Bioreactors
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Lecture - 15
Bioreactor env.par. (DO)

Welcome to lecture 15 NPTEL online certification course on Bioreactors.

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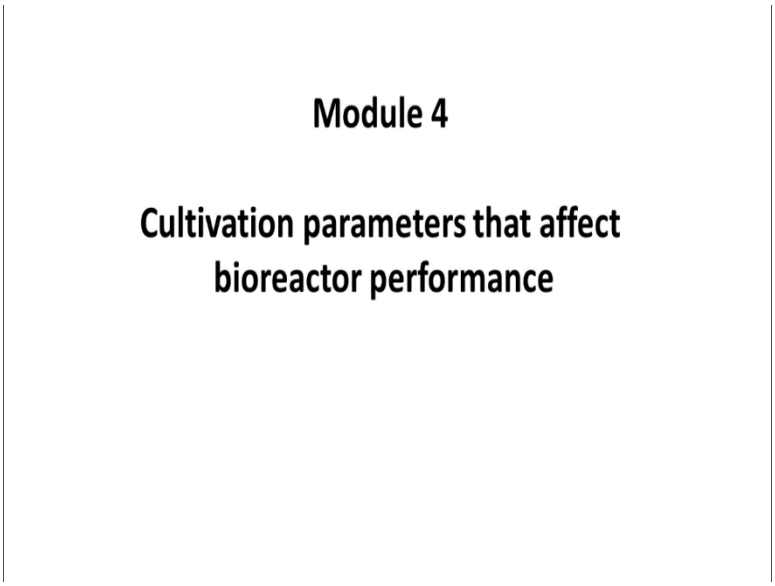

In the last lecture, we had started module 4, which is on Cultivation parameters that affect bioreactor performance. We saw a few cultivation parameters or bioreactor environment parameter as they are called that affect bioreactor performance, we will continue on that today.

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Many cultivation (environment) parameters affect the bioreactor performance. The common ones (there are some others) that are measured and controlled are:

- Temperature
- pH
- Medium composition (*we have seen some of this*)
- Aeration/Agitation
- Dissolved Oxygen (DO) level

What we saw in the previous lecture was that there are many cultivation variables that impact the performance of bioreactors. The few common ones that are measured and controlled are Temperature, pH, Medium composition, Aeration and Agitation as well as the Dissolved Oxygen level.

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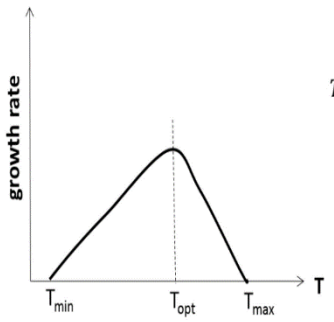
Temperature



$T_{max} - T_{min}$ Temperature range for growth

If $T_{max} - T_{min}$ is narrow: stenothermal organism

If $T_{max} - T_{min}$ is broad: eurythermal organism

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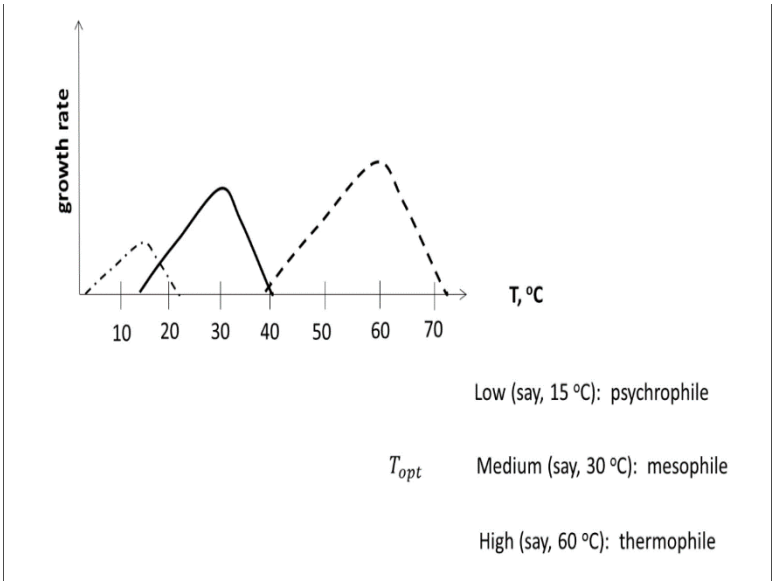

We had seen some details of temperature, the kind of organisms depending on whether their temperature range of growth is narrow: stenothermal or broad: eurythermal and whether the organism is Mesophilic depending on it is optimum temperature around 30-degree c, whether it is Psychrophilic optimum temperature 15-degree c or thermophilic optimum temperature 60-degree c.

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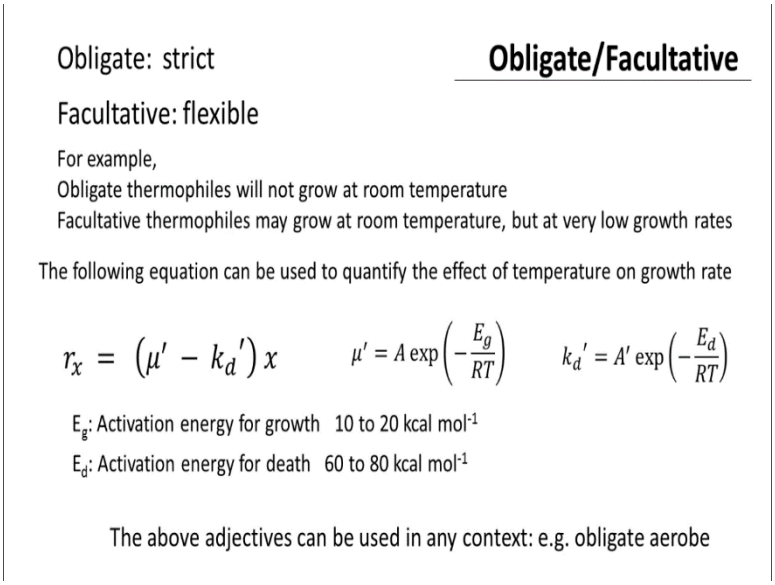

Then we also saw the adjective Obligate Facultative, that can be used in many different context including its temperature dependence, Obligate means strict and Facultative

means flexible. Then we saw a mathematical representation of the effect of temperature.

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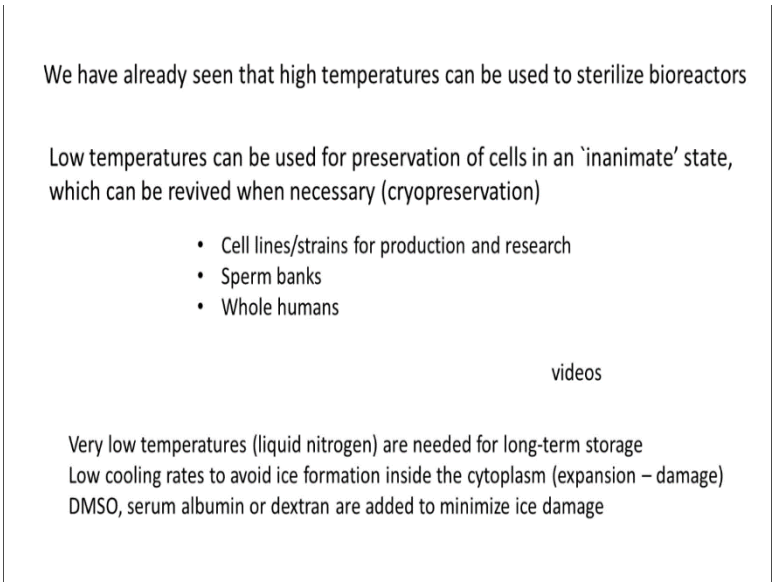

Then we saw the application of a low temperature to maintaining cell lines and strains for production and research in, for use in sperm banks as well as to keep whole humans in a state of suspended animation. There was, there was some reference to videos. I hope you have seen them, they are interesting videos.

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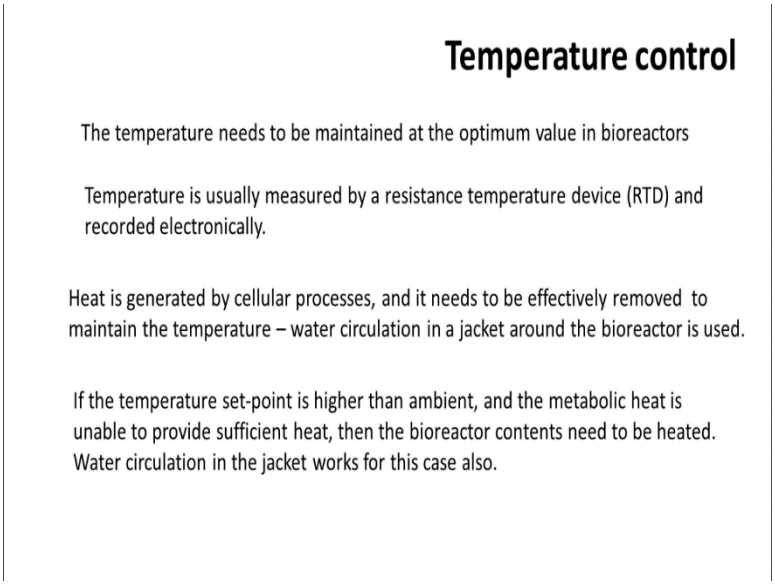

And then we looked at some aspects of temperature control.

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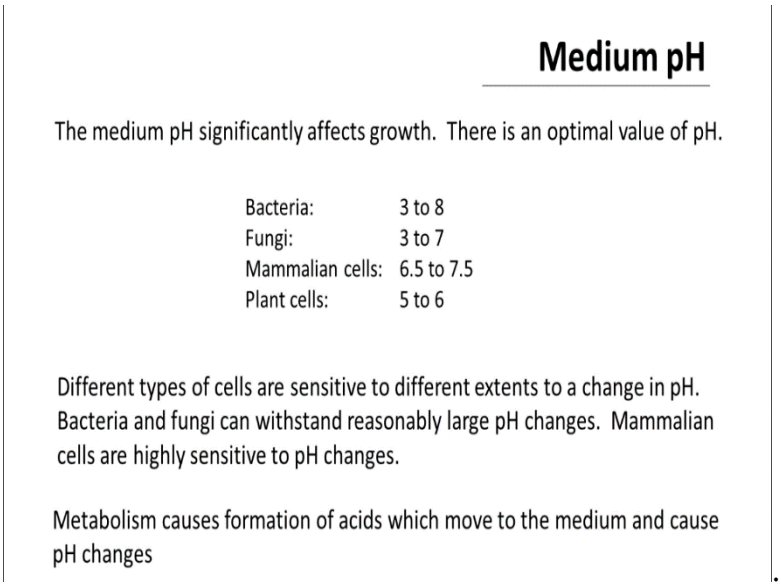

Then we went on to Medium pH. Some organisms can grow well over a wide range of pH, whereas some other organisms, some other cells such as Mammalian cells have a very narrow pH range for growth.

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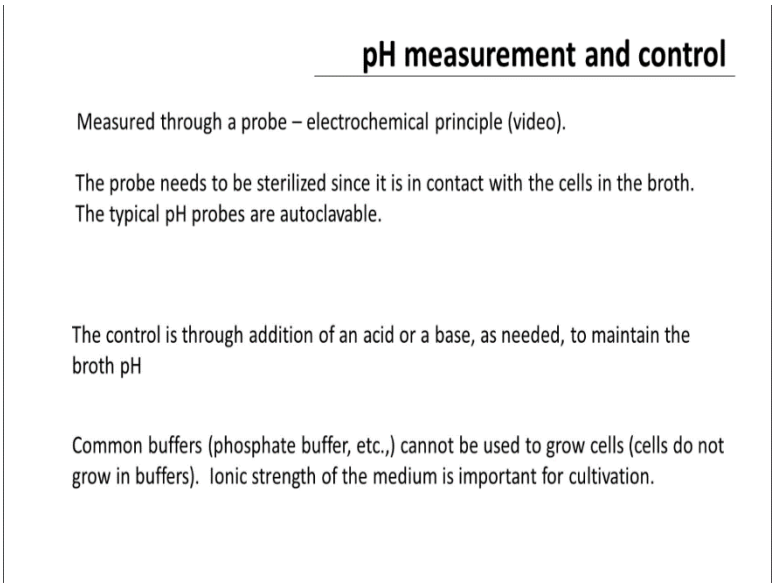

So, pH needs to be measured and controlled well in the bioreactor to get the best out of the bioreactor.

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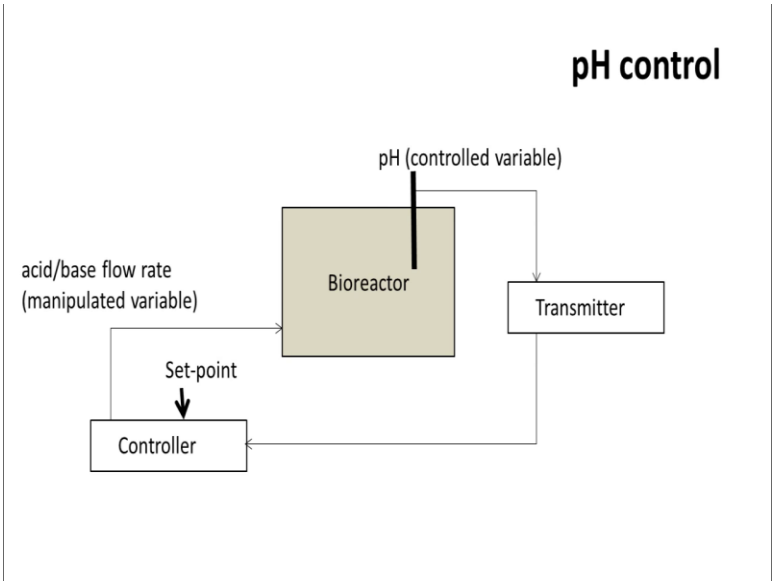

And we have also said that pH buffers supplemented with media components cannot be used for pH control, the cells just do not grow in them probably because of the high ionic strength of such media.

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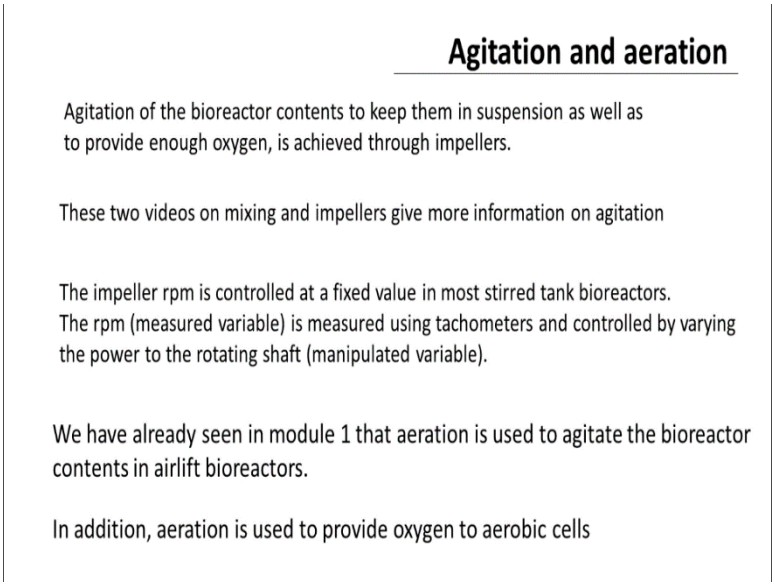

Then we looked very briefly at Aeration and agitation.

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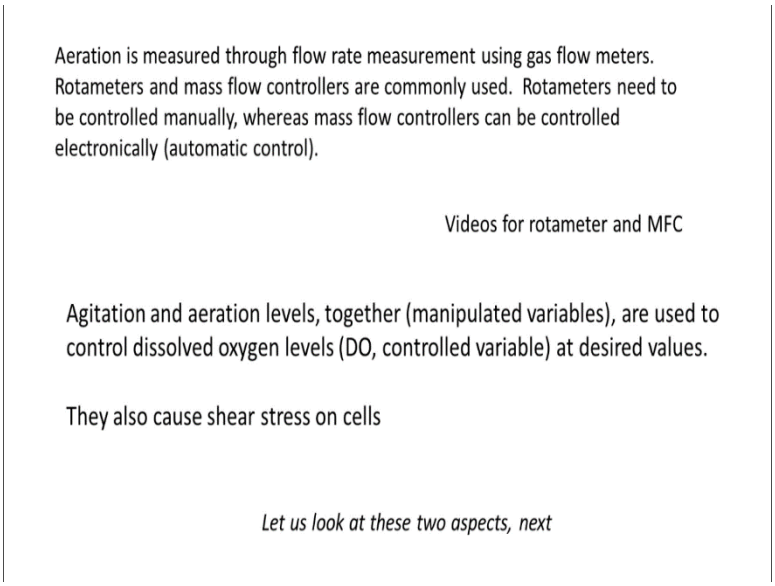

Then we said how they could be controlled, we said they also cause, they keep the cells in suspension, they also cause shear stress on cells. We said we will look at these 2 aspects starting in this lecture, let us go forward.

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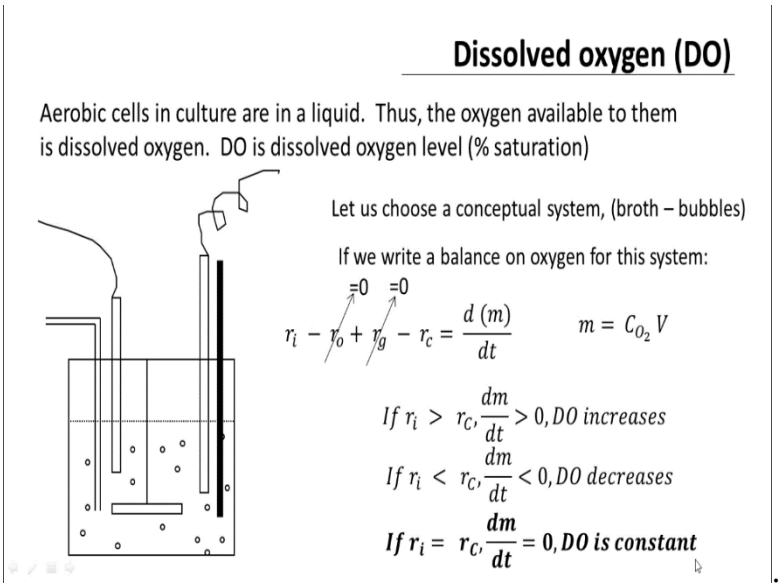

Dissolved oxygen or DO, is what we are going at look at next, we are going to look at this in some detail. Aerobic cells in culture are in the liquid. They are not in air, therefore, the oxygen that is available to them is the oxygen that is dissolved in the medium, dissolved in the water, predominantly in the medium. And DO is the level of

the dissolved oxygen and it is given as percentage saturation with air. Predominantly air is the source of oxygen for most bioreactors and therefore, it is been the practice to give dissolve oxygen levels as percentage air saturation. What it means is? You would have been introduced to the concept of equilibrium, let us say that we are considering water which is in contact with air. Oxygen which is in the air will transport to the water phase, the liquid phase, the gas phase, this will happen till there is a certain equilibrium that is achieved.

Microscopically speaking, there is a rate at which oxygen molecules move from the gas phase to the liquid phase and there is a rate at which the oxygen molecules move from the liquid phase to the gas phase, both keep happening and at a certain stage the concentrations are such, the rate of oxygen movement from the gas phase to the liquid phase equals the rate of oxygen molecule movement from the liquid phase to the gas phase. This is the condition we call as equilibrium.

The concentrations of course bear a certain relationship that is given by Henry's law and so on, when the equilibrium is achieved but they are not equal. You have 21 percent oxygen in the air, we have probably about around 8 ppm under standard conditions in water, oxygen is a sparingly soluble gas, it is only about 8 ppm, 8 milli grams per litre is what you find here, when you have 21 percent oxygen in the air. This is true for any species that is partitioning between 2 phases and so on, from thermodynamics you would know this. This particular example or this particular system is what is of interest in bioreactors therefore, we look at in some detail right.

Oxygen is partitioning at equilibrium you have a certain relationship between the dissolved oxygen concentration in the liquid and the concentration of oxygen in the air given in different units and so on. Now, when it is saturated with air oxygen, you have a certain amount of oxygen in the liquid. If you change the gas phase composition, of course the equilibrium concentration in the liquid is going to change. If you replace air with 100 percent oxygen, 100 oxygen gas, the oxygen concentration at equilibrium in the liquid is going to be very different. It is going to be about 40 ppm compare to 8 ppm when you have air which contains only 21 percent oxygen, you need to keep this in mind.

When use air, when it is completely saturated, you say that it is 100 percent saturated,

and we typically work between 0 and 100 in normal cases. Whereas you should keep in mind that when the gas concentration is changed, you can go above 100 percent air saturation, you can go up to a maximum of about 480 percent. You know this is 21 percent, that is 100 percent oxygen, if it is completely oxygen, that is in the gas phase then you can go up to 480 percent air saturation. This is the bioreactor here again. The schematic of it the stirrer as we have seen earlier, the medium in which cells are growing, maybe the DO probe, pH probe, the air inlet and a temperature probe. Let us choose a conceptual system, our systems as you know could be either real or conceptual. In this case we are going to choose conceptual system, why we are choosing such a system would become little clearer later, most likely I will point that out.

The system that we are going to choose is this broth minus the bubbles, the air bubbles that are going to come out here. The air bubbles are the ones that carry oxygen and provide oxygen to the medium which the cells in turn take up. We are going to a take the broth as a whole which contains everything including the gas bubbles and then we are going to conceptually remove the gas bubbles from it and that is going to be our system. If we write a balance on oxygen for this system, this is the overall balance equation we always start here. It is very fundamental.

$$r_i - r_o + r_g - r_c = \frac{d(m)}{dt}$$

So, input rate of oxygen minus the output rate of oxygen plus the generation of oxygen minus the consumption of oxygen equals the accumulation of oxygen, all in terms of mass per time.

We have chosen this particular system broth minus bubbles, so that we could actually set the output rate to be equal to 0, it simplifies our approach significantly. So the output rate is 0. Imagine this - we have broth minus bubbles, so only the liquid part of the broth is our system. For oxygen to go out of the liquid broth you need to reach super saturated conditions. Assuming that we are not going to operate at super saturated conditions, we will say that for our purposes the output rate of oxygen from our system, which is broth minus bubbles, our system, the output rate of oxygen from our system is going to be 0, that is how we set this to be 0. There is of course in input rate which is from the gas bubbles to our system which is the liquid broth. Let us assume that there is no reaction that generates oxygen in the, in our system and for now let us keep the consumption and

also the mass of oxygen let us write it in terms of the concentration of oxygen which is what is usually measured, which is measurable. Therefore, mass is concentration into volume and if we could replace this, we could write it in terms of concentration and so on. Now if the rate of input is greater than the rate of consumption, if you can provide oxygen at a much faster rate than the rate at which it is being consumed by the cell, the accumulation rate is going to be greater than 0,

$$If\ r_i\ >\ r_C,\ \frac{dm}{dt} > 0,\ DO\ increases$$

it is easy to see here if $r_i\ >\ r_C$, therefore $r_i\ - r_C$ is positive, therefore $\frac{dm}{dt}$ is going to accumulate. If this is the case then the DO, the dissolved oxygen level will increase, right from simple from balance. Alternatively if the rate of input is less than the rate of consumption by the cells, then the accumulation is less than 0, so the DO will get depleted or the DO will decrease.

$$If\ r_i\ <\ r_C,\ \frac{dm}{dt} < 0,\ DO\ decreases$$

And if the rate of input equals the rate of consumption, then the accumulation rate is 0, the DO will be a constant.

$$\boldsymbol{If\ r_i =\ r_C,\ \frac{dm}{dt} = 0,\ DO\ is\ constant}$$

It is a steady state situation. DO will be a constant, we strive to achieve this condition while operating bioreactors.

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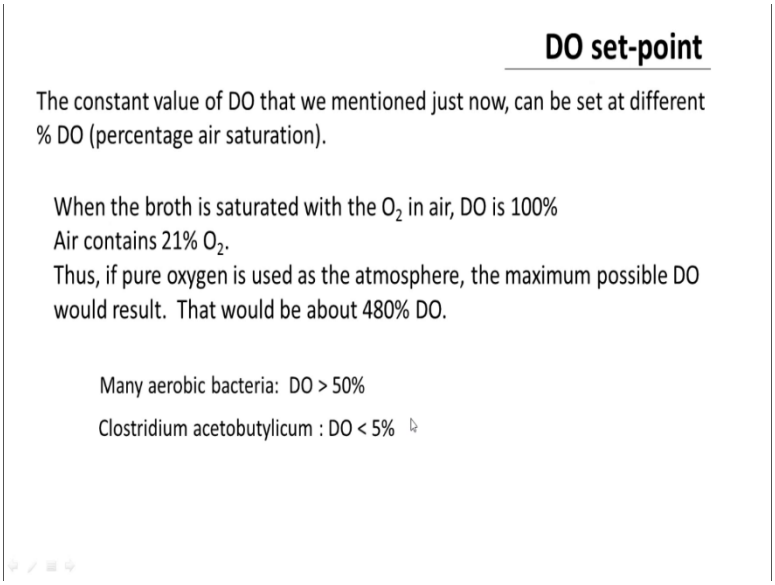

The DO set point, this is measured and it needs to be controlled. Let us see what the DO set point is? The constant value of DO that was mentioned just now can be set at different percentage, air saturation percentage DO. When the air, this is what we talked about in detail just a while ago let us a redo this just to get the point across. When the broth is saturated with the oxygen in air DO is 100 percentage, air contains 21 percentage oxygen. Thus, if pure oxygen is used as the atmosphere, the maximum possible DO would result that would be about 480 percentage. This is 21 percentage; this is 100 percentage oxygen. So, there would be corresponding change because from Henry's law p = hx, where p is the partial pressure of oxygen in the air phase and x is the appropriate concentration in the liquid phase, the mole fraction of the liquid phase, therefore, which is connected through the Henry's law constant h, and if we work that out, that is a linear relationship therefore, the 21 percent corresponding to 100, means that 100 percent would correspond to about 480 or so.

Now, why we are interested in this, see DO is nothing but the accumulated level of oxygen in the medium. The oxygen is being taken in by the cells, the oxygen is being supplied through aeration and so on, all those are fine. But the DO actually tells you the level of oxygen in the broth. What has been found is that many aerobic bacteria need a DO above 50 percent to do well, there are innumerable studies in the literature on the affect of DO on the growth and productivity of many different systems of interest, many different organisms of interest. Many aerobic bacteria have been found to prefer a

dissolved oxygen level of greater than 50 percent, that is why we are interested. But, some organisms such as *Clostridium acetobutylicum*, which is micro aerobic, requires low levels of oxygen and requires DO of less than 5 percent.

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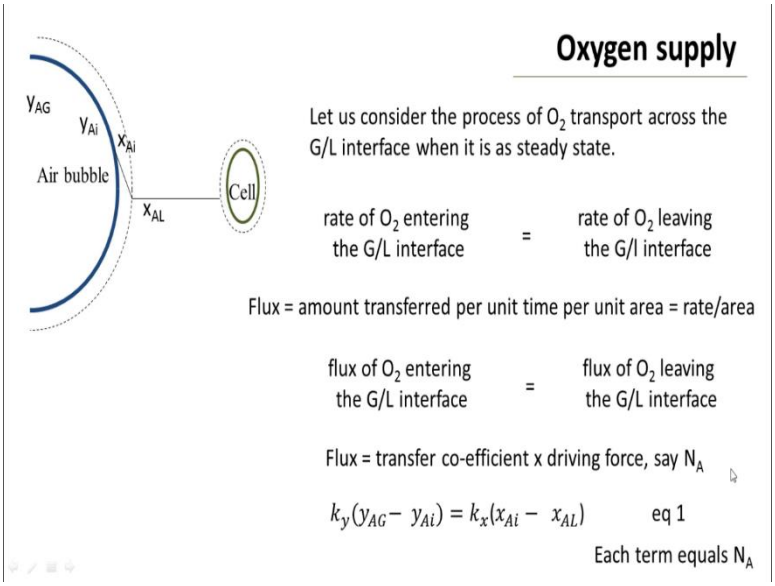

Now, let us look at the oxygen supply, before that let me emphasize it is because of this that we need to worry about the DO. DO directly affects the growth and productivity of organisms all though it is only a level of accumulation. Now let us look at the details of oxygen supply, I have artistically represented an air bubble by this hemisphere or a semicircle here, they are actually spheres approximately maybe I have just represented this by hemisphere, in 2 dimensions a semicircle. This is the air bubble and this is the cell, this is the liquid medium in which both are present our system actually.

What happens is oxygen from inside the air bubble moves to the liquid and then moves to the cell. In this process if we look microscopically at this process and if we look at the concentrations or the mole fractions for that matter, the mole fraction is going to be the highest inside the air bubble in terms of the gas mole fraction, then here at the interface this is gas concentration, this is liquid concentration it is going to be at equilibrium. People have found that it instantaneously achieves equilibrium at the interface and then all we concerned with for a while is the liquid phase mole fractions. So, x_{Ai} the liquid phase mole fraction is the equilibrium mole fraction with y_{Ai} in the gas phase.

Then there is a region very close to the air bubble, where the concentration of oxygen

decreases quite drastically. The mole fraction of oxygen decreases quite drastically in the liquid and this conceptual region is called the Gas liquid film. It actually does not, there is no film and so and so forth, it is a region, it is a, the film is the conceptual point. This is the region where the concentration decreases rapidly, and then in the liquid there is concentration or the mole fraction x_{AL}. And around the cell again because, there are 2 phases cell is some sort of a semi solid, semi liquid phase and this is the liquid phase therefore there is film around it where there will be significant drop in the concentration and the Cell C is the final concentration which it takes up for it its metabolic needs.

In fact, the only place where oxygen is required in the cell is as the final electron acceptor of the electrons released by various substrates in the oxidative phosphorylation pathway. That is the only place where it is required by aerobic organisms, but that is so crucial that it becomes essential. So, oxygen needs to be supplied to bioreactors to satisfy that particular need of the oxygen acceptor oh sorry, the electron acceptor. Let us consider the transport of oxygen across the gas liquid interface when it is at steady state. Let us say that the steady state has been reached and we are looking at the transport across this interface here right.

The rate of oxygen entering the gas liquid interface, must equal the rate of oxygen leaving the gas liquid interface for steady state to remain or for steady state to be achieved, so this is true at steady state.

i.e.

rate of oxygen entering the gas liquid interface = rate of oxygen leaving gas liquid interface

And now let us look at Flux,

Flux = amount transferred per unit time per unit area = rate/area

Flux is nothing but the amount transferred per unit area, per unit time. Or in other words it is rate per unit area, the area is perpendicular to the direction of transport. You would have seen a flux, mass flux, momentum flux and so on, in probably your transport course. We will deal with flux here, because it is much easier to see things here, this is an engineering meaning of flux. This slight difference in the way flux, the term flux is used

in some other context. There is one such context which comes in module 5; I will point that out when we see that. The rate is the same, the area is common therefore, the flux of oxygen entering the gas liquid interface must equal the flux of oxygen that is leaving the gas liquid interface.

i.e. flux of oxygen entering the gas liquid interface = flux of oxygen leaving the gas liquid interface

I have just divided this, these terms by area which also happens to be the same.

Flux can be seen as a transfer coefficient times driving force, this is the standard way of looking at flux it is seen as a product of a transfer coefficient times driving force. Driving force is the one that is necessary for the flux to occur. Let us say we call the flux as N_A in this case, we will develop it as A and then apply it to oxygen, this is a generic species A. The transfer coefficient is k_y, it is the mass transfer coefficient of oxygen. The transfer coefficient in the gas phase is k_y into the concentration difference that is the driving force here, that is this formulation, the transfer coefficient formulation where it is given as, where the flux is given as a product of the transfer coefficient and the driving force. The transfer coefficient k_y is here and the driving force is the difference in concentration or the difference in mole fractions $(y_{AG} - y_{Ai})$, this is the flux of oxygen reaching the interface. On the liquid side it is k_x, the transfer coefficient times the driving force $(x_{Ai} - x_{AL})$, the difference in mole fractions here. So, these two must be equal according to the condition for steady state. Let us call this equation 1 because, we are going to refer to this equation through this particular development.

$$k_y(y_{AG} - y_{Ai}) = k_x(x_{Ai} - x_{AL}) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

The flux needs to be the same therefore, each term equals N_A which we have defined as the flux.

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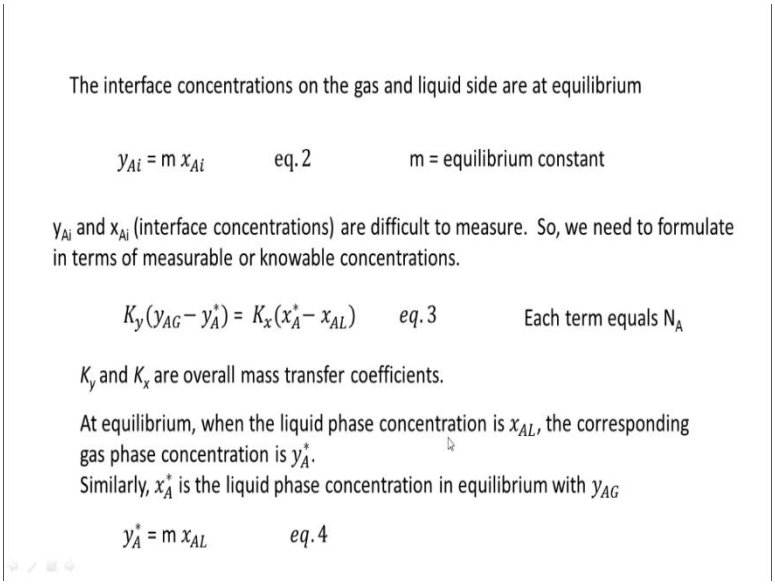

The interface concentrations on the gas and liquid side are at equilibrium, that is what we said earlier too, the $y_{Ai}$and x_{Ai} are at equilibrium. They are equilibrium concentrations and therefore, y_{Ai}can be written as some constant into x_{Ai}.

y_{Ai} = m x_{Ai} ……..(2)

From a larger perspective if you recall the equilibrium curves, it is typically y_{Ai} the mole fraction in one phase versus the mole fraction in the other phase this is usually a curve, so in certain regions the curve can be approximated to a straight line. That is essentially what this means, we are looking at piece wise linearity of the overall equilibrium curve and we are taking that to be a linear relationship between y_{Ai} and x_{Ai} in that small region, let us call this equation two, m is the equilibrium constant. This is usually the Henry's law constant. The interface concentrations $y_{Ai}$and x_{Ai} are usually difficult to measure. So, we need to formulate our equations in terms of measurable or knowable concentrations. This is very difficult to measure, some people have measured it through some specialized probes when they did studies and established that indeed equilibrium exists there, but we cannot do it on a regular basis.

So, if we write this in terms of the concentrations that can be measured, it is

$K_y(y_{AG} - y_A^*) = K_x(x_A^* - x_{AL})$……(3)

K_y and K_x are the overall mass transfer coefficients,

k_y and k_x are for the gas side alone and the liquid side alone respectively. Whereas if we are looking at transfer coefficient from here to here, we want to relate the transport, the flux with these concentrations as the driving force, the gas phase concentration and the liquid phase concentration. We cannot do that directly because this is gas phase, this is liquid phase. The gas phase composition is lets say, apples and the liquid phase composition is oranges, you cannot subtract apples from oranges or oranges from apples. So, we represent the concentration here, in terms of an equivalent gas phase concentration. The concentration in the liquid phase in terms of an equivalent gas phase concentration, and that is what y_A^* is all about. This is the concentration that is in equilibrium with the gas phase

At equilibrium with liquid phase concentration is x_{AL}, the corresponding gas phase concentration is y_A^*. So, we have replaced this individual side mass transfer coefficients with an overall mass transfer coefficient and measurable or knowable quantity such as the equilibrium value. Similarly, on the liquid side it is an overall mass transfer coefficient K x times $(x_A^* - x_{AL})$. x_A^* is the concentration that is in equilibrium with y_{AG}, the gas phase bulk concentration. y_A^* is the gas phase composition that is in equilibrium with the liquid phase bulk concentration. So, these are not actual quantities in terms of existing quantities, but these are conceptual quantities. They can be measured under conditions of equilibrium, but under those conditions under which transport is taking place these are conceptual quantities.

So, y_A^* is nothing, but m times x_{AL} in terms of the same equilibrium constant equation 4.

$y_A^* = m\, x_{AL}$ ……..(4)

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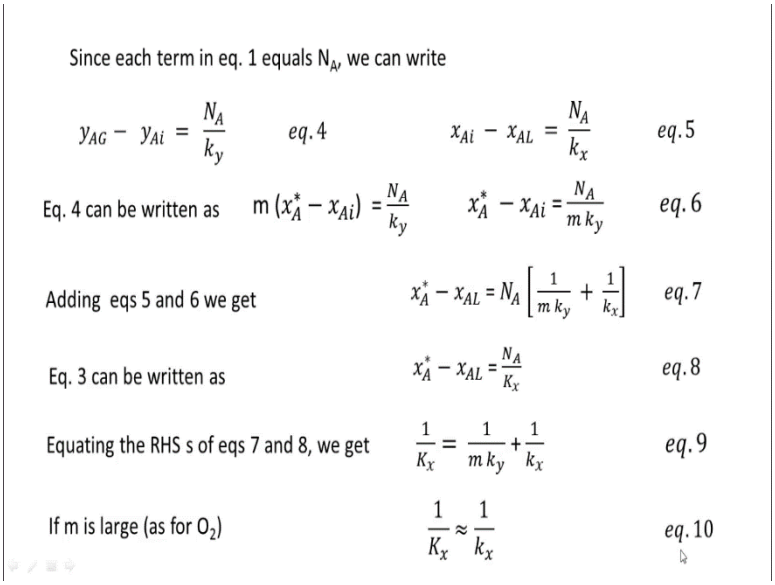

Since each term in (1) equals N_A, we can write,

$$y_{AG} - y_{Ai} = \frac{N_A}{k_y} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

$$x_{Ai} - x_{AL} = \frac{N_A}{k_x} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

Substituting, (5) can be written as

$m(x_A^* - x_{Ai}) = \frac{N_A}{k_y}$ and rearrange to get

$$x_A^* - x_{Ai} = \frac{N_A}{m k_y} \ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

Adding (6) and (7) we get,

$$x_A^* - x_{AL} = N_A \left[\frac{1}{m k_y} + \frac{1}{k_x}\right] \ldots\ldots\ldots\ldots\ldots\ldots\ldots (8)$$

(3) can be written as

$$x_A^* - x_{AL} = \frac{N_A}{K_x} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (9)$$

Equating the RHS s of (8) and (9), we get

$$\frac{1}{K_x} = \frac{1}{m\,k_y} + \frac{1}{k_x} \text{.....(10)}$$

If m is large (as for O_2), then

$$\frac{1}{K_x} \approx \frac{1}{k_x} \text{.....(11)}$$

For oxygen, the typical value of the Henry's law constant is 4.75 x 10^4, which is a large number. If m is large, as we can see, m is in the denominator we can conveniently ignore this, large compared to other values that is. But in any case if m is large, the denominator here is going to be large because, the other values are may be of the same order of magnitude and therefore, 1 by large value can be taken to be 0, and therefore we have equation 10.

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$$\text{Flux} = \frac{moles\ transferred}{area-time} = K_x(x_A^* - x_{AL})$$

$$\text{The mass transfer rate} = \frac{moles\ transferred}{time} = K_x A\,(x_A^* - x_{AL})$$

A = interfacial area

$$\frac{moles\ transferred}{volume-time} = K_x \frac{A}{V}(x_A^* - x_{AL}) = K_x a\,(x_A^* - x_{AL})$$

a = interfacial area per unit volume

$K_x a\ C_{O_2} = K_L a$ Volumetric oxygen transfer coefficient

$$r_i = K_L a\left(C_{O_2}{}^* - C_{O_2}\right) V$$

We saw that,

Flux = $\frac{moles\ transferred}{area*time} = K_x(x_A^* - x_{AL})$

The mass transfer rate = $\frac{moles\ transferred}{time} = K_x A\,(x_A^* - x_{AL})$, where A = interfacial area

$\frac{moles\ transferred}{volume*time} = K_x \frac{A}{V}\left(x_A^* - x_{AL}\right) = K_x a\,(x_A^* - x_{AL})$, where a = interfacial area per

unit volume

K_xa C_{O_2}=K_La = *Volumetric oxygen transfer coefficient*

$$\boldsymbol{r_i = K_L a\left(C_{O_2}^{*} - C_{O_2}\right) V}$$

The overall mass transfer coefficient on a mole fraction basis. This multiplied by the total oxygen concentration in the medium becomes gives the molar concentration basis. The molar concentration, the mass transfer coefficient of oxygen on a molar concentration basis on and also on a volumetric basis is K_La. So, it is called the Volumetric oxygen transfer coefficient or K_La. We did all this because we were writing a balance on oxygen, the oxygen is supplied from the air bubble into the liquid.

So, the rate of input we have derived to be $\boldsymbol{K_L a\left(C_{O_2}^{*} - C_{O_2}\right)}$**. W**e need to multiply it by the volume of our system to get on a mass basis. What we called as the DO is nothing, but $\boldsymbol{C_{O_2}}$. This is the concentration of the oxygen in the medium that is being measured at in any time, the accumulated amount of oxygen and that is our DO right. This is the relationship between concentration and DO.

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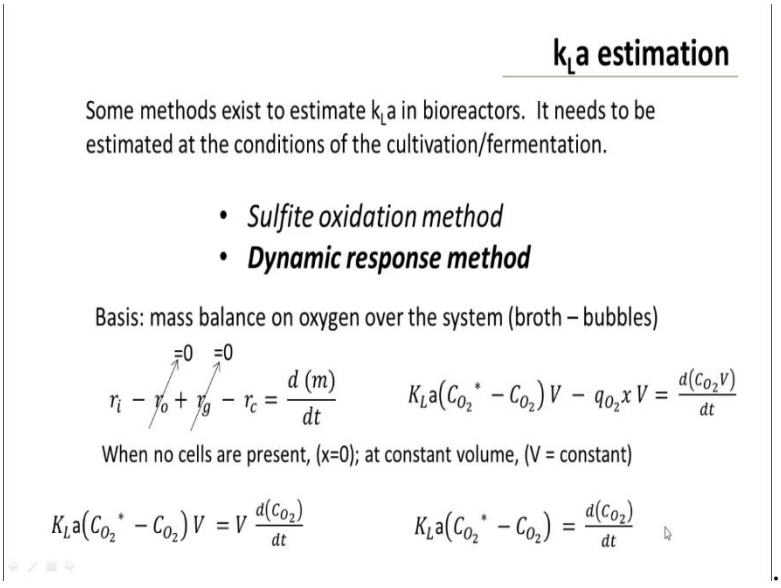

$\boldsymbol{K_L a}$ is an important parameter for the bioreactor and it needs to be estimated quite often, till we are sure that we have a good handle on the $\boldsymbol{K_L a}$. In fact, every time I used to run my bioreactor while I was doing my doctoral studies I used to estimate $\boldsymbol{K_L a}$ just before

running my bioreactor every time. So, in the industry you need to have a very good estimate of the K_La because that gives you direct measure of the oxygen transfer capacity of your bioreactor. There are some methods that exist to estimate K_La in bioreactors. It needs to be estimated at the conditions of cultivation and fermentation, because it is a function of so many different things temperature, pressure, composition, the flow rate, the agitation rate and so on so forth. So, many things affect the K_La therefore, what we do is? We fix all those and then finally estimate the K_La. There are 2 methods, the Sulfite oxidation method for K_La, and the Dynamic response method. The dynamic response method is one that is most preferred so this being an introductory course let us talk about this most common method the Dynamic response method.

For the background to this method let us again look at mass balance on oxygen over the system - broth minus bubbles. This is our mass balance here,

$$r_i - r_o + r_g - r_c = \frac{d\,(m)}{dt}$$

written on oxygen over the system broth minus bubbles. Output rate was 0 because of our, you know conveniently chosen system, broth minus bubbles. The generation rate is 0, there is no reaction that is generating oxygen in this case. The input rate we just saw was $K_L\mathrm{a}\left(C_{O_2}{}^{*} - C_{O_2}\right) V$ and if cells are present, then the consumption rate can be given as q_{O_2} times the cell concentration into the volume. $q_{O_2}$is the oxygen consumption rate per cell concentration, on a cell concentration basis this is the oxygen consumption rate therefore, you need to multiply it by the cell concentration to get mass per volume per time and further multiply it by the volume taken to get mass per time. The specific oxygen consumption rate is the name for q_{O_2}.

$$K_L\mathrm{a}\left(C_{O_2}{}^{*} - C_{O_2}\right) V - q_{O_2} x\, V = \frac{d\left(C_{O_2} V\right)}{dt}$$

When no cells are present, (x=0); at constant volume, (V = constant)

$$K_L\mathrm{a}\left(C_{O_2}{}^{*} - C_{O_2}\right) V = V\,\frac{d\left(C_{O_2}\right)}{dt}$$

$$K_L\mathrm{a}\left(C_{O_2}{}^{*} - C_{O_2}\right) = \frac{d\left(C_{O_2}\right)}{dt}$$

Solving this equation

$$\frac{d(C_{O_2})}{(C_{O_2}{}^{*} - C_{O_2})} = K_L a \ dt$$

From a table of integrals, we need the integral for the form $\frac{dx}{(a-x)}$

The solution is $\ln\left(\frac{C^*}{(C^*-C_{O_2})}\right) = k_L a \ t$

Thus, in a plot of $\ln\left(\frac{C^*}{(C^*-C_{O_2})}\right) \ vs \ t$, $k_L a$ is the slope.

This gives us the basis for the Dynamic response method. The dynamic response method is carried out in the absence of cells, the $k_L a$ is estimated in the absence of cells, in the actual broth that is okay, but there are no cells that are present.

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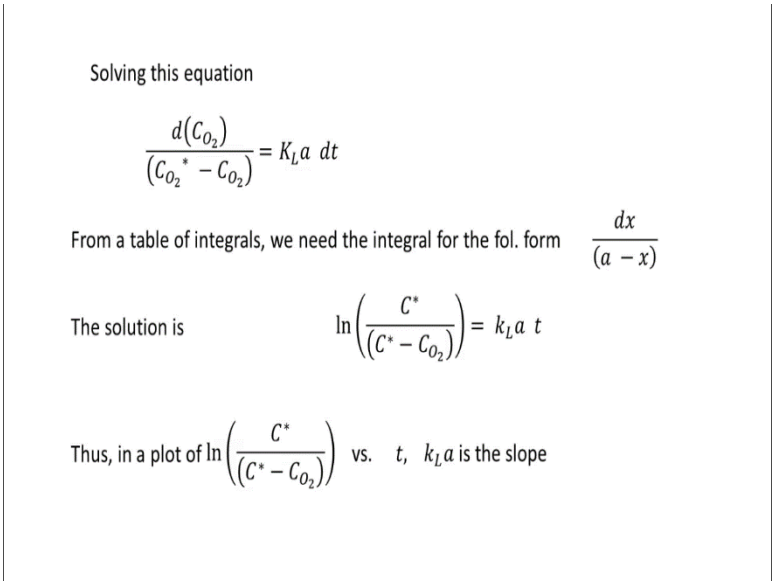

If we have data from an experiment that gives us the variation of the concentration of oxygen in the liquid verses time we will get $k_L a$ as the slope. That is what is done, that is the experiment that is performed to find $k_L a$ of the bioreactor.

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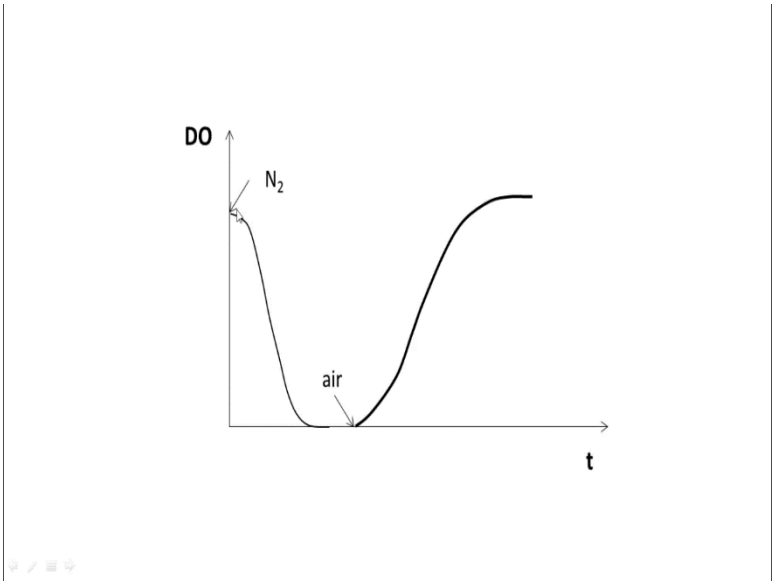

So, a typical experiment is something like this, we have a DO scale here, a time scale here, if initially of course the broth is going to contain oxygen because it is exposed to air, filtered air or sterilized air so we make sure there are no organisms. We first need to remove all the oxygen from the broth, to do that we use nitrogen, the bubble nitrogen through the broth. Nitrogen physically strips the broth of all the oxygen and if we follow the dissolved oxygen level with time after nitrogen is introduced, then it drops down to 0 after sometime. Make sure that it is at 0 definitely at 0, by seeing a 0 reading for a while and then if we introduce air it is going to be something like this. The dissolved oxygen is going to increase and reach the saturated value of dissolved oxygen. We are starting at slightly less than saturation here which is the usual case.

And from this we could a get the data for this kind of a plot: $\ln\left(\frac{C^*}{(C^*-C_{O_2})}\right)\ vs\ t$,

If we look at this, this assumes that the $\ln\left(\frac{C^*}{(C^*-C_{O_2})}\right)$ is a straight line, so this is valid only in the region where this graph is a straight line. So we choose the region where this graph is a straight line and then take this slope of it, that is how we find $k_L a$.

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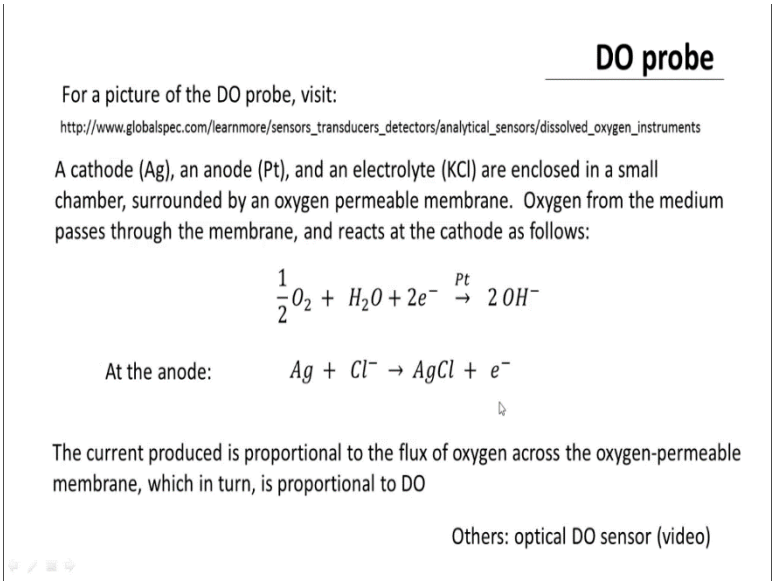

The DO probe, the probe that is used to measure the dissolved oxygen level. For a picture, you could visit the site mentioned in the video. It is an electrochemical probe therefore; you have a cathode which is platinum, an anode which is silver.

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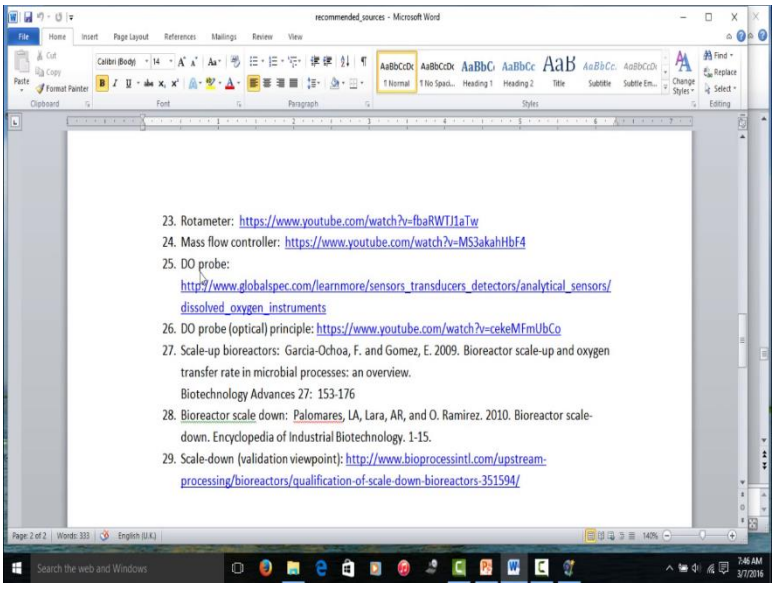

So, in an electro chemical probe so you need a cathode and an anode and an electrolyte. The electrolyte happens to be potassium chloride KCl. These are all enclosed in a small chamber surrounded by an oxygen permeable membrane, you have a cathode you have an anode in some form and then you have the electrolyte and an oxygen permeable

membrane, and this is dipped into the medium. Oxygen from the medium passes through the membrane and reacts at the cathode as follows, oxygen giving you hydroxyl ions in the presence of platinum which is the cathode,

$$\frac{1}{2}O_2 + H_2O + 2e^- \xrightarrow{Pt} 2\,OH^-$$

$$Ag + Cl^- \rightarrow AgCl + e^-$$

At the Anode you have a silver. Above are the 2 reactions that take place at the electrodes. When there is electrons being transferred at the electrodes, if you connect the electrodes with a wire you would see a current. The current produced is proportional to the flux of oxygen across the oxygen permeable membrane, which in turn is proportional to the dissolved oxygen that is the principle here. And then this is the principle here of the electrochemical probe. There are other optical probes too, I have shown you a link to this optical DO sensor which is a video, you can look at the video to find that out.

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Practice problem 4.1.

The following data was obtained during k_La determination of a stirred tank bioreactor operating at 500 rpm, 1 atm, and 37 °C by the dynamic response method. The oxygen source was air. A millivolt meter was used to read the dissolved oxygen level. Find the k_La of the bioreactor.

t, s	0	40	51	56	62	67	72	78	88	135	220
DO, mV	0.00	0.01	0.16	0.32	0.51	0.70	0.84	1.00	1.10	1.10	1.10

Let us finish this lecture with this practice problem 4.1, we will solve this problem in the next lecture. This is a problem for practicing the measurement of the K L a. 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 source of oxygen was air. A millivolt meter was used to read the dissolved oxygen level. Find the K L a of the bioreactor given this data. This is the typical data that

one gets, I think this is from my own experiment with the bioreactor during my doctoral days. Time verses DO, time in seconds DO in millivolts you have these various values, this is the data that is given you are asked to find the K L a. Please go ahead and do this. We will meet in the next lecture and we will solve this problem.