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Biosensor Development for Detection/Quantification of Phosphate in Hanford Contaminated Area

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ABSTRACT

Phosphate detection at the Hanford site is an essential tool for monitoring the stability of uranium in the field because it plays an important role in binding and precipitation. Since environmental factors can be influential for the stabilization of uranium via polyphosphate binding, the adsorption of phosphate in ground, soil and lake sediments must be determined. The stabilization of uranium via polyphosphate injection has been investigated by Wellman; et al (3). The study revealed that a reduced concentration of phosphate can reduce uranium stabilization. On the other hand, a high concentration of phosphate in water causes eutrification. In addition, when polyphosphate is added to the uranium contaminated area, stabilization of the uranium as apatite and sequestered as autunite has been reported by Wellman (4). Therefore, the investigation and quantification of phosphate species in this area is an essential resource for the uranium research, remediation and leaching processes. Despite the past research and previously developed phosphate detection devices, an improvement in design and field deployabiality of phosphate biosensors is still needed. For this reason, a new phosphate biosensor is being developed which employs higher technology products.

The objective of this task is to develop a biosensor that will detect the concentration of the phosphate species in the uranium contaminated area at Hanford. The method of detection is the immobilization of superior biocatalyst(s) on superconductive metal alloy(s) and micro-cantilever(s) for ground and lake sediments. Several phosphate related proteins were studied as biocatalysts to make enzymatic biosensors for the phosphate detection and quantification; however, the success is limited in terms of the activity and durability of the utilized enzymes, the conductivity of used materials, and the sensitivity of the biosensors (6). The detection mechanism of the biosensor was studied at Oak Ridge National Laboratories under the mentorship of Dr Thomas G. Thundat.

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1. INTRODUCTION

Uranium occurs in high concentrations at several contaminated sites in the United States (10). Due to the potential threat on human health, the U.S. Environmental Protection Agency (USEPA) has set a maximum contaminant level of 30 µg/L for uranium concentration in groundwater (11). Once released, the radionuclides persist in the environment and can have toxic effects on living organisms. However, by changing their chemical speciation, toxic and mobile species can be converted to nontoxic and immobile species (1). For this reason, in situ stabilization of uranium by polyphosphate amendments has gained popularity at some of the DOE sites contaminated with uranium (1).

Uranium in the environment can exist in two states of oxidation: +4 and +6. Uranium (VI) species are predominantly found in oxidizing environments and uranium (IV) prevails in reducing environments. Both of these species have a varying tendency towards complexation with other chemicals and are greatly affected by aquifer characteristics such as pH, redox status, ligand (fluoride, carbonate, sulfate, phosphate, and dissolved carbon) concentrations, and aluminum-oxide and iron-oxide mineral concentrations (7, 8, and 9). Environmental factors, such as pH, temperature, dissolved organic matter, and redox potential, have tremendous effects on both uranium and polyphosphates. For example, the hydrolysis of polyphosphates has been found to take place at lower pH ranges (8), which in turn can influence its reaction rate with uranium. The most commonly observed uranyl phosphate minerals can be formed by polyphosphate injection. The stabilization of uranium via polyphosphate injection has been investigated by Wellman, et al (3). The study revealed that reduced concentrations of phosphate can reduce uranium stabilization. On the other hand, a high concentration of phosphate in water causes eutrification. In addition, when polyphosphate is added to the uranium contaminated area, stabilization of the uranium as apatite and sequestered as autunite has been reported by Wellman. Since phosphate plays an important role in the soil and ground water sediments at Hanford, the amount of phosphate should be known in order to deploy the right quantity for remediation.

2. EXECUTIVE SUMMARY

The present research work has been supported by the DOE-FIU Science & Technology Workforce Initiative, an innovative program developed by the US Department of Energy's Environmental Management (DOE-EM) and Florida International University's Applied Research Center (FU-ARC). During the summer of 2009, DOE Fellow (Serkan Akar) spent 10 weeks performing a summer internship at Oak Ridge National Laboratory's Nano-Bio-Sciences Division under the supervision and guidance of Thomas George Thundat. This internship was coordinated by FIU's Applied Research Center, the Higher Education Research Experience program (HERE), and the Oak Ridge Institute for Science and Education (ORISE). The project was initiated from June 2, 2009 through August 8, 2009 with the objective to study the mechanism for detecting/quantifying the concentration of phosphate species for Hanford soil and groundwater sediments.

The objective of this task is to develop a biosensor that will detect the concentration of the phosphate species in the uranium contaminated area at Hanford. The method of detection is the immobilization of superior biocatalyst(s) on superconductive metal alloy(s) and micro-cantilever(s) for ground and lake sediments.

The most commonly observed uranyl phosphate minerals can be formed by polyphosphate injection. The stabilization of uranium via polyphosphate injection has been investigated by Wellman, et al (3). The study revealed that reduced concentrations of phosphate can reduce uranium stabilization. Techniques for phosphate-specific binding protein and the mechanism for detection were studied at ORNL. Preliminary data of electrochemically detection of phosphate has been gathered as a result of a series of successful experiments.

3. RESEARCH DESCRIPTIONS

The detection of phosphate has been an important tool for environmental and biological studies for decades. There has been many different detection methods investigated throughout the years. The sensitivity and response time of the analytical device have been the major issues in phosphate sensing. The uniqueness of the newly developed biosensor is that it uses a phosphate-specific protein. The protein only reacts with phosphate species. Therefore, the protein is immobilized on highly conductive and superconductive materials to compare the sensitivity of the device. Figure 1 shows the experimental setup for phosphate detection.

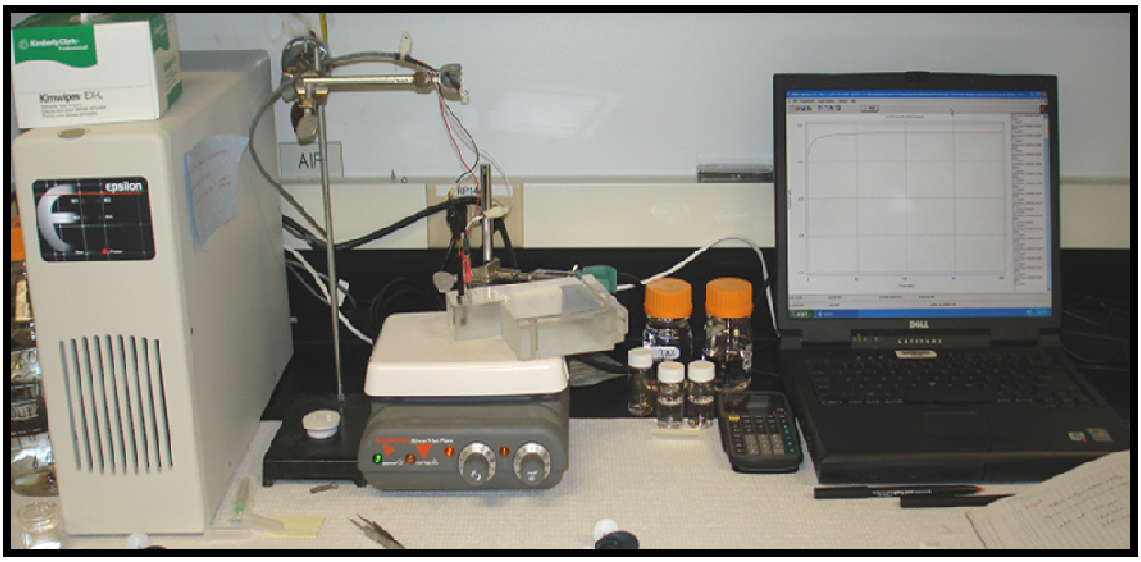

Figure 1. Setup for phosphate detection.

Phosphate (PO_4) has a specific binding affinity with proteins and this property is being used in this research. The detection method of the device is through electrochemical sensing techniques. The reaction below is used to for PO_4 detection.

$$Pyr + PO_4 + O_2 \xrightarrow{\text{Bio-catalyst}} \text{Acetyl P} + H_2O_2 + CO_2$$

PO_4 reacts with acid and produces hydrogen peroxide in the presence of oxygen and a bio-catalyst. The bio-catalyst makes the reaction extremely fast, which in turn increases the sensitivity and the response time. Thus, the construction of the biosensor will be built upon this verity. Figures 2 through 4 show the experimental cell and setup.

Figure 2. Cell made for experiment.

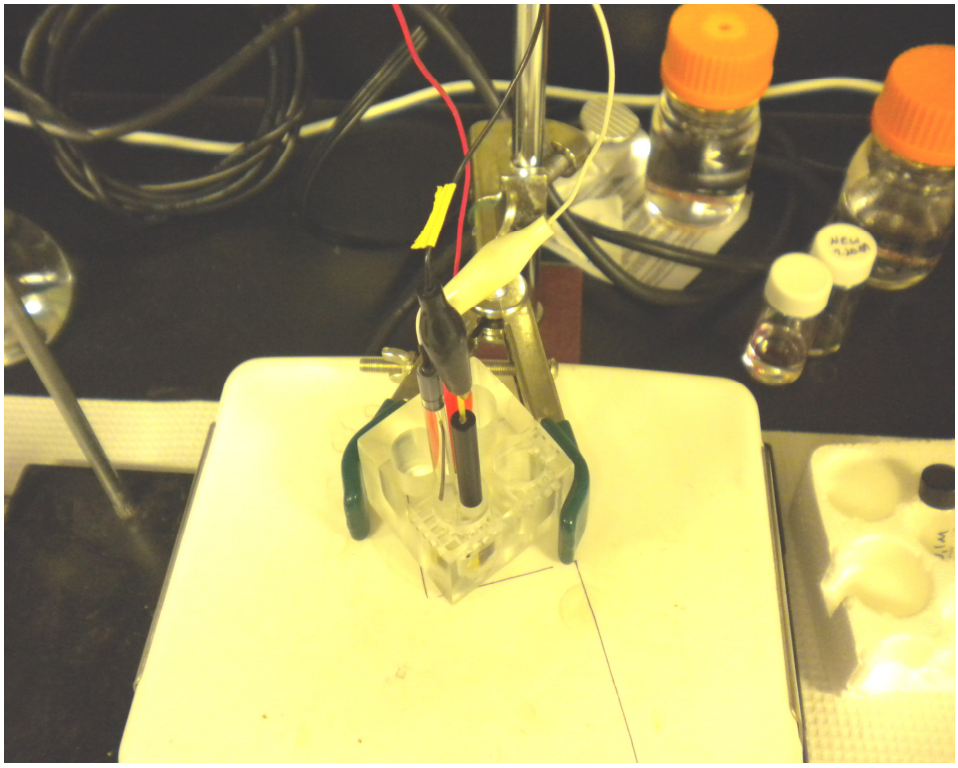

Figure 3. Experimental setup.

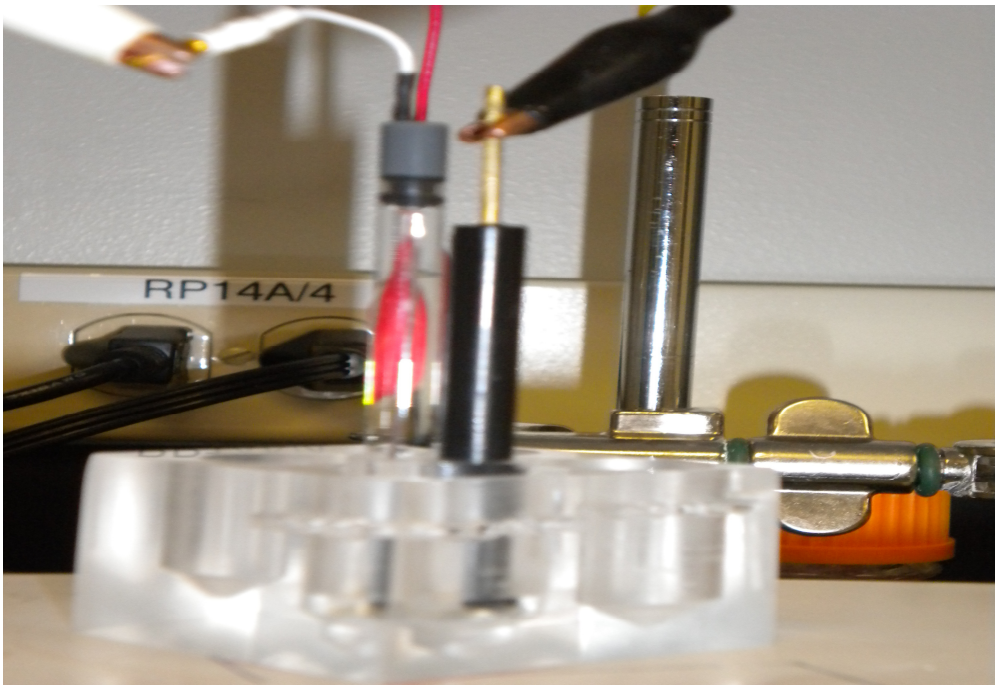

Figure 4. Experimental cell.

The development plan is outlined below:

1. Immobilization of biocatalyst on highly conductive metals (Carbon Nano-tubes with copper)
2. Immobilization of biocatalyst on super conductive metals (Multiwall Carbon Nano-tubes with Ni)
3. Compare the sensitivity of two metals

Immobilization of biocatalyst on metal substrate

First, the biocatalyst was biologically immobilized on highly conductive materials. The procedure of immobilization is as follows:

- Crosslinkers are moisture-sensitive; to avoid moisture condensation onto the product, the vial must be equilibrated to room temperature before opening (equilibration may require 30 minutes).
- Reconstitute the crosslinkers immediately before use. The NHS-ester moiety readily hydrolyzes and becomes nonreactive; therefore, do not prepare stock solutions for storage. Discard any unused reconstituted crosslinker.
- Hydrolysis of the NHS ester is a major competing reaction of the acylation reaction. Hydrolysis increases with increasing pH and occurs more readily in dilute protein or peptide solutions.

- Proteins that display biological activity (i.e., enzymes, antibodies etc.) may lose activity upon conjugation, which may be caused by conformational changes of the protein molecule when conjugated. Loss of activity may also occur when the crosslinker modifies lysine groups involved in binding substrate or an antigen.

After the immobilization process is completed, background buffer solution was placed into a homemade cell to run the experiment (Figure 5). Then, the immobilized metal is introduced to the system and phosphate is added. The phosphate is added in different concentrations to see the current change electrochemically.

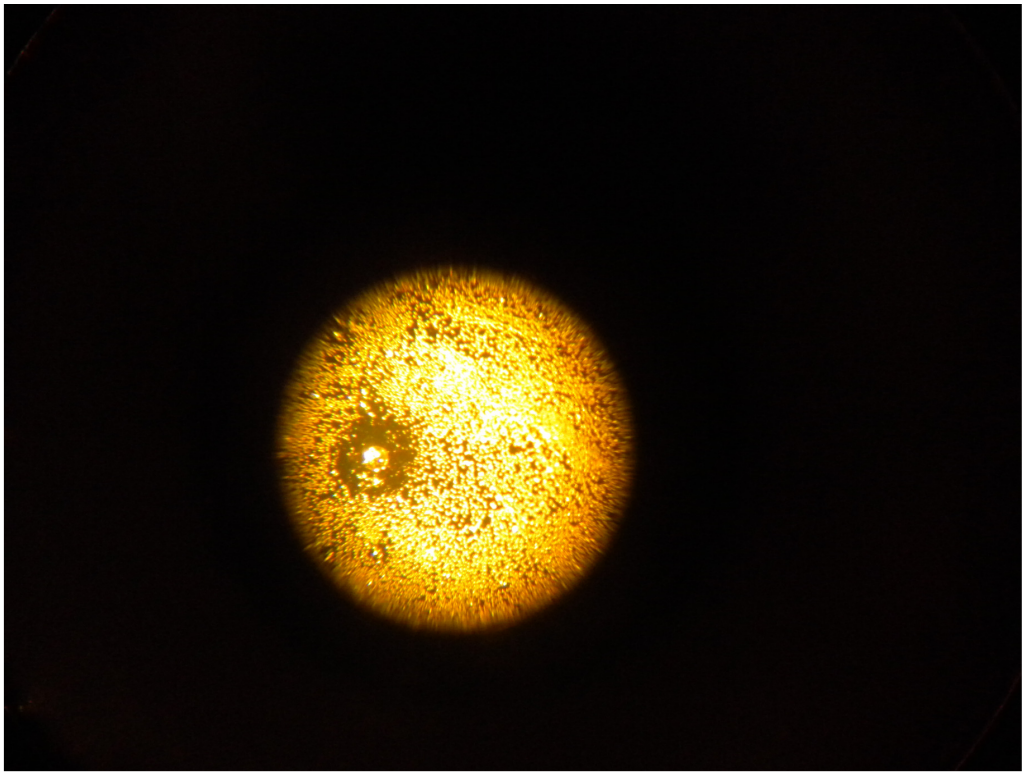

Figure 5. Microscopic view of immobilized bio-catalyst.

The immobilization process was done on a silicon based gold coated material. Silicon was coated with Au to obtain a substrate for proteins to attach. The proteins were conjugated for two reasons;

- Bio-catalyst (increases the rate of reaction)
- Specificity (chemical reaction occurs only in the presence of PO_4 and protein)

1. Immobilization of biocatalyst on highly conductive metals (Carbon Nano-tubes with copper)
2. Immobilization of biocatalyst on super conductive metals (Multiwall Carbon Nano-tubes with Ni)
3. Compare the sensitivity of two metals

Please note that, items 2 and 3 will be conducted at FIU/ARC as a continuation of this project due to the short time period of the internship.

4. RESULTS AND ANALYSIS

Method of detection of the biosensor is based on electrochemical analysis. Therefore experiments were done with the Epsilon electrochemical analyzer. The results of the electrochemical experiments shown in the figure 6-10 clearly indicated that the phosphate in soil and groundwater could be successfully detected and quantified. During the experiments, various concentrations of phosphate have been investigated and the table will be provided once the analysis of the data is done. Figures 6 through 10 show the graphs for some of the concentrations and Table 1 provides the results of experimental data.

In Figures 6 and 7, a background buffer solution is tested to make sure there is no other chemical is present in the solution. Cyclic Voltammetry is a diagnostic tool that indicates the existence of chemical composition in the solution tested (Figure 6). Amperometry on the other hand distinctively indicates how much of the addition of new chemical was performed in to the new solution. As figure 7 indicates that there was no addition of any chemical in to the background solution.

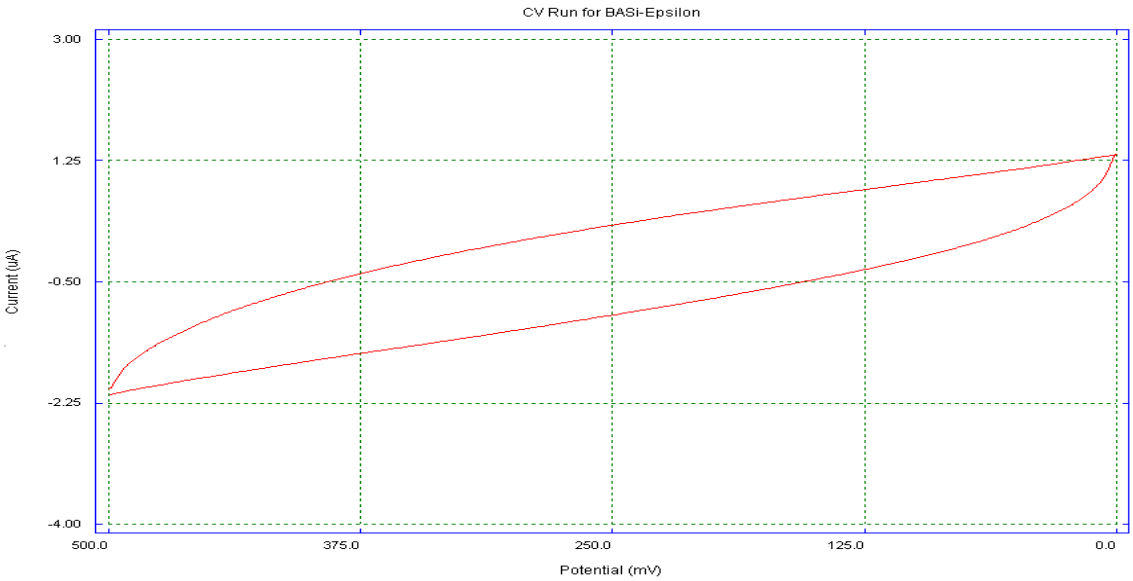

Figure 6. Cyclic voltammetry background for buffer solution.

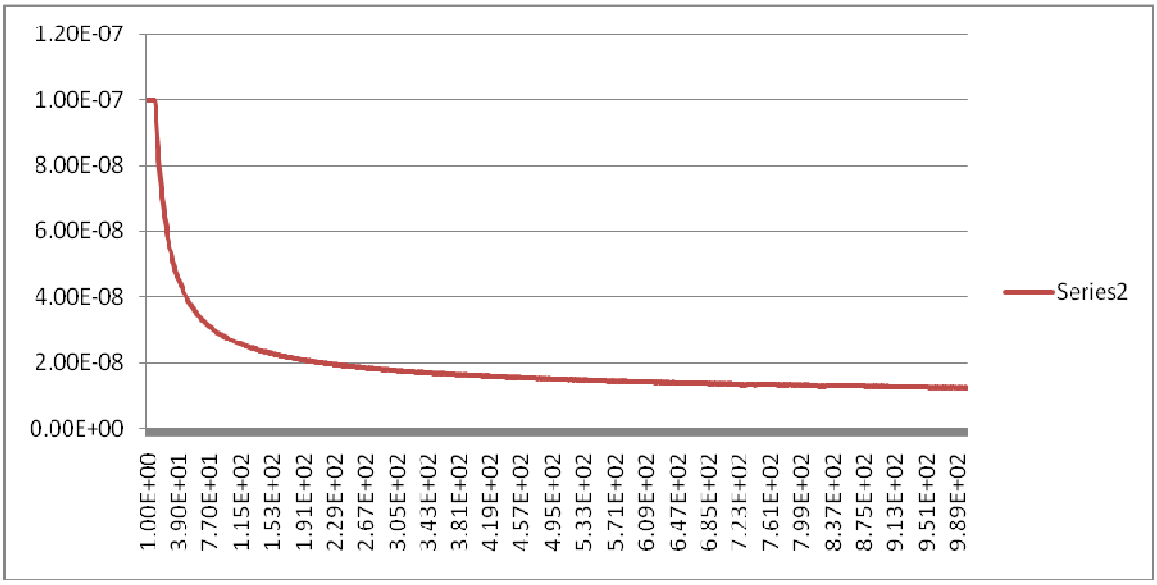

Figure 7. Amperometric background data.

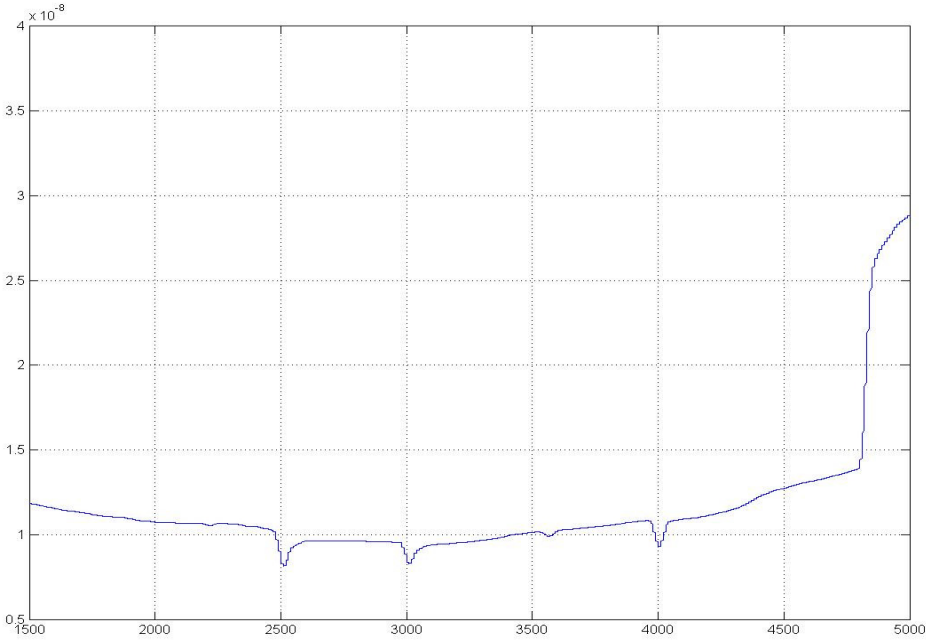

Figure 8. PO_4 addition points into buffer solution.

In Figure 8, an amperometric test was conducted and phosphate was added at different time periods. First the background solution gets disturbed and stabilizes back to the normal state. After certain addition points the concentration of the solution gets adopted to the new concentration point.

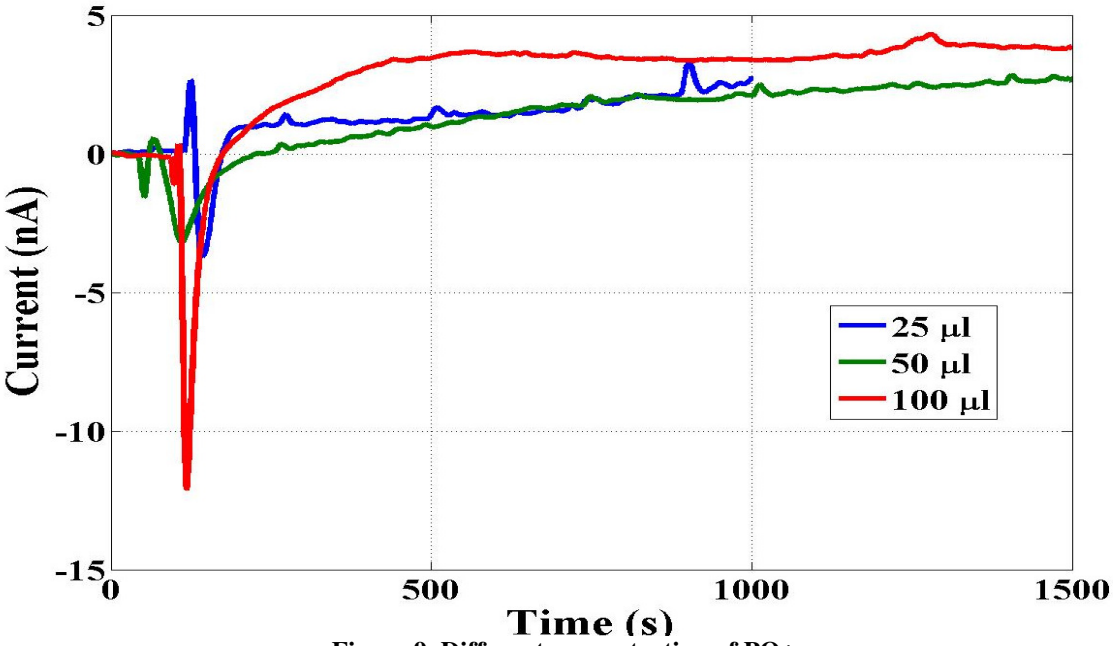

Figure 9. Different concentration of PO_4.

In Figure 9, different amount of phosphate addition is presented. The phosphate was added in to the background solution as 25µl, 50µl and 100µl. The slope of the different amount of phosphate graphs were analyzed and place on the same graph to identify the slope change. The slope is an indication of the concentration change which the calibration curve (Figure 10) was obtained based on the slope change.

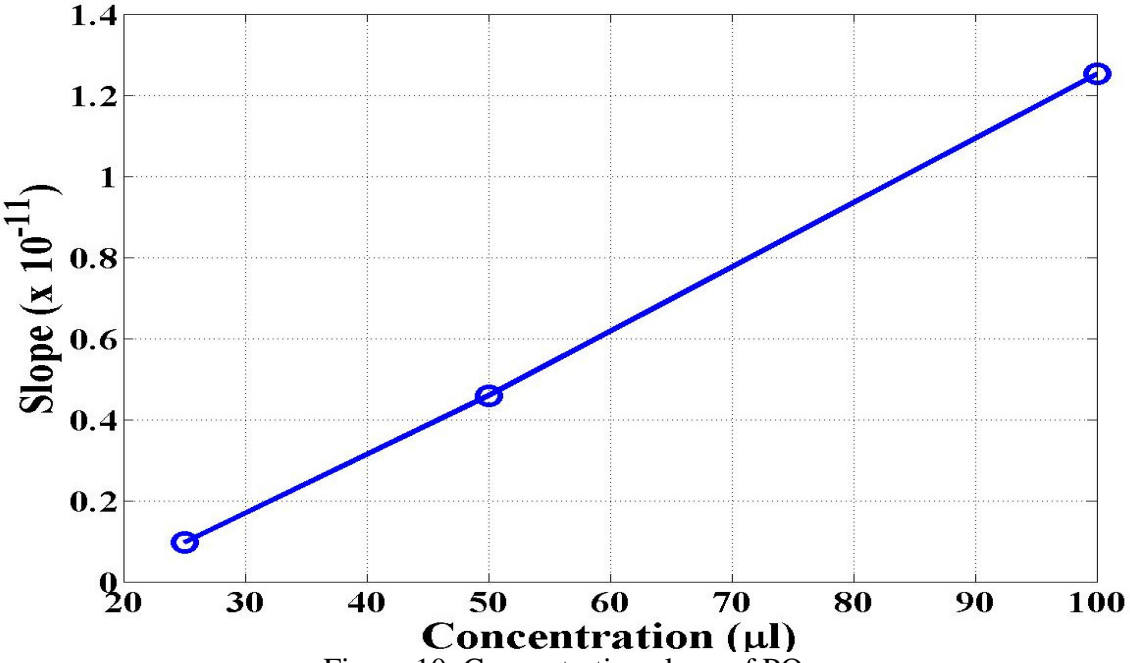

Figure 10. Concentration slope of PO_4

5. CONCLUSION

Phosphate solution was prepared in different concentration to establish a calibration curve. Then, it was added in to the background buffer solution and the change in graph was observed. From those concentration graphs, the calibration curve was obtained as a result of data analysis. Immobilization of biocatalyst and the experiments on super conductive metals (Multiwall Carbon Nano-tubes with Ni) will be conducted at FIU/ARC as a continuation of this project due to the short time period of the internship. The results of experiments and the analyzed data have clearly demonstrated that the phosphate is detected and quantified. When an unknown concentration of sample that contains phosphate is added to the background solution and a graph is obtained, the phosphate concentration can be calculated based on the calibration curve obtain. As a final step, water samples from Hanford will be tested and based on the calibration curve the phosphate will be detected and quantified.

6. REFERENCES

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7. APPENDIX

Table 1. Experiment Data

Experiment Type: DC Potential Amperometry (DCPA)
Title: DCPA Run for BASi-Epsilon
Data File Name: 25uL 1M Na2HPO4 and 25uL 1M Pyr mixed & added @1100sec in (100uL POD immob on Au in 1mL 50mM TRIS Buffer pH7),5 FScale100nA
Date & Time of the run : 8/1/2009 3:21:33 PM

Display Convention : IUPAC
Number of data points : 2000
# of points to skip : 0
Initial Potential : 450 (mV)
Time Limit : 2000 (Sec)
Current Full Scale : 100 nA
Filter : 100 Hz
Quiet Time : 0 (Sec)
Sample Interval : 1 sec

Analyst :
Solution :

Analyte :
Analyte Conc. :
Solvent :
Supporting Electrolyte :
S.E. Conc. :
pH :
Temperature :
Electrodes :

W.E. material :
W. E. area :
W. E. geometry :
W. E. radius :
W. E. conditioning :
Reference electrode :
Auxiliary electrode :
Notes :

1.00E+00	1.00E-07
2.00E+00	1.00E-07

3.00E+00	1.00E-07
4.00E+00	1.00E-07
5.00E+00	1.00E-07
6.00E+00	1.00E-07
7.00E+00	1.00E-07
8.00E+00	1.00E-07
9.00E+00	1.00E-07
1.00E+01	1.00E-07
1.10E+01	1.00E-07
1.20E+01	1.00E-07
1.30E+01	1.00E-07
1.40E+01	1.00E-07
1.50E+01	1.00E-07
1.60E+01	1.00E-07
1.70E+01	1.00E-07
1.80E+01	9.99E-08
1.90E+01	9.96E-08
2.00E+01	9.89E-08
2.10E+01	9.79E-08
2.20E+01	9.65E-08
2.30E+01	9.48E-08
2.40E+01	9.28E-08
2.50E+01	9.05E-08
2.60E+01	8.81E-08
2.70E+01	8.54E-08
2.80E+01	8.28E-08
2.90E+01	8.04E-08
3.00E+01	7.82E-08
3.10E+01	7.62E-08
3.20E+01	7.43E-08
3.30E+01	7.26E-08
3.40E+01	7.10E-08
3.50E+01	6.95E-08
3.60E+01	6.80E-08
3.70E+01	6.68E-08
3.80E+01	6.56E-08
3.90E+01	6.44E-08
4.00E+01	6.33E-08
4.10E+01	6.23E-08
4.20E+01	6.13E-08
4.30E+01	6.04E-08
4.40E+01	5.95E-08
4.50E+01	5.86E-08
4.60E+01	5.78E-08
4.70E+01	5.70E-08

4.80E+01	5.63E-08
4.90E+01	5.56E-08
5.00E+01	5.50E-08
5.10E+01	5.44E-08
5.20E+01	5.38E-08
5.30E+01	5.33E-08
5.40E+01	5.28E-08
5.50E+01	5.23E-08
5.60E+01	5.19E-08
5.70E+01	5.14E-08
5.80E+01	5.09E-08
5.90E+01	5.05E-08
6.00E+01	5.01E-08
6.10E+01	4.97E-08
6.20E+01	4.93E-08
6.30E+01	4.88E-08
6.40E+01	4.85E-08
6.50E+01	4.81E-08
6.60E+01	4.77E-08
6.70E+01	4.74E-08
6.80E+01	4.70E-08
6.90E+01	4.66E-08
7.00E+01	4.63E-08
7.10E+01	4.60E-08
7.20E+01	4.57E-08
7.30E+01	4.54E-08
7.40E+01	4.51E-08
7.50E+01	4.48E-08
7.60E+01	4.45E-08
7.70E+01	4.42E-08
7.80E+01	4.40E-08
7.90E+01	4.37E-08
8.00E+01	4.35E-08
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8.80E+01	4.21E-08
8.90E+01	4.19E-08
9.00E+01	4.18E-08
9.10E+01	4.17E-08
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1.08E+02	3.91E-08
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1.38E+02	3.61E-08
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