Transport Phenomena in Biological Systems
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Lecture-66
Bioreactor K_La Estimation

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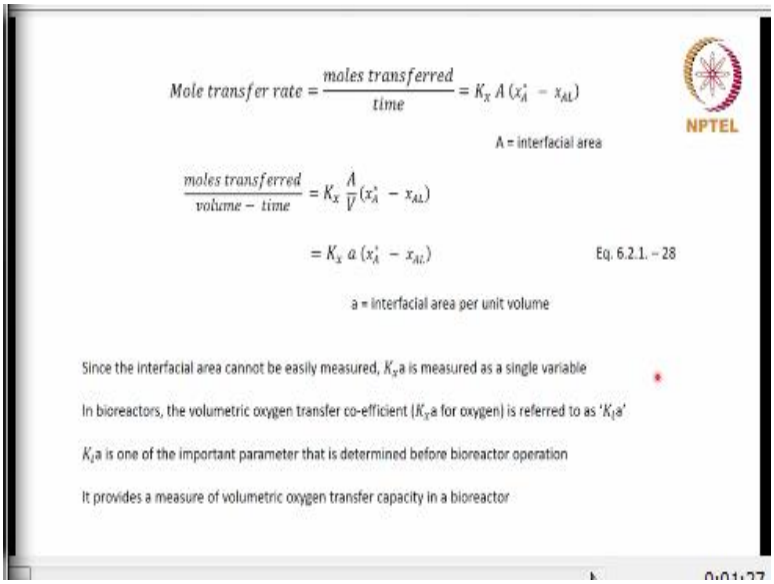

Welcome back, we are looking at a trans coefficient approach, we are taking this specific example of oxygen transfer in bioreactors, oxygen supplied to bioreactors. After a lot of discussion we have come up with this formulation, the moles transferred for you know volume per time is a certain K_xa times $X_A^* - X_{AL}$, X_A^* is the liquid side concentration of oxygen which is an equilibrium with the bulk gas side concentration.

And a is the interfacial area per unit volume, we said a is of course difficult to measure and therefore, K_xa is measured together. They say somewhat equivalent to K_La on the K_xa is written for mole fractions, K_La is usually used for concentrations. The coefficient concentration times the mole fraction as the concentration of the species. And therefore we use and K_La quite extensively when we deal with bioreactors.

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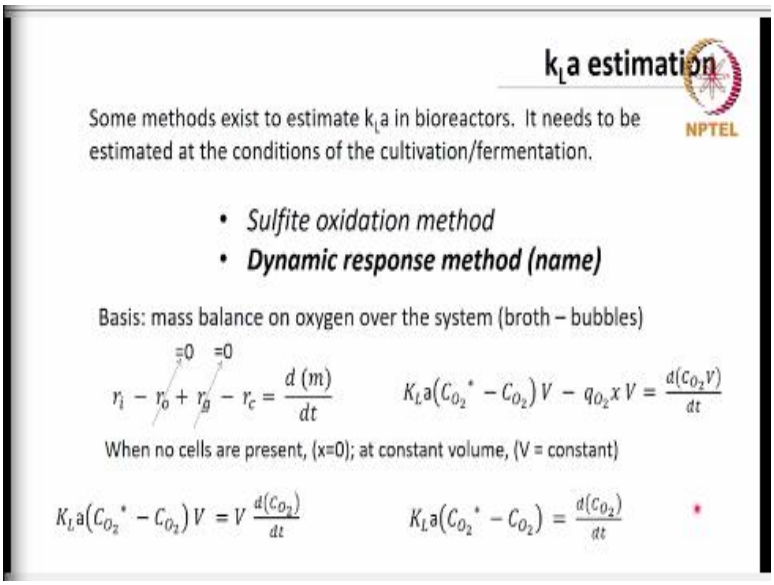

So, what I am going to do in this class, is to tell you how to estimate K_La, because it is important. And also we work out a problem that who would help you understand how to estimate K_La, ok, let us see how we are going to do about it. Some methods exist to estimate K_La in bioreactors some standard methods. And the K_La needs to be estimated at the conditions of the cultivation or fermentation, why because it depends on temperature, it depends on operation condition and so on so forth.

So, you fix everything, you fix the temperature at which you are going to operate, or fix the pH at which you are going to operate. And we fix the rpm and other things which we are going to operate and then estimate the k_La. There are 2 broad methods, sulfide oxidation method, dynamic response method, what I am going to call as dynamic response method here is a certain method, this name has been used in different ways in different textbooks, ok.

So, please be very careful when you come across this step, dynamic response method might refer to a slightly different method in some other textbooks. So, let me call the dynamic response method. Sulfide oxidation method can be used, but there are some limitations of this method. I just thought I mentioned that name to you, but there are some limitations for this method, I just thought I mention the name to you, but I am not going to tell you anything about this method.

So, let us look at the dynamic response method as it is called in this course. The basis for the dynamic response method is nothing but the mass balance on oxygen over the system, ok, that is also being used here. And that is the reason why we started looking at mass balances as some sort of a review to begin with ok. The system over which we are going to do the balances is the bioreactor broth and we are mentally going to remove the bubbles from a broth, ok.

So, broth minus the bubbles is going to be our system, that we can focus on, it can be a conceptual system right. So, this is going to be a system for analysis**,** this comes in handy, this we will know we need a experience. I did not mean it is certainly unrealistic to expect a knowledge to come up with this charts of a system right in the middle. This comes out of experience after you have been hit upon by various different difficult choices of systems for the purpose at hand.

And then it you come up with this elegant system or this way of choosing a system by which the analysis ok. So, take it on phase value, this we are going to do it on broth minus bubbles, we have taken the bioreactor broth, you have mentally removed the bubbles of it. The mass balance equation we all know by had now, input rate minus output rate plus generation rate minus consumption rate equals the accumulation rate and we are writing this on oxygen.

This is a bioreactor system broth minus volumes, the output rate is 0 of oxygen, you have to recall this is the bioreactor, bioreactor broth. The oxygen is being bubbled or the air is being bubbled through the bioreactor broth through the bubbles. And we are looking only at the liquid part of the broth. So, this is a system, oxygen does not get out of the liquid part of our system until super saturation conditions right.

That is the only way by which it can go out of the and be normally node operator of this conditions, that is the reason why this can be equal to 0. And this is essentially the reason why we chose this as a system and we mentally remove the bubbles, so that we can put around equal to 0. If we included the bubbles there is a lot of oxygen that was going with the bubbles, ok, we needed to account for that.

And that could have become a lot more difficult to do that, a lot more measurements in there, ok. So, we excluded the bubbles and we could put r_o equals to 0. The generation rate of course that is no reaction that is generating oxygen in the system. And of course input rate is there, consumption rate is there, the accumulation rate will be there, what is the input rate. Now we have an expression for the input rate in terms of K_La , K_La $(C_{O2}^* - C_{O2})V$.

K_La volumetric mass transfer coefficient times the concentration difference here ok. This is in terms of moles transfer per unit volume per time. We want each of these in terms of moles transfer per time, mass per time also time, we can refer moles per time and they will not be a big difficulty in this case, there is no reaction. So, we want moles per time for each of these remaining terms here.

This is K_La $(C_{O2}^* - C_{O2})$ and because this is in moles per volume per time, we need to multiply it by the relevant body which is the broth volume. So, now we have written it in terms of moles per time, ok, why because the input is across the interface between the gas bubbles and the liquid, so that is the input for the system ok. Now the consumption rate, we are going to write in terms of a certain oxygen uptake rate q_{O2} the units of q_{O2} are such that you need to multiply it by the cell concentration.

Because normalizing with concentration and also the volume to get it in terms of moles per time of oxygen that were q_{O2} oxygen uptake rate times the cell concentration times the volume equals suppose this is mass of oxygen, mass of oxygen is concentration times volume in the system, that we are looking at. Therefore $(dC_{O2}/dt)V$, the volume happens to be a constant and therefore that can be taken out of the derivative also.

Let us take the case when no cells are present in the bioreactor broth $x = 0$. So, when no cells are present $x = 0$ and add constant volume, V is a constant, we have K_La $(C_{O2}^* - C_{O2})V$, this time drops out $x = 0$. Now, V is a constant it can be taken out of the derivative $Vd(C_{O2}/dt)$. So, this V, V can be cancelled and K_La $(C_{O2}^* - C_{O2}) = d(C_{O2}/dt)$.

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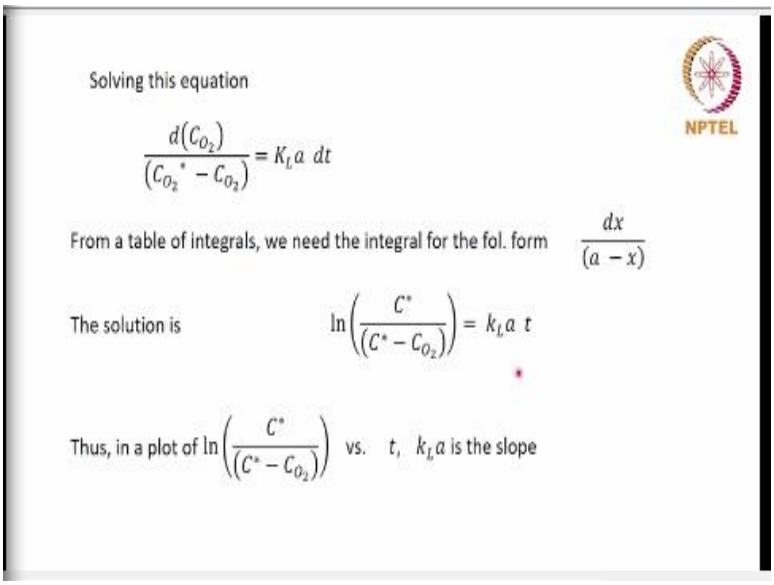

If you solve this equation, right, this is a differential equation right. If you solve this equation with an initial condition right you get d C_{O2}. This is a long term here recall, d C_{O2} in concentrations one side they have 1 by C_{O2}^* - C_{O2}, this C_{O2}. So, the long term arises because of this and this straightforward K_La dt, so t terms comes here. So, d [(C_{O2})/(C_{O2}^* - C_{O2})] = K_La dt.

And from the table of integrals, we need the integral for the form, this is equalent to dx by a - x in the table, ok, dx/(a – x) in the table. Therefore the solution of this form you can directly take from the table of integrals, you do not have to worry about working it out ln[C * / (C * - C_{O2})] = K_La t, so this is the solution from the table it is a standard form.

And what does this mean, this is of the form y = mx ok, where y is nothing but a ln[C * / (C * - C_{O2})] So, if plot ln[C * / (C * - C_{O2})] versus time, t you will get K_La as the slope, ok. So, this is the way of getting K_La in an actual experiment in the industry and so on so forth.

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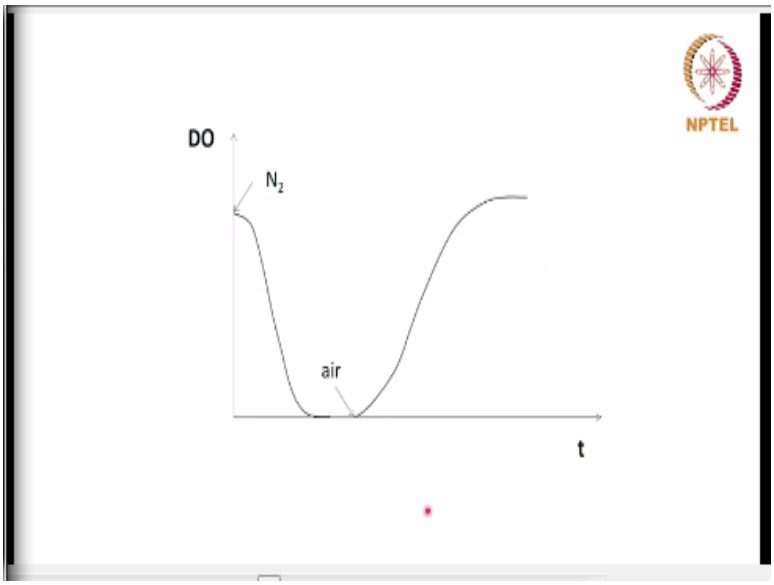

The way it is done is as follows, we plot, we look at, we measured that and we measure that plot dissolved oxygen in the broth versus time. And the experiment we do is as follows, we first purge the liquid with nitrogen to physically strip out all the oxygen that is present in the broth, ok. So, you pump it with nitrogen, that nitrogen replaces the oxygen on the broth, therefore the oxygen concentration in the broth drops gradually till it is zero.

Make sure it is indeed 0, wait for some time and then at time t = 0 introduced air, air is being introduced here. And then because of the oxygen coming into the broth, the DO level is going to increase, it will increase something like this and with a saturated value, ok.

So, once you have this data, you could plot this quantity $\ln[C^* / (C^* - C_{O2})]$ because you have C_{O2} verses time right. So, you can plot the entire data and you have that power of the set of data to get a good estimate of K_La it will be the slope of the curve $\ln[C^* / (C^* - C_{O2})]$ versus time . Let me present this problem to you and leave it to you to work out when we come back, I will present the solution ok, in the next class yeah.

I think that might be a little bit, ok, the following data was obtained during K_La determination of a stirred tank bioreactor operating at 500 rpm, 1 atmosphere pressure and 37 degrees C and the measurement is done by the dynamic response method, ok, mole time I mentioned about the

conditions of estimation being the same as that of operation. So, you need to fix these 500 rpm, 1 atmosphere pressure that is 37 C and measure K_La under these conditions itself.

The oxygen source is air, a millivolt meter was used to read the dissolved oxygen level in the labs in the industry also. Because the voltage is proportional to the concentration, the DO probe converts the concentration to a certain voltage and the voltage could be either directly measured or you could use some sort of an interface device to convert it into the concentration. We had DO's to this actual data from the lab and we have use some millivolt meter to read the dissolved oxygen level, find the K_La of the bioreactor.

This is the data actually that I had generated during my PhD case, ok, this is actual data from my PhD work, I use to run tons of bioreactors, large number of bioreactors when I work and this is during characterization. So, the data that is given is the time and seconds versus dissolved oxygen in millivolts. So, this is proportional to the concentration in either molar or in terms of percentage air saturation molar you want, ok.

So, you can take this problem, I have already given you the classic procedure for working out the plot $\ln[C^* / (C^* - C_{O2})]$ versus time, t and get K_La as a slope, when you try this out on your own, it is good because it is useful comes in handy for many different practical situations when we try this out.

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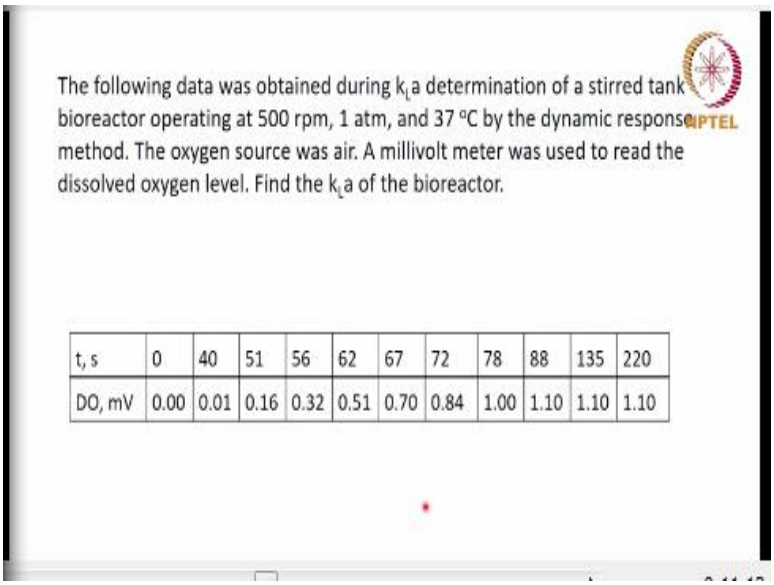

When we come back, I will give you the solution or I will write start out with the solution for this C line. Welcome back, in the previous class, we looked at a method of measuring $K_L a$, the dynamic response method and I had posed this problem to you and ask you to solve this as homework. And let us start by solving this. The following data was obtained during $K_L a$ determination of a stirred tank bioreactor operating at 500 rpm, 1 atmosphere pressure and 37 degree C by the dynamic response method.

The oxygen source was air or millivolt meter was used to read the dissolved oxygen level, find the $K_L a$ of the bioreactor, you have the data here, t in seconds, a DO in millivolts was t in seconds, ok. We already looked at problem solving the very beginning of this course.

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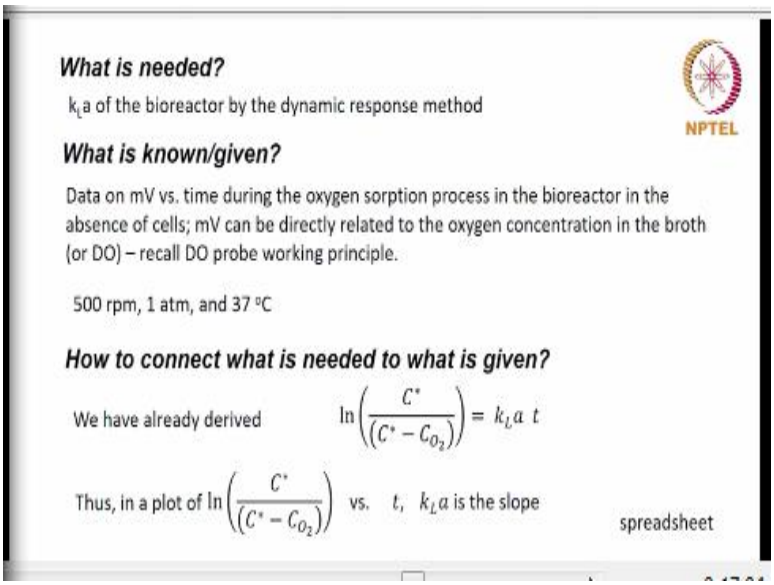

So, we look at what is needed first in this case, $K_L a$ of the bioreactor by the dynamic response method, what is known or given. We have data on millivolt versus time during the oxygen sorption process in the bioreactor in the absence of cells, millivolt can be directly related to oxygen concentration in the broth of DO.

If you want to look at the DO probe working principle from a reactor course or you can read up about the DO probe of principle, you will get why you can use DO in the case of actual concentrations. So, what is needed, what is known, yeah we are clear about, now how do we connect these 2. And of course also known as or the conditions on top operation that is fine and how to connect what is needed to what is given or we connect.

We have already derived that $\ln[C^* / (C^* - C_{O2})]$ versus time will give $K_L a$ transverse, so this is the connection ok. So, let us see it, I have already done that in a spreadsheet and so let me go to that directly.

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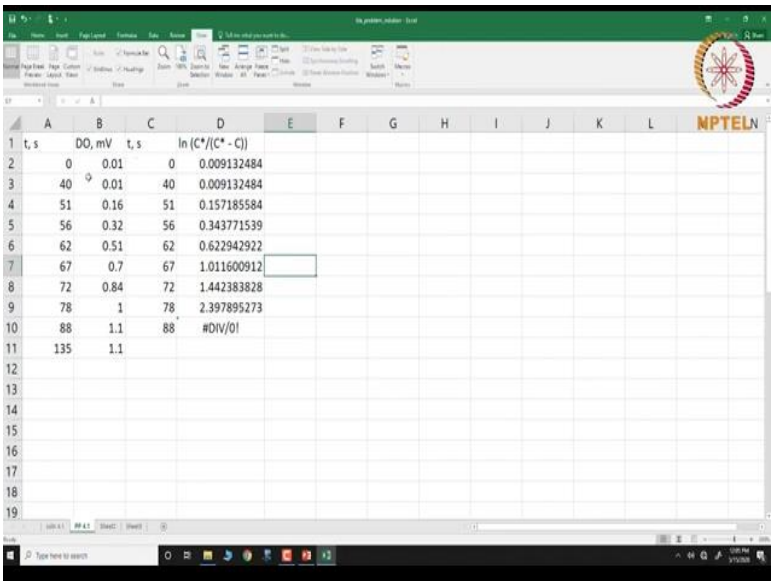

	A	B	C	D
1	t, s	DO, mV	t, s	ln (C*/(C* - C))
2	0	0.01	0	0.009132484
3	40	0.01	40	0.009132484
4	51	0.16	51	0.157185584
5	56	0.32	56	0.343771539
6	62	0.51	62	0.622942922
7	67	0.7	67	1.011600912
8	72	0.84	72	1.442383828
9	78	1	78	2.397895273
10	88	1.1	88	#DIV/0!
11	135	1.1		

Yes, you see here can I increase the view size there are views here yes, it is go to maximum, ok. Here we are, ok all these things that makes to do with a spreadsheet. This is the data that is given the first column you have time in seconds, in the second column you have millivolts, ok. I have just consider the relevant times here again for ease of plotting same columns here and here you have ln[C * / (C * - C_{O2})], ok.

You need to find the C * value, right, what is the C * value, it is going to be 1.1, why, go back to this. Here what is C *, this is the value that is in equilibrium with the gas phase concentration, right. So, you see here the data the millivolt itself 1, 1.1, 1.1, 1.1 it has reached saturation here, ok, or it has reach the equilibrium conditions here. So, this is the value that you are looking for, the value in the gas phase that is in equilibrium with the concentration in the liquid phase, right.

So, this is the value that you are looking for, so you have a measure of that itself, so you can verify with. If you do not have a measure of that you have to measure the saturated value and use that for C *. So, let us get back to excel, so I have calculated ln[C * / (C * - C)], the C is given in as various DO values here in millivolts. I am using all millivolts that of the you know it is a consistent here and the radiation has given here. And of course this does not make any sense therefore do not worry about that.

So, you can use this set of data, ln[C * / (C * - C)] versus time to get K_La as it is good. So, if you plot this ok.

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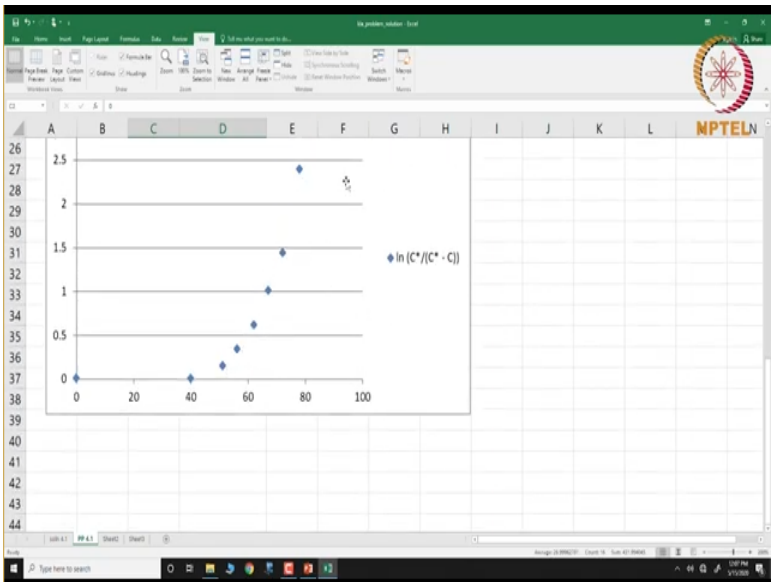

Now you take the linear region, you have 1.1, 7 links here, right. So, you need to avoid that and the linear even is somewhere here, you take a linear region because the relationship is where only in the linear region. The process itself goes over various regions of description where other different parts can be described by different ways, only one major part can be described by linear of variation.

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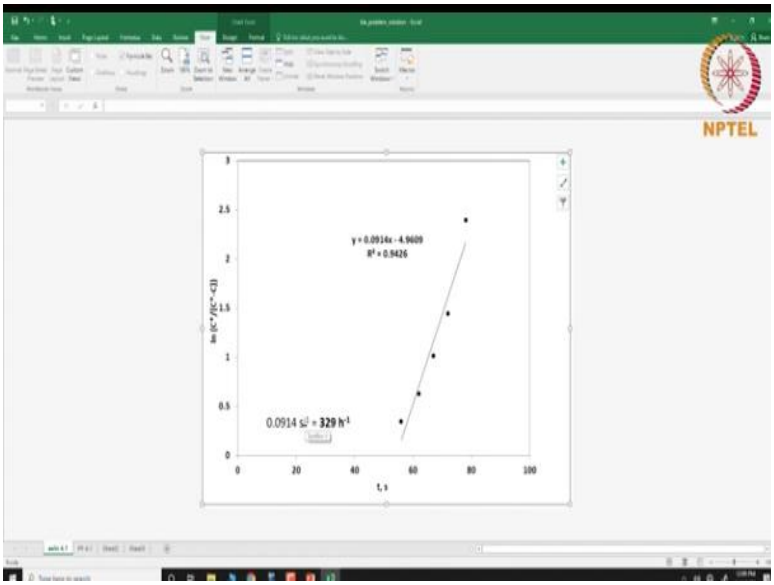
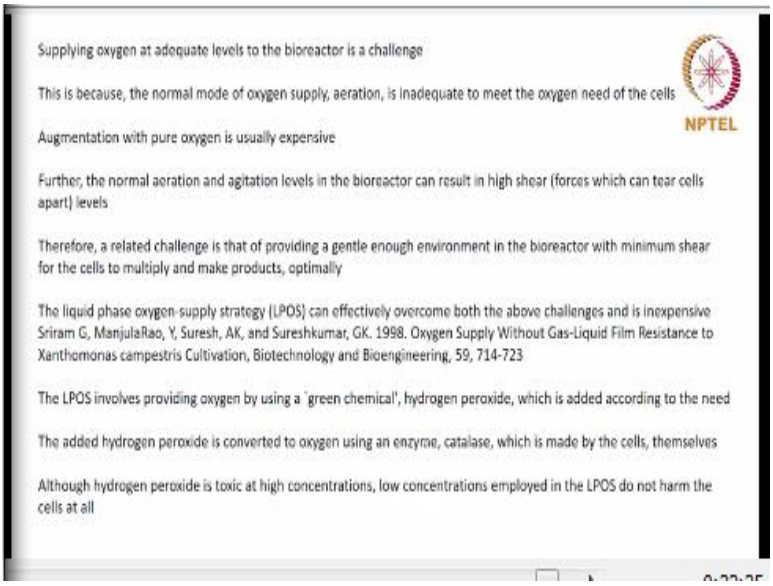


And if you do that is something like this, once again zoom in yeah, this is what it is, you have ln[C * / (C * - C)] versus time here, right. And they plot somewhere here we have neglected the other points where this linear relationship is not valid. Therefore we need to look at the data points only where the linear relationship is valid, you need not data, this is applying the model to the region where exactly K_La ok. So, we are not arbitrarily removing any plots, we are removing the points which does not fit which are not described by the model. So, you have this here, the equation turns out to be the fit 0.0914x - 4.9609, the fit is reasonable r square is 0.943.

And you take the slope here, it is going to be 0.0914, this is in second inverse. And in hour inverse we need to multiply by 3600, 329 hours, ok. So, 329 hour inverse is actually very high K_La of course we had used a diffuser or HPCL diffuser for diffusing out of bubbles, which have very fine bubbles that way. So, the surface area of bubble for you know very large and our rpm simply 450 or something like that, that is a yeast bioreactor and therefore this high K_La is acceptable. **(Refer Slide Time: 22:25)**



Fine, now should we do this here, I think why do not we take it up in the I know this is a short class. But why do not we take it up in the next class because we are going to discuss some papers from our research, I am going to tell you a lot of cases where how actually applying the transport principles in cutting edge research, thought would give you some examples, worked on a lot of this aspects, see you.