Utilization of anaerobic granular sludge for chromium (VI) removal from wastewater: optimization by response surface methodology

Yifan Hu, Changzhu Yang, Jinfeng Dan, Wenhong Pu and Jiakuan Yang

ABSTRACT

In this study, anaerobic granular sludge (AGS) was used as a novel adsorbent for hexavalent chromium (Cr (VI)) removal from aqueous solutions. Factor experiments were conducted to find out the effects of different variables on the biosorption process. Among these terms, the impact of three main independent variables (contact time, initial pH and AGS dosage) on the removal efficiency of Cr (VI) was modeled using a well-fitting polynomial equation ($R^2 = 0.9044$), by conducting 20 batch experiments designed by a central composite. The experimental isotherm data were successfully described by the Freundlich isotherm and the pseudo-second-order kinetic model was more suitable for explaining the kinetics process of adsorption. The AGS can be disposed using 0.1 M NaOH with 96.4% desorption efficiency. The results of the analyses (X-ray photoelectron spectroscopy and Fourier transformed infrared spectroscopy) suggested that Cr (VI) adsorption most likely involved electrostatic adsorption, redox reaction and complexation.

Key words | anaerobic granular sludge, biosorption, hexavalent chromium, response surface methodology

INTRODUCTION

Wastewater generated by industrial processes such as electroplating, textile dying and tanning always contains hexavalent chromium (Cr (VI)), which is mutagenic and carcinogenic to human beings and other living organisms. According to the World Health Organization (WHO), the maximum permissible concentration of Cr (VI) in surface water is 0.1 mg/L, but the chromium discharge by many industrial units is always beyond this limit.

Many techniques have been used to remove chromium from wastewater, including electrolysis (Wei et al. 2015), membrane separation (Erniza et al. 2016), chemical precipitation, ion exchange (Li et al. 2013), etc. Adsorption is the most popular method among them to remove heavy metals from liquid solution, due to the ease of design, the low operating cost and excellent removal efficiency of pollutants. Commercial activated carbon is the most popular and widely used adsorbent; however, its relatively high cost and losses during regeneration hamper its application. Accordingly, more and more attention has been paid to biosorption using biomass, such as agricultural waste (Rosales et al. 2016) and bacteria (Aryal & Liakopoulou-Kyriakides 2015).

Anaerobic granular sludge (AGS) is a complex ecological system: there are many acidifying bacteria and hydrolytic bacteria in the outer layer of the granules, while methanogens grow in the inner layer (Lim & Kim 2014). The biogas generated in the core of the granules is released to the surface and the nutrient substances need to be transferred into the core, so cavities and holes (Morgan et al. 2014) are formed as transporting channels, which result in a porous structure (Sun et al. 2011). Additionally, AGS contains many extracellular polymeric substances, with rich functional groups, such as carboxyl, amine, phosphoric and hydroxyl groups (van Hullebusch et al. 2006; Lin et al. 2012), which make AGS an effective adsorbent in the removal of heavy metals and hazardous organic pollutants, such as Pb (Guibaud et al. 2012), Co (van Hullebusch et al. 2006), methylene blue (Shi et al. 2014), malachite green (Cheng et al. 2008) and their mixture (Shi et al. 2015). In fact, AGS is more suitable for practical operation than other biosorbents such as fungus and activated sludge due to its excellent settling properties (Cheng et al. 2008). Above all, AGS is low cost, easily available and has excellent
performance for removing heavy metals. Furthermore, it is also a form of waste reuse. However, its performance in the removal of Cr (VI) has not been reported in previous studies.

Adsorption-coupled reduction and anionic adsorption are widely accepted mechanisms explaining the Cr (VI) adsorption process by biomaterials. Some researchers assumed that chromium (VI) was removed by ionic attraction between hexavalent chromium ion species and positively charged groups (Agarwal et al. 2006), while others claimed that if the contact time and protons were enough, Cr (VI) can be completely transformed into Cr (III) by biosorbents (Nakano et al. 2003; Park et al. 2004). Accordingly, it is necessary to determine which mechanism is responsible for the Cr (VI) removal by AGS.

The biosorption process is strongly affected by many factors, including the initial pH of the solution, dosage of the adsorbent, adsorbate concentration, contact time, temperature, etc. (Podstawczyk et al. 2015). In order to improve the efficiency of the biosorption process at a minimum cost and time, especially in the industrial application, it is necessary to take all variables into account. The traditional single-factor experiments cannot successfully predict the interaction effects between factors; thus, it is crucial to develop a method to study and predict the process of biosorption. Response surface methodology (RSM) is useful in designing experiments, optimizing complex processes, and predicting and evaluating the influence of related variables with minimum experimental runs. It has been used in many processes, such as food preservation, fermentation and extraction process, but has rarely been applied for adsorption.

The aim of the study was to investigate the performance of AGS as a biosorbent in the removal of hexavalent chromium from wastewater. In order to optimize the adsorption process, the effects of various parameters, such as contact time, AGS dosage, and initial pH, were studied by RSM. Kinetics and isotherm modeling were performed using various models. In addition, the mechanisms that govern hexavalent chromium removal by AGS were also investigated.

**MATERIAL AND METHODS**

**Materials**

The AGS used in this study was collected from a bench-scale expanded granular sludge bed reactor for treating brewery wastewater. The AGS was washed three times with distilled water to remove impurities, followed by drying at 65 °C for 24 h in an oven, and stored in a desiccator for the utilization in batch experiments.

All chemicals used in the study were analytically pure. A stock solution of chromium (1,000 mg/L) was prepared by dissolving a known amount of potassium dichromate (K₂Cr₂O₇, Tianjin Chemical Co., China) in distilled water and diluting it with deionized water to obtain the different working solutions at the required concentrations.

**Characterization methods**

The morphology and the chemical composition of the AGS surface were analyzed using a scanning electron microscope (SEM) instrument equipped with an energy dispersive X-ray (EDX) adapter (Nova NanoSEM 450, FEI, The Netherlands). Fourier transformed infrared (FTIR) spectroscopic (VERTEX 70, Bruker, Germany) analysis was performed over a wavelength range of 4,000–400 cm⁻¹ to identify the functional groups on the sludge. X-ray photoelectron spectroscopy (XPS) (AXIS-ULTRA DLD-600 W, Kratos, Japan) was used to determine the oxidation state of the chromium adsorbed on the biosorbent. Brunauer–Emmett–Teller (BET) analysis (BK112T-B, JWGB, China) was conducted to obtain the surface area of the AGS using N₂ adsorption, and the data collected were used to calculate the pore distributions. The Zeta potential was determined with a Zeta Potential Analyzer (Colloidal Dynamics, ZetaProbe, USA).

A density-pycnometer method was used to measure the particle density of the sludge. First, the pycnometer was calibrated with distilled water at 25 °C, then it was filled with the sample up to one fourth of its whole volume and the mass was determined using an analytical balance, followed by filling the pycnometer with distilled water to measure sand density (Freitas et al. 2017).

**Batch experimental design**

Adsorption equilibrium experiments were conducted by adding a predetermined amount of AGS (0.2–2.5 g) to 250 mL Erlenmeyer flasks containing 100 mL of Cr (VI) solution at various concentrations (5–100 mg/L) in the pH range 2 to 11. HCl (0.01 M) and NaOH (0.01 M) solutions were used to adjust the initial pH of the Cr (VI) solutions. Then the suspensions were sealed and shaken at a speed of 150 r/min, at different temperatures (25 °C, 35 °C, 45 °C), for a predetermined time. The concentrations of Cr (VI) before and after adsorption were analyzed by a standard method (APHA 1965) using a UV-visible
spectrophotometer (UV 1600 PC, MAPADA, China) at 540 nm wavelength. In order to determine the total Cr concentration in the filtrate, Cr (III) was converted to Cr (VI) using KMnO₄ (Tianjin Chemical Co., China). All measurements were conducted in triplicate.

The removal efficiency ($R$, %) of Cr (VI) from the solution was calculated from the following equation:

$$R(\%) = \frac{C_0 - C_t}{C_0} \times 100$$

(1)

The adsorption uptake ($q_t$, mg/g) of Cr (VI) in the sludge was calculated from the following equation:

$$q_t = \frac{(C_0 - C_t)}{m} V$$

(2)

where $C_0$ (mg/L) and $C_t$ (mg/L) are the Cr (VI) concentration before and after adsorption, respectively; $t$ (h) is time; $V$ (L) represents volume of the solution; $m$ (g) is the AGS dosage.

**Response surface methodology**

To reduce time and costs, the experimental design was crucial. In this study, the biosorption process of Cr (VI) by AGS was optimized with a minimum number of runs, as designed by the method of central composite design (CCD). Three of the factors (Contact time ($X_1$), AGS dosage ($X_2$) and Initial pH ($X_3$)) affecting the adsorption process were selected to model their impacts on the removal efficiency ($Y$) of Cr (VI). The predicted and experimental Cr (VI) removal efficiency and its change with coded and uncoded factor values are listed in Table 1. A second order polynomial equation (Equation (3)) was employed to fit the data using the least square method. To evaluate the precision of the model and the significance of the variables, analysis of variance (ANOVA) was performed. All calculations were conducted using MATLAB 8.3.

$$Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{3} b_{ij} X_i X_j$$

(3)

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Experimental runs of CCD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run</td>
<td>$X_1$: Contact time (min)</td>
</tr>
<tr>
<td></td>
<td>Uncoded</td>
</tr>
<tr>
<td>1</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>150</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
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<td>4</td>
<td>49</td>
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<tr>
<td>5</td>
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<tr>
<td>7</td>
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<tr>
<td>19</td>
<td>210</td>
</tr>
<tr>
<td>20</td>
<td>150</td>
</tr>
</tbody>
</table>
where $b_0$, $b_i$, $b_{ii}$, $b_{ij}$ represent constant, linear, quadratic and interaction coefficients, respectively.

RESULT AND DISCUSSION

Characterization of AGS

The AGS surface morphology before (a,b) and after (c,d) the biosorption can be observed in the SEM images presented in Figure 1. The micrograph (Figure 1(a)) showed that the sludge had a tight oval-shaped structure, which resulted in its excellent solid/liquid separation ability (a sludge volume index of 24 mL/g total suspended solids (TSS)). Cavities and holes of different sizes and shapes were observed on the surface of the granule (Figure 1(b)) as well. As shown in Figure 2(a), the predominant pore diameter was found to be within a mesopore range (Al Hinai et al. 2014). The BET surface area and average pore size were 457.3 m$^2$/g and 4.09 nm, respectively.

Some block-like structures (Figure 1(c) and 1(d)) were found on the surface of the AGS after adsorption and the density of the sludge changed from 1.286 g/mL to 1.306 g/mL, which might have been caused by the binding of chromium. This possibility was confirmed by the EDX analysis. As shown in Figure 2(c) and 2(d), the signal corresponding to Cr (before Cr (VI) biosorption) could not be observed; however, after the adsorption, the characteristic peak of Cr appeared.

As shown in Figure 2(b), the zeta potential of the sludge changed with the pH of solution. When the pH was below 2.47 (pH of zero-point charge, $\text{pH}_{zpc}$), the AGS was positively charged, indicating that negatively charged chromium species would be more easily adsorbed.

Result of batch experiments

Effect of AGS dosage

The effect of the AGS dosage on the Cr (VI) removal (initial Cr (VI) concentration of 50 mg/L at pH 4.5 for 150 min) is shown in Figure 3(a). The results indicated that Cr (VI) removal efficiency increased when the sludge dosage was
increased up to a value of 1.5 g. However, the removal efficiency was nearly constant with low sorption increment per increment of biosorbent dosage when the AGS dosage was higher than 1.5 g, due to the excess adsorption sites and fixed chromium quantity. According to this result, an AGS dosage of 1.5 g showed the best performance for the aim of this study, and was thus selected for the ensuing experiments.

**Effect of Cr (VI) concentration**

The effect of the initial Cr (VI) concentration on the biosorption capacity of AGS for chromium (AGS dosage of 1.5 g at pH 4.5 for 150 min) is presented in Figure 3(b). The amount of Cr (VI) adsorbed increased with the raising of the initial concentration of Cr (VI) in the range of 5–100 mg/L, because a higher concentration increased the number of collisions between the adsorbate and adsorbent, generating a driving force to overcome more mass transfer resistances between phases (Anupam et al. 2011).

**Effect of pH**

The pH is a prominent parameter affecting the adsorption process. As shown in Figure 3(c), when the pH was decreased from 6 to 2 (an initial Cr (VI) concentration of 50 mg/L, an AGS dosage of 1.5 g and a contact time of 150 min), the adsorption capacity of AGS for Cr (VI) increased rapidly, whereas there was no significant adsorption of chromium when the pH value was above 6. This might be due to the change of the charge of AGS by the pH of the solution (Figure 2(b)), which determines its electrostatic forces with negatively charged Cr (VI) species (Figure 3(d)), or the concentration of proton deciding the amount of Cr (VI) reduced to Cr (III) (Park et al. 2004).

**Effect of contact time**

The process of adsorption of Cr (VI) by the AGS occurred in two main stages (initial Cr (VI) concentration: 50 mg/L; AGS dosage: 1.5 g; pH: 4.5), as shown in Figure 4(a). In the initial stage, the adsorption rate was quickly increased due to the presence of enough free sorption sites and large...
transfer driving force. However after 40 min, the Cr (VI) adsorption began to slow down and moved towards equilibrium at 150 min. For practical reasons, a contact time of 150 min was selected for the ensuing experiments.

To reveal the transport mechanisms involved in the biosorption process, the experimental data were fitted using three models. The pseudo-first-order model was expressed as the following equation:

\[ \ln \left( \frac{q_e}{q_t} \right) = \ln \frac{q_e}{C_0} - k_1 t \]  

The pseudo-second-order model was defined as the following equation:

\[ \frac{1}{q_t} = \frac{1}{q_e} + k_2 t \]  

where \( q_e \) (mg/g) and \( q_t \) (mg/g) are the amount of Cr (VI) adsorbed at equilibrium and at time \( t \) (min), respectively. \( k_1 \) (min\(^{-1}\)) represents the pseudo-first-order model rate constant and \( k_2 \) (g/mg·min) represents the pseudo-second-order model rate constant. These parameters were obtained from the linear relationships based on experimental data (Figure 4(b) and 4(c)) and given in Table 2. The correlation coefficient (R\(^2\)) of the pseudo-second-order model was higher than that of the pseudo-first-order model, indicating that the second-order equation was more suitable for explaining the kinetics process.

To further analyze the rate-controlling step, intraparticle diffusion kinetic model was applied:

\[ q_t = k_i t^{1/2} \]  

where \( q_t \) (mg/g) is the amount of Cr (VI) adsorbed at time \( t \) (min), and \( k_i \) (mg/g·min\(^{0.5}\)) is the intraparticle diffusion kinetic model rate constant obtained from the slope of the straight line of \( t^{1/2} \) versus \( q_t \).

If intraparticle diffusion is rate-limited, the plot of Cr (VI) uptake \( q_t \) versus the square root of time \( t^{1/2} \) would result in a linear relationship. However, as shown in Figure 4(d), multi-linearity was observed, suggesting that more than one step took place. The first region was the instantaneous or external surface adsorption stage, followed
by the gradual adsorption stage, and the final region was the equilibrium stage (Shi et al. 2014).

### Adsorption isotherm

Langmuir (Equation (7)) and Freundlich (Equation (8)) isotherms were used for testing the equilibrium adsorption data. The results revealed that the Langmuir model gave a better fit than the Freundlich model at all temperatures (Figure 5), suggesting that AGS was not structurally homogeneous and the sorption types involved are not energetically equivalent (Wassie & Srivastava 2016). All parameters calculated are listed in Table 3. It was also found that the AGS has a higher adsorption capacity (of 13.19 mg/g) than other biosorbents (Table 4).

\[
q = \frac{q_{\text{max}} \cdot b_L \cdot C}{(1 + b_L \cdot C)}
\]  
\[q = K_F \cdot C^{1/n_F}
\]

where \(q_{\text{max}}\) (mg/g) is the maximum Cr (VI) uptake; \(b_L\) (L·mg\(^{-1}\)) and \(n_F\) are the adsorbate-biosorbent affinity; \(K_F\) (mg\(^{-1/2}\)L\(^{1/2}\)/mg/g) is the Freundlich adsorption isotherm constant, which is an indicator of adsorption capacity.

### Adsorption mechanisms

To investigate the adsorption mechanisms, XPS analysis was used to verify the oxidation state of the chromium adsorbed on the AGS. The XPS spectra of Cr 2P\(_{3/2}\) on the AGS surface after Cr (VI) adsorption are shown in Figure 6(a), where it can be observed that the chromium bound to the surface of the AGS was mostly or totally in the trivalent state, indicating that Cr (VI) was removed from the solution mainly by adsorption-coupled reduction (Park et al. 2007).
A comparison of the FTIR spectra of the sludge before and after the adsorption was performed to further determine the functional groups involved in the adsorption process (Figure 6(b)). All the functional groups in the AGS are listed in Table 5. The shift of the peak from 3,110 to 3,388 cm\(^{-1}\) verified the involvement of hydroxyl groups in chromate adsorption. When the proton concentration was enough, the hydroxyl groups could be regarded as an electron-donor (Nakano et al. 2001), by contacting with hexavalent chromium (HCrO\(_4\) is the main form (Figure 3(d)) in the acid solution); it reduced the hexavalent chromium to Cr (III) (Equation (9)). The reduction process could be the likely reason for the increase of the final pH after Cr (VI) adsorption. The shift from 1,636 to 1,645 cm\(^{-1}\), which is attributed to the complexation of Cr (III) with amino groups (Yao et al. 2016), was described by Equation (10).

\[ 7H^+ + HCrO_4^- + 3e \rightarrow 4H_2O + Cr^{3+} \]  

- NH\(_2^+ + Cr^{3+} \rightarrow -NH_2Cr^{3+} \]  

There were two possible ways (Wassie & Srivastava 2016) in which the reduction occurred: (i) Cr (VI) bound to the protonated groups (Jung et al. 2016) on AGS surface (Equations (11)–(14)) and then was reduced to trivalent chromium; (ii) Cr (VI) was directly reduced to Cr (III) in the aqueous phase.

- OH + H\(^+\) \rightarrow -OH\(^2-\)  
- OH\(^2-\) + HCrO\(_4^-\) \rightarrow -OH\(^-\) \ldots HCrO\(_4^-\)  
- NH\(_2^+ + H^+ \rightarrow -NH_3^+ \]  
- NH\(_3^+ + HCrO_4^- \rightarrow -NH_3^+ \ldots HCrO_4^- \]
Desorption experiments

The chromium loaded AGS might pose a disposal problem to the environment, as they are hazardous in nature. Such a problem may be overcome, to some extent, by desorption. Because the chromium bound to the surface of AGS was mostly or totally in its trivalent form, it is possible to convert Cr (III) to Cr (OH)$_3$ or Cr (OH)$_4$ in an alkaline medium. Accordingly, after adsorption, the adsorbents were filtered and placed into 50 mL 0.1 N NaOH solution for 2 h. The result showed that the desorption efficiency of chromium from the spent AGS was about 96.4%. Thus, it is clear that the AGS exhibited the better potential for application in the treatment of wastewater containing Cr (VI).

The amounts of Cr (VI) adsorbed on the AGS in successive adsorption-desorption cycles are shown in Figure 7. The amounts of adsorbed Cr (VI) decreased significantly from 12.9 to 9.5 mg/g in the first cycle, and then slowly decreased in the following cycles. The significant decrease in the first cycle may be due to the incomplete regeneration of the spent AGS.

### Table 5

The main absorbance bands in FTIR spectra and their assignments

<table>
<thead>
<tr>
<th>Peaks (cm$^{-1}$)</th>
<th>Functional groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,411</td>
<td>–OH and –NH stretching vibrations</td>
</tr>
<tr>
<td>2,922</td>
<td>C-H stretching vibration of alkyl structures</td>
</tr>
<tr>
<td>1,636</td>
<td>–NH bending vibration in –NH$_2$</td>
</tr>
<tr>
<td>1,545</td>
<td>C-N stretching vibration</td>
</tr>
<tr>
<td>1,450 and 1,386</td>
<td>–CH$_3$</td>
</tr>
<tr>
<td>1,076</td>
<td>C-O stretching vibration in COH</td>
</tr>
<tr>
<td>607</td>
<td>C-Cl stretching vibration</td>
</tr>
</tbody>
</table>

### Model by RSM

An optimal predictive multiple regression equation was developed by fitting the experimental data:

$$Y = 74.79 + 5.51X_1 + 9.07X_2 - 8.78X_3 - 3.79X_1^2 - 6.96X_2^2 - 1.31X_3^2 - 2.36X_1X_2 + 0.46X_1X_3 - 5.96X_2X_3$$  

### Table 6

ANOVA of the second-order polynomial equation

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>DF</th>
<th>MSS</th>
<th>F value</th>
<th>P value</th>
<th>R$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>3,756.62</td>
<td>9</td>
<td>417.4</td>
<td>10.51</td>
<td>0.001</td>
<td>0.9044</td>
</tr>
<tr>
<td>Residual</td>
<td>397.16</td>
<td>10</td>
<td>39.72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4,153.79</td>
<td>19</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
A negative or positive coefficient represents the corresponding impact of each factor on the response. According to the obtained ANOVA values (Table 6), the P value ($<0.001$) and $R^2$ ($=0.9044$) showed the high significance of the model results. Based on the sum of squares obtained from the ANOVA, the percentage of contributions (PC) for each individual term was calculated and is listed in Table 7. As the results in this table revealed, $X_2$ showed the highest level of significance with a contribution of 29.17% compared with other components. The Pareto chart (Figure 8) showed the influence of each factor investigated on the response. The vertical line in the figure represents a 95% test for significance. The results indicate that $X_2$ had a major effect on the adsorption of Cr (VI). The initial pH value was also found to be statistically significant. Among these significant variables, $X_1$ and $X_2$ had a positive effect and $X_3$, $X_2^2$, $X_2X_3$ and $X_3^2$ had a negative impact on the removal of Cr (VI).

The interaction between the AGS dosage and the initial pH is illustrated in Figure 9. It can be observed that the effect of the initial pH increased with the increase of the AGS dosage and vice versa, due to the change of the form of the functional groups on the AGS surface caused by the pH of the solution, which was responsible for the removal of Cr (VI) from the aqueous solution.

By conducting a small number of experiments designed by CCD using RSM, the main experimental conditions such as initial pH of the solution, contact time and AGS dosage were optimized and set at pH 2, contact time of 162 min and AGS dosage of 1.67 g. The predicted Cr (VI) removal efficiency under these conditions was 99.1%, while the experimental value was about 99.9%.
CONCLUSIONS

In this study, AGS was found to be a suitable adsorbent for Cr (VI) removal. The experimental isotherm data were successfully described by the Freundlich isotherm and a pseudo-second-order kinetic model was more suitable for explaining the kinetics process of adsorption. Through FTIR and XPS analyses, it was determined that the adsorption mechanism in the removal process possibly involved electrostatic adsorption, redox reaction and complexation. The obtained model based on RSM predicted a maximum Cr (VI) removal efficiency (99.1%) at pH 2, with 150 min of contact time and an AGS dosage of 1.67 g, while the experimental value was about 99.9%.

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