STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PILOT PLANT

TITLE: Phosphate Determination

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A. Scope

This SOP describes the procedure to measure orthophosphate concentration using the ascorbic acid method. The minimum detectable concentration is approximately 10 µg phosphate/L.

B. Safety and Training Requirements

Refer to UF lab safety policies regarding equipment listed in section D below before starting any process work.

Review the location of fire extinguishers, fire blankets, safety showers, spill cleanup equipment and protective gear before beginning any process work.

During operations in the Lab, the following safety gear will be utilized at all times:

- Lab Coat
- Safety Goggles
- Protective Gloves

C. Related Documents and SOPs

1. Thermo Spectronic 20D+ Manual
2. MSDS Binder

D. Equipment/Materials

1. Spectrophotometer
2. 50-mL Erlenmeyer flask
3. Phenolphthalein indicator
4. Concentrated sulfuric acid
5. Ammonium molybdate
6. Ascorbic acid
7. Potassium phosphate monobasic
8. Potassium antimonyl tartrate
9. DI water
10. Glass-stoppered bottles (3)

E. Detailed Procedure

1. Prepare the following solutions;
    a. 5 N sulfuric acid – dilute 70 mL concentrated H_2SO_4 to 500 mL with distilled water.

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b. Potassium antimonyl tartrate solution – dissolve 1.3715 g $K(SbO)C_4H_4O_6 \cdot {}^1/_2H_2O$ in distilled water in a 500 mL volumetric flask and dilute to volume. Store in a glass-stoppered bottle in the dark.
c. Ammonium molybdate solution – dissolve 20 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 500 mL distilled water. Store in a glass-stoppered bottle in the dark.
d. 0.1 M ascorbic acid – dissolve 1.76 g ascorbic acid in 100 mL distilled water. The solution is stable for about 1 week at 4 °C. Store in a glass-stoppered bottle in the dark.
e. Combined reagent – mix the above reagents in the following proportions for 100 mL of the combined reagent (**Note**: Mix after addition of each reagent. Let all reagents reach room temperature before they are mixed and mix in the order given. If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding. The reagent is stable for 4 h);
    i. 50 mL of 5N H_2SO_4,
    ii. 5 mL of potassium antimonyl tartrate solution,
    iii. 15 mL of ammonium molybdate solution, and
    iv. 30 mL of ascorbic acid solution.
f. 50 mg/L stock phosphate solution – dissolve in distilled water 219.5 mg anhydrous KH_2PO_4 and dilute to 1000 ml (Note: 1.00 ml = 50.0 μg PO_4^{3-}-P).
g. 2.5 mg/L standard phosphate solution – dilute 50.0 mL stock phosphate solution to 1000 mL with distilled water (Note: 1.00 mL = 2.50 μg PO_4^{3-}-P).

2. Pipet 20.0 mL of the sample into a clean, dry 50-mL Erlenmeyer flask.
3. Add 0.05 mL (1 drop) phenolphthalein indicator.
    a. If a red color develops add 5 N H_2SO_4 solution drop wise to just discharge the color.
4. Add 3.2 mL combined reagent and mix thoroughly.
5. After at least 10 min, but no more than 30 min, measure absorbance of each sample at 880 nm, using a distilled water blank with the combined reagent as the reference solution.
6. For highly colored or turbid waters,
    a. prepare a blank by adding all reagents except ascorbic acid and potassium antimonyl tartrate to the sample and
    b. subtract blank absorbance from absorbance of each sample.

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7. Prepare a series of six standards within the phosphate ranges indicated below.

Concentration (mg/L)	DI Water (mL)	Stock Phosphate Solution (mL)
0.01	19.996	0.004
0.10	19.96	0.04
0.25	19.9	0.1
0.50	19.8	0.2
0.75	19.7	0.3
1.00	19.6	0.4

8. Repeat steps E.2. – E.5. using the six standards instead of the sample
   a. Use a distilled water blank with the combined reagent to make the photometric readings.
9. Plot absorbance against phosphate concentration to give a straight line passing through the origin.
10. Test at least one phosphate standard with each set of samples.

NOTE: Arsenates react with the molybdate reagent to produce a blue color similar to that formed with phosphate. Concentrations as low as 0.1 mg As/L interfere with the phosphate determination. Hexavalent chromium and NO_2 interfere to give results about 3% low at concentrations of 1 mg/L and 10 to 15% low at 10 mg/L. Sulfide (Na_2S) and silicate do not interfere at concentrations of 1.0 and 10 mg/L

F. Data Archival and Analysis

1. Calculate the amount of phosphate in the sample.

$$\text{mg } PO_4^{3-}\text{-P/L} = \frac{\text{mg } PO_4^{3-}\text{-P (in approximately 23.2 ml final volume)} \times 1000}{\text{mL sample}}$$

2. Record all measurements in the laboratory notebook including the date, time, vessel, and batch number of the sample.