Metabolic Engineering
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Lecture – 12
Introduction to Metabolic Networks

Welcome to metabolic engineering course. So, today we are going to learn a very interesting topic that is the introduction to metabolic network.

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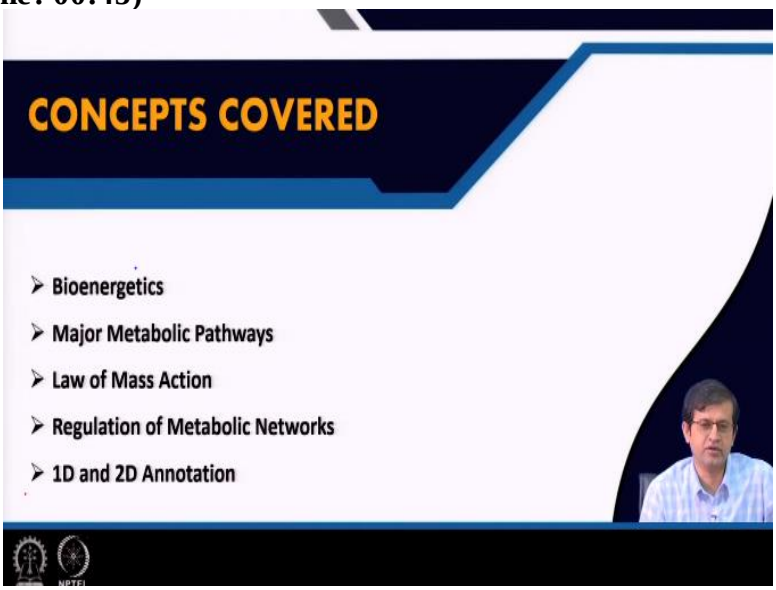

So, in this lecture I will give some background of metabolic networks like bioenergetics and then major metabolic pathways and then laws of mass action and the regulatory of metabolic network and then followed by 1 dimension and 2 dimension annotation of genome sequences. So, this will give you enough background to actually constructing the metabolic network it will give an introduction. So that you can understand or you can make the metabolic network on your own.

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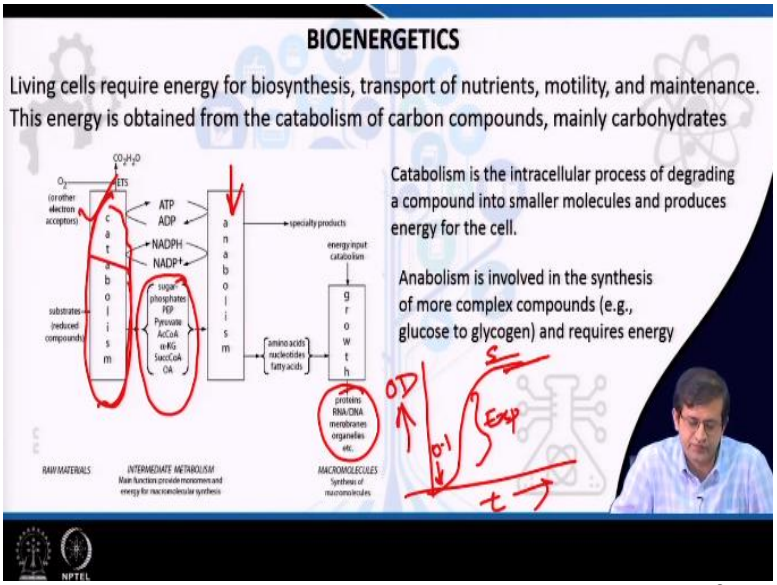

So, first I will start with and that the living cells require energy, so all of you agree that living cells require energy for biosynthesis transport of nutrients motility and maintenance, then how the cell gets energy? The cell actually gets energy from the catabolism of carbon compounds mainly the carbohydrate that we consume the microbial cell they consume only glucose.

So, they consume these substrates which is a carbohydrate and then and through catabolism on the left hand side you can see that the catabolism. So, through catabolism they are actually able to decompose the sugar into small molecules and in the process they get energy, the ATP, NADPH or genetic these are the energy molecule and this is required for all the processes the cell perform.

For example, the biosynthesis of protein, DNA, RNA, transport of nutrient, motility maintenance also all these functions are actually the cells are able to perform because of this energy which is generated from catabolism. So, catabolism is basically the intercellular process for degrading a compound into smaller molecule and also produce energy for the cell. It will not only produce a smaller molecule but also produce that is the essential part of the cell to produce energy.

So that cell conform all its functions and then also it produces key metabolites. So, on this region you can see that these are the key metabolites produced through catabolism and that is super phosphate PEP, pyruvate, acetyl coenzyme, alpha ketoglutarate, succinic, coenzyme,

oxaloacetate and so on. So, there are eleven key metabolites present inside the cell, these are the precursor for many of the compounds.

Or these are present almost in every cell right from microbial cell to the mammalian cell. And this catabolism the metabolic pathways are almost similar for all kinds of organisms right from bottom to top. And these molecules remain as a precursor molecule for anabolism. So, anabolism is a process which actually uses this small molecule and produce complex molecule.

For example, amino acids, nucleotide, fatty acid and those are required for the growth of the cell not only that, we have protein, RNA, DNA, these all macromolecules are actually synthesize through a process known as anabolism. This is already known I am just summarizing. The anomalism is involved in the synthesis are more complex compound that is from glucose to glycogen that is the storage of energy and these processes require energy.

So, the first I told catabolism which produces energy and the anabolism which required energy. So, whatever energy you get in this process, gets utilized in anabolism. So, this way the cell is actually creating its own energy and also utilizing that energy for many other processes. For example, formation of protein, DNA, RNA these are macromolecules synthesis these are the macromolecules which are present inside the cell and because of that we see the cell is growing the cell biomass is increasing.

So, we have I do not know whether you have seen how the cell is growing in a flask. So, if you have done culture or not but generally the cell grows like this. We have a curve like this and then it goes initially with time, this is x axis we have the time and the y axis we have growth and growth is measured in terms of OD optical density put the culture inside a spectrophotometer and then you can measure the optical density to know that how much it is concentrated.

So, initially when you start the culture here you will find the OD is almost point you start with 0.1 OD and then cell grows and the exponential phase we get increased OD which is around more than one 1.52 and then slowly it saturates. After some time you see that there it has reached the stationary phase. So, this is the exponential phase of the cell and this is the stationary phase.

So, this is you already know yes I am repeating it again, so that you make your revise this thing again. So, the cell grows and you see the growth or the biomass is increasing, OD is increasing when that means the concentration of the media is increasing at which the cell is growing. And this is because the cell is making protein, RNA, DNA membrane organelles. So, these are these cells are making through and these give rise to the growth. And then you see the cell biomass is increasing and you can measure the cell biomass using OD.

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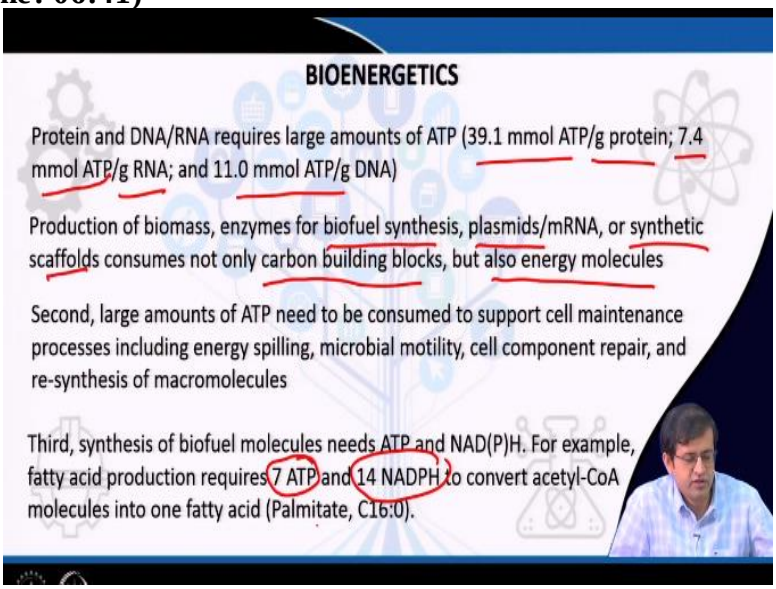

So, next you come to the bioenergetics part like how the protein DNA require large amount of ATP. For making protein, DNA cell requires around 39 millimole ATP per gram of protein then RNA we need 7.4 millmole ATP per gram of RNA and then for DNA we need 11 millimole ATP per gram of DNA. So, these are the ratio of ATP and the energy requirement we have in the cell.

So, the cell you know the protein is the important part of the cell which perform most of the function and that require the synthesis of protein require a lot of ATP molecule. The production of biomass enzyme for biofuels syntheses suppose you want to make a compound inside the cells through metabolic engineering that also requires energy. So, everywhere whatever you are going to make for example I am telling the biofuel production for the plasmid.

Suppose, you are putting a plasmid inside the cell or any synthetic scaffold you are putting inside the cell which is not natural you are expressing heterologously. Then also it not only

consume carbon building blocks the carbon is needed but also it require energy, 2 things are required. First the carbon is required the carbon in the sense that glucose it consumed that converted into carbon.

And then those carbons goes into the building block of this molecule, suppose you are producing biomass or when you are producing enzymes inside the cell. You put the plasmid inside the cell and then that get amplified inside the cell and it forms enzyme or protein or any synthetic scaffold you are putting. So, it not only requires carbon but also request energy molecule.

So, this you should keep in your mind that these are the 2 things you need because when I say that metabolic engineering or manufacturing any product inside the cell will require not only carbon but also energy. Second, large amount of ATP needed to be consumed to support or cell maintenance. As you know apart from building biomass enzymes biosynthesis, you also need ATP to support the cell maintenance.

Processes including energy spilling microbial motility cell component repair re-synthesis macro molecule all these processes require ATP. And third, the synthesis of biofuel molecule needs ATP and NADPH, for example fatty acid require 7 ATP. So, supposes you want to make fatty acid inside the cell it requires 7 ATP and 14 NADPH to convert acetyl coenzyme A into 1 fatty acid molecule that is palmitate that is C16 carbon as number of carbons present in palmitate is 16.

So, this enter process not only require carbon but also ATP molecule or NADPH molecule those are energy molecules which are required by the cell for making this compound in the form of a fatty acid inside the cell. So, these are the constraints the cell have first of all the carbon you are putting in and also the energy this way. If the cell is not having sufficient energy that is ATP molecule then yield will go low.

So that you should know even there you have sufficient carbon in the media but if the cell is actually not have energy then the yield of production will go low. So, these are the 2 factors you should look at when you try to make any compound.

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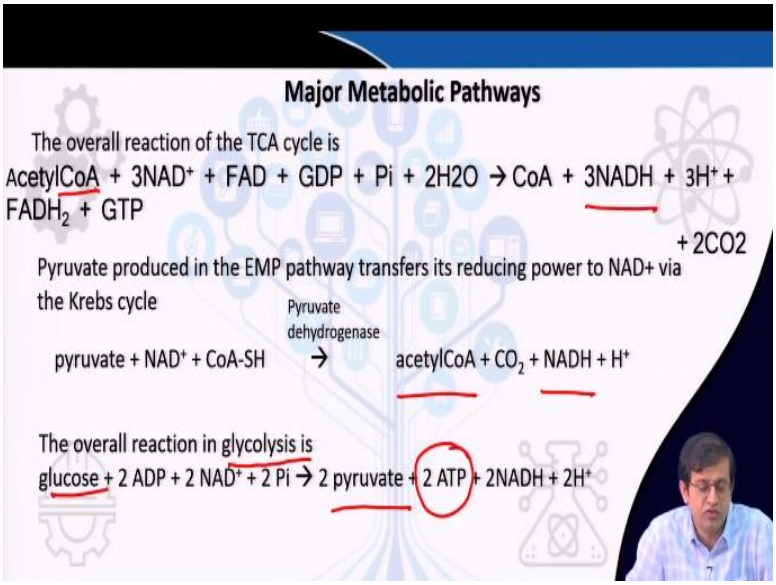

So, these are the major metabolic pathway, you must have gone through already those pathway first is the glycolysis process which is basically glucose is consumed and then 2 molecule of pyruvate it is made and you get 2 ATP molecule. So, in this process you get only 2 ATP molecules through glycolysis and then pyruvate goes into TCA cycle. So, the conversion of glucose to pyruvate is also known as EMP pathway.

And these empty after pyruvate is produced for EMP pathway it is transferred to the Krebs cycle or TCA cycle where in presence of NAD plus the pyruvate is converted into acetyl coenzyme and you have 1 molecules of NADH. Since we have 2 molecules of pyruvate therefore 2 molecules of pyruvate will give 2 molecules of NADH. And then we have the overall TCA cycle reaction which is shown over here the acetyl coenzyme-A, again it is converted into 3 molecules of NADH.

So, each molecule of acetyl coenzyme-A is converted into 3 molecule of NADH and then also produces CO 2. So, this I am showing just to tell you that how many energy molecules are produced per molecules of glucose.

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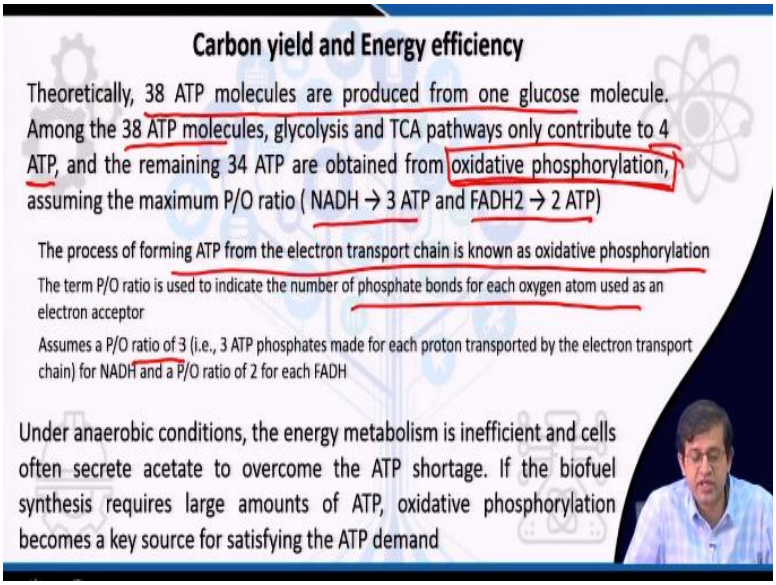

So, if you can estimate that the total theoretical yield that is 38 molecules are produced from one molecule of glucose. So, among these 38 molecule glycolysis and TCA only produced 4 molecules of ATP and remaining 34 ATP molecules are generated through oxidative phosphorylation. So oxidative phosphorylation is the process where you get a remaining 34 ATP molecule and what is oxidative phosphorylation? This is the process of forming ATP from the electron transport chain is known as oxidative phosphorylation.

And the term P / O that is the ratio is used to indicate the number of phosphate bond form for each oxygen atom used as an electron acceptor. So, we generally assume the P / O ratio as 3 when I do when the ratio is 3 then 3 ATP phosphates is made from each proton transport by the electron transport chain. So, it is using this we can assume that for NADH the P / O ratio is 3 that is, for every NADH molecule you generate 3 ATP molecules.

And for FADH you generate 2 NDAH to 2 ATP molecule, so this is the ratio you can use if the P / O ratio goes down then you get lesser ATP molecule. So, the here you can see when the P / O ratio is 3 then the number of ATP molecule for every NADPH is 3 and FDAH 2 we have 2 ATP. So, the combining this many number of ATP molecule you will see that 38 ATP molecules are produced for every one molecule of glucose.

Now under anaerobic condition the energy metabolism is insufficient and cells often secrete acetate to overcome the ATP shortage. If the biofuel synthesis requires a large amount of ATP oxidative phosphorylation become a key source for satisfying the ATP demand. So, the oxidative phosphorylation will become a very important mechanism by which the ATP

maintenance or the ATP requirement is satisfied when you want to synthesize a biofuel or a chemical or any by product from the cell.

So, it is always keep the P / O ratio to 3 but if it goes down then you there is a deficiency in energy, so your yield goes down. These are the constraints you have are the metabolic burden you have inside the cell when you try to make new molecule. You know anyone who tries to make a new molecule there is a metabolic burden inside the cell.

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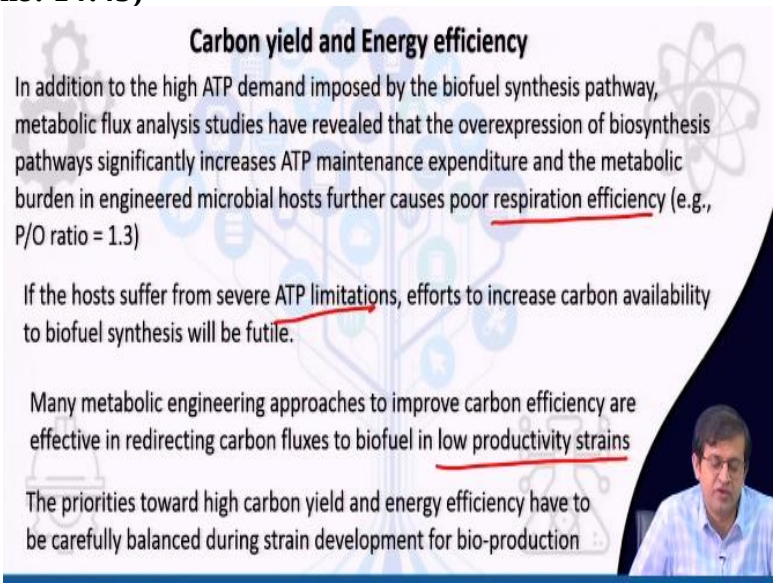

So, then, in addition to the high ATP demand imposed by the biofuel synthesis pathway or chemical synthesis pathway inside the cell metabolic flux analysis studies have revealed that the overexpression biosynthesis pathway significantly increases ATP maintenance expenditure and the metabolic burden in engineered microbe further causes poor respiration efficiency. So, as you add new metabolic pathway inside the cell or any heterologous pathway you are adding inside the cell then the ATP demand increases.

So, significantly ATP demand increases and because of that we have a poor respiration efficiency that is a phosphorylation process P / O ratio goes down from 3 it become 1.3. So, that also becomes a constraint in this the hosts suffer from severe ATP limitation the effort to increase carbon availability to biofuel synthesis will be useless. So, if there is cell is undergoing severe ATP limitation.

Then what will happen and as much as you keep substrate for the cell that is useless because the cell is actually suffering through ATP limitation. Many metabolic engineering approaches

were applied to improve carbon efficiency are effective redirecting the carbon to biofuel in low result in low productivity strain. So, you try to give as much carbon to the media but he actually said is not able to consume because of the energy limitation.

Because they lacks ATP inside the cell and that is why your biofuel productivity or the production level give rise to low productivity of the strains. So, the yield goes down so priority should be given, so that the carbon yield and the energy efficiency have to be carefully balanced, we have to balance these 2 things the carbon yield and also the energy efficiency that is the amount of ATP production said the cells should balance each other. So that you have better yield in terms of carbon, these 2 things you have to keep in mind when you design new cell form for production of new compounds through metabolic engineering.

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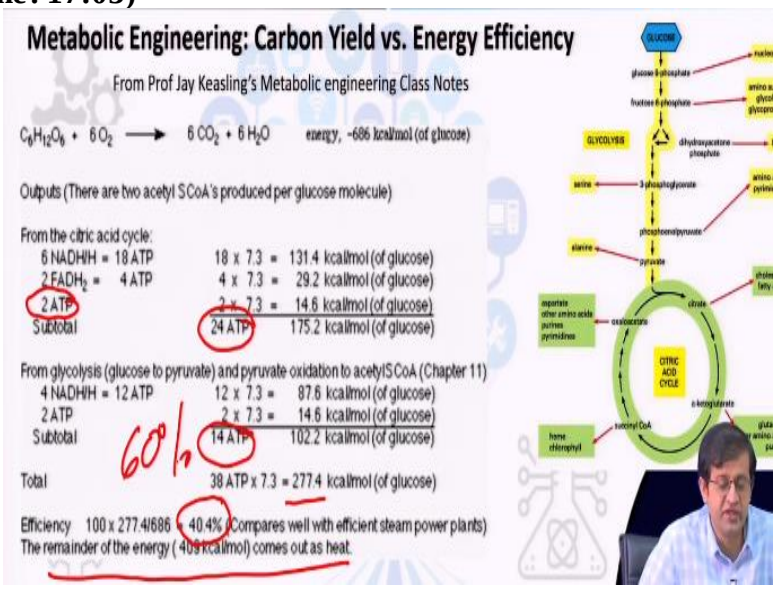

So, this is this slide which is taken from Professor Jay Keasling metabolic engineering class notebook and then you can see the first equation is the glucose is consumed inside the cell and it is producing 686 kilo calorie per mole. So, this much energy is produced when glucose is consumed. And then we have the ATP how many ATP molecules are produced you can see from glycolysis it is producing 2 ATP molecule.

And for each molecule you have 7.3 kilo calorie per mole and from glycolysis also it produces 4 NADH which give rise to 12 ATP, 4 into 3 12. So, we have 12 ATP coming from NADH very considered P / O ratio 3 and 2 ATP molecules gives rise to 14 ATP molecules is from the glycolysis and from the TCA cycle we have 2 ATP molecules as discussed earlier.

And then we have 6 NADH and 2 FADH combined we are getting a total 24 ATP molecule from TCA cycle.

So, if you some 24 + 14 what you get is basically 38 ATP molecule and each ATP molecule has energy around 7.3 kilo calorie per mole that give rise to total of 277.4 kilo calorie per mole that is generated from glucose. Then when you calculate the efficiency of the cell you will see that at 277.4, energy generated because of the ATP molecule divided by total energy produced by the glucose.

So, it only produced only 40% of the energy which is represented by ATP molecule inside the cell and remaining 60% of the energy which is coming as a heat. So, the remaining energy comes out as a heat, so 60% of the energy goes out as a heat. So, now we can think the cell is now not that very efficient in converting the energy only 40% of the energy is useful and remaining 60% goes out as a heat.

So, when you design the cell you can think of the major constant in the microbial cell it is not going to increase that 60% loss as a heat you cannot improve. So, when you consider a battery or lithium ion battery the energy they are designing new battery over time and they get much more efficient battery, the energy component conversion, conversion is better over time every day they are improving.

But in the microbial cell this constant will remain as it is not going to change over time it is not going to change. So, keeping this constant in energy, you have to think and design better microbial cells, so that the production is efficient and the efficiency is better.

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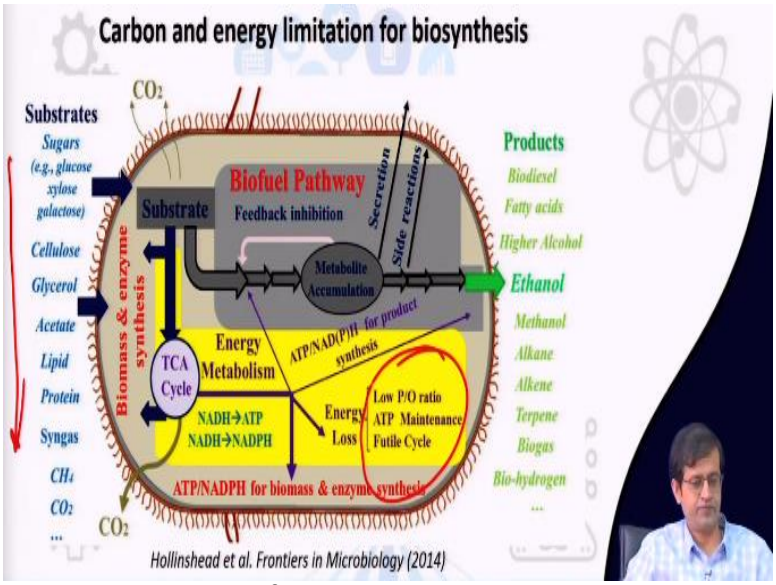

So, the carbon and energy limitation for biosynthesis is also you can see this cell where these are the substrate on the left hand side, the substance or like sugar which is in the form of glucose, xylose, galactose and also consume glycerol, acetate, lipid, proteins, syngas. So, these are the substrate the cell grows on and then the product you are looking from biodiesel, fatty acid and ethanol, methanol, alkenes.

So, these are the molecule as a product you can actually make but inside the cell you can see that the energy loss each glucose molecule it consume and the energy loss which is around 60%, the energy the 40% of the energy converted into ATP and NADPH molecule. So, this energy loss which are because of the low P / O ratio and the ATP maintenance and then several futile cycles this actually plays a major role when it comes to the amount of loss the cell is making.

So, this thing to keep in mind that the energy loss the cell is making for every glucose molecule is not going to improve over time, so how much ever engineering you do but the energy laws remain the same. And then that is the major bottleneck in microbial cell factories when you make your microbial as a cell factory, this thing you have to keep in mind.

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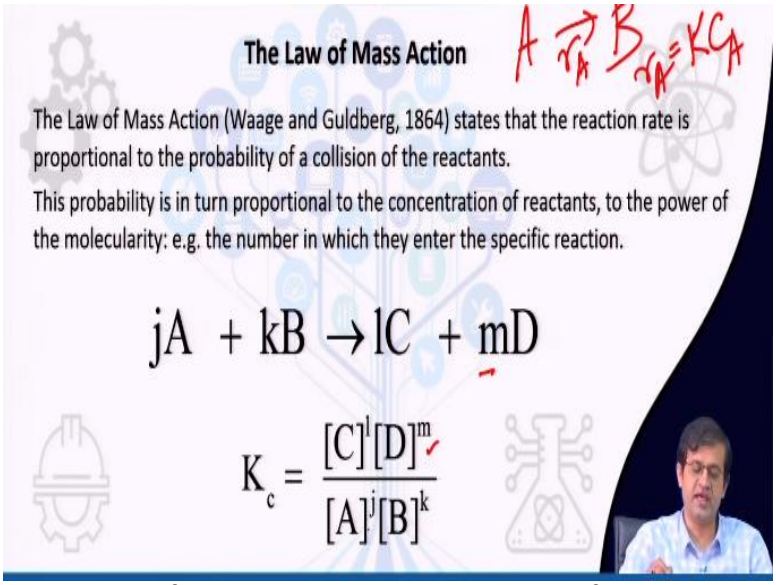

So, now I go into the law of mass action where the rate of reaction is proportional to the probability of the collision of the reactants. So, this is suppose I calculate the rate of a reaction that is A = B. So, I make the rate by which A is converted into B and we written it as r_A then the r_A become $K \times C_A$ that is a concentration. So, the rate of reaction will depend on the concentration of the reaction and the kinetic parameter K, the kinetic constant which is included in the rate of the reaction.

So, and this equilibrium constant you can get for any reaction for example, I have shown 2 reactants A and B and the product C and D for this reaction the equilibrium constant is given by the concentration C multiplied by the concentration D to the power the coefficient. So, D has a coefficient m. So that is why get the power here and then divided by the concentration of the reactant that is A multiplied by B. So, this way you get the constant and the rate of the reaction can be determined by $r_A = KC_A$ for these reactions while K is the equilibrium constant.

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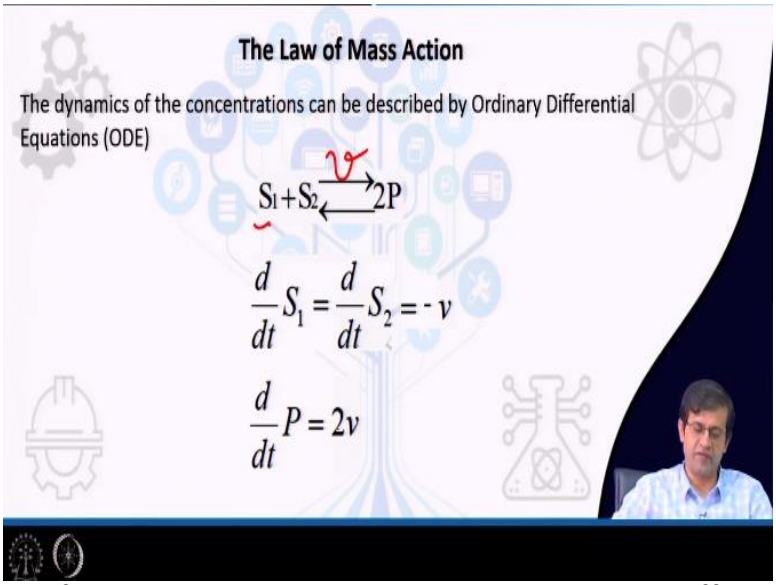

So, now the laws of mass action can also be represented by a differential equation, the previous one is a representation the other representation is through differential equation. Then the differential equation the change in concentration of the reactant S_1 and S_2 actually depend on the rate of the reaction that is v that is a flux. So, here we represent the reaction as v and that is the rate at which the mass is flowing.

And this rate v is basically the time derivative of the concentration of S_1 and S_2, so the change in concentration is actually minus of v that is the flux going through that reaction. The minus sign is coming because the concentration of S_1 is decreasing of time and the concentration of S_2 is also decreasing of time. So that is why you have $dS_1 / dt = - v$ and also dP / dt is equal to you have 2 multiply by v is the rate of the reaction is v going forward and also backward reactions also v then it is 2v. dP / dt that is the change in concentration of product P is equal to 2 multiplied by v ($P=2v$) where v is the rate of the reaction for this reaction.

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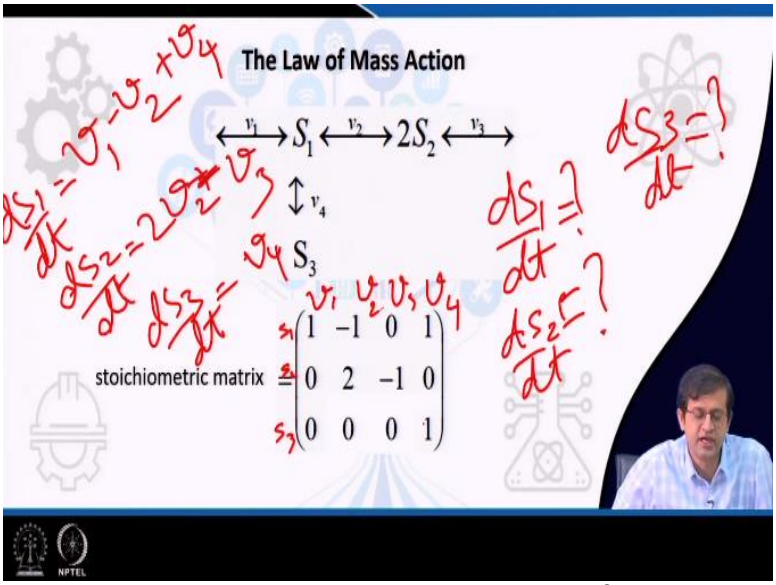

So, to do that you can actually make a stoichiometric matrix, for example you can calculate dS_1 / dt. So, this equation you can write then dS_2 / dt and dS_3 / dt. So, this reaction you can time derivative of the concentration you can write down any you can have equation and this equation you can represent it in a stoichiometric matrix, how stoichiometric matrix represented? It is basically you consider the column as the reaction.

So, the first column is v_1 and the second column is v_2 and the third column is v_3 and fourth column is v_4 and the metabolites you can represent as a row. So, first metabolic you can put S_1 and then S_2 and then this is S_3. Now we can see if you can write the equation for the dS_1 / dt, what is dS_1 / dt? dS_1 / dt is basically v_1 because it is v_1 is positive is $v_1 - v_2 + v_4$. So, similarly for dS_2 / dt you can write, so there is known v_1, so it is only v_2 and v_3, so you are getting $2V_2$ plus V_3.

So, this S is actually going in that direction is minus v_3 and similarly, for dS_3 / dt you have only plus v_4. So, if you can put this coefficient here for S_1 for the metabolites S_1 the coefficients are v_1 is 1 and v_2 is minus 1 and then v_4 is 1 and there is v_3 components here that is why v_3 is 0. Similarly for dS_2 / dt you have 0 that is v_1 is 0 coefficient for v_1 is 0 and then for v_2 is 2 and then v_3 we have minus 1 and for S_3 we have 0 0 1 in this way you can represent the stoichiometric matrix for any metabolic network.

This is a small metabolic network where you consider the metabolic S_1 S_2 S_3 and corresponding reaction flux that is v_1 v_2 v_3 v_4 are represented here and you construct a

stoichiometric matrix based on the network you design here. So, this is a simple representation of laws of mass action.

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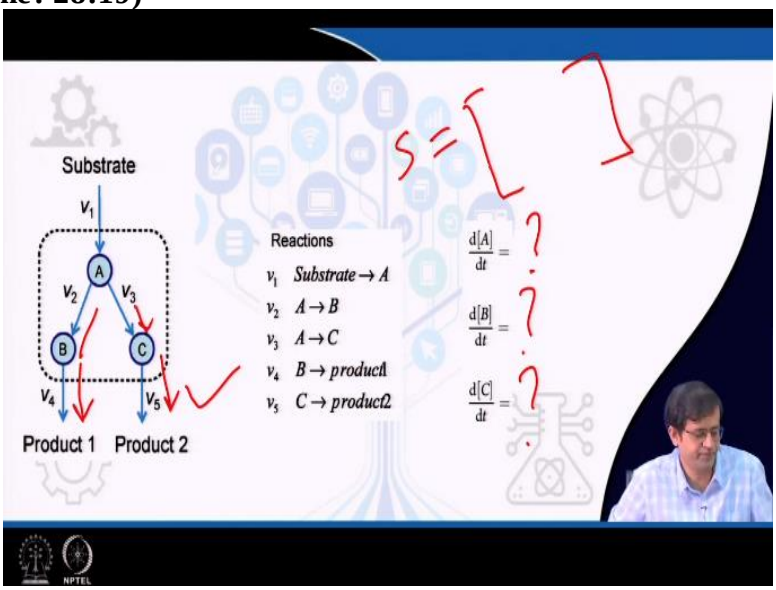

Similarly, you can actually consider a small network here and where the substrate is converted into a metabolite A, metabolite A is converted to metabolite B here and then metabolite A is converted into metabolite C and then we have the product 1 and product 2 that is going in this direction and in this direction. So, we have product 1 and 2 from metabolite B and C respectively.

Now we want to calculate what is dA / dt? What is dB / dt? And what is dC / dt? So, we calculate and make these stoichiometric matrix, these matrix which I discussed in the previous slide, can you make these stoichiometric matrix for this small network by constructing the time derivative of the concentration that is dA / dt, dB / dt and dC / dt and I give you this as exercise you construct the S matrix based only previous slide.

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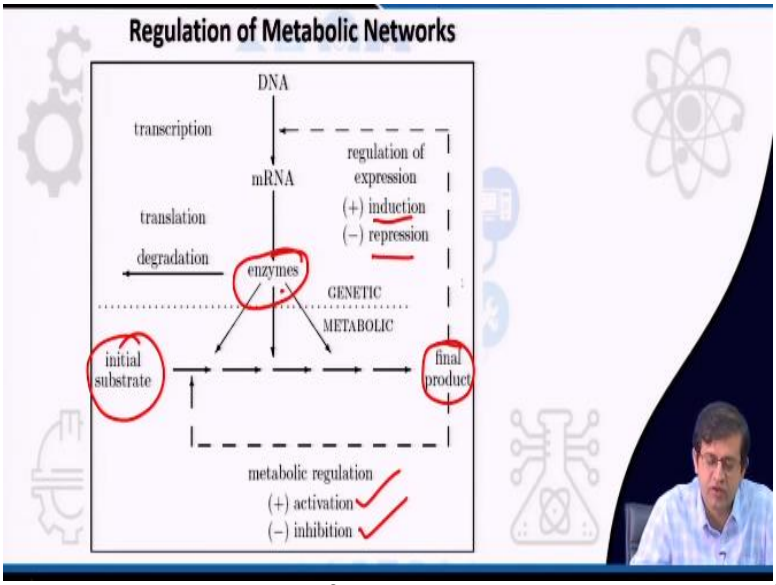

So, now we will go to the regulation of metabolic network, what are different regulation parameter you should remember learn metabolic regulation earlier, also but I am just going through it in a different scenario. So, here you can see that initial substrate is converted into a product. So, you have an enzyme from DNA you get the mRNA and from mRNA there is a protein and this protein is catalysing so many reactions.

So, this we have a chain of reaction and it is giving a final product, so from initial subset you are getting a final product. And then you can see this initial substrate can actually regulate in the sense that it can activate or inhibit the enzyme. So, the product it is forming it can actually act on the enzyme as well. So, it is, this kind of regulation is known as activation or inhibition.

And this goes through because of the allosteric mechanisms the allosteric mechanism of the protein you from allosteric mechanism; you know that the small molecule that the metabolite inhibit or they bind the binding site which is actually made for this the ligand. So, instead of original ligand, they bind on the protein surface and then try to inhibit the protein and this way you actually the activity of the enzyme is lost.

So, finally, what you see the activity of the enzyme is lost because of the metabolite which is produced from the reaction either the final product or the intermediate product. They act on the enzyme and then inhibitive and these inhibitions actually decrease the activity of the enzyme or it can activate also. So, there are 2 ways either it can activate or it can inhibit most of the time it gets inhibit and you do not see the final product.

And other way these metabolite and also interact with the DNA. So, it can bind the DNA and it can inhibit, it can regulate the expression the final product. So, this can be induction or repression, it can either it can induce expression of the gene or it can repress the expression of the gene. So, there are 2 ways it can happen either it can go interact with the enzyme or it can interact with the DNA itself.

And inhibit the expression or the repress the expression of the enzyme or inhibit the expression of the function of the enzyme. So, when these small molecules inhibit the enzyme and also this molecule can also repress the expression of that and DNA.

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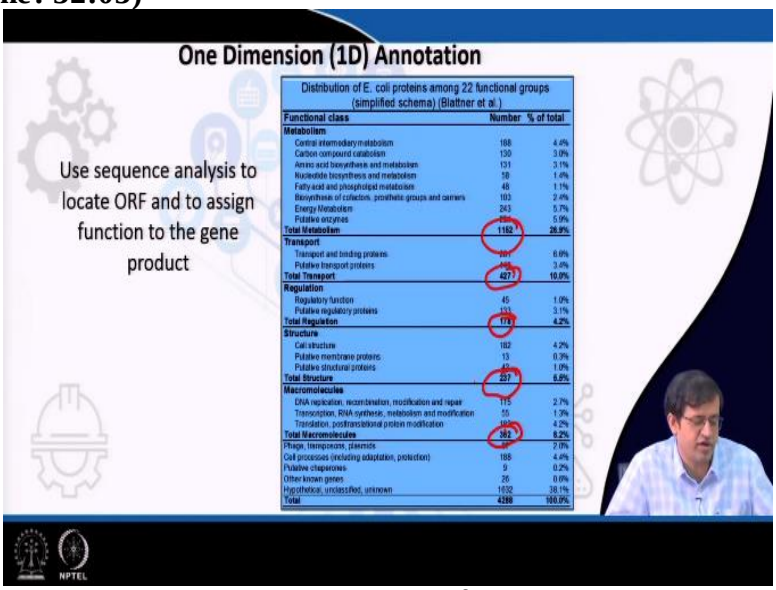

So, I will come to the one dimensional annotation of the genome sequence, so this is the one dimensional annotation, you can see that the metabolic any metabolic gene genome sequence can be annotated based on the open reading frame in the previous class I told that how you actually locate the open reading frame that is the start codon and the stop codon and based on that equation assign a function to the gene.

So, you can see the metabolism the functional class like we have the how many genes are actually involved in metabolism. So, we have around 1152 genes, then you go to the transport and how many proteins is actually involved in transport and then how many proteins genes are actually involved in the regulation. And so on. So, in macro molecule, how many genes are actually involved in macromolecules, how many genes are involved in structure cell structure?

So, you can actually assign function those gene, so in this way we annotate each and every gene, the first you identify how many genes are there and then give function to the gene and this is known as a 1D annotation or one dimension annotation.

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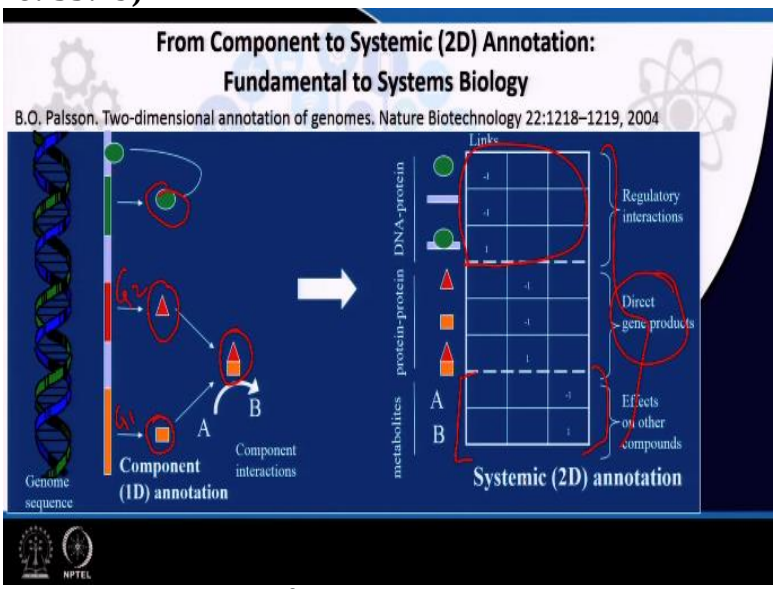

Now we come to the 2D annotation from the genome sequence, we start with the genome sequence and identify how many genes are there and then give function to this gene and then this is 1D annotation. So, then you try to see the interaction like how this gene product is actually involved in a reaction. So, you can see this is the protein 1 and protein 2 they form 2 subunits. So, in a protein you are seeing that there are 2 subunits and it is coming from 2 different gene.

So, gene 1 and gene 2, so these 2 gene giving rise to gene product or the peptide which formed the subunit of a given protein and then it is catalysing the reaction which goes from A to B. So, A is the metabolite and B is the metabolite and is converting metabolite A to B in presence of this enzyme and this enzyme is actually coming from 2 gene, gene 1 and gene 2 and it may happen that the protein is actually formed from the gene actually binding the DNA, so many possibilities are there.

Now this component interaction can be represented in a matrix form. So, as I have shown the stoichiometric matrix, this stoichiometric matrix is used for metabolic reaction but also you can make a matrix. So, in the bottom of this is stoichiometric matrix where I have shown where you have the metabolite A and metabolite B and the columns are the reaction.

And also you can represent protein-protein interaction, If the 2 proteins are interacting then it is 1 if it is not interacting it is actually inhibiting then it you can represent as minus 1 and also protein DNA interaction also you can represent that when protein this protein is interacting with the DNA then it is plus 1 and if it is not and it is not interacting then it is minus 1. So, these are some kind of mathematical representation you can make from the genome sequence from this you know annotated.

The genome sequence and find the component and how the components are interacting how they are linked together that you can represent in a mathematical form in the form of stoichiometric matrix, entire interaction in the genetic circuit can be represented in the mathematical form and in terms of plus 1 and minus 1. So, these are the regulatory interaction in the first part you can see, this is the regulatory interaction where you have the protein-DNA interaction are represented.

And the direct gene product interaction that is a protein-protein interactions are also represented in a matrix form and then their effect on the compound are also represented by the stoichiometric matrix.

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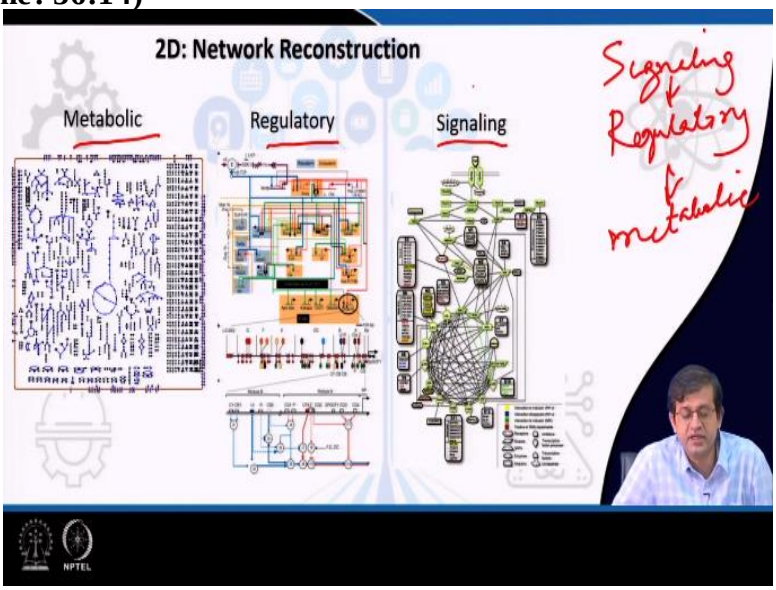

So, the 2D network reconstruction gives rise to metabolic network, so first one is a metabolic network we have the metabolites as node and the connections are the reaction and then you have the regulatory networks. The regulatory network is basically when you have the protein DNA interaction which is actually regulate the enzyme ultimately, you can actually make the regulatory network because of the 2D annotation.

2D annotation is basically the interaction of this component that is the gene product the how the gene product are interacting with the DNA or with the interaction within themselves, interaction or protein-protein interaction and then the protein DNA interaction can be represented in a regulatory network and then we have this signalling network, signalling network is again whuch very less understood.

Where you have the metabolite which gives us a stimuli inside the cell and this stimuli generate some kind of impulse and get an information is transferred inside the cell where some gene is expressed through signalling comes because of the impulse of certain metabolites present inside the cell which triggers some signalling. These 3 networks are actually form a cascade of networks.

So, first signalling and then regulation and then metabolic networks, so 3 networks can be placed one after another, first the signalling network and then we have the regulatory networks and then we have the metabolic networks. So, these 3 networks can be integrated together to in order to understand the model this enter cell, if you want to model this enter cell then these 3 networks the signalling network or the metabolic network, the regulatory network they can be combined.

Where you can understand the cellular behaviour better the entire because when you consider the metabolic network, there is known regulation you assume that all metabolites are available but once you apply the regulatory data we assume that the transcription factor which regulate the DNA where it binds. So, the regulatory part when you include then not all the metabolite form inside the cell and how the transcription factors are triggered is because of the signalling.

So, in this way, the entire mechanism can be represented in a 2D network reconstruction. So, this we will learn in more detail in subsequent class.

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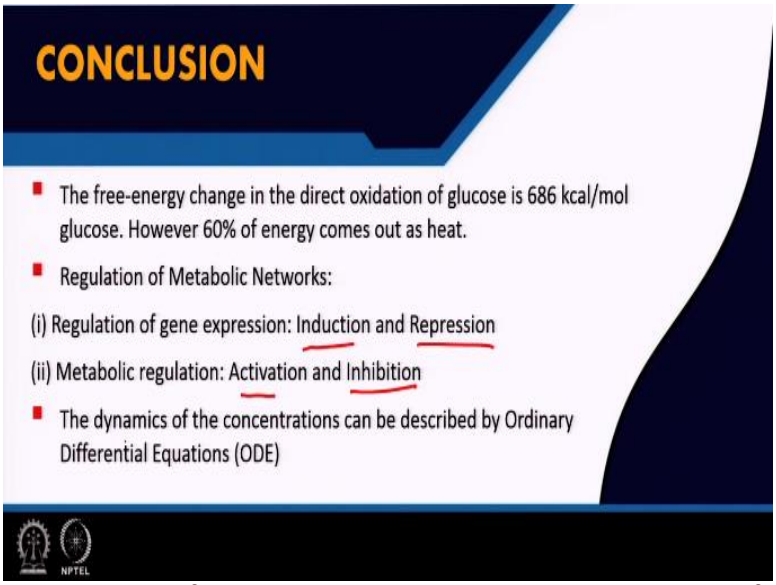

So, in conclusion, you see the free energy is change the direct oxidation of glucose is around 686 kilo calorie per mole of glucose for each molecule of glucose and then we saw that 60% of energy comes out as heat. And we also learn about the regulation of metabolic network the regulation can be at the gene expression level also at the metabolic regulation. That is in the gene expression regulation can be again classified into 2 components the induction and the repression.

And the metabolic regulation it can be divided into 2 parts that is activation and inhibition. And also we have seen that the dynamic of the concentration can be described by ordinary differential equation. So any metabolic network, a small metabolic network you can actually represented by ordinary differential equation and this is actually helpful for giving a mathematical representation in a network in terms of stoichiometric matrix. We will go into more into stoichiometric matrix in subsequent classes.

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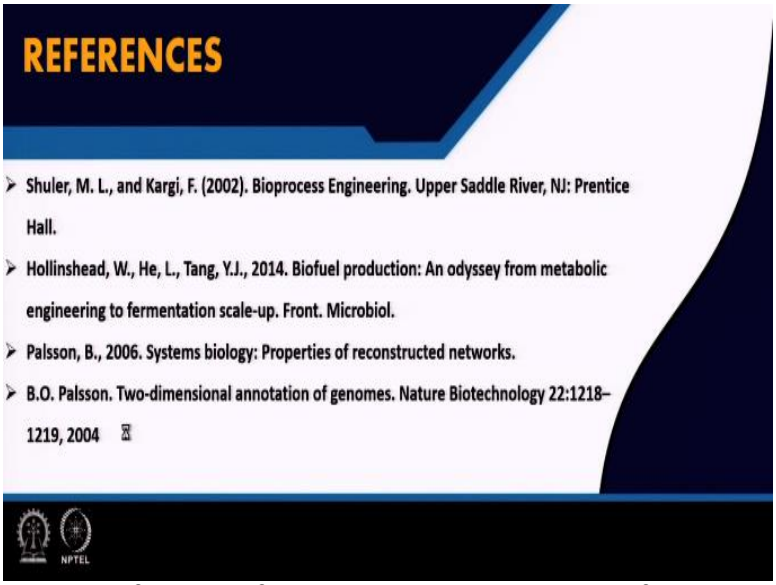

So, now, these are the references for this class where we can follow these references for further understanding and I conclude this lecture today. Thank you.