C3 - Biochemical Oxygen Demand Determination

1. Principle

BOD is expressed as weight of oxygen consumed per unit volume of water during 5 days at 20°C ; BOD is related to the amount of biodegradable organic matter in water sample ; during oxidative degradation of organic matter, aerobic micro-organisms which perform it, consume oxygen present in water as dissolved gas.

Water sample is diluted by a dilution solution (sometime containing bacterial seed) ; this sample is incubated during 5 days at 293°K and the consumed amount of oxygen is measured.
It is necessary to prepare many solutions corresponding to different dilutions in order to chose the one which presents an oxygen consumption equals to 40 to 60 % of the initial concentration of oxygen.

2. Procedure

2.1. Preparation of the dilution water

Dilution water can be prepared with either deionised water, or waste water, or surface water

A) In case of dilution water prepared with deionised water :
In one liter of deionised water, add :
5 mL of solution A
1 mL of solution B
1 mL of solution C
1 mL of solution D
1 mL of solution E

Solution A : [17 g Na_2HPO_4 , 12 H_2O + 2.8 g KH_2PO_4] in one litre of deionised water
Solution B : 20 g / L $MgSO_4$, 7 H_2O
Solution C : 25 g / L $CaCl_2$
Solution D : 1.5 g / L $FeCl_3$
Solution E : 2 g / L NH_4Cl

B) In case of dilution water prepared with surface water
Add to one litre of surface water, the solutions A, B, C, D and E in the same amount .

2.2. Preparation of the sample and the blank

Introduce the sample (sample volume : V_0) and the dilution water in a volumetric flask (volumetric flask volume : V_1) : this is the diluted sample. Then $F = V_1 / V_0$ is the dilution factor.
Fill the incubation flask with the diluted sample (be careful, the volumetric flask volume must be superior to the incubation flask volume) ; measure the oxygen concentration T_0 (about 10 mgO_2 / L, near saturation) and close hermetically the incubation flask : 5 days at 20°C.
Measure the oxygen concentration after 5 days (120 hours) : T_5

Blank : carry out this experiment only with dilution water (fill the incubation flask with dilution water) D_0 and D_5.

2.3. Interpretation of the results

Determinate the dilution such as :

$0.4\ T_0 \leq T_0 - T_5 \leq 0.6\ T_0$

$$\mathbf{BOD_5\ (mg\ O_2 / L) = F\ (T_0 - T_5) - (F - 1)\ (D_0 - D_5)}$$

☞ **summary** : the sample dilution must be such as T_5 is around 5 mg O_2 / L, since T_0 is around 10 mg O_2 / L ; you must know approximately the BOD of the sample and carry out the right dilution so that BOD in the incubation flask is around 5 mgO_2 / L.

☞ ***experiment examples :***
Raw water : 500 mg O_2 / L : dilution 100 times.
If the incubation flask volume is 250 mL : for example, use a 1000 mL volumetric flask : 10 mL of raw water + 990 mL of dilution water.
Measure oxygen concentration T_0 : it must be around 10 mg O_2 / L (otherwise, introduce a bubble aeration in dilution water).
Introduce the diluted sample in the incubation flask : 5 days at 20°C.
Measure T_5 : it should be around 5 mg O_2 / L (otherwise, try another dilution).
Blank : fill the incubation flask with the same dilution water : D_0 and D_5.
F = 1000 / 10 = 100
For example : if you measure :
a) D_0 = 9.6 mgO_2 / L
D_5 = 9.4 mg O_2 / L
T_0 = 9.5 mg O_2 / L
T_5 = 4.6 mg O_2 / L

$T_0 - T_5$ = 4.9
and 0.4 T_0 = 3.8 < 4.9 < 0.6 T_0 = 5.7

Then you can determinate BOD
BOD = 100 (9.5 – 4.6) – (40-1) ($D_0 - D_5$)
and BOD = 470.5 mg O_2 / L

b) D_0 = 9.7 mgO_2 / L
D_5 = 9.6 mg O_2 / L
T_0 = 9.9 mg O_2 / L
T_5 = 2.5 mg O_2 / L

$T_0 - T_5$ = 7.4
but 7.4 > 0.6 T_0 (5.94)

<u>You have to try another dilution (highest)</u>

b) D_0 = 9.8 mgO_2 / L
D_5 = 9.7 mg O_2 / L
T_0 = 9.8 mg O_2 / L
T_5 = 8.7 mg O_2 / L

$T_0 - T_5$ = 1.1
but 1.1 < 0.4 T_0 (3.92)

<u>You have to try another dilution (lowest)</u>

☞ **BOD standards :**
(150 mg of glucose + 150 mg of glutamic acid) / L : BOD = 220 +/- 18 mg O_2 / L

☞ **Bacterial seed** for industrial wastewaters : for example, introduce 10 % the volume of water dilution water with a suspension $10^3 - 10^6$ *Pseudomonas aeruginosa* / mL.

<u>3. Practical work</u>
Carry out the BOD determination of the "raw industrial wastewater" and the "treated industrial wastewater".
☞ Average BOD :
- raw water : 200 mg O_2 / L
- treated water : 50 mg O_2 / L.

<u>4. Report</u>

4.1. Explain the dilution of the raw and treated water you have carried out.

<u>5. Required material and reagents</u>

A) Preparation of dilution water
- preparation of solution A, B, C, D and E :
Solution A : [17 g Na_2HPO_4 , 12 H_2O + 2.8 g KH_2PO_4] in one litre of deionized water
Solution B : 20 g / L $MgSO_4$, 7 H_2O
Solution C : 25 g / L $CaCl_2$
Solution D : 1.5 g / L $FeCl_3$
Solution E : 2 g / L NH_4Cl

- Prepare 1L of suspension *Pseudomonas aeruginosa* $10^3 – 10^6$ / mL : solution **P**
- In 800 mL of deionized water, add :
 100 mL of solution **P**
 1 mL of solution A,
 1 mL of solution B,
 1 mL of solution C,
 1 mL of solution D,
 1 mL of solution E

- Complete to 1L with deionized water : flask noted **DW**

- Bubble DW and measure oxygen concentration : it must be saturated (around 9.2ppm at 20°C)

B) For one experiment :
- one litre of DW
- 100 mL of solution : (150 mg of glucose + 150 mg of glutamic acid) / $L_{\text{deionized water}}$
- two incubation flask (trial and blank)
- oxymeter
- 200 mL of diluted hypochlorite water in a beaker.