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Characteristics of a cobalt-based phosphate microelectrode for *in situ* monitoring of phosphate and its biological application

Woo Hyoung Lee¹, Youngwoo Seo², and Paul L. Bishop^{1,*}

¹ Department of Civil and Environmental Engineering, University of Cincinnati, Cincinnati, OH, 45221-0071, USA

² Department of Civil Engineering, University of Toledo, Toledo, OH, 43606, USA

Abstract

The *in situ* monitoring of phosphate has been of great importance in many environmental applications, particularly those involving biological treatment processes and eutrophication monitoring. A microelectrode with small tip size (~10 μm) was fabricated with cobalt wire, characterized and evaluated for *in situ* and *in vivo* environmental analysis of phosphate in biological applications. The electrochemical performance of this cobalt-based microelectrode was fully examined for its characteristics, including detection limit, response time, selectivity, reproducibility, life time, interference with pH, ions and dissolved oxygen (DO), and the stirring effect. The microelectrode showed excellent selectivity for the orthophosphate ions (HPO_4^{2-} , H_2PO_4^-) in various environmental conditions. Alkalinity and DO were found to interfere with electrode response to phosphate. The phosphate microelectrode was also evaluated with Scanning Electron Microscopy (SEM) and Cyclic Voltammetry (CV). The developed microelectrode was used for *in situ* monitoring of phosphate in microbial flocs. Well-defined phosphate profiles across the flocs were observed under anaerobic (phosphorus release) conditions. This full characterization and successful application showed that the cobalt-based phosphate microelectrodes can be a very useful tool for *in situ* measurement of phosphate in various environmental conditions, including within microbial flocs.

Keywords

Microelectrodes; Phosphate; Cobalt wire; Microbial flocs; *In situ* monitoring

1. Introduction

The *in situ* monitoring of phosphate has been of great importance in many environmental applications, particularly those involving biological treatment processes and eutrophication monitoring. Phosphate measurements also are needed to control the amount of phosphorus supplied to crops as fertilizer, and to determinate phosphate concentrations in body fluids for clinical diagnoses like hyperparathyroidism, vitamin D deficiency and Fanconi syndrome [1–3]. This paper describes a phosphate microelectrode developed to evaluate the wastewater phosphorus removal system. The enhanced biological phosphorus removal (EBPR) process is operated using poly-phosphate accumulating organisms (PAOs) that have the unique ability

*Corresponding author phone: (513)556-3675, fax: (513)556-2599, e-mail: Paul.Bishop@UC.edu.

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for luxury phosphate uptake and release during aerobic and anaerobic conditions, respectively. Many models have been developed to elucidate the phosphorus removal mechanisms in the EBPR process, including the ASM2 model [4]. However, these models have focused on macro-scale monitoring, including bulk solution analyses, operation mode, and modification of reactor type to enhance the phosphorus removal efficiency and to understand this mechanism. For better understanding of the phosphorus removal mechanism, there is a clear need for *in situ* monitoring of phosphate within the biofilms and/or activated sludge flocs, using microelectrodes with a very small diameter tip.

Many electroanalytical techniques have been developed to fabricate sensors, and significant effort has been given to the development of phosphate-selective electrodes [5–12]. In recent years, Xiao et al. [13] introduced cobalt as a phosphate-sensitive electrode material and showed that oxidized surfaces of metallic cobalt could be used as a selective potentiometric sensor for phosphate. They suggested a host-guest mechanism to explain the phosphate electrode response. Meruva et al. [14] found that the phosphate electrode response depended on pH, ionic strength, buffer salts and stirring rate. They suggested a mixed potential response mechanism, in which a cobalt oxide layer should be first generated on the surface of the cobalt in deionized (DI) water or buffer solution without phosphate ion, and then used to detect the phosphate ion in the sample or standard solution. This cobalt oxide reacts with phosphate ions in the solution and forms cobalt phosphate precipitates at the electrode surface, depending on pH values in the solution (Eq. (1)–(3)). These reactions give a voltage output which can be converted to phosphate concentration [13].

A fabricated microelectrode was evaluated with static potentiometric techniques, in which the potential of an electrochemical cell is measured at equilibrium when no electrolysis is occurring and hence the net current is zero [15].

This paper describes the performance of phosphate microelectrodes used in various environmental conditions and their application to actual environmental analysis. The electrochemical performance of a cobalt-based microelectrode with very small tip size (~10 μm) was fully examined for its characteristics, including detection limit, response time, selectivity, reproducibility, life time, interference with pH, ions and dissolved oxygen, and the stirring effect. The response to phosphate was also evaluated with Scanning Electron Microscopy (SEM) and Cyclic Voltammetry (CV). The developed microelectrode was applied for *in situ* monitoring of phosphate in microbial flocs in the anaerobic phase of the EBPR process.

2. Experimental section

2.1. Microelectrode fabrication

A section of cobalt wire (0.1 mm diameter, 99.995% pure, Aldrich Chemical Company), with an original diameter of 0.1 mm, was cut and etched in 2 M KCN until it reached 3 μm in

diameter. After heating and pulling a glass micropipette (O.D.: 1.2 mm, I.D.: 0.69 mm, 15 cm length, Sutter Instrument Co.) over the flame of a small burner, the etched cobalt wire was inserted into the pulled glass micropipette; the middle section of this micropipette was then melted to seal the cobalt wire in the glass using heat from a trough heating filament (Sutter Instrument Co.). The tip, which was wrapped with glass, was then beveled on a diamond abrasive plate (Sutter Instrument Co.) with a 45 ° angle to expose the cobalt surface. Next, the microelectrode was assembled with copper wire using bismuth alloy (44.7% bismuth, 22.6% lead, 19.1% indium, 8.3% tin and 5.3% cadmium). The copper wire was then connected to a Bayonet Neill-Concelman (BNC) cable. The finished phosphate microelectrode has a 5 ~ 10 µm tip diameter which allows for measuring the phosphate concentration in biofilm or activated sludge flocs without destroying the biofilm structure. (See Fig. S1, Supplementary data.) Fig. S1 shows that the cobalt wire was well exposed and coated with a glass body, with a 45 ° angle.

2.2. Pretreatment

The phosphate microelectrode was connected with an Ag/AgCl reference milli-electrode (MI-401, Microelectrodes Inc.) and immersed into DI water to form a cobalt oxide (CoO) layer on the surface of the microelectrode tip. Fig. 1 shows schematically the experimental setup for characterization and evaluation of the phosphate microelectrodes. The potential between the two electrodes was then monitored using a mill volt (mV) meter (Model 215, Denver Instruments). After a stable potentiometric response was obtained below -250mV, the microelectrode was removed from the DI water and immersed in 10⁻⁴ M KH₂PO₄ solution at pH 7.5 until a steady-state potential was observed. Through the pretreatment step, the cobalt-based phosphate ion microelectrodes achieved sensitivity towards the two main forms of phosphate ions (H₂PO₄⁻ and HPO₄²⁻) of interest.

2.3. Evaluation of microelectrode performance

Ortho-phosphate standard solution was prepared with KH₂PO₄, and the pH of each standard solution was adjusted by adding potassium hydroxide. A commercial Ag/AgCl milli-electrode was used as a reference electrode. Evaluation of the finished phosphate microelectrodes was conducted at ambient oxygen levels and at room temperature (23°C), and all calibration measurements at each experiment were replicated three times. Commercial oxygen mini-electrode (OM-4 Oxygen sensor, Microelectrodes Inc.) was used to verify the oxygen concentration in the standard solution during the DO interference experiment. A pH meter (Model 15, Accumet) with a pH/ATC combination electrode (Cat. No. 13-620-112, Accumet) was used to verify the pH value during the experiment. A mV meter (Model 215, Denver Instruments) was used to obtain potentiometric responses, and a Balance Talk SLTM (Labtronics Inc.) spread logger was used to continuously collect data. All experiments were conducted in a Faraday cage to minimize electrical noise and at 23°C room temperature, as illustrated in Fig. 1. An Analyst 300 Furnace and Flame Atomic Absorption Spectrometer (FAAS) with a graphite furnace were used to measure aqueous cobalt metal concentrations. MINEQL+ (Environmental Research Software, Hallowell, ME) chemical equilibrium modeling system software was used to investigate the dominant species of phosphate under different pH conditions [16].

2.4. Cyclic voltammetry

A BAS 100B electrochemical analyzer (Bioanalytical Systems Inc.) was used to record the cyclic voltammograms (CVs). An Ag/AgCl reference electrode was fitted with a Pt disk as the auxiliary electrode for voltammetry experiments. The scan rate was 50 mV/s when the total phosphate standard solution was varied from 10⁻⁵ to 10⁻² M in 0.05 M KHP buffer solution at pH 4.0.

2.5. Surface characterization

Scanning electron microscopy (SEM) studies with energy dispersive X-ray spectroscopy (EDS) were carried out with a XL30ESEM-FEG (Philips XL Series) with a 25keV beam energy for characterization of the surfaces of cobalt microelectrodes before and after exposure to phosphate standard solutions.

2.6. Operational conditions of SBR

A bench-scale sequencing batch reactor (SBR) with 4L volume was operated in the sequence of fill, mix, aerate, idle, settle, and decant phases for more than six months to provide environmental samples. The sludge retention time (SRT) was maintained at 10 days by wasting an appropriate amount of mixed liquor directly from the reactor at the end of the aerobic cycle. The hydraulic retention time (HRT) was 8 hours, including 2 hours in the anaerobic phase (mix) and 4 hours in the aerobic phase (aeration). Mixed liquor suspended solids (MLSS) was 1,900 ~ 2,000 mg/L. Synthetic wastewater of the following composition was used as the feed solution: 384.4mg/L CH_3COONa (200 mg/L as COD (Chemical Oxygen Demand) basis), 33.7 mg/L KH_2PO_4 (7.5mg/L as $\text{PO}_4^{3-}\text{-P}$), 93.3 mg/L $(\text{NH}_4)_2\text{SO}_4$ (20mg/L as $\text{NH}_4^+\text{-N}$), 10.5 mg/L CaCl_2 , 90 mg/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1mg/L yeast extract and 0.3 mL of nutrient solution per liter.

2.7. Micro-profile measurements

The microbial flocs were taken from the SBR reactor at the end of the anaerobic stage in the EBPR operation. The micro-profile measurements were carried out using an up-flow chamber with laminar flow conditions so that the floc was kept suspended but stationary while the microelectrode was inserted into the floc [17,18]. Positioning and movement of the tip of the microelectrode was accomplished using a three-dimension micromanipulator (Model 11N, Narisige, Japan) located outside the Faraday cage (TMC, MA). The measurement was monitored under stereo microscope with a CCD camera (Model JE-3662 HR, Javelin Elec., Torrance, CA). Feed solution with the same composition as in the anaerobic stage of the operation was continuously recycled using a peristaltic pump. Nitrogen gas was injected into the feed tank to establish anaerobic conditions. Several profiles were measured at 100 μm intervals into the flocs. The potential for phosphate was measured with a mV meter (Model 215, Denver Instruments). Phosphate concentrations of bulk solutions were measured using a Dionex (Sunnyvale, California) Model DX-120 ion chromatography apparatus equipped with Dionex AS14-4 and AG 14-4 columns and a conductivity detector.

3. Results and discussion

3.1 Phosphate microelectrode calibration

Fig. 2 shows the finished phosphate microelectrode. Seven different concentrations of standard phosphate solution were prepared with a range of $10^{-5.8}$ to 10^{-2} M KH_2PO_4 for calibration. To match the typical pH value as wastewater in the EBPR process, the pH of the standard solutions was adjusted to 7.5 by adding potassium hydroxide. At pH 7.5, MINEQL+ showed dihydrogen phosphate ion and hydrogen phosphate ion to be the dominant species, as shown in Table 1. Fig. S2 in the Supplementary data shows that the phosphate microelectrode performed linearly, and exhibited high sensitivity toward these two ions, with slopes of 33.1 mV/decade over a wide range of concentrations. The response time during the calibration was less than 1 minute. Fig. 3 describes the detection range of the phosphate microelectrode which was calibrated several times over two days. Even though a steady state potential during a continuous period of measurement was observed at a low concentration of 10^{-6} M, the standard deviations at concentrations below 10^{-6} were high at 26.2 (at 10^{-7} M) to 56.3 (at 10^{-6} M) mV. Therefore, the detection range of the phosphate microelectrode was confined to the range of 10^{-5} to 10^{-1} M, as shown in Fig. 3. The detection limit of the phosphate microelectrode was

found to be $10^{-5.1}$ M (7.9×10^{-6} M), which was calculated using the value of the blank signal (-250 mV), standard deviation (56.3) at 10^{-6} and the slope of the calibration curve.

3.2. Stirring effect

The effect of stirring on phosphate measurement was investigated to verify sensitivity and stability of response in the flowing condition at pH 7.5. A stirring plate was used for agitation at 200 rpm and the phosphate concentration in the test solution was 10^{-3} M. For *in situ* phosphate measurements, the convection of the bulk solution where the biofilms or activated sludge flocs are immersed can be a critical factor for measurement error. Theoretically, stir rate should have no effect on the recorded potential under potentiometric conditions. With stirring, the response time was shortened and the electrode response value was found to change less than 5 mV, which is within $\sim 3\%$ of the linear range of measurement (data not shown here). The response factor (slope) of the calibration curve with stirring was found to maintain its linear relationship with phosphate concentration. It can be concluded that the response was not influenced by stirring.

3.3. DO interference with phosphate measurement

When 0% and 21% DO concentrations were applied to the standard solution, the electrode response was highly affected by the concentration of DO; the electrode response changed from -580 mV to -330 mV in 10^{-4} M standard solution at pH 7.5 (data was not shown). This result indicated that dissolved oxygen has a significant effect on the *in-situ* measurement of phosphate. To provide a more detailed understanding, the DO interference was assessed at eight different DO concentrations ranging from 0% to 14% at 23°C . These DO range were based on the operational results from an EBPR process operating in our lab.

Fig. 4 shows changes in the calibration curve with different DO concentrations. The calibration curves show that the DO effect on the measurement of phosphate when the DO is above 2 % is minimal. But it becomes more significant below 2 % DO. The value of the Y-intercept decreased as DO decreased, with the slope ranging from 30.8 $\sim$ 40.7. These results indicate that oxygen interferes with the binding mechanism between cobalt oxide and phosphate so that the response decreases as dissolved oxygen is increased. Dissolved oxygen concentrations between 0% and 2% have a significant effect on the sensitivity of the phosphate measurement. While DO concentration shifted the Y-intercept, the response (slope) still showed good sensitivity toward phosphate ions. From the results, it is recommended that DO profiles should be measured simultaneously with phosphate in environmental applications, especially microbial flocs in which DO is known to gradually be depleted along depth into the floc [18].

3.4. pH interference with phosphate measurement

When comparing results at pH values ranging from high (pH 12.0) to low (pH 4.0), a linear calibration was successfully obtained at most pH values. However, the response was poor at the highest pH. Examination using MINEQL+ showed that $\text{Co}(\text{OH})_2$ is the dominant species above pH 11.0. It appears, due to formation of the precipitate $\text{Co}(\text{OH})_2$ ($K_{\text{sp}}: 1.6 \times 10^{-15}$) at the surface of the cobalt, the electrode response was decreased, as was reported by Chen et al [19]. Therefore, the microelectrode is predicted to have selectivity to phosphate ion below pH 11.0. The pH interference was further studied across a narrow pH range from 6.7 to 8.4 to correspond to conditions within the EBPR process (pH 7.2 $\sim$ 8.4). Fig. 5 shows that the phosphate microelectrode has a linear relationship toward the two phosphate ions (H_2PO_4^- , HPO_4^{2-}) according to the solution pH, with the phosphate microelectrode being slightly more sensitive at higher pH. The proportion of these phosphate ions at each tested pH values is shown in Table 1. Typical pH variation across the floc is usually within 0.5 pH units [18]. In this range, the effect of any pH change is minimal, and there is no need to measure pH

simultaneously. However, in a case of highly varied pH, it is recommended that the calibration curve should be acquired at the appropriate pH before measuring phosphate.

3.5. Ion interference with phosphate measurement

Both indirect and direct methods were used to investigate potential ion interferences. For the indirect method, interfering ions were kept constant, while phosphate ion was varied from 10^{-2} to 10^{-5} M phosphate. In the direct method, phosphate ion was kept constant, while interfering ions were varied. The four common anions (sulfate, chloride, nitrate, and acetate) in wastewater were tested; the concentrations of these ions were chosen based on a literature review [20–22,24] and preliminary experiments, and are shown in Table 2. The middle concentrations (9.1×10^{-4} M SO_4^{2-} , 5.4×10^{-3} M Cl^- , 5.1×10^{-4} M NO_3^- , and 2.6×10^{-4} M CH_3COO^-) among three concentrations of each ion in Table 2 indicated the typical concentration in wastewater [24].

Fig. 6(a)–6(d) show the results of the individual ion interference investigations at pH 7.5. Even though the presence of ions shifted the electrode response toward phosphate ions, ion interference in typical wastewater at pH 7.5 was demonstrated to be minor and the microelectrode successfully produced a linear relationship with phosphate ions. Fig. 6(e) shows the interference of alkalinity at higher alkalinity in the sample on the electrode performance. Anions such as CO_3^{2-} , and OH^- in the solution were reported to react with Co^{2+} to form precipitates such as CoCO_3 ($K_{\text{sp}}: 1.4 \times 10^{-13}$) and Co(OH)_2 ($K_{\text{sp}}: 1.6 \times 10^{-15}$) in the presence of cobalt [23]; other researchers explained that these precipitants of CoCO_3 and Co(OH)_2 can affect the cobalt electrode response [16]. From Fig. 6(e), alkalinity over 100 mg/L as CaCO_3 was found to be an interference factor due to CoCO_3 and Co(OH)_2 precipitates formed on the tip surface of the microelectrode. Fig. 6(a)–6(e) also indicate that the developed microelectrodes response followed the pattern of $\text{HPO}_4^{2-} > \text{H}_2\text{PO}_4^- > \text{HCO}_3^- > \text{Cl}^- > \text{SO}_4^{2-} > \text{NO}_3^- > \text{CH}_3\text{COO}^-$, and selectivity coefficients [11] and ion strengths in Table 2 support these patterns.

Fig. 7 shows the calibration with real bulk solution used for measuring the microprofiles in the activated sludge flocs, compared to calibrations with and without mixed ions. The bulk solution was taken from the end of the anaerobic phase in the EBPR process, where the COD had been reduced from 216 mg/L to 10 mg/L and phosphate had increased from 3 mg/L as P to 15 mg/L as P due to the phosphate release by the PAOs. Even though the calibrations in real wastewater had lower slopes than those in standard solutions, the calibration curve still had a good linear relationship with phosphate ions and it was possible to measure the phosphate concentration in the microbial flocs with depth.

3.6. Life time and regeneration

For long term stability testing, the microelectrode was immersed in a solution with a high concentration of phosphate ($10^{-3.8}$ M KH_2PO_4). Fig. S3 in the Supplementary data shows the response was relatively stable for a long period (12 days). However, at low concentration of phosphate the response was found to be changed over time. Fig. 8 shows electrode response changes and reliabilities for phosphate measurement at several contact times (0 to 10 days) at various phosphate concentrations. The freshly fabricated microelectrode was contacted with phosphate in a 10^{-4} M phosphate standard solution at pH 7.5, and the calibration curve was determined after 0, 2, 4, 6, and 10 days. Over longer contact times, the electrode response at the high concentration of $10^{-3.8}$ M phosphate did not change much, but a larger difference was observed in the potential at lower phosphate concentrations. It appears that cobalt phosphate precipitation ($\text{Co}_3(\text{PO}_4)_2$) from the reaction shown in Eq.(2) could decrease the response at low concentrations of phosphate. Results from SEM with EDS analysis showed that the phosphate weight percentage at the surface area increased after the phosphate reaction due to

the formation of cobalt phosphate. With additional contact time, a recess at the tip of the microelectrode developed due to corrosion. Fig. S4(a) in the Supplementary data shows a recess length of 20 μm was generated at the 10 μm diameter tip of the phosphate microelectrode after 12 days of exposure to phosphate ion.

From these results, the cobalt phosphate precipitation and diffusional reaction due to the recess generation could increase the variation of response between certain phosphate concentrations and make more detail quantitative analyses for phosphate possible. Therefore, generation of a recess manually by etching before measurement is recommended to increase the response range. These results also indicate that shelf life time is not affected by the storage period, but is a function of the exposure time. Fig. S4(b) shows that the microelectrode had a 20 μm tip diameter after rebeveling and recovered its original characteristics, with a similar slope in the calibration as shown in Fig. 9.

3.7. Corrosion reaction characteristics

Fig. 10 shows the results of CV testing, which was conducted to elucidate the recess generation mechanism. The peak current at 200 mV increased with phosphate concentration, indicating that phosphate was involved in the electrochemical oxidation process. However, reduction on the cathodic side was less than oxidation at the anode. This showed that the response to phosphate is not based on any ion-selective equilibrium binding mechanism, but rather on changes in the oxidation corrosion potential of the wire due to cobalt ion binding to phosphate ions. Therefore, it appears that the recess was generated due to corrosion after response to the phosphate ion.

SEM evaluations with EDS were carried out to analyze the microelectrode surface layer before and after the corrosion reaction between phosphate and cobalt metal. Fig. S5 in the Supplementary data shows SEM images before and after reaction of cobalt with phosphate. The microelectrode had a clear surface before the phosphate reaction (Fig. S5(a)). After reaction with phosphate ion, cobalt corrosion was observed, as shown in Fig. S5(b). This result supports the theory that phosphate response at the cobalt surface was based on a change in oxidation corrosion potential, and corrosion at the surface of the microelectrode can explain the possible disintegration of cobalt metal seen in Fig. S5(b) when the current was employed, even though the soluble cobalt concentration in the solution was not detected; this was probably due to the very low concentration, below the detection limit of FAAS.

3.8. In-situ monitoring of phosphate in the microbial flocs

The phosphate profiles with depth in flocs taken from the anaerobic phase of the EBPR process were measured. Fig. 11 shows the micro-profiles measured during the anaerobic phase. The phosphate concentration in the bulk solution at the end of the anaerobic stage was about 15.4 mg/L as P. The phosphate was released at about two-fold the amount of the inflow concentration (7.5 mg/L as P) by the PAOs. As the microelectrode penetrated the flocs, the phosphate concentration was seen to increase, finally slowing as it reached a peak value of about 19.5 mg/L at the floc center. The phosphate micro-profiles indicated that PAOs were probably distributed evenly throughout the flocs. The results show that the monitoring of phosphate was successful in defining spatial distributions of phosphate inside microbial flocs without destroying their structures.

4. Conclusions

The performance of a new cobalt-based phosphate microelectrode was investigated under different conditions (e.g., pH, DO, with interfering ions, and different alkalinities). The developed microelectrode performed linearly and exhibited high sensitivity toward phosphate

ions. The response time during the evaluation was less than 1 minute. The detection range of the phosphate microelectrode was in the range of 10^{-5} to 10^{-1} M and the detection limit of the phosphate microelectrode was found to be 7.5×10^{-5} M. The electrode response was not influenced by stirring. While DO was found to be a factor during calibration, the response still showed good sensitivity toward phosphate ions. DO profile measurements along with phosphate profiles were suggested for more precise phosphate analyses. Simultaneous measurement of DO and P profiles in one location in a sample is difficult, though, for technical reasons. Therefore, three to five DO and phosphate profiles measurements are recommended at nearby positions for the phosphate analyses. Ion interference experimental results showed alkalinity above 100 mg/L as CaCO_3 in the solution may be a limiting factor for measurement of phosphate. The recess at the tip of the microelectrodes due to corrosion and cobalt phosphate precipitation generated from the reaction between cobalt oxide and phosphate explained the decrease in response at low concentrations of phosphate. However, this condition made it possible to measure phosphate more quantitatively. For better results, generation of a recess manually before measurement by the etching process could possibly increase the response range. Phosphate profiles in the flocs taken from the anaerobic phase of an EBPR process were measured to demonstrate biological application. The results showed that the monitoring of phosphate was successfully conducted and could define spatial distributions of phosphate inside microbial flocs without destroying their structures.

Overall, this full characterization and successful application showed that the cobalt-based phosphate microelectrode could be a useful tool for *in situ* measurement of phosphate in various environmental samples, including within microbial flocs. These micro-profiles, combined with other constituent profiles including pH, DO, ammonia, and ORP, in the flocs can be used to elucidate the dynamic activity of microbial processes in the EBPR process and can be valuable for designing operating systems or modeling efforts for biological nutrient treatment.

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References

- Engblom SO. The phosphate sensor. *Biosensors & Bioelectronics* 1998;13:981–994. [PubMed: 9839387]
- Tissen, H. Phosphorus in the Global Environment: Transfers, Cycles and Management. John Wiley and Sons; Chichester: 1995.
- Kawasaki H, Sato K, Ogawa J, Hasegawa Y, Yuki H. Determination of inorganic phosphate by flow injection method with immobilized enzymes and chemiluminescence detection. *Analytical Biochemistry* 1989;182:366–370. [PubMed: 2514613]
- Henze, M.; Gujer, W.; Mino, T.; Matsuo, T.; Wentzel, MC.; Marais, GvR. Activated Sludge Model No.2. (IAWQ Scientific and Technical Report No. 3). London: IAWQ; 1995.
- Glazier SA, Arnold MA. Phosphate-selective polymer membrane electrode. *Analytical Chemistry* 1988;60:2540–2542. [PubMed: 3245590]
- Watanabe E, Endo H, Toyama K. Determination of phosphate ions with an enzyme sensor system. *Biosensors* 1988;3:297–306. [PubMed: 3149200]
- Liu J, Masuda Y, Sekido E. Response properties of an ion-selective polymeric membrane phosphate electrode prepared with cobalt phthalocyanine and characterization of the electrode process. *Journal of Electroanalytical Chemistry* 1990;291:67–79.
- Midgley D. Sulphate and phosphate ion-selective electrodes. *Ion-Selective Electrode Review* 1986;8:3–54.
- Carey CM. Association constant for potassium-acid-phosphate determined by K and HPO_4^- ion selective electrodes. *Journal of Dental Research* 1997;76:97.

10. Lui D, Chen WC, Yang RH, Shen GL, Yu RQ. Polymeric membrane phosphate sensitive electrode based on binuclear organotin compound. *Analytica Chimica Acta* 1997;338:209–214.
11. Marco RD, Phan C. Determination of phosphate in hydroponic nutrient solutions using flow injection potentiometry and a cobalt-wire phosphate ion-selective electrode. *Talanta* 2003;60:1215–1221. [PubMed: 18969148]
12. Engblom SO. Determination of inorganic phosphate in soil extract using a cobalt electrode. *Plant and Soil* 1999;206:173–179.
13. Xiao D, Yuan HY, Li J, Yu RQ. Surface-modified cobalt-based sensor as a phosphate-sensitive electrode. *Analytical Chemistry* 1995;67:288–291.
14. Meruva RK, Meyerhoff ME. Mixed potential response mechanism of cobalt electrodes toward inorganic phosphate. *Analytical Chemistry* 1996;68:2022–2026. [PubMed: 9027219]
15. Strobel, HA.; Heineman, WR. *Chemical Instrumentation: A Systematic Approach*. John Wiley & Sons; New York: 1989.
16. Schecher, WD.; McAvoy, DC. *MINEQL+, A Chemical Equilibrium Modeling System Workbook*. 2. Environmental Research Software; Hallowell, ME: 2003.
17. Ploug H, Jorgensen BB. A net-jet flow system for mass transfer and microsensor studies of sinking aggregates. *Marine Ecol Progr Series* 1999;176:279–299.
18. Li B, Bishop PL. Oxidation-reduction potential changes in aeration tanks and microprofiles of activated sludge floc in medium- and low-strength wastewaters. *Water Environment Research* 2004;76(5):394–403. [PubMed: 15523785]
19. Chen Z, Grierson P, Adams MA. Direct determination of phosphate in soil extracts by potentiometric flow injection using a cobalt wire electrode. *Analytica Chimica Acta* 1998;363:191–197.
20. Wang, J. PhD Dissertation. University of Cincinnati; Cincinnati, OH, USA: 2005. Microscopic Study of Phosphorus Removal Process in an Activated Sludge System.
21. Erdal UG, Erdal ZK, Randall CW. The competition between PAOs and GAOs in EBPR systems at different temperatures and the effects on system performance. *Water Sci Technol* 2003;47(11):1–8. [PubMed: 12906264]
22. Yu, T. PhD Dissertation. University of Cincinnati; Cincinnati, OH, USA: 2000. Stratification of microbial processes and redox potential change in biofilms.
23. Dean, JA. *Analytical Chemistry Handbook*. McGraw-Hill; New York: 1995.
24. Wang J, Bishop PL. Development of a phosphate ion-selective microelectrode and its use in studies of the enhanced biological phosphorus removal (EBPR) process. *Environmental Technology* 2005;26:381–388. [PubMed: 15906489]

Biographies

Woo Hyung Lee is a PhD candidate in the Department of Civil and Environmental Engineering at the University of Cincinnati. He received his MS degrees in Civil and Environmental Engineering from Korea University, Seoul, Korea in 2001. His current research interests include development and application of microelectrodes to evaluate the wastewater treatment process and nitrification in the chloraminated drinking water distribution system.

Youngwoo Seo is an assistant professor in the department of civil engineering at the University of Toledo, Toledo, OH. He received his PhD in the Department of Civil and Environmental Engineering at the University of Cincinnati, OH. Before joining the University of Toledo, he worked as an environmental consulting engineer at CDM Inc. His current research interests include microscale analysis of bacteria adhesion and *in-situ* monitoring of microbial activity with microsensors.

Paul L. Bishop is Associate Vice President for Research and Professor of Environmental Engineering at the University of Cincinnati, Cincinnati, OH. He is also currently a Program Director at the National Science Foundation. He received his PhD in 1972 from Purdue University, West Lafayette, IN. His current research interests are bioremediation of

contaminated soils, disinfection of drinking water distribution system biofilms, and development of integrated multi-analyte microelectrode sensors for in situ environmental monitoring.

FIGURE 1.
Experiment setup for characterization and evaluation

FIGURE 2.
The finished phosphate microelectrode with small tip diameter ($\sim 10\ \mu\text{m}$).

FIGURE 3.
Detection range of the phosphate microelectrode at pH 7.5 and ambient oxygen levels.

FIGURE 4.
Dissolved oxygen interference with the phosphate measurement at pH 7.5.

FIGURE 5.
pH interference of the phosphate microelectrode with varied pH at ambient oxygen levels.

FIGURE 6.

Evaluation of the selectivity of the phosphate microelectrode against major anions (a) Sulfate ion; (b) Chlorine ion; (c) Nitrate; (d) Acetate ion; (e) Alkalinity at pH 7.5 and ambient oxygen levels.

FIGURE 7.

Comparison of calibration in pH 7.5 standard phosphate solution with mixed ions and without ions to bulk solution in anaerobic phase of EBPR process.

FIGURE 8.

Evaluation of the relationship between contact time and response change at pH 7.5 and ambient oxygen levels.

FIGURE 9.
Evaluation of regeneration through tip re-beveling at pH 7.5 and ambient oxygen levels.

FIGURE 10.

Cyclic voltammograms of phosphate microelectrode in 0.05M KHP Buffer at pH 4.0 with varying total phosphate concentrations. (a) 0 M no phosphate (0.05M KHP buffer solution); (b) 10^{-5} M phosphate; (c) 10^{-4} M phosphate; (d) 10^{-3} M phosphate; (e) 10^{-2} M phosphate.

FIGURE 11.

Micro-profiles of Phosphate in flocs from EBPR process at the end of the anaerobic phase. Floc diameter was about 1,000 μm and the center of the floc is depicted as a depth of 0 μm .

TABLE 1Phosphate species^a at different pH values.

pH	H_2PO_4^-	HPO_4^{2-}
6.7	73.7 %	26.3 %
7.2	47.1 %	52.9 %
7.6	31.0 %	69.0 %
8.4	0.4 %	99.6 %

^abased on MINEQL+ analysis

TABLE 2

Ion concentration range, selectivity coefficient and ionic strength for ion interference evaluation.

Ion	Reagent	Range for measurement (M)		Selectivity Coefficient[11]	Ionic Strength
SO_4^{2-}	K_2SO_4	9.1×10^{-3}	9.1×10^{-4}	1×10^{-3}	1.82×10^{-3}
Cl^-	KCl	5.4×10^{-2}	5.4×10^{-3}	2×10^{-3}	2.68×10^{-3}
NO_3^-	NaNO_3	5.1×10^{-3}	5.1×10^{-4}	8×10^{-4}	0.25×10^{-3}
CH_3COO^-	CH_3COONa	2.6×10^{-3}	2.6×10^{-4}	1×10^{-3}	0.12×10^{-4}