Biochemical Engineering

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Module No. # 01

Lecture No. # 38

Manufacture of Biochemicals (Contd.) And Strategies for Biomolecules Separation

Students, so, in continuation of my last lecture, I would like to tell you that, in case of solid state fermentation, we have already seen that, in that particular fermentation medium, no free flow liquid was present. Now, here, when I had discussed about this fermentation, entire fermentation process, I have told that, the fermentation can be divided into two major groups; one is the submerge fermentation; another is the solid state fermentation. Now, when we are talking about the modified solid state fermentation, then, we have done some modification to the traditional fermentation process; that means, while yesterday, when I was discussing about the solid state fermentation, I have already mentioned you that, that heat generation is one of the major problem, in case of solid state fermentation. To overcome that particular problem, what the scientists, they have done, they have modified the reactor design in such a way, that instead of putting it inside the tray…What I have told you that, perforated trays are needed to dissipate the heat generation.

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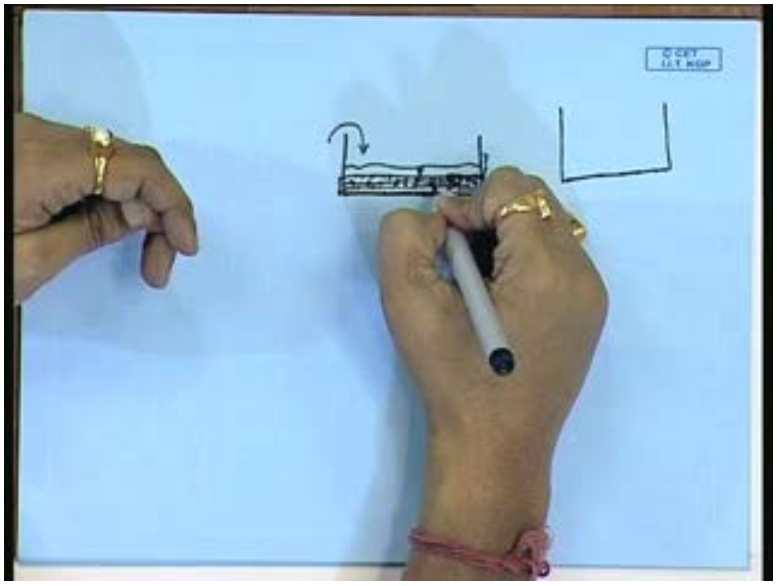

Now, in modified solid state fermentation what is done, the reactor vessel is constructed in such a way, that in this, this is the solid base pair and to which some shapes are, weird shapes are kept. Now, when it is kept, this shapes has got some pore size, definite pore size and, or, maybe the cotton wool; that shapes are there, to which the solids are kept. In the bottom, what it is done, the liquid nutrient medium is kept.

Now, when it is kept, when it is the liquid medium is there, this liquid medium has got a constant touch with this porous bed, to which the solid substrate is kept. Now, during fermentation, when heat generation is taking place within this bed volume, as this particular bottom side is continuously in touch with this liquid medium, continuously liquid is coming in contact with this bed, and heat is coming and getting absorbed to this bottom liquid. And, so, the heat generation within the bed is taken care of; it is controlled by the liquid which is there, present in the bottom of this reactor. And here, it has been seen that, with this type of modifications, the productivity, or the yield of a certain targeted product is enhanced many fold; and that is the reason, why it is called the modified solid state fermentation. Now, in case of yesterday, what I had told, that in the tray type of reactor, this is the perforated tray, which is present and then, we are doing, we used to do traditionally, the aeration, air passing, air circulation was done.

So, instead of doing that, this modification has been done to this tray type of reactor, and that is called the modified solid state fermentation. Now, here also, some problems are

there; that is, a handling problem; no, that problem of automation of this type of system. So, it is considered that, though there is no thumb rule, there is no definite, that, processes, which is the definite that a, what to tell, that, there is no such, there are definite rules for selection of any biological, or any microbiological processes; but, if we are handling any filamentous fungi, then, we are generally selecting the solid state, or modified solid state fermenter; and, if it is a bacterial system, in general, then, we are going for the submerge fermentation; as because, filamentous fungus is a thread like structure, and yesterday, I have told you the problems associated with that type of organism handling. And, as bacteria is unicellular in nature, and bacteria has got its cell wall and cell membrane within it, and it is very easy to handle, to produce the metabolite in the submerge type of fermentation.

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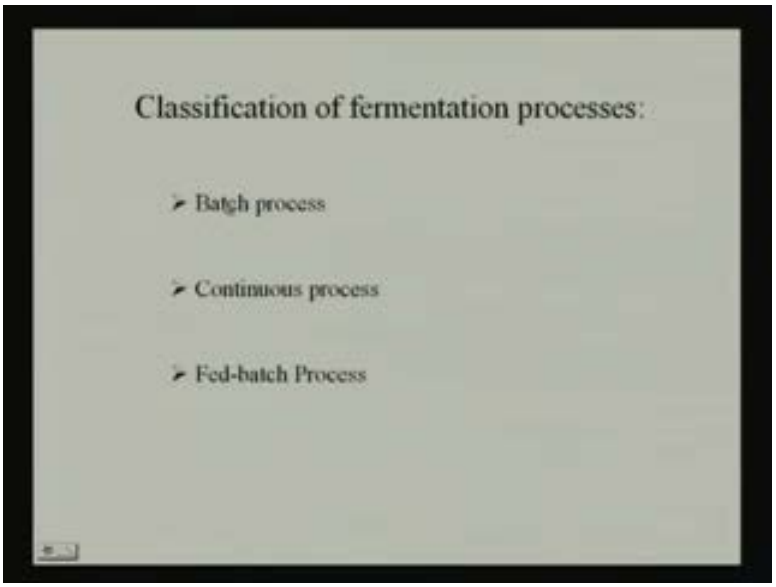

Now, when we are going for these submerge fermentation, then, we can classify this submerge fermentation into the batch process, fed-batch process and the continuous process.

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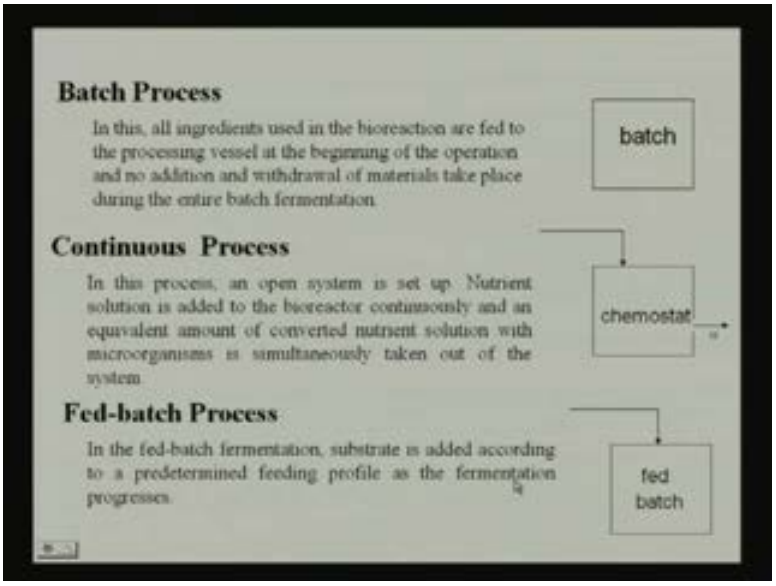

Now, what is the batch process? This batch process is that, in this particular system, all ingredients used in this bioreaction are fed to the processing vessel at the beginning of the operation, and no addition and withdrawal of materials take, take place during the entire batch fermentation; that means, whatever material we are just keeping, after the run is over, fermentation is over, the entire batch is getting dismantled and entire volume of liquid is taken out of this system; and this is the batch process.

While coming to this continuous process, what we are doing in this process, an open system is set up; nutrient solution is added to the bioreactor continuously and an equivalent amount of converted nutrient solution with microorganism is simultaneously taken out of the system; that means, if nutrient, this nutrient is continuously coming and continuously, your product, or the converted, that, that after biotransformation, after fermentation, that fermented broth is continuously going out of this system. The inflow rate and outflow rate is at a constant level, so that, the entire volume is maintained at a constant level. And here, the flow rate is in such a way that, the nutrient is getting the entire time of fermentation, conversion time, that nutrient, they are getting to convert to the desired product. In case of batch, fed-batch process of fermentation, substrate is added according to the predetermined feeding profile as the fermentation progress and this is the fed-batch type of fermentation system.

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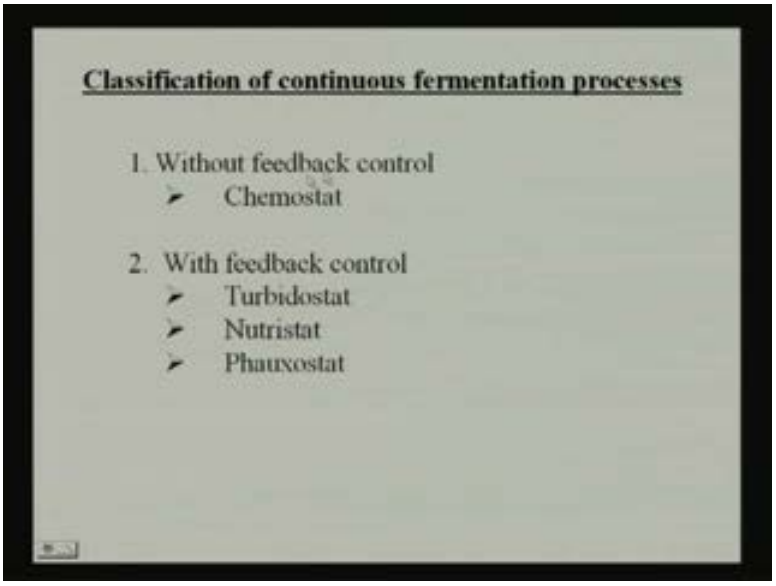

Now, if we see the continuous fermentation process, the continuous fermentation process can once again be divided as without feedback back control and with feedback control. Now, when we are coming to this without feedback control, we are mainly considering the chemostatic model.

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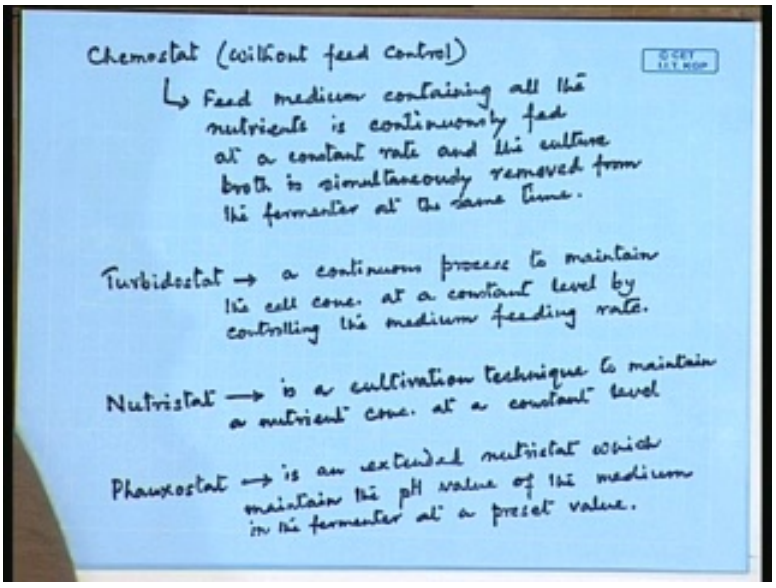

Now, here, with the chemostatic model, when we are considering this, the feed medium is containing all the nutrients is continuously fed at a constant rate and the culture broth is simultaneously getting removed from the fermenter at the same time; that, what I have

told you, the feeding rate, the, when the, the the, nutrient is coming inside this reactor and when it is going out, the flow rate of this inlet and outlet is maintained at a constant level, so that, that entire volume is getting maintained. Now, when we are coming to this with feedback control, it can be divided into three major group; one is called turbidostat; another is the nutristat; another is the phauxostat. Now, coming to this turbidostat, it is a continuous process to maintain the cell concentration at a constant level by controlling the medium, the feeding rate of the medium. So, this is the turbidostat. When we are coming to the nutristat, it is the cultivation technique to maintain the nutrient concentration at a constant level.

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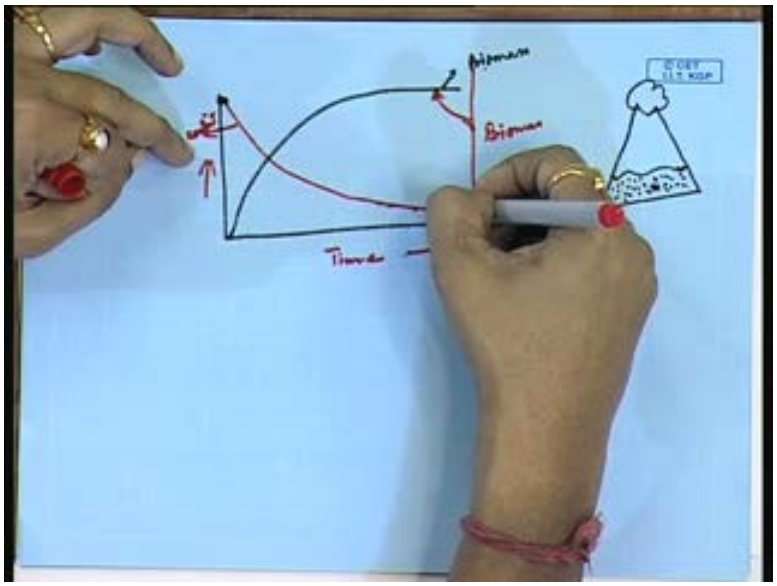

Now, what is happening, when we are talking about the different type of fermentation process, in case of batch process, what is there? When we are just taking any medium and we are inoculating with the live cells, then, fermentation, and we are just incubating, then, fermentation starts. Now, when fermentation starts, we are just expecting that, after a controlled time of fermentation, we will be getting our desired product. Now, in reality, inside the reactor, what is happening? Now, when we are giving this carbon, nitrogen, all this basic nutrients which are needed for this particular biological product formation, then, it is in the maximum that, at 0 minute, that it is the nutrient, carbon content of this media is 100 percent. Now, when it is, that inoculation is taking place. Now, when we are just inoculating this particular conical with some, some inoculums; this inoculum, that live cells are coming, and they starts their normal activities. When they will start

their normal activities, they will start consuming the carbon and nitrogen for their cell maintenance, for their growth, for their metabolic activities.

So, what is happening, when this metabolic activities of the cell started inside this reactor system, the nutrient, carbon and nitrogen and other minerals which are there inside this reactor, is continuously being uptaken by this microorganism; as a result, what is happening, the number of bacteria, or number of cells, or biomass concentration is gradually increasing, and carbon, nitrogen content of that medium, is gradually getting depleted. So, if we plot both these activities, then, how we can plot that; this way the biomass growth is taking place; this is the biomass growth, because media is very rich in nutrient; and, when we are considering the, the the carbon content of this medium (( )), we find that carbon content is continuously getting depleted. So, this is the carbon, that nutrient content. Now, after, after a certain time period, what is happening; when carbon concentration is gradually getting depleted, this microbial cells which are grown and present inside the reactor, is not getting sufficient nutrient; and, and, if this is the biomass and this is the carbon content. So, if this is the, if this is the graph, then, we can find that, after a certain time, there we there is a crises of nutrient inside the medium.

Now, in case of nutristat, it is a cultivation technique to maintain the nutrient concentration at a constant level inside the reactor, so that, there will, there will not be any crisis of the nutrient and cells will be proliferating; cells will be doing their normal activities. In case of phauxostat, what is happening is, it is the extended nutrient which maintain the pH value of the medium in the fermenter at a preset value. There is, there should not be any change in this particular pH, when the cells start proliferating. And, these are some of these continuous fermentation process.

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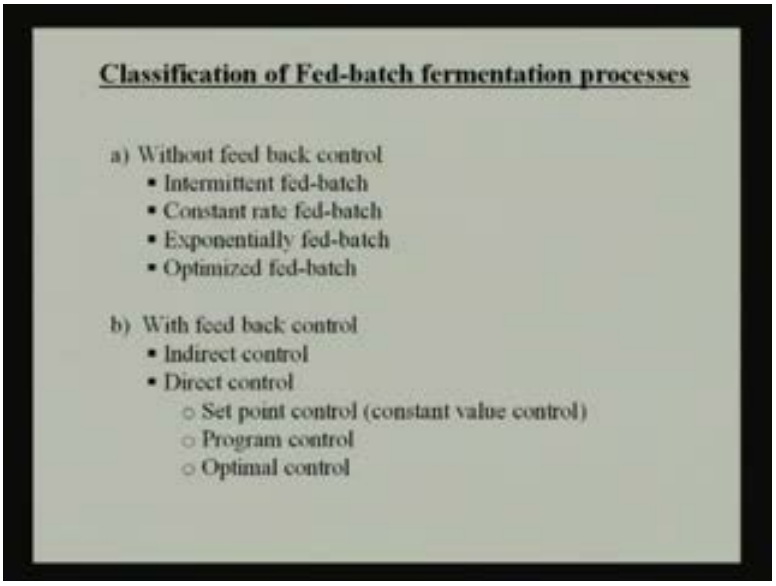

Coming to the fed-batch fermentation process, it is also without feedback control and with feedback control. When we are going for this feedback control, we can see that, it is intermittent fed-batch, it is constant rate fed-batch, exponentially fed-batch and optimized fed-batch. When we are further classifying to this with feedback control, then, indirect control and direct control; when we are, we are going for the direct control, then, we can go for the set point control, that is, constant value control and program control and optimal control.

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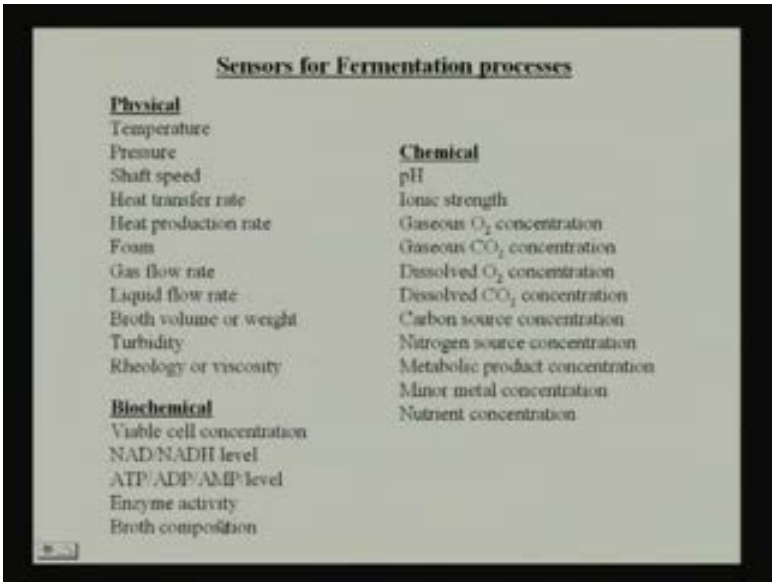

So, these are some of the technique, which I think, you have already learnt in the bioreactor design system. Now, here, this particular reactors, with all control systems are used for the manufacture of biologicals from any living cells, irrespective of the whether it is plant, animal, or microbial. Now, when we are going for all control system, the sensor is playing a significant role to sense a particular, a particular parameter. Now, here, in this particular, if we are coming to this sensor, then, we can divide it into physical sensor, chemical sensor and biochemical sensor. Under this physical sensor, we can go for this temperature sensor, pressure, shaft speed, heat transfer rate, heat production rate, foam generation, gas flow rate, liquid flow rate, broth volume or weight, turbidity, rheology or viscosity sensors which are there, and these are the parameters, which is influencing the product generation can be sensed in, with this automated reactor system.

When we are going for the chemical sensor, we can measure the pH, just I, as I have discussed about the phauxostat, the pH should be maintained throughout the reactor, throughout the reaction time, constant. So, when we are going for such type of fermentation, that means, there, there should not be any change in the pH. So, with the sensor, that, when it is, we are sensing that, there is a drop or increase in the pH, accordingly, the acid or alkali or buffer system, whatever maybe is kept, is added drop-wise to maintain the pH of that fermentation system. Now, here, besides this pH, ionic strength, gaseous oxygen concentration, gaseous carbon dioxide concentration, dissolved oxygen, dissolved carbon dioxide, carbon sources concentration, nitrogen sources concentration, metabolic product concentration, minor metal concentration and nutrient concentrations are playing a significant role, as far as this fermentation in the submerge and with controlled system is concerned. When we are going for the biochemical sensor, we are talking about the viable cell concentration. This is one of the very important parameter, as far as the biological product is concerned.

Now, here, suppose, we have inoculated our reactor system with the inoculums; but, we do not know what is the percentage of living cell and the dead cell. If the percentage of dead cell is present there, that is the waste; it does not have any role in the product formation. So, here, in some sensor are there, which, through which, we can go for the viable cell number or the concentration in the reactor system. We can also sense the NAD/NADH level of some of this particular cells; ATP ADP AMP levels are also can be

evaluated through the sensor; enzyme activity and broth composition can also get sensed with the sensor, which are already available and used in the fermentation system. Now, please note it that, whatever I am talking about this particular system are all aerobic process.

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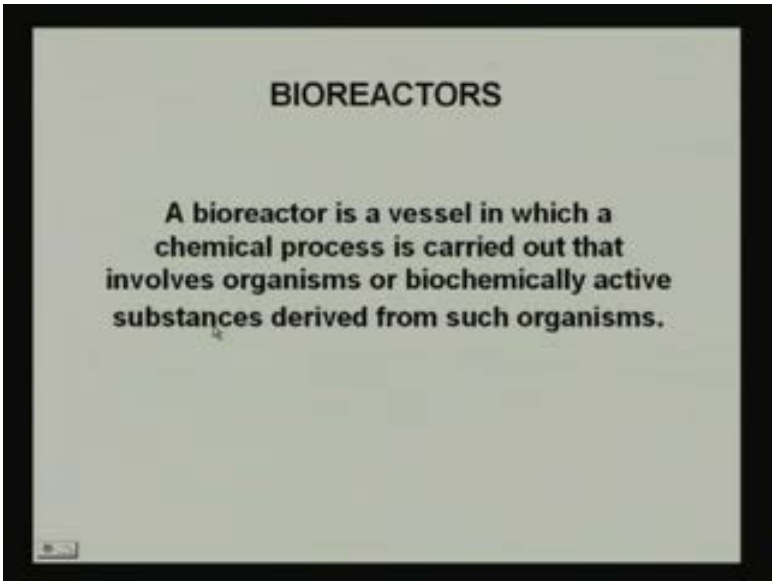

Now, when we are talking about the bioreactor, then, bioreactor is a vessel in which the chemical process is carried out, that involve the organisms, or biochemically active substances derived from such organisms.

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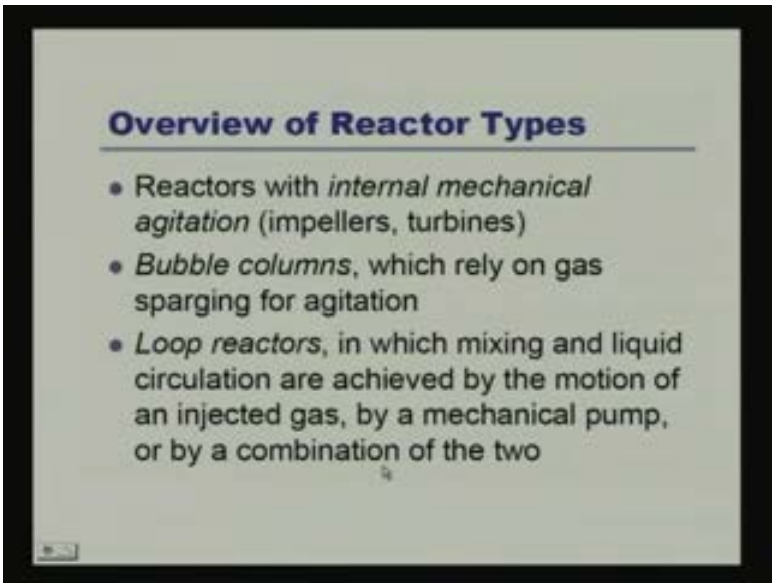

So, when we are going for that type of reactor selection, then, reactor with internal mechanical agitations, that is, impeller or turbine type of things are there; sometimes bubble columns, that is, which rely on the gas to sparge for agitation and sometimes, we are looking for the loop reactor, where we can tell that, this here, this mixing of the liquid circulation are achieved by the motion of an injected gas, by a mechanical pump, or by a combination of the two.

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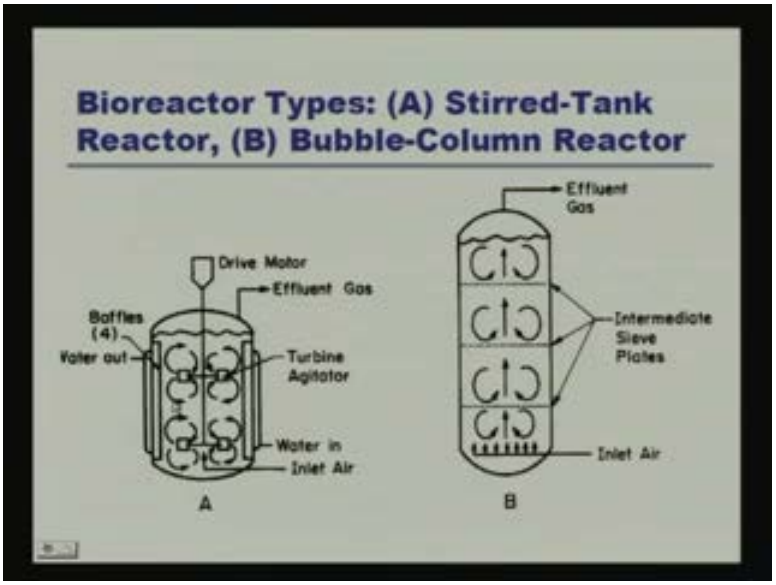

So, these are some of the configuration of the different types of reactor and I think, you have already learnt those things in some other classes, where you have gone for the engineering aspect of this particular reactor system. Here, today, I will be discussing the biological part of this, means, how this living cells are growing and what is the importance of each type of reactor, as far as the living cells are concerned. Now, here, when we are talking about the bioreactor, we mainly talked about the stirred tank reactor, or bubble column reactor, or some other type. So, here, the stirred tank reactor if we see, here, the main objective is that, aeration is there and we are just putting some impeller and that, that for uniform distribution of oxygen throughout this medium, we are just using this impeller to rotate, so that, uniform mixing will be there; uniform mixing not only for oxygen, but for nutrient and other parameters which are associated with this particular reaction type.

Now, when we are talking about the bubble column reactor, now, here, this particular reactor is little different from that type of, this c s t r, that continuous stirred tank reactor; why, here no impeller is there; and here, what is happening? This air is coming and air is just purging inside this reactor and through which the mixing is taking place, inside the reactor, which is satisfying the oxygen demand and the uniform distribution of the nutrient throughout the medium.

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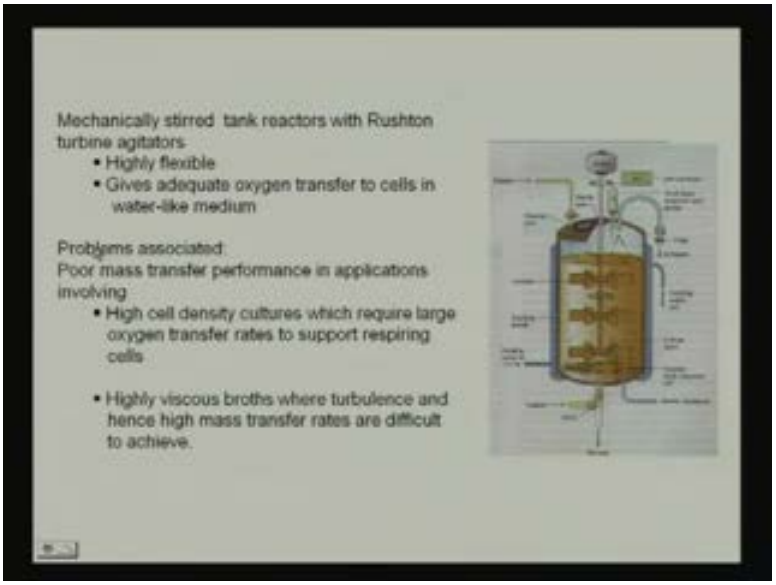

Now, mechanically stirred tank reactor with Rushton turbine agitators are highly flexible; it gives adequate oxygen transfer to the cell in water-like medium. Now, here, if the, the, the medium, which we are using is just like water, that means, viscosity, density, etcetera, are playing a significant role, as far as the cell growth and the product formation is concerned. Now, let us, let me give you one example; say, we are interested to produced gellan gum. Now, when we are going for that type of product formation, what we are doing? We are inoculating the bacteria to that particular environment and gradually, we can find, the, with the synthesis of this gum, this is a particular polysaccharide, which is coming extra cellular, which is coming out, secreted by the microorganism to the surrounding environment, and when this gum is being secreted through the wall of this bacteria to this surrounding environment, that liquid, the viscosity of that liquid is gradually getting changed, and after a certain time, it is so viscous, that we have to stop, and we have to take those gum out of this particular reaction type. Now, when such type of product formation is there, obviously, we have to

take the extra precaution. We have to, have the idea about that, how to purge oxygen, how to purge air, how to satisfy the organism, to sufficient nutrient supply and all.

So, here, this type of reactor, product formation, this, this, this selection of reactor system, is playing a significant role. Now, when we are talking about such type of reactor, or say, if we are going for the high cell density cultivation…Now, when we are going for the high cell density cultivation, we are, what we are talking about that, that cell concentration in normal cases, if suppose we are getting 10 milligram per liter; but, in case of high cell density cultivation, the cell concentration in the reactor is very many fold; it may go up to 110 milligram per liter. So, how many fold increase in that particular bacterial concentration, you just imagine; so, obviously, the viscosity of the nutrient medium which is there, is not same to that of this initial. So, when such type product formation is there, we have to achieve the oxygen supply demand, that the demand of oxygen for the microbes, for that type of product formation, and for this, sometimes, with the air, we have to purge the oxygen to satisfy that particular oxygen demand. And, when such type of product formation is there, obviously, the resultant broth, what is there inside the reactor, is totally having the different characteristics, than what it was initially. So, if we are talking about these problems, then, this high cell density culture which requires large oxygen transfer rate to support the respiring of the cells cannot be, get that, getting satisfied with this type of reactor system. Highly viscous broth, where turbulence and hence high mass transfer rates, are difficult to achieve, we should not use this type of reactor system.

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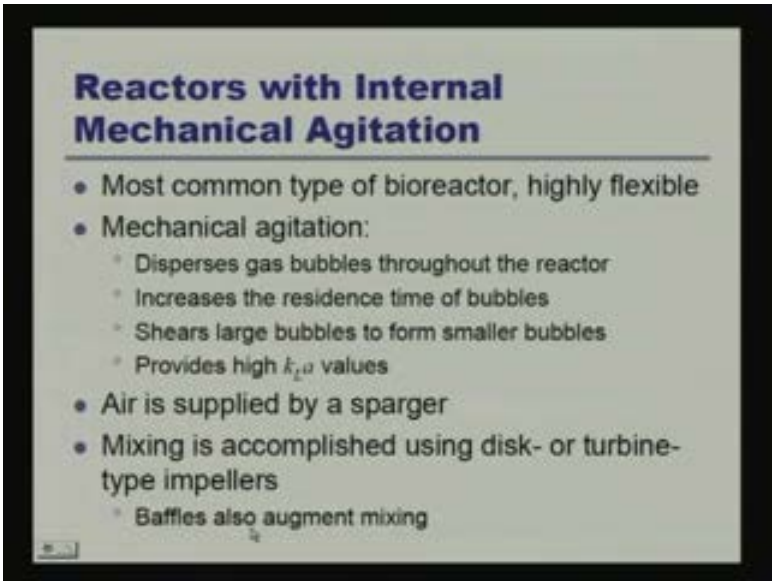

Now, there are some other reactor system, where most commonly, those bioreactors are highly flexible in nature, and which has got mechanical agitator that disperse the gas bubble throughout the reactor, increases the residence time of the bubble, shears large bubble to form the smaller bubble; it provides the high k L a value. Now, when this k L a is the mass transfer coefficient, and here, when we are getting the high mass transfer coefficient, obviously, we can think of that, yes, this is one of the efficient bioreactors system, and where, this, at a constant air flow, if mass transfer level is getting increased; so, air supply, air is supplied by the sparger, in such type of reactor system; mixing is accomplished using disk, or turbine type impeller; and, baffles also augment the mixing of such type of things.

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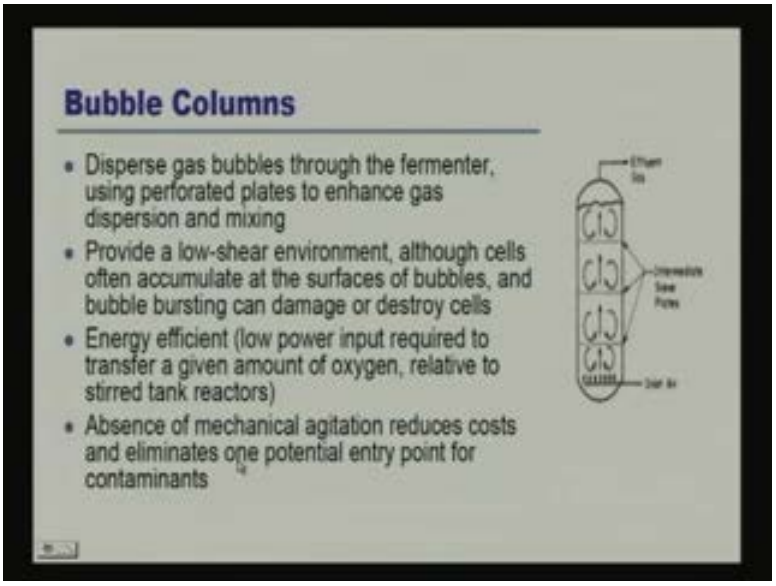

And, that is one of this reactor system. As, I have also, I earlier discussed that, it is a bubble column reactor. Now, here, the disperse gas bubble through the fermenter, which is using perforated plate to enhance the gas dispersion and mixing. It provides a low-shear environment, although cells often accumulate at the surface of the bubble, and bubble bursting can damage, or destroy the cells. So, these are some of this problems associated with this, but here, if the cells are very, very fragile cells, then, under no circumstances, we can use the agitator. So, how to satisfy this oxygen demand of this cells? So, this is one of the technique, through purging the air, we are just satisfying the oxygen demand of this cells. Energy efficiency, that is, low power input required to transfer a given amount of oxygen, relative to the stirred tank reactors; absence of mechanical agitation reduces the costs and eliminates one potential entry point for the contaminants. So, these are some of this, this, this positive points and the negative points of bubble column reactor.

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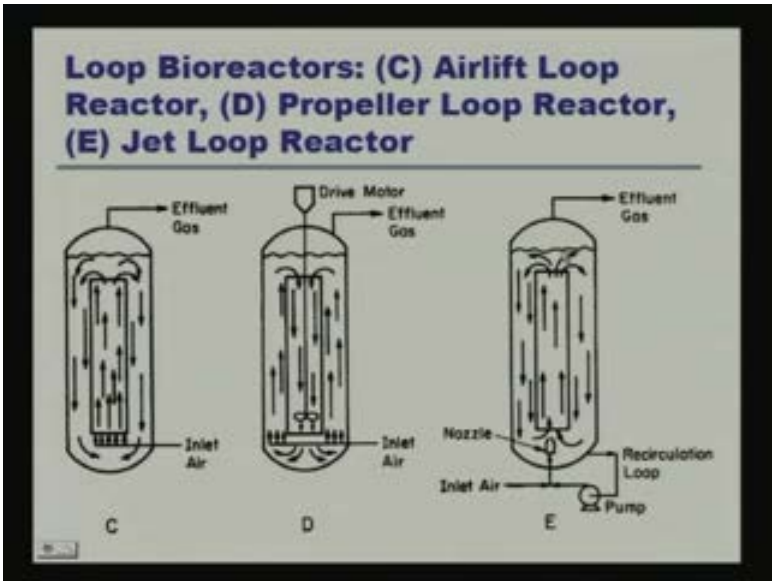

So, how to overcome the, the, the problems associated with the bubble column reactor? So, the alternative to this bubble column reactor is the loop type of reactor. This loop type of reactor can be the airlift loop, or propeller loop reactor, or jet loop reactor. Now, see, here this mechanism is same to that of the bubble column reactor. There also we purged the gas, and gas was just pushing this liquid out and it is, it was just getting mixed, in case of earlier. See, this is, gas is just pushing this liquid and liquid is getting mixed, and these all oxygen is coming in contact with this liquid, and oxygen, that liquid is getting dissolved oxygen; dissolved, this oxygen is getting dissolved and microorganism is taking that dissolved oxygen.

Here, now, see inside, one draft tube is there and through which, this, when this liquid is, this purging, that air, air is getting, passing inside this draft tube and see, it is pushing the liquid; and it has got one riser and one down comer and see, from this draft tube this liquid is coming out of this, the down comer and once again, it is getting circulated. So, obviously, this type of design has got little more advantages than the earlier reactor type. So, here, obviously, we can get the little better mixing efficiency; that dissolved oxygen, that k L a value is little improved, than the earlier one. Here, see here, this inside draft tube, this liquid, this gas, oxygen, air is coming and it is pushing. This, here, you see, not inside the draft tube, from the outside, air is going and it is just pushing the liquid and through the draft tube, once again, it is getting circulated. Here, through the nozzle that

air is coming and it is just pushing this liquid, at this mixing the air with this liquid coming in contact with this liquid, and it is just coming and getting it mixed.

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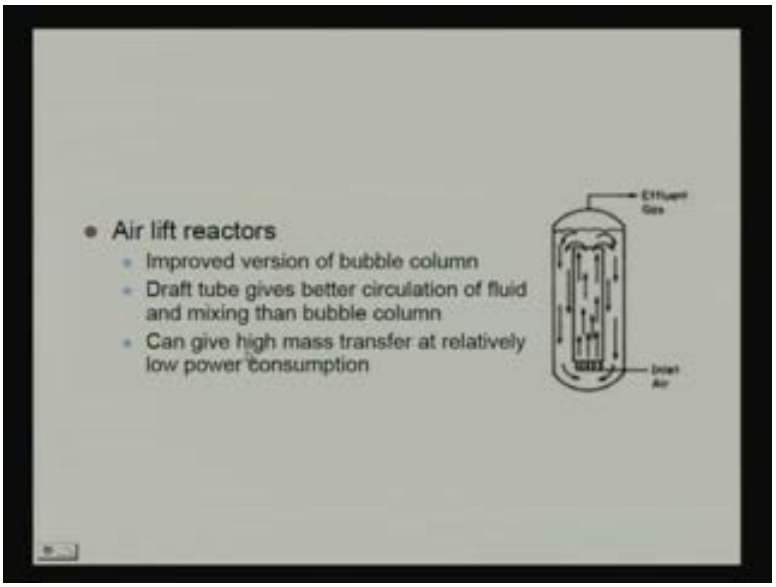

So, these are the different concept of air lift fermenter. So, it is improved version of bubble column reactor. Draft tube gives better circulation of fluid and mixing, than the bubble column. It can give high mass transfer and relatively low power consumption, what we have already discussed in this particular reactor system.

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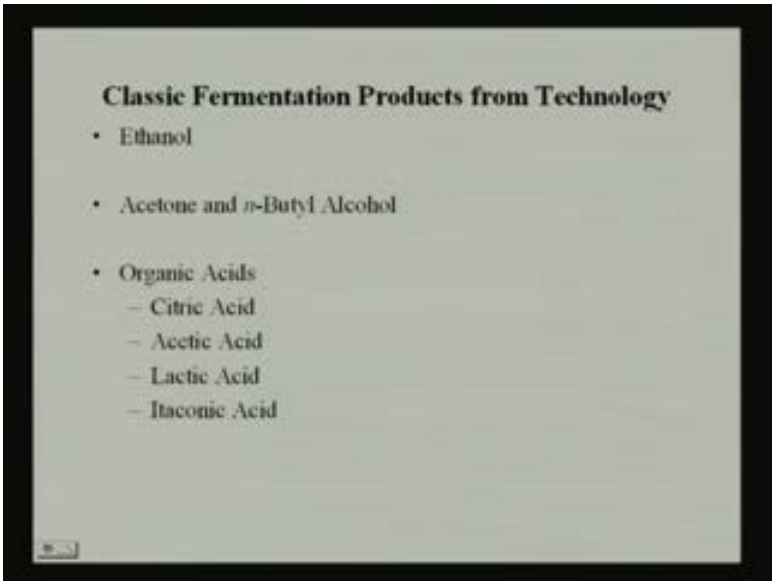

Now, when we are going for the any, any product generation, any product formation, the most popular fermented product is the ethanol. Ethanol is one of the most, it is the biggest fermentation process all over the world. Now, when we are going for this ethanol fermentation, now, we mainly concentrate, we are mainly talking about the edible ethanol. But now, it is, ethanol has got huge demand as far as biofuel is concerned. And, that is the reason, ethanol can be produced from different sources. One source is the, that from the edible, and another is the non-edible, or the ligno-cellulosic sources. Now, when we are producing ethanol from sugarcane…So, that process is one type, and because we are getting directly the sucrose, the sugar, and then, we are converting it to ethanol. When we are talking about ligno-cellulosics, that lingo-cellulosics to ethanol, it, that process is a little bit different from that of the other processes. Besides this ethanol, there are other solvents also, which can be produced with the microbiological intervention; that is, the acetone and n-butyl alcohol; some organic acids like citric acid, acetic acid, lactic acid, itaconic acid and so on.

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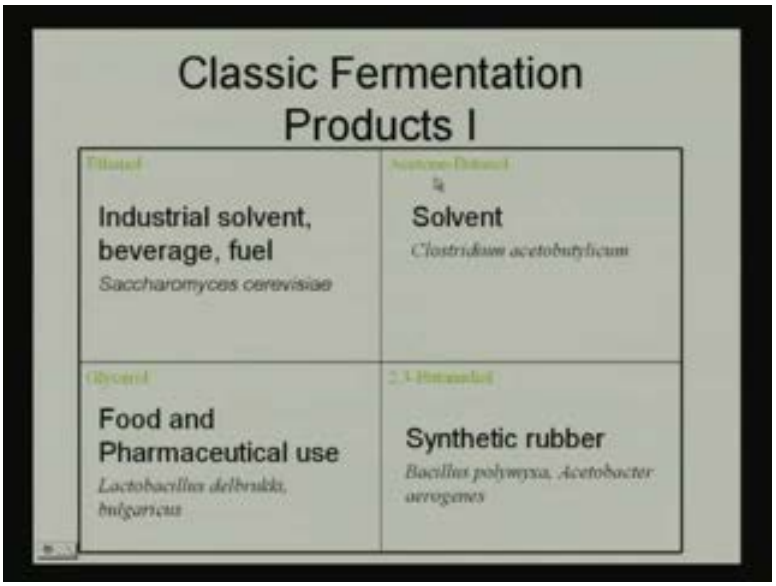

So many acids and alcohols can be produced by the fermentation system. now, when we are talking about this fermentation system, and, and the product formation, ethanol is one of the most popular fermentation; we can produce glycerol; we can produce acetone butanol; we can produce 2,3 butanediol and so on.

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Now, when we are talking about the fermentation with different, other products, then, it is little bit different, like acetic acid, which can be produced with the intervention of saccharomyces cerevisiae, saccharomyces species. Lactic acid, lactobacillus species are producing lactic acid. Citric Acid-Aspergillus niger and other species of aspergillus are also producing citric acid. Itaconic Acid-Aspergillus itaconicus. So, this is, these are some of the example, which are well established and well-known in the field of biotechnology.

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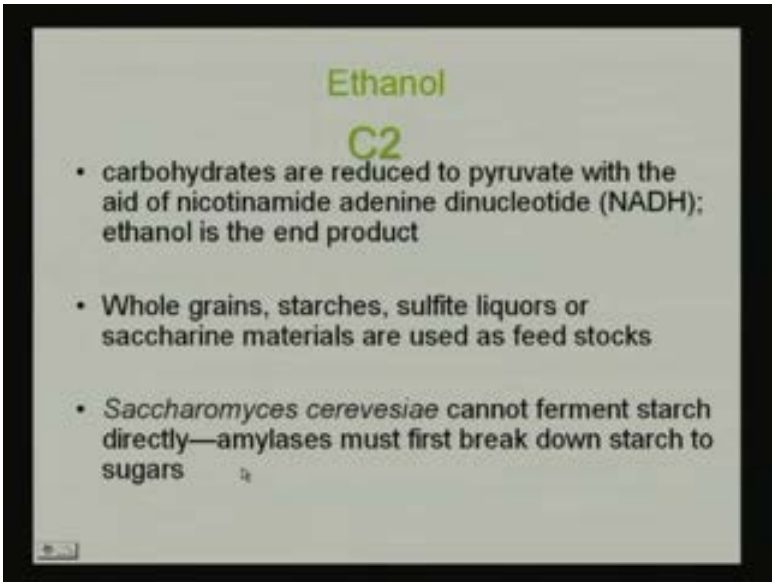

Let me give you one particular example of the biological, which are getting produced inside the microbial cell. Now, when we are talking about this ethanol, I told you that, ethanol is one of the biggest fermentation product, fermented product which are available in the global market. Now, carbohydrates are reduced to pyruvate with the aid of nicotinamide adenine dinucleotide and ethanol is the end product of this particular process. How this particular product is formed? We can use the whole grain, starchy material, sulphite liquors, or saccharine material, that are used as the feed stock for ethanol production. The organism which is actively participating in this particular conversion, is the saccharomyces cerevisiae, which cannot ferment starchy material directly, but, amylases must first break down to starch, and then, sugar is produced; with this sugar, the fermentation is taking place.

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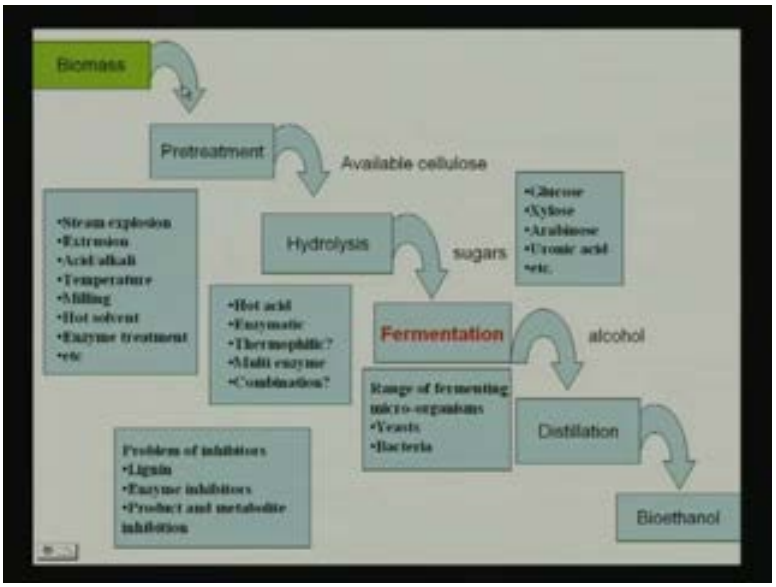

Now, when we are talking about such type of product formation, we are talking about C 2 product; that means, ethanol has got 2 carbon molecules. Now, here, if we see the overall production process…

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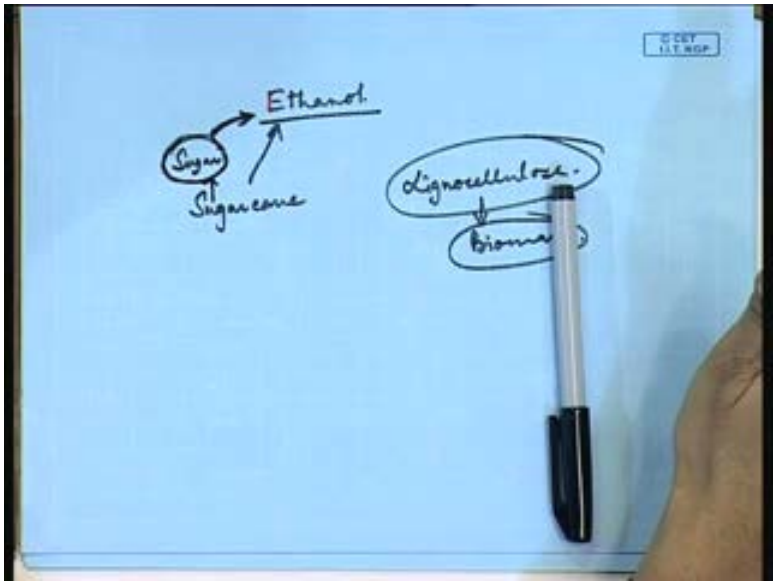

Now, as I have discussed you that, ethanol is the product. It can be produced from sugarcane. Now, when it is produced from sugarcane, what we are getting, sugar from sugarcane? We are getting sugar, which is sucrose, nothing else; this sucrose is getting converted to ethanol; that means, here sugar to ethanol conversion is taking place. Now, when we are going for this reducing that sugar to ethanol, this process is different from that of the process, if we are taking any ligno-cellulosics; that means, any biomass, any glassy materials. So, when we are talking about any ligno-cellulosics, when we are talking about any biomass, then, what we are talking about? We are talking about cellulose, hemicelluloses, lignin and other minerals, whatever is there, present, is there inside this reactor system. Now, pretreatment is playing a significant role in this particular conversion type. Now, when we are going…See, ultimate product is ethanol. What we are doing is, ethanol. Here, directly we are converting and we are getting this ethanol. But, our source is different; our source is lignocellulosics. The entire fermentation process is getting changed; just, here is the beauty, here is the freedom, here is the wisdom with the biotechnologist, who are designing, who are carrying out the experiment, and that is the reason, why biotechnology is so, is so interesting.

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See, we are going for the pretreatment process. Now, here we are getting the biomasses and biomass is pretreated. How we are going for this pretreatment? How we are doing

this pretreatment? We can go for the physical method; we can adopt chemical method; we can adopt biological method. Now, some of these examples are, this steam explosion, extrusion, acid alkali treatment, temperature, milling, addition of hot solvent, and if we are going for this biological means, we can fit it with the whole cell, microbes as a cell to delignify, or we can use some enzymes, that is the enzymatic treatment for removal of lignin from this lignocellulosics.

Now, when lignocellulosics, this lignin is removed from this biomass, we are getting available cellulose. This cellulose is undergoing hydrolysis. How we are going for this hydrolysis? We can take any other option, that is, either physicochemical process, or biological process; that means, we can use the hot acid, or we can use the, some enzyme, that maybe thermophilic in nature. Multi-enzyme systems can also be used and the combination of the enzymes, and some other techniques can also be taken for hydrolysis of the available cellulosic material, to convert this to glucose, or the sugar. When sugar is produced, what type of, what types of sugar we can expect? We can expect glucose; we can expect xylose, arabinose, uronic acid and so on. And, when we are going for, we are getting this sugars, we are going for this fermentation system.

Now, what type of fermentation? For ethanol production, it is anaerobic fermentation system. Now, when we are going for this anaerobic fermentation system, we are getting the product, which is called alcohol. Now, here, this range of fermenting microorganisms are yeast, as well as some bacteria. Now, yeast means, it is mostly the saccharomyces cerevisiae. As soon as this alcohol is produced, it is not the pure alcohol; alcohol along with some water molecule. So, we will have to remove this water from this alcohol, and ethanol and water. So, alcohol and water mixture is now, we are doing distillation. And, after distillation, we are getting the ethanol, which is having immense application in case of bioenergy, or biofuel is concerned. Because, this biomass to bioethanol, it has got plenty application as far as biofuel is concerned. Now, when we are talking about, this is one product, that is ethanol. Now, when I am giving you, this ethanol is produced from this fermentation system.

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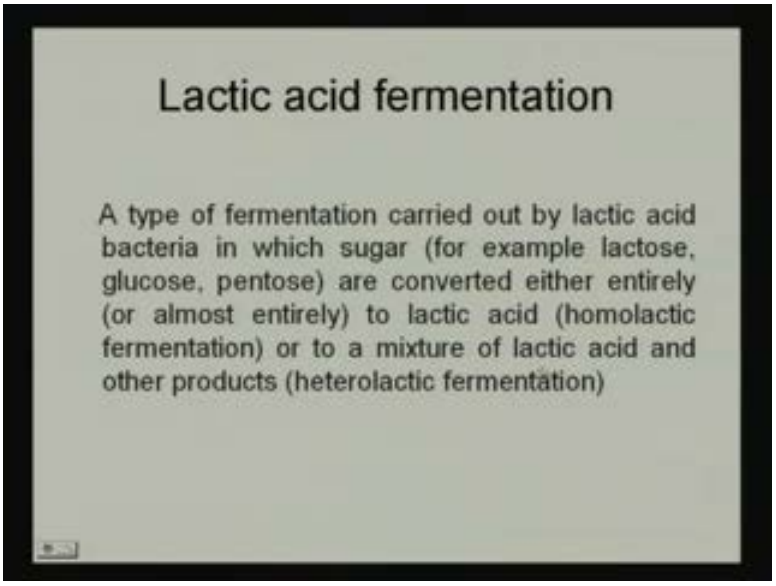

Another example I would like to give you, is the lactic acid fermentation. This is a type of fermentation which is carried out by lactic acid bacteria, in which, sugar, say for example, lactose, glucose, or any pentose sugar, sugars are getting converted, either entirely, or almost entirely, to lactic acid. And, when such type of conversions are there, we are calling these type of conversions as homolactic fermentation; or sometimes, we can also go for the mixture of lactic acid and some other product ,which we are calling it as heterolactic fermentation.

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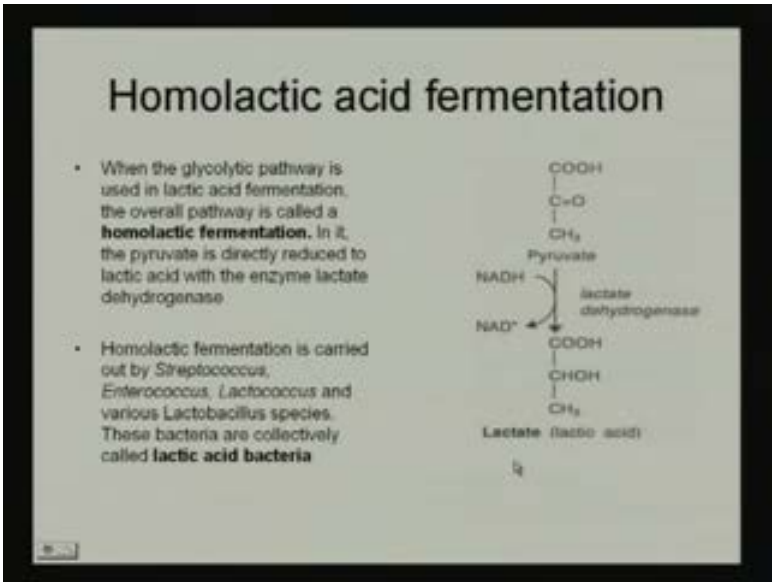

In case of homolactic fermentation, what we are doing? When this glycolytic pathway is used in this lactic acid fermentation, the overall pathway is called the homolactic fermentation. In it, the pyruvate is directly reduced to lactic acid with the enzyme lactate dehydrogenase. See, this is the pyruvate; in presence of lactate dehydrogenase, it is getting converted to lactic acid. Different microorganisms are participating in this particular conversions are streptococcus, enterococcus, lactococcus and different strains of lactobacillus, or the microorganism bacteria, which are efficiently producing homolactic acid.

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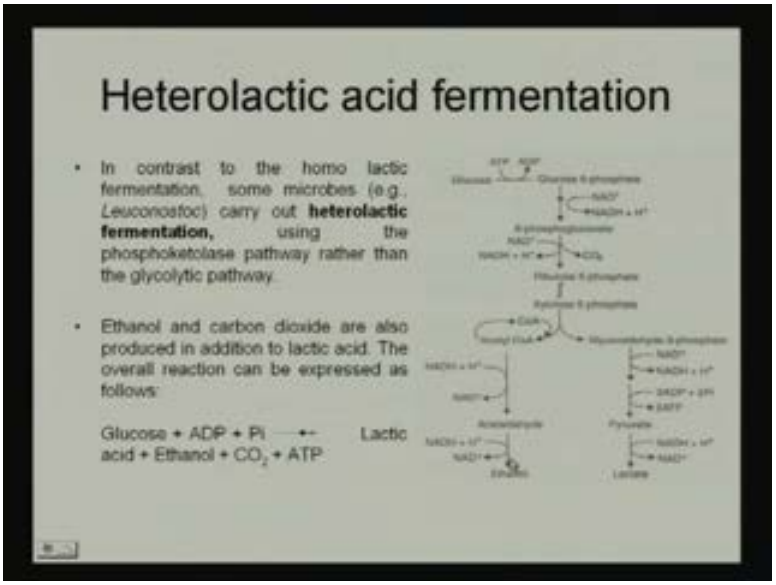

When we are talking about the heterolactic fermentation, we are talking about this, in contrast to homolactic, some microbes carry out this heterolactic fermentation, using the phosphoketolase pathway, rather than the glycolytic pathway. Now, here, see, you, glucose is converted to glucose 6 phosphate, to p p p pathways; see, 6 phosphogluconate to ribose 5 phosphate; ribulose five5 phosphate to xylulose 5 phosphate and one fraction is coming and producing glyceraldehyde 3 phosphate. If you remember p p p pathway, this same pathway is getting followed and ultimately pyruvate is getting converted to lactate. Here, some other by product, which is this xylulose, when it is coming in contact with this, getting converted to acetyl (( )); acetyl (( )) is getting converted to acetaldehyde and ethanol is produced. So, ultimately, in the reaction, we are getting, the, not only this lactic acid, but along with that, some amount of ethanol is also getting produced, along with the fermentation; that means, during fermentation, not only the

desired targeted product, but some by-product, or contaminated products are also found. So, that is the point of my telling, that during fermentation, though we are targeting for the production of single product, but along with this product, we may get some contaminants.

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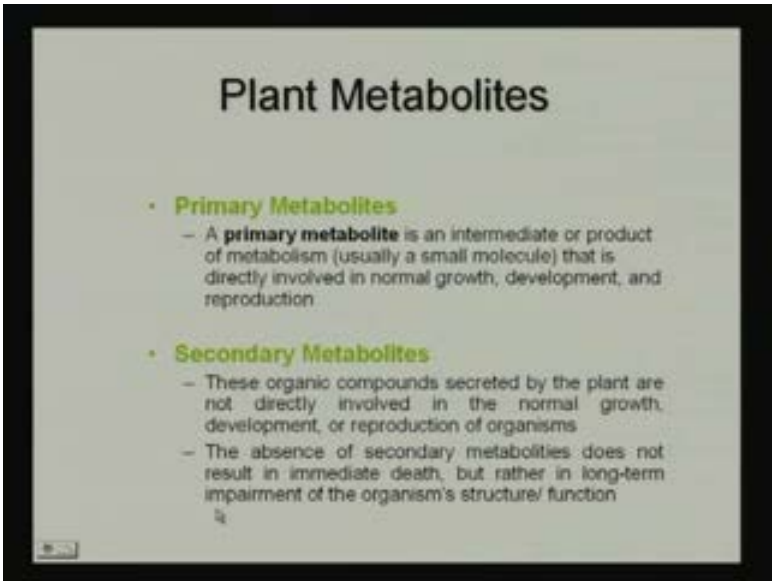

Say, for example, when we are talking about the plant metabolites, yesterday, I have already told you that, primary metabolites and secondary metabolites. A primary metabolites is an intermediate, or product of metabolites, which are usually a small molecule that is directly involved in a normal growth development and reproduction of a particular living cell; whereas, secondary metabolites, these are the organic compounds secreted by the plant; are not directly involved in the normal growth development and reproduction of this organism; but, the absence of the secondary metabolite does not result the immediate death, but rather, a long term impairment of the organisms structure and function.

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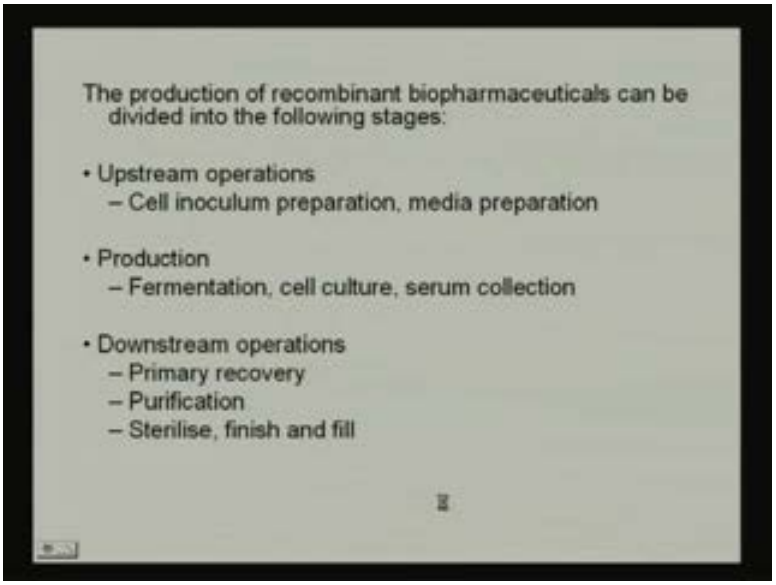

And, when we are talking about such type of product formation, then, we are talking, we are calling that, yes, along with this particular metabolite, major contaminants are there, which are present; because, inside the plant cell, along with our desired product x, some other products are also present inside this. And, this x is not, is not the only one which can be getting extracted when we are removing the cell wall of this particular plant; but, it will be, whatever is the intracellular metabolites, everything is coming out along with the our targeted molecule, which is x; and, that is the reason, which is, for which, we are very much interested to remove this contaminant from this cell.

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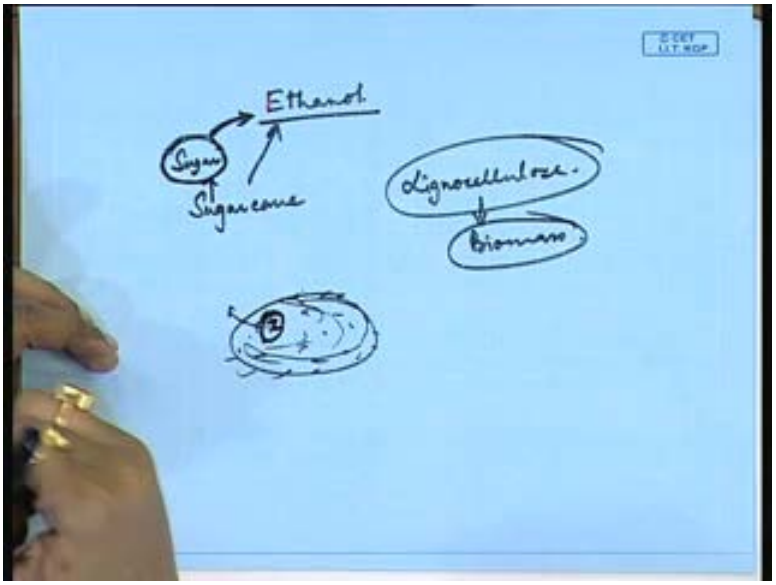

So, the production of recombinant biopharmaceuticals can be divided into the following strategies; that is, the upstream operation and production, and downstream operation. So, if we see, now, what I have already discussed till now, if I classify the entire lecture, what I have given, I have discussed with you, that entire production, that upstream, downstream.

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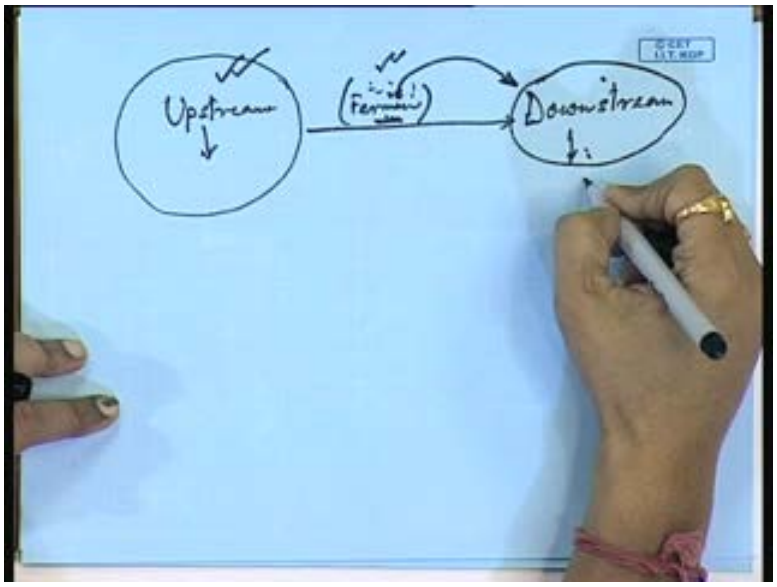

Now, if we are talking about this upstream and downstream, we can divide that cell inoculums preparation, media preparation, sterilization, cooling, etcetera, which are the process, are coming under this upstream processing. Then, we are going for this fermentation system, or the production of metabolites within the particular environment and we are calling it as fermentation; and this fermentation is the intermediate between the upstream and downstream. As soon as this fermentation is over, the product is produced. Now, we will be isolating and purifying this product for subsequent use. And, when we are talking about the subsequent purification of our targeted product from the mixture of this contaminants, we are talking about the downstream processing of that product. So, entirely, the entire process, starting from the manufacture of the metabolites to the purification, if we take this thing, the entire processes, then, we can divide it into upstream process, and then, the transition process, where metabolites are getting produced, targeted products are getting produced, which is the transition phase of upstream and downstream; and once the product is getting produced, we start the downstream processing.

So, here, this fermentation, cell culture, serum collections, whatever, whatever maybe the system, irrespective of the plant cell, animal cell, or microbial cell, when production of product is there, we are talking about the transition phase. And then, we are going for the downstream operation. Now, that is the reason, why the strategies of biomolecules separation is playing so important role, because this, if the targeted molecules are not getting separated with the appropriate concentration with high yield, then, we cannot make that process economically viable; and that is the reason, why downstream is so important. And when, while starting this downstream processing, we have to have the idea about the strategies of the downstream processing.

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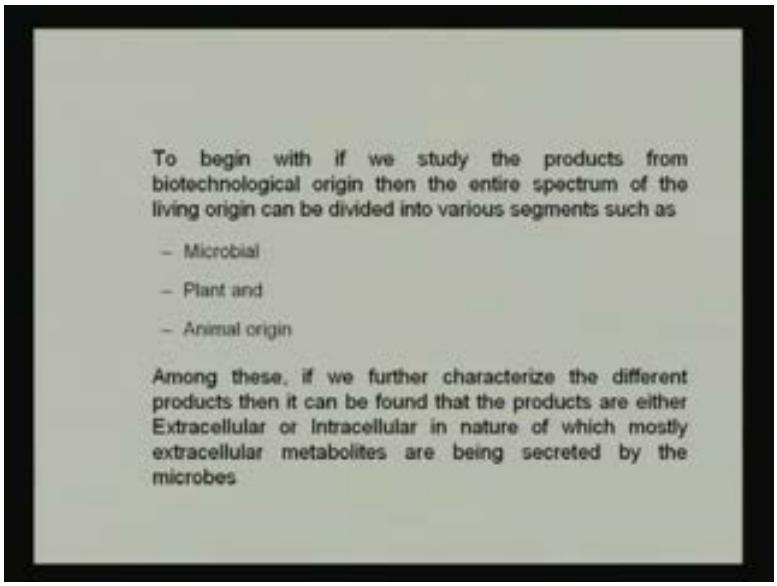

Now, when we are talking about this strategies, we are mainly talking about the two processes.

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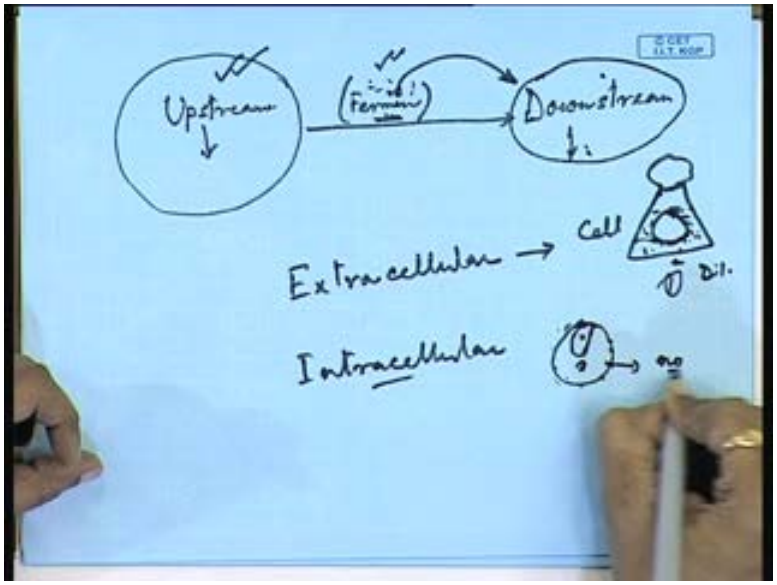

If this is a microbial process, we can get this microbial process into two different form. One is the extracellular metabolite; another is the intracellular metabolite. Now, whatever maybe the extracellular, the cell itself, from the cell wall, the product is being secreted to the surrounding medium, by this particular cell. And, that is the reason, why it is extra cellular. So, as soon as this metabolites are coming out of the cell, it is getting diluted inside the reactor. And, when it is getting diluted, that means, dilution problem is much; but though, the number of concentrate, the contaminants are not so high, compared to this intracellular. When, suppose, this is the cell and we are getting our product in the form of the inclusion body; and when we are, we want to get this product, we have to break this cell wall. When we will be breaking the cell wall, whatever maybe, the thousands and thousands of metabolites are coming out of this system and making the entire contaminants, along with our targeted products. So, thousands and thousands of contaminants are there. So, here, one problem is the dilution problem, and second problem here, the number of contaminants present inside the product. So, how, what strategy we will be taking to select the optimum process for downstream processing?

So, in the, in the next class, I will be talking about the strategies of downstream processing. Now, I think, I have made you clear that, how product is being produced inside the cell; what is upstream; what is transition phase and what is downstream; where from we can come to this downstream, and problems associated with the downstream processing. Thank you very much.