

Procedure with Winkler bottles

Method: Determination of the biochemical oxygen demand in 5 days (BOD₅) by using the dilution principle according to the German Standard Method DIN EN 1899-1-H51. The incubation of the samples is carried out in Winkler oxygen flasks. Determination of the dissolved oxygen is carried out similarly to the Winkler method (DIN EN 25813-G21) by photometric evaluation of the iodine colour.

Range: 2 – 3000 mg/l O₂ Method
8221

NANOCOLOR® reagent set: BOD₅ (REF 985 822)
436 nm

Wavelength: 436 nm

Requ. accessories: BOD₅ accessories set (REF 916 918), BOD₅ nutrient mixture (REF 918 994) or BOD₅ nutrient mixture PLUS (REF 918 995), piston pipettes with disposable tips, graduated cylinders (volumes 100 ml and 500 ml), water bath or incubator (alternative: a dark room with a temperature of about 20 °C)

Procedure:

Preparatory steps
<p>1. Preparation of samples First, 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 is formed, the sample should be homogenised very thoroughly.</p>
<p>2. Diluting water, BOD₅ nutrient mixtures and inoculating water The instructions supplied with the BOD₅ accessories set (REF 916 918) contain full details about the production and handling of the diluting water. Instructions for preparation and use of BOD₅ nutrient mixtures and inoculating water see leaflets of the test kits BOD₅ nutrient mixture (REF 918 994) or BOD₅ nutrient mixture PLUS (REF 918 995). Please observe the data given in the instructions.</p>
Step 1: Preparation of the control
<p>Fill a 1 l laboratory flask (from the BOD₅ accessories set) with 500 ml aerated diluting water and 2.5 ml nutrient mixture (1.25 ml R1 and 1.25 ml R2 from the BOD₅ nutrient mixture, REF 918 994/995), close the flask and mix by shaking vigorously (control).</p>
<p>Open 1 Winkler oxygen flask and 1 test tube, rinse both with several millilitres of the control and fill to the brim without air bubbles.</p>
<p>Close the Winkler oxygen flask without air bubbles, by slowly inserting the tapered glass stopper, and incubate in a water bath or an incubator for 5 days at 20 ± 1 °C in the dark.</p>
<p>Close the test tube without air bubbles and immediately start the measurement of dissolved oxygen according to step 3.</p>

Step 2: Sample						
Depending on the expected BOD ₅ of the sample, prepare in a 1 l laboratory flask the most suitable dilution according to the following table.						
<i>If there are no experiences regarding the expected BOD₅, at least two, preferably three different dilutions of the sample should be prepared to assure accuracy of the determination. For more reliable results, we recommend duplicate determinations.</i>						
expected BOD ₅ [mg/l O ₂]	Dilution	Examples for typical waters	Sample [ml]	Aerated diluting water [ml]	BOD ₅ nutrient mixture* [ml]	
					R1	R2
< 5	1 : 1	R	500	0	1.25	1.25
4 – 12	1 : 2	R, B	250	250	1.25	1.25
10 – 30	1 : 5	R, B	100	400	1.25	1.25
20 – 60	1 : 10	B	50	450	1.25	1.25
40 – 120	1 : 20	C	25	475	1.25	1.25
100 – 300	1 : 50	C, M	10	490	1.25	1.25
200 – 600	1 : 100	C, M	5	495	1.25	1.25
400 – 1200	1 : 200	M, I	2	398	1.0	1.0
800 – 2400	1 : 400	I	1	399	1.0	1.0
1000 – 3000	1 : 500	I	1	499	1.25	1.25
* BOD 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 water I = heavily polluted industrial waste water						
After preparation of the sample , close the flask and mix well by shaking vigorously.						
Open 1 Winkler oxygen flask and 1 test tube, rinse both with several millilitres of the sample dilution and fill to the brim without air bubbles .						
Close the Winkler oxygen flask without air bubbles , by slowly inserting the tapered 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 air bubbles and immediately start the measurement of dissolved oxygen according to step 3 .						
For all further dilutions of a sample or all further samples, the preparation is to be carried out in the same manner.						

Step 3: Measurement of dissolved oxygen

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

Measurement of dissolved oxygen at day 5: For measurement of the concentration of dissolved oxygen in the incubated Winkler flasks after **5 days** of incubation one (or, if twofold determinations are required, two) test tubes are filled to the brim with the sample to be tested. After filling, the test tubes are carefully closed **without air bubbles**, and the determination of dissolved oxygen is carried out as described in "Procedure".

Procedure:

Open test tube with control or sample dilution, add
2 drops BOD₅ R1 and
2 drops BOD₅ R2, close **without air bubbles** and shake.
 Wait **2 min**.

Open test tube, add
5 drops BOD₅ R3, close **without air bubbles**, shake to dissolve the flakes.
 Clean outside of test tube and measure.

Measurement:

Call up method **8221**.

Measure the concentration of dissolved oxygen in each test tube by pressing key **M**.

Write down all concentrations of dissolved oxygen measured at day 0 and after 5 days of incubation, because they are required for the subsequent evaluation.

Step 4: Evaluation

Oxygen consumption of the water for dilution 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 BOD₅:

$$BOD_5 [mg/l O_2] = D \times (O_s - O_c) + O_c$$

D = reciprocal value of the sample dilution (e.g. dilution = 1:200 → D = 200)

Analytical
 quality control:
 Reference:

NANOCONTROL BOD₅ (REF 925 82)

German standard methods for the examination of water, waste water and sludge (DIN EN 1899-1-H51 and DIN EN 25 813-G21)