Biochemical Engineering

Prof. Dr. Rintu Banerjee

Department of Agricultural and Food Engineering

Prof. Dr. Saikat Chakraborty

Department of Chemical Engineering

Indian Institute of Technology, Kharagpur

Module No. # 01

Lecture No. # 37

Manufacture of Biochemicals

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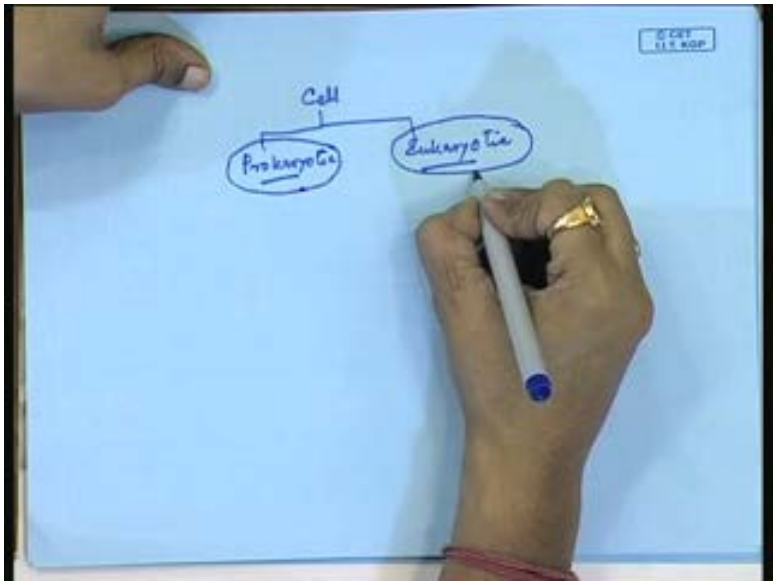

Good morning students. The topic of today's discussion is Manufacture of biochemicals. Now, in our earlier classes, we have already learnt about the cells and we have also learnt that, cells are of two types; one is the prokaryotic and another is the eukaryotic. So, we have also learnt that, how eukaryotic cells are different from that of the prokaryotic cells. We have also learnt that, prokaryotic cells are the primitive cells and eukaryotic cells are the advanced, or the modern cells, where all these organelles are performing different activities. And, when we have studied the cells and its organelles, we have also learnt that, how each and every organelles are participating in different activities of the cell.

Now, then, in some other classes, we have also learnt that, how the metabolic reactions are going on within the cell. And, we have also seen that, how this manipulation of cells, that means, that incorporation of one particular gene, can be inserted to another particular organism and with proper expression, how we can go for the over-production of any metabolites which can be produced in the cell. Now, today, in our, this particular class, we will be learning that, how this biochemicals can be manufactured and what are the different techniques and how one can produce this biochemicals for different activities, different applications.

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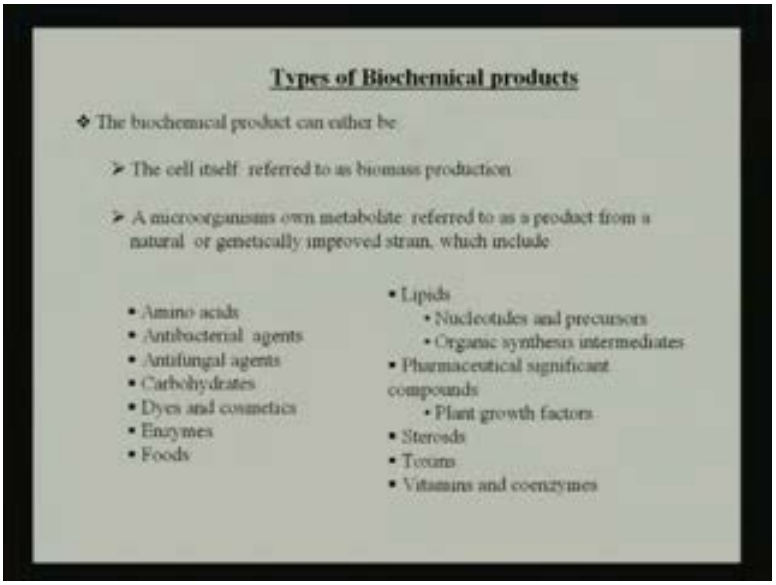

Now, here, coming to this biochemicals, if we are, if we see this biochemicals, then, we can find that, the cell itself can be used as a biomass and this biomass can be used for different purposes. And, the microorganism, with its own metabolites which is produced within the cell, or which are produced within the cell, can be referred as the product of our own interest, from the natural, or genetically improved strains.

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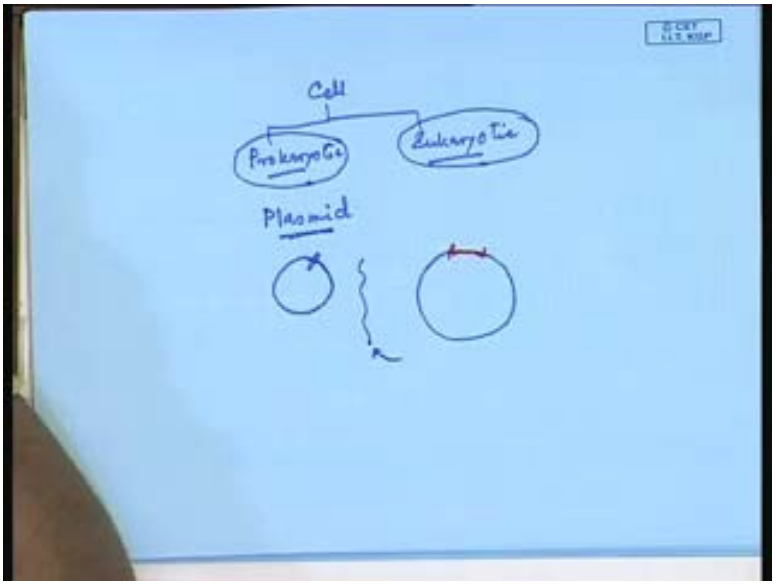

Now, here, in my earlier classes, I have all ready mentioned you that, in the prokaryotic cell, particularly in bacteria, there is a plasmid DNA. Now, if we remember that, what is that plasmid? Plasmid is a circular double helix structured DNA and it can be, with the restriction enzymes, we can make it a linear DNA; we can insert our targeted gene to this and once again, with the another enzyme called ligase, we can once again express this particular, this plasmid DNA, to any expression system; it maybe a bacteria, it maybe any higher organism, eukaryotic cell, yeast, or fungi, or anything.

So, if we see, and, why we are doing this type of genetically engineered organism? We are doing this type of genetically engineered organism, because of the over production, or improved productivity. Now, this products, products what I am talking about today, maybe amino acids, it maybe antibacterial agent, it maybe antifungal agent, it maybe carbohydrates, it maybe dyes and cosmetics ingredient, it maybe enzymes, foods, and so on; it can be that lipid, it can be the nucleotide, and its precursors; it can be organic synthesis and its intermediates, pharmaceutical significant compound, it maybe plant growth factors, steroid, toxins, vitamins, coenzymes, and so on. So, any metabolite which can be produced in any living cells can be considered as the biochemicals.

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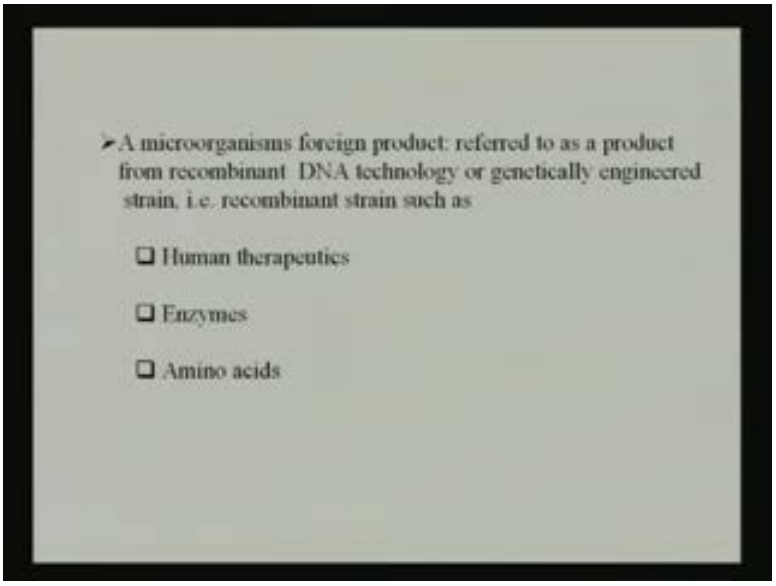

Now, how we are going for such type of things? We can use some lower group of microorganisms, what we have learned the architecture and different organelles, and we have already learnt that, how they behave and from that previous knowledge, we can now implement those knowledge, basic knowledge, for application oriented work. Now, here, when we are just talking about this particular insertion of one particular gene of our interest to any primitive organism, and if we are over producing this, all this activities are coming under the recombinant DNA technology, that is otherwise known as r DNA technology; or, we can tell that, these are the genetically engineered strains. Now, this over production can be done, either by genetically engineered strains, or sometimes, we can take the natural, or the wild strains too, where such type of metabolic productions or productivities are there. So, these types of strains can be used for human therapeutics; it can be used for enzymes; it can be used for amino acids and so on. So, any metabolites which are biological in nature, and produced by the cells, can be used for biochemical production.

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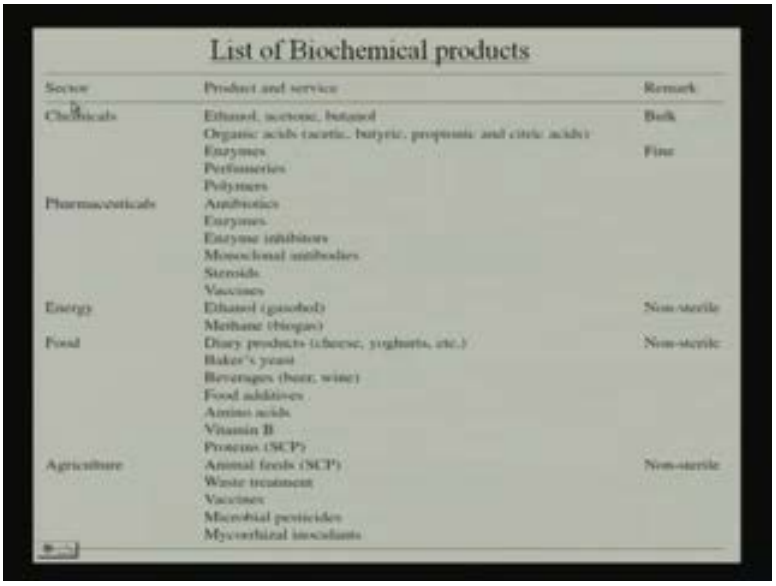

At a glance, if we see the different types of biochemical products, then, we can classify this, the different biochemicals, into different sectors, like that chemical sectors, pharmaceuticals, energy, food, agriculture and so on. Now, if we further classify the chemicals under each sector, then, we can find that, some of this chemicals like ethanol, acetone, butanol, etcetera, that are used as a bulk chemicals; organic acids like acetic acid, butyric acid, propionic acid, citric acids and so on; enzymes, polymers, and these are the product which are coming under the chemical sectors, which can be produced from the biological origins too.

Pharmaceuticals such as antibiotics, enzyme, enzyme inhibitor, monoclonal antibodies, steroids, vaccine, and so on, are coming under the pharmaceuticals, or the health care sector. When we are talking about the energy sectors, then, we are mainly talking about the ethanol that is, that is used as a gasohol and methane, that is the biogas, which has got immense applications, immense importance, as far as today's world is concerned. Food is also another important sector, where we can consider any dairy products, like cheese, yoghurts, any baker's yeast, beverages like beer, wine, food additives, amino acid, vitamins, single cell proteins, etcetera, which are coming under the food sector. When we are talking about the agriculture, then, animal feed, waste treatment process, vaccine, microbial pesticide, herbicides, etcetera, mycorrhizal inoculants, etcetera, these are the products which are very much coming under the agricultural sector.

So, here, I am just telling few names as a symbol that, any biological, or biochemicals, which are produced in any particular cells, any particular living cell, can be considered as the biochemicals, and we can go for the manufacture, or the production, or synthesis of that particular product in living cell.

Now, when we are talking about the cell, then, obviously, I have told you, the cells are of different types, and depending upon the development, we have categorized these cells into different sectors. One is the microbial cell; another is the plant cell; another is the animal cell. Now, while talking to this architecture and the cell organelles, I have also mentioned you that, animal cells are different from the plant cells are the different from microbial cell. So, each and every cell, irrespective of its origin, has certain similarities and certain differences; and those things, we have already learned.

Now, coming to the animal cell cultivation. Now, when we are talking about the animal cell culture, then, we are just taking those cells from the parent cell, or the donor cell. Now, when we are taking this, we are considering the animal cell cultivation. The cells which are removed from the animal tissues can be cultivated in nutritional medium outside of the donor's body; that means, we are taking those cells, and we are, we are making some environment in such a way, which will mimic the body conditions; that means, whatever is the body conditions, that, whatever is the physiological, pH, temperature, everything, we are simulating that, in any other environment and we are making that environment suitable for this animal cell cultivation.

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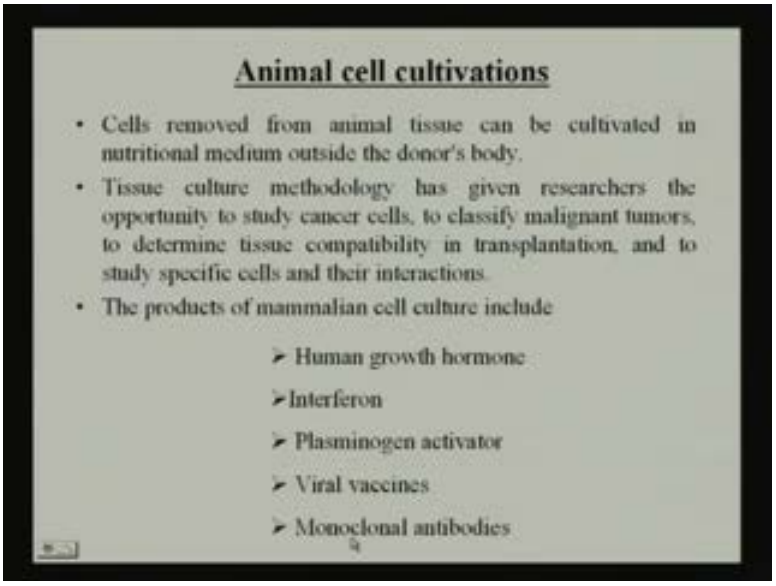

Now, this tissue culture methodology has given researchers the opportunity to study the cancer cell, to classify malignant tumors, to determine tissues compatibility in transplantation, and to study the specific cells and their interactions. So, when we are just going for these, so, different types of techniques are there. One of the techniques is that, one cell line, you just, normal cell line, you take, another cancerous cell line, you just take; and, you fuse both the cells together, and the resultant product, what we are getting is called the hybrid cell; that, one is the malignant, that cancerous, that malignant cell, another is the, one is the tumorous, another is the normal and they are getting fused and we are getting the hybridomas cell.

Now, this is one type of cell cultivation. There are very many types of cell cultivation. Now, why we are taking this cancerous cell for such type of activities? One of the reason is that, we already learnt that, this cancer cell has got one characteristics that, they do not have any control on its cell growth; n number of multiplication and cell divisions are going on, resulting in the formation of tumor. And, this particular characteristic, we would like to mimic, we would like to exploit for our metabolite production; and this way, when we are going for this cell cultivation, we can produce different types of biologicals, which can be synthesized through this technique. One of the very important products is your human growth hormone, interferon, plasminogen activators, viral vaccine, monoclonal antibodies and so on. These are some of the symbolic products I, I

am just showing you, just which has got immense applications, as far as human health is concerned.

Now, when, some mammalian gene products can also produced by this bacterial system using the recombinant DNA technology. Now, what we are doing? Now, mammalian cell culture, what we have seen? We have seen that, some of the cells we are taking from the donor cell and then, we are simulating some environment where it will mimic the body condition and cells will be proliferating; and this is the animal cell cultivation. In other way, I told you, very many techniques are there; that is one technique. Another technique is that, we can take the gene of our interest, which is giving our targeted product and we are just splicing that gene from that mammal's body and we are inserting that gene to any bacteria. Now, here, say, as I have told, this mammalian gene, can also get expressed in some microorganism, by taking some microbes, we can express that particular gene which is coming under the recombinant DNA technology.

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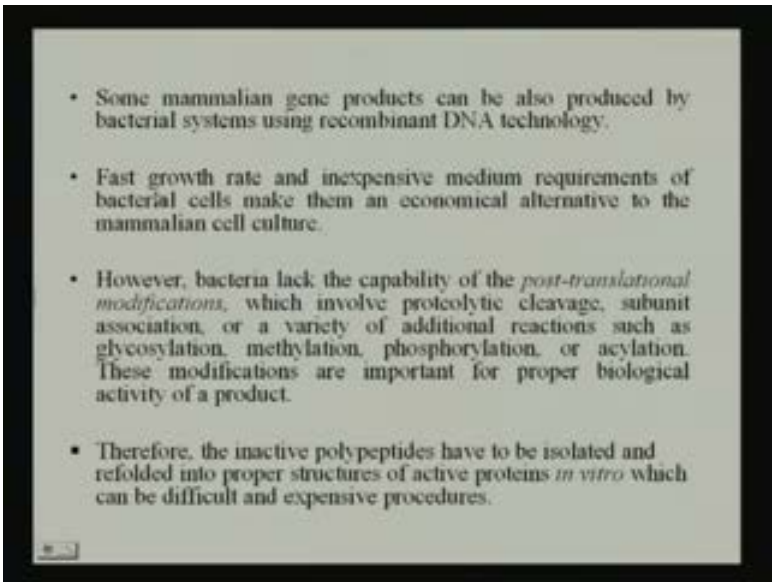

First, why, why we are going for such type of things, that mammalian gene, we are just taking and we are just getting it expressed in the very lower group of organism? Because, microorganisms can grow very fast. So, because of its fast growth rate and inexpensive medium required for the bacterial cells, make them an economical alternative to the mammalian cell culture. Now, just you imagine, if we are mimicking any body system, just see, how costly that particular affair is, that condition will be. So, here, if we can

take that gene out of this mammal, and if we can insert that to any bacteria, and if we can express, we can cultivate that particular bacteria for that particular product formation, then, what will happen; then, the production cost, medium requirement and everything will be the bacterial type; it is gene of mammal, but, that media, or the particular organism where we are expressing that particular gene, is the microorganism. So, we can use that microbial media for the microorganisms' growth. So, it is very easy; it is very cheaper than that mimicking the biological, that tissue or the body condition.

So, we can produce a cost effective product in this particular condition; however, bacteria lack the capability of post translational modification, which involve proteolytic cleavage, subunit association, or a variety of additional reactions, such as glycosylation, methylation, phosphorylation, acylation, and so on. These modifications are important for proper biological activities of the product. Now, as in my earlier classes, I have told you that, how simple a bacterial cell is and how complex the eukaryotic cell is compared to this prokaryotic cell.

Now, when we are taking some gene from a complex system to a simplified system, simple media, simple system, and we are just expressing that particular thing, it is happening that and we have also seen that, transcription, translation and all those, that central dogma which is going on for protein synthesis and entire body regulations, we have already learnt those things and now, we can understand that, what is going on within the cell. Now, when such type of activities are going on after translation, that process, when protein is being synthesized in higher organism, higher cells, that post translational modifications are needed, are done, which is very much lacking in the prokaryotic system; because in higher system, more organelles and more complexities are there; more modifications, or updated reactions are going on, which is not there in the bacterial system; and, that is the reason why, sometimes, after successful expression, some problems we have to face, which is very much needed for proper biological activities of that particular product. Therefore, an inactive polypeptide have been, had to be isolated and refolded into proper structures of active proteins in-vitro, which can be difficult and expensive procedures.

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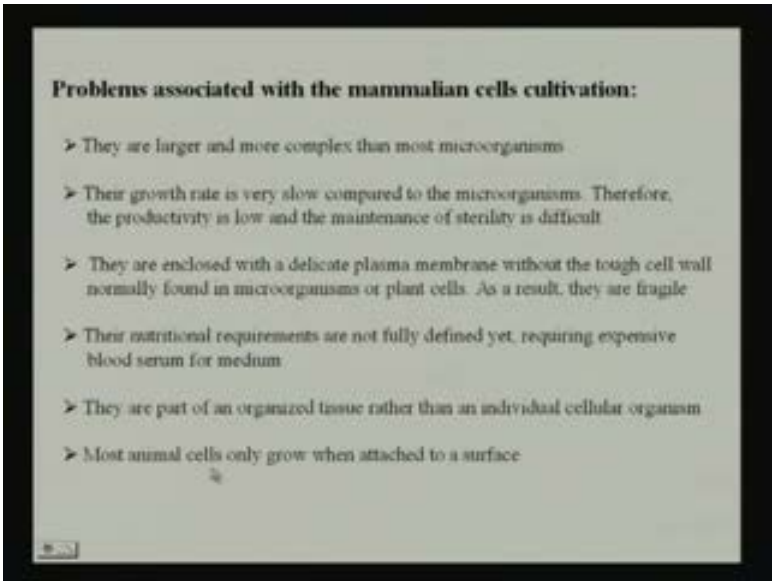

So, these are some of these problems and what are the actual problem associated with the mammalian cell cultivation process is that, if we see that cell cultivation, if we compare the mammalian cell with any microbial cell, we can find that, everything is different, starting from its architecture, to its behavioral properties, everything is different. Now, here, what we can see that, mammalian cells are larger and more complex than most of the microorganisms. Their growth rate is very slow compared to the microorganism. Therefore, the productivity is low and the maintenance of sterility is very, very difficult. This is one of the very important points, as far as animal cell culture is concerned.

Now, as they are growing in a very slow rate, we have to keep that particular, or we have to maintain that particular environment sterile, for a prolonged time. So, maintenance of sterility in that particular environment is a very difficult as far as the cell cultivation is concerned. They are enclosed with a delicate plasma membrane, without a tough cell wall normally found in microorganism and plant cells. As a result, they are very much fragile in nature. So, I have told you that, animal cells do not have any cell wall. So, as it does not have any cell wall, so, cell membrane is there; whereas, in microbial cell, we have learnt, the cell wall, cell membrane; in case of a plant cells also, cell wall is there. So, they are giving the extra protection and that protection is missing in case of animal cell; obviously, the handling of animal cell is very tough, because, because of its fragile nature; and that is the reason, why we are so much bothered about this animal cell cultivation process. The nutritional requirements are not fully defined yet, requiring

expensive blood serum for the medium; because we are mimicking the body condition. And, that is the reason why, this particular cell cultivation is so expensive. They are part of an organized tissue, rather than an individual cellular organism; most of the animal cells only grow, when attached to the surface.

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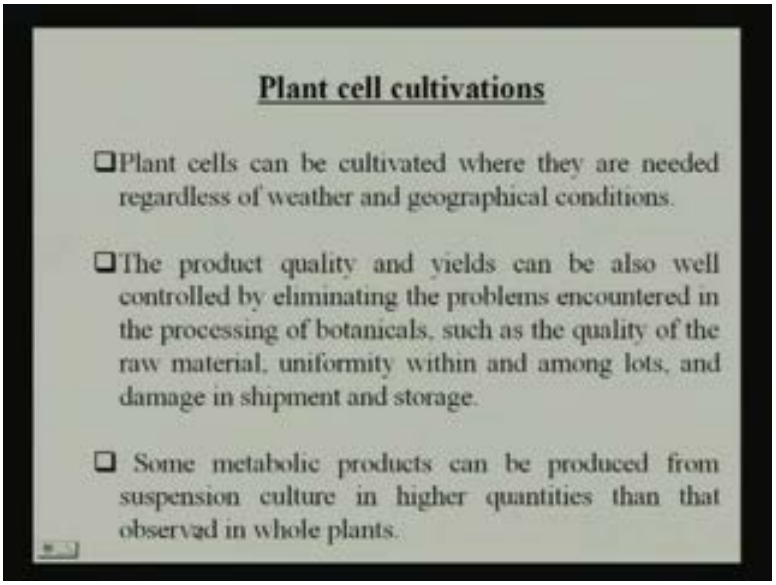

So, these are all about this animal cell cultivation. So, when we have compared the animal cells along with the plant and microbial cells, we have seen that, there is a significant difference the microbial cell and animal cell, mammalian cell and so on. Where we are considering the plant cell cultivation? Now, here, when we are talking about this plant cell, plant cell has got cell wall, which has got the similarity with the microbial cell; microbial cells also has got this, microbes, microbess has got cell wall. So, here, plant cells can be cultivated where they are needed, regardless of the weather and the geographical condition. The products, the product quality and yields, can also be well controlled by eliminating the problems encountered in the processing of botanicals, such as the quality of the raw material, uniformity within and among lots, and damage the shipment and storage. Some metabolic, metabolic products can also be produced from the suspension culture in higher quantities, than that is observed in the whole cell; and that is the reason, why we are going for this plant cell cultivation.

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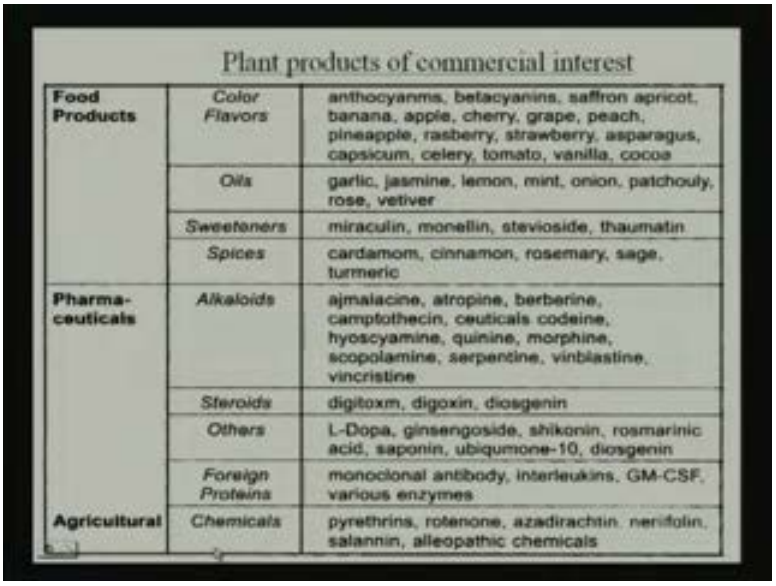

Now, here also, this same problem; when we are comparing the growth rate of the plant cell and any microbial cell, we generally find that, plant cells are growing in a much, much slower rate and maintenance of sterility is a big problem as far as animal cells, as well as plant cell cultivations are concerned. Now, if we see the different product which has got commercial interest, we can find that, the metabolites which are produced by the plant cells can be divided into two parts. One is called primary metabolites; another is the secondary metabolites. When we are talking about the primary metabolites, the production is of one type. When we are talking about the secondary metabolites, then only, we are going for the different types of cell cultivation, molecular, biological approaches and so on, or this macro propagation, and different techniques we are just talking about, for plant cell cultivations too.

Now, here, when we are talking about this food products, it maybe colors, flavors, it can be oils, it can be any sweetener, it can be any spices, that can be produced through plant cell cultivation. When we are talking about the pharmaceuticals, we are talking about the alkaloids, we are talking about steroids; we are, we are also talking about some foreign proteins and some other biochemicals, which has got immense application as far as today's world is concerned.

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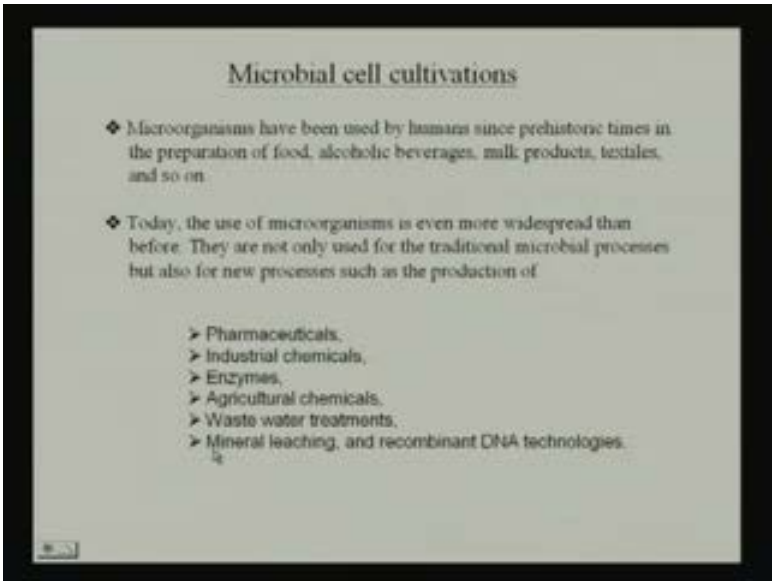

When we are talking about the agricultural sector, agro chemicals are playing a significant role, which can also be produced through plant cell cultivation. And here, I have given you some of the names, which are symbolic to this, plant tissue culture products. There are n number of such products, which can be produced, which are being produced and isolated and getting, and, and is applied, as far as today's modern world is concerned.

Coming, to the microbial cell cultivation, now, why I am talking about all this cultivation, this system, because, until and unless we know the plus point and the negative point of each and every system, it will be very, very difficult for us to choose the actual system for metabolite production; and until and unless we are selecting the system, we cannot go for the large quantity production of the metabolite, which is of our own interest. Now, when we are talking about this metabolic microorganisms as the means for cell cultivation, then we can find that, microorganisms have been used by humans since prehistoric times in the preparation of food, alcoholic beverages, milk product, textile and so on. Today the use of microorganism is even more widespread than before. They are not are only used for the traditional microbial processes, but also for new processes, such as production of pharmaceuticals, industrial chemicals, enzymes, agricultural chemicals, waste water treatment, mineral leaching, and recombinant DNA technology.

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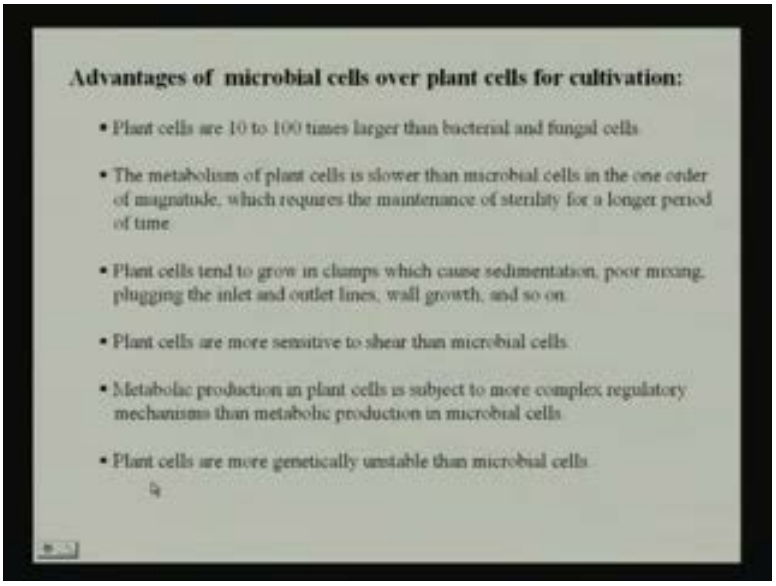

So, for any such, any products, which we have already learnt in today's class, can be possible through microbial intervention. And, that is the reason, why microbes or microbial system is so popular. Now, if we see the advantages of this microbial cells over the plant cell cultivation, then, we can find that, this plant cells are 10 to 100 times are larger, than bacterial or any fungal cell; that means, obviously, size is more; it is eukaryotic; bacterial or fungal system, it is mostly, this bacteria is prokaryotic in nature; and, if we see the organizational development, though fungus is coming under eukaryotes, but, their development is not that developed, compared to the plant cells.

Now, the metabolism of plant cells is slower than the microbial cells, as I have already mentioned you earlier; in the order of magnitude which requires the maintenance of sterility for a longer period of time, that I have already mentioned you. Cell culture tend to grow in clumps, which cause sedimentation, poor mixing, plugging of inlet and outlet line, cell wall, that wall growth and so on. So, it takes time; and maintenance of sterility is also very, very important as far as this plant cells are concerned. And, there should not be any microbial contamination, or any other contamination during this cell cultivation process. Plant cells are more sensitive to shear than the microbial cell; those cell walls are there in case of plants. Metabolic production in plant cells is subject to more complex regularity mechanism than metabolic production of microbial cells. Plant cells are more genetically unstable than the microbial cell, and that is the reason, why every time, the scientist, they are taking the microbial cell as one of the means to express, either animal

sources gene, or plant resource, the gene from plant, plant resource and expression media which is selected, is the microbial system.

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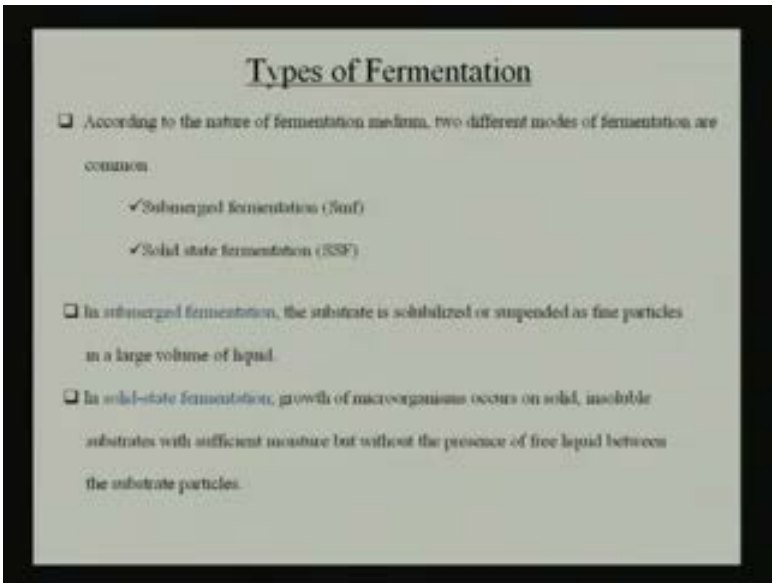

Now, when we are going for the cultivation of this microbes, so, that is the reason, the reason I have justified that, why microbes are generally considered for gene expression. Now, cloning is successfully done, but expression is not there; that system cannot be considered for further metabolite production. So, if we can successfully clone and express that particular metabolite in the microbial system, then only, we can go for, we can consider, that particular system for that metabolic production, or metabolite production. For this, if we are selecting any microbes, then, we are generally going for the fermentation system.

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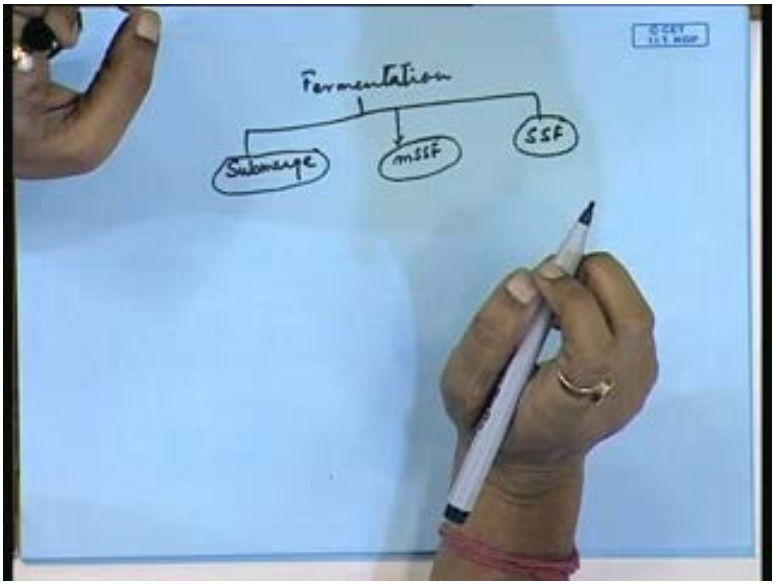

Now, in this fermentation system, we are, if we see, the entire fermentation system, then we can divide the fermentation process into two major categories. One is the submerge fermentation; another is the solid state fermentation. Nowadays, different types of fermentations are coming to picture. Some of these fermentations are called modified solid state fermentation; that means, whatever is this type of fermentation, some modification has been done and it is coming under this modified solid state fermentation. Now, what is submerge fermentation and what is solid state fermentation?

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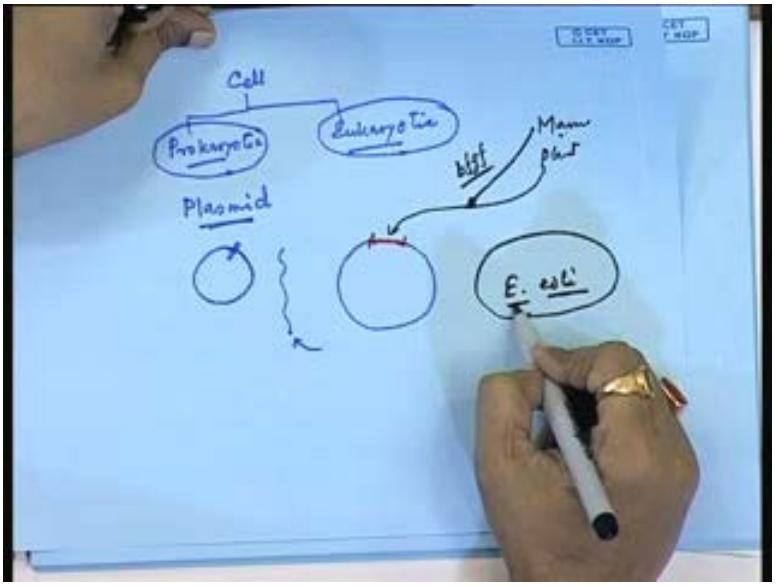

Now, suppose, we have taken a particular gene from a mammal, and from the, from that gene, or any plant, whatever maybe the sources, and we have taken that gene, and we have cloned it in a bacterial, in a plasmid DNA; and we express that thing in any bacterial system, say for example, E coli, is one of the example. So, suppose, we had taken that particular gene and we had expressed it for any metabolite production. Now, suppose, if it is from the animal origin, so, suppose bfgf, that basic fibroblast growth factor; so, suppose we, and this is the mammalian, that animal, this cell cultivation and it is a growth factor, and we can, we have taken this gene from the parent tissue, and we have expressed that thing in E coli; and we have seen that, this particular product is successfully getting expressed in the bacterial system. Now, when we are going for the large scale production of this particular metabolite, we are selecting the fermentation system.

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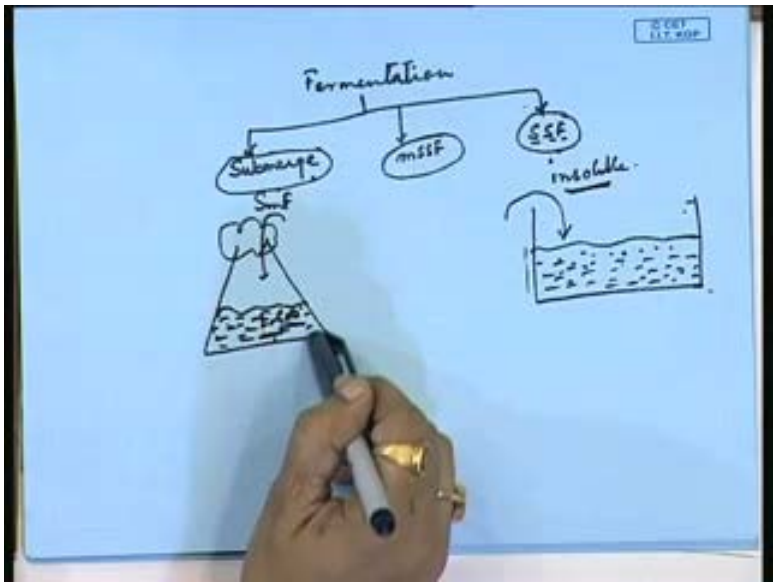

Now, when we are going for this fermentation system, we are just taking this particular, we know that, these are the different types of fermentation; one is the submerge; another is the solid state fermentation. So, this e coli can be grown in different system. What is submerge and what is solid state fermentation?

Now, we have to have some idea about this, what is submerge fermentation and what is solid state fermentation; then only, we can go for any modification, that is modified solid state and so on. Now, what is solid state and submerge fermentation? In submerge

fermentation, the substrate is solubilized, or suspended as fine particles in the large volume of liquid; that means, if we are considering that conical flask as one of the media, and here, if we are taking some of this media, liquid component of the media, and if we are just inoculating our, this E coli to grow in this, then, we are just calling it, when excess liquid is there; the media is liquid, that is called submerge fermentation; that means, everything is inside the surrounding liquid. When there is no free flow liquid within the system, fermentation area, medium, that, then we are calling it as solid state fermentation. In solid state fermentation, the major difference is that, here, the media component, or the constituents which are there, is in the soluble form. Here, we are taking insoluble biomasses, which are considered to be the, the, the substrate for the growth of the organism, and there is no free flow liquids are there.

So, if we are considering the tray as the bioreactor, then here, we are compiling the insoluble substrate and then, we are inoculating the microorganism to this, and then, we are just growing the microorganism and there is no free flow liquid. So, obviously, the aeration and whatever is there, through the surface diffusion; and here, as this is the liquid medium, we can take any type of vessel, any type of agitator, or any type of oxygen (( )) system, and with every control, we can go for the production of our metabolite through the submerge fermentation. So, here, we had the different types of different control system, and here, the oxygen transfer is mainly taking place through the diffusion process.

Now, here, some other problems which are associated with the solid state fermentation is that, when we are inoculating the microorganism to this insoluble substrate, the most of the reactions are exothermic in nature. Now, when this exothermic reactions are going on within the system, then, what we are find that, lot of heat is generated within this system; when heat is generated, then, temperature rise within the bed is taking place. Sometimes, it has been seen that, from the top layer to the bottom layer, a gradient of temperature which may exceed to 20 degree. Now, here, when such rise of temperature is there, the biochemicals which are being secreted, which are being produced by these living cells, microorganism, are getting denatured; because, it has been seen that, most of the biochemicals are heat sensitive. And, that is the reason, sometimes we have to have some water circulation, or some forced aeration, to move, remove the heat which is being

generated within this particular system. Now, these are some of the advantages and disadvantages of this submerge fermentation and solid state fermentation.

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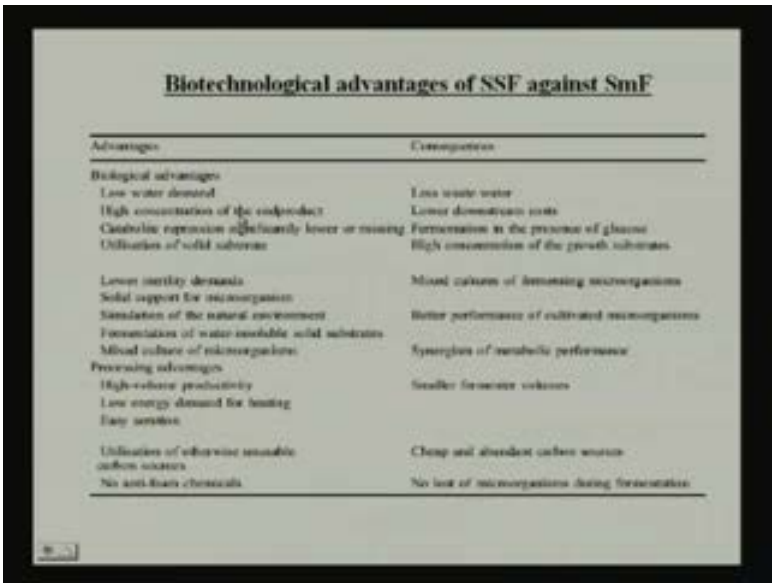

Now, here, if we see the advantages of solid state fermentation over submerge fermentation, then, we can find that, there are huge advantages of solid state fermentation, though it is suffering from some problems; but, if we compare the solid state fermentation and the submerge fermentation, then, we can see that, it is the low water demand, because no excess free flow liquid is there in this particular fermentation system, and obviously, less water, waste water is produced; high concentration of the end products are there, because here, whenever the metabolites, they are secreting the products in this particular media, it is remaining along with this insolubles; but here, as this is the liquid, this liquid, this, whatever is the metabolites, this metabolites are getting diluted, and diluted within the entire volume of this liquid; and that is the reason, why we have this dilution problem is so high, in case of the submerge fermentation system.

Now, here, if we see that, catabolite regressions significantly lower, or almost missing, in case of this solid state fermentation. Utilization of solid substrate is also there. We can use biomass as one of the raw material for any metabolite production; that means, this metabolite production can be the sources, is of cheaper, lower sterility demand, solid supports for the microorganisms are there; simulation of the natural environment is also there; fermentation of water insoluble solid substrates are generally taken as the raw

material; mixed culture of microorganisms can also be attempted for better productivity of the product. That processing advantages if we see, then, we can find that, high volume productivity; that means, smaller fermented volume is giving the high productivity. Low energy demand for heating, easy aeration, utilization of otherwise unstable carbon sources are also there, which are cheap and abundant carbon sources are being utilized in this process. No antifoam chemicals are to be added, because, no liquid is there; foaming problem is not there, which is very, very means, probable problem in case of the submerge fermentation.

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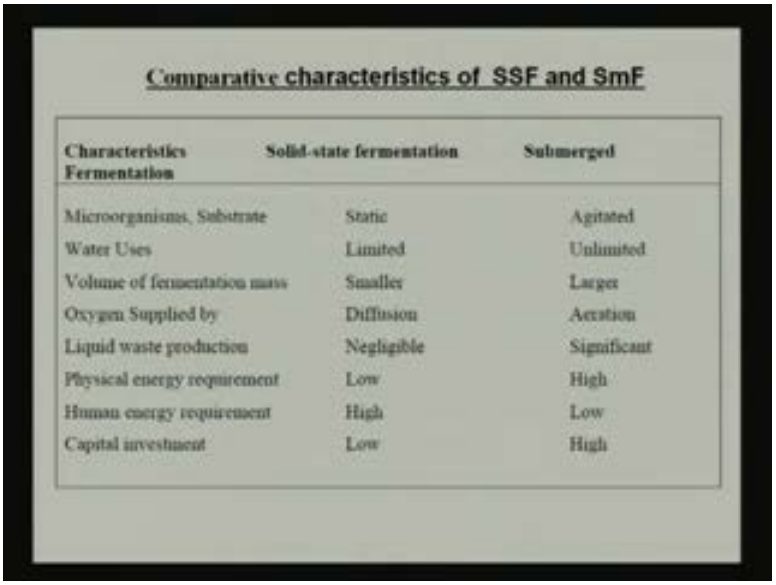

If we see the characteristics of solid state fermentation and submerge fermentation, then, further, we can divide the two processes like this. It is a static process, this one is the static process; we can go for agitation through, if we find that, the system is aerobic in nature. Water uses in this particular solid state fermentation is limited, and in case of submerge fermentation, the water use is unlimited. If we see the volume of fermentation mass, this, it is, in case of solid state fermentation, it is smaller; in case of submerge fermentation, a huge quantity of liquid handling is there. Oxygen supply, I have already told you that, through diffusion, and here, in case of submerge fermentation, it is through aeration. Liquid waste production is almost negligible in case of solid state fermentation, and in case of submerge fermentation, it is significant. Physical energy requirement is low in case of solid state fermentation, and in case of submerge fermentation, it is very high. Human energy requirement is, in case of solid state fermentation, is high, whereas,

in case of submerge fermentation, it is low. Capital investment is low in case of solid state fermentation, whereas, submerge fermentation, it is high.

Now, if we see, here, as this media is liquid, we can easily go for automation and with all mechanical means, we can use with the machines; but, here, as I have told you that, this process is being carried out through this solid state fermentation and here, the tray type of fermented and this here, this system mechanic, the mechanization of this particular system, is very, very difficult, and that is the reason, here, human intervention is very very needed; that is the reason, why manpower requirement, in case of solid state fermentation is so high, compared to the submerge fermentation. This is one of the disadvantage, disadvantages, what I have till now discussed. But, lot of advantages are there, on this solid state fermentation over the submerge fermentation.

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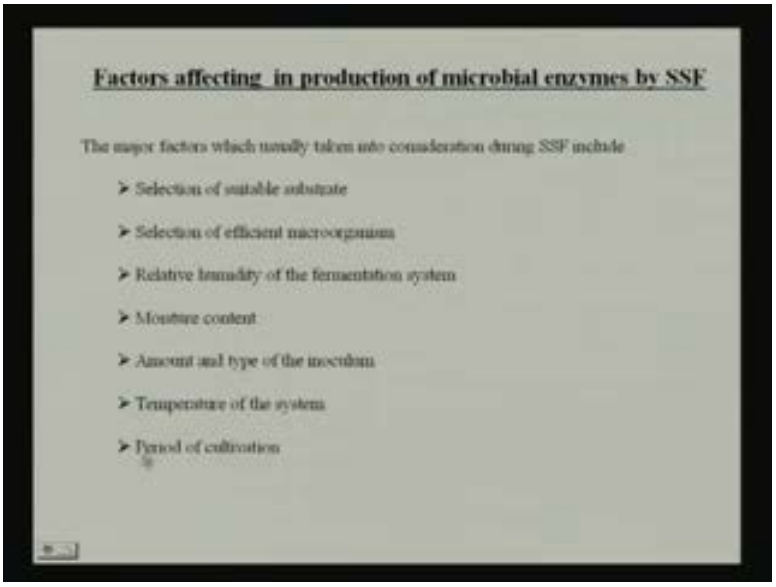

Now, if we see the fermentation, overall fermentation system, irrespective of solid state or submerge, we can find that, there are very many factors, which are playing a significant role, as far as this particular fermentation is concerned. Now, selection of suitable substrate, selection of efficient microorganism, that means, selection of microorganism, relative humidity of the fermentation system, moisture content, amount and type of inoculums which are used for this particular system, temperature of the system, production, that period of production, or the incubation time for the products

synthesis, these are some of the very, very important parameters, which are playing a significant role, as far as the fermentation is concerned.

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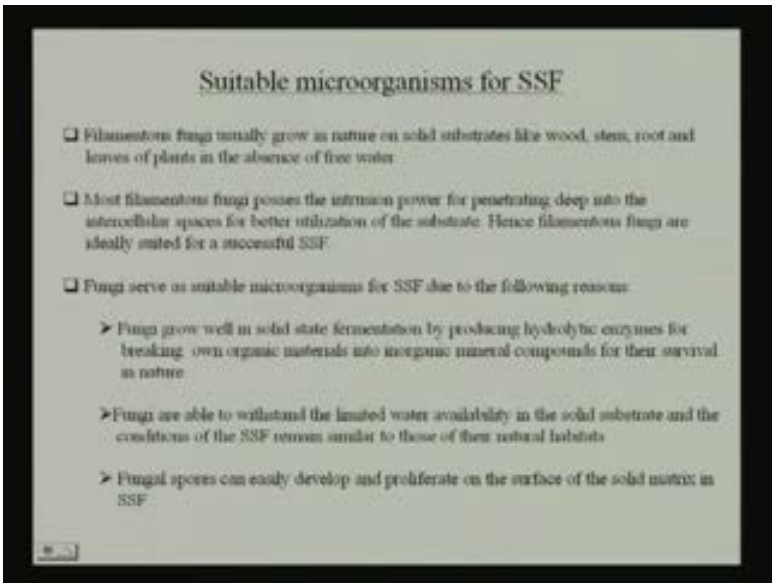

Now, if we see the suitable microorganism for a solid state fermentation, then, we can find that, in most of the cases, if we see the architecture of the bacterial cell, these are unicellular in nature; but, if we see the fungal cell, they are thread like hyaline structure; and this hair like structure, they are getting cracked, or it forms a lump when it is coming in contact with this liquid media. Sometimes, when, and this, this, this hair like structure of this fungus are so delicate, once, with very minute handling problem, there there that thread or the hifi may get cracked; once this hifi is getting cracked, all the intracellular materials are coming out of this cell and as we have already discussed, that lysozyme is there within the cell, and when lysozyme is there, lysozyme is one of the product which is coming out of the cell to this surrounding medium; and when this surrounding medium, this lysozyme is coming, it start damaging the intact cell; because that is the suicidal enzyme, and it damage the total cell. And, that is the reason why, while culturing the filamentous fungus, generally solid state fermentation is mostly preferred.

Now, here, this, this fungi, they serve a suitable microorganism for solid state fermentation, because of the following reason. Fungi grow well in solid state fermentation by producing hydrolytic enzyme and breaking their own organic material into inorganic minerals, mineral compounds for the survival in nature. Fungi are able to

withstand the limited water availability in the solid substrate, and, and the conditions of solid state fermentation remains similar to those of the natural habitat. Fungal spore can easily develop and proliferate on the surface of the solid matrix, in solid state fermentation.

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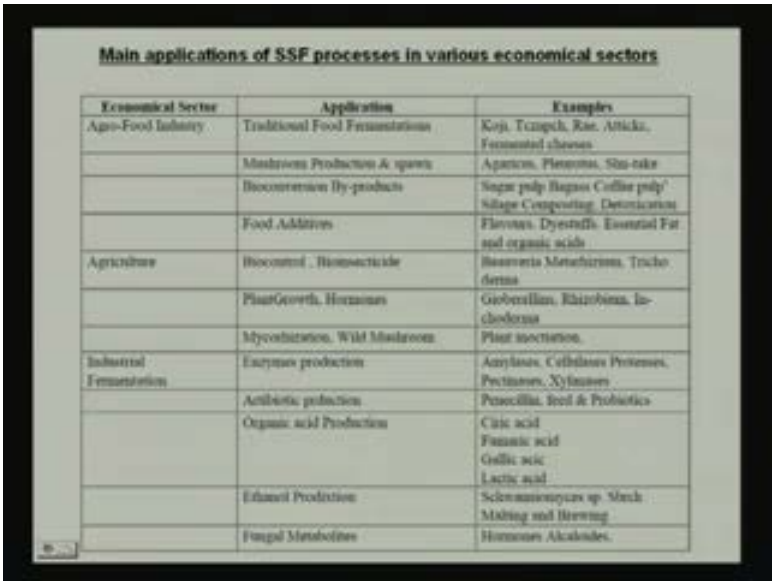

Now, these are some of these, this advantages, or the, the, the characteristics of some of these microorganisms, which are forcing us to select such type of fermentation system. And, when we are handling the filamentous fungi, mostly solid state fermentation system is selected. Now, this particular, this, this particular solid state fermentation is otherwise called as Koji fermentation.

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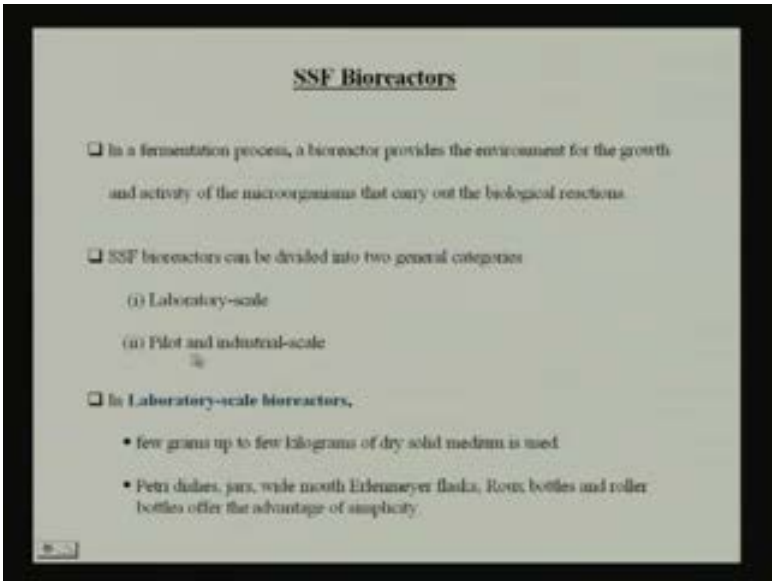

Now, when we are talking about the solid state bioreactor, now, here, for large scale production of such type of things, in the small tray, we can start, in small beakers or the Petri plate also, we can consider those things as the reactor, that tray type of reactor and we can start our work; and, when we are going for the large quantity, then, we are going for the tray type of reactor. Now, in the laboratory, we are just starting it in a laboratory scale, with the small tray, and when we are going for the pilot and the industrial scale, then, I told you that, number of trays are getting increased, for the increased amount, bulk production of the metabolites.

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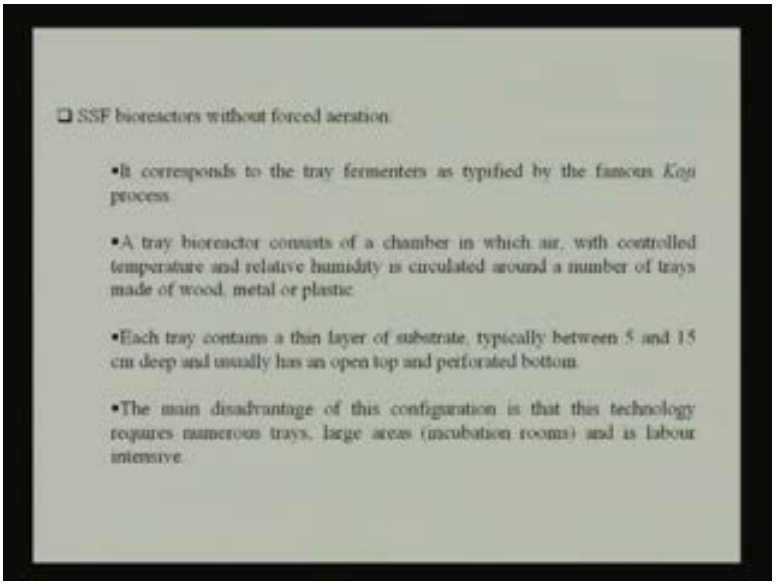

Now, when, in case of solid state fermentation, we are considering, it is without forced aeration is there. It corresponds to the tray fermented as typified by the famous Koji processes. A tray bioreactor consist of a chamber, in which the air is there with controlled temperature, and relative humidity is circulated around the number of trays made up of wood, metal or plastic. So, as I have told you that, as the metabolite, during metabolite production, lot of heats are, heat is being generated; to reduce the temperature, that water circulation is preferred. Each tray contain a thin layer of substrate, typically between 5 to 15 centimeter deep, and usually has an open top and perforated bottom. The main disadvantages of this configuration is that, this technology requires the numerous trays, large area, and it is labour-intensive; and that room which is there, that, in that particular room, the controlled temperature and humidity, both has to be maintained.

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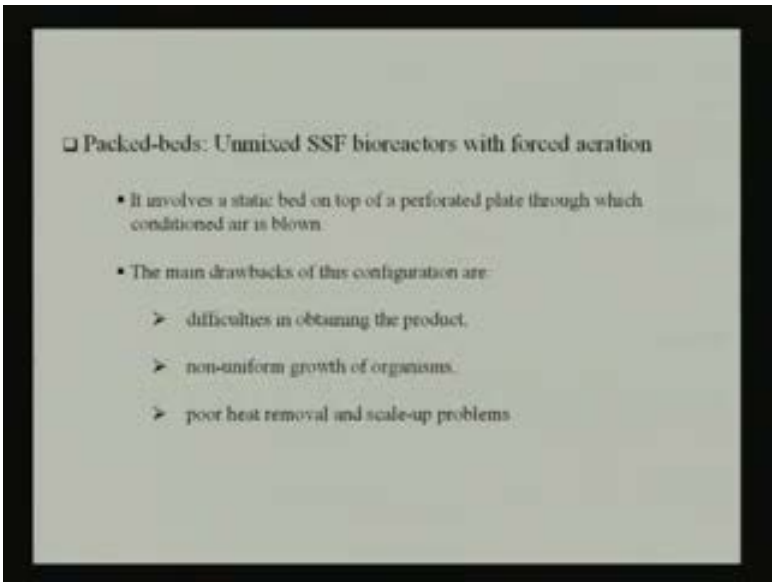

Now, when we are talking about the packed-bed unmixed solid state fermentations bioreactor, it involves the static bed on top of the perforated plate, through which the conditions air is blown. The main drawback of this configuration is that, it is difficult in obtaining the product; that means, leaching out the product is difficult; non-uniform growth of the microorganism is also there; poor heat removal and scaling up problem are associated with this.

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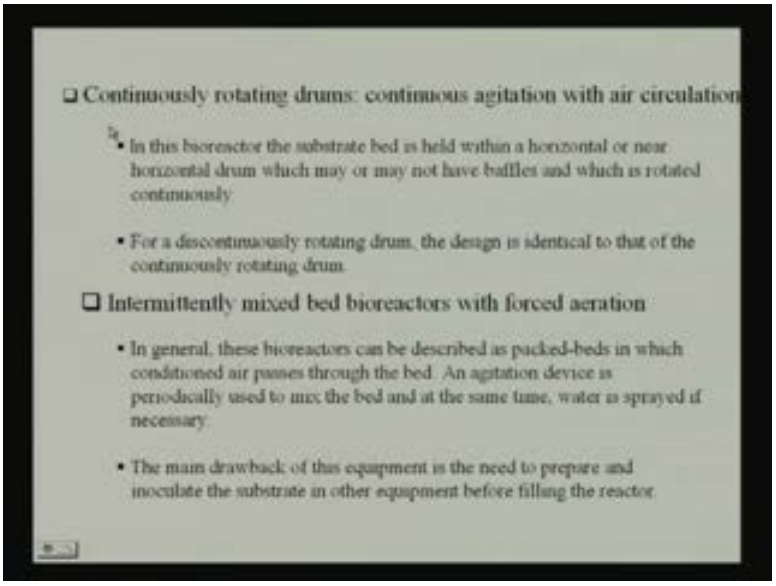

Now, when we are going for the continuously rotating drum and intermittent mixing bed bioreactor with forced aerations, we can overcome some of the problems associated with this type of static process. Now, in this rotating drum, drum is getting rotated in a very slow speed, and fermentation within that insoluble matrix are going on, and it is getting that rotation, and it is getting the aeration, and surface area also, it is getting throughout the drum, and that with a very low speed, the substrates are moving, and this, your, in a, in a horizontal drum, where, which may not have baffle, and which is rotated continuously, for such type of product formation. For a discontinuously rotating drum, the design is identical to that of a continuously rotating drum. Now, here, when we are going for the mixed bed bioreactor with forced aeration, now, here, we are just giving the aeration, forced aeration to drag the heat out of this particular bed.

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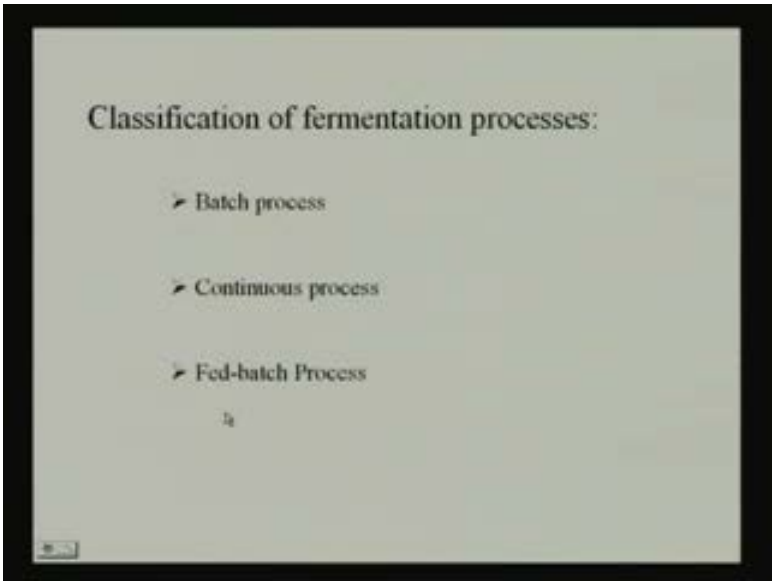

And, when we are going for this classification of this process, we can go for the batch process, continuous process, fed-batch process and so on. Now, in case of this solid state fermentation, fed-batch process, continuous process, is very, very difficult to maintain, for which, we can switch over to the submerge fermentation.

Now, when we are going for this submerge fermentation, this submerge fermentation is totally different from that of the solid state fermentation. Now, here, in case of continuous fermentation, one group of tray is entering, and one group of tray is going out; continuously there is a flow, and with a certain incubation time, that trays are going out continuously, and that is the continuous fermentation process. But, independent trays are being handled, if irrespective of whether, it is a continuous, or it is a batch process. In case of submerge fermentation, here, the control system is totally different from that of this solid state fermentation. So, in our next class, we will be discussing on the solid state, that is, a submerge fermentation and how this submerge fermentation is being taken care of by different types of reactor, for the production of different biochemicals of our own interest; we will learn that. Thank you very much.