Development of an in-situ total phosphorus analyzer

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Abstract— **A low cost autonomous, in-situ analyzer for orthophosphate and total phosphorus was developed and tested for near real time monitoring in aquatic and marine environments. The system applies the colorimetric stannous chloride-molybdenum blue method following oxidative digestion with acidic peroxodisulfate. A (fluorinated ethylene propylene) FEB tubing reactor coil is wrapped around a germicidal UV (254nm) light to accelerate the digestion reaction. A Nickel chromium wire (NiCr) wound between reactor coil wraps provides controlled heating for digestion. Reactor temperature is monitored continuously with a small temperature probe and controlled with a PID. A sequential injection analysis (SIA) method was chosen where the analyzer operates as a sequential batch reactor. Water sample and reagents are delivered through the microfluidics using syringe pump driven by linear actuators that are controlled with a Teensy microprocessor. To equalize pressures, reagent storage and waste are contained in collapsible reagent bags maintained at hydrostatic pressure outside the submersible housing. Following digestion and colorimetric reactions the sample is transferred to flow cell (5 cm path length) where a photodiode measures sample absorbance at 600 nm. For orthophosphate phosphorus measurements, the sample is not treated with digestion reagents, heat, or UV. Potassium dihydrogen phosphate and adenosine monophosphate (AMP) were used to assess analyzer performance for measuring orthophosphate and total phosphorus, respectively. The prototype analyzer produced a linear response during bench testing to total phosphorus with a precision of ±4.3% over a detection range concentration range of 30-200µg P/L. To evaluate the performance with natural waters, dilutions of algal culture with known phosphorus concentration were measured and found to be within 90% of nominal phosphorus concentrations. A preliminary cost analysis indicates that the operating components of the system (minus submersible housing) can be purchased for under $3000 compared to $30,000 for commercially available systems. At this low cost of construction, this sensor design may be a viable option for use in nutrient monitoring programs. Considering that system components have been designed to withstand internal pressures equivalent to a water depth of 100 m with a designed duty capacity of 2 weeks suggest the possibility of applications in coastal waters and relatively deep lakes.**

Keywords—phosphorus, in-situ, micro-fluidics, submersible

I. INTRODUCTION

The purpose of this research was to design and build a low-cost in-situ total phosphorus analyzer for use in aquatic and marine systems. Phosphorus is generally considered a limited nutrient for plant growth. Naturally occurring concentrations in aquatic systems generally range between 10-100 ug/L [1]. Each ecosystem has its own tolerance for phosphorus, but exceeding that level puts the system at risk of uncontrolled algae (phytoplankton) growth. As the excessive biomass die, dissolved oxygen is depleted via biomass oxidation and reduced photosynthetic activity [2]. In combination with physical water column structures, the occurrence of high nutrient loads enhances primary production that leads to hypoxia in the Gulf of Mexico [3].

There are three main forms of phosphorus in the natural environment including soluble reactive (ortho-phosphate), soluble unreactive, and particulate. Soluble reactive phosphorus, existing as inorganic phosphate ions, is readily available for primary biological production. Organic phosphorus includes components that have been assimilated into biomass and must first be subjected to a high temperature acid digestion step to liberate phosphorus to its ionic form prior to being measured. A third form includes particulate bound phosphorus. Both organic and particulate bound phosphorus must undergo digestion prior to measurement.

The distribution of phosphorus in surface waters can demonstrate a high degree of variability [2]. Thus, many samples must be analyzed to characterize the spatial distribution and inherent temporal frequency of phosphorus variability. The time and expenses associated with conventional sampling and laboratory analysis procedures justifies the development of an in-situ phosphorus analyzer. This paper presents the results from the development and testing of a prototype in-situ phosphorus analyzer designed to measure both reactive (soluble ortho-phosphate) and organic phosphorus.

The use of microfluidic systems has proven to be an essential part of nutrient sensor technology. Any instrument that requires a chemical reaction will most definitely contain some microfluidic components. Estela and Cerda [4] provide and in-depth review of different methods of flow analysis techniques used specifically for phosphorus testing. They describe the various methods of flow techniques including segmented flow analysis (SFA), flow injection analysis (FIA), and sequential injection analysis, batch injection analysis (BIA), and others. Traditional FIA systems are based on continuous flow of either sample and or reagents.

In Standard Methods [5], an automated method for phosphorus applies the ascorbic acid method in a system using switching valves. Gentle et al. [2] further developed this

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method by incorporating peristatic pumps for sample and reagent delivery and incorporated a UV-assisted heated digester for organic phosphorus. Lyddy-Meaney et al. [6] describe the use of inert gas pressure in combination with solenoid valves for reagent delivery.

Traditional phosphorus analysis is conducted in the laboratory using either automated instrumentation or wet chemistry techniques followed by spectrophometric analysis. Samples are usually retrieved from the field and stored in a refrigerator until tests can be conducted within 24 hours of sample collection [7]. These methods require large sample volumes and analysis time which translates to high unit data costs that can limit the number of samples analyzed. In response to such limitations, simplified chemical testing kits are provided for rapid field analysis of discrete samples [8]. These kits rely on prepackaged reagent and color comparison wheels to approximate phosphorus concentration. Thus, these methods are appropriate for collection of limited sample numbers where extreme precision is not required. Options available automated nutrient analyzers designed for field applications are limited. Some deployment methods applied in automated field units include ship board operation where the sample is delivered via continuous pumping such as the method employed by Gentle et al. [2]. While feasible, pumping limits the depth of sample collection. Other manufacturers have adopted submersible technologies with operational depths to 200m for ortho-phosphate (WETLabs, Cycle PO4). Combining the deployment capabilities of the WETLabs analyzer with the internal components required for total phosphorus detection has yet to be achieved in a cost effective manner.

The goal of this research is to develop a low cost in-situ total phosphorus sensor. Criteria applied to the prototype design (Fig. 1) include: field portable; submersible; automated sampling; cost effective; low environmental impact; low power consumption; ability for real time data transmission; and minimum two week duty cycle. To meet these criteria, a sequential flow injection protocol was applied to conduct a stannous chloride spectrophotometric absorbance method in a microfluidic system [2,5]. Benefits of microfluidic systems include reduced reagent and energy consumption. The prototype system (Fig. 1) developed through this research applies a sequential injection analysis (SIA) method derived from Ruzicka and Marshall [9]. Accurate and precise sample and reagent delivery is accomplished via computer controlled linear actuators to drive syringe pumps. Actuator control is provided by a PID algorithm coded in the C language on an 8 bit AVR 16 MHz (AT90USB1286) microprocessor. The PID control synchronizes pump operations with respect to start and stop time and flow rates to maintain consistent reaction stoichiometry between samples. The application of synchronized sample and reagent addition alleviates the need for flow reversals to facilitate mixing as suggested by Lyddy-Meaney et al [6] for conventional SIA methods. The use of check valves ensures unidirectional flow throughout the microfluidic system. Both sample inlet and sample/reagent outlet are exposed to hydrostatic pressure, ensuring that there is no flow through the system unless the pump is on. All sample and reagent waste are collected in a sampling bag housed at ambient pressure.

Analytical Flow Process

In the first step the sample and persulfate digestion reagent are mixed prior to entering the high temperature digester that was fabricated by wrapping a 6 watt germicidal UVC lamp (PLT, Part #G6T5) with 1.8 m FEB tubing having an Inside Diameter (I.D.) = 0.7mm. In the presence of UVC radiation, the reaction time for persulfate digestion can be reduced from 30 to as little as 10 minutes [2,5]. FEB tubing ensures the transmittance of UVC radiation to sample-persulfate mixture. The reactor heating element is fabricated by tightly wrapping Nickel chromium (NiCr) heating wire between each tubing wrap. Reactor temperature is monitored with a temperature probe (Dallas Semiconductor P/N 18b20) that allows feedback control heating element temperature. Once target digestion temperature (80 degrees C) is obtained, the UV light is switched on for 30 seconds and heater temperature is maintained for 13 minutes. Upon exiting the digester, sample pH is adjusted with the injection of NaOH prior to entering the cooling tube (2 meters FEB tubing with I.D. = 0.7mm) to help aid in rapid color development as suggested by Huagn and Zhang [10]. After leaving the cooling tube, colorimetric reagents, R1 and R2 are added simultaneously to the sample prior to entering the flow cell (Z-cell). Sample and reagent delivery span equivalent and simultaneous time intervals to ensure stoichiometric consistency and thorough mixing of sample and reagent throughout the sample plug. Linear actuator for the reagent and sample pumps is provided by a Proportional, Integrative, Differential (PID) algorithm coded in the C language on an 8 bit AVR 16 MHz (AT90USB1286) microprocessor. The Z-cell is configured with a 660nm LED light source (Mouser, C856) and a 635 nm peak photodiode (TAOS, TSL250R-LF). Phosphorus concentration is determined as a function of absorbance at 635 nm.

II. Materials and Methods

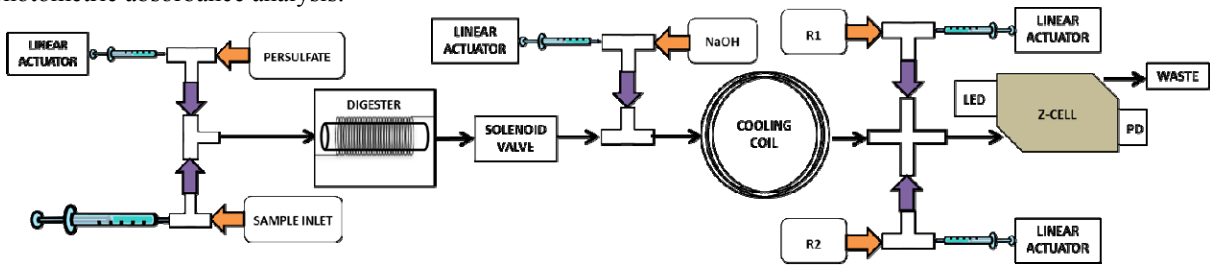
A. System Evaluation

Evaluation of the prototype system was conducted in the laboratory with both ortho-phoshphate (potassium dihydrogen phosphate) and organic phosphate (adenosine mono-phosphate). Dilutions of algal cultures with known phosphorus concentrations were used to evaluate system applicability in natural waters.

B. Reagents and Standards

All standards and reagents were prepared following procedures described in Gentle et al. [2]. Inorganic stock solutions (1 mg P/L) were prepared by dissolving 439 mg anhydrous KH_2PO_4 in 1000 ml reverse osmosis (RO) water.

Fig.1 Sensor schematic where the 3 main process steps include digestion, cooling, colorimetric reagent addition (R1 and R2), and spectrophotometric absorbance analysis.



Organic stock solutions (1 mg P/L) were prepared by dissolving 367.5 mg AMP (adenosine monophosphate) in 1000 ml RO water. Stock solutions were stored at 4° C in glass containers. Dilution series for both inorganic (ortho) and organic standards were prepared daily with RO water in acid-washed glassware.

Acidic persulfate digestion reagent was prepared by adding 40g potassium peroxidisulfate to 900ml H_2O. Then, 1.38 mil of concentrated H_2SO_4 was added and brought to a final volume of 1000 ml with RO water.

Ammonium molybdate reagent (R1) was prepared by adding 1.0 g ammonium molybdate to 50 ml H_2O, then adding 3.5 ml concentrated H_2SO_4 before bring the final reagent volume to 100ml with H_2O.

Stannous chloride reduction reagent (R2) was prepared by adding hydrazine sulfate and 0.02 g tin (II) chloride to 50 ml H_2O, then adding 2.8 ml concentrated H_2SO_4 before bringing final reagent volume to 100 ml with H_2O. Hydrazine sulfate is added to extend reagent stability [6].

The neutralizing reagent was prepared by adding NaOH to 50 ml H_2O and bringing the solution to a final volume of 100 ml with H_2O.

III. Results and Discussion

The digitized signal output from the photodiode was converted absorbance though application of the Beer-Lambert law

$$A=log_{10}I_0/I \qquad (1)$$

Where A is absorbance, I_0 is the radiant power transmitted through a matrix blank (i.e. water) and I is the radiant power transmitted though the sample. In this system the digitized signal was proportional to the radiant power.

Response curves for ortho-phosphate dilution series, ranging from 0-250 ug/L, were replicated three times. The combined data sets showed a linear absorbance response between 0-200 ug/L (R^2=0.9522) Fig. 2. Above 200 ug/l the signal was not linear. The average covariance (% STDEV/Mean) of the absorbance signal was 9%. A minimum detection limit of 34 ug/L was estimated from 5 times the mean standard deviation determined for absorbance measured at each concentration step.

Response curves for organic-phosphate dilutions series, ranging from 0-200 ug/L, were also conducted in triplicate. The combined data sets again showed a strong linear correlation (R^2=0.9522) Fig. 3. The average covariance (% SDEV/Mean) of the absorbance signal was 7.5 %. A minimum detection limit of 29 ug/l was estimated from 5 times the mean standard deviation for the absorbance measured at each concentration step. Comparison of linear regressions shows the slope for AMP to be roughly twice the slope determined for ortho-phosphate. This apparent increased sensitivity to organic is phosphorus is contrary to the decrease in sensitivity expected due to organic digestion in-efficiencies. An interference being investigated is the possibility of precipitate formation during the digestion step which would reduce radiant power transmission (i.e. increase absorbance).

To evaluate instrument response in natural waters the system was used to measure the total phosphorus concentrations in dilutions of algae culture (*Chorella protothecoides*) with known phosphorus concentrations ranging from 0-120 ug/L. Similar to the ortho- and organic phoshorus, the absorbance responses showed a linear correlation (R^2=0.9473) Fig. 4. Inspection of linear regressions show that the response slopes obtained from the algal cultures were approximately 20 and 40 % of the slopes obtain from the ortho- and organic phosphorus response curves. As the sample was unfiltered, it is suspected that turbidity resulting from the algal suspension may be interferring with the light transmission (i.e. increased absorbance) through the flow cell.

Fig. 2 Ortho-phoshate absorbance response curve

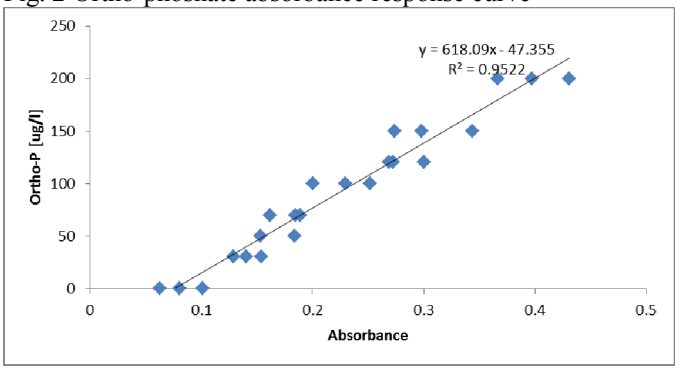


Fig. 3 Organic-phospate (AMP) absorbance response curve

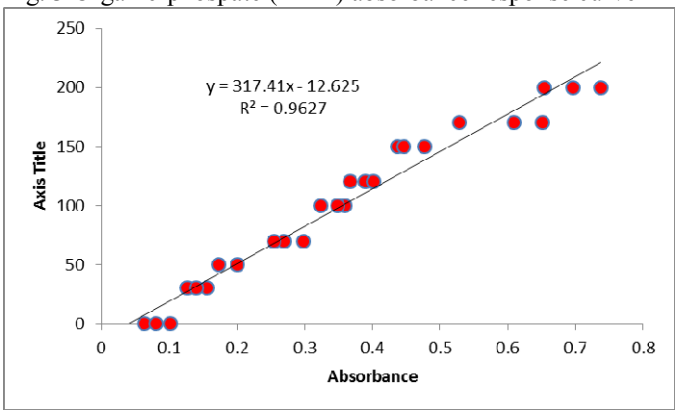
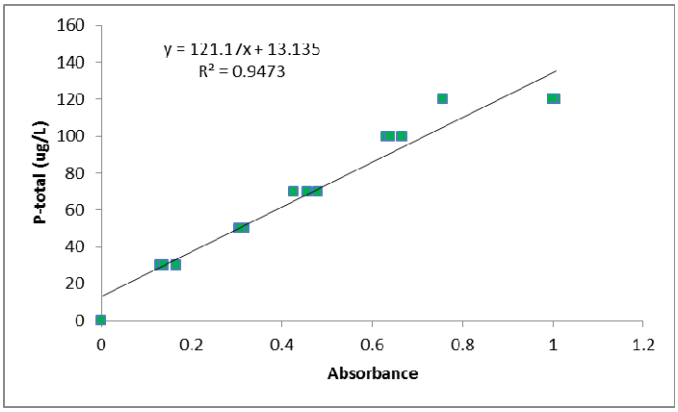


Fig. 4 Total phosphorus response curve obtained for algal culture dilution series



IV. Conclusions

The developed total phosphorus analyzer was designed, built, and evaluated with organic and inorganic standards in the laboratory. These preliminary evaluations demonstrated the ability to achieve linear absorbance responses over environmentally relevant concentrations between 30-200 ug/L. Linear absorbance responses were also demonstrated with live algal cultures to emulate instrument response in natural systems. While promising, these results have identified areas requiring further development.

To address turbidity effects, process flow routines are being developed to measure absorbance of the sample prior to reagent addition. Much effort continues to optimize process flow routines whereby feedback controls are implemented to ensure consistent reagent and sample stoichiometry.

Once the system has been thoroughly validated in the laboratory it will be subject to field trials as a submersible system. System designs have considered use in underwater applications to water depths of 5 atmospheres including: housing reagent and waste liquids in collapsible sampling bag as hydrostatic pressure and use the use of fluid handling components (i.e. tubing, syringes, fittings) with minimum burst pressures of 10 atmospheres.

A major design consideration is total system cost. Currently available options can cost in excess of $30,000 whereas the components used in the existing system can be obtained for ~$3,000. The use of enabling technologies including the application of open source software and low cost microprocessors will further reduce system and development cost.

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