Removal of organic matter and phosphate using ferrihydrite for reduction of microbial regrowth potential
Y. Yang, S. Takizawa, H. Sakai, M. Murakami and N. Watanabe

ABSTRACT
Dissolved organic matter (DOM) and phosphate in reclaimed water promote microbial growth in distribution systems and deteriorate water quality. In this study, we tested ferrihydrite (Fh) for its potential to remove both DOM and phosphate in order to control bacterial regrowth. Adsorption kinetics on Fh revealed that phosphate has a higher affinity with Fh than has DOM. The removal efficiency of DOM increased at lower pH. Fourier transform infrared spectroscopy (FT-IR) spectra of freeze-dried Fh showed that adsorbed DOM was rich in carboxyl/hydroxyl functional groups, indicating anion exchange at Fh surfaces to be a major mechanism, especially at low pH. Fh preferentially removed DOM greater than 1,000 Da. Specific ultraviolet absorption (SUVA) at 254 nm and DOC results suggest Fh adsorption removes more hydrophilic DOM than the coagulation–sand filtration process. Reduction of bacterial regrowth potential (BRP) by Fh was comparable to that of the coagulation–sand filtration process, which indicated that phosphorus was not the rate-limiting factor of microbial growth.

Key words | adsorption mechanism, bacterial regrowth potential (BRP) method, Fourier transform infrared spectroscopy (FT-IR), size exclusion chromatography (SEC)

INTRODUCTION
Wastewater reclamation and reuse are key processes for resolving worldwide water shortages. Microbial regrowth in reclaimed wastewater limits water reuse by biofilm formation in pipes and deteriorating water quality during storage and conveyance. Bacterial regrowth potential (BRP) is elevated by higher concentration of dissolved organic matter (DOM) and the nutrients in reclaimed water. Phosphorus is one of the essential inorganic elements for microbial growth. It is present in nucleic acids, phospholipids, lipopolysaccharides, and various metabolites present in the cytoplasm (Torriani et al. 1987); hence, phosphorous can be a rate-limiting element for microbial growth. Sathasivan & Ohgaki (1998) and Vrouwenvelder et al. (2010) reported that low phosphate concentrations can restrict microbial growth even at high DOM concentrations. Therefore, removing both phosphate and DOM is an effective way to reduce BRP.

Ferrihydrite (Fh) is iron-oxide particles (IOPs) that occur naturally in soils, sediments, and aquatic systems. Fh shows a large adsorption capacity for the phosphate, arsenate and DOM in aquatic systems as it has an extremely high surface area and reactivity. Among various microbial nutrients, phosphate and DOM have relatively strong affinity with Fh. Antelo et al. (2010) reported that 96% of phosphorus in groundwater was adsorbed on Fh. Kang & Choo (2010) also reported that a maximum dissolved organic carbon (DOC) removal rate of 50% by Fh adsorption was achieved using lake water. Choo & Kang (2003) have found that Fh is the most effective adsorbent among IOPs for the removal of DOM from secondary effluents. Uranyl binds preferentially on the edges of Fe octahedra of Fh with a bidentate complex, while phosphate forms double-corner bidentate complexes (Hiemstra et al. 2009). Although Fh has a high potential to remove both DOM and phosphate, few researchers have studied simultaneous adsorption of DOM and phosphate. This study aims to evaluate Fh as an adsorbent to remove both phosphate and DOM for controlling bacterial regrowth in simulated reclaimed water.

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MATERIALS AND METHODS

Preparation and characterization of ferrihydrite (Fh)

The two-line Fh was synthesized as an adsorbent following the method of Patrizia et al. (2001). FeCl₃·6H₂O (5.4 g) was dissolved in 100 mL Milli-Q water to prepare 0.2 M Fe(III) solution. Approximately 60 mL of 1 M NaOH was added dropwise into the Fe solution to bring pH to 7–8 under vigorous stirring with a magnetic stirrer. To remove sodium chloride, Fh solution was dialyzed for four days until water conductivity was less than 3 μS cm⁻¹. Synthesized Fh was kept as a slurry suspension at 4°C in the dark for later use as an adsorbent. A part of Fh was freeze-dried and stored in a desiccator for determination of surface area, pore diameter and volume by Belsorp mini-II (Bel Japan Inc.) as shown in Table 1. According to the IUPAC classification the synthesized Fh showed mostly small mesopores, and can adsorb high molecular weight (MW) molecules.

Bacterial regrowth potential (BRP) method

A BRP method reported by Sathasivan & Ohgaki (1998) was adapted after a minor modification. The indigenous bacteria in raw water were used as an inoculum for the BRP experiments. Water samples were sterilized by filtering through a 0.2 μm (GTTP, Millipore, USA) membrane, and 19 mL of samples were mixed with 1 mL inocula. After incubating for five days at 20°C, bacterial cell numbers were counted by the spread-plate colony counting method, and the growth was calculated by subtracting the numbers of bacteria in the inocula.

Analytical methods

DOC and phosphate concentrations were measured using a TOC-5000A (Shimadzu Corp., Japan) analyzer and a HACH DR/890 colorimeter, respectively. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR/FT-IR) 610 (Jasco International, Co. Ltd, Japan) was used to analyze the surface functional groups on freeze-dried Fh before and after adsorption of DOM. MW distribution of DOM was analyzed by high performance liquid chromatography (HPLC) (LC-10ADvp, Shimadzu Corp., Kyoto, Japan) with an online diode array detector using a Protein Pak60 column (Waters Corp., USA), packed with 10 μm diol-bonded silica beads. Size exclusion chromatography (SEC) grade polystyrene sulfonate (PSS) salt standards were used for MW calibration with the UVA detector. Before the analysis, pH of all the samples was adjusted to 6.8 by adding NaOH or HCl.

Adsorption of DOM and phosphate

A water sample from a river in Tokyo that receives wastewater effluents was used for the adsorption experiment after filtering through 0.45 μm PTFE membrane (JH, Millipore, USA). Sample pH was varied by adding HCl or NaOH before adsorption experiments. Stock Fh slurry was mixed with 100 mL water samples to make the Fh concentration of 50 mg-Fe/L, and adsorption experiments were conducted at 25°C while shaking at 100 rpm. Samples were taken at different reaction times and filtered through 0.45 μm PTFE filter (Dismic 45HP, Advantec, Japan) to remove Fh particles. Each adsorption experiment was repeated three times.

Adsorption kinetics

Adsorption kinetics were analyzed using the pseudo-second-order kinetic equation, which has been most widely used to describe time evolution of adsorption under non-equilibrium conditions (Ho et al. 2000):

\[
\frac{dq_t}{dt} = k_2(q_e - q_t)^2
\]

\[
\frac{1}{q_e - q_t} = \frac{1}{q_e} + k_2t
\]

\[
t = \frac{1}{k_2q_e} \frac{1}{q_e(t)}
\]

Table 1 Characteristics of prepared ferrihydrite (Fh)

<table>
<thead>
<tr>
<th>Property</th>
<th>Surface area (m²/g)</th>
<th>Pore volume (cm³/g)</th>
<th>Pore diameter (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrihydrite</td>
<td>278.1</td>
<td>0.1478</td>
<td>0.1294</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BET equation</td>
<td>BJH equation</td>
</tr>
<tr>
<td></td>
<td>2.13</td>
<td>2.58</td>
<td></td>
</tr>
</tbody>
</table>

where \( q_e \) and \( q_t \) are the adsorption capacity at equilibrium and at time \( t \), respectively (mg/g). \( k_2 \) is the rate constant of pseudo-second-order adsorption (g/(mg·min)). The initial adsorption rate, \( h_0 \) (mg/(g·min)), is given by taking \( q_t = 0 \) as:

\[
h_0 = k_2 q_e^2
\]

hence

\[
\frac{t}{q_t} = \frac{1}{h_0} + \frac{1}{q_e h_0 t}
\]  

(5)

By plotting \( t \) vs. \( t/q_t \), \( h_0 \) and \( q_e \) can be estimated.

RESULTS AND DISCUSSION

Fh adsorption effect with time and pH

Adsorption of both DOC and phosphate was fast and reached equilibrium in less than 5 min (Figure 1(a)). The values of \( q_e \), \( h_0 \), \( k_2 \) and \( R^2 \) of the pseudo-second-order model for Fh adsorption are shown in Table 2. The \( k_2 \) value of phosphate adsorption was higher than that of DOC indicating a higher adsorption rate of phosphate than that of DOC. Phosphate forms a very strong surface complex with hydroxyl groups (Gu et al. 1995) and has a higher affinity with Fh than has DOC.

The equilibrium adsorption, shown as \( q_e \), of DOC was higher than that of phosphate partly because the DOC concentration was higher than the phosphate concentration in the river water. Figure 1(b) shows reduction of UV absorbance at 254 nm (UV\(_{254}\)), which was higher than the removal of DOC. DOC represents the total amounts of DOM, while UV\(_{254}\) indicates UV-absorbing DOM including hydrophobic DOM such as aromatic DOM. The results shown in Figure 1 suggested that hydrophobic DOM was more preferentially removed by Fh than hydrophilic DOM. DOM forms complexes with mineral surfaces by ligand exchange (Gu et al. 1994). Kaiser & Guggenberger (2003) also reported that anion exchange took place between singly coordinated OH groups on mineral surfaces with carboxyl groups (COOH) and phenolic OH groups of the DOM to form strong organics–mineral associations, such as Fe–O–C bond. Anions possess one or more atoms with a lone pair of electrons and thus can act as an electron donor in a coordinate bond (Cornell & Schwertmann 2003).

Figure 2 shows DOC and specific ultraviolet absorbance (SUVA) after adsorption on Fh between pH 3.9 and pH 8.9.
The initial DOC and SUVA before Fh adsorption were 1.75 mg/L and 2.44 L/mg-m, respectively. At a Fh dose of 50 mg-Fe/L and a reaction time of 20 min, DOC and SUVA increased as pH increased from 3.9 to 8.9, which indicated that DOC and SUVA adsorption on Fh decreased. These results indicate that more hydrophobic organic compounds were adsorbed on Fh at acidic pH. When pH is lowered, the surface of Fh becomes more positively charged by protonation; and at further lower pH, DOM becomes less negatively charged. Hence, electrostatic interaction between Fh surface and DOM is increased when pH was lowered from 9 to 6. DOC reached a plateau below pH 6 because positive charge of Fh was offset by decreasing negative charge of DOM. Such pH-dependent adsorption of DOM by Fh was attributed to the surface complexation between negatively charged organic compounds and positively charged iron oxide surfaces (Arai & Sparks 2001).

On the other hand, hydrophobic interactions become more dominant at pH lower than 6 due to the protonation of hydroxyl and carboxyl groups of DOM and the suppressed ionization of the carboxyl groups (Kaiser & Guggenberger 2006).

The surface functional groups of Fh were also examined by ATR/FT-IR as shown in Figure 3. IR spectra of Fh were compared before and after DOM adsorption experiments at pH 6 and pH 7.7. The presence of the following functional groups is reported in the literature: 850 cm⁻¹ HPO₄²⁻ (Arai & Sparks 2001), 1,110 cm⁻¹ (C–O stretching of carbohydrates), 1,400 cm⁻¹ (aromatic rings stretching vibrations; C–H in-plane deformations; COO– stretching vibrations), 1,450 cm⁻¹ (C–H deformation of C–H₃ and C–CH₂), 1,620 cm⁻¹ (aromatic C=C and conjugated carbonyl), 1,640 cm⁻¹ (C=O stretching of quinines and conjugated ketones), and 3,000–3,600 cm⁻¹ (hydrogen-bonded –OH) (Kovac et al. 2002). Figure 3 shows that virgin Fh contains hydrogen bonded –OH. As reported in the previous studies, the structure of two-line Fh is α-FeOOH, and the surface functional groups are Fe-OH/O (Manceau & Gates; Zeng et al. 2008). Fh spectra after adsorption experiments showed adsorption of aromatic C=C and conjugated carbonyl (1,620 cm⁻¹) at pH 6, which is in agreement with a high removal rate of hydrophobic organics as shown by reduction of SUVA at lower pH (Figure 2). The complexation between –COOH of DOM and Fh surfaces was further evidenced by a strong band at 1,400 cm⁻¹, which was because of symmetrical stretching of carboxyl functional groups complexed with iron on the Fh surface (Gu et al. 1995). The relatively increased absorbance at 1,100 and 1,640 cm⁻¹ after DOM adsorption on Fh indicates that DOM adsorbed on Fh contained –COOH and –OH functional groups, which may also have interacted with Fh in the adsorption process. (Gu et al. 1994; Lutzow et al. 2006).

Comparison of removal efficiency with alternative treatment methods

The removal efficiencies of DOC, phosphate and BRP were compared with those of a water treatment plant, which takes water from the same river that was sampled for Fh adsorption experiments. Water samples were taken after coagulation–sedimentation–sand filtration, ozonation and biological activated carbon (BAC) processes. Table 3

![Figure 3](https://iwaponline.com/wst/article-pdf/66/6/1348/443372/1348.pdf)

**Figure 3** | FTIR spectra of Fh before and after adsorption.

**Table 3** | Water quality and operational conditions of different treatment processes in comparison with Fh adsorption

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Operational conditions</th>
<th>DOC (mg/L)</th>
<th>UV₂₅₄ (cm⁻¹)</th>
<th>SUVA (L/mg-m)</th>
<th>Phosphate (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw water</td>
<td>–</td>
<td>1.75</td>
<td>0.043</td>
<td>2.44</td>
<td>0.27</td>
</tr>
<tr>
<td>Coagulation–sand filtration</td>
<td>Coagulant: Poly-aluminium chloride; Dose: 22.9–25.1 mg/L</td>
<td>1.38</td>
<td>0.025</td>
<td>1.78</td>
<td>0.12</td>
</tr>
<tr>
<td>Ozonation</td>
<td>Contact time: 12 min; Dose: 0.075 mg/L</td>
<td>1.32</td>
<td>0.013</td>
<td>0.98</td>
<td>0.13</td>
</tr>
<tr>
<td>BAC</td>
<td>Filtration rate: 250 m/d; Filter bed height: 2.5 m</td>
<td>1.02</td>
<td>0.015</td>
<td>1.47</td>
<td>0.08</td>
</tr>
<tr>
<td>Fh</td>
<td>Dose: 50 mg/L; reaction time: 20 min; pH = 7.7</td>
<td>1.13</td>
<td>0.021</td>
<td>1.85</td>
<td>0.07</td>
</tr>
</tbody>
</table>
shows operational conditions, DOC, UV_{254}, SUVA and phosphate at each treatment process. The removal rates of DOC and phosphate by Fh adsorption were 35.4 and 73.3%, respectively. Both DOC and UV_{254} after Fh adsorption were lower than those after coagulation–sand filtration, but SUVA was higher after Fh adsorption than coagulation–sand filtration, which indicated that Fh preferentially removed hydrophilic organic matter that did not absorb UV_{254}. Ozonation was more effective than Fh adsorption in reduction of UV_{254} and SUVA, which indicated that ozonation decomposed aromatic organic matter and produced more hydrophilic smaller molecules. Fh had the highest removal of phosphate, which indicated higher affinity of phosphate with Fh than poly-aluminium chloride.

Differences in MW distribution were also observed between Fh adsorption and other water treatment processes (Figure 4). DOM in raw water ranged between 700 and 3,000 Da. Fh mainly removed DOM greater than 1,000 Da; and DOM greater than 2,000 Da was most efficiently removed. However, removal of DOM smaller than 1,000 Da was not very efficient by Fh adsorption. Ozonation and BAC showed a much higher removal of DOM smaller than 1,000 Da. These results may be attributed to the mesopores of Fh, which is more suitable than activated carbon for absorbing larger DOM.

BRP in the raw water decreased significantly by the coagulation–sand filtration process, but increased by ozonation due to disintegration of hydrophobic organic matter and production of hydrophilic organic matter (Figure 5). BRP reduction by Fh was only slightly better than the coagulation–sand filtration process. This suggests that phosphorus was not the limiting factor of microbial growth in the water samples used in this study. Fh adsorption may be useful as an alternative to coagulation–sand filtration, but further improvement of DOM and phosphorus removal by
Fh needs to be explored for better control of bacterial regrowth in reclaimed waters.

CONCLUSIONS

Both DOM and phosphate were rapidly adsorbed on Fh in batch experiments. Analysis of kinetic adsorption data using the pseudo-second-order adsorption model revealed that phosphate had a higher affinity with Fh than DOM. DOM rich in carboxyl/hydroxyl functional groups was preferentially adsorbed on Fh through the anion-exchange and ligand-exchange mechanisms, especially at low pH. Both hydrophobic and hydrophilic DOM were removed by Fh. Fh preferentially adsorbed DOM greater than 1,000 Da, possibly because of its mesoporous structure. More hydrophilic organic matter was adsorbed on Fh than in the coagulation–sand filtration process, which was indicated by the results that DOC after Fh adsorption was lower than that after coagulation–sand filtration, but SUVA was higher after Fh adsorption than after coagulation–sand filtration. BRP reduction by Fh was comparable to the coagulation–sand filtration process, which suggested that phosphorus was not a rate-limiting factor of BRP in the water sample used in this study.

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