BOD_5

Principle
Determination of ***5-day biochemical oxygen demand*** with inhibition of nitrification by ***5 mg/L allylthiourea***. The dissolved oxygen is analysed in an alkaline solution with a pyrocatechol derivative in the presence of Fe^{2+}, under which conditions a red dye is formed.

Range of Application
Surface water, low-load outflows of municipal or industrial sewage treatment plants with biological purification stage ***without*** additional inoculation.

Storage Information
The test reagents are stable at +2 to +8°C up to the expiry date given on the package.

Interferences
Peroxide compounds, powerful oxidizing agents, high chlorine concentrations and powerful reducing agents cause high-bias or low-bias results and interfere with the biochemical oxidation over 5 days.
Nitrite (NO_2^-) and Iron (Fe^{2+}) can interfere with the reaction if present in concentrations of at least 1 mg/L in the original sample.
The COD content should not exceed 25 mg/L in the original sample or 50 mg/L in the 1 : 2 dilution.
Samples with a high particulate content interfere with the determination – if necessary, carry out the analysis with the supernatant liquid after homogenizing and allowing the sediment to settle out.
Preserved or frozen samples can ***only*** be analysed with this procedure with the application (simplified inoculation procedure).
The measurement results must be subjected to plausibility checks (dilute and/or spike the water sample). This can be done with LCK 554 BOD_5 by means of a multiple determination.

Removal of Interferences
Samples that contain algae must be filtered before the analysis (1.2 µm filter; membrane filtration set LCW 904; to avoid high-bias results, rinse the filter with distilled water before use).

pH/Temperature
The pH of the water sample must be between pH 4 and pH 10.
The temperature of the water sample and the dilution water must be between 18 and 24°C.

For special attention
Samples of surface water that contain ***no municipal waste water*** are inoculated by means of a simplified procedure ***prior to the analysis*** (see application).

The transfer pipettes should be discarded after use. The beakers should be cleaned thoroughly with hot tap water after use, or, if strongly soiled, with a suitable cleansing agent.

Safety Advice
On grounds of quality and reliability, the analysis should be carried out only with original HACH LANGE accessories.

CADAS 100 (LPG 185 / ≥ LPG 210)
If this test is not already stored in your instrument please ask your HACH LANGE Agency for programming instructions.

Procedure I — LCK 554

Applies to all types of photometer

BOD_5

Edition 98/04

Sample Preparation
Please take note of the information under the heading "For special attention".

1. Original sample	
Introduce the magnetic stirring bar into a 100 mL beaker. Into the same beaker pipette	
Wastewater sample	40 mL
Place the beaker on the magnetic stirrer and stir for ***5 min*** at 500 – 750 rpm. This oxygenates the wastewater sample.	

2. Diluted sample	
Introduce the magnetic stirring bar into a 100 mL beaker. Into the same beaker pipette	
Wastewater sample chlorine-free drinking water (BOD_5 < 0.5 mg/L)	20 mL 20 mL
Place the beaker on the magnetic stirrer and stir for ***5 min*** at 500 – 750 rpm. This oxygenates the wastewater sample.	
The dilution factor must be taken into account when the BOD_5 content is calculated (measurement result x 2).	

Two sample cuvettes are needed for each wastewater sample. The ***sample cuvette 1*** is measured ***directly*** and the ***sample cuvette 2*** is measured after ***5 days***.
It is advisable to label the cuvettes:
e.g. ***A1*** = ***direct*** measurement
A5 = measurement after ***5 days***
A transfer pipette is used to fill the two sample cuvettes up to the brim in sequence with the prepared wastewater sample. Ensure that the ***sample cuvette 2 (measurement after 5 days)*** is ***free of air bubbles*** and seal it ***immediately***. Leave it for ***5 days at 20°C*** in the dark in a temperature-controlled cabinet or LT 20 dry thermostat.

Procedure II — LCK 554

Applies to all types of photometer

BOD_5

Edition 99/02

Procedure II: direct and after 5 days

1. Sample cuvette 1 (direct measurement) [see diagram A – I]
Place the funnel on the filled sample cuvette 1. ***Carefully*** pull the aluminium foil off the **DosiCap *Zip*** and pour the contents (tablets and glass beads) through the funnel ***into the sample cuvette 1***. Remove the funnel and immediately seal the ***sample cuvette 1*** with **DosiCap *Zip***, taking care that the cuvette contains ***no air bubbles***. ***NB:*** If the liquid meniscus falls below the cuvette opening when the funnel is removed, make up the volume by adding 2 to 4 glass beads. Repeatedly invert the sample cuvette 1 for ***3 min*** until the reagent tablets are completely dissolved. Wait ***3 min***, then thoroughly clean the outside of the cuvette again and evaluate immediate. ***Record the result!***
2. Sample cuvette 2 (measurement after 5 days) [see diagram A – I]
Open sample cuvette 2, which has been left to stand for ***5 days***, and place the funnel on it. ***Carefully*** pull the aluminium foil off the **DosiCap *Zip*** and pour the contents (tablets and glass beads) through the funnel ***into the sample cuvette 2***. Remove the funnel and immediately seal the ***sample cuvette 2*** with **DosiCap *Zip***, taking care that the cuvette contains ***no air bubbles***. ***NB:*** If the liquid meniscus falls below the cuvette opening when the funnel is removed, make up the volume by adding 2 to 4 glass beads. Repeatedly invert the sample cuvette 2 for ***3 min*** until the reagent tablets are completely dissolved. Wait ***3 min***, then thoroughly clean the outside of the cuvette again and evaluate immediate. ***Record the result!***

Data table — LCK 554

LP2W	***98/04***
BOD_5 • $F_1 = 0$ • $F_2 = 10.41$ • K = 0	
CADAS 30/30S/50/50S	***98/04***
BOD_5 • λ: 620 nm • Pro.: 1 • $F_1 = 0$ • $F_2 = 9.858$ • K = 0	
ISIS 6000/9000	***98/04***
BOD_5 • λ: 610 nm • Pro.: 1 • $F_1 = 0$ • $F_2 = 8.014$ • K = 0	
CADAS 100 / LPG 185	***98/04***
BOD_5 • λ: 620 nm • $F_1 = 9.63$ • $F_2 = 0$	
CADAS 100 / ≥ LPG 210	***98/04***
BOD_5 • λ: 620 nm • $F_1 = 9.63$ • K = 0	

Application — LCK 554

Applies to all types of photometer

BOD_5

Edition 99/02

Simplified inoculation procedure for surface water that is only ***minimally microbiologically*** contaminated. The procedure should be used for surface water that contains no municipal waste water.

Accessories
LZC 555 BioKit
LZP 065 reaction tubes with cap

Preparation of the inoculation solution

Add to ***reaction tube 1***	
inoculation material (from LZC 555) buffer solution (from LZC 555)	1 level dosing spoon (blue) 10 mL
Mix thoroughly for ***1 min*** and leave to settle for ***20 min***. Then pipette into ***reaction tube 2***	
solution from ***reaction tube 1*** chlorine-free tap water	0.2 mL 10 mL
and mix.	

Procedure I with cuvette test LCK 554

Fill ***sample cuvette 1*** with the sample or a diluted ***(1 : 2)*** sample, then analyse with ***procedure II (1. direct measurement)***.
Pipette 0.2 mL of the diluted solution from ***reaction tube 2*** into ***sample cuvette 2***, then top up with sample or diluted ***(1 : 2)*** sample. Leave to stand for ***5 days***, then evaluate with ***procedure II (2. measurement after 5 days)***.

Calculation of the BOD_5 content
Subtract 0.1 mg/L BOD_5 from the displayed result.

LASA 1 / plus **LCK 554**

BOD_5

Edition 98/04

Evaluation

1. Press "Mode" key and check program control number: **__ : 40**
2. Insert program filter **623 nm**.
3. Select test with "Mode" key.
4. Insert ***sample cuvette 1*** (direct measurement).
   Make a note of the displayed result (see table) "***A1***" resp.
   insert ***sample cuvette 2*** (measurement after 5 days).
   Make a note of the displayed result (see table) "***A5***".

Calculation of the BOD_5-concentration

A1 - A5 = mg/L BOD_5

Diluted samples:

The dilution factor must be taken into account when the BOD_5 content is calculated (measurement result x 2).

Parameter	Display	Meas. range
BOD_5	BOD5 LCK 554	0.5 – 12 mg/L

LASA 10 / 20 **LCK 554**

BOD_5

Edition 98/04

Evaluation

1. Press any key.
2. Check program control number: **__ : 40**
3. Select test with ↑ or ↓ key.
4. Insert ***sample cuvette 1*** (direct measurement).
   Make a note of the displayed result (see table) "***A1***" resp.
   insert ***sample cuvette 2*** (measurement after 5 days).
   Make a note of the displayed result (see table) "***A5***".

Calculation of the BOD_5-concentration

A1 - A5 = mg/L BOD_5

Diluted samples:

The dilution factor must be taken into account when the BOD_5 content is calculated (measurement result x 2).

Parameter	Display	Meas. range
BOD_5	BOD5 LCK 554	0.5 – 12 mg/L

LASA 30 **LCK 554**

BOD_5

Edition 98/04

Evaluation

1. Insert filter **605 nm**.
2. Select »Dr. Lange« mode.
3. Select test number (see below).
4. Control number must be **2**.
5. Insert ***sample cuvette 1*** (direct measurement) and press green key.
   Make a note of the displayed result (see table) "***A1***" resp.
   insert ***sample cuvette 2*** (measurement after 5 days) and press green key.
   Make a note of the displayed result (see table) "***A5***".

Calculation of the BOD_5-concentration

A1 - A5 = mg/L BOD_5

Diluted samples:

The dilution factor must be taken into account when the BOD_5 content is calculated (measurement result x 2).

Parameter	Test-No.	Meas. range
BOD_5	554	0.5 – 12 mg/L

LP2W **LCK 554**

BOD_5

Edition 98/04

Evaluation

1. Insert program filter **620 nm**.
2. Press "Tests" key until display (see below) appears.
3. Control number must be **6**.
4. Insert blank-value cuvette (distilled water) and press "Null" (zero) key.
5. Insert ***sample cuvette 1*** (direct measurement) and press "Ergebnis" (result) key.
   Make a note of the displayed result (see table) "***A1***" resp.
   insert ***sample cuvette 2*** (measurement after 5 days) and press "Ergebnis" (result) key.
   Make a note of the displayed result (see table) "***A5***".

Calculation of the BOD_5-concentration

A1 - A5 = mg/L BOD_5

Diluted samples:

The dilution factor must be taken into account when the BOD_5 content is calculated (measurement result x 2).

Parameter	Display	Meas. range
BOD_5	BOD5 LCK 554	0.5 – 12 mg/L

BOD_5

Edition 98/04

Evaluation

Insert ***sample cuvette 1*** (direct measurement).
Make a note of the displayed result (see table) "***A1***" resp.
insert ***sample cuvette 2*** (measurement after 5 days).
Make a note of the displayed result (see table) "***A5***".

Calculation of the BOD_5-concentration

A1 - A5 = mg/L BOD_5

Diluted samples:

The dilution factor must be taken into account when the BOD_5 content is calculated (measurement result x 2).

Parameter	Meas. range
BOD_5	0.5 – 12 mg/L

BOD_5

Edition 98/04

Evaluation

1. Check program control number:
   __ : 40 (CADAS 200)
   __ : 40 (ISIS 6000) ⇒ Select »CUVETTE TEST« mode.
2. Select test number (see below).
3. Control number must be **2**.
4. Insert ***sample cuvette 1*** (direct measurement) and press green key.
   Make a note of the displayed result (see table) "***A1***" resp. insert ***sample cuvette 2*** (measurement after 5 days) and press green key.
   Make a note of the displayed result (see table) "***A5***".

Calculation of the BOD_5-concentration

A1 - A5 = mg/L BOD_5

Diluted samples:

The dilution factor must be taken into account when the BOD_5 content is calculated (measurement result x 2).

Parameter	Test-No.	Meas. range
BOD_5	554	0.5 – 12 mg/L

BOD_5

Edition 98/04

Evaluation

1. Select »TEST« mode.
2. Select symbol (see below).
3. Check factors and measuring wavelength in memory "Mem" **(LPG 185)** or control number must be **8 (LPG 210)**.
4. Insert blank-value cuvette (distilled water) and press "NULL" (zero) key.
5. Insert ***sample cuvette 1*** (direct measurement) and press "MESS" (measure) key.
   Make a note of the displayed result (see table) "***A1***" resp. insert ***sample cuvette 2*** (measurement after 5 days) and press "MESS" (measure) key.
   Make a note of the displayed result (see table) "***A5***".

Calculation of the BOD_5-concentration

A1 - A5 = mg/L BOD_5

Diluted samples:

The dilution factor must be taken into account when the BOD_5 content is calculated (measurement result x 2).

Parameter	Symbol	Meas. range
BOD_5	554	0.5 – 12 mg/L

BOD_5

Edition 98/04

Evaluation

1. Select »Barcode Programs«.
2. Select test number (see below).
3. Control number must be **2**.
4. Insert ***sample cuvette 1*** (direct measurement) and press »Read«.
   Make a note of the displayed result (see table) "***A1***" resp. insert ***sample cuvette 2*** (measurement after 5 days) and press »Read«.
   Make a note of the displayed result (see table) "***A5***".

Calculation of the BOD_5-concentration

A1 - A5 = mg/L BOD_5

Diluted samples:

The dilution factor must be taken into account when the BOD_5 content is calculated (measurement result x 2).

Parameter	Test-No.	Meas. range
BOD_5	554	0.5 – 12 mg/L

Special note:
Measuring range for samples, not diluted: 0.5 - 6.0 mg/L BOD_5
Measuring range for diluted samples: 1.0 - 12.0 mg/L BOD_5

No.	Date	Sample and dilution	Result A1 Measuring direct	Result A5 Measuring after 5 days	Result BOD_5 (mg/L) A1 - A5
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					

HACH LANGE
UNITED FOR WATER QUALITY

Procedure
Applies to all types of photometer

Cuvette Test LCK 554

Procedure II direct and after 5 days