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

Lecture - 01
Introduction to Metabolic Engineering

Good morning. Today, we will be starting the first lecture of metabolic engineering course. Thank you for registration. Thank you for taking this course. This course will be taught by myself and Professor Pinaki Sar from biotechnology department. We will start with the introductory lecture, basic introduction about metabolic engineering, how the course will go. Overall we will give you an idea what will be covered in this course.

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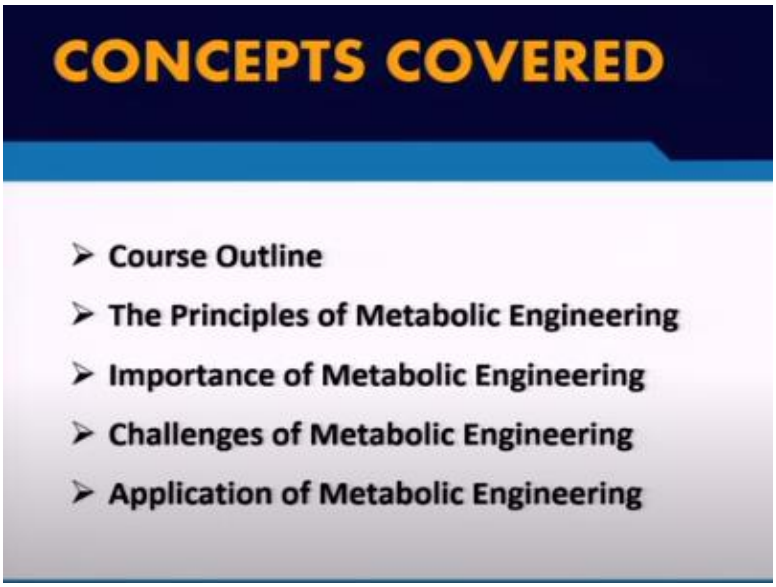

And we will take this introductory course into following points like course outline, what we will be learning from this course in several weeks and then followed by the principles of metabolic engineering, what are the steps followed in metabolic engineering and then followed by importance of metabolic engineering.

Why people do metabolic engineering, what is the need for metabolic engineering that will be discussed in a broad perspective. And then followed by challenges of metabolic engineering. So what are the problems, what are the issues when you do metabolic engineering and then finally, the application of metabolic engineering we will be learning in this lecture.

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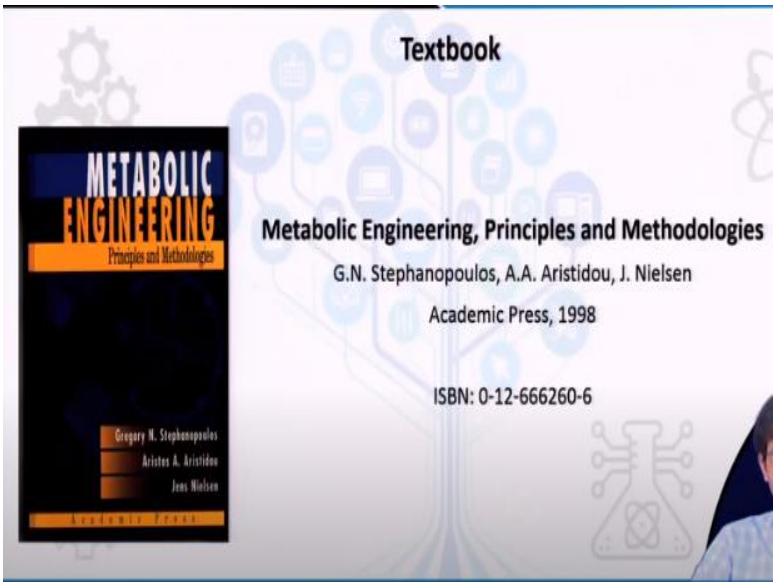

So the textbook we will be following, the name of the book is metabolic engineering, principles and methodologies. And this book is actually written by Gregory Stephanopoulos from MIT, USA coauthored by Aristidou and Jen Nielsen. So this was published in 1998 and is considered as one of the best book for metabolic engineering.

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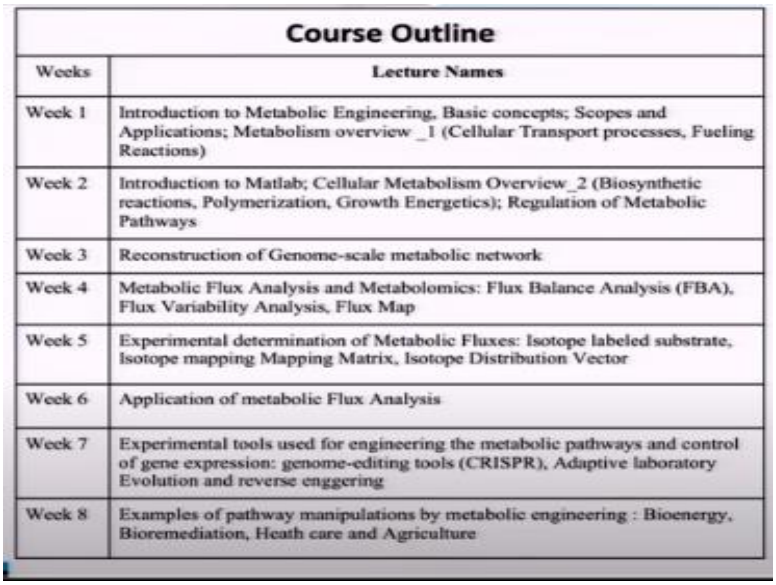

Course Outline

Weeks	Lecture Names
Week 1	Introduction to Metabolic Engineering, Basic concepts; Scopes and Applications; Metabolism overview _1 (Cellular Transport processes, Fueling Reactions)
Week 2	Introduction to Matlab; Cellular Metabolism Overview_2 (Biosynthetic reactions, Polymerization, Growth Energetics); Regulation of Metabolic Pathways
Week 3	Reconstruction of Genome-scale metabolic network
Week 4	Metabolic Flux Analysis and Metabolomics: Flux Balance Analysis (FBA), Flux Variability Analysis, Flux Map
Week 5	Experimental determination of Metabolic Fluxes: Isotope labeled substrate, Isotope mapping Mapping Matrix, Isotope Distribution Vector
Week 6	Application of metabolic Flux Analysis
Week 7	Experimental tools used for engineering the metabolic pathways and control of gene expression: genome-editing tools (CRISPR), Adaptive laboratory Evolution and reverse enggering
Week 8	Examples of pathway manipulations by metabolic engineering : Bioenergy, Bioremediation, Heath care and Agriculture

So in this course, myself and Professor Pinaki Sar from Department of biotechnology IIT Kharagpur will be teaching and we will cover mostly following topics. It will start with introductory to metabolic engineering, basic concepts, scopes, and then application, overview. For example, cellular transport processes, fueling reaction, and that will be covered in first week.

Followed by second week, we will have some introduction to MATLAB and then cellular metabolism overview, the biosynthetic reaction, polymerization, growth energetics, regulation or metabolic pathway. Further on this third week we will talk about reconstruction or genome scale metabolic network. This will have many sub topics as well.

Followed by we will have the metabolic flux analysis and metabolomics where we will learn about flux balance analysis, flux variability analysis, and then flux map and we will also learn about metabolomics. Then on the fifth week, we will learn about experimental determination of fluxes. That is through isotope level substrate, isotope mapping metrics, isotope distribution vector, those are the subtopics you will be learning.

And then on week six, we will be learning about application of metabolic flux analysis. On seventh week, we will learn about experimental tools used for engineering metabolic pathway, and then how to control gene expression, genome editing tool, adaptive laboratory evolution and reverse engineering. And on the last week, we will learn about some example of manipulation of metabolic engineering, bioenergy, bioremediation, healthcare and agriculture.

So we will cover as much as new topics toward the end where metabolic engineering is applied. So, we will start with some basic introduction, learn about metabolic pathways and then we will go to advanced topics like genome scale model, metabolic flux analyzes and then experimental determination of flux analysis like 13C MFA. Then application of metabolic flux analysis and also advanced genome editing tool which is used in synthetic biology.

You will also get an idea how you can actually apply this in metabolic engineering.

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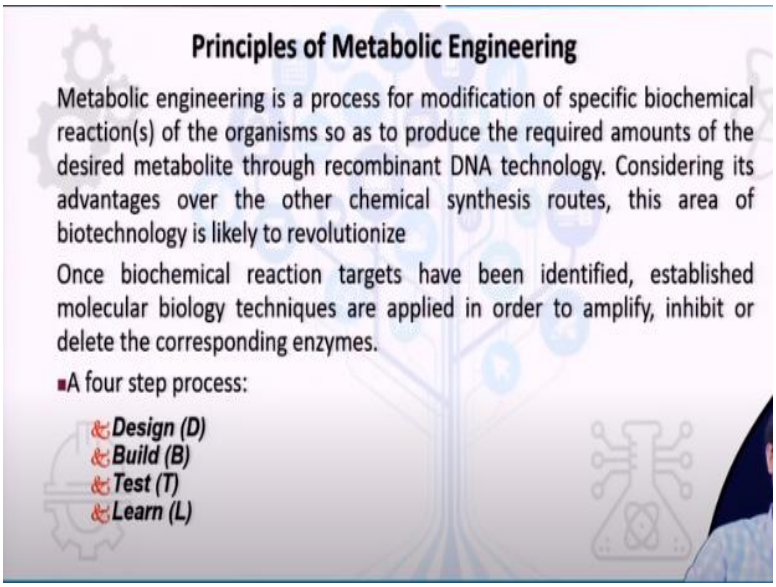

So come to the basic, like what is metabolic engineering? So. many of you are actually learning for the first time have no idea what is metabolic engineering. So I have defined it in such a way as metabolic engineering is a process for modification of specific biochemical reaction of the organism so as to produce required amount of the desired metabolite through recombinant DNA technology.

Considering advantage over the other chemical synthesis route, this area of biotechnology is likely to revolutionize in future. So, the main idea about the metabolic engineering came that you have to produce some metabolite or a compound or it can be biofuel, it can be some chemical, industrially important chemical which you want to make in the industry. So, it has a huge potential in that sense and most of the chemicals you get from raw petroleum.

So, most of the chemical which comes from raw petroleum are actually petroleum derived. So, to make this environment friendly, because more you depend on the petroleum, more carbon will be deposited in the atmosphere and that is a real concern in today's world. So if you can produce this chemical or biofuel from microbe by using metabolic engineering, then it will be environmental friendly.

And the way it is done is basically because of the revolution in genetic engineering. So, because we have a major development in genetic engineering, that is the recombinant DNA technology, you can actually make any chemical from the microbe.

Once biochemical reactions are targeted or identified or established, then molecular biology tools are applied in order to amplify, inhibit or delete corresponding enzyme.

So, firstly you have to identify the biochemical reaction from which you want to improve the production and then the metabolic engineering is applied. This involve four steps, that is design, build, test, and learn. So this is the latest routine followed in industry and as well as in academic labs.

These are the four steps one can follow, that is design, build, test and learn is also known as DBTL. So, DBTL is very popular nowadays for metabolic engineering. So what is DBTL?

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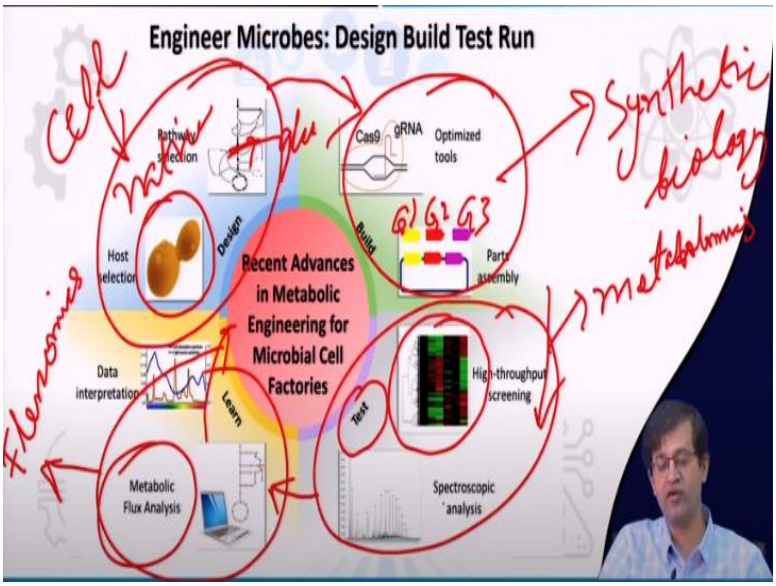

DBTL is basically the four steps followed in metabolic engineering, designed to build and to engineer the microbes for metabolite production. As I told metabolic engineering deal with production of some chemical, enzyme, protein, and anything which is industrially important in terms of its application, it can be a drug also.

So, it can be chemical, metabolites, you can think of any product or any commercially important chemical which is required in industry and also in daily usage. So to do that, the four steps design, build, test, and learn. First, in the design step you basically have to select the host. So, this is one of the major step in metabolic engineering, you have to choose a host organism.

Host organism is the organism that we will be using for metabolite production or chemical production. Any by-product you want to make, you have to choose the organism first. Now, these organisms are chosen based on the availability or the knowledge available for that organism. So generally this can be yeast or E. coli.

Because yeast and E. coli are very well known in terms of their molecular biology tool, in terms of their genetics, we know most of the things, not all of them, but approximately around 50 to 60% of the organism is known, because it has been studied for several decade.

And yeast is also metabolically engineered to produce different chemical used for bio production. And then E. coli is also used. So mostly the host organisms can be these two microbe, Saccharomyces cerevisiae and E. coli. And then you have to select the pathway. The pathway is selected based on the molecule you are targeting.

Suppose you want to make succinic acid. Suppose you want to make some molecule which is produced in the organism but not in a great amount. So the metabolic engineering deal with production of the chemical in huge amount. If it is producing naturally, it may not be sufficient for industrial production. So, for that you have to increase the production. Either you have to increase the production or you have to make a chemical which is not produced by the organism. That also you can do. So both way you can do the modification, if you want to improve the production, which naturally the organism is producing or in other the way.

So this is the yeast. You choose this host organism and then you choose for the metabolic pathway. Suppose, I look for some amino acid, then I need to improve the amino acid like the glutamate. So, for glutamate, you want to improve the production.

So we choose that pathway and then you build some gene cassette. So, these are the genetic parts you want to put in in the host organism. So this is the native organism. So, you have the native organism here and then you are building the parts. So, the building parts is also part of synthetic biology. So the metabolic engineering also deal with synthetic biology.

Many of you heard about synthetic biology. Synthetic biology deal with different biological part or the genetic parts. For example, promoter, gene, coding sequence, terminator. So, there are many things you can include in the part assembly. So, the part assembly is a process where you design the pathway, and then you integrate or you put it in the cell. So before it is actually put in the pathway you actually design it in the computer.

You have heard about synthesizing the DNA or you can synthesize the DNA or you can synthesize oligo. You do lot of analysis in designing the oligo that goes into assembly part. In another research, which comes in designing the assembly part is also known as biofoundry. So the biofoundry is another branch of study in the metabolic engineering.

They design different biological parts and try to assemble the pathway. Suppose you want to make a metabolic pathway inside the cell which has many genes like 10 genes 20 genes that you have to assemble in a proper way. Otherwise, what will happen? If you put randomly inside the cell, then the cell may not be effective in producing those protein from the gene.

So, you have to design in such a way that you can effectively produce those protein inside the cell. So, because the gene or the coding sequence you are putting inside the cell, it must be produced and the protein should be expressed from those genes. Otherwise, there is no purpose, right. So, the main purpose is actually to expose this genetic parts. Genetic parts are basically different genes.

This can be gene 1, gene 2, gene 3 like that. So, G1, G2 are the different genes. So, suppose the native organism, this is native, so the native organism do not have those genes. So, you want to set up a very new pathway that is heterologous pathway. Then you have to take out their gene from different sources and you design those genes. You have to put assemble those gene and put it into the host organism, so that the pathway is ready.

And therefore, for inserting the pathway, you have to use genome editing tool. So, right now you can insert the gene in the form of a plasmid or also you can integrate in

the chromosome. So in this course, we will be learning about CRISPR Cas9 tool where you can insert those gene in the chromosome, so that the host organism is permanently modified. You do not have to do many transformation.

So, this way if you design or buy those gene and put it into the organism through genetic engineering tool like CRISPR Cas9. So, here the concept of genetic engineering is coming, where with the help of recombinant DNA technology you want to put those genes into the chromosome or into the plasmid form. And once you put this assembly part inside the cell, then your pathway is ready.

But you do not know whether the pathway is actually doing anything, because inside the cell, you are not actually able to look. You can visualize that those proteins are formed or not, you are totally unaware of that. So to check that, the next step is test. So, test is very important, because in test you are actually verifying whether that pathway is actually making any sense or not.

Many times what happen, you put a metabolic pathway, then you may not be able to see any improvement of the chemical. So, the basic of test is that you have to test through high-throughput screening or through measuring the metabolite itself. So, in the spectroscopic analysis you can measure those metabolite. Suppose you are producing some metabolite, you have to measure it.

So, the cell is producing some metabolite, you want to measure it using some analytical method, maybe you can use a GC mass or LC mass or you can study the expression data, RNA seq data or gene expression data to see whether the inserted gene is express inside or not. So, this is a checking. You are checking that whether your heterologous genes are expressed or not.

Otherwise you will not be able to know what is happening inside the cell. So, proper checking is needed then you learn. After you test, after you verify that the metabolite is forming, and then you have to see how the carbons are flowing inside the cell. The metabolic flux analysis actually deals with that.

So, I will be going into the metabolic flux analysis in detail in our lectures. But just to tell you that the metabolic flux analysis deal with the flow of carbon, the flow of metabolite inside the cell. That is the rate of a reaction. For each and every reaction you can estimate the flux based on the engineering you are doing. The engineered cell can be characterized in terms of the flow of metabolite inside the cell.

And if you know that, then you can actually again go back and design. So, again you go back to the cycle, you start with it here, and in the next step add new genes and then you test it, that how much it is producing and then you look for fluxes. So, in the metabolic flux analysis you determine how much carbon is going in each of the metabolic pathway.

So this way, this entire process is known as DBTL that is design, build, test and learn. And today the metabolic engineering done using these four steps. Metabolic engineering has evolved for many years but right now these are the four steps followed to actually do metabolic engineering for microbes, and for bio-production.

Any kind of product you can manufacture from microbe provided you have the metabolic pathway. And this metabolic pathway, we will discuss more in subsequent lectures, that how you can actually select different metabolic pathway. And then we can also discuss about the spectroscopic analysis that is a part of the metabolomics. So, this goes as a part of metabolomics.

And this is a part of synthetic biology. And this is a branch of study known as fluxomics. So the metabolic engineering involve lots of branches of study as you can understand, it is very interdisciplinary field and first you have to know the cellular physiology, the cell physiology you have to know. The knowledge of this things are required for better metabolic engineering.

If you know all these four parts, then you are a better metabolic engineer. But it is not necessary that you should know each one of them. So you may not be familiar with metabolic flux analysis but still you will be able to do metabolic engineering. Suppose, you do not know the spectroscopic analysis, but still you will be able to do

metabolic engineering provided you outsource the measurement to another vendor or another lab.

So these four areas can be combined together for effective metabolic engineering.

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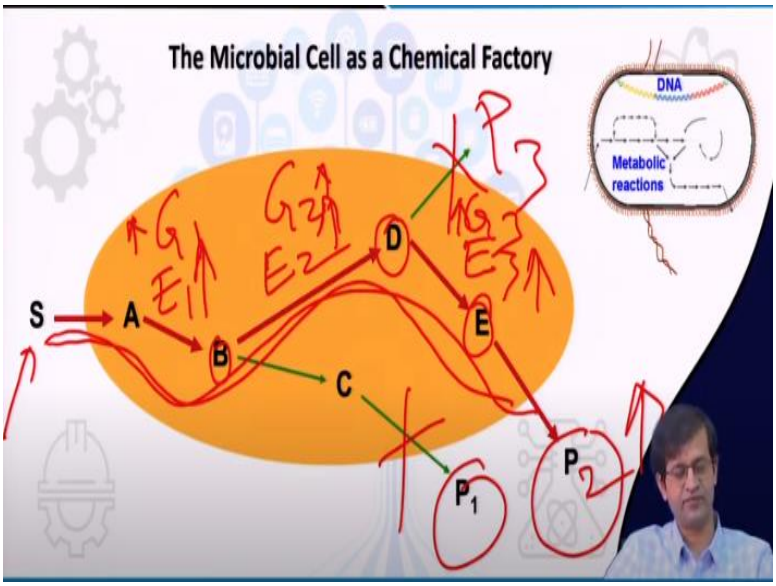

So in the next step, I would like to tell that the microbial cell is a chemical factory because the metabolite inside the cell can be considered as a chemical. But the amount which is present inside the cells is very small. So that is the only difference. If you consider the cell as a chemical factory, and these chemicals are actually produced inside the cell in very small amount, in microliter.

So, the purpose of metabolic engineering is actually to improve the production of this metabolite that is A, B, C, D, E. These are the metabolite inside the cell. So, S is basically a substrate. It is going inside. It can be glucose, galactose any kind of substrate the cell is eating up and then producing a product, P1 and P2.

This product can be ethanol, succinate and these are the product you are looking for basically. Here it can be P3. So, many product you can make from the cell. And suppose you want to increase the production of P, then you should look for this pathway. So, one particular pathway you have to look at. This P2, I want to improve. and P1, P3 you can remove.

I do not want them because I only want P2. So, this is the strategy used in metabolic engineering. When you target a given metabolite that is P2 and then you look for those metabolic pathways and the metabolic genes. So, these are basically metabolic genes which are catalyzing reaction like S to A, A to B, B to D and D to E.

So these are actually catalyzed by enzyme 1, enzyme 2, and enzyme 3 like that. So you have 1, 2, 3 enzyme which are actually involved in forming this final product. And this enzyme needs to be formed inside the cell. The enzymes are the bottleneck. If the enzymes are produced in enough amount inside the cell then you can actually make more product.

So the product will go up. So what you have done? You actually improve the enzyme production inside the cell and try to channelize more carbon along this pathway. So this is what followed in metabolic engineering. You try to get more enzyme inside the cell. How do you get more enzyme inside the cell? What you have to target?

It is very easy, because of the genomics, we know the sequence of these enzymes. Inside the cell you know which gene is actually correspond to this enzyme 1 and enzyme 2 and enzyme 3. So, because of this sequence of the genome you know which are the genes actually correlated with this enzyme 1, 2, and 3. And with this enzyme you can easily improve the production inside the cell by increasing the expression of the gene.

So, if you want to try to improve the expression of the gene, how do you that? Through molecular biology. Here, the genetic engineering is playing a role. Once you identify the pathway, and then you look for the enzyme which you want to improve inside the cell, and for doing that you need recombinant DNA technology or genetic engineering, where you actually up-regulate those genes.

If this enzyme correspond to gene 1, gene 2 and gene 3, assuming that each enzyme has a one to one correspondence, that is enzyme 1 correspond to gene 1 and then gene 2 correspond to enzyme 2, gene 3 correspond to enzyme 3. This way, if there is a one to one mapping, then by just up regulating these genes, so you try to up-regulate this gene 1, gene 2, gene 3, you automatically actually get more enzyme. More proteins

are there, so that reactions can be much more favorable. So more you have inside the cell in the sense that more metabolite B will be there, if you have more enzyme 2 producing the cell, then you have more metabolite D. And then more metabolite E and finally this metabolite E is exported outside the cell in the form of a product.

So, this is the way you can actually improve the production of certain product you are looking for. So this gives you an idea, what is the basis of metabolic engineering.

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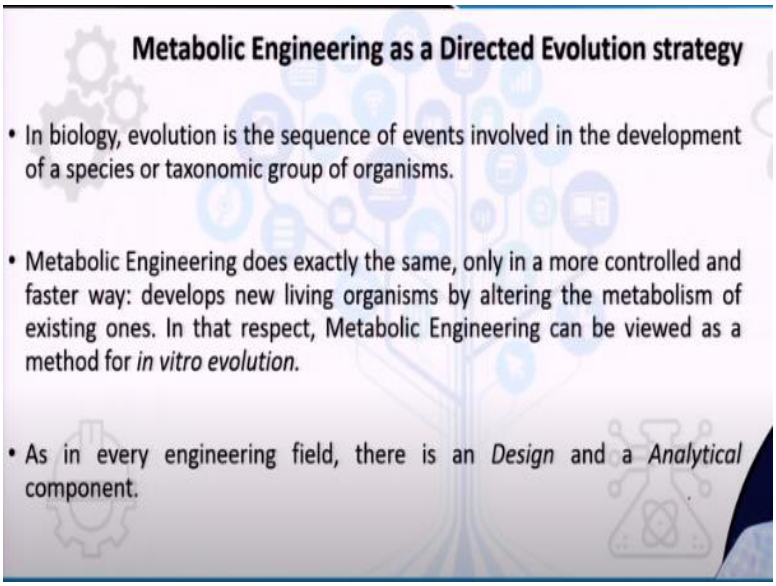

So metabolic engineering is also treated as a directed evolution strategy. The directed evolution strategy, where first you should know what is evolution. Evolution is the sequence of events involved in the development of a species or a taxonomic group of organism. So is basically a sequence of event where the species is actually evolving over a time, over several years so that it can perform particular function.

So, every cell if you see in the nature has developed certain function, certain characteristic and that is not a one day phenomena, it has actually evolved for several years to reach there. So this is very well-known, you already know. I do not have to talk much about it. But metabolic engineering does exactly the same. But it is in a more controlled and a faster way.

So, we do not have to wait for several years. So, we can actually do it in few days rather than evolution which has come up for several years. With the metabolic engineering you can do it in a more controlled way. So, you can develop new living

organism by altering the metabolism of the existing ones. In that respect metabolic engineering can be viewed as a method of in vitro evolution.

So, you are evolving the cell in vitro in the sense that you are doing in a directed fashion to achieve certain improvement of some byproduct. So, if you want to improve the production of byproduct, as in every engineering field, there is a design and analytical component. So, first you design and then there is analytical component where you analyze how the design you have made is actually performing.

So, metabolic engineering also a kind of a directed evolution where you try to evolve the strain so that you do not have to wait for many years.

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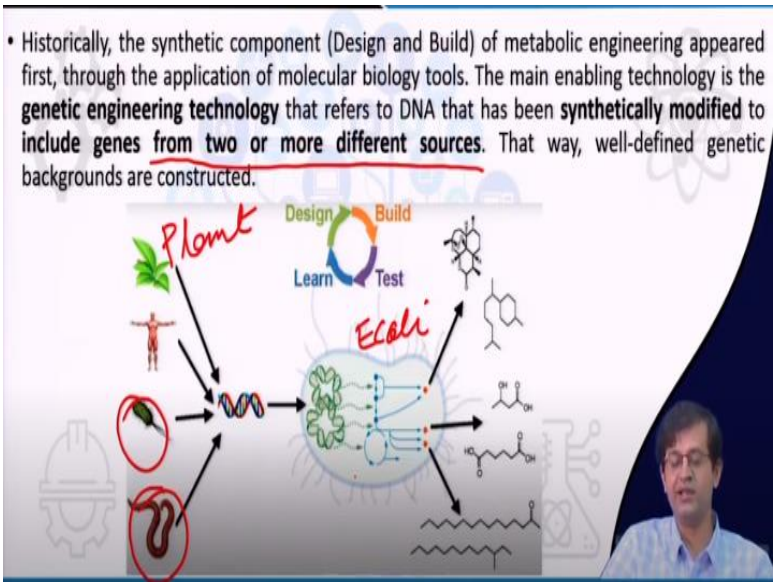

Historically, the synthetic component that is designed and build of metabolic engineering appeared first. So, in metabolic engineering the synthetic component actually came first where you can apply molecular biology tool. The main enabling technology is actually genetic engineering technology through which you are actually adding new genes.

So in this diagram, you can see microbe, suppose you consider this as an E. coli cell, E. coli. Why you have chosen E. coli? Because E. coli is easy to handle. You know most of the things in E. coli, when I say most of the things you know that means you know most of the genes in E. coli. Not all of them.

E. coli is the most well studied organism, one of the most well studied organism where you know most of the molecular biology tool and you can actually add new genes from different sources like from plant you can get a gene. So you can insert the gene inside the cell from the plant. Also from human being, from other microbe you can insert and from all the other mammalian organism also you can have those genes.

So through genomics, because of the revolution in genomic, you are able to do this, because lot of sequences are available right now. If you go to NCBI database, you will get several 100 organisms are available, whose metabolic pathway you want to put it in E. coli, because that particular product you want to make inside the cell. For example, I can give one example, like you have heard about anti-malarial drug, right?

Artemisinin is actually anti-malarial drug which is used to treat malaria patient. And also we saw a Nobel Prize for this molecule, the artemisinin drug where the person got the Nobel Prize for extracting this compound from this plant. So the plant is used to actually extract the drug. But this plant is actually not very easily available.

So that is why one scientist, what he has done? He actually inserted the gene which is required for making the artemisinin drug and put it into a microorganism and tried to make that compound through genetic engineering. So, through genetic engineering, he inserted those metabolic pathway for the production of the drug.

So this way, you can actually design the cell using the synthetic biology or the genetic engineering tool, where you can include two or more different sources. You can even include gene from two different sources. So, from two or more different sources you try to insert the gene in the microorganism like E. coli and try to generate the compound.

For example, the artemisinin drug was produced from yeast, Saccharomyces cerevisiae not from E. coli. So, this is just to tell you that you can get genes from various sources and during the design you try to insert those metabolic pathway in the chromosome of the cell so that you have a production.

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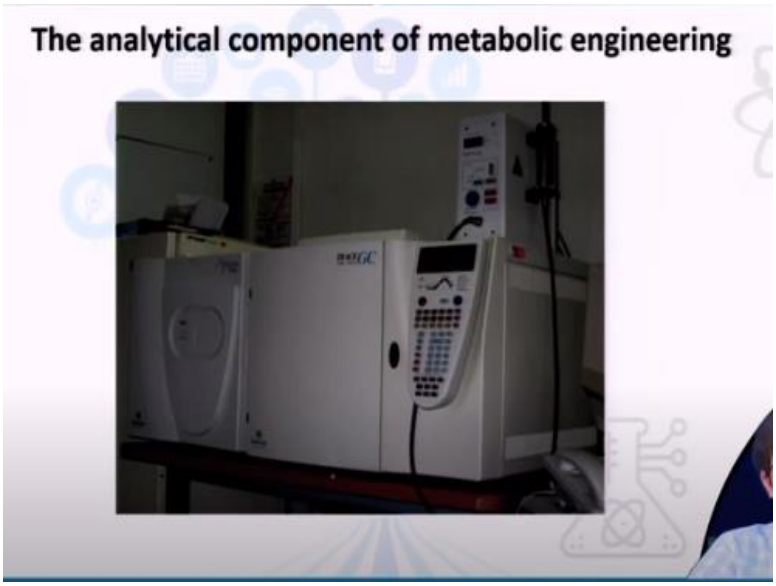

And then the analytical component of metabolic engineering is basically deal with measurement. So here I am showing one component that is GC mass, the gas chromatography set up for mass spectrometry. So it has a combination of gas chromatography and mass spectrometer, where you try to measure the compound, how much the cell is producing. This is a very important technique we use in metabolic engineering, where you try to measure the amount of product the cell is producing.

And this is a check, primary check which you can actually know whether your metabolic pathway is working or not. So learning this is also part of a metabolomics, where you try to actually measure the metabolite, not all the metabolite inside the cell, but some of the metabolite you can measure using GC mass.

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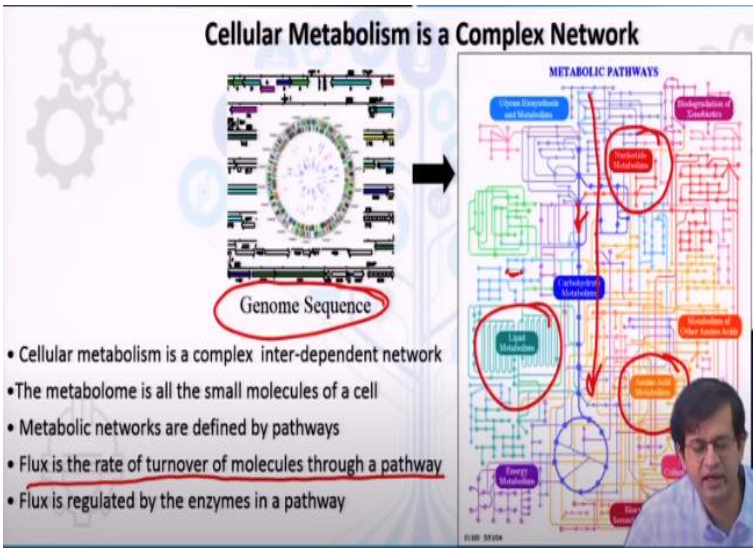

So, next we will come to another interesting topic that is the cellular metabolism. In cellular metabolism, you have to understand how the cell is actually producing different metabolite inside the cell. Here you can see the central metabolic pathway, which is going straight and that is the carbohydrate metabolism.

Suppose, the cell is growing on glucose then and then the glucose enter from this pathway, then it go to TCA cycle and then various amino acids are produced. Then we have lipid metabolism, nucleotide metabolism. So these are the blocks which are present. It is very well connected network, very complex network, is not very easy to actually understand how they are actually interconnected.

So, for that Professor Pinaki Sar will be talking more about different metabolic pathway that is involved in the metabolic reaction involving energy metabolism, the amino acid metabolism, nucleotide metabolism. So this pathways you will be learning in this course to some extent. But the basis of it and this enter metabolic pathway, you can actually derive from the genome sequence.

So, this is the technology which is available. This is a part of systems biology. So far I told about synthetic biology, where you can integrate gene from many other sources, and now you are coming to systems biology, where you can actually identify different metabolic pathway from its genome sequence. So if you know the sequence of the organism, then you would be able to actually identify what are the metabolic pathway this organism can have.

This is the biggest information you have when you start doing metabolic engineering. Because if you know what metabolic pathway the cell has, then you can do metabolic engineering much more easily. So this information is very much available for E. coli and yeast, Saccharomyces cerevisiae, and for many other non-conventional yeast also we have this information available.

So, what do you understand from the cellular metabolism, the cellular metabolism is a very complex network, which are interdependent. You can see that this metabolites are connected to each other. The metabolome is basically all the small molecule of a

cell. So the cell may have a lot of metabolite that constitute the metabolome of the cell.

The metabolic network are defined by pathways. In the metabolic network, this each node is basically a metabolite. And the connection between the two metabolite is basically a reaction. Any kind of network has two component, one is node and another is edges. Edges are basically the reaction.

So, you can identify this network directly from the genome sequence, because of the well-known technique which are available which you will learn in this course that is basically part of systems biology. So in this course, we will learn a little bit about systems biology, where you will be able to actually identify this kind of metabolic network or metabolic pathway directly from the sequence, and the flux and also.

Once you have the metabolic network, you can actually calculate the flux. Flux is the rate of turnover of molecule through a pathway. Suppose this reaction, there is a reaction going from here, and now we want to know how much carbon is flowing in this reaction. So, in this reaction, you can calculate and identify how much carbon is going through it.

The flux is regulated by the enzyme in a pathway. And this flux is also regulated and that is why you need to learn about the regulation of the metabolic pathway. So, in this course, we will also learn little bit about the regulation of the metabolic pathway, how much carbon it is flowing and how much it is regulated, that also you need some idea for doing the metabolic engineering.

Not just the pathway, you also need to know how these metabolic pathways are regulated inside the cell. This way, you would be able to actually have an idea how the carbons are flowing inside the cell, because this is very well connected. So this also we will learn about in detail in subsequent classes.

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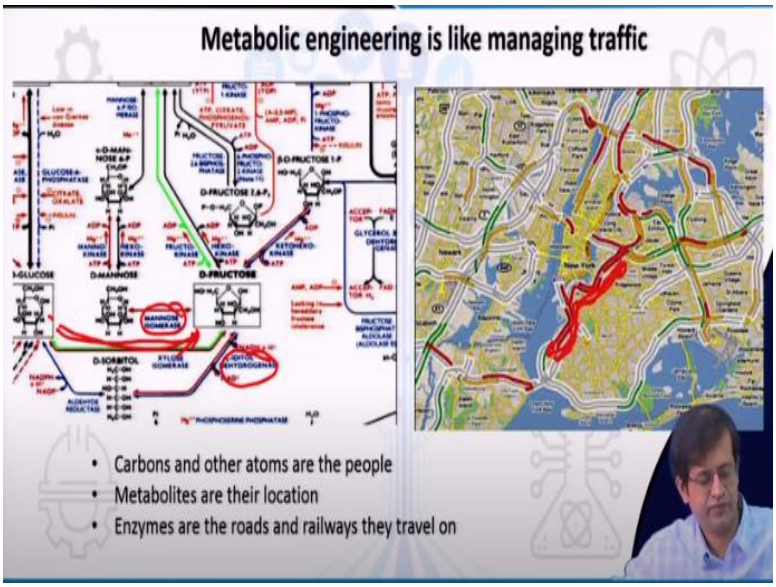

So the metabolic engineering is like managing a traffic. This slide gives you an overview of what is metabolic engineering. So in the left hand side, you can see that we have a metabolite and then there is a reaction, this metabolite is converted into this compound that is from glucose to D-fructose. And then each metabolites are connected through a reaction and there is an enzyme.

So this is the enzyme for this reaction, this is the enzyme for this reaction like that, they are connected in a network. It is even compared to traffic. Nowadays, if you see the Google map, if you open a Google map you will see that which roads are actually congested. So, the red regions are actually congested. That is there is a traffic jam. So, we do not go in that pathway.

And because of the Google, you get an online distribution of the traffic jam on the road. Similar thing you can see in the metabolic network, where the carbon atoms are the people. So you can consider the traffic and compare it to the metabolic network and the metabolites are the location. And enzymes are the road and the railway they travel on.

So, enzymes are basically the road. So, if you do not have enzyme then what happen this metabolite may not be converted into another metabolite. And the metabolites are the location. So suppose you want to go from here to here, these metabolites are basically the location and the roads are basically the enzyme. This way, you can actually understand what metabolic engineering is.

The carbons atoms are the people. So, one people is going through this road and then going from one place to the other, that is from metabolite 1 to metabolite 2, and then it is going through an enzyme. So this is what the analogy which is used in metabolic engineering to understand it better.

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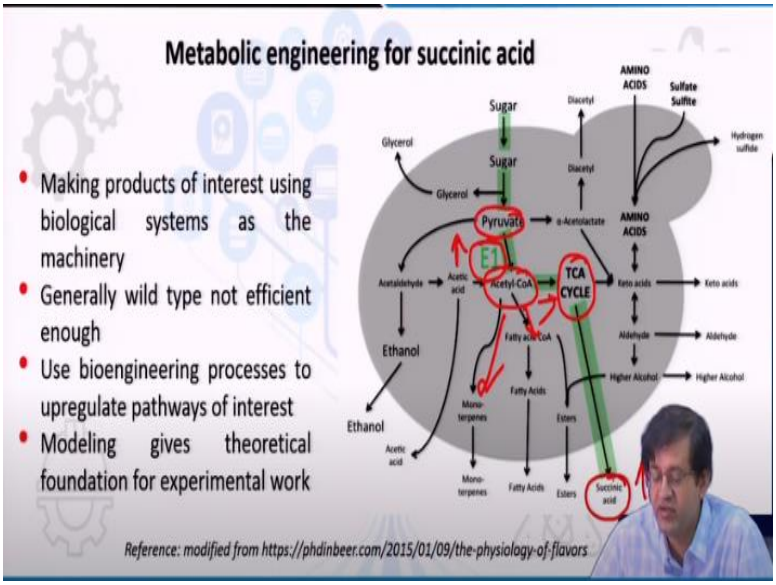

Now, I take a small example of metabolic engineering of succinic acid. So as you know the succinic acid is industrially relevant compound, which is very useful solvent in industry, the succinic acid. It is used in many purpose. It has an industry and has a high commercial value, and there are many ways you can make succinic acid. So using metabolic engineering by engineering the cell also you can make succinic acid.

Here, I have shown the metabolic pathway of succinic acid. So the sugar is entering the cell. It is coming from outside going inside the cell and then it is converted into pyruvate. And then from pyruvate to acetyl coenzyme A. And then from acetyl coenzyme A it goes to TCA cycle. And from TCA cycle you are getting the succinic acid. So this is the product you are looking for.

And these are the intermediate metabolite. It has first converted into pyruvate and then it should be converted into acetyl coenzyme A and then it should enter the TCA cycle and then it produce the succinic acid. Now my question is that if I want to improve the succinic acid production.

So, what I should do to improve the production of succinic acid? So, the straightforward way, you want to improve the production of enzyme 1. As I told in the previous slide that the enzyme production should improve. So to improve the enzyme production 1, do you think that the succinic acid will improve?

Suppose I increase the production of enzyme inside the cell by up regulating that gene, which is responsible for enzyme 1. So do we get the succinic acid production increase because now enzymes are produced more? The question is that if I increase the enzyme 1 should I get the improvement of succinic acid production also as well they are directly proportional or not?

So, whatever your answer, the answer is no, simply the answer is no. Because if you increase the production of enzyme 1, the succinic acid production may not go high because amount of acetyl coenzyme A produced is from pyruvate it is going to acetyl coenzyme A. And this acetyl coenzyme A is going into many other path.

We can see that acetyl coenzyme A is going into fatty acid pathway. Then it is going to monoterpene. And then only small portion of the flux goes into TCA cycle. So inside the cell even though you are producing more acetyl coenzyme A but only the fraction of acetyl coenzyme A which is produced more is going into TCA cycle. And then inside the TCA cycle small fraction of the TCA cycle flux is going into succinic acid.

So, this is a systems biology problem, because the metabolite which is produced inside the cell may not be converted totally into succinic acid. Suppose the carbon from the sugar is entering the cell and then the moment the enzyme 1 is more if you upregulate the enzyme 1, then more acetyl coenzyme A will be formed.

But the more acetyl coenzyme A may not enter into the TCA cycle. It may go into another reaction that is the idea. That is why when you do metabolic engineering this is a system biology problem. You have to understand with a system perspective where you have to consider the metabolic network and you have to see how much flux is actually going into different pathway.

So, when you are interested to make some product like succinic acid using biological system then these are the hurdle you will be facing, it is not very straightforward. And since you are improving succinic acid you are not happy with the wild type strain. So the wild type may not produce enough succinic acid. So that is why you are doing the metabolic engineering.

Using bioengineering, you want to upregulate this enzyme 1. So that is why you need a modeling technique to understand how actually the carbons are flowing and going into succinic acid. So this problem can be addressed using a systems biology perspective, where you want to understand how the carbons are flowing into different network pathways and you have to be very clear.

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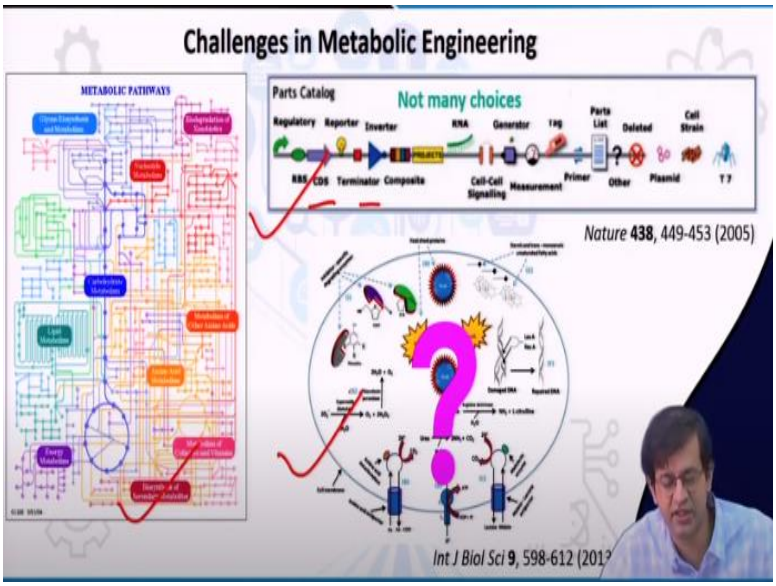

So, these are the challenges in metabolic engineering that you have to understand the problem in systems biology perspective. Not only that, if you know the metabolic pathway, then you want to actually make that pathway inside the cell, for that you need genetic parts. So, these are the genetic parts which are available, the coding sequence, and terminator, then plasmid, primer.

So all those thing you have know. And this is very much easier when you consider E. coli or Saccharomyces cerevisiae. But if you go to non-conventional organism, then the biological parts are less in unknown organism. So this are the parts you have to know when you do the metabolic engineering. And then not only the parts, you should know the molecular biology tool of that organism.

So whether that molecular biology tools are available because these genetic parts you have to actually insert in the organism. For that you need the molecular biology tool like transformation, cloning you would be able to do for that organism. And also you should know the genetic circuit. The genetic circuit is basically the regulation.

How those metabolic genes in which you are inserting inside the cell regulated, that you should know. These are the challenges in metabolic engineering in terms of biological parts, and also how these metabolic parts are actually regulated inside the cell once you insert them inside the cell. And also their molecular biology tool you should know.

So, if you know all those things, then your metabolic engineering is easy. Otherwise, it can be very time consuming if you do not know many of these things. The first is the metabolic pathway. You should be knowing the metabolic pathway and also the biological part and also the regulation of the metabolic genes which you are inserting or how it will affect when you insert a new gene.

If you know this three thing, then it is easier. Otherwise you have to do lot of study to understand this thing before starting the metabolic engineering.

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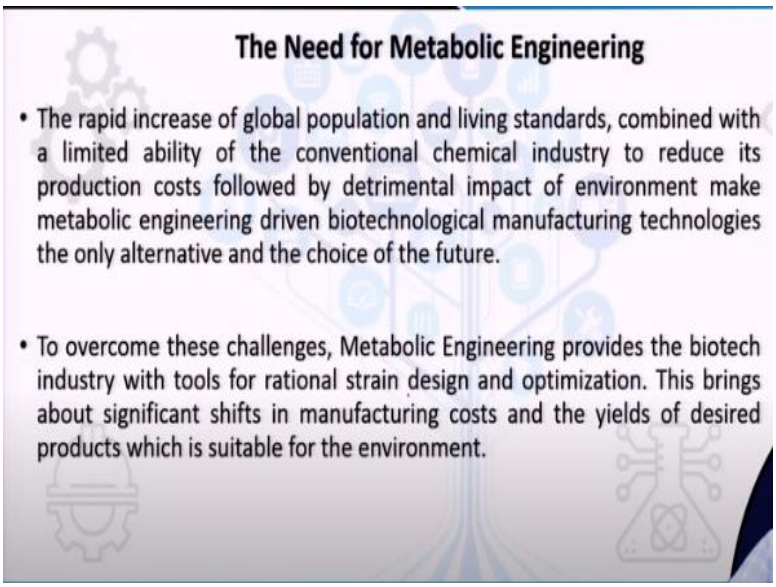

So, now I have come to the need for metabolic engineering. The rapid increase of global population and the living standard combined with limited availability of

conventional chemical industry to reduce the production cost followed by detrimental effect of environment made metabolic engineering driven biotechnological manufacturing technology the only alternative and the choice of the future.

Because right now, as I told before also that we mostly depend on petroleum derived products. So most of the chemical product you use is actually derived from petroleum resource. And metabolic engineering can help in this regard, because when you do metabolic engineering of the microbes, it is basically renewable way which is environmental friendly.

This is one of the factor for which the government is spending lots of money, a lot of industries are also spending money to actually make this product available through metabolic engineering. And other thing is basically, the cost of production. So the conventional way, what industry is producing these chemical may not be cost effective and also not environmental friendly.

So to overcome these challenges, you need metabolic engineering driven biotechnological manufacturing technology that can be the choice of the future and is alternative procedure, where you can get the higher production through metabolic engineering. To overcome this challenge metabolic engineering provides the biotech industry with tool of rational strain design and optimization.

This brings about significant shift in manufacturing costs, and yield of desired product which is suitable for the environment. So, using the metabolic engineering tool you can actually design the strain. Strain is basically nothing but the microbe, which you are actually engineering. You have to optimize that metabolic pathway so that the cost of production decreases.

So, several rounds of engineering you have to do through DBTL cycle that is design, build, test, and learn cycle to reduce the cost of production. So, one is the cost and another is environmental friendly. These are the two main motivation for metabolic engineering so that you get more yield, more product you can produce in this way you do not have to depend on the conventional chemical industry.

Why? The conventional chemical industries are actually not environmentally friendly. So you have to save your environment. When you do metabolic engineering, you are actually making this product in an environmental friendly way. And also you can improve the cost of production. So put together these are the challenges or these are the benefits you have for metabolic engineering.

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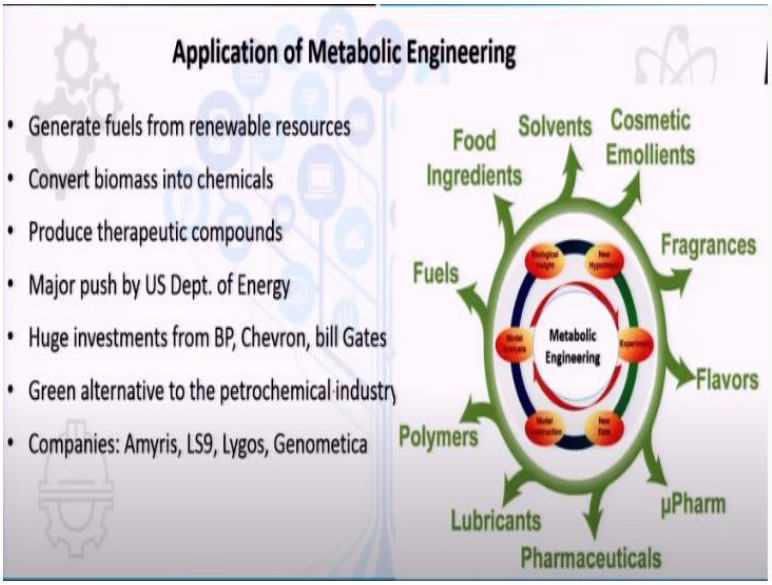

And the application of metabolic engineering. Also you can see that you can make lot of things, like you can make solvent, food ingredient, fuel, polymers, lubricant, pharmaceutical, flavors, and fragrance. So right now using metabolic engineering you can actually produce lot of things, not just fuel or solvent.

Several things can be produced using metabolic engineering. And the main, metabolic engineering is also used for fuel production for renewable sources, convert biomass into chemical. It can be used for producing therapeutic compound. There is a lot of funding from US Department of Energy and a huge investment came from companies like British Petroleum, Chevron, and then Bill Gates, they are investing lot of money for this kind of product.

Because this is green alternative to the petrochemical industry. From petrochemical industry also you get most of the compound, but it is not environmental friendly. That is a reason that we are getting lot of funding from other organizations who are supporting this kind of production. Also you can think of many other companies like

Amyris, LS9, Lygos, Genometica. They are actually dealing with metabolic engineering.

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So, many industries are successfully implementing metabolic engineering. For example, the genomatica. So, genomatica has produced bio-butane or butanediol. Bio-butanediol can be used for many purposes, where it can be a chemical feedstock that replaces petroleum based product. It is a raw precursor molecule for many compounds, which converts sugars into BDO that is butanediol through microorganism.

And right now 30,000 tons of renewable BDO has been produced per year. And it is actually benefits for local agriculture economy. A lot of people are also employed. And this genomatica company is based on California and the company which is actually producing in Italy and the plant has been opened in 2016.

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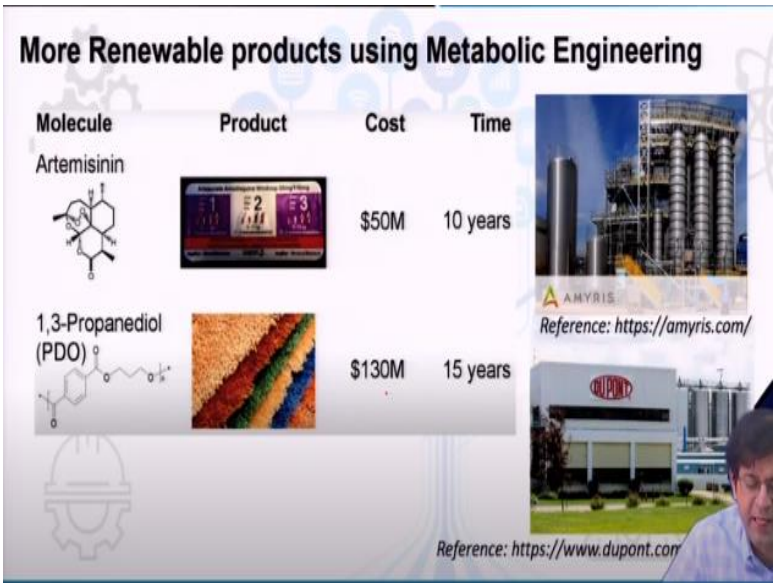

Many other product for example, artemisinin, which I have already told, it is a drug we use for malaria treatment is also produced from Saccharomyces cerevisiae, where it took almost 10 year. Right now the company which is actually involved in production of artemisinin is the Amyris. And this product took almost 10 years which goes from laboratory scale to the industrial scale.

And the project cost is around $50 million. And then we have the PDO, it is actually a fiber used for many purpose, it took almost 15 years to actually make this compound available in the market and the cost of the project is around 130 million and right now the product is manufactured from DuPont. DuPont is actually making this compound.

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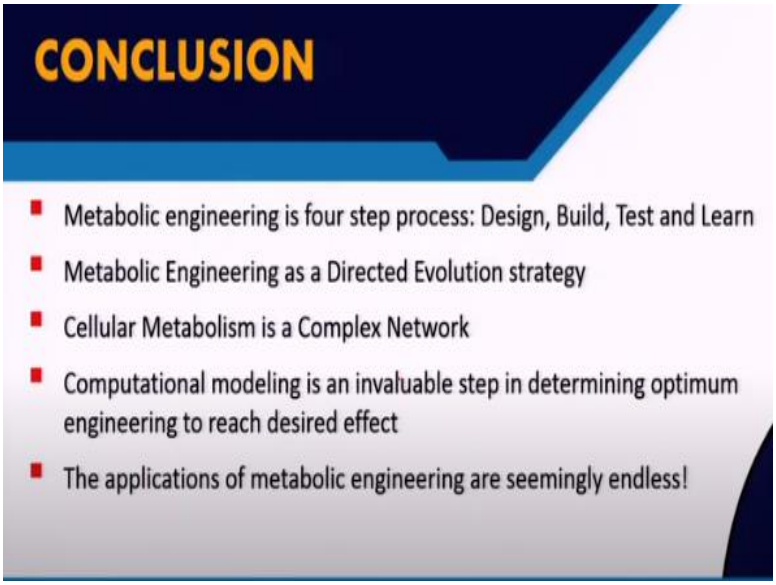

In conclusion, the metabolic engineering is actually a four step process; that is design, build, test and learn. So metabolic engineering as you know is a directed evolution strategy. So, you are evolving the strain in a smart way, in a less time. Through engineering technique, you are actually evolving the strain much quicker way so that you can produce your desired product.

And also you have seen that the cellular metabolism is a complex network, where the metabolites are interconnected and the reaction, enzyme all these are actually very well connected and the computational modeling is required because the production of metabolite is a systems biology problem. How these reactions are interconnected which I have already told you need computational modeling.

It is an invaluable step in determining the optimum engineering to reach desired product. Suppose you want to improve the desired product then you have to do some kind of computational modeling to understand how the carbons are moving or the flux is going from one reaction to the other. And finally, we also learnt what the applications of metabolic engineering are and the areas where metabolic engineering can be used .You can see the improved production of certain chemical or byproduct, which is helpful for the society.

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So, these are the references you can learn about the fundamentals of biochemistry by Voet & Voet. And also you can learn about genes by Benjamin Lewin. Also for

computational analysis of biochemical systems you can also learn this technique from the book given by Eberhard and Voit. Thank you.