Bioreactor Design and Analysis
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Lecture 03
Design of Batch Bioreactors - Part 1

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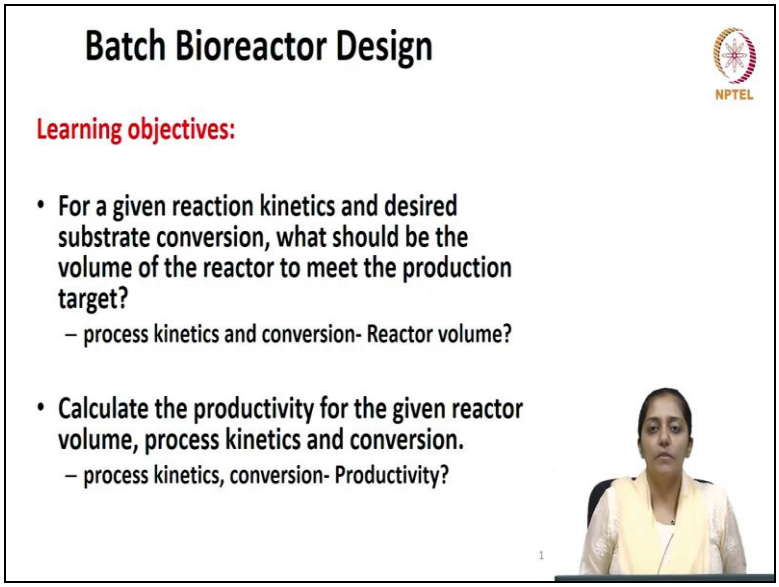

Welcome back students so after completing the introductory portion of the course let us begin with the batch bioreactor design. So today we are going to talk about what all is involved when we are designing batch bioreactors. So in the last class as I spoke about the different modes of cultivations and with the different modes of cultivation that is how the reactors are also classified; they are called as batch bioreactors or fed batch reactors or continuous cultivation reactors.

So let us study how to design the batch bioreactors. So the learning objectives for this particular module would be that for a given reaction kinetics and desired substrate conversion what should be the volume of the reactor to meet the production target? This is what one should be able to determine. The second thing is one should be able to determine the productivity of a given reactor when given the volume of the reactor the process kinetics and the conversion. Conversion here means substrate conversion desired.

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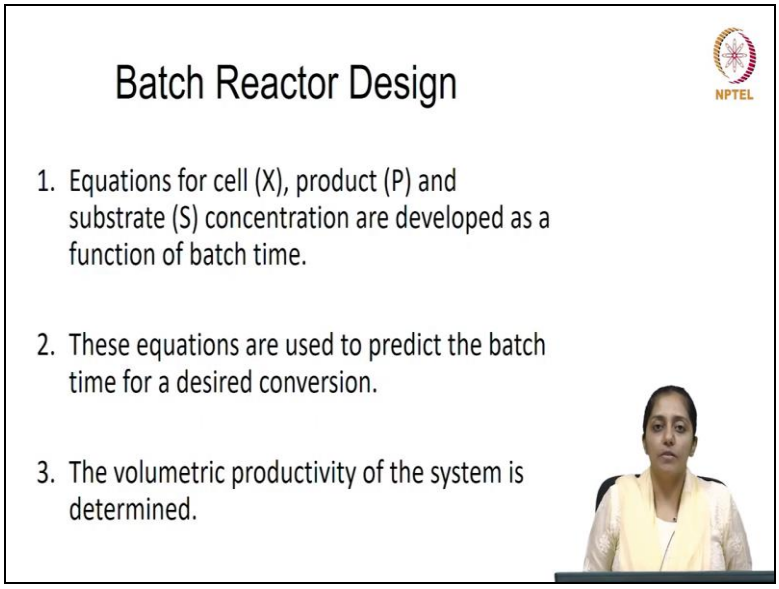

Now in order to find answers to the two objectives we must first formulate equations to determine the cell biomass, the product and the substrate concentration how it changes in the reactor with the batch time. These equations are used then to predict the batch time required for a desired substrate conversion. And then finally we can also determine using the same equations the volumetric productivity of the system.

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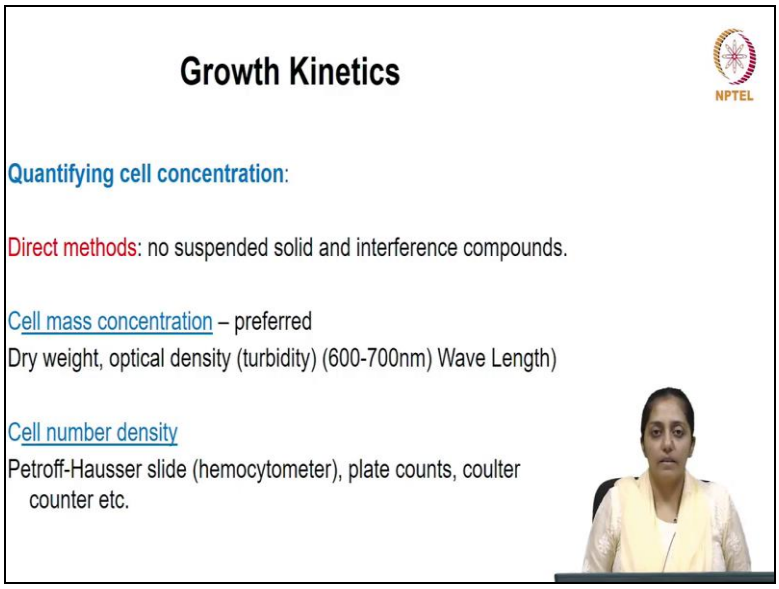

Now let us see how growth kinetics is generally determined in fermentations. Now anything changing with respect to time would be termed as kinetics of that variable. So here we are talking about the biomass or the cell concentration. Now how do we quantify cell concentrations? There are direct methods and there are indirect methods. In direct methods you can directly determine the cell mass concentration by determining the dry cell weight with respect to time or by correlating with the optical density of the culture broth.

Now here what we are measuring is the turbidity due to increase in the biomass concentration and the light scattered is measured at a wavelength generally for bacterial fermentations it is around 600 or 660 nanometers. The other ways can be determining the cell number the cell concentration as a function of cell number where the cell number density can be obtained from hemocytometer which is used for cell counting or coulter counter.

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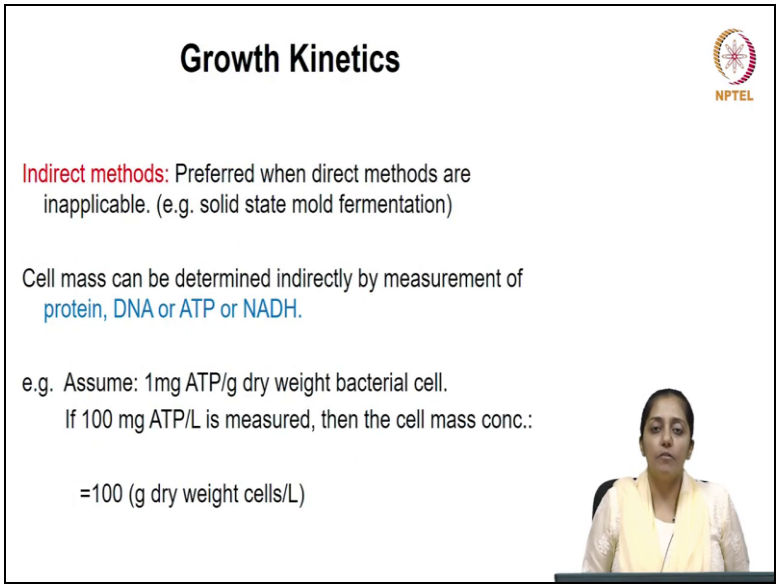

The indirect methods of biomass estimation involve correlating the biomass concentration or the cell number density with some intracellular parameters like the DNA or the ATP or NADH or proteins there are correlations in literature where people have found a mathematical correlation between the cell number densities. And how these intracellular parameters change during growth and they have been found to be directly correlated.

Like for example here if you see the example on the slide one milligram ATP per gram dry weight let us assume this is the amount of ATP present per gram dry weight of the cell then if 100 mg of ATP per litre is measured at any time then the corresponding cell mass concentration would be 100 grams dry weight per litre.

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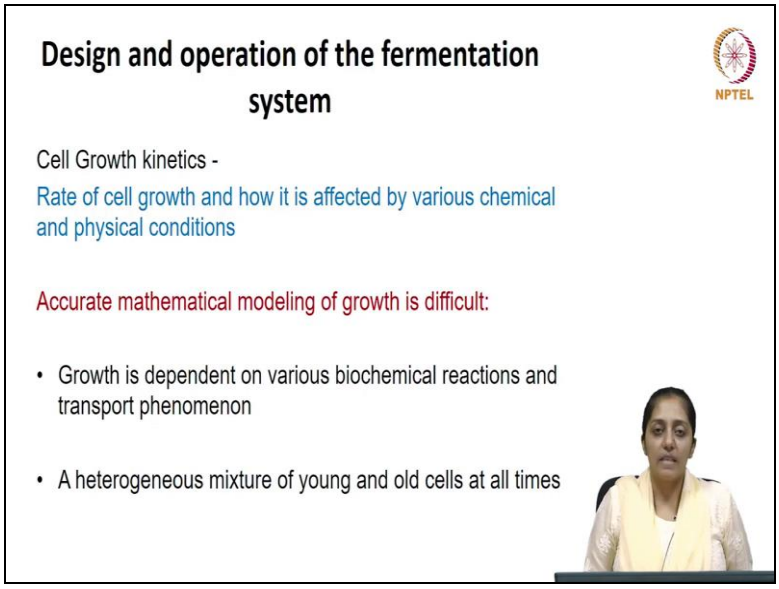

Now as I said earlier the cell growth kinetics or cell biomass growth kinetics is nothing but the rate of cell growth and we try to correlate it with various chemical and physical factors. How this rate is changing with those factors? Now when we determine this correlation it takes a form of a mathematical model although accurate mathematical modelling is difficult because there are numerous physical and chemical parameters which are going to affect the cell growth rate.

However we take assumptions for ease and there are various empirical models which are present which can correlate one or more of these growth rate limiting factors with the growth rate. So let us see growth is dependent we know on various biochemical reactions and the transport phenomena. And there is always a heterogeneous mass of young and old cells at all times in the liquid broth.

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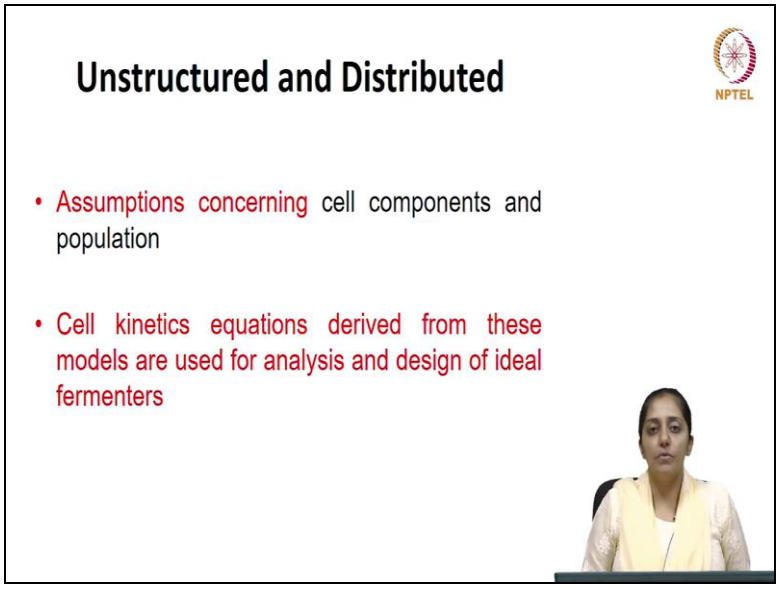

So for ease the most widely used kind of mathematical models fall under unstructured and distributed empirical models what does it mean? It means that there are assumptions concerning cell components and the cell population. The cell kinetic equations derived from these models can then be used for analysis and design of ideal fermenters.

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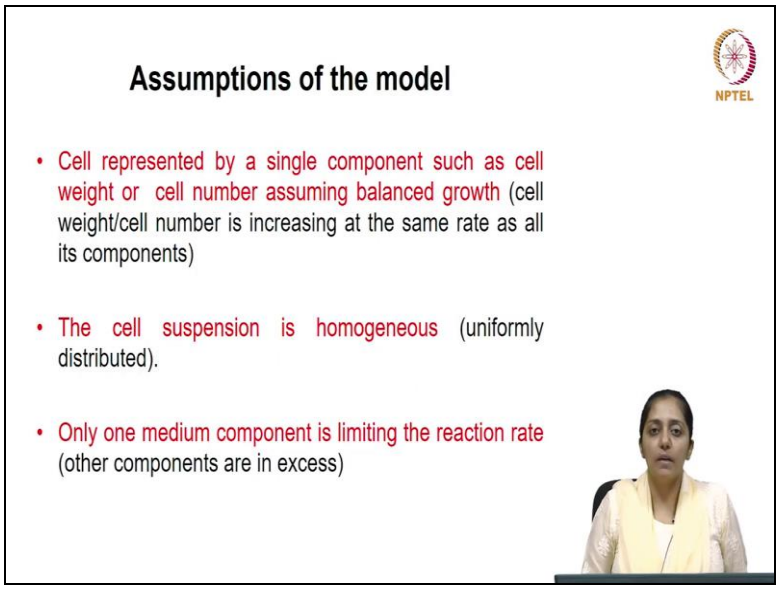

Ideal means we are assuming it is all homogeneous environment no mixing limitations. Now the assumptions in distributed and unstructured models these are cell is represented by a single component such as cell weight or cell number assuming balanced growth. Now what is balanced growth? Cell weight or the cell number is increasing at the same rate as all its component then we can term it as a balanced growth.

So when this assumption is in place the cell can be represented by a single component such as the cell weight or the cell number then the other assumption is that the cell suspension is homogeneous which means so that you can assume the variables are only changing with respect to time and there is no spatial variation. Third assumption only one medium component is limiting the reaction rate or the growth rate.

The other components here are therefore assumed to be present in excess throughout the batch time.

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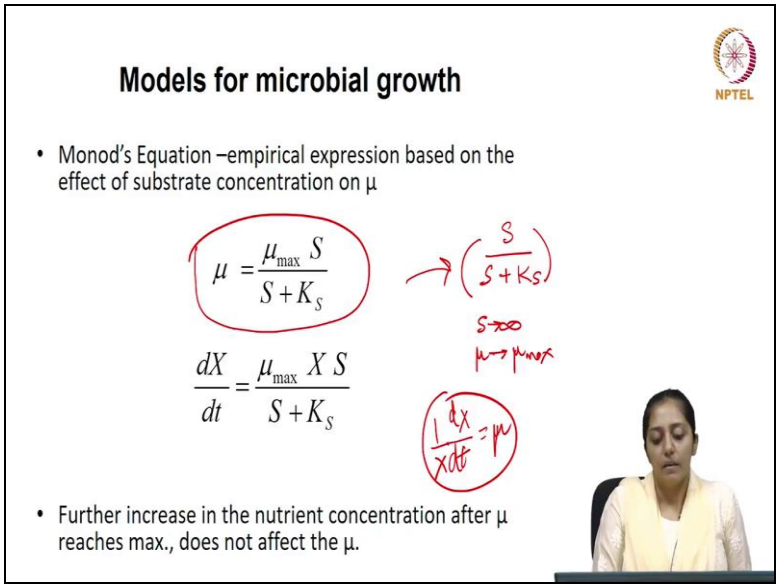

The most widely used unstructured distributed model is Monod's growth model. It is an empirical expression which is based on the effect of substrate concentration on specific growth rate of the culture. Where the specific growth rate as you can see on the slide can be represented as mu is equal to mu max times a limiting factor which is said as S upon S + K s. So this is the limiting factor.

So if you decipher from this equation one can make out clearly the culture will continue to grow at its maximum specific growth rate till the S is in excess. So which means you can see as S tends towards infinity your mu will tend towards mu max specific growth rate is given as 1 by x dx by dt. Now this is the rate of biomass accumulation inside a batch bioreactor which is equal to the specific growth rate of the culture.

So now in the second line the mu has been substituted by 1 by x dx by dt and the x has been taken to the right hand side of the equation towards mu max S upon S + K S. So now this

gives us a form how is the rate of change of biomass concentration is related to the limiting substrate and the biomass concentration at that time. So in the same equation one can see that any increase in the nutrient concentration after mu has reached mu max will no more affect the specific growth rate.

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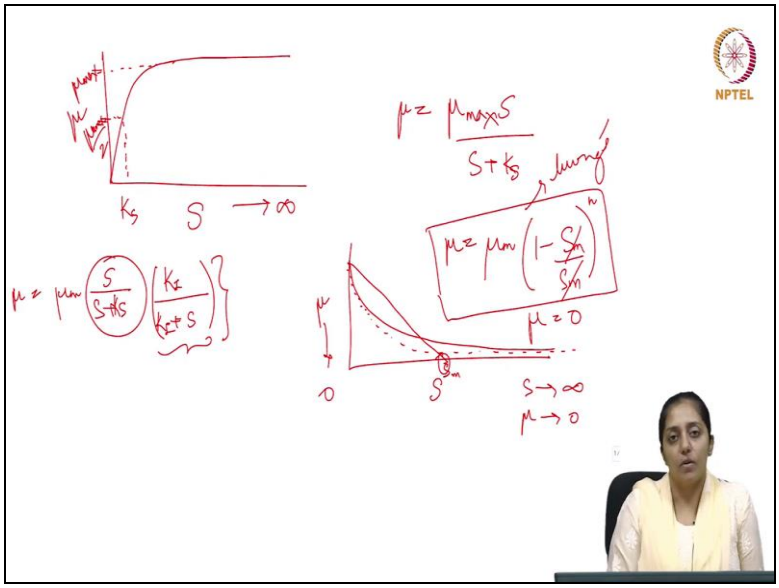

If you make a plot of mu versus S a culture following Monod's kinetics will show you a saturation curve like this which means as was mentioned in the point that as S is in excess the mu becomes equal to mu max. And what we call in Monod's model as K S which is called as Monod's saturation constant its value mathematically is that substrate concentration at which mu becomes equal to mu max by 2 half of its maxima.

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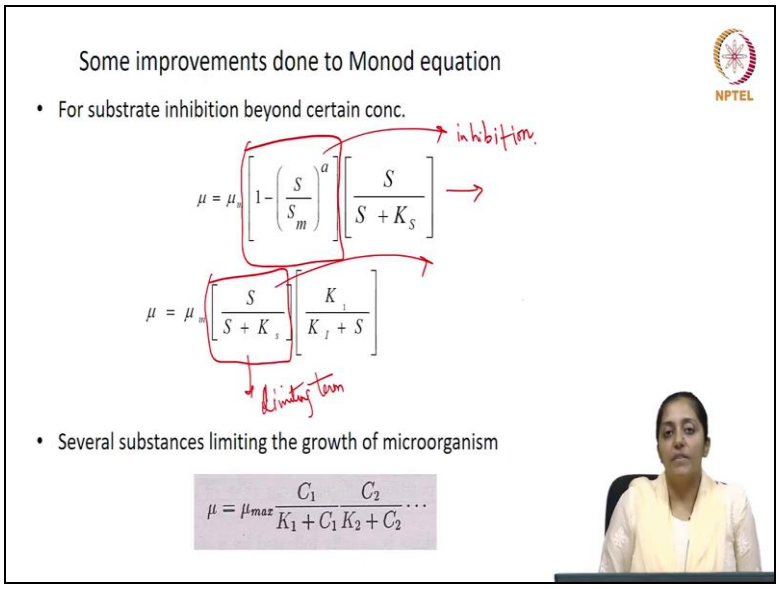

Now apart from Monod's model there have been other variations which have been made depending on the different other phenomena's when taken into account. Like for example Monod's model only takes care of the limiting effect of the rate limiting substrate. What if the substrate is showing an inhibitory effect as well then in that case there are other there are modifications which have been done to the Monod's model.

So as to bring the net specific growth rate of the culture as predicted by the model close to the actual value of the specific growth rate so for substrate inhibition into account you can see there are two well known type of equations which are used. Now these equations the first one this where the first term is what takes care of the inhibition substrate inhibition? Now can you make out how the first equation is different from the second equation?

Yes in the first equation if you see if you make a plot of how the specific growth rate is going to be inhibited by increase in the concentration of the inhibitory substrate you will see it can follow two different trends one is asymptotic and the other is inclined straight line with a negative slope. Let us assume in case of an inclined straight line where it cuts the x-axis at a value of substrate which we let us call it as S m then we may say that it is some defined value of substrate at which the specific growth rate is becoming nearly zero is not it.

So at this point the specific growth rate becomes zero. So this point is that substrate concentration at which we can say complete inhibition is happening complete inhibition of growth. So then if you remember the model it was something like this so if S becomes equal to S m will it not depict that mu becomes equal to 0. So this model is useful when there is a defined substrate concentration at which there is complete growth inhibition being observed.

So the equation said mu is equals to mu m S upon S + K S this was Monod's limiting factor. The other part of the equation talks about some K I upon K I + S this was your inhibition term. Now one I have already explained which is called as Luong's model is a well-known model which is used to determine substrate inhibition kinetics when there is a defined substrate concentration at which the specific growth rate becomes zero which means complete inhibition is happening.

But in case if you observe in your system that mu versus S curve the inhibitory substrate concentration is taking a form of an asymptote like what I drew here which means that as S

will tend to infinity the mu will tend to zero. So this kind of kinetics can be well determined by an equation of this sort K I upon K I + S. So one can make out how different model equations have been formulated to take into account the different physical phenomena's.

Now let us take another modification, if there are several substrates which are limiting the growth of the microorganism then how to incorporate the effect of change in the concentration of those substrates on mu. Then in that case let us assume there are different substrates let us call them as C 1 C 2 and so on. So then what you can see on the slide is one of the forms in which you can take into account the limiting effect of one or more of these substrates.

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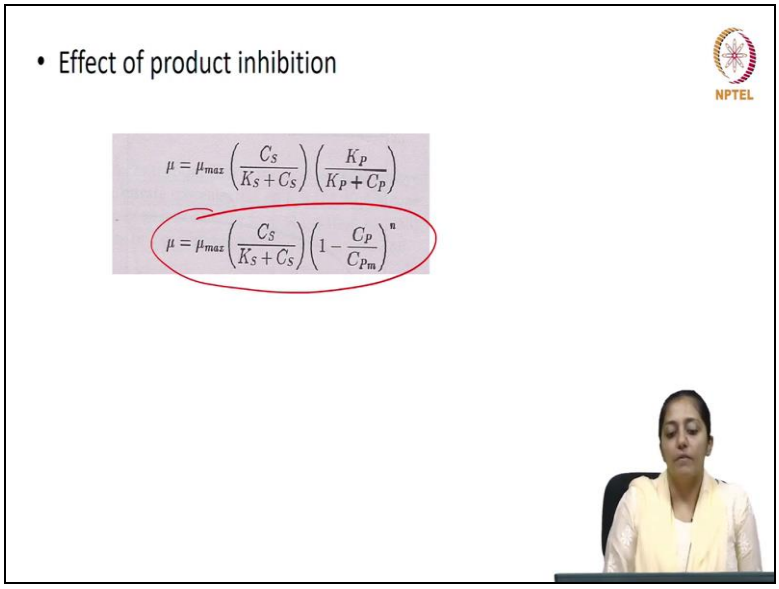

Now we were talking about substrate inhibition similarly if suppose we are observing that there is product inhibition in our system then how to take into account the effect of that product inhibition on the net specific growth rate. There are models present in literature like the one which are shown on the slide again based on the same principle. If you see a defined product concentration at which complete growth inhibition is happening then can you make out which among the two equations will be the right way to predict this phenomena?

Yes absolutely right I hope you can make out the second equation will be talking about that whereas the first equation will be an asymptotic behaviour of product inhibition.

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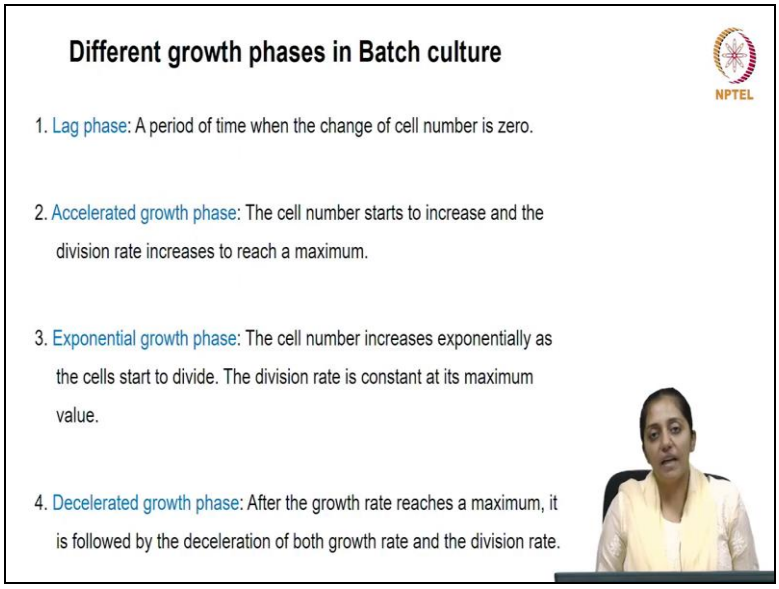

Now in batch culture it is important to know that there are different growth phases observed before we determine the growth rate of the culture. So there is a lag phase this is a period of time when the change of cell number is zero. So what is changing you can observe to some extent the change in cell volume but there is no change in the cell number. So there is no cell division happening during this phase the cells are trying to prepare themselves for subsequent cell divisions.

When I say prepare themselves which means the required enzymatic machinery or metabolites which are required they are being produced then comes the accelerated growth phase. This is the phase when the cell number starts to increase and the division rate increases to reach its maximum. So the cell number has started to increase the division rate rapidly increases to reach its maximum.

So during the acceleration growth phase the division rate reaches its maxima and the cell number continues to increase then comes the exponential growth phase. Now what is exponential growth phase this is the time when the cell starts dividing at maximum division rate and the cell number will change exponentially with time. The division rate is constant at its maximum. After this as the substrate starts getting depleted then we reach the deceleration phase.

Now; decelerated growth phase means that after the growth rate has reached its maximum. So now the growth rate has reached its maximum and at the exponential growth phase then in the deceleration growth phases both the division rate and the growth rate start coming down.

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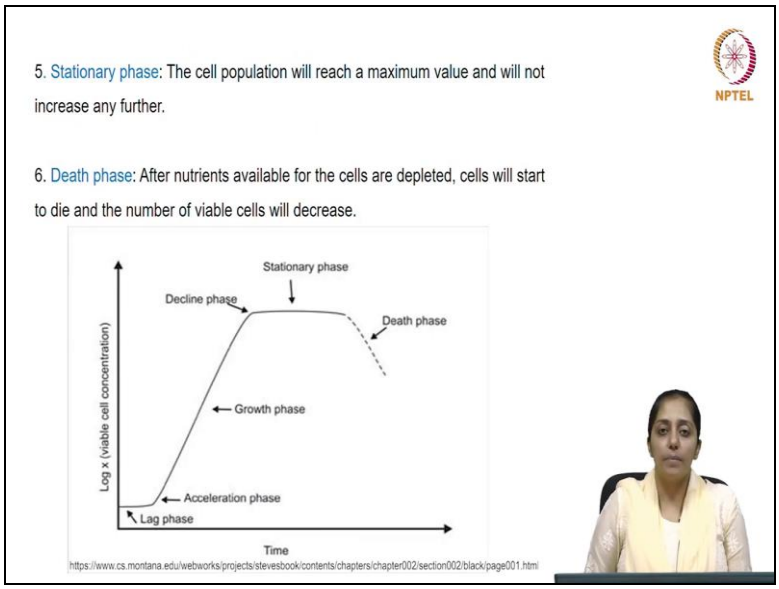

Finally when all the substrate has been depleted it comes to nearly complete depletion then the cell stops dividing. The cell population will reach its maximum value and there is no increase in cell number thereafter still if the batch cultivation is continued then there will be a time when the cell number starts decreasing this happens because of the cell lysis which could be due to the prolonged nutrient limitation.

So you can see on the slide the first is the lag phase where the y axis shows you the log of the cell concentrations. So there is no effective change so there is no change in the cell number this is your acceleration phase this is your deceleration phase and this is your exponential growth phase; this is your stationary phase and the death phase. So it is very clearly visible when the plot is made between the cell number versus time.

Generally when the plot is made between cell biomass concentration versus time then it is difficult to see the death phase because the y axis is based on the cell drive it estimation the cell mass concentration with time. So even if the cells get lysed the weight is incorporated. So the distinct phases which can be observed when the plot is made in terms of cell mass versus time then those are lag the log phase log phase is what is written here as growth phase and the stationary phase.