Application report

Respirometric BOD_5 determination of waste water polluted with organic or inorganic toxins or inhibitors

Foreword

By reason of their origin, some wastewater can be polluted with both inorganic and organic compounds that either act toxically on microorganisms, or can hamper their function in some other way. In order to assign a BOD value to such wastewaters, the samples must be displaced with a special biological dilution water to reduce the concentration of toxic (inhibitory) substances, on the one hand, and to guarantee optimum conditions for the microbiology on the other.
As organic toxins increase the BOD value, an additional reduction of the filling amount in the sample bottles is necessary here in order to measure the higher BOD values expected (see Notes for organically polluted wastewater).
Generally, a dilution series of the original sample is used for the measurement in both pollutant classes in order to determine the BOD and mathematically extrapolated from their data of the BOD value of the pure sample solution.

Measuring method

Respirometry

Measuring range

0-8,000 mg/l BOD

Note:
An extension of the measuring range for even higher BOD values is, in fact, theoretically conceivable through further dilution. This causes the amount of sample in the solution to sink, but to such low values that they can be superimposed by the blank solution of the dilution water. Many years of experience have indicated that the maximum possible measuring range is restricted to 8,000 mg/l BOD as a result.

Measuring equipment

OxiTop pressure measuring heads

Accessories

- Magnetic stirrer platform
- Thermostatic box (temp. = 20 °C ± 0.5 °C)
- Sample bottles brown with 510 ml nominal volume
- Stirring rods with stirring rod remover
- Overflow measuring beaker(s) V = 432 ml (250 ml for org. pollution)
- Rubber sleeve

- 3 l measuring flask
- 1 l measuring flask (4 pieces)
- 500 ml measuring flask (5 pieces)
- Opaque container for aerating the dilution water (V≈3.1 l)

Reagents

- Sodium hydroxide tablets
- N allylthiourea solution NTH 600 (β = 5 g/l)
- Potassium hydrogen phosphate
- Dipotassium hydrogen phosphate
- Disodium hydrogen phosphate heptahydrate
- Ammonium chloride
- Magnesium sulphate heptahydrate
- Calcium chloride
- Iron (III) chloride hexahydrate

Procedure

Producing the inoculated dilution water:

The dilution water is produced from various salt solutions and microbiologically inoculated with a wastewater sample later. Since the dilution water itself already has a low BOD value, this must be determined as a blank value and offset against the measured BOD values of the diluted solutions.

The salt solutions described here can be kept for approx. 6 months in opaque glass bottles at 0 – 4 °C without any loss of quality. The solutions must, however, be checked for precipitation or flocculation prior to use. If these are apparent, the solution must be discarded and replaced by a fresh one.

Note:

For producing all solutions and for filling the measuring flasks, only deionized water that is absolutely chlorine-free must be used as free chlorine can massively impede biological processes. If necessary, the chlorine must be removed by blowing it out with air.

Preparatory work:

Producing the salt solutions:

1. Phosphate buffer solution with pH 7.2 Dissolve
-8.5 g potassium dihydrogen phosphate (KH_2PO_4)
-21.75 g dipotassium hydrogen phosphate (K_2HPO_4)
-33.4 g disodium hydrogen phosphate heptahydrate ($Na_2HPO_4*7H_2O$) and

-1.7 g ammonium chloride (NH_4Cl) in approx. 500 ml water. Dilute to 1000 ml and mix.

Note: The pH value of this buffer solution should be 7.2 without further setting.

2. Magnesium sulphate heptahydrate, solution
22.5 g/l Dissolve
-22.5 g magnesium sulphate heptahydrate ($MgSO_4*7H_2O$) in water. Dilute to 1000 ml and mix.

3. Calcium chloride, solution
27.5 g/l Dissolve
-27.5 g water-free calcium chloride ($CaCl_2$) (or an equivalent amount if the hydrate is used
(e.g. 36.4 g $CaCl_2*2H_2O$)) in water. Dilute to 1000 ml and mix.

4. Iron (III) chloride hexahydrate, solution
0.25 g/l Dissolve
- 0.25 g iron (III) chloride hexahydrate ($FeCl_3*6H_2O$)
in water. Dilute to 1000 ml and mix.

Inoculation material:

- The wastewater sample used for biological inoculation should not exceed a BOD of 300 mg/l or a TOC of 100 mg/l.

- Before inoculation, the wastewater sample must be either decanted or filtered through a coarse folded filter so that it is free of suspended matter.

Producing the dilution water and inoculation.

- Pour 3 ml of each of the salt solutions no. 1 to 4 into a separate 3 l measuring flask and fill the flask up to the mark with deionized water.

- Transfer this solution into a somewhat larger opaque container and add 60 ml of the inoculation material to it.

- The dilution water obtained in this way must be aerated for several hours at approx. 20℃ with the light excluded to allow the biological activity in the new medium to start.

- The dilution water must always be freshly prepared at the start of the respective working day and discarded at the end of the day.

Preparation of the wastewater sample

-Measure the pH value of the undiluted wastewater sample. If this is outside of the pH 6-9 range, the sample must be neutralized with an HCl or NaOH solution until the pH value is inside the specified range.

-Add 20 drops of nitrification inhibitor NTH 600 to each liter of the waste water sample

Producing the series of dilutions:

Before preparing the sample solution of wastewater and dilution water, the wastewater sample must be homogenized on a magnetic stirrer until all deposited suspended matter is distributed evenly throughout the liquid. Only in this state is the removal of a representative sample for the preparing of the dilution series ensured which is why this process must be repeated each time before wastewater is removed from the sample for each sample solution of the dilution series.

- Use the COD value to estimate the expected BOD value of the wastewater sample in order to prepare the correct dilution series in accordance with the table shown below. If the ratio of COD to BOD is not known for the wastewater under examination, the assumption can be made that the BOD value is 50% of the COD value.

Expected measuring range of the wastewater sample in mg/l	Volume of wastewater sample to be pipetted into a 500 ml measuring flask in ml				
0-400	10	20	30	40	50
0-1600	2.5	5	7.5	10	12.5
0-8000	0.5	1	1.5	2	2.5

- Produce the dilution series according to the data in the table for the expected BOD measuring range by pipetting the specified amount of wastewater sample into a 500 ml measuring flask and fill this up to the calibration mark with **dilution water**.

- Measure a volume of 432 ml from each of the five sample solutions using a separate overflow measuring flask and transfer each of these amounts into separate brown sample bottles.

- Also measure an amount of 432 ml of the dilution water with an overflow measuring flask and transfer this amount into a separate brown sample bottle. This preparation is used to determine the blank value of the dilution water.

Note for organically polluted wastewater:

In the case of heavily organically polluted wastewaters it is necessary to reduce the filling quantity in the sample bottle in order to be able to detect the high BOD value to be expected with the measuring system. This makes it necessary to calculate in a filling quantity factor into the calculations that takes into account the lower filling quantity.
Because the sample itself is not diluted here, the effect of the possible overlay mentioned in the introduction does not occur due to the BOD value of the dilution water in this procedure.

-If your sample is heavily polluted with organic substances, use a 250 ml overflow measuring flask for measuring the sample solution into the sample bottle at this point instead of a 432 ml flask. A factor of 5 is used for the calculations for this filling quantity.

The blank value for the dilution water can continue to be measured with a filling quantity of 432 ml and also does not have to be multiplied with a factor under this precondition.

Measuring the dilution series

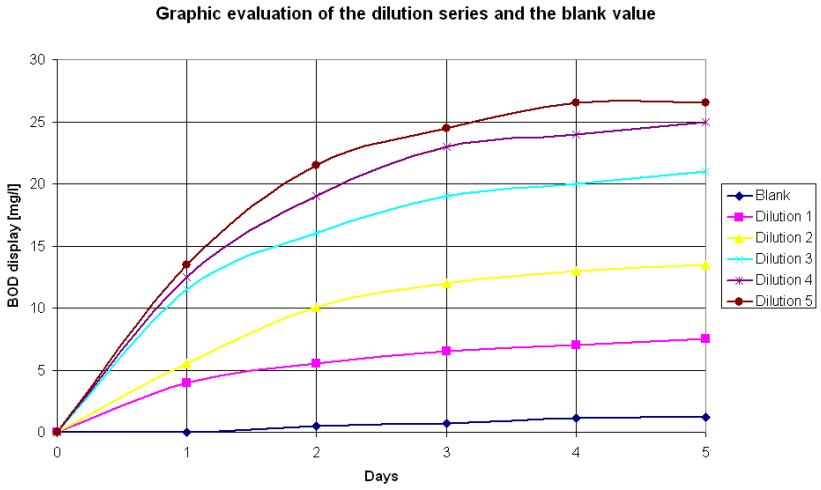
- Measure the temperature of the sample solutions in the sample bottles. If it lies outside the range of 15-21 ℃, the temperature of the samples must be adjusted before the measurement.

- Insert a magnetic stirring rod into each sample bottle and fit the bottle necks with the rubber sleeves that have been filled beforehand with sodium hydroxide tablets.

- Tightly screw the pressure measuring heads onto the bottles and start the measurement.

- Place the sample bottles in the incubator thermostatic box on a switched-on stirrer platform and incubate them at 20 ℃ ± 1 ℃ for 5 days.

- After incubation has finished, read the measured values from the pressure measuring heads and enter the BOD values on a diagram that covers the measuring days. This will result in a diagram similar to the one shown below.



- For each dilution and for the blank value, take the measured value of the fifth day and enter this value above the sample ratio in the sample solution in ml/l.

Example:

The middle one of the three dilution series is selected in order to cover a measuring range of 0-1600 mg/l BOD.
The following values result for the sample ratio in the sample solution in ml/l.

Dilution 1: 2.5 ml in 500 ml ⇒ 5 ml/l sample ratio

Dilution 2: 5 ml in 500 ml ⇒ 10 ml/l sample ratio

Dilution 3: 7.5 ml in 500 ml ⇒ 151 ml/l sample ratio

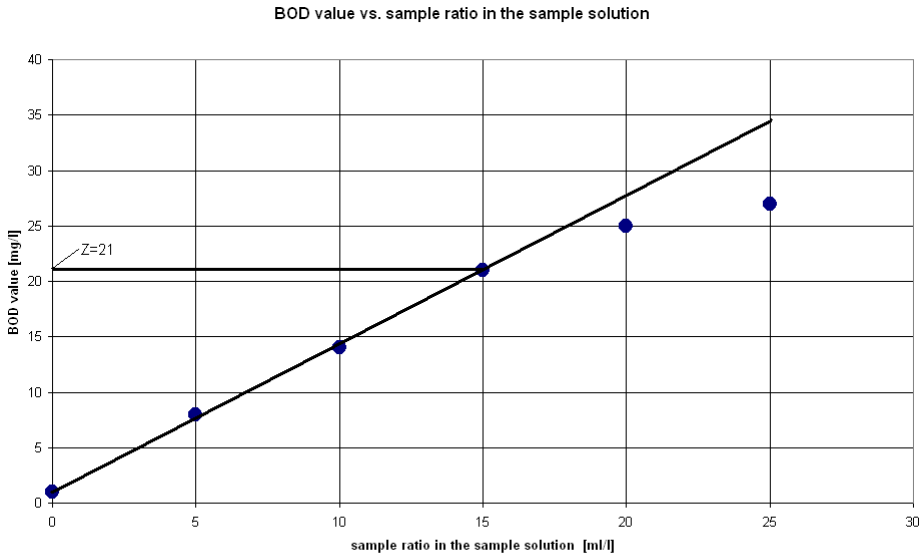
Dilution 4: 10 ml in 500 ml ⇒ 20 ml/l sample ratio

Dilution 5: 12.5 ml in 500 ml ⇒ 25 ml/l sample ratio

If these sample ratios are assigned to the respective BOD value for the fifth day of the measurement, this results in the following table:

Ratio of sample in the sample solution in ml/l	Associated BOD value of the fifth day in mg/l BOD
0 (blank value)	1
5	7.5
10	14
15	21
20	25
25	27

The BOD value plotted over the proportional volume of the sample in the sample solution results in the following graph:



The graphic shows clearly, that blank and the BOD values of the first three dilutions define a straight line, whereas the last two values of the sample solutions with the highest waste water concentration generally underlie, because the greater amount of toxic compounds are strongly hampering the biology. By this reason these values will not be taken to draw the straight line. Fundamentally it is insignificant for the result from which sample ratio the BOD value is taken. It is recommended however to use dilution 3 to keep the error of the extrapolation small (greatest real measuring point, represented by the straight line).

With these values given by the straight line, the BOD value [mg/l] for the pure sample can be calculated using the following formula

$$BOD_{5(rein)} = \frac{1000}{V_P} * (Z_M - BW) + BW$$

where:

$V_P =$ Volume of sample [ml] in 1000 ml sample solution (here 15 ml)

$\frac{1000}{V_p}$ = reciprocal value of the dilution factor to extrapolate the pure sample.

F = filling quantity factor (F = 1 with 432 ml, F = 5 with 250 ml sample solution in the sample bottle)

Z_M = BOD value of the dilution from the graphical evaluation [mg/l]

BW = BOD value of the dilution water [mg/l]

With the straight line coordinates for dilution 3 (15 ml/l; 21 mg/l), according to the formula, a BOD value of 1334 mg/l results for the pure sample.

-Proceed as described in the example to calculate the BOD value of your wastewater sample.

Note The water sample must be processed as soon as possible after it has been taken.

Bibliography Euro Norm EN 1899-1/ 1998

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.

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