinc, copper and cadmium was added as $Pb(NO_3)_2$, $NiCl_2.6H_2O$, $ZnCl_2$, $CuCl_2.2H_2O$, and $CdCl_2.H_2O$, respectively. All the biosorption tests were run in duplicate. Biosorptive capacity was calculated by using the equation;

$$q = V(C_o-C)/ m \qquad (3.1)$$

where:

q: the capacity, mg metal/g dry biomass, V: volume of the sample, L,

C and C_o: final and initial heavy metal concentrations in aqueous phase, mg/L,

m: is the amount of dry biomass, g.

Deionized water was used in all sorption tests to differentiate between the ions released and the ions existing in water with an exception of lead. In lead biosorption experiments, which were run at the beginning of this study, distilled water was used since deionized water was not available. Also, all glassware were washed in diluted HNO_3 and rinsed with distilled water before use in order to prevent metal contamination.

3.2.1 Sorption Kinetic Tests

Kinetic studies were conducted by mixing heavy metal solutions of 500 mL volume with the known amount of slurry biomass and taking samples (10 mL) at different time intervals for 6 h for Pb(II) and 4 h for the other metals beyond which there is no net sorption.

Three different initial metal concentrations were used for three C/N ratios in order to keep the initial metal to biomass concentration ratio constant at around 0.6 g/g. The initial Pb(II) concentrations were 2000, 3000, and 4000 mg/L and the initial metal concentrations for Zn(II), Ni(II), Cu(II), and Cd(II) were 200, 300, and 400 mg/L for the C/N ratios of 9, 21 and 43, respectively. Such high Pb(II) concentrations were deliberately selected in order to end up with the maximum

Pb(II) sorptive capacity that will provide a fair comparison of the results between Pb(II) and the other heavy metals. In order to keep the initial metal to biomass ratio constant, 50mL wasted sludge was used for the kinetic studies of nickel, zinc, copper and cadmium.

The biomass in the samples was removed by filtration through a 0.45 μm membrane filter and filtrates were analyzed by using flame atomic absorption spectrophotometer (AAS) (ATI Unicam 929), following pH adjustment to 2.0 using HNO_3. In all these measurements, the methods described in Standard Methods (APHA, 1995) were followed. Beside the Pb(II) ions absorbed, Ca(II) and Mg(II) ions released during the experiment were also analyzed by using AAS in order to investigate the mechanism of the process. At each sampling, the solution pH was measured in order to follow the change of pH during sorption.

3.2.2 Sorption Equilibrium Tests

To analyze sorption equilibrium, batch isotherm experiments were held using flasks of net volume 50 mL which are placed in a shaking incubator operating at a shaking rate of 200rpm and temperature of 25^{o}C. The amount of biomass added into each flask was approximately 0.1, 0.2 and 0.3 g as dry matter which were calculated by using MLSS concentration of sludge for C/N ratios of 9, 21 and 43, respectively. The heavy metal concentration varied from 50 to 4000 mg/L for Pb (II) and 10 to 1000 mg/L for other metals for each C/N ratio at an initial pH 4.0. Kinetic tests have shown that almost 100% of the metal removal was achieved within the first 2h of biomass-heavy metal contact. So, 4 h was employed as the equilibrium time for all metals. The sample taken at the end of this time was analysed for heavy metal, calcium and magnesium ions following the procedure described in Section 3.2.1. Also, the amount of carbohydrate released during the biosorption process was measured as described in Section 3.3.

3.3 Analytical Techniques

Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were determined gravimetrically as described by Standard Method 2540 (APHA 1995).

The amount of carbohydrate released during biosorption process was measured by using phenol sulphuric acid method (Dubois *et al.,* 1956). The principle of the method is the spectrophotometric measurement of the color formed upon the addition of phenol (50 μL) and sulphuric acid (5 mL) into a sample (2 mL) containing sugar in the test tubes. The absorbance of the characteristic yellow-orange color was measured at 480 nm using Jenway 6105 U.V./Vis. spectrophotometer. A previously prepared calibration curve given in Appendix A with alginate as the standard was then used to calculate the concentration of carbohydrate in each sample.

pH was measured by a EMAF EM78X pH meter.

CHAPTER 4

RESULTS

4.1 Sorption Kinetics

In order to investigate the time dependency of adsorption and also the influence of C/N ratio on the rate of adsorption, kinetic studies were held for five different metals and for each C/N ratio. These experimental studies were conducted by mixing heavy metal solutions, initial pH which of were set at 4.0, with the known amount of biomass and taking samples at different time intervals beyond which there is no net sorption. Both the initial metal and biomass concentrations are factors influencing rate of biosorption. So, due to differences in biomass concentration, three different initial metal concentrations were used for three C/N ratios in order to keep the initial metal to biomass concentration ratio constant at around 0.6 g/g.

4.1.1. Lead

The initial Pb(II) concentrations were 2000 mg/L, 3000 mg/L and 4000 mg/L for the C/N ratios of 9, 21 and 43, respectively. Figure 4.1 represents the time course of Pb(II) removal by activated sludge grown under three different C/N ratios for 6 h. It can be seen from the results that Pb(II) uptake was very rapid for all C/N ratios. Approximately, 85-90% of the biosorption occured in the first minute and in all cases, there was a sudden increase in Pb(II) adsorption capacity of the biomass during the first 5 min. After 5 min, the Pb(II) uptake rate gradually decreased and it remained nearly constant after 60 min for all C/N ratios.

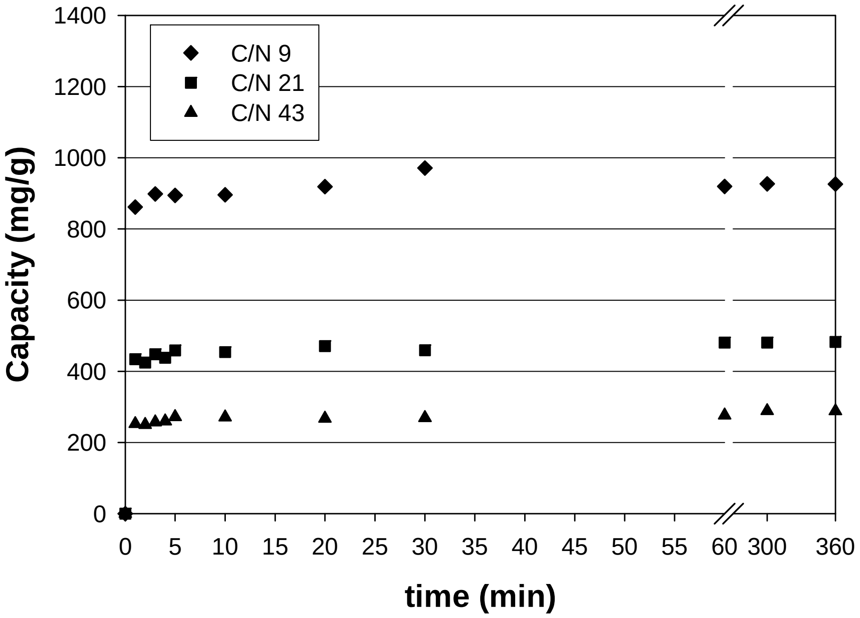


Figure 4.1. Time course of Pb(II) biosorption for three different C/N ratios

As it can be seen from Figure 4.1, the biosorption capacity of sludge increases with the decrease of C/N ratio. The capacities were approximately found as 950, 500 and 300 mg Pb(II)/g biomass for C/N ratios 9, 21 and 43, respectively.

As it was given in Figure. 4.2, in all kinetic experiments with Pb(II) there was a decrease in pH as biosorption proceeded. However, later on pH started to increase, and reached equilibrium after about an hour. It was seen that decrease in pH was much lower in C/N 43 compared with C/N 21 and 9. This decrease in pH implies that H^+ ions were released into the solution during biosorption but in different amounts for different C/N ratios.

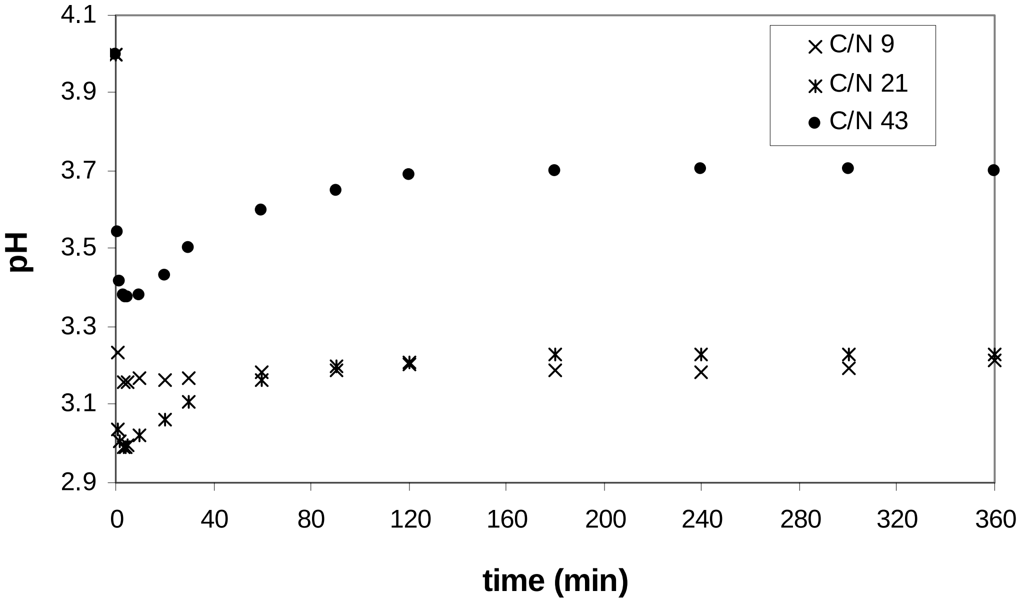
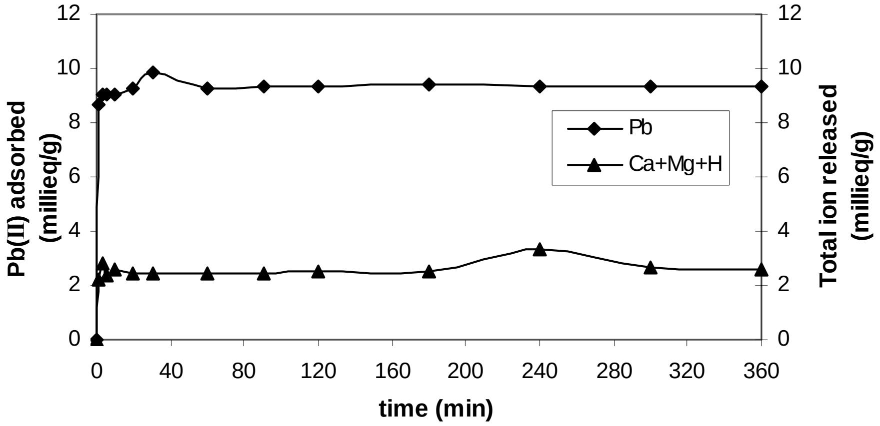
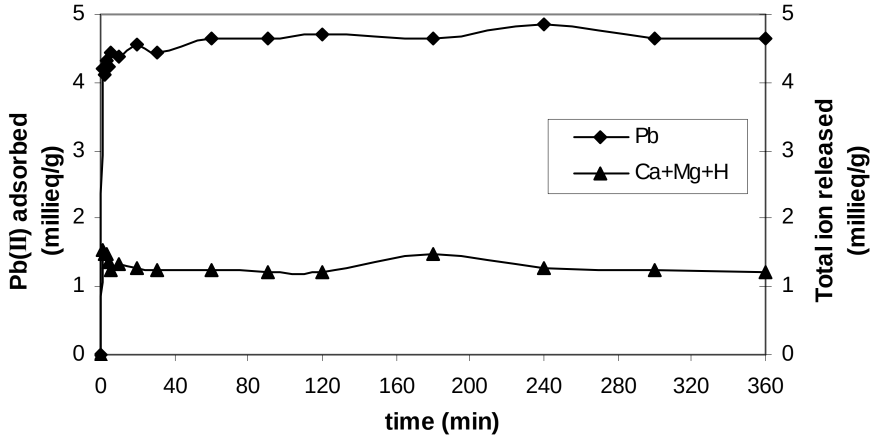


Figure 4.2. pH changes during Pb(II) biosorption for three different C/N ratios

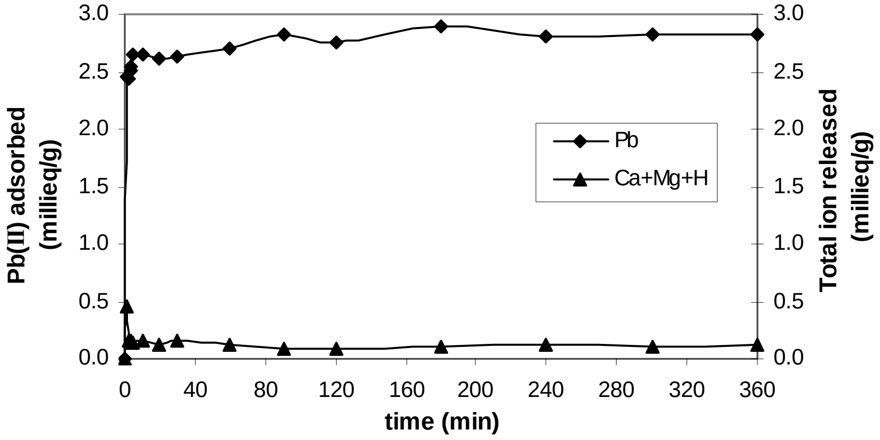
On the other hand, as Figure 4.3 shows, in parallel with H^+ ions release, there was also release of Mg(II) and especially Ca(II) ions with the uptake of the Pb(II). But, a comparison of the amount of Pb(II) ions adsorbed with the total ions (sum of Ca(II), Mg(II) and H) released show that Pb(II) adsorbed is always higher than the amount of total ions released at all C/N ratios. This indicates that an ion exchange mechanism is involved in biosorption but the whole Pb(II) removal can not be explained by ion exchange mechanism.



(a)



(b)



(c)

Figure 4.3. The change of the amount of Pb(II) ion adsorbed and total ion released (Ca(II), Mg(II)and H) with time a) C/N 9 b) C/N 21 c) C/N 43

It can be seen from Figure 4.3 that the amount of Pb(II) ion adsorbed and total ion released during biosorption remarkably decreases with the increase of C/N ratio but the ratio between them remain nearly the same for the ratio of C/N 9 and 21. This ratio increases when the C/N increases to 43.

4.1.2. Cadmium

The kinetic results of Pb(II) biosorption, at first, showed that the process was completed within 2 h. Beyond which there was no significant sorption. Thus, 4 h was selected as the duration of kinetic experiments for the other metals.

The initial Cd(II) concentrations were selected as 200 mg/L, 300 mg/L and 400 mg/L for the C/N ratios of 9, 21 and 43. Figure 4.4 represents results of Cd(II) biosorption kinetic tests by activated sludge for three different C/N ratios. It can clearly be seen from the results that Cd(II) uptake was again very rapid at the beginning for all C/N ratios. As it was observed in Pb(II) biosorption; approximately 80% of the biosorption occurred within the first minute and in all cases, there was a sudden increase in Cd(II) adsorption capacity of the biomass during the first 5 min. After 5 min, the Cd(II) uptake rate gradually decreased. The equilibrium was observed after 120 min for all C/N ratios which was rather long compared with Pb(II) biosorption.

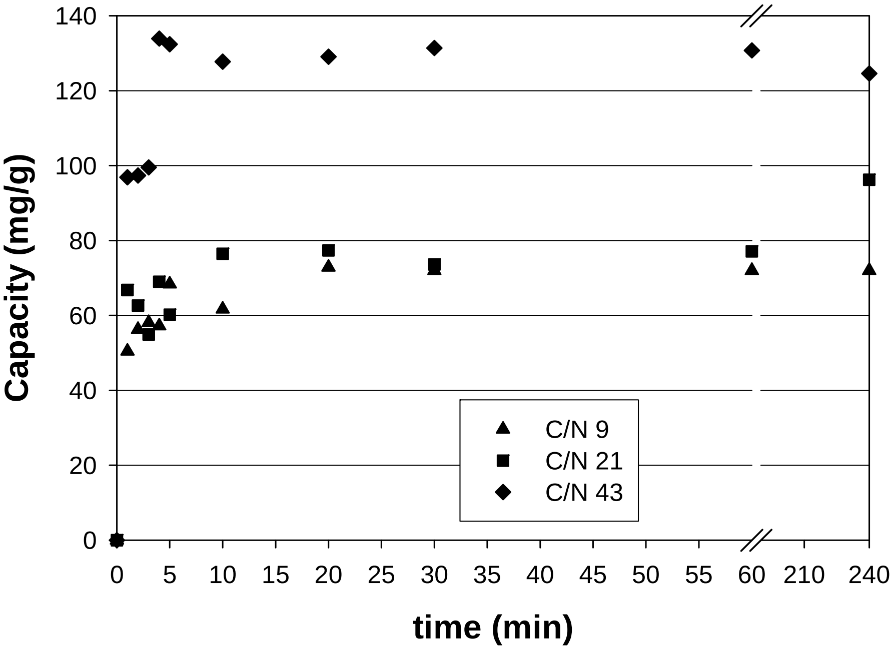


Figure 4.4. Time course of Cd(II) biosorption for three different C/N ratios

As it can be seen from Figure 4.4, in opposition to Pb(II), the biosorption capacity of activated sludge increased with the increase of C/N ratio. The capacities were approximately found as 70, 95 and 125 mg Cd(II)/g biomass for C/N ratios 9, 21 and 43, respectively. Figure 4.5 shows the pH behaviour of Cd(II) bisorption for all C/N ratios. It can be seen that for all kinetic experiments, first there was a sudden increase in pH from the initial value of 4.0 to 6.0 and after a small decrease during biosorption, the pH reached equilibrium approximately with a value of 5.8. Also, it was observed that for all C/N ratios, equilibrium pH values were very close to each other.

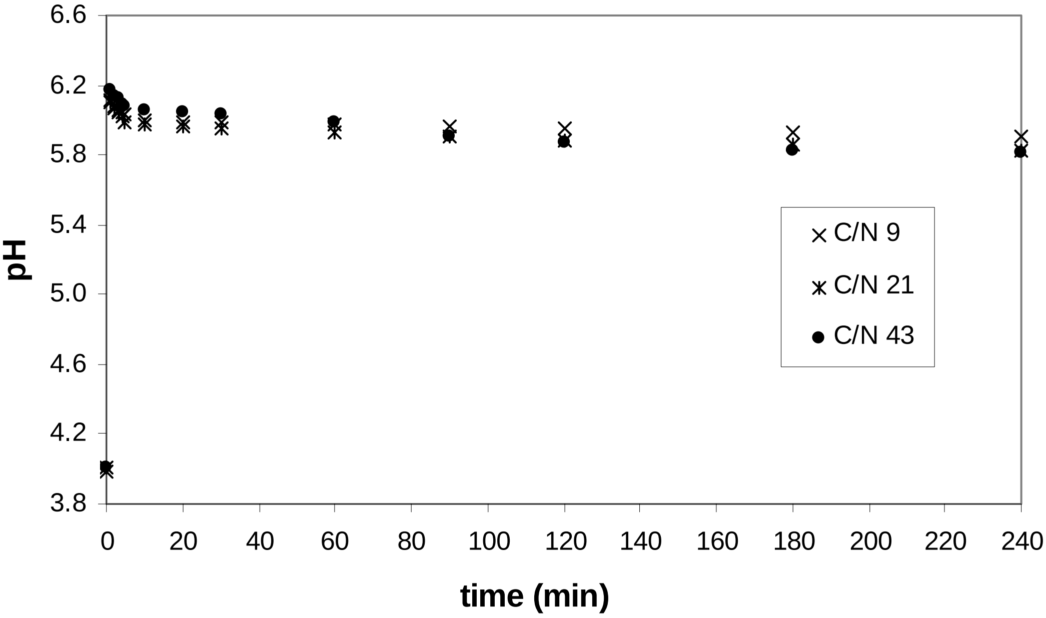
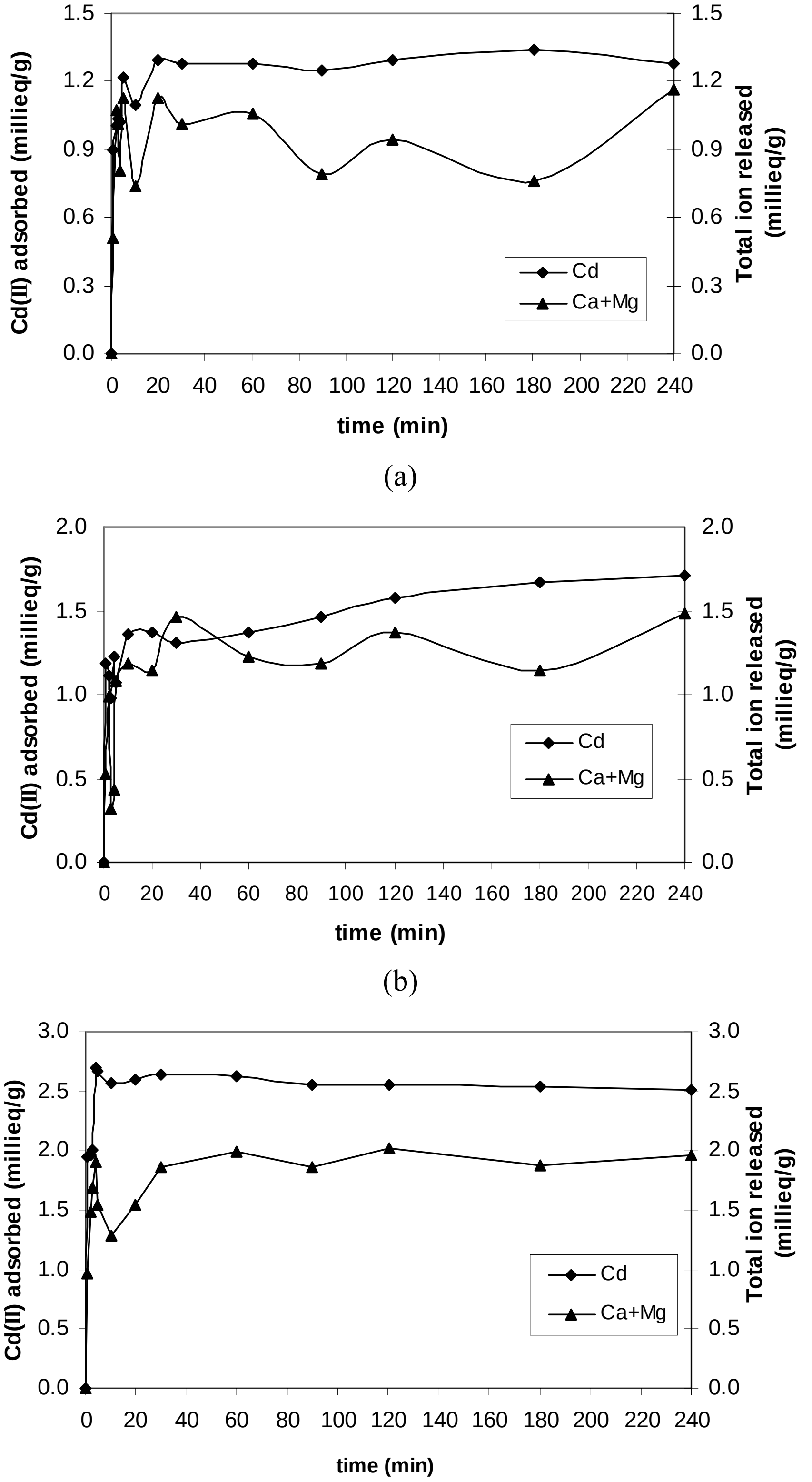


Figure 4.5. pH changes during Cd(II) biosorption for three different C/N ratios

When the amount of Cd(II) ions adsorbed was compared with the amount of Ca(II) and Mg(II) released (in this case there is no H release) during biosorption, again it was found that Cd(II) adsorbed is always higher than the amount of total ion released at all C/N ratios (Figure 4.6). However, the ratio of Cd(II) adsorbed to the total ions released is higher compared to that of Pb(II) adsorbed to total ions released. This indicates that in the sorption of Cd(II) ion exchange is a more predominant mechanism. Also, it can be seen from Figure 4.6, the amount of Cd(II) ion adsorbed and total ion released during biosorption increases with the increase of C/N and the ratio between them remain nearly the same for all C/N ratios.



(a)

(b)

(c)

Figure 4.6. The change of the amount of Cd(II) ion adsorbed and total ion released (Ca(II) and Mg(II)) with time a) C/N 9 b) C/N 21 c) C/N 43

4.1.3. Copper

For the kinetic studies of copper, the same amount of metal and biomass were used as it was described for cadmium uptake in Section 4.1.2. The results are shown in Figure 4.7. Similar initial rapid uptake was observed and again about 85 % of the biosorption occurred within the first minute. The process reached equilibrium after about 60 min for all C/N ratios. The equilibrium biosorption capacities were approximately found as 90, 90 and 50 mg Cu(II)/g biomass for C/N ratios 9, 21 and 43, respectively. As it can be seen easily, the biosorption capacity of reactors was nearly the same for the C/N ratios of 9 and 21. However, when the C/N ratio increased to 43, biosorption capacity decreased significantly.

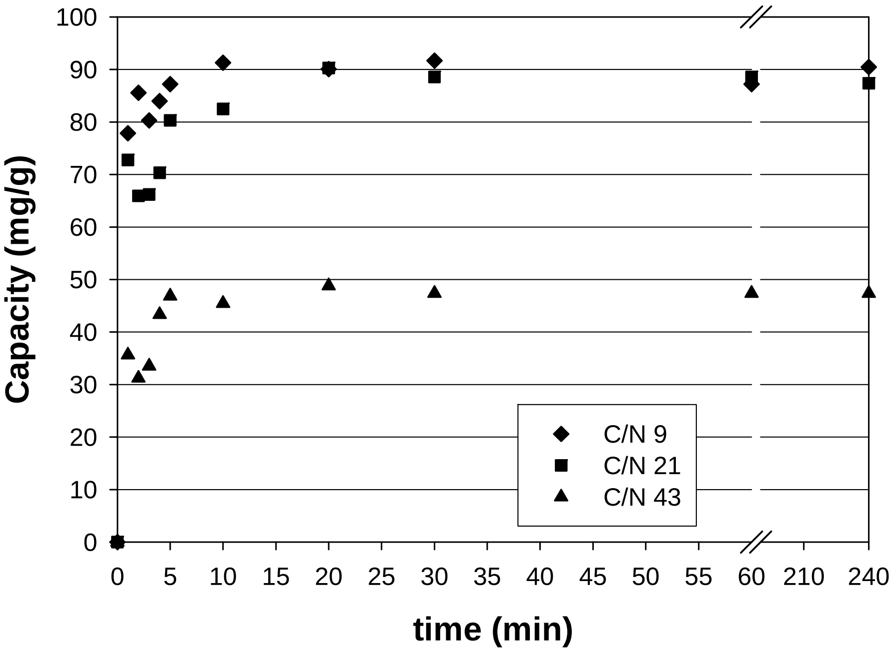


Figure 4.7. Time course of Cu(II) biosorption for three different C/N ratios

As it can be depicted from Figure 4.8, for all C/N ratios there was a rapid increase from the initial value of 4 to 4.8 within the first minute and with a small decrease during the process, the pH reached equilibrium at a value of approximately at 4.7. Also, it was observed for all C/N ratios that, obtained pH values are very close to each other as it was in Cd(II) biosorption.

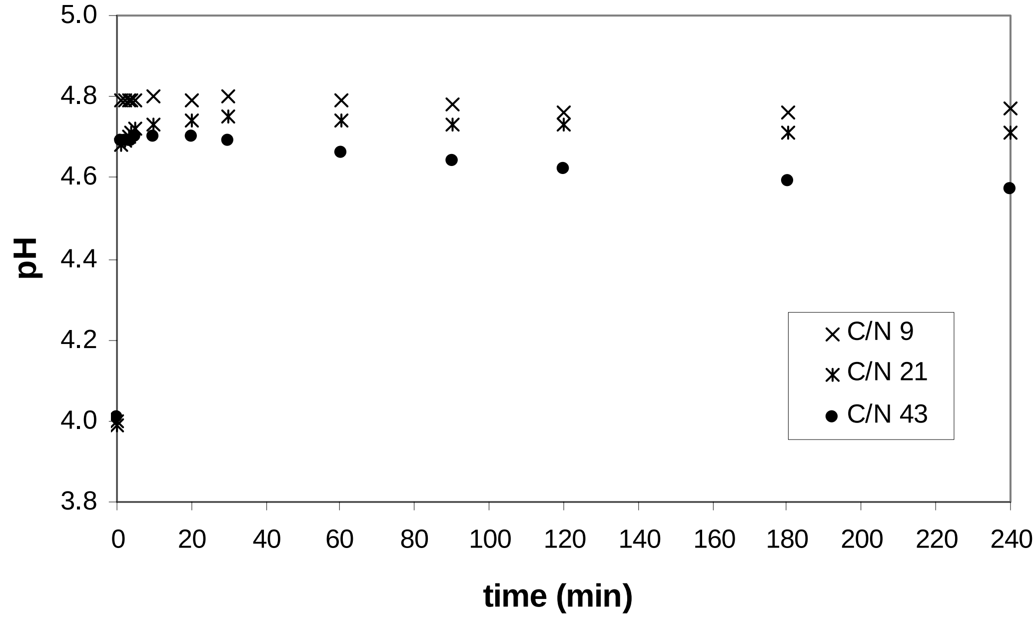
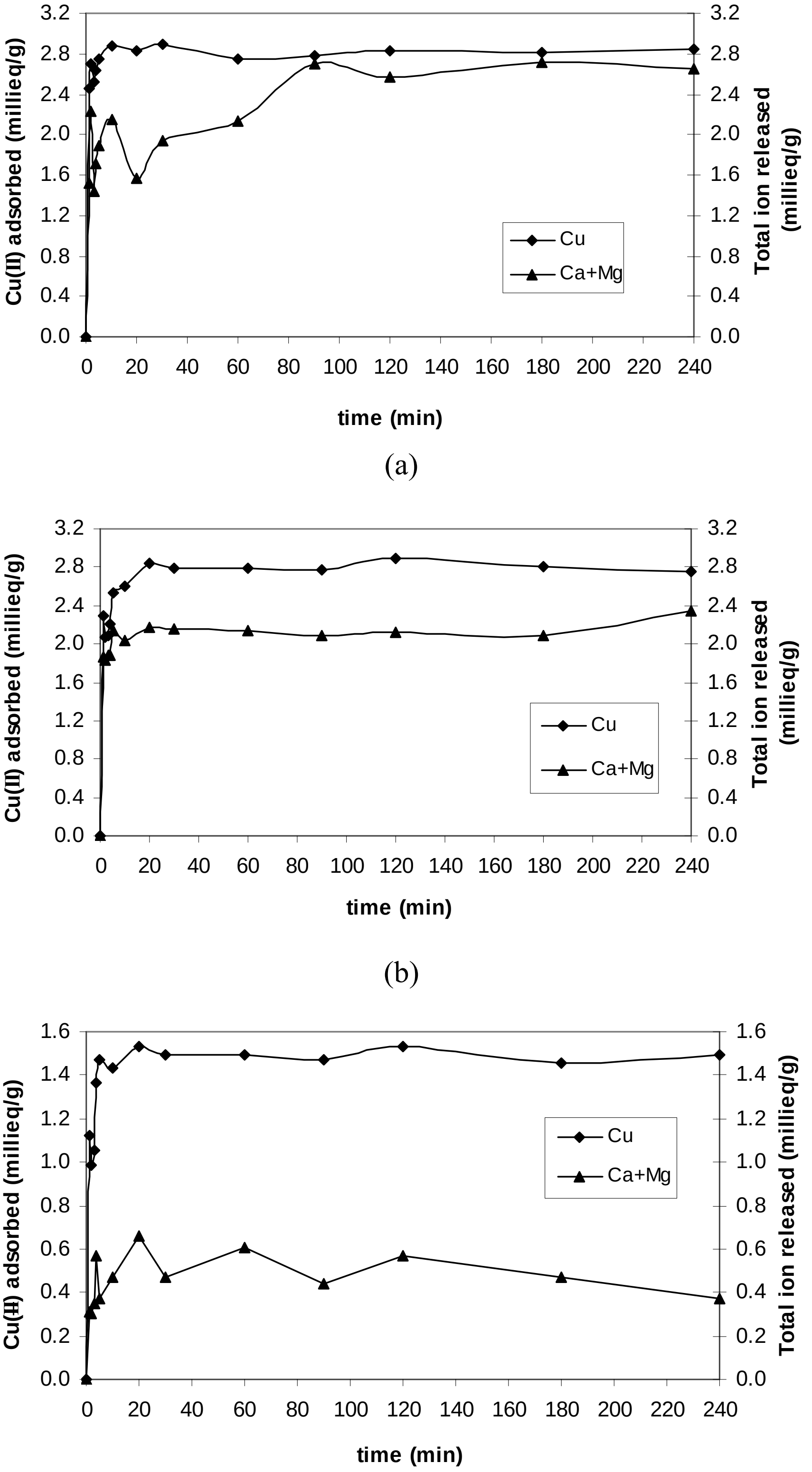


Figure 4.8. pH changes during Cu(II) biosorption for three different C/N ratios

When the amount of Cu(II) ions adsorbed were compared to the amount of Mg(II) and Ca(II) release, it was found that Cu(II) adsorbed is always higher than the amount of total ion released for the C/N ratios 21 and 43 (Fig. 4.9). For the C/N ratio 9, the amount of adsorbed and released ions is nearly same after 90 min. (Figs 4.9a). The amount of Cu(II) ion adsorbed and total ion released during biosorption decrease with the increase of C/N. Also, Cu(II) adsorbed to Ca(II) and Mg(II) released ratio is nearly the same for the C/N ratios 9 and 21; and higher for the C/N ratio 43.



(a)

(b)

(c)

Figure 4.9. The change of the amount of Cu(II) ion adsorbed and total ion released (Ca(II) and Mg(II)) with time a) C/N 9 b) C/N 21 c) C/N 43

4.1.4. Zinc

As presented in Figure 4.10, the time course of zinc biosorption which followed similar trend with other metals and metal uptake reached equilibrium approximately after 60 min for all C/N ratios. The biosorption capacities were approximately found as 60, 110 and 35 mg Zn(II)/g biomass for C/N ratios 9, 21 and 43, respectively, which indicated that when C/N increased from 9 to 21 biosorption capacity increased, on the other hand, with the increase of C/N ratio from 21 to 43, capacity decreases.

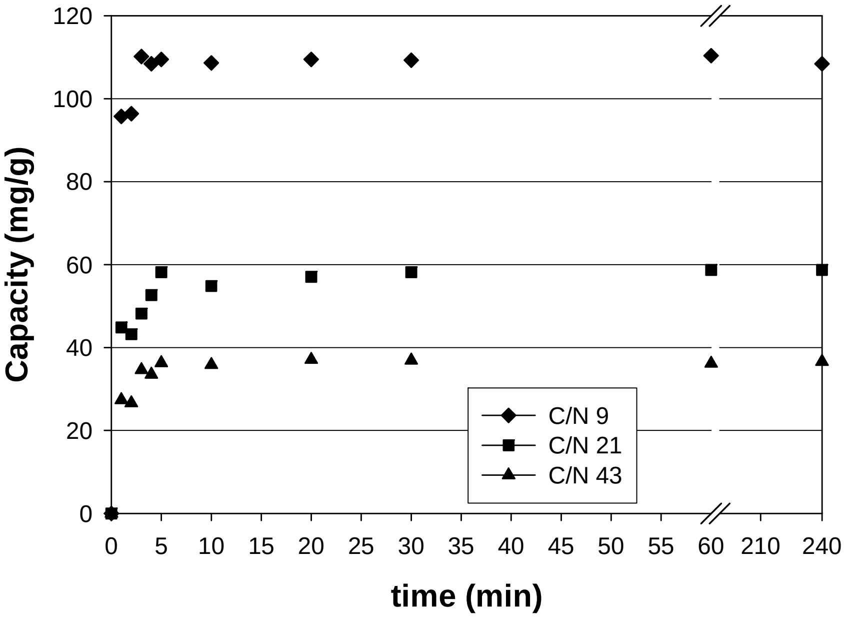


Figure 4.10. Time course of Zn(II) biosorption for three different C/N ratios

Also, time course of pH followed a similar trend with the metals of Cd(II) and Cu(II). For all C/N ratios, first there was a sudden increase from the initial value of 4.0 to 5.8 which was followed by an insignificant decrease during the process, the pH reached equilibrium at around 5.7. Also, it was observed that for all C/N ratios, equilibrium pH values are very close to each other (Figure 4.11).

When the amount of Zn ions adsorbed were compared to the amount of Mg(II) and Ca(II) release, the amount of Zn(II) ion adsorbed and total ion released during biosorption were observed highest at C/N 21 and lowest at C/N 43 like the capacity values but the ratio between them increases with the increase of C/N ratio (Figure 4.12).

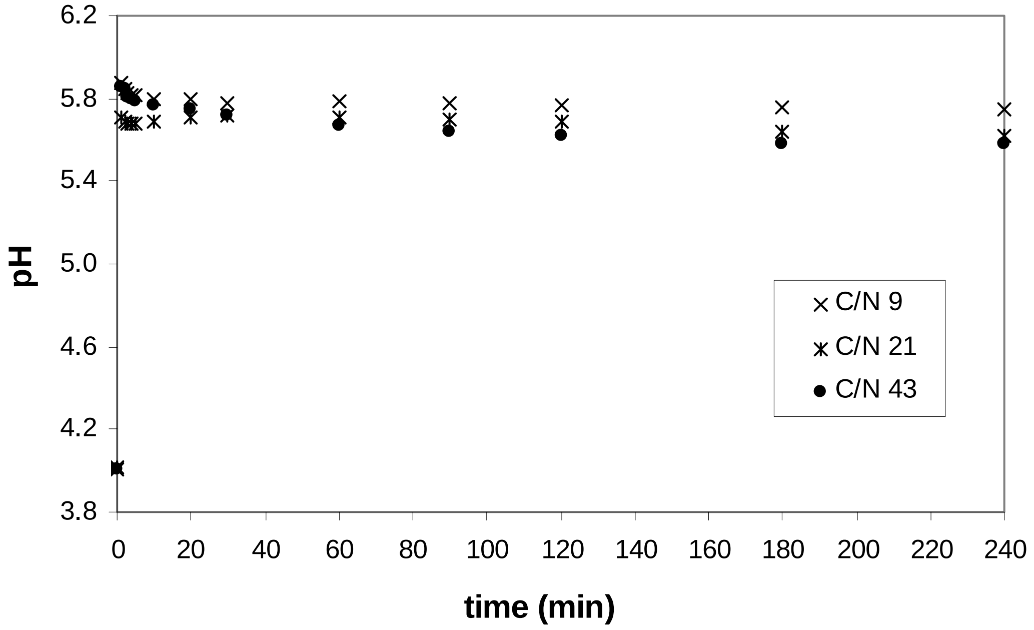
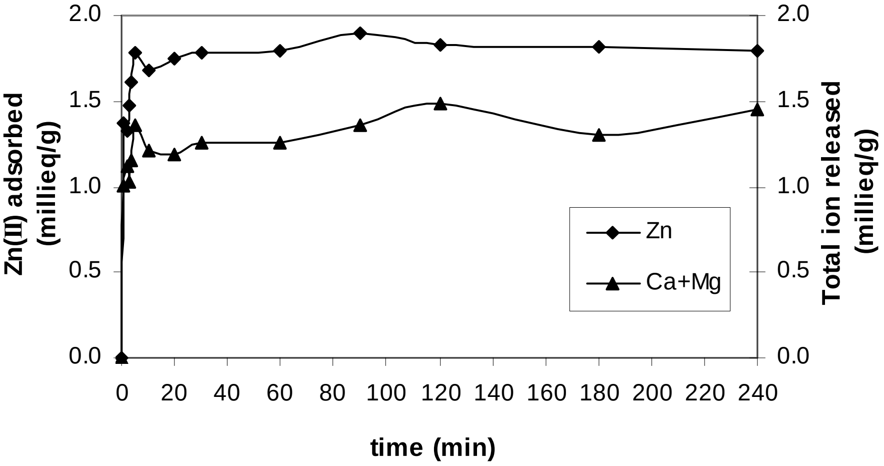
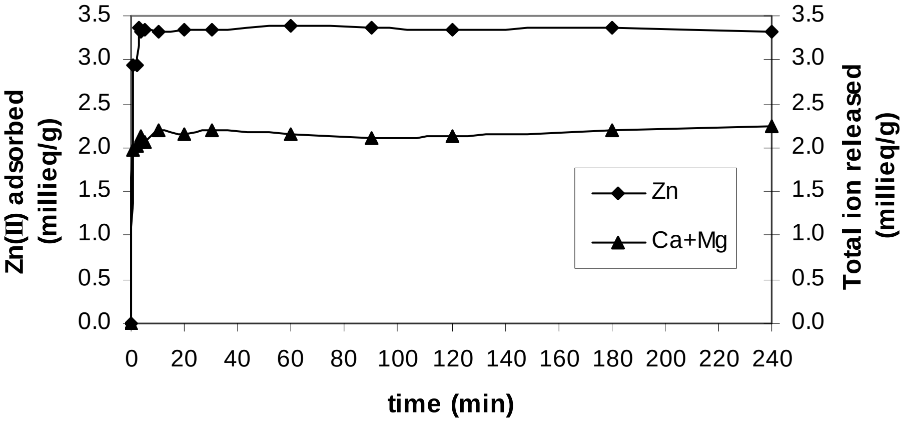


Figure 4.11. pH changes during Zn(II) biosorption for three different C/N ratios



(a)



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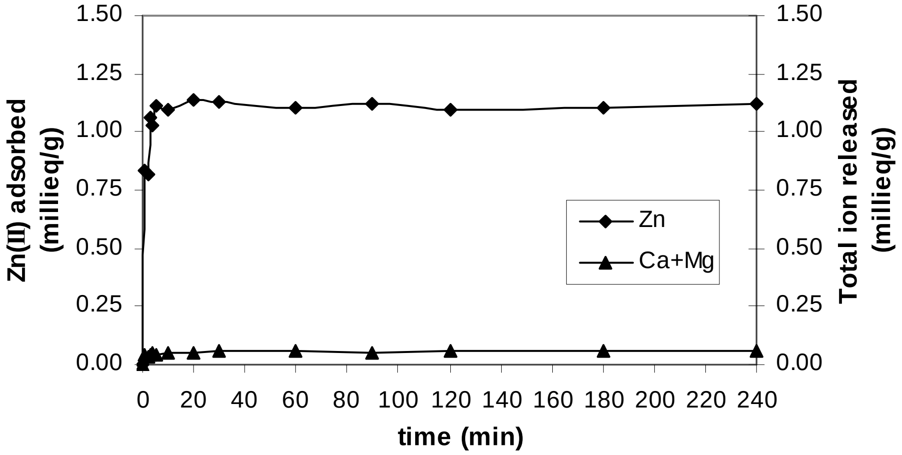


(c)

Figure 4.12. The change of the amount of Zn(II) ion adsorbed and total ion released (Ca(II) and Mg(II)) with time a) C/N 9 b) C/N 21 c) C/N 43

4.1.5. Nickel

As it can be readily seen from Figure 4.13, time course of nickel adsorption for all C/N ratios that nickel was unstable and there were consequent sorption and desorption cycles within a period of 2 h.

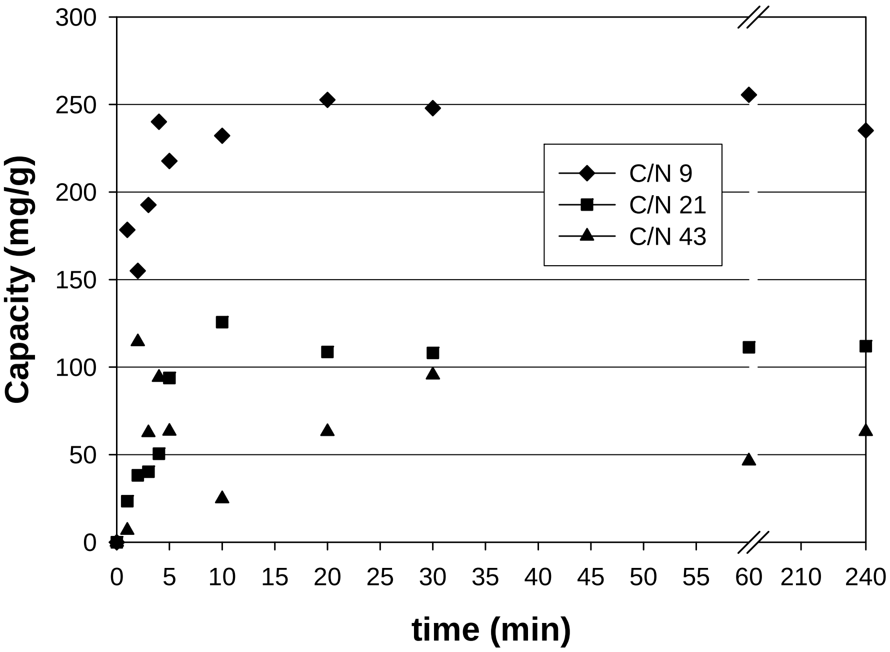


Figure 4.13. Time course of Ni(II) biosorption for three different C/N ratios

Following, a rapid uptake was observed in the first 5 min, equilibrium was established with the capacities of approximately 240, 135 and 40 mg Ni(II)/g biomass for C/N ratios 9, 21 and 43, respectively. Before the establishment of the equilibrium, system showed high fluctuations especially for C/N 43. This trend in time course of metal removal was specific to Ni(II).

In contrast to unstable biosorption capacities, system pH showed a stable behaviour. For all C/N ratios, firstly there was a sudden increase from the initial value of 4.0 to about 6.5, which then continued to increase during process until it reached equilibrium at about pH 7.0. Also, it was observed that for C/N ratios 9 and 21, obtained pH values were very close to each other but for C/N 43, pH was a little lower (Figure 4.14).

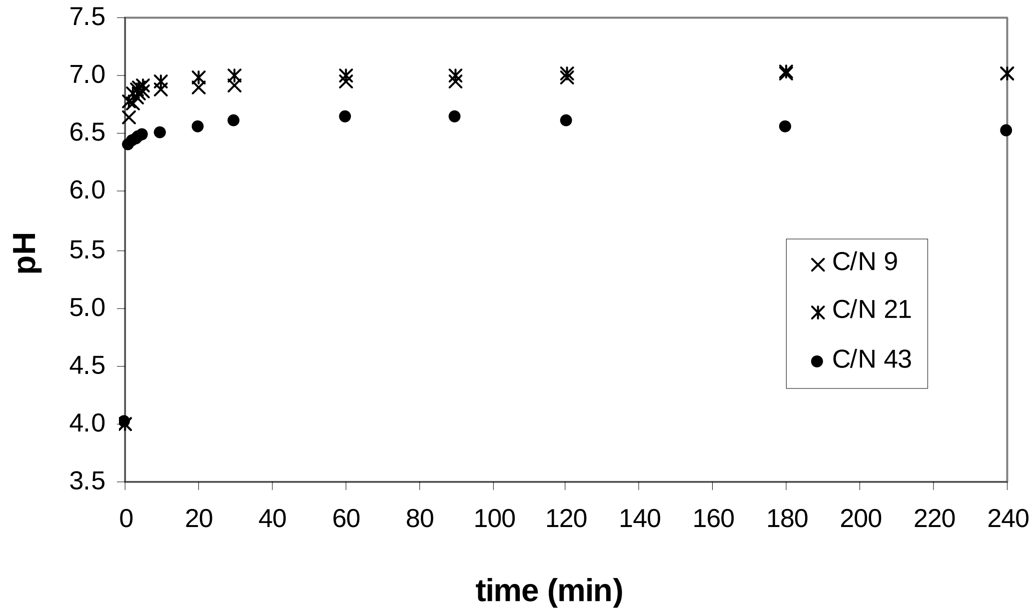
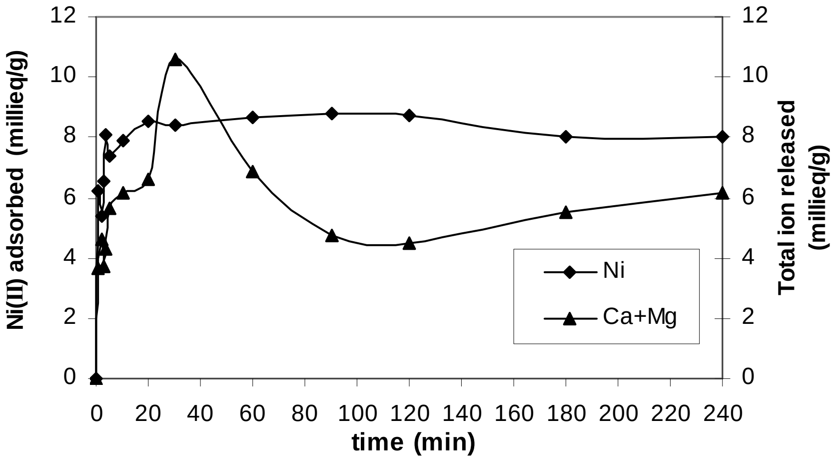
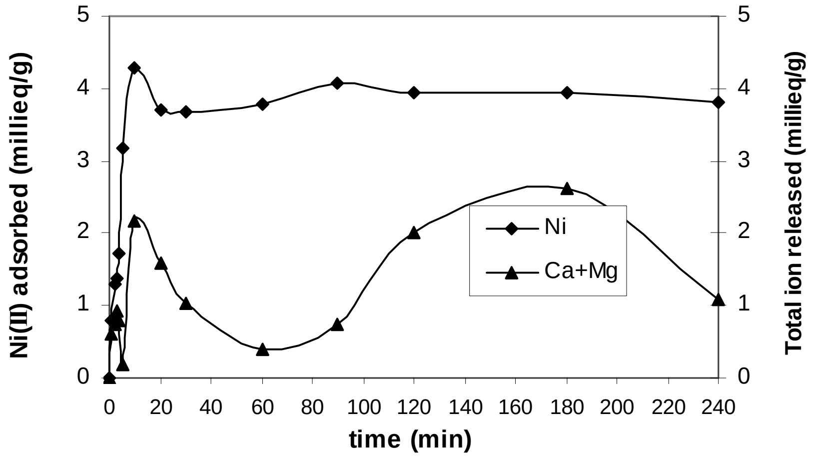


Figure 4.14. pH changes during Ni(II) biosorption for three different C/N ratios

As presented in Figure 4.15, the amount Mg(II) and Ca(II) released during biosorption were also fluctuating. Although these experiments were repeated several times to minimize the experimental errors and also to come up with reproducible data, it could not be possible to obtain a meaningful and stable data for nickel biosorption. It can be seen clearly that Ni(II) ions adsorbed and ions released during biosorption showed great fluctuations during the process. Even at some points released ions are higher than nickel ions adsorbed. So, it could not be possible to interpret this data or suggest a relationship between them.



(a)



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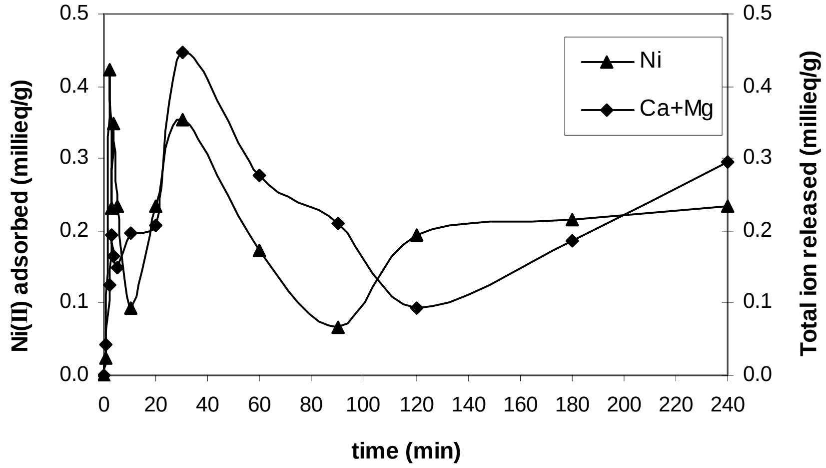


(c)

Figure 4.15. The change of the amount of Ni(II) ion adsorbed and total ion released (Ca(II) and Mg(II)) with time a) C/N 9 b) C/N 21 c) C/N 43

4.2 Sorption Equilibrium Tests

Sorption equilibrium tests were held in order to investigate the change in biosorption behaviour with initial metal concentration and to find the maximum biosorption capacity of five different metals for three different C/N ratios. Kinetic tests have shown that metal removal was almost completed within the first 2 h of biomass-heavy metal contact. So, to be on safer side, 4 h was employed as the equilibrium time for all metals.

4.2.1 Lead

The adsorptive capacity of the activated sludge grown under different C/N ratios was determined by examining the relationship between the amount of Pb(II) adsorbed by biomass (qe) and the metal concentration at equilibrium (C_e). The initial Pb(II) concentration was varied from 50 to 4000 mg/L while biomass concentration used was constant. The amount of biomass added for each metal concentration was 0.12, 0.21 and 0.31 g as dry matter for the C/N ratios of 9, 21 and 43, respectively. Adsorption isotherm data was presented in Figure 4.16. As shown, adsorption capacity was increasing with equilibrium metal ion concentration and reaching to a plateau which corresponds to maximum adsorptive capacity. The results showed that the maximum biosorptive capacities attained for activated sludge grown under different C/N ratios were very high. It was found as 995, 600 and 280 mg Pb(II)/g biomass, for the C/N ratios of 9, 21, and 43, respectively. It can clearly be seen that with the decrease of C/N ratio there was a remarkable increase in the Pb(II) sorption capacity of activated sludge. A two-fold increase in

C/N ratio from 21 to 43 resulted in 50 % decrease in the maximum adsorptive capacity. Similarly, with an increase in C/N ratio from 9 to 21, there was about 40% reduction in the maximum biosorptive capacity.

The pH measurements done in test beakers at equilibrium have shown that pH highly varies with the initial Pb(II) concentration. When equilibrium pH was plotted against initial Pb(II) concentration (Figure. 4.17), it was seen that in all cases, as the initial concentration increases, the equilibrium pH of the solution decreases. Especially for the C/N ratio of 9 and 21, there was a sharp decrease in equilibrium pH between the initial Pb(II) concentrations of 1000 mg/L and 1500 mg/L. This change in pH was thought to be due to a change in biosorption mechanism with the initial concentration of Pb(II). Since it is known that Pb(II) precipitate at pH values over 5, it could be stated that precipitation could take place at low initial metal concentrations because of high equilibrium pH values.

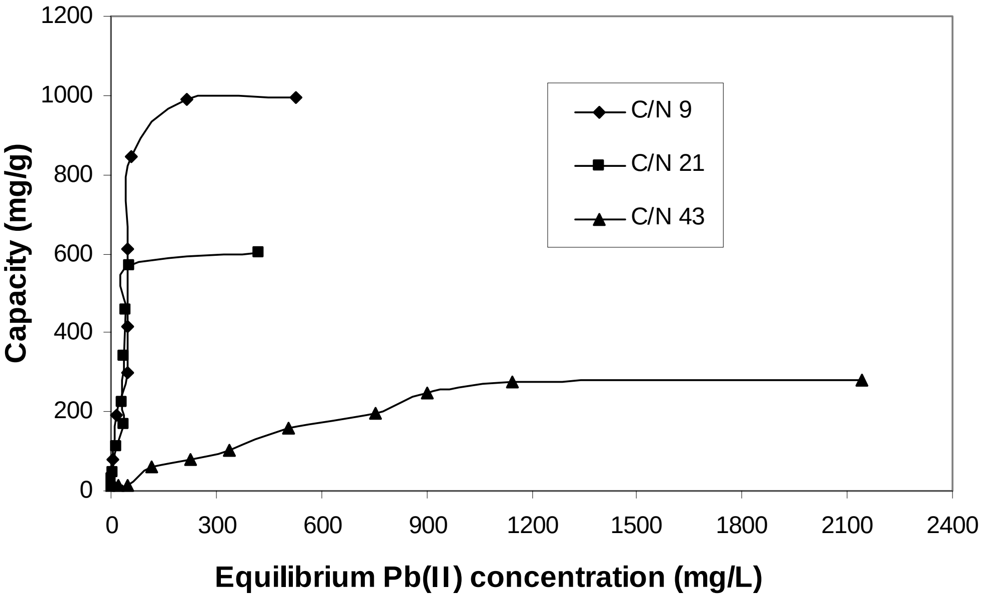


Figure 4.16. Adsorption isotherms of Pb(II) sorption for different C/N ratios

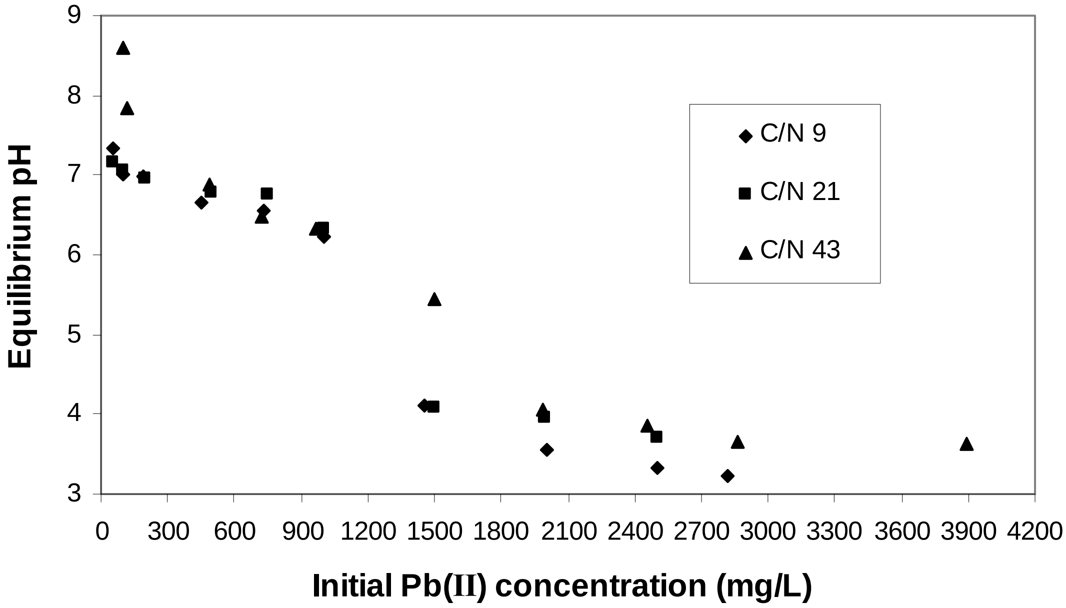
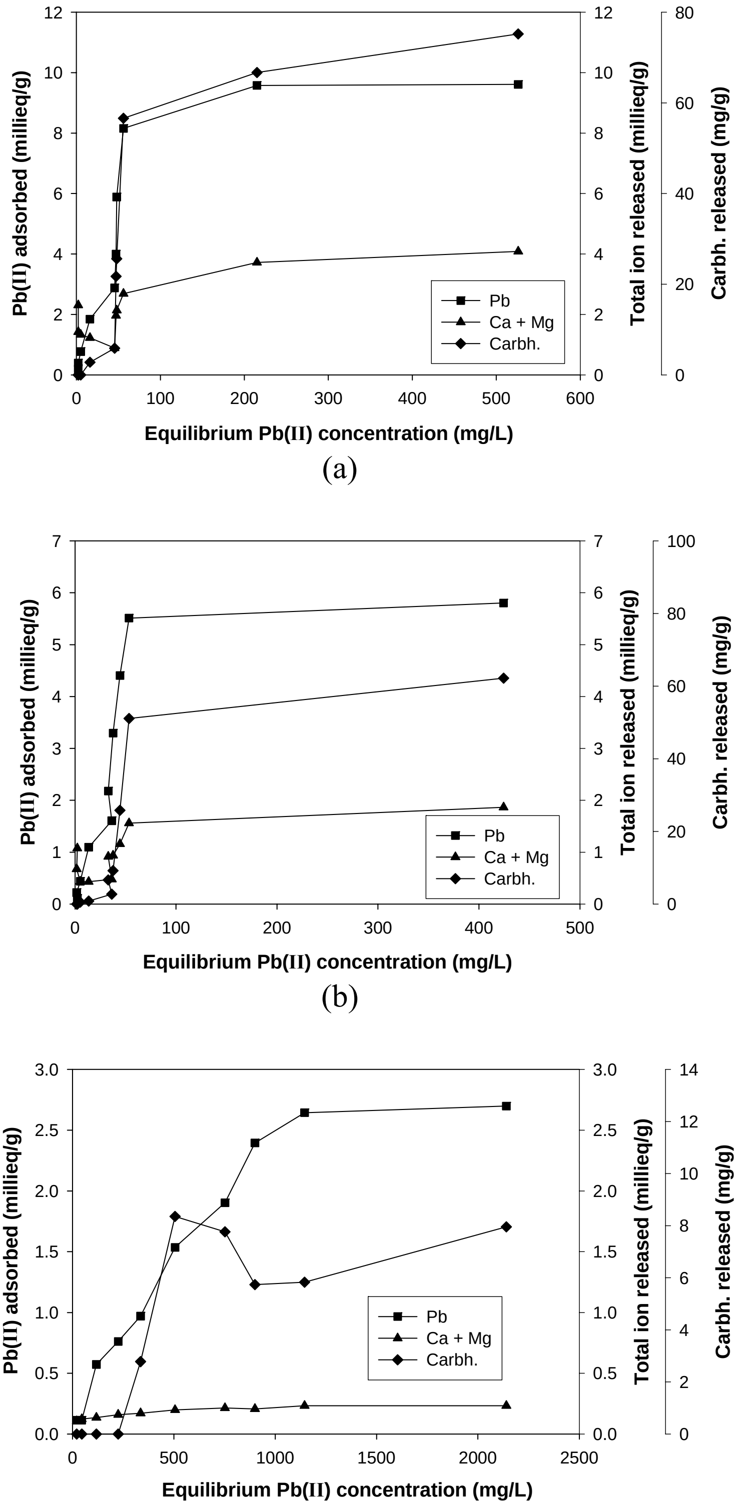


Figure 4.17. Effect of initial Pb(II) concentration on the equilibrium pH

In an attempt to investigate the biosorption mechanism, total amount of Ca(II) and Mg(II) ions released were also determined in isotherm experiments. In addition, the amount of carbohydrate released during biosorption was measured. It can be seen from Figure 4.18 that like the results from adsorption kinetic tests, some significant amount of cations are released from the surface. In all cases, at high equilibrium concentrations, the amount of Pb(II) adsorbed was higher than the amount of total ions released (Ca(II), Mg(II)). Also, at high Pb(II) concentrations it was observed that both adsorbed Pb(II) ions and total ions released during biosorption decreased with the increase of C/N ratio but the ratio of adsorbed Pb(II) ions to the total ions released remained nearly the same for all C/N ratios; whereas at low equilibrium concentrations, any regular relationship could not be observed between the adsorbed and released ions.



(a)

(b)

(c)

Figure 4.18. Amount of Pb(II) ion adsorbed and total ion (Ca(II), Mg(II))and carbohydrate released a) C/N 9 b) C/N 21 c) C/N 43

From Figure 4.18 it can clearly be seen that beside the Ca(II) and Mg(II) ions, for all C/N ratios also significant amount of carbohydrate was released into the solution during biosorption. Carbohydrate amount increased with the increase of equilibrium metal concentration and followed nearly the same trend with adsorbed and released ions with an exception of C/N 43. Also, similar to the adsorbed and released ions during biosorption, increase of C/N ratio resulted in a decrease of the carbohydrate amount released. Sludge extracellular polymers were observed to be very selective toward calcium ions. The selectivity to calcium is an important property of carboxylate containing polysaccharides (Bruus *et al.*, 1992). So, the release of carbohydrate could be attributed to this selectivity. It can be speculated that the release of calcium ions into the solution during biosorption could cause the release of some of the polysaccharides and an increase in the carbohydrate amount of solution.

4.2.2 Cadmium

Ten different initial metal concentrations used for the equilibrium tests of Cd (II), varied from 10 to 1000 mg/L. The amount of biomass added for each metal concentration was 0.11, 0.22 and 0.29 g as dry matter for C/N ratios of 9, 21 and 43, respectively. As it was in Pb(II) biosorption, the adsorption isotherm was of the standard L- shaped. The sorption capacity increased with equilibrium metal ion concentration and reached its maximum value after a certain concentration (Figure 4.19). As presented in the figure, the maximum Cd(II) biosorptive capacities were attained as 117, 140 and 173 mg Cd(II)/g biomass, for the C/N ratios of 9, 21, and

43, respectively. But in general, maximum capacities attained for Cd(II) were rather low when they were compared to the capacity values obtained for Pb(II) biosorption. Also, as it can be seen from the figure, the increase in C/N ratio resulted in an increase in the Cd(II) sorption capacity of activated sludge but the differences between attained capacities were not very different from each other.

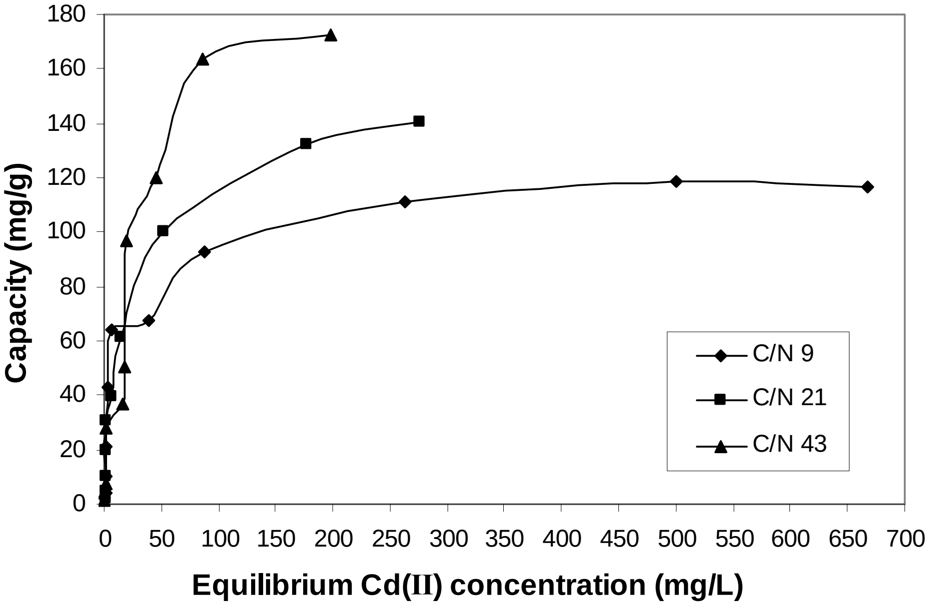


Figure 4.19. Adsorption isotherms of Cd(II) sorption for different C/N ratios

When the effect of initial Cd(II) concentration on equilibrium pH was investigated, it was observed that as the initial concentration increases, the equilibrium pH of the solution decreases which is similar to the Pb(II) biosorption. While the equilibrium pH values were nearly the same for the C/N ratios of 9 and 43, they were relatively higher for the C/N 21. As parallel to the observations with Pb(II), there was a significant decrease in equilibrium pH between the initial Cd(II) concentrations of 300 mg/L and 500 mg/L which pointed out a change in biosorption mechanism with the initial concentration of the metal (Figure 4.20).

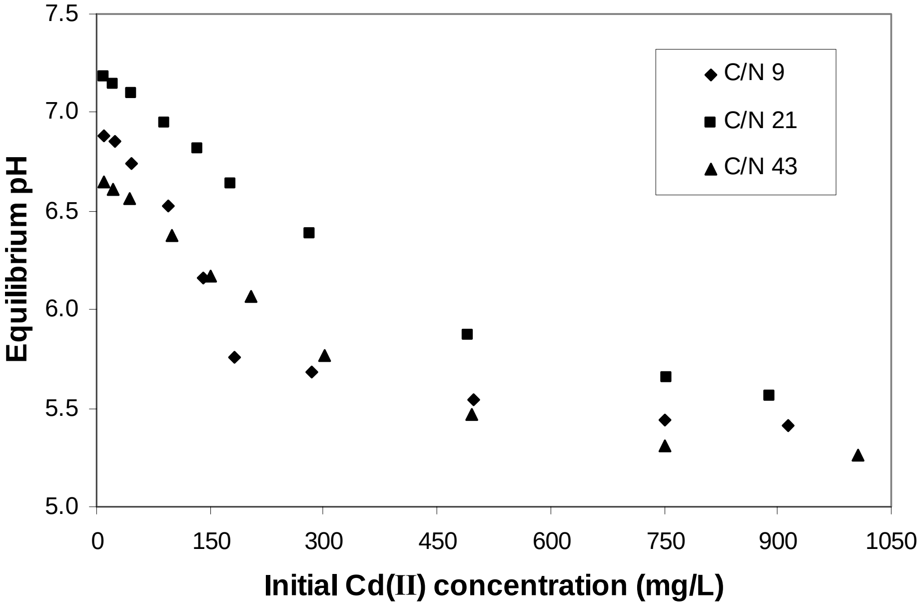
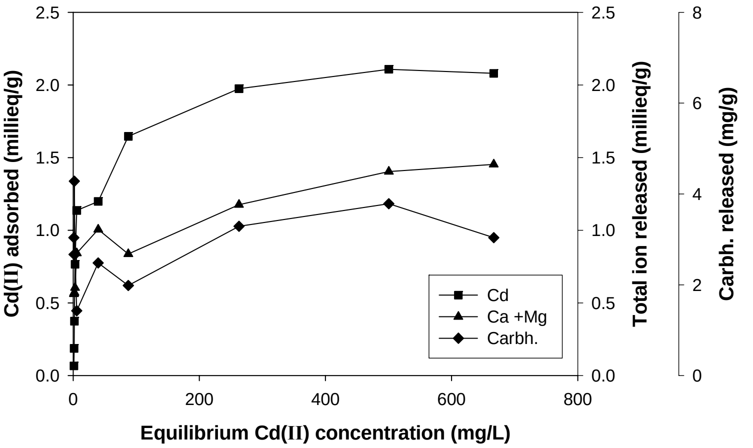
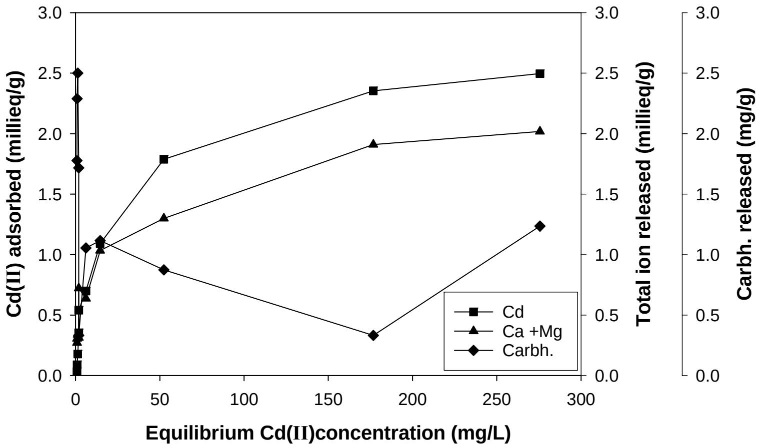


Figure 4.20. Effect of initial Cd(II) concentration on the equilibrium pH

Figure 4.21 represents the change in amount of Cd(II) ion adsorbed, sum of Ca(II)and Mg(II) released and also the amount of carbohydrate released with different equilibrium concentrations. At high equilibrium concentrations the amount of Cd(II) adsorbed was higher than the amount of total ions released (Ca(II), Mg(II)). As similar to Pb(II) sorption, at high equilibrium concentrations, the amount of Cd(II) ions adsorbed and total ions released were lowest at the C/N ratio of 9 and these values are very close to each other for the C/N ratios 21 and 43. But, the ratio of adsorbed Cd(II) ions to the total ions released remained nearly the same for all C/N ratios. Different from Pb(II) biosorption mechanism, Cd(II) biosorption is believed to be more ion exchange origin due to the closeness of released ion concentration and the adsorbed Cd(II) concentration.



(a)



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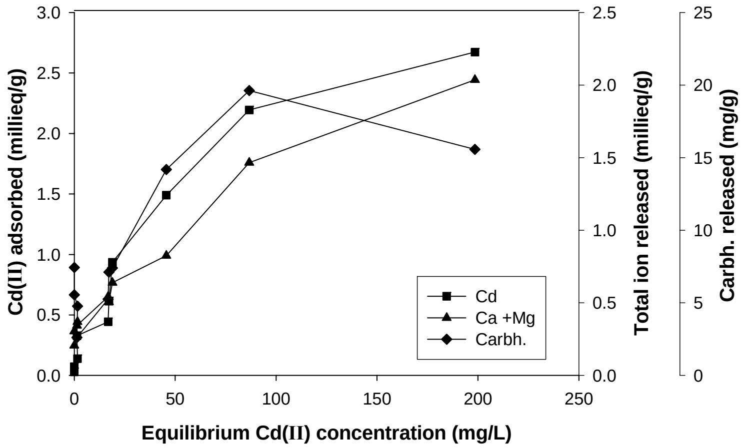


(c)

Figure 4.21. Amount of Cd(II) ion adsorbed and total ion (Ca(II), Mg(II))and carbohydrate released a) C/N 9 b) C/N 21 c) C/N 43

As it can be seen from Figure 4.21, similar to Pb(II) biosorption, there was a carbohydrate release during Cd(II) biosorption for all C/N ratios; but this time, a different tendency was observed for the released carbohydrate amount. Although, the amount of released ions increased when the C/N ratio increased from 9 to 21 and remained nearly same when the ratio increased from 21 to 43, the carbohydrate amount was very low at C/N 21 and significantly high at C/N ratio 43.

4.2.3 Copper

Ten different initial metal concentrations varying from 10 to 1000 mg/L were used for the equilibrium test of Cu(II). The amount of biomass added for each metal concentration was 0.1, 0.22 and 0.3 g as dry matter for C/N ratios of 9, 21 and 43, respectively. The maximum biosorptive capacities of activated sludge cultivated C/N ratios of 9, 21 and 43 were attained as 161, 90 and 45 mg Cu(II)/g biomass, respectively. In general, the Cu(II) biosorptive capacity of activated sludge was almost identical to Cd(II) sorption capacity and much lower than Pb(II). On the other hand, the variation in capacity with C/N ratio was quite similar to Pb(II) biosorption. There was a remarkable increase in the Cu(II) sorption capacity of activated sludge with the decrease of C/N from 21 to 9. However, further increase in C/N from 21 to 43, has resulted in a 40% reduction in maximum Cu(II) biosorption capacity of activated sludge (Figure 4.22).

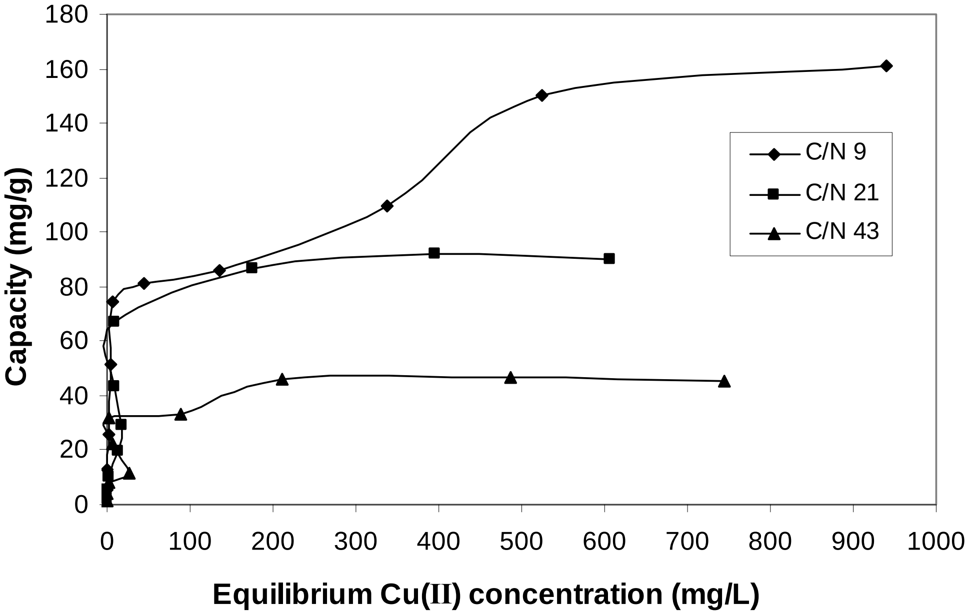


Figure 4.22. Adsorption isotherms of Cu(II) sorption for different C/N ratios

Figure 4.23 presents the change of equilibrium pH with initial metal concentration. The initial Cu(II) concentration effect on equilibrium pH followed the same pattern with Pb(II) and Cd(II) biosorption. As the initial concentration increased, the equilibrium pH of the solution decreased. For low Cu(II) concentrations higher equilibrium pH values were obtained for the C/N 21. However, at high initial metal concentrations the equilibrium pH values were nearly the same for all C/N ratios. On the other side, at all C/N values, there was a significant decrease in equilibrium pH between the initial Cu(II) concentrations of 300 mg/L and 500 mg/L.

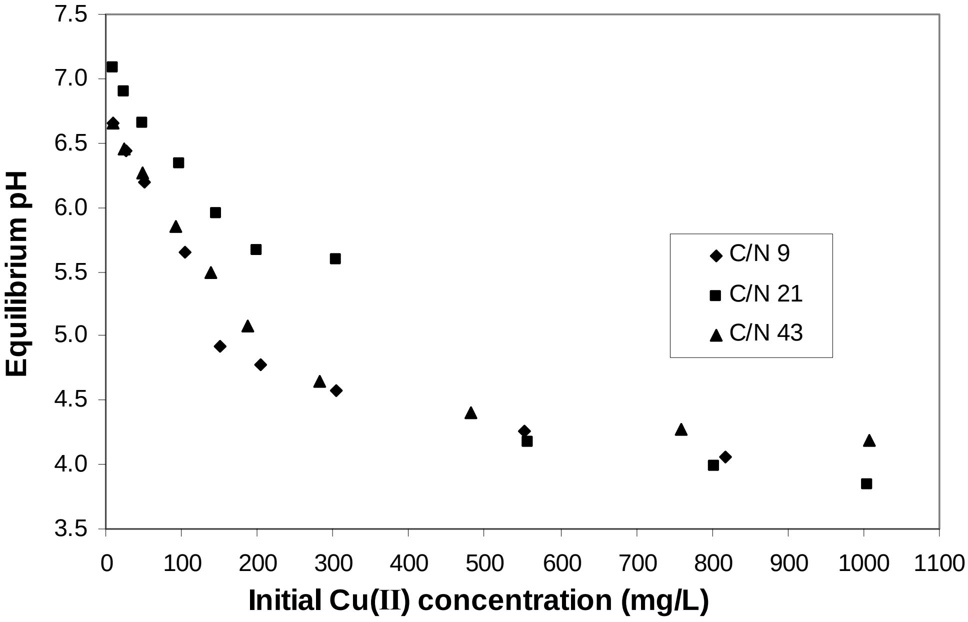
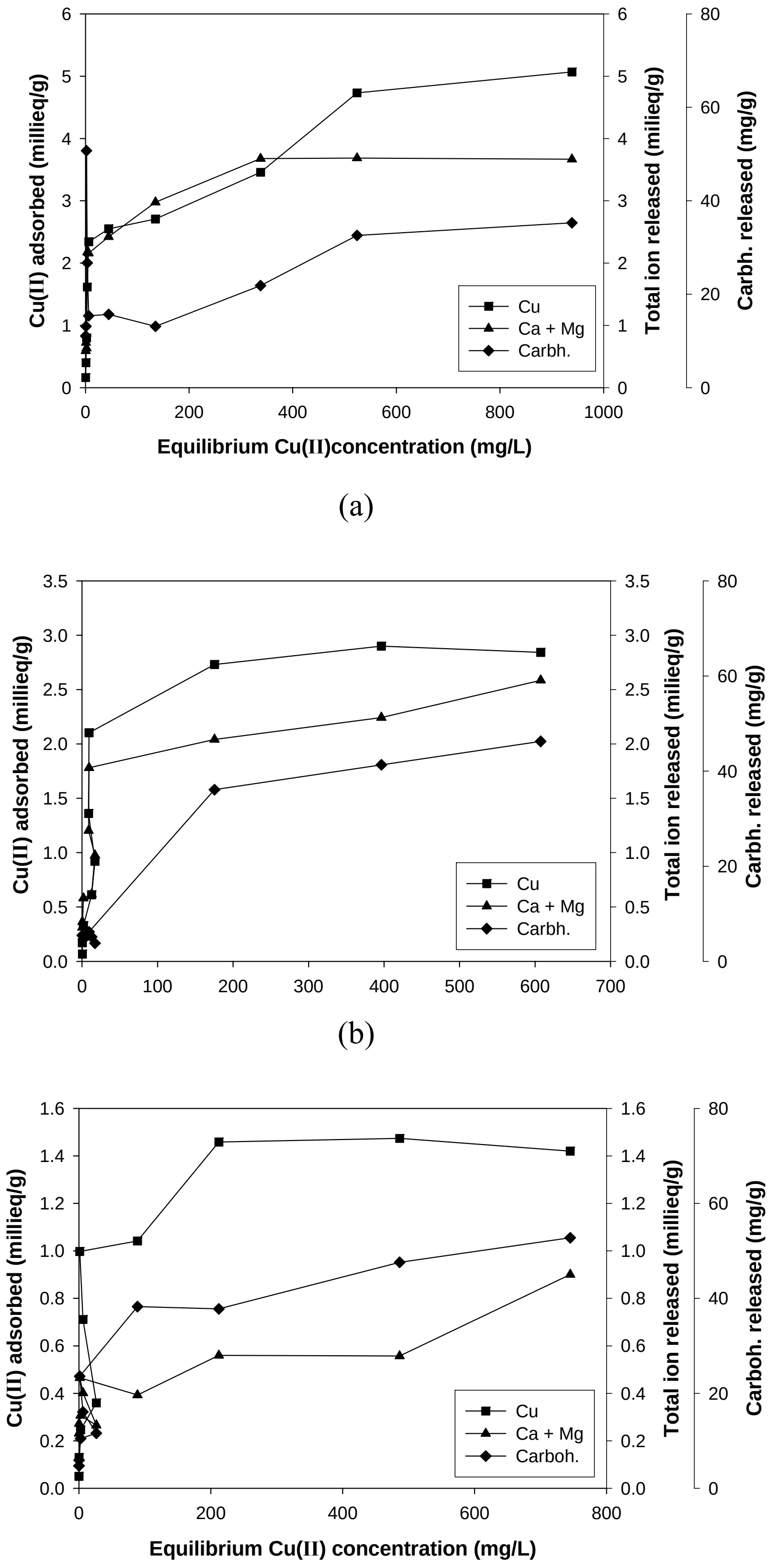


Figure 4.23.. Effect of initial Cu(II) concentration on the equilibrium pH

When the amount of Cu(II) ions adsorbed during biosorption was compared with the total ion released (Figure 4.24), it was observed that both the adsorbed and released ions decreased gradually with the increase of C/N ratio. Especially there was a significant decrease in the amount of released ions with the increase of C/N ratio to 43. According to these results, a decrease in carbohydrate amount was also expected with increase of C/N ratio but surprisingly the opposite was observed.



(a)

(b)

(c)

Figure 4.24. Amount of Cu(II) ion adsorbed and total ion (Ca(II), Mg(II))and carbohydrate released a) C/N 9 b) C/N 21 c) C/N 43

4.2.4 Zinc

The initial metal concentrations used for the equilibrium tests of Zn(II) were in the same concentration range with Cu(II) and Cd(II) studies. The amount of biomass added for each metal concentration was 0.1, 0.21 and 0.31g as dry matter for C/N ratios of 9, 21 and 43, respectively. The results are presented in Fig. 4.25, as isotherm plots. The maximum biosorption capacities for Zn(II) at different C/N ratios were attained as 90, 117 and 39 mg Zn(II)/g biomass, for the C/N ratios of 9, 21, and 43, respectively. A comparison of C/N effect on biosorption capacity indicates that Zn(II) behaves totally differently than the other metals investigated. When the C/N ratio was increased from 9 to 21, biosorptive capacity of activated sludge also increased a little, but an increase in C/N from 21 to 43 resulted in a drastic decrease in biosorption capacity, which was about 35% reduction.

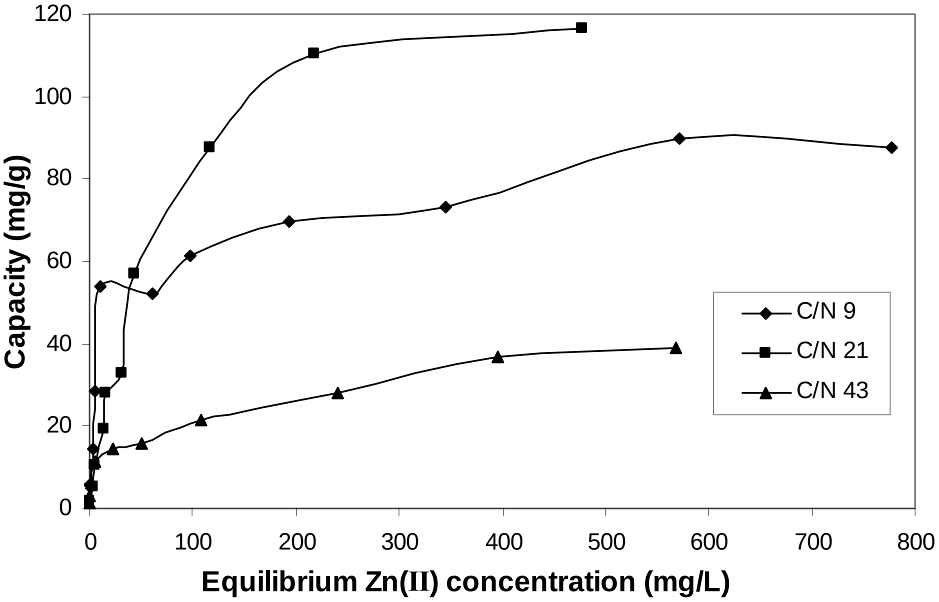


Figure 4.25. Adsorption isotherms of Zn(II) sorption for different C/N ratios

The decrease in equilibrium pH with the increase in initial metal concentration was also observed for the biosorption of Zn(II) at all C/N ratios (Fig 4.26). The equilibrium pH values obtained were nearly the same for all C/N ratios and as a similar trend with other metals investigated.

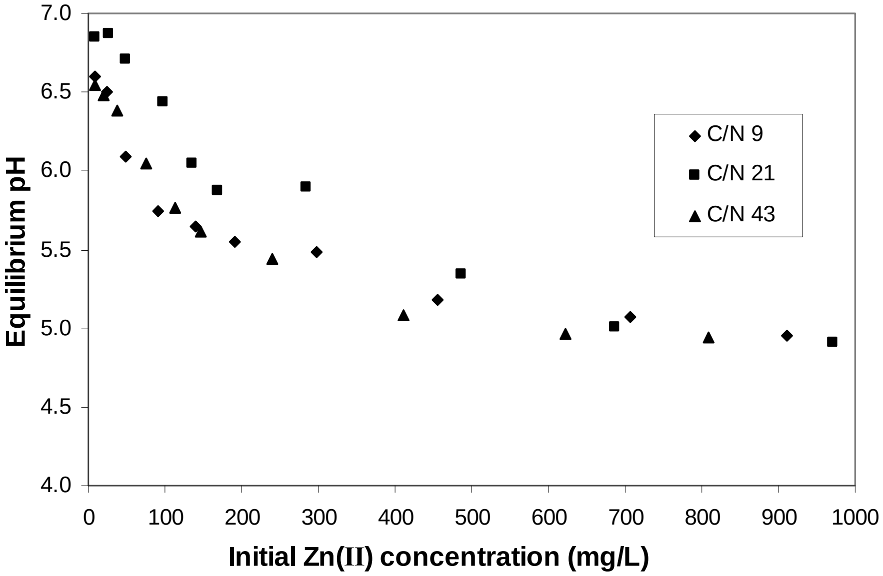


Figure 4.26. Effect of initial Zn(II) concentration on the equilibrium pH

The amount of Zn(II) ion adsorbed and Ca(II), Mg(II) and carbohydrate released for different equilibrium Zn(II) concentrations are presented in Figure 4.27. Like in biosorption capacities, the amount of Zn(II) ions increased with the increase of C/N ratio from 9 to 21. It then sharply decreased when the C/N ratio increased from 21 to 43. The release of Ca(II) and Mg(II) was seen highest at C/N ratio 21 and for C/N ratio 9 and 43 the values of total ion released were very close to each other whereas the ratio between the adsorbed and released ions was much higher in C/N 9 and nearly the same for other C/N ratios. When the amount of carbohydrate released was investigated, it was observed that carbohydrate release insignificantly increased with the increase of C/N ratio.

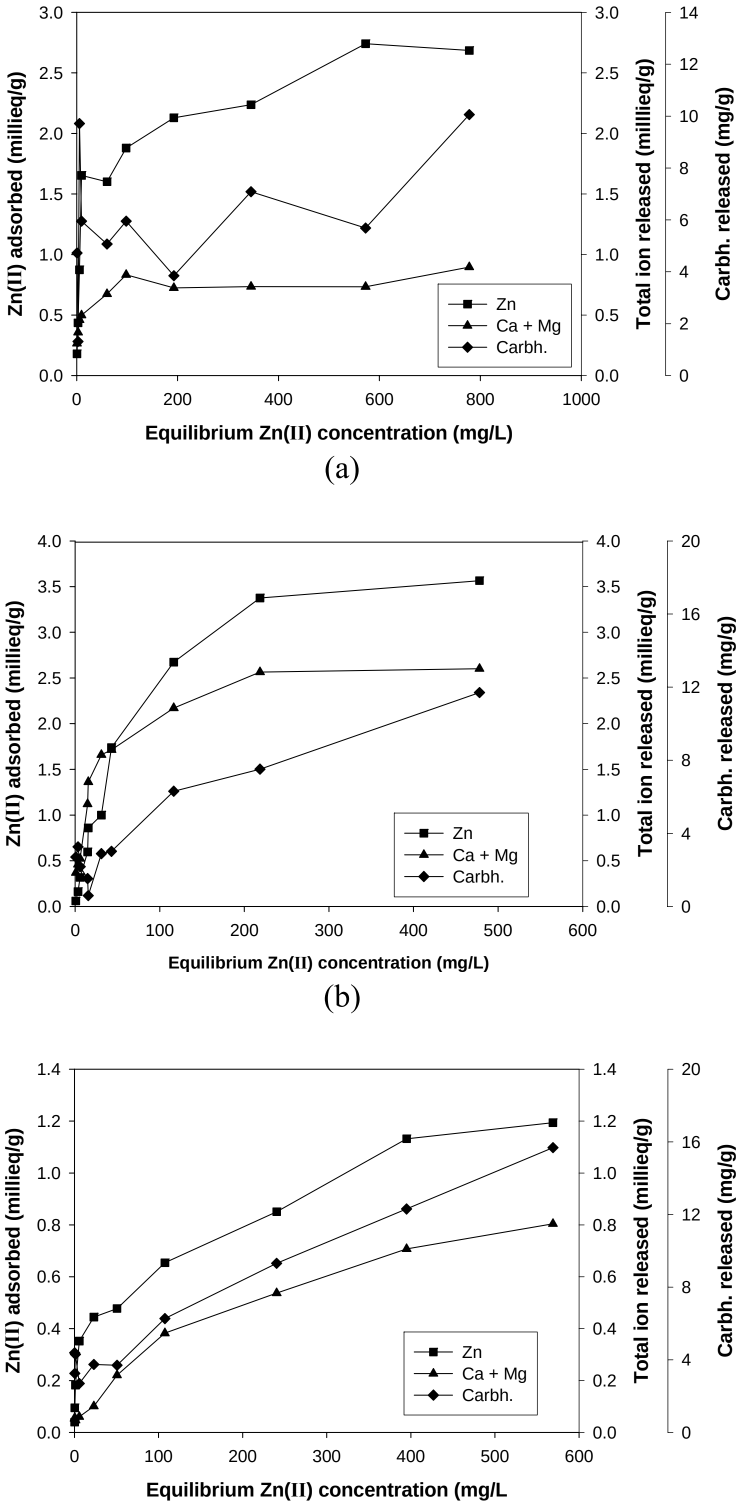


Figure 4.27. Amount of Zn(II) ion adsorbed and total ion (Ca(II), Mg(II)) and carbohydrate released a) C/N 9 b) C/N 21 c) C/N 43

4.2.5 Nickel

Figure 4.28 represents Ni(II) biosorption results. As it can clearly be seen from the figure, activated sludge cultivated under different C/N ratio conditions did not behave like a typical biosorbent. The isotherm plots presented in Figure 4.28 have indicated the variation of adsorptive capacity with Ni(II) concentration is highly complex. Although the experiments were repeated several times in order to minimize possible experimental errors, the sorption capacity values showed great fluctuations and an unstable behaviour was observed during the process. So, it should not be true to interpret the data collected and generate some conclusions out of them.

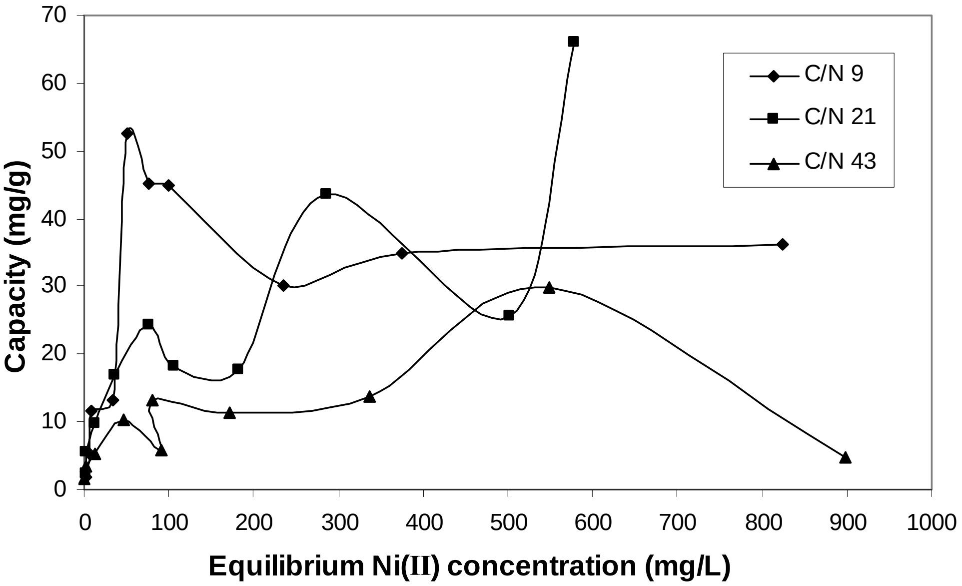


Figure 4.28. Adsorption isotherms of Ni(II) sorption for different C/N ratios

CHAPTER 5

DISCUSSION

5.1 Sorption Kinetics

The results of kinetic experiments conducted for five different heavy metals showed that biosorption of heavy metals by activated sludge is a fast process. For all metals, an initial rapid uptake was observed and nearly 85% of biosorption was completed within the first 5 min. After 5 min, the metal uptake rate gradually decreased and system finally reached equilibrium approximately after 60 min. Cheng *et al.* (1975) have proposed a two-step reaction model for metal uptake. The first stage is a rapid uptake phase of between 3 and 10 min, in which a large quantity of metal ions are adsorbed by the cell flocs. The second is a slow phase, which may extend over many hours. This behaviour implicates the fact that sorption occurs at two stages: the first is the rapid surface binding and the second is the slow intracellular diffusion, independent of the metabolic state of the cells (Yetis *et al.*, 2000).

Similar results were also suggested by many other researchers (Crist *et al.*, 1988; Bux *et al.*, 1996; Matheickal and Yu, 1996; Delgado *et al.*, 1998; Wong *et al.*, 2000; Gonzalez *et al.*, 2001; Leung *et al.*, 2001); while in some other studies single-step uptake was suggested for different biosorbents (Huang *et al.*, 1990; Guibal *et al.*, 1992).

Since biosorption of heavy metals involves inherently very a fast sorption reaction, mechanisms are based predominantly on chemisorption. The kinetics of such reactions is actually so fast that the experimental determination of metal uptake rates may represent a challenging problem (Volesky and Holan, 1995). Several authors have indicated that biosorption of heavy metals may be described by a first-order kinetic model. In theory, metal uptake kinetics should depend on the concentration of the metal and the concentration of the metal-binding sites. At low metal concentrations the availability of the binding sites was not limited. So, the uptake rate is expected to depend mainly on the metal concentration, thus exhibiting first order kinetics (Chang *et al.*, 1995).

The initial uptake rates of heavy metals in this study were also investigated by using a first order kinetic model. Semilog plots of concentration ratios versus time for first two minutes were studied for linear relations, as expected of first order reactions (Appendix B). An example of these plots is given in Figure 5.1 and comparison of first order rate constants (k), which were found for five different heavy metals and for all C/N ratios by using the plots stated above and the regression coefficients are given in Table 5.1.

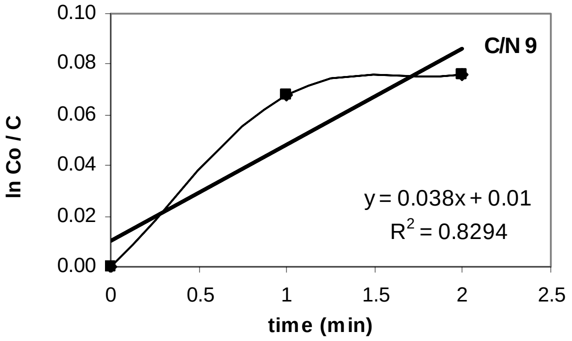


Figure 5.1. First order kinetic model for initial uptake rate of cadmium for C/N 9.

Table 5.1. First order rate constants (k) for all metals and C/N ratios

	Cadmium		**Copper**		**Lead**		**Nickel**		**Zinc**	
	k (min^{-1})	R^2	k (min^{-1})	R^2	k (min^{-1})	R^2	k (min^{-1})	R^2	k (min^{-1})	R^2
C/N 9	0.038	0.8294	0.061	0.8208	0.67	0.6865	0.091	0.6207	0.023	0.7205
C/N 21	0.055	0.6972	0.046	0.6693	0.49	0.7188	0.027	0.9862	0.079	0.7555
C/N 43	0.083	0.7542	0.024	0.6436	0.26	0.7407	0.088	0.7911	0.023	0.7278

It can be seen from Table 5.1, that the regression coefficients are not very high which means the rate of initial uptake can not be correlated well with the first order kinetic model. However, the correlation coefficient was never below 0.6 implying that heavy metal biosorption rate by activated sludge is highly related with the initial metal concentration and, probably increases linearly with it. For more sensitive determination of the order of sorption reaction, more frequent data points are required during the start of the process, which is almost impossible considering the nature of the experimental procedure adopted. Following the introduction of biomass solution to the metal solution, since the removal is almost instantaneous, it could not be possible to draw samples within smaller time intervals.

As presented in Table 5.1, Pb(II) biosorption always proceeded with the highest initial sorption rate as compared to other metals. Rate was the highest for the C/N of 9 and the lowest for 43, in parallel to the sorption capacity.

5.2 Sorption Equilibrium

For all metal ions, except nickel, the adsorption isotherms attained at different C/N ratios were L-shaped. This type of adsorption isotherm indicates favourable adsorption, where the adsorption capacity increases with equilibrium metal ion concentration. This increase continued until the active sites on the biomass surface are saturated. The highest capacities were observed for Pb(II) biosorption at all C/N ratios. In general, it was shown that activated sludge is an efficient biosorbent for metal removal especially for Pb(II). Pb(II) removal capacity of activated sludge was much higher than the capacity of some other biosorbents studied in literature (Matheickal and Yu, 1996; Chang *et al.*, 1997; Lopez-Delgado *et al.*, 1998; Schneider and Rubio, 1999; Yetis *et al*, 2000; Say *et al.*, 2001, Reddad *et al.*, 2002). From all the metals studied, the highest capacity was obtained for Pb(II) at C/N 9 with a value of 995 mg Pb(II)/g biomass and lowest for Zn(II) at C/N 43 with a value of 39 mg Zn(II)/g biomass.

For nickel biosorption, to obtain any meaningful data or to determine any maximum adsorptive capacity value for Ni(II) at any C/N ratio could not be possible. The capacity values showed great fluctuations and an unstable behaviour during the process One of the possible reasons for this unexpected behaviour was thought to be very low tendency of Ni(II) ions for biosorption among the heavy metal ions studied in literature (Brown and Lester, 1979; Rosin *et al.*, 1982; Kasan, 1993; Holan and Volesky, 1994; Bakkaloğlu *et al.*, 1998). Haytoğlu *et al.* (2001) reported that nickel was the one that had the weakest binding among the metals lead, copper, nickel and zinc. Also, Brown and Lester (1982) found that the sequence of solubilities of cadmium, manganese, cobalt and nickel is the reverse of the sequence of metal affinities for adsorption onto cells of *K aerogenes* indicating that the more soluble metals displayed the lowest removals.

On the other side, it was shown that nickel favors capsular polymers and cell walls instead of extracellular polymers in activated sludge and the role of extracellular polymers appears to be less specific in the removal of nickel by activated sludge than that of other metals (Battistoni *et al.*, 1993; Brown and Lester, 1982). Also, nickel was found to be associated predominantly with the soluble fraction of polymers (Rudd *et al.*, 1984). The stronger affinity of nickel for soluble polymers than for biomass could explain the lower binding capacity of activated sludge for nickel, and indicates that these soluble complexes may be unavailable for uptake by biomass (Solari *et al.*, 1996). So, inconsistent and unstable behaviour of nickel observed in this study could be attributed to the lowest biosorption tendency and weakest binding of nickel to the extracellular polymers of activated sludge.

It could be reported from the isotherm results that initial metal concentration is an important factor for biosorption. It affects equilibrium pH of the system as well as the mechanism of biosorption. At high concentrations, an ion exchange mechanism is involved in biosorption although the whole metal removal can not be explained by ion exchange. On the other hand, ion exchange phenomena could not be observed at low metal concentrations. A speculation for this can be as follows: at low metal concentrations, most metal bind to the already existing and available binding sites at the surface. Mechanism of this binding can be thought of as surface precipitation (may be some bulk precipitation for some metals due to relatively high pH at low metal concentrations), complexation and limited ion exchange. After a certain metal concentration, these available binding sites would be filled up leaving limited or no sites for the incoming excess heavy metal. At the same time, as the solution heavy metal concentration increases, there becomes a greater drive for the metal to exchange the cations already at the surface. This then initiates ion exchange starts to pick-up and take over the biosorption mechanism. The release of Ca(II) and Mg(II) ions along with carbohydrates and possibly the proteins (not measured in this study) as the heavy metals sorb are the indications of ion exchange.

5.3 Adsorption Isotherm Models

The results of isotherm studies were further examined in terms of conformity to the well-known Langmuir and Freundlich isotherm models to find out adsorption equilibrium model defining the biosorption of heavy metals by activated sludge for three different C/N ratios. The experimental data (q_e and C_e) obtained at different C/N ratios were fitted by linear regression method to evaluate the Freundlich and Langmuir model parameters by minimizing the sum of the squared deviations from experimental and predicted values.

The Freundlich constants K and n can be evaluated from linearized Freundlich isotherm by plotting log q_e vs log Ce on milimetric scale.

$$\text{loq } q_e = \log K + 1/n \log Ce \qquad (5.1)$$

The Langmuir constants Q^0 and b can be evaluated from linearized Langmuir isotherm by plotting Ce/q_e vs. Ce.

$$Ce/q_e = 1/Q^0b + Ce/Q^0 \qquad (5.2)$$

Linearized adsorption isotherm plots for all heavy metals (excluding Ni(II)) and C/N ratios are presented in Appendix. C. In Tables 5.2, 5.3, 5.4 and 5.5, the estimated values of the model parameters along with the regression coefficient (R^2) which permit the evaluation of the model goodness are given for heavy metals of lead, cadmium, copper and zinc respectively.

Table 5.2. Freundlich and Langmuir isotherm constants for Pb(II)

C/N	Freundlich			Langmuir		
	n	K (mg/g)(L/mg)$^{1/n}$	R^2	Q^0 (mg/g)	b (L/g)	R^2
9	1.39	22.33	**0.8941**	1111.11	0.0138	**0.9533**
21	1.29	13.31	**0.8546**	714.29	0.0145	**0.9106**
43	1.26	0.96	**0.9501**	454.55	0.00098	**0.8176**

Table 5.3. Freundlich and Langmuir isotherm constants for Cd(II)

C/N	Freundlich			Langmuir		
	n	K (mg/g)(L/mg)$^{1/n}$	R^2	Q^0 (mg/g)	b (L/g)	R^2
9	2.56	12.99	**0.7041**	120.48	0.053	**0.9977**
21	1.70	8.18	**0.7832**	153.85	0.038	**0.9665**
43	2.18	16.23	**0.9163**	192.31	0.039	**0.8884**

Table 5.4. Freundlich and Langmuir isotherm constants for Cu(II)

C/N	Freundlich			Langmuir		
	n	K (mg/g)(L/mg)$^{1/n}$	R^2	Q^0 (mg/g)	b (L/g)	R^2
9	2.75	16.51	**0.8029**	158.73	0.026	**0.9726**
21	2.21	7.89	**0.7915**	94.34	0.051	**0.9953**
43	5.153	12.02	**0.7867**	46.73	0.059	**0.9899**

Table 5.5. Freundlich and Langmuir isotherm constants for Zn(II)

C/N	Freundlich			Langmuir		
	n	K (mg/g)(L/mg)$^{1/n}$	R^2	Q^0 (mg/g)	b (L/g)	R^2
9	3.01	12.08	**0.8473**	89.29	0.036	**0.9913**
21	1.47	2.95	**0.9547**	136.98	0.139	**0.9899**
43	2.67	3.95	**0.9343**	39.37	0.027	**0.9651**

The adsorption data obtained for activated sludge grown in reactors with C/N 9 and 21 were fitted better to Langmuir model, whereas Freundlich model was more suitable for the biosorption with the activated sludge of C/N 43 for Pb(II) and Cd(II). Langmuir model seemed to better described adsorption equilibrium for all C/N ratios of Cu(II).

Correlation coefficients also showed that both of the models are very effective in describing the sorption of Zn(II) for C/N ratios 21 and 43 and there was a good fit of data to the Langmuir model for C/N ratio 9.

Freundlich constant K is an indicator of adsorption affinity. The values presented in Table 5.2 indicated that activated sludge grown at higher C/N ratios represent a lower affinity for Pb(II). The magnitude of K at the C/N ratio of 9 also showed the higher capacity of activated sludge at low C/N values which support the findings of adsorption studies of Pb(II) biosorption. However, since Freundlich model was not suitable for other metals, the values obtained by using the Freundlich isotherm model are in contradiction with the results of adsorption isotherm studies. Although the lowest capacity was observed at C/N 9 for Cd(II), the magnitude of K values showed the lowest capacity in reactors operated under C/N 21. Similarly, for copper the isotherm studies showed that sludge grown at C/N ratio 43 had the lowest capacity but when the K values in Table 5.4 are compared, it can be seen that K value obtained at C/N 21 is significantly lower than the others and for zinc the highest capacity was observed at C/N 21 but the lowest K values was also found at the same C/N ratio.

The Langmuir adsorption isotherm describes equilibrium between surface and solution as a reversible chemical equilibrium between species and assumes monolayer sorption. Constant b serves as an indicator of the strength or affinity of the material for the solute (Zhao *et al.*, 1999). For Pb(II) biosorption, a much higher b value obtained with activated sludge grown under the C/N ratio of 9 also confirmed the higher affinity of activated sludge at lower C/N ratios. Larger values of b for the C/N ratios of 9 and 21 also implied the strong binding of Pb(II) to the activated sludge cells at low C/N ratios. Similarly, higher b value was reached in reactors operated under C/N ratio 21 at which the highest capacity was also observed for Zn(II).

Langmuir or Freundlich sorption models are the most frequently used models for biosorption but they do not take into account electrostatic interactions or the fact that metal ion biosorption is largely an ion exchange phenomenon so these models are not very suitable for the prediction of the biosorption performance. Also, neither the Langmuir nor Freundlich isotherm model includes the pH values as one of the variables, although it is known that pH is one of the most important factors for heavy metal biosorption and has a very significant effect on metal removal (Schiewer and Volesky, 1995).

5.4 Effect of C/N ratio on Biosorption

The effect of C/N ratio on biosorption capacities for different metal ions is summarized in Figure 5.2. It can be easily seen from the figure that the effect of C/N ratio on heavy metal sorption capacity of activated sludge is highly variable. The metal ions show different biosorption affinities for microorganisms grown under different C/N ratios. Biosorption capacities for Pb(II) and Cu(II) ions increase with the decrease of C/N ratio whereas the increase in C/N results in an increase of

Cd(II) biosorption affinity. As for Zn(II) ion, a different behaviour was observed such as, the highest and lowest capacities have occurred at C/N ratio 21 and 43, respectively.

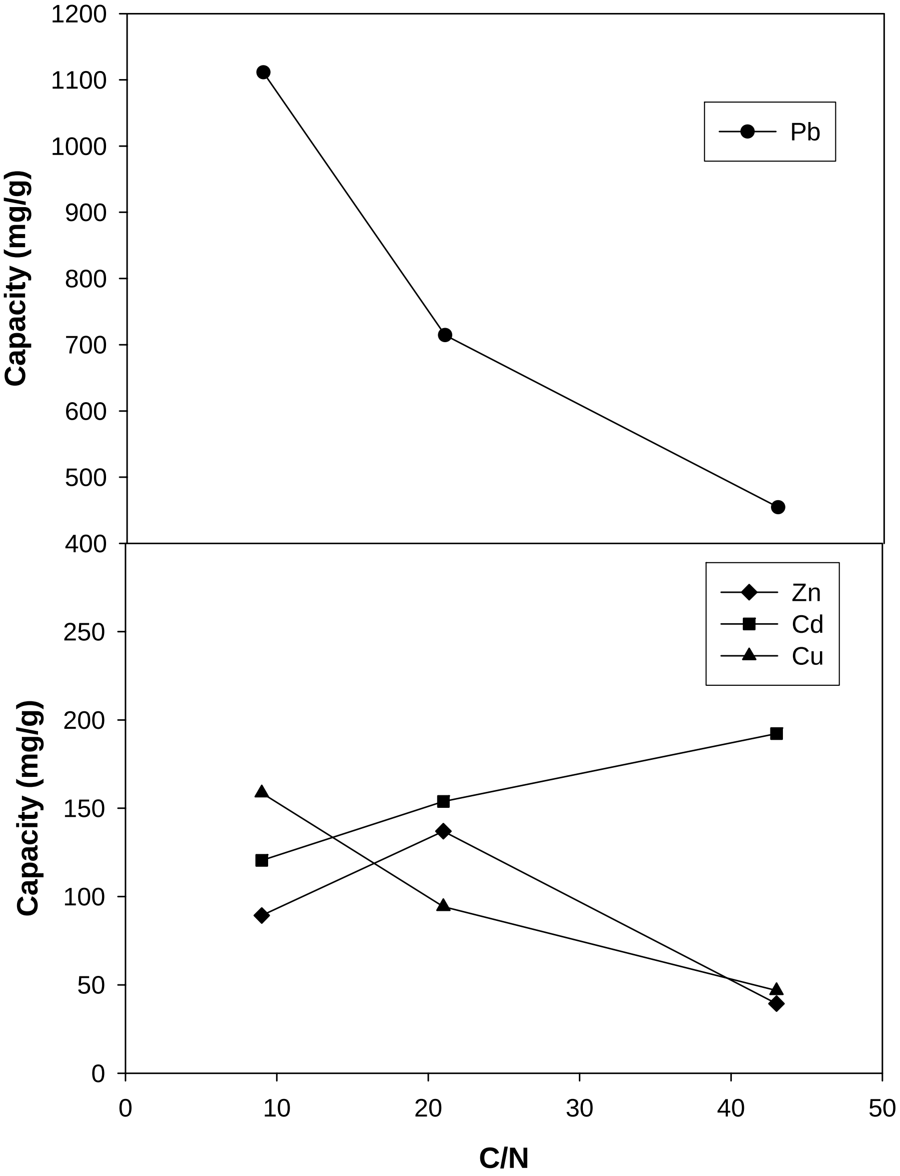


Figure 5. 2. The change of biosorption capacities of metal ions with C/N ratio

It is a well known fact that binding of heavy metals on the sludge surface can be attributed to the formation of surface complexes between metals and surface functional groups such as carboxyl, hydroxyl, phosphate, phenolic and amino groups of the ECP. The experimental findings of the present study indicated that biosorptive capacity of activated sludge is affected by the C/N ratio that is directly related with ECP structure of the sludge. It is known that C/N ratio of the feed is a major determinant of the composition of ECP in activated sludge. As the C/N ratio increases, the carbohydrate content of ECP increases, whereas the proteins decrease (Durmaz and Sanin, 2001).

Metals are known to favour specific binding sites which means in cases where several different types of metal-binding functional groups are present, different metals may associate preferentially with different specific types of functional groups. Ashkenazy *et al.* (1997) reported that carboxyl groups were the dominant species in the Pb(II) biosorption mechanism but also groups containing nitrogen interacted with Pb(II) cations. Doyle *et al.* (1980) and Holan and Volesky (1994) also suggested that carboxyl groups are mainly responsible for Pb(II) biosorption. Gonzalez *et al.* (2001) studied the mechanism of Cd(II) biosorption by dealginated seaweed waste and suggested that; the sorption of cadmium by dealginate is mainly due to an ion-exchange mechanism and indicated that carboxyl groups are largely responsible for sorption. Similar results were also reported by some other researchers like Muraleedharan and Venkobachar (1990) for copper biosorption, and Fourest et al. (1992) for zinc biosorption.

Gardea *et al.* (1990) observed that after the blocking of carboxyl groups of algal species by esterification, the binding capacity of Cu decreased. Fourest *et al.* (1996) used the same technique to demonstrate that the Zn binding by fungi was at least 30-70 % achieved by carboxyl groups.

Tobin *et al.* (1990), through a series of chemical treatments to *Rhizopus.arhizus*, mentioned that phosphate and carboxyl groups were important in biosorption, and amine groups did not play any significant role.

On the other hand, amino acid groups, present in the cell wall are suggested as the most active sites in the sludge-metal binding process (Tien and Huang, 1991). Artola *et al.* (1997) found that the main binding groups for metal (especially for copper) in the anaerobically digested sludge are of amino acid type. Also, Mameri *et al.* (1999) suggested that the carboxylic and amino groups are mainly involved in Zn(II) biosorption of a *Streptomyces rimosus* biomass.

The data of Kapoor and Viraraghavan (1997) concerning the cations of Cu(II), Cd(II) and Pb(II) showed that, in fungul biomass of *Aspergillus niger*, amine and carboxyl groups were important functional groups involved in lead, cadmium and copper biosorption. Lead biosorption was observed to be more sensitive to modifications of the carboxyl group than of the amine group, while cadmium and copper biosorption was found to be more sensitive to modifications of the amino group than the carboxyl group. Although, Akthar *et al.* (1996) indicated that the carboxyl group present in *Aspergillus niger* was important in biosorption of copper.

The studies stated above suggested that both carboxyl groups and amine groups play an important role in biosorption of metals. In general, many researchers reported that carboxylic groups are the main functional groups responsible for Pb(II) biosorption but the current results of the present study show that amine groups are also important for the biosorption of Pb(II). As it is stated above, with the increase of C/N ratio carbohydrate content of ECP –which means the carboxyl groups in the ECP structure – increases. On the other hand, Pb(II) biosorption capacity of activated sludge is enhanced with the decrease of C/N ratio so it can be said that Pb(II) uptake can not be attributed only to the carboxyl groups. Biosorption capacity is higher when the amine groups are dominant in the ECP structure. In theory, it is known that Pb(II) is a soft metal ion and it has higher affinity for the groups containing nitrogen and sulphur but as it was stated above, many studies in literature pointed out that carboxyl groups are mainly involved in Pb(II) biosorption. Beside amine groups there are also carboxyl groups in protein structure so it can be speculated that high biosorption capacities of Pb(II) could be due to the presence of both functional groups in protein structure.

This was also valid for Cu(II) biosorption; increase in amine groups in the ECP structure resulted in increase of Cu(II) biosorption capacity of activated sludge whereas an opposite tendency was observed for Cd(II) biosorption. When the C/N ratio increased which means carbohydrate amount of activated sludge increased, the Cd(II) biosorption capacity also increased which indicates the affinity of cadmium ions to carboxyl groups. For Zn(II) biosorption, a different behaviour was observed; the highest capacity was obtained at neither high nor low C/N ratios, the highest and lowest removals was seen at C/N 21 and 43 respectively. It can be speculated that both carboxylic and amino groups are mainly involved in biosorption and the ratio between them could also play an important role in uptake of Zn(II).

Nieboer and Richardson (1980) proposed a classification based on the atomic properties and the solution chemistry of the metal ions. They discriminated metal ions into three classes: oxygen-seeking (Class A), nitrogen- and sulphur-seeking (Class B), and borderline or intermediate class. In biological systems, class A ions show an almost absolute preference for binding to the type of ligands with oxygen as the donor atom. Consequently, class B metal ions seek out nitrogen and sulphur containing ligands in biological systems and often become irreversibly bound there. Borderline metal ions are able to form stable complexes with all categories of ligands. Preferences do exist which reflect the degree of class A or class B character of the particular borderline metal and the relative availability of the different ligands in a system.

There is a sharp separation between class A and borderline metal ions but the distinction between class B and borderline metal ions is less clear. Class B character increases in the order $Mn^{2+} < Zn^{2+} < Ni^{2+} < Fe^{2+} \cong Co^{2+} < Cd^{2+} < Cu^{2+} < Pb^{2+}$. Cd(II) falls among the borderline metals rather than in the class B group. Zn(II) is borderline with considerable class A character. Cu(II) and Pb(II) are the borderline ions with considerable class B character (Nieboer and Richardson, 1980).

This classification could easily explain the different biosorption capacities for different metals at various C/N ratios. Pb(II) and Cu(II) ions are included partly in nitrogen- and sulfur seeking and partly in borderline class which means Pb(II) and Cu(II) exhibit higher affinity for the $–NH_2$ and -NH groups than carboxyl groups. Since it was demonstrated earlier that at lower C/N ratios, ECP contained more proteins than carbohydrates (Durmaz and Sanin, 2001) higher adsorption of Pb(II) and Cu(II) at lower C/N ratio can be easily explained. Higher protein content means that there are more $–NH_2$ and –NH groups in ECP for Pb(II) and Cu(II) to bind yielding much higher biosorption capacities at lower C/N ratios. Similarly, Cd(II) ion is the borderline ion which exhibits higher affinity for carboxyl groups. Although, Zn(II) is borderline with considerable class A character and it is expected that Zn(II) to show preference for binding to carboxyl groups which should result in higher capacities at higher C/N ratio, it was observed that amine groups also have significant influence in Zn(II) biosorption.

CHAPTER 6

CONCLUSIONS

1. Activated sludge was found to be an efficient biosorbent for heavy metals namely Pb(II), Cd(II), Cu(II) and Zn(II). Especially, Pb(II) removal capacity of activated sludge was much higher than the capacity of some other biosorbents studied in literature.

2. Kinetic studies performed indicated that biosorption of heavy metals is relatively a rapid; two stage process of rapid surface binding followed by slow intracellular diffusion. For all metals and all C/N ratios, an initial rapid uptake was observed and nearly 85% of biosorption was completed within the first 5 min. After 5 min, the metal uptake rate gradually decreased and system reached equilibrium approximately after 60 min.

3. The initial uptake rates of heavy metals were investigated by using the first order kinetic model; but the rate of initial uptake can not be correlated well with this model. Only data of nickel uptake for C/N 21 fitted well to the model with a high regression coefficient.

4. The biosorptive capacity of activated sludge was found to be highly dependent on metal species and C/N ratio. The dependence of biosorptive capacity on C/N ratio was different for each metal; however at all C/N ratios, activated sludge exhibited the highest capacity for Pb(II).

5. The equilibrium pH attained was found to vary with metal and its initial concentration. In all cases, as the initial metal concentration increases, the equilibrium pH of the solution decreases.

6. For all metals and C/N ratios, there was a release of Ca(II) and Mg(II) during the process. At high concentrations, the amount of metal adsorbed was higher than the amount of total ions released (Ca(II), Mg(II)), which indicates an ion exchange mechanism for biosorption although the whole metal removal can not be explained by ion exchange. Ion exchange phenomenon could not be observed at low metal concentrations.

7. Beside Ca(II) and Mg(II) ions, carbohydrate release during the process was also observed which could be attributed to selectivity of carboxylate containing polysaccharides to calcium ions.

8. Amine and carboxyl groups were found to be mainly responsible for Pb(II) and Cu(II) biosorption whereas carboxyl groups are responsible for Cd(II). For Zn(II) biosorption, both carboxylic and amino groups are believed to be important.

9. The adsorption data obtained for activated sludge grown in reactors with different C/N ratios were fitted better to Langmuir model, whereas Freundlich model was more suitable for the biosorption with the activated sludge of C/N 43 for Pb(II) and Cd(II). Langmuir model seemed to better describe adsorption equilibrium for all C/N ratios of Cu(II). Both of the models were very effective in describing the sorption of Zn(II) for C/N ratios 21 and 43 and there was a good fit of data to the Langmuir model for C/N ratio 9.

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APPENDIX A

CALIBRATION CURVE

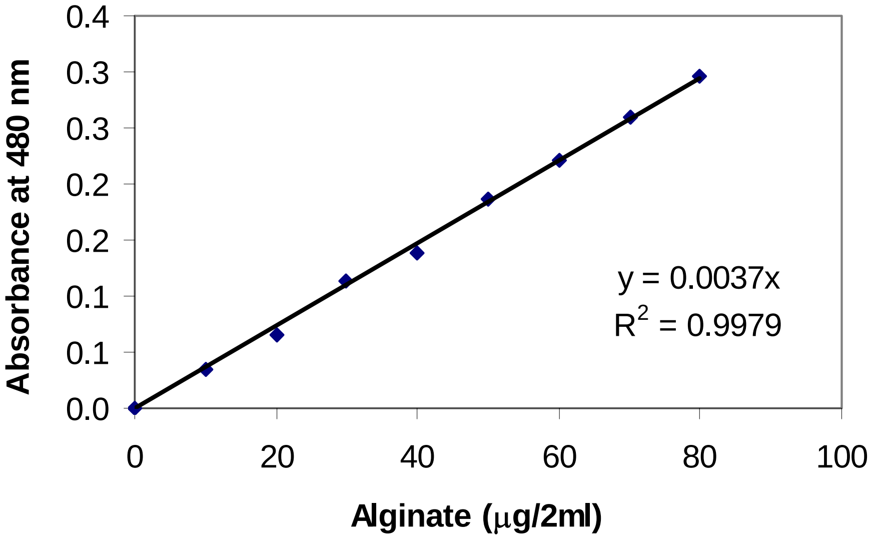


Figure A 1. Calibration curve for Dubois method

APPENDIX B

INITIAL UPTAKE RATES

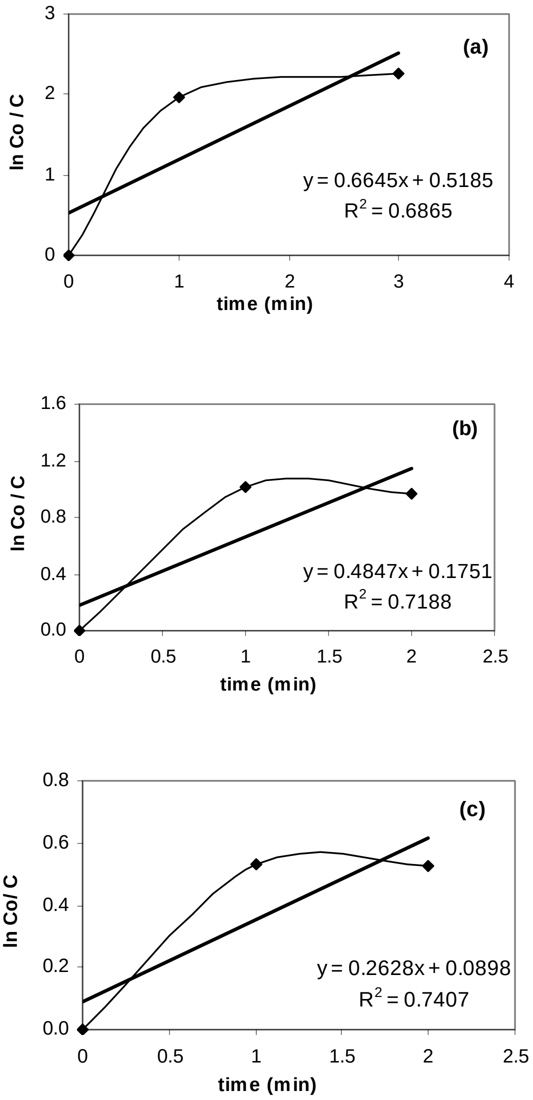
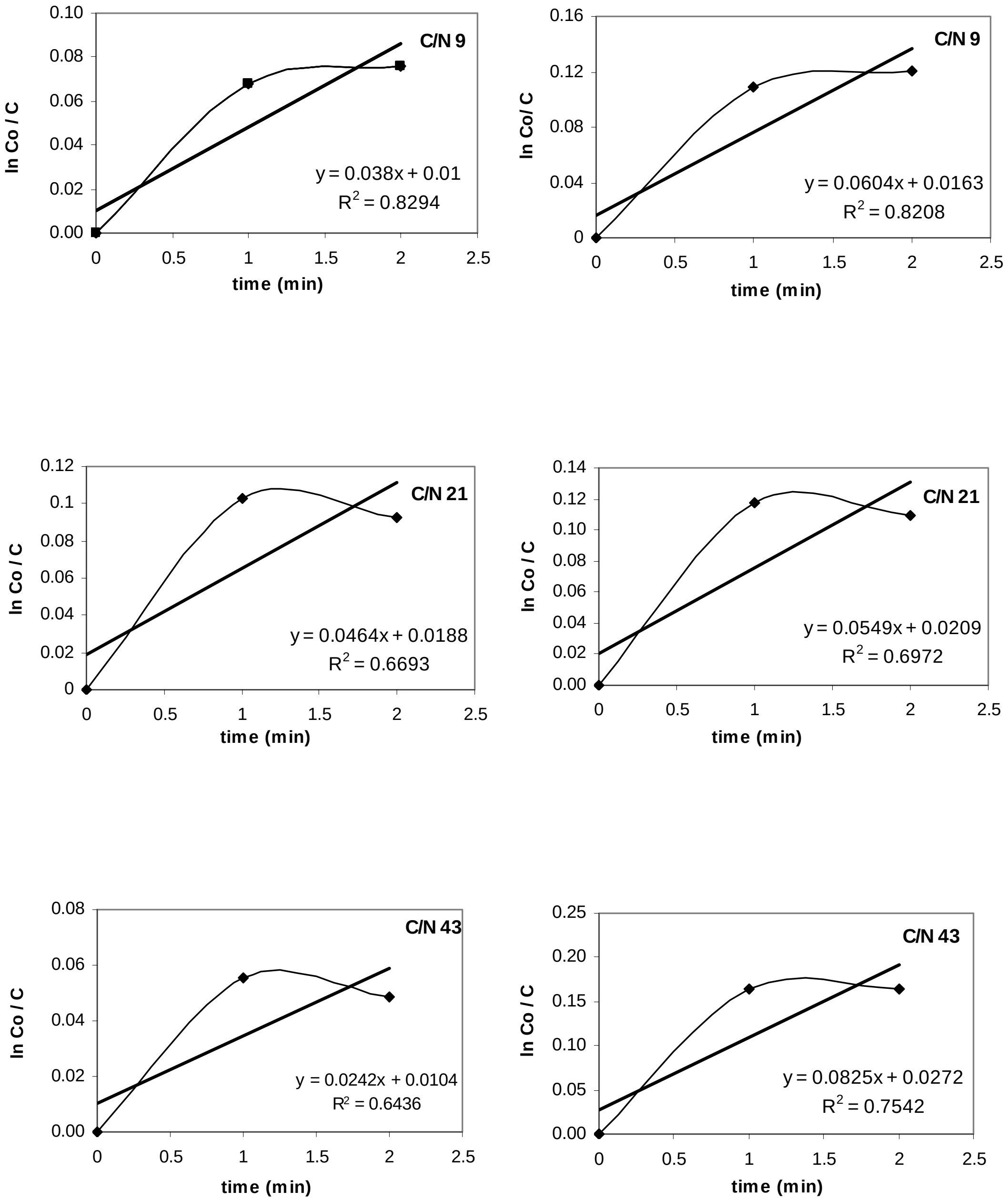


Figure B. 1. Semilog plots of concentration ratios versus time for lead at C/N a) 9, b) 21 c) 43



(a) (b)

Figure B. 2. Semilog plots of concentration ratios versus time for a) Copper b) Cadmium

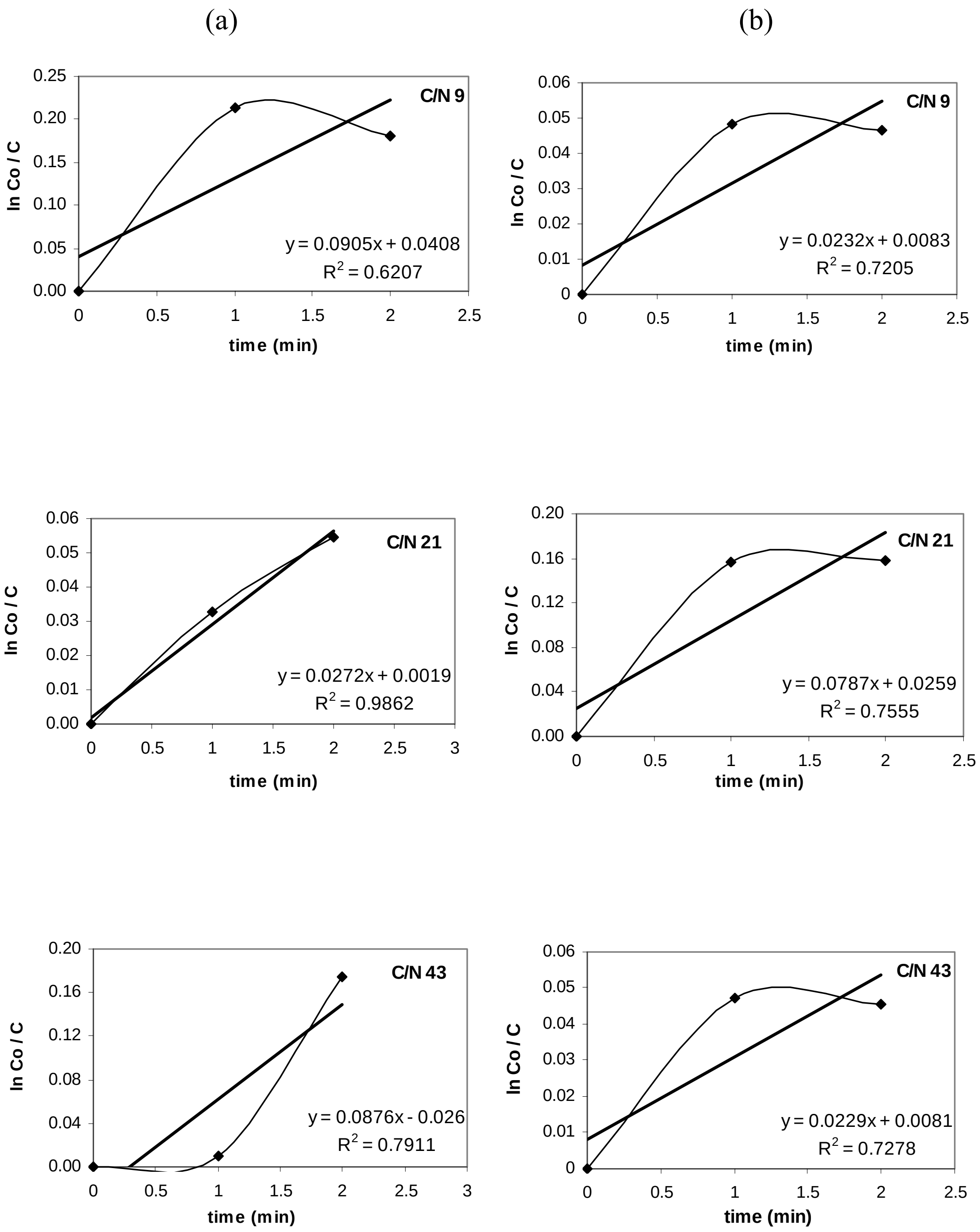


Figure B. 3. Semilog plots of concentration ratios versus time for a) Nickel b) Zinc

APPENDIX C

ADSORPTION ISOTHERM MODELS

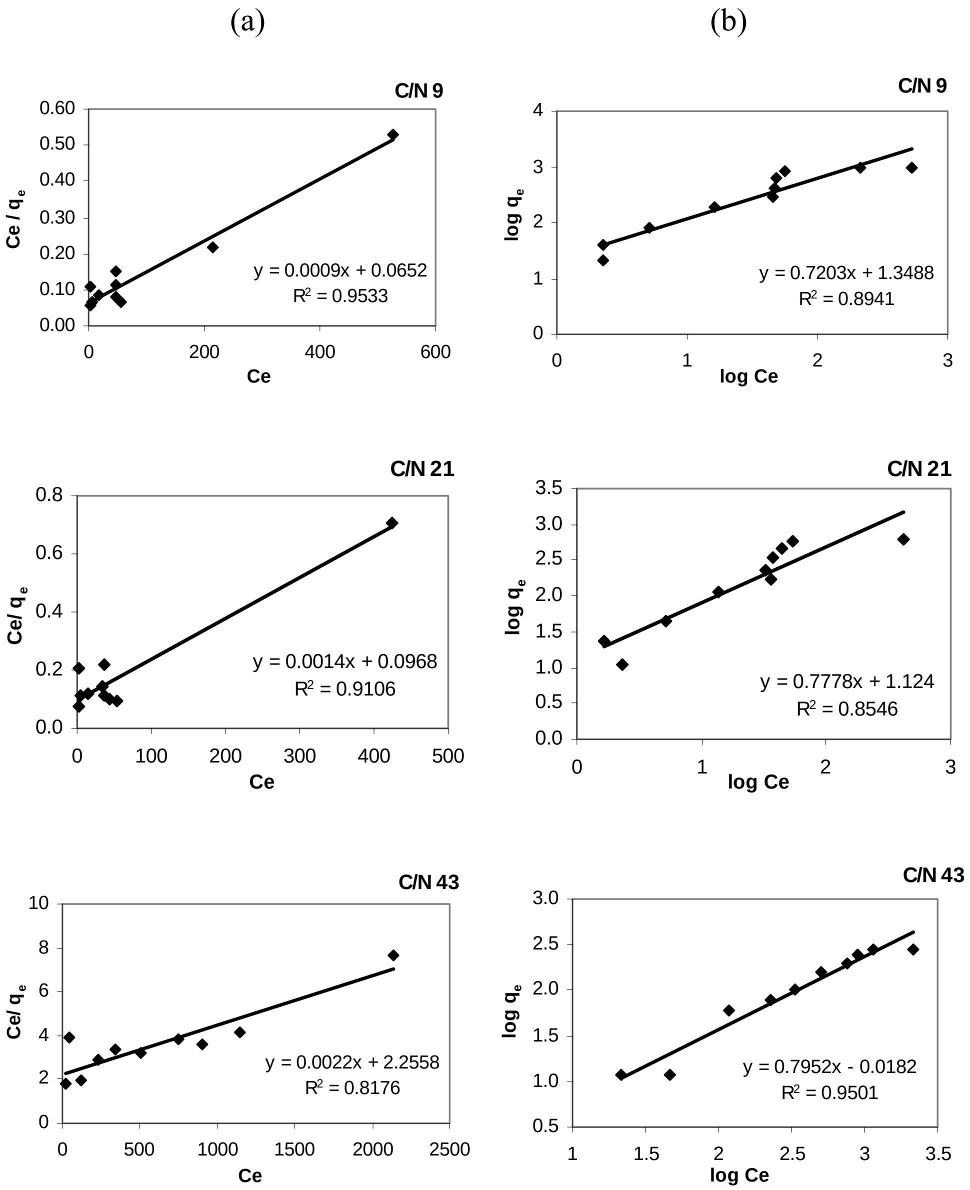


Figure C.1. Linearized a) Langmuir and b) Freundlich isotherms of lead for C/N ratios 9, 21 and 43

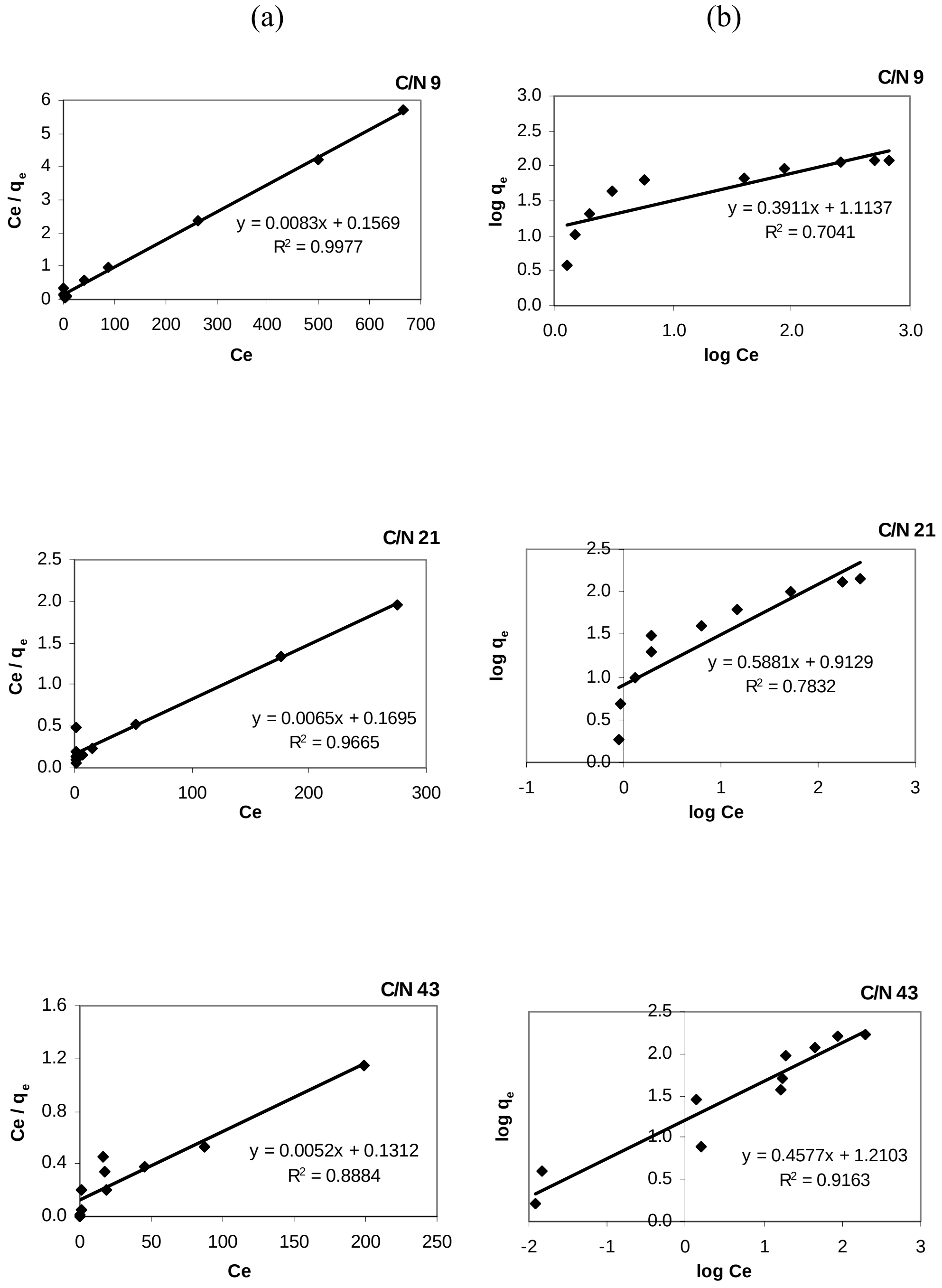


Figure C.2. Linearized a) Langmuir and b) Freundlich isotherms of cadmium for C/N ratios 9, 21 and 43

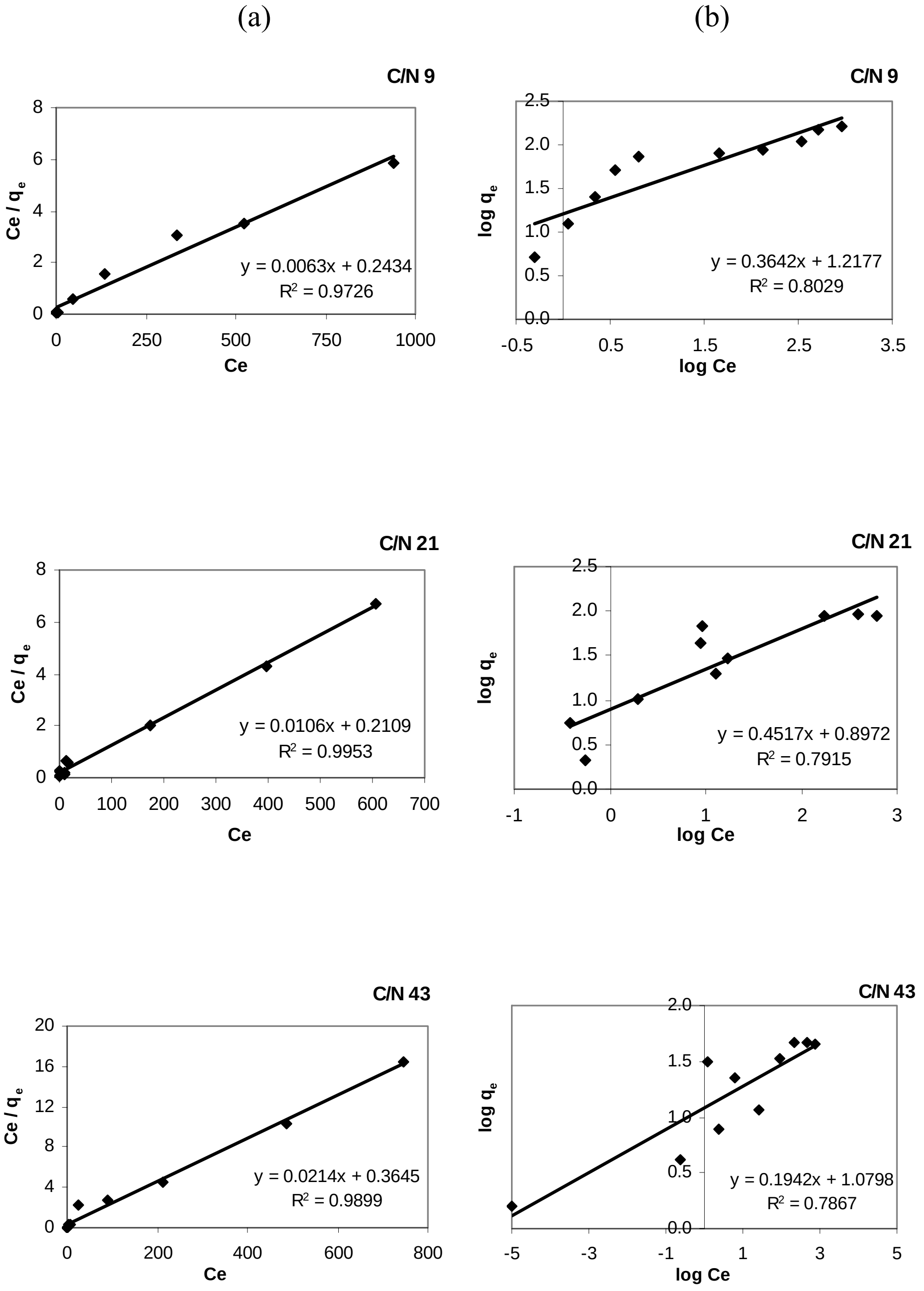


Figure C.3. Linearized a) Langmuir and b) Freundlich isotherms of copper for C/N ratios 9, 21 and 43

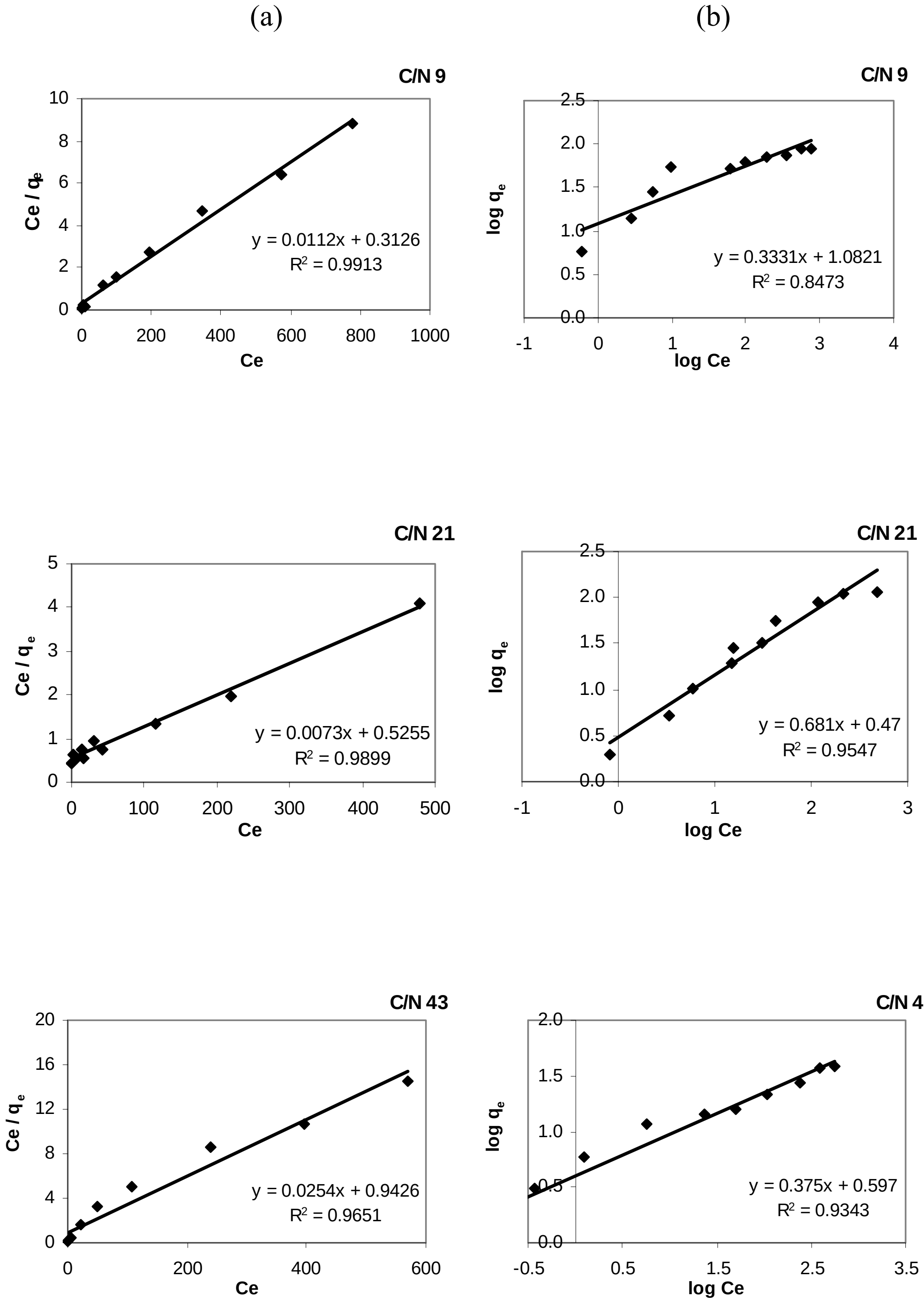


Figure C.4. Linearized a) Langmuir and b) Freundlich isotherms of zinc for C/N ratios 9, 21 and 43