Aspects of Biochemical Engineering
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Lecture – 32
Kinetics of Substrate Utilization, Product Formation and Biomass Production of Microbial Cells – II

Welcome back to my course Aspects of Biochemical Engineering, now in this last lecture I try to discuss that how the cell concentration can be expressed and I told you that 2 different type of cells are there, one is called the unicellular cell another is the multi cellular cell or we call it filamentous type of cells. Now in case of the unicellular cell number is proportional to mass. So, we can express the concentration of the cells both in terms of mass per unit volume or number of cell per unit volume and all are inter convertible.

But in case of this the filamentous cell or the number is not proportional to mass. So, we shall have to express only mass per unit volume. Now then I try to discuss that how the growth model can be expressed that you know that rate of growth of the cell can be expressed. So, that this model can be have 4 different types one is called segregated model, un segregated model, structured model and unstructured model. Now segregated model means as you know that we have in our if you look at the our human population, that we will find all the peoples there some way differ from each other they are not alike.

So, same thing happens to the microbial system, but also all cells they grow in a different way. So, if we considered if we put a marker to the each and every cell and we use we monitored the rate of growth of the individual cells and we write the growth equation for the individual cells, then we call it segregated model. But what is the un segregated model, when we assume that all the growth of the all cells are their uniform and this is a kind of ideal situation not the real situation. The real situation is that growth characteristics of the individual cells that should be different.

Now, in case of structured model I told you their living cell comprises of different type of bio molecules like RNA, DNA protein. Now if your model deals with how these bio molecules changes with respect to time, then individual and a rate of change of RNA

how it depends on the different parameters, rate of change of protein how they difference with the different parameters then we call this structure model.

Now, if we assume the rate of change of RNA, rate of change of DNA, rate of change of protein or other bio molecules, they are uniform they are not same then we call it unstructured models. So, we can easily visualize from that the unstructured and the un segregated model are simplified model, what do you call the ideal model and a real model is the about segregated and structured model. Now Monod similar to the Michaels menten equation they propose the equation that is mu equal to mu max s ks plus s. Now this is usually this is the unstructured and a un segregated model.

Now, this kinetic constants that mu max and ks we can easily determined with the help of line plotting the line plot, now then phase am I right and if it is active in the log phase our inoculation should be done in between mid log phase to the mid from mid log phase to late log face. So, this I discussed in the last class, now today I want to share with you with other type of models that proposed by different scientist and then we are going to discuss substrate inhibition and product inhibition and some toxic product toxic inhibition of the microbial system and then we should switch over to the Luedeking-Piret and the pert models. Now first let me start with that you know that other models that proposed by the different scientists, first models is the Blackman equation this equation is mu g equal to mu max and that is valid when S is greater than 2 case.

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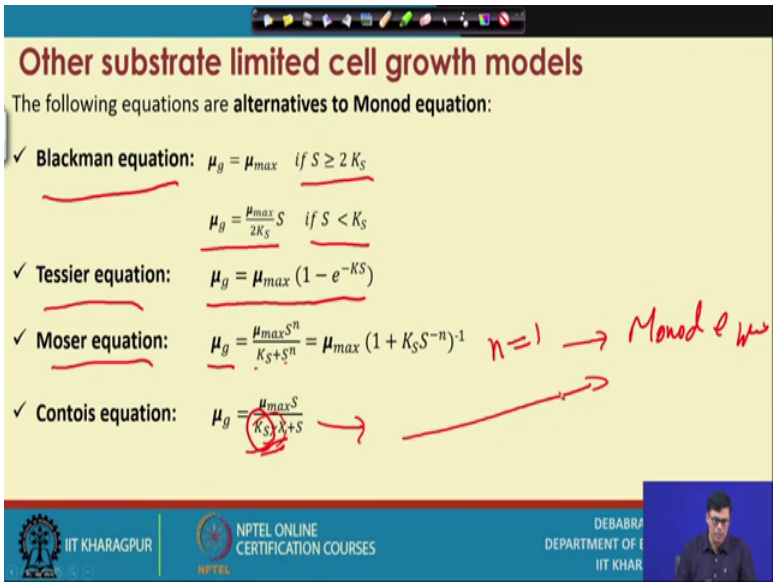

Now mu g; mu g is the specific growth rate then this is equal to a mu max twice Ks into S, if S is less than Ks. Tessier model is mu g equal to mu max 1 minus e to the power K into S and then that Moser model this is mu g equal to mu max S to the power n K S plus h to the power n; here I have seen that 1 we can find out if you 1 n equal to 1, then this equation tends to Monod equation am I right.

Now, here also we have seen the K S X, K S X we know saturation constant. Now this saturation constant is correlated with the cell mass concentration. Now if the cell mass concentration is constant then we can assume this as the constant, then also this will approach to the Monod equation.

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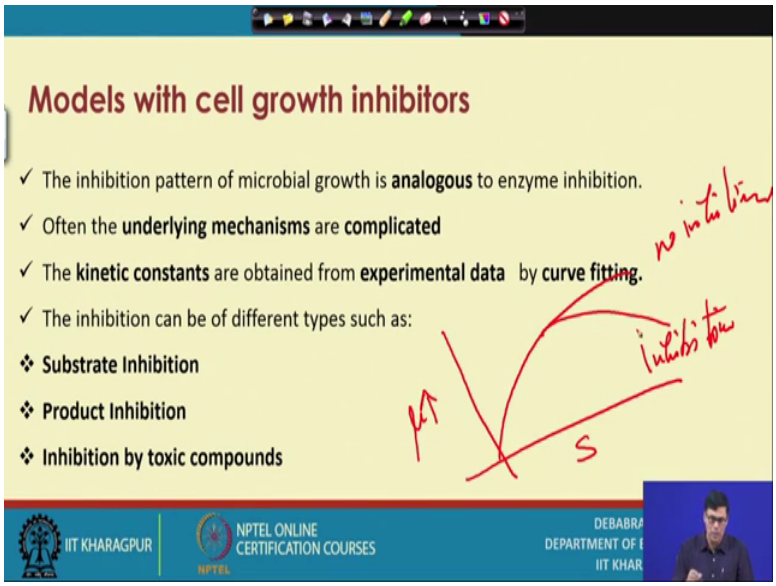

Now, as I told you that different type of inhibition that we have that I can give you a typical example, suppose when you plot mu versus S this we have this kind of plot this is no inhibition.

Now, in case of substrate inhibition what will happen this will be like this is with inhibition. Now why at the higher substrate concentration there will be inhibition, this we considered as the substrate inhibition..

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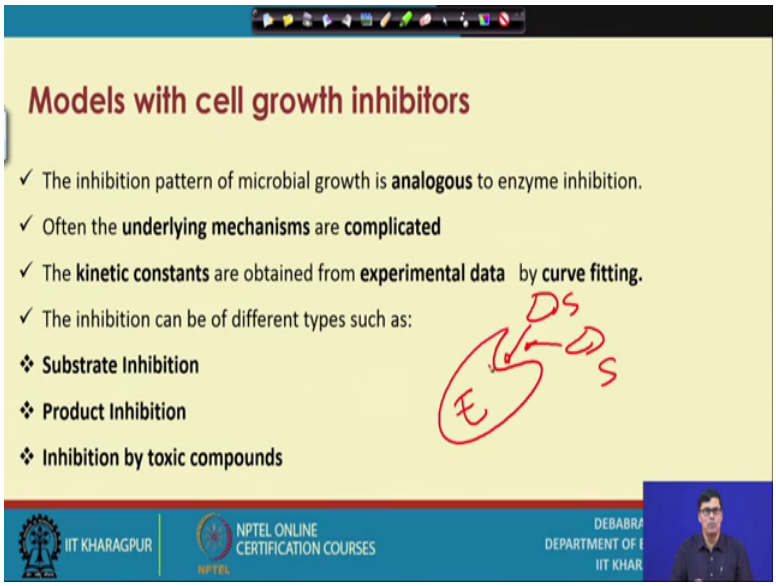

Now during when we discuss the inhibitic reaction kinetics we have we pointed out in case of enzyme, we know this is a substrate am I right. The enzyme also we have substrate inhibition when individual substrate they interact with the active side they give the product. But when I have 2 substrate try to interact the same active side, then we find some kind of inhibition take place. So, this we explained during the enzymatic reaction kinetics. The inhibition pattern of the microbial growth is analogous to the enzyme inhibition; this is exactly for that I want to explain.

Now, often underlying principle mechanisms are complicated. The kinetic constants are obtained from the experimental data per curve fitting, that we also we have we have found out then and we found out that you know there are 3 type of inhibition is substrate inhibition product inhibition and inhibition by the toxic compounds. So, you know that during the enzymatic reaction kinetics I have given one example that, how the enzymatic reaction kinetics can help you to determine the concentration of the toxic component like the pesticides.

So, this is the same similar type of things that is applicable to the microbial system also.

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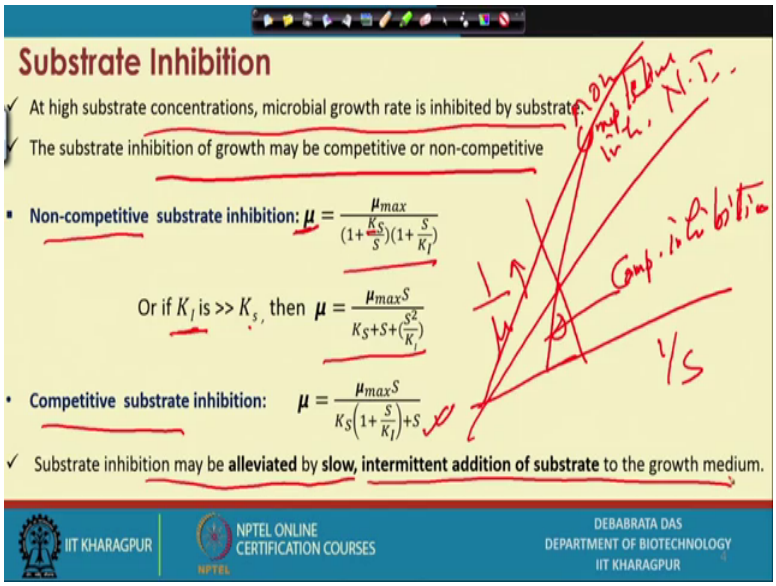

Now, first let me discuss about the substrate inhibition. At the high substrate concentration microbial growth is inhibited by the substrate because, this is the normal we have seen our case also we the human beings, when we start taking food we take excess of food our system also gets affected; though similar thing happened with the microbial system also. And this inhibition may be of 2 types, one is called compete non competitive inhibition another we have competitive inhibition.

Now, we have already seen that what do you mean by competitive inhibition and what is non competitive inhibition. Now what is the if you have a line plot like 1 by mu versus 1 by S plot, then here there is no inhibition this is no inhibition am I right. Now in case of non competitive inhibition this will be non competitive inhibition am I right and in case of competitive inhibition, what will be competitive inhibition? This is like this is competitive inhibition.

This we have already found in case of enzymatic reaction, similar thing is applicable here the equation is similar to that only the thing is that the different mu is the b is substituted by mu and km is substituted by K S that is the saturation constant. So, this is the can be written and this can be if K I is that enzyme and inhibitor complex constant that equilibrium constant is more than the K s, then we can write mu equal to mu max S ks plus S squared by K I and competitive inhibition we can similar to enzymatic reaction we can write like this.

Now, substrate inhibition may be elevated by slow and intermittent addition of substrate of the growth media. So, what does it mean that in the growth media if we add substrate slowly, then this substrate inhibition can be avoided. Now I can give a typical example that fade batch process, what is the fade batch process? That we can 2 type of feeding we can have in the reactor either intermediate feeding or constant volume feeding. So, like this we can find out that you know your system can use your organism, can use more substrate during the fermentation process.

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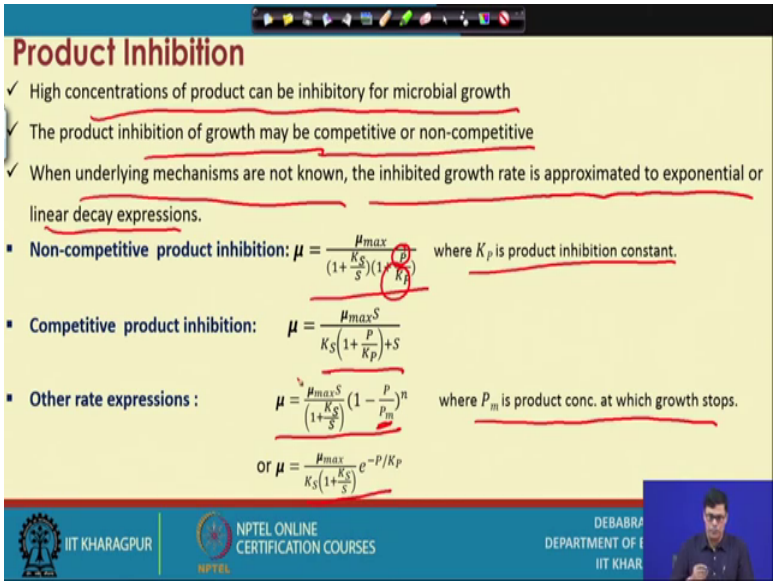

So, this is how we can partially overcome the substrate inhibition problem. Now product inhibition we it is similar to the substrate inhibition that high concentration of product and inhibitory effect on the growth of the cells. We have seen that particularly in case of a ethanol fermentation process, acetic acid fermentation processes. We have seen that the high concentration of product that they have some kind of inhibition effect. The product inhibition or growth may be competitive or non competitive and when underlying mechanism under known the inhibited the growth rate is approximated to exponential and linear decay expression.

So, you know that this is like we can have 3 different types, non competitive inhibition as we mentioned be before that this similar type of expression is there, only this is S is replaced by substrate inhibition it was this and it is replaced by p and K p is the product inhibition constant and in case of competitive inhibition we have similar type of

expression and in case of the other rate in linearized expression this we can have mu equal to that this exponential type; this is the mu max S 1 plus K S by S1 by 1 by P m maximum product to the power n a Pm is the concentration at which the growth stops.

When concentration is maximum at the maximum when growth no growth take place this can be modified in this form. So, this 3 different type of models we have this different type of equation, we can use just to describe the product inhibition.

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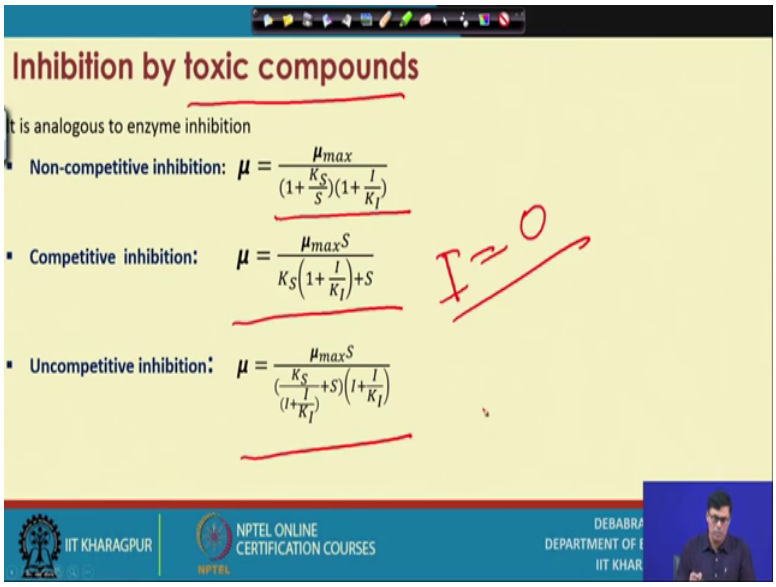

Now, I was talking at the inhibition with the respect to some toxic compounds, that may be produced during the fermentation process thought if you add some kind of toxic compounds, that you know that it can be expressed like this in case of non competitive inhibition it will be this.

In competitive inhibition will be this and uncompetitive inhibition the; similar to the enzymatic reaction all the cases, if you put I equal to 0 then all the models will be tends to the Monod equation. So, this is how similar to the enzymatic reaction I already explained, so I am not explaining again.

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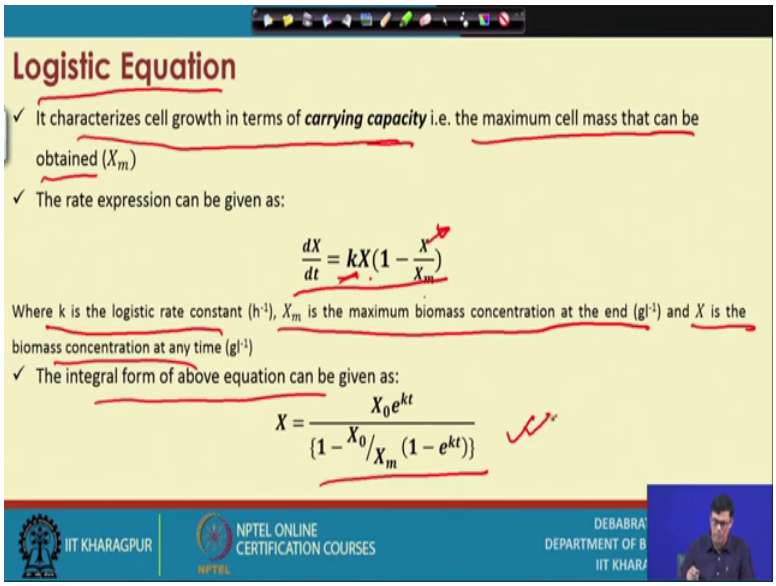

Now, that you know we discuss about the Monod equation for the cell growth kinetics. Now it can be explained with the help of other equation what you call logistic equation. The eta it character is the cell growth in time of carrying capacity, that is the maximum cell mass that can be obtained that you know; that means, this model or this equation based on that how much maximum cell can be concentration can be achieved on the basis of that, not on the basis of substrate concentration that.

So, basic difference mean with the Monod equation and the logistic equation is that, Monod equation based on in a concentration of limiting substrate, when logistic model depends on the maximum substrate concentration. So, this expression is that the rate of cell mass formation equal to k into X 1 minus x by x is the concentration of cell at any time t this is what he has written here in any time t and X m is the maximum biomass concentration at the end is the gram per liter and k is the logistic rate constant.

So, this can be explained like this now this integral form of the above equation can be written in this form, if we solve this equation it will come in this form this you can write in this form.

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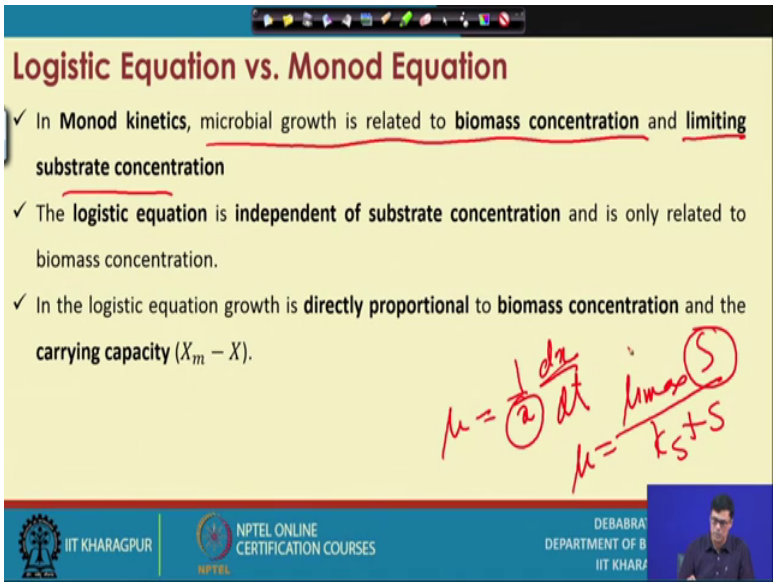

So, what is the difference between the logistic equation and the Monod equation? The Monod equation as they pointed out that the growth related with the biomass concentration and limiting substrate concentration am I right. Biomass concentration because when we considered mu equal to what 1 by x dx by dt, so it depends on the concentration of the cells and if you look at the Monod equation mu equal to what mu max S k S plus S.

So, it is the limiting substrate concentration. So, it is there and then in the logistic equation is the independent of substrate concentration, it does not depend on substrate is the only related to the biomass concentration. What we have already observed that how it is related to the biomass. In the logistic equation directly proportional to the biomass concentration and the carrying capacity that the X m minus X.

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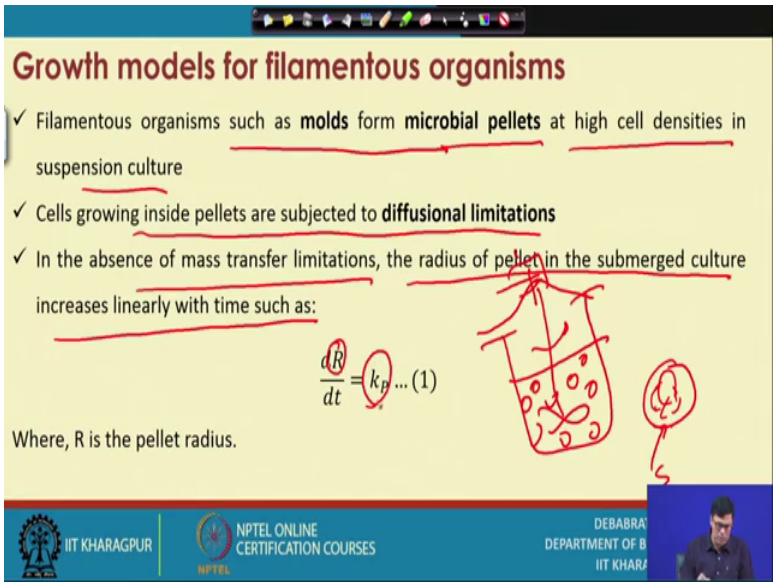

Now, in case of a unicellular cell we do not have much a problem because, but you know we have a typical type of growth characteristics in case of filamentous type of organism; as for example, as per gillas niggard. As per gillas armory when you grow in the fermentation media, that you know when they grow in the fermentation media what will happen? This you will find lot of pellet formation, the circular the spherical pellet formation.

The cells they will form the pellet inside the reactor. So, question comes how this we can growth model can be developed in case of filamentous organism, the filamentous organisms such as mold from the microbial pellet at the high cell density in suspension. So, this is under submerged condition they form the pellet. Now cell growth inside the pellet is subject to diffusion limitation; obviously, when there is a pellet at the concentration of the cell, then diffusion of the substrate diffusion inside the pellet is a problem.

The absence of mass transfer limitation that you radius of the pellet in submerge culture increases linearly with time as that dR by dt, dR is the change of radius of the pellet with respect we assumed to be constant the km, where R is considered as the radius of the pellet.

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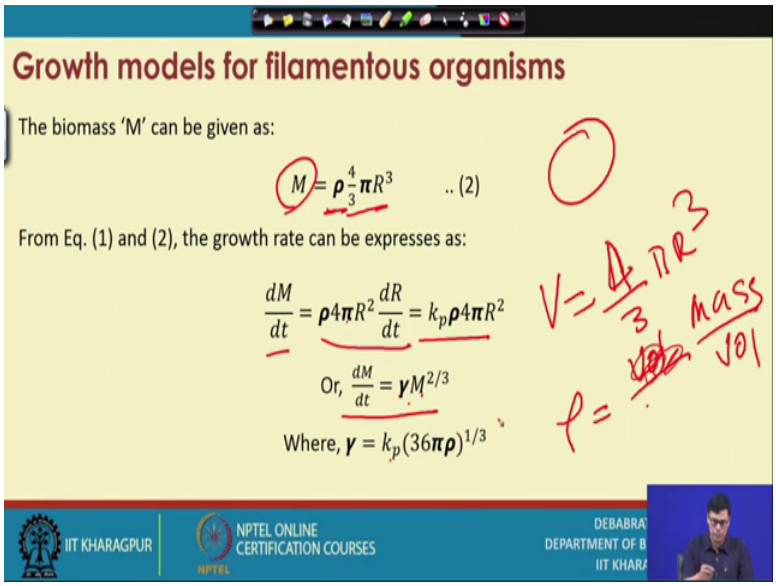

Now, here the mass of the cell the volume of a pellet; pellet is a we can consider this is a spherical in shape, what is the volume of the sphere 4 by 3 pi R cube am I right.

Now, what is the unit of rho is the volume per unit a mass per unit volume am I right. Now if you multiplied volume with mass that you know density. So, this will be obviously mass. Now if you differentiate with respect to time then we will get this equation by rho 4 pi R square dR by dt and we can write in this form and finally we can write dM by dt equal to gamma into M to the power 2 by 3, where gamma is equal to kp into 36 pi rho to the power 1 by 3, then we can write that the M equals coming in this form.

Now, if M we can differentiate we will get this equation and this I have already explained, then if you go to here this is dM by dt equal to M to the power 2 by 3 am I right.

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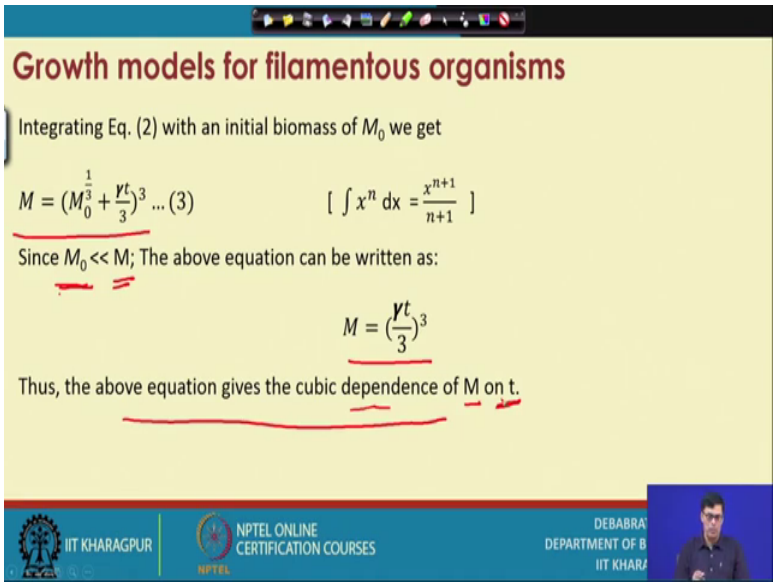

Now, if you write and the M integrate we will get form this is the M equal to M0 to the power 1 by 3 gamma t by 3 to the power 3, now if we assume M0 the initial mass of the pellet is negligible as compared to the final mass, then we can write this equation in this form. And this is how we can develop the correlation and the above equation gives the cubic dependence of the mass with respect to time. So, this is how you can develop the growth model in case of filamentous type of organism.

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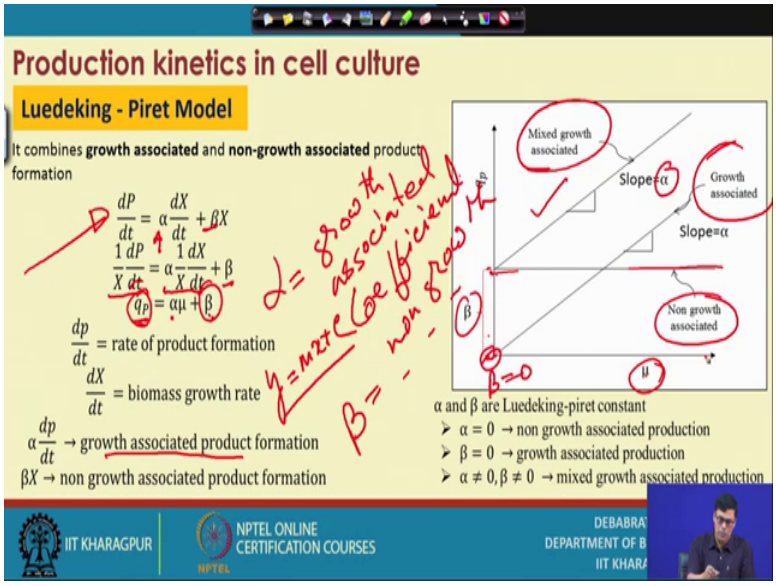

Now, as per microbial system is concerned, that up till now we talk about the rate of growth of the cells. Now what is about the product formation? And the rate of how the rate of product formation is correlated with the growth of the cells, the most of the microbial process that we grow the cell to get the desired amount of product I mentioned the basic difference between the biochemical process and the chemical process is what, that I told you that same substrate can produce the n number of products.

Here we only we change the type of organisms in the system, because glucose can be converted to citric acid, glucose can be converted to ethanol, glucose can be converted to acetic acid. So, question comes that how this product formation take place, how this product formation is correlated with the cell growth? Now this has been establish by the luedeking piret. The propose the equation like this that you can see here that dP by dt this is the equation they proposed dP by dt equal to alpha dX by dt into beta into x; where alpha is considered as the growth associated coefficient, you see that growth associated with this alpha is the growth associated coefficient and beta is the non growth associated coefficient.

So, I can write alpha is the growth associated coefficient am I right and beta is non growth associated coefficient. So, now if you divide by x then it will be specific product formation rate equal to alpha into a specific growth rate of the cell into beta. So, obviously we can write in the short form qp equal to alpha into mu into beta plus beta.

So, this is something equal to y equal to Mx plus c, this is equal to y into Mx plus c am I right it is straight line equation. So, what we have seen that, if you if this plot is the this is the beta intercept with the beta value and with the slope is the alpha value, the when in the growth model we have both alpha and beta value then we call it mixed growth associated product. Now in case of when beta is 0 the here beta will be equal to 0 am I right, this is passing through the origin.

Then we considered this as a growth associated product, now in case of non growth associated product the beta value should not change with respect to mu. Now you can see here that a here is specific rate of product formation it should be constant, this will not change with respect to mu value, then we call it non growth associated product. Now question comes that what are the different examples we have in case of growth associated product and non growth associated product.

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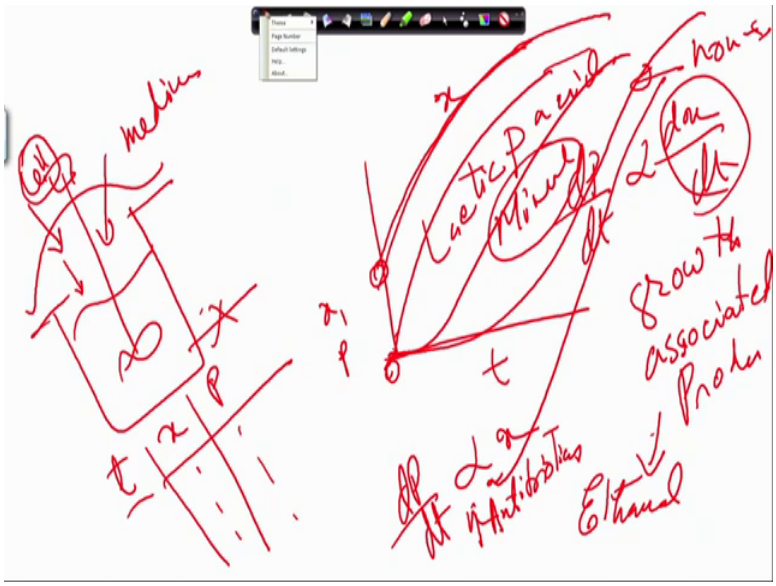

Now, what I want to point out suppose this is a batch fermentation process and here we first we put the media, then we proved the cell am I right, then time to time you can draw the sample. So, if you find out that you can find out the x, you can p at different times you can find out this is with respect to time, you know the x p if you plot it is if it is like this I needed like this, so this is P and this is x.

Now, 1 we should all remember the x should be little, it should not be 0 this is 0 am I right, because why you should not be 0 because you have to use the inoculums am I right, the cell you are adding to the media. So, there will be some initial cell mass concentration and from that the cell mass increase will take place and then if your product formation as your cells grow, if your product formation takes place simultaneously then what we can write dP by dt is proportional to dx by dt am I right. So, in that case we call it this is the growth associated product am I right.

Now, if suppose when your organism at in the Plato then, your product formation start then this is an example of non growth associated product. Now at that this situation dP by dt will be proportional to x, it will not be proportional to dx by dt. in case of non growth associated product your dp by dt will be proportional to x. Now in case of mixed growth associated product this is mixed growth that what will happen, both it neither it is growth associate nor it completely non a growth associated products.

So, both it depends on the growth of the cell and rate of growth of the cell and the cell mass concentration then we call it mixed growth associated the examples are several, but in case of growth associated product the examples I told you there is the ethanol fermentation am I right and in case of non growth associated product all the antibiotics like penicillin. Now in case of mixed growth associated product is the lactic acid, so this is the different fermentation process that we have.

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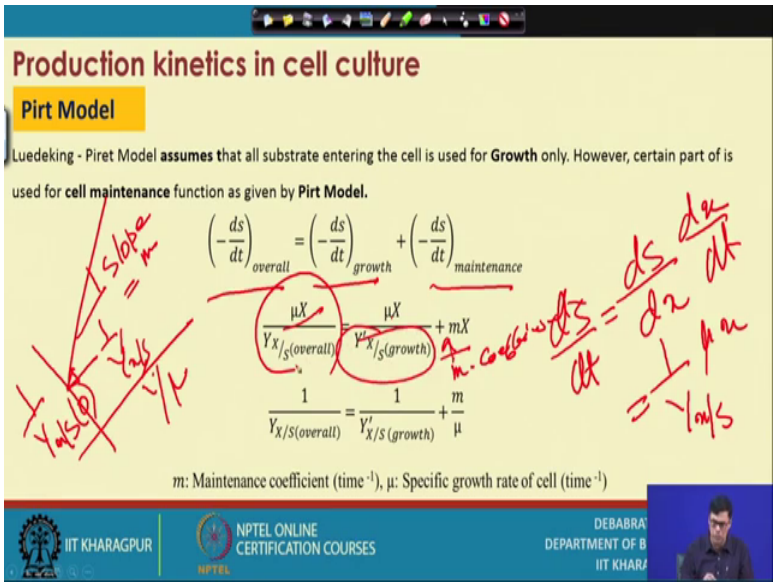

Now, next is the part equation; part equation where a cells when cell grow, it is some of the substrate use for the cell maintenance purpose. Now what do you mean my cell maintenance because in the solution if you see under the microscope, we will find cells are moving 1 place to others how they are moving they require some energy am I right. So, that is called the cell maintenance not only cells, that the cells sometimes due to mechanical studying and other purpose cell may get ruptured and for the recovery of the cell also they require some kind of energy and for the formation of some protein also they require energy.

So, the cell maintenance is a very important aspects that has been considered by part, now how they have considered what they are saying? The rate of substrate that is consumed in the system, it has dual purpose substrate is going for growth of the cells and substrate is going for the maintenance of the cells. Now when you say that a ds by dt when you write like the ds by dt, what I can write this is equal to ds by dt am I right. So,

this is equal to 1 by yx by S into mu into x. So, this is the exactly what he has written mu x into Yx by S the overall mu x y dash is a growth plus Mxa.

The why m X is a maintenance of the cells it depends on the proportional concentration of the cell more will be the maintenance less cell I will use dimension. So, this m is considered as the maintenance coefficient this is named end coefficient. Now if you divide by mu x then we have this correlation, now here if we plot 1 by YX by S overall and 1 by mu then we will get a straight line, the slope will give you the value of M and intercept will give you the 1 by Y dash x that is the this is called true yield coefficient. What is true yield coefficient? mass of cell produce the amount of cell produce from per a per gram of substrate consume, when substrate is used only for the cell growth not for other purpose that is called true grow thing and this is remain constant for a particular organism.

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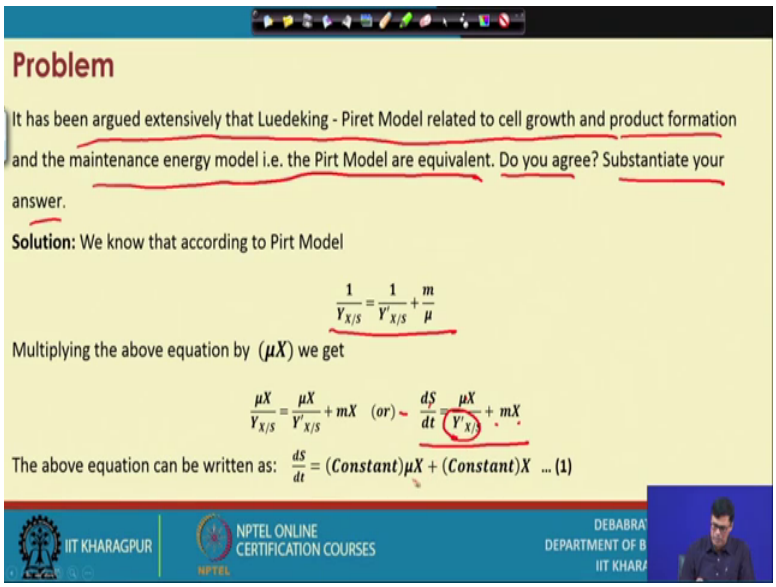

Now let us see the this very interesting problem, it has been extremely argued that Luedeking-Piret model related to the cell growth and product formation and maintenance of the energy model that is the part equation at equation equivalent do you agree and substantiate your answer. Now what does not mean that we by the time, we know that what is pirt equation deals with the maintenance of the cells and Luedeking-Piret model deals with the product formation.

Now, question comes are they equal are they equivalent not equal. Now how we can justify that? Now in the pirt equation we have this equate that the model, we have already derived and this has come from this am I right, that minus ds by dt equal to mu x why does this is the true yield coefficient m into X. Now why does x by S is constant am I right those we can write dS by dt is minus ds by dt is a constant into mu into X plus constant into x. Now when you go to come to the Luedeking-Piret model we can write the substrate use for 12 purposes.

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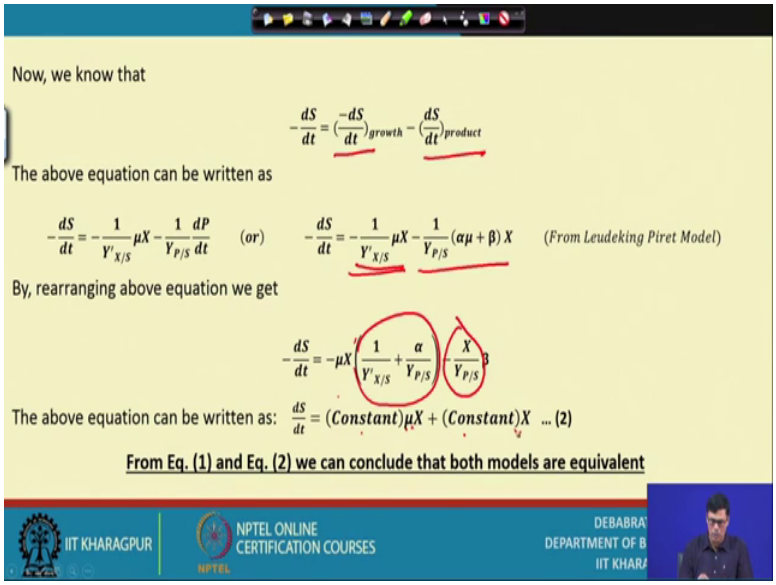

Similar to pirt equation 1 is for growth another is for product formation, now whatever the substrate use for growth I can write like this similar to a pirt equation and this is for this is for from the Luedeking-Piret model. Now we if we analyze this 2 equation then we can write minus d S by dt equal to this minus this, we can write that this is constant and this is also constant. So, we can write dx by dt is equal to constant into mu x and this is constant into mu x and we have found in case of pirt model also it is same. So, from that we can conclude that both are equivalent.

So, in conclusion I want to tell that different besides Monod equation, other scientists they propose the different type of growth model for the colding, how the substrate concentration related with the cell mass growth, and we find that you know some cases may be the case saturation constant is particularly the contrast models, the situation constant is related with the cell mass growth. If we cell mass grow this constant then cell

mass concentration is constant then it approaches to the Monod equation, then we try to explain the logistic equation and try to difference in logistic equation depends on the concentration of the biomass, it does not related with the concentration of the substrate and finally I try to discuss the Luedeking-Piret model and pirt the equation how they relate with the product formation and the maintenance of the cells.

Thank you very much.