BIOCHEMICAL OXYGEN DEMAND—ITS CHEMICAL REACTION, MATHEMATICAL INTERPRETATION AND METHODOLOGY

BY A. K. BASU

(Central Inland Fisheries Research Institute, Barrackpore)

Any one who studies water pollution and industrial effluents comes across the ever important three words—Biochemical Oxygen Demand, which means the amount of oxygen, in mg., taken up by 1 litre of the sample (usually diluted with sufficient well-oxygenated tap-water or synthetic dilution water made by aerating the distilled water and adding the nutrient salts) or the quantity of dissolved oxygen required to effect stabilisation by aerobic bacterial action that portion of the dissolved organic matter which can be oxidised in 5 days at 20° C. It has been observed that in India, Germany and U.S.A. the usual practice is to do B.O.D. tests at 20° C. (68° F.) but in England most of the B.O.D. experiments are done at 18·3° C. (65° F.) even though this is giving way to 20° C. rapidly. But in practice and interpretation the experiment is not so simple as that. In the present paper a comprehensive attempt to explain the basic methodology, chemistry and kinetics involved in B.O.D. experiment will be made.

DYNAMICS OF CHEMICAL REACTIONS AND ITS MATHEMATICAL DERIVATIONS

Sewage and industrial wastes containing significant amounts of putrescible organic matter will exert B.O.D. As a matter of fact the higher the putrescible organic matter, the higher is the B.O.D. It must be clearly known that only dissolved organic matter (oxidisable) which acts as food for bacteria and other organisms is responsible for exertion of B.O.D. The average composition of domestic sewage may be stated as follows:

- Carbohydrate .. 50%
- Nitrogenous compound .. 40%
- Fats and Grease .. 10%

For the purpose of brevity, sewage will be taken as a representative material to understand all biochemical reactions so far as B.O.D. is concerned. The organic matter in sewage has high energy value material and is utilised as
a source of energy by the living organisms as soon as they are discharged to
the nearest water course. Helped by their own peculiar enzymes these
living organisms break down the organic matter in the presence of oxygen
into products of lower energy and into stable inorganic substances.

\[
\text{Organic matter} + \text{Oxygen} + \text{Living organisms} \rightarrow \text{CO}_2, \text{NH}_3 \text{ and } \text{H}_2\text{O}
\]

As a result of a series of biochemical reactions both decay and decomposition
phenomena take place and a gradual stabilisation of putrescible matter is
achieved. This process of aerobic decomposition is reflected by gradual
deficit of dissolved oxygen from the solution with the consequent satisfaction
of B.O.D. in a certain number of days at standard temperature. It is well
known that whereas there is 21% by volume of oxygen in atmosphere the
dissolved oxygen in freshwater at 50° F. is only 0.5 to 1% (or 0.8%) by
volume. If, however, owing to excessive demand this D.O. is exhausted
then immediately aerobic type of bacteria is replaced by anaerobic type of
bacterial population to carry out the work. One of the peculiar behaviours
of this anaerobic bacterial population is that they can procure the oxygen
required for their life from oxygen bound in the organic matter itself and as
such they do not have to depend on available dissolved oxygen. The end-
point of all anaerobic digestion is somewhat different from that of the aerobic
one. Whereas in the case of aerobic digestion CO\textsubscript{2}, NH\textsubscript{3} and H\textsubscript{2}O are the
waste products available and are free from any disagreeable smell, in the case
of anaerobic digestion the reverse is true.
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The mechanisms and trend of synthesis and degradation of protoplasms in the natural condition may be shown as hereunder.

The entire system of aerobic and anaerobic digestion of organic matter may possibly be represented as follows:
The liquefaction, hydrolysis and decomposition of fats, nitrogenous matter and carbohydrates produce end products of alcohol, ketone (aldehyde) and organic acids in the anaerobic process of stabilisation. Figure 1, as is reported by Theriult (1927) shows that the progressive exertion of B.O.D. takes place in two stages: The first stage involves mainly the breaking down of carbonaceous matter while the second stage involves the oxidation of ammonium and ammonia to nitrite and nitrate. Even though complete stabilisation of organic substances requires approximately 100 days or so the reactions, as the curve indicates, are so nearly complete at the end of 20 days that the oxygen demand for that period may be considered indicative of the total demand. The rate of oxygen utilisation by the microbial organisms being greatest during the first part of the reaction period and physical conditions affecting sewage being not constant for more than a few days, it is acceptable to all concerned to define the oxygen requirements for the first 5 days as oxygen demand value. It has been estimated to be 68% of the total demand and is equivalent to stability for 5 days as shown by the methylene blue reduction test. Long-term B.O.D. or Ultimate Oxygen Demand (U.O.D.) requires more time and is a space-consuming experiment; however it can be calculated approximately by the use of the following formula:
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U.O.D. = 2·67·C + 4·57·N,

where C = organic carbon, N = sum of organic and ammoniacal nitrogen in mg./lit. Mathematically speaking B.O.D. is a time-temperature function and the rate of exertion of B.O.D. at any time is (i) independent of D.O. concentration of water (if D.O. is more than 4 mg./lit.), showing that the rate-determining steps is not in any way connected with oxygen, and (ii) proportional to the B.O.D. left out to be exerted at that particular time. Streeter and Phelps (1925) established a law for the first stage oxidation of carbonaceous matter in the B.O.D. reaction as follows:

"The rate of biochemical oxidation of organic matter is proportional to the remaining concentration of unoxidised substances, the concentration being measured in terms of oxidisability."

The B.O.D. equation

Let L = Total B.O.D. (Ultimate Oxygen Demand); L_t = B.O.D. remaining at t; and Y = B.O.D. satisfied at time: as such,

\[ L = Y + L_t \]

\[ \frac{L_t}{L} \] = fraction of B.O.D. remaining at time, t.

\[ 1 - \frac{L_t}{L} \] = fraction satisfied at time, t.

This relationship may be expressed as a differential equation,

\[ -\frac{dL}{dt} = KL \] (1)

where K is a constant expressing rate of oxidation.

Integrating (1),

\[ \log \frac{L_t}{L} = -Kt \] (2)

Putting it in common log,

\[ \log_{10} \frac{L_t}{L} = -kt \] (3)

\[ \frac{L_t}{L} = 10^{-kt} \] = fraction remaining at time, t.

\[ 1 - \frac{L_t}{L} = 1 - 10^{-kt} \] = fraction oxidised (4)

Multiplying (4) by L,

\[ L - L_t = L (1 - 10^{-kt}) \]
since \( L - L_t = Y \) the entire equation may be written as 
\[ Y = L (1 - 10^{-kt}) \]
where \( k = 0.4343 \times K \).

Phelps (1944) established a value for \( k = 0.1 \) at 20°C. and is supported by Theriult (op. cit.). The value for \( k = 0.1 \) corresponding to a daily oxidation rate of 20.6% of U.O.D. or total B.O.D. at the beginning of day. The value of \( k \) varies at different temperatures and an equation has been developed from the data which are as follows:
\[ k_T = k_{30} [1.047(T-20)] \]
where \( T \) is in °C.

This, along with others, signifies that temperature is one of the important factors in B.O.D. test. An increase (or decrease) by 1°C may mean a variation of ± 5% B.O.D. Theriult (op. cit.) has, however, suggested a modified formula,
\[ L_T = L_{30} (0.02T + 0.60) \]
But recent investigations showed that the total B.O.D., first stage, does not primarily depend on the temperature.
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The importance of $k$ could be easily judged from the above equation and it is only when $k$ of two or more sewage or trade effluents are identical they should be compared in terms of B.O.D. The deoxygenation rate constant, $k$, has special significance in reckoning the rate of self-purification of streams. In shallow and swift-flowing streams, $k$ may be greater than 0.1. When the value of $k$ is greater, then the time of flow required for a certain degree of purification is shorter. The values of 0.2 and 0.3 have been found for some rivers of medium size. In India, the $k$ value of river Gomti has been estimated as 0.15 (Bhaskaran, 1962). The values $k = 0.3$, 0.2 and 0.1 correspond to an oxidation each day of 75%, 54% and 30% of B.O.D. remaining respectively.

The successful experimentation of B.O.D. is dependent not only on organic matter and available oxygen but also upon biological life which promotes the rate of chemical reactions. It is imperative to have many different types of bacteria and a large number of aquatic micro-organisms. That is why while doing B.O.D. test of industrial wastes, it is necessary to inoculate the dilution water with stale sewage. Basically, there are two theories about the oxidation reaction of organic matter in course of B.O.D. reaction. One view is that bacteria in course of their multiplication feed upon and digest the organic matter. Protozoa and other micro-organisms feed upon the bacteria and by limiting their numbers permit their action to continue. As a matter of fact, without the work of these predators bacterial growth would soon reach maximum and cease before all the available organic
matter has been digested. The other view about this is that the enzymes or ferments which accompany bacterial growth accelerate or catalyse the oxidation and thus are responsible for the destruction of various forms of organic matter.

**Methodology**

The underlying principle, as is evident from the discussions above, is to determine the quantity of D.O. in mg./lit., required for stabilisation of decomposable organic matter by diluting suitable portion of the sample with aerated dilution water both immediately and after a period of 5 days' incubation at 20° C. (68° F.). The technique may seem simple yet with utmost care only an experienced analyst can do it. Quite many factors, such as temperature variation, dilution used, composition of dilution water, nitrification, presence of toxic substances, the nature and amount of bacterial seed, presence of anaerobic organisms, caustic alkalinity and mineral acidity may influence the test.

**Sampling**

Sampling of sewage (or industrial wastes) may be done by either "grab" method or "composite" method. It is always better to get a representative sample not only by collecting it hourly or so, but also by taking the volume of sample each time in proportion to the rate of flow of sewage or wastes at that time. Samples so collected should be preserved in ice box or a refrigeration unit at 3-4° C. in order to avoid any deterioration of samples by biochemical actions. Samples so collected should be neutralised to pH 7-0, if they are acidic or alkaline. Free chlorine or similar compounds present in the sample should be removed by addition of sodium thiosulphate or sodium sulphate taking care that only the minimal required amount is added.

**Apparatus**

B.O.D. incubator (20° C. ±0.5); B.O.D. bottles (300 ml. or so); Carboys—20 lit.; Burettes—50 ml.; Pipettes—different sizes; Conical flasks—500 ml.

**Reagents**

1. *Manganous sulphate*: Dissolve 480 gm. of MnSO₄·4H₂O (400 gm. MnSO₄·2H₂O or 384 gm. of MnSO₄·H₂O) in distilled water, filter and dilute to 1 lit. in volumetric flask.

2. *Alkaline iodide with azide*: Dissolve 40 gm. of NaOH, 15 gm. of potassium iodide and 1 gm. of NaN₃. Cool and make up to 100 ml. (This reagent should not give any colour with starch solution when diluted and
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acidified.) It is better to prepare the solution in 100 ml. amount than 1 litre amount.

(3) *Sulphuric acid:* 36 N.

(4) *Starch solution:* Make a paste of about 2 gm. starch in water. Pour into 200 ml. of boiling distilled water, stir and allow to settle overnight. Use the supernatant, which may be preserved by adding salicylic acid (1.25 gm./lit. supernatant solution).

(5) *Sodium thiosulphate:* Dissolve 6.205 gm. of $\text{Na}_2\text{S}_2\text{O}_3\cdot8\text{H}_2\text{O}$ in freshly boiled and cooled distilled water and dilute to 1 litre (1 ml. of 0.025 N sodium thiosulphate is equivalent to 0.2 mg. oxygen). Thiosulphate solution thus prepared may be preserved by adding 5 ml. chloroform and it is advisable that each day before titration the strength of thiosulphate be assessed.

(6) *Dilution water:* Surface water which is to receive the trade waste or sewage may be used as dilution water because it will give most significant results from the point of actual operating conditions since the biological growth available for the test would be same in concentration and type as will be available for further digestion of the discharged trade wastes or sewage. But any variations in the dilution water may cause disagreement in the results. Ordinarily, in the laboratory, to get comparable results it is customary to use synthetic dilution water by using double distilled water as base solution. The distilled water is taken in a carboy and to it is added 1 ml. each of (A), (B), (C) and (D) for each litre of dilution water.

(A) Dissolve 8.5 gm. $\text{KH}_2\text{PO}_4$, 33.4 gm. $\text{Na}_2\text{HPO}_4\cdot7\text{H}_2\text{O}$ and 1.7 gm. of $\text{NH}_4\text{Cl}$ in about 500 ml. of distilled water and dilute to 1 litre (pH 7.0).

(B) Dissolve 22.5 gm. $\text{MgSO}_4\cdot7\text{H}_2\text{O}$ in distilled water and dilute to 1 litre.

(C) Dissolve 22.5 gm. of calcium chloride in distilled water and dilute to 1 litre.

(D) Dissolve 0.25 gm. $\text{FeCl}_3\cdot6\text{H}_2\text{O}$ in distilled water and dilute to 1 litre.

In order to prevent inaccuracies in the dissolved oxygen determination, it is desirable that oxygen content of this dilution water should be brought to saturation limit by aerating the entire volume of dilution water. This also permits oxidation of any accidental presence of organic matter and allows dissolved oxygen to come to equilibrium with atmospheric oxygen at 20° C. (Tap water may also be used when distilled water is not available but it must be remembered that the tap water containing appreciable amounts of chlorine should be avoided). Presence of caustic alkalinity,
mineral acidity, chlorine, copper from the still or its condenser, and chromium from chromic acid mixtures used for cleaning the bottles may also have some inhibiting effects. The quality of dilution water and seed material may be checked by preparing a standard solution containing 300 mg./lit. of glucose or glutamic acid. The standard glucose solution should show B.O.D. of 224 ± 11 mg./lit., the standard glutamic solution should show a B.O.D. of 217 ± 10 mg./lit, and a mixture in equal proportions should have a B.O.D. of 220 ± 10 mg./lit.

(7) Seeding material.—Stale sewage or river or any surface water containing sufficient number of bacteria or other organisms and yet not enough to increase the oxygen demand of the dilution water is needed. (Stale sewage may be defined as that sewage which has been stored long enough to be septic and to provide an environment suitable for the development of biological flora). If there are nitrifying organisms in the seeding material, then the results after 5 days' incubation will be rather high.

Procedure

(1) To the entire volume of standard dilution water prepared earlier, add seeding material, if necessary (0·1-1·0% of settled sewage or 1-5% of surface water) so that oxygen depletion in the control bottles after 5 days' incubation at 20°C. is in the range of 0·2-0·8 mg./lit. Shake well and fill up four sets of control D.O. bottles.

(2) Prepare dilutions of the sample to cover the range of depletion of D.O. expected (usually 0·1 to 1·0% for strong trade wastes, 1·0 to 5·0% for moderately strong trade waste or sewage, 5·0 to 25·0% for stabilised effluents and 25·0 to 99·0% for polluted river water).

(3) Carefully siphon out about 500 ml. of dilution water in a litre volumetric flask without entrainment of air. Add the measured quantity of effluent carefully to make the desired dilution (dilution should be so provided that only 40·0 to 70·0% of the initial D.O. is used up and preferably the formal D.O. should never be less than 4·0 mg./lit.) and fill up the volumetric flask to 1 lit. mark. Mix well, avoiding entrainment of the least amount of air.

(4) Siphon the dilution into four bottles, very carefully, so that there are no air bubbles inside these bottles. Of these four bottles, two should be incubated for 5 days at 20°C. under water seal in order to avoid contamination of the samples by atmospheric oxygen during the period of incubation and the dissolved oxygen of other two bottles should be determined
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immediately by Alsterberg modification of the basic Winkler's method. Along with this incubate two control bottles in the incubator and find out the D.O. in other two blank (control) bottles immediately by azide-modification method.

(5) Take out the bottles from incubator at the expiry of 5 days and determine the dissolved oxygen content in the same manner.

Calculation

(A) When dilution water has been seeded,

\[
\text{B.O.D. in mg./lit.} = \frac{(S_1 - S_2) - (C_1 - C_2) \times 100}{\% \text{ dilution used}}
\]

(B) When seeding of dilution water has not been made (as in sewage),

\[
\text{B.O.D. in mg./lit.} = \frac{(S_1 - S_2) \times 100}{\% \text{ dilution used}}
\]

When \( S_1 = \text{D.O. of dilution water on 0 day}, \quad S_2 = \text{D.O. of diluted sample after 5 days' incubation}, \quad C_1 = \text{D.O. of the seeded dilution water (immediate D.O.)}, \) and \( C_2 = \text{D.O. of the seeded dilution water after 5 days' incubation at 20^\circ \text{C.}} \)

Note.—When \( C_1 - C_2 \) gives a low value then B.O.D. of the seed is to be assumed very small. In the event of that it is suggested that a higher concentration of seed should be taken and B.O.D. be re-estimated. If percentage of the seed in dilution water is \( A_1 \) and percentage of seed in the seed control is \( A_2 \) and the corresponding D.O. of seed control be \( B_1 \) and \( B_2 \) at start and after 5 days' incubation respectively, then the seed correction will be

\[
(B_1 - B_2) \times \frac{A_1}{A_2} = K.
\]

B.O.D. of the sample taking seed correction, in mg./lit.,

\[
= \frac{[(S_1 - S_2) - K] \times 100}{\% \text{ dilution}}
\]

The velocity constant and ultimate demand may be determined by graphic or statistical method from the standard B.O.D. test. The Biochemical Oxygen Demand is of paramount significance in all water pollution studies.
If the dissolved oxygen in any stream indicates the bank balance, then the Biochemical Oxygen Demand of the water indicates the liabilities. The excess of assets over liabilities is termed in this case as oxygen balance and if the stream is to be kept free from any nuisance, then there ought to be some oxygen balance left in the river or estuary at that instant. The 8th Report of the Royal Commission, (Vol. I, U.K.) gives the following classification of streams on the basis of 5 days’ Biochemical Oxygen Demand.

<table>
<thead>
<tr>
<th>Classification</th>
<th>5 days’ B.O.D. in ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very clean</td>
<td>... 1</td>
</tr>
<tr>
<td>Clean</td>
<td>... 2</td>
</tr>
<tr>
<td>Fairly clean</td>
<td>... 3</td>
</tr>
<tr>
<td>Doubtful</td>
<td>... 5</td>
</tr>
<tr>
<td>Bad</td>
<td>... 10</td>
</tr>
</tbody>
</table>

The B.O.D. of the mixture of sewage effluent plus river or estuarine water can be estimated by the use of the following formula, as suggested by the Royal Commission:

\[
\frac{(X + YZ)}{(Z + 1)}
\]

where \(X = \text{B.O.D. of effluent}\), \(Y = \text{B.O.D. of river water above outfall}\) and \(Z = \text{Dilution factor (proportion of river water to effluent discharge)}\).

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