Study on the adsorption of bacteria in ceramsite and their synergetic effect on adsorption of heavy metals
Shan Qiu, Fang Ma, Xu Huang and Shanwen Xu

ABSTRACT

In this paper, heavy metal adsorption by ceramsite with or without Bacillus subtilis (B. subtilis) immobilization was studied, and the synergetic effect of ceramsite and bacteria was discussed in detail. To investigate the roles of the micro-pore structure of ceramsite and bacteria in removing heavy metals, the amount of bacteria immobilized on the ceramsite was determined and the effect of pH was evaluated. It was found that the immobilization of B. subtilis on the ceramsite was attributed to the electrostatic attraction and covalent bond. The scanning electron microscopy results revealed that, with the presence of ceramsite, there was the conglutination of B. subtilis cells due to the cell outer membrane dissolving. In addition, the B. subtilis immobilized ceramsite showed a different adsorption capacity for different heavy metals, with the adsorption capacity ranking of \( \text{La}^{3+} > \text{Cu}^{2+} > \text{Mg}^{2+} > \text{Na}^+ \).

Key words | adsorption, bacteria, ceramsite, metal, synergetic effect

INTRODUCTION

The large amount of excess active sludge produced in wastewater purification is an increasing concern in today’s world. If not properly disposed of, it may become a potential threat to both the environment and human health due to heavy metals, organic pollutant, and pathogens. Sewage sludge partially removes heavy metals in wastewater through sedimentation of suspended solids, metal precipitation, and metal adsorption to biomass (Qiao & Ho 1996; Cenni et al. 1998; Krebs et al. 2001; Obrador et al. 2001; Wang et al. 2001). It is thus urgent to find a better sludge disposal method to prevent secondary pollution.

Bio-ceramsite technology is one of the most effective technologies in pretreatment of drinking water. This technology can be employed to remove heavy metals and organic pollutants from water (Liu 2005; Fuliana et al. 2004; Ahlberg et al. 2006; Feng et al. 2007). The structure of porous ceramsite is complicated. It has been reported that the porous structure of sludge-ceramsite improves mass transfer efficiencies (Zou et al. 2012). Generally, adsorption is the major mechanism accounting for the removal of heavy metals and organic pollutants by bio-ceramsite. It has also been demonstrated that, with the microorganism reproduction on the ceramsite surface which promotes ceramsite aging, the organic pollutant removal is due to the combined action of adsorption and bio-degradation (Rogalla & Sibony 1992; Wu et al. 2005). The pollutant removal efficiency is directly affected by adsorption capacities of ceramsite materials, which depend on their various structural characteristics. A multiplex porous structure can be formed on the ceramsite surface after alkaline hydrothermal reaction, which could obviously increase the superficial area and absorption efficiency. Bacteria can also adsorb considerable amounts of aqueous metal cations because of charged organic-acid functional groups. Several studies have proposed that electric double-layer interaction on the bacterial surface plays an important role in proton and metal binding. Different surface electric field models have been adopted to account for these effects.

Therefore, both bacteria and ceramsite can be used to remove toxic metals from contaminated wastewater and industrial effluents. How to improve pollutant removal efficiency and material reusability are the key points of concern. So far, there are no reports about the combination of bacteria and ceramsite. It is promising to study the co-effects of bacteria and ceramsite and their potential applications in removing toxic metals from sewage sludge.

doi: 10.2166/wst.2013.710
In bio-ceramsite technology for heavy metal removal, bacteria are generally absorbed in ceramsite first, and then heavy metals are absorbed by the bacteria immobilized ceramsite. To make clear the roles of the micro-pore structure of ceramsite and bacteria in removing heavy metals, B. subtilis was chosen as the model bacterium to be immobilized on the ceramsite in this study. B. subtilis is known for its capability to absorb metal ions (Beveridge & Murray 1980; Ellen & Caroline 2011). Also, B. subtilis has evolved a set of strategies that allow survival under harsh conditions. To study the adsorption capacity of B. subtilis in wastewater purification, the amount of bacteria adsorbed in ceramsite was measured and morphology was observed by scanning electron microscopy (SEM). The synergetic effect of bacteria and ceramsite on the adsorption of heavy metals was also evaluated and discussed.

EXPERIMENTAL

Materials

Several materials were used to prepare the ceramsite. Flyash with a density of 2.5 g/cm³ was provided by Harbin third Thermal Power Co. Ltd. Straw ash with a moisture content of 11% and ash content of 13% was provided by Harbin Printing Co. Ltd. Portland cement was purchased from Harbin Huilan Cement Co. Ltd. CaSO₄ with analytical grade was purchased from Huaxin Chem. Ltd. B. subtilis was provided by the Chinese Center for Type Culture Collections. All chemicals were of analytical grade. The medium contained NaCl 5 g, peptone 5 g, beef extract 5 g, and yeast extract 6 g per 1,000 mL (pH = 7.2). The medium was sterilized in high-pressure steam at 120 °C for 30 min.

NaCl, MgCl₂, CuCl₂, and LaCl₃ were purchased from Sigma-Aldrich, MO, USA. Deionized and doubly distilled water was used in all experiments.

Preparation of ceramsite

The raw materials were mixed and pelletized. The particle sizes were about 5–8 mm. These particles were left at a temperature of about 20 ºC for 3 days and then dried at 110 ºC in a blast roaster for 24 h. The temperature increased from 20 ºC at a rate of 8 ºC/min in a muffle furnace. The samples were subsequently soaked at 600 ºC for 10 min and at 1,000 ºC for 35 min, and cooled to room temperature.

The prepared ceramsite was characterized by an X-ray diffractometer (D/max-rB, Rigaku, Japan). The SEM analyses were carried out by using a SEM (S-570, JEOL, Japan). The particle surface areas were measured by an Accelerated Surface Area and Porosimetry system (ASAP 2020, Micromeritics, USA). After the samples were degassed at 393 K, their surface areas were analyzed with N₂ at 78.025 K between 0.05 and 0.20 P⁰, and the pore areas were analyzed at 0.995 P⁰.

Method

When the B. subtilis grew to primary-log phase at 37 ºC in a peptone culture, two 4 mL B. subtilis suspensions were centrifuged at 5,000 r/min for 2 min, then the precipitates were resuspended in 4 mL ultra-pure water. Using the same method, the B. subtilis was washed five times to thoroughly remove the culture medium.

Adsorption of B. subtilis

The adsorption of B. subtilis cells on the ceramsite were evaluated at the desired pH with 1.5 g/L B. subtilis suspension. Distilled water was used as the supporting electrolyte. 0.5 g ceramsite was taken into a 100 mL bunsen beaker which contained 50 mL B. subtilis suspension, and the suspension was stirred for 10 min with a magnetic stirrer at 400 r/min. After full adsorption, the suspension was filtered with a perforated sieve to separate the ceramsite from solution. The quantity of unabsorbed B. subtilis in solution was determined by spectrophotometer and the corresponding bacteria concentration was obtained through normal bacterial calibration curve.

After adsorption, the B. subtilis immobilized on the ceramsite was collected and the samples were processed for SEM observation. Glutaraldehyde and osmium tetroxide were used to fix and dehydrate the protein (or lipid) in the bacteria cells. Double fixation by oxidation after reduction made the most of the characteristics of the two different fixatives. Then the cells were dehydrated in a series of ethanol solutions (50, 60, 70, 80, 90, 95, 100%). After being fixed and dehydrated, the cells were observed by SEM (S-4800, Hitachi, Japan).

Adsorption of metal ions

Four metal ions (La³⁺, Cu²⁺, Mg²⁺, and Na⁺) were used separately to evaluate the adsorption capacity of the ceramsite with or without B. subtilis immobilization. Each test was
performed with 400 μg/mL metal ion solution containing ceramsite samples, which was continuously shaken for 3 days at room temperature. The mixture was then centrifuged at 3,000 r/min for 4 min. The metal concentrations in the supernatant were analyzed by an Inductively Coupled Plasma-Atom Emission Spectrometer (Optima 3000, Perkin-Elmer, USA).

RESULTS AND DISCUSSION

Preparation of ceramsite

Flyash, straw ash, and cement were the raw materials for the ceramsite preparation, and CaSO₄ was used as an additive agent. With the ceramsite characterization according to Dai (2010), Table 1 and Figure 1 show the characteristics of the prepared ceramsite. These properties were beneficial to improve the mechanical strength and solidification of heavy metals.

<table>
<thead>
<tr>
<th>Item</th>
<th>Results</th>
<th>Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broken rate, C₇/‰</td>
<td>2.3</td>
<td>≤6</td>
</tr>
<tr>
<td>Mud content, Cᵢ/‰</td>
<td>0.8</td>
<td>≤1</td>
</tr>
<tr>
<td>Solution rate in hydrochloride, Cᵤᵢ/‰</td>
<td>1.2</td>
<td>≤2</td>
</tr>
<tr>
<td>Porosity, υᵢ/‰</td>
<td>52.2</td>
<td>≥40</td>
</tr>
<tr>
<td>Surface area, Sₛ/cm²/g</td>
<td>13.8 × 10⁴</td>
<td>≥0.5 × 10⁴</td>
</tr>
</tbody>
</table>

Adsorption of bacteria

In the experiment, B. subtilis grew in a sterilized medium. If a mixed culture was used, for example, activated sludge, there would be competition for nutrition among the different bacteria. B. subtilis has evolved a set of strategies that allows survival in a stress and starvation environment. Therefore, B. subtilis will be more competitive than other bacteria in a mixed culture.

The addition of electrolyte will increase the counter-ion adsorption with the Stern layer close to the surface (Liu et al. 2009). The addition of base or acid can also influence electron charge on the bacteria surface. Figure 2 shows the fraction profile of unabsorbed bacteria with the changing pH. It was discovered that, in the pH range of 6–10 under neutral conditions, less than 10% of bacteria were adsorbed on the ceramsite. The amount of absorbed bacteria decreased with the increasing pH value. When hydrochloric acid (HCl) was added into the system to lower the pH value, the amount of absorbed bacteria increased significantly. Generally, the ceramsite built on the basis of the ionization degree with SiOH groups on the surface is characterized with negative charges under neutral conditions, as is the bacteria surface. As a result, the electrostatic repulsion exists between bacteria and ceramsite surface under neutral conditions. However, some cells were still found to be adsorbed on the ceramsite in this situation, indicating that there were other forces which functioned during the adsorption process. With the increasing pH values, the addition of OH⁻ in the medium increases the concentration of negative charges on the ceramsite surface, and the electrostatic repulsion enhances at the same time. Generally, the bacteria will be killed under...
conditions of pH higher than 10 and lower than 6. However, the charge density and chemical groups on the bacteria surface would not be changed. Consequently, the result would not be affected because electrostatic attraction and covalent bonds were attributed to the adsorption of bacteria.

With the addition of HCl, the binding of H\(^+\) with OH\(^-\) groups on the ceramsite surface will reduce the negative charge density. Therefore, negative charges on the ceramsite surface decrease gradually. Excessive H\(^+\) binding with OH\(^-\) groups or absorbing on the ceramsite surface could even make negative charges become positive ones. However, the negative charges on the bacteria surface are difficult to alter. Lipopolysaccharide (LPS), phospholipid bilayers, and protein formed on the outer layer, serving as buffer, can protect bacteria from damage due to the pH change. Therefore, with the decrease of pH, the electrostatic repulsion between the ceramsite surface and bacteria weakens and the attractive force enhances, resulting in the increase of the amount of absorbed bacteria.

To analyze the binding effect between bacteria and ceramsite, the surface groups of *B. subtilis* were measured. The attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) spectra of the native *B. subtilis* are shown in Figure 3. According to Figure 3, the sharpest and most prominent peaks at \(\sim 3,290, 1,639, 1,538 \text{ cm}^{-1}\) could be indexed as those of amide groups (Kiwi & Nadtochenko 2005; Nadtochenko et al. 2005, 2006). As we know, the envelope of *B. subtilis* consists of LPS, phospholipid bilayer, and protein. The protein is attached to the membrane. The outer layer of the phospholipid bilayers is hydrophilic and the intermediate part is hydrophobic. The amide groups showed the most significant peaks because they are the outermost layer. There were also prominent peaks at \(\sim 2,923\) and \(1,029 \text{ cm}^{-1}\), which were attributed to \(v_\text{a} (\text{CH}_2)\), phosphoric acid asymmetric vibration, and the \(-\text{C}-\text{O}-\text{C}-\) group in oligosaccharide which was bonded with protein to form glycoprotein, respectively. Small peaks at \(\sim 1,392\) and \(1,230 \text{ cm}^{-1}\) could be indexed as \(-\text{COO}^-\) in the hydrophobic glycerol end and symmetric vibration of \(\text{PO}_2\), respectively. After the bacteria were absorbed, there were changes of the spectral profile of *B. subtilis*. The amide peaks at \(1,639\) and \(1,538 \text{ cm}^{-1}\) weakened, indicating the bond formation of amide groups with the hydrophobic end of the phospholipids. The peak of symmetric vibration of \(\text{PO}_2\) at \(1,230 \text{ cm}^{-1}\) also weakened, suggesting that the hydrophobic end took part in the formation of bond.

To analyze the mechanism of *B. subtilis* immobilization on the ceramsite, *B. subtilis* cells were observed by SEM. Figures 4(a) and 4(b) show the native cells and cells growing on the ceramsite, respectively. The images revealed that the native cells grew separately, while cells in the presence of ceramsite conglomerated. As for the conglomeration of *B. subtilis* cells, the outer membrane of cells was found to dissolve to some extent. The outer membrane is the main
component of the cell wall. Because the surface groups of *B. subtilis* were damaged, the outer membrane of cells could not serve as the protective envelope any more. It was thus damaged, resulting in the conglutination of the *B. subtilis* cells.

**Synergetic effect on heavy metal adsorption**

As shown in Figure 5, compared to the abiotic solutions, the presence of bacteria improved the efficiency of metal removal up to 95%. The metal removal rate is rapid in both the abiotic and bacteria-bearing systems, especially in the first few minutes. The aqueous metal concentrations decreased in the first 5 h, and then slowly increased in the next 24–30 h.

Na\(^+\), Mg\(^{2+}\), Cu\(^{2+}\), and La\(^{3+}\) were chosen as the model metal ions to be absorbed on bacteria or ceramsite. They represent the metals in the first main group (Na\(^+\)), second main group (Mg\(^{2+}\)), the metals in a subgroup (Cu\(^{2+}\)), and inner transition elements (La\(^{3+}\)). The Cu atom has an incomplete d sub-shell, giving rise to cations with an incomplete d sub-shell. The La atom is characterized by an incomplete f sub-shell, and is one of the ‘inner transition metals’. Electrostatic attraction played an important role in metal ion adsorption. The metal ions with different electric charges were studied in detail to evaluate the effect of electric charge on adsorption.

Regarding the removal of Na\(^+\), Mg\(^{2+}\), Cu\(^{2+}\), and La\(^{3+}\), the metal adsorption amount under the same conditions was ranked as La\(^{3+}\) > Cu\(^{2+}\) > Mg\(^{2+}\) > Na\(^+\). It can be deduced from these results that the electrostatic attraction is the main reason for the heavy metal adsorption. As a result, the trivalent La\(^{3+}\) is easily adsorbed on the surface of ceramsite and bacteria, and Na\(^+\) is easily desorbed from the solid surface.

The metal ions could be adsorbed by both *B. subtilis* and ceramsite. As shown in Figure 5, the adsorption capacity of ceramsite was superior to that of *B. subtilis*, because the ceramsite has a larger surface area than *B. subtilis*. There existed adsorption competition between bacteria and ceramsite with the addition of metal ions. The ceramsite could also absorb bacteria, so that the force field of bacteria decreased. The metal ions were generally adsorbed by ceramsite prior to bacteria. They were absorbed on the bacteria until the metal ion adsorption was saturated on the ceramsite.

![Figure 5](https://iwaponline.com/wst/article-pdf/69/2/407/471890/407.pdf) | The dynamic process of adsorption in ceramsite for Na\(^+\), Mg\(^{2+}\), Cu\(^{2+}\), and La\(^{3+}\).
It should be noted that the electrostatic attraction is not a unique force between metal ions and solid surfaces. The covalent bond plays an important role in the process of adsorption. The covalent bond derives from the affinity of metal ions for the N, O, C terminals, especially –SH, terminals on the surface of bacteria and –OH on the ceramsite surface (Kiwi & Nadtochenko 2005; Nadtochenko et al. 2005, 2006). Compared with Mg$^{2+}$ and Na$^+$, Cu$^{2+}$ and La$^{3+}$ cations have valence electrons in d-orbitals and f-orbitals, which make it easier to bind with non-metallic elements. In the chemical interaction occurring between bacteria and metal ions, it is easier for Cu$^{2+}$ and La$^{3+}$ to form a coordination compound than Mg$^{2+}$ and Na$^+$, whose lone pair electrons are offered by O, N, P atoms on a bacterial surface.

**CONCLUSIONS**

In this study, B. subtilis and ceramsite were chosen to study the role of the micro-pore structure of ceramsite and bacteria in removing heavy metals with bio-ceramsite technology. For the pH values of medium influence on the surface charge of bacteria, it was difficult for B. subtilis to be adsorbed on the ceramsite under neutral conditions, while it could be considerably improved under the conditions pH < 6.

There existed conglomeration of B. subtilis cells in the presence of ceramsite due to the dissolving of the outer membrane of cells; and the surface groups of B. subtilis, such as amide and phospholipid, were affected by the ceramsite. The metal ions could be adsorbed by both B. subtilis and ceramsite, and the adsorption capability capacity of ceramsite was superior to that of B. subtilis. With regard to the removal of Na$^+$, Mg$^{2+}$, Cu$^{2+}$, and La$^{3+}$, the adsorption capacity for different metals was ranked in order as La$^{3+}$ > Cu$^{2+}$ > Mg$^{2+}$ > Na$^+$. The combination of bacteria and ceramsite was proven to be more efficient in removing metal ions than B. subtilis or ceramsite alone. This study is significant for the application of a bio-ceramsite technique in removing toxic metals. The reusability of the combination of bacteria and ceramsite should be further studied.

**ACKNOWLEDGEMENTS**

We gratefully acknowledge the financial support of the Open Project of the State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology (No. QA201206) and the Major Special Technological Programmes Concerning Water Pollution Control and Management (Grant No. 2012ZX07201002-3).

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First received 16 April 2013; accepted in revised form 10 October 2013. Available online 7 November 2013.