An investigation of heavy metal biosorption in relation to C/N ratio of activated sludge

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Abstract

The effect of C/N ratio of activated sludge on heavy metal biosorption was investigated. Three sets of semi-continuous reactors with different feed C/N ratios (9, 21 and 43 mg COD/mg TKN) were set up. Sorption equilibrium tests have indicated that the biosorptive capacity of activated sludge was highly dependent on metal species and the C/N ratio. The increase in C/N ratio resulted in an increase in the Cd(II) sorption capacity of activated sludge whereas it decreased the Cu(II) sorption capacity. As for Zn(II), a different behavior was observed such that, the highest and lowest capacities have occurred at C/N ratio of 21 and 43, respectively. For Ni(II) biosorption, isotherm tests produced greatly scattered data; so, it was not possible to obtain any plausible result to indicate the relationship between maximum adsorptive capacity and C/N ratio. The accompanying release of Ca(II) and Mg(II) ions and also carbohydrates into the solution during biosorption have indicated that ion exchange mechanism was involved however, was not the only mechanism during the sorption process.
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Keywords: Activated sludge; Biosorption; C/N ratio; Heavy metal; Sorption mechanism

1. Introduction

The increase in industrial activities has intensified environmental pollution with the accumulation of heavy metals. The main concern with the heavy metals is their toxicity and tendency to accumulate throughout the food chain, which is a serious threat to the environment, animals and mankind. Therefore, the need for cost effective methods for the removal of metals is vital. Commonly used methods are precipitation, ion exchange, electrochemical processes and membrane processes. However, the application of such processes is sometimes restricted because of technical or economic constraints. The search for new technologies, involving the removal of toxic metals from wastewaters has directed attention to biosorption, due to the metal-binding capacities of various biological materials [1].

The sequestering of metal ions by solid materials of biological origin is known under the general term "biosorption". It is a well-known fact that various types of algae, bacteria, fungi and yeast can readily adsorb or accumulate metal ions in significant quantities [2–8]. Furthermore, many researchers suggested that biomass from biological pollution control processes, especially activated sludge systems could be effective in removing heavy metals from polluted waters [9–14].

Factors, which may influence the biosorption process, include pH, concentration of metal present in the wastewater, and the composition and characteristics of wastewater and sludge. Metal removal by sludge is a consequence of interaction between metals in the aqueous solution and the bacterial cell surface; and follows complex mechanisms, mainly ion exchange, chelation and adsorption by physical forces [2].

The adsorption of heavy metals on the sludge surface is usually attributed to the formation of complexes between metals and as carboxyl, hydroxyl, and phenolic surface functional groups of the extracellular polymeric substances (EPS). These biopolymers which can be produced by many different species of bacteria isolated from activated sludge have been shown to be involved in the adsorption of metal ions from solution [9,15–21].

It has been suggested that the composition of the growth medium is important in the production of EPSs [9]. Feeding microorganisms with substrates at various carbon to nitrogen (C/N) ratios was shown to change the composition of EPS in

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Table 1
Characeristics of activated sludge samples cultivated at different C/N ratios [23]

C/N ratio (mg COD/mg TKN)	Total EPS (mg/g MLVSS)	Protein in EPS (mg/g MLVSS)	Cabohydrate in EPS (mg/g MLVSS)
9	41	33	8
21	49	28	21
43	58	12	46

terms of protein and polysaccharide content [22,23]. A summary of the results obtained from this earlier study conducted is given in Table 1. This table shows that at low C/N ratios the predominant fraction of EPS is proteins, whereas at high C/N ratios the predominant component of EPS is carbohydrates [23]. Considering that the carbohydrates and proteins have different functional groups that are effective in biosorption (carboxylic and amine groups in the case of proteins and mainly carboxylic functional groups in the case of carbohydrates), the C/N ratio of the feed can become a critical determinant of biosorption capacity. Therefore the purpose of this study is to identify the effect of growth conditions of activated sludge (in terms of C/N ratio) on biosorption process and biosorption mechanisms of four different heavy metals (Cu(II), Cd(II), Zn(II) and Ni(II)) onto activated sludge.

2. Materials and methods

2.1. Reactor operation

Mixed culture bacteria grown in semi-continuous reactors were used during the experiments. The microbial seed was obtained from the primary settling tank effluent of Ankara Wastewater Treatment Plant. Reactors had a working volume of 2 L and were operated at a mean cell residence time of 8 days. They were placed in a water bath where the temperature was kept at 20 °C. Required oxygen and complete mixing were provided by the use of air pumps. Dissolved oxygen concentration in reactors was kept at a minimum of 3 mg/L and pH was maintained at 7.0 ± 0.2.

Three sets of replica reactors were operated under three different C/N ratios. The first C/N ratio was selected as 21 (in terms of the ratio of chemical oxygen demand, COD to total Kjeldahl nitrogen, TKN). This set of reactors was operated to represent the typical operational conditions of activated sludge systems treating municipal wastewaters. The reactors were fed with the synthetic medium having the following composition (as mg/L): glucose (935), peptone (200), K_2HPO_4 (600), KH_2PO_4 (300), NH_4Cl (225), $MgSO_4 \cdot 7H_2O$ (112.5), $FeSO_4 \cdot 7H_2O$ (3.75), $ZnSO_4 \cdot 7H_2O$ (3.75), $MnSO_4 \cdot 7H_2O$ (3.75), $CaCl_2$ (15), $NaHCO_3$ (180). The second set of reactors was operated at a C/N ratio of 9 to represent a carbon-limited situation, whereas the third set of reactors was operated at a C/N ratio of 43, to represent nitrogen limited situation. The carbon content of the reactors with C/N ratio of 9 and 43 were adjusted by modifying the glucose amount in the feed.

Reactors were brought to steady state which was demonstrated by measuring mixed liquor suspended solid (MLSS) and mixed liquor volatile suspended solid (MLVSS) concen-

trations daily. Once the steady state was reached, biosorption tests were conducted using the sludge wasted daily from each reactor. Sludge was first centrifuged for 15 min, the supernatant was discarded and remaining biomass was resuspended in deionized water. This centrifugation–resuspension procedure was repeated once more; and finally, the MLSS concentration of biomass suspension used in biosorption tests was measured.

2.2. Biosorption tests

Before running sorption equilibrium (isotherm) tests, a series of sorption kinetic experiments was run in order to investigate the time dependency of sorption and determine the equilibrium time needed for isotherm tests. These experiments were conducted by mixing heavy metal solutions with a known amount of biomass and by taking samples from this mixture at different time intervals until there was no net sorption. These experiments were repeated at several initial metal concentrations to see the possible effect of initial metal concentration on sorption kinetics. In all these tests, the temperature and the initial pH of the metal solution were kept constant at 25 °C and 4.0, respectively. No buffers were used to keep the pH constant during the course of sorption in order not to affect the sorption process by buffers' components.

Biosorption isotherm tests were performed using flasks with a net volume of 50 mL which were placed in a shaking incubator operating at 200 rpm and 25 °C. The amount of biomass added into each flask was approximately 0.1, 0.2 and 0.3 g as dry matter, based on MLSS concentration of sludge with C/N ratios of 9, 21 and 43, respectively. The heavy metal concentrations varied from 10 to 1000 mg/L while the initial pH was set to 4.0 using 0.01 M HNO_3. The sorption isotherm tests were carried out at unadjusted pH conditions. Cd(II), Cu(II), Zn(II), and Ni(II) was added as $CdCl_2 \cdot H_2O$, $CuCl_2 \cdot 2H_2O$, $ZnCl_2$, $NiCl_2 \cdot 6H_2O$, respectively. When equilibrium was attained, biomass in samples was removed by filtration through a 0.45 μm membrane filter and filtrates were analyzed for the heavy metal content by using flame atomic absorption spectrophotometer (AAS) (ATI Unicam 929) [24]. Beside the adsorbed metal ions, released Ca(II) and Mg(II) ions were also analyzed using AAS. Also, the amount of carbohydrate release was measured. All the biosorption tests were run in duplicate and deionized water was used in all biosorption tests to differentiate between the ions released and ions existing in water. Biosorptive capacity was calculated by using the equation:
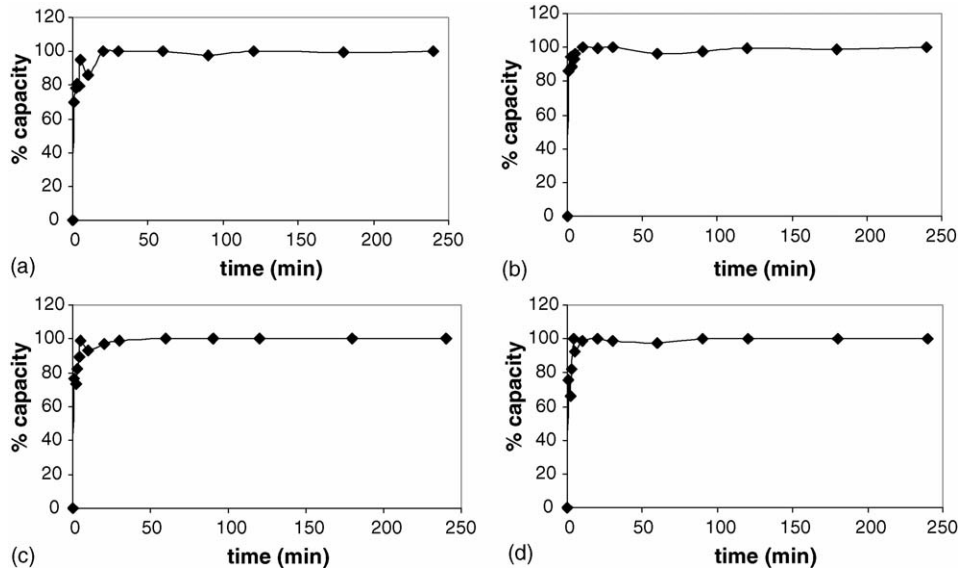
$$q_e = \frac{V(C_0 - C_e)}{m} \tag{1}$$

Fig. 1. Biosorption kinetics at C/N 9: (a) Cd(II); (b) Cu(II); (c) Zn(II); (d) Ni(II).

where q_e is the equilibrium capacity as mg metal/g dry biomass, V the volume of the metal solution, C_e and C_0 the equilibrium and initial metal concentrations in aqueous phase as mg/L, and m is the amount of dry biomass in grams.

2.3. Analytical methods

MLSS and MLVSS were determined according to Standard Methods for the Examination of Water and Wastewater [24]. The amount of carbohydrate release during biosorption process was measured by using phenol sulphuric acid method [25].

3. Results and discussion

3.1. Adsorption kinetics

The results of kinetic experiments conducted for four different heavy metals showed that biosorption of heavy metals by activated sludge is a fast process. For all metals, at all initial concentrations tested (200–400 mg/L), an initial rapid uptake was observed and nearly 85% of biosorption was completed within the first 5 min. After 5 min, the metal uptake rate gradually decreased and the system finally reached equilibrium approxi-
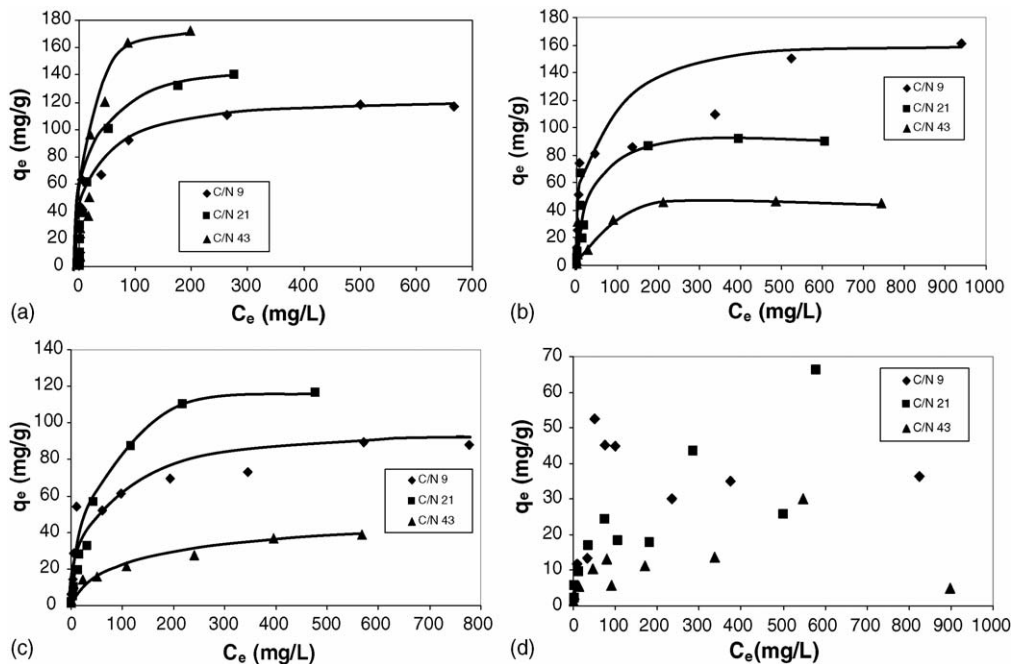

Fig. 2. Adsorption isotherms for different C/N ratios: (a) Cd(II); (b) Cu(II); (c) Zn(II); (d) Ni(II).

mately after 90 min (Fig. 1). This behavior implicates the fact that sorption occurs at two stages. First one is the rapid surface binding and the second is the slow intracellular diffusion [26–28]. Since almost 100% of metal removal was achieved within the first 90 min of biomass-heavy metal contact; an equilibrium time of 4 h was employed in the isotherm tests to make sure a complete equilibrium is reached.

3.2. Isotherm studies

The maximum adsorptive capacity of the activated sludge grown under different C/N ratios was determined by examining the relationship between q_e and C_e. As shown in Fig. 2, adsorption capacity increased with equilibrium metal ion concentration and reached a plateau which corresponded to maximum adsorptive capacity. For Cd(II), as presented in Fig. 2a, the maximum biosorptive capacities were attained as 120, 140 and 170 mg Cd(II)/g biomass, for the C/N ratios of 9, 21, and 43, respectively. As it can be seen from Fig. 2a, the increase in C/N ratio resulted in an increase in the Cd(II) sorption capacity of AS but not much. For Cu(II) biosorption, the maximum biosorptive capacities at C/N ratios of 9, 21 and 43 were attained as 160, 90 and 40 mg Cu(II)/g biomass, respectively (Fig. 2b). These findings show that biosorption capacity for Cd(II) increased with the increase of C/N ratio whereas the increase in C/N ratio resulted in a decrease in Cu(II) biosorption. There was a remarkable increase in the Cu(II) sorption capacity of activated sludge with the decrease of C/N from 21 to 9. An increase in C/N from 21 to 43, has resulted in about 40% decrease in maximum Cu(II) biosorption capacity of activated sludge. A comparison of C/N effect on biosorption capacity indicated that Zn(II) behaved totally different than the other metals investigated. When the C/N ratio was increased from 9 to 21, biosorptive capacity of activated sludge also increased some, but an increase in C/N from 21 to 43 resulted in a drastic decrease in biosorption capacity, about 65% (Fig. 2c).

The isotherm plots presented in Fig. 2d have indicated that for Ni(II), the adsorptive capacity of activated sludge cultivated under all three C/N ratios was highly complex. The biosorptive capacity was fluctuating with increasing C_e. One of the possible reasons for this unexpected behavior was thought to be very low tendency of Ni(II) ions for biosorption among the heavy metal ions studied in literature [29,30]. Also, nickel was found to be associated predominantly with the soluble fraction of polymers [16]. The stronger affinity of Ni(II) for soluble polymers than for biomass can explain the lower binding capacity of activated sludge for Ni(II), and indicates that these soluble complexes may be unavailable for uptake by biomass [31]. So, inconsistent and unstable behavior of Ni(II) observed in this study can be attributed to the lowest biosorption tendency and weakest binding of nickel to the EPS of activated sludge.

3.3. Biosorption mechanism

3.3.1. Ca(II) and Mg(II) release

Searching for the mechanism of sorption, the desorbed cations (Ca(II) and Mg(II)) from the sludge solids during sorp-
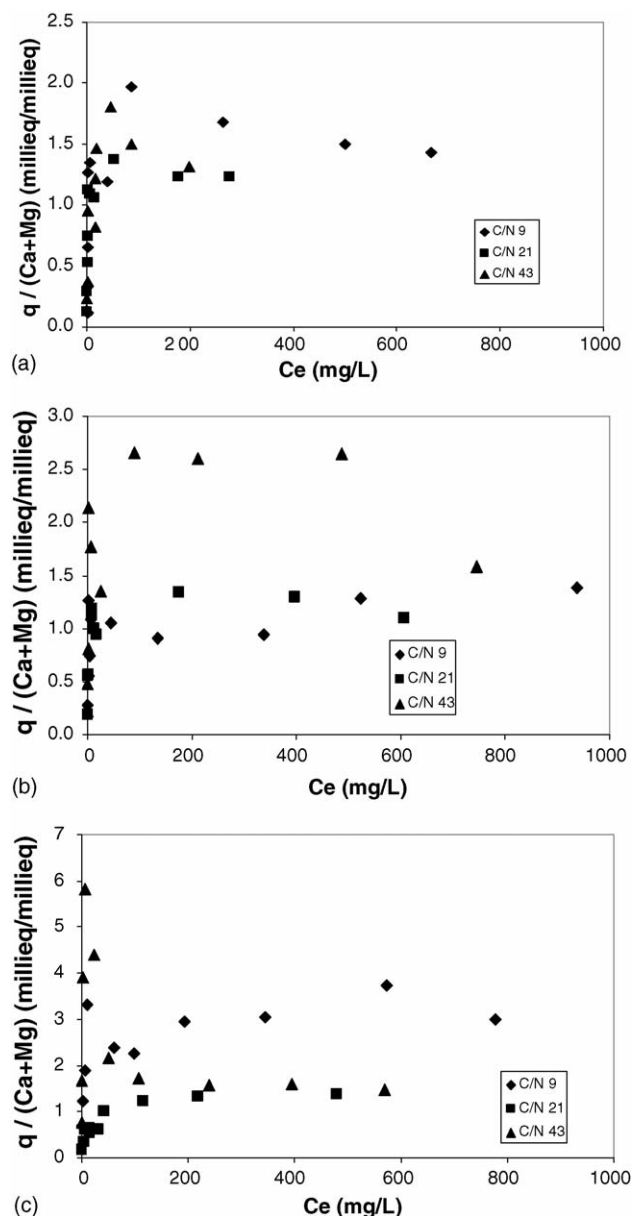

Fig. 3. The ratio of adsorbed to released ions: (a) Cd(II); (b) Cu(II); (c) Zn(II).

tion tests were also analyzed. Fig. 3 represents the ratio of adsorbed to released ions for three C/N ratios together. At all equilibrium metal concentrations, there was a concomitant Ca(II) and Mg(II) release with sorption indicating the existence of an ion exchange between Cd(II) and Ca(II) and Mg(II). At low metal concentrations it was observed that the ratio was highly dependent on C_e. On the other hand, at high metal concentrations, ratio of adsorbed to released ions reached nearly an equilibrium in all cases. At high equilibrium concentrations, the ratio of Cd(II) to Ca(II) + Mg(II) ions remained nearly the same for all C/N ratios whereas the ratio of Cu(II) to Ca(II) + Mg(II) ions was much higher for C/N 43. For Zn(II), highest ratio was seen at C/N 9 and the values were very close to each other for other C/N ratios.

The change in sorbed metal quantity (q) to Ca(II) + Mg(II) release ratio with equilibrium metal concentration indicates that

initial metal concentration plays an important role in biosorption mechanism. As the initial metal concentration and in turn C_e increases, there occurs a change in removal mechanism. As presented in Fig. 3, $q/(\text{Ca} + \text{Mg})$ ratio is smaller than 1.0 at C_e values below 50 mg/L. However, the $q/(\text{Ca} + \text{Mg})$ ratio is well above 1.0 at higher metal concentrations. This indicates that, although the whole metal removal cannot be explained by ion exchange alone, ion exchange mechanism appears to be involved in biosorption. For all metals except Ni(II), ion exchange accounted for at least 60% of the biosorption at high metal concentrations with only exception of Zn(II) sorption at C/N ratio of 9, which was at about 35%. However, an ion exchange phenomenon is not very obvious at low metal concentrations. It is speculated that at low metal concentrations, metals bind to the already existing and available binding sites at the surface. This binding could be thought as surface precipitation (for some metals as bulk precipitation due to relatively high solution pH at low metal concentrations), complexation and limited extent ion exchange. After a certain metal concentration is reached, these available binding sites would be filled up, leaving limited or no sites for the forthcoming metal ions. At the same time, as the heavy metal concentration in the solution increases, there becomes a greater drive for the metal to exchange the cations already at the surface. This then initiates ion exchange which starts to pick-up and take over the biosorption mechanism.

3.3.2. Carbohydrate release

From Fig. 4, it can clearly be seen that besides the Ca(II) and Mg(II) ions, a significant amount of carbohydrates was also released into the solution during biosorption of Cd(II) for all C/N ratios. Carbohydrate release into the solution per unit weight of biomass (as dry matter) increased with the increase of C_e and followed nearly the same trend with released ions. However, the amount of released ions increased when the C/N ratio increased from 9 to 21 and remained nearly the same when the ratio increased from 21 to 43. On the other hand, the released carbohydrate amount was very low at C/N ratio 21 and significantly higher at C/N ratio 43.

It was observed that the amount of total ion released during the biosorption of Cu(II) decreased gradually with the increase of C/N ratio, especially to 43. Since a positive correlation is expected between the released cations and carbohydrates, a decrease in carbohydrate amount was also expected with increase of C/N ratio. But surprisingly the opposite was observed (Fig. 5). For Zn(II) biosorption, the release of Ca(II) + Mg(II) was highest at C/N ratio 21 and almost half of it for C/N ratio 9 and 43. When the amount of carbohydrate release was investigated, it was observed that carbohydrate release increased slightly with the increase of C/N ratio (Fig. 6).

The reason of measurement of released carbohydrates during biosorption is our expectation that during an ion exchange process when the heavy metal exchanges with Ca(II) and Mg(II) on the sludge surface, some fraction of EPS should be released into the solution. Carbohydrate fraction is considered to be an indicator of polymer release and analyzed all throughout the tests. Our results clearly demonstrated the release of polymers from sludge surface during biosorption process possibly by the ion exchange
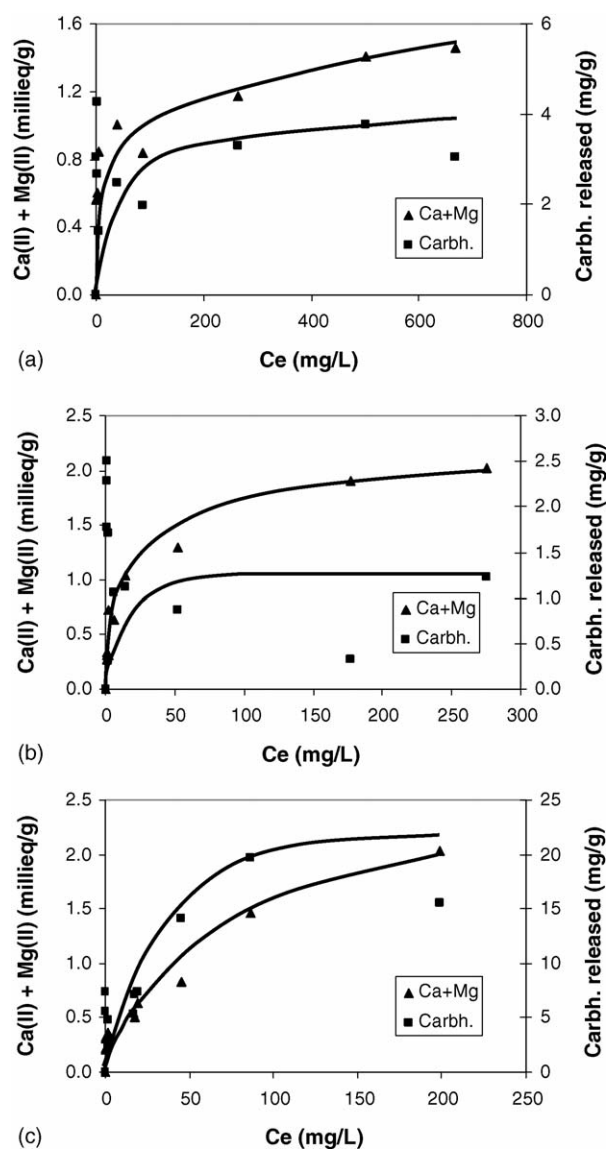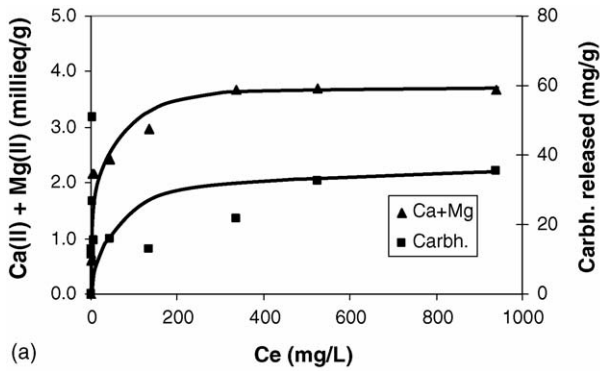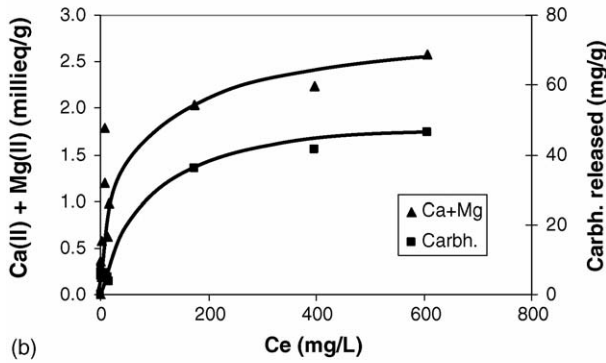

Fig. 4. Release of Ca(II) + Mg(II) and carbohydrates due to Cd(II) adsorption: (a) C/N 9; (b) C/N 21; (c) C/N 43.

mechanism. Ideally, a higher polymer release is expected with a higher amount of exchanged ions. However, there are some inconsistencies between the amount of Ca(II) + Mg(II) release and carbohydrate release for some heavy metals at some C/N ratios as mentioned above. The reason of inconsistencies is believed to be explainable by the unmeasured protein fraction release during biosorption process. Since Ca(II) and Mg(II) show affinity to carboxylic acids which are components of both carbohydrates and proteins, release of both fractions is expected during an ion exchange process. Because our results reflected only a part of the released polymers, some unexplainable trends existed in the results.
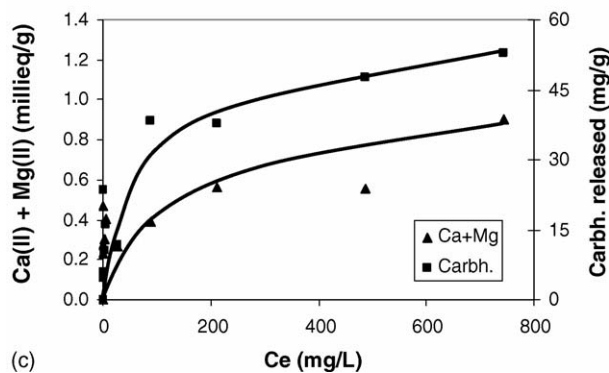
These arguments are in agreement with the recent studies that indicated the importance of calcium ions as a major contributer to bioflocculation [32]. Bruus et al. [32] further reported that when other ions (monovalent or divalent cations other than calcium and magnesium) are introduced

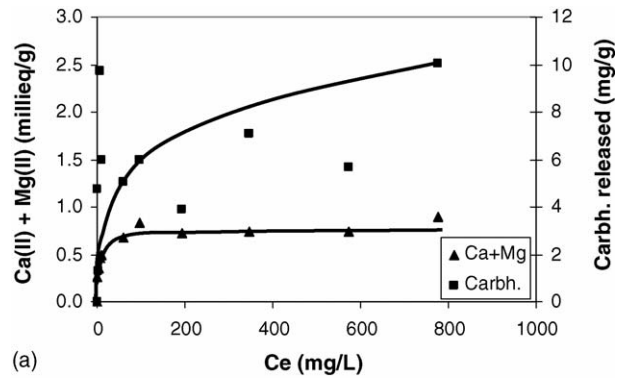Fig. 5. Release of Ca(II) + Mg(II) and carbohydrates due to Cu(II) adsorption: (a) C/N 9; (b) C/N 21; (c) C/N 43.

Fig. 6. Release of Ca(II) + Mg(II) and carbohydrates due to Zn(II) adsorption: (a) C/N 9; (b) C/N 21; (c) C/N 43.
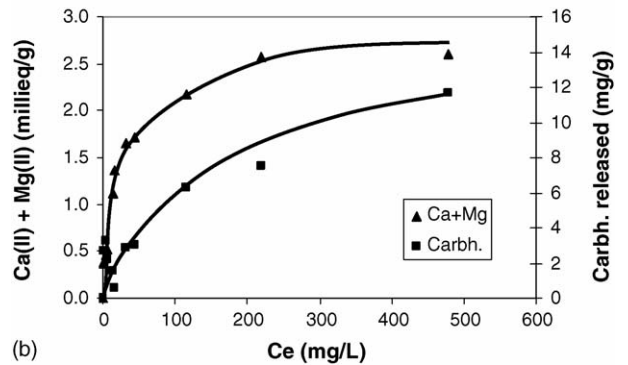
into the medium at high concentrations, calcium was released from the activated sludge flocs accompanied by a major release of extracellular polymers (EPS) (both carbohydrate and proteins).
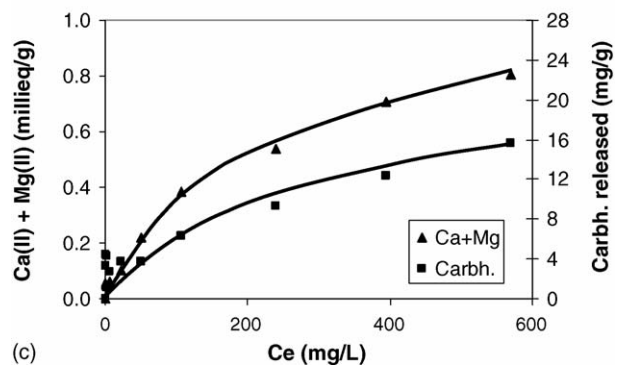
3.3.3. Equilibrium pH

As it can be seen from Fig. 7, the initial heavy metal concentration also had a remarkable effect on the equilibrium pH of the solution. In all cases, as the initial concentration increased, the equilibrium pH of the solution decreased. There is a sharp decrease in equilibrium pH between the initial metal concentrations of 300 and 500 mg/L for all metals. This substantial change could be related to the alteration in biosorption mechanism, initiated by the initial concentration of metal. A possible ion exchange mechanism between the metal ion and the hydrogen ions on the activated sludge surface can be suggested to explain this decrease in pH. The release of surface bound hydrogen ions into the solution causes a decrease in system pH. It is apt

here to note that for all metals studied, C/N has not a remarkable effect on equilibrium pH.

3.4. Overview of metal biosorption in relation to C/N

The effect of C/N ratio on biosorption capacities for three different metal ions is summarized in Fig. 8. It can easily be seen from the figure that the effect of C/N ratio on heavy metal sorption capacity of activated sludge is highly variable. The metal ions tested showed different biosorption affinities for microorganisms grown under different C/N ratios. Biosorption capacity for Cu(II) ions increased with the decrease of C/N ratio whereas the increase in C/N resulted in an increase in Cd(II) biosorption affinity. As for Zn(II) ion, a different behavior was observed such that, the highest and lowest capacities occurred at C/N ratio 21 and 43, respectively.

Highly variable effect of C/N ratio on the affinity of metals to bind activated sludge indicated that the composition of EPS,
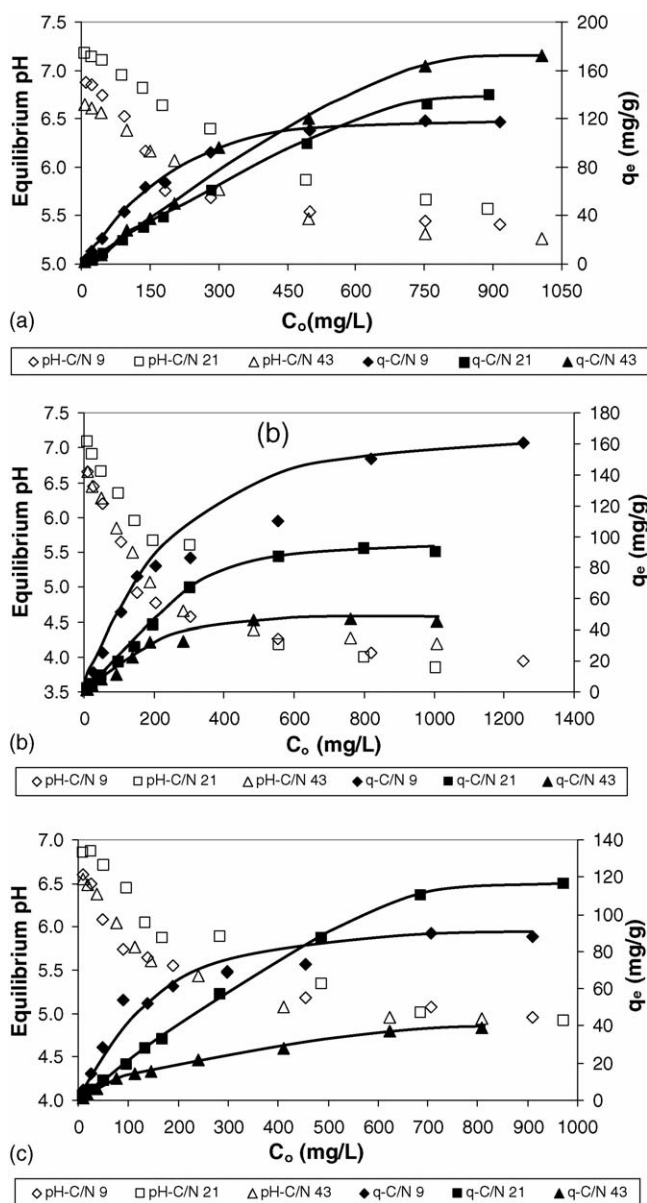
Fig. 7. Effect of initial metal concentration on the equilibrium pH: (a) Cd(II); (b) Cu(II); (c) Zn(II) (initial pH 4.0).
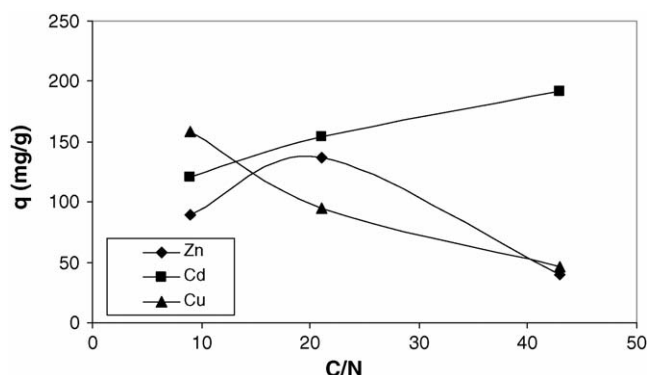

Fig. 8. The change of biosorption capacities of metal ions with C/N ratio.

which is known to be affected by C/N ratio [22], is an important parameter influencing biosorption. An increase in C/N ratio resulted in a decrease in protein but an increase in carbohydrate content of EPS. As indicated by several researchers, metals favor specific binding sites and therefore different metals may associate preferentially with different specific types of groups [27,33–35]. In line of this fact; Cd(II) appeared to have higher affinity for carboxylic groups, while Cu(II) did for amine groups. On the other hand, in the uptake of Zn(II) both groups were possibly equally involved as there is no increasing or decreasing trend with C/N ratio.

The classification of heavy metals (based on atomic properties and solution chemistry of metal ions) suggested by Nieboer and Richardson [36] further evidences that Cd(II) exhibits a higher affinity for O-containing groups than for N-containing groups. On the other hand, according to their classification, Cu(II) was a N- and S-seeking metal while Zn(II) was O-seeking. In parallel, Cu(II) biosorption increased with an increase in –NH content of EPS in activated sludge. However, Zn biosorption was not in accordance with this classification.

4. Conclusions

In this study, it is demonstrated that EPS composition is an important characteristic of activated sludge influencing heavy metal biosorption. Results indicate that heavy metal sorption onto activated sludge is a very fast process. The isotherm experiments conducted at constant temperature with three different C/N ratios (9, 21 and 43) showed that the biosorptive capacity of activated sludge was highly dependent on metal species and C/N ratio. The dependence of biosorptive capacity on C/N ratio was different for each metal. This was attributed to the presence of several different types of metal-binding functional groups which are favored by different metals. The measurement of the Ca(II) and Mg(II) ions which were released into the aqueous phase implied that ion exchange plays an important role in heavy metal biosorption but it is not the only mechanism responsible for removal.

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