Transport Phenomena in Biological Systems
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Lecture - 68
LPOS and Its Mechanism

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Welcome we are continuing from where we left off in the previous lecture. We are looking at some of the applications of transport phenomena transport processes and biological systems even to cutting edge research. We have looked at a lot of applications of industry related aspects and so on so forth. The right through the course, in fact they were used even introduce material and so on and so forth.

So in a sense the tutorial aspect of it, the practice aspect of it was integrated with the learning particles not separated out into just information and then practice. The practice was a part of the initial exposure itself that is the way the course is designed. However I feel that it was good to know some of the applications it will give you the scope, the wide scope of the applicability of it. I did start in the previous class with an application that we did application of transport phenomena to research.

That was made about 26 years ago; 25, 26 years ago in fact one of the first proposal that got funded

for, when I began my career as a faculty member in IIT Bombay was based on this and the paper that came out of it was what was discussed. I thought I will start there, give you a gist of what we talked about in the previous class and then look at other applications.

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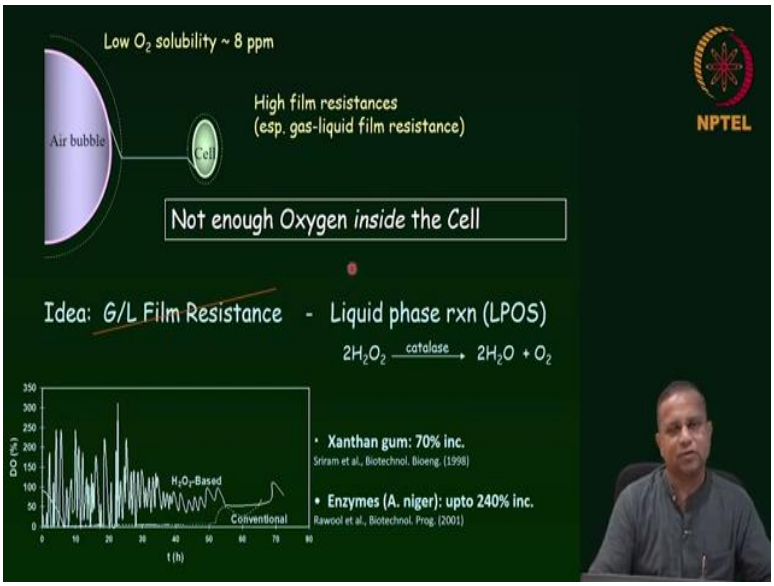

What we looked at was the transport of oxygen from an air bubble to a cell. The air bubble contains the gas, so it is gas phase. Cell is some sort of a semi liquids and is solid so it can be considered as a separate phase and the oxygen from the air bubble traverses to these cells through various resistances, there is transport of oxygen, so transport phenomena and we also said that if you plot the concentration of oxygen, on let us say a vertical axis corresponding to this distance on the horizontal axis.

Immediately after the gas liquid interface there is a region where the oxygen concentration drops significantly and then it kind of flattens out and then there is another region here. We are going to focus or we did focus on this region which we said was the conceptual gas liquid film. There is no physical film there it is just the region where the concentration decreases. So this film can be viewed as posing a resistance to the transport of oxygen from the gas phase to the liquid phase and ultimately to the cell.

So this a way or for the analysis, but this analysis this is the way of looking at the transport of oxygen and there are two fundamental limitations in this process and that is the reason why oxygen

supply at relevant rates to the bioreactor is a big challenge was a big challenge, it is still a big challenge to accept various things. So the high film resistances or you know here as well as here this happens to be the biggest resistance when you consider single cells and so on so forth.

The gas liquid film resistance, thereby you cannot supply it at rates that are necessary for these cellular culture as well as you cannot store oxygen. The solubility of oxygen is low it is about 8 ppm rule of thumb at typical conditions and therefore we are limited from a thermodynamic angle as well as a kinetic angle. As a result there is not enough oxygen inside the cell and our thinking was if we can get rid of the gas liquid filled resistance, then we completely obviate the difficulty that is posed by the film.

And as long as there is a gas and a liquid, as long as there are two phases it is physical reality that there will be a conceptual cell, so the only way to get rid of the gas liquid filing resistance is by not having the gas layer or at all because the cells are in the liquid phase. Gas phase at all and use a liquid phase reaction to generate oxygen and that is what we called as the liquid phase oxygen supply strategy.

The liquid phase reaction was the catalase decomposition of hydrogen peroxide added hydrogen peroxide to water and oxygen, these are the clean green products that arise as a result of this catalytic decomposition, and all aerobes are known to have catalysts. So that is the reason why we went into this and generalize the strategy. Then I told you about the performance here percentage air saturation of DO versus time when, we looked at a Xanthan Gum cultivation the conventional cultivation took the DO to 0 in about 6 and a half hours.

Whereas the LPOS maintained DO above the set point of 50% throughout the fermentation and that resulted in better yields. There were 70% increase in the volumetric productivity of Xanthan Gum, not just that we have applied this strategy to very many different cultivations. For example, we have shown up to 2.4 fold increase in highly commercial enzymes. From mold source A niger, Aspergillus niger.

And so and so, we have done, we have tested this with many different aerobic systems and it is

worked well. Microbial systems it is worked very well. So this I think is where we stopped last time, this is just a summary of what we saw all last time. It is nice to begin with the summary, it provides a certain continuity to the story, that is the reason why I began with this. Now, let me get to the next aspect of it which is rather interesting the initial part of transport.

There is some transport in the mechanistic aspects also I let you figure out what the transport aspects are but the mechanism was completely surprising to us. Whatever we started out, whatever assumptions we started completely went out of the window when we started investigating the mechanism by which the oxygen becomes available. So, let me present that next in this class. Let us take one by one slowly so that we have enough time to internalize and so on so forth.

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So let us discuss this paper today mechanism of oxygen availability from hydrogen peroxide to aerobic cultures as Xanthomonas campestris. This is published again in biotechnology by engineering highly reputed the most reputed journal in our field of biological engineering and do not worry about the abstract this time it is highly focused. So I let me read some parts of the introduction that will give you an idea.

And then I will tell you how this is indeed happening in kind of summarize it with a couple of slides at the end. I thought that would be a nice way of understanding this. Let me read oxygen is a necessary nutrient in aerobic bioreactors and insufficient oxygen supply is associated with low productivities is known and products of low quality. Efficient oxygen supply is difficult due to low oxygen solubility and high gas liquid mass transfer resistance.

Earlier we had proposed and demonstrated a methodology of oxygen supply to Xanthomonas campestris cultivation, which is viscous as well as a viscous and aerobic of course, which overcomes the gas liquid mass transfer resistance for oxygen supply. It uses the liquid phase decomposition by culture catalase ubiquitous and aerobic cells of periodically fed hydrogen peroxide.

The objective of the present work, this paper is to understand the mechanism of oxygen availability due to hydrogen peroxide decomposition by Xanthomonas campestris and to develop a kinetic

model for the same, not very too much about the kinetic model in this discussion. But I will talk to you about the previous one. An understanding of the mechanism of oxygen availability will contribute toward the optimization of the hydrogen peroxide-based oxygen supply methodology to cultivations of Xanthomonas campestris and other aerobic organisms.

Further the mechanism will also dictate the interpretation of DO values and oxygen uptake rates as clarified later. This is highly interesting we just wait for it towards the end of this lecture. Also studies on the kinetics of hydrogen peroxide decomposition by living cells in the range of concentrations employed has not been reported at the literature and so on so forth. In this paper we demonstrate that the decomposition of hydrogen peroxide by the cell is intracellular.

That is the key here, earlier we thought that we were adding hydrogen peroxide the cells are catalase and our visual thinking was there the cells will somehow sense catalase by we do not know how or we did not know how? We still do not know how it will somehow sense the hydrogen peroxide outside then it will somehow put out catalase. The catalase will break out in peroxide down and take in the oxygen.

Remember this picture was completely consistent with the data that we were getting. Only that picture turned out to be entirely wrong the data is still fine it can very well be explained or by whatever I am going to tell you now. The picture was completely wrong, it did not matter there, however, if you need to take things further, it will be in it will become important and there are a lot of transport aspects here you need to pay attention.

Look at how we are applying transport to cellular aspects at a fundamental level to be able to reasonably fundamental level to be able to generalize various aspects, that is the appreciation I would like you to have, is intracellular and also did use that within the cell the decomposition occurs most probably in the periplasmic space. In addition, it is shown that the entry of hydrogen peroxide flux into the cell comes here comes flux into the cell is controlled by the cell.

A mathematical model based on the postulate that the regulation of hydrogen peroxide flux into the cell is controlled by proton motor force predicts, the experimental data accurately. We will not

talk much about this module in this lecture. Further we have experimentally confirmed that the regulation of H_2O_2 flux into the cell is coupled to the proton motor force. All this has been shown however, let me just point out a few things here.

This type of experiments, the location of catalasic action. The discussion would be little beyond the scope of this course and therefore, let me not get into discussion. In other words, I will have give you a lot of background to get you up to speed here. I let you people are interested can go in read this paper. Let me see which aspects and information on the location, whether it is interesting or extracellular of the decomposition of hydrogen peroxide added externally to the cell suspension has not been reported of course.

This is way back in 2000 something, this information is particularly significant to the development of a model for the decomposition of external hydrogen peroxide by the cells. Further the interpretation of DO values will depend on the decomposition location. Then we discuss something about what comes from the literature data, then we discussed experiment very carefully thought out experiment.

And what we have plotted here are the oxygen evolution range versus time and we also looked at NADH fluorescence, which is called culture fluorescence. Let me read the headings to you that might be helpful. This was the location of catalasic action was one. Oxygen Efflux from periplasm to extracellular space when hydrogen peroxide is used as an oxygen source. So there is something about hydrogen peroxide coming in getting broken down here and then going out.

We have shown all those things, we have proved all those things to happen. What would be under the scope of this, oxygen uptake rates when hydrogen peroxide is used as an oxygen source? This is where we get into rates and so on. Then the model which I said, I will not get into in this particular lecture. And the coupling to the delta G and so on so forth the flux values are also given there.

See here the flux coming in and then experimental verification of the role of the proton motor force in hydrogen peroxide flux control and so on so forth. I think little beyond these scope of this

particular course. So, let me summarize whatever I said in easy terms. If I get into this it is a lot of detail yes, it is complete it gives you. I mean the questions that will arise in your mind as I give you a just will get answered by this.

So people who have such questions are directed to this paper first and if you do not understand it here, you can always raise queries, raise comments on in add comments onto the forum uncertainty answer there, discussion forum that is.

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Let us get back to our presentations here, which will summarize whatever I have just mentioned.

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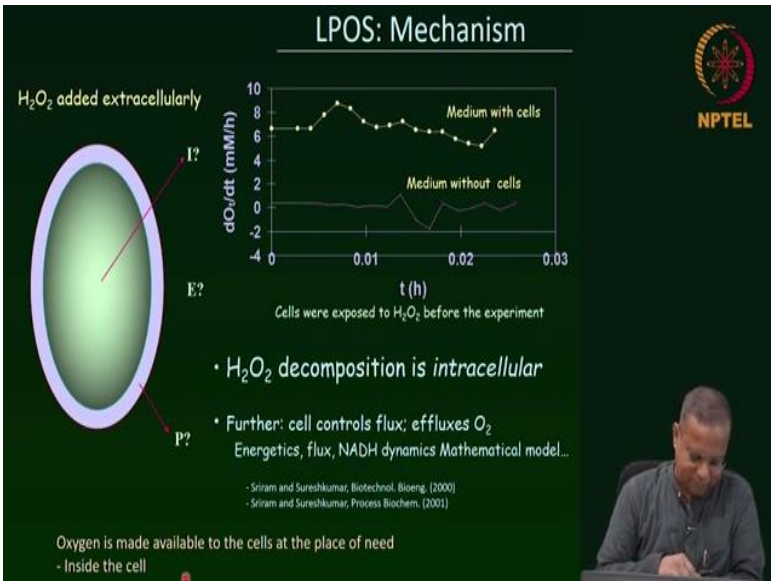

This paper addresses the mechanism of the liquid phase oxygen, supply strategy. So the basic question was this, the hydrogen peroxide is added extracellular in the extracellular space. This is an idealized representation of a cell some oval here. This is the extracellular space given as E this is the intracellular space given as I this is the very periplasmic space you all know this we are all biological people we have done enough biology to know that this exists.

What led me to start investigating this is the realization as usual I mean came when I was taking a shower one of those days at that time is that hydrogen peroxide is a small uncharged molecule. So if you are adding hydrogen peroxide here, there is nothing that prevents it from getting into the cell small uncharged. It is going to go into the cell through the membrane at reasonable rates.

And if catalase is localized in the cell. Why cannot the decomposition itself be happening inside the cell or something that is struck in and that is the reason why we started investigating this. And the question was whether the hydrogen peroxide added extracellularly is broken down in the interstellar space or in the extracellular space or in the intercellular space or more specifically in the periplasmic space.

So we did experiments with medium that contains cells, with medium that does not contain cells and look at the huge difference in oxygen evolution rates and of course the cells are exposed to hydrogen peroxide before the experiment and so on so. I am not getting into the experiment but this was necessary for a certain base comparison here. So there is a huge difference in the oxygen evolution rates.

And then further discussion NADH fluorescence investigations and so on so forth. Let us to the conclusion that hydrogen peroxide decomposition as predominantly intercellular. That was what came out with cell controls flux a flux the oxygen is coming out energetic studies, studies on flux. NADH dynamics mathematical model and so on and so forth is a published actually a couple of papers.

And the pictures is something like this; oxygen enters the cell. It gets broken down most likely in the periplasmic space whose Xanthomonas campestris, we did investigations only with that. This cell takes and whatever oxygen requires and by some mechanism it pushes out there is and the DO increases due to the oxygen that is coming out of the cell in excess of whatever has been consumed by the cell.

So the need of oxygen by the cell has completely met and whatever is not needed is actually what we are measuring as DO. So the interpretation of DO also needs to change in this case. Earlier the oxygen was provided in the liquid and then I mean the gas then liquid and then it went into the cell and so on so forth. So the DO the dissolved oxygen level directly gave us a measure of the ability or the possibility of oxygen going into the cell the level of aeration that can happen and so on so forth.

Here is just the reverse whatever is coming out of the cell is being measured. So all these very interesting things came out of the study. So oxygen has made available to the cells of the place of need inside the cell.

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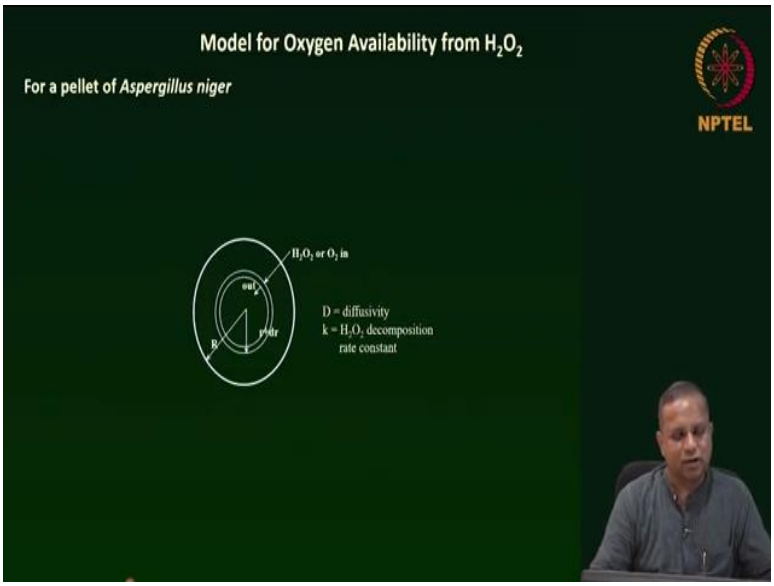

Let me see what I have here and then whatever is not needed is coming out and that is what we are actually measured. So let us, we can have short classes no it is actually quite long. For each paper, we will discuss a few paper so that we get an idea as to these scope or the wide scope of the applicability of the principles. Right from the idea to overcome on the fundamental challenges to understanding the mechanism, let us see what comes next. See you in the next class. Bye.