Metabolic Engineering
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Lecture - 19
Dynamic Flux Balance Analysis (DFBA) and Gene Deletion Algorithms

Welcome to metabolic engineering course, today we will talk about dynamic flux balance analysis and genetic gene deletion algorithms. So both this technique involves flux balance analysis what we learned in previous class? The optimization method which is using flux balance analysis can be extended. So, if you understood the flux balance analysis it will be actually helpful here.

So, I would recommend that you completely go through FBA. Once you have knowledge and proper understanding then this will be helpful. The dynamic flux balance analysis and gene deletion algorithms are only based on the extension of the flux balance analysis.

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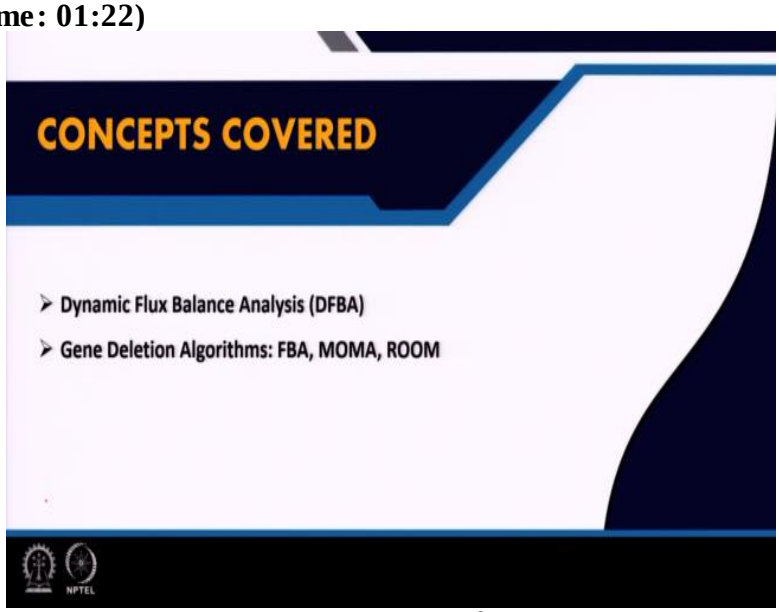

So, in this the concept will be covered the dynamic flux balance analysis that is DFBA. And gene deletion algorithm like FBA, MOMA, ROOM so, FBA can also be used for gene deletion prediction and MOMA and ROOM we will discuss how it is used.

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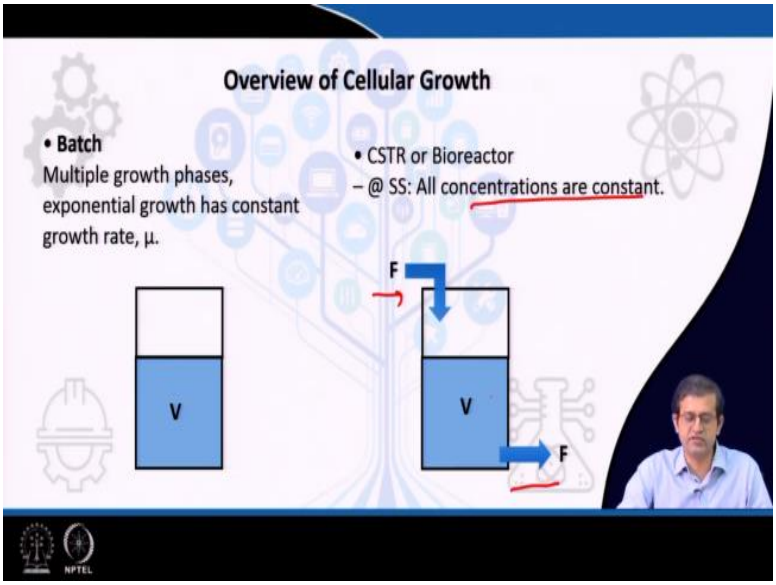

Today we will discuss about the batch culture and the CSTR. CSTR you know is actually continuous stirred tank reactor that is used for the bioreactor as well CSTR. And the batch culture you already know is a closed chamber there is nothing coming in or nothing coming out. So, it has multiple growth phases in batch culture. And it has an exponential growth has a constant growth rate that is mu, so the exponential the growth rate is constant.

And it is denoted by mu whereas, in CSTR we have flow and all the concentration are constant, its concentration are not changing with time. Whereas, in batch you have the concentration changing with time but the growth rate is fixed and the exponential growth rate and then you have a flow and then the outflow inflow and then the volume is also fixed in both the cases.

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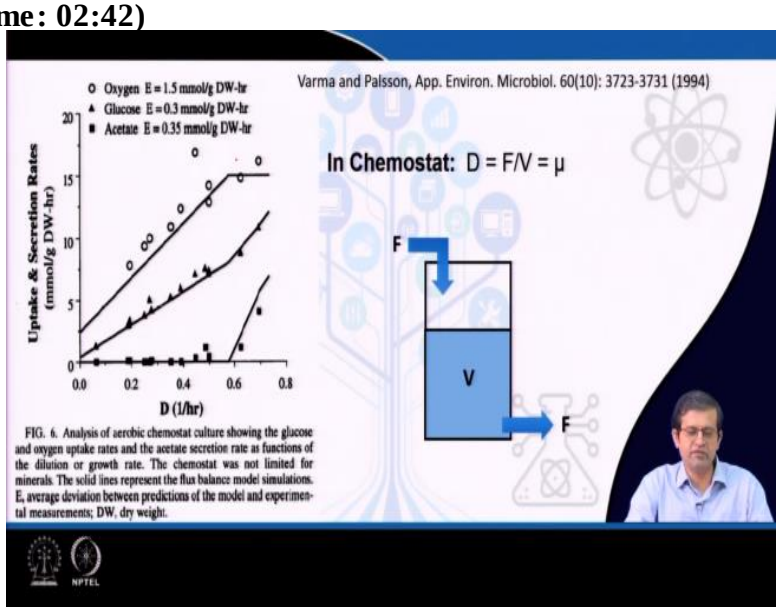

So, if you want to simulate using, the model which you have already discussed the metabolic network. This is a paper which is published in 1994 and this was the first work which is done by Bernard Palsson and Varma where they calculated they compared the growth profile and that is analysis of aerobic chemostat culture. So, in the glucose and oxygen uptake rates and acetate secretion rates has function of the dilation or growth rate has been shown over here.

So, the dots are basically the experimental values and these the continuous lines are basically the simulation data that has been plotted together in a NSM graph. The chemostat here was not limited to mineral the solid line represent the flux balance model simulation as I told. And E is the standard deviation or the average deviation between the prediction of the model and the experimental measurement. So, higher the deviation and then there is a difference between the experimental value and the model prediction.

So, the E value is actually 1.5 for oxygen uptake rate and then the E value for glucose uptake rate is around 0.3 and acetate is 0.35. So, both acetate and glucose the uptake values and the separation rates are actually very much comparing with the experimental value. So, this modeling and simulations are really useful where you can before actually doing the experiment. You can do it on the computer to find out how much it is producing you can have an idea and it is very much correlating also.

So, the diffusion you can get it from F by V where F is the flow and V is the volume and from there also you can get the specific growth rate. So, this way you can actually model the entire system where you can actually simulate and find out the dynamic nature of the growth rate.

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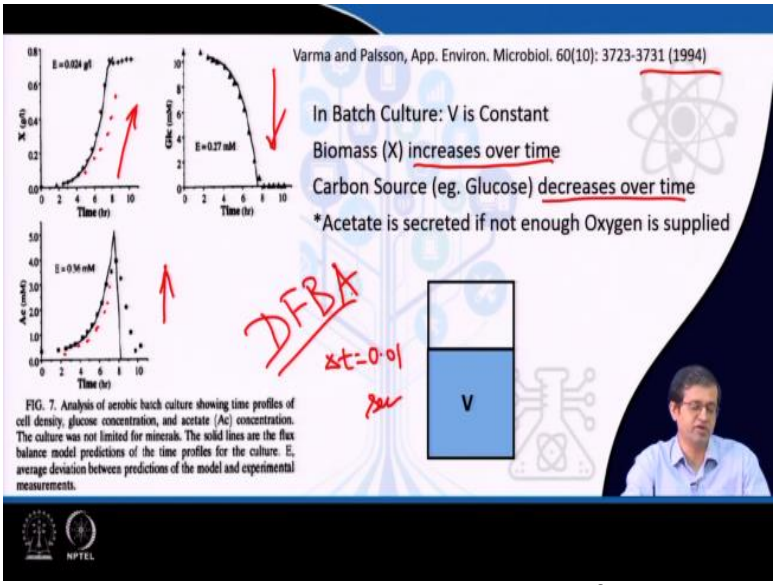

Then we come to the batch culture where V is the volume of the culture and then X is the biomass we want to actually measure over time so the biomass increases over time. Whereas in the CSTR we saw the all the concentration are fixed throughout the time but here the biomass is actually increasing over time and the carbon sources decrease over time. So the carbons which are taken up by the cell that is why there is increase in the biomass.

But the carbon source for example glucose it is decreasing over time and acetate is secreted at the same time so here you can see the biomass so the biomass is growing. So it is increasing over time after 8 hours you can see it is getting saturated. Whereas glucose you can see it is decreasing so it is decreasing over time. So initially we have almost more than 10 gram per liter 10 millimole. And then after 8 hours there is no glucose left in the media.

And you can see in the other case we see the acetate is increasing. And then after some time there the acetate is actually getting over. Because the cell is utilizing the acetate for its growth the moment the glucose is over then it grows on acetate. So, this way you can actually get the experimental value and compare with the model using the metabolic model. Here you can see that the model predicted very well when it comes to biomass concentration.

And the glucose consumption and the acetate secretion so both all the 3 things have been compared very well and this is the first study which is done in 1994 by Bernard Palsson. And he has shown for the first time the model using the metabolic model you can simulate and compare the batch culture which is very much which is based on dynamic FBA. So the method which is used is actually a dynamic FBA.

The computational method so dynamic flux balance analysis in dynamic FBA we actually solve this iteratively. You solve the FBA problem so basically each point you can take a delta t of 0.01 second. And then solve these FBA problem at different time points like that you have to go. So this is a dynamic FBA is actually a combination of all solving the FBA at different time points. And every time point the substrate uptake rate changes that is why you have different value of the growth.

So the biomass the maximum biomass is also changing because your uptake value is also changing. So every time point will have a different uptake value of the substrate. And the product formation is also changing simultaneously that is why we and how DFBA is implemented. We will discuss in subsequent slides it is very interesting that using the flux balance analysis you can do dynamic culture that is the culture which is changing with time you can simulate.

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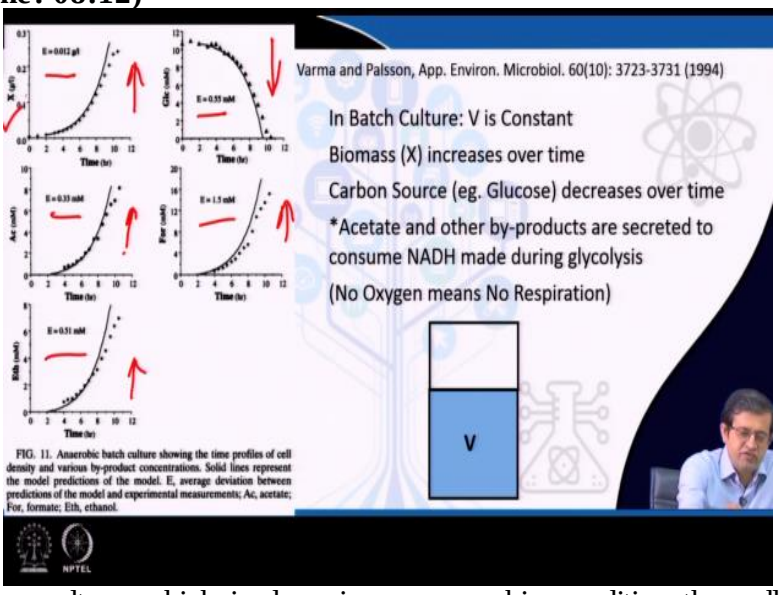

So, this is another culture which is done in an anaerobic condition the cell density and the byproduct concentration you can calculate theoretically. And the average deviation between the prediction and the experimental measurements are also shown over here. So this is the biomass which is increasing with time and then we have the substrate which is decreasing with time. And then we have the acetate which is increasing with time the formate is also increasing with time that these are the byproduct.

And the ethanol production is also increasing with time. So this is anaerobic culture whenever the acetate and other byproducts are secreted to consume NADH made during glycolysis. So the acetate actually consume NADH and which is producing glycolysis. We can be simultaneously use and no oxygen no respiration. So if you were using the batch culture you can compare and the correlation is also you can see the correlation value is also shown over here.

So E value is 0.012 means the average standard deviation. the deviation is very, very less 0.012, 0.33, 0.51. So only in case of formate we have the E value is around 1.5 otherwise most of them actually compared very well with the experiment the model simulation.

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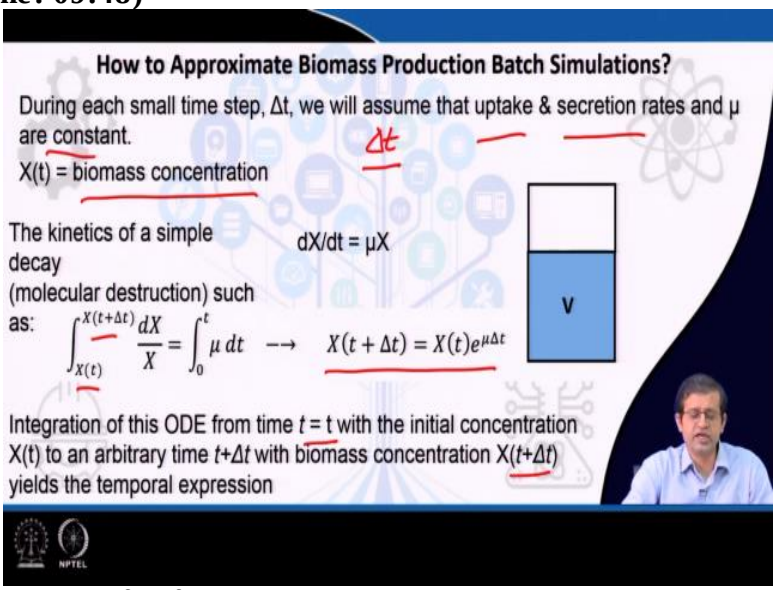

So then you saw the benefit of how the DBFA can be used actually to predict the growth rate secretion rate product formation substrate consumption rate all those things are compared very well. But how it is done in the computer? So, here I will go to the mathematics of how it is calculated. So we do simple kinetics of a simple decay for example during each small step delta t.

We assume that the uptake and secretion rates and mu are constant. So, the specific growth rate uptake rate, secretion rate are on a small interval of time like delta t. So using delta t need to be very small such that this uptake rate secretion rates are actually constant and then during that time. So using that approximation and using the kinetic decay simple decay which you have seen many times the dX by dt = mu X, mu is this specific growth rate and X is the biomass concentration. So basically the X is the biomass concentration.

And then you rearrange this equation what you will get is basically you integrate the equation from X the biomass at t = t. You calculate you have this biomass and t = t + delta t you have the final biomass. So that 2 different time points you integrate from t to t + delta t. And if you consider 0 to t the mu then what you will get is basically the delta t so once you integrate what you get?

You get this equation that is the biomass at t + delta t is equal to biomass at t = t multiplied by e to the power delta t. So this way you can actually calculate at any time point suppose provided you know the biomass concentration at t = t and you can calculate at any next time point, provided delta t is very small. So that the secretion and uptake rates are constant during that time interval you keep the delta t very, very small so that you can take this value the uptake rate, secretion rate to be constant and also the mu to be constant.

Using this approximation provided the delta t is very small, you can calculate the biomass at the next time point. And this way you can evolve the biomass concentration because the biomass you have to plot as a function of time or the biomass concentration. So that you can evaluate using this equation and solve the iteratively solve this equation and get a plot nice plot like this.

So you get a nice plot like this. These are the plots which are made using these equations, so this is just the biomass concentration and what about the substrate concentration? Substrate concentration also you have to calculate as a function of time for that also you have to integrate the equation of substrates.

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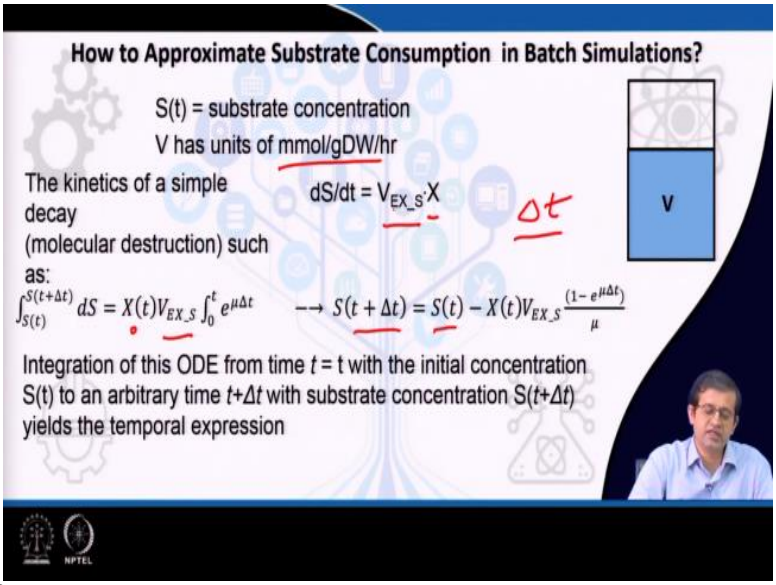

So the kinetic of the simple decay equation you can consider here also like we have dS by dt equal to the exchange rate of the substrate that is the rate at which the substrate is going inside the cell. So, this is the exchange rate at which the substrate is consumed for example glucose, galactose any substrate the cell is consuming you can define a exchange flux V that is the unit of the flux is actually millimole per gram dry weight per hour.

So this is a time derivative of the substrate concentration which is changing over time and you can actually get because V the substrate uptake rate is multiplied by the biomass concentration. So the X you have but V is actually constant we assume that at an interval of delta t the V EX underscore S is actually constant. So that you can bring this out or outside the integration you just integrate e to the power mu delta t.

And also you the Xt is basically t = 0 that is why is X 0 which is also constant so that is why we brought these 2 terms outside the integration. And then we integrated dS from and the substrate concentration is St at t = t and St + delta t when the substrate has the concentration at a different time point. And then we rearrange this equation what you get is equal to St + delta t = St - Xt that is the concentration of the biomass at t = t.

And then multiplied by the exchange flux for the substrate multiplied by 1 - e to the power mu delta t divided by mu, mu is the specific growth rate. So, this way you can actually calculate at a different time point that is how much substrate it is actually consumed as a function of time provided. You know the exchange uptake rate the exchange uptake rate you should know to actually calculate this.

So, if you know the substrate concentration at t = t then you can calculate at t + delta t in this way we evolve the substrate concentration as a function of time. And then you use that value to plot it and you make your delta t very, very small. So, that you can consider this as a constant over time and that when you integrate at the t = delta t then you can actually able to take it as a constant.

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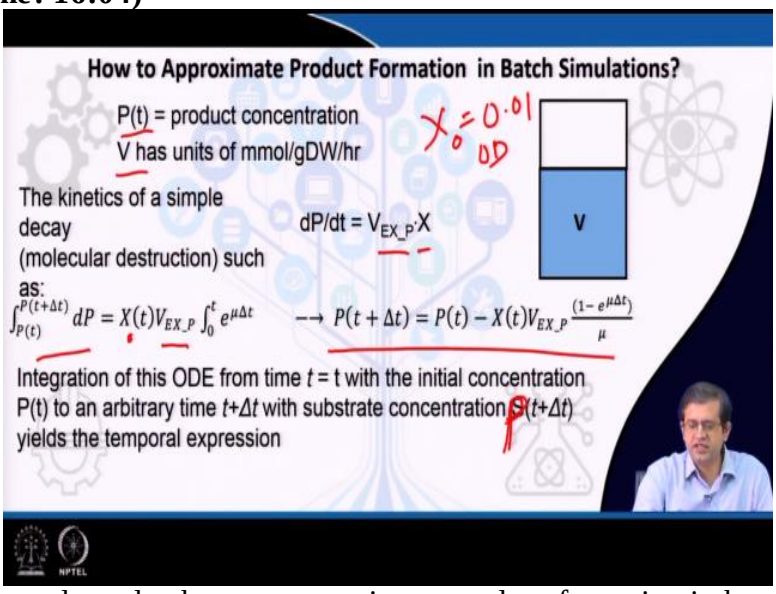

Similarly, for the product also how to approximate product formation in batch simulation also simultaneously you can calculate the product formation using this equation. The product formation you assume that is Pt that is a product. If the product can be acetate, ethanol, glycerol, formate and so on and any product which is coming outside the cell that you want to model using this equation.

And the V has a unit that is exchange flux that is the amount of product it is thrown outside the cell or secreted cell that is change in product is equal to exchange flux multiplied by the biomass. And that you can integrate as a change as the product changes from Pt to Pt + delta t. And also your exchange flux which is constant which is you can bring outside the integration and X 0 at t = 0.

Biomass is X 0 the initial concentration sometime X 0 you start it with the 0.01 at 0.1 OD. You start the initial concentration of the biomass that you can take at the time point. And then and you integrate from t = t to t + delta t and you get the product concentrations this will be

product. So, this way you can calculate the product formation t = t + delta t. If you know the product at t = t and also you should know the biomass at t = t.

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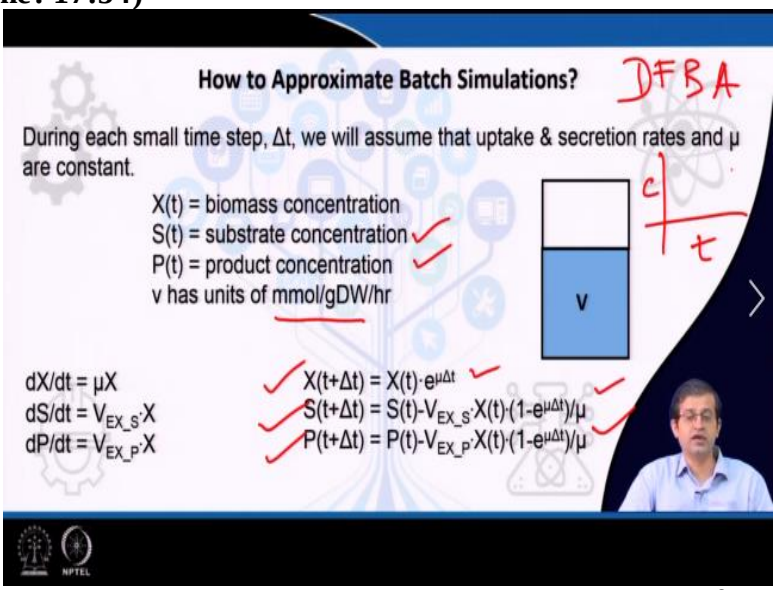

So, this way you can calculate all these. I have just given an equation for 1 product you can have P1, P2, P3 any number of product will obey this equation. Where you assume that Xt is the biomass concentration St is the substrate concentration and P is the product concentration. And then the flux unit is millimole per gram dry weight per hour in all the time, this is the equation using the DFBA.

So, dynamic flux balance analysis use these 3 equations to calculate the flux or the value of the concentration of the biomass because, DFBA is nothing but you are plotting the concentration versus time. So, you plot the concentration and the concentration you get it from here. So, using this equation using these 3 equation you get the concentration and as a function of time.

And then you plot it then you can compare with the experimental data to find out whether the growth curve or the production formation profile are matching or not. This is very useful the dynamic FBA is very useful which is used in many purposes to compare the experimental data. So, once you compare with the experimental data and you validate the model.

Then you can utilize the model to predict many new features in the many emergent properties you can predict which you do not have to do experiment. Just by the simulation you will be able to predict many new features and also you can validate the experimental data also.

Suppose you have some experimental data then you can validate with the model and your experimental data also get some theoretical support.

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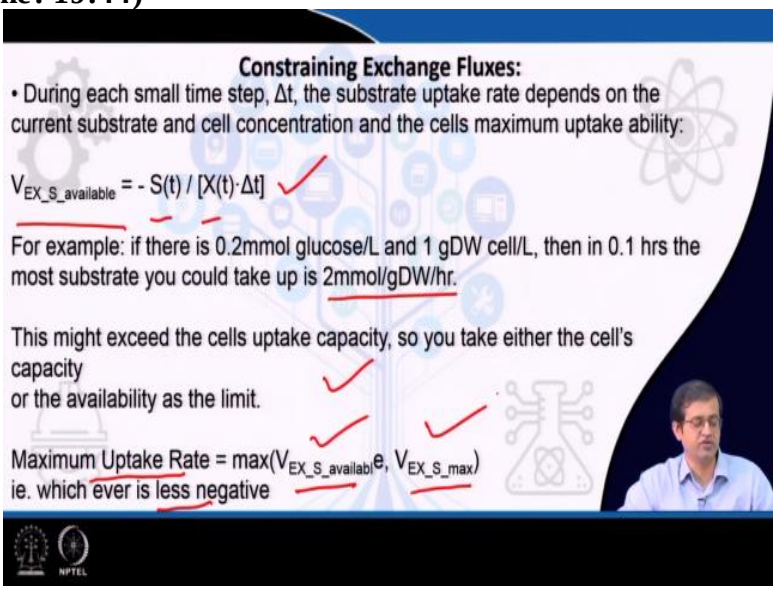

So now, one more thing when you do DFBA one more calculation you do that is the exchange rate available for the substrate. This is a one more thing you should keep in mind when you do dynamic FBA. The dynamic FBA you do is during each time step delta t is a substrate uptake rate is depends on the current substrate. And the cell concentration on the cell maximum uptake rate ability.

So, I told you that every time you have to choose the exchange rate, so, this V you have to actually calculate for every time step. So, every time step you have to calculate this one. So, for that you have to actually know what value should I choose? So, what value I should at that particular iteration what value should I choose? So, for that, there is a scheme there is a method what is the exchange uptake rate I should choose for a given concentration for that, you should know what is the current biomass?

The biomass at which, you are evaluating the cell concentration and also the current substrate that is the amount of substrate present in the media. So, the V exchange substrate available is nothing but the concentration of the substrate divided by the concentration of the biomass and also divided by the time delta t is that which you are integrating the equation of concentration.

So, that time you can for example, if there is a given one example why if you if I have only 0.2 millimole glucose per liter and 1 ground dry weight cell per liter then and then in 0.1 hour the most substrate you uptake is I basically 2 millimole per gram dry isolate per hour. So, we put this value 0.2 divided by 1 divided by 0.1 then we get actually 2 millimole per gram dry isolate per hour.

So, that is the exchange rate available in the media based on the media but that might exceed the cell uptake capability. So, this you have to see what is the maximum uptake rate? The cell has some capacity. So, every cell has a capacity to actually uptake some substrate. So, you need to take either the cell capacity or the availability. So, this is the available limit based on the media and only cell also has a limit.

So, what should we take whether the available limit or the cell capacity limit. So then we say that the maximum uptake rate is actually that is the maximum between the EX available. The exchange rate available in the media and is the exchange rate for maximum for the cell. So, whichever is less negative, because the exchange rates are actually negative for the substrate. The excess flux for the substrate is always negative V is always negative.

So you take whichever is less negative. So that is the maximum value you choose between these 2 exchange flux. So, this is the theory behind it, how will you select the exchange flux.

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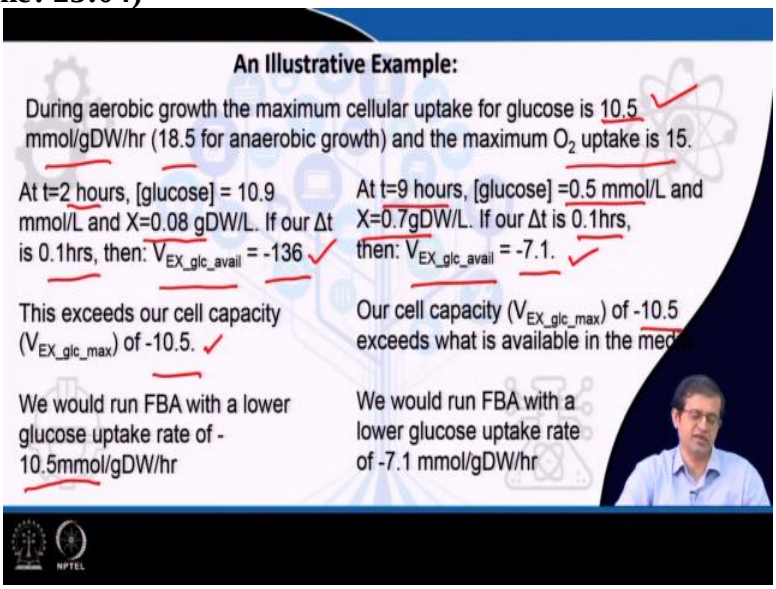

But I will give you more detail. So for example, if during aerobic growth the maximum cellular uptake rate for glucose is 10.5. Suppose you assume that the maximum uptake rate

for glucose for a given cell is 10.5 and for anaerobic, it is 18.5. And the maximum oxygen uptake rate is around 15. So at t = 2 hours. You see the amount of glucose present in the media is 10.9. And the biomass at that time point is basically at t = 2 hours is at 0.08.

If our delta t is 0.1 hour then V exchange glucose available is basically you just use this formula. So using this formula, you get V exchange glucose available is actually 136 minus 136. So which is quite very large, because that exceed the cell capacity, because cell capacity is only 10.5. The cell has the maximum capacity at which glucose can be uptake is only minus 10.5.

But your media is saying that it can go up to 136. So, which one cell will take this one or this one cell will take the minimum one that is 10.5. So we would run the FBA, where the glucose uptake rate is only 10.5, rather than 136. So this way, you can actually every iteration of integration, you have to use this condition, and then how much exchange flux is allowed in the cell.

And based on that you run the FBA because when you run the FBA when you calculate the concentration you need exchange flux, that is the glucose consumption exchange flux. And this is t = 2 hour then if we go t = 9 hour what you will see that the glucose concentration is 0.5 millimole and the biomass concentration is 0.7 so at that time point at t = 9 hours. If our delta t is 0.1 hour then you can calculate the exchange glucose flux which is available in the media as per the media how much exchange flux it can be what is the maximum?

So that you can calculate and found that it is 7.1 so which is much lower than the maximum capacity that is 18.5 so the media condition saying that it can only have 7.1. So but the cell capacity is 10.5. So which one cell we will take 7.1 or 10.5? So the FBA will be 7.1 because this is the maximum value available in the media. So you have to run the FBA with a lower glucose uptake rate that is 7.1 compared to 10.5. So this approximation is used when you do the simulation. So every iteration you have to check how much it is available the how much exchange flux available based on the media and also the capacity of the cell.

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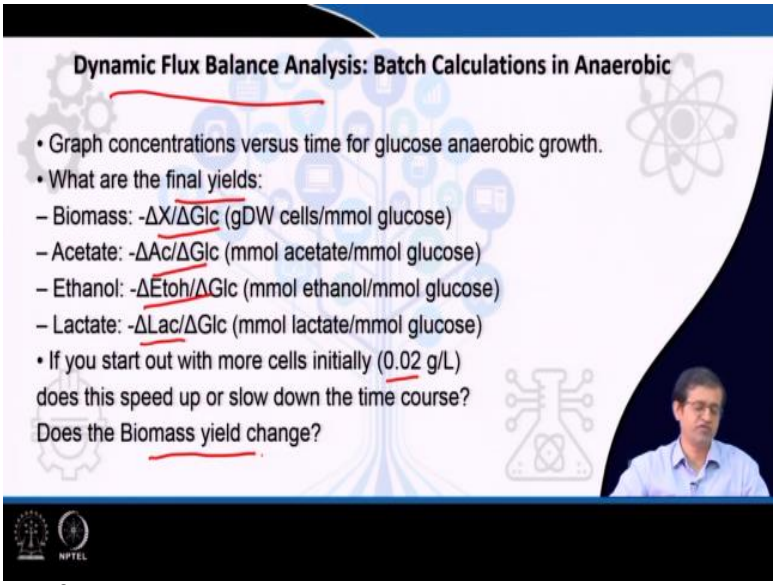

So during dynamic flux balance analysis batch culture calculation has been performed in anaerobic culture where you can also calculate the yield how much it is producing like biomass. You can see that delta X that is the change in biomass. How much biomass you are getting from t = 0 to at equal to some time you fix that that is a delta X you can calculate how much maximum biomass at t equal to infinity you can take.

When equal t = 0, t equal to infinity you calculate the change in biomass concentration divided by the how much glucose it has consumed. So that it becomes your yield of the biomass and similarly for acetate also the how much acetate it is produced divided by the amount of glucose consumed. Then amount of ethanol produced divided by the total amount of glucose consumed. For example lactate the total amount of lactate is consumed divided by the total number of glucose consumed.

So this way you can calculate the yield for the biomass acetate, ethanol and lactate and the yield is always actually millimole by millimole or gram by gram. Based on that you can found that how much efficiency the cell is working how much efficient it is. So this using dynamic FBA you can do a lot of things. So, using dynamic FBA you can find the yield of the biomass yield or acetate yield just by simulation. You do not have to go for experiments also so we can also change you can start with initial OD of 0.02.

And how does the yield is changing with if the starting OD is different that also you can check because you have to design an experiment. When you start a experiment you have to choose a initial OD and these initial OD you can simulate. And see how if you take a

different initial OD how we can actually the initial OD of the biomass. So how much starting OD you should take for running the experiment.

But if you can do some simulation like this it will help to choose which one is the starting point. So you can check whether if you start with 0.02 OD whether the biomass yield is changing or not, that also you can check. So this kind of experiment you can perform in computer and see what should be the starting OD.

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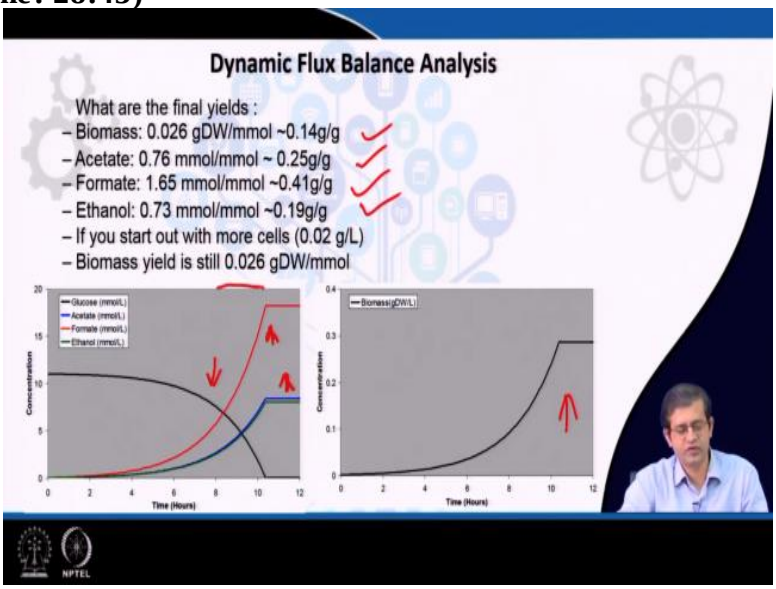

So this is a dynamic flux balance analysis have been performed for and they found out that the biomass yield is around 0.026, acetate yield is around 0.76, formate is 1.65, ethanol 0.73. So these are all simulation results the simulation results you can simulate it where you can see the substrate the glucose is going down. And then we have the formate which is going up and then you have the acetate and ethanol which is also going up so it is increasing with time.

And biomass you can see that the concentration of the biomass is increasing. So only the glucose is going down and other things are increasing. These are all entirely simulated results. And you can calculate the yield also; the yield for biomass acetate, formate, ethanol is also shown over here. And you can check you can vary the initial concentration of the cell to check whether the biomass is still remaining the same or not this is kind of experiment you can do.

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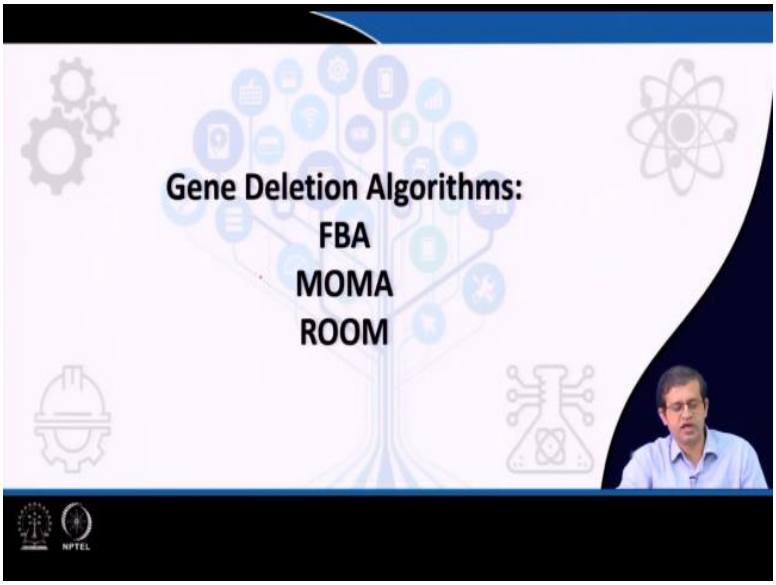

Now I come to gene deletion algorithm, gene deletion algorithm we will start with FBA.

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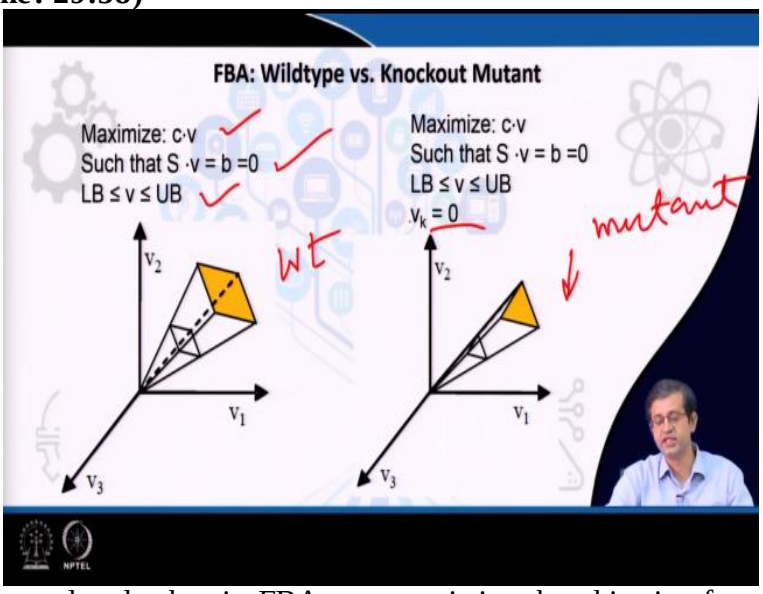

So FBA you know already that in FBA, we maximize the objective function. For example, you maximize any 1 flux, that can be a biomass, ATP or any other product that you want to maximize. And then you apply steady state condition S dot v = 0 and then put upper and lower bound of all fluxes, and that will allow the flux to be constrained in the solution space, where it is shown in the form of the cone all solution lies in the cone.

And then, in the mutant is, basically, you put one of the reaction to be 0 that we V k, that any reaction you want to mutate it. In that reaction we actually put the flux at the lower and upper bound to be 0 so it is basically lower bound of V k to be 0 in both the case in both lower bound and upper bound. This way, you can actually remove a reaction from the network.

Removing a reaction from the network is directly proportional to actually you can look back to the mapping GPR relationship you can see how many genes to be removed. So, suppose I want to remove a reaction, that is the flux for that reaction to be 0. You do not have to remove the reaction from the network you just put the flux going through their reaction to be 0. That you do by changing the lower bound and upper bound.

If you put lower bound and upper bound for any given reaction to be 0, then automatically that reaction is taken out from the network. And then also, you can see how many genes are actually removed. Because you are removing a reaction, that mapping, you have to design then you can go to the lab and knock off those genes to remove that reaction. So, this way, you can see that the moment you remove a reaction, then the solution space reduces.

Because you are putting a constraint. In the previous class also I told as you increase more number of constraint then you will see that your solution space reduces and you have a constraint solution. So here also you remove a reaction then you see the solution space also reduces. So your solutions space reduces over here and this is a wild type and this is the mutant.

So in the mutant, you see, they remove a reaction. So removing a reaction is also indirectly you are saying that you are removing a gene. Because every reaction has a mapping to a gene, it may be 1 gene, 2 gene, 3 gene that you have to see how many genes are you have to knockout to remove a reaction.

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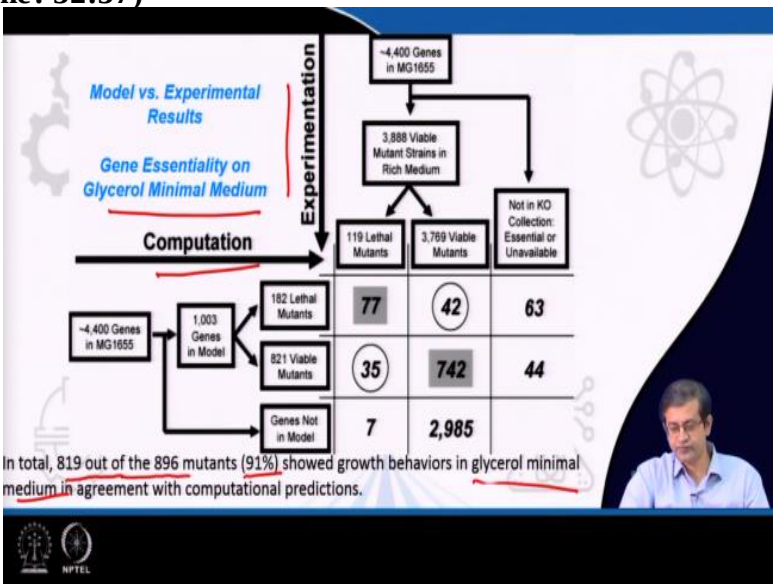

So, this is a model versus experimental result comparison where, you can see experimentally. You can actually mutate a gene and also through simulation or computational technique also you can remove a gene. And then you compare this to how it is performing. So in this a calculation were gene essentiality reality has been performed on a glycerol minimal media, where they use a strain that is MG1655 E. coli strain, which has 4400 genes and also you have taken a model which has 1003 genes.

So, the model has a lesser number of genes, and then you do in silico gene knockout one by one in knockout all genes. You see that 182 are the genes are actually lethal out of 1003 gene and 182 genes are actually lethal and 821 genes are actually viable. And this, when you compare with the experimental result, we see that 119 genes are actually lethal. And 3769 genes are actually viable those mutants so these are all single gene knockout.

And if you compare the model with the experiment, you see that 819 genes are actually it can predict correctly. So out of 896 mutants, it can predict correctly around 819 genes. So the accuracy of the model is 91%, which is very good in glycerol minimal media. In glycerol minimal media, they perform the experiment experimentally and also theoretically. And they found that 90% of the gene predictions are correct, using the model. So the models and these are all FBA mutants, every single gene knockout using FBA and they compare very well.

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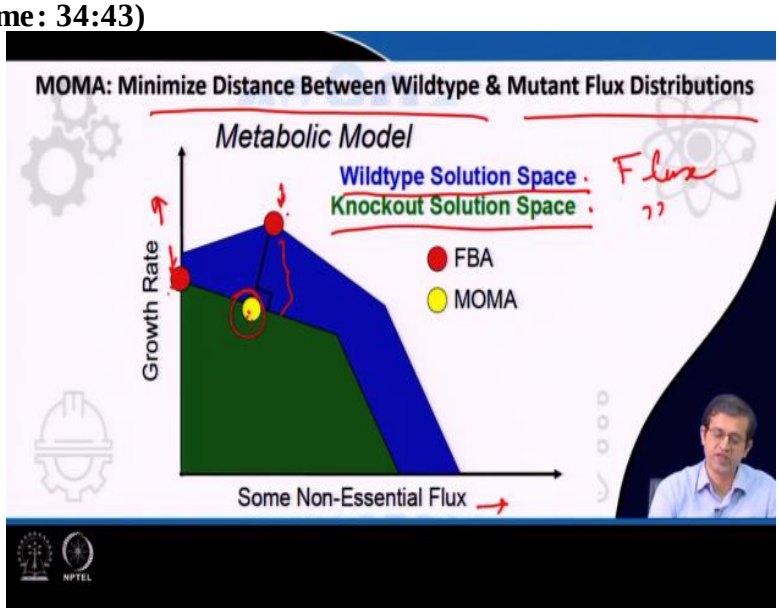

Now we will discuss about the MOMA, MOMA is another technique where you can actually predict the flux of the mutant, where you can actually predict the knockout fluxes. So, this is the blue one is basically the wild type solution space when there is no mutant and then the

green color is the knockout solution space. So, MOMA is basically minimizing the distance between the wild type and mutant flux distribution if you make a list of fluxes for the wild type strain.

So, if you have a flux distribution for the wild type and as well as for the mutant just single knockout using FBA and then you take a difference that how much difference it is there. So, this in this case you can see that you choose any non essential flux and then growth rate you can plot and you can see that the solution space for the mutant is smaller. So, is previously I told that as you put more constraints the solutions space will reduce compared to the wild type.

The wild type has a more bigger solution space but the mutant has a lesser solution space but MOMA gives a prediction that what is the MOMA? MOMA is another technique where you can get the prediction of the flux profile of the flux distribution you have for the wild type and for the knockout. But MOMA says no, the actual flux distribution for the knockout which is much more experimentally viable is not the one you get from the knockout flux profile by FBA.

But it is actually to draw a normal from this is the maximum growth rate you have in the wild type and this is the maximum growth rate you get in the mutant strain because this is where the biomass is maximum. So, these 2 optimal solutions you get for the knockout and the mutant. But MOMA says no this optimal solution is not here, but it is here. So, how did you get this solution space that the mutant solutions were there based on the MOMA prediction.

If you draw a normal from this point, and wherever it is hitting the solution space that is the point. If you draw a normal then that point become your optimal solution and that is the minimum distance. So, this is the minimum distance between the wild type flux distribution and the mutant flux distribution that is the minimum distance between 2 optima this is an optima you get from the wild type strain. And then you draw a normal and you get the most optimal prediction that you get from the MOMA.

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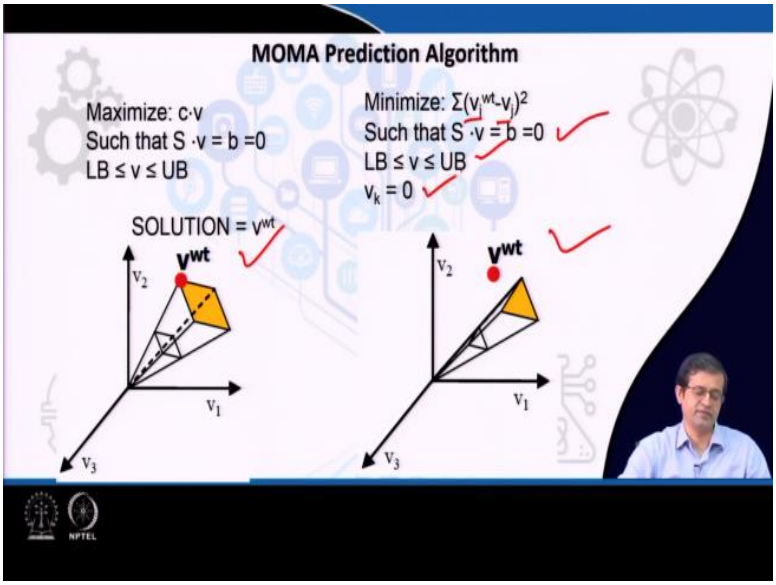

And that you can mathematically also you can calculate the MOMA prediction where you minimize the distance between the wild type flux distribution and the mutant distribution. So, mutant is the V j and the wild type is V j wild type. So, this is the wild flux distribution you get it from the just running simple FBA and also the mutant also you get it from FBA.

And then you take minimization problem or you take a difference with whole square and then the constraint remained the same that is S dot v = 0 and the upper bound and lower bound. And V k one of the reaction you have removed that is V k = 0. So, taking the difference, the minimize the distance between the wild type flux distribution and the mutant flux distribution you get the fluxes that is MOMA predicted fluxes, which is much more compared to the experiment.

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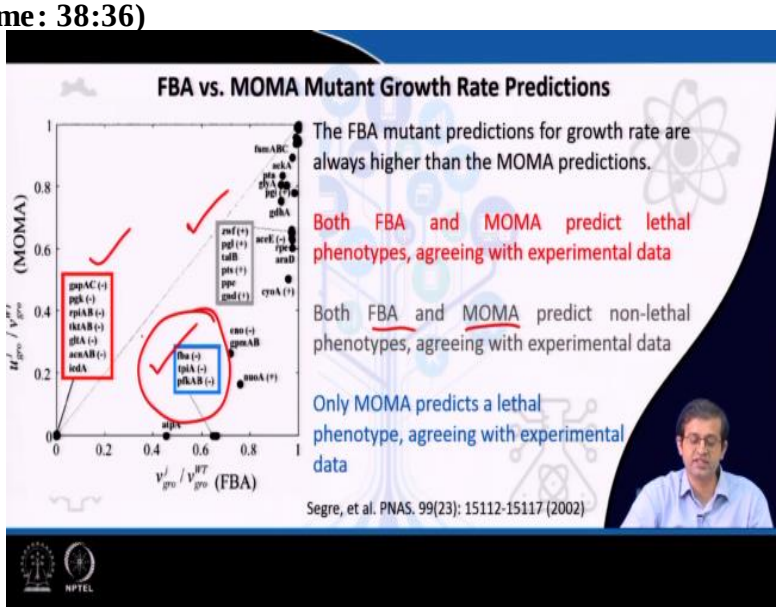

So, if you compare the FBA and MOMA that is a mutant growth rate prediction given by FBA and MOMA you will see that the predicted FBA and MOMA correctly predicted the knockout phenotype lethal knockout phenotype agreeing with the experimental data. So, these are the knockout which has been performed exponentially and it was found that FBA and MOMA predictions are actually exactly matching each other. And there are cases where FBA and MOMA predicted non lethal phenotype.

So, this is the cases where FBA and MOMA actually predicted zwf, pgl, talB, ppc, gnd and these genes are actually non lethal and then MOMA and FBA predicted in experimental data both these prediction are matching compared with the experimental data. But there are cases where you can see which is shown in blue color where the MOMA predict lethal phenotype agreeing with the experimental data where the FBA cannot predict. So, this is a case where there a MOMA prediction is much better. MOMA can predict much better because it can predict the lethal gene, which is experimentally shown to be true.

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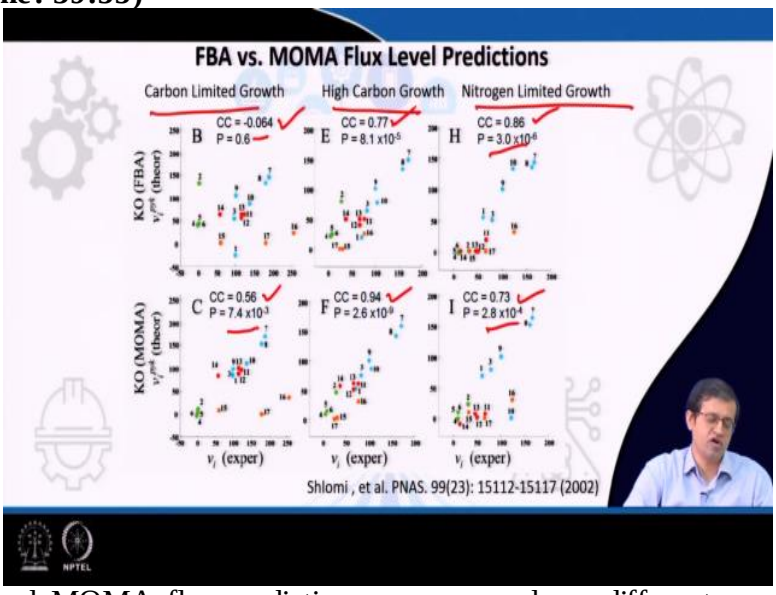

Similarly, FBA and MOMA flux prediction are compared are different growth condition like carbon limited growth and then high carbon growth, nitrogen limited growth. And they found the correlation coefficient CC is basically the correlation coefficient. And P is the correlation coefficient is actually low compared to the when you compare with the FBA prediction. Then correlation with 0.06 and the P value is 0.6 whereas, consider the knockout prediction the correlation coefficient is 0.56 but the P value is very, very less.

So, if the P value is very, very less than this is much more significant the results are much more significant. Whereas, for high carbon growth you see the correlation coefficient is 0.77. But, the MOMA prediction is much more much better like 0.94. So, in both the cases you can see 0.64 correlation coefficient is very low, but, the MOMA correlation coefficient is very high 0.56 and this case is very close to 1.

So, if it is very close to 1 then and then the experimental and the theoretical predictions are very much matching and here is the correlation coefficient for nitrogen limited growth is 0.86. But, for MOMA it is 0.73 in this case it has reduced a little bit, but the P value is very low, the P value is also very low for the FBA prediction. So, this way these are the 2 optimization tool FBA and MOMA that can be used to actually predict lethal knockout and that can be compared with the experimental data.

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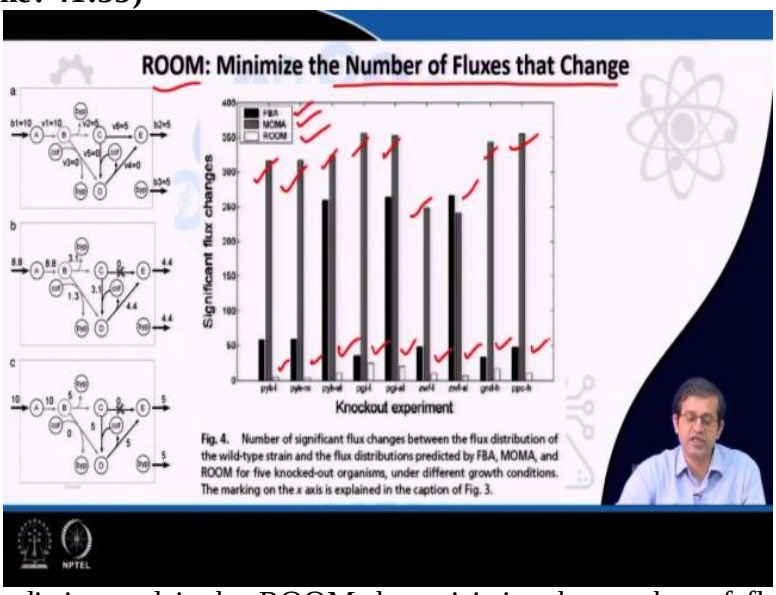

Now, another prediction tool is the ROOM that minimize the number of fluxes that change. So, what is the number of fluxes that change? There because the cell adjust within itself when you knockoff a gene, the cell will do minimum adjustments. So, the cell has a multiple optimal state and is very well connected network that is why this thing happened and when you remove a gene, then what happened that it will adjust within each cell so, that the minimum number of fluxes are changing.

So, this prediction ROOM algorithm will also work on this method where it changes minimum number of fluxes. So, these are the 3 prediction done in the FBA, MOMA and ROOM. You can see and then the knockout they have performed the number of significant

flux changes between the flux distribution of the wild type strain. And the flux distribution predicted by MOMA, FBA and ROOM for knockout organisms are shown over here.

So, they have done this many knockout you can see that the ROOM, the significant changes fluxes is maximum in FBA. So FBA is actually all the cases 1, 2, 3, 4, 5, 6, 7, 8 and 9. So, 9 knockout strain they have predicted and in all the 9 cases, you can see all the 9 knockouts have actually maximum flux change in FBA. But MOMA predicted little lesser much lesser than the FBA technique.

But ROOM you see, in all the situation the flux profiles are actually very, very less compared to the wild type. So, the number of fluxes changing is also very less. So, these 3 techniques are widely used to actually predict the flux profile of the knockout strain and each of the technique has their importance.

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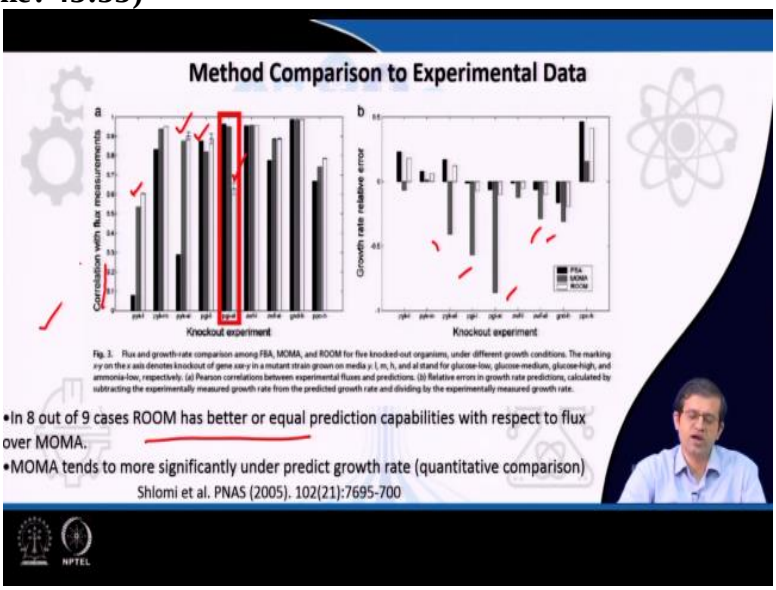

And then they got compared ROOM with experimental data. So, ROOM, MOMA and FBA have been compared here you can see the 9 knockouts. And the correlation coefficient with the flux measurement is plotted over here. The correlation coefficient is very high. So, 8 out of 9 cases ROOM was actually performed better. So, the ROOM actually out of 9 mutant 8 of the cases the ROOM actually perform better you see.

The correlation coefficient is more, more here, more here more only in this case, which is shown in red square box, rectangular box where we see there the prediction is low. So for

ROOM prediction the correlation coefficient is low. So, on all the cases except one case, why the ROOM is has a better prediction?

And if you compare the growth rate the relative error in the growth rate you can see that the MOMA is actually not performing better in the growth rate in all the cases you can see that the MOMA prediction is actually predicting, over predicting under predicting the growth rate. So, in all the cases the growth rate predicted by MOMA is not very good apart from correlation in the flux. So, in this way you can compare the growth rate and the flux profile you can compare to find out which of the method is actually performing better. The ROOM has performed better here, whereas, MOMA not perform well in terms of growth rate relative growth rate.

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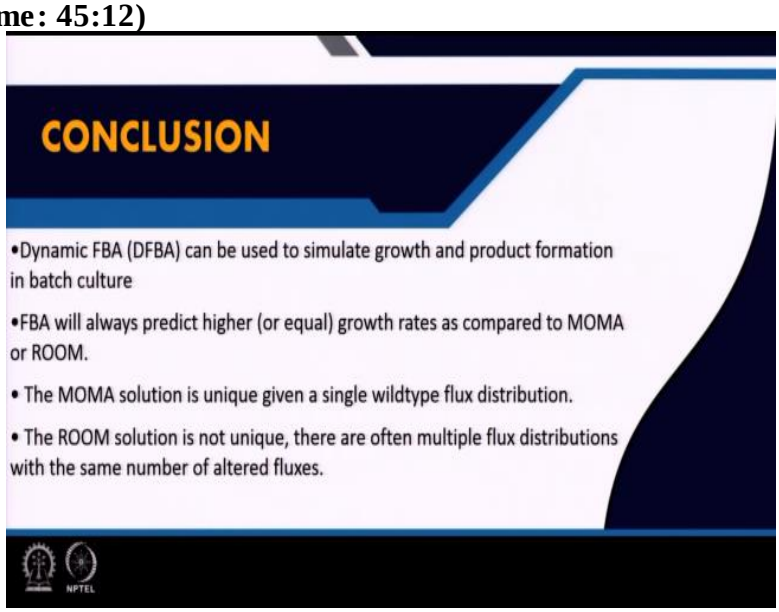

In conclusion, you can see how we have seen today that dynamic flux balance analysis actually can be used to simulate growth in batch culture. And you can simulate the product formation substrate consumption biomass formation those things you can actually calculate in a dynamic fashion. And you can compare with the experimental data and the FBA will always predict higher growth rate as compared to MOMA or ROOM.

So, in all cases we have seen that it is predicting more growth rate compared to a MOMA and ROOM. And the MOMA solution space is unique given a single wild type flux distribution, the MOMA solution space is unique. And then ROOM solution space is not unique. And they are often multiple flux distribution of the same number of altered fluxes.

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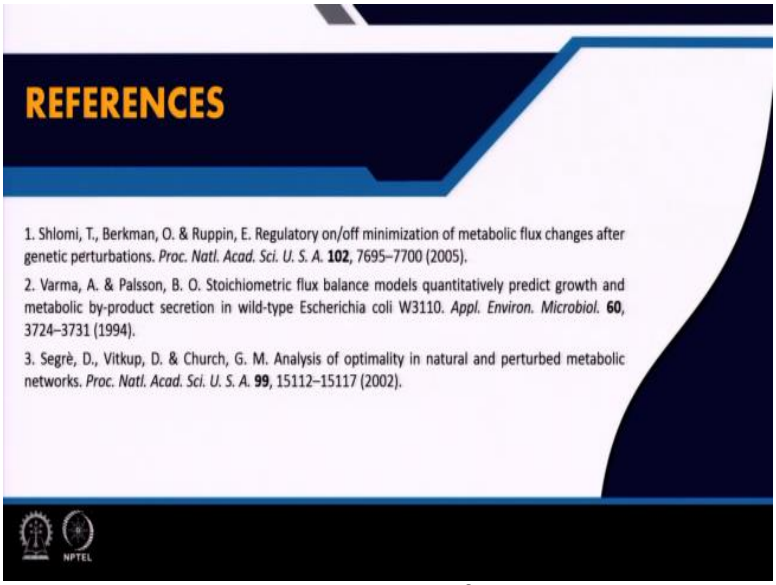

So these are the conclusion and then we have the references. Hope you enjoyed the class and thank you for listening.