

Wastewater Treatment and Recycling
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Lecture – 29

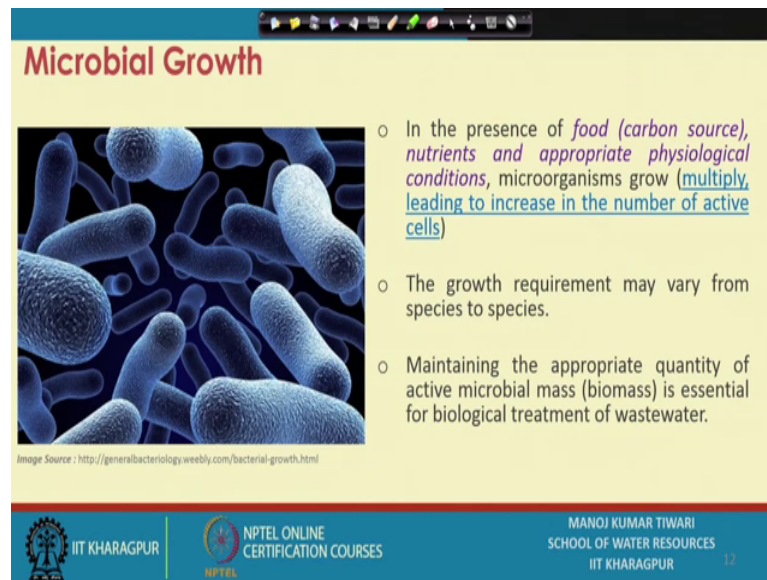
Biological Treatment of Wastewater : Microbial Growth and its Kinetics

Hello friends. So, we were discussing about the Biological Treatment of Wastewater in the previous lecture. The biological treatment essentially the, are used in the secondary treatment processes where our major objective is the removal of organic matter. So, in this particular lecture we are going to talk about some of the basic aspects which are essential to understand, how the biological treatment process works.

So, we will be basically talking about the microbial growth and it is kinetics and it is very important to understand this in order to design the biological treatment systems. Eventually the biological treatment systems are governed by the ability and actions of microorganisms to decompose and degrade the target organic contaminants. So, how they are going to degrade or decompose a specific type of contaminant or a type of particular type of organic matter, will depend on certain inherent properties of the microbial species which is involved in that degradation or decomposition process. And essentially, it will be basically how many cells are present; at what rate they can degrade or decompose the organic matter; what are the kinetics of substrate utilization or the pollutant degradation, what is the kinetics of biomass growth?

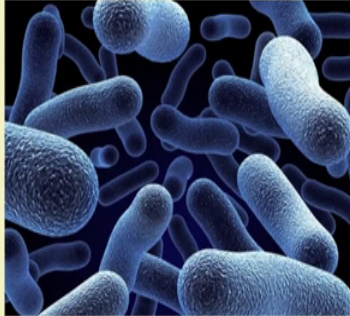
So, all these things are very essential in order to understand that how much contact time should be given, what time is enough. So, that will eventually help us in sizing the reactor, otherwise, how would we know what flow rate is good whether we should keep bacteria in contact with the water for half an hour or 2 hour or 4 hour. So, all those things will eventually govern by the, it is kinetics of the micro Microbial processes. So, that is what we are going to discuss in this particular lecture.

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The slide is titled "Microbial Growth" in red text at the top left. Below the title is a photograph of various blue, rod-shaped and spherical microorganisms. To the right of the image is a bulleted list of three points. The first point is underlined and includes the words "multiply" and "leading to increase in the number of active cells" in blue. The second point is a simple statement. The third point is also a simple statement. At the bottom of the slide, there is a footer with logos for IIT Kharagpur and NPTEL, and the name and affiliation of the presenter, Manoj Kumar Tiwari.

Microbial Growth



- In the presence of *food (carbon source), nutrients and appropriate physiological conditions*, microorganisms grow (multiply, leading to increase in the number of active cells)
- The growth requirement may vary from species to species.
- Maintaining the appropriate quantity of active microbial mass (biomass) is essential for biological treatment of wastewater.

Image Source : <http://generalbacteriology.weebly.com/bacterial-growth.html>

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To begin with the microbial growth, if we see essentially is the process where the, what is ever microbial cells are present, they utilize the food which is essentially carbon source. So, they utilize the food and the presence of all the essential nutrients and appropriate physiological conditions.

So, they have to have a suitable temperature, they have to have a suitable pH range, they have to have a all the essential nutrients. So, when all these things are present, when there are all the essential nutrients are present, the conditions are favorable, the environmental conditions including temperature pH or other physiological conditions are favorable and there is the source of food and energies available in the system, then microorganisms utilize that and they grow. So, how they grow? It is not the growth in size as we are a small kid and then slowly, slowly, slowly growing to the adults and that way the microbial growth essentially refers to the multiplication or like the microbes typically multiply.

So, how their cells are multiplying, ok? So, how many number of cells are increasing in a unit time? So, it is essentially the number of viable cells instead of the size of the cell or those kind of things.

So, when we say the microbial growth, we are essentially talking about the, the increase in the number of microbial cells.

So, this growth requirement may vary from the species to species. Some species will have a particular preference for organic matter, some may not work on that particular type of farming matter, some species works in a higher temperature range. As we discussed in the last class also that there are a species which works thermophilics, which works as a higher temperature mesophilic, which works at a moderate temperature then psychrophilic which works at a lower temperature. Similarly, we have aerobic species, anaerobic species depending on the presence of oxygen or the absence of oxygen, we have autotrophs, chemotrophs. So, what like from where they are getting the energy, what is the source of carbon for them?

So, all those things essentially suggest that the different species or different type of microorganisms, different class of microorganisms may essentially have different requirements for the growth, ok. And maintaining the appropriate quantity of microorganisms or rather we can say the viable microbial cells or the active microbial mass is very important, is very essential for the biological treatment of wastewater.

Why it is essential? Because this treatment is eventually been attained by the action of microorganisms. So, we have to have we have to kind of maintain a good healthy microbial consortium in the reactor in the system. So, that there are enough number of active microbial cells are present in order to take part in the decomposition or degradation process.

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Microbial Growth: Requirements

Nutritional Requirements For Microbial Growth

- ✓ **Carbon source:** For metabolism and synthesis of new cells – Depends on type and nature of microbes.
- ✓ **Minerals:** Principle elements – N, S, P, K, Mg, Ca, Fe, Na, etc. Trace elements – Zn, Mn, Mo, Se, Co, Ni, Cu, etc.
- ✓ **COD : N : P ratio:** 100:10:1-5 (for aerobic); 350:5:1 (for anaerobic)
C:N:P

The nutrient requirement is lower for anaerobic process due to lower growth rate of microorganisms as compared to aerobic process.

Image Source : Fundamentals of Microbiology, by Pommerville

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Now, if we see, as we are discussing the requirements for growth. So, the, there are different type of requirement, there are nutritional requirement. So, one needs a carbon source, microorganisms need a carbon source for metabolism and synthesis of new cell. Now this will depend on the type of nature; obviously, again depend on type and nature of the microbes, what kind of source they are using? So, like whether they are autotrophs or they are heterotrophs? If they are autotroph, so, whether they are photoautotrophs using sunlight or chemoautotrophs carbon? So, kind of for energy then whether they are using organic carbon, organic compounds or see you throw at that kind of like the different composition, different things can be made of. So, what are their requirement can actually be derived from that.

Then there are requirement of minerals. So, there are certain principal minerals, main minerals one needs of course, N, P, K is required, then sulphur is needed, magnesium, calcium, iron, sodium, chlorine. So, all these things are the principal elements which are essential for the microbial growth. Where are there are certain trace elements which are also needed in a very small quantities zinc, manganese, molybdenum, cobalt, nickel, copper. So, all those things, these kind of trace elements are needed and then the major nutrients are needed.

So, we can call that like macro and micro nutrients as well at times. Then there is a carbon, nitrogen and phosphorus ratio is needed. So, there has to be an appropriate C M P ratio, ok.

So, COD, essentially is the reflective of total organic carbon. So, that way we can say that COD N P ratio is essential. So, for aerobic one needs 100 is to 10 is to 1 to 5, while for anaerobic 350 is to 5 is to 1 is the one which generally or broadly is recommended.

The nutrient requirement is lower for anaerobic processes due to the lower growth rate of microorganisms. So, in anaerobic the rate of growth of microorganism is essentially very low as opposed to the aerobic processes. So, that is why, the requirement of nutrient is also very low, you can see here it is 100 verses 10 verses 1 to 5 whereas, this is 350 is to 5 is to 1.

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Microbial Growth: Requirements

Carbon and Energy Sources

		Energy source	
		Light (photo-)	Chemical compounds (chemo-)
Carbon source	Carbon dioxide (auto-)	Photoautotrophs <ul style="list-style-type: none">Plants, algae, and cyanobacteria use H₂O to reduce CO₂, producing O₂ as a by-productGreen sulfur bacteria and purple sulfur bacteria do not use H₂O nor produce O₂	Chemoautotrophs <ul style="list-style-type: none">Hydrogen, sulfur, and nitrifying bacteria, some archaea
	Organic compounds (hetero-)	Photoheterotrophs <ul style="list-style-type: none">Green nonsulfur bacteria and purple nonsulfur bacteria, some archaea	Chemoheterotrophs <ul style="list-style-type: none">Aerobic respiration: most animals, fungi, and protozoa, and many bacteriaAnaerobic respiration: some animals, protozoa, bacteria, and archaeaFermentation: some bacteria, yeasts, and archaea

Image Source : Microbiology, by Robert W. Bauman

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So; obviously, the nutrient requirement is lower in the anaerobic processes because of the lower growth rates. There is carbon and energy sources, ok. So, for carbon source whether it is using carbon dioxide as a carbon source or organic compounds.

So, if it is using organic compound, it is heterotrophs carbon dioxide from that way it is autotrophs, it is using from the light, so, then it is photoautotrophs, if it is using from the chemical compounds, it becomes chemoautotrophs and similarly we will have photo heterotrophs and chemo heterotrophs depending on what energy source and what carbon source the microbial consortium or the elements are using. So, that is about the need of the carbon source. Then there is temperature requirements, ok.

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Microbial Growth: Requirements

Temperature

- Temperature controls the rate of biochemical reactions and the integrity of protein structures, therefore, affects enzymatic activities.
- Enzyme activities increases with increasing temperature, however **high temperature kills microorganisms** by denaturing enzymes, by inhibiting transport carrier molecules or by change in membrane integrity.
- Various microbial species typically shows characteristic temperature dependence and possesses its own cardinal temperatures i. e. minimal, maximum and optimal growth temperatures.

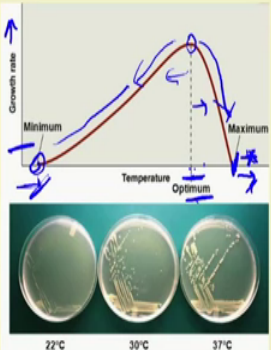


Image Source : Microbiology, by Robert W. Bauman

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So, temperature controls the rate of biochemical reactions, we have discussed this earlier as well. So or typical rate of biochemical reactions will be governed by temperature and various other physiological parameters, but temperature is one very important parameter in that. Further, it also sort of controls the integrity of protein structures. At high temperatures, there is disintegration of these protein structures leading to like this protein gets denatured and leading to sort of cells become dead because when the protein are exhaust cellular protein or intracellular protein becomes denatured, cells cannot survive that way.

So, that way temperature controls and in overall it affects the enzymatic activities because of the controlling the rate of biochemical reactions and controlling the sort of existence or the status of protein structures or various other that way. So, enzyme activities increased typically with increasing temperature. So, for a given set of microbial consortium, if we increase the temperature, normally what is seen is that they are, they become more active their enzymatic activities increases and at higher temperature the biochemical reaction rate also increases and that leads to the higher reaction rates eventually.

However, it is not just one way process because as we were saying that it all the temperature also sort of controls the integrity of the protein structures. So, when the temperature becomes too high, it can kill the microorganisms by denaturing enzymes, or

by inhibiting the transport carrier molecules or by change in the membrane integrity. So, these things get affected with the temperature and as a result at higher temperature to higher temperature, it could actually be sort of microbes cannot survive or could actually become dead as well.

So, that way if you see, there is a minimum temperature below which you would not see any microbial activities or any growth rate. So, if this is scale ease of or growth rate, so, below this temperature there is no growth rate. So, there has to be, has there have to have a minimum temperature at which the microbes can grow, ok. Then, there is a maximum temperature above which again you will not see any growth rate because the higher, at that higher temperature it can kill the microorganism. So, you will not see rather growth that way k or it can denature the enzymes or inhibit the protein these thing.

So, there are various ways through which the growth is prevented above certain temperatures. In between that if you increase the temperature, the growth rate is likely to increase, and it will be increasing to a optimum value and that temperature is actually the optimum temperature at which the microbial growth rate is the maximum.

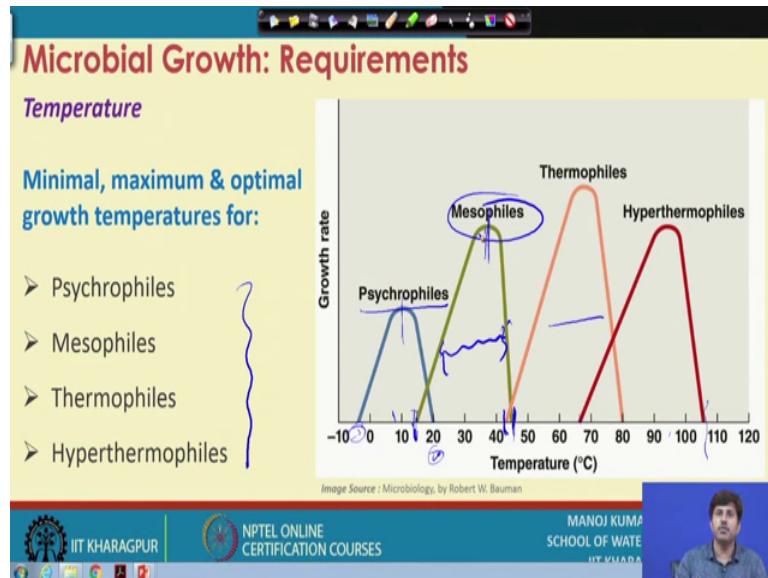
So, we can attain a temperature at which the microbial growth rate is maximum. So, that becomes our optimum temperature and either we move, we may move either side of the optimum temperature.

And what we will observe that growth rate is decreasing, because once you go above the optimum temperature, again this inhibition effects starts more prevailing and that leads to the decrease in the microbial growth rate beyond this optimum temperature. And if we move towards the lower temperature again the rate of biochemical reaction decreases and we will see the net decrease in the bio mass growth as well, growth rate as well.

So, there is optimum temperature which maintains. So, this way the various microbial species typically; so, this characteristic temperature dependence, ok and what typically, we call they have their own cardinal temperatures which is minimal temperature, maximum temperature and optimal growth temperatures, ok. So, this is the minimal temperature, optimum temperature and maximum temperature that way 3 different temperature characteristics are there which defines how the growth will be under certain ranges.

So, if you are at optimum temperature the growth is likely to be the highest, if we are at minimum lower than optimum temperature at towards minimum, we can have a kind of growth that to the minimum and below which there will not be any growth. Similarly, over and above maximum there will not be any growth, ok. So, that way we can see the growth pattern dependency on the temperature.

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Now, if we see the minimal, maximum and optimal growth temperatures for different type of species. So, as we discussed earlier 3 different type of species, the psychrophiles one which works at a lower range. So, you can see that maybe around minus 5 degree or so, is typically the minimal temperature for them, 20 degrees the maximum temperatures below, after 20 they will not see much of these things and around 10, 12 degrees the optimum temperature for psychrophiles. For mesophiles, it is like 14, 15 degrees the minimum and then close to 45 is the maximum and 37, 38 degree is normally the optimum temperature for the mesophilic group.

The mesophilic groups are the one which are most widely used because our normal ambient temperature relies in this range. So, we will see that our temperature in the winters goes as, as low as maybe 7, 8 somewhere here. But in most cases or in the water that way, it remains above 12, 15. And in winter, in summer sorry in winters and in summer it goes as high as 45 although that ranges. So, we get a large range for the large part of the year, we get a temperature suitable to this mesophilic range.

And that is why in the wastewater treatment processes or those kind of systems, we normally use mesophilic group of the microorganisms, ok, because their temperature range is what is suitable for us. If we go for other type of microorganisms let us say, if we go for thermophiles which have a optimum temperature somewhere close to 66, 67 degree Celsius.

So, we will have to provide a heating mechanism for the water which is which is going to be quite energy intensive, ok. So, that is why we avoid the other, other species, other species from the other temperature ranges and normally stick to the mesophilic range. In the thermophilic though you can see that there are minimum, minimal temperature is around 40 to 43, maximum is 80 and optimum somewhere as we were saying that is 65 to 70. And then there are a hypo thermophilic range, hyperthermophilic range which works at an even higher temperature where it can sustain close to the 95 or 100 degree for optimum and maximum it can sustain a at around hundred 708 degrees.

So, these are the different classes based on the temperature and their ranges of the temperature for minimal, maximum and optimal growth temperatures, ok. And mesophilic are the ones which are typically used and that is why we get the ideal performances or best performances when our temperature is approaching 35, 37 degree Celsius, because that is what is the optimal temperature for majority of the species falling under the mesophilic category or mesophilic range.

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Microbial Growth: Requirements

Other Requirements

- **pH** (*Neutrophiles vs Acidophiles vs Alkaliphiles*)
- **Oxygen** (*aerobic vs anaerobic vs facultative*)
- **Osmotic pressure**
- **Hydrostatic pressure**
- **Associations and Biofilms** (*attached growth vs suspended growth*)

The slide includes two hand-drawn diagrams: one of a single cell with internal organelles and another of a biofilm structure with multiple layers of cells.

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So, that is about temperature, then there are various other requirements for microbial growth, ok, there is requirement of pH. So, whether it is neutrophiles? So, neutrophiles are the one which actually pretty narrow range, ok. They work best in the neutral range of the P H, ok. So, around 6.5 to 7.8 or that is the typical range for neutrophilic, neutrophils or, so, the range is pretty narrow. Then there are acidophiles which work in the acidic ranges, which work better, or which has a higher growth rate in the acidic ranges. And similarly, we have alkaliphiles which actually work better in the basic range or alkaline ranges.

So, when your pH is higher than the neutral P H, you will see this kind of a species prevailing and they have the higher growth rate, when your P H is lower than the neutral P H or in the acidic range, the acidophiles are the one which actually survive or which grow better if while there are the neutral P H conditions, it is the neutrophils.

Then we have discussed in the previous lecture also there is requirement of oxygen. So, based on that will again depend on whether what kind of species it is, whether it is aerobic species, anaerobic species facultative species. So, for aerobic the oxygen is the electron acceptor.

So, it is very essential for the aerobic systems to have oxygen or particularly the dissolved oxygen kind of things present because that is what we act as an electron acceptor. And eventually a kind of, in the process of degradation of or in the process of oxidation of the organic matter that is what will be needed. So, in the aerobic we need oxygen, sufficient amount of dissolved oxygen should be present, oxygen being a nutrient it is not that the level of oxygen will only govern the rate of reaction, but there has to be adequate amount of oxygen present in the system. In the anaerobic system oxygen is not needed. In fact, for obligate anaerobes oxygen becomes toxic, ok.

So, this should be devoid of presence of the oxygen whereas, there are facultative ones which can may or may not need oxygen it, it does not matter actually, even if there is oxygen. So, they may use oxygen as a electron acceptor or if there is no oxygen present in the system, they can look for the alternate electron acceptor. So, they can survive both in presence and absence of oxygen whereas, obligate anaerobes cannot survive in the presence of oxygen and aerobic ones need oxygen for their survival or the obligated aerobic ones particularly.

Then there are certain other requirements like what is the osmotic pressure depending on the kind of species hydrostatic pressure. So, how much hydrostatic pressure one can leave? So, there are species which realize in the deep sea can sustain very, we can sustain like significant hydrostatic pressure, but most of the microbial species actually in the upper layer of water, if you put them deep down they probably they are not, there is deficiency might be deficiency of oxygen might be an ability to sustain the hydrostatic pressure or those kind of thing might lead to the sort of decay of those species and the growth will be affected. They probably cannot proliferate and cannot survive at the such hydrostatic pressures.

Then there is associations and biofilms. So, how the microbes are getting associated, whether they are forming any biofilm or not, whether the system is attached growth system or suspended growth system? So, this attached growth system versus suspended growth system gain is a will depend on the type of species and it is not like certain species can grow in only suspended growth system, the most of the species can grow either in a suspended growth system or attached growth system or in both in fact.

So, that chances are there. What essentially the attached growth system and suspended growth system means is that in a suspended growth system we have let us say water, the microbes are suspended in the water. So, they do not actually, they are not attached to any particular medium. So, if you are having let us say glass of water and the microbes growth, so, there might be some growth onto the surface of the glass which is kind of attached system because they are attached to the surface of the glass, but in the water medium also there would be lot of microorganisms present.

So, they are the suspended, they remain in the suspended in the water and this kind of when they grow within this suspended medium, this kind of growth is called suspended growth systems. When the microbes are multiplying and in the, when they are in the suspension in the water actually, they are not attached to anything.

And then there is attached growth system, so, we may see that we have a pipe with lot of packing materials, gravels, boulders, those kind of things. So, those kind of filters are there and when you are passing water from this one, so, the growth take place attached to certain surfaces. Now this could be the any, any sort of media, ok. There are, there are various different types of media which are used these days for attached growth system.

So, one can have any those kind of media, but whatsoever is the media that is secondary thing. There has to be has a surface on which the biomass gets attached and then growth, ok. So, that is what is referred to typically as attached growth system.

So, we have suspended growth system, we have attached growth system. This is very important because many of our, many of our treatment processes works on either of these principles.

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Microbial Growth: Requirements

Other Requirements

- **pH** (*Neutrophiles vs Acidophiles vs Alkaliphiles*)
- **Oxygen** (*aerobic vs anaerobic vs facultative*)
- **Osmotic pressure**
- **Hydrostatic pressure**
- **Associations and Biofilms** (*attached growth vs suspended growth*)

The slide includes a hand-drawn diagram in blue ink on the right side, showing a rectangular container with a layer of biomass (represented by a dense cluster of dots) attached to the bottom surface. Above the biomass, there are several small circles representing particles or nutrients in the liquid phase. The diagram illustrates the concept of attached growth versus suspended growth.

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So, like activated sludge process where we typically have water and then aviator. So, there the microbes remains in the suspension, they do not get attached to anything. And then we have trickling filter where there is a filter media or packing media is there and the water trickles on them. So, the here, whatever microbial growth is there it actually gets attached to the media available here and then grows. So, which one is the better? Whether attached growth system or suspended growth system, again it has to be basically sort of evaluation of pros and cons of both the system, ok.

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Microbial Growth: Requirements

Other Requirements

- pH (Neutrophiles vs Acidophiles vs Alkaliphiles)
- Oxygen (aerobic vs anaerobic vs facultative)
- Osmotic pressure
- Hydrostatic pressure
- Associations and Biofilms (attached growth vs suspended growth)

Handwritten notes on the slide:

- Substrate
- Degrade (pollutant)
- High Rate of Substrate Removal
- High Growth Rate
- More biomass
- Less Rate of Substr.
- Less biomass
- High Rate of Substrate Utilization

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So, particularly if we are talking about biological wastewater treatment system. So, there are two aspects, ok, one aspect what, what is our prime objective or what is our prime motto is to degrade or decompose the substance, degrade the pollutant. Now, this pollutant degradation, which is actually the substrate for the bacteria. So, our motto is to degrade the substrate and whatsoever is the rate of substrate degradation; however, high rate of substrate degradation it is better for the contaminant removal. So, we want a high rate of substrate removal.

Now, that is one aspect, so, we need high rate of substrate removal. And the second aspect is that since this removal or this degradation is being done by the microorganism, so, we need significant quantity of microorganisms as well so; that means, we need high growth rate of microorganism. So, that there are enough microorganisms growing, there are enough new viable cells are forming in the system. So, that substrate gets quickly utilized.

So, there are these two aspects; now, this point here is that attached growth system provides a higher growth rate bacteria, actually like to grow when they are attached to certain medium.

So, attached growth system typically has higher growth rate, but the substrate utilization or the rate at which the substrate is removed in attached growth system is slower. As opposed to the suspended growth system, the net growth in the suspended growth system

is smaller that is primarily for 2 reasons, one is in attached growth system when the microbes are attached to a let us say certain medium. So, when the flow takes place, you will not see that microbes too much of bacteria getting washed out from the system, ok.

So, because bacteria or microorganisms are attached to a medium and they remain attached with that medium. So, this washout thing is prevented and what you get out of from out of the system is predominantly water and most of the biomass retains in the system. But in case of suspended growth system when things are in the suspension and then when you take this water out of reactor, when water flows since microorganisms are in the suspense and so, they also flows along with the water.

So, there is lot of biomass wash out, there is lot of biomass wastage, biomass going in the effluent from the suspended growth system. And that is why the amount of biomass here is less, whereas, amount of biomass here is more. But at the same time attached growth system, let us say this is our surface and then there is bacteria, bacterial colonies are attached to this and then they will form another layer on that they might form another layer on that. So, kind of the biofilm formation that takes place here, the biofilm formation typically takes place in a ties growth system not too much in the suspended growth system.

So, what happens this kind of biofilm forms which sticks better which retains the microorganisms in this system and initially we may give you a very good result, but what happens over the time that you are, they say the meet your organic carbon is dissolved in the water, the substrate is dissolved in the water and for this survival for these microorganisms at the lower layers in the biofilm. They need to get the organic matter for their food source. So, for this organic matter has to diffuse through these layers, ok. If you see this organic matters essentially needs to be diffused to these layers.

Now, if it cannot diffuse to these layers, then what will happen? That the microorganisms at the lower level may not get the enough food and they may become dead. So, this diffusion becomes rate limiting process because as the thickness of the biofilm increases, the layers at the lower level may not get enough substrate because whatever substrate is coming. So, top layers utilize that and then next to the top layers and the next 2 top layers. So, they will probably consume the substrate and the lower layers will not get that much of substrate.

So, if you see the per unit biomass, the rate of substrate utilization here is low because substrate is being fed in basic through 1 direction and there is a diffusion substrate diffusion limitations. Whereas, the suspended growth system, if you are having microorganism suspended in the water and then water is having that organic matter. So, each microorganisms or each of these things are actually exposed to the water where there is enough amount of substrate available. So, the rate of substrate utilization is higher in, actually the suspended growth system because of the higher availability. So, this has less biomass, but high rate of substrate utilization, whereas, this has more biomass, but lesser rate of substrate utilization.

So, that is the problem, there is 1 good thing 1 bad thing here, 1 good thing 1 bad thing here also. So, it becomes the evaluation which system will work under which conditions in a better way and that can be sort of monitored by the proper analysis.

So, it is important to understand the difference between attached growth and suspended growth system in this way, that you are suspended growth system will have higher rate of substrate utilization, but lower biomass retention while attached growth system will have higher biomass retention, but lower rate of substrate utilization and may see a mass transfer limitations for the substrate or substrate transfer limitations to the inner layers in the bio in the biofilms, which are being formed and creating. Then this there might be possibility over a large period of time that these becomes dead and once they dead they lose the grid from the surface and this entire thing can actually float, ok. So, those are certain kind of issues that can come.

So, these are the key microbial growth requirement, other requirements, they will conclude this lecture here and in the next class we will have further discussions onto the kinetics of the growth biomass growth, how it takes place and what are the different systems or different units for the treatment of wastewater.

Thank you.