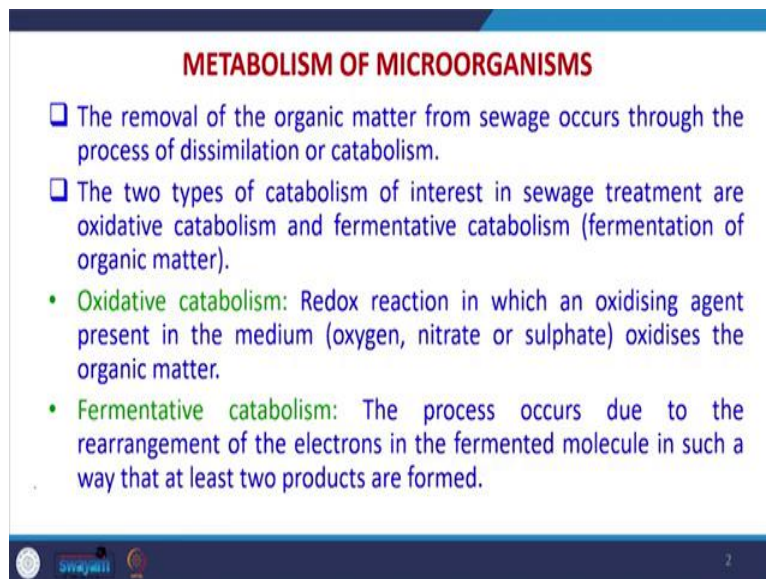


Biological Process Design for Wastewater Treatment
Professor Vimal Chandra Srivastava
Department of Chemical Engineering
Indian Institute of Technology, Roorkee
Lecture: 03
Fundamentals of Biochemical Operations

Good day everyone and welcome to this NPTEL online certification course on biological process design for wastewater treatment. So today we are going to further study the fundamentals of biochemical operation that we started studying in the previous class. And in the previous lecture, we studied regarding the microbiology and the biochemistry aspects and ecology aspects of biological systems which are used for wastewater treatment.

So today we are going to continue further in the same fundamentals and we are going to study further the metabolism of microorganism. How they generate they generate or get their energy and also the how these microorganism are used in the wastewater treatment in different ways. So we are going to start this lecture starting with the first section of metabolism of microorganism.

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METABOLISM OF MICROORGANISMS

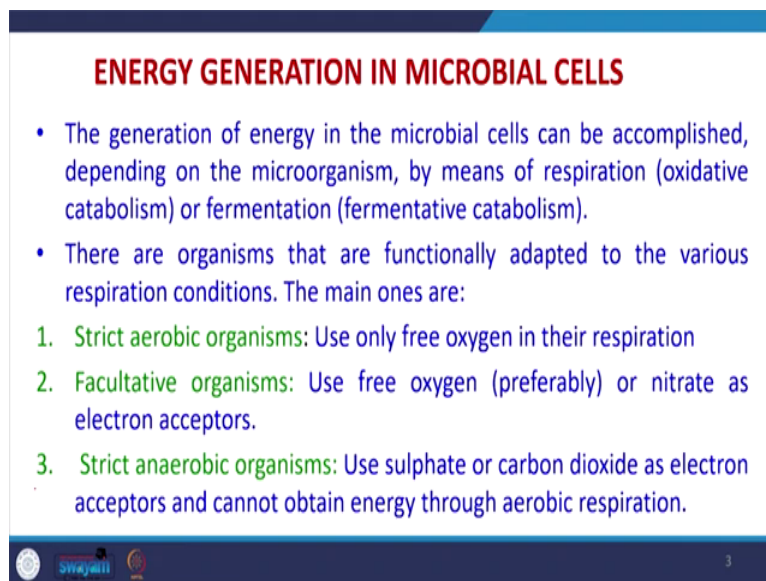
- The removal of the organic matter from sewage occurs through the process of dissimilation or catabolism.
- The two types of catabolism of interest in sewage treatment are oxidative catabolism and fermentative catabolism (fermentation of organic matter).
 - **Oxidative catabolism:** Redox reaction in which an oxidising agent present in the medium (oxygen, nitrate or sulphate) oxidises the organic matter.
 - **Fermentative catabolism:** The process occurs due to the rearrangement of the electrons in the fermented molecule in such a way that at least two products are formed.

So the removal of organic matter from sewage occurs through a process of either dissimilation or catabolism. There are 2 types of catabolism of interest in sewage treatment and one is the oxidative one and another is the fermentative one and the fermentation of organic matter via catabolism is very common. And similarly, oxidative catabolism is also common in the oxidative catabolism the redox reactions in which the oxidizing agents present in the medium they oxidize the organic matter.

So there are various types of oxidizing agents which may be present in the medium itself and these are like oxygen, nitrate, or sulfate which can actually oxidize the organic matter. So oxidative catabolism uses these oxidizing agents present in the medium themselves.

Similarly, in the fermentative catabolism the process occurs due to the rearrangement of electron in the fermented molecule in such a way that at least 2 products are formed. So we have some rearrangement of electrons so that the breakage of the organic matter takes place and we have 2 molecules getting formed from one raw material organic molecule and thus the catabolism occurs.

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ENERGY GENERATION IN MICROBIAL CELLS

- The generation of energy in the microbial cells can be accomplished, depending on the microorganism, by means of respiration (oxidative catabolism) or fermentation (fermentative catabolism).
- There are organisms that are functionally adapted to the various respiration conditions. The main ones are:
 1. **Strict aerobic organisms:** Use only free oxygen in their respiration
 2. **Facultative organisms:** Use free oxygen (preferably) or nitrate as electron acceptors.
 3. **Strict anaerobic organisms:** Use sulphate or carbon dioxide as electron acceptors and cannot obtain energy through aerobic respiration.

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Now how the energy generation takes place in the microbial cell. So this is very very important because the generation of energy in the microbial cell helps in the growth of microbial cells maintaining their various activities. So thus energy generation is very, very important aspect and this may happen depending upon the various environment also. So whether it is aerobic environment, facultative environment or anaerobic environment under all conditions the energy required. So that means there should be some different mechanisms via which the energy generation takes place under those conditions.

Now the energy generation in the oxidative catabolism takes place via respiration whereas in the fermentative catabolism it takes place via the fermentation process there are organisms that are functionally adapted to the various respiration conditions and depending upon presence of these organisms help in the generation of energy under various such condition. So there are 3 types of conditions possible one is aerobic condition, so under aerobic

condition that means the oxygen is available in plenty. So this oxygen itself is used in the respiration process for generating the energy which is used further.

Now in the facultative process the oxygen is not being supplied from outside but still some oxygen is available inside the system itself it may be free oxygen or nitrate etcetera. So they actually help in the generation of energy in the strict anaerobic condition where no oxygen is present inside the water either sulfate or carbon dioxide. They work as electron acceptor and they then we generate energy, microorganism generate energy via aerobic respiration. So energy generation in the microbial cells is very important for carrying out various activities of the microbial cell or their growth. So we required to generate energy.

Now these microbial cells may be present in the water under various conditions. These conditions are with respect to amount of oxygen present whether oxygen is present or not present. Now there are 3 conditions possible aerobic conditions under which the oxygen or air is present inside the water, there is second condition which is called facultative condition under which oxygen may be present inside the water but in limited quantity and the third condition anaerobic condition when there is no oxygen present inside the water and it is totally absent of oxygen.

Now microbial cells they can generate energy in these water conditions by various means and these means include respiration or fermentation. So in the oxidative catabolism respiration helps in the generation of energy, in the fermentative to catabolism the fermentation process helps in the generation of energy. Now organisms that are functionally adapted to these various conditions with respect to water, they generate energy under these conditions.

Now under strict aerobic conditions where strict aerobic organisms are present they actually use free oxygen for their respiration and thus generate energy. Similarly, facultative organisms they use free oxygen or nitrate as electron acceptors and thus generate energy. Similarly, in the strict anaerobic organisms which are present they cannot obtain energy via aerobic respiration does they use sulfate or carbon dioxide as an electron acceptors and thus generate energy inside the system.

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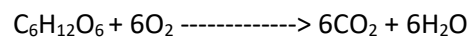
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- The main reactions for the generation of energy that occur in aerobic, anoxic and anaerobic conditions are:

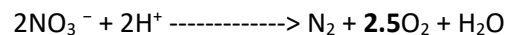
- ✓ **Aerobic conditions-**
$$\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \text{-----} \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O} + \text{Energy}$$
- ✓ **Anoxic conditions: nitrate reduction (denitrification) -**
$$2\text{NO}_3^- + 2\text{H}^+ \text{-----} \rightarrow \text{N}_2 + 2.5\text{O}_2 + \text{H}_2\text{O}$$
- ✓ **Anaerobic conditions: Sulphate reduction-**
$$\text{CH}_3\text{COOH} + \text{SO}_4^{2-} + 2\text{H}^+ \text{-----} \rightarrow \text{H}_2\text{S} + 2\text{H}_2\text{O} + 2\text{CO}_2$$

➤ The main reactions for the generation of energy that occur in aerobic, anoxic and anaerobic conditions are:

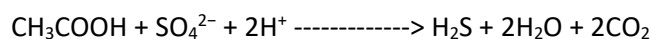
✓ **Aerobic conditions-**



✓ **Anoxic conditions: nitrate reduction (denitrification) -**



✓ **Anaerobic conditions: Sulphate reduction-**



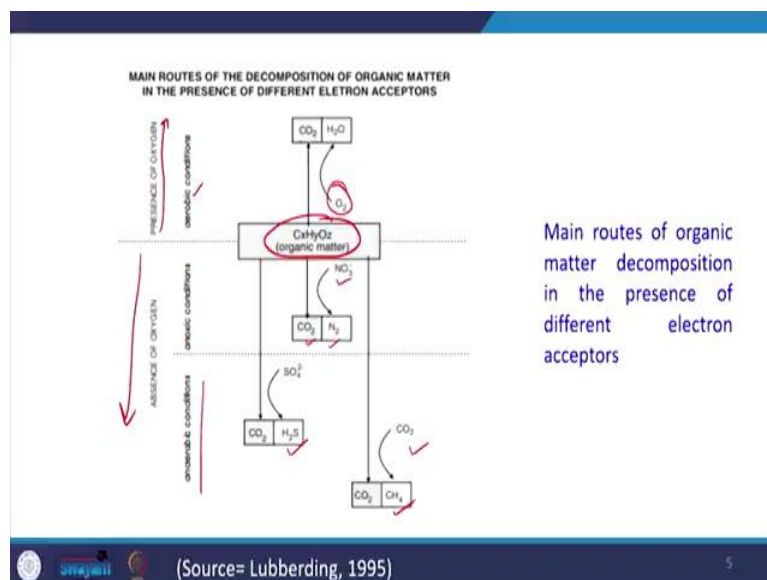
Now there are various reactions which are possible for generation of energy and under both the aerobic anoxic and anaerobic conditions. So under aerobic condition if any of the molecule is present so this molecule which is like glucose, fructose, any of these molecules or any hydrocarbon molecule that will be oxidized into CO₂ and H₂O, and will be having plus energy.

So we will be getting energy out of this system. Under anoxic condition where nitrate reduction or denitrification is one of the reaction. So here nitrate is getting reduced into nitrogen and oxygen and H₂O. So through this process we are getting energy. Similarly, under anaerobic conditions sulfate reduction is one of the examples which is given here. So we can see acetic acid plus sulfate when they are present, they actually getting reduced to H₂S.

So sulfate is getting reduced to H_2S and we are getting water as well as carbon dioxide molecule and plus energy. So we have there is one thing that under anoxic condition nitrogen will be produced, there is no CO_2 produced when the nitrate reduction is taking place under sulfate reduction condition we have H_2S which is getting produced and we can smell the odor from a distance also when the anaerobic conditions are prevailing, and when the strict anaerobic organisms are working for generating energy.

Similarly, under aerobic condition we get CO_2 and H_2O mainly and plus energy. So these are the main reactions which have which occur for the generation of energy inside various aerobic anoxic or anaerobic conditions.

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Now here again we can see the main roots of the decomposition of organic waste in the presence of different electron acceptors. So we have an organic matter which is present here, we can see $C_xH_yO_z$ a general organic molecule has been taken. Now whether we have oxygen present in the system or it is absent. So in the presence of oxygen we have aerobic conditions which are prevailing and under that this molecule will change into CO_2 and H_2O in presence of oxygen.

Now there are under anoxic conditions, the same molecule will reduce to CO_2 and N_2 when nitrate is present. So this is possible. Similarly, under strict anaerobic condition the sulfate is getting reduced to H_2S and similarly CO_2 is getting reduced to CH_4 . So we can have different molecules depending upon the conditions which are prevailing inside the water whether their

oxygen is present or oxygen is absent. So depending upon that these are the various roots of the decomposition of organic matter.

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Few important points

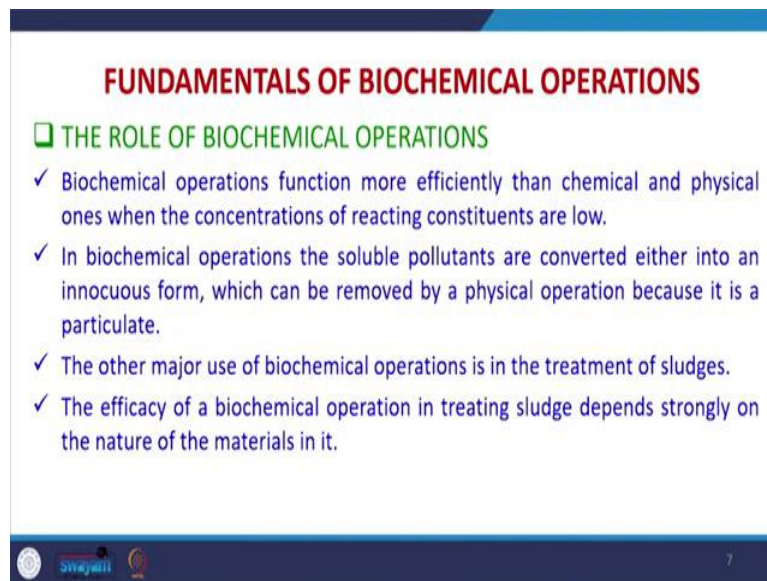
- ❖ Greater the oxidation state of final product, the greater the energy release.
 - ❖ The carbon in CO_2 is at its higher state of oxidation.
 - ❖ Therefore, oxidation reactions that oxidize the carbon in the substrate completely to CO_2 (aerobic respiration) release more energy than the reactions that produce, for example, ethanol (fermentation).
- ❖ The lower the oxidation state of the substrate, the greater the energy release.
 - ❖ For instance, the oxidation of acetic acid to CO_2 releases less energy than the oxidation of ethanol to CO_2 because the carbon in the acetic acid is at a higher oxidation state than in ethanol.
- ❖ CO_2 can never act as an energy source because its carbon is at the highest possible state of oxidation (CO_2 cannot be oxidized).

Now there are a few important points worth noting here, greater the oxidation state of final product greater is the energy release. So this has to be remembered that whatever is the oxidation state of the product, depending upon the amount of energy release is different. Now the carbon in CO_2 is at higher state of oxidation. So that is how we get energy released therefore oxidation reactions that oxidize the carbon in the substrate completely to CO_2 release more energy than reactions that produce like ethanol or CO etcetera.

So that means, the reaction where CO_2 is getting formed that will give more energy as compared to the reaction in which ethanol is getting formed that mean the fermentative process. So we have lesser release of energy the lower the oxidation state of the substrate greater the energy release. So that was the for instance the oxidation of acetic acid to CO_2 releases less energy than the oxidation of ethanol to CO_2 because carbon in acetic acid is at a higher oxidation state than the ethanol these 2 points are very important and they may be considered a general point which help in understanding that which reaction will be releasing more energy.

Now CO_2 can never act as an energy source because its carbon is at the highest state. So if CO_2 is present so CO_2 will not release any energy. So energy will be released when we have some carbonaceous source where $\text{C}_x\text{H}_y\text{O}_z$ some such structures are present. So CO_2 itself cannot be oxidized, this has to be noted.

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FUNDAMENTALS OF BIOCHEMICAL OPERATIONS

THE ROLE OF BIOCHEMICAL OPERATIONS

- ✓ Biochemical operations function more efficiently than chemical and physical ones when the concentrations of reacting constituents are low.
- ✓ In biochemical operations the soluble pollutants are converted either into an innocuous form, which can be removed by a physical operation because it is a particulate.
- ✓ The other major use of biochemical operations is in the treatment of sludges.
- ✓ The efficacy of a biochemical operation in treating sludge depends strongly on the nature of the materials in it.

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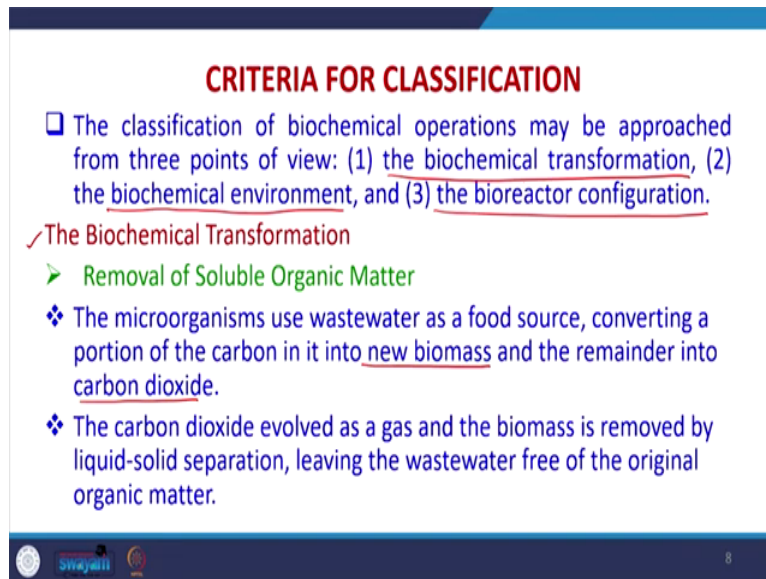
Now going further to the next section we will try to understand the fundamentals of some of the biochemical operations and the role of the biochemical operations. So biochemical operations function more efficiently than the chemical and physical ones when the concentration of the reactant constituents are low. So that means when a wastewater is having suppose very high amount of organic content.

So under those conditions it will be better maybe to use the physical or chemical system when the concentration of the organic molecule is lesser it is better to use the biochemical operations as compared to physicochemical processes. Also in the biochemical operations the soluble pollutants are converted either into innocuous form, which can be removed by physical operation because it is a particulate or something.

So the soluble pollutants are converted into form which is nontoxic and thus we can remove them and they do not affect any of the system. So it is easier to remove and they actually does the toxicity is getting reduced. The other major use of biochemical operation is in the treatment of sludge that we will be studying in the later section of this course the efficacy of biochemical operation in treating sludge depends strongly upon the nature of materials present in the sludge itself.

So in the biological treatment processes we have a lot of sludge which is getting generated and that is during primary treatment and also during biological treatment itself, whether aerobic or other types of anaerobic or facultative. So sludge will vary and the materials content in that sludge will also vary depending upon the operation that we are performing.

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CRITERIA FOR CLASSIFICATION

- ❑ The classification of biochemical operations may be approached from three points of view: (1) the biochemical transformation, (2) the biochemical environment, and (3) the bioreactor configuration.
- ✓ **The Biochemical Transformation**
 - **Removal of Soluble Organic Matter**
 - ❖ The microorganisms use wastewater as a food source, converting a portion of the carbon in it into new biomass and the remainder into carbon dioxide.
 - ❖ The carbon dioxide evolved as a gas and the biomass is removed by liquid-solid separation, leaving the wastewater free of the original organic matter.

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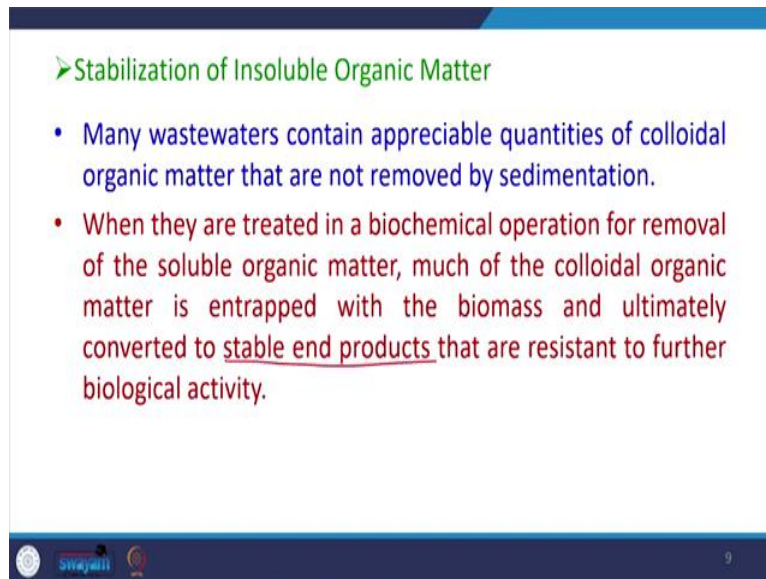
Now the biochemical operations can be classified into different processes and the classification depends upon the different point of views and these point of views are like the biochemical transformation process which is occurring and the biochemical environment where it is there. And bioreactor configuration. So these factors actually tell us that how we can classify the biochemical operations.

The biochemical transformation in the first so there are different possibilities which are there during the biochemical transformation. One is removal of soluble organic matter, the microorganisms which are present in the water use the organic matter inside the water as a food source and they convert a portion of the carbon present inside the water into new biomass.

So first thing that they generate new biomass and then the remainder is converted into carbon dioxide. So the soluble organic matter will generate new biomass as well as the carbon dioxide the carbon dioxide gets evolved as a gas and the biomass is removed by the solid liquid separation process leaving the wastewater free of the organic matter.

So any organic soluble organic matter which is present inside the water it is converted into new biomass plus carbon dioxide, carbon dioxide because of enhanced solubility we will go outside the water. And new biomass that is separated via liquid solid separation process.

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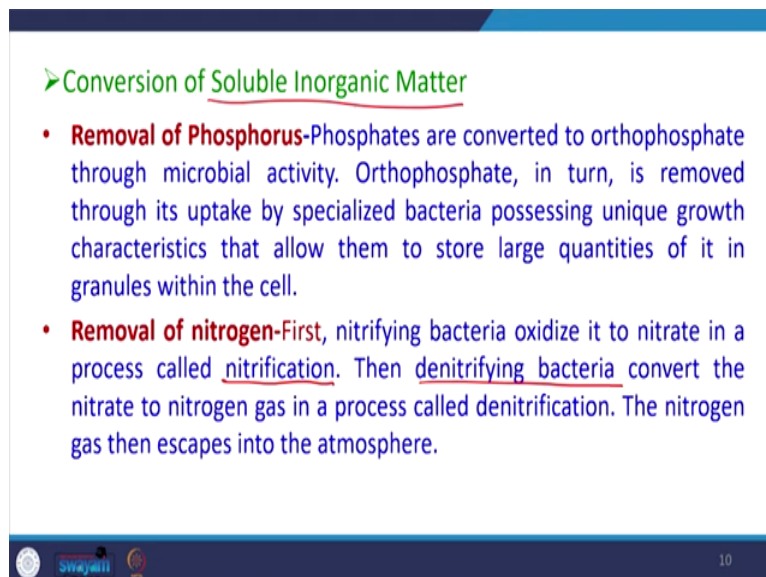
➤ **Stabilization of Insoluble Organic Matter**

- Many wastewaters contain appreciable quantities of colloidal organic matter that are not removed by sedimentation.
- When they are treated in a biochemical operation for removal of the soluble organic matter, much of the colloidal organic matter is entrapped with the biomass and ultimately converted to stable end products that are resistant to further biological activity.

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Now the insoluble organic matter present inside the wastewater it is also stabilized. So many wastewater contains very high quantity of colloidal organic matter that cannot be removed by sedimentation. So when they are treated in a biochemical operation for removal of soluble organic matter much of the collider organic matter is entrapped within the biomass and ultimately converted to stable end products that are resistant to further biological activity. So they become refractory in nature or they cannot further be converted into any other form. So they remain as such so they are separated out by a solid liquid separation process.

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➤ **Conversion of Soluble Inorganic Matter**

- **Removal of Phosphorus**-Phosphates are converted to orthophosphate through microbial activity. Orthophosphate, in turn, is removed through its uptake by specialized bacteria possessing unique growth characteristics that allow them to store large quantities of it in granules within the cell.
- **Removal of nitrogen**-First, nitrifying bacteria oxidize it to nitrate in a process called nitrification. Then denitrifying bacteria convert the nitrate to nitrogen gas in a process called denitrification. The nitrogen gas then escapes into the atmosphere.

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Now conversion of soluble inorganic matter so we have soluble organic matter now soluble inorganic matter. So what happens to this soluble inorganic matter now within this we can

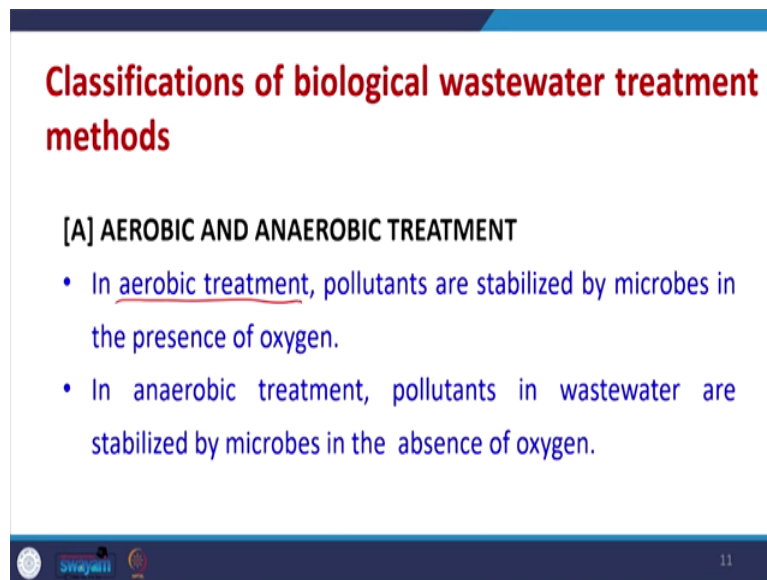
consider phosphorus we can consider nitrogen. Now phosphorus which may be present in the form of phosphates are converted into orthophosphate through microbial activity.

So orthophosphate is further removed through its uptake by specialized bacteria possessing unique growth characteristics and that allows them to store large quantities of it in the granules within the cell. So that is phosphates in the water are removed via conversion to orthophosphate and there is stabilization in some form of granules.

Now nitrogen has different ways of conversion first than nitrifying bacteria oxidizes the nitrogen into nitrate in a process which is called nitrification then the denitrifying bacteria convert the nitrate which is found into nitrogen gas in a process called denitrification. So there are 2 steps in the first step where we have nitrification happening where the nitrogen present inside any of the organic matter gets converted into nitrate.

In the second step denitrifying bacteria convert the nitrate which is formed into nitrogen gas and which ultimately escapes into the environment because of the presence of the nitrogen above the saturation limit. So this will ultimately escape into the atmosphere. So this is the 2 step process via nitrogen conversion inside the soluble inorganic matter happens.

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Classifications of biological wastewater treatment methods

[A] AEROBIC AND ANAEROBIC TREATMENT

- In aerobic treatment, pollutants are stabilized by microbes in the presence of oxygen.
- In anaerobic treatment, pollutants in wastewater are stabilized by microbes in the absence of oxygen.

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Now we can further classify the biological wastewater treatment methods into various categories. Now one of them the classification is aerobic and anaerobic treatment. So in the aerobic treatment the pollutants are stabilized by microbes in the presence of oxygen whereas in the anaerobic treatment pollutants in the wastewater are stabilized by microbes in the absence of oxygen.

And we have already seen that how the conversion of different organic matter happens in the presence of oxygen so any organic matter present inside the water gets converted into carbon dioxide and H₂O plus some amount of energy is released during aerobic treatment. So this is what happens but some of the organic matter is also used for further generating new biomass also.

So we have this process which happens in the aerobic. In the anaerobic treatments the pollutants inside the wastewater are stabilized by microbes in the absence of oxygen and we can have generation of different oxygen products at different gaseous products which may happen. So like we can generate H₂O as we can generate ammonia. So depending upon the quantity and quality of organic matter present we can have different types of molecules or gaseous molecules getting generated during the anaerobic treatment.

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Classifications of biological wastewater treatment methods

[B] SUSPENDED AND ATTACHED TREATMENT

- In suspended growth process, microbes are in suspension while converting organic matter to gases and cell tissue (Activated sludge). *Aerobic*
- In attached growth, microbes are attached to inert material like rocks or sand (trickling filter). *Aerobic*

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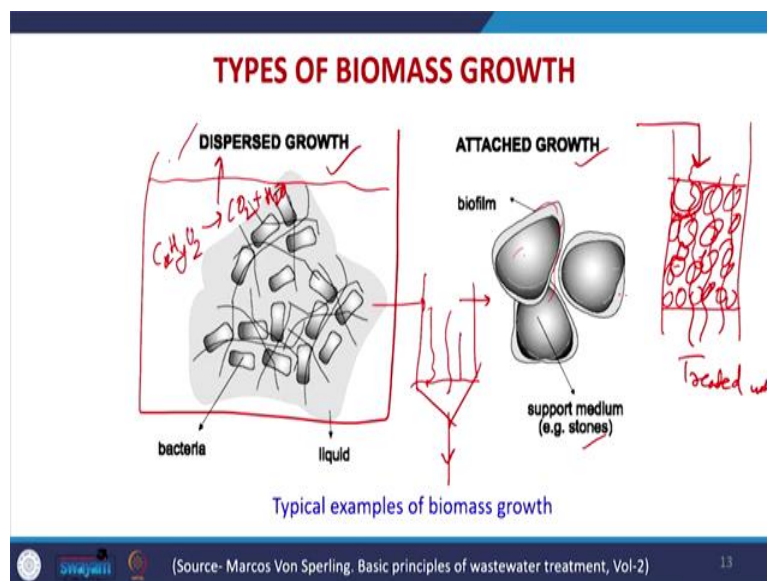
Now further the classification of biological wastewater treatment can be in the broader category of suspended and attached treatment what do they mean in the suspended growth process microbes are suspended inside the water. So that means they are in suspension while converting organic matter to gaseous and cell tissues. So this is activated sludge process and one of the example is activated sludge process which happens in the aerobic condition.

So we can have anaerobic conditions also but under aerobic condition activated sludge process is one of the most common treatment method for wastewater treatment. In the attached growth the microbes are attached to an inert material like rocks or sand. So for

aerobic treatment trickling filter is one of the examples under which the microbes are attached on an inert material it may be rock or it may be other types of packing material also.

And during that the water is actually this is dispersed from top and the water flows above these rocks and sands and the microbes which are attached to these inert molecules convert the organic matter inside the wastewater into CO_2 and H_2O . So both are aerobic conditions only but the method of treatment is different and the way of treatment is also different the efficiencies are different, there are certain advantages and disadvantages of both the systems.

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So types of biomass growth so we can see here the dispersed growth and the attached growth. So in the dispersed growth this whole system may be inside a wastewater treatment plant and this some reactor may be there. So this is one reactor we can consider and we can see the all the bacteria are dispersed all throughout this reactor. So this reactor will be here the growth of biomass will also occur as well as the organic matter present inside the system will get converted into CO_2 and plus H_2O and this CO_2 will be going off from the water surface.

So this is there and bacteria also grow during this process and this whole system is further taken and then we have a separation unit actually which will separate these biomasses the biomass will ultimately settle down and they will go below this separation unit which is called as a secondary clarifier.

So from here it is taken of and the clear water goes again so final treatment happens. In the attached growth process this we can see this biofilm of bacteria is all around this support

medium which may be stone. So the system works like this. So we have a packing system where these all these stones are packed. And so inside this suppose this is the packing system.

So we have different stones which are which are packed on all on these stones we have microbial film. So this microbial film will be grown and so water will be sent like from top and it will trickle through this system. While the water is trickling the biofilm which is there attached to this support medium between the stone or any other.

These biofilms likely oxidize the organic matter present inside the water and ultimately we get treated water here which is coming out. So this is how the system we have 2 types of system which is one is called dispersed growth or and another is called attached growth. So we have dispersed system are suspended system and other is attached system.

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SUSPENDED GROWTH

- **Dispersed growth:** The biomass grows in a dispersed form in the liquid medium, without any supporting structure
- **Systems:**
 - ❖ Stabilisation ponds and variants →
 - ❖ Activated sludge and variants → Aerobic
 - ❖ Up-flow anaerobic sludge blanket reactors (receiving wastewaters containing suspended solids).

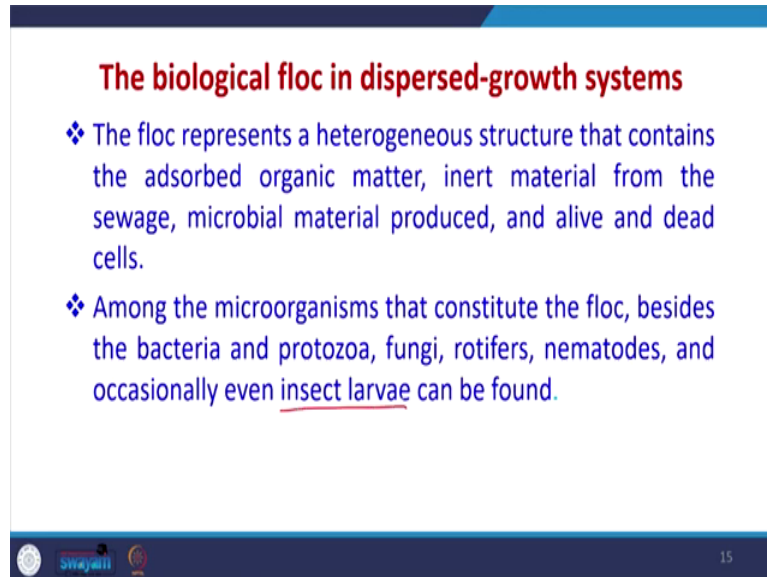
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Now in the suspended growth the biomass grows in that dispersed form in the liquid medium without any supporting structure. So we can call the dispersed system or suspended system they are same essentially the system examples include their stabilized ponds or its variants the activated sludge system or its variants up flow anaerobic sludge blanket reactor which actually works in the anaerobic condition whereas, these generally maybe aerobic systems.

Certainly the second one is aerobic system, this may change depending upon the depth of the water inside that treatment plant. So the system may be the pond may be aerobic or facultative anaerobic also if the depth is very high but generally it will be aerobic. So this is these are very common systems which we can see in our day to day life or these stabilization ponds are very common in our environment and we see very easily in villages and other

places. So they all use suspended growth system where the microorganism the biomass growth in the form of disperse form and here the treatment happens in that disperse phase itself.

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The biological floc in dispersed-growth systems

- ❖ The floc represents a heterogeneous structure that contains the adsorbed organic matter, inert material from the sewage, microbial material produced, and alive and dead cells.
- ❖ Among the microorganisms that constitute the floc, besides the bacteria and protozoa, fungi, rotifers, nematodes, and occasionally even insect larvae can be found.

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Then the biological floc, the floc which is present inside the dispersed growth systems. The floc represents a heterogeneous structure that contains the adsorbed organic matter inert material from the sewage microbial material produced during the treatment alive and dead cells also.

So among the microbes that constitute the floc besides the bacteria and protozoa, fungi, rotifers, nematodes and occasionally even insect larvae can also be present. So we have different microorganism which may be present in the biological floc overall floc biological floc which we present inside the disperse growth systems.

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ATTACHED GROWTH

- The biomass grows attached to a support medium, forming a biofilm.
- Systems with solid support for attachment:
 - ❖ Trickling filters ✓
 - ❖ Rotating biological contactors ✓
 - ❖ Submerged aerated biofilters ✓
 - ❖ Anaerobic filters ✓
 - ❖ Land disposal systems

Now there is another system which is called attached growth system. So they are various examples of attached growth systems the biomass growth attached to a support medium forming a biofilm. Now this attached inert material on which attachment happens this may be different this it may be a simple packing material, it may be rock, it may be some random packing material which is used in the chemical industry or it may be a simple plastic media also on which the attachment may be there.

So there are many examples like trickling filter the example I gave earlier then the rotating biological contractor submerged aerated bio filters, anaerobic filters also and some land disposal systems also. So we have all these systems may be used for treatment of wastewater depending whether attached growth is there or dispersed growth is there.

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Biofilm in attached growth systems

- ❖ Immobilisation is the attachment of the microorganisms to a solid or suspended supporting medium.
- ❖ The immobilization has the advantage of enabling a high biomass concentration to be retained in the reactor for long time periods.

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Now in the biofilm in the attached growth systems, it is immobilized and here the microorganisms are immobilized to a solid or suspended supporting medium. The immobilization has the advantages of enabling a high biomass concentration to be retained in the reactor for a longer time period.

However, there is this immobilization itself in the form of biofilm may take some time. So we will be studying all these suspended growth systems as well as attached growth systems for wastewater treatment in detail in the later section of this whole course.

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The diagram shows a cross-section of a biofilm. On the left is a grey block labeled 'support medium' with a red checkmark and the handwritten word 'React'. To its right is a vertical layer labeled 'biofilm', which is divided into an 'anaerobic' zone on the left and an 'aerobic' zone on the right. On the far right is 'wastewater'. Arrows indicate the movement of various substances: BOD, DO, and NH_4^+ move from the wastewater into the biofilm. NO_3^- moves from the aerobic zone to the anaerobic zone. NO_2^- , H_2S , and organic acids move from the anaerobic zone to the aerobic zone. A red double-headed arrow at the bottom indicates the overall flow of these substances.

Figure 5. Schematic representation of a biofilm

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Now we can see here the schematic representation of biofilm we can see this is a support medium which may be rock also and then we have a biofilm which is there. So inside the biofilm there may be aerobic conditions in the first stages and after that there may be anaerobic condition depending upon the thickness of the film. So we have wastewater which is it is BOD, DO, NH_4 etcetera.

And then the conversion happening and the products then coming out and going into the water itself. So this is how the treatment happens in the attached growth systems. So in today's lecture we studied regarding the how the microorganism generate energy. And then we learned about the aerobic, anaerobic and facultative processes and how the conversion happens under those conditions.

And later on we studied regarding the classification of the biochemical operations, whether it is attached growth or it is suspended growth. So there are various types of wastewater treatment systems, which work either of the 2 philosophy, so we will be studying in detail for biological treatment under aerobic condition under anaerobic condition, using suspended growth systems, as well as the attached growth systems.

Later on, we will be studying all these systems in detail. In the next lecture onwards we will be studying how to characterize waste water for it is various characteristics. So in the next lecture will start with the same thank you very much.