PHOSPHORUS

(PO_4)

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 of 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 and aggravating 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 2 mls of concentrated sulfuric acid (H_2SO_4). It should then be refrigerated at 4 degrees Centigrade (4°C) until analyzed.

Glassware Preparation

Phosphate contamination is common because of the tendency of phosphates to absorb 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 HCl 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.

Generally - Minimum of 9- 50 ml graduated cylinders
1- 1 ml pipet (for H_2SO_4)
1- 4 ml pipet (for ammonium molybdate solution)
1- 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.

The most common method used by Vermont wastewater analysts for the determination of Total Phosphorus is the Ascorbic Acid Method. That is the method described here.

Reagent

ASCORBIC ACID

0.1M - Dissolve 1.76 g ascorbic acid in 100 mls distilled water. Refrigerate at 4°C. This reagent must be prepared fresh weekly.

SULFURIC ACID

5N - Partially fill a 500 ml flask with approximately 400 mls distilled water. **Carefully** add 70 mls concentrated sulfuric acid (H_2SO_4). **Slowly and carefully** bring the volume to 500 mls with distilled water.

POTASSIUM ANTIMONYL TARTRATE SOLUTION

Partially fill (400 mls) a volumetric flask with distilled water. Dissolve 1.3715 g potassium antimonyl tartrate $K(SbO)\ C_4H_4O_6 \cdot \frac{1}{2}H_2O$ in the distilled water and then dilute to 500 mls with distilled water. This reagent should be stored in a glass-stoppered bottle.

AMMONIUM MOLYBDATE SOLUTION Dissolve 20 g Ammonium Molybdate $(NH_4)_6 MO_7O_{24} \cdot 4H_2O$ in 500 mls distilled water. This reagent should be stored in a glass-stoppered bottle.

COMBINED REAGENT

Allow all of the reagents listed above to reach room temperature. Then in a 1 liter beaker (or other large mouth container **ADD IN THIS ORDER**

50 mls 5N sulfuric acid (H_2SO_4) and 5 mls potassium antimonyl tartrate solution. Mix. Then add 15 mls Ammonium Molybdate solution. Mix. Add 30 mls 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 (H_2SO_4)
Concentrated Nitric Acid (HNO_3)
Phenolphthalein Indicator
1N Sodium Hydroxide Solution (NaOH)

Procedure

1) Pipet 50 mls of sample into a dry micro kjeldahl flask or tube

2) Add 1 ml conc H_2SO_4 and 5 mls conc HNO_3

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 mls of distilled water to solution

6) Add 1 drop (0.05 ml) phenolphthalein

7) Add 1N NaOH a drop at a time until the solution develops a slight pink color

8) Dilute this solution to 100 mls 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 mls of conc H_2SO_4 to 600 mls 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 mls 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 drop by drop 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 mls is reached (or heat for 30 minutes in autoclave at 98-137Kpa)

7) Cool to room temperature

8) Dilute to 30 mls 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 mls 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 drop by drop until red color disappears

4) Add 8.0 mls 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. 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.

$$mg\ P/L = mg\ P \frac{(approximately\ 58\ ml\ final\ volume)\ x\ 100}{ml\ sample}$$

Standards

EPA requires that at least one (preferably more) standards 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 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°C for 1 hour) KH_2PO_4 in distilled water and dilute to 1,000 mls. Make fresh each month.

1.0 ml = 0.1 mg of P

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

1.0 ml = 0.01 mg of P

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

1.0 ml = 0.001 mg of P

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

a. 0.10 mg/L: Dilute 1.0 mls of Solution B to 100 mls

$$\frac{.01 \text{ mg P}}{100 \text{ ml}} \times \frac{1000 \text{ ml}}{1 \text{ l}} = 0.10 \text{ mg/L}$$

b. 0.20 mg/L: Dilute 2.0 mls of Solution B to 100 mls

$$\frac{.02 \text{ mg P}}{100 \text{ ml}} \times \frac{1000 \text{ ml}}{1 \text{ l}} = 0.20 \text{ mg/L}$$

c. 0.40 mg/L: Dilute 4.0 mls of Solution B to 100 mls

$$\frac{.04 \text{ mg P}}{100 \text{ ml}} \times \frac{1000 \text{ ml}}{1 \text{ l}} = 0.40 \text{ mg/L}$$

d. 0.80 mg/L: Dilute 8.0 mls of Solution B to 100 mls

$$\frac{.08 \text{ mg P}}{100 \text{ ml}} \times \frac{1000 \text{ ml}}{1 \text{ l}} = 0.80 \text{ mg/L}$$

e. 1.00 mg/L: Dilute 10.0 mls of Solution B to 100 mls

$$\frac{.10 \text{ mg P}}{100 \text{ ml}} \times \frac{1000 \text{ ml}}{1 \text{ l}} = 1.0 \text{ mg/L}$$

f. 1.5 mg/L: Dilute 15.0 mls of Solution B to 100 mls

$$\frac{.15 \text{ mg P}}{100 \text{ ml}} \times \frac{1000 \text{ ml}}{1 \text{ l}} = 1.5 \text{ mg/L}$$

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 manufacturers instructions
	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 PHOSPHORUS

Document

*Supply Water Quality
- Conductivity
- Phosphate Free

Sampling

- Sample Type - (usually a composite)
- Sample Time - time sample collection started
- 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
	light path	≥2.5 cm
Photometer -	filter	Red
	light path	≥0.5 cm

- Service Records

Analytical Results

- Blank
  - What was used
  - How was it treated
  - Results
  - Frequency
- Standards
  - Number and concentrations used
  - Frequency of Use - at least 1/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:

References

Methods for the analysis of phosphorus can be found on page 356.3-1 of The Manual of Methods for chemical analysis of Water and Wastes and in the 18th Edition of Standard Methods for the Examination of Water and Wastewater page 4-115.