Bioreactor Design and Analysis
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Lecture 04
Design of Batch Bioreactors - Part 2

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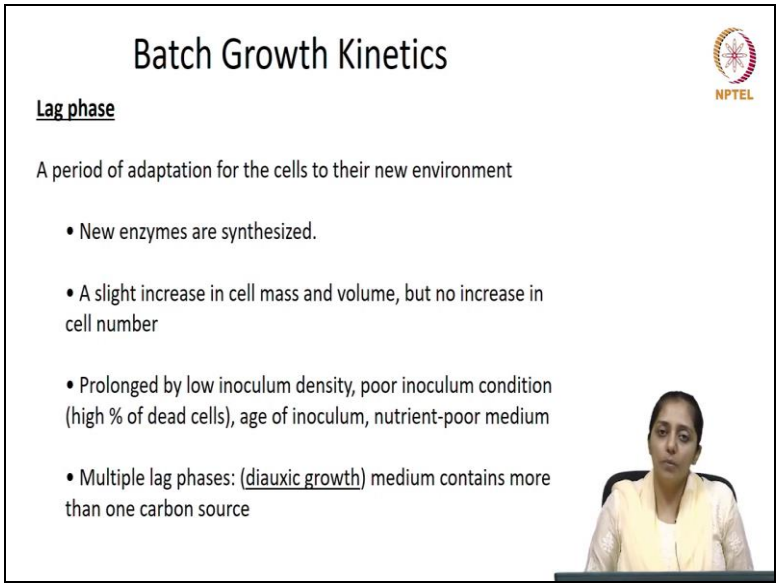

Now let us try to know a little more about the key phases in the growth batch culture growth curve. The lag phase as I said earlier it is a period of adaptation for the cells to their new environment. So you remember the lecture I was talking about a schematic where I spoke about a steed fermenter and I said steed means the starter culture. So generally starter cultures they are produced in rich medium and then they are brought to the production medium.

The starter culture now they are being grown in rich medium due to the fact so that they can be activated. Now when such cultures which have been grown on very nutrient rich medium are exposed to a defined production medium then you will observed lag phases. Now this is what is the reason for the lag phase. The adaptation of the cells in their new environment when suddenly the cells are brought from one environment which is very rich in nutrients to a limited environment then you observe the lag phase.

Now what do you mean by adaptation? Adaptation means formation of new enzymes which means synthesizing new enzymes. You may observe a slight increase in the cell mass or volume but definitely there is no increase in the cell number because the division has not yet

begin. The lag phase can be extended or can be prolonged if we use too low inoculum density. Inoculum density means the inoculum size the number of cells per unit volume or if the inoculum is not healthy which means that we know that all the cells are not at the same growth stage?

But if the majority of the cells are in the non growth phase in the inoculum then again you will have an extended lag phase. So that is the reason why the inoculum must have majority of the cells in their active growth phase which means the phase at which they are rapidly dividing or it should have less number of dead cells. Than the age of the inoculum they should be at a phase where they are rapidly multiplying.

Or if there is a large gap between the medium compositions in which the inoculum is prepared and the production medium composition I was talking about the starter culture so generally working cell bank it is prepared in a nutrient rich atmosphere then when the seed fermenter is used to prepare the inoculum for the production fermenter. This seed fermenter generally will have the same composition as the production medium.

So, that the activated culture is then acclimatized in the seed fermenter to multiply and prepare the inoculum for the production fermenter. So the nutrient composition because it is not much varying the period of adaptation is less. You must have heard about dioxic growth if not let us see in batch processes if there is a dioxic growth pattern then you will see more than one lag phases.

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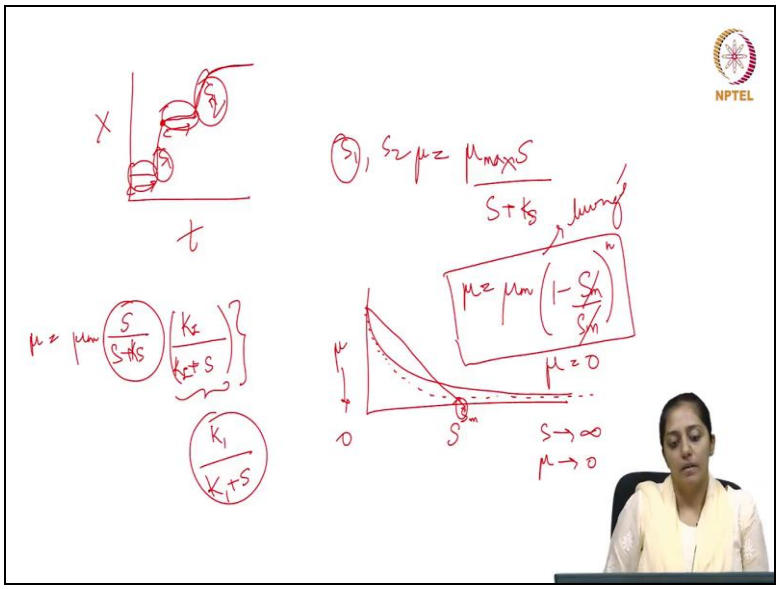

So this is the primary lag and this is your secondary lag phase. The primary lag we know why it happens it is a period of adaptation. Again there is another period of adaptation any guesses what can lead to this? Yes if there are more than one kind of substrates present in the medium. Suppose there are two different kind of carbon sources present in the medium. The culture generally prefers one carbon source over the other because it economizes its resources.

So it will continue to utilize S 1 till S 1 is present once S 1 depletes at this point it will start preparing its machinery to utilize S 2 and therefore the second lag before it begins utilization of S 2 for the second phase of multiplication. So this is dioxic growth pattern where you can observe multiple lag phases.

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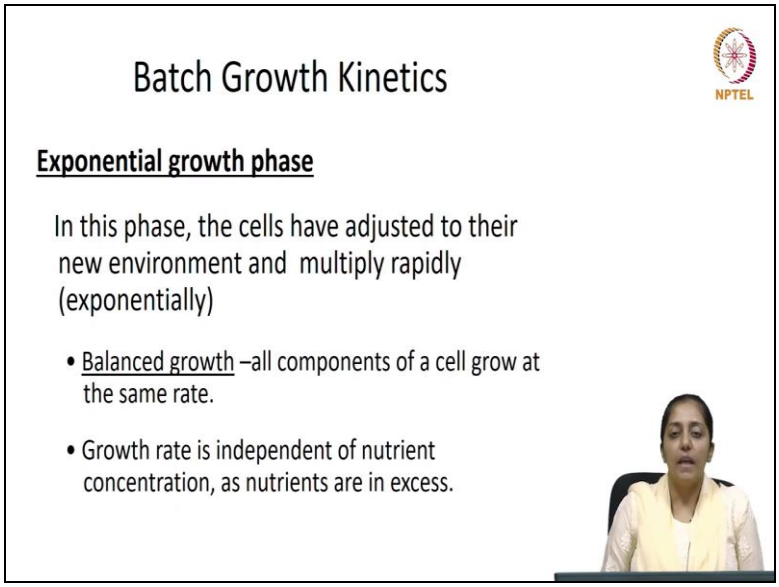

Then the exponential growth, so one key phase is your lag phase another key phase in batch growth curve is your exponential growth phase. Now in this phase the cells have adjusted to their new environment and they start multiplying rapidly or exponentially. Now here is when we can assume balanced growth. You remember balanced growth? Balanced growth means all components of the cell are growing at the same rate as the cell number or the cell mass.

And here is when growth rate is independent of nutrient concentration and as nutrients are present in excess. So which means the cell is growing at its maximum specific growth rate.

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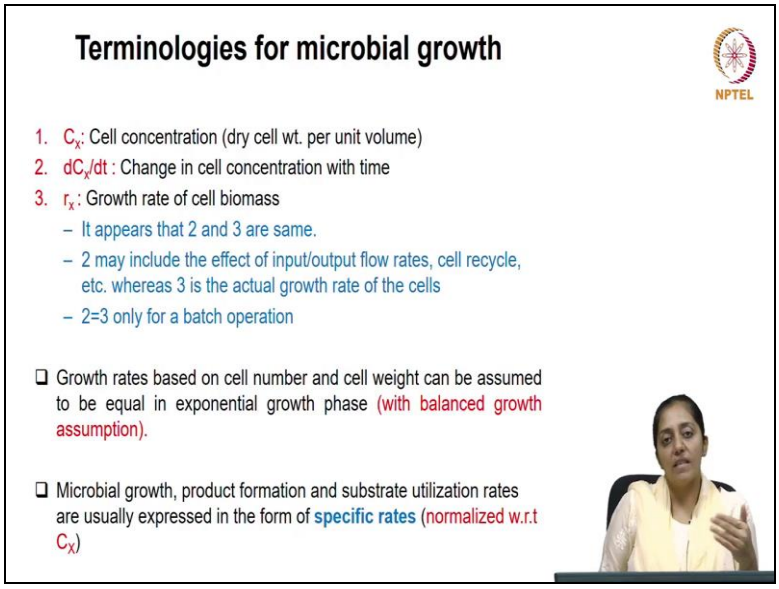

Now as the cell is growing in the exponential phase the substrate will continue to get depleted. So let us see the terminologies of microbial growth which we will be using later to formulate the equations. So C x will stand for cell concentration dC x by dt will mean change in cell concentration with time. Now cell concentration we will assume it is dry cell weight per unit volume where r x would be the growth rate of the cell biomass.

So I hope you can make out dC x by dt is not equal to r x so dC x by dt is the change in cell concentration with time inside the liquid broth and r x is the growth rate of the cell biomass which means the rate at which the cell is multiplying with time or the cell is growing. The cell number is changing with time. So when I say the two things it may appear to you that equation 2 is equal to 3.

Now 2 may include the effect of input and output flow rates or the cell recycle whereas 3 is the actual growth rate of the cell and this is how 2 may or may not be equal to 3 and 2 is equal to 3 only for a batch operation. Let us prove it. Now growth rates which are based on cell number or cell weight can be assumed to be equal in exponential growth phase because we can assume balanced growth during this phase.

Now microbial growth product formation and substrate utilization rates they are usually expressed in the form of specific rates. When we say specific rates here it means that we will be normalizing the rate of change of substrate or the product or the growth with respect to the cell concentration then it will be called as specific growth rate or specific substrate utilization rate or specific product formation rate.

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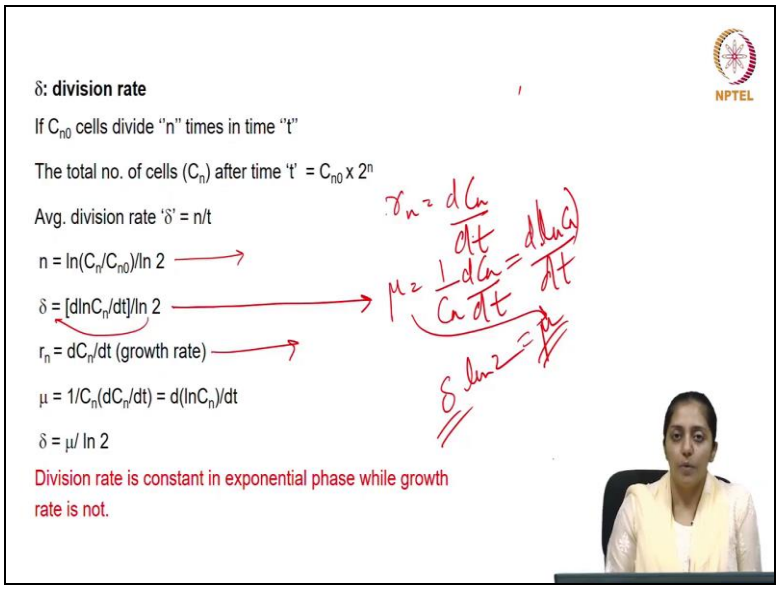

Now let us also see how is division rate correlated with the specific growth rate. You remember I spoke about division that during the accelerated growth phase the division rate of the salary becomes its maximum and during the exponential phase the division rate is maximum and the growth rate increases. Now let us try to see a correlation between the division rate and the specific growth rate.

So if C n0 are the cells initially present and they are dividing n times in time t. You can see on the slide so the initial number is C n0. The total number of C n of the cells after time t is C n. So C n is the cell number at time t and we know that the cell divides n times in time t so what will be the division rate? Division rate is the number of times the cell is dividing per unit time. So division rate is n by t.

Now if I need to determine n in terms of C n, so if you remember how is C n which is the number of cells after time t will be equal to C n0 multiplied by 2 to the power of n because after every division the cell will be becoming 2. So if there is one cell after one division it will become two cells after two divisions it will become 4. So after n divisions it will become C n0 into 2 to the power of n.

If rather than having one cell we have C n0 in the beginning. So now if you try to take a log of this equation it will become l n C n by C n0 n l n2 is not it. Now we know division rate is n by t so it becomes t l n C n by dt by l n2 is this clear. So division rate was the rate at which

the number of times the cell will divide. So it is dn by dt and your growth rate is what? So let us see from this equation.

So division rate is dn by dt so which will be l n C n by dt l n 2 is this clear. So now rate of growth is equal to d C n by dt this is your growth rate and if I normalize it with the cell number it becomes specific growth rate is not it. So if I substitute now mu as d l n C n by dt so this has been substituted as dl n C n by dt in this equation for clarity. So if you see this equation the rate of growth is d C n by dt in terms of cell number.

Specific growth rate will be 1 by C n d C n by dt now this can also be written as d l n C n by dt is not it. Now do you remember what was d l n C n by dt. From this equation if I do the substitution for d l n C n by dt the division rate times l n 2 will be equal to d l n C n by d t which then becomes equal to mu from this equation. So this is how mu and division rates are correlated because the specific growth rate during the exponential phase is constant at its maximum till the substrates are in excess.

So the division rate is also at its maximum however the growth rate which is d C n by dt will not be a constant will keep on increasing during the exponential phase.

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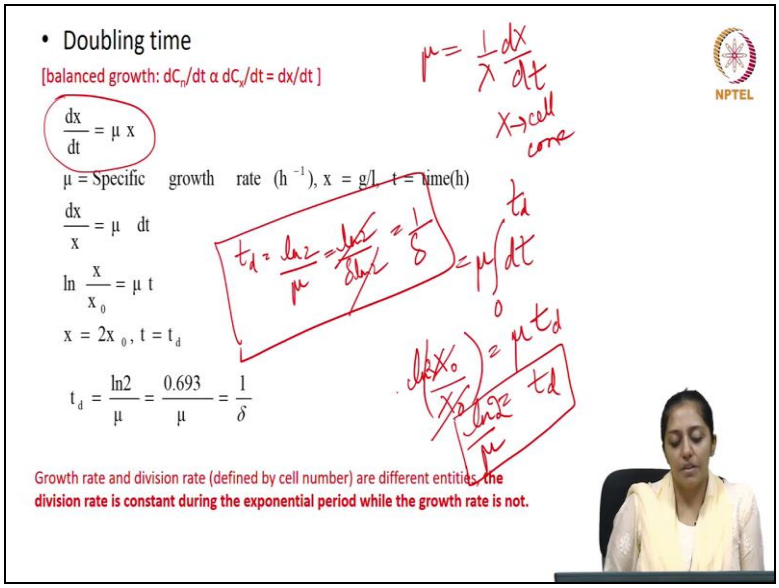

Let us try to find a correlation between the division rate and the doubling time as well as the specific growth rate. So this as you can see on the slide this is easy to understand I have told you earlier dx by dt is equal to mu x mu stands for your specific growth rate. So it has been

normalized with respect to the biomass concentration. Here x stands for cell concentration or biomass concentration.

So the doubling time means the time taken to double the cell concentration. So which means that if you integrate this equation; and put the limits as x 0 to 2 x 0 and we need to find that doubling time. So then it becomes l n x the 2 x 0 by x 0 is equals to mu t. So then your doubling time will become equal to then becomes l n2 by mu. Now we know how the division rate is related to the specific growth rate.

Division rate from here is equals to mu by l n2 so if we substitute doubling time was l n2 by mu and division rate was mu by l n2. So division rate times l n2 as so one can make out that doubling time is the inverse of the division rate. So the growth rate and division rate which is defined by cell number they are different entities and the division rate is constant during the exponential period while the growth rate is not.

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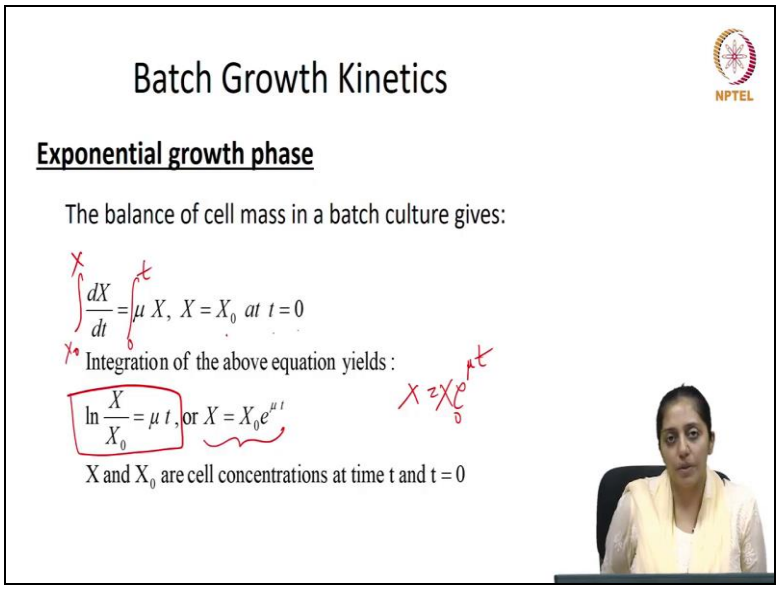

Let us see the exponential growth phase with the balance growth assumption. We know that for a batch culture the rate of change of cell biomass can be given as specific growth rate times the cell concentration. It is assumed that the cell concentration is x 0 at time t is equals to 0 which means at the beginning. Now if we integrate then from 0 to t and from x 0 to x we end up here it will be l n x by x 0 into is equal to mu times t.

Further changing into exponential form your x becomes an exponential function of time multiplied by the initial concentration. So this is how in batch growth your biomass concentration will be changing with time if the specific growth rate of the culture is mu.

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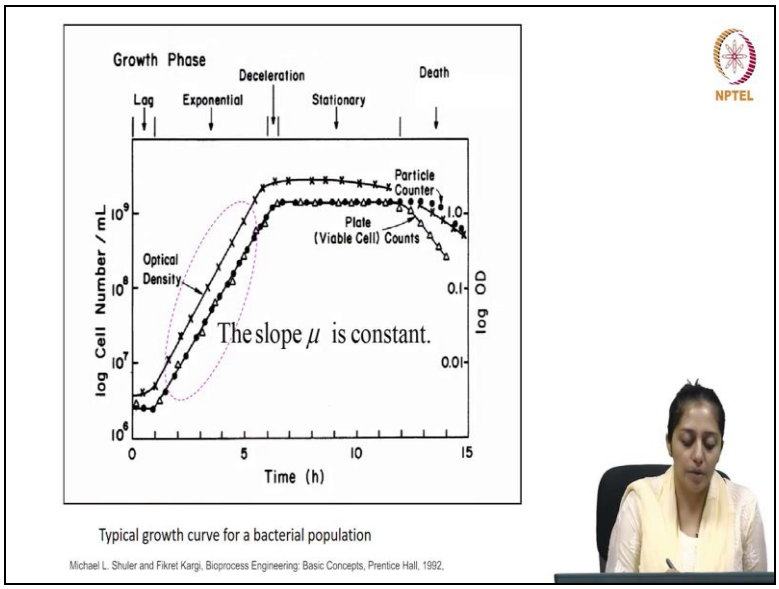

Now you can see the dotted curve this is the region which demonstrates constant slope. If you make a curve or a plot of log cell number versus time during the exponential phase this is an inclined straight line with a constant slope and it is showing the maximum slope in the growth curve. So this is what is your specific growth rate of the culture and as I have already told you we had already seen different phases of growth in a typical batch growth curve in terms of cell numbers.

You can see the death phase the stationary phase the decline phase the deceleration phase the exponential phase and the acceleration phase along with the lag phase.

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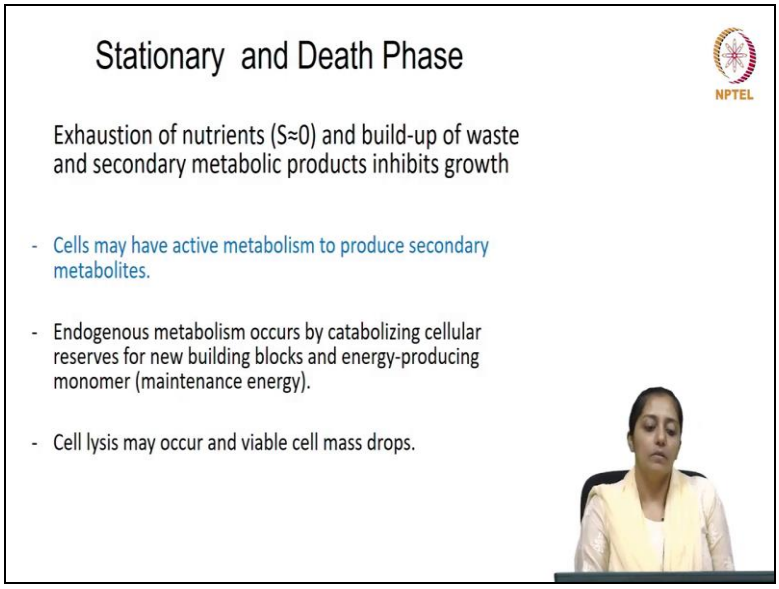

Now talking about the stationary and death phase they appear when there is exhaustion of neutrines which means the substrate is nearly completely consumed and there is buildup of waste and secondary metabolic products which might be inhibiting growth. So, in that case which can be clubbed under endogenous metabolism? Now cells may have active metabolism to produce secondary metabolites.

Endogenous metabolism occurs by catabolizing cellular reserves for new building blocks and energy producing monomers. Now this is all clubbed into maintenance energy. The cell lysis may occur in this phase and the viable cell mass or cell number will start decreasing.

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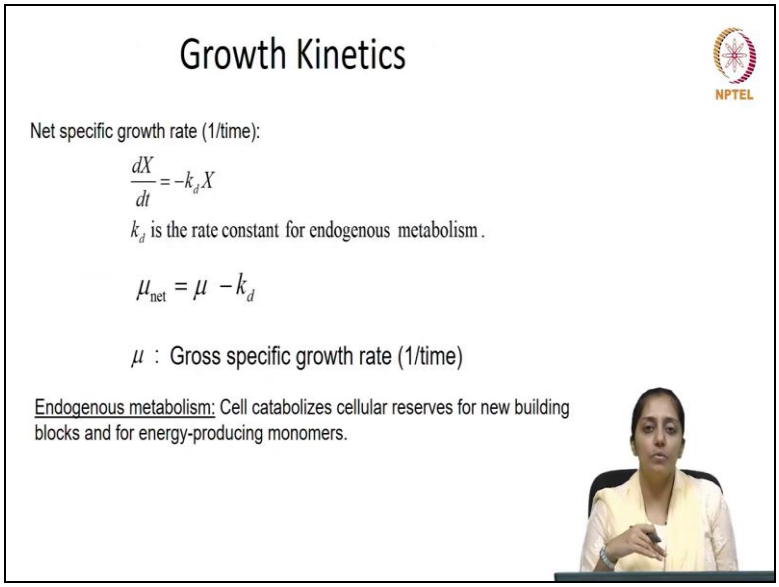

Now if we need to see the effect of the endogenous metabolism on the growth rate of the culture we need to take into account the effect of endogenous metabolism. Then the

endogenous metabolism can be defined by this equation. In which the net specific growth rate will then become the difference between the specific growth rate and the rate constant for the endogenous metabolism.

Which means the rate at which the cell number will be decreasing is proportional to the cells but the rate constant here is having a negative sign as the cell number will be decreasing. And this is given a notation k d which stands for the endogenous metabolism rate constant. And mu net will be your net growth rate. So in endogenous metabolism to repeat the cells start catabolizing the cellular reserves for new building blocks and for energy producing monomers.

So depending and this is termed under maintenance energy if the maintenance energy is high then the effect of k d on the net specific growth rate will be significant enough that you cannot ignore it. So some of the models take into account the maintenance factor and some models do not take into account depending on the significance of the maintenance energy or the value mathematically the value of k d the numerical value of k d.

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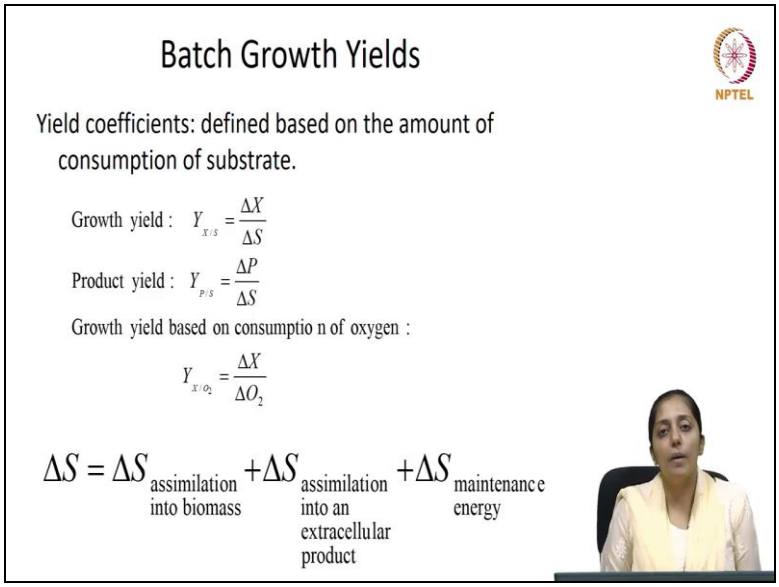

We will also come across some factors or culture parameters like yield coefficients. There are different yield coefficients it is based on the amount of consumption of substrate. So when we say the growth yield coefficient it is termed as Y x by S and it stands for and hence is given as delta X by delta S. Similarly product yield coefficient would be given a notation by P by s and it will stand for the amount of product produced per unit substrate consumed.

The growth yield based on consumption of oxygen can be written as Y x by O 2 where again it will mean the amount of biomass produced per unit oxygen consumed. So to have an idea about how the substrate is getting consumed during the batch cultivation some of the substrate gets accumulated for the growth which means for the production of biomass some of the substrates gets assimilated or used up for the production of the product which is coming out.

And some of the substrate is getting utilized for the endogenous metabolism or the maintenance energy.

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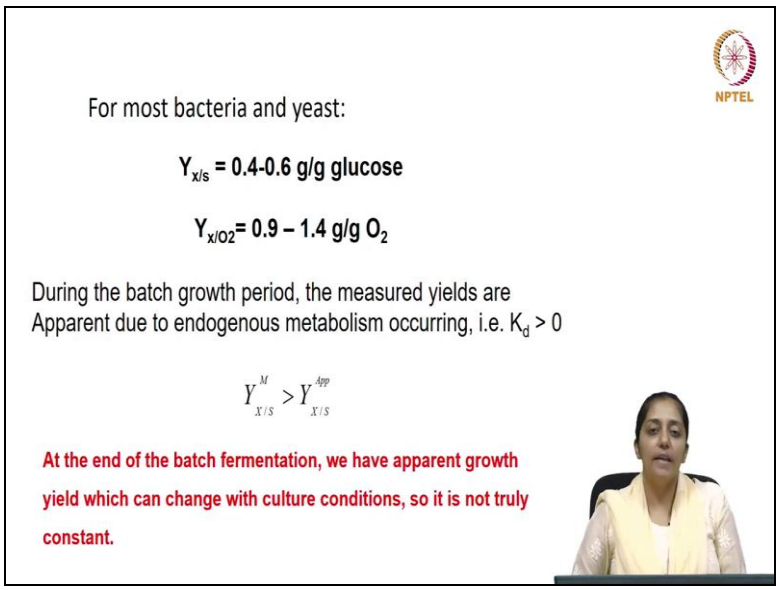

So for most bacteria in east this is an average range of Y x by s and Y x by O 2 which you can come across. During the batch growth period the measured yields are apparent due to endogenous metabolism occurring which means then in that case the k d value the numerical value of k d will be greater than zero. And in that case when the endogenous metabolism is high the theoretical yield coefficient will be higher than the actual or the experimentaly measured yield coefficients which will be based on the amount of biomass produced per unit substrate consumed.

So at the end of batch fermentation we have apparent growth yield which can change with culture conditions so it is not truly a constant.

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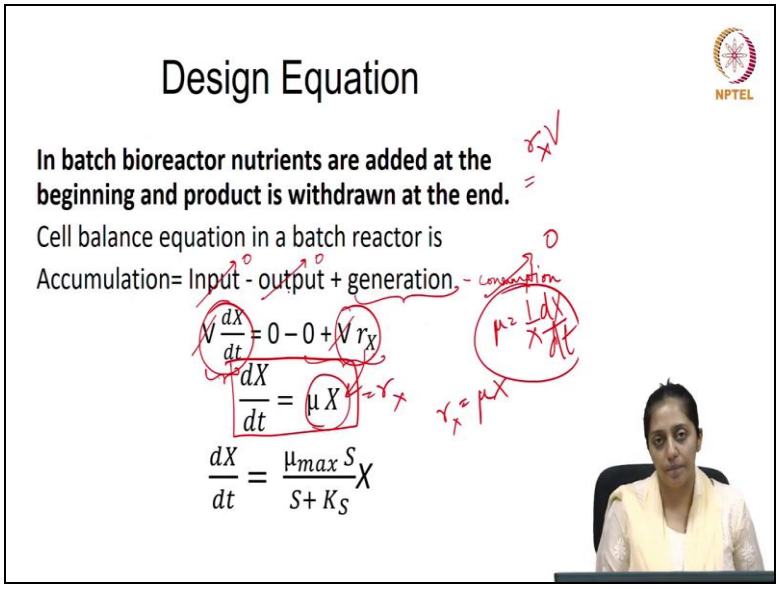

So now let us see the design equation for a batch bioreactor. Now let me draw a schematic here for a reactor. So as I said earlier it is a closed system let us assume X stands for the biomass concentration inside the reactor broth S stands for the substrate and P is for the product and V is your working volume inside the reactor. Now if we do a cell balance across this reactor the rate of accumulation inside the reactor will be equal to input, output any generation if it is happening or consumption.

In terms of biomass when we are doing a cell mass balance we know that there is no consumption of biomass which is taking place inside the reactor. So your LHS which stands for the biomass concentration in the broth at any given time. So dx by dt will stand for the rate at which the biomass is getting accumulated inside this reactor. Now it is a mass balance so if X is the concentration it is X times V, V is the volume of the batch.

Now V being a constant can be taken out from the differential and it is therefore represented as V times dx by dt on the LHS. Now input is zero in this reactor because it is a closed system the output is also zero is there a generation happening? Yes, because of the multiplication of the cells. So yes there is a generation of biomass but is there a consumption happening which they have not shown here.

The biomass is not getting consumed so therefore it has no meaning. So when we expand this equation your RHS your r x now you remember the equation number 3 where r x stands for the rate of growth of the culture. So rate of growth of the culture rate at which the cell mass

concentration is changing with time due to multiplication again it is a cell mass balance. So the V can be multiplied and can be taken out from the differential.

So it is V times r x it is the rate at which the mass is getting generated this is the rate at which the mass the cell mass is getting accumulated. So now if we try to correlate your V and V will get cancelled. Now this r x is the growth rate of the culture. So it can be written as in terms of specific growth rate if you remember it can be written as mu times x what was mu? It was 1 by x dx by dt. So your r x can be given as mu into x.

So that is what has been done here r x has been substituted as mu times x and here this x is your rate at which the accumulation of biomass the net change in the concentration of the biomass inside the reactor. So if you remember I said that for a batch dx by dt which here stands for the rate at which the biomass is accumulating inside the reactor is equal to the growth rate of the culture which is equal to r x.

It may or may not be the same in a fed patch or a continuous reactor I hope you can make out because the input the output streams will not be zero in those cases. So now if we assume that the culture is following Monod's kinetics or Monod's law, Monod's model for growth then we can substitute mu as mu max times S upon S + K S you remember this model which we saw few slides earlier this is Monod's model.

So they have substituted mu with mu max S upon S + K S where S is your limiting substrate. So now this becomes an equation which defines how the x inside the reactor inside the batch reactor is changing with time as a function of x and S where S is your limiting substrate.

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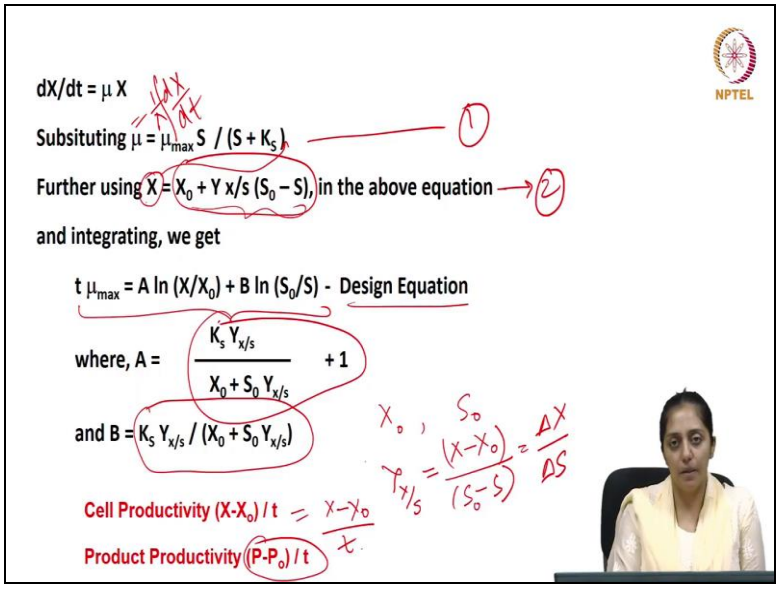

Some bit of rearrangement now another equation which we know is if X 0 is your initial biomass concentration in this fermentation and S 0 was your initial substrate concentration and Y X by S is your yield coefficient assuming no endogenous metabolism then Y X by S is equal to the amount of biomass produced to the total amount of substrate consumed which was delta X by delta S this is how yield coefficient is defined.

So yield X by S is can be written in the form of this equation. So now we have two equations which are correlating which two variables the X and the S. So now using these two equations where mu can be substituted as 1 by X dx by dt if we integrate after substituting X in terms doing the rearrangement so as to bring this X variable in terms of S and then substituting this X in terms of S in equation 1 and integrate where mu is 1 by X dx by dt.

So in order to get X you will then integrate this equation after substituting the X with in terms of S. Now when we integrate then we end up in this form which is called as the batch design equation where A is a constant given as this and B is another constant which can be given as given in the on the slide what will be the cell productivity? The cell biomass productivity can be determined as now this is volumetric productivity.

So what how is it defined the cell productivity would be the cell concentration achieved per unit time. So it will be for a batch X - X 0 to the batch time. Similarly product productivity in batch would be P - P 0 by t where X 0 and P 0 are the initial biomass and the product concentrations and X and P are the final concentrations and t is your batch time.