IS 3025-44 (1993): Methods of Sampling and Test (physical and chemical) for Water and Wastewater, Part 44: Biochemical Oxygen Demand (BOD) [CHD 32: Environmental Protection and Waste Management]

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IS 3025 (Part 44) : 1993
(Reaffirmed 2009)

भारतीय मानक

जल और अपशिष्ट जल नमूने लेने एवं परीक्षण (भौतिक एवं रसायन) की पद्धतियाँ

भाग 44 जैवरसायनिक आक्सीजन माँग

(पहला पुनरीक्षण)

Indian Standard

METHODS OF SAMPLING AND TEST (PHYSICAL AND CHEMICAL) FOR WATER AND WASTEWATER

PART 44 BIOCHEMICAL OXYGEN DEMAND (BOD)

(First Revision)

Fifth Reprint SEPTEMBFR 2007
(Including Amendment No 1)

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BUREAU OF INDIAN STANDARDS
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NEW DELHI 110002

March 1993

Price Group 2

FOREWORD

This Indian Standard (First Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Environmental Protection Sectional Committee had been approved by the Chemical Division Council.

Biochemical Oxygen Demand (BOD) is the most important parameter to determine the degree of pollution in lakes and streams at any time and their self purification capacities, assess the biodegradable organic load of the wastewaters for design of wastewater management system and thereafter to evaluate the efficiency of the same. As per definition BOD of a sample is defined as the amount of oxygen required by the micro-organisms to oxidise the organic matter by aerobic microbial decomposition to stable inorganic forms at some standard time and temperature. As per recommendations of the Royal Commission of Great Britain standard conditions are laid down as 20°C and 5 days. The standard temperature of 20°C is based on the average aquatic temperature of Great Britain and 5 days incubation period with an assumption that most of the carbonacious organic demand is satisfied during this period. The BOD test is being carried out with these standard conditions for nearly 3 decades throughout the world and our country is no exception.

However, it is felt that 20°C is not a universal average temperature and particularly for a tropical country like India where the temperatures of surface water in rivers, lakes, etc, vary from 20 to 35°C in different seasons and in different parts of the country. The average aquatic temperature in our country is around 27°C. Hence, to be more realistic to the Indian aquatic environment the technical committee responsible for formulation of this standard felt necessary to establish a higher temperature and thereby lower incubation period which would yield BOD values comparable to the standard conditions of 20°C and 5 days.

Biochemical oxygen demand (BOD) test uses standard laboratory procedures to determine the relative oxygen requirements of waters, wastewaters, effluents, etc. There are a number of variations to the oxygen demand test prescribed in this standard. These include using shorter or longer incubation periods, higher temperatures, etc.

In the perparation of this standard, considerable assistance has been derived from Standard Methods for Examination of Water and Wastewater published by American Public Health Association, Washington, USA, 16th edition. This standard supersedes Clause **12** of IS 2488 (Part 1) : 1966 Methods of sampling and test for industrial effluents, Part 1 and Clause **53** of IS 3025 : 1964 Methods of sampling and test (physical and chemical) for water used in industry.

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

AMENDMENT NO. 1 OCTOBER 2000
TO
IS 3025 (PART 44) : 1993 METHODS OF SAMPLING AND TEST (PHYSICAL AND CHEMICAL) FOR WATER AND WASTEWATER

PART 44 BIOCHEMICAL OXYGEN DEMAND (BOD)

(First Revision)

(*Page* 2, *clause* **8.1**, *lines* 11 *and* 12) — Substitute the following for the existing:

'Add 2 to 5 ml of treated sewage per litre of dilution water or use commercially available microbial seed mixture, as per manufacturer's direction, for seeding purposes.'

(*Page* 3, *clause* **10.2**, *title*) — Substitute 'When Dilution Water is not Seeded' for the existing title.

(*Page* 3, *clause* **10.3**, *lines* 11 to 16) — Substitute the following for the existing:

'f = ratio of seed in diluted sample to seed in control; [volume (ml) of seed in diluted sample/volume of seed in seed control],

P = sample volume (in ml) diluted to 1 litre with dilution water.'

(CHD 12)

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Indian Standard

METHODS OF SAMPLING AND TEST (PHYSICAL AND CHEMICAL) FOR WATER AND WASTEWATER

PART 44 BIOCHEMICAL OXYGEN DEMAND (BOD)

(First Revision)

1 SCOPE

This standard prescribes oxygen depletion method based on bio-assay procedure for measurement of biochemical oxygen demand.

2 REFERENCES

The following Indian Standards are necessary adjuncts to this standard:

IS No.	*Title*
3025 (Part 1) : 1986	Methods of sampling and test (physical and chemical) for water and wastewater: Part 1 Sampling (*first revision*)
3025 (Part 38) : 1989	Methods of sampling and test (physical and chemical) for water and wastewater: Part 38 Dissolved oxygen
7022 (Part 1) : 1973	Glossary of terms relating to water, sewage and industrial effluents, Part 1
7022 (Part 2) : 1979	Glossary of terms relating to water, sewage and industrial effluents, Part 2

3 TERMINOLOGY

For the purpose of this standard, definitions given in IS 7022 (Part 1) : 1973 and IS 7022 (Part 2) : 1979 shall apply.

4 PRINCIPLE

The biochemical oxygen demand (BOD) test is based on mainly bio-assay procedure which measures the dissolved oxygen consumed by micro-organisms while assimilating and oxidizing the organic matter under aerobic conditions.

The standard test condition includes incubating the sample in an air tight bottle, in dark at a specified temperature for specific time.

5 SAMPLING AND PRESERVATION

Sampling and sample preservation shall be done as prescribed in IS 3025 (Part 1) : 1986.

6 APPARATITS

6.1 Incubation Bottles

300 ml capacity narrow neck special BOD bottles with planed mouth with ground glass stoppers. New bottles should be cleaned with 5 N hydrochloric acid or sulphuric acid followed by rinsing with distilled water. In normal use, bottles once used for Winklers procedure should only be rinsed with tap water followed by distilled water.

During incubation (if incubator is used) to ensure proper sealing, time to time, add water to the flared mouth of the bottle.

6.2 Water Bath or Air Incubator

Air incubation with thermostatically controlled 27°C ± 1°C. Avoid light to prevent possibility of photosynthetic production of oxygen.

NOTE — Thermostatically controlled at 27°C ± 1°C water bath with continuous stirring may be preferred.

7 REAGENTS

7.1 Phosphate Buffer Solution

Dissolve 8.5 g potassium dihydrogen phosphate ($KH_2 PO_4$), 21.75 g potassium hydrogen phosphate (K_2HPO_4), 33.4 g disodium hydrogen phosphate ($Na_2HPO_4.7H_2O$) and 1.7 g ammonium chloride (NH_4C1) in about 500 ml distilled water and dilute to 1 litre. *p*H of the solution should be around 7.2 without any further adjustment.

7.2 Magnesium Sulphate Solution

Dissolve 22.5 g magnesium sulphate ($MgSO_4.7H_2O$) in distilled water and dilute to 1 litre.

7.3 Calcium Chloride Solution

Dissolve 27.5 g calcium chloride in distilled water and dilute to 1 litre.

7.4 Ferric Chloride Solution

Dissolve 0.25 g hydrated ferric chloride ($FeCl_3.6H_2O$) in distilled water and dilute to 1 litre.

7.5 Acid and Alkali Solution

1 N sodium hydroxide and 1 N sulphuric acid for neutralization of samples.

NOTE – Any of the above solutions showing any sign of biological growth may be discarded.

7.6 Glucose-Glutamic Acid Solution

Dry reagent grade glucose and reagent grade glutamic acid at 103°C for 1 hour. Add 150 mg of glucose and 150 mg of glutamic acid to distilled water and dilute to 1 litre. Prepare fresh immediately before use.

7.7 Other Reagents for Dissolved Oxygen Measurement

7.7.1 *Manganous Sulphate Solution*

Dissolve manganese sulphate (480 g of $MnSO_4.4H_2O$ or 400 g of $MnSO_4.2H_2O$ or 364 g of $MnSO_4.H_2O$) in freshly boiled and cooled water, filter and make up to 1 000 ml. The solution should not give blue colour by addition of acidified potassium iodide solution and starch.

7.7.2 *Alkaline Iodide Solution*

Dissolve 500 g of sodium hydroxide (or 700 g of potassium hydroxide) and 135 g of sodium iodide (or 150 g of potassium iodide) in freshly boiled and cooled water and dilute to 1 litre.

7.7.3 *Sulphuric Acid, Concentrated*

7.7.4 *Starch Indicator*

Dissolve 2 g of starch and 0.2 g of salicylic acid as preservative, in 100 ml of hot distilled water.

7.7.5 *Sodium Thiosulphate Stock Solution*

Dissolve approximately 25 g of sodium thiosulphate ($Na_2S_2O_3.5H_2O$) in boiled distilled water and make up to 1000 ml. Add 1 g of sodium hydroxide to preserve it.

7.7.6 *Standard Sodium Thiosulphate Solution*

Dissolve 250 ml of stock solution (**7.7.5**) in boiled distilled water and make up to 1 litre and standardize sodium thiosulphate against known standard before use.

8 PROCEDURE

8.1 Preparation of Dilution Water

Aerate the required volume of distilled water in a container by bubbling compressed air for 8 to 12 hours to attain dissolved oxygen saturation. Let it stabilize for 4 h at room temperature (around 27°C).

At the time of use, add 1 ml each of phosphate buffer, magnesium sulphate, calcium chloride and ferric chloride for each litre of dilution water.

Add 2 to 5 ml of treated sewage per litre of dilution water for seeding purpose.

NOTE — Seeding is not required for domestic sewage or surface water samples.

8.2 Dilution of Sample and Incubation

8.2.1 *Neutralization*

Neutralize the sample to *p*H around 7.0 using alkali or acid of such strength that the quantity of reagent does not dilute the sample by more than 0.5 percent.

9 PROCEDURE

9.1 Sample Volume and Dilution Techniques

On the basis of chemical oxygen demand (COD), determine expected BOD. Use the following formula for calculating sample volume:

$$\text{Sample volume in ml, per litre dilution} = \frac{X}{\text{expected BOD}} \times 1\,000$$

For keeping 2 dilutions take X = 2.5 and 4.0
For single dilution take X = 3.0 or 3.5.

Round off to nearest convenient volume fraction.

In case of high BOD samples, prepare primary dilutions with distilled water and then make the final dilution.

9.2 Take requisite quantity of sample in one litre volumetric flask. Dilute to the mark with the dilution water by siphoning from the container (**8.1**). Mix well. Rinse three BOD bottles with the diluted sample and fill up these bottles with the diluted sample. Stopper the bottles immediately after removing the air bubbles.

Samples of natural surface water bodies like river, lake and marine, generally do not require seeding and dilution due to naturally available microbiological population and low BOD values. For such samples which are likely

to have BOD less than 5 mg/l, BOD determination may be carried out as such (100 percent) without any dilution.

9.3 Determination of Initial Dissolved Oxygen (DO)

Determine initial DO for one bottle and keep two bottles for incubation at 27°C ± 1°C for 3 days. Prepare six blanks by siphoning out dilution water directly into the bottles. Determine initial DO in two bottles and incubate remaining four bottles at 27°C ± l°C for 3 days.

NOTE — DO shall be determined as per IS 3025 (Part 38) : 1989.

9.4 Determination of Final DO

After 3 days incubation at 27°C ± 1°C, determine final DO in incubated bottles.

NOTE — DO shall be determined as per IS 3025 (Part 38) : 1989.

10 CALCULATION

10.1 When Sample is Undiluted

BOD, mg/l = DO before incubation - DO after incubation.

10.2 When Dilution Water is Seeded:

$$\text{BOD, mg/1} = \frac{D_1 - D_2}{P} \times 1\,000$$

10.3 When Dilution Water is Seeded:

$$\text{BOD,mg/l} = \frac{D_1 - D_2 - (B_1 - B_2)f}{P} \times 1\,000$$

where

D_1 = Initial DO of sample in mg/l,

D_2 = DO of sample after incubation in mg/l,

B_1 = DO of seed control before incubation in mg/1,

B_2 = DO of seed control after incubation in mg/1,

f = ratio of seed in diluted sample to seed in control; (percent seed in diluted sample) : (percent seed in seed control),

P = percentage dilution of sample (sample volume in ml/10).

NOTE — f may be used only when seed correction is to be applied.

11 GLUCOSE GLUTAMIC ACID CHECK

BOD being a bioassay test, is greatly influenced by factors like toxicants, poor seeding, etc. For periodical checking of these factors, use a mixture of 150 mg glucose and 150 mg glutamic acid per litre as a standard check solution. Determine the 3 days 27°C BOD of 2 percent dilution of the glucose-glutamic acid standard check soution as in **9**. If the BOD value of the check is outside the range of 200 ± 37 mg/l, reject any BOD determinations made with the seed and dilute water and seek the cause of the problem.

12 EXPRESSION OF RESULTS

BOD is expressed as mg/1, 3 days at 27°C as given in **10** and round off the values as follows:

i) 0 to 10 up to first decimal

ii) Above 10 — whole number.

Bureau of Indian Standards

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