Pb(II) biosorption using anaerobically digested sludge

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Abstract

Removal of Pb(II) by using resting cells of anaerobically digested sludge (ADS) obtained from a nearby wastewater treatment plant was examined. Firstly, sorption kinetic and equilibrium experiments were conducted using agitated, thermostated (25 °C) batch reactors. The maximum Pb(II) sorption capacity was found to be very high (1750 mg/g dry ADS or 8.45 mmol/g dry ADS). At all initial Pb(II) concentrations tested, sorption resulted in neutralization with an increase in the solution pH from an initial value of 4.0–5.5 to an equilibrium value of 7.0–8.0, at which Pb(II) can precipitate as hydroxide. The removal of Pb(II) by ADS was found to involve bioprecipitation as well as biosorption. FTIR spectrometry highlighted carboxyl groups present on the surface of ADS as the major functional groups responsible for biosorption. Secondly, a three-stage semi-continuous pseudo-counter current reactor system was tested to reduce ADS requirement in comparison to a conventional single-stage batch reactor. At an initial Pb(II) concentration of about 200 mg/L, an effluent Pb(II) concentration of 1.3 mg/L was achieved in the three stage reactor, corresponding to a metal removal capacity of 682.7 mg/g dry ADS (3.30 mmol/g), in comparison to 1.9 mg/L and 644.0 mg/g dry ADS (3.10 mmol/g) for the single-stage batch reactor.
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Keywords: Anaerobically digested sludge; Lead; Removal; Sorption; Wastewater

1. Introduction

An alternative method for the removal of heavy metals from wastewaters has been the use of materials of biological origin [1] by means of biosorption and/or bioprecipitation [2]. Amongst the biological species tested, anaerobically digested sludge (ADS) biomass has taken relatively little attention with limited investigation. In the earliest study, Gould and Genetelli [3] examined heavy metal (Zn, Ni, Cu, and Cd) interactions with anaerobically digested sludge considering solution pH as the major parameter influencing the removal. They reported visual heavy metal precipitation after certain equilibrium pH values. On the other hand, in a later study, Alibhai et al. [4] indicated that the mechanism of attachment involves metal/surface ligand interactions. In a series of works, Artola et al. [5–7] also used anaerobic biomass and investigated the heavy metal binding to several types of sludge taken from a wastewater treatment plant. Artola et al. [6] found that the best Zn removal is achieved with thickened anaerobic and dewatered sludges.

Recently, Artola et al. [5] searched Cu removal in a contact settling tank, using anaerobically digested sludge (ADS) as biomass and reported a maximum metal uptake of 75 mg/g of total solids. In another recent study, Haytoglu et al. [8] have studied the Pb(II) removal using anaerobic biomass grown under laboratory conditions and observed very effective metal removal with a capacity of 1250 mg Pb(II)/g of biomass. All these results have pointed to the use of surplus anaerobic biological sludge from biological wastewater treatment plants as a cost-effective alternative to the currently used techniques for metal detoxification. However, single-stage batch sorption process requires a large quantity of sludge for a satisfactory metal removal due to the decrease in sorption capacity with a decrease in metal concentration. The objective of the present study is to investigate the removal of Pb(II) using ADS obtained from a municipal wastewater treatment plant in a semi-continuous counter-current reactor system. Such a reactor system was investigated to minimize the ADS requirement in the technological application of the metal removal using anaerobic biomass. At first, the Pb removal process has been analyzed through single-stage batch (SSB) experiments and then compared with three-stage semi-continuous (TSSC) pseudo counter-current system.

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Table 1
Characteristics of sludge from anaerobic digesters in Tatlar Wastewater Treatment Plant, Ankara, Turkey

Parameter	Value
pH	7.3–7.4
Alkalinity (mg/L as CaCO$_3$)	3500
Total solids (g/L)	2.7
MLSS (g/L)	2.7
MLVSS (% of MLSS)	45–47
Pb (mg/g)[a]	0.125–0.180
Cu (mg/g)[a]	0.171–0.363
Cr (mg/g)[a]	0.102–0.235

[a] *Source*: Bilgin et al. [9].

2. Methods

2.1. Materials

ADS samples were obtained from Tatlar Municipal Wastewater Treatment Plant in Ankara, Turkey. The treatment plant employs conventional activated sludge process as biological treatment, and anaerobic decomposition as sludge digestion process. The characteristics of the sludge from the anaerobic digestors are presented in Table 1. The samples were directly taken from the effluent of anaerobic digesters and kept refrigerated at 4 °C to make sure that there is no metabolic activity and thus no change in the characteristics of samples during storage. The ADS samples to be used in metal uptake experiments were prepared by discarding the supernatant after sufficient time of settling. The dry biomass content of the samples was determined gravimetrically as mixed liquor volatile suspended solids (MLVSS). Pb(II) solutions were prepared dissolving solid Pb(NO$_3$)$_2$ in distilled water.

2.2. Sorption kinetics and equilibrium tests

Sorption kinetics and sorption equilibrium (isotherm) tests were run at two initial pHs; the metal solution's original pH of 5.0 ± 0.2 and an adjusted pH of 4.0 ± 0.1 which has been reported as the optimum pH in Pb(II) removal by anaerobic sludge [10]. All sorption experiments were done in duplicate. The kinetic and equilibrium data presented is the arithmetic average of the data collected from the duplicate tests. The standard deviation for the duplicate samples was never above 10%.

In all sorption kinetics and sorption equilibrium tests, the temperature was kept constant at 25 °C. Metal sorption capacity was calculated by using the equation: $q = V(C_i - C)/m$, where q is the capacity as mg Pb(II)/g dry biomass; V the volume of the suspension; C_i and C the initial and equilibrium Pb(II) concentrations in aqueous phase as mg/L, respectively and m is the amount of biomass added as g dry weight.

In sorption kinetic tests, Pb(II) solution at 1250 mg/L initial concentration was placed in a beaker (2 L) and the biomass was added at a Pb(II) to biomass ratio of 1250 mg/g dry biomass. Then, the mixture was agitated at 200 rpm for about 5 h; until further depletion of the Pb concentration became insignificant.

In sorption equilibrium tests; flasks of 250 mL total volume were utilized. Pb(II) solutions (100 mL) of known concentration, varying from 500 to 2500 mg/L were placed in these flasks, initial pH was measured and ADS samples were added to yield a 0.1 ± 0.01 g biomass concentration. Afterwards, the flasks were agitated at 200 rpm to attain equilibrium. At the end, samples were taken from the flasks for the measurement of residual Pb concentration.

2.3. Analytical

Solution pH during the experiments was measured using a bench-top pH meter (Jenway Ltd., Essex, UK). The error in pH measurements was never above 2%.

All Pb measurements were conducted on samples which were filtered through 0.45 μm filter papers. To analyze Pb concentration, collected samples were acidified with concentrated HCl (5%, v/v) and kept at 4 °C until analyzed using an atomic absorption spectrophotometer (ATI Unicam Model 929). All measurements were done in accordance with Standard Methods [11].

2.4. FTIR analysis

In investigating the mechanism of biosorption, ADS samples before and after contact with Pb(II) solution (500 mg/L) were subjected to FTIR spectroscopic analysis. Original and Pb(II) contacted ADS solutions were filtered (0.45 μm), the biomass deposited over the filters were collected and dried at 60 °C overnight. Then, ground powder of each sample was prepared and FTIR spectrophotometric analyses were conducted using a Bruker Equinox-55 FTIR spectrophotometer with a Harrick Praying Mantiss ambient DRITS chamber sampling attachment.

2.5. Single-stage and three-stage sorption process

In testing SSB and TSSC pseudo counter-current sorption systems, continuously stirred reactors of 2 ± 0.1 L net volume, operating at room temperature was used. In SSB operation, Pb(II) solution (100 and 200 mg/L) was placed in the reactor and ADS solution was added to give a total MLVSS mass of 0.6 g. Then, the mixture was agitated for 2 h after which 1 h of sedimentation period was adopted. At the end, the supernatant was sampled for the final Pb(II) concentration measurement. Quantitative information on sludge settleability was obtained measuring sludge volume index (SVI) following the Standard Methods [11].

In TSSC system, pseudo counter-current flow of the Pb(II) solution and ADS streams was provided introducing the clear effluent (metal-laden solution) of the first reactor to the second reactor and that of the second reactor to the third reactor (Fig. 1). The amount of biomass added into each step was one-third of the amount added into the SSB reactor, that is 0.2 g MLVSS. Supernatant samples were collected from each stage for final Pb(II) concentration measurement.
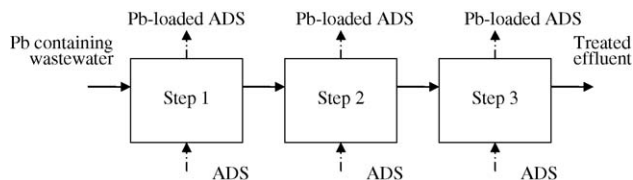
Fig. 1. Schematic presentation of the three stage semi-continuous (TSSC) sorption system.

3. Results and discussion

3.1. Sorption kinetic tests

Sorption kinetic tests were performed at the pHs of 4.0 ± 0.1 and 5.0 ± 0.2 and the results presented in Fig. 2 were obtained. As presented, there was a very rapid Pb(II) removal within the first 10 min of the biomass–metal contact, which corresponds to 52% and 68% removals with and without initial pH adjustment cases, respectively. In both cases, initial rapid Pb(II) removal was followed by a slower step and equilibrium was attained after 2 h. The equilibrium Pb(II) concentration attained at the initial pH of 5.0 ± 0.2, was around 15 mg/L while it was much higher; 250 mg/L at the initial pH of 4.0 ± 0.1. These values corresponded to 5.3 and 6.0 mmol of Pb(II) removal per g dry ADS, for the pHs of 4.0 ± 0.1 and 5.0 ± 0.2, respectively.

During these experiments, the time-course change in solution pH was also monitored and the data presented in Fig. 3 was obtained. As shown, for both initial pHs, the solution pH at equilibrium was higher than the initial pH. However, this increase was lower for the pH of 4.0 ± 0.1 than for 5.0 ± 0.2. As can be depicted from Fig. 3, at the initial pH of 4.0 ± 0.1, there was a sudden increase in pH up to 5.3; and then a decrease down to 4.8 within the proceeding 10 min. Afterwards, a gradual increase was observed in pH until equilibrium On the other hand; when the initial pH is 5.0 ± 0.2, solution pH was found to firstly increase to 5.2 and then decrease to 4.7. Afterwards, the pH increased with a relatively higher rate to an equilibrium value of 6.7 (Fig. 3). The initial increase observed in the solution pH may be explained in part, by the physiological activity of the
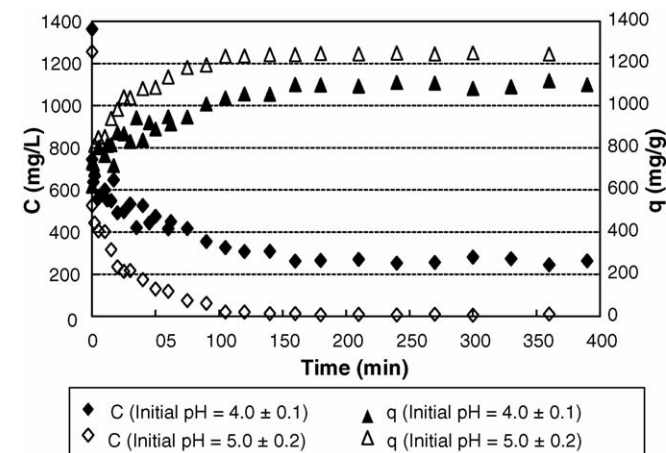

Fig. 2. Time course Pb(II) removal using ADS (initial Pb(II) concentration = 1250 mg/L, Pb(II) to biomass ratio = 1250 mg/g).
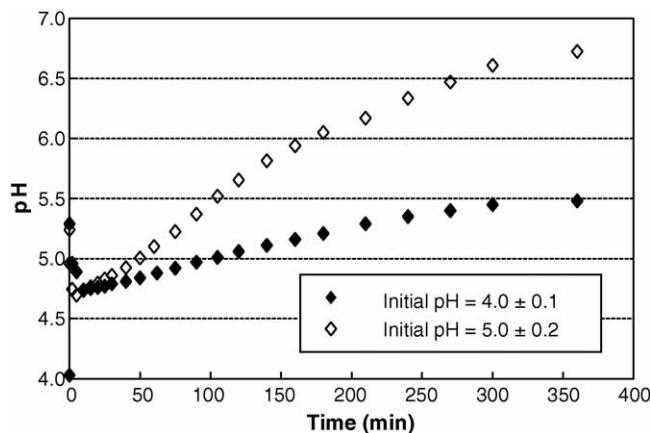

Fig. 3. Time course change of solution pH during Pb(II) removal using ADS (initial Pb(II) concentration = 1250 mg/L, Pb(II) to biomass ratio = 1250 mg/g).

resting cells in sludge. Anaerobic sludge reacts to agitation in open air and mixing with lead nitrate by expelling large amounts of CO_2 [5]. Also possible is the effect of alkalinity owned by the ADS used in the present study which is at around 3500 mg/L (Table 1). The alkalinity may cause a sudden increase in solution pH just after the addition of ADS into Pb(II) solution. As seen in Fig. 3, later, a sudden decrease was observed in pH possibly due to an exchange in between Pb(II) ions and protons released from the carboxylic acids in the cell wall of ADS bacteria [12]. In third phase, there was a gradual increase in pH in parallel to gradual decrease in Pb(II) concentration (Figs. 2 and 3). This gradual increase in pH was speculated as to originate from the interaction of amino groups of amino acids which are present in the cell wall of bacteria with Pb(II). The cell wall of both Gram-positive and Gram-negative bacteria consists of covalently linked polysaccharide and polypeptide chains, which form a bag-like structure that completely encases the cell [13]. Another possible cause of pH increase was speculated as metal binding by sulfide group (S^{-2}) as anaerobic sludge is high in sulfide content.

Thus, when ADS was mixed with Pb(II) solution, there was significant neutralization occurring; resulting in a solution pH of 6.7 at which lead hydroxide precipitation is possible. As one can clearly see from Fig. 4, when ADS was added to the beaker the solution became turbid and light (gray) colored clouds were formed besides the dark color clouds of the ADS. After mixing was stopped; sludge settled very rapidly within 20 min and a gray colored zone formed above the settled sludge layer. The formation of light gray color cloud was noted as an indicator of Pb precipitation which was thought to be one of the major mechanisms contributing to high Pb(II) removal. In a recent study, Naja et al. [12] reported similar findings and indicated that surface adsorption and micro-precipitation are sequential and possibly overlapping processes in lead biosorption by *Rhizopus arrhizus*.

3.2. Sorption equilibrium

Although precipitation appeared to be a major mechanism involved in Pb(II) removal; as discussed above, sorption was
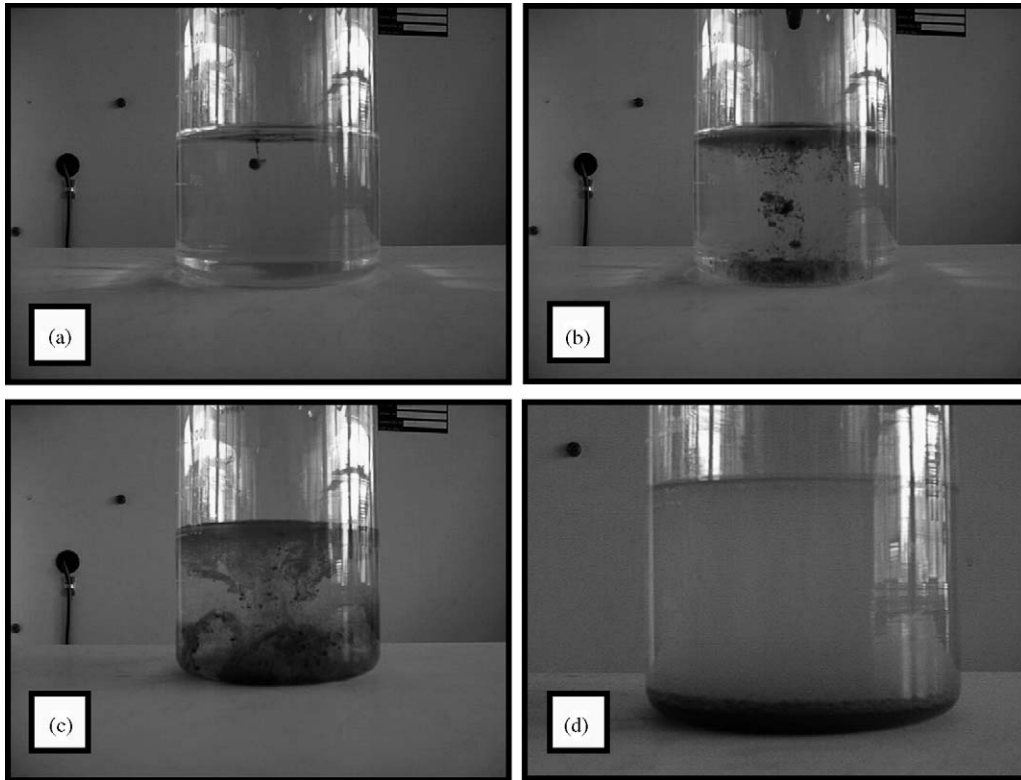
Fig. 4. Addition of ADS to Pb(II) solution (initial Pb(II) concentration = 500 mg/L and initial pH 5.0 ± 0.2). (a) First drop addition of ADS, (b and c) further addition of ADS and precipitate formation, (d) final suspension with settled sludge.
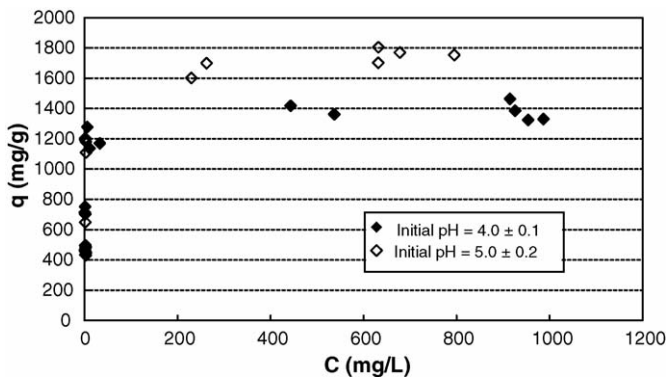

Fig. 5. Pb(II) sorption isotherms.

still considered to be another one. In order to search for possible involvement of sorption in the Pb(II) removal, a series of equilibrium tests have been performed and the results presented in Fig. 5 were obtained. As shown, there was an L-shaped relationship between Pb(II) removal capacity of ADS and equilibrium Pb(II) concentration. This implied that sorption is an important mechanism in Pb(II) removal. When Pb(II) removals obtained at initial pHs of 4.0 ± 0.1 and 5.0 ± 0.2 are compared, it is seen that the maximum Pb(II) uptake was reached almost at the same equilibrium Pb(II) concentration of 250 mg/L (Fig. 5). This indicates that all the available binding sites were filled at this Pb(II) concentration. On the other hand, when the maximum uptake values for these two cases were compared, it appears that the capacities
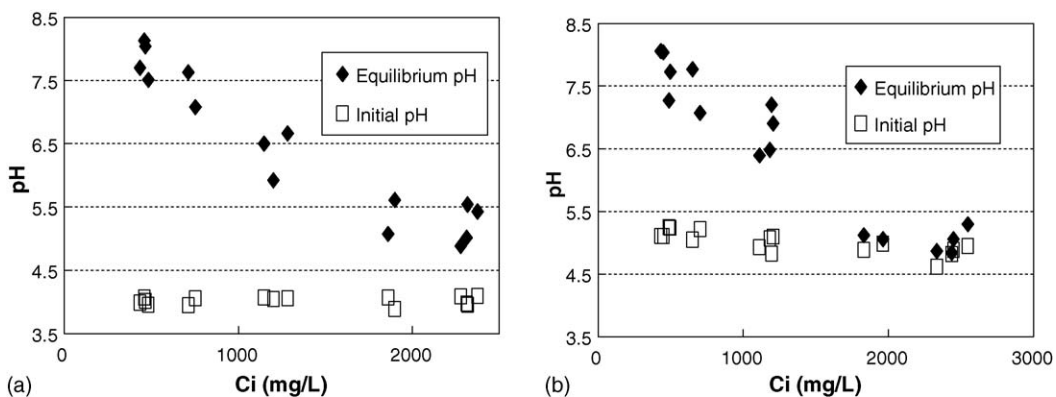

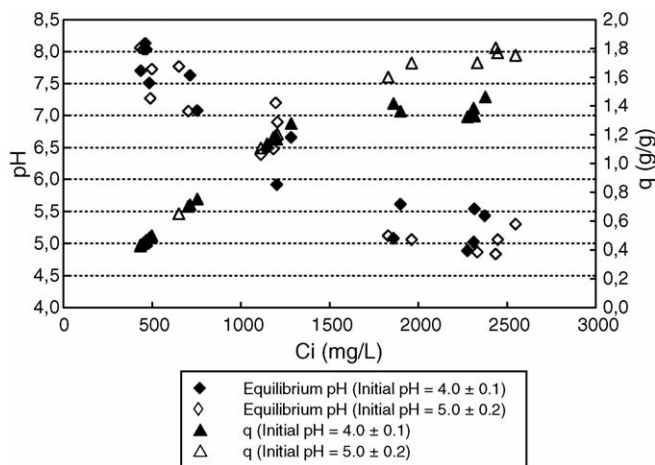Fig. 6. Equilibrium and initial pH values at the initial pH of: (a) 4.0 ± 0.1, (b) 5.0 ± 0.2.

Fig. 7. Pb(II) sorption equilibrium and the equilibrium solution pH.

Fig. 8. FTIR spectra for ADS before and after contact with 500 mg/L Pb(II) solution.

are different. When there is no initial pH adjustment (5.0 ± 0.2), the Pb(II) removal was 1750 mg Pb(II)/g ADS; while that for the initial pH of 4.0 ± 0.1 was 1400 mg Pb(II)/g biomass. This implies the initial pH is a factor that influences metal removal. On the other hand, both these capacity values are enormously high to be attributable to sorption as the only mechanism. The typical biosorptive capacities reported for other types of biomass is in the range of 0.1 mmol Pb(II)/g for *P. chrysogenum* fungus [14] to 3.7 mmol Pb(II)/g for *M. rouxii* filamentous fungus [15]. As seen from Fig. 6, which shows the initial and equilibrium pH values; there was an increase in solution pH with Pb(II) removal for both cases. For lower initial Pb(II) concentrations, equilibrium pH was around 7.5–8.0 which indicates again possible precipitation of Pb(II). Several investigators report that Pb(II) precipitates at pHs above 5.5 [2,16–18]. This fact clearly explains the very high Pb(II) removal obtained with ADS.

Interestingly, although the initial pH values were different, equilibrium pH values were almost the same at both initial Pb(II) concentrations (Fig. 7) with a concomitant trend in Pb(II) removal capacity up to an initial Pb(II) concentration of 1300 mg/L. Below this initial Pb(II) concentration; for both initial pHs, the equilibrium pH values were higher than 6.0, at which Pb(II) hydroxide precipitation can occur, indicating Pb(II) removal by precipitation. However, at initial Pb(II) concentrations above 1300 mg/L, equilibrium pH was below 5.5–6 at which Pb(II) precipitation does not occur. On the other hand, Pb(II) removal capacity was dependent on initial pH level and considerably less at the initial pH of 4.0 ± 0.1 than at 5.0 ± 0.2. Lower Pb(II) removal capacity at lower initial pHs can be attributed to the increase in protonation of the surface functional groups and possible competition between Pb(II) and H^+ ions. This indicated that at above 1300 mg/L Pb(II), the dominant Pb(II) mechanism is biosorption but not precipitation.

In order to investigate the functional groups responsible in biosorption, FTIR analysis of ADS sample before and after contact with 500 mg/L Pb(II) was performed and the FTIR spectra presented in Fig. 8 was obtained. The main difference observed between these two spectra was the presence of peaks at 679 and 838 cm^{-1} in the spectrum for Pb(II) containing sample. These
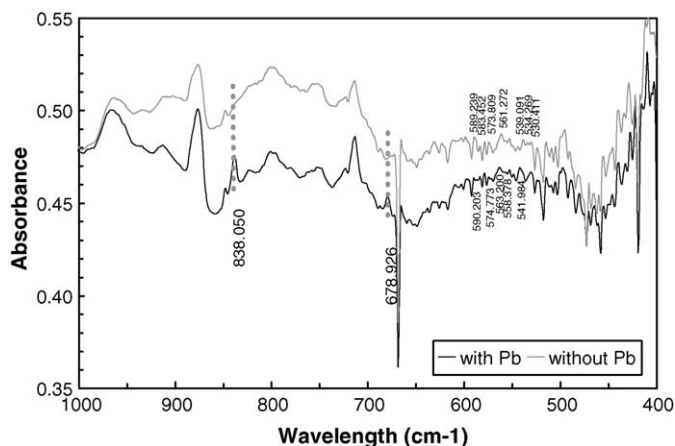
two peaks refer to the binding site of Pb(II) on the biomass structure. Also a shift of peaks was observed in the range of 650–525 cm^{-1}. These shifts were mainly due to the deformation of double carbonyl bonds from the carboxyl functional groups and formation of C–O bond. These observations were in agreement with the FTIR findings of many other researchers; whom indicated that the carboxyl functional groups are responsible for metal removal by several types of biomass [1,19–21]. These results indicated an interaction between the Pb(II) species and carboxyl groups present on the cell wall of the bacteria [22]. That also confirms with the possible interaction of amino groups of amino acids with Pb(II), as indicated before.

3.3. Reactor operation

Considering the potential full-scale application of ADS in the detoxification of metal bearing industrial effluents, a pseudo counter-current sorption reactor configuration was tested. In this selection, primary factor was the relatively low volumetric flow rate of Pb(II) bearing metal industry effluents and also the good settleability of the ADS after interaction with Pb(II). Pseudo counter-current sorption configuration that is composed of three stages was constructed and its Pb removal performance was compared with that of SSB. In fact, TSSC system was operated batch-wise with respect to ADS and semi-continuous-wise with respect to Pb(II) solution. The target in such a configuration was to optimize the Pb(II) removal process with respect to minimum biomass consumption and to see the possible improvement in the process efficiency with multi-stage reactor operation. Generally preferred packed-bed column reactor configuration was not selected due to high water content of ADS which makes it improper to be used as packing material.

Both SSB and TSSC reactor tests were performed at two different initial Pb(II) concentrations of about 80–90 mg/L and 200 mg/L and the results presented in Table 2 were obtained. As can be depicted, with an increase in the initial Pb(II) concentration, a better removal was obtained for both type of reactor configurations. However; in TSSC reactor application, the final Pb(II) concentrations were much lower than that in SSB appli-

Table 2
Results of single and three stage reactor applications

Parameter	SSB		TSCC	
Initial Pb(II) concentration (mg/L)	80.2	195.1	91.5	206.1
Final Pb(II) concentration (mg/L)	5.2	1.9	1.6	1.3
Pb(II) removal (%)	93.5	99.0	98.3	99.4
Initial pH	5.2	5.0	5.9	5.4
Final pH	7.6	6.5	8.5	7.3
q, mg Pb(II)/g dry ADS (mmol/g)	250.0 (1.21)	644.0 (3.10)	299.7 (1.45)	682.7 (3.30)
SVI (mL/g)	54	49	121	110

cation. At the lower initial Pb(II) concentration, the final metal concentration was 5.2 mg/L (0.025 mmol/L) from SSB reactor and 1.6 mg/L (0.008 mmol/L) from TSCC reactor. At about 200 mg/L initial Pb(II) concentration, the effluent concentration was 1.9 mg/L (0.009 mmol/L) and 1.3 mg/L (0.006 mmol/L), respectively. Also, the capacity of ADS was much more effectively used in TSSC reactor system than in SSB reactor. The Pb(II) removal capacity was as higher in the TSCC reactor (3.30 mmol/g ADS) than at SSB reactor (3.05 mmol/g) at the initial Pb(II) concentration of about 200 mg/L. These results imply that as the number of stages increases, there will be more efficient use of biomass.

Parallel to the improvement in Pb(II) removal capacity with TSSC pseudo counter-current reactor application, there was also an improvement in the settleability of sludge formed after the process. The SVI values in Table 2 are also confirming with this result.

4. Conclusions

In the present study, Pb(II) removal by ADS has been investigated. ADS was found to be very effective in the removal of Pb(II) with a maximum removal capacity of about 1760 mg (8.5 mmol) Pb(II) per g of biomass, corresponding to over 99% removal efficiency at the initial Pb(II) concentrations below 1000 mg/L. The pH attained after the process for this concentration range was in the range of 7.0 to 8.0 indicating effective neutralization, which makes the use of ADS more favorable in the treatment of acidic heavy metal bearing wastewaters. High equilibrium pH values attained also indicated that sorption onto the surface of biomass is not the only Pb(II) removal mechanism. Precipitation is another major mechanism.

Considering the precipitation that takes place during the process and also good settleability of sludge after contact with Pb(II), three stage semi-continuous pseudo counter-current reactor configuration was tested in comparison to a conventional single adsorption step process. Multiple stage reactor configuration appeared to be more effective than single stage.

Acknowledgement

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