

Research Article

A Method for Determination of Hydrocarbon and Nitrogenous Biochemical Oxygen Demands in Natural Waters by Manometric Method

Suren Sargsyan* 

Institute of Hydroecology and Ichthyology of the Scientific Center of Zoology and Hydroecology, National Academy of Science of Republic of Armenia, Yerevan, Armenia

Abstract

The aim of the work was the simultaneous determination of hydrocarbon biochemical oxygen demand - cBOD and nBOD (nitrogen) in the same water sample without using a nitrification inhibitor by the manometric method. Daily measurements of cBOD are determined by recording the pressure drop resulting from the absorption of carbon dioxide released by the oxidation of hydrocarbon organic matter by microorganisms with KOH (potassium hydroxide). Here, the processes of oxidation of mineral substances in water and especially the developing nitrification processes, which absorb oxygen dissolved in water and cause an additional pressure drop in the vessel, are a disturbing factor, for the measurement of which, in the daily 24-hour cycle of registrations, it is recommended to close the absorption hole of the vial with KOH for 8 hours. The 24-hour nBOD and cBOD results are calculated from the result of the pressure drop in these 8 hours. In the paper, in the form of a table, the detailed form of calculation of the recorded results of BODs at two pH-s of a natural water sample within 1-20 days is given. The possibility of anthropogenic impact assessment using this method is shown on the example of natural water. It is concluded that with the proposed method of measuring BODs records the complete and separate results of cBOD are recorded under conditions of developing nitrification in water. All information from both nitrification and BOD-full is recorded simultaneously, which gives a complete picture of the overall oxygen balance in the water. The proposed method is practically applicable and fully comparable with the results obtained by the standard manometric method.

Keywords

nBOD, cBOD, BOD Full, Method, Nitrification, Total Oxygen Balance

1. Introduction

In modern conditions, the use of improved and highly accurate research methods is becoming particularly relevant. Biochemical oxygen demand (BOD) studies are widely used in ecological water monitoring. BOD measurement reflects the processes of decomposition of organic substances in water.

It involves two phases: carbonic, during which oxygen is consumed for the microbial decomposition of carbon compounds (cBOD), and nitrogen, during which mineral nitrogen compounds are oxidized (nBOD) [1]. It reflects the degree of water pollution according to the amount of organic matter in

*Corresponding author: Sarsur@bk.ru (Suren Sargsyan)

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the water that can be oxidized by microorganisms [2]. The great advantage of the manometric method over other BOD determination methods [3, 4] is that it provides dynamic determination of practically all BOD from day 1 to day 20 or more days in the same water sample. However, the exact determination of cBOD (hydrocarbon) is always hindered by the oxidation processes of partial mineral substances (sulfides, iron, etc.), and especially the nitrification processes developing in water [5, 6], for the inhibition of which, inhibitor thiourea or allylthiourea is added to the water sample [7-10].

An attempt was made to quantitatively calculate nitrification in water with and without an inhibitor using manometric method by measuring BOD-5 [11]. Other researchers [12-14] have also conducted longer studies (30 days) in order to study in more detail the characteristics of the transformation of organic matter in natural waters, but using an inhibitor. Sometimes, especially in long-term experiments, the nitrifying bacteria adapt to the inhibitor and nitrification develops at some point, distorting the results. And increasing the concentration of the inhibitor suppresses the activity of saprophytic bacteria, which ensure the normal course of cBOD.

The last best method for clean determination of cBOD-5 (carbonic) without the use of an inhibitor by manometric method is the carbonate turbidimetric method [15]. It is based on the fact that no carbon dioxide is produced in nitrification and during the oxidation of minerals, and therefore only carbon dioxide separated by hydrocarbon oxidation is absorbed by potassium hydroxide. However, this method determines the specific cBOD-5 by stopping the experiment and recording the accumulated carbon dioxide. In this mode, there is no consecutive dynamic survey from the same water sample.

Another important aspect of the issue is that by passing the nitrification process or suppressing it with an inhibitor, we lose information about the amount of oxygen spent on that process, which plays a very large role in the overall oxygen balance of natural waters, for example in rivers [7, 16, 17].

In order to overcome these issues and to obtain full information about the total consumption of oxygen in water and all BODs, we made a technical innovation in the manometric method [18], the essence of which is to interrupt the absorption of carbon dioxide gas by potassium hydroxide in the system in a periodic discrete period and by computational method deriving nBOD and cBOD that was used in this study and is detailed here.

2. Method

2.1. Sample Collection

The investigated waters were taken from three observation points.

№1-observation point - Hrazdan River in the zone of Yerevan city under the Kievyan bridge, (9.03.2024), water temperature 6.5 °C, natural pH=8.3 and adjusted pH=7.0 with

sulfuric acid.

№2 - observation point - Hrazdan River before Bzhni vilage (23.06.2024), water temperature is 15.5 °C, natural pH=8.4.

№3 - observation point - Hrazdan River after Bzhni vilage (23.06.2024) water temperature is 15.6 °C, natural pH=8.4.

2.2. BOD Determination.

BOD tests were performed with the device whose block diagram is shown in Figure 1 below.

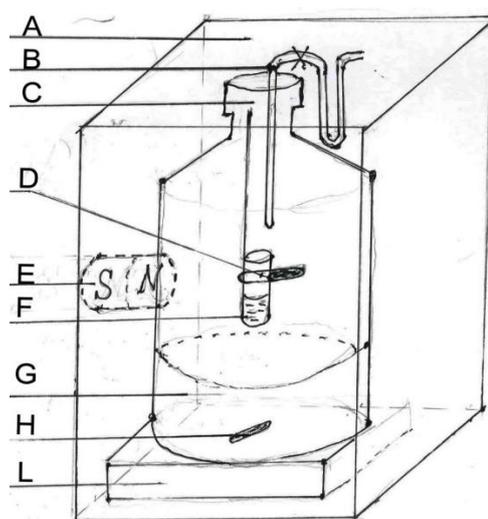


Figure 1. Block diagram of BOD measurement device. Explanation in the text.

The water sample is placed in a vessel (G) and hermetically sealed with a stopper (C) to which a manometer (B) is attached to measure the pressure in the vessel.

For the absorption of carbon dioxide in the vessel, a vial (F) containing 5-6 drops of 45% KOH is fixed in the air space under the stopper, at the entrance of which a magnetic valve (D) is fixed to which when a permanent magnet (E) is brought near (from the outside of the vessel) the KOH hole is closed. After being closed for 8 hours, the KOH hole opens when the magnet is moved from the opposite side of the vessel. After 16 hours it closes again for 8 hours and so on every day. Closing and opening the hole of the vial with KOH in the specified way does not at all break the hermeticity of the vessel with the water sample and the continuous recording of pressure differences. To enhance the gas exchange of carbon dioxide and oxygen in the water sample, the water sample is stirred with a magnet (H) on a magnetic stirrer (L). BOD was determined in a 188 ml water sample at 20.0 °C in the dark in incubator (A). All BOD results were recalculated to mg O₂/liter according to the formula given [15].

2.3. Sample Analyses

All waters were filtered through a Gas-38 filter to remove large zooplankton prior to determination. The amount of easily oxidizable organic matter in water samples was measured by determining the degree of permanganate oxidation (PO) of water by Kubel's method [19]. Total hardness (TH) of water was determined complexometrically [20]. The concentration of hydrogen ion in water was pH-measured with a pH-121 device. The results were processed statistically [21].

3. Results and Discussion

BOD-1-20 was analyzed in the water sample taken from observation point №1 at its own pH=8.3 and adjusted pH=7.0. In order to show in detail the calculation of BODs by the proposed method, the results obtained directly from two characteristic samples and the way of their calculation are given in the Table 1.

Table 1. The form of calculation of BOD (mgO₂/l) 1-20 in the waters of Hrazdan River № 1 observation point at two pH levels.

Column	pH=7.0					pH=8.3				
	1	2	3	4	5	6	7	8	9	10
Bod1-20	8-hournBod	24-hournBod	Bod full	Bod c	Bod Full st.	8-hournBod	24-hournBod	Bod full	Bod c	Bod Full st.
1	0.09	0.39	0.60	0.21	0.15	0.03	0.18	0.27	0.09	0.63
2	0.18	0.63	0.99	0.36	0.45	0.09	0.36	0.51	0.15	0.78
3	0.24	0.75	1.35	0.60	0.99	0.15	0.45	0.69	0.24	0.91
4	0.27	0.81	1.14	0.33	0.66	0.15	0.66	0.93	0.27	1.05
5	0.27	0.84	1.14	0.30	0.79	0.30	1.11	1.35	0.24	1.29
1-5	<->	3.42	5.22	1.80	3.00	<->	2.76	3.75	0.99	4.68
6	0.30	0.90	1.14	0.24	1.11	0.45	1.56	1.65	0.09	1.65
7	0.30	0.96	1.20	0.24	2.07	0.60	1.92	2.01	0.09	1.89
8	0.33	0.99	1.26	0.27	1.41	0.69	1.98	2.04	0.06	2.07
9	0.33	0.99	1.26	0.27	1.53	0.63	1.80	2.04	0.24	1.95
10	0.33	0.93	1.26	0.33	1.41	0.57	1.56	2.13	0.57	1.65
5-10	<->	4.77	6.12	1.35	7.53	<->	8.82	9.87	1.05	9.21
1-10	<->	8.19	11.3	3.15	10.5	<->	11.6	13.6	2.04	13.9
11	0.30	0.84	1.20	0.36	1.41	0.48	1.26	2.10	0.84	1.71
12	0.27	0.81	1.26	0.45	1.38	0.36	0.99	1.83	0.84	1.59
13	0.27	0.81	1.23	0.42	1.44	0.30	0.90	1.71	0.91	1.44
14	0.27	0.78	1.20	0.42	1.32	0.27	0.78	1.32	0.54	1.29
15	0.24	0.66	1.11	0.45	1.17	0.24	0.69	1.23	0.54	1.02
10-15	<->	3.90	6.00	2.10	6.72	<->	4.62	8.19	3.57	7.05
1-15	<->	12.1	17.3	5.25	17.3	<->	16.2	21.8	5.61	20.9
16	0.21	0.57	0.84	0.27	0.90	0.21	0.51	0.90	0.39	0.99
17	0.18	0.54	0.81	0.24	0.81	0.12	0.27	0.66	0.39	0.66
18	0.18	0.57	0.81	0.24	0.75	0.06	0.24	0.54	0.30	0.63
19	0.21	0.57	0.84	0.27	0.72	0.09	0.24	0.54	0.30	0.57
20	0.18	0.54	0.75	0.21	0.72	0.06	0.18	0.45	0.27	0.45

Column	pH=7.0					pH=8.3				
	1	2	3	4	5	6	7	8	9	10
Bod1-20	8-hournBod	24-hournBod	Bod full	Bod c	Bod Full st.	8-hournBod	24-hournBod	Bod full	Bod c	Bod Full st.
15-20	<->	2.79	4.05	1.23	3.90	<->	1.44	3.09	1.65	3.30
1-20	<->	14.9	21.4	6.48	21.2	<->	17.6	24.9	7.25	24.2

Note: the average statistical variation of the results is ± 0.02 , <-> it is not necessary the total calculation

Columns 1 and 6 of the table give direct readings of O₂ absorption (at pH=7.0 and pH=8.3, respectively) recorded with the hole of the KOH vial closed for 8 hours to show the form of nBOD (nitrogen) calculation.

Columns 2 and 7 show the calculated amount of absorbed O₂ in 24 hours with the hole of the KOH vial closed (conventional nBOD), which is theoretically equal to three times the value of absorbed O₂ in 8 hours. However for the most accurate calculation, it is necessary to take three times the arithmetic mean value of the results of 8 hours of closed KOH of that day and 8 hours of closed KOH of the next day, which was used in all the results. Such an averaged calculation more accurately reflects the tendencies (dynamics) of the possible development or decrease of nitrification in the results of the following days. In later stages of the records, when the results are slow to change or repeat, for example, for the 24 hours nBOD on day 20, three times of the 8 hours recorded result can be taken, as shown in Table 1 for BOD-20 only.

Columns 3 and 8 of the table give the BOD-full results, which directly show the change in the manometer reading of 8 hours before closing the hole in the KOH vial and the result recorded after 16 hours of opening the hole. In these 24 hours (8+16 hours), the result of the pressure drop caused by the absorbed O₂ includes, firstly, the absorption of O₂ in the water by autonomous oxidative processes, which are not accompanied by the release of CO₂ (oxidation of mineral substances, nitrification - conditional nBOD), and secondly due to the absorption of CO₂ gas by KOH, (which is equivalent to the absorption of the same amount of O₂) accumulated in the

vessel in the state of the closed hole of the KOH vial at 8 hours, and after opening the KOH hole for 16 hours, due to the cost of O₂ in cBOD.

Columns 4 and 9 of the table give cBOD values, which are calculated by the difference between the daily readings of BOD-full and nBOD.

Columns 5 and 10 of the table show the results of the BOD full (st.) standard recorded in the manometric method in days 1-20 in parallel water samples with the hole of the KOH vial always open.

This appears to be a test for the comparability of the BOD-full results (columns 3 and 8) measured with the KOH hole closed and open. According to the table they are completely comparable.

In table 1, from the observation of the dynamics of the results of nBOD (24hours) at pH=7.0 (2-column) and pH=8.3 (7-column), it can be seen that at pH=8.3 a strong pronounced outbreak of nitrification is observed from 5 to 15 days, with a sharp peak on the 8th day. This is a typical expressed dynamics of nitrification, which repeats the dynamics of NH₄⁺ (ammonium) ion transformation in river water during the days [22, 23].

At pH=7.0, nitrification also proceeds but with a weak expressed maximum.

Table 2 shows the average results of three experiments at the same pH-s from that same №1 observation point - with only consecutive 5-day cumulative BOD values within 20 days.

Table 2. The average results of BOD (mgO₂/l) of three experiments in the waters of observation point №1 of Hrazdan River at two pH levels and relative standard deviation in % (RSD%) for the BOD 1-20.

Bod days	pH=7.0				pH=8.3			
	Bod-full st.	Bod-full	nBod	cBod	Bod-full st.	Bod-full	nBod	cBod
1-5	3.84	5.04	3.15	1.82	6.63	6.42	5.77	1.65
5-10	6.78	6.72	4.83	1.89	9.45	9.30	7.47	1.83
1-10	10.62	11.76	7.98	3.78	16.08	15.72	12.24	3.48

Bod days	pH=7.0				pH=8.3			
	Bod-full st.	Bod-full	nBod	cBod	Bod-full st.	Bod-full	nBod	cBod
10-15	6.60	6.30	4.23	2.07	5.64	6.21	3.75	2.46
1-15	17.22	18.06	12.21	5.85	21.72	21.93	15.99	5.94
15-20	3.96	3.54	2.28	1.26	2.46	2.37	1.11	1.26
1-20	21.18	21.60	14.49	7.11	24.18	24.3	17.10	7.20
RSD%	3.4%	2.0%	2.3%	11.0%	6.2%	5.3%	9.0%	1.3%

As can be seen from the dynamics of the results of [Table 2](#) and the total results of BOD-1-20, at pH=8.3 the nitrification processes are noticeably developing and on the 20th day the total nBOD has significantly higher results than at pH=7.0 (about 12 %). The reason for this may be that pH=8.3 is close to the optimum of the nitrification process (pH=7.6-8.6). Comparing the cBOD recorded dynamics of the sum results of 1-5; 5-10; 10-15 and 15-20 at both pHs, the expressed peak cBOD in 10-15 is observed, which indicates that the water is dominated by hard-to-oxidize organic substances.

Comparison of BOD-full st. (KOH hole always open) 1-20 total results in the table with discrete records (KOH hole 8 hours closed and 16 hours open) with BOD-full 1-20 results at pH=7.0 (21.18 and 21.60 mg O₂/l, relative error 1.9%) and at pH=8.3 (24.18 and 24.30 mg O₂/l, relative error 0.5%) show very close comparable results. This confirms the comparability

of the proposed discrete method of BOD recording with the standard method of the manometric method. The RSD of this method is satisfactory and comparable to other methods.

In order to clarify the possibility of registering subtle changes in BOD in the proposed method, water quality indicators were studied before (№2 -observation point - not affected by anthropogenic influence) and after (№3 observation point, after the village was affected by anthropogenic influence) of Bzhni village of the Hrazdan River. Permanganate oxidation (PO) was equal to 2.20 mg O/liter and water hardness was 5.6 mg-eqv/l in observation point №2. BOD results are given in [Table 3](#). At the same time, permanganate oxidation in observation point №3 was equal to 3.80 mg O/l and water hardness was 5.8 mg-eqv/l. BOD results are given in [Table 3](#) measured at natural pH=8.4.

Table 3. BOD (mg O₂/l) results before (№2) and after (№3) Bzhni village and relative standard deviation in % (RSD%) for the BOD 1-20.

Bod Days	№2- observation point				№3- observation point			
	Bod-full st.	Bod-full	nBod	cBod	Bod- full st.	Bod-full	nBod	cBod
1-5	3.84	4.86	3.03	1.83	3.06	5.94	3.78	2.16
5-10	5.79	4.92	2.25	2.67	7.35	6.49	3.24	4.15
1-10	9.63	9.78	5.28	4.50	10.41	12.33	7.02	5.31
10-15	3.12	3.00	1.71	1.29	5.07	4.32	2.22	2.10
1-15	12.75	12.78	6.99	5.79	15.48	16.65	9.24	7.41
15-20	2.49	2.88	1.92	0.96	3.45	2.61	0.72	1.89
1-20	15.24	15.66	8.91	6.75	18.93	19.26	9.96	9.30
RSD%	2.6%	6.0%	3.7%	3.0%	8.2%	7.9%	4.1%	3.9%

In [Table 3](#), the comparison of the results of BOD-full st. (KOH hole always open) with discrete records (KOH hole 8 hours closed and 16 hours open) with the results of BOD-full 1-20 very close results are observed in both observation points,

the difference of which is 2.7% in observation point № 2 and 1.7% in № 3. At the same time, it can be seen from [Table 3](#) that the total oxygen balance calculated by the results of BOD-full 1-20 is 23% worse after Bzhni village (19.26 mg

O₂/l) than before Bzhni village (15.66 mg O₂/l). The results of cBOD 1-20 and nBOD 1-20 obtained by discrete registrations in Table 3 show that the reason for this bad oxygen balance (high oxygen consumption) after the village of Bzhni is caused by a significantly high level of cBOD (9.30 mg O₂/l) than before Bzhni village (6.75 mg O₂/l). It is a consequence of human factors, domestic and technical direct discharges into the river, as a result of which a significant increase in the PO indicator is also recorded - from 2.20 mg O/l to 3.80 mg O/l. Water hardness increases slightly. Deterioration of water quality in this observation point was also recorded in studies of previous years [24-26].

Table 3 also shows the pattern of cBOD dynamics in two observation points. Maximum values are recorded in cBOD 5-10 compared to cBOD 1-5; 10-15 and 15-20 testimonies. The nBOD results are also high after Bzhni village and show monotonically decreasing dynamics.

4. Conclusion

- 1) Performance of discrete periods of carbon dioxide absorption by potassium hydroxide in BOD measurements ensures complete and discrete cBOD results from the same water sample in intact water during 1-20 days of developing nitrification. Not using a nitrification inhibitor ensures the full development of natural microflora during experiments.
- 2) All information from both nitrification and BOD-full is recorded simultaneously, which gives a complete picture of the total oxygen balance in the water. Obtaining this information from the same water sample simplifies experiment setup, reduces water consumption and increases the reliability of results.
- 3) The proposed method is practically applicable and fully comparable with the results obtained by the standard manometric method.
- 4) The method allows evaluating changes in natural water quality after anthropogenic impacts. By the proposed method allows expanding the applicability of the manometric method.

Abbreviations

BOD	Biochemical Oxygen Demand
cBOD	Hydrocarbon Biochemical Oxygen Demand
nBOD	Nitrogenous Biochemical Oxygen Demand
BOD-full	Biochemical Oxygen Demand Full
KOH	Potassium Hydroxide
CO ₂	Carbon Dioxide
PO	Permanganate Oxidation
TH	Total Hardness

Author Contributions

Suren Sargsyan is the sole author. The author read and approved the final manuscript.

Conflicts of Interest

The author declares no conflicts of interest.

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