Indian Institute of Technology Kanpur

National Programme on Technology Enhanced Learning (NPTEL)

Course Title
Bioenergy

Lecture -12

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Welcome you all for the lecture series in bio energy over a span of two weeks. So in the first five lectures we talked about the energy economist, we talked about the basic concept of bio energy and second week we started with the most fundamental process by which the whole bio energetic machinery of the life-forms and the perennial source of energy the sun it works in the form of photosynthesis.

How the light energy is being utilized to synthesize molecules like carbohydrates and sugar. So in that section we talked about the simple reaction where carbon dioxide plus water making carbohydrate and as I end product as oxygen. And while dissecting the whole process we talked about the two set of reactions, the light reaction and the dark reaction.

So in the light reaction we talked about photo system 1, and photo system 2 and then we talked about the perennial source of electron in the form of water expecting clusters consisting of four manganese ions sitting at different oxidation state. And where we concluded is how that cluster traps water molecules to water molecules and convert it into protons, electrons and oxygen as a by-product.

And how such cluster or water splitting mechanism is inspiration for hydrogen energy and discussed that this kind of research will be discussed in the advanced optics. What we have not talked about photosynthesis the dark reaction another reaction where carbon dioxide is converted

into carbohydrate. As we have already told the oxygen is liberated by water and we have already through our lecture we have test for the mechanism.

But carbon dioxide gets converted into carbohydrate, the most fundamental reaction by which carbon is being captured or as a matter of fact the major source of bio fuels on earth. So today we are starting next three weeks in other word lecture our thrust area will be bio fuels. In the bio fuel section the way I have distributed initially I thought that in the second module itself we will talk about the origin of the bio fuels in terms of conversion of carbon dioxide to carbohydrate and rich large sugar molecules.

But then I kind of made a slight modification in it instead what we will do in these 15 lectures will talk about will distribute our lecture into two segments two broad segment the one segment where we will talk about how carbon dioxide is converted into carbohydrate large sugar moieties through the process of dark cycle and the involvement in the basic history of this how that happened how it all started.

And what word who are those landmark people who made this happen, role of one of the most abundant enzyme called Rubisco which catalyzes this Calvin cycle or a conversion of carbon dioxide to carbohydrate. Then we will talk about the photo respiration and how that compromises the formation of sugar molecules and from there we will move onto C3 and C4 plant.

So this part not one will constitute the origin of bio fuels next phase will be once this bio fuels are formed how we transform this bio fuels to form will for day-to-day use in automobiles or other industrial settings. So that we phase two so these 15 lectures will be kind of divided into two main fragments as I have told you the formation of bio fuels in the plant or in the Greenwood and part two will be the conversion of those biological fuel or biological materials into more efficient fuels by virtue of which they could be used in any kind of industrial setting as a readily available source of energy.

And in between or maybe we may go on advanced topics we will talk about what are the technologies which are involved in increasing the biomass or the bio fuel of the plant what are the different agro techniques which are employed all over the world.

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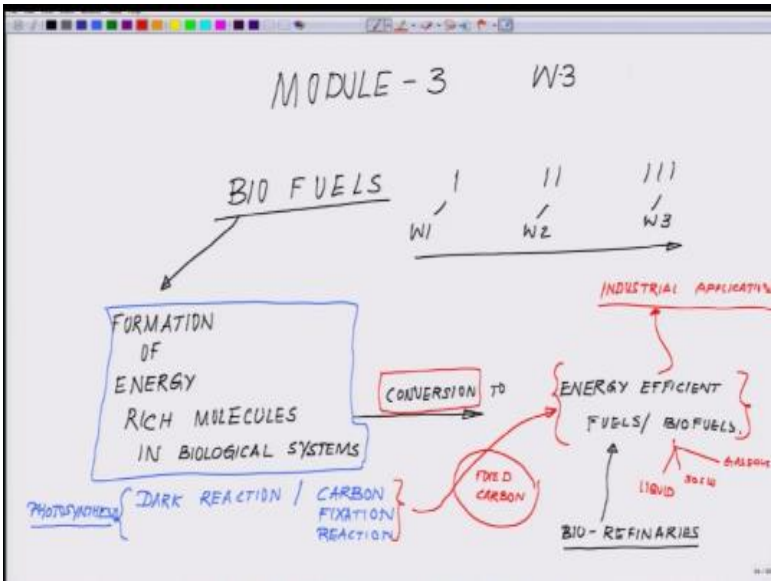

So we are into module 3 which is also week 3 and this is under the heading as you see in the curriculum as bio fuels 1, 2 & 3 which essentially week 1, week 2, and week3 this is how this whole thing is divided. In terms of its content so this is in terms of how this is going to preceding the time and in terms of its content we will talk about the formation of energy rich molecule in biological systems.

And once these are formed their conversion to energy efficient fuels or bio fuels, and there it is out here we will talk about the concept of bio refineries. Now in terms of the detailing and out so here in this segment we will talk about the dark reaction or carbon fixation reaction of photosynthesis.

Whereas in this process is to this conversion we talk about these fixed carbons which are formed, how they are converted into liquid and solid and gaseous fuels for industrial application. So this is the overall scheme of things what we are going to follow.

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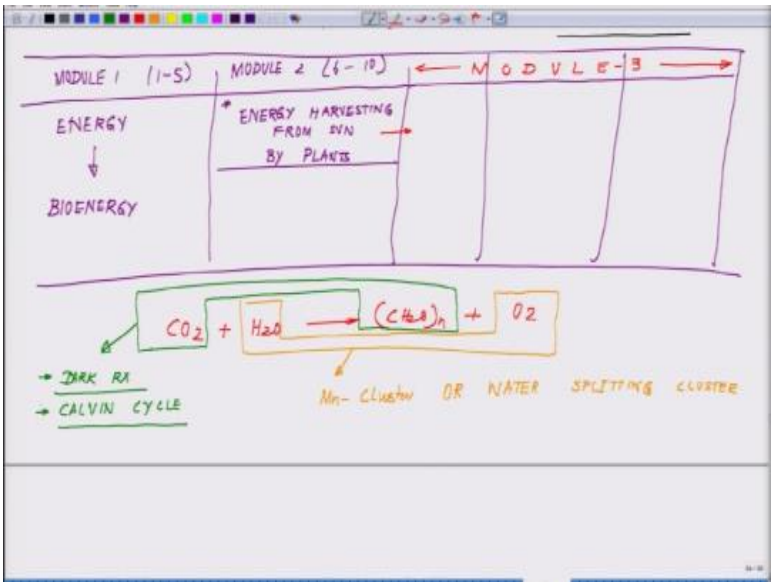

So just to follow up with give you how we are proceeding at this stage so that do not lose track of what all we have talked about in module 1 lecture 1 to 5, we have talked about energy to the concept of bio energy in module 2 which is lecture 6 to lecture 10, we have talked about energy harvesting that is energy harvesting from sun by plants this was the key thing. And now we are moving to module 3 or falls under one common module, module 3 where we will take it further forward as we have talked about the carbon fixation.

So now remember where we ended the last class so again getting back to the reaction where we ended the last class was CO_2 + H_2O forming CH_2O+ oxygen and we have talked about this reaction in our earlier sections and the role of manganese cluster or water splitting clusters. Now we will be talking about this part which is the dark reaction or also synonymous with Calvin cycle.

Now we are talking about this I told you that there are three things which are coming out of the process one you are having oxygen as a by-product.

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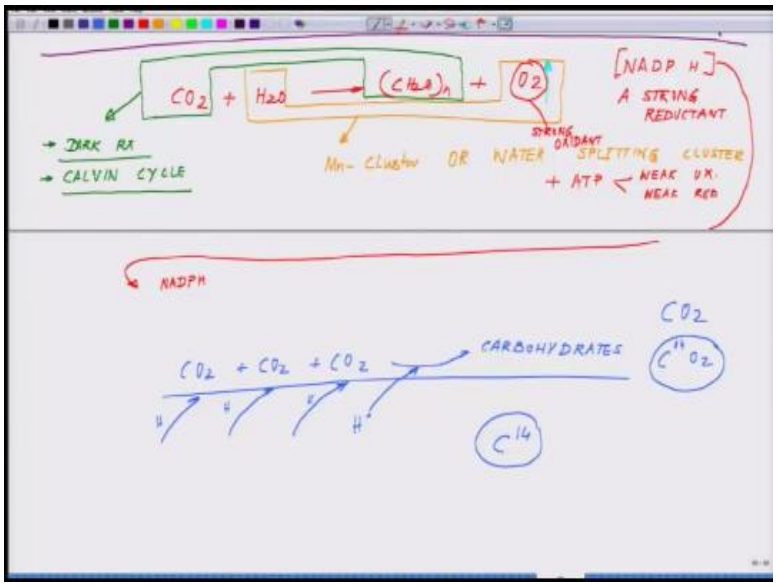

As I have already shown out here you could see that this is one of the by-product the other by-products which are coming out of this whole process we talked about was NADPH strong reductant all of you remember that and whereas oxygen is considered as the strong oxidant, apart from it we talked about their formation of ATP molecules which are weak oxidant and weak reductant.

So now our journey starts with this molecule, where this NADPH is fed. Now when you see this reaction it looks very simplistic, but to tell you the beginning of the century when this reaction of CO_2+CH+ water making CH_2O carbohydrates or plus the oxygen things were not clear how things are not clear, if you look at this reaction. So your first guess what will be is it carbon dioxide's are coming together.

And they are attaching like CO_2, CO_2, CO_2 is making carbohydrate or there is something else and if this thought strikes so what is happening here say for example, CO_2, CO_2, CO_2 and there is

some kind of a hydrogen coming through or in other words there is a reduction taking place like this, you know in the form of hydrogen and it is getting reduced is that the reaction it all started or there is something else and nothing was known.

So the way I am trying to put the story is that i wish you to think that what we read in the textbook like when a things are given all the form we are given that is not the way there is a history behind it and you can only appreciate this biomass formation and a bio energy, when you know the history right who were the people who laid the foundation stone for a field of very emerging field of bio energy.

So as for my wisdom the very first person who made an inroad into this area and rest of his life he is no more who dedicated his life on understanding this concept how CO_2 is getting converted into carbohydrate was Melvin Calvin. Melvin Calvin was an American chemist and around 1945 he joined in the radioactive lab in Berkeley and this was the time of Second World War. So if you guys remember when I showed you the history of things I told you it all started during 1800, 1700, 1800 likewise.

And 1900 for mankind it was one of the very interesting century essentially where radioactive isotopes were discovered followed by DNA structural illustrated much earlier that that during 1945 in the cyclotron there were some of those Radio isotopes which were being formed like C14 carbon that is basically a carbon 14 isotope. So whenever you write CO_2 so which assuming the carbon is 12 observation at 12 atomic number.

So you could have C labeled with C14 isotopes something like that. So you know that this carbon dioxide which has formed has form from C14 because it has a radioactive tag in the form of that additional epic number what you see out there additional mass what you see out there which helps it to be tagged at any point it gets incorporated to it.

And 45 soon after Japanese have surrendered and the whole world was in a turmoil post Second World War time it was during that time Melvin Calvin started his research at Berkeley. And the very thing what they did how this whole dark reaction concept started to pan out was very

interesting. So the organism or the system what they picked up was chlorella to write it down free system one second.

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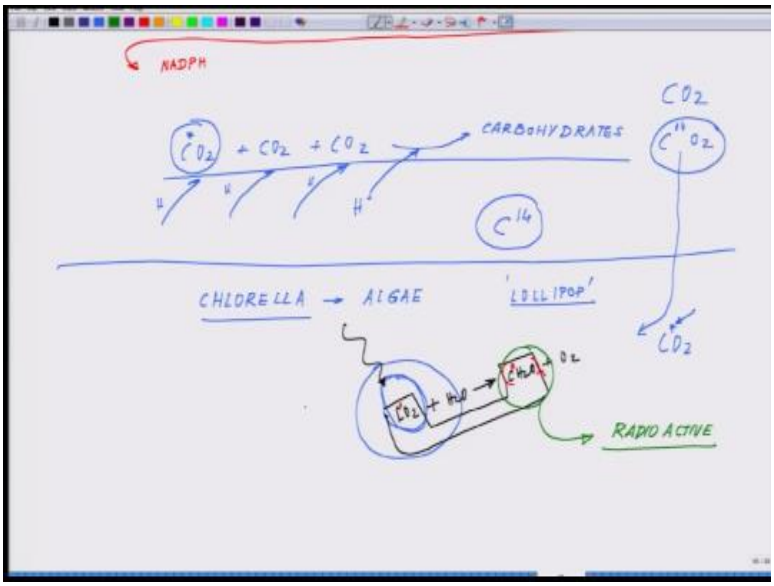

So chlorella is a algae and you can really culture chlorella in the lab and you could have a number of like generations of chlorella. So they picked up chlorella as their organelle of organism of choice to study the photosynthesis. The way they did it was very interesting and they developed apparatus which is lollipop.

So this apparatus essentially what they used to do is that so you have the chlorella there and in that you inject the radio isotope of radio isotopic carbon dioxide CO_2 which is already label I am just putting the level with aspects okay. So now if I told you the reaction just earlier than that if this CO_2 which is labeled now is being taken up whatever it will form if this you have to say so the reaction is like this if example here using of the chlorella okay.

Now this chlorella is it has chloroplast and everything so this chlorella is receiving the light and in that process our thing is that $CO_2 + H_2O$ is forming CH_2O + oxygen okay. So now we are not bothered about the second reaction H_2O_2 we are only bothered about this reaction. Now if this

reaction is taking place now what you will see is that if in the presence of, so if you culture them in the presence of I am just putting a red dot on the CO_2.

So that carbon if this carbon is labeled automatically the CH_2O which is formed will also be labeled. Now if this one is labeled if this molecule is labeled then this molecule will be radioactive because it is radioactive isotope. Now any form of carbohydrate which will be formed as a radioactive so the catch is something else, catch is how fast this reaction is happening like $CO_2 + H_2O$ CH_2+oxygen what they did was. So say for example, there are events which are happening no just help you to visualize what does that I mean.

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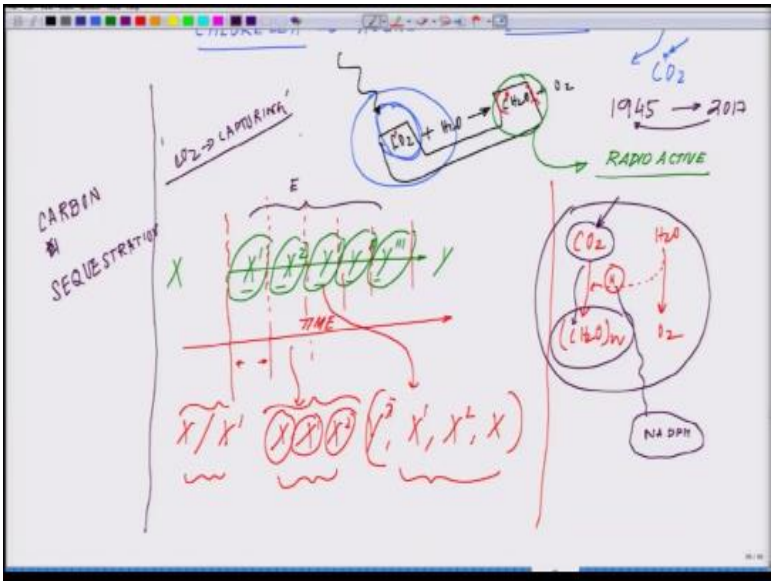

Say for example, this is the compound say X, X it forms Y we know this X forms Y but it does not form Y in one step it goes through a step like say X_1, X_2, Y_1, Y_2, Y_3 likewise and then it forms why say for example, these are the different state through which it passes and these states are function of time.

So on the x axis what you are seeing is a function of time. So now how you can isolate these different states you can only isolate different states if you can mark my word carefully if you can

stop this reaction from X to Y at different time point it stopped at this time point, at this time point, at this time point, at this time point at this time point freeze that moment in other word this particular time this reaction will move any further.

So what you will have in your reaction mixture will be X and X_1. Similarly if you freeze at that time you may have maybe some form of an X if at all it is there may be little of X_2 and Y_1 or X_2 or X_1 and X okay. Similarly if you freeze it at this zone and what we will have maybe you have of course Y_1 you will have X_1+X_2 maybe you may have X you do not know you cannot predict because we do not know how much of each one of this component has converted into the next one.

And now if you this simplistic reaction if you think of it is a very cumbersome process. So their journey was really cumbersome in the when they started it somewhere around 1945. So what they did so they grieve chlorella like this and they freeze the moment how they freeze the moment, so they allow this reaction so that your reaction is out here I am not anymore writing the other reaction.

And there is one more thing I just wanted to add for the simplicity sake I am adding this what is essentially happening CO_2 to CH_2N okay. Now whereas on the other hand what is other reaction H_2O to O_2 right. So this hydrogen is getting fed here which is essentially a reduction reaction this is a simplistic way you can visualize the whole thing and this is exactly what I was trying to tell you this hydrogen is fed in the form of NADPH.

And here what we are trying to figure out how this CO_2 with whom the CO_2 add because CO_2 either there are two options either CO_2, CO_2, to lot of CO_2 coming together by some XYZ enzyme which is present here is converting it into carbohydrate or CO_2 is attaching to somebody maybe there is another carbon source present in the plant or in the green leaves or in the chlorella what is the answer.

So this is the question or we are going to it because why this is so significant to understand this very core of the fundamentals because that is what you will help you to understand the whole

process of modern day what we talked about carbon sequestration or CO_2 capturing. So that is why instead of really going on a very applied aspects of it which is very easy for me to do I wanted to know bounce on you to think rationally that how possibly this kind of reactions are really taking place.

So with this technical how that CO_2 is getting reduced to form CH_2N this is the very, very first reaction which is happening. But then with whom CO_2 is attaching what they did. So how they freeze the moment this is very interesting to really realize it is 1945 always remember the time 1945 and today we are sitting in 2017 and you will look think with of and appreciation for the amount of research went on there.

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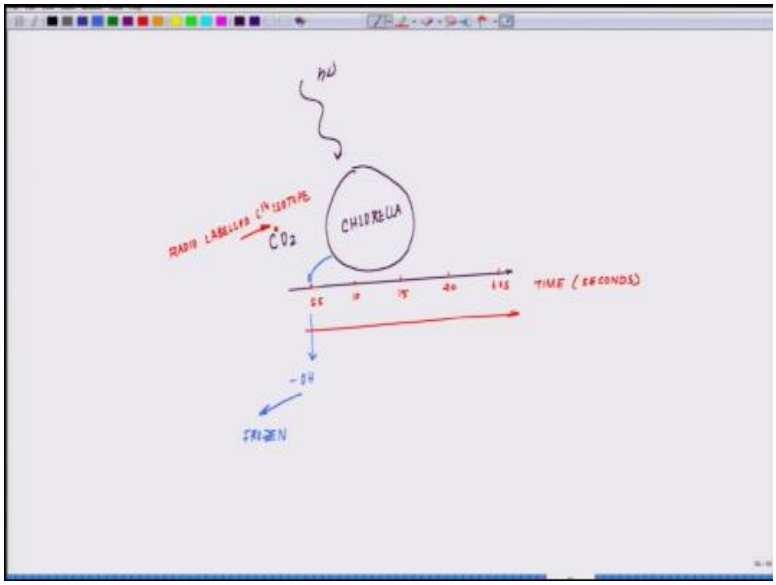

So they allow the reaction so you have the so they took the chlorella here is your chlorella expose it to sunlight Hν here you have the chlorella and allow the reaction to move assuming and of course this is going in the presence of CO_2 which is labeled CO_2 showing the radioactivity okay do not get confused with the free radical it is basically CO_2 which is a radio level so I am just marking it in case you forget radio level C14 isotope.

And you stop the reaction at say 5 seconds, 10 seconds, 15 seconds, 20 seconds, 60 seconds or one minute likewise. So just so how you are stopping the reaction so this is your time in seconds and how we are freezing the reaction. So the way they tried several ways to do it and I will recommend here and I has to recommend here I will supply you the normal lecture. So Melvin Calvin won the Nobel Prize 1965 probably 61 or 65 I have forgotten that well just get that for you read through another lecture.

So what they did at every time point they put the whole chlorella this thing into alcohol and that is where you are freezing everything gets frozen. So we are freezing this moment in alcohol I am just putting OH okay frozen we are freezing the moon I am not saying freezing the idea is the frozen the moment.

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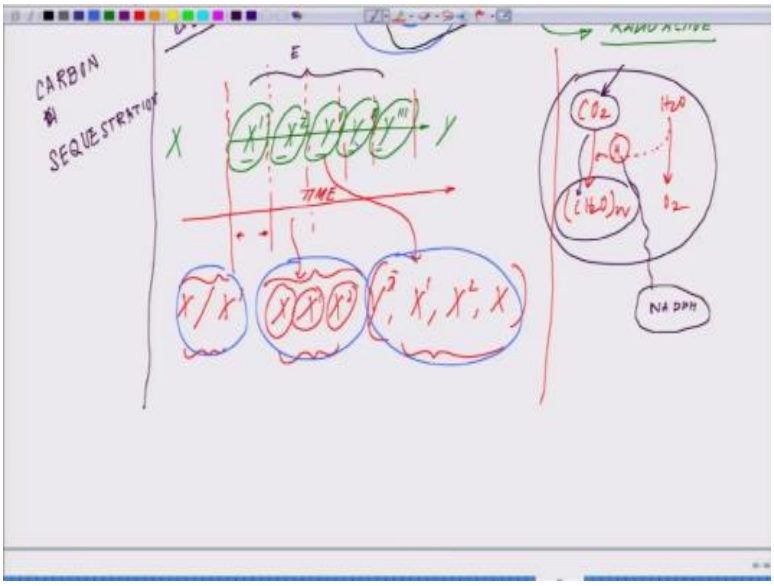

So that so if you follow me in the previous slide what I was trying to explain is something like this you are freezing this moment, you are freezing this moment, you are freezing this moment likewise you are freezing different moment.

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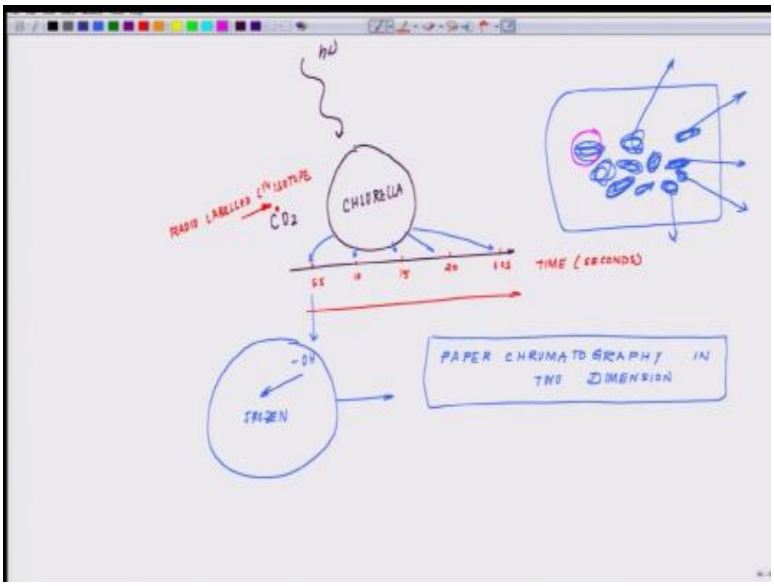

So here what exactly that is what they did they froze the different moment and they put in them al alcohol and then what they did they run them in each one of these whatever the product which is getting formed here they ran it on a paper chromatography column, paper chromatography is one of the techniques by which you can separate out the different product in two dimension okay.

Please go through this technique it is one of the very powerful separation techniques till this date everybody uses those who want to isolate the different molecules which are formed during a reaction. So using paper chromatography they started getting the different products on, but challenge starts there what are those products. Since 1945 for next ten years or so the journey was and if he to show you that the very, very first with the 60 seconds what they got or something if you see the graph it was extremely complex like this.

And a paper chromatographic plate it was, now think of it each one of them are scattered around with different atomic at different molecular mass. So now you have to figure out what this block represent what this represent, what this represents, what this represent, what this represent. So

the more you go on the time side the more larger time window you are looking you are going to get too many of this they wanted to shorten the time window.

So I will close in here today for this first lecture in this section then we will come which were the first one very, very first one they discovered and how they discovered this journey of discovering molecule by molecule and it is one of the most beautiful work which laid the foundation stone of today what we call about the biomass to bio energy and this whole field this is where it all started and from here we will talk about Rubisco.

We will talk about photorespiration, will talk about the C_3, C_4 and then comes how these are being processed okay I am closing here thank you.

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