Technical Assistance
HYDROLOGY PROJECT

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Training module # WQ - 16

Understanding dilution and seeding procedures in BOD test

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DHV Consultants BV & DELFT HYDRAULICS

with
HALCROW, TAHAL, CES, ORG & JPS

Table of contents

1. Module context

This module deals with understanding the need and procedures for dilution and seeding required for biochemical oxygen demand measurement. Modules in which prior training is required to complete this module successfully and other available, related modules in this category are listed in the table below.

While designing a training course, the relationship between this module and the others, would be maintained by keeping them close together in the syllabus and place them in a logical sequence. The actual selection of the topics and the depth of training would, of course, depend on the training needs of the participants, i.e. their knowledge level and skills performance upon the start of the course.

No.	Module title	Code	Objectives
1	*Basic water quality concepts*	**WQ - 01**	• Become familiar with the common water quality parameters • Appreciate important water quality issues
2	*Basic chemistry concepts*[a]	**WQ - 02**	• Convert units from one to another • Understand the concepts of quantitative chemistry • Report analytical results with correct number of significant digits
3	*How to prepare standard solutions*[a]	**WQ – 04**	• Recognise different types of glassware • Use an analytical balance and maintain it • Prepare standard solutions
4	*Understanding the chemistry of dissolved oxygen measurement*[a]	**WQ - 11**	• Appreciate significance of DO measurement • Understand the chemistry of DO measurement by Winkler method
5	*How to measure dissolved oxygen (DO)*[a]	**WQ - 12**	• Measure dissolved oxygen in water samples
6	*Understanding biochemical oxygen demand test*[a]	**WQ - 15**	• Understand the significance and theory of BOD test
7	*Understanding chemical oxygen demand test*	**WQ - 18**	• Appreciate significance of COD measurement • Understand the chemistry of COD measurement

a – prerequisite

2. Module profile

Title	:	Understanding dilution and seeding procedures in BOD test
Target group	:	HIS function(s): Q1, Q2, Q3, Q5
Duration	:	1 session of 60 min
Objectives	:	After the training the participants will be able to*:* • Understanding the need and procedure for dilution and seeding in BOD measurement
Key concepts	:	• Significance • Dilution • Seed and seed correction
Training methods	:	Lecture, exercises and open discussion
Training tools required	:	OHS
Handouts	:	As provided in this module
Further reading and references	:	• Standard Methods: for the Examination of Water and Wastewater, APHA, AWWA, WEF/1995. APHA Publication • Chemistry for Environmental Engineering, C.N. Sawyer, P.L. McCarty and C.F. Parkin. McGraw-Hill, 1994

3. Session plan

No	Activities	Time	Tools
1	***Preparations***		
2	***Introduction*** • Recapitulate BOD reaction • Introduce the lecture topics	5 min	OHS
	Dilution • Explain limited solubility of oxygen in water, its influence on reaction rate and need for dilution • Explain requirements for dilution water and dilution procedure	5 min 20 min	OHS OHS
	Seeding • Explain the meaning and need for seeding • Ask participants for examples where seeding would be required • Describe how an appropriate seed may be obtained • Describe the need and procedure for seed correction	5 min 10 min 10 min	OHS OHS OHS
	Conclusion • Briefly review the material covered in the lecture and invite comments	5 min	

4. Overhead/flipchart master

OHS format guidelines

Type of text	Style	Setting
Headings:	OHS-Title	Arial 30-36, with bottom border line (not: underline)
Text:	OHS-lev1 OHS-lev2	Arial 24-26, maximum two levels
Case:		Sentence case. Avoid full text in UPPERCASE.
Italics:		Use occasionally and in a consistent way
Listings:	OHS-lev1 OHS-lev1-Numbered	Big bullets. Numbers for definite series of steps. Avoid roman numbers and letters.
Colours:		None, as these get lost in photocopying and some colours do not reproduce at all.
Formulas/Equations	OHS-Equation	Use of a table will ease horizontal alignment over more lines (columns) Use equation editor for advanced formatting only

Recapitulate

- Biochemical oxygen demand
- Measures oxygen equivalence of aggregate organic matter

$$\xrightarrow{\textit{micro-organisms}}$$

$$\textbf{Organic matter} + O_2 \Rightarrow H_2O + CO_2$$

- Both oxygen and micro-organisms are necessary for the test

Necessity and procedure for

- Dilution
- Seeding

Dissolved Oxygen

- Min. conc. during incubation period 1 mg/L
- Lower conc. limits BOD reaction rate
- Limited solubility in fresh water

Temp.^{o}C	Conc. mg/L
10	11.1
20	9.1
25	8.3
27	8.0
30	7.5

Dilution of samples

- BOD should be < 7 mg/L
- Min. DO consumption 2 mg/L for reliability
- Grossly polluted waters & wastes need dilution
- Unpolluted surface waters have BOD < 7 mg/L

Dilution water

- Should not contain organic matter
- Prepared from distilled water
- Contains buffer and inorganic nutrients
- Saturated with DO

Dilution procedure

- % mixture = (vol. of sample/ vol. of diluted sample)x100
- Two procedure
  - *separate container*
  - *pipetting into BOD bottle*

Using separate container	
Range of BOD	% mixture
1,000 - 3,500	0.2
400 - 1,400	0.5
200 - 700	1.0
100 - 350	2.0
40 - 140	5.0
20 - 70	10.0
10 - 35	20.0
4 - 14	50.0
0 - 7	100.00

Direct pipetting into 300mL bottles	
Range of BOD	mL sample
1,200 - 4,200	0.5
600 - 2,100	1.0
300 - 1,050	2.0
120 - 420	5.0
60 - 210	10.0
30 - 105	20.0
12 - 42	50.0
6 - 21	100.0
0 – 7	300.0

Choice of dilution

- BOD = (DO consumed/ % mixture) x 100
- When in doubt, use more than one dilution
- Example: when three dilutions were used

S No	Dilution % mixture	Initial DO mg/L	Final DO mg/L	O_2 Con. mg/L	BOD mg/L
1	50	7.5	2.5	5.0	10.0
2	20	7.5	5.2	2.3	11.5
3	10	7.7	6.9	0.8	80

Reject result at 3, why?

Preparing dilution

- Incubate bottles in duplicate
- Example: Estimated BOD, 30 mg/L, use 10% dilution (why not 20%?)
  - *prepare 1000 mL dilute sample*
  - *0 day 1 bottle = 300 mL*
  - *3 day 2 bottles = 600 mL*
  - *there will be some spillage, make 1L diluted sample*
  - *mix 100 mL sample with 900 dilution water*

Seeding

- Provides micro-organisms to oxidise organic matter
- Not required for
  - *municipal wastes, biologically treated effluents, surface water samples*
- Some industrial wastes may require seeding
  - *exotic or difficult to degrade, high temperature, extreme pH, toxic substances*

Source of seed

- Settled sewage
- Soil culture
- Receiving water

Seed correction

- Organic matter transferred with the seed
- Seed control
- Corrected BOD value of the sample

$$BOD_{3,27}, mg.l^{-1} = \frac{(D_0 - D_T) - f \times (B_0 - B_T)}{P}$$

D = DO in diluted & seeded sample, 0 & T days

B = DO in seed control, 0 & T days

f = (% seed in diluted sample)/(% seed in seed control)

P = % mixture/100

Example

- Seed control
  - *estimated BOD 150 mg/L, dilution 2%*
  - *B_O & B_T = 7.4 & 4.4 mg/L, respectively*
- Sample
  - *estimated BOD 800 mg/L, dilution 0.5%*
  - *seed 4 mL/1000 mL or 0.4%*
  - *D_O & D_T = 7.3 & 2.3 mg/L, respectively*

Example contd.

$$P = 0.5/100 = 0.005$$

$$f = 0.4/2.0 = 0.2$$

$$BOD = \frac{(7.3 - 2.3) - 0.2(7.4 - 4.4)}{0.005}$$

$$= 880 \text{ mg/L}$$

5. Evaluation sheets

6. Handout

Recapitulate

- Biochemical oxygen demand
- Measures oxygen equivalence of aggregate organic matter

micro-organisms

Organic matter + $O_2 \Rightarrow H_2O + CO_2$

- Both oxygen and micro-organisms are necessary for the test

Necessity and procedure for

- Dilution
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- Two procedures
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100 - 350	2.0
40 - 140	5.0
20 - 70	10.0
10 - 35	20.0
4 - 14	50.0
0 - 7	100.0

Direct pipetting into 300mL bottles	
Range of BOD	**mL sample**
1,200 - 4,200	0.5
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30 - 105	20.0
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Choice of dilution

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- When in doubt, use more than one dilution
- Example: when three dilutions were used

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1	**50**	**7.5**	**2.5**	**5.0**	**10.0**
2	**20**	**7.5**	**5.2**	**2.3**	**11.5**
3	**10**	**7.7**	**6.9**	**0.8**	**80**

Reject result at 3, why?

Preparing dilution

- Incubate bottles in duplicate
- Example: Estimated BOD, 30 mg/L, use 10% dilution (why not 20%?)
  - *prepare 1000 mL dilute sample*
  - *0 day 1 bottle = 300 mL*
  - *3 day 2 bottles = 600 mL*
  - *there will be some spillage, make 1L diluted sample*
  - *mix 100 mL sample with 900 dilution water*

Seeding

- Provides micro-organisms to oxidise organic matter
- Not required for
  - *municipal wastes, biologically treated effluents, surface water samples*
- Some industrial wastes may require seeding
  - *exotic or difficult to degrade, high temperature, extreme pH, toxic substances*

Source of seed

- Settled sewage
- Soil culture
- Receiving water

Seed correction

- Organic matter transferred with the seed
- Seed control
- Corrected BOD value of the sample

$$\text{BOD}_{3,27}, \text{mg.l}^{-1} = \frac{(D_0 - D_T) - f \times (B_0 - B_T)}{P}$$

D = DO in diluted & seeded sample, 0 & T days
B = DO in seed control, 0 & T days
f = (% seed in diluted sample)/(% seed in seed control)
P = % mixture/100

Example

- Seed control
  - *estimated BOD 150 mg/L, dilution 2%*
  - *B_O & B_T = 7.4 & 4.4 mg/L, respectively*

- Sample
  - *estimated BOD 800 mg/L, dilution 0.5%*
  - *seed 4 mL/100 mL or 0.4%*
  - *D_O & D_T = 7.3 & 2.3 mg/L, respectively*

Example contd.

P = 0.5/100 = 0.005
f = 0.4/2.0 = 0.2

$$BOD = \frac{(7.3 - 2.3) - 0.2(7.4 - 4.4)}{0.005}$$

$$= 880 \text{ mg/L}$$

Add copy of Main text in chapter 8, for all participants.

7. Additional handout

These handouts are distributed during delivery and contain test questions, answers to questions, special worksheets, optional information, and other matters you would not like to be seen in the regular handouts.

It is a good practice to pre-punch these additional handouts, so the participants can easily insert them in the main handout folder.

8. Main text

Contents

Understanding dilution and seeding procedures in BOD test

1. Dilution

In the determination of BOD in the laboratory it is necessary that excess dissolved oxygen is available during the period of incubation of the sample. In case the BOD value is more than the available oxygen, the BOD reaction will come to a stop and it will not be possible to estimate the total oxygen demand during the period of incubation. Tests in which at least 1 mg/L DO remains after 3 days of incubation period and at least 2 mg/L DO is consumed give the most reliable results. This ensures that the rate of BOD exertion is not limited by the available DO concentration and the difference in the initial and final DO concentration is sufficiently large to be statistically reliable.

The solubility of atmospheric oxygen at 27 ^{o}C, the temperature of incubation of the sample, is only about 8 mg/L. Therefore, samples that consume more than 7 mg/L DO during the incubation period of 3 days will not fulfil the condition that excess DO is always present. Such samples should be diluted. This is the case with sewage and many other waste liquids. Grossly polluted surface waters may have a BOD of more than 7 mg/L. Ordinarily, though, surface water samples do not require dilution if they are saturated with DO.

Care has to be taken that the water used for dilution does not contribute any BOD. Dilution water is prepared from distilled water that has been supplemented with phosphate buffer pH 7.2, and salts containing nitrogen, magnesium, calcium and iron as nutrients to provide an environment conducive to growth of micro-organisms. It is also aerated for a significant period of time to raise the concentration of DO to near saturation value.

The degree of dilution depends upon the expected BOD value of the sample. There are two procedures for dilution:

Using percent mixtures: A 1 to 2 L graduated cylinder is half filled with the dilution water, a predetermined volume of the sample is added and the volume is made up to the desired level with more dilution water. The dilution is expressed as percent mixture given by:

Percent mixture = {(Volume of sample)/(Volume of dilute sample)} x 100

Direct pipetting: A predetermined sample volume is pipetted directly into an empty BOD bottle of known capacity, usually 300 mL. The bottle is then filled with dilution water.

Based on knowledge of the approximate BOD of the sample, the required dilution can be determined from Table 1. In case nothing is known about the sample, more than one dilution may have to be tried. An idea of the strength of the waste may be obtained from the COD value of the waste.

If D_o and D_T are DO values initially and after time T, respectively, the BOD of the sample is given by:

$$BOD = \{(D_o - D_T)/(\text{Percent mixture})\} \times 100$$

Table 1 Dilutions for samples having different BOD values.

Using separate container		*Direct pipetting into 300mL bottles*	
Range of BOD	% mixture	Range of BOD	mL Sample
1,000 - 3,500	0.2	1,200 - 4,200	0.5
400 - 1,400	0.5	600 - 2,100	1.0
200 - 700	1.0	300 - 1,050	2.0
100 - 350	2.0	120 - 420	5.0
40 - 140	5.0	60 - 210	10.0
20 - 70	10.0	30 - 105	20.0
10 - 35	20.0	12 - 42	50.0
4 - 14	50.0	6 - 21	100.0
0 - 7	100.0	0 – 7	300.0

Example 1

In a test for determination of BOD of a sample from a polluted river stretch, three dilutions were used. The observations and results of the calculations are given below.

S No	Dilution % mixture	Initial DO mg/L	Final DO mg/L	O_2 Con. mg/L	BOD mg/L
1	50	7.5	2.5	5.0	10.0
2	20	7.5	5.2	2.3	11.5
3	10	7.7	6.9	0.8	80

Accept the results at 1 and 2. Report the average value of 10.7 mg/L as BOD of the sample. Result at 3 is not acceptable since it does not meet the condition of minimum consumption of 2 mg/L.

Example 2

The BOD of a sample is estimated to be 30 mg/L. What dilution would you recommend? Calculate the quantities of the sample and the dilution water that should be mixed for the suggested dilution to conduct the test.

From Table 1, two dilutions giving 10 or 20 % mixture seem to be suitable. However, if 20% dilution is used and the BOD turns out to be around 35 mg/L, the condition of the minimum DO of 1 mg/L at the end of the incubation period may not be met. Therefore adopt a 10% dilution.

It is advisable to incubate the diluted sample in duplicate. Therefore, for 3 BOD bottles (one to be titrated initially and the remaining two at the end of the incubation period) each of 300 mL capacity, a total of 900 mL diluted sample will be needed. Since some amount may be wasted, prepare 1000mL of dilution water. Therefore mix 100 mL of sample with 900 mL dilution water.

2. Seeding

The purpose of seeding is to introduce a biological population capable of oxidising the organic matter in the sample. Seeding would not be necessary for domestic and municipal sewage, unchlorinated treated effluents and surface waters. When there is a reason to believe that the sample contains very few micro-organisms, for example as a result of chlorination, high temperature, extreme pH or because of the specific composition of some industrial wastes, the dilution water should be seeded.

Most often, the supernatant of fresh, settled sewage may be used as a seed. In cases where the sample may contain organic matter, which is hard to degrade, the seed may be developed by adding a small amount of soil to a portion of the sample and aerating it for 24 to 48 hours. Soil is a medium that supports a wide variety of micro-organisms capable of metabolising many different types of organic matter. Alternatively, water from a body of water receiving the waste may be used as a source of seed. A small volume of seed added to the dilution water, 4 – 6 mL per litre, would contain a sufficient number of micro-organisms adapted to the waste to carry out the oxidation of the organic matter.

Correction must be carried out to account for the oxygen consumed in oxidation of organic matter carried with the seed. The volume of seed added to the dilution water should be recorded and parallel seed control test should be run to determine the BOD of the seed.

If the seeding and dilution methods are combined, the following general formula is used to calculate the BOD:

$$BOD_{3,27}, mg.l^{-1} = \frac{(D_0 - D_T) - f \times (B_0 - B_T)}{P}$$

where:

D_0	=	DO of diluted sample initially, mg/L
D_T	=	DO of diluted sample after 3 day incubation at 27°C, mg/L
P	=	decimal volumetric fraction of sample used (% mixture/100)
B_0	=	DO of seed control initially, mg/L
B_T	=	DO of seed control after incubation, mg/L
f	=	ratio of %seed in diluted sample to %seed in seed control

Example 3

A seeded BOD test was conducted on an industrial waste, estimated BOD 800 mg/L, using fresh settled sewage as the source for seed micro-organisms. The BOD of the seed was estimated as 150 mg/L.

Seed control
Dilution used from Table 1 = 2%
Initial DO = 7.4 mg/L
Final DO = 4.4 mg/L
Sample
Dilution used from Table 1 = 0.5%
Amount of seed used for seeding the dilution water for the sample = 4 mL/L or 0.4%
Initial DO = 7.3 mg/L
Final DO = 2.3
Calculation
P = 0.5/100 = 0.005

f = 0.4/2.0 = 0.2

$$BOD = \frac{(7.3 - 2.3) - 0.2(7.4 - 4.4)}{0.005} = 880 \text{ mg/L}$$

3. Problems

1. Explain how a proper seed might be obtained in order to determine the BOD of an industrial waste that is not readily oxidised biologically.
2. Calculate the BOD_3 at 27°C of a wastewater sample for the following data:
   Bottle capacity = 300 mL
   Volume of waste added to the bottle = 5 mL
   Initial DO in bottle containing waste and dilution water = 7.8 mg/L
   DO in bottle containing waste and dilution water after 3 days = 4.2 mg/L
3. Calculate the BOD_3 at 27°C of a wastewater sample for the following data:
   Bottle capacity = 300 mL

<u>seed control</u>

   Volume of seed added to the bottle = 6 mL
   Initial DO in bottle containing seed and dilution water = 8.20 mg/L
   DO in bottle containing seed and dilution water after incubation = 4.80 mg/L

<u>sample</u>

   Volume of seed added to bottle = 2 mL
   Volume of sample added to bottle = 30 mL
   Initial DO in bottle containing seed, sample and dilution water = 8.10 mg/L
   DO in bottle containing seed, sample and dilution water after incubation = 3.20 mg/L