# UNIT 6
# MICROBIAL FLORA OF WATER

## WATER POLLUTION

Polluted water is water whose composition has been changed to the extent that it is unusable. In other words, it is toxic water that cannot be drunk or used for essential purposes like agriculture, and which also causes diseases like diarrhoea, cholera, dysentery, typhoid and poliomyelitis, etc.
The main water pollutants include bacteria, viruses, parasites, fertilisers, pesticides, pharmaceutical products, nitrates, phosphates, plastics, faecal waste and even radioactive substances. These substances do not always change the colour of the water, meaning that they are often invisible pollutants.

Microbes play a special role in treating waste/ sewage water.

- The purpose of waste water treatment is to remove contaminant from water so that the treated water can meet the acceptable quality standard. The quality standard usually depends whether the water will be reused or discharged into river.
- Methods of waste water treatment depends on composition of waste water and required quality for treated water. Treatment process are broadly classified as physical, chemical and biological treatments.
- Physical treatment methods utilizes physical separation of pollutant such as by filtration etc.
- Chemical treatment methods utilizes chemical characteristics of pollutant for purification. For e.g. Coagulation etc
- Biological treatment methods utilizes biological characteristics of pollutants such as bacteria, viruses by purification.
- Other purpose of waste water treatment includes;
  - To reduce strength of sewage
  - To make waste water less offensive
  - To prevent public health from toxic effect of pollutant
  - To conserve nature

Steps of sewage treatment process:

I. Preliminary treatment of wastewater:

- The main objective of preliminary treatment is to remove gross solids (such as plastics, cloths, cans, dead body of animals etc), grits and fats from waste water.
- Some of the treatment technique applied for preliminary treatment purpose are;

a. Screening:

- Screening is used to remove gross solid waste like plastics, cloths, dead animals from waste water.
- For this purpose waste water is passed through a metal screen which consists of vertical or inclined steel bars usually set 5 cm apart.

- The removes gross solids are disposed by burning or composting.

b. Grit removal:

- Grits are small, non-biodegradable particles which are heavier than suspended organic matters.
- Grits are removed by carefully regulating the flow velocity of sewage in grit removal tank

c. Skimming:

- Skimming is the process of removal of fatty and oily material from sewage.
- In this method, sewage is placed in skimming tank and it is aerated from bottom so that fats and oils are collected at top of the liquid which are then removed by skimming.

II. Primary treatment of wastewater:

- After removal of gross solids, grits and fats, next step in treatment is removal of remaining suspended solids as much as possible.
- The main objective of primary treatment is to reduce strength of sewage by removing suspended materials.
- Some common technique applied for primary treatment of sewage are:

a. Sedimentation:

- Sedimentation tank is used for removal of suspended solids and some organic matters.
- There are different types of sedimentation tank.
- Common example is rectangular horizontal flow tank. In this tank sewage flow very slowly (1-2 feet/min) such that solids present is waste water settle at bottom.
- Settled solids are periodically removed by sludge scrapper.
- This technique removes about 90% of suspended solids and about 40% of organic matters from sewage.

b. Mechanical flocculation:

- In this method sewage is paced in a flocculation tank, then sewage is rotated at an speed of 0.43m/sec with the help of rotating paddles.
- Flocs are aggregates of bacterial cells and fungal filaments.
- While sewage rotates in circular motion, small size dissolved solids attached to each other to form large size solids and settles at the bottom which is then removed out.

c. Chemical flocculation:

- In this method, sewage is placed in coagulation tank and then some precipitating agents such as alum is added.
- Alum forms precipitate of $Al(OH)_3$, suspended solids attached to the precipitate such that size of precipitate increase gradually to settle down at bottom.

d. Neutralization:

- If sewage is highly acidic or basic, it is neutralized by adding base or acid to facilitate growth of microorganisms during secondary treatment process.

III. Secondary treatment of waste water:

- In secondary treatment, dissolved or colloidal organic matters are present in sewage are removed by utilizing microorganisms. In this steps, microorganisms utilizes organic matter and converts them into inorganic minerals.
- Following changes occurs in sewage during secondary treatment;
    - Organic matter (carbon) is oxidized into $CO_2$ and $H_2O$
    - Organic nitrogen compounds are first converted into $NH_3$ and then into $NO_3$
    - Colloidal matters are coagulated or precipitated out.
- Thus main purpose of secondary treatment of sewage is to reduce BOD level.
- Various techniques are used in secondary treatment of sewage. Some of them are:

a. Trickling filter:

- Trickling filter consists of filtering bed, spraying arm and water collecting chamber.
- Filtering bed consists of well graded gravel, broken stone of size (40-150mm diameter).
- Effluent or sewage from primary treatment tank is sprayed uniformly over the filter bed. During filtration a gelatinous layer of bacteria, algae, protozoa and some fungi is produced on the surface of filter bed. This layer is called Zoogleal layer.
- As the water trickles through the filter bed, organic matter present in it are oxidized by microorganism of zoogleal layer.
- Although trickling filter is classified as aeration process of sewage treatment, it is facultative system. It is because aerobic bacteria lies on the top of the filter bed whereas anaerobic bacteria lies in middle or bottom of filter bed.
- Trickling filter can reduce BOD of sewage by about 65-85% depending on the rate of filtration.

b. Oxidation ditch:

- Oxidation ditch consists of circular canal with inlet and outlet.
- In this method, sewage from primary treatment plant is placed in oxidation ditch and then it is agitated with the help of mechanical rotator and then left for a period of about 12-24 hours.
- During the period of oxidation, microorganism present in sewage oxidize the organic matter.
- Finally the sewage is removed from oxidation ditch through outlet for tertiary treatment.
- Oxidation pond or lagoon:

- Oxidation pond is also known as lagoon or reduced pond or stabilization pond.
- It is an aerobic method of sewage treatment technique.
- In this treatment method, sewage from primary treatment plant is placed in an oxidation pond and left there for 10-40 days.
- During this period in oxidation pond, microorganisms oxidize the organic matter present in sewage. Oxygen released by algae during photosynthesis is utilized by microorganism for oxidation of organic compounds. During oxidation $CO_2$ and $H_2O$ are released which are utilized by algae for photosynthesis. Therefore there is mutually beneficial relationship between algae and bacteria.
- Some oxygen is also derived from atmosphere for oxidation because oxidation pond is open system.
- The oxidation pond remains aerobic during day time and first hours of night. During this period oxidation of organic compound (aerobic decomposition) takes place. During rest hours of night condition become anaerobic and anaerobic decomposition of organic compound takes place.
- Advantage of oxidation pond;
  - It is very simple and easy technique
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  - Treated sewage can be utilized for irrigation
- Limitation of oxidation tank:
  - Holding time is very long (10-40 days)
  - It require large area
  - It creates bad odor. Furthermore it may become breading place for mosquitoes and other vectors
  - It is influenced by seasonal temperature. It is effective only in warm climate but not in cold and rainy season.

c. Activated sludge system:

- Activated sludge system, consists of aeration tank, settling tank and sludge return system.
- At first sewage from primary treatment plant is mixed with sludge drawn from previous batch, which is known as activated sludge or return sludge.

- The activated sludge contains large number of microorganisms and serves as inoculum of microorganisms.
- After mixing of activated sludge, sewage is placed in aeration tank. In aeration tank. Sewage is continuously aerated for 6-8 hours. During this period microorganisms oxidizes the organic compounds to form CO2, H20 and NO3 etc.
- After oxidation, sewage is passed to settling tank and left undisturbed for 2-3 hours. Sludge settle to the bottom. This sludge is called activated sludge which is fully oxidized and is very offensive. This activated sludge can be used as inoculum for next batch of sewage.
- Most of the sludge is removed and some is returned to aeration tank for next round of treatment.
- By sludge digestion process, BOD of sewage is reduced by 5-15%.

d. Septic tank:

- Septic tank is used for disposal of content of toilet where sewage system is not available for disposal.
- Septic tank is prepared under the ground.
- Sewage along with toilet content is placed into septic tank where heavier solid wastes settle down to from sludge whereas lighter solids including fats form layer on top of sewage called scum.

- In septic tank organic compounds in sewage is anaerobically digested by anaerobic microorganisms such as Methanogenic bacteria.
- After anaerobic decomposition, the sludge become stable and inoffensive whereas liquids in sewage percolates into soil from septic tank.

IV. Tertiary or final treatment of waste water:

- Tertiary treatment of waste water is final treatment process in which all the chemical and biological agents are completely removed from sewage before disposal into river.
- The main objectives of tertiary treatment process;

a. Removal of suspended solids:

- Suspended solids are removed by two methods:
    - *Microstraining:*
        - In this method, sewage is placed in rotating drum filter of pore size 25-35 $\mu$m and then drum is rotated,
        - During rotation, clear water comes out of drum and suspended solids remains inside drum.
    - *Chemical coagulation and filtration:*
        - In this method, precipitating agents such as alum is added in sewage. Fine

suspended solids adsorbs to the surface of Al(OC)3 precipitate, finally precipitate with adsorbed solids are separated by filtration.

b. Removal of dissolved solids like salts:

- Various technique are used for this purpose such as adsorption by activated carbon, reverse osmosis
    - *Adsorption by activated carbon:*
        - Dissolved solids can be removed by filtering the water through filter containing activated carbon particle.
    - *Reverse osmosis:*
        - Reverse osmosis removes dissolved solids like NaCl and microbial cells

c. Removal of nitrate and phosphate

- If sewage after treatment is to be discharge into river, nitrate and phosphate should be removed from sewage before disposal. It is because nitrate and phosphate causes eutrophication.
- These plant nutrients are removed by biological process. At first sewage is placed in a tank containing nitrifying bacteria. These bacteria converts ammonium salt and nitrite into nitrate
- Then the sewage is placed into second tank containing denitrifying bacteria. These bacteria. These bacteria converts nitrate into Nitrogen gas that leaves the sewage.
- Phosphate is also removed by bacteria by microbial assimilation process.

d. Killing of microorganisms

- Finally microorganisms in sewage are killed by disinfection like chlorination.

BOD: The amount of oxygen that would be consumed if all the organic matter in one litre of water were oxidized by bacteria; is called biological oxygen demand. Higher BOD means higher level of pollution in water. Higher BOD shows higher level of organic matter in water. When the BOD of wastewater is significantly reduced, the effluent is sent to a settling tank. In this tank, the bacterial 'flocs' are allowed to settle at the bottom. This sediment is called activated sludge. A small part of the activated sludge is pumped back into the aeration tank to serve as inoculums. Remaining part of the sludge is sent to large tanks called anaerobic sludge digesters. In this tank, anaerobic bacteria digest the bacteria and fungi in the sludge. During this process, a mixture of methane, hydrogen sulphide and carbon dioxide is produced. These gases form the biogas. Biogas is used as a source of energy. The effluent from the secondary treatment plant is usually released into natural water bodies.

Determination of BOD in a water sample:

Principle

The Biochemical Oxygen Demand (B.O.D.) of sewage or of polluted water is the amount of oxygen required for the biological decomposition of dissolved organic matter to occur under aerobic condition and at the standardised time and temperature. Usually, the time is taken as 5 days and the temperature 20°C as per the global standard.

The B.O.D. test is among the most important method in sanitary analysis to determine the polluting power, or strength of sewage, industrial wastes or polluted water. It serves as a measure of the amount of clean diluting water required for the successful disposal of sewage by dilution. The test has its widest application in measuring waste loading to treatment plants and in evaluating the efficiency of such treatment systems.

The test consists in taking the given sample in suitable concentrations in dilute water in B.O.D. bottles. Two bottles are taken for each concentration and three concentrations are used for each sample. One set of bottles is incubated in a B.O.D. incubator for 5 days at 20°C; the dissolved oxygen (initial) content ($D_1$) in the other set of bottles will be determined immediately. At the end of 5 days, the dissolved oxygen content ($D_2$) in the incubated set of bottles is determined.

$$\text{Then, mg/L B.O.D.} = \frac{(D_1 - D_2)}{P}$$

where,

P = decimal fraction of sample used.

$D_1$ = dissolved oxygen of diluted sample (mg/L), immediately after preparation.

$D_2$ = dissolved oxygen of diluted sample (mg/L), at the end of 5 days incubation.

### Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD) is used to determine the quantity of pollution in water after wastewater treatment. The higher value of chemical oxygen demand indicates the higher organic pollution in the water sample. Only chemically digest able matter can be determined by the COD test.

COD determination takes less time than the Biological Oxygen Demand test. COD is recommended where the polluted water has toxicity and organic matter can't be determined by biological oxygen demand and useful in water effluent treatment plants.

## Principle:

The organic matter, present in the water sample is oxidized by potassium dichromate in the presence of sulfuric acid, silver sulfate and mercury sulfate to produce carbon dioxide (CO2) and water (H2O). The quantity of potassium dichromate used is calculated by the difference in volumes of ferrous ammonium sulfate consumed in blank and sample titrations. The quantity of potassium dichromate used in the reaction is equivalent to the oxygen (O2) used to oxidize the organic matter of wastewater.

**Preparation of Potassium dichromate (K2Cr2O7) Solution:**

Add 6.13 gm Potassium dichromate (previously dried at 105 °C for at least two hours) into 800 ml distilled water. Shake the flask well to dissolve the content and make up the solution to 1000 ml and mix well.

**Preparation of Silver sulfate-Sulfuric acid Solution:**

Dissolve 10 gm Silver sulfate ($Ag_2SO_4$) in 500 ml concentrated sulfuric acid and make up the solution to 1000 ml swirl the flask to mix well. Allow standing the solution for 24 hours before use.

**Preparation of Mercury sulfate Solution:**

Dissolve carefully 0.1 gm of $HgSO_4$ in 5 ml of concentrated Sulfuric acid.

**Preparation of Ferrous ammonium sulfate Solution (0.025 M):**

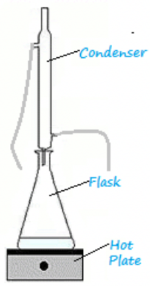
Dissolve 9.8 g ferrous ammonium sulfate in a solution of 100 ml of distilled water and 20 ml concentrated Sulfuric acid. Cool the solution and make up the solution to 1000 ml of distilled water. Standardize the solution to determine the actual concentration to calculate the chemical oxygen demand.

**Preparation of Ferroin Indicator:**

Add 3.5 gm of Iron Sulfate heptahydrate and 7.5 gm of Phenanthroline monohydrate to 400 ml of distilled water. Mix well to dissolve and make up to 500 ml of distilled water.

## Test for Chemical Oxygen Demand:

1. Take 10 ml of sample into a round bottom reflex flask.
2. Add some glass beads to prevent the solution from bumping into the flask while heating.
3. Add 1 ml of Mercury sulfate ($HgSO_4$) solution to the flask and mix by swirling the flask.
4. Add 5 ml of Potassium dichromate ($K_2Cr_2O_7$) solution.
5. Now add slowly and carefully 15 ml Silver sulfate- Sulfuric acid solution.
6. Connect the reflex condenser and digest the content using a hot plate for 2 hours.
7. After digestion cools the flask and rinses the condenser with 25 ml of distilled water collecting in the same flask.
8. Add 2-4 drops of ferroin indicator to the flask and titrate with 0.025 M ferrous ammonium sulfate solution to the endpoint.
9. Make the blank preparation in the same manner as sample using distilled water instead of the sample.



Calculate the chemical oxygen demand by following formula:

$$COD = \frac{8 \times 1000 \times DF \times M \times (V_B - V_S)}{\text{Volume of sample (in ml)}}$$

Where,

DF – Dilution Factor (if applicable)

M – Molarity of standardized Ferrous Ammonium Sulfate solution

$V_B$ – Volume consumed in titration with blank preparation

$V_S$ – Volume consumed in titration with sample preparation

**What is TDS**

TDS stands for **Total Dissolved Solids** and refers to the total concentration of dissolved substances in drinking water. TDS comprises inorganic salts and a small amount of organic matter as well. Inorganic salts are made up of the positively charged cations (calcium, magnesium, potassium and sodium) and negatively charged anions (carbonates, nitrates, bicarbonates, chlorides and sulphates). The TDS level is how much of the total dissolved solids are present in the water.

Importance of TDS in Drinking Water

TDS in drinking water originates from places like natural sources, sewage, urban run-offs, industrial wastewater, chemicals in the water treatment process, chemical fertilizers used in the garden and plumbing. Water is a universal solvent and easily picks up impurities and can absorb and dissolve these particles quickly. Although elevated levels of TDS in drinking water is not a health hazard, it does lend the water a bitter, salty, or brackish taste. Calcium and magnesium, two minerals commonly found in TDS, can also cause water hardness, scale formation and staining.

**What are Different TDS levels**

The TDS level helps indicate whether the drinking water is fit for consumption, requires filtration or is highly contaminated. Parts per million (PPM) is the measurement used for measuring TDS level in the water.

TDS Level Chart for Drinking Water

| TDS in Water (measured in PPM) | Suitability for Drinking Water |
|---|---|
| Between 50-150 | Excellent for drinking |
| 150-250 | Good |
| 250-300 | Fair |
| 300-500 | Poor, not good for drinking |
| Above 1200 | Unacceptable |

Naturally, mineral water has no smell or taste. A change in the TDS level changes the texture and taste, making the water unfit for consumption. Some of the reasons why one should measure the TDS level of their drinking water are:

*Taste (high TDS level can make the water salty and/or bitter).

*Health Concerns (water with high TDS level will not have a drastic impact on your health but the high level of lead or copper can make you fall sick).

*Cooking (TDS level above 1000 PPM can change the way the food tastes).

**TOC**: Total Organic Carbon (TOC) is a measure of the total amount of carbon in organic compounds in pure water and aqueous systems. TOC is a valued, analytical technique that is applied by organizations and labs to determine how suitable a solution is for their processes. Unless it's ultra-pure, water will naturally contain some organic compounds. TOC has become an important parameter used to monitor overall levels of organic compounds present. This has happened despite the lack of any direct quantitative correlation between total organic carbon and the total concentration of organic compounds present and reflects the importance of having an easy-to-measure, general indicator of the approximate level of organic contamination. In many cases, the TOC is used as an on-going monitor of change or lack of change in organic content.

When completing TOC analysis, the following is measured:

- TC – Total Carbon

- TIC – Total Inorganic Carbon

- POC – Purgeable Organic Carbon

- NPOC – Non-Purgeable Organic Carbon

- DOC – Dissolved Organic Carbon

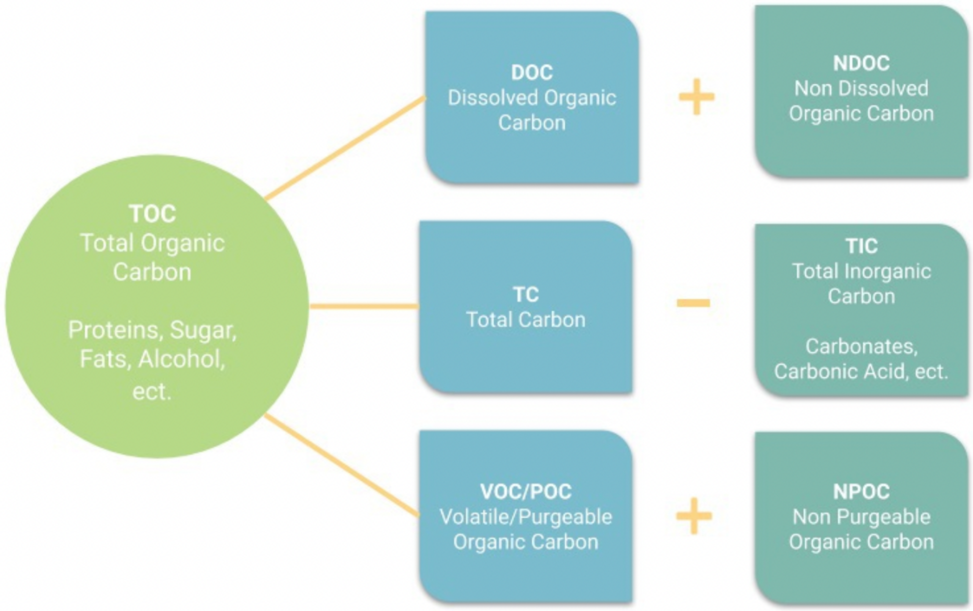
- NDOC – Non-Dissolved Organic Carbon

To calculate TOC, one can subtract the total amount of inorganic carbon from total carbon found. Alternatively, one can add Purgeable and Non-Purgeable Organic Carbon, or Dissolved and Non-Dissolved Organic Carbon. As sums, they look like:

TOC = TC - TIC

TOC = POC + NPOC

TOC = DOC + NDOC




**How is Total Organic Carbon Measured?**

TOC is measured at very different concentrations in a very wide range of systems. The table below gives an indication of Total Organic Carbon levels in various types of water. Levels vary widely within each type, but, broadly, they range from sub-ppb levels in ultra-pure water for laboratory and microelectronic applications up to hundreds of ppm in effluents and process streams.

For many of these systems, the TOC alone does not provide enough information. The carbon-containing compounds can be present in different forms and the proportions of each can be critical. A breakdown of some of these divisions is shown in the above diagram.

Dissolved organic carbon (DOC) is generally taken as that which will pass through a 0.45um filter. Large particle size TOC is classified as particulate or non-dissolved (NDOC). About 50 to 75% of DOC in natural waters is in the form of polymeric organic acids - fulvic and humic acids.

About 10% of the TOC is in colloids, mainly humic acids and various minerals. A further 10 to 20% are small molecules from the decomposition of organic matter.

| Water | Typical TOC (Range) | Typical TOC Present as Particulates |
|---|---|---|
| Bog | 33 ppm (10 to 60) | 4 ppm |
| Marsh | 17 ppm (10 to 60) | 3 ppm |
| Eutrophic Lake | 12 ppm | 3 ppm |
| Oligotrophic | 2.2 ppm | 0.2 ppm |
| River | 7.0 ppm (1 to 10) | 2.5 ppm |
| Precipitation | 1.1 ppm | 0.1 ppm |
| Ground Water | 700 ppb | |
| Sea Water | 500 ppb | 50 ppb |
| Waste Water | Up to 1000 ppm | |
| Process Waters | Very Wide Range | |
| Drinking Water | 100 ppb to 10 ppm | |
| Purified Water | 1 ppb to 500 ppb | |
| Ultrapure Water | 0.1 ppb to 10 ppb | |

Table Showing Total Organic Carbopn (TOC) Levels in Various Types of Water

**Total Organic Carbon Analysis**

TOC is universally measured by oxidizing the organic compounds present to forms which can be quantified.

A variety of oxidation and detection methods are used depending on the nature and concentration of TOC being measured and the analytical requirements (e.g. speed, sensitivity).

1. High-temperature combustion at 1,200 °C in an oxygen-rich atmosphere. The CO2 produced is passed through scrubber tubes to remove interferences and measured by non-dispersive infrared absorption (NDIR).
2. High-temperature catalytic oxidation at 680°C in an oxygen-rich environment inside tubes filled with a platinum catalyst followed by NDIR.
3. Thermo-chemical oxidation with heat and a chemical oxidizer, usually a persulphate.
4. Photo-chemical oxidation with UV and a chemical oxidizer, usually a persulphate.

5. Photo-oxidation by ultra-violet (UV) light alone or with a catalyst. The UV oxidation method offers the most reliable, low maintenance method of determining TOC in ultra-pure waters.