Application report

BOD measurement: Determination of the ready biodegradability of chemicals acc. to OECD 301 F (manometric respirometry)

Measuring method Respirometry, differential pressure measurement

Measuring range 0-100% biological degradability; pressure: 500-1350 hPa

Measuring equipment Pressure measurement heads

Note: The pressure measurement heads used must provide the option of outputting the differential pressure. This is not the case with all systems.
Please consult the manufacturer of your instrument regarding this possibility prior to the measurement.

pH meter:
- Minimum resolution of the pH value display: 0.01

pH electrode:

- Membrane: cylindrical, spherical or conical membrane

- Diaphragm: split ring or hole diaphragm

Accessories

- Magnetic stirrer platform for sample bottles
- Sample bottles, brown, with nominal volume of 510 ml (min. 4 pieces)
- Stirring rods with stirring rod remover
- Thermostat cabinet (temp. = 20 °C± 1°C or temperature specified by oneself)
- Rubber sleeves
- Opaque container for aerating the biological inoculation solution (V≈1 l)
- Filter or decanter
- Laboratory scales
- Aerating pump with frit
- Centrifuge for processing a sludge sample if necessary
- Variable pipettes with V = 20 ml and other attachments as required
- 1000 ml measuring flasks (7 pieces)

Reagents

- Sodium hydroxide pellets
- 0.1 M NaOH solution
- 0.1 M HCl solution
- HCl concentrate
- NaEDTA
- Potassium dihydrogen phosphate
- Dipotassium hydrogen phosphate
- Disodium hydrogen phosphate dihydrate
- Ammonium chloride
- Magnesium sulfate heptahydrate
- Calcium chloride
- Iron (III) chloride hexahydrate
- Additives for dissolving test substance where necessary
- Technical buffer solutions, pH 7.00 and 10.0

Description of the measuring process

The substance whose biological degradability is to be analyzed, is dissolved in a microbiologically inoculated mineral medium and incubated at a constant temperature for 28 days. The decomposition processes due to the bacteria cause oxidization of the test substance in the measurement solution where part of the oxygen is consumed from the gas phase of the measuring bottle. The resulting carbon dioxide is bound by an absorbent (mostly Na OH pellets) and thus removed from the gas phase of the bottle. The slowly accumulating negative pressure is directly proportional to the oxygen consumed and is, hence, a characteristic key indicator for the decomposition rate of the microbiology employed.
By calculating the theoretically expected oxygen consumption for the oxidation of the complete test substance, the decomposition rate of the microorganisms in the period under observation can be specified as a percentage of the theoretical complete decomposition.
If calculation of the theoretical oxygen demand is not possible, the less definitive ratio of the real measured oxygen consumption to the COD of the measurement solution can still be specified as the biological degradability.
This specification must, however, be especially marked by the addition of "x% of COD".

Requirements of deionate or distillate for preparation of the mineral medium

Only deionized or distilled water that is free of toxic substances such as copper or solvents is suitable for preparation of the mineral medium. Such toxic impurities can already be present both in the untreated water from which the deionate or distillate is produced and also introduced by the treatment process such as filtering through an ion exchanger, for example. If there is an excess spread in the values and abnormally low decomposition rates, the water should subsequently also be examined using suitable analytical methods in case of uncertainty.

Also, the proportion of organic carbon in the mineral medium must not be higher than 10% of the organic carbon content introduced by the test substance.

In order to check adherence to this threshold, it is recommended to carry out a measurement based on the LC-OCD method (Liquid Chromatography - Organic Carbon Detection).
Water from the same process batch should always be used for each test series in every case.

Definition of terms

Test substance:
Substance whose biological degradability is to be tested.

Mineral medium:
Salt solution that is produced according to a specified recipe and is used to set the osmotic conditions for the microorganisms. This solution is required for the dilution of all preparations.

Inoculum:
Solution which can be produced from a wide range of sources such as activated sludge or surface water samples and which contains the microorganisms required for the decomposition.

Measurement solution: Mixture of mineral medium, inoculum and stock solution that is filled into the measuring bottles where the actual measurement takes place.

Stock solution: Solution of the test substance in mineral medium that is used for ensuring homogeneous entry of the test substance into the measurement solution.

Inoculum blank:
Solution of mineral medium and inoculum that can be used through calculation to compensate for oxygen absorption due to any organic impurities from the inoculum and is also used as a medium for insoluble substances.

Preparatory work:

Inoculum:

The biological inoculum can be obtained from a wide range of sources. A sludge sample from the biological stage of a wastewater treatment plant is thus just as conceivable as a sample from surface water.

It is only important here that the sample is sufficiently aerated during transport and during lengthy dwell times.

When using sludge, care must also be taken that it is filtered prior to inoculation or decanted to free it as far as possible of organic material that may falsify the measurement.

For the preparation recommended here where the 1 l measurement solution contains 20 ml of inoculum, the concentration of organic materials in the **inoculum** must not exceed 1.5 g/l.

If solutions of different composition are used, the concentration of organic materials must be limited to 30 mg/l in the **measurement solution**.

If this cannot be clearly guaranteed after filtering or decanting, the sludge must be dried and the final proportion of solid material weighed and the inoculum diluted with mineral medium according to the specifications prior to adding to the measurement solution.

A number of treatment options for different sources of biological inoculum are shown in [1].
Concrete detailed instructions are given here for concentration and dilution, for washing, centrifuging, homogenizing and conditioning of sludges to the conditions of use, especially for treatment where inhibiting or toxic substances are suspected. If your inoculum requires specific pretreatment due to its property (e.g. too viscous, impure or not adapted to the conditions for measurement), please refer to the detailed instruction in this literature.

Conditioning the biology:

To acclimatize the microorganisms to the osmotic conditions in the measurement solution, it may be necessary to condition them prior to use. This is achieved by producing a solution in the concentration range of approx. 3-5 g inoculum to one liter of mineral medium. This mixture is aerated for 5-7 days in an opaque vessel and, as a now conditioned inoculum, can then be used for directly inoculating the mineral medium used for the measurement.
If sludge is used for this preparation, the solution must be diluted prior to inoculation once again by a factor of 5 to ensure that the solids introduced into the measurement solution is under 30 mg/l in each case. The dry mass does not need to be determined in this case as the solids content has been reduced accordingly by the high dilution.

Conditioning can also be used in certain cases to increase the accuracy of the overall process if greater statistical deviations are noted during the analysis.

Preparation of the mineral medium:

Four salt solutions are required for the preparation of the mineral medium.

Note:
Only use water that fulfils the above-mentioned requirements and reagents of analytical quality for producing the following salt solutions.

Solution 1: Phosphate buffer solution with pH 7.4
Add 8.5 g potassium dihydrogen phosphate (KH_2PO_4),
21.75 g dipotassium hydrogen phosphate (K_2HPO_4),
33.4 g disodium hydrogen phosphate dihydrate
($Na_2HPO_4*7H_2O$) and
0.5 g ammonium chloride (NH_4Cl)

to a 1000 ml measuring flask filled with approx. 500 ml of water and then mix by shaking gently.
Fill to the calibration mark and shake again.

Note: The pH value of this buffer solution should be 7.4± 0.2 without further adjustment.

Solution 2: Calcium chloride, 27.5 g/l solution
Add 27.5 g anhydrous calcium chloride ($CaCl_2$) (or an equivalent amount if the hydrate is used (e.g.
36.4 g $CaCl_2*2H_2O$))
to a 1000 ml measuring flask filled with approx. 500 ml of water and then mix by shaking gently.
Fill to the calibration mark and shake again.

Solution 3: Magnesium sulfate heptahydrate, 22.5 g/l solution
Add 22.5 g magnesium sulfate heptahydrate ($MgSO_4*7H_2O$)
to a 1000 ml measuring flask filled with approx. 500 ml of water and then mix by shaking gently.
Fill to the calibration mark and shake again.

Solution 4: Iron (III) chloride hexahydrate, 0.25 g/l solution
Add 0.25 g iron(III) chloride hexahydrate ($FeCl_3*6H_2O$)
to a 1000 ml measuring flask filled with approx. 500 ml of water and then mix by shaking gently.
Fill to the calibration mark and shake again.

Note: To guarantee long term stability of solution 4, add a drop of concentrated HCl or 0.4 g NaEDTA.

Then fill a 1000 ml measuring flask with approx. 800 ml of water, add 10 ml of solution 1 by pipette and then mix this solution by gently shaking to produce the mineral medium. Then add 1 ml each of solutions 2-4 and fill the measuring flask to the calibration mark with water.

Stock solution of the test substance:

If possible, the test substance should be dissolved in a stock solution before adding it to the measurement solution in order to guarantee homogeneous introduction into the measurement solution.

Ensure best possible homogeneity before producing the stock solution, especially when analyzing solid mixtures in which the concentration of test substance is particularly low. All other constituents contained in a mixture must also be tested separately for their biological degradability to guarantee that the oxygen consumption occurs solely through decomposition of the test substance.

Emulsifiers and other additives which guarantee solubility of the test substances can be used if they are neither decomposable nor toxic to the biology and cause no other impairments of the measurement process such as extreme foaming or overheating due to exothermal reactions.

The use of solid carrier substances for solid materials to be tested is not advisable, although with oily test substances the use of such additives can be useful.

In any case when using an additive, another preparation of the test series must be added that only contains the additive in the inoculated mineral medium. If several additives are used, each of these must be tested. The additives are only permitted if they do not exhibit degradability in these blank values.

With the aid of the permissible mixing methods, an aqueous (with mineral medium) stock solution of the test substance of approx. 1-10 g/l concentration is produced where this value should be regarded as a rough guideline only since lower values may be possible depending on the solubility, for example.
If only a small amount of the test substance is available, correspondingly small amounts of a stock solution are to be prepared.
However, the exact concentration of the test substance in the stock solution must be known, in any case.

If the test substance is fully insoluble in water, even with the permitted additives, the details in the section entitled "Procedure for fully insoluble test substance" must be followed.

Calculation of the theoretical oxygen demand ThOD

The theoretical oxygen demand can only be calculated if the chemical composition of the test substance is known.

If this is not known, the COD of the test substance must be determined [2] and the described calculations carried out with this value instead of the ThOD. In this case, it is imperative to mark the result as "x % COD".

For a substance with the chemical formula $C_c H_h Cl_{cl} N_n Na_{na} O_o P_p S_s$, the ThOD is calculated according to the formulas given below where the capital letters stand for the respective chemical elements and the small index capitals for the number of atoms in one molecule of the test substance. If one type of atom is not present in its molecule, the value of its index must be set to zero.

M stands for the molar mass of the test substance in $\frac{g}{mol}$.
The data in the square brackets represent the units of the values and are only listed for completeness.
The unit of the ThOD is interpreted as mg of oxygen per mg of test substance used.

Applies for oxidation without nitrification:

$$ThOD = \frac{16\left[\frac{g}{mol}\right] \cdot \left(2c + \frac{1}{2}(h - cl - 3n) + 3s + \frac{5}{2}p + \frac{1}{2}na - o\right) \cdot \left[\frac{mg}{mg}\right]}{M\left[\frac{g}{mol}\right]}$$

Applies for oxidation with nitrification:

$$ThOD = \frac{16\left[\frac{g}{mol}\right] \cdot \left(2c + \frac{1}{2}(h - cl) + \frac{5}{2}n + 3s + \frac{5}{2}p + \frac{1}{2}na - o\right) \cdot \left[\frac{mg}{mg}\right]}{M\left[\frac{g}{mol}\right]}$$

Select the formula according to the expected decomposition process.

Implementation

1 liter of a measurement solution comprising inoculum, stock solution and mineral medium must be produced where the precise concentration of test substance in the measurement solution must be calculated.

Use the concentration of the stock solution you produced to calculate the volume of this solution that needs to be pipetted into a 1000 ml measuring flask in order to determine a concentration of 100 mg/l test substance in the measurement solution.

Example:

The guideline is: a concentration of 100 mg/l of test substance in a 1000 ml measuring flask should be produced in the measurement solution.
$\Rightarrow$ c=100 mg/l and V=1 l

For mass m, the formula $c = \frac{m}{V}$ results in

$m = c * V$ =100 mg/l * 1 l =100 mg = 0.1 g.

In other words, there must be 0.1 g of the pure test substance in the measurement solution.

The test substance is however present in the stock solution, hence the corresponding volume of stock solution must be pipetted into the flask of the measurement solution.

If your stock solution has a concentration of c= 5 g/l, for example, then:

$$c = \frac{m}{V} \Rightarrow V = \frac{m}{c} = \frac{0.1g}{5\frac{g}{l}} = 0.02l = 20ml$$

In this case, therefore, you need to pipette 20 ml of the stock solution into the 1 l measuring flask in order to obtain a concentration of 100 mg/l of test substance in the measurement solution after filling.

Pour approx. 500 ml mineral medium into a 1 l measuring flask and pipette the calculated amount of stock solution into it.
Then add 20 ml of the inoculum and fill the flask with mineral medium up to a few cm below the mark.

Measure the pH value of the solution.
If necessary, adjust the pH value with a few drops of a 0.1 M NaOH solution or 0.1 M HCl solution to a value of pH 7.4 ± 0.2 .
Then fill the flask up to the calibration mark with mineral medium.

As a double determination of the measurement solution is recommended for each measurement step, the measurement solution that is used must be divided into 2 measuring bottles. Fill each of two measuring bottles with 300ml of the measurement solution. This volume has been chosen so that when the bottle is sealed, the measurement solution still has a sufficient gas volume.

Note:
If the **abiotic decomposition** of the test substance is to be analyzed, prior to incubation the measurement solution must be mixed with a substance that is toxic for microorganisms such as a sufficient concentration of copper ions. It is, of course, recommended here to dispense with inoculum even when preparing the measurement solution.

To allow for any oxygen absorption due to decomposition of organic substances in the inoculum in the calculation, an inoculum blank must be prepared. Double determination is also recommended here.

Pour 20 ml of the inoculum into a 1 l measuring flask and fill the flask with mineral medium up to a few cm below the mark.

At this point it is absolutely crucial that the same inoculum in the same amount is used as for the inoculation of the measurement solution because the concentration of microorganisms and of organic impurities is not known. This blank value is deducted from the oxygen absorption of the measurement solution and must reflect the oxygen consumption of the organic substances from the inoculum contained in the measurement solution as closely as possible.

Now measure the pH value of the inoculum blank.
If necessary, adjust the pH value with a few drops of a 0.1 M NaOH solution or 0.1 M HCl solution to a value of pH 7.4 $\pm$ 0.2 . Then fill the flask up to the mark with mineral medium.

Fill each of two measuring bottles with 300 ml of the inoculum blank.

Place 2-3 NaOH pellets into the neck of each of the 4 measuring bottles fitted with rubber sleeves and screw the measuring heads onto the bottles.

Incubate the measuring bottles prepared in this way for 28 days at 20 $\pm$ 1°C.

Note:
Other measuring temperatures can be selected depending on requirements, but must be kept constant within an interval of ± 1°C during the measurement. OECD Guideline 301 specifies a temperature range of 20 – 24 °C although the operating temperature range of the measuring heads (see Technical data) ultimately dictates their use.
With WTW measuring heads, the permissible range of use is between +5°C and +50°C.

Procedure for total insolubility of the test substance

If the test substance cannot be made into an aqueous solution even with the use of additives, it must be weighed directly into an inoculated mineral medium as a measurement solution. A double determination of the measurements is also recommended here.

Produce an inoculated mineral medium as described in the previous sections and calculate the weighed amount of test substance needed to achieve the required concentration of 100 mg/l test substance in the 300 ml of the measurement solution. The possible introduction of solids through sludge must be limited to a maximum of 30 mg/l in the measurement solution.

Pour 300 ml of the inoculated blank into each of the two measuring bottles.

Place the precisely weighed amount of test substance directly into the measuring bottles.

Ensure there is also prior homogenization here if the test substances are contaminated.

Seal the measuring bottle with a suitable lid and make certain that the test substance has been fully absorbed by the measurement solution by shaking several times and that there are no residues sticking to the bottle walls.

Use a double blank value here too.

Put 2-3 NaOH pellets into the neck of each measuring bottle fitted with rubber sleeves and screw the measuring heads onto the bottles.

Incubate the measuring bottles prepared in this way for 28 days at 20 ± 1°C.

Note:
Other measuring temperatures can be selected depending on requirements, but must be kept constant within an interval of ± 1°C during the measurement. OECD Guideline 301 specifies a temperature range of 20 – 24 °C; however, the operating

temperature range of the measuring heads (see Technical data) ultimately limits use.
With WTW measuring heads, the permissible range of use is between +5°C and +50°C.

Analysis:

If there is no negative pressure in the measuring bottle throughout the entire time period, microbial toxicity of the test substance must be assumed. If this is not plausible, the measuring system must be checked for leaks.

As mentioned at the beginning under measuring equipment, the measuring head must be able to output the differential pressure as a measured value from which the oxygen consumption can be calculated. This is the only way to relate the oxygen consumption directly to the weighed amount of test substance.

The oxygen consumption (mass m_{O_2}) is calculated from the

measured differential pressure (Δp) according to the following formula:

$$m_{O_2} = \frac{\Delta p * V * M}{R * T}$$

where:

V = volume of the gas phase occurring above the measurement solution.
When using the standard brown measuring bottles, the volume is calculated as follows:

V = bottle volume – volume of the measurement solution

= 0.510 l – 0.3 l = 0.21 l

If you use a different measuring bottle, the volume of the gas phase must be calculated accordingly.

M = molar mass of the oxygen = 32 g/mol
R = Universal Gas Constant = 8.314472 $\frac{J}{mol * K}$
T = absolute temperature for the measurement

The incubation temperature of 20°C recommended here equates to an absolute temperature of 293.15 K.

If you have selected a different incubation temperature, the absolute temperature must be calculated according to the following formula:

T=$\upsilon + 273.15K$ with =temperature in °C

Now use the specified formula to calculate the mass of oxygen consumed for each differential pressure measured.

Then calculate the average value from the two oxygen consumption values of the blank solutions.

$$b_m = \frac{b_1 + b_2}{2}$$

Average values cannot be calculated from the oxygen consumption values for the measurement solutions; one BOD value is determined from the two values each time and the 2 different degradabilities calculated from the two values which are both specified as the results.

The BOD is calculated according to the following formula:

$$BOD = \frac{A}{m_{Testsubs\,\tan z}}$$

The BOD [mg/mg] is to be understood here as mg of oxygen per mg of test substance used.

A= corrected oxygen absorption of the measurement solution:

A = m_{O_2} - b_m

m_{O_2} = oxygen consumption value of the measurement solution

b_m = average value of the oxygen consumption of the blank solutions in [mg]

$m_{Testsubs\,\tan z}$ = mass of test substance in the measuring bottle (mg)

$$m_{Testsubs\,\tan z} = c_{Messlösung} * V_{MF}$$

where

$c_{Messlösung}$ = concentration of test substance in the measurement

solution (100 mg/l here)

V_{MF} = volume of the measurement solution in the measuring

bottle (300 ml here)

Calculate a BOD for each of the two oxygen consumption values obtained from the double determination.

Thus, a BOD_1 and a BOD_2 value are obtained that have equal

standing for as long as they fulfill the condition:

$$| BOD_1 - BOD_2 | < 0.1 * (BOD_1 + BOD_2)$$

If this condition is not met, the measurement must be repeated.

Biological degradability D is calculated according to the formula:

$$D = \frac{BOD}{ThOD} * 100\ [\%]$$

Calculate the biological degradability for each of the two BOD values. The two decomposition values have equal standing as the result.

Information on the validity of the measurement

The oxygen absorption of the inoculum blank solution normally moves in a range of 20-30 mg/l and should not exceed a value of 60 mg/l in 28 days. If the values are higher than these, take a critical look at the procedure and check the data. In case of doubt, a more intensive preparation of the inoculum according to [1] is recommended. If the pH value based on the measurement is outside the pH 6-8.5 range and the degradability is below 60% at the same time, the test must be repeated with a lower concentration of test substance in the measurement solution.

An absolute oxygen consumption of more than 900 mg in a flask must not be exceeded in one measuring step. If the ThOD for the weighed substance amount exceeds this value, a lower amount must be used.

Literature

[1] OECD Guideline for testing of Chemicals, Ready Biodegradability, 301F Manometric Respirometry, paragraphs 9 to 15, 1992
[2] Deutsche Einheitsverfahren für Wasser-, Abwasser- und Schlammuntersuchung; Band 6 G-H; Abschnitte H41, H43, H44 , H45; VCH

Note

The information contained in our application reports is only intended as a basic description of how to proceed when using our measurement systems. In isolated instances or if there are special general conditions on the user side, exceptional properties of the respective sample can, however, lead to a change in the execution of the procedure or require supplementary measures and may, in rare cases, lead to a described procedure being unsuitable for the intended application.

In addition, exceptional properties of the respective sample such as special general conditions can also lead to different measurement results.

The application reports have been prepared with the greatest possible care. Nevertheless, no responsibility can be accepted for the correctness of this information.

The current version of our general terms of business applies.

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