Transport Phenomena in Biological Systems
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Lecture - 69
LPOS for Mold Cultivations

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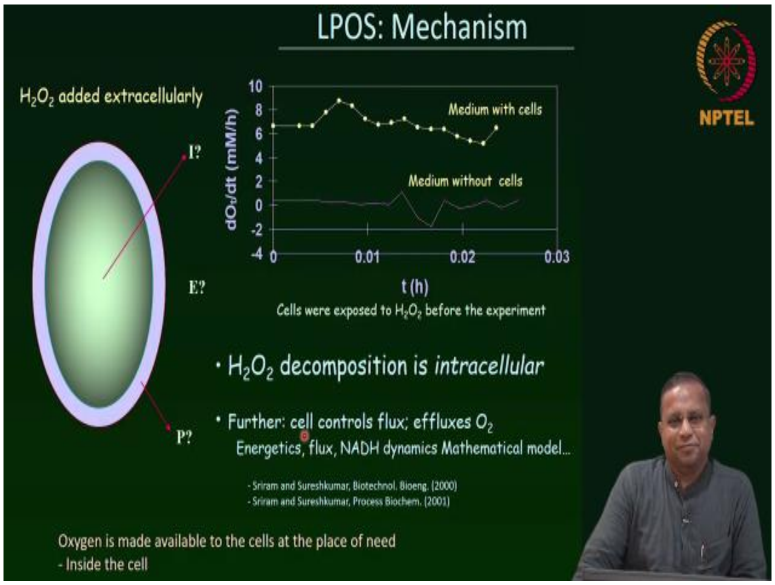

Welcome back, let us continue with another application of transport to research again from our group. This is also related to liquid phase oxygen supply strategy but in this case we are going to look at the LPOS as applied to a mold, you all know what a mold is, it has is highly filamentous in this case *Aspergillus niger* was the mold, that we looked at bread mold you would know the thing that grows on your bread and so on so forth.

I do not need to tell you this you are all biological engineers anyway so the, it so happens that *A niger* is a versatile system it produces a lot of products industrial industrially used products enzymes, and so on so forth. High turnover kind of rather very high amounts of those products are used worldwide the value is also high, billions of dollars, and so on so forth per year. So we looked at the application of this. Let me go directly to the paper and then come back to a summary of that information.

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So that paper was published in 2001 in biotechnology progress this is again a reputed journal in my mind it is second in the pecking order. So the title is improvement in enzyme productivities from mold cultivations using the liquid phase oxygen supply strategy. The authors are Subodh Rawool, who was MTech student who did his MTech with me, Susmitha was my PhD student, K krishnamurthy Rao was my colleague and myself.

They said it was 2001, I think I changed the spelling of my name in 2002 so this is still the old spelling the work was done at IIT Bombay. Let me go to the introduction again abstracts tend to be a little too, what should I say little too focused and may not be very appropriate for an initial exposure although all researchers read the abstract first to get an idea they are in the field so that is fine.

Mold cultures are extensively used in the bio industry for important products such as enzymes acids and antibiotics. *Aspergillus niger* is an industrially important and versatile mold which is used to produce enzymes such as protease catalase glucose oxidase alpha amylase lipase neurogenesis and organic acids such as citric acidic gluconic and fumaric acids. However adequate oxygen supply to mold cultures and addressing their shear sensitivity.

You know they have hyphae if you start providing high RPMs using an impeller to improve oxygen uptake rates or oxygen supply rates to the bioreactor then it is going to shear off the hyphae of the mold. Then the productivity is going to go down so it is a double-edged problem there. However adequate oxygen supply to mold cultures and addressing their shear sensitivity continue to be important challenges, this was in 2001.

For example, both morphological forms of the mold namely the pellet forms a pellet sometimes productive form is a pellet form and the filament is formed. They both suffer from inadequate oxygen availability when aeration is used to supply oxygen. In the case of filamentous growth, the oxygen mass transfer coefficient k_La diminished. These are details let us not get into that, thus methods to improve oxygen availability without increasing the shear level can be expected to improve productivity.

That is why we got into this earlier work from our lab which we also discussed showed, that the application of a liquid-phase oxygen supply strategy to a viscous fermentation of *Xanthomonas campestris* producing Xanthan Gum, improved oxygen availability and product formation. The oxygen supply strategy employs the decomposition of hydrogen peroxide added in pulses on need-bases by the enzyme catalase which is available from the culture itself to provide the necessary oxygen.

Also in the case of the bacterium *Xanthomonas campestris* the decomposition of extracellularly added hydrogen peroxide occurred intracellularly and thus the oxygen requirement of the cell was effectively and completely met. In addition to the above, there are isolated reports in the use of hydrogen peroxide for oxygen availability but they have not addressed the fundamental aspects such as elimination of gas-liquid transport resistance or the mitigation of shear sensitivity which are possible through this strategy and thus making its applicability much broader.

In this work, the growth and enzyme production by *A niger* cultivated with hydrogen peroxide-based oxygen supply strategy and with aeration were studied because we need to compare. The enzymes considered were catalase protease and glucose oxidase also the oxygen availability from hydrogen peroxide to a pellet of *A niger* was represented using a mathematical model and compared with the oxygen availability representations when aeration is used.

In fact this aspect is what we are going to discuss in this lecture. This is just direct transport aspects, so that is what we are going to see here. What we are going to see here in detail to repeat is also the oxygen availability from hydrogen peroxide to a pellet of *A niger* was represented using a mathematical model and compared with the oxygen availability representations when aeration was used.

And then in addition some insights on the mechanism of oxygen availability from hydrogen peroxide to the mold *A niger* were obtained. See here mathematical representation let me spend a little bit of time here, the mathematical representation. The objective is to represent the concentration profiles, this is cutting edge work at that time when it was published, we were looking at concentration profiles.

The same thing that we picked up as a part of the mass flux chapter in this course, represent the concentration profiles of hydrogen peroxide and oxygen the substrate and the product. Inside a pellet of *A niger* cells when either hydrogen peroxide or air is the oxygen source. Let us consider a spherical pellet of radius r of *A niger* cells placed in the medium containing hydrogen peroxide or dissolved oxygen.

When hydrogen peroxide is present in the medium it diffuses into the pellet and gets decomposed by the catalyst from cells to yield oxygen. When dissolved oxygen is present in the medium through aeration oxygen diffuses into the pellet and is consumed by the cells. So either way, it is diffusion into the pellet and then the cells inside the pellet are going to consume it. Let us consider the mass balances over a thin spherical shell I hope you are able to recall whatever we did.

Let us consider the mass balances over a thin spherical shell I am doing shell balances here because the new system I am not when I would like to have a better understanding therefore I am doing shell balances here or a thin shell of thickness dr at a distance r from the pellet center. Assuming uniform pellet density uniform catalase activity in the pellet and the decomposition of hydrogen peroxide to be first order with respect to hydrogen peroxide.

Under steady-state conditions, the mass balance for hydrogen peroxide on simplification would yield this. In other words in this case, since it was quite straightforward, you could do shell balances and arrive at something I think we just took the equation for a spherical coordinate system you do shell balances will get the same thing or we did shell balances I do not remember. But either case you could also take the equation from your table there; from the table of equation of equations of continuity.

The spherical coordinate tables this is a case with reaction at steady state and so on you will end up with this equation. The boundary conditions are at r equals 0 C_H is finite this is the hydrogen peroxide C_H is a hydrogen peroxide concentration it is finite r equals 0 that is the center, this is the center C_H is finite. C_H is finite means the physical for the physical reality to hold the hydrogen peroxide concentration either needs to go through a minima or a maxima.

In this case, minimum because the hydrogen peroxide concentration outside is higher therefore it will go through a minima there and therefore the derivative (dC_H/dr) is going to be 0 we have already seen this, so directly applied here and at r equals R at the surface the hydrogen peroxide composition is some C_H^R is a hydrogen peroxide composition of the pellet surface r is a pellet radius and so on so forth.

And analytical solution was obtained by using this trick. Let me not get into the math part of it you can read it from the paper. And we get the term R_A consists of the generation of oxygen due to decomposition of hydrogen peroxide which came out like this and the above equation was solved numerically, because it became difficult to get an analytical solution therefore resolved numerically using the shooting method or solution of non-linear ODE's.

When you need you need to go and pick this up, just take it on face value here that is good enough for this course. We did not look at numerical methods in this course at all. It is very important they are very important and then again on one side they are very important; they are brute force methods and on the other side they are not very you know straightforward or easy. There are challenges that every single application of those will have to take care of that.

It is not a cakewalk is what I mean, then this is these are the equations for aeration and then this is what I wanted to show you. You see here, these four plots are the ones that I wanted to discuss with you. Here you see the oxygen concentration with the pellet radius this is when aeration was used. So this is the oxygen concentration in millimolar and this is the pellet radius 0.5 mm radius or 1 mm diameter pellet we are taken.

And this is the point this is a 1 mm radius pellet therefore 2 mm diameter pellet. So here you can see that the oxygen concentration as a result of the oxygen diffusion into the cell at the same time the cells inside the pellet are consuming oxygen and so on so forth. Because of that it goes down from about 0.25 millimolar to 0 at this location. Therefore this part of the pellet from 0 to 0.2 mm the cells there are completely devoid of oxygen.

That is the inside this gave us, direct transport and gave us this. And here if you see it is even worse with the all the oxygen is gone by whatever is provided by aeration was completely gone by about 0.88 mm. So the majority of the pellet in terms of the radius was starved of oxygen which is not a very happy situation for production, for growth of a production. Whereas when we used hydrogen peroxide these are hydrogen peroxide concentrations and the oxygen can also be found out but we had plotted the hydrogen peroxide to give you an idea here.

Is that is what is coming in similar to oxygen coming in here. So hydrogen peroxide concentration goes from here to here and this would get taken up by the cells and broken down, so nowhere near 0. Similarly here it has gone down a little bit this is 7.53 this is 7.38 that is the only difference. So it makes things you know you are able to get insights into what is happening in the system as long as you model things in a representative fashion.

And that modeling was based entirely on transport principles that we saw in the mass flux chapter in this course, application to cutting edge aspects. When we come back in the next class we look at another aspect of transport applied to one of our research problems see you.

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