Transport Phenomena in Biological Systems
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Lecture - 72
Pseudo-Steady State Approximation Applied to Bio-oil Production

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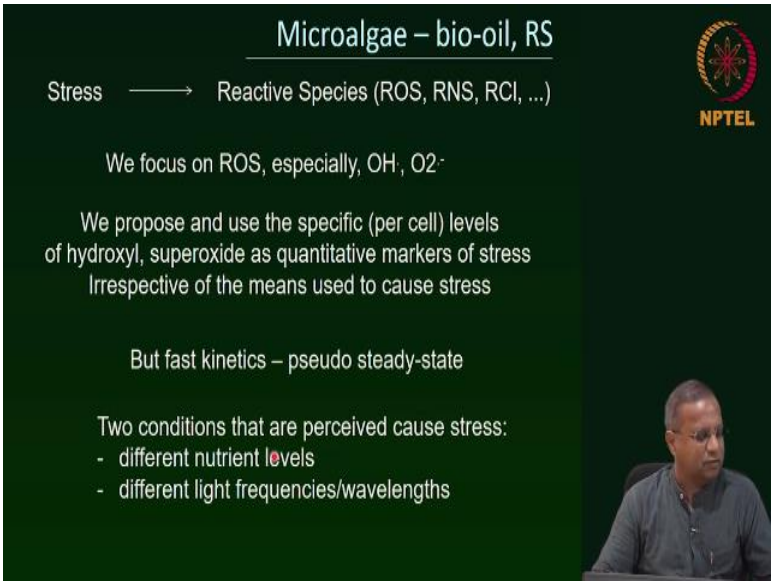

Welcome, in this class we would look at another application of transport especially in these two classes; the last two classes of this kind I am going to look at the application of Pseudo Steady State principle in our research and that has led to so much insight so much benefit in terms of the knowledge generated for improving by oil productivity on one side and even dealing with cancer or managing cancer, treating cancer those aspects.

But for that matter managing any disease this insight is going to be helpful. So let me first talk about the bio oil aspect sometime in the middle of the previous decade or let me say 2004-2005 the oil prices shut up and the interest in the alternative energy aspects came up again it typically happens in cycles and there is a lot of interest in bio oil from micro algae biodiesel from micro algae bio oil from micro algae and so on so forth.

Let me show you how the principle of Pseudo Steady State was effectively used for the system in our study to give you a little bit of background in terms of molecule molecular aspects. You all know vegetable oils; vegetable oils are the ones that we use for cooking and so on so forth. Where did they come from? They come from vegetables; what aspect of the vegetables leads to oil?

They are the lipids in those vegetables that result that our oil that are extracted out by pressing them and so on so forth and they are used. Similarly, you could get oils from microalgae, same way same kind of molecules that contribute to oil from vegetable sources similar molecules contribute to oil from microalgae sources. The crude oil that we get out of the earth has a different composition but at certain levels it can contribute to these fractions also.

You get a wide variety of fractions there and the difficulty is with respect to crude oil and the pollution the carbon dioxide you know, it is a greenhouse gas there is a lot of climate change aspects that are caused by a heavy use and indiscriminate use of crude oil essentially you are taking out carbon that was formed millions of years ago and putting it out into the atmosphere and so on so forth.

The major difficulties which where viral in about 13 years ago so all these things came and we also started looking at it from the reactive species angle which happens to be a research core line if you want to call it that. The idea here is something like this microalgae bio oil from microalgae and reactive species related aspects. We already know that these stresses cause reactive species in the cell reactive oxygen species, reactive nitrogen species reactive halogen or chloride species and so on so forth.

We of course focus on hydroxyl and superoxide here which are ROS and we proposed and used the specific or the per cell levels of hydroxyl and superoxide as quantitative markers of stress irrespective of the means that were used to cause these stress and this is where the pseudo ratio comes in, why? Because the kinetics of these reactive species is very fast, hydroxyl radicals 10^{10} per molar per second.

The superoxide radicals anywhere from 10^3 onwards higher and so on so forth. So when you are interested in processes related to growth which happen over time scales of about an hour or more and so on so forth doubling. The reactive species reactions can be considered to be at steady state. In other words we do not worry about the unsteady nature associated with the reactive oxygen species reactions.

When the interest is in the much slower process which is a growth rate, if you do that then it becomes there it provides you with significant insight and you could actually use the Pseudo Steady State levels of hydroxyl and superoxide as quantitative markers of stress and we showed that it was they are fundamental mediators of stress irrespective of the means that that are used to cause a stress this paper and other papers.

Irrespective of the means that are caused the stress they are caused by the imbalance between the production and destruction of the reactive oxygen species in the same are the amounts accumulated lead to stress. So by using the Pseudo Steady State principle we had a means by which we could address these aspects quantitatively. So we saw two conditions that were perceived to caused stress. Let me switch to the paper at this stage.

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This was again published in biotechnology in bioengineering in 2013 stress induced lipid production in Chlorella vulgaris relationship with specific intracellular reactive species levels fundamental mediators of stress, that is what we establish here. Kavya Menon was my MS student, Ranjini Balan my PhD student and myself and I think I will read the, that is it, it is here. So I will read the introduction the abstract tends to be a little too focused for an undergraduate course.

Attainment of sufficient cell concentrations and sufficient specific lipid accumulation levels seem to be two major challenges in the commercialization of algae cultivations for bio oil production. Stresses can be used to improve bio oil productivity in microalgae and many studies on the effects of stresses on algae such as UV treatment, temperature, pH, salinity, nitrogen deficient growth media have been reported other stresses that is light and nutrients symbiotic relations have been found to induce changes in the cellular composition and so on so forth.

But have not been exploited well toward improved cell yields, although the effects of many stresses have been investigated the understanding of their effects seems to be fragmented, possibly because a unifying view has not been employed this is where we contributed, we contributed a unifying view in terms of the reactive oxygen species the reactive oxygen species are known as the known to be signaling molecules for various cellular events.

And it is reasonably well accepted that ROS are the molecular mediators of various stresses, we propose to use the specific interest in the reactive oxygen species in level as a quantitative marker for the stress irrespective of the means to induce the stress especially because easy quantification of si ROS are possible through fluorimetry. It is known that si ROS levels are higher under stress although only a few studies have quantified such levels.

Our lab is one such lab few labs that quantifies it, even in the studies that quantify ROS levels the common quantification is of total ROS that includes many species such as superoxide anion radicals, hydroxyl radicals, hydrogen peroxide, hypochlorous acid, peroxyl, alkoxyl singlet oxygen and per oxynitrate and all these are measured by something called DCFH-DA. It measures some mix of these species.

In our lab we measured the specific levels of individual reactive oxygen species say hydroxyl alone or superoxide alone separately which we believe would facilitate a better understanding of the various roles of ROS and their effects on many different aspects of cell function. One of the perceptions that seemed to discourage, this is where we come to transfer directly. One of the perceptions that seem to discourage the measurements of the actual amounts of ROS.

Either as concentrations or as specifically levels in the culture is that the species are short-lived and hence may not contribute significantly to the information on cell status. Nevertheless, we have found in our earlier studies in our lab, that actual amounts do provide significantly useful and quantitative information. This dilemma could be resolved when the rates of the various relevant processes are considered.

It can be seen that the fast rates associated with ROS reactions makes it possible to consider them as being a Pseudo Steady State in comparison with the much slower metabolic processes or growth. This is the crux here when viewed in the context of Pseudo Steady State the net specific ROS levels can be considered as an indicator of cell status or the stress levels in the cells at a particular time point.

Then this, let me read this and then summarize this work addresses the effect of stresses induced by nutrient and light on micro algal growth and lipid production nutrient levels and light wavelength higher frequencies and hence higher energies are perceived to cause present cells, in addition to the investigation we did something else also possible. So, these are the materials and methods these are the results and discussion in a certain framework which may not be very relevant for our discussion here.

What I do is I would go directly; you could read this if you have an interest certainly there is a very interesting set of results that we have here.

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And we go to the summary directly, we looked at two conditions that are perceived to cause stress one is different nutrient levels and different light frequencies or wavelengths. The model system was chlorella vulgaris a micro alga.

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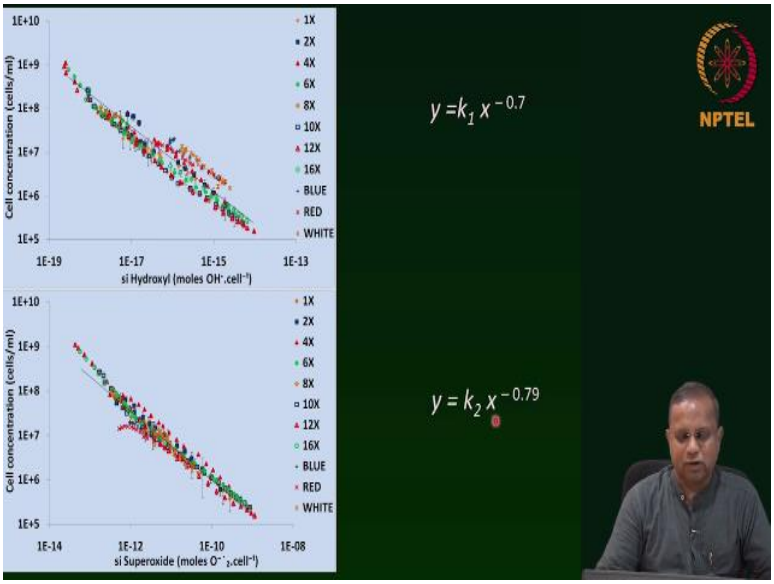

And these were the results, you see cell concentration on the y-axis cells per ml versus specific intracellular hydroxyl radical level in moles of hydroxyl per cell. You see here this is the various nutrient levels 1X, 2X, 4X and so on and so forth compared to a certain control and this corresponds to the data with blue light, this corresponds to the data with red light and this corresponds with the data with white light.

All this data of cell concentration versus hydroxyl radical levels nicely falls in this region. It is a beautiful relationship which shows the fundamental nature of the effect of hydroxyl radical levels higher the hydroxyl radicals lower the cell concentrations. This is irrespective of even the various phases of the batch growth that he went to under these conditions, all of them nicely line up here.

And here you have cell concentration versus specific intracellular superoxide radicals this is hydroxyl radical highly reactive and so on the superoxide highly reactive as well as it plays signaling roles in this cell. You could see as these levels increase the cell concentration goes down, beautiful relationship and this was actually quantified also. As see this is a log-log scale, so $y=k_1x^{-0.7}$ was the line that corresponds to this equation corresponding to this line and $y=k_2x^{-0.79}$. It is the equation that corresponds to this line and more interestingly.

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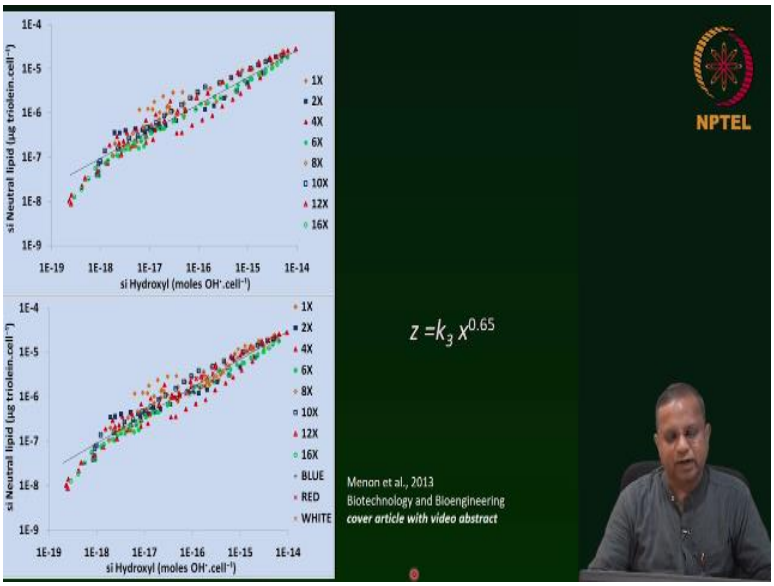

This is the effect on specific intracellular neutral lipid level in the cell which is nothing but the one that is oil, the neutral lipid is the one that will give you oil. It was measured in some terms and so

on so forth do not worry too much about this. So this is specific intracellular neutral lipid level. This is specific intracellular hydroxyl radical level, beautiful linear relationship with all the data put together.

And linear relationship this was $Z= k_3X^{0.65}$ the nexus x axis and so on so this is actually published as a cover article with video abstract where considered prestigious at that time when it was first introduced and it was published in 2013 in biotechnology and bioengineering.

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Welcome to abstract we all know that bio oil from microalgae is an area of current research interest. The process of making bio oil from microalgae is quite simple to describe, nevertheless there are no commercial plants across the world that utilize this process to produce bio oil from microalgae that is essentially because there are very many challenges to this particular process.

If we focus on the upstream side we can consider getting sufficient cell concentrations as well as getting each cell to have a sufficient amount of bio oil in it are two major challenges. In this particular work Kavya Menon, Ranjini Balan and I have looked at some aspects of this. Now let us look at stress, people have considered various kinds of stresses to improve the yield of bio oil or the productivity of bio oil from microalgae.

When we think of stress the first thing that comes to mind is its relationship to the reactive species in this work we focus on the reactive oxygen species and especially the hydroxyl species and the superoxide species. In fact we propose to use the specific level of these reactive species or the amount per cell as quantitative markers of stress irrespective of the means that I used to create this stress.

And one of the first questions that comes to mind is the reactive species, react very fast so if the reactions are so fast how can they be considered as markers or something. Nevertheless, we have found that these are provided as very useful quantitative information in studies done earlier and this difficulty can be resolved by considering the Pseudo Steady State aspects, since these reactions are so fast.

You can consider them to be at Pseudo Steady State levels in comparison with the kinetics of slower processes such as metabolic processes or growth process. In this work we have considered two conditions that are perceived to cause stress one is different nutrient level and also different frequencies or wavelengths of light causing stress. The model system in this particular study is chlorella vulgaris cell concentration with was a specific hydroxyl and specific superoxide reasonably linear.

Something interesting happens in the case of neutral lipid levels to let is just look at neutral different levels versus specific hydroxyl levels when all the data are put on the same graph there is a relationship that we see and there are other interesting results in the manuscript the findings provide a step for us to rationally design the stress based strategies for improving bio oil yields from microalgae a possibly and personally there are more interesting results you know such as questioning what exactly stresses the very notion of stress.

Kavya, Ranjini and I warmly invite you to read the paper in fact we have made special efforts to make the paper easily readable the title is; Stress Induced Lipid Production in Chlorella vulgaris: Relationship with Specific Intracellular Reactive Species Levels and I need to acknowledge the funding that was provided by the Department of Biotechnology Government of India the Department of Science and Technology Government of India.

And the Divashri fellowship by Srikumar Surya Narayan, a distinguished alumnus of IIT Madras happy reading. We all need fuel to get around and as America takes steps to improve our energy security. Homegrown fuel sources are more important than ever. The Energy Department is researching one of the fuel sources of the future found here in algae, have a look at this algae farm.

These large man-made ponds are called raceways and they cultivate a new crop of algae every few weeks. You see algae or more correctly microalgae are very small aquatic organisms that convert sunlight into energy, some of these algae store energy in the form of natural oils. Under the right conditions algae can make a lot of oil that can be converted into biofuels algae could potentially produce up to 60 times more oil per acre than land-based plants.

Extract that oil and you have the raw materials to make fuel for cars, trucks, trains and planes. In the future anything that runs on gasoline and diesel could also use biofuel from algae. The oil is extracted by breaking down the cell structure of the algae. This can be done by using solvents or sound waves. After the oil is extracted then it is further processed at an integrated bio refinery or in the future at a traditional oil refinery.

Another great benefit of algae consider this like plants algae needs carbon dioxide to grow and that is good for the environment, since it takes CO_2 out of the atmosphere making it a nearly carbon neutral fuel source. There may even be opportunities to build algae farms next to power plants that use fossil fuels, actually using CO_2 exhausts to feed algae ponds. There are over 100,000 different strains of algae some grow better in different climates or in freshwater saltwater or even wastewater.

So scientists are testing different algae under many different conditions, to find the best strains and develop the most efficient farming practices. While commercial production is still a way off algae holds great promise to become a reliable homegrown fuel source to reduce our nation is reliance on foreign oil. In Iowa where corn is almost a religion a new faith may be taking root. This is algae growing out of solution looks just like a cornfield we run the harvester, this our harvester reminds us of a combine pulling off the algae.

Algae is not that something we usually try to get rid of, you can actually crack the code on actually growing and harvesting algae and getting this biomass the applications for this biomass is really incredible. You can use it to feed animals you can use it to feed people you can take it is very high value in proteins you can use it to fuel your cars you and get the oil out of that. So when you look at algae that is why so much interest is in it because it has such a wide application in so many different sources.

In addition to its biofuel possibilities algae is already being used in all kinds of ways you might not be aware of food products, baby formula or nutritional supplements like spirulina. Algae contains the all-important nutrient omega-3 fatty acids. It is all about omega-3 and the world is

short omega-3 they are long Omega 6 and Omega 7 but they are short omega-3 and algae may have the best ability to solve the shortage of Omega-3 in the world at the highest quantity.

Quantity that is what they are trying to tackle here and Shenandoah Iowa in an unusual pairing a traditional corn ethanol plant is supporting algae production and next generation biofuels. It turns out corn has what algae needs. The third of the kernel is starch being converted into fuel a third of the kernel is fiber being converted into animal feed and there is a third left and all that is mean today is being converted in the CO_2 in the atmosphere.

So we could actually take that other third of the kernel that we are basically emitting into the atmosphere capture it and create a whole another product around how we convert CO_2 warm water waste heat and sunlight in algae CO_2 waste water and heat all byproducts of producing corn ethanol exactly what algae needs and this joint project called bio process algae is the result of an unlikely partnership.

This whole process has been serendipitous, Todd Becker CEO of Green Plains Renewable Energy, a major producer of corn ethanol and Tim burns CEO of bioprocess H_2O a water treatment company whose technology has been used to get algae out of water. How to keep algae out of wastewater systems we have not lot of knowledge and that knowledge gave us the opportunity to bring it in to how we can grow algae and we knew that with our system.

The heart of it is attached growth, we have a system in which we provide a lot of surface area for the algae basically think of a condominium for the algae to reside on and that condominium provides a lot of surface area. So, we have a big mass transfer devices think of it that way. So on a typical open pond system which algae traditionally grown on we would be about 40 times the surface area of the system so it gives us a lot of opportunity to be more cost-effective.

Let is say an acre of land an acre of land that produces corn today in the United States produces 7 tons per acre our goal in these reactors is to get 15 to 40 tons per acre of product, and instead of a once a year harvest algae is harvested several times a week. The idea of getting gas and oil from

algae is really not new in fact it is millions of years old. All our oil today is ancient algae deposits compaction of hundreds of millions of years of riverbeds and compaction of algae already.

What we are trying to do is accelerate with the process and to what Mother Nature has done so effectively and start to industrialize it and produced by our process, the stumbling block is cost. But recent breakthroughs promise to reduce that cost at the Pacific Northwest National Laboratory researchers have developed a technology that uses extreme pressure and high temperatures to accomplish in minutes what nature is done over millions of years convert algae into oil still to be cost-effective algae needs to be grown in high volume.

And co-locating algae production with a corn ethanol plant might the way. You basically can use free inputs the sunlight, waste water, warm water, heat and CO_2 and there is a lot of all of that available and so if you actually combine all that, you actually take something that is really free and you are going to create something with a lot of value and in the process they just might be teaching the rest of the world a new way to look at that greenhouse gas CO_2 instead of a pollutant it could become a product.

If you think about the ability to utilize greenhouse gases and CO_2 algae in my opinion is the only profitable use of CO_2 currently on the market so if you are able to profitably use that CO_2 portion that is going to give you opportunity to mitigate the rest of the CO_2 emission. Reducing CO_2 emissions while growing a renewable and sustainable fuel source that could mean less expensive gas that is the promise of algae it may be a while before algae de-thrones corn in this part of the world but as this remarkable experiment is demonstrating there is no reason they cannot get along.
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