REF 985 822

Test 8-22 09.13

NANOCOLOR® BOD_5

en

Method:
Tube test for the determination of the biochemical oxygen demand in 5 days (BOD_5) by using the diluting principle according to the German Standard Method DIN 38409-H51. The incubation of the samples is carried out in Winkler oxygen flasks. The determination of oxygen dissolved in water at day 0 and after 5 days is carried out similarly to the Winkler Method DIN EN 25813-G21 by photometric evaluation of iodine-color.

Range:	**2–3000 mg/L O_2**	
Factor:	**007.0**	**007.6**
Wavelength (HW = 5–12 nm):	**436 nm**	**445 nm**
Reaction time:	**5 days**	
Reaction temperature:	**20 ± 1 °C**	

Contents of reagent set:
3 empty test tubes
15 mL BOD_5 R1
15 mL BOD_5 R2
30 mL BOD_5 R3

Hazard warning:
Reagent R1 contains manganese(II) chloride 25–83 %, reagent R2 contains sodium hydroxide solution 20–55 %, reagent R3 contains sulfuric acid 51–80 %.
H314 Causes severe skin burns and eye damage.
P260, P280, P301+330+331, P303+361+353, P304+340, P305+351+338 Do not breathe vapors. Wear protective gloves / eye protection. IF SWALLOWED: rinse mouth. Do NOT induce vomiting. IF ON SKIN (or hair): Remove / Take off immediately all contaminated clothing. Rinse skin with water / shower. IF INHALED: Remove to fresh air and keep at rest in a position comfortable for breathing. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. For further information ask for a safety data sheet.

Interferences:
Changes in pH-value, accumulation of special microbial metabolites and compounds, which are toxic to microorganisms (e. g. mycotoxines, free chlorine, heavy metals) can cause a decrease of substrate metabolism and a reduction of the oxygen consumption. Iron(II) salts, sulfur dioxide and sulfur hydrogen consume oxygen and falsify the BOD_5-results, meaning they cause false negative results, also. If algae or nitrified microorganisms are present, increased results could occur.

Sample preparation:
At the beginning, the sample is adjusted to room temperature. Then the pH-value is checked. The pH-value of the sample should be between pH 6 and 8, and has to be adjusted, if necessary. If, in this case, a precipitate has been developed, the sample should be homogenized very well or filtrated (membrane filtration kit, REF 916 511). In case of samples containing algae, filtration may also be necessary in order to avoid exaggerated results. Remove free and / or bounded chlorine by addition of sodium sulfite.
Remark: Store the sample in a tightly closed bottle full to the brim at a temperature of 0–4 °C immediately after taking the sample until carrying out the analysis. Start the BOD_5 determination as soon as possible or within 24 hours of taking the sample. Samples may also be frozen to keep longer. Homogenise frozen samples after thawing and always use inoculated BOD_5-Nutrient Solution (see BOD_5 Nutrient Mixture, REF 918 994, or BOD_5-Nutrient Mixture PLUS, REF 918 995).

Diluting water, BOD_5-Nutrient Solutions and inoculating water:
Details on the preparation and handling of diluting water are given in the BOD_5-Accessories Set (REF 916 918). For the use and application of BOD_5-Nutrient Solutions and inoculating water, refer to the instructions for reagent sets BOD_5-Nutrient Mixture (REF 918 994) or BOD_5-Nutrient Mixture *PLUS* (REF 918 995). Make sure to observe the data specified there.

Determination of BOD_5:
Requisite accessories: BOD_5-Accessories Set (REF 916 918), BOD_5-Nutrient Mixture (REF 918 992) or BOD_5-Nutrient Mixture *PLUS* (REF 918 995), graduated cylinders (volume 100 mL and 500 mL), piston pipettes with tips, equipment for incubation with thermostat for 20 ± 1 °C (e. g. water bath or incubator) or as an alternative a dark room with a room temperature of about 20 °C

Step 1: Control (oxygen consumption of the diluting water)

Fill in a 1 L laboratory flask (BOD_5-Accessories Set, REF 916 918)
500 mL aerated diluting water and
2.5 mL nutrient solution (1.25 mL R1 + 1.25 mL R2 from reagent set BOD_5-Nutrient Mixture, REF 918 994, or BOD_5-Nutrient Mixture *PLUS*, REF 918 995), close the vessel and mix to enrich the oxygen content by shaking vigorously for a few seconds (**Control**).

Open
1 Winkler oxygen flask and
1 test tube, wash both with several milliliters of the control and fill to the brim without letting air bubbles in.

Close the Winkler oxygen flask, **without** letting **air bubbles** in, by slowly pressing in the obliquely cut glass stopper and incubate in a water bath or an incubator for **5 days** at **20 ± 1 °C** in the dark.

Close the test tube **without** letting **air bubbles** in and immediately start the measurement of dissolved oxygen according to **step 3**.

Step 2: Sample dilutions

*Depending on the expected BOD_5 of a sample, the most suitable dilution in accordance to the following table must be prepared in a 1 L-laboratory flask (BOD_5-Accessories Set, REF 916 918). If there is no experience regarding the expected BOD_5 of a sample, at least two, preferably three, different dilutions of this sample should be prepared to assure accuracy of the determination. For more reliable results, we recommend **duplicate determinations**.*

Expected BOD_5 [mg/L O_2]	Dilution	Examples for typical waters	Sample [mL]	Aerated diluting water [mL]	Nutrient Solution* [mL] R1	R2
< 5	–	R	500	0	1.25	1.25
4–12	1 + 1	R, B	250	250	1.25	1.25
10–30	1 + 4	R, B	100	400	1.25	1.25
20–60	1 + 9	B	50	450	1.25	1.25
40–120	1 + 19	C	25	475	1.25	1.25
100–300	1 + 49	C, M	10	490	1.25	1.25
200–600	1 + 99	C, M	5	495	1.25	1.25
400–1200	1 + 199	M, I	2	398	1.0	1.0
800–2400	1 + 399	I	1	399	1.0	1.0
1000–3000	1 + 499	I	1	499	1.25	1.25

* BSB_5-Nutrient Mixture (REF 918 994) or BOD_5-Nutrient Mixture *PLUS* (REF 918 995)
R: River water
B: Biologically suitable biomass from a sewage plant
C: Clarified biomass from a sewage plant or mildly polluted industrial waste water
M: Raw municipal sewage
I: Heavily polluted industrial waste water

After preparation of the sample dilution based on the above table, close the laboratory flask and mix to enrich the oxygen content by shaking vigorously for a few seconds.

Open
1 Winkler oxygen flask and
1 test tube, wash both with some milliliters of the sample dilution and fill to the brim without letting air bubbles in.

Close the Winkler oxygen flask, **without** letting **air bubbles** in, by slowly pressing in the obliquely cut glass stopper and incubate in a water bath or in an incubator for **5 days at 20 ± 1 °C** in the dark.

Close the test tube **without** letting **air bubbles** in and immediately start the measurement of dissolved oxygen according to **step 3.**

Proceed in the same way for all other samples or sample dilutions.

Remark: The added laboratory flask in the BOD_5-Accessories Set can be used for all preparations of any water samples to be tested (control, sample dilutions). Before using, the flask must be washed thoroughly by using tap water, after every preparation and before every new preparation, respectively.

Step 3: Measurement of dissolved oxygen

Preliminary remarks:
The added test tubes in the reagent set NANOCOLOR® BOD_5 can be used for all measurements of dissolved oxygen. Before using for a new determination of dissolved oxygen the test tube is directly be emptied down the drain and thoroughly washed with tap water. Additional empty test tubes (REF 916 80) can be ordered at MACHEREY-NAGEL.

Measurement of dissolved oxygen on day 0: The measurement of dissolved oxygen in test tubes filled at the beginning of the test (day 0) has to be started immediately.

Measurement of dissolved oxygen on day 5: The measurement of the concentration of dissolved oxygen in the incubated Winkler flasks after 5 days of incubation starts with the filling of one empty test tube (for double determinations two empty test tubes) to the brim, with the water sample to be tested (control and sample dilutions). After the filling, the test tubes are carefully closed without letting air bubbles in, and the determination of dissolved oxygen is carried out as in the following chapter "Procedure" described.

Procedure:

Open test tube, filled with control or sample dilution, add
2 drops BOD_5 R1,
2 drops BOD_5 R2, close **without** letting **air bubbles** in and shake.
Wait **2 min**.

Open test tube, add
5 drops BOD_5 R3, close **without** letting **air bubbles** in and shake to dissolve the flakes.
Clean outside of test tube and perform measurement.

Measurement:
For *NANOCOLOR*® photometers and PF-11 / PF-12 see manual, test 8-22.

Photometers of other manufacturers:
For other photometers check whether measurement of round glass tubes is possible. Verify factor for each type of instrument by measuring standard solutions.

Step 4: Evaluation

Important:
The BOD_5 value is only calculated for samples in which the residual oxygen concentration after 5 days incubation still amounts to at least 2 mg/L O_2 and where the oxygen consumption lies between 2 and 6 mg/L O_2.

Oxygen consumption of the diluting water O_C (control):

$O_C = O_{C0} - O_{C5}$

O_{C0} = oxygen concentration in the control at the beginning of the test (day 0)
O_{C5} = oxygen concentration in the control at the end of the test (day 5)

Oxygen consumption of the sample O_S (sample dilution):

$O_S = O_{S0} - O_{S5}$

O_{S0} = oxygen concentration in the sample dilution at the beginning of the test (day 0)
O_{S5} = oxygen concentration in the sample dilution at the end of the test (day 5)

Calculation of BOD_5:
$BOD_5 = D \times (O_S - O_C) + O_C$

D = reciprocal value of the sample dilution
(e. g. sample dilution 1+199 ---> V = 200

Presentation of the results:
The BOD_5 is given in mg/L O_2 and noted as follows:

< 10 mg/L O_2	rounded up to mg/L (e.g. 6.7 mg/L O_2 is rounded up to 7 mg/L O_2)
10–1000 mg/L O_2	reported with two significant digits (e. g. 314 mg/L O_2 is reported as 310 mg/L O_2)
> 1000 mg/L O_2	reported with three significant digits (e. g. 1578 mg/L O_2 is reported as 1580 mg/L O_2)

Analytical Quality Control:
NANOCONTROL BOD_5 (REF 925 82)

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Table for evaluation for *NANOCOLOR*® BOD_5 - Test 8-22

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Sample: _____ Date: _____

COD [mg/L O_2]: _____ pH-value: _____

total Phosphate [mg/L P]: _____ Ammonia-N [mg/L NH_4-N]: _____

Nitrite-N [mg/L NO_2-N]: _____ Nitrate-N [mg/L NO_3-N]: _____

Results of the determination of BOD_5:

Date of test beginning (day 0): _____ Date of test end (day 5): _____

Control:	O_{C0} [mg/L O_2]	O_{C5} [mg/L O_2]	O_C [mg/L O_2] = (O_{C0} - O_{C5})

Sample dilutions:	Dilution D	O_{S0} [mg/L O_2]	O_{S5} [mg/L O_2]	O_S [mg/L O_2] = (O_{S0} - O_{S5})	BOD_5 [mg/L O_2] = [D x (O_S - O_C) + O_C]

Ø BOD_5 [mg/L O_2]: _____

O_C = Oxygen consumption of the control after an incubation period of 5 days
O_{C0} = Oxygen concentration in the control at the beginning of the test (day 0)
O_{C5} = Oxygen concentration in the control at the end of the test (day 5)
D = Reciprocal value of the sample dilution (e. g. sample dilution 1+199 --> V = 200)

O_S = Oxygen consumption of the sample dilution after an incubation period of 5 days
O_{S0} = Oxygen concentration in the sample dilution at the beginning of the test (day 0)
O_{S5} = Oxygen concentration in the sample dilution at the end of the test (day 5)

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