Phosphorus

VT WSMD Wastewater Program Lab Manual Section #15

Table of Contents

Phosphorus

Background

Phosphorus in wastewater is almost always present in the form of phosphates. There are three major classifications of phosphates. They are: (1) Orthophosphates: Fertilizers are the major source of this class of phosphates; (2) Polyphosphates: (Pyro-, meta-, etc.). Detergents and commercial cleaning agents comprise the major source of these; and (3) Organic Phosphates: These are formed mainly by biological processes, their major source being body wastes and food residues. Organic phosphates may also be formed from orthophosphates during biological treatment. Analysis of Total Phosphorus includes all these forms of phosphates.

It is very useful to monitor the amount of phosphorus present in the waste stream because of its extreme importance in the growth of organisms. In fact, phosphorus can often be the limiting factor in the growth of organisms. Phosphorus plays an important part in "algal blooms", a common problem in Vermont lakes and streams.

Sampling and Preservation

Samples for phosphorus analysis should be taken from a composite sample into a glass or plastic bottle. If the sample will not be processed the same day, it should be preserved by adding a sufficient volume of concentrated sulfuric acid (H_2SO_4) to lower the sample to pH 2 or less. It should then be refrigerated at 6°C until analyzed.

Glassware Preparation

Phosphate contamination is common because of the tendency of phosphates to adsorb onto glass surfaces. It is extremely important, therefore, to clean all glassware used in the analysis of phosphorus very carefully. All glassware should be acid-washed with a **hot** 1:1 HC1 solution and then thoroughly rinsed with distilled water. **It is an extremely good idea to use this glassware for phosphorus determination only!** After use, the glassware should be washed rinsed with distilled water and covered until its next use. If the glassware is filled with distilled water until used again, the acid washing is needed less frequently.

Equipment

- Spectrophotometer capable of measuring at 880 nm with a light path of 2.5 cm or longer.
  OR
  Filter photometer with a red color filter and a light path of 0.5 cm or longer.
- 1 cm cell or cuvette (for phosphorus concentrations of 0.3 to 1.2 mg/L)

Glassware

This depends on the method of digestion, etc. See specific method descriptions.

Generally:

- Minimum of 9 50 mL graduated cylinders
- 1 mL pipet (for H_2SO_4)
- 4 mL pipet (for ammonium molybdate solution)
- 2 mL pipet (for ascorbic acid)

* **REMEMBER**: All glassware used in the analysis of Phosphorus must be acid washed and should be dedicated to this analysis only.

Methods

There are three colorimetric methods for analysis of Total Phosphorus listed in the 23rd edition of Standard Methods for The Examination of Water and Wastewater. They are:

1. The vanadomolbdophosphoric acid method. This method is good for phosphorus in the range of 1-20 mg/L P (4500 - P. c)
2. The stannous chloride method (4500 -P. d)
3. The ascorbic acid method This is an excellent method for Phosphorus in the range of 0.01 to 6 mg/L as P.

The ascorbic acid method is the preferred method in the great majority of Vermont wastewater facilities. The preferred means of performing this method is with the use of Hach T 'n T method 8190 for analysis of Total Phosphorus.

HACH® TEST'N TUBE METHOD

The most common method used by Vermont wastewater analysts for the determination of Total Phosphorus is Hach Test 'n Tube method. This method has proven to yield good results, is relatively easy to perform, safe if performed according to HACH instructions and negates the need for preparing dangerous chemicals onsite.

We have provided a link to the Hach method 8190 Test 'N Tube method 8190 for Total Phosphorus. The method is applicable for phosphorus concentrations of 0.02 to 1.10 mg/l P. (0.06 to 3.5 mg/L PO_4^{3-}
Note: The State does recommend the use of at least two standards to be run every time the analysis is performed. These standards should bracket the expected phosphorus concentration of samples being analyzed.

https://www.hach.com/asset-get.download.jsa?id=7639983838

Note: There are other kits available for the analysis of Total Phosphorus from companies other than Hach®. This link is provided because at this time most Vermont facilities have the Hach® equipment necessary to perform the analysis. There are also other digestive methods available from Hach® and other companies

WET CHEMISTRY ASCORBIC ACID METHOD

The wet chemistry method described here is EPA accepted but not commonly performed at Vermont laboratories. The method is cumbersome, requires the use of dangerous chemicals and is no longer recommended. There are more "automated" ascorbic acid methods including the Hach® Test 'N tube ascorbic acid method that are safer and CWA acceptable. The method can detect phosphorus in the range of 0.01 to 6 mg/L as P.

Reagent

- Ascorbic Acid
  0.1M - Dissolve 1.76 g ascorbic acid in 100 mL distilled water. Refrigerate at 6 °C. This reagent must be prepared fresh weekly.
- Sulfuric Acid
  5N - Partially fill a 500 mL volumetric flask with approximately 400 mL distilled water. **Carefully** add 70 mL concentrated sulfuric acid (H_2SO_4). **Slowly and carefully** bring the volume to 500 mL with distilled water.
- Potassium Antimonyl Tartrate Solution
  Partially fill (400 mL) a 500 mL volumetric flask with distilled water. Dissolve 1.3715 g potassium antimonyl tartrate K(SbO) $C_4H_4O_6$½H_2O in the distilled water and then dilute to 500 mL with distilled water. This reagent should be stored in a glass-stoppered bottle.
- Ammonium Molybdate Solution
  Dissolve 20 g Ammonium Molybdate $(NH_4)_6$ Mo_7O_{24} • $4H_2O$ in 500 mL distilled water. This reagent should be stored in a glass-stoppered bottle.
- Combined Reagent
  Allow all the reagents listed above to reach room temperature. Then in a 1-liter beaker (or other large mouth container **ADD IN THIS ORDER:**
  - 50 mL 5N sulfuric acid (H_2SO_4) and 5 mL potassium antimonyl tartrate solution. Mix.
  - Then add 15 mL Ammonium Molybdate solution. Mix.
  - Add 30 mL of the 0.1M Ascorbic Acid Solution. Mix.
  - **This reagent must be used within 4 hours**.

ASCORBIC ACID METHOD

Procedure

Before colorimetric determination of phosphorus, all wastewater samples and standards used for calibration curve must be properly digested using one of the two methods described here.

NOTE: The perchloric acid digestion method, although acceptable is not mentioned here because of the inherent danger and special equipment associated with that method.

Digestion Method #1 - Sulfuric Acid - Nitric Acid Digestion

Equipment

- Fume Hood
- Digestion Rack
- Micro-Kjeldahl digestions flasks

Reagents

- Concentrated Sulfuric Acid (H2SO4)
- Concentrated Nitric Acid (HNO3)
- Phenolphthalein Indicator
- 1N Sodium Hydroxide Solution (NaOH)

Procedure

1) Pipet 50 mL of sample into a dry micro Kjeldahl flask or tube
2) Add 1 mL conc H2SO4 and 5 mL conc HNO3
3) Heat slowly on digestion rack until there is approximately 1 mL of solution left. Continue digestion carefully until the solution becomes colorless
4) Cool to room temperature
5) Add about 20 mL of distilled water to solution
6) Add 1 drop (0.05 mL) phenolphthalein
7) Add 1N NaOH one drop at a time until the solution develops a slight pink color
8) Dilute this solution to 100 mL with distilled water

Digestion Method #2 - Persulfate Digestion

Equipment

- Hot Plate
- Glass scoop

Reagents

- Phenolphthalein indicator
- Sulfuric Acid Solution:
    - Dilute concentrated sulfuric acid by slowly and carefully adding 300 mL of conc H_2SO_4 to 600 mL of distilled water. Then continue dilution with distilled water to 1 liter
- Ammonium persulfate $(NH_4)_2S_2O_8$ Solid
  OR Potassium persulfate $K_2S_2O_8$ Solid
- Sodium Hydroxide (NaOH) 1N.

Procedure

1) Pour 50 mL of well mixed sample into a suitable container (200 mL beaker)
2) Add 1 drop (.05mL) phenolphthalein indicator
3) If a red color develops, add sulfuric acid solution one drop at a time until red disappears
4) Add 1 mL of the sulfuric acid solution and 0.4 g solid $(NH_4)S_2O_8$ **or** 0.5 g solid $K_2S_2O_8$
5) Add one boiling chip (bead)
6) Gently boil the solution on the hot plate for 45 minutes or until a final volume of about 10 mL is reached (or heat for 30 minutes in autoclave at 98-137Kpa)
7) Cool to room temperature
8) Dilute to 30 mL with distilled water
9) Add 1 drop (.05mL) phenolphthalein indicator
10) Add sodium hydroxide solution (NaOH) until the sample develops a slight pink color
11) Dilute this sample to 100 mL with distilled water

Total Phosphorus Determination: Ascorbic Acid Method

Procedure

1) Pipet 50.0 mL of digested sample into a 125 mL Erlenmeyer flask.
2) Add 1 drop (.05mL) phenolphthalein indicator solution
3) If sample turns red add 5N sulfuric acid solution one drop at a time until red color disappears
4) Add 8.0 mL of the combined reagent and mix the solution thoroughly
5) After 10 minutes, but not more than 30 minutes, measure the absorbance at 880 nm

NOTE: The color should be stable for about an hour, but it is highly recommended that the absorbance be read within 30 minutes after the addition of the combined reagent.

Calculation

Prepare a standard curve by plotting the absorbance values of standards versus the corresponding phosphorus concentrations on linear graph paper or a program with graphing ability. This standard curve must be prepared at least once a year using at least 6 standard concentrations.

Obtain concentration value of sample directly from prepared standard curve. Report results as P, mg/L.

Standards

EPA requires that at least one (preferably more) standard be run to calibrate the spectrophotometer each time. Below are instructions for making three stock solutions, and directions for making dilutions of the stock solutions for a set of six appropriate standards. The standards chosen here to encompass a wide range of likely results. They can be modified to suit specific situations. Use Class A volumetric glassware for all solutions.

Solution A: Stock phosphate solution of 100 mg/L. Dissolve 0.4393 g of predried (105 ℃ for 1 hour) KH2PO4 in distilled water and dilute to 1,000 mL. Make fresh each month.

$$1.0\ mL = 0.1\ mg\ P$$

Solution B: Stock phosphate solution of 10 mg/L. Dilute 100.0 mL of Solution A to 1,000 mL with distilled water.

$$1.0\ mL = 0.01\ mg\ P$$

Solution C: Stock phosphate solution of 1 mg/L. Dilute 100.0 mL of Solution B to 1,000 mL with distilled water.

$$1.0\ mL = 0.001\ mg\ P$$

The set of standards (0.1, 0.2, 0.4, 0.8, 1.0, 1.5 mg/L) is then made:

1. 0.10 mg/L: Dilute 1.0 mL of Solution B to 100 mL

$$\frac{0.01\ mg\ P}{100\ mL} * \frac{1000\ mL}{1\ L} = 0.10 \frac{mg}{L}$$

2. 0.20 mg/L: Dilute 2.0 mL of Solution B to 100 mL

$$\frac{0.02\ mg\ P}{100\ mL} * \frac{1000\ mL}{1\ L} = 0.20 \frac{mg}{L}$$

3. 0.40 mg/L: Dilute 4.0 mL of Solution B to 100 mL

$$\frac{0.04\ mg\ P}{100\ mL} * \frac{1000\ mL}{1\ L} = 0.40 \frac{mg}{L}$$

4. 0.80 mg/L: Dilute 8.0 mL of Solution B to 100 mL

$$\frac{0.08\ mg\ P}{100\ mL} * \frac{1000\ mL}{1\ L} = 0.80 \frac{mg}{L}$$

5. 1.00 mg/L: Dilute 10.0 mL of Solution B to 100 mL

$$\frac{0.10\ mg\ P}{100\ mL} * \frac{1000\ mL}{1\ L} = 1.0 \frac{mg}{L}$$

6. 1.5 mg/L: Dilute 15.0 mL of Solution B to 100 mL

$$\frac{0.15\ mg\ P}{100\ mL} * \frac{1000\ mL}{1\ L} = 1.5 \frac{mg}{L}$$

Total Phosphorus Ascorbic Acid Method Troubleshooting Guide

PROBLEM	MOST LIKELY CAUSE	SOLUTION
Inconsistent or abnormally high phosphorus results	Contaminated Glassware	Acid wash all glassware used in the analysis and use only dedicated glassware
	Fingerprints on sample cell or Improper placement of cell	Make sure to handle cell so as to avoid fingerprints in light path - **Be careful** to place the cell into measuring device described in manufacturer's instructions. **Clean nitrile gloves should be worn when handling cells.**
	Sample phosphorus concentration too high	Dilute the sample prior to digestion
	Highly colored or turbid sample	Use a blank which consists of a sample to which all reagents except the ascorbic acid and antimonyl potassium tartrate have been added. The blank absorbance is then subtracted from the absorbance of each sample.
		Dilute sample before digestion

Quality Control for Total Phosphorus

Document the Following (Including but not limited to):

Supply Water Quality

- Conductivity < 10 micro siemens
- Phosphate Free

Sampling

- Sample Type - (usually a composite)
- Sample Time - time sample collection started and ended
- Duration of Composite - 8 hour, 24 hour
- Type of Composite
    - Time/Flow - include discrete sample volumes
    - Flow - include volume/sample per volume of discharge
    - Straight - document <10% change in flow rate during sampling event
- Sample Location

Glassware

- Acid washed - Distilled water rinses
- Dedicated to Phosphorus Analysis ONLY

Equipment

- Spectrophotometer
    - wavelength 880 nm
    - light path < or = 2.5 cm
- Photometer
    - filter Red
    - light path < or = 0.5 cm
- Service Records

Analytical Results

- Blank
    - What was used
    - How was it treated
    - Results
    - Frequency
- Standards
    - Lot #s, preparation and expiration dates
    - Number and concentrations used
    - Frequency of Use - at least one per set up
    - Results
    - Graph - Plotted standard results – annual

Duplicate

- Replicate schedule

Reporting Total Phosphorus Data

TOTAL PHOSPHORUS BENCH SHEET
SAMPLE TYPE:
SAMPLING TIME AND DATE:
SAMPLE VOLUME:
SAMPLE PRESERVATION:
ANALYST:
ANALYSIS TIME AND DATE:
METHOD:
BLANK RESULTS (include calculation if applicable):
STANDARDS
Concentrations Used:
Results (include calculations if applicable):
SAMPLE RESULTS
Raw Data and Calculations:

Orthophosphate Colorimetric Method Troubleshooting Guide

PROBLEM	MOST LIKELY CAUSE	SOLUTION
Inconsistent or abnormally high orthophosphate results	Contaminated Glassware	Acid wash all glassware used in the analysis and use only dedicated glassware. Rinse the sample vial and **CAP** before analysis
	Fingerprints on sample cell or Improper placement of cell	Make sure to handle cell so as to avoid fingerprints in light path - **Be careful** to place the cell into measuring device described in manufacturer's instructions. **Nitrile gloves should be worn when handling cells**
	Sample orthophosphate concentration too high	Dilute the sample prior to digestion

References

Methods for the analysis of orthophosphate can be found on page 356.3-1 of The Manual of Methods for chemical analysis of Water and Wastes and in the 23rd Edition of Standard Methods for the Examination of Water and Wastewater section 4500-P

Hach® Methods can be found via links provided.

Orthophosphate/Reactive Phosphorus for Process Control

Phosphates that can be measured with colorimetric analyses **without** first being digested are referred to as reactive phosphorus. This portion of the phosphorus in wastewater usually consists of mostly orthophosphate but can contain other condensed phosphates. Most Vermont wastewater facilities are interested in orthophosphate concentrations as a quick indicator of Total Phosphorus concentrations for process control purposes. The methods described here are designed for that purpose. If orthophosphate appears in a facility's permit as an NPDES required parameter, the sample will have to be filtered immediately upon collection through a 0.45-micron filter before analysis. For process control purposes filtering is not necessary.

The most common method of analysis of orthophosphate/reactive phosphorus in Vermont wastewater facilities is the Hach ascorbic acid TNT method. This method can be performed using the same colorimeter as used for Total Phosphorus without the need for the digestor / reactor.

Here is a link for the Hach orthophosphate/reactive phosphorus method.

https://www.hach.com/asset-get.download.jsa?id=7639983836

Note: Other companies may provide kits acceptable for this analysis. This link is provided because at this time the majority of Vermont wastewater facilities have the Hach equipment necessary to perform this analysis.

Quality Control

Because in most cases orthophosphate is measured for process control only, there are no required quality control procedures. However, it is generally a good idea to measure standard(s) in approximating the expected concentration of the sample in order to be sure the results generated are as accurate as possible. Typically, a 1.0ppm standard is purchased. Dilutions are prepared from that standard.

Recommended Quality control:

Supply Water Quality

- Conductivity < 10 micro siemens
- Phosphate Free

Sampling

- Sample Type – usually a grab but a check can be done on composites
- Sample Time -
- Sample Location

Glassware

- Acid washed - Distilled water rinses

- Dedicated to Phosphorus Analysis ONLY

Equipment

- Spectrophotometer
  - wavelength 880 nm
  - light path < or = 2.5 cm
- Photometer
  - filter Red
  - light path < or = 0.5 cm
- Service Records

Analytical Results

- Blank
  - The sample is used to "zero" the instrument
- Standards
  - Number and concentrations used
  - Frequency of Use - at least 1/set up
  - Results

Duplicate

- Replicate schedule

Reporting:

Orthophosphate is recorded as mg/L as P. Be sure that (either) the instrument used converts the result from orthophosphate as PO_4 to orthophosphate as P. If the instrument used does not convert the result automatically there is a simple conversion that can be used. Simply multiply the mg/L as PO4 result by 0.326. For example: If the instrument used gives a reading of 2.0 mg/L PO4, simply multiply the result by 0.326 to yield a result of 0.652 mg/L as P. **Be sure to record the result as xx mg/L as P.**

Reporting Orthophosphate Data

ORTHOPHOSPHATE BENCH SHEET
SAMPLE TYPE: (typically a grab)
SAMPLING TIME AND DATE:
SAMPLE VOLUME: (volume used in analysis, typically 10 mL.)
SAMPLE PRESERVATION: N/A for process control (filtered for NPDES required reporting)
ANALYST:
ANALYSIS TIME AND DATE:
METHOD: (ex: Hach method 8048)
STANDARDS
Concentrations Used:
Results (include calculations if applicable):
SAMPLE RESULTS
Raw Data and Calculations:

References:

Standard Methods for Analysis of Water and Wastewater 23rd edition Section 4500-P

Hach®methods can be found via links provided