Biosorption of Cu(II) by powdered anaerobic granular sludge from aqueous medium
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ABSTRACT
Copper(II) biosorption processes by two pre-treated powdered anaerobic granular sludges (PAGS) (original sludges were methanogenic anaerobic granules and denitrifying sulfate removal (DSR) anaerobic granules) were investigated through batch tests. Factors affecting the biosorption process, such as pH, temperature and initial copper concentrations, were examined. Also, the physico-chemical characteristics of the anaerobic sludge were analyzed by Fourier transform infrared spectroscopy, scanning electron microscopy image, surface area and elemental analysis. A second-order kinetic model was applied to describe the biosorption process, and the model could fit the biosorption process. The Freundlich model was used for describing the adsorption equilibrium data and could fit the equilibrium data well. It was found that the methanogenic PAGS was more effective in Copper(II) biosorption process than the DSR PAGS, whose maximum biosorption capacity was 39.6% lower. The mechanisms of the biosorption capacities for different PAGS were discussed, and the conclusion suggested that the environment and biochemical reactions during the growth of biomass may have affected the structure of the PAGS. The methanogenic PAGS had larger specific surface area and more biosorption capacity than the DSR PAGS.

Key words | biosorption, copper, isotherm, kinetic model, powdered anaerobic granular sludge

INTRODUCTION
Heavy metal pollution has become one of the most serious environmental problems nowadays. The industrial wastewater from manufacturing enterprises such as electroplating, mining and smelting contain various heavy metal ions, including Cu, Zn, Pb, Cd, Ni, etc. All these heavy metal ions are detrimental to the ecosystem, especially to the water body (He et al. 1998). Furthermore, many health problems of humans are indirectly caused by wastewater containing heavy metals. Heavy metals can be easily accumulated in the human body and induce long-term health risks (Singh et al. 2010).

Many technologies have been developed in the last decade to remove heavy metals from wastewater, among which the biosorption process is attractive due to its low cost and easy application (Kratochvil & Volesky 1998; Wang & Chen 2009). Many biomaterials have been used as adsorbents, such as algae and fungi, of which a lot of work has focused on characterizing them (Kapoor & Viraraghavan 1995; Chen et al. 2008a; Liu et al. 2009).

Recently, waste sludge is used more and more as a bio-adsorbent. Waste sludge is a byproduct of wastewater treatment processes, which makes it easy to obtain for the treatment of heavy metal polluted water. On the other hand, the elimination of heavy metals with waste sludge was also valuable as a waste recycling process (Hawari & Mulligan 2006).

Many researchers found the basic mechanism of the biosorption process could be attributed to the functional groups abounding in sludge, such as carboxylic acid and hydroxyl, which could bind metal ions and thus remove them from solution (Hammami et al. 2007; Cui et al. 2011; Sun et al. 2011). According to their research, pH, temperature as well as the concentration of metal ions in liquid are the most important parameters influencing the biosorption process (Kargi & Cikla 2006; Kliç et al. 2008). pH level affects the ionization of the functional groups in sludge. Temperature could influence the thermodynamic characteristics of the reaction between functional groups and metal ions.
Differences in concentrations of metal ions change the mass transfer efficiency between solid and liquid phases.

However, most previous work has focused on the maximum adsorption capacity of a certain kind of waste sludge. These studies failed to investigate the source and characteristics of the sludge used for the biosorption process. In fact, different sludges often have very different physico-chemical properties due to their different composition and structure during their formation process. However, only a few studies compared the adsorption process by different waste sludges (Lister & Line 2001).

Among different kinds of waste sludge, anaerobic granular sludge often has higher biomass content. Furthermore, the treatment of such sludge is still a problem for many wastewater treatment plants. The sludge is suitable for the biosorption process because of its lower cost and physical properties (Hawari & Mulligan 2006). Obviously, characteristics of such waste sludge might depend on the treatment processes in many ways. To our knowledge, no research has compared the biosorption capacity of different kinds of powdered anaerobic granular sludge (PAGS) and the associated mechanisms.

Anaerobic granular sludge was the most widely used anaerobic granular sludge which treats wastewater with high concentrations of soluble organic matter (Wu et al. 1991; Fukuzaki et al. 1995). Denitrifying sulfide removal (DSR) granular sludge was cultivated and applied in a newly developed simultaneous denitrification and desulfurization process, which was fed with high-loading synthetic wastewater containing sulfide and nitrate, and it converted them into nitrogen and elemental sulfur (Reyes-Avila et al. 2004; Chen et al. 2008b). The purpose of this study is to investigate the copper biosorption capacities and analyze the adsorption mechanisms of two typical PAGS. In this study, the biosorption capacity of two PAGS, i.e. methanogenic and DSR PAGS, was measured in batch tests. The adsorption kinetics and models under different conditions, and their physical-chemical properties, were also studied.

MATERIAL AND METHODS

Preparation of sludge and chemicals

The methanogenic sludge granules were obtained from a full-scale anaerobic digester treating brewery wastewaters (Harbin, China). The volatile suspended solids (VSS) and suspended solids (SS) concentrations were 26.65 and 40.41 g/L, respectively. The DSR granules were obtained from an expanded granular sludge bed (EGSB) reactor treating high-loading sulfide and nitrate wastewater used by Chen et al. (2008b). The concentrations of VSS and SS of the fed granules were 117 and 137 g/L, respectively.

Both types of granular sludge were washed with distilled water three times and dried at 30°C. The granules were then grind and sieved to make sure that their particle size was below 200 μm. The powdered sludge granules were treated with 100 mL 1% H₂O₂ for 1 g of sludge; thus some functional groups on the surface of the particles could be activated for better adsorption effect (Pamukoglu & Kargi 2007).

Analytical grade Cu(NO₃)₂·3H₂O was used as the adsorbate which was dissolved with distilled water. The stock solution of Cu(II) was 1,000 mg/L.

Characterization of PAGS

Fourier transform infrared spectroscopy (FT-IR) analysis of the two PAGS was performed using a spectrum one FT-IR spectrometer (Perkin Elmer, USA) in the range 400–4,000 cm⁻¹ by KBr pellets method. ASAP 2020 Surface Area and Porosity Analyzer (Micromeritics, USA) was applied to analyze the specific surface area of the dried anaerobic sludge. S-4700 scanning electron microscopy (SEM) (Hitachi Ltd., Japan) was used to examine the microstructure of the dried anaerobic sludge particles’ surface.

Batch adsorption experiments

The biosorption characteristics of Cu(II) by two kinds of PAGS were investigated by batch experiments. For each batch test, PAGS were mixed with Cu(II) solution to achieve the concentration of 1,000 mg/L PAGS and different levels of Cu(II). Two hundred milliliters of mixture solution with pretreated sludge and Cu(II) were sealed in screw-capped flasks (250 mL). The flasks were then placed onto a shaking table at 150 rpm. The pH was adjusted with 0.1 M NaOH and HCl.

The effect of pH on biosorption was examined from pH 2 to 6 at 25°C, the effect of temperature ranging from 25 to 45°C, and initial concentrations of Cu(II) were 100–400 mg/L Cu²⁺ at pH 4. All the batch experiments were performed for 480 min and repeated three times. In addition, a control experiment without addition of PAGS was performed with initial concentrations of 200 mg/L Cu²⁺.

Chemical analysis

The concentration of Cu(II) in the aqueous solution was measured by the Optima 5300 DV optical emission spectrometer (Perkin Elmer, USA). The element content of C...
and N was measured by Vario EL cube Elementar Analysen systeme GmbH (Elementar, Germany). Elemental sulfur in the DSR anaerobic granular sludge was determined following the sulfite method (Jiang et al. 2009). SS/VSS of the sludge were measured by APHA standard methods (APHA 1998).

RESULTS AND DISCUSSION

Effect of pH

Figure 1 shows the effect of pH on the removal rate of Cu(II) by the two PAGS. The highest adsorption efficiency of the methanogenic PAGS was 22.74 mg-Cu/g-PAGS at pH 4, which was much higher than 13.7 mg-Cu/g-PAGS by the DSR PAGS. The copper uptake increased slightly from pH 2 to 3 and rose sharply from pH 3 to 4. Thus, the optimum pH for the adsorption process should be around 4. This result is similar to other biosorption materials (Gulnaz et al. 2005).

When the pH level was below 4, the adsorption efficiency was much lower than the condition when the pH value was above 4, possibly due to the competition between Cu(II) and H⁺ for active sites on the surface at lower pH (Wang et al. 2010). When the pH value was above 5, Cu(II) hydroxides could be formed as precipitate (Pamukoglu & Kargi 2006). The K_{sp} of Cu(OH)₂ is 2.2 \times 10^{-20} between 18 and 25°C (Speight 2005).

Effect of temperature

Figure 2 shows the effect of temperature on the biosorption efficiency by DSR PAGS and methanogenic PAGS at 25°C, 35°C and 45°C with initial Cu(II) concentration of 200 mg/L and pH 4. Clearly, the adsorption efficiency of both PAGS decreased significantly when the temperature was increased.

The biosorption efficiency of methanogenic PAGS was higher than DSR PAGS at all the three temperatures. The lowest biosorption capacity of dried powdered methanogenic granular sludge was 7.29 mg-Cu/g-PAGS at 45°C, while the biosorption capacity of dried powdered DSR sludge was only 5.48 mg-Cu/g-PAGS. By comparing adsorption at 25°C to that at 45°C, the biosorption amount of Cu^{2+} by methanogenic PAGS decreased by 68%, while DSR PAGS decreased by 60%.

Similar phenomena were reported by many other researchers (Gulnaz et al. 2005; Wang et al. 2006). It was widely recognized that the decrease of biosorption efficiency with elevated temperature was due to the decrease of liquid viscosity; this increased adsorbate diffusion on the adsorbent and reduced combinations between the metal ion and function groups from sludge (Al-Qodah 2006).

Effect of initial Cu(II) concentration on sorption kinetics

Three different initial concentrations of Cu(II) were tested at 25°C and pH 4. Figure 3 shows that the biosorption efficiency was improved with increasing initial Cu(II) concentrations. When the initial concentration of Cu(II) was 100 mg/L, the adsorption amount of copper by methanogenic PAGS and DSR PAGS was only 17.65 and 8.71 mg-Cu/g-PAGS. When the initial concentration of Cu(II) increased to 400 mg/L, the adsorption amount of
copper by methanogenic PAGS and DSR PAGS were increased to 39.01 and 27.94 mg-Cu/g-PAGS, respectively. Higher initial concentration could improve the transfer of metal ions from solution to solid phase (Aksu & Akpınar 2003).

A second-order kinetic model was used to describe the effect of initial concentrations on the biosorption process.

The pseudo second-order kinetic is expressed as:

\[ \frac{t}{q_t} = \frac{1}{kq_e^2} + \frac{1}{q_e} t \]

where \( q_t \) and \( q_e \) are the adsorbed metal concentration on the adsorbent at time \( t \) and the equilibrium metal concentration (mg-Cu/g-PAGS), respectively. \( k \) (g-PAGS-min/mg-Cu) is the rate constant of second-order kinetic models.

The correlation coefficients and rate constants of the second-order kinetic models were obtained at 25 °C with initial pH of 4, and the results are shown in Figure 4 and listed in Table 1. The pseudo second-order kinetic model had a considerably good correlation with all three initial Cu(II) concentrations, indicated by all of the correlation coefficients (>0.99). Therefore, the pseudo second-order kinetic model was favorable for describing the biosorption process.

As Table 1 shows, the pseudo second-order kinetic model had a considerable correlation coefficient with all three initial Cu(II) concentrations; the pseudo second-order kinetic model agreed well with both kinds of biomass because all of the correlation coefficients calculated from both biosorption processes were higher than 0.99. Therefore, the pseudo second-order kinetic model was favorable for describing the biosorption process.

### Adsorption isotherms

The linear form of the Freundlich adsorption isotherm was used to describe the Cu(II) adsorption and equilibrium uptake in this work. The Freundlich model could be linearized as below:

\[ \ln q_e = \frac{1}{n} \ln C_e + \ln K \]

where \( q_e \) and \( C_e \) are the equilibrium metal concentration on adsorbent and in solution (mg/g and mg/L), respectively. \( n \) is the Freundlich exponent and \( K \) is the Freundlich constant; both of them are related to the characteristics of the adsorbate/adsorbent system at a certain temperature.

The Freundlich constants at pH 4, 25 °C are given in Table 2. The Freundlich isotherm could fit both kinds of PAGS' biosorption data well (see its high correlation coefficient). The Freundlich exponent related to the biomass capacity of Cu(II) adsorption. Therefore, the DSR PAGS might have a heterogeneous surface with various metal binding functional groups rather than a homogenous monolayer.

### Characteristics of PAGS

FT-IR analysis was performed to identify functional groups on the two kinds of PAGS (Figure 5). According to the results for methanogenic PAGS, the peak at 3,405 cm\(^{-1}\) could be assigned as \(-OH\) or \(-NH\) stretching vibrations, the peak at 2,925.3 cm\(^{-1}\) could be attributed to asymmetric
stretches of the C=O bond from the amide I band of proteins (Gulnaz et al. 2005; Wang et al. 2010), the peak at 1,545.7 cm⁻¹ could be stretching vibrations of C-N of the peptide bond from proteins, and the peak at 1,095.3 cm⁻¹ could be C-O-C and O-H stretching vibrations from polysaccharide (Gulnaz et al. 2005). The peak at 1,537.3 cm⁻¹ could be C-N stretching vibration of the peptide bond from proteins.

According to the analysis for DSR PAGS, the peaks at 3,399.9 cm⁻¹ could be –OH or –NH stretching vibrations, the peaks at 2,925.6 cm⁻¹ could be asymmetric stretching vibration of CH₂, the peak at 1,431.9 cm⁻¹ was attributed to the –CH stretching, the peak at 1,642.6 was attributed to C=O stretching and C-N (amide I) peptide bond of protein (Wang & Chen 2013). According to the FT–IR analysis, on the surfaces of both kinds of PAGS, hydroxyl (–ROH) and amine (–NH₂) existed. However, carboxyl (–COOH) only appeared in methanogenic PAGS, thus it could be a reason for its better biosorption capacity than DSR PAGS.

Characteristics of the sludge biomass

The microbial community of anaerobic methanogenic granules treating brewery wastewater mainly consisted of methanogens, syntrophic acetogens and hydrolytic-fermentative bacteria. Thus the granules could convert soluble organic matter into methane and CO₂ in an anaerobic environment (Wu et al. 1991). The internal structure of the granules was porous, which could promote its treatment effect and excrete gas easily (Alphenaar et al. 1995). Furthermore, according to Diaz et al. (2006), the methanogenic granules had a multilayer structure.

On the other hand, the DSR anaerobic granular sludge mainly consisted of heterotrophic and autotrophic denitrifying bacteria. The simultaneous removal of sulfate, nitrate and acetate in DSR anaerobic granules was performed. During the process, much less gas was produced compared to the methane-producing process (Chen et al. 2008b).

Table 2 | Freundlich parameters for biosorption of Cu(II) at 25 °C, pH 4

<table>
<thead>
<tr>
<th>Copper(II) (mg/L)</th>
<th>100</th>
<th>200</th>
<th>400</th>
</tr>
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<tbody>
<tr>
<td>k (g-PAGS-min/mg-Cu)</td>
<td>0.0051</td>
<td>0.0020</td>
<td>0.0009</td>
</tr>
<tr>
<td>r²</td>
<td>1.000</td>
<td>0.999</td>
<td>1.000</td>
</tr>
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Table 1 | The correlation coefficient and rate constant of second-order kinetics model with different initial concentrations of Cu(II) at 25 °C, pH 4

<table>
<thead>
<tr>
<th>Copper(II) (mg/L)</th>
<th>100</th>
<th>200</th>
<th>400</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanogenic PAGS</td>
<td>k (g-PAGS-min/mg-Cu)</td>
<td>0.0031</td>
<td>0.0020</td>
</tr>
<tr>
<td>DSR PAGS</td>
<td>0.0016</td>
<td>0.0011</td>
<td>0.0008</td>
</tr>
<tr>
<td>r²</td>
<td>1.000</td>
<td>0.999</td>
<td>0.999</td>
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Figure 5 | FT–IR spectra of methanogenic PAGS (a) and DSR PAGS (b).
Also, many researchers reported that a mass of elemental sulfur was accumulated in the sludge after the operation of the desulfurization process (Krishnakumar et al. 2005; Zhou et al. 2011). Furthermore, Janssen et al. (1999) found the elemental sulfur particles produced during the sulfide oxidation process could secrete and accumulate on the surface of cells. Therefore, different from methanogenic sludge, a much more compact structure in the DSR granules was formed which mainly consisted of elemental sulfur; the percentage content of elemental sulfur was 66.7% for the DSR granular sludge used in this work, which could reduce the mass transfer efficiency. Similarly, compared to the methanogenic granules, lower concentration of biomass in the DSR granules also reduces the content of functional groups in sludge and thus lowers the biosorption efficiency.

Effect of sludge characteristics on biosorption processes

The PAGS’ particle diameters had been considered an important factor influencing the biosorption process (Gulnaz et al. 2005). In addition, the characteristics of the PAGS’ natural surface texture and structure were never discussed on its effect on the biosorption process before. Although both kinds of biomass were anaerobic granules, their characteristics such as growth environment, microbial community and physical structure were different; these characteristics were believed to affect the biosorption capacity largely.

The characteristics of different kinds of anaerobic granular sludge mentioned in the last section were in the PAGS after pre-treatment. The elemental analysis indicated that the DSR PAGS still mainly consisted of S, and the concentration was 67.8%. As the SEM photos show (Figure 6), the DSR PAGS have a much smoother surface than methanogenic PAGS, which indicates that although nitrogen and CO₂ were also produced during the DSR treatment process, this could not change the surface structure a lot due to the accumulation of bio-produced sulfur. Furthermore, according to the analysis of specific surface area (Figure 7), the dried powdered methanogenic granules (6.7957 m²/g) had much more surface area than the powdered DSR granules (5.7889 m²/g). Obviously, the methanogenic PAGS, which had less smooth structure and more surface area, could have a better biosorption capacity.

In summary, the biochemistry process during the growth of both kinds of anaerobic granulars affected their physical properties such as surface properties and structure. These physical properties were also kept in PAGS after the pre-treatment process, and affected the PAGS’ biosorption efficiency greatly.

CONCLUSIONS

The biosorption of Cu(II) in aqueous solution by two types of pre-treated powdered anaerobic granules was investigated in this work. The optimum pH for the biosorption process by both PAGS was around 4. The adsorption reaction was exothermic, whose equilibrium could be described by the Freundlich equation. A pseudo second-order model could fit the adsorption kinetics well.

Methanogenic PAGS had a much higher biosorption efficiency of Cu(II) than DSR PAGS, which might be due to the biochemical reaction during its growth process. The methanogenic process produced gas, which facilitated the formation of a more porous structure and larger surface area of the granules than the DSR process. Furthermore, the methanogenic PAGS had more different functional
groups on the surface than the DSR PAGS. Thus, the environmental conditions and biochemical reactions during the growth process of anaerobic granular sludge were important factors for the formation of a granular structure with high biosorption efficiency. The findings should also be considered for the engineered application of waste sludge for the biosorption process.

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