Study of orthophosphate uptake by diffusive gradient in thin film (DGT) system with Co-loaded chitosan bentonite biocomposite binding gel and the effect of its anion interferences on the phosphate response

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Abstract. The binding gel in DGT has been modified with Co-loaded chitosan-bentonite (Co-CSBent) biocomposite in order to enhance the phosphate binding capacity. The comparison was also performed by Chitosan-bentonite (CSBent). The binding and diffusive gel for this method were made from acrylamide, ammonium persulfate, and N,N-methylenebisacrylamide. The synthesized binding gel and biocomposite were characterized using Fourier Transform Infrared (FTIR), X-Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM). Characterization results showed that biocomposites had been synthesized successfully. For control, DGT Co-CSBent and CSBent were tested with 2 ppm phosphate and without anions, C\text{CSBent} of orthophosphate value of 1.9127 µg/mL and 1.6643 µg/mL were obtained respectively. Both binding gels in DGT were examined with various anions including Cl, SO\text{3}, HCO\text{3}, and NO\text{3} in the concentration ranging from 0.5 mg/L to 2.5 mg/L. At higher SO\text{3} inhibitor concentration, C\text{CSBent} value of 1.0153 µg/mL (CSBent) and 1.2736 µg/mL (Co-CSBent) were obtained. Whereas, at higher Cl inhibitor, C\text{CSBent} value of 1.2934 µg/mL (CSBent) and 1.9584 µg/mL (Co-CSBent) were obtained. At higher HCO\text{3} inhibitor concentration, C\text{CSBent} value of 0.7371 µg/mL (CSBent) and 0.8628 µg/mL (Co-CSBent) were obtained. And, at higher NO\text{3} inhibitor concentration, C\text{CSBent} value of 0.459 µg/mL (CSBent) and 0.5889 µg/mL (Co-CSBent) were obtained. Based on the obtained data, Cl and SO\text{3} do not affect the C\text{CSBent} of orthophosphate value. However, NO\text{3} and HCO\text{3} anions gradually reduced the C\text{CSBent} of orthophosphate value compared with C\text{CSBent} of the control value. Phosphate binding by biocomposite is controlled by ion exchange, electrostatic force, and Lewis metal ion complexation.

Keywords: orthophosphate, diffusive gradient in thin film, biocomposite, anion interference, chitosan bentonite

1. Introduction

Phosphorus is a macronutrient for the growth of aquatic plants and their metabolism [1]. In the aquatic system, phosphate is divided into colloid, particular, and dissolved. Dissolved phosphate is divided into organic phosphate (dissolved organic phosphate, DOP) and inorganic phosphate (dissolved inorganic phosphate, DIP), which consists of orthophosphate and polyphosphate [2]. Rumhayati [3] subdivided the phosphate ability of filtration process and concentration based on its reactivity to molybdate reagents. The reactive filtrated phosphate of the molybdate reagent is called the filterable reactive phosphate (FRP) containing orthophosphates and polyphosphates and organic phosphates, which are readily hydrolyzed by acids. Meanwhile, the concentration of the filtered organic phosphate (FOP) is determined by the oxidation step before it is reacted with molybdate reagent [3]. Phosphorus in waters comes from a variety of sources, including geological deposits, industrial waste, agricultural activities, household waste, agriculture and mining [4]. The tolerance limit of the phosphate content in
water is 0.5 mg/L [5], and many aquatic systems have exceeded the threshold, thereby causing eutrophication of reservoirs, rivers, and lakes.

The diffusive gradient in thin film (DGT) technique has been performed on the measurement of the dissolved phosphate in waters [6]. This DGT apparatus can be used for phosphate analysis in water, soil, or sediment. The principle of DGT apparatus is based on a simple device that accumulates phosphate on a binding agent (binding gel) after passing through a hydrogel that acts as a diffusion layer. It depends on the steady-state concentration gradient of the bonding agent [6]. Accumulation occurs when contacting with phosphate. The accumulated analytes are eluted and determined by using spectrophotometry using the blue phosphomolybdenum method. The capacity of DGT can be determined by using equations derived from Fick’s First Law [7–8]

\[ C = \frac{M \times \Delta g}{D \times t \times A} \]

The capacity of DGT depends on the adsorbent used on the gel binding. In this study, biocomposite adsorbent was used to be included in gel binding on DGT. The biocomposite was prepared with three main ingredients, including bentonite (in this study is Na-Bentonite), chitosan, with (Co-CSBent) or without Co\textsuperscript{2+} (CSBent). The three compounds have their respective ability to absorb phosphate. But the phosphate is absorbed very little and absorption can only be done under certain conditions. To increase the phosphate sorption capacity and eliminate the deficiencies of each compound, the three compounds are synthesized into biocomposites. It is expected that the compounds formed is stable and created adsorbents that are able to bind phosphates effectively.

2. Experimental

2.1. Materials
Chitosan, Bentonite (PT Madani Alam Lestari), cellulose nitrate membrane (GE Healthcare Life Science), Glacial Acetic Acid (Merck), N,N’-methylenebisacrylamide, Acrylamide (Sigma), Ammonium Persulphate (Merck), N,N,N’,N’-tetramethylethylenediamine (TEMED) 99 % (Sigma), Pottasium Antimonyl Tartrate (Sigma), Ammonium Molybdate (Merck), H2SO\textsubscript{4} p.a (Merck), and Ascorbic Acid.

2.2. Synthesis of Co-CSBent and CSBent biocomposites
2 g of chitosan (CS) was dissolved in 2 % acetic acid solution at 40 °C and stirred. At the same time, 6 g of Na-Bentonite (Bent) was dispersed in 50 mL of distilled water for 2 hours. The bentonite dispersion was added slowly to chitosan solution prepared over 24 hours with strong magnetic stirring at 60 °C. After colloid formation of CSBent biocomposite, metal ions were filled by adding CoCl\textsubscript{2}·6H\textsubscript{2}O 0.3 % (w/v) solution. Stirred for 1 hour with a strong stirring and obtained biocomposite Co-CSBent in the form of a slurry. The Co-CsBent was stored for 24 hours, then filtered using filter paper and washed with distilled water. Co-CSBent biocomposites were dried in a hot air oven at 45-50 °C until dry to obtain the fine powder. Then, the biocomposite was sieved to obtain a consistent size.

2.3. Preparation of diffusive gel and binding gel (CSBent and Co-CSBent)
The 5 mL gel solution was prepared based on Zhang et al. [6] procedure. This gel solution contains 15 % acrylamide solution and 0.3 % cross-linker. Then, added with 35 μL of ammonium persulfate solution, and added 10 μL of TEMED solution into the container. Stirred until homogeneous (approximately 15–20 seconds), immediately the solution was put into a glass mold. The solution which has been inserted into the mold was then heated in an oven at 45 °C for 45 minutes to an hour until the gel formed. The formed gel was washed with distilled water then soaked for 24 hours for hydration. For binding gel preparation, the procedure is almost the same as above, but the gel solution added to biocomposite as much as 2 g.

2.4. DGT component installation
For installation of DGT apparatus, gel sheets (diffusive and binding) were cut in 25 mm diameter, and the filter membrane was soaked in distilled water. DGT devices were washed and rinsed with distilled water. The binding gel pieces were placed first on DGT molding followed by a diffusive gel and membrane filter. The DGT apparatus was perfectly closed.
2.5. DGT uptake test
DGT test in this study used 2 ppm phosphate with various circumstances. That included 4 variables, homogeneous binding gel, contact time, pH, and anion with various concentrations.

2.6. Binding gel elution
DGT apparatus that have absorbed the phosphate were dismantled and binding gel that has absorbed the phosphate was taken. The binding gel was immersed in a 0.25 M 6 mL H$_2$SO$_4$ solution for 16 hours. The elution solution was phosphate, analyzed by blue phosphomolybdenum method.

3. Results and discussion
3.1. Characterization of biocomposite
After biocomposite was made, biocomposites were characterized by Fourier Transform Infrared (FTIR). FTIR results of bentonite, chitosan, CSBent, and Co-CSBent are presented in figure 1. FTIR results from bentonite exhibit a peak at 1049 cm$^{-1}$ which is a stretch of Si-O-Si. The peak at 3632 cm$^{-1}$ is the stretched vibration of OH bound to Al (or Mg) in bentonite [9]. The peak chitosan FTIR results at 1041 cm$^{-1}$ show the presence of C-O-C bonds indicating the presence of ether in the chitosan compound. At peak 1306 cm$^{-1}$ is secondary carbon, that is CH$_2$-OH, while 1380 cm$^{-1}$ is tertiary carbon, which is CH-OH. Chitosan contains the basic characteristics of the peak at 1585 cm$^{-1}$ and 2895 cm$^{-1}$ because of the amide bond and the presence of the alkyl chain [10]. The small peak at 519 cm$^{-1}$ is the bent and spin vibration of Si-O [11]. The peak at 906 cm$^{-1}$ is a bentonite material that enters the biocomposite, which is Al-O(OH)-Al and Al-Al-OH in the prolonged state [12]. The amide group shifts its peak to 1523 cm$^{-1}$ because the amide is protonated to NH$_3$+. In the band 3628 cm$^{-1}$, there are OH and NH groups derived from chitosan [13]. Based on the FTIR data, it is certain that chitosan and bentonite are integrated with the synthesized biocomposites.

Scanning Electron Microscopy-Energy Dispersive X-Ray Spectroscopy (SEM-EDS) characterization of synthesized biocomposites is given in figure 2. This characterization is to determine the surface of biocomposite. Co-CSBent biocomposites have irregular and large pores characterized by a yellow circle. The pores function is for phosphate uptake in the sample. On the surface morphology, macromolecular compounds is visible, it is caused by impurities during synthesis or when drying in the oven. The pores of the Co-CSBent biocomposite are covered by phosphates.
Figure 2. SEM-EDS characterization of biocomposites (a) before phosphate uptake and (b) after phosphate uptake at magnification: (i) 1000 x, (ii) 2500 x and (iii) EDS result.

composites characterized by a yellow circle. This happens because the biocomposite uptake the phosphate in the sample. EDS characterization show the presence of Co on biocomposite and phosphate element is detected after the uptake reaction. Based on EDS results, Co-CSBent biocomposite had successfully uptake the phosphate.

3.2. Characterization of gels

After gels had been synthesized, gels were characterized by FTIR. FTIR results from acrylamide, diffusive gel, and binding gels are presented in figure 3. On the raw material of acrylamide spectrum, there is a visible peak at 800 cm$^{-1}$, which is the bending of the NH$_2$ group out of plane (oop). On the other hand, the 1150 cm$^{-1}$ peak is a deformation vibration of the C-N bond [13]. At 1340-1420 cm$^{-1}$ is a C-C bond presents in the acrylamide compound. Stretching and deformation vibrations corresponded to the C=O appear at 1673 cm$^{-1}$ [14]. The largest peak at 3180-3350 cm$^{-1}$ is the stretching of the NH group having the characteristic of 2 identical peaks. In diffusive peak gel appears almost the same as acrylamide, only different in the transmittance. Significant differences occur at the peak of 1612 cm$^{-1}$, in diffusive gel peak that wavenumber disappears. It proves that the C=C bond disappears and it can be concluded that the synthesis of acrylamide to polyacrylamide was successful. Diffusive gel, when
Figure 3. FTIR characterization of acrylamide, diffusive gel, and binding gel (CSBent and Co-CSBent) materials

![FTIR Characterization of Materials](image)

Figure 4. The possible mechanism of phosphate sorption onto biocomposites compared with gel binding, did not change significantly. This is because the biocomposites contained in the binding gel is a minor compound, so it is not detected in FTIR.

3.3. Phosphate uptake mechanism

The significance of phosphate sorption onto biocomposites was achieved by electrostatic attraction between adsorbent and adsorbate interface, complexation of Lewis metal ions associated with $\text{H}_2\text{PO}_4^-$ and ion-exchange [15], which are shown in figure 4.

3.4. DGT uptake test on various experimental variation

This test was performed to determine the ability of DGT-Co-CSBent and DGT-CSBent while ensuring that the binding gel was made to phosphate uptake in various variations. DGT uptake test on various experimental variations is presented in figure 5.

Based on the data on the graph, DGT Co-CSBent and CSBent were tested with 2 ppm phosphate and without anions. The $C_{\text{max}}$ of orthophosphate value obtained for three repetitions is almost the same with average 1.9127 $\mu$g/mL (DGT-Co-CSBent) and 1.6643 $\mu$g/mL (DGT-CSBent). This indicates that...
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Figure 5. The result of DGT uptake test on various experimental variations:  
(a) homogenous binding gel test (control), (b) deployment time and (c) pH of solution

Figure 6. The result of anion interferences effect on the DGT phosphate uptake of  
(a) DGT-CSBent and (b) DGT-Co-CSBent

The gel binding made has been quite homogeneous. DGT-Co-CSBent is superior in absorbing phosphate due to the reaction of Lewis metal ion complexation associated with HPO$_4^{2-}$ [15]. The optimum deployment time obtained in this experiment was at 24 hours, after that time $C_{\text{DGT}}$ was almost stagnant. That is because of the lack of empty sites available on the binding gels that have reached the equilibrium stage. A decrease in $C_{\text{DGT}}$ at the elevated pH occurs because an increase in pH value will increase the electrostatic repulsion between phosphate and OH$^-$ ions. Later, phosphates and OH$^-$ ions will compete to be adsorbed by the empty space in the binding gel. While low pH causes electrostatic pull between the opponent of oxyanion of phosphate and the surface of protonated sorbent [16] so as to facilitate phosphate to be bound by the sorbent.

3.5. DGT uptake test on anion effect

This test was performed to determine the effect of DGT-CSBent and DGT-Co-CSBent phosphate uptake on anion interferences, which is widely present in water. DGT uptake test on anion effect is presented in figure 6. Based on the obtained data, Cl$^-$ and SO$_4^{2-}$ do not affect the $C_{\text{DGT}}$ of orthophosphate value. However, a significant decrease of the $C_{\text{DGT}}$ of orthophosphate value from the interferences of HCO$_3^-$ and NO$_3^-$ ions is because the two anions are the hardest bases compared with the other anions. The hardness sequence of an anion according to the theory of HSAB is NO$_3^->$ HCO$_3^->$ SO$_4^{2-}>$PO$_4^{3-}>$Cl$^-$. This DGT has a good phosphate uptake in the anion interference of SO$_4^{2-}$ and Cl$^-$. 

4. Conclusions

DGT binding gel Co-CSBent and CSBent biocomposites are able to bind phosphates. The capacity of Co-CSBent binding gel is greater than CSBent binding gel for the phosphate uptake. It is seen from $C_{\text{DGT}}$ from DGT-Co-CSBent is higher than DGT-CSBent. The effects of Cl$^-$ and SO$_4^{2-}$ ions are not observed in $C_{\text{DGT}}$ of orthophosphate value, whereas NO$_3^-$ and HCO$_3^-$ ions obviously affects the $C_{\text{DGT}}$ of
orthophosphate value. The results obtained in accordance with HSAB theory which became the basis of this research.

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