Material and Energy Balances
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Module No # 07
Lecture No # 33
Flux balance analysis – Part 2

So welcome back the let us look at most important aspect of FBA or what is potentially the most deviated aspect of flux balance analysis. How do you pick this up objective functions in your school problem of chairs and tables objective function are very clear you wanted to maximize the profit of industry or whatever right.

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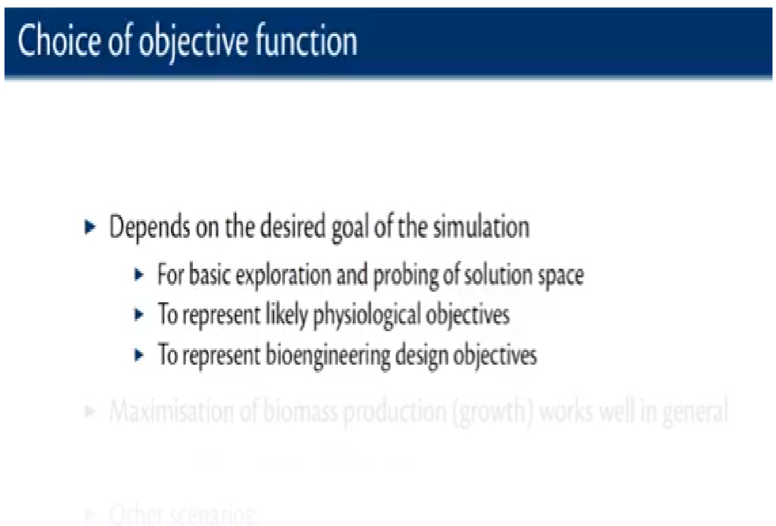

In this case how do you what is the objective function that you pick and this is where I want you to have a more fundamental understanding of FBA and let us see what that is? One obvious rate of objective function is based on the desired goal of simulation may be for basic explanation and probing of the solution space or to represent likely physiological objectives or to represent bioengineering design objectives.

So let us just look at the last two that you can imagine what exploring the solutions spaces right. You just try different objectives functions to see what kinds of configuration are admissive in the cell. But let us remain ourselves what we have been doing.

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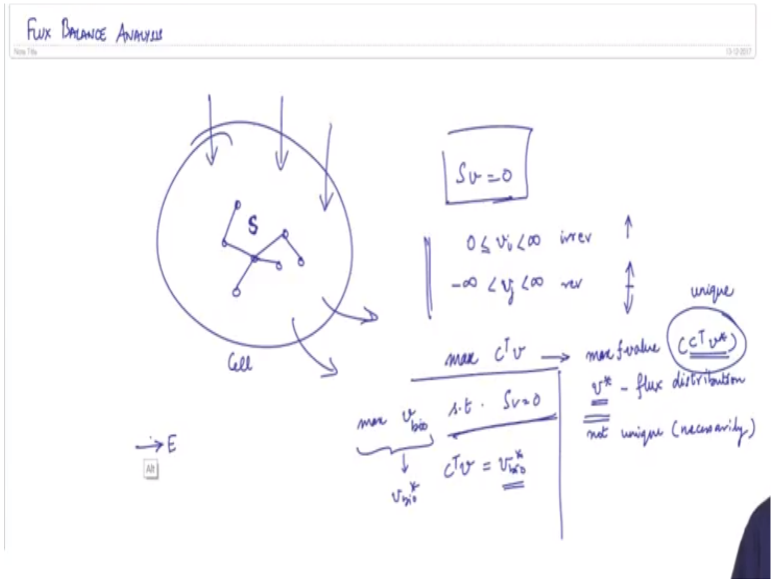

You have a cell which takes several metabolites gives out some metabolites and there is a complex metabolic network inside of it. This is the metabolic network essentially represent as S or stoichiometric matrix and what you do is SV=0 this is not you cannot violate this constraint right this is a fundamental constraints why it arises from stoichiometry?

You can only produce one mole of fructose from one mole of glucose no other choice right and this is thermodynamics reversible and irreversible reactions and let us actually not say infinity, but typically some large value right we know this as to be only in this direction and this can be in either direction reaction right the values. So right this can be 0 of course so this is what we have so far and you said you cannot solve this system and ambiguously one unique solutions.

So to pick one solutions we said let us now impose this objective function maximize C transfers to be what does this give you? It gives you two things when you solve you will get the max value or what we call the max function value this is basically C transfers into the V best and which is basically or flux distribution what can you now let me about the uniqueness of V star and C transpose V star.

Have you studied linear programming before you may need to call in some logic of linear programming here. It is interesting to note that this is unique whereas this is not unique this has major implication where everything we do it with FBA. And one thing it is easy to understand the math of FBA but as always in this course what we want to understand is you know the ramification that any mathematical technique that we have has for modeling any given system.

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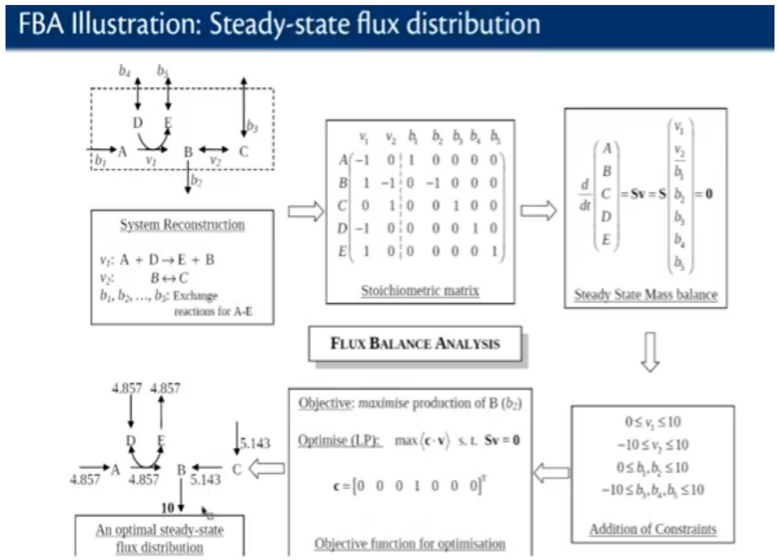

So let us look at a simple illustration of FBA how does a FBA actually work so you start with a system or you reconstruct a system how do you reconstruct a system go back to couple of modules (()) (05:20) databases literature all the hard work essentially send a long list of biochemical reactions occur with in equal eye of any organism of interest. Given this is your system now how do you set up the what is the next step you would do here for flux balance analysis.

DX / DT = S into V what is the stoichiometric matrix what is going to be size of the stoichiometry matrix hmm here what is M and what is R? M is 5 this is one reactions right this basically A + D getting E + B this is how you nominate that (())(06:19) right ATB becoming ADP is something like that. So that makes it 7 right so you have internal reactions V1 and V2 and you have a bunch of exchange V1 and V2, V5 fair enough so the stoichiometric matrix therefore be 5 cross seven and it looks like this fair enough.

So now you can see the balance this nicely V1 is B1 which really make sense right sorry you will find that V1 yeah – V1 + V1 = 0 balance for A and B inverse in three reactions so V1 – V2 – B2 = 0. Whatever flow comes in split across these right this V2 in turn depends on the V3 this V1 is in turn depend on something and on it depends on this and so on you will find all of these right. But overall because V1 also linked to be 4 V1 is also linked to B5 and like that.

So one thing here is it is a little there is a bit of catch here I cannot have reversible arrows here because if I say this is -5 I have to commit to one direction in the first place right. So here it is a reversible arrow is shown the sake of highlighting the reversible reaction but in a sense when you start even writing a stoichiometric matrix you have to understand B4 in a particular direction V5 in a particular direction right so what is B5 here? What is this is a reaction right it is E on the right hand side alone which is basically it is say something like no so what I am saying is?

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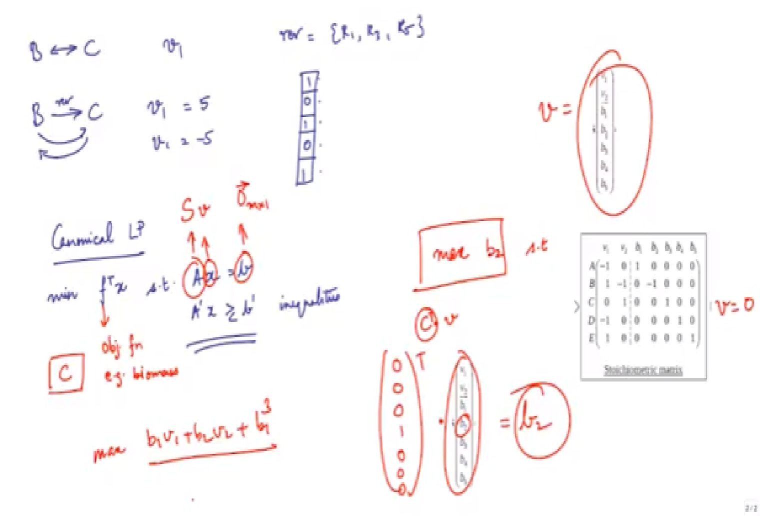

What was the reaction take the same reaction? Have a reaction like this if I say V what is it? This is the flux of the power reaction and the backward reaction so it make sense if I say this is a reversible reaction with V1 has the flux right. So now if V1 is 5 it means B is going to C if V1 is -5 it means C is going to B yeah. So invariably all the reaction you keep the reversibility information separate.

So you say reversibility R1, R3, R5 something like that what are the all the list of reversible reaction or typically you keep a Boolean vector which says 1, 0, 1, 0 and 1 this says R1 is reversible R2 is not R3 is reversible R4 is not R5 is reversible right. So when we do lab session you will get familiar with this because to even fill up this column I need to know what the reaction is this reversible.

I still need to know what is the forward and backward reaction right so you can assume that all the reaction are the forward reaction and if you get a negative flux you get in the reverse direction. But what do I even be by forward direction is this the forward or the backward right so I need to commit to 1 arrow direction. So assuming that we have this so here in fact you should this should have been a -1 right he should be going out for example otherwise you just have a V1 + B5 = 0.

So they are going to be of opposite science for sure so now you set up this equation DX / DT = SV. And you say SV equals 0 so now you have to solve the equations is basically reads – B1 +V1 = 0 V1 – V2 – B2 = 0, B2 + B3 = 0, - B1 + B4 = 0 and so on. Essentially multiplying this with this matrix multiplication and note that this is just a partition and try to show it might seem like a divided by or something, but it is a partition to show that there are internal fluxes and there are some exchange fluxes in the same vector.

So the next step you put in other constraints from the knowledge of biology or whatever here that toy constraints I say 0 less than equals V1 less than equals 10 -10 less than equals V2 less than equals 10. So typically for any irreversible reversible reaction I would have the constraint in both the reactions for the irreversible reaction it is only in one direction. What is the next step? I have assembled the constraints I have to do the optimization so I say my product of interest is V.

So I maximize the secretion of B outside this cell how much V is coming out of this cell right which means maximize B2 which means maximize 0, 0, 1, 0, 0, 1 and 0, 0, 0 into this vector right. I am just putting that back into canonical form right. So what is the canonical LP you know canonical quadratic equation is X square + BX square + C = 0 right. So the canonical LP is minimize F transpose X such that you can even have in equalities right.

This is an equality constraints you can have inequality constraint as well right this will be a proper linear program. Now if you map it back to our system what is A? What is B? What is X? What is F? projective function like biomass which is basically your C this is the name we gave and it is useful know the variable names as well because we saw the same things that are used in the mat lab tool box for solving flux balance and other such problems what is A the equivalent of A the stoichiometric balance.

The equivalent of B 0 vector what is the size of the O vector M cross 1 very good what is X? Is this clear? If you want me to repeat it I will repeat it because this can be a little confusing and it is important to fix say this ideas before we go any further which is why I drop the discussion on objective functions and jumped back to an example prevail toy be a problem. So basically start with system the extract the stoichiometric matrix out then we write this mass balance equations.

DX/ DT = SV this is the mass balance equation a steady state this becomes 0 right and then we had other constraints base on knowledge biology capacity whatever and then set up the optimization problem because this is going to give us infinitely many solutions still right. So then we set up the optimization problem and we compute the best V like we shall give you the max C transpose V this is C.V right or the same just the dot product but these two vectors.

Such that X3 = 0 and you see that 0 is also bold meaning that is also a vector this should give you a solution one possible solutions set is this so you have 4.857 of A coming in 4.8 of A coming in 5.143 of B coming in giving. But if you see this is one just arbitrary solution there are many equivalent solutions you might be able to come up with we could just have 0, 0, 0, 0, 10, 10, 10, 10.

This is the objective functions I said maximize I said max B2 such that S into V = 0 right and what is V? V is nothing but this. So now this is your LP problem this is your LP objective function so this as to be return as some C dot V so what is the C vector that will multiple this B2 it will be 0, 0, 0, 1, 0, 0, 0. So now C transpose this transpose into this vector good it just seems like little contract it is not just familiar with you know linear programming and linear algebra but essentially this is what it is right.

I set this up as my objective function of choice and I have to give my objective function as some linear combination of all these variables and then linear combination of 0 of all variables except B2. As some linear combination of V which is when remain linear programming I can come up you can say you are very well in and then you have a rights to say maximize B1V1 + B2V2 this is quadrature this is B1Q non-linear.

You can go in for any objective function right your constraints are same as SV = 0 linear constraints of course this is therefore no longer linear programming we will see example of these that are very interesting formulations and non-linear programming based there are non-linear optimization based we will give you interesting results. So let us get back here we see that they are all there are different possible solutions you can have right.

So clearly 0, 0, 0, 0, 10, 10, 10, 10 is one solutions or simply 5, 5, 5, 5 is another solutions right of course this will be 10. And this will max out at 10 because that is a constraint that you have yourself imposed here is 0 less than equals B1B2 less than equals 10. So now I want to draw your attention back to the fact that the solution is unique or the objective function value is unique whereas the possible flux distribution are many.