Industrial Biotechnology.
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Lecture-34.
Citric Acid Production (Continued).

Welcome back to my course Industrial Biotechnology, now in the last lecture I tried to cover this citric acid fermentation process and now I try to mention that the citric acid has tremendous potentiality in the industry, because it has lot of use and citric acid actually this technology has been marketed by John and Starch Limited UK throughout the world. And initially the it was the technology work established by Pfizer and then this it usually produce by aspergillus niger and this aspergillus niger why it is used because the citric acid concentration is very high in the fermentation broth.

And we observe the TCA cycle how the citric acid accumulation take place, we we shall have to inhibit isocitrate dehydrogenase then and only then the citric acid accumulation will take place. We told you that if the pH is maintained as 4 or 6 we will get more level of gluconic acid and oxalic acid but if you if you do not control the pH allow the pH to drop down will get the accumulation of citric acid.

We found that in normal citric acid fermentation process that first 24 hours there is no accumulation of citric acid after 48 hours there is a little accumulation of citric acid, inhibition of isocitrate dehydrogenase but after 96 hours there will be total inhibition of aconitase and isocitrate dehydrogenase. The two type of fermentation that is used can be used for citric acid fermentation one is surface fermentation another in submerge fermentation.

Surface fermentation the organism grow on the surface of the media and submerge fermentation the organism grows throughout the medium. So in the industry mostly we use the submerge fermentation process but initially we should have some information on the solid fermentation process because 20 per cent citric acid still yet to produce through the surface fermentation process.

Now let me continue that this is I also explain in the last lecture what are the different composition of the medium, how plays very important role I mentioned that not only the sugar content is very important because your 1 mole of sugar 1 mole of glucose rather is produced 1 mole of citric acid and citric acid is available in two different forms. One is citric

acid anhydrous and citric acid monohydrate. So when you when you calculate the yield it is coming about in case of monohydrate citric acid monohydrate is 123 per cent.

In case of citric acid anhydrous it is 112 per cent so it is more than 100 per cent so now but you know this citric acid production that depends on the minerals contents in the fermentation media, it has been found iron and manganese plays very important role, because I told you this two that metal ions plays important role in the metabolic pathway. The manganese if we reduce the concentration that will produce free ammonia in the fermentation medium that and trigonise the inhibition effect of citric acid to phosphofroctukinase.

But the irons since it is the co-factor of accumulated enzyme, so if you remove that the activity of the accumulated enzymes will be reduced so you have more accumulation of citric acid in the fermentation broth.

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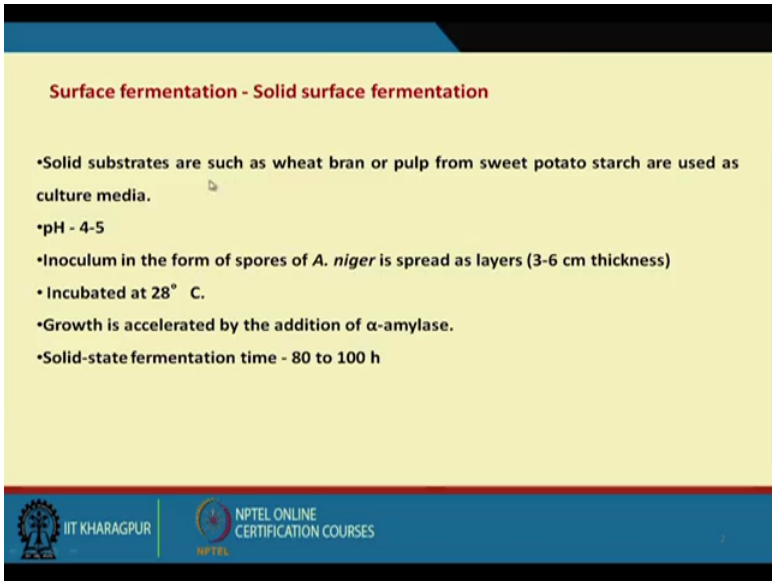

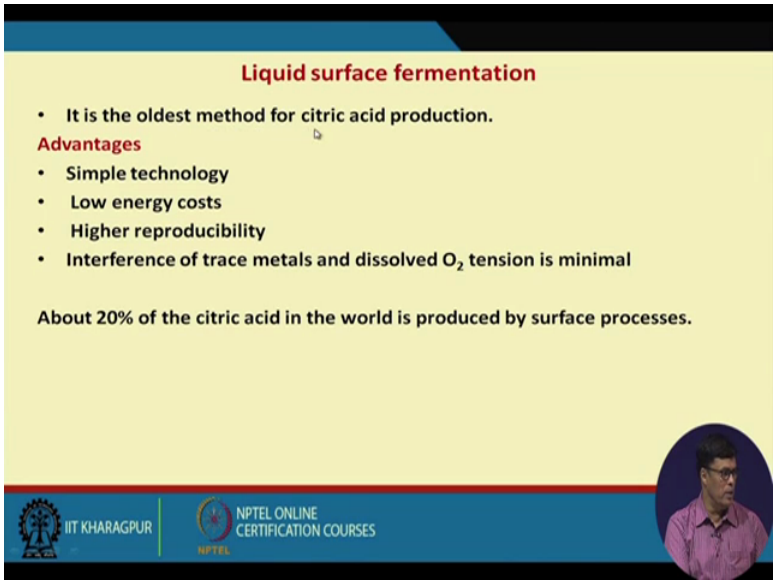

So this is now let me talk about the solid state fermentation process the solid substrate such as the wheat bran or pulp from sweet potato starch are used for the culture media. And then pH is 4 to 5 inoculum in the form of spores of aspergillus niger spread as layer 3 to 4 centimetre thick layer formation is there then incubation at 28 degree centigrade. Growth is accelerated by the addition of alpha amylase solid state fermentation take time is about batch fermentation is 80 to 100 hours. The old liquid surface fermentation process because solid surface liquid surface both are surface fermentation process but in liquid surface fermentation medium would be liquid.

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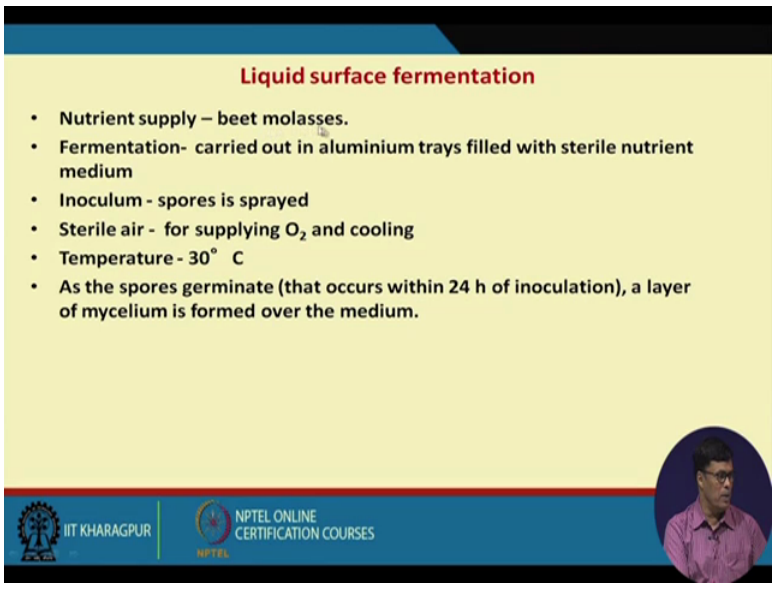

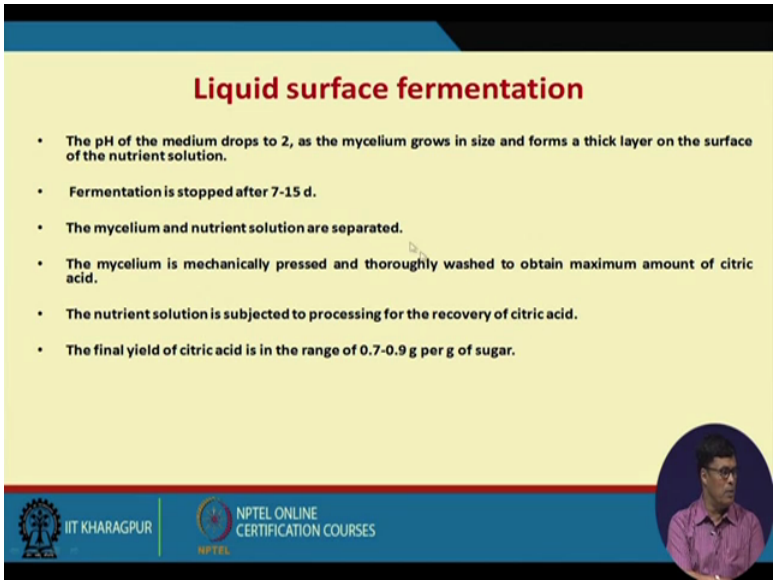

And solid surface fermentation the medium will be solid and here this is the oldest method of citric acid production simple technology, low energy cost, high reproducibility and interference of the trace element and dissolved oxygen tension is minimum . And 20 per cent citric acid in the world is produced by surface fermentation process by using liquid media. The nutrient supply is the beet molasses that is used fermentation carried out in the aluminium tray filled with sterile nutrient medium then you look spores as sprayed sterile air was supplied and then temperature is 30 degree centigrade.

Spores germination takes place and layer of mycelium forms on the surface the pH drop to 2 and mycelium grow in size and forms the thick layer on the surface of the nutrient the fermentation stops after 7 to 15 days the mycelium and nutrient solution were separated and mycelium is mechanically pressed and thoroughly washed to obtain the maximum amount of citric acid.

The nutrient solution is subjected to processing for the recovery of citric acid, final yield of citric acid is 0.5 to 0.7 per gram of sugar. This is yield what we had in the liquid surface fermentation process. Now let me give you the more information on the submerged fermentation process with largely in operation in the industry and I worked with Citrozia biochemicals we used the submerge fermentation process for the production of citric acid.

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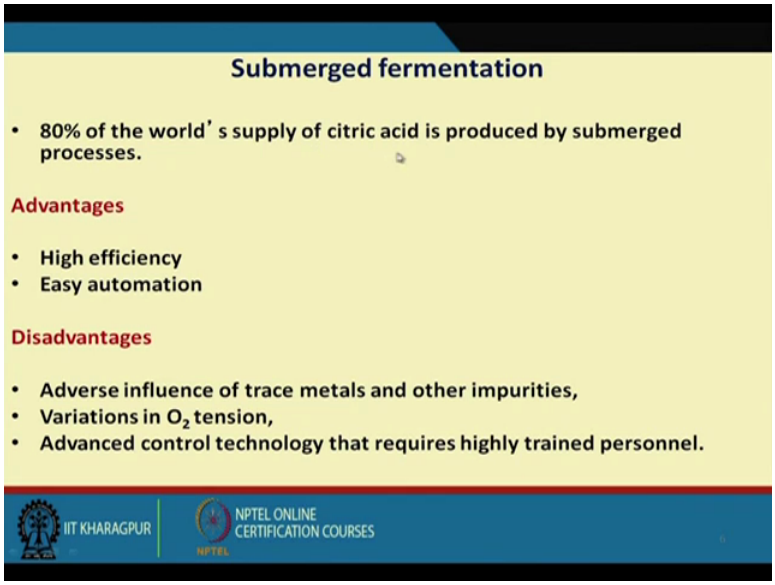

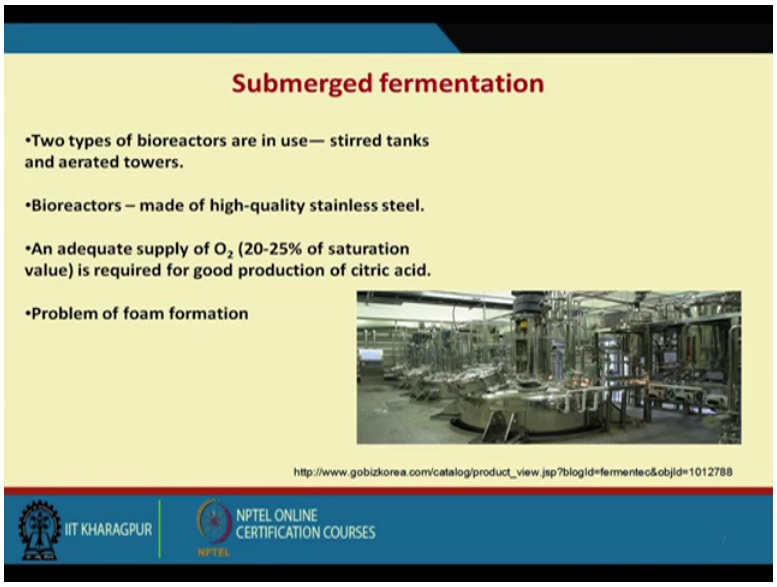

Now 80 per cent of the world's supply citric acid is produced by the submerged fermentation process it is considered as a highly efficient and easy automation. This advantages the adverse influence of the trace metals and other impurities and variation of oxygen tension, advance control technology that require high trained personnel , because because this is the kind of since it is the fast process so you require little bit trained personnel for the operation of this process.

Now that two types of bioreactors are used one is we have stirred tank reactor, another aerated tower reactor what you call I told you that the air lifter fermenter is used but it is less used in the industry for citric acid production. Bioreactors is made of high quality stainless steel because the reasoning is that the citric acid has some corrosion properties so the quality

of the stainless steel is usually used high quality stainless steel usually used, when I discussed about the material of construction I told you that how the metal ion plays important role for improving the characteristics of stainless steel.

Particularly chromium and molybdenum that increase the acid resistance and corrosive characteristics of the stainless steel. So this is this is the stainless steel 317 is usually used for the citric acid fermentation process the adequate supply of oxygen 22 to 25 per cent saturation is required for the good production of citric acid. Now problem of foam formation that is quite common because since it it is the aerobic fermentation, the foam fermentation is quite common . So to have this to subside the foam we shall have to use the anti foam oil and and for sterile antifoam oil we put in the reactor to subside the foam.

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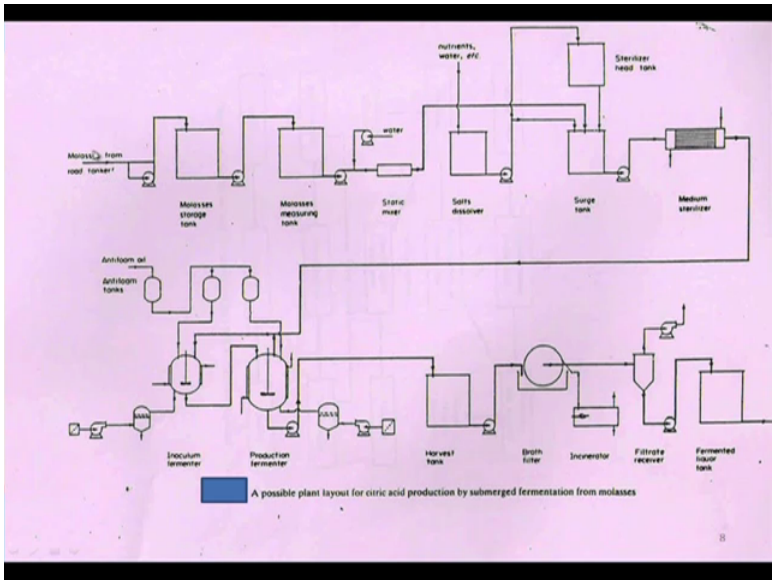

Now this is the flow diagram for how the citric acid fermentation process is usually carried out thus the molasses from the from the road tanker because it is come in a tanker and from the tanker I told you that the molasses storage tank what you call MST that is located close to close to the main gate of the factory and where this is a very big tank where we stored the molasses for throughout the for whole year, because molasses is a seasonal product and since it is a good raw materials for the citric acid production we need this.

But we have we observed we have acquired scarcity, off season we have the acquired scarcity of cane molasses because in India cane molasses also used largely for the ethanol production. So we we replace the cane molasses by using the hydrol, hydrol is the by product of the glucose industry that you know after crystallization of glucose that that in the mother liquor

contains about the 50 per cent of sugar that is replaced by the molasses. So we successfully replaced a portion of the cane molasses during the off season and we find suitable for the citric acid production.

Then this is molasses measuring tank what you call MMT that is located in the fermentation plant and so whatever day to day requirement is there we use to pump this to the to the fermentation plant and this pump is the special type of pump I told you because this is since this is the very viscous liquid we use the gear pump to drag the cane molasses from NST to MMT.

And after that it contains about the 50 per cent of sugar so you have to dilute it and because in the fermentation medium the citric acid concentration is about 10 to 11 per cent so we know that so so so initially we shall have to use sufficient amount of water for the losing and maintain the sugar concentration. Then we shall have to add some kind of nutrients as for as for cell must production is require where we require sufficient nitrogen source and other material.

So we put it here the and this is this we recycle again and again to ensure that this medium is perfectly mixed you know it is homogenise. After ensure that this is perfectly homogenised then we pass through the medium sterilizer in the medium sterilizer we take it here so we have two vessels we have inoculum fermenter we have production fermenter we can take it to the inoculum vessel and we another we can take it to the production fermenter. Now here I want to again point out I already mentioned in my previous lecture mineral concentration in the fermentation media plays very important role.

So cane molasses contains significant amount of iron and manganese, magnesium so that is to be removed before we use this cane molasses for the production fermenter. For the inoculum vessel we do not report any type of pre treatment but for the production fermenter we shall have to remove the iron and manganese and here you can see the anti foam oil tanker this we have sterilize anti foam oil.

This is we have this is used we use for subside the foam and this anti foam tanker is also connected with this mechanical seal I told you when when I explained the bioreactor this mechanical seal that shaft also it is like this lubricated with the help of this anti foam oil, so that friction between the shaft and the mechanical cell is minimum. Then after the that so

what happens in this fermentation process that that you know that this this inoculum vessel and production fermenter they are located in the fermentation plant.

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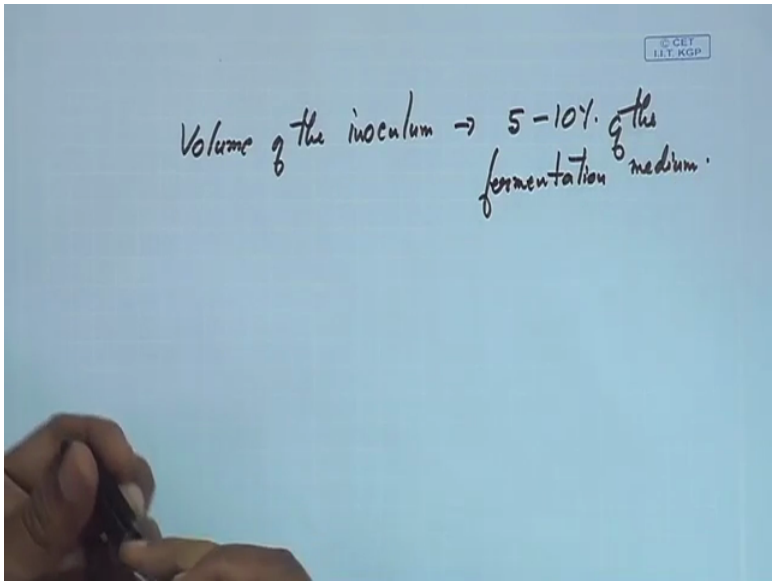

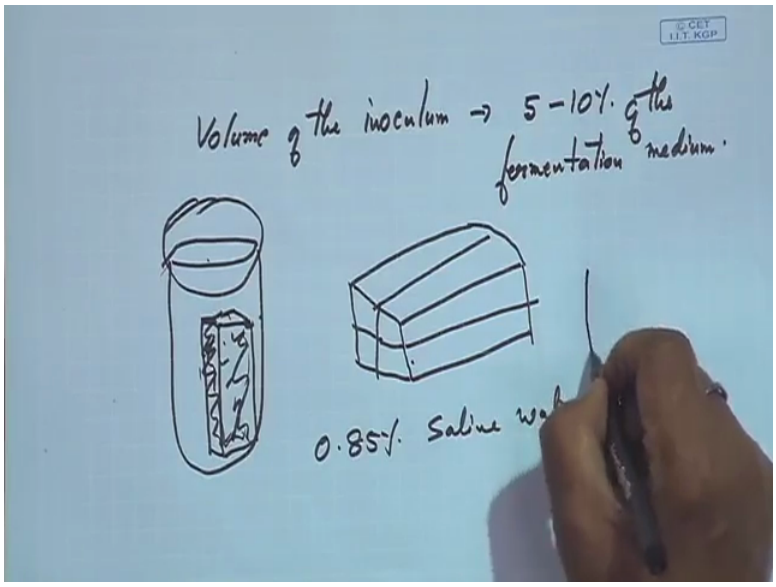

So and usually the volume of the inoculum volume of the inoculum is usually 5 to 10 per cent of the fermentation medium, so the the Citrozia biochemicals where I worked the capacity of this reactor was 200 cubic metres. So that this should be 10 to 20 litres so 10 to 20 cubic metre our side of the medium was we find the 40 cubic metre is good enough for the for this production fermenter.

Now question comes that very interesting question that how we prepared the seed culture in the lab for this fungal fermentation process because we now that you know fungi can not only use citric acid fermentation process but also used for the in different anti biotic fermentation

process. Now all the fungi most of the fungi they are filamentus growth and very difficult to we have to quantification.

Quantification of the organism very important plays very important role, in case of yeast cultivation what we can do we can we can count the yeast cells so we can we can number this we can what number through R and D we research and development we try to find out what number is will be most suitable for the production fermenter so that number we can we can easily in case of (())(15:16) .

Now question comes how we can do in case of fungal fermentation process where the cell has the filamentas growth. So what we do in this fungal fermentation process we used to do this correlation of the fungi because why is correlation of the fungi because spores you can count we can under hemocytometer you can count the spore and correlation of the fungi take place under stress condition, what is the stress condition we maintain mostly the moisture is the stress condition.

So I can show you the technique that is used in our industry how we prepared the seed culture in the lab so it is very simple I can tell you that you know we can we have bread that the kind of bread we have available in the market is like this that is used during the breakfast this kind of bread small bread we can have so we can cut it with the help of knife we can cut it. We can we can just cut it like this so we can afford pieces you know that you know small bread we can cut it like this we can have four pieces and then their white mouth, this that test tube that is cotton plug this is cotton plug then here we put this we put this bread.

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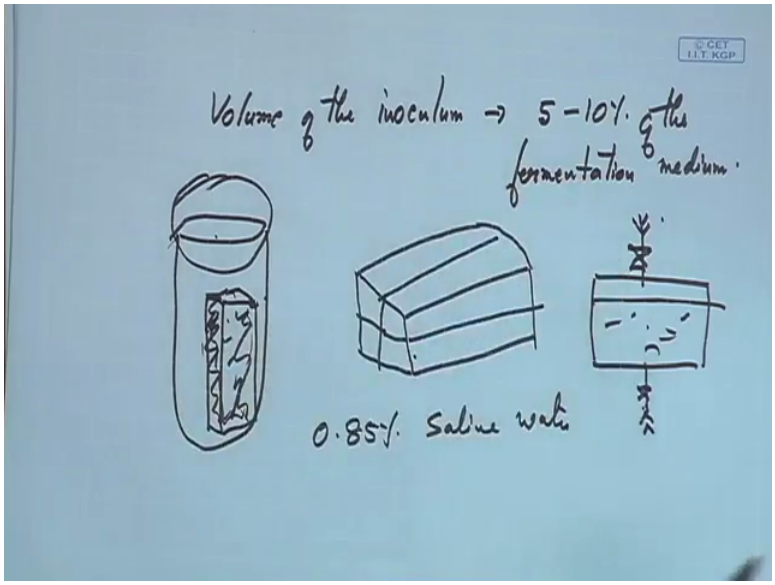

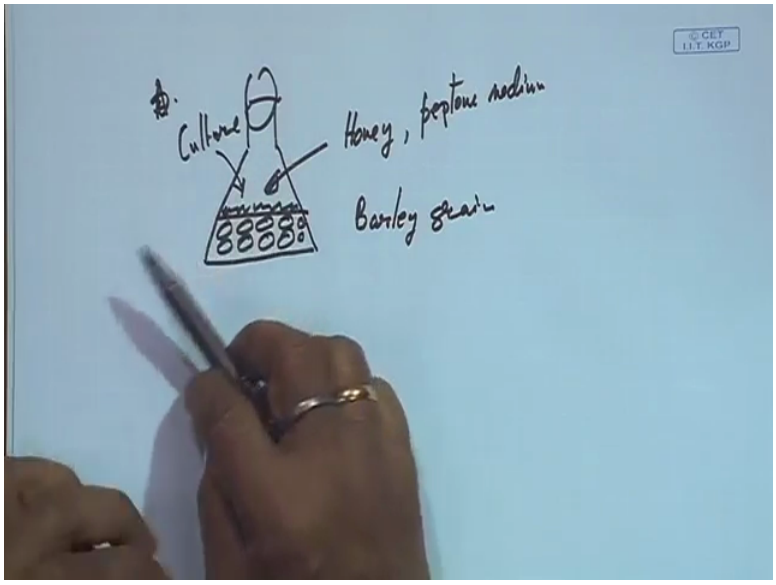

And then we inoculate the organism here. Now here in the bread do you I told you bread is produced by using yeast because yeast is used and yeast has vitamin B which is required for the growth of the required for the growth of asperagillus niger. So when you keep it and inoculate this organism so it has the limited moisture content so after some time this will be totally totally covered with spores. This this totally cover with then we can we can make we can we can make a suspension in point 85 per cent saline water, saline water and then this we can we can find out that what particular concentration particular concentration of spores is required for the inoculum vessel.

We develop the particular concentration of spores and then we put it in the seed can I showed you the seed can how it looks, seed can it looks like this. Now here we have a we have a valve here and we have a valve then we have here we have nut and bolt arrangement when you take it to the inoculum vessel this nut and bolt arrangement we connected and we sterilize this line and aseptically we transfer the spores.

This has the spores inside then and again we sterilize the line and open this and close this valve and open this nut and bolt and take it to the laboratory. This is how it can be done now another thing I want to show that I did my Ph.D. from IIT delhi and during my stay there was a international conference and there we demonstrated one experiment how to develop the spores by using different types of fungal culture and what we use that there is the interesting thing that I want to show you.

There is very simple technique through which we can develop the spores. Here we can take the barley grains this is the barley grains we can take. This is barley grains and here we put

the honey peptone medium then we inoculate the cells culture asperagillus niger or anything whatever fungi culture we inoculate this, now we keep it in the incubator for sometime after sometime you will find mat formation this Is at the surface there will be mat formation.

So what we can do we can take the culture that you know bottle out and we can strike the hand like this because since it is a solid particle as soon as we strike then this particle will be this mat that here is formed here that will be disturbed and this cell will be this will be coated on the surface of the barley grain. Then again we keep it in the incubator after 6 hours again we take it out again we strike it, like this after sometime we find that each barley grains is totally covered with several spores.

Is covered with several spores then we make a solution we prepare the 0.85 per cent saline water and shake it and take the spores out we can we can easily any organism we can produce spores like this, though we can use particularly might be avert that penicillin is produced by penicillin cyzo yenum that is also very good colour of spores you know green colour in case of asperagillus niger we produce a black colour of spores different fungi they have different colour of spores production is there.

So this is after that so you can here you can see that since the capacity here 5 to 10 per cent as compared to production fermenter we have different capacity air filter we use for the different purpose. Here it should be 10 to 20 times higher higher than that of that inoculum vessel then after the fermentation is over you take it to the harvest tank. Now this unit this whole unit remain in the fermentation plant but after the fermentation is over I told you how we take the fermentation broth to the harvesting tank.

We just here we have air outlet, in the air outlet if we close then there will be air pressure here with this air pressure even you do not have to use pump without using pump you can drag the liquid from production fermenter to the harvesting tank. And when it comes to the harvesting tank this is located in the recovery section or the purification section then it first it passes through the rotary vacuum filter you can see this is the rotary vacuum filter. When it passes through this cell mass is to be separated after the during the fermentation process cell mass is very important because cell mass plays major role for the production of citric acid. After the fermentation process the cell mass is to include in the reaction mixture so you have to remove the cell mass first by using rotary vacuum filter that I shall explain in details.

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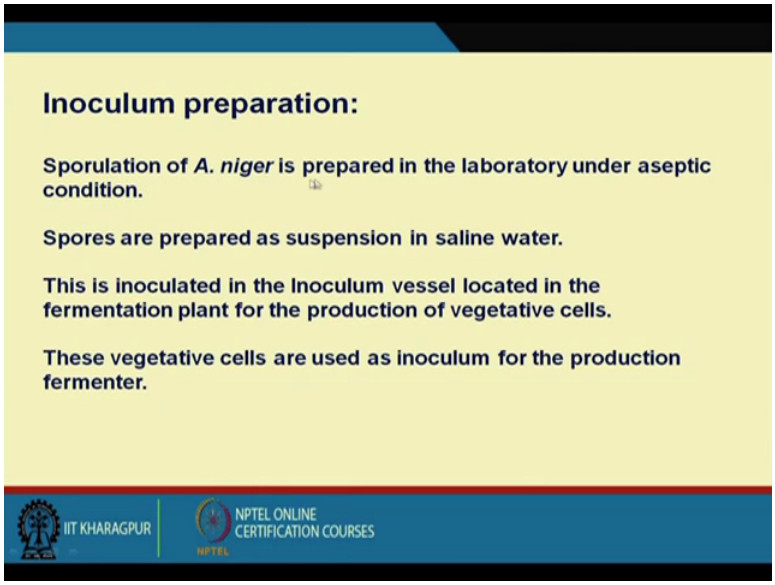

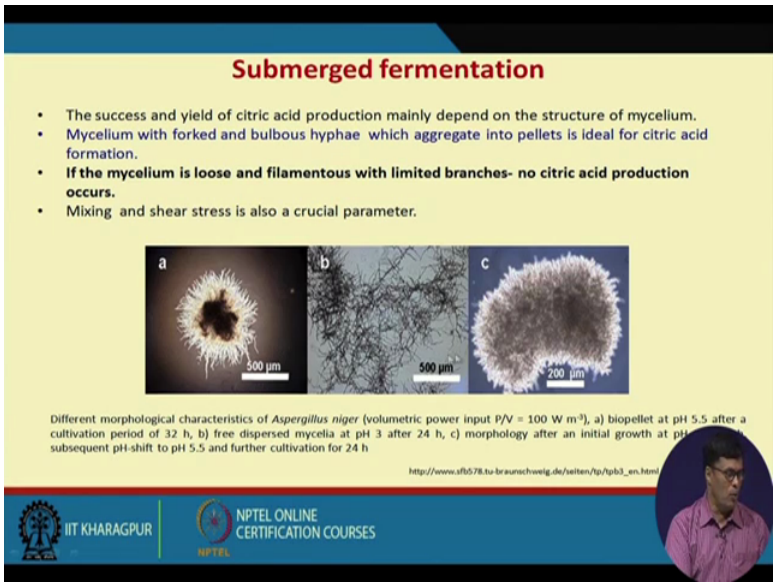

Then that filtrate you take it out and put it in the filter reservoir and that you put in vacuum so that you know the air content here, it should be oxidation do not take place, then then we further purification is taking place I shall discuss later. Now inoculum preparation I was discussing that sporulation of asperagillus niger is prepared in the laboratory under aseptic condition. Spores are prepared in suspension in saline water this is inoculated in the inoculum vessel located at the fermentation plant for the production of vegetative cells because when we do the inoculation in the production fermenter that should be vegetative cell that should not be the spores.

And these vegetative cells are used as the inoculum for the production fermenter, so here there is some important aspect of the morphology of the of the cells that the success and yield

of citric acid production mainly depends on the structure of the mycelium. The mycelium with forked and bulbous that hyphae which aggregate the pellets and is ideal for citric acid formation.

So this is this says that if the mycelium is loose and filamentous is limited branch no citric acid production take place so the mixing and shear stress is also crucial parameterisation before that one typical characteristics of citric acid fermentation or fungal fermentation process is that after there at the initial stage of fermentation you have to keep the agitator mechanical agitator ideal for sometime.

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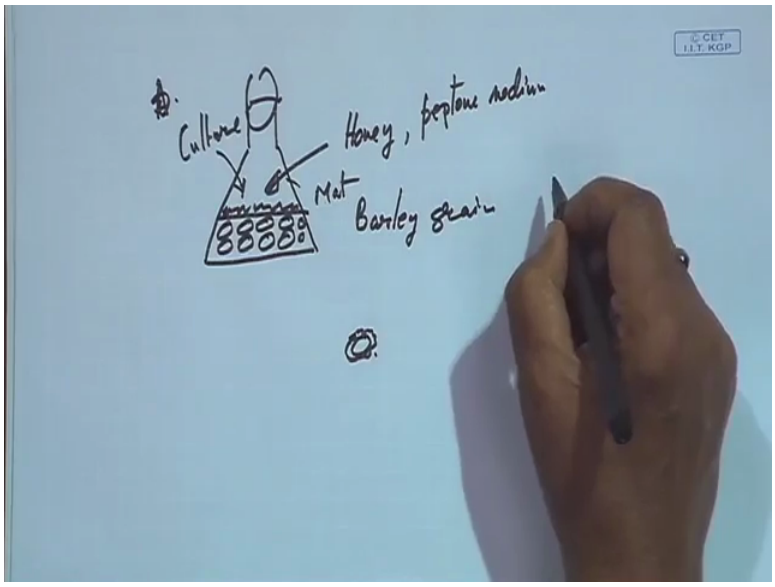

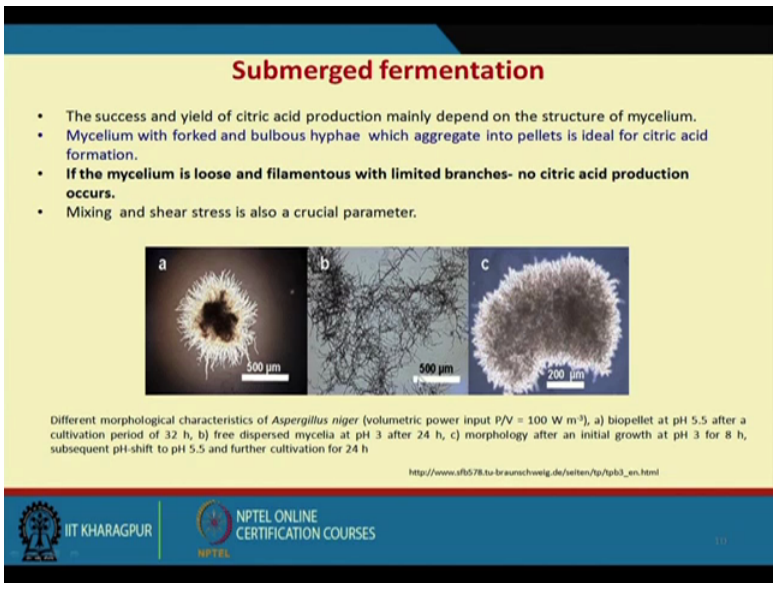

I can give the example that usually the inoculum vessel the time of fermentation is 33 hours and you know ideal time is 10 hours approximately 10 hours is the ideal time. Now in case of

production fermenter I told you that time is 124 hours to 144 hours so and here the ideal time is 30 hour, this is the ideal time here the ideal time. This is the total fermentation time. So this this time you have to give if you give the time then and only the mycelium will build up and then then the purpose of agitator to keep the cell in suspension in the fermentation medium so they feely interact with the medium and give the products.

So that is that is why it is required so different morphological characteristics of the aspergillus niger is this bio pellet at 5.5 after after cultivation of 32 hours this is you can see and pH disperse medium at pH 3 after 24 hours is like this mold branches is there and morphology after the initial growth pH as 3 for 8 hours subsequent shifted to 5.5 and subsequent 24 hours is like this. So you can see how the morphology of the organism they differ from time to time.

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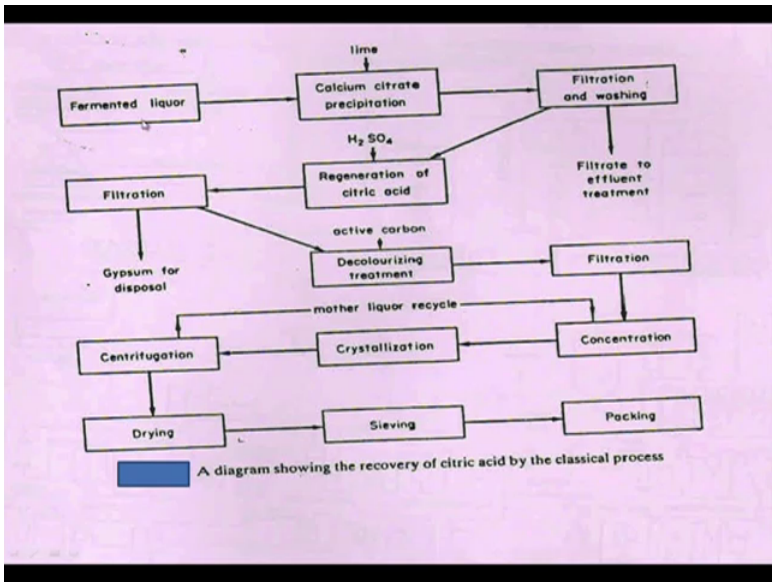

Now if you look at the citric acid production process it can be broadly differentiated in these steps that the crude raw material we take we take suppose we take cane molasses here then we do the upstream processing, upstream processing means we have include the air sterilization, medium sterilization and pre treatment of the raw materials or preparation of the raw material, then we take it to the fermentation process then after fermentation process we get the good product and through downstream processing we purify the product we get the citric acid.

Now this is this is what I want this is the downstream processing of the purification process of the citric acid industry, the fermented liquor we take it here. In the first I told you that in

the previous slide I I mentioned that I can show you there that here I told you the after the removal of that cell must we take it to the filtered reservoir.

And after this we take this is the fermented liquor this is the filtered reservoir filtered material we take it here. This filtered material we first we treat with lime why we treat with lime because when we treat with lime, lime is nothing but calcium hydroxide calcium hydroxide this calcium hydroxide react with citric acid and precipitate in the form of calcium citrate.

And this is the solid mass so so as soon as the calcium citric is precipitated out then we pass it through the filtration, this is called pannevis I shall show you how it works and (())(27:27) and we wash this with water and then we we discard the filtrate. Filtrate is the industrial effluent and now I want to point out is this is the typical type of effluent because since we are treating with calcium hydroxide, the this filtrate will contain lot of calcium iron. There is the possibility that the effluent contains lot free calcium iron.

So this is the typical character not only organic material but also calcium iron content will be very high then then that the solid material calcium citrate we take it to the treat with concentrated H2 SO4 and then it react with H2 SO4 and produce citric acid and and gypsum calcium sulphate to H2O and after that calcium sulphate is a insoluble mass. You pass through the the gypsum filter where we can we can get the solid gypsum which is which is required for the cement industry.

And then this filter at contains the contains the citric acid since it is produced from cane molasses there is the possibility that it has lot of colour and this citric acid crystal is perfectly clear so it initially it is required that we should remove the colour from that. So we use the activated carbon just to remove the colour just to remove the colour which has the bleaching property and when this after the treatment we we remove this calcium we remove the activated carbon.

I told you carbon is used very fine particles and to remove the fine particles sometimes we shall have we shall use we use some kind of filtrate because one filtrate I can tell you pannevis which is cellulosic material that absorbs this fine particle on the surface there is a bigger particle that we can easily separate it out. After that we concentrate it pass it through the evaporator and it is the tubler evaporator and when because this this contains about 22 per cent citric acid.

This 22 per cent citric acid when passed through the evaporator we get the 60 per cent citric acid. And then we cool it down and we it undergo the crystallization takes place solid crystal formation then we pass it through the centrifugation process, after centrifugation process we separate out the crystal and mother liquor that you take it back to the evaporator again just to concentrate further and the solid crystal you wash it first and then we dry it with the help of drum dryer. There is a drying technique is there then we use some kind of sieving machine so that different size of crystals can be separated out, so obviously the higher size of larger size of crystals will be having higher market value as compared to those small size of solid particles then we pack it in the polythene pouch and sell it in the market. This is the downstream processing of the of the citric acid industry so I shall continue in the next class. Thank you.