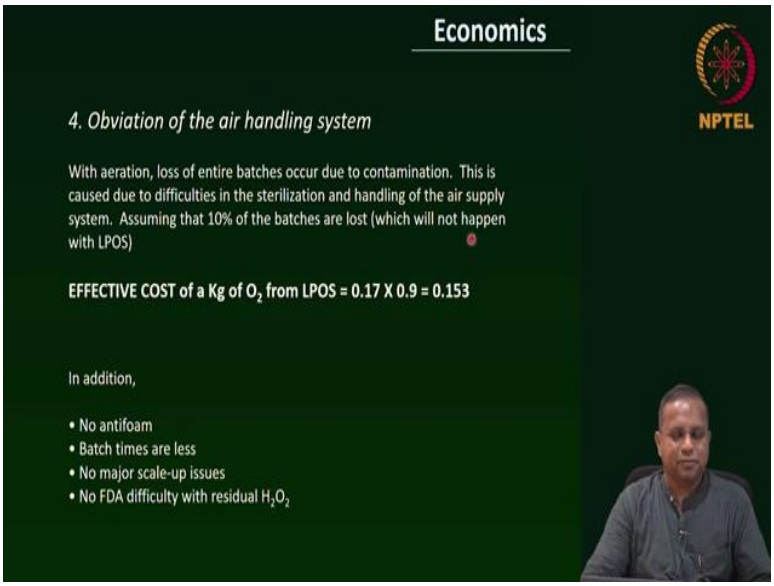
Transport Phenomena in Biological Systems
Prof. G.K. Suraishkumar
Department of Biotechnology Bhupat and Jyoti Mehta School of Biosciences building
Indian Institute of Technology, Madras

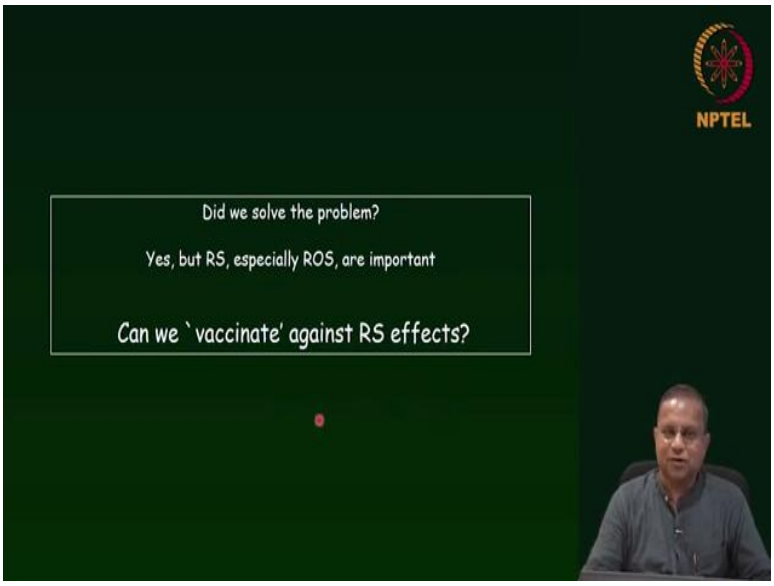
Lecture - 71
Couette Flow Cultivations

(Refer Slide Time: 00:16)



Welcome back we are looking at the application of some transport principles in cutting-edge research. The previous class we looked at the liquid phase oxygen supply strategy details and I showed you some details of the economics whereby the cost is brought down to about 15 paise or even less per kilogram of oxygen.

(Refer Slide Time: 00:50)



At that stage we were something at thoughts were something like this did we solve the classic problem of oxygen supply to bioreactors? It was a classic problem, yes because as you saw oxygen was made available to the cell at the place of need and whatever the oxygen does not need seems to come out of the cell.

So that is as complete a solution as you can get, yes we did solve but what I did not tell you very clearly was that this strategy affects the growth, why? Because we are using hydrogen peroxide although hydrogen peroxide has made in the cell as a part of its metabolism. It is something called a reactive species and a reactive species can affect the growth of cells, the metabolism of cells in very many different ways.

And that is the reason why whenever we use this although the per cell productivity is higher, the cell yields were lower in the case of LPOS and this we had already noticed and we were figuring out means by which we can offset that, the thinking was if it can somehow offset that since the per cell productivity is higher if the cell concentration is also brought up simultaneously then the productivity will go up that much better, the productivity will become that much higher.

That was the thinking behind that approach then when we started probing this this was way back in 1995. We realized that we are adding hydrogen peroxide there is iron in the medium and you have the classic Fenton reaction that is happening the reaction between hydrogen peroxide and

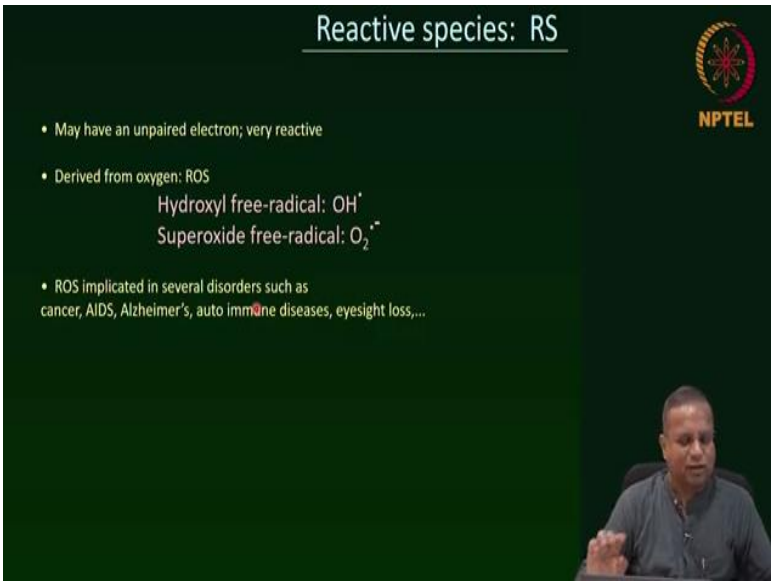
iron to give you hydroxyl radicals and so on and so forth and those reactive oxygen species much more reactive much more deleterious reactive oxygen species that are generated from hydrogen peroxide could be the reason why this cell concentrations were in low.

Such we are using low enough concentrations of hydrogen peroxide but still whatever little is used there is a deleterious effect and that is cumulative and that results in lower cell age and this is what we started looking into way back in 1995, the first PhD my first PhD student Mandhana Rao joined with a reactive oxygen species background in January 1996 and she started looking at this problem.

Her approach was her thoughts were can we vaccinate against this reactive species effects. She started thinking in that direction the idea was we will somehow make the cells immune to the negative effects of reactive species and thereby when we use the LPOS the cells are more robust and therefore they will not die as much or their growth will not be affected as much. So this was her thought and we did a lot of work in that entire in direction of research change of using LPOS or viewing LPOS from a reactive species point of view.

And coming up with entire strategy is based on reactive species themselves. It so happens that if you induce reactive species, this is what we found out when they went and characterized this. If you induce reactive species you actually improve productivity by a totally different mechanism in the cell, that is not highly transport related, so all the very interesting I will not get into that in this particular lecture or in this particular course.

(Refer Slide Time: 04:42)



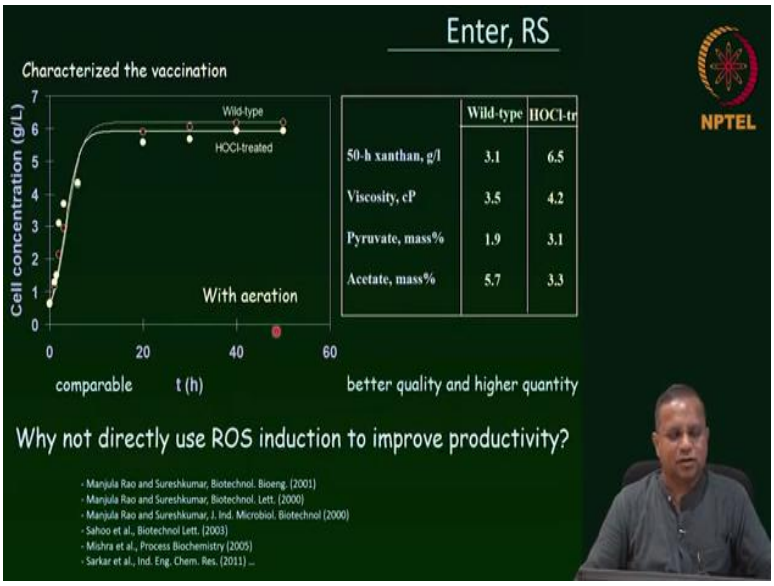
Some background on reactive species for you many of you may not know what they are many they may have an unpaired electron, may not. They are very reactive in comparative terms all the way there is second order rate constant for hydrogen peroxide with iron is only 76 mol^{-1} s^{-1}. The hydroxyl radical rates typically of 10^9, 10^{10} mol^{-1} s^{-1} the second-order reaction takes.

So it spans a wide range, so it is only by practice will know what to call as what the categories is reactive oxygen species are not, so the reactive species are general the whichever have whichever may have an unpaired electron and are reactive. The ones that are derived from oxygen are called reactive oxygen species. You could derive them from nitrogen, you could derive them from halogen and so on and so forth.

Ones that we focused on which are most important in fact because of their reactivities hydroxyl free radicals this is a highly reactive one. This is the most reactive one in fact hydroxyl radicals $\dot{O}$H dot the dot is on the O, the unpaired electron is on the O. The superoxide free radicals $\dot{O_2^-}$ this is nothing new the reactive oxygen species have been implicated in very many different diseases.

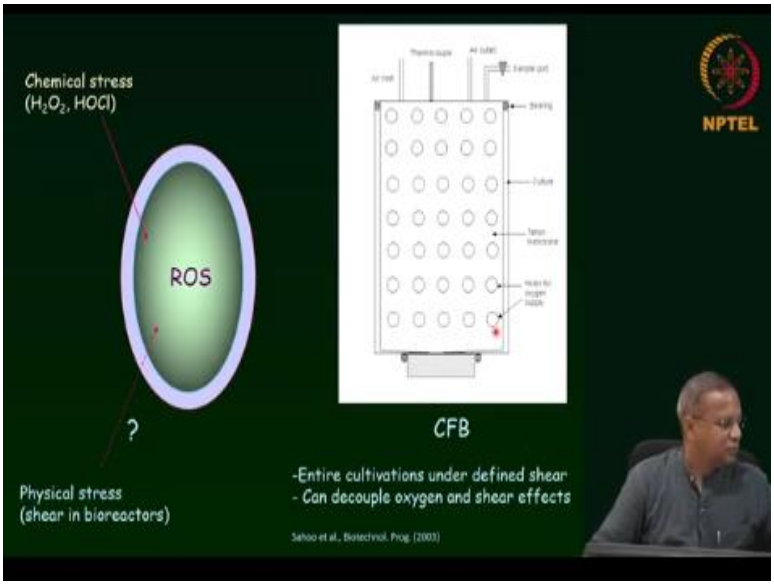
In fact I have an entire course in elective and reactive species in a medical and related technology and that is a whole different field altogether.

(Refer Slide Time: 06:24)



So this is what I mentioned that I will not get into in great detail that our whole direction change when he started looking at reactive species and we showed that to work in very many different systems and so on.

(Refer Slide Time: 06:46)



The today's paper is on this aspect, we were looking at chemical stresses to this cell such as hydrogen peroxide addition as a part of LPOS, HOCL addition to induce reactive species in the cell and so on so forth which I have not discussed much in this course. So when you add these something happens we wanted to see we saw that the effects of the chemical stress are mediated through the reactive species.

We wanted to see whether the effects of a physical stress is also mediated through reactive species because physical stress shear in bioreactors is common, that is we were working on their bioreactors at that time very relevant. And we wanted to see whether they are mediated by a reactive species and that is how we got into this and as I very briefly mentioned we had to come up with a means of growing cells at defined shear levels.

We had used a device here something like this now let me switch to the actual paper and that paper to discuss.

(Video Start Time: 08:07)

So this paper published again in 2003 in biotechnology progress, macro level and genetic level responses of Bacillus subtilis to Shear stress. Authors are Susmita Sahoo my third PhD student Rajesh Kumar Varma my Mtech student and all these are colleagues A.K Suresh, K Krishnamoorthy Rao, Jayesh Bellare and myself, here the spelling was different. So here some parts of the introduction for you; Hydrodynamic or Shear stress everything is transferred now add sub lytic levels significantly affects the macro level cell responses such as morphology growth and productivity. This is known, nothing new.

The macro level responses result from altered genetic level responses to the shear stress. All the shear is normally perceived to be deleterious it can also be beneficial since shear effects these cells affect cells at a fundamental level a better understanding of its effects on the cells in culture could lead to the design of appropriate bio reactors or the operation of existing bio reactors and favorable regimes.

This is way back 17 years old, it is published the work was done two years before that. Studies on the effects of and cell responses to shear are available predominantly on mammalian plant and insect cells. However, information on shear responses of bacterial cells which are widely used in a variety of industries including those producing high-value products such as pharmaceuticals and other recombinant products is not as abundant.

The information on genetic level responses of bacterial cells to shear is not available, so that is what we started that is there was a motivation to get into this work. Many studies on shear effects

have been reported in stirred tank bioreactors in which it is difficult to decouple the effects of shear and oxygen transfer, everything is transferred that they are seen here, effects of sheer oxygen transfer k_La.

For example, increase in RPM to increase shear rate would also result in an increase in the volumetric oxygen transfer coefficient, further it is difficult to quantify the shear level in the stirred tank. Because why we have already seen the velocity profiles are completely undefined right difficult to measure also and we know that shear stress is based on the velocity gradient therefore you have the velocity profile you know you can figure out the shear stress profile.

And the average shear may not be a representative of the actual range of shear levels to which the cells are exposed in the vessel. However, shear studies in a Couette flow regime which is achieved in the space between two concentric cylinders one of the ways of achieving that rotating at different angular velocities are available. Although the defined and uniform shear field in Couette flow facilitates analysis.

It is non-trivial to supply oxygen to aerobic cultures at adequate rates without affecting the flow field for the entire duration of growth. This is the challenge that we contributed to overcome; one is therefore constrained to use device short term experiments or to introduce oxygen air in a manner that might compromise the defined shear field. There are some details there in this work the Couette flow bioreactor has been designed and fabricated to cultivate cells and at well-defined laminar shear conditions for the entire duration of growth.

This was our contribution, earlier you could do for short times as long as oxygen did not become limiting till that time you could do but not complete activations. The CFB the Couette flow bioreactor was designed to ensure operation in the Couette flow regime by using a thin annulus as well as to ensure oxygen supply without compromising the laminar flow profiles. So that long-term cultivations of an aerobically growing organism can be carried out in a defined shear environment.

The problem of oxygen supply with minimum disturbance to the flow field was addressed by having oxygen inside the hollows inner cylinder and transferring it to the annular space containing the culture through an oxygen permeable membrane. So let us describe this here, this is the longitudinal section of the Couette flow device you can see the inner cylinder here with a tapered end about half a degree or one degree or something like that.

And you have the outer cylinder here and of course this is the bearing to ensure appropriate rotation and so on so forth. There is an auto bearing here to ensure at a maximum of 1500 RPM here. So this is the space the annular space where the culture is grown. How do you supply oxygen to this, so that you could carry out cultivations over the entire length. What we did was we cut holes into the surface of the inner cylinder.

And then covered the outer surface of the inner cylinder in other words the surface that faces the culture with a Teflon membrane. So the oxygen that is provided here will pass through these holes that is not a problem pass through the membrane that is covering the holes and get into the annular space where the cells are grown and thereby it supplies oxygen continuously to these cells.

And then we had to characterize it and so on so what we found that it can provide oxygen to significant levels required levels by the cells and then we had to adjust the cell concentrations and so on so forth. So this entire thing there is nothing but all transport, we had to for example here let me show you this, where was it designed here, Shake flask cultivation, used as control, two types of devices have been used in the present cultivations the Couette flow bioreactor at several speeds of rotation to vary the shear rate and the Shake flask.

For a meaningful comparison of the two devices we need methods of calculating shear rates and these two devices the shear rates and the CFB can be exactly calculated from the velocity field you know we derived this in this class. We derive the exact same expression in this class and that you remember this gamma dot I mean I would have used a different symbol here the shear rate

$$\dot{\gamma}(r) = 2\Omega(KR/r)^2/(k^2-1).$$

This way it actually derived in this class and this is we are using this in research. Look at the direct application of something we derived in an undergraduate course being used directly in research, so that is the power of it and then of course there are various other aspects of transport Reynolds number power, number here k_L effective which is the we know the k_La so k_L can be estimated separately and so on so forth for this case.

So for all that we have these expressions that have been looked at derived and so on so forth or taken from the literature and then this was used to get some very interesting results. So here one does that let me show you here and then go back to the presentation to show you the other results. This is the SEM the scanning electron micrograph of the cells that are grown in the Shake flask.

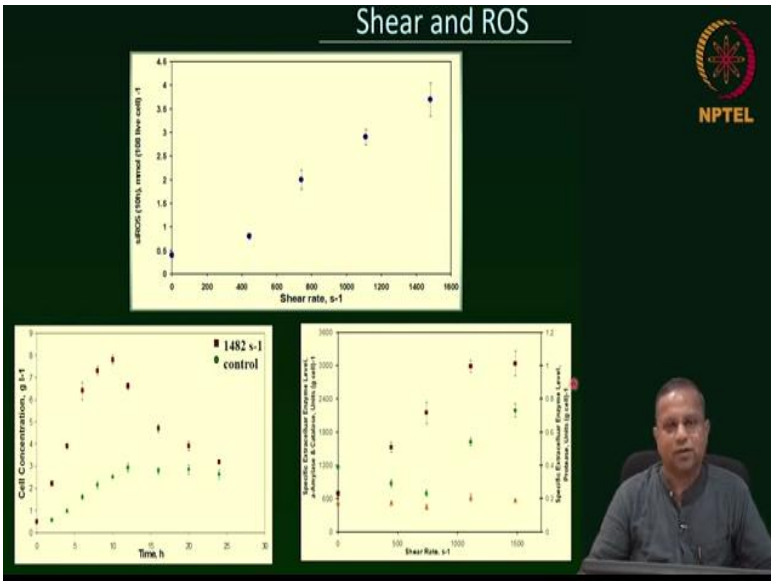
The cells that are grown in the Shake flask with minimal shear are nice and long alright you see here whereas that are grown in the CFB had 1500 RPM 1482s^{-1} shear rate. It is much smaller when you employ shear you expect the cell to elongate and so on and so forth. Here what we find is completely opposite, completely counterproductive, this is a very interesting result for us.

And there were other interesting results some results, let me show you as in that presentation that we were looking at let me close this now.

(Video End Time: 17:41)

So here this is the reason why we got into it we wanted to look whether the reactive oxygen species mediate the effects of physical stresses also we designed and fabricated a device entirely based on the principles of transport phenomena. We had used the expressions that we have derived in this course and this such a device could do entire cultivations and a defined shear stress it can decouple oxygen and shear effects and so on.

(Refer Slide Time: 18:12)

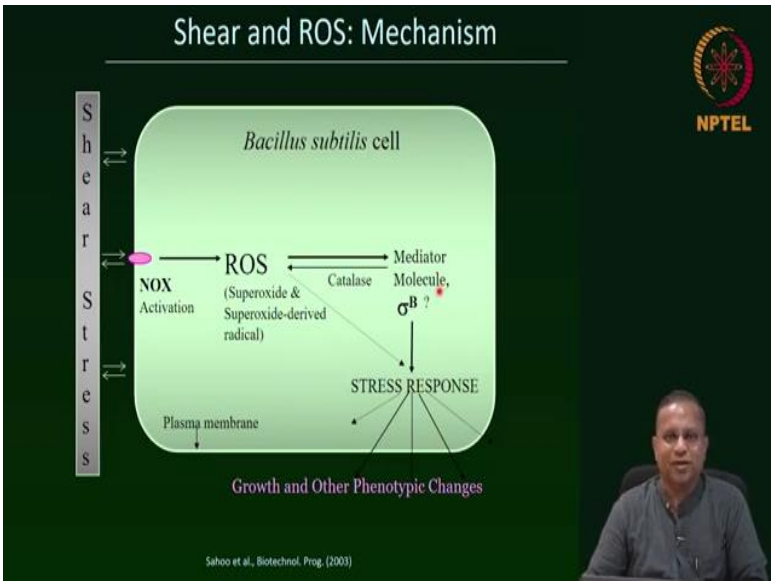


And these were some of the very interesting results you see specific intracellular ROS at a certain point versus shear rate. As the shear rate increases the specific intracellular ROS increases the reactive oxygen species. The effect of shear is being mediated through the reactor, it is not possibly being mediated we have actually shown it is mediated this data shows that it is possibly being mediated by specific intracellular reactive oxygen species.

Then we looked at the growth the cell concentration profiles cell concentration was sustained with the control it is this green dots it is standard in the shake flask but when it was grown at 1482 s^{-1}, look at the increase in cell concentration maximum cell concentration and the growth rate you could calculate the growth rate from this you know how to do that. So both were different right much higher when the shear was employed.

And also the specific intracellular enzyme level of 3 different enzymes this is extracellular and this is intracellular enzymes and with the shear rate there are changes and 2 of these enzymes so all these were interesting and then.

(Refer Slide Time: 19:32)



Susmita also worked out the mechanism by which this happens and she proved some parts of it, what she showed was the shear stress activates our shear stress rate it activates the NADH oxidase which is an enzyme on the membrane. So NOX NADH oxidase it is activated that causes the induction of reactive oxygen species such as superoxide and superoxide derived radicals.

And then a lot of molecular biology happens here there is a sigma B transcription factor that gets involved and so on and so forth. So this stress response, in other words a lot of responses to the stress get manifest; such as changes in growth and other phenotypes of this cell and one of those phenotypes is increased production of products of interest to us. So that is what is shown as a part of this paper we saw the application of transport in this.

And in the next class I think I will present probably a couple more and maybe finish with them what you say? I think maybe 2 classes or 1 class depending on how it goes we will take up two other examples of this and one would be pretty much current the work that was published recently and so on so forth. See you in the next class, bye.