NANYANG TECHNOLOGICAL UNIVERSITY

School of Civil and Environmental Engineering

CV2701: Laboratory 2A

Laboratory Manual

For

Experiment Lab 2A-6(ENV)

Wastewater Quality Analysis

Location : Environment Laboratory, N1-B3c-49

Session 2004/2005

WASTEWATER QUALITY ANALYSIS
- DO, BOD, COD & TOC ANALYSIS

1. OBJECTIVES

(i) To correctly perform DO measurements and BOD_5, COD, and TOC analyses for a given set of wastewater samples.

(ii) To evaluate the BOD_5/COD, TOC/COD, and TOC/BOD_5 ratios for a given set of wastewater samples.

2. THEORY

2.1 Dissolved Oxygen (DO)

All gases of the atmosphere are soluble in water to some degree. Oxygen is classified as poorly soluble, and its solubility is affected both by atmospheric pressure, and physical and chemical properties of water such as temperature, salinity, pollutants, etc. The solubility of atmospheric oxygen in fresh waters ranges from 14.6 mg/L at 0^oC to about 7 mg/L at 35^oC under 1 atm. of pressure. Most of the critical conditions related to dissolved-oxygen deficiency, both in natural waters and biological wastewater treatment, occur during the warmer months when temperatures are high and solubility of oxygen is at a minimum. The low solubility of oxygen is a major factor limiting the purification capacity of natural waters. In aerobic biological treatment processes, the limited solubility of oxygen is also of great importance, because it governs the rate at which oxygen will be absorbed by the medium and therefore the cost of aeration. Hence, DO analysis is a key test both in natural waters and water pollution control practice.

2.2 Biochemical Oxygen Demand (BOD)

Biochemical oxygen demand (BOD) is defined as the amount of oxygen required by bacteria while stabilizing decomposable organic matter under aerobic conditions. The BOD test is widely used to determine the pollutional strength of domestic and industrial wastewaters in terms of the oxygen that they will require if discharged into natural watercourses in which aerobic conditions exist. The test is one of the most important both in regulatory work and in studies designed to evaluate the purification capacity of receiving water bodies. Its disadvantage is the long time required by the test, generally taking 5 days.

2.3 Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD) is another parameter used widely to measure the pollutional strength of domestic and industrial wastewaters. COD is defined as the amount of oxygen required to oxidize organic matter chemically. Potassium dichromate ($K_2Cr_2O_7$) is generally chosen for this purpose due to its strong chemical oxidizing capability. Almost all organic compounds (except for ammonia, aromatic hydrocarbons, pyridine and their related compounds) can be oxidized by dichromate under heated acidic and $AgSO_4$-catalysed conditions, equivalent to 95 – 100% of the theoretical values.

One of the main limitations of the COD test is its inability to differentiate between biologically oxidizable and biologically inert organic matter. Nor can it provide any evidence of the biological decomposition rate that proceeds either in natural or man-made conditions. The major advantage of COD test is the short time required for evaluation. The determination can be made in about 3 hr rather than the usual 5 days required for the measurement of BOD.

2.4 Total Organic Carbon (TOC)

TOC measures the organic carbon concentration in the water and wastewater. The test can be performed very rapidly (only several minutes) and conveniently (by TOC instrument) and is becoming more popular. For a given wastewater, if a repeatable empirical relationship is established between its TOC and BOD or COD, then measurements in TOC can be used to estimate the accompanying BOD or COD. However, this relationship must be established independently for each set of conditions, such as at various points in a treatment process.

3. EQUIPMENT

3.1 Dissolved Oxygen Probe

The use of DO probes or electrodes which allow in-situ measurements to be made has become standard practice in recent years. They are especially useful for taking DO profiles of reservoirs and streams, for monitoring DO levels in aerobic biological wastewater treatment processes, and for conducting BOD analyses.

An inert metal such as gold or platinum serves as the cathode, and silver is used for the anode. These are electrically connected with a potassium chloride solution, and the cell is separated from the sample by means of a gas-permeable membrane, usually polyethylene. The membrane shields the cathode and anode from contamination by interfering liquids and solids. When a potential of about 0.5 to 0.8 volt is applied across the anode and cathode, any oxygen which passes through the membrane will be reduced at the cathode, causing a current to flow. The magnitude of the current produced is proportional to the amount of oxygen in the sample.

Dissolved-oxygen electrodes are very sensitive to temperature, and thus either temperature measurements must be made along with dissolved-oxygen measurements so that a correction can be applied, or else instruments which are equipped with a thermistor or other device to compensate automatically for temperature changes must be used.

3.2 Biochemical Oxygen Demand

The BOD test is essentially a bioassay procedure involving the measurement of oxygen consumed by living organisms (mainly bacteria) while utilizing the organic matter present in a waste, under conditions as similar as possible to those that occur in nature. The requirements of the environmental conditions for the test can be summarized as follows:

- Sufficient nutrients, e. g. N, P, S, K, Na, and certain trace elements.
- Free from toxins.
- Presence of a mixed culture of microorganisms (seed).
- Dissolved oxygen must be available in the sample throughout the period of the test.
- No interference due to re-aeration.
- 20^oC incubation.

It is possible to interpret BOD data in terms of organic matter, as well as the amount of oxygen used during its oxidation. This concept is fundamental to an understanding of the rate at which BOD is exerted.

Typical BOD_5 values in mg/L for various wastewaters and sewage are given below:

Raw Sewage	- weak	110
	- medium	220
	- strong	440
	- typical in Singapore	300 – 350
Primary effluent		150 – 200
Secondary effluent		20 – 50
Tertiary effluent		5 –10
Raw reservoir water	- Kranji	5 –15
	- Upper Pierce	3 –7

3.3 Chemical Oxygen Demand

In this test, potassium dichromate is used to oxidize the organic matter. In order for the potassium dichromate to oxidize organic matter completely, the solution must be strongly acidic and is refluxed with a silver catalyst at an elevated temperature.

3.4 Total Organic Carbon

TOC analyser is used for measuring the TOC concentration of water and wastewater samples. The analysis actually measures the total carbon (TC) and inorganic carbon (IC) of the sample separately, and the TOC concentration is obtained by difference, i.e. TOC = TC – IC.

To measure the TC content, the sample for analysis is homogenized (for total TC) or filtered (for soluble TC) and diluted as necessary and a micro-portion of known quantity is injected into a heated reaction chamber packed with an oxidative catalyst such as cobalt oxide. The water is vaporized and the organic carbon is oxidized to CO_2 and H_2O. The CO_2 is measured by means of a non-dispersive infrared analyzer.

The IC content is measured by injecting another micro-portion of the sample into a separate reaction chamber packed with phosphoric acid-coated quartz beads which releases inorganic carbon as CO_2.

4. <u>EXPERIMENT</u>

Four wastewater samples are to be analysed in this laboratory session. Each batch of students is required to carry out either total BOD_5 and COD or soluble BOD_5 and COD tests on a particular sample. TOC test will not be conducted but the procedures will be demonstrated to the students. The allocation of samples to each batch is given in Table 1:

Table 1 Sample allocations

Wastewater sample	Batch number	
	Total BOD_5 & COD	Soluble BOD_5 & COD
Raw sewage	1	2
Combined water	3	4
Primary effluent	5	6
Secondary effluent	7	8

4.1 Dissolved oxygen measurements using DO probe

(i) Remove glass stopper of the BOD bottle, and place DO probe into the sample.
(ii) Switch on the stirrer of the DO probe.
(iii) Read the DO value in mg/L when the meter reading has stabilized.

4.2 BOD measurements

The following procedure applies to both total BOD_5 and soluble BOD_5 determinations.

No dilution is required for samples whose 5-day BOD is less than 6 mg/L. Standard dilution technique should be applied to samples with DO depletion greater than 6 mg/L. The analyst has to decide what dilution should be set for determination of BOD. In most instances, three dilutions will be sufficient to cover the possible range of a sample with unknown strength.

The BOD value is not affected by oxygen concentrations greater than 0.5 mg/L. Dilutions that produce a depletion of oxygen less than 2 mg/L should not be used. Hence, it is customary to base calculations of BOD on samples that produce a depletion of at least 2 mg/L and have at least 0.5mg/L of dissolved oxygen remaining at the end of the incubation period. This restriction usually means a DO range of 2 to 6 mg/L.

Table 2 presents suitable dilutions prepared by direct pipetting into bottles of about 300 mL capacity. It is customary to estimate the BOD of a sample and set one dilution based upon the estimate. Two other dilutions, one higher and one lower, are also set up. For example, a sample is estimated to have a BOD of 1000 mg/L. From Table 2, a 0.5% mixture should be used. If a 0.2 and a 1.0% mixture are included, the range of measurable BOD is extended from 200 to 3000 mg/L and should compensate for any errors in the original estimate.

Table 2 BOD measurable with various dilutions of samples.

Using percent mixtures		By direct pipetting into 300 mL bottles	
% mixture	Range of BOD (mg/L)	mL	Range of BOD (mg/L)
0.01	20,000 - 60,000	0.02	30,000 - 90,000
0.02	10,000 - 30,000	0.05	12,000 - 36,000
0.05	4,000 - 12,000	0.10	6,000 - 18,000
0.1	2,000 - 6,000	0.20	3,000 - 9,000
0.2	1,000 - 3,000	0.50	1,200 - 3,600
0.5	400 - 1,200	1.0	600 - 1,800
1.0	200 - 600	2.0	300 - 900
2.0	100 - 300	5.0	120 - 360
5.0	40 - 120	10.0	60 - 180
10.0	20 - 60	20.0	30 - 90
20.0	10 - 30	50.0	12 - 36
50.0	4 - 12	100	6 - 18
100.0	0 - 6	300	0 - 6

In the direct-pipetting technique, preliminary dilutions should be made of all samples that require less than 0.5 mL of the sample, so that amounts added to the bottles can be measured without serious error. The volumes of all bottles must be known in order to allow calculation of the BOD when this method is used.

4.2.1 Procedure for Standard Dilution Technique

(i) Prepare dilution water by adding the following per litre of required dilution water, then aerate to oxygen saturation (approx. 1 hour),

- 1 mL phosphate buffer,
- 1 mL magnesium sulfate solution,
- 1 mL calcium chloride solution,
- 1 mL ferric chloride solution,
- 2 mL of settled raw sewage seed.

(ii) Set up three seeded dilution water blanks. Always siphon dilution water into BOD bottles to avoid entrapping air bubbles.

Note, BOD_5 of seeded dilution water should range between 0.6~1.0 mg/L.

(iii) Prepare three dilutions for each sample.

(iv) Measure the initial DO of each diluted sample and blank using a calibrated DO probe.

(v) Incubate blanks, the remaining samples at 20^{o}C for five days.

(vi) After five days incubation, measure DO in each bottle by DO probe, and calculate BOD_5 as follows:

$$BOD_5 \text{ as } mgO_2/L = \frac{(D_1 - D_2) - (B_1 - B_2)f}{P}$$

where: D_1 = initial DO of sample, mg/L
D_2 = Final DO of incubated sample after 5 days, mg/L
B_1 = DO of seed control before incubation, mg/L
B_2 = DO of seed control after incubation, mg/L
P = Decimal volumetric fraction of sample used

$$f = \frac{\%\text{ seed in } D_1}{\%\text{ seed in } B_1} = \frac{\text{volume of dilution water in sample}}{\text{volume of BOD bottle}}$$

Note: Only consider dilutions where: (1) depletion is $\geq$ 2.0 mg/L, and
(2) final DO $\geq$ 1.0 mg/L.

If more than one dilution satisfies (1) and (2) above, selects dilution with greatest DO depletion.

4.2.2 Calculation Worksheet

Typical examples of worksheet for calculating BOD concentration are shown in Table 3. Enter all your results in the BOD calculation worksheets provided, and attach them with your batch report.

4.3 Chemical Oxygen Demand (using the closed reflux colorimetric method)

4.3.1 Preparation of Samples

(i) Turn on the COD reactor. Preheat to 150^{o}C.

(ii) Remove the cap of a COD digestion reagent vial (Vial type 0-1500 mg/L). Hold the vial at a 45^{o} angle. Pipette 2 mL of sample into the vial. For greater accuracy, a minimum of three replicates per sample should be analyzed and the results averaged.

(iii) Close the vial cap tightly. Hold the vial by the cap and invert gently several times to mix the contents.

(iv) Place the blank and sample vials in the preheated COD reactor. Heat the vials for 1 hour.

(v) After 1 hour, turn the reactor off. Invert each vial several times while still warm. Place the vials into a rack and cool for 1 hour at the room temperature.

4.3.2 Preparation of Calibration Curve

(i) Preparation of Potassium hydrogen phthalate (KHP) standard solution: Lightly crush and dry Potassium hydrogen phthalate (KHP) powder to constant weight at 110^{o}C.

(ii) Dissolve 425mg of dry KHP in distilled water and dilute to 500mL. The solution has a theoretical COD of 1000 mg/L.

(iii) Prepare at least five standards from this standard solution with COD equivalents to cover each concentration range (50, 100, 250, 500 and 1000 mg/L). Use distilled water for the calibration blank.

(iv) Repeat procedure as in Section 4.3.1 for preparation of samples.

4.3.3 Sample measurement

(i) Switch on visible spectrophotometer and set wavelength to 600nm. Enter the value for “K factor” to 2941. The constant K factor is derived from the standard calibration curve.

(ii) Pour blank into the glass cell and place it in the sample compartment. Press “Autozero” key to set the zero absorbance.

(iii) Discard blank and place the cell containing the digested sample in the measuring position. Close sample compartment lid and press “Start” key to measure the absorbance. The result will show the direct COD concentration reading of sample measured as mg/L or ppm.

4.4 Total Organic Carbon (This will be demonstration only)

(i) If a sample contains gross solids or insoluble matter, homogenize until satisfactory replication is obtained.

(ii) Prepare standard organic and inorganic carbon series by diluting stock solutions to cover the expected range in samples. Inject these standards into analyser.

(iii) Withdraw a portion of prepared sample using a syringe. Inject samples into analyzer and determine sample carbon concentration.

(iv) Subtract the inorganic carbon from the total carbon and TOC is determined by the difference. Apply the appropriate dilution factor when necessary.

5 <u>REPORT</u>

Enter the test results in the relevant worksheets as in Tables 4 to 6. Submit these results together with the batch report, which includes completion of the following discussion, on the specified submission day.

6. DISCUSSION

(1) Using results of the entire group (not batch), calculate the BOD_5, COD, and TOC removal efficiencies of:

- (a) the primary treatment process based on raw sewage (input) and on primary effluent (output).
- (b) the secondary treatment process based on primary effluent (input) and on secondary effluent (output).
- (c) the sewage plant's overall treatment efficiency.
- (d) comment on the results obtained.

Note: assess the treatment efficiency as $\frac{C_{input} - C_{output}}{C_{input}} \times 100\%$

(2) Compute the BOD_5/COD, TOC/COD, and TOC/BOD_5 ratios for the different wastewater samples and comment on the results.

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LAB 2A-6(ENV).doc

Table 3 Examples of Worksheet for Calculating BOD Concentration

Project: Mass Balance - BOD

$$BOD_5,\ \text{as}\ mgO_2 / L = \frac{D_1 - D_2 - (B_1 - B_2)\, f}{P}$$

$$f = \frac{\text{Vol of dilution water in sample}}{\text{Vol of BOD bottle}}$$

Initial Blanks		Final Blanks	
B1-1	: 7.4	B2-1	: 6.6
B2-2	: 7.8	B2-2	: 6.7
B3-3	: 7.7	B3-3	: 6.7
Ave B1	: 7.7	Ave B2	: 6.7

Sample	Raw Sewage			Secondary Effluent			Combined Sewage		
Sample No.	1	2	3	4	5	6	7	8	9
Sample Vol (mL)	2	5	10	20	50	100	2	5	10
Bottle Vol (mL)	300	300	300	7.3	300	300	300	300	300
D_1	7.8	7.9	7.7	5.8	7.3	6.9	7.0	7.0	6.9
D_2	5.0	2.3	<1.0´	1.5´	3.9	1.5	5.0	3.0	1.0
Sample Deplet. (D_1-D_2)	2.8	5.6			3.4	5.4	2.0	4.0	5.9
Average B_1	7.7	7.7			7.7	7.7	7.7	7.7	7.7
Average B_2	6.7	6.7			6.7	6.7	6.7	6.7	6.7
Blank Deplet, (B_1-B_2)	1.0	1.0			1.0	1.0	1.0	1.0	1.0
(D_1-D_2)-(B_1-B_2)f	1.8	4.6			2.6	4.73	1.0	3.0	4.9
BOD, mg/L	$\frac{(1.8)(300)}{2}$	$\frac{(4.6)(300)}{5}$			$\frac{(2.6)(300)}{50}$	$\frac{(473)(300)}{100}$	$\frac{(1.0)(300)}{2}$	$\frac{(3.0)(300)}{5}$	$\frac{(4.9)(300)}{10}$
BOD, mg/L	270	276			15.4	14.2	150	180	147
Reported BOD_5, mg/L	276			14.2			147		

Table 4 Worksheet for Calculation of BOD_5 Concentration

Group:______________________________ Date: ____________

$$BOD_5 \text{ as } mgO_2/L = \frac{(D_1 - D_2) - (B_1 - B_2)f}{P}$$

$$f = \frac{\%\text{ seed in } D_1}{\%\text{ seed in } B_1} = \frac{\text{volume of dilution water in sample}}{\text{volume of BOD bottle}}$$

Blank/Sample	Blank			Sample		
Blank/Sample No.	1	2	3	1	2	3
Sample Vol (mL)						
Bottle Vol (mL)						
D_1						
D_2						
Sample depletion ($D_1 - D_2$)						
Average B_1						
Average B_2						
Average blank depletion (B_1–B_2)						
(D_1–D_2) – (B_1–B_2)f						
BOD_5, mg/L						
Reported BOD_5, mg/L						

Table 5 Combined test results of BOD_5, COD, and TOC of samples from a water reclamation plant

Group:____ Date: ______________

Sample	Total (mg/L)		Soluble (mg/L)		
	BOD_5	COD	BOD_5	COD	TOC
Raw Waste					
Combined Water					
Primary Effluent					
Secondary Effluent					

Table 6(a) Percent removal efficiencies for total samples

Process	Total BOD_5 removal (%)	Total COD removal (%)
Primary treatment process based on raw sewage and primary effluent		
Secondary treatment process based on primary and secondary effluent		
Overall treatment plant		

Discussion on percent removal efficiency of total BOD_5 and COD:

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Table 6(b) Percent removal efficiencies for soluble samples.

Process	Soluble BOD_5 removal (%)	Soluble COD removal (%)	Soluble TOC removal (%)
Primary treatment process based on raw sewage and primary effluent			
Secondary treatment process based on primary and secondary effluent			

Discussion on percent removal efficiency of soluble BOD_5, COD, and TOC:

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Table 7 BOD_5, COD, and TOC ratios of wastewater samples

Sample	Total BOD_5 to COD ratio	Soluble BOD_5 to COD ratio	Soluble TOC to COD ratio
Raw Waste			
Combined Water			
Primary Effluent			
Secondary Effluent			

Discussion on BOD_5, COD, and TOC ratios:

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