Biochemical Engineering

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Module No. # 01

Lecture No. # 25

Effect of Multiple Substrates and Inhibition on Microbial Growth

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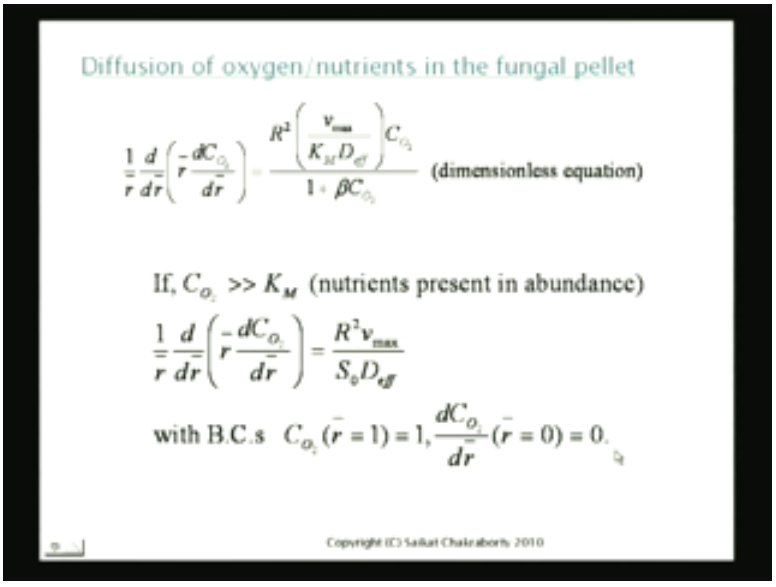

So, from where we left, which is that effective diffusion in the fungal pellet and if we go to the slide, now, what we see is that, we wrote this equation, if you remember, which is the diffusion equation. So, on your left hand side is the diffusion of oxygen, and on your right hand side is the reaction that occurs because of the growth of the fungus, fungal pellet. So, it is, the fungal pellet essentially has to grow. What was the major assumption out here? The major assumption was upto the steady state approximation. So, the idea was that, though the fungal pellet is actually growing in radius, but, the rate of growth of the radius is much smaller than the rate of diffusion of oxygen, as a result. And, how did we conclude this? We concluded this through time scale analysis. We measured what is the time scale of the growth of the radius of the fungus, and we measured, what is the

time scale for diffusion of oxygen in the fungal pellet. And, what we found was that, the time scale for the growth of the radius is much, much larger than the time scale for the growth of the fungal, of the diffusion of the oxygen; or, in other words, the diffusion of oxygen is a much faster process, as compared to the actual growth of the radius of the fungal pellet.

So, as the result of which, we can write the pseudo, steady state approximation, or in other words, we can assume that, the diffusion of oxygen is the steady state process, as compared to the growth of the radius, fine. So, based on that, we write this equation out here, and what you see over here is that, the temporal term in oxygen concentration is missing, because of the pseudo steady state approximation, fine. So, this is the radial diffusion, excuse me; this is the radial diffusion, and this is the reaction term and this is written in dimensionless coordinate; all of it is written in dimensionless coordinate; and, I am not redefining these dimensionless coordinates. The reason I am not redefining is that, we already did this same, that immobilized enzyme case, you know, the last example we did in, in the immobilized enzyme, already had the same variables and same dimensionless coordinates.

So, you can go back and look up; (( )) so, I am not redefining it. Now, the assumption here is that, C oxygen is much, much greater than K M, which is a very reasonable assumption, because, if you want to grow something, you know, fungus is growing for example, oxygen is typically in abundance, as compared to the k m. So, that is a very straightforward assumption. Now, when you make that assumption, what you find is that, this, this equation, which is a Michaelis-Menten kinetics, reduces to a first order kinetics over here; is that clear? So, the Michaelis-Menten kinetics over here, reduces to a zeroth order, sorry, reduces to a zeroth order kinetics over here, because, in the, when C O is much larger than K M, then, what happens? C O in the numerator and the denominator cancel out, and this is what you have.

So, this is, this is what we got over here, and you know S 0 is all of this combined, K M and beta and so on, combined. So, R square, r is a radius of the mold, of the fungal mold, at that point of time; is that very clear? R, ideally, R is a function of time; it is increasing with time, but, since we are doing a pseudo steady state approximation, so, R over here is the radius of the fungal, at that point of time, fine. So, we write this equation; then, this is a very straightforward zeroth order, on the right hand side, and you can integrate it

straight away, and what are my boundary conditions? Some are written on the screen, which is that, the oxygen concentration at the outside radius r equals 1, or r equals big r, whatever it is, is 1 and d d d d r of C O 0 is 0, ok.

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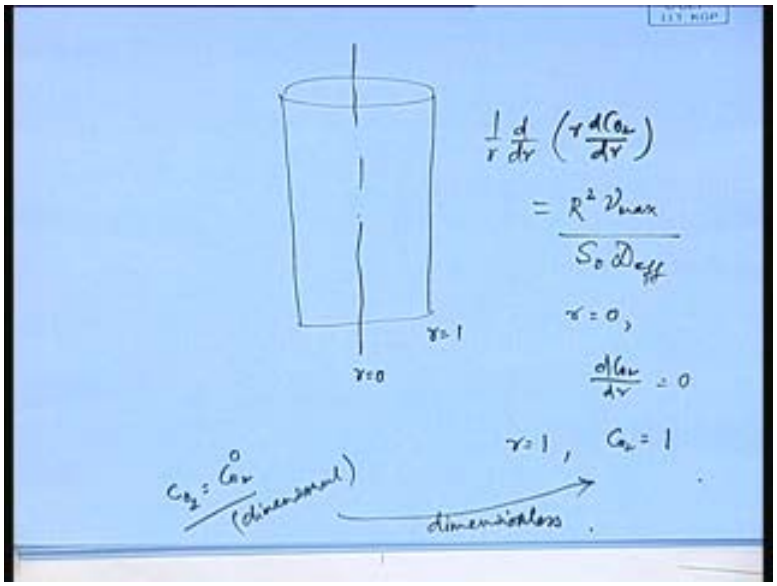

So, let me show you this by assuming this assumption. So, this is my cylindrical mold; my reaction equation over here is 1 over r d d r of r; nu max I used, instead of r max; the reason being that, r, now, we are using for the overall radius of the mold; that is why, I changed the…D effective; S 0 is that K M over beta, that you, K M times beta, sorry. So, this is my equation. The boundary condition inside is…So, this is my r; this is r equals 0; this is r equals 1. So, r equals 0, boundary condition is symmetry, right. So, equals 0, r equals 1; I am saying that, oxygen is present in a certain concentration. So, C O 2 equals 1; 1 is the dimensionless coordinate, which means that, actual value of concentration is something, like some value C, C O 0, or something like that. So, C O 2 is equal to C O 0 in dimensionless; dimensional, when you turn into dimensionless, this is what you get, right. So, this is a boundary conditions we have. We can straightaway integrate this. This is a straight forward integration and we can get the profile of concentration within the mold. So, you know, so, why are we doing this and the reason would be obvious, you know, some of, one of you, I think, asked this question the other day, and the reason would be obvious, once we come up with the solution.

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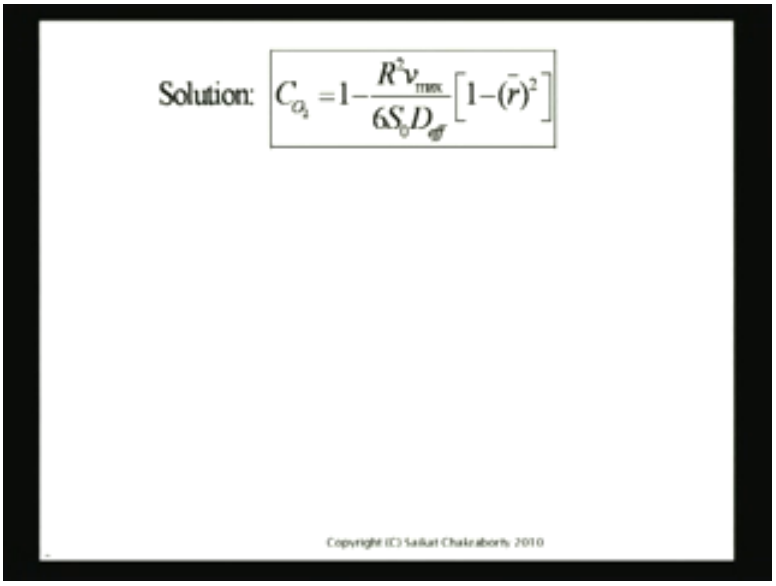

So, look at the solution. So, this is how it goes as, you know, it goes quadratically; essentially, as you see, it increases with radius, right. It increases quadratically with radius. So, it is some constant plus another constant times r square; is that clear. So, it is one constant, some a, plus b times r square. So, it increases quadratically with the radius. Now, what you know, what you know is that, the concentration at the outside, at this interface between the fungus and the air is a constant, which means, in other words, it decreases quadratically, as you go inside the fungus; is that clear. So, what will happen is, just try to, you know, invasion this. As you, as you keep increasing the size of the mold, what will happen? The gradient will get steeper and steeper, right, because the concentration that you provide at the outside, that is the interface between the mold and the, and air is a constant. So, as the radius keeps increasing, the gradient will get steeper and steeper, fine; and there, will come a point in time…

So, this is a steady, pseudo steady, under a pseudo steady state approximation, we are making this; but remember, this r is actually a function of time. So, when you make a pseudo steady state approximation, how do you do that? (( )) how do you enforce that? So, first you solve the steady… So, if I ask you to solve a problem using pseudo steady state approximation, so, what is the idea? You decouple the state, the two, two phenomena. How do you decouple the two phenomena? By first showing that, the time scales of these two phenomena are different. So, the time scale which is much smaller is under steady state. So, you write a steady state equation like, just like we did, and solve

for it; then, you solve the unsteady state equation and get how radius varies with time. For example, here, if you have your d r d t, if I remember, here d r d t equals K, fine.

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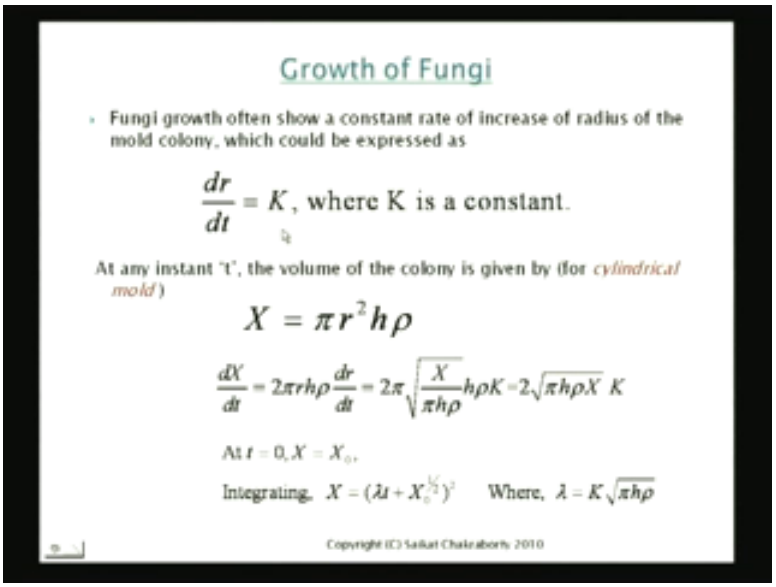

So, if this is what you have, then, you got your x as a function like this, and you can also integrate d r d t equals K over here, right away, and get how radius varies with time. So, radius here, would vary in some, you know, linearly, for example, here it is varying, some K t, fine. So, what you do here is that, after we have solved for the steady state, you impose that temporal dependence of radius on this equation; or, in other words, if radius, for example, varies linearly with time over here, or, radius varies exponentially with time over here, then, you go and put that into this equation; is that clear? Let me write, if it is not clear.

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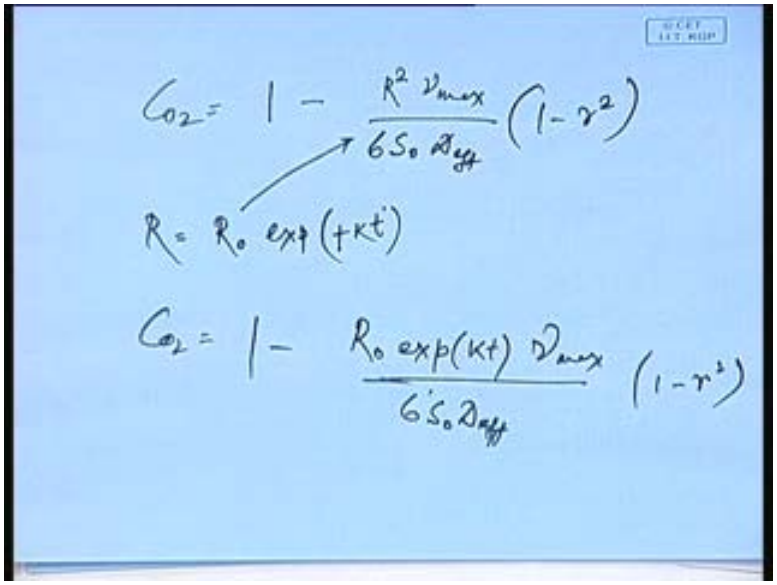

So, your C 0, the solution that you get here is, C 0 minus R square nu max, 1 minus r square. Now, if R equals some R 0 at t equal 0 time, let us say, exponential; we are just assuming; exponential minus, you know, K t, something like this, sorry; it should be plus K t, in this case, if it is increasing with time. So, what you do is, you put…So, this is your, essentially your R, at any point of time. So, you put that over here, and what you get is, 1 minus R 0 exponential K t, fine. So, if this (( )) exponential K t is some linear dependence and that will come in. So, this is a way to, is that, is that clear? This is the way to solve the pseudo approximation, when you have a pseudo steady, steady state, or there is the decoupling of state, two states, then, this is the way to solve it. First, go and solve for the steady state, and then, impose; then, solve for the unsteady state and impose that unsteady state condition on the steady state solution; is that clear? This is the way to do it, fine.

Now, excuse me. So, what you find over here, what you find is that, if just by looking at this equation that, as the radius keeps increasing, the concentration gradient becomes steeper. Now, the concentration outside is a constant, remember; if the concentration outside will not a constant, if you are increasing it, then, no problem; but, because the concentration outside the mold...So, concentration here is a constant, is a constant and this radius is keeps increasing, what will happen? There, there will come a point in time, when the center of the mold would stop getting any oxygen at all; is that clear? There will come a point in time, when the center of the mold will stop getting any oxygen at

all, and that means that, the center of the mold can grow, no longer grow; and, that is my critical radius.

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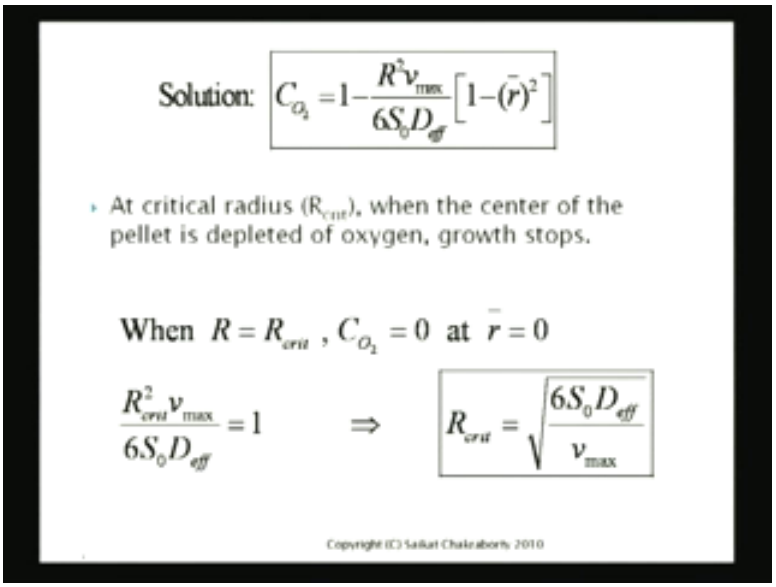

So, let me show you the definition of that. So, at critical radius, the critical radius is defined as that radius, when the center of the mold is depleted of oxygen. And, if the center of the mold is depleted of oxygen, then, the growth stops; because, this thing has to push from outside, you know; so, this is a, this is a radius that we want to evaluate; and how would you evaluate that, from this expression? We put r equals, this r equals what? What, how do we get this R critical?

(( )).

C.

(( )) concentration of (( )).

Yes, but, there, that is fine. This side is 0; but, there is a variable out here; critical radius is a constant and r over here, is a variable. So, C O 2 equals 0, I agree; what else?

Sir, that will happen at this center.

Yes; so?

That (( )) will also have to be 0.

Yes. So, this r has to be 0; this r, remember big r, is the external radius; this radius at r equals 0. Thus C O 2 has to be 0. So, you put 0 over here, 0 over here and you will simply get the critical radius right away, as square root of 6 S 0 D effective over nu max. So, I hope this answers your question from last time that, fungus cannot grow, grow indefinitely and there will come a point, when it will stop growing and that is the time it will try to divide itself, so that, it can continue to grow. So, this is the limit of the colony. So, it is a colony, that kind of, it keeps expanding, but, it does not expand indefinitely; it expands only up till the time, that the radius equals the critical radius; so, which is given over here, square root of 6 S 0 D effective over nu max.

So, once this critical radius is, is attained, then, the funguses stop growing and it will, maybe divide and allow for future growth; is it clear? So, what we looked at is, you know, we are looking at different, different things, at the same time. So, first, we looked at the normal growth kinetics, and you know, how the kinetics changes, and what is the dynamics of the kinetics, and how different models had been proposed, to sort of quantify these dynamics, and what we figured is this, that, of all the kinetics model, kinetic models that have been proposed, it (( )) the Monod model, or the modified Monod model, which are the most useful models.

Then, we looked at the effective mass transfer, gave you a couple of examples to look at the effect of mass transfer, and just as we had seen that, in the case of, case of a immobilized enzymes, mass transfer effects kinetics; or, in other words, kind of decelerates kinetics. Similarly, if mass transfer effects here are going to decelerate, or slow down the growth process; but, there is no running away from it, because, for the growth process, just as in the enzymatic process, you need the substrate; you need to provide the oxygen, and the nitrogen, and the hydrogen, and the carbon, just as we showed in the first, I think, the first slide of this whole chapter. You need all of these substrates to be available to the, to the cell and without this, there is no way you can have cell growth.

Now, as soon as you need all of these substrates to be available to the cell, you immediately need, you know, mass transfer effect, would, all the mass transfer limitations would automatically come in. So, there is no running away from it. So, we looked at some of these and with the effect of, with the, for the case of normal growth of single cells and the growth of fungus. So, what we said was that, the major difference

between the growth of cells, cell colony, and the growth of fungus is that, a cell, cell in, when a cell colony grows in general, it is like a cell is surrounded by the fluid and therefore, by the substrate, and therefore, it has direct effect, direct contact with the substrate; whereas, a fungus would always grow as a pellet, or a mold, you know, as, as the, as a lump of colony and therefore, the cells inside the fungus do not have any contact with the nutrients.

So, the only way the nutrients can come in, is through the interface, from the outside, it is slowly diffuse it and this is the basic difference. And therefore, we did, looked at the mass transfer effect for a single cell and then, we looked at the mass transfer effect for the fungus mold. So, we are at this point. Now, what we are going to do for the rest of the lecture today, is look at some other effects, and those effects are the effects of multiple substrates and the effects of inhibition, which means that, if you have more than one substrate, right. So, you can provide substrates in different forms, and both can lead to cell growth; then, what happens, right. So, for example, you can give your, your sugar, you know, both in terms of sucrose and glucose, you know, maybe two different form.

So, fructose, sucrose or glucose, you know, two or three different forms. Now, all of these substrates, they have different kinetics, remember; each of them will have different kinetic, but they, all of them lead to growth of the cell, fine. So, how do we account for that, a; b is that, what would be the effective inhibition, just like we did in the enzyme case. So, there is, just as you have substrate, there could be inhibitory things that are there, a and b is that, when you give too much of substrate, that, one of the thing that we talked about, I think, earlier, you can have, what is known as substrate inhibition. So, how do these affect the cell growth process and we are just going to look at that. I hope we can finish this today. Then, we can start a new chapter; but anyway, we are not going to hurry through it; we are going to do it very slowly.

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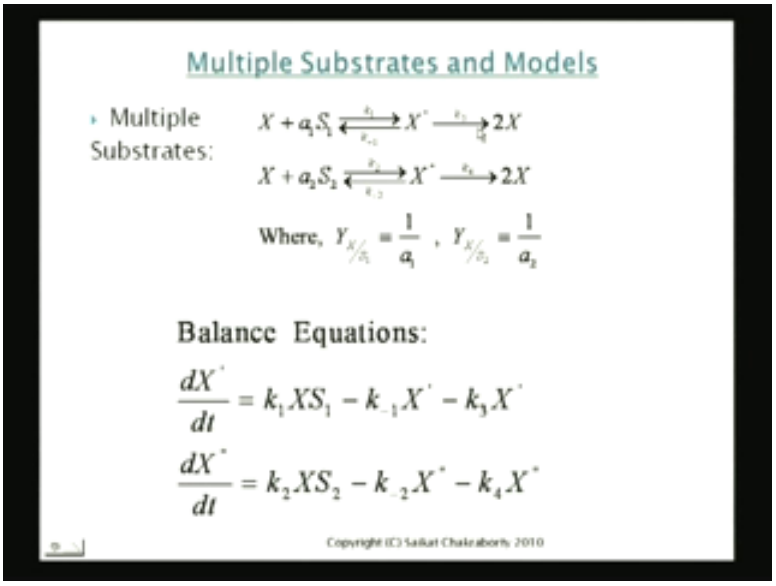

The first thing we will look at, is the effect of multiple substrates. So, multiple substrate means that, you have cells which are exposed to two different substrates, and both these substrates will lead to the product formation, that is daughter cells, but through different kinetic routes. So, this is, what you see on the screen is, x is your cell, starting cell, and S 1 is substrate number one and you get the daughter cell x, 2 x; but, through the formation of the intermediate compound, intermediate cell x prime, which is one route, with a set of reaction coefficients k 1, k minus 1 and k 3; and then, you have another substrate, that is, they are parallelly in the system. So, you have, might have, you know, given two substrates to the system. So, that gives again, the daughter cells, 2 x, but through different route, x double prime; and the reaction rate constants are k 2, k minus 2 and k 4. Now, I talked about the yield yesterday, if you remember, in the last class, I talked about yield and so, the yield is the amount of daughter cells produced per, per mole of the substrates, ok.

So, for case one, what is your yield? One daughter cell is produced; from two, two cells, you get, one cell, you get two cells, which means, one, effectively one extra cell has been produced for a one mole of the substrate. So, the yield of x over S 1 is 1 over a 1; and for case two, the yield of x over S 2, that is, one daughter cell has been produced for a 2 moles of S 2. So, yield is 1 over a 2, fine. Now, how do we find…So, what we are trying to find is, what the, how would we find the overall growth rate of the system? So, what is the goal, that has to be very clearly defined. So, we did…What did we do? We have done

only one single substrate before, and we have found the growth rate; so, which is d x d t equals nu, nu, nu times x, where nu is given by the Monod, Monod kinetics, S 0 over K plus S 0, some r max S 0, double K plus S 0.

So, here we are trying to find out, what would be the form of the growth, overall growth of the system, because, what we are in, essentially interested at the end of the day is the overall growth. I start with 5 million cells; how many cells do I get after 24 hours or so, that is what I am interested; excuse me. So, the balance equations, excuse me, the balance equations over here, are this. So, what I am writing is, I am writing a balance for the intermediate species; the del x t, del x prime del t and as you see over here, the first term is k 1 x S 1 which is the term, because of this forward reaction over here. See the, where the arrow is. So, this forward reaction over here, we (( )) this term. The second term is minus k minus 1 x prime, which is the result of the backward reaction over here. The first reaction, the backward reaction you had; and the third term over here, is minus k 3 x prime, which is the result of the formation of the product, of the daughter cells. Is it clear, these three terms? It is very straightforward; and then, the same thing is repeated for x double prime, ok. So, k 2 x times S 2 minus k minus 2 x double prime minus k 4 x double prime; is it clear to all? So, is there a question?

(( )).

No; power, no, no, no, no; yes; that, I will explain that. See, this is not an elementary reaction. See, when do you have the power? Let us, let me explain that, if there is any doubt.

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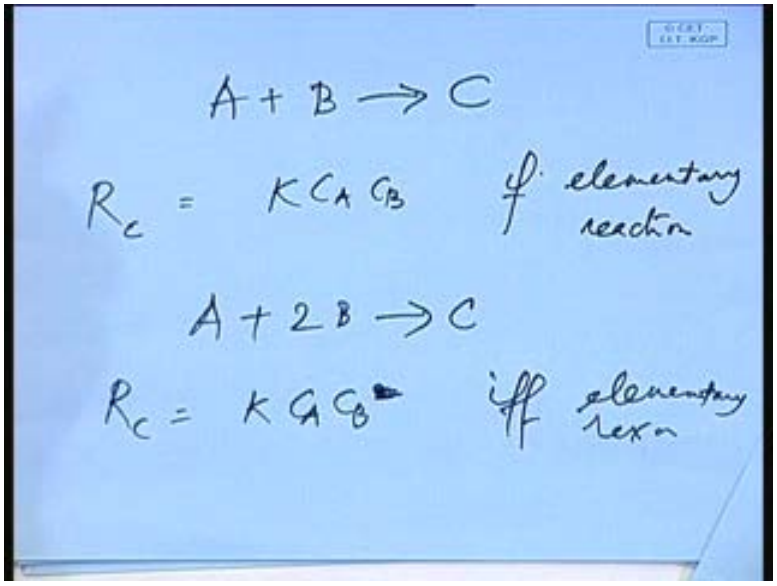

A plus B giving C; then, rate of formation of, of C is K C A C B; if it is an elementary reaction; A plus 2 A, 2 B giving C, the rate is, could be given as K C A C B square, only if, if and only if, it is an elementary reaction; is that clear? If it is not an elementary reaction, there is, nobody said that, that is a, that is a rate. So, it could be this.

(( )).

This one?

No; no, no, A plus 2 B (( )) equals to C.

Yes, elementary reaction means, the definition of the elementary reaction is that, the rate of the reaction is written exactly as the same way in which the molar concentration, composition of the reactants react; that is the definition. See this reaction for example; it is defined, an elementary reaction is, will be defined only if…So, if this, this molar concentration is 1 is to 2 mole, it reacts in 1 is to 2 mole ratio and the reaction rate is also given as C A C B square; but, it is not necessary that, exactly the, the mole ratio in which they react, will be the ratio in which the, the rate of the reaction is measured; because there, see that, the elementary reaction means that, there are no other effects, apart from the molecular collision between the, between the two molecules that govern the rate of the reaction. Do you understand what I am saying?

So, if there are no other effects…So, it is…See, this is very unlikely that, this will be an elementary reaction. Why, because, the probability of 2 molecules of B and 1 molecules of A, coming and colliding and forming 1 molecule of C, is very, very small. So, typically, a elementary reactions are of the, or either first order, or second order. So, you know, when we talk about first order reaction, so, it cannot be an elementary reaction; because, 1 molecule cannot combine with your, itself; there has to be a molecular collision. So, what is the elementary reaction mean that, the way 2 molecules collide with each other is exactly the way the reaction is represented, as also, when the reaction rate is written. So, do not assume that, whenever you write a...So, this is the molar ratio; this is its stoichiometry.

So, do not confuse stoichiometry with the power of the reaction. The stoichiometry would be same as the power of the reaction only, and only if, it is an elementary reaction; elementary reaction means that, all other effects are ignored. So, it is directly that, the 2 molecules are combining and that is forming the reaction; but, in the most cases, it is not like that. You know that, there are complex steps out there; for example, this step that I have written over here, as I, as we do in the enzyme, they are not necessarily elementary reactions, which means that, it is not that this step alone is occurring; there are multiple steps that are, that are probably occurring over here, and they are summarized as these reversible reactions. And, elementary reaction means that, one single step, single step reaction and in that ratio. So, the molar ratio, the stoichiometric ratio that is there; so, they combine, these 2 molecules, it combine and form. So, this is something very fundamental; you should not make a mistake about it.

So, if I want to say, tell you that, this is the rate of the reaction, then, I will say that, this is the reaction and which reaction, one is an elementary reaction, which means that, in this case, you will raise to the, raise this to the power a 1; and, but then, elementary reaction, if a 1 is not an integer, then, it cannot be an elementary reaction also; do you understand what I am trying to say? If a, a is say 0.75, it cannot be a elementary reaction, because 0.75 of a molecule cannot combine with, you know, another molecule. So, for elementary reaction a 1 has to be a number, typically, 1 or at best 2; even 2 is hard, but, at best 2; but, not more than 2; because, 3 molecule, the probability of 3 molecules combining with each other goes down. You know, if you have read some of (( ))

mechanics, you will understand that, the probability goes down exponentially. So, anyhow, now, so, this is the reaction rate and…

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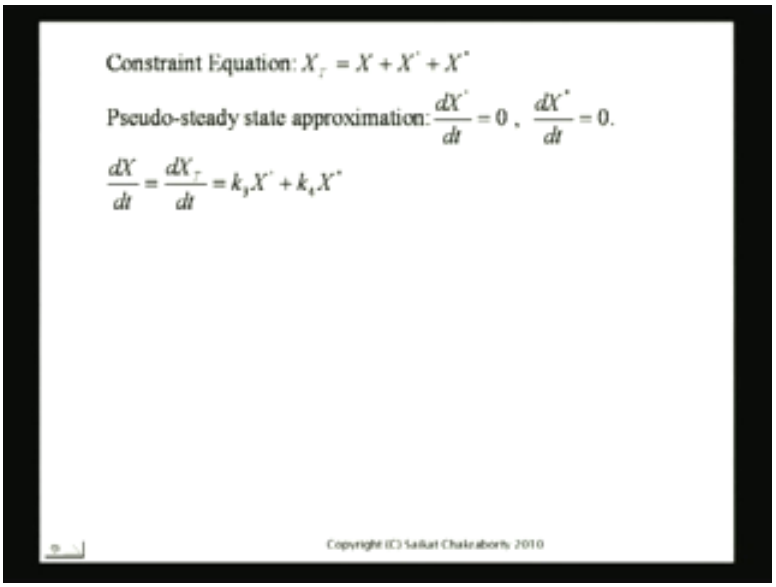

So, the next step, what we do is, we write the constraint equation like we did before, which is the total amount of cells present in the system. So, x t is the total amount of cells present in the system. So, the total amount of cells present in the system is either in the form of x, which is a daughter or the mother cell, or in the form of any of the intermediate species x prime or x double prime. So, the, this, this is my constraint equation. Next, what I do is, use a pseudo steady state approximation, the quasi steady state approximation like we did before; that del del t of x prime equals 0 and del del t of x double prime equals 0, fine. Now, let me explain this a little bit, from what is written in the last line.

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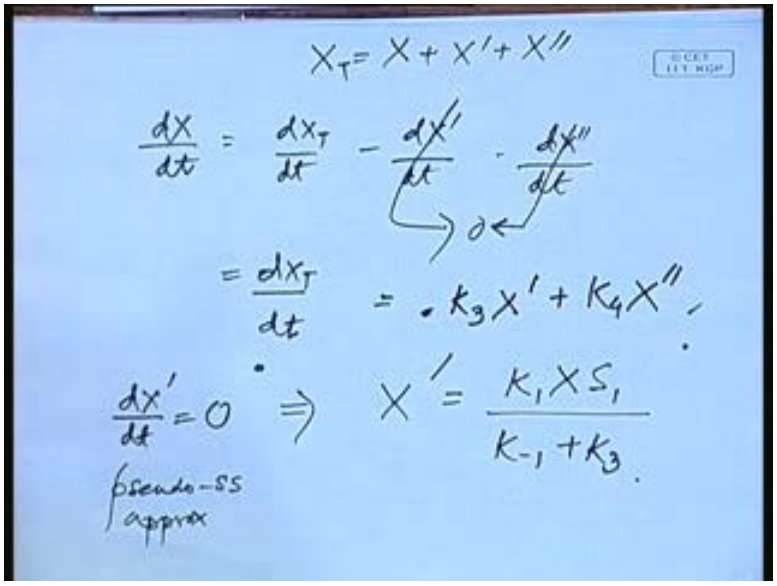

So, d x d t. So, x, my x equals, x t equals, x plus x prime plus x double prime. So, d x d t would be d x t d t, minus d x prime and d t minus, d x double prime d t, right; but, these are 0, because of pseudo steady state approximation. So, you get this as d x t d t, fine; you get this as d x t d t.

Now, this equals k 3 x prime plus k 4 x double prime. Can anybody tell me, why is that? d x t d t equals k 3 x prime plus k 4 x double prime?

(( )).

Right. So, x t is what? x t is the overall amount of cells that are present. Now, see, the only difference here from what we did in the enzyme case, if I go, if I am allowed to go back to this slide. So, look at this. So, this is the reaction that is occurring because of the formation of the daughter cells that are occurring through the, from x prime or x double prime to 2 x. So, what is the major difference from what we are doing here, from what we are doing in the constraint? This x t is not a constant; that is the only difference. In the enzymes case and all the previous cases we had done, this x t was a constant, right. But here, the x t is not a constant. So, that is a major difference that, that is there; why, because, so, there is a overall growth, growth of the cells. And, how is this overall growth quantify, if I come here. So, what is the overall growth? If you look at this system, for example, the overall growth is, 1 cell, 1 x, 1 cell, 1 daughter cell grows from this, ok.

So, the total growth for the system d x t d t, d d t of x t equals, so, k 3 times x prime plus k 4 times x double prime. So, one daughter cell growth, each of these reactions; first reaction, one daughter cell growth, and second reaction, one daughter cell growth; is it clear to everybody? This is a little tricky part. So, this is what we have. So, I hope you agree with me. Now, d x prime d t equals 0. So, x prime equals, if you go back, you know, to your notes and check the, I, I can go back to the slide in a minute, but, just let me write this; this is what I get here, if you go back to your notes and the pseudo steady state; this is a pseudo steady state approximation; pseudo steady state approximation; this is what we meant. So, you will see that, d x prime d t equals 0 would give you this and if you have a doubt, I will go back to it in the, here. So, look at this. So, what you have is k 1 x S 1 minus k minus 1 x prime minus k 3 x x prime, ok.

So, from here, you can, if this side, the left hand side is 0, you can straightaway express your x prime as a function of x and S 1; clear to all of you? So, the same thing you can do over here, with x double prime. If your d x, d d t of x double prime is 0, then, you can express your x double prime as a function of x and S 2; yes, S 2, right. So, this is what we do over here.

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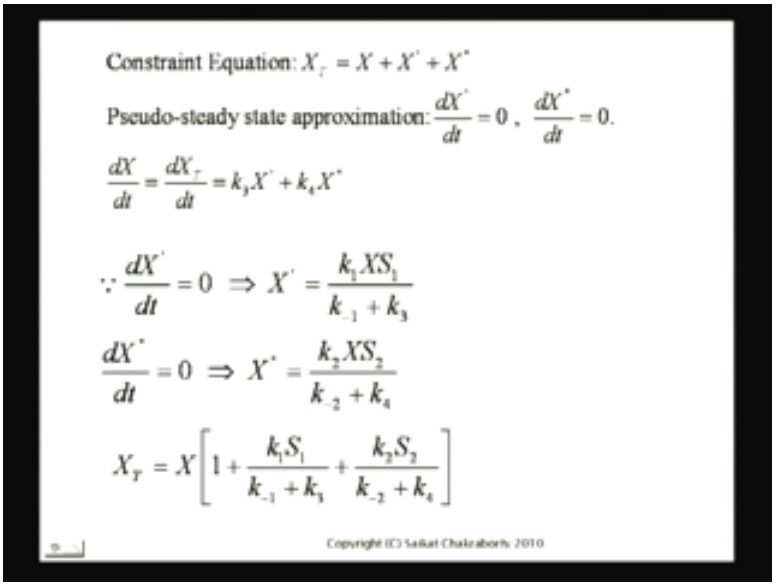

So, x, x, d d t of x prime is written as 0, and x prime is given by this number, and x double prime is written as this number. So, why we are doing it because, we intend to substitute these two into our, our constraint equation, just like we had done before. So,

when we substitute these two into our constraint, our constraint equation is here at the top and what we do is, we substitute these two into our constraint equation. So, this is what I get. So, x t equals x times…So, x is the first term, then, x prime here. So, you take x out of the parameters, it is common, out of the parameter; so, you get this and get this. But, so, only thing that you have to keep in mind again that, x t is not a constant over here; it is varying with time. So, that is the major difference between what we are doing now, to what we did in the previous constraint equation. Now, we can take a derivative of this; I will, I will continue, as soon as you finish writing. Shall I go ahead?

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$$\frac{dX_T}{dt} = k_3 X^* + k_4 X^{**} = X\left[\frac{k_3 k_1 S_1}{k_{-1} + k_3} + \frac{k_2 k_4 S_2}{k_{-2} + k_4}\right]$$

$$= X_T\left[1 + \frac{k_1 S_1}{k_{-1} + k_3} + \frac{k_2 S_2}{k_{-2} + k_4}\right]^{-1}\left[\frac{k_3 k_1 S_1}{k_{-1} + k_3} + \frac{k_2 k_4 S_2}{k_{-2} + k_4}\right]$$

$$= \mu X_T$$

Now, we take the derivatives. So, d x this, this was already something that I have written d x d t, d d t of x t is k 3 x prime plus k 4 x double prime. So, you do the same thing again; you replace your x prime by what you got from the pseudo steady state approximation; you replace your x double prime by what you got from the pseudo steady state approximation, fine. Just write the first step, then, I will show the, the next step; just write the first line, then, I will show you the, you know, how to get the second line. You probably know that already, but, I will still show you.

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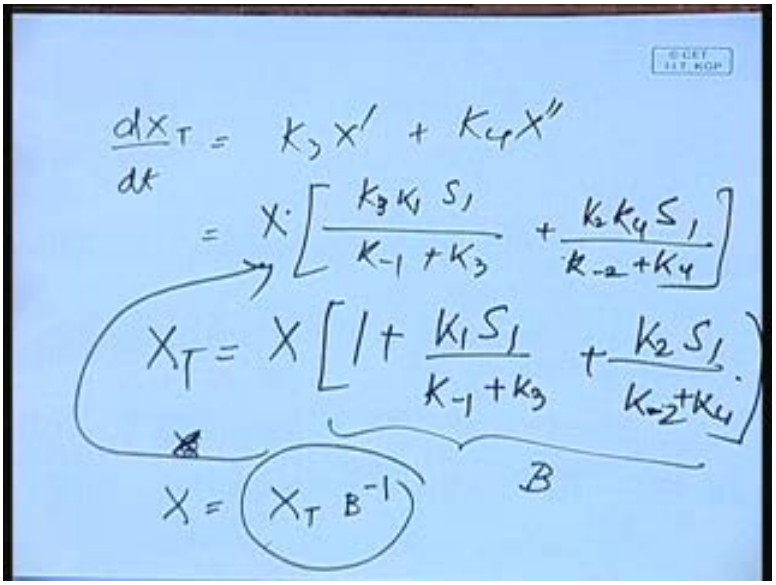

So, d x d t equals k 3 x prime plus k 4 x double prime. So, this, you replace; as soon as you replace, you get x times this number k 3, fine. Now, what you do is, let us go to this slide. So, here, you can have the…Here, the last line, you have the, you have the relationship between x t and x. So, because, why I am doing this because, we, we, we want to find everything, in terms of the total amount of cell that is present in the system. So, because it does not matter to us, which one is the, is a pseudo steady state, which is the daughter, which is the, because, what you can measure essentially at the end of the day is, a total amount of cells; you know, you, under microscope, you cannot differentiate that, this is in the intermediate species, this is the final and this is the initial species. So, what you can measure, is the total amount of cell. So, that is why, all our calculations, we want to do it in terms of the total amount of cell. So, x t over here is 1 plus k 1 S 1 k minus 1 k 3 plus k 2 S 1 k minus 2 plus k 4, fine. So, this quantity is a little too big, so, let me call it, this B or something. So, if x is x t times B inverse, then, you can put it over here, into this equation, this whole thing. So, x t is… So, then, you can get it in that form and let us call this, this thing A; this, this term is A; this term is B; then, what you have is…So, what I did was, I just said, this term is A; this term is B.

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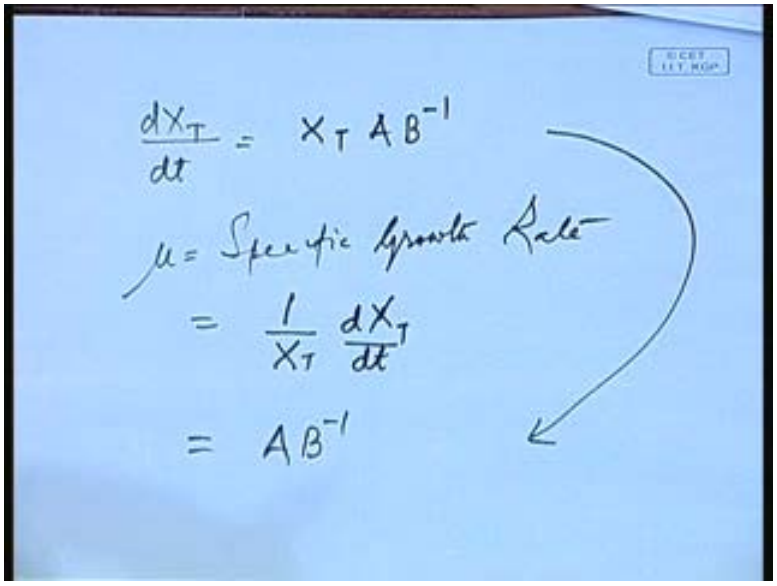

And then, what you have is d d, d x d t equals x t times A times B inverse, fine. Now, what is my mu? mu is the specific growth rate, fine. How is it defined? 1 over x t times d x t d t; take the first order rate constant. So, from this, what we will get from this equation, from here? So, what I get, this equals A times B inverse. So, I got my specific growth rate.

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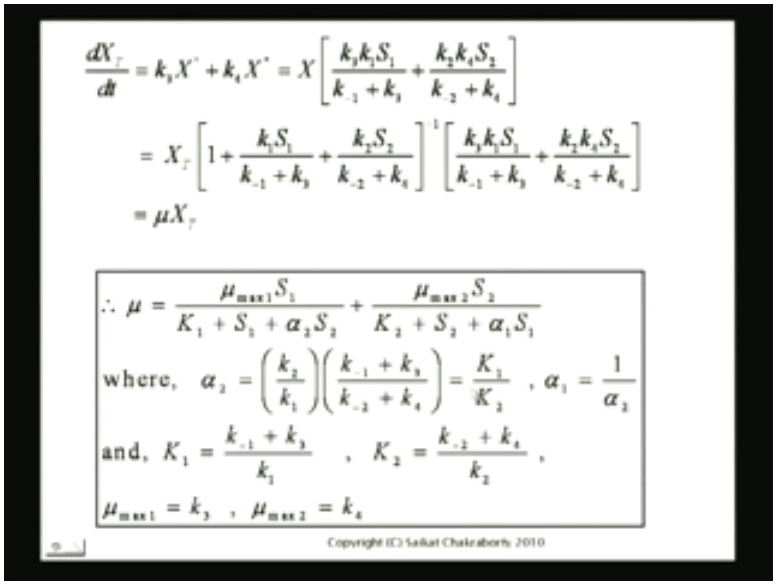

Now, the specific growth rate for the Monod model, if you remember, were some S, you know, K S times r x r max S times K over K plus S, right; that was the Monod growth

model, mu, right. So, now, you can…So, this is what you get. So, the exactly the, you know, once we do the, the steps that we did, did in, on paper, this is what you get and mu is your, mu, a nu rate, specific growth rate. So, once you do the simplification, which we are not doing because of lack of time…So, this is your mu; this inverse time this. And, there is, if you multiply both of these, and you know, you can do a little bit of simplification, then, you see, find out that, mu comes out to be in a very nice compact form, and what is interesting over here is that, the, it is interesting to note is that, the substrates influence each other.

So, the specific growth rate of the two substrates put together, is not equal to the specific growth rate of substrate one, and the, plus the specific growth rate of substrate two. They are not independent; the substrate, the, the, the two growths are not independent of each other, and what you find over here is that, the, this is specific growth rate of substrate one; but, it is being influenced; in the presence of substrate two, is, there is an influence out here, alpha 2 S 2. Similarly, the specific growth rate of substrate two is being influenced by this specific growth rate of substrate one and that is influenced out here in terms of alpha 1 s 1. And, alpha 1 and alpha 2 are constants, obviously. So, again, when you come to alpha 1 and alpha 2, interestingly, we will see that, alpha, alpha 2 and alpha 1, both of them involve all the rate constants, all the rate constants of the reactions.

So, k 1, k minus 1, k 3, k 2, k minus 2, k 4. So, all the six rate constants of the two reactions are involved in alpha 1 and alpha 2. And not surprisingly, what you find that, mu max 1 equals k 3 and mu max 2 equals k 4. Why is that not surprising, because, the maximum rate possible is the one, you know, in the absence of everything, just the straight forward production rate. And, why I am saying that is interesting, is because, you know, you, we did not presume anything; we just went ahead and did the calculation, and when you got the final results, you can make some physical sense out of those results. So, k 1 is the specific, you know, the rate constants in, involved in the reaction one; k 2 are the rate constants involved in reaction two. And, as I said that, the mu max, maximum growth rate of reaction one is k 3; the maximum growth rate of reaction two is k 4, fine.

So, this is an important result and, and you know, you might want to at least remember, if not remember it completely, at least, have a sense of what is going on. And, this is not difficult to remember actually, because, this is a Monod growth kinetics and you just add

one term in the denominator and this is a Monod growth kinetics, add one term in the denominator; and what are the difficult to remember, might be these constants. So, those might be difficult to remember. So, the next thing we do is, the effect of inhibition. So, here, first thing we looked at, is the effect of multiple substrates; but the next thing that we do, is the effective inhibition.

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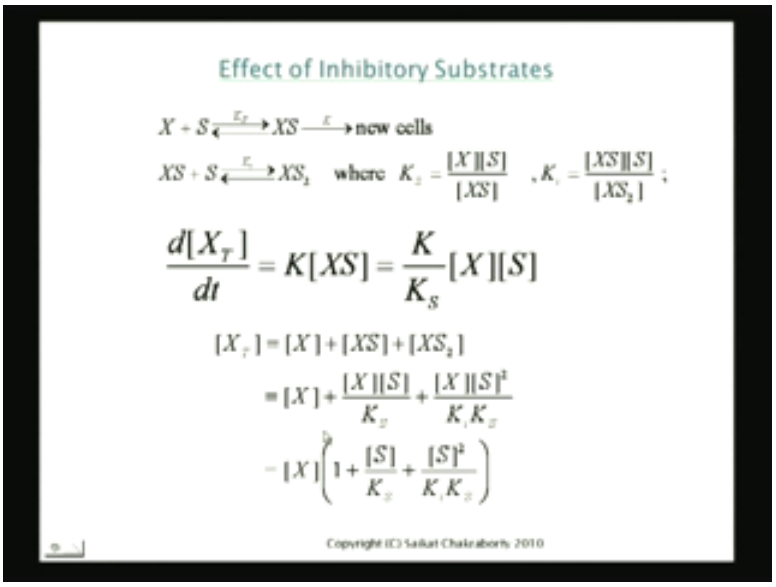

So, what is the substrate? You know, if you have one single substrate, but, instead of the substrate, along with the substrate producing the cells, the substrate inhibits. So, this is exactly the same, if you remember, as substrate inhibition that we did for the case of enzyme kinetic; exactly the same. So, the kinetics is also the same. So, in there also, we had the thing X plus S giving X S, which you know, in, in earlier case, gives products, but, in this case, our product is the new cell. So, X plus S giving X S gives new cell and this is the inhibition. So, X plus S, more substrate is there, that present over there; instead of, you know, giving new cells, it gives this complex X S 2, which does not lead to product formation. So, we have to, you know, go through the similar set of steps that we did for inhibition in case of enzyme kinetics, except that, in these, in this case, the difference being that, this is varying with time. So, x t is not a constant, but, x t is varying with time, ok.

So, x t over, d x t, d d t of x t over here is K times X S, right; is that clear to everybody? Why is that, because, this second reaction is not leading to any growth of cell. So, you

know…So, that is not considered over there. So, d d t of x t is K times S. Now, K, K, K times X S, sorry. Now, from here, from the first reaction, the reversible first reaction, what you find is, if this is assumed to have attained equilibrium, then, X S could be written as, X times S could be written as, K S times X S; is it clear to everybody? Very straightforward. So, we can replace my X S that I have over here, by X times S divided by K S, fine. So, you have K over K S times X times S, ok.

Now, the other things to take care of, this X S 2. So, my constraint equation again, is the total amount of cell that is present. Cells are present as parent cells and daughter cells, in, as X, in terms of the complex, intermediate complex X S, and in terms of the complex that you have is X S 2. So, the cells are presented in these three different forms, fine. Again, I want to reiterate that, x t is not a constant, but, it varies with time. So, d d t of x t is not 0; but, what we do over here is that, this, from this inhibition, in inhibition reaction, the substrate inhibition reaction, this, we again assume to have gained equilibrium, fine. We had assumed the substrate inhibition reaction given here to have attained the equilibrium and as the result, we can write K i as X S times X S times X S 2, clear. Then, X S, again, we can write from this equation as, X times S over K S, clear. So, that is what we do. So, we write this X plus X S plus X S 2. So, X plus X S, I have, I have substituted from here. So, X times S over K S and X S 2, I have substituted from here. So, X S 2 is, from here is, X S times S over K i, fine; where, X S could again be substituted by, X times S over K S, right. So, I can write this like this, and then, X, then, I can take X common, out of the whole thing, and this is, this is the part I get. I will just show you, if there is any problem with this.

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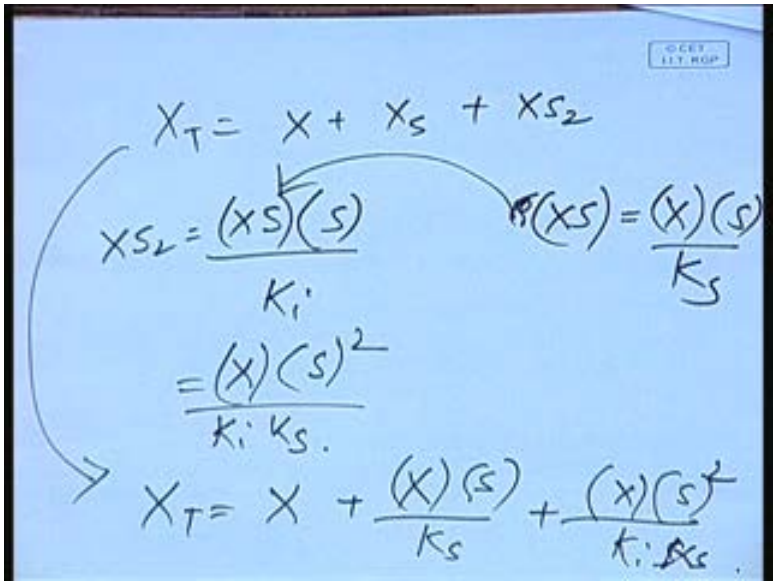

So, x t equals X plus X S plus X S 2. I am just doing away with the bracket. So, X S 2 equals X S times S over K i. Now, X S equals, this is from the X times S over K S and replace this over here. So, what I get is, X times S square over K i K S. So, from, if I replace it over here, I get X plus X S X times S over K S plus X times S square over K i K i K S. So, you can take it out.

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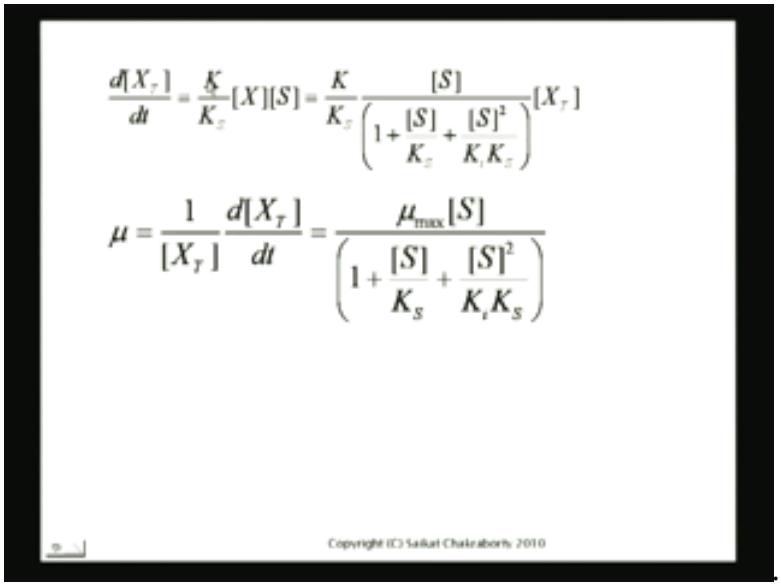

So, what you, what you get over here is that, d x d t equals K S, K over K S times X times S; the reason being that, this is the final reaction. This is the only reaction, only thing that generates products, fine. Is that clear?

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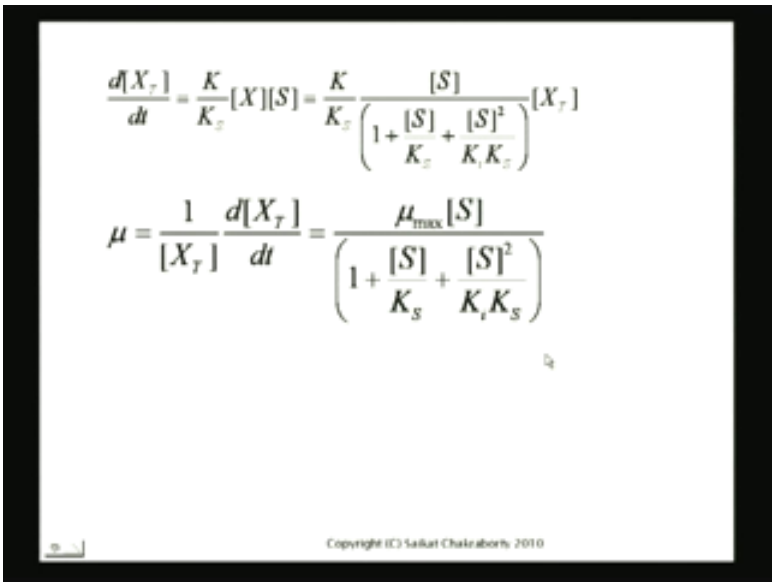

So, this is the only, this is the only step that generates product. So, you have K over K S times X S and then, you can substitute your X, X in terms of x t that we got. So, x in terms of x t is, we got over here; you substitute that back from here and you get in terms of x t. Now, my specific growth rate is 1 over x t d d t of x t. So, what is that? That is now, my mu over here, fine. So, what you find is mu, is this. So, what is the difference? The major difference that you find over here is, as compared to the mu that you have before, was that, the mu before, had something which is similar to a Monod growth kinetics; but, this is no way similar to the, close to the Monod growth kinetics. And, if you remember what we did from last time, what, what is going to be the characteristic of the mu?

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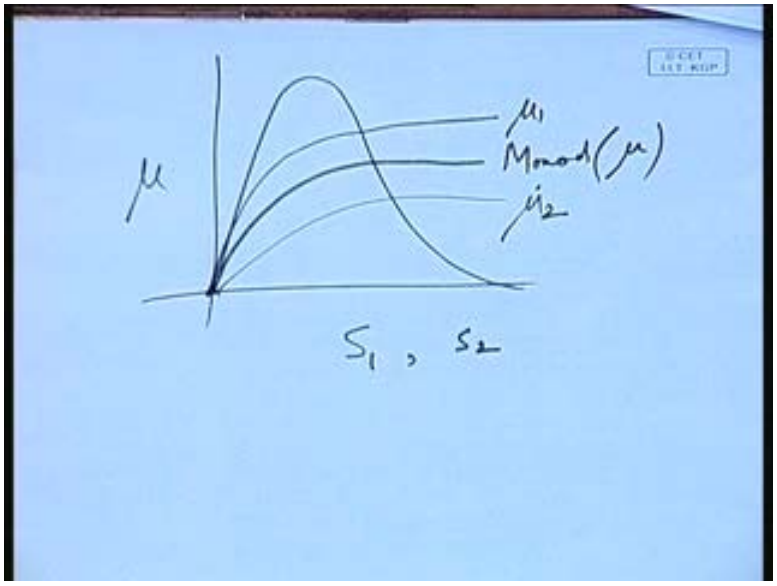

How is this? You know, how is this mu going to be different from mus' in the last case, graphically? Let us think graphically. So, you know, mu over S; in the Monod growth kinetics, it is going to be something like this, right. So, Monod. So, for multiple, you will have S 1 and S 2. So, say mu 1 is something like this; mu 2 is something like this, for multiple (( )); this is mu. Now, how is this (( )).

(( )).

Yes. So, it will go through a maximum and then, go down to 0. So, this one starts at 0 and saturates; this one starts from 0 and goes to 0 through a maximum. Why is that? If you look at the expression itself, you will see that, that, it will, it will saturate out in the absence. So, how do you obtain the maximum? So, they are simply differentiated and do a del mu del x and get that. So, we did a del mu del x to get the maximum value, and the maximum specific growth rate turns out to be, S critical is square root of K i K S; square root of K i K S. It is similar, if you remember from last time, in the case of the enzymatic growth, it is exactly similar, to what we did in the last case. (( )) when you are done, (( )) last case (( )). For the last case, you can do some calculations on your own also. So, there is a mu; just do, do a del mu del x and get this. So, shall we move on?

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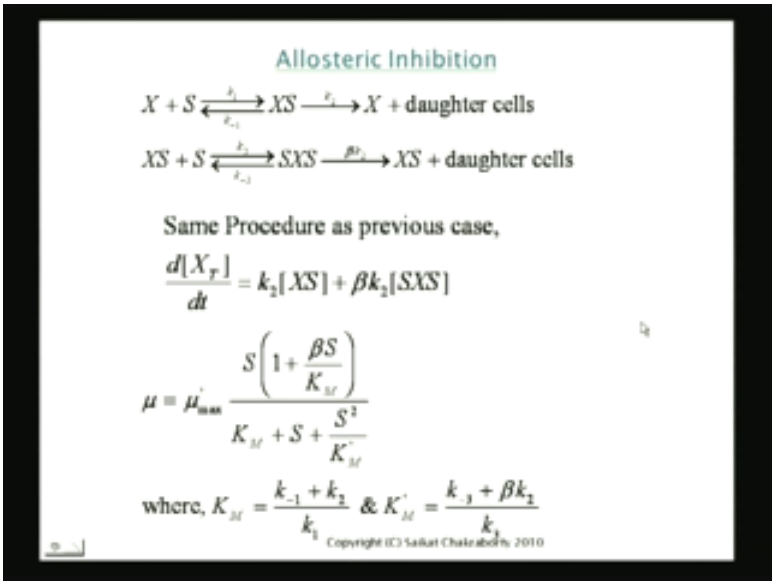

So, the last thing that we are going to do is, in today's lecture is, allosteric inhibition and this is the last thing that we are going to do in this chapter also. So, in this kind of inhibition, what happens is that, instead of stopping here…See, what is the difference between the last case and here, is set, last time, we stopped here, X S 2 and we stopped here. In allosteric inhibition, what happens is that, this also gives rise to daughter cells. We do not stop here completely. We say that, this also gives a rise to, rise to daughter cells. So, in a way, it is the combination of substrate inhibition and multiple substrates. Come to think of it, it is a, do you agree with me? So, in a way, it is a combination substrate inhibition. In substrate inhibition, we had everything like, we had, but, we stopped over here, right. So, there is no daughter cells produced from the second reaction. But, in the multiple substrate, we did not have the same substrate, or combining, but, we had daughter cells from both the substrates.

So, in a way, it is like a combination of multiple substrates and substrate inhibition. So, I would not do all the steps for it. I will do some steps and you can go ahead and do the rest, either here, or later, maybe at home, as an assignment. So, the procedure is that, the same procedure is as before; the only difference that is going to happen is that, del del t of x t is K 2 times X S plus this beta K 2 or X K 3, K 4 also we can call it. But, we just call it beta K 2, just for the sake of algebra, times X S 2; is that clear? So, I think, there, there is nothing hard in it. So, and then, you have to go and do the rest of the algebra, which is the x t, you have to, you know, calculate the x t, which is the X plus X S plus X

S S, as before, and do the del del t of x t. And then, take the reversible for both cases, and this is a final result that you will come up with for mu. mu is defined as 1 over x t del d d t of x t and this is the final result, you will come up with. So, the difference between, yes, the difference between last time and this time is that, last time, we had the denominator and you had one term in the numerator; you have, you have another term in the numerator. So, it is quadratic, both in the numerator and the denominator and when you, again, it will have a lot more interesting kinetics.

So, what I would like you to do is, probably (( )) slide here. So, what I would like you to do is, actually go and plot this; because, this is not necessarily going to have the same kinetics as, as that and it can even have multiple peaks. I am not sure, but, once you differentiate it, you will be able to figure out that, if it has multiple peaks. So, what I want you to do is, as a little assignment is that, do the steps; steps are very straight forward; but, do the steps and check the answer. And then, once you have got this, and I want you to plot this a and b c. The first thing you should actually do is, go and differentiate it; find the, find the maxima and the minima; because, once you get the maxima and the minima, you can plot it; very straight forward. Otherwise, you know, it is like a black box, trying to plot this. So, go and find the maxima and the minima; one of the maxima is going to be like what we had before; but, there in, there is a quadratic term. So, it could lead to some other interesting effect.

So, I want you to see that. So, this is, this is a something, very common that happens and this is, this is the specific growth rate in, in, in that case and we need to understand how this works, in order to be able to figure out. So, the reason we are doing this is, like, you know, what we are going to do in the next chapter and these, all these cases that we did today are important, because, I am going to quiz you on, on those things, later in the exams.

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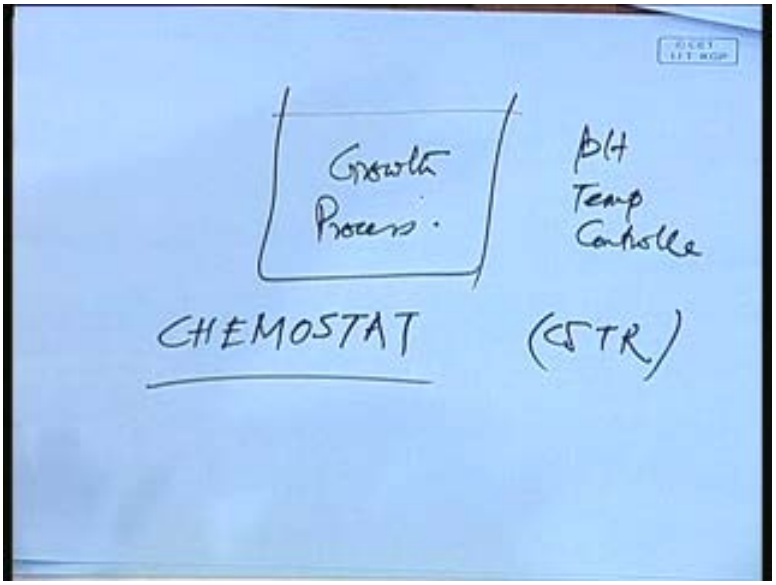

So, in the next chapter, what we are going to look at, is the process, the dynamics of cell growth. See, we are looking at the kinetic sub cell growth. But, what happens is that, the cell growth process happens in a, in a reactor like this, you know. So, it could be a batch reactor; it could be a chemostat, continuous third tank reactor.

So, one of the things that we are going to look at is, starting next class, it is a new chapter, is chemostat ,which is a C S T R, C S T R for a biological reaction. So, temperature is controlled and you know, pH is controlled. So, pH and temperature controlled and then, we look at the growth process inside this reactor. So, that is when all the kinetics that we are doing in this class and the previous classes in the mass transfer effects and so on, come into play. So, right now, we are looking at…So, this is all connected. So, right now, we are looking at the kinetics in itself; just isolating the kinetics and looking at it. Then, we put, check the kinetics out and put into the reactor, and look at what the effects are, and this, these are, I am, I am assure you that, some of these effects are going to be very interesting. So, we, we will work with, you know, some, probably, what we will start with is, not even this, the very straight forward case here, the Monod kinetics and then, we will go and slowly do what I will probably ask you to do, what the effects of multiple reactions are, the effects of inhibition and the effects of allosteric inhibition. So, we will stop here, and I will see you in the next class. Thanks.