Aspects of Biochemical Engineering
Prof. Debabrata Das
Department of Biotechnology
Indian Institute of Technology, Kharagpur

Lecture - 55
Operation of Industrial fermenter and material analysis

Welcome back to my course Aspects of Biochemical Engineering. Now till now why I was discussing different aspects the, of the biochemical engineering and, now today I am going to discuss a very new topic, that is the Operation of Industrial fermenter and the material analysis. Because whatever we have covered up till now that try to understand how we can analyze the process. Now this is regarding application of that because, how when we applied in the fermenter how the industrial process that is in operation. So, so this will give you the information that how the industrial fermenter is operated and how you can how we can do the material analysis.

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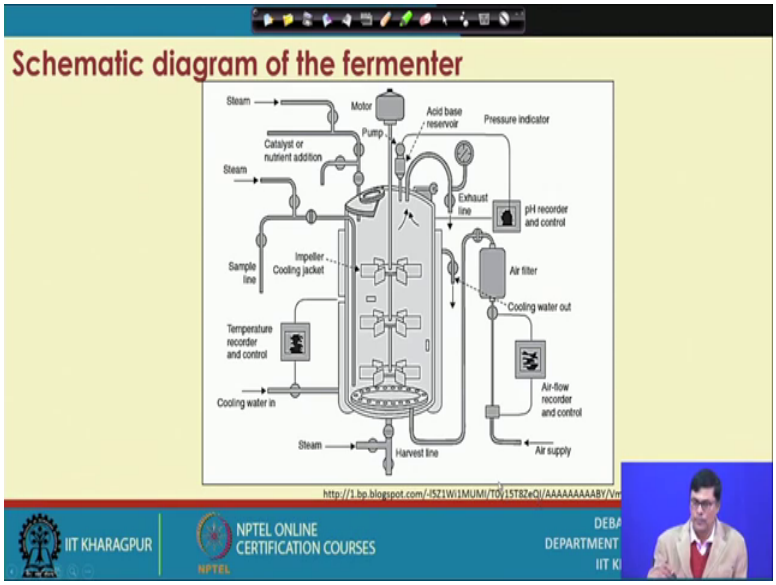

So, now you hope this particular lecture will be very useful for you and first let me start with this the schematic diagram of the fermenter, if you look at the fermenter that you know that ferme this is I told you to type of things, that we have rea we have reacted am I right, what is the reactor how you define reactor. The reactor is a vessel in which the reaction take place.

Now we have in biochemical industry we have bioreactor. What is bioreactor? Bioreactor also same thing that in the in the vessel in which the reaction take place with the help of some bio molecules, that is why we call it bioreactor. So, only the difference between the chemical and biochemical bioreactor and chemical reactor is that, in the bioreactor mostly we shall have to we are more concerned about the stability of the reactor, but in the chemical process they are not very much careful about the sterility of the reactor that is not that does not plays very important role. So, this is the, this the main constant that we have.

Now, let us see what are the other things that we have, now here I told you this is the agitator shaft am I right, this is called agitator shaft and I told you this should be perfectly straight. And there in the world there will be hardly 2 3 companies, they manufacture this shaft then this should be particularly straight. Now here the major problem that we have here, this is the connection between the top of the lead and the shaft. Because there we put some kind of mechanical seal, what you call mechanical seal.

And the mechanical seal is a we put in a manner. So, that no air can enter into the system that is that is why we because, air prisons lot of microorganism in which we allow there to enter into the system then condemnation will take place. Now there are other lot of things that we have that we have some temperance that that you know this is impeller I have already discussed, this impeller that is at us with the shaft for the starting purpose.

And different height we shall have to provide this because, you know why where to make the mixture perfectly homogeneous in nature and, then the another very important thing is the temperature, you have to maintain the temperature how you for maintaining temperature two thing we shall have to do by passing the you can pause the heating hot water, or cold water through which you can maintain the temperature because, you is since you are using the stirrer, if you move then move the stirrer with the high velocity, then we can assume the heat transfer will be very good and you know that the temperature can be maintained.

So, this is at the, that this is the temperature recorded we have from where we can maintain the temperature. So, here this is connected with the controller. So, if the temperature rises the chilled water that pipeline that is that a pump will be on and it will bring the chilled water, it over to reduce the temperature is temperature is low, then hot

water pump will be on and no hot water will be circulated through the jacket this is a jacket. Now one thing I want to tell you that this kind of jacket is not used by the industry. So, what do we use in the industry because this is the vessel, now what we what we use we put some kind of you know that tubes like this, with we with the re pipe the wrap around the surface of the of the reactor.

So, that you when you pass the water, they need you ensure that water is circulated through the periphery of the, this reactor, but when you pass the liquid here suppose there is some kind of deposition here due to some other reason. So, the water will do they have the channeling effect, if the channeling effect will be there then the, that the temperature control will be a problem. So, this is this is the one of the reason why we use the that pipe we wrap around the surface of the reactor.

So, and for heating with some time, we use the stream some time we use the hot water and here, you can use the sampling purpose this portion is very interesting in the lab, how we can do the sampling we can in the lab we can do the sampling very easily because, there is the outgoing that the air outlet am I right in the exhaust this is the exhaust line. Now, here in this the exhaust line if you valve if you close this valve, then there will be a pressure here, the air pressure here and due to the air pressure the automatically if you open this valve open this valve, then you can draw the sample and collect the sample. And this way you can do it very easily.

So, here we have you see that we are if this is this some kind of pH probe is there, it is connected here dissolve oxygen probe that will be connecting here, then air filter is there air filter we for air sterilization purpose this is the air flow recorder, we record this flow rate how we maintain the air flow by using rotameters that we can find out what is the flow rate of air, then we can we have this is a Spurger that we have. So, you know that and then and after the fermentation is the over, then we draw the sample like this through the harvesting the pipeline. Now here I want to point out the different type of valve, we use this is the, you can this is the valve and the same type of valve is not use for the all purpose.

As for example, for harvesting we always use the gate valve am I right and, in case of sampling suppose in the sampling of what we use we use ball valve because, the why ball valve because we can instantaneously open and intensely instantaneously you can

close, that is why you can use that. So, this is this is the kind of this is the pH recorder, with the help of pH probe, you can this is connected with the record, you can you can you can record that that you know but p h. So, this is the again connected with acid tank and alkali tank. So, as the suppose you say to a particular page pH 7.

So, if you neutral want to operate, if the pH increases then the pump connected with the acid tank, suppose this is the acid and this is alkali. So, this acid pump connected with this acid that will be on and this will take the liquid that will acid in to increase the decrease the temperature. Now did not get decrease the pH. Now if the pH is less then it is connect with the pump is connected with the alkali tank that will be on and bring it to there to increase the pH. So, this is how we can control the different operational parameter in the fermenter.

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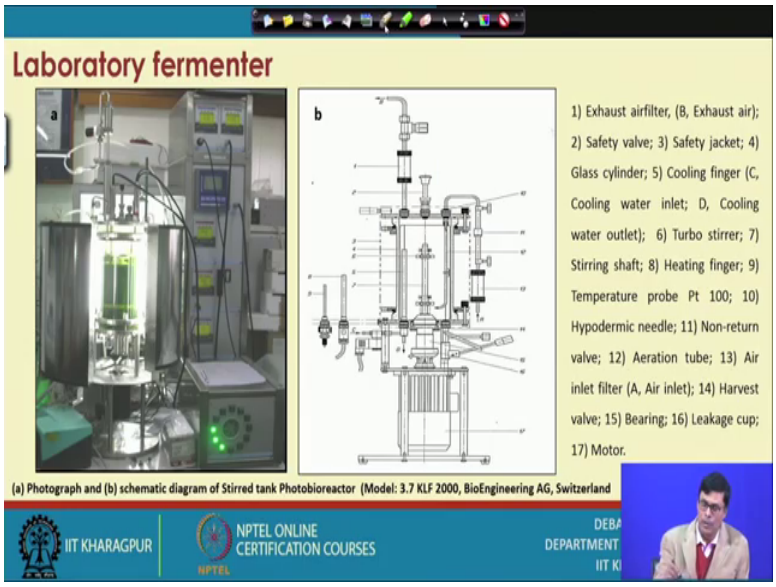

Now, this is the in the laboratory because how we operate because, you know I am in I am from IIT Kharagpur, we have bioengineering a g fermented this is bioengineering is this is the control modules this is called control module, what is the purpose of the control module, is to is to is to call here is the call different type of control controllers, we have and recorded different type recorded different type of control as we can.

So, we can monitor that what is going on inside the reactor, that we have different type of sensor here, with the help of sensor and probe we can monitor as for example, temperature we use some kind of thermistor that is collected and that we find out how

much temperature is there, we have pH probe that measure the pH, we have dissolve oxygen probe we measured the that dissolve oxygen concentration the fermentation fermentation liquid. So, these are all plays very important roles.

So, here you can see that the different different things that, we have we have exhaust air filter that is 1 that you can see this is exhaust air filter. Now question comes why we put the air filter at the exhaust, the reason is that that when suppose you your running a fermented, non obvious all of a sudden the power is off, then there is the every possibility there air present in the air can enter into the exhaust line and, enter into this fermentation fermenter, then if we enter into the fermenter your fermentation will be spoiled.

So, we want to have the exhaust filter, then we have safety valve because this is the safety valve that we have because, there is some other reason if you pressure increases there is every possibility of some kind of disaster that can be avoided we have safety jacket because, in the lab this is made of glass. So, if it is made of glass and 2 type of sterilization technique, we have one is called insitu and the invitro. .

So, insitu means that the fermenter, wherever is it look at it here itself we do the sterilization and we know the for sterilization you have to maintain the temperature 121 degree centigrade. And for that we shall have to increase the stream pressure to 15 p s i. So, to so somehow that if they if the pressure of the stream increases, that call may causes some kind of disaster to avoid that we put some kinds of jacket here.

The steam this stainless steel jacket we put across this fermenter show that, they even some the bar if by their glass vessel bust it will not affect you very much, then you have this glass cylinder is the reactor cooling finger used for cooling purpose cooling water inlet, cooling water outlet and turbo stirrer, the then stirring shaft heating finger to a temperature probe, then higher hypodermic needle for, aseptic temperature that you know sampling purpose we can do that. The non return valve this is very important because, the non return valve which is located here, you can see that why the non return valve is recovery is required here, non return valve means valve looks like this.

So, here is it like this it can go one direction it cannot come other direction, the one direction. So, the why it is like this suppose if there is a power pillar, then if the liquid due to comes out there due to some other reason and, then here you have filtered and if

you drape your liquid comprises of lot of organic material, if you enter into the filter then filter will be spoilt and because it will be contaminated.

So, your air cannot be sterilized. So, to avoid that we should have this you know non return valve, or one way valve the aeration tube, then air inlet filtered and harvesting valve and bearing is required for any kind of moving safety as you know that we require bearing leakage crab and mortar, but one thing I think it is not mentioned here that here, we use another thing that is we call that foam controller.

We have foam sensor that you know we insert here, that you know that the as soon as foam formation is there that is the normal characteristics of the fermentation process, that is touched the foam then it is connected with a tank of antifoam oil and it will take the antifoam oil and put him in the reactor. So, that foam will subside. So, this is how the things is operated.

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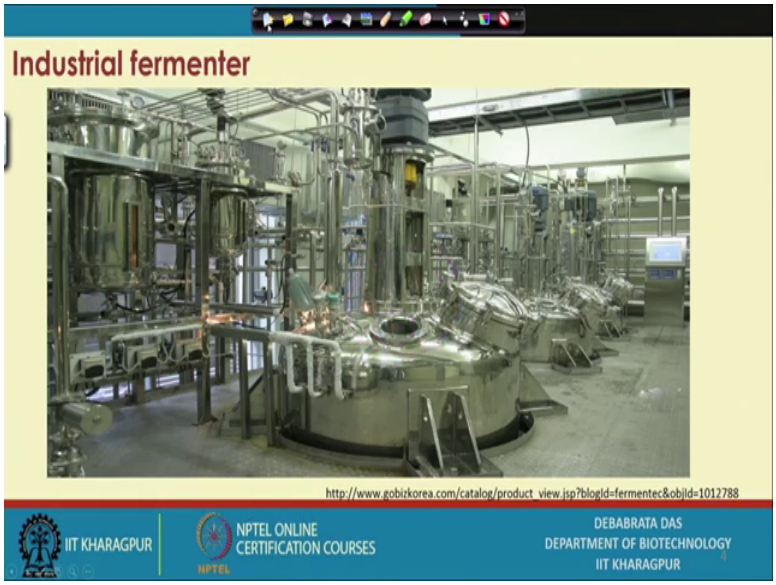

Now, industrial fermented looks like that this is the bigger form of lack fermentation process, this is this is the mechanical seal I was to telling you, that you know this is the this is the very very important portion, through which we can we can have the contamination. So, this should be properly designed and, this is the watching glass you can that the big fermenter we have two watching glass, one this way and there is the other way, one way you can put the light another way you can see that exactly what is happening in the fermenter. So, you can have you can see the antifoam wall tankard and

other things that is located here, different other things that is located here similar to your lab fermentation process.

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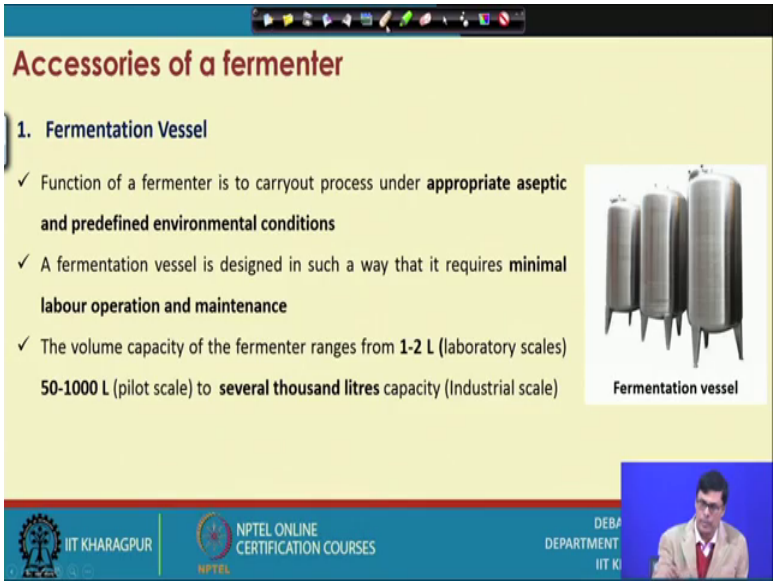

Now, later let us let us give you some information about the different accessories, that we have in the fermentation. That first is the fermentation vessel am I right the function of the fermenter is to carry out the appropriate aseptic and predefined environmental conditions, a fermentation vessel is designed in such a way it requires the minimum labor of operation and maintenance, that the volume capacity range from 1 to 2 liters in the lab scale and, 50 to 10 1000 liter pilot scale and several 1000 liters capacity in the industrial scale.

So, different capacity that we have required and main purpose main thing that you shall have to consider that minimal labor operation and maintenance particularly, I want to point out that that material of construction should be that, there should not be any kind of damage to the surface of the material construction and most of the material is made of stainless steel. And different type of stainless steel as you know that is the available in the market one is the SS 314 one is the SS 316 one is the SS 317 the different type of depending on your requirement, whether in fermentation industry we usually go for SS 316.

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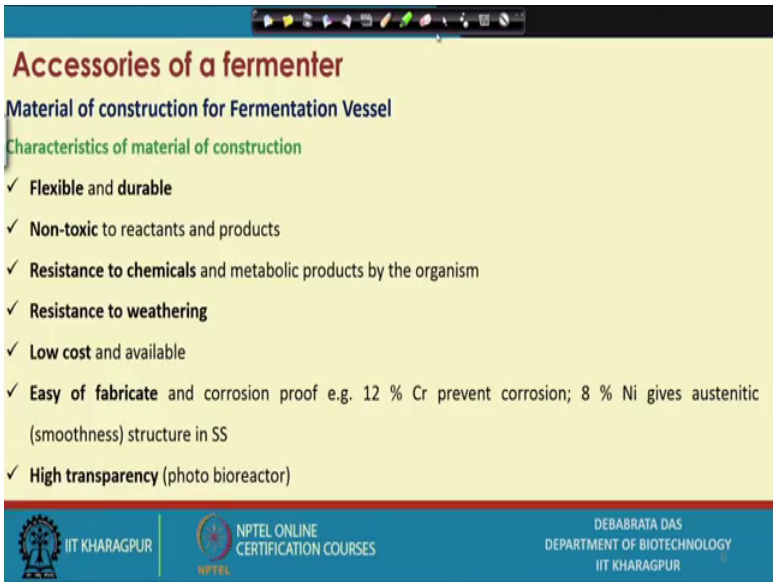

Now, material of construction is it should which should be flexible and durable that I told you nonkcin non toxic and to the reactant and product, because whatever in the media of whatever component is there should not react with the material of construction, resistance to chemicals and metabolic products, by the organism resistance to weathering that low cost and available, they should be available and easy to fabricate and corrosion probe.

As for example, because I was because I told you I told you that stainless steel easily used by the material of construction, what is stainless steel stainless steel is a call alloy steel, that is the mixture of metals though iron with the iron we mix with different metals, that makes a stainless steel. So, it has been found if the alloy steel cor the stainless steel content 12 percent of chromium, it prevents the corrosion. Not only that if you have suppose, I work with citric acid industry and citric acid is more corrosive to the material of construction. So, we can go for a little bit higher is in that case we add some kind of molybdenum to have the mode acid resistant property of the stainless steel.

Now, the nickel and that is give the austenitic structure, or strategic structure means, this is the smoothness of the structure. The if the if the material of construction is too smooth, then what will happen your organism may grow on the surface of the of the reactor and, the your process will be hampered. So, that is undesirable and, high transparency or for photo bioreactor, but not for all reactor photo bio reactor we require the polycarbonate

material we use because, polycarbonate you transparent say will be more high. So, this is how the material cop construction, we can we can we can we can choose.

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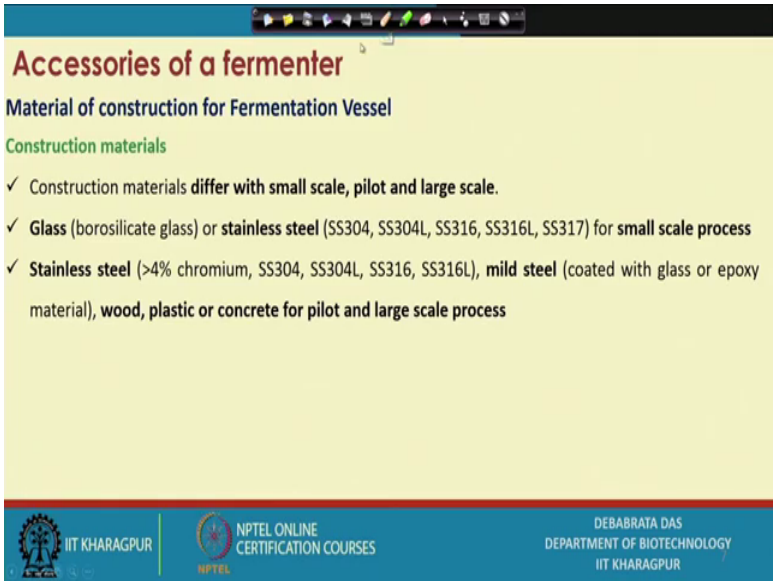

Now, construction of material, different from the small scale from the small scale small scale pilot scale and the large scale, I told you that we have different type of stainless steel, that we have this is 301 30L the 316 316L and 31 SS 317 for the small process.

And reversed stainless steel this is we use that, this the same thing we more or less we use and mild steel coated with the epoxy material, epoxy we know epoxy is kind of it to protect the acid and S to S effect that can be we can we can protect us to the mild steel from that, there will sometime we use the epoxy coating material with the mild steel. And wood and plastic and concrete of pilot and large scale, we use this is some cases we use mostly, but mostly as I told you mostly we prefer the stainless steel material.

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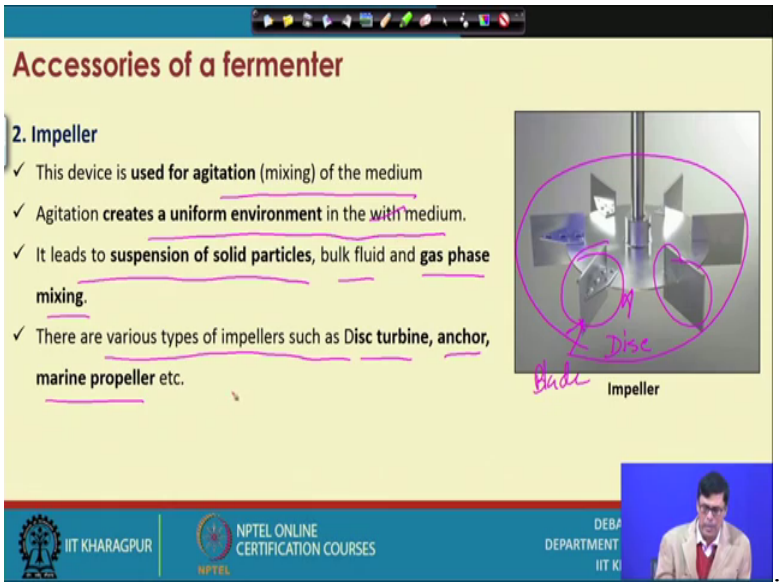

Now next that very important that I already discussed, that the impellers this impellers it looks like this and, you see that this is the this is called disc, this is the disc and this is the blade am I right. And you can remember I told you this number of blades that this kind of discs we have a different height and, this we have seen this number of blades very it plays very important role, in the vibration of the fermenter the in the in the industry we shall have to work who do the trial error, just to optimize the number of blade in the disk, that device is used for the agitation and mixing and create uniform in environment, were in the media in the media it leaves the suspension of the solid particles bulk fluid and gas phase mixing, there are various type of impeller disc turbine, anchored and marine propeller I have I already discussed that. So, I do not like to repeat here again.

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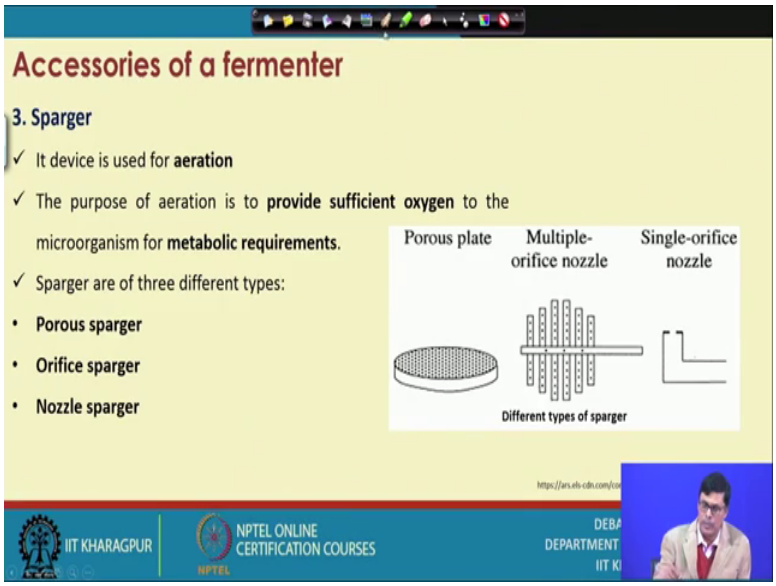

Then this is the different type of sparger because, sparger just to you have a porous plate type, this is multiple that the orifice nozzle and single nozzle type. The different type of three different type of aeration that the things we required, that we put at the bottom of the fermenter.

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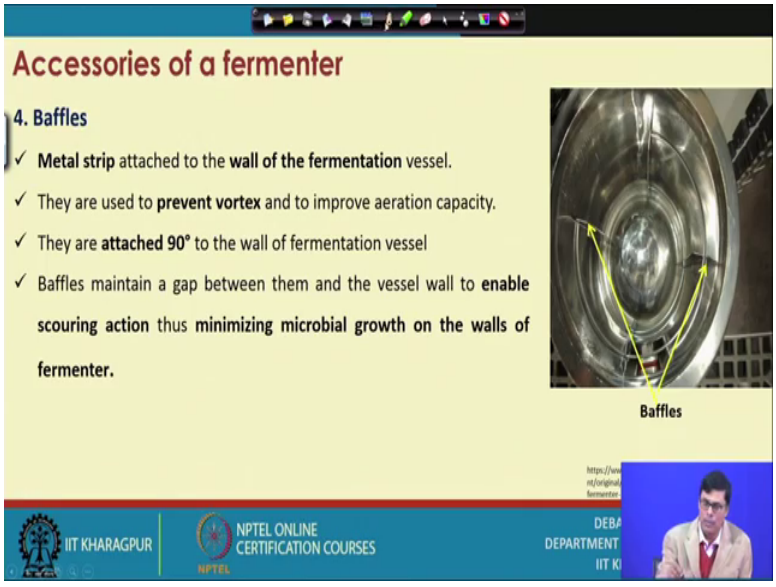

Now, baffle I told you can you can here, you can see this is the baffle am I right this is the baffle and, why we use the baffle to stop the vortex, I mention you that suppose you do the agitation, if you do not use the baffle it will be like this. Now if you in the in the

reactor if you have baffles like this and, you have if agitator then your level is like this. So, there should not be any kind of any kind of vortex formation and this is desirable.

So, this is the purpose why you use the baffle. Now in case of sometimes we in the big fermenter, this we use the hollow type of baffles, where why we use the hollow because, we use for cooling purpose because we pass the water through this because, in the dual since we are in the tropical country, then in the summer temperature of the environment rises to a great extent. So, we required more cooling effect. So, we use the baffle for cooling purpose that is that is why we use.

So, so this is the this is the metal strip at us with the wall of the fermentation of the baffle, they are used to prevent the vortex to improve the aeration capacity, there at 90 degree with a wall of the fermentation vessel, baffle with this remains maintain a gap between them and the vessel wall is enable to squaring souring this than action and, thus minimizing the microbial growth on the wall of the fermenter. So, this is the, this is how the baffles is why the baffle is we have in this.

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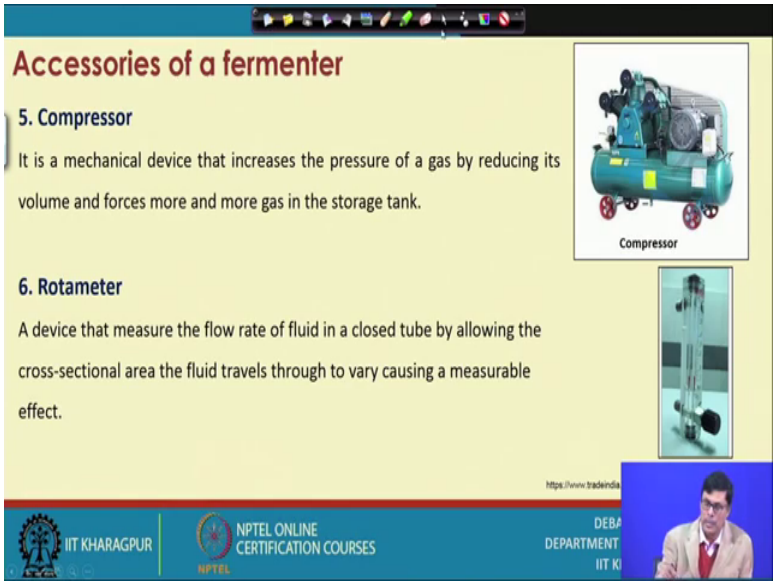

And other things that we have with the fermentation industry the compressor, this is to supply air that you know this is the, this is the how the compressor losses gives very very big noise in the fermented a fermentation industry.

And then we have rotameter; rotameter so, it looks like this and this is this is rotameter is use the usually used for low flow rate, it divides to measure the flow rate of the fluid. So, closed tube by allowing the cross section area and, the fluid travels through a wave to vary the causing the measurable effect. Now here we can this is the, these are knob by opening and closing we can controls the flow rate of air, that can be done very easily this is the rotameter is easy to handle.

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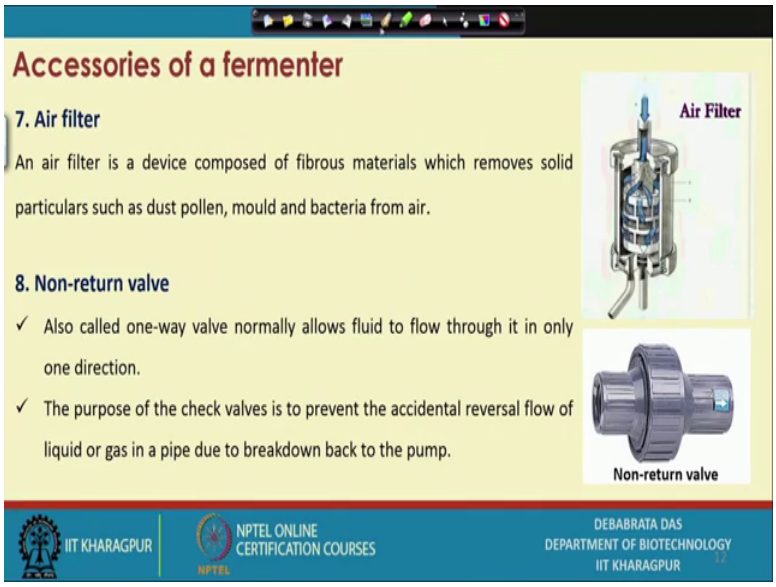

This is the air filter that we have I have already discussed the air sterilization process, we use the for air filter, we use the fibrous type of material, particularly we use the glasswool, glasswool fiber we use fiber we use as a for air sterilization purpose, remove the solid particles dust pollen mould and bacteria from the air. We had to I was talking about non return valve non return valve is looking like this, it goes one direction you can see that they have given the direction, the liquid cannot go flow cannot go flow this direction. So, only it can flow one direction that is why we call it non return valve.

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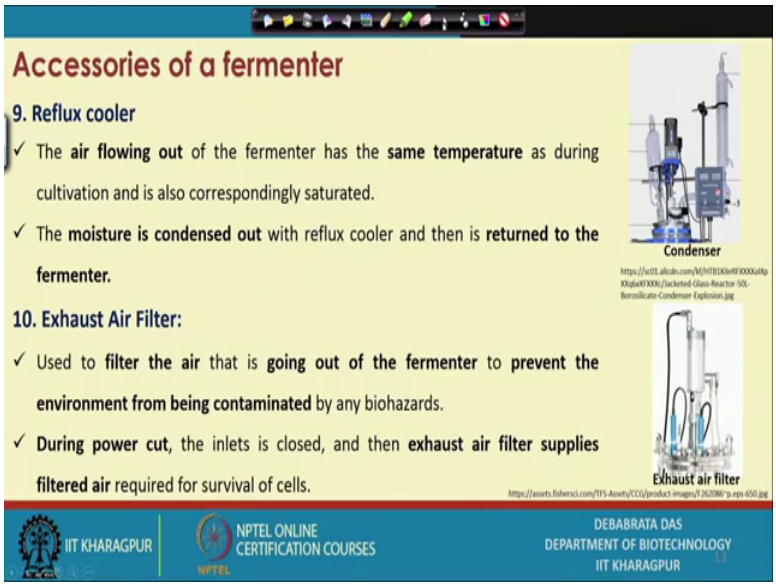

Then we have reflux cooler that I told you why the reflux cooler is there, that is also we should understand that why reflux cooler is there, because in the fermented this suppose this is a fermented. And now what we are doing we are sparing here am I right, we are we are passing air in and this is the thing and here this is the air out.

Now, what is happening when here you are sparging your air is the not a saturated, that the unsaturated, but when sparger this through this is it will be saturated. So, when it will be it takes some kind of moisture from the fermenter. So, you will find if air if you allow the air to go out like this, we will find with respect to time the volume of the liquid volume of the fermenter will keep on decreasing. So, what we do we put a condenser here. So, that they know that whatever l the vapor is going down there should be condensed back to the fermenter. So, this is what we the exhaust air filter is there, we I told you to avoid the contamination that we can use that.

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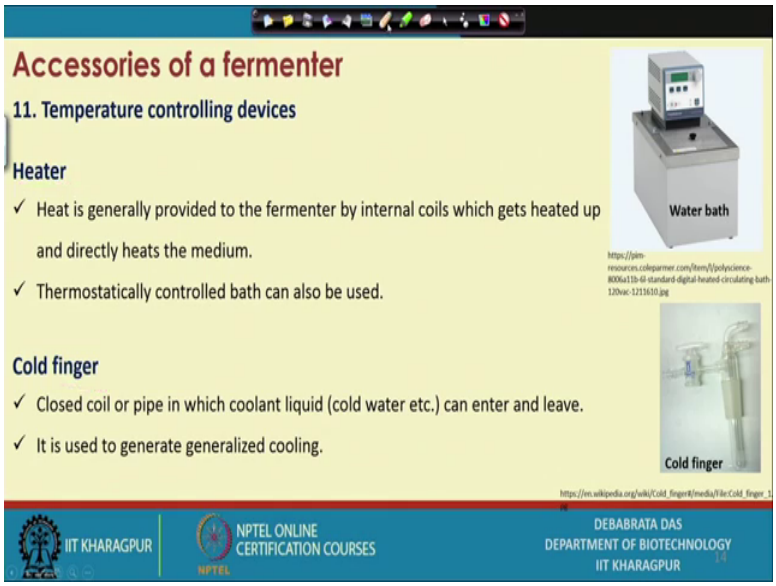

Then we have temperature controller device, we have heaters, we can have heaters, we can have cooler cool cool finger that way different type of a arrangement we can have.

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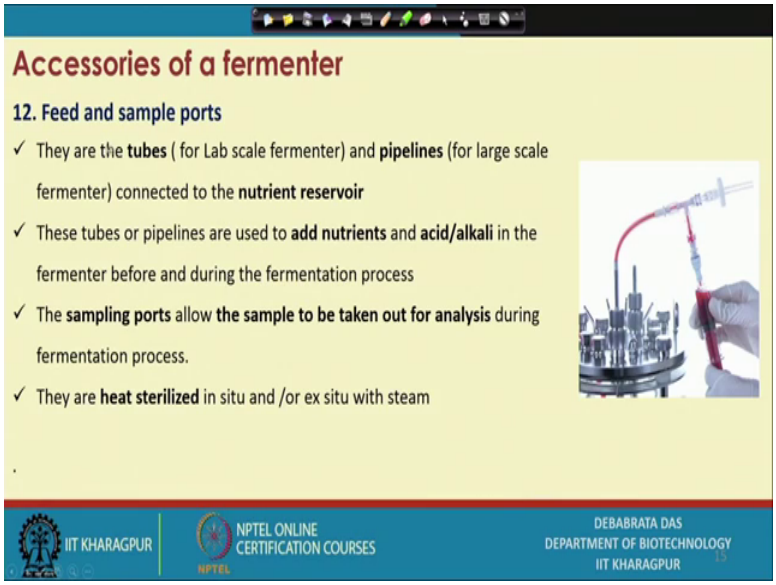

Then this is the feed and sample port that I showed you with the if we increase the pressure air pressure, you can automatically draw the sample again and close the valve.

But this is how we can do there. So, this is this is the tubes and, pipelines and the connected with the nutrition reservoir. And this should be valve is I told you this is valve is use, just to which can be instantaneously open and instantaneously close. And this

tubes are pipelines are used adding mutants sometimes I use for adding the nutrient also has it, then alkali fermenter before and during the fermentation process sampling port sample taking out for analysis and heat sterilization in situ and ex situ to with stream that is that is what we use here.

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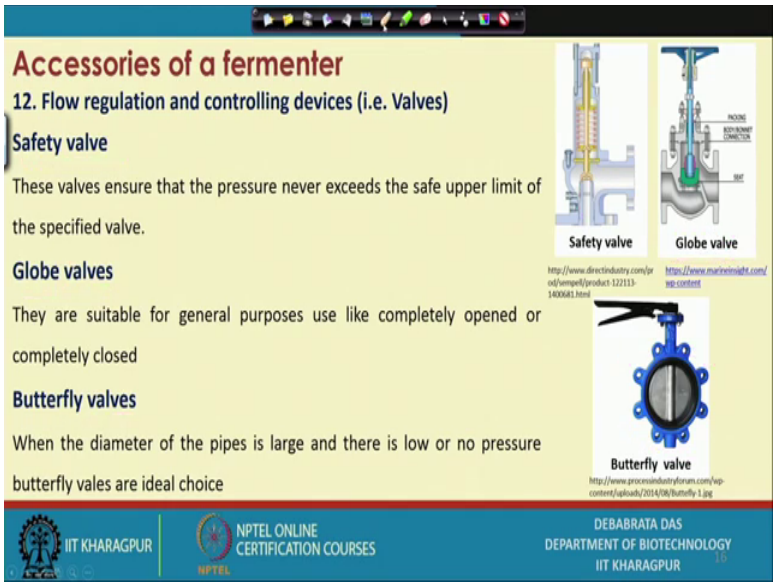

Now, flow regulation we have if you flow regulation and control we have safety valve I told you safety valve located at the top of the fermenter, justly if you somehow the pressure buildup take place. So, that before it causes any kind of damage, if the safety valve will blow so that the air will go out like this. So, that you know that the damage can be avoided, we have globe valve, we have butterfly valve different layer type of valve with that, globe valve is looks like this. This is mostly used in the water basin you can see that will clo because, slowly slowly you can open slowly slowly you can close, but butterfly valve can be used for sampling purpose also along with the ball valve, this we can instantaneously open and instantaneously you can close.

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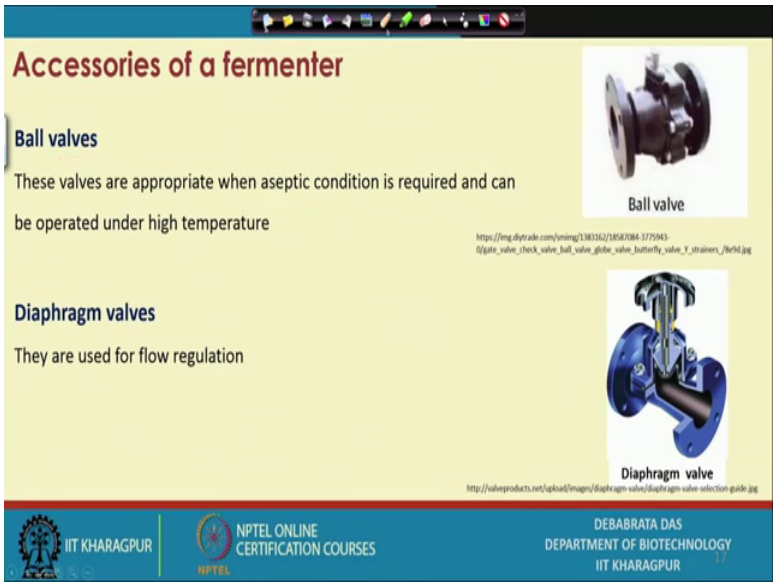

Now this is the ball value that I was talking about this is used largely for sampling purpose, the aseptic when aseptic condition we required can be operated the high temperature, diaphragm valve also you can be used for sampling purpose.

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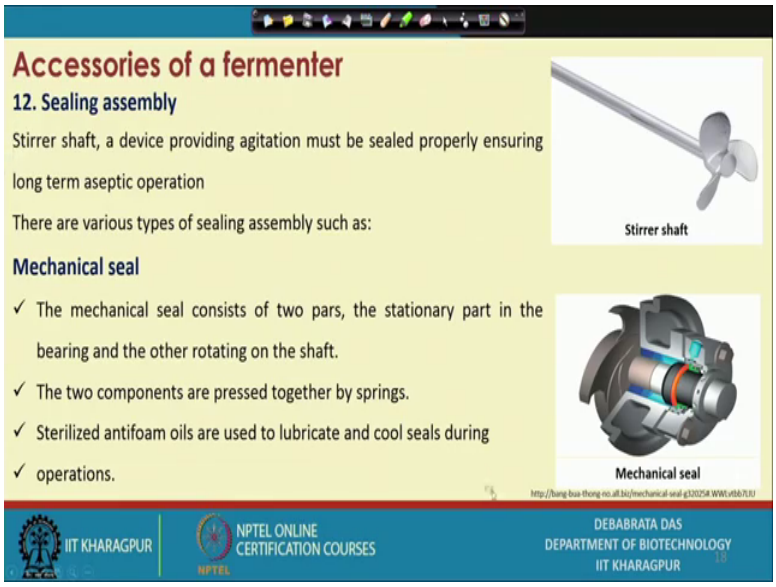

Then we have sealing at assembly this is the mechanical seal, I was telling about that this mechanical seal which is located at the top of the fermenter that plays very important roles. So, that air from outside cannot be should not enter into the system.

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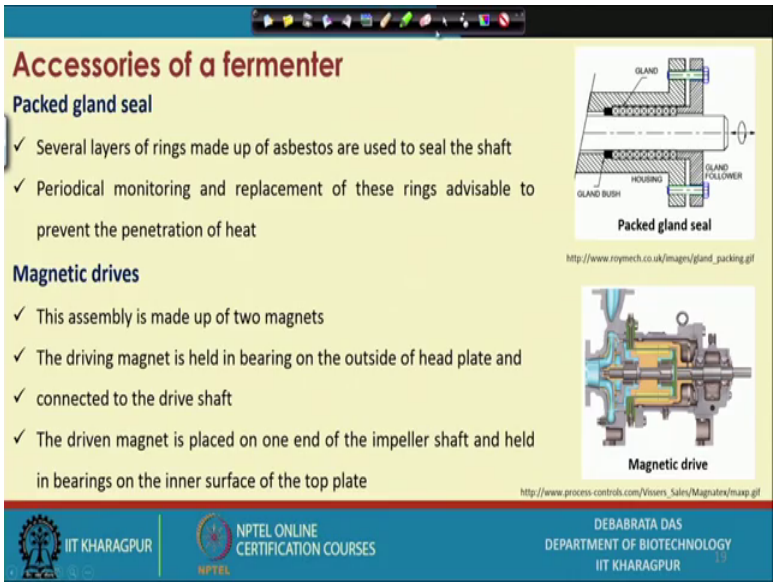

That that plays very important role, now packed a gland inside this is that inside this is that mechanical seal we have some packing material that that is shown here this is the shaft that we have. The several layers of ring made of asbestos are used as a seal to seal, this shaft periodical monitoring and replacement, only one thing I want to highlight that review this is the mechanical seal, then in case of biochemical industry we put some kind of line. So, that we can the antifoam oil antifoam oil. .

And you know this is this is realized antifoam oil with the help of pump, we put it inside and just to lubricate the shaft and, if we lubricate the shaft the friction, across the mechanical seal will be less and since we are passing at the high velocity. So, there will be always a positive pressure and if there is a positive pressure no air can enter into the system that is why we use the magnetic type. Now it is used to avoid any kind of contamination problem. So, this is the another and the recent development we have.

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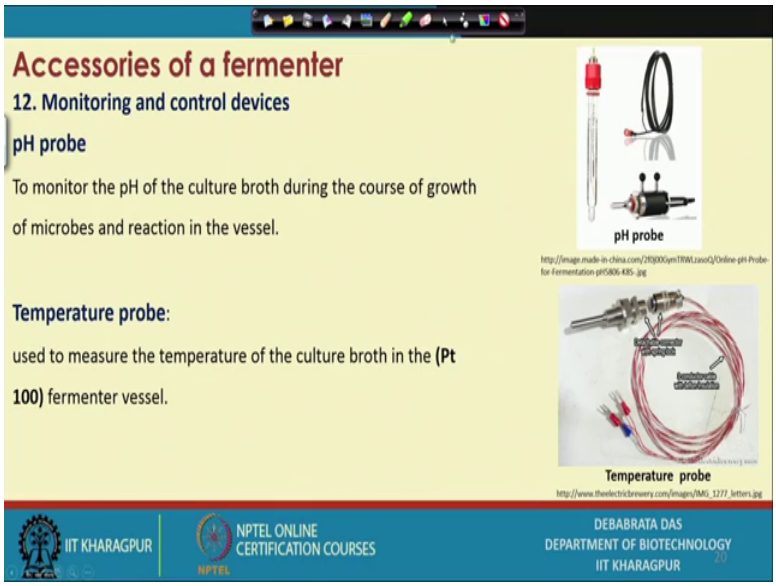

Then for monitoring we require the pH probe I told you, whenever you use any kind of probe first you have to calibrate, the pH probe whenever use the, we first we still have to calibrate with respect to our standard pH that is the pH solution. And temperature probe also that is the that is the temperature is the culture is measured and, this is made of bimetallic, we know that bimetallic it is not like mercury thermometer through is the metal that the temperature is a bimetallic, through the resistance difference in the resistance we measured the temperature.

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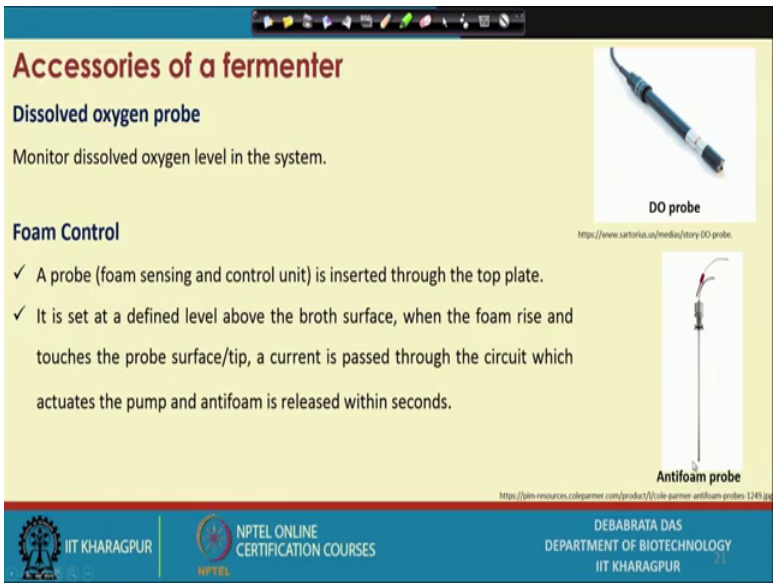

Now, dissolve oxygen probe that is also used for measuring, the dissolve oxygen from control I told you.

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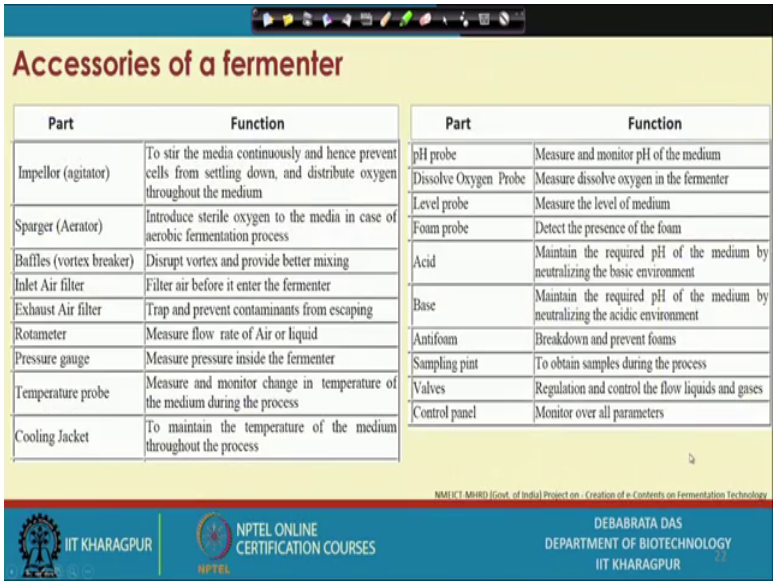

This is the sharp needle that that is present at the top of the fermenter which is just when your foam touch, suppose the this is the foam touch here, then it is connected with some your pump is connected with some antifoam oil tankard. And it will draw the draw the liquid and antifoam oil antifoam oil and, it will it will take the liquid and put it in the fermenter to subside the foam that is the how the foam control take place.

So, the this is the different type of this is the summary of the different parts that present in the fermented, this is the function of the fermented I do not like to go in details again, I already explained and this is the different parts and that is explained here. So, this is kind of repetition of that.

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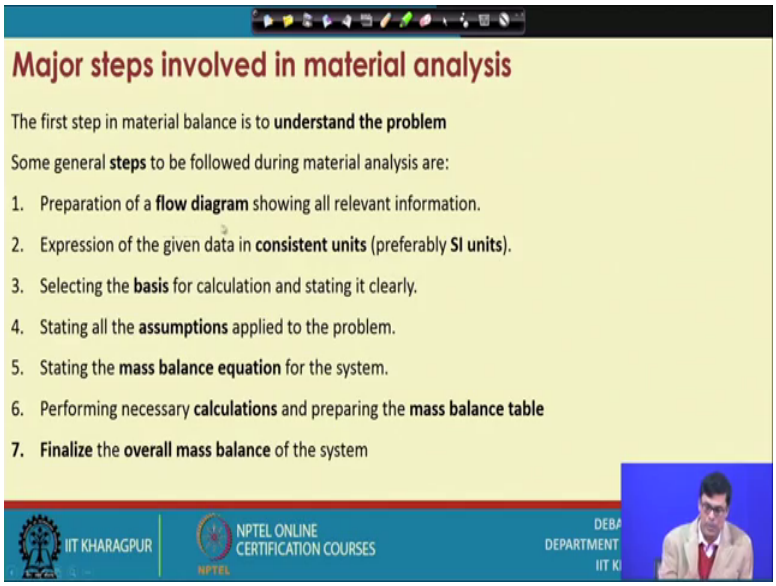

Now, let them one important thing is the material analysis of the process that that is the first we shall have to prepare the flow diagram, this is the flow diagram of the process that you know.

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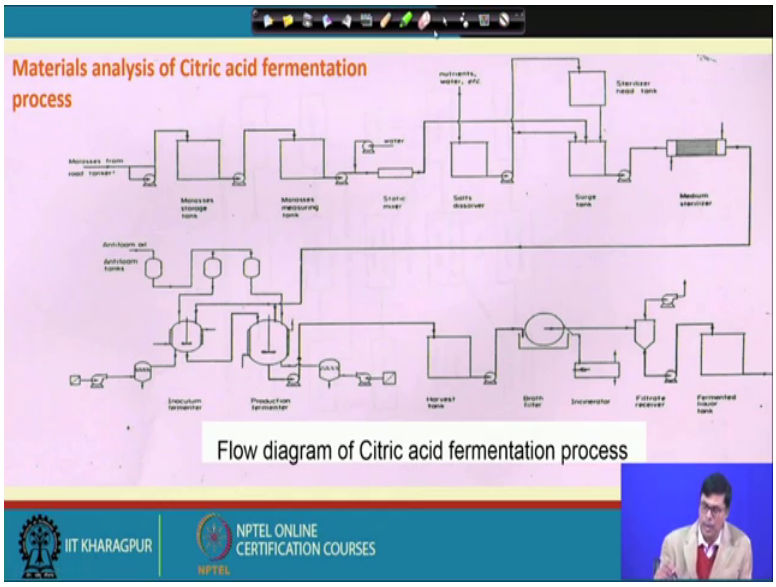

This is the I have given the examples of the citric acid fermentation process, where cane molasses is use as the raw material and, this is first store in the middle of molasses storing storage tank they need. This is located at the gate of the industry, but the molasses measuring tank and look at it at the fermenter fermentation plant and, then they form this

do you take the desired amount of cane molasses here, then we this contents about the 50 percent of sugar, we diluted with the water and with the nutrient.

And then we mix thoroughly before, it passes through the sterilizer and then after sterilization, we take it in the, this is inoculum vessel inoculum vessel for (Refer Time: 32:11) of the culture and, this is the production fermenter for production of the media. And this is the air sterilizer, I told you the capacity of the air sterilizer is 10 times higher, in production permitted as compared to the inoculum vessel and, after the fermentation is over, you take the harvesting tank this is the all the then it contents the cell mass also.

So, first you have to remove the cell mass with the help of rotating disc biology that rotary vacuum filtered and, rotary vacuum filter we use RVF, then we get the solid material cell we can burn it and, we can get the energy or and then the liquid material we take it and store it at the filters to reservoir, then we pass it through the purification unit for purification of the product.

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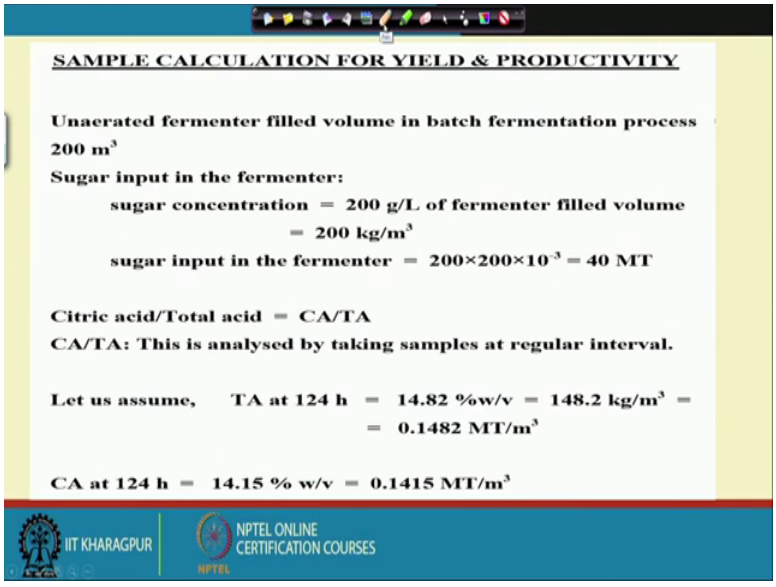

Now, let us see how we can do the analysis of this process, suppose unaerated unaerated fermented filled volume in the batch fermentation process is 200 cubic meter. And a sugar concentration is 200 gram per liter, then this is 200 gram per liter. So, I have already showed you that how we can convert the unit, this is the I can write that if you want to convert into k g. So, how what I can write that how we can convert to k g that 1 k g is equal to how much 1000 gram.

So, gram will cancel then you can convert to k g, the liter you can call convert to cubic meter, how we can convert to cubic meter per cubic meter 1 cubic meter means how much 1000 liter, the liter will cancel. So, 100, 1000, 1000 will cancel; that means, that 20 gram per liter is equal to 20 k g per cubic meter, you can write like this. So, sugar input we can easily calculate we know that that 200 cubic meter so, if you multiply that will get the 40 made metric tons of sugar.

Now, carbon that you know that every industry, when we work with any kind of industry that we have two type of analytical devices for finding out the product concentration, one is a very very first analyzing device and another is exact and analysis of your product. So, we have seen most of the if you want to determined the product concentration exact product concentration only they take lot of time, particularly for citric acid estimation it takes more than one time, one year one day. So, we what we do in the industry that we have a titration method with respect to dilute sodium hydroxide solution by using methyl orange as the indicator, we find out how much sodium hydroxide is required to consume that you know the sodium hydroxide solution and, that is co correlated with total acidilled.

And then from that we can find out and, and that is why you can see that we have a ratio of sighting acid and total acidille. So, now total acid as the 250 degree centigrade is 14 point and, this is this a 14.82 percent weight by volume well; that means, 40 148.2 k g per cubic meter. So, you can easily find out that what is the in terms of metric tons per cubic meter and, we also similarly if you know the concentration of citric acid, you can find out in matrix and so, if you multiplied by 200 cubic meter, you can easily find out the how much metric ton of citric acid is produced by your industry, this is how we can we can do the material analysis, in your say in your in your in your production fermenter.

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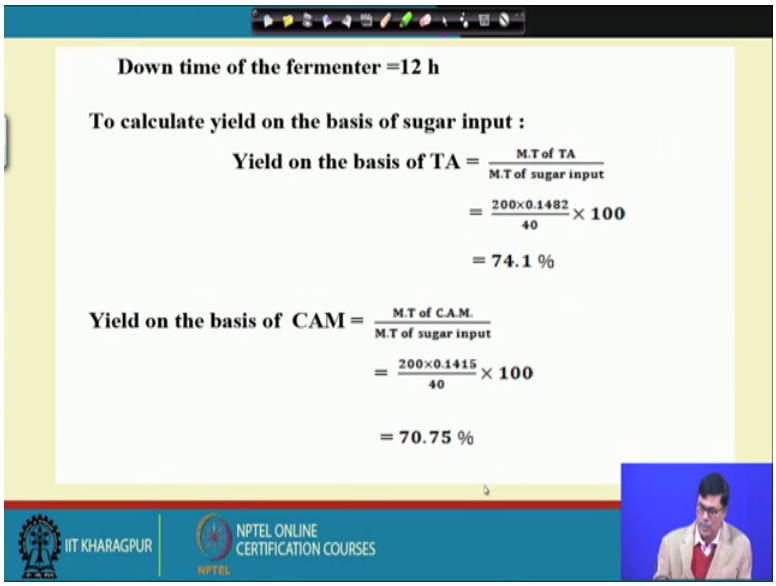

Then as the suppose your downtime is downtime is 12 hours, and time of fermentation is suppose the 140 hours the total time will be 140 plus 12 hours that will be total time, then now how we can calculate the yield, the metric tons this is the matrix tons of the total acid and, this is the metric tons of sugar yield sugar that is the input in the system, then the we calculate this 74.1 percent. The conversion efficiency any kind of industry plays very important role, similarly the citric acid available in two different forms one is cam, another is a citric acid anhydrides. Now cam, if you we have already seen the cam concentration is this, and if this is the sugar yield so, we can easily find so, if you compare the total acidille and total citric acidile always compatibly it will be less as compared to that.

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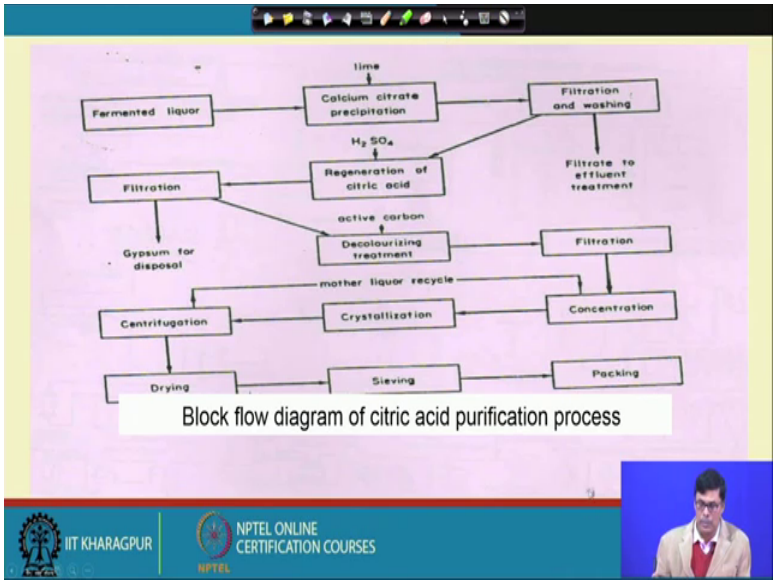

Now, question comes that how you can analyze the downstream processing, this is the fermentation block, then we you treat with lime and, then to just to precipitate the citric acid in the form of calcium citrate, then you filter it out you take out the calcium citrate, then hydrolyze with sulfuric acid and, then it pa it passes through the filter filtration process then where the gypsum calcium sulfate will be separated out here is the your citric acid you he is contain a lot of color, we use the activated charcoal to remove the color. Then we again do the filtration to remove the activated charcoal, then we concentrate with the help of evaporator that evere that evaporation, then after evaporation we cool it down and, do the crystallization to separate the crystals and, then we pass it through the centrifugation machine.

And finally, we dry the sample after drying receiving, but the crystal size will be different and then, we pack it in the in the polythene polythene bag. .

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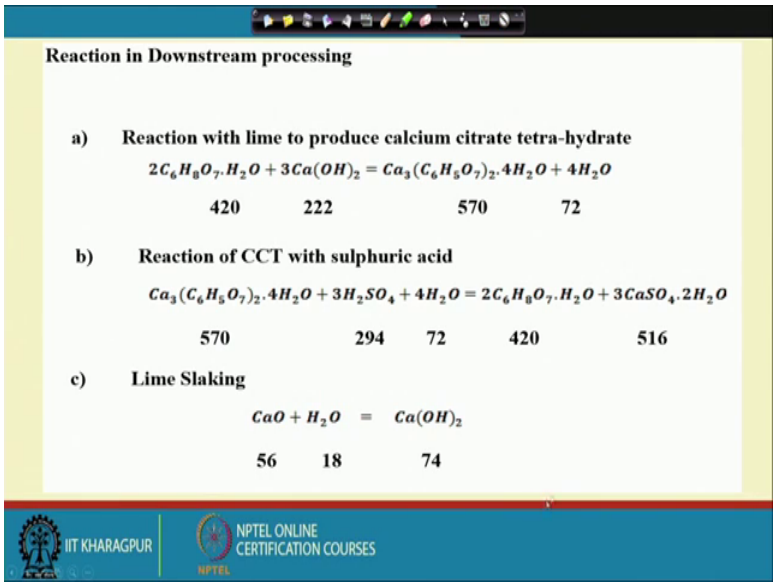

This is the how we can we can we can we can do that now, question come how we can do the the material analysis of the process. Now for citric acid calcium sulfate citric calcium citrates citrate formation how much lime is required. Now as you know the lime is produced this calcium hydroxide is produced from where calcium oxide, this is the H 2 O is gives like this. Now how calcium oxide is formed from calcium carbonate, if you calcium carbonate if you heat, it if it will calcium oxide and cal carbon dioxide.

So, this is this is the available in the in the mines. So, this is the you can easily calculate that how much if you know the stoichiometry, you can find out how much kazon lime is required and, if you know how much calcium citrate is form, you can find out how much acid is required. And if you if you know this is how much the acid is required, you can you may also find out how much gypsum means this is called gypsum, gypsum means produces a good ingredients for the cement producing industry.

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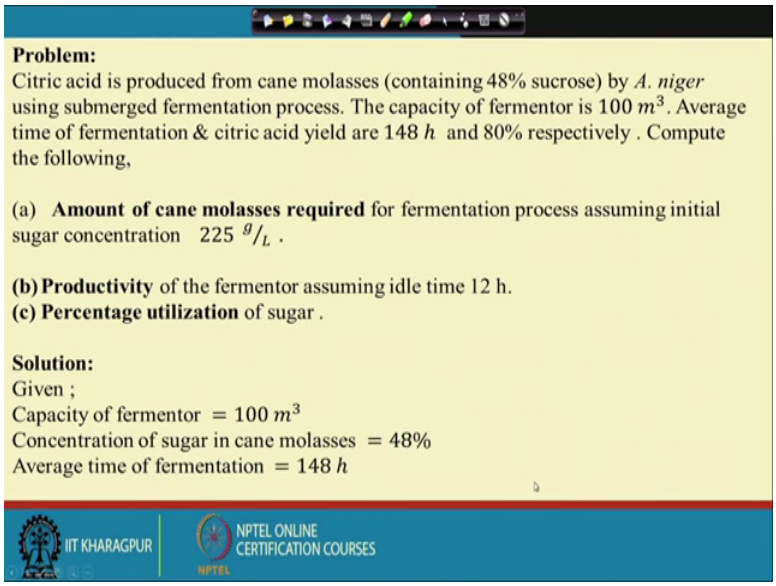

So, this is how we can do the material analysis, and this is the two problems I have given here, I hope you can you can do it by yourself.

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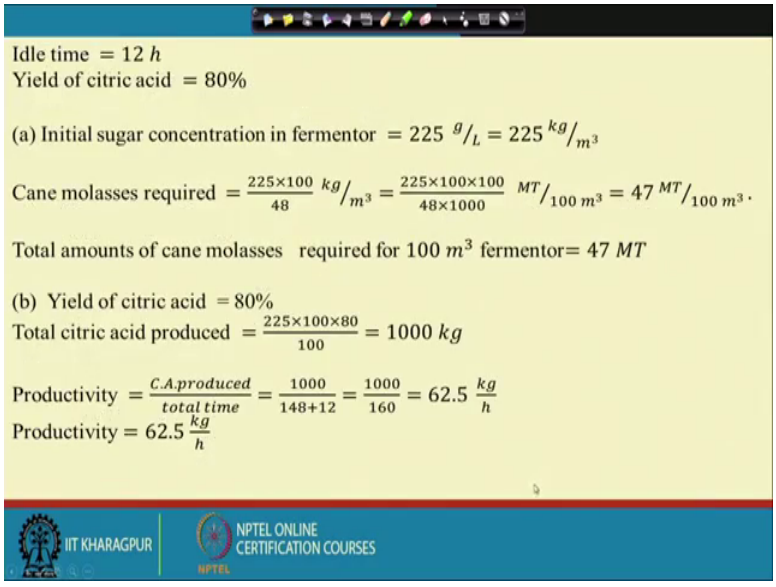

There since time I have little less.

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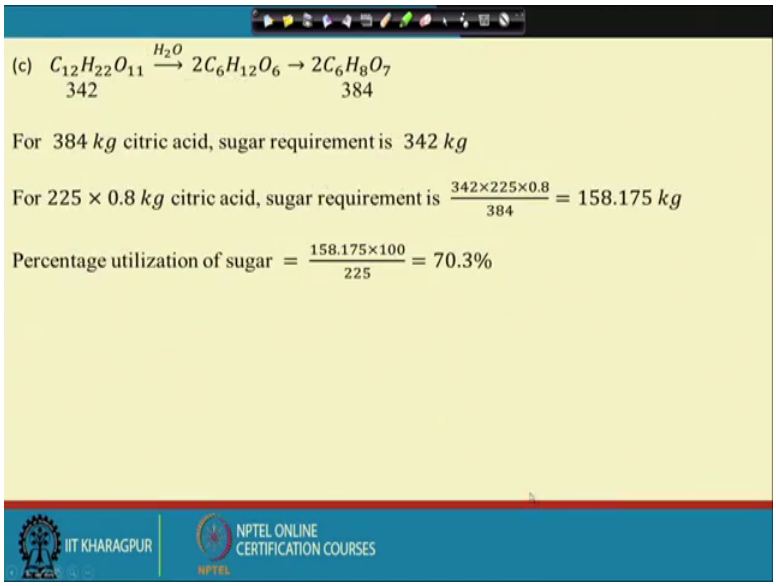

So, I I am just leaving it to you just you solve try to calculated by yourself.

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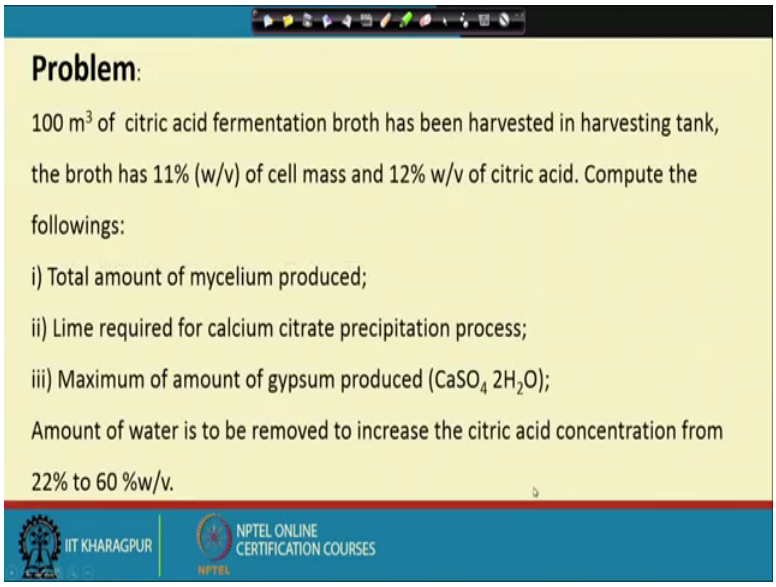

I hope you understand that. Now so, in conclusion I want to point out that that how I try to give you the glimpse how the fermentation industrial, fermentation processes are operated and, what are the different accessories present in the fermentation process and, main our main criteria is to maintain the stability of the system. So, that no contaminants can inside can enter into the system and, how the temperature, how the pH, how the dissolve oxygen concentration can be monitored, how the sample can be drawn by the

fermenter, all this thing I try to point out and finally I try to show you, this material analysis with respect to citric acid fermentation process.

Thank you very much.