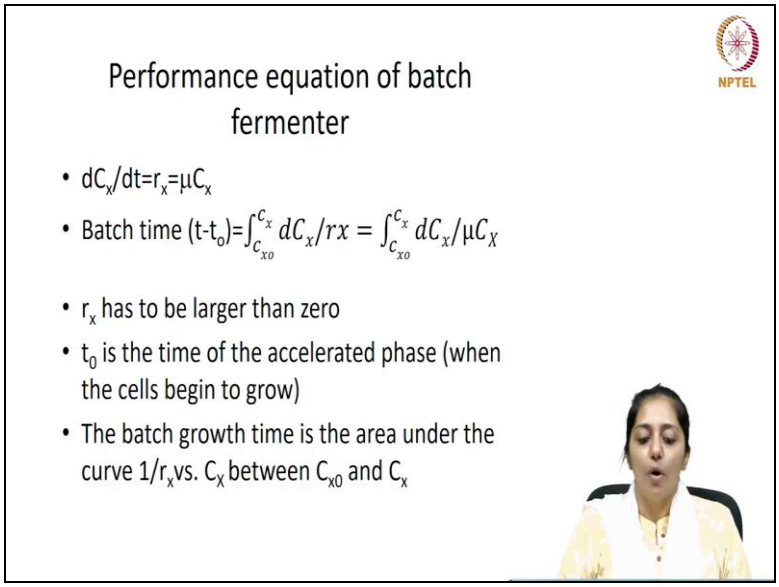
Bioreactor Design and Analysis
Dr. Smita Srivastava
Department of Biotechnology
Indian Institute of Science – Madras

Lecture 06
Design of Batch Bioreactors - Part 4

(Refer Slide Time: 00:17)



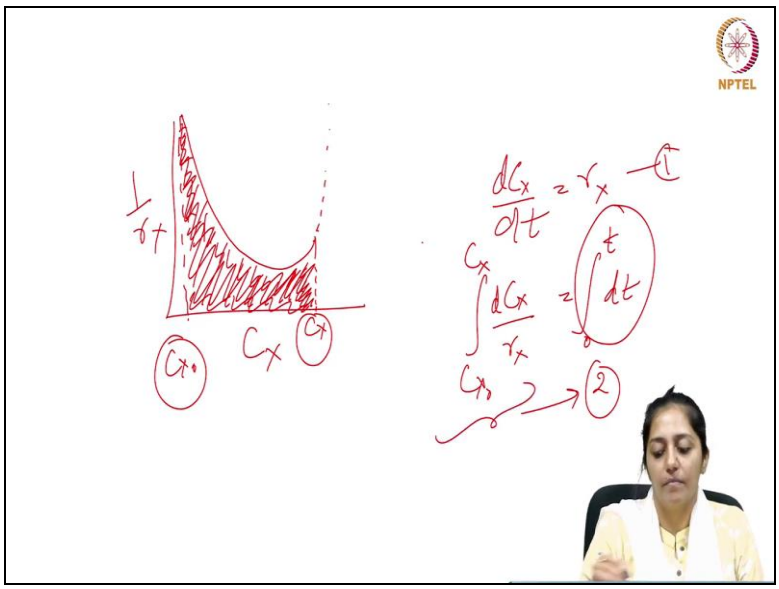
Now to draw a performance equation for a batch reactor we know the first equation which demonstrates how the cell concentration will change with time or the growth rate of the culture in a batch which is equal to mu times C x let us call this as equation 1. So if we want to determine the batch time we can integrate let us assume the starter culture concentration is C x0 and the batch time is t.

So then if we integrate equation 1 with the boundary conditions of x0 C x0 and C x for the biomass and t and t0 for the time so then your batch time which would be t - t0, t0 is generally 0 then rx which is given in equation one has been further substituted as mu times C x as again shown in equation one and you apply the limits after integration. So this is how you can calculate the batch time.

Now t0 is generally equal to 0 when we assume that there is no lag phase. But if there is a lag phase so you remember last class we were talking about the lag phase in which there is a phase which is called as the acclimatization period where the cell division is not happening. So if suppose there is a lag phase and it is of sometime t0 then the batch time would be a

difference between the time of harvest to that t0 that is why it is given in equation 2 as t - t0 where if there is no lag phase then t0 can be substituted as 0.

(Refer Slide Time: 02:50)

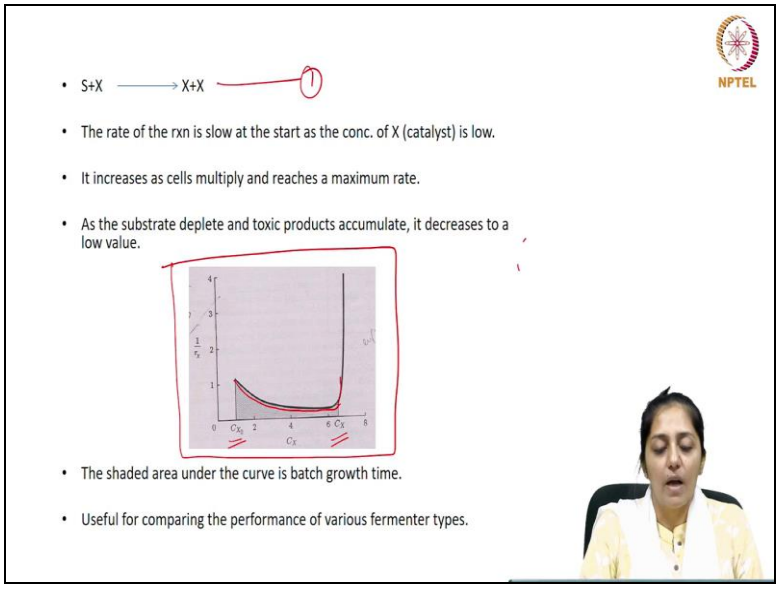


Now if you see equation 2 and if we make a plot of 1 by rx versus C x. So if you remember the previous equation it is d C x by d t equals to rx is not it, rx was your growth rate. So we can write it applying integration as equation two. Now with this equation 2 if you make a plot of 1 by rx versus C x then it looks like this and if this is your harvest time where your final concentration is C x and this was your beginning which is your c x0.

So can you see that it takes a u shaped curve. Now in order to understand why it is taking a u-shaped curve let us see. If C x is very less then the growth rate is less initially as the biomass is getting produced because the growth rate is improving. So your 1 by rx during the long phase comes down till the culture is growing and then suddenly after depletion of the nutrients the one by rx will go towards 0.

So for a batch if starting was C x0 and the final is C x and if you make a plot of 1 by rx versus C x then if given these limits the area under this curve with these limits will demonstrate something what will it demonstrate? It will demonstrate the batch time as given in equation 2.

(Refer Slide Time: 05:47)

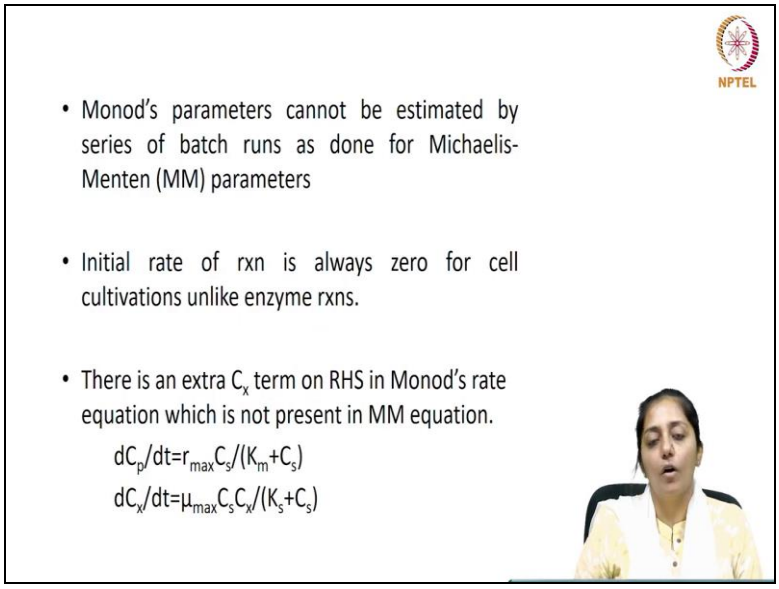


So now biological reactions or cell formation in a reactor it is termed as an auto catalytic reaction where the biomass is said to be catalyzing further production of the biomass as shown in equation on top. So S stands for substrate so S is combining with the biomass which means the biomass is consuming the substrate and producing more biomass. So the rate of reaction is slow at the start as the concentration of X which is the catalyst here keeps on increasing the rate of reaction also keeps on improving and until it reaches the maximum rate.

So if you can see the schematic shown here it demonstrates a batch kinetics in which the initial biomass concentration which is the concentration of your starter culture C x0 we put in the medium and at the harvest which is the maximum cell concentration is C x. So a plot of 1 by rx versus C x is shown to with growth it is increasing the biomass it reaches its maximum the rate and then it suddenly goes to very high value which means nearly going to 0.

So as the substrate depletes the toxic products accumulate and it will decrease the value of rx and therefore the 1 by rx value suddenly increases. So as I said in the earlier lawn the area under this curve which is shown here as a shaded region is what will give us the batch time. The time in which the biomass concentration achieved becomes C x. So this technique can be used to compare the efficiency of different reactors different in the sense efficiency especially when you want to compare.

(Refer Slide Time: 08:26)



A batch reactor with a continuous reactor or a combination of reactors we will take an example as we move ahead. Now Monod's parameters if you remember Monod's equation in order to make the Monod's model for a system we will have to define the values of these constants in the Monod's model which we call as the maximum specific growth rate and the Monod's saturation constant K S.

How to determine the value of these? You can use batch culture to find the value of mu m and K S but that may not be the right estimate. We will see how we can do and once we take the continuous fermentations we will be talking about the limitations of this procedure as well but for a guess for a rough guess or for initial guess people do use this technique. So in which if you do a rearrangement or you do some experiments where you collect the specific growth rate values at different substrate concentrations.

And you make a plot of 1 by mu versus 1 by S then you will get an inclined straight line which we call as Lyme Weaver Berg plot. Now you must have heard about the Lyme Weaver Berg plot during calculation of the kinetic parameters in enzyme kinetics following Michaelis-Menten equation. So do you remember Michaelis-Menten; Michaelis-Menten was the rate of enzymatic reaction V max multiplied by S versus K m + S.

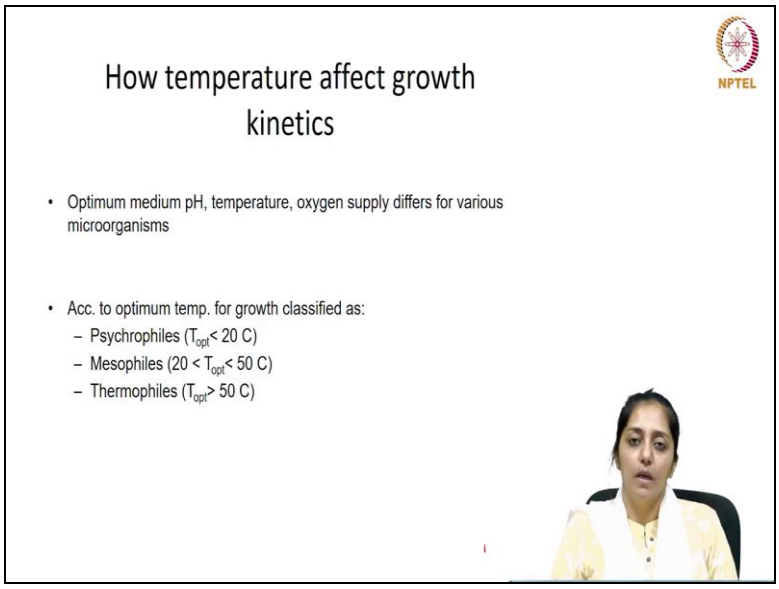
But here it is the rate of enzymatic reaction and here it is specific growth rate. So, direct implementation of Lyme Weaver Berg plot which we generally use in enzyme kinetics which we call as 1 by V versus 1 by S plot may not give the two representation of the Monod's model parameters. The reason being the initial rate of reaction is always 0 for cell cultivations

unlike enzyme reactions there is an extra C x term on RHS in monon's rate equation which is not present in the Michaelis-Menten equation.

So if you see equation 1 and equation 2 your equation 2 where we have given for growth rate d C x by dt it is equals to mu max they have substituted Monod's model which is C S by K S + C S multiplied by mu max and this C x term has come from the LHS which was because mu is equals to 1 by C x d C x by dt. So this C x has been taken to the other side. So if you now compare Michaelis-Menten equation which is the rate of reaction is the maximum rate multiplied by the substrate concentration by K S + C S.

But there is an extra C x term which is not there in the Michaelis-Menten equation. So this is how they are different.

(Refer Slide Time: 13:18)



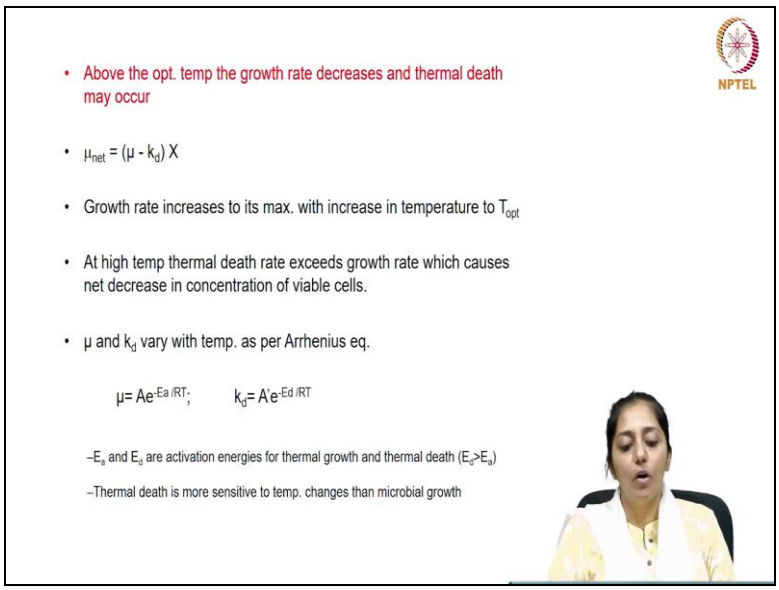
Now in Monod's equation there are certain assumptions which we take like being a controlled bioreactor system. We assume that the fermentation is running at the optimum temperature and ph which is being continuously maintained throughout the cultivation period hence the effect of temperature and ph is negated or is not taken into account on the specific growth rate. There is only one substrate which is limiting the specific growth rate however apart from substrate.

There are other factors physical and chemical which are also having effect on the specific growth rate of the culture like medium ph temperature oxygen supply where the significance of each of these factors may differ from organism to organism. Like for example

psychrophiles. Psychrophiles these are organisms which generally grow at temperatures less than 20 degrees C or at lower temperatures or mesophiles where the optimum temperature ranges between 20 to 50 ambient temperatures.

Thermophiles which only survive at very high temperatures. So then depending on the organism the effect of temperature may vary on the kinetics of the growth kinetics of the culture.

(Refer Slide Time: 14:52)



Now above the optimum temperature the growth rate would decrease and thermal death rate may begin. Let us talk about cultures mesophiles they grow at ambient temperatures. So at very low temperatures you will find the growth rate slow and at very high temperatures suddenly you will find that the cells begin to die because the cell starts lysing and the internal components starts getting denatured.

So then to take into account the thermal death rate as the temperature is high where then the apparent growth rate will have to be a club of the actual growth rate with the simultaneous death rate. So here if you observe equation 1 it is taking into account the growth rate and the reduction due to the death rate. So the net specific growth rate is given as a difference between the growth rate the specific growth rate of the culture minus the death rate of the culture the specific death rate of the culture which is termed here as k d.

Now growth rate increases to its maximum with increase in temperature to T optimum. So the plot may be more like a hill where you will observe that the growth rate of the culture will

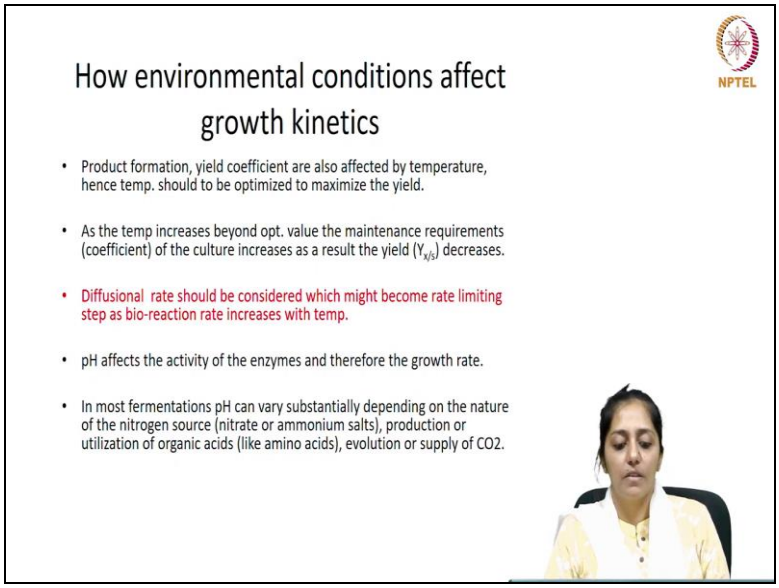
increase as the temperature is increased this behaviour is generally found to follow a well-known model which is called as Arrhenius equation which demonstrates the effect of temperature on the growth rate of the culture.

And the thermal death rate after that optimum temperature if we continue to increase then you will observe that the growth rate of the culture will start which is the net will start decreasing which is due to the thermal death rate which can again it is analogous to the Arrhenius equation the difference is that the death rate would be now having a negative sign and it is demonstrated as k d the specific death rate.

Now E d and E a I hope you remember Arrhenius equation. So E a is your activation energy for thermal growth and E d stands for the activation energy for thermal depth and it is observed that E d is generally higher than E a because as the temperature is increasing. So beyond the optimum it will start decreasing so the activation energy for thermal breadth is much higher than the activation energy required for growth.

So, thermal depth is more sensitive to temperature changes than the microbial growth because of this increased factor E d.

(Refer Slide Time: 18:26)



So now how environment conditions affect growth kinetics product formation your yield coefficient they are also affected by temperature and hence temperature should be optimized to maximize the yield. As the temperature increases beyond optimum value the maintenance requirements and therefore the value associated with the maintenance coefficient of the

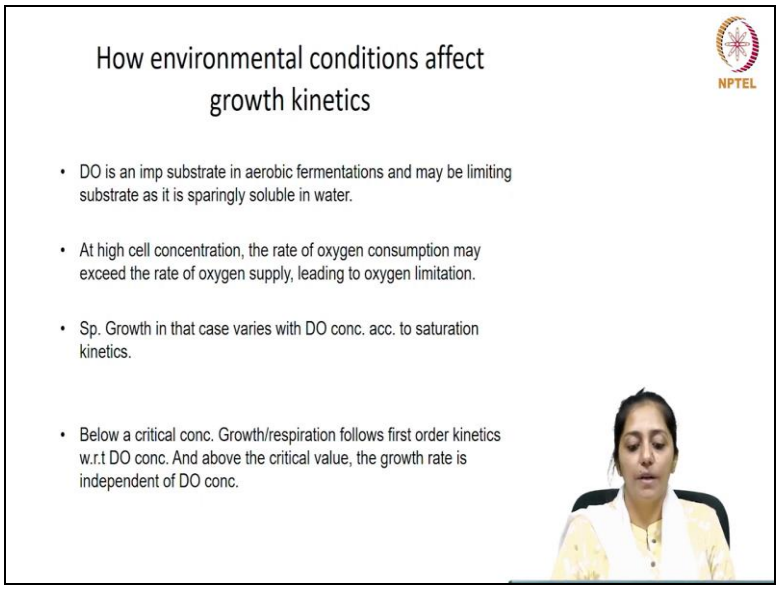
culture increases as a result your Y x by s which is your yield coefficient starts decreasing. So if you remember in the last class we talk about in what all different aspects the substrate gets consumed?

It not only gets consumed for growth but it also gets consumed for endogenous metabolism which is a club of the maintenance energy. So when the maintenance requirements are high there is a large amount of substrate also getting utilized for the maintenance requirements. So your net yield coefficient which is a club of all these factors the yield coefficient for the growth will start decreasing. The diffusional rate should be considered which might become rate limiting step as bioreaction rate increases with temperature.

So now as we know that the growth rate is a function of temperature now as the temperature increases the growth rate will keep on improving till it reaches its optimum temperature desired temperature. Now as the growth rates are improving then the rate limiting step could become the rate at which the substrate is being made available to the cell where diffusional limitations have to be considered, ph affects the activity of the enzymes and therefore the growth rate of the culture in most fermentations the ph can vary substantially depending on the nature of the nitrogen source.

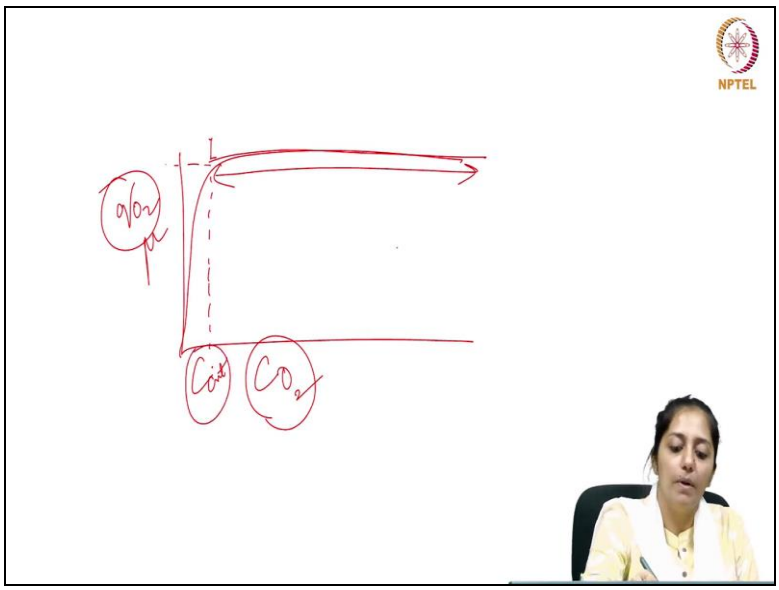
So generally nitrogen source are provided in the form of nitrates or ammonium salts. So production or utilization of organic acids like amino acids or evolution or supply of carbon dioxide all these factors may play a role in the change in the medium pH during fermentation.

(Refer Slide Time: 21:13)



Now dissolved oxygen as an important substrate in aerobic fermentations and can become limiting substrate as it is the sparingly soluble component in the fermentation broth. At high cell concentrations the rate of oxygen consumption may exceed the rate of oxygen supply inside the reactor which can then lead to oxygen limitations. Specific growth rate in that case starts getting affected by the dissolved oxygen concentration as a saturation kinetics which means that rather than substrate which is readily available carbon nitrogen medium components in the medium.

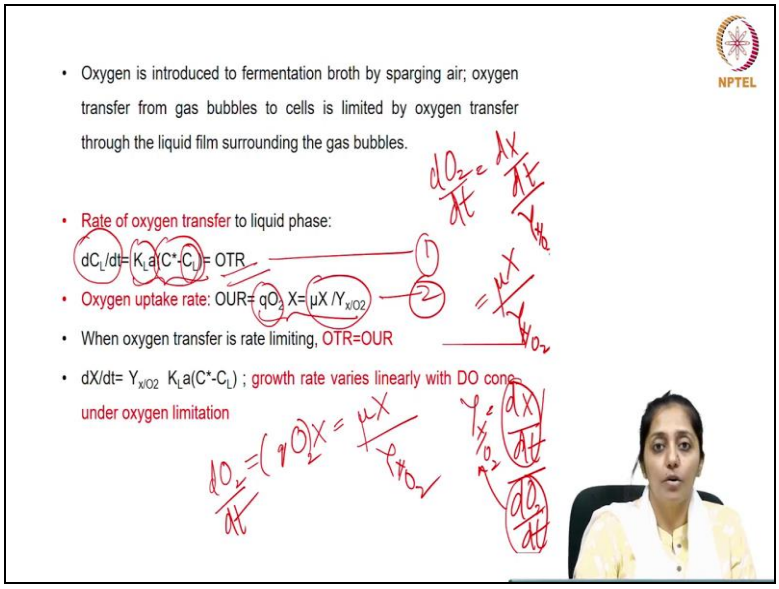
(Refer Slide Time: 22:09)



If it is oxygen limited it will follow this saturation kinetics in terms of oxygen concentrations in the medium. Now below a critical concentration the growth or the respiration follows first order kinetics with respect to the dissolved oxygen concentration and above the critical concentration the growth rate is independent of the dissolved oxygen. So if you see the plot your critical oxygen concentration would be somewhere here so above this you may observe you may call this as q O 2 your specific growth rate or specific product formation rate which is biomass here with respect to your oxygen.

So here you will observe that the growth rate of the culture will become independent of the concentration of oxygen above this critical value but below this critical value it varies mostly linearly.

(Refer Slide Time: 23:26)



So generally oxygen is introduced into the fermentation broth by sparging air. Now oxygen has to be transferred from the gas bubbles to the cells which can get limited by the oxygen transfer through the liquid film which is surrounding the gas bubble. So your equation one given on the slide demonstrates the rate at which the oxygen is getting transported to the through that liquid film. So dC L by dt is your rate of transfer of oxygen which is given by K L a this is called as volumetric mass transfer coefficient.

And the second term is the driving force which is the difference between the maximum solubility of oxygen under the given conditions of the fermentation and C L is the concentration of oxygen in the broth in the bulk. So this equation is called as OTR which is the oxygen transport rate equation. Similarly the rate at which the oxygen is getting consumed in the fermentation broth can be given as q O 2 multiplied by X where what is q O2?

Your q O 2 stands for the specific oxygen demand of the culture which means the amount of oxygen consumed per unit biomass per time so to understand oxygen uptake rate as q O2 which is specific oxygen uptake rate. So if we convert it into oxygen uptake rate we need to multiply it with the biomass so that it becomes oxygen uptake rate. The rate at which oxygen is getting consumed for growth which is be equal to mu x by Y x by O2.

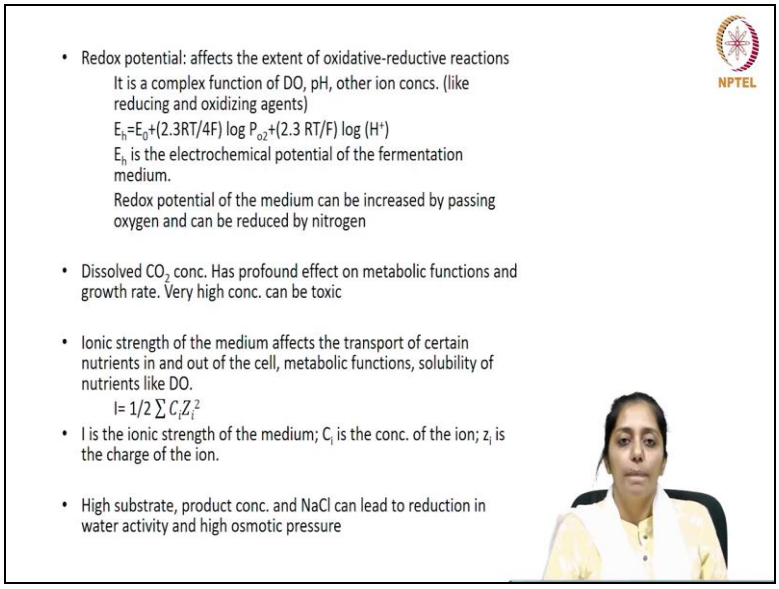
Now what was Y x by O 2? It can be written as dx by dt by d O2 by dt the amount of oxygen which has been consumed for the growth. So if you multiply if you need to know the amount of oxygen consumed the rate at which it is getting consumed for growth you can write it as dx

by dt by by Y x by O 2. Now this dx by dt is again substituted as mu times x and then you get your equation 2. Now when oxygen transfer is rate limiting it means that the rate at which the oxygen is coming inside the reactor is exactly equal to the oxygen demand which means the rate at which the oxygen is getting consumed in the reactor.

If this is happening there is no oxygen accumulation taking place inside the reactor. So in that case your oxygen transfer starts becoming rate limiting factor and your growth rate can then be made a function of the oxygen transfer taking a form of equation 3 here where your dx by dt can be given as yield coefficient multiplied by the oxygen transport rate which is K La multiplied by c star minus C L as given in equation one.

We will be taking oxygen transfer much more in detail when we talking about mass transfer operations in bioreactors.

(Refer Slide Time: 28:21)



So Redox potential this also affects the extent of oxidative and reductive reactions in the fermentation it is said to be a complex function of dissolved oxygen pH and other ion concentrations like reducing or oxidizing agents if present in the medium. So if you see equation one it is one of the ways in which the Redox potential has been demonstrated where E h is the electrochemical potential of the fermentation medium. The Redox potential of the medium can be increased by passing oxygen and can be reduced by nitrogen.

So if you see the term P o2 which stands for partial pressure your Redox potential can be improved as the partial pressure can be increased by passing in oxygen and can be reduced by

removing oxygen by purging in nitrogen. Dissolved carbon dioxide concentration also has a profound effect on metabolic functions and growth rate especially in case of plant cells or animal cells and very high concentrations can also be toxic.

Then ionic strength of the medium this can affect the transport of certain nutrients in and out of the cell its metabolic functions solubility of nutrients like that of dissolved oxygen. So then ionic strength can be defined as given in equation two for the medium where C i stands for the concentration of a particular ion and Z i will be the charge of that ion in the medium. So it is a club of all the ionic species and their associated charges which make up the ionic strength of the medium.

High substrate and product concentration and sodium chloride can lead to reduction in water activity and high osmotic pressure.