Metabolic Engineering
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Lecture - 02
Essence of Metabolic Engineering

Welcome to the NPTEL online certification course on metabolic engineering. In today's lecture, I am going to discuss about the essence of metabolic engineering.

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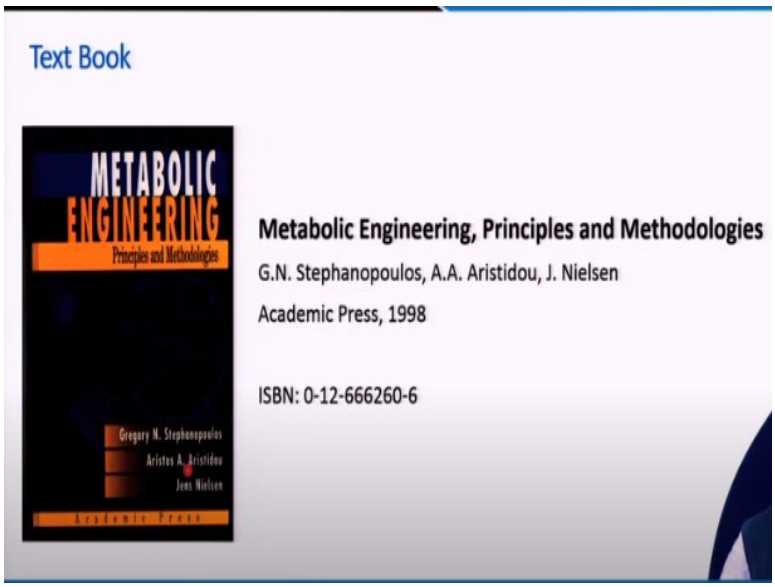

And during this lecture or maybe afterwards also for this course in general I am going to use this book as the textbook, which is metabolic engineering principles and methodologies by Stephanopoulos et al.

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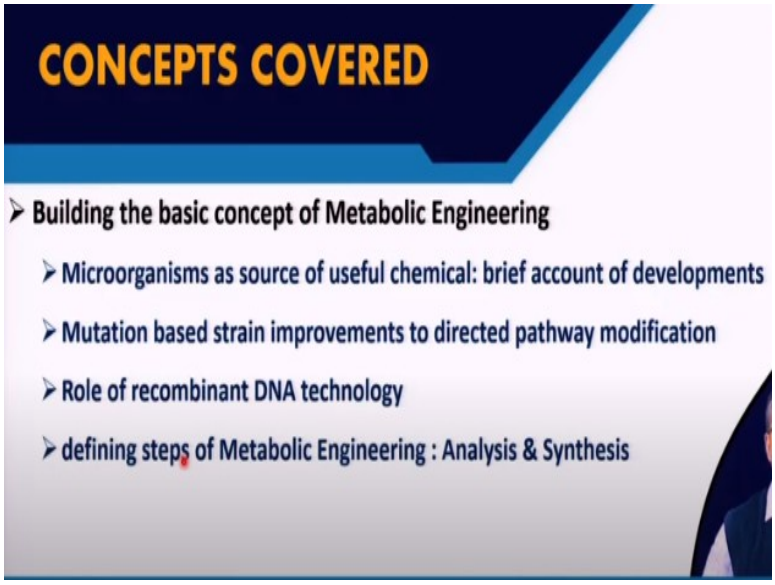

During this lecture the following topics will be covered. We are going to present the basic concept of metabolic engineering with the following major aspects like microorganisms as the source of useful chemicals and a brief account of developments towards metabolic engineering will be discussed. Mutation based strain improvements to directed pathway modification will be followed.

Role of recombinant DNA technology in developing this metabolic engineering will be discussed briefly. And then finally, we would like to highlight the defining steps of metabolic engineering like the analysis and synthesis.

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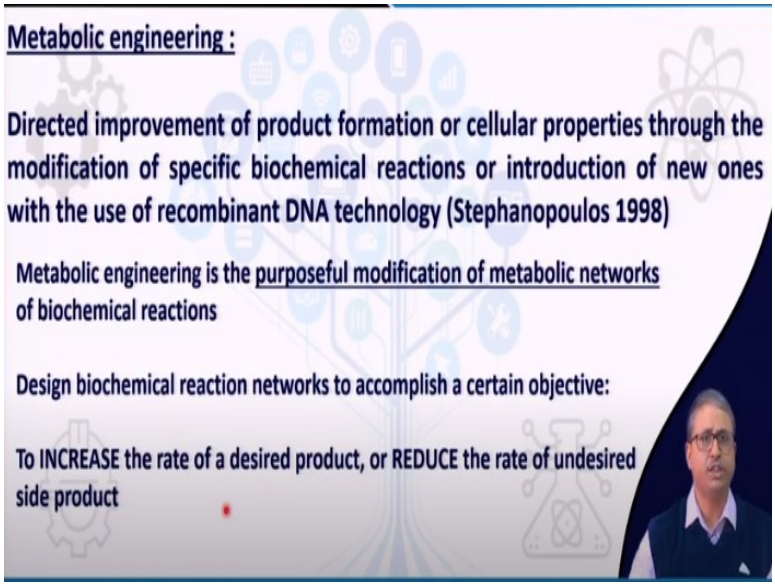

Now metabolic engineering is defined as a directed improvement of product formation or cellular properties.

So it includes both improvement of the cellular that is the mostly microbial cell properties as well as the formation of different products including the existing or natural products and products which are new to the microbial system through the modification of specific biochemical reactions or introductions of new ones with the use of recombinant DNA technology.

This definition was coined by Stephanopoulos in 1998. Metabolic engineering as we understand and as it has been developed over the past few decades, is the purposeful modification of metabolic networks of biochemical reactions. So it is important to understand that it is going to consider the metabolic function or metabolic reaction considering the networks of several reactions going to be involved or are involved in a particular reaction or a particular pathway.

Next is the design biochemical reaction network to accomplish certain objectives. That is to increase the rate of desired product which is already produced by the organism or reduce the rate of undesired side product.

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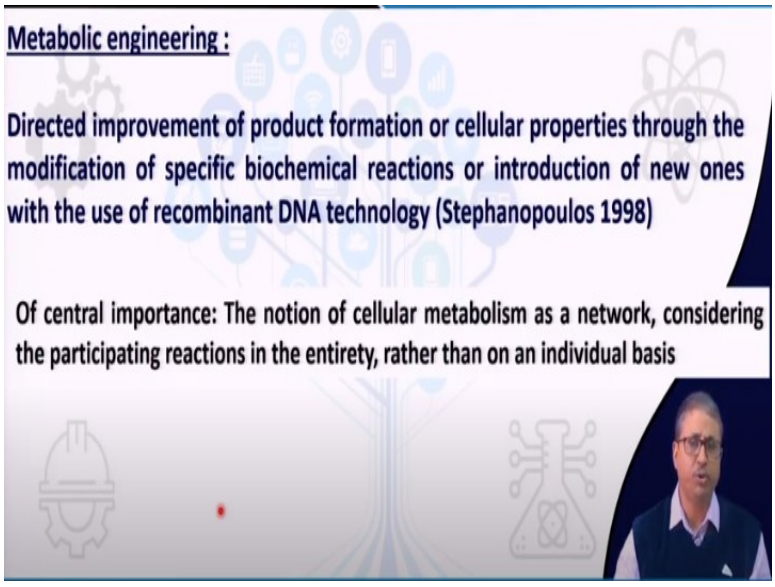

Now with respect to this, I would also like to emphasize here that metabolic engineering has evolved over the past several decades with central importance having into the notion of cellular metabolism as a network considering the participating reactions in the entirety rather than on an individual basis. So we are going to discuss all these points, including the individual reaction and the networks of reactions.

And what do you understand by the entirety of the process in detail during our subsequent lectures.

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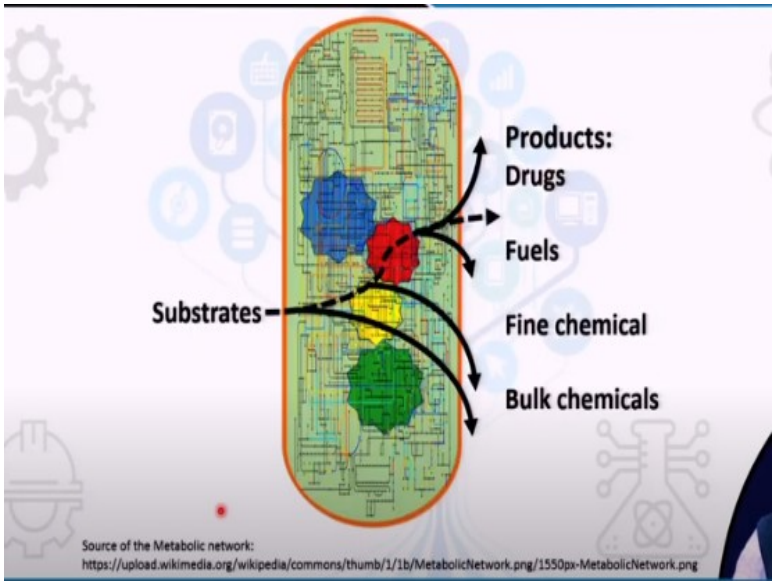

So the basic concept of metabolic engineering as all of us understand is relying on microbial cells, microbial systems to produce different products. So we generally use different substrates to produce these products. And these products might include drugs, fuels, fine chemicals and bulk chemicals.

But the most notable point over here is that this road to the products from the substrates is not a very straightforward unidirectional road naturally. It is often endowed with numerous metabolic reactions, forming the metabolic networks and any substrate which is specific to a particular pathway is processed metabolically through that complex set of reactions present within the metabolic network and then eventually lead to the production of different products.

So any kind of metabolic engineering, which actually target towards the alteration of the metabolic pathways would require to emphasize and trust on this complexity of the networks or the complexity of metabolic reactions leading towards the formation of the desired products.

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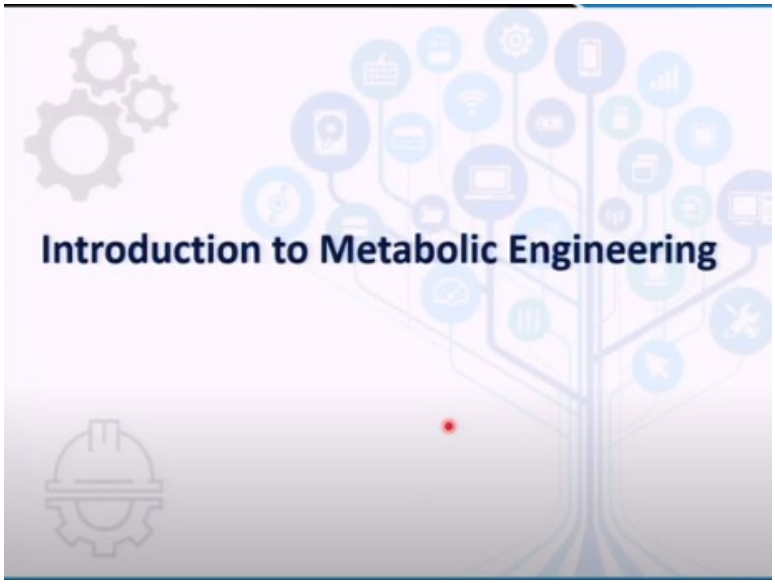

Now in the next few slides, I would like to introduce the subject metabolic engineering with a brief background of its development.

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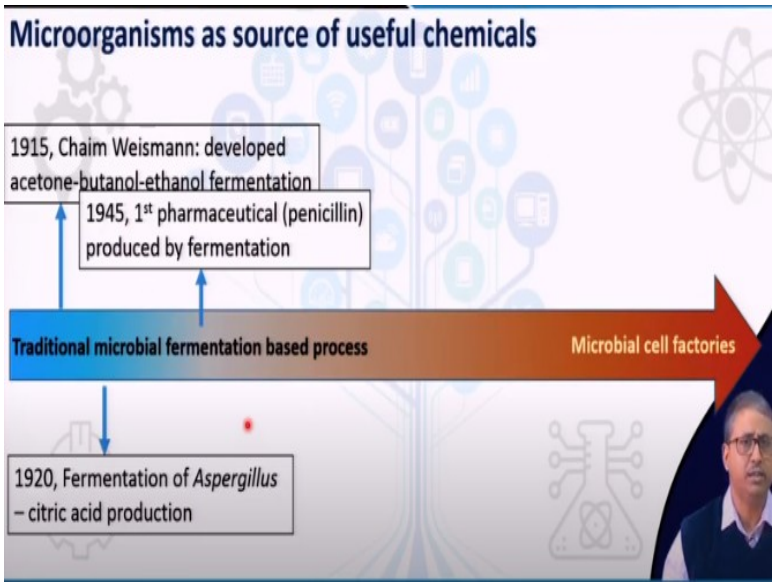

Microorganisms as source for useful chemicals have been used in our society for a very long period of time. And our society has always dependent on biomass derived carbon and energy for nutritional and other survival. We have evidence that more than 8000 years, we have been using microbial systems towards the production of different useful compounds like the food or beverage.

And if we want to look at this traditional processes, which perhaps we are well understood, since last 90 years or close to 100 years, we have made a significant progress in terms of the development of the subject or development of this field. So in

order to understand the subject metabolic engineering, we need to understand that how this subject or how this research field or this field of industrial biotechnology has evolved.

So during World War I, Chaim Weismann developed the acetone-butanol-ethanol based fermentation. That was in the year of 1915. And for next 50 years or so even more than 50 years, this fermentation process developed by Weisman was successfully used towards the production of acetone. And then in recent years, the same process is being revived towards the production of other compounds like 1-butanol.

Subsequently, fermentation of fungus, *Aspergillus niger* towards the production of citric acid was developed and it was scaled up for different commercial applications. And the same technology using the filamentous fungus *Aspergillus* in 1945 the first antibiotic that is the penicillin was produced by industrial scale fermentation. And during World War II, this fermentation derived penicillin played a very important role.

In the subsequent time, we have seen a great deal of interest both on the fundamental research as well as from the industrial microbiology and biotechnology research in improving the production of natural products by microbial cells or microorganisms.

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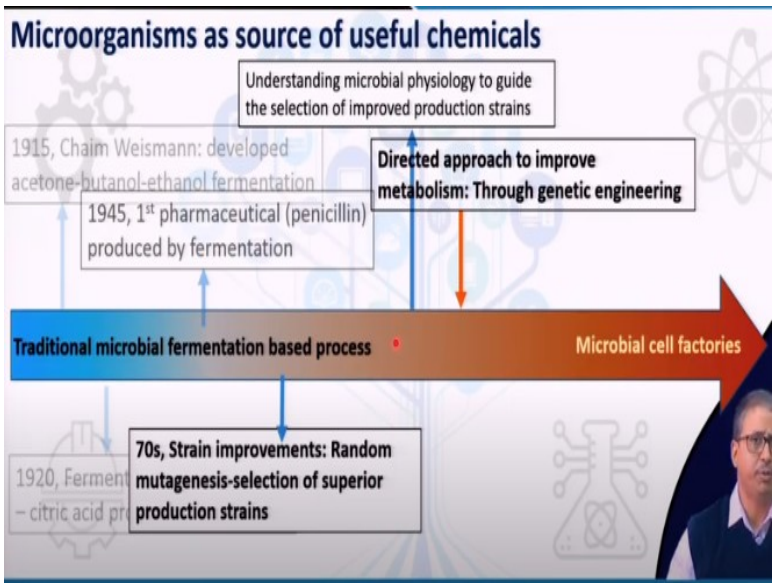

Particularly during the 1970s, strain improvement using random mutagenesis followed by selective screening played a very important role in selecting superior production strains. So a large number of strains were isolated, screened, mutated and superior production strains were developed.

And one of the achievements was that through these kind of random mutagenesis and selective screening, the antibiotic penicillin production ability of natural *Aspergillus* strain was improved out up to 10,000 fold. Subsequently, understanding the microbial physiology to guide the selection of improved production strains was realized and incorporated into the research plan.

So in addition to the mutation and screening of natural organisms capable of producing antibiotics mainly or other useful compounds during that time like citric acid or acetone or ethanol microbial physiology was studied in order to provide a better growth conditions and an overall improved production condition.

However, during the time of 1980s to 1990s, we observe a very significant change in the approach of metabolic engineering, so called metabolic engineering because the terminology was not defined during that period of time, which is basically through the genetic engineering. Because by that time the DNA structure of microorganisms and some other information were available.

So more directed approach towards improvement of the metabolism was observed.

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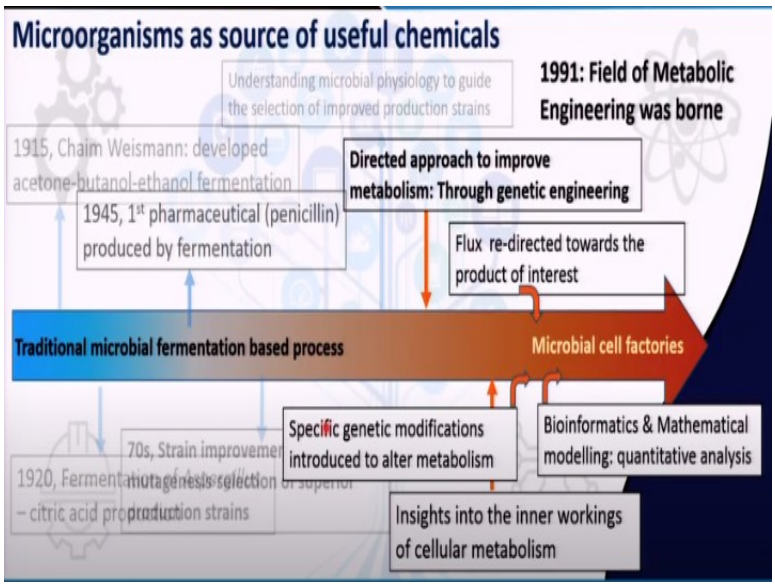

So through incorporation of these genetic engineering tools, new insights into the inner workings of cellular metabolisms were obtained. And with the further development of bioinformatics and mathematical modeling, quantitative analysis of the pathways involved in the product formation were achieved along with identification of specific genes.

And their modification through different recombinant DNA technology enabled us to introduce specific modifications in the DNA structure, which allowed us to modify the pathway and obtain the improved strains further. Eventually, through this bioinformatics, mathematical modeling and modification of specific genes and pathways, flux redirection based approaches, emerged.

Flux redirected towards the product of interest enabled the scientist and investigators to produce further improved and highly efficient microorganisms and which eventually culminated into a new field of industrial biotechnology or industrial microbiology, which we called the field of metabolic engineering.

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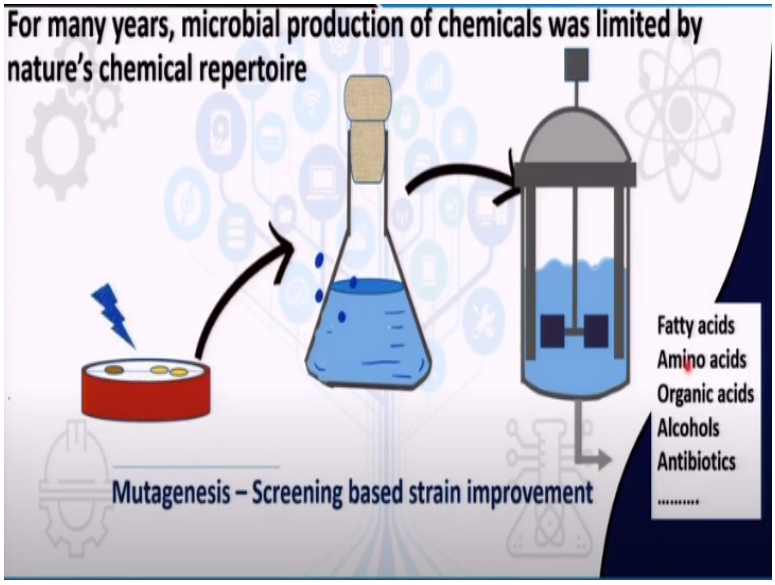

So before we go into the details of this development, which enabled us to understand metabolic engineering, I would like to discuss very briefly over here that what was this screening and production of natural molecules like antibiotics or ethanol or citric acid from microorganisms like bacteria and fungi, which were naturally available.

So for many years, microbial production of chemical was limited by nature's chemical repertoire because we were relying on what microorganisms can naturally do. Like, you can see that we used to grow the microbes like bacteria or fungi into laboratory condition, used to get the cultures or pure cultures.

And those pure cultures were grown in the lab culture conditions followed by their growth in the bioreactors and a range of products were successfully obtained. Subsequently, as we talked about the strain improvement, different mutation studies were done.

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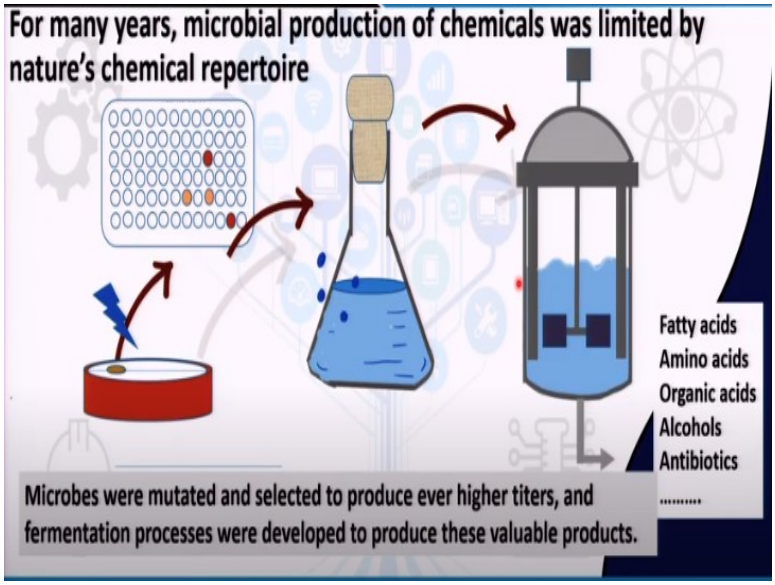

And following these mutations or mutagenic treatment, we could obtain a number of mutant strains and followed by different types of screening procedure, those strains were selected further for their laboratory growth, and they are scale up through the different bioreactor configuration.

So this enabled us as I mentioned earlier, to produce a large concentration of the product, which we call the titer, particularly the example of antibiotic production is considered to be one of the remarkable examples because as I mentioned, with this mutation followed by selective screening, the natural producing ability was enhanced up to 10,000 fold.

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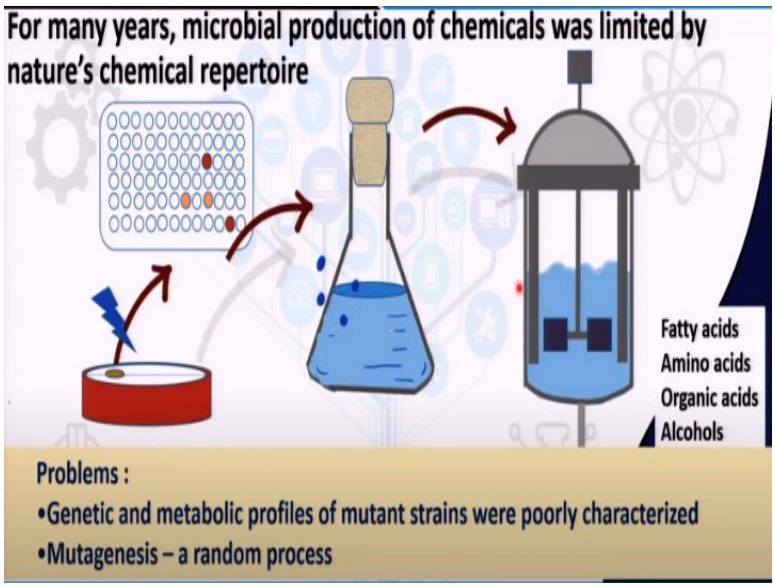

But this mutation and screening based method is having some intrinsic problems. The problems are that the genetic and metabolic profiles of the strains, the mutant strains in particular, were poorly characterized. And, in general, the mutagenesis was a random process.

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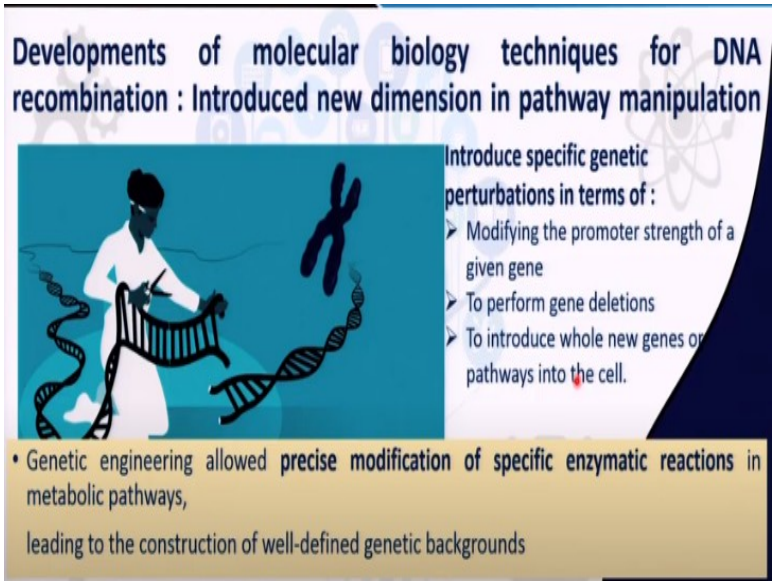

Eventually, during the late 80s, or mid 80s, as the development of molecular biology techniques for DNA recombination came into forefront that introduced a new dimension in pathway manipulation. We were able to modify genes or we were allowed to do precise modification of specific enzymatic reactions that leads to the construction of well defined genetic backgrounds.

So in contrast to the random mutagenesis, the recombinant DNA technology allowed us to firstly precisely modify the specific reactions and also to obtain genetically well-structured organisms. The genetic background of the organisms were clearly known to the scientist, so that they were available for further modifications and improvement.

Now introduction of these genetic engineering tools allowed us to perform different alterations in terms of modifying the promoter strength of the given gene, in terms of performing gene deletions, in terms of introducing a whole new gene or a pathway into the cell.

Even in today's time, we were able to transfer or express genes and pathways entirely from other domain like from eukaryotic organism to the prokaryotic microorganisms,

we can introduce new genes or pathway and enable them to function towards the production of the desired molecule.

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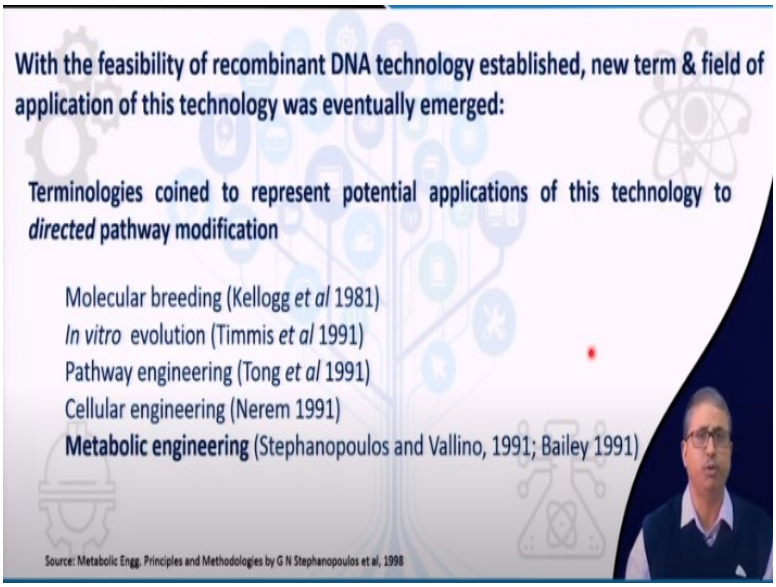

Now with the feasibility of recombinant DNA technology established the new term of field of application of this technology was eventually emerged. And there are different terminologies which were initially proposed, including the molecular breeding to in vitro evolution, pathway engineering, to cellular engineering. And finally, the metabolic engineering terminology was proposed originally by Stephanopoulos and Vallino in 1991 and also by Bailey in 1991.

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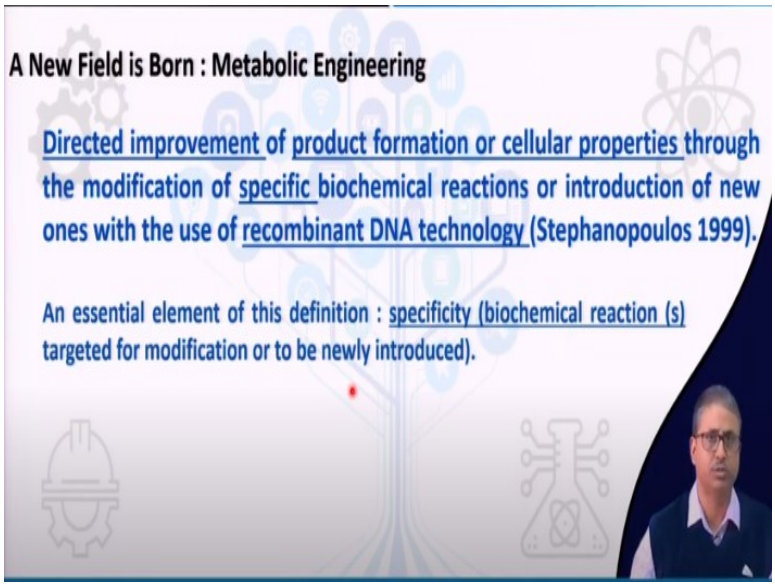

So

So a new field was born, which is metabolic engineering. It is defined as the directed improvement of product formation or cellular properties through the modification of specific biochemical reactions or introduction of new ones with the use of recombinant DNA technology. Now this definition has several components within it. Number one, is the directed improvement.

It is not a random process, it is a very focused, very targeted, it is a directed improvement. Directed improvement of what? Directed improvement of product formation, specific product or even cellular properties, like different physiological properties were often targeted for microorganisms to allow them to sustain different industrial scale operations.

Now these improvements are done through modification of specific biochemical reaction. So the specificity is there in terms of the biochemical reaction, which we are targeting or the metabolic engineering is targeting and also the specificity in its strategy that how these improvements are going to be implemented.

Now the modification could be in terms of specific biochemical reaction or it could be introduction of entirely a new one, if it is not there in the natural organism with the use of recombinant DNA technology. So the methodological aspect for improving the biochemical pathway of the organism is the recombinant DNA technology.

And the selection of the specific pathway, or the introduction of the pathway would be done following a very rational analysis of the entire process. And the overall thing will be highly directed towards the product formation or similar properties. It is not going to be a random process anymore like the mutagenesis followed by the selective screening. Now as I discussed an essential element of this definition is the specificity.

The specificity of the biochemical reaction, so it is not a random process. Now this specificity of the biochemical reaction or multiple reactions targeted for modification or to be newly introduced. So we need to be very much focused regarding the particular biochemical reaction or reactions that we are going to alter or modify or we plan to incorporate it.

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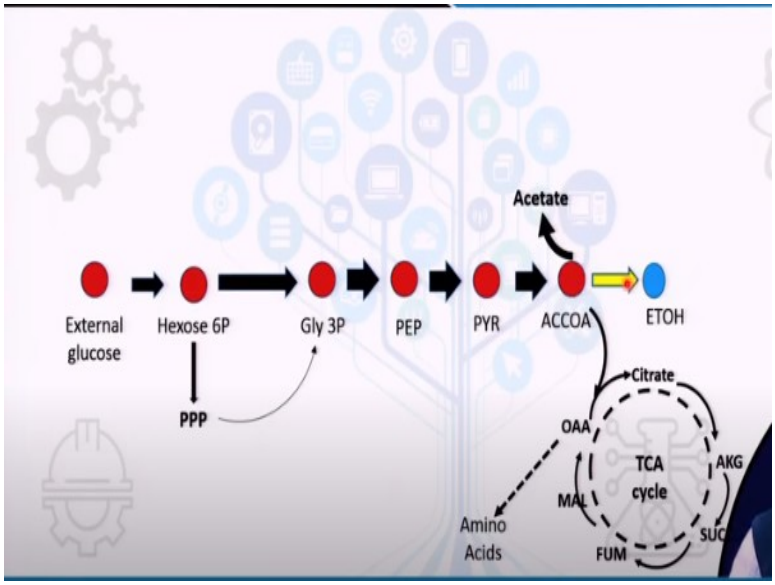

Incorporate it from a different host or different organism which is called heterologous expression of the genes. Now let us see a very simple metabolic pathway, a set of reactions where glucose is processed towards pyruvic acid and then ethanol could be produced. So you can see the arrows are of different thickness and the thickness of the arrow indicates the relative flux that is the rate of flow of the metabolites like glucose in this case.

So we can see very clearly that the glucose is processed within the metabolism of glycolysis up to pyruvic acid and then up to acetyl-CoA. And then following acetyl-CoA, there are multiple options like one pathway goes to the TCA cycle, another pathway goes to the alcohol, ethyl alcohol production. Another pathway goes to the acetate production.

Now in case we want this pathway to be utilized or used for alcohol production, ethyl alcohol production, we need to identify the specific reaction which is involved in taking the flux or taking the carbons towards ethyl alcohol that is this particular reaction. So that is how out of so many reactions we need to identify the particular reaction which is targeted towards the desired product formation.

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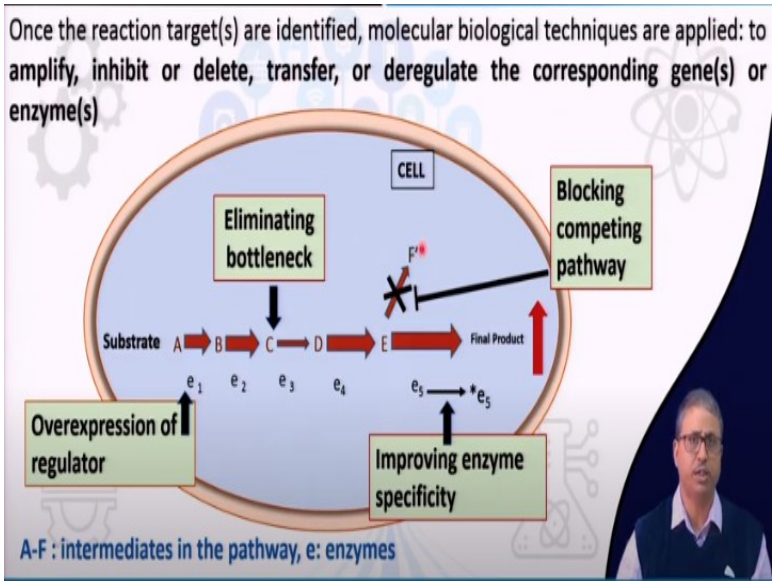

And once such reaction targets because the reaction targets could be many. It could be amplification of a target or it could be deletion of a target, unnecessary targets may be down regulated. So once the reaction targets are identified, molecular biology techniques are applied to amplify, inhibit, delete, transfer or deregulate the corresponding genes or they are enzymes eventually.

So in this diagram, you can see that there is a substrate A, which is converted to different intermediates B, C, D, E. And then finally, the product F is produced. You have a kind of branch point where E can be diverted towards another product which is F prime and also there is a slow process or maybe the enzyme is inefficient over here, the conversion of C to D is not compatible compared to the other reaction steps.

So in similar type of situations, we first identify the metabolic pathway, identify the specific genes which we need to alter and then implement different strategies including overexpression of some of the regulators, which might improve the function of the important or critical enzymes. Eliminate the bottlenecks. Suppose, in this case C to D there are seems to be some kind of reaction bottlenecks are there.

So those bottlenecks can be removed. Improving the enzyme specificity. We can improve the specificity of the enzyme towards certain substrate or certain intermediate and converting the specific intermediate or the substrate to a particular product. Or we can block the competing pathway also. Like in this case, the

competing pathway which was taking the carbons from the other or the intermediates from the E towards F prime is a side product is blocked.

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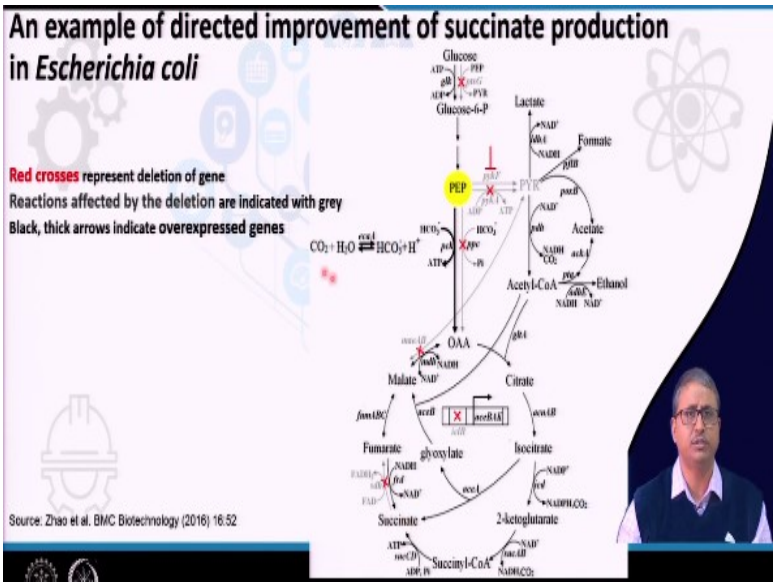

Now there are several examples of identifying the target reactions and then manipulating them. So here I just present you one of the examples where succinate production in E. coli is improved by altering the central carbon metabolism. As you can see the red crosses are the reactions or the enzyme reactions which are deleted or inhibited. And the gray lines are the reactions which are negatively impacted due to this deletion of these reactions.

And the black thick arrows indicate the overexpressed gene. So here you can see a particular gene is overexpressed and followed by or coupled with a number of deletions that allowed the production of a higher concentration of succinic acid by this recombinant Escherichia coli.

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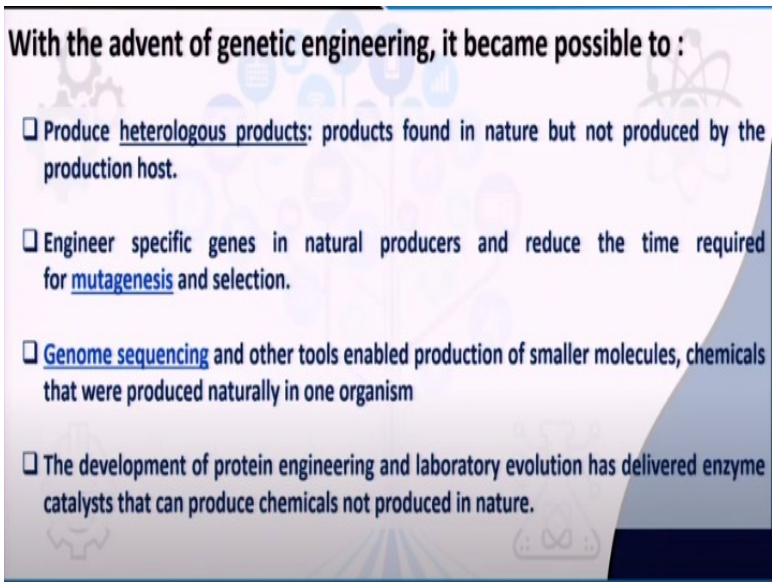

Similar examples are numerous in number. Now with the advent of genetic engineering, it became possible to do a number of things including to produce heterologous products that is the products found in nature but not produced by a particular production host. Engineer specific genes in natural producer and reduce the time required for mutagenesis and selection.

So you can engineer a specific gene which is existing in the natural host. We can look at the genome sequence like the entire whole genome sequence. And other tools enable the production of small molecules. And development of protein engineering and laboratory evolution that has actually delivered a number of improved enzyme catalyst that can be produced through chemical but not produced naturally.

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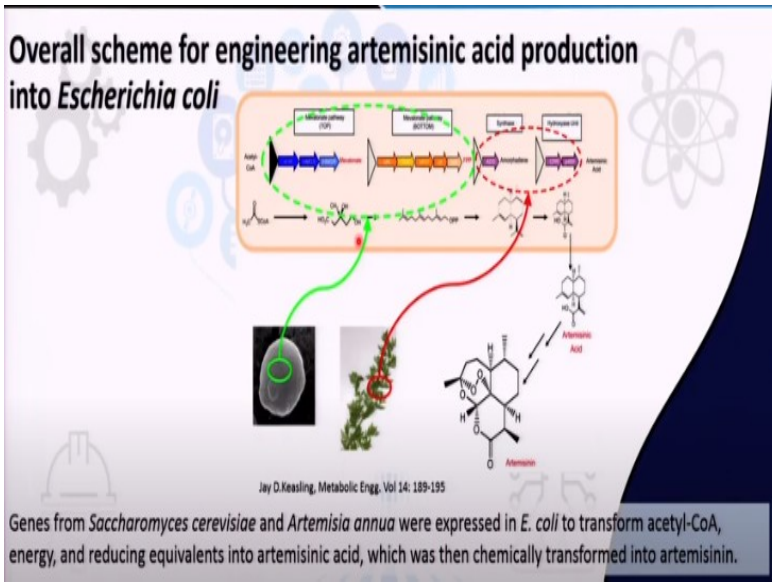

So here is the example for the heterologous expression and the synthesis of Artemisia, one of the very important and useful drug for combating the problem of malaria. So this Artemisia is basically anti-malarial drug. So using the genes from yeast and the plant, Artemisia, the E. coli host is engineered to produce this Artemisia.

So we will discuss in detail about this production of these Artemisia during some of our application lectures. So here we are just trying to emphasize that heterologous genes from different organisms can be brought together and can be placed in a production host like E. coli because the system of E. coli is well understood and well optimized for different bioreactor use.

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The next is the directionality in effort, which is the focal theme for metabolic engineering. Directionality in terms of the target selection, experimental design and also the data analysis.

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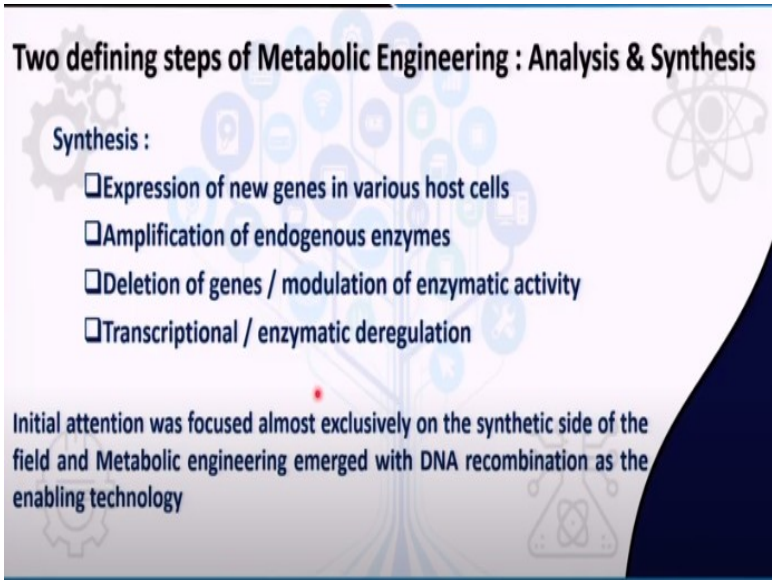

Finally, there are two defining steps of metabolic engineering. One is the analysis and another is the synthesis. In the synthesis part, we try to express the new genes in various host cells. Amplify the endogenous genes and enzymes which are preexisting or already there in the natural organism. Deletion of genes or modulation of the enzymatic activity. And transcriptional or enzymatic deregulation in order to control the metabolic pathway function.

Now initially, the synthesis part was under focus, because the advent of recombinant DNA technology allowed the scientists who are working on metabolic engineering to synthesize a number of recombinant or improved strains by incorporating the ideas and by virtue of the methods and tools which were available through recombinant DNA technology.

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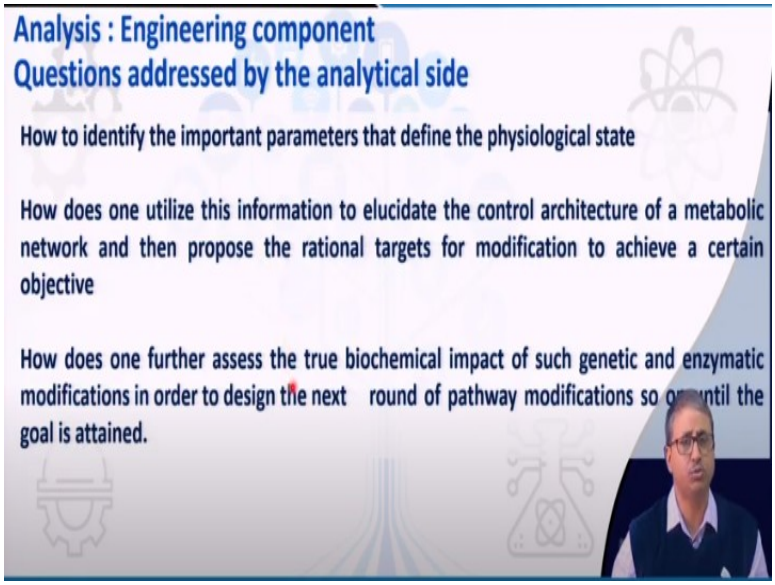

Following synthesis, that means once the recombinant organism is produced, the analysis is done. So this is basically considered to be an engineering component where questions addressed from an engineering point of view. For example, how to identify the important parameters that define the physiological state. And how does one utilize the information to elucidate the control architecture of a metabolic network.

Because, as I mentioned earlier, metabolic reactions are highly coordinated and they are in a network. So how one can utilize the information that these are the important parameters and these important parameters are regulating the metabolic network, how it is regulating. And then propose a rational target that these particular reaction or these particular reactions could be altered.

And finally, how does one further assess the true biochemical impact of such genetic and enzymatic modifications.

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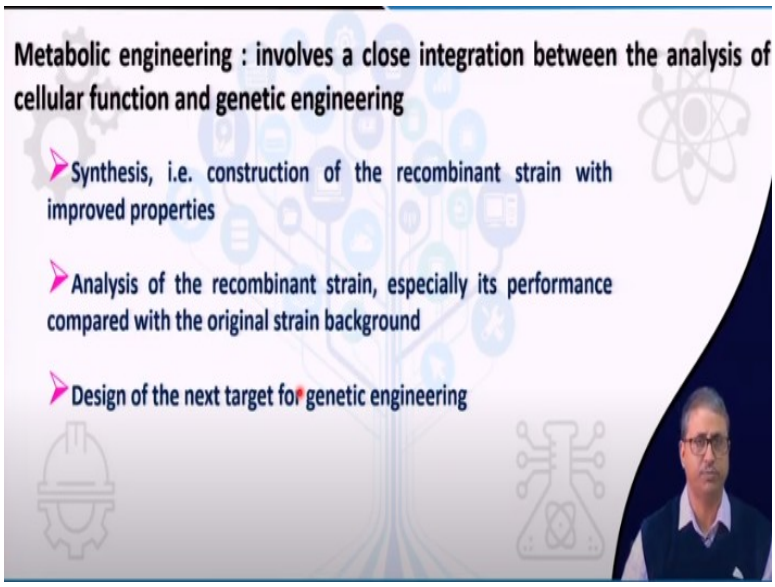

Now metabolic engineering essentially involves these two steps, close integration between the analysis of the cellular function and genetic engineering. So we have a synthesis part where we construct the recombinant strain with improved properties. Then we analyze the recombinant strain, especially their performance compared to the original or the native strain background. And then we design further changes until the desired outcome is reached.

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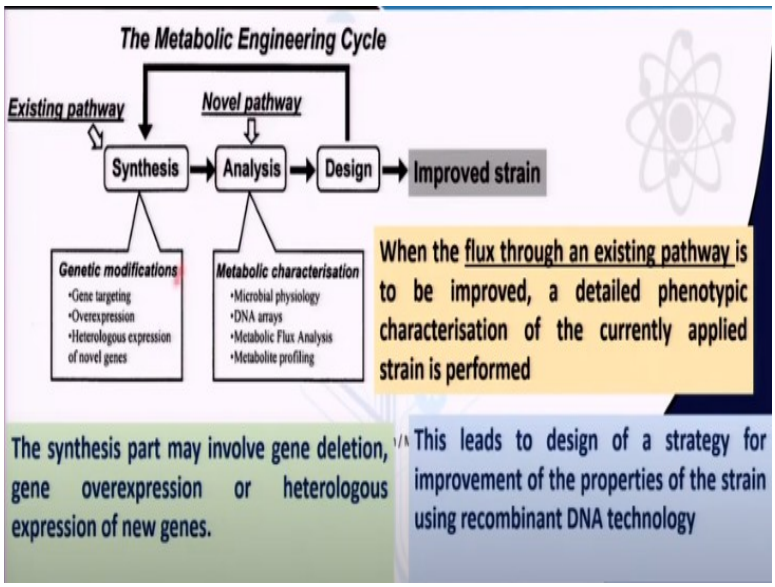

So in overall, we see that we have existing organism. We try to know how it is synthesizing. Then we try to analyze that what are the processes it is performing, what are the physiological and genetic processes, how the flux etc., are being processed within the system. And then we do the designing part. Following designing, we make a strategy and then the strategy is implemented towards synthesis again.

And this is the kind of iterative process. So multiple cycle of the synthesis, analysis, design is conducted towards to achieve the improved strain. So when the flux through an existing pathway is to be improved, a detailed phenotypic characterization of the currently applied strain is performed.

So whenever we have a strain with us, we characterize the strain both physiologically, phenotypically as well as the flux were calculated to find out that, how the particular organism, the natural organism is processing the substrates and what are the possible means, by which we can improve it. Then, we design a strategy that is a part of the design component which enable us to actually improve the properties of the strain using the specific methods of recombinant DNA technology.

So we analyze the strain, then make the strategy and then implement the strategy that is under the part of the synthesis. So we analyze, following analyze, we design the strategy and these designed strategies are implemented through recombinant DNA technology.

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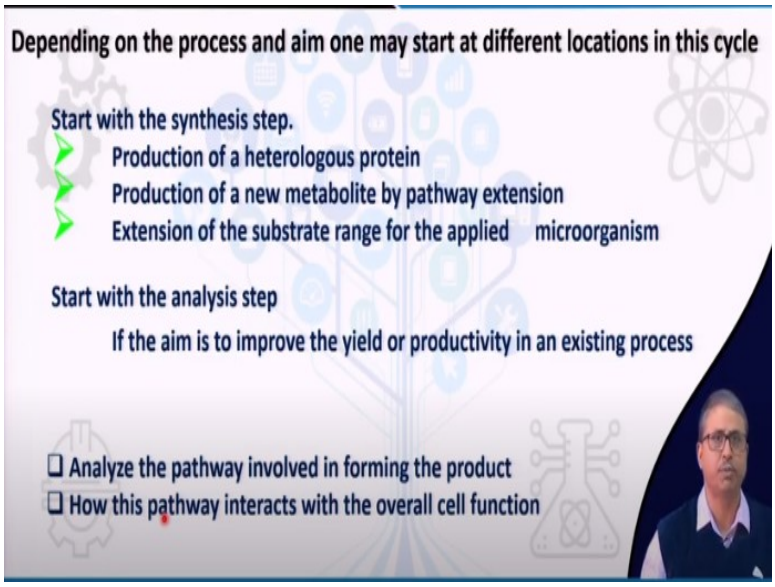

Now depending upon the process and aim, one may start at different locations as well. Like it is not mandatory that we have to start with the synthesis step. Synthesis step is usually considered as the start, if we are working on the production of heterologous protein or production of a new metabolite by pathway extension or extension of the substrate range of the applied microorganism.

However, we can start with the analysis step, if the aim is to improve the yield or the productivity of the existing process, where we know that, we need to actually analyze the pathway involved in forming the product and how this pathway interacts with the overall cell function.

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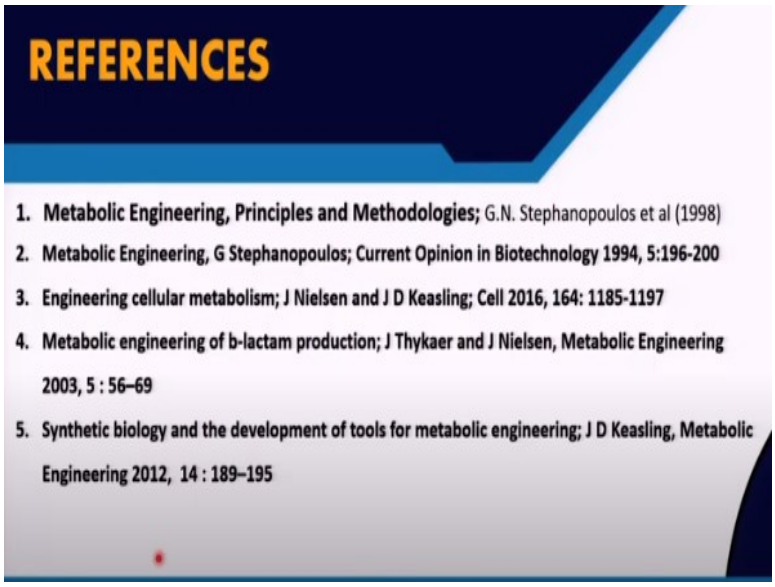

So for this part of my lecture, I have used these following references, which includes the metabolic engineering textbook and couple of useful research articles including the review papers.

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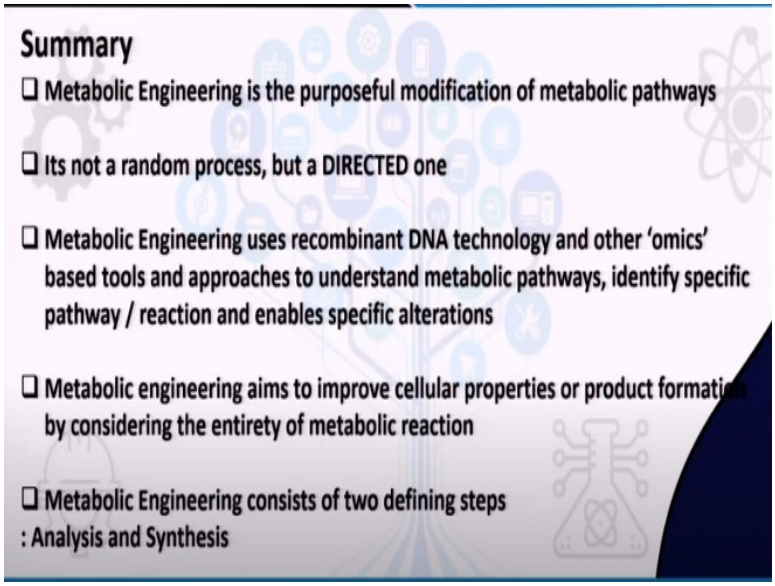

And the summary of the today's lecture is that metabolic engineering is the purposeful modification of metabolic pathways. And it is not a random process, but a

directed one. Metabolic engineering uses recombinant DNA technology and other omics based tools and approaches to understand metabolic pathways, identify specific pathway reaction and enables specific alterations.

Metabolic engineering aims to improve the cellular properties or product formation by considering the entirety of metabolic reaction. And lastly, metabolic engineering consists of two defining steps, analysis and synthesis. Thank you.