Computational Systems Biology
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Lecture – 66
Understanding FBA

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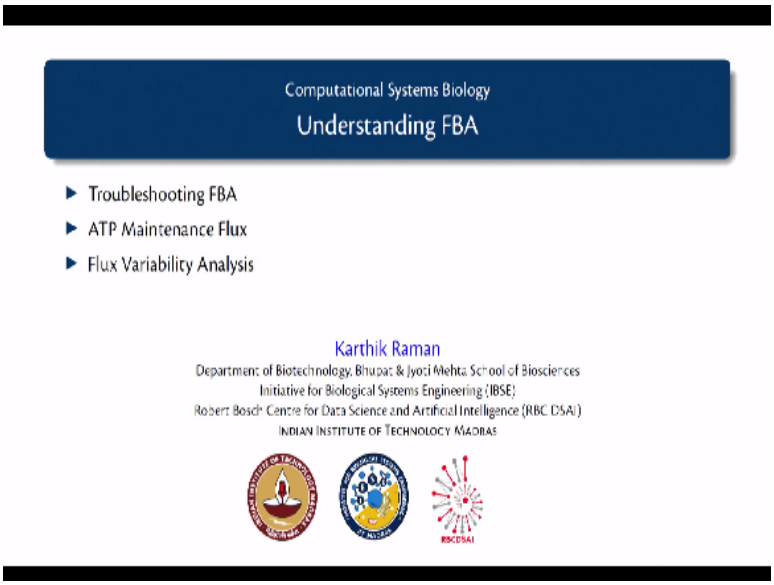

In this video, we will continue with building our understanding, our practical understanding of FBA. We will look at, you know, how you go about troubleshooting an FBA. So the classic problem is you have a model, it does not show growth, how can I fix it, right? So what are the things that one might need to watch out for?

What are the possible sources of this error and we will also look at the concept of ATP maintenance flux and this very interesting concept known as flux variability analysis which tries to tell you what are the minimum and maximum fluxes that any reaction can admit or any flux can, values a flux can take under a given set of constraints.

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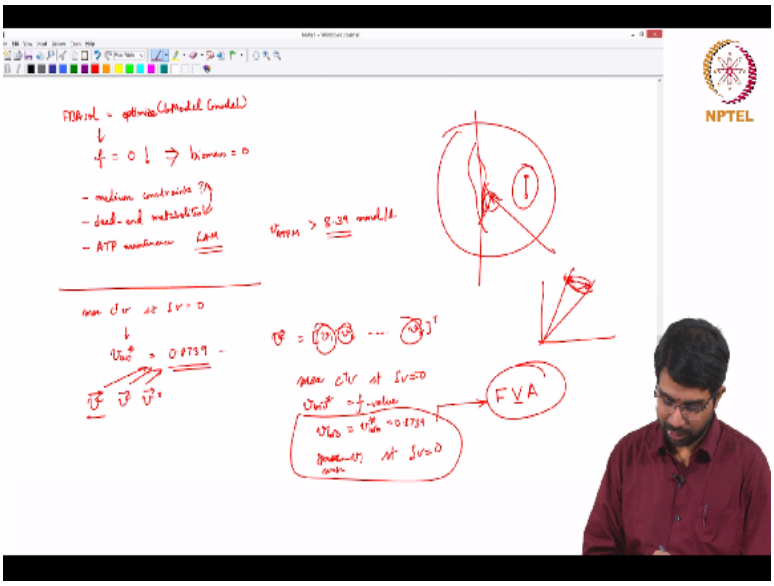

So you now understand the concept of a gap, a blocked reaction and dead-end metabolites and also how to put a band-aid fix for dead-end metabolites, right. So the most important thing you need to really worry about when you are performing FBAs, the first thing you will encounter is, you take, you build a model or you download a model and you do optimize CD model. You will find that you get 0 growth rate.

How do you fix it, right? So, so let us say we say, I have a problem that everybody encounters. Practically what do you think this could be because of? What are the reasons that might give you 0 growth rate? So the first thing would be, are my median constraints making sense or not? So one way of looking at is your, see the obvious, okay, the obvious reason why this happens, immediate reason consequence of this or this is an immediate consequence of the biomass reaction not being able to carry a flux.

Why does the biomass reaction not carry a flux, that is what we are looking at essentially. So the other issue could be that what we just discussed, we could have dead-end metabolites spoiling the show or… blocked reactions do not effect. Blocked reactions are, you know, unnecessary baggage, you know, they exist, they do not carry a flux but they do not affect your simulation as such.

There may mistakes in the model or gaps in the model, that is something we will have to

diagnose later but for now, why would we have 0 flux? It could be because your median constraints are wrong or may be there are some dead-end metabolites in the model which do not have a source or sync but typically these are 2 major things. The third thing could be that there is usually an additional flux known as flux for ATP, right.

So this can also go wrong. What is this? So you have something known as growth-associated maintenance, right. There is obviously some error, some ATP that is going into your biomass, right. We, we did see that in thee biomass equation in the previous class but in addition to this the cell may require some other ATP, some more ATP for sustaining other cellular function, right.

So if the cell is really decelerated like we were doing earlier reducing the amount of glucose to a very low value and so on, may be the cell is not producing enough, sufficient ATP to sustain life. It is able to sustain metabolism theoretically but it cannot support other important functions, right. So this is usually bundled up as another constraint which says from the ATP maintenance>something… for E. coli, it is something of this sort. So this is actually a fit value to make sure that your lethality results agree nicely with, or your growth rates agree nicely with experimental values.

"Professor - student conversation starts" Sir blocked reactions are because of dead-end metabolites. Blocked reactions are because of dead-end metabolites, yes. The second thing you can just tell that because blocked reactions (()) (05:16). Well not exactly. That is only if it is always blocked. So you could have, yes. **"Professor - student conversation ends."** So you could have, you know, several path rates like this and you can, this could be a blocked reaction not causing any damage.

If one of these reactions is blocked, if this were blocked, then you have trouble because the biomass gets blocked in turn because everything is like sort of interconnected. **"Professor - student conversation starts"** Yes sir, (()) (06:00) known as dead-end metabolites, right. These are dead-end metabolites but they are not going to affect the, so their fluxes are going to drop to 0. It is not going to affect the rest of the fluxes.

But if the, if some important metabolite here is dead-end, that is where the problem comes. So all these are actually interconnected which means that, you know, your median was wrong or something is not been specified, right. So you need to open an exchange flux for this. So you need this from outside then it all works. **"Professor - student conversation ends."**

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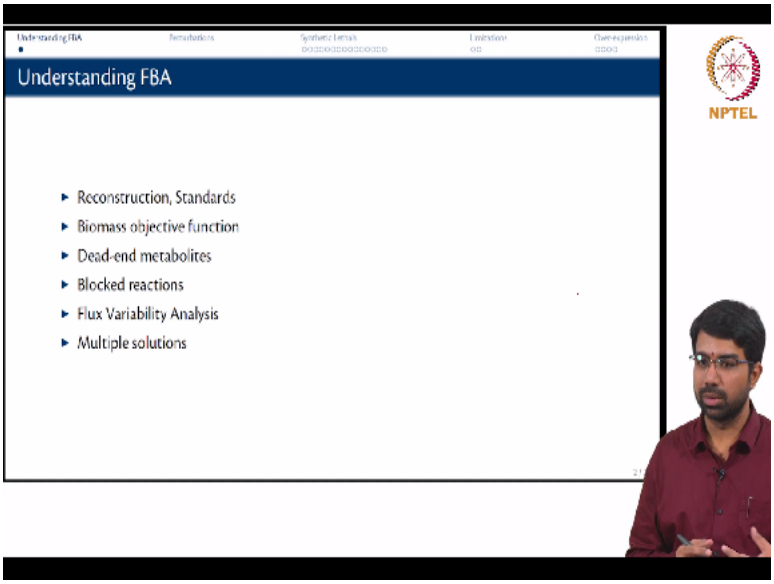

And let us again revisit the concept of multiple solutions, right. We have been delivering that over the last few classes and the previous lab session and so on, right. So this is what you are solving, right. So let us say the solution, let us say you got the V bio as V bio*. This is like the growth rate, let us say this is your 0.8739 some units that we just saw earlier in for the E. coli model, right.

So now if you use a different solver or, you know, something else, what you guarantee to get is, the same value for V bio* but the V vector itself could be anything, could have multiple V vectors that give the same growth rate. So this being the case, it is very interesting to understand how much variation can it admit or rather this V is going to be something like, right.

So how much can, what is the leeway that V1 has for variation? What is the leeway that V2 has for variation? What is the leeway that Vr has for variation? So can you think of what is the maximum value of V1 that is possible? Can you set up a simulation to figure that? What is the maximum value of V1 that is admissible by the cell?

“Professor - student conversation starts” (()) (08:18) upper bound. See that is the upper bound, right but what does the cell… See we even saw earlier in the, in one of the examples that we said that the upper bound of oxygen was -100 or something. But the real value of oxygen uptake rate was -31 or something like that. Yes but that was one, one solution. You see all the solution, right.

Yes, so, but that might actually be the, you do not know that could very well have been the maximum oxygen uptake bound under those conditions. So that is what we are getting at. **“Professor - student conversation ends.”** So how would you formulate this? How would you find the maximum value of V1? So you can basically sort of reframe your objective function. Now you do the same thing.

You first to max C transpose V such that SV0. You compute your V bio* with this, right. This is the f value, optimal growth rate. Then you add a condition V bio=V bio* and now maximize V1 such that SV=0 and V bio=V bio*. You can also minimize it. This is known as flux variability analysis. It tells you what are the boundaries of the flux cone essentially. We were talking about a cone, right.

So you have several fluxes and there is a cone, so what is the boundary in some sense? So each flux, how much can it vary? V1 can vary between so much. So this tells you, so suppose you know that, so what is a blocked reaction in light of FBA? **“Professor - student conversation starts”** The boundary, maximum value is 0. Max and min are both 0, right. So that is the blocked reaction. Very simple way to define it.

So V bio stands 0.8739. Correct. You want to maximize C transpose V. We have already maximized C transpose V to get this. C will be having only 1 value, only 1 (()) (10:56). Yes. So whatever value of V might be, to maximize it, that particular thing is if it is becoming more than some, let say if it is, that particular (()) (11:08) becomes more than everything, then we can tell this maximum, right.

We will not care about the remaining n-1 element. But they are all constraints, no, where V=0. So everything is intimately tied. So no, almost no variable can vary freely. V bio is connected to several other things. **“Professor - student conversation ends.”**

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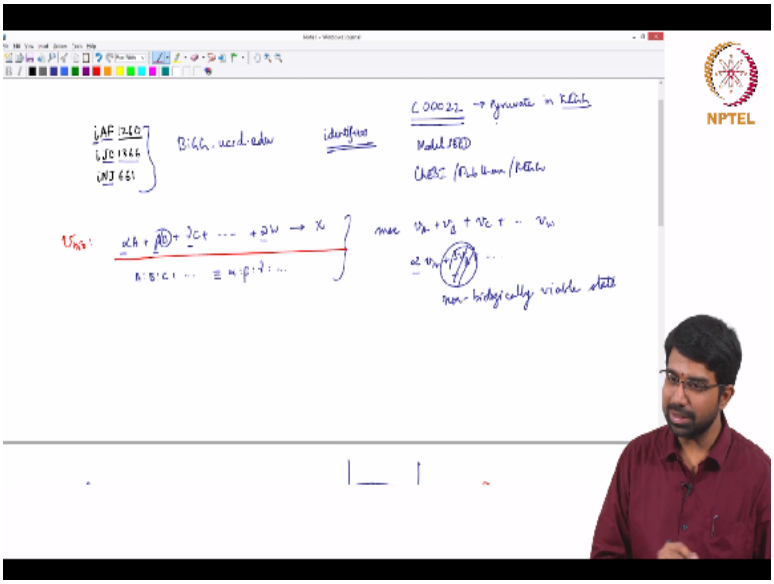

It is connected to everything in that, in this equation, right. This is your V bio, right. So it is connected to everything in this equation. So you will find that all the fluxes are actually interlinked by SV=0. **“Professor - student conversation starts”** Sir we did V1*, so this will be the max V1 (()) (11:49) how will that maximize for V bio, well you get (()) (11:56) with that constraint. Yes, yes, yes. That will be valid. **“Professor - student conversation ends.”**

Because we know that this is an admissible, feasible solution. So unless you say that, suppose you solve x+y=10 and you got x as 5, right and now you, you put that value back into x+y=10 and find out the value for, it will still be feasible, right. Unless you put in an arbitrary value, now you put -10 and then try to figure out a something, then you would have a problem. So this has very interesting implications, right.

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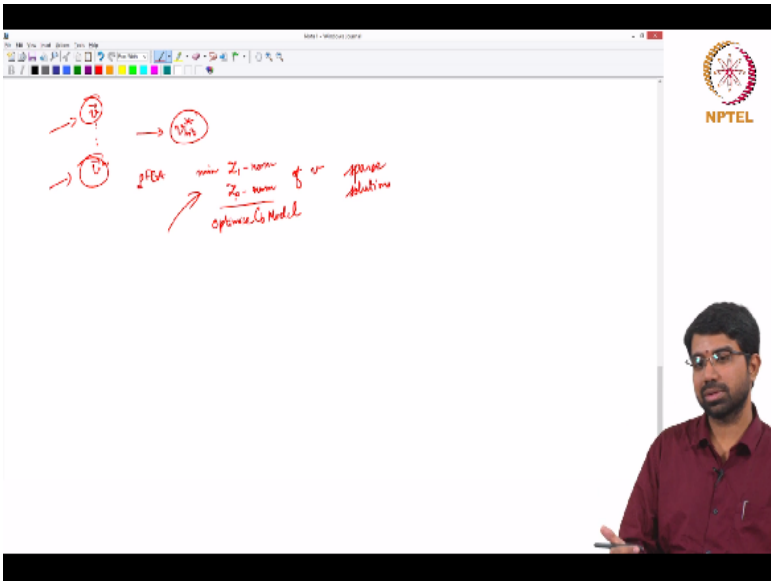

So now you are absolutely convinced that there are many vectors, right which can give you the same V bio. Which of these vectors is more biologically valid, right? So one argument was this idea called parsimonious FBA which is you will be, you know, parsimonious with the vector. So if you can drive something to 0, please drive it to 0, right. So which means that you minimize the, we can talk about minimizing the L1 norm or the L0 norm of V.

So of all possible V's, that solve this. Can you find that which minimizes the L1 norm or L0 norm and in fact, this is built in to optimize the V model as well. This will be like a par solutions. So parsimony is the same as parsity. So these are some very important points that one needs to keep in mind while working with flux balance models or with metabolic models and studying flux balance analysis.

"Professor - student conversation starts" But why some, most pars that does the most biological flux? Because, you know, there is some economy in biology, right. So the cell tries to expend its resources very wisely. It would not be wastefully expending reactions that, you know, driving reactions in a rate higher than its necessary. But, well and mathematically speaking you can have a few cycles or loops that are taking arbitrary values so you can drive them to a low value or even cancel them out.

So this is just that you nullifies as many reactions as possible. Yes. And this is also some,

somewhat related to solver artefact and so on. The solver will try to give you, so each solver depending upon the algorithm will arrive at one of these. So this gives you a handle to get a more unique solution. It means, still not be unique. There is no guarantee about that. What is guaranteed of course is still your V bio* is unique, right but these solutions may still vary.

So that problem where the bio react, for bioreactor design, we have to find some (()) (15:17) between the cells maximum, the cell maximizing its growth rate and you want take a maximize metabolite. If your answer is not to work, you can just excuse this, you can get maximum cell rate, cell growth rate and then find the maximum admissible metabolite and then adjust it. Very well. So that is bilevel optimization that people normally do to kind of find the optimal rate at.

So what is the max product for the max biomass. **"Professor - student conversation ends."**

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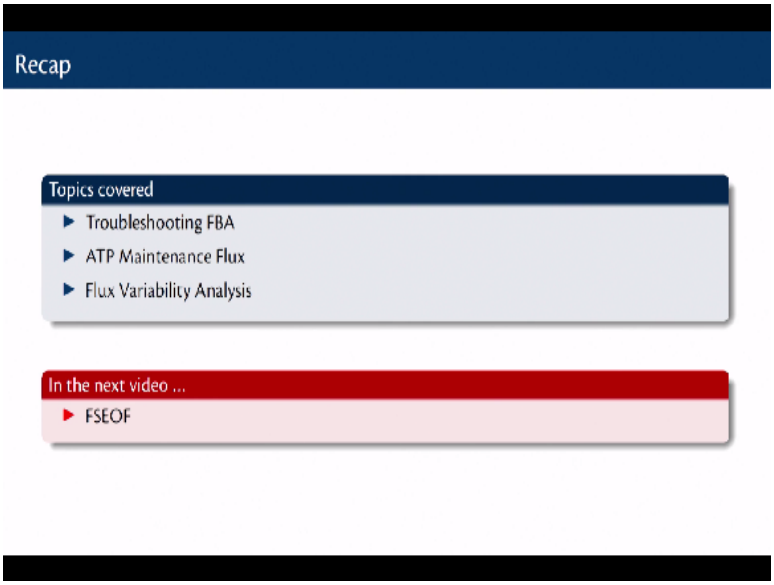

So in this video, I hope you got a good overview of how one goes about troubleshooting an FBA simulation and we also studied the concept of ATP maintenance flux and the very interesting concept of flux variability analysis. In the next video, we will go back to how we perturb these metabolic networks and look at the other kind of perturbation. We have already looked at deletions previously. Now let us look at over expression which involves this interesting technique known as FSEOF which stands for flux scanning based on enforced objective flux.