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LABORATORY TRAINING MANUAL FOR SEWAGE TREATMENT PLANT PERSONNEL

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INTERSTATE SANITATION COMMISSION
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TABLE OF CONTENTS

		PAGE
INTRODUCTION		
I.	Gravimetric Determination of solids	1
	A. Total Solids	
	B. Total Volatile Solids	2
	C. Total Suspended Solids, Non-Settleable Solids, And Settleable Solids	2
	D. Volatile Solids	4
	E. Example Calculation	6
	F. Sludge Solids	10
	G. Sludge Volume Index	11
II.	Biochemical Oxygen Demand	12
	A. Equipment	12
	B. Reagents	12
	C. Procedure	14
	D. Supplementary Sheet For Biochemical Oxygen Demand Test	22
III.	Volatile Acids	24
	A. Equipment	24
	B. Reagents	24
	C. Procedure	25
	D. Calculations	26
IV.	Chemical Oxygen Demand	27
	A. Equipment	27
	B. Reagents	27
	C. Procedure	28
	D. Calculations	29

INTRODUCTION

The efficient operation of wastewater treatment plants, whether involving simple primary treatment or advanced tertiary processes, is contingent upon the proper application of chemical and/or physical methodology. The treatment plant operator should not only have a thorough understanding of these processes but should be well versed in the application of certain basic laboratory tests so as to insure effective plant performance.

The Interstate Sanitation Commission has developed a laboratory training program tailored specifically to the needs of treatment plant personnel. The present program includes an introduction to basic laboratory techniques and a description of tests normally utilized in treatment plants; i.e., BOD, Suspended Solids, and Volatile Acids. The methodology for each of the analyses are in accordance with "Standard Methods for the Examination of Water and Wastewater". The program is conducted by trained Interstate Sanitation Commission staff members in the Commission's mobile laboratory. The instruction is on an individual basis and the operator benefits by performing the various analyses with realistic samples, equipment, and conditions.

The context of each training session is quite flexible and can be easily tailored to the requirements of the individual operator. Generally though, the program requires a two to three-day visit.

I. GRAVIMETRIC DETERMINATION OF SOLIDS

Sewage Solids may be classified in several ways. Those discussed in the manual are the following:

1) Total Solids - all solid material remaining after evaporation of the water from sewage. Total Solids include both dissolved and suspended Solids.

2) Total Suspended Solids (T.S.S.) - all the solid particles which are carried in sewage.

3) Settleable Solids (S.S.) - the solids which settle from sewage when it is held in a quiscent state for one hour.

4) Non-Settleable Solids (N.S.S.) - solids which remain in suspension after the sewage has been held in a quiescent state for one hour.

5) Volatile Solids - solids which combust at 550°C. Volatile Solids are classified under several categories: Total Volatile Suspended Solids (T.V.S.S.), Volatile Settleable Solids (V.S.S.), Volatile Non-Settleable Solids and Total Volatile Solids.

A. Total Solids

a) Procedure

1. Heat a porcelain dish at 550°C in a muffle furnace for 1/2 hour.

2. Cool for 30 minutes in a desiccator and weigh.

3. Slowly evaporate 100 ml of homogeneous sample in the porcelain dish.

4. Dry for one hour at 103°C; cool in a desiccator for 1/2 hour and weigh.

b) Calculations

1. Weight (in grams) of dish and dried sample - Initial Weight (in grams) of dish = grams of Total Solids/ 100 ml.

2. g./100 ml x 10,000 = mg/l Total Solids

B. Total Volatile Solids (T.V.S.)

a) Procedure (This test is a continuation of the previous one.)

1. Place the porcelain dish with the dried sample (Section A, Step 4) in a muffle furnace at 550°C for 15 minutes.

2. Cool in a desiccator for 30 minutes and weigh.

b) Calculations

1. Weight (in grams) of the dish after evaporation - Weight (in grams) after 550°C = g./100 ml Total Volatile Solids.

2. g./100 ml x 10,000 = mg/l T.V.S.

C. Total Suspended Solids, Non-Settleable Solids and Settleable Solids

a) Equipment

1. Porcelain crucibles, 25 ml capacity, with a 2.1 cm bottom inside diameter.

2. Glass fiber filters, 2.1 cm diameter.

3. Muffle furnace at 550°C. CAUTION: Temperatures above 550°C will melt glass fiber filters.

4. Drying oven at 103°C.

5. Glass cylinder, of at least 9 cm diameter, and of at least one liter capacity (20 cm depth).

6. Desiccator, equipped with a drying agent (i.e., $CaCl_2$).

7. Tongs.

8. Balance capable of weighing to at least .0001 g.

9. Siphon

b) Preliminary Procedure

1. Place a glass fiber filter in each of 8 numbered

crucibles making sure that the pad is covering all the holes.

2. Set each crucible in a suction flask which is attached to a vacuum pump designed to draw approximately 28 psig.

3. Apply vacuum and rinse each crucible with about 100 mls of distilled water.

4. Dry in an oven for one hour at 103°C.

5. Cool in a desiccator for 30 minutes and weigh.

c) Procedure

1. Obtain at least one liter of sample from the influent and one liter from the effluent of the treatment plant.

2. Shake the influent sample and pour a 50 ml sample into each of two beakers (100-150 ml size).

3. Same as #2 except for the effluent sample.

4. Place the remaining samples into two cylinders and let settle for one hour.

5. During this one-hour period, filter the 4 samples from Steps 2 and 3 through the pre-weighed crucibles. Wash each flask twice with distilled water and add the rinse water to the crucibles.

6. Dry the 4 crucibles in an oven at 103°C for one hour.

7. Cool and weigh.

8. After the samples from Step 4 have settled for one hour, fill the siphon with distilled water and pinch the end.

9. Place the siphon into the influent cylinder. The siphon should be placed about half-way down beneath the surface of the liquid.

10. Withdraw about 25 mls into a waste beaker or until the sample completely fills the siphon.

11. Siphon 250 mls into a 400-500 mls beaker.

12. Repeat Steps 10 and 11 for the effluent sample.

13. Stir the influent beaker and pour out two 50 ml samples into 100-150 ml beakers.

14. Repeat Step 13 for the effluent sample.

15. Filter these 4 samples according to the procedure in Step 5.

16. Dry these crucibles at 103°C for one hour, cool in a desiccator, and weigh.

d) Calculations

1. Total Suspended Solids (T.S.S.) - Use the results from Section C, Steps 2, 3, and 5.

$$(\text{Crucible weight in grams after filtration} - \text{Crucible weight in grams before filtration}) \times \frac{1{,}000{,}000}{\text{ml sample}} = \frac{\text{mg}}{\text{l}}\ \text{T.S.S.}$$

2. Non-Settleable Solids (N.S.S.) - Use the results from Section C, Steps 8-16.

$$(\text{Crucible weight in grams after filtration} - \text{Crucible weight in grams before filtration}) \times \frac{1{,}000{,}000}{\text{ml sample}} = \frac{\text{mg}}{\text{l}}\ \text{N.S.S.}$$

3. Calculated Settleable Solids (C.S.S.)

Calculated Settleable Solids (mg/l) = Total Suspended Solids (mg/l) - Non-Settleable Solids (mg/l).

D. Volatile Solids

To determine the Total Volatile Suspended Solids, the Volatile Non-Settleable Solids, and the Calculated Volatile Settleable Solids, the previous procedure may be applied with the following additions:

a) Preliminary Setup: After preparation of the crucibles, muffle them at 550°C for 15 minutes. Allow them to cool

in a desiccator for 30 minutes and weigh. Muffle, cool, and weigh as many times as is necessary to attain constant weight.

b) Procedure: After completion of the T.S.S., N.S.S., and C.S.S. tests from Section C, muffle the crucibles at 550°C for 15 minutes. Cool for 30 minutes and weigh.

c) Calculations:

Total Volatile Suspended Solids in mg/l = (weight after 103°C in grams - weight after 550°C in grams) x 1,000,000 /ml sample.

Volatile Non-Settleable Solids in mg/l = (weight after 103°C in grams - weight after 550°C in grams) x $\frac{1,000,000}{\text{ml sample}}$

Calculated Volatile Settleable Solids in mg/l = Total Volatile Suspended Solids in mg/l - Volatile Non-Settleable Solids in mg/l.

E. Example Calculation:

Given the following data, calculate T.S.S., T.N.S.S., C.S.S., T.V.S.S., V.N.S.S., and calc. V.S.S.

Influent: sample volume = 50 ml

	Data Before Settling			Data After Settling for 1 hr.		
	Initial Weight (gms)	Wt. 103°C (gms)	Wt.550°C (gms)	I_o (gms)	Wt. 103°C (gms)	Wt. 550°C (gms)
Trial I	18.4921	18.4980	18.4931	19.3112	19.3132	19.3115
Trial II	17.3911	17.3972	17.3920	16.3217	16.3237	16.3221

Effluent: sample volume = 50 ml

	Initial Weight (gms)	Wt. 103°C (gms)	550°C (gms)	I_o (gms)	Wt. 103°C (gms)	Wt. 550°C (gms)
Trial I	14.2111	14.2130	14.2113	17.1410	17.1418	17.1412
Trial II	16.2123	16.2138	16.2126	16.4102	16.4109	16.4103

a) Total Suspended Solids

Inf.

Trial 1

18.4980 gms
-18.4921 gms
.0059 gms

Trial 2

17.3972 gms
-17.3911 gms
.0061 gms

.0059 x 20,000* = 118 mg/l
.0061 x 20,000* = 122 mg/l

$$\text{AVER.} = \frac{122+118}{2} = 120 \text{ mg/l T.S.S.}$$

Eff.

Trial 1

14.2130 gms
-14.2111 gms
.0019 gms

Trial 2

16.2138 gms
-16.2123 gms
.0015 gms

.0019 gm x 20,000 = 38 mg/l
.0015 gm x 20,000 = 30 mg/l

$$\text{AVER.} = \frac{30+38}{2} = 34 \text{ mg/l}$$

NOTE: All weighings should be performed after the crucible has reached ambient temperature.

$$\% \text{ Removal} = \frac{\text{INF. T.S.S. mg/l} - \text{EFF. T.S.S. mg/l}}{\text{INF. mg/l}} \times 100$$

$$= \frac{120-34}{120} \times 100 = 72\%$$

*NOTE: The factor 20,000 was obtained by dividing 1,000,000 by 50 ml sample size.

b) Non-Settleable Solids

Inf.

Trial 1	19.3132 gms	Trial 2	16.3237 gms
	-19.3112 gms		-16.3217 gms
	.0020 gms		.0020 gms

.0020 gms x 20,000 = 40 mg/l
.0020 gms x 20,000 = 40 mg/l

$$\text{AVER.} = \frac{40+40}{2} = 40 \text{ mg/l}$$

Eff.

Trial 1	17.1418 gms	Trial 2	16.4109 gms
	-17.1410 gms		-16.4102 gms
	.0008 gms		.0007 gms

.0008 gms x 20,000 = 16 mg/l
.0007 gms x 20,000 = 14 mg/l

$$\text{AVER.} = \frac{14+16}{2} = 15 \text{ mg/l}$$

$$\% \text{ Removal} = \frac{40-15}{40} \times 100 = 63\%$$

c) Calculated Settleable Solids

Inf.	Eff.
120 mg/l T.S.S.-40 mg N.S.S.=80 mg/l C.S.S.	34 mg/l TSS-15 mg/l NSS=19mg/l C.S.S.

$$\% \text{ Removal} = \frac{80 \text{ mg/l} - 19 \text{ mg/l}}{80 \text{ mg/l}} \times 100 = 76\%$$

d) Total Volatile Suspended Solids

Inf.

Trial 1

18.4980 gms
-18.4931 gms
.0049 gms

Trial 2

17.3972 gms
-17.3920 gms
.0052 gms

.0049 gms x 20,000 = 98 mg/l
.0052 gms x 20,000 = 104 mg/l

$$\text{AVER.} = \frac{98+104}{2} = 101 \text{ mg/l}$$

Eff.

Trial 1

14.2130 gms
-14.2113 gms
.0017 gms

Trial 2

16.2138 gms
-16.2126 gms
.0012 gms

.0017 gms x 20,000 = 34 mg/l
.0012 gms x 20,000 = 24 mg/l

$$\text{AVER.} = \frac{24+34}{2} = 29 \text{ mg/l}$$

$$\% \text{ Removal} = \frac{101-29}{101} \times 100 = 71\%$$

e) Volatile Non-Settleable Solids

Inf.

Trial 1

19.3132 gms
-19.3115 gms
.0017 gms

Trial 2

16.3237 gms
-16.3221 gms
.0016 gms

.0017 gms x 20,000 = 34 mg/l
.0016 gms x 20,000 = 32 mg/l

$$\text{AVER.} = \frac{34+32}{2} = 33 \text{ mg/l}$$

Eff.

Trial 1

17.1418 gms
-17.1412 gms
.0006 gms

Trial 2

16.4109 gms
-16.4103 gms
.0006 gms

.0006 gms x 20,000 = 12 mg/l
.0006 gms x 20,000 = 12 mg/l

$$\text{AVER.} = \frac{12+12}{2} = 12 \text{ mg/l}$$

$$\% \text{ Removal} = \frac{33-12}{33} \times 100 . = 64\%$$

f) <u>Calculate Volatile Settleable Solids</u>

<u>Inf.</u>

101 mg/l T.V.S. - 33 mg/l V.N.S.S. = 68 mg/l C.V.S.S.

<u>Eff.</u>

29 mg/l T.V.S.S. - 12 mg/l V.N.S.S. = 17 mg/l C.V.S.S.

$$\% \text{ Removal} = \frac{68-17}{68} \times 100 = 75\%$$

E. Sludge Solids

a) Equipment

1. Porcelain evaporating dish with a loose fitting cover

2. Balance

3. Steam bath

4. Oven (103°C)

5. Desiccator

6. Muffle furnace (550°C)

b) Procedure

1. Muffle a clean evaporating dish at 550°C for 15 minutes.

2. Cool and weigh

3. Pour about 50 mls of sample into the dish and weigh

4. Evaporate to dryness, heat in an oven at 103°C for one hour, cool in a desiccator and weigh.

5. Cover the dish and muffle at 550°C for one hour.

6. Cool in a desiccator.

7. Remove the cover and add any ash from the cover back into the dish.

8. Weigh

c) Calculations

1. Wt. of dish and wet sample - wt. of dish = weight of wet sample.

2. Wt. of dish and dry sample - wt. of dish = weight of dry sample.

$$\% \text{ Solids} = \frac{\text{wt. of dry sample}}{\text{wt. of wet sample}} \times 100$$

3. Wt. of dish and dry solids - Wt. of dish after ignition = Wt. of volatile matter

$$\% \text{ Volatile Matter} = \frac{\text{wt. of volatile matter}}{\text{wt. of dry solids}} \times 100$$

% Ash = 100% - % Volatile Matter

F. Sludge Volume Index (SVI)

The SVI is a measure of the settleability of activated sludge and is an indication of sludge bulking. It is defined as the volume in mls of one gram of activated sludge after the mixed liquor has settled for 30 minutes.

a) Procedure

1. Pour 1000 mls of activated sludge (mixed liquor taken from the effluent of the aeration tank) into a graduated cylinder and settle for 30 minutes.

2. Record the volume of sludge.

3. Using another sample, determine the Suspended Solids.

b) Calculations

$$SVI = \frac{\text{ml settled sludge} \times 1000}{\text{mg/l Suspended Solids}}$$

II. BIOCHEMICAL OXYGEN DEMAND

The B.O.D. test is an empirical test used to evaluate the amount of oxygen that an organic waste will deplete from its surroundings. Bacteria metabolize organic material as food and in so doing utilize oxygen and produce carbon dioxide and other gases much as a human being does. Relying on this principle, the test relates the amount of biologically decomposable material in wastewater to the amount of oxygen consumed by micro-organisms in stabilizing the organic waste.

A. Equipment:

a) Incubation bottles or B.O.D. bottles, 300 ml, ground-glass stoppered bottles, with a special flared mouth for maintaining a water seal.

b) Incubator, maintained at 20°C.

c) A large bottle

d) General laboratory equipment

B. Reagents

a) Ferric Chloride, dissolve 0.25 grams of $FeCl_3 \cdot 6H_2O$ in distilled water and dilute to one liter.

b) Calcium Chloride, dissolve 27.5 grams of $CaCl_2$ in distilled water and dilute to one liter.

c) Magnesium Sulfate, dissolve 22.5 grams of $MgSO_4 \cdot 7H_2O$ in distilled water and dilute to one liter.

d) Ammonium Phosphate Buffer, dissolve 8.5 grams KH_2PO_4 (potassium dihydrogen phosphate), 33.4 grams $Na_2HPO_4 \cdot 7H_2O$ (disodium hydrogen phosphate heptahydrate), 21.75 grams K_2HPO_4 (dipotassium hydrogen phosphate) and 1.7 grams of NH_4Cl (ammonium chloride) in distilled water and dilute to one liter. If properly prepared, the solution will be buffered at pH 7.2.

e) Manganous Sulfate, dissolve 480 grams $MNSO_4 \cdot 4H_2O$ (or 400 grams of $MnSO_4 \cdot 2H_2O$) in distilled water. Filter the solution through Watman #40 filter paper and dilute to one liter.

f) Alkaline Iodide Azide

1. To 600 mls of distilled water, add, with constant stirring, 500 grams of NaOH. CAUTION: Use a heat resistant container since this solution gives off much heat. Continue stirring until all the NaOH dissolves. Now add 135 grams of NaI (or 150 grams KI) and stir. Allow the solution to cool and dilute to one liter.

2. Dissolve 10 grams NaN_3 (sodium azide) in 40 mls of distilled water and add to the solution prepared in Step 1.

g) Concentrated Sulfuric Acid (36N), reagent grade.

h) $^N/_{40}$ Sodium Thiosulfate, dissolve 6.205 grams of $Na_2S_2O_3 \cdot 5H_2O$ (sodium thiosulfate, pentahydrate) in distilled water and dilute to one liter. The normality of this solution is approximately 0.025N. This solution is not stable and must be standardized frequently (refer to Procedure, Section C, part f, for the standardization procedure). Preserve with 5 ml of chloroform. (If a $^N/_{10}$ stock solution is purchased already prepared, dilute 250 mls of stock solution to a liter with distilled water to obtain a 0.0250N solution.)

i) Starch.- Mix 5 grams of soluble starch with 50 mls of distilled water. Add this suspension to 900 mls of boiling water. Dissolve the starch suspension by allowing it to boil for a few minutes. Cool and settle overnight, Preserve with 1.25 grams salicylic acid per liter.

j) Standard Potassium Dichromate Solution (0.0250N) Dry a few grams of potassium dichromate at 103°C for 2 hours. Cool in a desiccator for 30 minutes. Weigh accurately 1.226 grams of potassium dichromate on an analytical balance. Dissolve, carefully, in distilled water and dilute to one liter in a volumetric flask. Extreme care should be taken in the preparation of this solution.

k) Sodium Sulfite Solution - Dissolve 1.6 grams of anhydrous sodium sulfite (Na_2SO_3) in one liter of distilled water. Prepare fresh daily.

1) Seed - Place 500 mls of unchlorinated domestic waste water in a jar, aerate for 12 hours, and settle. The purpose of a seed is to be sure that bacteria are present to oxidize organic matter in the sample.

C. Procedure (Azide Modification of Winkler Method)

a) Preliminary Setup

1. Place 300 mls of distilled water in a bottle for each B.O.D. bottle to be filled.

2. Add 1 ml of ferric chloride solution, 1 ml of calcium chloride solution, 1 ml of magnesium sulfate solution, and 1 ml of ammonium phosphate buffer for every liter of water in the bottle. For example, if 2 liters of water are used then 2 mls of each solution must be added. This solution is now called dilution water.

3. Aerate the dilution water for 20 minutes.

4. Set up the B.O.D. bottles in the following manner:

TABLE I

	Seed		Influent		Effluent	
Initial D.O.	(1)	(2)	(3)	(4)	(5)	(6)
	Seed		Influent		Effluent	
Final D.O.	(7)	(8)	(9)	(10)	(11)	(12)

In the above Table, bottles 1-6 are used to determine the initial dissolved oxygen; bottles 7-12 are incubated for 5 days at 20°C. The seed bottles are used to correct for the extra amount of B.O.D. contributed by the seed . (This diagram is based upon two dilutions used per sample. It should be noted that two dilutions per sample is the minimum and more dilutions should be prepared if necessary.)

b) Pretreatment of Sample

1. If chlorine is present, place 100 mls of sample in each of two flasks. Into one flask, add 10 mls Acetic Acid (1-1),(5 mls concentrated Acetic Acid, 5 mls Distilled Water),10 mls KI solution (10 grams per 100 ml), and 1 ml of starch. If chlorine is present, the solution will turn blue. Titrate with

Sodium Sulfite until the blue color disappears. Record this amount and discard the test sample. To the second sample, add only the amount of Sodium Sulfite that was determined by titration. This should be sufficient to dissipate the chlorine in the sample. The treated sample is used to prepare the B.O.D. dilutions.

2. If the sample is either strongly alkaline, or acidic, neutralize the sample to pH 7 with dilute H_2SO_4 if alkaline or dilute NaOH if acidic.

c) Seed Correction

1. Place 10 mls of seed in each of the four bottles designated as seed in Table I.

2. Fill these bottles with dilution water and stopper. Care should be taken to insure that no air bubbles are trapped in the bottles.

3. To the remaining dilution water, add 3 ml of seed for each liter of dilution water remaining.

d) Dilution Preparation

1. To bottles 3 and 9, add one quantity of treated raw sewage and to bottles 4 and 10 add another quantity. The amount of sample used depends upon the nature of the sewage. Enough sample should be added to give a utilization of at least 2 mg/l of oxygen, while having at lease 1 mg/l of oxygen remaining in solution after 5 days. The following table will help in deciding the dilutions.

B.O.D. MEASURED WITH VARIOUS DILUTIONS OF SAMPLE.*

ml of sample	Range of B.O.D.
0.5	1,200-4,200
1.0	600-2,100
2.0	300-1,050
3.0	200- 700
4.0	150- 525
5.0	120- 420
6.0	100- 350
10	60- 210
15	40- 140
20	30- 105
25	25- 84
30	20- 70
40	15- 65
50	12- 42
100	6- 21
300	0- 7

*All samples are directly pipetted into 300 ml bottles for each dilution selected.

It should be noted again that the use of two dilutions represents a minimum and should only be used if the analyst is familiar with the range of B.O.D. within the sample. If the range is not known, it will be necessary to use three or more dilutions.

2. Prepare bottles #5, 6, 11, and 12 in a similar manner using treated effluent as a sample.

3. After all the samples have been pipetted into the bottles, fill them carefully with seeded dilution water being careful not to overfill or trap any air bubbles.

4. Place bottles #7-12 in the incubator at 20°C for 5 days. It is important that a water seal be maintained in the neck of the B.O.D. bottles throughout the incubation period. If the seal is not maintained, air bubbles will form in the bottles, invalidating the results.

e) Determination of Dissolved Oxygen

1. Add to each of the remaining bottles (1-6) 2 mls of manganous sulfate solution and 2 mls of alkaline-iodide-azide solution. Be careful to add the reagents

well below the surface of the liquid solution. Replace the glass stoppers, rinse with water, and shake vigorously. After letting the precipitate settle for a few minutes, shake once again.

2. Again let the precipitate settle until at least 1/2 of the bottle is clear.

3. Add 2 mls of concentrated sulfuric acid and rinse. Shake until the precipitate completely dissolves.

f) Standardization of the Sodium Thiosulfate

1. Add the following to an Erlenmeyer flask: 2 grams KI, 100 mls distilled water, 10 mls sulfuric acid (1+9: 1 ml H_2SO_4 & 9 ml H_2O), and 25 mls of standard potassium dichromate solution. Dilute to 200 mls with distilled water. Titrate this solution with thiosulfate to a pale straw color. Add 1 ml of starch and continue titrating until the blue color just disappears. Twenty-five mls of the thiosulfate should be used if the normality is exactly 0.0250N.

2. If more or less than 25 mls is used, a calculation is necessary. To calculate the normality of the thiosulfate use the formula: $N_1 \times V_1 = N_2 \times V_2$.

   Where N_1=the normality of the potassium dichromate (0.0250N), $V1$ =the volume of dichromate (25 mls), N_2=the normality of the thiosulfate (unknown) and $V2$ =the volume of thiosulfate that was titrated. For example, assume that 25.62 mls of sodium thiosulfate are needed for the titration. The normality of the dichromate (0.0250N) times the amount used (25.00 mls) will equal the normality of the thiosulfate (unknown U) times the amount used (25.62 mls from the titration). Substituting the numbers into the formula:

$$0.0250 \times 25.00 = U \times 25.62$$
$$25.62U = 0.625$$
$$U = 0.0244N$$

   Note that because it took slightly more than 25 mls of the thiosulfate to react completely with the iodine, its normality is slightly less than the potassium dichromate.

g) Titration

1. If the normality of the sodium thiosulfate is exactly 0.0250N, then titrate 203 mls of sample from bottles 1-6 to a pale straw color, add 1 ml of starch, and continue titrating until the blue color disappears. Record the number of mls of thiosulfate used on a suitable data sheet. (Note: The volume of 203 mls accounts for the addition of 4 mls (2 mls each) of manganese sulfate and alkali-iodide-azide reagents; i.e., $\frac{200x300}{300-4} = 203$ mls.)

2. If the normality of the sodium thiosulfate is some value other than 0.0250N (as in our example), then a calculation is necessary to insure that 1 ml titrated can be read directly as mg/l of dissolved oxygen. Referring to the previous example, the normality of the thiosulfate was 0.0244, so that the amount of sample to be titrated will be:

$$\frac{0.0250}{203} = \frac{0.0244}{U}$$

See Table III

$$(0.0250)(U) = (203)(0.0242)$$

$$U = 198 \text{ mls}$$

h) 5 Days Later

1. The B.O.D. bottles (7-12) are now ready for titration. Follow the procedure described previously (Section C, parts f and g).

i) Calculations

1. Seed Correction. Calculate the amount of oxygen used by the seed by subtracting the final D.O. (B_2) from the initial D.O. (B_1) (bottles 1, 7, and 2, 8) and average the two results. This average, when multiplied by the seed correction factor, will give the amount of B.O.D. contributed by the seed only in each sample. The seed correction factor can be calculated as follows:

$$f = \text{ratio of the seed in sample to seed in control} = \frac{\%\text{ seed sample}}{\%\text{seed in control}}$$

% Seed in Sample: Each liter of dilution water contains 3 mls of seed and each ml of dilution water 0.003 ml of seed. The amount of seed in each sample bottle is equal to (300-mls of sample)(0.003). The % of seed in the sample is equal to the mls of seed in the sample divided by 300 mls or

$$\frac{(300\text{-mls of sample})(0.003)}{300} = \text{\% Seed in Sample}$$

% Seed in the Control is also equal to the mls of seed in the control divided by the control volume (300 mls). Since 10 mls of seed was used in the control, the % seed in the control equals 10/300. Finally in equation form:

$$\text{Seed Connection Factor} = f = \frac{\frac{(300\text{-mls of sample})(0.003)}{300}}{\frac{10}{300}} = \frac{\text{\% of seed in sample}}{\text{\% of seed in control}}$$

The following three examples show what this factor would be if samples sizes of 1, 2, and 3 mls were used:

$$\frac{\frac{(300-1)(0.003)}{300}}{10/300} = 0.0897$$

$$\frac{\frac{(300-2)(0.003)}{300}}{10/300} = 0.0894$$

$$\frac{\frac{(300-3)(0.003)}{300}}{10/300} = 0.0891$$

The seed correction can now be calculated by multiplying $f(B_1-B_2)$. Note that the seed correction, as well as the correction factor are not constant but vary with sample size. Therefore, a new calculation must be performed for each sample volume.

2. B.O.D.

The B.O.D. as mg/l O_2 can be determined by using the formula:

$$mg/l\ B.O.D. = \left[(D_1 - D_2) - f(B_1 - B_2)\right] P$$

D_1 = initial D.O.
D_2 = final D.O. after 5 days
$f(B_1-B_2)$ = seed correction

$$P = \frac{300}{\text{ml of sample}}$$

j) Example

Given the following results, calculate the B.O.D.:

Seed Control

Trial I , B_1(initial D.O. of the seed control) = 7.50
Trial II, B_1(" " " " " ") = 7.60
Trial I , B_2(final " " " " ") = 3.50
Trial II, B_2(" " " " " ") = 3.60

Influent sample

Trial I , Volume of sample = 4 mls
D_1(initial D.O.) = 800
D_2 (final D.O.) = 5.25

Trial II, Volume of sample = 6 mls
D_1 = 7.96
D_2 = 4.18

Effluent Sample

Trial I , Volume of Sample = 20 mls
D_1 = 8.10
D_2 = 6.10

Trial II, Volume of sample = 30 mls
D_1 = 8.00
D_2 = 5.00

Seed Control

Trial #1 (B_1-B_2) = 7.50-3.50 = 4.00
Trial #2 (B_1-B_2) = 7.60-3.60 = 4.00

Average = 4.00

$$f(4\ ml\ sample) = \frac{\frac{(300-4)(0.003)}{300}}{10/300} = 0.0888 \text{ (See Table II)}$$

Y(seed correction) = $f(B_1 - B_2)$ = 0.0888(4.00) = 0.36

f(6 ml sample) = $\frac{\frac{(300-6)(0.003)}{300}}{10/300}$ = 0.0882 (See Table II)

Y = (0.0882)(4.00) = 0.35

f(20 ml sample) = $\frac{\frac{(300-20\text{ ml})(0.003)}{300}}{10/300}$ = 0.0840 (See Table II)

Y = (0.0840)(4.00) = 0.34

f(30 ml sample) = $\frac{\frac{(300-30)(0.003)}{300}}{10/300}$ = 0.0810 (See Table II)

Y = (0.0810)(4.00) = 0.32

Influent Sample

mg/l B.O.D. = $\left[(D_1-D_2)-f(B_1-B_2)\right]$ P

Trial I $\left[(8.00-5.25)-(0.36)\right]\left[300/4\right]$ = 179 mg/l B.O.D.

Trial II $\left[(7.96-4.18)-(0.35)\right]\left[300/6\right]$ = 172 mg/l B.O.D.

Average B.O.D. = $\frac{179 + 172}{2}$ = 176 mg/l

Effluent Sample

Trial #1 = $\left[(8.10-6.10)-(0.34)\right]\left[300/20\right]$ = 25

Trial #2 = $\left[(8.00-5.00)-(0.32)\right]\left[300/30\right]$ = 27

Average B.O.D. = $\frac{25 + 27}{2}$ = 26 mg/l

3. Calculation of Removal and % Removal

Removal = Influent B.O.D. - Effluent B.O.D.
176-26 = 150 mg/l B.O.D. removal

% Removal = $\frac{\text{Influent B.O.D. - Effluent B.O.D.}}{\text{Influent B.O.D.}}$ x 100

$\frac{150}{176}$ x 100 = 85% Removal

4. On the following page is a data sheet containing all the values shown in the previous example.

D. Supplementary Sheet for Biochemical Oxygen Demand Test

1. To convert from other than a 5-day BOD to a 5-day BOD because of a holiday or other circumstances, use the following factors:

To convert from:

a) 4-day BOD to a 5-day BOD...multiply by 1.149
b) 6-day BOD to a 5-day BOD...multiply by 0.909
c) 7-day BOD to a 5-day BOD...multiply by 0.85

2. The following is a table of (f) factors which are applied in the seed correction based on the amount in mls of sample pipetted into the BOD bottle.

TABLE II

Dilution H_2O*	Sample	Factor	Dilution H_2O*	Sample	Factor
300	+ 0	.0900	288	+ 12	.0864
299	+ 1	.0897	287	+ 13	.0861
298	+ 2	.0894	286	+ 14	.0858
297	+ 3	.0891	285	+ 15	.0855
296	+ 4	.0888	280	+ 20	.0840
295	+ 5	.0885	275	+ 25	.0825
294	+ 6	.0882	270	+ 30	.0810
293	+ 7	.0879	265	+ 35	.0795
292	+ 8	.0876	260	+ 40	.0780
291	+ 9	.0873	255	+ 45	.0765
290	+10	.0870	250	+ 50	.0750
289	+11	.0867	200	+100	.0600

*NOTE: The dilution water contains 3 mls of seed per liter of dilution water.

3. The following table is presented to deduce some of the calculations required in determing the BOD. Column (1) represents the number of mls of thiosulfate actually used by the analyst in standardizing this solution against 25 mls of 0.025N Potassium Dichromate solution. Knowing this value, the analyst may read directly from the table the normality of the thiosulfate solution (column (2) and the mls of sample to be used in the BOD determination (column 3).

B.O.D. LABORATORY RECORD

Plant SAMPLE DATA

Date Incubated ____________ Date of Analyses ____________ By ________

5 Day B.O.D. at 20°C

SEED

D. O. Bottle Number	B.O.D. Bottle Number	Sample	Samp. Vol. ml	D.O. (mg/1) Initial B_1	Incub. B_2	B_1-B_2	Avg. of (B_1-B_2)
1	7	Seed	10	7.50	3.50	4.00	4.00
2	8	Seed	10	7.60	3.60	4.00	

SAMPLE

Dechlorination Required

	Yes	No
Inf.	X	
Eff.	X	

D.O. Bot. No.	BOD Bot. No.	Sample	Samp. Vol. ml	D.O. (mg/1) Initial D_1	Incub. D_2	X= (D_1-D_2)	f*	Y=Avg. (B_1-B_2) x f	X-Y	BOD mg/1
3	9	Inf.	4	8.00	5.25	2.75	.0888	0.36	2.39	179
4	10	"	6	7.96	4.18	3.78	.0882	0.35	3.43	172
		"								
		"								
5	11	Eff.	20	8.10	6.10	2.00	.0840	0.34	1.66	25
6	12	"	30	8.00	5.00	3.00	.0810	0.32	2.68	27
		"								
		"								

*The dilution water contains 3 ml of seed per liter.

(5) Day 20°C BOD, mg/l x (R) = 5-Day 20°C BOD, mg/l

Avg. Inf. ____________ x(   ) = Avg. Inf. 176

Avg. Eff. ____________ x(   ) = Avg. Eff. 26

Removal 150 mg/l 85 %

TABLE III

MILLILITERS OF THIOSULFATE	NORMALITY OF THIOSULFATE	MLS. OF SAMPLE TO BE TITRATED
26.08 - 26.00	0.0240	195
25.98 - 25.90	0.0241	196
25.88 - 25.78	0.0242	197
25.76 - 25.68	0.0243	197
25.66 - 25.58	0.0244	198
25.56 - 25.48	0.0245	199
25.46 - 25.38	0.0246	200
25.36 - 25.26	0.0247	201
25.24 - 25.16	0.0248	201
25.14 - 25.06	0.0249	202
25.04 - 24.96	0.0250	203
24.94 - 24.86	0.0251	204
24.84 - 24.76	0.0252	205
24.74 - 24.68	0.0253	205
24.66 - 24.58	0.0254	206
24.56 - 24.48	0.0255	207
24.46 - 24.38	0.0256	208
24.36 - 24.28	0.0257	209
24.26 - 24.20	0.0258	210
24.18 - 24.10	0.0259	210
24.08 - 24.00	0.0260	211

III. VOLATILE ACIDS

Volatile acids are produced by saprophytic bacteria in the decomposition of organic material. Volatile acids consist of short-chain fatty acids, such as acetic, propionic, and butyric. They are termed volatile acids because they can be distilled at atmospheric pressure. In well balanced anerobic digesting units, methane forming bacteria utilize volatile acids and convert them to methane and carbon dioxide.

Overproduction by the saprophytic bacteria produces a buildup in volatile acids, a decrease in the pH of the digester and a destruction of methane forming bacteria. This will tend to upset the digester operation. Therefore it is important to check the volatile acid concentration of the digester and keep it between 100-250 mg/l as acetic acid.

A. Equipment

a) centrifuge or filter assembly
b) Gooch crucibles or fritted glass crucible
c) separatory funnel

B. Reagents

a) Silicic acid (50-200 mesh):

1. Wash the acid several times with distilled water.

2. Dry in an oven at 103°C for 1 hour.

3. Store in a desiccator.

b) Chloroform, butanol reagent:

1. Carefully add 60 mls chloroform, 20 mls n-butanol, and 16 mls 0.5N H_2SO_4 into a separatory funnel and shake.

2. Allow the layers to separate and filter the lower organic layer into a clean and dry bottle.

c) Phenolphthalein Indicator: Dissolve 80 mg of phenolphthalein in 100 mls absolute methanol.

d) Thymol Blue Indicator: Dissolve 80 mg thymol blue in 100 mls absolute methanol.

e) Sulfuric Acid

f) Standard Sodium Hydroxide 0.02N:

1. Weigh 20 grams of NaOH, dissolve in CO_2 free distilled water and dilute to 100 mls in a volumetric flask. This is a 5N stock solution.

2. Place 4 mls of the NaOH stock solution in a 1000 ml volumetric flask and q. s. with absolute methanol. This solution is (0.02N) and is not stable and must be standardized before use.

3. Prepare an 0.0200N potassium biphthalate solution by dissolving 4.085 grams (dried at 103° for 1 hour) in water and dilute to one liter in a volumetric flask.

4. Place 25 mls of 0.0200N potassium biphthalate in a beaker and titrate this with 0.02N NaOH solution to a phenolphthalein endpoint.

5. Calculate the Normality of the NaOH with the formula $N_1 \times V_1 = N_2 \times V_1$ (N=Normality and V=Volume).

C. Procedure (Column - Partition Chromatography)

a) Obtain 15 mls of clear sample by either centrifuging or filtering a sample of sludge.

b) Add a few drops of thymol blue solution and acidify the sample with 1:1 H_2SO_4 until the indicator turns from red to blue.

c) Prepare a Gooch crucible by adding 10 grams silicic acid and packing the crucible by applying suction.

d) Place 5 mls of sample evenly over the surface.

e) Apply 5 psi of vacuum for a few seconds. Be careful not to draw any sample past the crucible.

f) Add 50 mls chloroform-butanol reagent to the crucible and as before, apply 5 psi of vacuum. Draw the reagent through the crucible until the last of it reaches the surface of the silicic acid, and then quickly remove the vacuum. Be careful not to draw all the reagent through the crucible.

g) Add a few drops phenolphthalein solution to the flask and titrate with 0.02N NaOH. Take care not to aerate the sample. (For more accurate results, bubble N_2 through the solution while titrating. If N_2 is not available, air scrubbled with ascarite may be substituted.)

h) Repeat the procedure using 5 mls of distilled water as a sample. This is a blank. (Note: Use a new column for each sample to be analyzed.)

D. Calculations

Organic acids in mg/l acetic acid

$$= 60 \times 1000 \times N \times (a-b)/B$$

Where N = normality of the NaOH, a = mls of NaOH used for the sample, b = ml of NaOH used for the blank, and B = ml of sample, 60 = equivalent weight of acetic acid.

IV. CHEMICAL OXYGEN DEMAND

The chemical oxygen demand test is used as one means of determining the strength of sewage and industrial waste. An advantage of this test is that the analyses time is short (approximately 3 hrs.) as opposed to the biochemical oxygen demand test, 5 days. The chemical oxygen demand test is based on the fact organic material (with some exceptions) can be oxidized to carbon dioxide and water by potassium dichromate (a strong oxidizing agent) in an acid medium. The test requires that an excess of potassium dichromate be present in all samples in order to ensure that all the organic matter is oxidized. The excess is measured by titration with ferrous ammonium sulfate (a reducing agent), using Ferroin as an indicator.

The major drawback to the C.O.D. test is that all the oxidizable material in a sample (including metals & chloride ion) will give a C.O.D., thus interfering with the analysis. A correction can be made for the effect of Cl^- ion by adding mecuric sulfate to tie up the ion. However, this method is ineffective above Cl^- concentrations of 2000 mg/l.

A. Equipment

1) 500 ml Erlenmeyer flasks with ground glass 24/40 neck.

2) 300 mm Liebig condenser with 24/40 ground glass joint.

3) hot plates.

4) water supply to condensers

5) boiling chips

6) general laboratory equipment including ring stands and clamps.

B. Reagents

a) standard potassium dichromate solution: 0.250N, -- dissolve 12.259 of predried (at 103°C) primary reagent grade, $K_2Cr_2O_7$ in distilled water and dilute carefully to 1 liter in a volumetric flask.

b) sulfuric acid: dissolve 25 grams silver sulfate per 9-lb. bottle of H_2SO_4 (it may take several days until all the reagent dissolves).

c) standard ferrous ammonium sulfate titrant; 0.25N, dissolve 98g. $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water, add 20 ml concentrated sulfuric acid, cool, and dilute to 1000 ml.

d) ferroin indicator: dissolve 1.485 grams of 1, 10-phenanthroline monohydrate, 0.695 gram of $FeSO_4 \cdot 7H_2O$ in water and dilute to 100 mls.

5) mecuric sulfate

C. Procedure

1) If the sample contains chloride ion add 1.0 gram mecuric sulfate to a 500 ml erlanmyer flask.

2) Add, with a volumetric pipet, 50 mls of sample or an aliquot dilluted to 50 mls with distilled water; it may be necessary to prepare several samples of different volumes.

3) Prepare a blank, in a similar manner, with 50 mls of distilled water.

4) Add, carefully 25 mls of potassium dichromate with a volumetric pipet, mix well, and add five boiling chips.

5) Slowly and cautiously add, with a graduated cylinder 75 mls of sulfuric acid reagent - to avoid a violent reaction add the acid in small increments, allowing the sample to cool, somewhat, between additions (Caution: If sample turns green, discard and start again using less sample volume)

6) Assemble condensers and connect to cold tap water supply.

7) Reflux samples for two hours.

8) While samples reflux standardize the ferrous ammonium sulfate titrant in the following manner: Add, 25 mls of standard potassium dichromate and 200 mls distilled water to a 500 ml erlenmyer flask, Slowly and cautiously add 30 mls of sulfuric acid; Cool to room temperature, Add 5 drops of ferroin indicator and titrate with ferrous ammonium sulfate; the color change is from blue-green to redish-brown - the titration should be as fast as possible.

9) Cool the samples for 15 minutes, if the samples have turned green, the excess of potassium dichromate has been depleted, start again using less sample.

10) Wash condensers down into the flask with 200 mls distilled water.

11) Cool flasks to room temperature (Hot solutions will decompose the ferroin indicator)

12) Add 5 drops ferroin indicator

13) Titrate with standard ferrous ammonium sulfate - the color change is from blue-green to redish-brown; the titration should be as fast as possible.

D. Calculations

1. Normality of ferrous ammonium sulfate

$N_1 X V_1 = N_2 X V_2$

N_1 = Normality of potassium dichromate = 0.250N
$V1$ = Volume of potassium dichromate = 25 mls
N_2 = Normality of ferrous ammonium sulfate = unknown
V_2 = Volume of ferrous ammonium sulfate = titration

$$N_2 = \frac{6.25}{V_2}$$

2. Calculation of C.O.D.

$$\text{mg/l C.O.D.} = \frac{(a-b)c \text{ X } 8000}{\text{ML of sample}}$$

a = blank titration
b = sample titration
c = normality of ferrous ammonium sulfate

3. Example see data sheet (p.)

a) standardization titration = 25.90 mls
b) blank titration = 24.9 mls
c) sample titration = 19.8 mls
d) mls of sample = 25 mls

Calculate C.O.D.

$N_2 = 6.25/_{25.90} = 0.241$

$$\text{mg/l C.O.D.} = \frac{(24.9 \text{ mls} - 19.8 \text{ mls}) \ 0.241 \text{ X } 8000}{25 \text{ mls}}$$

mg/l C.O.D. = 393

INTERSTATE SANITATION COMMISSION
C.O.D. LABORATORY RECORD

Plant__________________________

Date Collected____________

Investigation No._________

Date of Analysis _________

By:________________________

	Refluxing			Titration			C.O.D.
Flask No.	Sample	Sample Vol.	Dist. H_2O	0.250N $K_2Cr_2O_7$	Vol. of Fe Comp. ml vs. Samp.	mgs of Cl of samp.	mg./l C.O.D.= $\frac{(a-b)c \times 8000}{e}$
-	-	e	-	-	a=blank b=samp.	-	
1	Blank	None	50.00	25.00	24.9		
2	Sewage	25.0	25.0	25.00	19.8		mg/l= $\frac{(24.9=19.8)0.241X8000}{25}$=393
				25.00			
				25.00			
				25.00			

Ag_2SO_4 Used Yes ___ No ___

STANDARDIZATION of
$Fe(NH_4)_2(SO_4)2.6\ H_2O$

Flask	ml of 0.250N $K_2Cr_2O_7$	$Fe(NH_4)_2(SO_4)2.6H_2O$	Average	Normality of $Fe(NH_4)_2(SO_4)_2\ 6H_2O$ (C)
A	25.00	25.80	25.90	6.25/Average = 0.241 N
B	25.00	26.00		