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S.Y. B.Sc. (Sem. IV) (CBCS)

MICROBIOLOGY

[401]: APPLIED AND ENVIRONMENTAL MICROBIOLOGY

Unit 4

MICROBIOLOGY OF DRINKING WATER AND WASTE WATER

Prepared By

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Introduction

- Microbiology of water comprises the study of nature, distribution, and activities of microorganisms in fresh, estuarine, and marine waters. The earth's moisture is in continuous circulation.
- This process of circulation of moisture is known are as water cycle or hydrologic cycle which has different stages atmospheric, surface, and ground water. The volume of moisture and the nature of microorganisms in each stage extremely variable.
- The microbial flora of atmospheric moisture is contributed by the air along with particles of dust. It is removed from the earth's surface in large numbers by rain and hail. The surface water includes waters of lakes, streams, rivers, and oceans.
- These waters are susceptible to periodic contamination with microorganisms from atmospheric water by precipitation, surface washings of soil, and any wastes that are dumped into them.
- the composition of microbial flora in the surface water is dependent upon the microbial nutrients that are present in the water, geographical, biological, and climatic conditions Ground water is subterranean water that occurs in the soil or rock - containing materials.
- Depending upon the permeability of the soil and the depth to which the water penetrates, the microbes are removed by filtration and as such the groundwater may be free from any microbes.
- population in a body of surface water is largely determined by the physical and chemical conditions which prevail in that habitat.
- Some of these conditions are temperature, hydrostatic pressure, light, salinity, turbidity, pH, and in organic and organic constituents.
- Again, optimum growth of microbes depends upon the interaction between these conditions and the nature of surface water (fresh or marine). Besides these, the distribution of microorganisms varies according to the depth of water.
- In deep waters, the top layers and the bottom sediments harbor the higher concentration of microorganisms than the rest. The aggregation of floating and drifting microbial group in the surface of water is known as plankton which when comprising.
- primarily of algae is phytoplankton, when protozoa and other minute animals predominate it is zooplankton, and when there is a mixture of both plant and animal life it is zoo phytoplankton.
- Microbial inhabitants of the bottom region (benthic zone) of a body of water are the benthic organisms, richest in an estuarine - marine system. In the benthos two distinct life forms are found, one attached and non - motile, the other unattached and capable of horizontal and vertical movements.
- The attached species growing on rock or stone surfaces are epilithic, on plants are epiphytic, and on animals are epizoic. Owing to the intensive concentration of biomass of benthic organisms, it forms one of the most important grazing grounds for protozoa, vegetarian fish, etc. between the benthic zone and the zone of producers (photosynthetic algae) is the pro - fundal zone of open water where photosynthetic activity decreases progressively.
- In fresh water, the pro - fundal zone and benthic zone are largely populated by heterotrophic organisms. Whereas, in marine water, they are between the upper strata and the area just above the sea floor is relatively barren, a vast microbiological oceanic desert region.

MICROBIOLOLGYOF DRINKING WATER

- Monitoring and detection of indicator and disease - causing microorganisms are a major part of sanitary microbiology. Bacteria from the intestinal tract generally do not survive in the aquatic environment, are under physiological stress, and gradually lose their ability to form colonies on differential and selective media Their die - out rate depends on the water temperature, the effects of sunlight, the populations of other bacteria present, and the chemical composition of the water.
- Procedures have been developed to attempt to " resuscitate " these stressed coliforms using selective and differential media A wide range of viral, bacterial, and protozoan diseases result from the contamination of water with human and other animal fecal wastes.
- Although many of these pathogens can be detected directly, environmental microbiologists have generally used indicator organisms as an index of possible water contamination by human pathogens. Researchers are still searching for the deal " indicator organism to use in sanitary microbiology. These are among the crested criteria for such an indicator
    - The indicator bacterium should be suitable for the analysis of all types of water: tap, river, ground, impounded, recreational, estuary, sea, and waste.
    - The indicator bacterium should be present whenever enteric pathogens are present.
    - The indicator bacterium should survive longer than the hardest enteric pathogen.
    - The indicator bacterium should not reproduce in the contaminated water and produce an inflated value
    - The assay procedure for the indicator should have great specificity, in other words, other bacteria should not give positive results. In addition, the Procedure should have high sensitivity and detect low levels of the indicator.
    - The testing method should be easy to perform.
    - The indicator should be harmless to humans. The level of the indicator bacterium in contaminated water should have some Great relationship to the degree of fecal pollution.

- **Potable** - (clean) water – free of all **objectionable** material, including pathogens, tastes, odors, colors, toxins, radioactive material, organisms, oils, gases, etc.
- **Fresh** – non-salt or sea water
- **Pollution** – anything that makes it non-Potable
- **Sewage** – the community waste or garbage that mother nature and we dump onto sewers or land

Sr.No.	QUESTION	ANSWER
1	Clean water is known also as____.	Potable water
2	Define pollution in terms of water.	anything that makes it non-Potable
3	Disease caused by which microorganisms from contaminated water?	Bacteria, protozoa and virus
4	Monitoring and detecting indicator microorganisms is known as_____.	Sanitory microbiology

5	Which are types of surface water?	Marine and fresh water

Indicator organisms

- Index of possible water contamination by human pathogens.
  - *E. coli,*
  - *Enterobacter aerogenes,*
  - *Klebsiella pneumonia,*
  - *Clostridium perfringes,*
  - Coliforms – They are defined as facultatively anaerobic, non-sporing, gram negative, rod-shaped bacteria that ferment lactose producing gas within 48 hours at 37°C.
  - *Coli* – A faecal coliform found in faeces.
  - *Aerogenes, K.pneumoniae* – Non-faecal coliforms found in soil and vegetation.
  - Faecalis – Found in marine and brackish water (More Salinity then fresh water)
  - Perfringes – Long survival pathogen. Thus, an indicator of past pollution.

Nuisance bacteria

- They cause changes in color, odor and taste of water
- Slime – forming bacteria – Produce gummy or slimy conditions.
- S (sulfur) bacteria – Produce sulphuric acid and hydrogen sulphide which makes water very acidic and imparts foul odor. Precipitated S ions damage pipelines.
- Fe bacteria – Transform soluble Fe compounds into insoluble form. Precipitate of Fe damage pipelines.
- Algae – Turbidity, discoloration and unpleasant odor and taste.

Sr.No.	QUESTION	ANSWER
1	Give an example of indicator microorganisms.	E.coli
2	Example of human pathogen.	Clostridium perfringes
3	Turbidity, discoloration and unpleasant odor and taste is due to _____-.	Algae
4	Produce sulphuric acid and hydrogen sulphide.	S (sulfur) bacteria
5	A faecal coliform found in faeces is known as ___.	Coli

Typical Water Quality Standards

- Drinking Water
  - No coliforms contamination acceptable
  - Recreational water: (Contaminated water Swimming pool, hot tubes, Decorative

Fountains,Lack,River)

- 200 fecal coliforms /100 ml
- Fish and wildlife habitat
- 5000 fecal coliforms/100 ml
- Shellfish
- 14 fecal coliforms/100m

Coliforms	Gram-negative, non spore-forming, oxidase-negative, rod-shaped facultativeanaerobic bacteria
Thermo tolerant coliforms	produce acid and gas from lactose at 44.5±0.2°C within 24±2h, also known as faecal coliforms
Escherichia coli	Most appropriate group of coliforms to indicate faecal pollution from warm-blooded animals.
Faecal streptococci	Gram-positive, catalase-negative cocci from selective media.
Enterococci:	resistance to 60°C for 30 min and ability to reduce 0.1% methylene blue. The enterococci are a subset of faecal streptococci .
Sulphite-reducing clostridia (SRC)	Gram-positive, spore-forming, non-motile, strictlyanaerobic rods that reduce sulphite to H_2S.
Clostridium perfringens	reduce nitrate,hydrolyse gelatin and produce lecithinase and acid phosphatase.
Bifidobacteria	Obligately anaerobic, non-acid-fast, non-spore-forming, non-motile,Gram-positive bacilli most numerous groups of bacteria in the faeces of warm blooded animals.

- **Bacteriophages (phages):** These are bacterial viruses., most interesting somatic coliphages, male-specific RNA coliphages

- **Coliphages:** Somatic coliphages attack E. coli strains via the cell wall and include spherical phages. The F-RNA coliphages attack E. coli strains via the sex pili (F factor) and are single-stranded.

Bacteriological analysis & Sampling techniques of water

- Microbiological assays for possible water contamination include:
- The standard platecounts
- The most probable number (MPN)procedure
- The membrane-filter method, and
- The ONPG and MUG test

- **Standard plate count (SPC) technique**

➢ which is used to assay the total bacterial population, aliquot of water sample is spread over the surface of **tryptone glucose extract agar** plates (or nutrient agar plates) and incubated at 20°C and 37°C for 24 and 72 hours respectively.

➢ The number of colonies that develop provide an estimate of the total viable population of bacteria

The most probable number (MPN) procedure

➢ The most probable number (MPN) analysis is a statistical method based on the random dispersion of microorganisms per volume in a given sample.

➢ In this method, measured volumes of water are added to a series of tubes containing a liquid indicator growth medium.

➢ The media receiving one or more indicator bacteria show growth and a characteristic color

➢ change. The color change is absent in those receiving only an inoculum of water without indicator bacteria.

➢ From the number and distribution of positive and negative reactions, the MPN of indicator organisms in the sample may be estimated by reference to statistical tables.

➢ MPN test is completed in three steps:

- Presumptive test
- Confirmed test
- Completed test

1. Presumptive test

➢ This test, a specific enrichment procedure for coliform bacteria, is conducted in fermentation tubes filled with a selective growth medium (MacConkey lactose broth), which contains inverted Durham tubes for the detection of fermentation gas.

➢ A series of lactose broth tubes are inoculated with measured amounts of the water sample to be tested.

➢ The series of tubes may consist of three or four groups of three, five, or more tubes.

➢ The main selective factors found in the medium are lactose, sometimes a surfactant such as Na-lauryl sulfate or Na-taurocholate (bile salt), and often a pH indicator dye for facilitating detection of acid production, such as bromcresol purple or brilliant green.

➢ The selective action of lactose occurs because many bacteria cannot ferment this sugar, whereas coliform bacteria and several other bacterial types can ferment it.

➢ The surfactant and dye do not inhibit coliform bacteria, whereas many other bacteria, such as the spore formers, are inhibited.

2. Confirmative test

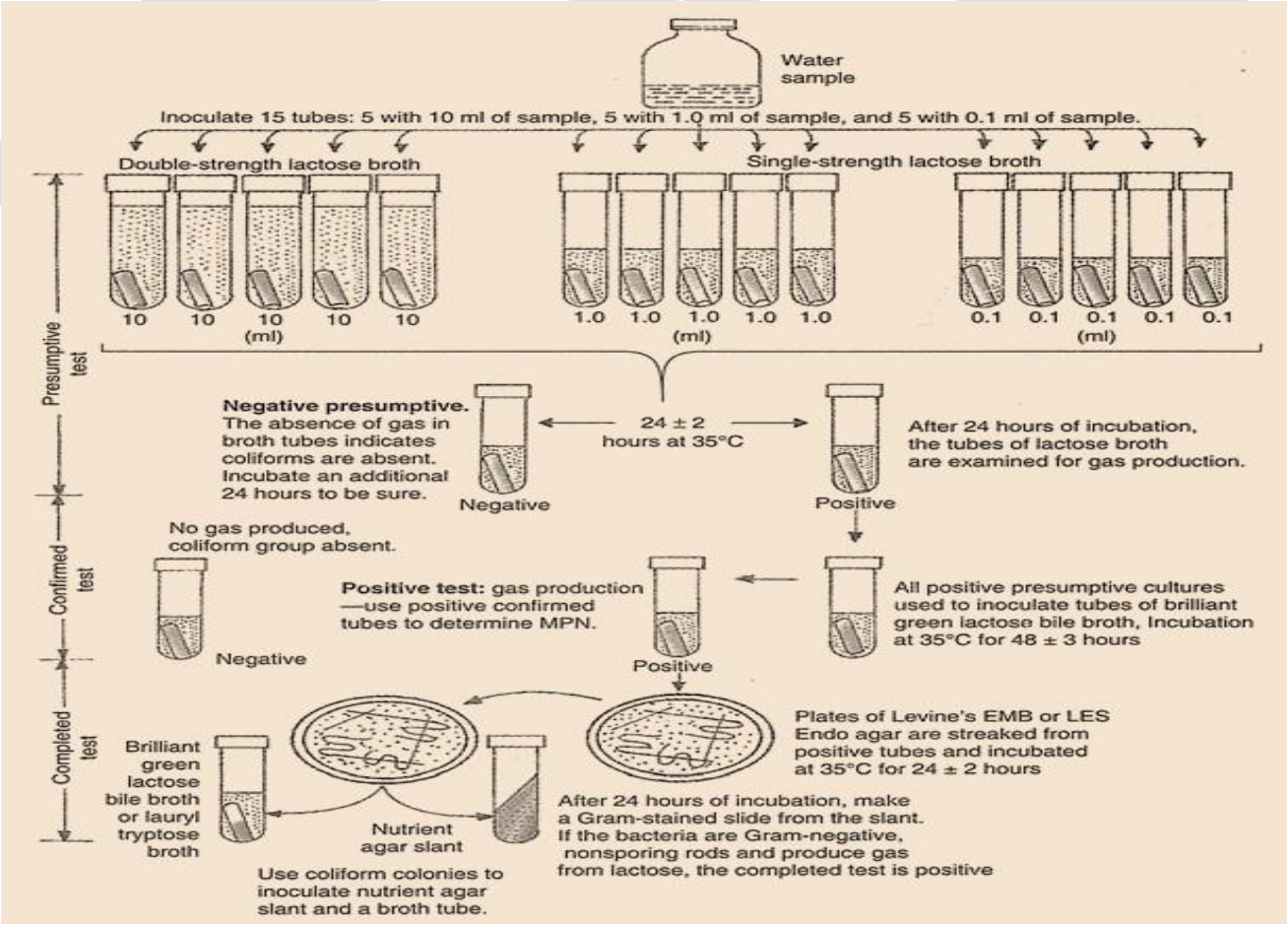
➢ This test serves to confirm the presence of coliform bacteria when either a positive or doubtful presumptive test is obtained.

➢ A loopful of growth from such a presumptive tube is transferred into a tube of brilliant green lactose bile (BGLB) 2% broth (or other lactose broth) and incubated at 35°C for 48 hours.

- This is a selective medium for detecting coliform bacteria in water, dairy, and other food products.
- A selective agent in the medium is lactose. The broth tube also contains a Durham tube to detect gas production.
- A plate of LES Endo agar (or EMB agar) is streaked with a loopful of growth from a positive tube and incubated at 35°C for 18–24 hours.
- Typical coliform bacteria (*E. coli* and *Enterobacter aerogenes*) exhibit good growth on this medium and form red to black colonies with dark centers or a sheen.
- *Salmonella typhi* exhibits good growth but the colonies are colorless. *S. aureus* growth is inhibited altogether.

3.Confirmed test

- This test helps to further confirm doubtful and, if desired, positive confirmed test results.
- A typical coliform colony from an LES Endo agar plate is inoculated into a tube of brilliant green bile broth and on the surface of a nutrient agar slant.
- They are then incubated at 35°C for 24 hours. After 24 hours, the broth is checked for the production of gas, and a Gram stain is made from organisms on the nutrient agar slant.
- If the organism is a Gram-negative, non-spore- forming rod and produces gas in the lactose tube, then it is positive that coliforms are present in the water sample.



Objective of MPN technique

- To enumerate the number of bacteria, present in the drinking water by the MPN method.
- To identify the bacteria, present in the drinking water sample.

Sr.No.	QUESTION	ANSWER
1	How many test are used for detecting pathofens?	Four
2	Full form of MPN.	Most probable number
3	How many stages of MPN techniques?	Three.
4	What is the full form op SPC	Standard plate count
5	How many sets are used for MPN technique.	Three

The Membrane Filter Technique

- The Membrane Filter (MF) Technique was introduced in the late 1950s as an alternative to the Most Probable Number (MPN) procedure for microbiological analysis of water samples.
- It involves the use of membrane filters, which are thin porous sheet structures composed of cellulose esters or similar polymeric materials.
- They act essentially as two-dimensional screens and as such, all particles, both biological and non-biological, which exceed the pore size, are retained upon the surface of the filter from fluids passing through.
- Bacteria-tight membrane filters capable of retaining microorganisms larger than 0.45 micrometer (µm) are frequently used for analysis of water.

Principle of membrane filter technique

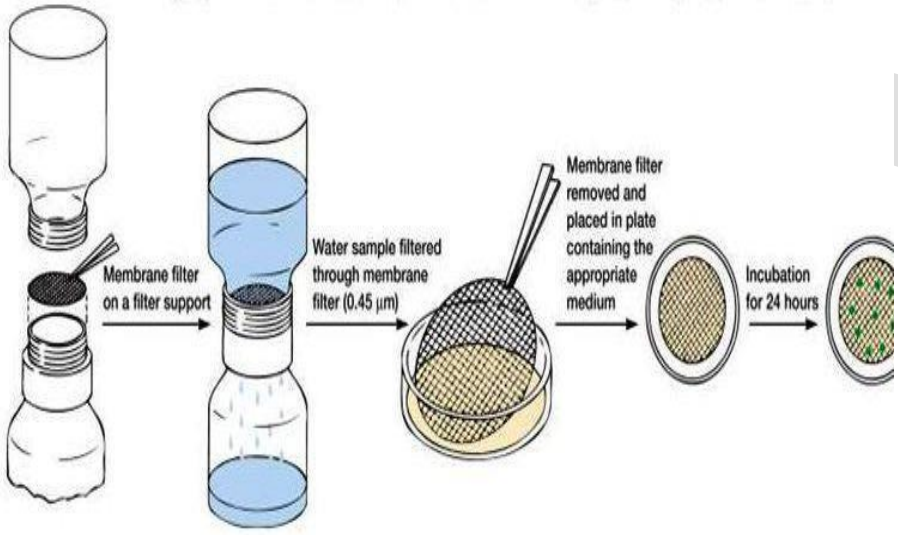
- Membrane filters have a known uniform porosity of predetermined size (generally 0.45 µm) sufficiently small to trap microorganisms.
- A water sample is passed through a sterile membrane filter that is housed in a special filter apparatus contained in a suction flask.
- Following filtration, the filter disc that contains the trapped microorganisms is aseptically transferred to a sterile Petri dish containing a pad saturated with the appropriate medium.
- The passage of nutrients through the filter during incubation facilitates the growth of organisms in the form of colonies, on the upper surface of the membrane.
- Membrane filtration and colony count techniques assume that each bacterium, clump of bacteria, or particle with bacteria attached, will give rise to a single visible colony.
- Each of these clumps or particles is, therefore, a colony forming unit (CFU) and the results are expressed as colony forming units per unit volume.

- Discrete colonies thus formed can be easily transferred to confirmation media.
- Following incubation, the colonies present on the filter are counted with the aid of a microscope.

Use of membrane filter technique

- It is used to analyze a series of dilutions of water samples collected upstream and downstream from an outlet of a sewage treatment plant.
- EPA-approved guidelines for the determination of fecal contaminating organisms (EPA Method 1103.1) are routinely utilized worldwide to examine water samples before treated water is released into a nation's waterways.
- A total count of coliform bacteria determines the potability of the water source.

1. Delicate media components that cannot withstand steam sterilization by autoclaving (e.g., serum, certain carbohydrate solutions, certain antibiotics, and other heat-labile substances) can be sterilized by membrane filtration.
2. The pharmaceutical and cosmetics industries typically focus on monitoring their process water for Pseudomonas species.



Merits of membrane filter technique

- Results are available in a shorter period of time
- Larger volumes of sample can be processed
- Because of the high accuracy of this method, the results are readily reproducible.
- Allows isolation and enumeration of discrete colonies of bacteria
- Allows for removal of bacteriostatic or bactericidal agents that would not be removed in Pour Plate, Spread Plate, or MPN techniques.
- It involves less preparation than many traditional methods, and is one of a few methods that will allow the isolation and enumeration of microorganisms.

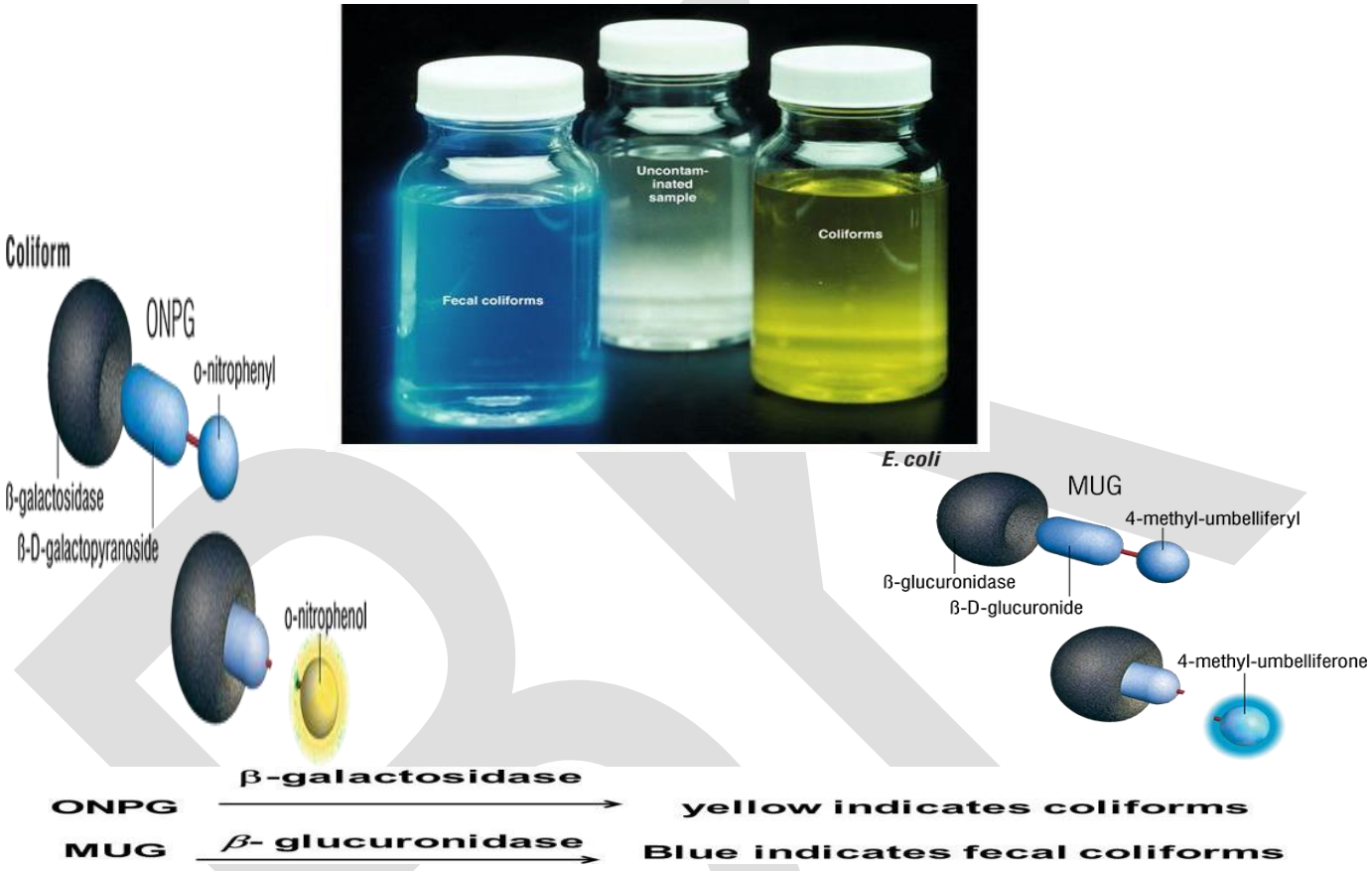
Demerits of membrane filter technique

- During the processing of turbid specimens that contain large quantities of suspended materials; particulate matter clogs the pores and inhibits passage of the specific volume of water.
- When small quantities of sample (for example, of sewage effluent or of grossly polluted

surface water) are to be tested, it is necessary to dilute a portion of the sample in sterile diluent to ensure that there is sufficient volume to filter across the entire surface of the membrane.

ONPG and MUG test

- This is based on the secretion of enzymes by the coliforms which leads to the formation of a product and which can be detected simply by the color change.
- ONPG (o-nitrophenyl-(3-D-galactopyrano-side)
- MUG (4- methylum-belliferyl-p-D-glucuronide).



Characteristics of a Useful Indicator

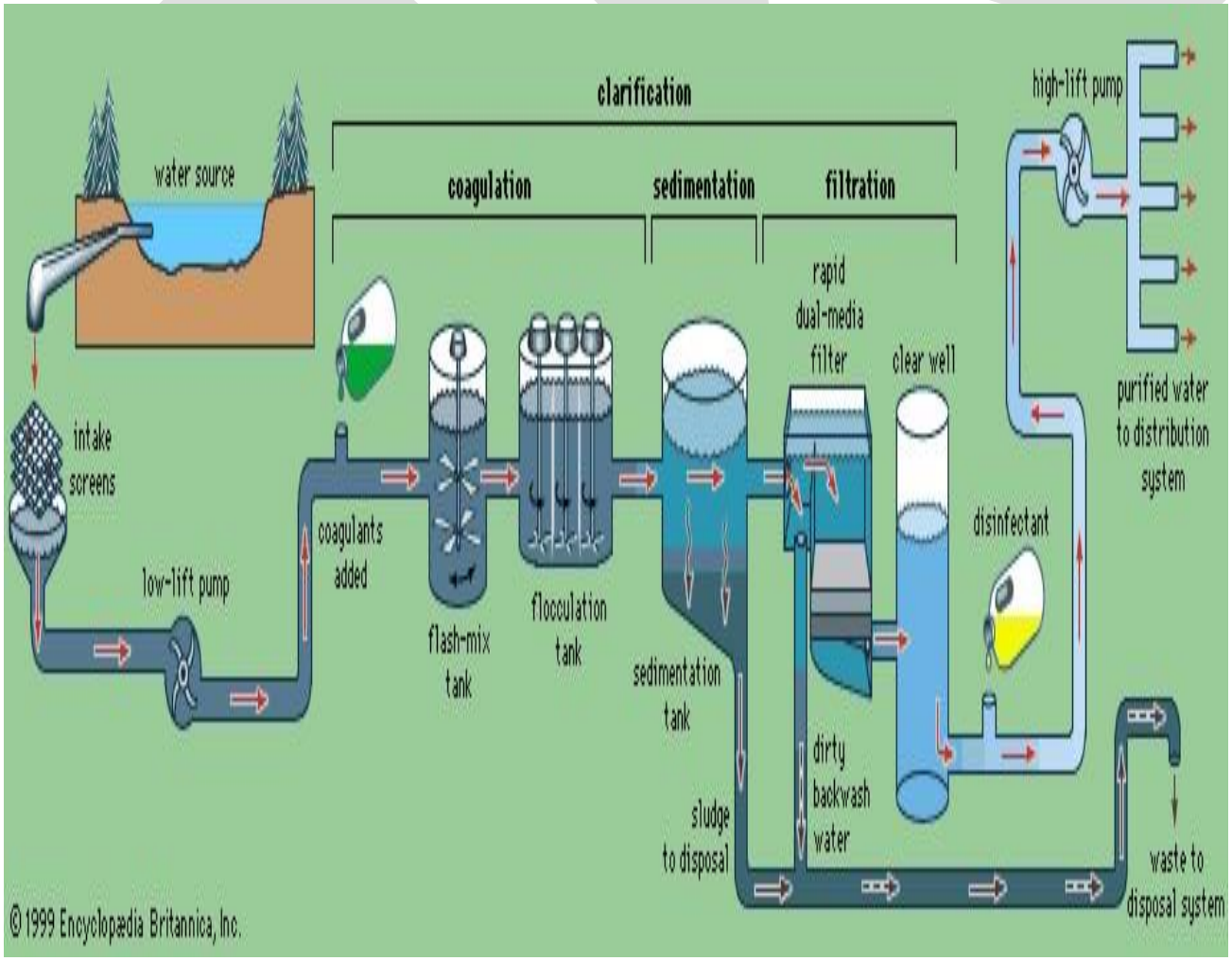
- Always present when pathogens are present
- Not present in the absence of the pathogen
- Correlated with degree of pollution
- More easily detectable than a pathogen
- Survive longer than the pathogen
- Not dangerous to work with

Sr.No.	QUESTION	ANSWER
1	Full form of ONPG	o-nitrophenyl-(3-D-galactopyrano-side
2	Which color indicate positive result for ONPG test?	Yellow

3	Full form of MUG.	4- methylum-belliferyl-p-D-glucuronide).
4	Which color indicate positive result for MUG test?	Blue
5	ONPG and MUG test is used to detect _____ organisms.	Coliform

Water purification

- It's an process by which undesired Chemical compounds, organic and inorganic materials, and biological contaminants are removed from water.
- That process also includes distillation (the conversion of a liquid into vapour to condense it back to liquid form)
- deionization (ion removal through the extraction of dissolved salts).
- The purification procedure reduces the concentration of contaminants such as suspended particles, parasites, bacteria, algae, viruses, and fungi.
- Pretreatment may include the addition of chemicals to control the growth of bacteria in pipes and tanks (prechlorination) and a stage that incorporates sand filtration.
- which helps suspended solids settle to the bottom of a storage tank.



- Sedimentation
- Filtration use of Sand filters
- Disinfection

Sedimentation

- Sedimentation is done when water consist of large sized organic materials such as leaves, and gravels which have run off from the soil. Suspended settle down depending on their size and weight and condition of stored water. Sedimentation is done in large reservoir or in restrict area of settling tank.
- The process includes coagulation, a step in which chemicals are added that cause small particles suspended in the water to clump together.
- Flocculation follows, which mixes the water with large paddles so that coagulated particles
- can be brought together into larger clumps that slowly settle on the bottom of the tank or basin.
- **Most commonly used Coagulants in Water Treatment**
- Alum (aluminum sulfate), $Al_2(SO_4)_3$. Still, the most common coagulant used in conjunction with cationic polymers.
- Polyaluminum chloride, this is efficient in some waters, requiring less pH adjustment and producing less sludge.
- Ferric chloride, $FeCl_3$. This may be more effective than alum in some applications.
- Ferric sulfate, $Fe_2(SO_4)_3$. It is effective in some waters and more economical in some locations.
- Ferrous Sulfate, $FeSO_4$. It costs bit more compare with ferric chloride but result would be better than that
- Sodium Aluminate, $Na_2Al_2O_4$
- Cationic polymers can be used alone as the primary coagulant or in conjunction with aluminum or iron coagulants.

Filtration

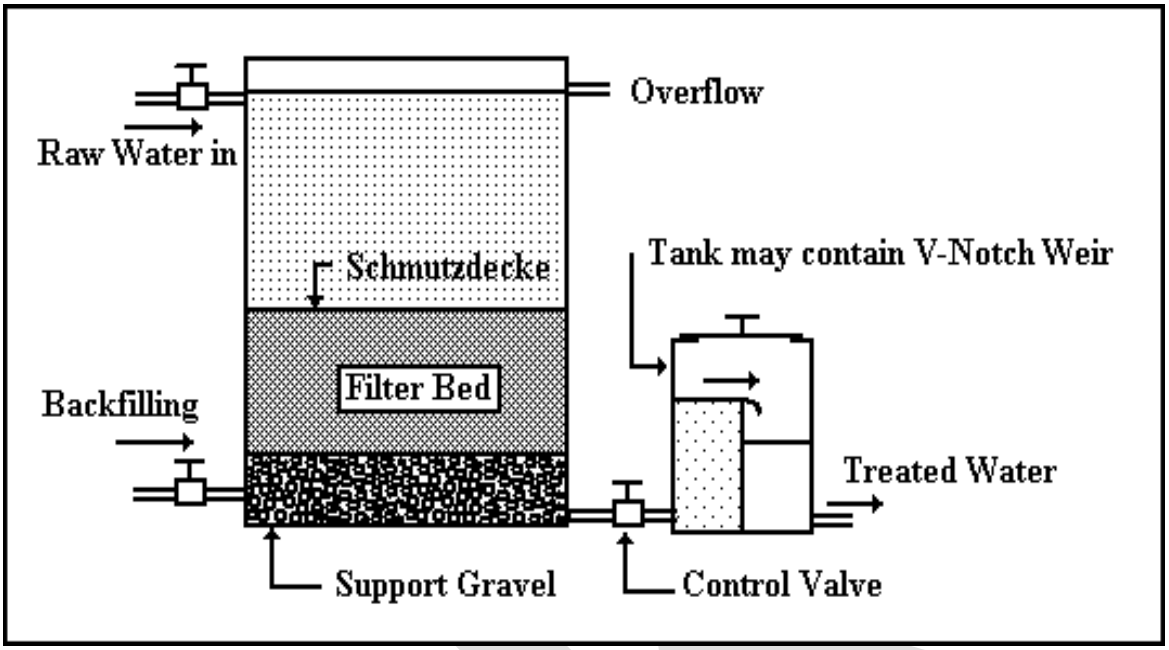
- After separating most floc, the water is filtered as the final step to remove remaining suspended particles and unsettled floc.
- The most common type of filter is a rapid sand filter. Water moves vertically through sand which often has a layer of activated carbon or anthracite coal above the sand. The top layer removes organic compounds, which contribute to taste and odor.
- Membrane filtration is essentially a thin film of synthetic polymer through which there are pores of fairly uniform size. Their specified pore size they are quite thin and so liquid flow through them fairly rapidly.

Types of sand filter

Slow sand filter

- In these filtrations plant the rate of filtration of water is slow; hence the plant requires a

considerable area. This plant consists of a concreate floor containing drainage tiles. The tile is covered with first coarse sand and finally 2 to 1 feet of sand at the top of plant.



- Water purification is done not by physical action but by physiological mechanisms supported by microorganisms. In the surface of layers of fine sand, a colloidal material, consisting of bacteria, algae and protozoa is attached. This mucilaginous material makes the pores more effective by closing the pores between the sand grains.
- Sand grains have positive charges and bacterial cell wall have negative charge. Therefor bacteria are adsorbed on the surface of sand. Protozoa ingest bacteria, due to intense microbial interaction chemical concentration of water is reduced. When filtration efficiency of the plant is reduced, due to deposition of thick mucilaginous material, the plant is taken out for cleaning.

Rapid sand filter

- Similar to slow sand filter, the rapid sand filter is also constructed. This plant consists of layers of sand, gravel and rock. before filtration, water is treated with alum or ferrous sulphate in a settling tank where precipitates settle down.
- Then water is allowed to pass through rapid sand filter plant. This plant depends on physical trapping of fine particles and floes or coagulants. The pores of the plants are soon clogged. It is cleaned by forcing cleaned water backward.
- About 99% bacteria are removed by this plant. Some pathogens are removed by this plant.
- In addition, rapid sand filter plant operates about 50 times faster than slow sand filter, and can delivers about 150 to 200 million gallons of water per acre per day.
- It requires less land area, less cost and less maintenance. Therefore, many plants are constructed in a chain, if one plant is being cleaned, the others are under operation.

Disinfection

- The final step in water purification.
- During that step, harmful microbes, such as bacteria, viruses, and protozoa, are killed through the

addition of disinfectant chemicals.

- Disinfection usually involves a form of chlorine, especially chloramines or chlorine dioxide
- Chlorine is a toxic gas, resulting in some danger from release associated with its use.
- To avoid those risks, some water-treatment plants use ozone, ultraviolet radiation, or
- hydrogen peroxide disinfection instead of chlorine.
- UV radiation (light) is very effective at inactivating cysts, as long as the water has a low level of color so the UV can pass through without being absorbed

Sr.No.	QUESTION	ANSWER
1	How many steps of water purification?	Three
2	Which are stages of water purification?	Sedimentation Filtration, disinfection
3	Which light is used for disinfection process?	UV radiation
4	Ozon is used for which purpose?	Disinfection
5	How many types of sand filter are used in water purification?	Two

MICROBIOLOLGYOF WASTE WATER

- Liquid effluent derived from domestic sewage or industrial sources that cannot be discarded in untreated form into lakes or streams due to public health, economic, and aesthetic considerations
- Commonly containing potentially harmful inorganic and organic compounds as well as pathogenic microorganisms

Domestic waste water: waste water or sewage that drains of a home.
Industrial waste water: such as acids, oils, greases matter discharges.

Characteristics of waste water

- Physical characteristics
- Chemical characteristics &
- Biological (Microbiological) characteristics.

Physical characteristics

- **Volume:** It consist of **99%** of sewage waste water while remaining **1%** is solid.
- **Odor**: Odor in domestic waste water usually caused by gases produced by the decomposition of organic matter. The fresh domestic sewage has soapy or oily smell.
- **Color:** fresh water is normally brownish gray color. anaerobic condition develops, the color of waste water changes sequentially **gray** to **dark gray** & ultimately to **black.**

- **Temperature:** the temperature of waste water is commonly higher than that of local water supply. In addition, oxygen is less soluble in warm water than in cold water.
- **Turbidity:** Turbidity of water shows presence of solid matter in suspension. Degree of turbidity may be taken as intensity of pollution measurement.
- **Solids:** The most important physical characteristics of waste water is its total solid contents, which is composed of **floating matter**, **settle able matter**, **colloidal matter** & matter in solution
- **pH:** pH of waste water is generally acidic as a result of different chemical reaction taking place in it.
- **Dissolved oxygen:** pure water has a dissolved oxygen concentration of 9mg/L at 20°C. Fresh water sewage, DO is zero. Therefore, aerobic organisms will die but anaerobe organisms will be able to survive.

Chemical characteristics

Sewage may contain chemical pollutants like – organic waste, inorganic waste, radioactive pollutant & thermal pollutants.

- **Organic pollutants:** Nitrogenous compounds like **urea, amino acids, amines & proteins.**
- Non nitrogenous compounds like **carbohydrates, fats & soaps**.
- **Inorganic pollutants:** It is majorly contributed by industries like paper & pulp industries, textile industries, pharmaceutical industries fertilizers, refineries, etc.
- it includes inorganic salts of nitrates, nitrites, sulfates, phosphates, mineral acids like HCL, H_2SO_4, metal compounds, etc. polyphosphates are major source of phosphate & serve as algal nutrients

Radioactive pollutants

- Radioactive materials in nuclear weapons.
- Use of radioactive materials in nuclear power plants
- Use of radioisotopes in medical, industrial & research applications
- Used in mining & processing of ores (Naturally occurring minerals or rocks) to produce usable radioactive substances.
- It destroys biological immune system making body less resistant to various diseases.
- Causes serious skin cancer, breast cancer, leukemia, DNA breakage, etc.
- Trace amount may lead to increase in rate of mutation in plants.
- Produces set of symptoms such as nausea, vomiting, diarrhea, lethargy (Lack of Energy) & general weakness – radiation sickness.

Thermal pollutants

- These pollutants include the waste form atomic, nuclear, & thermal power plants.
- Reduction in dissolved oxygen, thus suffocation arises,
- Changes in physical & chemical properties of water.
- Excessive eutrophication (Excess amount of nutrient)
- Migration of aquatic biota. (Organism living in or depending on aquatic environment)

Sr.No.	QUESTION	ANSWER
1	Which are physical properties of waste water?	Odor, smell
2	Which are chemical properties of waste water?	Organic and inorganic materials
3	Which are biological properties of waste water?	BOD
4	Which are thermal pollutants in waste water?	atomic, nuclear, & thermal power plants
5	What is the color of waste water?	Dark gray, brown

Biological characteristics

- BOD (Biochemical oxygen demand)
- COD (Chemical oxygen demand)

B.O.D. Biochemical Oxygen Demand (BOD) refers to the amount of oxygen that would be consumed if all the organics in one liter of water were **oxidized** by bacteria and protozoa. The first step in measuring BOD is to obtain equal volumes of water from the area to be tested and dilute each specimen with a known volume of distilled water. which has been thoroughly shaken to insure oxygen saturation. an oxygen meter is used to determine the concentration of oxygen within one of the vials. The remaining vial is than sealed and placed in darkness and tested five days later.

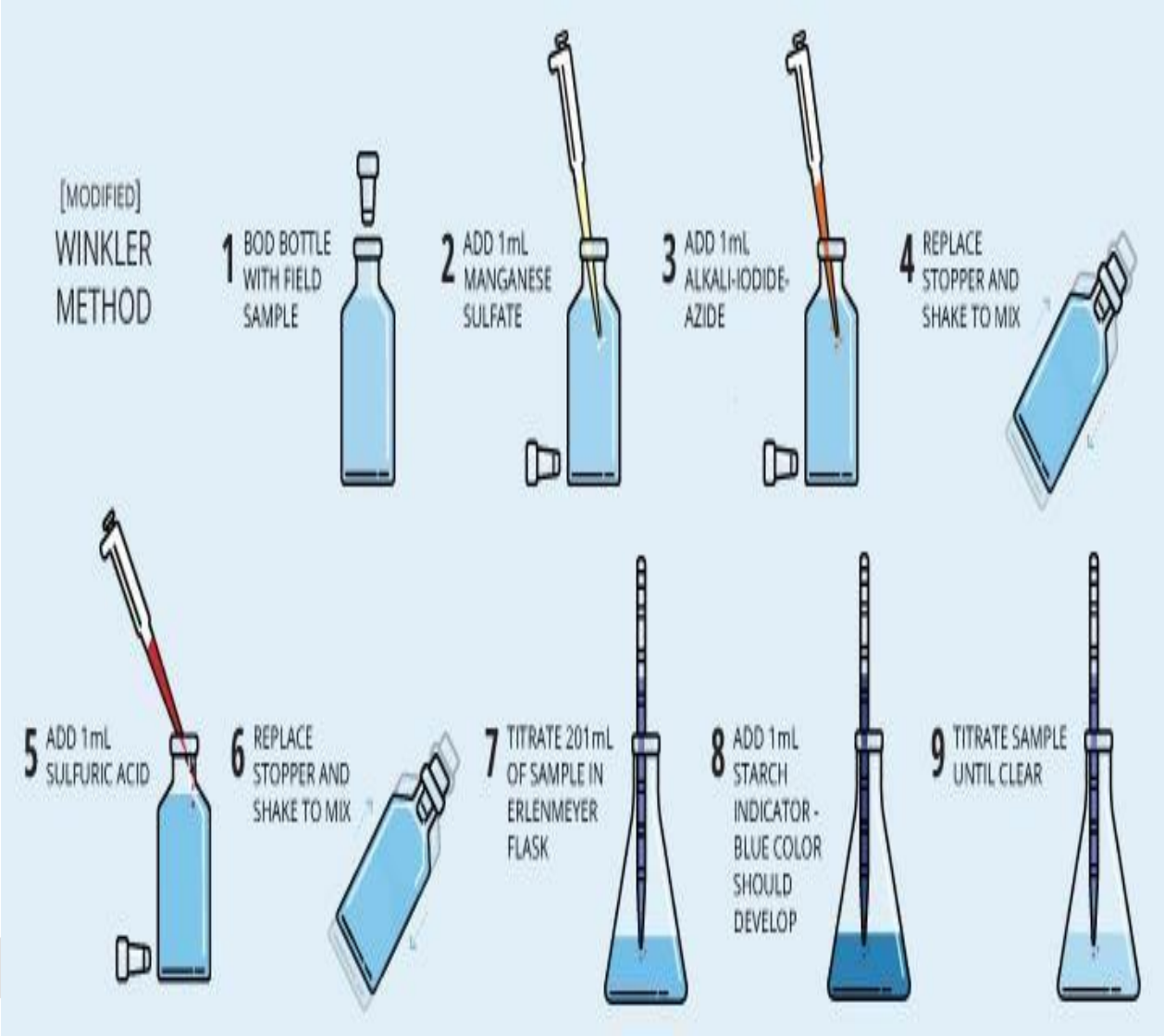


Table: BOD and Water Quality	
BOD (in ppm)	Water Quality
1-2	Very Good There will not be much organic waste present in the water supply
3-5	Fair: Moderately Clean
6-9	Poor: Somewhat Polluted Usually indicates organic matter is present and bacteria are decomposing this waste.
10 or greater	Very Poor: Highly Polluted Contains organic waste.

Chemical oxygen demand (COD)

The amount of oxygen that can be consumed by reactions in a measured solution. Mass of oxygen consumed over volume of solution which in SI units is milligrams per liter (mg/L). A COD test can be used to easily quantify the number of organics in water. The most common application of COD is in quantifying the amount of oxidizable pollutants found in surface water (e.g., lakes and rivers) or wastewater. The basis for the COD test is that nearly all organic

compounds can be fully oxidized to carbon dioxide with a strong oxidizing agent under acidic conditions. The amount of oxygen required to oxidize an organic compound to carbon dioxide, ammonia, and water. **COD**: **Chemical Oxygen Demand;** oxidation by strong chemical oxidant, usually **$K_2Cr_2O_7$** (potassium dichromate) in the presence of sulfuric acid at elevated temperatures (~ 150 ºC), during 2 hours.

Correlations between COD/BOD.

- Ratios for COD to BOD of **0.5 to 2** are usually found in potable water or exceptionally clean surface or groundwater.
- Ratios of COD to BOD of **2 to 4** are usually seen in routine domestic/municipal sewage wastes.
- Ratios of COD to BOD of **4 to 6** are usually indicative of industrial type wastes.

Sr.No.	QUESTION	ANSWER
1	Full form of BOD	Biological oxygen demand
2	Full form of COD	Chemical oxygen demand
3	Si unit of COD?	Gm/L
4	Which reagent is used to measure COD?	**$K_2Cr_2O_7$** (potassium dichromate
5	Which acid is used in BOD?	Sulfuric acid

Waste water treatment

- **Single Dwelling Process**
- **Municipal Treatment**
  - Primary Treatment,
  - Secondary Treatment,
  - Advanced & final treatment

Waste water treatment is required

- Prevent destruction of aquatic life
- Avoid spreading of pathogen
- Eliminate danger of contamination natural water, rivers, streams etc.
- Avoid accumulation of toxic material which cause ecological imbalance.
- Avoid any undesirable or harmful effect on ecosystem.

Single dwelling unit

- Its Contain domestic waste which includes human excreta such as urine. faeces, soapy material,

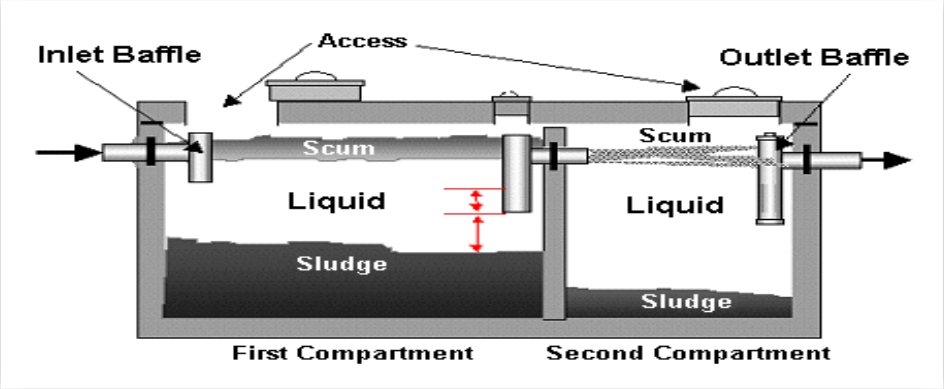
food, waste paper, dirt etc.

- sewage is collected from a locality & taken to centralized located sewage treatment plants.
- Treatment carried out by anaerobic digestion by aerobic metabolism processes.

Septic tank

- To provide satisfactorily disposal of sewage from small buildings, institutes, hotels etc.
- Septic tank is sewage settling tank which retain the sold of the sewage that enter to it & decompose it anaerobically.
- Septic tank has 2 major functions to be carried out-sedimentation of sludge & anaerobic digestion of sewage.
- size varies from 8-10ft. in diameter & depth is 25-30ft.

Double



compartment septic tanks

Sr.No.	QUESTION	ANSWER
1	How many treatment used to treat waste water?	Two
2	Which are types of waste water?	Domestic and municipal
3	Which treatment is used to treat domestic waste water?	Septic tank
4	How many steps of municipal waste water treatment plant?	Three
5	In which step microorganisms is involved?	Secondary treatment

Municipal waste water treatment

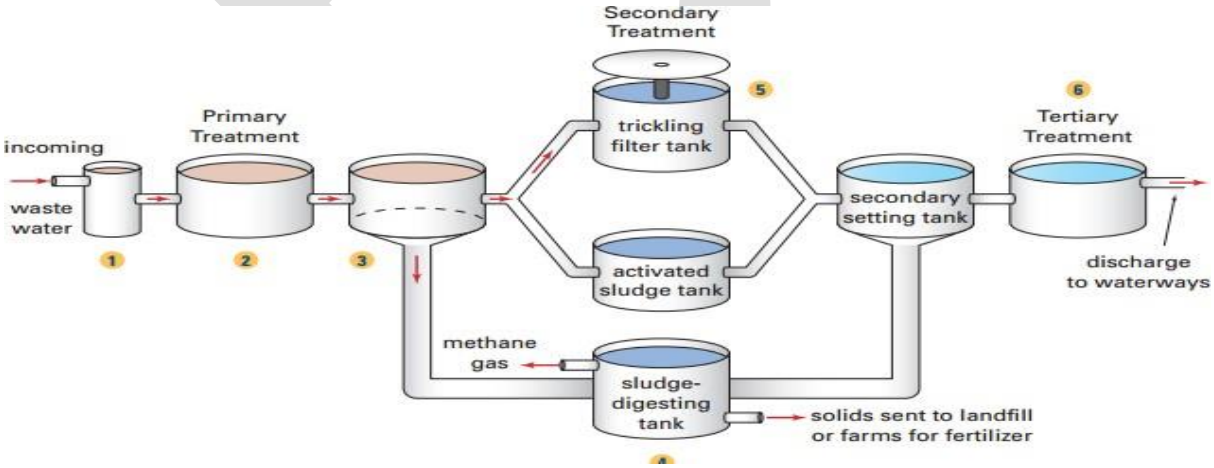
- Municipal treatment plants carry out a series of treatment processes which are-
- Primary treatment: To remove coarse solids i.e., removal of settable solids.
- Secondary treatment (Biological Treatment): Adsorb & oxidize organic matter of Wastewater i.e., reduces BOD.
- Tertiary treatment process: Further reduce BOD by removal of nutrients such as P & N.
- Final treatment Process: To disinfect & dispose of effluent (Liquid remaining)

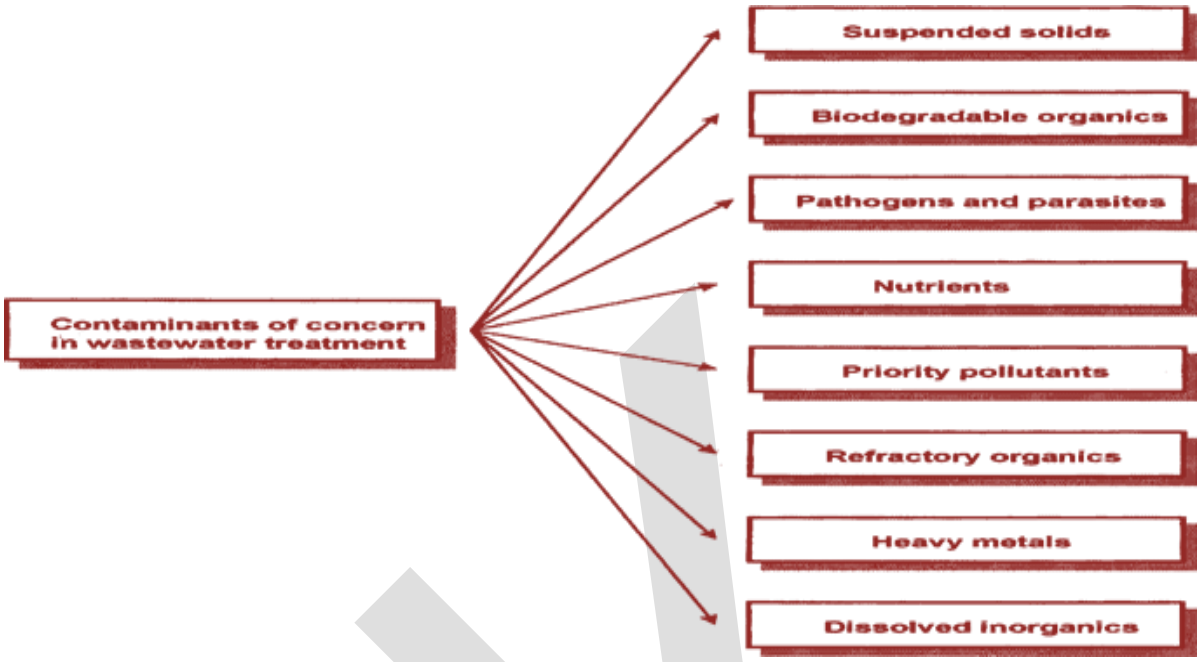
Primary treatment

- To remove coarse solids i.e., removal of settable solids.
- Secondary treatment (Biological Treatment):
- Adsorb & oxidize organic matter of Wastewater i.e., reduces BOD.
- Tertiary treatment process/ Final treatment Process
- Further reduce BOD by removal of nutrients such as P & N.
- To disinfect & dispose of effluent (Liquid remaining)

Table Major Steps in Primary, Secondary, and Tertiary Treatment of Wastes

treatment Step	Process
Primary	Removal of insoluble particulate materials by settling, screening, addition of alum and other coagulation agents, and other physical procedures
Secondary	Biological removal of dissolved organic matter Trickling filters Activated sludge Lagoons Extended aeration systems Anaerobic digesters
Tertiary	Biological removal of inorganic nutrients Chemical removal of inorganic nutrients Virus removal/inactivation Trace chemical removal





Primary Treatment

- It removes 20-30% of BOD.
- In this treatment particulate matter is removed & resulting solid is called Sludge.

Secondary Treatment

- It promotes biological oxidation of dissolved organic matter forming biomass of microbes & CO2.
- 90-95% of BOD & many bacterial pathogens are removed.
- Settable stable flocs will form.

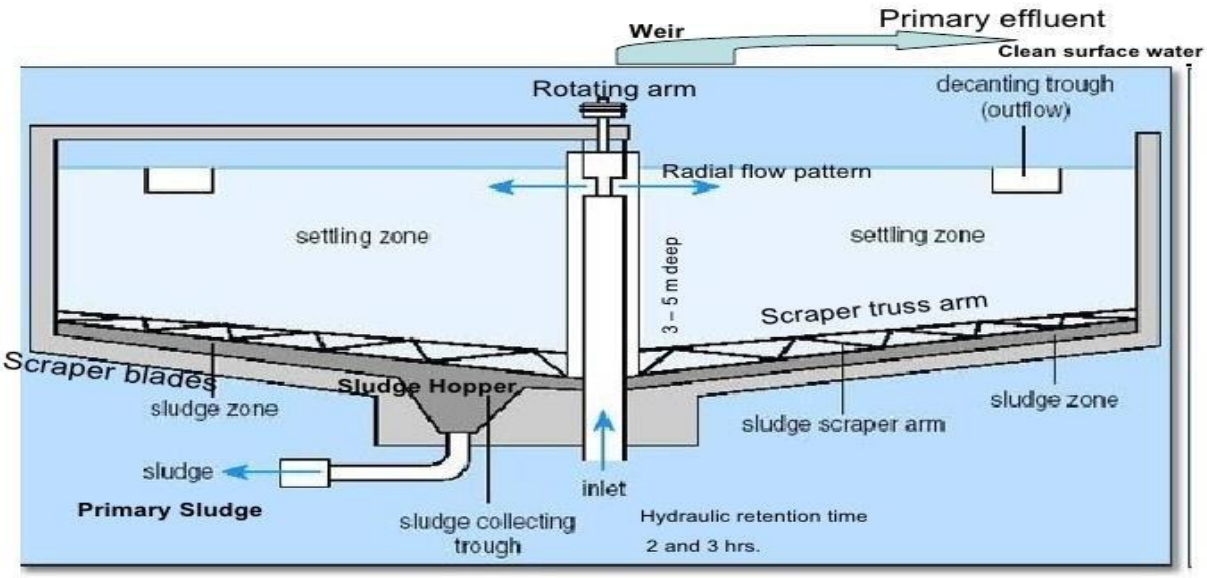
Primary Treatment

- Removal of solid waste (sludge) Includes:
- **Screening:** Coarse solid which may clog the mechanical equipment and pipes.
- **Comminution**: Grinding of coarse solids into smaller and more uniform particles.
- **Flotation**: Separation of suspended and floatable solids particles by air bubbles.
- **Grit removal**: Sand, ash, egg shell etc., of less diameter than 0.2 mm. – inorganic cannot be broken down by biological treatment process.

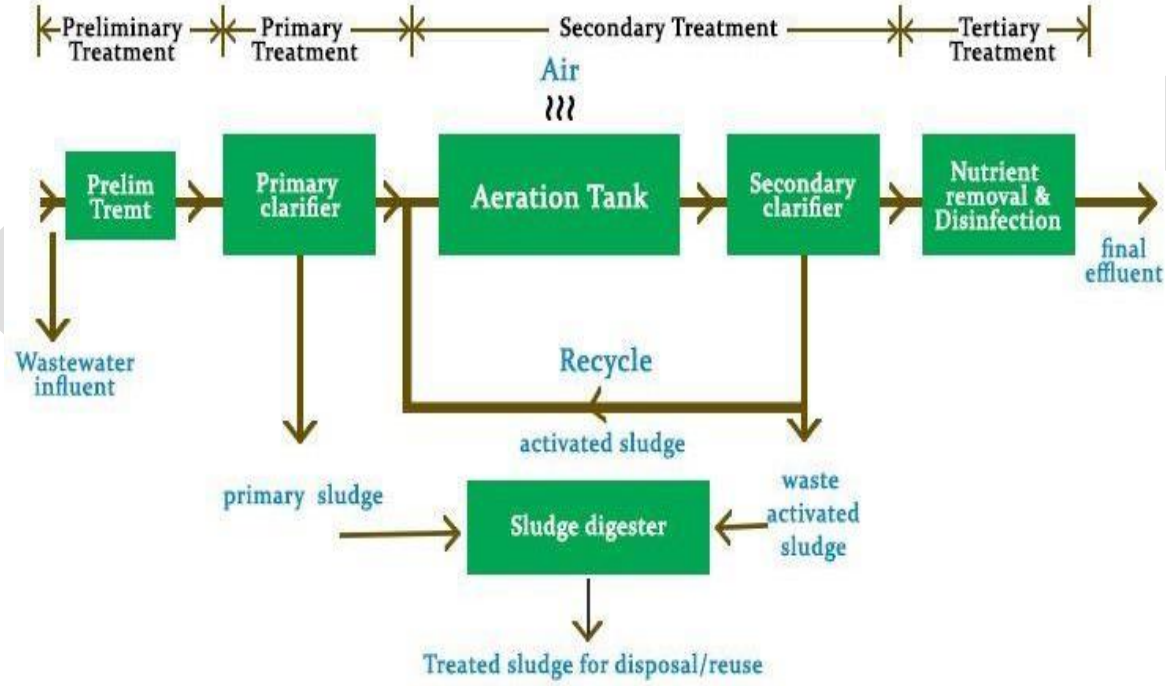
Secondary Treatment

- Biological treatment process
- Dissolved and non-settling organic solids from the primary effluent are removed.
- Microbes plays an important role

Primary Sedimentation tanks (or) Clarifiers (or) rectangular basins



30% BOD, 50-70% Total Suspended Solids and 65% of the oil and grease are removed during primary treatment. And some organic and phosphorus also.



Sr.No.	QUESTION	ANSWER
1		
2		
3		
4		

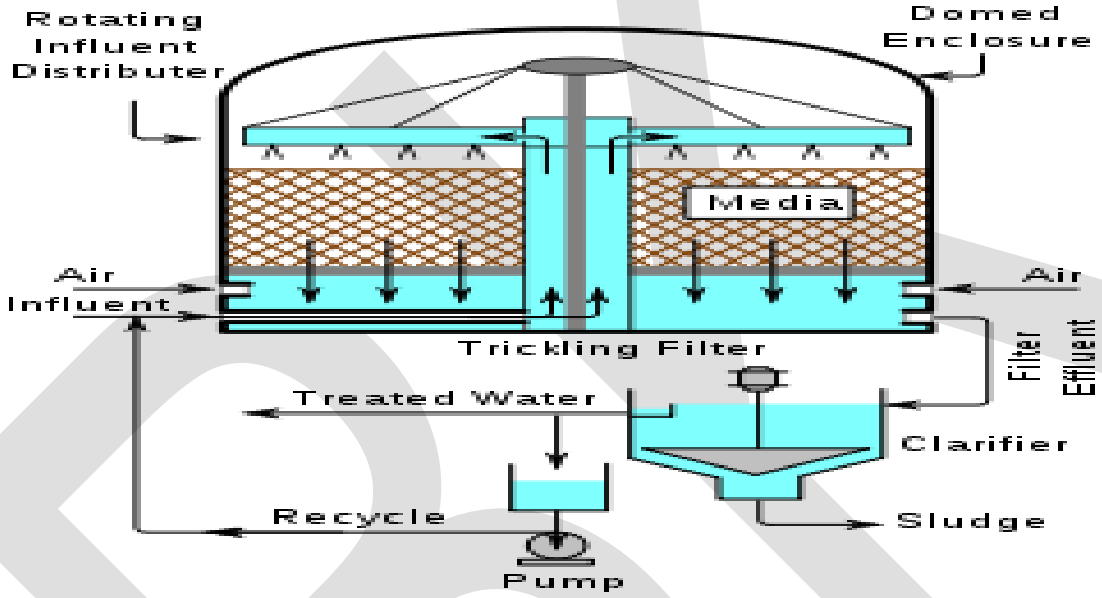
5		

Biological treatment of waste water

- Majorly classified into two types and are as follows:
  - Biological Aerobic Treatment (in presence of oxygen)
  - Biological Anaerobic Treatment (in absence of oxygen)

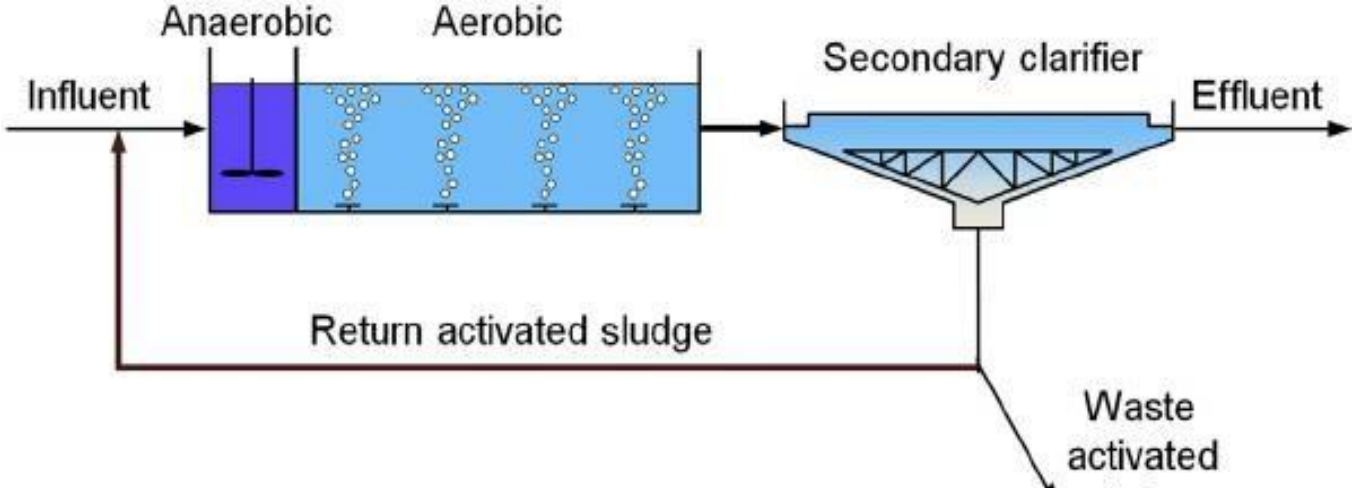
Biological Aerobic Treatment

- Aerobic wastewater treatment is a biological process that takes place in the presence of oxygen. It is the rapid and the most efficient biological waste treatment which remove up to 98% of organic contaminants.

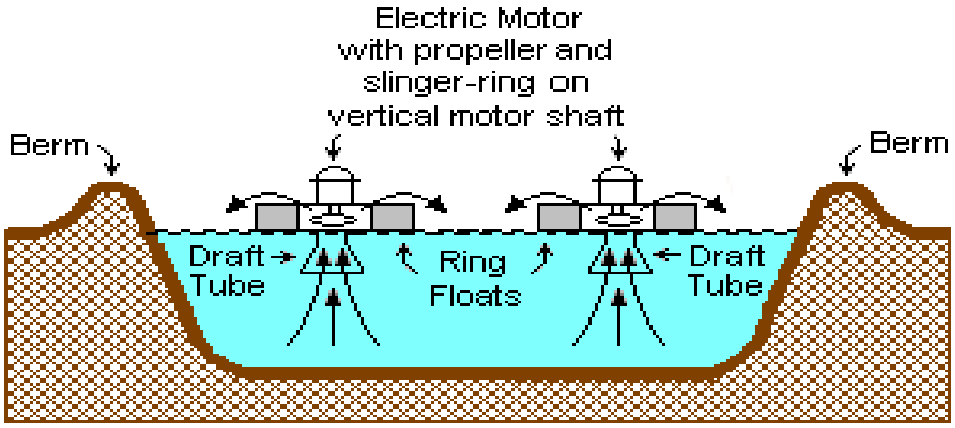


Activated Sludge Process: The activated sludge process is the most widely used biological waste treatment in secondary stage of wastewater treatment. An activated sludge process refers to a multi-chamber reactor unit that makes use of highly concentrated microorganisms to degrade organics and remove nutrients from wastewater to produce a high-quality effluent.

Activated Sludge Process



- Aerobic conditions are maintained by diffusion, and either by forced-air flowing through the bed or natural convection of air if the filter medium.



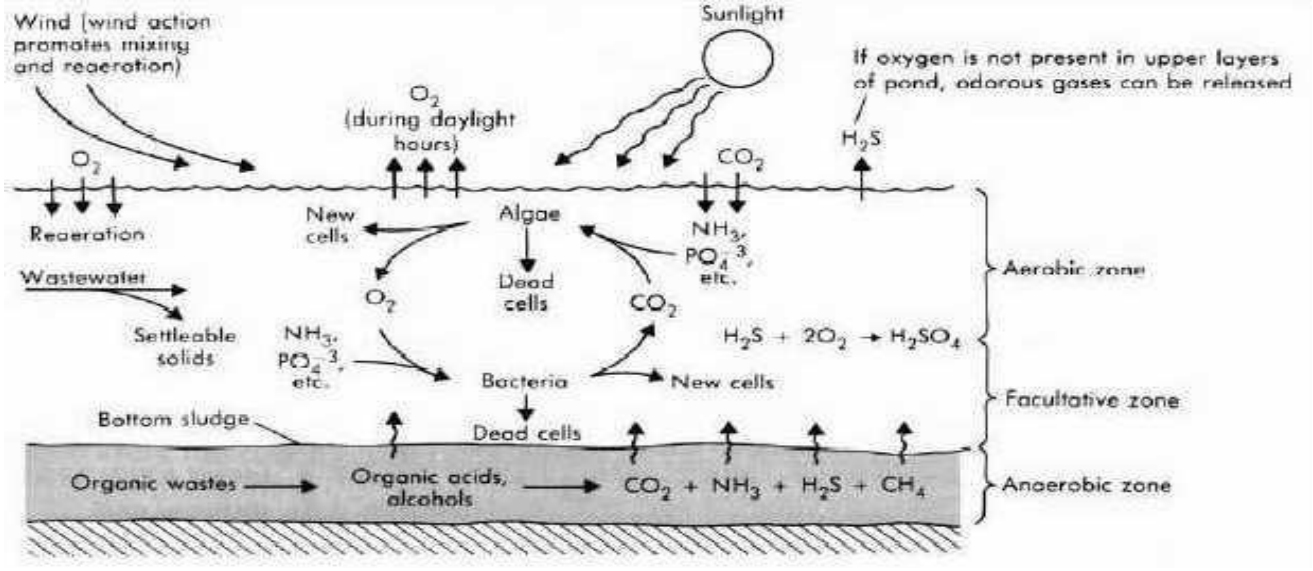
A TYPICAL SURFACE – AERATED BASIN

Aerated Lagoons:

- It is one of the aerobic biological wastewater or waste treatment process.
- An aerated lagoon is a treatment pond that is provided with mechanical aeration that introduces oxygen into the pond in order to promote the biological oxidation of the wastewater.
- The effluent of aerated ponds may be reused or used for recharge, but settled sludge requires a further treatment.

Oxidation Pond:

- Oxidation ponds are large and shallow; a typical depth would range from 1-2.5m. The ponds are composed of microorganisms, which feed on the organic matter received from primary effluent.
- Algae are a key feature in the oxidation pond system.
- Algae are much like the aeration tank in the activated sludge system; they deliver a steady flow of oxygen to the bacteria.
- The algae require sunlight to produce oxygen via photosynthesis, reaeration created by wind delivers air flow when sunlight is not available.
- Overall the process is slow and requires large areas of land.



Tertiary treatment

- The main function of tertiary treatment is to decrease the load of nitrogen & phosphorous compound present in the effluents.
- It further reduces the BOD
- Unit processes have been developed to remove nutrients. simple organic substances & complex synthetic organic compounds.
- Different process employed includes biological treatment & also physical – chemical treatments which predominates ammonia removal by nitrification (aerobic process):

ammonia-oxidizing bacteria:

$NH_4^+ + 1.5O_2 \longrightarrow NO_2^- + H_2O + 2H^+$

nitrite-oxidizing bacteria: $NO_2^- + 0.5O_2 \longrightarrow NO_3^-$

- **Net: $NH_4^+ + 2O_2 \longrightarrow NO_3^- + H_2O + 2H^+$**
- denitrification (anaerobic process): facultative heterotrophic bacteria:
  organic substrates + $NO_3^- \longrightarrow N_2$
- note **nitrogen *removal* occurs by nitrification + denitrification**
- removal of ammonia and nitrogen by anaerobic ammonia oxidation ("anammox"):
  anaerobic bacteria $NH_4^+ + NO_2^- \longrightarrow N_2 + 2H_2O$
- biological phosphorus removal: facultative heterotrophs
- under anaerobic conditions, hydrolyze stored polyphosphate to accumulate intracellular organic polymer (*e.g.*, polyhydroxy butyrate)
- under aerobic conditions, oxidize stored organic polymer to accumulate phosphate as intracellular poly-phosphate
- The following types of tertiary treatment further processes are used.
  - Suspended solids removal
  - Carbon adsorption
  - Ion exchange
  - Reverse osmosis
  - Chemical oxidation.

Suspended solid removal: There are basically 3 methods used for such solid removal. **Coagulation:** Coagulation is carried out using alum, lime, polyelectrolyte & other chemical agents. **Filtration:** Filtration is carried out by sand filters, membrane filters, diatomeoues earth filter. **Micro screening:** Micro screening removers 70-90 % suspended solids.

Carbon Adsorption: Adsorption is accumulating or concentrating a solute at the surface of a solid. Activated carbons have been widely used as adsorbents in waste water treatment, to remove taste & odor causing organics. Activated carbon is prepared from a carbonaceous material such as wood, lignite, coal etc. which are treated with the thermal activation which yields a very porous structure with large surface area. This is carried out by heating carbon at about 1700°F. waste water is now adsorbed on the activated carbon.

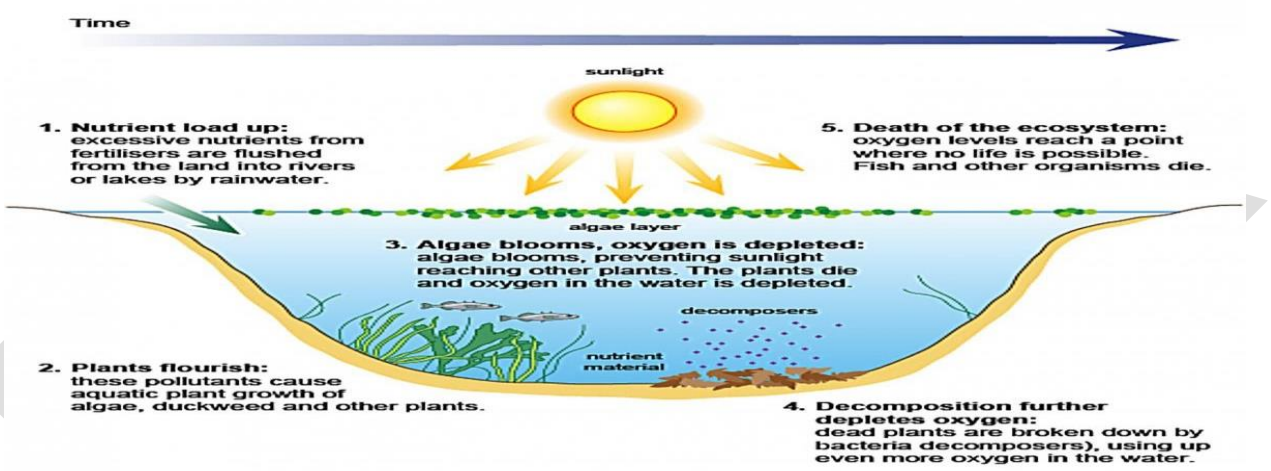
Ion exchange: Chromatographic technique is used for separation of molecules based on

reversible exchange of ion in solution. Exchange of ions occurs between liquid & solid phase (resin). Ion exchange process is where ions are held to functional groups of resin by electrostatic forces are exchanged. Complete demineralization can be achieved. Resin's particles are of 2 types: **Cation exchange** is negatively charged & will attack positively charged cation. **Anion exchange** is positively charged & attracts negatively charged anions.

Reverse osmosis: In waste water treatment contaminated water effluent is placed in contact with the suitable membrane at excess (very high) osmotic pressure. Under these conditions water with a very small amount of contaminant permeates the semi permeable membrane & dissolved contaminants are concentrated in the waste water compartment.

Chemical oxidation: Chlorination: used for the purpose of disinfection, reduction in BOD, elimination of color & odor, oxidation of metal ions & harmful cyanides. Ozonization: it reacts with unsaturated organics on waste water & helps in its degradation.

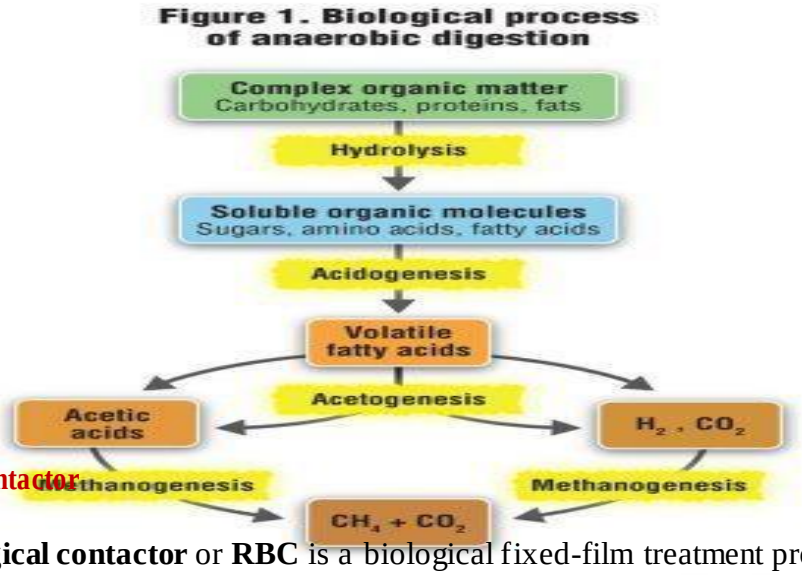
Nutrient removal: Eutrophication.



Sr.No.	QUESTION	ANSWER
1	How many types of waste water treatment?	Two
2	Which are types of waste water treatments?	Aerobic and anaerobic treatm,ent plant.
3	aerobic treatment remove how much % of organic waste?	98% organic waste
4	Nutrient removal was carried out by which process?	Eutrophication
5	Which process required aerobic as well as anaerobic treatment?	Activated sludge process.

Biological Anaerobic Treatment& Sludge Digestion

- Anaerobic treatment is a slow process and it occurs in many different stages. Anaerobic digestion is biological process which is used in wastewater treatment plants for sludge degradation and stabilization. Biogas is produced as the bacteria feed off the biodegradable material in the anaerobic process. Overall, the process converts about 40% to 60% of the organic solids to methane (CH_4) and carbon dioxide (CO_2).



Rotating biological contactor

- A **rotating biological contactor** or **RBC** is a biological fixed-film treatment process used in the treatment of wastewater following primary treatment.
- The primary treatment process involves removal of grit, sand and coarse suspended material through a screening process, followed by settling of suspended solids. The RBC process allows the wastewater to come in contact with a biological film in order to remove pollutants in the wastewater before discharge of the treated wastewater to the environment, usually a body of water (river, lake or ocean).
- A rotating biological contactor is a type of secondary (Biological) treatment process. It consists of a series of closely spaced, parallel discs mounted on a rotating shaft which is supported just above the surface of the waste water. Microorganisms grow on the surface of the discs where biological degradation of the wastewater pollutants takes place.

Aerobic treatment Composting

- The organic portion of solid waste can be biodegraded by composting, the process by which solid heterogeneous organic matter is degraded by aerobic, mesophilic and thermophilic microorganisms.
- Composting is a microbial process that converts organic waste materials into a stable, sanitary, humus-like product. reduced in bulk. It can be used for soil improvement.
- Composting, like incineration, requires sorting of the solid waste into its organic and inorganic components.
- This can be accomplished at the source by the separate collections of garbage (organic waste) and trash (inorganic waste) or at the receiving facility by using magnetic separators to remove ferrous

metals and mechanical separators to remove glass, aluminum, and plastic materials.

- The remaining largely organic waste is ground up. Mixed with sewage sludge or bulking agents such as shredded newspaper or wood chips and then composted.
- The addition of dehydrated sewage sludge to domestic garbage improves the carbon/nitrogen balance because sewage sludge is high in nitrogen and therefore enhances microbial biodegradation activities, as well as providing a means of disposing of some sewage sludge waste and supplying a considerable number of decomposer microorganisms.
- The addition of 10% by weight sewage sludge lo the material being composted improve its porosity. This is important because 30% air space is needed to optimize the availability of oxygen for microbial respiration in the aerobic compost process.
- It also is important because water must drain out of the composting material to prevent waterlogging and the development of anaerobic conditions.
- In a compost of domestic garbage and sludge, numerous microbial species that come from soil, water, and fecal matter are present.
- The relatively high moisture content of the compost material favors the development of bacterial rather than fungal populations.
- In the composting of solid organic wastes, the process is initiated by mesophilic heterotrophs, which, as the temperature rises, are replaced by thermophilic microorganisms.
- The initial temperature increase is probably due to the growth of mesophilic bacteria in the interior portion thermophilic microorganisms prominent in the composting process include the bacteria *Bacillus stearothermophilus* -
- *Thermomonospora spp*., *Thermoactinomyce*.s *spp*., and *clostridium Thermophila* and the fungi *Geotrichum candidum, Aspergillus fumigatus, Mucor pusillus , Chaetomium thermophile, Thermoascus auranticus* and *Torula thermophila*.
- In the continuous- reactor composting process, the reactor is maintained continuously at thermophilic temperatures by using the heat produced within the reactor by the biodegradation of the organic Matter.
- Control of several conditions is critical for achieving optimal composting. Temperatures needed to achieve maximal rates of organic matter decomposition are in the range of 50 c to 60°C.
- Self-heating typically raises the temperature inside a static compost pile to 55°C to 60°C or above in two to three days under favorable conditions but after a few days at this optimal level the temperature gradually declines unless the pile is turned, to resupply oxygen and ensure that the thermophilic process occurs throughout the pile instead of only at the core.
- Moisture must be adequate; 50% to 60% water content is optimal but excess moisture- 70% or above-interferes with aeration and lowers self-heating because of water's large heat capacity.

- The carbon-to-nitrogen ratio must not be greater than 40:1.
- A lower nitrogen content precludes the formation of a sufficient microbial biomass, and a greater nitrogen concentration and lowering the usefulness of the compost product as a fertilizer.
- Although compost is a good soil conditioner and supplies some plant nutrients, it cannot compete with synthetic fertilizers for use in agricultural production.
- The sale of compost effectively reduces the cost of the waste disposal operation but generally does not render the waste disposal operation self-supporting.
- When sewage sludge is used as a major component of the original compost mixture, however, the finished product may contain relatively high concentrations of potentially toxic heavy metals, such as cadmium, chromium, and thallium.
- Because little is known about the behavior of these metals in agricultural soils, the use of sewage sludge-derived compost in agriculture is not widely practiced. Compost does find extensive applications in parks and gardens for ornamental plants, in land reclamation (particularly after strip mining) and as part of highway beautification projects.

Sr.No.	QUESTION	ANSWER
1	Which treatment is used for anaerobic digestion?	Sludge digestion
2	Which treatment is used for aerobic solid waste management?	Composting
3	Composting is how many days treatment?	30-90 days
4	How much moisture content is required for composting?	40-60%
5	Which microorganism give musty smell to compost?	Actinomycetes