Effectiveness of anaerobic biomass in adsorbing heavy metals

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Abstract This study focuses on the effectiveness of waste anaerobic dead biomass (ADB) in adsorbing heavy metals, namely Pb(II), Cr(VI), Cu(II), Ni(II) and Zn(II). The metal uptake capacity of ADB was investigated and compared with the values for various biomass types from the literature. The biomass, which was grown under laboratory conditions using a synthetic wastewater, was used throughout the study after sterilization. The maximum metal adsorptive capacities were evaluated by running isotherm tests at 25°C and initial pH of 4. It was observed that Pb(II) was adsorbed with the highest capacity. The maximum adsorptive capacity of ADB for Pb(II), Zn(II), Cu(II), Ni(II) and Cr(VI) was determined as 1250, 625, 357, 227 and 384 mg/g dry biomass, respectively. These values were significantly higher than the corresponding capacities reported in the literature for other types of biomass. In describing the adsorption equilibrium, both the Langmuir and Freundlich isotherm models were examined. The experimental data for Pb(II), Zn(II), Cr(VI), and Ni(II) fitted both the Langmuir and Freundlich models with correlation coefficients of 0.80–0.99 while Cu(II) only fitted the Langmuir model with a correlation coefficient of 0.99. Therefore, different and distinct aspects of the interactions between the cell surfaces and the metal ions might have occurred for Cu(II) and the rest of the metals. The equilibrium pH values attained were all higher than the initial pH value of 4.0, and this indicated that both the type and the initial concentration of the metal influenced the equilibrium pH. Furthermore, there was a decrease in equilibrium pH with increasing initial metal concentration at varying levels.

Keywords Anaerobic sludge; binding capacity; biosorption; heavy metals; pH

Introduction
Increasing heavy metal usage and mankind’s technological advances have resulted in metal polluted receiving environments; especially polluted bays, ground waters, soils, etc. Besides esthetical problems the main concern is the toxicity and tendency of heavy metals to accumulate throughout the food chain, which is a serious threat to the environment, animals and humankind. Therefore, efficient and cost-effective heavy metal removal/recovery methods become vital.

Biosorption may be defined as the removal of metal or metalloid species and particulates from solution by biological material. This technology, which uses microbial cells as biosorbent for heavy metal removal and recovery is a potential alternative to existing conventional removal methods. Metal accumulation by biosorption is generally considered to be a rapid physical/chemical phenomenon that uses biomass. The main mechanisms involved in biosorption can be listed as complex formation, chelation of metals, ion exchanging, adsorption, inorganic micro precipitation and translocation of metal into the cell (Volesky, 1990). All these mechanisms, except the last one, are metabolism independent, which may also occur with dead cells. Factors that influence biosorption include the composition and characteristics of wastewater and biomass, the concentration of metal present, temperature and pH (Artola et al., 1997).

There are several studies in the literature which investigated the biosorption of heavy metals using activated sludge, fungi, algae and yeast as biosorbent, in either living or non-living form (Crist et al., 1981, 1990, 1992; Rudd et al., 1984; Beveridge and Fyfe, 1985; Aulenbach et al., 1987; Gourdon et al., 1990; Aksu et al., 1990; Zhou and Kiff, 1991;
Battistoni et al., 1993; Corder and Reeves, 1994; Churchill et al., 1995; Imai and Gloyna, 1996; Yang and Volesky, 1996; Butter et al., 1998; Kapoor and Viraraghavan, 1998; Matheickal et al., 1999). In these studies the mechanism of metal ion uptake was found to vary depending on the metal ion under study and the nature and condition of the biomass used. But little attention has been paid to anaerobic bacteria biosorption especially in non-living conditions (Solari et al., 1996). Previous studies on heavy metal removal by anaerobic biomass, has been mainly focused on the effect of metals on biogas formation, the relative toxicity of metals and the effect of chemical treatment on biosorption (Mehrotra et al., 1987; Katsiri et al., 1988; Hickey et al., 1989; Mueller and Steiner, 1992; Bhattacharya et al., 1995; Alkan et al., 1996; Fang, 1997; Lin and Chen, 1997; Leighton and Forsters, 1997). Few reports exist dealing with the binding mechanism or capacity of anaerobic biomass and the operational factors influencing them (Alibhai et al., 1985; Solari et al., 1996; Artola et al., 1997).

The use of anaerobic dead biomass (ADB) as the biosorbent has several advantages such as:

- Bacteria make excellent biosorbent because of their high surface-to-volume ratios and anionic cell walls (Beveridge and Fyfe, 1985).
- Dead biomass neither requires nutrients nor is affected by environmental conditions.
- The increase in commercial use of anaerobic digesters results in tonnes of anaerobic waste biomass.

In the present work, the adsorptive capacity of ADB in removing five heavy metals, namely Ni(II), Cu(II), Cr(VI), Zn(II), and Pb(II) was investigated.

Materials and methods

Organism, media and growth conditions

The biomass used in adsorption studies was collected from a laboratory-scale anaerobic reactor. This main anaerobic reactor has been operating for 11 months, at 20 days of sludge age, in a hot room where temperature was kept constant at 35 ± 2°C. The reactor with 16 L of effective volume was continuously stirred and operated in fed-batch mode. The composition of the synthetic feed was as follows (concentrations of the constituents are given in brackets as mg/L): Dog food (2000), NH₄Cl (1200), MgSO₄·7H₂O (400), KCl (400), Na₂S·9H₂O (300), CaCl₂·2H₂O (50), (NH₄)₂HPO₄ (80), FeCl₂·4H₂O (40), CoCl₂·6H₂O (10), KI (10), MnCl₂·4H₂O (0.5), CuCl₂·2H₂O (0.5), ZnCl₂ (0.5), AlCl₃·6H₂O (0.5), NaMoO₄·2H₂O (0.5), H₃BO₃ (0.5), NiCl₂·6H₂O (0.5), NaWO₄·2H₂O (0.5), Na₂SeO₃ (0.5), Cysteine (10), NaHCO₃ (6000). Dog food served as the carbon source for the microbial growth. The chemicals in the synthetic wastewater ensured the required nutrients, alkalinity and osmotic pressure necessary for anaerobic growth (Takashima and Speece, 1989).

Initial COD of this synthetic wastewater was 21,000 ± 1,000 mg/L.

The concentrations of volatile fatty acid (VFA), bicarbonate alkalinity (HCO₃⁻), COD and mixed liquor suspended solids (MLSS) in the main reactor were monitored. These parameters varied between 0–500 mg/L for VFA, 2,500–3,500 mg/L for HCO₃⁻ and 5,900–7,000 mg/L for MLSS until steady state was reached. At steady state VFA, HCO₃⁻ and MLSS were stable around 200, 3000 and 5,900 mg/L, respectively. COD removal efficiency was between 65–73%. VFA and alkalinity were taken as the primary indicator of steady state conditions and was controlled twice a week.

The waste biomass of the main anaerobic reactor that was used in the metal uptake experiments was concentrated by settling. Then, the microorganisms were deactivated by sterilisation in an autoclave at 121°C for 1 h. Several different methods are available for inactivating microbial biomass like sterilisation, chemical treatment and heat treatment. Published information suggests that the way the cells are inactivated affects their respec-
tive metal-binding capacity, and that sterilisation (autoclaving) improves the binding
capacity of biomass (Corder and Reeves, 1994). For this reason, sterilisation was chosen
for inactivating the anaerobically digested biomass. After sterilisation, the biomass was
concentrated once again by settling in an Imhoff cone and the concentration of the final bio-
mass suspension to be used in sorption tests was measured.

**Metal uptake experiments**
The ability of ADB to take up Cu(II), Zn(II), Ni(II), Pb(II), and Cr(VI) was investigated by
running batch sorption tests. Metal salts used were analytical grade, CuCl$_2$.2H$_2$O, ZnCl$_2$,
NiCl$_2$.6H$_2$O, Pb(NO$_3$)$_2$ and K$_2$Cr$_2$O$_7$. The metal concentrations tested were in the range of
300–1,500 mg Cu/L, 250–2,500 mg Zn/L, 400–2,000 mg Ni/L, 500–2,500 mg Pb/L,
150–1,500 mg Cr/L. For each metal, series of batch sorption reactors containing 100 mL
metal salt solutions were prepared. Initial pH of 4 was adjusted using either concentrated
or dilute NaOH or HNO$_3$, and 5 mL of sample from each reactor was taken for initial metal
concentration determination. Then 0.1 g dry weight ADB was added to the solutions and
reactors were placed in an orbital shaker (Gallenkamp NTI 200) operating at 25°C and 160
rpm, for 24 h. Preliminary studies have shown that 90% metal removal was achieved within
the first 6 h of biomass-metal solution contact and no desorption was observed until 24 h.
So, 24 h was employed as the equilibrium time. The biomass was removed by filtration
through a 0.45 µm membrane filter (Millipore) and the filtrates were analysed for metal
concentrations.

**Analytical techniques**
VFA and HCO$_3^-$ were analysed by simple acid titration method (Anderson and Yang,
1992). Biomass concentration was measured as MLSS (Standard Methods, 1995). The pH
measurements were done using a pH meter (Jenway 3010) and a pH probe (Cole Palmer
Instrument Co.). Initial and final metal concentrations were measured using ATI Unicam
929 atomic absorption spectrometer.

**Calculations**
The metal uptake by dead biomass was calculated from the results of samples analysed
using the formula: "$q = (C_o - C_e) \times V/M$" where $q$ is the adsorptive capacity (mg/g); $C_o$ is
the initial metal concentration (mg/L); $C_e$ is the equilibrium metal concentration attained in
solution at the end of the adsorption process (mg/L); $V$ is the solution volume (L) and $M$
the amount of biosorbent (g dry weight).

**Results and discussion**
The data obtained by equilibrating the anaerobic sludge with the five heavy metals present
in the aqueous phase is shown in Figure 1. The results are presented as simple isotherms. As
shown, there are significant differences in the behaviour of ADB in adsorbing different
heavy metals. Lead was best sorbed and the other metals followed with an order of
Pb(II)>Zn(II)>Cu(II)>Ni(II)>Cr(VI) in the high concentration range (>1,500 mg/L). In the
low concentration range, variance between the adsorption capacities of five metals were
even more striking. The equilibrium data was also examined as Langmuir and Freundlich
isotherms and the best fit parameters were evaluated by regression analysis (Table 1).
Linearized adsorption isotherm plots indicated that there is a good fit of data to the
Langmuir model for the metals; Ni(II), Cu(II), Cr(VI) and Pb(II). However, Cu(II) sorption
equilibrium seems to be poorly described by both Freundlich and Langmuir models.
In all cases, Langmuir model better described the adsorption equilibrium than Freundlich
model. Correlation coefficients presented in Table 1 also show that the models are equally
effective in describing the sorption of Zn(II), Cr(VI) and Ni(II) by ADB. Although the validity of these sorption models does not necessarily reflect the physico-chemical underlying principles of the sorption process; these findings suggest that the removal of heavy metals by ADB may be to monolayer surface sorption. Meanwhile, Volesky (2001) reports that the monolayer deposition considered for deposition of sorbates implies surface-based adsorption which is not the case for biosorption. Biomaterials are gel-like and transparent to ions which are extremely small and easy to penetrate.

On the basis of isotherm parameters, in particular \( b \) and \( n \), which offer some quantification of the intensity of binding, it is possible to derive a set of binding series (Alibhai et al., 1985). Tables 2 and 3 show that anaerobic biomass possesses the highest affinity and the highest capacity for Pb(II) as reflected by the highest \( b \) and \( q_{\text{max}} \) values, respectively. This result is in agreement with an earlier work by Leighton and Forster (1997) who found lead to bind most strongly to the acidogenic sludge. The values for Cu(II) are not used in the present comparison considering the very poor fit of data to the Freundlich isotherm model. As presented, the series are the same whether derived from \( b \) or \( n \). On the other hand, the capacity series based on \( K \) is also the same. Only the series derived from \( q_{\text{max}} \) differs for the metals Ni(II) and Cr(VI); but, not for Pb(II) and Zn(II). These findings imply that there are different and distinct aspects of interactions between ADB and the metals Ni(II) and Cr(VI). This results in a higher capacity for Cr(VI) than for Ni(II), but a lower strength of binding for Cr(VI) than for Ni(II). Nickel was the one that had the weakest binding among the metals; lead, copper, nickel and zinc. Metals are known to favour specific binding sites (Leighton and Forster, 1997). For example, Battistoni et al. (1993) have reported that cadmium is bound to the extracellular polymers and nickel favours capsular polymers and cell walls in activated sludge.

Table 1  Freundlich and Langmuir isotherm constants for heavy metals tested

<table>
<thead>
<tr>
<th>Metals</th>
<th>Freundlich*</th>
<th>Langmuir**</th>
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<tbody>
<tr>
<td></td>
<td>( K )</td>
<td>( 1/n )</td>
</tr>
<tr>
<td>Zn(II)</td>
<td>74.50</td>
<td>0.16</td>
</tr>
<tr>
<td>Ni(II)</td>
<td>12.79</td>
<td>0.35</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>217.52</td>
<td>0.06</td>
</tr>
<tr>
<td>Pb(II)</td>
<td>519.50</td>
<td>0.11</td>
</tr>
<tr>
<td>Cr(VI)</td>
<td>0.58</td>
<td>0.78</td>
</tr>
</tbody>
</table>

* \( K \) values are given in (mg/g)(l/mg)^{1/n} and \( 1/n \) is unitless
** \( q_{\text{max}} \) and \( b \) values are given in mg metal/g biomass and l/g, respectively
Maximum adsorption capacities attained for Pb(II), Zn(II), Cr(VI), Cu(II) and Ni(II) metal ions were 1,250, 625, 384, 357, 227 mg metal/g biomass, respectively, as derived from Langmuir constant, $q_{\text{max}}$. The differences of weight-based metal sorption capacities were within more than one order of magnitude. When compared with the other biosorbents reported, ADB was found to be a very effective biosorbent (Alibhai et al., 1985; Aulenbach et al., 1987; Aksu et al., 1990; Zhou and Kiff, 1991; Holan and Volesky, 1993; Brierley and Brierley, 1993; Corder and Reeves, 1994; Churchill et al., 1995; Leusch et al., 1995; Imai and Gloyna, 1996; Solari et al., 1996; Artola et al., 1997; Leighton and Forster, 1997; Kapoor and Viraraghavan, 1998). These high capacities may be explained by a hypothesis that states a two-step mechanism of metal adsorption to biomass. The first step is the stoichiometric interaction between the metal ion and the active site on the wall. This interaction acts as the nucleation site for the deposition of more metal ion. The deposition therefore grows in time within the intramolecular spaces between wall polymers until it is physically constrained by the wall meshwork. The end result is a bacterial wall which contains copious amounts of metal which are not easily replaced by water (Beveridge, 1986). The way the cells are inactivated is also reported to affect their respective biosorptive capacity (Corder and Reeves, 1994). Autoclaving employed in the present study, might have resulted in the rupture of cell walls which resulted in the exposure of the interior of the cell to the metal.

The mechanism of heavy metal removal by anaerobic biomass can involve four distinct processes: adsorption, incorporation into cells, precipitation and the subsequent entrapment of the precipitate in the biofloc and chelation of soluble metal species by organic and inorganic ligands (Cheng et al., 1975). The possible interaction of metal with anions like carbonate, sulphide etc., can precipitate metal and compete with the metal surface interaction (Alibhai et al., 1985). The extremely high metal adsorption capacities attained in this study, confirms metal precipitation. Removal by ion exchange is a function of the ionic valency and the ionic radius. On the basis of valency, Cr(VI) should be adsorbed more strongly whereas, based on the ionic radius, the removal of Pb(II) should be the most intense. Figure 2 shows that, the adsorption capacity (as mmol metal/g biomass) follows an increasing general pattern with ionic radius, except for Cr(VI). A similar trend has been reported by Volesky (1990) for R. arrhizus. This might be due to the nature of the chromium species existing in solution. The charge, size and the nature of the ionic species will decide the overall ionic exchange process. The calculated ionic potential for Cr(VI) is excessively higher that the other ions studied. Ions with high ionic potential attract more water molecules and form hydrated ions that are larger than those of ions having lower ionic potentials (Faure, 1991). This might be the reason why Cr(VI) behaves differently.

The Irving–Williams series describes a descending order of divalent metal-ion complexes stabilities formed regardless of the nature of the complexing agent: Pb>Cu>Ni>Co>Zn> Cd>Fe>Mn>Mg (Churchill et al., 1995). The series is based upon metal-cation parameters of hydrated ionic radius, valence, and ionisation potential. The observed affinity series for the divalent cations is; Pb>Zn>Ni. This follows the Irving–Williams series with the exception of Zn. The diverse nature of anaerobic biomass may be responsible for this deviation. Leighton and Forster (1997) pointed out the very
different behaviour of zinc in its biosorption by mesophilic and thermophilic anaerobic sludge and attributed this to the different surface characteristics of the two sludges. The solvent, stereochemistry, and electronic configurational factors present are reported to influence the tendency of metals to form covalent bonds with a particular site (Churchill et al., 1995).

Earlier studies have indicated that the solution pH is an important parameter affecting biosorption of metal ions (Matheickal et al., 1999; Artola et al., 1997). The reasons explaining the behaviour of the biomass relative to solution pH, are: acidic functional groups present in the cell wall (Artola et al., 1997), and metal chemistry in solution. The equilibrium pH values attained at the end of isotherm tests, were all higher than the initial pH value of 4.0, and there was a strong dependency of the equilibrium pH on the initial metal concentration (Figure 3). It is also worthy to note that there was a decrease in equilibrium pH with increasing initial metal concentration at varying levels. The equilibrium pH in Pb(II) sorption varied between 7.4 and 5.3 for initial metal concentrations less than 1,200 mg/L. For higher initial Pb(II) concentrations, the equilibrium pH did not change significantly. Similarly, a drastic pH increase was observed in Cr(VI) and Cu(II) biosorption. Cu(II) and Zn(II) ions had almost the same behaviour, decrease in equilibrium pH as the initial metal ion concentration increases. These findings agree with the results of other researchers. Artola et al. (1993) pointed out that equilibrium pH values were lower for copper than for zinc, nickel and cadmium; as is the case for this study. In a recent study, Artola et al. (1997) further investigated the heavy metal binding to anaerobic sludge as reference to equilibrium pH and concluded that the main binding groups for metals in sludge are of amino acid types.

On the other side, the magnitude increase in pH from its initial to equilibrium value was variable for the same initial metal concentration of these five metals (Figure 3). This further confirms that there are different and distinct aspects of interactions between ADB and different metals. Although the main binding site of anaerobic sludge for metals is shown to be amino acid groups (Artola et al., 1997), there may be some other groups in the cell which are capable of metal uptake. The presence of different types of metal species with changing pH, of course, further contributes to pre-mentioned different and distinct interactions between ADB and metals.

Table 3 Capacity series derived from the Langmuir and Freundlich parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Capacity series</th>
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<tr>
<td>$K$</td>
<td>Pb(II) &gt; Zn(II) &gt; Ni(II) &gt; Cr(VI)</td>
</tr>
<tr>
<td>$q_{max}$</td>
<td>Pb(II) &gt; Zn(II) &gt; Cr(VI) &gt; Ni(II)</td>
</tr>
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Figure 2 The relationship between the Freundlich parameter, $q_{max}$ and the ionic radius of the adsorbed metals
Conclusion

In the present study, waste biomass from an anaerobic digester operated under laboratory conditions, was used in batch adsorption tests of five heavy metals: Ni(II), Cu(II), Cr(VI), Zn(II) and Pb(II). When compared with the other biosorbents reported, ADB was found to be a very effective biosorbent, considering its abundance and excellent biosorptive capacity. The maximum adsorption capacities attained were 1,250, 625, 315, 230 and 225 mg metal/g dry biomass; for Pb(II), Zn(II), Cu(II), Ni(II), and Cr(VI), respectively. The incredibly high sorptive capacity of ADB for metals was partly attributed to the autoclaving employed in the biomass preparation stage, which might have resulted in the exposure of both the interior and the exterior of the cell to the metal. The extent to which this biomass preparation stage contributes to the biosorptive capacity appears to be of some considerable significance and will require further study.

The equilibrium pH values attained were found to vary with the metal and its initial concentration and they were all higher than the initial pH value of 4.0. All these findings have confirmed that there are different and distinct aspects of interactions between ADB and different metals.

References


