Study of orthophosphate detection method using DGT (diffusive gradients in thin films) device with modified binding layer Fe-chitosan-bentonite biocomposite in aquatic system

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Abstract. In this work, a study of orthophosphate detection technique in the aquatic system has been conducted. The detection technique used in this study was Diffusive Gradients in Thin Films (DGT) with Fe-Chitosan-Bentonite (Fe-CSBent) biocomposite as the binding agent. The phosphate species diffused through the polyacrylamide gel and then bound by Fe-CSBent as the binding agent on the binding layer. The adsorption of phosphate by Fe-CSBent is through electrostatic attraction, inner sphere complexation, and ion-exchanged. The synthesized Fe-CSBent biocomposite was characterized using Fourier Transform Infrared (FTIR), Scanning Electron Microscopy (SEM), Energy Dispersive X-Ray Spectroscopy (EDS) and X-Ray Diffraction (XRD) analysis, and the synthesized diffusive gel and binding gel were characterized using FTIR analysis. In this experiment, we examined the DGT-Fe-CSBent capability in orthophosphate binding with parameters such as the deployment time, pH of the solution and the presence of co-existent anion i.e. sodium tripolyphosphate (STPP). The mass of bound orthophosphate can be calculated after eluted the binding gel with acid and then measured with the molybdenum blue method using UV-Vis spectrophotometry. Based on the experiment, DGT-Fe-CSBent bound the orthophosphates more efficiently in concentration of 2.2970 μg/mL with 90.208 % efficiency, compared to DGT-CSBent in concentration of 1.7333 μg/mL with 79.874 % efficiency. It was also known that the presence of STPP influenced the amount of orthophosphate concentration bound by Fe-CSBent in the binding gel. DGT-Fe-CSBent device that was made to be selective in predicting bioavailable phosphate, but the presence of other phosphate species such as sodium tripolyphosphate may be influenced orthophosphate bound by DGT.

Keywords: phosphate, diffusive gradients in thin films (DGT), biocomposite, chitosan, bentonite

1. Introduction

Phosphate is an important compound for aquatic ecosystems because it is one of the nutrients needed for the growth of aquatic plants [1]. Eutrophication is a high nutrient level in aquatic systems, such as nitrogen (N) and phosphate (P) causing excessive algal blooms. This can cause the low content of dissolved oxygen in the water resulting in the demise of life in the aquatic ecosystem. The high phosphates concentration in aquatic ecosystems can come from agricultural fertilizers, detergents, soaps, and industrial activity [2]. The limit of phosphate tolerance in water is <0.5 mg/L [3]. That is why an effective technique for the detection of phosphates in aquatic systems is needed.
Various technologies of phosphate detection have been found and used, such as microbial remediation, adsorption, ion exchange, and others. The adsorption technique is more desirable because it is more effective, easy to operate, economical and selective against pollutants in aquatic systems [4]. Another approach for phosphate detection in the environment is the diffusive gradients in thin films (DGT) system. DGT is a system made for the measurement of available phosphate (bioavailable) where the DGT can absorb bioavailable phosphate in water in accordance with phosphate uptake carried out by biota such as algae. The DGT system has been used for the measurement of the dissolved phosphate in-situ in waters. This is due to the interaction of phosphate species in water, which can cause the concentration of phosphate species to change when the sample is stored [5]. A DGT device comprises three layers: cellulose nitrate membrane, diffusive layer in the form of polyacrylamide gel, and binding layer containing a binding agent or adsorbent in a polyacrylamide gel. The mechanism of DGT system, i.e. phosphate diffused through cellulose nitrate membrane and diffusive gel, then bound by the binding agent or adsorbent in the binding gel. The success of the DGT system is determined by the performance of the binding gel containing the adsorbent according to the analyte studied [6].

In this study, we used DGT system for phosphate binding with Fe-Chitosan-Bentonite (Fe-CSBent) biocomposite as binding agent in binding layer. Bentonite is a type of smectite clay, which has an isomorphic replacement of Si with Al on tetrahedral sites and Al with Fe and Mg on octahedral sites, which produces net electrical charge on its surface [7]. Chitosan is a biopolymer derived from natural crustacean shells. Chitosan is a derivative product of chitin compounds obtained from the process of deacetylation of chitin under alkaline conditions (NaOH) [8]. To increase the capacity of phosphate uptake in acidic medium, modification of active amine group (−NH) was modified in chitosan such as functionalization, grafting, cross-linking and biocomposite forms [4]. In this study, Chitosan-Bentonite biocomposite adsorbents were used and loaded with Fe to improve the stability of biocomposite and phosphate uptake capacity in water. The synthesized Fe-CSBent biocomposite were characterized using Fourier Transform Infrared (FTIR), Scanning Electron Microscopy (SEM), Energy Dispersive X-Ray Spectroscopy (EDS) and X-Ray Diffraction (XRD) analysis, and the synthesized diffusive gel and binding gel were characterized using FTIR analysis. Phosphate binding test was conducted on DGT-Fe-CSBent with various parameters, i.e. pH and presence of co-existent anion i.e. sodium tripolyphosphate (STPP). The mass of bound phosphate can be calculated after eluted the binding gel with acid and then measured with molybdenum blue method using UV-Vis spectrophotometry.

2. Materials and methods

2.1. Materials

Chitosan, bentonite (PT. Madani Alam Lestari), cellulose nitrate membrane (GE Healthcare Life Sciences), FeCl₃·6H₂O, glacial acetic acid, N,N′-methylenebisacrylamide, acrylamide, ammonium persulphate, N,N,N′,N′-Tetramethylethlenediamine (TEMED) 99 %, potassium antimony tartrate, ammonium molybdate, H₂SO₄, (p), and ascorbic acid.

2.2. Synthesis of Fe-CSBent biocomposites

The preparation of Fe-CSBent biocomposites is based on the procedure of Kumar and Viswanathan [4]. Chitosan (CS) was dissolved in 2% acetic acid solution and stirred for 2 hours at 40 °C. Na-Bentonite (Bent) was dispersed with double distilled water then stirred for 2 hours. Slowly added the bentonite dispersion into the chitosan solution, then heated at 60 °C for 24 hours with high intensity stirring. After 24 hours, the metal ion was loaded by adding FeCl₃·6H₂O 3 % (w/v) and stirred for 2 hours. The obtained Fe-CSBent biocomposite slurry was kept for 24 hours for the aging process, followed by filtered the slurry and washed with double distilled water. The biocomposite was dried and mashed into powder and sieved to obtain a uniform biocomposite size.

2.3. Gel preparation and mounting

Preparation of gel solution was based on Zhang et al. [5] procedure. Combined acrylamide and crosslinkers N, N′-methylenebisacrylamide until homogeneous then added initiator ammonium persulfate and catalyst TEMED. The gel solution was immediately casted between two glass plates separated by 0.1 mm wide glass and heated at 42 ± 2 °C for 45 minutes. The binding gel was made by mixing the Fe-CSBent biocomposite and gel solution and the same procedure was performed as
diffusive gel. Gel diffusive and binding gel were hydrated with double distilled water for at least 24 hours before use. Throughout this hydration, the gel will be expanded to a steady shape and also washed the impurities on the gel. The diffusive gel and binding gel were stored in 0.1 M NaNO₃ and double distilled water, respectively.

Prior to the installation of the DGT component, the gels (diffusive gel and binding gel) was cut with a 25 mm diameter DGT cutter and the cellulose nitrate membrane was soaked in double distilled water. The binding gel was placed first on the DGT device, followed by diffusive gel and cellulose nitrate membrane. The DGT device was then closed properly.

2.4. Measurement of DGT-Fe-CSBent performance and response
Orthophosphate uptake by DGT-Fe-CSBent and DGT-CSBent used 2 ppm KH₂PO₄ solution. The DGT device was inserted into the solution for 24 hours (optimum time). DGT-Fe-CSBent performance test included 4 parameters, homogeneous of the binding gel, deployment time, pH of the solution, and presence of co-existent anion i.e. sodium tripolyphosphate (STPP).

2.5. Analytical methods
After immersion of the DGT device, the binding gel was eluted in H₂SO₄ 0.25 M for 16 hours. Initial phosphate concentrations and eluted concentrations were measured with the molybdenum blue method using UV-Vis spectrophotometry. The concentration of phosphate in the DGT can be calculated using the equation derived from Fick’s First Law [5]:

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

2.6. Characterization
The synthesized biocomposites were characterized by Fourier Transform Infrared (FTIR), X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDS).

3. Results and discussion

3.1. Characterization of biocomposites
FTIR spectra of bentonite, chitosan, CSBent and Fe-CSBent are shown in figure 1a. The band observed around 3600 cm⁻¹ indicated the presence of O–H stretch of bentonite, CSBent and Fe-CSBent. At 2895 cm⁻¹ indicated the presence of C–H stretch band of chitosan, CSBent, and Fe-CSBent. In the chitosan spectra, it showed that N–H bending band at 1585 cm⁻¹ but it showed in the CSBent and Fe-CSBent spectra that the N–H bending band is at 1523 cm⁻¹. This is due to −NH₂ in chitosan protonated into −NH₃⁺ during biocomposite synthesis [9]. At 1041 cm⁻¹, it showed the presence
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Figure 2. SEM Image of (a) Fe-CSBent before adsorption phosphate 1000 x magnification, (b) Fe-CSBent after adsorption phosphate 1000 x magnification, (c) Fe-CSBent before adsorption phosphate 2500x magnification, and (d) Fe-CSBent after adsorption phosphate 2500 x magnification.

of C–O–C band in chitosan, CSBent, and Fe-CSBent [10]. The presence of Al–Al–OH (stretching deformation) and Al–O(OH)–Al (stretching vibration) bands are found in 906 cm$^{-1}$ [11] and Si–O–Al (vibration bands) and Si–O– (bending and stretching vibration) bands are found in 519 cm$^{-1}$ in CSBent and Fe-CSBent [12].

Based on diffraction pattern that are shown in figure 1b, it showed that the peak of bentonite shifted between bentonite before and after synthesized into biocomposite. According to Bragg’s law, the smaller of $\theta$ (degree) the greater the value of $d$. This explains the shift in the diffraction degree of bentonite at $2\theta = 6.96625^\circ$ and synthesized CSBent at $2\theta = 5.74633^\circ$ which caused by the increasing interlayer distance between atoms in bentonite after being synthesized into biocomposite. This proves that the expansion of bentonite structure after synthesis.

The SEM image in figure 2a and figure 2c show the presence of pores on the surface morphology of Fe-CSBent before phosphate adsorption with 1000x and 2500x magnification, respectively. Figure 2b and figure 2d show that on the surface of Fe-CSBent after phosphate adsorption is asymmetrical and believed to be succeeded in phosphate adsorption. The SEM-EDS results (figure 3a and figure 3b) show an increase in element O and the presence of element P in the Fe-CSBent sample indicated that the phosphate was successfully adsorbed by Fe-CSBent.

3.2. Characterization of diffusive and binding gel

The FTIR spectra of acrylamide, diffusive gel, CSBent gel, and Fe-CSBent gel are shown in figure 4. The FTIR spectra of acrylamide show the characteristic bands at 3180–3350 cm$^{-1}$, 1673 cm$^{-1}$, 1612 cm$^{-1}$, and 1299 cm$^{-1}$ indicating the presence of N–H stretch, C=O stretch, C=C stretch, and C–N stretch, respectively. The FTIR spectra of diffusive gel, CSBent gel, and Fe-CSBent gel show the characteristic bands at 3180 cm$^{-1}$, 2900 cm$^{-1}$, 1673 cm$^{-1}$, and 1296 cm$^{-1}$, indicating the N–H stretch, C–H stretch, C=O stretch, and C–N stretch, respectively. In the acrylamide FTIR spectra, it is shown the existence of the C=C band but no existence of the C=C band in the diffusive gel and binding gel. This is because polymerization of polyacrylamide gel has been successfully performed.
Figure 3. SEM-EDS of (a) Fe-CSBent before adsorption phosphate and (b) Fe-CSBent after adsorption phosphate.

Figure 4. FTIR characterization of acrylamide, diffusive gel, CSBent gel, and Fe-CSBent gel

3.3. Mechanism of phosphate binding by DGT-Fe-CSBent

The mechanism of phosphate binding by DGT is the phosphate species diffused through the diffusive layer and then bound by the adsorbent in the binding gel. The use of cross-linker N, N'-methylenebisacrylamide is to create gel that has pores, so only orthophosphates can be diffused through the gel. The diffused orthophosphate is then bound by Fe-CSBent. Figure 5 shows the binding of phosphates by Fe-CSBent through three mechanisms: electrostatic interaction between the adsorbent surface and the phosphate, complexation of Lewis metal ion with the phosphate, and ion exchange. The chitosan surface (−NH₂) at the low pH condition (pH<5.0) will be protonated (−NH₃⁺),
Figure 5. Mechanism of phosphate binding by DGT-Fe-CSBent.

which causes the chance of electrostatic interaction between phosphate ions and the surface of the adsorbent [10,13]. The metal ion, in this case Fe is considered to act as Lewis acid that will combine with negatively charged phosphate species that are considered as strong Lewis bases. The inner sphere complex formation between HPO₄²⁻ and the hydrated metal oxide in the synthesized biocomposite, Fe(OH)₃ could be occurred [14]. The hydroxyl group (−OH) in bentonite will be replaced with phosphate ion by ion-exchange in Fe-CSBent biocomposite, the possible mechanism of phosphate binding by DGT-Fe-CSBent [4].

3.4. DGT-Fe-CSBent performance and response
Homogeneity tests were performed to determine whether the binding gel used in the test was homogeneous or not. Based on the experiment, Cₒ value from the four repetitions on both gels obtained have almost the same results (figure 6a). The average orthophosphate concentration bound by DGT-CSBent was 1.7333 μg/mL with standard deviation of 0.1784 and 79.874 % efficiency. The average orthophosphate concentration bound by DGT-Fe-CSBent was 2.2970 μg/mL with a standard deviation of 0.1105 and 90.208 % efficiency. DGT-Fe-CSBent binds orthophosphates more efficiently compared to DGT-CSBent. It can be concluded that both CSBent and Fe-CSBent gels were homogeneous enough.

The optimum deployment time of DGT obtained in this experiment was at 24 hours, after 24 hours the Cₒ of orthophosphate values were almost stagnant because of the lack of empty sites available on the Fe-CSBent binding gels so that have reached the equilibrium stage (figure 6b). The influence of pH solution was done to determine the effect of pH on phosphate binding and to determine the tolerable pH range in phosphate binding by DGT. Phosphate binding test done at pH 3–6 showed good results. At pH>6, there was a decrease in phosphate binding efficiency by DGT due to the electrostatic repulsion between negatively charged phosphate ions and hydroxyl ions (OH). At pH<6, electrostatic attraction occurred between the negatively charged phosphate ions and the protonated biocomposite surface resulting in maximum phosphate binding [4].

3.5. Effect of co-existing anion (sodium tripolyphosphate) on orthophosphate binding by DGT
Phosphates in nature are available in the form of orthophosphates, polyphosphates (pyro-, meta-, etc.), as well as organic phosphates. Phosphate analysis can be classified into two methods, first is the total phosphate analysis, i.e. conversion of phosphate form to orthophosphate by oxidation destruction process and second is soluble phosphate analysis, i.e. measuring the phosphate from orthophosphate without destruction [15]. The destruction process used strong acid that is H₂SO₄ and HClO₄. The destruction process will hydrolyze the polyphosphate into orthophosphate, which its concentration as the total phosphate can be determined by the molybdenum blue method [16]. Research using DGT method is a method made for the measurement of bioavailable phosphate where DGT can absorb the bioavailable phosphate in water in accordance to the phosphate uptake by biota such as algae.

It can be seen that Cₒ of orthophosphate (without destruction) increases by the addition of STPP concentration (figure 7). This is due to the STPP which may have diffused through the diffusive gel and bound by the Fe-CSBent on the binding gel. To prove that STPP diffused through the diffusive gel and bound by Fe-CSBent on the binding gel, a comparison of Cₒ of orthophosphate (without
Figure 6. DGT performance and response with various parameters (a) homogeneous of the binding gel, (b) deployment times, and (c) pH of solutions.

Figure 7. Effect of co-existing anion (STPP) on orthophosphate binding by DGT destruction) and total phosphate (with destruction) was performed. It appears that the total phosphate concentration (orthophosphate and polyphosphate of STPP) obtained from the destruction analysis is greater than the orthophosphate concentration (without destruction). It can be concluded that in the elution solution there is also STPP bound to Fe-CSBent in the binding gel. Although STPP diffuses into binding gel, orthophosphate is the bioavailable phosphate in the aquatic system in accordance to phosphate uptake by biota such as algae.
4. Conclusions
Modification of binding gel with biocomposite in DGT can bind with phosphate. DGT-Fe-CSBent binds with orthophosphates more efficiently compared to DGT-CSBent. DGT-Fe-CSBent device, which was made to be selective in predicting the bioavailable phosphate, but the presence of other phosphate species such as polyphosphate, may influence the orthophosphate binding by DGT.

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