Transport Phenomena in Biological Systems
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Lecture-67
Liquid Phase Oxygen-Supply Strategy

Welcome, in the previous class or in a few of the previous classes we saw the transfer coefficient approach for the mass transfer of especially oxygen from the gas phase to the liquid phase. And we also looked at how to measure K_La in the bioreactors at the formulation part is for K_La, the volumetric mass transfer coefficient of oxygen in the bioreactor. And we also saw a problem has to how to measure the scaling ok, let us take things further in this class.

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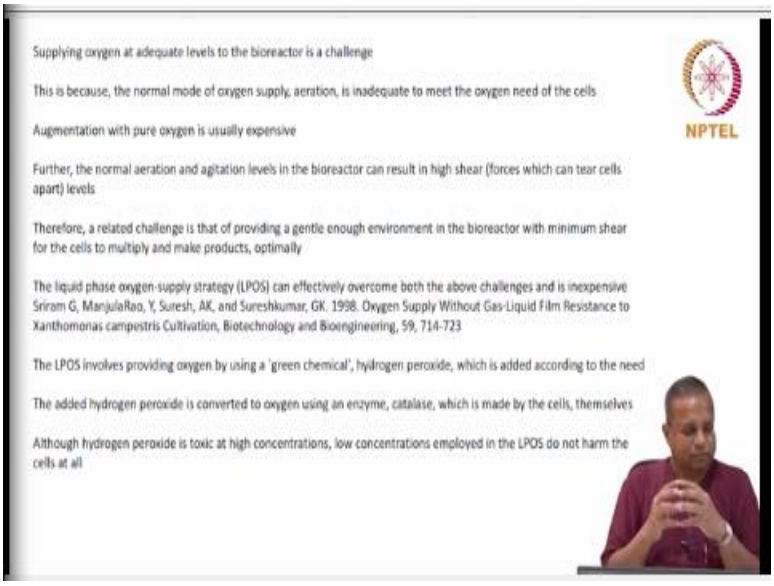

Supplying oxygen at adequate levels to the bioreactor is always a challenge ok, the bacterial or the aerobic microorganisms gobble up oxygen ok. And to supply at adequate rates is always a challenge, this is because a normal mode of oxygen supply which is aeration is inadequate to meet the oxygen need of the cells ok, that is the reason here. And augmentation with pure oxygen is usually expensive you cannot use oxygen cylinder, oxygen cylinders are expensive.

Further the normal aeration and agitation levels in the bioreactor can result in high shear right, if you increase the aeration rate, the bubbles are going to come out at much higher rates, they are going to break it much higher rates. That breakage would release a lot of energy which can shear

the cells. So, the shear we all know are the forces that can tear the cells apart. And therefore a related challenge is that of providing a gentle enough environment in the bioreactor with minimum shear for the cells to multiply and make products optimally.

The liquid phase oxygen supply strategy LPOS ok liquid phase oxygen supply strategy. LPOS can effectively overcome both the above challenges and is an expensive, this we have shown. In fact this is something that we generalized and we have shown, this was published as a paper in 1998 Sriram, ManjulRao, AK Suresh and myself I was a corresponding author here oxygen supply without gas liquid film resistance to Xanthomonas Campestris cultivation.

This was published in biotechnology and bioengineering which happens to be the most prestigious journal in our field of Biological Engineering in 1998, the details are given here, ok. And in this lecture as well as in probably the next lecture, we are going to see some of our research work in which we have applied the transport principles very effective ok. So, the principles can be applied anywhere in this case to cutting edge research.

The liquid phase oxygen supply method provides oxygen using a green chemical hydrogen peroxide which is added according to need ok, that is the way we can go about doing this. The added hydrogen peroxide is converted to oxygen using an enzyme catalyst which is made by the cells themselves. And although hydrogen peroxide is toxic at high concentrations, low concentrations employed in the LPOS do not have the sense at all, ok.

This is the LPOS method based on hydrogen peroxide to provide oxygen, this is something like this ok, where are the papers, ok I will come to the paper or before I get to the papers let me tell you this. We have I just lost my train of thought here, we have I was going to tell you the idea, right. See we have the oxygen or the air bubble from which oxygen gets applied to the liquid, right.

That is the case of aeration, the standard way by which oxygen is supplied. So, here if you have all this, you need to go from the gas phase to the liquid phase. In other words you are overcoming a certain resistance, we can view it that way, you are overcoming a certain resistance for the

transfer of oxygen from the gas phase to the liquid phase. And if you look at it closely, that resistance is the biggest resistance for transfer of oxygen in the situation, ok.

So, our thinking was can we get rid of this biggest resistance to oxygen transfer from the gas phase to the liquid phase. If we can get rid of this resistance then the transfer would be that much simpler, because this was a classic challenge a long time ago, I think it is still a classic challenge. So, the thinking was something like this, as long as there are 2 phases, as long as there is a separate gas phase and there is a separate liquid phase.

There will certainly be a gas film liquid are gas liquid film resistance and if we can get rid of the gas liquid film then we can overcome this resistance. And the only way to get rid of gas liquid film is not to have 2 phases have a single phase alone ok that was the thing. Or in other words we were going to generate oxygen in the liquid phase itself as a part of a liquid phase reaction, thereby the oxygen species would be in the liquid phase itself and that would supply oxygen to the cells ok.

Note this concept of or this notion of oxygen being a gas, that is fine it is a gas under normal temperature and pressure. But it is a species it is a molecule it can be in the gas phase, it can be in the liquid phase and so on depending on the conditions or even the solid phase depending on the conditions. In this case normally when it is present in air it is present as a gas phase, when it gets into that liquid it is dissolved oxygen, it is in the liquid phase and that is what the cells take up.

Therefore the idea was we can directly provide it in the liquid phase then the cells can take up much, or the product the supply rates can be high enough and so on, that was the idea that we tried out, ok.

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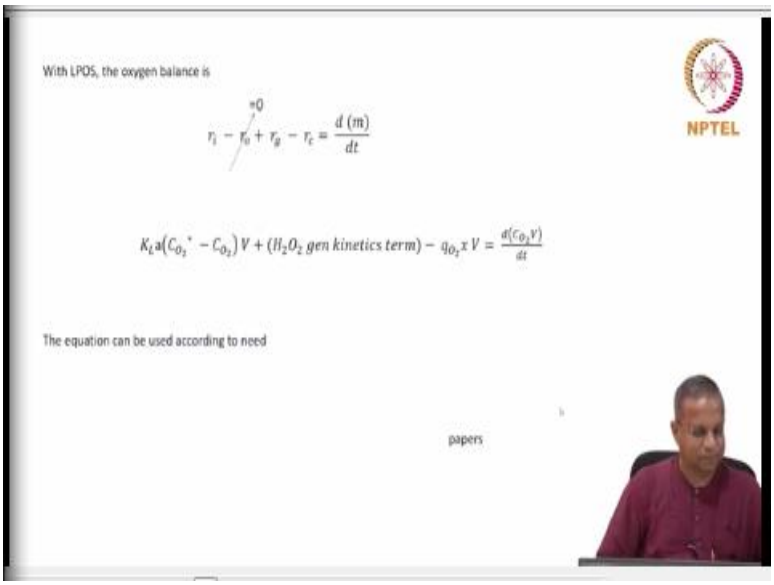

With LPOS, if we write the oxygen balance it all comes down to the basic oxygen balance now, the mass balance. Input rate minus output rate plus generation rate minus consumption rate equals the accumulation rate. The output rate is 0 because we are looking at broth minus bubbles. The generation rate is there in this case because, we generating oxygen using hydrogen peroxide. The input rate is $K_L a(C_{O2}^* - C_{O2})V$.

So, volume is needed to convert this into moles per time. This is from a hydrogen peroxide generation kinetics, we will have to put in the appropriate term here. This is the uptake rate in terms of oxygen uptake rate, we need to multiply this is given usually in units of the form that we need multiply it by the cell concentration and the volume to get moles per time equals the accumulation rate.

So, this is the mass balance and this equation can be used according to need when you do not have any sense this goes to 0, you could use you know then this becomes irrelevant, therefore these 2 terms need to be taken together. The catalyst that is necessary for generation of oxygen from hydrogen peroxide actually comes from the cells, therefore these 2 terms usually go together.

I thought I will present to you some papers of us which initially the first paper would talk about this liquid phase oxygen supply strategy and then later papers will talk of other things, all related to transport as applied to cutting edge biological engineering aspects, so let us do that. And then

this is what I have here again, let us start doing that, yeah. **(Video Starts: 09:28)** So, this is the I think this is big enough for us to look at, is not it.

So, this paper published in biotech bioeng oxygen supplied without gas liquid film resistance to xanthomonas campestris cultivation, G. Sriram, Y. ManjulaRao, AK Suresh and myself, I used to spell my name already definitely there. So, 1998 change it around 2001, 2002, so after that it is Suraishkumar, ok. Let me read out the abstract to you and then explain a few things here, ok. Alternative methods of oxygen supply are of crucial importance, remember this is way back in 1988, 22 years.

Alternative methods of oxygen supply are of crucial importance, especially in viscous fermentations and shear sensitive fermentations. A method of oxygen supply that completely eliminates the gas liquid transport resistance has been presented. The method involves a need based liquid phase decomposition of hydrogen peroxide to provide the necessary oxygen. When xanthomonas campestris was cultivated, this is a viscous for cultivation using this method of oxygen supply.

Dissolved oxygen or DO levels were maintained above the set point of 50% throughout the cultivation. Whereas the conventional cultivation was able to meet the culture oxygen demand only for about 6 hours in a 72 hour fermentation. Furthermore, the maximum specific growth rate and Xanthan yields in the novel cultivation were 89% and 169% respectively of those obtained and convention cultivation.

A mathematical model was also developed to simulate and predict results and fermentations employing the present in methodology. In addition studies with HOCL pretreatments indicated that mono functional catalyst maybe responsible for the decomposition of hydrogen peroxide. These are fundamental details if you want you could read those and we also could back out some constants which are fundamental and so on, ok.

I will probably read some parts of the introduction to present the base and then we will talk about what we did, ok. The performance of aerobic bioreactors especially on large scale may be

suboptimal due to inadequate oxygen supply rates. Insufficient oxygen supply could lead to suboptimal productivities as well as products of low quality, all these are well known. Inadequate oxygen supply results due to low solubility of oxygen in the medium 8 ppm at equilibrium.

And the large gas liquid film resistance for oxygen transport when aeration is used, ok. For people who do not know about the film, if you look at the concentrations of oxygen near the interface just beyond the interface into the liquid side. Assuming that it goes from the gas phase to the liquid phase, just beyond the interface on the liquid side, there is going to be a huge drop in concentration, ok then the drop is going to be much, much shallower.

So, the region where the oxygen concentration drops is the conceptual film nothing else ok. It is a conceptual film, there is actually no physical film there, it is a region where there is a huge drop in the oxygen concentration. Researchers have tried to eliminate the gas liquid oxygen transport to meet the oxygen requirement of the culture through several means, some of the means are given here.

The methods described here have attempted to improve the oxygen transport rates but still retain the basic limitation of gas liquid oxygen transport, it is always from the gas phase to the liquid phase. Hence they have been only partially successful or partly successful and may not meet the requirement of large scale bioreactors and thus may make the systems partly anaerobic. This could lead to formation of undesirable products in the system which could question the economic viability of the process itself.

Further, if either the fermentation is viscous or the microorganisms are shear sensitive. Such methods of enhancing oxygen availability may be ineffective, ok because the productivity will go down. Therefore if oxygen transport can be achieved with complete elimination of the gas liquid transport, it would greatly improve the economics for the process. The methodology demonstrated in this article to overcome gas liquid transport resistance is through a liquid phase conversion of hydrogen peroxide to oxygen and water.

This reaction is catalyzed by the enzyme catalyst available from the culture itself, no external catalyst is needed. In this case the oxygen molecule being in the liquid phase is ready for consumption by cells and hence gas liquid oxygen transport is nonexistent. Although hydrogen peroxide is used to kill cells we all know, you know 4.5% is used for a mouthwash and so on. The concentrations required for killing cells or in the percent solution range.

Whereas the methodology presented here employs hydrogen peroxide on a need basis at concentrations at least 150 times lower. Therefore cell death may not be a concern and we have also demonstrated that cell death is not a concern. The particular reaction for oxygen evolution was chosen because it occurs naturally. Hydrogen peroxide is generated in the cell either directly by the divalent reduction of oxygen or indirectly by the dismutation of superoxide radicals.

The generated hydrogen peroxide is scavenge through catalyst, also most aerobes contain catalyst, which is used as a defense against hydrogen peroxide generated in this cell, ok. So, we are using something that is already present in the cell to take care of this. Thus for hydrogen peroxide has not been used as an oxygen source of bacterial cultivation and so on so forth. And the scope of the paper is given here followed by some materials and methods and then some modeling details, ok.

Let us look at some results and discussion here, let us probably ok. This is some basic characterization DO was sustain when we pulsed it with experimental and simulation values of DO variations xanthomonas campestris during aeration, ok, that is the varies will not get into that. And then feasibility demonstration of oxygen supply using hydrogen peroxide, ok, what we did was let me go to the graph here.

We took the medium with cells standard bioreactor broth and also did an experiment where we considered a medium without cells, ok. Our thinking was that somehow the cells would sense hydrogen peroxide, put out catalyst and then break things down, that completely changed as I tell you the next class. Here with that idea we went into this experiment, when we added a 200 micro molar pulse of hydrogen peroxide to the medium with cells.

The dissolved oxygen level went up as expected, as soon as we added the pulse the DO level went up, ok. This very clearly told us that the oxygen is becoming available in the broth ok, we thought oxygen is becoming available immediately in the broth it is the cells can take up, that was different later. Then when we did it with the medium without the cells of course there is no availability of oxygen in the broth, no oxygen became available in the broth, no increase in DO took place.

Or this mild increase in DO is due to surface aeration, ok, that over a 60 minute period that we can explain through surface aeration alone, ok. So, this was feasibility demonstration for the availability of oxygen from hydrogen peroxide decomposition by cells, the system. Then we did the cultivation with hydrogen peroxide pulses, ok, graduate students sat and did this experiment over a long period of time or a few graduate students did this.

This prediction will not get into that, here we are looking at dissolved oxygen versus time for xanthomonas campestris cultivation. In the conventional aeration case the conventional method of providing oxygen supply aeration. We started at 100% by the way this is percentage air saturation, in air you have 21% oxygen. So, if you reach 21% oxygen in air you have 100% air saturation, ok.

So, in theory you can go up to 480% air saturation alright because if you go to 100% oxygen it will be almost 5 fold, 21, you are talking 21 and 100. So, almost 544.89 fold of the air saturation value and therefore, it can go from 0% DO to 480% DO because this percent air saturation. So, here we are looking at if you start out with 100 for xanthomonas campestris cultivation done with air, the DO drops to 0 and remains at 0 pretty much slow out, let us not get into this region.

Whereas if you use pulses of hydrogen peroxide, you add a pulse the DO goes up and then it comes down, when it comes down because the 0 you add another pulse then it goes up comes down, something like this. You add DO was sustained, you add a pulse it goes up comes down, when it comes down here you again at a pulse it goes up comes down and so on and so forth. So, by continuously measuring the DO and adding the pulse when the DO is close to 0.

Kept the DO value above the 50% DO level which we set for this case, ok. So, here we found that in the case of conventional cultivation, the growth rate which is important because growth rate will

determine the cell concentration, higher the cell concentration, higher the productivity and therefore this is important, ok. So, the growth rate is comparable between the 2, so this method of cultivation is not affecting the growth rate significantly, 0.111 h^{-1}, 0.098 h^{-1}.

Then the yield of per unit substrate, the product concentration divided by the substrate concentration 0.165, 0.287 it is higher in the case of hydrogen peroxide base cultivation. Yield per unit biomass P by X is almost 9 fold ok. Huge each cell is producing 9 fold higher the xanthomonas campestris, ok. And the final xanthomonas concentration also is much higher than expected.

So, using this cultivation has significantly improved the xanthan yield from this. We thought it was due to a better supply of oxygen at or a better supply rate of oxygen in that situation. Also this viscous fermentation it is difficult normally speaking to supply oxygen to this fermentation. So, this method can become very useful in supplying oxygen to both challenging fermentations as well as regular fermentations.

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We also worked out the costs that are involved and if you consider everything, the cost per kilogram of oxygen turned out to be 15 paisa, compared to about 2 rupees per kilogram when you consider aeration as a source by although air is free. You need to go through appropriate filtration, appropriate sterilization and so on so forth. And that is expensive, ok and of course the compressor costs are there, you know compressor costs here, so many other parameters on which we did the comparison, it was 15 paisa per kilogram of oxygen, that was 2 rupees per kilogram of oxygen.

I think we will stop here for this class, when we come back let me present a few more applications of transport in our work ok. So, that you can appreciate the width of application and even to cutting edge aspects, ok, see you in the next class, bye.