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Chemical Removal of Total Phosphorus from Wastewater to Low Levels and Its Analysis

by

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Bachelor of Science, Honors Co-operative Chemistry, University of Waterloo, 2012

THESIS

Submitted to the Department of Chemistry

in partial fulfilment of the requirements for

Master of Science

Wilfrid Laurier University

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Abstract

Numerous studies have been conducted on the removal of inorganic phosphorus (P) from wastewater, but a push towards lower effluent targets necessitates the additional removal of organic phosphorus as well. This study tested the ability of manganese oxide nanoparticles and iron oxide as potential catalysts for conversion of organic P into more readily removable inorganic forms, as well as the role of iron(III) chloride as coagulant to subsequently allow P to be removed by solids/liquid separation. Removals of 99-101% were obtained for model compounds at pH 5-7, 0.05-0.5 M H₂O₂, and Fe:P molar ratio of 5:1. Presence of H₂O₂ was found necessary to remove phosphonates in particular, increasing removal from 17 to 101%. Tests in real wastewaters containing organic P also showed higher removal with peroxide addition. Due to interference from H₂O₂, the standard method for P analysis in wastewater, colorimetry, could not be used as the primary analytical tool. An accurate and sensitive protocol using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) capable of low-level P detection was developed instead and compared to colorimetry using model organic P compounds and real wastewater samples. Detection limits for colorimetry and ICP-OES were 0.002 and 0.09 mg P/L respectively. ICP-OES gave analytical recoveries closest to 100% for model organic P compounds, but both methods gave highly variable data at concentrations below 0.15 mg P/L. ICP-OES seems promising for TP measurements given its high recoveries for model compounds, but more work is needed to improve its detection limit and sensitivity.

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I want to give a shout-out to all the lab members from both groups that I got to work with. You guys made working in the lab and attending meetings so much more fun. Big thanks to Holly for great advice on several aspects of this project, and to Rabia for great writing tips. To Elissa, Giselle and Rabia, I am especially grateful for making work seem enjoyable and stress-free, and for all the fun get-togethers. Another shout-out to Ammi, Abbu, Betty and Al who have been extremely proud of me and cheered me on.

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Preface

Due to its tendency to cause eutrophication, phosphorus concentrations are regulated in many wastewater treatment plants. A trend of phosphorus regulations towards lower concentrations is driving the need to study ways of reaching these targets. Low phosphorus concentrations also necessitate sensitive and precise phosphorus determination methods. The research described here was undertaken to address parts of both of these issues (i.e. treatment and phosphorus analysis).

This thesis is composed of five chapters. Chapter 1 is intended to provide the necessary background needed to understand the objectives of the thesis. It discusses the role of phosphorus in the natural environment and how it acts as a contaminant, its sources, speciation, regulations, current treatment practices and chemical analyses. It also highlights the gap in literature regarding treatment methods and defines the overall research objectives of this thesis. Chapters 2-4 provide the experimental details, results and discussion related to the research objectives. These chapters are presented as drafts for submission to scientific journals, and include their own abstract, introduction, conclusions and references. Each of these chapters also have 'supplementary information' sections, which are meant to be included in the journal articles. Chapters 2 and 3 also have appendices. These contain data that are not meant to be included in the journal articles. Chapter 2 pertains to the analytical methods for phosphorus measurement. Chapters 3 and 4 provide details related to the development of a treatment protocol for achievement of low level phosphorus removal. Chapter 5 concludes the results of the whole project and their significance, and provides suggestions for future study.

1 Introduction

1.1 Phosphorus in the environment

Phosphorus (P) is a ubiquitous naturally occurring element and is ranked as the 11th most abundant element in the lithosphere and 13th in seawater¹. It is present in rocks, soils, ocean, all living cells, blood, bones and teeth². Having important roles in biological functions such as photosynthesis and nerve function make phosphorus an essential element for sustaining life in all animals and plants².

Phosphorus is used for many man-made substances. Fertilizers constitute the largest application (about 85%), while others include detergents, pesticides, surfactants, and flame retardants². The main commercial source of the element is mining from phosphate mineral rocks, specifically minerals in the apatite ($\text{Ca}_5(\text{PO}_4)_3(\text{F},\text{Cl},\text{OH})$) group, which contain about 95% of all phosphorus in the Earth's crust^{1,2}.

In the natural environment, mineralization, weathering, erosion and runoff are responsible for transferring dissolved and particulate phosphates from land to ocean over thousands of years¹. The dissolved fraction is very small owing to the very low solubility of phosphates¹. An even lower fraction of the transferred dissolved phosphate remains in soluble form, since aluminum and iron precipitate or co-precipitate out phosphate in waters with low pH, and in alkaline waters, calcium removes the phosphate¹. Because such little phosphate is available to aquatic biota, it becomes the limiting nutrient in many environments.

1.2 Phosphorus as a contaminant

In relatively recent years, anthropogenic causes have increased the amount of dissolved phosphorus reaching water bodies, leading to unrestricted algal growth (*eutrophication*)^{3,4}.

Eutrophication is a phenomenon where a water body (such as a lake, river or groundwater) becomes enriched with nutrients, leading to an increase in the biological activity within the ecosystem, resulting in oxygen depletion, increased aquatic animal mortality, poor water quality, and aesthetic decline⁴. In freshwater systems, phosphorus has been identified as the limiting nutrient^{4,5}. (In marine systems, nitrogen is identified as the key limiting nutrient^{4,5}.) Eutrophication can potentially result from dissolved phosphorus inputs of as low as 10 µg/L, at which point water may appear turbid¹. Increasing inputs of phosphorus lead to increasingly turbid waters, with deoxygenation starting to occur at 50 µg/L¹.

Anthropogenic factors that have increased the amount of phosphorus flowing into natural water bodies include agricultural runoff, increased use of inorganic fertilizers, and industrial and urban waste discharge¹. Various attempts are in place to control phosphorus loading, one of which is regulating the phosphorus concentration in wastewater effluent^{1,6,7}.

1.3 Speciation

In wastewater, phosphorus is present in both dissolved and particulate forms⁸. The dissolved fraction is defined as that which passes through a 0.45 µm membrane, while the particulate fraction is the difference between total and dissolved⁸. These fractions are subdivided into reactive, acid-hydrolysable and organic components⁸. Figure 1 shows a summary of the phosphorus fractions and some representative compounds of each category. Classification of compounds under these categories is based on their reactivity to chemical analyses involving colorimetry and specific digestion methods⁹.

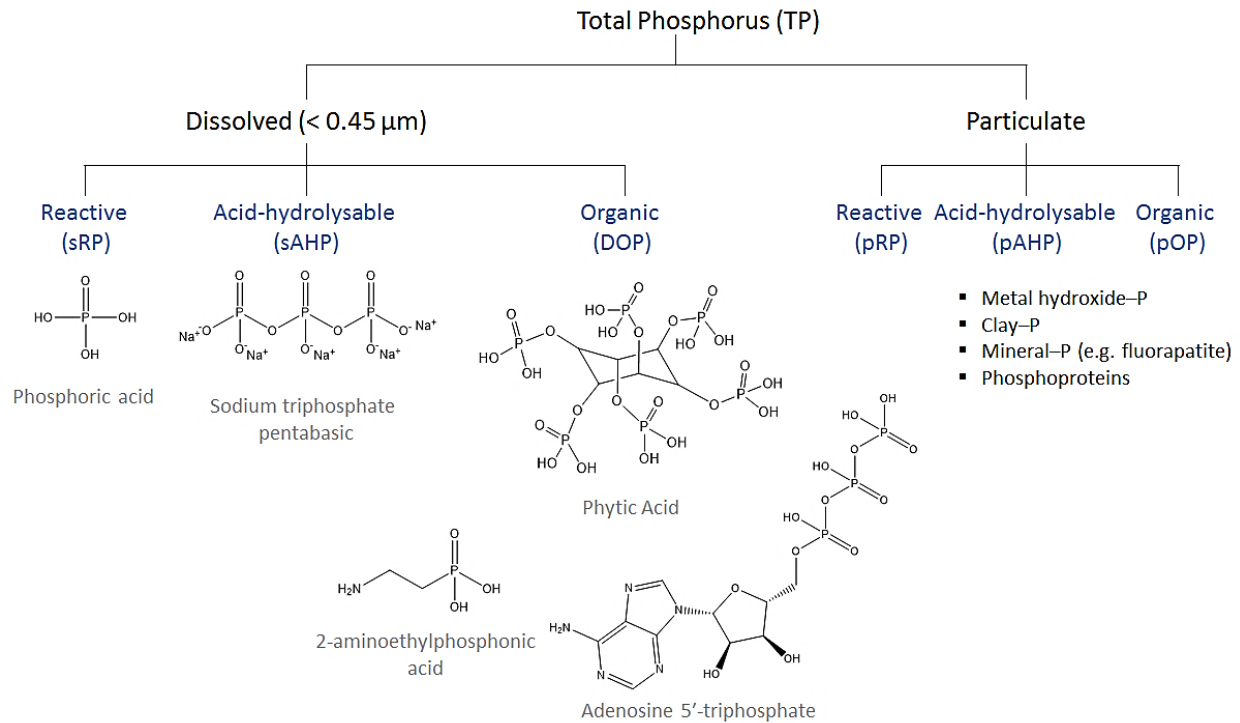


Figure 1. Phosphorus fractions and examples of compounds that fall under each category^{8,10}.

Dissolved compounds that respond directly to colorimetry are termed soluble reactive phosphorus, sRP⁸. These consist primarily of orthophosphates in protonated and deprotonated forms and are the most abundant and bioavailable of the forms of phosphorus¹¹. Compounds that can be converted to reactive phosphorus after acid addition are termed soluble acid-hydrolysable phosphorus, sAHP⁸. These consist of condensed phosphates with P-O-P bonds and are used in detergents¹⁰. An example of a compound in this category is sodium triphosphate pentabasic (Figure 1). Dissolved organic phosphorus (DOP) are those compounds that can convert to orthophosphates under harsher digestion (i.e. oxidation) processes⁸. Compounds that fall in this category include phytic acid (Figure 1) (a type of inositol phosphate), adenosine triphosphate (ATP) (Figure 1) and phosphonates¹⁰. Phytic acid is a major component of organic phosphorus in many soils¹²; ATP is produced by all plants and animals¹⁰, and phosphonates are used in scale formation inhibitors¹³, pesticides and pharmaceuticals¹⁴.

Particulate forms of phosphorus (Figure 1) consist of the soluble forms attached to particles, for example, orthophosphate bound to clays or metal hydroxides¹⁰. The particulate forms may also include phosphorus in minerals such as fluorapatite ($\text{Ca}_5(\text{PO}_4)_3\text{F}$) and large organic molecules that precipitate out of solution such as phosphoproteins¹⁰.

1.4 Regulations

Phosphorus regulations for wastewater treatment plants (WWTPs) are typically based on total phosphorus (TP)⁵. Unlike traditional toxic pollutants, there are no generic criteria (e.g. Minimum Contaminant Levels, MCLs) established for phosphorus, since the effect of nutrients depends on site-specific conditions such as soil, hydrology, species diversity and climate⁵. Instead, each waste discharger is individually assessed and issued a permit that specifies the effluent limit¹⁵. The limits depend on the influent volume and are usually stated as total loads in units of lbs/day or lbs/year, from which the concentration in mg/L as monthly or weekly averages is calculated. In Ontario, the effluent limits are specified on Certificate of Approvals (COA)¹⁵. The following table shows some of the requirements of specific WWTPs around Ontario.

Table 1. Phosphorus regulations for some Ontario cities.

| WWTP Location | Discharge limit (mg/L as TP) | Reference |
|--|------------------------------|-----------|
| Tilbury (Municipality of Chatham-Kent) | 0.5 | 16 |
| Mt. Albert (discharges to Lake Simcoe) | 0.1 | 17 |
| Barrie (discharges to Lake Simcoe) | 0.18 | 17 |
| Beaver River (discharges to Lake Simcoe) | 0.3 | 17 |
| New Hamburg (Region of Waterloo) | 0.3 | 18 |
| Elmira (Region of Waterloo) | 0.5 | 18 |
| Hespeler (Region of Waterloo) | 1 | 18 |

In the coming years, the threat of eutrophication is expected to increase due to increasing nutrient pollution¹⁹. One of the main drivers of this change is population growth which ultimately leads

to increased meat consumption and a subsequent increase in intensive agriculture, and fertilizer consumption¹⁹. In response to these threats, many treatment utilities are pre-emptively upgrading their systems to meet future phosphorus loadings. Moreover, improvements in treatment technologies and dissatisfaction with the quality of freshwater lakes are driving environmental agencies all over the world to set stricter phosphorus regulations. In the US, limits in the range of 0.009-0.05 mg/L have been proposed²⁰. In a WWTP in Germany, limits of 0.05-0.12 mg/L have been proposed²¹.

1.5 Treatment methods

1.5.1 Wastewater treatment process overview

Conventional wastewater treatment methods use combinations of physical, chemical and biological processes to remove large solids, small particles, organic matter, nutrients and other contaminants from wastewater. The treatment is done in various stages, termed preliminary, primary, secondary, and tertiary. An example of one basic treatment train is shown Figure 2 below.

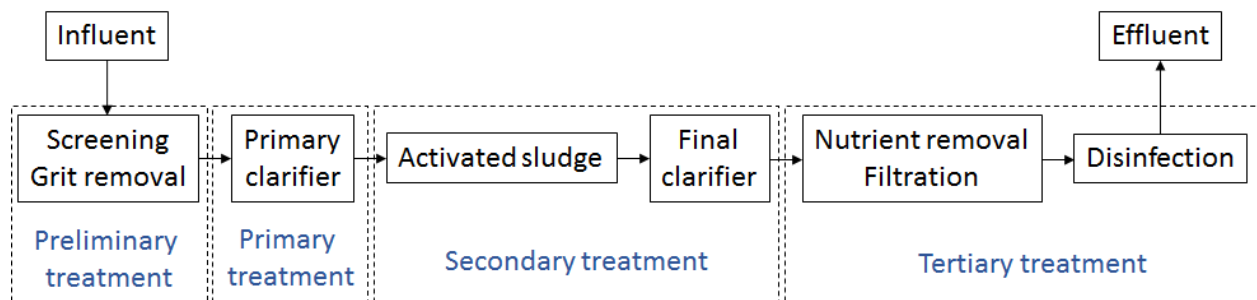


Figure 2. Schematic of a typical wastewater treatment plant^{22,23}.

Preliminary treatment (Figure 2) gets rid of large solids, inorganic grit, and scum with the help of screens and grit chambers^{22,23}. Primary treatment (Figure 2) removes suspended and floating material, and some organic matter using fine screens, sedimentation tanks and/or flotation^{22,23}. Secondary treatment (Figure 2) involves biological treatment of organic matter and consists of stabilization ponds,

trickling filters and/or activated sludge processes^{22,23}. Tertiary treatment (Figure 2) is a blanket term for various purposes and its process selection depends on the treatment problem²². For example, it can include disinfection, nutrient removal, and fine particle removal. Apart from these steps, the treatment process also has stages for sludge removal and disposal. The design of WWTPs varies widely and depends on influent wastewater quality, flow rate, resources available and targeted effluent quality. For example, not all plants employ tertiary treatment.

Phosphorus removal can occur at the primary, secondary or tertiary stage¹⁵ and can include using physical, chemical or biological processes, or engineered wetlands⁵. The following sections describe these processes in more details.

1.5.2 Physical/chemical phosphorus removal

Physical/chemical methods of phosphorus removal involve two main steps: formation of stable precipitates, followed by solids-liquid separation. The first step is achieved by addition of multi-valent metal salts, mainly aluminum, iron(III) or calcium²⁴. These are called coagulants. Magnesium, iron(II), as well as coagulant aids are also sometimes used. In Ca-mediated removal, direct precipitation of calcium phosphate compounds is the dominant removal mechanism⁵. In Al- and Fe(III)- mediated removal, the dominant process is co-precipitation (defined as: “the simultaneous precipitation of a normally soluble component with a macro-component from the same solution by the formation of mixed crystals, by adsorption, occlusion or mechanical entrapment.”²⁵) Addition of Al or Fe(III) to waters with sufficient alkalinity results in rapid precipitation of hydrous ferric or aluminum oxides (HFOs or HAOs)^{5,26}. The removal of phosphorus can occur by the following mechanisms: adsorption onto or co-precipitation with HFO/HAO^{5,26}; precipitation of ferric or aluminum phosphates, (ferric phosphate only precipitates at pH below 5, not typically found in wastewater)^{5,26}; and precipitation of mixed cation phosphates (i.e. Ca,

Mg, Fe, Al phosphates, or hydroxyphosphates)^{5,26}. Chemical addition can take place at multiple locations in the treatment train, including before or after primary treatment, directly to activated sludge reactor, before final clarifier, or as a polishing step at the tertiary stage^{15,27,28}. The last of these is gaining popularity with municipalities as effluent limits move towards low levels¹⁵. The second step in chemical phosphorus removal, solid-liquids separation, is usually achieved by sedimentation, filtration and/or membranes⁵.

1.5.3 Biological phosphorus removal

In biological phosphorus removal, microorganisms are used to store phosphorus in cells and these organisms are later removed as part of solid sludge, a process called *Enhanced Biological Phosphorus Removal* (EBPR). All microorganisms require phosphorus for growth and so have the ability to naturally remove the element from wastewater⁵. The EBPR process 'enhances' this effect by selectively accelerating the growth of microorganisms that can accumulate phosphorus in their cells in excess of their growth needs⁵. These microorganisms are called *Phosphorus Accumulating Organisms* (PAOs)⁵. They are inoculated into sludge and are then subjected to cycles of carbon-rich anaerobic and carbon-poor aerobic zones²⁴. In the anaerobic zone, PAOs store readily biodegradable organic compounds from the wastewater intracellularly in the form of glycogen and polyhydroxyalkanoates (PHA), the energy for which is derived from converting polyphosphate (ATP) into orthophosphate^{5,24}. In the subsequent aerobic zone, the PAOs metabolize the stored glycogen and PHA, and the energy released from this process is used to take up orthophosphate back into the cell and convert it to polyphosphate^{5,24}. The amount of orthophosphate taken up in the aerobic zone ends up being more than what was released in the anaerobic zone, thus reducing the overall phosphorus concentration of the wastewater^{5,24}. A settling period allows the microbial population to sediment²⁴. The sludge, now

high in organic matter and microbial content, is wasted, recycled or used for biogas production²⁴. As with chemical phosphorus removal, the sludge is removed using a solid/liquid separation step.

1.5.4 Comparison of the physical /chemical and biological phosphorus removal

Both methods have several advantages when designed well. EBPR uses less chemicals, produces less sludge, produces biogas (which can be used for energy generation), and has higher potential for phosphorus recovery for use in land application¹⁵. Chemical phosphorus removal is found to be more reliable, less complex and protects solids processing equipment better¹⁵. Both methods can achieve TP as low as 0.1 mg/L under favorable conditions; however, for WWTPs requiring TP removal below 0.05 mg/L, chemical treatment appears to be the better choice¹⁵. Ultimately a combination of the two methods, for example, EBPR at the secondary level and chemical treatment at the tertiary level, will convey the benefits of both options¹⁵.

1.5.5 Engineered wetlands

Engineered wetlands employ all biological, physical and chemical processes with the help of plants and soils to remove both soluble and particulate phosphorus from wastewater. These systems require minimal operating and maintenance costs, but also require large land area, and are best suited for rural areas or developing countries⁵. Their performance can be as good as 98% phosphate removal from sewage²⁹, but is highly dependent on environmental conditions and wastewater quality⁵.

1.6 Importance of organic phosphorus

Compared to reactive phosphorus (which is mainly orthophosphates and inorganic in nature), organic phosphorus has received little attention in wastewater treatment until very recently. The treatment methods described in the previous section target the removal of orthophosphate or

compounds that can be easily converted to orthophosphates³⁰. As the limits for effluent TP decrease, the removal of other fractions – specifically organic phosphorus and some refractory acid hydrolysable forms – is becoming more important^{5,30}. In a 2012 study by Gu et. al., the sum of dissolved organic phosphorus and soluble acid hydrolysable phosphorus (collectively called soluble non-reactive phosphorus, sNRP) ranged from 0.009 – 0.054 mg P/L in secondary wastewater effluents, and from 0.003 – 0.042 mg P/L in tertiary wastewater effluents⁸. sNRP can therefore have a significant impact when TP regulations are set close to 0.01 mg P/L.

Knowledge of phosphorus compounds that persist in effluents after treatment is rare in the literature, but some examples of possibly persistent compounds include refractory compounds such as phosphonates, inositol phosphates and in some cases, condensed organic and inorganic phosphates³¹. Phosphonates have C-P bonds which are very resistant to oxidation and hydrolysis³¹. This property, combined with their high solubility in water, makes them good candidates for use in pesticides and pharmaceuticals. The compounds can dissolve in soil or blood, for example, and remain stable even in complex matrices. The weed killer, Roundup, contains the phosphonate glyphosate and has been recently detected in wastewater treatment plant effluent³². Pharmaceuticals, which are a source of organic phosphorus, in particular phosphonates, are also often not eliminated by wastewater treatment³³. In 1998, Nowack reported the sum of three phosphonates in a Swiss wastewater treatment plant influent receiving input from textile industries to be 0.352 mg P/L (value converted from μ mol/L for the Herisau WWTP, analyzed using HPLC)³⁴.

Moreover, while reactive phosphorus has historically been considered the most abundant and readily available for phytoplankton and bacterial uptake, more studies are surfacing on the bioavailability of organic phosphorus and its ability to contribute to algal blooms^{11,35}.

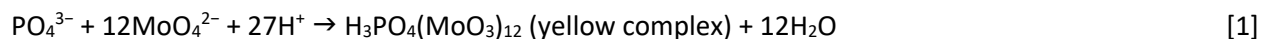
Studies on removal of these compounds from wastewater are rare in the literature, creating a gap in the research area. This gap in knowledge is the focus of this thesis. It has been hypothesized here that organic phosphorus compounds can be converted to orthophosphate, and thus be made chemically reactive to already established methods for phosphate removal. This conversion and subsequent removal of total phosphorus was assessed by using oxidants including hydrogen peroxide and bleach, potential catalysts for the oxidation, and iron(III) chloride as coagulant.

1.7 Chemical analysis

Low effluent TP concentrations not only demand effective treatment methods, but also robust analytical methods, which should be accurate, precise, sensitive and have low detection limits.

The standard method for measuring phosphorus in wastewater is a colorimetric determination of orthophosphates^{9,36,37}. Fractions other than orthophosphates can be detected after pre-treatment and digestion using methods involving heat and acid to release the orthophosphate groups⁹. The digestion process is time-consuming, especially when several samples need to be measured¹⁰. Moreover, despite the harsh conditions of concentrated acid and high temperatures, not all of the orthophosphate may be released from the more refractory organic phosphorus compounds¹⁰.

There are a few variations of the colorimetry method, the most common of which is based on the formation of a blue complex³¹. Phosphate and ammonium molybdate first react in an acidic medium to form a yellow phosphomolybdate heteropolyacid (Equation 1)³¹. Using ascorbic acid, this complex is then reduced to phosphomolybdenum blue with potassium antimony tartrate as a catalyst (Equation 2)³¹. The intensity of the blue color of phosphomolybdenum blue is measured spectrophotometrically and is proportional to phosphorus concentration.



Because this method relies on a reducing agent to form the blue complex, it could not be used as the primary analytical tool in this study, since the hydrogen peroxide (an oxidizing agent) used for the wastewater treatment protocol (Section 1.6) interferes directly with the ascorbic acid. This interference can be overcome by adding a quencher or heating the sample to remove excess peroxide, but adds an extra time-consuming step to the whole method. As a result of this interference, alternatives to standard TP analysis had to be evaluated for the present study. Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) and Energy Dispersive X-Ray Fluorescence (ED-XRF) were selected as possible alternatives.

With ICP-OES, samples are heated to extremely high temperatures close to 6000 K, which break down molecules into individual elements. The elements are then measured by spectroscopy. This method is more expensive than colorimetry, but is extremely time efficient due to combining the digestion and detection methods. In ED-XRF, samples are targeted by X-rays causing them to fluoresce. The emitted fluorescence is detected by a spectrometer. In this case, no digestion step is required. The method is cheap and fast.

Over the course of this project, ICP-OES was successfully adopted for TP measurements, but ED-XRF was found to give unreliable calibration curves and was not considered for further testing. Details of TP measurements using ICP-OES have been presented in Chapter 2. Details of attempted ED-XRF calibrations are briefly mentioned in Appendix to Chapter 2.

ICP-OES is being used in some laboratories for phosphorus analysis but it has not achieved a 'standard' status for water and wastewater analyses³⁷. Furthermore, limited studies were found in the scientific literature citing use of ICP-OES for wastewater samples specifically, or for analysis of organic phosphorus compounds commonly found in wastewater. Studies on techniques related to ICP-OES or for matrices other than wastewater were found and are discussed in the introduction section of Chapter 2 (Section 2.1). Using ICP-OES for TP determination in this study also created the opportunity to perform a thorough comparison of the method to colorimetry, and contribute data to literature to establish ICP-OES as a potential alternative to the standard method for TP determination.

Detection limits for colorimetry for TP determination are in the range of 5-10 µg P/L³⁸. Detection limits for ICP-OES in natural waters have been reported as 1-20 µg P/L³⁹. Even though low detection limits for ICP-OES are reported in the literature, the limits obtained in preliminary studies for this research were much higher – around 1000 µg P/L. In order to meet other goals of the project, one of the objectives of this research (discussed further in section 1.8) was to reduce the detection limit to at least 50 µg P/L – the value to which WWTPs are increasingly being regulated.

1.8 Research objectives

The objectives of this research can be summarized as follows:

- 1. Develop an interference-free analytical protocol to reliably measure low concentrations of total phosphorus (particularly refractory organic phosphorus compounds) in treated wastewater samples.**

'Reliable' in this context implies that the method should be accurate, sensitive, have a low detection limit (at least 50 µg P/L), and be relatively easy to use. As was discussed in Section 1.7, ICP-

OES and ED-XRF were evaluated in this study. Because the main chapters of this thesis have been presented as papers for submission to scientific journals, the data related to this objective (i.e. development of the ICP-OES protocol resulting in detection limit of 50 µg P/L) has been included as an appendix to Chapter 2, instead of as a dedicated chapter. The final 'successful' protocol is part of the second objective (see below), and has been included in the methods section of Chapter 2.

2. Compare total phosphorus measurements using ICP-OES and colorimetry in terms of accuracy, precision, sensitivity and detection limits in order to contribute data for establishment of ICP-OES as a new standard for total phosphorus measurements.

Total phosphorus measurements using ICP-OES were compared to those made using colorimetry. Two commonly used digestion procedures were evaluated: sulfuric acid-nitric acid and persulfate. First, the analytical recoveries of model organic phosphorus compounds were measured using both techniques and digestion procedures. High recoveries with low deviations would indicate the technique is capable of making accurate and precise measurements. Recoveries of 100% are ideal. Second, total phosphorus concentrations of wastewater samples were compared using ICP-OES and colorimetry using the better digestion method, to determine if the techniques give similar results.

3. Amend the process of chemical phosphorus removal by iron(III) chloride, to enable removal of both inorganic and organic forms of phosphorus to low levels (≥ 98% phosphorus removal).

Iron(III) chloride is one of the main coagulants used for chemical phosphorus removal. As was mentioned in Section 1.6, the process only targets phosphate removal. The objective here was to modify treatment by iron(III) chloride to enable removal of organic phosphorus as well. This was accomplished by converting organic phosphorus to phosphates with the help of oxidants and oxidation catalysts. Other variables that can modify removal were also tested, including pH and metal dose. Iron(III) chloride

was chosen as the main coagulant due to experience with the chemical. As the project progressed, iron(III) chloride was found to be a catalyst (as well as a coagulant) for oxidation of organic phosphorous, and aluminum chloride was used to test this claim. Details of this can be found in Chapter 3.

To simplify the research, the study was first performed on solutions of model phosphorus compounds in pure water and then in synthetic wastewater. (Tests on real wastewaters are part of the next objective.)

'Low level' for this objective was defined as greater than or equal to 98% phosphorus removal. The detection limit of the ICP-OES could not consistently be brought down to 0.05 mg P/L (50 µg P/L), and ranged from 0.01-0.09 mg P/L. The tests on model compounds were done on 5 mg P/L solutions. Therefore, the maximum removal hypothetically achievable given the detection limit was 98-99.8%. (Details can be found in Chapter 3.)

4. Determine if the phosphorus removal protocol using iron(III) chloride with an oxidizing agent can be applied to real wastewater samples.

The main conclusions drawn from Chapter 3 were applied to real wastewater samples to determine if they hold true in complex real matrices. The protocols were modified further to get residual phosphorus concentrations below 0.1 mg P/L. This objective is addressed in Chapter 4.

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2 Comparison of ICP-OES and Colorimetry for the Measurement of Total Phosphorus in Water and Wastewater Samples

2.1 Abstract

The current standard method for total phosphorus (TP) measurement in wastewater samples is colorimetry with a digestion step. ICP-OES is being adopted as a faster, more efficient method for TP measurement, however, there is limited data comparing the two methods for wastewater samples. The objective of this work was to compare ICP-OES and colorimetry using the ascorbic acid method followed by persulfate digestion and sulfuric acid – nitric acid (SANA) digestion. This was done using three organic phosphorus model compounds prepared in pure water: adenosine 5'-triphosphate (ATP), phytic acid and 2-aminoethyl phosphonate (AEP), at low concentration of 0.15 mg P/L and high concentration of 4 and 5 mg P/L. The detection limit for colorimetry was 0.002 mg P/L and 0.09 mg P/L for ICP-OES. Highest recoveries were found for ICP-OES (between 89-98%), followed by persulfate digestion (93-106% for high concentration, and 71-77% for low concentration), and lastly by SANA digestion (90-91% for ATP, 11% for phytic acid at high concentration, and the remaining samples were not detected). ICP-OES and colorimetry followed by persulfate digestion were also compared for 91 wastewater samples. The linear equation for the correlation was found to be $y = 1.12x + 0.16$, with an r^2 value of 0.94 and $p < 0.05$. ICP-OES seems promising for TP measurements given its high recoveries for model compounds, but more work is needed to improve its detection limit and sensitivity.

2.2 Introduction

Due to its eutrophication tendency, phosphorus (P) concentration is a parameter monitored by many wastewater treatment plants in many parts of the world¹⁻⁴. Regulations for P in effluents are based on total phosphorus (TP) levels⁵ and are increasingly moving towards concentrations lesser than 0.05 mg P/L⁶. Currently, the standard method for TP determination in wastewater effluents is colorimetric analysis^{7,8}. Since it is largely orthophosphates (and some condensed phosphates) that respond directly to the colorimetric analysis, a digestion step is required to oxidize all forms of P in the sample to phosphates⁸. Colorimetry itself has several advantages: quick to perform, inexpensive, requiring simple equipment and easy to automate, but a digestion step renders the method time-consuming and more sophisticated to automate. Moreover, digestion procedures do not always guarantee complete conversion of all phosphorus species into orthophosphate, especially for hard-to-digest samples such as phosphonates which have refractory C-P bonds⁹. In a 2005 review by Worsfold et. al, recoveries for various model P compounds and standard samples in a wide variety of liquid matrices are listed¹⁰. Recovery for sodium tripolyphosphate in a sewage matrix was 85%. Recoveries for 2-aminoethylphosphonate (2-AEP) in various matrices ranged from 77-108%. Incomplete digestion of P compounds can potentially result in underestimation of TP levels.

Other techniques for instrumental analysis of P in liquid samples include inductively coupled plasma with optical emission spectroscopy (ICP-OES) or mass spectrometry (ICP-MS), high pressure liquid chromatography (HPLC), and X-Ray fluorescence (XRF)¹¹. HPLC requires extensive method development for each P compound being analysed and is not suitable for routine TP analysis. XRF has high detection limits¹². Still other methods exist (voltammetry, potentiometric titration, fluorimetry, etc.¹¹) but are not commonly used for P analysis. TP analysis by ICP-OES has historically not been common due to its high detection limits, but advances in ICP technologies are making their use for low

level P analysis more attractive, and they are already being used in some laboratories⁷. In the agriculture industry, ICP-MS and ICP-OES are being adopted for TP determination in soil extracts and several comparisons have been made between colorimetry and ICP¹³⁻¹⁶. However, for ICP measurements for TP in wastewater samples, little data exists in the literature. Manzoori et. al. (1999) have shown the possibility of using ICP-OES for wastewater samples, including a treated sewage sample¹⁷. Their comparisons with standard colorimetric methods and ICP-OES showed very good correlation (8.0 and 7.6 µg/mL measured for TP using ICP and standard colorimetric method respectively), however their analysis was done in conjunction with a molybdate-based colorimetric flow analyzer, whereby the samples eluting from the manifold were introduced into the ICP-OES, and so the matrix of the wastewater sample was modified.

The aim of this paper is to describe a comparison between TP measurements made using ICP-OES directly and standard colorimetry methods in terms of accuracy, precision and detection limits, to determine if ICP-OES can replace colorimetry as the standard method for TP analysis. The objectives were addressed by first using representative model P compounds and testing their analytical recoveries, and then comparing TP in real water and wastewater samples obtained from various sources.

2.3 Materials and methods

2.3.1 Model compounds

Three model compounds were used in this study to test for P analytical recovery, including: (1) adenosine 5'-triphosphate disodium salt hydrate, 99%, from Sigma Aldrich, (abbreviated ATP) (an organic condensed phosphate with C-O-P and P-O-P bonds), (2) phytic acid sodium salt hydrate from rice, 22% P, from Sigma Aldrich (abbreviated PhyA) (a phosphate ester with a refractory C-O-P bond), and (3) 2-aminoethyl phosphonic acid, 99%, from Sigma Aldrich (abbreviated AEP) (a phosphonate, with

a refractory C-P bond). These compounds were chosen as they have a range of chemical bonds and resistance to hydrolysis, and are representative of naturally occurring P compounds¹⁰.

A certified reference material (CRM) (H_3PO_4 , Sigma Aldrich, 1002 ± 4 mg P/L, Lot BCBM9148V) was also included in the tests as a check standard.

Stock solutions of 100 mg P/L were prepared for the model compounds using 18 M Ω -cm Milli-Q[®] (Millipore Corporation) de-ionized water. Each stock solution and the CRM were diluted to 0.15 and 4-5 mg P/L. These values were chosen to represent a low and high range for effluent TP concentrations. Values below 0.15 mg P/L were not chosen so as to be well above the detection limit of both methods.

2.3.2 Water and wastewater samples

A total of 91 samples obtained from various anonymous wastewater sources were tested in this study for TP using ICP-OES and colorimetry with persulfate digestion. These included: (1) 6 samples from different locations in US and Canada, including: 2 filtered reverse osmosis concentrates from different municipal water recycling facilities, 1 filtered secondary effluent from a municipal WWTP, 1 filtered tertiary final effluent from a municipal WWTP, 1 unfiltered final effluent from a municipal WWTP, and 1 unfiltered final effluent from an automotive industrial WWTP. Speciation of the samples is given in Table 3 (supplementary data). (2) 18 wastewater effluents run through metal oxide based sorbents and anion exchange resin sorbents for various times; (3) 67 samples passed through Purifics Photo-Cat[®] reactor, ranging in sample matrix (synthetic wastewater, natural river water spiked with KH_2PO_4 , and some WWTP effluents listed in set 1), pH, and addition of TiO_2 nanoparticles. (A description of the Photo-Cat[®] reactor is provided by Gerrity et. al. 2009, p. 1599¹⁸.) Samples listed in (2) and (3) are parts of other phosphorus related studies^{19,20}.

2.3.3 Colorimetry analysis

For total P measurements, the sulfuric acid-nitric acid digestion and persulfate digestion procedures described in Standard Methods for the Examination of Water and Wastewater were used (Methods 4500-P B, Parts 4 and 5)⁸. Deviations and exact volumes are described in sub-section 2.3.3.1. After digestion of samples, colorimetric determination was done using Seal Analytical AutoAnalyzer Method No. G-103-93 Rev. 10 (multitest MT8, p.11). This method is based on the ascorbic acid method described in Standard Methods (Method 4500-P E)⁸. Absorption measurements were made at 660 nm.

2.3.3.1 Digestion

Digestion was done on 10 mL samples in 20 mL glass vials. For sulfuric acid-nitric acid digestion, 0.2 mL concentrated sulfuric acid and 1 mL of concentrated nitric acid were added. For persulfate digestion, 0.2 mL of 30% sulfuric acid and 80 mg/L ammonium persulfate was added. The samples were placed on a hot plate in the fume hood for 2 hours at 105 °C or until the volume had been reduced to 1 mL. After removing from the heat and cooling to room temperature, 1 drop of phenolphthalein was added followed by 5N sodium hydroxide until a faint pink color persisted. The samples were re-diluted to 10 mL by weight.

2.3.3.2 Standards, blanks and quality control

Standards in the 0-6 mg P/L range were made from a 1000 mg P/L stock solution prepared using KH_2PO_4 (99%, from BDH). 18 M Ω ·cm Milli-Q[®] (Millipore Corporation) de-ionized water was used for all solution preparation. Blanks (prepared using ultrapure water) and standards were run through the digestion steps.

2.3.4 ICP-OES instrumentation and analysis

The ICP-OES model used was Perkin-Elmer Optima 8000 dual-view, equipped with a Perkin-Elmer S10 autosampler. A gemcone nebulizer with an argon humidifier, cyclonic spray chamber, and ceramic torch were used. Pump tubes were 1.14 mm internal diameter and were made of PVC. Autosampler tubes were 1.14 mm internal diameter and made of PharMed. The software used for data processing was WinLab32 Version 5.5.0.0714. Phosphorus was measured in the axial mode at 213.6 nm. Other operating conditions used include: power: 1500 W; pump flow rate: 2 L/min; plasma gas (Ar): 15 L/min; auxiliary gas (Ar): 0.5 L/min; nebulizer gas (Ar): 0.6 L/min; viewing height above induction coil: 15 mm.

Calibration solutions were prepared using KH_2PO_4 in Milli-Q water. These were checked against calibrations done in synthetic wastewater matrix and were found not to be different (Figure 7 in supplementary data). The carrier solution used was 2% HNO_3 . All samples were acidified with nitric acid to pH 2-3 prior to analysis.

2.4 Results and discussion

2.4.1 Uncertainty in ICP-OES results

The detection limit (defined as three times the standard deviation of the blank sample for $n \geq 5$) for colorimetry was measured to be 0.002 mg P/L. For ICP-OES, the value was found to vary with each analysis date and ranged from 0.01-0.09 mg P/L.

Uncertainty in ICP-OES measurements can be estimated from Figure 3. There is high variation for samples at and below 0.15 mg P/L.

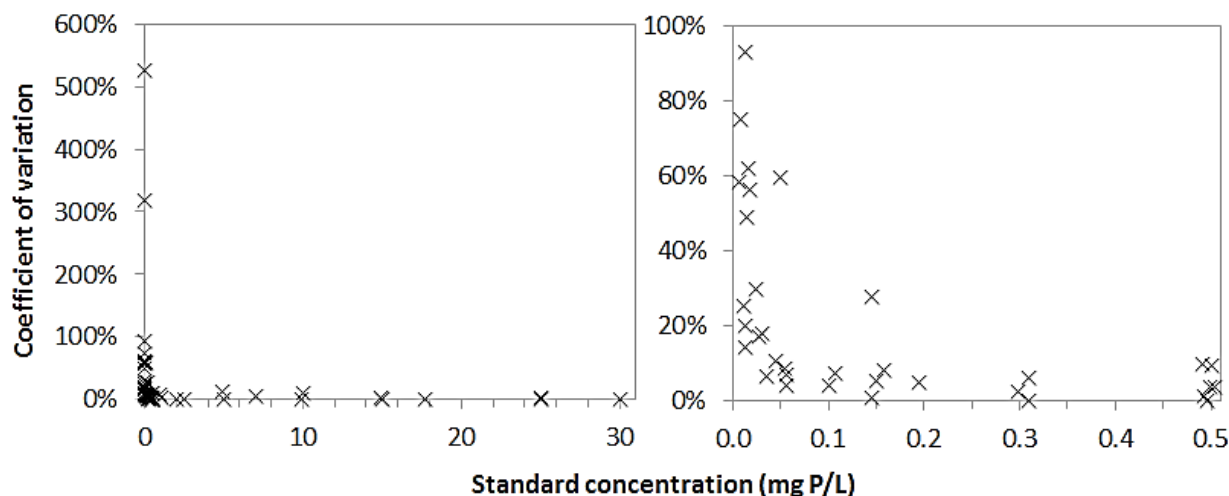


Figure 3. Estimation of uncertainty in ICP-OES. Both plots show the same data on different scales. Coefficient of variation = standard deviation in measurement/mean value.

2.4.2 Determination of TP recoveries in model compounds

Table 2 shows the TP recovered in ATP, PhyA, AEP and the CRM, for low and high concentrations, by ICP-OES as well as colorimetry with persulfate digestion and sulfuric acid-nitric acid (SANA) digestion.

Table 2. Analytical recoveries (reported as percentages) for the model compounds. Values in **blue** are considered 'good' (90-100%); values in **green** are considered 'moderate' (70-89% or 111-130%), values in **red** are considered 'poor' (0-15%). DL = detection limit; n = number of samples measured.

| Compound | ICP-OES | Persulfate digestion | SANA digestion | ICP-OES | Persulfate digestion | SANA digestion |
|--|-------------------------------------|----------------------------------|----------------------------------|---------------------------|---------------------------|---------------------------|
| | 0.15 mg P/L, unless noted otherwise | | | 4-5 mg P/L | | |
| H₃PO₄ CRM | 91 ± 5 (n = 3) | (1 mg P/L) 123 (n = 1) | (1 mg P/L) 124 (n = 1) | 93 ± 3 (n = 3) | 109 (n = 1) | 107 (n = 1) |
| ATP | 93 ± 2 (n = 3) | 77 ± 3 (n = 3) | 91 ± 5 (n = 3) | 93 ± 12 (n = 4) | 99 ± 1 (n = 3) | 90 ± 1 (n = 3) |
| PhyA | 89 ± 14 (n = 3) | 71 ± 2 (n = 3) | < DL (n = 3) | 91 ± 4 (n = 3) | 93 ± 1 (n = 3) | 11 ± 2 (n = 3) |
| AEP | 98 ± 3 (n = 3) | 75 ± 2 (n = 3) | < DL (n = 3) | 93 ± 4 (n = 3) | 106 ± 6 (n = 3) | < DL (n = 3) |

ICP-OES gave essentially full recoveries at both low and high concentrations for all compounds tested. The recoveries for the 0.15 mg P/L samples were 89-98%, and the recoveries for the 4 mg P/L samples were 91-93%.

The recoveries for colorimetry with persulfate digestion were also good for the 4 mg P/L samples (93-109%). However, for the 0.15 mg P/L, the values were only 71-77% for the model compounds, and were much higher than expected for the CRM: 123%. The difference in the behavior of low- and high- concentration samples is not immediately clear.

The SANA digestion worked well for ATP and CRM only for 4 mg P/L samples (with recoveries of 90 and 107% respectively). At the low concentration, only ATP was recovered well (91%). The CRM was unexpectedly high (124%). Recoveries for both PhyA and AEP were very low, for both low and high concentrations (11% for PhyA for the high concentration, and below detection for all other samples). The results for the ATP, PhyA and AEP are not surprising. Condensed phosphates (e.g. ATP) are easier to break down than other organic P compounds. It is likely that the conditions used for SANA digestion were not harsh enough for breaking down the more refractory AEP and PhyA, but they were for persulfate digestion.

In this study, persulfate digestion appears to be better than SANA digestion for breaking down organic P compounds, and ICP-OES appears to be better than colorimetric methods of TP determination.

2.4.3 Wastewater samples

A total of 91 wastewater samples were analyzed for TP by ICP-OES and colorimetry with persulfate digestion. Due to poor recoveries for model compounds obtained by SANA digestion, this method was not tested on wastewater samples.

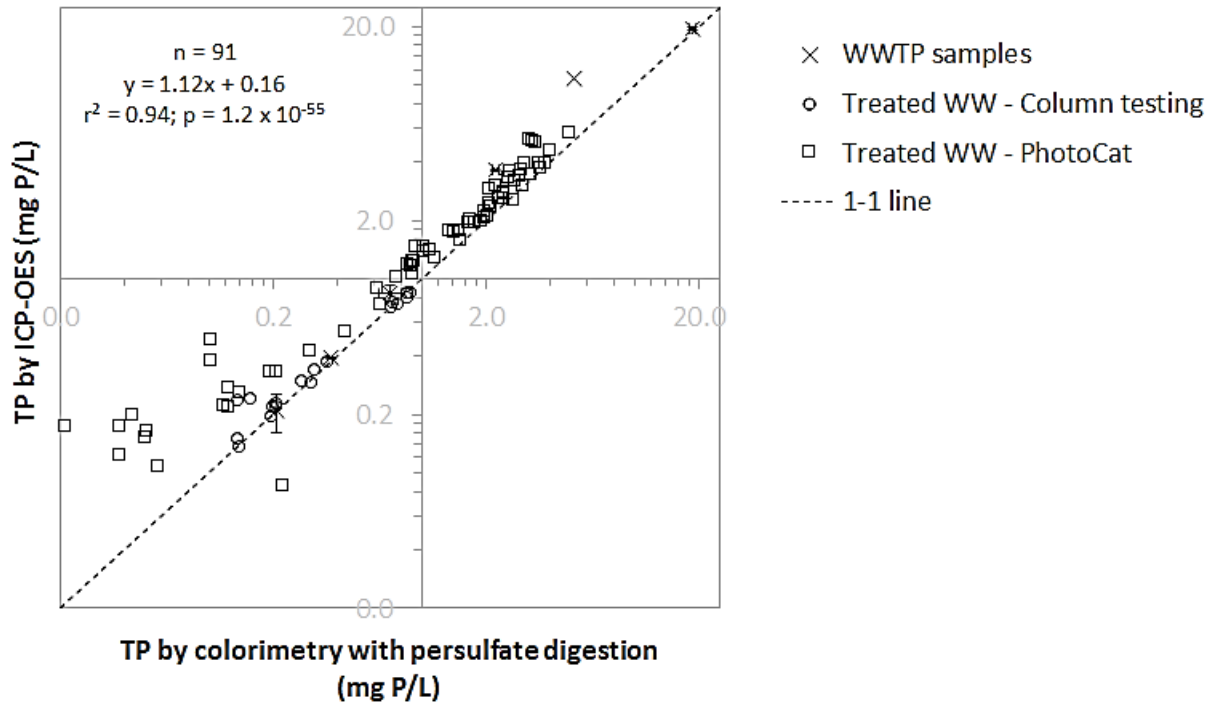


Figure 4. Linear regression on wastewater samples. The x- and y- axes are in logarithmic scale, and have been greyed to view the data points easily.

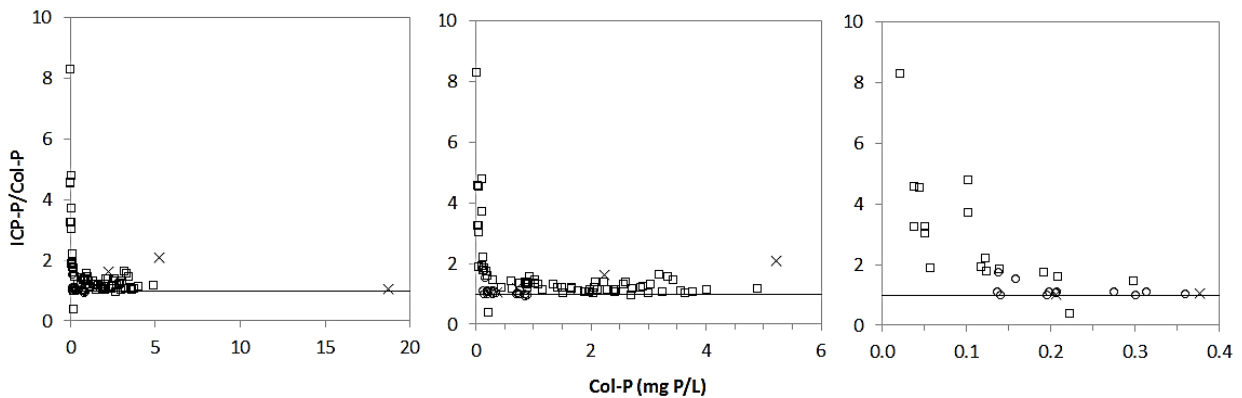


Figure 5. Relationship between ICP-P/colorimetry-P and colorimetry-P (col-P) for all wastewater samples. The different graphs show the same data with different x-axis scales. The black horizontal line represents ICP/Colorimetry = 1.

A linear regression and correlation (Figure 4) was performed on the wastewater samples to determine if the results using both methods are comparable. A highly significant relationship was found

between the two methods. When all samples were analyzed together, the linear equation obtained was $y = 1.12x + 0.16$ with $r^2 = 0.94$ and $p < 0.05$. The 95% confidence intervals for the slope were 1.06-1.18 and 0.00-0.32 for the intercept. As the slope and the intercept are higher than 1 and 0 respectively, the regression analysis implies that the TP measured by ICP is consistently higher than that measured by colorimetry.

The relationship of ICP-P/colorimetry-P to colorimetry-P (Figure 5) indicates that the deviation from 1:1 is greater for concentrations below 0.15 mg P/L. There are two possible reasons for this: (1) ICP-OES may not be as sensitive at concentrations below 0.15 mg P/L. Figure 3 shows high uncertainty in ICP-OES measurements below 0.15 mg P/L. (2) Persulfate digestion does not completely recover all the TP in the samples. Poor recoveries for lower concentration samples using persulfate digestion were shown in Table 2.

To observe if the amount of organic P in wastewater samples affects the results of TP analysis in ICP-OES and colorimetry, an analysis of ICP-P/Colorimetry-P versus the organic P content in the wastewater samples was performed (Figure 6).

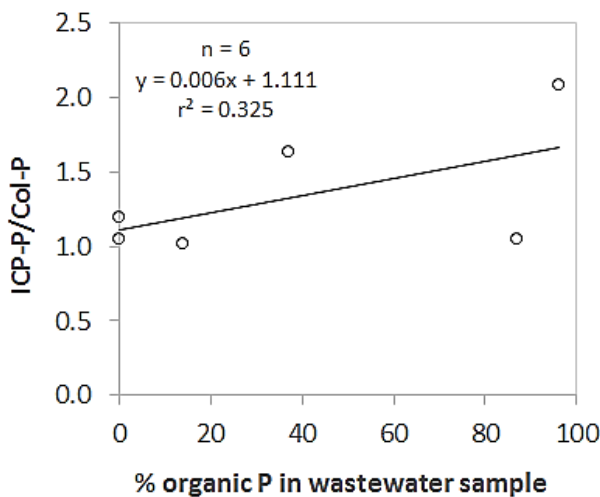


Figure 6. Effect of %organic P in the wastewater sample on the ICP-P/colorimetry-P ratio.

It was hypothesized that digestion (for colorimetric analyses) may not break down all organic P and hence give lower colorimetry-P values, whereas ICP will be able to measure all TP in the samples. Hence, as the percent organic P in samples increases, the ICP-P/Col-P ratio should increase. However, Figure 6 shows a very weak relationship between the two ($r^2 = 0.325$). A low, albeit, positive slope was obtained and the difference between ICP-OES and colorimetry was as high as twice in some cases (sample with 96% organic P in Figure 6). It must be emphasized that this analysis was only performed on 6 samples and for more conclusive results, a greater number of samples should be analyzed.

2.5 Conclusions

Analytical recoveries of three naturally occurring organic phosphorus compounds in water were compared between ICP-OES and the ascorbic acid colorimetric method (Standard Methods 4500-P E). Digestions for colorimetry were done using the sulfuric acid-nitric acid (SANA) and persulfate methods. The compounds tested were adenosine 5'-triphosphate (ATP), phytic acid (PhyA) and 2-aminoethyl phosphonic acid (AEP). Of these, PhyA and AEP are refractory compounds and resistant to hydrolysis. Comparisons between ICP-OES and colorimetry with persulfate digestion were also made using 91 samples with wastewater or wastewater-like matrices.

The detection limit for colorimetry was measured to be 0.002 mg P/L, and 0.01 - 0.09 mg P/L for ICP-OES.

ICP-OES recovered all model compounds to > 89% at both low (0.15 mg P/L) and high (4 and 5 mg P/L) concentrations. Persulfate digestion recovered all compounds to > 93% at high concentrations, but to $\leq 75\%$ at low concentrations. SANA digestion recovered $\geq 90\%$ ATP, but $\leq 10\%$ PhyA and 0% AEP. Since recoveries of ICP-OES were closest to 100% particularly at the low concentration, the results

suggest ICP-OES is the most accurate and reliable method for measuring total phosphorus in refractory compounds.

The uncertainty in ICP-OES measurements, estimated from standard deviations for known phosphate standards, was high for concentrations below 0.15 mg P/L making the technique insensitive to low measurements.

A strong correlation was found between ICP-OES and persulfate digestion followed by colorimetry, with $p < 0.05$. The linear equation obtained was $y = 1.12x + 0.16$ with $r^2 = 0.94$. The slope indicated that ICP-OES consistently measured higher phosphorus than colorimetry. The disagreements in the measurements were much greater for concentrations below 0.15 mg P/L. It is not clear whether the anomaly is due to ICP-OES recovering higher phosphorus, and/or due to the persulfate method giving incomplete digestions. The difference was also greater (as much as twice) for samples with high organic phosphorus.

More work is needed for both methods before low phosphorus concentrations can be accurately made and compared. Based on the high recoveries for organic phosphorus model compounds, ICP-OES seems very promising.

2.6 Acknowledgements

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2.8 Supplementary data

Table 3. Characterization of wastewater samples. NOTE: % inorganic P = (RP/TP) x 100; % organic P = 100 - % inorganic P.

| Sample No. | Sample description | P Speciation | | | |
|------------|--|----------------------|----------------------|---------------|-------------|
| | | RP (mg P/L) | TP (mg P/L) | % inorganic P | % organic P |
| 1 | Reverse osmosis concentrate from municipal water recycling facility (filtered) | 21.43 ± 1.61 (n = 5) | 19.58 ± 0.33 (n = 3) | 100%* | 0% |
| 2 | Reverse osmosis concentrate from municipal water recycling facility (filtered) | 2.29 ± 0.04 (n = 3) | 3.65 ± 0.02 (n = 3) | 63% | 37% |
| 3 | Secondary effluent from a municipal WWTP (filtered) | 0.19 ± 0.01 (n = 3) | 0.21 ± 0.00 (n = 2) | 91% | 9% |
| 4 | Tertiary final effluent from a municipal WWTP (filtered) | 1.34 ± 0.00 (n = 3) | 1.60 ± 0.02 (n = 2) | 84% | 16% |
| 5 | Final effluent from a municipal WWTP (unfiltered) | 0.05 ± 0.00 (n = 2) | 0.31 ± 0.00 (n = 2) | 16% | 84% |
| 6 | Final effluent from an automotive industrial WWTP (unfiltered) | 0.40 ± 0.02 (n = 2) | 8.96 ± 0.22 (n = 2) | 4% | 96% |

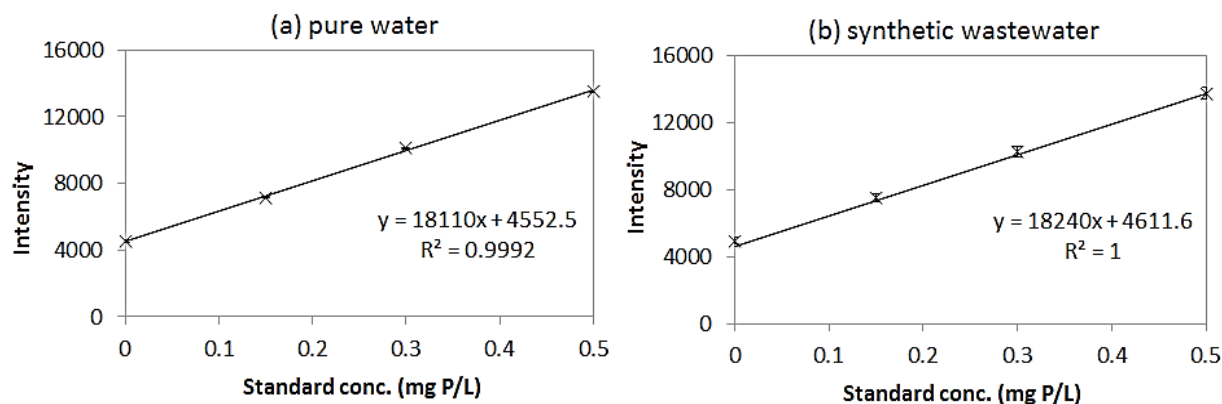


Figure 7. Comparison of calibration curves made using pure water and synthetic wastewater. The synthetic wastewater composition used is shown in Table 4.

Table 4. Synthetic wastewater composition (prepared in aqueous medium). Adapted from Jung e. al. (2005)²¹.

| Compound | Concentration (mg/L) |
|---|-----------------------------|
| Magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) | 24.0 |
| Calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) | 2.4 |
| Sodium bicarbonate (NaHCO_3) | 300.0 |
| Sodium acetate (CH_3COONa) | 820.3 |

3 Chemical removal of total phosphorus from synthetic wastewater to low levels

3.1 Abstract

The most abundant form of phosphorus in wastewater, inorganic phosphates, has been well studied for P removal to prevent eutrophication in natural waters, but less attention has been given to the examination of removal of organic forms of P. This work tested the ability of manganese oxide nanoparticles and iron oxide as potential catalysts for conversion of organic P into inorganic forms, as well as the roles of iron and aluminum oxides as coagulants and adsorbents to subsequently allow P to be removed by sedimentation. The chemical conditions under which the metal oxides can efficiently achieve these roles were studied, including addition of hydrogen peroxide and sodium hypochlorite as oxidation aids, and varying metal dosage and pH. Adenosine 5'-triphosphate (ATP), 2-aminoethylphosphonic acid (AEP) and phytic acid (PhyA) were used as organic P model compounds, representing a range of organic forms of P. ICP-OES was used as the primary analytical tool to detect phosphorus removal. Essentially complete removals of 99-101 % were obtained for the model compounds at pH 5-7, 0.05-0.5 M H₂O₂, and Fe:P molar ratio of 5:1. Tests in synthetic wastewater showed that presence of possible interfering ions did not reduce the removal efficiency, demonstrating great potential of removing organic P from real wastewater.

3.2 Introduction

The chemical removal of inorganic phosphorus from wastewater has been widely studied due to it being the most bioavailable phosphorus form¹, and has been successfully achieved to low levels²⁻⁵. In contrast, the removal of organic phosphorus (OP) in wastewater has received little attention. As regulations for P effluent limits are moving towards lower concentrations, and evidence of abundance and bioavailability of OP is surfacing¹, the need to remove OP is becoming increasingly significant.

While other studies have attempted to remove OP from wastewater, their focus has been on a specific P compound^{6,7}, and an opportunity still exists for the need to study P removal for a variety of functional groups present in wastewater matrices. The objective of this study was to find a protocol capable of removing all different OP forms, using a combination of technologies and approaches used in the wastewater treatment industry.

Examples of organic forms of phosphorus potentially present in wastewater include condensed phosphates such as adenosine triphosphate (ATP), phosphate esters such as inositol phosphates, and phosphonates, such as 2-amino ethyl phosphonate. These compounds can enter wastewater from various sources. ATP is generated by plants and animals⁸. Inositol phosphates are synthesized by plants and are a major component of OP in many soils from which they can leach into water⁹. Phosphonates are used in detergents, inhibitors of scale formation and other industrial applications, from which they can be discharged into wastewater treatment plants^{10,11}. Of these compounds, phosphonates are the hardest to remove due to having a C-P bond which is much more stable than C-O-P bonds in the other OP compounds.

It has been hypothesized in this study that organic P compounds can be converted to inorganic forms, specifically orthophosphates, and thus be made chemically reactive to already established

methods for phosphate removal. To this end, the use of oxidants including hydrogen peroxide and bleach were assessed, as well as manganese oxide nanoparticles and iron oxides as potential catalysts for the oxidation of organic P compounds. Iron, and in some cases, aluminum chlorides were then used to precipitate out inorganic P (the breakdown product of oxidation), which later becomes part of the solids. pH, metal, and oxidant dosages were also varied in order to achieve high removals.

The treatments were initially done in a pure water matrix. These tests were used to screen for a protocol with the highest P removal, which was subsequently tested in a synthetic wastewater matrix. The results of various treatment methods assessed in both pure water and synthetic wastewater are presented in this article.

Phosphorus measurements were performed using ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry). Though colorimetry with an acid digestion is the standard method used as a measure of total P in aquatic samples¹², it was discovered that peroxide interferes with the ascorbic acid method used for colorimetry. Moreover, it was suspected that the conditions used in acid digestion would not be harsh enough to break down all the organic P compounds successfully, particularly phosphonates, thereby reducing the analytical recoveries of the phosphorus compounds. After measuring %P recoveries for model organic phosphorus compounds by both ICP-OES and colorimetry with persulfate digestion, and finding similar or better recoveries on the ICP-OES, the latter was recognized as the more appropriate method for this study's analyses.

3.3 Materials and Methods

Five chemicals were selected to represent a mixture of organic phosphorus compounds in wastewater, and were chosen due to their varying structures and high possibility of being present in

wastewater¹³. These 'model compounds' are listed below, along with their source and abbreviations as used in this paper.

1. Potassium phosphate monobasic, KH_2PO_4 (BDH, 99%) – included as a control
2. Adenosine 5'-triphosphate disodium salt hydrate (Sigma Aldrich, 99%) (*abbr.* ATP)
3. Phytic acid sodium salt hydrate from rice (Sigma Aldrich, 22% P) (*abbr.* PhyA)
4. 2-aminoethyl phosphonic acid (Sigma Aldrich, 99%) (*abbr.* AEP)
5. Sodium triphosphate pentabasic (Fluka, 98%) (*abbr.* NaTP)

Thirteen different treatment protocols were attempted with each model compound varying in metal dosage, pH, types and amounts of oxidants used (full details present in Table 5 in Section 3.4). The tests were initially done in a pure water matrix to screen for the protocol giving the highest P removal. This protocol was then tested in synthetic wastewater to assess whether the presence of other chemicals interferes with the P removal process. The synthetic wastewater was prepared using a modified protocol from Jung et. al. (2005) and contained magnesium sulfate, calcium chloride, sodium bicarbonate and sodium acetate in water (exact composition shown in Table 6, supplementary data)¹⁴.

3.3.1 Treatment and solid-liquid separation

Stock solutions of 100 mg P/L were prepared for compounds 2-5. Stock solution of 1000 mg P/L was prepared for KH_2PO_4 . These were then diluted to 5 mg P/L (0.16 mM P). For treatment in pure water, 18 M Ω ·cm Milli-Q® (Millipore Corporation) de-ionized water was used for the dilution. For treatment in synthetic wastewater, water prepared using the recipe in Table 6 was used for dilution. To 10 mL of each 5 mg P/L solution in separate glass vials, hydrogen peroxide (Sigma Aldrich, 30-32 wt.% in water) was added to achieve a final concentration of 0.01-0.5 M. (The exact concentration for each trial is indicated in Table 5 in Section 3.4.) 0.5 M was initially chosen as a concentration to start with. This

concentration was lowered in subsequent trials down till 0.05 M until no loss in removal efficiency was observed. Bleach (Clorox, 7.4 wt.% sodium hypochlorite) was used as the oxidant instead of hydrogen peroxide in three of the treatment protocols. 1 mL of bleach was added to 10 mL samples to get a final concentration of 0.1 M NaOCl. Manganese (III,IV) oxide nanoparticles (*abbr.* MnO_{2-x} NPs), prepared in-house (composition shown in Table 7), were added to the samples after the addition of peroxide or bleach, to get a final metal concentration of 0.4 mM Mn. Finally, iron(III) chloride (ferric chloride hexahydrate, Fluka, 99-102%) was added to achieve an Fe concentration of 0.4-0.8 mM. In two of the treatment protocols, aluminum(III) chloride was used instead of iron. The final concentration of Al in these samples was 0.8 mM. These concentrations were chosen to allow the metal to be well in excess of the phosphorus. The samples were stirred throughout the addition of each chemical. pH was adjusted the following day (after 18-24 hours) using HCl (Fluka Analytical, 1N standard solution) or NaOH (Sigma Aldrich, 5N volumetric standard) to values between 4 and 9 (exact pH values are shown in Table 5). After pH adjustment, the samples remained unstirred overnight, after which the solids were separated from the liquid using either centrifugation or filtration using syringe filters with 25 mm polyethersulfone membranes with 0.45 µm pore size.

3.3.2 Phosphorus Analysis

The liquid fraction from the treated samples was acidified using HNO₃ (Fisher Scientific Canada, Certified ACS Plus) to 2% by volume, and then analyzed for total P using ICP-OES. The ICP-OES model used was Perkin-Elmer Optima 8000 dual-view, equipped with a Perkin-Elmer S10 autosampler. A gem-tip cross flow nebulizer, Scott spray chamber, and quartz torch were used. Pump tubes were 1.14 mm internal diameter and were made of PVC. Autosampler tubes were 1.14 mm internal diameter and made of PharMed. The software used for data processing was WinLab32 Version 5.5.0.0714. Phosphorus was measured in the axial mode at 213.6 nm. This wavelength was chosen due to having the highest

sensitivity. (Results for wavelength selection from 177.4, 178.2 and 214.9 nm in axial modes, as well as 213.6 nm in radial mode are included in Appendix A.) Other operating conditions used included: power: 1500 W; pump flow rate: 2 L/min; plasma gas (Ar): 15 L/min; auxiliary gas (Ar): 0.5 L/min; nebulizer gas (Ar): 0.6 L/min; viewing height above induction coil: 15 mm.

Calibration solutions were prepared from a 1000 mg P/L stock of KH_2PO_4 in Milli-Q water. For determination of P in samples treated in pure water, standards were prepared from the stock using pure water. For samples treated in synthetic wastewater, the dilutions were done in synthetic wastewater.

The line at 213.6 nm was checked to ensure absence of interference from FeCl_3 , MnO_{2-x} NPs, H_2O_2 , bleach and synthetic wastewater. Duplicate, blank and check standards were run every 10-12 samples.

The %P removed from the treated samples was used as the main measurement for assessment of the treatment methods and was calculated as follows:

$$\%P \text{ removed} = \frac{1 - [P] \text{ measured in liquid fraction (mgP/L)}}{[P] \text{ added to the sample (mgP/L)}} \times 100 \quad [1]$$

3.4 Results and discussion

3.4.1 Tests in pure water

Results from the various treatment protocols (summarized in Table 5 and Figure 8) showed highest P removals for a combination of the following conditions: pH 5, Fe:P molar ratio of 5:1 and H_2O_2 0.1 M (serial number [abbr. S. No.] 13, Table 5). It was found that when all variables were kept constant and the peroxide concentrations alone were changed from 0.5 M to 0.05 M, the %P removal remained the same. For this reason, the concentrations in S. Nos. 6, 12 and 13 are shown as a range. Where pH

was not adjusted, the value measured 18-24 hours after addition of ferric chloride is shown in brackets.

The following sub-sections discuss the effect of each variable on the %P removed in detail.

Table 5. Results for %P Removal for each P compound using various treatment methods, done in pure water. The uncertainties in the table represent standard deviations for n number of replicates. '---' indicates no MnO_{2-x} NPs or oxidant added.

| S. No. | pH | Mn:P | Fe:P | Al:P | [Oxidant] (M) | %P Removed | | | | |
|--------|--------|------|------|------|---|---------------------------------|-------------------|---------------------|----------------------|---------------------|
| | | | | | | KH ₂ PO ₄ | ATP | NaTP | AEP | PhyA |
| 1 | (3.0) | --- | 5 | --- | H ₂ O ₂ 0.1 | 8 | 8 | 8 | 8 ± 0.4 (n = 2) | --- |
| 2 | (10.2) | --- | 2.5 | --- | NaOCl 0.1 | 20 | 18 | --- | 13 | --- |
| 3 | (9.1) | 2.5 | 2.5 | --- | NaOCl 0.1 | 23 | 18 | 25 ± 2 (n = 2) | 3 | --- |
| 4 | 6 | --- | 2.5 | --- | NaOCl 0.1 | 39 ± 5 (n = 2) | 48 | --- | 15 | 94 |
| 5 | (3.8) | 2.5 | 2.5 | --- | --- | 54 | 41 | 34 | 86 ± 0.6 (n = 2) | --- |
| 6 | (3.3) | 2.5 | 2.5 | --- | H ₂ O ₂ 0.1-0.5 | 91 ± 4 (n = 3) | 88 ± 2 (n = 3) | 86 ± 0.5 (n = 2) | 84 ± 2 (n = 2) | --- |
| 7 | 6 | 2.5 | 2.5 | --- | H ₂ O ₂ 0.5 | 92 | 82 | --- | --- | --- |
| 8 | 6 | --- | 2.5 | --- | --- | 96 | 92 ± 1 (n = 2) | --- | 14 ± 3 (n = 3) | --- |
| 9 | 5 | --- | 5 | --- | --- | 98 ± 1 (n = 2) | 98 ± 2 (n = 2) | 99 ± 1 (n = 2) | 18 ± 1 (n = 3) | --- |
| 10 | 5 | --- | --- | 5 | --- | 100 | 100 | 100 | 17 | 99 |
| 11 | 5 | --- | --- | 5 | H ₂ O ₂ 0.1 | 100 | 99 | 99 | 46 | 99 ± 0.1 (n = 2) |
| 12 | 5 | --- | 2.5 | --- | H ₂ O ₂ 0.05-0.5 | 97 ± 2 (n = 3) | 95 ± 1 (n = 2) | 97 | 96 ± 2 (n = 2) | 94 |
| 13 | 5 | --- | 5 | --- | H ₂ O ₂ 0.05-0.1 | 99 ± 2 (n = 2) | 99 | 101 | 101 ± 0.3 (n = 2) | 96 ± 1 (n = 2) |

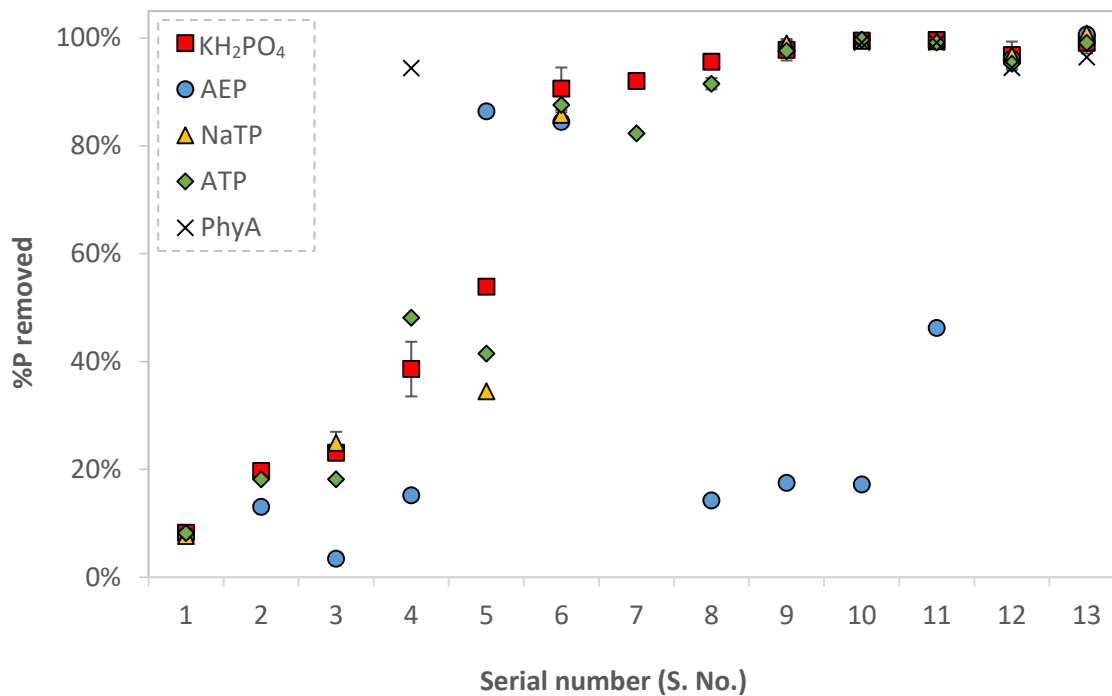


Figure 8. %P removed for each compound using various treatment methods, performed in pure water. The serial number corresponds to the numbers in the first column in Table 5.

3.4.2 Effect of pH

pH has a significant effect on P removal at extremely low and high values. Figure 9 shows the %P removed for all the P compounds used in this study as pH increases from 4 to 9. The tests were done in pure water, with Fe:P molar ratio of 5:1, and 0.05 M H₂O₂. The curve shows a characteristic 'U-shape' of %P removed versus pH, and suggests that the optimal pH for removal of these compounds is between 5 and 7, with removal efficiency decreasing rapidly outside these ranges.

Since it is hypothesized that the organic P compounds liberate phosphate groups on oxidation, the P removal curves of these compounds should be similar to that of orthophosphate. This is found to be true in the dataset presented here, up to pH 7. The data is also consistent with a previous study

performed by Szabo et. al. (2008) who studied the factors affecting phosphate removal and showed pH 5-7 as the best range for achieving low soluble orthophosphate¹⁵.

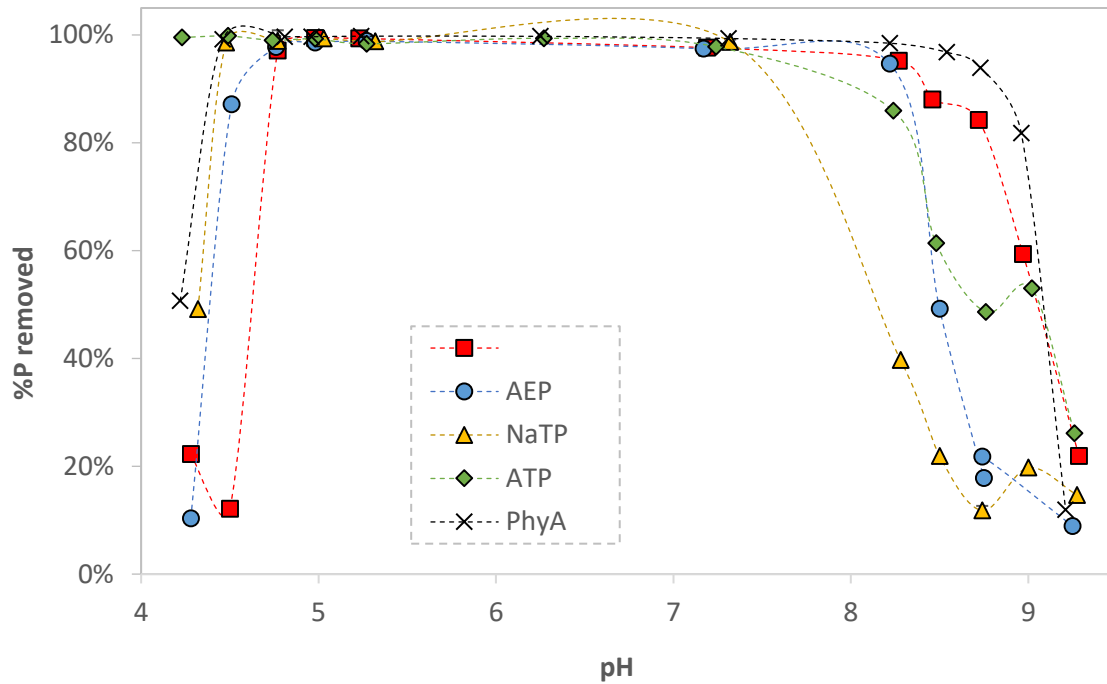


Figure 9. Effect of pH on %P removed for various compounds. The dashed lines are included only as a guide to trace trends. Data for residual-P is shown in Figure 11 (supplementary data).

Smith et. al. (2008) who developed a surface complexation model for phosphate, showed that removal of orthophosphate involves two main mechanisms: co-precipitation of phosphate into HFO (hydrated ferric oxide) flocs, and adsorption of phosphate onto HFO¹⁶. HFO flocs are formed during neutralization of iron chloride solution, which is initially acidic¹⁶. At low pH values, iron hydroxides are not formed. As pH increases, iron hydroxides start to precipitate (formation of HFO flocs). As the pH increases further, iron hydroxides start to resolubilize, owing to a build-up of negative charge on their surface¹⁶. Thus, when the pH is not highly acidic or basic, the formation of solid iron hydroxides occurs, capable of removing phosphate during co-precipitation and further by adsorption onto the precipitates.

The pH range 5-7 is convenient for wastewaters that are slightly alkaline with pH around 8; the formation of iron hydroxides upon addition of iron chloride to the wastewater releases protons, which offsets the alkalinity and brings the pH close to 6, at which efficient removal occurs.

3.4.3 Effect of oxidants

Hydrogen peroxide did not affect P removal for KH_2PO_4 , ATP and NaTP; however, for AEP, addition of just 0.05 M peroxide increased the removal from 18% to a 101% when Fe:P molar ratio was 5:1 and pH was adjusted to between 5 and 7 (s. nos. 9 and 13). The same effect was observed when a lower Fe:P molar ratio of 2.5:1 was used (s. nos. 8 and 12). KH_2PO_4 was not expected to be affected by peroxide as it is an orthophosphate and does not require oxidation. For ATP and NaTP, the P removal was already high with no addition of peroxide (possibly due to hydrolysis in the acidic conditions upon addition of ferric chloride) and therefore not affected by peroxide as well. AEP which is a phosphonate and has the refractory C-P bond was barely removed without peroxide addition, but the removal increased from 18% to 101% with addition of 0.05 M peroxide.

Compared to hydrogen peroxide, sodium hypochlorite did not work as effectively under similar conditions. The decrease in effectiveness may have been less due to oxidation potential of the hypochlorite and more due to decrease in formation of flocs as there was a significant drop in KH_2PO_4 removal observed, which is an orthophosphate and is not expected to be affected by oxidation (s. No. 4). It is possible that the negatively charged hypochlorite ion competes with phosphate for adsorption sites onto iron hydroxides. It should also be noted that the sodium hypochlorite used was not reagent grade and may contain other interfering impurities.

3.4.4 Effect of iron dose

Two different iron doses were tested: Fe:P molar ratio of 2.5:1, and 5:1. The higher iron dose increased P removal by 2% (s. nos. 8, 9, 12, and 13). Szabo et. al. (2008) and Smith et. al. (2008) also showed a decrease in residual soluble $\text{PO}_4\text{-P}$ on application of higher metal doses^{15,16}. Increasing the amount of coagulant forms more HFO flocs, into which an increased amount of phosphate can co-precipitate with and adsorb onto, thereby removing more P from solution. While higher metal doses give better P removals Fe:P molar ratios greater than 5 were not tested as these values are outside engineering practice, due to production of higher amounts of sludge.

3.4.5 Aluminum(III) versus iron(III)

Without peroxide added, aluminum worked as well as iron for all compounds tested (s. nos. 9 and 10), with a metal:P ratio of 5. Removal for KH_2PO_4 , ATP and NaTP were 98-100%, while removal for AEP was only 17-18% using both metals. With peroxide added (s. nos. 11 and 13), there was a marked increase in the removal of AEP with both metals, however treatment with Fe(III) was far superior. %P removed with Al(III) was 46%, while with Fe(III) was 101%.

Iron can exist in +2 and +3 oxidation states and hence participate in redox chemistry with peroxide. This reaction catalyzes the C-P bond breakage in AEP. Since Al has only one stable oxidation state of +3 in the soluble form, it does not participate in redox chemistry. The slight increase from 17 to 46% with peroxide addition in the case of aluminum treatment, was likely just due to breakage of C-P bond from peroxide itself.

3.4.6 Effect of MnO_{2-x} nanoparticles catalyst

MnO_{2-x} NPs gave good P removals (85-90%) even at acidic pH (s. no. 6). In this case, Fe:P molar ratio was 2.5:1 and Mn:P ratio was also 2.5:1, so that the total metal:P ratio was 5:1. Peroxide was 0.1-

0.5 M, and pH was not adjusted, but was measured to be ~3. The adjustment of pH to 5-7 was only tested for KH_2PO_4 and ATP and did not seem to have a significant effect on P removal (s. no. 7).

The removal with iron alone as the coagulant was found to be better than with MnO_{2-x} NPs, in both cases when the total metal:P molar ratio was 2.5:1 or 5:1, however the pH had to be adjusted to 5-7. At acidic pH values, FeCl_3 alone could not achieve high P removal. Therefore, the addition of MnO_{2-x} NPs may be good for treatment of acidic wastewaters. For treatment of slightly basic or neutral wastewater, iron alone would work better.

3.5 Phosphorus removal in synthetic wastewater

The conditions that enabled the most efficient P removal in pure water were tested in a synthetic wastewater recipe. These conditions included adjustment of pH to 5-7; Fe:P molar ratio of 5:1; and H_2O_2 0.05 M. Almost complete removal, 96-98%, was obtained with each compound (Figure 10), indicating the treatment method works well in the presence of increased ionic strength and potentially interfering anions in wastewater including chloride, sulfate, bicarbonate and acetate.

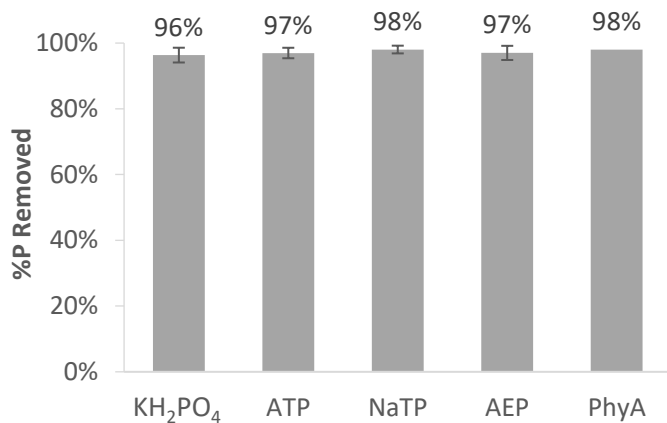


Figure 10. %P Removed for different phosphorus compounds in synthetic wastewater. The error bars represent standard deviations for n=5.

3.6 Conclusions

Experiments were conducted using organic and inorganic model phosphorus compounds in order to achieve high P removal percentages. These compounds included: potassium phosphate monobasic, sodium triphosphate, adenosine 5'-triphosphate, phytic acid, and 2-aminoethyl phosphonic acid. The experimental parameters varied included adjustment of pH, use of H₂O₂ and bleach as oxidants, MnO_{2-x} nanoparticles as oxidation catalyst, and ferric or aluminum chloride as the coagulant.

Essentially complete P removals of 99-101% were obtained with a combination of: Fe:P molar ratio 5:1, pH 5-7, and [H₂O₂] = 0.05-0.5 M. This combination worked as efficiently in the presence of other chemicals found in wastewater, including magnesium sulfate, calcium chloride, sodium bicarbonate and sodium acetate. Presence of H₂O₂ was found necessary to remove phosphonates in particular. Addition of just 0.05 M H₂O₂ increased the removal of 2-aminoethyl phosphonic acid from 18% to 101% when ferric chloride was used. When aluminum chloride was used, the increase was 17% to 46%.

Highest P removals occurred for a broad range of pH from 5 to 7, when the only metal added was Fe(III). The removal efficiency decreased rapidly at very acidic and basic pH. P removal in acidic samples can be significantly improved by addition of MnO_{2-x} nanoparticles.

3.7 References

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3.8 Supplementary data

Table 6. Synthetic wastewater composition (prepared in aqueous medium). Adapted from Jung e. al. (2005)¹⁴.

| Compound | Concentration (mg/L) |
|---|----------------------|
| Magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) | 24.0 |
| Calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) | 2.4 |
| Sodium bicarbonate (NaHCO_3) | 300.0 |
| Sodium acetate (CH_3COONa) | 820.3 |

Table 7. Composition of MnO_{2-x} NPs (prepared in aqueous medium).

| Compound | Concentration (mM) |
|------------------------------|--------------------|
| Manganese chloride | 1.6 |
| Sodium polystyrene sulfonate | 1.6 |
| Guanidine carbonate | 1.28 |
| Hydrogen peroxide | 32 |

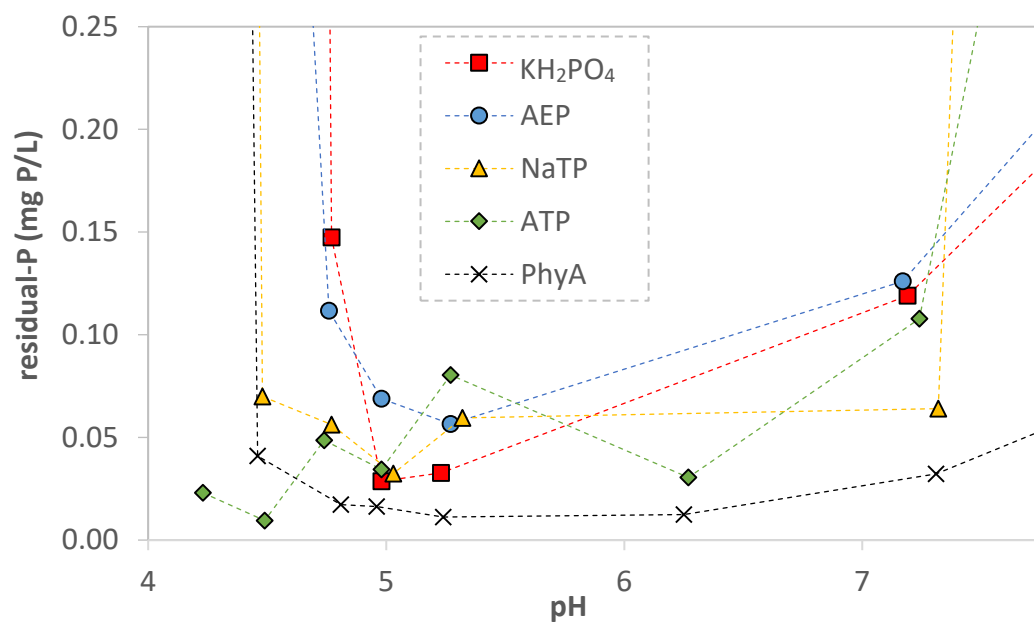


Figure 11. Effect of pH on residual-P. Note: For KH_2PO_4 , AEP and PhyA, the LOD was 0.05 mg P/L, for NaTP and ATP, the LOD was 0.12 mg P/L. Dashed lines are only included as a guide for trends.

4 Chemical removal of total phosphorus from real wastewaters to low levels

4.1 Abstract

Phosphorus exists in wastewater as both inorganic and organic forms. The removal of the inorganic fraction has been the focus of many studies due to it being the more abundant form. Increasingly stricter phosphorus regulations, however, make the removal of organic forms of phosphorus more relevant. In this study, chemical phosphorus removal was attempted with seven wastewater samples with the goal of achieving residual phosphorus concentrations below 0.1 mg P/L. Iron(III) and aluminum chlorides were used as the coagulants, and hydrogen peroxide was used to oxidize and convert organic phosphorus in the samples to orthophosphate. The samples varied significantly in phosphorus concentration (0.21-19.58 mg P/L as TP) and % organic P (0-96%). Residuals of 0.1 mg P/L were achieved in all cases, except one, where residual of 0.3 mg P/L was achieved. Other main findings of the study were: (1) With addition of hydrogen peroxide, samples with high organic phosphorus showed higher removals than with no peroxide addition, when FeCl_3 was used as the coagulant. The enhanced removal with peroxide was not observed with AlCl_3 . (2) Treatment in two stages (splitting the total metal dose between two steps, and filtering to remove the solids each time) can give significantly improved removals.

4.2 Introduction

Chemical phosphorus removal using aluminum and iron salts is a common practice in wastewater treatment plants and has been the focus of many studies in the literature¹⁻⁴. As the major form of P in wastewater is orthophosphates⁵, many of these studies aim to remove this form alone from wastewater. This strategy can result in non-compliance in wastewater treatment plants (WWTPs) when effluent P limits are set to very low levels, since other minor, but refractory, forms can become more relevant⁶⁻⁸. Increasingly, there is a push for lower P effluent limits with values as low as 0.05 mg P/L (as total phosphorus - TP) being proposed^{9,10}. These low targets create a need to investigate the applicability of chemical phosphorus removal methods to the removal of various forms of P.

Phosphorus exists in wastewater in both soluble and particulate forms which can be organic or inorganic in nature¹¹⁻¹³. Inorganic forms of phosphorus include orthophosphates (PO_4^{3-}) and some condensed phosphates (e.g. tripolyphosphates)^{12,14}. Organic forms of phosphorus consist of phosphonates and orthophosphates attached to an organic moiety (e.g. phytic acid and adenosine 5'-triphosphate)^{12,14}. Phosphonates are refractory compounds and have C-P bonds that are very resistant to oxidation and hydrolysis¹². Phytic acid has also shown resistance to hydrolysis¹². These compounds have been detected in soils from which they can leach into water bodies¹². Phosphonates can be found in pesticides and pharmaceutical products as well, and have been detected in effluents¹⁵⁻¹⁷.

The objective of this study was to remove phosphorus from wastewaters with varying amounts of inorganic and organic P, in order to achieve residual phosphorus below 0.1 mg P/L. The research presented in this paper is a continuation of the studies from Chapter 3, "Chemical removal of total phosphorus from synthetic wastewater to low levels," in which the same objective was achieved using model P compounds added to pure water and waste-water like matrices. The conclusions from the

preceding study were applied in this chapter to real wastewater effluents obtained from various municipal and industrial water and wastewater treatment plants. The treatment was done using ferric chloride as the coagulant, and was compared to treatment with aluminum chloride in selected cases. The pH used for the treatment was 5-6. As the coagulants do not directly remove all forms of P, hydrogen peroxide was added as an oxidant to convert organic P forms into orthophosphate and make them chemically reactive to the coagulants. Manganese (III,IV) oxide nanoparticles were added to some of the treatment steps and assessed as a potential catalyst to the aforementioned oxidation.

4.3 Methods

4.3.1 Wastewater samples

A total of seven wastewater samples obtained from various anonymous locations in US and Canada were studied in this project. The samples included: 2 reverse osmosis concentrates from two different municipal water recycling facilities (labelled *ROC-1* and *ROC-2* in this paper), 1 secondary effluent from a municipal WWTP (labelled *WW-1*), 1 tertiary final effluent from a second municipal WWTP (labelled *WW-2*), 1 sample from a pilot-scale anaerobic membrane bioreactor (labelled *AMBR*); 1 final effluent from a third municipal WWTP (labelled *WW-3*), and 1 final effluent from an automotive industrial WWTP (labelled *AUTO*). The sample *AUTO* was influent to *WW-3*.

Samples *ROC 1*, *ROC 2*, *WW-1*, *WW-2* and *AMBR* were filtered through 0.45 μm polyethersulfone membranes (25 mm). Samples *AUTO* and *WW-3* were kept unfiltered. The characterization for the samples is shown in Table 8 in Section 4.4.

4.3.2 Treatment and solid-liquid separation

The samples were treated in glass vials using 10 mL aliquots. For samples that were treated using oxidant, hydrogen peroxide (Sigma Aldrich, 30-32 wt.% in water) was added to achieve a final concentration of 0.05 or 0.1 M. (The concentration for each trial is indicated in Table 9 in Section 4.4.) Manganese(III,IV) oxide nanoparticles (*abbr.* MnO_{2-x} NPs), prepared in-house (composition shown in Table 10), were added to the samples after the addition of peroxide, to get a final metal concentration of 0.4 mM Mn. Iron(III) chloride (ferric chloride hexahydrate, Fluka, 99-102%) or aluminum(III) chloride was then added to achieve an Al/Fe concentration of 0.4-0.8 mM (exact metal used and concentration are shown in Table 9). The samples were stirred throughout the addition of each chemical. pH was adjusted the following day (after 18-24 hours) using HCl (Fluka Analytical, 1N standard solution) or NaOH (Sigma Aldrich, 5N volumetric standard) to values between 4 and 9 (exact pH values are shown in Table 9). After pH adjustment, the samples remained unstirred overnight, after which the solids were separated from the liquid using either centrifugation or filtration with 25 mm 0.45 µm polyethersulfone membranes.

4.3.3 Chemical analysis

4.3.3.1 Total phosphorus

The liquid fraction from the treated samples was acidified using HNO₃ and then analyzed for total P using ICP-OES. The ICP-OES model used was Perkin-Elmer Optima 8000 dual-view, equipped with a Perkin-Elmer S10 autosampler. A gemcone high salts nebulizer, argon humidifier (to prevent salt build-up at the nebulizer), cyclonic spray chamber and ceramic torch were used. The pump tubes were 1.14 mm internal diameter and were made of PVC. Autosampler tubes were 1.14 mm internal diameter and made of PharMed. The software used for data processing was WinLab32 Version 5.5.0.0714. P was

measured in the axial mode at 213.6 nm. Other operating conditions were: power: 1500 W; pump flow rate: 2 L/min; plasma gas (Ar): 15 L/min; auxiliary gas (Ar): 0.5 L/min; nebulizer gas (Ar): 0.6 L/min; viewing height above induction coil: 15 mm.

Calibration solutions were prepared from a 1000 mg P/L stock of KH_2PO_4 in 18 M Ω ·cm Milli-Q® (Millipore Corporation) de-ionized water. These were checked against calibrations done in synthetic wastewater matrix and were found not to be different. The carrier solution used was 2% HNO_3 . All standards and blanks were acidified with nitric acid to pH 2-3 prior to analysis. Duplicate, blank and check standards were run every 10-12 samples. Samples with high standard deviations were remade and retested.

The % P removed from the treated samples was used as the main measurement for assessment of the treatment methods and was calculated as follows:

$$\% P \text{ removed} = \frac{1 - [P] \text{ measured in liquid fraction (mg P/L)}}{[P] \text{ measured in untreated sample (mg P/L)}} \times 100 \quad [1]$$

The limit of detection (LOD) for the ICP-OES was calculated using $n \geq 5$ blank samples (de-ionized water acidified with nitric acid) and was defined as “three times the standard deviation of the blank concentration.” The limit of quantification (LOQ) was defined as “ten times the standard deviation of the blank concentration.”

4.3.3.2 Reactive phosphorus

Reactive phosphorus (RP) was measured using colorimetry. Colorimetric determination was done using Seal Analytical AutoAnalyzer Method No. G-103-93 Rev. 10 (multitest MT8, p.11). This method is based on the ascorbic acid method described in Standard Methods (Method 4500-P E). The measurements were made at 660 nm.

4.3.3.3 Total/Dissolved Organic Carbon analysis

Measurements were made using a Shimadzu Total Organic Carbon Analyzer TOC-L. For filtered samples, the measured value was reported as dissolved organic carbon (DOC), and for unfiltered samples, the total organic carbon (TOC) was reported.

4.4 Results & Discussion

4.4.1 Wastewater samples characterization

Table 8 shows the pH, P speciation, and organic carbon content of the seven wastewater samples. The samples vary significantly in the amount of P (ranging from 0.21 to 19.58 mg P/L as TP), P speciation (ranging from 96% organic P to 100% inorganic P) and organic C (from 9 to 585 mg C/L).

Table 8. Characterization of wastewater samples. NOTE: % inorganic P = (RP/TP) x 100; % organic P = 100 - % inorganic P.

*For ROC-1, the % inorganic P was determined to be 109%, but is reported as 100%.

| Sample | pH | P Speciation | | | | TOC/DOC (mg C/L) |
|--------|-----|-------------------------|-------------------------|---------------|-------------|---------------------|
| | | RP (mg P/L) | TP (mg P/L) | % inorganic P | % organic P | |
| ROC-1 | 8.2 | 21.43 ± 1.61 (n = 5) | 19.58 ± 0.33 (n = 3) | 100%* | 0% | 147 (DOC) |
| ROC-2 | 8.2 | 2.29 ± 0.04 (n = 3) | 3.65 ± 0.02 (n = 3) | 63% | 37% | 65 (DOC) |
| WW-1 | 8.0 | 0.19 ± 0.01 (n = 3) | 0.21 ± 0.00 (n = 2) | 91% | 9% | 9 (DOC) |
| WW-2 | 6.9 | 1.34 ± 0.00 (n = 3) | 1.60 ± 0.02 (n = 2) | 84% | 16% | 37 (DOC) |
| WW-3 | 7.3 | 0.05 ± 0.00 (n = 2) | 0.31 ± 0.00 (n = 2) | 16% | 84% | 12 (TOC) |
| AMBR | 7.8 | 4.27 ± 0.05 (n = 3) | 4.85 ± 0.07 (n = 2) | 88% | 12% | 11 (DOC) |
| AUTO | 9.9 | 0.40 ± 0.02 (n = 2) | 8.96 ± 0.22 (n = 2) | 4% | 96% | 585 (TOC) |

4.4.2 Treatment results

Table 9 summarizes the % P removed for all the protocols tested using the seven wastewater samples. The effect of each parameter varied is discussed in detail in sections 3.2.2-3.2.5.

Table 9. Composition and results of all protocols attempted. Note 1: sample was treated in 2-steps; Note 2: sample was unfiltered. Numbers in bold: below LOQ. LOD and LOQ changed with each analysis date.

| S. No. | pH | Mn:P | Fe:P | Al:P | [H ₂ O ₂] (M) | Notes | P _{initial} (mg P/L) | P _{residual} (mg P/L) | % P removed |
|-------------|----|------|------|------|---|-------|----------------------------------|-----------------------------------|--------------------|
| WW-1 | | | | | | | | | |
| 1 | 5 | --- | 5.4 | --- | 0 | | 0.204 | < 0.07 | > 64 |
| 2 | 5 | --- | 5.4 | --- | 0.1 | | 0.204 | < 0.07 | > 64 |
| 3 | 5 | --- | --- | 5.4 | 0 | | 0.206 | < 0.07 | > 64 |
| 4 | 5 | --- | --- | 5.4 | 0.1 | | 0.204 ± 0.000 (n = 2) | < 0.07 (n = 2) | > 64 (n = 2) |
| WW-3 | | | | | | | | | |
| 5 | 5 | --- | 6.4 | --- | 0 | 2 | 0.386 | 0.167 | 57 |
| 6 | 5 | --- | 6.4 | --- | 0.1 | 2 | 0.383 | 0.074 | 81 |
| 7 | 5 | --- | 12.7 | --- | 0 | 2 | 0.384 | 0.104 | 73 |
| 8 | 5 | --- | 12.7 | --- | 0.1 | 2 | 0.380 | < 0.04 | > 90 |
| 9 | 5 | 3.2 | 3.2 | --- | 0 | 2 | 0.379 | 0.220 | 42 |
| 10 | 5 | 3.2 | 3.2 | --- | 0.1 | 2 | 0.375 | 0.138 | 63 |
| 11 | 5 | --- | 8.1 | 8.1 | 0.1 | 2 | 0.300 | 0.084 | 72 |
| 12 | 5 | --- | --- | 6.4 | 0 | 2 | 0.386 | 0.184 | 52 |
| 13 | 5 | --- | --- | 6.4 | 0.1 | 2 | 0.382 | 0.184 | 52 |
| 14 | 5 | --- | 12.8 | --- | 0 | 1,2 | 0.383 | 0.056 | 85 |
| 15 | 5 | --- | 12.8 | --- | 0.1 | 1,2 | 0.380 ± 0.001 (n = 2) | < 0.04 (n = 2) | > 90 (n = 2) |
| 16 | 5 | --- | 16.2 | --- | 0.1 | 1,2 | 0.300 | < 0.07 | > 76 |
| 17 | 5 | --- | 6.2 | --- | 0 | | 0.251 | 0.167 | 33 |
| 18 | 5 | --- | 6.2 | --- | 0.1 | | 0.248 ± 0.001 (n = 3) | 0.115 ± 0.050 (n = 3) | 53 ± 20 (n = 3) |
| WW-2 | | | | | | | | | |
| 19 | 5 | --- | 3.2 | --- | 0 | | 1.556 | 0.156 | 90 |
| 20 | 5 | --- | 3.2 | --- | 0.1 | | 1.546 | 0.132 | 91 |
| 21 | 5 | --- | 24.5 | --- | 0 | | 1.010 | 0.041 | 96 |

| | | | | | | | | | |
|--------------|---|-----|------|------|------|---|--------------------------|--------------------------|-------------------|
| 22 | 5 | --- | 24.5 | --- | 0.05 | | 1.005 | 0.033 | 97 |
| ROC-2 | | | | | | | | | |
| 23 | 5 | --- | 2.5 | --- | 0 | | 3.730 | 0.550 | 85 |
| 24 | 5 | --- | 4.9 | --- | 0 | | 3.763 | 0.147 | 96 |
| 25 | 5 | --- | 4.9 | --- | 0.01 | | 3.763 | 0.204 | 95 |
| 26 | 5 | --- | 4.9 | --- | 0.05 | | 3.749 | < 0.01 | > 99.7 |
| 27 | 5 | --- | 4.9 | --- | 0.5 | | 3.597 | < 0.01 | > 99.0 |
| 28 | 6 | --- | 3.3 | --- | 0 | | 3.610 ± 0.000 (n = 2) | 0.354 ± 0.010 (n = 2) | 90 ± 0 (n = 2) |
| 29 | 6 | --- | 3.3 | --- | 0.05 | | 3.586 ± 0.010 (n = 2) | 0.530 ± 0.054 (n = 2) | 85 ± 1 (n = 2) |
| 30 | 6 | --- | 4.1 | --- | 0 | 1 | 3.761 ± 0.000 (n = 2) | 0.290 ± 0.019 (n = 2) | 92 ± 1 (n = 2) |
| 31 | 6 | --- | 5.0 | --- | 0 | | 3.600 ± 0.000 (n = 2) | 0.275 ± 0.018 (n = 2) | 92 ± 0 (n = 2) |
| 32 | 6 | --- | 5.0 | --- | 0.05 | | 3.583 ± 0.000 (n = 2) | 0.452 ± 0.029 (n = 2) | 87 ± 1 (n = 2) |
| 33 | 6 | --- | 10.0 | --- | 0 | | 3.584 | 0.205 | 94 |
| 34 | 6 | --- | 10.0 | --- | 0.05 | | 3.560 | 0.224 | 94 |
| AMBR | | | | | | | | | |
| 35 | 5 | --- | 4.6 | --- | 0 | | 5.281 | 0.038 | 99 |
| 36 | 5 | --- | 4.6 | --- | 0.05 | | 5.267 | 0.104 | 98 |
| 37 | 5 | --- | 5.5 | --- | 0 | | 4.743 | < 0.07 | > 98 |
| 38 | 5 | --- | 5.5 | --- | 0.1 | | 4.701 | < 0.07 | > 98 |
| 39 | 5 | --- | --- | 5.5 | 0 | | 4.752 | < 0.07 | > 98 |
| 40 | 5 | --- | --- | 5.5 | 0.1 | | 4.697 | < 0.07 | > 98 |
| AUTO | | | | | | | | | |
| 41 | 5 | --- | 13.1 | --- | 0 | 2 | 10.251 | 6.682 | 35 |
| 42 | 5 | --- | 13.1 | --- | 0.1 | 2 | 10.159 | 5.099 | 50 |
| 43 | 5 | --- | 24.3 | --- | 0.1 | 2 | 8.229 | 0.586 | 93 |
| 44 | 5 | --- | 28.5 | --- | 0 | 2 | 9.878 | 0.251 | 97 |
| 45 | 5 | --- | 28.5 | --- | 0.1 | 2 | 9.793 | 0.215 | 98 |
| 46 | 9 | --- | 8.7 | --- | 0.1 | 2 | 10.415 | 9.735 | 7 |
| 47 | 9 | --- | 8.7 | --- | 0 | 2 | 10.507 | 9.872 | 6 |
| 48 | 5 | 2.2 | 2.2 | --- | 0 | 2 | 7.199 | 7.014 | 3 |
| 49 | 5 | 2.2 | 2.2 | --- | 0.1 | 2 | 7.151 | 6.691 | 6 |
| 50 | 5 | --- | 12.1 | 12.1 | 0.1 | 2 | 8.208 | 0.672 | 92 |
| 51 | 5 | --- | 17.3 | 17.3 | 0.1 | 2 | 8.063 | 0.101 | 99 |

| | | | | | | | | | |
|--------------|---|-----|------|-----|------|-----|---------------------------|--------------------------|--------------------|
| 52 | 5 | --- | --- | 4.4 | 0 | 2 | 10.485 | 10.335 | 1 |
| 53 | 5 | --- | --- | 4.4 | 0.1 | 2 | 10.399 | 10.919 | -5 |
| 54 | 5 | --- | 8.7 | --- | 0 | 1,2 | 10.338 | 1.417 | 86 |
| 55 | 5 | --- | 8.7 | --- | 0.1 | 1,2 | 10.249 ± 0.000 (n = 2) | 1.384 ± 0.172 (n = 2) | 86 ± 2 (n = 2) |
| 56 | 5 | --- | 24.3 | --- | 0.1 | 1,2 | 8.178 | < 0.07 | > 99 |
| 57 | 5 | --- | 7.9 | --- | 0 | | 8.220 | 7.473 | 9 |
| 58 | 5 | --- | 7.9 | --- | 0.1 | | 8.125 ± 0.029 (n = 2) | 6.493 ± 0.977 (n = 2) | 20 ± 12 (n = 2) |
| ROC-1 | | | | | | | | | |
| 59 | 5 | --- | 2.5 | --- | 0 | | 14.500 | 0.659 | 95 |
| 60 | 5 | --- | 4.9 | --- | 0 | | 14.572 | 0.495 | 97 |
| 61 | 5 | --- | 4.9 | --- | 0.01 | | 14.508 | 0.466 | 97 |
| 62 | 5 | --- | 4.9 | --- | 0.05 | | 14.455 | 0.536 | 96 |
| 63 | 5 | --- | 4.9 | --- | 0.5 | | 13.711 | 0.283 | 98 |
| 64 | 6 | --- | 5.2 | --- | 0 | | 18.782 ± 0.005 (n = 2) | 0.692 ± 0.013 (n = 2) | 96 ± 0 (n = 2) |
| 65 | 6 | --- | 5.2 | --- | 0.05 | | 18.667 ± 0.018 (n = 3) | 0.622 ± 0.082 (n = 3) | 97 ± 0 (n = 3) |
| 66 | 6 | --- | 10.0 | --- | 0.05 | | 18.248 | 0.370 | 98 |
| 67 | 6 | --- | 5.7 | --- | 0 | 1 | 14.151 ± 0.027 (n = 2) | 0.550 ± 0.027 (n = 2) | 96 ± 0 (n = 2) |

4.4.2.1 Uncertainty in the results

The LOD for the ICP-OES was calculated for each analysis date and was found to vary between 0.01 and 0.07 mg P/L.

For treated samples that gave residual-P below detection limit, the % P removal was reported as:

$$\%P \text{ removed} > \frac{1 - \text{LOD for the analysis date (mg P/L)}}{[P] \text{ measured in untreated sample (mg P/L)}} \times 100 \quad [2]$$

From all the replicates with the treated wastewater samples, the standard deviation in the % P removed was found to be in the range of 0.1-4.4% (see Table 11 in supplementary data for all the values). Standard deviations of 12% and 20% were also found for two data points, but based on previous

experience with the ICP-OES measurements, these samples are thought to be outliers. (These samples are discussed briefly in Section 4.4.2.2). These standard deviations account for uncertainties in sample preparation as well instrument noise. When comparing % P removals across various series in Table 9 in the following discussions, differences of less than 5% in the P removals were not considered significant. For example, 96% and 97% are not considered different, whereas 96% and 90% are. When comparing any two values across Table 9, differences in phosphorus *concentrations* were also checked to make sure they were not directly within error of one another, based on data in Figure 12. For example, the values 0.167 and 0.074 mg P/L are accepted as 'different', but 0.156 and 0.132 mg P/L are not.

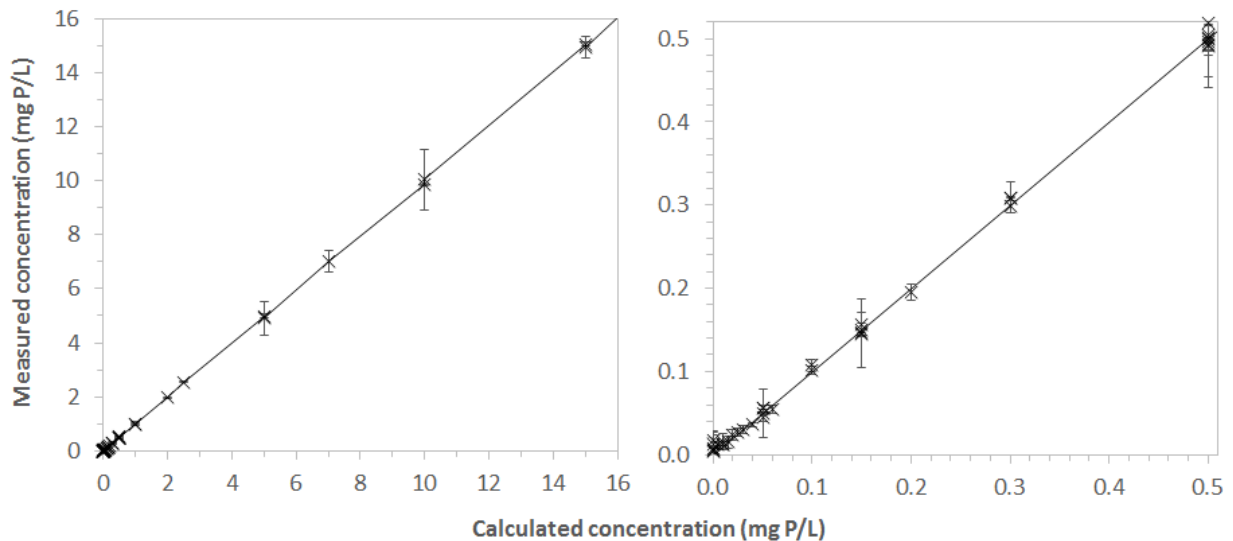


Figure 12. Measured versus calculated (expected) concentrations for standards prepared using KH_2PO_4 in pure water. The error bars represent standard deviations in the measurements. ('n' varied for each data point). The black solid line represents the 1:1 ratio.

4.4.2.2 Effect of oxidant

For samples with low organic P (ROC-1, AMBR and WW-2, 0%, 12% and 16% organic P respectively), the % P removed was high without the aid of peroxide (97%, > 98% and 90% respectively);

S. Nos. 60, 37 and 19) and there was little room for increased removals. For ROC-2 which had 37% organic P, the P removal was also high without any peroxide added (96%, S. No. 24). Samples with very high organic P (WW-3 and AUTO), however, showed marked differences. % P removal for WW-3 increased from 57 to 81% on addition of 0.1 M peroxide when pH was 5 and Fe:P ratio was 6.4 (S. Nos. 5 and 6). When the Fe:P ratio was increased to 12.7, the same trend was found and % P removal increased from 73 to > 90% (S. Nos. 7 and 8). For AUTO, the % P removed increased from 35 to 50% when Fe:P ratio was 13.1 and pH was adjusted to 5 (S. Nos. 41 and 42).

Since WW-3 and AUTO were the only unfiltered samples, to ensure that the enhanced effect of peroxide on these samples was not due to any particulate fraction, the tests were repeated on filtered aliquots of WW-3 and AUTO. % P removal for filtered WW-3 increased from 33 to 53% on addition of 0.1 M peroxide (S. Nos. 17 and 18), and from 9 to 20% for filtered AUTO (S. Nos. 57 and 58). Note that S. Nos. 18 and 58 had high standard deviations, which could have resulted from inconsistencies in filtration. Unfortunately, all the aliquots needed for these samples were not filtered at the same time, increasing the chances of them being nonhomogeneous. These samples were also hard to pass through a 0.45 μm membrane and required several membranes to get 10 mL aliquots.

The enhanced removal with peroxide was not observed for samples treated with AlCl_3 . For WW-3, the removal remained at 52% (S. Nos. 12 and 13) and for AUTO, the removal remained at 0-1% (S. Nos. 52 and 53). It is likely that the increased P removal by peroxide with iron treatment is due to catalysis by iron. Iron can exist in the +2 and +3 oxidation states and hence participate in redox chemistry with peroxide. This reaction catalyzes the breakdown of organic P compounds. Since Al does not have stable oxidation states other than +3 in the soluble form, it does not participate in redox reactions.

4.4.2.3 *Effect of metal*

Four combinations of metals were tested with and without peroxide addition, including: (1) Fe(III) as total metal, (2) Al(III) as total metal, (3) half Fe(III) and half Mn, and (4) half Fe(III) and half Al(III).

For WW-3, when no peroxide was added and pH was adjusted to 5, Fe and Al worked similarly, giving removals of 57 and 52% respectively (S. Nos. 5 and 12). Replacement of Fe with half Mn also did not show a significant difference (S. Nos. 5 and 9); the % P removal dropped from 57 to 42%, but the difference in the residual P concentration was only 0.05 mg P/L. Without peroxide addition then, all metal combinations attempted gave comparable results. When peroxide was added, Al treatment did not show any advantages over absence of peroxide (S. Nos. 12 and 13), but Fe showed a significant improvement (S. Nos. 5 and 6), as already discussed in the previous section. Mn+Fe treatment also showed improvement upon peroxide addition, with an increase to 63% phosphorus removed from 42% (S. Nos. 9 and 10). This may have been due to presence of Fe alone or expected Mn redox activity. Relative to treatment with peroxide and Fe as the only metal though, Mn+Fe with peroxide did not show much higher removal. Data for Al+Mn treatment would be interesting to have to assess treatment by Mn. With peroxide addition, iron(III) appears to be the best metal.

Other samples for which Al and Fe were compared included WW-1, AMBR and AUTO. For WW-1 and AMBR, the residual-P from treatment with both metals, with and without peroxide addition, were below detection limit and meaningful comparisons cannot be drawn between the different protocols. For AUTO, the Al:P ratio used was very low (S. Nos. 52 and 53) and similar data for Fe-treatment is not available.

Replacement of half the iron with aluminum yielded no loss in removal efficiency when tested on AUTO (S. Nos. 43 and 50). For WWTP operators that prefer to use Al over Fe, either due to lower cost of the metal or greater experience with it, this result suggests that they may not have to completely discard Al.

It should also be noted that treatment with ferric chloride at various pH values showed pH 5 to result in lowest residual-P (see following section). Treatment with aluminum was also done at pH 5 but other pH values should be tested to determine which gives the lowest phosphorus residual.

Increasing the Fe:P increased the P removal efficiency. For ROC-2, the removal increased by 11% when Fe:P was increased from 2.5 to 5, with no peroxide added and pH adjusted to 5 (S. Nos. 23 and 24). For AUTO, with pH adjusted to 5 and no peroxide added, the removal increased by 63% when Fe:P increased from 13.1 to 28.5 (S. Nos. 41 and 44). When 0.1 M peroxide was added, the increase was 48% (42 and 45). Very high Fe:P molar ratios (13.1-28.5, S. Nos. 41-45) had to be used for AUTO to get at least a 50% P removal. These created a large amount of precipitated solids, and in actual engineering practice would increase the cost of sludge handling. To reduce the metal dosage, addition of the metal in two steps as opposed to one can result in increased removal efficiency. This is discussed further in section 4.4.2.5.

4.4.2.4 Effect of pH adjustment

pH was not extensively tested in this study, but based on the available results from treatment of the samples at pH values of 5, 6 and 9, highest removals were found for pH 5 in presence of peroxide. In absence of peroxide pH 5 and 6 gave similar removals. Figure 13 summarizes the effect of pH adjustment to 5 and 6 for two samples.

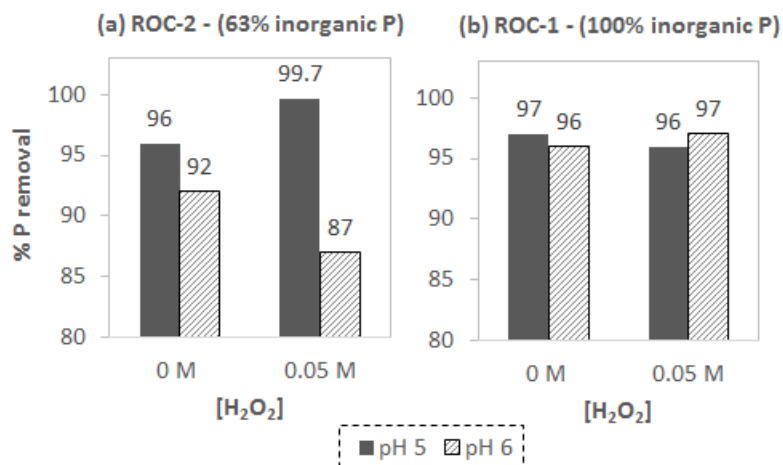


Figure 13. Effect of pH on ROC-1 and ROC-2.

For ROC-2, when the Fe:P ratio was 5 and no peroxide was added, removal at pH 5 was similar to that of removal at pH 6 - 96% and 92% respectively (S. Nos. 24 and 31). With 0.05 M peroxide present, however, the removal at pH 5 was > 99.7% (S. No. 26) while removal at pH 6 was 87% (S. No. 32).

Treatment of ROC-1 did not show any significant changes with pH. When Fe:P ratio was 5, and no peroxide was added, the removal at pH 5 was 97% (S. No. 60) while removal at pH 6 was 96% (S. No. 64). When Fe:P ratio was 5, and 0.05 M peroxide was added, removal at pH 5 was 96% (S. No. 62) while removal at pH 6 was 97% (S. No. 65).

For AUTO, % P removal was tested at pH 9 at Fe:P ratio of 8.7. The removal for treatment with and without peroxide were very low – 6 and 7% respectively (S. Nos. 46 and 47). Directly comparable data at pH 5 or 6 is not available.

4.4.2.5 Two-step treatment

Performing the treatment in two steps instead of one was found to enhance the % P removal significantly. When the Fe:P ratio was 13.1 for AUTO and 0.1 M peroxide was used, the % P removed was 50% (S. No. 42). When the total metal added was reduced to 8.7 times P and added in two steps instead of all together, the removal was much higher – 86% (S. No. 55). Similarly, when the Fe:P ratio was 24.3 the removal was 93% (S. No. 43), but when the same amount was added in two separate steps, the removal was 100%. (S. No. 56). For WW-3, when Fe 12.8 times P was added in one step, the removal was 73% (S. No. 7), but increased to 85% when iron was added in two steps (S. No. 14).

Treatment in two stages is more efficient because in the second stage, metal dose is being applied to a reduced phosphorus level. The Fe/P ratio is therefore higher for the second stage despite the same iron dose being applied in both stages. Szabo et. al. (2008) showed that increasing the Fe/P molar ratio results in lower residual P¹⁸. It should be noted that the two-step treatment not only involved splitting of the total metal added into two steps, but also involved filtering the samples twice, and it is possible that the increased removals occurred due to additional filtration.

4.5 Conclusions

Seven wastewater samples varying considerably in initial phosphorus concentration (from 0.21-20 mg P/L as TP) and percent organic phosphorus (from 0-96%) were subjected to phosphorus removal treatment using ferric and aluminum chloride as coagulants. The samples were also tested to assess whether hydrogen peroxide enhances the phosphorus removal. Phosphorus in all samples except one was reduced to less than 0.1 mg P/L. The 20 mg P/L sample was reduced to below 0.3 mg P/L. Addition of hydrogen peroxide to samples with high organic phosphorus increased the % P removal from 57 to 81% in one case. The enhanced removal with peroxide was observed only for samples treated with

iron(III) chloride, and not with aluminum chloride. Without addition of hydrogen peroxide, iron(III) chloride gave similar phosphorus removal to aluminum chloride. Treatment in two stages as opposed to one (i.e. splitting the total metal dose between two steps, and filtering to remove the solids each time) gave significantly improved removals (increase from 35 to 86% was found in one case).

4.6 References

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4.7 Supplementary data

Table 10. Composition of MnO_{2-x} NPs (prepared in aqueous medium).

| Compound | Concentration (mM) |
|------------------------------|--------------------|
| Manganese chloride | 1.6 |
| Sodium polystyrene sulfonate | 1.6 |
| Guanidine carbonate | 1.28 |
| Hydrogen peroxide | 32 |

Table 11. List of standard deviations measured in the %P removed in all replicates.

| S. No. | Standard deviation in samples | n |
|--------|-------------------------------|---|
| 1 | 0.1% | 2 |
| 2 | 0.2% | 2 |
| 3 | 0.3% | 2 |
| 4 | 0.3% | 2 |
| 5 | 0.4% | 3 |
| 6 | 0.5% | 2 |
| 7 | 0.5% | 2 |
| 8 | 0.8% | 2 |
| 9 | 1.5% | 2 |
| 10 | 1.7% | 2 |
| 11 | 4.4% | 2 |
| 12 | 11.7% | 2 |
| 13 | 20.0% | 3 |

5 Conclusions

5.1 Objectives of this research

The movement of phosphorus regulations for wastewater treatment plants towards low levels creates two problems: (1) upgrading current wastewater treatment methods to enable removal of phosphorus to extremely low levels, and (2) being able to measure low levels of phosphorus reliably. These problems were addressed in this study by defining the following objectives, which were stated in Chapter 1.

1. Develop an interference-free analytical protocol to reliably measure low concentrations of total phosphorus (particularly refractory organic phosphorus compounds) in treated wastewater samples.
(Addressed in Chapter 2)
2. Compare total phosphorus measurements using ICP-OES and colorimetry in terms of accuracy, precision, sensitivity and detection limits in order to contribute data for establishment of ICP-OES as a new standard for total phosphorus measurements.
(Addressed in Chapter 2)
3. Amend the process of chemical phosphorus removal by iron(III) chloride, to enable removal of both inorganic and organic forms of phosphorus to low levels ($\geq 98\%$ phosphorus removal).
(Addressed in Chapter 3)
4. Determine if the phosphorus removal protocol using iron(III) chloride with an oxidizing agent can be applied to real wastewater samples.
(Addressed in Chapter 4)

5.2 Key findings of study and significance

Several conclusions were successfully drawn from the results for all objectives. These are outlined in detail in the conclusion sections of Chapters 2-4. The key findings from the whole study are summarized below.

1. The primary analytical tool selected for the study was ICP-OES. Factors such as instrument parameters, wavelength selection, and data processing were optimized resulting in detection limits of 0.01-0.09 mg P/L. The phosphorus measurements were found to be sensitive, accurate (recoveries for certified reference material were complete) and free of interference from other chemicals used in the treatment, including hydrogen peroxide, bleach, manganese(III, IV) oxide nanoparticles, and iron(III) chloride.

Being able to measure phosphorus reliably, particularly at concentrations below 0.1 mg P/L, enabled successful completion of other objectives in this study.

2. ICP-OES gave complete recoveries (89-98%) for adenosine 5'-triphosphate (ATP), phytic acid (PhyA) and 2-aminoethylphosphonate (AEP) (model organic P compounds) at both low (0.15 mg P/L) and high concentrations (4-5 mg P/L). Colorimetry with persulfate digestion gave complete recoveries (93-106%) for high concentrations, but recoveries for low concentration samples were moderate (71-77%). Colorimetry with sulfuric acid-nitric acid digestion gave complete recoveries for adenosine 5'triphosphate only (90-91%). Recoveries for phytic acid and 2-aminoethylphosphonate were extremely low (11%) or not detected.

ICP-OES is more accurate than colorimetry at both low and high concentrations. The persulfate digestion method is not reliable at low concentrations and should be avoided. Sulfuric acid-nitric acid digestion

method should be avoided even for measuring high concentrations, especially when refractory compounds are being used.

3. Comparison of total measurements by ICP-OES and colorimetry with persulfate digestion showed a very strong correlation ($y = 1.12x + 0.16$, $r^2 = 0.94$, $p < 0.05$); however, values measured by ICP-OES were consistently slightly higher. The correlation deviated more for values below 0.15 mg P/L, the reason for which is not clear. The difference in measurements by both methods was also greater (as much as twice) for samples with high organic phosphorus.

Comparisons between ICP-OES-generated TP values to those measured by colorimetry with persulfate digestion can be done confidently only for samples that are known to be mostly inorganic, and have high phosphorus concentrations. For TP measurement of samples with a high amount of organic phosphorus in them, persulfate digestion should be avoided. For samples with low total phosphorus (less than 0.15 mg P/L), further work is needed to reduce uncertainties in both methods.

4. For the phosphorus removal studies, when 5 mg P/L solutions of potassium phosphate monobasic, sodium triphosphate, adenosine 5'-triphosphate, phytic acid, and 2-aminoethyl phosphonic acid in pure water were tested, essentially complete phosphorus removals of 99-101% were obtained with a combination of: Fe:P molar ratio 5:1, pH 5-7, and $[H_2O_2] = 0.05-0.5$ M. Presence of H_2O_2 was found necessary to remove phosphonates in particular. The effect of peroxide was much more enhanced with $FeCl_3$ as the coagulant, than with $AlCl_3$.

5. For wastewater samples that are known to contain phosphonates (which are present in many pharmaceuticals and pesticides, as was discussed in Section 1.6), testing the effect of hydrogen peroxide addition to achieve low residual TP will be highly beneficial.

6. Using modifications to the protocol developed in Chapter 3, residual phosphorus concentrations of less than 0.1 mg P/L were attained in six real wastewater samples that had initial P of 0.21-8.96 mg P/L (TP). A seventh sample with 20 mg P/L was reduced to below 0.3 mg P/L. Fe:P ratios as high as 28.5 had to be used to achieve low residual P, however, the metal dose can be significantly reduced by employing a two-stage treatment. Even lower residual P can be attained by addition of hydrogen peroxide to samples.

5.3 Recommendations for future work

Due to limits of scope of this project, the questions in the following list could not be addressed, and are included here as potential directions for future studies:

1. Can ICP-OES instrument parameters and data processing methods be optimized further to consistently achieve detection limits of at least 0.05 µg P/L?
 2. What is the optimum amount of hydrogen peroxide needed for phosphorus removal? Does adding too much peroxide hinder removal? What is the lowest amount of peroxide needed to save costs?
 3. What is the effect of Total/Dissolved Organic Carbon (TOC/DOC) on the phosphorus removal process?
 4. What is the effect of other ions (e.g. Ca^{2+} , Mg^{2+} , SO_4^{2-} , etc.) on the removal process?
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Appendix To Chapter 2

A.1 – Development of an accurate and sensitive ICP-OES protocol for low level P detection

A.1.1 – ICP-OES calibration at different wavelengths

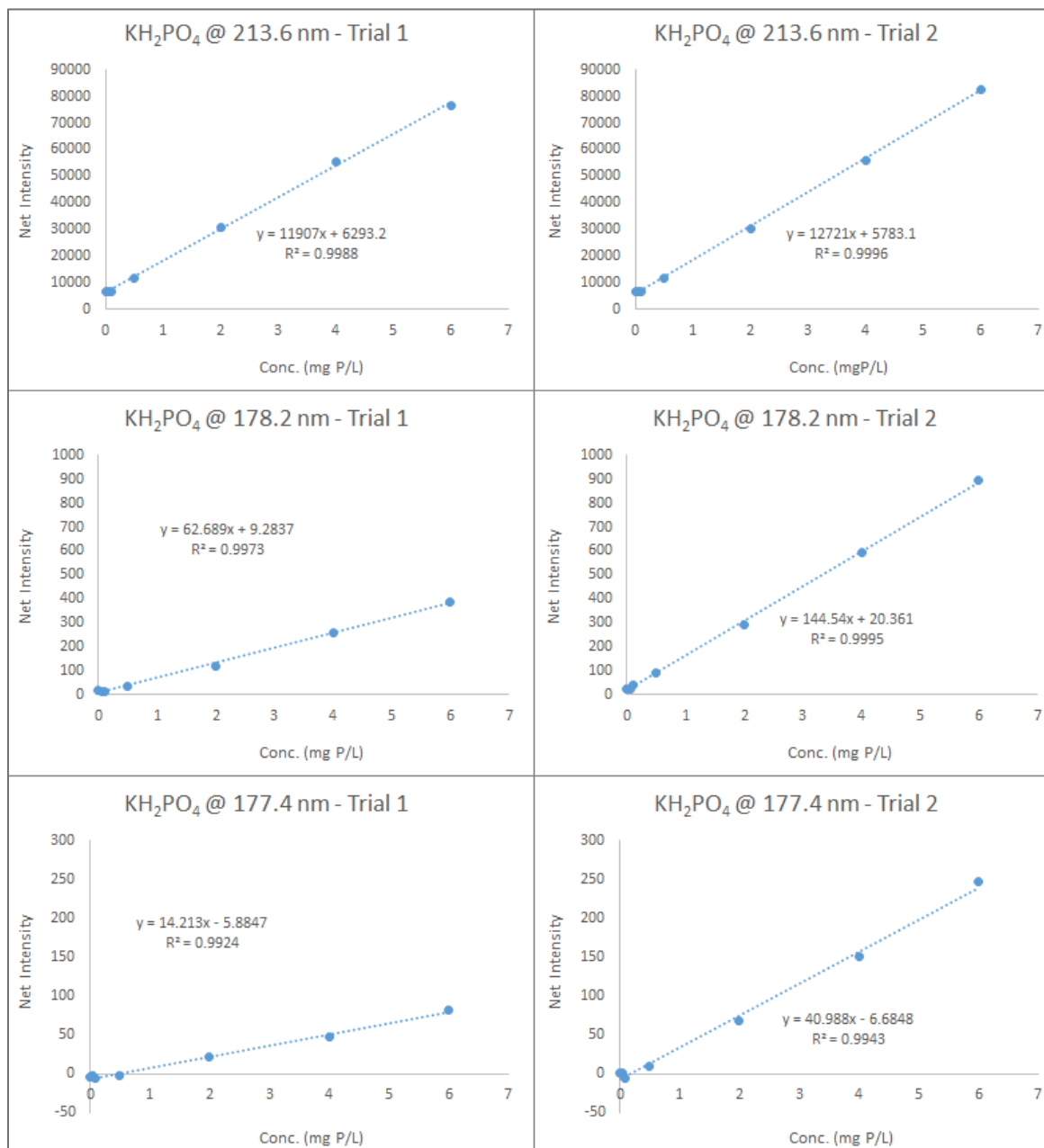


Figure 14. Calibration curves prepared using KH_2PO_4 on ICP-OES at 213.6, 178.2 and 177.4 nm. The line at 213.6 nm was much more sensitive than lines at 178.2 and 177.4 nm. The lines at 178.2 and 177.4 nm were also less consistent (compare slopes for either trial), which made them not relevant for low level P analysis.

A.1.2 – ICP-OES radial vs. axial calibration

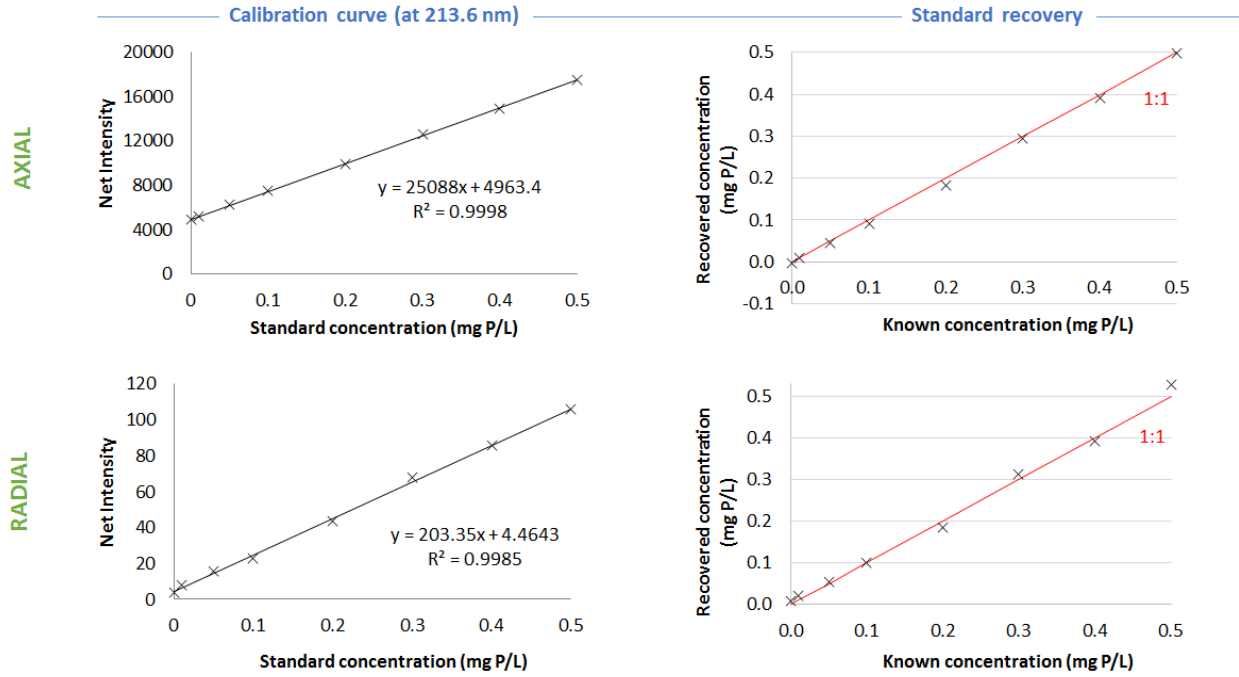


Figure 15. Axial versus radial calibrations. Both worked well at lower concentrations. Axial calibration was chosen over radial due to being much more sensitive.

A.1.3 – Screenshots of spectra for low P samples

A.1.3.1 – Axial calibrations

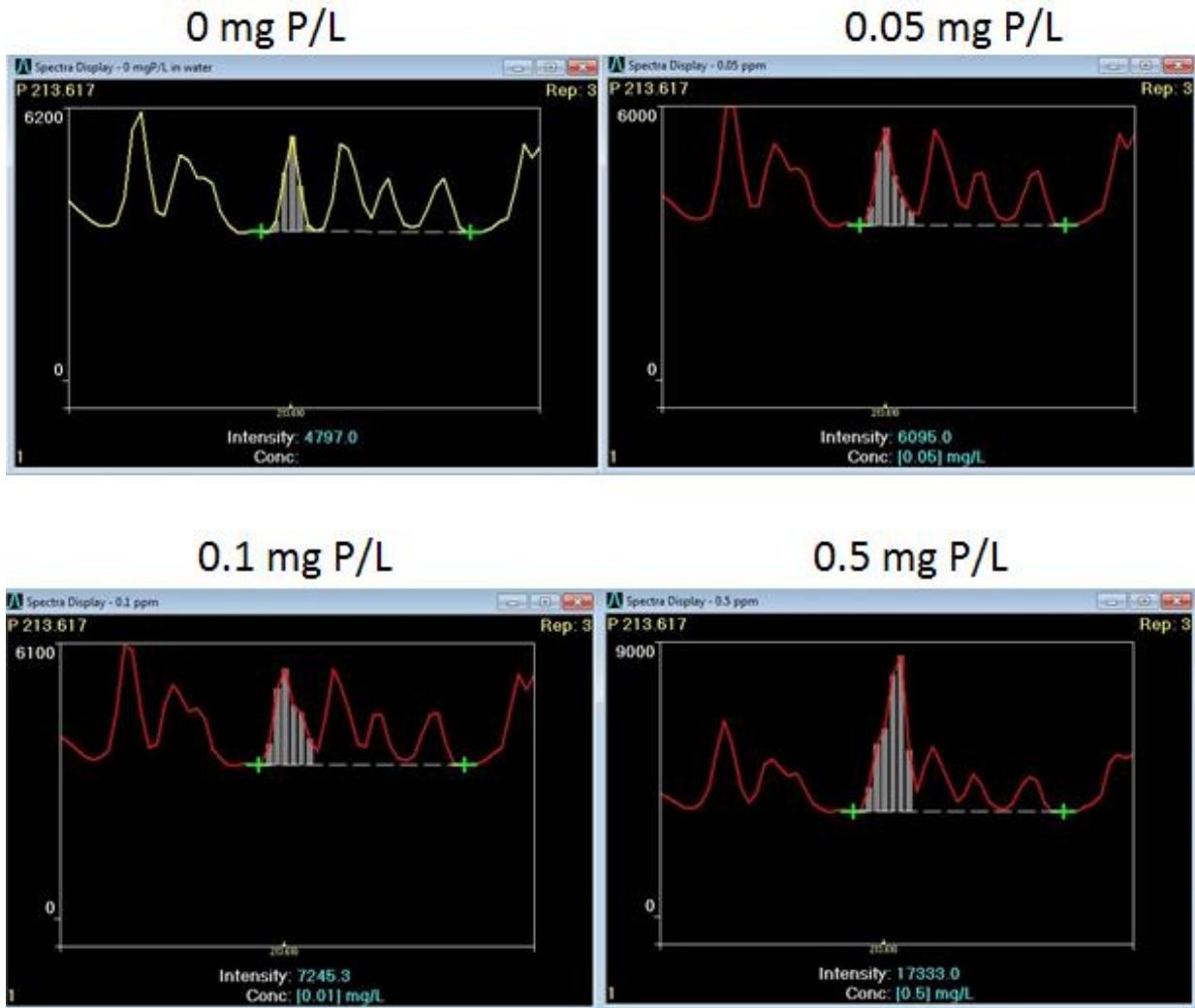


Figure 16. Sample spectra for low phosphorus samples obtained using axial viewing on the ICP-OES.

A.1.3.2 – Radial calibrations

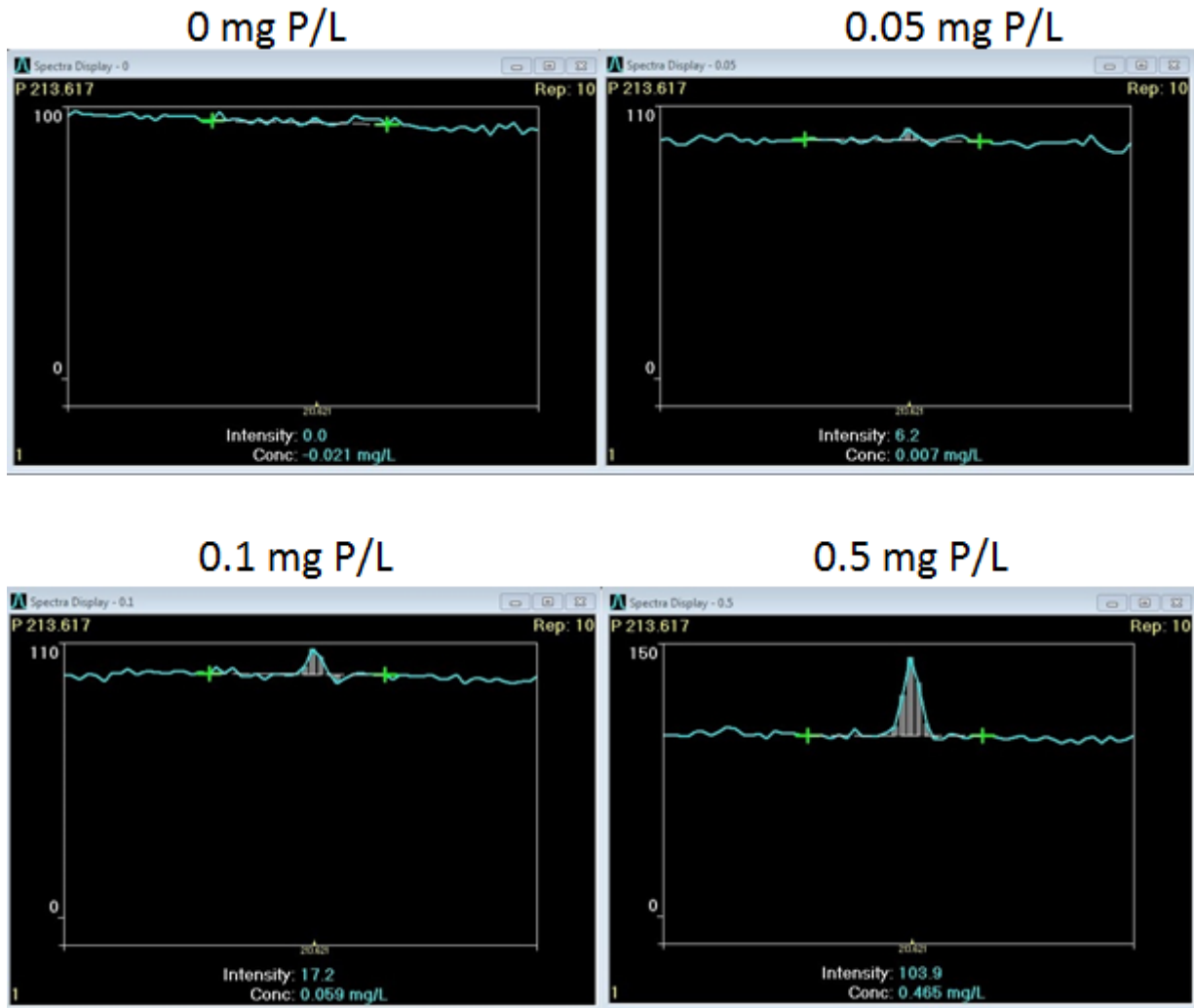


Figure 17. Sample spectra for low phosphorus samples obtained using axial viewing on the ICP-OES.

A.2 – Assessment of interference

Table 12. Concentration of P measured in compounds used in the treatment protocol, other than the model compounds.

Manganese(III,IV) oxide nanoparticles (MnO_{2-x} NPs) and bleach had a small amount of phosphorus, but it was insignificant compared to the initial P concentration (5 mg P/L) used in the protocol in Chapter 3. Sample WW-3 in Chapter 4 had MnO_{2-x} NPs in some of the protocols (see S. Nos. 9 and 10 in Table 9), but the amount of the NPs added to the sample was a quarter of that used in the measurement below.

| Blank sample | [P] mg P/L measured on ICP-OES |
|---|---------------------------------------|
| MnO_{2-x} NPs + FeCl_3 + H_2O_2 | < 0.05 |
| MnO_{2-x} NPs | 0.19 |
| FeCl_3 | < 0.05 |
| H_2O_2 | < 0.05 |
| FeCl_3 + H_2O_2 | < 0.05 |
| Bleach | 0.28 |

A.3 – ED-XRF Calibrations

Instrument used: PANalytical Epsilon 3 bench-top energy dispersive X-Ray fluorescence spectrometer

Standards: 75, 100, 250, 500, 750 and 1000 mg P/L solutions prepared from KH_2PO_4 (99%, from BDH) in 18 $\text{M}\Omega\cdot\text{cm}$ Milli-Q® (Millipore Corporation) de-ionized water

Sample volume: 5 mL

Measurement time: 60 s

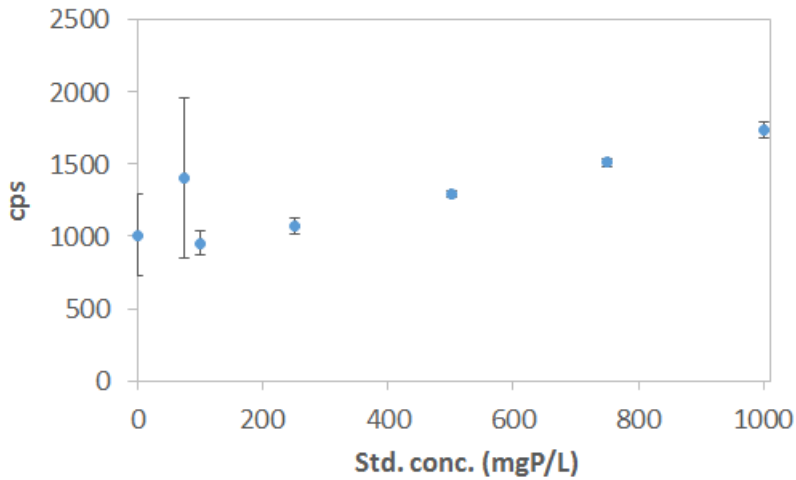


Figure 18. Averaged results of three different ED-XRF calibrations, performed on different days. Error bars represent standard deviations. The very high standard deviations at the lower end made ED-XRF not relevant for low-level P analyses.

Appendix To Chapter 3

A.4 – Raw data from all protocols

Table 13. Initial-P and residual-P for all samples treated in pure water and synthetic water. Notes: 1 = filtered through 0.45 µm instead of centrifugation. 2 = filtered through 0.2 µm instead of centrifugation. 3 = UV-radiated samples, 365 nm. 4 = UV-radiated samples, 408 nm. 5 = overnight stirring with open lid. Values in **blue** = below LOD; values in **red** = below LOQ. LOD and LOQ varied for each analysis date. A '---' indicates the metal was not added. 'Not adj.' indicates pH was not adjusted.

| S. No. | pH | Mn:P | Fe:P | Al:P | [H ₂ O ₂] (M) | Notes | P _{initial} (mg P/L) | P _{residual} (mg P/L) | % P removed |
|-------------------------------------|--------|------|------|------|---|-------|----------------------------------|-----------------------------------|---------------|
| KH₂PO₄ | | | | | | | | | |
| 1 | (3.0) | --- | 5 | --- | 0.1 | | 4.91 | 4.51 | 8% |
| 2 | 4 | --- | 5 | --- | 0.05 | | 4.93 | 4.61 | 6% |
| 3 | 6 | --- | 2.5 | --- | 0 | | 4.95 | 0.22 | 96% |
| 4 | 5 | --- | 2.5 | --- | 0.05 | | 4.94 | 0.12 | 98% |
| 5 | 5 | --- | 2.5 | --- | 0.1 | | 4.91 | 0.05 | 99.0% |
| 6 | 6 | --- | 2.5 | --- | 0.1 | | 4.91 | 0.34 | 93% |
| 7 | 6 | --- | 2.5 | --- | 0.1 | 1 | 4.90 | 0.14 | 97% |
| 8 | 7 | --- | 2.5 | --- | 0.1 | | 4.91 | 0.28 | 94% |
| 9 | 5.4 | --- | 2.5 | --- | 0.5 | | 4.71 | 0.28 | 94% |
| 10 | 6 | --- | 2.5 | --- | 0.5 | | 4.73 | 0.37 | 92% |
| 11 | 5 | --- | 5 | --- | 0 | 3 | 4.93 | 0.15 | 97% |
| 12 | 5 | --- | 5 | --- | 0 | 5 | 4.93 | 0.06 | 99% |
| 13 | 5 | --- | 5 | --- | 0.05 | | 4.91 | 0.11 | 97.7% |
| 14 | 5 | --- | 5 | --- | 0.05 | 2 | 4.90 | 0.07 | 99% |
| 15 | 5 | --- | 5 | --- | 0.05 | 1 | 4.90 | 0.02 | 99.7% |
| 16 | 6 | --- | 5 | --- | 0.05 | | 4.90 | 0.09 | 98.1% |
| 17 | 8 | --- | 5 | --- | 0.05 | | 4.91 | 0.19 | 96% |
| 18 | 5 | --- | 5 | --- | 0.1 | | 4.88 | -0.03 | 100.6% |
| 19 | 6 | --- | 5 | --- | 0.1 | 2 | 4.88 | 0.04 | 99% |
| 20 | 6 | --- | 5 | --- | 0.1 | | 4.87 | 0.06 | 98.8% |
| 21 | 6 | --- | 5 | --- | 0.1 | | 4.87 | -0.12 | 102.4% |
| 22 | 7 | --- | 5 | --- | 0.1 | | 4.87 | 0.17 | 96.5% |
| 23 | 5 | --- | --- | 5 | 0 | | 4.94 | 0.02 | 99.6% |
| 24 | 5 | --- | --- | 5 | 0.1 | | 4.88 | 0.02 | 99.7% |
| 25 | (3.74) | 2.5 | 2.5 | --- | 0 | 4 | 4.93 | 2.28 | 54% |
| 26 | (3.34) | 2.5 | 2.5 | --- | 0.1 | | 4.89 | 0.43 | 91% |

| | | | | | | | | | |
|------------|--------|-----|-----|-----|--------------|---|------|--------------|---------------|
| 27 | (3.25) | 2.5 | 2.5 | --- | 0.5 | | 4.71 | 0.67 | 86% |
| 28 | (3.31) | 2.5 | 2.5 | --- | 0.5 | 4 | 4.71 | 0.27 | 94% |
| 29 | 5.7 | 2.5 | 2.5 | --- | 0.5 | | 4.70 | 0.37 | 92% |
| 30 | (10.2) | --- | 2.5 | --- | Bleach 0.1 M | | 4.98 | 4.00 | 20% |
| 31 | 6 | --- | 2.5 | --- | Bleach 0.1 M | | 4.21 | 2.43 | 42% |
| 32 | 6 | --- | 2.5 | --- | Bleach 0.1 M | | 4.52 | 2.66 | 35% |
| 33 | (8.88) | 2.5 | 2.5 | --- | Bleach 0.1 M | | 4.49 | 3.43 | 23% |
| AEP | | | | | | | | | |
| 34 | (3.0) | --- | 5 | --- | 0.1 | | 4.91 | 4.55 | 7% |
| 35 | 6 | --- | 2.5 | --- | 0 | | 4.95 | 4.37 | 12% |
| 36 | 5 | --- | 2.5 | --- | 0.1 | | 4.92 | 0.26 | 95% |
| 37 | 6 | --- | 2.5 | --- | 0.1 | | 4.90 | 0.51 | 90% |
| 38 | 6 | --- | 2.5 | --- | 0.1 | 1 | 4.93 | 0.26 | 95% |
| 39 | 7 | --- | 2.5 | --- | 0.1 | | 4.92 | 0.46 | 91% |
| 40 | ~5 | --- | 2.5 | --- | 0.5 | | 4.72 | 0.12 | 97.4% |
| 41 | 6 | --- | 2.5 | --- | 0.5 | | 4.73 | 0.62 | 87% |
| 42 | 5 | --- | 5 | --- | 0 | 3 | 4.92 | 4.09 | 17% |
| 43 | 5 | --- | 5 | --- | 0 | 5 | 4.92 | 4.00 | 19% |
| 44 | 6 | --- | 5 | --- | 0.05 | | 4.90 | 0.07 | 98.5% |
| 45 | 5 | --- | 5 | --- | 0.1 | | 4.88 | -0.03 | 100.7% |
| 46 | 6 | --- | 5 | --- | 0.1 | 2 | 4.88 | 0.07 | 99% |
| 47 | 6 | --- | 5 | --- | 0.1 | | 4.89 | 0.03 | 99.4% |
| 48 | 6 | --- | 5 | --- | 0.1 | | 4.89 | -0.03 | 100.6% |
| 49 | 7 | --- | 5 | --- | 0.1 | | 4.87 | 0.02 | 99.7% |
| 50 | 5 | --- | --- | 5 | 0 | | 4.93 | 4.08 | 17% |
| 51 | 5 | --- | --- | 5 | 0.1 | | 4.87 | 2.62 | 46% |
| 52 | (3.63) | 2.5 | 2.5 | --- | 0 | 4 | 4.94 | 0.67 | 86% |
| 53 | (3.26) | 2.5 | 2.5 | --- | 0.1 | | 4.89 | 0.82 | 83% |
| 54 | (3.23) | 2.5 | 2.5 | --- | 0.5 | | 4.71 | 0.57 | 86% |
| 55 | (10.2) | --- | 2.5 | --- | Bleach 0.1 M | | 4.98 | 4.33 | 13% |
| 56 | 6 | --- | 2.5 | --- | Bleach 0.1 M | | 4.55 | 3.52 | 15% |
| 57 | (9.43) | 2.5 | 2.5 | --- | Bleach 0.1 M | | 4.49 | 4.31 | 3% |
| ATP | | | | | | | | | |
| 58 | (3.0) | --- | 5 | --- | 0.1 | | 4.91 | 4.51 | 8% |
| 59 | 4 | --- | 5 | --- | 0.05 | | 4.93 | 3.96 | 20% |
| 60 | 6 | --- | 2.5 | --- | 0 | | 4.95 | 0.42 | 92% |
| 61 | 5 | --- | 2.5 | --- | 0.1 | | 4.91 | 0.27 | 94% |
| 62 | 6 | --- | 2.5 | --- | 0.1 | | 4.90 | 0.28 | 94% |

| | | | | | | | | | |
|-------------|--------|-----|-----|-----|--------------|---|------|--------------|---------------|
| 63 | 6 | --- | 2.5 | --- | 0.1 | 1 | 4.91 | 3.20 | 35% |
| 64 | 7 | --- | 2.5 | --- | 0.1 | | 4.90 | 0.24 | 95% |
| 65 | 5 | --- | 2.5 | --- | 0.5 | | 4.72 | 0.18 | 96% |
| 66 | 6 | --- | 2.5 | --- | 0.5 | | 4.73 | 0.49 | 90% |
| 67 | 5 | --- | 5 | --- | 0 | 3 | 4.93 | 0.18 | 96% |
| 68 | 5 | --- | 5 | --- | 0 | 5 | 4.92 | 0.05 | 99% |
| 69 | 5 | --- | 5 | --- | 0.05 | 2 | 4.90 | 0.02 | 99.6% |
| 70 | 5 | --- | 5 | --- | 0.05 | 1 | 4.90 | 0.03 | 99.4% |
| 71 | 6 | --- | 5 | --- | 0.05 | | 4.92 | 0.06 | 98.8% |
| 72 | 5 | --- | 5 | --- | 0.1 | | 4.89 | 0.04 | 99.1% |
| 73 | 6 | --- | 5 | --- | 0.1 | 2 | 4.88 | 0.50 | 90% |
| 74 | 6 | --- | 5 | --- | 0.1 | | 4.89 | 0.02 | 99.7% |
| 75 | 6 | --- | 5 | --- | 0.1 | | 4.88 | 0.09 | 98.1% |
| 76 | 7 | --- | 5 | --- | 0.1 | | 4.88 | 0.23 | 95.3% |
| 77 | 8 | --- | 5 | --- | 0.05 | | 4.91 | 0.78 | 84% |
| 78 | 5 | --- | --- | 5 | 0 | | 4.94 | 0.01 | 99.7% |
| 79 | 5 | --- | --- | 5 | 0.1 | | 4.87 | 0.04 | 99.2% |
| 80 | (3.69) | 2.5 | 2.5 | --- | 0 | 4 | 4.94 | 2.89 | 41% |
| 81 | (3.43) | 2.5 | 2.5 | --- | 0.1 | | 4.89 | 0.71 | 85% |
| 82 | (3.26) | 2.5 | 2.5 | --- | 0.5 | | 4.71 | 0.56 | 88% |
| 83 | (3.33) | 2.5 | 2.5 | --- | 0.5 | 4 | 4.71 | 0.50 | 89% |
| 84 | 6 | 2.5 | 2.5 | --- | 0.5 | | 4.70 | 0.83 | 82% |
| 85 | (10.2) | --- | 2.5 | --- | Bleach 0.1 M | | 4.98 | 4.08 | 18% |
| 86 | 6 | --- | 2.5 | --- | Bleach 0.1 M | | 4.55 | 2.14 | 48% |
| 87 | (9.22) | 2.5 | 2.5 | --- | Bleach 0.1 M | | 4.49 | 3.65 | 18% |
| NaTP | | | | | | | | | |
| 88 | (3.0) | --- | 5 | --- | 0.1 | | 4.91 | 4.54 | 8% |
| 89 | 5 | --- | 2.5 | --- | 0.5 | | 4.72 | 0.15 | 96.8% |
| 90 | 6 | --- | 2.5 | --- | 0.5 | | 4.73 | 0.98 | 79% |
| 91 | 5 | --- | 5 | --- | 0 | 3 | 4.92 | 0.08 | 98.4% |
| 92 | 5 | --- | 5 | --- | 0 | 5 | 4.93 | 0.02 | 99.6% |
| 93 | 6 | --- | 5 | --- | 0.05 | | 4.91 | 0.06 | 98.8% |
| 94 | 5 | --- | 5 | --- | 0.1 | | 4.88 | -0.04 | 100.8% |
| 95 | 6 | --- | 5 | --- | 0.1 | 2 | 4.88 | 0.03 | 99.4% |
| 96 | 6 | --- | 5 | --- | 0.1 | | 4.89 | -0.08 | 101.7% |
| 97 | 7 | --- | 5 | --- | 0.1 | | 4.88 | 0.09 | 98.2% |
| 98 | 5 | --- | --- | 5 | 0 | | 4.94 | 0.02 | 99.5% |
| 99 | 5 | --- | --- | 5 | 0.1 | | 4.89 | 0.03 | 99.4% |

| | | | | | | | | | |
|---|----------|-----|-----|-----|--------------|---|------|--------------|---------------|
| 100 | (4.08) | 2.5 | 2.5 | --- | 0 | 4 | 4.94 | 3.23 | 34% |
| 101 | (3.47) | 2.5 | 2.5 | --- | 0.1 | | 4.89 | 0.71 | 85% |
| 102 | (3.34) | 2.5 | 2.5 | --- | 0.5 | | 4.71 | 0.65 | 86% |
| 103 | (9.03) | 2.5 | 2.5 | --- | Bleach 0.1 M | | 4.49 | 3.37 | 24% |
| PhyA | | | | | | | | | |
| 104 | 5 | --- | 2.5 | --- | 0.05 | | 4.94 | 0.27 | 94% |
| 105 | 5 | --- | 5 | --- | 0.05 | | 4.91 | 0.17 | 96% |
| 106 | 5 | --- | --- | 5 | 0 | | 4.94 | 0.04 | 99.2% |
| 107 | 5 | --- | --- | 5 | 0.1 | | 4.89 | 0.04 | 99.2% |
| 108 | not adj. | 2.5 | 2.5 | --- | 0.5 | | 4.71 | 0.57 | 88% |
| 109 | 6 | --- | 2.5 | --- | Bleach 0.1 M | | 4.21 | 0.23 | 94% |
| DEP – diethyl(hydroxymethyl) phosphonate | | | | | | | | | |
| 110 | 5 | --- | 5 | | 0 | | 4.74 | 2.84 | 40% |
| 111 | 5 | --- | 5 | | 0 | | 4.92 | 3.42 | 31% |
| 112 | not adj. | --- | 5 | | 0.1 | | 4.91 | 4.60 | 6% |
| 113 | 4 | --- | 5 | | 0.05 | | 4.92 | 4.12 | 16% |
| 114 | 5 | --- | 5 | | 0 | | 4.92 | 3.94 | 20% |
| 115 | 5 | --- | 5 | | 0.1 | | 4.89 | -0.02 | 100.3% |
| 116 | 6 | --- | 5 | | 0.05 | | 4.89 | 0.11 | 97.8% |
| 117 | 6 | --- | 5 | | 0.1 | | 4.87 | 0.13 | 97% |
| 118 | 6 | --- | 5 | | 0.1 | | 4.89 | 0.03 | 99.3% |
| 119 | 6 | --- | 5 | | 0.1 | | 4.87 | 0.02 | 99.5% |
| 120 | 7 | --- | 5 | | 0.1 | | 4.86 | 0.23 | 95.2% |
| 121 | 8 | --- | 5 | | 0.05 | | 4.89 | 0.39 | 92% |
| 122 | 5 | --- | 2.5 | | 0.1 | | 4.91 | 0.89 | 82% |
| 123 | ~5 | --- | 2.5 | | 0.5 | | 4.73 | 1.45 | 69% |
| 124 | 6 | --- | 2.5 | | 0 | | 4.97 | 3.40 | 32% |
| 125 | 6 | --- | 2.5 | | 0.1 | | 4.89 | 1.46 | 70% |
| 126 | 6 | --- | 2.5 | | 0.1 | | 4.91 | 0.76 | 85% |
| 127 | 6 | --- | 2.5 | | 0.5 | | 4.73 | 2.26 | 52% |
| 128 | 7 | --- | 2.5 | | 0.1 | | 4.90 | 1.12 | 77% |
| 129 | not adj. | 2.5 | 2.5 | | 0 | | 4.94 | 4.12 | 17% |
| 130 | not adj. | 2.5 | 2.5 | | 0.1 | | 4.89 | 4.07 | 17% |
| 131 | not adj. | 2.5 | 2.5 | | 0.5 | | 4.71 | 3.71 | 21% |
| 132 | not adj. | --- | 2.5 | | Bleach 0.1 | | 4.98 | 3.94 | 21% |
| 133 | 6 | --- | 2.5 | | Bleach 0.1 | | 4.54 | 3.35 | 19% |
| 134 | not adj. | 2.5 | 2.5 | | Bleach 0.1 | | 4.49 | 3.92 | 12% |