Application Report

Determining Biochemical Oxygen Demand (BOD) with Lovibond® OxiDirect

- manometric method[2] -

Introduction

The **B**iochemical **O**xygen **D**emand over a testing period of n days **(BOD_n)** is precisely defined and associated with experimental standards. It represents the quantity of oxygen aspirated in the course of aerobic breakdown of organic substances by microorganisms.
BOD is thus an substantial feature in determining the effect of discharged effluents on the oxygen content of a water-course or on the oxygen demand of an effluent treatment plant. BOD levels are stated in mg/l of oxygen and are usually measured over a period of 5 days **(BOD_5)**.

Principle of measurement

Microorganisms[1] feed on the organic compounds contained in a water sample, which they consume in the presence of oxygen (O_2) - that is, the compounds are biochemically oxidised and thus broken down, either partially or completely. The complete breakdown of organic materials ($C_{org.}$) results in their oxidation to carbon dioxide (CO_2) and inorganic salts (mineralisation), as covered by expression 1:

$$(1)\ C_{org.} + O_2 \xrightarrow{\text{microorganisms}} CO_2 + \text{Salts}$$

The manometric method for BOD-determination[2] is based on the fact that the oxygen which is converted to carbon dioxide is removed from the gas phase of the sample by the use of potassium hydroxide KOH (Hütter, 1984). Therefore, in the closed system BOD-flask/BOD-sensor, a drop in pressure occurs, which is proportional to the amount of oxygen consumed.

Lovibond® OxiDirect

With the OxiDirect, the change in pressure resulting from the consumption of oxygen is measured in the flasks by electronic pressure sensors and calculated directly in terms of mg/l BOD.

This method is outstandingly suitable for routine analysis work and provides a range of advantages in comparison to the dilution method for BOD determination[3]:

- the sample can usually be used without predilution;
- individual measurement ranges are much wider;
- all measured values are stored automatically;
- the BOD-graph (see Fig. 1) is easily drawn up and
- there is considerably less work involved.

Selecting the Measurement Range

The BOD value of a sample depends on the level of bio-available organic substances contained. The range of the measurement system should be selected to ensure that the expected readings will be roughly within the upper half of the scale. Thus, where BOD values of 250 mg/l are expected, the range 0-400 mg/l would be ideal (see Table 1). For samples where BOD-values are unknown, they could be estimated by taking 80 % of the COD[4] level as the *maximum* BOD value. **Important!** Note that, if the measurement range is exceeded, no BOD-(end-) value will be obtained! However, the individual daily values may be used to make an estimate of the final figure (see Fig. 1).

Table 1: Measurement ranges with the associated sample volumes and the required amount of nitrification inhibitor (ATH) from Lovibond®.

measurement range mg/l BOD	sample volume ml	ATH drops
0 - 40	428	10
0 - 80	360	10
0 - 200	244	5
0 - 400	157	5
0 - 800	94	3
0 - 2000	56	3
0 - 4000	21,7	1

The BOD-values for samples with a BOD in excess of 4000 mg/l[5] can be determined by pre-treatment with the use of so-called dilution water (see Lovibond® Application Report).

[1] bacteria, fungus, archaea and protozoa
[2] to DIN 38 409 - H 52
[3] to DIN 38 409 - H 51
[4] **C**hemical **O**xygen **D**emand (COD)
[5] reserve of measurement range till 5000 mg/l BOD

Preparing the Sample

- **pH value** of the sample: for biochemical oxidation the most suitable pH value is between pH 6.5 and pH 7.5. If the pH value of a sample is outside this range, it should be set within range, since any greater deviation results in an underestimation of the BOD value. Too high, a pH value can be reduced with 1-N-sulphuric acid, while too low, a pH value can be increased with 1-N-sodium hydroxide solution.
- **Homogenisation:** the sample should be homogenised or pre-treated to any special requirements for obtaining the total BOD of a sample, including contained particles. Comparable BOD values can only be obtained if the pre-treatment of each sample is carried out similar.
- **Volume** of the homogenised sample: the sample volume can be determined from Table 1, depending on the measurement range required. It can then be measured precisely, using the relevant overflow vessel and poured into the sample flask. We recommend that three, or at the very least, two determinations should be made for each sample.
- **Inhibiting of nitrification:** to suppress this considerable source of irritation, the nitrification inhibitor N-allylthiourea (ATH) from Lovibond® should be added in drops to the sample, as detailed in Table 1.
  Nitrification is caused by two groups of nitrifying bacteria: the first group oxidises ammonium (NH_4^+) to nitrite (NO_2^-), representing the substrate for the second group, which forms nitrate (NO_3^-); see expression 2:

(2) $NH_4^+ \xrightarrow{+1\,1/2\,O_2} NO_2^- \xrightarrow{+1\,1/2\,O_2} NO_3^-$

  This conversion requires 4.57 mg/l O_2 per mg of NH_4^+ and has a significant effect on the BOD, which is intended to determine only the oxygen consumed in the course of carbon oxidation (C-BOD).
- **Sealing the sample flasks:** to ensure correct gas exchange by agitation during the incubation period, a magnetic stirring rod[6] from Lovibond® must be inserted into the sample. A dry, grease-free gasket is filled with two drops of potassium hydroxide solution from Lovibond® and inserted into the neck of the flask. The vessel is then sealed by screwing a sensor onto the BOD-bottle.
- **Tempering the sample:** the Auto-Start-Function[7] allows to use the sample without pre-tempering, provided the sample temperature is not more than 5°C below the incubation temperature selected (generally 20°C). **Important!** To eliminate artificially high readings, samples which are warmer than the selected incubation temperature need to be cooled down before starting the measurement! Thus, where the selected incubation temperature is 20°C, samples which are warmer than 20°C must be cooled and samples cooler than 15°C should be heated to between 15°C and 20°C. This can be achieved, for example, by placing the stirred sample in a Lovibond® incubator or in a tempered water bath.

Starting & Evaluating Measurements

The process is started as described in the operating instructions for the equipment. The sample is then incubated in a thermostatically controlled cabinet for the selected incubation period (5 days in the case of BOD_5 measurement) and at the selected incubation temperature (generally 20°C). The sample is agitated constantly in order to ensure oxygen delivery from the gas phase of the measurement system into the water sample, in which oxygen is consumed. **Important!** The incubation temperature ($T_{ink.}$) must be maintained within the range of $T_{ink.} \pm 1$ °C - otherwise, errors of up to 10 % BOD per 1 °C can occur!
The BOD value is displayed in mg/l in the display of the equipment. Should slight deviations occur within the parallel samples (normally < 10 %), then usual the mean value of measurements is taken.

Cleaning

We recommend repeated rinsing in hot water to clean every item which get in contact with a sample, to prevent contamination by materials such as tensides[8] which would affect the BOD measurement. In the case of severe contamination a cleaning agent should be used; the equipment must then be rinsed very thoroughly with distilled water.

[6] of defined volume

[7] for details see instruction manual of the device

[8] cleaning agents

Advice on Evaluation of Results

- BOD values do not increase in a linear manner; after a day they must always be higher than on the previous day but the daily increase in mg/l BOD becomes ever smaller (see Fig. 1).
- if BOD readings become linear, the sample is outside the measurement range (overflow). To obtain BOD values, a higher measurement range must be chosen.
- if BOD readings suddenly increase during the measurement period, it is possible that nitrification has started (see above).
- if BOD readings fall in the course of measurement, the system may have developed a leak, or the sample material has become problematic (for example, anaerobiosis).

Interpretation

BOD_n values can be used to reach conclusions regarding the characteristics of a water body, as well as the biological activity of the incubated microflora. For example, the introduction of effluents with a high level of oxygen consumption (high BOD value) can lead to an oxygen starvation of the water-course (fish killing). In an other case, the performance of an effluent treatment plant can be checked by comparing the BOD levels before and after an effluent treatment.
In general, the following conclusions may be drawn:

- high BOD reading indicates a high content of biodegradable organic materials in the sample - in other words, without further pretreatment, this sample will cause stress on the oxygen level of a water course.
- a low BOD reading in the sample indicates either a low content of organic materials (that means low stress on the oxygen level of a water course), or substances which are difficult to break down, or various functional problems (the sample may contain poisons or inhibiting substances, or have an extremely high pH, etc.). This can be evaluated in detail by the comparison with the results of other analyses, as explained below.
- the BOD graph (see Fig. 1) provides further information on the significance of the measurement (conformance with the measurement range; errors; kinetics of the biological degradation process).
  The BOD gains informative value if evaluated in association with other parameters, such as COD, DOC, POC, TOC. An example is provided by comparing the obtained BOD value with the corresponding COD value:
- a small difference indicates that a large proportion of the organic substances can be broken down.
- a large difference suggests either that the organic substances are not easily biodegradable, or that there is an error.

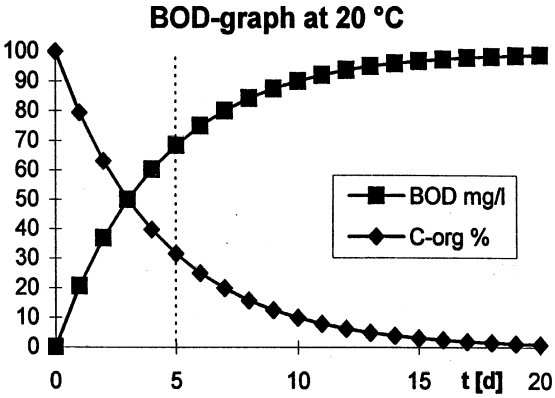

Fig. 1: Idealised BOD-graph at 20 °C (to Habeck-Tropfke, 1992) compared with the proportional reduction in biodegradable organic compounds ($C_{org.}$). After 5 days incubation, approx. 70 % of the $C_{org.}$ has been broken down: this is the equivalent of the BOD_5 value.

Note

The comments and explanations set out in this paper refer to regular samples and conventional reactions of microorganisms in the course of a BOD measurement and cover the majority of all samples. Thus, this method is used with success and without problems in practically all municipal effluent treatment plants.
Special cases are always a possibility, however, and arise from specific, local circumstances. For example, therefore, underestimated BOD values might be the result of a severe inhibition, or the presence of certain, disturbing constituents in the sample, or maybe even the result of special effluent treatment processes in front of the site the sample was taken from. Extreme conditions are frequently encountered with industrial effluents. They often contain very high or very low BOD loadings, as well as oxidising or toxic materials. Cases of this kind must be analysed with care and the problems which arise must be treated on an individual basis (please ask for Lovibond® Application Report of BOD determination of strongly-loaded organic waste water).

Bibliography

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