Application report

Supervision of BOD measuring systems according to DIN/ISO 9000 and GLP

Foreword

The microbiological decomposition process is one of the most important processes in the wastewater and waste treatment as well as soil measurements and, therefore, must be controlled in a suitable way.
The checking of the measuring equipment required for this for correct functioning and detecting any measured value deviation present are among the basic tasks that have to be taken into account during this work.
Four different methods for monitoring this system are presented here that can be selected and combined according to your own requirements. Whichever method is used the frequency, the form and the documentation of the results must always be adapted to the relevant regulations such as DIN/ISO 9000 and GLP and a detailed test plan worked out. As the time intervals of the tests are oriented to the operating conditions of the instruments, it is not possible to specify a general procedure for this. The time progression for each scenario must be specified, controlled and carried out under the personal responsibility of the operator in writing.
The documentation of the results is clearly regulated by the guidelines of the DIN/ISO 9000 and the GLP:
The type and identification number of the instrument to be inspected must be entered together with the measurement results, date and signature of the performing person in a log book which must be kept available at all times for inquiry. It is, however, also unavoidable here that concrete, individual work instructions are specified by the operator, taking into account the publications mentioned.

If any deviation of the measured value or failure of the measuring system is determined during the examinations, the measuring system must be sent back to the manufacturer for processing as no adjustment of the measuring system can be undertaken by the user himself.

Measuring method

Creation of a known negative pressure and comparison with the display

Measuring range

0-4.000 mg/l BOD, Δp_{max} = 142 mbar = 14200 Pa

Measuring equipment

OxiTop® pressure measuring heads

Accesories

General:
- Magnetic stirring platform
- Thermostat cabinet (temp. = 20 °C± 0.5 °C)
- Sample bottles, brown, with nominal volume 510 ml
- Stirring rods with stirring rod remover
- Overflow measuring flasks, V = 432 ml and V = 164 ml
- Rubber sleeves
-1l measuring flasks (7 pieces)
- Pipettes, V = 1 ml
- Pipette, V = 0.5 ml
- Opaque container for aerating the dilution water (V≈1.1l)

Manometer method:
- Vacuum pump
- Adapter piece
- Manometer
- Hose connectors
- Seals

Reagents

General:
- Sodium hydroxide pellets
- N-allylthiourea solution NTH 600 (β = 5 g/l)

Tablet method:
- Calibration tablets

Glutamic acid glucose method
- D(+) glucose
- L glutamic acid
- Potassium hydrogen phosphate
- Dipotassium hydrogen phosphate
- Disodium hydrogen phosphate heptahydrate
- Ammonium chloride
- Magnesium sulphate heptahydrate
- Calcium chloride
- Iron (III) chloride hexahydrate

Implementation

1. Quick test for checking the measuring system for leakage:

Foreword:
Any leakage of the entire measuring system consisting of bottle and measuring head is of vital importance as even the smallest leaks over the relatively long measuring periods can massively falsify the measured values. For this reason, it is important to check the leakage of the system at regular intervals.

Implementation:

Fill a measuring vessel half full with hot water at a temperature of approx. 50℃. When cooled to a constant 20℃, a stable negative pressure should be reached.

2. **Manometer method:**

Terminology explanation:

Absolute pressure: The pressure that is measured against vacuum (most frequent display of a pressure gauge).

Differential pressure: Difference between the variable outside air pressure and the absolute pressure in the bottle.

Foreword:

As a quick test for checking the function and for estimating any deviation of the measured display value, the checking of the measuring head with the aid of a manometer has proven reliable.
In this method a known negative pressure is created with a vacuum pump and the display on pressure measuring head is converted to the corresponding difference to the outside air pressure.
Practical execution of this requires an adapter which is used to connect the pump to the measuring head and, thus, can compare its display with the manometer of the pump. Such an adapter can mostly be obtained from the manufacturer of the pressure measuring head from whom the constant for converting from the display value to pressure can also be obtained.

One digit on the display on the OxiTop measuring heads from WTW is equal to a differential pressure of 3.55 hPa to the outside air pressure OxiTop®-i heads have the possibility to measure pressure directly in hPa.

Implementation:

- Make an airtight connection of the pressure measuring head with a vacuum pump that has a display for the differential pressure it creates to atmospheric air pressure.

- If the pressure display only shows the absolute pressure, the differential pressure must be calculated by subtraction from the prevailing air pressure and the absolute pressure displayed on the pump.

- The leak tightness of the measuring setup must be ensured in any case. If necessary, check this by applying a negative pressure and observing the pressure display overnight. If the specified negative pressure has not significantly changed by next morning, leak tightness of the system can be assumed.

- Generate differential pressures at intervals of 10 hPa to approx. 140 hPa below the prevailing air pressure by means of the pump and note the display value on the measuring head for each of the selected pressures.

- Calculate the display values in differential pressures with the aid of the conversion constant and compare them with the pressures given on the pump.

- If the specified differential pressure deviates from the calculated differential pressure by less than 4 hPa, it can be assumed that the measuring system is functioning correctly. If the deviation is greater than that, the instrument must be sent in for checking.

- For a quick check of the measuring equipment, a lower number of specified differential pressures can also be set and checked.

3. Calibration tablets (leakage test and calibration):

One possibility for testing the leak tightness of the system and carrying out the calibration of the measuring head at the same time is provided by calibration tablets that contain an oxygen depleting substance and so can generate a defined negative pressure in the bottle.

Note:

Distilled water must always be used for this method and not biological dilution water as in other cases. Because the oxygen depletion is caused here by a chemical substance and not by micro-organisms, any biological impurities can lead to results that are too high. Only when it is ensured that the negative pressure can be traced back solely to the calibration substance can a professional calibration be carried out.

Implementation:

- Before use, the bottles must be thoroughly rinsed with distilled water and any impurities removed with a brush.
- The volume of distilled water (mostly 164 ml) that should be filled in the bottle is specified in the package insert of the calibration tablets.

- In contrast to the normal BOD determination, the measuring process on the measuring head is already started before adapting the temperature and screwing onto the bottle.

Note: An own routine for checking the leakage and calibration with the aid of the tablets is stored in the program of some measuring heads. Check this using the operating instructions of your measuring head and, if necessary, proceed as described there. If no routine is available in your measuring head, follow the procedure described in this application report.

- Activate the measurement and place the bottle filled with distilled water and the measuring head **not screwed on the bottle** in the thermostat cabinet, adjust the temperature and leave both for approx. 4 hours at 20 °C ± 0.5 °C.

- After expiry of the preliminary temperature adjusting time **one** calibration tablet is completely dissolved in the distilled water of the bottle. Make sure that the whole tablet is introduced into the bottle as the oxygen depletion of the substance depends on the amount added. If a tablet is no longer complete, it must be discarded and a new complete tablet must be used.

- Make sure that **no** absorber has been added in the sleeve and close the bottle tightly with the measuring head. The sleeve must nevertheless be inserted in the bottle as it is also used as a seal between the measuring head and bottle.

- Make sure when adding the tablet that the liquid temperature cannot drop excessively. The best way is not to take the bottle out of the thermostat cabinet at all when putting in the tablet. If the temperature of the liquid after adding the tablet is outside the range of 15 – 21 °C, the temperature must be readjusted.

- Incubate the bottles closed with the measuring heads in the thermostat cabinet for 5 days at 20 °C ± 0.5 °C.

- After the 5 days has elapsed, the final value of the oxygen consumption can be read on the measuring head.

- The set value and the tolerance of the calibration tablets are batch-dependent and are given on the label of the box of tablets. If the displayed measured value is within the specified tolerance, the overall measuring system is leaktight and the calibration was successful.
If it lies outside the specified tolerance, the instrument must be sent to the manufacturer for checking.

4. BOD standard solution of glutamic acid and glucose: (Checking the biological effectiveness and calibration)

Foreword:

In this method a liquid with a defined BOD is produced with the aid of a glutamic acid and glucose solution and inoculated with biological dilution water and diluted. This test is primarily intended for checking the effectiveness of the biology present at the place of use relative to the decomposition performance. The micro-organisms react differently to the substances to be decomposed so that the decomposition performance always differs depending on the microbiology and composition of the wastewater.
The decomposition performance of the microbiology can be checked in-situ with the aid of a specified standard on an organic material and compared with the data of other biologies. Hence, it is also possible here to calibrate the measuring head using the specified BOD standard.

Production of the inoculated dilution water:

The dilution water is produced from various salt solutions and subsequently microbiologically inoculated with a wastewater sample. As the dilution water itself already has a low BOD value, it must be determined as a control sample and then subtracted from the measured BOD value of the standard solution.
The salt solutions described here can be stored in opaque glass bottles at 0 – 4 ℃ for approx. 6 months without a loss in quality. However, prior to using the solutions, they need to be checked for sediments or flocculation. If any of these are detected, the solution must be discarded and replaced by a fresh one.

Note:

Only deionised water that is absolutely chlorine-free must be used for producing all solutions and for filling the measuring flask as free chlorine can significantly impair the biological processes. The chlorine must be removed by blowing out by air if necessary.

Preparation:

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.

Comment: The pH-value of this buffer solution should be 7.2 without further adjustment.

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 anhydrous 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 COD of 300 mg/l or a TOC of 100 mg/l.

- Prior to inoculation the wastewater sample must either be decanted or filtered with a coarse pleated filter so that it is free of suspended matter.

Production of the dilution water and inoculation.

- Add 1 ml of each of the salt solutions numbers 1 to 4 to a 1 l measuring flask and fill the flask to the mark with deionized water.

- Transfer this solution into a somewhat larger opaque vessel and add 20 ml of the inoculation material.

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

Production of the standard solutions for the measurement:

1. Standard solution: Dilution water for blank value.

- 0.5 ml of the inhibitor allylthiourea solution are pipetted into a 1 l measuring flask and the remainder filled with **dilution water** up to the mark.

Note:
If, as recommended, a 3-x determination of the blank value is carried out, approx. 1.5 l of this solution is needed.

2. Standard solution: Glucose glutamic acid solution.

- Weigh

150 mg D(+) glucose and

150 mg L glutamic acid

respectively into a 1 l measuring flask and mix with 0.5 ml of the inhibitor allylthiourea solution.

The flask is filled with **dilution water** up to the mark.

The solids take some time to dissolve and this can be accelerated by heating **gently** and mixing on a magnetic stirrer.

Measurement:

It is recommended to carry out 3x determination for both standard solutions respectively.

- Using an overflow measuring flask, measure out

432 ml of the standard solution 1 and
164 ml of the standard solution 2

and put each of the solutions into **separate** brown sample bottles.

For a 3x determination this procedure must be repeated twice more.

- Insert a magnetic stirring rod in each bottle and insert the respective rubber sleeve with 2 sodium hydroxide pellets in the neck of the bottle.

- If the temperature of the liquids is outside the range of 15-21 ℃, the temperature of the bottles must be adjusted before beginning

the measurement.

- Screw the measuring heads tightly onto the bottles and start measurement as described in the manual of the measuring heads.

- Place the bottles on the stirrer platform in the thermostat cabinet and let them incubate for 5 days.

Evaluation:

- Determine the average value of the multiple determinations from the BOD values of the 5th day for the two standard solutions.

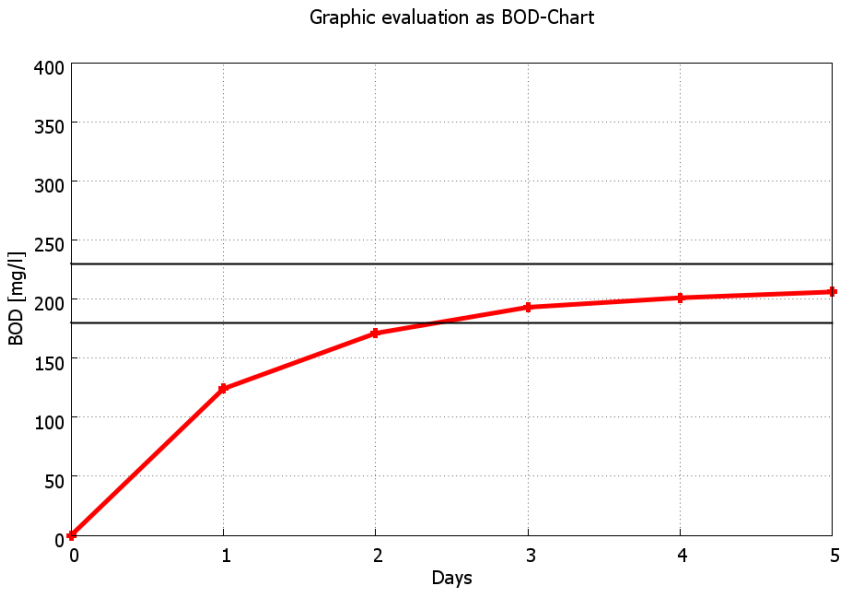
- The value of the 2nd standard solution must be by multiplied by a factor of 10 because of the dilution.

- The blank value that is given by BOD value of the standard solution 1 must be subtracted from this result.

- Hence the final result is:

[(BOD$_5$ standard solution 2)*10] - (BOD$_5$ standard solution 1)

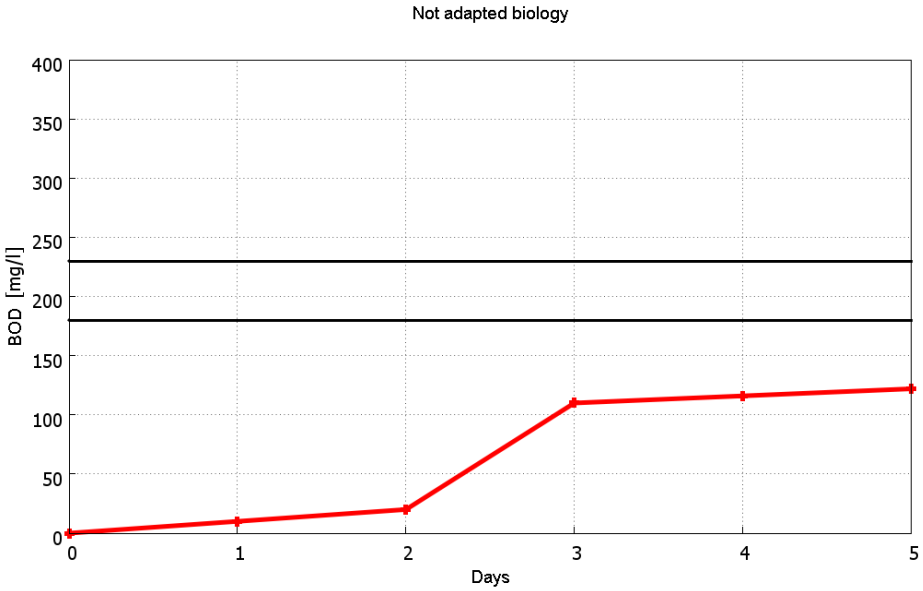
If the final result lies between 180 and 230 mg/l, the effectiveness of the decomposition process for the biology used is acceptable and the measuring heads are working properly.
The charted evaluation of the measurements results in the chart shown below.



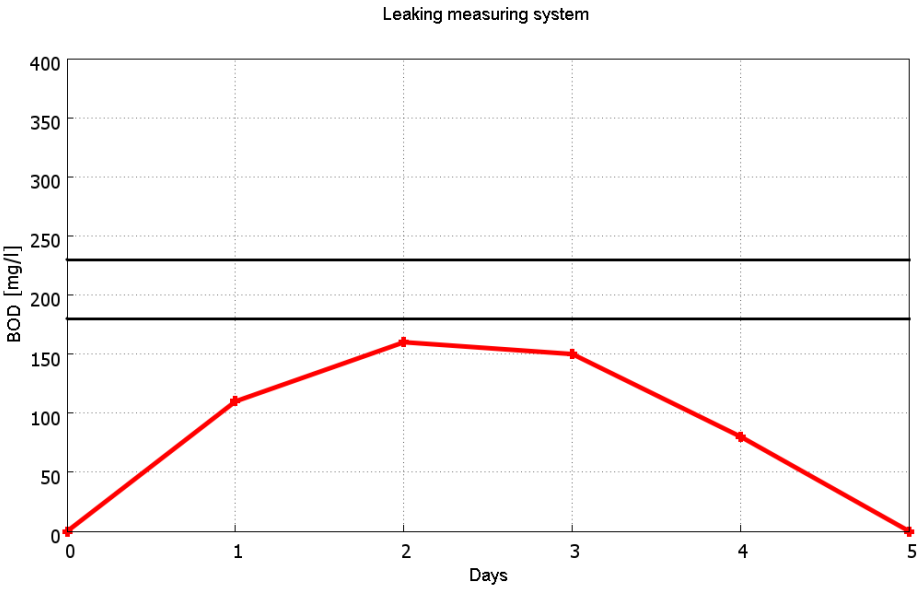
It is plain to see that the BOD value on the 5th day is within the specified limits of 180 – 230 mg/l

Interpretation

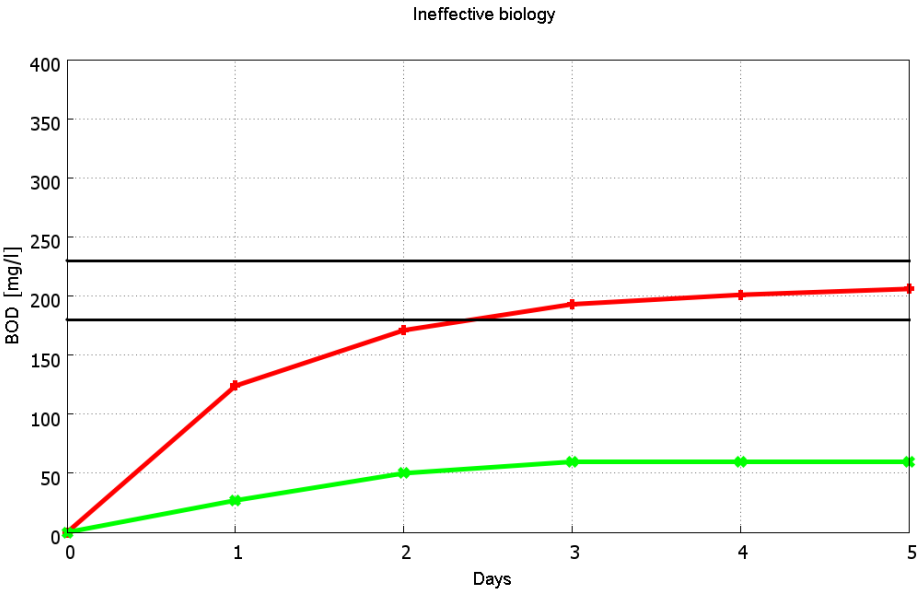
If microbiological organisms are placed in an environment that is unfamiliar to them, they mostly react with shock to the new environmental conditions and it is not possible to predict how long they will need to adapt to the new living conditions.
In the production of dilution water for biochemical measurements, under certain condition this can lead to curve progressions that deviate strongly from the ideal curve form.
In this case, it is important to correctly interpret the results in order to take suitable measures for error correction. The following charts serve here as an aid to decision-making and give instructions of how to proceed.



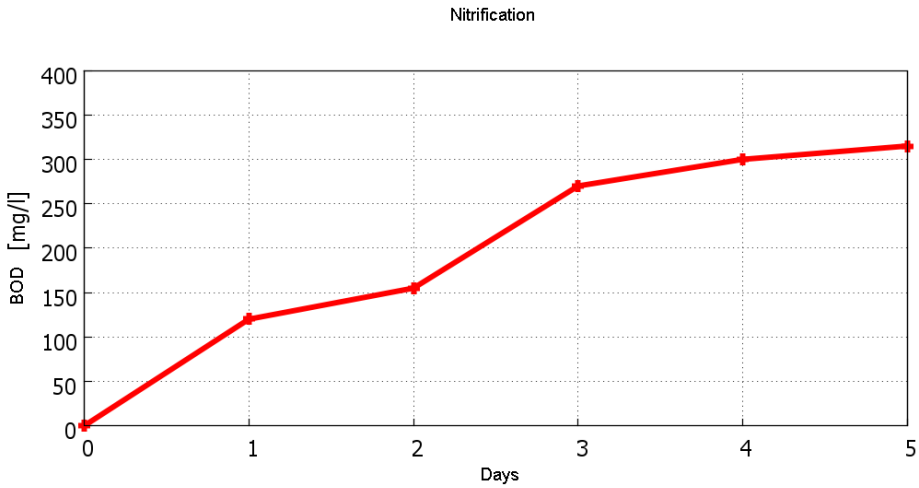
The biology has not yet adapted to the new conditions. Repeat the measurement with the dilution water that has aged in the meantime.



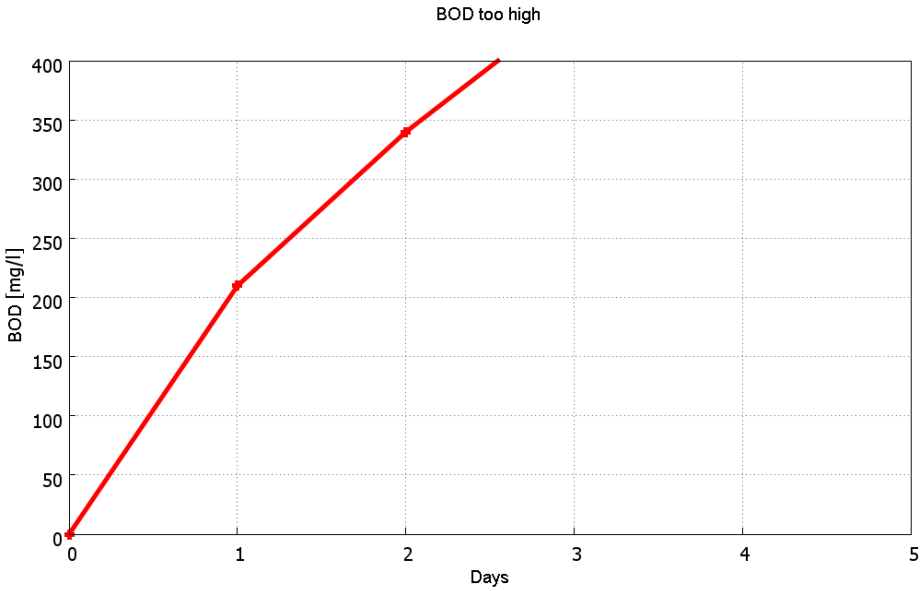
Leaking measuring system. Replace the rubber sleeve or the seal on the measuring head.



The upper curve shows the normal progression of a BOD measurement. In the lower curve the biology employed was not effective enough. It may have been inhibited by wastewater constituents. Carry out the BOD determination according to the application report [Inhibiting and toxic substances].



until approx. 2nd day: carbon oxidization. 2nd day up to 5th day: Oxidization of carbon and nitrate. Measurements without nitrification inhibitor or with already decomposed and therefore ineffective inhibitor.



Too high BOD; predilute the sample or use a smaller sample volume.

Bibliography

[1] BSB-Fibel, Grundlagen der Messtechnik, WTW Eigenverlag

[2] DIN/ISO 9000 ff

[3] OECD-Grundsätze der GLP, Umweltdirektorat der OECD, Paris 1999

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.

Any further questions? Please contact our Technical Support

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