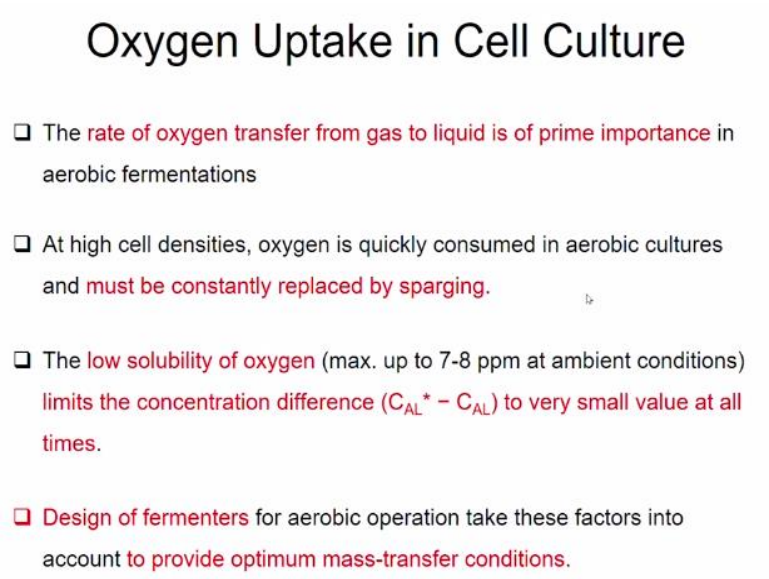
Bioreactor Design and Analysis
Prof. Dr. Smita Srivastava
Department of Biotechnology
Indian Institute of Technology - Madras

Lecture – 20
Mass Transfer in Bioreactors – Part 2

(Refer Slide Time: 00:16)



Hello students, so in the last class we were discussing about mass transfer in bioreactors. So during mass transfer operations in bioreactors, we then came across that oxygen mass transfer which is a gas liquid mass transfer is really crucial for aerobic fermentations in bioreactors. Then we characterized how to describe mathematically the oxygen transport rate in bioreactors and today we are going to begin with how to characterize the oxygen uptake rate in bioreactors.

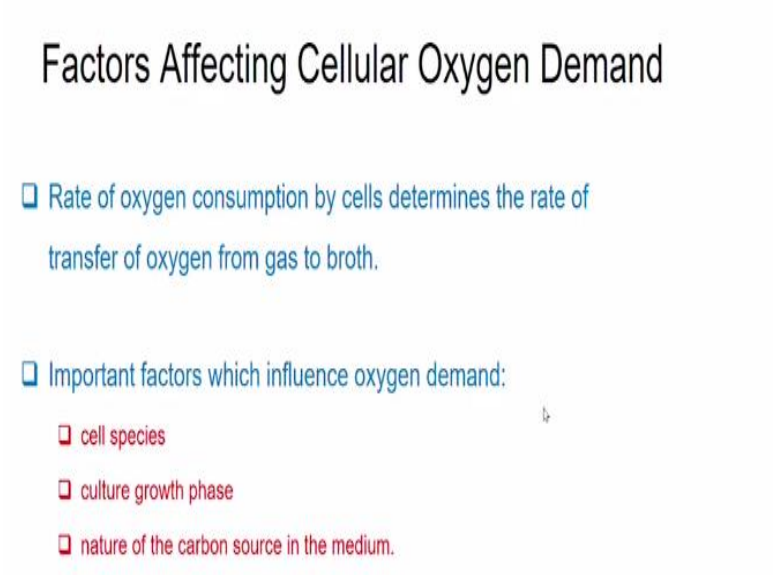
Now we know that oxygen is being taken up in the bioreactors by the culture, which means by the cells which are suspended in the broth. So, now we will see how we can describe mathematically the rate of oxygen uptake in fermentations. So, the rate of oxygen transfer from gas to the liquid we have now understood is of prime importance in aerobic fermentations and at high cell densities the oxygen gets very quickly consumed in aerobic cultures.

And therefore must be constantly replaced by sparging. Now the limitation is the low solubility of oxygen which is just nearly 7 to 8 ppm under ambient conditions. Now because

the solubility is so low, the driving force which is the concentration difference ends between the equilibrium concentration of oxygen which is called as the saturation concentration of oxygen which is nearly 8 ppm.

And the actual oxygen concentration in the medium this difference is always very small in absolute terms. So, therefore in order to compensate for this reduced difference or this small difference the reactor design is manipulated so as to affect the mass transfer coefficient which is k L a.

(Refer Slide Time: 02:38)



Now the factors which can affect oxygen demand which is the oxygen uptake rate involves the types of cell species with which we are working with, then the growth of the culture whether it is in active growth phase or resting cells so accordingly the oxygen demand also changes. Then the nature of carbon source in the medium like for example if it is a easily metabolizable sugar like glucose then the oxygen uptake rates are higher or the oxygen demand is higher, complex sugar then the uptake rate is slow.

(Refer Slide Time: 03:19)

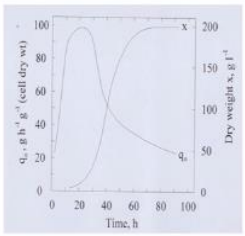
In a batch culture:

- Rate of oxygen uptake varies as the concentration of cells increases during the cultivation period

  Rate of oxygen uptake is proportional to the number of cell present.

- Specific oxygen uptake rate also varies during the cultivation period.

  Specific oxygen uptake rate increases to max. at early log phase and then decreases gradually

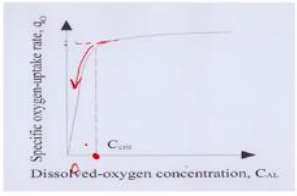


Pauline Doran, Bioprocess Engineering Principles, 2nd Edition, Academic Press 2012

Now in batch cultures, the rate of oxygen uptake it varies as the concentrations of cells increase during the cultivation period. Obviously because it is a function of X, so as the X would change the oxygen uptake rate would also change. Now rate of oxygen uptake is proportional to the number of cells present at any time. Now specific oxygen uptake rate this is also said to be varying with the cultivation period where the specific oxygen uptake rate it increases to its maximum during the early log phase and then decreases gradually.

(Refer Slide Time: 04:10)

- If Q_O is the oxygen uptake rate per volume of broth and q_O is the specific oxygen uptake rate: $Q_O = q_O X$
- The inherent demand of oxygen depends primarily on the biochemical nature of the cell and its nutritional environment.
- As the level of dissolved oxygen in the broth falls below a certain point, the specific rate of oxygen uptake is also dependent on the oxygen concentration in the broth.



Pauline Doran, Bioprocess Engineering Principles, 2nd Edition, Academic Press 2012

So, if you can see on the slide if Q 0 can be defined as the oxygen uptake rate per unit volume of the broth and this is capital Q 0 and small q 0 is defined as the specific oxygen uptake rate means per unit biomass the demand of oxygen, then capital Q 0 and small q 0 can be related with the X, X stands for the biomass concentration here. The inherent demand of oxygen depends primarily on the biochemical nature of the cell and its nutritional environment.

Which means we are talking about the specific oxygen demand. Now as the level of dissolved oxygen in broth it falls below the critical oxygen level, this inherent demand of oxygen which we call a specific rate of oxygen uptake starts getting dependent on the oxygen concentration of the broth which is mostly linear. So, if you see the plot shown on the slide which is between specific oxygen uptake rate and critical oxygen concentration.

Then you see that it follows a saturation curve where beyond the C critical value where I have put the dot on the x axis, beyond this oxygen concentration you will observe that the specific oxygen demand is constant. And below this value it rapidly drops almost as a linear function of the concentration of oxygen present in the medium.

(Refer Slide Time: 06:06)

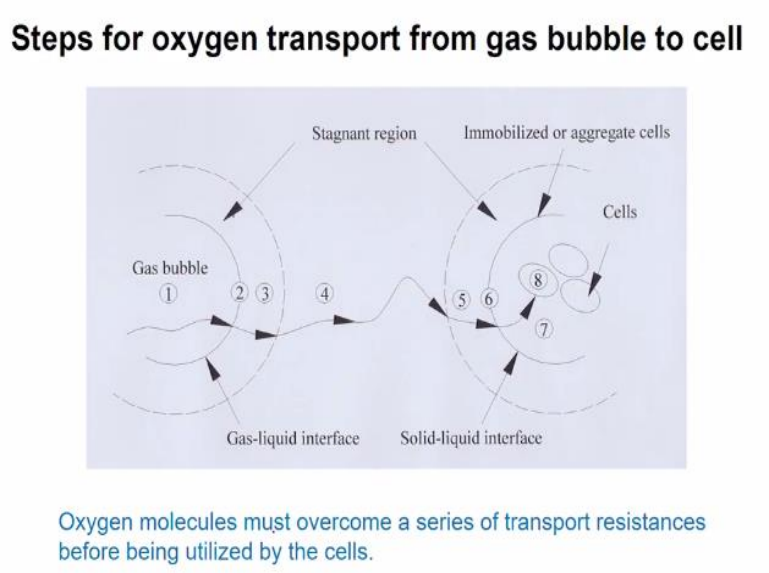
- To eliminate dissolved oxygen limitations and allow cell metabolism to function at its optimum, the dissolved oxygen concentration at every point in the fermenter must be above C_{crit}.
- The exact value of C_{crit} depends on the organism, but under average operation conditions usually falls between 5~10% of air saturation.
- For cells with relatively high C_{crit} level, the task of transferring sufficient oxygen to maintain $C_{AL} > C_{crit}$ is always more challenging than for cultures with low C_{crit}.
- Choice of substrate for the fermentation can also significantly affect oxygen demand.
- As glucose is generally consumed more rapidly than other sugars or carbon-containing substrates, rates of oxygen demand are higher when glucose is used.

Now in order to eliminate dissolved oxygen limitations and therefore allow cell metabolism to function at its optimum, the dissolved oxygen concentration at every point in the fermenter must be kept above the C critical value. So, the exact value of C critical it is culture dependent depends on the organism, however in general under standard operating conditions it usually falls between 5 to 10% of the air saturation.

Now for cells with relatively high C critical level the task of transferring sufficient oxygen in order to maintain at all times the bulk oxygen concentration greater than the C critical is quite challenging in comparison to the cultures which have low C critical values. Choice of substrate for fermentation can also have significant impact on oxygen demand. Like for

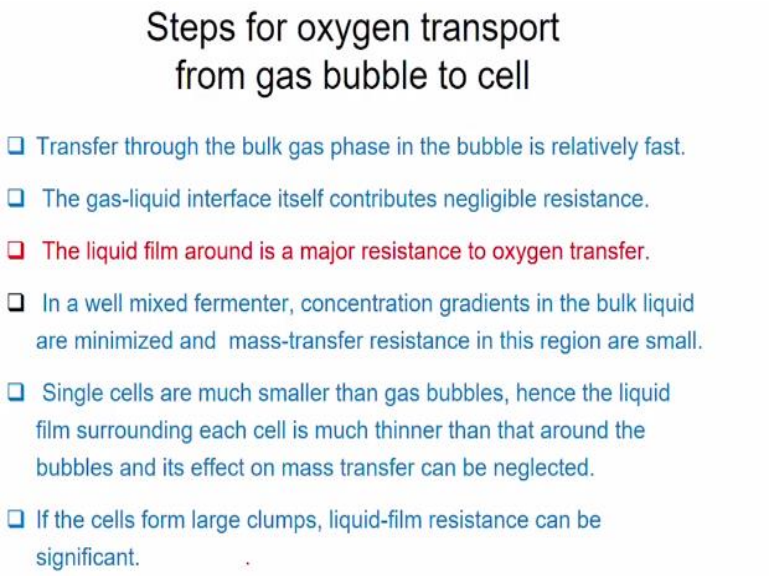
example glucose it is generally consumed more rapidly than other sugars or carbon containing substrates and the rates of oxygen demand are therefore higher.

(Refer Slide Time: 07:32)



So, steps of oxygen transport from the gas bubble to the cell let us see. As shown on the slide one side we have a gas bubble, the other side we have cells and surrounding the gas bubble we have a gas liquid interface and surrounding the cell aggregate or the immobilized cell structure we have solid liquid interface. So, the oxygen travel for oxygen to reach to the cells inside the immobilized bead the oxygen molecules must overcome a series of transport resistances before it can be utilized by the cells present inside the immobilized structure.

(Refer Slide Time: 08:19)



So, let us try to break it down into the different steps for successful oxygen transfer to happen from the gas bubble to the cell. Now first the oxygen has to transfer through the bulk gas

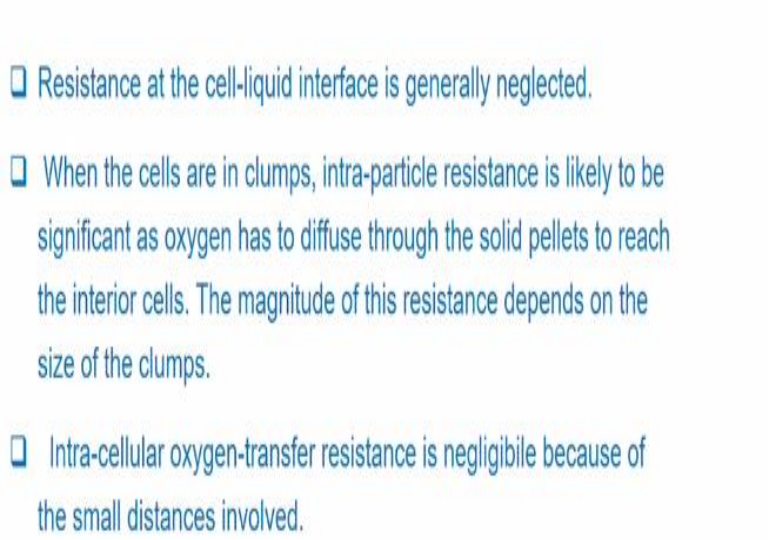
phase in the bubble to the interface. Now this transfer from the bulk gas to the interface is relatively fast. Then the gas-liquid interface this contributes negligible resistance. So, across the gas-liquid interface there are two films.

One is the gas film towards the bubble side and the other is the liquid film towards the bulk liquid side. So, gas film itself it contributes negligible resistance. It is the liquid film around this gas liquid interface which poses the major resistance to oxygen transfer. Now in a well-mixed fermenter, the concentration gradients are absent in the bulk. They are minimized and the mass transfer resistance in this region also can be expected to be minimum or negligible.

Now single cells they are very small than the gas bubbles and if we are assuming that it is a homogeneous mixture finely suspended cells in the bulk, then the liquid film surrounding each cell can be assumed to be much thinner than around the bubbles and therefore its effect on the mass transfer can be neglected or the resistance of this film is negligible. Now if the cells they are forming clumps depending on the kind of fermentations.

Like in case of plant cells which tend to aggregate or in case of immobilized cell systems, then these cells can form a bigger clump where then the liquid film resistance can be significant and cannot be neglected or ignored.

(Refer Slide Time: 10:36)

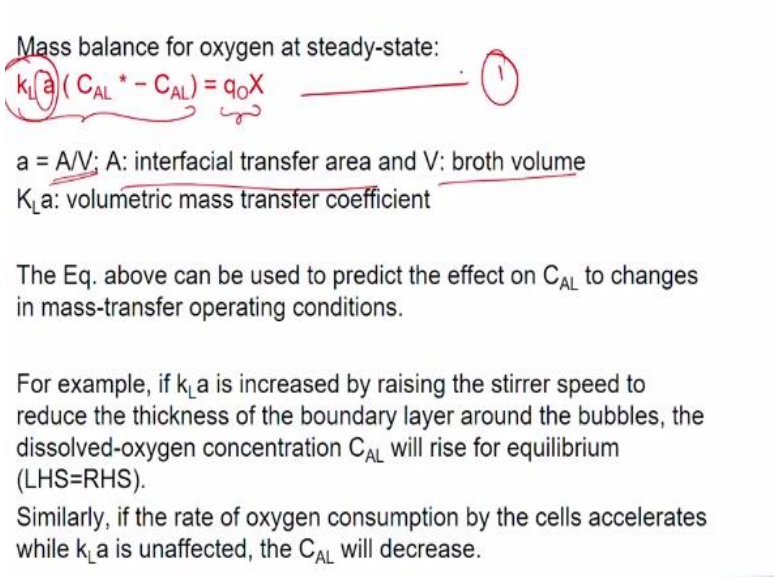


So, resistance at the cell-liquid interface is generally neglected in microbial fermentations which are uniformly suspended and homogeneous in nature. When the cells are in clump, the intraparticle resistance is likely to be significant as oxygen will have to diffuse from the

surface to inside of the pellet till it reaches the reaction center of the cell surface. So, it has to diffuse through the solid pellet to reach the interior cells.

The magnitude of this resistance depends on the size of the clump. Intracellular oxygen transfer resistance is negligible because of the small distances involved. Even in immobilized systems now once it has reached to a cell surface traveling from the cell surface to the reaction center those distances are negligible considering the size of a single cell. So, therefore this resistance can be neglected.

(Refer Slide Time: 11:48)



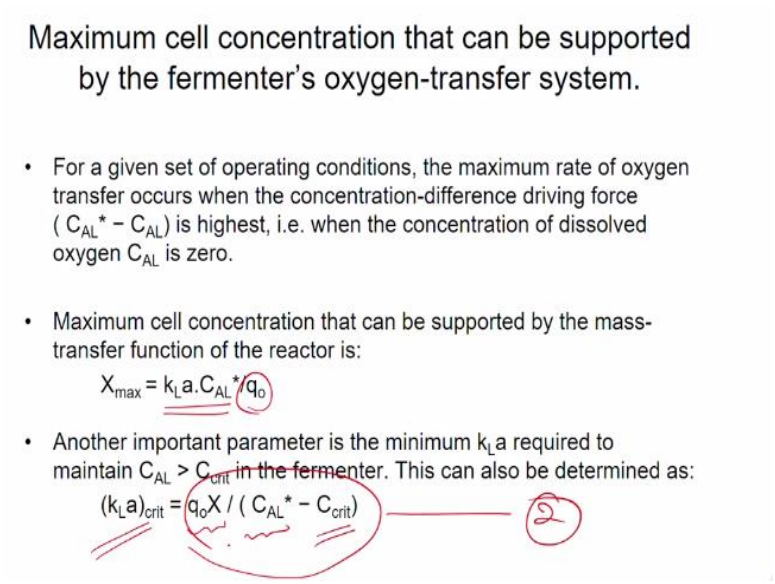
So now at steady state if you do a mass balance for the oxygen, then oxygen uptake rate will become equal to the oxygen transfer rate so that there is no accumulation of oxygen in the bulk, so which is steady state. So, we know that if you can see on the slide we know the RHS of this equation k L a C star – C AL, this defines the transport rate of oxygen in the medium from the gas. The LHS is q 0 X.

Now this q 0 X is the oxygen uptake rate where small q 0 stands for the specific oxygen demand of the culture and X is the biomass concentration. Now this small a in this is given as capital A by V where A is the interfacial transfer area and V is the broth volume and k L a together as constant is called as volumetric mass transfer coefficient. Now this equation given above here the equation 1, so the equation 1 can be used to predict how the mass transfer operating conditions.

If change will bring about a change in the bulk oxygen concentration. Like for example if k L a is increased by raising the stirrer speed to reduce the thickness of the boundary layer around the bubbles, so which means the resistance can be reduced and therefore the mass transfer coefficient can be improved if you remember the two-film theory. Now the dissolved oxygen concentration in this case will therefore then rise to maintain the equilibrium.

And when this happens so therefore you will observe of equation 1, your c AL will increase so that the difference between the C star – C AL reduces and the steady state is maintained. Similarly, if the rate of oxygen consumption by the cells accelerates while this k L a is unaffected, then the C AL will decrease.

(Refer Slide Time: 14:30)



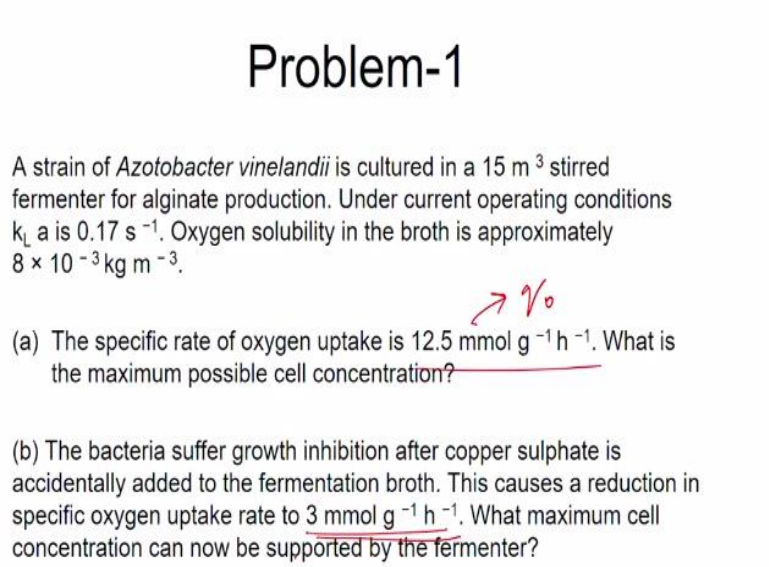
Now if we need to determine maximum cell concentration that can be supported by a fermenter system then how do we determine that? For a given set of operating conditions the maximum rate of oxygen transfer occurs when the concentration difference which is the driving force is highest. For a given oxygen transfer system the maximum rate of oxygen transfer will occur when the concentration difference is maximum.

So, when will this be maximum? When the C AL is 0, so then only the C star – C AL will be maximum. So then if we need to find out the maximum cell concentration that can be supported for the given oxygen transfer system, then we can find out having the maximum oxygen transport rate which is given here as k L a times C AL star divided by the specific oxygen demand of the culture.

So, this way we can find out what can be the maximum biomass which can be supported under a given oxygen transfer system. Another important parameter is the minimum k L a which might be required to maintain the bulk oxygen concentration always above the C critical level in the fermenter. So how do we determine that? Again, the same thing k L a can now be written as k L a critical and your C AL bulk is nothing but your C critical and X is your biomass.

So, if we need to find out for a given biomass what can be the minimum k L a which is required to maintain the concentration of the oxygen in the bulk above the C critical then we can use the equation 2 here. So, C AL has been replaced by C critical given the value of X, q0 and C AL star. So, this will be the critical k L a required for ensuring that the bulk oxygen concentration is above C critical always given the value of biomass concentration.

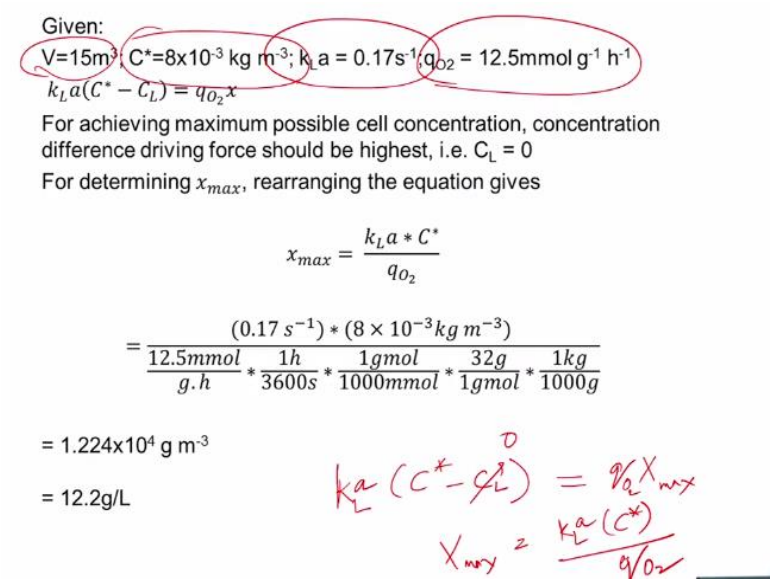
(Refer Slide Time: 16:57)



Let us discuss a problem here. A strain of Azotobacter it is cultured in a 15meter cube stirred fermenter for alginate production. Under current operating conditions the volumetric mass transfer coefficient is given as 0.17 second inverse. The oxygen solubility in the broth is approximately given as 8 ppm. Now the specific rate of oxygen uptake is given to us which means the small q 0 value and we need to determine what can be the maximum biomass concentration which can be supported by this oxygen transfer?

So, as we had done earlier, we will talk about the approach and you can solve the problem. So, let us see how to solve the first part.

(Refer Slide Time: 18:02)

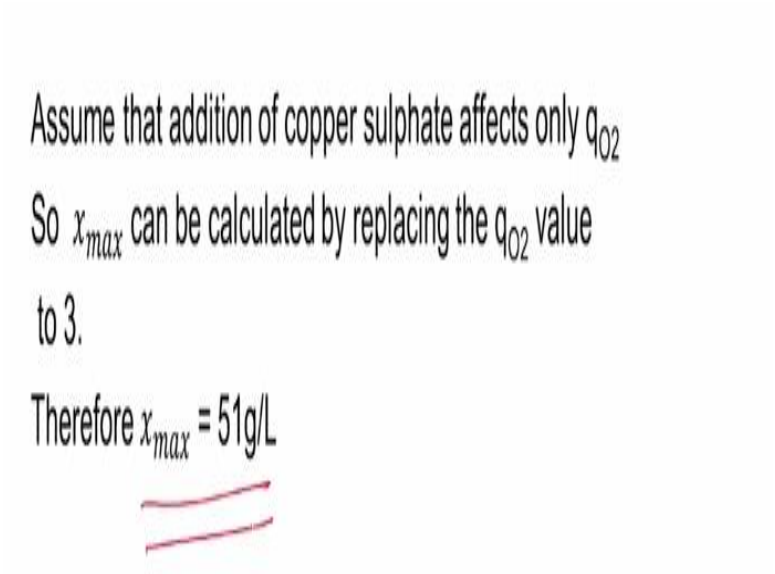


So, what has been given to us? The volume which is 15 meter cube, the saturation concentration the equilibrium concentration of oxygen which is 8 times 10 to the power of -3 kg per meter cube, volumetric mass transfer coefficient is given, the specific oxygen demand is also given. Now under steady state we know that oxygen transport rate which is C star – CL times k L a = q O 2 times X.

So here X can stand for the X maximum and C AL so C L is 0. So, your maximum biomass which can be supported can be given as, so this is what has been done here. Let us see the second part. The bacteria suffer growth inhibition after copper sulfate is accidentally added to the fermentation broth. This causes a reduction in the specific oxygen uptake rate to 3 millimolar per gram per hour.

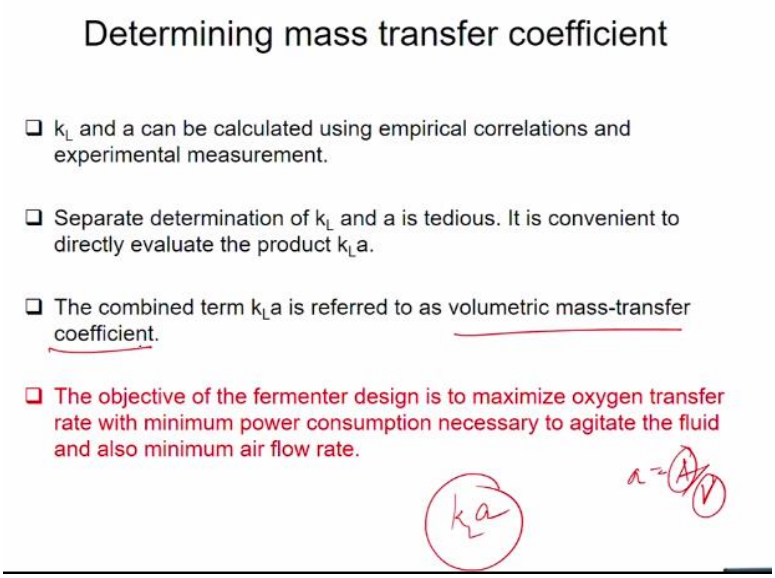
So now the specific oxygen demand has reduced to 3 millimolar per gram per hour. What maximum cell concentration can now be supported by the fermenter? So rather than using 12.5, we will have to use 3 millimolar value.

(Refer Slide Time: 20:11)



So, if we do the substitution then the X max changes to 51 grams per liter. So now because the specific oxygen demand has reduced so your amount of biomass concentration which can be supported with this under the same conditions is increased.

(Refer Slide Time: 20:20)



Now how do we determine the value of volumetric mass transfer coefficient? Now volumetric mass transfer coefficient is a club of 2 constants the mass transfer coefficient and the small a factor which is nothing but A by capital V where A is the area for mass transfer and V is the volume. Now k L and a they can be calculated using very empirical co-relationships and also via experimental measurements.

Separate determination of the mass transfer coefficient and the small a value might be very challenging and tedious, so it is convenient to directly evaluate the entire constant of

volumetric mass transfer coefficient which is club of both k L and a. So, the combined term k L a is referred to as volumetric mass transfer coefficient. The objective of the fermenter design is to maximize the oxygen transfer rate with minimum power consumption necessary to agitate the fluid and also minimum air flow rate.

So that should be the objective so that in minimum cost we are able to provide maximum oxygen transfer for growth. So, the two things please remember, minimum power consumption necessary to agitate the fluid and also minimum air flow rate to maximize the oxygen transfer rate.