Microbiology of water and sewages

For Post graduate Civil Students

Course Code: CVE 614

By

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Introduction

Water is considered as a nutrient-poor environment, but it allows growth and reproduction of microbes. The term “Aquatic Microbiology” is a branch of science that deals with microorganisms that found in all types of water. According to the World Health Organization, there are about two and a half billion people do not have access to clean and potable water. More than five million people die every year from using unclean and contaminated water, from them, 50% are due to microbial diseases such as cholera; the most sever one.

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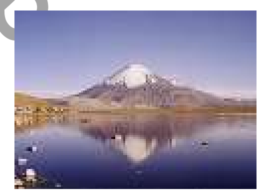
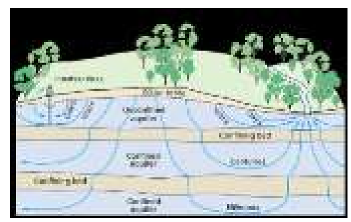
<u>Types and Sources of Water and Wastewater</u>

<u>Sources of Water</u>



1- **Surface water:** Surface water is water in a river, seas, oceans, or lake.



2- **Groundwater:** is fresh water located in the subsurface pore space of soil and rocks. It is also water that is flowing within aquifers below the water layers.

<u>World Demand for Water:</u>

How much water do humans use? The answer depends on where they live and on their socioeconomic status. Under primitive conditions a person will consume three to five gallons per day for drinking and subsistence farming. In a city where water is also used for cleaning, manufacturing, and sanitation, per capita use is around 150 gallons per day. In the United States, which has among the highest water consumption rates in the world, each person uses an average of 1,340 gallons of water per day. Table 1 shows how much water is required to produce common goods and services.

Table 1. Average water requirements.

Item	Gallons used
1 pound of cotton	2,000
1 pound of grain-fed beef	800
1 loaf of bread	150
1 car	100,000
1 kilowatt hour of electricity	25
1 pound of rubber	100

Natural characteristics of drinking water

- **Color**: The color must be acceptable, not exceeding 50 units on the platinum cobalt scale.
- **Taste**: Be acceptable and palatable.
- **Odor**: The healthy water for drinking does not smell any odor.
- **Turbidity**: It must be clear. The maximum amount of turbidity in the treated water is 5 units, and in the ground water 52 units.
- **Dissolved oxygen** at 25 °C: be 5-8 mg / L
- **Dissolved carbon dioxide** at 25 °C: 2-3 mg / l degree
- **Electrical conductivity** at 18 °C: 0.0004 µm / cm^2.
- **Thermal conductivity** at 40.8 °C: 1.555 W / m.
- **The refractive index**: at 20 °C 1.33 units.
- **Steam pressure** at 20 °C: 17.62 mm Hg.
- **Specific heat** at 1 °C: 1.00 kJ / kg. Degree.
- **Specific temperature** at 20 °C: 0.99 kJ / kg. Degree.
- **The density** at 20 °C: 0.99823 g / cm 3.
- **Freezing Point:** 0 °C.
- **Boiling point**: is 100 ° C.
- **The latent heat of evaporation** at 20 °C: 584.9 g. Calorie / gram
- **Surface tension** at 20 °C: 72.75 Dyne / cm.

What are types of wastewater?

 Industrial sources: Petrochemical, dairy, food, pharmaceutical, metallurgical, etc.

 Domestic sources: Form households and non-industrial businesses

 Domestic sewage: Sinks, toilets, and showers. Sewage is 99.9% water and 0.02-0.04% solids

Types of Water Pollutants:

Composition of wastewater:

Many different types of contaminants can pollute water and render it unusable. Untreated wastewater, domestic or industrial, may contain harmful pathogenic organisms or toxic substances which may cause serious health hazards. The strength of sewage is reflected by its nuisance potential of offensive odours and biodegradable organic matter. This strength is determined by the oxygen demand (biochemical oxygen demand or BOD) and is measured in milligrams per litre (mg/litre). The higher the BOD, the stronger the sewage is.

Wastewater must be safe trough proper treatment and disposal of the effluent. To be able to carry out such treatment effectively, the characteristic s of waste must be known. There are three categories of waste characteristics: namely physical, chemical and biological.

1- Physical Characteristics

Fresh sewage has a soapy grey color and with insignificant odor, however, when it gets old (septic or stale), the color changes to black, and it emits a very offensive odor. This is due to biological oxidation which results in depletion of the available dissolved oxygen. The temperature of sewage is slightly higher than that of ordinary water. This is due to bacterial action. Furthermore, increase in temperature enhances bacterial activity and causes an increase in oxygen utilization by micro-organisms.

Wastewater may include suspended and dissolved solids. Solids in wastewater must be identified in order to determine the methods of treatment of waste. Solids may

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be classified as settle able solids or suspended solids. Settle able solids can be settled and removed through sedimentation. However, the suspended solids may be coarse, fine or colloidal in nature and can be removed in preliminary and primary treatment. An Imhoff Cone Test is used to determine the amount of solids in wastewater. This test determines the amount of settable solids and necessity of using a sedimentation tank for treatment. It also monitors the efficiency of primary sedimentation by measuring settle able solids of influent and effluent. Approximately 35-40 % of domestic wastewater treatment may be achieved through sedimentation of settle able solids.

2- Chemicals Characteristics

Wastewater contains numerous chemicals. The composition and nature vary depending on whether the wastewater is domestic or industrial. These chemicals may be organic (proteins, carbohydrates, urea and grease) or inorganic (salts and metals). Fresh sewage is alkaline in nature and enhances bacterial action; however, stale (old) sewage is acidic and difficult to treat. These chemicals may include:

- ***Disinfectants***: water disinfection byproducts including chlorine, bromate, and chlorite.
- ***Inorganic chemicals:*** such as arsenic, cadmium, lead, and mercury.
- ***Organic chemicals***: such as benzene, dioxin, and vinyl chloride.

*** Many inorganic and most major organic pollutants are emitted from industrial facilities, mining, and agricultural activities such as fertilizer and pesticide application.

- ***Radio elements***: including uranium and radium. It occurs naturally in geologic deposits.

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3- Biological Characteristics

All organic matter contains large numbers of micro-organisms. Those found in wastewater include bacteria, algae, fungi and protozoa. Bacteria are the most active and are present in large numbers in sewage. For example, 4.5 litres of sewage may contain 20-25 billion bacteria. Coli forms, the organisms that inhabit the human intestinal tract, act as indicator organisms in water analysis. Presence of coli forms in a water sample indicates faecal contamination. (we will study the microbiology of wastewater in more details later)

Methods of treatment

Water can be treated through several methods according to the types of pollutants:

Sedimentation: Water passes into a large basin where a chemical substance is added to the aluminum so that the floating impurities become heavier than the water and are deposited in a bottom and are regularly pulled out.

Filtration: The water passes after the disposal of the sediment through a layer of sand ranging in thickness between 80 cm, 150 cm Water is extracted from the rest of the particles

Sterilization: 3 methods are performed

1- Chlorination 2- Ozone sterilization 3- Sterilization with ultraviolet radiation

Desalination: is an artificial process by which saline water (generally sea water) is converted to fresh water. The most common desalination processes are distillation and reverse osmosis. It is usually only economically practical for high-valued uses (such as household and industrial uses) in arid areas. However, there is growth in desalination for agricultural use.

Biological treatment: Bacteria act to convert organic substances into dissolved inorganic substances which can be deposited by other materials. (we will study the this type of treatment in more details later)

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Wastewater Microbiology

Microbiology: is the scientific study of microorganisms; their growth and characteristics.

Microorganisms: are the organisms that are too small to see with the naked eye, they include bacteria, algae, fungi, and viruses. They can vary in size from less than 20 nm to several microns.

1- **Bacteria**: They are unicellular (single-cell) organisms each have a different form and can be found inside or outside the human body. They might be harmful or useful.

2- **Algae**: Plant-like organisms living in a humid environment and derive their energy from the heat of sun to make their food in a process similar to photosynthesis.

3- **Fungi**: Plant-like organisms (not green), some are unicellular (e.g yeast) and some are multicellular (e.g. mushrooms).

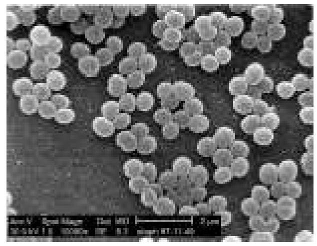
4- **Viruses**: Viruses are not living organisms. They can only be reproduced by using a host cell; inside bacteria or inside the cells of animals or human body. Because they lack various properties that we use to define living organisms, they are not even technically considered alive, and they are not affected by antibiotics.

** Microbiologists study these organisms using:

- ✓ **Microscopes**: to magnify microbial cells.
- ✓ **Genetics**: to study the relationships between microbes and their habitats.
- ✓ **Microbial culture**: is a method of multiplying microbial organisms by letting them reproduce in suitable medium under controlled laboratory conditions. It is used to determine the type of organism, its abundance in the sample being tested, or both.

Aquatic Microbiology: is a branch by which microbiological examination of water, water purification, and biological degradation of waste are studied.

Petri dish containing bacteria



A kind of bacteria

A kind of algae

Mushroom

Yeast

Swine flu virus

Microorganisms in water and diseases

Microorganisms occupy the aquatic environment of all kinds. They may be suspended in fresh water or seawater or live in deposits in groundwater, or on plants. Various types of bacteria/viruses are categorized as pathogens (disease-causing organisms) that can be found in water.

Raw sewage generally contains millions of microorganisms per mL. Many of them are pathogenic, bad odor and taste producing. It is essential that the sewage be treated to remove pathogenic and offensive odor and taste causing microorganisms. This is done by chemical treatment, oxidizing organic material and destroying pathogenic microorganisms.

How to discover that there is microbe in water?

There are the microorganisms called **indicator micro-organisms**: they are microbes that, their presence in water is considered an indicative of the presence of pathogenic microbes. Main types: E.coli and Enterobacter

Characteristics of indicator micro-organisms :

1. Present only in polluted water.
2. Associated with pathogenic microbes.
3. There is relative relationship between their numbers and water pollution with microbes.
4. They have the ability to live longer than the pathogenic microbes in water.
5. Harmless to humans.
6. Easy to detect in a laboratory for its presence in large numbers compared to the pathogenic microbes.

Bacteria in water:

Water bacteria can be classified as:

1- **Endemic bacteria**:

They are the bacteria that are constantly present in water. they include:

a) Self-feeding bacteria: They are classified by their ability to absorb the infrared into the purple bacteria and the green bacteria.
b) Organic chemical bacteria: They are considered as anaerobic bactericidal bacteria.
c) Chemical self-feeding bacteria: These bacteria get the energy from the oxidation processes of inorganic materials present in water.

2- **Exotic bacteria:**

They are the bacteria coming from other environments such as soil, air or those coming from the industrial and municipal sewage.

<u>Fungi in water:</u>

The fungi are found in most types of water, lakes, and rivers. There are no fungi in the dry places because the grains of the land are working to filter the ground water there is no nutrients for these fungi.

What is the importance of fungi?

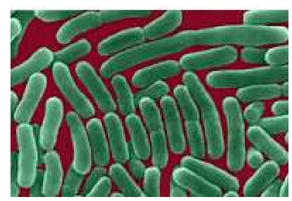
1. It plays an important role in the balance of the aquatic environment, especially freshwater, as it analyzes many organic pollutants.
2. It plays an important role in the biological degradation of some contaminations, and it contributes in the food cycle of aquatic organisms.

<u>Algae in water:</u>

Algae can improve the water quality as it produces oxygen by photosynthesis in surface water. Thus it helps in the aerobic microbial degradation of the dissolved organic matter - ammonia oxidation - removing sulfur from the byproducts of chemical industries and contributes in self-purification.

Examples of some microbes present in water:

1. **<u>Salmonella typhi</u>**



They are microscopic living organisms living in the intestines of humans and animals, they migrate to water through sewage and can live for weeks in water or soil under suitable conditions of heat, humidity and acidity. Salmonella produces endo-toxins, which cause two kinds of diseases: typhoid and gastrointestinal infections.

Symptoms of the infection appear in the form of fever, diarrhea and vomiting, and this continue for 2-5 days then it is cured.

2- Vibrio cholerae bacteria

They live in water at 40 ° C and at pH of 9-10. They cause Cholera by the excretion of extracellular toxins.

Symptoms: of cholera start in the form of severe diarrhea exceeding a liter / hour, thirst, muscle pain, general weakness decline in blood circulation and severe dehydration.

3- Shigella spp bacteria

They spread as a result of the contamination of drinking water with sewage water, and they produce cellular toxins causing bacillary dysentery disease (الدوسينتاريا).

Symptoms: fever, fatigue, loss of appetite, abdominal pain, blooded stool because the bacteria penetrate the wall of intestine causing infections and bleeding in the colon, then death!!

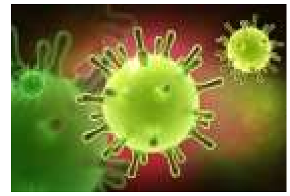
4- HAV virus

This virus is transmitted to humans through contaminated food and drink from human stool. It is frequent in autumn and winter, where the temperature is suitable for the survival of the virus, and it causes hepatitis.

Symptoms: underweight, fever, abdominal pain, diarrhea and itching. The death rate is about 1% of the infected persons.

5- polio virus

It causes of polio, its problem is that more than 90% of the infected do not appear symptoms. The virus invades nerve cells and reaches the central nervous system causing a tumor and muscle spasm.



6- **SARS virus**

It is a Corona group virus that travels through poorly treated contaminated water.

Symptoms: fever, cough and inflammation of the pharynx, intestinal problems and muscle pain.

Factors influence the growth and distribution of micro-organisms in aquatic environments

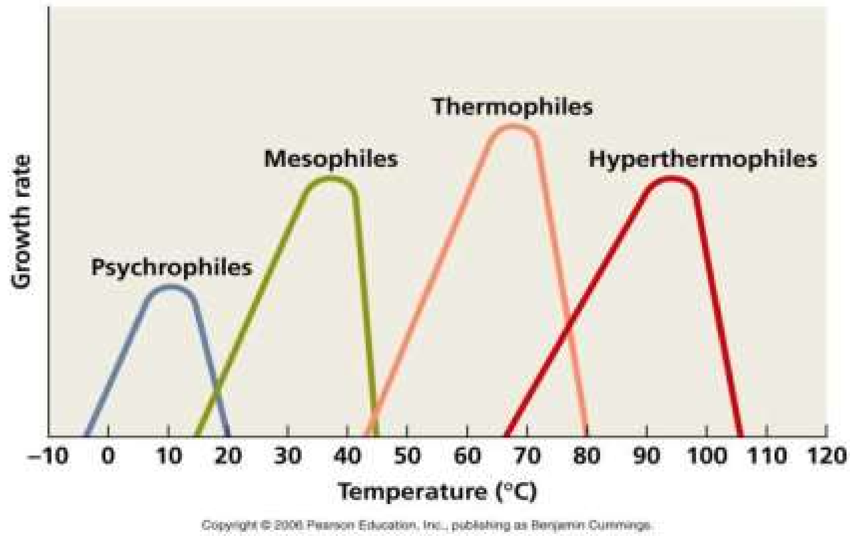
The growth of microorganisms is influenced by various physical and chemical factors of their environment.

- Physical factors: Temperature, Light, movement (agitation), pH, osmotic pressure, and hydrostatic pressure.
- Chemical factors: Oxygen, carbon, nitrogen, phosphorus, sulfur, etc.

Physical factors

1. *Temperature*:

Temperature is the most important factor that determines the rate of growth, multiplication, survival, and death of all living organisms. In general, the higher the temperature, the more easily microorganisms can grow up to a certain point. Very high and very low temperatures both prevent most microorganisms to survive.



2. *Light*:

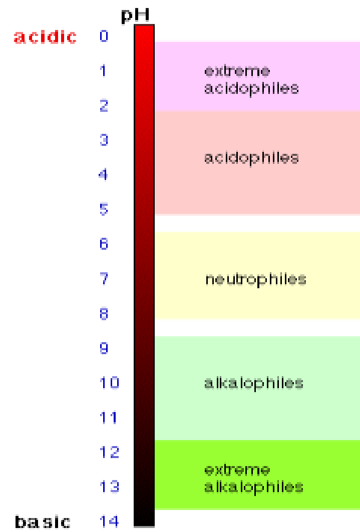
Visible light is beneficial for microorganisms because it is the source of energy for photosynthesis. The amount of light penetrating the water layers depends on: the position of the sun, turbidity, the depth, and color of the water. The sea water is clearer and less polluted than the land water because the sunlight penetrates the land water with a depth of 10 - 150 meters while in the sea by more than 150 meters.

3. *Water movement:*

The greater the movement of water, the more the distribution of heat within the water, and this leads to increase the balance of the chemical contents.

4. *pH of water:*

Microbial growth is strongly affected by the pH of the medium. Most bacteria live in a pH ranges from 6.5 to 8.5. Changes in pH can be observed in environments that have certain contaminants. Increasing in pH to about 10 will affect living aggregates.



5. *Osmotic pressure:*

Osmotic pressure is the minimum pressure which needs to be applied to a solution to prevent the inward flow of water across a SPM (semi-permeable membrane), and it increases as the concentration of salt in water increases.

Some bacteria grow in high osmotic pressure, and others grow at low osmotic pressure.

Microbiological Water Testing

It is a method of analyzing water to estimate the type and concentration of microorganisms present. According to the type and concentration of the detected microbes, the quality of water was determined.

• **Sample specifications for laboratory testing**

1. The sample must be in sterile bottle.
2. Must be representative of the source (take from different places).
3. Avoid its contamination after taking it.
4. The examination should be done immediately after taking the sample.

Types of water microbiological tests

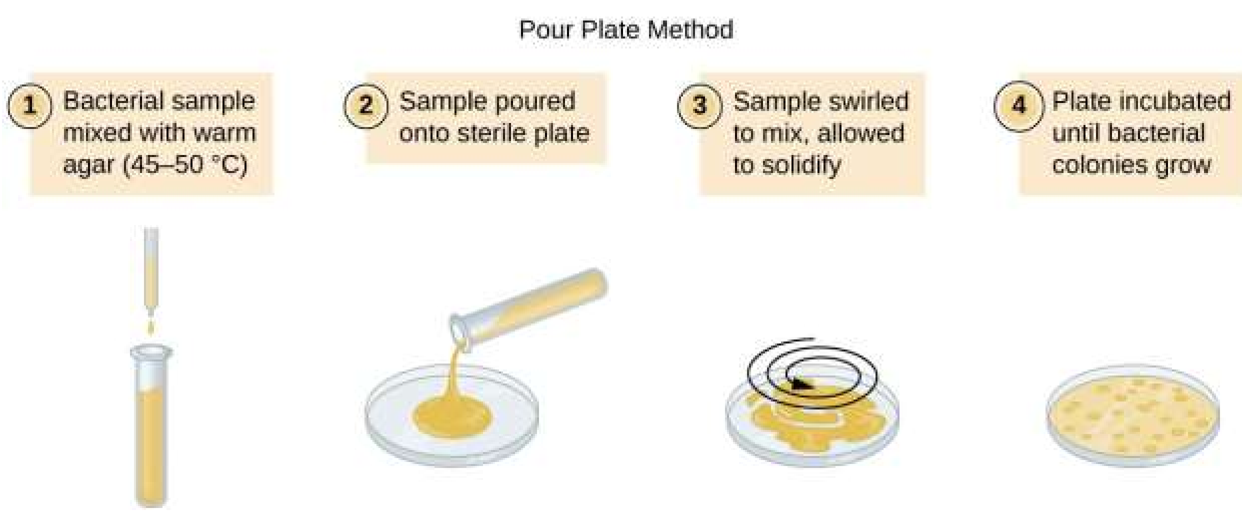
There are two main methods used to detect the microorganisms in water, and to detect the water portability:

1- Direct plates count method:

This method relies on bacteria growing as a colony on a nutrient medium (agar) so that the colony becomes visible to the naked eye and the number of colonies on a plate can be counted.

- The laboratory experiment includes serial dilution of the sample (1:10, 1: 100, 1: 1000, etc ...) in sterile water then pouring the sample into nutrient agar in a closed dish and then incubated.

  *** The dilution is required to obtain 30-300 colonies in the dish. Less than 30 colonies are considered insufficient for counting and more than 3,000 colonies make counting more difficult. So, several dilutions have been done to obtain an appropriate number of colonies for counting. All testing tools should be sterilized.



- After 24-48 hours, count all the colonies. A magnifying colony counter can aid in counting small colonies.
- Finally, calculate CFU/mL using the formula:

CFU/mL = (No of colonies * dilution factor) / volume of culture plate

CFU: Colonies forming unit: is a measure of viable bacterial or fungal cells.

Disadvantages of plate count method

1. Preparation for pour plate method is time consuming.
2. Loss of viability of heat-sensitive organisms coming into contact with hot agar
3. Embedded colonies are much smaller than those which happen to be on the surface. Thus, one must be careful to score these so that none are overlooked.
4. Reduced growth rate of obligate aerobes in the depth of the agar.

2- <u>Most probable number (MPN) analysis for quantifying coliforms:</u>

Coliform Bacteria: rod-shaped Gram-negative bacteria that can ferment lactose with the production of acid and gas when incubated at 35–37°C. They are a commonly used indicator of sanitary quality of foods and water.

How can Coliform bacteria reach the water sources?

When water is contaminated with sewage, it is usually found in warm-blooded intestines and its presence in the water indicates the contamination of water by the pathogens, and it is not suitable for drinking or human use in general.

Their analysis includes three sub-tests:

- Presumptive test - Confirmed test - Completed test

Presumptive test

Purpose of the test:

It is performed to test the ability of coliform bacteria in the sample to ferment the lactose sugar aerobically to acid and gas.

Test steps:

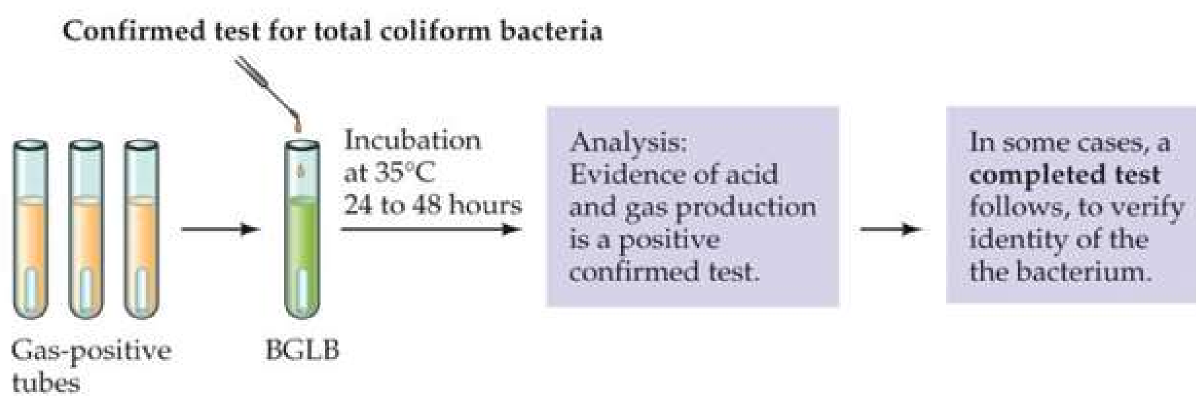
1. Preparing the lactose solution and mix it with an indicator in an inverted Durham tube (purple colored).
2. Inject the solution with the sample of water to be tested
3. If the sample does not contain Coliform bacteria, the color of the detector remains as it is (purple) and does not contain any gas.
4. If the sample has coliform bacteria, then aerobic fermentation of the sugar is taken place, and the acid is produced due to the decrease in PH. The color of the solution is changed to yellow (the middle tube).
5. Some species produce hydrogen gas during the fermentation process and are stored inside the inverted Durham tube (right tube).

Confirmed test

Purpose of the test:

To ensure the positive presence of the coliform bacteria by incubating the sample of the previous test into brilliant green lactose bile broth (BGLB) tube.

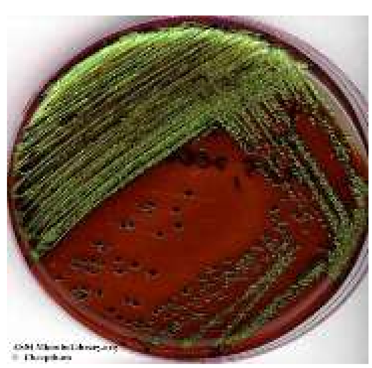
The test should be performed in the case of samples showing a positive or doubtful result of the first test (Presumptive), i.e, the appearance of any amount of gas after 48 hours of incubation.



Completed test

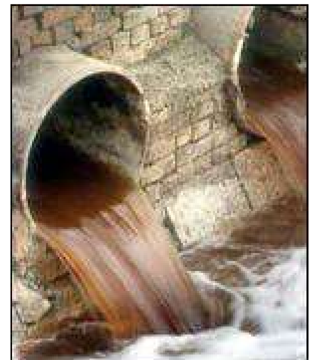
Positive tubes from the confirmed test are analyzed by using Eosin Methylene Blue (EMB) plates – Incubated at 35°C for 24-48 hrs.

– Only coliforms will turn dark with a metallic green sheen.



Low pH: dye turns metallic green

Biological Wastewater treatment



Untreated sewage is dangerous to public health because it contributes pollution to water, land and air, and this facilitates transmission of disease, depreciation of land and degradation of the environment. The waste must be thoroughly treated to produce safe and environmentally acceptable water.

Wastewater should be treated to:

1. Reduce environmental pollution.
2. Reduce costs of treatment of water supplies.
3. Remove rotten materials, e.g., sludge, harmless.
4. Allow for recovery of useful by-products e.g., effluent for irrigation or dry sludge as fertilizers.
5. Kill pathogenic organisms.

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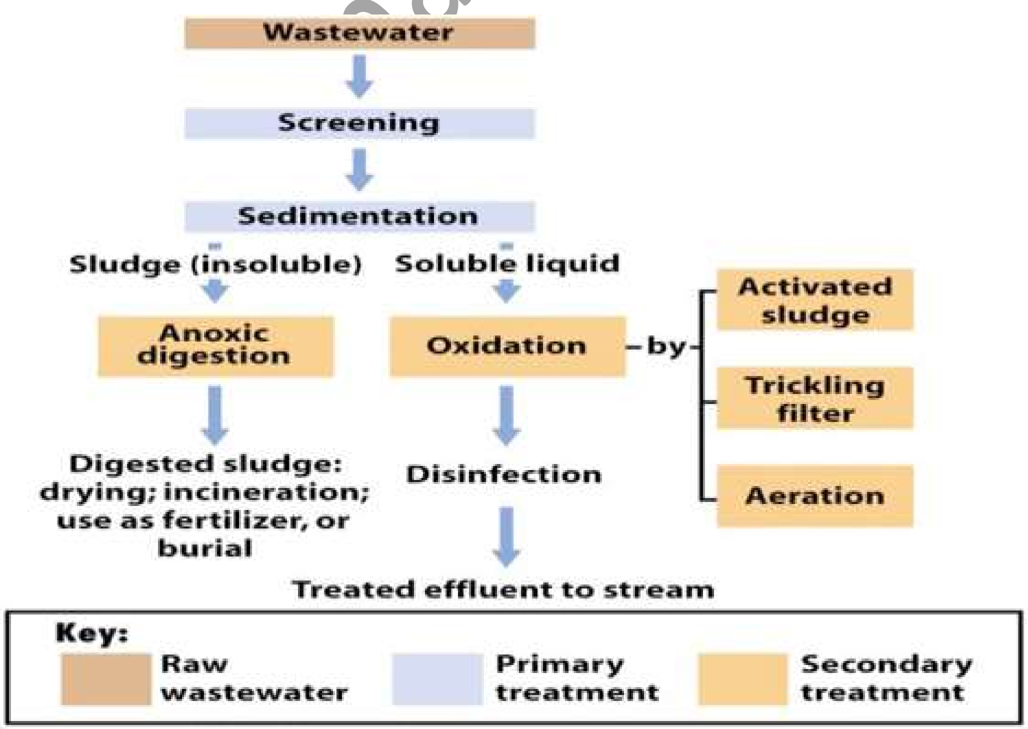
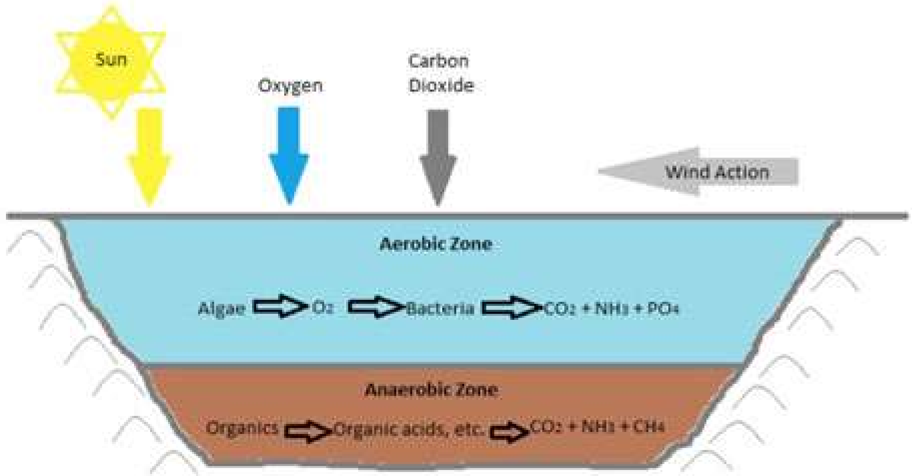
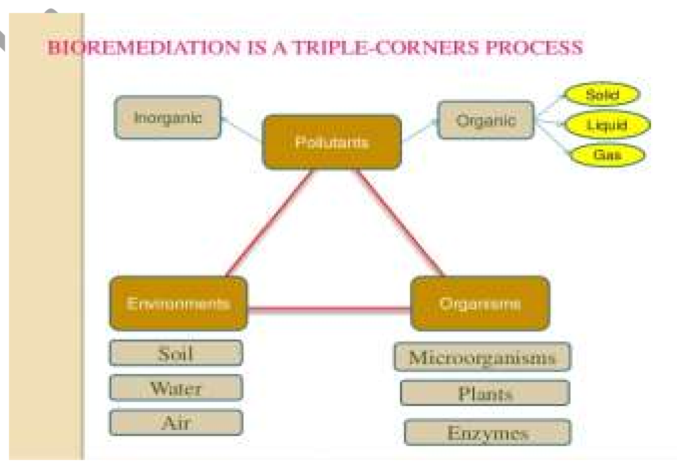


Biological treatment of wastewater is a secondary treatment process of water using a wide variety of microorganisms, primarily bacteria. These microorganisms convert biodegradable organic matter contained in wastewater into simple substances and additional biomass.



It has another term called "Bio-remediation" which is a natural process that relies on bacteria, fungi, and plants to alter organic contaminants by carrying out their normal life functions. Metabolic processes of these organisms are capable of using chemical contaminants as an energy source, rendering the contaminants harmless or less toxic products in most cases. This will result in a high quality amendment for soil rejuvenation and environmental remediation programs.



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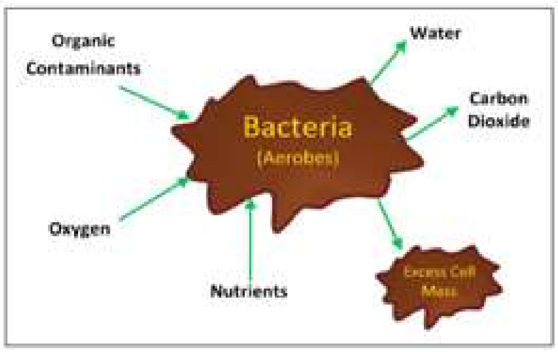
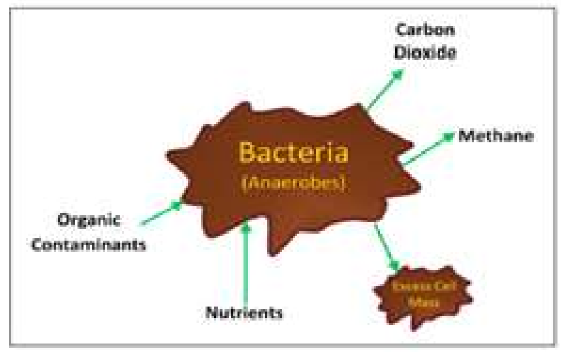
In general, biological treatment is an important and integral part of any wastewater treatment plant that treats wastewater from either municipality or industry having soluble organic impurities. Economically, biological treatment has cemented its place in any integrated wastewater treatment plant compared to other treatment processes such as chemical oxidation; thermal oxidation etc.

The biological treatment processes and techniques can be classified as *Aerobic & Anaerobic treatments*. The differences between them are listed in the following figures and table.



Aerobic Treatment Principle



Anaerobic Treatment Principle

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Parameter	Aerobic Treatment	Anaerobic treatment
Process Principle	• Microbial reactions take place in the presence of molecular/ free oxygen • Reactions products are carbon dioxide, water and excess biomass	• Microbial reactions take place in the absence of molecular/ free oxygen • Reactions products are carbon dioxide, methane and excess biomass
Applications	Wastewater with low to medium organic impurities (COD < 1000 ppm) and for wastewater that are difficult to biodegrade e.g. municipal sewage, refinery wastewater etc.	Wastewater with medium to high organic impurities (COD > 1000 ppm) and easily biodegradable wastewater e.g. food and beverage wastewater rich in starch/sugar/ alcohol
Reaction Kinetic	Relatively fast	Relatively slow
Net Sludge Yield	Relatively high	Relatively low (generally one fifth to one tenth of aerobic treatment processes)
Post Treatment	Typically direct discharge or filtration/ disinfection	Invariably followed by aerobic treatment
Foot-Print	Relatively large	Relatively small and compact
Capital Investment	Relatively high	Relatively low with pay back
Example Technologies	Activated Sludge e.g. Extended Aeration, Oxidation Ditch, MBR, Fixed Film Processes e.g. Trickling Filter/Biotower, BAF, MBBR or Hybrid Processes e.g. IFAS	Continuously stirred tank reactor/digester, Upflow Anaerobic sludge Blanket (UASB), Ultra High Rate Fluidized Bed reactors e.g. EGSBTM, ICTM etc.

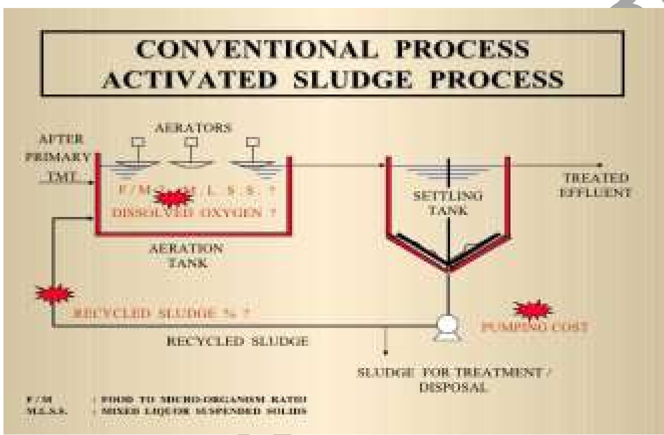
Table 1: Major Differences in Aerobic and Anaerobic Treatment

Aerobic Biological Treatment Technologies

There are several common techniques which are:

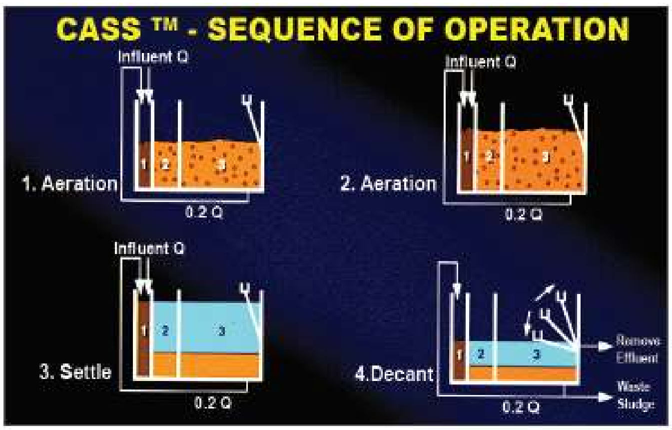
A. *Conventional Activated Sludge Process (ASP) System:*

This is the most common and oldest bio-treatment process used to treat municipal and industrial wastewater. Typically wastewater after primary treatment (suspended impurities removal) is treated in an activated sludge process based biological treatment system comprising aeration tank followed by secondary screen.



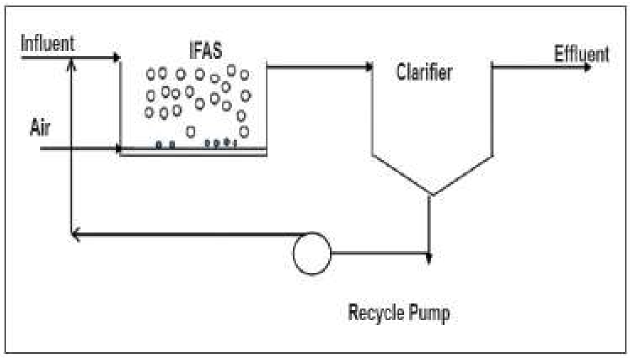
B. *Cyclic Activated Sludge System (CASSTM)*

As its name, it is one of the most popular sequencing batch reactor (SBR) processes employed to treat municipal wastewater and wastewater from a variety of industries including refineries and petrochemical plants.



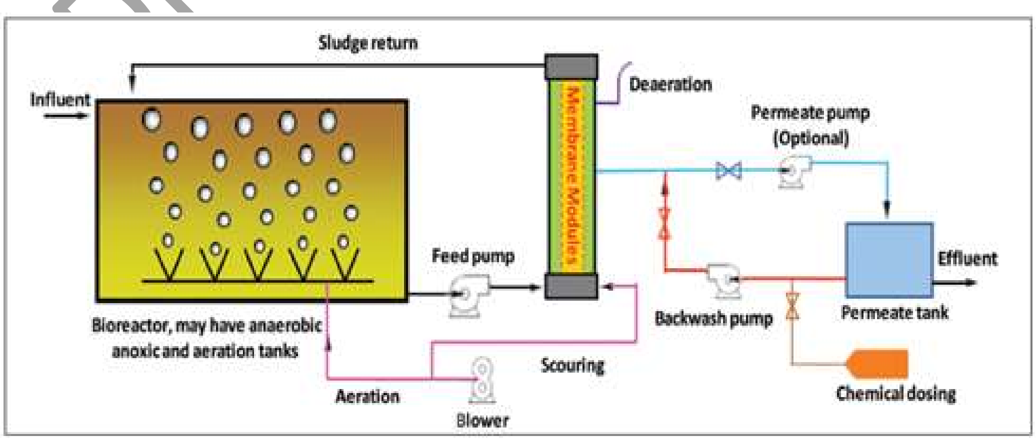
C. <u>Integrated Fixed Film Activated Sludge (IFAS) System</u>

There are several industrial installations where two stage biological treatment comprising stone or plastic media trickling filter (also known as packed bed bio-tower) followed by activated sludge process based aeration tank, followed by secondary clarifier have been in operation.



D. <u>Membrane Bioreactor (MBR)</u>

It is the latest technology for biological degradation of soluble organic impurities. It is used extensively for domestic sewage treatment, but it is limited for industrial waste treatment applications. The MBR process is very similar to the ASP in that both have mixed liquor solids in an aeration tank. The difference between the two processes lies in the method of separation of bio-solids. In the MBR process, the bio-solids are separated by means of a polymeric membrane, as against the gravity settling process in the secondary screen in ASP.



****Comparison between Aerobic Biological Treatment Technologies:

Parameter	Conventional ASP	CASS™	IFAS	MBR
Treated Effluent Quality	Meets specified discharge standards with additional filtra-tion step	Meets/ exceeds specified discharge standards without additional filtra-tion step	Meets/ exceeds specified discharge standards with additional filtration step	Exceeds specified discharge stan-dards without additional filtration step. Very good for recycle provided TDS level permits
Ability to adjust to variable hydraulic and pollutant loading	Average	Very good	Very good	Very good
Pretreatment Requirement	Suspended impurities e.g. oil & grease and TSS removal	Suspended impurities e.g. oil & grease and TSS removal	Suspended impurities e.g. oil & grease and TSS removal	Fine screening for suspended impurities like hair and almost complete oil & grease removal
Ability to cope with ingress of oil	Average	Good	Average	Poor & detrimental to membrane
Secondary Clari-fier Requirement	Needed	Aeration Basin acts as clarifier	Needed	Clarifier is replaced by Membrane filtration
Complexity to operate & control	Simple, but not operator friendly	Operator friendly	Operator friendly	Requires skilled operators
Reliability & Proven-ness of Technology	Average	Very good	Very good	Limited references in industrial applications
Capital Cost	Low	Low	High	Very High
Operating Cost	Low	Low	High	Very High
Space Requirement	High	Low	Average	Low

Table 2: Comparison of Aerobic Biological Treatment Options

References:

General Microbiology, C.P Powar

Microbiology, P.D. Sharma

Handbook of Water and Wastewater Microbiology, M. Duncan and H. Nigel