Facile and effective synthesis of adsorbent – utilization of yeast cells immobilized in sodium alginate beads for the adsorption of phosphorus in aqueous solution

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ABSTRACT

We compared the adsorption efficiency of phosphates onto Ca-alginate immobilized yeast and freely suspended yeast under different conditions of pH and temperature. The results clearly demonstrated that the adsorption efficiency onto Ca-alginate immobilized yeast was better than that of freely suspended yeast, and reached a maximum at pH 9.17 and 35 °C. Scanning electron microscopy was used to characterize the morphology of Ca-alginate immobilized yeast. Fitting the adsorption equilibrium data to existing models showed that the Freundlich isotherm model described the process better than the Langmuir model, and the process of adsorption followed pseudo-first-order kinetics. During the initial period of experiment, external diffusion was a key rate-controlling step, and intraparticle diffusion also contributed to the mass transport. The thermodynamic properties (Gibbs free energy change of –15.143 kJ/mol, enthalpy change of 274.118 kJ/mol, and entropy change of 290 J/(mol K)) indicated that the adsorption process was endothermic.

INTRODUCTION

Water eutrophication is a growing problem and phosphorus is a key nutrient that leads to eutrophication of freshwaters (Huang et al. 2016). Therefore, it is crucial to control the content of phosphorus in effluent that enters bodies of water.

Current methods of phosphorus removal include chemical, biological and ecological processes (Sukačová et al. 2015). Chemical processes are reliable and easy to operate. Many raw minerals and industrial waste products including zeolite, waste oyster shell, calcium, and iron oxides (Li et al. 2015; Yan et al. 2015) have been tested for use in phosphorus sequestration. However, the large amounts of chemicals applied in the chemical process may produce chemical sludge which can cause secondary pollution. Some physical methods, such as reverse osmosis or electro-dialysis, are added to remove phosphate from water; however, these approaches are most costly than other processes.

In recent years, much emphasis has been focused on the use of immobilized cells in the degradation of wastewater pollutants. Immobilized cells are cells or cell remnants that are prevented from moving freely to all parts of an aquatic system by natural or artificial means (Tampion & Tampion 1987). For example, Gonzalez et al. (2001) used immobilized Pseudomonas putida to absorb phenolic material from industrial wastewater in a fluidized bed bioreactor. Israeli researchers (Abu-Salah et al. 1996; Massalha et al. 2010) also employed immobilized cells to remove phenol from an aquatic environment. Quinoline was adsorbed by immobilized cells of Burkholderia pickettii, as reported by Zhang et al. (2002). Bai & Abraham (2003) investigated the adsorption of Cr(VI) by immobilized fungal biomass. Moreover, immobilized microbial cell systems may provide advantages over freely suspended cells (Tan & Ting 2012). The use of immobilized cells in continuous operation allows the retention of high cell density in the reactor, even beyond wash-out conditions. Because the biomass immobilization is an inert reaction, it is non-toxic to the biomass. In addition, immobilization cells can limit effects on microorganisms due to xenobiotic toxicity, or water and nutrient stress, allowing improved microbe survivability (Hori et al. 2015). Sodium alginate is a linear, unbranched copolymer which has a unique property in that stable gels can be formed in the presence of metal cations like Ca2+; thereby Ca-alginate can be used to immobilize yeast cells facilely and effectively. Furthermore,
Ca-alginate beads applied for adsorption are confirmed to have great adsorption capacity (Mahmood et al. 2015), so Ca-alginate with entrapped yeast may have better adsorption effect than freely suspended yeast.

The ability of living alginate immobilized yeast cells to adsorb phosphorus was investigated in present study. The removal efficiency of Ca-alginate immobilized cells was compared with that of freely suspended cells. Two factors (pH and temperature) were investigated to determine how these factors affected phosphorus removal efficiency in synthetic effluents. The isotherm and kinetic parameters were calculated and fitted to models in order to determine the adsorption mechanism.

MATERIALS AND METHODS

Yeast and media

Yeast cells (Saccharomyces cerevisiae, 2.3875) were supplied from China General Microbiological Culture Collection Center. The cells were subcultured and incubated in sterilized medium (pH 7) for 2 days at 32°C. The composition of growth medium was (in grams per liter): beef extract (5), peptone (10), NaCl (5), agar-agar (20). The yeast was cultured on the culture medium (pH 7). The yeast suspension was divided into two parts. One was freely suspended yeast and the other was used for immobilized yeast.

Preparation of immobilized cell beads

Ca-immobilized cell beads were prepared by dropping a mixture of sodium alginate solution and yeast cells into 4% (w/w) CaCl₂ solution under slow magnetic stirring at 25 ± 3.0°C. The resulting beads were 5 ± 0.2 mm in diameter and stirred in this solution for 30 min. They were then collected by filtration, washed three times with sterile and deionized water, and stored in Tris–HCl buffer (pH 7) at 4°C.

Experimental methods

The adsorption of phosphorus by Ca-alginate immobilized cells of yeast and freely suspended yeast from water were investigated in a solution with 9.75 mg/L initial phosphorus concentration. The adsorbent dosage was 1.0 g/L. A series of experiments were conducted by varying the pH (4.36–9.17) and temperature (25°C to 35°C) to determine the optimum conditions for adsorption. All the volumes for phosphorus solution were 100 mL and three repetitions were carried out for all experiments. The pH was adjusted by addition of 0.1 mol/L HCl or NaOH at 30°C. Equilibrium isotherm and kinetic models were used to determine the adsorption process characteristics.

Analytical methods

The removal of phosphorus (as PO₄³⁻) was determined by measuring the concentration of phosphorus in the aqueous phase using a UV–visible spectrophotometer (UV-1201, Beifen-Ruili Analytical Instrument Co. Ltd, China) at 540 nm. All the experiments were carried out with three independent repetitions and the mean values were used. In the case of significant differences, comparisons of means were performed using SPSS software (SPSS Inc.). Statistical significance was set at the level of $p < 0.05$.

The phosphorus adsorption ratio (%) onto Ca-alginate immobilized yeast and freely suspended yeast was determined by using the following equation:

$$\eta = \frac{C_0 - C}{C_0} \times 100\%$$  \hspace{1cm} (1)

where $\eta$ is the adsorption ratio of phosphorus, $C_0$ is the initial concentration of the phosphorus in the solution (mg/L) and $C$ is the final concentration of the phosphorus in the solution (mg/L).

Scanning electron microscopy

Samples of Ca-alginate and yeast immobilized beads were coated under vacuum with a thin layer of gold and examined by scanning electron microscopy (SEM, S4800, Hitachi Ltd, Japan).

RESULTS AND DISCUSSION

Adsorption of PO₄³⁻

The SEM images of pure Ca-alginate and Ca-alginate immobilized yeast are shown in Figure 1(a) and 1(b). Figure 1(a) shows that the gel structures of Ca-alginate are relatively tight; tight gel structures may insure that yeast cells could be immobilized and preserved in Ca-alginate beads and Ca-alginate could protect yeast cells from water and nutrient stress in aqueous solution (Daemi & Barikani 2012). Figure 1(b) illustrates interiors of Ca-alginate
immobilized yeast beads, and yeast cells are clearly visible, suggesting yeast cells have been immobilized in Ca-alginate.

There are many Ca\(^{2+}\) ions and pores distributing on the surface of Ca-alginate beads (Liu et al. 2002), so that PO\(_4^{3-}\) could either combine with Ca\(^{2+}\) ions or enter the Ca-alginate beads via the pores, as the diameters of PO\(_4^{3-}\) (8 × 10\(^{-10}\) m) (Huheey et al. 2006) are much smaller than diameters of apertures of the Ca-alginate (0.2 × 10\(^{-6}\) to 0.5 × 10\(^{-6}\) m). Phosphorus ions which enter the interior of Ca-alginate could be metabolized by yeast inside the immobilized beads (Massalha et al. 2007; Farbo et al. 2016). Thus, phosphorus ions could be removed by surface Ca\(^{2+}\) and the inside yeast, and the schematic diagram for phosphorus removal is shown in Figure 2.

**Effect of pH**

Figure 3 shows that the removal of PO\(_4^{3-}\) generally increased with increasing pH values. The adsorption ratio of the Ca-alginate immobilized yeast was maintained at 60–85% between pH 4.36 and pH 9.17, but the freely suspended yeast exhibited a removal ratio of 38–55% in a pH range from 4.36 to 9.17. The amount of PO\(_4^{3-}\) adsorption onto the Ca-alginate immobilized yeast was higher than freely suspended yeast at pH values above 4.36. At pH 4.36, the yeast and Ca-alginate both contributed to adsorption, for a rapidly increasing adsorption ratio. At pH 9.17, the maximum phosphorus adsorption on the Ca-alginate immobilized yeast was 85.71% and the maximum removal ratio of PO\(_4^{3-}\) by freely suspended yeast was only 54.69%. This result showed that Ca-alginate immobilized yeast enhanced the functionality of the yeast over different ranges of the pH.

It is well known that PO\(_4^{3-}\) in water is easy to hydrolyze and the hydrolytic reaction equations are:

\[
\begin{align*}
\text{PO}_4^{3-} + H_2O & \rightleftharpoons \text{HPO}_4^{2-} + OH^- \\
\text{HPO}_4^{2-} + H_2O & \rightleftharpoons \text{H}_2\text{PO}_4^- + OH^-
\end{align*}
\]

Figure 1 | The SEM micrograph for the plain Ca-alginate (a) and internal Ca-alginate immobilized yeast (b).

Figure 2 | The scheme of PO\(_4^{3-}\) adsorption onto the Ca-alginate immobilized yeast.
Medium pH affects the hydrolysis of PO$_4^{3-}$. In the acidic and mildly acidic environment, the hydrolysis products are mainly HPO$_4^{2-}$, H$_2$PO$_4$ and H$_3$PO$_4$ (Zhou et al. 2015), as determined by Visual MINTEQ (Gustafsson 2011), a software with chemical equilibrium model for the calculation of metal speciation, as shown in Figure 4 theoretically. Phosphorus in aqueous solution could react with Ca$^{2+}$ and form CaHPO$_4$ and Ca(H$_2$PO$_4$)$_2$. But both CaHPO$_4$ and Ca(H$_2$PO$_4$)$_2$ can easily dissolve in acidic solution. This may explain why, as the pH increased from 4.36 to 6.58, the amount of phosphorus removal increased slowly to 72.58% with Ca-alginate immobilized yeast, and the adsorption ratio significantly increased to 89.21% as the pH increased from 6.58 to 9.17.

### Effect of temperature

The temperature of wastewater has a significant effect on the efficiency of the phosphorus adsorption process of microorganisms. As shown in Figure 5, the adsorption of PO$_4^{3-}$ by Ca-alginate immobilized yeast and freely suspended yeast appears to be temperature-dependent. The average removal ratio of PO$_4^{3-}$ by the Ca-alginate immobilized yeast was 67.66% at 25°C, 68.01% at 30°C and 77.81% at 35°C. The average removal ratio of PO$_4^{3-}$ by the freely suspended yeast appeared to be 49.88, 52.16, and 55.03% for 25, 30, and 35°C, respectively. Significant differences ($p < 0.05$) are indicated by different letters in Figure 5. There were no significant differences of removal ratio between 25°C and 30°C ($p = 0.980$ for immobilized yeast; $p = 0.105$ for freely suspended yeast), yet significant differences of removal ratio were found between 25°C and 35°C ($p = 0.003$ for immobilized yeast; $p = 0.001$ for freely suspended yeast), as well as between 30°C and 35°C ($p = 0.004$ for immobilized yeast; $p = 0.047$ for freely suspended yeast). These data indicated that as the temperature gradually increased, ion diffusion increased (Wu et al. 2016) and the chance of interactions between negative ions (PO$_4^{3-}$) and positive ions (Ca$^{2+}$) also increased. Additionally, the amount of PO$_4^{3-}$ that contacted the Ca-alginate immobilized yeast via the pores may also increase, contributing to the

\[
\text{H}_2\text{PO}_4 + \text{H}_2\text{O} \leftrightarrow \text{H}_3\text{PO}_4 + \text{OH}^-
\]
higher adsorption by Ca-alginate immobilized yeast compared to freely suspended yeast.

Equilibrium isotherm studies

The adsorption isotherm data of PO\textsubscript{4}\textsuperscript{3-} onto Ca-alginate immobilized cells of yeast and freely suspended yeast at different temperatures were analyzed using Langmuir and Freundlich equations.

The Langmuir model assumes monolayer sorption of a solute from a liquid solution. The linearized form of the Langmuir equation is given by Equation (5):

\[
\frac{C_e}{q_e} = \frac{1}{q_{\text{max}}K_c} + \frac{C_e}{q_{\text{max}}}
\]

where \(q_{\text{max}}\) is the maximum specific uptake of PO\textsubscript{4}\textsuperscript{3-} corresponding to site saturation (mg/g), \(K_c\) is an equilibrium constant (L/mg), \(C_e\) is the equilibrium concentration (mg/L), and \(q_e\) is the amount of PO\textsubscript{4}\textsuperscript{3-} uptake at equilibrium (mg/g).

The essential characteristics of a Langmuir isotherm can be represented in terms of the dimensionless constant separation factor \(R_L\) given as:

\[
R_L = \frac{1}{1 + \frac{K_cC_0}{q_{\text{max}}}}
\]

where \(R_L\) indicates if the type of the isotherm describes a reaction that is unfavorable \((R_L > 1)\), linear \((R_L = 1)\), favorable \((0 < R_L < 1)\) or irreversible \((R_L = 0)\) (Tofighy & Mohammadi 2011).

The Freundlich model is based on sorption on a heterogeneous surface. The Freundlich isotherm model is represented as Equation (7):

\[
q_e = K_fC_e^n
\]

where \(K_f\) and \(n\) are the Freundlich constants of adsorption capacity and adsorption intensity, respectively.

Table 1 shows equilibrium isotherm constants obtained from fitting the experimental results to the Langmuir and Freundlich models. The experimental data fitted best with the Freundlich isotherm model. The value of \(q_{\text{max}}\) increased with increasing temperature, indicating that the adsorption of PO\textsubscript{4}\textsuperscript{3-} onto Ca-alginate immobilized cells of yeast became more favorable at higher temperature. \(R_L\) values calculated for adsorption of PO\textsubscript{4}\textsuperscript{3-} onto Ca-alginate immobilized cells at various temperatures were between 0 and 1, indicating favorable adsorption. Values of \(K_f\) and \(n\) obtained from the Freundlich isotherm model also increased with temperature, indicating that PO\textsubscript{4}\textsuperscript{3-} was favorably adsorbed by Ca-alginate immobilized yeast cells as temperatures increased.

Kinetic modeling of adsorption

In the removal of PO\textsubscript{4}\textsuperscript{3-} from water by Ca-alginate immobilized yeast, it is critical to investigate the mechanisms of the adsorption process, including mass transfer and chemical adsorption. To do this, kinetic models are used to test the experimental data. This paper uses two kinetic modeling equations, pseudo-first-order and pseudo-second-order, to fit the experimental results. The relevant equations are as follows:

pseudo-first-order model: \(q_t = q_e(1 - e^{-k_1t})\) (8)

pseudo-second-order model: \(q_t = \frac{q_e^2k_2t}{1 + q_ek_2t}\) (9)

where \(k_1\) and \(k_2\) are the rate constant (g/(md-d)) for the pseudo-first-order and pseudo-second-order model, respectively, and \(q_t\) (mg/g) is the PO\textsubscript{4}\textsuperscript{3-} uptake at time \(t\).

Table 1 | Isotherm constants obtained from Langmuir and Freundlich models for the adsorption of PO\textsubscript{4}\textsuperscript{3-} onto Ca-alginate immobilized yeast and freely suspended yeast

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>Immobilized yeast</th>
<th>Freely suspended yeast</th>
<th>Langmuir</th>
<th>Freundlich</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(R_L)</td>
<td>(q_{\text{max}}) (mg/g)</td>
<td>(K_c) (L/mg)</td>
<td>(R^2)</td>
</tr>
<tr>
<td>25</td>
<td>0.86</td>
<td>55.51</td>
<td>0.016</td>
<td>0.9921</td>
</tr>
<tr>
<td>30</td>
<td>0.81</td>
<td>79.76</td>
<td>0.024</td>
<td>0.9465</td>
</tr>
<tr>
<td>35</td>
<td>0.85</td>
<td>95.95</td>
<td>0.018</td>
<td>0.9258</td>
</tr>
<tr>
<td>25</td>
<td>0.89</td>
<td>56.26</td>
<td>0.012</td>
<td>0.9930</td>
</tr>
<tr>
<td>30</td>
<td>0.88</td>
<td>59.24</td>
<td>0.013</td>
<td>0.9869</td>
</tr>
<tr>
<td>35</td>
<td>0.85</td>
<td>67.65</td>
<td>0.018</td>
<td>0.9743</td>
</tr>
</tbody>
</table>
The kinetic parameters for the reaction with either Ca-alginate immobilized yeast or freely suspended yeast are listed in Table 2. Figure 6(a) and 6(b) show the fitting results according to the pseudo-first-order and pseudo-second-order models, respectively. The values of the correlation ($R^2$) from Table 2 implied that the pseudo-first-order model better described the kinetic experimental data than the pseudo-second-order data. The values of $k_1$ and $q_e$ indicated that the adsorption ratio and adsorption amount onto Ca-alginate immobilized yeast were significantly better than those of freely suspended yeast. Overall, $\text{PO}_4^{3-}$ was much faster and better adsorbed by Ca-alginate immobilized yeast compared with freely suspended yeast. Ca-alginate could also provide extra driving force that prompted the adsorption of $\text{PO}_4^{3-}$.

The external diffusion and intraparticle diffusion processes were investigated to determine the rate-controlling steps. The Spahn and Schlunder model was used to depict the procedure of external diffusion, and the equation was as follows:

$$\ln \frac{C_t}{C_0} = -k_{\text{ext}}t$$

(10)

where $k_{\text{ext}}$ is the constant (1/d) and $C_t$ is the concentration of $\text{PO}_4^{3-}$ at any time.

If the adsorption process is controlled by an external hindrance, the relationship of $\ln C_t$ versus time $t$ should be described by the equation above.
The relationship is depicted in Figure 7, and demonstrates that external diffusion was the critical factor in controlling the initial adsorption rate of both Ca-alginate immobilized yeast and freely suspended yeast.

The intraparticle diffusion is another important step of adsorption. The equation is as follows:

$$q_t = f(t^{0.5})$$  \hspace{1cm} (11)

The plot of $q_t$ against $t^{0.5}$ might be multilinear, which indicated two or more steps occurring in the adsorption process. The plots of $q_t$ versus $t^{0.5}$ for immobilized and freely suspended yeast can be observed in Figure 8. The value of the rate parameter $k_{p,i}$ ($i =$ stage number) was equal to the slope of the lines for each stage. The parameters $k_{p,1}$ and $k_{p,2}$ represent the stage of intraparticle diffusion and equilibrium, respectively. The results indicate that the values of $k_{p,1}$ were obviously larger than $k_{p,2}$, both for Ca-alginate immobilized yeast and freely suspended yeast, because the active sites of the adsorbent were almost vacant at the start of adsorption, resulting in rapid adsorption. The adsorption rate then gradually deceased as the active sites became filled (Letterman 1999). As shown in Figure 8, the first line did not pass through the origin of coordinates, suggesting that the intraparticle diffusion was not the only limiting factor in this process (Chen et al. 2011).

### Thermodynamic studies

The thermodynamic parameters such as Gibbs free energy change ($\Delta G^0$), enthalpy ($\Delta H^0$), and entropy change ($\Delta S^0$) must be considered in order to determine whether the processes occur spontaneously. In order to determine the thermodynamic parameters of the PO$_4^{3-}$ adsorption, experiments were carried out at different temperatures between 25–35 °C. The Gibbs free energy change of the sorption reaction is given as:

$$\Delta G^0 = -RT \ln K_c$$  \hspace{1cm} (12)

$$\ln K_c = \frac{\Delta S}{R} - \frac{\Delta H}{RT}$$  \hspace{1cm} (13)

where $T$ is the temperature (K), $R$ is the gas constant (8.314 J/(mol K)), and $K_c$ is the equilibrium constant obtained from the Langmuir isotherm. The enthalpy and entropy change can be determined from the slope and intercept of a graph of $\ln K_c$ versus $1/T$. The results of these thermodynamic calculations are shown in Table 3.

The calculated $\Delta G^0$ values for the adsorption of PO$_4^{3-}$ onto immobilized cells of yeast were $-12.24$, $-13.87$, and $-15.14$ kJ/mol, respectively, as the temperature increased from 25 to 35 °C. The values of $\Delta H^0$ and $\Delta S^0$ were 274.118 kJ/mol and 290 J/(mol K), respectively. A positive value of $\Delta H^0$ indicates that the endothermic nature of the adsorption process and the positive value of

<table>
<thead>
<tr>
<th>$T$ (°C)</th>
<th>$\Delta G^0$ (kJ/mol)</th>
<th>$\Delta H^0$ (kJ/mol)</th>
<th>$\Delta S^0$ (J/(mol K))</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>$-12.24$</td>
<td>274.12</td>
<td>290</td>
</tr>
<tr>
<td>30</td>
<td>$-13.87$</td>
<td>$-42.18$</td>
<td>133.57</td>
</tr>
<tr>
<td>25</td>
<td>$-15.14$</td>
<td>290</td>
<td>133.57</td>
</tr>
</tbody>
</table>

![Figure 8](image-url)
ΔS° suggests an increased randomness at the solids/solution interface during the adsorption of PO₄³⁻ onto the adsorbent (Sun et al. 2015).

CONCLUSIONS

PO₄³⁻ uptake by Ca-alginate immobilized cells of yeast was observed to be more efficient than by freely suspended cells. Ca-alginate immobilized yeast was successfully used as the adsorbent for the removal of PO₄³⁻ ions from aqueous solution. The removal ratio of phosphorus by the Ca-alginate immobilized yeast increased rapidly at higher pH values. The temperature affected the removal capacity and the maximum adsorption capacity was achieved at 35 °C. Isotherm models were fitted to the experimental data and the Freundlich model better described the adsorption results compared to the Langmuir model. The kinetic data fitted better to the pseudo-first-order model than the pseudo-second-order model. During the initial fast adsorption stage and in the initial time of cultivation, external diffusion was the rate-controlling step. Intraparticle diffusion also contributed to controlling the rate. Thermodynamic studies indicated that the adsorption process was endothermic.

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REFERENCES


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