REMOVAL OF LEAD USING ANAEROBIC BIOMASS

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
THE MIDDLE EAST TECHNICAL UNIVERSITY

BY

EMRE TOKCAER

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

THE DEPARTMENT OF ENVIRONMENTAL ENGINEERING

SEPTEMBER 2003

Approval of the Graduate School of Natural and Applied Sciences

_____

Prof. Dr. Canan Özgen

Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

_____

Prof. Dr. Ülkü Yetiş

Head of Department

This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

_____

Prof. Dr. Ülkü Yetiş

Supervisor

Examining Committee Members

Prof. Dr. Celal Gökçay _____

Prof. Dr. Ülkü Yetiş _____

Prof. Dr. Gülay Özcengiz _____

Assoc. Prof. Dr. F. Dilek Sanin _____

Dr. İpek İmamoğlu _____

ABSTRACT

REMOVAL OF LEAD USING ANAEROBIC BIOMASS

Tokcaer, Emre

M.Sc., Department of Environmental Engineering

Supervisor: Prof. Dr. Ülkü Yetiş

September 2003, 80 pages

Use of anaerobically digested sludge (ADS) in heavy metal removal, was researched. The raw and dewatered ADS samples collected from the effluent of anaerobic digesters and mechanical dewatering units of Ankara City Wastewater Treatment Plant were used. Sorption kinetic and equilibrium tests were conducted using raw ADS at initial pH of 2.0, 4.0 and without adjusting the initial pH. The highest Pb(II) removal capacity was observed as, 8.5 mmol (or 1760 mg) Pb(II) per g of biomass, when the initial pH was not controlled. When dewatered ADS was used Pb(II) removal capacity of ADS was found to drastically decrease to 2.5 mmol (or 518 mg) Pb(II) per g of biomass.

Both biomass samples resulted in an increase in the solution pH from an initial value of 4 – 5 to an equilibrium value of 7 – 8. Large floc particles settling rapidly were formed after the ADS samples contacted with Pb(II) solution. The high Pb(II) removal capacities, and visual observations during the experiments indicated that precipitation is a dominant mechanism especially at low initial Pb(II) concentrations.

FTIR studies showed that carboxyl groups present in the biomass surface of raw ADS were major functional groups in biosorption of Pb(II). The low capacity values attained at initial pH 2.0 indicated that there was a competition between Pb(II) species and hydrogen ions for carboxyl groups.

Single and 3-stage fed-batch reactor systems were operated using raw ADS at different initial Pb(II) concentrations. The efficiency of reactor systems increased when 3-stage fed-batch configuration was used and an effluent Pb(II) concentration below 2 mg/L was reached from an initial value of about 200 mg/L.

Key Words: Anaerobic sludge, biosorption, heavy metals, lead, precipitation, reactor operation.

ÖZ

ANAEROBİK BİYOKÜTLE KULLANARAK KURŞUN GİDERİMİ

Tokcaer, Emre

Yüksek Lisans, Çevre Mühendisliği Bölümü

Tez Yöneticisi: Prof. Dr. Ülkü Yetiş

Eylül 2003, 80 sayfa

Bu çalışmada ağır metal gideriminde anaerobik çürütme çamurunun (AÇÇ) kullanımı incelenmiştir. Kullanılan ham ve susuzlaştırılmış AÇÇ örnekleri, Ankara Şehri Atıksu Arıtma Tesisi içindeki anaerobik çürütücü ve mekanik susuzlaştırma ünitelerinin çıkışından alınmıştır. Başlangıç pH sı 2.0, 4.0 ve başlangıç pH sı ayarlanmamış koşullarda ham AÇÇ kullanılarak sorpsiyon kinetik ve denge testleri yapılmıştır. En yüksek Pb(II) giderim kapasitesi 8.5 mmol (ya da 1760 mg) Pb(II) / g biyokütle olarak başlangıç pH sının kontrol edilmediği koşullarda gözlenmiştir. Susuzlaştırılmış AÇÇ kullanıldığı zaman Pb(II) giderim kapasitesi 2.5 mmol (ya da 518 mg) Pb(II) / g biyokütle olarak bulunmuştur.

Her iki biyokütle de çözelti pH sında artmaya neden olmuş, başlangıç değeri 4 – 5 arasında olan pH dengede 7 – 8 olarak ölçülmüştür. Ayrıca AÇÇ örnekleri Pb(II) çözeltisi ile karıştırıldıktan sonra çabuk çökebilen, iri ve iyi topaklaşan parçacıklar oluşmuştur. Yüksek Pb(II) giderim değerleri ve deneyler sırasındaki gözlemler biyosorpsiyonun yanı sıra kimyasal çökelmenin özellikle düşük başlangıç Pb(II) konsantrasyonlarında baskın mekanizma olduğunu göstermiştir.

FTIR çalışmalarında, ham AÇÇ biyokütlesi yüzeyindeki karboksil gruplarının Pb(II) biyosorplanmasındaki temel fonksiyonel gruplar olduğu bulunmuştur. Başlangıç pH 2.0 da elde edilen düşük kapasite değerleri, karboksil grupları için Pb(II) ile hidrojen iyonları arasında bir rekabet olduğunu göstermektedir

Ham AÇÇ kullanılarak farklı Pb(II) başlangıç konsantrasyonları için tek ve 3 basamaklı kesitli-beslemeli reaktör sistemleri çalıştırılmıştır. Üç basamaklı reaktör düzeneği kullanıldığında reaktör sisteminin verimliliğinin arttığı görülmüş ve yaklaşık 200 mg/L başlangıç Pb(II) konsantrasyonunda 2 mg/L nin altında çıkış konsantrasyonu elde edilmiştir.

Anahtar Kelimeler: Anaerobik çamur, ağır metaller, biyosorpsiyon, çökelme, kurşun, reaktör çalışması.

ACKNOWLEDGMENTS

I would like to express my deepest gratitudes to Prof Ülkü Yetiş for giving me the opportunity to research on what I was curious about. Her guidance and our discussions have encouraged me to make this study.

I gratefully acknowledge the financial support from the Turkish Scientific and Technical Research Council (TUBITAK), under Grant No. ICTAG / A033 (102/056).

I would like to acknowledge the help of Dr. Gürkan Karakaş for the FTIR analysis of samples and interpretation of the results. Also thanks to Mr. Kemal Demirtaş for his help in laboratory studies, especially for AAS analysis of the samples.

Thanks to my friends Niğmet, Tuba, Esra, Gökşen, Burcu, Serkan, Volkan and Bilgen. It was always nice to have their technical and spiritual support throughout this study.

Finally thanks to my parents and my sister for always being with me.

TABLE OF CONTENTS

LIST OF TABLES

TABLE

LIST OF FIGURES

FIGURE

LIST OF SYMBOLS

B: Langmuir constant related to energy of sorption

C: Pb(II) concentration

Ce: Equilibrium Pb(II) concentration

Ci: Initial Pb(II) concentration

K: Freundlich constant

K_a: Acidity constant

N: Freundlich constant related to energy of sorption

pHe: Equilibrium pH

pHi: Initial pH

q: amount of Pb(II) adsorbed per unit biomass

Q_{max}: Langmuir constant showing maximum sorption sites available

T: Temperature

ABBREVIATIONS

ADS: Anaerobically Digested Sludge

h: Hour

min: Minute

s: Second

SVI: Sludge Volume Index

CHAPTER 1

INTRODUCTION

Many of the heavy metals were used prior to this century. They have been used for structural, medical and cosmetic purposes (Fergusson, 1990). Today as a result of industrial activities and technological development the use of heavy metals has increased continuously. Consequently the discharge of heavy metal bearing wastewaters has also increased. The danger of such discharges comes from the toxicity of heavy metals and their tendency to accumulate to higher concentrations throughout the food chain, thus raising the hazard involved and endangering human health (Volesky, 1990).

Due to serious health hazards and environmental problems caused, removal techniques have been developed for heavy metals for a long time. Well known conventional technologies include chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, membrane technologies and evaporation recovery. Most of these technologies are well established and well studied for use in treatment of industrial wastewaters (Patterson, 1985; Lanouette, 1977; Cadman and Dellinger, 1974). However, the commercial value of some metals, as well as the pressure of public and media due to environmental awareness, in addition to stricter regulations and legislations applied by governments, necessitates industries to research and develop new technologies (Tsezos et al., 1997), as the use of conventional technologies is becoming inefficient and/or very expensive (Ceribasi and Yetis, 2001).

In Turkey, Water Pollution Control Regulation (WPCR) sets Pb discharge limits for several industries. The limit varies depending on the type of industry. However, the highest allowable value for industries dealing with lead is 2 mg/L while the lowest limitation is set as 0.3 mg/L (Turkish Water Pollution Control Regulation, 1988).

In recent years, the search for an effective, simple and cost-effective treatment technology has lead to the investigation of materials of biological origin, as potential metal sorbents (Gonzalez et al., 2001). A variety of biological species like bacteria, algae, fungi, and yeasts were tested and it has been demonstrated that some biological materials have the ability of sequestering metals from solutions. This led to the development of biosorption process, which is defined as "a non-directed physicochemical interaction that takes place between metal/radionuclide species and the cellular compounds of biological specimens" (Figueira et al., 1999). The mechanisms associated with metal sorption by microorganisms are known to include extracellular and intracellular metal binding and are complex and dependent on the metal ion and the biological system (Sağ and Kutsal, 1995).

Amongst the biological species tested as biosorbents, anaerobically digested sludge (ADS) biomass is the one that has taken relatively little attention with limited investigation. The earliest study on heavy metal removal using anaerobic sludge was reported by Gould and Genetelli (1978). Alibhai et al. (1985) also studied zinc, lead, iron and chromium binding by digested sludge. Artola et al. (1992) investigated the heavy metal binding to several types of sludge taken from a wastewater treatment plant and reported the anaerobically digested, dewatered and thickened sludge samples to be the best types for use in heavy metal removal depending on the conditions. In their further studies; Artola et al. (1997 and 2001) preferred to use anaerobically digested sludge. Recently, Haytoglu (2000) have studied the Pb(II) removal using anaerobic biomass and found that ADS exhibits a very high capacity for Pb(II). In the study with ADS, Haytoglu (2000) used autoclaved anaerobic sludge which was grown under laboratory conditions and reported a Pb(II) removal capacity of 1250 mg Pb(II)/g biomass which is much higher than the values reported earlier in the literature for almost all types of biomass.

One of the main objectives of the present study is to further investigate the use of ADS in heavy metal removal and contribute to the basic understanding of metal biosorption mechanism by ADS. As metal species, Pb(II) was selected; due to its hazardous nature to the various ecosystems and human health, and properties like easy accumulation on the microorganisms (Suh et al., 2001). As biomass, to be more realistic, ADS from a full-scale wastewater treatment plant; Ankara Wastewater Treatment Plant was utilized. Sludge samples, taken from the outlet of the anaerobic sludge digester and also from the outlet of belt filter were tested. The aim in testing these two different types of ADS was to investigate the effect of dewatering on the biosorptive characteristics of ADS and more importantly to contribute to the research on the practical application of biosorption using ADS.

Another objective of this study was to search for a suitable reactor configuration to be used in the practical application of Pb(II) removal by ADS. For this purpose, single and multiple stage completely mixed fed-batch reactor systems were operated at two different influent Pb(II) concentrations. Fed-batch reactor configuration was selected instead of commonly adopted fixed bed column configuration, considering the characteristic properties of ADS.

CHAPTER 2

LITERATURE REVIEW

2.1. BIOSORPTION

It has been known for a long time that the biological materials are able to bind heavy metals; either by active or passive uptake. In the active uptake of metals, which is called bioaccumulation, the metabolic activity of the biomass is responsible for the removal of metals from solution. On the other hand, the passive binding of heavy metals to a certain biomass is referred as biosorption (Volesky, 1990). As it results from the passive uptake of biomass the process becomes more favorable, because the biomass is not required to be alive. Usage of dead (or inactive) biomass brings numerous advantages like; no need of nutrients or other growth supplements, and the toxicity of metals cannot affect the sorption capacity of the biomass (Tsezos et al., 1997). Another advantage of the process is the possible recovery of the precious metals and the recycle of the biomass for reuse; which can be obtained in a multi-stage process where biosorption is followed by elution and electrolysis steps. Such a process scheme suggested by Butter et al. (1998) is given in Figure 2.1. Although, the research on biosorption as a defined process begins from 1980s with the investigation of kinetics and equilibrium of biosorption in a systematic way using models and equations from the field of adsorption (Tsezos, 2001); some researchers had reported the ability of some strains of yeast and different species of bacteria to remove heavy metals at the end of 1970s (Cadman and Dellinger, 1974).

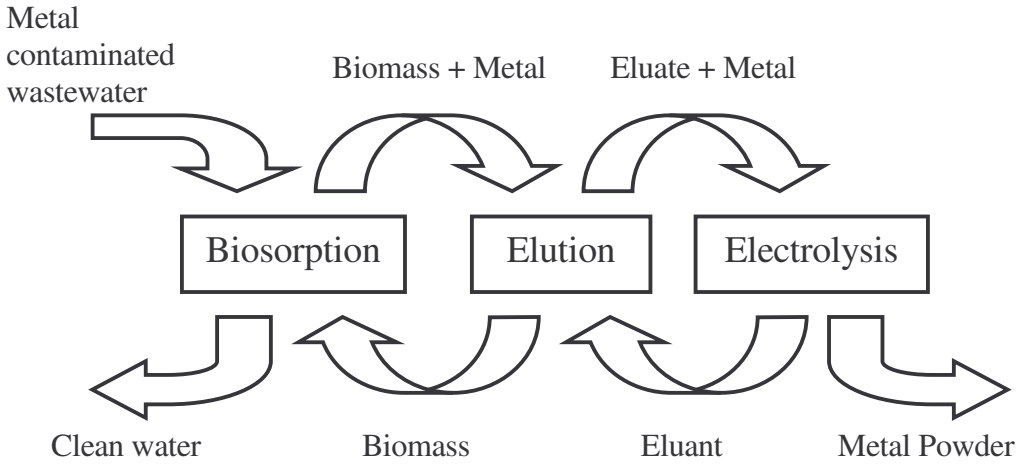

Figure 2.1. Multi-stage biosorption process scheme for removal and recovery of heavy metals (From Butter et. al., 1998).

In the past, biosorption of heavy metals has been studied extensively. In one of the comprehensive studies; Schiewer (1996), for example, have reviewed the roles of major operational factors in biosorption and indicated that the efficiency of biosorption depends on the type of biomass, the physico-chemical characteristics of the targeted metals and the environmental characteristics of the solution such as; temperature, pH, presence of ligands and other cations.

It is obvious that the type of biomass will effect the removal of metals, as separate biomass types show different cell wall structures. Due to variations in the cell wall structure the amount or type of available sorption sites as well as the functional groups responsible for metal binding will be different, thus the biosorption capacity will be affected (Holan and Volesky, 1994; Williams et al., 1998; Gardea-Torresday et al., 1996; Davis et al., 2000). In their study Williams et al. (1998) used four different biosorbents (*E. maxima*, dealginate, alginate and *L. usitatissimum*) for heavy metal removal and showed that residual metal concentrations obtained using different biosorbents varied from each other and alginate was reported as the most effective

biosorbent. In a similar study, different species of Sargassum were investigated for their Cu and Cd uptake capacities and *S. vulgare* was found to have the highest uptake capacity in comparison to *S. fluitans* and *S. filipendula* species (Davis et al., 2000).

Physico-chemical characteristics of the targeted metal will determine its ”behavior” in the solution, for example the oxidation state of a metal will be important in formation of stable bonds (Fergusson, 1990) and therefore will affect the biosorption capacity. As regards the role of physico-chemical characteristics of the targeted elements; extensive studies have been reported to date (Holan and Volesky, 1994; Alibhai et al., 1985; Figueria et al., 1999). For example, if iron is oxidized from ferrous to ferric form it can easily form hydroxide precipitate, which was reported to be the case in a study with anaerobically digested sludge (Alibhai et al., 1985). Such partial oxidation of Fe(II) to Fe(III) was also reported by Figueira et al. (1999), and the XPS analysis of Fe(II) and Fe(III) contacted biomass samples showed some differences indicating that the removal mechanism could be affected by the oxidation state of metal species.

Holan and Volesky (1994) investigated different types of marine algae for removal of lead and nickel and reported the sequence with respect to taxonomy in decreasing capacity order. The genus sequence for lead uptake was *Fucus* > *Ascophyllum* > *Sargassum* > *Padina* and for nickel it was as *Ascophyllum* > *Sargassum* > *Fucus*. The change in order of sequence for different heavy metals showed that the biosorption capacity was not only affected by the biomass types but also the physico-chemical characteristics of the targeted metal.

The temperature of the system was also reported to affect the biosorption process. Dölek (1997) and Haytoglu (2000) studied the effect of temperature on Pb(II) biosorption in their studies. Dölek (1997) reported 25 °C and 35 °C to be the optimum temperatures for biosorption while Haytoglu (2000) reported 25 °C to be the optimum value. However, overall effect of temperature was reported to be little as compared to other influencing effects (Schiewer, 1996). This coincides with results of Alibhai et al. (1985) indicating that the biosorption capacities for different metals (Pb, Zn and Cr) were the same at temperatures of 20° C and 30° C.

On the other hand, many of the researchers in biosorption studies have investigated the pH effect on metal removal (Esposito et al., 2002; Volesky, 1990; Schiewer, 1996; Delgado et al., 1998; Marques et al., 2000). Artola et al. (1992) have indicated that the solution pH is "the single most important factor" in metal adsorption. In general, metal removal capacity of biomass was reported to increase as pH increases (Waihung-Lo et al., 1999; Gonzalez et al., 2001; Delgado et al., 1998; Gardea-Torresday et al., 1996; Esposito et al., 2002). At lower pH values active sites are protonated and the metals compete with protons, thus the capacity decreases. On the contrast, the active sites are deprotonated as pH increases, thus being more attractive for metals as they are negatively charged and as a result biosorption capacity increases (Waihung-Lo et al., 1999; Gonzalez et al., 2001; Delgado et al., 1998; Gardea-Torresday et al., 1996; Esposito et al., 2002; Tsezos et al., 1995; Kratochvil and Volesky, 1998; Schiewer, 1996). However, extreme pH values are reported to affect the biosorption adversely, since they may cause deformation of the surface of biomass (Kapoor, 1998).

Another role of solution pH is that it determines the speciation / hydrolysis of metals in the solution. Therefore, in several of the biosorption studies, the solution pH is controlled in order to avoid generation of metal hydroxylation and precipitation, as many of the heavy metals are known to precipitate over the pH value of 5.5 (Kratochvil and Volesky, 1998; Holan and Volesky, 1994; Patterson et al., 1977).

On the other hand, change in solution pH due to biosorption was also observed. The solution pH was reported to either increase (Tsezos et al., 1995) or decrease (Schiewer 1996) depending on mechanism involved in biosorption. Several mechanisms involved in biosorption are discussed in Section 2.2.

Several workers have reported that presence of other cations could have an adverse effect on biosorption of heavy metals due to competition between metals and other cations as well as hydrogen ions (Schiewer, 1996; Tobin et al., 1988). Tobin et al. (1988) showed that the biosorption of Ag was reduced in the presence of Cd, La and UO_2, while the biosorption of these metals and UO_2 was also reduced in the presence of Ag. Therefore, heavy metals are reported to compete with each other especially if

the binding sites responsible are the same and thus, the biosorptive capacity for a specific metal is decreased in the presence of another metal compared to single metal solution case.

2.2. MECHANISMS OF BIOSORPTION

Biosorption of heavy metals is a complex phenomenon. The sequestering or uptake of the metals by the biomass surface can occur via complexation, coordination, chelation of metals, ion exchange, adsorption, inorganic microprecipitation and/or a combination of the above mechanisms (Volesky, 1990). The possible interactions involved in the mechanism are well represented by Schiewer (1996) in a schematic way in Figure 2.2. It should be noted that for a given system these interactions / mechanisms may operate simultaneously or in sequence, some mechanisms could be the preliminary steps for other mechanisms to take place.

Three major sorption mechanisms represented in Figure 2.2 are ion exchange, adsorption and micro-precipitation. These mechanisms are known to result either from interactions of sorbate – sorbent, metal – ligand and solute – solvent or from binding of complexes and/or chelates, formed due to sorbate – sorbent and metal – ligand interactions, to the biomass surface. In biosorption, ion exchange refers to the replacement of another cation, which occupies the binding site, by the metal in solution. Therefore, cations like Ca^{+2} and Mg^{+2} are supposed to be released to the solution if ion exchange takes place (Schiewer, 1996). On the other side, adsorption refers to the binding of metals to free sites.

The sites available for binding the metals through adsorption or ion exchange are called as the functional groups. Potential functional groups over the biomass surface have been suggested as carboxyl, sulfate, phosphate, amine and hydroxyl groups (Fourest and Volesky, 1996; Tobin et al., 1990; Waihung-Lo et al., 1999; Gonzalez et al., 2001; Figueira et al., 1999; Urasa and Macha, 1999). Among these functional groups; carboxyl group was reported as the major group responsible for metal binding (Crist et al., 1988; Tobin et al., 1984; Gonzalez et. al., 2001; Figueira et. al., 1999).

However, Fourest and Volesky (1996) indicated that the solution pH could be significant on the contribution of different functional groups in biosorption.

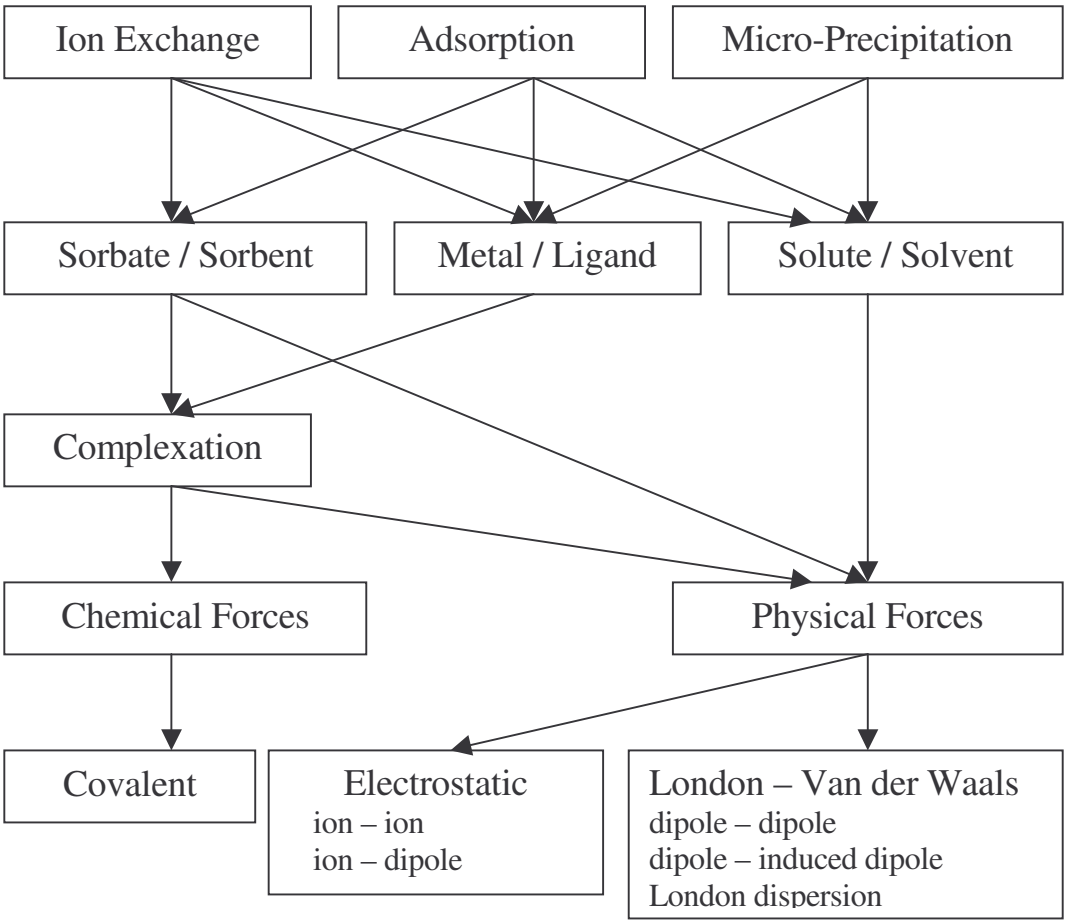

Figure 2.2. Biosorption Mechanisms (From Schiewer, 1996).

Another major metal removal mechanism; micro-precipitation, also called as surface precipitation, is the condensation or precipitation of heavy metal hydroxides onto surface of the biomass. The deposition of heavy metals within the diffuse part of double layer is favored if the solid surface is negatively charged. As a result, although the solubility limit in the bulk solution is not exceeded, the solubility limit at the

surface of biomass, being less than the solubility limit in bulk solution, could be exceeded and surface precipitation could take place (Schneider et al., 2001).

Complex formation of metals with ligands occurs as ligands attach to the metal ion (central atom), thus surrounding the metal ion. The directly attached atoms of the ligand to the metal ions are referred as the coordination atoms and determine the coordination number of the complex. If one ligand is attached to the metal ion through two or more coordinating atoms, then the complex is called chelate (Schiewer, 1996).

The final binding of metals or their complexes may be through chemical or physical forces. Chemical forces include covalent bonding and physical forces include electrostatic or Van der Waals forces (Schiewer, 1996).

Major analytical methods used to investigate the mechanisms responsible for biosorption of heavy metals include titration experiments and some spectrophotometric analysis tools like; Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Fourier Transform InfraRed (FTIR) Spectroscopy and different X-Ray spectroscopy studies.

In several of the studies investigating the functional groups involved in biosorption, titration has been used. After comparing the pKa values obtained in titration curves with values reported for inorganic acids, acidic groups over the surface of the biomass were determined (Schiewer, 1996; Gonzalez et al., 2001; Fourest and Volesky, 1996). In their study with dealginated seaweed, Gonzalez et al. (2001) titrated the biomass using HCl or NaOH to investigate the possible binding sites available. Two pK_a values were obtained from the titration curve. These values; 3.63 and 9.09 corresponded to pK_a values reported for carboxylic acids and saturated thiol or amine groups, respectively. Thus, the carboxyl and thiol or amine groups were determined as the possible functional groups responsible for biosorption of Cd. With further research, it was found that the metal uptake of dealginated seaweed decreased from 95 % to 17 % indicating that the carboxyl group was the major functional group involved in the uptake of Cd by dealginated seaweed.

On the other side, SEM and TEM techniques were used to show the location of metal sorption/uptake places throughout the cell structure (Volesky and May-Phillips, 1995; Waihung Lo et al., 1999; Templeton et al., 2003; Tsezos et al., 1997). Waihung Lo et al. (1999) analyzed Pb(II) biosorption by *Mucor rouxii* using SEM coupled with X-Ray Energy Dispersion Analysis (XEDA), and after contact with Pb(II) found a Pb(II) peak while the Ca and K peaks were observed to decrease, which indicated a decrease in the concentration of these cations on the biomass surface. Depending on these results, they suggested ion exchange to be the dominant mechanism for the uptake of Pb(II) by *Mucor rouxii*.

In a recent study, Tsezos et al. (1997) used TEM coupled with XEDA, which is a similar instrumental technique, to investigate the uptake of different heavy metals; Ag, Ni and Pd by different microbial strains. The microbial strains used in the study were labeled as; BP 7/26: *Arthrobacter sp.*, ER 121: *Alkaligenes eutrophus* and AS 302: *Pseudomonas mendocina*. The location of biosorbed Ag, Ni and Pd were observed using these microbial strains, which were reported to have very different characteristics. The results showed that Ag formed distinct clusters located outside the cells whereas Pd was retained inside the microbial cells. The location of Ni could not be determined as all the strains biosorbed only little amount of Ni. So, it was concluded that the observed differentiation in the location of biosorbed metals was most probably dependent on the metal rather than the microbial strain.

FTIR and X-Ray spectroscopy studies also yield very useful structural and analytical information on metal biomass interactions with simple sample preparation. The use of both methods is common in determination of biosorption mechanism. FTIR studies are especially used for determination of functional groups while X-Ray analyses are performed to understand the form of metal after its sorption to the surface (Fourest and Volesky, 1996; Figueira et al., 1999; Gonzalez et al., 2001; Templeton et al., 2003).

Fourest and Volesky (1996) investigated the FTIR spectrum of Cd bounded and protonated *Sargassum fluitans* biomass. The absorbance peak at 1738 cm^{-1} corresponding to the carbonyl double bond of carboxyl group was observed in the

spectra of protonated biomass. However, in the spectra of Cd bound biomass, another absorbance peak at 1630 cm^{-1} was observed instead of the peak at 1738 cm^{-1}. These different peaks were interpreted as the deformation of double bond and formation of a single C – O bond due to complexation of carbonyl group by dative coordination with Cd.

Figueira et al. (1999) also used FTIR analysis to investigate the metal uptake by *Sargassum fluitans*. They have obtained nearly the same results as Fourest and Volesky (1996) in the FTIR spectrum, an absorbance peak at 1738 cm^{-1} for protonated biomass and instead of that peak new absorbance peaks were observed for Fe(II) and Fe(III) contacted protonated biomasses at 1639 cm^{-1} and 1642 cm^{-1}, respectively. The results were again interpreted as the complexation of carbonyl group by dative coordination with Fe species. The study also included X-ray Photoelectron Spectroscopy (XPS) analysis. The results indicated that after contacted with Fe(II) sulfate solution, the biomass contained both Fe(II) and Fe(III) species whereas it only contained Fe(III) after exposure to Fe(III) sulfate solution.

In an earlier work (Urasa and Macha, 1999), metal uptake by composted sludge was reported to involve phosphates. The investigators postulated that Pb was first adsorbed on the surface of the apatite found in organic matter, and then cation exchange occurred between Ca in the apatite and Pb in solution. Urasa and Macha (1999) have also indicated that composted sludge had a high affinity for Pb with a retention capacity of 15 % of its weight. On the other hand, Alibhai et al. (1985) reported a Pb retention capacity below 0.1 % for anaerobically digested sludge and indicated that possible Pb removal mechanism was adsorption. As opposed, in the earliest study on heavy metal removal by anaerobic sludge, Gould and Genetelli (1978) reported that weakly acidic ligands were involved in heavy metal complexation. Thus, the results from different works are contradicting and implies that to describe the general behavior of heavy metals in the presence of anaerobically digested sludge or specifically of Pb which is the target metal in the present study, it is essential to have a detailed review of the related literature. In the following section, a detailed literature review on Pb removal by anaerobic biomass is presented.

2.3. LEAD REMOVAL BY ANAEROBIC BIOMASS

Lead is one of the metals of primary importance in biosorption studies due to its frequent occurrence and also its pronounced removal. Table 2.1 gives a summary of the biosorption studies with lead. As can be seen clearly, lead uptake capacity of different biomass types is highly variable. However, as reported by Dölek (1997); although the metal affinity of biosorbents depends on the surface structure and type of biomass, the biosorption capacities reported for lead for most of the biosorbents are much higher as compared to other heavy metals. In a recent study, Haytoglu (2000) further confirmed this and reported the highest Pb removal capacity for anaerobically digested sludge. She found a capacity of 1250 mg Pb(II) / g biomass using anaerobic biomass grown under laboratory conditions.

Effects of environmental factors on lead biosorption such as initial pH and temperature were investigated in several of the previous studies. The optimum pH values for different types of biomass used are found to be in the range of 4 – 6. Dölek (1997) tested the effect of pH on biosorption of lead with *Phanerochaete chrysosporium* and found that in the range of 2 – 7, the sorption capacity is maximum at the value of 5.0.

Kapoor (1998) also worked over the pH range of 2 – 6 using *Aspergillus niger.* He observed a sharp increase in capacity when the pH was increased from 3 to 4, which he refers as the “adsorption edge”. The optimum pH range in his studies was 4 – 6 and he preferred to study at the pH value of 5 during his experiments (Kapoor and Viraraghavan, 1997; Kapoor, 1998). In parallel, Waihung et al. (1999) reported the optimum pH as 6.0 for the biosorption of lead by *Mucor rouxii*. Using anaerobically digested biomass Haytoglu (2000) indicated pH 4.0 as the optimum in Pb removal with anaerobic biomass when she investigated the removal in the pH range of 2 to 5.

Table 2.1 Lead uptake values for different biomass types.

Biomass Class	Biomass Class	Capacity (mg/g)	Reference
Sphaerotilus natans	Bacteria	135	Pagnanelli et al., 2003
Streptomyces noursei	Filament. Bact.	55	Mattuschka et al., 1993
Bacillus subtilis	Biosorbent	601	Brierley et al., 1986
Bacillus subtilis	Biosorbent	189	Brierley and Brierley, 1993
Fungal biomass	Biosorbent	373	Brierley et al., 1986
Ascophllum nodosum	Brown marine algae	270 – 360	Holan and Volesky, 1994
Fucus vesiculosus	Brown marine algae	220 – 370	Holan and Volesky, 1994
Sargassum natans	Brown marine algae	220 – 270	Holan and Volesky, 1994
Durvillaea potatorum	Marine algae	331	Matheickal and Yu, 1999
Sargassum fluitans	Brown seaweed	220	Fourest and Volesky, 1996
Absidia orchidis	Fungus	351	Holan and Volesky, 1995
Penicillum chrysogenum	Fungus	25	Nemec et al., 1977
Penicillum chrysogenum	Fungus	122	Niu et al., 1993
Penicillum chrysogenum	Fungus	93	Holan and Volesky, 1994
Phanerochaete chrysosporium	Fungus	80	Dölek, 1997
Rhizopus arrhizus	Fungus	91	Tobin et al., 1984
Rhizopus arrhizus	Fungus	55	Fourest and Roux, 1992
Rhizopus nigricans	Fungus	166	Holan and Volesky, 1995
Streptomyces longwoodensis	Fungus	100	Friis and Myers-Keith, 1986
Mucor rouxii	Filament. Fungus	769	Waihung et al., 1999
Saccharomyces uvarum	Yeast	48.9	Ashkenazy et al., 1997

Although there have been plenty of studies investigating biosorption of lead (Holan and Volesky, 1994; Fourest and Volesky, 1996; Kapoor, 1998; Waihung Lo et al., 1999; Ashkenazy et al., 1997; Pagnanelli et al., 2003; Matheickal and Yu, 1999), there is only a few study using anaerobic biomass as the biosorbent (Haytoglu, 2000). The rare use of anaerobic biomass (sludge from wastewater treatment plants) is reasoned for the mixed and heterogeneous microbial populations involved, which makes biosorption researches difficult and doubtful (Volesky, 2001). As mentioned in the previous section, the earliest study on heavy metal removal by anaerobic biomass dates back to 1978 (Gould and Genetelli, 1978), where the mechanism of metal (Ni, Zn, Cu, Cd) removal was investigated considering pH as the major parameter influencing the removal. The investigators reported visual heavy metal precipitation after certain equilibrium pH values below which there occurs metal removal by sorption. On the other hand, in a later study, Alibhai et al. (1985) indicated that the mechanism of attachment involves metal/surface ligand interactions.

In a series of recent works, Artola et al. (1992, 1997, 2001) also used anaerobic biomass in biosorption. In 1992, Artola and Rigola investigated the optimum sludge for Zn removal from a variety of sludge samples collected from a wastewater treatment plant and determined the best type of sludges to be thickened anaerobic and dewatered sludges. Afterwards Artola et al. (1997) the possible binding mechanism of Ni to anaerobically digested sludge and the responsible surface groups in the process. They reported the functional groups to be of the amine type due to similarities observed between glycine-metal and sludge-metal titration curves. In their further study, Artola et al. (2001) researched the Cu removal in a contact settling tank, again using anaerobically digested sludge as biomass.

2.4. REACTOR BIOSORPTION STUDIES

Before examining the literature on biosorption in reactors it is preferred to give brief information about the reactor configurations used in adsorption studies as these configurations are also applicable to biosorption studies. These reactor configurations can be divided into two main groups; slurry contact and column contact applications. Slurry contact applications include; single stage batch, multiple stage batch, multiple

stage countercurrent and continuous applications. Column contact applications are fixed beds and moving or pulse beds (Bernardin, 1985).

In single stage batch configuration the heavy metal solution is contacted with fresh biomass for a determined contact time. When the heavy metal concentration reaches the desired level, biomass is separated from solution. While the treated solution is discarded, used biomass is disposed or reused after recovery of metals. The results from sorption experiments can be directly used in this configuration.

In multiple stage batch applications the heavy metal solution is contacted with additional fresh biomass in each stage. In this configuration less amount of biomass can be utilized for the same amount of metal removal. At the end of every stage biomass is separated from the solution and the effluent enters the next stage.

Multiple stage countercurrent application provides cost effectiveness of biomass. In a two-step countercurrent application for example, the heavy metal solution is contacted with once used biomass in the first step. After a determined contact time passed the biomass is separated from the solution and disposed or reused after metal recovery. The treated solution enters the second step where it is contacted with fresh biomass. After the desired concentration of metal is obtained, the biomass is separated from the solution, becoming once used biomass. The once used biomass is sent to the first step to contact with new untreated heavy metal solution. The treated solution with desired effluent concentration of metal is discharged. Such a reactor configuration is preferred where the steepness of the isotherm curve or very low discharge standards point out the use of single batch treatment will not be cost effective.

In continuous applications plug flow conditions are created in the reactor through the use of stirred reactors in series or adjusting baffles. The volume of the tank is designed to give enough contact time for metal removal. When desired metal concentration is achieved the biomass is separated and the treated solution is discharged. The use of countercurrent biomass flow with multiple stages is also possible during continuous

flow operations through installation of proper additional equipment and may provide cost effectiveness of biomass.

In fixed bed column applications, the columns are filled with biomass and the flow is passed through process until the biomass is exhausted. When this happens, new columns are installed. On the other hand, in pulse bed applications the exhausted portion of biomass is spent from the reactor and fresh biomass is added to replace that portion. Countercurrent biomass addition can be utilized in both types of column applications (Bernardin, 1985).

Since concentration difference is the driving force in sorption experiments, plug flow regime results in better efficiency compared to complete mix conditions (Volesky, 2001). Therefore; most preferred type of reactor configuration in biosorption studies has been the fixed bed column applications (Kratochvil and Volesky, 2000; Figueira et al., 2000; Yan and Viraraghavan, 2001). In the proceeding paragraphs, a number of previous studies are summarized.

Recently, Matheickal and Yu (1999) investigated the biosorption of Pb(II) in a packed bed column by calcium treated biomasses of *Durvillaea potatorum* and *Ecklonia radiata*, referred as DP95Ca and ER95Ca, respectively. A column of packed with 2 g of biomass, was fed with 2.4 mM Pb(II) solution at a flowrate of 1.5 mL/min. About 1000 mL Pb(II) solution was passed through the column before breakthrough and the Pb(II) concentration in the effluent of the column was below 0.02 mg/L for both DP95Ca and ER95Ca biomasses. The calculated capacities after saturation of the column were 1.6 and 1.3 mmol/g respectively, for DP95Ca and ER95Ca.

In another recent study, Yan and Viraraghavan (2001), used a column of 1.27 cm diameter and 40 cm height to investigate the biosorption of Pb, Cd, Ni and Zn metal solutions. The column was filled with 4.5 g of immobilized biomass beads of *Mucor rouxii*, and the height of packed bed was 29 cm. Influent concentration for each metal was approximately 10 mg/L and the initial pH was 6. The column performed its maximum efficiency at a flow rate of 2.28 mL/min with Pb(II) for which the

breakthrough was observed after 20 bed volumes of Pb(II) was passed through the column. The breakthrough for Cd and Zn was obtained at 4 and 5 bed volumes, while the Ni concentration in the effluent immediately differed from zero even at the first bed volume. The calculated capacity values for Pb, Cd, Ni and Zn were as 4.06, 1.25, 0.36 and 1.36 mg per g of bead, respectively.

In a comprehensive study, Volesky et al. (2003) investigated the biosorption of Cu in a packed bed column filled with biomass of *Sargassum filipendula*. The column had an inner diameter of 2.5 cm and a height of 50 cm, 38 g dry biomass was placed in the column, which gave a biomass height of 41 cm and packing density of 189 g/L. Afterwards the column was operated at a flowrate of 15 mL/min to treat the influent Cu solution of 35 mg/L initial concentration and pH of 5. The breakthrough time was determined to be the time at which the effluent Cu concentration reached 1 mg/L. After saturation of biomass the column was washed with 1 % $CaCl_2$ tap water solution (pH = 3) for desorption and regeneration processes. The capacity of biomass, breakthrough time and mass of biomass in the column was followed during 10 consecutive sorption/desorption cycles. Although small deviations were observed the capacity was reported to be constant at approximately 38 mg/g. On the other hand, the breakthrough time decreased continuously from 25.4 h to 12.7 h from first to last cycle. And the mass of *Sargassum filipendula* also decreased from 38 g to 29.8 g.

Although packed-bed column applications have been preferred due to their high efficiency in sorption studies, some other types have also been used considering the type or form of biomass to be used. For example, Artola et al. (2001) preferred to operate a contact settling type of reactor while studying the removal of Cu by anaerobically digested sludge. The total volume of the reactor was 0.0365 m^3 with a diameter of 0.29 m and a height of 0.72 m. The reactor was configured with 3 operational zones; a clarification zone at the top, a mixing zone for metal-sludge contact and a conic shaped sedimentation zone at the bottom. The reactor was fed with Cu solution from the bottom and sludge was introduced at a flowrate of 3.7×10^{-4} dm^3/s from the top of the reactor. The optimum Cu/sludge feed ratio was investigated changing the influent Cu concentration in the range of 50 – 250 mg/L during the tests.

Maximum metal uptake was reported to be 75 mg/g at Cu/sludge feed ratios above 90 mg Cu per g of total solids.

In a recent study, Pümpel et al. (2001) introduced MERESAFIN (MEtal REmoval by SAnd Filter INoculation) process. MERESAFIN, a patented moving bed Astrasand® filter, was used to treat the industrial effluent from a metal plating company in Vienna. The sand filter was inoculated by a mixture of selected microorganisms whose ability in biosorption or bioprecipitation was tested before. The wastewater of the company contained organic acids and inorganic phosphates in addition to nickel. With the selection of best types of bacteria responsible for biosorption, bioprecipitation and biodegradation the process worked successfully with such a complex wastewater. A regenerating biofilm was obtained in the sand filter, which was reported to constitute the major part of the biomass even after eight months of first inoculation. While organic complexes formed by nickel were completely degraded, the released nickel was biosorbed by the biomass but a much higher extend was precipitated in the biofilms.

CHAPTER 3

MATERIALS AND METHODS

3.1. MATERIALS

Raw and dewatered biomass samples collected from the Ankara Sincan Municipal Wastewater Treatment Plant (that employs conventional activated sludge as biological treatment, and anaerobic decomposition as sludge digestion process) were utilized. Raw ADS samples were directly taken from the effluent of anaerobic digesters and dewatered ADS samples were collected from the outlet of mechanical dewatering units following conventional completely mixed anaerobic digesters. ADS samples that were brought to the laboratory from the treatment plant were kept refrigerated at a temperature of 4°C to make sure that there is no metabolic activity and thus no change in the characteristic of samples. Raw ADS to be used in metal uptake experiments was concentrated by emptying the supernatant after sufficient time of settling, while dewatered biomass was utilized without any pre-treatment.

Pb(II) solutions used in the experiments were synthetically prepared using $Pb(NO_3)_2$ salt supplied by PANREAC (Montplet and Esteban SL) and distilled water.

3.2. ANALYTICAL METHODS

To determine the solid and organic content of ADS samples total solids (TS), total volatile solids (TVS), mixed liquor suspended solids (MLSS) and mixed liquor volatile

suspended solids (MLVSS) measurements were done in accordance with the methods described in Standard Method 2540 (Standard Methods, 1995). The amount of biomass to be added was determined with respect to the organic content of the sludge samples; which is measured as MLVSS content for raw ADS, and TVS content for dewatered ADS.

Solution pH during the experiments was measured using a bench-top pH meter (Jenway Ltd., Essex, UK) and a general-purpose pH electrode.

The Pb(II) concentration of the samples were analyzed using a flame atomic absorption spectrophotometer (AAS) (ATI Unicam Model 929). Measurements were done in accordance with Standard Method 3500-Pb B (Standard Methods, 1995). A calibration curve prior to sample measurements were prepared using stock solution of Pb(II). The samples with Pb(II) concentrations exceeding upper detection limit were diluted by a convenient factor by distilled water. Prior to AAS analysis, the pH of the samples was adjusted to approximately 2.0 using HNO_3.

To measure the settleability of sludge formed after biosorption, it was decided to use sludge volume index (SVI) as an indicator. However, since the available volume of sludge samples was not sufficient to run the standard SVI test, a modified method was developed. Thirty minutes after the beginning of sedimentation period in 2 L beakers, the level of settled sludge was marked. When the reactors were emptied water was filled up to marked level, and the volume of this water was measured pouring that amount of water to a graduate cylinder. SVI was then calculated by dividing this volume by total amount of MLSS added to the reactor.

3.3. BIOSORPTION TESTS

The ability of ADS to take up Pb(II) was investigated by running batch biosorption experiments that were mainly composed of sorption kinetics and sorption equilibrium (isotherm) tests. These tests were carried out with both raw and dewatered ADS samples. In the experiments with raw ADS samples, kinetics and equilibrium tests were carried out with and without initial pH adjustment. With dewatered ADS

samples, all tests were performed without any pH adjustment. During the experiments with pH adjustment, HNO_3 was used for pH adjustment. In preparing Pb(II) solutions, a stock $Pb(NO_3)$ solution of 25000 mg/L concentration was utilized. In all sorption experiments, tests were run in duplicate and the temperature was kept constant at 25°C. Biosorptive capacity was calculated by using the equation;

$$\left[q = \frac{V(C - C_0)}{m} \right]$$

where q is the capacity as mg Pb(II) per g dry biomass, V is the volume of the sample, C and C_o are the final and initial Pb(II) concentrations in aqueous phase as mg/L and m is the amount of biomass added as g.

3.3.1. Sorption Kinetics Tests

Kinetic experiments were conducted with both raw and dewatered ADS. Lead solution at the initial concentration of 100, 200 and 1250 mg/L was placed in a beaker of 2 L volume and the biomass was added. For all kinetic tests, a Pb(II) to biomass ratio of 1250 mg/g biomass was kept. After the addition of biomass, the mixture was agitated for 5 h and metal removal was followed. At certain time intervals, samples (10 mL) were taken and the solution pH was measured in order to follow up the time course change of pH during sorption. After 5 h, stirring was stopped and a final sample was taken following a settling period of 1 h. All samples were filtered through 0.45 μm membrane filters and filtrates were analyzed for Pb(II) concentration following pH adjustment to 2.0 using HNO_3. In the kinetic tests with initial pH adjustment, solution pH was adjusted to 4.0±0.2 or 2.0±0.4 using different normalities of HNO_3 solution.

3.3.2. Sorption Equilibrium Tests

In sorption equilibrium tests, flasks of 250 mL total volume placed in a shaking incubator, were utilized as batch reactors. Pb(II) solutions (100 mL) of known concentration, varying from 100 to 2500 mg/L were placed in these flasks and when necessary, the pH of the solutions was adjusted to the desired value using HNO_3. Then, raw or dewatered ADS samples were added to the flasks to yield 0.1±0.01 g

biomass concentration. Afterwards, to reach equilibrium the flasks were incubated in a shaking incubator for 24 h at 25°C and 200 rpm. At the end, 10 mL samples were taken from the flasks, filtered through 0.45 μm membrane filters and the Pb(II) concentration of filtrates was determined. In the sorption tests with initial pH adjustment, solution pH was adjusted to 4.0±0.2 or 2.0±0.4.

3.4. FTIR SPECTROSCOPY ANALYSIS

In investigating the mechanism of biosorption, FTIR spectroscopy technique was adapted and raw ADS samples before and after contact with Pb(II) solutions were analyzed. A sample of ADS with a solids concentration of 0.1 g/L MLVSS was added to both 100 mL solutions of distilled water and 500 mg/L Pb(II). The mixtures were then placed in a shaking incubator at 25°C and 200 rpm. After 24 h, the mixtures were filtered through 0.45 μm membrane filters and the biomass deposited cakes were dried at 60°C overnight, then ground powder of each sample was prepared and FTIR spectrophotometric analyses were conducted using a Bruker Equinox-55 FTIR spectrophotometer device with a Harrick Praying Mantiss ambient DRITS chamber sampling attachment.

3.5. REACTOR OPERATION

Single stage and three stage serial reactor combinations were tested for the initial Pb(II) concentrations of 100 and 200 mg/L. Continuously stirred fed-batch reactors of 2L±0.1 volume were used in all these experiments. Raw ADS was selected as the biomass to be used in reactor operation. The experiments were performed at room temperature and reactor content was mixed rapidly using a magnetic stirrer. Two hours of mixing was followed by a 1 h sedimentation period in fed-batch reactors.

3.5.1. Single Stage Reactor Operation

After the reactor was filled with the desired concentration of Pb(II) (100 or 200 mg/L), sufficient time for mixing was allowed to have a homogenous solution and samples were collected to measure initial Pb(II) concentration. Then the biomass was added to

give an MLVSS amount of 0.6 g. After 2 h, mixing was stopped and 1 h of sedimentation was allowed and supernatant samples were collected for final Pb(II) concentration measurement.

3.5.2. Three Stage Reactor Operation

First stage of the tests was carried exactly as described above for single stage tests, except, the amount of biomass added was one third, that is 0.2 g MLVSS. After the first stage was completed and supernatant sample was collected the supernatant was taken into another reactor for second stage without disturbing the settled sludge. At the beginning of second stage the same amount of biomass (0.2 g MLVSS) was added again. After 2 h of mixing and 1 h of sedimentation, sampling was done for Pb(II) concentration measurement. The supernatant was again taken into another reactor for the third stage showing care not to disturb the settled sludge. Biomass was added to give an MLVSS amount of 0.2 g. Two hours of mixing and 1 h of sedimentation was performed. Supernatant samples were collected for final Pb(II) concentration measurement.

Solution pH was monitored and SVI tests were performed at every stage of the reactor tests. All samples were filtered through 0.45 μm membrane filters and filtrates were analyzed for Pb(II) concentration following pH adjustment to 2.0 using HNO_3.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. SORPTION TESTS USING ADS

4.1.1. Sorption tests with raw ADS at initial pH of 4.0

In the previous study by Haytoglu (2000), the optimum operational conditions for biosorption of Pb(II) using anaerobic biomass grown under laboratory conditions were found to be an initial pH of 4.0 and a temperature of 25°C. The maximum capacity of the biomass under these conditions was reported to be 1250 mg Pb(II)/g biomass. For the purpose of verifying this high Pb(II) sorption capacity value obtained and to assess the effect of using biomass taken from a wastewater treatment plant instead of biomass grown under laboratory conditions, the first set of experiments were conducted under the same operational conditions as those of Haytoglu (2000).

After the addition of ADS of sufficient amount to yield 1g biomass/L concentration in the solution Pb(II) concentration and the pH of the solution were followed with respect to time, and the results shown in Figure 4.1 were obtained. As shown, the Pb(II) concentration in the solution decreased with time and reached equilibrium after about 2h. In fact, the removal of Pb(II) from the solution occurred in two steps; a rapid initial removal step followed by a slow one. While the Pb(II) concentration decreased from 1350 mg/L to 650 mg/L within the first 10 min, a steady state concentration of 250 mg/L was measured in the solution after 2.5 h. There was a time course increase in the

calculated capacity values and the capacity obtained at equilibrium was 5.3 mmol Pb(II)/g biomass (Figure 4.1).

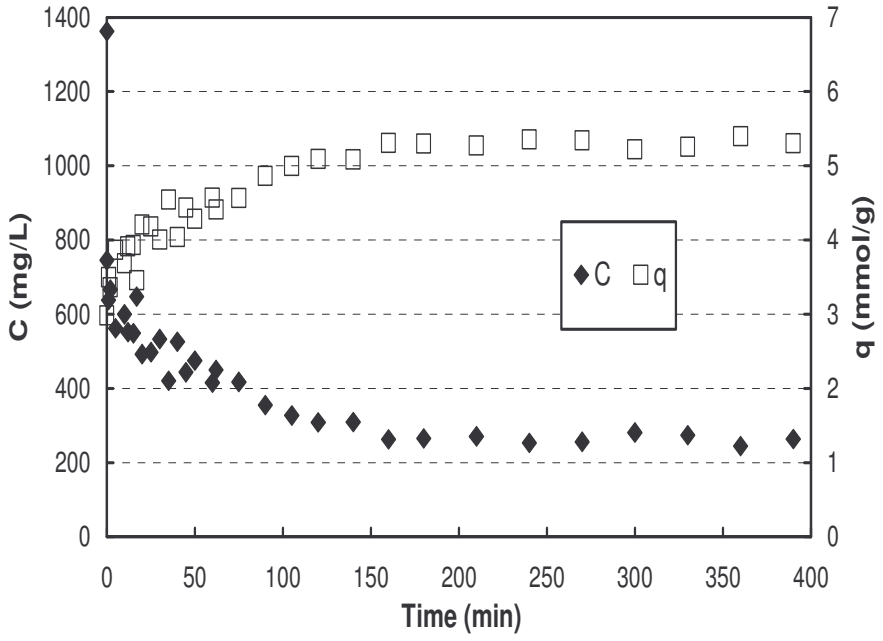

Figure 4.1. Time course Pb(II) removal by raw ADS at initial Pb(II) concentration of 1250 mg/L (Initial pH = 4.0, T = 25°C).

The pH of the solution was also monitored during the experiments and the data presented in (Figure 4.2) was obtained. There was a sudden increase in the pH, up to 5.3 just after the addition of biomass; then the pH decreased for 10 min to a value of 4.8, afterwards a continuous increase was observed till the end of the experiment, where the pH reached the value of 5.5.

Finally during 1 h sedimentation period, adopted after 2h sorption period; it was seen that the biomass particles settled very fast, forming large floc structures.

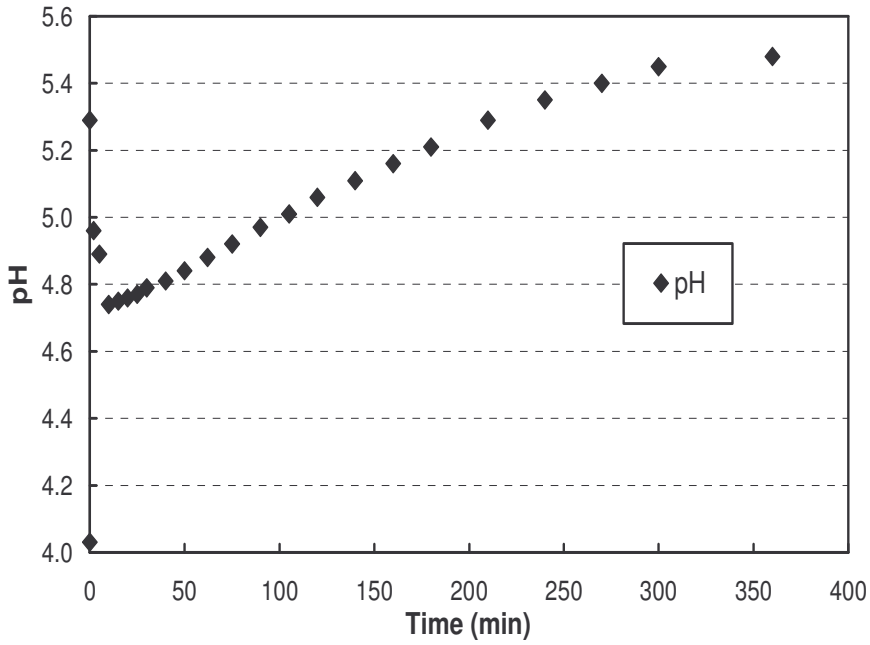

Figure 4.2. Time course pH change during Pb(II) removal using raw ADS at an initial Pb(II) concentration of 1250 mg/L (Initial pH =4.0, T=25°C).

Following the Pb(II) sorption kinetic studies by raw ADS, sorption equilibrium (isotherm) tests were conducted to determine the maximum capacity of the biomass at the initial solution pH of 4.0 and the results presented in Figure 4.3 were obtained. As can be seen, the sorption isotherm was of the classical L-type indicating favored sorption of Pb(II) by ADS. The maximum capacity of raw ADS appeared as 6.7 mmol Pb(II)/g biomass at a Pb(II) equilibrium concentration of about 1000 mg/L. An evaluation of the equilibrium and initial Pb(II) concentrations have indicated that, especially for the samples having an initial Pb(II) concentration of 750 mg/L or less, measured equilibrium concentrations of Pb(II) in the solutions were very low (< 3mg/L). As expected, an increase was observed in Pb(II) removal capacity until an equilibrium concentration of about 400 mg/L is reached; beyond which capacity is independent of equilibrium Pb(II) concentration.

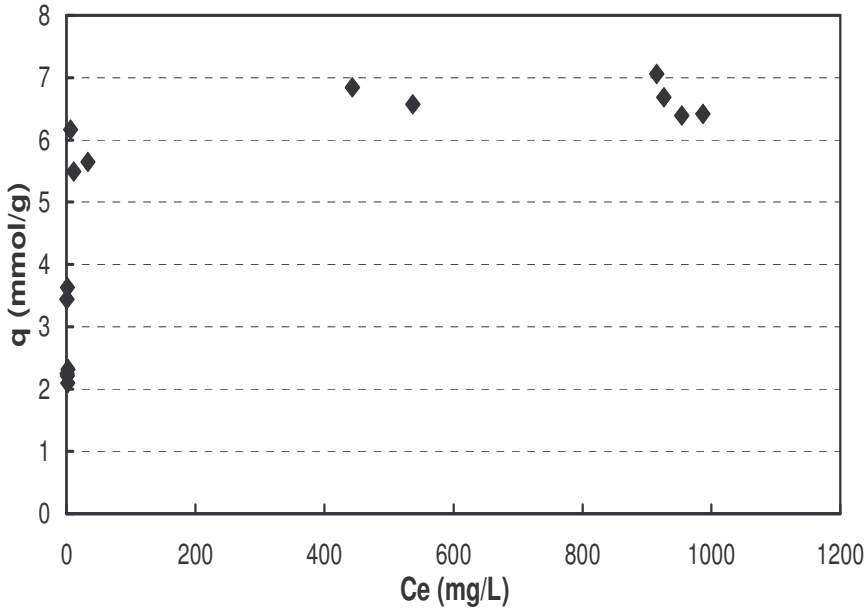

Figure 4.3. Pb(II) sorption isotherm using raw ADS (Initial pH = 4.0, T=25°C).

When the isotherm data were fitted to Langmuir and Freundlich models in order to see if these models can describe the sorption behavior or not, it was observed that Langmuir model is quite satisfactory in describing the Pb(II) sorption under the present conditions, but not the Freundlich model (Table 4.1). A very low correlation coefficient of 0.702 indicated that Freundlich model was not proper for the description of the phenomenon.

Table 4.1. Langmuir and Freundlich model constants for sorption isotherm tests with raw ADS at initial pH of 4.0.

	Langmuir Model	
Q_{max} =1429 mg/g (=6.9 mmol/g)	B = 0.700 L/mg	R^2 = 0.9977
	Freundlich Model	
N = 7.553	K = 598 $(mg/g)(L/mg)^{1/n}$	R^2 = 0.7021

After 24 h of equilibration time, it was observed that the solution pH of all the ADS – Pb(II) solution mixtures increased with sorption. Thus, the equilibrium pH was higher than the initial pH for all test mixtures (Figures 4.4 and 4.5). The increase in the solution pH was higher in samples with low initial Pb(II) concentration while it was lower in samples with high initial Pb(II) concentration. For example; the equilibrium pH of the sample with 500 mg/L initial Pb(II) concentration was measured to be 8.0 whereas that of the sample with 1750 mg/L initial Pb(II) concentration increased to 5.5 only.

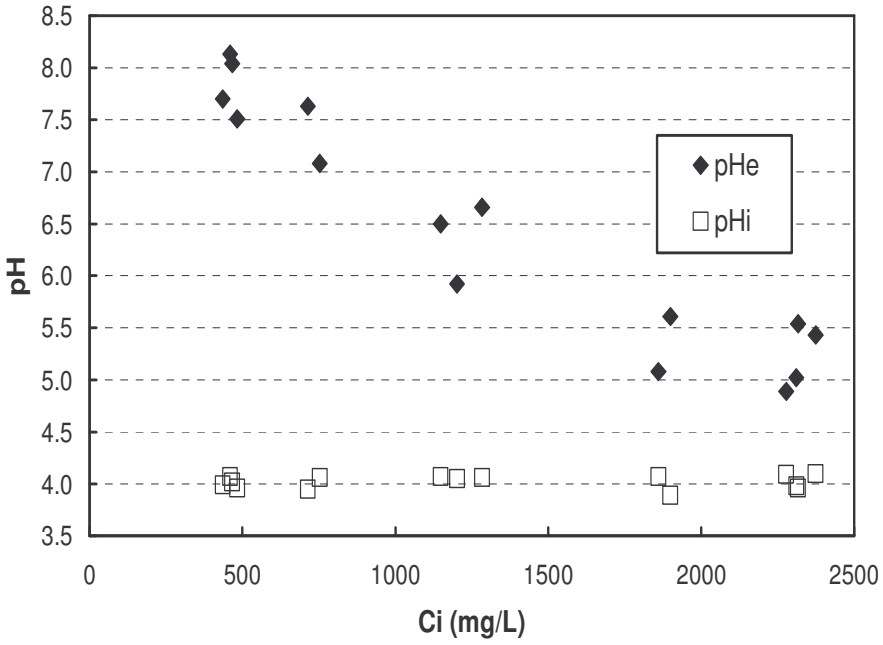

Figure 4.4. Initial and equilibrium pH versus initial Pb(II) concentration for isotherm tests with raw ADS (Initial pH = 4.0, T=25°C).

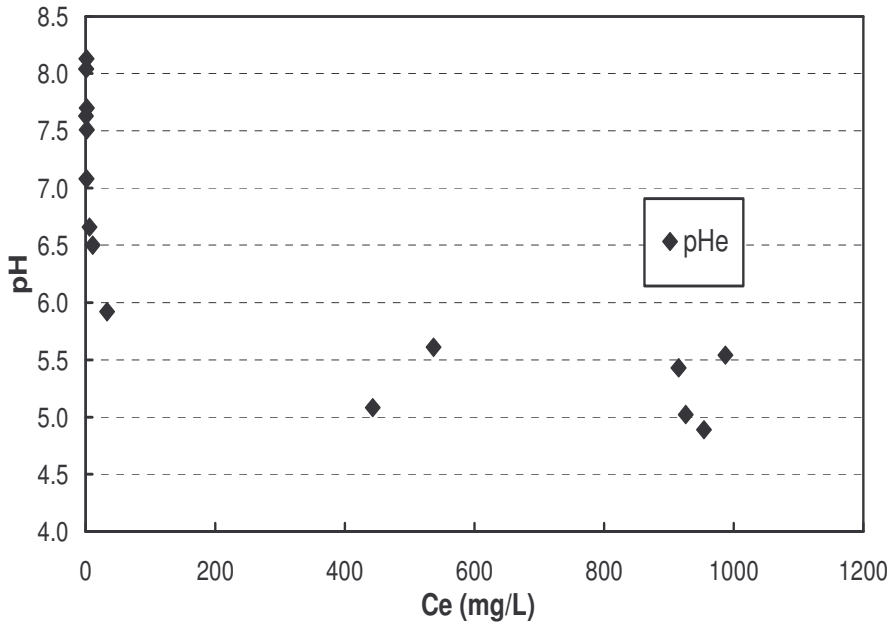

Figure 4.5. Equilibrium pH versus equilibrium Pb(II) concentration using raw ADS (Initial pH = 4.0, T=25°C).

High equilibrium pH values obviously had an effect on Pb(II) removal efficiency as over 99 % Pb(II) removal was achieved when equilibrium pH was over 6.5. The percentage Pb(II) removal at equilibrium pH of 6.0 is 97 % and it drastically reduced to 60 – 75 % when equilibrium pH was below 5.5. However, the Pb(II) sorption capacity showed a reverse relation with respect to equilibrium pH. At equilibrium pHs below 5.5, the Pb(II) sorption capacity was between 6.5 – 7.0 mmol Pb(II)/g biomass, whereas, above equilibrium pH of 7.0 the capacity range was 2- 3.5 mmol Pb(II)/g biomass (Figure 4.6).

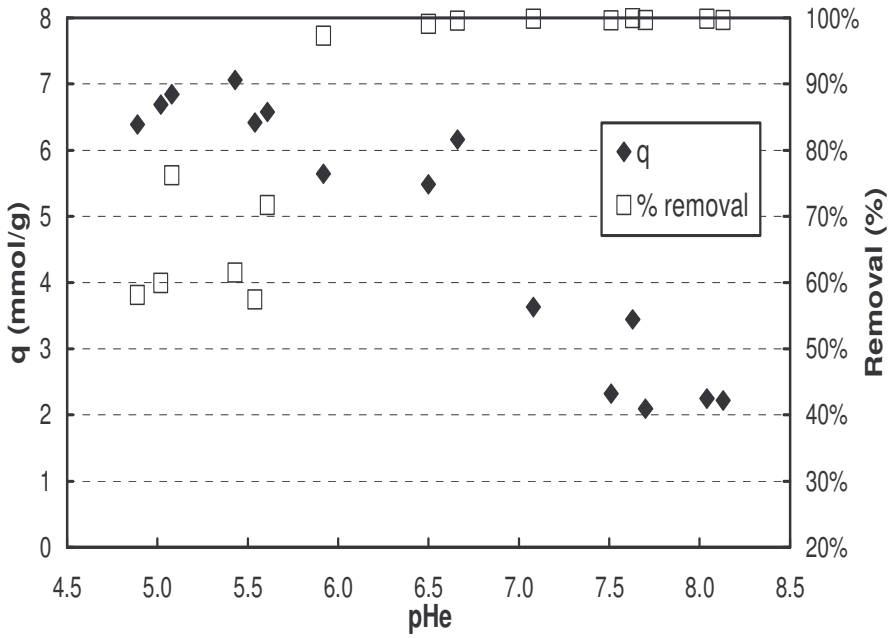

Figure 4.6. Capacity and % removal values using raw ADS with respect to equilibrium pH (Initial pH = 4.0, T=25°C).

In effect, the enormously high capacity of 1250 mg Pb(II)/g of ADS biomass, reported by Haytoglu (2000) was of the primary concern and also the origin of the present study. In order to adopt more "realistic" conditions; in the present study, ADS coming from a real wastewater treatment plant was utilized. Besides the high Pb(II) removal capacity of 1388 mg (6.7 mmol) Pb(II)/g biomass, findings such as; an increase in solution pH and high equilibrium pH values, higher increase of pH values for lower initial Pb(II) concentrations and the decrease in capacity with increasing pH indicated similarity with observations of Haytoglu (2000). Differences observed in numeric values were supposed to originate from the source of biomasses used. Haytoglu (2000) used autoclaved anaerobic biomass grown under laboratory conditions, whereas the biomass used in this study was obtained from the outlet of an anaerobic digester tank of a wastewater treatment plant.

4.1.2. Sorption tests with raw ADS at pH 2.0

The high capacity value obtained at initial pH 4.0 using raw ADS has been very promising for the treatment of heavy metal containing wastewaters, specifically Pb(II). However, since heavy metal containing wastewaters are known to have lower pH values due to high concentration of cations present, it was decided to investigate metal removal capacity of ADS at low pH values. Towards this end, sorption tests with initial pH adjusted to 2.0 were performed, and the Pb(II) removal performance of ADS at low pH was investigated.

At this low initial pH value, sorption kinetic tests were firstly performed with the same initial Pb(II) concentration of 1250 mg/L and biomass concentration of 1 g biomass/L. The pH of the Pb(II) solution was adjusted to 2.0 by using HNO_3 before adding the biomass. Time course change in Pb(II) concentration and solution pH was monitored during the tests, and the results shown in Figure 4.7 were obtained. As shown, the Pb(II) concentration in the solution decreased with time and reached an equilibrium after about 2h. The decrease in Pb(II) concentration occurred again in two steps; a rapid removal step followed by a slow removal step. While the Pb(II) concentration decreased from 1150 mg/L to 900 mg/L within the first 10 min, after 10 min the Pb(II) concentration first decreased slowly to 850 mg/L till the end of 60 min. Then an increase was observed and a steady state concentration of 1000 mg/L was measured in the solution after 2 h. The corresponding Pb(II) sorption capacity values suddenly increased and reached values around 1.5 mmol Pb(II)/g biomass. Afterwards, a decrease was observed and finally the capacity reached its equilibrium value at 0.7 mmol Pb(II)/g biomass (Figure 4.7). This equilibrium capacity value was much lower than that obtained at pH 4.0. At the pH of 4.0, the capacity was 5.3 mmol Pb(II)/g biomass (Figure 4.1).

The pH of the solution was also monitored during this experiment. Following the addition of biomass there was a sudden initial increase in pH, up to 2.3. However; soon after, a decrease was observed in pH till the end of the experiment. The solution pH at equilibrium was 2.2 (Figure 4.8). After 1 h sedimentation period, sludge was observed to settle rapidly, leaving a relatively clear supernatant.

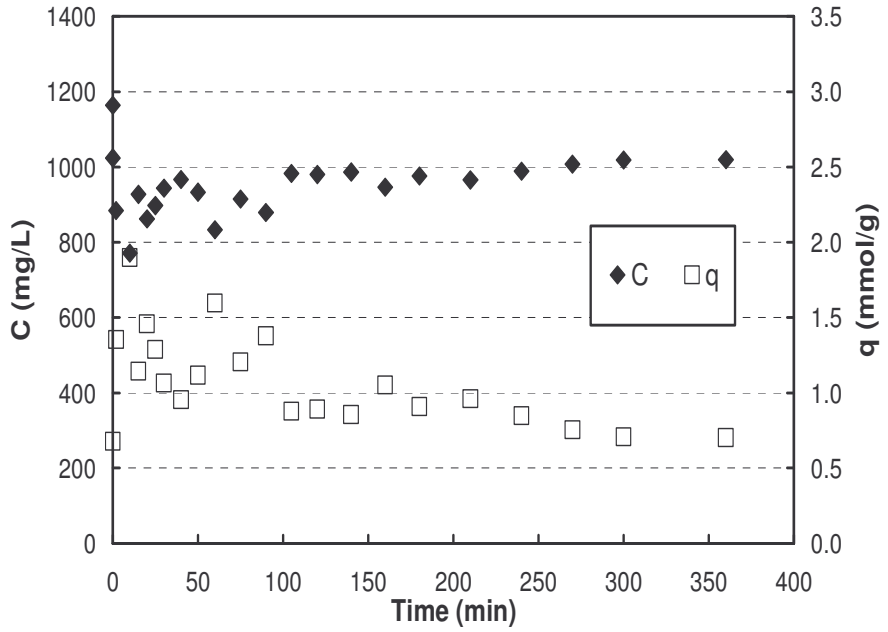

Figure 4.7. Time course Pb(II) removal by raw ADS at initial Pb(II) concentration of 1250 mg/L (Initial pH = 2.0, T=25°C).

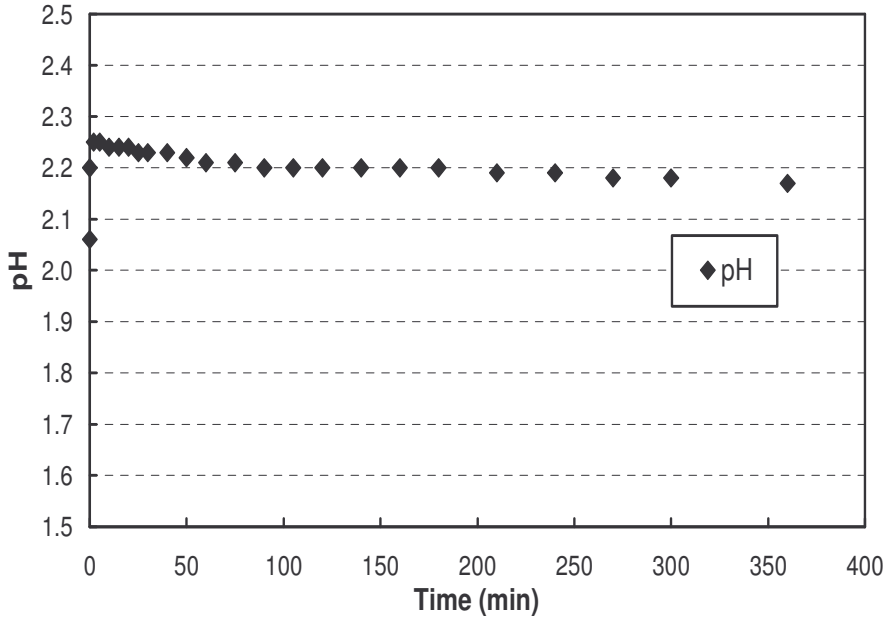

Figure 4.8. Time course pH change during Pb(II) removal using raw ADS at initial Pb(II) concentration of 1250 mg/L (Initial pH =2.0, T=25°C).

It is known that Pb(II) concentration in heavy metal containing wastewaters is generally in the range of 100 – 200 mg/L. While the highest concentration is around 400 mg/L in typical TV tube manufacturing industry effluents (Patterson, 1985). Therefore, to represent the real wastewater conditions and also to understand the effect of initial Pb(II) concentration on rate of biosorption, kinetic tests with initial Pb(II) concentrations of 100 and 200 mg/L were performed while keeping the initial pH at 2.0. During the tests, the Pb(II) concentration and the solution pH were monitored. In these experiments; to keep the biomass to Pb(II) concentration ratio constant at 1 g biomass/1250 mg Pb(II), the biomass concentration was adjusted to 0.08 and 0.16 g biomass/L for the initial Pb(II) concentrations of 100 and 200 mg/L, respectively.

As can be depicted from Figure 4.9, in the kinetic tests with 83 mg/L initial Pb(II) concentration, the Pb(II) concentration decreased from 83 mg/L to 77 mg/L in the first 5 min and then fluctuated in the range of 74 – 81 mg/L. The concomitant sorption capacity values were also fluctuating, within the sorption capacity range of 0.3 – 0.6 mmol Pb(II) / g biomass. On the other side, there was a gradual decrease in solution pH from an initial value of 2.1 to 1.9 as biosorption proceeded (Figure 4.10).

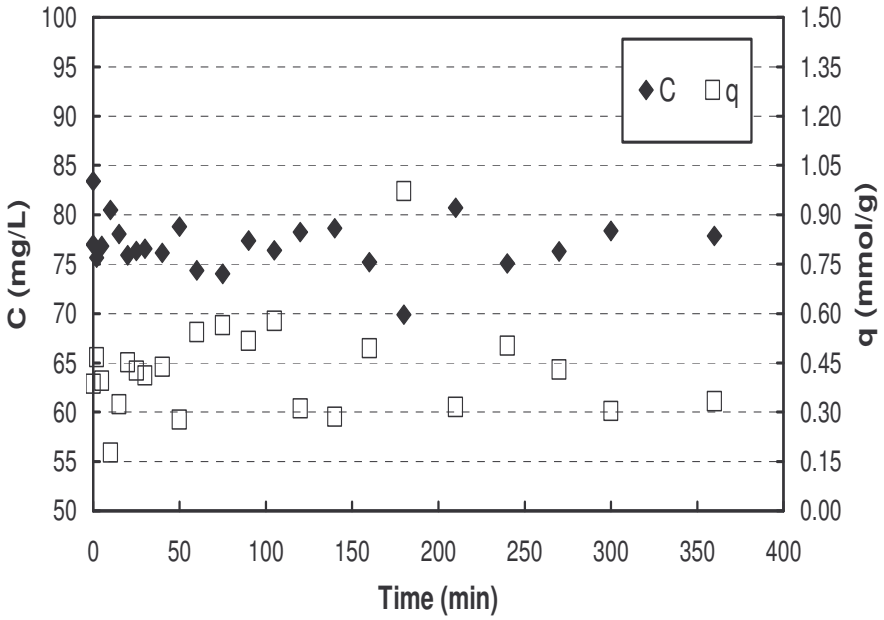

Figure 4.9. Time course Pb(II) removal using raw ADS at initial Pb(II) concentration of 83 mg/L (Initial pH = 2.0, T=25°C).

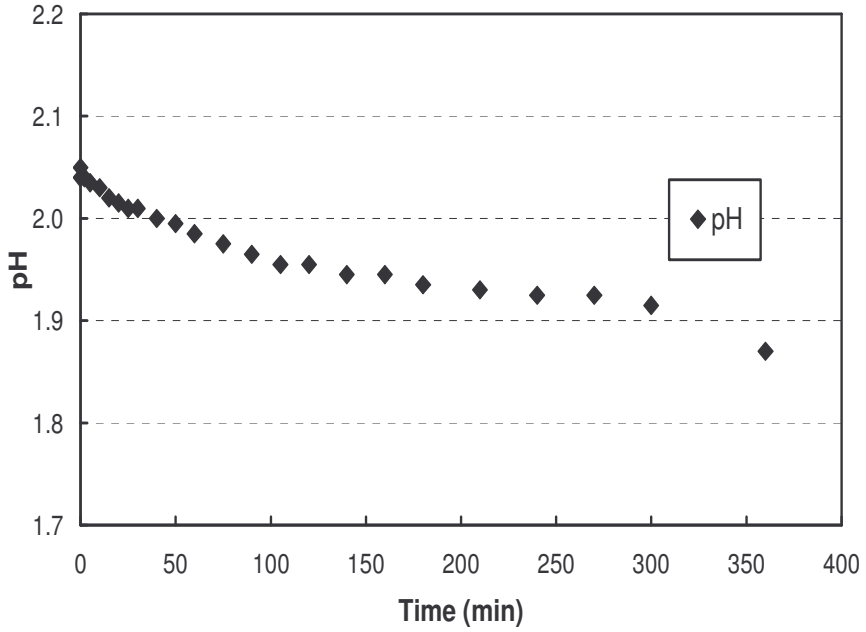

Figure 4.10. Time course pH change during Pb(II) removal using raw ADS at initial Pb(II) concentration of 83 mg/L (Initial pH =2.0, T=25°C).

At the highest Pb(II) concentration tested, which is about 159 mg/L; there was a sudden decrease down to 139 mg/L within the first 5 min. Afterwards, there was very little change in solution Pb(II) concentration and actually no more decrease after about half an hour. The calculated sorption capacity values increased and reached an ultimate value of about 0.7 mmol Pb(II) / g biomass (Figure 4.11). At this initial Pb(II) concentration, there occurred a sudden slight increase in solution pH just after the addition of biomass to metal solution and pH increased to above 2.1 from its initial value (Figure 4.12). However, right after a couple of minutes, the solution pH started to decrease gradually and stabilized at about 1.9.

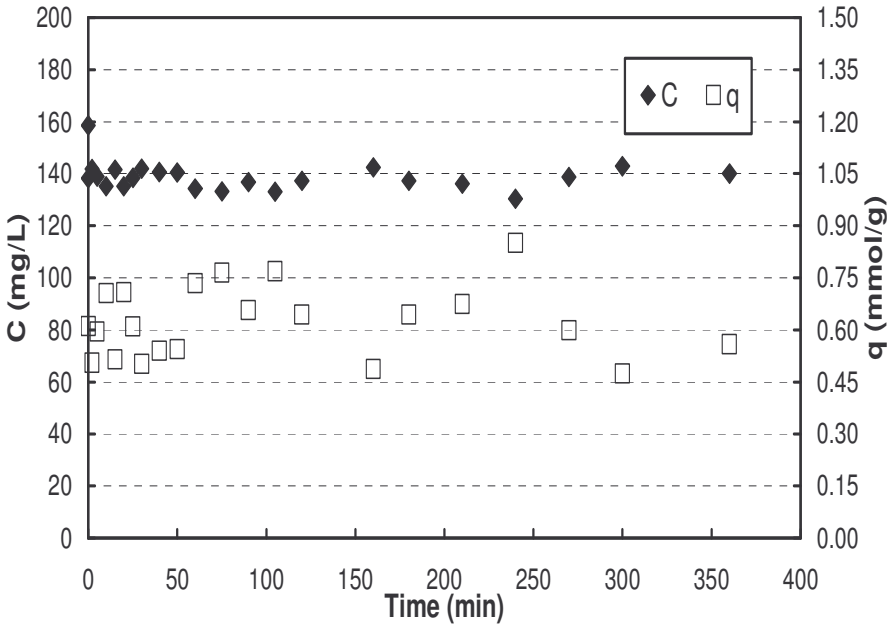

Figure 4.11. Time course Pb(II) removal using raw ADS at initial Pb(II) concentration of 159 mg/L (Initial pH = 2.0, T=25°C).

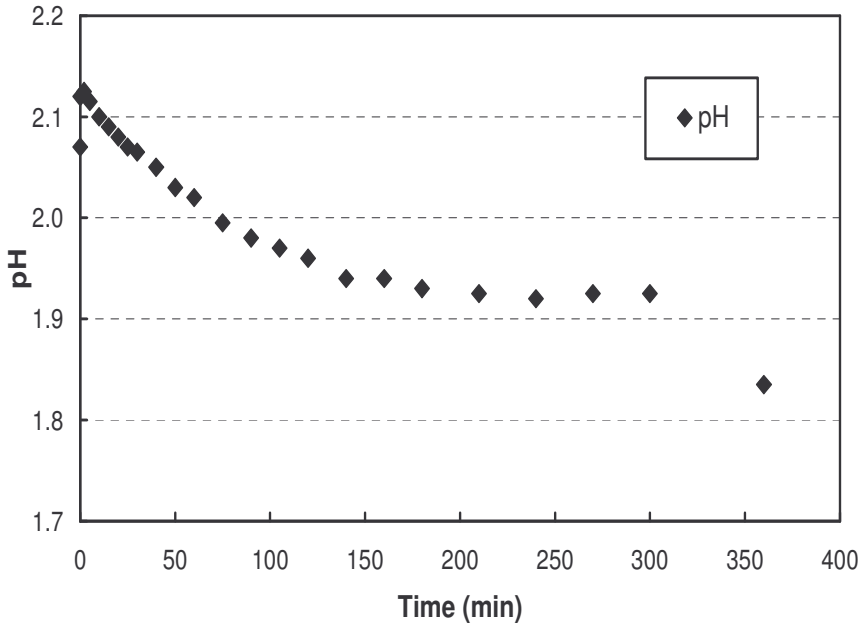

Figure 4.12. Time course pH change during Pb(II) removal using raw ADS at initial Pb(II) concentration of 159 mg/L (Initial pH =2.0, T=25°C).

When the effect of initial Pb(II) concentration on Pb(II) uptake by ADS was considered, it was seen that Pb(II) removal increased with increasing initial Pb(II) concentration. The combined results of experiments presented in Figure 4.13 show that for initial Pb(II) concentrations of 83 and 159 mg/L, the removal rates were approximately around 10 and 14 %, respectively. For 1250 mg/L initial Pb(II) concentration the Pb(II) concentration decreased to 75 % of the beginning value within the first 50 min but after that time Pb(II) concentration increased and the removal rate at steady state was around 15 %.

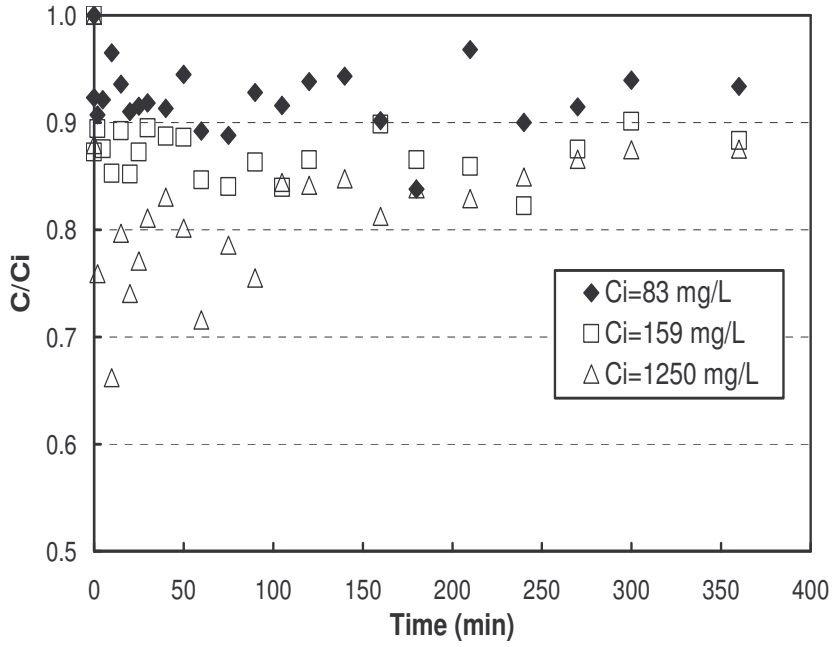

Figure 4.13. Effect of initial Pb(II) concentration on time course Pb(II) removal using raw ADS (Initial pH =2.0, T=25°C).

After sorption kinetic tests at different initial Pb(II) concentrations, isotherm experiments were conducted to determine the maximum capacity of raw ADS at the initial solution pH of 2.0. As shown in Figure 4.14, isotherm obtained was not of the L-shape but the S-shape. The S-shaped curve was reported to be an indicator of competitive sorption, possibly a competition between H^+ and K^+ ions present in

solution. Therefore, it is suggested that at pH 2 the high H^+ concentration resulted in strong competition with Pb(II) species and decreased the Pb(II) sorption capacity. On the other hand, L-shaped curves indicated a reduction in the number of available binding sites as Pb(II) concentration in the solution increased (Arıcan, 1998). The sorption capacity values were observed to increase exponentially with increasing equilibrium Pb(II) concentration. The rate of increase was slow at low equilibrium concentrations of Pb(II) and fast at high equilibrium concentrations of Pb(II). The highest capacity value observed was 1.8 mmol Pb(II) / g biomass for the equilibrium Pb(II) concentration of 2116 mg/L. For the samples with equilibrium Pb(II) concentrations less than 2000 mg/L, the capacity values calculated were below 0.6 mmol Pb(II) / g biomass, while the capacity values for samples with equilibrium Pb(II) concentrations higher than 2000 mg/L were above 1.2 mmol Pb(II) / g biomass.

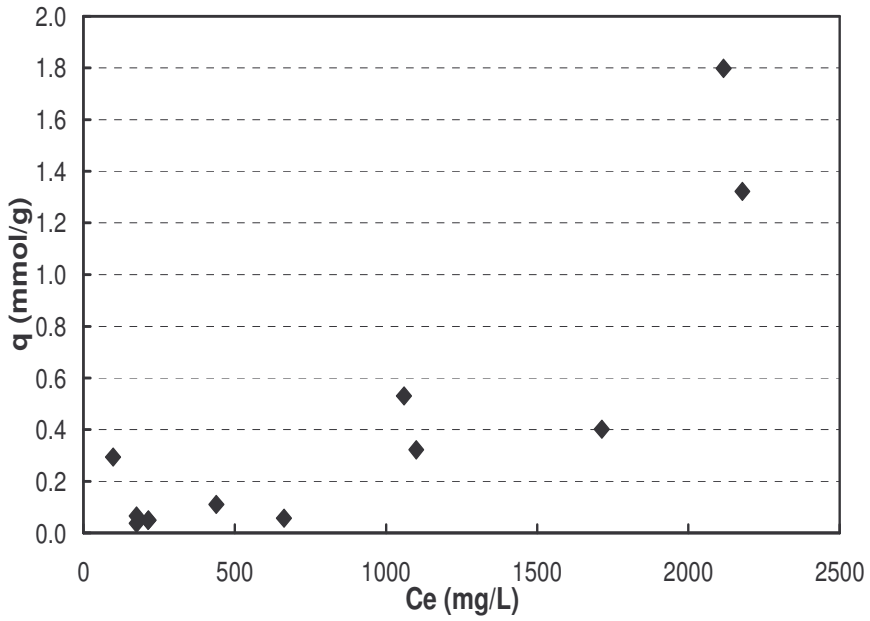

Figure 4.14. Pb(II) sorption isotherm using raw ADS (Initial pH = 2.0, T=25°C).

When isotherm data were applied to Langmuir and Freundlich models in order to see if these models can describe the data or not, it was observed that both of these models

were not satisfactory in describing Pb(II) sorption (Table 4.2). The correlation coefficients were very low.

Table 4.2. Langmuir and Freundlich model constants for sorption isotherm tests with raw ADS at initial pH of 2.0.

Langmuir Model		
Q_{max} = -250 mg/g	B = -0.0002 L/mg	R^2 = 0.0510
Freundlich Model		
N = 1.085	K = 0.122 $(mg/g)(L/mg)^{1/n}$	R^2 = 0.5693

A comparison of equilibrium pH values with initial pH values, has indicated that for all the samples, the equilibrium pH values were less than the initial pH values. The pH values ranged between 1.6 – 1.9 and the difference between the initial and equilibrium pH values were nearly the same for all samples (Figure 4.15). When equilibrium pH values were plotted against equilibrium Pb(II) concentration, it was observed that the equilibrium pH slightly higher for higher equilibrium Pb(II) concentration (Figure 4.16).

On the other hand, percentage Pb(II) removal efficiency and Pb(II) sorption capacity of the biomass was independent of equilibrium pH (Figure 4.17). However, Pb(II) capacities attained were all lower than the capacities attained when initial pH was adjusted to 4.0.The equilibrium pH varied in the range of 1.6 – 1.9 while the Pb(II) removal efficiency was less than 20 % in almost all samples.

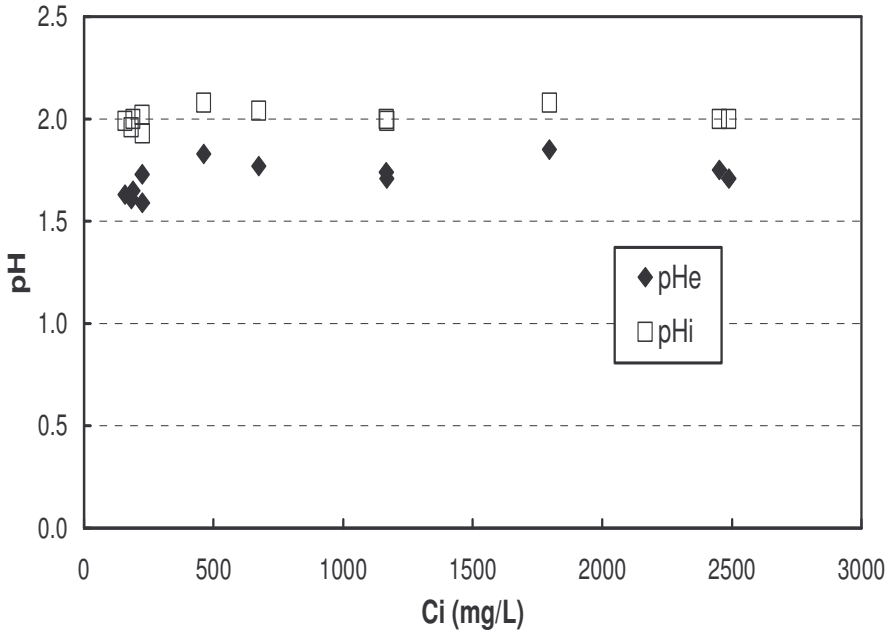

Figure 4.15. Initial and equilibrium pH versus initial Pb(II) concentration for isotherm tests with raw ADS. (Initial pH = 2.0, T=25°C).

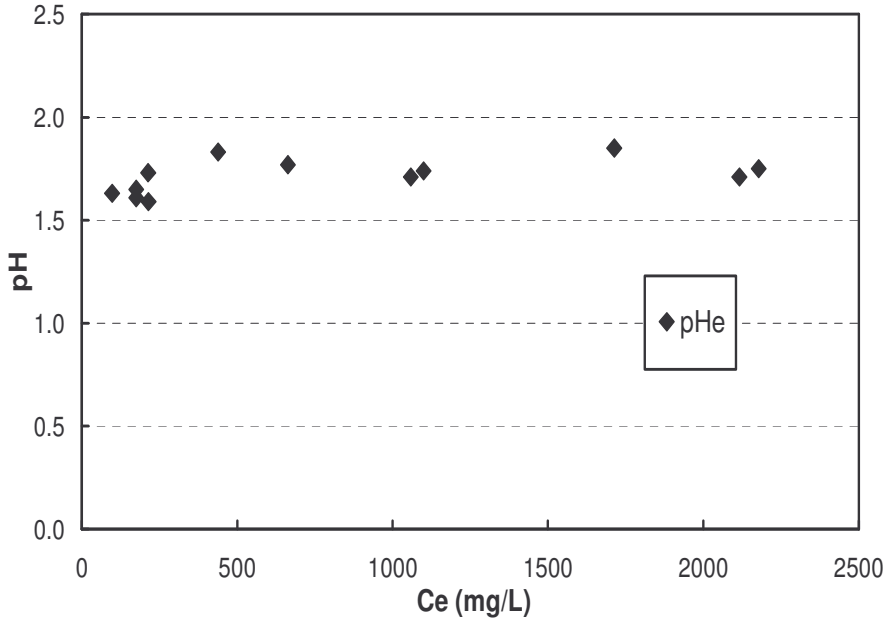

Figure 4.16. Equilibrium pH versus equilibrium Pb(II) concentration using raw ADS. (Initial pH = 2.0, T=25°C).

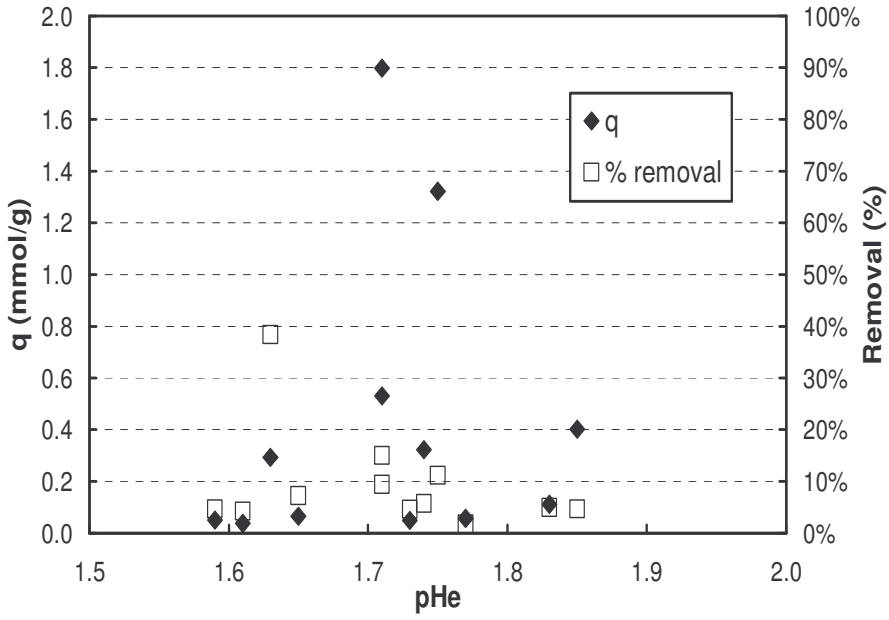

Figure 4.17. Capacity and % removal values with respect to equilibrium pH using raw ADS (Initial pH = 2.0, T=25°C).

When the results obtained are compared with those obtained with raw ADS at initial pH 4.0, the first thing to notice was very low Pb(II) sorption capacities, which were calculated to be less than 0.6 mmol Pb(II)/g biomass with the exception of only two samples. It was also observed that the shape of isotherm curve for these different initial pHs was different, indicating separate removal mechanisms. Another difference was in the trend of solution pH. The solution pH had increased in experiments with initial pH 4.0 while it decreased in experiments with initial pH 2.0.

Low sorption capacity values at pH 2 have also been reported in the literature for different types of biomasses (Waihung Lo et al., 1999; Gonzalez et al., 2001; Delgado et al., 1998; Gardea-Torresday et al., 1996; Esposito et al., 2002). The phenomenon was related to protonation of functional groups and possible destruction of the surface structure (Waihung Lo et al., 1999; Gonzalez et al., 2001; Delgado et al., 1998; Gardea-Torresday et al., 1996; Esposito et al., 2002; Tsezos et al., 1995, Kratochvil and Volesky, 1998; Schiewer, 1996). Due to competition between protons and metal ions in the solution the sorption capacity was reported to decrease significantly.

4.1.3. Sorption tests with raw ADS without initial pH adjustment

In search for any possible adverse effects of HNO_3 added for adjusting the initial pH of solution either to 4.0 or 2.0, another series of experiments were performed without adjusting the initial pH.

Kinetic tests performed at an initial Pb(II) concentration of 1250 mg/L and biomass concentration of 1 g biomass/L, have revealed the time course change in Pb(II) concentration presented in Figure 4.18. The Pb(II) concentration in the solution decreased suddenly with time and finally reached equilibrium. The removal of Pb(II) from the solution occurred again in two steps; a rapid removal step followed by a slower one. While the Pb(II) concentration decreased from 1250 mg/L to 400 mg/L within the first 10 min, steady state was reached after 2 h at an equilibrium Pb(II) concentration of about 15 mg/L. There was a concomitant time course increase in the calculated Pb(II) sorption capacity values and the equilibrium capacity obtained was 6.0 mmol Pb(II)/g biomass (Figure 4.18).

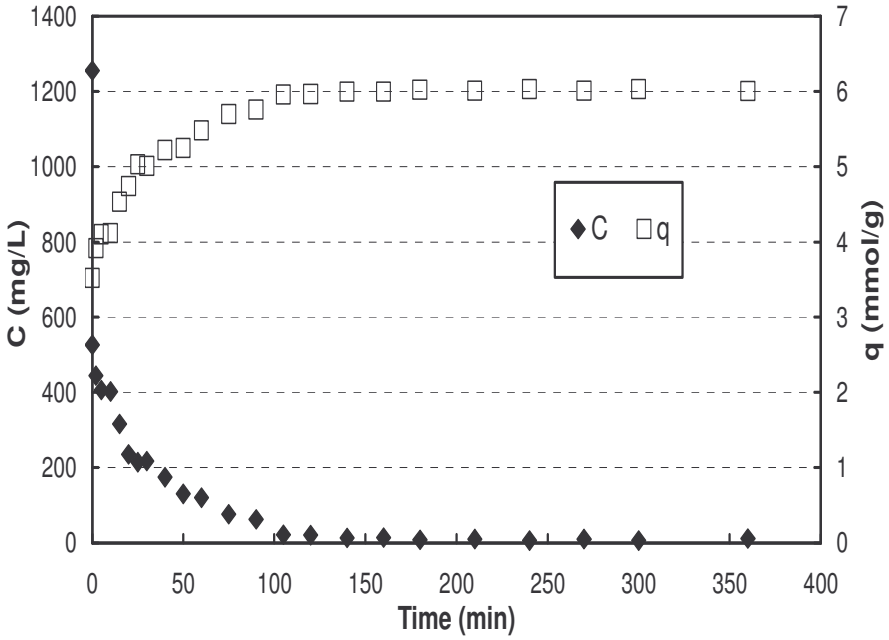

Figure 4.18. Time course Pb(II) removal using raw ADS at initial Pb(II) concentration of 1250 mg/L (No initial pH adjustment, T=25°C).

Meanwhile, solution pH showed an initial sudden increase up to 5.2 from an initial value of 5.0 when the biomass was added. Then, the pH decreased for about 5 min to a value of 4.7, and afterwards a continuous increase was observed till the end of the experiment, where the pH reached the value of 6.7 (Figure 4.19). Finally, during 1 h sedimentation period, it was seen that the biomass particles settled very fast, forming large floc structures.

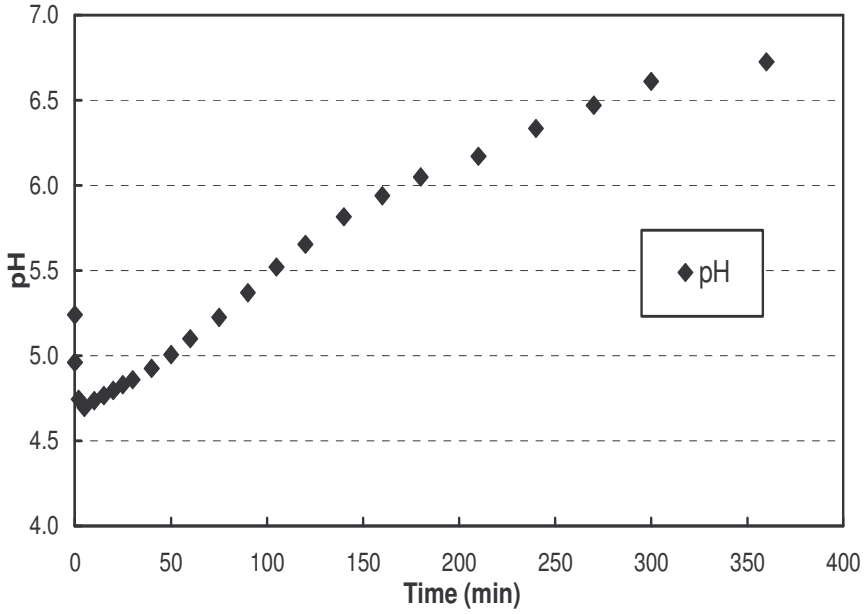

Figure 4.19. Time course pH change during Pb(II) removal using raw ADS at initial Pb(II) concentration of 1250 mg/L (No initial pH adjustment, T=25°C).

After the kinetic tests, isotherm test was conducted to determine the maximum capacity of the biomass without adjusting the initial solution pH and the results presented in Figure 4.20 were obtained. The maximum capacity of raw ADS appeared as 8.5 mmol Pb(II)/g biomass at an equilibrium Pb(II) concentration of about 800 mg/L.

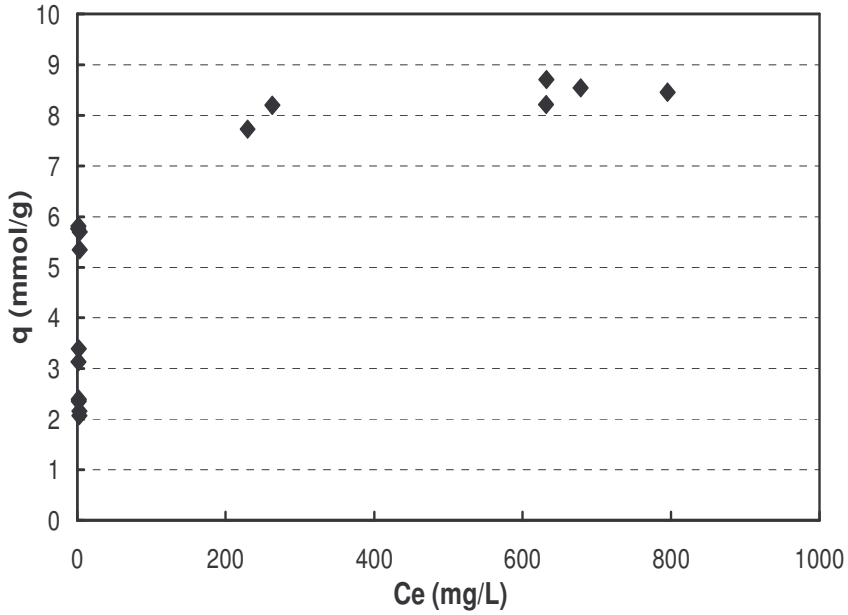

Figure 4.20. Pb(II) sorption isotherm using raw ADS (No initial pH adjustment, T=25°C).

From the isotherm plot given in Figure 4.20, it is seen that the Pb(II) sorption capacity of raw ADS increased as the equilibrium Pb(II) concentration increased and finally reached a plateau. Thus, the isotherm plot is of the classical L-type, indicating that the Pb(II) sorption capacity is independent of equilibrium Pb(II) concentration above 200 mg/L equilibrium concentration.

When isotherm data were fitted to Langmuir and Freundlich models in order to see if these models could describe the sorption behavior, it was observed that Langmuir model was quite satisfactory in describing the Pb(II) sorption under the present conditions (Table 4.3). However, as it can clearly be seen from the very low value of correlation coefficient, Freundlich model was not proper for the description of the phenomenon.

Table 4.3. Langmuir and Freundlich model constants for sorption isotherm tests with raw ADS at no initial pH adjustment.

Langmuir Model		
Q_{max} = 1667 mg/g (= 8 mmol/g)	B = 0.200 L/mg	R^2 = 0.9991
Freundlich Model		
N = 6.477	K = 651 $(mg/g)(L/mg)^{1/n}$	R^2 = 0.5911

In an attempt to compare the initial and equilibrium pH values with the initial Pb(II) concentration, Figure 4.21 was drawn. As shown, the pH of all samples increased during contact with Pb(II) and thus the equilibrium pH values were all higher than the initial pH. The amount of increase in the solution pH was higher in samples with low initial Pb(II) concentration while it was lower in samples with high initial Pb(II) concentration. For example; equilibrium pH of the sample with 500 mg/L Pb(II) concentration was measured to be around 7 – 8, whereas the equilibrium pH value of samples with 1800 mg/L and higher Pb(II) concentrations were below 5.5. The same relationship was valid between equilibrium pH and equilibrium Pb(II) concentration (Figures 4.22).

It was again observed that the high equilibrium pH values effect the removal of Pb(II) as over 99 % Pb(II) removal efficiency was observed when equilibrium pH was above 6.5. The removal efficiency of Pb(II) at equilibrium pH below 5.5 was between 70 – 90 %. However, the capacity values showed a reverse relation with respect to equilibrium pH. Below the equilibrium pH of 5.5 the capacity values calculated were between 7.7 – 8.7 mmol Pb(II)/g biomass, whereas, above equilibrium pH of 7.0 the capacity range was 2- 3.5 mmol Pb(II)/g biomass (Figure 4.23).

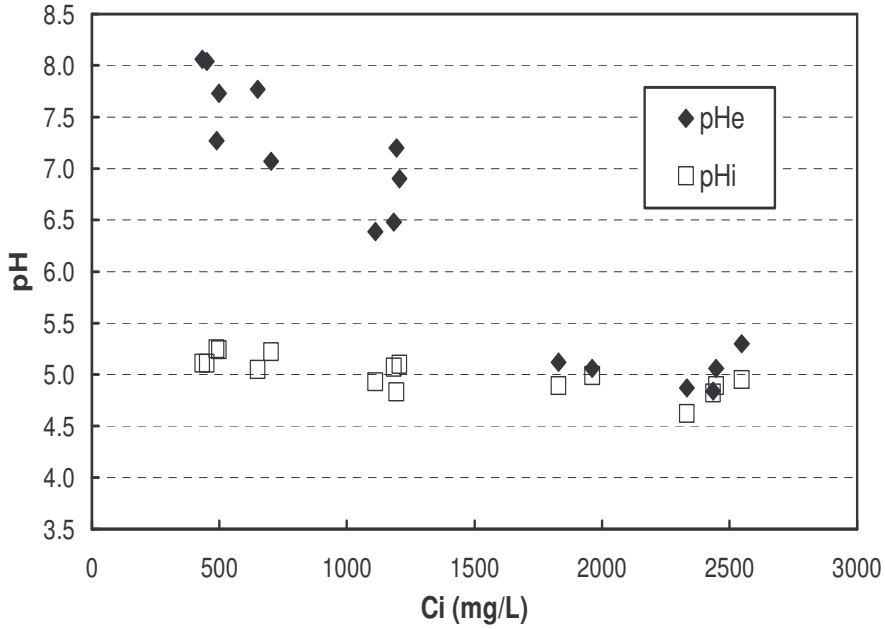

Figure 4.21. Initial and equilibrium pH versus initial Pb(II) concentration for isotherm tests with raw ADS (No initial pH adjustment, T=25°C).

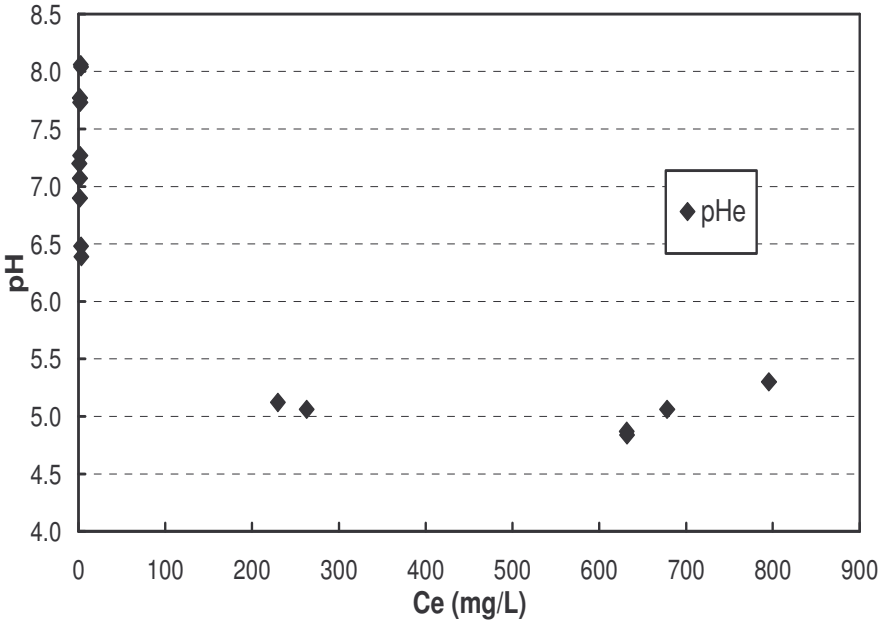

Figure 4.22. Equilibrium pH versus equilibrium Pb(II) concentration using raw ADS (No initial pH adjustment, T=25°C).

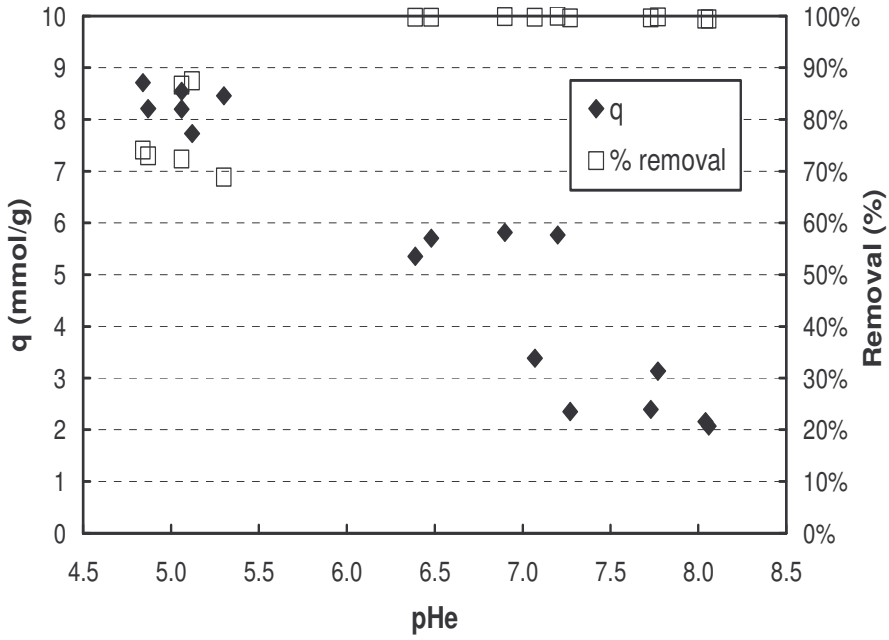

Figure 4.23. Capacity and % removal values using raw ADS with respect to equilibrium pH (No initial pH adjustment, T=25°C).

When compared with previous series of experiments, the same removal trend was noticed between the results of experiments without initial pH adjustment and those performed at initial pH 4.0. Besides the enormously high removal capacity, again an increase in solution pH and high equilibrium pH values, greater increase of pH values for lower initial Pb(II) concentrations and the decrease in capacity with increasing pH were observed. Thus, the results also indicated similarity with observations of Haytoglu (2000). However, the Pb(II) removal capacity was even higher when the initial pH was not adjusted and a better Pb(II) removal efficiency was obtained especially at higher initial Pb(II) concentrations.

Compared to other Pb(II) sorption capacities reported in literature, this huge removal capacity; 1760 mg Pb(II)/g biomass, was still questionable; was biosorption the only mechanism responsible for Pb(II) removal or was there any precipitation effect? In order to find an answer to this question, equilibrium pH values measured and the equilibrium capacities calculated from the two experimental sets, initial pH 4.0 and no initial pH adjustment, were plotted together with respect to initial Pb(II) concentration

in Figure 4.24 (In experiments with no initial pH adjustment, the initial pH of the samples was in the range of 4.5 – 5.0). There were two interesting outcomes of this figure; first, the equilibrium pH's measured for both experimental sets were approximately the same for the same initial Pb(II) concentration although the initial pH values were different. Secondly observation of nearly the same Pb(II) removal capacity up to a certain initial Pb(II) concentration (1200 mg/L), despite the expected shift of capacity curves due to differentiation in protonation/deprotonation of the surface functional groups which would be a result of different initial pHs. The expected shift, however, was seen only to the end of the curves.

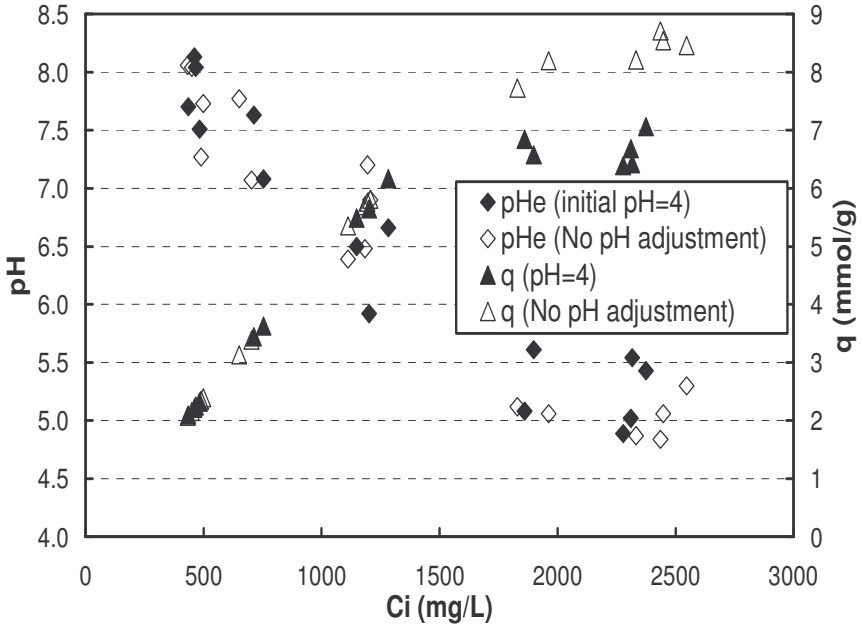

Figure 4.24 Combined results of raw ADS isotherm tests. Equilibrium pH and capacity versus initial Pb(II) concentration.

As precipitation of lead is known to take place at pH values higher than 5.5, above that pH value the solubility of lead will determine the equilibrium lead concentration. Therefore, in Figure 4.24, below initial Pb(II) concentration of 1300 mg/L or, in an other way, above equilibrium pH of 6.0, precipitation can be suggested as the

dominant mechanism. Since the equilibrium pH values were observed to be approximate at the same initial Pb(II) concentration of both sets, the solubility of those samples (thus the equilibrium Pb(II) concentrations) should also be approximate, which explains the coinciding removal capacities for those samples.

On the other hand, the shift observed in removal capacities above initial Pb(II) concentrations of 1300 mg/L, or below equilibrium pH of 6 was suggested to result from biosorption. As indicated before, such a shift was expected due to protonation/deprotonation of surface functional groups responsible for the biosorption of Pb(II). So, the shift in removal capacity showed biosorption was more effective for initial Pb(II) concentrations higher than 1300 mg/L.

Pictures of Pb(II) removal by raw ADS were taken to visualize the process. These pictures are presented in Figures 4.25, 4.26 and 4.27. The effect of precipitation can also be observed in these pictures. While ADS was added to the beaker it was observed that the solution became turbid and gray colored clouds were formed besides the brown color of the sludge (Figure 4.25c, 4.25d, 4.25e). This gray color was noted as the indicator for precipitation of Pb(II). When Figure 4.26, which shows the sedimentation of the sludge, is considered; it can be seen that large floc particles were formed just after the mixing was stopped and the sludge settled rapidly. Even after 20 min nearly all sludge settled. A gray colored zone was also noticed after 2 h over the settled sludge. Finally comparing the pictures of initial Pb(II) solution with treated effluent in Figure 4.27, it was seen that although the Pb(II) was removed from the system the initial Pb(II) solution seems much more appreciable in terms of turbidity. Thus, it can be concluded that prior to discharge this effluent should be filtered in order to remove precipitated lead.

In their studies Artola et al. (1992, 1997, 2001) also reported an increase in solution pH during both batch and reactor experiments. They decided to study at pH “evolving freely” as that will decrease the operational costs for chemical requirement (Artola et al., 1992). An equilibrium pH range of 6 – 8 was reported for Cu, Cd and Ni systems in contact with anaerobically digested sludge (Artola et al., 1997). Also in pilot plant

biosorption reactor studies Artola et al. (2001) found the pH of treated effluent to be varying between 6.0 and 6.8, while the pH of the influent Cu solution was 5.0 – 5.3. The biomass used in this study was also anaerobically digested sludge.

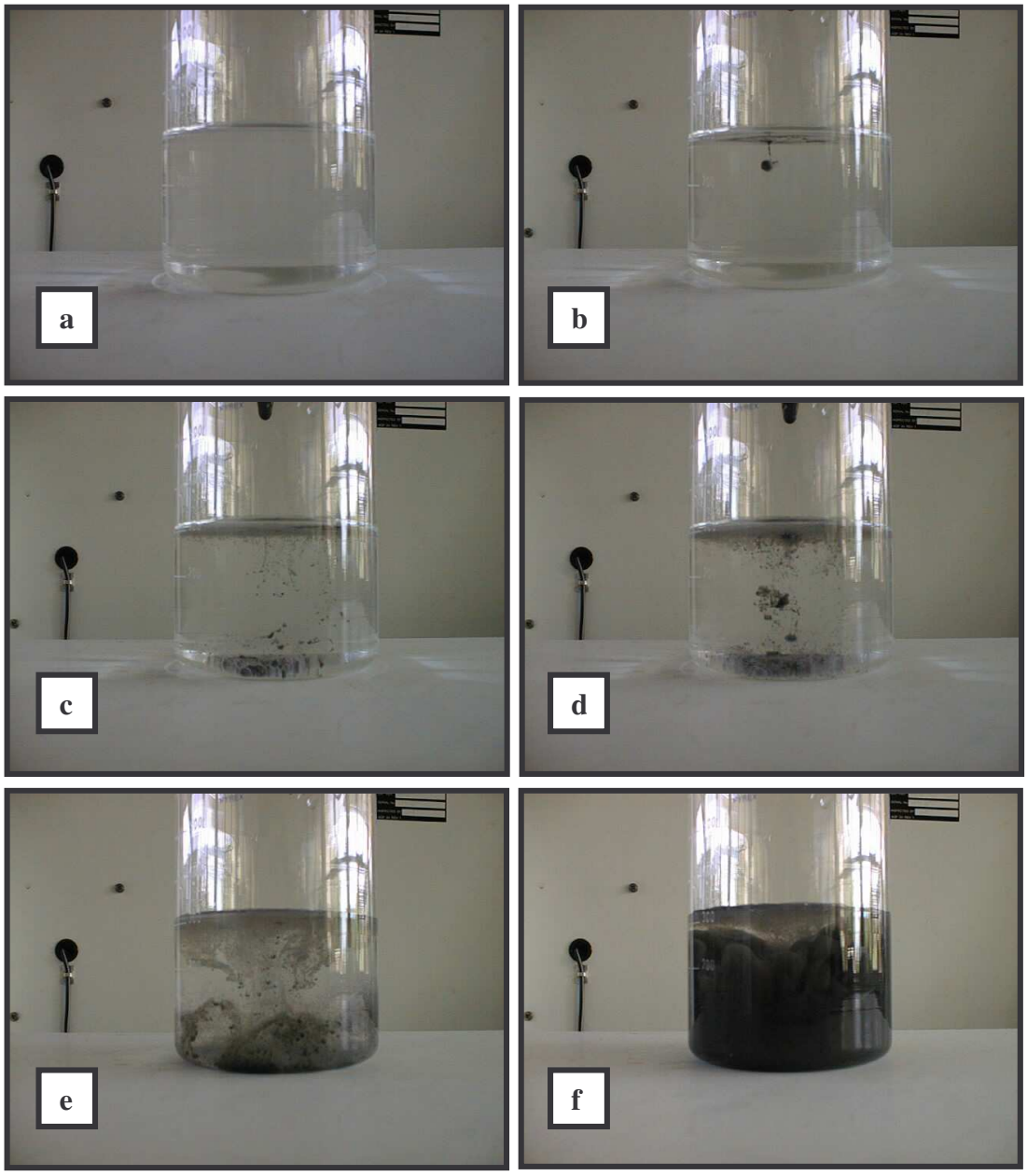

Figure 4.25. Addition of raw ADS to Pb(II) solution (Initial Pb(II) concentration = 500 mg/L and initial pH is between 4-5).

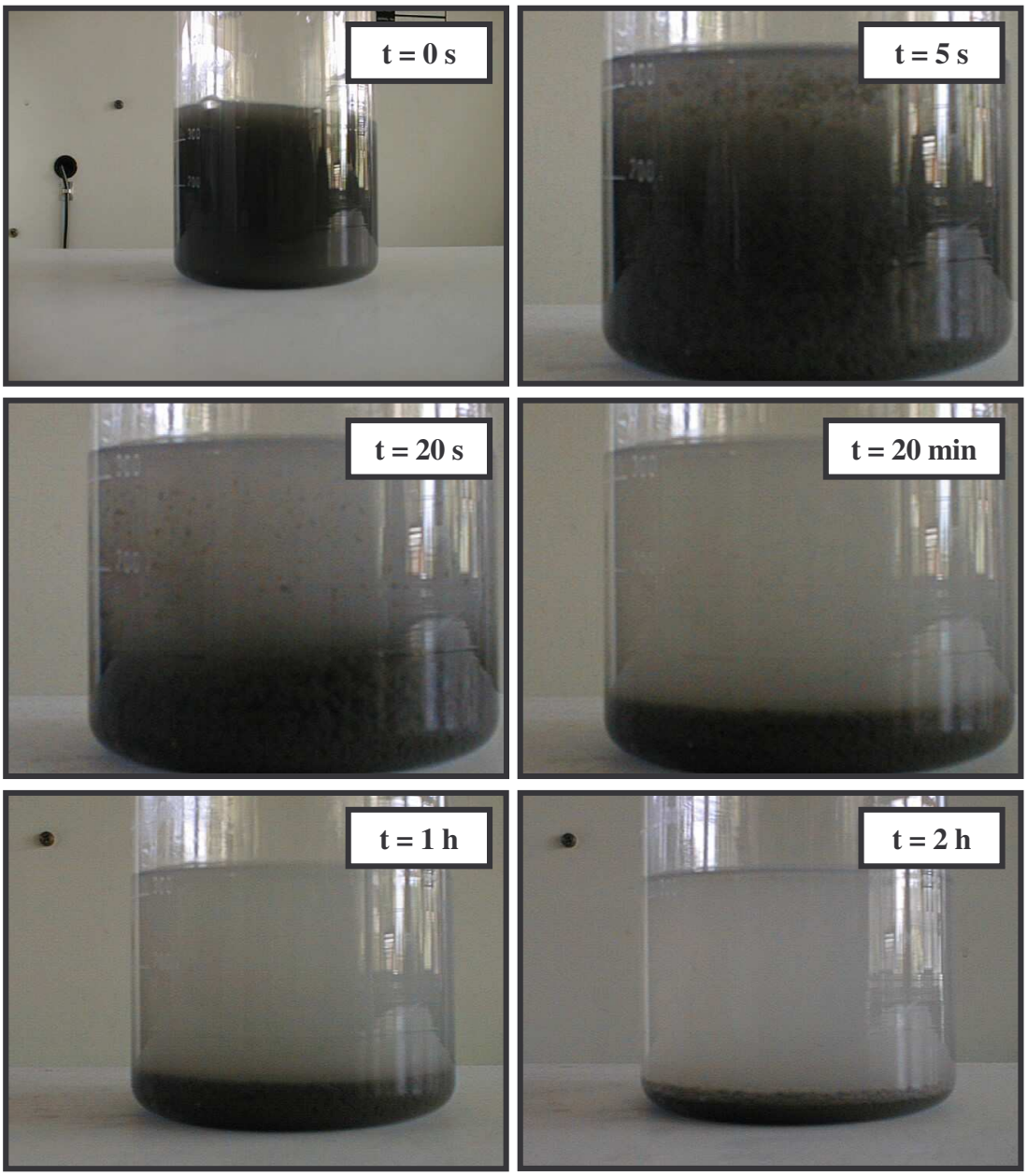

Figure 4.26. Floc formation and sedimentation of sludge (Initial Pb(II) concentration = 500 mg/L and initial pH is between 4-5).

Figure 4.27. Initial Pb(II) solution (a) versus treated effluent (b).

Although most of the metals were known to form precipitates above pH 5.5 (Kratochvil and Volesky, 1998; Holan and Volesky, 1994; Patterson et al., 1977), Haytoglu (2000) and Artola et al. (1992; 1997; 2001) did not consider precipitation in their studies. Artola et al. (1997), after comparing sludge metal systems to glycine metal systems, suggested the major functional group on the sludge surface to be of amino acid type due to similar pH behaviors of the two systems. On the other hand, Haytoglu (2000) did not test the pH values above 5, recognizing the precipitation effect, while investigating for optimum pH value. But, somehow did not notice any possible precipitation for the equilibrium pH values between 5.3 and 7.4 (Haytoglu, 2000). In another study, Holan and Volesky (1994) did not evaluate capacity values for lead concentrations above 250 mg/L due to observation of visual precipitation.

However, they did not bring any explanation related to equilibrium pH values, which were reported to be higher for concentrations below 250 mg/L (Holan and Volesky, 1994).

Generally the precipitation of heavy metals has been suspected to be in the form of hydroxide (Kratochvil and Volesky, 1998; Holan and Volesky, 1994; Patterson et al., 1977). However, for this study, the form of precipitation is not thought to be in the hydroxyl form but in carbonate form. The reason for this expectation has been the presence of bicarbonate (HCO_3^-) ions in anaerobically digested sludge. The bicarbonate ion was reported as the major source of buffering capacity in anaerobic digesters for the optimum pH range of 6.5 – 7.6. It was also noted that 1000 to 5000 mg/L bicarbonate concentration as $CaCO_3$ was required to keep the digester at the optimum pH range (Parkin and Owen, 1986). The bicarbonate ion concentration in the anaerobic digesters of Ankara Municipality Wastewater Treatment Plant, where the raw ADS samples were collected from, was reported to be in the range of 2500 – 3500 mg/L as $CaCO_3$ (Personal communication).

Here it may be worth noting that in a study investigating removal of Cu(II), Cd(II) and Pb(II) using biomass collected from a brewing industry, Marques et al. (2000) reported the medium pH to rise from an initial value of 4.5 to a final between 7.0 to 8.0 if the biomass was used without any pretreatment. A different pH profile was observed when biomass was previously incubated and washed in distilled water. Comparing removal rates for both situations, it was observed that excreted intracellular products were responsible for 80 % Pb(II) precipitation (Marques et al., 2000). It is well known that brewery products contain carbonate alkalinity (carbondioxide, bicarbonate or carbonate) as a result of fermentation process.

In a recent study, Barkay and Schaefer (2001) has defined the formation of insoluble metal precipitates by the interaction of metals and microbial metabolic products as biomineralization. Biomineralization, a “hot” research topic, generally has been searched for the purpose of bioremediation and investigations are now directed

towards altering the biological pathways or metabolic activities that are responsible for reducing the toxic effects of metals and radionuclides (Barkay and Schaefer, 2001; Lloyd and Lovley, 2001).

In another recent study, Templeton et al. (2003) showed that "biomineralization" counted for 90 % of the Pb uptake by *Burkholderia cepacia* for pH values less than 4.5 and for 45 – 60 % of total uptake for pH values around neutral. It was reported that Pb accumulated near the outer membrane of the biofilm as pyromorphite. The data represented "an example of simultaneous adsorption and precipitation" under conditions where bulk precipitation was not expected. It was also suggested that formation of pyromorphite would decrease for higher concentrations of Pb as a result of toxicity or microorganisms running out of phosphate sources (Templeton et al., 2003).

4.1.3.1. FTIR Analysis of raw ADS

For investigating the functional surface groups responsible in biosorption of Pb(II) FTIR analysis on raw ADS sample before and after contact with 500 mg/L Pb(II) solution was performed. The FTIR spectra obtained was given in Figure 4.28.

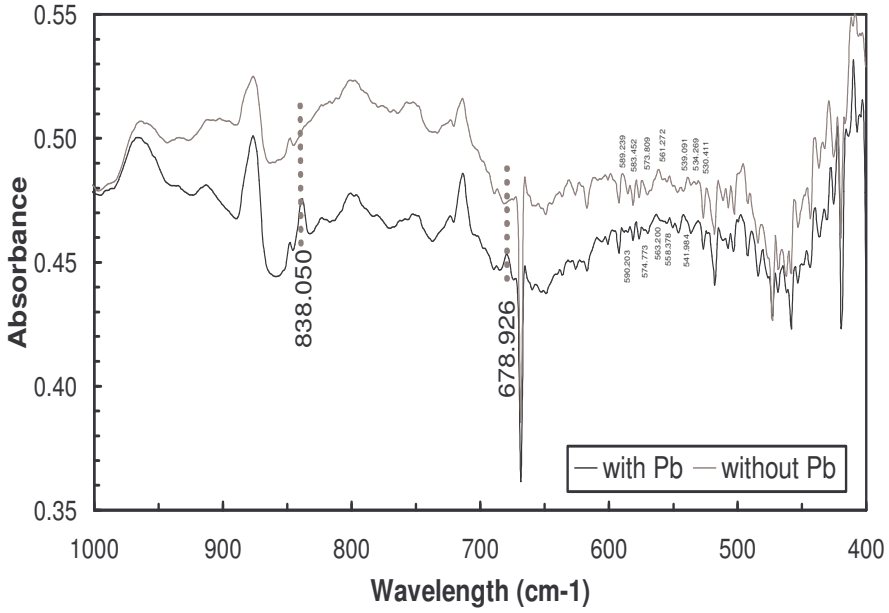

Figure 4.28. FTIR spectra for raw ADS before and after contact with 500 mg/L Pb(II) solution.

The main difference observed between these two spectra was the presence of peaks at 679 and 838 cm^{-1} in the spectrum for Pb(II) containing sample. These two peaks refer to the binding site of Pb(II) on the biomass structure. At 1740 cm^{-1}, another peak (not shown in Figure 4.28) was also seen in the FTIR spectrum of Pb(II) containing sample. Also a shift of peaks was observed in the range of 650–525 cm^{-1}. These shifts were mainly due to the deformation of double carbonyl bonds from the carboxyl functional groups and formation of C-O bond. These results indicated an interaction between the Pb(II) species and carboxyl groups present over the surface of the biomass (www.nist.gov). Another observation from the comparison of spectra for ADS samples before and after contact with Pb(II), was a decrease in the amount of OH^- ions bound to the biomass surface. This was in agreement with the pH increase observed during the sorption tests.

4.1.4. Sorption tests with dewatered ADS without pH adjustment

The most common reactor type used in biosorption research is the continuous flow column reactor, packed with biomass due to its high efficiency and ease of application (Figueira et al., 2000; Yun et al., 2001; Kratochvil and Volesky, 2000). It was obvious that raw ADS which had a solids concentration of 27,000 mg/L was not appropriate for use in packed-bed column applications since it was in slurry form. On the other hand; considering its high solids concentration, dewatered ADS seems to be proper for use in packed-bed columns. To this end, sorption studies using dewatered ADS were conducted and Pb(II) removal capacity of dewatered ADS was compared with that of raw ADS. All the tests with dewatered ADS were carried out without controlling the initial pH of the test mixture, taking into account higher Pb(II) removal capacity of raw ADS at the uncontrolled initial pH tests.

Kinetic tests with an initial Pb(II) concentration of 1250 mg/L and with a biomass concentration of 1 g biomass/L were conducted and time course change in the solution Pb(II) concentration and the solution pH were monitored. Experimental findings are presented in Figure 4.29.

As can be depicted from Figure 4.29, following a sudden decrease, the solution Pb(II) concentration decreased gradually with time and after about an hour reached an equilibrium value. Thus, as in the previous cases, there were two distinct steps in Pb(II) removal; a rapid removal step followed by a slow one. While the Pb(II) concentration decreased from 1000 mg/L to 750 mg/L within the first 10 min, steady state was reached after about 1 h and the solution Pb(II) concentration was stabilized at 700 mg/L. Correspondingly, the Pb(II) removal capacity increased with time and finally reached the equilibrium capacity at a value of 1.4 mmol Pb(II)/g biomass (Figure 4.29).

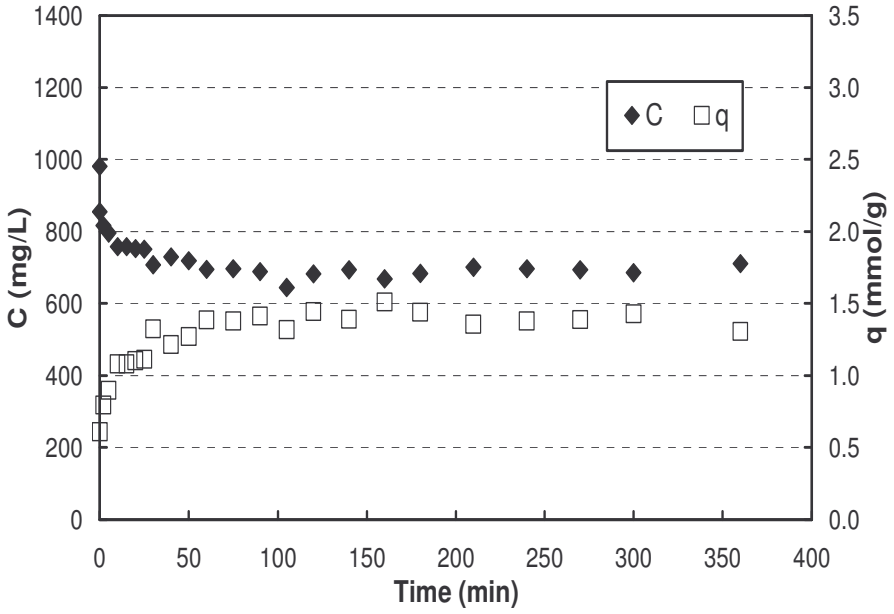

Figure 4.29. Time course Pb(II) removal using dewatered ADS at initial Pb(II) concentration of 1250 mg/L (No initial pH adjustment, T=25°C).

As presented in Figure 4.30, as Pb(II) removal progresses, there occurred a sudden increase in pH up to 5.5 from an initial value of 5.0 and then a gradual decrease from this level back to 5.0 at equilibrium. During 1 h sedimentation period the biomass particles were observed to settle rapidly, forming large floc structures.

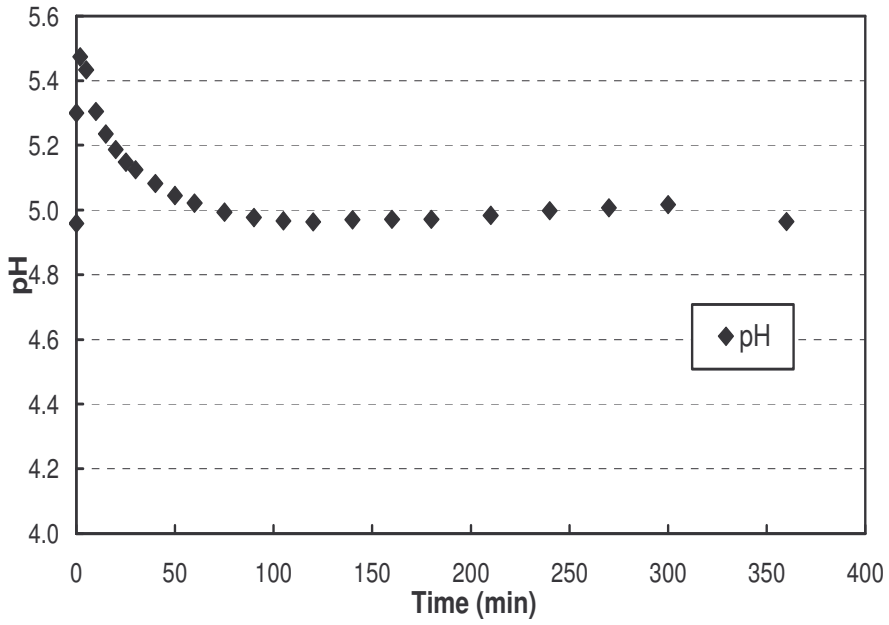

Figure 4.30. Time course pH change during Pb(II) removal using dewatered ADS at initial Pb(II) concentration of 1250 mg/L (No initial pH adjustment, T=25°C).

When isotherm test was carried out to investigate the Pb(II) removal by dewatered ADS, it appeared that the capacity is quite low as compared to raw ADS (Figure 4.20 and Figure 4.31). The maximum Pb(II) sorption capacity value for dewatered ADS was determined to be about 2.5 mmol Pb(II)/g biomass. An evaluation of the equilibrium and initial Pb(II) concentrations have indicated that, especially for the samples having an initial Pb(II) concentration below 250 mg/L, measured equilibrium concentrations of Pb(II) in the solutions were less than 5 mg/L.

The isotherm plot in Figure 4.31 was of the L-shape, indicating that the capacity increased with an increase in equilibrium Pb(II) concentration for equilibrium concentrations lower than 70 mg/L and was independent of the change in equilibrium Pb(II) concentration for equilibrium concentrations above 200 mg/L. When isotherm data were applied to Langmuir and Freundlich models in order to see if these models could fit to the data or not, the correlation coefficients presented in Table 4.4 were obtained. The values clearly indicate that both models are not appropriate for the

description of Pb(II) sorption. Even, Freundlich model was totally inappropriate with a correlation coefficient of 0.0322.

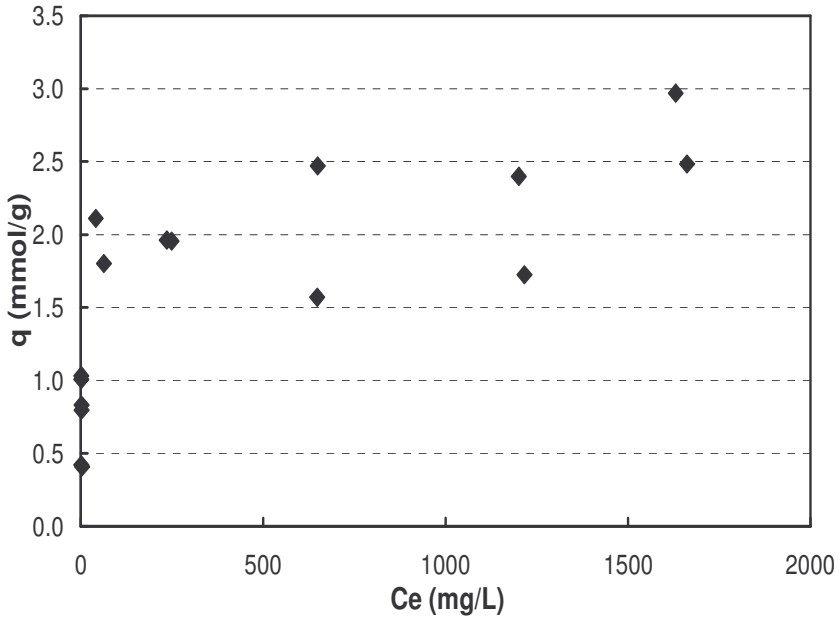

Figure 4.31. Pb(II) sorption isotherm using dewatered ADS (No initial pH adjustment, T=25°C).

Table 4.4. Langmuir and Freundlich model constants for sorption isotherm tests with dewatered ADS at no initial pH adjustment.

Langmuir Model		
Q_{max} = 294 mg/g (= 1.4 mmol/g)	B = -0.015 L/mg	R^2 = 0.6286
Freundlich Model		
N = -17.513	K = 508 $(mg/g)(L/mg)^{1/n}$	R^2 = 0.0322

In the isotherm tests the equilibrium pH of all samples were found to be higher than the initial pH of the samples as shown in Figure 4.32. The amount of increase in the solution pH was higher in samples with low initial Pb(II) concentration and it was lower in samples with high initial Pb(II) concentration. For samples with initial Pb(II) concentration of 500 mg/L and less, the equilibrium pH values up to 7.5 were observed, whereas the equilibrium pH value of samples with 1000 mg/L and higher Pb(II) concentrations were below 5.5. The increase in equilibrium pH was also higher for low equilibrium Pb(II) concentrations and lower for high equilibrium Pb(II) concentrations (Figures 4.33).

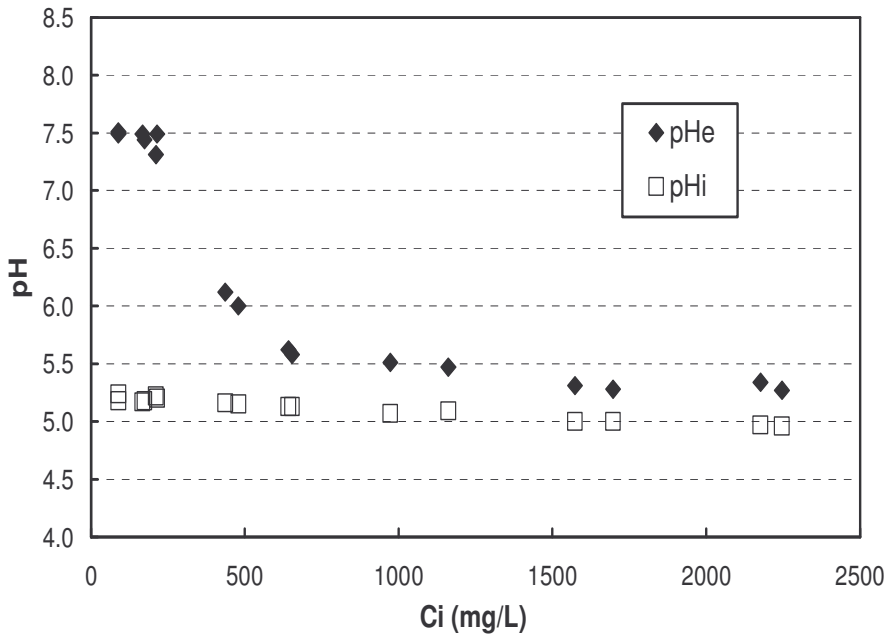

Figure 4.32. Initial and equilibrium pH versus initial Pb(II) concentration for isotherm tests with dewatered ADS (No initial pH adjustment, T=25°C).

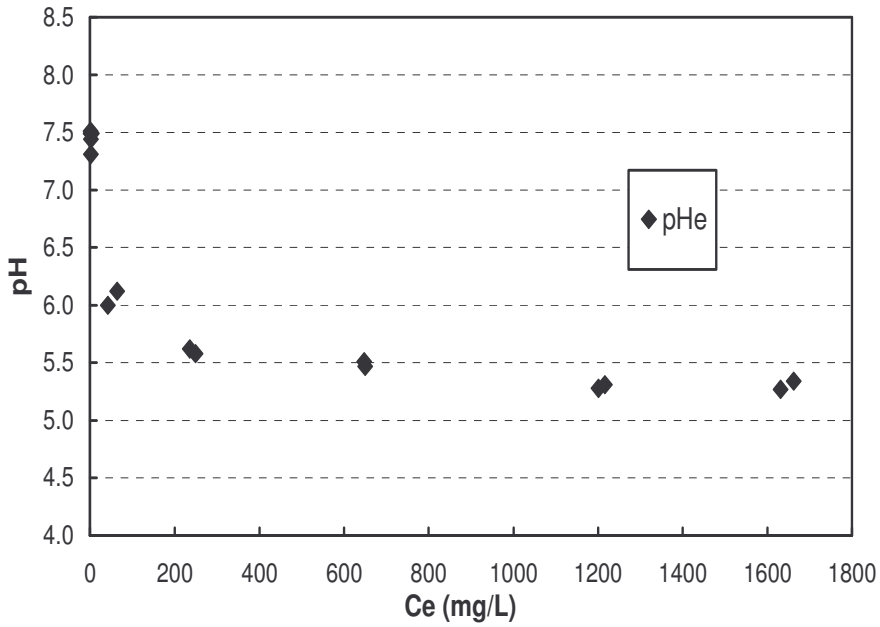

Figure 4.33. Equilibrium pH versus equilibrium Pb(II) concentration using dewatered ADS (No initial pH adjustment, T=25°C).

As regards the effect of equilibrium pH on Pb(II) sorption capacity and percentage Pb(II) removal Figure 4.34 was plotted. As shown, over 95 % Pb(II) removal was observed when equilibrium pH is equal to or greater than 6.5. The removal rate of Pb(II) at equilibrium pH of 6 was 90 % and for equilibrium pH below 5.5 the removal rate was between 20 – 45 %. However, the Pb(II) sorption capacity had a reverse relation with respect to equilibrium pH. Below the equilibrium pH of 5.5 the capacity values calculated are between 2.0 – 3.0 mmol Pb(II)/g biomass, whereas, above equilibrium pH of 7.0 the highest capacity value calculated was 1.0 mmol Pb(II)/g biomass.

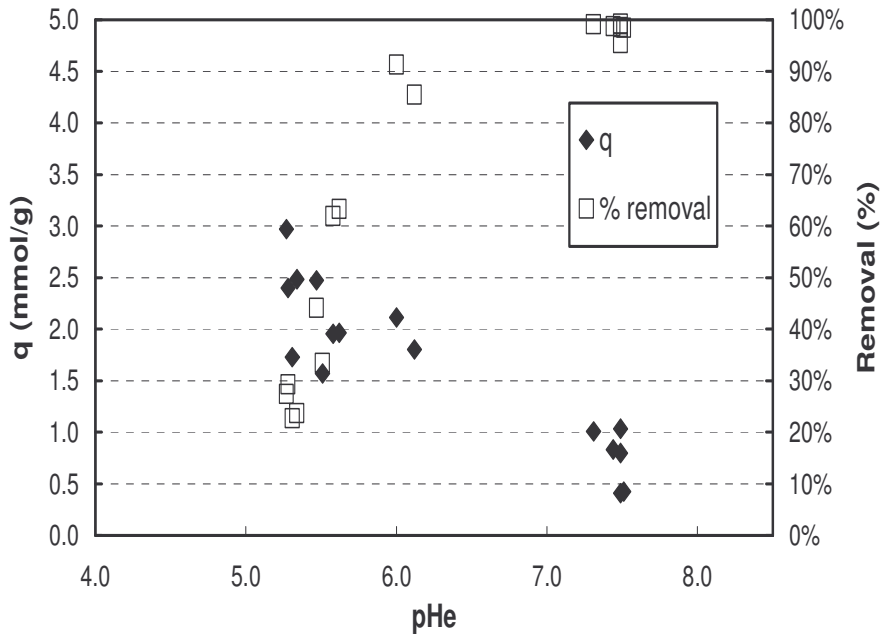

Figure 4.34. Capacity and % removal values using dewatered ADS with respect to equilibrium pH (No initial pH adjustment, T=25°C).

The removal of lead using dewatered ADS showed similarity with results of raw ADS. Again an increase in solution pH and high equilibrium pH values, greater increase of pH values for lower initial Pb(II) concentrations and the decrease in capacity with increasing pH were observed during the experiments with dewatered ADS. However the removal efficiency of dewatered ADS, 518mg (2.5 mmol) Pb(II)/g biomass, was very low compared to raw ADS.

On the other hand, the high equilibrium pH values obtained indicated that precipitation was again involved in the Pb(II) removal mechanism. Thus, the difference between the capacities of raw and dewatered biomass was supposed to be as a result of loosing bicarbonate ions and soluble ligands possibly present in raw ADS during the process of dewatering. It was also suggested that the addition of polymers to enhance dewatering could have affected the availability of functional groups or changed the surface characteristics of raw ADS. Measuring lower equilibrium pH values in the tests with

dewatered ADS also supported the idea that bicarbonate concentration for this biomass was lower than raw ADS.

Although similarities were observed during isotherm tests, comparison of the time course pH values for both biomasses showed the mechanisms involved or more probably the effect of precipitation had been different (Figure 4.35). When kinetic tests with raw and dewatered ADS samples were considered, it was observed that a rapid removal occurred in the first 10 minutes and it was followed by a rather slow removal stage. These results were compared with monitored pH values to establish some relationship between solution pH and removal of Pb(II). A sharp increase in pH was observed just after the addition of biomass (Figure 4.35) this increase was expected to be a result of addition of biomass containing bicarbonate ions. Afterwards a decrease of pH, very sudden especially in raw ADS studies, was measured which falls within the time range of the rapid removal of Pb(II). The decrease in pH being higher for raw ADS and low for dewatered ADS corresponds well with higher removal capacities obtained with raw ADS. Therefore it was considered that the sorption of metal takes place during this period due to interaction with carboxyl groups available on the surface of biomass. So the main mechanism for sorption is speculated as ion exchange and the decrease in pH was supposed to result from the replacement of Pb(II) ions by other cations previously available on the biomass surface.

With further increase in pH, observed only in raw ADS studies, the solubility of Pb(II) decreases. The decreased solubility enhances both the sorption and precipitation of Pb(II) and results in better removal capacities for raw ADS compared to dewatered ADS.

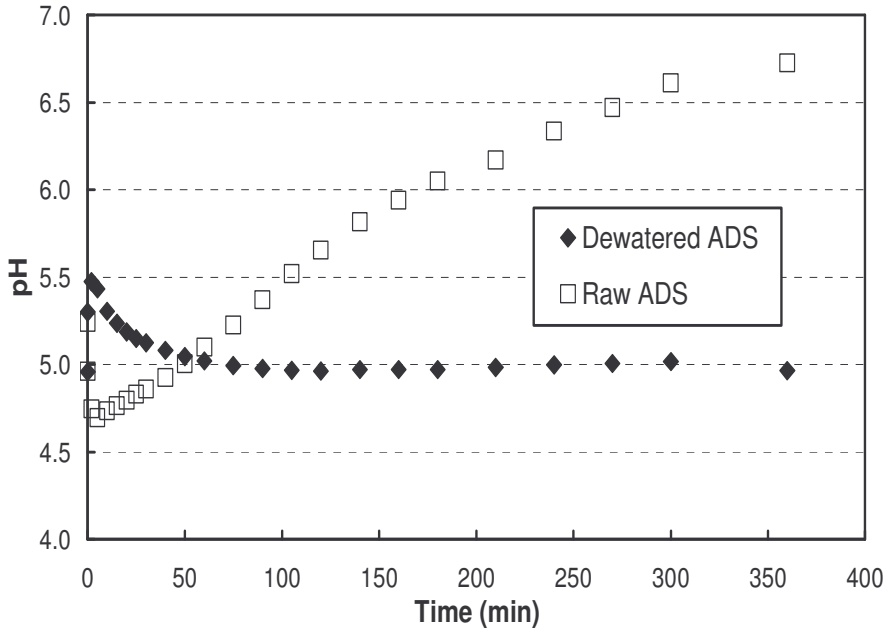

Figure 4.35. Compared time course change in pH during removal of Pb(II) with raw and dewatered ADS.

Considering the isotherm data it was found that the isotherm curve was S-shaped only for sorption equilibrium experiments with initial pH 2, all other isotherm curves were L-shaped. As mentioned before, L-shape indicates reduction of available binding sites and S-shape refers to a competition between the metal and other cations present in the system.

Calculated values of Langmuir and Freundlich constants for isotherm sorption tests were summarized in Table 4.5. The correlation coefficients in Table 4.5 indicated that Freundlich model was not able to identify the isotherm data for any of the equilibrium tests while Langmuir model was able to approximate the data, excluding those obtained for initial pH of 2. Such a result also confirms that L-shaped isotherm data is well represented by the Langmuir model.

Although the Langmuir model seemed to fit experimental data, one should not except that these models could approximate the system, as precipitation was a part of the

removal mechanism. Even, in several of the biosorption works, Langmuir and Freundlich models have been reported not to be proper to model biosorption equilibria. When the major mechanism responsible for biosorption is accepted as ion exchange, the validity of these models, which were generated for sorption, could be considered (Kratochvil and Volesky, 1998). However, when processes other than sorption are included, these models could not be valid. In the past, new models, including the effect of pH and ionic strength have been reported and researched (Esposito et al., 2002; Pagnanelli et al., 2003; Schiewer, 1996).

Table 4.5. Calculated isotherm constants for Langmuir and Freundlich Models.

BIOMASS	FREUNDLICH			LANGMUIR		
	N	K $(mg/g)(L/mg)^{1/n}$	R^2	Q_{max} (mg/g)	B (L/mg)	R^2
Raw ADS (Initial pH = 4.0±0.2)	7.553	598	0.7021	1429	0.700	0.9977
Raw ADS (Initial pH = 2.0±0.2)	1.085	0.1223	0.5693	-250.000	-0.0002	0.0510
Raw ADS (No pH adjustment)	6.477	651	0.5911	1667	0.200	0.9991
Dewatered ADS (No pH adjustment)	-17.513	508	0.0322	294	-0.015	0.6286

4.2. REACTOR OPERATION

Considering potential full-scale application of biosorption in the detoxification of metal bearing industrial effluents, batch type reactor configuration was tested. In this reactor type selection; primary factor was the relatively low volumetric flow rate of Pb(II) bearing metal industry effluents and also the well-settling property of the ADS biomass after Pb(II) biosorption. The generally preferred packed-bed column reactor configuration was not selected due to high water content of raw ADS which makes it improper to be used as packing material. On the other side, dewatered ADS was not

tested as packing material in a packed-bed reactor or in any other reactor configuration due to its much lower Pb(II) sorption capacity than raw ADS.

Single stage and three stage serial combinations of batch reactors were adopted. In single stage batch reactor tests, whole biomass was added at once (0.6 g biomass/L) to the reactor whereas in three stage serial reactor tests, one third (0.2 g biomass/L) of the same amount of biomass was added at each step. In three stage reactor application, reactors were operated batch wise; the effluent of first reactor introduced to the second reactor as the influent and the effluent of the second reactor entered as the influent of the third reactor. The objective in such an application was to optimize the Pb(II) removal process with respect to minimum biomass consumption.

The reactor tests were performed at two different initial Pb(II) concentrations of 100 and 200 mg/L. There was no pH adjustment done during the tests. Therefore, the initial pH was around 5.0 – 6.0. In all the batch reactor tests, 2 h of mixing was provided that was followed by 1 h of sedimentation period. Solution pH was monitored during the tests and SVI measurement was conducted as described before to have an idea about the settleability of biomass.

4.2.1. 100 mg/L initial Pb(II) concentration

The results obtained from single stage reactor application are given in Table 4.6. As observed, a high Pb(II) removal efficiency of 94.0 % was obtained. The removal corresponded to a decrease of Pb(II) concentration from the initial value of 80.2 mg/L to 5.2 mg/L. However, the effluent Pb(II) concentration was not able to satisfy the Pb(II) discharge limit set by Turkish Water Pollution Control Regulation. Parallel to isotherm tests, during single stage reactor experiments, the solution pH increased continuously from an initial value of 5.2 to 7.6 as shown in Figure 4.36. The Pb(II) removal capacity attained after 3h (2h mixing and 1h settling) was calculated as 1.150 mmol Pb(II)/g biomass, and the SVI was measured as 54 mL/g.

Table 4.6. Results of single stage reactor with initial Pb(II) concentration of 80.2 mg/L.

Parameter	Value
Initial Pb(II) concentration (mg/L)	80.2
Final Pb(II) concentration (mg/L)	5.2
Pb(II) removal (%)	94.0
q (mmol Pb(II)/g biomass)	1.150
SVI (mL/g)	54

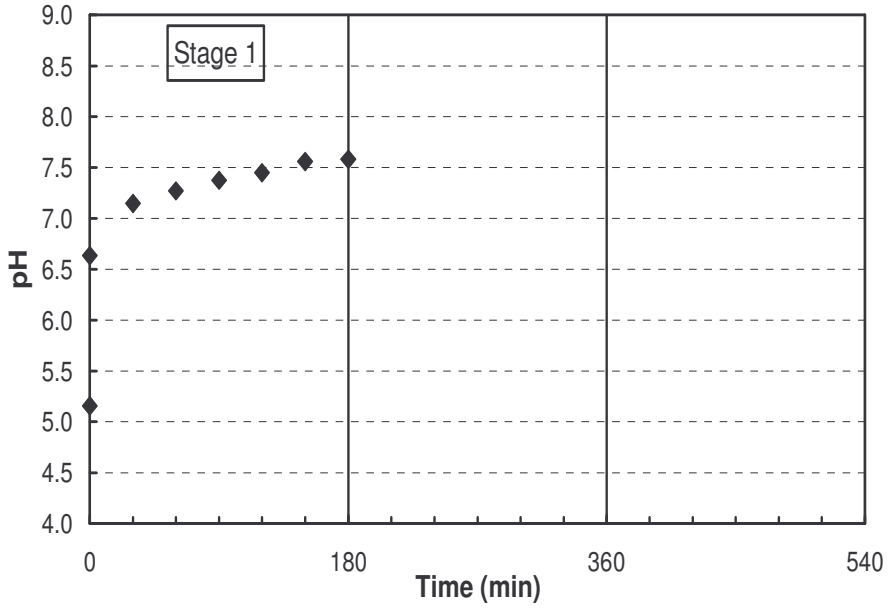

Figure 4.36. Time course change of solution pH during single stage reactor tests (Initial Pb(II) concentration = 80.2 mg/L).

When three stage reactor application was tested for an initial Pb(II) concentration of 91.5 mg/L, the results presented in Table 4.7 were obtained. During the first stage of the three stage reactor, the pH showed a sudden increase from 5.9 to 6.8 right after the addition of biomass and then decreased reaching an equilibrium value of 6.1 as shown in Figure 4.37. Meanwhile a 78.3 % removal was observed in Pb(II) from the solution; decreasing from an initial value of 91.5 mg/L to a final value of 19.9 mg/L. The

capacity was calculated as 3.271 mmol Pb(II) / g biomass and the SVI was measured as 110 mL/g.

In the second stage the pH increased continuously from 6.2 to 7.8 (Figure 4.37). The Pb(II) concentration decreased further from 19.9 mg/L to 1.9 mg/L, that is a removal of 90.3 % was observed. The capacity was calculated as 0.821 mmol Pb(II) / g biomass and the SVI was measured as 142 mL/g (Table 4.7).

Finally in the third stage solution pH again increased continuously from 7.8 to 8.5 (Figure 4.37). And the Pb(II) concentration decreased from 1.9 mg/L to 1.6 mg/L which corresponds to a removal efficiency of 16 %. The Pb(II) removal capacity was calculated as 0.017 mmol Pb(II) / g biomass and SVI was estimated as 111 mL/g (Table 4.7).

Table 4.7. Results of single stage reactor with initial Pb(II) concentration of 91.5 mg/L.

Parameter	**Value**		
	STAGE 1	STAGE 2	STAGE 3
Initial Pb(II) concentration (mg/L)	91.5	19.9	1.9
Final Pb(II) concentration (mg/L)	19.9	1.9	1.6
Pb(II) removal (%)	78.3	90.3	16.0
q (mmol Pb(II)/g biomass)	3.271	0.821	0.017
SVI (mL/g)	110	142	111

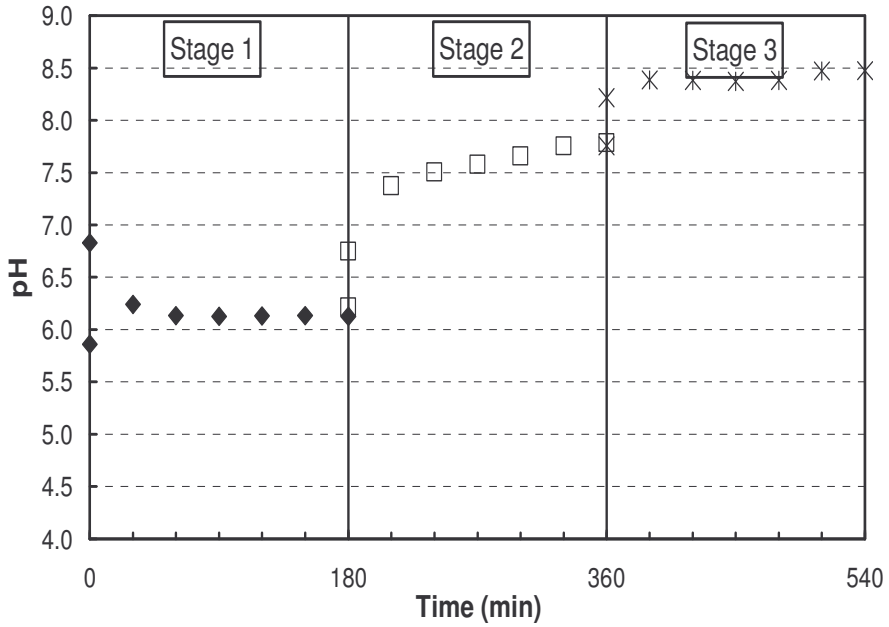

Figure 4.37. Time course change of solution pH during three stage reactor tests. Initial Pb(II) concentration = 91.5 mg/L.

In the whole system, the Pb(II) concentration decreased from 91.5 mg/L to 1.6 mg/L with a removal efficiency of 98.3 % and the final effluent concentration was satisfactory in meeting the Turkish Water Pollution Control Regulation standard of 2 mg/L. As shown in Figure 4.37, the pH of the solution was increased from 5.9 to 8.5, thus neutralization was also achieved.

4.2.2. 200 mg/L initial Pb(II) concentration

Single stage reactor operation with initial Pb(II) concentration of about 200 mg/L (195.1 mg/L) yielded the results given in Table 4.8. The Pb(II) removal efficiency was calculated as 99.0 % with the effluent Pb(II) concentration of 1.9 mg/L. During the tests, solution pH was also monitored, which increased continuously from an initial value of 5.0 to 6.5 as shown in Figure 4.38. The calculated Pb(II) removal capacity was 3.051 mmol Pb(II)/g biomass and the SVI was measured as 49 mL/g.

Table 4.8. Results of single stage reactor with initial Pb(II) concentration of 195.1 mg/L.

Parameter	Value
Initial Pb(II) concentration (mg/L)	195.1
Final Pb(II) concentration (mg/L)	1.9
Pb(II) removal (%)	99.0
q (mmol Pb(II)/g biomass)	3.051
SVI (mL/g)	49

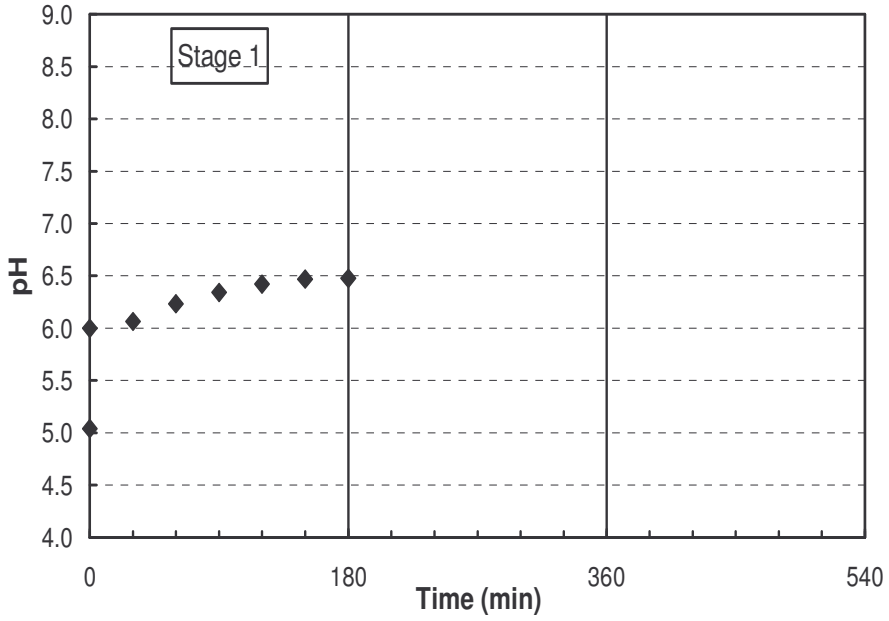

Figure 4.38. Time course change of solution pH during single stage reactor tests. Initial Pb(II) concentration = 195.1 mg/L.

Results obtained in three-stage reactor tests are presented in Table 4.9. During the first stage solution pH showed a sudden increase from 5.4 to 6.2 just after the addition of biomass and then decreased reaching an equilibrium value of 5.7 (Figure 4.39). Meanwhile, a 52.9 % removal was observed in Pb(II) from the solution. The Pb(II) concentration decreased from an initial value of 206.1 mg/L to a final value of 97.0

mg/L. The capacity was calculated as 5.165 mmol Pb(II) / g biomass and the SVI was measured as 128 mL/g.

In the second stage the pH increased continuously from 5.7 to 5.9 (Figure 4.39). The Pb(II) concentration decreased further from 97.0 mg/L to 16.2 mg/L, that is a removal of 83.4 % was observed. The capacity was calculated as 3.832 mmol Pb(II) / g biomass and the SVI was measured as 112 mL/g.

Table 4.9. Results of single stage reactor with initial Pb(II) concentration of 206.1 mg/L.

Parameter	**Value**		
	STAGE 1	STAGE 2	STAGE 3
Initial Pb(II) concentration (mg/L)	206.1	97.0	16.2
Final Pb(II) concentration (mg/L)	97.0	16.2	1.3
Pb(II) removal (%)	52.9	83.4	92.2
q (mmol Pb(II)/g biomass)	5.165	3.832	0.706
SVI (mL/g)	128	112	89

Finally in the third stage solution pH again increased continuously from 5.9 to 7.3 (Figure 4.39). And the Pb(II) concentration decreased from 16.2 mg/L to 1.3 mg/L with a removal efficiency of 92.2 %. The capacity was calculated as 0.706 mmol Pb(II)/g biomass and SVI was measured as 89 mL/g.

In the overall, the Pb(II) concentration decreased from 206.1 mg/L to 1.3 mg/L with a removal efficiency of 99.4 %. The pH of the solution was increased from 5.4 to 7.3 as shown in Figure 4.39, thus an effective pH neutralization was achieved.

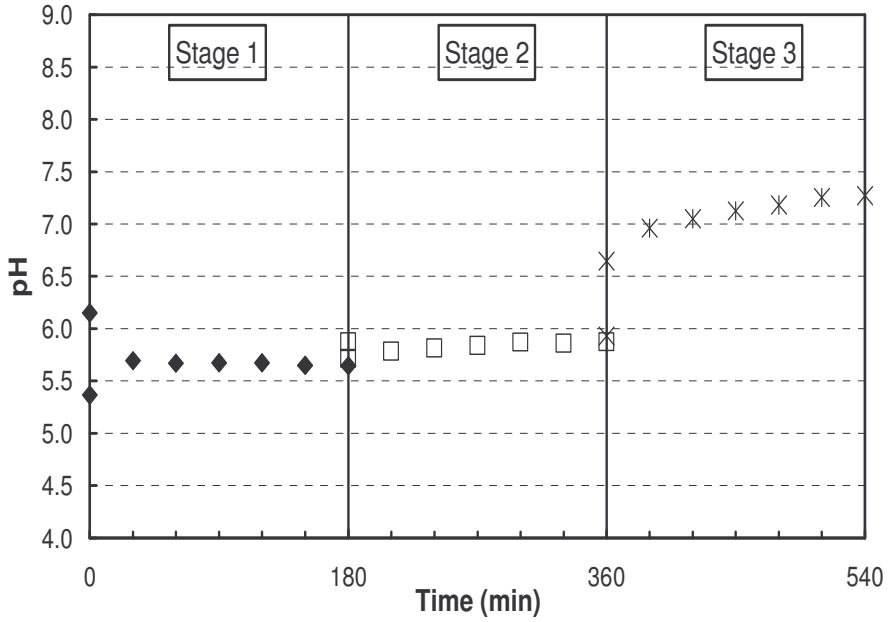

Figure 4.39. Time course change of solution pH during three stage reactor tests (Initial Pb(II) concentration = 206.1 mg/L).

All of the final Pb(II) concentration values, except the one for single stage system starting with the initial Pb(II) concentration of 100 mg/L, were below the Pb(II) discharge limits, 2 mg/L. That means both reactor configurations can be used to meet the requirements of Turkish Water Pollution Control Regulation for the initial Pb(II) concentration of 200 mg/L; while, multiple stage configuration is required to satisfy the limitation if the initial Pb(II) concentration is 100 mg/L.

Thus, a general comparison of single stage application with multi stage reveals that the capacity of biomass is much more effectively used in multiple stage configuration. That is an expected result, as this type of configuration provides a better use of available capacity on biomass. In this way, multiple stage reactor configuration minimizes the biomass amount to be used for a desired level of treatment by maximizing the sorption capacity consumption. As seen from results, with initial Pb(II) concentration of 100 mg/L, although the same amount of biomass was used in both configurations, the desired effluent level could not be achieved in single-stage configuration. The effluent Pb(II) concentration achieved in single stage configuration

could be obtained after 2 stages in three stage configuration while the biomass used was only 2/3 of the first case.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

In the present study, Pb(II) removal by ADS has been investigated. Raw ADS was found to be very effective in the removal of Pb(II). The maximum removal capacity was about 1760 mg (8.5 mmol) Pb(II) per g of biomass, with over 99 % removal efficiency for initial Pb(II) concentrations below 1000 mg/L. The pH values attained after biosorption for this concentration range were between 7.0 – 8.0 indicating neutralization effect of the process, which makes the use of raw ADS in heavy metal removal more favorable.

Sorption tests using dewatered ADS indicated that dewatering causes about a three fold decrease in Pb(II) removal capacity of ADS; only 518 mg (2.5 mmol) Pb(II) per g of biomass was removed using dewatered ADS. The reduction in Pb(II) removal capacity was attributed to possible loss of bicarbonate ions and other soluble ligands during dewatering of ADS. On the other hand, still high removal efficiencies (> 95%) could be obtained with dewatered ADS but, for initial Pb(II) concentrations below 500 mg/L. A neutralizing effect was again observed with final pH values around 6.0 – 7.5 for initial Pb(II) concentrations below 500 mg/L.

For both raw and dewatered ADS, Pb(II) sorption was found to be a very rapid process. Within the first 10 min, 70 % of the Pb(II) removal was complete using raw ADS.

High equilibrium pH values indicated that biosorption was not the only mechanism involved in removal of Pb(II). Especially for equilibrium pH values above 6 the major mechanism seemed to be carbonate precipitation. Therefore, the removal of lead by raw ADS is suggested to involve biomineralization/bioprecipitation as well as biosorption.

On the other side, FTIR studies indicated that Pb(II) biosorption by ADS involves an interaction between Pb(II) and carboxyl groups available on the biomass surface. Thus, ion exchange through the carboxyl functional groups was supposed to be effective for the biosorption of Pb(II).

Considering that precipitation takes place during the process, single and multiple stage fed-batch reactor configurations were tested. Multiple stage (3 stages in this study) reactor configuration appeared to be a proper reactor configuration for Pb(II) removal by raw ADS. The selection of this configuration was in fact based on the physical characteristic of raw ADS, nature of the removal process i.e. and very good settleability of the sludge formed after the removal process.

In future studies, for a better evaluation of the process, it is recommended that heavy metal removal from a typical metal bearing industrial wastewater would be investigated using the multi stage reactor configuration tested in the present study. Also, the contribution of biosorption and biomineralization/bioprecipitation mechanisms to the total removal could be investigated by a multi-disciplinary team in a more focused and detailed research on the mechanism of the process.

REFERENCES

1. Alibhai K.R.K., Mehrotra I., Forster C.F. (1985). Heavy metal binding to digested sludge. Water Research, 19(12), 1483-1488.

2. APHA, AWWA, WEP (1995) Standard Methods for Examination of Water and Wastewater. 19th edition. United Book Press. Baltimore.

3. Arıcan B. (1998) "Nickel sorption by activated sludge." An M.Sc. thesis submitted to graduate school of Natural and Applied Sciences of METU.

4. Artola A., Balaguer M. D., Rigola M. (1997). Heavy metal binding to anaerobic sludge. Water Research, 31(5), 997-1004.

5. Artola A., Martin M. J., Balaguer M. D., Rigola M. (2001). Pilot plant biosorption in an integrated contact settling system: application to Cu(II) removal by anaerobically digested sludge. Journal of Chemical Technology and Biotechnology, 76, 1141-1146.

6. Artola A., Rigola M. (1992). Selection of optimum biological sludge for zinc removal from wastewater by a biosorption process. Biotechnology Letters, 14(12), 1199-1204.

7. Ashkenazy R., Gottlieb L., Yannai S. (1997). Characterization of acetone washed yeast biomass functional groups involved in lead biosorption. Biotechnology and Bioengineering, 55(1), 1-10.

8. Barkay T., Schaefer J. (2001). Metal and radionuclide bioremediation: issues, considerations and potentials. Current Opinion in Microbiology, (4), 318-323.

9. Bernardin F. E. (1985) "Experimental design and testing of adsorption and adsorbates." Adsorption Technology Ed. Slejko F. L. 37-90.

10. Brierley C. L., Brierley J. A. "Immobilization of biomass for industrial application of biosorption." Biohydrometallurgical Technologies Ed. Torma A. E., Apel M. L., Brierley C. L. Warrendale, PA: The Minerals, Metals & Materials Society, 1993. 35-44.

11. Brierley J. A., Brierley C. L., Goyak G. M. "AMT-BIOCLAIM: A new wastewater treatment and metal recovery technology." Fundamental and Applied Biohydrometallurgy Ed. Lawrance R. W., Branion R. M. R., Ebner H. G. Amsterdam: Elsevier, 1986. 291-304.

12. Butter T. J., Evison L. M., Hancock I. C., Holland F. S., Matis K. A., Philipson A., Sheikh A. I., Zouboulis A. I. (1998). The removal and recovery of cadmium from dilute solutions by biosorption and electrolysis at laboratory scale. Water Research, 32(2), 400-406.

13. Cadman T. W., Dellinger R. W. (1974) "Techniques for removing metals from process wastewater." Industrial Wastewater and Solid Waste Engineering Ed. Vincent Cavaseno New York: McGraw-Hill Publications, 109-115.

14. Ceribasi H. I., Yetis U. (2001). Biosorption of Ni(II) and Pb(II) by *Phanerochaete chrysosporium* from a binary metal system - Kinetics. Water SA, 27(1), 1-6.

15. Crist R. H., Oberholser K., Schwartz D., Marzoff J., Ryder D., Crist D. R. (1988). Interactions of metals and protons with algae. Environmental Science and Technology, 22, 755-760.

16. Davis T. A., Volesky B., Vieira R. H. S. F. (2000). *Sargassum* seaweed as biosorbent for heavy metals. Water Research, 34(17), 4270-4278.

17. Delgado A., Anselmo A. M., Novais J. M. (1998). Heavy metal biosorption by dried powdered mycelium of *Fusarium flocciferum*. Water Environment Research, 70(3), 370-375.

18. Dölek A. (1997) "Biosorption of lead by a white-rot fungus." An M.Sc. thesis submitted to graduate school of Natural and Applied Sciences of METU.

19. Dündar İ. V. (2003) "Characteristic properties and operational parameters of anaerobic sludge digesters in Ankara Municipal Wastewater Treatment Plant." (Personal communication).

20. Esposito A., Pagnanelli F., Veglio F. (2002). pH-related equilibria models for biosorption in single metal systems. Chemical Engineering Science, 57, 307-313.

21. Fergusson J. E. (1990) "The Heavy Elements: Chemistry, Environmental Impact and Health Effects." Pergamon Press.

22. Figueira M. M., Volesky B., Mathieu H. J. (1999). Instrumental analysis of iron species biosorption by Sargassum biomass. Environmental Science and Technology, 33(11), 1840-1846.

23. Figueira M. M., Volesky B., Zarian K., Ciminelli V. S. T. (2000). Biosorption column performance with a metal mixture. Environmental Science and Technology, 34(20), 4320-4326.

24. Fourest E., Roux J. C. (1992). Heavy metal biosorption by fungal mycelial by-products: mechanisms and influence of pH. Applied Microbiology and Biotechnology, 37, 399-403.

25. Fourest E., Volesky B. (1996). Contribution of sulfonate groups and alginate to heavy metal biosorption of the dry biomass of *Sargassum fluitans*. Environmental Science and Technology, 30(1), 277-282.

26. Friis N., Myers-Keith P. (1986). Biosorption of uranium and lead by *Streptomyces longwoodensis*. Biotechnology and Bioengineering, 28, 21-28.

27. Gardea-Torresday J. L., Tiemann K. J., Gonzalez J. H., Cano-Aguilera I., Henning J.. A., Townsend M. S. (1996). Removal of nickel ions from aqueous solution by biomass and silica-immobilized biomass of *Medicago sativa* (alfalfa). Journal of Hazardous Materials, 49, 205-216.

28. Gonzalez M. E. R., Williams C J., Gardiner P. H. E. (2001). Study of the mechanisms of cadmium biosorption by dealginated seaweed waste. Environmental Science and Technology, 35(14), 3025-3030.

29. Gould M.S., Genetelli E.J. (1978). Heavy metal complexation behavior in anaerobically digested sludges. Water Research, 12, 505-512.

30. Haytoglu B. (2000) "Heavy metal biosorption by anaerobic biomass." An M.Sc. thesis submitted to graduate school of Natural and Applied Sciences of METU.

31. Holan Z. R., Volesky B. (1994). Biosorption of lead and nickel by biomass of marine algae. Biotechnology and Bioengineering, 43, 1001-1009.

32. Holan Z. R., Volesky B. (1995). Accumulation of Cd(II), Pb(II) and Ni(II) by fungal and wood biosorbents. Applied Biochemistry and Biotechnology, 53(2), 133-146.

33. Kapoor A. (1998) "Removal of heavy metals from aqueous solution by fungi *Aspercillus niger*." A Ph.D. thesis submitted to faculty of Graduate Studies and Research of University of Regina.

34. Kapoor A., Viraraghavan T. (1997). Heavy metal biosorption sites in *Aspergillus niger*. Bioresource Technology, 61, 221-227.

35. Kratochvil D., Volesky B. (2000). Multicomponent biosorption in fixed beds. Water Research, 34(12), 3186-3196.

36. Kratochvil D., Volesky B. (1998). Advances in the biosorption of heavy metals. Trends in Biotechnology, 16(7), 291-300.

37. Lanouette K. H. (1977) "Heavy metals removal." Industrial Wastewater and Solid Waste Engineering Ed. Vincent Cavaseno New York: McGraw-Hill Publications, 116-123.

38. Lloyd J. R., Lovley D. R. (2001). Microbial detoxification of metals and radionuclides. Current Opinion in Biotechnology, (12), 248-253.

39. Marques P. A. S. S., Rosa M. F., Pinheiro H. M. (2000). pH effects on the removal of Cu(II), Cd(II), and Pb(II) from aqueous solution by waste brewery biomass. Bioprocess Engineering, 23, 135-141.

40. Matheickal J. T., Yu W. (1999). Biosorption of lead(II) and copper(II) from aqueous solutions by pretreated biomass of Australian marine algae. Bioresource Technology, 69, 223-229.

41. Mattuschka B., Straube G. (1993). Biosorption of metals by waste biomass. Journal of Chemical Technology and Biotechnology, 58, 57-63.

42. Nemec P., Prochazka H., Stamberg K., Katzer J., Stamberg J., Jilek R., Hulak P. "Process of treating mycelia of fungi for retention of metals." U. S. Patent 4 021 368 1977.

43. Niu H., Xu S. X., Wang J. H. (1993). Removal of lead from aqueous solutions by *Penicillum* biomass. Biotechnology and Bioengineering, 42, 785-787.

44. Pagnanelli F., Esposito A., Toro L., Veglio F. (2003). Metal speciation and pH effect on Pb, Cu, Zn and Cd biosorption onto *Sphaerotilus natans*: Langmuir type empirical model. Water Research, 37, 627-633.

45. Parkin G. F., Owen W. F. (1986). Fundamentals of anaerobic digestion of wastewater sludges. Journal of Environmental Engineering, 112(5), 867-920.

46. Patterson J. W. (1985) "Lead." Industrial wastewater treatment technology Ed. Patterson J. W. 175-189.

47. Patterson J. W., Allen H. E., Scala J. J. (1977). Carbonate precipitation for heavy metals pollutants. Journal of Water Pollution Control Federation, 49(12), 2397-2410.

48. Pümpel T., Ebner C., Pernfuss B., Schinner F., Diels L., Keszthelyi Z., Stankovi A., Finlay J. A., Macaskie L. E., Tsezos M., Wouters H. (2001). Treatment of rinsing water from electroless nickel plating with a biologically active moving-bed sand filter. Hydrometallurgy, 59, 383-393.

49. Sag Y., Kutsal T. (1995). Biosorption of heavy metals by *Zoogloea ramigera*. Use of adsorption isotherms and a comparison of biosorption characteristics. The Chemical Engineering Journal, 60, 181-188.

50. Schiewer S. (1996) "Multi metal ion exchange in biosorption." A Ph.D. thesis submitted to faculty of Graduate Studies and Research of McGill University.

51. Schneider I. A. H., Rubio J., Smith R. W. (2001). Biosorption of metals onto plant biomass: exchange adsorption or surface precipitation? International Journal of Mineral Processing, 62, 111-120.

52. Suh J. H., Kim D. S., Song S. K. (2001). Inhibition effect of initial Pb(II) concentration on Pb(II) accumulation by *Saccharomyces cerevisiae* and *Aureobasidium pullulans*. Bioresource Technology, 79, 99-102.

53. Templeton A. S., Trainor T. P., Spormann A. M., Newville M., Sutton S. R., Dohnalkova A., Gorby Y. Brown G. E. Jr. (2003). Sorption versus biomineralization of Pb(II) within *Burkholderia cepacia* biofilms. Environmental Science and Technology, 37(2), 300-307.

54. Tobin J. M., Cooper D. G., Neufeld R. J. (1988). The effects of cation competition on metal adsorption by Rhizopus Arrhizus biomass. Biotechnology and Bioengineering, 31, 282-286.

55. Tobin J. M., Cooper D. G., Neufeld R. J. (1990). Investigation of the mechanism of metal uptake by denatured *Rhizopus arrhizus* biomass. Enzyme and Microbial Technology, 12(8), 591-595.

56. Tobin J. M., Cooper D. G., Neufeld R. J. (1984). Uptake of metal ions by *Rhizopus arrhizus* biomass. Applied and Environmental Microbiology, 47, 821-824.

57. Tsezos M. (2001). Biosorption of metals. The experience accumulated and the outlook for technology development. Hydrometallurgy, 59, 241-243.

58. Tsezos M., Remoudaki E., Angelatou V. (1995). A systematic study on equilibrium and kinetics of biosorptive accumulation. The case of Ag and Ni.. International Biodeterioration and Biodegradation, 129-153.

59. Tsezos M., Remoudaki E., Angelatou V. (1997). Biosorption sites of selected metals using electron microscopy. Comp. Biochem. Physiol., 118A(3), 481-487.

60. Turkish Water Pollution Control Regulation (1988). Official Gazette #19919.

61. Urasa I. T., Macha S.F. (1999). Investigation into heavy metal uptake by wastewater sludges. Water, Air and Soil Pollution, 109, 207-218.

62. Volesky B. (1990) "Removal and recovery of heavy metals by biosorption." Biosorption of heavy metals Ed. Volesky B. CRC Press.

63. Volesky B. (2001). Detoxification of metal-bearing effluents: biosorption for the next century. Hydrometallurgy, 59, 203-216.

64. Volesky B., May-Phillips H. A. (1995). Biosorption of heavy metals by *Saccharomyces cerevisiae*. Applied Microbiology and Biotechnology, 42, 797-806.

65. Volesky B., Weber J., Park J. M. (2003). Continuous-flow metal biosorption in a regenerable *Sargassum* column. Water Research, 37, 297-306.

66. Waihung Lo, Hong Chua, Kim Hung Lam, Shu-Ping Bi (1999). A comparative investigation on the biosorption of lead by filamentous fungal biomass. Chemosphere, 39(15), 2723-2736.

67. Williams C. J., Aderhold D., Edyvean R. G. J. (1998). Comparison between biosorbents for the removal of metal ions from aqueous solutions. Water Research, 32(1), 216-224.

68. www.nist.gov (2002) "Official web page of National Institute of Standards and Technology."

69. Yan G., Viraraghavan T. (2001). Heavy metal removal in a biosorption column by immobilized *M. rouxii* biomass. Bioresource Technology, 78, 243-249.

70. Yun Y. S., Park D., Park J. M., Volesky B. (2001). Biosorption of trivalent chromium on the brown seaweed biomass. Environmental Science and Technology, 35(21), 4353-4358.