## Biological Treatment Process:

A process consists of the application of a controlled natural process in which microorganisms remove soluble and colloidal organic matters from the waste water and are in turn removed themselves.

Most domestic and industrial effluents are heterogeneous, and contain both dissolved and suspended matters, both organic and inorganic. Treatment of such effluent or wastewater involves the removal of contaminants to prevent any adverse effects on the receiving water or allow its reuse.

Three treatment processes are usually fallowed:

1. Primary treatment: This is essentially a physio-chemical process where sedimentation, chemical coagulation and precipitation are fallowed to remove the coarse and fine suspended solids.
2. Secondary treatment: The process involves the removal of colloidal and dissolved organic substances and some toxic chemicals. It is essentially a biological treatment process.
3. Tertiary treatment: This is also called advanced treatment, where further purification is needed depending on the reuse of that water. At this stage, excess nutrients like nitrogen, phosphorous and toxic elements like metals are removed.

Biological treatment or secondary treatment mainly based on: The catabolic activities of microbes including bacteria, algae, fungi, protozoa, rotifers and nematodes.

Principle requirements of secondary treatment include: adequate supply of microbes, ensuring the contact of bacteria with influent, and oxygen availability.

Secondary treatment or biotreatment usually applied in: Controlled environment or bioreactor (offers possibility of shortening the treatment time). Although there could be natural system of treatment in lagoons and stabilization ponds, using both the microbes and higher plants.

**Techniques used in biological treatment**:

Two techniques used in this application with the same principles:

1. Attached film growth → like trickling filter.
2. Suspended growth process → like activated sludge.

## ACTIVATED SLUDGE PROCESS

## 8.1 INTRODUCTION

Activated sludge is a suspended-growth process that began in England at the turn of the century. This process has since been adopted worldwide as a secondary biological treatment for domestic wastewaters. This process consists essentially of an aerobic treatment that oxidizes organic matter to CO2 and H2O, NH4, and new cell biomass. Air is provided by using diffused or mechanical aeration. The microbial cells form flocs that are allowed to settle in a clarification tank.

## 8.2 DESCRIPTION OF THE ACTIVATED SLUDGE PROCESS

### 8.2.1 Conventional Activated Sludge System

A conventional activated sludge process includes (Fig. 8.1) the following:

**.** Aeration tank. Aerobic oxidation of organic matter is carried out in this tank. Primary effluent is introduced and mixed with return activated sludge (RAS) to form the mixed liquor, which contains 1500–2500 mg/L of suspended solids.

Aeration is provided by mechanical means. An important characteristic of the activated sludge process is the recycling of a large portion of the biomass. This makes the mean cell residence time (i.e., sludge age) much greater than the hydraulic retention time. This practice helps maintain a large number of microorganisms that effectively oxidize organic compounds in a relatively short time.

The detention time in the aeration basin varies between 4 and 8 hours.

**.** Sedimentation tank. This tank is used for the sedimentation of microbial flocs (sludge) produced during the oxidation phase in the aeration tank. A portion of the sludge in the clarifier is recycled back to the aeration basin and the remainder is wasted to maintain a proper F/M (food to microorganism's ratio).

**Process variables used in control of the biological processes**:

**8.2.1.1 Mixed Liquor Suspended Solids (MLSS).** The content of the aeration tank in an activated sludge system is called mixed liquor. The MLSS is the total amount of organic and mineral suspended solids, including microorganisms, in the mixed liquor. It is determined by filtering an aliquot of mixed liquor, drying the filter at 105 ºC, and determining the weight of solids in the sample. MLSS= Mixed liquor suspended solids in the tank measured in unit (mg/L).

**8.2.1.2 Mixed Liquor Volatile Suspended Solids (MLVSS).** The organic portion of MLSS is represented by MLVSS, which comprises nonmicrobial organic matter, as well as dead and live microorganisms and cellular debris. The MLVSS is determined after heating of dried filtered samples at 600–650 ºC, and represents approximately 65–75 percent of MLSS.

**8.2.1.3 Hydraulic Retention Time (HRT).**

Hydraulic retention time (HRT) or aeration period:

This is the average time spent by the influent sewage in the aeration basin tank. It is calculated as the tank volume ($m^3$) divided by the flow rate:

$HRT(t) = \frac{V}{Q}$ Where: t= Aeration period (h), V= Tank volume ($m^3$)

Q= average daily flow rate ($m^3$/d).

The higher the inflow rate (Q) the sooner the sewage influent will reach the outlet and therefore the lower will be the residence time or hydraulic retention time.

**8.2.1.4 BOD loading**: this can be calculated by kg BOD applied to the tank per day divided by the liquid volume in the aeration tank.

$BODload = \frac{Q.BOD}{V}$ Where: Q, raw waste water flow ($m^3$ /day)

BOD load= kg BOD/ $m^3$. day.

BOD= daily flow of BOD (mg/L)

**8.2.1.5 Food-to-Microorganism Ratio (F/M).** The food-to-microorganisms (F/M) ratio indicates the organic load into the activated sludge system and is expressed in kilogram BOD per kilogram of MLSS per day. It is expressed as:
Food to microorganism's ratio (F/M): It is calculated as the daily flow of BOD divided by the total MLSS in the aeration tank.

$F/M = \frac{Q.BOD}{MLSS.V}$ Where:

F/M= food to microorganism’s ratio (kg BOD/day. kg MLSS).

BOD= raw wastewater BOD (mg/L).

V= volume of aeration basin or tank ($m^3$).

MLSS= Mixed liquor suspended solids in the tank (mg/L).

The food-to-microorganism ratio is controlled by the rate of activated sludge wasting. The higher the wasting rate the higher the F/M ratio. For conventional aeration tanks the F/M ratio is 0.2–0.5 (kg BOD/day. kg MLSS), but it can be higher (1.5) for activated sludge using high purity oxygen. A low F/M ratio means that the microorganisms in the aeration tank are starved (مجوّع), generally leading to a more efficient wastewater treatment.

The higher the biomass growth in the tank, the higher rate of BOD removal. As the ratio of food (BOD) to microorganism's increases, so the rate of BOD removal, growth rate, and respiration rate increase.

$$BOD \quad efficiency = \frac{quantity \quad of \quad BOD \quad removed(effluent)}{quantity \quad of \quad BOD \quad entering(iffluent)} \times 100\%$$

**8.2.1.6 Sludge Age.** Sludge age is the mean residence time of microorganisms in the system. While the hydraulic retention time may be in the order of hours, the mean cell residence time may be in the order of days. This parameter is the reciprocal of the microbial growth rate $\mu$ (see Chapter 2).
Sludge age may vary from 5 to 15 days in conventional activated sludge. It varies with the season of the year and is higher in the winter than in the summer season.

**Principles of suspended growth:**

- Raw wastewater flowing into the biological reactor (aerated tank) containing organic matter (BOD) as a food supply.
- Bacteria metabolize the waste solids, producing new growth while taking in dissolved oxygen and releasing $CO_2$.
- Protozoa graze on bacteria for energy to reproduce. Some of the new microbial growth dies releasing cell contents, to solution for resynthesis.
- After the addition of a large population of microorganisms, aerating raw wastewater for a few hours removes organic matter from solution by synthesis into microbial cells.
- Mixed liquor (ML), is continuously transferred to a clarifier for gravity separation of the biological floc and discharge of the calrifiered effluent.
- Settled floc (activated sludge) is returned continuously to the aeration basin for mixing with entering raw waste as a seed or inoculums (Figure 8.1).

**The important parameters controlling the operation of an activated sludge are** organic loading rates, oxygen supply, and control and operation of the final settling tank. This tank has two functions: clarification and thickening. For routine operation, one must measure sludge settleability by determining the sludge volume index (SVI).

**Biological treatment by activated sludge:**

<u>Source of wastewater:</u>

Wastewater comes from two major sources: as human sewage and as process waste from manufacturing industries (wastewater from industry is about 7 times that of domestic sewage). If the wastewater untreated, and discharged directly to the environment, the receiving waters would become polluted and water-borne diseases would be widely distributed.

Biological treatment simply: involves applying naturally occurring bacteria at very much concentration in tanks.

<u>The concept of treatment is very simple:</u> The bacteria remove small organic carbon molecules by eating them. As a result, the bacteria grow, and the wastewater is cleansed.

The control of the biological treatment process is very complex, because of the large number of variable affect it, these include:

- Changes in the composition of the bacterial flora in the tank.
- The influent of the sewage to the process show variation inflow rate.
- Chemical composition and pH and temperature.
- Industrial wastewater contains the resistant chemicals that the bacteria degrade only very slowly.
- Toxic chemicals that inhibit the functioning of activated sludge bacteria.
- High concentration of toxic chemicals can produce a toxic shock that kills the bacteria.

When this happens the system may pass untreated effluent direct to the environment.

**The nature and composition of waste water:**

Domestic sewage mainly contains:

1. Organic carbon, either in solution or as particulate matter
2. About 60% is in particulate form may settle or suspended in the tank.
3. Particles of 1 nm to 100 μm remain in colloidal suspension and during treatment become adsorbed on to the flocs of the activated sludge.

<u>Organic matter is easily biodegradable consisting of:</u>

1. Protein, amino acid, peptides, carbohydrates, fats and fatty acid.
2. The average carbon to nitrogen to phosphorus ratio (C: N: P ratio) stated as approximately 100:20:6 which is ideal to growth of activated sludge bacteria.

While, industrial waste water are more variable in composition (such as pulp and paper) industries for example, are deficient in nitrogen and phosphorous. These nutrients need to be added to achieve correct ratio for microbial growth.

**Soft and hard organic matter (BOD) digestion:**

The time for the removal of organic carbon varies with the ability of activated sludge bacteria to ingest it:

- Soft BOD: this group of compounds is often referred to as the readily biodegradable or soft BOD, which is small molecular weight compounds will start to be removed from the sewage immediately after it has entered the activated sludge tank. Their removal may be completed in 1-2 hours.
- Hard BOD: higher molecular weight compound will take several hours to be degraded and removed. Compounds are more recalcitrant, and may still be present several days. This less readily biodegradable BOD is often referred to as hard BOD (figure 3-1)

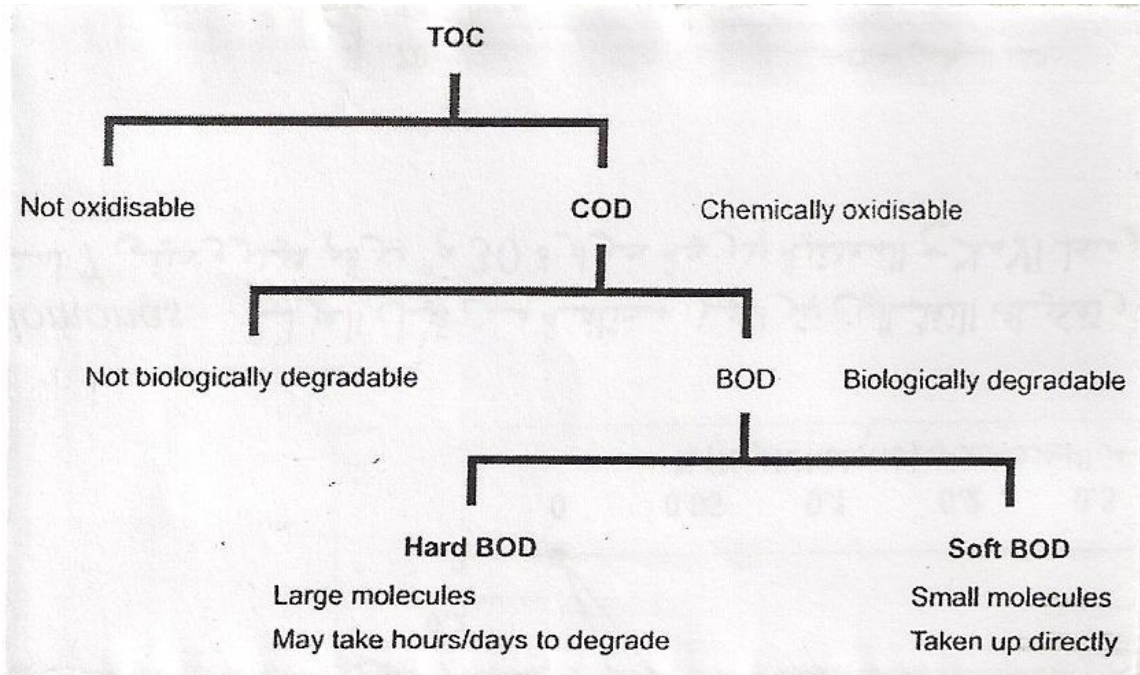


Figure (3-1): the relationship between the organic carbon fractions and their degradability.

## 8.3 BIOLOGY OF ACTIVATED SLUDGE

There are two main goals of the activated sludge system:

1. Oxidation of the biodegradable organic matter in the aeration tank (soluble organic matter is thus converted to new cell mass).
2. Flocculation, that is, the separation of the newly formed biomass from the treated effluent.

**Microbial ecology:**

Activated sludge bacteria:

The activated sludge of the aeration basin of a waste water treatment works is a complex of competing organisms. The dominant organisms are the bacteria, of which there may be 300 species present. Bacteria are amongst the smallest and most abundant living organisms. Single cell, varying in size from about 0.5-2 µm the interior of the cell contains the cytoplasm and the thousands of different chemicals, whose reactions are regulated by enzymes. Most bacteria are spherical, but some may be rod shaped or spiral form and filamentous bacteria.

**- Digestion process by bacteria:**

Small molecular weight compound diffuse into the bacteria (ingestion) through the cell wall. At the same time, some large complex molecules that have been synthesized within the bacteria pass outwards. This process is referred to as secretion. The secretions include slimes and gels that may bond the bacteria together and also enzymes. The enzymes break down large organic molecules into smaller monomers that are small enough to be ingested.

Then bacteria use the ingested molecules for the synthesis of new molecules, in the process of growth, reached normal size, and divided into two, and the process is repeated.

- **Types of bacteria in activated sludge**:

There are two types of bacteria according to the nutrition mode:

1. The heterotrophic or carbonaceous bacteria, which are the predominant groups of organisms. Feeding mainly on organic carbon molecules rather than inorganic ones.
2. The autotrophic bacteria, which take inorganic chemicals, and use these in the synthesis of organic carbon. For example, nitrifying bacteria that remove ammonia from the waste water. There are few species of autotrophs, they have low growth rates, and they cannot compete by the faster – growing heterotrophs.

**Bacterial flocs:**

The flocs are formed from aggregates of non-living organic polymers that are probably secreted by bacteria. They have open porous structure; vary in size from less than 10 µm up to 1 mm (1000 µm).

The bacteria are adsorbed on to the internal and external surfaces of the floc. Immediately after the waste water enters the aeration tank, the fine particulates, colloidal particles and large molecules, adsorb to the floc material. The enzymes that are secreted by the bacteria into the water will facilitate their digestion. For the bacteria living on or inside of the floc, a mixed liquor oxygen concentration of 1.2-2 mg $O_2$/l may be required, below this range the center of the flocs may become oxygen depleted and colonized by facultative anaerobic bacteria. The outer surface of the activated sludge flocs are colonized by microorganisms of a higher trophic level such as, protozoa and rotifers. These feed on bacteria and particulate material in the waste water

## Bacteria aggregation by intracellular and extracellular products:

* The production of an intracellular storage product, ploy- hydroxybutyric acid, was first thought to be responsible for bacterial aggregation. Extracellular polysaccharides in the form of capsules and loose extracellular slimes produced by Zooglea ramigera and other activated sludge microorganisms play a leading role in bacterial flocculation and floc formation. It is now accepted that the extracellular polymeric substances (EPS) produced by some activated sludge microorganisms are mainly responsible for floc formation.
These polymeric substances are composed of:

- Carbohydrates (e.g., glucose, galactose), amino sugars, uronic acids (glucuronic, galacturonic acids), proteins, lipids, and small amounts of nucleic acids, and are refractory to biodegradation.
- Recently, it was reported that proteins were the most important polymeric components found in extracted EPS. Most of these components contribute to the negative surface charge of flocs. Various probes are available for EPS components.

* Since EPS have both hydrophobic and hydrophilic properties, some investigators have demonstrated that hydrophobic interactions are also involved in microbial floc formation and in the adhesion of bacteria to the flocs. A positive correlation was found between relative hydrophobicity of the flocs and their flocculating ability, suggesting that surface charge is less important than hydrophobic binding. The adhesion of E. coli to activated sludge flocs increases with hydrophobicity of the cell surface. Hydrophobic *Serratia marcescens* attached in higher numbers to activated sludge flocs than hydrophilic E. coli.

* Sludge retention time (SRT) influences the composition of EPS and the physicochemical properties (hydrophobicity and surface charge) of the flocs. As SRT increases, the floc surface is less negatively charged and more hydrophobic.

* **Divalent cations, mostly** Ca and Mg, bridge the negatively charged groups of EPS, play an important role in the flocculation of activated sludge and retain the biopolymers in the floc. Conversely, monovalent cations such as Na and NH4 negatively affect the settling properties of activated sludge and help release biopolymers in the suspending medium. It appears that floc stability is affected by the ionic strength of the influent wastewater. A suggested structure of the activated sludge floc is displayed in Figure 8.18. However, excessive production of EPS can be responsible for bulking (see Chapter 9), a condition consisting of loose flocs that do not settle well. Poor sludge settleability, as indicated by a high sludge volume index (SVI) was associated with the amount of total EPS. This condition (nonfilamentous bulking), contrasts with filamentous bulking, which is caused by the excessive growth of filamentous bacteria. Microbial flocculation can be enhanced by adding commercial polyelectrolytes or by adding iron and aluminum salts as coagulants (see Chapter 9).
Extracellular polymers are also responsible for removing phosphorus in activated sludge. Scanning electron microscopy combined with energy dispersive spectrometry (EDS) showed that EPS alone contained, on average, between 27 and 30 percent phosphorus.

**Metabolism of bacteria:**

Treatment of the sewage in the aeration tank involves removal of organic carbon from the mixed liquor by ingestion by the bacteria. Once inside the bacteria, the carbon compounds are metabolized. Metabolism comprises the thousands of simultaneous chemical reactions that are going on at one time inside the bacteria. In each of these reactions, a substrate in the presence of an enzyme (which acts as a catalyst), is converted into product.

Substrate $\xrightarrow{\text{enzyme}}$ product.

The product then becomes the substrate for the next step in the chain, and is almost immediately converted in the presence of another specific enzyme, into a different product.

The major divisions of metabolism are:

- Catabolism or energy metabolism: this comprises a serious of reactions in which carbon compounds are broken down to yield cellular energy. This is biological oxidation and involves oxygen uptake by the bacteria. This process referred to as respiration.
- Anabolism: this is a series of biosynthetic reaction in which small molecules are joined together to form large molecular weight macromolecules. This requires an input of energy from catabolism, and is the basis of the process of growth.

**Microbial processes:**

We can identify the three major processes that are relevant to the biological treatment:

1. ingestion
2. respiration
3. growth and division

These processes in an ingle bacterial cell can be shown in figure (3-2).

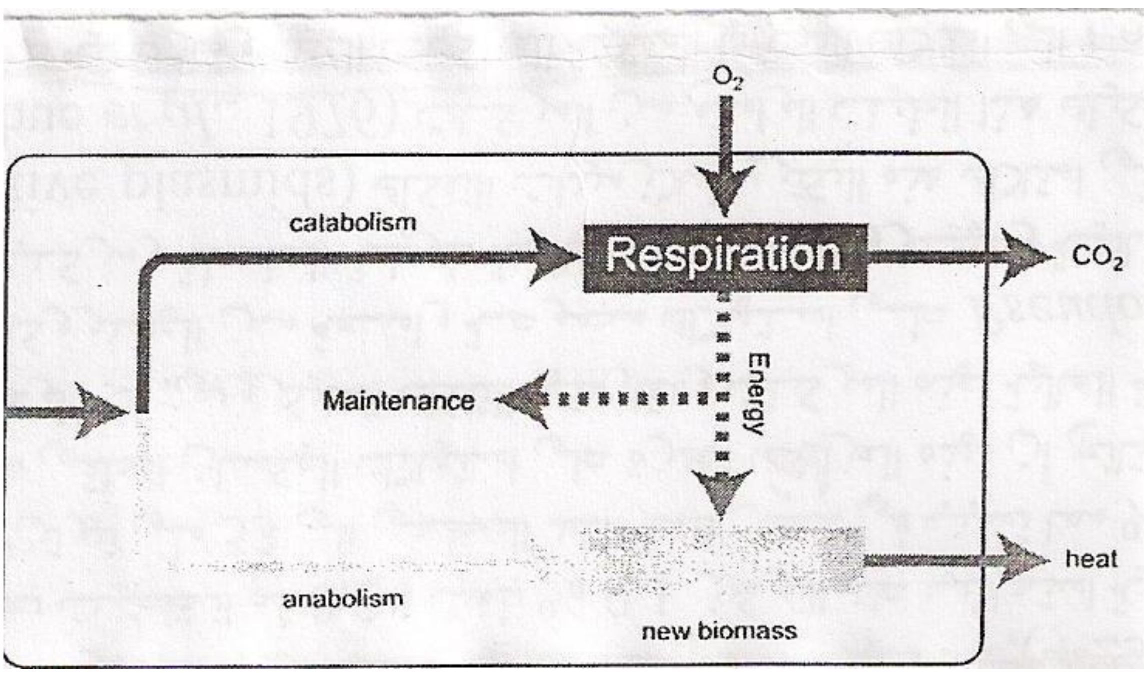


Figure (3-2): Respiration of a single bacterium showing the relationship between the three processes.

The three processes, ingestion, respiration and growth are very highly coupled. No one process can go faster than the other. It will be noted that the 3 processes correspond to the major processes that we shall see during the operation of the treatment system. They can be summarized as:

| Bacterial process | Treatment plant process |
|---|---|
| Ingestion | Biodegradation |
| Respiration | Aeration requirement |
| Growth and division | Biomass production |

**Ingestion:**

This involves the passage of organic carbon compounds, other molecules and ions from the mixed liquor into the bacterium, they have pass through the cell wall and inner membrane. Ions such as sodium diffuse in because the concentration in the mixed liquor is higher than inside the bacterium.

Small organic molecules: similarly pass in a long concentration by various mechanisms located in the inner membrane.

Large molecules: are excluded, in order to use these for their nutrition and growth. The bacteria secrete enzymes into the water to digest them into small monomers, which can then pass into the cell.

The bacterium required the presence of the particular chemical compound in the water to switch on the genes for the synthesis of the enzyme required for its digestion. This process also calls adaptation or acclimation.

**Growth of bacteria:**

Growth rate is measured as the increase in number of cells with time. Some bacteria may double their biomass in as little as 20 minutes, provided they have the right conditions of temperature, pH and an abundance of organic carbon, other nutrients, trace elements etc.

The growth rate observed is a result of both: genetic and environmental factors. The shape of the growth curves and the maximum rate of growth under optimal conditions are genetically determined.

T**he effects of environmental factors on microbial growth:**

1. **Substrate concentration:**

The main substrate for growth is the BOD or degradable organic carbon in the mixed liquor. With increase in the concentration of substrate, the growth rate increase exponentially and then levels off. So with further increase in concentration of substrate in the medium, there is no further increase in growth. So the bacteria are at their maximum growth rate.

**Availability of the nutrients:**

The major substrate requirement is for carbon, growth also dependent on the intake of:

a. Macronutrients: like nitrogen and phosphorous. The optimum ratio of C: N: P in the mixed liquor is generally thought to be 100:5:1. The ratio of these nutrients in domestic sewage reported as 100:20:6. This indicates that nitrogen and phosphorous will not be limiting for growth of bacteria.
b. Micronutrients (trace) components: which include S, Ca, Mg, K and Fe. These elements are available in abundance in domestic sewage. By contrast, the waste water from pulp and paper, and food processing industries can be deficient in N and P. Nutrients therefore need to be added to the mixed liquor to obtain maximum bacterial growth and treatment. Lack or an insufficiency of a nutrient may result in incomplete treatment, because the bacteria are unable to grow optimally.

**2. Oxygen levels:**

If oxygen levels in the mixed liquor are too low, respiration will be inhibited and hence energy will not be available for growth. The higher the oxygen concentration in the water, the larger amount of oxygen inter to the inside of the bacterial cell. Oxygen is no limiting above concentration about 1.5 – 2 mg $O_2$/l for bacterial in flocs. Below these critical concentrations, the respiration rate falls rapidly due to the unavailability of oxygen.

**3. Temperature:**

As the temperature increases, the rate of growth, and hence requirement for oxygen for respiration increases. The respiration and growth rate approximately doubles for every 10 ˚C increase in temperature within optimum range. However, the solubility of oxygen in water decreases with increase in temperature. Optimum aeration becomes more difficult as the temperature in the tank rises. It is for this reason that most thermophilic plants, operating at 40 - 60 ˚C, have to use pure oxygen for aeration.

4. **Toxicity:** toxic chemical in the waste water can inter the bacteria and inhibit one or more enzymes of the pathways involved in either anabolism or catabolism. When pathway actually inhibited, all three processes of ingestion, growth and respiration will be similarly inhibited. Immediately after feeding, the respiration rate rises rapidly to its maximum value. When toxic waste water is introduced, the respiration rates falls to a new lower level. The difference between this new rate and the maximum rate is measure of the inhibition. The percentage of inhibition increases with increase in concentration of the toxic chemical in the mixed liquor.

### 8.3.1 Survey of Organisms Present in Activated Sludge Flocs

The activated sludge flocs contain mostly bacterial cells as well as other microorganisms, and inorganic and organic particles. Floc size varies between, 1 mm (the size of some bacterial cells) to >1000 mm.

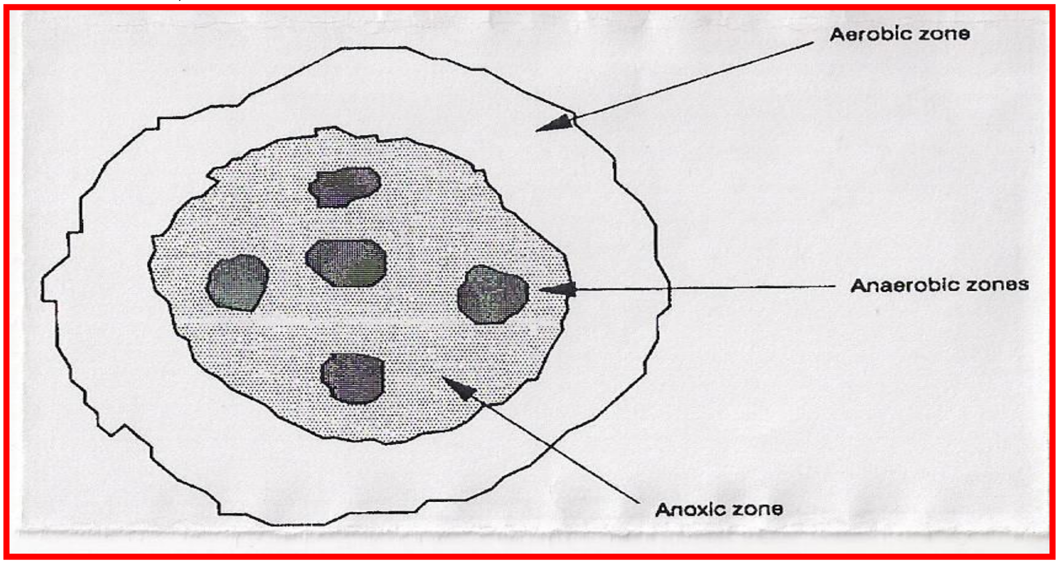


Figure 8.4: Microbial community in activated sludge flocs

Activated sludge flocs contain a wide range of prokaryotic and eukaryotic microorganisms, and many of them can be routinely observed with regular phase-contrast microscopy.

**8.3.1.1 Bacteria**. As the oxygen level in the flocs is diffusion-limited, the number of active aerobic bacteria decreases as the floc size increases. Anoxic zones can occur within flocs, depending on the oxygen concentration in the tank. They disappear when the oxygen concentration exceeds 4 mg/L. The inner region of relatively large flocs favors the development of strictly anaerobic bacteria such as methanogens or sulfate-reducing bacteria (SRB). The presence of methanogens and sulfate-reducing bacteria can be explained by the formation of several anaerobic pockets inside the flocs or by the tolerance of certain methanogens and SRB to oxygen (Figure 8.5). Thus, activated sludge could be a convenient and suitable seed material for starting anaerobic reactors.

Bacteria, particularly the gram-negative bacteria, constitute the major component of activated sludge flocs. Hundreds of bacterial strains thrive in activated sludge but only a relatively small fraction can be detected by culture-based techniques. They are responsible for the oxidation of organic matter and nutrient transformations, and produce polysaccharides and other polymeric materials that aid in the flocculation of microbial biomass.

The total aerobic bacterial counts in standard activated sludge are in the order of 108 CFU/mg of sludge. When using culture-based techniques, it was found that the major genera in the flocs are *Zooglea, Pseudomonas, Flavobacterium, Alcaligenes, Achromobacter, Corynebacterium, Comomonas, Brevibacterium, Acinetobacter, Bacillus spp.*, as well as filamentous microorganisms. Some examples of filamentous microorganisms are the sheathed bacteria (e.g., *Sphaerotilus*) and gliding bacteria (e.g., *Beggiatoa, Vitreoscilla*), which are responsible for sludge bulking (التضخّم). *Pseudomonas* species.

Zoogloea is exopolysaccharide-producing bacteria that produce typical finger-like compounds and are found in wastewater and other organically enriched environments (Fig. 8.7). These finger-like projections consist of aggregates of Zooglea cells surrounded by a polysaccharide matrix (Fig. 8.8). They are isolated by using enrichment media containing m-butanol, starch, or m-toluate as the carbon source. They are found in various stages of wastewater treatment but their numbers comprise only 0.1–1 percent of the total bacterial numbers in the mixed liquor. Although, through the use of 16S rRNA-targeted probes, their level was found to be as high as 10 percent.

Activated sludge flocs also harbor autotrophic bacteria such as nitrifiers (*Nitrosomonas, Nitrobacter*), which convert ammonium to nitrate (see Chapter 3). The use of 16S rRNA targeted probes showed that *Nitrosomonas* and *Nitrobacter* species occur in clusters and are in close contact in activated sludge flocs and in biofilms.

**8.3.1.2 Fungi.** Activated sludge does not usually favor the growth of fungi, although some fungal filaments are observed in activated sludge flocs. Fungi may grow abundantly under specific conditions of low pH, toxicity, and nitrogen-deficient wastes. The predominant genera found in activated sludge are *Geotrichum, Penicillium, Cephalosporium, Cladosporium, and Alternaria*. Sludge bulking may result from the abundant growth of *Geotrichum candidum*, which is favored by low pH from acid wastes. Laboratory experiments have shown that fungi are also capable of carrying out nitrification and denitrification. This suggests that they could play a role in nitrogen removal in wastewater under appropriate conditions. Some advantages of a fungi-based treatment system are the ability of fungi to carry out nitrification in a single step, and their greater resistance to inhibitory compounds than bacteria.

**8.3.1.3 Protozoa.** Protozoa are significant predators of bacteria in activated sludge as well as in natural aquatic environments. Protozoan grazing on bacteria (i.e., bacterivory) can be experimentally determined by measuring the uptake of 14C- or 35S-labeled bacteria. Such grazing can be significantly reduced in the presence of toxicants (e.g., heavy metals). For example, *Aspidisca costata* grazing on bacteria in activated sludge is reduced in the presence of cadmium.

Protozoa may also graze on Cryptosporium oocysts and thus help in the dispersion and transmission of protozoan parasites.

**. Ciliates**

The cilia that give the organisms their name are used for locomotion and for pushing food particles into the mouth. Ciliates appear to be the most abundant protozoa in activated sludge plants. They are subdivided into free, creeping (الزاحف), and stalked ciliates (المطارد). Free ciliates feed on free-swimming bacteria. The most important genera found in activated sludge are *Chilodonella, Colpidium, Blepharisma, Euplotes, Paramecium,* *Lionotus, Trachelophyllum, and Spirostomum.* Creeping ciliates graze on bacteria on the surface of activated sludge flocs.

**. Flagellates**

These protozoa move via one or several flagella. They take up food via the mouth or via absorption through their cell wall. Some important flagellates found in wastewater are **Bodo ssp., Pleuromonas spp., Monosiga spp., Hexamitus spp.,** and a colonial protozoa *Poteriodendron spp.*

**. Rhizopoda (amebae)** Amebae move slowly via pseudopods (false feet), which are temporary projections of the cells. This group is subdivided into ameba (e.g., Amoeba proteus) and thecameba, which are surrounded by a shell (e.g., Arcella). Flagelated protozoa and free-swimming ciliates are usually associated with high bacterial concentrations (> $10^8$cells/mL), whereas stalked ciliates occur at low bacterial concentrations (< $10^6$/mL). Protozoa contribute significantly to the reduction of BOD, suspended solids, and numbers of bacteria, including pathogens (Curds, 1975). There is an inverse relationship between the number of protozoa in mixed liquor and the COD and suspended solids concentration in activated sludge effluents. Changes in the protozoan community reflect those of the plant operating conditions, namely F/M ratio, nitrification, sludge age, or dissolved oxygen level in the aeration tank. The protozoan species composition of activated sludge may indicate the BOD removal efficiency of the process. For example, the presence of large numbers of stalked ciliates and rotifers indicate a low BOD. The ecological succession of microorganisms during activated sludge treatment is illustrated in Figure 8.11.

**Rotifers**

Rotifers are metazoa (i.e., multicellular organisms) with sizes varying from 100 mm to 500 mm. Their body, anchored (مرسو) to a floc particle, frequently "stretches out" from the floc surface. The rotifers found in wastewater treatment plants belong to two main orders, Bdelloidea (e.g., *Philodina spp., Habrotrocha spp.) and Monogononta (e.g., Lecane spp., Notommata spp*.). The four most common rotifers found in activated sludge and trickling filters are illustrated in Figure 8. The role of rotifers in activated sludge is twofold:

1. They help remove freely suspended bacteria (i.e., nonflocculated bacteria) and other small particles and contribute to the clarification of wastewater. They are also capable of ingesting Cryptosporidium oocysts in wastewater and can thus serve as vectors for the transmission of this parasite.
2. They contribute to floc formation by producing fecal pellets surrounded by mucus.

The presence of rotifers at later stages of activated sludge treatment is due to the fact that these animals display a strong ciliary action that helps in feeding on reduced numbers of suspended bacteria (their ciliary action is stronger than that of protozoa).

### 8.3.2 Organic Matter Oxidation in the Aeration Tank

Domestic wastewater has a C:N:P ratio of 100:5: 1, which satisfies the C, N, and P requirements of a wide variety of microorganisms. Organic matter in wastewater occurs as soluble, colloidal, and particulate fractions (see Chapter 7). The soluble organic matter serves as a food source for the heterotrophic microorganisms in the mixed liquor. It is quickly removed by adsorption, co flocculation, as well as absorption and oxidation by microorganisms. Aeration for only a few hours leads to the transformation of soluble BOD into microbial biomass. Aeration serves two purposes: (1) supplying oxygen to the aerobic microorganisms, and (2) keeping the activated sludge flocs in constant agitation to provide an adequate contact between the flocs and the incoming wastewater. An adequate dissolved oxygen concentration is also necessary for the activity of heterotrophic and autotrophic microorganisms, especially nitrifying bacteria. The dissolved oxygen level must be in the 0.5–0.7 mg/L range. Nitrification ceases when DO is, 0.2 mg/L.

### 8.3.3 Sludge Settling

The mixed liquor is transferred from the aeration tank to the settling tank where the sludge separates from the treated effluent. A portion of the sludge is recycled to the aeration tank and the remaining sludge is wasted and transferred to an aerobic or anaerobic digester for further treatment. Flocculation or aggregation of cells is generally a response of microorganisms to low nutrient conditions in their environment. They provide a more efficient utilization of food due to the close proximity of cells. Products released by one group of microorganisms can serve as a growth substrate for another group. Thus, sludge settling depends on the F/M ratio and sludge age. Good settling occurs when the sludge microorganisms are in the endogenous phase, which occurs when carbon and energy sources are limited and when the microbial specific growth rate is low. Good sludge settling with subsequent efficient BOD removal occurs at low F/M ratio (i.e., high MLSS concentration). Conversely, a high F/M ratio is conducive to poor sludge settling. In municipal wastewaters, the optimum F/M ratio is 0.2–0.5. A mean cell residence time of 3–4 days is necessary for effective settling. Poor settling can also be caused by sudden changes in physical parameters (e.g., temperature, pH), absence of nutrients (e.g., N, P, micronutrients) and presence of toxicants (e.g., heavy metals), which may cause a partial deflocculating of activated sludge.

The conventional way of monitoring for sludge settleability is by determining the sludge volume index (SVI). Mixed liquor drawn from the aeration tank is introduced into a 1 L graduated cylinder and allowed to settle for 30 min. Sludge volume is recorded.
Sludge volume index, which is the volume occupied by 1 g of sludge, is given by:

$$SVI(mg/l) = \frac{SV.1000}{MLSS}$$

Where SV = volume of the settled sludge in the graduated cylinder (mL), and MLSS = mixed liquor suspended solids (mg/L).
In a conventional activated sludge plant (with MLSS < 3500 mg/L) the normal range of SVI is 50 –150 mL/g.

## NUTRIENT REMOVAL BY THE ACTIVATED SLUDGE PROCESS

### 8.4.1 Nitrogen Removal
### 8.4.1phosphorous removal

For nitrogen and phosphorous removal see the previous lecture.

## 8.5. PATHOGEN AND PARASITE REMOVAL BY ACTIVATED SLUDGE

Both components (aeration and sedimentation tanks) of the activated sludge process affect, to some extent, the removal/inactivation of pathogens and parasites. During the aeration phase, environmental (e.g., temperature, sunlight) and biological (e.g., inactivation by antagonistic microorganisms) factors, possibly aeration, have an impact on pathogen/parasite survival. Floc formation during the aeration phase is also instrumental in removing undesirable microorganisms. During the sedimentation phase, certain organisms (e.g., parasites) undergo sedimentation, while floc-entrapped microbial pathogens settle readily in the tank. As compared with other biological treatment processes, activated sludge is relatively efficient in removing pathogenic microorganisms and parasites from incoming primary effluents.

### 8.6.1 Bacteria
Activated sludge is generally more efficient than trickling filters for the removal of indicator (e.g., coliforms) and pathogenic (e.g., *Salmonella*) bacteria. The removal efficiency may vary from 80 percent to more than 99 percent. Bacteria are removed via inactivation, grazing by ciliated protozoa (grazing is particularly effective for free - swimming bacteria), and adsorption to sludge solids or encapsulation within sludge flocs, or both, followed by sedimentation.

### 8.6.2 Viruses
The activated sludge process is the most efficient biological process for virus removal from sewage. It appears that most of the virus particles (.90 percent) are solids-associated and are ultimately transferred to sludge.

The ability of activated sludge to remove viruses is related to the capacity to remove solids. Thus, many of the viruses found in the effluents are solids-associated. Viruses are also inactivated by environmental and biological factors.

Attempts have been made to estimate the contribution of both associations to solids and inactivation to virus removal in activated sludge. After 10 h of aeration, 25 percent are removed by adsorption to sludge flocs, and 75 percent are removed by inactivation. Therefore, inactivation alone is not sufficient for removing most of the viruses with a retention time varying from 6 to 12 h.

In summary, virus removal/inactivation by activated sludge may be due to the following:

. Virus adsorption to or encapsulation within sludge solids (this results in the transfer of viruses to sludge);

. Virus inactivation by sewage bacteria (some activated sludge bacteria may have some antiviral activity);

. Virus ingestion by protozoa (ciliates) and small metazoan (e.g., nematodes).

### 8.6.3 Parasitic Protozoa

Protozoan cysts such as those of *Entamoeba histolytica* are not inactivated (معطل) under the conditions prevailing in the aeration tank of an activated sludge process. They are, however, entrapped in sludge flocs and are thus transferred to sludge after sedimentation. Similar removals were observed for both *Entamoeba histolytica and Giardia* cysts ز More than 98 percent of *Giardia* cysts are removed and become concentrated in sludge. Cysts numbers at various treatment stages of a California wastewater treatment plant are shown in Table 8.4. More than 99 percent of Giardia cysts were removed by the activated sludge treatment and most of them were transferred to sludge.

### 8.6.4 Helminth Eggs

Because of their size and density, eggs of helminthes parasites (e.g., *Taenia, Ancylostoma*, *Necator*) are removed by sedimentation during primary treatment of wastewater and during the activated sludge treatment, thus they are largely concentrated in sludge's.