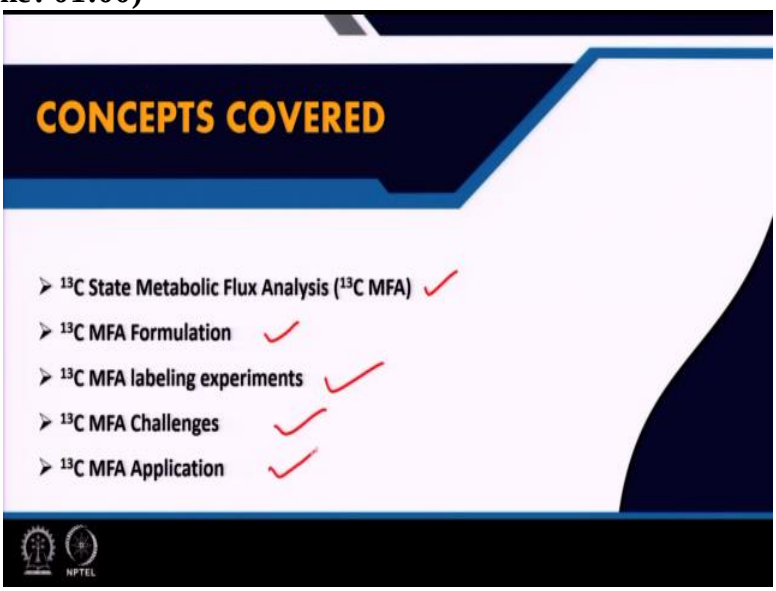
Metabolic Engineering
Prof. Amit Ghosh
School of Energy Science and Engineering
Indian Institute of Technology - Kharagpur

Lecture - 24
13 C Metabolic Flux Analysis (13 C MFA)

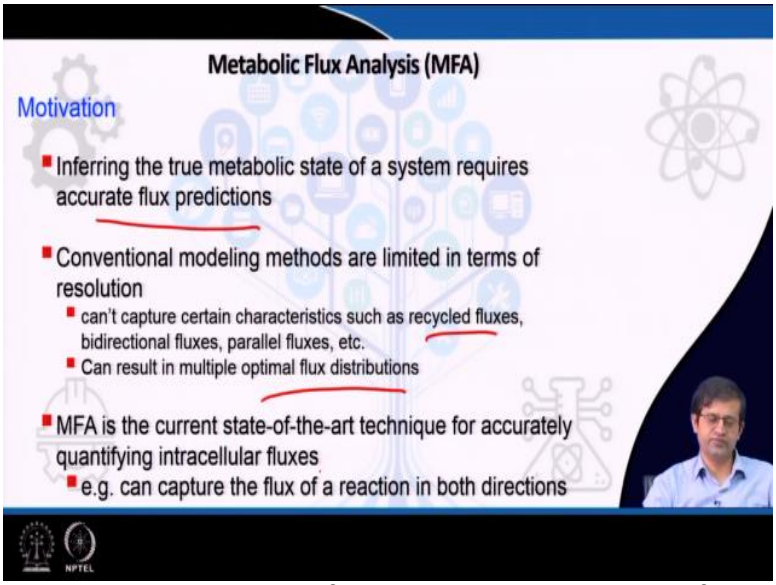
Welcome to metabolic engineering course today we will talk about 13 C metabolic flux analyses. 13 C metabolic flux analysis, so far we learned about flux balance analysis. So, in this class we will learn about 13 C metabolic flux analysis. So this is an experimental way to determine the fluxes. So, in FBA we basically did not do any experiment, but in this case we in 13 C MFA we will deal with doing labeling experiment.

(Refer Slide Time: 01:00)



So, let us discuss about this topic today. And the topics will be covered in this part of the lecture will involve 13 C MFA. And then 13 C MFA formulation, 13 C MFA labeling experiment, 13 C MFA challenges and 13 C MFA application.

(Refer Slide Time: 01:19)

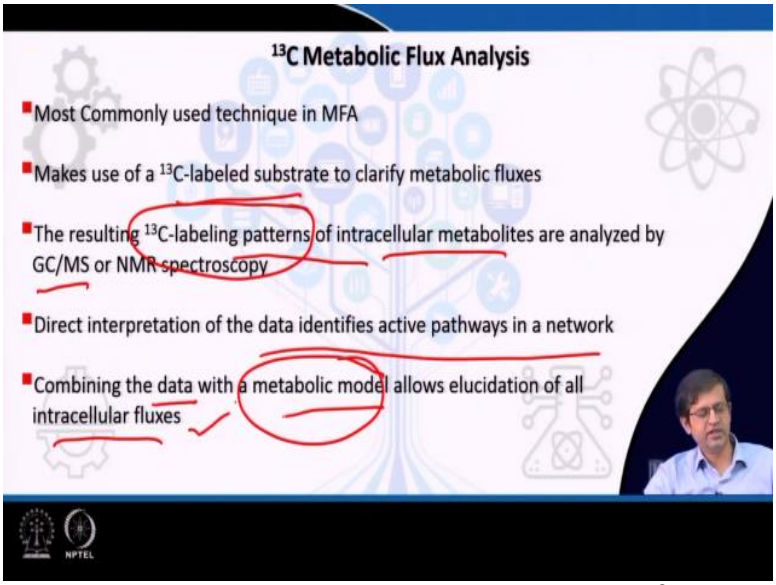


Mostly we will start with the metabolic flux analysis. So, metabolic flux analysis is a wide subject where you want to infer the metabolic fluxes accurate metabolic flux prediction. As I told the metabolic fluxes cannot be measured directly from the experiment. So, we need proper metabolic modeling and then we can accurately predict fluxes. So, rather than measuring fluxes we actually predict fluxes or inferring the true metabolic state of a system.

So, metabolic fluxes are actually the true phenotype of a cell. So, whatever the genomics, transcriptomics, metabolomics, ultimately if you know the metabolic flux, that is the ultimate phenotype of the cell, so, nothing better than flux. So, it involves modeling as I told you, they are conventional metabolic modeling are limited in terms of resolution cannot capture certain characteristics such as recycled fluxes, bidirectional fluxes can result in multiple optimal flux distribution.

So, FBA generally give multiple flux distribution that those are the limitation in metabolic flux analysis. MFA is the current state of the art technique for accurately quantifying the intracellular fluxes. Using metabolic flux analysis you can actually accurately quantify intracellular fluxes and that is can capture the reaction in both direction. So, both forward and backward reaction can be a measure can be inferred experimentally also.

(Refer Slide Time: 03:05)



So, the 13 C MFA mostly commonly used in MFA 13 C metabolic flux analysis makes use of 13 C labeled substrate. So, here you allow the cell to grow in a labeled substrate. So, the labeled substrate is quite expensive than normal glucose. So, that is why these experiments are actually very expensive experiments, not only expensive experiment, once you extract the metabolite through different protocol.

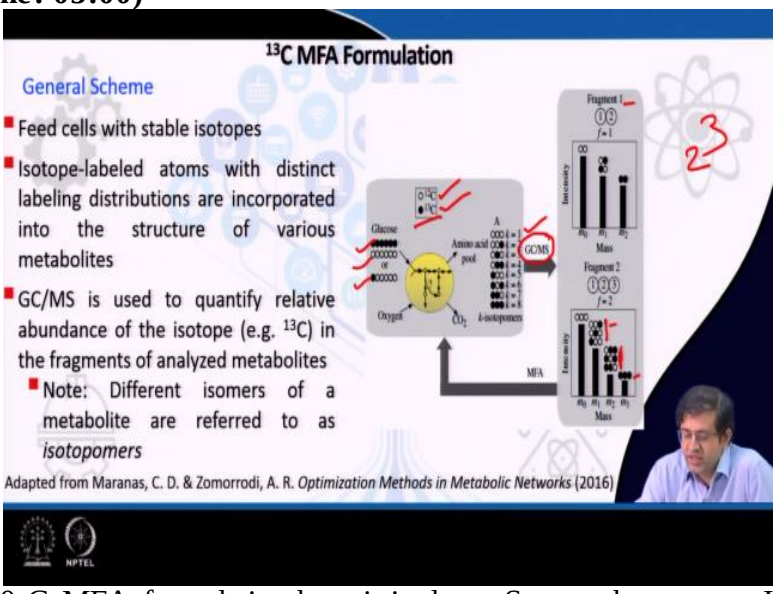
Ultimately, you have to feed the intracellular have to measure the intracellular labeling pattern and that is done in GC mass or NMR spectroscopy. The different mass spectrometers are available today, which can be effectively used to measure the labeling pattern of intracellular metabolites. So, any metabolite which is inside the cell you have to measure either you can measure intracellular metabolites or proteogenic amino acid is also considered for labeling measurement.

Direct interpretation of the data; identify the active pathway in a network. So, this way you can identify which pathway is actually active through 13 C metabolic flux analyses. You would be able to identify the active pathway in a cell. Also the labeling pattern also sometimes able to give you active pathway and those type of analysis is known as 13 C fingerprinting.

The technique used widely to identify which are reaction active, combining the data with the metabolic model always helps you to elucidate all intracellular fluxes. So, you have to combine the metabolic model along with the labeling data. So, 13 C labeling data you have to

combine with the metabolic model in order to get the intracellular fluxes in 13 C MFA. So, this way you can actually measure much more accurate fluxes.

(Refer Slide Time: 05:00)



So, this is the 30 C MFA formulation how it is done. So, you have to as I told you how to feed the cell with a stable isotope labeled glucose. So, you can see the labeled glucose either the glucose is fully labeled or no labeled. So, this is fully labeled glucose and this is no labeled glucose any dark circle means that the glucose is labeled and that is shown over here. The white circle gives the normal glucose this one and the filled circle with black color gives you the 13 C labeled carbon.

So, glucose has 6 carbons that is why we have 6 circle and 6 circle can be filled or unfilled the filled circle is basically 13 C carbon. And the glucose can be fully labeled and the glucose can be unlabeled that is the second one and the last one basically, we have 1 carbon labeled. So, the carbon which is actually the first one not only 1 carbon labeled that is the first carbon labeled. Position is also matter in 13 C MFA. In 13 C MFA we have we use 3 kinds of glucose fully labeled glucose and unlabeled glucose and first carbon labeled glucose.

So, if the cell grows on these types of carbon sources, then what will happen? Then your metabolite will also be labeled. So, the amino acid are generally targeted for measurement you can see the amino acid which has 3 carbon. For example, alanine has 3 carbon, these 3 carbons can have this many possible iso-topomer. What are isotopomer? Isotopomer is basically the carbon atoms which are labeled like how many carbon atoms are labeled k = 1 means none of the carbons are labeled.

So, it is basically normal alanine and k = 2 is basically 1 carbon labeled and then k = 3 you have again 1 carbon labeled but the position is different, k = 4, 2 carbon labeled k = 5, 1 carbon labeled, but the first carbon is labeled. And then k = 6, 2 carbon labeled but the positions are different from k = 4 and then k = 7 we have first 2 carbons are labeled and k = 8 all the carbons are labeled.

So, for a molecule of 3 carbon we have 2 to the power 3 isotopomer, 8 isotopomers are present for a given alanine amino acid alanine has 3 carbon. And these 3 carbons we can give. So, if you put it in a mass spectrometer that is GCMS. Then what will happen? A single peak since it is labeled, if it was not labeled you would get a single peak, but because of the labeling, and then you will get 4 peaks.

So 4 peaks you know the property of the GCMS. GCMS is actually measured the molecule based on the mass, m by z ratio. So m by z ratio assumes the charge to be constant. And then for a given alanine the charge is constant, but the mass, there is a difference there is a difference in the mass. Because 13 C and 12C normal carbons are there is a difference in the 1 unit of mass.

So that is why m 0 means we have a different peak, because the mass of m 0 is different from m 1, m 2, m 3. So the isotopomer and then there is a mass distribution vector mass distribution which you can get from the GCMS is shown over here. So here you can see the m 0 means a the normal alanine that has 3 carbon and m 1 means the 1 carbon labeled, that is why it is m 1. So, all the 3 molecules shown over here is basically having 1 carbon labeled. So they grouped into 1 peak.

So because the mass spectrometer will not be able to differentiate, this 3 molecules, which are having different isotopomer because the mass are same that is why they will be grouped into m 1. Similarly for m 2 if you see these are all 2 carbon labeled. So that is why they are grouped into m 2. So we have 2 carbon labeled par molecules. So that is why it will give us single peak.

And m 3 if you see there is only 1 and all the 3 carbons so these all m 0, m 1, m 2, m 3 are different because they have a different mass. That is why they show different peaks in the GC

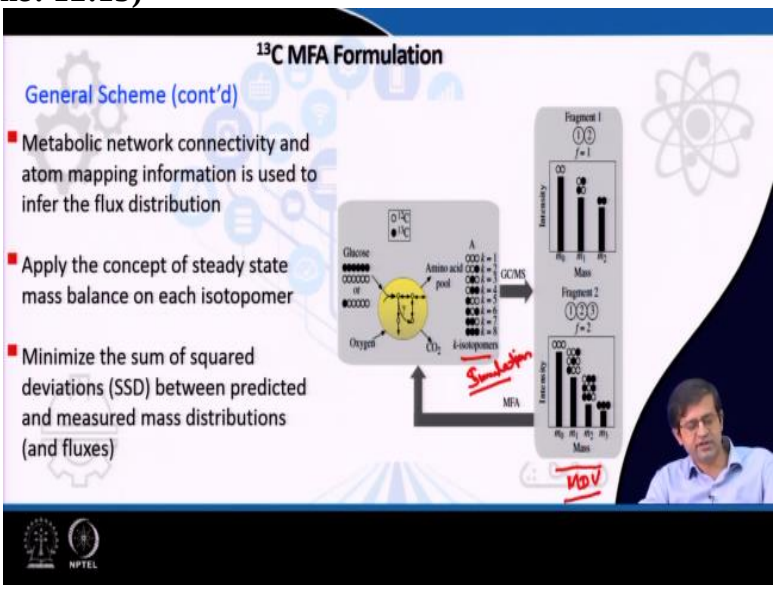
mass. Similarly, for a fragment of 2 carbons you will have 3 peaks. In this way if I have a 4 carbon fragment, why we are calling fragment? In the GCMS different metabolites can be treated as a fragment that can be measured in the GCMS fragment 1, fragment 2 and it will differentiate based on the mass of the molecule it is measuring.

So, out of k = 1 to 8 isotopomer we have 4 peaks. So, for k isotopomer k = 8 we have 4 peaks m 0, m 1, m 2, m 3. So, if you feed cell with isotope labeled glucose and they will get this split in the peak and the isotope labeled atom with distinct labeling distribution are incorporated into the structure of various metabolites. So, isotopes labeled are actually incorporated in most of the metabolites and then you can measure.

GCMS is used to quantify relative abundance of the isotopes in the fragment analyzed. So, the GCMS is used to actually characterize or get a relative abundance of the isotope present in the sample and the difference isomers of metabolites are referred to as isotopomer. So, these are basically isomers of metabolite, this is same metabolite, but they differ based on the carbon atom which are at labeled or unlabeled and they are known as isotopomers.

So, these isotopomers are actually can be measured in a GCMS but you do not find the difference, if they are of same mass. So, for example, this one you see that all of them all the 3 molecules have same mass that is why they are grouped into same pic. And similarly, for this one, you will see that they are grouped into same pic.

(Refer Slide Time: 12:15)

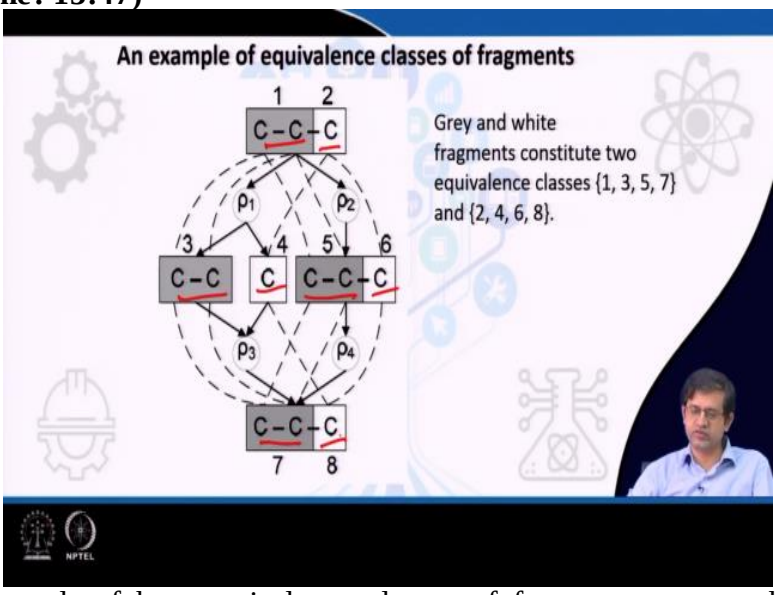


So, the metabolic network connectivity and atom mapping information is used to infer the flux distribution. So, to measure the fluxes or calculate the fluxes you need to actually connect, you have to make an isotope atom mapping matrix or atom mapping information is used to infer the flux that can map between isotopomer and the mass distribution vector that you get from MDV, you get from GC mass.

So, these are the 2 information these you can compute theoretically. So, through simulation you actually have a isotopomer and then MDV that you get it from experiment. So, the MDV is very much connected to isotopomer. So, there is a mapping matrix which can calculate this information. And then you can compare and get most important thing that is the fluxes, so, minimize the sum square division between the predicted.

So, here we have the predicted through simulation we have a predicted distribution of isotopomer. And then you have the MDV that you get it from experiments. So, this difference, if you can measure the difference and then you would be able to calculate the fluxes, so, how it is done we will discuss in more detail in subsequent slides.

(Refer Slide Time: 13:47)



So, this is an example of how equivalence classes of fragment can we characterize. So, I started with a 3 carbon system where first 2 carbons are labeled. So, this one is labeled this one is labeled which is shaded in grey color. And in another class we have 2 that is not labeled because I have a molecule in which the first 2 carbon are labeled and the third carbon is not labeled.

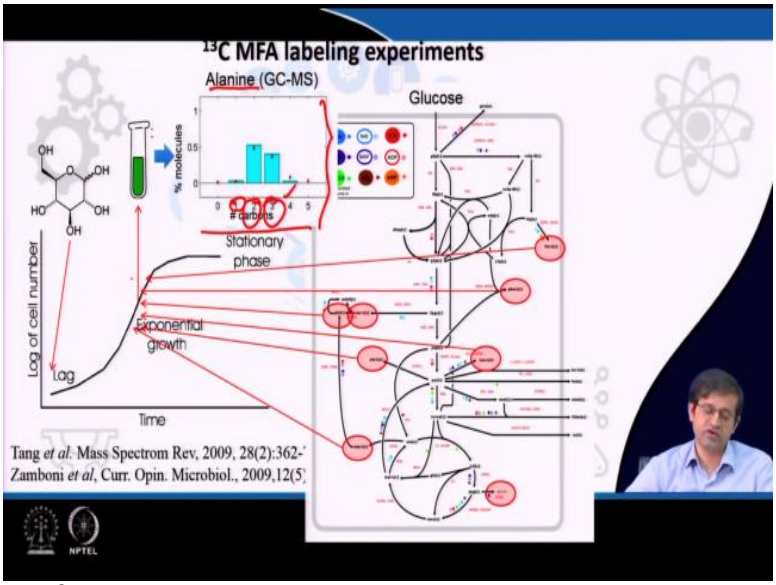
Then I call this part as 1 this part as 2 and then it goes through a reaction and it produces 2 molecules. Suppose I have a molecule that has 3 carbon and the first 2 carbons are labeled. So, after reaction what happened that these 2, 3 carbon broken into 2 fragment where we have first 2 carbons are actually labeled which comes as a molecule 3 and then this is unlabeled one as molecule 4.

So, we can see how the labeling carbons are distributed, when it goes to a metabolic network or in a metabolism, how they get separated. And we have another reaction where you can see that these 3 carbon are remain intact there is no change. So, in this reaction, the metabolites are not broken. So, they are not separated in terms of carbon and then what happen?

We have another reaction it just retain the same and in another reaction you can see in the row 3, you can see that these 2 carbon again joined together to become an original molecule. So, this way you can see how the fragments, they join each other and then they broken and they separate each other. And they form a different molecule and that you have to track. So, in 13 C MFA labeled, you track this labeled carbon in order to identify how the carbons are flowing in the network.

On that also give you an idea how much carbon how much flux the reaction carry, you can identify. And this network can be grouped into 2 classes that 1, 3, 5, 7 and 2, 4, 6, 8. 1, 3, 5 and 7. So, this all have 2 carbon labeled that is why they are belong to the same class and 2, 4, 6, 8 has one carbon 2, 4, 6, 8 they have 1 unlabeled carbon. So, these belong to a different class. So, we have 2 classes in this network and in one class where we have 2, 4, 6, 8, have unlabeled carbon and 1, 3, 5, 7 have labeled carbon.

(Refer Slide Time: 16:38)



So, 13C metabolic flux analysis start with an experiment, so, here you have to do the experiment where you allow the cell to grow in a labeled glucose. So, culture the cell where in the media you have labeled glucose this is not a normal glucose is a labeled. Where the first carbon is actually labeled the first carbon atom of the glucose molecule is labeled, and it allow the cell to grow in that labeled media.

So, what will happen after sometime? Since the glucose is labeled. So as the cells eat up this glucose which is labeled glucose and then slowly the metabolite will also be labeled. So, most of all metabolites will get some labeling incorporation, depending on the reaction it is having. And in the previous slide, I have told you how the reaction happens like 1 metabolite get separated into 2 metabolites and again 2 metabolite join each other to form 1 metabolite.

So, all those type of association dissociation reactions, which are happening inside the cell, depending on the type of reaction it get, incorporate the labeling. So, the incorporation of the labeling is not random, that is what I want to say. It basically says that the type of reaction it is undergoing, if the reactant and product have same number of carbons, there is no change in the labeling pattern.

So, if we have a reaction A to B, if A has a labeling pattern, and B has a labeling pattern A and B labeling pattern will remain the same, because there is no association and dissociation in the reaction. These occur only when the reactants and products undergo association or dissociation then only we see that the labeling pattern changes.

And now we want to identify which are the metabolites you want to measure for labeling measurement, that is very important like once you have the cell cross the exponential phase here. So, during the exponential phase, where the cell is growing, and where you assume steady state approximation, that is the time you take out the cell and lyse the cell and get all the amino acid. So, amino acids are considered as the end metabolite of the cell. So, you lyse the cell and get the metabolites.

Now, the amino acids that you get from the cell it can be intracellular amino acid or proteogenic amino acid. So, nowadays, because of the technology in metabolomics we are able to measure the intracellular amino acid also that can also be used for metabolite measurement. So, you take out this culture and feed it into GCMS. So in this GCMS, you can measure the labeleding pattern of the amino acid since amino acids are the end metabolites.

So, once the amino acids are formed there is no dissociation, the amino acids are not broken. Most of the amino acids are not broken because they go for protein synthesis. So these proteogenic amino acid or the proteins can be collected from the cell and then you can do trypsin digestion to get all the amino acid from the protein and then you can feed it into GCMS and you can get it a profile like this.

So, this is the metabolite profile so, what is this? This is very important. In the y axis you see the percentage of the molecule which are labeled so y axis is basically the percentage of the molecule which is labeled. And these are the molecule these are the carbon like the number of carbon which are labeled. So, 0 means, the fraction of the carbon which is 0 carbon labeled.

So, here you can see there is no peak means there is no molecule which has 0 carbon labeled so, alanine molecule. So, we have considered the labeling pattern of alanine, the alanine molecule has none of the carbon the percentage of the molecule where none of the carbon molecules are labeled so, that is 0 and then I have the fraction 1, 1 means that is 1carbon only 1 carbon labeled.

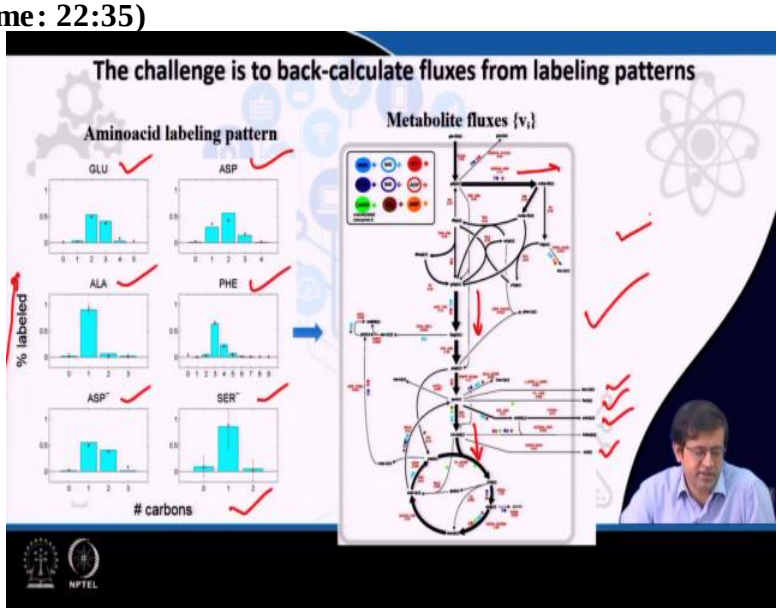
And this position can be anywhere because you are measuring through GCMS it cannot differentiate the position. So, only a fraction of the molecule which is actually 1 carbon labeled that is with 13 C and then we have 2 that is a fraction of the molecule which is

actually 2 carbon labeled and we have 3 carbon labels since alanine has 3 carbon, so, we get a maximum of 3 carbon.

But when we measure through GCMS then there is a probability that you may get an error and also that is why the some 4 carbon labeled is also showing, but it is actually because of the error. So, this way you can actually measure the metabolite profile the labeling profile of different amino acid and then this amino acid labeling are required for calculating the fluxes so, this experiments are done in a flask.

So, you can use a flux where you provide a labeled media and allow the cell to grow in a labeled media and when it is in the exponential phase you take out the culture. Significant amount of culture is taken out from the flask and then you lyse the cell and get the protein outside the cell and do trypsin digestion to get all the amino acid or you can also use intracellular amino acid also proteogenic amino acid intracellular amino acid both you are used for flux measurement.

(Refer Slide Time: 22:35)

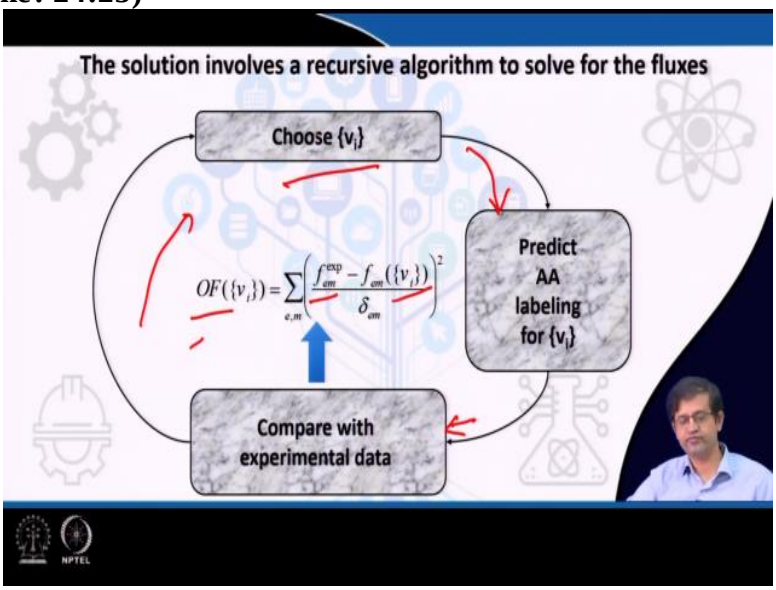


So, these are the amino acid labeleding pattern you can see this is glutamate, aspartic acid, alanine, phenyl alanine, aspartate, serine and on the x axis you can see that the number of carbons labeled. 0 means none of the carbons are labeled 1 means 1 carbon labeled 2 means 2 carbon labeled and 3 means 3 carbon labeled and their percentage labeling is shown in y axis and the y axis you can see that how much fraction of the molecule it is labeled.

So, it is basically a probability you take 1 maximum labeling it can be 1 and then based on that, you make a ratio of how many carbons are labeled. And once you measure the labeled then you can using that labeling pattern you can get a flux profile like this on the right hand side, you see a flux profile that you obtain from 13 C MFA. So, what it says that the glucose is entering the broadening of the arrows are basically the amount of carbon that is flowing.

Most of the flux goes through glycolysis and some part of the flux goes through the ppp pathway shown over here. Some of the flux goes in glycolysis and then it goes into TCA cycle and then from the TCA cycle you can see different metabolites are synthesized like glutamate from acetyl coenzyme A. You can see acetate is synthesized and then the formate, lactate which goes outside the cell and ethanol. So, most of the metabolites you can characterize using the labeling pattern. So, the input for this flux analysis is the labeling amino acid labeling pattern and output is basically a flux profile which is shown over here.

(Refer Slide Time: 24:25)



So, the solution how it actually solves this problem is very interesting how the fluxes are determined? So, in 13 C MFA the technique is different from FBA. So, FBA, we have seen that you maximize the biomass and you get a flux profile. But in 13 C MFA we do not have a biomass equation. So most of the time, we do not actually include biomass equation in the metabolic network. And so what to optimize when we do not have an objective function.

So, here we actually get the flux profile based on labeling pattern. So, the experimental data that we get, so, this is the experimental data we have. Using this experimental data, you want

to obtain the flux profile and for that you also take a metabolic network, so metabolic network metabolic model is also needed to actually measure the flux.

So, to measure the flux, what you do? You choose a distribution of the flux. So, the distribution of flux is chosen and then there are mathematical algorithms or equations set of equation where you can use the flux profile to predict the amino acid labeling. So, for given v i so, if I have a distribution of fluxes you can actually predict the amino acid labeling.

So, there are a set of equation which is completely theoretical. If you have a flux profile then you can predict the amino acid labeling. So, the amino acid labeling can also be predicted from the flux profile and this amino acid labeling that you predict and that is from the simulation you can compare with the amino acid labeling you are getting from the GCMS that is the experimental data.

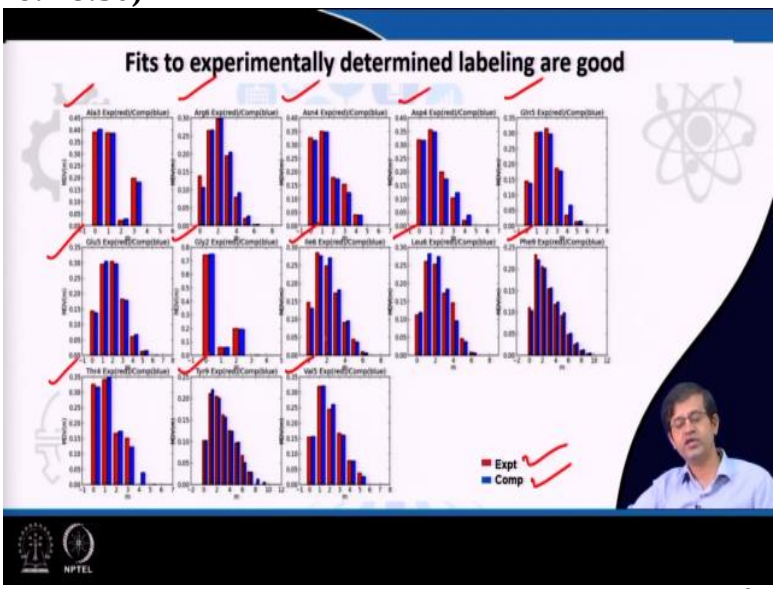
So, that is why 13 C MFA is also a procedure to fit the computational data with experimental data is a fitting procedure. So, they what do you do is basically you predict the amino acid labeling from the flux profile and then compared with the experimental data. So, it is basically recursive algorithm. The recursive algorithm says that you have an error function, the objective function is basically error function.

The error function is defined in such a way that the experimentally determined metabolic labeling profile that is a mass distribution vector can be compared with the computationally derived labeling provide. This one is basically experimental one which is shown and this is the simulated mass distribution vector. And then if the difference is very, very small, then your objective function become very, very easy, very close to 0, 10 to the power minus 10, 10 to the power minus 20 like that.

And that is why you stop your calculation otherwise, it is an iterative procedure. So, that is why it is a recursive algorithm. So, if I give you an edge flux profile, you calculate the amino acid labeling and then compare with the experimental data and if the comparison that is the error function between experiment and the simulation or the computational methods are actually very, very close, then your ability function becomes very, very small, very much close to 0.

And that is where you stop the calculation so, it will not go in iterative procedure and it will stop. So, that is why the flux determination using the 13 C MFA takes a lot of time, because it is a iterative procedure and iteration for many cycles and once it is equal then the calculation is stopped.

(Refer Slide Time: 28:30)

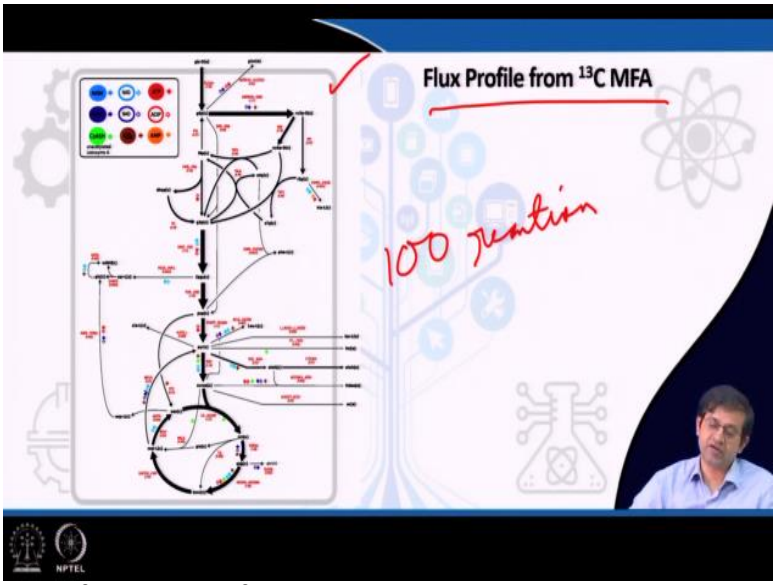


So, once you fit the data with the experimental labeling, these are the fitted values shown over here the experiments shows red color and the blue is basically the computation. So, the experimental values and the computational values are shown over here. So, these are values are compared for alanine so, the alanine, arginine asparagines, aspartate, glutamine, glutamate, lysine, isoleucine, leucine, phenylalanine, threonine, tyrosine, valine.

So all these amino acids have been compared the labeling pattern have been compared with the experimental value if the fitting is good, then that is where the calculation stop and what you get you get the flux. So, then you can tell okay this is the labeling patterns are matching with the experimental data this flux distribution must be experimentally close. So, the strain that you are using that is the time point you are using.

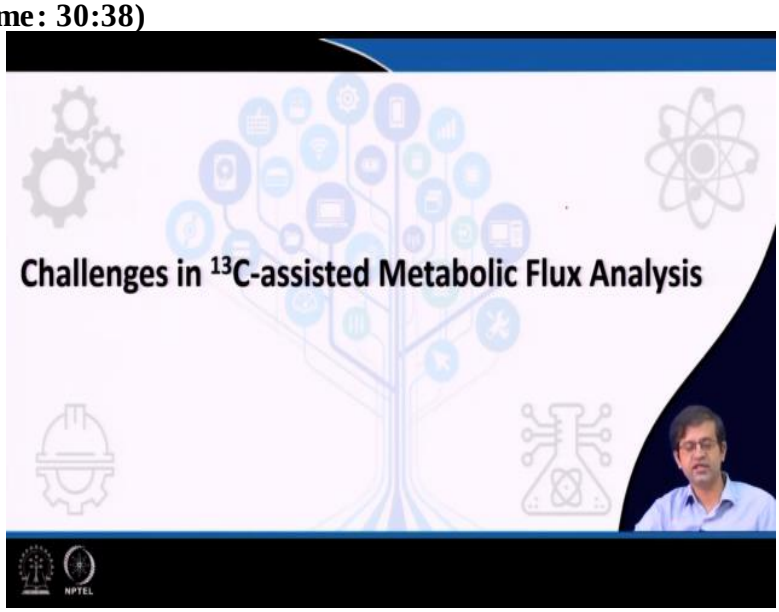
So, all those says that during that time point you since you collected the sample during that time point, where the labeling patterns are very close to each other and you say that this is much more close to the experimentally measured flux. So, this way get experimentally infer flux from the labeling pattern.

(Refer Slide Time: 29:53)



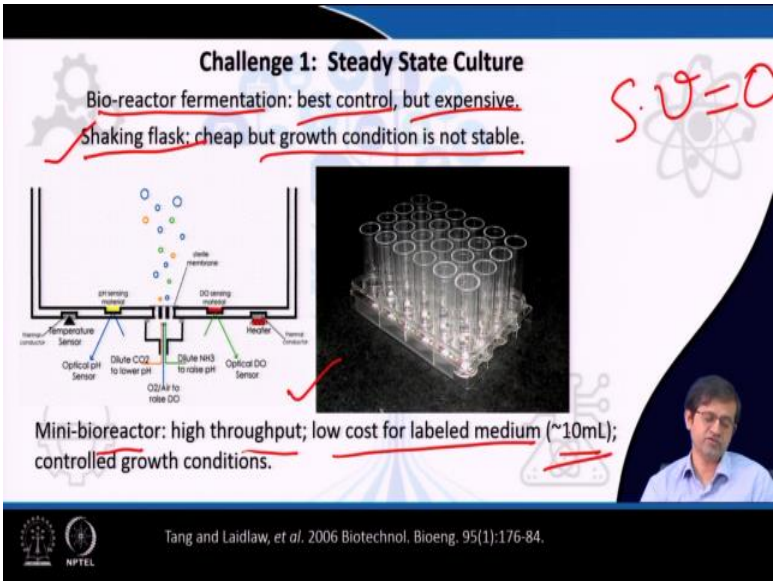
So, this is the flux profile you get from 13 C MFA. So, generally that MFA flux analysis has 50 to 60 or 100 reaction. So, only the central metabolic network is considered. So, if you include more reaction what happened? Your calculation becomes very time intensive. Also it may happen that it will more error in flux estimation. So, that is why 13 C MFA flux profile are calculated for not more than 100 reactions. You make your network very small so that your calculation is not very time intensive.

(Refer Slide Time: 30:38)



So, the challenges in 13 C MFA, there are various challenges in 13 C MFA assisted metabolic flux analysis.

(Refer Slide Time: 30:45)

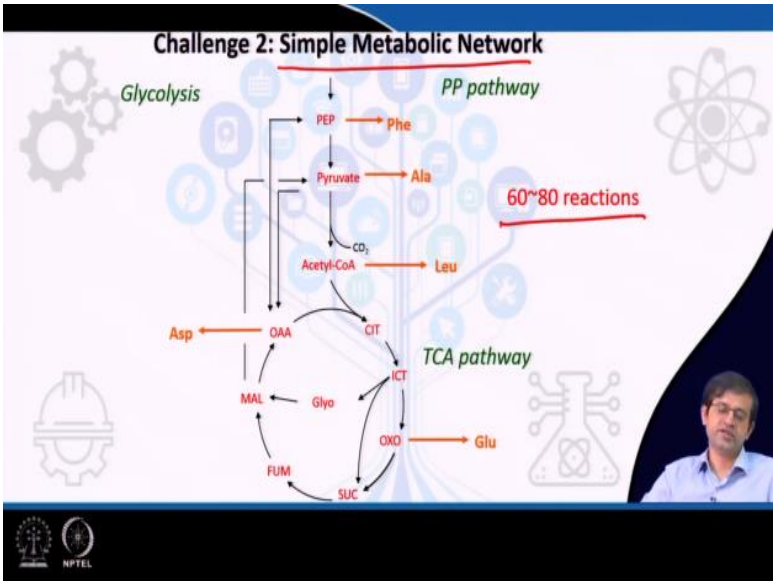


The first one is basically this steady state culture. So, we assume the steady state approximation S dot v = 0 that we saw in FBA flux balance analysis, the steady state approximation is also applied in 13 C MFA. But reaching a steady state is also time consuming, it takes a lot of time to reach to the steady state. So, that is those are the bottleneck in 13 C MFA and the 13 C MFA are done in shake flask experiment, shake flux is cheaper, but the good condition is not stable.

So, that makes the experiment a little erroneous. And then you can have bioreactor fermentation that is much more important if you can buy bioreactor for doing these kind of steady state culture, but they are expensive, then the control is better because in a bioreactor, we have a better control compared to this shake flask. So, the mini bioreactors are also available, where you can generate high throughput data low cost for labeling media.

This is a mini bioreactor shown over here, if the volume of the culture is very less like suppose 10 mL, then you save a lot of money, because 13 C MFA require labeled glucose media and the volume of the media is very small like if you start a culture with 30 mL, then you need more labeled glucose. But if you have a smaller volume, like 10 mL, then you have less volume. So your cost of experiment will reduces if you use a mini bioreactor. So, these are the challenges you face, you will face when you do 13 C MFA experiment.

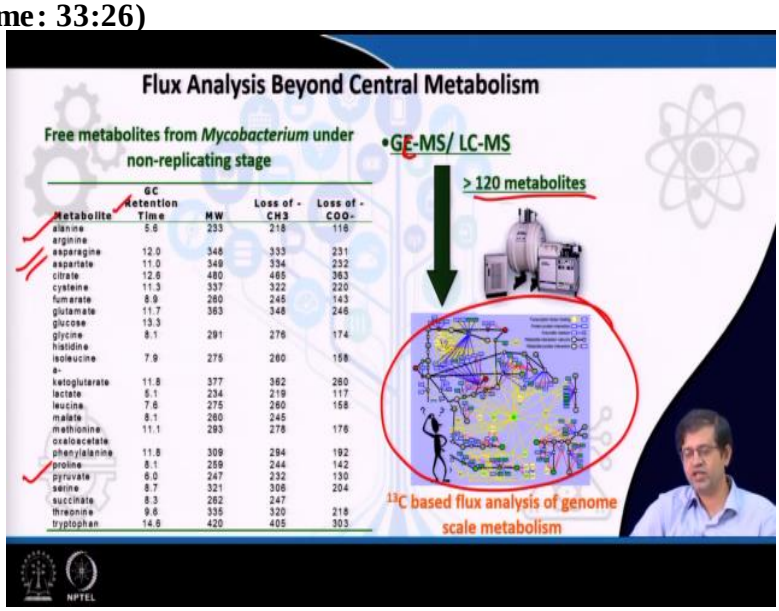
(Refer Slide Time: 32:29)



Then the second challenge is the simple metabolic network. So it is generally have 60 to 80 reactions. As I told the central metabolic network is considered for 13 C MFA calculation. So maybe you want to make your calculation simpler. That is why you take 60 to 80 reactions, it is not only simpler, if you have many reaction, then the error rate will be more in this system. Because 13 C MFA deal with nonlinear equation.

So FBA is simple because the linear equation, so your calculations are very, very simple. You can do calculation in FBA in a fraction of minute. But in 13 C MFA, the equations are nonlinear. And that is why it takes a lot of time. So, in order to reduce the cost of calculation, you reduce the network. So that is the simplest way so, you have a simple metabolic network, so that your calculations are easier.

(Refer Slide Time: 33:26)

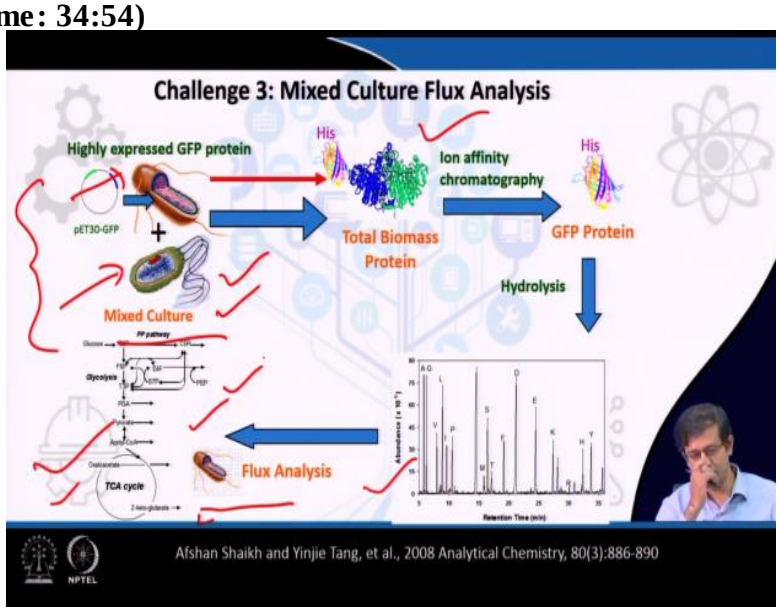


So, the flux analysis and beyond central metabolism you can go beyond the central metabolism for that you have to measure different metabolites which retention times are given for different metabolites, which you can measure in GC. And then you can get a genome scale metabolic flux analysis. So if you can do genome scale metabolic flux analysis, the benefits are more than you can do cofactor balancing or consumption and production of different metabolites can be done at a genome scale labeled.

So there are a lot of benefits if you can go from central metabolic network to genome scale labeled. Because FBA are done in a genome scale labeled but 13 C MFA are not done at a genome scale labeled. So there is a bottleneck. So these are the challenges people are trying where they can actually do genome scale metabolic flux analysis using 13 C MFA. And where in center the genome scale network can be consider.

For that you can measure different metabolite alanine, arginine, asparatate all different amino acid can be measured, pyruvate can also be measured. So that there are 120 metabolites you can measure today using LC mass. GC mass is available, where many metabolites can be measured, but in LC mass we have much more degree of freedom or you can measure many metabolites.

(Refer Slide Time: 34:54)



So then we have the challenges with mixed culture. So mixed culture is basically we have 2 microbes, microbe 1 and microbe 2 and 13 C MFA can only be done in a pure culture. So, how 13 C MFA can be extended to include mixed culture is also another challenge. So, that

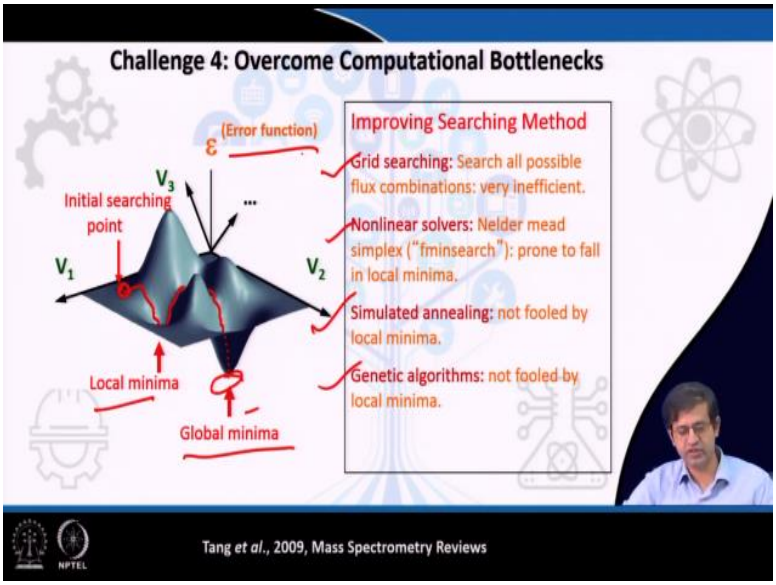
challenge you can do by measuring the flux using peptide rather than metabolite. So, far I discussed that, the metabolites can be used to measure the fluxes.

But when you consider the consortia or a mixed culture then the metabolite cannot be used for flux estimation for that you generally use protein the total biomass protein can be used to actually calculate the fluxes. So, you hydrolyze the protein and then get the amino acid and after hydrolysis you can use a peptide rather than amino acid for measuring fluxes. So, these are the challenges we have where mixed consortia or mixed culture can also be used to actually measure the fluxes.

So, here you have the fluxes organism specific fluxes. you can start a culture with a mixed consortia and then you identify the protein do hydrolysis and peptides specific organism can be chosen where you can have a labeling profile for peptide and the peptide is unique for a given organism so, that you can get organism specific metabolic fluxes. So, suppose I have 2 organisms.

Then I can design a peptide such as that which is unique for these 2 organisms. And peptide labeling can be also be used to calculate the fluxes because peptides are also made up of amino acid. So, in your calculation you can break the peptide into amino acid to calculate the fluxes for individual organism. So, you start with a culture with a mixed cell and then you collect the protein based on the organism. And then you can hydrolyze the protein and to get the peptide and this peptide can be used to calculate the fluxes. So, this kind of technique can also be developed for measuring the fluxes in a mixed culture.

(Refer Slide Time: 37:24)

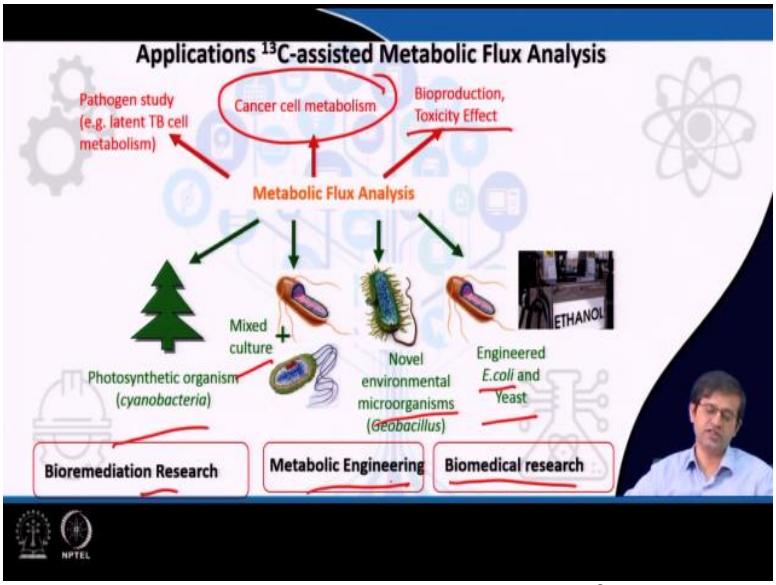


Then we have another challenge that is a computational bottleneck. The computational bottlenecks are basically search algorithm you start with a point. And then you have to reach global minima here where the search algorithm, but you if you are stuck in a local minima, then you are not reaching the final solution. So, these are the problem in flux analysis where the local minima is, if you are stuck, then how to overcome that.

So, for that we have different search algorithm like grid search algorithm, nonlinear solver like those are simulated annealing genetic algorithm. These are the algorithms which are available to solve this problem so, that you can reach the global minima. So, many algorithms are very powerful to reach the global minima which you have to see and the objective function here is basically an error function.

The objective function which you are choosing here is basically 13 C MFA is an error function this can be used to actually overcome the challenges different such algorithm can be used where you can minimize the local minima.

(Refer Slide Time: 38:44)

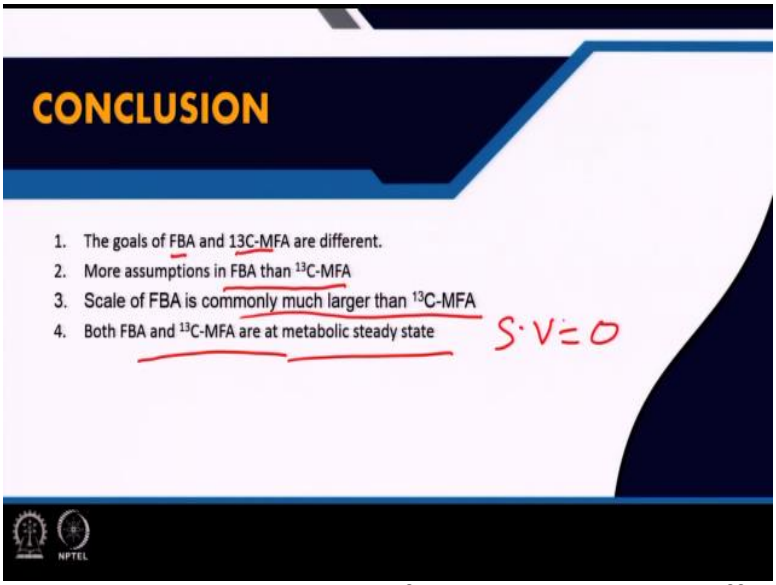


So, what are the application in 13 C MFA? The application of 13 C MFA is very wide and diverse. So, the metabolic flux analysis can be used for photosynthetic organism for measuring the fluxes in photosynthetic organism like cyanobacteria or it can be used for mixed culture or it can be used for environmentally important of microorganism like Geobacillus or it can be used for engineered E. coli and yeast for biofuel production or metabolite production.

So, the applications are very wide and the metabolic flux analysis are really useful nowadays. So, not only in metabolic engineering not only in understanding cyanobacteria, but it can also be used for cancer cell metabolism and also be used for bio production and toxicity effector are also be studied pathogen study can also be done. So, those are used bio remediation.

So, 13 C MFA are used in bioremediation, biomedical research and metabolic engineering. So, a lot of applications are there for 13 C MFA. The main factor in 13 C MFA is that it does not have maximize the biomass. So, that that is the main important factor that plays an important role in13 C MFA.

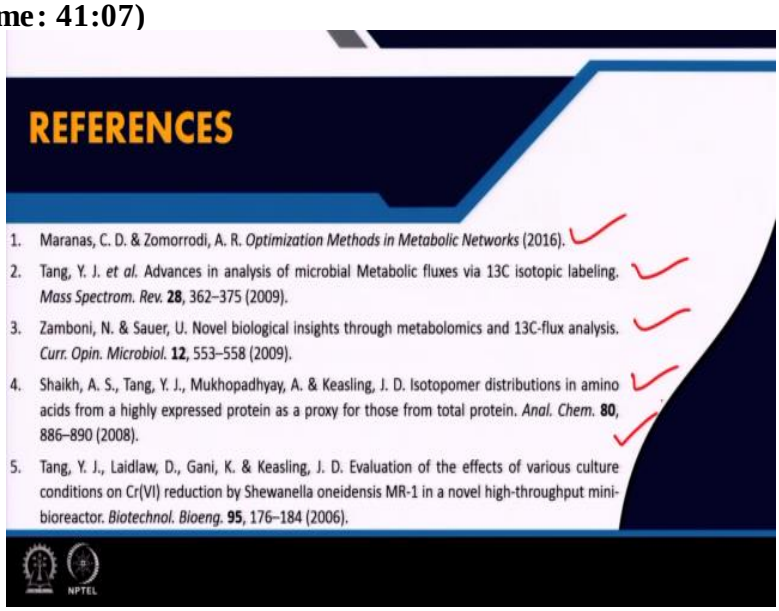
(Refer Slide Time: 40:13)



So, in conclusion, you can see that the goal of FBA and 13 C are different. So FBA are generally use for a big metabolic network or genome scale metabolic network where, 13 C MFA we use for small metabolic network here you do not have any biomass objective function, where FBA has a biomass operating function. More assumptions are there in FBA than 13 C MFA. So that is why 13 C MFA is much more preferable because they use less assumption in 13 C MFA.

Scale of FBA is commonly larger than 13 C MFA. So, the metabolic network is larger in FBA, both FBA and 13 C MFA are at a metabolic steady state, so, they use S dot v = 0, the metabolic steady state in both flux calculation. So, this is an overview of what we learned about 13 C MFA and FBA.

(Refer Slide Time: 41:07)



And then these are the reference you can study more and it is a book that is optimization method in metabolic network by Costas Maranas. And then we can read many more references which are given here about 13 C MFA to get more idea about this method. Thank you, thank you for listening we will close here. Next class we will discuss more about 13 C MFA, thank you. Thank you for listening.