Removal of Phosphate Ions by PGAF (Poly-γ-Glutamic Acid and Flocculants)

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ABSTRACT
Poly-γ-glutamic acid and flocculants (PGAF) were prepared for the removal of phosphate ions from aqueous solution. Scanning electron microscopy images of PGAF were obtained, and the amounts of calcium and oxygen on the PGAF surface were confirmed by electron probe microanalysis. The removal percentage of phosphate ions by PGAF was 98.2% within 10 min. Hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂) and calcite (calcium carbonate) were produced by adding PGAF to a phosphate ion solution. These products were collected using poly-γ-glutamic acid and precipitated in aqueous solution. The results suggest that the removal rate of phosphate ions is very fast, and solid/liquid separation is achieved very easily using PGAF. Moreover, the removal of phosphate ions by PGAF increased as the amount of adsorbent was increased. The optimal agitation speed for the removal of phosphate ions was 60 – 120 rpm, whereas the temperature and solution pH did not affect the removal of phosphate ions. Therefore, PGAF may be useful for the removal of phosphate ions from aqueous solution.

Keywords: calcium hydroxide, PGAF, phosphate, poly-γ-glutamic acid

INTRODUCTION
Phosphorus is an important nutrient for agricultural applications. Chemical fertilizers containing phosphate can considerably increase food production. However, dissolved phosphates from fertilizers engender eutrophication, and has a strong effect on ecological systems in aquatic environments (Yagi and Fukushi, 2012; Filippelli, 2008). Eutrophication can result in fish death and habitat degradation with the loss of plant and animal species (Hakanson et al., 2007). Moreover, increased phosphate concentrations lead to bloom of phytoplankton and degradation of water quality (Das et al., 2006). Therefore, there is currently an urgent demand for phosphate removal methods.

Several techniques such as chemical precipitation (Donnert and Salecker, 1999), biological processes (De-Bashan and Bashan, 2004), adsorption (Kang et al., 2003), ion exchange (Blaney et al., 2007), membrane technologies (van Voorthuizen et al., 2005), and constructed wetlands (Kurniadie and Kunze, 2000), have been used to remove phosphate ions from aqueous solution. Of these available approaches, the adsorption method is believed to be one of the most economical, effective, and reliable. This method allows the use of nutrient-loaded filters as phosphate fertilizers and soil conditioners (Hylander et al., 2006).

Poly-γ-glutamic acid is an anionic polypeptide synthesized by the Bacillus species through a fermentation process, and is composed of numerous repetitive glutamic acid units connected by an unusual γ-amide bond, leaving the side-chain α-carboxyl groups free for conjugation with a wide variety of compounds...
(-[-NH-CH(COOH)-(CH_2)_2-CO-]_n-) (Inbaraj et al., 2008). In several previous studies, poly-γ-glutamic acid (γ-PGA) has shown to be an effective adsorbent for the removal of various dyes and metals (Inbaraj and Chen, 2011; Wang et al., 2010; Inbaraj et al., 2009; Siao et al., 2009). However, its application as an adsorbent for the removal of phosphate ions remains to be explored. Therefore, the objective of this study is to evaluate its phosphate removal capacity in a batch-mode adsorption process.

MATERIALS AND METHODS

Materials
Poly-γ-glutamic acid and flocculants (PGAF) were purchased from Nippon Poly-Glu Co., Ltd., Japan (Fig. 1). It is seen that the surface was quite rugged. The particle size and the specific surface area of PGAF is < 840 μm and 2.8 m²/g, respectively. The storage stability and durability of PGAF is more than three years. Poly-γ-glutamic acid (MW: 200 – 500 or 1,500 – 2,500 kDa) and calcium hydroxide were purchased from Wako Pure Chem. Ind. Ltd., Japan. Scanning electron microscopy (SEM) was carried out with a JSM-5500LV instrument (JEOL, Japan). X-ray diffraction analysis was performed using a RINT 2100V diffractometer (Rigaku, Japan). The amounts of calcium, oxygen, and phosphorus on PGAF were measured with an electron probe micro analyzer (EPMA, JXA-8530F, JEOL, Japan), with an accelerating voltage of 45.0 kV and beam diameter of 5 μm.

Effect of agitation on the removal of phosphate ions
Adsorbent (PGAF, 0.5 g) was added to phosphate solution (300 mL; 100 mg/L). The initial pH was approximately 5.0. The suspension was agitated at 30 – 600 rpm for 15 min under ambient conditions. Subsequently, the solution was filtered with a 0.45-μm membrane filter (GC-50, Toyo Roshi Kaisha, Ltd., Japan), and the concentration of phosphate ions was measured with a DR/890 colorimeter (HACH, USA).

The amount of phosphate removed was calculated from equation (1):

\[ R = \frac{C_e}{C_0} \times 100 \]  

where \( R \) (%) is the residual percentage, \( C_0 \) (mg/L) is the initial concentration, and \( C_e \) (mg/L) is the equilibrium concentration.
Effect of contact time on the removal of phosphate ions
Each adsorbent (PGAF, γ-PGA, and calcium hydroxide; 0.5 g) was added to phosphate solution (300 mL; 100 mg/L). The initial pH was approximately 5.0. The suspensions were agitated at 60 rpm for 0.5 – 15 min under ambient conditions. The amount of phosphate removed was calculated from equation (1).

Effect of dosage on the removal of phosphate ions
Adsorbent (PGAF, 0.1 – 0.5 g) was added to phosphate solution (300 mL; 100 mg/L). The initial pH was approximately 5.0. The suspension was agitated at 60 rpm for 15 min under ambient conditions. The amount of phosphate removed was calculated from equation (1).

Effect of temperature on the removal of phosphate ions
The experiment was conducted in a batch mode. Adsorbent (PGAF, 0.1 g) was added to phosphate solution (50 mL) with a concentration of 100 – 1,000 mg/L. The initial pH was approximately 4.6 – 5.1. The suspension was agitated at 100 rpm for 1 h at 5 – 45°C. The amount of phosphate removed was calculated from equation (1).

Effect of pH on the removal of phosphate ions
The experiment was conducted in a batch mode. Calcium hydroxide (0.1 g) or PGAF was added to phosphate solution (50 mL; 100 mg/L). The initial pH was 3 – 12, which was adjusted with hydrochloric acid or sodium hydroxide solutions. The suspensions were shaken at 100 rpm for 24 h at 25°C, and then filtered with a 0.45-µm membrane filter. The amount of phosphate removed was calculated from equation (1). The solution pH after phosphate removal was measured with a digital pH meter (Mettler-Toledo International Inc., Japan).

RESULTS AND DISCUSSION
Figure 2 shows the effect of agitation on the removal of phosphate ions by PGAF; the removal rate increased with increasing agitation speed. The removal percentage of phosphate ions at 600 rpm was 99.0% within 15 s. However, a dispersion of flocculant production was observed with increasing agitation speed. On the other hand, the sample solution was not uniformly agitated at 30 or 45 rpm. The removal of phosphate ions was slower, and the residual percentage of phosphate ions was still 14.3% and 7.4% for 30 and 45 rpm, respectively after 5 h. It was thus elucidated that agitation speed is an important factor for phosphate removal from aqueous solution, with the optimal agitation speed found to be 60 – 120 rpm. Therefore, the experimental condition (agitation is 60 rpm) was chosen and used for the following adsorption experiments of phosphate ions.
Figure 3 shows the residual percentage of phosphate ions according to the adsorbents used. The two kinds of poly-γ-glutamic acid shown in Fig. 3 did not show any removal capacity for phosphate ions from aqueous solution, and the amount of phosphate removed onto the poly-γ-glutamic acid was not dependent on its molecular weight. The removal percentage of phosphate ions by PGAF was 98.2% after 10 min, and that by calcium hydroxide was 99.4% after 2 min. In a previous study, we confirmed that the adsorption of phosphate ions reached equilibrium quickly at about 40 – 90 min. The removal rate of phosphate ions by PGAF was higher than those found for other adsorbents (Yang et al., 2013; Yang et al., 2014). Moreover, different flocculants (calcium sulfate, aluminum sulfate, and aluminum hydroxide) did not show any removal capacity for phosphate ions from aqueous solution (data not shown). These results indicate that the removal capability of phosphate ions by PGAF can be attributed to the calcium hydroxide and not to poly-γ-glutamic acid. We confirmed that calcium and oxygen were present on PGAF before the adsorption of phosphate ions (Fig. 4). Thus, we elucidated that calcium hydroxide is one of the flocculant components of PGAF.
Figure 5 shows the XRD patterns of PGAF and Ca(OH)₂ before and after the removal of phosphate ions. It can be seen that hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂) and calcite (calcium carbonate) were produced from both PGAF and Ca(OH)₂ (Miyoshi et al., 2011). Hydroxyapatite is produced according to equation (2):

\[
10\text{Ca}^{2+} + 2\text{OH}^- + 6\text{PO}_4^{3-} \rightarrow \text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6
\]

The reaction between phosphate ions and calcium hydroxide was affected by the solution pH. A previous study showed that calcium hydrogen phosphate, calcium hydrogen phosphate mixed with hydroxyapatite, and hydroxyapatite alone were produced at pH 4.0 – 6.0, pH 6.0 – 7.0, and above pH 7.0, respectively (Kato and Matsuoka, 1991). Moreover, calcium carbonate was produced from the reaction between calcium hydroxide and carbon dioxide in air (Ca(OH)₂ + CO₂ → CaCO₃ +...
H\textsubscript{2}O). These results indicate that phosphorus was removed from the aqueous solution, and hydroxyapatite and calcite were produced on the PGAF surface.

Figures 6 and 7 show the concentration of phosphate ions on the PGAF surface and SEM images of PGAF, respectively, before and after the removal of phosphate ions from the aqueous solution. We confirmed that phosphorus was present on the PGAF surface after adsorption (Fig. 6). Moreover, we could observe the crystallite produced on the PGAF surface (Fig. 7).

Fig. 5 - XRD patterns of PGAF and Ca(OH)\textsubscript{2} before and after the removal of phosphate ion.

Fig. 6 - Amount of phosphate ion onto PGAF surface.
Figure 8 shows the sample solution after the removal of phosphate ions by PGAF and Ca(OH)$_2$. Precipitation was observed within 1 min after stopping the agitation after PGAF addition. On the other hand, precipitation was not observed within 24 h after stopping the agitation after the addition of calcium hydroxide. The solution became cloudy using calcium hydroxide. These results suggest that the removal of phosphate ions is very fast, and solid/liquid separation is very easy using this treatment compared with that using other adsorbents. Poly-$\gamma$-glutamic acid is an extracellular biopolymer and a by-product produced by common soil microbes such as Bacillus subtilis and Bacillus licheniformis through fermentation. It is an anionic polypeptide composed of many D- and/or L-glutamic acid units, which are linked through $\gamma$-amide linkages between $\alpha$-amino and $\gamma$-carboxylic acid functional groups ([-NH-CH(COOH)-(CH$_2$)$_2$-CO-])$^\gamma$ (Inbaraj et al., 2009). Flocculant products (hydroxyapatite and calcite) were produced upon the addition of PGAF to the phosphate solution. These products were collected by poly-$\gamma$-glutamic acid, and precipitated in aqueous solution.

The effect of the dosage on the removal of phosphate ions by PGAF is shown in Fig. 9. The residual percentage of phosphate ions decreased with an increasing amount of PGAF (0.1 – 0.5 g). Equilibrium adsorption was reached within 10 min, and the removal percentage of phosphate ions onto PGAF was 99.6%. In this study, 0.1 g of adsorbent was used for 300 mL of sample solution. On the other hand, Yang et al. (2014) reported that 0.1 g of adsorbent was used for 25 mL of sample solution. The ratio of adsorbent/solution in this study is therefore twelve times lower than that in the previous work (Yang et al., 2014). These results indicate that the treatment method used in this study could provide an adsorbent capable of keeping a high treatment capacity and water quality.
The adsorption isotherms of phosphate ions onto PGAF are shown in Fig. 10. It is seen that the temperature did not affect the amount of phosphate adsorbed. These results suggest that PGAF is useful for the removal of phosphate ions at 5 – 45°C. The maximum adsorption amounts of phosphate ions onto PGAF at 5, 25, and 45°C were 152.3, 156.5, and 169.2 mg/g, respectively. Thus, PGAF possesses a removal capacity superior to other adsorbents reported in the literature (Table 1). Notably, the high removal capacity of PGAF was achieved at 100 – 1,000 mg/L (Li et al., 2014; Köse and Kıvanç, 2011; Krishnan and Haridas, 2008; Liu et al., 2008; Lam et al., 2007; Li et al., 2006).
Fig. 10 - Adsorption isotherms of phosphate ion onto PGAF.

Table 1 - Comparison of the phosphate ion removal capacities of the various adsorbents.

<table>
<thead>
<tr>
<th>Adsorbent</th>
<th>Removal capacity (mg/g)</th>
<th>References</th>
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<tbody>
<tr>
<td>Fe-Cu binary oxide</td>
<td>39.8</td>
<td>Li et al., 2014</td>
</tr>
<tr>
<td>Al oxide hydroxide</td>
<td>35</td>
<td>Lam et al., 2007</td>
</tr>
<tr>
<td>Mesoporous ZrO$_2$</td>
<td>29.7</td>
<td>Liu et al., 2008</td>
</tr>
<tr>
<td>Calcined waste eggshell</td>
<td>23.0</td>
<td>Köse and Kivanc, 2011</td>
</tr>
<tr>
<td>Iron impregnated coir pith</td>
<td>70.92</td>
<td>Krishnan and Haridas, 2008</td>
</tr>
<tr>
<td>Red mud</td>
<td>113.87</td>
<td>Li et al., 2006</td>
</tr>
<tr>
<td>Fly ash</td>
<td>63.2</td>
<td>Li et al., 2006</td>
</tr>
<tr>
<td>PGAF</td>
<td>169.2</td>
<td>This study</td>
</tr>
</tbody>
</table>

Table 2 shows the effect of pH on the removal of phosphate ions by PGAF and Ca(OH)$_2$. Generally, pH is considered to be an important parameter in physicochemical reactions at the water/solid interface. The pH values after phosphate removal using PGAF and Ca(OH)$_2$ were 6.5 – 9.0 and 12.6 – 12.9, respectively. The removal percentages of phosphate ions onto PGAF and Ca(OH)$_2$ were 97.6 – 99.1% and 99.5 – 100%, respectively; therefore, the initial pH of the aqueous solution essentially has no effect on the phosphate removal by PGAF and Ca(OH)$_2$. The maximum removal of phosphate ions using PGAF occurs in the pH range 3 – 12. The pH of natural water or wastewater is in the range 6 – 9. Within this range, the percentage of phosphate removal by PGAF was over 97.6%. Thus, in view of practical applications, the pH would not need to be adjusted; therefore, PGAF would be more useful for phosphate removal from aqueous solution than Ca(OH)$_2$. 

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CONCLUSIONS
Poly-γ-glutamic acid and flocculants (PGAF) were used for the removal of phosphate ions from aqueous solution. Calcium and oxygen were found to be present on the surface of the PGAF. The removal percentage of phosphate ions by PGAF was 98.2% within 10 min. The XRD patterns obtained in this study showed that hydroxyapatite and calcite were produced when PGAF was added to the aqueous solution. These products were collected using poly-γ-glutamic acid and precipitated in aqueous solution. This treatment is very efficient for solid/liquid separation. Moreover, the removal percentage of phosphate ions onto PGAF increased with increasing amount of adsorbent. The optimal agitation speed for the removal of phosphate ions was found to be 60 – 120 rpm; the temperature and solution pH did not affect the amount of phosphate removed.

REFERENCES


Yagi S. and Fukushi K. (2012) Removal of phosphate from solution by adsorption and
