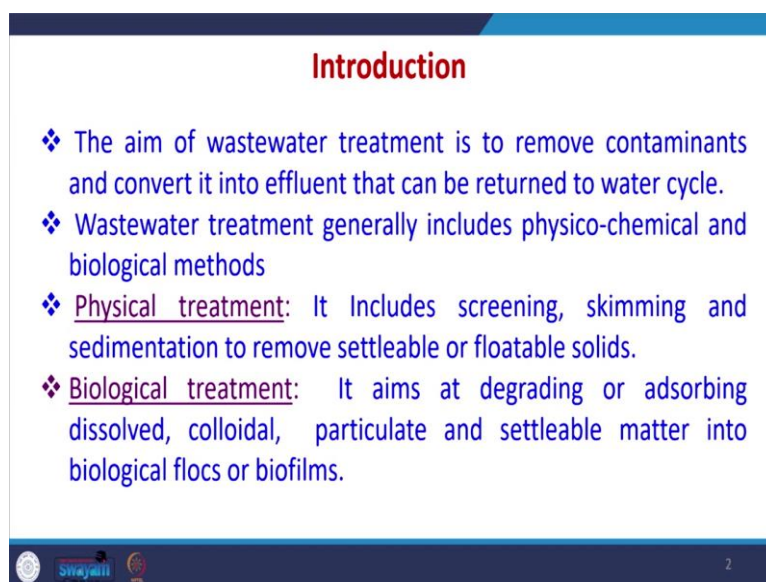


Biological process design for wastewater treatment
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Lecture: 02
Microorganisms in Biological Wastewater Treatment

Welcome to the second lecture in this NPTEL online certification course on Biological Process Designed for Wastewater Treatment. So, today we will try to learn regarding the microorganisms which are used in the biological wastewater treatment, will learn the basics of these microorganisms little bit and how they work in the biological wastewater treatment. So, before going ahead, we should understand what is the difference between the physicochemical treatment and biological treatment of wastewater.

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Introduction

- ❖ The aim of wastewater treatment is to remove contaminants and convert it into effluent that can be returned to water cycle.
- ❖ Wastewater treatment generally includes physico-chemical and biological methods
- ❖ Physical treatment: It Includes screening, skimming and sedimentation to remove settleable or floatable solids.
- ❖ Biological treatment: It aims at degrading or adsorbing dissolved, colloidal, particulate and settleable matter into biological flocs or biofilms.

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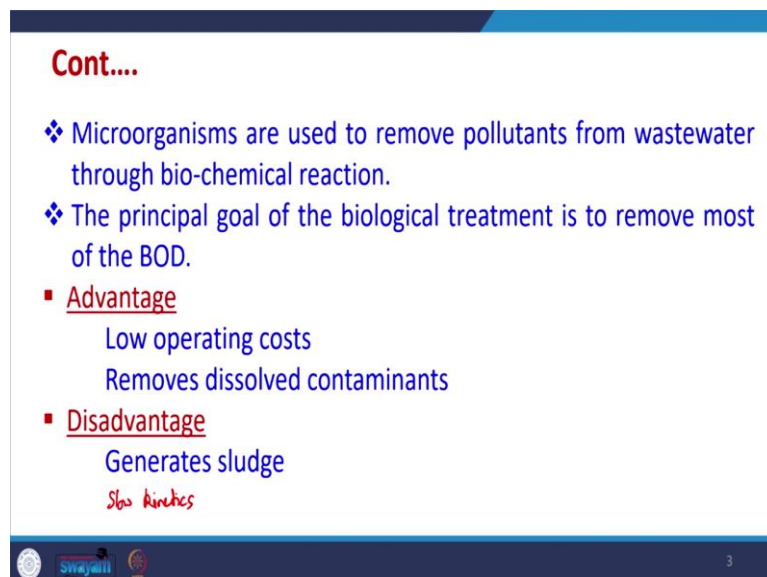
So, in the last lecture, we already understood what is the difference between water treatment and wastewater treatment. Now, for water treatment, which is used for domestic purposes, the water which is available from various sources like river or lake, reservoir et cetera from in those wastewater the organic compounds present in the water may be less. So, we generally do not require any biological treatment to happen for those wastewater treatment units, where only the solid content, some pathogens et cetera have to be removed, whereas in the industrial wastewater they contain lot of organic compounds and these organic compounds have to be totally degraded into carbon dioxide and water. So, that the oxygen demand which is there with respect to degradation of these compounds, may not happen in the river.

So, ultimately what we are doing is that we are supplying the oxygen beforehand, so, that all the organics gets converted into CO₂ and H₂O and that demand does not incur during the usual treatment in any river. So, we need to remove all the organic compounds contaminants present in the effluent before they are discharged or before it is reused. Now, wastewater treatment units generally include both physico-chemical and biological methods and physical treatment involves like screening, skimming, sedimentation, et cetera to remove the floatable or settleable solids and ultimately after physical treatment, we have to go for biological treatment.

And in the biological treatment, we want to remove the various types of organic matter present, they may be dissolved, they may be colloidal, they may be particulate in nature, they may be settleable or they may be colloidal themselves and we want to convert these compounds or these matter into CO₂ and H₂O using certain means and that certain means maybe some flocs, some biofilms, some activated sludge, et cetera. So, we always use microorganisms for biological treatment, and that treatment may be anaerobic, aerobic that thing will study.

So, little bit of the basic of these things, we should understand the how the water is treated, more detailed design of that will come later.

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- ❖ Microorganisms are used to remove pollutants from wastewater through bio-chemical reaction.
- ❖ The principal goal of the biological treatment is to remove most of the BOD.
- Advantage
 - Low operating costs
 - Removes dissolved contaminants
- Disadvantage
 - Generates sludge
 - Slow kinetics*

swajani 3

Going further the microorganisms in the biological treatment processes are used to remove pollutants from wastewater through biochemical reaction. So, that means the micro-organism has inherent characteristic to use these pollutant as substrate, actually they use these pollutant

as their food and via their own biochemical reaction inside their body, they convert these pollutant into CO_2 and H_2O using them as nutrient for their growth. So, principle, overall, the principal goal of the biological treatment process is to remove most of the biological oxygen demand that may happen later on, if we do not treat that water and it goes into directly into any aquatic body including river or reservoir. So, this is possible.

There are many advantages of biological treatment process. First thing its very operating cost is much lower as compared to physico-chemical treatment. It will remove most of the dissolved contaminants very easily, but disadvantage is that it will generate lot of sludge because micro-organism themselves will grow and that means, they have to be removed the growth which happens. So, extra all those extra micro-organism which have grown they have to be separated out so, that the micro-organism concentration inside the reactor remains the same.

Second disadvantage with respect to the use of biological treatment process is that they have slow kinetics that means, they work much slower than the usual physico-chemical treatment processes. So, under that condition same amount of wastewater has to be treated and first it will be treated in physical system then in the biological system. So, because the kinetics is slow that means, the volume will increase. So, if suppose, physico-chemical treatment requires 5 hours of treatment, whereas, biological treatment requires 30 of hours of treatment. So, that means, the volume of the biological treatment system will be approximately 6 to 10 times higher or larger than then that of physico-chemical treatment.

So, this is one of the major disadvantage, they require lot of space and volume for the unit to be installed. However, they have lot of inherent advantage that they naturally degrade the water. So, in this way, it is very, very important to understand the biological treatment of wastewater.

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Microbiology and ecology of wastewater treatment

Why study the microbiology of sewage treatment?

$\text{Organic} + \text{O}_2/\text{Air} \xrightarrow{\text{Micro}} \text{CO}_2 + \text{H}_2\text{O}$

- ❖ In a water body, organic matter is converted into inert mineralized products by purely natural mechanisms. In terms of water quality, **microorganisms play an essential role.**
- ❖ The understanding of the microbiology of sewage treatment is essential **for optimizing the design and operation of biological treatment systems.**

Swayam 4

Now, we are going to study the microbiology and ecology of wastewater treatment. And this is very important with respect to understand the microbiology of this treatment process. However, if you want to study in very great detail, then you will have to take a separate subject on microbiology and ecology. Here in the subject we will be doing only basic knowledge of micro-organisms and the micro biology and ecology aspects. So, if you want to study in detail, you will have to take maybe some other subject or course for related to this subject.

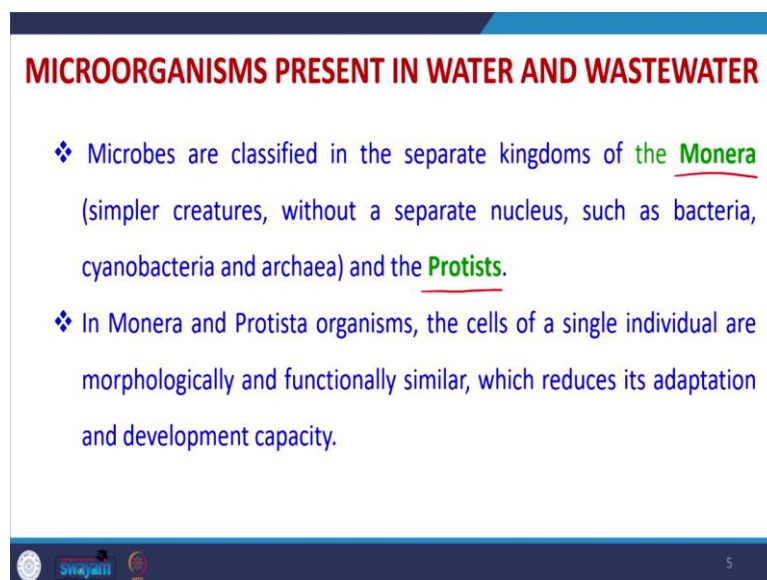
Now, in the water bodies, organic matter is converted into inert mineralized products by natural mechanisms. So, in any water body if suppose, we discharge anything or we excrete, so, all the micro-organisms which are present in the natural environment will naturally convert that organic matter into inert mineralized product or plus CO_2 and H_2O . So, any contaminant, any organic matter, what will happen, will be generally be converted into CO_2 and H_2O and it will require certainly oxygen or we can call it air, depending upon whether it is aerobic process or anaerobic process. And certainly all different types of micro-organisms should be present during this reaction when it is happen.

Now, this role of micro-organism is very important during this degradation process, it will depend upon what type of organic compounds are present in the water, whether they are highly aromatic or aliphatic whether they contain halogens or not, what type of other elements are present. So, depending upon that, whether sulfur is present or not, depending upon that the micro-organism required will be different. So, if you want to understand that, it is very essential that we should understand that how the microorganisms work under different

conditions, what is the pH at which they are going to work, what is the temperature. So, temperature, pH, concentration and the type of organic pollutants present in the water et cetera play a role.

The understanding of microbiology of sewage treatment or wastewater treatment is essential for optimizing also the design and operation of biological treatment system. Because if we can understand that, then only we can know whether that what will be the pH will be required. If suppose the optimum pH is 6 and we are operating the plant at 4 pH or 9 pH the system will not work. So, that means we will have to adjust the pH before taking the water into the system. That means we have to optimize the design and operation so that the system works efficiently at those conditions. So, that is why the microbiology is very very important for understanding.

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MICROORGANISMS PRESENT IN WATER AND WASTEWATER

- ❖ Microbes are classified in the separate kingdoms of the Monera (simpler creatures, without a separate nucleus, such as bacteria, cyanobacteria and archaea) and the Protists.
- ❖ In Monera and Protista organisms, the cells of a single individual are morphologically and functionally similar, which reduces its adaptation and development capacity.

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There are very types of micro-organisms or micros, which may be present in any other natural environment or in the wastewater and these may be classified into 2 categories. One is Monera and another is Protists. Now Monera is like simple creatures which have a separate without a separate nucleus, and these includes like bacteria, cyanobacteria, archaea et cetera, then Protista include like virus protozoa et cetera. And the in both Monera and Protista organisms, the cells of a single individual are morphologically and functionally similar, they were very similar and their morphology is simpler, but they have different, within their structures, they are different and their adaptation level may also be different and their working mechanism may also be different. So, that is why we have to understand this.

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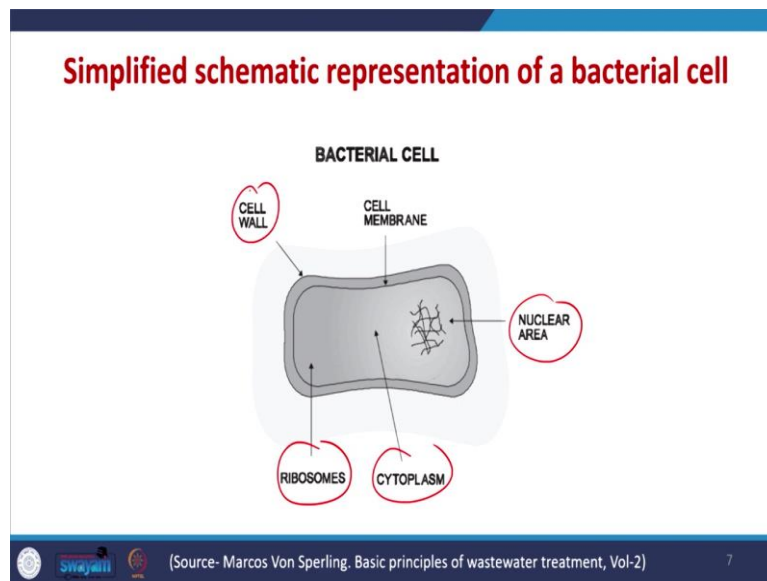
Basic characteristics of the main groups of microorganisms					
	MONERA (PROKARYOTES)		PROTISTS (EUKARYOTES)		
CHARACTERISTIC	Bacteria ✓	Cyanobacteria ✓	Algae ✓	Protozoa ✓	Fungi ✓
Nuclear membrane	Absent	Absent	Present	Present	Present
Photosynthesis	Minority	Majority	Yes	No	No
Movement	Some	Some	Some	Mobile	Immobile

Source: adapted from La Rivière (1980)

The different types of main group of micro-organisms which are used in the wastewater treatment plant or these types of micro-organisms may be present in the wastewater treatment plant, these include bacteria and cyanobacteria, which fall into the category of Monera and similarly, algae, protozoa and fungi, which fall into the category of Protists. Now, these micro-organisms have different characteristic, and this is highlighted here, like nuclear membrane is absent in the Monera, but it is present in the Protists.

Similarly, the photosynthesis activity is it happens in some of the micro-organisms it does not happen in in some of the type of micro-organisms. In protozoa and fungi there is no photosynthesis, whereas, in algae there is lot of photosynthesis. Cyanobacteria also has a good amount of photosynthesis whereas, bacteria has very minor amount of photosynthesis activity. The movement also is different in different micro-organisms, like fungi is totally immobile whereas, the protozoa is mobile and the bacteria cyanobacteria algae have some mobility. So, there is some difference in their basic characteristics of all these main group of microorganisms.

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Now, this diagram shows simplified schematic of a bacterial cell, what are the different sections of a bacterial cell and little bit basics of these we are going to study. So, a bacterial cell may be having a cell membrane, a nuclear area, a cytoplasm section and the ribosomes et cetera. Some of them may be having cell wall. Some may not be having cell walls. So, bacteria has a cell wall but other types of microbes them do not have cell wall also. So, it will depend upon the different types of micro-organisms that the schematic representation may vary a little.

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BIOLOGICAL CELLS

Structure of cell

- 1. Cell membrane**
 - ✓ The cells generally have as an external boundary a cell membrane.
 - ✓ The membrane is semi-permeable and therefore exerts an important role in selecting the substances that can leave or enter the cell.
- 2. Cytoplasm**
 - ✓ The cell's interior contains organelles and a colloidal suspension of proteins, carbohydrates, and other complex forms of organic matter.

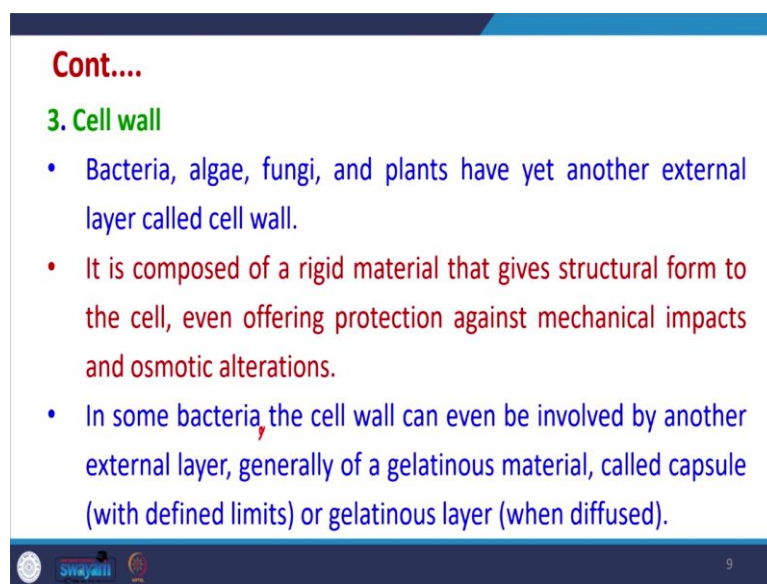
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Now, in the biological cells, the structure of cells include a many things. First thing is cell membrane, the cell membranes, the cells generally have an essential external boundary,

which is called cell membrane and it is generally semi-permeable and therefore, it exerts an important role in selecting the substance that can leave or enter the cell. So, depending upon the type of biological micro-organism, we are using, and type of cell membrane their treatment efficiency and the release mechanisms may be different.

Similarly, cytoplasm is another important section of the structure of cell. The cells interiors contain organelles, and a colloidal suspension of proteins carbohydrate and other complex forms of organic matter which actually fall into the cytoplasm section of the structure of the cell.

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3. Cell wall

- Bacteria, algae, fungi, and plants have yet another external layer called cell wall.
- It is composed of a rigid material that gives structural form to the cell, even offering protection against mechanical impacts and osmotic alterations.
- In some bacteria, the cell wall can even be involved by another external layer, generally of a gelatinous material, called capsule (with defined limits) or gelatinous layer (when diffused).

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Similarly, cell wall may be present in some of the micro-organism it may not be present in some. So, bacteria, algae, fungi and plants have cell walls. It is composed of a rigid material that give structural form to the cell even offering protection against mechanical impacts and osmotic alteration because of the change in osmotic pressure et cetera. So, cell wall is there. In some bacteria the cell wall can even be involved by another external they are generally of a gelatinous material called capsule. So, in some of the bacteria, there can be another layer maybe there which is called as capsule, with defined limits or gelatinous layer which may be diffused in nature.

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4. Nucleic acids

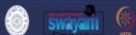
- Each cell contains nucleic acids, a genetic material vital for reproduction. Ribonucleic acid (RNA) is important for synthesizing proteins and is found in the ribosome present in the cytoplasm.
- The cytoplasm of the prokaryotic cells frequently contains DNA in small structures called plasmids.



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ECOLOGY OF BIOLOGICAL WASTEWATER TREATMENT

- The role played by microorganisms in sewage treatment depends on the process being used.
 - The microbial mass involved in the aerobic processes consists mainly of bacteria and protozoa.
 - Other organisms, such as fungi and rotifers, can also be found, but their importance is lower.
- Rotifers (also called wheel animalcule) are efficient in consuming dispersed bacteria and small particles of organic matter.
 - Their presence in the effluent indicates an efficient biological purification process.



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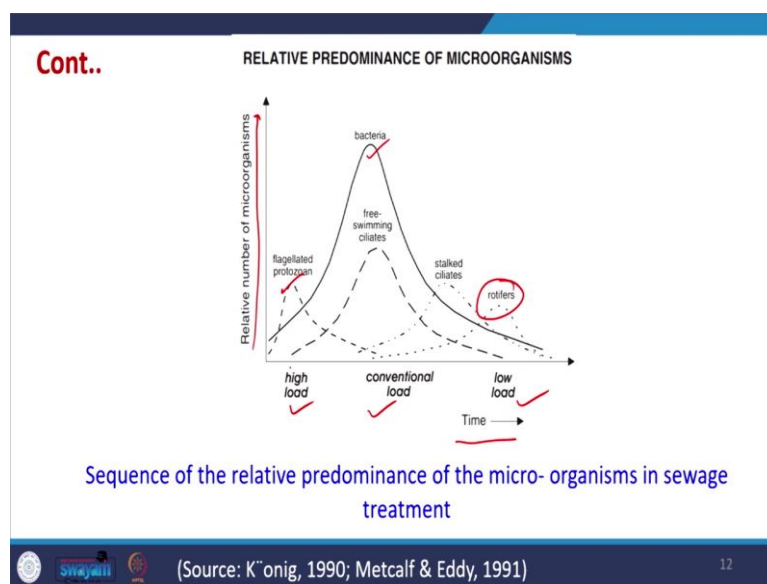
Now going further nucleic acid is another section, each cell contains nucleic acids, a genetic material vital for reproduction. So, RNA is important for synthesizing proteins and is found in ribosome present inside the cytoplasm. So, the cytoplasm of the prokaryotic cells frequently contains DNA in a small structure called plasmid. So, these are the different sections of micro-organism or bacterial cells et cetera. Going further what is the ecology of biological wastewater treatment, since we are concentrating on the biological wastewater treatment, microbiology and ecology both are important. The role played by micro-organism in sewage treatment plants depend upon the process being used.

So, whether we are using activated sludge process or biological which type of process we are using, whether it is aerobic, anaerobic, so, depending upon that the treatment efficiency may

be different. The microbial mass involved in aerobic processes consists of bacteria and protozoa. So, this if aerobic treatment is happening, these microorganisms will be involved, other micro-organisms such as fungi and rotifers can also be found, but their importance is lower.

Now, rotifers which are called as wheel animalcule, if they are efficient in consuming the dispersed bacteria and small particles of organic matter and if they are present in the effluent which is coming out of the treatment indicates that efficient biological process has happened during the treatment in the plant itself.

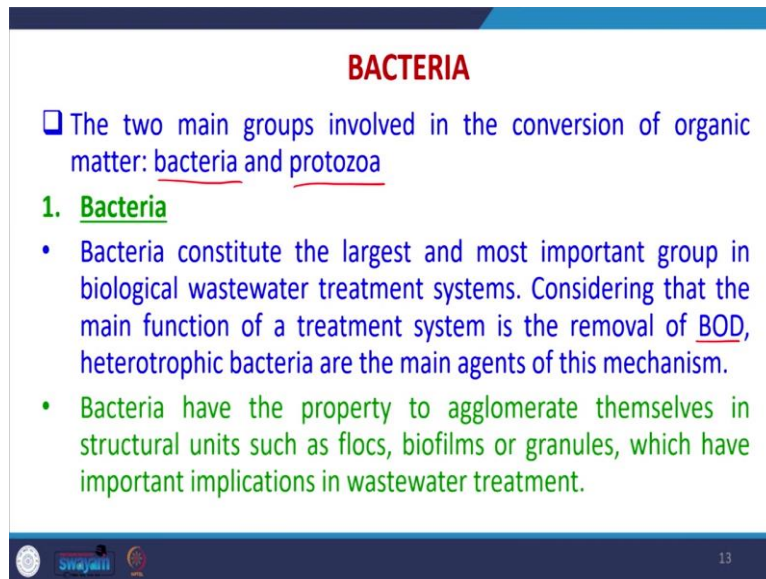
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Now, this is how with time the relative number of micro-organism change. So, initially it will depend upon the high load, conventional load and low load also. So, it will vary depending upon the type of load and the time and so, we can have protozoa initially present then we can have bacteria, which will first increase and then decrease. Ultimately, we will be having rotifers. So, that means, if you have rotifers, it means in the effluent that the overall biological process has been working well. So, this is the overall sequence of the relative predominance of the micro-organism with time in the sewage treatment plant. And it will certainly depend whether we have high load, conventional load or low load.

High load will be there more in the industrial wastewater, and low load will be there where scarcity of people, are there not much committees there, but water generated is more. So, this is what there. Now little bit of the micro-organism, we are going to study.

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BACTERIA

- The two main groups involved in the conversion of organic matter: bacteria and protozoa

1. Bacteria

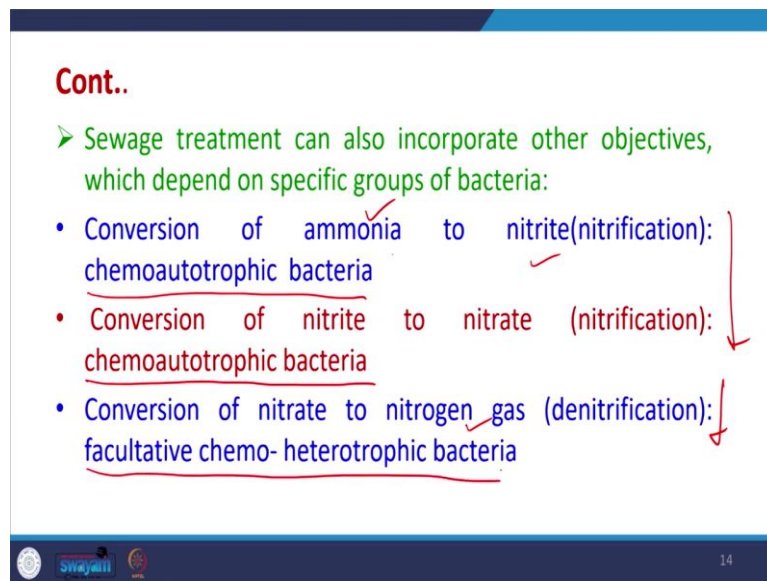
- Bacteria constitute the largest and most important group in biological wastewater treatment systems. Considering that the main function of a treatment system is the removal of BOD, heterotrophic bacteria are the main agents of this mechanism.
- Bacteria have the property to agglomerate themselves in structural units such as flocs, biofilms or granules, which have important implications in wastewater treatment.

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The 2 main groups which are involved in the conversion of organic matter, they are bacteria, and protozoa. Now we will try to understand little bit of bacteria and protozoa in today's lecture. Bacteria actually constitute the largest and most important group in the biological wastewater treatment system. And their main function is to remove the BOD. BOD means biological oxygen demand or biochemical oxygen demand. And we will understand regarding BOD later on during the characterization and these bacteria actually helping the removal of BOD. Heterotrophic bacteria are the main agents for this mechanism.

Bacteria have the property to agglomerate themselves and they form in structure unit such as flocs so, they will actually come together and form a bigger floc, biofilm or granules. So, depending upon the type of treatment system, they may be present in the form of floc and in the form of biofilm like in trickling filter, we use biofilm rotating disk we use biofilm, they may be also in the form of granules in the activated sludge process. So, bacteria are one of the most important class of micro-organism which help in the conversion of organic matter.

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➤ Sewage treatment can also incorporate other objectives, which depend on specific groups of bacteria:

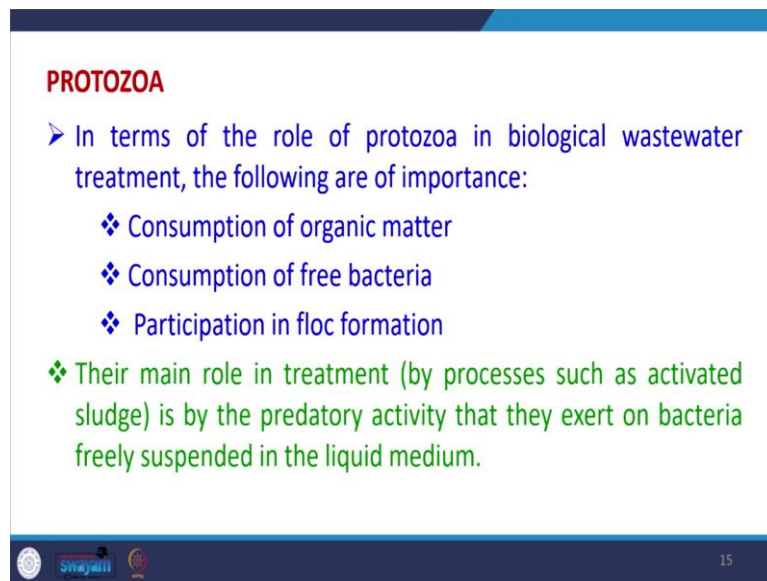
- Conversion of ammonia to nitrite (nitrification):
chemoautotrophic bacteria
- Conversion of nitrite to nitrate (nitrification):
chemoautotrophic bacteria
- Conversion of nitrate to nitrogen gas (denitrification):
facultative chemo- heterotrophic bacteria

The slide includes handwritten red annotations: checkmarks above 'ammonia' and 'nitrite', underlines under the bacterial groups, and red arrows pointing downwards from the right side of each bullet point.

Continued further the bacteria a sewage treatment can also incorporate other objectives depending upon the specific group of bacteria, what type of bacteria we are using. Suppose the water contains nitrogenous compounds also. So, that means will generate ammonia, nitrite et cetera. So, if you want to convert ammonia into nitrite that means, this process is called nitrification. So, we need to have chemoautotrophic bacteria. Now, if you want to convert nitrite into nitrate that means nitrification again, again the chemoautotrophic bacteria will be required.

However, if we want to convert a nitrate into nitrogen gas further that means, first nitrification is happening then denitrification is happening. If suppose we want to convert ammonia to nitrogen gas then we require facultative chemo-heterotrophic bacteria. So, depending bacteria not only convert carbon into degrade the carbonaceous compound they also help in the treatment of those wastewater which contain lot of nitrogenous compounds. So, this is there.

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PROTOZOA

- In terms of the role of protozoa in biological wastewater treatment, the following are of importance:
 - ❖ Consumption of organic matter
 - ❖ Consumption of free bacteria
 - ❖ Participation in floc formation
- ❖ Their main role in treatment (by processes such as activated sludge) is by the predatory activity that they exert on bacteria freely suspended in the liquid medium.

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Now, protozoa in terms of the role of protozoa and biological wastewater treatment, there are many important things. They help in the consumption of organic matter, they actually consume the free bacteria which is available, they participate in the floc formation or granule formation. So, they help in that process. So, it overall helps in the treatment efficiency. Their main role in the treatment such as activated sludge is by predatory activity that they exert on the bacteria freely suspended. So, this way, they actually remove these bacteria which are freely suspended, which are not working. So, this way they are helping in the overall improving the efficiency of the treatment process.

Now, there are certain aspects which are important for microbial cells, what is the if they have to survive and they have to grow they require carbonaceous source or substrate materials. Similarly, they require energy for their growth.

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ENERGY AND CARBON SOURCES FOR MICROBIAL CELLS

- In terms of the carbon source, there are two fundamental organism types:
 - ✓ *Autotrophic organisms*- Carbon source: carbon dioxide (CO₂)
 - ✓ *Heterotrophic organisms*-Carbon source: organic matter ✓
- In terms of the energy source, there are two basic organism types:
 - ✓ *Phototrophic organisms*-Energy source: light ✓
 - ✓ *Chemotrophic organisms*-Energy source: energy from chemical reactions.

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So, what are the energy and carbon sources for microbial cells? So, this is there. In terms of carbon source there are 2 fundamental organism type. So, one is autotrophic organism. The carbon source may be carbon dioxide. Then there is a heterotrophic organism the carbon source is organic matter. So, if we are going for wastewater treatment heterotrophic organisms which require organic which use organic matter as carbon source are very important.

Similarly, with respect to energy source because they have to grow and they require food and as well as energy. So, the energy so, there are 2 types of basic organism types. One is phototrophic another is chemotrophic. So, phototrophic use light as the energy source whereas, the chemotrophic use chemical reactions as the energy source. So, we it is in the wastewater treatment depending upon the treatment study, generally, the chemotrophic organisms will be favorable because they are using chemicals for their energy and they are using chemical and they are converting them in getting energy via chemical reactions using all those organic matters which are in a way substrate to these materials, these micro-organism.

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- In most sewage treatment processes light does not penetrate significantly due to the high turbidity of the liquid. So the presence of microorganisms that have light as energy sources is extremely limited.
- Therefore, the organisms of real importance, in this case, are the chemo-autotrophs (responsible, for example, for nitrification) and the chemo-heterotrophs (responsible for most of the reactions that occur in biological treatment).



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ENERGY AND CARBON SOURCES FOR MICROBIAL CELLS

- ☐ In terms of the carbon source, there are two fundamental organism types:
 - ✓ *Autotrophic organisms*- Carbon source: carbon dioxide (CO₂)
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- ☐ In terms of the energy source, there are two basic organism types:
 - ✓ *Phototrophic organisms*-Energy source: light ✓
 - ✓ *Chemotrophic organisms*-Energy source: energy from chemical reactions. ✓



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In most sewage treatment processes, light does not penetrate significantly due to high turbidity. So, any wastewater generally is opaque in nature, it is not transparent, so, that means water cannot penetrate because of the high turbidity. So, the presence of micro-organism that have light as energy source is extremely limited. So, we always try to see that we use chemotropic micro-organism. The organism of real importance in this case are the chemo-autotrophs responsible for example for nitrification. Chemo-heterotrophs responsible for most of the reaction that occur in the biological treatment.

So, we always will go for chemo-heterotrophs, chemo from here, which use chemical reactions as energy source and heterotopic which use organic matter as carbon source. In some other places where the research is going on for utilizing CO₂ et cetera autotropic

organisms, which use carbon dioxide have more importance, but in the wastewater treatment, generally, the chemotropic and heterotrophic organisms have more important.

So, in today's lecture with a little bit is studied regarding the micro-organisms which are present in the wastewater. There is a lot of importance of microbiology and ecology to be studied. In general, the bacteria are the most important for treatment of wastewater for biological wastewater treatment and within bacteria, there are different types of bacteria. So, we require heterotrophic bacteria, which require carbon as a organic matter as a carbon source.

And similarly, which chemotropic bacteria hetero chemotrophic bacteria which require chemical reactions for generating energy. In the next lecture, we will be studying regarding the metabolism of these micro-organisms and also some more important aspects before going further for characterizing the understanding the characteristics of wastewater with respect to biological aspects. So, today we will end this lecture with this. Thank you very much.