

**ENV 3001L**

**Environmental Engineering  
Laboratory Manual**

Spring 2008

Department of Civil and Environmental Engineering  
Florida International University

**ENV 3001L: Environmental Engineering Laboratory  
FLORIDA INTERNATIONAL UNIVERSITY**

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**EXERCISE 1  
Preparation of Reagent and Sample Solutions**

**Purpose**

To prepare stock and standard reagent solutions.

**Definitions**

The term "water" used in these laboratory exercises refers to distilled or demineralized (or deionized) water.

A Normal Solution contains one gram equivalent weight of solute per liter of solution.

A Molar Solution contains one gram molecular weight of solute per liter of solution.

The Equivalent Weight of a compound is that weight of the compound which contains one gram of available hydrogen or its chemical equivalent.

A Standard Solution is one whose strength or reacting volume per unit volume is known.

A Stock Solution is a relatively concentrated solution. It is prepared first, and then an exact dilution of this may be made to the desired strength.



**Preparation of Reagents**

**1. Stock Sulfuric Acid, approximately 0.1 N**

Prepare a stock 0.1 N  $\text{H}_2\text{SO}_4$  solution by diluting 3 milliliters of concentrated  $\text{H}_2\text{SO}_4$  volumetrically to 1 liter with distilled water. Pour this into a plastic container and label. Confirm that 3 mL of concentrated  $\text{H}_2\text{SO}_4$  is indeed required.

2. Standard Sulfuric Acid, 0.02 N

Measure 200 milliliters of stock 0.1 N H<sub>2</sub>SO<sub>4</sub> into a graduated cylinder. Dilute volumetrically to 1 liter with distilled water. Do not remove from 1-liter container! You will need to standardize this H<sub>2</sub>SO<sub>4</sub> solution.

3. Stock Sodium Hydroxide, approximately 0.2 N

Dissolve 8.0 grams of NaOH in distilled water and dilute volumetrically to one liter with distilled water. Pour into plastic container and label. Confirm required grams of NaOH.

4. Standard Sodium Hydroxide, 0.02 N

Dilute 100 milliliters of 0.2 N NaOH to 1 liter with distilled water. Do not remove from 1-liter container.

5. Standard Sodium Carbonate, 0.0200 N

Dissolve 1.060 grams anhydrous Na<sub>2</sub>CO<sub>3</sub> in water and dilute to 1 liter. Confirm required grams of Na<sub>2</sub>CO<sub>3</sub>.

6. Magnesium Sample Solution, 100 mg/L Mg

Prepare solution that will contain 100 mg/L Mg using MgCl<sub>2</sub>·6H<sub>2</sub>O. Calculate how many grams of the magnesium salt are required to prepare this solution, and check with instructor before you proceed to prepare solution.

7. Calcium Sample Solution, 100 mg/L Ca

Prepare solution that will contain 100 mg/L Ca using CaCl<sub>2</sub>. Calculate how many grams of the calcium salt are required to prepare this solution, and check with instructor before you proceed to prepare solution.

8. Sodium Bicarbonate Solution, 0.1 M

Calculate the mass of NaHCO<sub>3</sub> required to prepare a 0.1 M solution. Prepare solution after confirming calculations with instructor.

**Standardization of Sulfuric Acid, 0.0200 N**

- (a) Using a graduated cylinder, measure 100 mL of the approximate 0.02 N H<sub>2</sub>SO<sub>4</sub> solution. Record the volume of 0.02 N H<sub>2</sub>SO<sub>4</sub> remaining. Rinse and fill a 50-mL burette with the 100 mL of measured H<sub>2</sub>SO<sub>4</sub>.
- (b) Volumetric pipette 25 mL of the 0.0200 N Na<sub>2</sub>CO<sub>3</sub> standard solution into a 250-mL Erlenmeyer flask and add about 30 mL of water. Add two to four drops of phenolphthalein solution.
- (c) Titrate with the 0.02 N H<sub>2</sub>SO<sub>4</sub> solution until the disappearance of the pink color. Record the number of mL of H<sub>2</sub>SO<sub>4</sub> as "A".
- (d) Add about two to four drops of methyl orange indicator solution and continue titration to a salmon pink color.
- (e) Record the total mL of H<sub>2</sub>SO<sub>4</sub> as "B". The value of "2A" should equal or nearly equal "B". Use "B" to calculate normality.
- (f) Determine the normality of the acid using the following relationship:

$$V_1 \times N_1 = V_2 \times N_2$$

where V<sub>1</sub> and N<sub>1</sub> are the volume and normality, respectively, of the H<sub>2</sub>SO<sub>4</sub>, and V<sub>2</sub> and N<sub>2</sub> are the

volume and normality, respectively, of the  $\text{Na}_2\text{CO}_3$ .

Solving for the normality of the  $\text{H}_2\text{SO}_4$ :

$$\begin{aligned}N_1 (\text{H}_2\text{SO}_4) &= (V_2 \times N_2)/V_1 \\ &= (25 \times 0.0200)/B\end{aligned}$$

(g) Adjustment of Normality:

If the normality falls outside the range  $0.0200 \pm 0.0002$ , adjustment of the solution is necessary.

If normality is above 0.0202 go to (1)

If normality is below 0.0198 go to (2)

(1)  $(V_1 \times N_1)_{\text{analyzed}} = (V_2 \times N_2)_{\text{desired}}$

where  $V_1$  = volume remaining in the flask (900 mL)

$N_1$  = analyzed normality greater than 0.02

$V_2$  = total final volume

$N_2$  = desired normality = 0.02

Therefore:

$$V_2 = (N_1 \times 900)/0.02$$

Volume of water to be added, mL =  $V_2 - 900$

(2) If the normality is less than 0.0198:

$$V_3 = (0.02 - N_1) \times (900)/0.08$$

where

$V_3$  = volume of 0.1 N  $\text{H}_2\text{SO}_4$  to be added to 900 mL of  $N_1$

$N_1$  = analyzed normality less than 0.0198

The examples given assumed that 900 milliliters of the dilute  $\text{H}_2\text{SO}_4$  remain in the flask. If the volume remaining is different from 900, substitute the actual volume for 900 in the relationships given. The solution must be standardized after each adjustment.

### Standardization of Sodium Hydroxide, 0.0200 N

- Using a graduated cylinder, measure 100 mL of the 0.02 N NaOH solution. Record volume of 0.02 N NaOH remaining in the 1-liter flask.
- Volumetrically pipette 25 mL of 0.02 N NaOH solution into a 250-mL Erlenmeyer flask. Add two to four drops of phenolphthalein solution and titrate with the previously standardized 0.0200 N  $\text{H}_2\text{SO}_4$ . Record mL required to titrate to colorless.
- Use the  $V_1 \times N_1 = V_2 \times N_2$  relationship as described in the previous section (g) to calculate normality.
- Adjust the normality within the range of  $0.0200 \pm 0.0002$  N.  
If the normality is greater than 0.0202, go to section (g-1).  
If the normality is less than 0.0198, then use:  
$$V_3 = (0.02 - N_1) \times (900)/0.18$$
- If adjustment is necessary, the solution must be standardized again.
- Store standardized 0.02 N  $\text{H}_2\text{SO}_4$  and 0.02 N NaOH in labeled plastic containers.

### Questions to be answered in laboratory reports:

- Show that 1 milliliter of the 0.02 N  $\text{H}_2\text{SO}_4$  solution is equivalent to 1.0 milligram  $\text{CaCO}_3$ .

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**EXERCISE 2**  
**Biotic Indicators of Stream Health**

**Purpose**

To familiarize the student with the concept of stressed stream analysis using biotic indicators.

**Theory**

The Clean Water Act of 1972 seeks to maintain the physical, chemical and biological integrity of the nation's waters. Most of the water quality criteria that environmental engineers are familiar with involve species concentrations, e.g., bacterial pollution, suspended sediments, nutrients, and biochemical oxygen demand (BOD). However, excellent water quality in itself does not ensure biological integrity. Other factors such as a degraded habitat or the presence of non-toxic chemicals may adversely impact biotic communities. Evaluating the quantity and quality of biotic community indicators such as fish and invertebrates is a more accurate measure of the biological health of natural water, and has been used by regulatory agencies to establish water quality criteria. This exercise seeks to familiarize students with some of the indicators of stream health that are commonly used in assessing the biological integrity of a natural water.

Such indices include the community diversity and similarity indices. Higher species diversity is generally indicative of a more complex and healthier community allowing for more species interactions and greater system stability, and therefore indicates good environmental conditions. Species diversity has two parts: richness refers to the number of species found in a community, and evenness refers to the relative abundance of each species. A community is said to have high species diversity if many nearly equally abundant species are present. A community with only a few species is considered to have low species diversity even if those species are present in abundant numbers.

**Methodology**

In this exercise you will enter the data for fish collected in Northrup Creek into a spreadsheet, create formulas and calculate indices to compare the fish communities above and below a wastewater treatment plant discharge. You will use your analysis to reach conclusions about the similarity and diversity of the two communities. The following details the methodology to be used in your analysis:

1. Calculating the Proportionality Index of Community Similarity

A good way to compare communities in different places or at different times is to examine community similarity. The Percent Similarity is calculated as the sum of the lowest percent values of a species between two communities, and illustrated in the following example. The higher the value for the Percent Similarity Index the more similar the two communities.

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*This exercise is adapted from the exercise on Stressed Stream Analysis presented by Dr. James Haynes at the NSF Workshop on "New Approaches and Techniques for Teaching Science: Addressing Environmental Problems to Stimulate Undergraduate Learning", July 19–23, 2002, Coastal Carolina University.*

The following example illustrates how to calculate the Percent Similarity Index:

Species	Community 1	Community 2
1	50/93 = 54%	0/112 = 0%
2	25/93 = 27%	7/112 = 6%
3	12/93 = 13%	15/112 = 13%
4	6/93 = 6%	30/112 = 27%
5	0/93 = 0%	60/112 = 54%
Total	93 fish sampled	112 fish sampled

Percent Similarity (PS) = □ (lowest percent value of species between communities)

In this case, PS = 0% + 6% + 13% + 6% + 0% = 25%

## 2. Simpson's Index of Diversity

Simpson's Index calculates the probability that two organisms sampled from a community will belong to different species, i.e., the more even the abundance of individuals across species, the higher the probability that the two individuals sampled will belong to different species. Simpson's Index values range from 0 to 1, with 1 representing perfect evenness (all species present in equal numbers). The formula for Simpson's Index is:

$$D_s = 1 - \frac{\sum n_i(n_i - 1)}{N(N - 1)} \quad \text{where}$$

$n_i$  = number of individuals in the  $i$ -th species collected, and

$N$  = total number of organisms in the sample

For example, if you collected three species with 40, 25, and 15 individuals, respectively:

$$D_s = 1 - \frac{40(39) + 25(24) + 15(14)}{80(79)} = 1 - \frac{2370}{6320} = 1 - 0.375 = 0.625$$

## 3. Determining Statistically Significant Differences in Simpson's Diversity Index

Inevitably the  $D_s$  value that you calculate for each community will be different. In order to determine if the difference is statistically significant, you need to calculate the variability in the data collected. The formula to calculate the variance of Simpson's Index ( $s^2$ ) is:

$$s^2 = \frac{4 \left[ \sum p_i^3 - \left( \sum p_i^2 \right)^2 \right]}{N} \quad \text{where}$$

$p_i$  = proportion of organisms in  $i$ -th species ( $n_i$ ) to total number of organisms in sample ( $N$ ) =  $n_i/N$

For the example above you should obtain  $s^2 = 0.0008$

You will then use a  $t$ -test to determine whether or not Simpson's diversity values are different for the fish communities above and below the wastewater discharge point. The  $t$ -statistic for sample data is computed as:

$$t = \frac{D_1 - D_2}{\sqrt{s_1^2 + s_2^2}}$$

The calculated  $t$ -statistic is compared to the value listed in  $t$ -table. For any calculated  $t$ -statistic greater than listed value, the difference in diversity between the two communities is considered to be significant.

Data are provided on the following page.

**Data for Computing Community Similarity and Diversity Indices**

In order to assess the impact of a wastewater effluent on Northrup Creek, fish communities were sampled and analyzed at two different locations: 75 km upstream and 75 km downstream of the wastewater treatment plant. Northrup Creek is a slow moving sandy bottom headwater stream in Monroe County, New York. The following data on fish communities was collected at the two locations. Analyze the data according to the methodology discussed and present your conclusions about the two communities and whether you think the wastewater effluent has an adverse effect on biological integrity.

Part 1: Calculating the Proportional Index of Community Similarity

Question: What is the percent similarity of the fish communities above and below the WTP?

Species	Numbers	
	Above WWTP	Below WWTP
Creek chub	170	75
Hornyhead chub	60	1
Bluntnose minnow	26	0
Fathead minnow	41	1
Blacknose dace	2	0
Unidentified minnows	5	4
White sucker	41	2
Tadpole madtom	1	0
Rock bass	24	0
Pumpkinseed	3	1
Johnny darter	5	14
Tessellated darter	13	2
Central mudminnow	10	8
Brook stickleback	9	1
Total	410	109

Part 2: Calculating Simpson's Index of Diversity

Part 3: Distinguishing Significant Differences in Community Diversity Values

Additional information on t-tests is available in standard statistical texts and the following web pages:

<http://www.union.edu/PUBLIC/BIODEPT/t.html>

<http://www.georgetown.edu/departments/psychology/researchmethods/statistics/inferential/ttestdep.htm>

<http://www.statsoft.com/textbook/stbasic.html>

<http://bmj.com/collections/statsbk/7.shtml>

<http://home.clara.net/sisa/t-thlp.htm>

**EXERCISE 3**  
**pH and Acid-Base Titration Curves**

**Purpose**

To familiarize the student with the use of a pH meter and to study the interaction of acids and bases.

**Theory**

A titration is the procedure by which a measured amount of a chemical reagent is added to a solution in order to bring about a desired and measured change. The study of acid-base titration involves consideration of the reactions that occur between acids and bases. Acids and bases may be classified as "strong" when they are completely dissociated into ions in solution; or "weak" when they are partially dissociated. At the equivalence point of a titration, the equivalents of base (or acid) added equal the equivalents of acid (or base) in the original solution. The end point of a titration is an operational approximation of the equivalence point. To determine the endpoint of an acid-base titration either a pH meter or an acid-base indicator can be used. A titration curve for an acid-base titration may be obtained by plotting measured pH against volume of acid (or base) added.

The reaction of a strong base with a strong acid involves the combination of  $H^+$  and  $OH^-$  to form  $H_2O$  and is governed by the ionic product of water. Thus the equivalence point of such a titration at  $25^\circ C$  is at pH 7.0. The titration of a weak acid with a strong base (or a weak base with a strong acid) involves at least two sequential reactions. The pH of the equivalence point depends on the degree of dissociation of the weak acid (or base) and its concentration, as well as on the ionization constant of water.



**Measurement of pH**

There are two Orion Model 420A pH/mV/ $^\circ C$  meters available for use in the laboratory. Each meter is equipped with Orion Triode Combination Electrode/ATC Probe. The meter allows two-point auto-calibration with three buffer

solutions. The three buffer solutions provided are pH 4, pH 7, and pH 10, color-coded for easy identification (i.e., pH 4 is pink, pH 7 is yellow, and pH 10 is blue). This laboratory exercise involves calibrating the pH meter and then measuring the pH of a lake sample. Follow the procedure indicated in the instrument manual; remember also to preserve/handle the probe in an appropriate manner.

### Acid-Base Titrations

Titration curves will be prepared for the following two acid-base reactions:

- (i) 50 mL of approx. 0.1 M HCl with 0.5 M NaOH ( $pK_a \approx 3$  for HCl)
- (ii) 50 mL of approx. 0.1 M acetic acid with 0.5 M NaOH ( $pK_a = 4.7$  for  $CH_3COOH$ )

### Procedure

1. Prior to the laboratory class calculate the equivalence points for the above titrations.
2. Add 2 drops of an appropriate indicator to 50 mL of the  $\sim 0.1$  M HCl solution in a beaker. Observe and record the resulting color. Insert the pH electrode and observe and record pH.
3. Titrate the stirred solution with 0.5 M NaOH from a buret, taking sufficient readings of titrant volume and pH to enable the construction of a well-defined titration curve. Carry out the titration to a point at least 2 mL beyond the point at which the indicator changes color. Observe and record titrant volumes and pH values at which indicator color changes occur.
4. Repeat parts 2 and 3 for the titration of the  $\sim 0.1$  M acetic acid solution with 0.5 M NaOH using appropriate indicator.

### Data Analysis

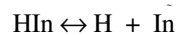
(Use a spreadsheet for this part of your lab report submittal)

1. Plot the following titration curves for each titration performed: (i) pH vs. titrant volume, and (ii) pH vs. moles of OH<sup>-</sup> added per liter of solution ( $C_B$ ).  
Note:  $C_B = (\text{molarity of base}) (\text{titrant volume}) / (\text{sample volume})$   
Compute measured strength (concentration) of acids.
2. Graphically determine and plot the buffer intensity,  $\beta$ , vs. pH using the above plot. Buffer intensity is defined as  $\beta = \Delta C_B / \Delta pH$
3. Determine the volume of titrant required to reach the equivalence point (inflection point on the titration curve). Compare this volume with value required to reach endpoint indicated by color change.

### Cautionary Notes

Sodium hydroxide and the two acids you will be using are corrosive, and will cause damage to your skin or clothes on contact. Therefore, please use gloves and wear your least favorite T-shirts for the performance of this laboratory exercise. The individual adding titrant to the sample is also advised to use safety goggles to protect against accidental spills/splashes.

Acid-base indicators are usually organic acids containing one or more ionizable protons. The protonated and deprotonated forms of these molecules have different colors, so the color change of the indicator molecule can be used to indicate a specific  $H^+$  activity. The behavior of an indicator in solution can be represented as follows:



where HIn represents the acid form of the indicator and In<sup>-</sup> is the basic form. In "acid" solution or when the solution pH is less than the indicator pK<sub>a</sub>, HIn is the predominant form; for pH > pK<sub>a</sub>, In<sup>-</sup> is the predominant form. Based on their pK<sub>a</sub> values it is possible to select an indicator that changes color near the equivalence point of a reaction of interest. Table I below lists characteristics for selected acid-base indicators. Care must be taken when adding indicators since excessive amounts of these organic acids can introduce significant error.

### Questions

Assuming that a 0.01 M indicator in the form HIn is added to the HCl, what volume of indicator would have to be added to introduce a 1% error relative to the amount of HCl present?

**Table I. Acid-Base Indicators**

Indicator	Acid color	Basic color	pH range
Thymol blue (acidic)	red	yellow	1.2 - 2.8
Methyl orange	red	yellow-orange	3.1 - 4.6
Methyl red	red	yellow	4.4 - 6.2
Bromthymol blue	yellow	blue	6.0 - 7.6
Thymol blue (alkaline)	yellow	blue	8.0 - 9.6
Phenolphthalein	colorless	purple	8.2 - 9.8

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*The following sections are adapted from the Water Chemistry Laboratory Manual by D. Jenkins, V.L. Snoeyink, J.F. Ferguson, and J.O. Leckie, AEEP (1973)*

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**EXERCISE 4**  
**Hardness Determination for Water Samples\***

**Purpose**

To introduce the concept of complex formation and stability and to illustrate the analytical application of this concept to the measurement of hardness in water.

**Theory**

Hardness of water is a property caused by the presence of polyvalent metal cations, primarily  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in natural waters. Hardness is undesirable in a water supply because it results in scale formation and in soap wastage. Refer to the text and other references for further details.

Hardness is most frequently measured through the application of the principle of chelation. Most metal ions are capable of sharing electron pairs with a donor (a species which has a "free" electron pair) to form a coordination bond. If a molecule or ion has more than one "free" electron pair which can be shared with a metal ion or similar species, it is called a chelating agent. The complex is termed a chelate.

Hardness determination uses one of the most common chelating agents: ethylene-diamine-tetra-acetic acid (EDTA). EDTA is a tetraprotic acid ( $\text{pK}_{\text{a},1} = 2.0$ ,  $\text{pK}_{\text{a},2} = 2.8$ ,  $\text{pK}_{\text{a},3} = 6.2$ , and  $\text{pK}_{\text{a},4} = 10.3$ ). In the completely deprotonated form, EDTA can form coordination bonds at six sites, and the resulting metal complexes are very stable. If the deprotonation of EDTA is not complete, these chelates are not as stable.

In the determination of hardness with EDTA, several competing equilibria are involved. The sample solution is buffered at a  $\text{pH} \approx 10$  as a compromise between chelate stability (EDTA chelate stability increases with increasing pH) and the need to prevent precipitation of metal ions being analyzed (e.g., as  $\text{CaCO}_3(\text{s})$  or  $\text{Mg}(\text{OH})_2(\text{s})$ ). The ammonia buffer used in the test helps to prevent precipitation of metal ions since ammonia can form weak complexes with them. Since EDTA and its hardness complexes are not colored, an additional chelating agent, Eriochrome Black T (EBT), is used to facilitate endpoint detection.

EBT exists as a blue-colored anion at pH 10. A small amount of EBT is added to the test solution prior to titration with EDTA and a red-colored complex is formed with  $\text{Mg}^{2+}$  present in solution (i.e., the titration starts with red color). The buffer is frequently "spiked" with a trace of  $\text{EDTA} \cdot \text{Mg}^{2+}$  to facilitate endpoint detection in the unlikely event that the sample does not contain  $\text{Mg}^{2+}$ .

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*This experiment is adapted from the Water Chemistry Laboratory Manual by Jenkins et al.*

As EDTA is added to the solution, it combines first with  $\text{Ca}^{2+}$ , and then with  $\text{Mg}^{2+}$  because the  $\text{EDTA}\cdot\text{Ca}^{2+}$  complex is more stable than the  $\text{EDTA}\cdot\text{Mg}^{2+}$  complex. The metals are readily removed from their ammonia complexes because the EDTA-metal ion chelate is much more stable. After EDTA has complexed all of the free  $\text{Mg}^{2+}$ , it will remove  $\text{Mg}^{2+}$  from the red  $\text{EBT}\cdot\text{Mg}^{2+}$  complex causing a color change from red to blue.

### Procedure

1. Measure 50 mL sample into a 250 mL beaker.
2. Add 1-2 mL buffer solution. The pH should be  $10.0 \pm 0.1$ .
3. Add 1-2 drops EBT indicator.
4. Titrate to a blue color. The duration of the titration should not exceed 5 minutes.
5. Perform titration on each of samples provided.
6. Titrate 50 mL of distilled water to which buffer has been added to obtain a blank.

### Questions

Report the total hardness of each sample.

What would be the effect of performing the test at pH 9 instead of pH 10?

### Samples

1. 100 mg/L Mg (prepared in earlier laboratory exercise)
2. 100 mg/L Ca (prepared in earlier laboratory exercise)
3. 50 mg/L each of Ca and Mg (by mixing equal volumes of samples 1 and 2)
4. Tap water  
and any other water sample you may wish to analyze for total hardness.

### Reagents

- Standard EDTA solution, 0.01 M (1 mL = 1 mg hardness as  $\text{CaCO}_3$ ): Dissolve 3.723 g disodium-ethylene-diamine-tetraacetate-dihydrate in distilled water and dilute to 1 liter. (Approximately 200 mL per student group).
- Buffer solution: Dissolve 16.9 g ammonium chloride ( $\text{NH}_4\text{Cl}$ ) in 143 mL concentrated ammonium hydroxide; add 1.25 g of the di-magnesium salt of EDTA and dilute to 250 mL with distilled water. Keep in a tightly stoppered plastic or resistant glass container. (Approximately 50 mL per student group).

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**EXERCISE 5**  
**Determination of Alkalinity**

**Purpose**

To determine, with previously prepared reagents, the concentrations of the different forms of alkalinity contained in prepared samples and in natural and treated waters.

**Theory**

Alkalinity is defined as the capacity of a water to neutralize an acid. In most natural and treated waters, alkalinity is imparted to the water by the carbonate system. Total alkalinity, T, and phenolphthalein alkalinity, P, are defined as follows:

$$T = (\text{HCO}_3^-) + (\text{CO}_3^{2-}) + (\text{OH}^-) \quad (\text{i})$$

$$P = \frac{1}{2}(\text{CO}_3^{2-}) + (\text{OH}^-) \quad (\text{ii})$$

where  $(\text{HCO}_3^-)$ ,  $(\text{CO}_3^{2-})$ , and  $(\text{OH}^-)$  are the bicarbonate, carbonate, and hydroxide ion concentrations, respectively, expressed as equivalents per liter or as mg/L as  $\text{CaCO}_3$ . Accordingly, T and P are the respective alkalinity concentrations expressed as equivalents per liter or as mg/L as  $\text{CaCO}_3$ .

Alkalinity is determined by titration of a sample with an acid of known concentration. For P alkalinity, titration is carried out to the P endpoint (pH ~8.3) or the disappearance of the pink phenolphthalein color, if present. The T alkalinity is determined by titration to an endpoint pH value of ~4.5, as determined by the methyl orange color change from yellow to peach.

From the titration results, the concentrations (as equivalents/L or mg/L as  $\text{CaCO}_3$ ) of the different forms of alkalinity may be calculated as follows:

Result of Titration	Hydroxide (OH <sup>-</sup> )	Carbonate (CO <sub>3</sub> <sup>2-</sup> )	Bicarbonate (HCO <sub>3</sub> <sup>-</sup> )
P = 0	0	0	T
2P < T	0	2P	T - 2P
2P = T	0	2P	0
2P > T	2P - T	2(T - P)	0
P = T	P	0	0

**Reagents**

0.02 N  $\text{H}_2\text{SO}_4$ , phenolphthalein indicator, and methyl orange indicator

### Solutions

- (1) Dilute 15 mL of 0.2 M NaOH to 1 liter with distilled water ( $[\text{OH}^-] = 3 \times 10^{-3} \text{ M}$ ).
- (2) Dilute 15 mL of 0.2 M NaOH solution and 30 mL of 0.1 M  $\text{NaHCO}_3$  solution to 1 liter with distilled water ( $[\text{CO}_3^{2-}] = 3 \times 10^{-3} \text{ M}$ ).
- (3) Dilute 30 mL of 0.1 M  $\text{NaHCO}_3$  solution to 1 liter with distilled water ( $[\text{HCO}_3^-] = 3 \times 10^{-3} \text{ M}$ ).
- (4) Dilute 25 mL of 0.2 M NaOH solution and 30 mL of 0.1 M  $\text{NaHCO}_3$  solution to 1 liter with distilled water ( $[\text{OH}^-] = 2 \times 10^{-3} \text{ M}$ ;  $[\text{CO}_3^{2-}] = 3 \times 10^{-3} \text{ M}$ ).
- (5) Dilute 15 mL of 0.2 M NaOH solution and 40 mL of 0.1 M  $\text{NaHCO}_3$  solution to 1 liter with distilled water ( $[\text{HCO}_3^-] = 1 \times 10^{-3} \text{ M}$ ;  $[\text{CO}_3^{2-}] = 3 \times 10^{-3} \text{ M}$ ).

### Procedure

1. Measure 50 mL of each sample into a 250 mL beaker. (Use graduated cylinder).
2. Rinse and fill buret with standardized 0.02 N  $\text{H}_2\text{SO}_4$ .
3. Add 2-4 drops of phenolphthalein indicator to the sample and titrate to endpoint with 0.02 N  $\text{H}_2\text{SO}_4$ ; record the mL required. This is P-alkalinity.
4. Add 2-4 drops of methyl orange indicator to the same sample, and titrate to pH 4.5 endpoint or to peach color. This is T-alkalinity.
5. If no color develops on addition of phenolphthalein, record the pH, immediately add 2-4 drops methyl orange indicator, and titrate as above.
6. Continue with other samples following the same procedure.
7. Calculate P and T-alkalinity in eq/L, mg/L as  $\text{CaCO}_3$ , and moles/L  $\text{CaCO}_3$ .
8. Calculate hydroxide, carbonate and bicarbonate concentrations using the given table. Express as eq/L and mg/L  $\text{CaCO}_3$ , and moles/L of  $\text{OH}^-$ ,  $\text{CO}_3^{2-}$ , or  $\text{HCO}_3^-$ .
9. Use tabular form where possible.

### Report

For each sample, report the total alkalinity, P-alkalinity, and the hydroxide, carbonate, and bicarbonate concentrations as eq/L and as mg/L as  $\text{CaCO}_3$ .

Also compare the experimentally-derived alkalinity values with the theoretical values and report on the accuracy of your measurements

**EXERCISE 6**  
**Microscopy**

**Water Quality and Biological Indicators\***

Water quality affects the abundance, species composition and diversity, stability, productivity, and physiological condition of indigenous populations of aquatic organisms. Therefore, an expression of the nature and health of the aquatic communities is an expression of the quality of the water. Biological methods used for measuring water quality include the collection, counting, and identification of aquatic organisms; biomass measurements; measurement of metabolic activity rates; measurement of the toxicity, bioaccumulation, and biomagnification of pollutants; and processing and interpretation of biological data (Standard Methods, 1975). Plankton and periphyton are among the aquatic communities that are considered indicative of water quality.

Plankton refers to those aquatic forms having little or no resistance to transport by currents and living free-floating and suspended in open water. Planktonic plants are referred to as phytoplankton and animals as zooplankton. The phytoplankton (microscopic algae and bacteria) occur as unicellular, colonial, or filamentous forms; many perform photosynthesis and are grazed upon by zooplankton and other aquatic organisms. The zooplankton in fresh water comprise principally protozoans, rotifers, cladocerans, and copepods; a much greater variety of organisms is encountered in marine waters. Plankton, particularly phytoplankton, have long been used as indicators of water quality. Some species flourish in highly eutrophic waters while others are very sensitive to chemical contamination. Because of their short life cycles, plankton respond quickly to environmental changes, and hence the standing crop and species composition indicate the quality of the water mass in which they are found. The attached plates depict various species commonly encountered in natural waters.

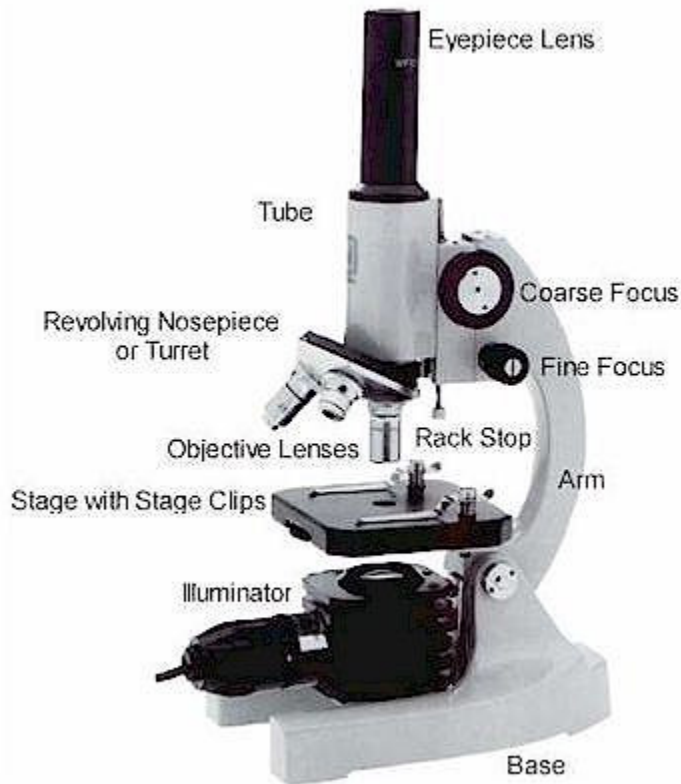
Communities of microorganisms growing on stones, sticks, and other submerged surfaces are greatly influenced by water quality and are very useful in assessing the effects of pollutants on lakes and streams. This group of organisms, known as periphyton, include filamentous bacteria, attached protozoa, rotifers and algae, and also the free-living microorganisms found swimming, creeping, or lodged among the attached forms. The periphyton show dramatic effects immediately below pollution sources, and observations of their condition are generally very useful in assessing conditions in surface waters.

**The Microscope**

The biological examination of water may be accomplished by the use of microscopy. The microscope is a precision instrument; please handle it carefully and learn to use it properly. Remember: never touch the lenses or force the instrument. All adjustments should work easily and freely, and no force is required. Reduced to its simplest terms, a simple microscope is a magnifying glass held on an adjustable stand. However, you are using a compound microscope. It differs from the simple microscope in that it has two separate lens systems; the objective lens system, the one nearest the specimen, which magnifies the specimen a definite amount; and the eyepiece lens system which magnifies the image formed by the objective. Therefore, the image seen by the eye has a magnification equal to the product of the two systems.

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\*The material in this section is taken from *Standard Methods for the Examination of Water and Wastewater*.



### *The Microscope*

#### Parts and Specifications

The simplest optical microscope is the magnifying glass and is good to about ten times (10X) magnification. The **compound** microscope has two systems of lenses for greater magnification,

- 1) the ocular, or eyepiece lens that one looks into
- 2) the objective lens, or the lens closest to the object.

**Eyepiece Lens:** the lens at the top that you look through. They are usually 10X or 15X power.

**Tube:** Connects the eyepiece to the objective lenses

**Arm:** Supports the tube and connects it to the base

**Base:** The bottom of the microscope, used for support

**Illuminator:** A steady light source

**Stage:** The flat platform where you place your slides.

Stage clips hold the slides in place, you are able to move the slide around by turning two knobs. One moves it left and right, the other moves it up and down.

**Revolving Nosepiece or Turret:** This is the part that holds two or more objective lenses and can be rotated to easily change power.

*\*The material in this section is taken from Standard Methods for the Examination of Water and Wastewater.*

**Objective Lenses:** Usually you will find 3 or 4 objective lenses on a microscope. They almost always consist of 4X, 10X, 40X and 100X powers. When coupled with a 10X (most common) eyepiece lens, we get total magnifications of 40X (4X times 10X), 100X, 400X and 1000X.

**Condenser Lens:** The purpose of the condenser lens is to focus the light onto the specimen.

**Diaphragm or Iris:** Many microscopes have a rotating disk under the stage. This diaphragm has different sized holes and is used to vary the intensity and size of the cone of light that is projected upward into the slide.

**How to Focus Your Microscope:** The proper way to focus a microscope is to start with the lowest power objective lens first and while looking from the side, crank the lens down as close to the specimen as possible without touching it. Now, look through the eyepiece lens and **focus upward only** until the image is sharp. If you can't get it in focus, repeat the process again. Once the image is sharp with the low power lens, you should be able to simply click in the next power lens and do minor adjustments with the focus knob. If your microscope has a fine focus adjustment, turning it a bit should be all that's necessary. Continue with subsequent objective lenses and fine focus each time.

### Questions

What are the magnifications of the microscope you are using?

Comment on the water quality based on the algal species identified.

Remember a few details when using the microscope:

- Because you have a binocular body in the microscope, you must use both eyes.
- Always start by using the low power objective.
- If the field is too bright, partially close the diaphragm or reduce the light.
- After placing the slide, look through the ocular and, using the coarse adjustment, bring the specimen into view. Then sharpen the image by using the fine adjustment slowly while looking through the microscope with both eyes open.
- Make sure to adjust the interpupillary distance to fit your eyes.
- When preparing your own specimen, make sure you use a cover glass. The cover glass is necessary to flatten the surface so that the light enters the objective properly.

In this exercise, you should familiarize yourself with the microscope and microscopically examine surface water samples. The surface water samples may be collected from at least two lakes in the campus: you could attempt to sample both attached and suspended microorganisms. Use a dropper to place a small water sample on slide and cover with cover glass. Identify at least five algal species: clearly sketch them and label them. If possible, identify and sketch any rotifer or crustacean you may detect in your water samples. Based on the algal identification try to determine the quality of the water samples. Please include sketches drawn in the lab in your report.

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*\*The material in this section is taken from Standard Methods for the Examination of Water and Wastewater.*

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**EXERCISE 7**  
**The Spectrophotometer and Beer's Law\***

**Purpose**

To demonstrate the operation of a spectrophotometer and to determine whether Beer's Law applies to a particular solution.

**Theory**

Optical methods of analysis can be used to determine satisfactorily the concentration of many dissolved substances. The law relating the amount of light transmitted by a solution to the concentration of a light-absorbing constituent is the Beer-Lambert Law, also known as Beer's Law:

$$\log \frac{I_0}{I} = A = abC$$

where

$I$	=	intensity of monochromatic light transmitted through the test solution
$I_0$	=	intensity of light transmitted through the reference solution - the blank
$A$	=	absorbance (dimensionless)
$b$	=	light path length (usually in cm)
$a$	=	absorptivity, a constant for a given solution/system and a given wavelength
$C$	=	concentration of solute, g/L.

Beer's Law states that for a given solution, absorbance is directly proportional to light path length and concentration of absorbing substance. In the instrumental measurement of color intensity, the light transmitted through the solution is measured. The transmittance of a solution (T) is defined as  $I/I_0$ , and %T as  $I/I_0 \times 100$ .

The colorimeter, or visible wavelength spectrophotometer, is an instrument that makes possible a quantitative measure of light passing through a clear solution. This instrument is capable of supplying light with a narrow wavelength bandwidth and is equipped with light-sensitive phototubes to measure light intensity. The light source for an instrument such as the Bausch and Lomb (Milton Roy) Spectronic 20 is a tungsten lamp. Light from this is dispersed by a diffraction grating (or prism) and the desired wavelength region is selected by passing through a slit. This system produces a wavelength bandwidth of about 20 nm. The selected wavelength band is then passed through the sample solution. That light which is not absorbed by the solution is received by the phototube, the signal from which is displayed on the instrument scale. The light source does not emit the same light intensities at all wavelengths, and the phototube is not equally responsive at all wavelengths. Additionally, the solution medium and the cuvette may absorb light of certain wavelengths. The intensity control dial on the spectrophotometer is used to compensate for such effects.

The sensitivity of analysis for a particular solution and the degree of adherence to Beer's Law depends on the choice of wavelength. Wavelength selection is based primarily on an evaluation of the solution absorption spectrum that

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\*Taken from the Water Chemistry Laboratory Manual, Third Edition, by Jenkins et al., 1972: AEEP.

describes the absorption characteristics as a function of wavelength. Maximum sensitivity, or the largest absorptivity (a), is found at wavelengths where maximum light absorption occurs. Similarly, minimum absorptivities are found where minimum light absorption occurs. Since adherence to Beer's Law assumed a constant absorptivity and since the bandwidth produced by the Spectronic 20 is about 20 nm, it is necessary to choose a nominal wavelength where the absorptivity is approximately equal to that of the immediately adjacent wavelengths. Thus, if possible a wavelength corresponding to a relatively flat portion of the absorption spectrum is generally chosen. Other portions of the spectrum may be used if a reproducible curve of absorbance versus concentration can be obtained.

Deviations from Beer's Law occur for a number of reasons. For example, because a band of wavelengths is passed through the sample a deviation can occur, particularly if the molar absorptivity for this band of wavelengths differs significantly. This deviation from Beer's Law usually becomes larger at higher concentrations. In some instances chemical reactions of solute, temperature effects, stray or scattered light, fluorescence of solutes, and other factors can also cause deviations.

### Procedure

1. Turn on the spectrophotometer and allow it to warm up for approximately 20 minutes.
2. Prepare a p-nitrophenol solution (approximately  $3 \times 10^5$  M) by diluting the stock p-nitrophenol solution with deionized or distilled water. Add 0.1 M NaOH dropwise so that the pH of the diluted solution is at least 9.0. (Note: If the yellow color does not become more intense after one or two drops of NaOH are added, the pH is already above 9.)
3. Insert an opaque light block in the cuvette holder (done automatically in Spectronic 20) and adjust the amplifier control knob until the needle indicates 0% T.
4. Select one of the cuvettes for the blank solution (in this case distilled or deionized water). Do not interchange it with the other cuvettes. Do not handle the lower portion of the cuvette through which the light passes. Always rinse the cuvette with several portions of the solution you are using (deionized water or distilled water in this case) before taking a measurement. Place approximately 3 mL of solution in the cuvette for analysis. Wipe the outside of the cuvette with Kimwipes or equivalent - never use a handkerchief or towel for this purpose because they will leave lint on the surface. To avoid scratching the cuvette, insert it into the cell holder with the index line facing you. After the cuvette is seated, line up the index lines exactly.
5. Turn the wavelength control knob to 530 nm.
6. Rotate the light intensity control knob until the needle indicates either 80% T or the maximum possible %T at this wavelength. Rotate the wavelength control knob and note how the response varies with wavelength. The variation is due to light source characteristics, differing absorption of light at various wavelengths by the cuvette and the distilled water, and variation in phototube response.
7. Determine the absorption spectrum of the solution prepared in part 2 making measurements at 350, 375, 400, 425, 450, 475, 500, 525, and 550 nm in the following manner for each wavelength:
  - a. Set 0% T when no cuvette is in cell holder (light path is blocked with an opaque insert).
  - b. Set 100% T when the blank is in the cell holder.
  - c. Insert the cuvette containing the sample - read and record %T and A.
8. Plot the absorption spectrum. In the region of the absorbance maximum take additional readings at 5 nm intervals. Select the best wavelength for quantitative analysis and check this wavelength with the instructor before proceeding.
9. Empty the cuvettes and rinse them thoroughly with water.
10. Prepare 5 dilutions of the stock solution, which will give approximately equally spaced absorbance readings between 0.1 and 0.7 at the wavelength you have selected. (Note: It may be necessary to make two consecutive dilutions of the stock solution to avoid having to work with very small volumes (< 1 mL) of stock solution. Graduated cylinders should not be used to make the dilution. To adjust pH, add NaOH drop wise

- until no further color change occurs, and dilute to the mark.)
11. For the quantitative analysis it is necessary to determine a cell correction that accounts for the differences in the blank and sample cell characteristics. To do this place deionized or distilled water in the blank cuvette and set the instrument to 100% T. Then place distilled water in the sample cuvette and measure the difference in absorbance and %T (if any). The difference is called the cell correction and should be applied to the test solution data.
  12. Measure the absorbance of each dilution at the selected wavelength, resetting 0 and 100% T before each measurement. Rinse the cuvettes thoroughly between measurements. Begin measurements with the lowest concentration and proceed to the highest concentration.
  13. Adjust absorbance and %T values with the appropriate cell corrections, then plot absorbance vs concentration on arithmetic paper and %T vs concentration on semi-log paper.
  14. Determine whether Beer's Law is followed and, if so, determine the values of the molar absorptivity,  $\epsilon$ , where
 

$\epsilon$	=	$a M$ , liter mole <sup>-1</sup> cm <sup>-1</sup>
$a$	=	absorptivity, liter g <sup>-1</sup> cm <sup>-1</sup>
$M$	=	molecular weight, g mole <sup>-1</sup>
  15. Determine the concentration of the unknown p-nitrophenol solution in moles/L.

### Questions

1. Can a soluble substance be analyzed spectrophotometrically if its light absorption varies with concentration but it does not obey Beer's Law?
2. Assuming that more than one peak or plateau occur in an absorption spectrum, what would be the advantages, if any, in choosing a wavelength at an absorbance peak other than the maximum?
3. If a solution has a green color, what wavelength and color of light is being absorbed by the solution?

### Apparatus

Spectrophotometer  
 Cuvettes: 1 cm path length  
 Pipettes, volumetric flasks and beakers

### Reagents

0.1 M NaOH (~ 30 mL in a dropper bottle per student group)  
 10<sup>-3</sup> M p-nitrophenol: add 0.139 g p-nitrophenol to 500 mL of distilled or deionized water. Adjust pH to >9 with 0.1 M NaOH and dilute to 1 liter with distilled/deionized water. (~250 mL per student group)  
 p-nitrophenol, unknown concentration

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**EXERCISE 8**  
**Chemical Oxygen Demand\***

**Purpose**

To determine the chemical oxygen demand (COD) of a standard glucose-glutamic acid mixture, and to determine the COD values for given aqueous samples (e.g., Coke and Diet Coke).

**Theory**

COD is used as a measure of the oxygen equivalent of the organic matter content of a sample that may be oxidized by a strong chemical oxidant. For samples from a specific source, COD can be related empirically to BOD, organic carbon, or organic matter. COD measurements generally involve the dichromate reflux method: oxidation of most organic compounds is 95 to 100% of the theoretical value. Most types of organic matter are oxidized by a boiling mixture of chromic and sulfuric acids. A sample is refluxed/digested in strongly acidic solution with an excess amount of potassium dichromate ( $K_2Cr_2O_7$ ). After digestion, the remaining unreduced  $K_2Cr_2O_7$  is titrated with ferrous ammonium sulfate to determine the  $K_2Cr_2O_7$  consumed, and the oxidizable organic matter is calculated in terms of oxygen equivalent. The standard reflux time is 2 hours, although reduced times may be used if it has been shown that a shorter period yields the same results. Reagents used include standard potassium dichromate solution (0.0417 M), sulfuric acid reagent (containing  $Ag_2SO_4$ ), ferroin indicator solution, and standard potassium ferrous ammonium sulfate titrant.

The COD test will permit the measurement of the oxygen demand of the unknown samples, e.g., Coke and Diet Coke. Results from the COD test will be used to calculate required dilutions for the BOD test to be performed next.

**Procedure**

Ampules and culture tubes with premeasured reagents are available commercially, and instructions furnished by the manufacturer should be followed. This exercise will involve the use of Hach reagents and the Hach COD reactor. The Hach instructions will be followed in performing this experiment.

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\*The material in this section is taken from *Standard Methods for the Examination of Water and Wastewater*.

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**EXERCISE 9**  
**Biochemical Oxygen Demand\***

**Purpose**

To determine the 5-day BOD ( $BOD_5$ ), the BOD rate constant and the ultimate BOD of a standard glucose-glutamic acid mixture, and to determine the  $BOD_5$  values for given samples (e.g., Coke and Diet Coke).

**Theory**

The BOD test is a bioassay in which the rate (and extent) of the aerobic degradation of organic matter is assessed in terms of the amount of oxygen consumed during its degradation. The complex reactions involved can be summarized as follows:



Since the assay is designed to measure organic matter degradation, none of the other factors influencing the rate of degradation should be limiting. To ensure the presence of excess dissolved oxygen (DO) throughout the test, it is stipulated that a DO residual of 1 mg/L should be present after 5 days incubation for the test to be valid. To ensure a measurable DO uptake it is stipulated that there be a DO uptake of at least 2 mg/L during 5-day incubation for the test to be considered valid. Since water at 20°C, the common temperature of the BOD test, contains only about 9 mg DO/L, samples for BOD measurement usually must be diluted. Dilution is carried out with a water supplemented with all the inorganic minerals necessary for microbial growth and buffered at a physiological pH value. Oxygen is supplied by saturating the sample or dilution water with air. Microorganisms are supplied by seeding the dilution water with an appropriate inoculum (usually sewage, treated sewage, or in some cases microorganisms acclimated to the particular substrate of interest).

The rate of oxidation of carbonaceous organic matter in the BOD test has been approximated by a first order equation:

$$y = L_0 (1 - e^{-kt}) \quad (2)$$

or

$$y = L_0 (1 - 10^{-k't}) \quad (3)$$

where

- $L_0$  = "ultimate" (limiting) amount of DO consumed (ultimate BOD), mg/L
- $y$  = amount of DO consumed at time  $t$  (BOD at time  $t$ ), mg/L =  $BOD_t$
- $t$  = time, days
- $k$  = pseudo first-order rate constant (base  $e$ ),  $\text{day}^{-1}$
- $k'$  = pseudo first-order rate constant (base 10),  $\text{day}^{-1}$

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\*The material in this section is taken from *Standard Methods for the Examination of Water and Wastewater*.

Evaluation of this equation is complicated because both  $L_0$  and  $k$  (or  $k'$ ) are generally unknown. However, if suitable data are available, procedures exist for determining both of these parameters. One simplified method for finding  $k$  and  $L_0$  is based on the following linear approximation of Eq. (1):

$$\left(\frac{t}{y}\right)^{\frac{1}{3}} = (kL_0)^{-\frac{1}{3}} + \left(\frac{k^{2/3}}{6L_0^{1/3}}\right)t \quad (4)$$

A plot of  $(t/y)^{1/3}$  vs  $t$  is a straight line with slope  $b = k^{2/3}/(6L_0^{1/3})$  and intercept on the  $(t/y)^{1/3}$  axis of  $a = (kL_0)^{-1/3}$ . It can then be shown that  $k = 6b/a$  and  $L_0 = 1/(ka^3)$ . Experimental values of  $y > 0.9L_0$  should not be used in this procedure because in this range the assumptions made in developing this relationship no longer hold.

Nitrogen-containing organic compounds can be degraded in the BOD test and the ammonia released, together with the ammonia already present, can be oxidized to  $\text{NO}_2^-$  and  $\text{NO}_3^-$  with significant oxygen consumption. The degree to which ammonia is oxidized in the standard 5-day BOD test is largely dependent on the seeding organisms. If the seed contains significant numbers of nitrifying bacteria, ammonia oxidation will occur at once. However, if the seed does not contain large populations of these microorganisms, then nitrification may not occur during the 5-day period of the test because the nitrifiers grow relatively slowly and may not have time to develop a significant population in 5 days.

### Procedure

1. Incubate at 20°C, a series of BOD bottles containing given sample, diluted with aerated dilution water which has been seeded with reconstituted Polyseed culture, for a series of daily time intervals between 1 and 7 days, inclusive. Keep the water seals filled with water during incubation.
2. Prepare these dilutions so that between 40 and 70% oxygen depletion occurs for each incubation time. In preparing dilutions, it will be helpful to assume  $k$  and  $L_0$  values to compute an estimated BOD at the various incubation times. (For example, you may use  $k' = 0.16 - 0.19/\text{day}$ .) To ensure a 40 to 70% oxygen depletion at each incubation period prepare several different % dilutions for each incubation period.
3. Check you proposed % dilutions with the instructor before proceeding.
4. Determine initial DO for the dilution water in triplicate. Assume that this is the initial DO of the mixture of sample and dilution water because these samples do not have an initial oxygen demand and have been saturated with DO. (The same quantity of seed is added to each BOD flask at the start of the BOD test.)
5. Remove dilutions from the incubator at 1, 2, 3, 4, 5, 6, and 7 days. Determine DO. Select for data analysis those dilutions showing at least 1 mg/L residual DO and 2 mg/L DO depletion.
6. Incubate blanks for DO analysis on each day that you are measuring sample DO. These blanks contain only the seeded dilution water and represent the oxygen demand exerted by the settled sewage seed. The measured DO for the blank is subtracted from sample DO measurements for that incubation period.
7. The given samples are:
  - glucose-glutamic acid standard
  - 0.1% Coke solution in deionized water
  - 1% Diet Coke solution in deionized water

The BOD value for a particular sample on a particular day is computed as:

$$y = BOD_t = \frac{(DO_0 - DO_t) - (1 - P)(B_0 - B_t)}{P} = \frac{DO_{\text{blank}} - DO_{\text{sample}}}{P} \quad (5)$$

where  $DO_0$  and  $B_0$  are the initial dissolved oxygen levels measured in the particular sample and the blank, respectively (i.e., the DO levels for day 0), and  $DO_t$  and  $B_t$  are the DO levels measured in the sample and blank on day  $t$ .

### Data analysis

- (i) Report in tabular form the 5-day BOD values for the various samples.
- (ii) Calculate  $k$  and  $L_0$  for the glucose-glutamic acid standard. How do these values compare with those cited in Standard Methods and other references?

### Questions

1. Assume the glucose-glutamic acid solution contained a toxic material. How would you experimentally demonstrate the presence of this toxicity? How would you eliminate its effects in the BOD test?
2. What would you expect ultimate BOD value of the glucose-glutamic acid standard to be if a nitrifying seed had been used to determine its BOD?
3. Explain the differences observed in  $BOD_5$  values measured for Coke, Diet Coke, and glucose-glutamic acid standard. Are the results reasonable?
4. In general for a particular wastewater which of the parameters would you expect should be higher: the measured BOD or COD? Why?

**Apparatus:** 300-mL capacity BOD bottles (~30 per group); a darkened incubator ~20°C; DO meter

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**EXERCISE 10**  
**Streeter-Phelps Model of the Dissolved Oxygen Sag Curve**

**Purpose**

To understand the effects of oxygen-demanding wastes on natural water quality and to use a simple model for the dissolved oxygen in a stream

**Background**

The amount of dissolved oxygen (DO) in water is one of the most commonly used indicators of a river's health. As DO drops below 4 mg/L many life forms including game fish are unable to survive. A number of factors control the DO level in streams: oxygen-demanding wastes remove DO; algal photosynthesis adds DO during the day but algae remove DO at night during respiration; and the respiration of other aquatic organisms also removes DO due to respiration. In addition, DO levels increase when surface waters are re-aerated by equilibration with atmospheric oxygen. Temperature also plays a role in regulating stream DO levels with higher summer temperatures generally reducing oxygen solubility.

The simplest DO model for a river focuses on two key processes: oxygen depletion due to biodegradation of wastes, and oxygen replenishment due to reaeration. In this simple model it is assumed that there is a continuous discharge of waste at a given location on the river, that the water and wastes are uniformly mixed at any cross-section of the river, and that there is no dispersion of wastes in the direction of flow. This is referred to as the point-source plug flow model.

The rate of deoxygenation due to biological degradation is assumed to be proportional to the BOD remaining at that point:

$$\text{Rate of deoxygenation} = k_d L_t$$

where

$k_d$  = the deoxygenation rate constant ( $\text{day}^{-1}$ )

$L_t$  = the BOD remaining  $t$  days after the wastes enter the river (mg/L)

BOD consumption by biodegradation may be modeled as a first-order reaction so that BOD remaining after time  $t$ ,  $L_t = L_0 e^{-k_d t}$  where  $L_0$  = the BOD of the mixture of river and wastewater at point of discharge.

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*This exercise is adapted from the text "Introduction to Environmental Engineering and Science" by G.M. Masters, Prentice-Hall, Inc. (1998).*

The rate at which oxygen is replenished is assumed to be proportional to the difference between the actual DO in the river at any given location and the saturated value of dissolved oxygen. This difference is the oxygen deficit  $D$ :

$$\text{Rate of reaeration} = k_r D$$

where

$k_r$  = reaeration rate constant ( $\text{day}^{-1}$ )

$D$  = dissolved oxygen deficit =  $(\text{DO}_s - \text{DO})$

$\text{DO}_s$  = saturated value of DO

DO = actual DO at given location downstream

The reaeration rate constant  $k_r$  depends on the particular conditions in the river, e.g., fast-moving versus slow, shallow depth versus deep, etc. Typical reaeration rate constants are listed below:

Water body	Range of $k_r$ at 20°C ( $\text{day}^{-1}$ )
Small ponds and backwaters	0.10 – 0.23
Sluggish streams and large lakes	0.23 – 0.35
Large streams of low velocity	0.35 – 0.46
Large streams of normal velocity	0.46 – 0.69
Swift streams	0.69 – 1.15
Rapids and waterfalls	>1.15

The deoxygenation caused by biodegradation of wastes and oxygenation by reaeration are two competing processes that are simultaneously removing and adding oxygen to the stream. Combining the above equations yields:

Rate of increase of deficit = Rate of deoxygenation – Rate of oxygenation, or

$$\frac{dD}{dt} = k_d L_0 e^{-k_d t} - k_r D$$

which has the solution

$$D = \frac{k_d L_0}{k_r - k_d} (e^{-k_d t} - e^{-k_r t}) + D_0 e^{-k_r t}$$

Since the deficit  $D$  is the difference between the saturation value of DO and actual value of DO, we can write the equation for the DO =  $\text{DO}_s - D$ ,

$$\text{DO} = \text{DO}_s - \frac{k_d L_0}{k_r - k_d} (e^{-k_d t} - e^{-k_r t}) - D_0 e^{-k_r t}$$

representing the classic Streeter-Phelps oxygen sag equation first described in 1925.

The problem to be addressed for this lab exercise involves the use of spreadsheet to plot DO versus distance and answer pertinent questions about the Streeter-Phelps oxygen sag model.

**Methodology/Problem Statement**

The ultimate BOD of a river just below a sewage outfall is 50 mg/L and the DO is 8 mg/L ( $DO_s = 10$  mg/L). The deoxygenation rate coefficient  $k_d$  is 0.3/day and the reaeration rate coefficient  $k_r$  is 0.9/day. The river is flowing at the speed of 48 miles per day. The only source of BOD in the river is this single outfall.

- (a) Plot DO as a function of distance downstream from the outfall
- (b) Find the critical distance downstream at which DO is minimum
- (c) Report the minimum DO
- (d) If a wastewater treatment plant is to be built, what fraction of BOD would have to be removed from the sewage to assure a minimum DO of 5 mg/L everywhere downstream?

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**EXERCISE 11  
Color Adsorption using Activated Carbon**

**Purpose**

To demonstrate the adsorption of organic compounds on activated carbon

**Material Available**

Powdered activated carbon, granular activated carbon, pulverized rubber, 0.05% methyl orange solution



**Procedure**

1. Place a magnet in a 250-mL erlenmeyer flask; add 100 mL of distilled water; add 1 mL of the 0.05% methyl orange solution.
2. Place flask on magnetic stirrer and stir at low speed for 5 seconds.
3. Add 2 grams of powdered activated carbon and continue stirring for 2 minutes. Avoid forming a vortex; if one forms, break it by inserting a glass rod on its side. This disrupts flow pattern and increases turbulence considerably.
4. Vacuum filter contents of flask into a clean flask. Label and set aside.
5. Repeat steps 1 through 4 using 2 grams of granular activated carbon instead of powdered activated carbon.
6. Repeat steps 1 through 4 using 2 grams of pulverized rubber.
7. Prepare a control sample receiving no adsorbent and filter as before.
8. Compare the four filtrates obtained.

**Questions**

Which adsorbent is better at removing color from solution and why?

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**EXERCISE 12**

**Removal of Turbidity using Aluminum Sulfate**

**Purpose**

To demonstrate the removal of turbidity from water by coagulation, flocculation, and sedimentation processes.

**Material Available**

Aluminum sulfate (check and record the amount of water in the formula weight)  
Six-paddle stirrer used for jar tests  
Turbidimeter

**Procedure**

1. Prepare a bucket of turbid water by adding soil and a little clay to a bucket of tap water.
2. Prepare a 5% alum solution.
3. Fill each of six 1-liter beakers with the turbid water making sure that the same volume of water is added to each beaker, and that the water is of uniform turbidity.
4. Place the beakers on the six-paddle stirrer, and stir the contents of all beakers at 100 rpm.
5. Rapidly add varying doses of alum to the beakers. Record time. Stir at 100 rpm for one minute (Rapid mixing).
6. Reduce the speed to ~50 rpm and stir for 5 minutes. Reduce speed to ~20 rpm and stir for another 5 minutes. (This promotes floc formation.)
7. Compare the floc sizes and characteristics in the six beakers.
8. Stop the stirrer and observe the settling of floc particles. Compare the clarity of water in different beakers.
9. Measure and record the turbidity of settled water in each beaker.
10. Plot measured turbidity as a function of coagulant dose.

**Questions**

1. What is the optimum dose for coagulation/flocculation of your water?
2. Why is it necessary to change the speed of the paddle mixers?

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**EXERCISE 13**

**Air Pollution: Aerosol Particle Sampling**

**Purpose**

To demonstrate the aerosol sampling from cigarette smoke to determine particle concentration.

**Material Available**

- Cigarette
- Filter paper
- Filter paper holder
- Diltion tuneel and Flask
- Air pump
- Hood
- Balance (0.0001g)
- Stopwatch
- Desiccator

**Procedures**

- Prepare flask and tunnel and adjust desired flow rate
- Obtain a single cigarette for observation and obtain weight (in grams)
- Obtain a filter paper and get initial weight (in grams) after keeping in a desiccator at least 1 hour.
- Place the filter paper in the filter holder.
- Take the cigarette and place in holding flask under the hood.
- Turn on pump to begin suctioning of cigarette and light it.
- Collect the particles on the filter paper the time needed for completion of cigarette burning
- Record the air sampling time.
- After sampling the particles, turn off the pump; remove the filter paper carefully from the filter holder, than place it inside the desiccator for 15 minutes.
- Then after weight the filter paper with particles, record the value.
- Determine particle matters collected on the filter paper.
- Determine particle concentration in the sampling air.