Water and Wastewater Engineering
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Lecture-18
Introduction to Microbiology

In the last class we discussed about the wastewater treatment that is we discussed about primary treatment, secondary treatment and tertiary treatment. In fact we also discussed further on the various units that are present in the primary treatment and certain design aspects. The systems that are units or the systems that are present in the primary treatment are screens, grid chamber and sedimentation tank. These are very important units. These units are designed and some of the design aspects we have seen. In today's class we will discuss about the secondary treatment.

Secondary treatment is very very important because the objective of the secondary treatment is; number one, to remove biodegradable organic matter and this biodegradable organic matter is in terms of two things; one is in colloidal form as well as dissolved form. So the secondary treatment is aimed at removing the organic matter which is biodegradable and that organic matter which is biodegradable may be present in colloidal form or in dissolved form. So this organic matter if not removed from the wastewater and if we discharge the wastewater with this organic matter into the river then there will be oxygen depletion.

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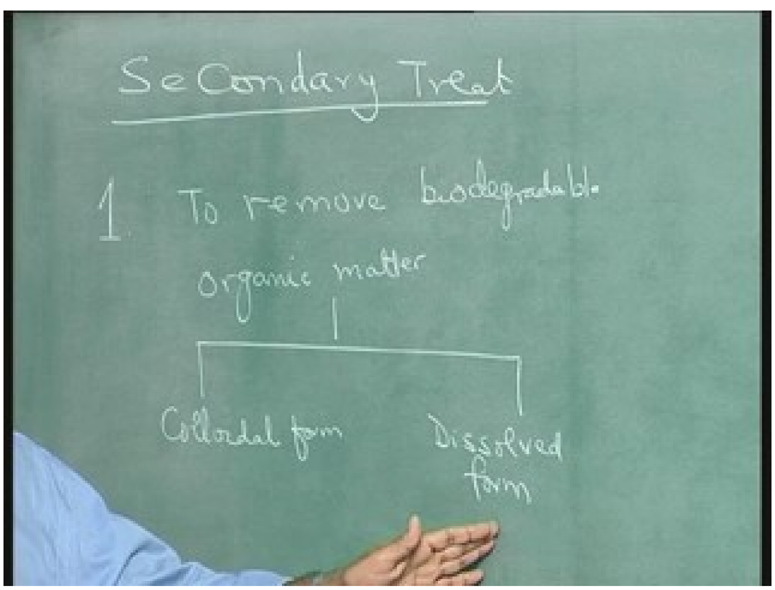

So discharge of wastewater containing organic matter is not permitted we have to remove it. In order to remove it what we do is we employ biological waste treatment systems or biological waste treatment plants. The purpose of biological treatment plant is to remove the organic matter that is the colloidal form as well as in the dissolved form. So the biological waste treatment plants obviously employ the microorganisms. Microorganisms are important or most important components of a biological wastewater treatment system.

So, as the microorganisms do the work for us that is cleaning up the environment we should know something more about the microorganisms or the microbes. In fact the microbes are used to clean up the environment. We are using the microorganisms to clean up the environment. I will write 'env' for environment so the environment could be three types. One is what is called it could be lithosphere, it could be hydrosphere, it could be atmosphere.

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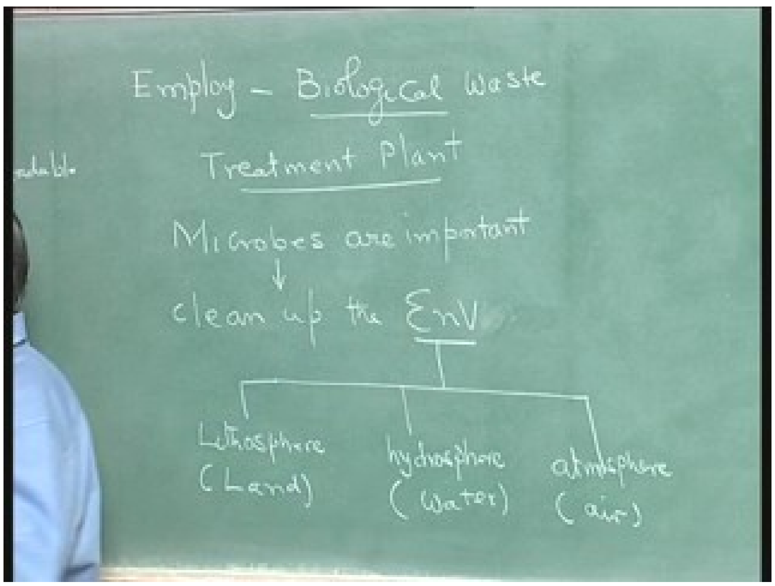

Lithosphere means land. These three components of the environment are; one is lithosphere that is the land environment where we live in then the hydrosphere which is the water environment where we use water for various beneficial purposes and the third component of the environment is the atmosphere that is air by which we breathe and so on. That means if the environment is polluted with biodegradable organic matter what we try to do is we clean up the environment using the microorganisms.

If I clean up the lithosphere land, clean up of land if I call it using the microorganisms, again everywhere I am using the microorganisms for cleaning up then it is called bioremediation. Bioremediation is a process by means of which I can clean up the pollutants present in the land using the microorganisms. In the river water if I want to clean up the river I employ what is called wastewater treatment plants. These are designed to clean up the waste before it enters into the rivers. So cleaning up the river is by employing the wastewater treatment plant, treat the wastewater and discharge into the rivers that are the clean up of the environment as far as the hydrosphere is concerned, the river is the hydrosphere.

The third type of thing is atmosphere. So air pollutants that are present in the atmosphere can be removed using the microorganisms. In such a case we use what is called bio trickling filters. Using bio trickling filters we can sort of clean up the air. The air may contain oxides of sulphur, oxides of nitrogen SO_x and NO_x. So SO_x and NO_x can be removed using the biological reactors.

In other words the microorganisms are very versatile, they can be used to remove the pollutants from the land, they can be used to remove the pollutant from the river, from the water, they can be used pollutant from the air, all three things are present. So now it is the basic requirement for us to know about the microorganisms since we employ the microorganisms to do work for us to clean up the environment. So to know about the microorganisms first we should know something about the microbiology to some extent.

I will give a brief introduction for microbiology. We will discuss only that part of microbiology that is required for the waste treatment or clean up of the environment. So, microbiology means in this particular thing what I will be discussing is the types of microorganisms we like to employ in the wastewater treatment or in the treatment of the land or in the treatment of the air.

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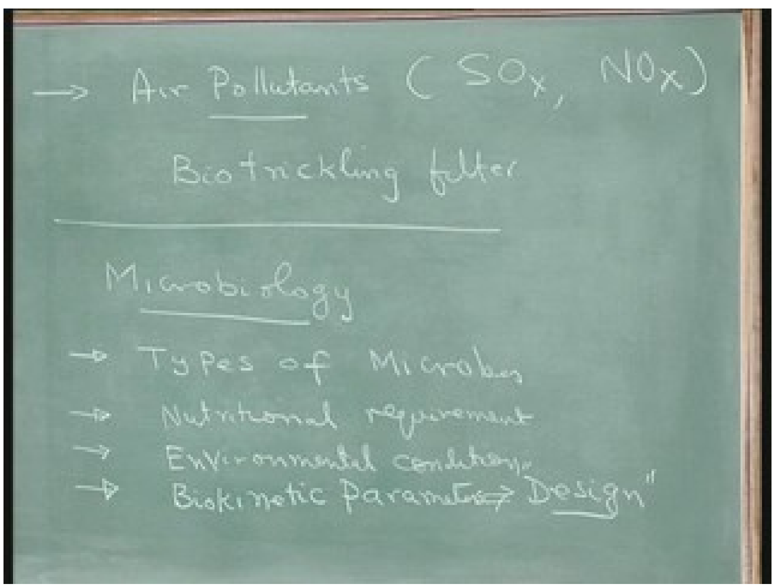

So the type of microorganisms we will discuss and then their nutritional requirements, nutritional requirements of microorganisms which is important and we will also discuss about environmental conditions which affect the growth of microorganisms and we will also discuss about what is called bio kinetic parameters for the microorganisms. The bio kinetic parameters are required for the design of the treatment system, so for the design purposes this is required.

So, for the design purposes the bio kinetic parameters that are required are the following. for example, I would like to know the growth rate of microorganisms that is again writing bio kinetic parameters, the rate at which the microorganisms are growing of microbes in a given organic matter microbial growth rate, BOD removal rate we will study and decay rate of microorganisms. We will study all these things in the bio kinetics parameters.

All these parameters are to be found out in order to effectively design the treatment system. In a series of lectures we are going to discuss these things and finally I also would like to discuss types of bio reactors that are employed to treat the wastewater and also the oxygen requirements by the microbes will also be discussed in this particular thing. Finally I will discuss the treatment units like activated sludge, tickling filter and thirdly anaerobic system. We will discuss the design of these things and at the end of the series of lectures you should be in a position to design given the wastewater and it characteristics and the quantity of wastewater or the flow rate of wastewater. I should be able to choose which of the treatment systems I should employ and how do design the particular treatment system. That is what we are going to do in series of lectures from today onwards.

Now coming back to the type of microorganisms there are many ways by means of which the microorganism can be classified. I will use a method of classification which is based on the nutritional requirement, nutrient requirements. For that matter any living system including us would require the following things: Number one, we require an energy source. In the food we eat we require energy source. Energy source is required in order to produce the energy for the microorganisms so we also require what is called a carbon source. That means the food we eat should contain carbon compounds, the food we eat should contain a compound which produces energy. So this energy that is produced from the energy source will be utilized for two purposes; one is for the maintenance and the other thing is for biosynthesis.

Maintenance energy that is the energy required for the cell to move around, cell to conduct certain activities so that is the maintenance energy and this is the biosynthesis for producing more biomass. So this is the carbon source, carbon source is required for the growth of microorganisms, growth of microbes.

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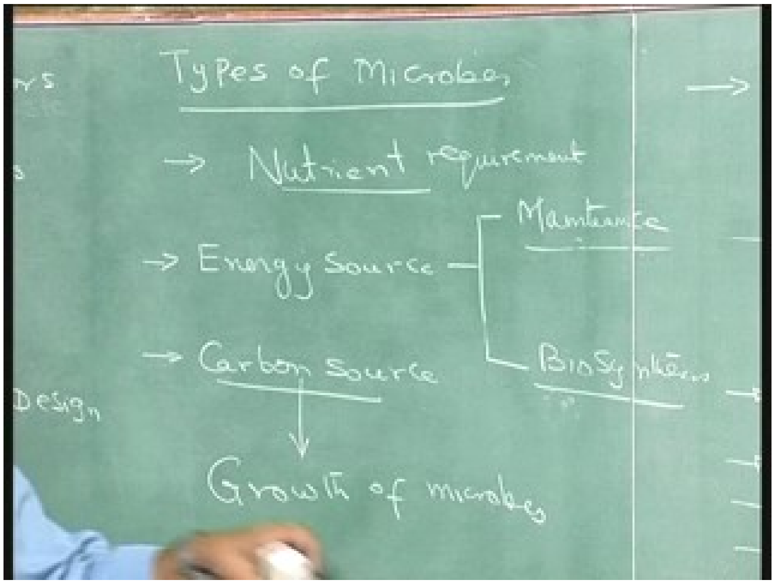

For the growth of microorganisms we require the carbon source. That means the food we eat or the organic matter I am providing to the microorganisms should contain a carbon source, it should contain an energy source. The microorganisms can utilize different components different compounds for the energy source. For example, for energy if microorganisms use or the microbes use organic carbon compounds for energy then it is called heterotrophic. It will use organic compound for energy. And this heterotrophic organism uses organic compounds for energy, it also uses organic compound for biosynthesis as carbon source. So the same organic compound can be used as a carbon source as well as the energy source by heterotrophic organisms. These are the majority of organisms we encounter in nature; the majority of microbes we encounter in nature are heterotrophic in nature.

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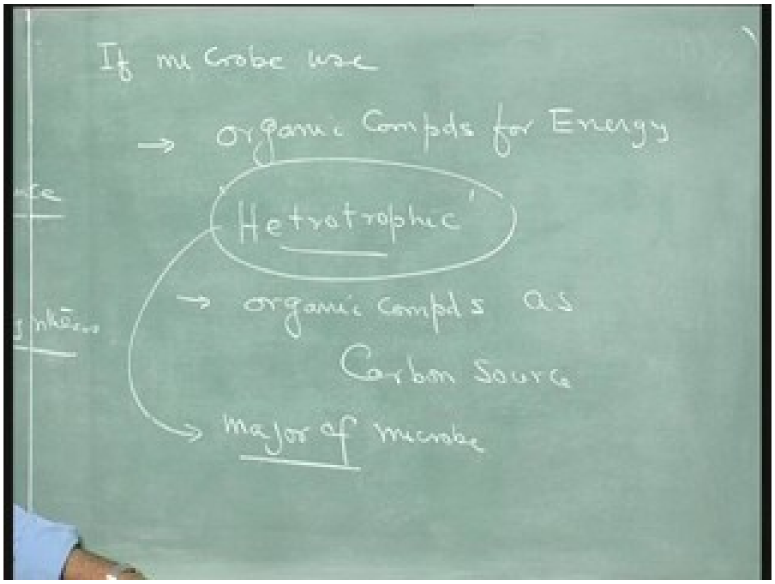

In fact heterotrophic organisms are those organisms or the organisms we employ mostly in the wastewater treatment so these are employed in the wastewater treatment plant. Suppose if the microorganisms instead of organic compounds for energy purpose if they utilize inorganic compounds for energy purpose they are known as chemo-autotrophic organisms. so the second group of organisms are chemo-autotrophs, autotrophs is self dependent so they are independent organisms, they are depending on self only, self depended organisms and they depend on the chemo, chemo means chemical, they depend on the chemicals that is the chemicals are inorganic chemicals.

For example, energy source for the chemo-autotrophs is inorganic compound like ammonia. So this inorganic compound like ammonia is oxidized to nitrite and nitrate to produce energy so this produces energy. This energy that is produced is in the form of ATP adenosine triphosphate. This ATP is adenosine triphosphate. Adenosine triphosphate is an energetic compound and it gives out the energy whenever energy is required so ATPs are produced. These organisms which utilize this oxidized ammonia to nitrite to nitrate in the presence of oxygen where oxygen is required for this are called nitrosomonas and this organism here is called nitrobactor. These are the two organisms which utilize the inorganic compounds for energy purpose.

The second one is what is the carbon source for them? The carbon source could be inorganic carbon compound. So these organisms which utilize the inorganic compounds for energy and inorganic carbons as carbon source, and the carbon source is required for the growth of microorganisms, this is used for the growth, this is the energy source and this is the carbon source these are known as autotrophic organisms. These are special organisms, we use them specifically for the nitrification to occur in tertiary treatment because I discussed about the nitrification when I discussed about the tertiary treatment. So in the tertiary treatment the nitrification occurs and I want nitrification to take place so this will occur because of these microorganisms.

The third type of organisms is what is called photo autotrophic organisms. Photo autotrophic organisms are those which utilize sunlight, the energy source in this case is sunlight, they get energy from sunlight and utilize the carbon dioxide as a carbon source. The carbon source is carbon dioxide here. The simple reaction I can write here is; they utilize carbon dioxide plus water and this is the sunlight in the form of h nu, h nu is the sunlight plant constant and this is the energy sunlight energy (Refer Slide Time: 23:13) and these are the photosynthetic plants and an example of microscopic photosynthetic plant is algal cell.

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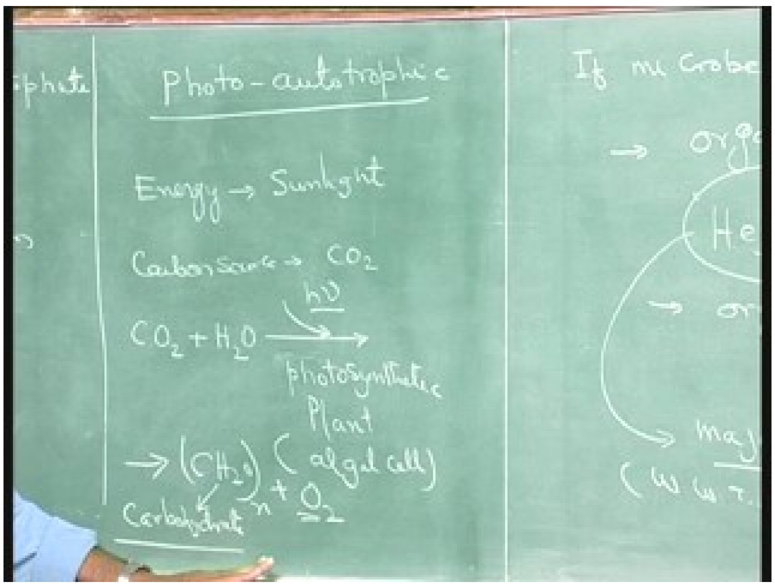

So with this reaction what would happen is that, the resultant of this reaction is H_2O_n plus oxygen. H_2O_n is nothing but a carbohydrate this is the carbohydrate they prefer carbohydrate and oxygen is produced by the photosynthetic cell. This is the photosynthetic reaction what we are writing. So we utilize photo autotrophic organisms like algae in the wastewater treatment especially in oxidation ponds. The example where we use this particular thing is oxidation ponds. Example of photosynthetic or photo autotrophic organism in oxidation ponds: that is what we use. We use the photosynthetic autotrophic organisms. That means we use heterotrophic organisms, we use chemo autotrophic organisms and we also use photoautotrophic organisms for the wastewater treatment and depending upon the type of reactors we are designing.

So basically most commonly used wastewater treatment or commonly used microorganisms in wastewater treatment as I already indicated are heterotrophic organisms. Again I emphasize that the classification of these organisms are based on the nutritional requirements that is the carbon source and then the energy source that is what I have been discussing particularly. There is certain other classification; people have so many other classifications. Another classification I can tell you is based on the temperature. We have what is called as cycrophilic microorganisms.

Cycrophilic microorganisms are those microorganisms which grow under low temperatures. For example, these organisms grow in the refrigerators also; they spoil the food stored in the refrigerator. So the organism that grow around four degrees or to ten degrees Celsius they are cycrophilic organisms. The second type of organisms is mesophilic organisms.

Mesophilic organisms are those organisms which grow in the medium temperature that is about say 27 to 40 degree Celsius, then we have got thermophilic organisms. Thermophilic organisms are those organisms which grow at higher temperature that is about 40, 45 degrees to 60 degree Celsius. This is yet another classification based on the temperature, the tolerance of the microorganisms to the temperature.

However, that classification is one classification, this is another classification based on the nutritional requirements. We will move forward with the heterotrophic organisms because these heterotrophic organisms are employed very widely in the wastewater treatment. So these are widely employed in wastewater treatment and most of the treatment plants are based on the activity of heterotrophic organisms and hence I should try to find out what is called growth curve for heterotrophic organisms. For these organisms I should know what the growth curve is.

What we mean by growth curve?
Growth curve shows, with respect time how the growth of microorganisms take place in a reactor.

Let me consider a batch reactor. What is a batch reactor?
Batch reactor is a reactor where there is no inflow or outflow or output from the reactor that is batch. Now let me consider a batch reactor. This is (Refer Slide Time: 28:10) sort of a batch reactor where I have the liquid and this batch reactor is completely mixed; I am mixing it completely, a completely mixed reactor that means the contents are mixed thoroughly. That means wherever I take the sample, if I take the sample over here or over here wherever I take the sample the concentration of the contents will be the same that is what is called the batch reactor.

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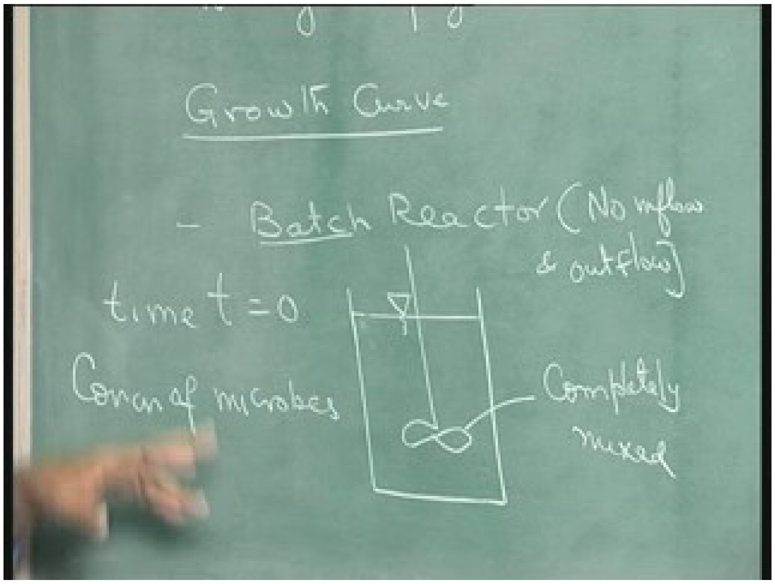

Let us say in this batch reactor I have a time t = 0 to start with. I have what is called a concentration of microorganisms, these microorganisms are heterotrophic microorganisms, this concentration microorganism is X_0, that means X_0 is the concentration of microorganisms in the reactor and then there is also food. What is the food? Food is the energy source and carbon source. That is, for the heterotrophic organisms organic compounds recalling these organic compounds are both energy source and carbon source.

Organic compounds provide energy as well as the carbon to the microorganisms. So such organic compounds which give energy as well as carbon for biosynthesis for the growth of microorganisms is known as the substrate. Let us call this as substrate. Substrate is also biodegradable organic matter so substrate also can be called BOD biochemical oxygen demand, oxygen that is required to decompose or to oxidize organic matter organic matter, substrate under aerobic conditions where oxygen required is nothing but the BOD.

We can say the BOD is same thing as the substrate. So let us take time t = 0, concentration of microorganism is X_0 and concentration of substrate I will go back here substrate is equal to S_0. So in this reactor I have S_0 and at time t = 0 the conditions are; X equal to x_0 and S = S_0. X is the biomass microbes concentration or biomass concentration it is also called as biomass and S is the substrate concentration.

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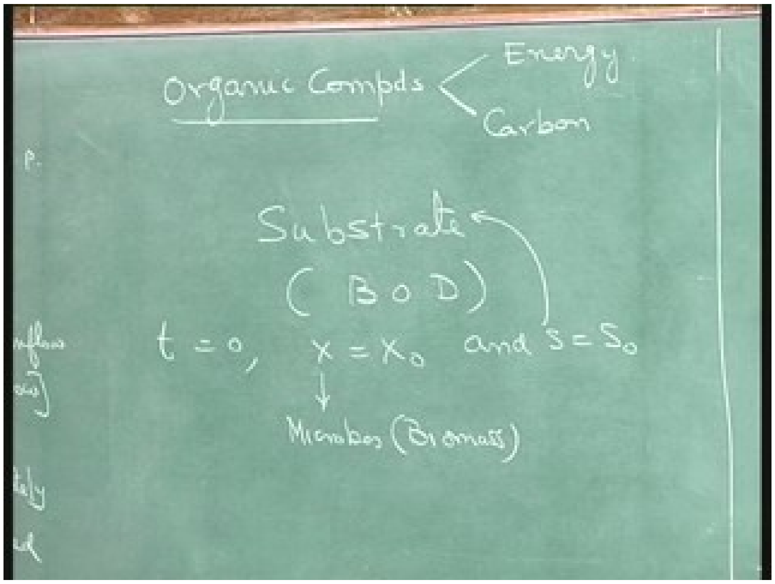

What I do is that I now bring in contact the microorganisms and the substrate in the reactor time t = 0 and I am mixing completely and during this mixing what is happening is that I am also providing what is called aeration oxygen supply. I am also providing aeration or oxygen supply is also there in this reactor. So because of this particular thing what would happen is the organisms utilize the organic matter present in the wastewater or present in this reactor. So, for example, if I say that organic compounds can be called as glucose I will take a simple example $C_6H_{12}O_6$ is the glucose this is a representative of organic matter in wastewater or this is nothing but the substrate for me now, substrate plus oxygen, this is mixing or aeration (Refer Slide Time: 32:18) and aeration is providing the oxygen and then I have microorganisms.

And what type of microorganisms I have?
We can say that it had heterotrophic microorganisms. The microorganisms use organic compounds for energy purpose as well as for the carbon source or for biosynthesis. So when these things happen what will happen is that the reaction will take place with the production of carbon dioxide plus water, that is the carbon is converted into carbon dioxide, hydrogen to water plus we will have energy. This energy is in the form of ATP adenosine triphosphate as already indicated. So this energy is used by the microorganisms to produce more cells so that is what it is going to happen. That means now what is happening is I am giving food to the microorganisms I am giving substrate to the microorganisms, the microorganisms utilize the organic matter and increase in the number or increase in the BOD weight. So as a result as the time progresses, as the time increases X is going to increase, the biomass concentration increases and S is going to decrease. There is decrease in the organic matter because microorganisms are utilizing organic matter and they increase in number that means X is increasing as the time progresses.

So, if I were to plot a graph of this particular thing then the time is on X axis, and micro milli concentration on the y axis then at time t = 0 I have certain amount of microorganisms that is x_0 to start with, in fact time t = 0 whatever organisms are presented or introduced in the system they are called seed microorganisms present in the seed and if you try to find out the concentration of microorganisms as a function of time then initially you may have something like this and then afterwards it will move up and then it will go and then it will be some sort of stationary and then over a long period of time may be I will break it here (Refer Slide Time: 34:59) over a long period of time then it would be declining. So there are four distinct segments of the curve. This curve is known as bacterial growth curve when I am trying to grow the organisms in a batch process.

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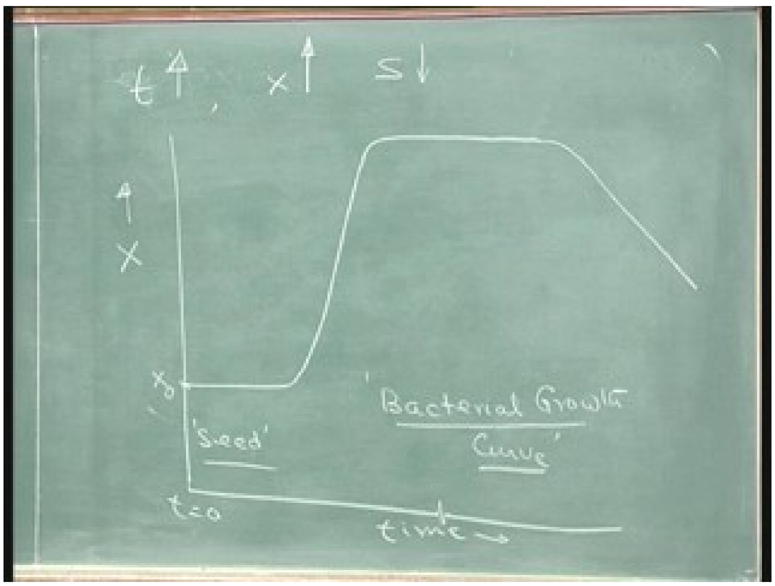

Batch process is the process where we do not have in flow and out flow, the organisms are growing within a closed environment, so closed eco system the microorganisms are growing. So let me call it as number one segment, this is number two segment (Refer Slide time: 35:45) number three segment, number four segment. There are four segments in the bacterial growth curve. This bacterial growth curve indicates how the micro organisms are utilizing the food and growing in numbers.

Therefore in segment number one what is happening is, if you see there is no increase in the number of microorganisms, there are almost same microorganisms up to this time. This particular period is called lag period. The lag period is there because the microorganisms are put into a new environment I am putting the microorganisms in the new environment and may be the substrate, the food is new to them as a result of which they take sometime to get acclimatized to the new environment and also to the new substrate. So during this particular process even though there is no net growth of microorganisms lots of activities are taking place so the organisms are physiologically very active, they are very very active physiologically. This means that they are producing new enzymes in their BOD in the cells so as to act on the substrate and also to adjust to the new environmental conditions. So this is a physiologically active condition, physiologically active state. Lots of activities are taking place. Enzymes are produced; they know how to degrade the substrate the food so that they can increase in the number. Having done that segment number two is called log growth phase.

Log growth phase is where the organisms are multiplying or increasing exponentially. They are increasing and the growth rate is exponential. That is the reason why we have got a sudden increase here of microorganisms. As the microorganisms are increasing very rapidly or as the microbial concentration is increasing rapidly what is happening to the substrate concentration, if the microorganisms are increasing the substrate should be decreasing, so there is decrease in the substrate concentration number one. Number two is, because of this there are more microorganisms and less food so in this particular case what is happening is there are more microbes and less food that means there is more competition for food.

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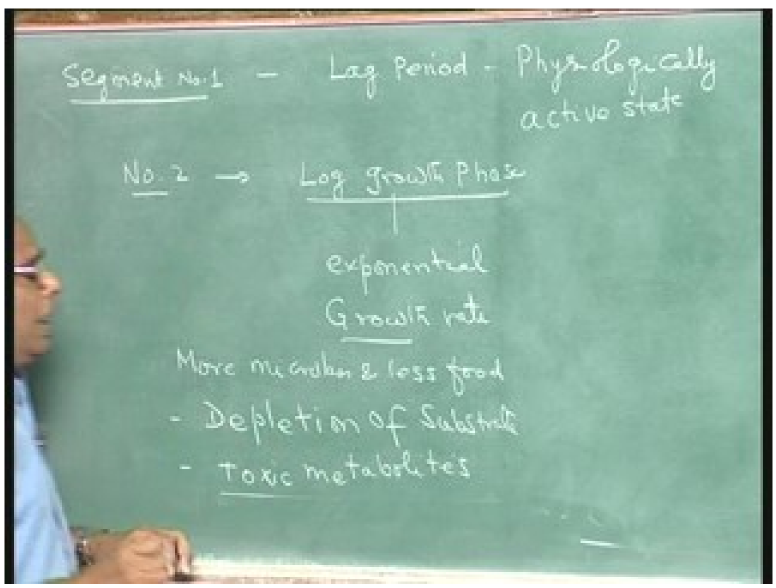

I told you there is a closed environment, a closed eco system, in this closed eco system there is no new food coming in as a result of which the food is limited and the micro organisms are full as the result of which there is competition for food and because of that competition the growth rate starts decreasing.

For example, if you take, there is a point of inflection here if you go straight like this if it is exponential it should have been like this but there is a point of inflection from this point onwards there is a change in the slope of this particular curve, the slope of the curve starts decreasing so it goes like this. So the point of inflection is occurring because of the depletion of food. This is depletion of substrate or food and again I tell you that it is a closed environment. So the toxic end product, metabolic end product starts accumulating in the reactor. So accumulation of toxic end product becomes harmful to the microorganisms. We are not removing any material from this so toxic end products accumulate and these end products are a result of metabolites. The toxic metabolites accumulate as a result of which the growth rate decreases, so as a result of these two things (Refer Slide Time: 41:10) their net result is decrease in the growth rate.

This is number three segment and this decrease in growth rate is called stationary phase. This is the stationary phase where there is no increase in the microbial concentration. That means this microbial concentration remains constant here, it has reached a plateau it will not increase further so this is called the stationary phase.

In stationary phase there is no net growth of microbes. Now, if you reach number four segment, what is happening number four segment? The fourth segment is the segment where the microbial concentration is decreasing with time. As the time passes this is decreasing. That means there is no growth. In fact there is destruction of microbes; this is the result of destruction of microorganisms. Microorganisms are decreasing with time and that is what it is happening in this particular thing.

Why it is happening?
The food is almost exhausted. number one, the reason for this is the substrate is exhausted I will put it here number one and number two reason for this particular thing is that accumulation of toxic metabolize toxic end product. So these are the reasons which are responsible for the declining growth phase.

If you look at the microbial growth rate or bacterial growth rate curve we have got these four segments and these four segments are depending upon the availability of the substrate and growth of microorganisms. So these are the things, this particular curve is very very important for us in understanding what exactly is happening in the wastewater treatment plant.

Now let us move forward. After understanding the growth curve we will try to follow what exactly happens to the reactor. We will try to put mathematical equations for the growth curve. In fact there are several mathematical models to describe the rate growth rate of microorganisms.

Number one is a very simple model based on Monod's model and people also have applied another model which is what is called a logistic growth model to describe the growth curve or the behavior of growth of microorganisms.

Now we will try to use both the models and then see how exactly we can formulate some of the mathematical models. So now I will try to look at the fundamentals of the mathematical models. Now, again I go back to the reactor this reactor at time $t = 0$ $X = X_0$ $S = S_0$. Mind you, both S and X are measured in the same units so the milligrams per liter of biomass and milligrams per liter of substrate both are measured in terms of milligrams per liter X and S are units measured in the same units.

Now here I can write down; dx by dt rate of growth rate of microbes proportional to the microorganisms present at that time. This is the one simple equation we are taking about, the growth rate is proportional to X, X is the microorganisms present at that time. In other words this can be dx by dt is equal to mu into X removing the proportional to constant with the mu.

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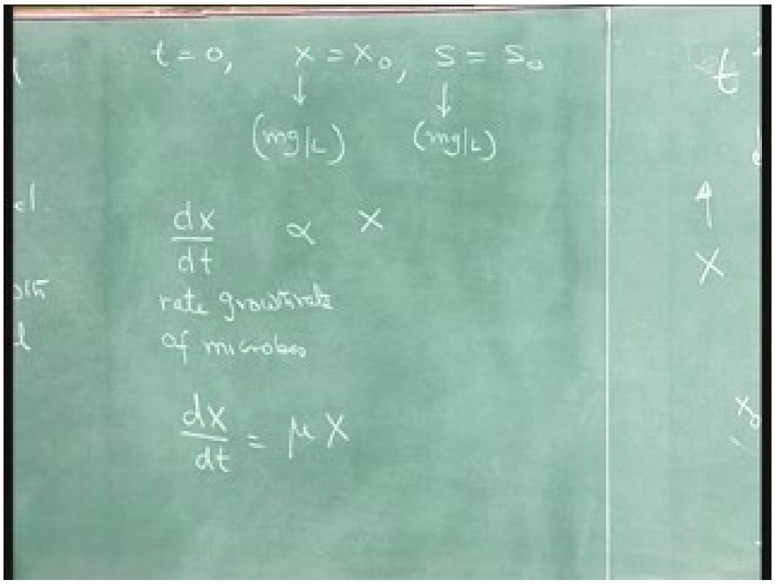

Here mu is known as the specific growth rate and the units of mu you take it, the units of X here and X here are the same they cancel out and mu will take a unit of t inverse time inverse, mu has the unit of time inverse, mu is the specific growth rate. Now, this specific growth rate mu is not a constant.

So, what is mu?
mu is the specific growth rate of microorganisms that should depend on the concentration of the substrate. In other words mu is not a constant but mu is a function of substrate concentration S that is what I will write. It is a function of concentration and also it is a function of environmental conditions. Let us say that environmental conditions are kept very conducive for the growth of microorganisms so we are not going to change that particular thing.

So now what is happening is that the relation between mu and S is given by mu is equal to mu max multiplied by S over K_s plus S. So suppose I want to plot a graph between S that is the substrate concentration and then mu here mu is the specific growth rate and then the substrate concentration on this particular thing when the substrate concentration is zero there is no substrate concentration then what is going to happen to the growth rate is the growth rate is also zero, no food, no growth rate. Then as the substrate concentration increases growth rate also increases like this and then finally it would go and then it will become sort of asymptotic or it could be stationary, it becomes stationary to this.

As the S increases this particular thing is almost constant. So this constant thing is called as mu max. Maximum growth rate has occurred at this particular thing. now again going back; another substrate concentration is increasing mu also increases and this follows this trace of this curve (Refer Slide Time: 49:23), this is the curve and then beyond this particular concentration of the substrate whether the substrate is this or substrate is this whatever be the substrate the mu value is constant. That is, the mu value when it takes the mu max value then that is independent of substrate concentration. Up to this particular period up to this particular point probably the two are a function of substrate concentration and beyond this particular thing it is independent of substrate concentration and that is what we can see in this particular thing so that is the mu max. The mu max is the maximum growth rate constant.

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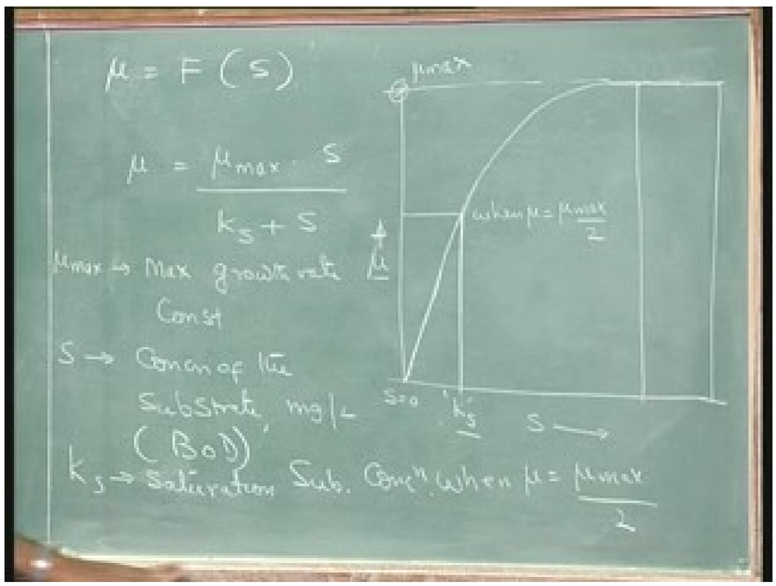

But only now I am including the word ‘constant’ because growth rate is a constant. Here it is only specific growth rate and here it is a constant because that is the only one unique value for a particular microorganism for a particular substrate. For a particular wastewater as well as for particular group of microorganisms mu max will be the only one value that is why it is a constant and S is the concentration of the substrate and milligram per liter so this also can be called as BOD whatever BOD I have in the wastewater.

Now the K_s is the term which I have to say K_s is nothing but the value of S this is Ks (Refer Slide Time: 51:02) what is K_s? K_s is the value of S when mu is equal to mu max by 2 that is half of mu max. Now I know the mu max value, half of mu max value I take and corresponding S value is equal to K_s. So Ks is a saturation substrate concentration when mu is equal to half of mu max that is what it is. These are all terms indicated here. In other words mu is the function of the substrate. So, substituting back in this equation I have; dx by dt the growth rate of microorganisms is equal to mu into, mu is given by mu max multiplied by over K_s plus multiplied by the X. So you can see here that the growth rate of microorganisms is a function that depends upon a constant mu max, another constant K_s, K_s is also a constant so I can write it here this is also a constant, K_s is also a constant it depends upon the substrate concentration and it depends upon the microbial concentration so this is the actual equation which governs the growth of microorganisms and then growth rate curve.

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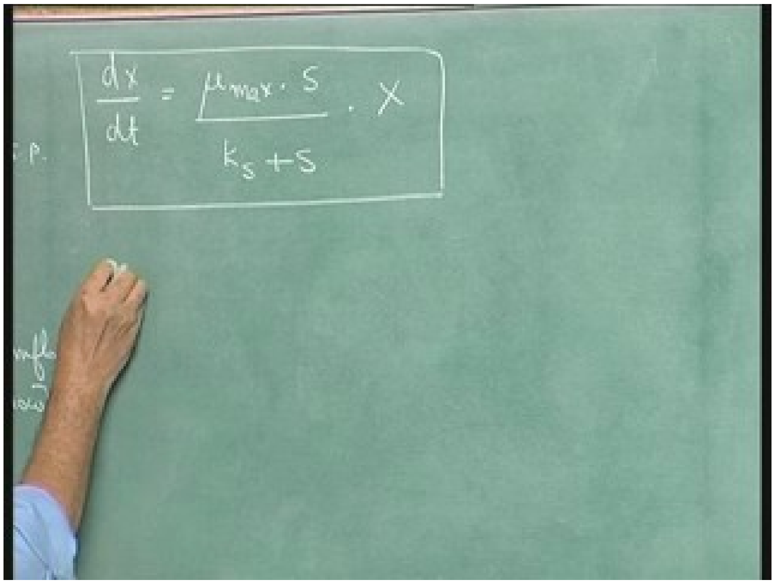

Now let us take case one. I will try to simplify this particular equation with certain conditions. Let us take case one. Case one is that when substrate S is unlimited.

What does it mean?
When the substrate is unlimited means if I go to this particular graph somewhere here I am there is very high substrate concentration, substrate unlimited means very high concentration of substrate. So when is the high substrate concentration present in the reactor is during the initial periods of the batch reactor. In the batch reactor during the initial periods we have this particular thing. So, that S is very very high compared to K_s; K_s is small, S is very high and hence I can say K_s plus S is approximated to S. In the denominator Ks plus S can be approximated to S because S is very great compared to K_s. That means if I take that equation now and substitute this condition that the substrate is unlimited dx by dt is equal to mu max multiplied by S over S because K_s plus S is S multiplied by X. So this S and S cancels out dx by dt is equal to mu max into X.

Can I solve this equation now? Yes I can solve this equation. I could not have solved the equation dx by dt equal to mu into X; mu is the specific growth rate and mu max is the maximum specific growth rate constant. This is a constant (Refer Slide Time: 54:49) that is why I can differentiate dx by X = mu max into dt. I can solve this equation when t = 0, $X = X_0$ and when t = t; X = X. That means the solution of this particular thing is nothing but applying X is equal to X_0 into e to the power of mu max multiplied by t. So this is the solution of this. So this particular equation is telling me that X equal to X_0 into e to the power of mu max into t. The mu max is a positive value so all these are positive value, X is a positive value multiplied by X_0 and hence it is a positive value.

In other words this particular thing describes the growth of microorganisms at exponential growth rate. That means this equation describes the exponential growth rate or this is the log growth rate of the curve.

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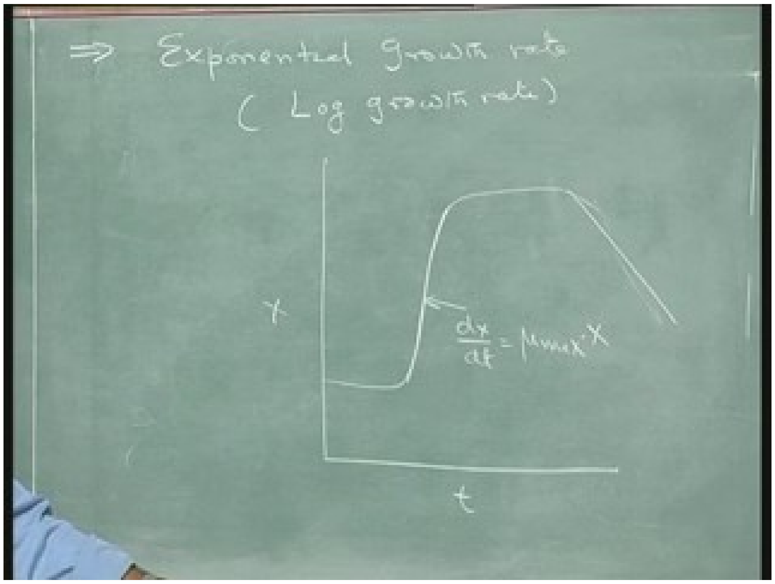

Suppose if I plot this curve again, t versus X so I have this, this is the curve what we have so this particular thing is represented by dx by dt is equal to mu max into X. This is the curve, exponentially it goes that means this is exponential curve. That means this exponentiality is valid only up to certain level, afterwards it is not valid. We will continue in the next class when the substrate is limited. In this, unlimited substrate we have taken but when the substrate is limited what is going to happen is what we will discuss in the next class.