Early-BOD Test Kit

Applications Guide

Version 1.2

CheckLight Ltd.
P.O Box 72 Qiryat-Tiv`on 36000, Israel.
Tel: (972)-4-993-0530, Fax: (972)-4-953-3176
www.checklight.biz, info@checklight.biz

1. Technology Overview

1.1 Why is it important to determine BOD in water?

The Biochemical Oxygen Demand (BOD) test aims to determine the concentration of oxidizable and biodegradable organic compounds in (mainly heavily polluted) water. The BOD test is widely used to determine the degree to which a waste stream will contribute to pollution of receiving waters by depriving organisms in those waters (fish) of their source of oxygen. The BOD test is of prime importance in regulatory programs and in determining the overall health of receiving waters. Because of the 5-day lag, BOD5 results are seldom of any use for real time process adjustment or decision making.

1.2 The principle of the Early-BOD test

The active reagent is a freeze-dried preparation of *Vibrio harveyi*. The non-assimilable organic compounds in water (including carbohydrates, proteins, and complex nutrients) are first hydrolyzed by a mild acid pre-treatment. This treatment breaks down polymers into assimilable oligomers and monomers. Once exposed to the treated water sample, the hydrated bacteria will undergo prompt induction of luminescence providing the sample contains assimilable organic compounds.

Luminescence increases with time, with an intensity dependent on the concentration of the organic compound. Sub-ppm concentrations of different kinds of organic compounds can be determined within 2-3 hours. The testing procedure is simple as well: Only 1mL of sample is necessary to run the assay, making collection, storage, and disposal of sample material easy and inexpensive.

Comparative studies have found a high correlation between the standard 5 days long procedure and the Early-BOD test (Figure 1).

1.3 What is unique about CheckLight's Early-BOD test?

CheckLight developed a unique technology for determination of BOD in water in a **3-4 hours** long procedure.

In addition to the clear advantages of rapidity and simplicity, the test has additional benefits:

1. Acid hydrolysis of the tested water converts all the water flora into biodegradable nutrients (a potential oxygen consuming nutrient source not measured in the standard BOD method).

2. The test determines only the consumption of 0_2 due to bio-oxidation of organic carbon sources (rather than reduced inorganic compounds utilized by some litho-autotrophic bacteria).

3. The high sensitivity of the test allows extensive dilution of the sampled water before testing, thus eliminating possible inhibitory effects stemming from sample turbidity or the presence of toxic agents.

Acquiring this vital information in real time enables timely corrective measures in wastewater processing, lowering running costs, and mitigating the risk of heavy fines due to exceeding the BOD permit limit.
The test can serve as a powerful tool in municipal and industrial wastewater monitoring units in plants & environmental supervising authorities.

1.4 Comparison to Other Methods

Table 1.1 - BOD testing – Comparative Analysis

Test Feature	CheckLight Early-BOD	Standard BOD5 (biochemical oxygen demand)	COD (chemical oxygen demand)	TOC (total organic carbon)
Oxidant	Microbial luminescence as a respiratory by-product	Respiration by microbes	$K_2Cr_2O_7$	O_2, $KMnO_4$
Best use of method	Predicting wastewater treatment plant operations and receiving stream loads	Predicting wastewater treatment plant operations and receiving stream loads	Testing potentially toxic industrial wastes (gives a measure of the pollutant strength)	Total amount of carbon in organic pollutants
Possible interference by inorganics	No	No	Yes	Yes
Response time	3-4 hours	5 days (at least)	3-5 hours	Minutes to hours
Measures only waste that can be degraded	Yes	Yes	No	No
Test volume	Few milliliters	Hundreds of milliliters	Few milliliters	Few milliliters

Table 1.2 - BOD testing – Comparative Analysis (continued)

Test Feature	CheckLight Early-BOD Test	Standard BOD5 (biochemical oxygen demand)	COD (chemical oxygen demand)	TOC (total organic carbon)
Accuracy	Good	Poor	Varies	Good (although knowledge of total C is of limited use)
Reliability	Good	Questionable	Good	Good
Sensitivity to nitrification	No	Yes	No	No
Detection Limit	About 0.1mg/L	About 3 mg/L	About 5 mg/L	About 1 mg/L
Reagents generate toxic waste	No	No	Yes	No
Correlation with Standard	Yes	The Standard Method	Sometimes	Sometimes
Sensitivity to toxins	Low- due to high dilution	Medium- high (depends on dilution)	None	None
All organics are oxidized	Yes	Not always	Not always	Not always
Precision	Good	Poor	Good	Good

1.5 Repeat Dispensing in the Early-BOD Test- Recommended Products

All of CheckLight's **lab test procedures** include the dispensing of very low volumes (10 microliters) of hydrated bacterial suspension into the assay mix.
Since the overall time span of each test is short and light emitted by the bacteria in the sample is compared to light emitted in the negative control, one has to ensure rapid dispensing. Moreover, as each 10 microliter aliquot holds about one million cells, dispensing 9 or 11 microliter leads to a dramatic change in emitted light, and, hence, to skewed results. It is therefore essential to ensure accurate dispensing.

Given the above, the use of an automatic pipettor or repeat dispenser provides the optimal solution and should be regarded as an essential tool.

There are numerous products on the market. Among the recommended options for highly reliable products are:

1. Finnpipette Stepper model 4540 from Thermo Scientific-
http://www.thermo.com/com/cda/product/detail/0,1055,19353,00.html

2. Ripette electronic pippetor from Ritter -
http://www.ritter-online.de/e/medical_care/ripette/index.php

A less sophisticated line of products is the syringe-less version.

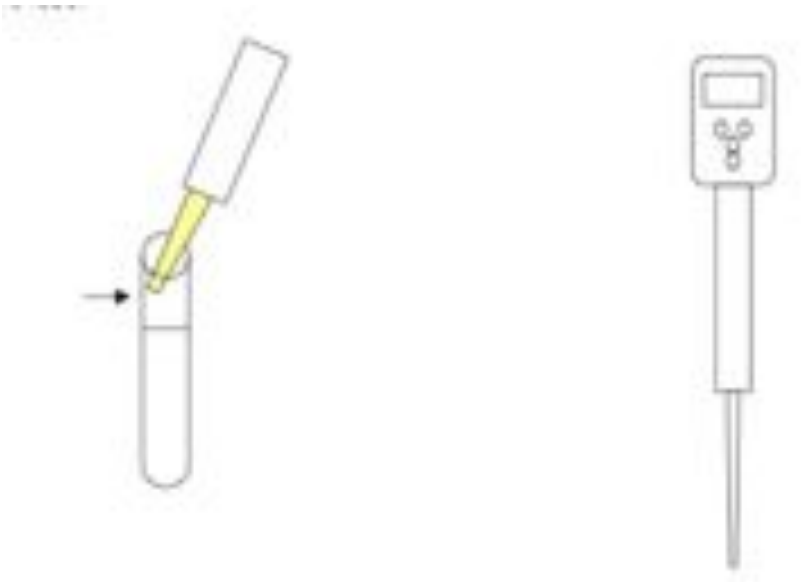
We provide a product manufactured by Microlit -
http://www.microlit.com/elec.htm

Instructions for use are provided in the next page.

A short training video clip on pipetting is also avaliable for download on our web site.

1.5.1 Instructions For Operating The Electronic Micropipette (Repeat Dispenser)

1. The device has 3 operation modes to choose from. The one relevant for use with CheckLight's kits is **CASE III – Stepper Mode**.
2. Follow the instructions for setting the Stepper Mode in the provided Operation Manual.
3. During the dispensing phase, it is very important to **touch the inner side of the tube with the edge of the dispensing tip** in order to ensure that each drop is released and captured in the tube.



1.6 Tips for preparing accessory solutions (not provided in the kit)

Sample pre-treatment requires the use of the following solutions which are not provided with the kit-

1. 7N NaOH. CAS Number: 1310-73-2
2. 7N HCl. CAS Number: 7647-01-0
3. 0.1M MOPS (4-Morpholinepropanesulfonic acid sodium salt), pH 6.8
   CAS Numbers: 71119-22-7 & 1132-61-2 (sodium salt & acid forms)

1. To prepare the sodium hydroxide solution the following steps should be carried out:

Weigh 28gr of NaOH pellets into 100ml final volume of water.

2. To prepare the hydrogen chloride solution the following steps should be carried out:

The concentration of HCl in the commercially available stock solution is 37%.
HCl concentration 37 % has a density of =1.19 g/ml. Hence, to obtain 7N HCl, use 58 ml of the 37% stock and adjust volume with water to 100ml with a graduated cylinder.

3. To prepare the MOPS buffer the following steps should be carried out:

Dissolve 1.156gr of MOPS sodium salt with 1.156gr of MOPS (acid form) in 90ml of double distilled water (<5ppb TOC). Adjust with concentrated HCl or NaOH to reach pH 6.8. Complete final volume to 100ml using a graduated cylinder.

Keep all stock solutions at room temperature. The NaOH and HCl are very stable (at least one year); the MOPS soluton should be kept in a light protected bottle and discarded once it turns yellowish.

2. Application of the Early- BOD Test in a Municipal Wastewater Treatment Plant

2.1 Overview

In heavily polluted waters (e.g., municipal wastewater), biochemical oxygen demand (BOD) and chemical oxygen demand (COD) tests are traditionally used for assessing the effectiveness of biological treatment. The BOD test is widely used to determine the degree to which a waste stream will contribute to pollution of receiving waters by depriving organisms in those waters (fish) of oxygen. The BOD test is of prime importance in regulatory programs and in determining the overall health of receiving waters.
The BOD test measures the ability of naturally occurring microorganisms to digest organic matter, usually in a 5 day incubation at 20°C, by analyzing the depletion of oxygen that measures biodegradable organic matter. The BOD analysis is an attempt to simulate the effect a waste will have on the dissolved oxygen of a stream by a laboratory test. It gives an indication of the amount of oxygen needed to stabilize or biologically oxidize the waste.
Chemical Oxygen Demand measures the ability of hot chromic acid solution to oxidize organic matter. This analyzes both biodegradable and non-biodegradable (refractory) organic matter.

The main disadvantage of the standard BOD test is the 5 day time lag and the difficulty in obtaining consistent repetitive values. The COD test, on the other hand, can be performed in a few hours, but the results of the test are usually higher that the corresponding BOD test for several reasons. Many organic compounds which are dichromate oxidizable are not biochemically oxidizable; Certain inorganic substances, such as sulfides, sulfites, thiosulfates, nitrites and ferrous iron are oxidized by dichromate, creating an inorganic COD, which is misleading when estimating the organic content of the wastewater.

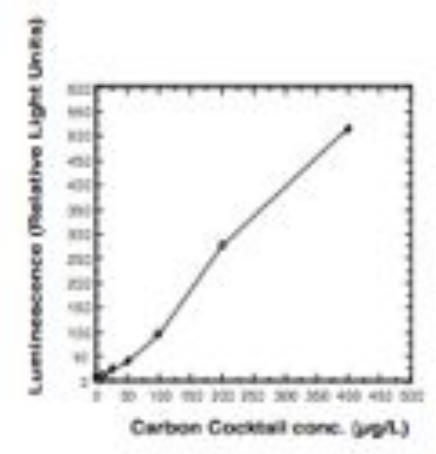
2.2 Case Study

CheckLight was able to demonstrate that its proprietary Early-BOD test kit could provide the means to measure in real time BOD values in industrial and municipal wastewater.
Unlike the COD test, it measures the concentration of biodegradable organic matter. Acquiring this vital information in real time enables timely corrective measures in wastewater processing.

2.2.1 The Method

The non-assimilable organic compounds in water (including carbohydrates, proteins, and complex nutrients) are first hydrolyzed by a mild HCl pre-treatment (0.7N HCl, 1 hour at 100°C). This treatment breaks down polymers into assimilable oligomers and monomers. CheckLight's specially formulated reagent is then exposed to the treated sample, and luminescence is recorded after 2-3 hours of incubation at ambient temperature. The level of luminescence is proportional to the concentration of utilizable organic carbon in the sample. Figure 1 shows the resultant measurements of the standard Carbon Cocktail used as reference and positive control in the test system.

Figure 1-



CheckLight Ltd.
P.O Box 72 Qiryat-Tiv`on 36000, Israel.
Tel: (972)-4-993-0530, Fax: (972)-4-953-3176
www.checklight.biz, info@checklight.biz

Data analysis is straightforward: **the higher the luminescence, the higher the concentration of utilizable carbon.**

2.2.2 The Results

In order to verify the accuracy and reliability of the CheckLight test, multiple samples were collected from a large municipal wastewater treatment plant and tested in parallel with both standard test (5 days long; measured in mg/L O_2) and Early-BOD Test (3 hours; measured in mg/L carbon). As can be seen in Figure 2, a high correlation was found between the tests.

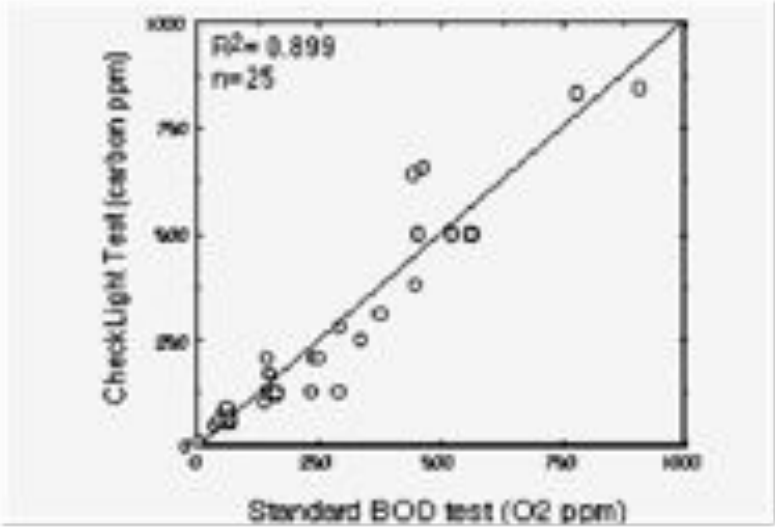
The operators noted that in addition to the clear advantages of rapidity and simplicity, the test has additional benefits:

1. Acid hydrolysis of the tested water converts all the water flora into bio-degradable nutrients (a potential oxygen consuming nutrient source not measured in the standard BOD method).

2. The test determines only the consumption of O_2 due to bio-oxidation of organic carbon sources (rather than reduced inorganic compounds utilized by some litho-autotrophic bacteria).

3. The high sensitivity of the test allows extensive dilution of the sampled water before testing thus minimizing possible inhibitory effects stemming from sample turbidity or the presence of toxic agents.

Figure 2:



CheckLight Ltd.
P.O Box 72 Qiryat-Tiv`on 36000, Israel.
Tel: (972)-4-993-0530, Fax: (972)-4-953-3176
www.checklight.biz, info@checklight.biz

Whitewater Group

2.2.3 The Outcome

The wastewater treatment plant operators were pleased with the results from the comparison and can now regularly use the **CheckLight** kit to monitor treatment plant efficiency thus enabling rapid response to changing conditions.

CheckLight kits are favoured for the following reasons:

- **Time saving** - accurate and meaningful results of dozens of samples obtained within 3-4 hours. No need for sample transportation to external labs.
- **Reliable** - data highly correlative with standard test.
- **Easy to use** – only basic laboratory skills are required to use kits.
- **Cost effective**- enabling frequent monitoring for rapid response to changing conditions in water quality.

3. Frequently Asked Questions

Q: Are luminous bacteria dangerous? Do I need to be a trained microbiologist in order to be able to conduct CheckLight's assays?

A: Luminous bacteria are not pathogenic and are harmless. No special skill is required to carry out the different tests other than basic laboratory techniques (pipetting, dilutions etc) and equipment (pipettor, tips, luminometer).

Q: What are the benefits of obtaining rapid information on BOD levels?

A: Acquiring this vital information in real time enables timely corrective measures in wastewater processing, lowering running costs, and mitigating the risk of heavy fines due to exceeding the BOD permit limit.

Q: What are the advantages of using this test over the standard COD test?

A: Chemical Oxygen Demand measures the ability of hot chromic acid solution to oxidize organic matter. This analyzes both biodegradable and non-biodegradable (refractory) organic matter. The COD test can be completed in a few hours, but the results of the test are usually higher that the corresponding BOD test for several reasons. Many organic compounds which are dichromate oxidizable are not biochemically oxidizable- certain inorganic substances, such as sulfides, sulfites, thiosulfates, nitrites and ferrous iron are oxidized by dichromate, creating an inorganic COD, which is misleading when estimating the organic content of the wastewater. Moreover, the COD test is limited in sensitivity and requires the addition of toxic chemicals such as mercury to eliminate interferences such as chloride between estimated BOD and measured BOD.

The luminescence-based BOD test truly measures the ability of the bacteria to digest the organic matter present in the sample.

Q: Why is there a control in each assay?

A: Readings of the negative control are needed in order to obtain the background reading of the cells without the sample. In addition, a set of positive controls is run in order to calibrate the system and provide the proper "translation" of light units to carbon equivalent units.

Q: Can I "play around" with the volumes of bacteria, buffers and other assay conditions?

A: No. It is extremely important to follow the test protocol instructions to the word. Since the test is very sensitive, any seemingly minor variations result in poor reliability.

Q: Can I reuse the provided test vials?

A: Due to the high sensitivity of the assay, care should be taken to keep all vials, plastic tips, and pipettes extremely clean. Do not reuse test vials and do not wash glassware, pipettors, or pipette tips with detergent, acid, or solvents.

Q: What is the shelf life of the reagents?

A: The shelf life of the freeze dried bacteria is one year when stored in a deep-freezer (-10°C to -20°C). Reagent should not be stored in a self-defrosting freezer, which defrosts by warming up periodically. The assay buffers should be stored in a regular refrigerator (~4°C) and under no circumstances should they be frozen.

CheckLight Ltd.
P.O Box 72 Qiryat-Tiv`on 36000, Israel.
Tel: (972)-4-993-0530, Fax: (972)-4-953-3176
www.checklight.biz, info@checklight.biz

4. Bibliography

1. Lenore S. Clescerl, Arnold E. Greenberg, Andrew D. Eaton (1999). *Standard Methods for Examination of Water & Wastewater* (20th ed.). Washington, DC: American Public Health Association. ISBN 0-87553-235-7.
2. U.S. Environmental Protection Agency (EPA). Washington, DC. "Secondary Treatment Regulation." *Code of Federal Regulations,* 40 CFR Part 133.