Metabolic Engineering
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Lecture - 21
Robustness Analysis and Phenotypic Phase Planes

Welcome to metabolic engineering course, today we will discuss about the robustness analysis and phenotypic phase plans for the metabolic network.

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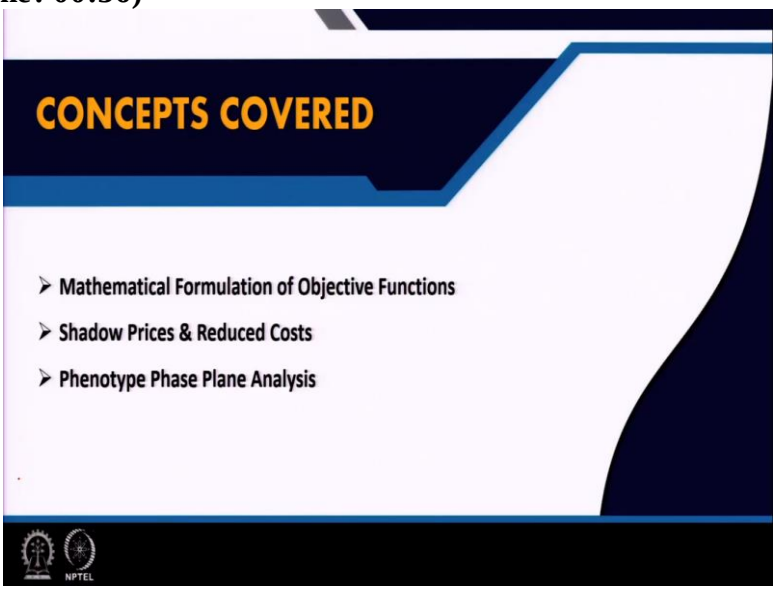

So, the topics will be covered today is about the mathematical formulation of objective function and then shadow prices and reduced costs analysis, phenotype phase plane analysis. From this network analysis the different optimization scheme will be used to actually understanding the overall network how you can actually design and how you can bring out new properties?

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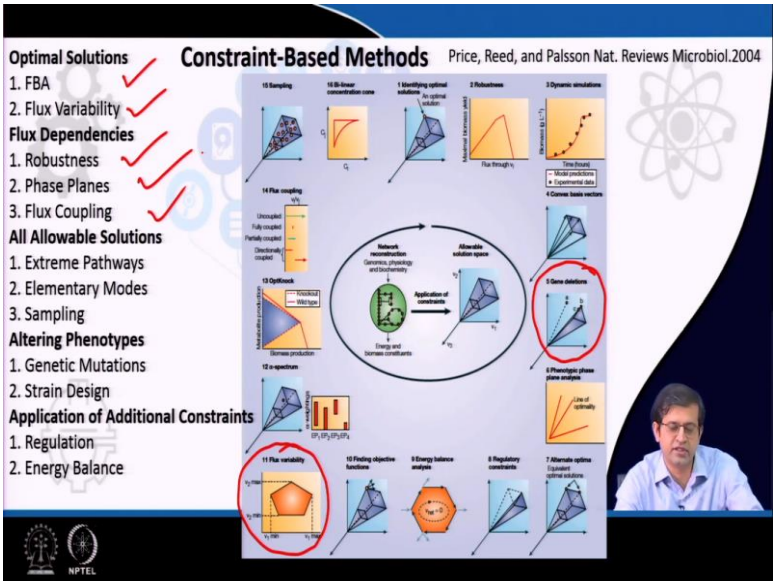

So, these are the constant based methods which are already available in the previous class we discussed about flux variability analysis. So, this one we discussed. So, we actually have a range of fluxes that is v 1 min and v 1 max, v 2 min and v 2 max. So, these ranges of fluxes we can estimate because there are many multiple solutions which exist in the biochemical network.

So, given an objective function we can have a range value of fluxes which can give raise to the objective function that is the biomass objective function value which is unique. So, the biomass objective function for a given network is unique just the all other internal fluxes may have a range which can goes from low minimum value to maximum value and any value within that range can give rise to the maximum biomass.

And then we discuss about gene deletion algorithm, this we have discussed in the previous class we learned about the FBA deletion, MOMA deletion and ROME. So, these are the 3 techniques we learned. So, many things you can do by constraint based method FBA that we learned the flux variability analysis and today we are going to learn about robustness, phase plane, flux coupling we already learned in the previous class. So, as we progress we learn more or more of these constraint based method. Using the metabolic network we can do various analyses constraint based analyses to understand the network.

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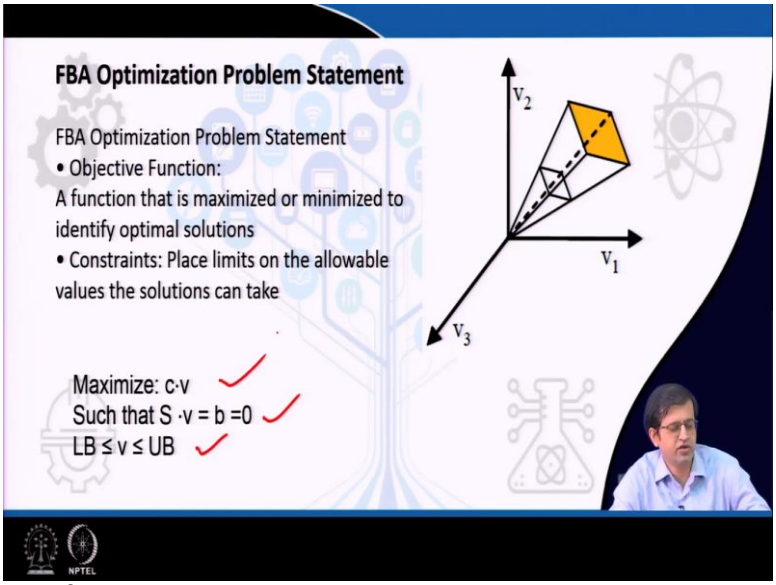

So, this is the FBA formulation that we discussed previously the FBA optimization problem for every objective formulation actually starts with the objective function. So, a function that can be minimized or maximized to identify the optimal solution and the constraint you put on the allowable fluxes that is the lower bound and upper bound of different regulatory constraints in terms of fluxes you can put and then you have the steady state approximation that there is no metabolite accumulation and then you maximize the objective function.

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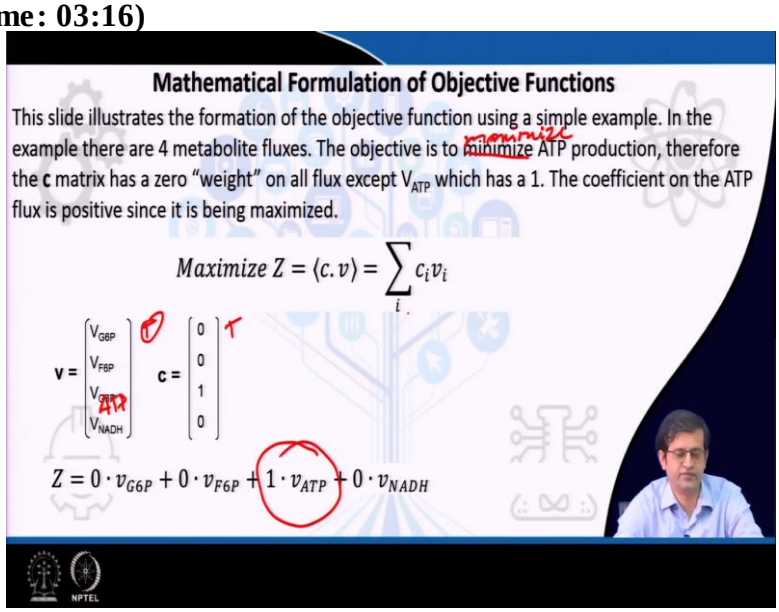

So, this formulation of maximization is basically is represented in terms of four reaction. So, we have seen the slide illustrates the formation of objective function using simple example. So, there are 4 metabolite fluxes, so there are more metabolite fluxes we have and the objective function you have to design is actually maximize the ATP production this will be maximized, to maximize ATP production therefore, the c has 0 for all other the c you define a

column vector c that have all the components 0 except where ATP is there, we have the V ATP.

So, the only V ATP that position we have 1 and other values are 0. So, when you multiply the c dot v, we actually get the 0 multiplied by they should be transposed. So, then if you multiply c dot v then this would be a column vector. Sorry, this is transpose, we can cancel this. So, when you multiply c dot v, then what you get basically 0 multiplied by v G6P and then 0 multiplied by flux vector F6P and 1 multiplied V ATP.

So, all other terms get 0 because you are multiplying by zero with all other fluxes except V ATP. So, you maximize since I want to define an objective function where I want to maximize ATP production. So that is why I kept the c to be nonzero at V ATP position. So, the coefficient of ATP flux is positive since it is being maximize. So, this way you define the objective function and remaining constraint S dot v = 0 and a boundary condition you already know.

This way you can formulate the objective function. So, any biomass you can optimize any flux also you can maximize or minimize. So, in flux variability you have seen that you are minimizing and maximizing fluxes provided and your objective function that is growth rate you are keeping fixed to that maximum value, so this way you can formulate an objective function and define cv, the v is basically is a column vector and c is a row vector.

So, c is a row vector. So, when you multiply c and v then you get one value that is V ATP that you want to maximize. This one you want to maximize or maximize V ATP provided you have c vector and v vector which is basically summation of c and v.

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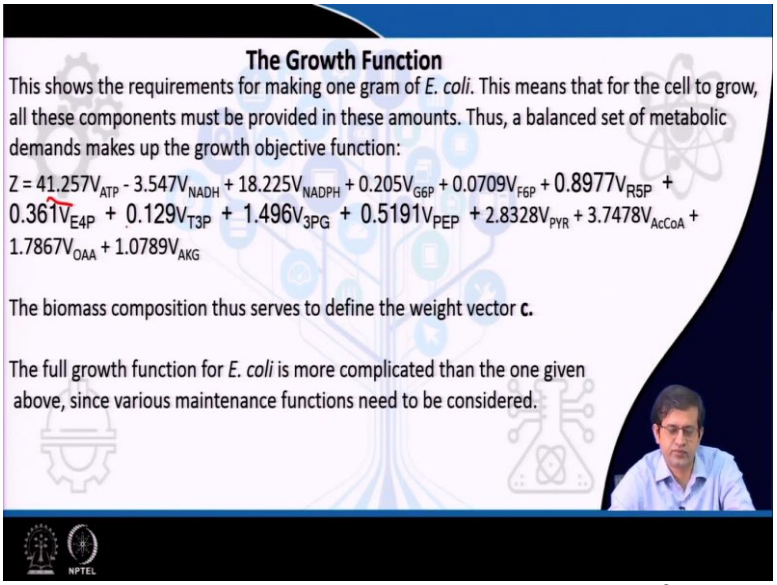

So, the growth function which you have already know the growth function that required and this shows the requirement for making 1 gram of E. coli. So, this is the growth functions which have been defined here, see the Z. So, Z we are maximizing here. So, Z you can define as a linear combination of different component in the biomass. So, we need V ATP multiplied by the coefficient this coefficient you get it from experiment.

So, this coefficient you get it from experimental value for 41.257, 3.547 for NADH. So, these are the component you have to see for each cell it varies from cell to cell. So, this is defined for E. coli but suppose if you use Saccharomyces cerevisiae this component will change the coefficient, the coefficient value will be changing. So, this means that for cell to grow all the components must be provided in this amount for getting some growth in the cell you have to this much flux that is the coefficient value you should be able to the cell should provide otherwise it will not grow.

So, thus a balance set of metabolic demand makes up the growth objective function which is shown over here the biomass composition thus solve to define the weight vector c, the full growth function of E. coli is much more complicated than one which is shown over here. Since various maintenance functions need to be considered. For simplicity we consider a simple equation where the coefficients are shown over here which is experimentally determined and then you put it as objective function for the growth.

So, this the NADH, how much NADH? How much NADPH? G6P, F6P the amount it is required that comes in as a coefficient in the biomass equation.

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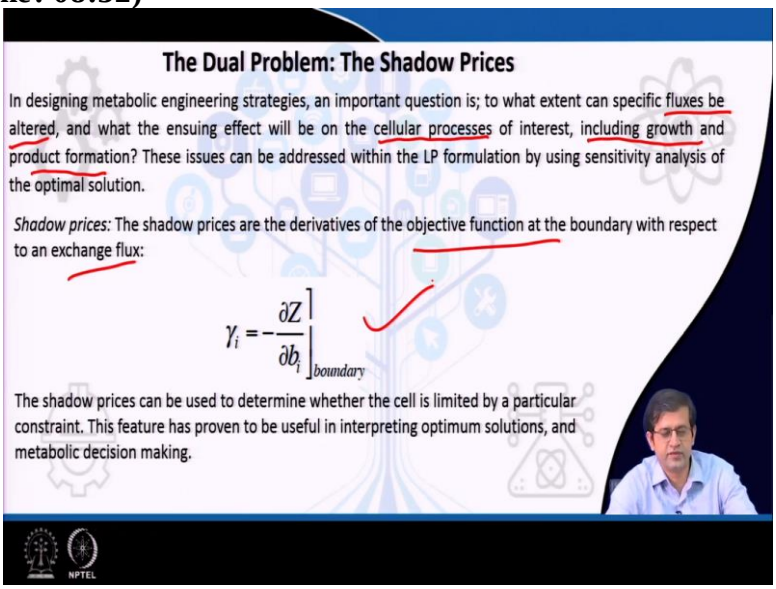

So, now, we will come to another concept that is the shadow prices that is dual problem. what is shadow prices? In designing metabolic engineering strategies an important question is that what extent can specific fluxes be altered because you are doing metabolic engineering you are basically changing the fluxes the inter cellular fluxes are changed when you do metabolic engineering what happened you either removing a gene or adding a gene or up regulating or down regulating gene.

And then what happened the fluxes inside the cell are going to altered, the whatever genetic perturbation you do a ultimately the fluxes get altered. This is a regular phenomena and you have make sure the effect will be on this cellular process of interest including growth and product formation. So, whatever perturbation you do at the genetic level, it should affect on the cellular process.

If it is not affecting the cellular process then your effort like you have generally this metabolic engineering strategies are actually labour intensive and it takes a lot of time. Suppose, you want to remove a gene it takes 2 to 3 months or even more depending on the organism you are changing. So, this includes growth and product formation. So, your main aim is to actually product formation and also including the growth.

So, if the fluxes are not affecting growth and product formation then the genetic perturbation is not of use. So, you have to design in such a way that the specific fluxes with that you want to alter has an effect on cellular processes like growth and product formation, these issues can

be addressed within the LP formulation the linear programming which we discussed in previous classes.

That linear programming formulation can be used, where the sensitivity analysis can be performed for the optimal solution. So, the sensitivity analysis can be calculated in terms of shadow prices, this is another term which is used to actually calculate the sensitivity of the metabolic network, the shadow prices are the derivative of the objective function. So, what is shadow price is basically a derivative of the objective function at the boundary with respect to exchange flux.

So, it is basically a derivative which is given by gamma i. So, Z is the objective function you already know is the biomass of objective function and then you do a derivative of the objective function with respect to exchange flux. So, not internal fluxes, it is the exchange flux that you make a derivative and these shadow prices shows that it can be used to determine whether this cell is limited by a particular constraint or not.

That is the exchange flux that the flux which is entering the cell, whether it has a limitation or a constraint in the cell growth that you can check. This feature has been proven to be very useful in interpreting optimal solution and metabolic decision making then especially in metabolic engineering. So, whether the excess flux is actually important for the cell or not that you can check using this equation.

Using this equation the shadow price precisely define the incremental change in the objective function, if a constraining flux is the incrementally change, shadow price may change discontinuously as the excess flux is varied, these shadow prices can be used to determine whether a optimal functional state of a network is limited by the availability of particular compound. So, whether the network is actually constrained by any compound that is up taking or producing that you can check.

Sometime what happen the many products which are synthesizing the cell are actually constraining the network? So that using shadow prices, you can even check whether it is limited whether the availability of a particular compound is actually constraining the network or not. So, the shadow price calculation is important to check the limitation of the network or the robustness of the network you can check very easily on the sensitivity of the network.

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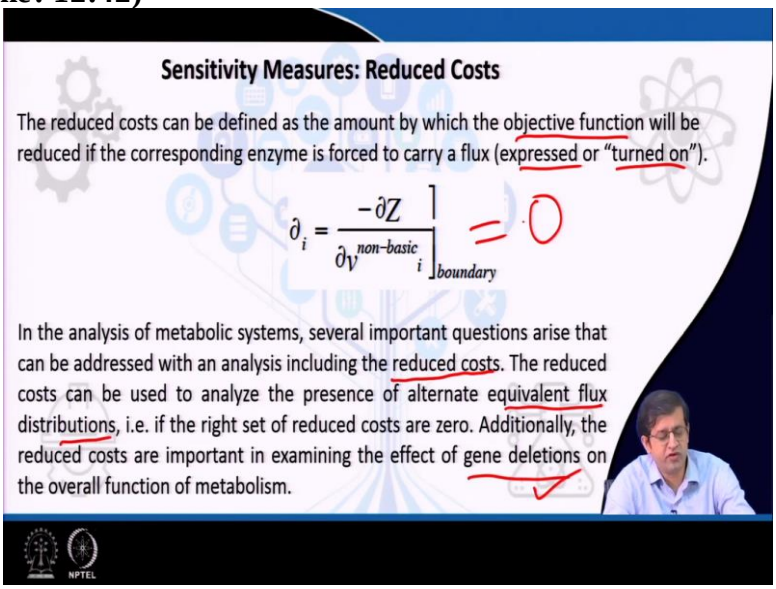

So, another term we introduced is the reduced cost the sensitivity measure. The reduced cost can be defined as the amount by which the objective function can be reduced if the corresponding enzyme is forced to carry a flux which was not carrying a flux before. So, you turned on it or express that gene, so that it carry a flux. So, when metabolic engineering also this is very crucial where you add a gene and all of a sudden it carries a flux.

But as soon as the reaction carry a flux because of the availability of the enzyme, then how much the objective function is going to reduce whether the objective function will reduce or not? The objective function is basically the growth rate whether there is a effect on the growth rate or not? Moment you add a gene. So that also you can check using the metabolic model in the analysis of metabolic systems several important questions arise that can be addressed with an analysis including the reduced costs.

Using a reduced cost you can check you can do metabolic system analysis and you can address several questions that may arise because of the metabolic engineering. The reduced costs can be used to analyse the presence of alternate equivalent flux distribution. So, whether it has a equivalent flux or not? If the right set of reduce costs are 0 or not that also you can check.

Additionally, the reduced costs are important in examining the effect of gene deletion. As I told in metabolic engineering, we do a lot of gene deletion whether this the deletion of genes actually affecting the overall function of the metabolism that you can also change from the

reduced cost. So, reduced cost analysis is another parameter, the reduced costs can be defined as the amount by which the objective function will change with flux level through an internal reaction that is not in the basis solution.

That is the flux that have a 0 net flux. Several important questions that can be addressed using reduced costs, the reduced costs can be used to analyse the presence of alternate equivalent flux distribution. If a reduced cost is 0, it means that the flux level through the corresponding reaction does not change the objective function, so if the reduced cost is 0. Suppose, I get a 0 for that reaction that means he does not actually correspond to does not change the objective function.

So, your objective function that is the growth rate or the biomass equation is not changing, the growth rate is not changing. Thus reduce costs can be useful in examining the effect of gene deletion. So that is why the gene deletion and other experiment you want to perform on the bench you can check before if the value is 0 by adding a gene then it is not affecting the growth, then it is beneficial for metabolic engineering similarly, for deletion also you can check. So, this reduce cost measure is actually sensitivity of the metabolic network that you are designing or the phenotype you are planning.

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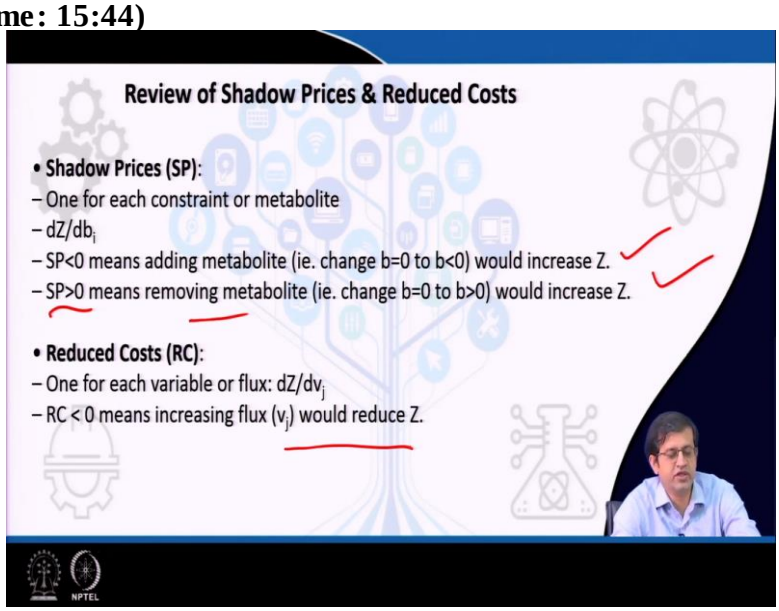

So, in summary, the shadow prices and reduced costs what we learned in shadow prices, we defined dZ / db for each constraint or metabolite that is importing or exporting. So, dZ / db. If the shadow price is less than 0 means the metabolite is required by the cell and it will

increase the objective function. If shadow price is greater than 0 that means that metabolite is not required by the cell and you can remove the metabolite from the network.

So, these are the 2 important conclusion from the shadow price, the shadow price is less than 0 that means that metabolite is required by the cell and if you provide more of the metabolite and then what will happen your growth will increase that is Z will increase. Similarly, the reverse is the case where the shadow price is greater than 0, when shadow price is greater than 0 that means that metabolite not required by for the cell growth.

That means by removing the metabolite would increase the Z. So, if you remove that metabolite and it will increase. And the reduced cost what we see that if reduced costs is less than 0 that means increasing flux would reduce Z. So if the reduced cost is increasing means if it is less than 0 that means the flux if you increase the flux, so that reaction that will increase Z. So, these are the 2 important conclusions that you draw from the shadow prices and the reduced cost.

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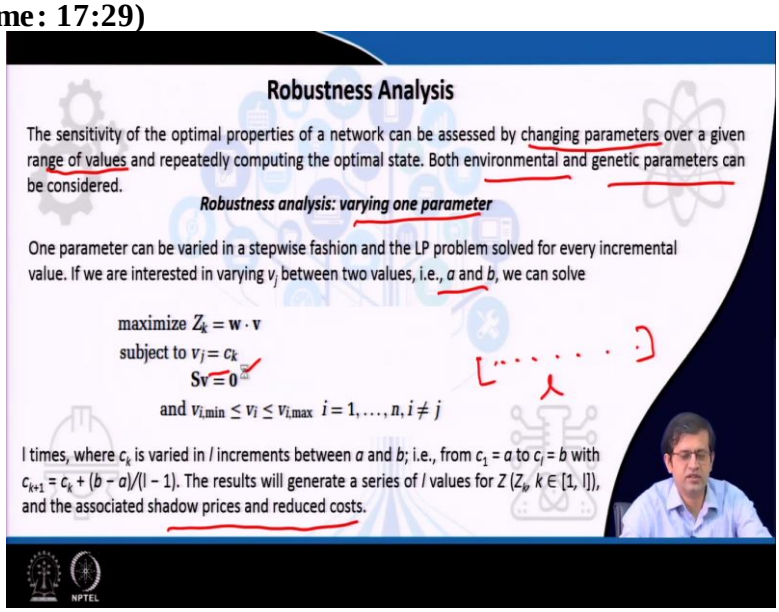

Now, we will discuss about the robustness analysis, what is robustness analysis, the sensitivity of the optimal property of a network can be assessed by changing parameter over a given range of value and repeatedly computing the optimal state. Both environment and genetic parameters can be considered. So, here in this what will happen you change the sensor, check the sensitivity of the optimal properties of the network like the growth rate by changing some parameters over a range of values.

So, you change one of the parameters, what a range of values like from 0 to 10 you change. Those parameter can be environmental or genetic parameter that can be considered because robustness you want to check how the network is changing with changing one parameter at a time. So, you can change one parameter at a time. And then you see how one parameter variation in one parameter how the network is changing.

So, one parameter can be varied in a stepwise manner. And the LP problem solved for every incremental value if you are interested in varying v j between 2 values, suppose you want to change the flux v j any reaction j between a and b, then what happened you solve the growth maximization problem, the FBA problem provided the flux value that is the v j value to be c k, you choose v j, and c k actually varied in l increment.

So, between a and b, I divided into l points, l number of points. So, c 1 will start from a and c l the last point c l will be c b because that range you are considering and in between a and b you have that many number that is l number of points. So that is given by c k + (b – a) divided by l - 1. So, you feed that many number of points and that many number of v j you choose and calculate the maximum value of biomass that is the growth rate.

This result generates a series of value of l for Z and the associated shadow prices and reduces costs you can calculate. By using that you can calculate the shadow prices and then you can check the robustness of the network. So, you are varying the one parameter that is v flux v j and you choose flux v j between a range, so the range you can choose between a and b. So, you know the maximum and minimum value of flux v j.

And then you are creating range of points suppose you have created l points l number of points, so, c k will go from the value of c k that will vary for l increment and will rise between a and b. So, this way you can check how your objective function that is the growth is changing the biomass is changing with variables when you are changing one parameter at a time.

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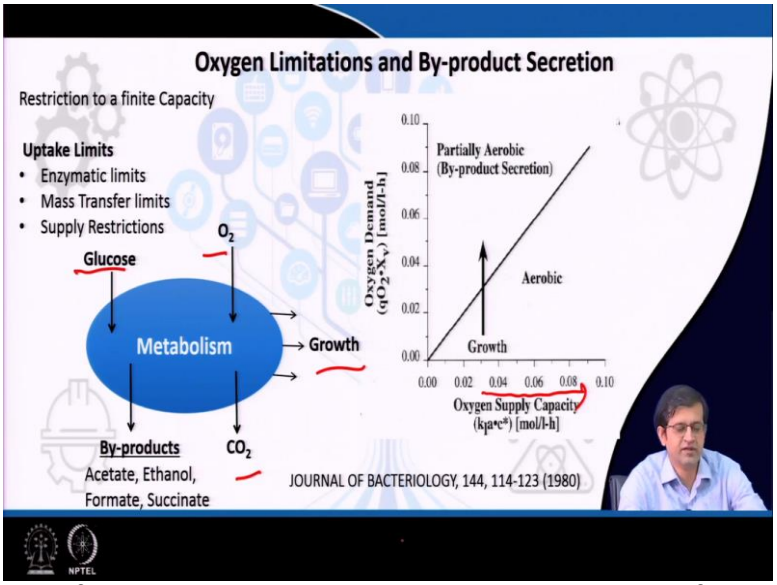

Now you can think of a cell which is growing in glucose. So, you for that the robustness analysis that vary the single parameter flux value and how for that I have given an example. So, this is an example where you can see that the uptake limit the enzyme limit and the mass transfer limit and supply restriction how it is changing when the cell is going on a glucose. So, glucose will supply and then oxygen you supply then produces carbon dioxide produce carbon.

And you see a growth in the cell and the by-product which is form Acetate, Ethanol, Formate, Succinate. Now you want to see that how it is changing. The parameters suppose you want to change the oxygen supply. So, here the very variable parameter that you have chosen is the oxygen supply. So, you keep on increasing oxygen supply initially it was 0 and then slowly you increase the oxygen supply and how the cell growth is changing that you want to observe.

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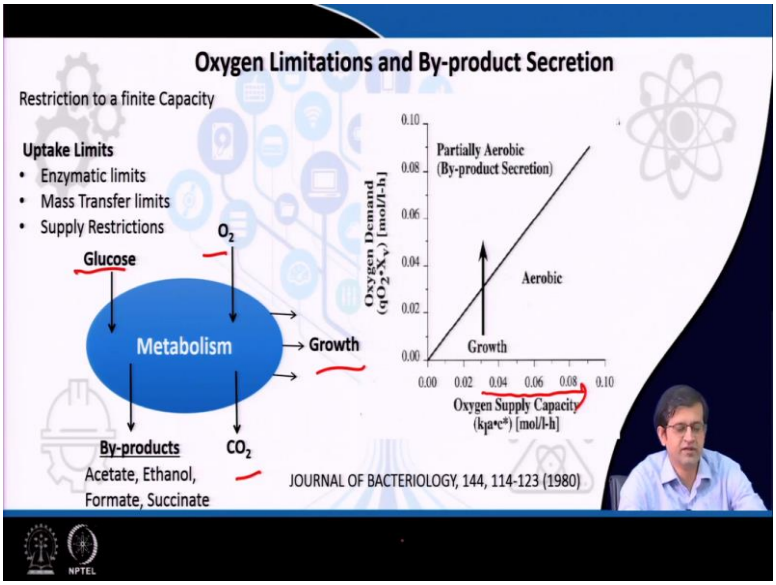

So, I broken down into 4 phases; phase 1, phase 2, phase during the growth. So, initially it was anaerobic because there is no oxygen 0. So, slowly you are increasing the oxygen supply which is denoted by millimole per gram dry weight per hour that you want to. Since you are increasing oxygen so, it is fully anaerobic it is going to aerobic and how the formate, ethanol are fromed in different phase like phase 2 phase 1 phase 2 phase 3 that we will discuss.

In this example, we vary the maximum allowable uptake rate of oxygen the whole range of oxygenation is shown from fully aerobic to fully anaerobic condition. The growth rate is graphed in the upper panel and the by production secretion are actually shown in the lower panel in this panel you can see the growth rate is increasing. And then in the lower panel you can see that you have the acetate production and then formate production and then the ethanol production; these are the by-product which are formed.

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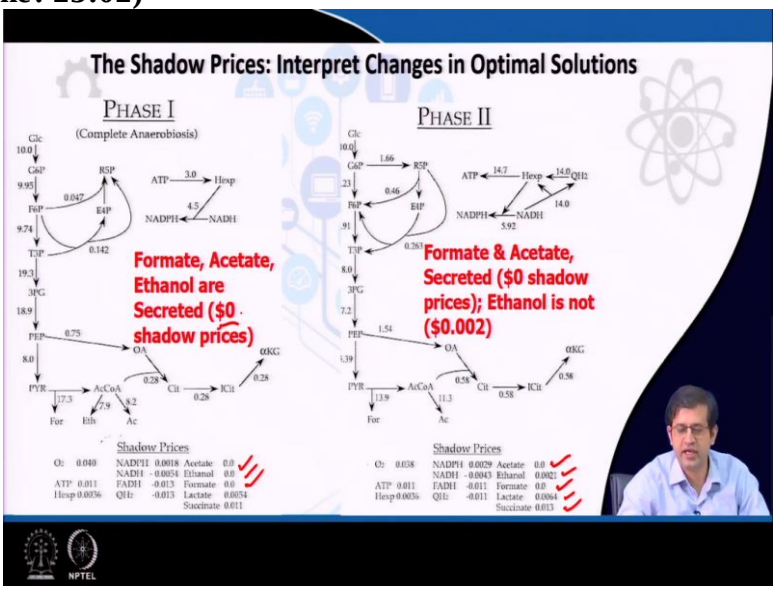

So, in the shadow price calculation, you can see that by the shadow prices has been calculated for phase 1 where it is completely anaerobic. So, you can see in fully anaerobic the acetate ethanol and formate productions are shown over here. And you can see the shadow price value which is positive, negative for ethanol, formate and lactate and the shadow prices value is 0, 0 for ethanol and formate it whereas for lactate and succinate it is positive.

Since the shadow prices are 0, the formate, acetate, ethanol are secreted, so shadow price as I told that whenever the shadow prices is 0 that means the cell actually do not require for the growth. So that is why it is secreted out. So, you can see that in the phase 1 these metabolites are secreted and when you go to phase 2, then what happened?

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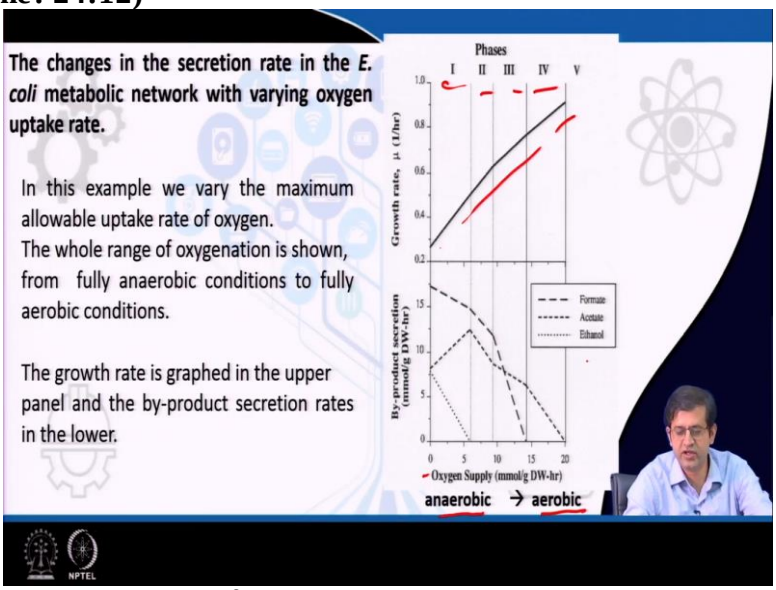

So, in this case you can see that formate, acetate, ethanol are produce and since the shadow prices are 0 that's why the cell does not require this metabolite and in their secreted out. An in phase 2 what happened? The shadow price for acetate is 0. And then formate is also 0 but ethanol and lactate, succinate they all have the shadow prices are positive. So, the shadow prices are positive for them and ethanol is 0.002, so the shadow prices for ethanol is 0.002.

So, the formate and acetate are secreted ethanol is not. So, ethanol is not secreted in the second phase. So, this shows that the changes in the secretion rate and the shadow prices of key metabolite in the E. coli core metabolic data with varying oxygen as the number given and when the metabolite is in 0 shadow price actually do not affect the value of the objective function. So, whenever the shadow price is 0, then it is not affecting the objective function that is the growth rate.

But the metabolite with the negative shadow price can increase the objective transfer and thus they are not secreted. So, the shadow prices value is actually negative those are not secreted. So, ethanol is not secreted in phase 2. Whereas as a formate and acetate are secreted.

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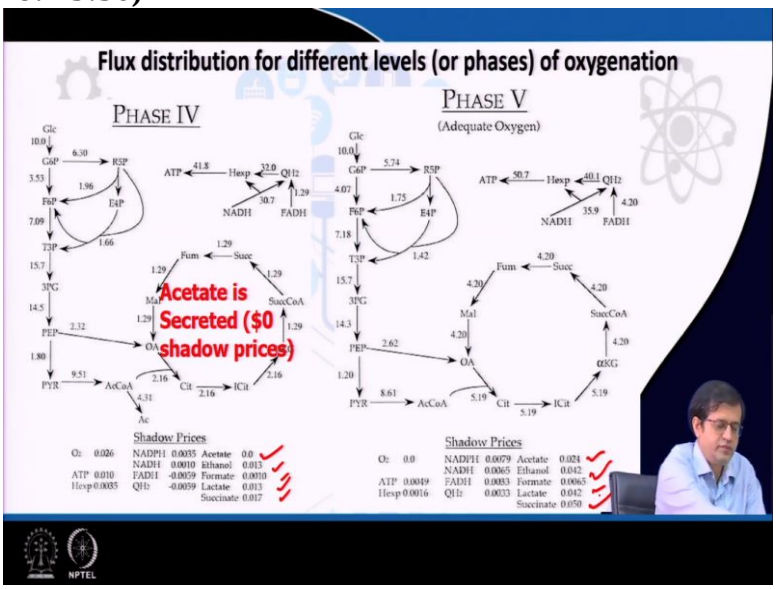

So, in phase 4 and phase 5 what you see that acetate is secreted because the shadow prices is 0. So, the shadow price is for acetate is 0 because you may have the cell does not require acetate in phase 4. So, acetate shadow prices are 0, whereas the shadow prices for formate, ethanol, lactate, succinate are actually negative. So, the negative value which is shown over here.

The ethanol, formate acetate, lactate are actually having a negative value. That is why they are not secreted. But in phase 5, what do you see that the shadow price value for none of the metabolites is 0. So, none of the metabolites are 0. So, they are not secreted. So that is why you do not get any metabolite secretion when you have adequate oxygen supply. So, by observing the shadow prices, you will be able to calculate which are the metabolites secreted or not secreted.

So, whenever the shadow price is negative, then you know that that metabolite is not secret because that is utilized for the cell growth and it is not a secreted outside the cell. So, now, we will go to phase plane analysis that is varying the multiple flux simultaneously in previous slides what you saw in only oxygen uptake rate is actually changing one parameter is varied

but in this case in phenotype phase plane analysing what you will see is basically multiple fluxes will change simultaneously.

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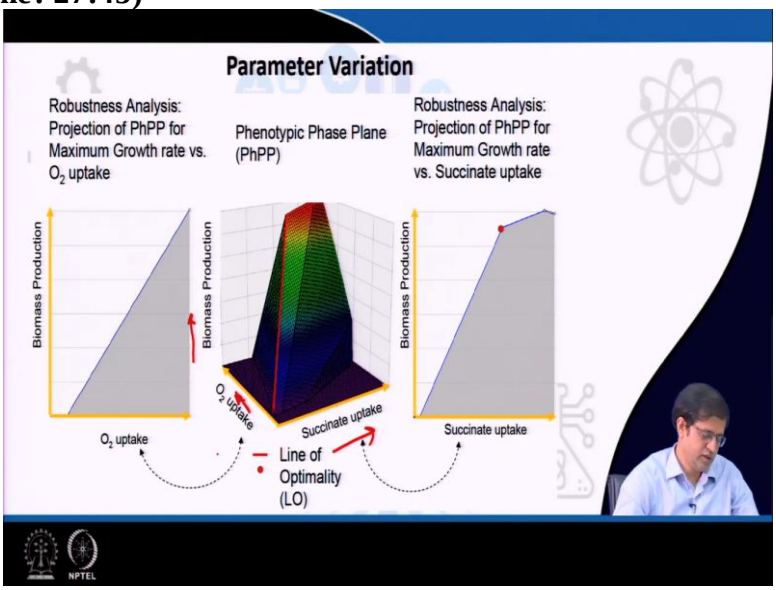

And the parameter shown over here the robustness analysis that is a projection of phenotype phase plane and that is the maximum growth rate versus the oxygen uptake rate. So, in this case you can see there are 2 variables that is the biomass production and the oxygen uptake rate on the right hand side. You can see the robustness analysis projection of phenotype phase plane for maximum growth rate versus succinate uptake.

So, here we have 2 variables the oxygen uptake rate and the succinate uptake rate, 2 variable they have used for phenotypic phase plane and on the y axis what you see is the biomass, so biomass by default it is there and then you check how these 2 variables suppose the product you are looking for you want to produce succinate production producing from the cell and you are supplying oxygen.

And that you want to check by varying the oxygen after and the succinate uptake rate. So, this 2 parameter you can see how much succinate the cell is using and the how much oxygen the cell is using and how the biomass is changing with time that you can plot using in the metabolic network.

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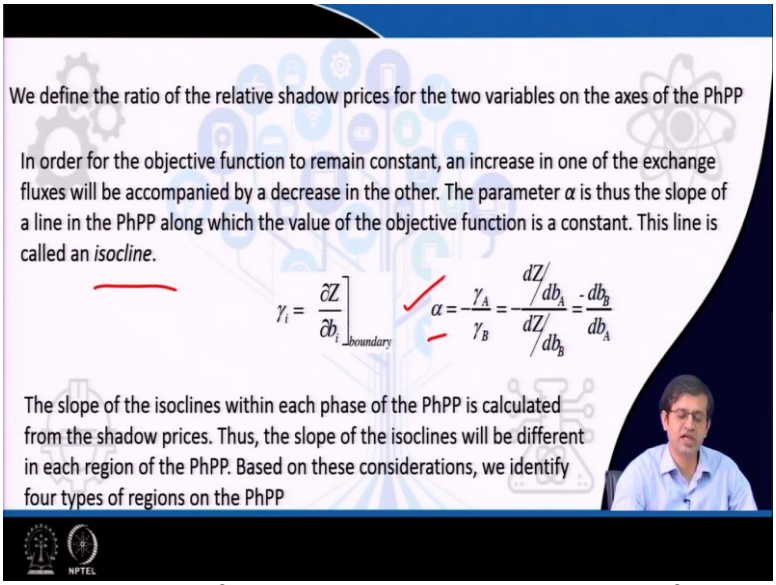

So, we define 2 ratio here we define the ratio relative shadow price for the 2 variables on the axes of phenotype phase plane, you know what for the objective function to remain constant, an increase in one of the exchange fluxes will be accompanied by a decrease in other and the parameter alpha is used to actually denote this parameter we define a parameter alpha. The alpha says that if you increase one of the exchange flux.

So, suppose you increase one of the exchanges flux will be accompanied by decrease of the other, so that your alpha remain constant. So, in order for the objective function it is to be constant. Since the objective Z is constant when for both the points A and B when Z is constant then dZ dZ is cancel and what you get is db B by db A. And, the slope of the parameter alpha is the slope of a line in the phenotype phase plan.

Along with the value of the objective function that is constant and this line is known as Isocline. So, Isocline is formed where your maximum objective function that is the biomass is constant in that line and you call it is Isocline and the slope of the Isocline within each phase or phenotype phase plane is calculated from the shadow price, thus the slope of the Isocline will be different in each region of the phenotype phase plane based on these considerations, you identify 4 types of region on the phenotype phase plane.

So, based on alpha, we defined in the previous slide we have shown the phenotype phase plane, now we want to actually break the phenotype phase plane into 4 regions. So, if you change oxygen uptake rate and succinate uptake rate what do you get a profile like this. So, you solve the FBA problem and get points. So, you plot those points and you get a plot like

this which is very easy FBA formulation says that if you have uptake rate on the oxygen and the succinate uptake rate and you get it you maximize the biomass.

Then what you get it is the profile like this and you plots it. Now we want to characterize the phenotype phase plane that is PhPP into 4 region based on the value of alpha and the alpha says that in order for the objective function to be remained constant, an increase in one of the exchange flux can be accompanied by decrease in other. So, if you keep a Z fix, then what happen if you increase one of the parameters and that is the exchange flux db.

So, db is basically change in one of the exchange flux and db A is another exchange flux. If the change in exchange flux in one of the exchange flux is equal to the change in exchange flux of another like A then, what happened is alpha remains constant and this becomes your isocline, isoclines are formed and based on the number of isocline you actually defined 4 regions in the metabolic network. What are those 4 regions?

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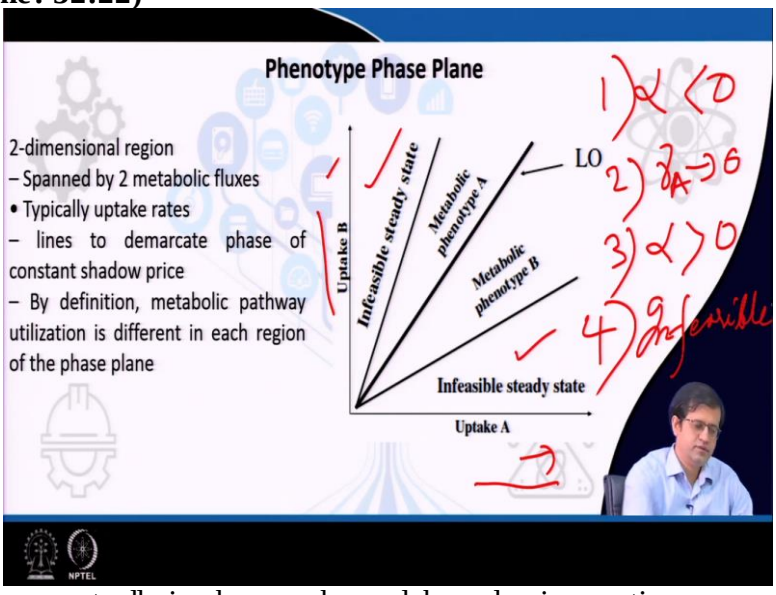

So, the 4 regions are actually in phases where alpha value is negative, so, you choose alpha as negative that is less than 0. So, this is one case. So, alpha is less than 0. So, when alpha value is negative, there is a dual limitation of the substrate based on the absolute value of alpha the substrate will a greater contribution toward obtaining the objective function can be identified. If the absolute value of alpha is greater than unity, the substrate along the x axis is more valuable to our obtaining the objective function.

Whereas, if the absolute value of alpha is less than unity, this substrate along the y axis is more valuable to the objective function. So, these become the substrate which is in the y axis that we can valuable when alpha is less than unity. And when it is greater than unity then, this substrate along the x axis is more valuable to obtaining the objective function. So, this is what you get when alpha is less than 0.

Then we have another condition that the phases where the isoclines are either horizontal or vertical are phases of single substrate limitation. The alpha value in these phases will be 0 or infinity. So, the phase plane why the isoclines plans are either horizontal or vertical that means the ratio I told it can be if alpha becomes 0 then it alpha, the gamma become 0, this becomes 0 then what happened? The alpha becomes infinity that means it will become vertical and then also you have the alpha can be horizontal also.

The isoclines can be either vertical or horizontal then what happened? The alpha value in these phases will be 0 and infinity and these phases arise when the shadow prices one of the subset goes to 0. And thus has no value to the cell. So, the shadow price that is gamma in the 2 the shadow price for A one of the uptake rate goes to 0. So, when shadow price goes to 0 then it has no value to the cell. And in the third case, what happened? The phases in the phenotype phase plane can have a positive alpha value.

So, alpha in this case we consider alpha to be greater than 0. So, in these phases, one of the substrate is innovated it towards obtaining the objective function and this substrate will have a positive shadow price. So, this is when you consider alpha greater 0 what happened is one of the shadow price become positive. The metabolic operation in this ways is a waseful, in that it consumes substrate that is not needed to improve the objective function.

The post peaks, the phases with positive alpha values are expected to be physiologically unstable. For example, under selection presser cell would move their phenotype state out of the phase. So, in this case, what happened? It consumed substrate that is not needed to improve the objective function, so that you can remove that metabolite the alpha. When alpha is positive, the phases with positive alpha are expected to be physiologically unstable. And then the fourth one is basically finally due to stoichiometric limitation.

There are infeasible steady state phases in the phenotype phase plane, if the substrate are taken up at the rate represented by this point the metabolic network is not able to obey the mass energy and redox constant while generating the biomass, the metabolic network can only transiently operate in such a region. So, it is basically this is the region the infeasible region where it is the stoichiometric; this is due to limitation of the stoichiometric network that creates infeasible steady state phases in the phenotype phase plane. So, this is a region is in feasible region.

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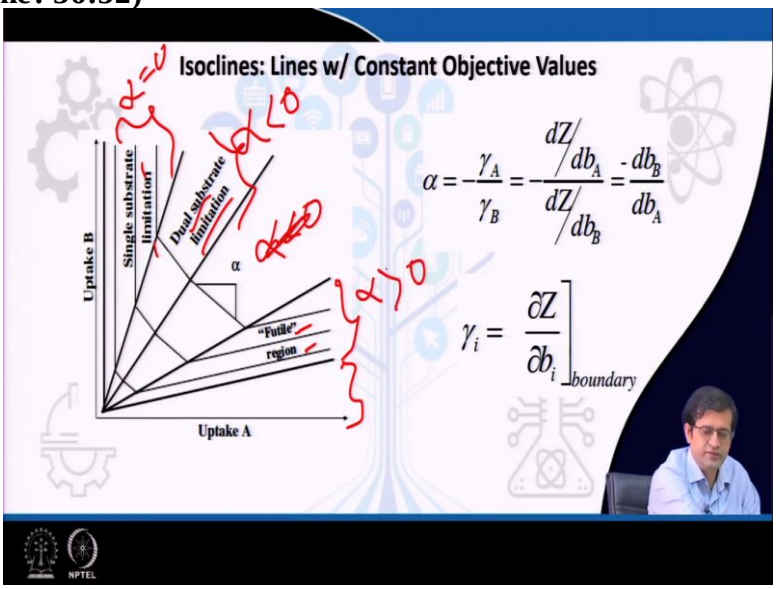

So, what we have is basically the one alpha is actually negative and the alpha one is positive and these are the region which are formed when the alpha become positive or negative we have the futile region and we have the limitation region these single substrate limitation and the futile region and the dual substrate limitation is comes under these are these are the 4 region which is shown over here.

So, these are the infeasible region which you are not important and then we have the futile region and the substrate limitation region and this single substrate limitation region, this you can calculate from the phenotype phase plane.

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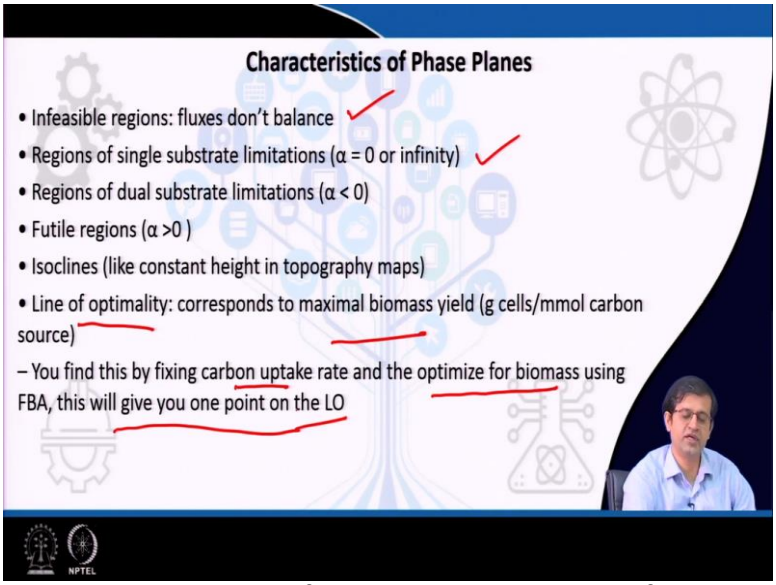

So, in summary what you get is the infeasible region where the flux do not balance, so the stoichiometric. This is the limitation of stoichiometric and the region of single substrate limitation where alpha = 0. So, if you go to previous one where the substrate limitation happened and this is the substrate limitation where alpha = 0 or alpha = 0 or infinity. In this case the substrate inhibition happened and then we have a region where alpha less than 0 and this is the dual substrate limitation.

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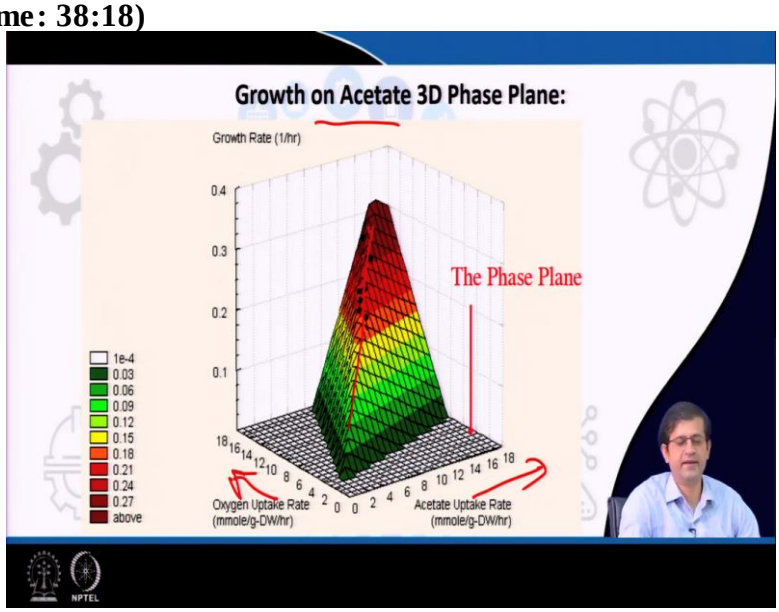

So, this is the region where you have the dual substrate alpha less than 0. So, alpha less than 0 that is the dual substrate. So, this is the alpha less than 0. So that is the region we have the dual substrate in limitation and then we have futile region where alpha is greater than 0, So, this is the case we have the alpha greater than 0 and the line of optimality the isoclines the constant height in topography maps and the line of optimality correspond to the maximal biomass yield.

The line of optimality will lies in the region where we will get the maximum biomass, line of optimality correspond to the maximal biomass yield that you are observing by fixing the carbon updated and optimize the biomass using a FBA, this will give you the one point of the optimality. So, once you fix the carbon uptake rate and the biomass and after you optimize the biomass you will get a single point on the line of optimality.

And that is the region you have to operate to get the maximum biomass and also you will see that they go at that carbon uptake rate, you will be able to get the maximum biomass while doing the metabolic engineering. So, this is the growth phenotype phase plane that you can get for a while growing the cell on acetate, the E. coli cell on acetate but you can see the oxygen uptake rate which is changing with time and then the acetate uptake rate will be change.

And this is the line of optimality which is shown over here where the growth is maximum, this is the region where we want to operate you can see that this is the region if you can operate the cell in that region then you can get the maximum this you can see that is my biomass is actually greater than 0.27 and this is where you want to operate the cell.

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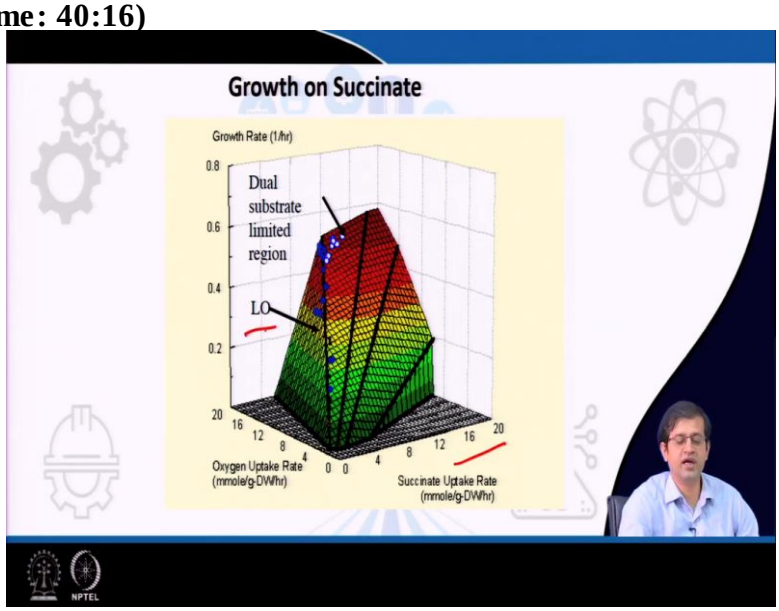

Similarly, if you want to grow the cells on succinate, then you can see the line of optimality where the growth rate is maximum shown over here and this is the region you want to offer the cell this is the end and then beyond that, you have the dual substrate limitation. This way you can do the sensitivity analysis and reverse analysis in terms of 2 uptake rate that is

oxygen uptake rate and the substrate uptake rate. So, varying these 2 parameters, we will be able to draw the phenotype explain and try to calculate the line of optimality where the growth rate are maximum.

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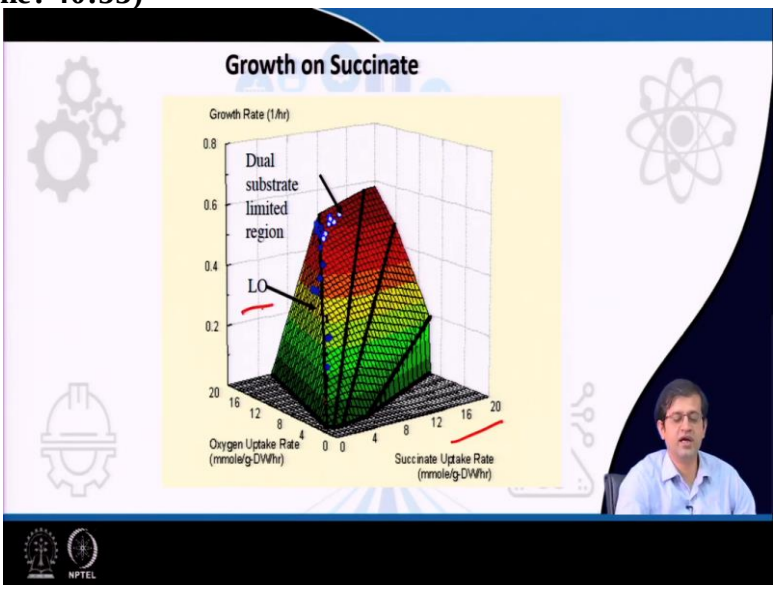

So, in conclusion the flux balance and the capacity constant form a closed polyhedral sub spaces. So, basically when you do the flux balance analyse you get cone kind of structure this polyhedral is basically contain all the flux balance solution and the linear programming can be used to find the optimal solution in this space. So, using the linear programming, you will be able to get the optimal solution is a single point where you have the maximum growth rate.

And also you can shadow prices and reduced costs are used to characterize the optimal solution. So, using the shadow prices and the reduced costs formula that we discussed today can be characterize the optimal solution how the optimal solution is good or bad that you can characterize and all possible combination of the value of 2 parameters can lead to the definition of a phase plane.

So, from the network you would be able to considering 2 parameters like oxygen uptake rate and the substrate uptake rate you can draw a phase plane and the boundaries in the phase plane edges only the polyhedral cone. So, the boundaries we already get from and you can get it from the polyhedral cone and thus the boundaries represent the systematic pathway.

So, this is what we learn today. And for references you can read the book written by Bernard Palsson is that systems biology properties of reconstructing network and also you can read

about the nature review in microbiology. Also you can read the Journal of bacteriology. These are the references you can read for you for your further study. Thank you for listening. Hopefully you enjoyed the class.