DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

REMOVAL OF HEAVY METALS FROM WASTEWATER BY BIOSORPTION USING EXCESS SLUDGE

by
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REMOVAL OF HEAVY METALS FROM WASTEWATER BY BIOSORPTION USING EXCESS SLUDGE

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We have read the thesis entitled **"REMOVAL OF HEAVY METALS FROM WASTEWATER BY BIOSORPTION USING EXCESS SLUDGE"** completed by **M. YUNUS PAMUKOĞLU** under supervision of **PROF. DR. FİKRET KARGI** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Doctor of Philosophy.

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REMOVAL OF HEAVY METALS FROM WASTEWATER BY BIOSORPTION USING EXCESS SLUDGE

ABSTRACT

Activated sludge obtained from a paint industry wastewater treatment plant (DYO-Izmir) was found to be the most suitable among the others tested resulting in the highest biosorption capacity (50 mg g^{-1}). Pre-treatment by 1% hydrogen peroxide solution was found to be superior to the other methods yielding the highest biosorption capacity (65 mg g^{-1}).

Effects of operating parameters on batch biosorption kinetics of copper (II) ions onto pre-treated powdered waste sludge (PWS) were investigated. Batch isotherms of biosorption of Cu(II) ions were investigated and the langmuir isotherm was found to fit the experimental data better than the other isotherms tested.

Biosorption of Cu(II) ions onto pre-treated powdered waste sludge (PWS) was also investigated using a fed-batch operated completely mixed reactor. Breakthrough curves describing variations of effluent copper ion concentrations with time were determined for different operating conditions.

In order to investigate the adverse effects of Cu(II) ions on performance of an activated sludge unit, synthetic wastewater containing Cu(II) ion was treated in an activated sludge unit and COD, Cu(II), toxicity removals were investigated. Copper ion toxicity on COD removal performance of the activated sludge unit was partially eliminated by operation at high sludge ages (30 days) and HRT's (25 hours).

Copper (II) ion toxicity onto activated sludge organisms was eliminated by addition of powdered waste sludge (PWS) to the feed wastewater for removal of Cu(II) ions by biosorption before biological treatment. Box-Behnken experimental design method was used to investigate Cu(II), chemical oxygen demand (COD) and toxicity removal performance of the activated sludge unit under different operating conditions. Optimum conditions resulting in maximum Cu(II), COD, toxicity removals and SVI values were found to be SRT of nearly 30 days, HRT 15 hours, PWS loading rate 3 g h^{-1} and feed Cu(II) concentration of less than 30 mg l^{-1}.

Keywords: Activated sludge, biological treatment, biosorption, Box-Behnken experimental design, chemical oxygen demand (COD), copper (II) ions, fed-batch reactor, isotherms; kinetics; mathematical model, operating conditions, powdered waste sludge (PWS), pre-treatment, toxicity

TOZ ARITMA ÇAMURU KULLANILARAK BİYOSORPSİYONLA ATIKSULARDAN AĞIR METALLERİN GİDERİMİ

ÖZ

Test edilen diğer toz aktif çamurlarla karşılaştırıldığında en iyi biyosorpsiyon kapasitesi DYO boya endüstrisi atıksu arıtma tesisinden elde edilen toz aktif çamuru ile elde edilmiştir (50 mg g^{-1}). En etkin ön yıkama işlemi, DYO İzmir boya endüstrisi aşırı çamurunun %1 H_2O_2 çözeltisi ile yıkanmasıyla elde edilmiştir (65 mg g^{-1} TAÇ) olarak elde edilmiştir.

Kesikli biyosorpsiyon deneyleri ile ön arıtılmış toz arıtma çamurunun (TAÇ) bakır iyonları biyosorpsiyon kapasiteleri incelenmiştir. Cu(II) iyonlarının biyosorpsiyon izotermleri araştırılmıştır ve test edilen diğer izotermler arasında Langmuir izotermi, deney sonuçlarıyla daha yüksek bir korelasyon göstermiştir.

Toz arıtma çamuru (TAÇ) kullanılarak Cu(II) iyonlarının biyosorpsiyonu kesikli beslemeli işletilen tam karışımlı bir reaktör kullanılarak da araştırıldı. Çıkış bakır iyonları konsantrasyonlarının zamanla değişimleri farklı işletme şartlarında araştırılmıştır.

Cu(II) iyonu içeren sentetik atıksu bir aktif çamur ünitesinde arıtıma tabii tutulmuş ve Cu(II) iyonlarının, KOİ, Cu(II) ve toksisite giderimine etkileri araştırılmıştır. Aktif çamur ünitesi yüksek çamur yaşlarında (30 gün) ve yüksek hidrolik bekleme sürelerinde (25 saat) işletildiğinde Cu(II) iyonu toksisitesi kısmen elimine edilmiş ve daha yüksek KOI giderimleri elde edilmişdir.

Biyolojik arıtımdan önce biyosorpsiyonla Cu(II) iyonlarının giderimi için besleme atıksuyuna toz arıtma çamuru (TAÇ) ilavesiyle aktif çamur üzerindeki Cu(II) iyonu toksisitesi elimine edilmiştir. Farklı işletme şartlarında çalıştırılan aktif çamur reaktörünün toksisite, kimyasal oksijen ihtiyacı (KOİ) ve Cu(II) giderim verimlerinin incelenmesi amacıyla Box-Behnken deneysel tasarım metodu uygulanmıştır. Maksimum Cu(II), KOİ, toksisite giderimleri ve minimum çamur hacim indeksleri elde etmek için gerekli optimum işletme şartları, çamur bekleme süresi için 30 gün, hidrolik bekleme süresi için 15 saat, TAÇ yükleme hızı için 3 g sa^{-1} ve giriş Cu(II) konsantrasyonu için ise 30 mg l^{-1}’ den az olarak saptanmıştır.

Anahtar sözcükler: Aktif çamur, bakır (II) iyonu, biyolojik arıtım, biyosorpsiyon, Box-Behnken dizayn, işletim şartları, izotermler, kesikli beslemeli reaktör, kimyasal oksijen istegi (KOI), kinetik, matematiksel model, ön arıtım, toksisite, toz arıtma çamuru (TAÇ)

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CHAPTER ONE
INTRODUCTION

1.1 The Problem Statement

Presence of heavy metals in wastewater is of interest because of their known toxic effects on the receiving environment and also on the performance of biological treatment processes. On the other hand, the performance of wastewater treatment processes in terms of metal removal is also of great importance in determining the quantity of heavy metals discharged into receiving waters, especially in areas where water re-use is practiced. Therefore, recovery of heavy metals from wastewater became an important environmental issue, recently.

Heavy metals present in many industrial wastewaters such as automobile, metal finishing, leather tanning, electroplating, petroleum and textile dying are known to have toxic effects to the receiving environment. Heavy metal containing wastewaters cause detrimental effects on all forms of life upon direct dicharge to the environment. (Fergusson, 1990; Volesky, 1990; Kratochvil and Volesky, 1998; Aksu 2005). Copper, zinc, lead, mercury, chromium, cadmium, iron, nickel and cobalt are the most frequently found heavy metals in industrial wastewaters (Fergusson, 1990).

Trace amounts (µg l^{-1}) of some metal ions such as copper, zinc, cobalt, iron, nickel are required by some organisms as cofactors for the enzymatic activities. However, heavy metal ion concentrations at ppm (mg l^{-1}) level are known to be toxic to the organisms because of irreversible inhibition of many enzymes by the heavy metal ions. Toxicity of heavy metal ions on activated sludge bacteria varies depending on the type and concentrations of heavy metal ions and the organisms as well as the environmental conditions such as pH, temperature, dissolved oxygen (DO), presence of other metal ions, ionic strength and also the operating parameters such as sludge age (SRT) and hydraulic residence time (Dilek *et al.*, 1998).

Heavy metals in the wastewater have increased because of industrial and human activities. Mine drainage, metal industries, petroleum refining, tanning, photographic processing and electroplating are some of the main sources of heavy metals. Erosion of surface deposits of metal minerals, agricultural runoff, and acid rain contribute to heavy metals in wastewater naturally (Dean *et al.* 1977). Types of metals released by various industries are shown in Table 1.1

Table 1.1 Types of metals released by different industries (Dean *et al.* 1977)

	Al	Ag	As	Cd	Cr	Cu	Fe	Hg	Pb	Ni	Sn	Zn
Pulp,paper mills					⊗	⊗		⊗	⊗	⊗		⊗
Organic chemicals, petrochemical	⊗		⊗	⊗	⊗		⊗	⊗	⊗		⊗	⊗
Chlorine, inorganic chemicals	⊗		⊗	⊗	⊗		⊗	⊗	⊗		⊗	⊗
Fertilizers	⊗		⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗		⊗
Petroleum refining	⊗		⊗	⊗	⊗	⊗	⊗		⊗	⊗		⊗
Basic steel works, foundries			⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗
Motor vehicles,finishing	⊗	⊗		⊗	⊗	⊗		⊗		⊗		
Textile products					⊗							
Leather tanning					⊗							

Activated sludge is an effective biosorbent for heavy metal ions removal because of its low cost and availability (Aksu and Akpınar, 2001). Heavy metals at trace concentrations are known to have no detrimental effect on microorganisms in activated sludge process, and are taken up by microbial cells as essential micronutrients, involving ion exchange, adsorption and complexation. The metal uptake mechanisms include passive adsorption on the binding sites of cell walls, and metabolically mediated active transport (Arican et.al., 2002).

Large numbers of microorganisms have been used as sorbents for heavy metals (Davis T., *et al.*, 2003). Biocatalytic activity of microorganisms, for example, those belonging to the genus *Thiobacillus*, are primarily responsible for generation of acid mine drainage (AMD) from metal sulfide ores (Tsukamoto and Miller, 1999).

Biosorption modeling considering two metals systems can be carried out using either empirical equations or chemical-physical mechanistic models (Pagnanelli *et* *al.*, 2003). Empirical models are more or less simple mathematical equations with some adjustable parameters, which can be fitted to the experimental data. In some cases, models contain parameters obtained only from isotherms of one metal system (Al-Asheh *et al.*, 2003).

Simple sorption isotherm curves are usually constructed as a result of studying equilibrium batch sorption behavior of different biosorbent materials. These curves enable quantitative evaluation of biosorption performance of these materials for only one metal. However, when more than one metal at a time is present in a sorption system, evaluation, interpretation, and representation of biosorption results become much more complicated. With two metals in solution, instead of two-dimensional biosorption isotherm curves the system evaluation results in a series of three-dimensional sorption isotherm surfaces. This novel approach is very useful particularly because it permits a complete control over the values of the final concentrations of both sorbates present in the system (Figueira *et al.*, 2000).

1.2 Treatment Techniques for Heavy Metal Removal

Different methods were developed for the removal of heavy metals from wastewater. Conventional techniques commonly applied for the removal of heavy metals from wastewater include chemical and physical methods. Chemical methods are chemical precipitation /neutralization, coagulation /flocculation, solvent extraction. Physical methods are electrodialysis, ion exchange, membrane separation, adsorption and filtration. In chemical precipitation, chemicals such as ferrous sulfate, lime, caustic and sodium carbonate are commonly used. However, these chemical and physical methods have significant disadvantages, including incomplete metal removal, producing large volume of sludge, requirements for expensive equipment and monitoring systems, high reagent or energy requirements and generation of toxic sludge or other waste products that require disposal. New technologies are required that can reduce heavy metal concentrations to environmentally acceptable levels at affordable costs (Atkinson *et al.*, 1998).

Adsorption process is an affective option for the removal of heavy metals from wastewater. An economical and easily available adsorbent would make the adsorption-based process an attractive alternative for the removal of heavy metals from wastewaters. Activated carbon is the most widely and effectively used adsorbent. A typical activated carbon particle, whether in a powdered or granular form, has a porous structure consisting of a network of interconnected macropores, mesopores, and micropores that provide a good capacity for the adsorption of heavy metals due to its high surface area of 600-1000 m^2 g^{-1} (Aksu Z. et. al., 2002). In spite of these characteristics, activated carbon suffers from a number of disadvantages. It is quite expensive and the higher the quality, the greater the cost. Both chemical and thermal regeneration of spent carbon is expensive, impractical on a large scale, produces additional effluent, and results in considerable loss of the adsorbent.

Because of these disadvantages, a biological treatment that is called biosorption based on organisms or plants could be an alternative method to clean up industrial wastewaters containing heavy metals. Biosorption is essentially the passive and physicochemical binding of chemical species to biopolymers. (Su M. et. al., 1995, Yetis U. et. al., 1998). The advantages of biosoption process over chemical and physical methods can be summarized as follows:

1. Utilization of excess sludge for removal of toxic heavy metal ions from wastewater
2. Low cost of the biosorbent and possibility of reutilization
3. Efficient, rapid and cheap separation of the biosorbent from solution
4. High selectivity of metal adsorption and desorption
5. Possibility of operation under a broad range of conditions (pH, temperature)

Activated sludge is a well-known biomass used for the treatment of some industrial and domestic wastewaters. Part of the microorganisms over grown in such wastewater systems can be separated and utilized for removal of heavy metal ions as an abundant and cheaper biosorbent. Activated sludge contains both bacteria and protozoa. The cell wall of bacteria essentially consists of various organic compounds such as carboxyl, acidic polysaccharides, lipids, amino acids and other components.

The protozoa are unicellular, motile, relatively large eucaryotic cells that lack cell walls. Protozoa can absorb components through their outer membranes that contain proteins and lipids.

Most of the biosorption studies were carried out in batch systems. Fed-batch operation of completely mixed adsorption reactors has the following advantages over the adsorption columns.

1. Provides better contact between the adsorbent and the adsorbate since the adsorbent particles are not in contact with each other due to complete mixing
2. Reduces transport limitations (liquid film resistance) encountered in adsorption columns to minimum level
3. Improves the rate of adsorption due to complete mixing and better contact between the phases
4. Provides a homogenous mixture and therefore, better control of the environmental conditions
5. Eliminates hydrodynamical problems such as flow channeling, dead regions, radial dispersion encountered in adsorption columns.

1.3 Metal Ion Uptake and Interaction of Metal Ions with Microorganisms

Heavy metals can be present in wastewater in two different forms: particulate and solubilized form. Heavy metals of solubilized form exist as free metal ions or as complexed ions by forming metal-ligand complex with inorganic or organic ligands. Heavy metals in the form of particulate include heavy metals in colloidal form and heavy metals adsorbed on particulate matter.

Heavy metals can be taken up by microorganisms in many different ways (Brierley, 1990). Microbial metal uptake involving the rapid, metabolism-independent uptake of metals to cell walls and other external surfaces is called passive uptake. In living microorganisms, under the effect of cell metabolic cycle, some heavy metal ions will go through cell membrane and enter cells. This metal uptake is referred to as active uptake or intracellular uptake. Metal uptake by both active and passive (dead) cells can be termed as bioaccumulation. Cell structures compose of mainly three parts, i.e. extracellular polymeric substances (EPS) surrounding the cell, a cell membrane (CM) and cell content (CC). HIE is the heavy metals incorporated in EPS. HIM is the heavy metals incorporated in cell membrane. HIC is referred to heavy metals incorporated in cell content. MN is metabolic need of microorganisms for metal ions. Biosorption of metal ions consisting of HIE and HIM is defined as extracellular uptake. The uptake of heavy metals by cell contents (CC) is HIC, which is referred to as intracellular uptake (Figure 1.1). Metal uptake by dead cells is through passive uptake and metal uptake by live cell involves both passive and active uptakes (Fuhrmann and Rothstein, 1968).

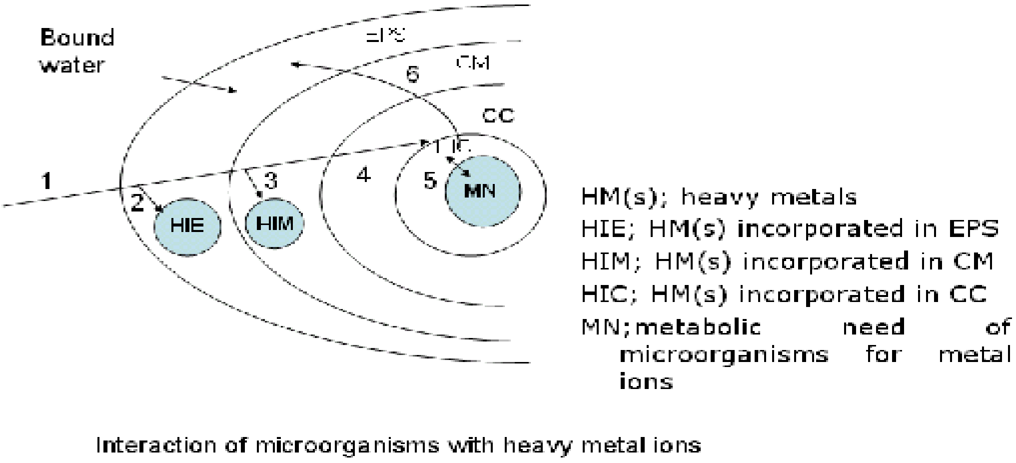


Figure 1.1 Metal ion uptake and interaction of metal ions (Fuhrmann and Rothstein, 1968).

1.4 Biosorption Process

Accumulation of dissolved substances at interfaces or between phases is called as adsorption. Adsorbate is the dissolved substance adsorbed on solid phase which is called as adsorbent. Adsorption of dissolved substances on microbial cell surfaces has been defined as biosorption. Metallic cations are attracted to negatively charged sites at the surface of the cell, a number of anionic ligands participate in binding the metal. Ion exchange, complexation, chelation, physical adsorption, micro-precipitation, and oxidation/reduction are all possible processes that contribute to the biosorption of heavy metals. Ion exchange is recognized as the principal mechanism of heavy metal biosorption. The polymeric structure of biomass surfaces consists of proteins, carbohydrates, lipid, and is of a negative charge because of the ionization of organic groups. The functional groups of cell wall constituents, such as carboxyl, amino, phosphoryl and sulfo groups, are referred as the probable sites for ion exchange (Bux and Kasan, 1994).

In biosorption, either live or dead microorganisms or their derivatives are used, which complex metal ions through the functioning of ligands or functional groups located on the outer surface of the cell (Bolton and Gorby, 1995). The use of dead microbial cells in biosorption is more advantageous for water treatment. Since dead organisms are not affected by toxic wastes, they do not require a continuous supply of nutrients and they can be regenerated and reused for many cycles. However, the use of dead biomass in powdered form has some problems, such as difficulty in the separation of biomass after biosorption, mass loss after regeneration and low strength and small particle size, which make it difficult to use in column applications. To solve these problems, dead biomass can be immobilized in a supporting material. Much of the bioremoval literature deals with artificially immobilized biomass. Researchers have recognized that immobilizing nonliving biomass in a biopolymeric or polymeric matrix may improve biomass performance, biosorption capacity, increase mechanical strength and facilitate separation of biomass from metal-bearing solution. Immobilization also allows higher biomass concentration, resistance to chemical environments and column operations and immobilized systems may be well suited for non-destructive recovery. Indeed, the use of immobilized biomass has a number of major disadvantages. In addition to increasing the cost of biomass pre-treatment, immobilization adversely affects the mass transfer kinetics of metal uptake. When biomass is immobilized, the number of binding sites easily accessible to metal ions in solution is greatly reduced since the majority of sites will lie within the bead. So a good support material used for immobilization should be rigid, chemically inert and cheap, should bind cells firmly, should have high loading capacity and should have a loose structure to overcome diffusion limitations.

Different methods were developed for quantification of metal ion toxicity on activated sludge systems. Some of those methods are based on inhibition of enzymatic activities (Strotmann *et al.*, 1992); respiratory activities of the bacteria (Vankova *et al.* 1999); and kinetics of bacterial activities (Cabrero *et al*., 1998). One of the toxicity assessment methods used is the 'resazurin assay' which is relatively simple, inexpensive and rapid method for assessment of the toxicity of chemical compounds and water samples (Liu, 1986; Brouwer, 1991; Strotmann *et al.*, 1993). Basic principle of the method is the measurement of percentage of inhibition on dehydrogenase activity of bacteria in the presence of toxic compounds. Toxicity values obtained with the resazurin assay were reported to be comparable to the more commonly used biological methods such as *Daphnia magna*, and Microtox TM (Farre and Barcelo, 2003).

1.5 Microorganisms Used as Biosorbents

The use of microorganisms as biosorbents for heavy metals offers a potential alternative to existing methods for detoxification and recovery of these components from industrial wastewaters and is subject of extensive studies. Such industrial process can serve as an economical and constant supply source of biomass for use as an adsorbent material for removal of heavy metal ions from wastewater. The special surface properties of microorganisms enable them to adsorb heavy metal ions from solutions. This passive bioaccumulation process (biosorption) has distinct advantages over the conventional methods: the process does not produce chemical sludges (i.e. nonpolluting), it could be highly selective, more efficient, easy to operate and hence cost effective for the treatment of large volumes of wastewaters containing low pollutant concentrations. Industrial applications of biosorption often make use of dead biomass, which does not require nutrients and can be exposed to environments of high toxicity (Strotmann *et al.*, 1992).

1.6 Objectives and Scope of this Study

Objectives of the proposed study can be summarized as follows:

1. To determine biosorption capacity of different waste sludges obtained from different wastewater treatment plants for Cu(II) ion removal. Selection of the most suitable sludge.

2. To examine the effects of various pre-treatment methods on biosorption of Cu(II) ions by different waste sludges. Selection of the most suitable pre-treatment method.

3. To investigate the effects of environmental parameters on batch biosorption of Cu(II) ions. Effects of the following parameters would be investigated:

   - pH and zeta potential
   - Cu(II) ion concentration
   - Powdered waste sludge (PWS) concentration
   - Surface area or particle size of the PWS
   - Temperature
   - Agitation speed (rpm)

4. To determine the effects of operating parameters such as pH, temperature, Cu(II) ion and the adsorbent concentrations and particle size on batch biosorption kinetics of copper (II) ions onto pre-treated powdered waste sludge (PWS).

5. To establish the adsorption isotherms for Cu(II) adsorption onto PWS and to determine the most suitable isotherm and the constants

6. To investigate the effects of operating parameters on the performance of a fed-batch operated biosorption system and to determine the biosorption

capacity of PWS and the rate constant for Cu(II) ion biosorption by using the modified Bohart-Adams equation. Following parameters will be investigated:

- Effect of feed flow rate
- Effect of feed copper (II) ion concentrations
- Effect of the amount of adsorbent (PWS)

7. To investigate the adverse effects of Cu(II) ions on performance of an activated sludge unit treating synthetic wastewater containing Cu(II) and to determine the COD, Cu(II), toxicity removals.

8. To investigate the effects of hydraulic residence time (HRT) and the sludge age (solids retention time, SRT) on the performance of an activated sludge unit treating synthetic wastewater containing Cu (II) ions.

9. To develop a mathematical model describing the Cu (II) ion inhibition on COD removal in an activated sludge unit.

10. To develop a new operational approach for operation of activated sludge with removal of Cu(II) ions by biosorption before biological treatment

11. To investigate Cu(II), chemical oxygen demand (COD) and toxicity removal performance of the activated sludge unit under different operating conditions using the modified Box-Behnken experimental design method.

12. To develop suitable mathematical models for biosorption of Cu(II) ions onto PWS in batch, fed-batch and continuous activated sludge systems and to determine the model constants.

CHAPTER TWO
LITERATURE REVIEW

A number of studies were reported in literature for the removal of heavy metals from wastewater by biosorption. Major studies may be summarized as follows:

Su M., Cha D. & Anderson P. (1995) compared the ability of metal removal using an aerobic selector activated sludge system and a conventional CSTR system. Heavy metals studied in this research were zinc, cadmium and nickel. Results of experimental data revealed that metal biosorption by activated sludge was rapid and about 70% of the soluble metals in solution was removed during the first 30 min. Metal biosorption behavior closely followed a Freundlich isotherm model for equilibrium concentrations above 0.05 mg l^{-1}. Results of the Freundlich model suggested that the adsorption capacity of sludge from the aerobic selector was significantly higher than that of the CSTR system. (Su M., Cha D. & Anderson P., 1995).

Byerley J. & Scharer M. (1997) examined Uranium(VI) biosorption from process liquor by *Streptomyces levoris, Rhizopus arrhizus,* mixed culture (activated sludge), *Saccharomyces cerevisiae and Chlorella vulgaris.* A Langmuir-type isotherm adequately described the sorption equilibrium data. At pH 5 and 20 °C, the maximum equilibrium sorption capacities ranged from 146 mg U^{VI} g^{-1} (dry weight) biomass for the mixed culture to 240 mg U^{VI} g^{-1} (dry weight) biomass for *Rhizopus arrhizus.* Thermal inactivation had only a marginal effect on the sorption equilibrium. Increasing the sorption temperature from 4 °C to 35 °C, however, enhanced biosorption by 40% to 90% for both inactivated and viable cells (Byerley J. & Scharer M., 1997).

Bakkaloglu I. *et al.* (1998) developed an innovative heavy metal removal process composed of biosorption, sedimentation and electrolysis. This study covers the comparison of various types of waste biomass including bacteria (*S. rimosus*), yeast (*S. cerevisiae*), fungi (*P.chrysogenum*) and activated sludge as well as marine algae (*F.vesiculosus* and *A.nodosum*), for their efficacy in the biosorption, sedimentation and desorption stages in the removal of zinc, copper and nickel ions. In the biosorption studies carried out with single metal solutions, *A. nodosum, S.rimosus* and *F.vesiculosus* proved to be the best biosorbents for zinc, copper and nickel ions respectively. The sedimentation efficiencies were proportional to the biosorption efficiencies. Desorptions were not effective, hence recycling did not yield good results. Overall, among the biomass tested, *A.nodosum, S.rimosus, F.vesiculosus* and *P. chrysogenum* had the highest potential for use in the heavy metal removal process. (Bakkaloglu I. *et al.*, 1998).

Yetis Ü. *et al.* (1998) determined heavy metal biosorption potentials of two white-rot fungi, *Polyporous versicolor* and *Phanarochaete chrysosporium.* It was found that both *P. versicolor* and *P. chrysosporium* were the most effective in removing Pb(II) from aqeous solutions with maximum biosorption capacities of 57.5 and 110 mg Pb(II)g^{-1} dry biomass, respectively. With *P. versicolor*, the adsorptive capacity order was determined to be Pb(II)>Ni(II)>Cr(III)>Cd(II)>Cu(II) whereas the order was Pb(II)>Cr(III)>Cu(II)>Cd(II)>Ni(II) with *P. chrysosporium.* As a general trend, metal removal efficiency with these fungi decreased as the initial metal ion concentration increased (Yetis Ü. *et al.*, 1998).

Bux F., Atkinson B. & Kasan H. C (1999) investigated the biosorptive capacity of two waste products of the wastewater treatment industry i.e., waste activated and waste digested sludge. Surface charge of each was determined in order to relate electronegativity with biosorptive potential. Activated sludge was found to be more effective than digested sludge for removal of zinc from a metal plating effluent, viz., 5.9 mg Zn (g sludge)$^{-1}$ as opposed to 4.0 mg g^{-1}, respectively, as well as producing a higher net negative charge. It was also noted that as initial zinc concentrations in solution increased there was a concomitant increase in sludge biosorption capacity (Bux F., Atkinson B. & Kasan H. C., 1999).

Aksu Z. *et al.* (1999) investigated the ability of the dried activated sludge to bind phenol and nickel (II) ions simultaneously from phenol–nickel (II) bearing solution and the results were compared with single component adsorption. The optimum initial biosorption pH was determined as 4.5 for nickel(II) ions and as 1.0 for phenol. Multi-component biosorption studies were performed at these two initial pH values. It was observed that the equilibrium uptakes of phenol and nickel(II) ions were changed due to the initial biosorption pH and the presence of other components (Aksu Z. *et al.*,1999).

Utgikar V., *et al.* (2000) studied the equilibrium biosorption of Zn(II) and Cu(II) by nonviable activated sludge in a packed column adsorber. Equilibrium metal uptakes from solutions containing single metal ion were 2.5 mg g(dry biomass)$^{-1}$ and 3.4 mg g(dry biomass)$^{-1}$ for Zn(II), and 1.9 mg g(dry biomass)$^{-1}$ and 5.9 mg g(dry biomass)$^{-1}$ for Cu(II) at pH 3.0 and 3.8, respectively. In binary mixture studies with Cu(II) and Zn(II), equilibrium was reduced by about 30% for each metal, indicating some competition between the two metals with Cu(II) preferentially adsorbing to achieve a higher equilibrium uptake of 1.93 mg g(dry biomass)$^{-1}$ [0.03 mmol g(dry biomass)$^{-1}$] as compared to 1.5 mg g(dry biomass)$^{-1}$ [0.02 mmol g(dry biomass)$^{-1}$] for Zn(II). Results of this study shows that non-viable biomass, a waste product of wastewater treatment plants, can be used as a biosorbent for the treatment of acid mine drainage. (Utgikar V., *et al.* 2000).

Sag Y., Yalcuk A. & Kutsal T. (2000) studied the biosorption of Pb(II), Ni(II) and Cu(II), in single component, binary and ternary systems using *R. arrhizus* in continuous-flow stirred-tank contactor (CFST). The continuous system mass balance was written for single and multi-metal solutions and batch system mass balance was written for the solid phase and was solved simultaneously. The relative capacities in the ternary metal mixtures were in the order Pb(II)>Ni(II) >Cu(II), in agreement with the single and dual component data. At higher metal ion concentrations the driving force in CFST is greater, in comparison with batch reactor, forcing the biosorbent to adsorb greater amounts of metal ions. For that reason, in CSTR higher concentrations of multi-metal mixtures can be studied and higher biosorption yields can be obtained. For the treatment of large volumes of industrial wastewaters, continuous treatment was recommended (Sag Y., Yalcuk A. & Kutsal T. 2000)

Sağ Y. & Kutsal T (2000) studied the biosorption of Fe(III), Cr(VI), Pb(II), Cu(II) and Ni(II) ions on *Zoogloea ramigera* (activated sludge bacterium) and *Rhizopus arrhizus* (filamentous fungus) as a function of temperature and initial metal ion concentration. Langmuir adsorption isotherms obtained at different temperatures for each metal–microorganism system were used. Binding capacities for Fe(III), Cr(VI) and Pb(II) of *Z. ramigera* and *R. arrhizus* increased as the temperature increased from 15 to 45°C indicating that this biosorption was endothermic. Maximum equilibrium uptake of Cu(II) and Ni(II) ions by *Z. ramigera* and *R. arrhizus* were obtained at 25°C, then the amount of adsorption decreased rapidly as the temperature increased from 25 to 45°C. The biosorption of Cu(II) and Ni(II) ions by *Z. ramigera* and *R. arrhizus* has been determined to be exothermic (Sağ Y. & Kutsal T., 2000).

Aksu Z & Akpinar D (2001) investigated the ability of dried anaerobic activated sludge to adsorb phenol and chromium(VI) ions in a batch system. The effects of initial pH and single- and dual-component concentrations on the equilibrium uptakes were studied. The optimum initial biosorption pH for both chromium(VI) ions and phenol was determined as 1.0. It was observed that the equilibrium uptakes of phenol and chromium(VI) ions changed due to the presence of other component. The mono-component adsorption equilibrium data fitted very well to the non-competitive Freundlich and Redlich–Peterson models for both the components. The results showed that the cells of dried anaerobic activated sludge bacteria may find promising applications for simultaneous removal and separation of phenol and chromium(VI) ions from aqueous effluents (Aksu Z & Akpinar D, 2001).

Say R., Denizli A. & Arıca M (2001) studied the biosorption of heavy metals (Cd(II), Pb(II) and Cu(II) from artificial wastewaters of onto the dry fungal biomass of *Phanerochaete chryosporium* in the concentration range of 5±500 mg l^{-1}. The maximum absorption of different heavy metal ions on the fungal biomass was obtained at pH 6.0 and the biosorption equilibrium was established after about 6 h. The experimental biosorption data for Cd(II), Pb(II) and Cu(II) ions were in good agreement with those calculated by the Langmuir model (Say R., Denizli A. & Arıca M., 2001).

Gabriel J. *et al.* (2001) investigated the biosorption of copper to the pellets of different wood-rotting fungal species. Copper sorption was studied in both batch and column arrangements. The optimum pH for copper sorption was between 3.5 and 4. In 100 mg l^{-1} Cu (II), maximum q_e values were found for *Oudemansiella mucida* (8.77 mg g^{-1} dry wt), *Lepista nuda* (6.29 mg g^{-1}), *Pycnoporus cinnabarinus* (5.08 mg g^{-1}) and *Pleurotus ostreatus* (4.77 mg g^{-1}). Both biomass yield and specific sorption were influenced by the composition of the fermentation broth. The results of column experiments showed that mycelial pellets of wood-rotting fungi can be used as promising biosorbent material for copper removal (Gabriel J. *et al.*, 2001).

Liu Y. *et al.*(2002) evaluated the effects of initial Zn(II) and aerobic granule concentrations on the kinetics of Zn(II) biosorption on aerobic granule surfaces. Acetate-fed aerobic granules with a mean diameter of 1. mm were used as biosorbents. Results showed that the kinetics of Zn(II) biosorption on the aerobic granule surfaces were related to both initial Zn(II) and granule concentrations. The maximum biosorption capacity of Zn(II) by aerobic granules was 270 mg g^{-1} (Liu Y. *et al.*, 2002).

Gourdon R., Diard P. & Funtowicz N (2002) designed an automated bench-scale countercurrent biosorption system (CBS) for the removal of metals from aqueous effluents. The system has been tested with activated sludge microorganisms as a biosorbent and lead and copper as model metals. Nearly 5 1 of a lead nitrate solution at 100 mg l^{-1} of lead have been treated down to a final concentration of 0.1 mg l^{-1} (99.9% removal) by using 4.8 g of dry biosorbent. Under similar conditions, copper chloride solutions at 100 mg 1^{-1} of copper were treated down to a final concentration of 35–45 mg l^{-l} representing 60% removal (Gourdon R., Diard P. & Funtowicz N., 2002).

In a study by Arican B., Gokcay C. & Yetis U(2002), the effect of sludge age on Ni^{2+} removal characteristics was investigated by using biomass from activated sludge reactors operating at different dilution rates (0.09, 0.16, and 0.24 h^{-1}) by batch adsorption tests. The kinetic studies have indicated that sludge grown at all dilution rates, exhibits both active and passive uptake of Ni^{2+}. The data were evaluated in the form of adsorption isotherms. Linear adsorption isotherms were obtained at all dilution rates, indicating the presence of equilibrium between biomass and the free nickel species (Arican B., Gokcay C. and Yetis U, 2002).

Hammaini A., *et al.*(2002) investigated the effect of the presence of Pb on the biosorption efficiency of activated sludge for Cu, Cd and Zn. Simple isotherm curves had to be replaced by three-dimensional sorption isotherm surfaces, to evaluate the two-metal sorption system efficiency. In order to describe the isotherm surfaces mathematically, three Langmuir-type models were evaluated. The isotherms indicated a competitive uptake of the different metals with Pb that was preferentially adsorbed (Hammaini A., *et al.* 2002).

Aksu Z., Gönen F. and Demircan Z (2002) examined the potential use of Mowital®B30H resin immobilized dried activated sludge as a substitute for granular activated carbon for removing chromium(VI) in a continuous packed bed column. The effect of operating parameters (flow rate and inlet metal ion concentration) was investigated on the sorption characteristics of each sorbent. From the batch system studies the optimal sorption pH value was determined as 1.0 for both sorbents and therefore, the packed bed sorption studies were performed at this pH value. The total amount of sorbed chromium (VI) and equilibrium chromium (VI) uptake decreased with increasing flow rate and increased with increasing inlet chromium (VI) concentration for both immobilized dried activated sludge and granular activated carbon systems. The suitability of the Freundlich and Langmuir adsorption models to the column equilibrium data was also investigated for each chromium (VI)-sorbent system. The results showed that the equilibrium data for both the sorbents fitted the Langmuir model best within the concentration range studied (Aksu Z., Gönen F. and Demircan Z., 2002).

Aksu Z. *et al.*(2002) studied biosorption of chromium (VI) and nickel (II) ions, both singly and in combination, by dried activated sludge in a batch system as a function of initial pH and single-and dual-metal ion concentrations. The optimal initial pH values for single chromium (VI) and nickel (II) biosorptions were determined as 1.0 and 4.5, respectively. Adsorption isotherms were developed for both the single- and dual-metal ion systems at these two pH values and expressed by the mono- and multi-component Langmuir and Freundlich adsorption models and model parameters were estimated by the non-linear regression. It was concluded that multi-component Freundlich model agreed well with the experimental results for the initial concentration range tested for both studied pH values (Aksu Z. *et al.*, 2002).

Dias M.A. *et al.*(2002) investigated the biosorption of chromium, nickel and iron from metallurgical effluents, produced by a steel foundry, using a strain of

Aspergillus terreus immobilized in polyurethane foam. *A. terreus* UFMG-F01 was immobilized in polyurethane foam and subjected to biosorption tests with metallurgical effluents. Maximal metal uptake values of 164.5 mg g^{-1} iron, 96.5 mg g^{-1} chromium and 19.6 mg g^{-1} nickel were obtained in a culture medium containing 100% of effluent stream supplemented with 1% of glucose, after 6 d of incubation. In this work, a strain of *A. terreus* was successfully used as a metal biosorbent for the treatment of metallurgical effluents (Dias M.A. *et al.*, 2002).

Lui Y. *et al.*(2003) studied the feasibility of aerobic granules as a novel type of biosorbent, for cadmium removal from industrial wastewater. Batch tests were carried out at different initial Cd^{2+} and granule concentrations. Based on experimental data, a kinetic model was developed to describe Cd^{2+} biosorption by aerobic granules. Results showed that the Cd^{2+} biosorption on aerobic granule surface was closely related to both initial Cd^{2+} and granule concentrations. The maximum biosorption capacity of Cd^{2+} by aerobic granules was 566 mg g^{-1}. This study for the first time shows that aerobic granules have a high biosorption capacity to Cd^{2+} and can be used as an effective biosorbent for the removal of cadmium or other types of heavy metals from industrial wastewater (Liu Y., 2003).

Sağ Y. *et al.*(2003) investigated the biosorption of Pb(II) and Cu(II) ions in single component and binary systems using activated sludge as biosorbent in batch and continuous-flow stirred tank reactors. The activated sludge in three different phases of the growth period was used in biosorption experiments: growing cells; resting cells; dead or dried cells. The Freundlich model described the experimental equilibrium uptake of Pb(II) and Cu(II) ions by the resting activated sludge better than the Langmuir model. Using a mathematical model based on continuous system mass balance for the liquid phase and batch system mass balance for the solid phase, the forward rate constants for biosorption of Pb(II) and Cu(II) ions were 0.793 and 0.242 l $(\text{mmol min})^{-1}$, respectively (Sağ Y. *et al.*, 2003).

Ozdemir, G., *et al.*(2003) investigated the removal of chromium, cadmium and copper ions applying a dead exopolysaccharide producing bacterium, *Ochrobactrum anthropi*, isolated from activated sludge. Optimum pH values of chromium(VI), cadmium(II) and copper(II) were 2.0, 8.0 and 3.0 respectively. Both the Freundlich and Langmuir adsorption models were suitable for describing the short-term biosorption of chromium(VI), cadmium(II) and copper(II) by *O. anthropi*. (Ozdemir, G., *et al.*, 2003).

Hammaini A., *et al.* (2003) investigated the adsorption of three metal ions, Cu, Cd and Zn, in a bicomponent mixture system by activated sludge. In order to describe isotherm surfaces mathematically, three empirical models were used. The isotherms indicated a competitive uptake with Cu being preferentially adsorbed followed by Cd and Zn. For instance, at C_f[Cd]=0.9 mmol l^{-1} and with 0.5 and 2 mmol l^{-1} Cu in the system, the Cd uptake was 0.17 and 0.08 mmol/g, respectively. Therefore, a 53% decrease of Cd biosorption was observed when Cu was present at the highest concentration. On the other hand, 0.9 mmol/l Cd caused a reduction of 54% on the Cu uptake [q(Cu)=0.28 mmol g^{-1}] in comparison with the results obtained when Cd was present at 0.225 mmol l^{-1} [q(Cu)=0.13 mmol g^{-1}]. A 83% of the total metal uptake was due to Cu when the residual concentrations of Cu and Zn were the same (e.g., either 0.5 or 1 mmol l^{-1} each one). On the other hand, when the residual concentrations of Cd and Zn were the same (e.g., either 0.5 or 1.5 mmol l^{-1} each one), about 71% of the total metal uptake was due to Cd uptake (Hammaini A., *et al.*, 2003).

Galli E. *et al.* (2003) determined the optimum conditions for copper (Cu) biosorption by *Auricularia polytricha* mycelium in view of its immobilization in polyvinyl alcohol (PVA). The adsorption of Cu(II) onto *A. polytricha* was studied in batch with respect to initial pH, temperature, adsorption time, initial metal ion and biomass concentration. At optimal adsorption conditions, biomass was immobilized in PVA in column and a biosorption capacity of about 90% was obtained indicating that. *Auricularia polytricha* strain could successfully be used as Cu biosorbent (Galli E. *et al.*, 2003).

Volesky B., Weber J. and Park J.M. (2003) investigated metal biosorption behavior of raw seaweed *S. filipendula* in ten consecutive sorption–desorption cycles in a packed-bed flow-through column during a continuous removal of copper from a 35 mgl^{-1} aqueous solution at pH 5. The elutant used was a 1% (w/v) $CaCl_2$/HCl solution at pH 3. The sorption and desorption was carried out for an average of 85 and 15 h. The weight loss of biomass after this time was 21.6%. The Cu biosorption capacity of the biomass 38mg Cu/g based on the initial dry weight. The column service time, considered up to 1 mg Cu l^{-1} in the effluent, decreased continuously from 25.4 h for the first to 12.7 h for the last cycle. Regeneration with $CaCl_2$/HCl at pH 3 provided elution efficiencies up to 100% (Volesky B., Weber J. and Park J.M. 2003).

A biomatrix was prepared from rice husk, a lignocellulosic waste from agro-industry, for the removal of several heavy metals as a function of pH and metal concentrations in single and mixed solutions. The ultimate maximum adsorption capacity obtained from the Langmuir isotherm increases in the order (mmol g^{-1}): Ni (0.094), Zn (0.124), Cd (0.149), Mn (0.151), Co (0.162), Cu (0.172), Hg (0.18) and Pb (0.28) (Krishnani K. *et al.*, 2008).

Bhainsa K. and D’Souza S. investigated the removal of copper ion using NaOH treated *Rhizopus oryzae* biomass in a batch reactor. The copper biosorption by viable and pretreated fungal biomass fit well to a Lagergren’s pseudo-second order reaction in comparison to pseudo-first order kinetics. Investigation on effect of pH indicated improved performance in the range of pH 4-6 in alkali treated biomass. Copper uptake exhibited by viable biomass was highest at 21 °C, unlike pretreated biomass that showed maximum uptake across the range of temperature 21-55 °C. The maximum copper loading capacity of the viable and pretreated biomass according to Langmuir isotherm was 19.4 and 43.7 mg g^{-1}, respectively. Copper uptake decreased with an increasing dose of biosorbent, although enhancement in the total metal ion removal was observed at higher dose (Bhainsa K. and D’Souza S. 2008)

Three biomass, birch wood *Betula* sp., marine brown alga *Fucus vesiculosus,* and terrestrial moss *Pleurozium schreberi,* have been compared as raw materials for preparation of biosorbents for removal of copper ions from diluted water solutions. Small sample doses (0.5 g/100 ml) of the biosorbents prepared from alga and moss enabled more than 90% removal of Cu(II) ions from diluted water solutions (5-20 mg l^{-1}). A pseudo-second-order rate model properly described the experimental kinetic data for the biosorbents. The maximum sorption capacities (X_m) determined from the experimental equilibrium isotherms by applying the Langmuir model showed that the alga had the best copper-binding ability (X_m = 23.4 mg g^{-1}), followed by the moss (X_m =11.1 mg g^{-1}), and the sawdust (X_m = 4.9 mg g^{-1}) (Grimm A. *et al.*, 2008).

Apiratikul R.and Pavasant P. investigated the biosorption of Cu(II), Cd(II), and Pb(II) by a dried green macroalga Caulerpa lentillifera. The maximum sorption capacities of the various metal components on C. lentillifera biomass could be prioritized in order from high to low as: Pb(II) > Cu(II) > Cd(II). The sorption energies obtained from the Dubinin-Radushkevich model for all sorption systems were in the range of 4-6 kJ mol^{-1} indicating that a physical electrostatic force was potentially involved in the sorption process. Thomas model could well describe the breakthrough data from column experiments. Ca(II), Mg(II), and Mn(II) were the major ions released from the algal biomass during the sorption which revealed that ion exchange was one of the main sorption mechanisms (Apiratikul R.and Pavasant P., 2008).

Marin A. *et al.* studied the biosorption of several metals (Cd(II), Zn(II) and Pb(II)) by orange wastes in binary systems. Experimental sorption data were analysed using an extended multicomponent Langmuir equation. The maximum sorption uptake was approximately 0.25 mmol g^{-1} for the three binary systems studied. The reliability of the proposed procedure for obtaining the equilibrium data in binary systems was verified by means of a statistical F-test (Marin A. *et al.*, 2008).

In none of the literature studies, biosorption of Cu(II) ions on activated sludges from different treatment plants (domestic, industrial and municipal wastewater) was studied extensively. Also, the effects of different pre-treatment methods on biosorption characteristics of different powdered waste sludge (PWS) samples were not investigated. For this reason, the major objective of the first part of thesis was to determine the most suitable PWS and the pre-treatment method maximizing the biosorption of copper (II) ions and to investigate the effects of particle size (or specific external surface area), biosorbent and copper ion concentrations and also the pH on the rate and extent of copper ion biosorption on pre-treated PWS.

Most of the reports on biosorption of metal ions were on the isotherm studies with limited information on the kinetic analysis. In none of the literature studies the kinetics of biosorption of Cu(II) ions onto pre-treated powdered waste sludge (PWS) was investigated as a function of operating parameters such as pH, temperature, Cu(II) and adsorbent concentrations and the particle size. Therefore, the another objective of the first part of thesis was to study the kinetics of Cu(II) biosorption on pre-treated PWS at different operating conditions and to determine the most suitable kinetic model. Variations of the kinetic constants with the operating conditions were also quantified and the most suitable operating conditions maximizing the rate and the extent of Cu(II) biosorption were determined.

Limited number of studies was reported on adsorption operations in continuous stirred tank reactors (CSTR) which may result in loss of adsorbent by elutriation due to lack of sedimentation phase during the operation. No reports were found in literature on the use of fed-batch reactors for adsorption with a sedimentation period. Therefore, one of the objectives of the second part of thesis was to investigate the biosorption of Cu(II) ions onto pre-treated powdered waste sludge (PWS) in a completely mixed reactor operating in fed-batch mode. The performance of the system was evaluated by operation under different experimental conditions such as variable feed flow rate, feed copper ion concentrations and the amount of adsorbent in the reactor. The data were used to establish the breakthrough curves where breakthrough times were determined to reach a certain effluent adsorbate (copper) concentration. A modified Bohart-Adams equation was used to estimate the adsorption capacity of PWS and the rate constant.

Most of the literature studies on biosorption of heavy metal ions were performed using batch or continuous systems and quantified the metal ion toxicity on microorganisms by enzyme inhibition, respiration activity and biomass yields. Toxic effects of Cu(II) on COD removal, biomass yield and sludge settling characteristics (SVI) in an activated sludge unit were not investigated, modelled and reported in literature. Therefore, the objective of the third part of this thesis was to investigate the performance of an activated sludge unit treating Cu(II) containing synthetic wastewater to quantify toxic effects of Cu(II) and also to develop a mathematical model describing the Cu(II) inhibition on COD removal rate.

There was no sound mathematical model describing the inhibitory effects of metal ions on performance of activated sludge units. Therefore, another objective of the third part of thesis was to develop a sound mathematical model describing the Cu(II) ion inhibition on COD removal in an activated sludge unit treating Cu(II) containing synthetic wastewater.

Removal of heavy metals from wastewater by biosorption has been studied extensively by many investigators (Strotman *et al.*, 1992; Aksu *et al.*, 2002; Liu *et al.*, 2003; Sag *et al.*, 2003; Kargi and Cikla, 2006; Pamukoglu and Kargi, 2006). Studies on integration of biosorption processes into biological treatment systems for heavy metal ion removal before treatment are very limited (Pamukoglu and Kargi, 2007c). Therefore, the objective of the fourth part of thesis was to investigate the utilization of pre-treated powdered waste sludge (PWS) for removal of Cu (II) ions from wastewater by biosorption before biological treatment in order to improve the performance of an activated sludge unit.

One of the major practical objectives of the study was to investigate the utilization of pre-treated powdered waste sludge (PWS) for removal of Cu (II) ions from wastewater by biosorption in an activated sludge unit under different operating conditions. A Box-Behnken experimental design method was used by considering the operating parameters such as sludge age, hydraulic retention time, feed Cu(II) concentration and PWS loading rate as the independent variables while percent Cu(II), chemical oxygen demand (COD), toxicity (TOX) removals and SVI values were the objective functions. The operating conditions maximizing Cu(II) , COD and toxicity removals were determined.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Experimental System

3.1.1 Batch Shake Flask Experiments

Batch shake flask experiments were performed using a gyratory shaker at 150 rpm and room temperature (25^{o}C). Erlenmeyer flasks of 500 ml were charged with 200 ml of tap water. The flasks were incubated in a gyratory shaker (Gallenkamp) at 25 ^{o}C for 24 hours.

In variable particle size experiments, copper ions (in form of $CuSO_4$) and PWS of different particle sizes (53-338µm) were added to the flasks to yield 100 mg l^{-1} Cu(II) ions and 1 g l^{-1} PWS in the solution and the initial pH was adjusted to 5. In experiments with variable copper ion concentrations, PWS was 1 g l^{-1} with a particle size of 64 µm at pH =5 while copper ion concentrations were varied between 50 and 400 mg l^{-1}. Powdered activated sludge (PWS) concentration was varied between 0.25 and 3 g l^{-1} in experiments with variable PWS concentrations while initial copper ion concentration was 200 mg l^{-1} at pH =5 with PWS particle size of 64 µm. Finally in variable pH experiments, the PWS concentration was 1 g l^{-1} with a particle size of 109 µm and copper ion was 100 mg l^{-1} while pH was varied between 2 and 6. In variable pH experiments pH was adjusted to 2 to 6 by using dilute H_2SO_4 and NaOH solutions.

Samples were removed from the flaks every hour for analysis. A control flask free of PWS with 100 mg l^{-1} Cu(II) ions was used to determine the extent of non-adsorptive copper removal from the solution.

3.1.2 Experiments with Fed–Batch Operation

The experimental system is depicted in Figure 3.1 which consisted of a feed reservoir, a feed pump and a completely mixed reactor placed on a magnetic stirrer. The adsorption reactor was made of plexiglass with 15 cm diameter and 30 cm height with a total volume of 5.3 l. The feed reservoir contained the copper ion solution at pH =5 to avoid copper ion precipitation which was fed to the reactor with a desired flow rate while the reactor containing the adsorbent (PWS) was mixed. The feed flow was stopped when the reactor was full and the reactor contents were allowed to settle for one hour. After the clear supernatant was removed a new fed-batch cycle was started with a fresh adsorbent solution.

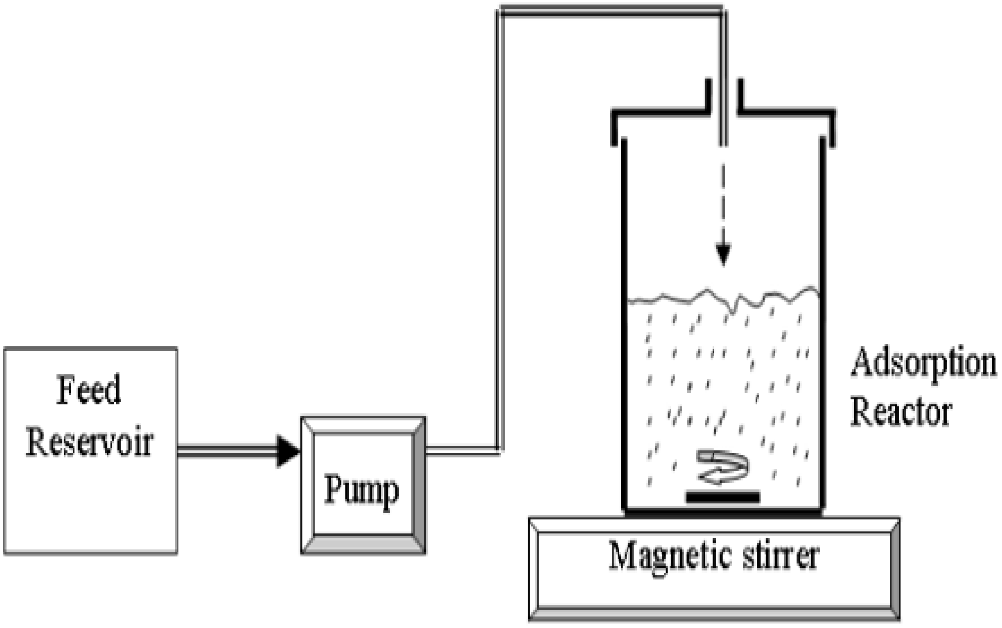


Figure 3.1 A schematic of the experimental set-up used in fed-batch adsorption experiments

3.1.3 Activated Sludge Experiments

The laboratory scale activated sludge unit used throughout the study consisted of an aeration tank of volume 8.5 l and a sludge settling tank of 1.5 l made of stainless steel. A schematic diagram of the experimental setup is depicted in Figure 3.2. The aeration and sludge settling tanks were separated by an inclined plate which allowed passage of the wastewater from the aeration to the settling tank through the holes on the plate. The inclined plate had a 3 cm gap at the bottom which allowed the passage of the settled sludge from the settling to the aeration tank. Aeration tank was vigorously aerated by using an air pump and several porous diffusers. Synthetic wastewater was kept in a deep refrigerator at 4 ^{o}C to avoid any decomposition and was fed to the aeration tank with a desired flow rate by a peristaltic pump (Watson-

Marlow 505 D_i / l). The effluent was removed from the top of the settling tank by gravitational flow. Temperature, pH and dissolved air (DO) concentrations in the aeration tanks were around T =25 ± 2 ^oC (room temperature), pH=7. ± 0.2 and DO = 2 ± 0.5 mg l^{-1}, respectively. Initial biomass concentration was adjusted nearly 4 g l^{-1}.

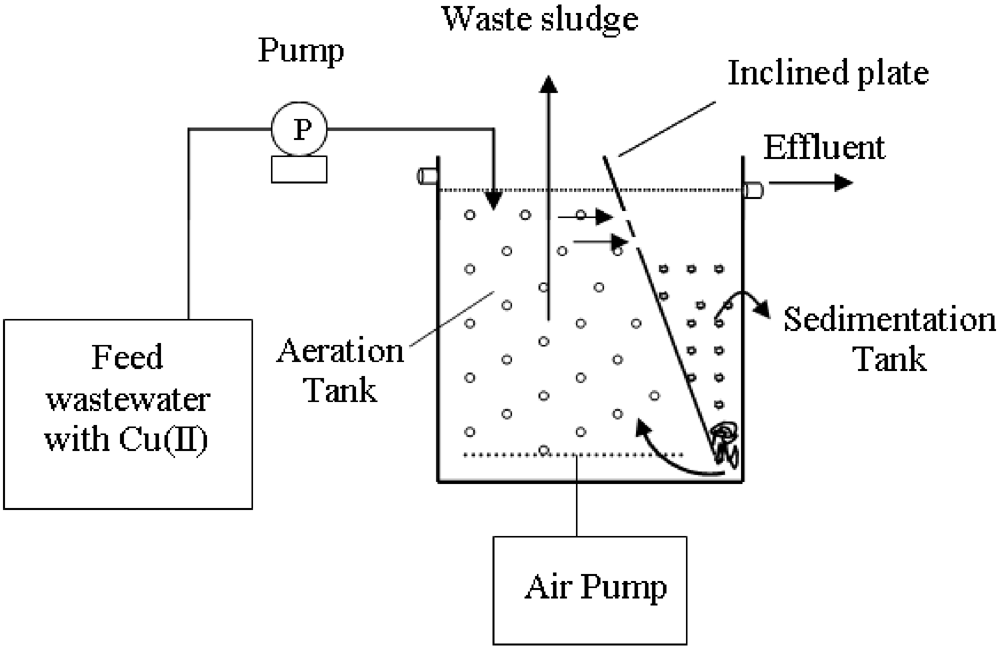


Figure 3.2 A schematic diagram of the experimental setup

3.1.4 Activated Sludge Experiments with PWS Addition

A schematic diagram of the experimental setup is depicted in Figure 3.3 which consisted of a feed reservoir, a mixing tank placed on a magnetic stirrer, a primary sedimentation tank and an activated sludge unit. The feed reservoir (50 l) was placed in a deep refrigerator at 4 ^oC to avoid any decomposition in the feed. The mixing tank placed on a magnetic stirrer was made of plexi-glass (3 l). The primary sedimentation tank was (1.5 l) was made of stainless steel. The activated sludge system consisted of an aeration tank of volume 8.5 liter and a sludge settling tank of 1.5 liter made of stainless steel. The aeration and sludge settling tanks were separated by an inclined plate which allowed passage of the wastewater from the aeration to the settling tank through the holes on the plate. The inclined plate had a 3 cm gap at the bottom which allowed the passage of the settled sludge from the settling to the aeration tank. The aeration tank was vigorously aerated (approx. 5 $vol.vol^{-1}min^{-1}$) by using an air pump and several porous diffusers. Synthetic wastewater was kept in a deep refrigerator at 4 ^{o}C to avoid any decomposition and was fed to the mixing tank with a desired flow rate (0.85 l h^{-1}) by a peristaltic pump (Watson- Marlow 505 D_i / l). Powdered waste sludge (PWS) was added automatically to the mixing tank with the aid if a feeder and timer with a desired loading rate (0.1-1.g PWS h^{-1}). Hydraulic residence time (HRT) in the mixing tank was 3.5 hours to allow adsorption of Cu (II) ions in the feed onto PWS. The effluent of the mixing tank was fed to the sedimentation tank where the PWS with adsorbed Cu (II) was separated from the feed wastewater by sedimentation (HRT_{ST} = 1.8 hour). Solids (PWS containing adsorbed Cu(II)) were removed from the bottom of the sedimentation tank everyday to avoid desorption of Cu(II) ions. The activated sludge unit was fed with the effluent from the top of the sedimentation tank (0.85 l h^{-1}) by gravitational flow. Temperature, pH and dissolved oxygen (DO) concentrations in the aeration tank were T =25 ± 2 ^{o}C, pH = 7. ± 0.2 and DO = 2 ± 0.5 mg l^{-1}. Initial biomass concentration was adjusted nearly 4 g l^{-1}.

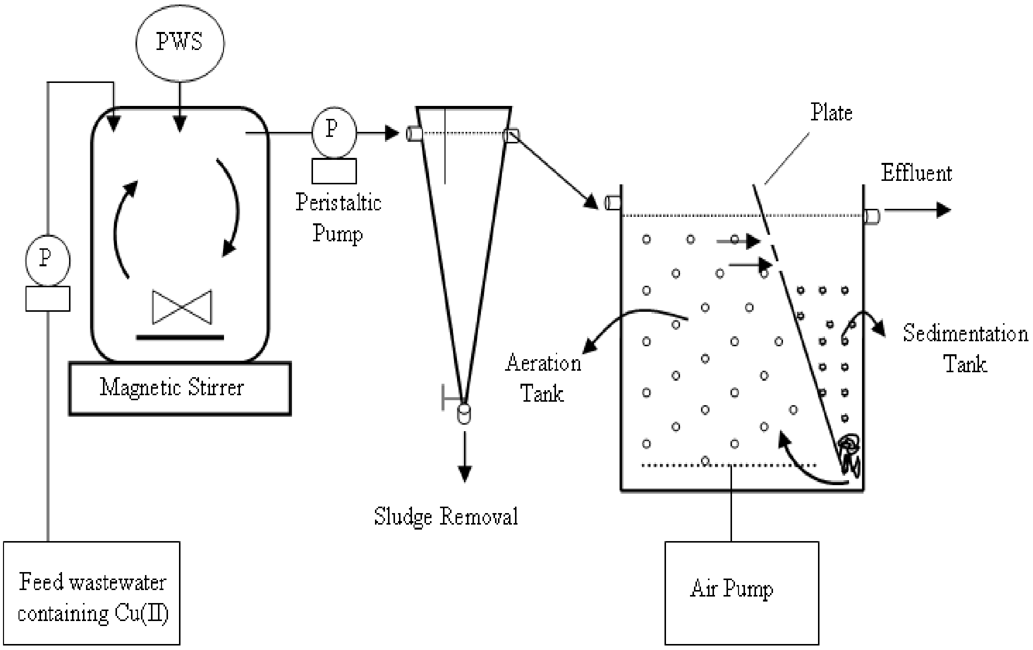


Figure 3.3 Schematic diagram of experimental system

3.2 Experimental Procedure

3.2.1 Batch Shake Flask Experiments

3.2.1.1 Selection of the Most Suitable PWS

Five activated sludge samples were obtained from different wastewater treatment plants. Two samples were from industrial treatment plants (PAK MAYA Bakers yeast and DYO paint companies). The other two samples were from domestic (Guzelbahce and Kisikkoy) and one sample from municipal wastewater treatment plant (Cigli) in Izmir, Turkey. The samples were dried at 80 ^{o}C until constant weight, ground and sieved below -70 mesh (D_p < 212 µm) before use in adsorption experiments. Waste activated sludge samples obtained from different wastewater treatment plants were tested for their Cu(II) ion biosorption capabilities without pre-treatment and the sludge obtained from DYO paint industry in Izmir, Turkey was found to be superior to the other sludges tested. The raw sludge from the paint industry contained 16 µg l^{-1} Cr, 58 µg l^{-1} Zn, 2 µg l^{-1} Cu, 1 µg l^{-1} Pb, 80 µg l^{-1} Fe, 20 µg l^{-1} Mn, and no detectable Ni and Cd with a density of 1.2 g cm^{-3}. The sludge was ground and pre-treated with 1% H_2O_2 solution in order to remove impurities from the PWS surface and activate the functional groups for binding metal ions. The sludge pre-treated with 1% H_20_2 had better biosorption capacity for Cu(II) ions as compared to the other pretreatment methods with 1% H_2SO_4, NaOH, NaOCl and ethanol. 200 ml 1% H_2O_2 solution was mixed with 2 g of PWS in an 500 ml erlenmeyer flask and placed on a gyratory shaker at 150 rpm and 25 ^{o}C for 6 hours for pre-treatment. Pre-treated PWS was washed with deionized water on a filter paper until the filtrate pH was neutral. Washed PWS was dried at 80 ^{o}C, reground and sieved by using sieve plates of sizes between 40 and 270 mesh to yield six different size fractions with average particle sizes between 53 and 338 µm. Only the size fraction between 200 and 270 mesh with an average particle size of 64 µm was used in kinetic and isotherm studies reported in this thesis. The BET surface area of dried sludge with a particle size of 64 µm was 69 m^2 g^{-1} after pre-treatment with 1% H_2O_2. Pre-treated and washed sludge did not release any copper ions in control experiments indicating no copper interference from the PWS. Pure $CuSO_4$ was used to adjust initial copper ion concentrations in solution to desired levels. Dilute sulfuric acid and NaOH (1%) solutions were used to adjust the initial pH to the desired levels.

3.2.2 Experiments with Fed–Batch Operation

Waste activated sludge samples from a paint industry wastewater treatment plant (DYO, Izmir, Turkey) was used after pre-treatment in fed-batch biosorption experiments since this sludge was found to be superior to the other sludge samples tested for biosorption of copper ions. The sludge was dried, ground and sieved to desired particle size to obtain the powdered waste sludge (PWS) samples. The PWS samples were pre-treated using five different pre-treatment solutions of 1% H_2SO_4, NaOH, ethanol, NaOCl and H_2O_2. A 200 ml of pre-treatment solution was mixed with 2 g of PWS in a 500 ml erlenmeyer flask and placed on a gyratory shaker at 150 rpm and 25 ^oC for 6 hours for pre-treatment. Pre-treated PWS was washed with deionized water on a filter paper until the filtrate pH was neutral. Pre-treatment with 1% H_2O_2 was found to be superior to the other methods yielding the highest biosorption capacity (65 mg Cu. g PWS^{-1}). Pre-treated and washed PWS samples were dried at 80 ^oC, reground and sieved to different mesh sizes. PWS samples with particle size of 64 µm were used in fed-batch adsorption experiments.

Fed-batch operation was used to provide an alternative to adsorption column operations. In fed-batch operation, the feed Cu(II) ion solution was fed to a completely mixed tank containing the adsorbent (PWS) with a desired flow rate and no effluents were removed until the tank was full. Liquid volume in the tank increased linearly with time with a constant feed flow rate. The PWS in the tank was allowed to settle for an hour at the end of the operation and the clear supernatant free of copper ions was removed from the tank. The fed-batch operation was repeated with a new PWS solution. A control tank free of PWS was operated under the same conditions to determine the copper ion concentrations in the absence of adsorbent. Difference between the copper ion concentrations in the control and the experimental tank was considered when calculating solid phase copper ion concentrations. The biosorbed copper ion concentrations in the adsorbent (solid) phase were determined by using the eqn 3.1.

$$q = \frac{V\,(C_c - C)}{m_{ads}} = \frac{(V_o + Q\,t)\,(C_c - C)}{m_{ads}} \qquad \text{Eqn 3.1}$$

where q is the biosorbed copper ion concentration in the solid phase (mg g^{-1}); V is the liquid volume in the adsorption tank which increased linearly with time (l); C_c and C are the zinc ion concentrations in the control and the adsorption reactors, respectively (mg l^{-1}); Q is the flow rate of the feed zinc ion solution (l h^{-1}); and m_{ads} is the mass of adsorbent in the adsorption reactor (g).

Three sets of experiments with different experimental conditions were performed. The first set of experiments was used to investigate the effects of the amount of adsorbent (which corresponds to the column height in adsorption column operations) on the performance of the system. The amount of adsorbent in the adsorption reactor was changed between 1 and 6 g while the feed flow rate and the copper ion concentration in the feed were constant at Q = 0.25 l h^{-1} and C_o = 200 mg l^{-1} in this set of experiments. Feed flow rate was changed between 0.075 and 0.325 l h^{-1} while the amount of adsorbent and the feed copper ion concentrations were constant at m_{ads} = 3 g and Co = 100 mg l^{-1} in the second set of experiments. The variable was the feed copper ion concentration in the third set of experiments which was varied between 50 and 300 mg l^{-1} while the feed flow rate and the amount of adsorbent were constant as Q = 0.1 l h^{-1} and m_{ads} = 3 g. Control experiments were performed under the same conditions as that of the adsorption experiments. Every experiment was operated until the adsorption reactor was full up to 5 litre.

Experiments were started by placing 1 litre tap water and desired amount of PWS in the adsorption reactor. The pH in the feed and the reactor was adjusted to pH= 5 at the beginning which remained almost constant during the operation (pH = 5 ± 0.2). The average particle size of PWS was 64 µm. Copper ion solution was fed to the reactor with a desired flow rate while the reactor contents were completely mixed by a magnetic stirrer. The operation was performed at the room temperature of T = 25 ± 2 ^{o}C.

3.2.3 Activated Sludge Experiments

About 7 liter of the synthetic wastewater was placed in the aeration tank and was inoculated with 1 liter of the activated sludge culture. The system was operated in batch mode for three days to obtain a dense culture of the activated sludge before starting the continuous operation. Feed wastewater was fed to the reactor with a desired flow rate and removed with the same rate. Sludge age (SRT) was kept constant at 10 days by removing 10% of the sludge from the aeration tank everyday. Hydraulic residence time was kept constant at 10 hours by adjusting the feed flow rate to 0.85 l h^{-1}. Experiments were performed in the order of increasing feed Cu(II) concentrations from 0 to 22 mg l^{-1}. In variable sludge age experiments, sludge age (SRT) was varied between 5 and 30 days while HRT was constant at 10 hours. Sludge age was adjusted by removing certain fraction of sludge from the aeration tank everyday using a syringe. For example, 10% of the sludge was removed from the aeration tank everyday to adjust the sludge age to 10 days. In variable hydraulic residence time (HRT) experiments, HRT was varied between 5 and 25 hours while the sludge age was constant at 10 days. Hydraulic residence times were adjusted to desired level by changing the flow rate of the feed wastewater. Feed COD and Cu (II) concentrations were constant at 2000±100 mg l^{-1} and 14 mg l^{-1}, respectively throghout the experiments. Experiments were performed in the order of increasing sludge age and hydraulic residence times. The same sets of experiments were performed with Cu (II)-free wastewater to determine the performance of the system in the absence of Cu (II) ions. Every experiment was continued until the system reached the steady-state yielding the same COD, Cu(II) and toxicity levels in the effluent for the last three days. Average time elapsed for each experiment was about fifteen days. Three experiments were repeated twice to test the reproducibility of the results. Due to good reproducibility of the repeated experiments, the other experiments were not repeated. The samples collected from the feed and effluent wastewater at the steady-state were analyzed for COD, Cu(II), biomass concentrations and also for toxicity by the resazurin method after centrifugation.

3.2.4 Activated Sludge Experiments with PWS Addition

Experiments were started as batch wise. About 7 liter of the synthetic wastewater was placed in the aeration tank and was inoculated with 1 liter of the activated sludge culture. The system was operated in batch mode for several days to obtain a dense activated sludge culture before starting the continuous operation. Feed wastewater was fed to the system with a desired flow rate and removed with the same rate. Temperature, pH and DO were approximately T = 25 ± 2 ^{o}C, pH = 7.0±0.2, DO = 2 ±0.5 mg l^{-1} throughout the experiments. Two sets of experiments were performed with the feed Cu (II) of 14 and 22 mg l^{-1}. The loading rate of PWS was varied between 0.1 and 0.6 g h^{-1} with the feed Cu (II) of 14 mg l^{-1} and between 0.2 and 1 g h^{-1} when the feed Cu (II) was 22 mg l^{-1}. The sludge age (SRT) and HRT was kept constant at 10 d and 10 h, respectively throughout the experiments. Sludge age was adjusted to 10 days by removing 10% of the sludge from the aeration tank everyday. Hydraulic residence time (HRT) in the aeration tank was kept at 10 hours by adjusting the feed flow rate to 0.85 l h^{-1}. Experiments were performed in the order of increasing PWS loading rate. Every experiment was continued until the system reached the steady-state yielding the same COD, Cu (II) and toxicity levels in the effluent for the last three days. Average time elapsed for each experiment was two to three weeks. Two experiments (the lowest and the highest PWS loadings) from each set were repeated twice to test the reproducibility of the results. Due to good reproducibility of the repeated experiments (standard deviations of less than 5%), the other experiments were not repeated. The samples collected from the feed and effluent of the mixing and sedimentation tanks and also from the activated sludge unit at the steady-state were analyzed for COD, Cu (II), biomass concentrations and also for toxicity by the resazurin method after centrifugation.

3.2.5 Box-Behnken Experiment Design

Experiments were started as batch wise. About 7 liter of the synthetic wastewater was placed in the aeration tank and was inoculated with 1 liter of the activated sludge culture. The system was operated in batch mode for several days to obtain a dense activated sludge culture before starting the continuous operation. Feed wastewater was fed to the system with a desired flow rate and was removed with the same rate. The hydraulic residence time (HRT) was adjusted to the desired level by adjusting the feed flow rate while the sludge age was adjusted by removing certain amount of sludge from the aeration tank everyday. For example, 10% of the sludge was removed everyday from the aeration tank to adjust the SRT (Θc) to 10 days. Temperature, pH and DO were approximately T = 25 ± 2 ^{o}C, pH = 7.0 ±0.2, DO = 2 ±0.5 mg l^{-1} throughout the experiments. The steady-state operation was assumed when the same effluent COD and Cu(II) concentrations were realized in the effluent for the last three days. Average time elapsed for each experiment was about three weeks to reach quasi steady-state. The samples collected from the feed and effluent of the mixing and sedimentation tanks and also from the activated sludge unit at the steady-state were analyzed for COD, Cu (II), biomass concentrations and also for toxicity by the resazurin assay after centrifugation.

3.3 Analytical Methods

3.3.1 Batch Shake Flask Experiments

The samples (5 ml) withdrawn from the erlenmeyers every hour were centrifuged at 8000 rpm (7000 g) to remove solids. The clear supernatants were analyzed for copper ion contents using an Atomic Absorption spectrometer (ATI Unicam 929 AA Spectrometer) at 324.8 nm wavelength. Zeta potentials on PWS particle surfaces were measured by using a Zeta-Meter System 3.0 (Zeta-Meter Inc, NY, USA).

3.3.1.1 Surface Area Measurement by Methylene Blue Method

Surface area was determined by methylene blue method as summarized below (Santamarina *et al.*, 2002).

- methylene blue (MB) solution was prepared by mixing 1.0 g of dry powder MB with 200 ml of deionized water

- 2.0 g of oven-dry MB specimen was mixed with 200 ml of deionized water

- the methylene blue (MB) solution was added to the PWS solution and was continually mixed for about 2 h, then the mixture was left overnight to reach absorption equilibrium and allow particle settlement

- 5 ml of the fluid sample was removed, placed in a test tube and was centrifuged

- the residual MB concentration was measured using a spectrometer (Aqualythic spectrophotometer, wavelength = 600 nm)

- more MB solution was added and the previous steps were repeated (for convenience, a set of suspensions can be prepared in advance)

- the amount of MB added was plotted versus the amount of absorbed MB and the point of complete cation replacement was identified

The specific surface area ($m^2\ g^{-1}$) was computed from the amount of absorbed MB at the optimum point using the Eqn 3.2;

$$S_s = \frac{m_{MB}}{319.87} A_v A_{MB} \frac{1}{m_s}$$

Eqn 3.2

where m_{MB} is the mass of the adsorbed MB at the point of complete cation replacement, and m_s is the mass of the PWS, A_v is Avogadro's number (6.02×10^{23} mol^{-1}), and A_{MB} is the area covered by one MB molecule (typically assumed to be 130 $Å^2$).

3.3.2 Experiments with Fed–Batch Operation

Samples (5 ml) were removed from the adsorption and the control reactor every hour which were centrifuged at 8000 rpm (7000g) to remove solids before analysis. Clear supernatants after centrifugation were analyzed for Cu(II) ion concentrations using an Atomic Absorption Spectrometer (ATI Unicam 929 AA Spectrometer) at a wavelength of 324.8 nm. Every sample was analyzed for three times to test the accuracy of the measurements.

3.3.3 Activated Sludge Experiments

Samples were withdrawn everyday for analysis and centrifuged at 8000 rpm (7000 g) for 20 minutes to remove biomass from the liquid phase. Clear supernatants were analyzed for COD and Cu (II) ion contents. Chemical oxygen demand (COD) was determined using the closed reflux method according to the Standard Methods (Greenberg AE *et al.*, 1989). Cu (II) ion concentrations in the clear supernatants were analyzed by using an Atomic Absorption Spectrometer (ATI Unicam 929 AA Spectrometer, Cambridge, UK) at 324.8 nm wavelength. Biomass concentrations were determined by filtering the samples through 0.45μ milipore filter and drying in an oven at 105 ^{o}C until constant weight. COD and Cu (II) analyses were carried out in triplicates with less than 3% standard deviations from the average. Sludge volume index (SVI) was determined by settling 1 litre sludge in an Imhoff cone for 30 minute and determining the settled biomass concentration (X_r) by filtering and drying. The SVI is calculated by using the relationship of SVI = 10^6 / X_r where X_r is in mg l^{-1} and SVI is ml g^{-1}.

Resazurin reduction method was used to determine the toxicity of the feed and effluent wastewater (Liu D., 1986 - Farre M. and Barcelo D., 2003). The test organisms (washed activated sludge) to be subjected to the toxic feed and effluent wastewater were cultivated on nutrient broth before using for determination of the toxicity of wastewater samples. The test cultures were transferred every day to new medium to keep the sludge age constant during the course of toxicity measurements. In the presence of active bacterial culture with dehydrogenase enzyme activity, resazurin changes color from blue to pink forming the reduced compound resorufin. Inactive bacteria do not cause any change in the color of resazurin and the color remains blue. Therefore, the color of the resazurin solution is an indicator of bacterial activity. A visible spectrometer was used to determine the color at 610 nm. Percent toxicity removal was calculated by using the following equation:

$$E = 1 - (TOX_e / TOX_o) \qquad \text{Eqn 3.3}$$

where TOX_e and TOX_o are the toxicities of the effluent and the feed wastewaters which were determined with respect to the test organisms dehydrogenase activity unexposed to Cu (II) ions.

3.3.4 Activated Sludge Experiments with PWS Addition

The same analytical methods were used as previous section (*3.3.3 Activated Sludge Experiments*)

3.3.5 Box Behnken Experiment Design

The same analytical methods were used as previous section (*3.3.3 Activated Sludge Experiments*)

3.4 Wastewater Composition

Synthetic wastewater composed of diluted molasses, urea, KH_2PO_4 and $MgSO_4$ with a COD/N/P ratio of 100/8/1.5 was used throughout the study. Typical composition of the feed wastewater was $COD_o = 2000 \pm 100$ mg l^{-1}, $N_T = 160 \pm 10$ mg l^{-1}, PO_4-P = 30 ± 1 mg l^{-1}, $MgSO_4$ = 50 mg l^{-1} and Cu(II) concentrations between 0 and 22 mg l^{-1}. Concentrated molasses was diluted by a factor of 500 to obtain dilute molasses with a COD content of nearly 2,000 mg l^{-1}. pH was nearly 6.9 in the feed wastewater which increased to pH>7.5 in the aeration tank because of ammonia released from biodegradation of urea. pH of the aeration tank content was adjusted manually to pH 7 by addition of dilute sulfuric acid several times a day.

3.5 Organisms

The activated sludge culture obtained from PAK MAYA Bakers Yeast Company wastewater treatment plant in Izmir, Turkey was used as the seed culture. The activated sludge culture was grown in the aeration tank using the same synthetic wastewater in the absence of Cu (II) ions.

CHAPTER FOUR
THEORETICAL BACKGROUND

4.1 Batch Biosorption Kinetics

During the adsorption period the concentration of adsorbate remaining in solution decreases until a dynamic equilibrium is reached. The time needed to reach the equilibrium is called equilibrium time. In the analysis of data obtained in biosorption of heavy metal ions, first and second order reaction rate models were used to describe the adsorption kinetics.

Lagergren (1898) developed a model for adsorption kinetics based on the first order reaction rate. The model has the following form:

$$\log(q_e\text{-}q_t) = \log(q_e) - (k_L/2.3)t \qquad \text{Eqn 4.1}$$

where, k_L is theLagergren rate constant for adsorption (h^{-1}); q_e is the amount of metal ion adsorbed at equilibrium (mg g^{-1}); and q_t is the amount of metal ion adsorbed (mg g^{-1}) at any given time t (h).

A pseudo-second order reaction rate equation rate was developed and used by Ho *et al.* (2004) to study the kinetics of adsorption of heavy metals on peat. Equation 4.2 expresses the model (Ho's model).

$$\frac{t}{q_t} = \frac{1}{2K' q_e^2} + \frac{t}{q_e} \qquad \text{Eqn 4.2}$$

where, K' is the second order reaction rate constant for adsorption (g $mg^{-1}.h^{-1}$); q_e is the amount of metal ion adsorbed at equilibrium (mg g^{-1}) ; and q_t is the amount of metal ion adsorbed (mg g^{-1}) at any given time t (h).

4.2 Batch Biosorption Isotherms

The equilibrium established between adsorbed component on the biosorbent and unadsorbed component in solution can be represented by adsorption isotherms. An adsorption isotherm represents a relationship between solid phase concentration of an adsorbate or the amount of adsorbate adsorbed per unit weight of adsorbent and solution phase concentration of an adsorbent at an equilibrium condition under a particular temperature. Several different forms of isotherms are shown in Figure 4.1. Type I isotherm is also called convex-shaped isotherm in which equilibrium solid phase concentration of an adsorbate increases sharply from a low to high equilibrium concentration. The adsorption is said to be favorable. When an isotherm has the concave shape, adsorption is unfavorable and isotherm is called Type III isotherm. If equilibrium solid phase concentration of an adsorbate increases linearly with equilibrium concentration of an adsorbate in the liquid phase, the isotherm is called linear or a Type II isotherm (Weber and Morris, 1982).

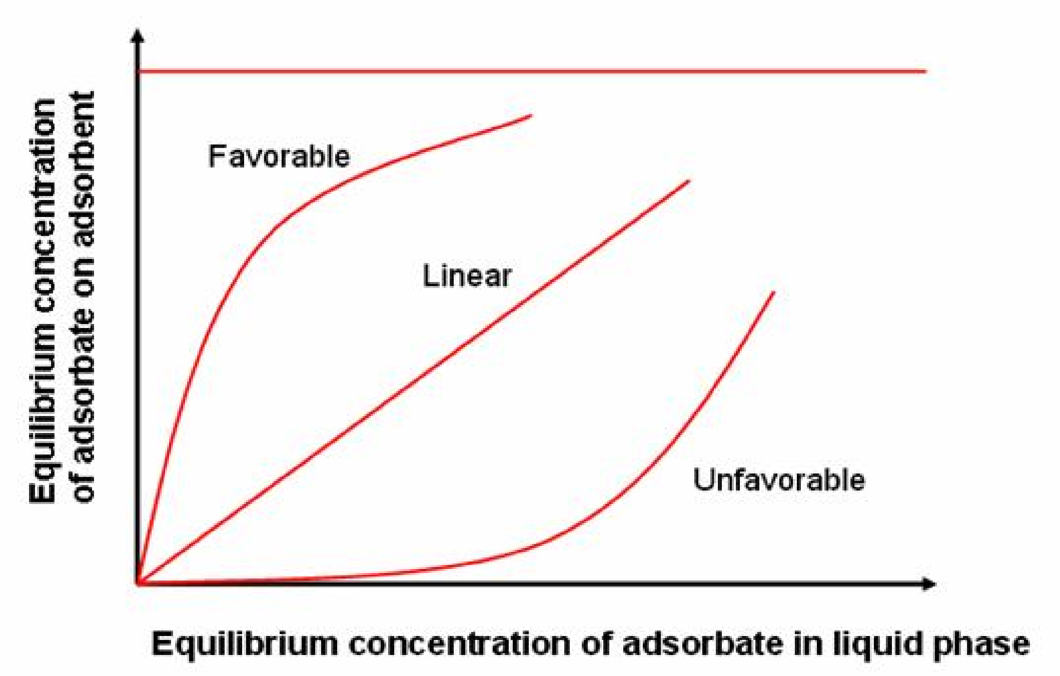


Figure 4.1 Plot of various kinds of isotherms (Weber and Morris, 1982)

A number of adsorption isotherms have been developed to describe adsorption equilibrium. The common isotherm models used in environmental engineering are the Langmiur, the Freundlich and the Brauner, Emmett and Teller (BET) models. These models were developed to describe the adsorption of gases on to solids and are commonly used in describing the equilibrium of adsorption of pollutants from water or wastewater.

The most widely used isotherm equation for modelling equilibrium is the Langmuir equation which is valid for monolayer sorption onto a surface with a finite number of identical sites which are homogeneously distributed over the sorbent surface and is given by Eq. 4.3.

$$q_{eq} = \frac{Q^0 b C_{eq}}{1 + b C_{eq}}, \qquad \text{Eqn 4.3}$$

where q_{eq} is the amount of metal ion bound to per gram of dried biomass at equilibrium and C_{eq} is the residual (equilibrium) metal ion concentration left in solution after binding, respectively. Q^0 is the maximum amount of metal ion per unit weight of sorbent to form a complete monolayer on the surface bound at high C_{eq}; and b is a constant related to the affinity of the binding sites. Q^0 and b can be determined from a plot of q_{eq} versus C_{eq}.

The Freundlich expression is an empirical equation based on sorption on a heterogeneous surface suggesting (as expected) that binding sites are not equivalent and/or independent. The mono-component Freundlich equation is given below:

$$q_{eq} = K_F\, C_{eq}^{1/n} \qquad \text{Eqn 4.4}$$

where K_F and n are the mono-component Freundlich constants related to the sorption capacity and sorption intensity of the sorbent, respectively. Eq. 4.4 can be linearized in logarithmic form and Freundlich constants can be determined. Both models were developed for a single-layer adsorption. However, the Freundlich model physically provides a more realistic description of adsorption by organic matter because it accounts for different binding sites. However, in most cases, both equations fit the data set reasonably well for the experimental data over moderate ranges of concentration.

The BET isotherm describes multilayer adsorption at the adsorbent surface, with an assumption that the Langmuir isotherm applies to each layer (Weber, 1972). It is also assumed that initial adsorbed molecules are adsorbed to a surface with energy comparable to the heat of adsorption for monolayer attachment; for subsequent layers adsorption place as condensation reactions. The BET isotherm has the following form:

$$q = \frac{BC_e Q^0}{(C_S - C_e)\left[1 + (B-1)\left(\frac{C_e}{C_S}\right)\right]}$$ Eqn 4.5

where, C_s is the saturation concentration of the adsorbate (mg l^{-1}); Q^0 is the amount of adsorbate adsorbed per unit weight of adsorbent for monolayer adsorption (mg g^{-1}), and B is the constant relating to energy of interaction with surface.

Another isotherm used to describe adsorption equilibrium data is the generalized adsorption isotherm equation which has the following form

$q = (q_m.C^n)/(K+C^n)$ Eqn 4.6

where K is the saturation constant (mg l^{-1}); n is the cooperative binding constant; q_m is the maximum adsorption capacity of the adsorbent (mg g^{-1}); q (mg g^{-1}) and C (mg l^{-1}) are the equilibrium heavy metal ion concentrations in the solid and liquid phases, respectively.

One of the difficulties in describing the adsorption of pollutants from waste streams is that wastewaters contain not one, but many kinds of pollutants. When several components are present, interference and competition phenomena for adsorption sites occur and lead to a more complex mathematical formulation of the equilibrium. Therefore, multi-component isotherms attempt to express relationships between the adsorbed quantity of one component and the concentrations of all other components, either in solution or already adsorbed. Several isotherms have been proposed to describe equilibrium for such systems. These isotherms range from simple models related to the individual isotherm parameters only (non-modified adsorption models) to more complex models related to the individual isotherm parameters and to correction factors (modified adsorption models).

4.3 Fed-Batch Biosorption Kinetics & Design Equations

Since the fed-batch operation used in this study is different from the conventional adsorption column operations, the well known Bohart-Adams equation used for design of adsorption columns was modified. The liquid volume in the reactor increased with time with a constant feed flow rate. No effluent was removed from the completely mixed reactor operated in fed-batch mode until the tank was full.

The Bohart–Adams equation used to predict the performance of adsorption columns has the following form (Eckenfelder, 1990; Cooney, 2000):

$$t_b = \frac{N_o X}{C_o V_o} - \frac{1}{K C_o} \mathrm{Ln}\left[\frac{C_o}{C_b} - 1\right] \qquad \text{Eqn 4.7}$$

where, t_b is the operation time to reach the breakthrough concentration C_b in the effluent (h); C_o is the feed adsorbate(copper ion).concentration (kg.m^{-3}); C_b is the the effluent adsorbate concentration at the break-through point (kg.m^{-3}); N_o the adsorption capacity of the adsorbent (kg adsorbate.m^{-3} of bed); X the depth of the packed section of the column (m); V_o the linear velocity of fluid (Q/A, m h^{-1}); K is the adsorption rate constant (m^3 kg^{-1}.h^{-1}). The following definitions were used to modify eq.4.7.

$$N_o = q_s\,(1-\varepsilon) \qquad V_{bed} = X\,A_o = \frac{V_{ads}}{(1-\varepsilon)} \qquad \text{Eqn 4.8}$$

where, q_s is the capacity of the adsorbent per unit adsorbent volume (kg adsorbate. m^{-3} adsorbent); (1-ε) is the adsorbent (solid) volume fraction (m^3 adsorbent.m^{-3} bed) in the reactor or in the bed; A_o is the cross section area of bed (m^2), and V_{ads} is the volume of the adsorbent in the reactor or in the bed (m^3). Bohart–Adams equation takes the following form after substitution of eq. 4.8 into eq.4.7.

$$t_b = \frac{q_s(1-\varepsilon)}{C_o V_o A_o} (X A_o) - \frac{1}{K C_o} \text{Ln} \left[\frac{C_o}{C_b} - 1\right] \qquad \text{Eqn 4.9}$$

Further arrangement yields,

$$t_b = \frac{q_s(1-\varepsilon)}{C_o Q} \frac{V_{ads}}{(1-\varepsilon)} - \frac{1}{K C_o} \text{Ln} \left[\frac{C_o}{C_b} - 1\right] \qquad \text{Eqn 4.10}$$

where Q is the volumetric flow rate of the feed solution ($m^3 h^{-1}$)

Adsorbent volume is defined as

$$V_{ads} = \frac{m_{ads}}{\rho_{ads}} \qquad \text{Eqn 4.11}$$

Substitution of eq.4.11 into eq.4.10 yields

$$t = \frac{q_s}{C_o Q} \frac{m_{ads}}{\rho_{ads}} - \frac{1}{K C_o} \text{Ln} \left[\frac{C_o}{C_b} - 1\right] \qquad \text{Eqn 4.12}$$

where, m_{ads} is mass of the adsorbent (kg), ρ_{ads} is the density of dry adsorbent (kg adsorbent.m^{-3} adsorbent).

By defining the capacity of the adsorbent per unit mass of adsorbent ($q_s^{'}$) as follows

$$q^{'}_s = \frac{q_s}{\rho_{ads}} \qquad \text{Eqn 4.13}$$

where $q^{'}_s$ is the adsorption capacity per unit mass of adsorbent (kg adsorbate. kg^{-1} adsorbent), one can write the eq Eq.4.13 as follows

$$t_b = \frac{q'_s}{C_o Q} m_{ads} - \frac{1}{K C_o} \mathrm{Ln}\left[\frac{C_o}{C_b} - 1\right] \qquad \text{Eqn 4.14}$$

where, QC_o/m_{ads} is the specific loading rate of solute (adsorbate) per unite mass of adsorbent (L_s = kg adsorbate.kg^{-1} adsorbent. h^{-1}) which is inversely proportional with the operation time (t_b) as expected.

A plot of experimental data in form of break-through time (t_b) versus $m_{ads}/ Q C_o$ results in a line with a slope of q_s' and intercept of - $(1/KC_o)$ Ln $(C_o/ C_b - 1)$ for constant C_o and C_b values.

4.4 Activated Sludge Kinetics with Metal Ion Inhibition

Activated sludge design equations were modified for Cu (II) ion containing wastewaters by considering Cu (II) inhibition on COD removal rate. Copper ion inhibitions on both the maximum specific substrate removal rate and on the saturation constant were considered.

In an activated sludge unit, a COD balance over the aeration tank yields

$$Q\,(COD_o - COD_R) = U_{COD}\, X\, V \qquad \text{Eqn 4.15a}$$

$$\text{or} \quad U_{COD} = \frac{Q\,(COD_o - COD_R)}{V\,X} = \frac{COD_o - COD_R}{\Theta_H\, X} \qquad \text{Eqn 4.15b}$$

where Q is the flow rate of wastewater to the aeration tank (l h^{-1}), COD_o and COD_R are the COD concentrations in the feed and in the reactor (or effluent) at the steady-state (mg l^{-1}); U_{COD} is the specific rate of COD removal (mg COD mg^{-1}X h^{-1}); X is the total biomass concentration in the aeration tank at steady-state (mg l^{-1}); V is the volume of the wastewater in the aeration tank (7.6 l); Θ_H is the hydraulic residence time (V/Q, h).

In the presence of Cu (II) ions the specific rate of COD removal (U_{COD}) may be written as follows

$$U_{COD} = \frac{k_{app}\,COD_R}{K_{s,app} + COD_R} \qquad \text{Eqn 4.16}$$

where k_{app} and $K_{s,app}$ are the apparent specific rate and saturation constants for COD removal which can be written as follows with the Cu (II) inhibition constants.

$$k_{app} = \frac{k}{(1+ Cu / K_{Cu})} \quad \text{and} \quad K_{s,app} = K_s\left(1 + \frac{Cu}{K'_{Cu}}\right) \qquad \text{Eqn 4.17}$$

where, k is the maximum specific rate constant for COD removal in the absence of Cu (II) ions (h^{-1}); K_{Cu} is the Cu (II) ion inhibition constant for k (mg l^{-1}); Cu is the total Cu (II) concentration in the feed and also in the aeration tank at steady-state (mg l^{-1}); K_s is the saturation constant for COD removal in th absence of Cu (II) (mg l^{-1}); K'_{Cu} is the Cu inhibition constant for K_s (mg l^{-1}).

Combination of eqns 4.15, 4.16 and 4.17 yields the following equation which may be used for design purposes.

$$U_{COD} = \frac{k_{app}\,COD_R}{K_{s,app} + COD_R} = \frac{[k / (1+ Cu / K_{Cu})]\,COD_R}{K_s (1+ Cu / K'_{Cu}) + COD_R} = \frac{COD_o - COD_R}{\Theta_H\ X} \qquad \text{Eqn 4.18}$$

In the absence of copper (II) ions, eqn 4.18 reduces to the following equation,

$$U_{COD} = \frac{k\,COD_R}{K_s + COD_R} = \frac{COD_o - COD_R}{\Theta_H\ X} \qquad \text{Eqn 4.19}$$

or in double reciprocal form,

$$\frac{1}{U} = \frac{\Theta_H X}{COD_o - COD_R} = \frac{1}{k} + \frac{K_s}{k} \frac{1}{COD_R} \qquad \text{Eqn 4.20}$$

A plot of 1/U versus $1/COD_R$ (or 1/S) yields a straight line with a slope of K_s / k and y-axis intercept of 1/k.

Similarly, in the presence of Cu (II) ions eqn 4.20 can be written in double reciprocal form as follows

$$\frac{1}{U} = \frac{\Theta_H X}{COD_o - COD_R} = \frac{1}{k_{app}} + \frac{K_{s,app}}{k_{app}} . \frac{1}{COD_R} \qquad \text{Eqn 4.21}$$

In this case, a plot of 1/U versus $1/COD_R$ (or 1/S) yields a straight line with a slope of $K_{s,app} / k_{app}$ and y-axis intercept of $1/k_{app}$.

The most important operating variable affecting the performance of an activated sludge unit or the effluent quality is the sludge age or the solids retention time (SRT). In the presence of Cu (II) ions, the steady-state effluent COD concentration (or the aeration tank COD) is related to the sludge age as follows

$$\frac{1}{\Theta c} = Y_{app} U_{COD} - b_{app} = Y_{app} \left(\frac{COD_o - COD_R}{\Theta_H \ X} \right) - b_{app} \qquad \text{Eqn 4.22}$$

where Y_{app} is the apparent growth yield coefficient ($gX \ g \ COD^{-1}$) ; b_{app} is the apparent death rate constant (d^{-1}) including Cu (II) inhibitions and Θ_c is the sludge age (SRT, d). A plot of $1/ \Theta_c$ versus U yields a line with a slope of Y_{app} and a y-axis intercept of b_{app} in the presence of Cu (II) ions. Inhibition effects of Cu (II) ions on the growth yield coefficient and on the death rate constants can be expressed as follows:

$$Y_{app} = \frac{Y_M}{(1 + Cu / K_Y)} \qquad \text{Eqn 4.23}$$

$$b_{app} = b\left(1 + \frac{Cu}{K_b}\right) \qquad \text{Eqn 4.24}$$

where, Y_M is the maximum growth yield coefficient (gX g S^{-1}) and ‘ b’ is the death rate constant (d^{-1}) in the absence of Cu (II) ions; K_Y and K_b are the inhibition constants on the growth yield coefficient and death rate constants in the presence of Cu (II) ions.

Combination of eqns 4.22, 4.23 and 4.24 yields the following design equation

$$\frac{1}{\Theta_c} = \frac{Y_M}{(1 + Cu/ K_Y)} U_{COD} - b\left(1 + \frac{Cu}{K_b}\right) \qquad \text{Eqn 4.25}$$

In the absence of Cu (II) ions eqn 4.25 can be written as follows,

$$\frac{1}{\Theta_c} = Y_M U_{COD} - b = Y_M\left(\frac{COD_o - COD_R}{\Theta_H \; X}\right) - b \qquad \text{Eqn 4.26}$$

A plot of 1/ Θ_c versus U yields a line with a slope of Y_M and a y-axis intercept of (b).

4.5 Box-Behnken Statistical Experiment Design

Box-Behnken statistical experiment design method was used to determine the effects of operating parameters such as SRT ,HRT, feed Cu(II) concentration and PWS loading rate on percent COD, Cu(II), toxicity removals and SVI values in an activated sludge unit treating Cu(II) containing synthetic wastewater. Four important operating parameters; SRT (X_1), HRT (X_2), feed Cu(II) concentration (X_3) and PWS loading rate (X_4) were considered as independent variables. The low, center and high levels of each variable designated as -1, 0, and +1, respectively are presented in Table 4.1

Table 4.1 The levels of independent variables in Box-Behnken statistical experiment design

Variable	Symbol	Coded variable level		
		Low level	Center level	High level
		-1	0	+1
Sludge age, SRT (d)	X_1	5	17.5	30
Hydraulic residence time, HRT (h)	X_2	5	15	25
Feed Cu (II) concen. (mg l^{-1})	X_3	0	25	50
PWS loading rate (g h^{-1})	X_4	0	2	4

The Box-Behnken design is an independent, rotatable quadratic design with no embedded factorial or fractional factorial points where the variable combinations are at the midpoints of the edges of the variable space and at the center. Among all statistical experiment designs, Box-Behnken design requires fewer runs than the others, e.g., 29 runs for a 4-factor experimental design.

Response functions describing variations of dependent variables (percent Cu(II), COD, toxicity removals and SVI values) with the independent variables (*X*;) can be written as follows:

$$Y = \overbrace{\underbrace{b_o+\Sigma}}\ b_i*\overbrace{\underbrace{X_i+\ \Sigma}}\ b_{ij}*X_i*\overbrace{\underbrace{X_j+}}\ \Sigma\ b_{ii}*X_i^2 \qquad \text{Eqn 4.27}$$

Linear interaction squared

where *Y* is the predicted response (percent Cu, COD, toxicity removals and SVI values), b_0 is the offset term and b_i is the linear effect while b_{ii} and b_{ij} are the square and the interaction effects, respectively.

Table 4.2 Experimental data points used in Box-Behnken statistical design in the order of increasing feed Cu(II) concentrations.

Run	X_1 SRT (d)	X_2 HRT (h)	X_3 FeedCu(II) (mg l^{-1})	X_4 PWS (g h^{-1})
1	17.5	15	0	0
2	17.5	15	0	4
3	17.5	5	0	2
4	17.5	25	0	2
5	5	15	0	2
6	30	15	0	2
7	5	5	25	2
8	30	5	25	2
9	5	25	25	2
10	30	25	25	2
11	5	15	25	0
12	30	15	25	0
13	5	15	25	4
14	30	15	25	4
15	17.5	5	25	0
16	17.5	25	25	0
17	17.5	5	25	4
18	17.5	25	25	4
19	17.5	15	25	2
20	17.5	15	25	2
21	17.5	15	25	2
22	17.5	15	25	2
23	17.5	15	25	2
24	17.5	15	50	0
25	17.5	15	50	4
26	17.5	5	50	2
27	17.5	25	50	2
28	5	15	50	2
29	30	15	50	2

Experimental data points used in Box-Behnken statistical design are presented in Table 4.2. The response function coefficients were determined by regression using the experimental data and the Stat-Ease Design Expert 7.0.3 (trial licence) computer program. The response functions for percent Cu, COD, toxicity removals and SVI values were approximated by the standard quadratic polynomial equation as presented below.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4 + b_{11}X_1^2 + b_{22} X_2^2 + b_{33} X_3^3 + b_{44} X_4^4 \qquad \text{Eqn 4.28}$$

CHAPTER FIVE
RESULTS AND DISCUSSION

5.1 Batch Shake Flask Experiments

In this set of experiments, biosorption capacities of different waste sludges obtained from different wastewater treatment plants were examined for the selection of the most suitable sludge. The effects of various pre-treatment methods on biosorption of Cu(II) ions by different waste sludges for the selection of the most suitable pre-treatment method. Also, the effects of environmental parameters such as pH and zeta potential, Cu(II) ion concentration, powdered waste sludge (PWS) concentration, surface area or particle size of the PWS, temperature, agitation speed (rpm) on biosorption of Cu(II) ions were experimented. Adsorption isotherms were established for copper (II) ions and the most suitable isotherm and its constants were determined. And finally, kinetics of biosorption of copper ions onto PWS were investigated.

5.1.1 Selection of Powdered Waste Sludge (PWS)

The first set of batch biosorption experiments were performed with PWS samples from different sources without any pre-treatment. Figure 5.1 depicts variation of biosorbed copper (II) ion concentrations (mg g^{-1}) with time for different PWS samples (pH =5, $D_p < 212$ µm). Biosorption of copper ions increased with time as shown in Figure 4.1 and reached saturation level after 6 hours of incubation. PWS samples obtained from DYO paint company resulted in the highest biosorption capacity of 50 mg Cu(II) g PWS^{-1} followed by that obtained from the Kisikkoy domestic WW treatment plant (37 mg Cu(II) g PWS^{-1}). The other PWS samples resulted in lower than 30 mg Cu g PWS^{-1} biosorption capacity. There were no biosorption in the control flask free of PWS samples. PWS sample obtained from DYO paint company was the most suitable one among the others tested yielding the highest biosorption capacity without any pre-treatment.

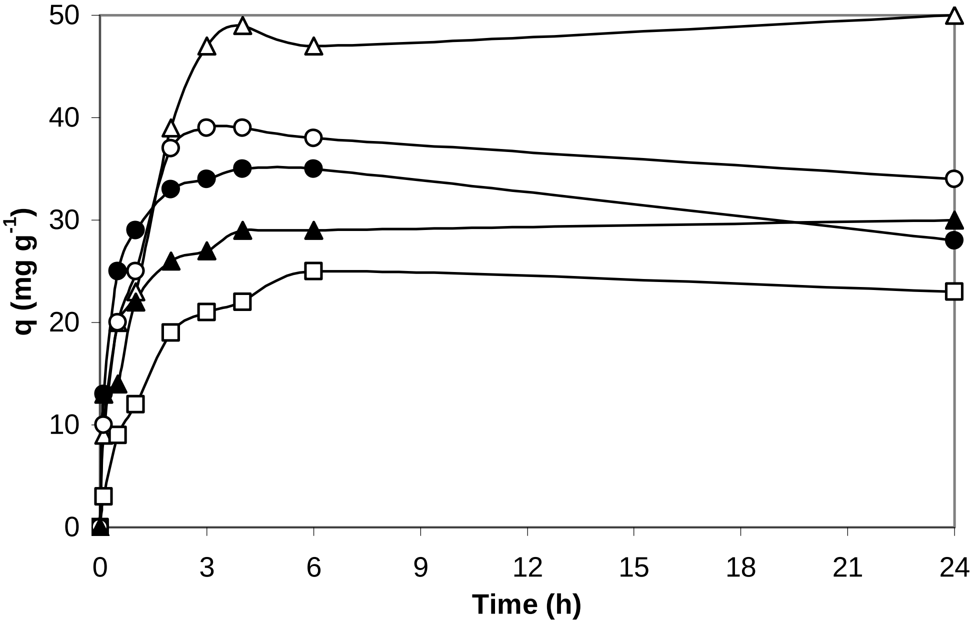
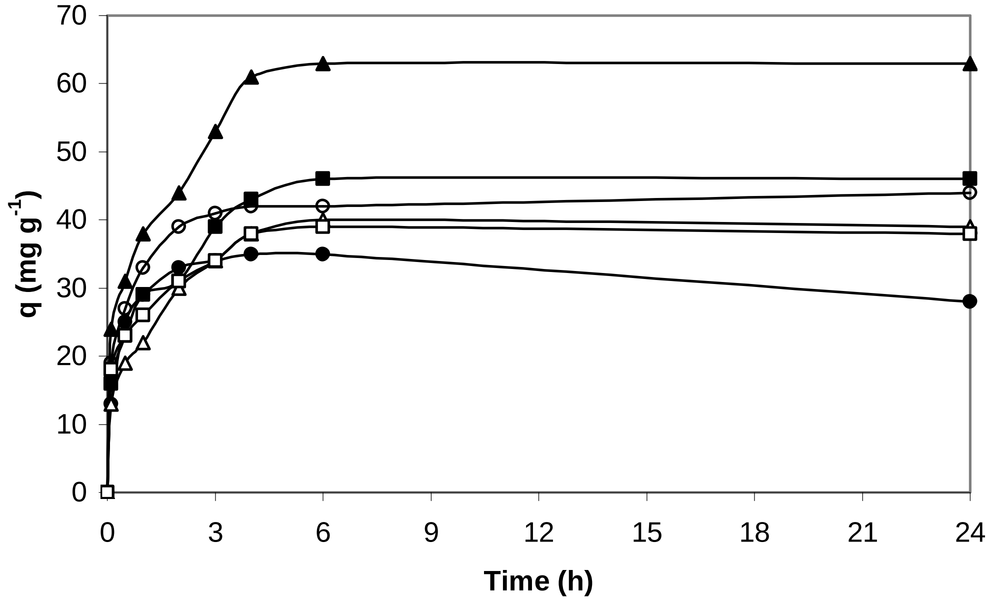


Figure 5.1 Variation of biosorption capacities of PWS samples from different wastewater treatment plants. ● Pakmaya Bakers Yeast Ind., Δ DYO Paint Ind., □ Guzelbahce, domestic, ○ Kısıkkoy, domestic, ▲ Cigli, Municipal (pH =5, $D_p < 212$ µm)

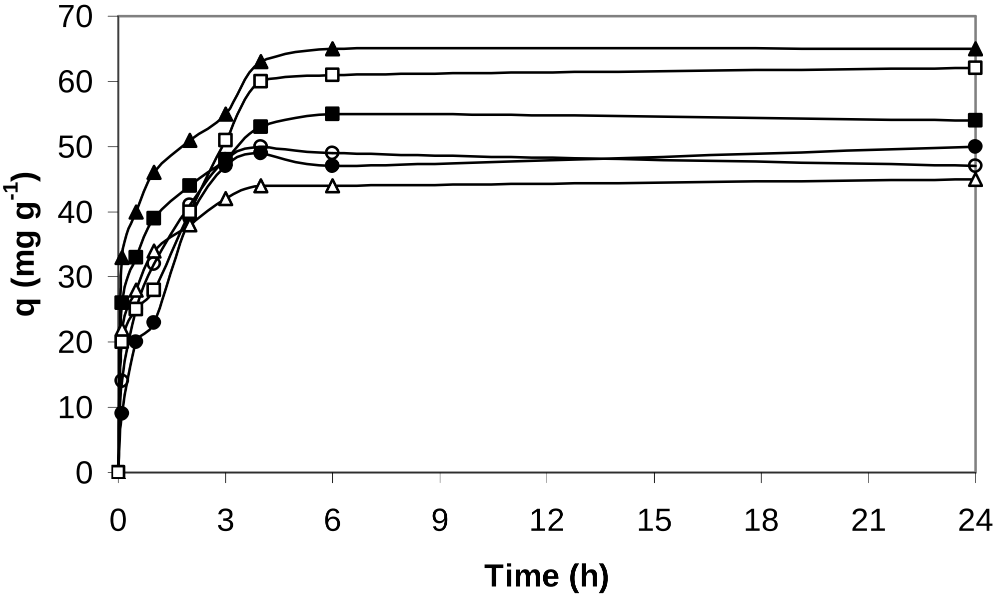
5.1.2 Selection of Pre-treatment Method

In the second set of experiments, the effects of different pre-treatment methods on biosorption of copper ions onto different PWS samples were investigated. Every PWS sample was subjected to the aforementioned pre-treatments and used for biosorption of copper ions under the same experimental conditions (pH = 5, D_p< 212 µm). Figures 5.2.a to 5.2.e depict variation of biosorbed copper ion concentrations (mg g^{-1}) with time for different PWS samples when different pre-treatment methods were used. Figure 5.2.a shows experimental results when different pre-treatment methods were used for the activated sludge obtained from PAK MAYA Bakers Yeast company WW treatment plant. Obviously pre-treatment with 1% H_2O_2 solution improved the biosorption capacity the most as compared to the other pre-treatment methods yielding 62 mg Cu(II) g PWS^{-1} as compared to 30 mg Cu(II). g PWS^{-1} without any pre-treatment. When the same procedure was used for the activated sludge samples obtained from DYO paint industry, the results presented in Figure 5.2.b were obtained. Again pre-treatment with 1% H_2O_2 resulted in the highest biosorption capacity of nearly 65 mg Cu(II) g PWS^{-1} as compared to 50 mg

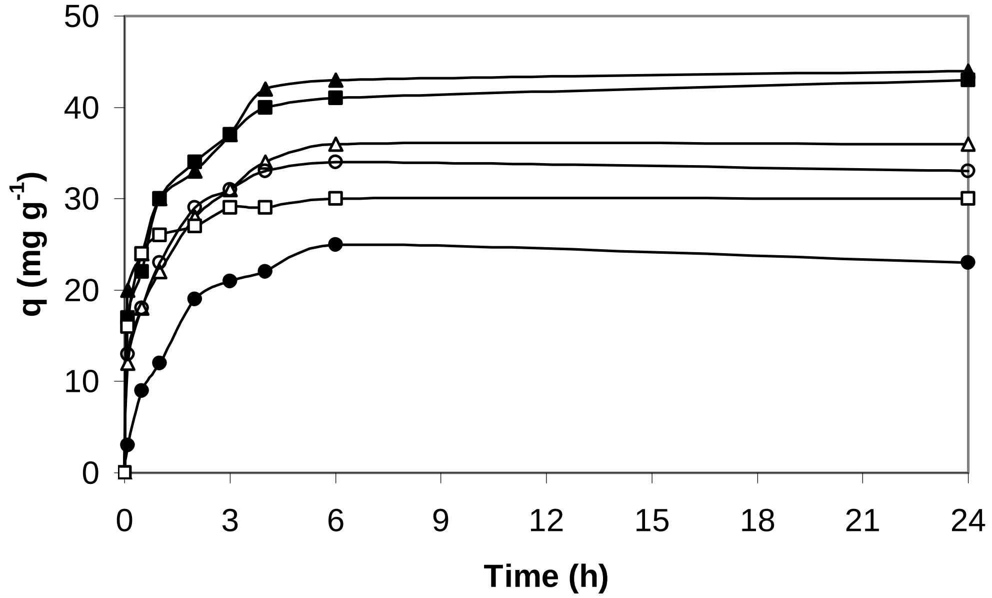
g^{-1} biosorption capacity with no pre-treatment. Biosorption characteristics of the two domestic waste sludges are depicted in Figures 5.2.c and 5.2.d when different pre-treatment methods were used. Biosorption capacities of those sludges varied between 30 and 45 mg Cu(II) g PWS^{-1}. Again the most suitable pre-treatment method was with 1%H_2O_2 yielding biosorption capacities of 45 mg g^{-1} for Kısıkkoy PWS and 43 mg g^{-1} for Guzelbahce PWS samples. Pre-treatment with 1% NaOCl solution resulted in the highest biosorption capacity (42 mg g^{-1}) for the PWS from Cigli municipal treatment plant followed by 1% NaOH (38 mg g^{-1}) and 1% H_2O_2 (35 mg g^{-1}) pre-treatments (Figure 5.2.e).



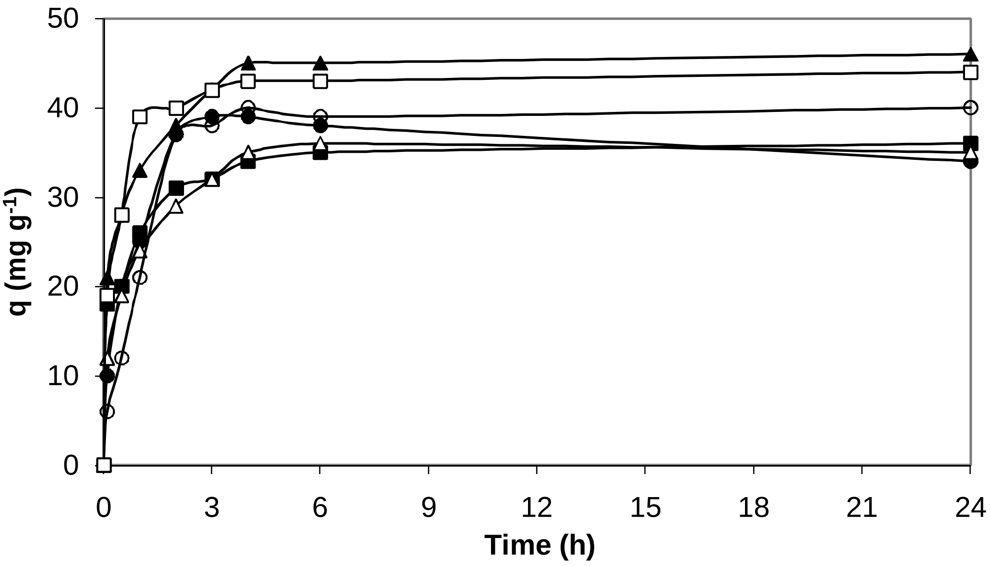
(a)


70
60
50
40
30
20
10
0
q (mg g^{-1})
0
3
6
9
12
15
18
21
24
Time (h)

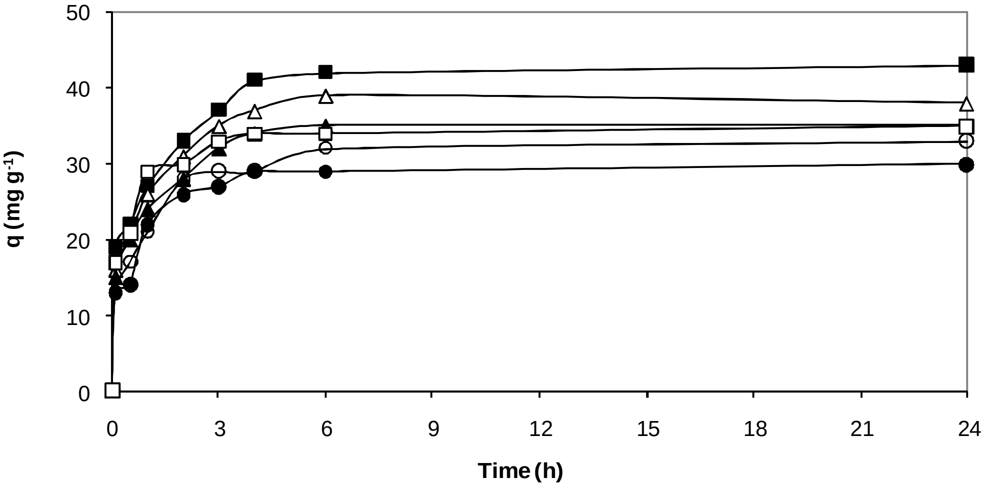
(b)


50
40
30
20
10
0
q (mg g^{-1})
0
3
6
9
12
15
18
21
24
Time (h)

(c)



(d)



(e)

Figure 5.2 Effects of different pre-treatment methods on biosorption capacity of PWS from different treatment plants. ● No pre-treatment, Δ NaOH, □ Ethanol, ○ H_2SO_4, ▲ H_2O_2, ■ NaOCl. (a) PWS from Pakmaya Bakers Yeast WW treatment plant, (b) PWS from DYO Paint Industry WW treatment plant, (c) PWS from Guzelbahce domestic WW treatment plant, (d) PWS from Kısıkkoy domestic WW treatment plant, (e) PWS from Cigli municipal WW treatment plant (pH = 5, D_p< 212 µm)

The results of the PWS and pre-treatment method selection experiments are summarized in Figure 5.3 in form of bar diagrams. Percent copper ion removals by biosorption are depicted for different activated sludge samples and pre-treatment methods. Industrial sludges from PAK MAYA Bakers Yeast and DYO paint companies WW treatment plants performed much better than those obtained from domestic and municipal treatment plants. Since the industrial sludges were exposed to heavy metals in the treatment plants, their biosorption capacities were higher as a result of adaptation. Also, the composition of microbial flora in industrial wastewater treatment plants may be more suitable for heavy metal biosorption as compared to the domestic sludges. The highest precent copper removal (65%) was obtained by using PWS from DYO paint industry WW treatment plant with 1% H_2O_2 pre-treatment. PWS from PAK MAYA Bakers yeast treatment plant yielded 63 % copper ion removal when pre-treated with 1% H_2O_2. Therefore, the most suitable sludge and pre-treatment methods were the sludge from DYO paint company and the pre-treatment with 1% H_2O_2. Probably H_2O_2 oxidized or activated some functional groups on the surfaces of bacterial biomass and provided more suitable surface characteristics for attachment of copper ions.

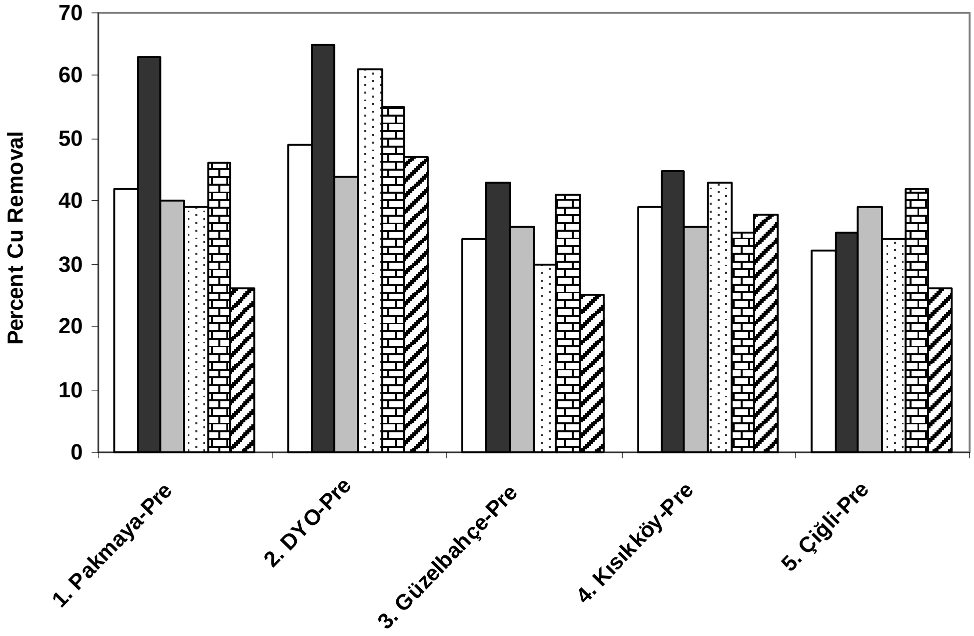


Figure 5.3 Variation of percent copper ion removal by biosorption for different PWS samples with different pre-treatment methods. (pH = 5, D_p< 212 µm)

Pretreatment-H_2SO_4 □ , Pretreatment-H_2O_2 ■ , Pretreatment-NaOH ▒
Pretreatment-Ethanol ░ , Pretreatment-NaOCl ▦ , No Pretreatment ▨

5.1.3 Effects of Environmental Conditions on Biosorption of Cu(II) Ions onto PWS

In this set of experiments, the effects of operating parameters such as pH, temperature, Cu(II) ion and the adsorbent concentrations, temperature, agitation speed and particle size on biosorption of copper (II) ions onto pre-treated powdered waste sludge (PWS) were investigated.

5.1.3.1 Effect of Particle Size or External Surface Area of PWS

Average particle size of PWS was varied between 53 and 338 µm in this set of experiments. Figure 5.4 depicts time course of variations of biosorbed (solid phase) copper ion concentration for different particle sizes over a period of 24 hours. Biosorbed copper ion concentration (mg Cu(II) $gPWS^{-1}$) increased with time for all particle sizes. However, small particle sizes (large external surface area) resulted in higher rates and extent of copper ion removal. Biosorption was almost complete within the 6 hours of incubation. Equilibrium solid phase copper ion concentrations were 46, 65 and 96 mg g^{-1} for the average particle sizes 338, 178 and 53 µm, respectively. Apparently, reductions in particle size resulted in increases in external surface area of PWS particles yielding more binding sites for copper ions and therefore, more efficient biosorption. The results are in agreement with literature studies reporting increased biosorption of copper and lead ions with decreasing particle size (Gulnaz O. *et al.*, 2005 - Bektas N and Kara S., 2004). However, increase in the extent of copper ion biosorption with decreasing particle size is much larger in our study as compared to the literature results indicating high biosorption capacity of the adsorbent (pre-treated PWS) used in our study. No copper ion removal was observed in the control flask.

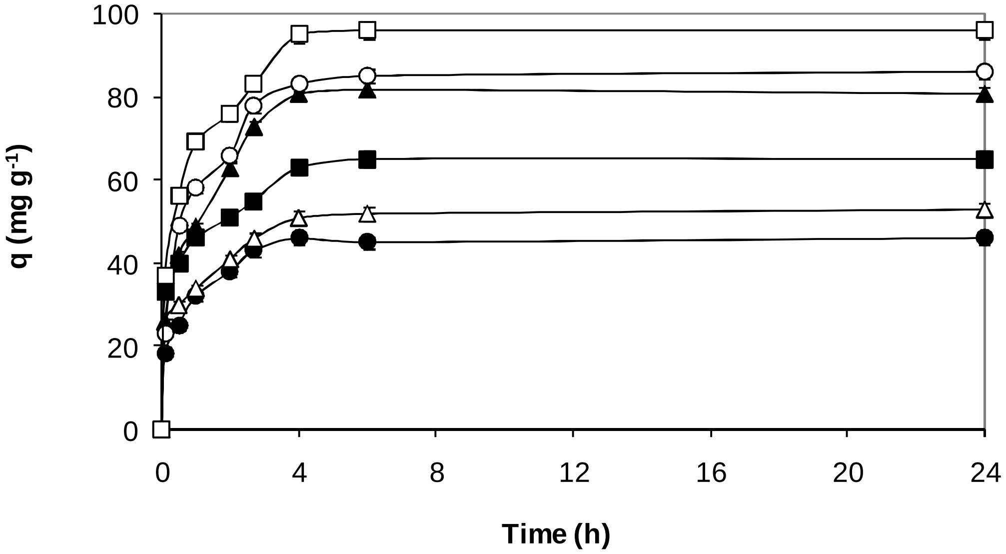


Figure 5.4 Variation of biosorbed copper ion concentration with time for different particle sizes of PWS (PWS=1 g/l, Cu_0=100 mg/l, pH=5)

. □ 53 μm , ○ 64 μm , ▲ 109 μm, ■ 178 μm , Δ 231 μm , ● 338 μm

The relationship between the particle size and the specific external surface area of PWS (cm^2g^{-1}) can be written as follows by assuming spherical particles:

$$a_s = \frac{N \Pi D_p^2}{N (\Pi/6) D_p^3 \rho} = \frac{6}{D_p \rho} \qquad \text{Eqn 5.1}$$

where a_s is the specific external surface area of PWS (cm^2g^{-1}), N is the number of particles, D_p is the average diameter of the spherical particles (cm) and ρ is the density of dry PWS (1.2 g cm^{-3}). Specific surface areas of PWS were calculated for each particle size by using eqn 5.1. Percent biosorption and the final Cu(II) concentrations were plotted against specific external surface area (a_s) of PWS for each size fractions (Figure 5.5). Final copper ion concentrations in solution decreased and percent biosorption increased with increasing specific external surface area as shown in Figure 5.5. Increases in percent biosorption or decreases in the final copper concentrations were much steeper at low specific surface areas (large particle sizes). Apparently, biosorption of copper ions onto PWS particles was limited by the availability of biosorbent surface area. Percent biosorption of Cu(II) increased from

46% to 95% and the final Cu(II) decreased from 54 mg l^{-1} to 5 mg l^{-1} ($Cu(II)_o$ = 100 mg l^{-1}) when the specific external surface area (a_s) increased from 148 cm^2 g^{-1} to 943 cm^2 g^{-1}.

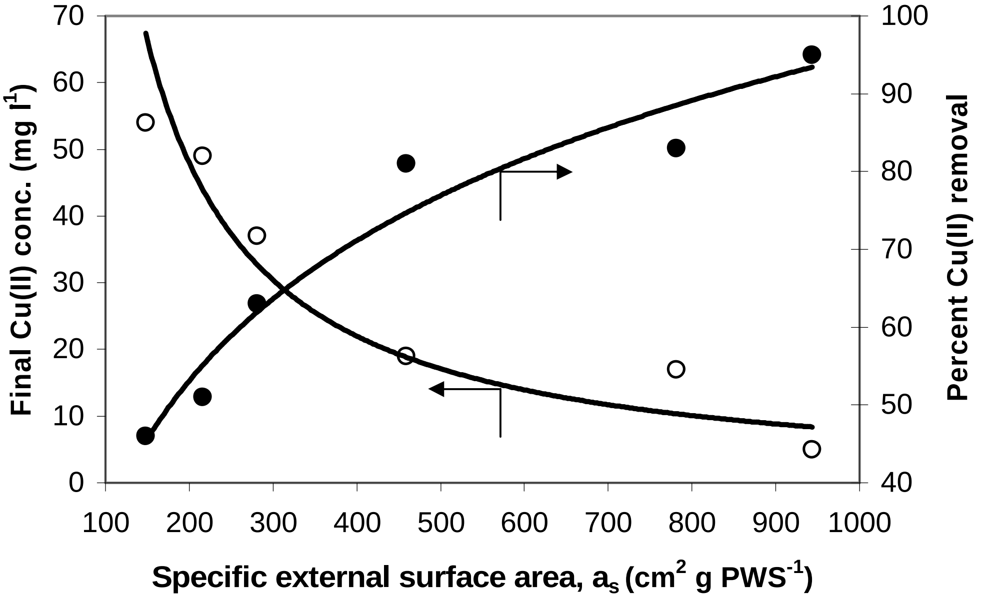


Figure 5.5 Variation of final copper concentration and percent copper removal with the specific external surface area of PWS. ○ Final Cu(II) concentration (mg l^{-1}) ● Percent Cu(II) removal

The rate of biosorption is an important parameter in selecting the right adsorbent and determining the optimal operating conditions yielding maximum biosorption. The surface rates of biosorption were determined by using the following equation:

$$R_s = R_v / a_v \qquad \text{Eqn 5.2}$$

where, R_s is the rate of Cu(II) removal per unit external surface area of PWS particles (surface biosorption rate, mg Cu(II) cm^{-2}. h^{-1}); R_v is the rate of Cu(II) removal per unit volume of the solution (volumetric rate of biosorption, mg Cu(II) l^{-1} h^{-1}); a_v is the external surface area of PWS particles per unit volume of the reaction mixture (cm^2 l^{-1}). R_v values were calculated using the experimental data as the initial biosorption rate for the first one hour (ΔCu(II) / 1, mg l^{-1} h^{-1}). a_v values were determined using the following equation:

$$a_v = a_s . \rho_{pulp} \qquad \text{Eqn 5.3}$$

where ρ_{pulp} is the pulp density of PWS in reaction mixture (g PWS. l^{-1}) which was 1g PWS l^{-1} in variable particle size experiments. Figure 5.6 depicts variation of initial surface and volumetric biosorption rates (R_s and R_v) with the specific external surface area of PWS particles (a_s). The initial volumetric biosorption rate (R_v) increased, but the surface biosorption rate (R_s) decreased with the increasing specific surface area of PWS. Specific surface area of PWS is inversely proportional to the particle diameter (D_p) as given in eqn 5.1. Decreases in particle size or increases in the specific external surface area resulted in larger reaction surface and therefore, higher volumetric biosorption rates (R_v). However, the surface rate of biosorption (R_s) is inversely proportional to the specific surface area (a_s) as given in eqns 5.2 and 5.3. Therefore, the surface biosorption rate decreased with increasing specific surface area (a_s) or decreasing particle size (D_p). Decreases or increases in biosorption rates were much steeper at large particle sizes or low specific external surface areas because of surface area (binding site) limitations. The volumetric and surface biosorption rates were 32 mg $l^{-1}h^{-1}$ and 0.22 mg cm^{-2} h^{-1} at a specific surface area of 148 cm^2 g^{-1}. When the specific surface area increased to 943 cm^2 g^{-1} the volumetric rate increased to nearly 70 mg l^{-1} h^{-1} and the surface rate decreased to 0.073 mg cm^{-2} h^{-1}. At large a_s values above 780 cm^2g^{-1} or small particle sizes below 64 µm, the rates of biosorption did not change considerably with the particle size because of availability of sufficient surface area or binding sites. The rate of biosorption was limited by the availability of surface area or binding sites at large particle sizes (low surface area). However, when large adsorbent surface area was available for copper ion binding (small particle sizes <64 µm) the rate of biosorption was limited by the concentration of Cu(II) ions in solution.

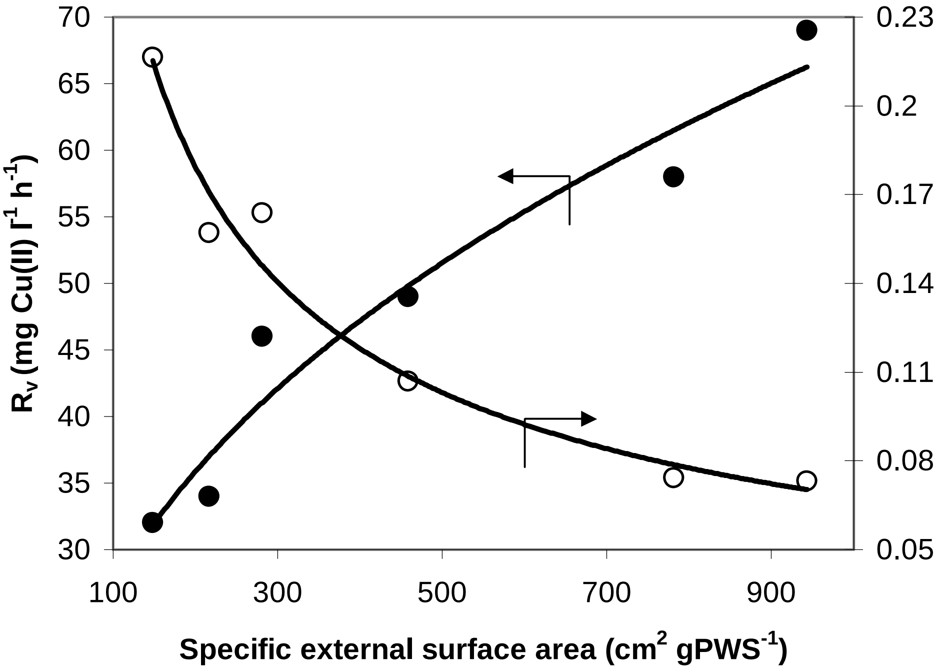


Figure 5.6 Variation of volumetric and surface biosorption rates with the specific external surface area of PWS. ○ Surface rate of biosorption, R_s, ● Volumetric rate of biosorption, R_v

5.1.3.2 Effect of Initial Copper (II) Ion Concentration

Copper (II) ion concentrations were varied between 50 and 400 mg l^{-1} in this set of experiments while the PWS concentration, particle size and pH were constant at 1 g l^{-1}, 64 µm and pH = 5, respectively. Variations of the solid phase (biosorbed) Cu(II) concentrations with time are depicted in Figure 5.7 for different initial Cu(II) concentrations. Biosorbed copper ion concentrations increased with time and reached equilibrium after six hours of incubation for all initial copper ion concentrations tested. At low initial Cu(II) concentrations such as 50 and 100 mg l^{-1}, the extent of biosorption was limited by copper ion concentrations yielding 46 mg g^{-1} and 84 mg g^{-1} equilibrium solid phase concentrations. However, at high initial Cu(II) concentrations such as 300 and 400 mg l^{-1}, the extent of biosorption was limited by the concentration of the PWS yielding high equilibrium Cu(II) concentrations in the solid phase as 113 and 117 mg g^{-1}, respectively. The results are in agreement with the literature studies reporting high metal ion biosorption at high metal ion concentrations (Gulnaz O. *et al.*, 2005 - Bektas N and Kara S., 2004). At constant adsorbent (PWS) concentration, initial copper ion concentration should be high enough to maximize copper ion biosorption.

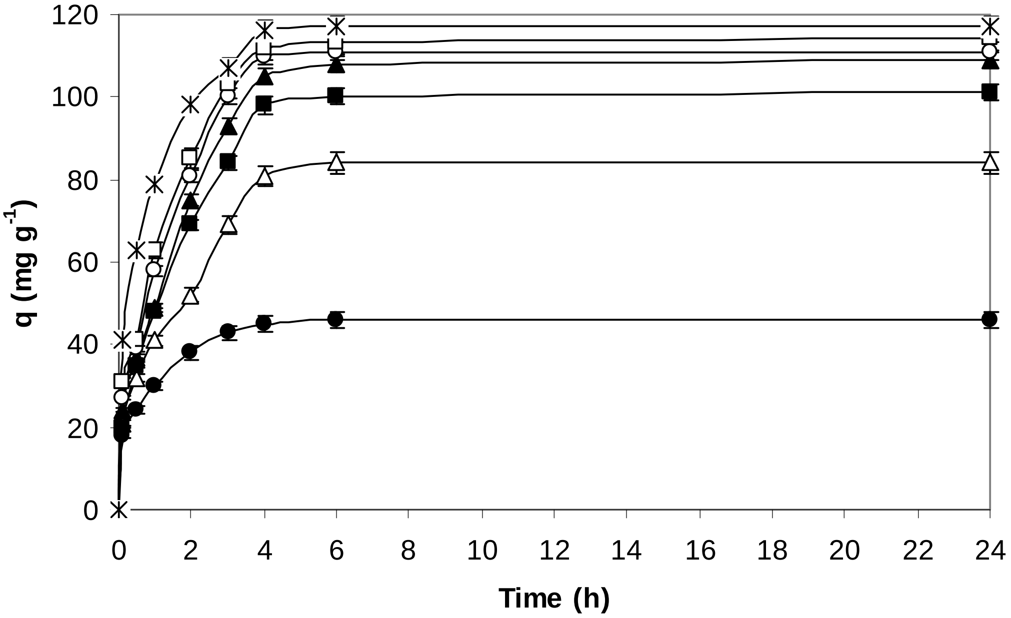


Figure 5.7 Variation of biosorbed copper ion concentration with time for different initial copper ion concentrations. (PWS=1 g/l, pH=5, Dp=64 μm) ● 50 mg l^{-1}, Δ 100 mg l^{-1}, ■ 150 mg l^{-1}, ▲ 200 mg l^{-1} , ○ 250 mg l^{-1}, □ 300 mg l^{-1}, ⁎ 400 mg l^{-1}

Table 5.1 Variation of percent copper removal and biosorbed copper concentrations with the initial copper ion concentration at the end of 6 hours of incubation (D_p = 64 μm, pH =5, PWS = 1 g l^{-1}, T = 25 °C)

Initial Cu(II) conc. C_o (mg l^{-1})	Final Cu(II) conc. C, (mg l^{-1})	Percent Cu(II) removal	Biosorbed Cu(II) conc. q (mg g^{-1})
50	4	92	46
100	16	84	84
150	50	67	100
200	92	54	108
250	139	44	111
300	187	38	113
400	283	29	117

Table 5.1 summarizes data on variations of percent removal and the final copper ion concentrations with initial Cu(II) concentrations. Percent copper ion removal decreased from 92% to 29% and the final Cu(II) concentration increased from 4 mg l^{-1} to 283 mg l^{-1} when the initial Cu(II) concentration was raised from 50 to 400 mg l^{-1}. At low initial Cu(II) concentrations such as 50 and 100 mg l^{-1}, all copper ions were biosorbed onto binding sites on PWS surfaces since binding sites were in excess of copper ions yielding low copper ions in solution such as 4 and 16 mg l^{-1}, respectively. However, at high initial copper ion concentrations such as 300 and 400 mg l^{-1}, a large fraction of binding sites on PWS surfaces were occupied by copper ions since the copper ions were in excess of the binding sites yielding high Cu(II) concentrations in the solution at equilibrium such as 187 and 283 mg l^{-1}, respectively. At a PWS concentration of 1 g l^{-1}, the initial Cu(II) concentration should be lower than 100 mg l^{-1} to obtain more than 85% Cu(II) removal.

One of the important parameters in biosorption of heavy metal ions is the equilibrium solid phase (biosorbed) metal ion concentration. Variations of equilibrium solid phase Cu(II) concentration (q_e, mg Cu $gPWS^{-1}$) with initial Cu(II) are also summarized in Table 5.1. As expected the equilibrium solid phase copper ion concentration increased with increasing initial Cu(II) concentration. A larger fraction of the binding sites on PWS surfaces were occupied by Cu(II) ions as the initial copper ion concentration increased yielding higher solid phase Cu(II) concentrations at equilibrium since the adsorbent (PWS) concentration was constant at 1 g l^{-1}. The biosorbed copper ion concentration increased from 46 to nearly 117 mg g^{-1} when initial Cu(II) concentration was increased from 50 to 400 mg l^{-1}.

5.1.3.3 Effect of Biosorbent (PWS) Concentration

The biosorbent (PWS) concentration was varied between 0.25 and 3.0 g l^{-1} in this set of experiments while the initial copper ion concentration, PWS particle size and pH were constant at 200 mg l^{-1}, 64 µm and 5, respectively. Variations of the solid phase (biosorbed) Cu(II) concentrations with time are depicted in Figure 5.8 for different biosorbent (PWS) concentrations. Biosorbed copper ion concentrations (q, mg g^{-1}) increased with time and reached equilibrium after six hours of incubation for all biosorbent (PWS) concentrations tested. At low PWS concentrations such as 0.25

and 0.5 g l^{-1}, the available binding sites on biosorbent surfaces were occupied by copper ions, since copper ion concentrations exceeded the binding sites yielding high equilibrium biosorbant Cu ion concentrations such as 156 and 126 mg g^{-1}, respectively. However, at high biosorbent (PWS) concentrations such as 2.5 and 3.0 g l^{-1} large number of binding sites were available on PWS surfaces and only a fraction of those binding sites were occupied by Cu(II) ions yielding low solid phase Cu(II) concentrations such as 50 and 52 mg g^{-1}, respectively. The extent of biosorption was limited by Cu(II) ion concentration at high PWS concentrations. At constant initial metal ion concentrations the biosorbent PWS concentration should be low to maximize solid phase metal ion concentration at equilibrium. Our results are in agreement with literature reports indicating lower biosorbed metal ion concentrations (q) at high adsorbent concentrations (Esposito A. *et al.*, 2001). However, the extents of copper ion biosorption obtained in our studies are larger than tose reported in literature studies (Esposito A. *et al.*, 2001) indicating high biosorption capacity of the adsorbent (pre-treated PWS) used.

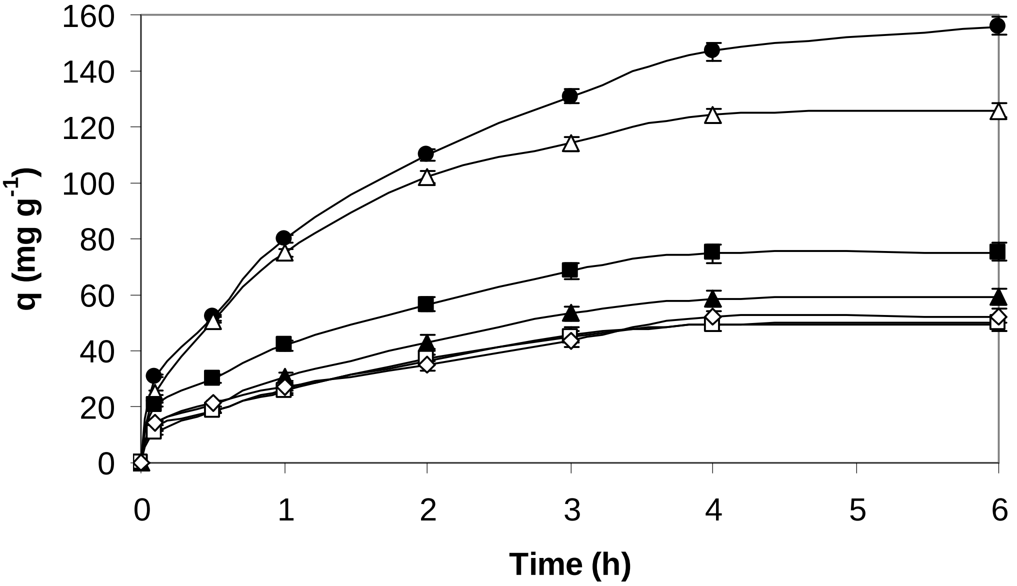


Figure 5.8 Variation of biosorbed copper ion concentration with time for different PWS concentrations. (pH=5, Cu_0=200 mg/l, Dp=64 µm) PWS (g l^{-1}): ● 0.25 , △ 0.5, ■ 1, ▲ 1.5, ○ 2, □ 2.5, ◇ 3

Table 5.2 Variation of percent copper removal and biosorbed copper concentrations with the initial PWS concentration at the end of 6 hours of incubation ($(Cu(II))_o$ = 200 mg l^{-1}, pH = 5, D_p = 64 µm, T = 25 ^oC)

PWS conc. (g l^{-1})	Final Cu(II) conc. (mg l^{-1})	Percent Cu(II) removal	Biosorbed Cu(II) conc. q, (mg g^{-1})
0.25	161	20	156
0.5	137	32	126
1	125	38	75
1.5	111	44	59
2	101	50	50
2.5	75	63	50
3	43	79	52

Variations of percent removal and the final copper ion concentrations with the biosorbent (PWS) concentration are summarized in Table 5.2. Percent copper ion removal increased from 20% to 79% and the final Cu(II) concentration decreased from 161 mg l^{-1} to 43 mg l^{-1} when the biosorbent (PWS) concentration was raised from 0.25 to 3.0 g l^{-1}. At low biosorbent concentrations such as 0.25 and 0.5 g l^{-1}, the extent of copper biosorption at equilibrium was limited by the availability of the binding sites on the biosorbent (PWS) yielding low percent copper ion removal such as 20 and 32% and high residual copper ions in solution such as 161 and 137 mg l^{-1}, respectively. However, at high biosorbent concentrations such as 2.5 and 3.0 g l^{-1}, the binding sites on the biosorbent surfaces exceeded copper ions in solution and large fractions of copper ions were biosorbed onto the binding sites resulting in nearly 63 and 79% copper ion removal with residual copper ions of 75 and 43 mg l^{-1} in solution, respectively. At an initial Cu(II) concentration of 200 mg l^{-1}, the biosorbent (PWS) concentration should be higher than 3 g l^{-1} to obtain more than 80% Cu(II) removal.

Table 5.2 also summarizes equilibrium solid phase Cu(II) concentrations (q_e, mg Cu(II) g PWS^{-1}) at different initial biosorbent (PWS) concentrations at a constant initial Cu(II) concentration of 200 mg l^{-1}. The equilibrium solid phase copper ion concentration decreased with increasing biosorbent concentration. A smaller fraction of the binding sites on PWS surfaces were occupied by Cu(II) ions as the initial PWS concentration increased yielding lower solid phase Cu(II) concentrations at equilibrium, since initial copper ion concentration was constant at 200 mg l^{-1}. At low PWS concentrations larger fractions of binding sites on biosorbent surfaces were occupied by Cu(II) ions yielding high biosorbed (solid phase) Cu(II) concentrations. The biosorbed copper ion concentration decreased from 156 to 52 mg g^{-1} when initial PWS concentration was increased from 0.25 to 3.0 g l^{-1}.

5.1.3.4 Effects of pH and Zeta Potential

pH is an important parameter affecting the rate and the extent of biosorption of metal ions onto biosorbents such as PWS. Variation of pH may affect the surface charge of biosorbent and also the solubility of metal ions. Some metal ions such as copper and zinc are known to precipitate in form of hydroxides at high pH values equal or greater than 6 (Sawyer N.C. and McCarty P.L., 1978). For this reason, the effects of initial pH on biosorption of Cu(II) ions onto PWS was investigated for pH values between 2 and 6. PWS and Cu(II) concentrations were 1 g l^{-1} and 100 mg l^{-1}, respectively and the PWS particle size was 109 µm, in this set of experiments.

Figure 5.9 depicts variation of solid phase (biosorbed) copper ion concentrations with time for different initial pH values. Biosorbed Cu(II) concentration increased with time at all pH levels tested and equilibrium was reached after six hours of incubation. At low pH values such as pH = 2 and 3, due to high (H^+) ion concentrations in solution the surfaces of PWS were neutralized (which are negatively charged at neutral pH) yielding slightly negative zeta potentials (-5 and -13mV, respectively) and thus prohibiting binding of positively charged Cu(II) ions onto the surfaces of PWS. As a result, the extent of biosorption was rather low at low pH values. However, the equilibrium solid phase Cu(II) ion concentration increased with increasing pH, because of increasingly negative charges or highly negative zeta potentials on the surfaces of the PWS until pH 5 which attracted positively charged Cu(II) ions more strongly. The only mechanisms for copper ion removal at pH values above 5 is not biosorption, but also precipitation of Cu(II) ions in form of $Cu(OH)_2$ (Aksu Z. *et al.*, 1999). The solubility constant for $Cu\ (OH)_2$ at 25 °C is $K_{sp} = 2.\ 10^{-20}$ which yields only 13 mg l^{-1} soluble Cu(II) ions in solution at pH=6, when the initial

Cu(II) is 100 mg l^{-1}, whereas copper ions are completely soluble for pH values lower than or equal to 5. In fact, a white-blue precipitate was observed in solution at pH values 6 and above. Therefore, it can be said that the optimum pH for biosorption of copper ions is about pH =5 when initial copper was 100 mg l^{-1}, above which Cu(II) ions precipitate in form of $Cu(OH)_2$.

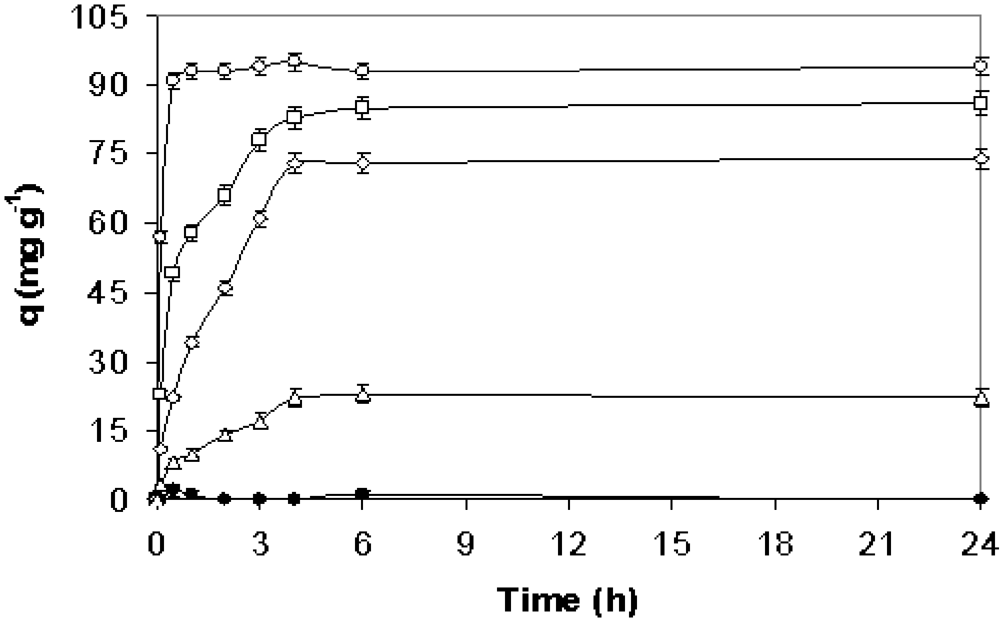


Figure 5.9 Variation of biosorbed Cu(II) concentration with time at different pH's. (PWS=1 g/l, Cu_0=100 mg/l, D_p=64 µm) pH : ● 2, Δ 3, ◊ 4, □ 5, ○ 6

Figure 5.10 depicts variation of maximum biosorption capacities and the initial rate of biosorption (within the first one hour) with pH. Both the biosorption capacities and the rate of biosorption increased with pH. Rate of increase in biosorption capacities at pH values larger than 5 is not only by biosorption, but also by precipitation of copper ions in form of $Cu(OH)_2$. For this reason, the maximum biosorption capacity was obtained at pH=5 as 90 mg g^{-1} without any copper ion precipitation. The initial rate of biosorption at pH 5 was nearly 60 mg l^{-1} h^{-1}.

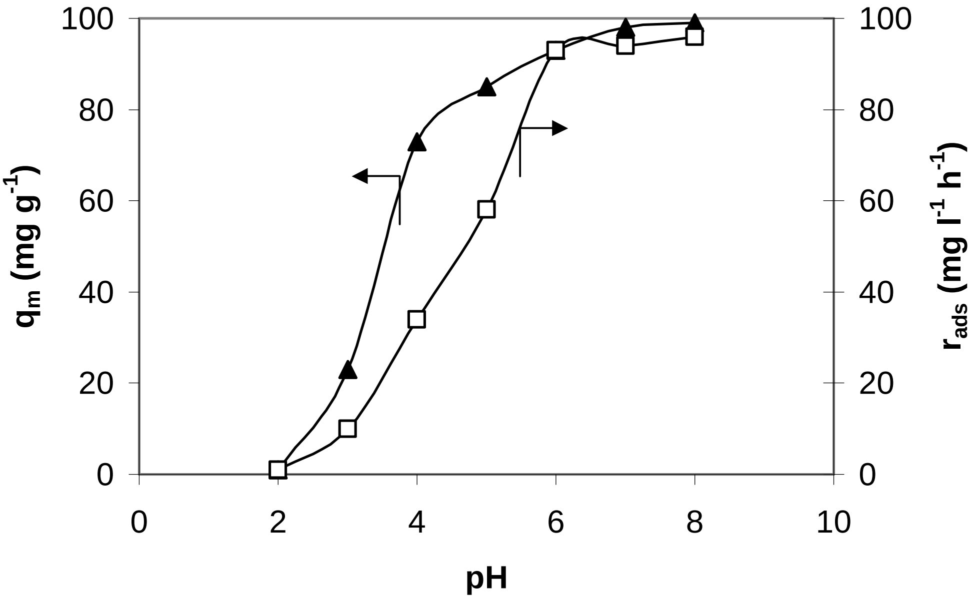


Figure 5.10 Variation of biosorption capacity and the initial rate with pH

Zeta potential is defined as the potential at the interface of Stern and diffuse layers on the surface of PWS particles. Figure 5.11 depicts variation of zeta potential with pH in 1 g l^{-1} PWS solution before and after Cu(II) ion addition. The surfaces of PWS particles are negatively charged at high pH levels yielding highly negative zeta potential. As the pH decreases H$^+$ ions cover the surfaces of PWS particles yielding less negative zeta potential. The zeta potential increased from -8mV to -42 mV when when the pH increased from 2 to 8. Larger (more negative) zeta potentials at high pH levels yielded higher rate and extent of copper ion biosorption as shown in Figure 4.14. After the copper ions addition (100 mg l^{-1}), the surfaces of PWS particles would be partially covered by Cu(II) ions yielding less negative zeta potential as shown in Figure 5.11. The zeta potentials at pH=5 were -30 and -16 mV in the absence of presence of copper ions in 1 g l^{-1} PWS solution. In the presence of Cu(II) ions, the zeta potential became more negative as the pH increased from 2 and 5. For pH values above 5, Cu(II) ions partially precipitated in form of $Cu(OH)_2$ and covered the surface of PWS particles leading less negative zeta potentials. Copper ion removal for pH levels above 5 is not only by biosorption but also precipitation. Therefore, the most suitable pH maximizing the rate and extent of Cu(II) biosorption was pH =5 with a zeta potential of -30 mV.

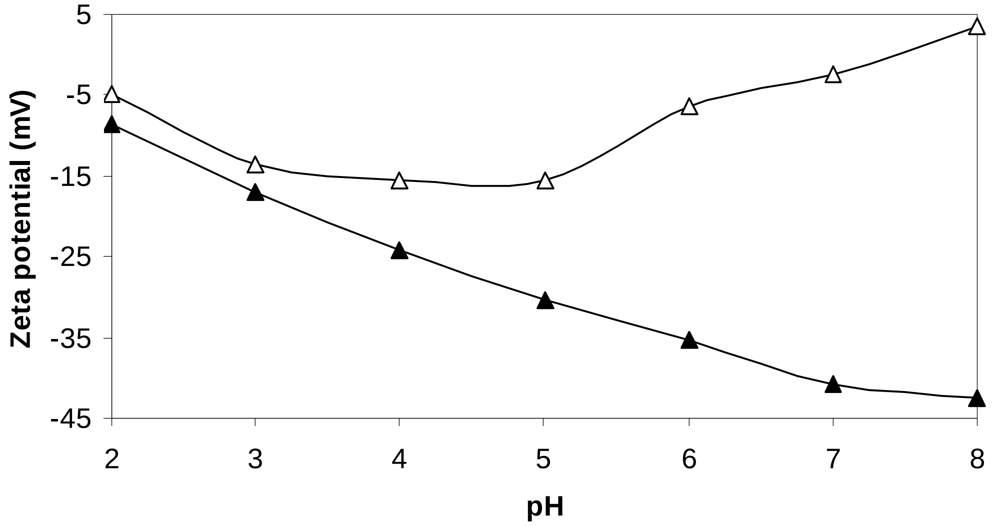


Figure 5.11 Variation of zeta potential with the pH in PWS solution. ▲ before Cu(II) addition, Δ after Cu(II) addition

Most of the literature studies on biosorption of heavy metals were carried out by using pure cultures (Galli E. *et al.*, 2003 - Sağ Y. and Kutsal T., 2000) and binary metal ion mixtures (Sağ Y. *et al.* 2003 - Hammaini A. *et al.* 2002). There are very few of studies on biosorption of copper ions onto powdered waste sludge (PWS). As compared to a recent study by Gulnaz et al, the PWS we have used has larger biosorption capacities at pH 4 and 5. Furthermore, considerable improvements in biosorption capacity were obtained by reducing the particle size of PWS in our study as compared to Gulnaz et al (Gulnaz O. et al, 2005). This is mainly because of the selection and pre-treatment procedure we have used for the PWS in our study. The results indicated that when operated at optimal pH of 5 and particle size of 53 µm, the pre-treated PWS had better biosorption performance for copper (II) ions as compared to the literature studies.

5.1.3.5 Effects of Temperature

Batch biosorption experiments were performed at different temperatures between 30 and 50 ^{o}C with 1 g l^{-1} PWS of particle size 64 µm, Cu(II) of 100 mg l^{-1} at pH 5. Figure 5.12 depicts variation of biosorbed Cu(II) ion concentrations with time for different temperatures. Biosorbed Cu(II) concentration increased with time and reached equilibrium after 6 hours of incubation at all temperatures tested. Equilibrium solid phase Cu(II) concentration increased from 82 mg g^{-1} to 90 and further to 97 mg g^{-1} when the temperature was increased from 30 to 40 ^{o}C and further to 50 ^{o}C.

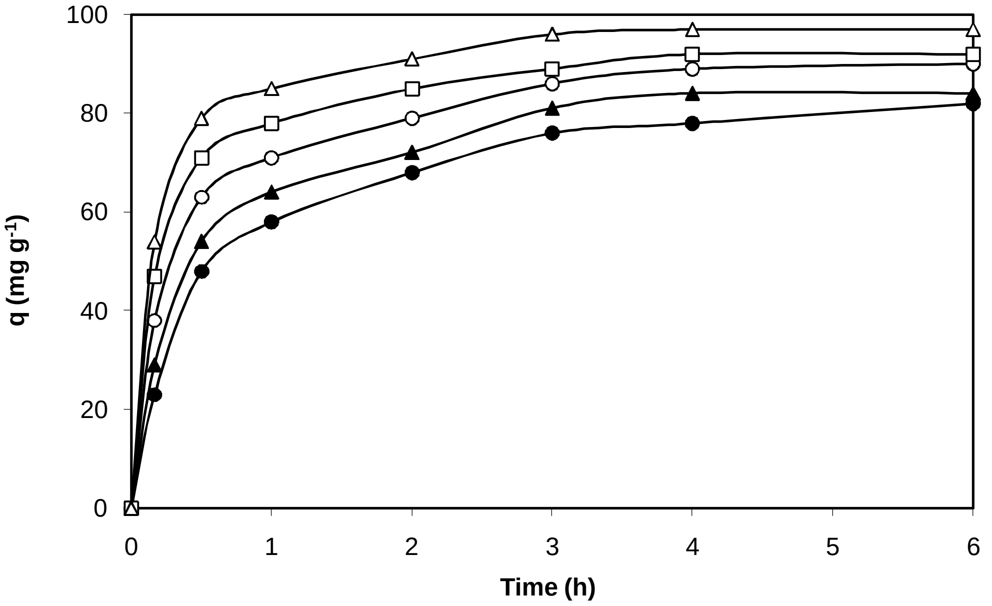


Figure 5.12 Variation of biosorbed Cu(II) ion concentration with time at different temperatures (PWS=1 g l^{-1} , D_p=64 µm, Cu(II)=100 mg l^{-1} , pH=5) Temperature (^{0}C): ● 30, ▲ 35, ○ 40, □ 45, Δ 50

Variations of percent Cu(II) removals with time are depicted in Figure 5.13 for different temperature. The percentage of adsorption increased from 82 to 97 % with the rise in temperature from 30 to 50 ^{o}C.

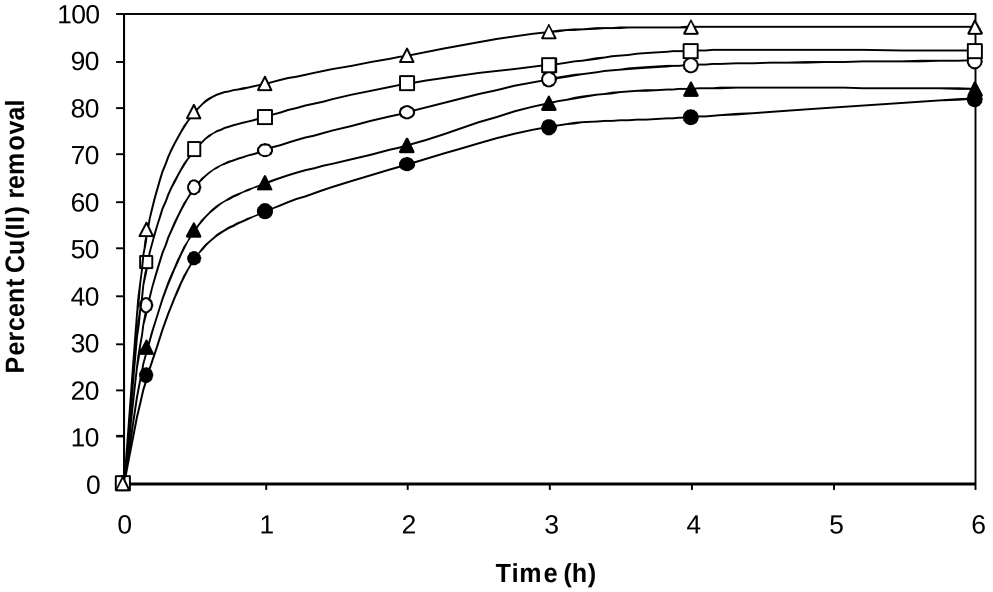


Figure 5.13 Variation of percent Cu(II) removals with time for different temperature (PWS=1 g l^{-1}, D_p=64 µm, Cu(II)=100 mg l^{-1}, pH=5) Temperature (^{0}C): ● 30, ▲ 35, ○ 40, □ 45, Δ 50

5.1.3.6 Effects of Agitation Speed (rpm)

Pretreated PWS of 1g l^{-1} and Cu(II) of 100 mg l^{-1} were used in this set of experiments at different agitation speeds ranging from 50 to 200 rpm. This set of experiments were done with 64 µm particle size of PWS at pH=5 and 25 ^{o}C and the effects of agitation speed on adsorption of Cu(II) ions were investigated.

Variations of the biosorbed Cu(II) ion concentrations with time are depicted in Figure 5.14 for different agitation speeds. Biosorbed copper ion concentrations increased with time and reached equilibrium after six hours of incubation for all agitation speeds. As shown in Figure 5.14, the uptake of copper(II) ion increased with the increasing agitation speed. At low agitation speed such as 50 and 75 rpm, some of the biosorbent precipitated at the bottom of the shake flask yielding low solid phase Cu(II) ion concentrations such as 35 and 48 mg g^{-1} because of the insufficient agitation speed. At agitation speeds such as 150 and 200 rpm, the best homogeneity for the mixture suspension was observed and high solid phase Cu(II) concentrations such as 82 and 85 mg g^{-1} were obtained.

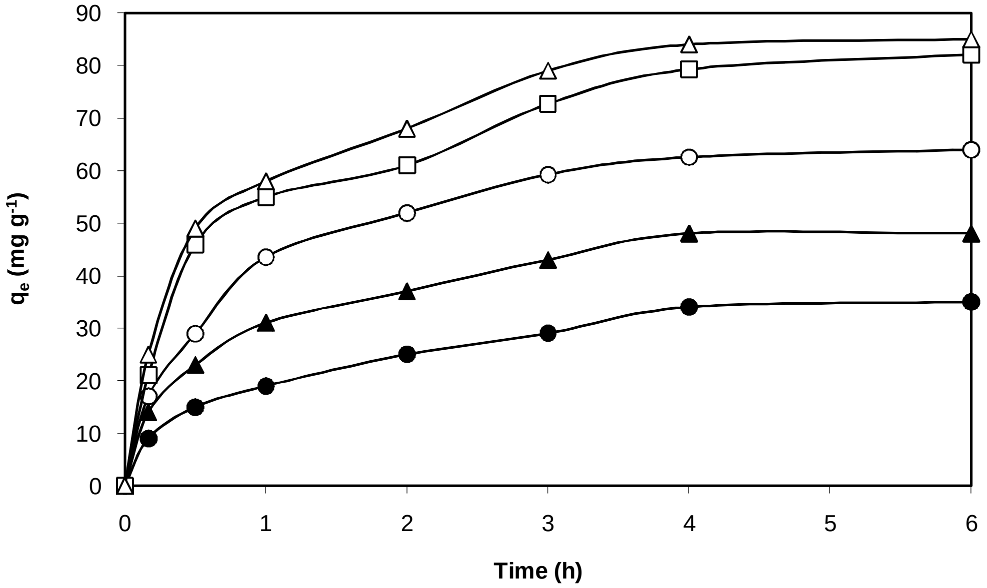


Figure 5.14 Variation of biosorbed Cu(II) ion concentration with time for different agitation speed. (PWS=1 g l^{-1}, D_p=64 µm, Cu(II)=100 mg l^{-1} , pH=5) ● 50 rpm, ▲ 75 rpm, ○ 100 rpm, □ 150 rpm, Δ 200 rpm

Percent Cu(II) removals with time for each agitation speed as plotted in Figure 5.15. Percent Cu(II) removals increased with increasing agitation speed. The highest Cu(II) ion removal capacity of PWS (85 %) was obtained at the agitation speed of 200 rpm. Increasing agitation speed prevents the precipitation of biosorbent and provides a good homogeneous mixing and more than Cu(II) ions get biosorbed on PWS surfaces.

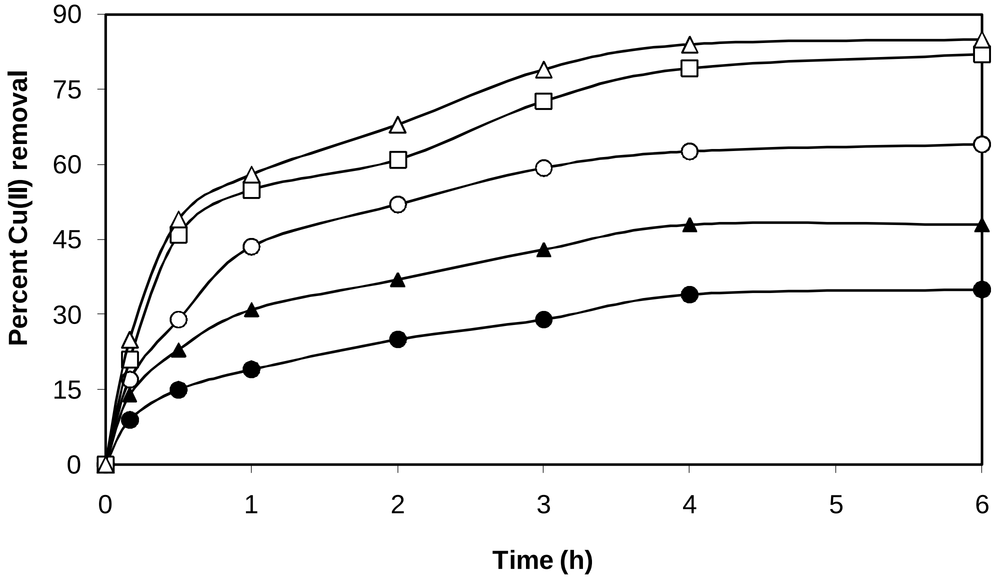


Figure 5.15 Variation of percent Cu(II) removals with time for different agitation speed (PWS = 1 g l^{-1} , D_p=64 µm, Cu(II)=100 mg l^{-1} , pH=5) ● 50 rpm, ▲ 75 rpm, ○ 100 rpm, □ 150 rpm, Δ 200 rpm

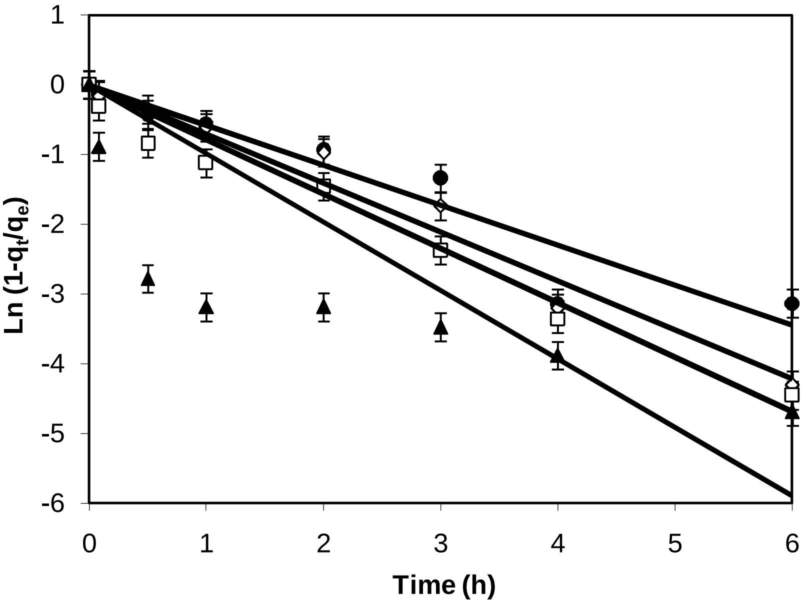
5.1.4 Kinetics of Biosorption of Copper (II) Ions onto PWS

In this part of experiments, the effects of operating parameters such as pH, temperature, Cu(II) ion and the adsorbent concentrations and particle size on batch biosorption kinetics of copper (II) ions onto pre-treated powdered waste sludge (PWS) were investigated. Pseudo-first and second order kinetics were applied for correlation of pre-equilibrium biosorption data and the rate constants were determined. The pseudo-second order kinetics were found to be more suitable for representation of the kinetic data for almost all cases.

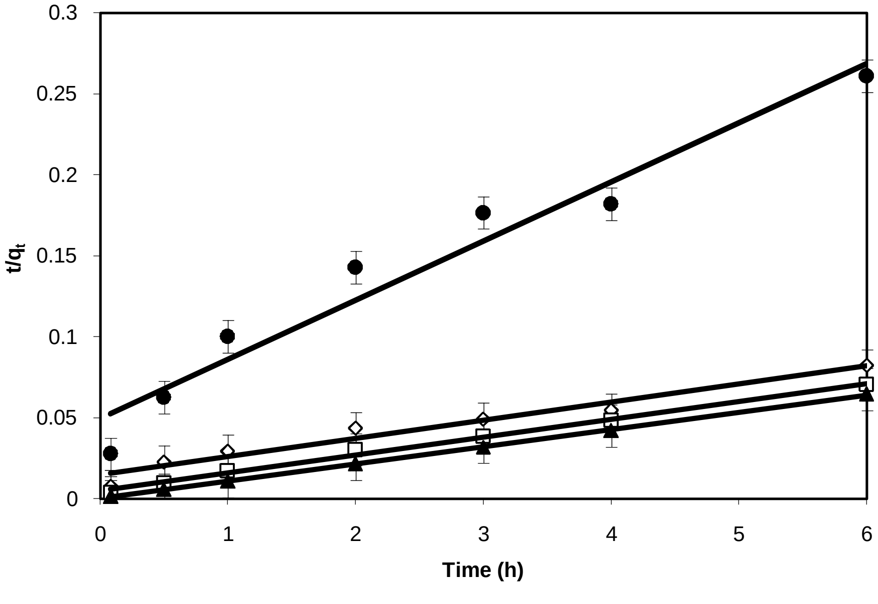
5.1.4.1 Effects of Initial pH

pH is an important parameter in biosorption of metal ions since variations in pH affect the surface charge of biosorbent and also the solubility of metal ions. Cu(II) ions are known to precipitate in form of hydroxides at pH values above 5. For this reason the effects of initial pH on biosorption of Cu(II) ions onto PWS were investigated for pH values between 3 and 6 while PWS and Cu(II) concentrations were 1 g l^{-1} and 100 mg l^{-1}, respectively with PWS particle size of 109 µm. pH variations during biosorption experiments were negligible (± 0.2 pH units).

The kinetic constants for the pseudo-first and second order models were determined from the slopes and intercepts of the lines in Figure 5.16 for different pH values. Deviations from a line at pH 6 is due to precipitation of $Cu(OH)_2$. The pseudo-second order kinetic model (R^2 = 0.95-0.99) was found to fit the experimental data better than the first order model (R^2 = 0.79-0.95) because of higher correlation coefficients. Pseudo-second order model was found to be more suitable since adsorption of Cu(II) ions onto PWS involves both the adsorbent and the adsorbate molecules and the rate depends upon concentrations of both the adsorbent and the adsorbate.



(a)



(b)

Figure 5.16 (a) A plot of Ln (1- q_t / q_e) versus time according to the pseudo-first order kinetics, (b) A plot of t/q_t versus time according to the pseudo-second order kinetics at different pH's. pH: ● 3, ◊ 4, □ 5, ▲ 6

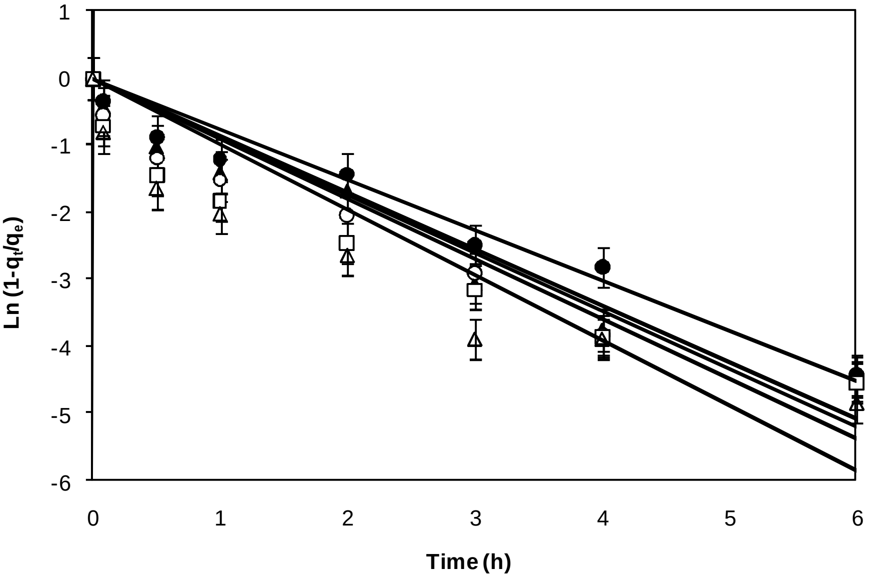
Table 5.3 Variation of the pseudo-first and second order rate constants with pH

pH	3	4	5	6
k_1 (h^{-1})	0.47	0.58	0.85	1.22
R^2	0.88	0.94	0.95	0.79
k_2(mg/g)$^{-1}$ h^{-1}	0.012	0.013	0.024	0.097
R^2	0.95	0.98	0.99	0.99

Variations of the first and second order rate constants (k_1 and k_2) with pH are presented in Table 5.3. Both rate constants increased with increasing pH due to increasingly negative charges on PWS surfaces and lower (H^+) ion concentrations competing with the Cu(II) ions at high pH values. The first and the second order rate constants were 0.85 h^{-1} and 0.024 $(mg/g)^{-1}.h^{-1}$, respectively at pH 5 which dropped to 0.47 h^{-1} and 0.012 $(mg/g)^{-1}.h^{-1}$ at pH 3. Considerable increases in the rate constants were observed at pH 6 and above due to precipitation of Cu(II) ions along with biosorption.

5.1.4.2 Effects of Temperature

Batch biosorption experiments were performed at different temperatures between 30 and 50 ^oC using a temperature controlled gyratory shaker with 1 g l^{-1} PWS of particle size 64 µm, Cu(II) of 100 mg l^{-1} at pH 5. The kinetic constants for the pseudo-first and second order models were determined from the slopes and intercepts of the lines in Figure 5.17 for different temperatures. On the basis of the correlation coefficients, the pseudo-second order kinetic model (R^2 = 0.99) was found to represent the experimental data better than the first order model (R^2 = 0.76-0.9). Second order model represented the data better because of interactions between the adsorbate and adsorbent like a bimolecular reaction.



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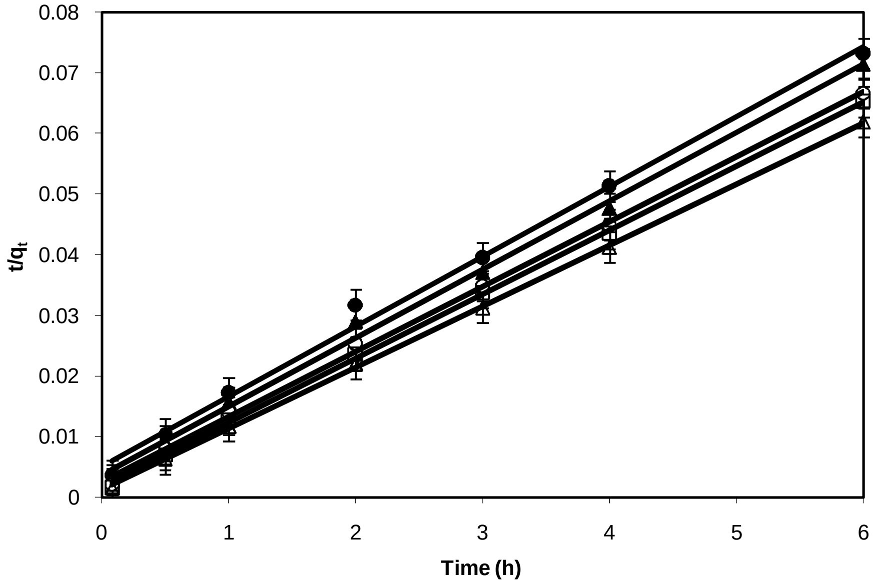


(b)

Figure 5.17 (a) A plot of Ln(1-q_t/q_e) versus time according to the pseudo-first order kinetics. (b) A plot of t/q_t versus time according to the pseudo-second order kinetics at different temperatures. Temperature (^{o}C): ● 30, ▲ 35, ○ 40, □ 45, Δ 50

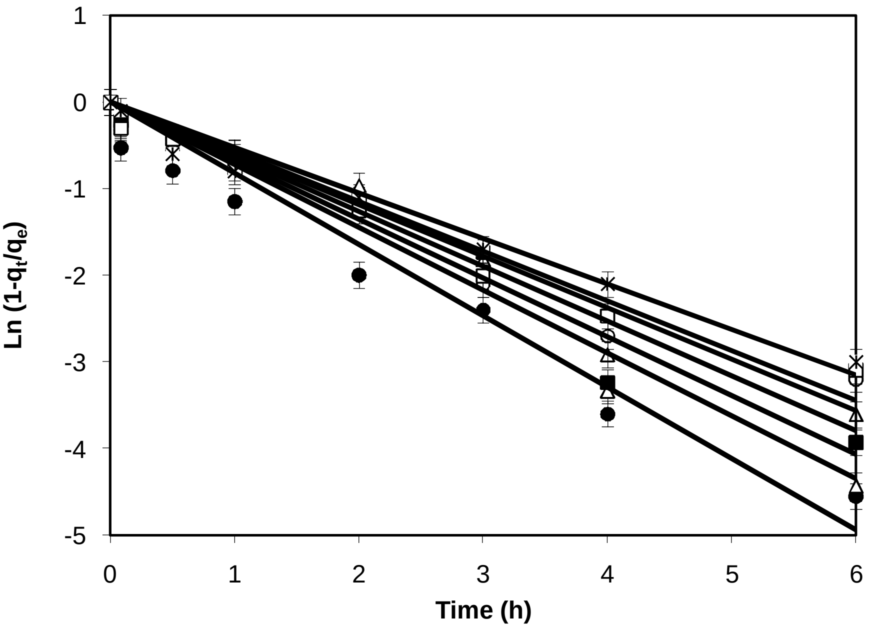
Table 5.4 Variation of the pseudo-first and second order rate constants with temperature

Temperature(^oC)	30	35	40	45	50
k_1 (h^{-1})	0.87	1.08	1.12	1.19	1.34
R^2	0.88	0.89	0.9	0.81	0.76
$k_2(mg/g)^{-1}$ h^{-1}	0.026	0.035	0.044	0.066	0.085
R^2	0.99	0.99	0.99	0.99	0.99

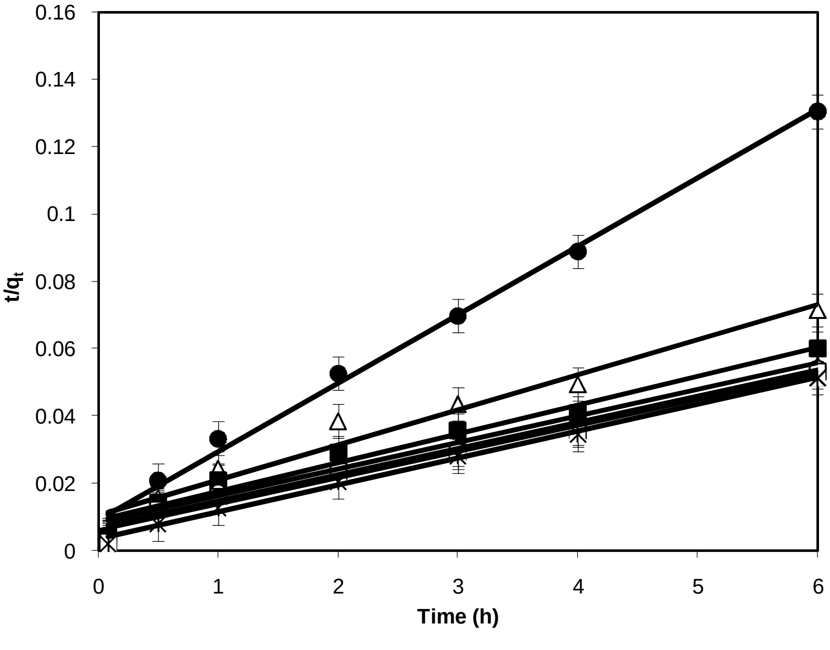
Variations of the first and second order rate constants (k_1 and k_2) with temperature are presented in Table 5.4. Both rate constants incrased with increasing temperature due to higher frequency of interactions (i.e, high energy levels) among the Cu(II) ions and the adsorbent particles at high temperatures. The first and the second order rate constants increased from 0.87 h^{-1} and 0.026 $(mg/g)^{-1}.h^{-1}$ to 1.34 h^{-1} and 0.085 $(mg/g)^{-1}.h^{-1}$ when the temperature was increased from 30 to 50 ^oC, respectively. The activation energies for the biosorption were determined from the slopes of the lines of Ln k versus 1/T plots (Arrhenius plots) which were 3.750 cal mol^{-1} (3.75 kcal mol^{-1}) and 11,690 cal mol^{-1} (11.69 kcal mol^{-1}) for the pseudo-first and second order kinetics, respectively. Apparently, second-order kinetics was more sensitive to temperature variations as compared to the first-order kinetics, since the rate depended upon concentrations and energy levels of of both the adsorbate and the adsorbent.

5.1.4.3 Effects of Copper(II) Concentration

Copper(II) ion concentrations were varied between 50 and 400 mg l^{-1} in this set of experiments while the PWS concentration, particle size and pH were constant at 1 g l^{-1}, 64 µm and pH = 5, respectively. The kinetic constants for the pseudo-first and second order models were determined from the slopes and intercepts of the lines in Figure 5.18 for different Cu(II) concentrations. Both pseudo-first and second order kinetic models were found to be suitable for representation of the experimental data with comparable correlation coefficients for variable Cu(II) ion concentrations.



(a)



(b)

Figure 5.18 (a) A plot of Ln (1-q_t/q_e) versus time according to the pseudo-first order kinetics, (b) A plot of t/q_t versus time according to the pseudo-second order for different Cu(II) ion concentrations. ● 50 mg l^{-1}, ∆ 100 mg l^{-1}, ■ 150 mg l^{-1}, ▲ 200 mg l^{-1}, ○ 250 mg l^{-1}, □ 300 mg l^{-1}, ◊ 400 mg l^{-1}.

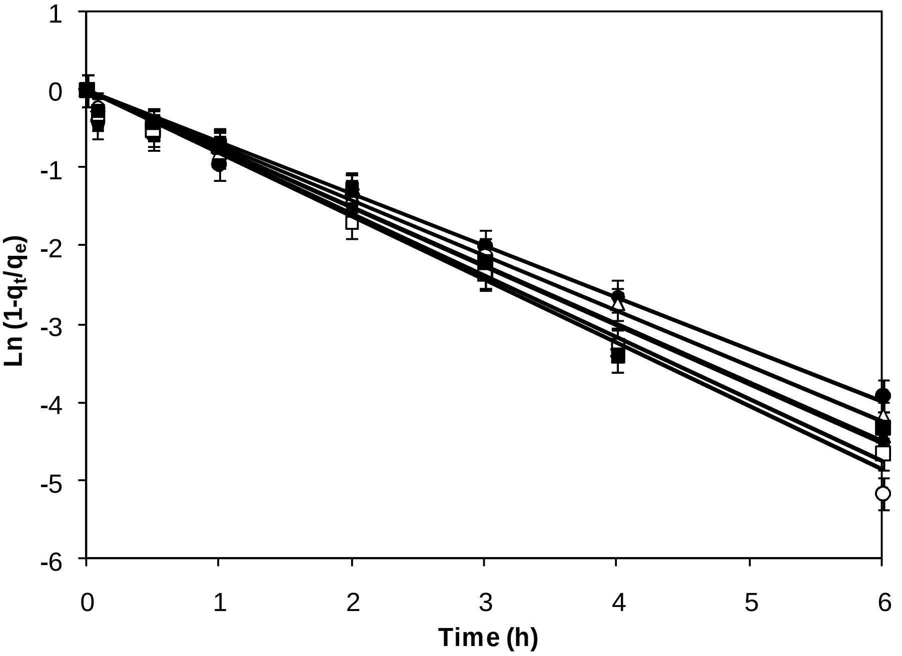
Table 5.5 Variation of the pseudo-first and second order rate constants with Cu(II) concentration.

Cu(II) mg l^{-1}	50	100	150	200	250	300	400
k_1 (h^{-1})	0.822	0.724	0.677	0.632	0.594	0.574	0.525
R^2	0.95	0.96	0.97	0.98	0.96	0.96	0.97
k_2(mg/g)$^{-1}$ h^{-1}	0.0591	0.0232	0.0136	0.0113	0.0110	0.0109	0.0100
R^2	0.99	0.93	0.94	0.91	0.92	0.93	0.99

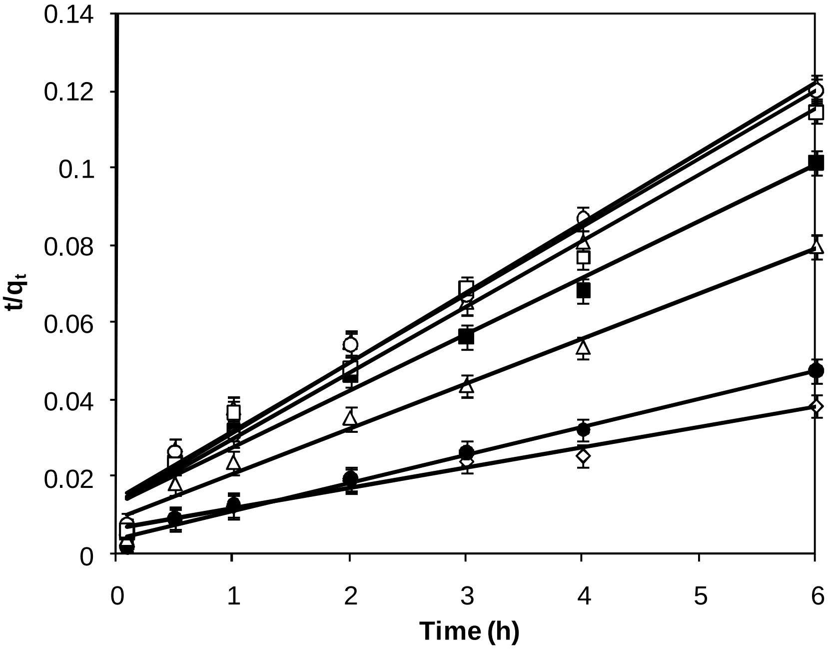
Variations of the pseudo first and second order rate constants with initial Cu(II) concentration are summarized in Table 5.5. The rate constants for both the first and second order models decreased with increasing initial Cu(II) concentrations due to competition among the Cu(II) ions for the same binding sites on PWS surfaces at high Cu(II) concentrations (hindering effect). The first and second order rate constants decreased from 0.822 h^{-1} and 0.059 (mg/g)$^{-1}$ h^{-1} to 0.525 h^{-1} and 0.010 (mg/g)$^{-1}$ h^{-1} when the Cu(II) concentration increased from 50 to 400 mg l^{-1}.

5.1.4.4 Effects of Adsorbent (PWS) Concentration

The biosorbent (PWS) concentration was varied between 0.25 and 3.0 g l^{-1} in this set of experiments while the initial Cu(II) ion concentration, PWS particle size and pH were constant at 200 mg l^{-1}, 64µm and pH 5, respectively. The kinetic constants for the pseudo-first and second order models were determined from the slopes and the intercepts of the lines in Figure 5.19 for different PWS concentrations. The second order kinetic model (R^2 = 0.98-0.99) represented the experimental data better than the first order model (R^2 = 0.79-0.95) with higher correlation coefficients since adsorption involves interactions between the adsorbate and the adsorbent.



(a)



(b)

Figure 5.19 (a) A plot of Ln (1-q_t/q_e) versus time according to the pseudo-first order kinetics, (b) A plot of t/q_t versus time according to the pseudo-second order kinetics for different PWS concentrations. PWS (g l^{-1}): ◊ 0.25, ● 0.5, Δ 1, ■ 1.5, ▲ 2, ○ 2.5, □ 3.

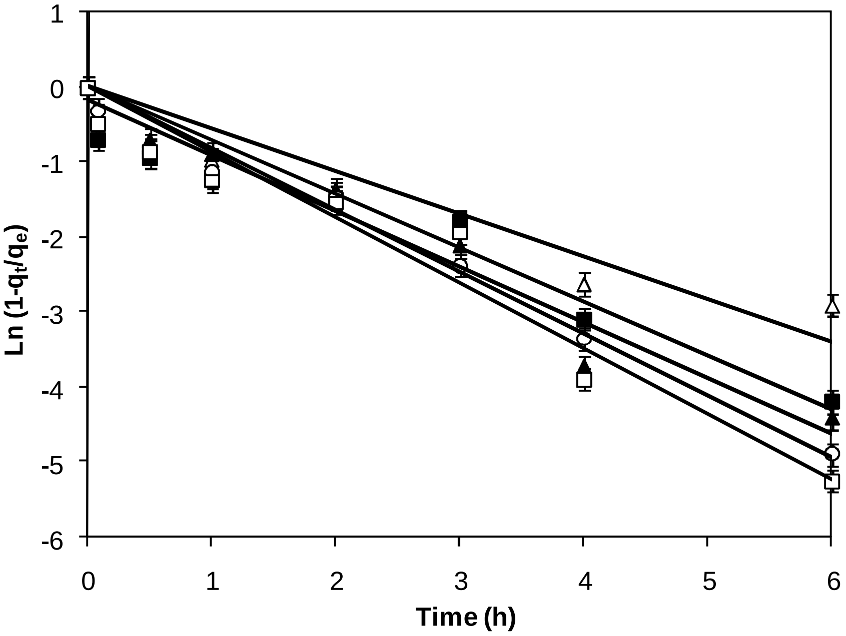
Table 5.6 Variation of the pseudo-first and second order rate constants with the adsorbent (PWS) concentration

PWS, gl^{-1}	0.25	0.50	1.0	1.5	2.0	2.5	3.0
k_1 (h^{-1})	0.586	0.663	0.707	0.748	0.754	0.811	0.792
R^2	0.95	0.96	0.94	0.92	0.92	0.93	0.79
$k_2(mg/g)^{-1}\,h^{-1}$	0.0042	0.0126	0.0149	0.0166	0.0217	0.0246	0.0223
R^2	0.99	0.99	0.98	0.98	0.98	0.99	0.98

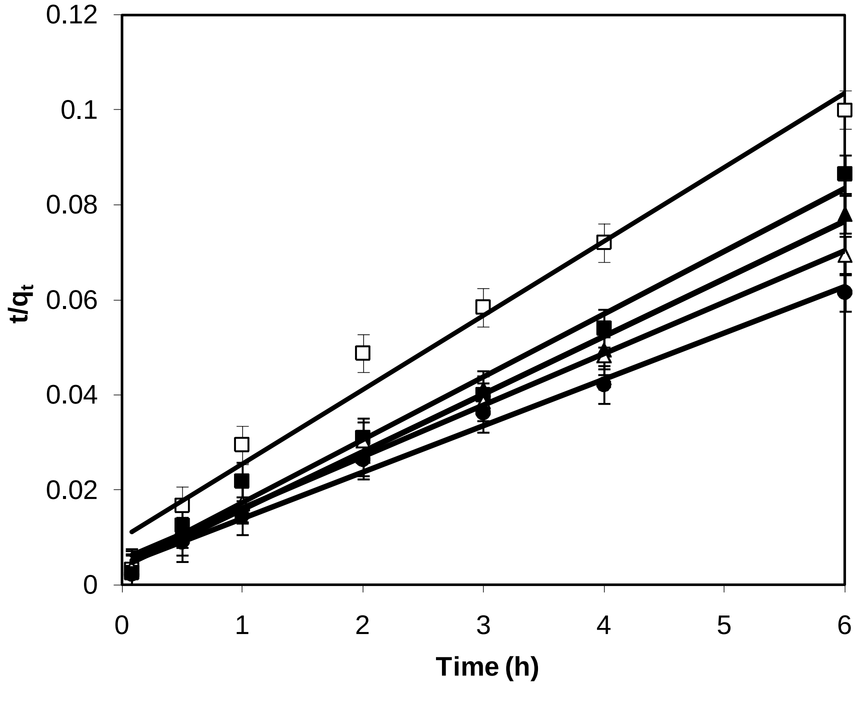
Variations of the first and second order rate constants (k_1 and k_2) with PWS concentrations are summarized in Table 5.6. Both the first and the second order rate constants increased with the adsorbent (PWS) concentration due to increased binding sites at high adsorbent concentrations. The first and second order rate constants increased from 0.586 h^{-1} and 0.0042 to 0.792 h^{-1} and 0.0223 $(mg/g)^{-1}\,h^{-1}$, respectively with the equilibrium biosorption capacities (q_e) of 188 and 59 mg g^{-1}, as the PWS concentrations increased from 0.25 to 3 g l^{-1}.

5.1.4.5 Effects of Particle Size of the Adsorbent

The kinetics of biosorption of Cu(II) ions onto pre-treated PWS samples were investigated for five different particle sizes of lower then 53µm, 64µm (53-75µm), 109µm (75-143µm), 178µm (143-212µm) and 231µm (212-251µm). The kinetic constants for the pseudo-first and second order models were determined from the slopes and the intercepts of the lines in Figure 5.20 for different partical sizes of PWS. Again the second order kinetic model (R^2 = 0.98-0.99) was found to fit the experimental data better than the first order kinetic model (R^2 = 0.83-0.96) since adsorption involves interactions between the adsorbent and the adsorbate like biomolecular reactions.



(a)



(b)

Figure 5.20 (a) A plot of Ln (1-q_t /q_e) versus time according to the pseudo-first order kinetics. (b) A plot of t /q_t versus time according to the pseudo-second order kinetics for different particle sizes. D_p(μm): ● 53, Δ 64, ▲ 109, ■ 178, □ 231μm.

Table 5.7 Variation of the pseudo-first and second order rate constants with the adsorbent (PWS) particle size (D_p)

D_p(µm)	53	64	109	178	231
k_1 (h^{-1})	0.808	0.791	0.783	0.718	0.560
R^2	0.93	0.96	0.96	0.91	0.83
$k_2(mg/g)^{-1}\ h^{-1}$	0.0309	0.0293	0.0287	0.0224	0.0223
R^2	0.98	0.99	0.99	0.99	0.99

Variations of the pseudo- first and second order rate constants (k_1 and k_2) with the particle size are presented in Table 5.7. The rate constants for both models increased with decreasing particle size due to larger total surface area of particles at small particle sizes. The highest first and second order rate constants were 0.808 h^{-1} and 0.0309 $(mg/g)^{-1}h^{-1}$ respectively with a particle size of 53µm (for which q_e was 96 mg g^{-1}) which dropped to 0.560 h^{-1} and 0.0223 $(mg/g)^{-1}h^{-1}$ for an average particle size of 231µm (for which q_e was 53 mg g^{-1}).

As compared to the literature studies on Cu(II) biosorption onto different adsorbents, the Cu(II) biosorption capacity of the pre-treated waste sludge (PWS) used in this study is superior to the others reported (Arican B. *et al.*, 2002 - Bux F., *et al.*, 1999 - Kargi F. & Cikla S., 2006). This is due to pre-treatment by 1% hydrogen peroxide and particle size reduction or surface area enlargement of the PWS used. It is thought that as a strong oxidant, H_2O_2 activates some functional groups on the surface of PWS and therefore improves the biosorption capacity of PWS surfaces for Cu(II) ions.

The rate and extent of biosorption depends on concentrations of adsorbent and the adsorbate as well as the environmental conditions (pH, temperature, ionic strength, particle size). At high Cu(II) and low PWS concentrations the rate is limited by the availability of PWS surfaces or binding sites. On the contrary, the limitation is due to low Cu(II) concentration at high PWS and low Cu(II) concentrations. There is usually an optimal adsorbate/adsorbent ratio maximizing the rate and extent of biosorption. The optimal adsorbate/adsorbent ratio or the highest Cu(II) biosortion capacity (156 mg Cu g^{-1}PWS) was obtained with PWS and Cu(II) concentrations of

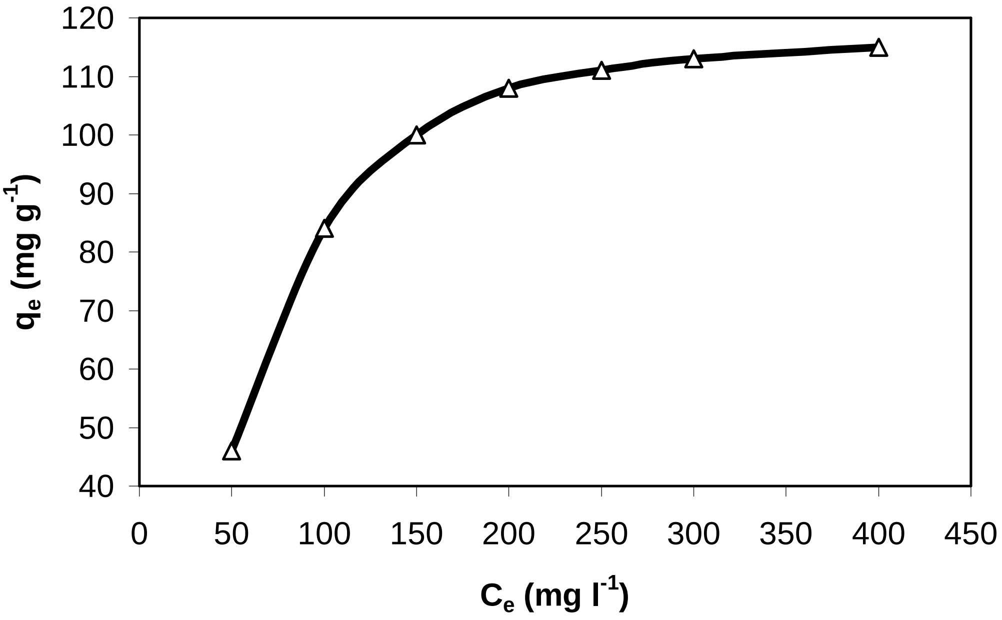
0.25 g l^{-1} and 100 mg l^{-1} with the lowest particle size (< 53 μm). The optimum initial adsorbate/adsorbent ratio was 100/0.25 = 400 mg Cu (II) g PWS^{-1}

5.1.5 Isotherm Studies

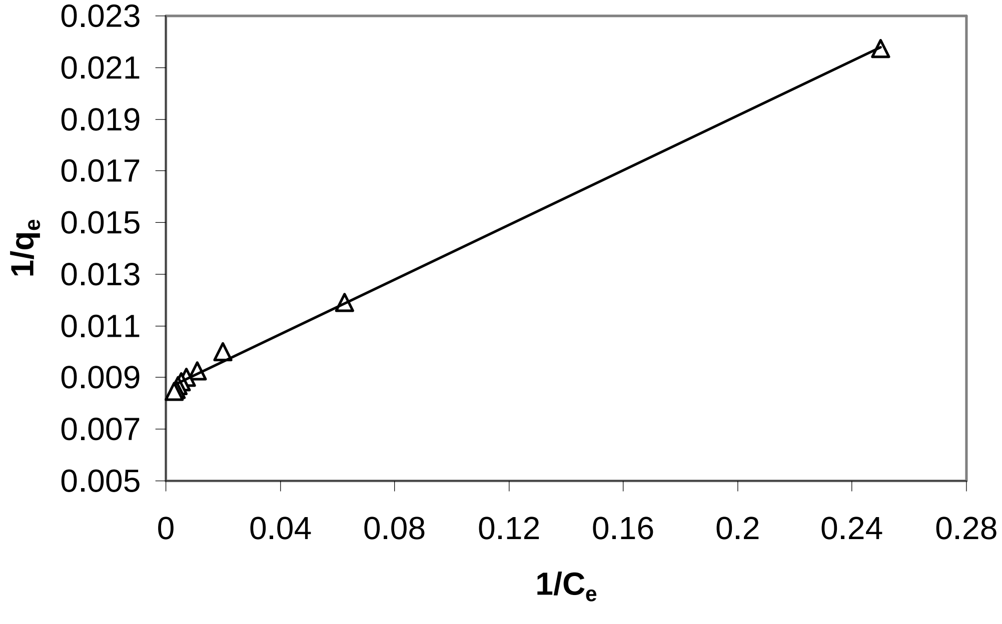
The equilibrium data were correlated with the Langmuir, Freundlich and the generalized isotherms. The isotherm data were obtained with variable Cu(II) concentrations while the PWS concentration was constant. The plots of equilibrium concentrations of Cu(II) ions in the solid and aqueous phases, that is q_e versus C_e and also $1/q_e$ versus $1/C_e$ are presented in Figures 5.21.a and 5.21.b, respectively to test the validity of the Langmuir isotherm. From the slope and intercept of the line in Figure 5.21.b, the Langmuir constants were found as

$$q_m = 116.3 \text{ mg g}^{-1}, \quad K = 6.14 \text{ mg l}^{-1} \qquad (R^2 = 0.998)$$

Apparently, equilibrium biosorption data of Cu(II) ions fit to the Langmuir isotherm very well ($R^2 = 0.998$). In fact, the hyperbolic shape of the graph (q_e versus C_e) in Figure 5.21.a also indicated that the Langmuir isotherm represented the equilibrium data quite well.



(a)



(b)

Figure 5.21 (a) Variation of equilibrium biosorbed Cu(II) ion concentration with the equilibrium aqueous phase Cu(II) concentration. (b) Langmuir isotherm plot of $1/q_e$ versus $1/C_e$. (PWS=1 g l^{-1}, D_p=64µm, pH=5)

Figure 5.22 depicts a plot of Ln q_e versus Ln C_e for Cu(II) ion biosorption onto the PWS to test the validity of the Freundlich isotherm. From the slope and intercept of the line, the following Freundlich isotherm constants were found,

K= 40 mg g^{-1} (mg $l^{-1})^{1/n}$, 1/n = 0.21 (n= 4.76) (R^2 = 0.89)

Apparently, the Freundlich isotherm did not represent the equlibrium data as well as the Langmuir isotherm.

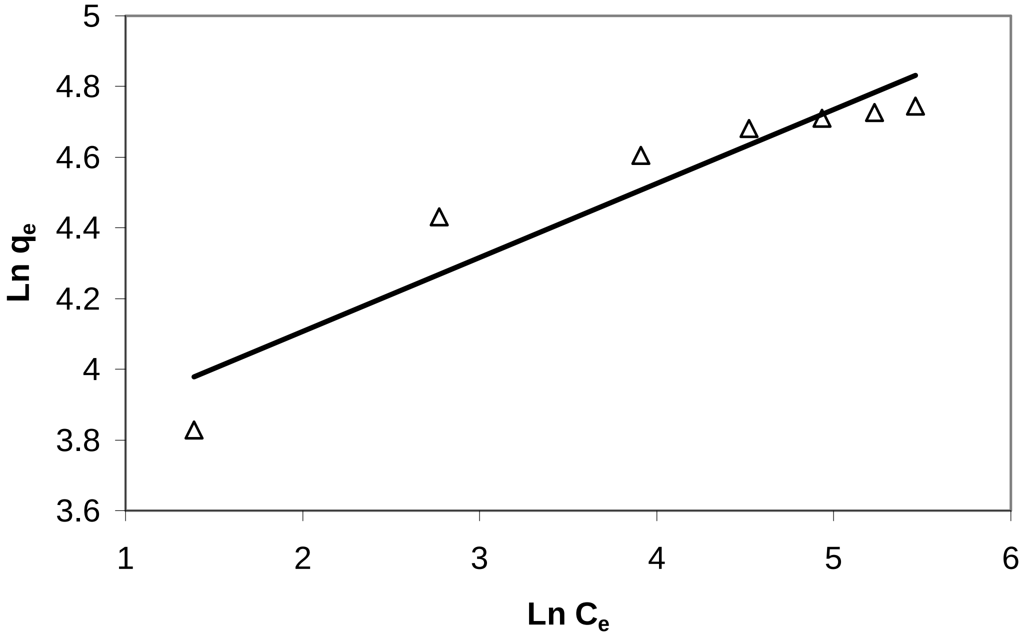


Figure 5.22 Freundlich isotherm plot of Lnq_e versus LnC_e for biosorption of Cu(II) ions onto pre-treated PWS. (PWS=1 g l^{-1}, D_p=64µm, pH=5)

A plot of the equilibrium data in form of Ln ((q_m / q_e) -1) versus Ln C_e is depicted in Figure 5.23 to test the validity of the generalized isotherm equation. The q_m value was taken as q_m = 116.3 mg g^{-1} as determined from the Langmuir isotherm. From the slope and intercept of the line presented in Figure 5.23, the following values were found for the constants of the generalized biosorption isotherm.

K = 17.65 (mg $l^{-1})^n$, n = 1.152 (R^2 = 0.987)

Apparently, the generalized adsorption isotherm represents the equilibrium data reasonably well, but the fit is not as good as the Langmuir isotherm. Since the exponent (n) was close to 1 (n = 1.15), the generalized isotherm approximates to the Langmuir expression.

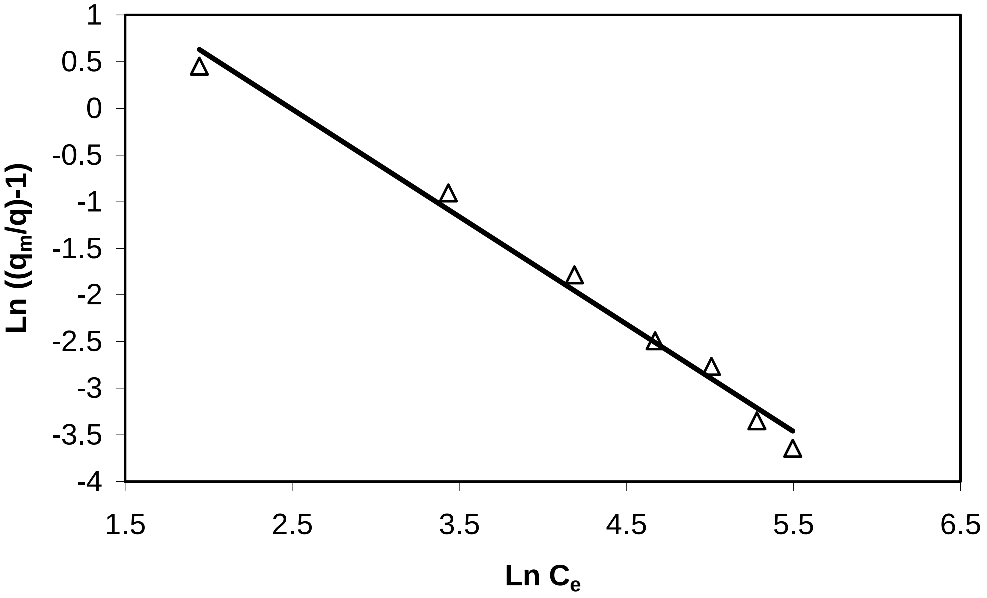


Figure 5.23 Generalized biosorption isotherm plot of Ln((q_m/q))-1) versus Ln C_e for Cu(II) ion biosorption onto PWS. (PWS=1 g l^{-1}, D_p=64µm, pH=5)

Table 5.8 Summary of the isotherm constants and the correlation coefficients for different isotherms

Parameter	**Langmuir Isotherm**	**Freundlich Isotherm**	**Generalized Isotherm**
q_m (mg g^{-1})	116.3		116.3
K	6.14	40	17.65
n		4.76	1.152
R^2	0.998	0.89	0.987

Table 5.8 summarizes the results of the isotherm constants for the three different equilibrium isotherms tested. On the basis of the correlation coefficients (R^2), Langmuir isotherm seemed to represent the equilibrium adsorption data with better fit as compared to the other isotherms. However, the generalized isotherm was also satisfactory for representation of the equilibrium data.

As compared to the literature studies on biosorption of Cu(II) ions onto waste activated sludge (R. Gourdon et al, 1994; Hammaini A. et al, 2002; Bakkaloglu I. et al, 1998; Gulnaz et al, 2005) the maximum biosorption capacity (q_m) obtained in this study (116 mg g^{-1}) is superior to the literature values which vary between 10 and 80 mg g^{-1}. The saturation constant for the Langmuir isotherm (K= 6.14 mg l^{-1}) is also considerably lower than those reported in literature on copper biosorption (Bakkaloglu I. et al, 1998) indicating high affinity of the waste sludge used for Cu(II) biosorption. This is probably because of the selection of a better waste sludge, pre-treatment with 1% H_20_2 and also the small particle size (64µm) of PWS used in this study.

5.2 Experiments with Fed–Batch Operation

In this part of experiments, the effects of operating parameters on the performance of a fed-batch operated biosorption system (Figure 3.1) were investigated. These operating parameters were biosorbent content, feed flow rate, feed Cu(II) ion concentrations and the amount of adsorbent (PWS). Both biosorption capacity of PWS and the rate constant were determined for the Cu(II) ion biosorption by using the modified Bohart-Adams equation.

5.2.1 Effect of Biosorbent Content on the Performance of the System

A set of fed-batch biosorption experiments were carried out with six different amounts of adsorbent (PWS) between 1 and 6 g while the feed Cu(II) ion concentration, and the feed flow rate were constant at 200 mg l^{-1}, 0.25 l h^{-1}, respectively throughout the experiments yielding a constant adsorbate (Cu ion) loading rate of 50 mg h^{-1}. A control tank was operated under the same conditions as the adsorption tank in the absence of PWS with no copper ion adsorption. Percent copper ion removals in the experimental tank were based on the copper ion concentrations in the feed ($E = 1- C/C_o$).

Figure 5.24 depicts break-through curves describing variations of copper ion concentrations with time in the adsorption reactor for different adsorbent (PWS) contents in fed-batch experiments. Copper ion concentrations increased with time due to increased occupation of binding sites on PWS surfaces for all PWS concentrations. The effluent copper ion concentrations decreased with increasing amounts of the adsorbent (PWS) due to larger surface area or binding sites on PWS surfaces at high PWS concentrations. Final copper concentration at the end of 10 h operation decreased from 145 mg l^{-1} to 60 mg l^{-1} when PWS was increased from 1 g to 6 g. The extent of copper biosorption was limited by the availability of the binding sites on the biosorbent (PWS) surfaces yielding high residual copper ions in solution at low biosorbent contents such as 1 and 2 g. At high biosorbent contents such as 5 and 6 g PWS, the binding sites on the biosorbent surfaces were in excess of copper ions in solution and large fractions of copper ions were biosorbed onto PWS surfaces resulting in low residual copper concentrations of 69 and 60 mg l^{-1}, respectively. Figure 5.24 was used for determination of breakthrough times for different operations to obtain C_b= 10 mg l^{-1} in the effluent.

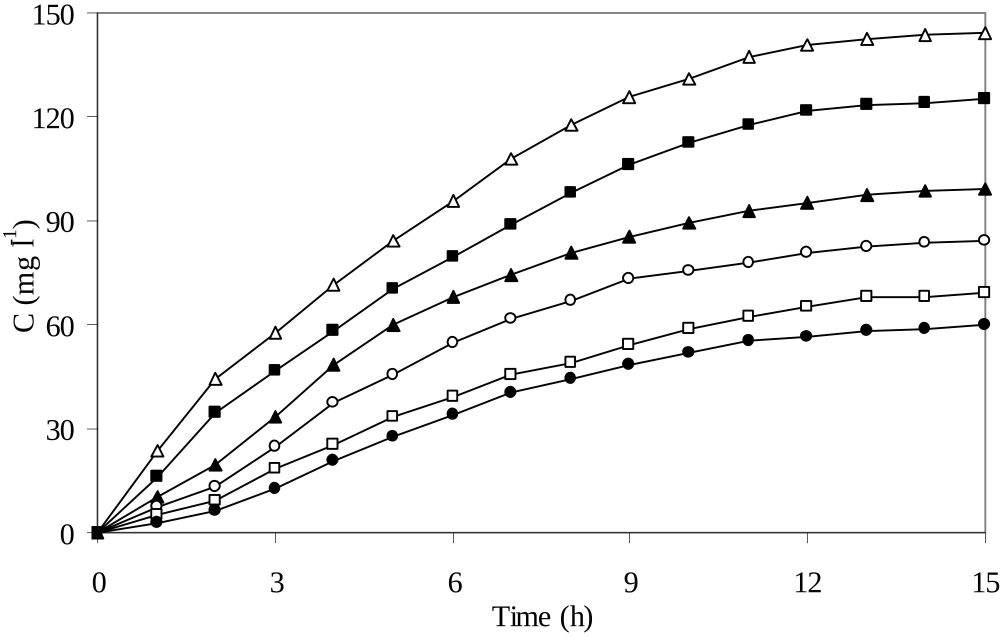


Figure 5.24 Breakthrough curves for copper ion adsorption onto PWS for different PWS contents in fed-batch operation. (C_o = 200 mg l^{-1}, Q = 0.25 l h^{-1}) PWS (g): Δ 1, ■ 2, ▲ 3, ○ 4, □ 5, ● 6

Variations of solid phase copper ion concentrations and percent copper ion removals at the end of 10 hours of operation with the amount of adsorbent (PWS) used are depicted in Figure 5.25. Percent copper ion removals increased with increasing mass of PWS due to increased binding sites on the adsorbent. Unlike percent copper ion removals from the aqueous phase, biosorbed copper ion concentrations (mg g^{-1}) decreased with increasing mass of biosorbent. This is because of decreased fractional occupation of large surface area of the adsorbent at high adsorbent concentrations while the copper ion loading rate was constant.

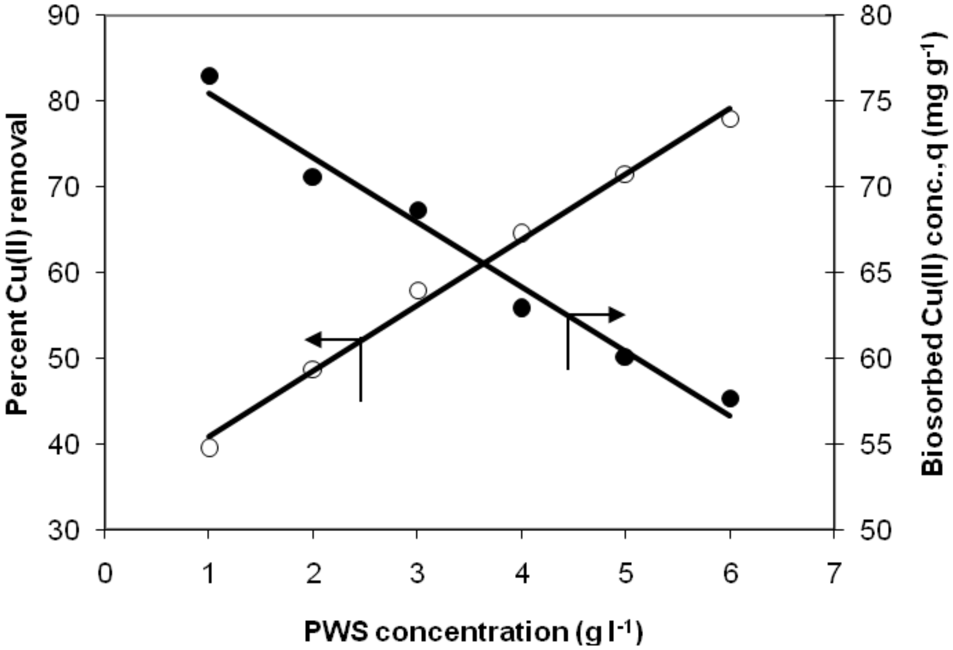


Figure 5.25 Variation of percent copper ion removals and biosorbed (solid phase) copper ion concentrations with the amount of the adsorbent (PWS) at the end of 10 hours fed-batch operation (C_o = 200 mg l^{-1}, Q = 0.25 l h^{-1}).

5.2.2 Effect of Feed Flow Rate on the Performance of the System

In this set of fed-batch adsorption experiments, the feed flow rate was changed between 0.075 l h^{-1} and 0.325 l h^{-1} at six different levels while the feed copper ion concentration and the amount of PWS were constant at 100 mg l^{-1} and 3 g, respectively. A control tank was operated under the same conditions of the experimental tank in the absence of PWS.

Breakthrough curves describing variations of copper ion concentrations in the adsorption reactor with time for different feed flow rates are depicted in Figure 5.26. Copper ion concentrations in aqueous phase increased with time due to increased occupation of binding sites on PWS surfaces. The effluent contained high concentrations of copper ions at high feed flow rates or high copper ion loading rates due to limited number of binding sites on PWS surfaces at constant PWS content of 3 g. Final copper ion concentrations at the end of 10 h operation increased from 1.5 mg

l^{-1} to 41 mg l^{-1} when the feed flow rate was increased from 0.075 to 0.325 l h^{-1}. At low feed flow rates or low copper loading rates (QC_o), the extent of copper biosorption was limited by the availability of copper ions in aqueous phase yielding low residual copper ions in solution since the adsorbent concentration was constant. However, when the feed flow rate or the copper ion loading rate was increased, binding sites on PWS surfaces became saturated yielding high copper ion concentrations in the liquid phase. Figure 5.26 was used for determination of break-through times for different operations to obtain C_b= 10 mg l^{-1} in the effluent.

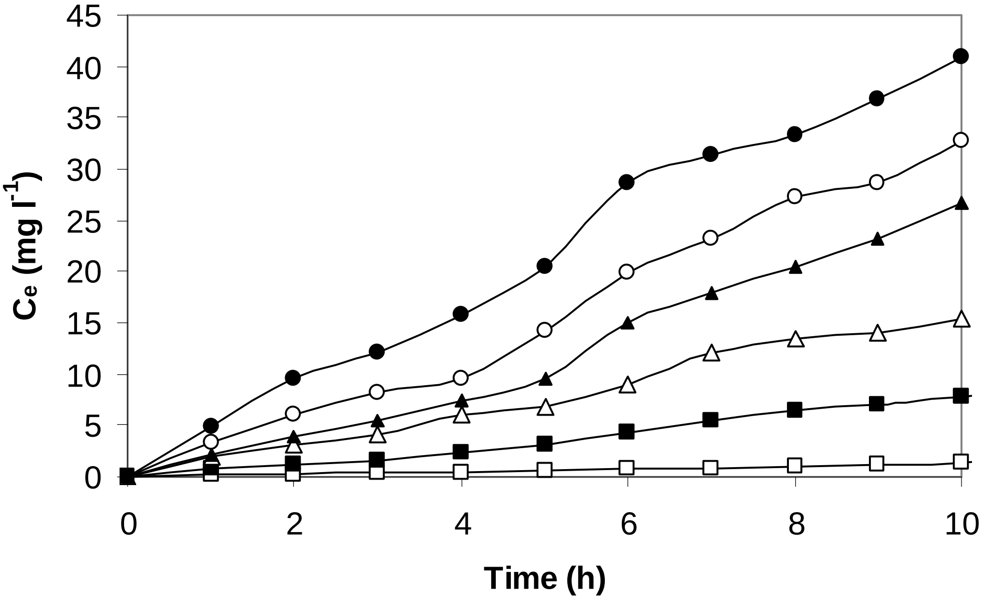


Figure 5.26 Breakthrough curves for copper ion adsorption onto PWS for different feed flow rates of Cu(II) solution in fed-batch operation (C_o=100 mgl^{-1}, PWS=3 gl^{-1}). Flow rates (l h^{-1}): □ 0.075 , ■ 0.125 , Δ 0.175 , ▲ 0.225 , ○ 0.275 , ● 0.325.

Variations of solid phase copper ion concentrations and percent copper ion removals with the feed flow rate at the end of 10 hours of operation are depicted in Figure 5.27. Biosorbed (solid phase) copper ion concentrations increased, but percent copper ion removals decreased with increasing feed flow rate or copper ion loading rate. At low feed flow rates and constant PWS of 3 g, adsorption of copper ions was limited by the concentration of copper ions because of excess binding sites available on PWS surfaces yielding high percent copper ion removals, but low solid phase copper concentrations. However, at high feed flow rates due to high copper ion loading rates the binding sites on PWS surfaces became saturated yielding low percent copper removals from the aqueous phase, but high solid phase copper ion concentrations. In this case, the extent of adsorption was limited by the availability of the binding sites on the adsorbent (PWS) surfaces.

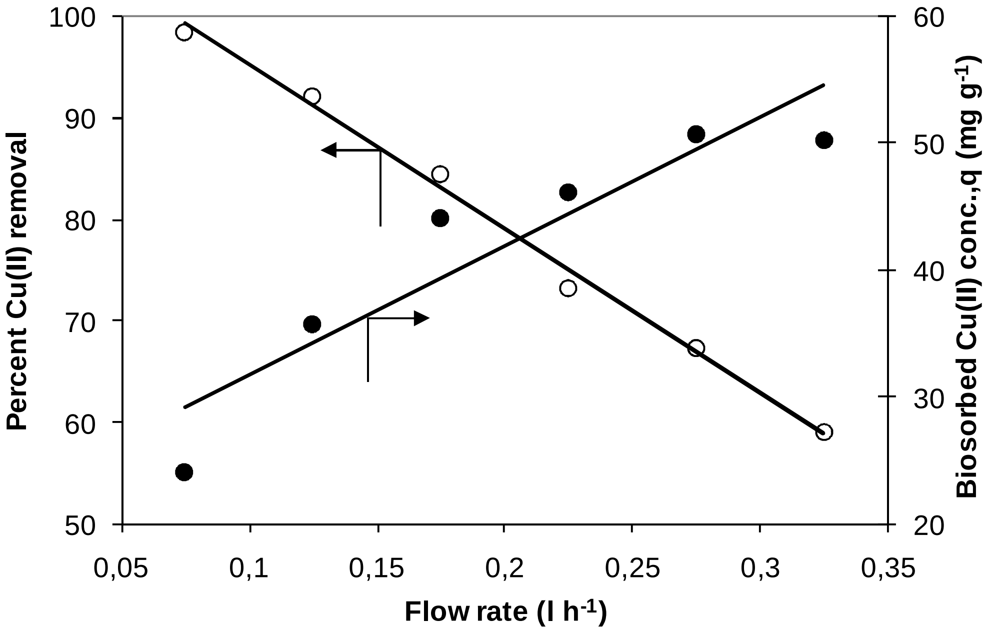


Figure 5.27 Effect of feed flow rate on percent copper ion removals and solid phase Cu(II) concentrations at the end of 10 hours of operation (C_o=100 mgl^{-1}, PWS=3 gl^{-1})

5.2.3 Effect of Feed Cu (II) Ion Concentration on the Performance of the System

In the last set of fed-batch adsorption experiments, the feed copper ion concentration was changed between 50 and 300 mg l^{-1} at six different levels while the feed flow rate and the amount of PWS were kept constant at 0.1 l h^{-1} and 3 g, respectively. A control tank was operated under the same conditions of the experimental tank in the absence of PWS. Percent copper ion removals in control experiments were considered to be zero in calculations.

Breakthrough curves describing variations of copper ion concentrations in the adsorption reactor with time for different feed copper ion concentrations are depicted in Figure 5.28. Copper ion concentrations in aqueous phase increased with time due to continuous feeding and increased occupation of binding sites on PWS surfaces with time. The effluent contained high concentrations of copper ions at high feed copper ion concentrations or high copper ion loading rates (Q C_o) due to limited number of binding sites on PWS surfaces at constant PWS content of 3 g. Final copper concentration at the end of 10 h operation increased from 3 mg l^{-1} to 97 mg l^{-1} when the feed copper ion concentration increased from 50 to 300 mg l^{-1}. At low feed copper ion concentrations or low copper loading rates (QC_o), the binding sites on the adsorbent (PWS) surfaces were in excess of copper ions available in aqueous phase yielding low effluent copper ion concentrations in solution. However, when the feed copper ion concentration or the copper ion loading rate was increased, copper ion concentrations exceeded the binding sites on PWS surfaces yielding high copper ion concentrations in solution. Figure 5.28 was used for determination of breakthrough times for different operations to obtain C_b= 10 mg l^{-1} in the effluent.

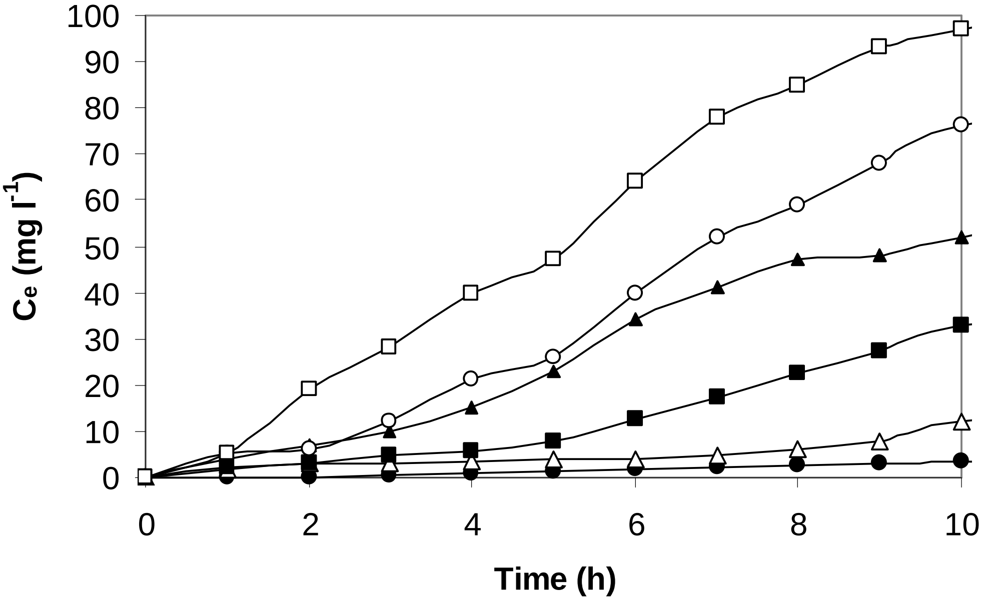


Figure 5.28 Breakthrough curves for copper ion adsorption onto PWS for different feed copper ion concentrations. ● 50 mg l^{-1}, Δ 100 mg l^{-1}, ■ 150 mg l^{-1} ▲ 200 mg l^{-1},○ 250 mg l^{-1}, □ 300 mg l^{-1}. (Q = 0.1 l h^{-1}, PWS = 3 g l^{-1})

Variations of solid phase copper ion concentrations (q) and percent copper ion removals at the end of 10 hours of operation with the feed copper ion concentration are depicted in Figure 5.29. Biosorbed (solid phase) copper ion concentrations increased, but percent copper ion removals decreased with increasing feed copper ion concentration due to increased loading of copper ions and limited number of binding sites on PWS surfaces. At low feed copper ion concentrations and constant PWS of 3 g, adsorption of copper ions was limited by the availability of copper ions in solution since the binding sites available on PWS surfaces were in excess of copper ions present in solution. However, at high feed flow rates due to high copper ion loading rates the binding sites on PWS surfaces were occupied and extent of adsorption was limited by the availability of the binding sites on the adsorbent (PWS) surfaces yielding low percent removals, but high biosorbed copper ion concentrations.

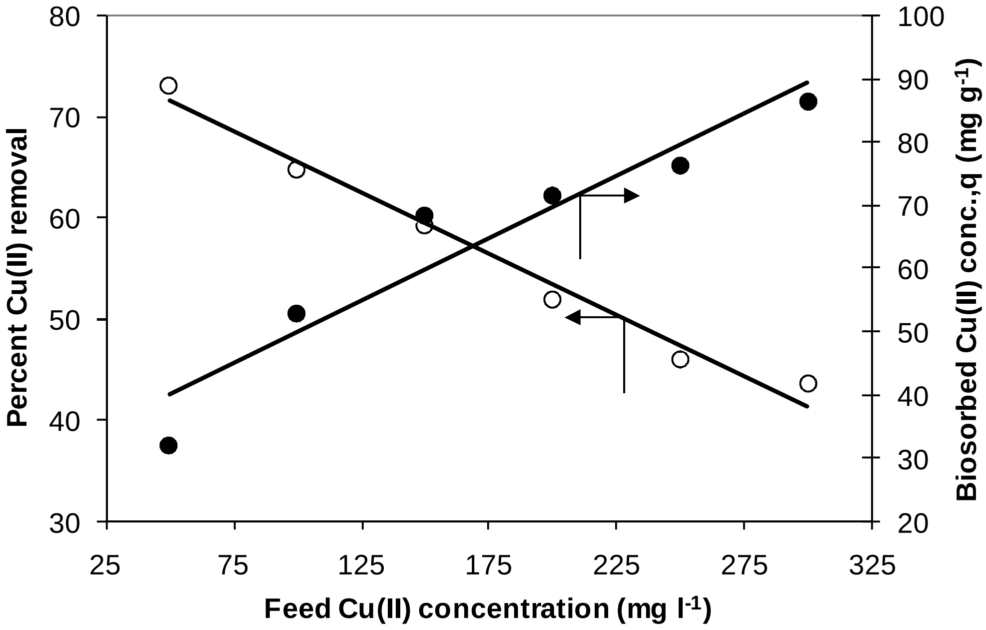


Figure 5.29 Effect of feed copper ion concentration on percent copper ion removals and solid phase copper ion concentrations at the end of 30 hours of operation. (Q = 0.1 l h^{-1}, PWS = 3 g l^{-1})

5.2.4 Determination of the Adsorption Capacity & the Rate of Adsorption

Experimental data obtained from the experiments with variable contents of the adsorbent, feed flow rate and feed copper ion concentrations were used to determine the constants of modified Bohart-Adams equation, namely the adsorption capacity (q_s') and the adsorption rate constant (K) by iteration using the STATISTICA 5 program. Table 5.9 summarizes the data used for iteration. The adsorption capacity and the adsorption rate constant were found to be

$q_s' = 0.0277$ kg Cu.kg PWS^{-1} = 27.7 g Cu(II) kg PWS^{-1} and K = 15.1 m^3 kg^{-1} h^{-1}, (R^2=0.88)

Table 5.9 Fed-batch operation experimental data used in estimation of the parameters of the modified Bohart-Adams equation.

C_o (kg m^{-3})	**Q (m^3 h^{-1})**	**m_{ads} (kg)**	**m_{ads}/ (Q C_o), (h)**	**t_b (h)**
0.1	0.075 x 10^{-3}	3 x 10^{-3}	600	20
0.1	0.125 x 10^{-3}	3 x 10^{-3}	300	9.5
0.1	0.175 x 10^{-3}	3 x 10^{-3}	150	5.5
0.1	0.225 x 10^{-3}	3 x 10^{-3}	100	3
0.1	0.275 x 10^{-3}	3 x10^{-3}	75	2.6
0.1	0.325 x 10^{-3}	3 x10^{-3}	60	1.4
0.05	0.1 x 10^{-3}	3 x 10^{-3}	400	20
0.1	0.1 x 10^{-3}	3 x 10^{-3}	200	12.2
0.15	0.1 x 10^{-3}	3 x 10^{-3}	120	7
0.2	0.1 x 10^{-3}	3 x 10^{-3}	85.7	5.2
0.25	0.1 x 10^{-3}	3 x 10^{-3}	66.67	4.2
0,3	0.1 x 10^{-3}	3 x 10^{-3}	54.55	2.2
0,2	0.25 x 10^{-3}	1 x 10^{-3}	20	0.3
0.2	0.25 x 10^{-3}	2 x 10^{-3}	40	0.7
0.2	0.25 x 10^{-3}	3 x 10^{-3}	60	1.2
0.2	0.25 x 10^{-3}	4 x 10^{-3}	80	1.5
0.2	0.25 x 10^{-3}	5 x 10^{-3}	100	2.2
0.2	0.25 x 10^{-3}	6 x 10^{-3}	120	2.7

As compared to the literature studies, the adsorption capacity of pre-treated PWS for copper ions was superior to those obtained by using modified rice husk for copper and lead ion adsorptions in column operations (Wong *et al.*, 2003). Also, the adsorption capacity of PWS was comparable with those reported in literature for powdered activated carbon (PAC) (Eckenfelder, 1989; Cooney, 2000). However, the adsorption rate constant obtained in this study was an order of magnitude higher than those obtained in PAC adsorption columns as reported in literature. This is because of elimination of mass transfer limitations encountered in adsorption columns by using a completely mixed adsorption reactor in our studies. Therefore, utilization of a completely mixed adsorption reactor operating in fed-batch mode was proven to be more advantageous as compared to the adsorption columns.

5.3 Activated Sludge Experiments

In the first phase of the activated sludge experiments, the toxic effects of Cu(II) ions on activated sludge system were investigated by changing the feed Cu(II) concentration while keeping the feed COD, HRT and SRT constant at 2000 mg l^{-1},10 h and 10 d, respectively. Raw experimental data are presented in Table 5.9 which summarizes COD, Cu(II) , toxicity removals as well as the biomass concentrations in the aeration tank and the sludge volume indexes (SVI) at different feed Cu(II).

Table 5.10 Raw data on COD, Cu(II), toxicity removals, biomass concentration and SVI for variable feed Cu(II) concentrations

$Cu(II)_O$ (mg l^{-1})	**$Cu(II)_R$ (mg l^{-1})**	**%Cu(II) removal**	**COD_o (mg l^{-1})**	**COD_R (mg l^{-1})**	**%COD removal**
0	0.0	0	2060	113	95
5	1.1	78	2120	328	85
10	2.4	76	1987	477	76
15	5.2	65	2038	693	66
17	8.6	49	2040	1264	38
22	13.1	40	2022	1557	23
$\%TOX_O$	$\%TOX_R$	%TOX removal	X (mg l^{-1})	X_r (mg l^{-1})	SVI (ml g^{-1})
0	0	-	4050	7350	136
15	10	33	3550	7120	140
29	17	41	2520	4850	206
44	21	52	1530	3400	294
57	26	54	1120	3000	333
69	31	55	870	2140	467

5.3.1 Effluent Cu(II) Ion Concentrations

Figure 5.30 depicts variation of the effluent Cu(II) concentrations and also percent Cu(II) removals from the feed wastwater as function of the feed Cu(II) concentration. Cu(II) concentration in the feed was varied between 5 and 22 mg l^{-1}. The effluent Cu(II) concentrations increased with increasing feed Cu(II) content yielding 1.1, 5.2 and 13.1 mg l^{-1} Cu(II) concentrations in the effluent when the feed Cu(II) were 5, 15 and 22 mg l^{-1}, respectively. Percentage of Cu(II) biosorbed onto bacterial cells decreased from 78% to 40% when the feed Cu(II) increased from 5 to 22 mg l^{-1}, respectively since the biomass had certain biosorption capacity. Apparently, lower fractions of Cu(II) were biosorbed onto the biomass at high Cu(II) concentrations yielding high free Cu(II) in the solution. Effluent Cu(II) concentrations increased sharply for the feed Cu(II) concentrations above 10 mg l^{-1}.

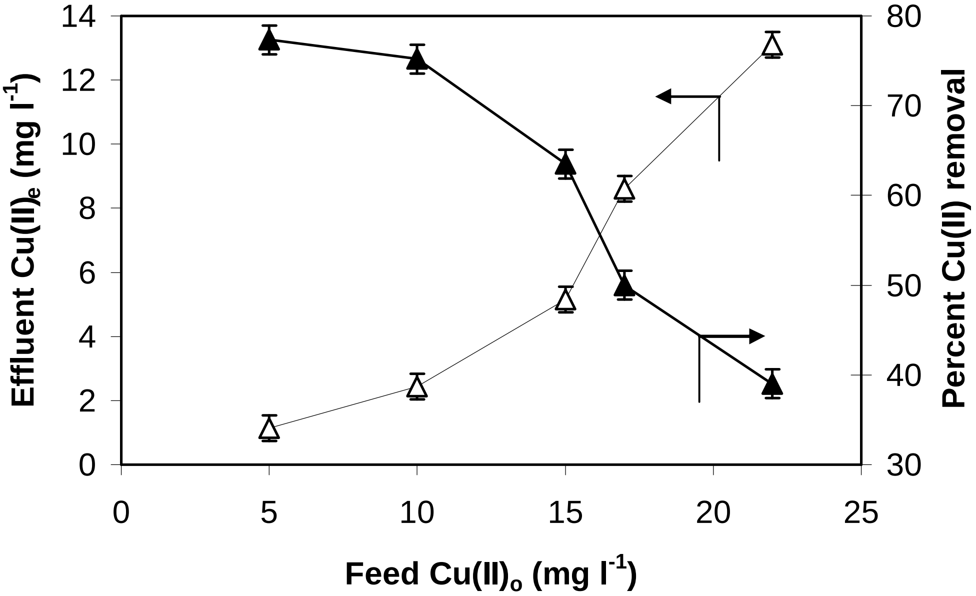


Figure 5.30 Variation of the effluent Cu(II) and percent Cu(II) removal with the feed Cu(II) concentration in the activated sludge unit. (COD_o = 2000 mg l^{-1}, HRT = 10 h, SRT = 10 d)

Figure 5.31 depicts variation of the equilibrium solid phase (biosorbed) Cu(II) ion concentrations with the aqueous phase Cu(II) in the aeration tank of the activated sludge unit, in other words the equilibrium isotherm. Solid phase Cu(II) concentrations were calculated by using the following equation (Pamukoglu and Kargi, 2006).

$$q_e = V(C_o - C_e) / VX = (C_o - C_e) / X \qquad \text{Eqn 5.4}$$

where, q_e is the equilibrium Cu(II) concentration on the biomass (mg Cu(II) g^{-1} biomass); V is the wastewater volume in the aeration tank (8.5 L); C_o and C_e are the feed and aeration tank (or effluent) Cu(II) ion concentrations (mg l^{-1}); and X is the biomass concentration in the aeration tank (g l^{-1}). Biosorbed Cu(II) concentration increased with the equilibrium aqueous phase Cu(II) concentration, as expected. Three different adsorption isotherms (the Langmuir, Freundlich and generalized isotherms) were tested to determine the most suitable one. The Langmuir isotherm was found to be the more suitable than the other tested isotherms with the following coefficients.

$$Q = \frac{Q_m\ C}{K + C} \qquad \text{Eqn 5.5}$$

where $Q_m = 25\ \mathrm{mg\ g^{-1}}$ and $K = 18.8\ \mathrm{mg\ l^{-1}}$, ($R^2 = 0.99$)

The hyperbolic shape of the curve also indicated that the Langmuir isotherm would represent the equilibrium data better than the other isotherms.

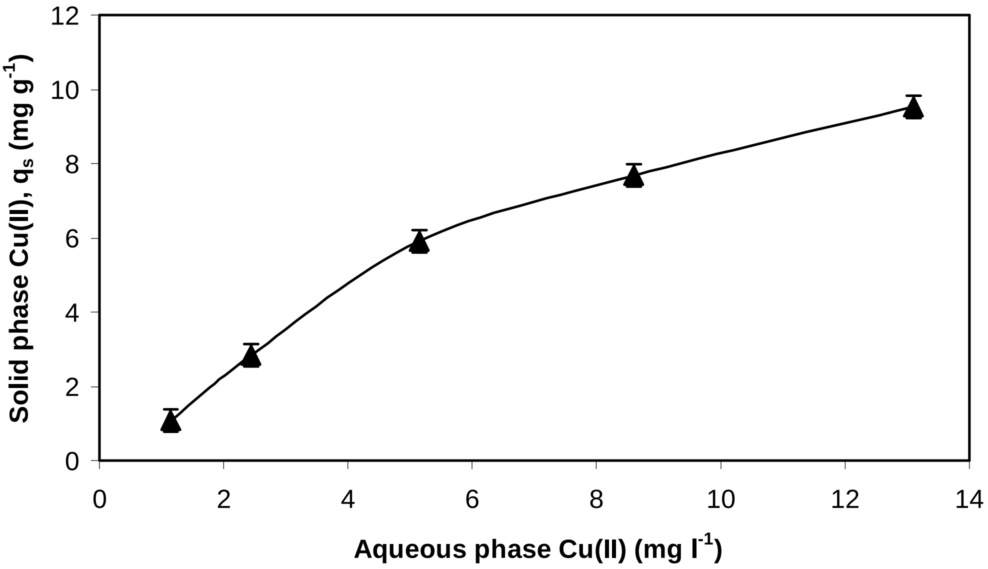


Figure 5.31 Variation of the equilibrium Cu(II) concentrations in aqueous and solid phases in the activated sludge unit (COD_o = 2000 mg l^{-1}, HRT = 10 h, SRT = 10 d).

5.3.2 Effects of Feed Cu(II) on COD & Toxicity Removals

Figure 5.32 depicts variation of the effluent COD and percent COD removals with the feed Cu(II) concentrations. Effluent COD increased and percent COD removal decreased with increasing feed Cu(II) concentration due to toxic effects of Cu(II) on the microorganisms. Percent COD removal with Cu(II)-free wastewater was nearly 95% with an effluent COD of 113 mg l^{-1} which decreased to 66% with an effluent COD of 693 mg l^{-1} for the feed Cu(II) of 15 mg l^{-1} and further to 23% with an effluent COD of 1557 mg l^{-1} for the feed Cu(II) of 22 mg l^{-1}. The decrease in percent COD removal was much steeper for the feed Cu(II) concentrations larger than 10 mg l^{-1} due to toxic effects of Cu(II) ions on the microorganisms at high Cu(II) concentrations. A feed Cu(II) concentration of 5 mg l^{-1} yielded 1.1 mg l^{-1} free Cu(II) level in the effluent which was not toxic.

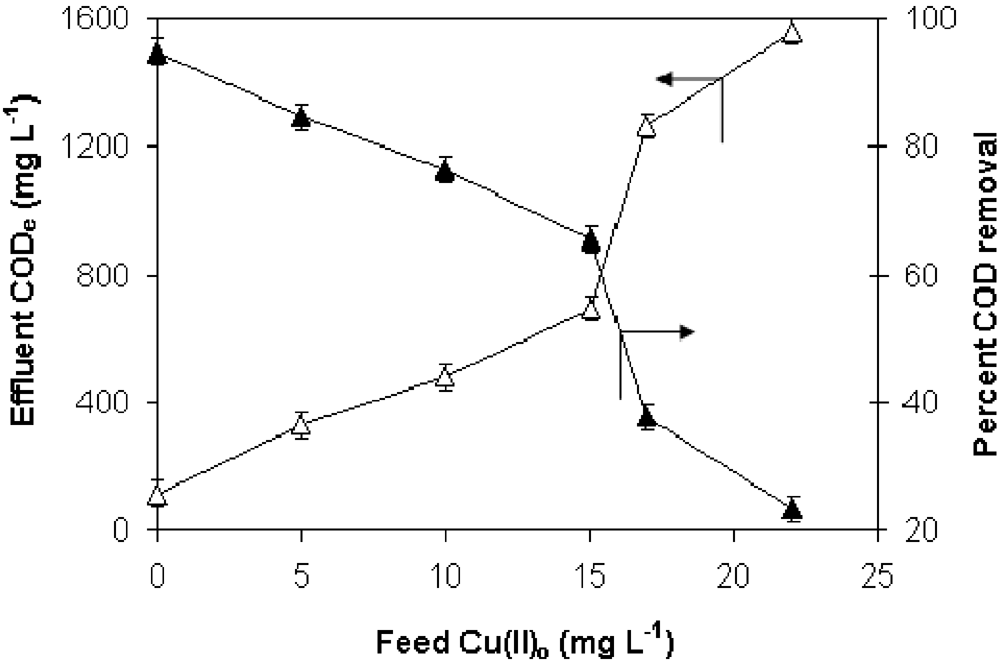


Figure 5.32 Variation of the effluent COD and percent COD removal with the feed Cu(II) concentration (COD_o = 2000 mg l^{-1}, HRT = 10 h, SRT = 10 d).

Figure 5.33 depicts variations of percent feed and the effluent toxicity with Cu(II) concentrations. Feed and effluent toxicities increased with Cu(II) concentrations almost linearly. Feed wastewater toxicities were much higher than the effluent toxicities because of higher free Cu(II) concentrations in the feed as compared to the effluent. Part of the Cu(II) in the feed were removed from the wastewater by biosorption onto the activated sludge microorganisms and resulted in lower free Cu(II) levels and lower toxicities in the effluent. In other words part of the Cu(II) ions and the toxicity remained in the solid biomass phase leaving lower Cu(II) and toxicities in the effluent wastewater. Toxicities of the feed and the effluent were nearly 15 and 10% for the feed and the effluent Cu(II) of 5 and 1.1 mg l^{-1}, respectively which increased to 69 and 31% for the feed and the effluent Cu(II) of 22 and 13.1 mg l^{-1} almost linearly.

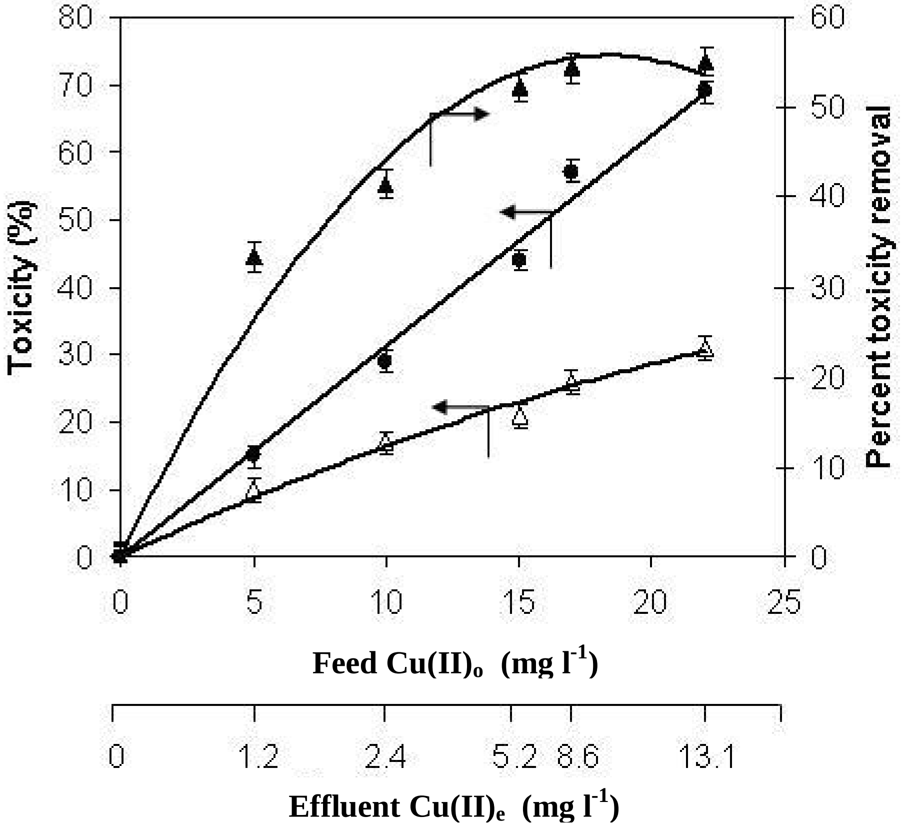


Figure 5.33 Variation of the feed and effluent toxicities and percent toxicity removal with the feed and effluent Cu(II) concentrations. ● feed , Δ effluent, ▲ percent removal

Variations of the feed and effluent wastewater toxicities with the feed Cu(II) concentration were determined by linear curve fitting to the experimental data and the following equations were developed.

Percent feed wastewater toxicity = 3.13 $Cu(II)_o$ ($R^2 = 0.98$)

Percent effluent wastewater toxicity = 1.48 $Cu(II)_o$ ($R^2 = 0.96$)

As shown in Figure 5.33, the difference in the feed and the effluent toxicities increased with increasing feed Cu(II) concentrations due to larger concentrations of free Cu(II) ions in the effluent at high feed Cu(II) contents. Percent toxicity removals from the feed wastewater increased with the feed Cu(II) concentrations because of larger percent Cu(II) removals at high feed Cu(II) concentrations. Percent toxicity removal from the feed wastewater was nearly 33% with the feed Cu(II) of 5 mg l^{-1} which increased to nearly 55% with the feed Cu(II) of 22 mg l^{-1}.

5.3.3 Biomass Concentrations & the Sludge Volume Index

Figure 5.34 depicts variations of steady-state biomass concentrations in the aeration tank and also the sludge volume index (SVI) with the feed Cu(II) concentration. Biomass concentrations decreased and the SVI increased with increasing feed Cu(II) content. Steady-state biomass concentration with Cu(II) free wastewater was 4,050 mg l^{-1} which decreased to 2,520 mg l^{-1} at 10 mg l^{-1} feed Cu(II) and further to 870 mg l^{-1} at 22 mg l^{-1} Cu(II) concentrations due to toxic effects of high Cu(II) contents. High biomass concentrations at low Cu(II) contents resulted in low SVI values or good settling sludge characteristics. The SVI of the sludge was 136 ml g^{-1} when the wastewater was free of Cu(II) ions which increased to 206 ml g^{-1} when the feed Cu(II) was 10 mg $L^{-1}$and further to 467ml g^{-1} at the feed Cu(II) of 22 mg l^{-1}. Sludges with SVI values above 200 ml g^{-1} are known to be bulking sludges which cause anomalies and low treatment performances in activated sludge processes. Therefore, the feed Cu(II) concentration should be below 10 mg l^{-1} in order to avoid bulking sludge formation and preferably below 5 mg l^{-1} to obtain good settling sludge.

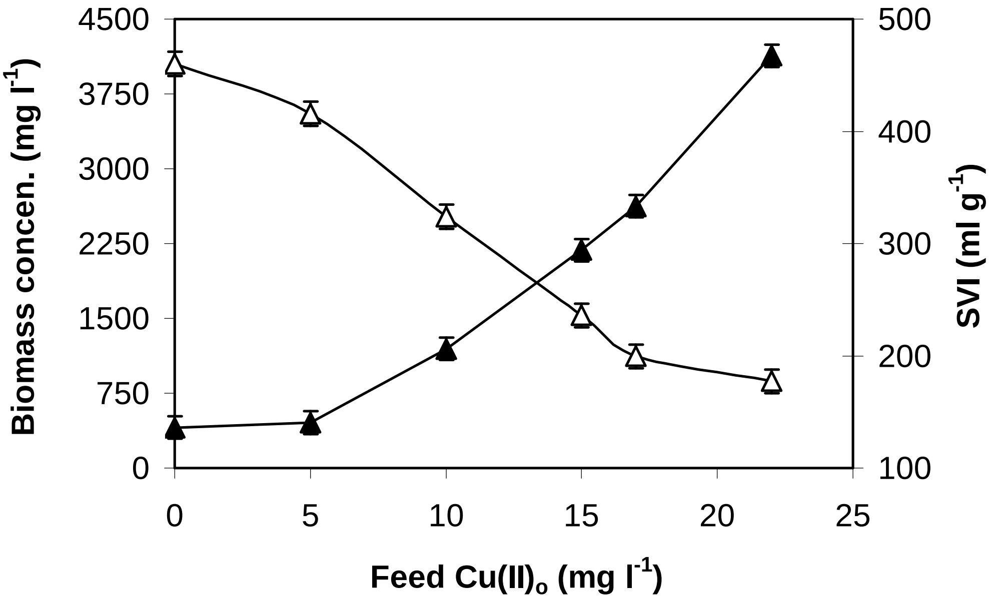


Figure 5.34 Variation of steady-state biomass concentration (X) and the sludge volume index (SVI) with the feed Cu(II) concentration (COD_o = 2000 mg l^{-1}, HRT = 10 h, SRT = 10 d).

5.3.4 Copper Ion Inhibition on COD Removal Rate

COD data presented in Figure 4.28 were used to estimate the rate of COD removal by the organisms at different feed or reactor Cu(II) ion concentrations. The aeration tank (reactor) and the effluent concentrations were the same since the aeration tank was completely mixed. The following non-competitive inhibition equation was used in modelling copper ion inhibition on COD removal rate in the activated sludge unit (Pamukoglu and Kargi, 2007).

$$R = \frac{Q\,(COD_o - COD_e)}{V} = \frac{(COD_o - COD_e)}{\theta_H} = R_o \frac{K_{CU,I}}{(Cu)_R + K_{Cu,I}} \qquad \text{Eqn 5.6}$$

where R and R_o are COD removal rates with and without Cu(II) in the aeration tank (mg COD l^{-1} h^{-1}); Q is the flow rate of the wastewater (l h^{-1}); COD_o and COD_e are the feed and effluent COD concentrations at the steady-state (mg l^{-1}); V is the volume of wastewater in the aeration tank (L); θ_H = V/Q is the hydraulic residence time (HRT, h) which was 10 hours; $(Cu)_R$ is the Cu(II) concentration in the aeration tank at the steady-state (mg l^{-1}); $K_{Cu,I}$ is the Cu(II) inhibition constant for COD removal (mg l^{-1}).

In double-reciprocal form eqn 4.6 can be written as follows,

$$\frac{1}{R} = \frac{1}{Ro} + \frac{(Cu)_R}{Ro\ K_{CU,I}} = \frac{\theta_H}{(COD_o\text{-}COD_e)} \qquad \text{Eqn 5.7}$$

Experimental data on COD removal obtained at different feed and therefore, the reactor Cu(II) concentrations were plotted in form 1/ R versus $(Cu)_R$ as shown in Figure 5.35. From the slope and the intercept of the best-fit line the following coefficients were found.

R_o = 270 mg COD l^{-1} h^{-1} and $K_{CU,I}$ = 2.85 mg l^{-1} (R^2 = 0.93)

The value of Cu inhibition constant ($K_{CU,I}$ = 2.85 mg l^{-1}) indicates that when the aeration tank $Cu(II)_R$ concentration iss 2.85 mg l^{-1}, the rate of COD removal decreases to one-half of that obtained with the Cu(II)-free wastewater. Therefore, Cu(II) concentration in the aeration tank should be less than 1.1 mg l^{-1} or the feed Cu(II) less than 5 mg l^{-1} in order to obtain nearly 90% of the COD removal rate obtained with Cu(II) free wastewater. Feed Cu(II) concentrations of above 10 mg l^{-1} resulted in the aeration tank Cu(II) of above 2.5 mg l^{-1} causing severe inhibitions on COD removal rate. Copper ions must be removed from the wastewater before biological treatment to reduce the concentration below 5 mg l^{-1} in order to overcome adverse effects of Cu(II) ions on performance of the activated sludge unit.

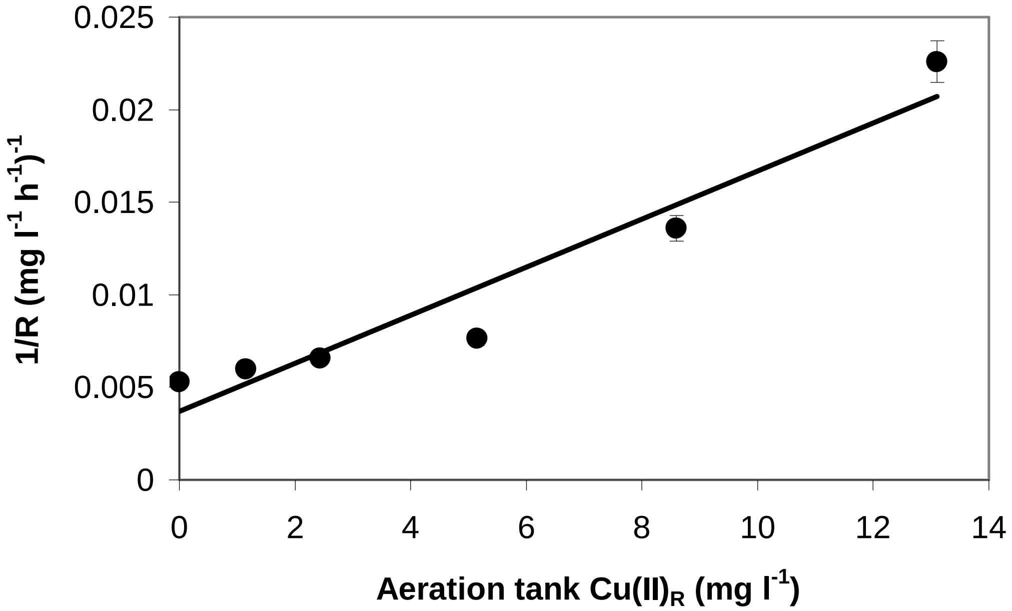


Figure 5.35 A plot of 1/R versus aeration tank Cu(II) concentration for determination of Cu(II) inhibition constant.

5.3.5 Copper(II) Ion Toxicity as Function of Operating Parameters

In this set of experiments the effects of operating parameters such as the HRT, SRT and the feed Cu(II) concentration on COD, Cu and toxicity removals were investigated in an activated sludge unit.

5.3.5.1 Effect of Sludge Age on Cu(II) Ion Toxicity

Figure 5.36 depicts the variation of the concentration of Cu (II) in the aeration tank (reactor) or in the effluent with the sludge age (SRT) while the feed Cu (II) was constant at 14 mg l^{-1}. The Cu(II) concentration inside the reactor increased from 2.4 mg l^{-1} to 4.1 and further to 6.2 mg l^{-1} when the sludge age was decreased from 30 days to 20 and further to 5 days. Due to increased biomass concentrations in the aeration tank with increasing sludge age, more Cu (II) ions were adsorbed onto the biomass at high sludge ages leaving lower concentrations of free Cu (II) ions in solution. Since the feed Cu (II) concentration and therefore, Cu (II) ion loading rate was constant throughout the experiments, larger fractions of Cu (II) ions were adsorbed onto the high concentrations of biomass at high sludge ages.

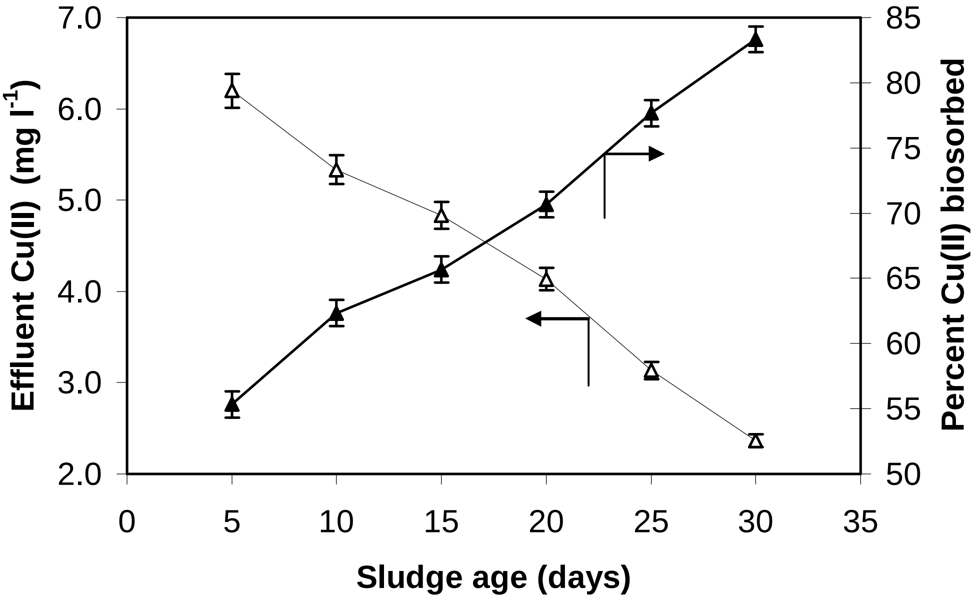


Figure 5.36 Variations of effluent Cu(II) and percent Cu(II) removals with the sludge age (COD_o = 2000±100 mg l^{-1}, $Cu(II)_o$ = 14 mg l^{-1}, HRT = 10 hours).

Figure 5.37 depicts variation of biomass concentrations in the aeration tank at steady-state and the sludge volume index (SVI) with the sludge age for both Cu (II)-free and Cu (II) containing wastewater. Biomass concentrations increased with increasing sludge age for both cases as expected. However, biomass concentrations in the absence of Cu (II) ions were higher than those in the presence of 14 mg l^{-1} Cu (II). Lower biomass concentrations in the presence of Cu(II) ions are due to Cu(II) ion toxicity on the microorganisms. Biomass concentrations in the aeration tank for Cu(II)-free wastewater were 2200, 5600 and 6900 mg l^{-1} while the concentrations were 980, 4350 and 6700 mg l^{-1} for Cu (II) containing wastewater at the sludge ages of 5, 20 and 30 days, respectively. Copper (II) ion toxicity on the organisms was more pronounced at low sludge ages (5-15 days) as compared to those at high sludge ages (25-30 days) due to low biomass concentrations at low sludge ages. At a sludge age of 30 days biomass concentrations in the aeration tank were almost the same for both Cu (II)-free and Cu (II)-containing wastewaters indicating the fact that Cu (II) ion toxicity can be overcome by operation at high sludge ages yielding high biomass concentrations. Sludge volume index (SVI) value is a measure of the settling characteristics of the sludge. Low SVI values below 100 ml g^{-1} indicate well settling sludge while SVI values greater than 150 ml g^{-1} means bulking sludge. SVI values decreased with increasing sludge age for both cases due to high biomass concentrations at high sludge ages (Figure 5.37). However, the SVI values were lower in the absence of Cu (II) as compared to those in the presence of Cu (II) due to toxic effects of Cu (II) ions on the organisms reducing the biomass concentration. However, at high sludge ages (25-30 days) the difference in SVI values for both cases become negligible. The lowest SVI value obtained at a sludge age of 30 days were 69 ml g^{-1} and 74 ml g^{-1} for Cu (II)-free and Cu (II)-containing wastewaters, respectively. The system should be operated at sludge ages of 25 days and above in order to overcome Cu (II) toxicity and to obtain good settling sludge

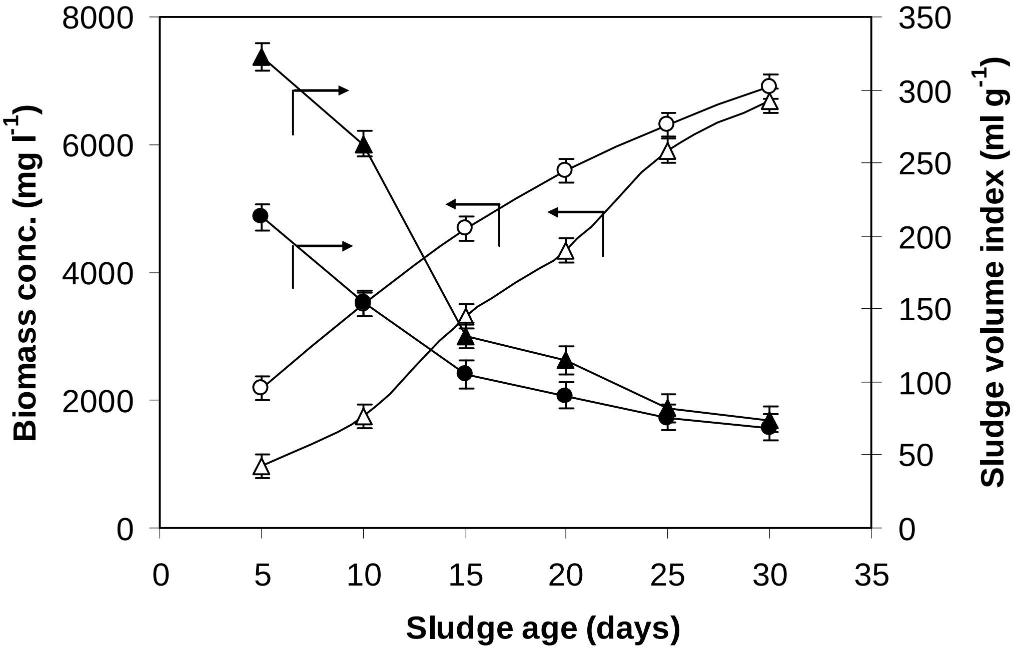


Figure 5.37 Variations of aeration tank biomass concentration and the sludge volume index (SVI) with the sludge age (COD_o = 2000±100 mg l^{-1}, HRT = 10 hours). ●,○ Cu-free wastewater, ▲, Δ Cu-containing wastewater (14 mg l^{-1})

Figure 5.38 depicts variation of effluent COD and percent COD removals with sludge age in the presence and absence of Cu (II) ions in the feed wastewater. The effluent COD decreased and percent COD removal increased with the sludge age for both cases. However, percent COD removals were higher and the effluent COD concentrations were lower for Cu (II)-free wastewater as compared to Cu (II)-containing wastewater at all sludge ages except 30 days.

Lower COD removals in the presence of Cu (II) are due to Cu (II) ion toxicity on the organisms reducing their oxidation capabilities. Percent COD removals for Cu (II)-free wastewater were 68, 94 and 99% while the percent removals were 53, 83, and 99 % for Cu(II)-containing wastewater at the sludge ages 5, 20 and 30 days, respectively. The difference in percent COD removals or the effluent COD concentrations between the two cases decreased with increasing sludge age due to high biomass concentrations at high sludge ages. At a sludge age of 30 days the effluent COD and percent COD removals were the same for both Cu (II)-free and Cu (II)-containing wastewaters indicating the fact that Cu (II) ion toxicity can be overcome by operation at high sludge ages due to high biomass concentrations.

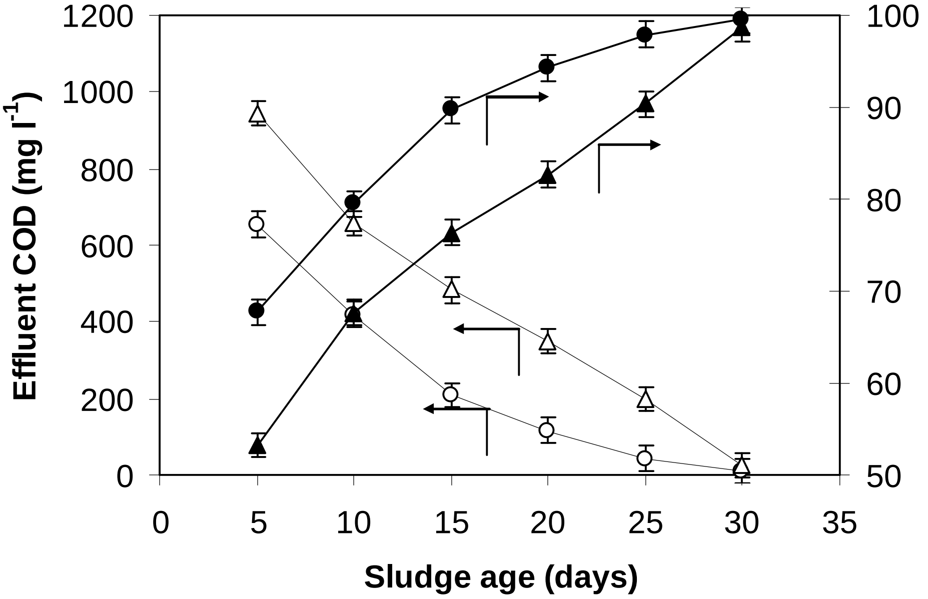


Figure 5.38 Variations of effluent COD and percent COD removal with the sludge age (COD_o = 2000±100 mg l^{-1}, HRT = 10 hours). ●,○ Cu-free wastewater, ▲, Δ Cu-containing wastewater (14 mg l^{-1}).

Figure 5.39 depicts variation of the effluent toxicity and percent toxicity removals with the sludge age in the presence of Cu (II) ions (14 mg l^{-1}). Feed wastewater toxicity was considered to be zero for Cu (II)-free wastewater. Percent effluent wastewater toxicity decreased and percent toxicity removal ($E = 1- (TOX)_e /(TOX)_o$)

increased with increasing sludge age due to high biomass concentrations and also low free-Cu (II) concentrations at high sludge ages. Percent toxicity removal from the feed wastewater increased from 30% to 70 and further to 95% when the sludge age was increased from 5days to 20 and further to 30 days. Operation at high sludge ages of 30 days and above is recommended to overcome Cu (II) toxicity on the organisms.

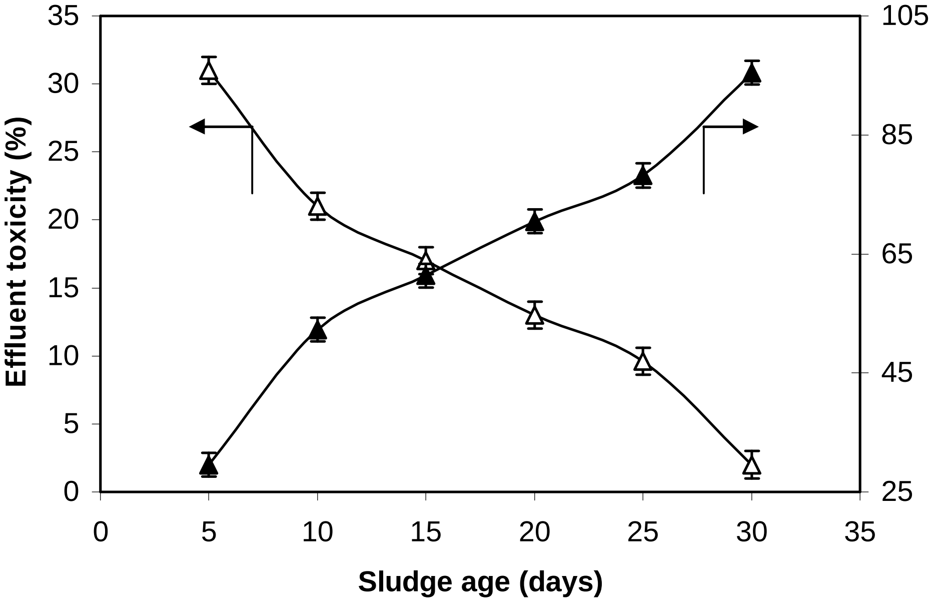


Figure 5.39 Variation of effluent toxicity and percent toxicity removal with the sludge age (COD_o = 2000±100 mg l^{-1}, $Cu(II)_o$ = 14 mg l^{-1}, HRT = 10 hours).

5.3.5.2 Effect of Hydraulic Residence Time on Cu(II) Ion Toxicity

Figure 5.40 depicts variation of aeration tank or effluent Cu (II) concentration with the hydraulic residence time (HRT) while the feed Cu (II) was constant at 14 mg l^{-1}. The aeration tank Cu (II) concentration decreased from 6.2 mg l^{-1} to 4.6 and further to 3.2 mg l^{-1} when the HRT was increased from 5 hours to 15 and further to 25 hours. More Cu (II) ions were adsorbed onto the biomass at high HRT levels, due to increased biomass concentrations in the aeration tank with increasing HRT, resulting in lower levels of free Cu (II) ions in solution. Cu(II) ion loading rate (L = Q Cu_o) decreased with increasing hydraulic residence time or decreasing feed flow rate yielding lower levels of free Cu (II) ions in the reactor.

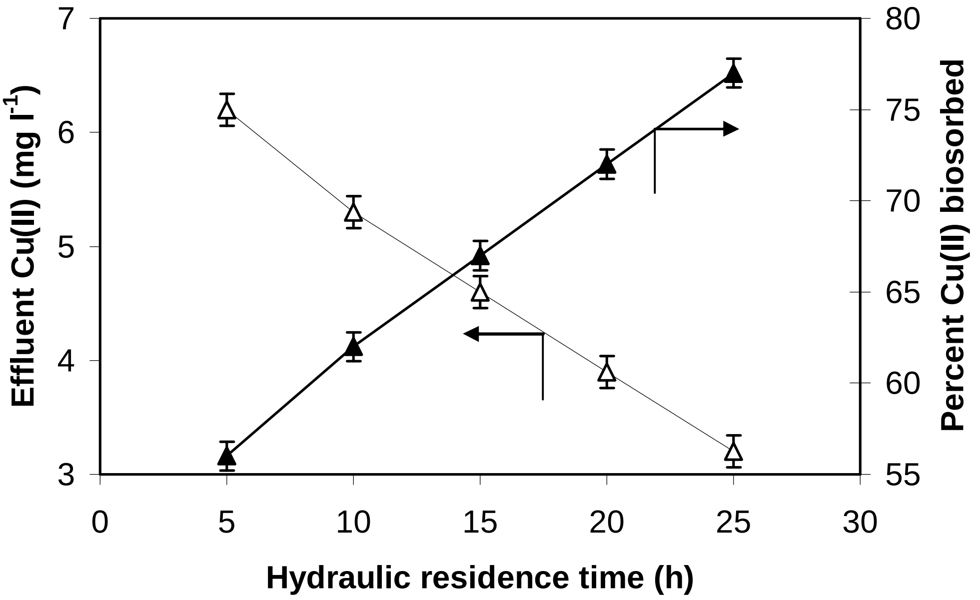


Figure 5.40 Variations of effluent Cu(II) and percent Cu(II) removals with hydraulic rsidence time (COD_o = 2000±100 mg l^{-1}, $Cu(II)_o$ = 14 mg l^{-1}, SRT = 10 days).

Figure 5.41 depicts variation of biomass concentrations in the aeration tank at steady-state and the sludge volume index (SVI) with the hydraulic residence time (HRT) for both Cu (II)-free and Cu(II)-containing wastewater. Biomass concentrations increased with increasing HRT for both cases as expected. However, biomass concentrations for the Cu (II)-free wastwater were higher than those for the Cu (II)-containing (14 mg l^{-1}) wastewater. Lower biomass concentrations in the presence of Cu (II) ions are due to Cu (II) ion toxicity on the organisms. Biomass concentrations in the aeration tank for Cu (II)-free wastewater were 2200, 4450 and 6600 mg l^{-1} while the concentrations were 1100, 3150 and 5350 mg l^{-1} for Cu (II) containing wastewater at the HRT levels of 5, 15 and 25 hours, respectively. Copper (II) ion toxicity on the organisms was more pronounced at low HRT's (5-10 hours) as compared to those at high HRT's (20-25 hours) due to low biomass concentrations at low hydraulic residence times (HRT). Unlike the the biomass concentrations at high sludge ages, even at high HRT levels biomass concentrations for Cu (II)-free wastewater was considerably higher than those of the Cu(II)-containing wastewater, indicating the fact that sludge age (SRT) has a much more pronounced effect on biomass concentrations as compared to HRT and therefore, on Cu (II) ion toxicities.

Sludge volume index (SVI) values decreased with increasing HRT for both cases due to high biomass concentrations at high HRT levels (Figure 5.41). However, SVI values in the absence of Cu (II) were lower than those in the presence of copper ions due to toxic effects of Cu (II) ions on the microorganisms. The SVI values decreased from 71 to 41 ml g^{-1} and from 83 to 45 ml g^{-1} in the absence and presence of Cu (II) ions, respectively when the HRT was increased from 5 to 25 hours. Good settling sludges were obtained at all HRT levels for both cases although sludge settling characteristics were adversely affected from the presence of 14 mg l^{-1} Cu (II) ions.

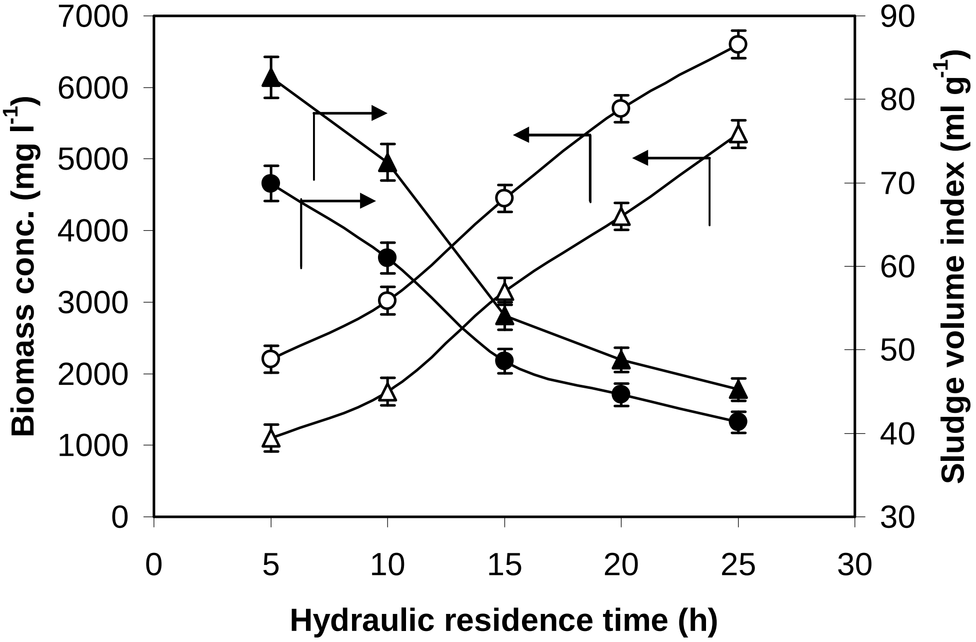


Figure 5.41 Variations of aeration tank biomass concentration and the sludge volume index (SVI) with hydraulic residence time (COD_o = 2000±100 mg l^{-1}, SRT = 10 days). ●,○ Cu-free wastewater, ▲, Δ Cu-containing wastewater (14 mg l^{-1})

Figure 5.42 depicts variations of percent COD removals and the effluent COD's with HRT in the presence and absence of Cu (II) ions in the feed wastewater. The effluent COD's decreased and percent COD removals increased with increasing HRT for both cases. However, percent COD removals were higher and the effluent COD concentrations were lower for Cu(II)-free wastewater as compared to Cu (II)-containing wastewater at all HRT levels. Lower COD removals in the presence of Cu (II) are due to Cu (II) ion toxicity on the organisms. Percent COD removals from Cu

(II)-free wastewater were 60, 86 and 97% while the percent removals were 42, 76 , and 91% from Cu (II)-containing wastewater at HRT levels of 5, 15 and 25 hours, respectively. The difference in percent COD removals or the effluent COD concentrations between the two cases decreased with increasing HRT due to high biomass concentrations at high HRT levels. However, even at an HRT of 25 hours percent COD removal from Cu (II)-free wastewater (97%) was significantly higher than that of the 14 mg l^{-1} Cu (II)-containing wastewater (91%). The HRT levels must be much higher than 25 hours in order to overcome Cu (II) ion toxicity.

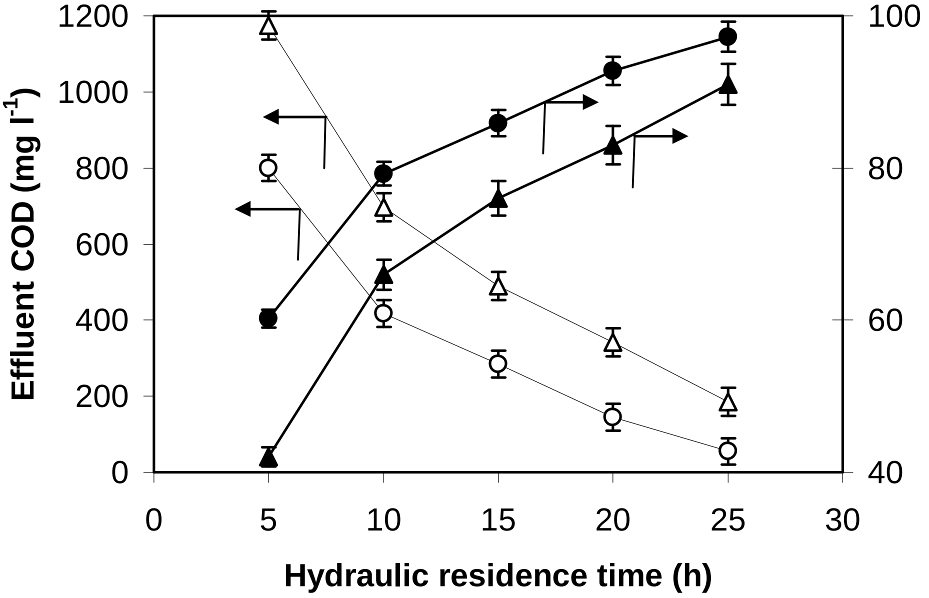


Figure 5.42 Variations of effluent COD and percent COD removal with hydraulic residence time (COD_o = 2000±100 mg l^{-1}, SRT = 10 days). ●,○ Cu-free wastewater, ▲, Δ Cu-containing wastewater (14 mg l^{-1})

Figure 5.43 depicts variation of the effluent toxicity and percent toxicity removals with HRTin the presence of 14 mg l^{-1} Cu (II) ions. Feed wastewater toxicity was considered to be zero for Cu(II)-free wastewater. Percent feed wastewater toxicity decreased and percent toxicity removal (E = 1- $(TOX)_e$ /$(TOX)_o$) increased with increasing HRT due to high biomass concentrations and also low free-Cu (II) concentrations at high HRT levels. Percent toxicity removal from the feed wastewater increased from 34% to 64 and further to 84% when the HRT was increased from 5 hours to 15 and further to 25 hours. Operation at high HRT's of above 25 hours is recommended in order to partially overcome Cu (II) toxicity on the organisms.

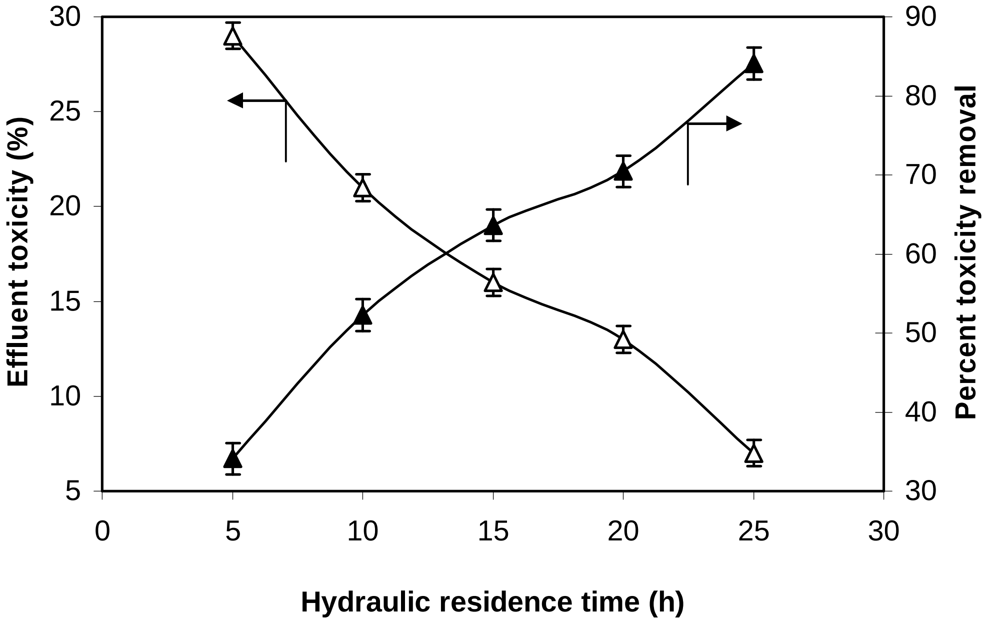


Figure 5.43 Variation of effluent toxicity and percent toxicity removal with hydraulic residence time (COD_o = 2000±100 mg l^{-1}, $Cu(II)_o$ = 14 mg l^{-1}, SRT = 10 days).

There are no literature studies investigating the effects of Cu (II) ion toxicity on activated sludge performance extensively under different operating conditions. Most of the studies were based on batch or continuous experiments measuring the Cu (II) toxicity by respirometric methods or by quantifying the biomass concentrations at different Cu (II) concentrations. Utilization of dehydrogenase activity (Resazurin) essay for quantification of Cu(II) ion toxicity was only used by Wong (Wong PK., 1999). Our study was performed with a constant feed Cu(II) at different SRT and HRT levels. Free and biosorbed Cu (II) were quantified along with COD, and toxicity removals under different operating conditions. Like the litrature studies our study also indicated that Cu (II) ion is toxic to the activated sludge organisms at soluble Cu (II) concentrations as low as 2 mg l^{-1}. However, the total Cu (II) concentration should be considered in quantifying the Cu (II) toxicity since both the soluble and adsorbed Cu (II) ions contribute to the toxic effects.

5.3.6 Mathematical Modeling of Copper(II) Ion Inhibition on COD Removal

A mathematical model was developed to describe the Cu (II) ion inhibition on COD removal from synthetic wastewater containing 15 mg l^{-1} Cu (II) in an activated sludge unit. Experimental data obtained at different sludge ages (5-30 days) and hydraulic residence times (5-25 h) were used to determine the kinetic, stoichiometric and inhibition constants for the COD removal rate in the presence and absence of Cu (II) ions.

5.3.6.1 Performance of the System in the Absence of Cu (II) Ions

Table 5.11 Data for variable SRT (θ_c) and HRT θ_H experiments in the absence of Cu (II) ions

θ_c (d)	**θ_H (h)**	**COD_0 (mg l^{-1})**	**COD_e (mg l^{-1})**	**%COD Rem**	**X (mg l^{-1})**
30	10	2020	10	99	6900
25	10	2010	43	98	6300
20	10	2040	117	94	5600
15	10	2015	208	90	4700
10	10	2035	418	79	3500
5	10	2020	654	68	2200
10	25	2030	55	97	6600
10	20	2020	145	93	5700
10	15	2015	284	86	4450
10	10	2020	417	79	3020
10	5	2010	800	60	2200

Experimental data obtained at different hydraulic residence times (HRT= 5-25 h, SRT = 10 d) and the sludge ages (SRT = 5-30 d, HRT = 10 h) in the absence of Cu (II) ions are presented in Table 5.11. The data obtained at variable HRT (5-25 h) and constant SRT (10 d) were plotted in form of 1/U versus 1/ S (or 1/ COD_R) in Figure 5.44.

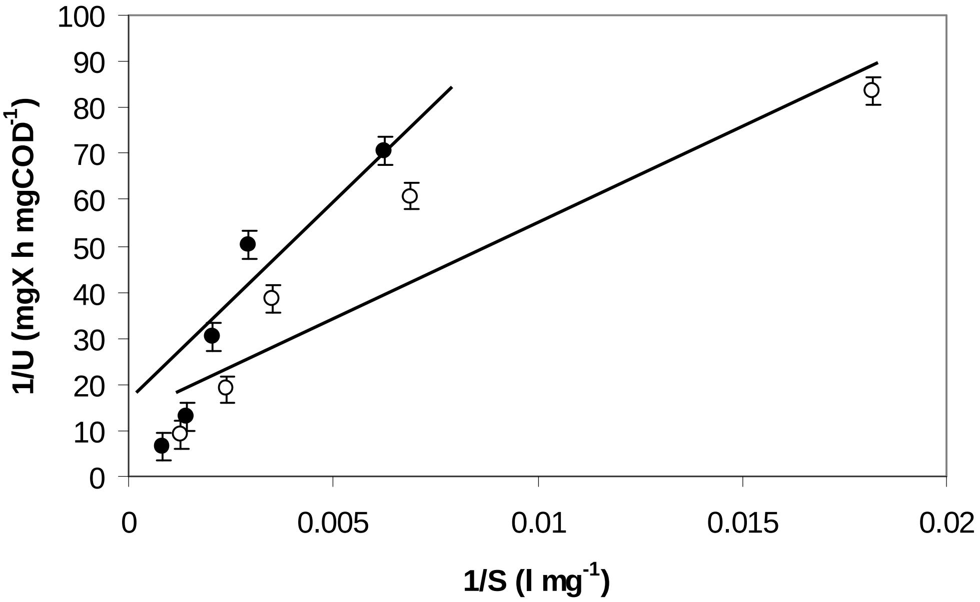


Figure 5.44 Plots of 1/U versus 1/S for variable HRT experiments in the presence (●) and absence (○) of Cu (II) ions (SRT = 10 d, COD_o = 2000 mg l^{-1}). ○ Cu-free experimental data, ● experimental data with 15 mg l^{-1} Cu (II) in the feed. Continuous lines are the model predictions.

From the slope and the intercept of the best-fit line the following constant were obtained by using the eqn.4.20

$k = 0.0625\ h^{-1} = 1.5\ d^{-1}$, $K_s = 254$ mg l^{-1} (R^2= 0.85)

Similarly, the experimental data obtained with variable SRT (5-30 d) at constant HRT (10 h) were plotted in form of 1/ Θ_c versus U according to eqn. 4.26 and the following constants were determined from the slope and the intercept of the best-fit line (Figure 5.45).

$Y_M = 0.23$ gX g^{-1}COD , b = 0.138 d^{-1} (R^2 = 0.96)

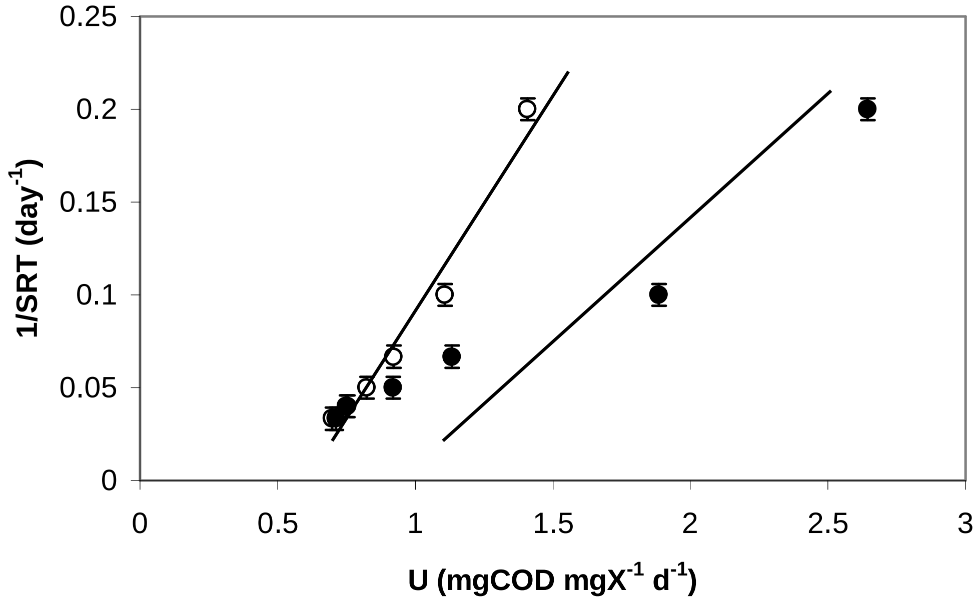


Figure 5.45 Plots of 1/θc versus U for variable SRT experiments in the presence (●) and absence (○) of Cu (II) ions (HRT = 10 h, CODo = 2000 mg l^{-1}). ○ Cu-free experimental data, ● experimental data with 15 mg l^{-1} Cu (II) in the feed. Continuous lines are the model predictions.

The kinetic and stoichiometric constants determined for Cu (II)-free wastewater are comparable with the literature reports where k is between 1-5 d^{-1}, K_s is 100-300 mg l^{-1}, Y_M is 0.2-0.5 g X g^{-1}S and b is 0.01-0.15 d^{-1}. The maintenance coefficient (m, gCOD gX^{-1}.h^{-1}) defined as m = b / $Y_{X/S}$ was calculated to be m= 0.6 g COD g^{-1}X d^{-1} = 0.025 g S g^{-1}X h^{-1} in the absence of Cu(II).

Specific rates of COD removal (U) were calculated using eqn 4.19 with the predicted model constants and were depicted in form of 1/U versus 1/S as a continuous line along with the experimental data points in Figure 5.44. The agreement between the model predictions (continuous line) and the experimental data (open circles) was reasonable in the absence of Cu (II). The experimental data and model predictions were compared in Figure 5.45 in form of 1/θc versus U in the absence of Cu (II) ions. The model predictions were in good agreement with the experimental data.

5.3.6.2 Performance of the System in the Presence of Cu (II) Ions

The same experiments as listed in Table 5.12 were performed with 15 mg l^{-1} Cu (II) ions in the feed wastewater. Some Cu (II) ions were adsorbed onto the biomass in the activated sludge yielding aqueous Cu (II) ion concentrations lower than 15 mg l^{-1}. Since both the free and adsorbed Cu (II) would cause inhibition on microbial activities, the total Cu (II) ion concentration was considered in the model.

Table 5.12 Data for variable SRT(θ_c) and HRT (θ_H) experiments in the presence of Cu (II) ions

θ_c (d)	**θ_h (h)**	**Cu_0 (mg l^{-1})**	**Cu_e (mg l^{-1})**	**%Cu biosorbed**	**COD_0 (mg l^{-1})**	**COD_e (mg l^{-1})**	**%COD Rem**	**X (mg l^{-1})**
30	10	15	2.4	84	2017	27	99	6700
25	10	15	3.1	79	2050	198	90	5900
20	10	15	4.1	73	2015	349	83	4350
15	10	15	4.8	68	2043	484	76	3300
10	10	15	5.3	65	2032	657	68	1750
5	10	15	6.2	59	2025	945	53	980
10	25	15	3.2	79	2055	185	91	5350
10	20	15	3.9	74	2011	342	83	4200
10	15	15	4.6	69	2041	490	76	3150
10	10	15	5.3	65	2047	696	66	1750
10	5	15	6.2	59	2025	1175	42	1100

The experimental data obtained with the feed Cu (II) concentration of 15 mg l^{-1} are presented in Table 5.12 for variable HRT and SRT experiments along with effluent Cu (II) concentrations. The effluent Cu (II) decreased and percent biosorption of Cu (II) by microorganisms increased with increasing HRT and SRT. The experimental data obtained with variable HRT (5-30 h) and constant SRT (10 d) were plotted in form of 1/U versus 1/S (or $1/COD_R$) as shown in Figure 5.44. From the slope and the intercept of the best-fit line the following apparent coefficients were obtained (eqn 4.21)

$$k_{app} = 0.054\ h^{-1} = 1.3\ d^{-1}, \quad K_{s,app} = 463\ \text{mg}\ l^{-1} \qquad (R^2 = 0.90)$$

By using the definitions of k_{app} and $K_{s,app}$ in eqn 4.16 and substituting the values of $k = 1.5\ d^{-1}$, $K_s = 254$ mg l^{-1} and Cu = 15 mg l^{-1} into eqn 4.17, the following inhibition constants were determined.

$K_{Cu} = 97$ mg l^{-1} , $K'_{Cu} = 18$ mg l^{-1}

Apparently, Cu inhibition on the saturation constant (K_s) was more pronounced than that on the rate constant (k), since K_{Cu} was larger than K'_{Cu}. The type of Cu inhibition on COD removal rate is non-competitive resulting in lower rate and higher saturation constants in the presence of Cu (II) ions. There are no similar kinetic modeling studies in the literature on Cu (II) ion inhibition in activated sludge operations with the determined kinetic and inhibition constants to compare our results with.

Similar to the experiments in the absence of Cu (II) ions, the experimental data obtained with variable sludge ages (SRT = 5-30 d) at a constant HRT of 10 h in the presence of 15 mg Cu l^{-1} were used to estimate the inhibition constants on 'Y' and 'b'. Equation 4.25 was used to correlate the experimental data using the values of Y_M = 0.23 gX g^{-1}S , b = 0.1385 d^{-1} , Cu = 15 mg l^{-1}. STATISTICA 5 program was used for determination of the inhibition constants by iteration and the following constant were determined by using the Quasi Newton-Simplex approximation.

$K_Y = 27$ mg l^{-1} , $K_b = 210$ mg l^{-1} ($R^2 = 0.85$)

Apparently, Cu (II) ion inhibition on the growth yield coefficient (Y) was more pronounced than that on the death rate coefficient (b) since K_Y was smaller than K_b.The growth yield coefficient decreased to Y_{app} = 0.15 gX g^{-1}COD and the death rate coefficient increased to b_{app} =0.15 d^{-1} in the presence of 15 mg l^{-1} Cu (II) ions. Therefore, the maintenance coefficient in the presence of 15 mg Cu l^{-1} was $m_{app} = b_{app}/\ Y_{app} = 1\ d^{-1} = 0.041$ gCOD g^{-1}X. h^{-1}. The maintenance coefficient increased by 64% in the presence of 15 mg l^{-1} Cu (II) as compared to Cu-free wastewater. In the presence of Cu(II) ions, specific rates of COD removal (U) were calculated using eqn 5.8 with the predicted model constants and were depicted in form of 1/U versus 1/S as a continuous line along with the experimental data points (closed circles) in Figure

5.44. The agreement between the model predictions (continuous line) and the experimental data (closed circles) was reasonable at high 1/S or high HRT levels, but not that good at low 1/S or HRT values in the presence of Cu (II). The experimental data and model predictions were compared in Figure 5.45 in form of $1/\theta_c$ versus U in the presence of Cu (II) ions. Again the agreement between the model predictions and the experimental data was reasonable at low SRT or high U values, but not that good at high SRT levels in the presence of Cu (II).

With the determined kinetic and inhibition constants, the design equations (eqns 4.18 and 4.25) in the presence of Cu (II) ions take the following form

$$U_{COD} = \frac{[0.0625 / (1+ Cu / 97)]\, COD_R}{254\,(1+ Cu / 18) + COD_R} = \frac{COD_o - COD_R}{\Theta_H\ X} \qquad \text{Eqn 5.8}$$

where Cu, COD_R and biomass (X) concentrations are in mg l^{-1}, U is in mg COD $mg^{-1}X\ h^{-1}$ and Θ_H is in hours.

$$\frac{1}{\Theta_c} = \frac{0.23}{(1+ Cu/ 27)} U_{COD} - 0.1385\left(1+ \frac{Cu}{210}\right) \qquad \text{Eqn 5.9}$$

where Cu is in mg l^{-1}, U is in mg COD $mg^{-1}X\ d^{-1}$ and Θ_c is in days.

Equation 4.27 was used to predict the variations of effluent COD (or COD_R) with HRT and the results are depicted in Figure 5.46. The model predictions are in reasonable agreement with the experimental findings especially at high HRT (greater than 15 h) levels where inhibition is less pronounced due to high biomass concentrations.

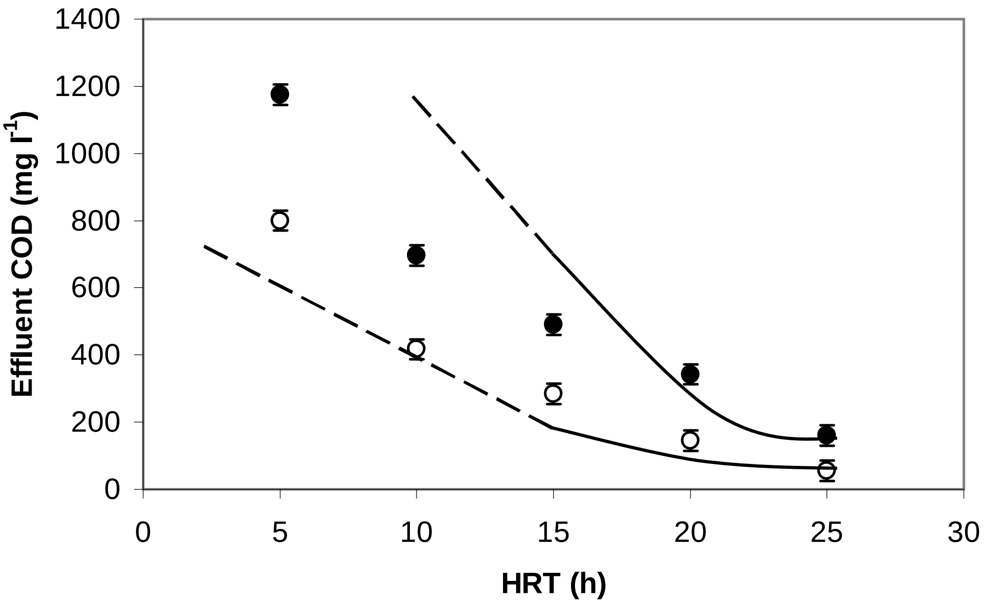


Figure 5.46 Variations of effluent COD concentration with hydraulic residence time (HRT). ○ Cu-free feed, ● Cu-containing feed (15 mg Cu l^{-1}) experimental data. Continuous lines are the model predictions.

5.4 Activated Sludge Experiments with PWS Addition

In this set of experiments PWS was continuously added to the feed wastewater containing Cu(II) in a mixing tank for biosorption of Cu(II). The effluent was settled in a sedimentation tank to remove Cu containing PWS and the effluent was fed to the activated sludge unit as depicted in Figure 3.3.

5.4.1 Experiments with 14 mg l^{-1} Cu (II) in the Feed

The results of the continuous experiments with PWS addition to the feed wastewater are depicted in Figure 5.47 to Figure 5.50 where the feed PWS loading was varied between 0.1 and 0.6 g h^{-1} while the Cu (II) loading was constant at 12 mg Cu h^{-1}. Figure 5.47 depicts variation of percent Cu (II) removals with PWS loading rate in the effluents of the mixing tank and activated sludge unit. Cu (II) concentrations in the effluents of the mixing and the sedimentation tanks were approximately the same since adsorption of Cu (II) ions onto PWS took place in the mixing tank. Cu (II) ions in the effluent of the mixing tank decreased from 12 to 3.5

mg l^{-1} and further to 0 with 16%, 76% and 100% Cu (II) removals when the PWS loading rate was increased from 0.1 to 0.4 and further to 0.5 g h^{-1} due to increased adsorption of Cu (II) ions onto PWS. Cu (II) concentrations in the effluent of the activated sludge unit were considerably lower than those of the mixing and sedimentation tank effluents due to further adsorption of Cu (II) ions onto activated sludge organisms. Cu (II) concentrations in the effluent of the activated sludge unit decreased from 8.4 mg l^{-1} (41% removal) to zero (100% removal) when PWS loading rate was increased from 0.1 to 0.5 g h^{-1} due to increased adsorption of Cu (II) onto biomass in the activated sludge unit. Approximately 1 g h^{-1} PWS addition was required for 28 mg h^{-1} Cu (II) loading for complete removal of Cu (II) ions in the mixing tank in order to obtain Cu-free feed for the activated sludge unit.

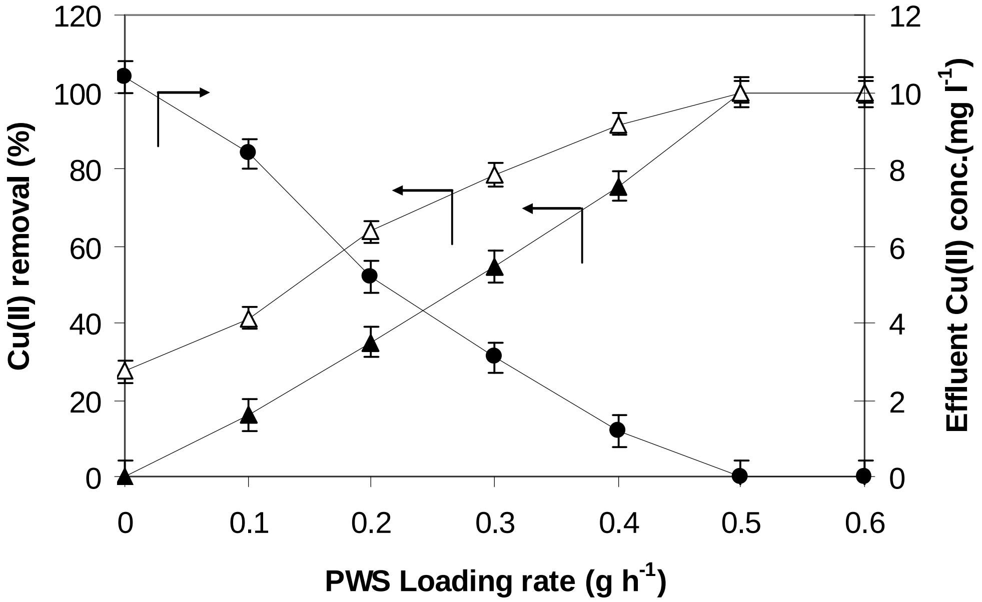


Figure 5.47 Variations of effluent Cu (II) and percent Cu (II) removals with the PWS loading rate. ▲ Mixing tank effluent, Δ, ● Activated sludge effluent. Cu_o = 14 mg l^{-1}

Figure 5.48 depicts variation of COD removal in the activated sludge unit with PWS loading rate when COD_o = 2000 mg l^{-1} and Cu_o = 14 mg l^{-1}. Effluent COD concentration decreased and percent COD removal increased with increasing PWS loading rate due to increased removal of Cu (II) ions by adsorption onto PWS. Percent COD removal was 45% and 77% with effluent COD's of 1100 and 470 mg l^{-1} at PWS loadings rates of 0.1 and 0.4 g h^{-1} which increased to 80% with an effluent COD of 420 mg l^{-1} at a PWS loading of 0.5 g h^{-1}. Further increases in PWS loading rates did not improve COD removal performance since complete removal of Cu (II) was achieved with a PWS loading of 0.5 g h^{-1} corresponding nearly 1 g PWS requirement for 28 mg Cu removal.

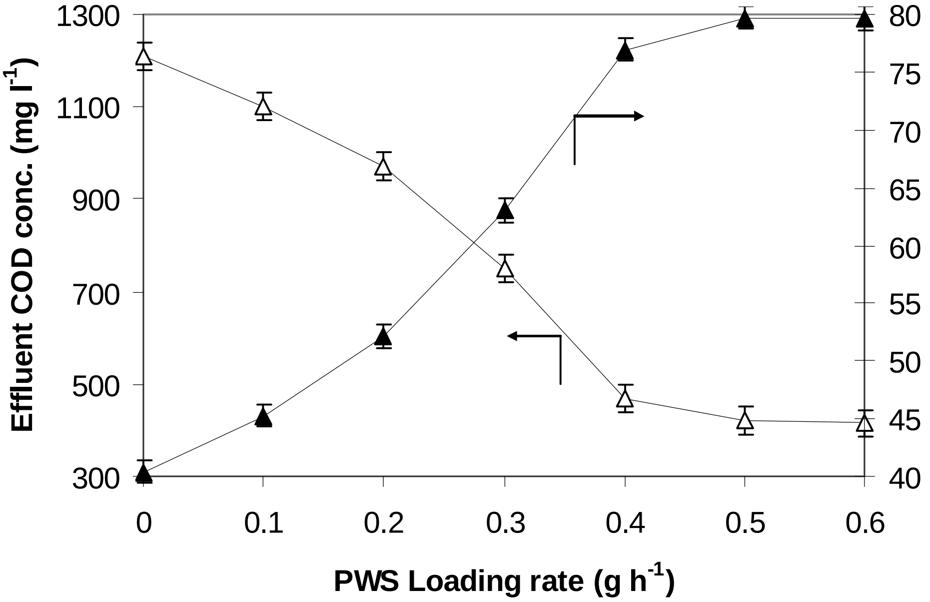


Figure 5.48 Variations of effluent COD and percent COD removals with the PWS loading rate. Δ Effluent COD, ▲ Percent COD removal. Cu_o = 14 mg l^{-1}

The extent and the rate of COD removal in the activated sludge unit is closely related with the biomass concentration in the aeration tank. Biomass concentration in the aeration tank increased with increasing PWS loading rate as shown in Figure 5.49 due to elimination of Cu (II) toxicity on activated sludge bacteria with PWS addition. Biomass concentration in the aeration tank increased from 2500 mg l^{-1} to 3200 mg l^{-1} and further to 3820 mg l^{-1} when PWS loading rate was increased from 0.1 g h^{-1} to 0.4 g h^{-1} and further to 0.6 g h^{-1}, respectively yielding higher rates and extent of COD removal. Settled biomass concentration in the sedimentation tank (X_r) also increased with increasing PWS loadings yielding better settling sludge with lower sludge volume index (SVI) at high PWS loadings. SVI decreased from 208 ml g^{-1} to 125 ml g^{-1} with increases in X_r values from 4800 mg l^{-1} to 8000 mg l^{-1} when PWS loading was increased from 0.1 g h^{-1} to 0.5 g h^{-1}. PWS loading rate of 0.5 g h^{-1} eliminated Cu

(II) ions from the feed wastewater yielding high biomass concentration and better settling sludge with low SVI values.

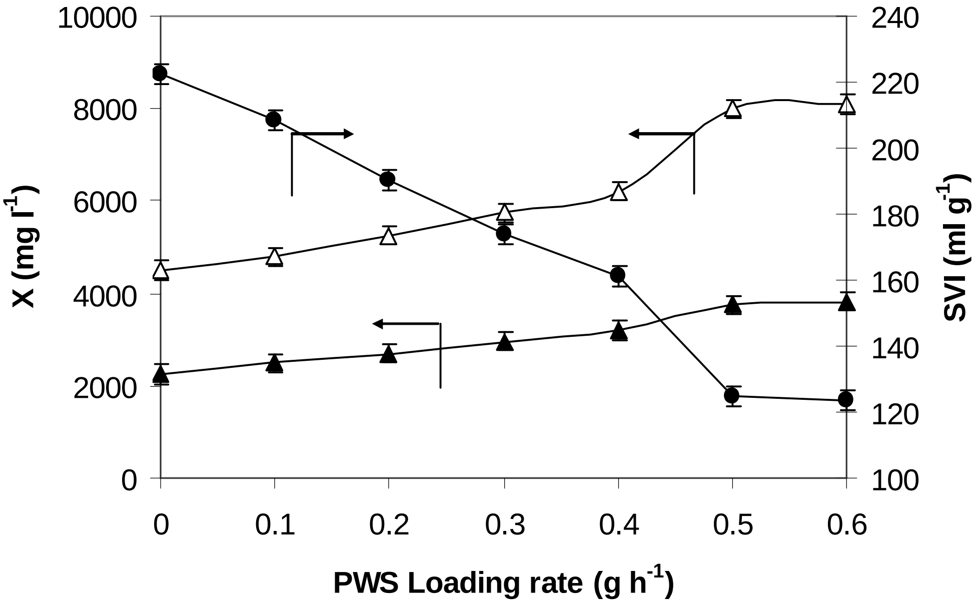


Figure 5.49 Variations of biomass concentrations and the sludge volume index (SVI) with the PWS loading rate. ▲ Biomass in the aeration tank (X), Δ Settled biomass concentration (X_r), ● Sludge volume index (SVI). Cu_o = 14 mg l^{-1}

Removal of Cu (II) toxicity is another important parameter investigated and depicted in Figure 5.50. Toxicities of the mixing tank and the activated sludge effluents decreased and percent toxicity removals increased with increasing PWS loading rate due to removal of Cu (II) from the feed by PWS addition. Percent toxicity of the feed with Cu (II) = 14 mg l^{-1} was 44% with respect to Cu (II)-free water which decreased to 41% in the mixing tank effluent and further to 32% in the effluent of the activated sludge unit when PWS loading rate was 0.1 g h^{-1}. Percent toxicity removal in the activated sludge effluent as compared to the feed increased from 27% to 80% and further to 100% when PWS loading was increased from 0.1 g h^{-1} to 0.4 and further to 0.5 g h^{-1}. Further increases in PWS loading rates did not result in any improvement in toxicity removal since Cu (II) was completely removed with the addition of 0.5 g PWS h^{-1} corresponding 1 g h^{-1} PWS addition per 28 mg h^{-1} Cu (II) loading.

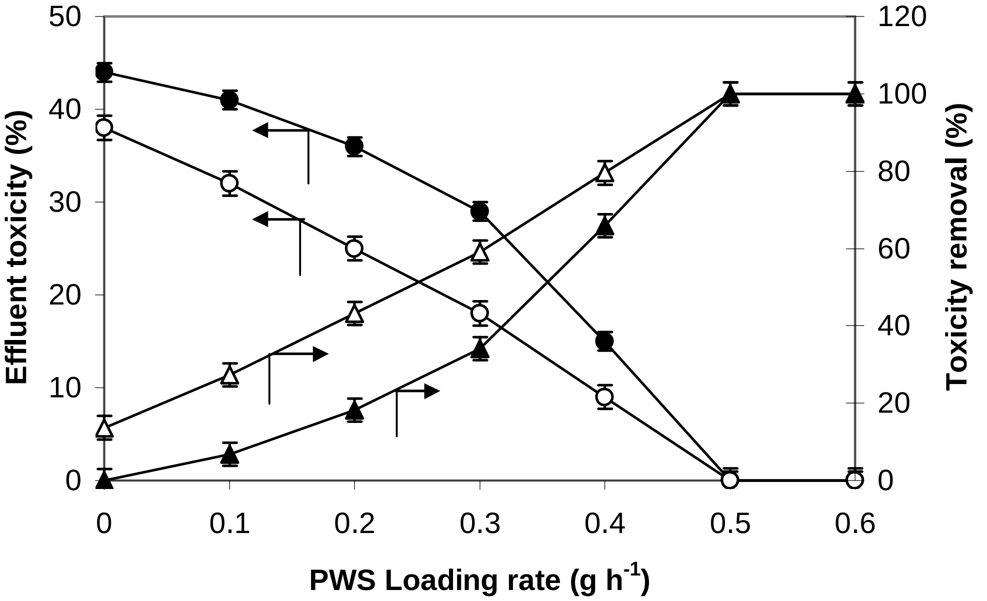


Figure 5.50 Variation of effluent toxicities and percent toxicity removals with the PWS loading rate ● ▲ Mixing tank effluent, ○ Δ Activated sludge effluent. Cu_o = 14 mg l^{-1}

5.4.2 Experiments with 22 mg l^{1} Cu (II) in the Feed

Similar experiments were performed with the feed Cu (II) concentration of 22 mg l^{-1} where the PWS loading rates were varied between 0.2 and 1 g h^{-1} while HRT and SRT were constant at 10 h and 10 days, respectively. Figure 5.51 depicts variation of percent Cu (II) removal with PWS loading rate in the effluents of the mixing tank and activated sludge unit. Cu (II) concentrations in the effluents of the mixing and the sedimentation tanks were approximately the same since adsorption of Cu (II) ions by PWS took place in the mixing tank. Cu (II) ions in the effluent of the mixing tank decreased from 17 to 7.7 mg l^{-1} and further to zero with 24%, 65% and 100% Cu (II) removals when the PWS loading rate was increased from 0.2 to 0.6 and further to 0.8 g h^{-1} due to increased adsorption of Cu (II) ions onto PWS. Further adsorption of Cu (II) ions onto activated sludge organisms reduced Cu (II) concentrations in the effluent of the activated sludge unit as compared to the mixing and sedimentation tank effluents. Cu (II) concentrations in the effluent of the activated sludge unit decreased from 13.2 mg l^{-1} (40% removal) to zero (100% removal) when PWS loading rate was increased from 0.2 to 0.8 g h^{-1} due to increased adsorption of Cu (II) onto biomass in the activated sludge unit. Again, approximately 1 g h^{-1} PWS addition was required for 28 mg h^{-1} Cu (II) loading for complete removal of Cu (II) ions in the mixing tank in order to obtain Cu-free feed for the activated sludge unit.

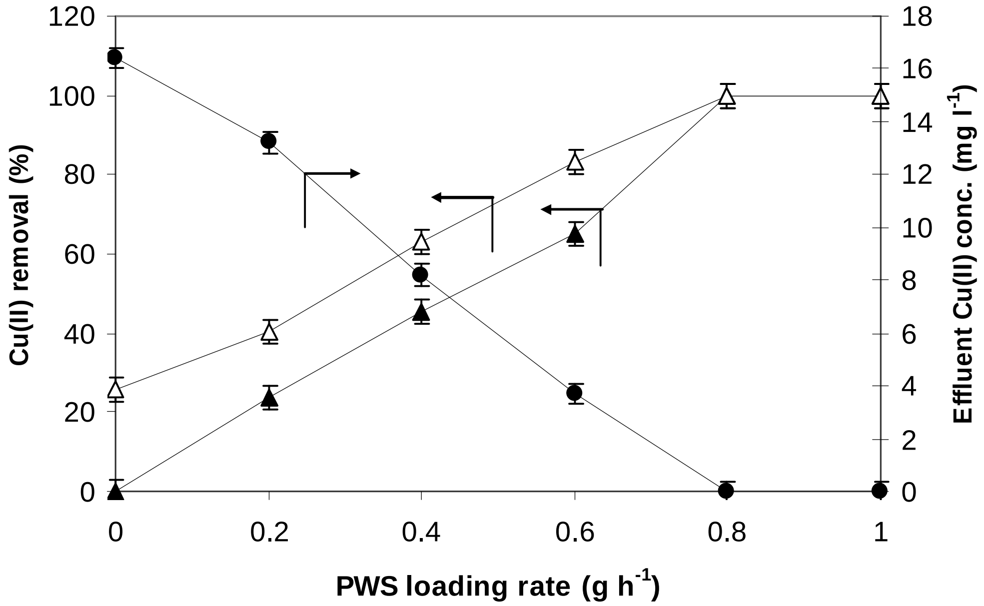


Figure 5.51 Variations of effluent Cu (II) and percent Cu (II) removals with the PWS loading rate. ▲ Mixing tank effluent, Δ , ● Activated sludge effluent. Cu$_o$ = 22 mg l^{-1}

Variations of effluent COD and percent COD removal in the activated sludge unit with PWS loading rate are depicted in Figure 5.52 when COD$_o$ = 2000 mg l^{-1} and Cu$_o$ = 22 mg l^{-1}. Effluent COD concentration decreased and percent COD removal increased with increasing PWS loading rate due to increased removal of Cu (II) ions by adsorption onto PWS. Percent COD removal was 33% and 58% with effluent COD's of 1340 and 860 mg l^{-1} at PWS loadings of 0.2 and 0.6 g h^{-1} which increased to 79% with an effluent COD of 420 mg l^{-1} at a PWS loading of 0.8 g h^{-1}. Further increases in PWS loading rates did not improve COD removal performance since complete removal of Cu (II) was achieved with a PWS loading of 0.8 g h^{-1} corresponding nearly 1 g PWS requirement for 28 mg Cu removal.

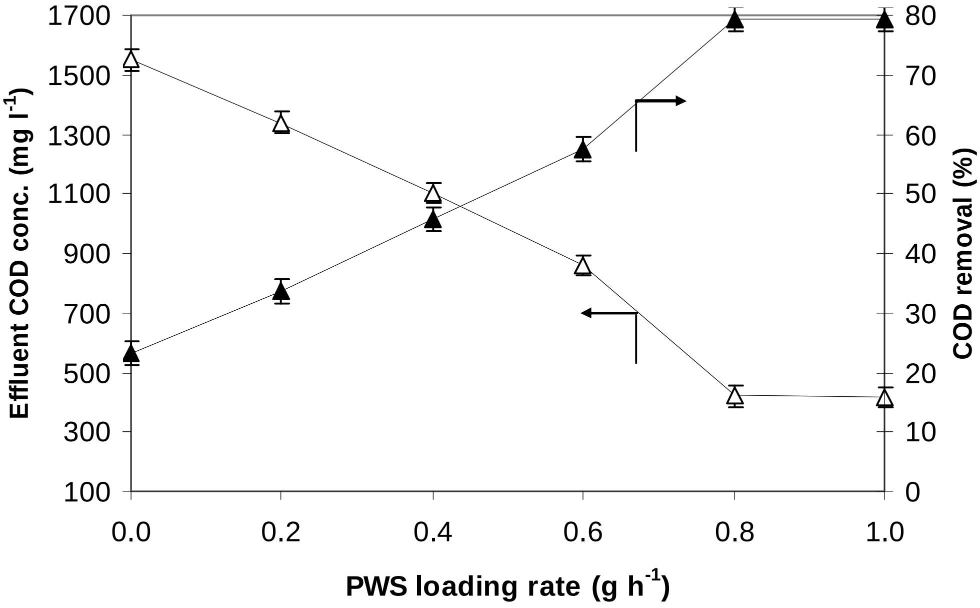


Figure 5.52 Variations of effluent COD and percent COD removals with the PWS loading rate, Δ Effluent COD, ▲ Percent COD removal. $Cu_o = 22$ mg l^{-1}

Biomass concentration in the aeration tank of the activated sludge unit is an important parameter affecting the rate and extent of COD removal which increased with increasing PWS loading rate as shown in Figure 5.53 due to elimination of Cu (II) toxicity on the activated sludge bacteria with PWS addition. Biomass concentration in the aeration tank increased from 2030 mg l^{-1} to 3040 mg l^{-1} and further to 3900 mg l^{-1} when PWS loading rate was increased from 0.2 g h^{-1} to 0.6 g h^{-1} and further to 1. g h^{-1}, respectively yielding higher rates and extent of COD removal. Settled biomass concentration in the sedimentation tank (X_r) also increased with increasing PWS loadings yielding better settling sludge with lower sludge volume index (SVI) at high PWS loadings. SVI decreased from 247 ml g^{-1} to 122 ml g^{-1} with increases in X_r values from 4050 mg l^{-1} to 8200 mg l^{-1} when PWS loading was increased from 0.2 g h^{-1} to 1. g h^{-1}. PWS loading rate of 0.8 g h^{-1} completely eliminated Cu (II) ions from the feed wastewater yielding high biomass concentration and better settling sludge with low SVI values.

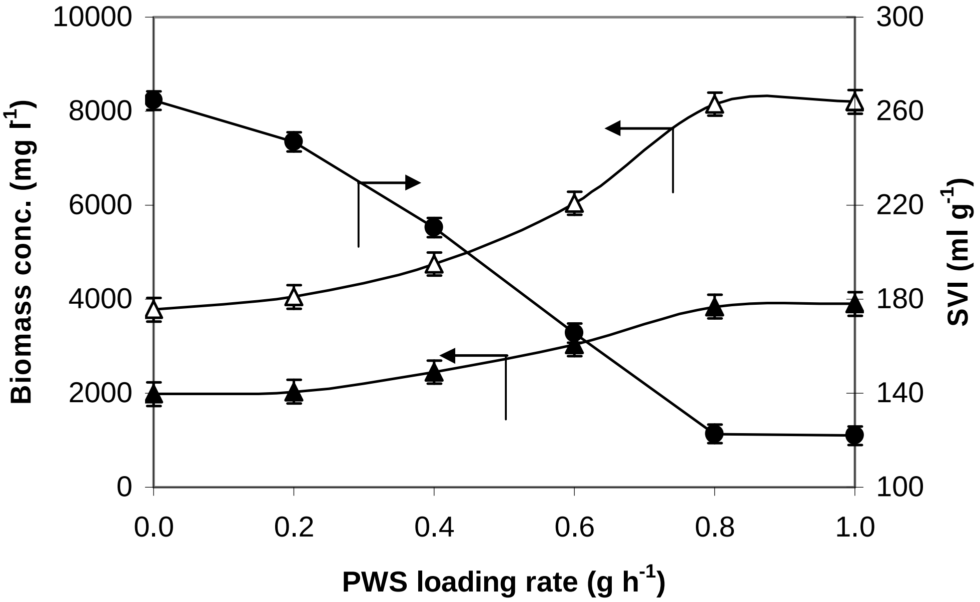


Figure 5.53 Variations of biomass concentrations and the sludge volume index (SVI) with the PWS loading rate. ▲ Biomass in the aeration tank (X), Δ Settled biomass concentration (X_r), ● Sludge volume index (SVI). Cu_o = 22 mg l^{-1}

Variations of effluent toxicity and percent toxicity removals with PWS loading rate are depicted in Figure 5.54. Toxicities of the mixing tank and the activated sludge effluents decreased and percent toxicity removals increased with increasing PWS loading rate due to removal of Cu (II) from the feed by adsorption onto added PWS. Percent toxicity of the feed with Cu_o = 22 mg l^{-1} was 69% with respect to Cu-free water which decreased to 47% in the effluent of the mixing tank and further to 37% in the effluent of the activated sludge unit when PWS loading rate was 0.2 g h^{-1}. Percent toxicity removal in the activated sludge effluent as compared to the feed increased from 46% to 80% and further to 100% when PWS loading was increased from 0.2 g h^{-1} to 0.6 g h^{-1} and further to 0.8 g h^{-1}. Further increases in PWS loading rates did not result in improvement in toxicity removal since Cu (II) was completely removed with the addition of 0.8 g h^{-1} PWS corresponding 1 g h^{-1} PWS requirement for 28 mg h^{-1} Cu (II) loading rate.

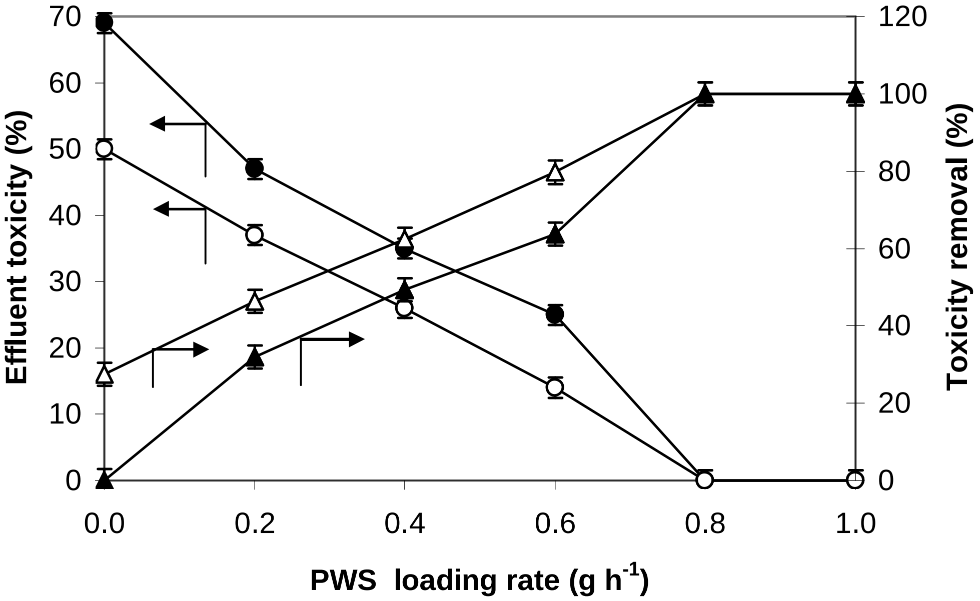


Figure 5.54 Variation of effluent toxicities and percent toxicity removals with the PWS loading rate ● ▲ Mixing tank effluent, ○ Δ Activated sludge effluent. Cu_o = 22 mg l^{-1}

There are no reported studies on removal of metal ions from wastewater by biosorption before biological treatment in order to eliminate metal ion toxicity. Most of the literature studies are on the adverse effects of metal ions on activated sludge performance (F. B. Dilek, 1998; Pamukoglu M.Y. & Kargi F., 2007) or biosorption of metal ions on different biosorbents including PWS in batch systems with no biological treatment (Arican B., 2002; Pamukoglu M.Y. & Kargi F., 2006). Since the toxic effects of metal ions on activated sludge bacteria are well documented in literature, this study provides a good option to eliminate metal ion toxicity in activated sludge systems. Although the biosorption capacity of PWS for Cu(II) were reported to be higher in some batch biosorption studies (Pamukoglu M.Y. & Kargi F., 2006; Pamukoglu M.Y. & Kargi F., 2006), the difference in experimental conditions and longer duration of biosorption in batch studies could be responsible for this difference.

5.4.3 Box-Behnken Experiment Design

Box-Behnken experimental design method was used to investigate Cu(II), chemical oxygen demand (COD) and toxicity removal performance of the activated sludge unit treating synthetic wastewater containing Cu(II) ions with PWS addition. The effects of four different independent variables: SRT (5-30 days), HRT (5-25 hours), feed Cu(II) concentration (0-50 mg l^{-1}) and PWS loading rate (0-4 g h^{-1}) on percent Cu(II), chemical oxygen demand (COD), toxicity (TOX) removals and sludge settling characteristics (SVI) were investigated. The experimental data of the Box-Behnken design are presented in Table 4.2. The data were correlated with response functions for determination of the coefficients for each independent variable by iteration. Different response functions were used to correlate the experimental data and the most suitable one was determined by using the analysis of variance (ANOVA) program. The results of the ANOVA test for Cu(II) removal are presented in Table 5.13. ANOVA test for different response function models indicated that the quadratic model provided the best fit to the experimental data with the lowest standard deviation, the highest correlation coefficient (0.99) and the lowest *p*-value. The computed F value was much greater than that of the tabular $F_{0.01\ (14,\ 14)}$ value of 3.70 suggesting that the fit is highly significant. P values of less than 0.05 for any parameter in ANOVA test indicated a significant effect of the corresponding variable on the response.

The estimated coefficients of the response functions are presented in Table 5.14. The predicted values of the experimental data as determined by the response functions with the estimated coefficients are compared with the experimental results in Table 5.15. Response function predictions were in good agreement with the experimental data.

Table 5.13 ANOVA regression analysis for Cu(II) removal efficiency

Source	Sum of squares	df	Mean square	*F* value	*p*-value Prob >*F*	
Cu (II) Removal						
Mean vs. Total	1.219E+005	1	1.219E+00			
Linear vs. Mean	18358.49	4	4589.62	4.90	0.0050	
2FI vs. Linear	1285.49	6	214.25	0.18	0.9782	
Quadratic vs.	20941.41	4	5235.35	279.87	< 0.0001	Suggested
Cubic vs.	213.65	8	26.71	3.32	0.0805	
Residual	48.24	6	8.04			
Total	1.627E+005	29	5610.77			
Lack of fit tests						
Linear	22486.52	20	1124.33	1978.97	< 0.0001	
2FI	21201.04	14	1514.36	2665.49	< 0.0001	
Quadratic	259.62	10	25.96	45.70	0.0011	Suggested
Cubic	45.97	2	22.99	40.46	0.0022	
Pure Error	2.27	4	0.57			

Source	Std Dev	R^2	Adj. R^2	Press	
Model summary					
Linear	30.61	0.4494	0.3577	32803.36	
2FI	34.32	0.4809	0.1925	59311.40	
Quadratic	4.33	0.9936	0.9872	1498.97	Suggested
Cubic	2.84	0.9988	0.9945	6623.49	

Table 5.14 Response function coefficient for percent Cu(II), COD, toxicity removals and SVI

	B_O	B_1	B_2	B_3	B_4	B_{12}	B_{13}	B_{14}	B_{23}	B_{24}	B_{34}	B_{11}	B_{22}	B_{33}	B_{44}
$Y_{Cu(II)}$ $R^2=0.99$	-26.016	0.84	0.92	4.85	16.95	4.5x 10^{-3}	8.2x 10^{-3}	-5.4x 10^{-2}	7.0x 10^{-3}	-7.3x 10^{-2}	0.35	-2.1x 10^{-2}	-2.9x 10^{-2}	-8.9x 10^{-2}	-3.51
Y_{COD} $R^2=0.99$	68.0893	-0.11	0.07	-0.32	6.45	3.8x 10^{-2}	-1.1x 10^{-2}	1.0x 10^{-1}	-1.1x 10^{-2}	7.7x 10^{-2}	2.5x 10^{-1}	3.7x 10^{-3}	-5.1x 10^{-3}	-8.9x 10^{-3}	-2.25
Y_{TOX} $R^2=0.99$	-14.81	0.33	0.58	4.36	10.58	2.0x 10^{-2}	2.0x 10^{-2}	1.4x 10^{-2}	1.0x 10^{-2}	-3.6x 10^{-2}	0.31	-1.9x 10^{-2}	-2.8x 10^{-2}	-8.9x 10^{-2}	-2.14
Y_{SVI} $R^2=0.91$	117.24	-0.44	0.04	1.79	-24.46	-5.2x 10^{-2}	6.1x 10^{-3}	-2.9x 10^{-2}	-9.9x 10^{-4}	-9.0x 10^{-3}	-1.12	7.8x 10^{-3}	6.8x 10^{-3}	4.0x 10^{-2}	8.43

Table 5.15 Comparison of experimental and predicted TCP, COD and toxicity removals

Run	Exp. COD (%)	Pred COD (%)	Exp. Cu(II) (%)	Pred. Cu(II) (%)	Exp.TOX (%)	Pred. TOX (%)	Exp. SVI	Pred. SVI
1	82	77	0	0	0	0	98	100
2	81	79	0	0	0	0	98	135
3	76	79	0	4	0	2	100	91
4	91	94	0	4	0	2	89	78
5	76	78	0	3	0	2	108	98
6	97	97	0	4	0	2	81	72
7	71	71	86	85	72	74	120	113
8	75	75	95	91	86	82	112	104
9	71	71	88	88	75	75	118	112
10	95	93	99	96	99	92	84	77
11	52	52	55	55	51	50	171	184
12	58	60	62	64	57	62	150	163
13	75	73	97	96	90	88	112	107
14	91	92	99	99	97	99	89	84
15	51	51	55	57	51	53	169	180
16	56	58	61	63	55	60	154	166
17	76	75	98	98	96	92	108	102
18	87	88	99	99	97	96	92	88
19	77	77	96	96	86	87	100	100
20	77	77	95	96	84	87	100	100

Table 5.15 Continued.

Run	Exp. COD (%)	Pred COD (%)	Exp. Cu(II) (%)	Pred. Cu(II) (%)	Exp.TOX (%)	Pred. TOX (%)	Exp. SVI	Pred. SVI
21	78	77	97	96	88	87	100	100
22	78	77	97	96	88	87	100	100
23	77	77	96	96	87	87	100	100
24	20	22	22	24	18	14	345	294
25	70	74	92	97	81	84	120	104
26	57	54	70	68	48	49	154	173
27	61	58	77	75	59	60	142	158
28	54	54	67	65	42	42	160	175
29	62	61	78	77	68	67	141	157

Variations of percent Cu (II) removal with the feed Cu (II) and PWS loading rates at constant SRT (15 d) and HRT (15 h) are depicted in Figure 5.55. Percent Cu (II) removal increased with the feed Cu(II) concentration up to 30 mg l^{-1} corresponding Cu(II) loading rate of 17 mg h^{-1}. Further increases in Cu(II) concentration or loading rates caused decreases in percent Cu(II) removal due to insufficient binding sites on PWS surfaces for Cu(II) biosorption at high Cu(II) loadings. At low feed Cu(II) loadings or concentrations biosorption of Cu(II) onto PWS surfaces is limited by Cu(II) concentrations since the binding sites on PWS surfaces are in excess of Cu(II) ions in solution. However, at high Cu(II) loadings biosorption of Cu(II) onto PWS surfaces is limited by the binding sites on PWS surfaces since Cu(II) ions are in excess of the binding sites. In the absence of PWS addition, nearly 60% Cu(II) removal was observed due to biosorption of Cu(II) ions onto biomass in the activated sludge unit. Percent Cu(II) removal increased with the addition of PWS. Percent Cu(II) removal increased from 60% to 85% and further to 100% with the of 1g h^{-1} and 2 g h^{-1} PWS loading rates, respectively as compared to no PWS addition at the peak value when the feed Cu(II) was 30 mg l^{-1}. Further increases in PWS loading did not improve Cu(II) removal since Cu(II) loadings were balanced with PWS additions at 2 g h^{-1} PWS loadings. The optimal SRT/HRT/PWS/feed Cu(II) ratio yielding the highest Cu(II) removal (99%) was 15 d/15 h/2 g h^{-1}/30 mg l^{-1} corresponding to the optimum PWS loading /Cu(II) loading ratio of 0.118 gPWS/ mgCu(II) under the specified experimental conditions.

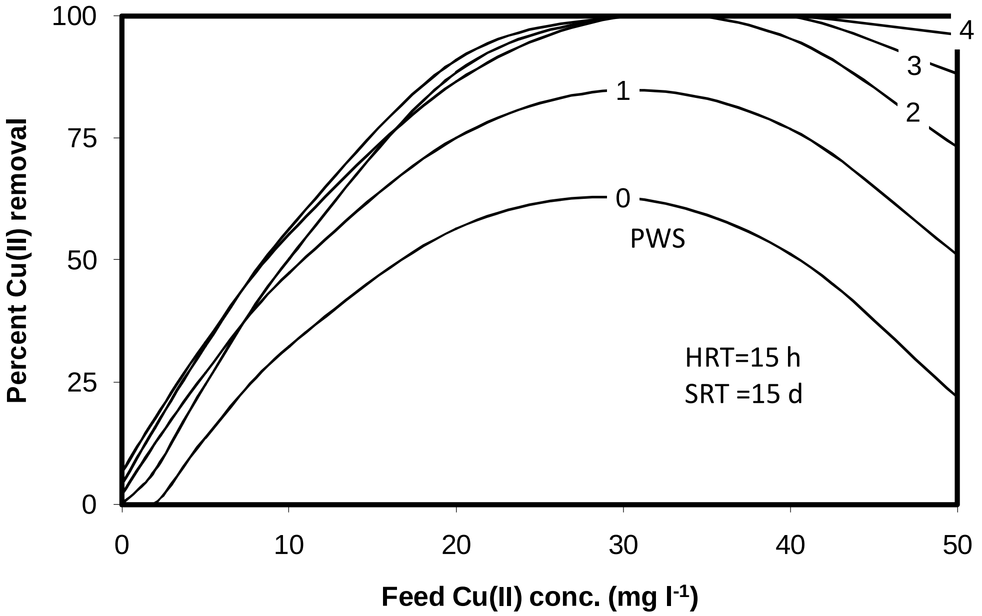


Figure 5.55 Variation of percent Cu(II) removal with the feed Cu(II) concentration at different PWS loadings. HRT = 15 hours, SRT = 15 days.

Effects of SRT and PWS loading rates on percent Cu (II) removals are depicted in Figure 5.56 at constant feed Cu(II) concentration (25 mg l^{-1}) and HRT (10 h). Percent Cu (II) removals increased with increasing SRT for all PWS loading rates up to 20 days and leveled off at higher SRT levels. The optimum SRT was 20 days under the specified operating conditions. Increases in PWS loading rates yielded increases in percent Cu(II) removals at all SRT values up to 3g h^{-1} PWS loading rate. In the absence of PWS addition, the maximum percent Cu(II) removal was 60% which increased to 80% and further to 93% when PWS was added with loading rates of 1 and 2 g h^{-1}. At low PWS loadings, the extent of Cu (II) biosorption was limited by the availability of the binding sites on PWS surfaces. Therefore, percent Cu(II) removal or biosorption increased with increasing PWS loadings up to 3 g h^{-1}. Further increases in PWS loadings did not yield higher Cu(II) removals since Cu(II) loading rate ($L_{Cu(II)}$ =Q $Cu(II)_o$) was balanced with the adsorbent (PWS) loading rate of 3 g h^{-1}. At a PWS loading rate of 2 g h^{-1} percent Cu(II) removals were 87%, 94% and 93% for SRT's of 5, 20 and 30 days, respectively at a feed Cu(II) concentration 25 mg l^{-1} and HRT of 10 h. The optimal SRT/HRT/PWS/feed Cu(II) ratio yielding the highest Cu(II) removal (99%) was 20 d/10 h/3 g h^{-1} / 25 mg l^{-1} corresponding to the optimal loading ratio of L_{PWS} / $L_{Cu(II)}$ = 0.141 gPWS/ mg Cu(II) under the specified operating conditions. The ANOVA analysis indicated that all four variables SRT, HRT, feed Cu(II) concentration and PWS loading rate and their interactions (X_1, X_3, X_4, X_3X_4, X_3^2,X_4^2) played important roles for the removal of Cu(II).

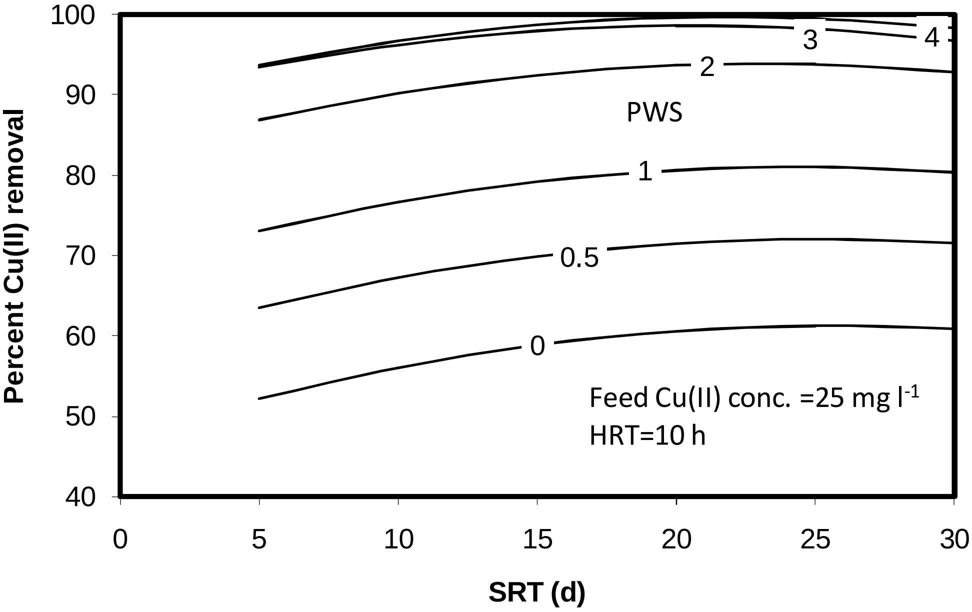


Figure 5.56 Variation of percent Cu(II) removal with the sludge age (SRT) at different PWS loadings. $Cu(II)_o$ = 25 mg l^{-1}, HRT = 10 hours.

Figure 5.57 depicts variation of percent COD removal with the PWS loading rate and feed Cu(II) concentration at constant HRT (15 h) and SRT (15 d) values. As expected, percent COD removal increased with increasing PWS loading rate for all feed Cu(II) concentrations due to elimination of Cu(II) by biosorption onto PWS surfaces in the mixing tank. COD removals increased slightly with PWS addition at low feed Cu(II) concentrations below 5 mg l^{-1} and reached a maximum level at a PWS loading rate of 2 g h^{-1}. However, at high feed Cu(II) contents above 10 mg l^{-1} COD removal steadily increased with increasing PWS loading rates due to availability of more binding sites on PWS surfaces at high PWS loading rates. In the absence of Cu(II) loading COD removal was the highest as 85% and low Cu(II) concentrations in the feed such as 5 mg l^{-1} did not affect COD removals because of Cu(II) removal by biosorption onto PWS surfaces. However, COD removal decreased with increasing feed Cu(II) concentrations or Cu(II) loadings due to insufficient Cu(II) removals by the PWS additions. At a feed Cu(II) concentration of 50 mg l^{-1} or loading rate of 28.4 mg Cu(II) h^{-1}, percent COD removal was nearly 22% in the absence of PWS loading which increased with increasing PWS loadings and reached nearly 67% at a PWS loading rate of 4 g h^{-1}. Percent COD removals reached the maximum level of nearly 70% at high PWS loadings of 4 g h^{-1} at all feed Cu(II) concentrations due to availability of high binding sites on PWS surfaces. At a constant feed Cu(II)(II) concentration of 30 mg l^{-1}, HRT and SRT of 15 hours and 15 days, percent COD removals were 49%, 73% and 80% at PWS loading rates of 0, 2 and 4 g h^{-1}, respectively. At a low feed Cu(II) concentration of 5 mg l^{-1}, the optimal SRT/HRT/PWS/feed Cu(II) ratio yielding the highest percent COD removal (82%) was 15 d/15 h/2 g h^{-1}/5 mg l^{-1}, while at a high feed Cu(II) concentration of 50 mg l^{-1}, this ratio was 15 d/15 h/4g h^{-1}/ 50 mg l^{-1}. The optimal loading rate ratio was L_{PWS} / $L_{Cu(II)}$ = 0.141 g PWS/ mg Cu(II) when the feed Cu(II) was 50 mg l^{-1} under the specified operating conditions.

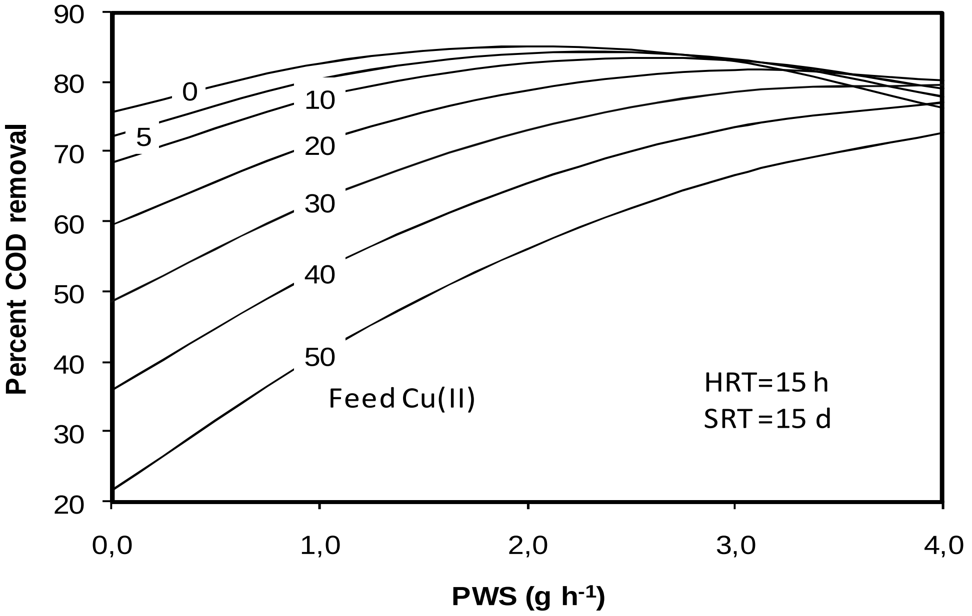


Figure 5.57 Variation of percent COD removal with PWS loading rate at different fed Cu(II) concentrations. HRT = 15 hour, SRT = 15 days.

Figure 5.58 depicts variations of percent COD removal with HRT at different PWS loading rates and a constant feed Cu(II) concentration (30 mg l^{-1}) and SRT (15 h). Percent COD removal increased with increasing HRT and PWS loading rates. The lowest COD removal was obtained in the absence of PWS addition which also increased with increasing HRT. Cu(II) removal in the absence of PWS was due to biosorption onto biomass in the aeration tank which was not sufficient. Cu(II) removal increased with increasing PWS additions and yielded higher COD removals due to removal of Cu(II) ions resulting in lower levels of Cu(II) toxicity on the microorganisms. Maximum COD removals (> 80%) were obtained at an HRT of 25 h and PWS loading rates of 3 or 4 g h^{-1}. Increases in COD removals by increasing PWS loading from 3 g h^{-1} to 4 g h^{-1} were marginal and 3 g h^{-1} PWS loading rate was sufficient. When PWS loading rate was 2 g h^{-1}, percent COD removals were 69%, 73% and 76% at HRT of 5, 15 and 25 hours, respectively at a feed Cu(II) concentration of 30 mg l^{-1} and SRT of 15 day. That is increasing HRT from 15 to 25 h yielded marginal improvements in COD removals and 15 h HRT may be sufficient for satisfactory COD removals. The optimal loading rate ratio was $L_{PWS} / L_{Cu(II)}$ = 0.29 g PWS/ mg Cu(II) at an HRT of 25 h. Operations at high HRT levels such as 25 h increases percent COD removal at the expense of high PWS loading requirements. The ANOVA analysis indicated that all four variables SRT, HRT, feed Cu(II) concentration and PWS loading rate and their interactions (X_1, X_2, X_3,X4, X_1X_2, X_1X_3, X_2X_3, X_3X_4, X_3^2,X_4^2) played important roles for the removal of COD.

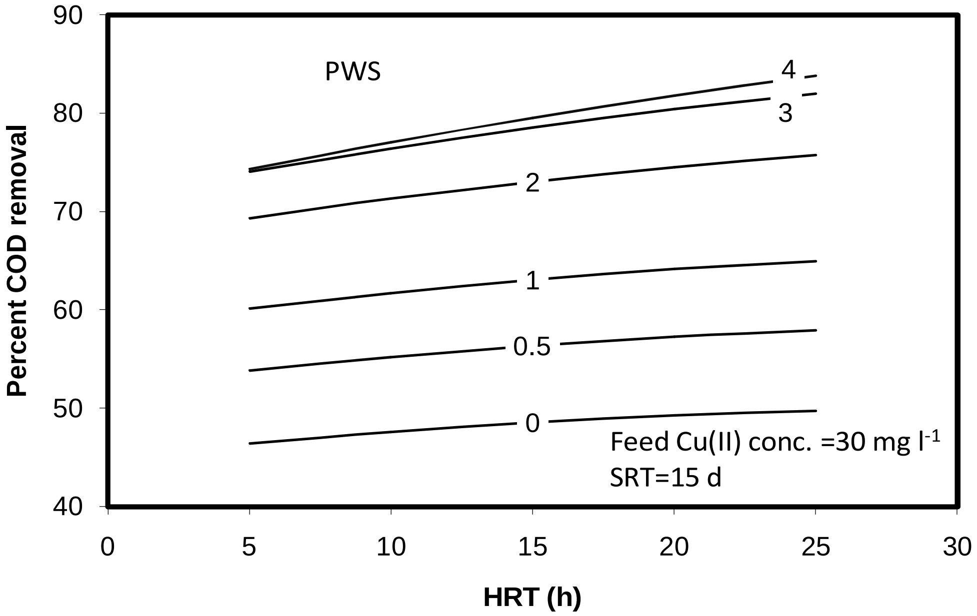


Figure 5.58 Variation of percent COD removal with HRT at different PWS loading rates. $Cu(II)_o$ = 30 mg l^{-1}, SRT = 15 days.

Variation of percent toxicity removal with the feed Cu(II) and PWS loading rates at constant SRT (15 d) and HRT (15 h) as depicted in Figure 5.59 shows similar trends with percent Cu(II) removals (Figure 5.55) since the only source of toxicity is Cu(II) ions. Percent toxicity removal increased with the feed Cu(II) concentration up to 30 mg l^{-1} corresponding Cu(II) loading rate of 17 mg h^{-1}. Further increases in Cu(II) concentration or loading rates caused decreases in percent toxicity removal due to insufficient removal of Cu(II) at high Cu(II) loadings. In the absence of PWS addition, nearly 55% toxicity removal was observed due to biosorption of Cu(II) ions on to biomass in the activated sludge unit. Percent toxicity removal increased with the addition of PWS. The peak percent toxicity removal increased from 55% to 75% and further to 90% with 1g h^{-1} and 2 g h^{-1} PWS loadings, respectively as compared to no PWS addition. Percent toxicity removals were lower than that of Cu(II) removals due to residual toxic effects of Cu(II) ions adsorbed onto biomass in the activated sludge. The maximum (100%) toxicity removal was obtained with 4 g h^{-1} PWS loading when the feed Cu(II) was 30 mg l^{-1} although 3 g h^{-1} PWS loading yielded comparable toxicity removals. The optimal SRT/HRT/PWS/Feed Cu(II) ratio yielding the highest toxicity removal (100%) was 15 d/15 h/3 g h^{-1}/30 mg l^{-1} corresponding to optimum PWS loading /Cu(II) loading ratio of 0.176 g PWS/mg Cu(II) under the specified experimental conditions.

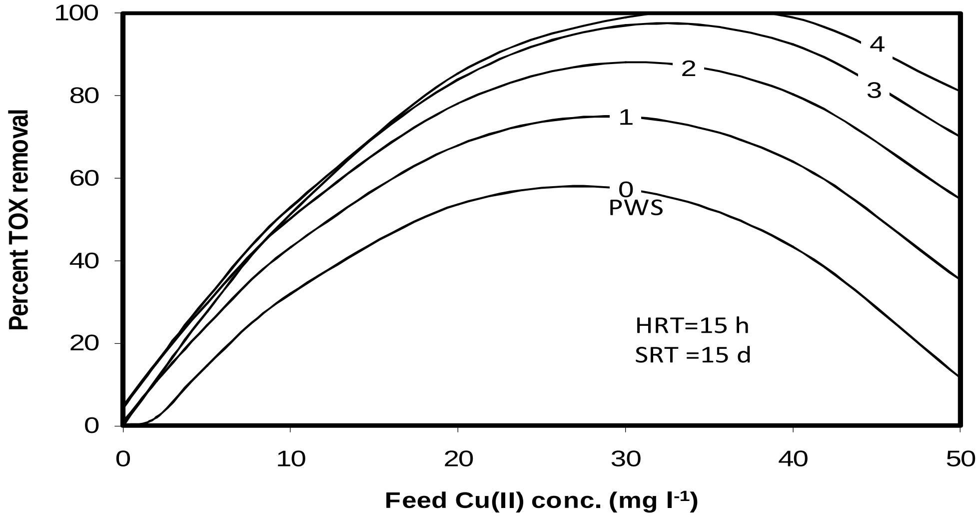


Figure 5.59 Variation of percent toxicity removal with the feed Cu(II)o concentrations at different PWS loadings. HRT = 15 hours, SRT = 15 days.

Sludge volume index (SVI) value is a measure of the settling characteristics of the sludge. Low SVI values below 100 ml g^{-1} indicate well settling sludge while SVI values greater than 150 ml g^{-1} means bulking sludge. Variations of sludge volume index (SVI) with HRT at different PWS loading rates and a constant feed Cu(II) concentration (30 mg l^{-1}) and SRT (15 h) are depicted in Figure 5.60. SVI decreased with increasing HRT and PWS loading rates since percent copper removal increased yielding lower levels of toxicities at high HRT and PWS loadings. When HRT was increased from 5 h to 10 and 15 hours at a constant PWS loading rate of 2 g h^{-1}, SVI values decreased from 117 ml g^{-1}, to 111 and 106 ml g^{-1}, respectively. SVI also decreased with increasing PWS loadings due to effective removal of toxic Cu(II) ions at high PWS loadings yielding high biomass concentrations in the aeration tank. The lowest SVI (90 ml g^{-1}) was obtained with 3 g h^{-1} PWS loading since Cu(II) removal was maximum with this PWS loading. When the system was operated with feed Cu(II) of 30 mg l^{-1} and SRT of 20 days, the HRT and PWS loadings should be 25 h and 3 g h^{-1} in order to obtain minimum SVI and good settling sludge.

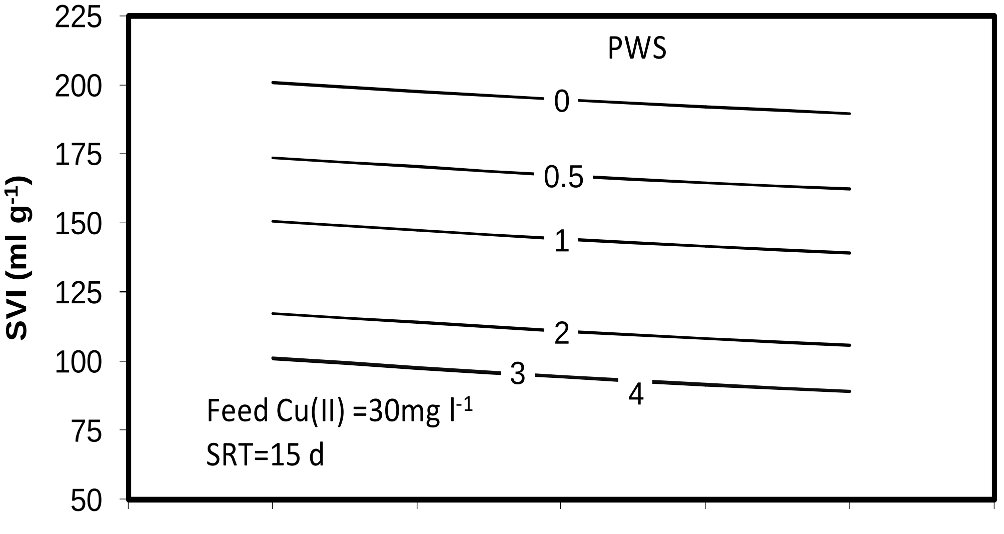


Figure 5.60 Variation of sludge volume index (SVI) with hydraulic residence time (HRT) at different PWS loading rates. $Cu(II)_o$ = 30 mg l^{-1}, SRT = 15 days.

Consequently, the data were correlated with a quadratic response function and the coefficients were determined by regression analysis. Percent Cu(II), COD, toxicity removals and SVI values calculated from the response functions were in good agreement with the experimental results. Cu(II), COD and toxicity removals increased with increasing PWS loading rate and SRT and decreased the feed Cu(II) concentration and HRT. Optimum conditions resulting in maximum Cu(II), COD, toxicity removals and SVI values were found to be SRT of nearly 30 days, HRT 15 hours, PWS loading rate 3 g h^{-1} and feed Cu(II) concentration of less than 30 mg l^{-1}. The optimal PWS loading/ Cu loading ratio was found to be 0.14 g PWS/ mg Cu at an SRT of 20 days and HRT of 15 hours.

CHAPTER FIVE
CONCLUSIONS

Powdered waste sludge (PWS) was used for removal of Cu(II) ions from synthetic wastewater by biosorption. In order to determine the most effective activated sludge for copper(II) ion removal from wastewater by biosorption, five different activated sludges obtained from different wastewater treatment plants were tested for their Cu(II) biosorption capacity in form of dry powder. The sludge sample obtained from the paint industry wastewater treatment plant (DYO Paint Ind., Izmir, Turkey) was found to be superior to the others tested with a biosorption capacity of nearly 50 mg Cu(II) g PWS^{-1}. Various pre-treatment solutions were used in order to improve biosorption capacity of the selected powdered activated sludge (PWS). Hydrogen peroxide (1%, v/v) pre-treatment was found to be the most effective one as compared to the other methods. The biosorption capacity has improved to nearly 65 mg g^{-1} after 1% H_2O_2 pre-treatment.

Using the waste sludge from the paint industry after pre-treatment with 1% H_2O_2, isotherms of biosorption of Cu(II) ions onto pre-treated powdered waste sludge (PWS) were studied. Experiments were carried out by varying initial Cu(II) ion concentrations between 50 and 400 mg l^{-1} while the adsorbent (PWS) concentration and the particle size were constant at 1 g l^{-1} and 64 µm at pH 5. Three different biosorption isotherms namely the Langmuir, Freundlich and the generalized isotherms were used to correlate the equlibrium experimental data. The constants of each isotherm were determined by using the equilibrium biosorption data. The Langmuir isotherm was found to fit the experimental data better than the other isotherms tested although the generalized isotherm also represented the data reasonably well. The maximum biosorption capacity of pre-treated PWS for the Cu(II) ions was found to be nearly 116.3 mgCu(II) g PWS^{-1}. The paint industry waste sludge used for Cu(II) ion biosorption was found to be superior to the waste sludges used by other investigators yielding higher biosorption capacity (q_m) and lower saturation constant (K) for the Langmuir isotherm.

Biosorpion of copper ions from aqueous solution onto powdered waste sludge (PWS) was investigated as functions of important operating variables such as particle size or external surface area of PWS, concentrations of adsorbent (PWS) and adsorbate (Cu(II) ions) and also medium pH. The extent of biosorption of copper ions increased and the final Cu(II) concentration in solution decreased with decreasing particle size (D_p) because of larger external surface area of PWS at small particle sizes. Six hours of incubation was sufficient to reach biosorption equilibrium. Percent biosorption increased from 46 to 95 % when particle size decreased from 338 to 53 µm yielding effluent Cu(II) concentrations as 54 and 5 mg l^{-1}, respectively at the end of 6 hours incubation. The initial rate of biosorption was also affected by the changes in particle size. The volumetric biosorption rate (R_v) increased and the surface rate (R_s) decreased with decreasing particle size or increasing external specific surface area. Changes in biosorption rate with the particle size were steeper at large particle sizes which became rather insignificant at small particle sizes below 64 µm.

Initial copper ion concentration also affected the extent of biosorption at constant PWS concentration and the particle size. As the copper ion concentration increased more binding sites on PWS surfaces were occupied by Cu(II) ions yielding larger solid phase Cu(II) concentrations (q_e, mg g^{-1}) at equilibrium. The maximum biosorbed Cu(II) concentration was found to be 117 mg g^{-1} with initial Cu(II) and PWS concentrations of 400 mg l^{-1} and 1 gl^{-1}, respectively. Increases in PWS concentrations resulted in increased binding sites on the biosorbent when initial copper ion concentration was constant at 100 mg l^{-1}. Therefore, lower fractions of PWS surfaces were occupied by Cu(II) ions as the PWS concentration increased which yielded lower solid phase Cu(II) concentrations (q_e). The equilibrium biosorbed Cu(II) concentration (q_e) decreased from 156 to 52 mg g^{-1} when the PWS concentration was increased from 0.25 to 3 g l^{-1}.

Medium pH affected the zeta potentials or surface charges of PWS and also the solubility of the copper ions. At low pH values, or high (H^+) ion concentrations, the surfaces of PWS particles -which would be negatively charged at neutral pH- were partially neutralized yielding less negative surface charges and therefore lower attraction forces between the PWS surfaces and Cu(II) ions. The extent of biosorption was negligible at low pH levels which increased with increasing pH as a result of more negative charges or zeta potentials on PWS surfaces attracting Cu(II) ions. Copper ions precipitated in form of $Cu(OH)_2$ at pH levels at and above 6 resulting in very fast removal of Cu(II) ions from solution. Therefore, the optimal pH yielding maximum extent of biosorption without any copper ion precipitation was pH 5, which resulted in minimum zeta potential of -15.6 mV and a solid phase Cu(II) concentration of 85 mg g^{-1}.

Batch biosorption kinetics of Cu(II) ions onto powdered waste sludge (PWS) under different operating conditions such as variable pH, temperature, Cu(II) ion concentrations, PWS concentration and the particle size were investigated. The following conclusions were reached on the basis of the experimental results.

- Second-order kinetics was found to be more suitable as compared to the first-order due to inclusion of both adsorbent and adsorbate species in biosorption of Cu(II) ions onto PWS.
- The rate constants of both models increased with increasing pH from 3 to 6 due to increasing negative surface charges of PWS and also less competition with the Cu(II) ions by the lower H^+ concentrations in solution. The biosorption should be carried out at pH 5 to obtain high rates.
- The rate constants increased with increasing temperature due to higher energy levels and frequency of interactions among the adsorbate and the adsorbent species at high temperatures. The activation energy according to the second-order kinetic model was 11.69 kcal mol^{-1} indicating high degree of sensitivity of the biosorption rate to temperature changes.
- The rate constants for both kinetic models decreased with increasing initial Cu(II) concentrations due to competition among the Cu(II) ions for the same binding sites on PWS surfaces at high Cu(II) concentrations (hindering effect). Cu(II) concentrations below 100 mg l^{-1} is preferred for high rates of biosorption.

- The rate constants increased with the increasing adsorbent (PWS) concentration due to increased binding sites at high adsorbent concentrations. High PWS concentrations are preferred for high rates of biosorption. However, due to adsorbent particle agglomeration adsorbent concentrations above 3 g l^{-1} are not reconmmended.
- The rate constants for both models increased with decreasing particle size due to larger total surface area of particles at small particle sizes. Biosorption should be carried out at low particle sizes of the adsorbent.

A completely mixed adsorption tank operated in fed-batch mode was used in the second set of experiments to compare with the batch biosorption experiments. Fed-batch experiments were performed with variable feed flow rates (0.075-0.325 l h^{-1}), feed copper ion concentrations (50- 300 mg l^{-1}) and the amount of adsorbent (1-6 g). Breakthrough curves were obtained for each set of experiments. Percent copper ion removals from the feed solution increased with increasing mass of PWS, but decreased with increasing feed flow rate and feed copper ion concentration or increasing copper ion loading rate. Solid phase (biosorbed) copper ion concentrations (mg g^{-1}), increased with increasing feed flow rate and copper ion concentrations at constant PWS of 3 g. A modified Bohart-Adams equation was used to correlate the breakthrough time (t_b) with the mass of adsorbent (m_{ads}) and also with the copper ion loading rate (Q C_o). Experimental data was used to determine the constants of the modified Bohart-Adams equation by iteration. The adsorption capacity of PWS (27.7 gCu. kg^{-1}PWS) used was comparable with those obtained with the powdered activated carbon (PAC) adsorption columns (30-80 gCu. kg^{-1}PAC) and superior to some of the other adsorbents reported in literature. The adsorption rate constant determined in our study (15.1 m^3 kg^{-1} h^{-1}) was an order of magnitude larger than those obtained in PAC adsorption column studies (0.5-1.5 m^3 kg^{-1} h^{-1}). This is mainly because of the use of completely mixed adsorption tank instead of an adsorption column and therefore, elimination of mass transfer limitations encountered in adsorption columns. A completely mixed adsorption reactor operated in fed-batch mode was proven to be more advantageous as compared to the adsorption columns.

The third set of experiments involve activated sludge system operated with and without the addition of PWS. Copper(II) ion toxicity on COD removal performance of an activated sludge unit treating Cu(II) containing synthetic wastewater was investigated at different feed Cu(II) concentrations varying between 0 and 22 mg l^{-1}. Increases in the feed Cu(II) concentration caused increases in the aeration tank aqueous phase Cu(II) concentrations which resulted in inhibition of microbial activities. Part of the Cu(II) in the feed was biosorbed onto the surfaces of the organisms resulting in lower free Cu(II) levels in the aqueous phase. Low biomass concentrations and microbial activities at high Cu(II) concentrations due to Cu(II) toxicity resulted in low COD removals from the wastewater. Toxicity levels of the feed and the effluent wastewater increased almost linearly with the Cu(II) concentration indicating severe toxicities for the feed Cu(II) concentrations of above 10 mg l^{-1}. Sludge settling was also unsatisfactory yielding bulking sludge with SVI values above 200 mg l^{-1} when the feed Cu(II) was 10 mg l^{-1} with a $Cu(II)_R$ of 2.5 mg l^{-1}. A kinetic model was developed to describe the inhibitory effects of Cu(II) ions on COD removal rate and the Cu(II) inhibition consant was determined as 2.85 mg Cu(II) l^{-1} by using the experimental data.

In order to investigate Cu (II) ion toxicity on the organisms at different operating conditions, synthetic wastewater containing 14 mg l^{-1} Cu (II) ions and 2000±100 mg l^{-1} COD was treated in an activated sludge unit at different solids (SRT) and hydraulic retention times (HRT). Part of the Cu (II) ions in the feed wastewater was biosorbed onto the biomass in the activated sludge unit. The extent of Cu(II) biosorption increased and the free-Cu (II) in solution decreased with increasing sludge age (SRT) and HRT since high SRT and HRT levels resulted in high biomass concentrations in the aeration tank. As a result of high biomass concentrations at high sludge age and HRT levels Cu (II) ion toxicity decreased, percent COD and toxicity removals increased with increasing SRT and HRT. However, SRT had a much more pronounced affect on biomass concentrations and therefore, on the COD and toxicity removals as compared to HRT. Sludge volume index values also decreased yielding well settling sludges at high SRT (30 days) and HRT (25 hours) levels. Copper ion toxicity was more pronounced at low SRT (5-10days) and HRT (5-10 hours) levels which was overcome at high sludge ages of 30 days.

Design equations of activated sludge units were modified for Cu (II) containing wastewater to include Cu (II) ion inhibition on COD removal rate and the saturation constants. Activated sludge experiments were performed with synthetic wastewater containing 15 mg l^{-1} Cu (II) ions and 2000 mg l^{-1} COD at different solids (SRT) and hydraulic retention times (HRT) in order to quantify Cu (II) ion inhibition on COD removal rate at different operating conditions. The same experiments were repeated with Cu (II)-free wastewater. The kinetic and stoichiometric constants obtained in the absence of Cu (II) ions were, k = 1.5 d^{-1}, K_s = 254 mg l^{-1}, Y_M = 0.23 gX g^{-1} COD, b = 0.1385 d^{-1} which are comparable with the literature values. Copper ions present in the feed wastwater (15 mg l^{-1}) affected both the rate (k) and the saturation constants (Ks) adversely yielding lower 'k' and higher 'Ks'values with a non-competitive inhibition pattern. The inhibition constants for the rate and the saturation constants were found to be K_{Cu} = 97 mg l^{-1} and K'_{Cu} = 18 mg l^{-1} indicating more pronounced inhibition on K_s. The growth yield coefficient decreased to 0.15 gX g^{-1}S and the death rate constant increased to 0.15 d^{-1} in the presence of 15 mg l^{-1} Cu (II) ions. The inhibition constants on the growth yield (Y) and the death rate constant (b) were K_Y = 27 mg l^{-1} and K_b = 210 mg l^{-1} indicating more pronounced Cu (II) inhibition on the growth yield coefficient or higher maintenance requirements in the presence of Cu (II) ions. Model predictions were compared with the experimental data and a reasonable fit was observed especially at high SRT and HRT levels where Cu (II) inhibition was less pronounced due to high biomass concentrations.

In order to overcome the inhibition effects of Cu(II), powdered waste sludge (PWS) was added to synthetic wastewater containing 14 or 22 mg l^{-1} Cu (II) for removal of Cu (II) ions by adsorption before biological treatment in an activated sludge unit. Feed wastewater containing Cu (II) and PWS were mixed in a mixing tank and the mixture was settled in a sedimentation tank to separate PWS from the wastewater. The effluent of the sedimentation tank with low Cu (II) content was fed to the activated sludge unit for COD removal. The system was operated with different PWS loading rates while the HRT and SRT were constant at 10 h and 10 days, respectively. Cu (II) was effectively removed from the wastewater by adsorption onto PWS before biological treatment reducing the toxicity of the feed wastewater and hence improving the performance of the activated sludge unit. Cu

(II) removal from the feed wastewater increased with increasing PWS loading rates yielding high biomass concentrations in the activated sludge unit and therefore high COD removals. Cu (II) removal by PWS addition also improved the settling characteristics of the activated sludge bacteria resulting in SVI values as low as 122 ml g^{-1}. The minimum PWS loading rate was found to be 1 g h^{-1} per 28 mg h^{-1} Cu (II) loading rate to achieve complete Cu (II) removal from the feed wastewater to eliminate Cu (II) toxicity.

A Box-Behnken experimental design method was used considering four different independent variables: SRT (5-30 days), HRT (5-25 hours), feed Cu(II) concentration (0-50 mg l^{-1}) and PWS loading rate (0-4 g h^{-1}). Effects of those variables on percent Cu(II), chemical oxygen demand (COD), toxicity (TOX) removals and sludge settling characteristics (SVI) were investigated. Different response functions were correlated with the experimental data using the ANOVA test and a quadratic polynomial equation was found to be the most suitable one with the highest correlation coefficients and the lowest *p*-values. Operations at high SRT (>20 days) and HRT (>15 h) increased the biomass concentration and reduced the toxic effect of Cu(II) ions yielding high COD and toxicity removals. Cu(II) ion removal from the wastewater was accomplished by PWS addition before biological treatment. Cu(II) removal increased with increasing PWS loading rates yielding low effluent toxicities and high COD removals. The required PWS loading rates varied with Cu(II) loadings and the operating conditions. The optimal PWS loading/ Cu loading ratio was found to be 0.14 g PWS/ mg Cu at an SRT of 20 days and HRT of 15 hours.

The following recommendation are made for further study:

- In this study, single heavy metal ion (Cu(II)) was removed by biosorption onto PWS. However, in reality municipal and industrial effluents contain more than one type of metal ion. PWS can be used for removal of metal ions from real wastewaters containing a number of metal ions.

- The combined effects of heavy metals on activated sludge may not be same as the single metal ion effects. Therefore, it is worth to extend the study to investigate the combined effects of different heavy metals on the activated sludge. The combined effects on activated sludge, and the competitive adsorption among different metals are of practical importance and will provide useful information for actual industrial applications.

- Similar studies can be conducted with the other heavy metals individually and in combination to determine which heavy metals should be the greatest concern.
- Determination of synergistic or antagonistic relationships between heavy metals would be especially usefull in real wastewater treatment with PWS.
- The effectiveness of PWS for the removal of organics such as dyestuffs and pesticides can be investigated.
- PWS can be used as an effective adsorbent for the other treatment processes for heavy metal ion removal such as, anaerobic treatment, nutrient removal and sequencing batch reactors.
- The feasibility of using powdered waste sludge (PWS) in adsorption of heavy metals could be further investigated using adsorption columns.

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APPENDIX

RAW EXPERIMENTAL DATA AND FIGURES

A.1 Raw Data For Batch Shake Flask Experiments

A.1.1 Raw Data for Selection of Powdered Waste Sludge (PWS)

Table A.1 Raw data for variation of biosorption capacities of PWS samples from different wastewater treatment plants (No Pre-treatment)

No Pre-treatment	**Time (h)**								
Wastewater Treatment Plant	0	0.083	0.5	1	2	3	4	6	24
1. Pakmaya	0	13	25	29	33	34	35	35	28
2. DYO	0	9	20	23	39	47	49	47	50
3. Güzelbahçe	0	3	9	12	19	21	22	25	23
4. Kısıkköy	0	10	20	25	37	39	39	38	34
5. Çiğli	0	13	14	22	26	27	29	29	30

A.1.2 Raw Data for Selection of Pre-treatment Method

Table A.2 Raw data for the effects of different pre-treatment methods on biosorption capacity of PWS from different treatment plants (a- Pretreatment - H_2SO_4, b- Pretreatment - H_2O_2, c- Pretreatment – NaOH, d- Pretreatment – Ethanol, e- Pretreatment - NaOCl

(a)

Pretreatment - H_2SO_4	**Time (h)**								
Wastewater Treatment Plant	0	0.083	0.5	1	2	3	4	6	24
1. Pakmaya	0	19	27	33	39	41	42	42	44
2. DYO	0	14	25	32	41	48	50	49	47
3. Güzelbahçe	0	13	18	23	29	31	33	34	33
4. Kısıkköy	0	6	12	21	37	38	40	39	40
5. Çiğli	0	13	17	21	28	29	29	32	33

(b)

Pretreatment - H_2O_2	**Time (h)**								
Wastewater Treatment Plant	0	0.083	0.5	1	2	3	4	6	24
1. Pakmaya	0	24	31	38	44	53	61	63	63
2. DYO	0	33	40	46	51	55	63	65	65
3. Güzelbahçe	0	20	24	30	33	37	42	43	44
4. Kısıkköy	0	21	28	33	38	42	45	45	46
5. Çiğli	0	15	20	24	28	32	34	35	35

(c)

Pretreatment - NaOH	**Time (h)**								
Wastewater Treatment Plant	0	0.083	0.5	1	2	3	4	6	24
1. Pakmaya	0	13	19	22	30	34	38	40	39
2. DYO	0	22	28	34	38	42	44	44	45
3. Güzelbahçe	0	12	18	22	28	31	34	36	36
4. Kısıkköy	0	12	19	24	29	32	35	36	35
5. Çiğli	0	16	20	26	31	35	37	39	38

(d)

Pretreatment - Ethanol	**Time (h)**								
Wastewater Treatment Plant	0	0.083	0.5	1	2	3	4	6	24
1. Pakmaya	0	18	23	26	31	34	38	39	38
2. DYO	0	20	25	28	40	51	60	61	62
3. Güzelbahçe	0	16	24	26	27	29	29	30	30
4. Kısıkköy	0	19	28	39	40	42	43	43	44
5. Çiğli	0	17	21	29	30	33	34	34	35

(e)

Pretreatment - NaOCl	**Time (h)**								
Wastewater Treatment Plant	0	0.083	0.5	1	2	3	4	6	24
1. Pakmaya	0	16	23	29	31	39	43	46	46
2. DYO	0	26	33	39	44	48	53	55	54
3. Güzelbahçe	0	17	22	30	34	37	40	41	43
4. Kısıkköy	0	18	20	26	31	32	34	35	36
5. Çiğli	0	19	22	27	33	37	41	42	43

Table A.3 Raw data for variation of percent copper ion removal by biosorption for different PWS samples with different pre-treatment methods

Percent rem. of Cu(II) (%)	**Pretreatment- H_2SO_4**	**Pretreatment- H_2O_2**	**Pretreatment- NaOH**	**Pretreatment- Ethanol**	**Pretreatment- NaOCl**	**No Pretreatment**
1. Pakmaya	42	63	40	39	46	26
2. DYO	49	65	44	61	55	47
3. Güzelbahçe	34	43	36	30	41	25
4. Kısıkköy	39	45	36	43	35	38
5. Çiğli	32	35	39	34	42	26

A.1.3 Raw Data for The Effects of Environmental Parameters on Biosorption of Cu(II) Ions onto PWS

Table A.4 Raw data for variation of biosorbed copper ion concentration with time for different particle sizes of PWS.

Biosorbed Cu(II) conc. (mg g^{-1})	**Time (h)**								
Average Particle size (µm)	0	0.083	0.5	1	2	3	4	6	24
338	0	18	25	32	38	43	46	45	46
231	0	26	30	34	41	46	51	52	53
178	0	33	40	46	51	55	63	65	65
109	0	26	42	49	63	73	81	82	81
64	0	23	49	58	66	78	83	85	86
53	0	37	56	69	76	83	95	96	96

Table A.5 Raw data for variation of final copper concentration and percent copper removal with the specific external surface area of PWS.

Spec. ext. surface area of PWS (m^2 g^{-1} PWS)	**Final Cu(II) conc. (mg l^{-1})**	**Percent Cu(II) removal (%)**
147.929	54	46
216.4502	49	51
280.8989	37	63
458.7156	19	81
781.25	17	83
943.3962	5	95

Table A.6 Raw data for variation of volumetric and surface biosorption rates with the specific external surface area of PWS

Spec. ext. surface area of PWS (m^2 g^{-1} PWS)	**Volumetric biosorption rate, R_v (mg Cu(II) l^{-1} h^{-1})**	**Surface biosorption rate, R_s (mg Cu(II) cm^{-2} h^{-1})**
147.929	32	0.21632
216.4502	34	0.15708
280.8989	46	0.16376
458.7156	49	0.10682
781.25	58	0.07424
943.3962	69	0.07314

Table A.7 Raw data for variation of biosorbed copper ion concentration with time for different initial copper ion concentrations

Biosorbed Cu(II) conc. (mg g^{-1})	**Time (h)**								
Initial Cu(II) concentrations (mg l^{-1})	0	0.083	0.5	1	2	3	4	6	24
50	0	18	24	30	38	43	45	46	46
100	0	21	32	41	52	69	81	84	84
150	0	20	35	48	69	84	98	100	101
200	0	24	37	49	75	93	105	108	109
250	0	27	39	58	81	100	110	111	111
300	0	31	41	63	85	103	112	113	114
400	0	41	63	79	98	107	116	117	117

Table A.8 Raw data for variation of biosorbed copper ion concentration with time for different PWS concentrations

Biosorbed Cu(II) conc. (mg g^{-1})	**Time (h)**							
PWS concentrations (mg l^{-1})	0	0.083	0.5	1	2	3	4	6
0.25	0	31	52	80	110	131	147	156
0.5	0	25	51	75	102	114	124	126
1.0	0	21	30	42	57	69	75	75
1.5	0	14	21	31	43	53	59	59
2.0	0	13	19	27	37	46	50	50
2.5	0	11	19	26	37	45	50	50
3.0	0	14	21	27	35	44	52	52

Table A.9 Raw data for variation of biosorbed Cu(II) concentration with time at different pH's

Biosorbed Cu(II) conc. (mg g^{-1})	**Time (h)**								
pH	0	0.083	0.5	1	2	3	4	6	24
2	0	0	2	1	0	0	0	1	0
3	0	3	8	10	14	17	22	23	22
4	0	11	22	34	46	61	73	73	74
5	0	23	49	58	66	78	83	85	86
6	0	57	91	93	93	94	95	93	94

Table A.10 Raw data for variation of biosorption capacity and the initial rate with pH

pH	Biosorption capacity, q_m (mg g^{-1})	Initial rate, r_{ads} (mg l^{-1} h^{-1})
2	1	1
3	23	10
4	73	34
5	85	58
6	93	93
7	98	94
8	99	96

Table A.11 Raw data for variation of zeta potential with the pH in PWS solution

pH	Zeta potential before Cu(II) addition	Zeta potential after Cu(II) addition
2	-8.6	-5.0
3	-17.1	-13.6
4	-24.3	-15.6
5	-30.4	-15.6
6	-35.3	-6.4
7	-40.8	-2.4
8	-42.6	3.5

A.1.4 Raw Data for the Kinetics of Biosorption of Copper Ions onto PWS

Table A.12 Raw data; (a) a plot of Ln (1- q_t / q_e) versus time according to the pseudo-first order kinetics at different pH's, (b) A plot of t/q_t versus time according to the pseudo-second order kinetics at different pH's

Pseudo-first order kinetics	**Time (h)**						
pH	0.083	0.5	1	2	3	4	6
3	-0.13976	-0.42744	-0.57054	-0.93827	-1.34373	-3.13549	-3.13549
4	-0.16093	-0.35282	-0.61519	-0.97186	-1.73912	-3.20545	-4.30407
5	-0.31121	-0.84343	-1.12214	-1.45862	-2.37491	-3.35574	-4.45435
6	-0.88583	-2.78295	-3.18842	-3.18842	-3.4761	-3.88156	-4.69

Table A.13 Raw data; (b) a plot of t/q_t versus time according to the pseudo-second order kinetics at different pH's

Pseudo-second order kinetics	**Time (h)**						
pH	0.083	0.5	1	2	3	4	6
3	0.027667	0.0625	0.1	0.142857	0.176471	0.181818	0.26087
4	0.007545	0.022727	0.029412	0.043478	0.04918	0.054795	0.082192
5	0.003609	0.010204	0.017241	0.030303	0.038462	0.048193	0.070588
6	0.001456	0.005495	0.010753	0.021505	0.031915	0.042105	0.064516

Table A.14 Raw data; (a) a plot of Ln(1-q_t/q_e) versus time according to the pseudo-first order kinetics at different temperatures.

Pseudo-first order kinetics	**Time (h)**						
Temperature (^{o}C)	0.083	0.5	1	2	3	4	6
30	-0.3245	-0.86349	-1.19996	-1.42311	-2.47293	-2.8094	-4.41884
35	-0.4173	-1.00866	-1.39813	-1.67006	-3.05636	-3.7495	-4.44265
40	-0.54057	-1.17865	-1.51513	-2.02595	-2.90142	-3.81771	-4.51086
45	-0.70396	-1.44156	-1.82455	-2.45316	-3.14631	-3.83945	-4.5326
50	-0.80078	-1.64053	-2.02002	-2.63906	-3.89182	-3.89182	-4.85

Table A.15 Raw data; (b) a plot of t/q_t versus time according to the pseudo-second order kinetics at different temperatures

Pseudo-second order kinetics	**Time (h)**						
Temperature (^{o}C)	0.083	0.5	1	2	3	4	6
30	0.003609	0.010417	0.017241	0.031746	0.039474	0.051282	0.073171
35	0.002862	0.009259	0.015625	0.028986	0.037037	0.047619	0.071429
40	0.002184	0.007937	0.014085	0.025316	0.034884	0.044944	0.066667
45	0.001766	0.007042	0.012821	0.023529	0.033708	0.043478	0.065217
50	0.001537	0.006329	0.011765	0.021978	0.03125	0.041237	0.061856

Table A.16 Raw data; (a) a plot of Ln(1-q_t/q_e) versus time according to the pseudo-first order kinetics for different Cu(II) ion concentrations.

Pseudo-first order kinetics	**Time (h)**						
Cu(II) ion conc. (mg l^{-1})	0.083	0.5	1	2	3	4	6
50	-0.5261	-0.7885	-1.1451	-1.9924	-2.3979	-3.6000	-4.5500
100	-0.2877	-0.4796	-0.6696	-0.9651	-1.7228	-3.3322	-4.4308
150	-0.2183	-0.4203	-0.6360	-1.1285	-1.7346	-3.2387	-3.9318
200	-0.2436	-0.4055	-0.5824	-1.1260	-1.8192	-2.9178	-3.6109
250	-0.2703	-0.4187	-0.5931	-1.1253	-2.0971	-2.7000	-3.2000
300	-0.3018	-0.4224	-0.7538	-1.2528	-2.0065	-2.4700	-3.1000
400	-0.1000	-0.6000	-0.8000	-1.1000	-1.7000	-2.1000	-3.0000

Table A.17 Raw data; (b) a plot of t/q_t versus time according to the pseudo-second order kinetics for different Cu(II) ion concentrations.

Pseudo-second order kinetics	**Time (h)**						
Cu(II) ion conc. (mg l^{-1})	0.083	0.5	1	2	3	4	6
50	0.0046	0.0208	0.0333	0.0526	0.0698	0.0889	0.1304
100	0.0040	0.0156	0.0244	0.0385	0.0435	0.0494	0.0714
150	0.0042	0.0143	0.0208	0.0290	0.0357	0.0408	0.0600
200	0.0035	0.0135	0.0204	0.0267	0.0323	0.0381	0.0556
250	0.0031	0.0128	0.0172	0.0247	0.0300	0.0364	0.0541
300	0.0027	0.0122	0.0159	0.0235	0.0291	0.0357	0.0531
400	0.0022	0.0104	0.0147	0.0213	0.0283	0.0351	0.0522

Table A.18 Raw dat; (a) a plot of Ln(1-q_t/q_e) versus time according to the pseudo-first order kinetics for different PWS concentrations.

Pseudo-first order kinetics	**Time (h)**						
PWS concentrations (mg l^{-1})	0.083	0.5	1	2	3	4	6
0.25	-0.1075	-0.4023	-0.7124	-1.0860	-1.5597	-2.4500	-3.5500
0.5	-0.4049	-0.5655	-0.9466	-1.3500	-2.0000	-2.6400	-3.9100
1.0	-0.3174	-0.4457	-0.8044	-1.3689	-2.3383	-2.7400	-4.1800
1.5	-0.2744	-0.4257	-0.7248	-1.2730	-2.1972	-3.4012	-4.3175
2.0	-0.2942	-0.4506	-0.7332	-1.2577	-2.3224	-3.2387	-4.4300
2.5	-0.2346	-0.4557	-0.6971	-1.2786	-2.1073	-3.2387	-5.1600
3.0	-0.3067	-0.5150	-0.7251	-1.7000	-2.3500	-3.2771	-4.6600

Table A.19 Raw data; (b) a plot of t/q_t versus time according to the pseudo-second order kinetics for different PWS concentrations.

Pseudo-second order kinetics	**Time (h)**						
PWS concentrations (mg l^{-1})	0.083	0.5	1	2	3	4	6
0.25	0.0052	0.0096	0.0125	0.0192	0.0242	0.0256	0.0385
0.5	0.0020	0.0091	0.0129	0.0196	0.0263	0.0323	0.0476
1.0	0.0040	0.0183	0.0238	0.0353	0.0437	0.0536	0.0796
1.5	0.0058	0.0240	0.0323	0.0463	0.0563	0.0682	0.1014
2.0	0.0064	0.0270	0.0377	0.0548	0.0652	0.0808	0.1212
2.5	0.0078	0.0268	0.0310	0.0543	0.0670	0.0870	0.1200
3.0	0.0059	0.0234	0.0366	0.0480	0.0687	0.0769	0.1146

Table A.20 Raw data; (a) a plot of Ln(1-q_t/q_e) versus time according to the pseudo-first order kinetics for different particle sizes.

Pseudo-first order kinetics	**Time (h)**						
Average Particle size (μm)	0.083	0.5	1	2	3	4	6
231	-0.6400	-0.7885	-0.9628	-1.3683	-1.8101	-2.6210	-2.9087
178	-0.6931	-0.9316	-1.1939	-1.4816	-1.7918	-3.0910	-4.1897
109	-0.3758	-0.7053	-0.8925	-1.4231	-2.1163	-3.7257	-4.4188
64	-0.3112	-0.8434	-1.1221	-1.4586	-2.3749	-3.3557	-4.9000
53	-0.4804	-0.8611	-1.2425	-1.5302	-1.9357	-3.8816	-5.2500

Table A.21 Raw data; (b) A plot of t/q_t versus time according to the pseudo-second order kinetics for different particle sizes.

Pseudo-second order kinetics	**Time (h)**						
Average Particle size (μm)	0.083	0.5	1	2	3	4	6
231	0.0032	0.0167	0.0294	0.0488	0.0584	0.0720	0.1000
178	0.0025	0.0125	0.0217	0.0310	0.0400	0.0540	0.0864
109	0.0032	0.0119	0.0170	0.0270	0.0411	0.0494	0.0780
64	0.0036	0.0102	0.0172	0.0303	0.0385	0.0482	0.0693
53	0.0022	0.0089	0.0145	0.0263	0.0361	0.0421	0.0615

A.1.5 Raw Data for Isotherm Studies

Table A.22 Raw data for variation of equilibrium biosorbed Cu(II) ion concentration with the equilibrium aqueous phase Cu(II) concentration, langmuir isotherm plot of $1/q_e$ versus $1/C_e$, freundlich isotherm plot of Lnq_e versus LnC_e, generalized biosorption isotherm plot of $Ln((q_m/q))-1)$ versus Ln C_e (PWS=1 g l^{-1}, D_p=64µm, pH=5).

Equil. aqueous phase Cu(II) conc., C_e (mg l^{-1})	**Equil. biosorbed Cu(II), q_e (mg g^{-1})**	**$1/q_e$**	**$1/C_e$**	**Ln q_e**	**Ln C_e**	**$Ln((q_m/q))-1)$**
4	46	0.0217	0.2500	3.8286	1.3863	0.4480
16	84	0.0119	0.0625	4.4308	2.7726	-0.9045
50	100	0.0100	0.0200	4.6052	3.9120	-1.7819
92	108	0.0093	0.0109	4.6821	4.5218	-2.4941
139	111	0.0090	0.0072	4.7095	4.9345	-2.7636
187	113	0.0088	0.0053	4.7274	5.2311	-3.3499
283	115	0.0087	0.0035	4.7449	5.6454	-4.7622

A.2 Raw Data for Experiments with Fed–Batch Operation

Table A.23 Raw data for the breakthrough curves for copper ion adsorption onto PWS for different PWS contents in fed-batch operation. (C_o=200 mg l^{-1},Q = 0.25 l h^{-1})

Time (h)	**1 g l^{-1} PWS**	**2 g l^{-1} PWS**	**3 g l^{-1} PWS**	**4 g l^{-1} PWS**	**5 g l^{-1} PWS**	**6 g l^{-1} PWS**
0	0	0	0	0	0	0
1	23.92	15.92	10.43	7.22	5.24	2.71
2	44.64	34.64	19.71	13.04	9.45	6.07
3	57.47	46.93	33.57	24.88	18.33	12.47
4	71.77	58.18	48.63	37.28	25.51	20.86
5	84.50	70.37	59.84	45.47	33.68	27.72
6	95.65	79.35	68.25	54.77	39.38	33.98
7	108.16	88.73	74.46	61.68	45.66	40.13
8	117.47	98.32	80.72	66.68	49.26	44.62
9	125.58	106.36	85.49	73.47	54.44	48.21
10	131.21	112.51	89.46	75.68	58.61	52.08
11	137.58	117.88	92.68	77.72	62.25	55.63
12	140.89	121.67	95.37	80.96	65.42	56.79
13	142.61	123.19	97.52	82.58	67.85	58.31
14	143.48	124.28	98.44	83.45	68.27	59.10
15	144.32	125.44	99.13	84.02	69.31	60.00

Table A.24 Raw data for variation of percent copper ion removals and biosorbed (solid phase) copper ion concentrations with the amount of the adsorbent (PWS) at the end of 10 hours fed-batch operation (C_o = 200 mg l^{-1}, Q = 0.25 l h^{-1}).

Adsorbent (PWS) (g l^{-1})	Percent Cu(II) removal	Biosorbed Cu(II) (mg g^{-1})
1	40	77
2	49	71
3	58	69
4	65	63
5	72	60
6	78	58

Table A.25 Raw data for the break-through curves for copper ion adsorption onto PWS for different feed flow rates of Cu(II) solution in fed-batch operation (C_o=100 mgl^{-1}, PWS=3 gl^{-1}).

Time (h)	0.075 l h^{-1}	0.125 l h^{-1}	0.175 l h^{-1}	0.225 l h^{-1}	0.275 l h^{-1}	0.325 l h^{-1}
0	0	0	0	0	0	0
1	0.1	0.8	2	2.1	3.4	4.8
2	0.2	1.2	3.2	3.8	6	9.5
3	0.3	1.6	4	5.5	8.2	12
4	0.4	2.4	6	7.5	9.5	15.7
5	0.6	3.2	6.9	9.5	14.3	20.5
6	0.7	4.2	9	15	19.8	28.6
7	0.76	5.4	12.1	18	23.2	31.4
8	1	6.5	13.5	20.5	27.3	33.4
9	1.2	7.1	14.1	23.2	28.6	36.8
10	1.4	7.7	15.4	26.6	32.7	40.9

Table A.26 Raw data for the Effect of feed flow rate on percent copper ion removals and solid phase Cu(II) concentrations at the end of 10 hours of operation (C_o=100 mgl^{-1}, PWS=3 gl^{-1})

Flow rate (l h^{-1})	**Percent Cu(II) removal**	**Biosorbed Cu(II) (mg g^{-1})**
0.075	98.6	24
0.125	92.3	36
0.175	84.6	44
0.225	73.4	46
0.275	67.3	51
0.325	59.1	50

Table A.27 Raw data for the breakthrough curves for copper ion adsorption onto PWS for different feed copper ion concentrations. (Q = 0.1 l h^{-1}, PWS = 3 g l^{-1})

Time (h)	**50 mg l^{-1}**	**100 mg l^{-1}**	**150 mg l^{-1}**	**200 mg l^{-1}**	**250 mg l^{-1}**	**300 mg l^{-1}**
0	0.0	0.0	0.0	0.0	0.0	0.0
1	0.1	1.7	2.1	4.0	5.0	5.0
2	0.2	3.0	3.2	7.0	6.0	19.0
3	0.4	3.2	4.9	10.0	12.0	28.0
4	0.8	3.3	5.8	15.0	21.0	40.0
5	1.2	3.7	8.0	23.0	26.0	47.0
6	1.6	4.1	12.7	34.0	40.0	64.0
7	2.0	4.9	17.3	41.0	52.0	78.0
8	2.5	6.1	22.7	47.0	59.0	85.0
9	2.9	8.0	27.3	48.0	68.0	93.0
10	3.3	12.0	32.7	52.0	76.0	97.0

Table A.28 Raw data for the effect of feed copper ion concentration on percent copper ion removals and solid phase copper ion concentrations at the end of 30 hours of operation. (Q = 0.1 l h^{-1}, PWS = 3 g l^{-1})

Feed Cu(II) conc. (mg l^{-1})	**Percent Cu(II) removal**	**Biosorbed Cu(II) (mg g^{-1})**
50	73	32
100	65	53
150	59	69
200	52	72
250	46	77
300	44	87

A.3 Raw Data for Activated Sludge Experiments

Table A.29 Raw data for variation of the effluent Cu(II) and COD, percent Cu(II) and COD removals, feed and effluent toxicities with the feed Cu(II) concentration in the activated sludge unit. (COD$_o$ = 2000 mg l^{-1}, HRT = 10 h, SRT = 10 d)

Feed Cu(II) conc. (mg l^{-1})	**Percent Cu(II) removal**	**Efluent Cu(II) conc. (mg l^{-1})**	**Effluent COD$_e$ (mg l^{-1})**	**Percent COD removal**	**Feed toxicity (%)**	**Effluent toxicity (%)**
0	0	0	113	95	0	0
5	77	1.14	328	85	15	10
10	75	2.44	477	76	29	17
15	64	5.15	693	66	44	21
17	50	8.60	1264	38	57	26
22	39	13.10	1557	23	69	31

Table A.30 Raw data for variation of the equilibrium Cu(II) concentrations in aqueous and solid phases in the activated sludge unit (COD_o = 2000 mg l^{-1}, HRT = 10 h, SRT = 10 d).

Aqueous phase Cu(II) (mg l^{-1})	**Solid phase Cu(II), q_s (mg g^{-1})**
0	0
1.14	1.09
2.44	2.84
5.15	5.92
8.60	7.68
13.10	9.54

Table A.31 Raw data for variation of steady-state biomass concentration (X) and the sludge volume index (SVI) with the feed Cu(II) concentration, Feed Cu(II) concentration with $1/R_{COD}$ (COD_o = 2000 mg l^{-1}, HRT = 10 h, SRT = 10 d).

Feed Cu(II) conc. (mg l^{-1})	**Biomass concen. (mg l^{-1})**	**Sludge volume index, SVI (ml g^{-1})**	**$1/R_{COD}$ (mg l^{-1} h^{-1})$^{-1}$**
0	4050	136	0.053
5	3550	140	0.060
10	2520	206	0.066
15	1530	294	0.077
17	1120	333	0.136
22	870	467	0.226

Table A.32 Raw data for variations of effluent Cu(II), biomass concentration, the sludge volume index (SVI)and percent Cu(II) removals with the sludge age (COD_o = 2000±100 mg l^{-1}, $Cu(II)_o$ = 14 mg l^{-1}, HRT = 10 hours).

Sludge age (day)	Efluent Cu(II) conc. (mg l^{-1})	Percent Cu(II) Removal	Biomass conc.-Cu free(mg l^{-1})	SVI-Cu free (ml g^{-1})	Biomass conc.-Cu 14 mg l^{-1} (mg l^{-1})	SVI-Cu Cu 14 mg l^{-1} (ml g^{-1})
30	2.4	83	6900	69	6700	74
25	3.1	78	6300	76	5900	82
20	4.1	71	5600	91	4350	115
15	4.8	66	4700	105	3300	132
10	5.3	62	3500	154	1750	263
5	6.2	55	2200	213	980	323

Table A.33 Raw data for variations of effluent COD, percent COD removal, effluent toxicity and percent toxicity removal with the sludge age (COD_o = 2000±100 mg l^{-1}, HRT = 10 hours).

Sludge age (day)	Effluent COD-Cu free (mg l^{-1})	Percent COD removal -Cu free	Effluent COD-Cu 14 mg l^{-1} (mg l^{-1})	Percent COD removal - Cu 14 mg l^{-1}	Effluent toxicity (%)	Percent toxicity removal
30	10	99	27	99	2	95
25	43	98	198	90	10	78
20	117	94	349	83	13	70
15	208	90	484	76	17	61
10	418	79	657	68	21	52
5	654	68	945	53	31	30

Table A.34 Raw data for variations of effluent Cu(II), biomass concentration, the sludge volume index (SVI)and percent Cu(II) removals with hydraulic residence time (COD_o = 2000±100 mg l^{-1}, SRT = 10 days).

Hydraulic residence time (h)	Efluent Cu(II) conc. (mg l^{-1})	Percent Cu(II) removal	Biomass conc.-Cu free(mg l^{-1})	SVI-Cu free (ml g^{-1})	Biomass conc.-Cu 14 mg l^{-1} (mg l^{-1})	SVI-Cu Cu 14 mg l^{-1} (ml g^{-1})
25	3.2	77	6600	41	5350	45
20	3.9	72	5700	45	4200	49
15	4.6	67	4450	49	3150	54
10	5.3	62	3020	61	1750	72
5	6.2	56	2200	70	1100	83

Table A.35 Raw data for variations of effluent COD, percent COD removal, effluent toxicity and percent toxicity removal with hydraulic residence time (COD_o = 2000±100 mg l^{-1}, $Cu(II)_o$ = 14 mg l^{-1}, SRT = 10 days).

Hydraulic residence time (h)	Effluent COD-Cu free (mg l^{-1})	Percent COD removal -Cu free	Effluent COD-Cu 14 mg l^{-1} (mg l^{-1})	Percent COD removal - Cu 14 mg l^{-1}	Effluent toxicity (%)	Percent toxicity removal
25	55	97	185	91	7	84
20	145	93	342	83	13	70
15	284	86	490	76	16	64
10	417	79	696	66	21	52
5	800	60	1175	42	29	34

A.3.1 Raw Data for the Mathematical Modeling of Copper(II) Ion Inhibition on COD Removal

Table A.36 Raw Data; plots of 1/U versus 1/S for variable HRT experiments in the presence and absence of Cu (II) ions (SRT = 10 d, COD_o = 2000 mg l^{-1}).

1/S (l mg^{-1}) - Cu free	**1/U (mgX h mgCOD^{-1}) - Cu free**	**1/S (l mg^{-1}) -Cu 15 mg l^{-1}**	**1/U (mgX h mgCOD^{-1}) -Cu 15 mg l^{-1}**
0.018182	83.5443	0.00625	70.58047
0.006897	60.8	0.002924	50.32954
0.003521	38.56153	0.002041	30.46422
0.002398	18.83968	0.001437	12.95337
0.00125	9.090909	0.000851	6.470588

Table A.37 Raw Data; plots of 1/θc versus U for variable SRT experiments in the presence and absence of Cu (II) ions (HRT = 10 h, CODo = 2000 mg l^{-1}).

U (mgCOD mgX^{-1} d^{-1}) - Cu free	**1/SRT (day^{-1}) - Cu free**	**U (mgCOD mgX^{-1} d^{-1}) - Cu 15 mg l^{-1}**	**1/SRT (day^{-1}) - Cu 15 mg l^{-1}**
0.69913	0.033	0.712836	0.033
0.749333	0.040	0.753356	0.040
0.824143	0.050	0.919172	0.050
0.922723	0.067	1.133818	0.067
1.1088	0.100	1.885714	0.100
1.407273	0.200	2.644898	0.200

Table A.38 Raw Data for variations of effluent COD concentration with hydraulic residence time (HRT).

Hydraulic residence time (h)	Effluent COD-Cu free (mg l^{-1})	Effluent COD- Cu 15 mg l^{-1} (mg l^{-1})
25	55	160
20	145	342
15	284	490
10	417	696
5	800	1175

A.4 Raw Data for Activated Sludge Experiments with PWS Addition

Table A.39 Raw data for variations of effluent Cu (II) and percent Cu (II) removals with the PWS loading rate (Cu_o = 14 mg l^{-1}).

PWS loading rate (g h^{-1})	Percent Cu (II) removal (Activated sludge)	Percent Cu (II) removal (mixing tank)	Activated sludge effluent Cu(II) conc. (mg l^{-1})
0.6	100	100	0
0.5	100	100	0
0.4	92	76	1.2
0.3	78	55	3.1
0.2	64	35	5.2
0.1	41	16	8.4
0	27	0	10.4

Table A.40 Raw data for variations of effluent COD, percent COD removals, biomass concentrations and the sludge volume index (SVI) with the PWS loading rate (Cu_o = 14 mg l^{-1}).

PWS loading rate (g h^{-1})	Effluent COD conc. (mg l^{-1})	Percent COD Removal	Biomass conc., X (mg l^{-1})	Settled biomass conc., X_r (mg l^{-1})	sludge volume index, SVI (ml g^{-1})
0.6	415	80	8100	3820	123
0.5	420	80	8000	3750	125
0.4	470	77	6200	3200	161
0.3	750	63	5750	2950	174
0.2	970	52	5250	2700	190
0.1	1100	45	4800	2500	208
0	1210	40	4500	2250	222

Table A.41 Raw data for variation of effluent toxicities and percent toxicity removals with the PWS loading rate (Cu_o = 14 mg l^{-1}).

PWS loading rate (g h^{-1})	Effluent toxicity (mixing tank) (%)	Percent toxicity rem. (mixing tank)	Effluent toxicity (Activated sludge) (%)	Percent toxicity rem. (Activated sludge)
0.6	0	100	0	100
0.5	0	100	0	100
0.4	15	66	9	80
0.3	29	34	18	59
0.2	36	18	25	43
0.1	41	7	32	27
0	44	0	38	14

Table A.42 Raw data for variations of effluent Cu (II) and percent Cu (II) removals with the PWS loading rate (Cu_o = 22 mg l^{-1}).

PWS loading rate (g h^{-1})	**Percent Cu (II) removal (Activated sludge)**	**Percent Cu (II) removal (mixing tank)**	**Activated sludge effluent Cu(II) conc. (mg l^{-1})**
1	100	100	0
0.8	100	100	0
0.6	83	65	3.7
0.4	63	45	8.2
0.2	40	24	13.2
0	26	0	16.4

Table A.43 Raw data for variations of effluent COD, percent COD removals, biomass concentrations and the sludge volume index (SVI) with the PWS loading rate (Cu_o = 22 mg l^{-1}).

PWS loading rate (g h^{-1})	**Effluent COD conc. (mg l^{-1})**	**Percent COD Removal**	**Biomass conc., X (mg l^{-1})**	**Settled biomass conc., X_r (mg l^{-1})**	**sludge volume index, SVI (ml g^{-1})**
1	415	79	3900	8200	122
0.8	420	79	3840	8150	123
0.6	860	58	3040	6040	166
0.4	1100	46	2450	4750	211
0.2	1340	33	2030	4050	247
0	1550	23	1980	3780	265

Table A.44 Raw data for variation of effluent toxicities and percent toxicity removals with the PWS loading rate (Cu_o = 22 mg l^{-1}).

PWS loading rate (g h^{-1})	**Effluent toxicity (mixing tank) (%)**	**Percent toxicity rem. (mixing tank)**	**Effluent toxicity (Activated sludge) (%)**	**Percent toxicity rem. (Activated sludge)**
1	0	100	0	100
0.8	0	100	0	100
0.6	25	64	14	80
0.4	35	49	26	62
0.2	47	32	37	46
0	69	0	50	28

A.4.1 Raw Data for Box-Behnken Experimental Design

Table A.45 Raw data for variation of percent Cu(II) removal with the feed Cu(II) concentration at different PWS loadings. HRT = 15 hours, SRT = 15 days.

	Percent Cu(II) removals				
Feed Cu(II) conc. (mg l^{-1})	**0 g PWS h^{-1}**	**1 g PWS h^{-1}**	**2 g PWS h^{-1}**	**3 g PWS h^{-1}**	**4 g PWS h^{-1}**
0	0	2	6	4	0
2	0	12	18	16	7
5	14	27	33	32	25
10	32	47	55	56	50
20	56	75	87	91	88
30	63	85	100	100	100
40	51	77	95	100	100
50	22	51	73	88	96

Table A.46 Raw data for variation of percent Cu(II) removal with the sludge age (SRT) at different PWS loadings. $Cu(II)_o$ = 25 mg l^{-1}, HRT = 10 hours.

	Percent Cu(II) removals					
Sludge age (SRT) (day)	**0 g PWS h^{-1}**	**0.5 g PWS h^{-1}**	**1 g PWS h^{-1}**	**2 g PWS h^{-1}**	**3 g PWS h^{-1}**	**4 g PWS h^{-1}**
5	52	64	73	87	94	93
10	56	67	77	90	97	96
15	59	70	79	92	99	98
20	61	72	81	94	100	99
25	61	72	81	94	100	98

Table A.47 Raw data for variation of percent COD removal with PWS loading rate at different feed Cu(II) concentrations. HRT = 15 hour, SRT = 15 days.

	Percent COD removals						
PWS loading rate (g h^{-1})	**0 mg Cu(II) l^{-1}**	**5 mg Cu(II) l^{-1}**	**10 mg Cu(II) l^{-1}**	**20 mg Cu(II) l^{-1}**	**30 mg Cu(II) l^{-1}**	**40 mg Cu(II) l^{-1}**	**50 mg Cu(II) l^{-1}**
0.00	76	72	68	59	49	36	22
1.00	83	80	78	71	63	53	41
2.00	85	84	83	79	73	66	56
3.00	83	83	83	82	79	74	67
4.00	76	78	79	80	80	77	73

Table A.48 Raw data for variation of percent COD removal with HRT at different PWS loading rates. Cu(II)$_o$ = 30 mg l^{-1}, SRT = 15 days

	Percent COD removals					
HRT (h)	**0 g PWS h^{-1}**	**0.5 g PWS h^{-1}**	**1 g PWS h^{-1}**	**2 g PWS h^{-1}**	**3 g PWS h^{-1}**	**4 g PWS h^{-1}**
5	46	54	60	69	74	74
10	48	55	62	71	76	77
15	49	56	63	73	79	80
20	49	57	64	75	80	82
25	50	58	65	76	82	84

Table A.49 Raw data for variation of percent toxicity removal with the feed Cu(II)o concentrations at different PWS loadings. HRT = 15 hours, SRT = 15 days.

	Percent TOX removals				
Feed Cu(II) conc. (mg l^{-1})	**0 g PWS h^{-1}**	**1 g PWS h^{-1}**	**2 g PWS h^{-1}**	**3 g PWS h^{-1}**	**4 g PWS h^{-1}**
0	0	1	5	4	0
2	2	11	15	15	11
5	15	24	30	31	28
10	32	43	50	53	51
20	54	68	78	84	85
30	57	75	88	97	99
40	43	64	80	92	99
50	12	35	55	70	81

Table A.50 Raw data for variation of sludge volume index (SVI) with hydraulic residence time (HRT) at different PWS loading rates. Cu(II)$_o$ = 30 mg l^{-1}, SRT = 15 days.

	SVI (ml g^{-1})					
Sludge age (SRT) (day)	**0 g PWS h^{-1}**	**0.5 g PWS h^{-1}**	**1 g PWS h^{-1}**	**2 g PWS h^{-1}**	**3 g PWS h^{-1}**	**4 g PWS h^{-1}**
5	201	174	151	117	101	101
10	198	170	147	114	97	98
15	195	167	144	111	94	95
20	192	165	142	108	91	92
25	190	162	139	106	89	89