Theory:

BOD is Biochemical Oxygen Demand referred to as a “standardized laboratory procedure used to determine the relative oxygen requirements of wastewaters, effluents and polluted water. It is a measure of the molecular oxygen utilized during a specific incubation period for the biochemical degradation of organic material.

Bacteria and other microorganisms use organic substances for food as they metabolize organic material, they consume oxygen. Organics are broken down into simpler compounds such as CO\textsubscript{2} and H\textsubscript{2}O and energy released is used for growth and reproduction.

When this process occurs in water, the oxygen consumed is dissolved oxygen.

If oxygen is not continuously replaced, the DO level will decrease as the organics are decomposed by microbes.

Organic waste in sewage is one of the major types of water pollutants.

Impractical to isolate and identify each specific organic chemical and determine its concentration. Complete decomposition of organic material by microorganisms takes time: (approx. 60 - 90 days under normal conditions).

The total amount of oxygen required to “stabilize” all biodegradable organic compounds is the UBOD or BODL.

The 60 - 90 days is too long to wait for lab results (hard to manage discharge from sewage treatment plants with such a wait). More than 2/3 of BOD exerted in first 5 days, the BOD is a function of time and the BOD\textsubscript{5} is the common BOD used.

**BOD\textsubscript{5} Measurements**

1) Winkler method:

The standard method for measuring BOD\textsubscript{5} depends on incubation of 300 mL glass BOD bottles including portions of the sample for 5 days.

Two DO readings: initial and after 5 days’ incubation in dark at 20\textdegree C.

\[
\text{BOD}_5 = \frac{\text{DO}_1 - \text{DO}_5}{P}
\]
P = \frac{\text{volume taken from the sample}}{\text{volume of the bottle (300 ml)}}

2) Respirometric method

Same method as used in lab Standard methods or alternative ones, **This method provides a direct** measurement of oxygen consumed by microorganisms from an air or oxygen-enriched environment in a closed vessel under conditions of constant temperature and agitation.

Respirometry measures oxygen **uptake continuously over time** Oxygen reservoir is the air column above the sample.

As with Standard Methods, dilution may be required.

Sample bottles are sealed to prevent interference from external atmospheric pressure. Oxygen is provided by constant agitation (magnetic stir bar helps diffuse oxygen into sample).

Carbon dioxide is generated as heterotrophic bacteria oxidize organic material.

As CO₂ is generated, it is removed from the system by sodium hydroxide absorption and a pressure change is recorded.

Change in pressure is proportional to CO₂ generated. i.e., O₂ consumed, the final reading is converted to equivalence of BOD value.
Measuring ranges and sample volumes

The BOD level of a sample depends on the quantity of organic matter present, which can vary considerably. The Orbeco-Hellige Oxi700 is calibrated for the various sample volumes and the corresponding measuring ranges listed in the table below. The overall measuring range of the system is 0 – 4000 mg/l. For all measuring ranges, BOD is shown directly in mg/l.

<table>
<thead>
<tr>
<th>Expected BOD value, mg/l</th>
<th>Amount of sample to be used, ml</th>
<th>Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 40</td>
<td>432</td>
<td>1</td>
</tr>
<tr>
<td>0 – 80</td>
<td>365</td>
<td>2</td>
</tr>
<tr>
<td>0 – 200</td>
<td>250</td>
<td>5</td>
</tr>
<tr>
<td>0 – 400</td>
<td>164</td>
<td>10</td>
</tr>
<tr>
<td>0 – 800</td>
<td>97</td>
<td>20</td>
</tr>
<tr>
<td><strong>0 – 2000</strong></td>
<td><strong>43.5</strong></td>
<td><strong>50</strong></td>
</tr>
<tr>
<td>0 – 4000</td>
<td>22.7</td>
<td>100</td>
</tr>
</tbody>
</table>

Procedure:

1) Take the appropriate amount from a homogenous sample correctly consistent with the expected range of BOD, which can be estimated by the following equation \((\text{COD} / 2)\), (in our experiment the volume is 43.5 ml)

2) Pour the sample in the incubation bottle.

3) Put the magnetic stirrer in the bottle.

4) Add drops of nitrification inhibitor if necessary.

5) Add appropriate amount of Sodium hydroxide solution in its place.

6) Select the appropriate range from the sensor.

7) Close the bottle by the sensor.

8) Zero the sensor as instructions, and incubate the bottle in the BOD incubator at 20 C° for 5 days.

Automatic storing of measured values

Beginning with the start time of the measurement, the OxiTop® automatically stores one value every 24 hours. The individual measured values can be called up by actuating the push button, “S”. In
this case, the number of the measured value that corresponds to the day (1-5) appears first and is followed by the stored value. This is possible both during the measurement as well as after it.

**Measurement results**

A value is stored every 24 hours for the BOD5 determination using the OxiTop® measuring system. The display is in digits. The conversion into actual BOD values is performed by multiplying with factors and, as a result, subject to the sample volume. After 5 days, 5 values have been stored. Entered on an evaluation sheet or on graph paper, they reproduce the oxygen degradation curve of the sample. The fifth value is the required BOD5 value. Graphical evaluation has the advantage that the type of depletion can be recognized more easily. It is often readily apparent from the five daily values whether, for example, an inhibition or nitrification was present in the measurement of the sample.

**The necessary factor is calculated according to the BOD equation:**
At this point, the measuring principle should be mentioned again. The OxiTop measures the difference in pressure and calculates the BOD according to the equation mentioned above. As a result, the experimental conditions must fulfill the prerequisites of the equation! That is to say, the bottle volume must be 510 mL, an allowable filling volume (432 mL, 365 mL, 250 mL...) must be used and the measuring temperature must be 20°C. If this is not the case, an incorrect BOD value will inevitably result.