MICROBIOLOGY OF WASTE WATER

Every community produces both liquid and solid wastes. The liquid waste of community life is termed sewage it is also called wastewater. It is essentially the water supply of the community after it has been fouled by variety of uses.

Sewage includes all the water from a household that is used for washing and toilet wastes. Rain water flowing into street drains and some industrial wastes. Wastewater that does not contain human excreta is termed Sallage. Term garbage is applied to all types of solid and semisolid wastes, such as waste food, decayed fruits and vegetables, grass, leaves, paper, plastics, metal pieces, etc.

Sources (or origin) of sewage-Domestic, Agricultural and Industrial

Most communities generate wastewater from both residential and nonresidential sources. The components that make up the wastewater flow from a community may include the following:

1. Domestic (or sanitary) sewage- It is the residential wastewater discharged from residences and from commercial, institutional and similar facilities.

There are two types of domestic sewage –

a) **Blackwater** (waste water from toilets)

b) **Graywater** (waste water from all sources except toilets)

Blackwater and graywater have different characteristic, but both contain pollutants and disease causing agents that require treatment.

2. Agricultural waste water- refers to biotic and abiotic byproducts of farming practices that result in contamination or degradation of the environment and surrounding ecosystems, and/or cause injury to humans and their economic interests. The pollution may come from a variety of sources, ranging from point source water pollution (from a single discharge point) to more diffuse, landscape-level causes, also known as non-point source pollution. Management practices play a crucial role in the amount and impact of these pollutants. Management techniques range from animal management and housing to the spread of pesticides and fertilizers in global agricultural practices.

3. Industrial wastewater- It is the nonresidential waste water in which industrial waste predominate. Many industries produce waste water high in chemical and biological pollutants

Stormwater – it is nonresidential source. It is the surface water run off that occurs after storms or floods. It carries trash and other pollutants from streets as well as pesticides and fertilizers from farm yards and agriculture fields

Sewer or sewerage system

A system of pipes used to collect and transport sewage from its source to treatment facility is called sewer or sewerage system .

sewer systems are generally classified into following types according to the type of the wastewater flowing through them –

• Sanitary sewer systems
• Storm sewer system and
• Combined sewer systems

1. Sanitary sewer systems carry three major components-domestic wastewater, industrial wastewater and infiltration /inflow water. Infiltration is the extraneous water (for example groundwater) that enters sewer system through leaky joints, cracks and breaks,
or porous walls. Inflow is storm water that enters the sewer system from storm drain connections or through manhole over's.

2. Strom sewer system is designed to carry surface runoff water that occurs after storms or floods.

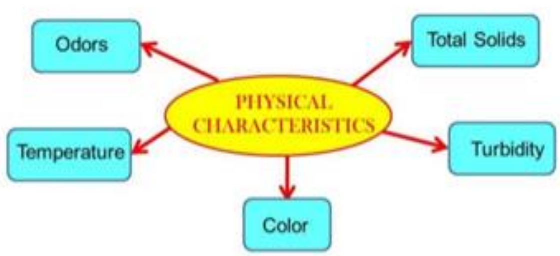
3. combined sewer system if the sewer system carries all forms of wastewater domestic, industrial and storm water.

Unlike the water supply system, waste water flows through sewer pipes by gravity rather than by pressure. The pipe must be sloped to permit the wastewater to flow at the velocity of atleast 0.5m/sec, because at lower velocities the solid material tends to settle in the pipe.

Physical, Chemical and Microbiological characteristics of waste water

Wastewater is characterized in terms of the physical properties and the chemical, and biological composition.

Physical characteristics of sewage
The important physical characteristics of wastewater includes temperature, color, odour, turbidity and total solids



Temperature
Temperature of waste water is usually higher than that of the water supply because of the addition of warm water from households and industrial activities. It is also due to exothermic reactions of microbial activities.
Wastewater temperature is important for two reasons-
1. Biological processes are temperature dependent and dissolved oxygen levels
2.Optimum temperature for biological activity are in the range of temperature rises to 50^0C.
When the temperature of waste water drops below 5^0C, virtually all biological activity become

dormant (lying inactive as a sleep) Oxygen is less soluble in warm water than in cold water. The increase in the rate of biochemical reactions that t accompanies and increase temperature can cause depletion in dissolved oxygen concentration in wastewater. Wastewater temperatures also affect receiving water. Hot water is example, a byproduct of many industrial processes when discharged in large quantities; it can increase the temperature of receiving water locally and disrupt the balance of aquatic life.

Odour-

Odour of wastewater depends on its age. Fresh domestic wastewater has a distinctive, somewhat diagredable odour, which is less objectionable than the odour of stak (or septic) wastewater. The hydrogen sulphide produced by anaerobic microorganisms that reduce sulphate to sulphide. Industrial waste water may contain either odorous compounds or compounds that produce odour during the process of waste water treatment.

Odours at low concentrations cause psychological stress in humans. Offensive odours can cause poor appetite for food, lowered water consumption, impaired respiration, nausea and vomiting and mental perturbation. In extreme situations, offensive odours can affect the socio-economic status of the community .

Colour

Like odour, color of the waste water also depends on its age. Fresh domestic waste water has light brownish – gray color. However, as the travel time in sewer systems increases and more anaerobic conditions develop, the color of the waste water changes sequentially from gray to dark gray and ultimately to black. When the color is black, waste water is often described as septic. Some industrial wastes may also add color to domestic wastewater. In most cases, the gray, dark gray and black color of the waste water is due to the formation of metallic sulphides.

Total solids

Wastewater contains very small amount of solids (about 0.1 %) in comparison to large amount of water (99.9%). On the percentage basis it appears too small. However, large amount of (several million liters) sewage handled by a major municipal sewage treatment plant contains few tons of solids.

Solid materials in waste water can consists of organic and/ or inorganic materials and organisms. The solids must be significantly reduced by treatment, if not they can increase BOD levels when discharged to receiving waters and provide places for microorganisms escape disinfection. Proper solid analysis is important for the control of physical and biological waste water treatment processes and possess compliance with effluent quality limits.

Analytically, the total solids content of a waste water is defined as the residual matter left after evaporation at 103 to 105^0C.

It includes total suspended solids and total dissolved solids.

Suspended solids are non filterable solids which remain on the filter when waste water is passed through a filter. The suspended solids are further divided into settleable and non-settleable solids. Settleable substances, such as sand, grit and heavier organic and inorganic materials, settle out from the rest of the waste water during the preliminary stages of treatment. Knowing the amount of settleable solids in the waste water provides information on how much sludge will be created in the sedimentation tank. Materials that resist settling may remain suspended in waste water or float on the surface as scum. Suspended solids in waste water must be treated, or they will clog soil absorption systems or reduce the effectiveness of disinfection system.

Dissolved solids are non-filterable solids which do not remain on the filter when wastewater is passed through a filter. This fraction of total solids consists of colloidal particles and organic and inorganic materials which dissolve like salt in water. Excessive amounts of dissolved solids in waste water can have adverse effects on the environment. Volatile and fixed solids are two classes of solids in waste water differentiated on the basis of their volatility at $550\pm 50^{0}C$. Volatile solids are driven off as gas at this temperature. The volatile solids are generally organic materials. Solids which can remain behind as ash at this temperature are fixed solids. Fixed solids are inorganic or mineral matter. Each type of solids is processed differently. Settleable and some suspended solids are usually removed by filtration or settling. Other suspended solids and dissolved solids are treated by biological processes or chemical precipitation. Dissolved solids such as metals and chloride can only be removed by distillation or reverse osmosis.

Turbidity

Turbidity, a measure of light transmitting property of water is used to indicate the quality of wastewater with respect to its colloidal and suspended solid matter content. Colloidal matter will scatter or absorbed light and thus prevent its transmition. Therefore, higher the concentration of colloidal matter greater will be the turbidity of wastewater. In gene there is no relationship between turbidity and the concentration of suspended solids in untreated waste water. There is, however, a reasonable relationship between turbidity and suspended solids the settled secondary effluent.

Chemical characteristics of sewage

Waste water is mostly water by weight other materials make up only a small portion of wastewater, but are large enough to endanger public life and environment. They are organic and inorganic matter in filterable and non-filterable forms added into used water as domestic, industrial and agricultural wastes. Chemical constituents of waste water are subjected to variation between communities and within a community at different times.

Organic matters

Organic materials in waste water originate from plants animals or synthetic organic compounds and enter into used water in human excreta, paper products, detergents, cosmetics, foods, and from agricultural, commercial, industrial sources

The principal groups of organic substances present in wastewater are proteins, carbohydrates and oil and grease. urea is an important nitrogenous organic substance present in fresh domestic waste. These organic substances are biodegradable.

1.When Proteins are subject to many forms of microbial decomposition. When proteins are present in large quantities, extremely foul odours are produced by their decomposition.

2. Among carbohydrates present in waste water, sugars are readily decomposed aerobically or anaerobically by microbial activities

3. Starches are more stable, unless converted by microbial activities

4. Cellulose and wood fibers being more resistant to bacterial activity are degraded most by fungi under acidic condition fatty organic materials from vegetables, animals and petroleum are not quickly decomposed by bacteria

Large amounts of biodegradable materials can cause pollution. In fat, too much organic matter in waste water can be dangerous to receiving waters (lakes, streams, rivers, and ocean) because microorganisms use dissolved oxygen in the water to breakdown the waste. This can reduce or deplete the supply of oxygen in the water needed by aquatic life resulting in fish kills, odours and overall degradation of water quality. The amount of oxygen microorganism need to breakdown wastes in wastewater is referred to as biochemical oxygen demand (BOD). when large amounts of oil and grease are discharged to receiving waters from community system, apart from increasing BOD levels when they float to the surface and harden can trap trash, plants and other materials causing foul odours and attracting flies and mosquitoes and other disease vectors Some organic compounds are more stable than others and cannot be broken down by microorganisms. Their presence in wastewater uses an additional challenge for treatment. This is true of many synthetic organic compounds developed for agriculture and industry.

For example

1. Surfactants of synthetic detergent factories and domestic waste origin. Alkyl -benzene-sulphonate (ABE) used in synthetic detergents as surfactant resists biological breakdown therefore, nowadays, linear – alkyl -sulphonate a biodegradable surfactant is used in synthetic detergents.
2. Priority pollutants are organic and inorganic substances known as suspected to be carcinogenic, mutagenic or toxic. Benzene, toluene, ethyl benzene, chloro benzene, dichloro methane are some of the organic priority pollutants present in industrial and commercial discharges.
3. Agricultural chemicals such as pesticides, insecticides and herbicides (collectively called biocides) can be significant contaminants of receiving waters when carried through surface runoff water from agricultural fields. In receiving waters, they kill or contaminate fish, making them unfit to each many of these chemicals (endrin, lindane, toxaphene, silvex, methoxychlor, etc) are classified as priority pollutants.

MEASUREMENT OF ORGANIC CONTENT OF WASTEWATER

A number of different tests have been developed to determine the organic content of wastewater. the laboratory methods used to measure gross amounts of organic matter in wastewaters include

1. Biochemical oxygen demand (BOD)
2. Chemical oxygen demand (COD)
3. Total organic carbon (TOC).

BIOCHEMICAL/BIOLOGICAL OXYGEN DEMAND (BOD)

Microorganisms such as bacteria are responsible for decomposing organic waste. When organic matter is present in water the bacteria will begin the process of breaking down this waste. When this happens, much of the available dissolved oxygen (DO) is consumed by aerobic bacteria, robbing other aquatic organisms of the oxygen they need to live. therefore, biochemical oxygen demand is a measure of the oxygen used by microorganisms to decompose the organic matter in water/ wastewater.

If there is a large quantity of organic waste in the water there will also be a lot of bacteria present to decompose this waste. In this case, the demand for oxygen will be high (due to all the bacteria) so the BOD level will be high. As the waste is consumed BOD level will begin.

The dilution water is seeded with a bacterial culture when treated waste water is being tested Record the DO level of diluted sample from one bottle immediately using the DO test kit (dissolved oxygen meter).

Place the second bottle containing diluted sample in an incubator at 20^0C for 5 days. After 5 days of incubation period record the DO level using the DO test kit .

Subtract the day 5 reading from the day 1 reading to determine the BOD level. Record the value in ppm or mg/ltr.

$$\text{BOD mg/ltr} = \frac{D1-D2}{P}$$

Where, D1 = dissolved oxygen of diluted sample immediately after preparation (ppm or mg/ltr)
D2 = dissolved oxygen of diluted sample after 5 days incubation at 20^0C (ppm or mg/ltr)
P= volumetric fraction of sample used

LIMITATIONS OF BOD TEST

- BOD test is a time-consuming test as it requires long period of time to obtain result.
- Only biodegradable organics present in water sample are measured by this test.
- 5 days incubation period may not be sufficient for microbial degradation of the total biodegradable organic matter present in the water sample
- Pretreatment or use of inhibitory agents is necessary to eliminate the interference by nitrifying bacteria. Inspite of these limitations
- BOD5 test will continue to be used until a better, alternative and rapid test method is developed.

INORGANIC MATTER

Waste water contains inorganic substances (minerals, metals and their compounds) added from both residential and nonresidential sources. Most inorganic substances are relatively stable and cannot be broken down easily by microorganisms in waste water.

Large amounts of many inorganic substances can contaminate soil and receiving waters. Because concentration of various inorganic constituent can greatly affect the beneficial uses of water, it is necessary to examine the nature of some of the constituents, particularly those added to surface water. pH is an important quality parameter of both natural waters and waste water to decline.

Therefore, BOD value indicates the microbiologically oxidizable organic matter present in water/ waste water. In other words, the magnitude of BOD is related to the amount of organic matter present in water sample
The strength of water/ wastewater due to the presence of organic matter is expressed in terms of its BOD level:

BOD level (in ppm)	Water Quality
1-2	Very good (there will not be much organic waste)

3-5	Fair-moderately clean
6-9	Poor- somewhat polluted (usually indicates organic matters is present)
100 or greater	Very poor- very polluted (contains organic waste in large quantity)

Raw domestic waste water has BOD values in the range of 100400 ppm. Wastewater from food processing industries have BOD values in range of 100-10000ppm. One can imagine the problems created if untreated strong wastewaters with high BOD are relised into surface water.

Aerobic microorganisms use up all the dissolved oxygen creating anaerobic condition in the water body. Very soon fishes and other aquatic organisms die due to lack of dissolved oxygen.

Foul odour are produced because of incomplete oxidation of organic matters. At this point the water body is almost dead biologically, although microorganisms are still present and functioning. Such a water becomes unfit for drinking, recreation and other beneficial uses. Therefore, wastewater must be given proper treatment to reduce its BOD level to safe limits before discharging into a natural water body.

BOD TEST PROCEDURE

The BOD test measures the amount of the DO used by microorganisms to degrade organic wastes in wastewater. This test is important for evaluating both how much waste water is likely to require and the potential impact that it can have on receiving waters. The most widely used parameter of organic pollution applied to both wastewater and surface water is the 5day BOD (BOD5) test, it is performed by using a DO (dissolved oxygen) test kit. Take equal quantity of waste water sample in two BOD bottles. Dilute the sample with specially prepared water containing dissolved oxygen (dilution water).

pH value less than 7.0 may indicate the state (septic) conditions of waste water. It is difficult to treat waste water with pH value less than 5.0 and more than 10.0 by biological means as it inactivates or kills those microorganism’s pH of waste water needs to remain between 6.0 and 9.0 in order to protect beneficial microorganisms. if the pH is not altered to the range before discharge, the waste water may alter the pH of the receiving waters and adversely affect the aquatic organisms present.

Alkalinity in waste water results due to the presence of hydroxides, carbonates and bicarbonates of Ca, Mg, Na or K. borates, silicates, phosphates and similar compounds can also contribute to the alkalinity in waste water. Abnormal alkalinity indicates the presence of industrial wastes in wastewater.

Wastewater c contains chlorides added through domestic industrial and agricultural wastes. Human excreta contribute about 6.0g of chlorides per person per day into waste water. backwash from hard water softening process may add large quantity of calcium chloride into waste water.

Wastewater often contains large amounts of nitrogen and phosphorous in the form of nitrate and phosphate respectively – the nutrients that promotes plant growth. Organisms only require small amount of nutrients in the biological treatment, so there normally is an excess of available nutrients in treated waste water. excess of nitrogen and phosphorous can result in eutrophication, the nutrient enrichment of water bodies causing excessive growth of aquatic plants (algae, cyanobacteria, rooted aquatic vegetation). When aquatic plants die and are decomposed by aerobic bacteria, the dissolved oxygen in the water becomes depleted. the oxygen depletion can reduce the population of fishes and other oxygen consuming aquatic organisms excess of nitrogen in nitrate form in drinking water may contribute to miscarriages and is the cause of a serious illness in infants called methemoglosinemia or blue baby syndrome.

Sulphur is present as sulphate ions in waste water. Sulphate is reduced biologically under anaerobic conditions to hydrogen sulphide. hydrogen sulphide is released to the atmosphere above the waste water in sewers that are not flowing full and thus accumulates in the sewer pipes the accumulated H2S can be oxidized biologically to sulphuric acid which is corrosive to sewer pipes.

Large amount of many inorganic substances can contaminate soil and water. Some are toxic to animals and humans. For this reason, extra treatment steps are often required to remove inorganic materials from industrial wastewater sources. Heavy metals, for example which are discharged with many types of industrial waste waters, are difficult to remove by conventional treatment methods.

Gases commonly found in untreated wastewater include nitrogen, oxygen, carbon dioxide, hydrogen sulphide, ammonia and methane the first three are common gases of the atmosphere the latter 3 are derived from the decomposition organic matter present in waste water. Presence of dissolved oxygen in wastewater is desirable because it prevents the formation of foul odours.

Certain gases in waste water can cause odours, affect the treatment or are potentially dangerous. methane, for example is combustible. hydrogen sulphide and ammonia can be toxic and pose asphyxiation hazaras. Wastewater odours (because of hydrogen sulphide and ammonia) can affect the mental well-being of residents, lower property values and affects the local economy.

BIOLOGICAL CHARACTERISTICS OF WASTEWATER

Wastewater contains various types of microorganisms. They are mainly of domestic, foot and dairy industries in origin. The types and quantity of microorganisms will vary depending on the nature of the wastewater, bacteria, protozoa, viruses, helminths and fungi are the principle group of microorganisms present in wastewater.

BACTERIA

Wastewater consist of vast quantities of bacteria. Raw waste water may contain millions of bacteria per milliliter. These bacteria include coliforms, facal streptococci, anaerobic spore forming bacilli, the proteus group and other types of gastrointestinal origin. The so-called sewage fungi present on the sides and bottom of sewage pipes and tanks forming slimy growth are not fungi but bacteria belonging to the genera sphaerotilus, crenothrix, beggiatoa and Rhodospirillum. The waste water may also contain methanogenic bacteria.

Most of the bacteria present in waste water are saprophytic and are harmless to man. They are responsible for the decomposition of organic matter and stabilization of wastewater. However, pathogenic (disease causing) bacteria of typhoid, dysentery and other intestinal disorders may be present in wastewater. Test for total coliform and fecal coliform nonpathogenic bacteria are used to indicate the presence of pathogenic bacteria.

Bacteria present in wastewater can be classified into aerobic, anaerobic and facultative types according to their dissolved oxygen requirement. The predominant physiological types of bacteria may change during the course of decomposition of organic matter in wastewater. In the begin, aerobic and facultative anaerobic bacteria dominate and decompose organic matter present in wastewater (ex: Enterobacter, alcaligenes, escherishia, pseudomonas, etc.,). Finally, obligate anaerobes- the methanogenic bacteria (methanococcus, methanosarcina, etc,) will take over the process.

PROTOZOA

Protozoa present in wastewater include amoebas, flagellates and free swimming and stalked ciliates. They feed on bacteria, other microscopic microorganisms and particulate organic matter present in wastewater. They presence in wastewater is essential in the operation of biological treatment processes and in the purification of streams. Therefore, protozoa in wastewaters are considered as sewage polishers.

Number of protozoan present in waste water are also pathogenic and are of great concern in drinking water supplies.

VIRUSES

Wastewater often contains viruses that may produce diseases. They enter into wastewater mainly through human excreta. Wastewater may also contain bacteriophages.

FUNGI

Along with bacteria, fungi are the principal group of microbes present in wastewater. Saprophytic fungi present in it are responsible for the decomposition of organic matter (cellulose and lignin in particular). Without the presence of fungi to break down organic material, the carbon cycle would soon cease to exist in nature and organic matter would start to accumulate.

PATHOGENIC MICROORGANISMS PRESENT IN WASTEWATER

Wastewater is a potential source pathogenic microorganism. Many disease-causing bacteria, viruses, protozoa and helminths are present in wastewater. These pathogens often originate from people and animals that are infected with or are carriers of a disease. For example, blackwater from domestic source contain enough pathogens to pose a risk to public health other likely sources in communities include hospitals, schools, farms and food processing industries.

Because the numbers of pathogenic microorganisms present in wastewaters and polluted waters are few and difficult to isolate and identify, the Coliform organism, which is more numerous and more easily tested for, is commonly used to indicate the presence of pathogenic organisms. Some illness from wastewater- related sources are relatively common. The usual pathogenic bacteria that may be excreted by man cause diseases of gastrointestinal tract such as typhoid fever (*Salmonella typhi*), cholera (*Vibrio cholerae*), Shigellosis(*Shigella*), Leptospirosis (*Leptospira*

sp), Salmonellosis (*Salmonella sp*), Yersinios (*Yersinia enterolitica*) and gastroenteritis or Diarrhorea (*Escherichia coli*).
The common viral diseases from wastewater related sources infectious Hepatitis (*Hepatitis A*), Polio (*Poliomyelitis virus*), and respiratory diseases (*Adeno virus*).

Amoebiasis (*Entamoeba histolytica*), Giardiasis (*Giardia lambia*), cryptosporidiosis (*Cryptosporidium sp*) and Balantidiasis (*Balantidium coil*) are some of the protozoan diseases of waste water origin.

Some of the diseases caused by helminths, present in waste water are-Ascariasis (or round worm infestation) (*Ascariasis lumbricoides*), Enterobiasis (*Enterobius vericularis*), Taeniasis (*Taenia seginata* the beef tape worm), Taeniasis (*Taenia solium*-the pork tape worm) and Trichuriasis (*Trichuris trichiura*-the whip worm).

Out breaks of these diseases can occur as a result of drinking water polluted by waste water, eating contaminated fish or recreational activities in polluted waters. Some illness can be spread by animals and insects that come in contact with waste water. Because these microorganisms are highly infectious, they are responsible for many thousands of deaths each year in areas with poor sanitation, especially in tropics. For this reason, waste water treatment is as important to public health as drinking water treatment.

USE OF INDICATOR ORGANISMS
The numbers of pathogenic organisms present in waste and polluted waters are few and difficult to isolate and identify.
Intestinal tract of man contains countless rod-shaped bacteria known as coliforms in addition to other forms of bacteria.

Because of coliforms are more numerous and more easily tested for, they are commonly used as indicator organisms. thus, the presence of coliforms organisms in waste and polluted water is taken as an indication that pathogenic organisms may also be present. Their absence is taken as an indication that the sample is free from disease producing organisms. The coliforms include Escherichia and Enterobacter. Apart from human wastes, they can also grow in soil. thus, the presence of coliforms does not always mean contamination with human wastes. Apparently, E. coli are entirely of fecal origin. There is difficulty in determining E.coli to the exclusion of the soil coliforms.

As results, the entire coliform group is used as an indicator of fecal contamination.
Other microorganisms that have been proposed for use as indicators of pollution are- fecal streptococci, enterococci, clostridium perfringens, etc.

There are two methods for determining the presence and density of coliform bacteria in waste and polluted waters.
• The membrane filter (MF)technique-provides a direct count of cells trapped and then cultured on a suitable medium.

• The multiple tube fermentation method – provides an estimate of the most probable number (MPN) per 100 milliliters from the number of test tubes in which acid and gas are formed after incubation at 35-37 degree Celsius for 24-48 hours.

***Coliform* tests are useful**

1. To determine the presence or absence of pathogenic organisms in waste and polluted waters.
2. For determining whether waste water has been adequately treated and
3. Whether water quality is suitable for drinking and recreation

RATIO OF FECAL *COLIFORMS* AND FECAL *STREPTOCOCCI*

The quantities of fecal coliforms (FC) a fecal streptococci (FS) that are discharge by human beings are significantly different from the quantities discharged by animals. Therefore, it has been suggested that the ratio FC count to the FS count in a sample can be used to show whether the suspected contamination derives from human or from animal waste.

The FC/FS ratio for domestic animals is less than 0.

If the ratios are obtained in the range of 1-2, interpretation is uncertain. If the sample is collected near the suspected source of pollution, the most likely interpretation is that the pollution derives equally from human and animal sources.

WASTE WATER TREATMENT (SEWAGE TREATMENT PROCESS)

Waste water treatment processes are many and varied. The methods in which physical forces predominate are known as unit operations. The methods involving chemical or biological reactions are termed unit processes. Both unit operations and processes are employed together to provide complete treatment of waste water.

The choice of waste water treatment method is based on the area to be served and the density of population. The methods best for the country side (rural areas) are not applicable to urban and industrial areas and vice versa. Waste water treatment is managed by
1. On small scale -as in single dwelling units and rural areas and
2. On large scales in towns and cities by municipal bodies.

Small scale treatment of waste water

Single dwelling units and small communities without public sewers often use cesspools or septic tanks for the disposal of domestic waste water.

Cesspools

Human waste is thrown in cesspools in many homes. It is constructed in underground part with concrete in such a way that it contains wall of cylindrical rings with pores (Fig.).

Its opening is near the ground level. Wastewater (sewage) enters the cesspool through the inlet pipe. The bottom of cesspool remains open. Therefore, the suspended solid material falls on the bottom of cesspool and forms sludge after getting deposited in huge amount.

Water passes out through the open bottom of cesspool and through pores into the surrounding soil. The organic materials of the sludge are decomposed by anaerobic bacteria resulting in release and deposition of breakdown products on the ground.

Thus the amount of breakdown products exceeds; it forms thick layers which need to be cleaned by using strong acids. Dried bacterial preparation of Bacillus subtilis or yeast cells should be added at intervals. These accelerate the decomposition of sludge deposited at the bottom of cesspool.

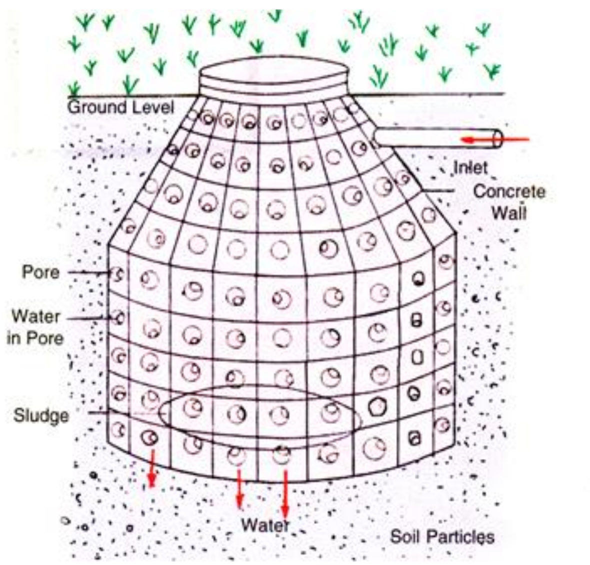


Diagram of cesspool. Wastes enter through inlet. Water passes into surrounding soil through pores of wall and bottom. Solid materials accumulate at the bottom as sludge.

Imhoff Tanks

Imhoff tank is an improved septic tank. It basically consists of a two storey tank in which the sedimentation (settling) occurs in the upper tank while the digestion of the settled solids takes place in the lower compartment (Fig.).

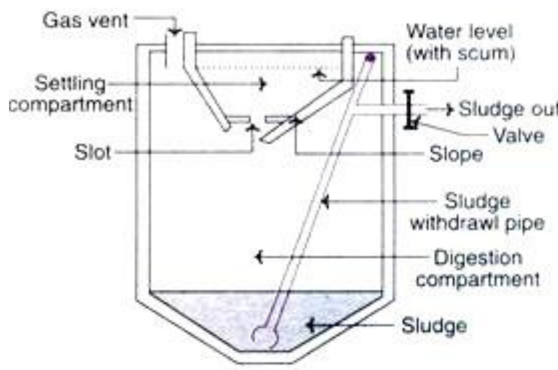


Fig. 57.24 : A diagrammatic view of imhoff tank.

As the sewage enters the sedimentation tank, the solids settle down to the bottom and the sewage flows into the digestion tank through slopes and slot of the sedimentation tank. Gas produced in the digestion process escapes through gas vent. The sedimentation tank is designed in such a way that the gases and the gas-buoyed sludge particles raising from the sludge layer do not enter into it. The sludge collection at the bottom can be withdrawn periodically.

Septic tanks

Septic tanks are recommended for individual houses and for small communities and institutions with a contributing population around 300. Septic tanks work on the principle of anaerobic digestion. This occurs as the solids of the sewage settle at the bottom of the tank. Under anaerobic conditions, the biodegradable organic matter is converted to gases (CH_4, CO_2, H_2S etc.) and liquid compounds. This results in a drastic reduction in the volume of sludge.

A thick crust of scum formed on the surface of septic tank maintains anaerobic conditions. The effluent coming out of the septic tank contains some organic solids and pathogens. Therefore, disposal of the effluent from septic tank has to be dealt with very carefully. The septic tanks are dislodged and cleaned at regular intervals, usually once in 2-5 years (depending on the tank size and its use).

Construction and operation of septic tanks

Septic tanks are usually constructed with bricks, or stone masonry. Thick-wall polythene and fibre glass tanks are also in use in recent years. Whatever may be the construction material used, the septic tank must be water-tight and must function efficiently. The size of the tank is variable, depending on the number of users. For a family of five members, a tank with a length of 1.5 m and a breadth of 0.75 m is recommended. For such a tank, the cleaning interval is 2-3 years.

Nowadays, polyethylene or fiber glass tanks are used regardless of the material used, the septic tank mostly have water inlet and structurally round. The tank should be 2 to 3mm length than width and 4 to 6 ft deep.

Septic tank has inlet (influent) and outlet (effluent) pipes at its opposite ends. Ells Tees may be used at inlet and outlet connections. Instead of ells or tees, wooden influent and scum baffles may be used. Influent baffle controls the flow rate the influent (waste water flowing into the treatment plant).

Scum baffle prevents scum from entering outlet pipe along with effluent (treated waste water flowing out of the treatment plant). Manholes with lids should be provided in the roof of the tank over the inlet and outlet pipe for cleaning purpose. a vent pipe may be provided at the roof of the tank to facilitate the escape of gas produced during anaerobic digestion of organic matter.

The outlet pipe is connected to a distribution (diversion) box buried underground. Diverging from the distribution box are perforated clay pipes embedded in shallow trenches filled porous materials (gravel). the underground area of soil embedding distribution box and clay pipes is called disposal field (leach field, absorption field or drain field). It is through the disposal field the effluent from the septic tank infiltrates into the surrounding soil.

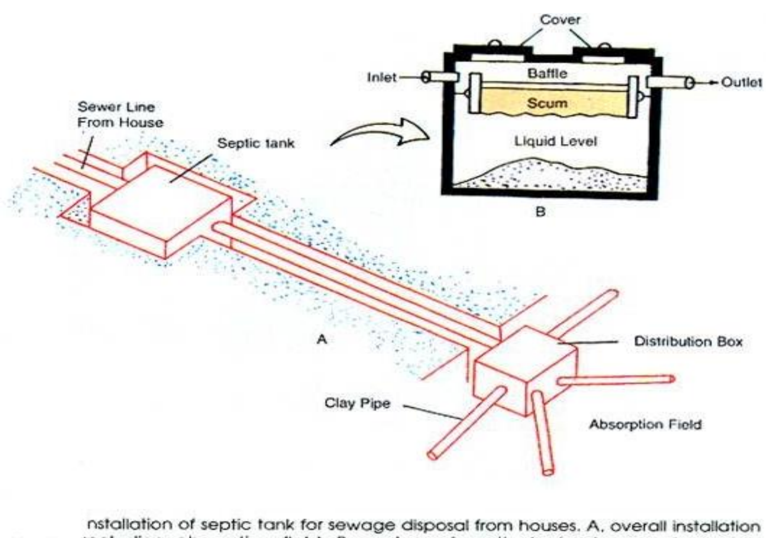
Process description- domestic waste water (sewage) flows into the septic tank through inlet pipe. It is retained in the tank for very long time during which the suspended solids (about 60 to 70% of organic matter) in the sewage sink to the bottom of the septic tank as sludge (any solid or semi solid).

waste settles to the bottom of sedimentation tanks of septic tanks) and float to the surface of waste water as scum (composed of oil and fat bodies and grease) when fresh waste water is released into the septic tank, the fed and skimmed waste water(effluent) flows out from the clear space between the sludge and scum layer through the outlet pipe into the distribution box by overflow mechanism. The distribution box may be provided with the central wall to regulate the release of effluent into the disposal field.

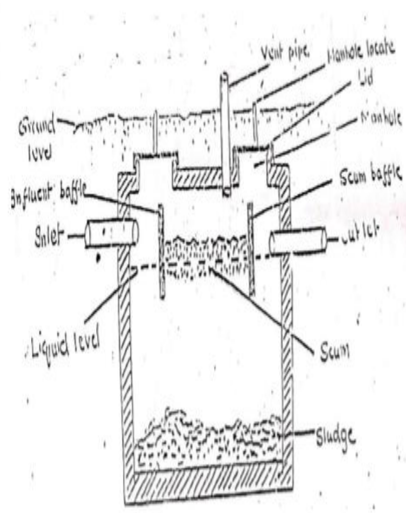
The effluent from septic tank has offensive odour and contains contaminants including pathogenic microorganisms. Therefore, it should not be released into open drain or natural water bodies without further treatment. When released into disposal field, the effluent slowly infiltrates into the soil. Meanwhile the soil bacteria decompose and stabilize the organic matter present in the effluent.

The sludge and the scum accumulated in septic undergo anaerobic digestion by the activity of anaerobic and facultative anaerobic bacteria present in the sewage into a mixture of gases (CO_2,CH_4, H_2S , H_2, etc) and humus. The gas escapes out through vent pipe.

Long-term accumulation of sludge and scum can reduce the effective volumetric capacity of the septic tank. Therefore, sludge and scum must be pumped out periodically. When applied onto field the sludge serves as a good source of humus. Small amount of sludge may be reintroduced into the septic tank as starter culture.



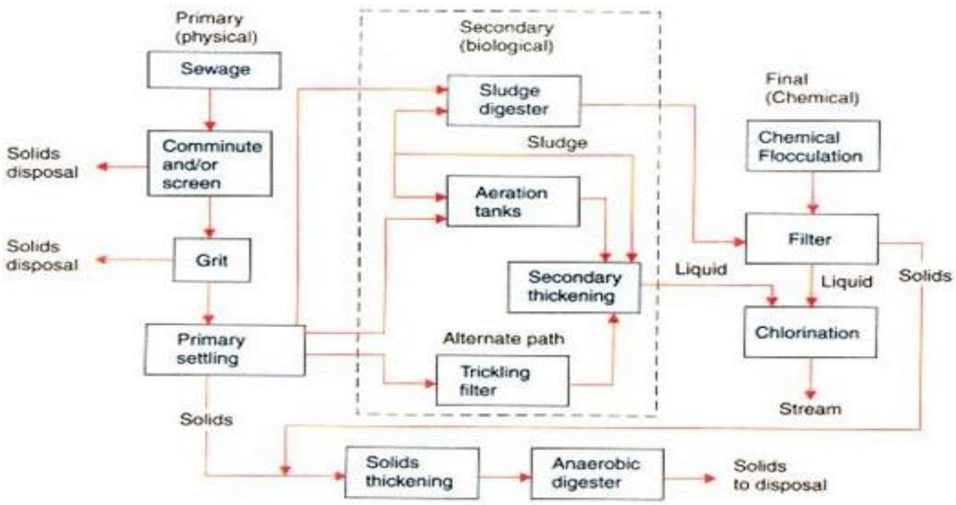
nstallation of septic tank for sewage disposal from houses. A. overall installation including absorption field; B. a view of septic tank where water enters through inlet and solids accumulate as sludge.



As the sewage enters the septic tank, the solids settle to the bottom while grease and other light materials float on the surface, and form a scum. The bottom-settled organic material undergoes facultative and anaerobic decomposition to form more stable compounds and gases (CH_4, CO_2, and H_2S). In this way, there is a continuous reduction of solids of sewage entering the septic tank. However, sludge accumulates at the bottom of the tank. Desludging of septic tank has to be carried out periodically (once in 2-5 years).

LARGE SCALE WASTE WATER TREATMENT (MUNICIPAL WASTE WATER TREATMENT)

Sewage treatment on a large scale of populations of city is known as large scale sewage treatment. In cities sewage and garbage are generated in massive amount per day which is treated by municipal plants. A schematic view of waste treatment by a municipal plant is shown in Fig.



Flow chart of different stages of sewage treatment.

Treatment Steps	*Processes*
Primary	Screening and removal of insoluble particulate materials, addition of alum and other coagulants
Secondary	Biological removal of dissolved organic matter through trickling filters, activated sludge, lagoons, extended aeration systems and anaerobic digesters
Tertiary	Chemical removal of inorganic nutrients, virus removal/inactivation, trace chemical removal

Waste water (sewage) from large population of cities is carried out in a large scale by mechanized municipal waste water treatment plants. The overall process can be divided into two major steps:

i) Primary treatments.

ii) Secondary treatments.

• Primary treatment- accomplishes the removal of course and suspended settleable and floatable solids from the wastewater by physical methods.

• Secondary treatment – is a biological treatment process to reduce the organic matter content of the wastewater by microbial decomposition

• Tertiary treatment- is used after primary and secondary treatment to improve effluent quality (to produce effluent) by removing nutrients and pollutants. It is accomplished mostly by physical methods and chemical processes. iii) Wastewater form some cities also require a step called tertiary treatment.

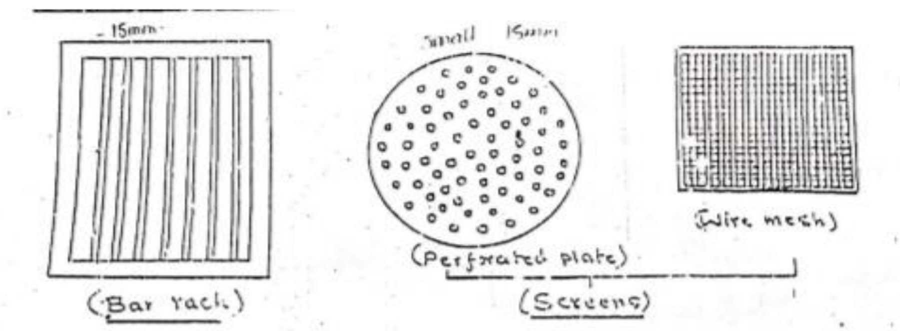
Primary treatment
The waste water that enters a treatment plant contains floating and suspended large small and dissolved, organic and inorganic solids. Removal of a portion of these by physical methods is termed as primary treatment. It is carried out by screening, grinding, degritting and sedimentation.

Large and course solids such as rags, plastics, wooden pieces, gritt, etc might clog or damage the pumps and treatment machinery. These are removed by screening, griding and degritting-collectively called preliminary treatment.

Preliminary treatment: is used to condition the wastewater to aid the processing that occurs downline. Rags, plastics, wooden pieces, grit, etc are removed at this stage to protect the pumps and other equipment in the treatment plant. Different stages of preliminary treatment are;

1. Screening: at a treatment plant, wastewater first passes through a device called screen. It trapes all large floating or suspended solids such as rags, wooden pieces, plastics, stones, etc.

There are two main types of screens:
a) Bar racks or bar screens of parallel rods or bars
b) Screens consisting of perforated plates or wire meshes. The space between the bars is 15mm or more in bar racks. Perforation plates are smaller than 15mm in size. The material trapped from the waste water by the screens are known as screenings.
Smaller openings on bar racks or screens, greater will be the screenings collected. Screenings are removed manually or mechanically. The screenings are disposed off as a solid waste either by
1. Burring in the ground
2. By incineration (burning).



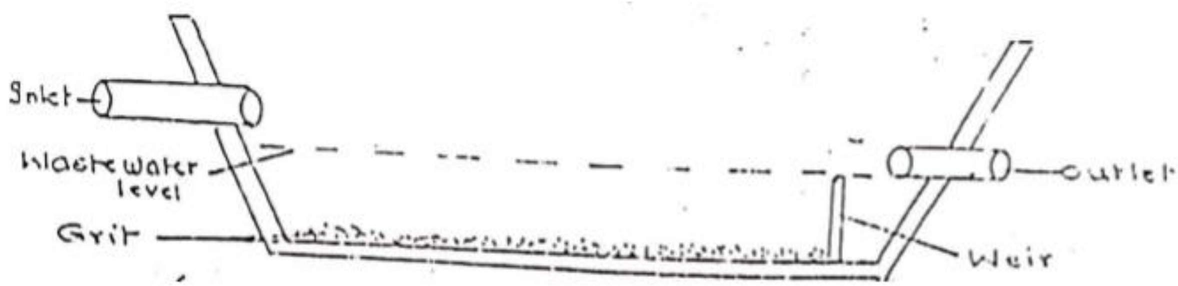
2) **Grit removal (degritting):** wastewater contains inert and dry materials called grit. Grit consists of silt, sand, gravel, glass pieces, cinder etc. that have specific gravity or settling velocity greater than the organic solids present in wastewater.
Removal of grit is one of the preliminary steps in sewage treatment. Grit being inorganic in nature cannot be removed in the biological treatment processes. If grit is not removed prior to downline processes of sewage treatment

a) It causes excessive wear on the equipment.
b) It forms heavy deposits in pipelines
c) It combines with the organic matter present in the sludge and create problem in sludge digestion and
d) Increase the frequency of cleaning of sedimentation tanks and sludge digesters.

Grit is removed in a device called grit chamber or grit tank.
There are three general types of grit chambers:

a) Horizontal flow type grit chamber
b) Aerated grit chamber and
c) Vortex- type grit chamber.

Horizontal flow type grit chambers are rectangular or long and narrow channel shaped settling tanks. The grit allowed to settle in a grit chamber by slowing the velocity of the wastewater flow to approximately one foot per second. The inorganic grit settles at this velocity while most of the organic solids that remain in the in-suspension pass through.



Longitudinal section of a horizontal flow type of grit chamber

The grit is removed from the tanks and washed to remove the residual organic material. it is disposed off as a solid waste in a landfill.
Grit chambers are most commonly installed next to screening facilities and before primary sedimentation tanks.

C) grinding or comminution – as an alternative to bar racks or screens, grinders (also called comminutors) are used in the preliminary treatment of waste water. Grinders are used to chop or grind up the coarse solid without removing them from the waste water. The chopped or grinded solids are returned to the flow stream of waste water for removal in the subsequent treatment steps.
Grinding comminution eliminates the problems caused by solids of different sizes while pumping the waste water and improves the efficiency of waste water treatment processes.

Primary sedimentation

The objective of sewage treatment by sedimentation is to physically separate suspended solids from the waste water. it is based on the principle, that when a liquid containing solids in suspension is placed in a tank relatively undisturbed state for some time, those solids having higher specific gravity than the liquid will tend to settle while those with lower specific gravity will float. physical separation of suspended solids from screened and degritted waste water as a last step in primary treatment is called primary sedimentation or primary settlement. This is usually carried out by designing radial flow type, upward flow type, or horizontal flow type tanks namely- primary sedimentation tank, primary settling tank or primary clarifier.

The screened and degritted waste water contains finely divided and colloidal solids (organic materials) in suspended state. This waste water flows into primary sedimentation tank Wastewater is held in the tank for few hours allowing many of the readily settleable suspended solids with higher specific gravity than that of water to sink to the bottom as a muddy material called sludge.

Meanwhile readily floatable suspended solids such as oil and grease having lower specific gravity than that of water flow to the surface and form a layer of scum. the process of primary sedimentation can remove about 40 to 60 percent of the suspended organic solids present in the waste water that enters the sedimentation tank. This is approximately equal to reduction in BOD5 of the incoming waste water by about 20 to 40% .

The suspended solids present in screened and degritted wastewater are mostly in an incompletely flocculated state. They can be subjected to flocculation by eddying (circular or spiral) motion of the waste water in the primary clarifier. It is present through the coal scence of fine particles about their collision into aggregates or fibres. They are allowed to separate from waste water.

For some industrial wastewaters, coagulation process is incorporated by adding chemicals such as aluminium sulphate (alum), ferric chloride, or poly electrolytes collectively called coagulants. coagulants change the surface characteristics of the suspended solids so that they attach to one another (coagulate) and precipitate.
Coagulation and flocculation can remove more than 80 % of suspended solids present in the wastewater entering primary sedimentation tank.

As and when the suspended solids settle to the bottom of the sedimentation tanks to form sludge and float to the surface as scum the wastewater gets clarified (becomes more and more clean).

A tank designed both for clarification and flocculation purpose called the clarificatory.

The sludge is drawn off from the bottom of the sedimentation tank and the serum is skimmed and subjected to further treatment. The clarified wastewater effluent (the settled and skimmed waste water) flows out through the effluent pipe by overflow mechanism on to the next stage of waste water treatment.

Flotation

Flotation is an alternative used in the treatment of solid waste water, air is forced into the wastewaters under pressure of 1.75 to 3.5kg /sq. cm. the wastewater, super saturated with air is then discharged to the surface, where they are removed. Floatation can remove more than 75 percent of the suspended solids. Chamber into the lower digestion chamber. Other suspended solids such as oil and fat bodies and greasy substances present in the wastewater float to the surface of the wastewater as scum.

The solid matter (sludge) collected in the digestion chamber undergoes anaerobic digestion by the activity of anaerobic and facultative anaerobic microorganisms (ex: bacteria).

The process of sludge digestion in Imhoff tank is better than that in septic tank because the sludge is not allowed to mix with the influent.

A mixture of gases produced in the digestion chamber by anaerobic digestion of the sludge is collected in the gases vent a narrow portion in Imhoff tank besides sedimentation chamber.

Gases are allowed to escape into atmosphere. Because the principal gas produced is methane it can be collected through a vent pipe and used for heating and lighting purposes.

Because of the overhanging tip in the bottom of the sedimentation chamber, gases and gas-buoyed sludge particles rising from sludge layer in the digestion chamber are prevented from entering into sedimentation chamber.

A mixture of raw and partially digested sludge is removed periodically (every two to three months) from the Imhoff tanks water hydrostatic pressure for further treatment through the slude withdrawal pipe. When applied to field it contributes to humus.

The settled and skimmed wastewater (effluent) is refused through the outlet pipe by over flow mechanism from the sedimentation chamber. The effluent typically goes into leaching field or land application. It can be released into natural water body after final treatment.

The Imhoff tank delivers better effluent than that obtained from septic tank.
The conventional Imhoff tank are rectangular in shape where anaerobic digestion of the sludge is carried out in the unheated digestion chamber. Modified designs of Imhoff tanks are provided with devices for heating the digestion chamber.

SECONDARY TREATMENT OF WASTEWATER (BIOLOGICAL TRATMENT OF WASTEWATER)

Wastewater (sewage) after it has undergone primary treatment contains about 40 to 60 % of organic matter mostly in dissolved state. Wastewater is then subjected to secondary treatment which removes most of this organic matter (85 to 90 percent) and reduces the BOD5 to and extends of 90%. Therefore, secondary treatment processes are employed to reduce the BOD of the wastewater to reasonable levels before releasing it to natural waterways. This is achieved through mineralization of a small fraction of the biodegradable organic matter and the conversion of the remaining to settleable solids .

1) The secondary treatment of wastewater relies on microbial activity and therefore is known as biological treatment of wastewater. The process may be conducted in a large variety of devices by aerobic or anaerobic methods.

2) Bacteria among the microorganisms present in wastewater have a key role in the secondary treatment of the same.

3) Aerobic bacteria in the presence of oxygen convert organic matter to stable end products such as CO2, NO3, PO4 and SO4. It is an orderly, inoffensive and rapid process.

Secondary treatment of wastewater by aerobic method is carried out

a) In attached – growth (fixed film) treatment system, such as trickling filter and rotating biological contactors

b) In suspended- growth treatment systems, such as, activated sludge system and oxidation ponds

4) Aerobic bacteria purify organic matter to products such as, CO3, NH3, CH4, H2S, amino acids and various organic acids. It is a disorderly and offensive process. It is slower than aerobic process wastewater treatment in septic tanks, Imhoff tanks and other anaerobic digesters work on the principle of putrefaction of organic matter .

Eukaryotic microorganisms from among fungi, protozoa, and algae also contribute their might to the process of secondary treatment of wastewater

TRICKLING FILTER

1) It is an aerobic, attached growth (fixed film) wastewater treatment installation. It is employed to remove the organic matter present in the settled wastewater from primary sedimentation tank partly by mineralization and partly by coagulation

2) In trickling filter process, the waste water is sprinkled intermittently over a bed of some type of porous medium as the wastewater percolates through this porous bed called filter bed, a fraction of the organic matter present in it is absorbed on the gelatinous film of microorganisms that coats the medium and is mineralized by the microbial activity. The major portion of remaining organic matter coagulates into sludge forming solids.

3) In most of the installations, trickling filter are built in a circular shape. A trickling filter consist of:

a) A filter bed of high porous medium such as, crushed stones, gravel, slag or synthetic (plastic / PVC) materials the depth of the filter bed ranges from 1 to 12 meters depending on the materials used.

b) A rotatory distributor suspended over the filter bed. It consists of 2 or hollow arms that are mounted on a pivot in the centre of the filter. The arms have a row each of nozzles along their length through which the wastewater is sprinkled over the filter bed when this distributor revolves in a horizontal plane. The distributor may be driven either by dynamic reaction of the wastewater discharging from the nozzles or by an electric motor. Clearance of 6 to 9 inches should be allowed between the distributor arm and the top of the filter bed to facilitate uniform distribution of wastewater when sprinkled on the porous medium.

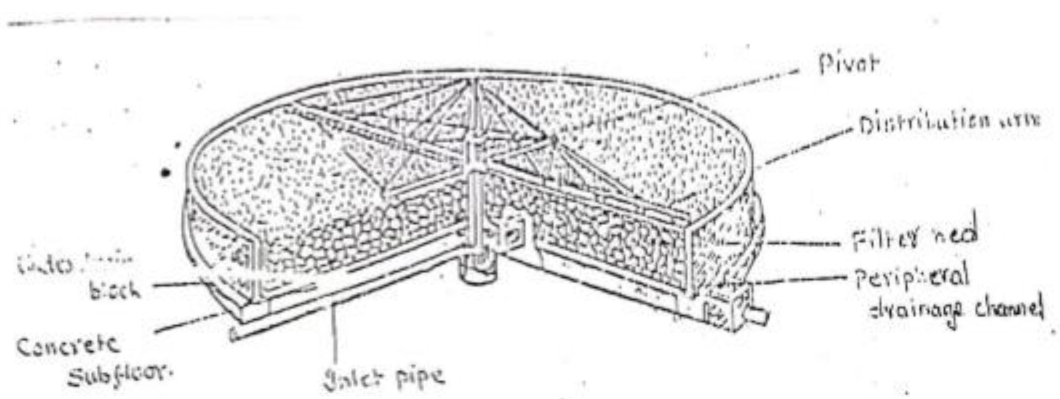
c. An inlet (influent) pipe situated below the filter through which the waste water is pumped into the rotatory distributor.

d) An underdrain which acts as a collection system for treated waste water in a trickling filter. The underdrain system has precast blocks of vitrified clay or fibre glass grating laid on a reinforced concrete subfloor. the floor and underdrain block slope to central or peripheral drainage channel at a 1 to 5 % grade. the underdrains also ventilate the filter, providing the air for the microorganisms that live in the filter slime.

Process description- The partially treated wastewater (primary settled wastewater) is sprayed on the filter media through the nozzles on the horizontally revolving arms of the rotatory distributor. The spraying saturates the wastewater with the atmospheric oxygen. Intermittent application of wastewater maintains aerobic condition in the filter bed.

When a new trickling filter is operated former wastewater treatment within few weeks' time a greenish- yellow or blackish slimy layer zoogloeal (zoogleal) film (slime) develops on the filter media. Zoogleal film is a biological layer of aerobic, anaerobic, facultative anaerobic bacteria, fungi, algae and protozoans. Worms, insect larvae and snails are also present.

a) The bacteria – Zoogleal ramigera predominate trickling filter along with, *Flavobacterium, Pseudomonas* and *Thermophilus* and *Sphaerotilus natuns* and Baggiatoa in the lower regions of the filter has nitrifying bacteria- *Nitrosomonas* and *Nitrobacter*.
b) The fungi- *Fusarium, Mucor, Penicillium, Sporatichum* and various yeasts are present in the tricking filter. The over growth of mycelial fungi may clog the filter and restrict ventilation.
c) Algae such as *Phormidium, chlorella* and *Ulothrix* are commonly found in the upper surface of trickling filters. Generally, algae do not take direct part in waste degradation, but during day time add oxygen to the percolating waste water. Algae become troublesome when they clog, they filter surface which produces odours.
d) The ciliate protozoa such as *Opercularia* and Epistylis present in filter do not stabilize the waste but control the bacterial population.



A cutaway view of trickling filter

Whe
n the wastewater percolates through the porous media, the organic matter present is degraded by the population of microorganisms attached to the filter media. The organic material present in the wastewater is absorbed on the zoogleal film. In the cutter portion of the zoogleal film, the organic material is degraded by aerobic microorganisms to stable and products. This accounts to only a fraction of the organic material present in the wastewater. The remaining portion of the

organic materials coagulate to form sludge forming solids. As the slime layer increases in thickness, it may be sloughed off from the filter media by the percolating wastewater and a new slime layer starts to grow.
The underdrain collection system collects treated wastewater containing sludge forming solids and sloughed off slime layer is released into secondary sedimentation tank. Sludge forming solids sediment in the secondary sedimentation tank from where the sludge is transported to a sludge digester for microbial decomposition the effluent may be carring waterbody after disinfection.
The trickling filter process, where proceeded by sedimentation can remove about 85% of BOD5 of wastewater entering the plant.

ADVANTAGES OF TRICKLING FILTER ARE
1) BOD5 of the treated wastewater is very much reduced
2) Needs less electric power for running the mechanical equipment
3) Mechanical wear and tear are limited
4) Needs know skilled super vision .

DISADVANTAGES OF TRICKLING FILTER ARE
1) Installation requires large land area
2) Construction cost is high
3) Raw sewage cannot be treated
4) Process develops fly nuisance.

ACTIVATED SLUDGE PROCESS

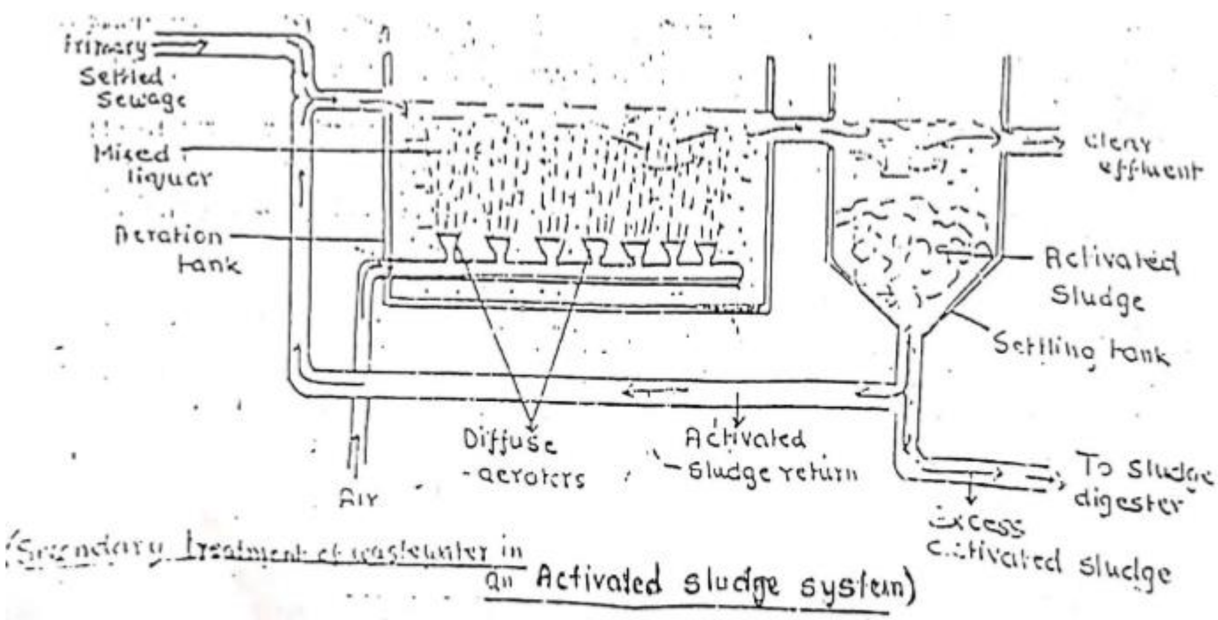
1) Activated sludge system is a popular aerobic suspended growth type of secondary (biological) wastewater treatment system.
2) The process is based on the aeration of wastewater from primary treatment with flocculating biological growth, followed by separation of treated wastewater from this growth. Usually, the separation of the growth from the treated wastewater is done by settling (gravity separation) or by floatation and other methods.
3) The flocs composed of actively growing sewage metabolising microorganisms and organic matter mostly soluble form) sediment to form activated sludge. Therefore, the process is termed activated- sludge process. a part of the activated sludge is added to the incoming waste water as inoculum. The remainder is wasted and sent on to a sludge treatment process.
4) The activated sludge process is a continuous, or semi continuous aerobic method for biological wastewater treatment. The treatment system consists of
a) An aeration tank
b) Secondary settling tank.

Aeration tanks may be square or rectangular in shape. They are usually constructed of reinforced concrete and are left open to the atmosphere. Influent and effluent pipes with valves are provided at their opposite ends. Aeration is provided by means of diffuse aerator, mechanical aerators or by a combination of diffused and mechanical aerators.

Process description: after primary settling the wastewater, mainly composed of dissolved organic matter, is introduced into an aeration tank through the influent pipe. It is mixed with some of the sludge from the previous batch to improve the efficiency of the process. This mixture is called mixed liquor.
The mixed liquor is aerated and agitated by oxygen or pure air injection through diffused aerators and/ or by mechanical aerators. The aeration and agitation are continued for 4-8 hrs. during the holding period in the aeration tank, vigorous development of heterotrophic microorganisms takes place in the wastewater.

The microbial community includes bacteria such as, zoogloeal, *Escherichia, Enterobacter*, *Pseudomonas*, *Achromobacter*, *Flavobacterium, Nocardia, Nitrosomonas, Nitrobacter,Sphaerotilus, Beggiatoa*, etc, and few filamentous fungi *Cephalosporium, Penicillium* and *Gladosporium* and some yeasts. Protozoa present are mainly represented by ciliates

Bacteria present play dominant role in activated sludge process. The activity of aerobic bacteria oxidises much of the wastewater into carbon dioxide and water. Fungi play a subordinate role in the activated sludge process. The remaining organic matter and the microorganisms aggregate to form activated sludge particles, known as flocs. These are held in flocs by bacterial slimes. ciliate protozoa adhere to the flocs but feed on the bacteria in suspension. Therefore, they act as effluent polishers.



The contents of aeration tank after aeration for 4 to 5 hours depending on requirement are transferred to a secondary sedimentation tank, where the flocs settle out, removing much of the organic matter as activated sludge. occasionally the sludge will float rather than settle out: this phenomenon is called bulking. When this happens, the floc flows out with the discharge effluent. The bulking is generally caused by the overgrowth of filamentous microorganisms which produce loosely packed flocs that fail to settle out. Bulking of sludge can be avoided by

a) Chlorination
b) Prolonged aeration or
c) Increasing pH of wastewater to 8 or more liming

The well aerated activated sludge is golden brown in colour unlike the light brown under aerated and overaerated muddy brown activated sludge.
A portion of the settled activated sludge recycled for inoculation of the incoming wastewater (return sludge). the remainder is wasted and onto a sludge treatment process (by anaerobic digestion and/or composting) or subjected to incineration or disposed in landfills.
The clear effluent is disinfected and disposed. Combined with primary settling, the activated sludge process can remove 85 to 95 % of BOD from the wastewater.

ADVANTAGES OF ACTIVATED SLUDGE PROCESS

- It provides well clarified effluent
- ➢The effluent is free from offensive odour
- ➢BOD of the wastewater is reduced to as low as 5 to 15 % and
- ➢Reduction in intestinal pathogens

DISADVANTAGES OF ACTIVATED SLUDGE

- ➢Skilled supervision and constant check on the return sludge is necessary.
- ➢Large amount of sludge formed poses disposal problems
- ➢Wastewater from soaps and detergents factories when aerated and agitated produce foams which requires anti foaming,
- ➢Only primary settled wastewater can be treated .

OXIDATION POND (STABILIZATION POND OR LAGOON):

1) oxidation ponds, also called stabilization ponds or lagoons are low cost suspended growth type biological wastewater treatment systems. These are shallow earthen basins which hold wastewater for long duration to allow for the natural degradation of organic matter. The system takes advantage of natural aeration and microorganisms in the wastewater to renovate sewage. Many small communities and many industries use oxidation ponds, for wastewater treatment. oxidation pond requires a large land area and thus is usually located in rural areas. 2) there are 3 types of stabilization ponds- a) aerobic stabilization ponds (oxidation pond) b) facultative stabilization pond and c) anaerobic stabilization pond.
Facultative stabilization ponds or lagoons that function in mixed conditions in which stabilization of wastewater is brought about by a combination of aerobic, anaerobic and facultative microorganisms, are the most common. 3) Oxidation ponds are shallow earthen basins having 2 to 5 ft depth with a surface area of several acres. These are used to treat raw or partially treated industrial wastewater or a mixture of industrial and domestic wastewater. 4) There are 3 zones in an oxidation pond – a) The photic zone favourable for algal growth b) Aerobic heterotrophic zone of aerobic microorganisms c) The anaerobic zone 5) Aerobic

treatment of waste water in oxidation ponds is carried out by the combined activities of algae and aerobic bacteria

Oxygenation of wastewater accomplished is mainly through wind action and also to the photosynthetic activity of algae. 6) The organic materials present in wastewater are decomposed by aerobic heterotrophic bacteria into stable

and simple end products such as CO2, H2O, are mineral matter.

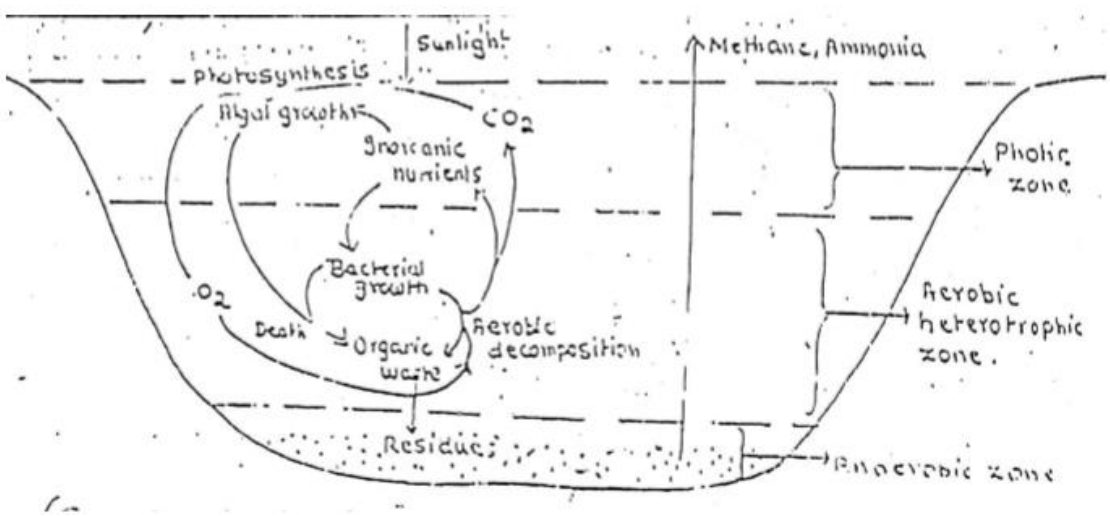
Simple and stable end products (mineral matters) produced by the activity of aerobic microorganisms (mostly bacteria) support the growth of algae, such as chlorella (C.pyvenoidosa and C.ellipsoides), Scenedesmus (S.quadiricauda and S.acutus), spirulina platencis, etc, climatic conditions such as sunlight and temperature also favour the growth of algae.

7) Thus, there is a simultaneous increase in algal and bacterial biomass in wastewater stored in oxidation ponds. CO2 evolved by aerobic decomposition of organic matter is utilised by algae for photosynthesis in the presence of sunlight. The oxygen released during photosynthesis keeps the wastewater in aerobic condition.

8) it has been observed that the aerobic treatment of wastewater is efficient provided, plenty of sunlight is available, when the temperature is high and the depth of the oxidation pond rise between 3-4 feet. The rain fall should be low.

9) dead algal and bacterial cells are added to the decomposable organic matter in wastewater. The unused organic matters sediment to the bottom of the pond as residues these are subjected to anaerobic decomposition and as a result methane, ammonia and other substances are produced. After settling most of the algal and bacterial biomass, the effluent containing oxidised products is periodically removed.

The effluent must be subjected to final treatment before its disposal.



By aerobic treatment of wastewater in oxidation ponds a reduction in BOD of 75 to 85 % can be attained .

ADVANTAGES : Oxidation ponds are less expensive and simple biological treatment devices. o Skilled supervision is not necessary. o Coliforms are very much reduced in the treated water.

DISADVANTAGES: The temperature cannot be controlled o Although BOD is reduced by the aerobic decomposition of organic matter, it is probably small because the waste is transformed into other forms of organic material – the algal and bacterial biomass

Tertiary Treatment (Reverse Osmosis, Ion Exchange method and Electro-dialysis)

Tertiary treatment is the final treatment meant for abolishing the secondary effluents and removal of fine suspended solids, traces of organics and bacteria. The sewage effluent from secondary treatment plant is introduced into a flocculation tank where lime is added to eliminate calcium phosphate.

The solution then enters the NH3 stripping tower. Nitrogen present in waste water exists as NH+4 which is converted to gaseous ammonium ion at high pH(ll). Phosphorus is removed by adding ferric chloride or aluminium sulphate. The remaining organic materials are removed by desalination, ion exchange and finally chlorination is used for disinfection.

The toxic, non-biodegradable chemicals in industrial waste water can be removed by adsorption (on activated charcoal), ion exchange, ultra-filtration, reverse osmosis and electrodialysis.

Reverse Osmosis

Reverse osmosis which is also commonly referred to as RO is a type of filtration method used for the removal of molecules and ions from a certain solution.

Reverse osmosis involves the application of pressure (usually greater than the osmotic pressure) on one side of the solution where a semipermeable membrane is placed in between the solutions. This membrane is used to filter out contaminants down to the smallest particles. The contaminants are often referred to as RO concentrate.
Reverse osmosis is a membrane treatment process primarily used to separate dissolved solutes from water. Reverse osmosis is most commonly known for its use in water purification particularly with regard to removing salt and other effluent materials from water molecules.

Reverse osmosis is one of the oldest and most popular separation techniques used mainly for the purification of water. The process was mainly adopted for desalination of seawater in the year 1950, where the whole process was relatively slow and limited to certain laboratories. However, after a lot of research and advancements in technology, there were significant developments especially in the field of polymers and the production of efficient membranes.

Today, this technique is extensively used by many around the world to purify water for industrial, residential, commercial and scientific purposes.

RO membranes

Common membrane materials include polyamide thin film composites (TFC), cellulose acetate (CA) and cellulose triacetate (CTA) with the membrane material being spiral wound around a tube, or hollow fibres bundled together.

Hollow fibre membranes have a greater surface area and hence capacity but are more easily blocked than spiral wound membranes.

RO membranes are rated for their ability to reject compounds from contaminated water. A rejection rate (% rejection) is calculated for each specific ion or contaminant as well as for reduction of total dissolved solids (TDS).

TFC membranes have superior strength and durability as well as higher rejection rates than CA/CTA membranes. They also are more resistant to microbial attack, high pH and high TDS. CA/CTA's have a better ability to tolerate chlorine.

Sulphonated polysulphone membranes (SPS) are chlorine tolerant and can withstand higher pH's and are best used where the feed water is soft and high pH or where high nitrates are of concern.

Reverse Osmosis Principle

To break down the process further, due to the presence of membrane, large molecules of the solute are not able to cross through it and they remain on the pressurized side. The pure solvent, on the other hand, is allowed to pass through the membrane. When this happens the molecules of the solute start becoming concentrated on one side while the other side of the membrane becomes dilute. Furthermore, the levels of solutions also change to some degree.

In essence, reverse osmosis takes place when the solvent passes through the membrane against the concentration gradient. It basically moves from a lower to a higher concentration.

Reverse Osmosis Process

Osmotic pressure is the minimum pressure required to stop solvent flow through the semipermeable membrane. Therefore, when the solution side (the side where the solute concentration is high) is subjected to a pressure greater than the osmotic pressure, the solvent particles on the solution side move through the semipermeable membrane to the region where the solute concentration is low. Such inverse solvent movement through the semipermeable membrane is called reverse osmosis.

It is important to note that the pressure applied to the solution side must be higher than the osmotic pressure for the reverse osmosis process to proceed. Osmotic pressure is a colligative property, which depends on the concentration of the solution. In water purification, the reverse osmosis process is very important. Many water purifiers used today use reverse osmosis in the purification process as one of the steps.

Benefits of Reverse Osmosis

1. This process can be used to effectively remove many types of dissolved and suspended chemical particles as well as biological entities (like bacteria) from the water
2. This technique has a wide application in treating liquid wastes or discharge
3. It is used in purifying water to prevent diseases
4. It helps in the desalinating seawater
5. It is beneficial in the medical field.

Advantages of Reverse Osmosis

- Reverse Osmosis has several advantages including the following:
- Bacteria, viruses and pyrogen materials are rejected by the intact membrane. In this respect RO water approaches distilled water in quality.
- Available units are relatively compact and require little space. They are well suited to home dialysis.
- In average use, the membrane has a life of a little more than one to two years before replacement is necessary.
- Periodic complete sterilization of the RO system with formalin or other sterilant is practical.

Disadvantages of Reverse Osmosis

- The disadvantages of RO systems include the following;
- Cellulose acetate membranes have limited pH tolerance. They degrade at temperatures greater than 35^{o}C. They are vulnerable to bacteria. They eventually hydrolyze.
- Polyamide membranes are intolerant of temperature greater than 35^{o}C. They have poor tolerance for free chlorine.
- Thin-film composites are intolerant of chlorine. High flux polysulfones require softening or deionization of feed water to function properly.

Ion Exchange method

As the name indicates, ion-exchange involves the displacement of one ion by another. The exchange occurs between the ions of insoluble exchange material (ion-exchange materials) and the ions of different species in solution (i.e. waste water for advanced treatment).
The ion-exchange process is carried out by employing two types of ion-exchange materials—cation exchangers and anion exchangers (Fig. 57.14). The synthetic resins with strong acidic (H^+) and basic (OH^-) functional groups serve as ion exchangers. The cation exchangers (with H^+ or Na^+) can replace the positively charged ions (Ca^{2+}, Mg^{2+}) in water by hydrogen ions. This is what is done for removing the hardness of water.

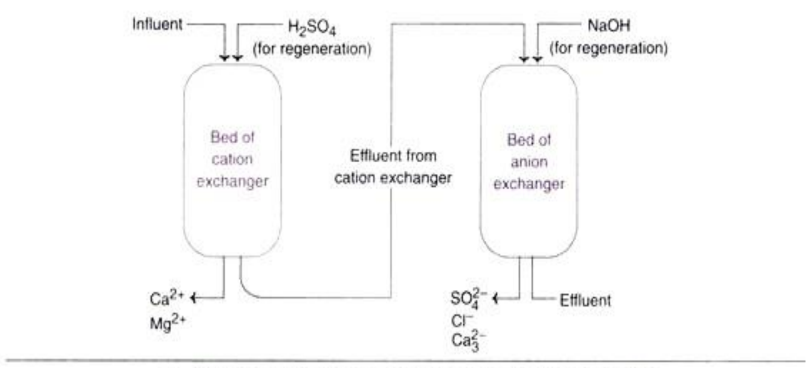


Fig. 57.14 : Ion-exchange process for the removal of dissolved solids.

The anion exchangers (with OH^-) can remove negatively charged ions (SO_4^{2-}, NO_3, and CO_3^{2-}). The waste water is first passed through a cation exchanger and then through an anion exchanger packed in two separate columns. When the ion exchange capacity of the resin is exhausted, it has to be regenerated for further use. For cation- exchange resins, regeneration can be done with strong acids (H_2SO_4, HCI), while for anion exchange resins, alkali (NaOH) is used. For an effective removal of dissolved solids by ion exchange, the waste water should not contain high concentration of suspended solids as they block the ion exchange beds.

Electro-dialysis

Electro Dialysis (ED) is a membrane process, during which ions are transported through semi permeable membrane, under the influence of an electric potential.

The membranes are cation- or anion-selective, which basically means that either positive ions or negative ions will flow through. Cation-selective membranes are polyelectrolytes with negatively charged matter, which rejects negatively charged ions and allows positively charged ions to flow through.

By placing multiple membranes in a row, which alternately allow positively or negatively charged ions to flow through, the ions can be removed from wastewater.

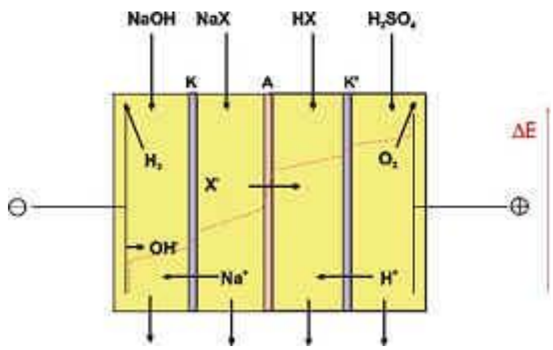
In some columns concentration of ions will take place and in other columns ions will be removed. The concentrated saltwater flow is circulated until it has reached a value that enables precipitation. At this point the flow is discharged.

This technique can be applied to remove ions from water. Particles that do not carry an electrical charge are not removed.

Cation-selective membranes consist of sulphonated polystyrene, while anion-selective membranes consist of polystyrene with quaternary ammonia.

Sometimes pre-treatment is necessary before the electro dialysis can take place. Suspended solids with a diameter that exceeds 10 µm need to be removed, or else they will plug the membrane pores. There are also substances that are able to neutralize a membrane, such as large organic anions, colloids, iron oxides and manganese oxide. These disturb the selective effect of the membrane.

Pre-treatment methods, which aid the prevention of these effects are active carbon filtration (for organic matter), flocculation (for colloids) and filtration techniques.



An electrodialysis system

Microbes in extreme environments: Microbes thriving at High and Low temperatures, pH, High hydrostatic and Osmotic pressure, Salinity and Low nutrient level

The extremophiles are the organisms which grow under extreme environmental conditions like temperature, salinity, pH, anaerobic conditions (sensitive to oxygen), extreme atmospheric pressure, water stress and others under which other organisms will generally not grow or the conditions that may kill other organisms.
The term ‘extremophile’ has generally not been used in the old literature as it came up with the discovery of a unique group of prokaryotes from extreme environments. It is from extreme environments. It is from Latin (extremus = extreme, and Greek phila = love or friend of)
The extremophiles include extreme psychrophilic (cold loving) extreme thermophiles, (heat loving) alkalophiles (bacteria that live at very high pH or alkaline conditions), acidophiles (microorganisms) which show preference for growth at low pH, approximately 2.0), methanogens (methane producing microorganisms) osmophiles (the organisms which grow optimally in or on media of high osmotic pressure), barophiles (atmospheric pressure lovers) and sulphur metabolizers. Archaeoglobus profundus a sulphur reducer bears optimum growth temperature of 82°C (Fig. 6.1)

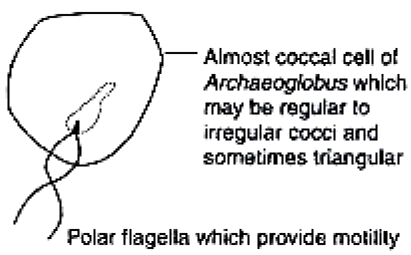


Fig. 6.1. Electron micrograph of *Archaeoglobus* the only genus of sulphate reducing archaea.

The first extremophile to have its genome sequence was Methanococcus jannaschii, an archaeobacterium which lives near sea level where temperature reaches boiling point of waters and pressure enough to crush an ordinary submarine. These atmospheric pressure lovers are called barophiles. It exhibits high growth rate and high enzymatic activities at elevated temperatures.

High temperature: Thermophiles and Hyperthermophiles

Thermophiles

Microorganisms that can grow at temperatures of 55°C or higher (the minimum being usually around 45°C, the optimum being between 55-65°C, maximum being 80°C).

They fluorish in habitats including composts, self-heating hay stacks, hot water lines, and hot springs. *Thermoplasma acidophilum* (minimum 45°C, optimum 59° C. maximum 62°C), *Bacillus stearothermophilus* (minimum 30°C, optimum 60-65°C. maximum 75°C), *Methanosarcina thermophila*, *Methanobacterium wolfei*, *Methanobacterium* *thermoautotrophicum, Archaeglobus profundus, Alicyclobacillus acidoterrestris, A. acidocaidarius,* etc. are the examples of thermophiles.

(i) Physiology:

Thermophile Mode of adaptation

a. Proteins

- The enzymes and other proteins in these organisms are much more stable to heat than those in mesophiles.
- It is seen that a critical amino acid substitution on one or a few locations in the enzymes allows them to fold in such a way that it is consistent with heat stability.
- Besides, certain solutes such as diinositol phosphate and monosylglycerate are produced in significant quantities that help to stabilize the proteins against thermal degradation.

b. Cell membrane

- In addition to enzymes and other components of the cell, cytoplasmic membranes of thermophiles need to be heat stable.
- Thermophiles typically have lipid-rich in saturated fatty acids, thus allowing the membrane to remain stable and functional at high temperatures.
- Saturated fatty acids form a stronger hydrophobic environment than unsaturated fatty acids, which also increases membrane stability.

c. Nucleic acid

- Thermophiles contain special DNA binding proteins that arrange the DNA into globular particles that are more resistant to melting.
- Also, another factor that is common in all thermophiles is the presence of a unique DNA gyrase enzyme that acts to introduce positive supercoils in DNA, providing considerable heat stability.

Hyperthermophiles

Microorganisms that have growth optima between 80°C and about 113°C are called hyperthermophiles. They usually do not grow well below 55°C. *Pyrococcus abyssi* and *Pyrodictium occultum* exemplify marine hyperthermophiles isolated from hot areas of the seafloor. *Thermoproteus uzoniensis, Staphylothermus marinus, Pyrococcus furiosus, Hypothermus butylicus, Pryococcus woesei, Pyrodictium brockii,* , etc.

Hyperthermophile Mode of adaptation

a. Proteins

- Hyperthermophilic archaea used structure-based physical mechanisms to increase the thermostability of its proteins in the process of its thermophilic adaptation.
- The stability of proteins and enzymes in hyperthermophiles is improved as a result of an increased number of salt bridges (cations that bridge charges between amino acid residue) in the structure.
- Besides, an increase in the number of ionic bonds between the positive and negative charges of various amino acids also stabilizes proteins.
- The increased ionic bonds create a densely packed highly hydrophobic interior of the proteins, which resists the unfolding of proteins under high temperatures.
- Some hyperthermostable proteins even employ an alternative, sequence-based mechanism of their thermal stabilization.

b. Membranes

- Most hyperthermophilic archaea do not possess any fatty acids in their membranes; instead, they have lipids with branches hydrocarbon chains.
- These chains are composed of repeating units of a five-carbon compound, isoprene, bound to each other by ether linkages.
- The ether linkages are more stable linkages, which in turn, stabilizes the membranes against thermal breakage, and the branching reduces the membrane fluidity.
- The overall structure of membranes forms a lipid monolayer which is much more heat resistant than the lipid bilayer of mesophilic organisms.

(ii) Molecular Adaptation:

These bacteria contain heat-stable enzymes and proteins which regulate various macromolecular functions at high temperature. The critical amino acids substituted in one or more locations in these enzymes allow them to fold in a different manner and thereby withstand the denaturing effect of heat resulting into the survival of these organisms.

Further, the cytoplasmic membrane contains lipids rich in saturated fatty acids, thus allow the membrane to remain stable and functional at high temperature. The thermophilic archaea do not contain fatty acids in their lipids, neither its membrane has ester linkages with glycerol phosphate. This imparts more rigidity to its membrane systems.

Applications

Most of the microorganisms that thrive above the boiling point of water belong to archaea. The enzymes of thermophiles are of great interest. Hyperthermophiles have focused on thermostable enzymes from vent. The proteins (chaperons) were also discovered.

Enzymes

1. New enzymes from hyperthermophiles have reduced the number of steps needed to transform starch into fructose syrup. The amylase, glucoamylase, pullunases and glucosidases are the enzymes used in starch industry.
2. Taq polymerase is very important enzyme used in molecular biology for the amplification of DNA using polymerase chain reaction (PCR). This enzyme found in Thermus aquaticus is active at 80°C at pH 8.
3. The DNA ligase has been characterized from *Thermus thermophilus*.
4.Topoisomerases type I purified from *Sulfolobus acidocaldarius, Desulfurococcus amylolyticus, Thermoplasma acidophilum, Fervidobacterium islandicum, Thermotoga maritima*, and **Methanopyrus kandleri,**
5. Topoisomerase II has so far been isolated from *Sulfolobus acidocaldarius*.
6. *Thermotoga maritima* contains of both gyrase and reverse gyrase enzymes.
7. Repair of extensive DNA damage caused by ionizing-radiation at 95°C has been demonstrated in *Pyrococcus furiosus*.

Chaperons

The chaperons are the proteins which express under stress conditions such as elevated temperatures. They are involved in protein folding. These are detected in *S. shibate* and *S. Solfactaricus*.

Low temperature: Psychrophiles

Microorganisms that grow well at 0°C possess an optimum growth temperature of 15°C or lower and the maximum temp, for growth is around 20°C. They are usually found growing in Arctic and Antarctic habitats because 90% of the ocean is 5°C or colder.

Psychrophiles are widespread among bacterial genera such as *Pseudomonas, Vibrio, Alcaligenes, Bacillus, Photobacterium, Arthrobacter*. *Chlamydomonas nivalis* is a psychrophilic alga that produces bright red spores which turn the show field pink in appearance.

Physiology

Psychrophiles produce enzymes that function optimally in the cold. Its cell membrane contains high content of unsaturated fatty acid which maintains a semi-fluid state at low temperature. The lipids of some psychrophilic bacteria also contain polyunsaturated fatty acids and long chain hydrocarbons with multiple double bonds.

Molecular Adaptation:

The active transport in such organisms occurs at low temperature. It indicates that the cytoplasmic membranes of psychrophiles are constructed in such a way that low temperature does not inhibit membrane function. The membrane contains polyunsaturated fatty acids in their lipids which maintain the rigidity at low temperature and organisms thus are able to survive.

Applications

(a) Source of pharmaceuticals

An antitumor polysaccharide has been isolated as narinactin from marine actinomycetes. A mixture of protease and amylase isolated from Bacillus subtilis removes the dental plaque.

(b) Bacterial ice nucleating agents

There are several uses for ice-nucleating agents (INA) produced by bacteria. They are being used in artificial snow-making, in the production of ice creams and other frozen foods.

These are also used in immunodiagnostic kits as a conjugate to antibodies and as a substitute for silver iodide in cloud seeding.

(c) Fermentation industry

Mesophilic yeasts containing unsaturated fatty acids in membranes (lipids) have been found to be resistant between -80 and -20°C. These are preferred for its storage in baking and other processing industries. Fermentation at 6-8°C reduces the inhibitory effect of ethanol on cell membrane of the yeast cells.

(d) In microbial leaching

Currently microbial leaching operations involve oxidative solubilization of copper and uranium ores. Leaching operation in temperate countries is carried out at very low ambient temperature. Microbial leaching operation from sulfide ores is carried out at 4-37°C.

(e) In bioremediation

Psychrophiles have ability to degrade various compounds in their natural habitat. They are used in bioremediation of several pollutants at low temperature. The bacterial strains were found to mineralize dodecane, hexadecane, naphthalene, toluene, etc.

It has been demonstrated in laboratory and field experiments using specific bacterial strains. A psychrophilic bacterium, Rhodococcus sp. strain Q15 has been studied for its ability to degrade n-alkanes and diesel fuel at low temperature.

(f) Denitrification of drinking water sources

The presence of high nitrate concentration in water has become a major problem in many countries. The most widely used practices for removal of NO3 is the biological denitrification. Most of the denitrification processes are carried out at 10°C.

(g) Anaerobic digestion of organic wastes

Methanogenium frigidum isolated from Ace lake (Antarctica) grows optimally at 15°C. This bacterium is found to produce methane from hydrogen and carbon dioxide.

pH

1. Acidophiles

Microorganisms that have their growth optimum between about pH 0 and 5.5. Several species of Thiobacillus and archaebacterial genera including Sulfolobus and Thermoplasma are acidophilic. Many fungi also grow optimally at pH 5 or below and a few grow well at pH values as low as 2.

(i) Physiology:

Obligate acidophiles have an optimum pH for growth which remains extremely low (1 to 4). To shield the intracellular enzymes and other components from low to medium pH, the organ-isms maintain a large pH gradient across the mem-brane. Special forms of lip-ids are present in their mem-brane which may minimize the leakage of H+ down the pH value.

For instance, the presence of certain fatty ac-ids has been reported to pro-vide special adaptations to growth and survival at extremely low pH. Acidophiles maintain the cytoplasmic pH around 6.5. In these organisms, the pH remains generally 1-2 which is lower in comparison to neutrophiles and alkalophiles.

In acidophiles the pH is compensated by positive inside electric potential which is opposite to that present in neutrophiles. The reversed electric potential is generated by electrogenic K+ uptake which allows the cells to extrude more H+ and thus maintain the internal pH.

(ii) Molecular Adaptation:
Most critical factor for obligate acidophily lies in the cytoplasmic membrane. When the pH is raised to neutrality, the cytoplasmic membrane of obligately acidophilic bacteria actually dissolve and the cells lyse. It is suggested that the high concentration of hydrogen ions are required for stability of membrane that allows bacteria to survive.

(iii) Applications:
Potential applications of acid-tolerant extremozymes range from catalysts for the synthesis of compounds in acidic solutions to additives for animal feed which are intended to work in animal stomach.

When added to feed, the enzymes improve the digestibility of expensive grains, therefore avoiding the need for more costly food. Rusticyanin proteins from acidophiles help in acid stability. Expression of heterogenous arsenic resistance genes in the iron-oxidizing Thiobacillus ferrooxidans has been established as biotechnological approach of bioremediation.

2. Alkalophiles:
Microorganisms that prefer the pH range of 8.5 to 11.5 for their growth and survival are called alkalophiles. Alkalophiles live in soils laden with carbonate and in Soda lakes, and most of them are aerobic or facultative anaerobic. Bacillus alkalophilus, B. firmus RAB. B. sp. No. 81 and B. sp. No. C-125 are some alkalophiles.

(i) Physiology:
The cell surface of alkalophiles can maintain the neutral intracellular pH in alkaline environment of pH 10-13. The recommended concentration of NaOH for large scale fermentation is 5.2% depending upon organism. The pH should remain 8.5-11. Sodium ions (Na+) are required for growth, sporulation and also for germination. The presence of sodium ions in the surrounding environment has proved to be essential for effective solute transport through the membranes.

In the Na+ ion membrane transport system, the H+ is exchanged with Na+ by Na+/H+ antiport system, thus generating a sodium motive force (smf). This drives substrate accompanied by Na+ ions into the cell.

The incorporation of α-aminobutyrate (AIB) increased two fold as the external pH shifts from 7 to 9, and the presence of Na+ ions significantly enhance the incorporation. Molecular cloning of DNA fragments conferring alkalophily was isolated and cloned. This fragment is responsible for Na+/H+ antiport system in the alkalophily of alkalophilic microorganisms.

(ii) Molecular Adaptation:
Alkalophiles contain unusual dither lipids bonded with glycerol phosphate just like other archaea. In these lipids, long chain, branched hydrocarbons, either of the phytanly or biphytanyl type, are present.

The intracellular pH remains neutral in order to prevent alkali-labile macromolecules in the cell. The intracellular pH may vary by 1-1.5 pH units from neutrality which helps these organisms to survive in highly alkaline external environment.

(iii) Applications:
Some alkalophiles produce hydrolytic enzymes such as alkaline proteases, which function well at alkaline pH. These are used as supplements for house hold detergents.

For example an alkaline protease called subtilisin has been produced from B. subtilis which is used in detergent. The stone washed denim fabric is due to the use of these enzymes. These enzymes soften and fade fabric by degrading cellulose and releasing dyes.

Some extremozymes and their applications.

Extremozyme	*Uses*
Thermozymes	Required for DNA amplification reactions and industrially important product formation
Halozymes	Proteases, alkaline phosphatases, lipases and amylases are used in industry for manufacturing of detergents
Acidozymes	Sulphate oxygenase, *Thiobacillus* dehydrogenase, rusticyanin (acid stable e^- carrier) and thromopsin
Psychozyme	Pectinase, lipase, cellulase, amylase for detergents, Food processing (cheese making, meat tendering, lactate hydrolysis), Biosensors (environmental applications), biotransformations and contact lens cleaning solutions
Alkalozymes	Protease (detergents), amylase (starch industry), cyclomaltodextrin glucanotransferase (chemical and pharmaceutical), pullunases (detergents), xylanses (pulp and paper industry), pectinases (paper production)

High hydrostatic pressure

Barophiles/ piezophile (from Greek "piezo-" for pressure and "-phile" for loving)

Barophiles are those bacteria that grow at high pressure at 400-500 atmosphere (atm) on 2 to 3°C. Such conditions exist in deep-sea habitat about 100 metre in depth. Many are barotolerant and do not grow at pressures above 500 atm. but some live in the gut of invertebrates (amphipods and holothurians).
Photobacterium shewanella and Colwella inhabit more rapidly. Some thermophilic archaea are barophiles e.g. Purococcus spp. and Methanococcus jannaschii. Barophiles adapt the extreme pressure (200-600 bars) involving macromolecular structures in cells. Increasing pressure makes structures more compacts, and this tendency has been the principle of microscopic ordering.

(i) Physiology
There are variations in membrane structure and function. The amount of mono- unsaturated fatty acids in the membrane increases due to increase in the pressure. The organism is thereby able to circumvent the loss of membrane fluidity imposed by increasing the pressure. As the pressure decreases, membrane fluidity presumably increases and the cells respond by decreasing the level of mono-unsaturated fatty acids.
It is evidenced that increased pressure decreases the binding capacity of enzymes for their substrates. Thus the enzymes must be folded in such a way as to minimize these pressures in

barophiles. It is not known whether H^+, Na^+ or both are used as coupling ions in energy transduction in these organisms.

(ii) Molecular Adaptation

In the cytoplasmic membranes of high pressure tolerant microbes, the amount of unsaturated fatty acids is more which allows the adaptative significance. Further, the adaptativity may also be due to changes in protein composition of the cell wall outer membrane called OmpH protein, a type of porin.

The porins are structural proteins meant for diffusion of organic molecules through the outer membrane and in to the periplasm. It is observed that OompH system is pressure-dependent and required for growth at high pressure.

(iii) Applications

Barophiles are the major source of unsaturated fatty acids or polyunsaturated fatty acid. The microbial barophilism is helpful in enhancing the mining. Underground mining operations usually occur at increased pressures and temperatures and barophilic thermophiles are better adapted under such situations.

Recently, vacant salt mine area has been worked out as fermenters for the biological gasification of pretreated lignite or agricultural crops based on the involvement of extremophiles endowed with adaptation to high pressure and temperature besides salinity.

Osmotic pressure

The microorganisms which can tolerate or prefer high concentrations of organic solutes as sugars are called osmotolerant or osmophiles. Some of the noted habitats of osmophilic microorganisms are honey, sap flows, nectar of flowers, molasses and sugary syrups. Some yeasts like Debaromyces hansenii and Zygosaccharomyces rouxii are good examples of osmophiles. The moulds Aspergillus and Penicillium also are osmotolerant.

Mode of adaptation

a. Osmoprotectatnts

Osmophiles produce different osmoprotectants like alcohols and amino acids that prevent the change in osmotic pressure inside the cell.

These solutes increase the osmotic pressure inside the cell so as to balance the turgor pressure on the cell from the outside environments.

b. Enzymes

Proteins and enzymes in osmophiles have more protein charges and hydrophobicity that protects them against the change in the solute composition in the cytoplasm.

The unfavorable interactions that disrupt internal microbial proteins caused by dehydration may be averted by modulating their net charge.

Salinity

Halophiles

- Halophiles are a group of extremophiles that require high salt concentrations for their survival and growth.
- Halophiles are of two types; obligate halophiles that require NaCl concentration of 3% or more and halotolerant that survive at both average salt concentrations and higher.
- Halophilic microorganisms constitute the natural microbial communities of hypersaline ecosystems, which are widely distributed around the world.
- The general features of halophilic microorganisms are low nutritional requirements and resistance to high concentrations of salt with the capacity to balance the osmotic pressure of the environment.
- The salt requirement in halophiles is classified into three groups; low (1-3%), moderate (3-15%), and extreme (15-30%).
- Salt requirement depends on factors like temperature, pH, and growth medium.
- They are physiologically diverse; mostly aerobic and as well anaerobic, heterotrophic, phototrophic, and chemoautotrophic.
- Ecologically, the halophilic microorganisms inhabit different ecosystems characterized by a salinity higher than seawater that range from hypersaline soils, springs, salt lakes, sabkhas to marine sediments.
- These organisms are found in all three domains of life, i.e., Archaea, Bacteria, and Eukaryota.
- Halophilic bacteria are more abundant in specific phylogenetic subgroups, most of which belong to Halomonadaceae, a family of Proteobacteria.

Prokaryotic genera of extremely halophilic species.

Halobacteria	*Methanogens*	*Bacteria*
Halobacterium salinarium	*Methanobacterium* sp.	*Acetohalobium* sp.
Halobacterium halobium		
Haloferax mediterranei		*Actinopolyspora* sp.
Haloarcula sp.		*Ectothiorhodospira* sp.
Halococcus acetoinfaciens		
Halococcus agglomeratus		
Natronobacterium gregoryi		
Natronococcus sp.		

(i) Physiology:

Halophilic bacteria lack peptidoglycan in cell walls and contain ether-linked lipids and archaean type RNA polymerases but Natrobacterium is extremely alkalophilic as well. Former also contains diether lipids not present in other extreme halophiles.
They are chemoorganotrophic bacteria that require amino acids, organic acids and vitamins for optimum growth. Sometimes they oxidize carbohydrates as energy source. Cytochromes a, b and c are present but membrane mediated chemiosmosis generates proton motive force. They also require sodium for Na^+ ions.
Halobacterium exceptionally thrives in osmotically stressful environment and does not produce compatible solutes. Peptidoglycan is absent in their cell wall. Aspartate and glutamate (acidic amino acids) are present.
The negative charges of the carboxyl groups of these amino acids are shielded by Na^+ ions. The ribosomes of Halobacterium requires high K^+ ions for stability, which is a unique feature as no other group of prokaryotes requires it for internal components.

The membrane lipids of these archaea are composed of diphytanylglycerol, diether analogues of glycerophospholipids. The extreme halophiles contain high intracellular concentration of Na^+ and K^+ and their proteins seem to have adapted to this high salt concentration by having a higher fraction of acidic amino acid residues and a more compact packing of a polypeptide chain than protein from non-halophilic bacteria. In the halophilic bacteria generally a Na^+/H^+ antiporter is used to pump Na^+ outwards and solute uptake has been shown to be Na^+ coupled in several halobacterial species.

(ii) Molecular Adaptation:
In such bacteria K^+ ions inside the cell is more than Na^+ ion outside the cell which act as its solute. Hence, the cells maintain cellular integrity. Halobacteria lack peptidoglycans in their cell walls and contain ether-linked lipids and archaean type RNA polymerases which maintain the rigidity at salty conditions. These changes in cytoplasmic membrane allow such bacteria to survive.

(iii) Applications:
(a) Bacteriorhodopsin:
The retinal proteins of halobacteria have been observed as integral proteins of the purple membrane, containing one of the proteins called bacteriorhodopsin. This protein is light-driven, proton translocator and converts sunlight to electricity.

(b) Bioplastic or polyhydroxy alkanoates (PHA):
This kind of heteropolymer is biodegradable. It exhibits total resistance to water and degraded in human tissues; hence it is biocompatible. It has pharmaceutical and clinical importance, including the use in delayed drug release, bone replacement and surgical sutures.

(c) Polysaccharides:
Microbial exopolysacchrides are used as stabilizers, thickness, gelling agents and emulsifiers in the pharmaceutical industries, paint and oil recovery, paper, textile and food industry.

(d) Microbially enhanced oil recovery:

Residual oil in natural oil fields can be extracted by injection of pressurized water down in a new well. The bacterial biopolymers are of interest in enhanced oil recovery because of their bio-surface activity and properties of bio-emulsifiers.

(e) Cancer detection:

A protein (84 kDa) has been used from Halobacterium halobium as an antigen to detect antibodies against the human e-myc oncogene product in the sera of cancer patient suffering from pyrolytic leukaemia cell line (HL-60). The use of halobacterial antigens as probe for some types of cancer seems to be promising.

(f) Drug screening:

Plasmid, pGRB-1 of Halobacterium strain GRB-1 used in the pre- screening of new antibiotics and anti-tumor drugs affect eukaryotic type IIDNA topoisomerase and quinotone drugs which act on DNA gyrase. Such drug causes DNA cleavage of small plasmid from halophilic archaea in vivo.

(g) Liposomes:

Ether-linked lipid of the halobacteria is used in liposome preparation having great value in the cosmetic industry. Such liposomes would be more resistant to biodegradation, good shelf-life and resistance to other bacteria.

(h) Enzymes:

Proteases and amylases from Halobacterium salinarium, H. halobium, and lipases from several halobacteria have been reported. A site-specific endonuclease activity has been reported in H. halobium.

(i) Bioremediation:

Bertrand (1990) observed that the halobacterial strain EH4 isolated from a salt-mark was found to degrade alkanes and other aromatic compounds in the presence of salt.

(j) Gas vacuoles or vesicles:

Some Halobacterium spp. produce intracellular gas filled organelles called vacuoles of gas vesicles which provide buoyancy. In the future, the genes of such properties are possible to engineer in other microorganisms to produce gas vacuoles to float in water.

(k) In food:

A sauce called 'nam pla' is prepared in Thai from fish fermented in concentrated brine that contains a large population of halobacteria responsible for aroma production. Because they produce salt-stable extracellular proteases. It has importance in the fermentation and the flavour and aroma producing processes.

(l) Other products:

Moderate halophiles remove phosphate from saline environment. Isolation of stable antimicrobial-resistant mutants is due to the presence of cloning of the genes for over-production of interesting industrially important compounds.

Large scale cultivation of Spirulina platensis in Israel uses brackish water which is unsuitable for agriculture and the Spirulina biomass is marketed as a healthy food. Spirulina grows optimally in alkaline lakes with a salt concentration ranging from 2 to 7%.

Low nutrient level

- Xerophile Definition and Characteristics
- Xerophiles are a group of extremophiles that are capable of surviving in environments with low availability of water or low water activity.
- Generally, xerophilic organisms are capable of growing at a_w values lower than xerotolerant organisms (a_w below 0.8).
- Two major types of the environment provide habitats for the most xerophilic organisms, namely foods preserved by some form of dehydration or organic solute-promoted lowering of a_w and saline lakes, where low a_w values are a consequence of inorganic ions.
- In environments where little water is available, organisms must take up and maintain sufficient water against extreme concentration gradients to support cellular processes.
- Xerophiles are of different types belonging to different groups of living beings. Xerophilic fungi represent a large group of xerophilic organisms.
- Eukaryotic organisms like plants capable of surviving at low water condition, called xerophytes are also xerophiles.
- Xerophiles are closely related to halophiles as halophilic environments tend to have low water activity.
- Even though water is crucial for many biomolecular processes in living beings, xerophiles have intricate means to survive in conditions with low water activity.

Xerophile Mode of adaptation
a. Dormancy
One of the most common responses of prokaryotes to low water conditions is a reversible form of dormancy.
These organisms under a temporary period of dormancy in the form of spores so that they reduce metabolic activity and resume normal metabolism when appropriate conditions are available.
The formation of spores and reduction in metabolic activities provide long periods of survival for many microorganisms as well as larger eukaryotes.

b. Extracellular polysaccharides and biofilm formation
Various xerophilic organisms form biofilms as it allows the survival of organisms in habitats with low moisture content.
These biofilms consist of microbial aggregates and extracellular polysaccharides produced by those organisms.
The extracellular polysaccharides in the biofilms are hydrophilic, which contributes to rapid water absorption rates and restoration of photosynthetic activity.

Biofilm formation also reduces the need for large quantities of water as they occupy less space and have less metabolic activities.

c. Cell membrane

The cell membrane of xerophilic organisms tends to have an increased ratio of fatty acids which creates a tighter lipid packing that preserves the membrane during desiccation.

Increased cyclopropane fatty acid content in the membrane also reduces the membrane permeability to protons which thus, helps in balancing the intracellular pH.

Xerophilic microorganisms adapt to low water activity by increasing the concentration of negatively charged phospholipids that facilitates the preservation of membrane bilayer structural integrity.

d. Proteins

In the case of xerophytes, a range of proteins that counteract the effects of low water activity is produced.

These proteins are rich in glycine and have a highly hydrophobic backbone that transitions into the ordered structure under desiccating conditions, preventing denaturation.

Xerophilic cyanobacteria code for various shock response genes on dehydration that regulate the utilization of water in metabolic processes during desiccation.

Xerophile Examples

Some common examples of xerophiles are *Aspergillus penicillioides, Cereus jamacaru, Deinococcus radiodurans, Aphanothece halophytica, Anabaena, Bradyrhizobium japonicum, Saccharomyces bailli*, etc.

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