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# Determination of Biochemical Oxygen Demand (BOD) Without Nitrification and Mineral Oxidant Bacteria Interferences by Carbonate Turbidimetry.

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**ABSTRACT**

Biochemical Oxygen Demand (BOD) is a generalized parameter in the environmental monitoring of water and wastewater. In the conventional methods, oxidation of mineral materials such as sulfides and ferrous iron as well as nitrification processes could consume dissolved oxygen and cause the interferences in the BOD measurement. Based on carbonate turbidimetry, a new simple method for determination of BOD in aqueous samples was developed to eliminate these interferences. In this method, BOD is determined by indirect measuring of carbon dioxide produced from degradation of biodegradable organic compounds by microorganisms. Simplicity, low cost and availability of required equipment in most water laboratories are the advantages of the presented method. In the experimental section, optimization of influencing parameters, including pH and initial organic load of synthetic solutions was performed. The results of BOD analysis for municipal and industrial wastewater samples by the proposed method were comparable with those of the standard manometric method.

**Keywords:** BOD determination, carbonate turbidimetric method, Water and wastewater pollution, environmental analysis

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## INTRODUCTION

Discharge of municipal and industrial wastewaters as well as agricultural and industrial drainage containing organic materials in the water resource cause the reduction of dissolved oxygen (DO) [1, 2]. Biochemical oxygen demand (BOD) is an empirical parameter that refers to the amount of required oxygen for microorganisms to oxidize organic materials in wastewater, effluent, and polluted water samples [3]. Dilution and manometric methods are the two most conventional methods for measuring the BOD. In these methods, BOD is calculated through the changes in the amount of DO in water samples or by pressure drop, which is the result of oxygen consumption in a bottle containing the sample [4].

In the dilution method, samples are incubated n-days (5 days as $BOD_5$ in the standard test) at 20 °C in a dark bottle and the initial and final amount of oxygen is determined using an amperometric sensor or iodometric titration [5, 6]. The BOD is calculated by the following equation:

$$BOD_n = [(C_1 - C_2) - (V_t - V_e)(C_3 - C_4) / V_t]V_t/V_e$$

Where $C_1$ is the initial oxygen concentration of sample, $C_2$ is the oxygen concentration after n-day of incubation, $C_3$ is the initial oxygen concentration of blank, $C_4$ is the blank oxygen concentration after n-day incubation, $V_e$ is the initial sample volume, and $V_t$ is the final sample volume after dilution.

Dilution method is a standard test method for BOD. However, it has some disadvantages such as necessity for aeration of some samples (for example, the samples containing hydrogen peroxide). Dilution is a critical step in this method and the accurate estimation of dilution order is very important. Moreover, DO sensors always show some uncertainties in the samples with high electrical conductivity.

In the manometric method, the pressure drop due to oxygen consumption in a bottle containing sample is measured by a manometer. The produced carbon dioxide is absorbed into a potassium hydroxide solution. BOD is calculated through following equation:

$$BOD_n = \frac{M(O_2)}{R.T_m}\left(\frac{V_{tot}-V_l}{V_l} + \alpha\frac{T_m}{T_0}\right).\Delta p(O_2)$$

Where $M(O_2)$ is Molecular weight of oxygen (32000mg/mol) , $R.T_m$ is Gas constant (83,144L.hPa/(mol.K)), $V_{tot}$ is Bottle volume [ml], $V_l$ is Sample volume [ml], α is Bunsen absorption coefficient (0.03103), $\Delta p(O_2)$ Difference of the partial oxygen pressure [hPa], $T_m$ is Measuring temperaturefor BOD and $T_0$ is Temperature (273.15 K).

In spite of having a lot of advantages, manometric measurements need some requirements that must be met. One of the disadvantages of this method is the impact of temperature on the gas pressure. A little change in temperature can lead to a change in pressure difference and consequently, the BOD amount. On the other hand, for instruments based on measuring pressure reduction, to equalize temperature of sample and incubator, reading of initial pressure is performed 20 minutes after inserting the sample bottle into the incubator. This could be an origin of error in measurements. Also, entering a little amount of air into the bottle sample during incubation period causes all measurements not to be valid.

Generally, the nitrification processes could consume dissolved oxygen and cause the interferences in the BOD measurement [7, 8]. Moreover, the BOD is affected by oxidation of inorganic materials such as sulfides and ferrous iron [5, 9, 10]. Because of the importance of BOD measurement in the environmental monitoring, many attempts have been made to develop new rapid methods for determination of BOD [6, 11, 12]. However, most of these methods cannot be regarded as standard methods and suffer from some limitations [13, 14]. In order to eliminate the noted interferences, a method basically identical to that of manometric one is presented, in which the BOD is determined by indirect measuring of carbon dioxide. Turbidity produced by converting carbon dioxide to carbonate in the presence of barium chloride is determined and related to the BOD amount. One of the most important advantages of this method, unlike the two mentioned standard methods, is that the oxygen consumed in nitrification and other oxidant process such as mineral oxidant bacteria has no quota in calculations. This is because the products of inorganic biooxidation do not enter to the absorber solution and consequently not any precipitation with barium ion. The method is

performed only with a turbidity meter or a spectrometer that can be found in most laboratories and does not need DO sensors or expensive sensitive manometers.

## MATERIALS AND METHODS

*Sample preparation*

In the proposed method, the sample is incubated at 20 °C for a given period of time in a dark bottle. As organic materials are consumed by microorganisms, carbon dioxide is released and then absorbed by potassium hydroxide solution on the headspace of the sample as the following reactions:

$C_xH_yO_zN + O_2 \rightarrow CO_2 + H_2O + NH_3 + Energy$ Catabolism

$CO_2 + KOH \rightarrow K_2CO_3 + H_2O$ Absorption

Then carbonate concentration is measured turbimetrically as barium carbonate:

$$Ba^{2+} + CO_3^{2-} \rightarrow BaCO_3\ (s)$$

Produced carbonate amount is in proportion to the amount of consumed oxygen for oxidizing biodegradable organic materials.

*Chemicals and Supplements*

All chemicals used in the experiments were in the analytical reagent grade and were purchased from Merck Co. The solutions were prepared in distilled water. The biochemical oxygen demand measurements were performed by a commercial BOD meter (model Aqualytic Al 606). Turbidity determination was performed by a commercial WTW turbidity meter (model Turb 550). A commercial Metrohm pH meter (model 827) was used for all pH measurements.

Four nutrient solutions were prepared according to the following instructions:

*Phosphate buffer solution*: 21.75 g $K_2HPO_4$ , 8.5 g $KH_2PO_4$, 33.4 g $Na_2HPO_4$ , and 1.7 g $NH_4Cl$ was dissolved in water and then diluted to a final volume of 1000 mL.

*Calcium chloride solution*: 27.5 g $CaCl_2$ was dissolved in water and then diluted to a final volume of 1000 mL.

*Magnesium sulfate solution*: 22.5 g $MgSO_4.7H_2O$ was dissolved in water and then diluted to a final volume of 1000 mL.

*Iron chloride solution*: 0.25 g $FeCl_3.6H_2O$ was dissolved in water and then diluted to a final volume of 1000 mL.

*Potassium hydroxide (absorber)*: 40 g potassium hydroxide rinsed by deionized water to remove absorbed carbon dioxide and diluted to a final volume of 100 ml after dissolution and stored in a closed bottle away from air contact.

*Experimental procedure*

1000 ml of water sample was placed in a 2 L Becker, and then 1ml of each nutrient solution was added to the Becker. The samples pH was adjusted at 6.5 to 7.5. Then 224 ml of the prepared sample was placed in an opaque glass bottle containing a magnet. Five droplets of a 40% KOH solution were placed into the container mounted on the cap to absorb carbon dioxide produced in the period of incubation and the bottle cap was tightly fastened so that no air could diffuse into it. The bottle was incubated at 20 °C for 5 days in an incubator while the sample was being agitated on a stirrer to accelerate carbon dioxide and oxygen transfer (Figure 1).

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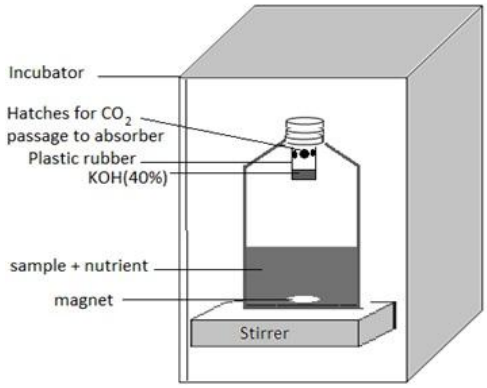


**Figure 1: Schematic apparatus used for BOD measurement.**

After five days, potassium hydroxide solution was moved to a 25 ml balloon and diluted to volume. The carbonate content of absorption solution was determined by turbidity meter using barium chloride as a suspension reagent based on calibration curve.

*Calculations*

BOD value is obtained from the concentration of carbonate according to the following equation:

$$BOD = [C_{carbonate}\ (32.00/60.01)]V_{balloon}/V_{sample}$$

Where C carbonate is carbonate concentration (mg/L) in balloon after absorber dilution which obtained from calibration curve, $V_{balloon}$ is balloon volume used to absorber dilution, $V_{sample}$ is incubated sample volume (ml).

## RESULTS AND DISCUSSION

*Optimization of pH*

To obtain the optimum pH range of the method, the synthetic seeded solutions of glucose with four different pH levels were prepared and the $BOD_5$ was analyzed by manometric and turbidimetric methods (Table. 1).

**Table 1: Optimization of pH for $BOD_5$ (mg $O_2$/L) measurement**

| | $BOD_5$ in different pHs | | |
|---|---|---|---|
| **Method** | **6.5** | **7.0** | **7.5** |
| Manometric (Mean $BOD_5$) | 54 | 48 | 47 |
| Carbonate turbidimetric ($BOD_5$) | 56, 47, 42, 61, 53 (14*) | 39, 53, 40, 55, 51 (16*) | 43, 50, 41, 52, 45 (10*) |
| Carbonate turbidimetric (Mean $BOD_5$) | 51.8 | 47.6 | 46.2 |
| * RSD% | | | |

As shown in Table 1, the optimum pH for the measurements is between 6.5 to 7.5 and in this range the mean value of BOD, obtained by carbonate turbidimetric method, is very close to that of manometric. However, at pH=8.0, a negative significant deviation was observed. This could be due to the solubility of the carbon dioxide in aqueous solution of sample at high pH, which results in the formation of soluble forms of carbonate and bicarbonate ion. The relative standard deviations (%) of the Results of proposed method are shown in Table 1. The RSD of this method is satisfactory and comparable to other methods. It has been

reported the RSD between the respirometric test, iodometric method, and oxygen sensor were 1–35% [15, 16]. These deviations can be explained due to the heterogeneity of the samples.

*Dynamic range of the method*

To investigate the linear response range of the method, glucose solutions were prepared in the range of 1-1000 mg $O_2$/L. To prevent the shortage of oxygen in the period of incubation, a lower volume of sample having higher BOD was chosen. All sample bottles were 500 ml and measurements were performed in pH= 7.

**Table 2: $BOD_5$(mg $O_2$/L) Results for dynamic range of proposed method compare to manometric standard method**

| BOD5 value in the sample volume (ml) | | | | | |
|---|---|---|---|---|---|
| **Method** | **244** | **244** | **244** | **157** | **94** |
| Manometric (Mean $BOD_5$) | 5.7 | 42 | 97 | 320 | 878 |
| Carbonate Turbidimetric ($BOD_5$) | 6, 4, 4,7,4 | 39, 53, 34, 55, 51 | 95, 77, 81, 84, 85 | 263, 278, 261, 281, 257 | 650, 677, 631, 643, 659 |
| Carbonate Turbidimetric ($BOD_5$) | 5.2 (-0.7*) | 46.4 (9.5*) | 84.4 (-14.9*) | 268 (-19.4*) | 652 (-40.48) |
| *Relative error (%) | | | | | |

As shown in Table 2, the mean value of BOD, obtained by carbonate turbidimetric method, is very close to that of manometric in BOD lower than 50 mg $O_2$/L. However, at BOD>50, a negative significant deviation in relative error was observed. Therefore, using this method, a linear range was obtained between generally in the range of 1–50 mg $O_2$/L.

*Real sample analysis*

To assess the feasibility of the application of the proposed method for the analysis of environmental samples, three different matrix wastewater samples were analyzed by both manometric and turbidimetric methods. Nutrient solutions were added and the pH of water and waste water samples was adjusted by the addition of diluted sulfuric acid or sodium hydroxide and buffer solutions before incubation. Table 3 shows that the results of presented method are comparable with standard manometric method and it is suitable for the determination of BOD in urban and industrial water and waste water samples.

One of the advantages of the turbidimetric method, as compared with the conventional manometric and dilution methods, is that the nitrifying bacteria and oxidation of sulfide and iron have no influence on the experimental results. The metabolites products from microorganisms' activities include nitrate, sulfate and some minerals which are soluble in the water, are not absorbed by potassium hydroxide solution at the top of the sample bottle [8]. Therefore, contrary to the manometric or dilution method, no inhibitor reagents are needed to add to the samples. Also, the results of BOD are not affected by the errors from sensitive apparatus of DO meter and manometer. On the other hand, the required equipment for turbidimetric method is usually found in every water and wastewater laboratory.

**Table 3: BOD determination by both manometric and turbidimetric methods in real samples**

| Sample | Carbonate turbidimetric method | Manometric standard method |
|---|---|---|
| Urban wastewater | 558 (60*) | 492 |
| | 1670 (30*) | 1842 |
| | 2480 (30*) | 2752 |
| Treated petrochemical wastewater | 42 (2*) | 46 |
| | 92 (2*) | 83 |
| | 105 (2*) | 136 |
| Untreated petrochemical wastewater | 97 (8*) | 119 |
| | 255 (8*) | 231 |
| | 337 (8*) | 309 |
| *Dilution factor | | |

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## CONCLUSION

In this study, we described a new, simple and inexpensive method for determination of BOD in environmental samples. Optimum pH range for measurement, like other conventional methods, was between 6.5 -7.5. The samples with BOD in the range of 1-50 mg $O_2$/L can be directly analyzed and those with higher concentrations will be analyzed after an appropriate dilution. The results and RSD of presented method are comparable with standard manometric method and it can be applied for the BOD analysis of real water effluents such as urban and industrial wastewater samples.

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