Unit 2 - Microbiology of Water and Waste water

2.1 Types of Waters and sources of microbes in it

Types of waters range from the vast ocean reaches to lakes and flowing bodies of water, such as rivers and streams. Roughly 71% of the Earth's surface is occupied by water, 97% of which is contained in the world's oceans. Less than 1% of water is found in streams, rivers, and lakes.

Distribution of Water on Earth's Surface

Water Source	Percentage of Total Water
Oceans	97.24
Ice caps, glaciers	2.14
Groundwater	0.61
Freshwater lakes	0.009
Inland seas	0.008
Soil moisture	0.005
Atmosphere	0.001
Rivers	0.0001
Total	**1 0 0**

Natural waters are commonly grouped into four classes : (1) atmospheric waters (2) surface waters, (3) stored waters and (4) ground waters.

1) Atmospheric waters

Rain, snow and hail which fall on land tend to carry down particles of dust, soot, and other materials suspended in the air. These often bear bacteria and other microorganisms on their surface. The number of organisms depends upon local conditions. After heavy rain or snow the atmosphere is washed free of organisms.

2) Surface waters

As soon as rain or snow reaches the earth and flows over the soil, some of the soil organisms are gathered up by the water. Bodies of water such as streams, rivers, and oceans represent surface water. Microbial populations depend upon their numbers in the soil and, also, upon the kinds and quantities of food material dissolved out of the soil by water. Climatic, geographical and biological conditions bring about great variations in microbial populations of surface waters. Rivers and streams show their highest count during the rainy period Dust blowing into rivers and streams also contributes many microorganisms. Animals also make considerable contribution to the microbial flora of the surface waters. They bathe and often drop their excreta in the water.

3) Stored waters

Inland waters held in ponds, lakes, or reservoirs represent stored waters. Storage generally reduces the numbers of organisms in water. A certain degree of purity and stability is established.

4) Ground waters

As water seeps through the earth, microorganisms as well as suspended particles are removed by filtration in varying degrees, This depends on the permeability characteristics of the soil and the depth to which the water penetrates. Ground water brought to the surface by springs or deep wells contains very few organisms, To construct a well the nature of the soil and underlying porous strata, the nature of the water table, and the nature, distance, and direction of the local sources of pollution must be taken into consideration.

Sources of microbes in water

Pathogens may enter waters through point and non-point sources, while others may occur naturally in the environment. Some point sources are wastewater treatment facilities and combined sewer overflows. Non-point sources include land and road runoff, human sewage from recreational boats, and septic systems. The major sources of bacterial contamination are due to the point sources. Treatment facilities have greatly reduced the number of pathogens that are released into the environment though disinfectant processes. However, treatment is not always 100% effective and breakdowns in facilities sometimes occur. During heavy rains, there is too much water for the sewage treatment plants to handle, and some untreated or partially treated water may be disposed into the Bay. Wildlife, domestic animals and birds may also contribute pathogens to the environment.

Combined Sewer Overflows (CSO's) are the largest contributor of bacteria and viruses to the Bay. CSO's combine residential, commercial and industrial wastes which carries pollutants in the form of sewage solids, metals, oil, grease and bacteria. During periods of heavy rain, the water in the CSO's combine with the storm water running over the land. The CSO then becomes overwhelmed with water which forces it to discharge untreated or partially treated wastewater into Narragansett Bay through the combined sewer overflow. Each year, billions of gallons of untreated sewage from combined sewer outflows are released into the Bay and its tributaries.

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Microbial communities in natural water

Water can support the growth of many types of microorganisms. Some are advantageous and some are disease causing. A variety of microorganisms live in fresh water. The region of a water body near the shoreline (the littoral zone) is well lighted, shallow, and warmer than other regions of the water. Photosynthetic algae and bacteria that use **light** as energy thrive in this zone. Further away from the shore is the limnitic zone. Photosynthetic microbes also live here. Saltwater presents a different environment to microorganisms.

Cyanobacteria, protozoa, **algae**, and tiny animals such as rotifers can be important in the food chain that forms the basis of life in the water. For example, the microbes called cyanobacteria can convert the **energy** of the **sun** into the energy it needs to live. The plentiful numbers of these organisms in turn are used as food for other life. The algae that thrive in water is also an important food source for other forms of life.

1) **Bacteria: -** Bacteria which are used in food fermentation are found in water also bacteria that live in the intestinal tracts of humans and other warm blooded animals, such as ***Escherichia coli***, ***Salmonella, Shigella,* and *Vibrio***, can contaminate water if feces enters the water.

As the water deepens, temperatures become colder and the **oxygen** concentration and light in the water decrease. Now, microbes that require oxygen do not thrive. Instead, purple and green **sulfur** bacteria, which can grow without oxygen, dominate. Finally, at the bottom of fresh waters (the benthic zone), few microbes survive. Bacteria that can survive in the absence of oxygen and sunlight, such as methane producing bacteria, thrive.

The higher **salt** concentration, higher **pH**, and lower **nutrients**, relative to **freshwater,** are lethal to many microorganisms. But, salt loving (halophilic) bacteria abound near the surface, and some bacteria that also live in freshwater are plentiful (i.e., *Pseudomonas* and *Vibrio*). Also **archaebacteria** is one of the dominant forms of life in the **ocean**. The role of archaebacteria in the ocean food chain is not yet known, but must be of vital importance.

2) **Viruses: -**The intestinal tract of warm-blooded animals also contains viruses that can contaminate water and cause disease. Examples include rotavirus, enteroviruses, and coxsackievirus.

3) **Protozoa**: **-** The two protozoa of the most concern are *Giardia* and *Cryptosporidium*. They live normally in the intestinal tract of animals. *Giardia* and *Cryptosporidium* form dormant and hardy forms called cysts during their life cycles. If ingested in drinking water they can cause debilitating and prolonged diarrhea in humans.

4) Algae: - Another microorganism found in saltwater are a type of algae known as dinoflagellelates. The rapid growth and multiplication of dinoflagellates can turn the water red. This "red tide" depletes the water of nutrients and oxygen, which can cause many **fish** to die. As well, humans can become ill by eating contaminated fish.

2.2 Determining sanitary quality of water

One of the most important aspects of aquatic microbiology is related to several human diseases transmitted via water. The design and development of epidemiological surveillance studies have led to the awareness of the magnitude of human morbidity and mortality associated with waterborne infectious diseases.

Sewage water pollution is one of the major problems in cities. The sewage water is drained off into rivers without treatment. The careless disposal of sewage water leads to a chain of problems, such as spreading of diseases, eutrophication, increase in Biological Oxygen Demand (BOD), etc.

The waste water that flows after being used for domestic, industrial and other purposes is termed as sewage water. In ideal situations, the sewage water is channeled or piped out of cities for treatment. Bulk of the sewage contains water as the main component, while other constituents include organic wastes and chemicals.

Improper handling of waste water is the main reason behind the pollution of water. The sewage is drained off in large quantities into rivers. It slows down the process of dilution of the constituents present in the water; which in turn, stagnate the river.

Draining off the water without treatment is also a reason behind sewage water pollution. These effluents contain innumerable pathogens and harmful chemicals. The detergents that release phosphates in water help the growth of algae and water hyacinths.

Fecal pollution of water is the contamination of water with disease-causing organisms (pathogens) that may inhabit the gastrointestinal tract of mammals. Ingestion of water contaminated with feces is responsible for a variety of diseases important to humans via what is known as the fecal-oral route of transmission

Bacteriological evidence of fecal pollution

Each gram of human feces contains approximately 12 billion (1.2×10^9) bacteria, among them may include pathogenic bacteria, such as *Salmonella*, associated with gastroenteritis. In addition, feces may contain pathogenic viruses, protozoa and parasites. If ingested, these organisms would cause disease.

The presence of fecal coliform bacteria in aquatic environments indicates that the water has been contaminated with the fecal material of man or other animals. Fecal coliform bacteria can enter rivers through direct discharge of waste from mammals and birds, from agricultural and storm runoff, and from untreated human sewage. Individual home septic tanks can become overloaded during the rainy season and allow untreated human wastes to flow into drainage ditches and nearby waters. Agricultural practices such as allowing animal wastes to wash into nearby streams during the rainy season, spreading manure and fertilizer on fields during rainy periods, and allowing livestock watering in streams can all contribute fecal coliform contamination.

At the time this occurs, the source water may be contaminated by pathogens or disease producing bacteria or viruses, which can also exist in fecal material. Some waterborne pathogenic diseases include ear infections, dysentery, typhoid fever, viral and bacterial gastroenteritis, and hepatitis A. The presence of fecal coliform tends to affect humans more than it does aquatic creatures, though not exclusively. While these bacteria do not directly cause disease, high quantities of fecal coliform bacteria suggest the presence of disease causing agents. The presence of fecal contamination is an indicator that a potential health risk exists for individuals exposed to this water. During high rainfall periods, the sewer can become overloaded and over flow, bypassing treatment. As it discharges to a nearby stream or river, untreated sewage enters the river system. Runoff from roads, parking lots, and yards can carry animal wastes to streams through storm sewers

Indicators of fecal pollution

The direct detection of pathogenic bacteria and viruses, and cysts of protozoan parasites requires costly and time-consuming procedures, and well-trained labor. The task would be enormous if one contemplates the monitoring of hundreds of pathogens and parasites on a routine basis in water and wastewater treatment plants, receiving waters, soils, and other environmental samples. Therefore, indicators of fecal pollution were much needed. As early as 1914, the U.S. Public Health Service (U.S.P.H.S.) adopted the coliform group as an indicator of fecal contamination of drinking water.

The criteria for an ideal indicator organism are the following:

i. It should be a member of the intestinal microflora of warm-blooded animals.
ii. It should be present when pathogens are present, and absent in uncontaminated samples.
iii. It should be present in greater numbers than the pathogen.
iv. It should be at least equally resistant as the pathogen to environmental factors and to disinfection in water and wastewater treatment plants.
v. It should not multiply in the environment.
vi. It should be detectable by means of easy, rapid, and inexpensive methods.
vii. The indicator organism should be non pathogenic

Proposed or commonly used microbial indicators are discussed below

i. Coliform Bacteria
ii. Fecal Streptococci
iii. Anaerobic bacteria
iv. Bacteriophages

i. Coliform Bacteria

Characteristics of the coliform group

The total coliform group belongs to the family Enterobacteriaceae and includes the aerobic and facultative anaerobic, gram-negative, non-sporeforming, rod-shaped bacteria that ferment lactose with gas production within 48 hours at 35^{o}C. Total coliforms include *Escherichia coli, Enterobacter, Klebsiella,* and *Citrobacter*. These coliforms are discharged in relatively high numbers in human and animal feces, but not all of them are of fecal origin. These indicators are useful for determining the quality of potable water, shellfish harvesting waters, and recreational waters.

Fecal coliforms are thermotolerant bacteria that include all coliforms that can ferment lactose at 44.5oC. The fecal coliform group comprises bacteria such as *Escherichia coli* or *Klebsiella pneumonae*. The presence of fecal coliforms indicates the presence of fecal material from warm-blooded animals. Some investigators have suggested the sole use of *E. coli* as an indicator of fecal pollution as it can be easily distinguished from the other members of the fecal coliform group

ii. Fecal Streptococci

This group comprises *Streptococcus faecalis, S. bovis, S. equinus and S. avium.* Since they commonly inhabit the intestinal tract of humans and warm-blooded animals, they are used to detect fecal contamination in water. Members of this group survive longer than other bacterial indicators but do not reproduce in the environment.

iii. Anaerobic Bacteria

Some of the anaerobic bacteria are important part of fecal flora. We will cover three important anaerobic bacteria that have been proposed as indicators of fecal contamination.

a. Clostridium perfringens
b. Bacteroides spp.
c. Bifidobacteria

iv. Bacteriophages

Bacteriophages have a basic structure similar to that of animal viruses. They infect a wide range of bacteria. They initiate a lytic cycle, which results in the production of phage progeny and the destruction of the bacterial host cells.

v. Bacterial Spores

Aerobic spores are non pathogenic, ubiquitous in aquatic environments, occur at much higher concentrations than the parasitic protozoan cysts, do not grow in environmental waters There is still an ongoing debate among the public health community on which microorganism should be used as an indicator, as there is a weak relationship between some indicators and the pathogens or parasites they are supposed to represent. There is probably no universal ideal indicator microorganism that fulfills all the criteria outlined earlier

The best among these is *E. coli* because *Streptococci* and *Bifidobacteria* survive for a very short period and detection of anaerobes, spores and viruses is difficult and expensive.

IMViC REACTIONS

IMViC reactions are a set of four useful reactions that are commonly employed in the identification of members of family enterobacteriaceae. The four reactions are: Indole test, Methyl Red test, Voges Proskauer test and Citrate utilization test. The letter 'i' is only for rhyming purpose.

INDOLE TEST:

Principle: Some bacteria can produce indole from amino acid tryptophan using the enzyme typtophanase.Production of indole is detected using Ehrlich's reagent or Kovac's reagent. Indole reacts with the aldehyde in the reagent to give a red color. An alcoholic layer concentrates the red color as a ring at the top.

Procedure: Bacterium to be tested is inoculated in peptone water, which contains amino acid tryptophan and incubated overnight at 37oC. Following incubation few drops of Kovac's reagent are added. Kovac's reagent consists of para-dimethyl aminobenzaldehyde, isoamyl alcohol and con. HCl. Ehrlich's reagent is more sensitive in detecting indole production in anerobes and non-fermenters. Formation of a red or pink coloured ring at the top is taken as positive.

METHYL RED (MR) TEST:

Principle: This is to detect the ability of an organism to produce and maintain stable acid end products from glucose fermentation. Some bacteria produce large amounts of acids from glucose fermentation that they overcome the buffering action of the system. Methyl Red is a pH indicator, which remains red in color at a pH of 4.4 or less.

Procedure: the bacterium to be tested in inoculated into glucose phosphate broth, which contains glucose and a phosphate buffer and incubated at 37oC for 48 hours. Over the 48 hours the mixed-acid producing organism must produce sufficient acid to overcome the phosphate buffer and remain acid. The pH of the medium is tested by the addition of 5 drops of MR reagent. Development of red color is taken as positive. MR negative organisms produce yellow color.

VOGES PROSKAUER (VP) TEST:

Principle: While MR test is useful in detecting mixed acid producers, VP test detects butylene glycol producers Acetyl-methyl carbinol (acetoin) is an intermediate in the production of butylene glycol. In these test two reagents, 40% KOH and alpha-naphthol are added to test broth after incubation and exposed to atmospheric oxygen. If acetoin is present; it is oxidized in the presence of air and KOH to diacetyl. Diacetyl then reacts with guanidine components of peptone, in the presence of alphanaphthol to produce red color. Role of alpha-naphthol is that of a catalyst and a color intensifier.

Procedure: Bacterium to be tested is inoculated into glucose phosphate broth and incubated for at least 48 hours.0.6 mls of alpha-naphthol is added to the test broth and shaken. 0.2 ml of 40% KOH is added to the broth and shaken. The tube is allowed to stand for 15 minutes. Appearance of red color is taken as a positive test. The negative tubes must be held for one hour, since maximum color development occurs within one hour after addition of reagents.

CITRATE UTILIZATION TEST:

Principle: This test detects the ability of an organism to utilize citrate as the sole source of carbon and energy. Bacteria are inoculated on a medium containing sodium citrate and a pH indicator bromothymol blue. The medium also contains inorganic ammonium salts, which is utilized as sole source of nitrogen.Utilization of citrate involves the enzyme citritase, which breaks down citrate to oxaloacetate and acetate. Oxaloacetate is further broken down to pyruvate and CO_2.Production of Na_2CO_3 as well as NH_3 from utilization of sodium citrate and ammonium salt respectively results in alkaline pH. This results in change of medium's color fromgreen to blue.

procedure: Bacterial colonies are picked up from a straight wire and inoculated into slope of Simmon's citrate agar and incubated overnight at 37oC. If the organism has the ability to utilize citrate, the medium changes its color from green to blue.

Bacterium	**Indole**	**MR**	**VP**	**Citrate**
Escherichia coli	+	+	-	-
Enterobactor aerogens	-	-	+	+

Eijkman's elevated-temperature test

The physiological basis of the Eijkman elevated-temperature test for differentiating fecal from nonfecal coliforms was investigated. Manometric studies indicated that the inhibitory effect upon growth and metabolism in a nonfecal coliform at 44.5 degrees involved cellular components common to both aerobic and fermentative metabolism of lactose. A temperature increase from 35 to 44.5 degrees drastically reduced the rates of glucose uptake in nonfecal coliforms, whereas those of fecal coliforms were essentially unchanged.

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2.3 Bacteriological examination of water

Bacteriological analysis of water includes the

A) Qualitative analysis

B) Quantitative analysis.

A) Qualitative analysis

By qualitative analysis, we can conclude about the presence of coliform bacteria in water. If they are abundant in water sample, this a strong evidence that the intestinal waste have polluted the water. *Escherichia coli* is an indicator bacterium because its survival period is more than other bacteria and the detection of it is easier and less expensive.

Qualitative analysis consists of following three types of tests.

1. Presumptive test
2. Confirmed test
3. Completed test

1. Presumptive test:

In this test selective medium is used. It contains lactose, bile salt (Sodium taurocholate which allow the growth of coliform bacteria). Coliforms ferment lactose into acid and gas. The gas is collected in Durham's tube but sometimes *Staphylococcus aureaus* and *Proteus vulgaris* synergistically ferment lactose to acid and gas. Hence sometimes we get false positive test. Therefore, further confirmation is to be done by confirmed test. For this test LBB (Lactose Bile Broth), LTB (Lauryl Tryptose Broth) or MacConkey's broth media are used

Medium is distributed in the test tubes. Durham's tubes are placed in inverted position and air in the Durham's tube is removed completely. The tubes are plugged with cotton and are wrapped with paper and autoclaved by steaming for 30 to 40 minutes.

Requirements:

i. Sterile LBB or LTB or MacConkey's broth tube

ii. Water sample

Procedure:

One ml of given water sample is added with sterile pipette into LBB or LTB or MacConkey's broth tube and incubated at 37 ^{o} C for 24 hours. If gas is observed in Durham's tube, test is positive. If gas is not observed test is negative and further testing is not done.

2. Confirmed test:

If presumptive test is positive, then sample from this is streaked on selective medium like EMB (Eosin Methylene Blue) or Endo agar or sample is added in BGLBB (Brilliant Green Lactose Bile Broth).

EMB agar contains eosin and methylene blue, which give typical characteristics to the colony of E. coli. Endo agar contains Basic fuchsin dye, which binds with acetaldehyde which is produced during fermentation of lactose by E. coli. Thus the E. coli colonies become red coloured while other are colourless.

Typical colony	Atypical colony
1. Small, nucleated	1. Large, nucleated
2. Dark red in colour	2. Pink
3. Well isolated	3. Mucoid
4. With or without metallic sheen	4. Dull
5. Ex. *Escherichia coli*	5. Ex. *Enterobactor aerogens*

BGLBB Medium is distributed in the test tubes. Durham's tubes are placed in inverted position and air in the Durham's tube is removed completely. The tubes are plugged with cotton and are wrapped with paper and autoclaved by steaming for 30 to 40 minutes.

Requirements:

Sterile Endo agar plate or EMB agar plate or BGLBB tube
Suspension from positive presumptive tube

Procedure:

Loopful suspension from the positive presumptive test is streaked either on Endo or EMB agar plate or one loopful is inoculated in BGLBB tube. Incubation is done at 37^{o} C for 24 hours. If red colour colonies on Endo agar or typical colonies on EMB agar or gas production in BGLBB tube are observed, confirmed test is positive.

Endo agar contains Basic fuchsin dye, which binds with acetaldehyde which is produced during fermentation of lactose by E. coli. Thus the E. coli colonies become red coloured while others are colourless.

3. **Completed test:**

This test is done to test whether E. coli in confirmed test can again give a positive presumptive test. Sample from typical colony from EMB agar or red colony from Endo agar or from BGLBB tube is inoculated in LBB or LTB or MacConkey's broth tube. Also one loopful suspension is streaked on nutrient agar slant and incubated at $37\ ^{o}$ C for 24 hours.

If acid and gas is produced, the test is positive and if suspension from slant showing Gram negative, rod shaped, and motile bacteria then test is positive.

Requirements:

i. Sterile LBB or LTB or MacConkey’s broth tube

ii. Sterile Nutrient agar slant

Procedure:

The suspension of red colour colony on Endo agar or typical colony on EMB agar or loopful from BGLBB positive tube is inoculated in Sterile LBB or LTB or MacConkey’s broth tube and also streaked on Nutrient agar slant. Incubation is done at 37^{o} C for 24 hours.

If gas is produced and suspension from slant is showing Gram negative, rod shaped and motile bacteria then completed test is positive.

B) Quantitative analysis of water

1. MPN
2. SPC
3. Membrane filter technique

1. MPN

Drinking water may be contaminated with sewage, human excreta. It may cause outbreak of intestinal infections like Typhoid, Dysentery, Cholera, Gastro etc. Coliform bacteria are commonly found in human intestine and considered as indicators of fecal pollution.

The coliform bacteria are able to ferment lactose with production of acid and gas within 24 hours at 37^{0} C. Estimation of number of coliforms in water sample is made by adding various quantities of water from 0.1 ml to 10 ml in MacConkey’s broth. MacConkey’s broth contains pH indicator, which indicates the change in pH in the medium. It also contains Durham’s tube for collection of gas.

In this way it is possible to test quality of water samples containing coliform and then to express the degree of contamination from number of tubes showing acid and gas. By referring MacCardy’s or Swaroop’s table, it is possible to find out number of coliforms present in 100 ml of water sample.

After getting the results i.e. coliform count for 100 ml, we can determine the quality of water and say about the potability of water.

Sr. No.	Quality of Water	Coliform Count / 100ml
1	Excellent	0
2	Satisfactory	1 to 3
3	Intermediate	4 to 9
4	Unsatisfactory or Non potable	More than 10

Requirements:

1. Water sample to be tested
2. Sterile MacConkey's single strength broth tubes (10) each containing 5 ml of medium.
3. Sterile MacConkey's double strength broth tubes (5) each containing 10 ml of medium.
4. Sterile pipettes 10 ml and 1 ml capacity.

Note: In double strength medium the ingredients are added in double amount except water. When 10 ml water sample is added in this tube the medium becomes single strength. Therefore double strength medium is used.

Procedure:

1. In the first set of five test tubes of double strength medium, 10 ml of water sample is added in each tube.
2. In second set of five test tubes of single strength medium, 1 ml of water sample is added in each tube.
3. In third set of five test tubes of single strength medium, 0.1 ml of water sample is added in each tube.
4. All these additions are done in sterile condition and after shaking the tubes, they are incubated at 37 ^{0} C for 24 hours.
5. The number of tubes showing acid and gas production from each set are recorded and statistical estimation of number of coliforms per 100 ml of water is done from the MacCardy's or Swaroop's table.

2. Bacteriological examination of water by Standard Plate Count (SPC)

Water provides the nutrients for the growth of variety of microorganisms. These microorganisms can be pathogenic, non-pathogenic and saprophytic. They remain viable and even multiply in the water. The pathogenic microorganisms may cause many serious epidemics if consumed by the human beings and the animals. Therefore it is very essential to analyze the water microbiologically.

SPC is one of the microbiological examination methods. By this method we can count the viable number of microorganisms in the given water sample. The series of dilutions of given water sample is prepared to get the countable colonies.

Advantages of SPC: -

1. It counts viable organisms only.
2. We can identify the organisms present in the water.

Disadvantages of SPC: -

1. For each cell to produce colony, the bacteria would have to be well separated otherwise many cells form single colony and count will be less.
2. All kinds of microorganisms cannot be grown on the supplied medium.
3. Some rapidly growing species inhibit the growth of other species.
4. Method is time consuming, expensive and requires skilled persons.

Requirements: -

1. Sterile nutrient agar
2. Sterile 9 ml saline tubes
3. Sterile empty petri plates
4. Water sample

Procedure: -

1. Different dilutions of given water sample are prepared in saline.
2. 0.1 ml of each dilution is added in sterile empty petri plates aseptically.
3. Sterile molten and cooled at 45 ^{0} C nutrient agar is poured over the sample.
4. Plates are shaken well to mix the sample, allowed to solidify and incubated at 37 ^{0} C for 24 hours.
5. Colonies are counted form each plate and calculations are done.

3. MEMBRANE FILTER TECHNIQUE

By growing and counting colonies of fecal coliform bacteria from a sample of water, the amount of bacteria originally present can be determined.

Membrane filtration is the method of choice for the analysis of fecal coliforms in water. Samples to be tested are passed through a filter of particular pore size (generally 0.45 micrometre). The microorganisms present in the water remain on the filter surface. When the filter is placed in a sterile petri dish and saturated with an appropriate medium, growth of the desired organisms is encouraged, while that of other organisms is suppressed. Each cell develops into a separate colony, which can be counted directly, and the results calculated as microbial density. Sample volumes of 1 ml and 10 ml will be used for the water testing, with the goal of achieving a final desirable colony density range of 20 to 60 colonies per filter. Contaminated sources may require dilution to achieve a "countable" membrane.

A 100 ml volume of a water sample is drawn through a membrane filter (0.45 µm pore size) through the use of a vacuum pump. The filter is placed on a petri dish containing M-FC agar and incubated for 24 hours at 44.5 °C (112.1 degrees F). This elevated temperature heat shocks non-fecal bacteria and suppresses their growth. As the fecal coliform colonies grow they produce an acid (through fermenting lactose) that reacts with the aniline dye in the agar thus giving the colonies their blue color.

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2.4 Water purification methods

Water purification is the process of removing undesirable chemicals, materials, and biological contaminants from contaminated water. The goal is to produce water fit for a specific purpose. Most water is purified for human consumption (drinking water) but water purification may also be designed for a variety of other purposes, including meeting the requirements of medical, pharmacology, chemical and industrial applications. In general the methods used include physical processes such as filtration and sedimentation, biological processes such as slow sand filters or activated sludge, chemical processes such as flocculation and chlorination and the use of electromagnetic radiation such as ultraviolet light.

The purification process of water may reduce the concentration of particulate matter including suspended particles, parasites, bacteria, algae, viruses, fungi; and a range of dissolved and particulate material derived from the surfaces that water may have made contact with after falling as rain.

The standards for drinking water quality are typically set by governments or by international standards. These standards will typically set minimum and maximum concentrations of contaminants for the use that is to be made of the water.

1. Screening

The first step in purifying surface water is to remove large debris such as sticks, leaves, trash and other large particles which may interfere with subsequent purification steps. Most deep groundwater does not need screening before other purification steps.

2. Flocculation

It is a process which clarifies the water. Clarifying means removing any turbidity or colour so that the water is clear and colourless. Clarification is done by causing a precipitate to form in the water which can be removed using simple physical methods. Initially the precipitate forms as very small particles but as the water is gently stirred, these particles stick together to form bigger particles. Many of the small particles that were originally present in the raw water adsorb onto the surface of these small precipitate particles and so get incorporated into the larger particles that coagulation produces. In this way the coagulated precipitate takes most of the suspended matter out of the water and is then filtered off, generally by passing the mixture through a coarse sand filter or sometimes through a mixture of sand and granulated anthracite (high carbon and low volatiles coal). Coagulants / flocculating agents that may be used include:

1. Iron (III) hydroxide. This is formed by adding a solution of an iron (III) compound such as iron(III) chloride to pre-treated water with a pH of 7 or greater. Iron (III) hydroxide is extremely insoluble and forms even at a pH as low as 7. Commercial formulations of iron salts were traditionally marketed in the UK under the name Cuprus.

2. Aluminium hydroxide is also widely used as the flocculating precipitate although there have been concerns about possible health impacts and mis-handling led to a severe poisoning incident in 1988 at Camelford in south-west UK when the coagulant was introduced directly into the holding reservoir of final treated water.
3. PolyDADMAC is an artificially produced polymer and is one of a class of synthetic polymers that are now widely used. These polymers have a high molecular weight and form very stable and readily removed flocs, but tend to be more expensive in use compared to inorganic materials. The materials can also be biodegradable.

3. Sedimentation

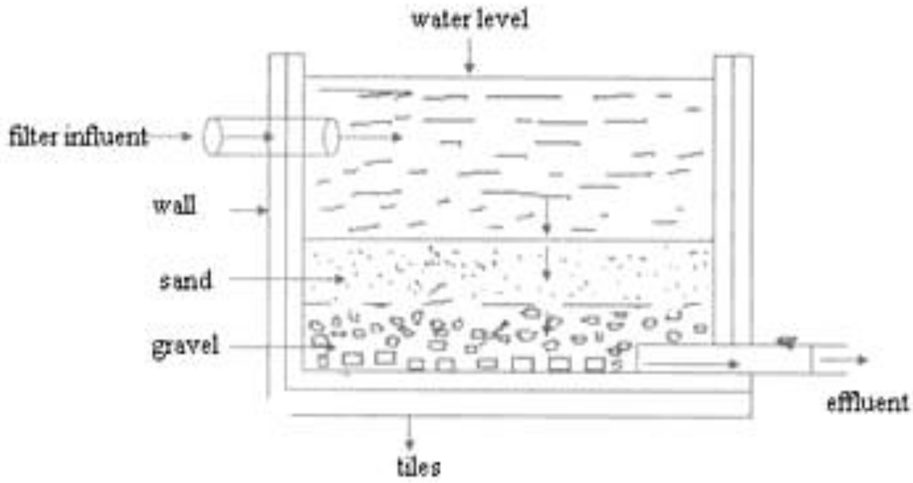
Waters exiting the flocculation basin may enter the sedimentation basin, also called a clarifier or settling basin. It is a large tank with slow flow, allowing floc to settle to the bottom. The sedimentation basin is best located close to the flocculation basin so the transit between does not permit settlement or floc break up. Sedimentation basins may be rectangular, where water flows from end to end or circular where flow is from the centre outward. Sedimentation basin outflow is typically over a weir so only a thin top layer—that furthest from the sediment—exits. The amount of floc that settles out of the water is dependent on basin retention time and on basin depth. The retention time of the water must therefore be balanced against the cost of a larger basin. The minimum clarifier retention time is normally 4 hours. A deep basin will allow more floc to settle out than a shallow basin. This is because large particles settle faster than smaller ones, so large particles collide with and integrate smaller particles as they settle. In effect, large particles sweep vertically through the basin and clean out smaller particles on their way to the bottom.

4. Filtration

After separating most floc, the water is filtered as the final step to remove remaining suspended particles and unsettled floc.

Slow sand filter

Slow sand filters may be used where there is sufficient land and space as the water must be passed very slowly through the filters. These filters rely on biological treatment processes for their action rather than physical filtration. The filters are carefully constructed using graded layers of sand with the coarsest sand, along with some gravel, at the bottom and finest sand at the top. Drains at the base convey treated water away for disinfection. Filtration depends on the development of a thin biological layer, called the zoogleal layer or Schmutzdecke, on the surface of the filter. An effective slow sand filter may remain in service for many weeks or even months if the pre-treatment is well designed and produces water with a very low available nutrient level which physical methods of treatment rarely achieve. Very low nutrient levels allow water to be safely sent through distribution system with very low disinfectant levels thereby reducing consumer irritation over offensive levels of chlorine and chlorine by-products.



Cutaway view of a typical slow sand filter

Rapid sand filter

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 odour. The space between sand particles is larger than the smallest suspended particles, so simple filtration is not enough. Most particles pass through surface layers but are trapped in pore spaces or adhere to sand particles. Effective filtration extends into the depth of the filter. This property of the filter is key to its operation: if the top layer of sand were to block all the particles, the filter would quickly clog.

Some water treatment plants employ pressure filters. This work on the same principle as rapid gravity filters, differing in that the filter medium is enclosed in a steel vessel and the water is forced through it under pressure.

Advantages:

- Filters out much smaller particles than paper and sand filters can.
- Filters out virtually all particles larger than their specified pore sizes.
- They are quite thin and so liquids flow through them fairly rapidly.
- They are reasonably strong and so can withstand pressure differences across them of typically 2–5 atmospheres.
- They can be cleaned (back flushed) and reused.

Membrane filtration

Membrane filters are widely used for filtering both drinking water and sewage. For drinking water, membrane filters can remove virtually all particles larger than 0.2 um—including *giardia* and *cryptosporidium.* Membrane filters are an effective form of tertiary treatment when it is desired to reuse the water for industry, for limited domestic purposes, or before discharging the water into a river that is used by towns further downstream. They are widely used in industry, particularly for beverage preparation (including bottled water). However no filtration can remove substances that are actually dissolved in the water such as phosphorus, nitrates and heavy metal ions.

5. Disinfection

Disinfection is accomplished both by filtering out harmful microbes and also by adding disinfectant chemicals in the last step in purifying drinking water. Water is disinfected to kill any pathogens which pass through the filters. Possible pathogens include viruses, bacteria, including *Escherichia coli*, *Campylobacter* and *Shigella*, and protozoa, including *Giardia lamblia* and other *cryptosporidia*. In most developed countries, public water supplies are required to maintain a residual disinfecting agent throughout the distribution system, in which water may remain for days before reaching the consumer.

A) Chlorine disinfection

The most common disinfection method involves some form of chlorine or its compounds such as chloramine or chlorine dioxide. Chlorine is a strong oxidant that rapidly kills many harmful micro-organisms. Because **chlorine is a toxic gas**, there is a danger of a release associated with its use. This problem is avoided by the use of **sodium hypochlorite,** which is a relatively inexpensive solution that releases free chlorine when dissolved in water. Chlorine solutions can be generated on site by electrolyzing common salt solutions. A solid form,

calcium hypochlorite exists that releases chlorine on contact with water. Handling the solid, however, requires greater routine human contact through opening bags and pouring than the use of gas cylinders or bleach which are more easily automated.

The generation of liquid sodium hypochlorite is both inexpensive and safer than the use of gas or solid chlorine. All forms of chlorine are widely used despite their respective drawbacks. One drawback is that chlorine from any source reacts with natural organic compounds in the water to form potentially harmful chemical by-products trihalomethanes (THMs) and haloacetic acids (HAAs), both of which are carcinogenic in large quantities.

The minimum recommended concentration of free chlorine is 0.5 mg/l for one hour. The free residual chlorine provides a margin of safety against subsequent microbial contamination, such as may occur during storage and distribution. The sum of the chlorine demand of the specific water plus the free residual chlorine of 0.5 mg/l constitutes the correct dose of chlorine to be applied.

Action of Chlorine: - When chlorine is added to water, there is formation of hydrochloric and hypochlorous acids. The hydrochloric acid is neutralised by the alkalinity of the water. The hypochlorous acid ionises to form hydrogen ions and hypochlorite ions as follows:

$H_2O + Cl_2 \Leftrightarrow HCI + HOCI$
$HOCI \rightarrow H^+ + OCl-$

The disinfecting action of chlorine is mainly due to the hypochlorous acid, and to a small extent due to the hypochlorite ions. The hypochlorous acid is the most effective form of chlorine for water disinfection. It is more effective (70-80 times) than the hypochlorite ion

B) Ozone disinfection

O_3 is an unstable molecule which readily gives up one atom of oxygen providing a powerful oxidizing agent which is toxic to most waterborne organisms. It is a very strong, broad spectrum disinfectant that is widely used in Europe. It is an effective method to inactivate harmful protozoa that form cysts. It also works well against almost all other pathogens. Ozone is made by passing oxygen through ultraviolet light or a "cold" electrical discharge. To use ozone as a disinfectant, it must be created on-site and added to the water by bubble contact. Some of the advantages of ozone include the production of fewer dangerous by-products (in comparison to chlorination) and the lack of taste and odour produced by ozonization. Another of the main disadvantages of ozone is that it leaves no disinfectant residual in the water.

C) Ultraviolet disinfection

Ultraviolet light is very effective at inactivating cysts, in low turbidity water. UV light's disinfection effectiveness decreases as turbidity increases, a result of the absorption, scattering, and shadowing caused by the suspended solids. The main disadvantage to the use of UV radiation is that, like ozone treatment, it leaves no residual disinfectant in the water; therefore, it is sometimes necessary to add a residual disinfectant after the primary disinfection process. This is often done through the addition of chloramines, discussed above as a primary disinfectant. When used in this manner, chloramines provide an effective residual disinfectant with very few of the negative aspects of chlorination.

SEWAGE

The wastewater resulted from different sources is called as sewage.

Sewerage System: -

The wastewater of the city is collected through a system of pipes called as sewerage system, which carries the used water to its ultimate point of treatment and disposal.

There are three types of sewerage systems –

a) Sanitary sewers: - Which carry domestic and industrial wastewater.

b) Storm sewers: - Which carry surface and storm (atmospheric) waste water.

c) Combined sewers: - Which carry all the waste water (sewage) through a single system of sewers.

CHEMICAL COMPOSITION OF SEWAGE

Chemical characteristics: -

Domestic wastewater or sewage consists of approximately 99.9 % water, 0.02 to 0.03 % suspended solids and other soluble organic and inorganic substances.

On a percentage basis the amount of solids appears small, however the tremendous volume of material handled daily by a major municipal plant (e.g. several hundred millions of gallons) contains as much as hundred tons of solids.

The chemical constituents, present in low concentration, never the less are extremely important and are subject to variations, between communities as well as within a community, even from hour to hour.

Inorganic chemicals initially present in water supply will like wise are present in the sewage.

Organic compounds are contributed through human excrement and other domestic wastes, and both organic and inorganic compounds are added by industrial wastes. E.g. slaughter houses, sugar factories, paper mills and creameries add organic substances, mines and metal industries contribute acids and salts by metals and other inorganic wastes.

The organic compounds in sewage are classified as nitrogenous and non-nitrogenous. The principle nitrogenous compounds are urea, proteins, amines and amino acids. The non-nitrogenous substances include carbohydrates, fats and soaps.

Modern technology may produce significant change in sewage characteristics. The increased use of household garbage disposal units has increased the total organic load. Some synthetic detergents displacing soaps are resistant to microbial degradation.

The 0.02 to 0.03 % suspended solids consists of following composition in percentage approximately.

i) Lipids	30 %
ii) Proteins	25%
iii) Carbohydrates--	
Cellulose	4 %
Hemicelluloses	3 %
Lignin	6 %
iv) Amino acids, Starch, Pectin	8 %
v) Alcohols	5 %
vi) Ash	20 %

WASTE TREATMENT PROCEDURES

(SEWAGE TREATMENT PROCEDURES)

Wastewater treatment is necessary before wastewater can be disposed off without producing significant undesirable or even harmful effects. However some communities and municipalities still dispose off inadequately treated wastewater into natural bodies of water, either because they are indifferent to the consequences or because it is assumed that the body of water is sufficiently large and so located that dilution prevents hazards. But disposal of inadequately treated wastewater leads to: -

i) Greater possibility for dissemination of pathogenic microorganisms.

ii) Increased danger in using natural bodies of water for drinking supplies.

iii) Contamination of oysters and other shellfish by the pollution, making them unsafe for human consumption.

iv) Large losses in the waterfowl population, chargeable to pollution of their winter-feeding grounds.

v) Increased danger of swimming in the water and diminished value of the water for other recreational purposes.

vi) Depletion of oxygen supply of water by unstable organic matter in sewage, killing aquatic life.

vii) Creation of miscellaneous objectionable conditions such as offensive odors and accumulation of debris, which decreases property values.

viii) Accumulation and dissemination of toxic chemicals that endanger ecosystems and threaten public health.

[“DILUTION IS NOT THE SOLUTION OF POLLUTION” but the treatment is the solution of pollution.]

WASTEWATER TREATMENT PROCESS

These processes are many and varied. We will discuss the treatment processes as they are applicable to two separate situations.

1. A single dwelling or unit structure
2. A community or municipality

1. **A single dwelling or unit structure: -**

   Treatment and disposal of wastewater and sewage from individual dwellings or other unit structures (e.g. some motels or shopping center) can be accomplished by anaerobic digestion and / or by aerobic metabolic processes. One of the more common installations used to accomplish this is the septic tank an anaerobic digesting system.

2. **A community or municipality: -**

   Municipal wastewater treatment procedures carry out a series of treatment processes, which may be summarized as follows –

   The liquid and solid wastes resulted are treated and some useful byproducts are resulted.

A) PRIMARY SEWAGE (WASTE) TREATMENT

I) Sedimentation by Septic and Imhoff tank
II) Floatation
III) Coagulation by alum or other agents

B) SECONDARY SEWAGE (WASTE) TREATMENT

I) Aerobic degradation (Treatment of liquid waste)

1) Trickling filter

2) Activated sludge digestion

3) Oxidation ponds (Lagoons)

II) Anaerobic degradation (Treatment of solid waste)

III) Solid waste disposal (Composting and Landfill technology)

IV) Liquid waste disposal – Evapotranspiration system

C) TERTIARY SEWAGE (WASTE) TREATMENT

1) Biological removal of inorganic nutrients

2) Chemical removal of inorganic nutrients

3) Trace chemical, odor and colour removal

4) Removal of microorganisms by Chlorination, Ozonization, Ultraviolet radiations and filtration.

****

A) PRIMARY SEWAGE (WASTE) TREATMENT

The first step is removal of large floating solid particles like branches, rags, leaves etc by screening through a metal board or mesh. The pore size of the mesh is about 2 to 5 cm. The sewage is passed through this mesh and the large particles are removed and burned.

The primary treatment is carried out by either of three processes.

I) Sedimentation by Septic and Imhoff tank

II) Floatation

III) Coagulation by alum or other agents

I) Sedimentation by Septic and Imhoff tank: -

The principle of this method is that the particles in sewage settle at the bottom according to their specific gravity. The settling depends up on the size, shape and weight of the particles. Larger particles settle immediately while lighter particles or floccules settle slowly by adhering to each other.

The sedimentation procedure is done in the tanks. For individual dwelling units in houses or motels, septic tanks, which are small, are used while for community or municipal waste big tanks and imhoff's tanks are used.

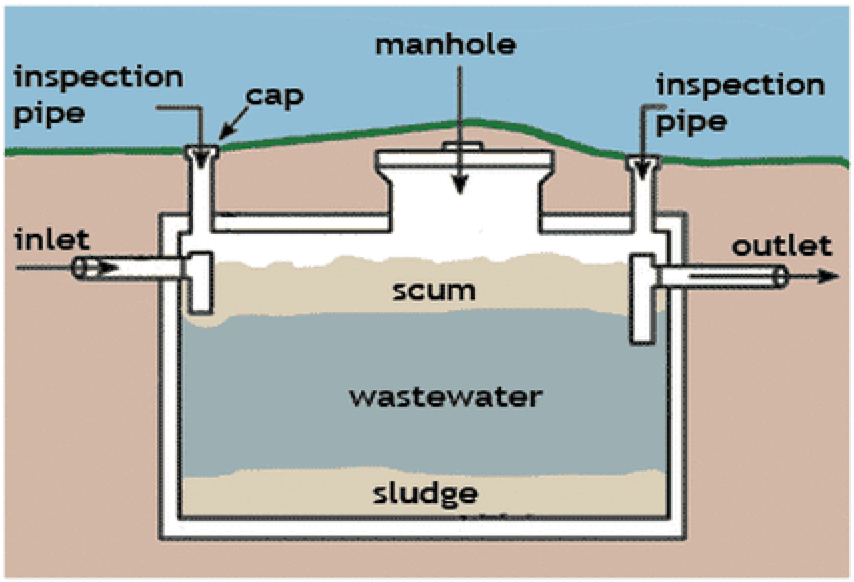
i) Septic tank
ii) Imhoff tank

i) Septic tank: -

This type of disposal system is widely used by individual families in rural areas, where public sewers are not available.

It is a metal or concrete vat placed below the ground level near the dwelling, to which all the domestic waste flow (fecal matter) is collected. Roughly 750 gallons tank (3' X 5' X 5') will handle the average domestic sewage of a family of four member.

Sewage entering the tank is retained long enough to permit adequate decomposition. Thus sedimentation and biological degradation of sludge by microorganisms are accomplished. Much of the organic material is hydrolyzed and fermented by anaerobic bacteria. The products include sugars, alcohols, organic acids, amino acids, amines, glycerol, fatty acids and other chemicals and the gases H_2S, CH_4, CO_2 and Hydrogen.



The end products are still very unstable i.e. high in BOD and odors. The effluent from the tank is distributed under the soil surface (a disposal field) through perforated pipes.

The process, however, does not eliminate pathogenic microorganisms carried in the sewage; hence precaution should be taken to avoid mixing of sewage water with drinking water supply. Hence septic tanks should be far away from the drinking water supply.

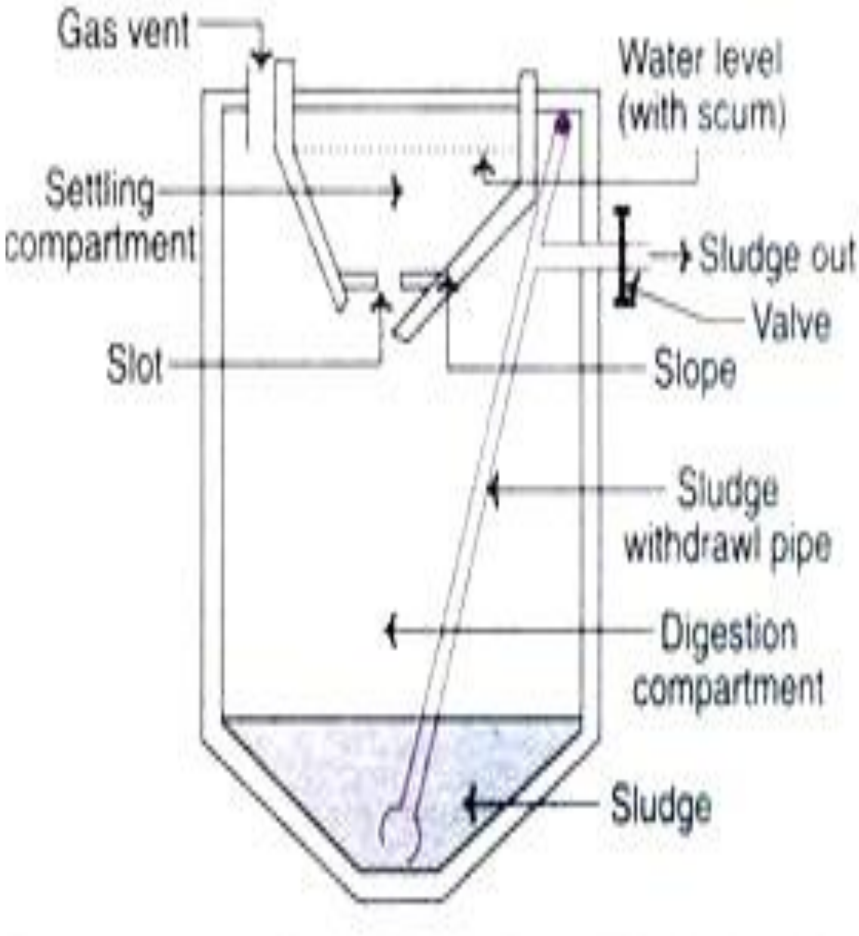
In the tank there are some materials, which are quite resistant to decomposition. The undigested materials, which resist microbial action, are termed as SLUDGE. This has to be taken out periodically by pumping, to prevent the tank and the drain field from becoming plugged. The sludge can be used as fertilizer.

ii) Imhoff tank: -

The Imhoff tank process is closely related to the septic tank, but is on a larger scale. It is used for treating the sewage of a community or small city. Imhoff tank is vertical while septic tank is horizontal.

Thus Imhoff tank is a sewage-settling tank in which solids are settled at the bottom and sewage is added from the top. In septic tank influent and effluent flow is horizontal.

The solids after settling are removed from the bottom from time to time. Imhoff tank is made up of metal or concrete, which has tapering bottom. The bottom has opening, which can be closed and opened. There are pipes fitted in inclined position for the removal of water after settling of solids. The solids are removed from the tapering bottom opening.



Imhoff tank

In some Imhoff tank, another small, vertical Imhoff tank is fitted. First time sewage is added in the small tank, allowed to settle, the water from this tank is removed. The remaining settling solids are added into big tank. In small tank sludge digestion is not so much while in bigger tank sludge digestion is more anaerobically.

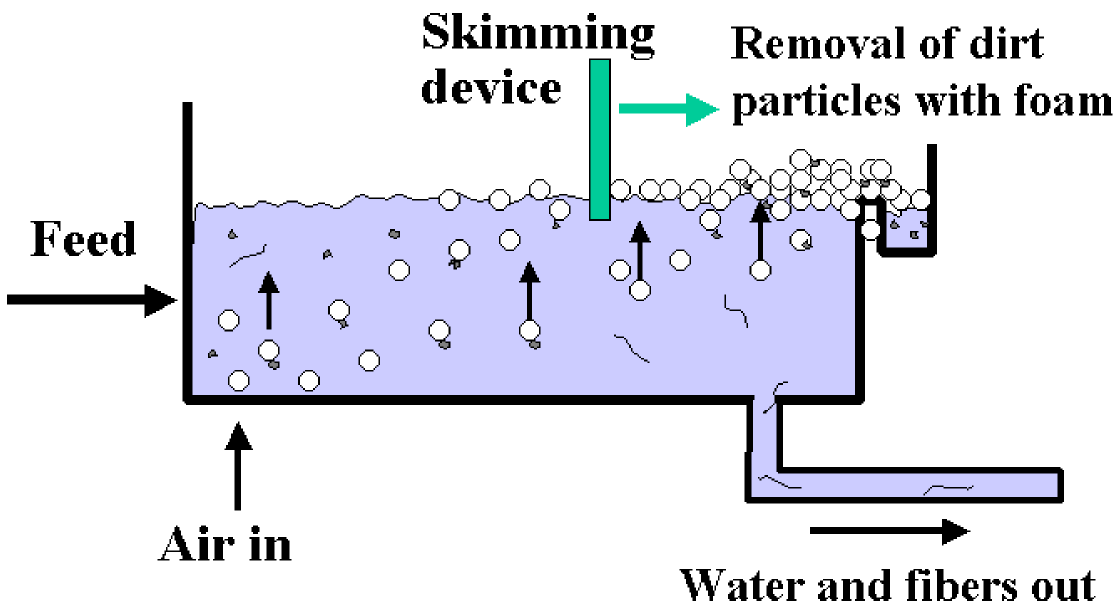
The sludge is removed periodically and taken for further treatment.

II) FLOATATION: -

In this method, suspended solids of lower densities are removed which are not settled at the bottom in sedimentation procedure.

Sewage (effluent) from settling tank is added in a tank and bubbles of air are generated at the bottom of the tank by passing air under pressure.

The air bubbles carry the suspended solids to the top of the sewage liquid. At the top, a layer is formed which is called as “SCUM”. This scum is then removed and further treatment is done.



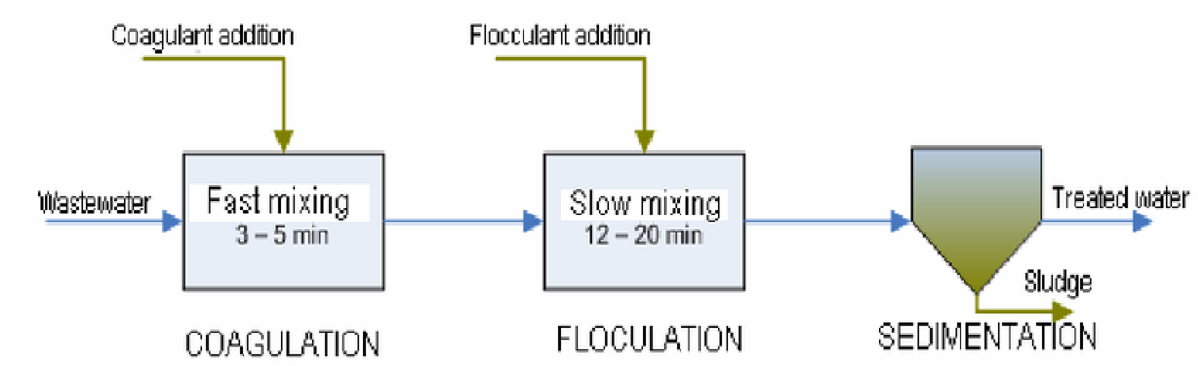
FLOATATION TANK

III) COAGULATION BY ADDING ALUM OR OTHER AGENTS: -

This is the sewage settling method by the sedimentation process in which mainly colloidal particles (dense particles) are settled.

Generally colloidal particles are light in weight hence they do not settle according to own density. The sewage containing colloidal particles (effluent from sewage settling tank) is taken in a tank. Then metal salts are added in alkaline condition e.g. $Al_2(SO_4)$ (Alum), $CaSO_4$, Fe_2SO_4, $Fe_2(SO_4)_3$, $FeCl_3$ etc.

These salts are dissolved at different pH in sewage, mixed well and allowed to settle. The ionic forms of the metals like Al^{+3}, Fe^{+3}, Fe^{+2} etc. attract negatively charged particles and then are settled.



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B) SECONDARY WASTE (SEWAGE) TREATMENT

This process mainly involves biological process and is involving two mechanisms -- Aerobic and Anaerobic degradation. The activities involve mainly microbial degradation including hydrolysis. Oxidation, reduction reactions etc..

Secondary treatment consists of –

I) Aerobic degradation (TREATMENT OF LIQUID WASTE)

1) Trickling filter
2) Activated sludge digestion
3) Oxidation ponds (Lagoons)

II) Anaerobic degradation (TREATMENT OF SOLID WASTE)

III) Solid waste disposal

1) Composting
2) Landfill technology

IV) Liquid waste disposal

Evapotranspiration system

I) Aerobic degradation (TREATMENT OF LIQUID WASTE)

After the removal of sludge, the soluble components present in the remaining wastewater are removed by these processes.

1) Trickling filter
2) Activated sludge digestion
3) Oxidation ponds (Lagoons)

1) <u>Trickling filter</u>

The trickling filter consists of a bed of crushed stone, gravel, slag or synthetic material over which sewage trickles (flows). Trickling filter tank has drains at the bottom.

The liquid sewage is spread over the surface of the bed either by rotating arm or through nozzles. The spraying saturates the liquid with oxygen.

Intermittent application of sewage permits maintenance of aerobic conditions in the bed. The filtering medium of the tank becomes coated with microbial flora, the "**Zoogloeal film**", which consists of bacteria, fungi, protozoa and algae. As the sewage seeps over these surfaces, the microorganisms adsorb and metabolize the organic constituents to more stable end products. This operation may be regarded as a stationary microbial culture (on the stones) fed by continuous supply of nutrients (organic constituents of the sewage). A newly constructed bed must acquire the zoogloeal film before it can function efficiently. This requires operation over a period of few weeks.

In the zoogloeal film following microorganisms are present—

A. **Slime producing bacteria**: -

Which produce slime due to which film is formed and which is sticked to the rocks.

B. **Filamentous bacteria and fungi: -**

Which help to maintain the structure of the film and also oxidize organic matter.

C. **Protozoa: -**

Which eat the bacteria keeping the film from becoming more thicker, otherwise the flow of the sewage will be blocked.

D. **Invertebrate animals: -**

E.g. rotifers and nematodes, which eat protozoa. Thus the overeating of bacteria by protozoa is controlled.

E. **Autotrophic bacteria: -**

Which oxidize ammonia and H_2S liberated during decomposition into NO_3 and SO_4.

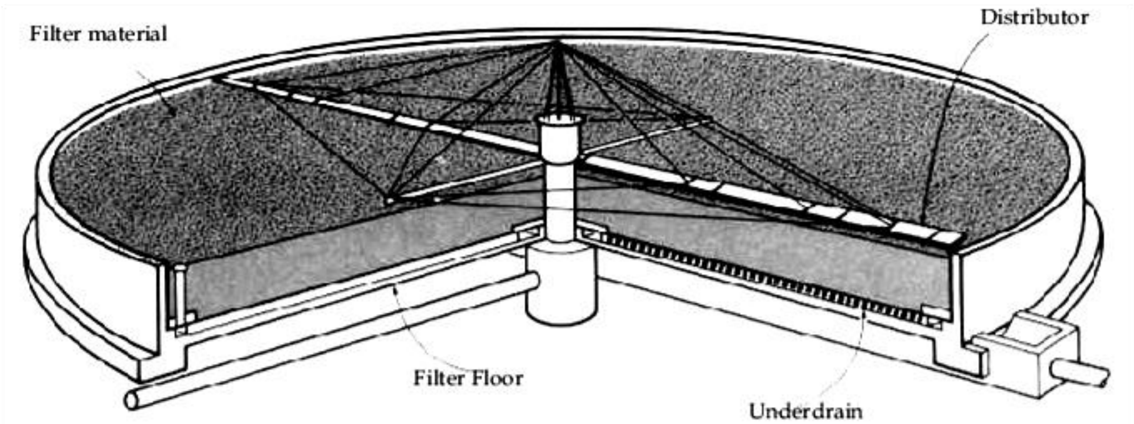
Thus in this filter the final result is conversion of organic matter into inorganic matter. This process is called as mineralization.

Organic Carbon $\longrightarrow$ CO_2

Organic Nitrogen $\longrightarrow$ NH_3 $\longrightarrow$ NO_3

Organic Sulfur $\longrightarrow$ H_2S $\longrightarrow$ SO_4

Organic phosphorus $\longrightarrow$ PO_4



Trickling filter

About 90 to 95 % of BOD and many bacterial pathogens are removed by this process.

2) Activated sludge digestion (Aeration by aerators)

The activated sludge process is one of the most efficient and popular systems in the purification of sewage by biological treatment.

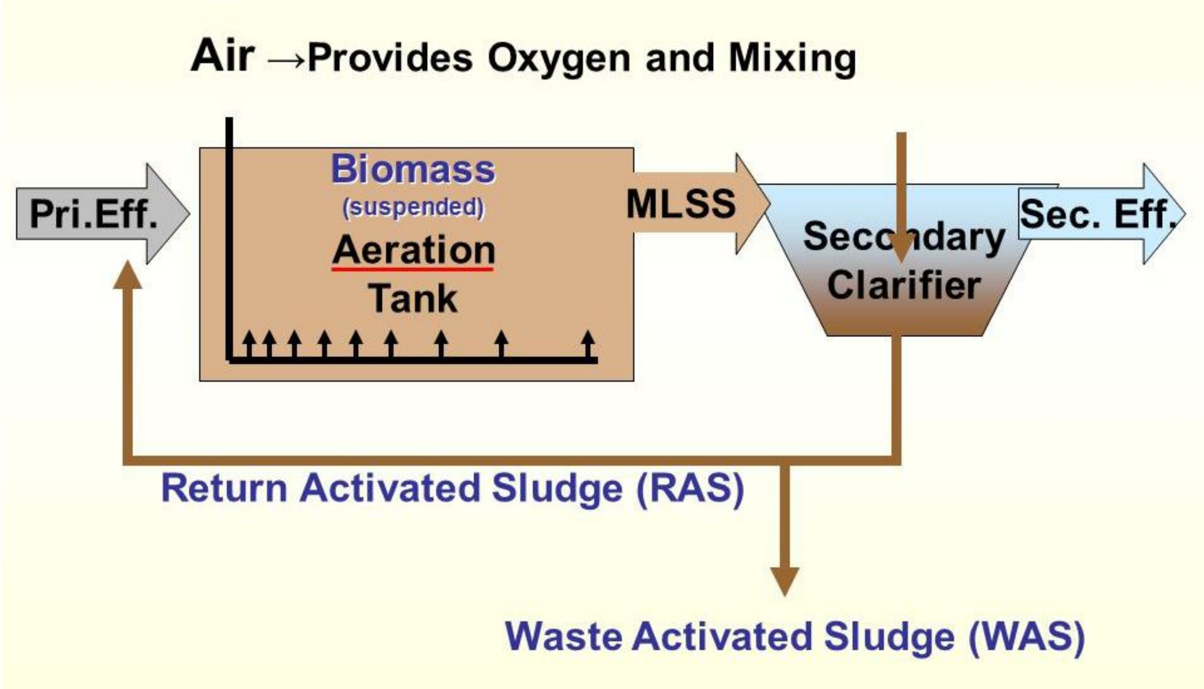
In this process vigorous aeration of the sewage is done. This result in the formation of the floc, finely suspended and colloidal matter of the sewage forms aggregates designated as floccules. If this floc is permitted to settle and then added to fresh sewage that is again vigorously aerated, flocculation occurs in a shorter time than before.

By repetition of this process i.e. addition of sedimented floc to fresh sewage, aeration, sedimentation, addition of sediment to fresh sewage, aeration etc. a stage is reached where complete flocculation of the fresh sewage occurs very quickly, e.g. a few hours. These particles of floc i.e. activated sludge contain large numbers of very actively metabolizing bacteria together with yeasts, molds and protozoa. This combination of microbial growth is very effective in the oxidation of organic compounds.

A poor settlement of activated sludge flocs adversely affects performance of a sewage treatment plants. The sludge becomes more voluminous and is difficult to control. The principal reason for poor settling (Bulking) of activated sludge is the growth of filamentous microorganisms. Many different species of microorganisms have been isolated from sludge in this condition.

The use of activated sludge is of great importance in wastewater treatment. This process usually employs an aeration period of 4 to 8 hours, after which a mixture is piped to a sediment tank. The effluent from these tanks represents wastewater treated to secondary levels, there is a considerable reduction of suspended solids and BOD is reduced.

Some of the advantages of this method are that relatively little land space is needed and the quality of final effluent is such that it does not require high dilution for disposal.



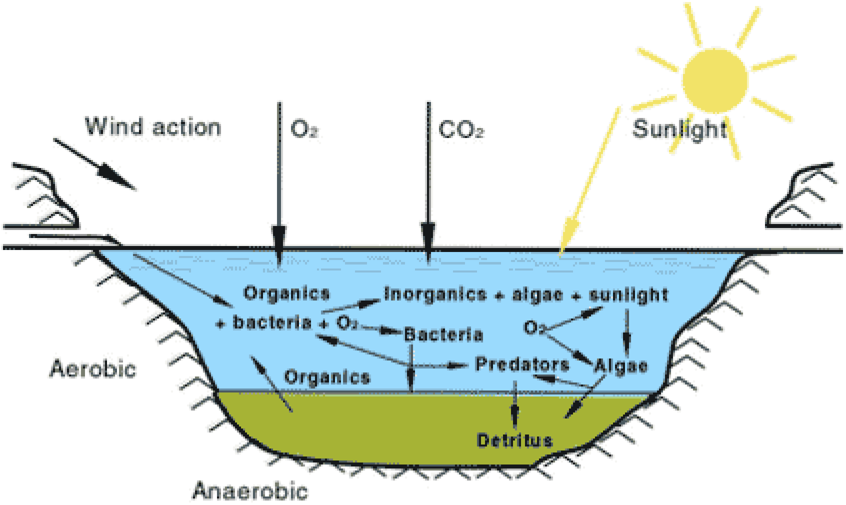
3) OXIDATION PONDS (LAGOONS) :-

These are shallow ponds with a depth of about 5 feet, used mainly for aerobic stabilization of wastes.

Oxygenation of wastewater is accomplished through wind action. An additional important source of oxygen is the photosynthetic activity of algae. The interaction of bacteria & algae is an important factor in the stabilization process.

Bacteria digest and oxidize the organic material in sewage. Algae utilize CO_2 and other substances resulting from bacterial action & through photosynthesis, produce oxygen needed for aerobic bacterial action.

Because of this interaction and the long detention time, oxidation ponds reduce BOD by 75 to 90 %. This process is recommended for small communities in rural areas where suitable and sufficient land is available.



Oxidation pond (lagoon)

II) Anaerobic degradation (Treatment of solid waste): -

The solids, which accumulate during sedimentation, are pumped into a separate tank designed especially for the digestion of sludge under controlled conditions. Solids recovered from the aerobic treatment processes may also be returned to the sludge digester.

The microbial action on the constituents of sludge (proteins, organic fibers of cellulose, carbohydrates, fats) is termed as **sludge digestion**.

Anaerobic digesters are large tanks designed to operate anaerobically with continuous input of untreated sludge and removal of the final, stabilized sludge product. The anaerobic and facultative types of bacteria are active. These microorganisms bring about a decrease in organic solids by degrading them to soluble substances and gaseous products.

Large amounts of gases consisting mainly of

Methane (CH_4) ----- 60 to 70 %

CO_2 ----- 20 to 30 %

H_2 & N_2 ----- 5 to 10 %

This gas mixture can be used as a fuel for heating purposes and for electricity production. Conditions affecting microbial growth and metabolism will be reflected in the degree of digestion of the sludge.

e.g. - inoculums, pH and temperature.

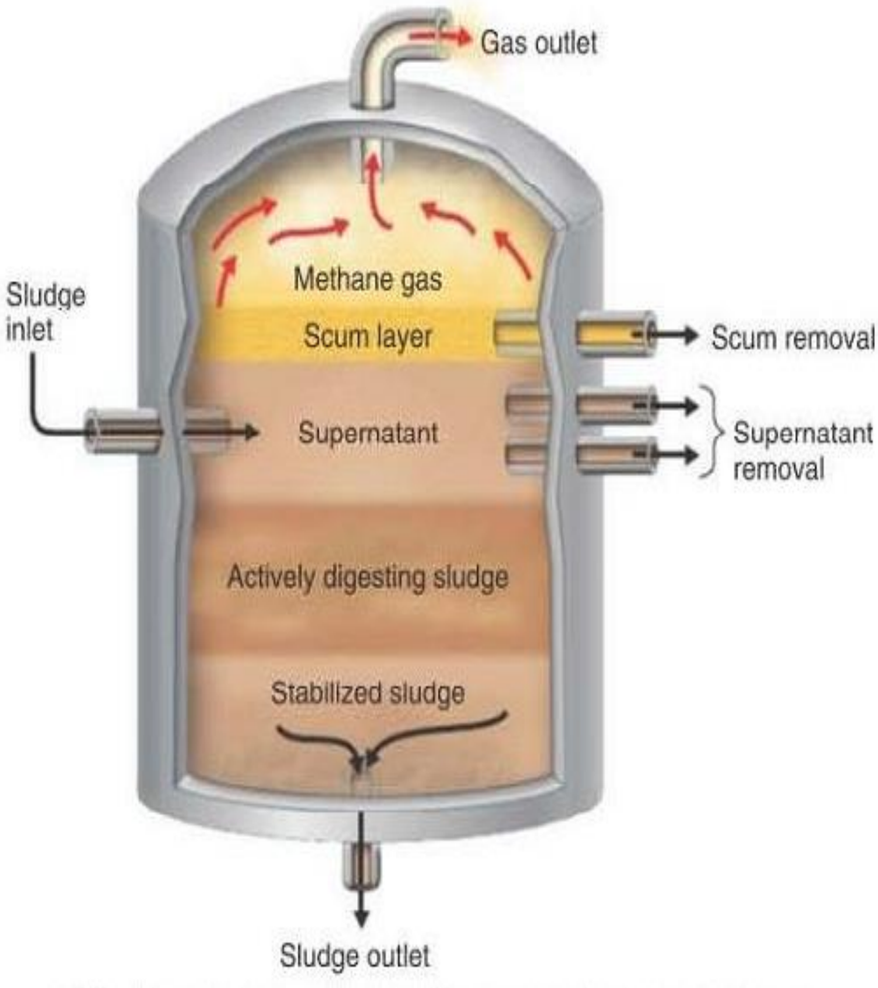
The proportion of inoculums used and the thoroughness of mixing with the fresh sludge are important. Most rapid digestion of sludge occurs at temperatures which favor growth of bacteria (50 to 60 ^{0} C). At neutral pH (7) is optimum for sludge digestion.

The temperature is maintained around 30 ^{0}C, which is below the optimum stated above, because it has not been found practical to operate at thermophilic temperatures. Complete digestion requires 2 to 3 weeks or even longer.

The pH and temperature must be maintained at optimum level. If hydrogen and organic acids accumulate, methane production can be inhibited, resulting in a stuck digester.

The digestion process involves three steps –

1) The fermentation of sludge components to form organic acids, including acetate.
2) Production of methanogenic substrates: acetates, CO_2 and H_2.
3) Methanogenesis (Methane production) by the methane producers.



<u>Anaerobic sludge digester</u>

Table: - sequential reactions in the anaerobic digestion

Sr. No.	**Process step**	**Substrates**	**Products**	**Major microorganisms**
1	Fermentation	Organic polymers	Butyrate, propionate, Lactate, Succinate, Ethanol, Acetate, H_2, CO_2	Clostridium, Bacteroids, Peptostreptococcus, peptococcus, eubacterium, lactobacillus
2	Acetogenic reactions	Butyrate, propionate, Lactate, Succinate, Ethanol,	Acetate, H_2, CO_2	Syntrophomonas, Syntrophobacter, acetobacterium.
3	Methanogenic reactions	Acetate, H_2, HCO_3	$CH_4 + CO_2$ CH_4	Methanosarcina, Methanothrix, Methanobrevibacter, Methanomicrobium, Methanogenium, Methanobacterium, Methanococcus, Methanospirillum.

III) Solid waste disposal: -

The undigested material remained in secondary waste treatment is removed from plant, dried and it is either

→ Buried

→ Burned

→ Used as an agricultural soil build or compost

The most commonly used techniques for disposal of undigested sludge are

1) **Composting / Sludge as fertilizer**
2) **Landfill technology**

1) Composting / Sludge as fertilizer

Composting is a process where dewatered sludge undergoes decomposition, usually within a thermophilic temperature range. Dewatered sludge is mixed with a bulking agent such as wood chips. The bulking material is added to enhance circulation of air throughout the sludge to improve the stabilization process.

The mixture of sludge and bulking material is placed in aerated piles. Oxygen is furnished by forced aeration. The mixture is allowed to "cure" or biologically decomposed for a period of time. For effective stabilization this period of time is normally considered to be 21 days.

After 21 days, the bulking agent is separated from the sludge and the sludge is allowed to cure further for several weeks.

Upon final curing, the sludge has been transformed to humus type material and is suitable for use as soil conditioner.

Biosolids : - These are treated sewage sludge. Biosolids are carefully treated and monitored. They are nutrient-rich organic materials resulting from the treatment of domestic sewage in a treatment facility. When treated and processed, these residuals can be recycled and applied as fertilizer to improve and maintain productive soils and stimulate plant growth.

Once the wastewater reaches the plant, the sewage goes through physical, chemical and biological processes which clean the wastewater and remove the solids. If necessary, the solids are then treated with lime to raise the pH level to eliminate objectionable odors. The wastewater treatment processes sanitize wastewater solids to control pathogens (disease-causing organisms, such as certain bacteria, viruses and parasites) and other organisms capable of transporting disease.

After treatment and processing, biosolids can be recycled and applied as fertilizer to improve and maintain productive soils and stimulate plant growth. The controlled land application of biosolids completes a natural cycle in the environment. By treating sewage sludge, it becomes biosolids which can be used as valuable fertilizer, instead of taking up space in a landfill or other disposal facility.

Biosolids may have their own distinctive odor depending on the type of treatment it has been through. Some biosolids may have only a slight musty, ammonia odor. Others have a stronger odor that may be offensive to some people. Much of the odor is caused by compounds containing sulfur and ammonia, both of which are plant nutrients.

The application of biosolids reduces the need for chemical fertilizers. Biosolids are used to fertilize fields for raising crops. Agricultural use of biosolids, that meet strict quality criteria and application rates, have been shown to produce significant improvements in crop growth and yield. Nutrients found in biosolids, such as nitrogen, phosphorus and potassium and trace elements such as calcium, copper, iron, magnesium, manganese, sulfur and zinc, are necessary for crop production and growth. The use of biosolids reduces the farmer's production costs and replenishes the organic matter that has been depleted over time. The organic matter improves soil structure by increasing the soil's ability to absorb and store moisture.

The organic nitrogen and phosphorous found in biosolids are used very efficiently by crops because these plant nutrients are released slowly throughout the growing season. This enables the crop to absorb these nutrients as the crop grows. This efficiency lessens the likelihood of groundwater pollution of nitrogen and phosphorous.

2) **Landfill technology: -**

In this system, in a dug, the undigested sludge is added and compacted by roller and then some soil is added and again the undigested sludge is added and then again compacted by roller.

Thus about 4 to 10 layers can be performed. On this soil layer we can prepare gardens, parks by planting trees.

Sometimes sludge remaining is dried and burned in an incinerator and ash ia produced. This ash has less volume and can be used in landfill technology.

Generally landfills prevent decomposing of solid waste because they are dry and anaerobic. In some landfills, methane produced can be recovered for an energy source.

IV) Liquid waste disposal: - Evapotranspiration system, Irrigation and dilution)

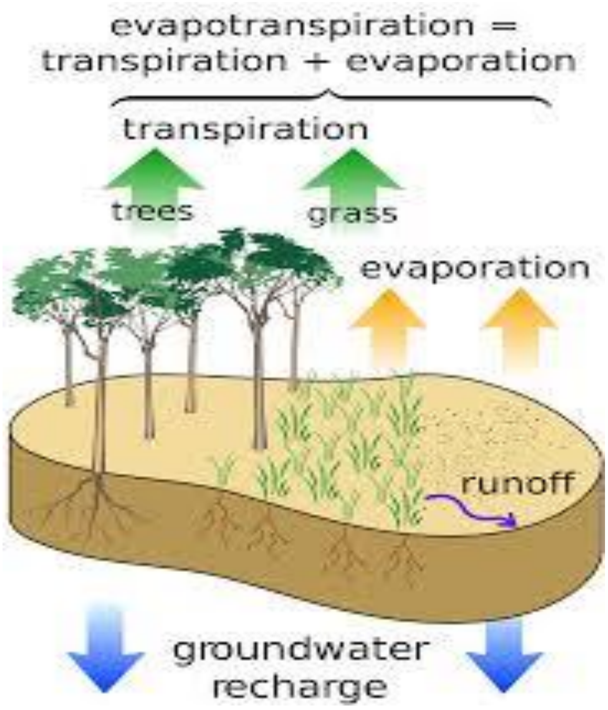
After settling of sewage soils, upper clear liquid from tank is given out, which is called as effluent which is further treated by aerobic processes such as trickling filters, oxidation ponds etc.

This effluent is used in evapotranspiration system, Irrigation and dilution.

Evapotranspiration system: -

This system consists of porous bed over water impermeable solid bottom. On this bed trees are planted and the effluent is spread over the land.

The water from effluent is absorbed by the plants and evaporated through the leaves of plants. This process is called as transpiration. Also water from bed is directly evaporated and the process is called as evaporation. This problem of disposal of effluent will be somewhat solved but precaution should be taken to avoid mixing of effluent with drinking water through the percolation in soil.



Evapotranspiration system

Irrigation:

Generally this is disposal by irrigation. This involves spreading the wastewater over the surface of the ground, generally by irrigation ditches. There is some evaporation, but most of the wastewater soaks into the ground and supplies moisture with small amounts of fertilizing ingredients for plant life. This method is largely restricted to small volumes of wastewater from a relatively small population where land area is available and where nuisance problems will not be created. It has its best use in arid or semi-arid areas where the moisture added to the soil is of special value. If crops are cultivated on the disposal area, the growth of vegetation, often must be excluded from wastewater.

Dilution

Disposal by dilution is the simple method of discharging wastewater into a surface water such as a river, lake, ocean, estuaries or wetlands. This results in the pollution of the receiving water. The degree of pollution depends on the dilution, volume and composition of the wastewater as compared to the volume and quality of the water with which it is mixed. When the volume and organic content of the wastewater is small, compared with the volume of the receiving water, the dissolved oxygen present in the receiving water is adequate to provide for aerobic decomposition of the organic solids in the wastewater so that nuisance conditions do not develop. However, in spite of the continued aerobic status of the receiving water, microbial pollution remains a health menace and floating solids in the wastewater, if not previously removed, are visible evidence of the pollution.Where the dissolved oxygen in the receiving water is inadequate to maintain aerobic decomposition, anaerobic decomposition takes place and putrefaction with objectionable conditions results. It is not so much the volume of wastewater that is the critical factor as the amount of readily decomposable organic matter in the wastewater. Thus a volume of of wastewater that has been treated to remove or reduce this organic matter can be discharged to a natural surface water without creating objectionable conditions while the same volume of raw or untreated wastewater might produce a nuisance. The dissolved oxygen in the receiving water is the determining factor.

C) Tertiary waste treatment

Tertiary sewage treatment purifies sewage more than is possible with primary and secondary treatment. The goal is to remove such pollutants as non-biodegradable organic material (e.g. polychlorinated biphenyles) heavy metals and minerals. It is particularly important to eliminate the salts of nitrogen and phosphorous compounds that can promote eutrophication. (Eutrophication means over growth of algae or cyanobacteria and the eventual death of other organisms due to increase in phosphorous).

Microorganisms present in water cause problems of disease production, when such water is used for drinking purpose. So tertiary sewage treatment also include killing of microorganisms.

The effluent from secondary treatment plants contains some residual BOD. It also contains about 50 % of original nitrogen and 70 % of original phosphorus. Tertiary treatment depends less on biological treatment than on physical and chemical treatment. However some systems use biological treatment.

Tertiary treatment consists of

1. Biological removal of inorganic nutrients

2. Chemical removal of inorganic nutrients

3. Trace chemicals, odours and colour removal

4. Removal of microorganisms by chlorination, ozonization, Ultraviolet radiation and filtration.

1. Biological removal of inorganic nutrients: -

In these processes nitrogen and phosphorus are removed by using microorganisms.

-→ Nitrogen removal: - A widely used process for nitrogen removal is denitrification. Here nitrate produced under aerobic condition is used as an electron acceptor under conditions of low oxygen with organic matter added as an energy source. Nitrate reduction is nitrogen gas and nitrous oxide (N_2O) as the major products.

→ Phosphorus removal: - For this, aerobic and anaerobic conditions are used alternately in a series of treatments and phosphorus accumulates in specially adopted microbial biomass as polyphosphate. This 'phosphostrip' process is used in many parts of the world.

2. Chemical removal of inorganic nutrients: -

-→ Nitrogen removal: - Excess nitrogen may be removed by chemical methods by "Stripping" volatilization as NH_3 at high pH. Ammonia itself can be chlorinated to form dichloramine, which then converted to molecular nitrogen.

→ Phosphorus removal: - It is usually precipitated as calcium or iron phosphate by combining with lime, alum and ferric chloride.

3. Trace chemicals, odours and colour removal: - These are removed by filters of fine sands and activated charcoal.

4. Removal of microorganisms by chlorination, ozonization, Ultraviolet radiation and filtration: - Though nitrogen, phosphorus, trace chemicals, odour and colour are removed from effluent, still microorganisms remain in water. Some times pathogenic bacteria, viruses and protozoa may be present in such water and if such water is used or mixed with drinking water bodies, diseases may be caused.

Hence these microorganisms are removed or killed by Chlorination, Ozonization, Ultra violet radiation and by Filtration.

Thus tertiary treatment provides water that is suitable for drinking but the process is extremely costly.

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