Name of the project:	**Digitization of chemistry experiments to improve the quality and support chemistry teaching in secondary schools**
Acronym:	**ChemIQSoc**
Project number:	**2021-1-SK01-KA220-VET-000027995**

Co-funded by the Erasmus+ Programme of the European Union

Tittle: Determination of biochemical oxygen demand (BOD_5)

Work instructions

Task: Determinate the BOD_5 in wastewater.

Theory

Biochemical oxygen demand after *n* days (BOD_n) is the mass concentration of dissolved oxygen consumed under specified conditions of biochemical oxidation of organic and inorganic substances in water.

Value *n* represents the incubation time equal to 5 or 7 days.

The water sample is adjusted and diluted with various amounts of dilution water with a high amount of dissolved oxygen and with inoculating aerobic microorganisms, with suppression of nitrification. Incubation takes place at a temperature of 20°C for a defined time of 5 or 7 days, in the dark, in a filled closed container.

The concentration of dissolved oxygen before and after incubation is determined. The difference is used to calculate the amount of oxygen consumed per litre of sample. For expected low values up to 6 mg/l of oxygen, the sample is not diluted. The range for performing the test according to this document is in the interval: (3-3,500) mg/l with an expanded uncertainty U ($k = 2$) 5.5%.

Equipment:

Incubation containers

BOD containers, with lids–jars with a volume from 250 ml to 300 ml or from 100 ml to 125 ml are preferred, with lids, it is preferable to use containers with straight walls or other equivalent flasks.

Incubator

Capable of maintaining a temperature of 20°C ± 1°C.

Device for determining the concentration of dissolved oxygen

Cooling devices

From 0°C to 4°C, for transport and storage of the sample.

Dilution container

Sealable glass bottle with a capacity dependent on the volume of the diluted sample, equipped with a scale divided between 2.5 ml and 10 ml, or another suitable container in which dilution can be carried out.

Aeration device

Bottle with compressed air or compressor. The quality of the air must be such that aeration does not cause contamination, especially by adding organic substances, oxidizing, or reducing materials or metals. If there is suspicion of contamination during aeration, the air must be filtered and rinsed.

Chemicals:

- inoculation water (river water containing municipal wastewater),
- phosphate buffer solution (pH 7.2),
- magnesium sulfate heptahydrate (solution 22.5 g/l),
- calcium chloride (solution 27.5 g/l),
- iron chloride hexahydrate (solution 0.25 g/l),
- dilution water,
- inoculated dilution water,
- hydrochloric acid or sulfuric acid (solution $c(HCl) \approx 0.50$ mol/l, $c(H_2SO_4) \approx 0.25$ mol/l, or as appropriate),
- sodium hydroxide (solution, approximately 20 g/l or as appropriate),
- sodium sulfite (solution, approximately 50 g/l or as appropriate),
- glucose–glutamic acid, control solution,
- allylthiourea (ATM) (solution, 1.0 g/l).

Procedures:

Preparation of dilution water

1. Add 1 ml of solutions to approximately 500 ml of distilled water: phosphate buffer solution (pH 7.2), magnesium sulfate, calcium chloride and iron chloride (according to KR). The solution is made up to a volume of 1000 ml and stirred.
2. The solution is kept at 20°C ± 2°C; it is aerated for at least 1 hour using a suitable device to achieve a concentration of dissolved oxygen of at least 8 mg/l.
3. The water must not be supersaturated with oxygen, before use it is left to stand for 1 hour in an open container.

Inoculated dilution water

1. Depending on the origin of the inoculation water, add 5-20 ml of inoculation water per litre of dilution water. The resulting inoculated water is stored at 20°C and is always prepared just before use.

Sample preparation

1. If necessary, the sample is neutralized by adding a solution of HCl ($c \approx 0.5$ mol/l) or NaOH ($c \approx 20$g/l) to a pH between 6 and 8.
2. Free and chemically bound chlorine is removed by adding the necessary volume of sodium sulfite solution (c ≈ 50 g/l) so that it is not in excess.
3. Samples containing algae are filtered through a suitable filter (e.g. 1.6 µm). Filtration can significantly affect the final BOD values and is only used when it is considered necessary for water quality assessment. If the sample contains large particles and a large dilution factor is required, the sample must be homogenized.

Preparation of test solutions

1. The sample is heated to 20°C ± 2°C and shaken in a half-filled container to eliminate possible supersaturation of the solution with oxygen. A known volume of the sample is poured into the dilution container, add 2 ml of allylthiourea solution (c = 1.0 g/l) per litre of diluted sample and the container is filled up to the mark with inoculated dilution water.
2. Stir gently to prevent trapping air bubbles.
3. The blank is carried out in parallel with the determination, using inoculated dilution water with 2 ml of allylthiourea solution. The consumption for the blank should not exceed 1.5 mg/l of dissolved oxygen.

Determination of dissolved oxygen by electrochemical method

1. Each diluted sample is poured into an incubation container so that the liquid slightly overflows. During filling, changes in the amount of oxygen in the liquid must be prevented. Adhesively held air bubbles on the walls of the jar are allowed to escape.
2. The concentration of dissolved oxygen at zero time in each container is determined by immersing an oximeter probe. The containers are closed so that no trapped air bubbles remain inside.
3. Containers with diluted test solutions are placed into an incubator and left in the dark for n days ± 4 h. After this time, the concentration of oxygen is determined again.

Management of chemical substances

Chemicals	**Form**	**H-statements**	**P-statements**
Phosphate buffer solution (pH 7.2)	Liquid	---	---
$MgSO_4 \cdot 7H_2O$	Solid	---	---
$CaCl_2$	Solid	H319	P301, P312, P280, P305, P351, P338

Chemicals	Form	H-statements	P-statements
HCl	Liquid	H314, H335	P261, P280, P305, P351, P338, P304, P340, P310
H_2SO_4	Liquid	H314	P280, P305, P351, P338, P301, P330, P331, P310
NaOH	Solid	H314, H290	P280, P310, P305, P351, P338
Na_2SO_3	Solid	H319	P305, P351, P338
Glucose	Solid	---	---
Allylthiourea	Solid	H301	P301 + P310

Sources of risk and assessment of risk severity

There is no risk when following all the principles for working with chemicals and using personal protective equipment (gloves, goggles, lab coat).

Waste management method

Chemicals are disposed of in designated collection containers.

Risk reduction measures

Use of personal protective equipment (goggles, gloves, lab coat).

References

1. Horáková, M.: *Analytika vody*. VŠCHT, Praha, 2000, 283 s.
2. Ilavský J.: *Chémia vody – Laboratórne cvičenia*. STU, Bratislava, 2015, 189 s.

Worksheet

Experimental data

1. Study the workflow in detail and record the most important working parameters of the method.

2. Record the measurement results.

Sample number	1	2	3	Blank 1
$c(O_2)$ v mg/l in zero time				
$c(O_2)$ v mg/l after 5 days				

Calculations

1. BOD_5 in milligrams of oxygen per litre, with dilution, is calculated according to the Eq:

$$\boldsymbol{BOD_5 = \left[(c_1 - c_2) - \frac{V_t - V_e}{V_t} \cdot (c_3 - c_4)\right] \cdot \frac{V_t}{V_e}}$$

c_1 is the dissolved oxygen concentration in one of the test solutions at time zero in mg/l
c_2 is the dissolved oxygen concentration in the same solution after n days in mg/l
c_3 is the dissolved oxygen concentration in the blank solution at time zero in mg/l
c_4 is the dissolved oxygen concentration in the blank solution after n days in mg/l
V_e is sample volume used for preparation of test solution in ml
V_t is total volume of test solution in ml

BOD_5 in milligrams of oxygen per litre, without dilution, is calculated according to the Eq:

$$\boldsymbol{BOD_5 = c_1 - c_2}$$

c_1 is the dissolved oxygen concentration in one of the test solutions at time zero in mg/l
c_2 is the dissolved oxygen concentration in the same solution after n days in mg/l

Results are given in milligrams per litre. Results lower than 10 mg/l shall be rounded to the nearest mg/l, results between 10 mg/l oxygen and 1 000 mg/l oxygen shall be given to two significant figures. Results above 1 000 mg/l shall be given to three significant figures.

2. Indicate the BOD values

Sample number	1	2	3	Blank 1
BOD_5				

Questions

1. 1. What is the BOD_5 value.

2. Why is concentration of O_2 value an important indicator of water purity or pollution?

3. On what principle the oximeter works?

4. Indicate the sources of error in the determination of BOD_5. Suggest possible solutions.

Conclusion

Briefly summarize the objective of the experiment, the main results and compare them with the expected values.

Disclaimer