Industrial Biotechnology.
Professor Debabrata Das.
Department of Biotechnology.
Indian Institute of Technology, Kharagpur.
Lecture-35.
Citric Acid Production (continued).

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Welcome back to my course Industrial Biotechnology I was discussing about the citric acid fermentation process in the last lecture I tried to point out that that how the surface fermentation liquid surface fermentation that works and in this lecture also I discussed about the submerged fermentation process which is largely used by the industry and then what is the that flow diagram of this whole process.

A process flow diagram that you know that I I tried to discuss both process flow and block flow diagram and we have come across that that for we have we have three part we have in the in any industry we have upstream processing then we have bioprocess then we have downstream processing. Upstream processing mostly deal with for the for the preparation of the medium, air sterilization, medium sterilization and inoculum preparation and downstream processing usually deals with the purification of the products.

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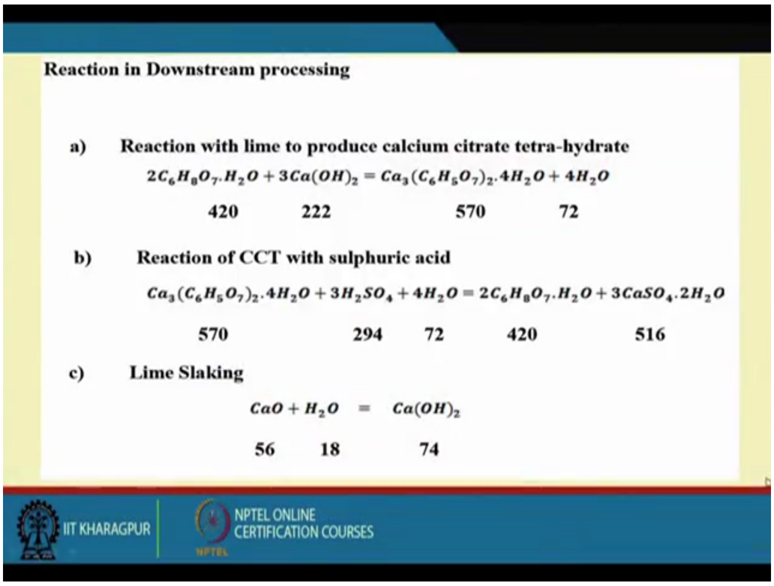

Now in case of citric acid industry there is so many downstream processing involved now we will discuss those process in details. Let me first let me discuss that that how the how the calcium citrate precipitation takes place, this is this is citric acid and this is, this is calcium hydroxide lime when in combination with this it produce the calcium citrate and then give this calcium citrate when we hydrolyse hydrolyse with the H2 SO4 concentrated H2 SO4 it produce again citric acid and calcium sulphate H2O this is called gypsum.

And calcium hydroxide is usually produced from calcium carbonate calcium carbonate if you heat it it produce calcium oxide and carbon-di-oxide and then with calcium oxide when we make a slurry in water and filter it out then we produce the calcium hydroxide. This is kind of stoichiometry of this process this will be very much required to know that how much calcium hydroxide is required for the precipitation of lime.

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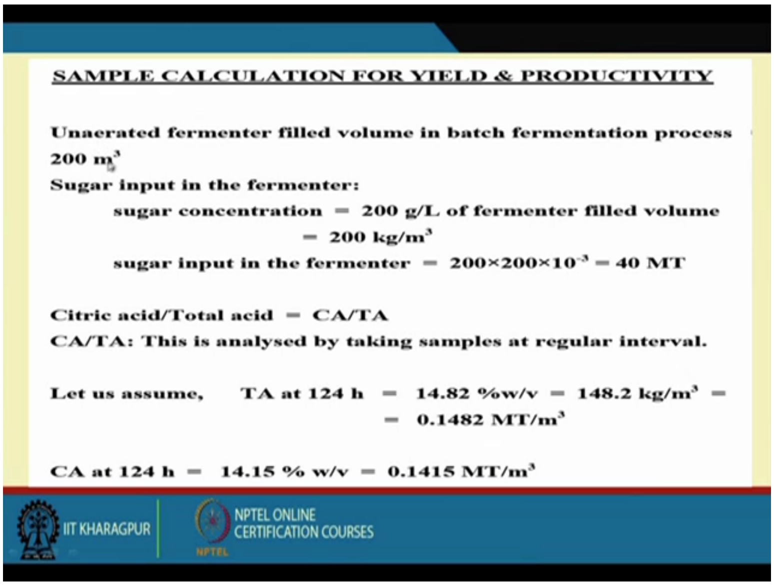

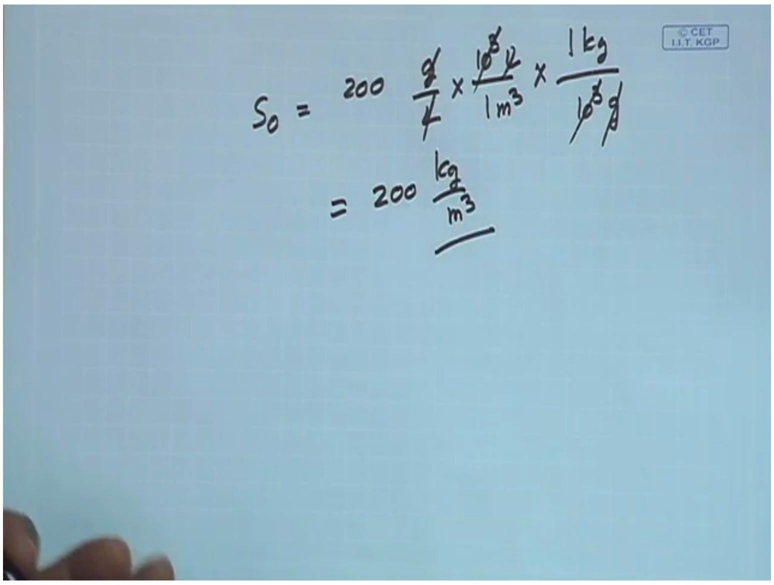

We can also find out how much of sulphuric acid required we can also calculate to find out how much gypsum is produced in the fermentation process. Now another another sample calculation we I want to show you in the in the particular citric acid fermentation process. Suppose we are using 200 cubic metre of this batch fermentation process and sugar concentration is 200 grams per litre, 200 grams per litre that means I want to I I showed you before that if I if I write the initial substrate concentration is 200 gram per litre you can always convert it to kg per cubic metre.

How we can do that I can write 1 cubic metre is equal to 1000 litres and 1 kg is equal to 10 to the power 3 gram so this gram this gram will cancel, this litre this litre will cancel, this will this will cancel, so this would be equal to 200 kg per cubic metre. So you can easily convert to that unit what we have done in here and then sugar input in the fermenter this is the volume 200 cubic metre then 200 you can multiply and if you divide by thousand then you will get the this will be if you multiply you will get the kg but if you divide by thousand because 1 metric tonne MT is the metric tonne is equal to 1000 kg.

So you can this is the total metric tonne of cane molasses required for one batch is about 40 metric tonnes. Now in the fermentation process I forgot to mention that all the fermentation process we should have a rapid estimation technique. I can give the example of citric acid industry actual citric acid estimation process takes bonding but when your plant is in operation it is if you if you take your decision after one day it is too much, it is not you will be having lot of loss in the in process.

So we shall have to find out a rapid estimation technique it is it is not exact estimation but it gives you some information whether your citric acid concentration during the fermentation is increasing or it is coming already in the plateau. So for that what technique we use we use the titrimetric technique we use some we use some sodium hydroxide solution and we try to titrate that and we find out that you know how much sodium hydroxide one normal sodium hydroxide or 0.1 normal sodium hydroxide solution required to increase the pH to 8.6.

We have a standard chart from that we can find out that if this much millimetres is required this is equivalent to this much of acid. So from that we can easily calculate how much acid is there, now if you during the and this this residue hardly takes couple of minutes so this will help us to find out that whether acid formation is increasing the fermentation process or not. When you will find that it is almost coming constant then we stop the operation and do the harvesting.

So I want to point out that total acid concentration we usually estimate in the industry. So here we have we have try to find out that how we calculate the citric acid and total acid residue now this is regular sample this is analysed by taking the sample as the regular interval total acid concentration citric acid concentration finally we do that, let us assume the total acids concentration after 124 hours of fermentation is 14.82 per cent.

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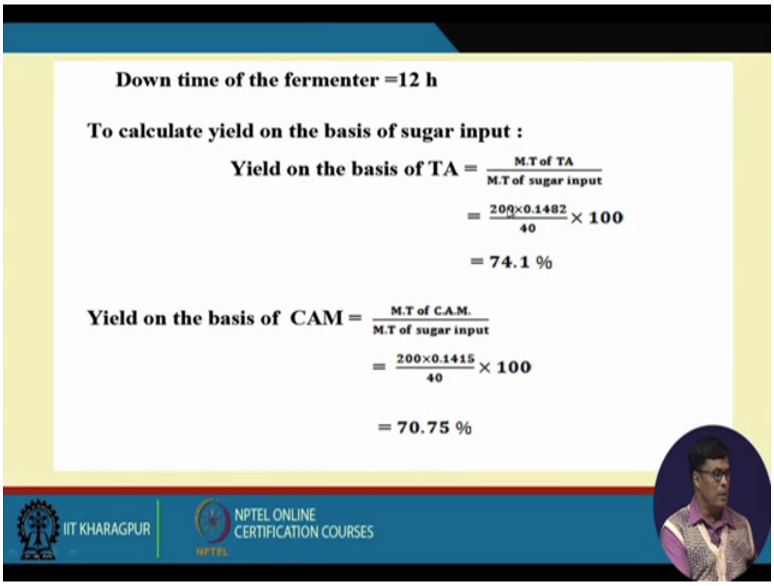

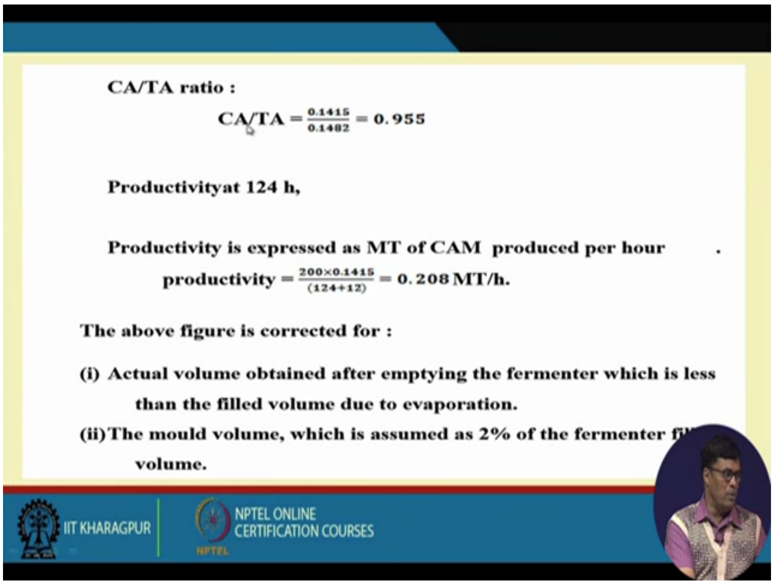

Now this is weight by volume that means if you actually it will come 148.2 kg per cubic metre and that is equal to point 1482 metric tonnes per cubic metre. If the citric acid concentration is 14.15 per cent because it will be little bit less than that than which is coming this is the typical estimation value estimated value in the industrialization I am showing you and then if we assume the down time of the fermenter is 12 hours and then we can easily calculate that what is the yield of total acid.

How we can calculate the yield of total acid total acid production will be what this into 200 cubic metre, this is the total acid am I right. So total acid this is multiplied by an amount of, an amount of that sugar how much input is there 40 metric tonnes, so you can easily calculate and multiply by 100 you can find out how much this that yield is there percentage yield you can you can find it out. Then yield of CAM that citric acid monohydrate we can also calculate similarly we multiply this as 70.72474.

So this is the how we can calculate total acid yield and CAM yield in the citric acid industry now we can also find out what is the ratio of CA and TA. This you can find out this is 0.95 the productivity now how we can calculate the productivity at 124 hours that also we can calculate we know this is the total amount of this citric acid monohydrate that is produced and this time of fermentation this is 12 hour the ideal time. If you divide by that you will get point 208 metric tonnes per hour, so this is how you can calculate the productivity of this.

Now here I want to point out two different things the actual volume of obtained after emptying the fermentation which is less than the yield volume due to evaporation because since we do the aeration in the fermentation process there is every possibility some due to the saturation of the yield some of the water present in the medium that will be evaporated out so volume will be decreasing.

The mould volume also assume to be 2 per cent of the fermented filled volume because mould that that fungal cells also occupy some kind of volume in the in the medium that also that is to be corrected. So these are the couple of things. Now let me go the details of the this downstream processing how the cell mass separation how the calcium citric separation all these things is taking place one by one.

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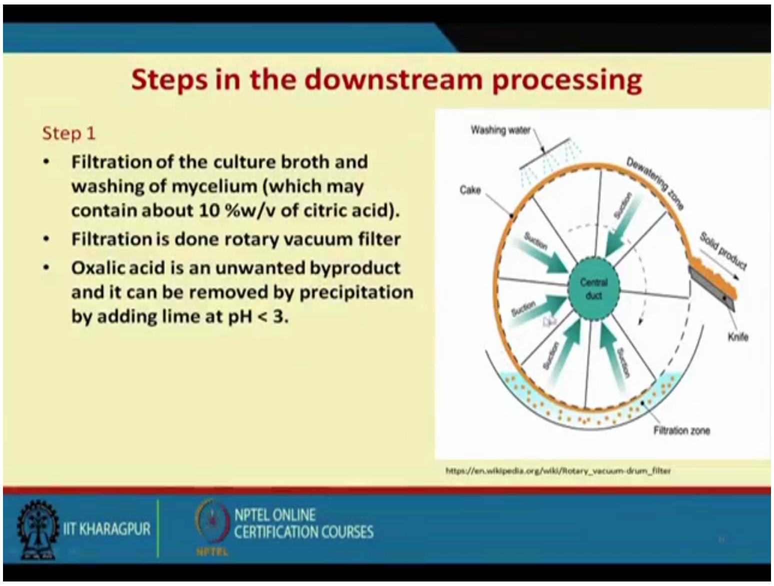

Now this is first I want to show you the rotary vacuum filter, the filtrate of the culture broth and washing mycelium which may be contains about 10 per cent weight by volume citric acid so what is happening we take the take the fermentation broth here in the trough this is the trough and this is the rotating disk and now here this is the pipe line and I told you this is the fine muslin cloth. This is dotted one is a fine muslin cloth and when and this pipe we we apply vacuum here.

So as soon as the vacuum here the water will be sucked and since we are sucking water contains cell mass the cell mass will adhere on the surface of the muslin cloth. Then you can also clean it with the washing water so that some citric acid which is attached to the cell mass that also go that also we can recover. Then when it comes here it will be perfectly dry because we since we are applying vacuum it will almost dry not completely dry that almost little lot of moisture will be taken out.

Then at night will touch the muslin cloth here and this will separate the solid now here we have a screw conveyer which can take the take the material from here to the wagon because in the below the this unit there might be the railway wagon where we can put this this this cell mass there and this cell mass is for the production of raw quality paper so this is a this this can be used for the raw quality paper formation.

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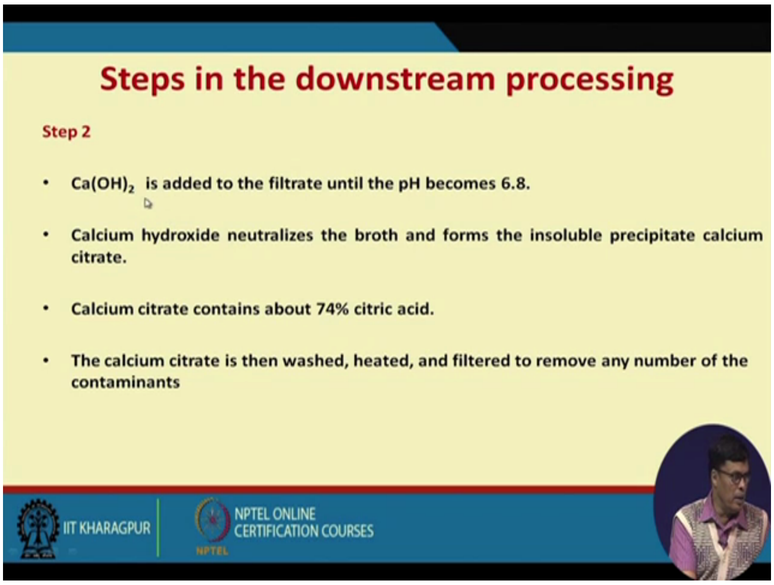

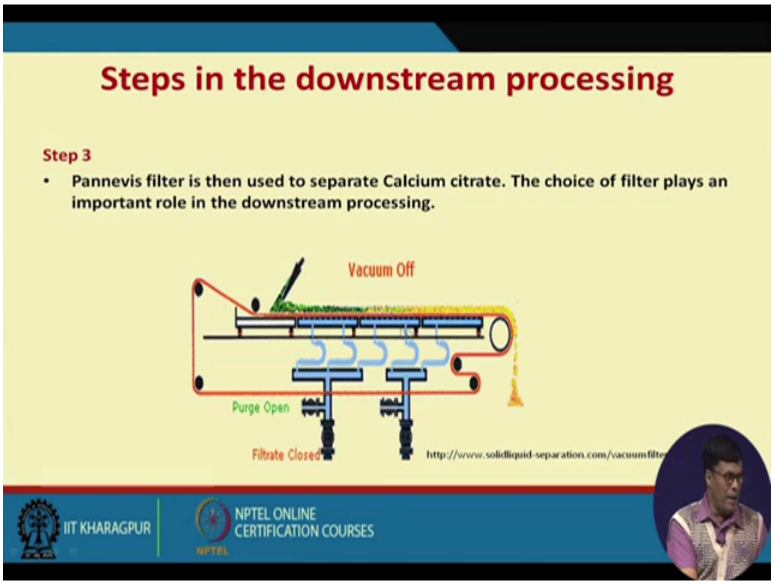
Steps in the downstream processing
Step 3
• Pannevis filter is then used to separate Calcium citrate. The choice of filter plays an important role in the downstream processing.
Vacuum Off
Purge Open
Filtrate Closed
http://www.solidliquid-separation.com/vacuumfilte
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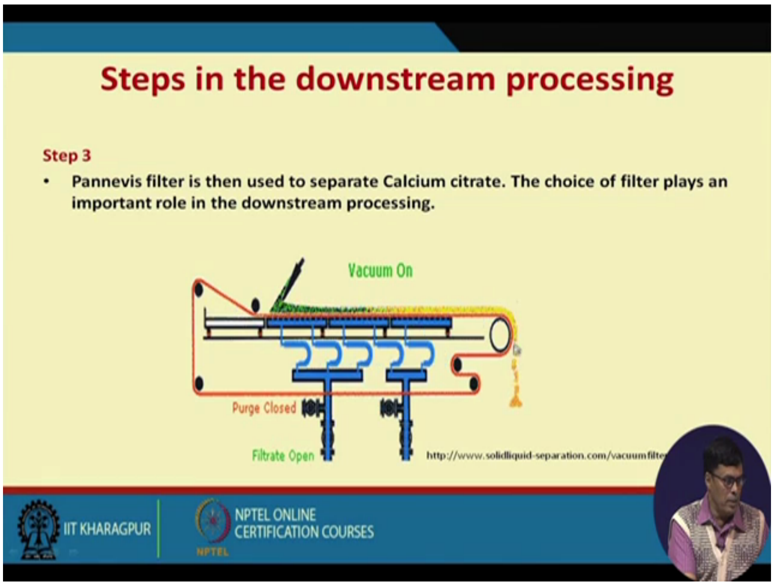
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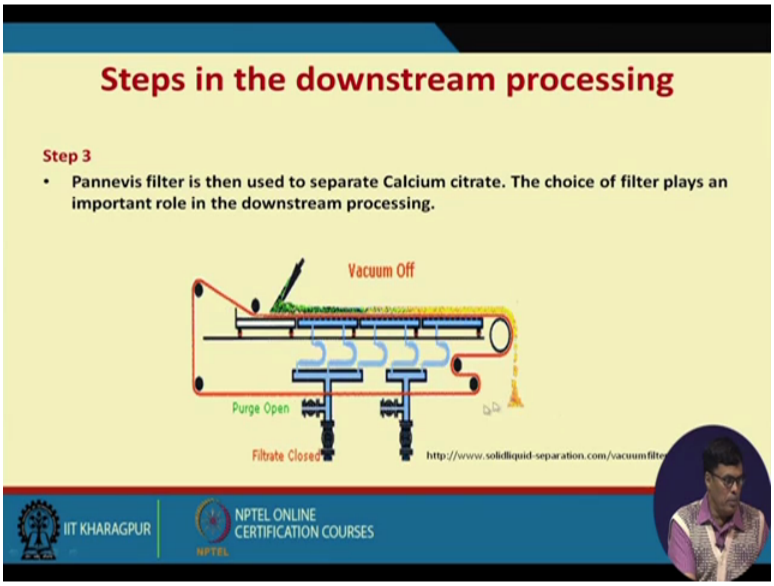
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Now then another step is the calcium citrate precipitation I mentioned that calcium hydroxide is added until unless the pH is 6.8 and calcium hydroxide neutralizes the broth and form the insoluble precipitate of calcium citrate thus calcium citrate contains about the 74 per cent of citric acid. The calcium citrate then washed, heated and filtered now how it taking place is like this, this is called pannevis filter.

Now let me explain it this is thick cotton pad this is the white one is the thick cotton pad and inside you can see this one, this is stainless steel this is made up of stainless steel and there we apply the vacuum here we apply the vacuum. When when your pannevis when the here we put the slurry containing calcium citrate and when you when you put it here then what will happen here we apply the vacuum.

Since we apply the vacuum it sucks the water suck the liquid here and this is the filter and this is the industrial effluent and then this is the solid material you can this is solid material and this is the calcium citrate this (())(13:01) fall it down then with the help of screw conveyer we can take the material one from one point to other, because screw conveyer it looks like this, this is this is shaft and the screw is like this.

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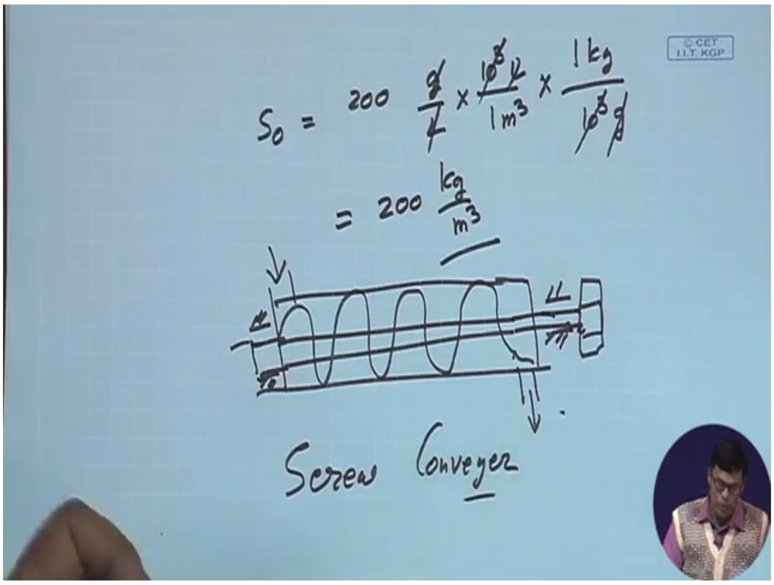
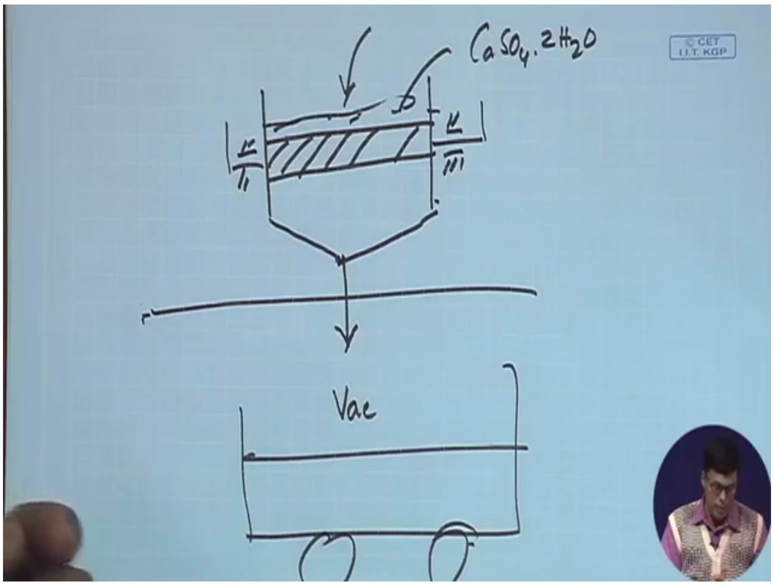


Steps in the downstream processing

Step 4

- H_2SO_4 is added at 60°C.
- Calcium citrate then reacts with sulphuric acid to form calcium sulphate (Gypsum)and citric acid
- Further, filtration is done to separate Calcium sulfate (gypsum, $CaSO_4.2H_2O$)
- Name of the filter is Gypsum filter



Steps in the downstream processing

Step 5

- The final steps for citric acid recovery are — treatment with activated charcoal and crystallization.
- The dilute citric acid is treated with activated charcoal to decolorize the solution.
- Evaporation is done to produce crystals of citric acid.

This is on the surface like this so this is inside this vessel so so here if I put it here and we put it here so it is connected with a motor like this we have motor here so and here we have also bearing this is all bearing that we have so we can have the bearing. So the material will fall here and take the material out h used as a to to transfer the material from one place to other so it is called screw conveyer, screw conveyer this is called screw conveyer this is largely used by the industry.

Then then I told you this is treated with sulphuric acid and the kind of start time reactor and calcium citrate react with citric sulphuric acid from calcium sulphate and citric acid and this citrate was separated with the help of gypsum filter. Now let me show you the little bit on gypsum filter how it looks, gypsum filter is like this so here we have again we have thick cotton pad here here.

So here we applied vacuum so if we put the put the slurry here then then what will happen that calcium sulphate will be here this is the calcium sulphate 2H2O now there is a handle here look at handle here this is mould with some kind of bearing here this is also kind of bearing here so you can tilt this so you can tilt this and below below you might be having the wagon here. So this material will fall down automatically the wagon this is relay wagon and take the material out.

And this is I told you this gypsum is used as a used in the cement industry then then this citric acid contains some kind of colour material this colour material is removed with the help of activated charcoal and then after after activated yeast passes through the evaporator and I explained how the evaporator it is tube evaporator, it is like this. So here we have steam this is the steam for heating purpose and here we have citric acid so it comes here like this then ultimately it we throw it in a particular kind of vessels like this.

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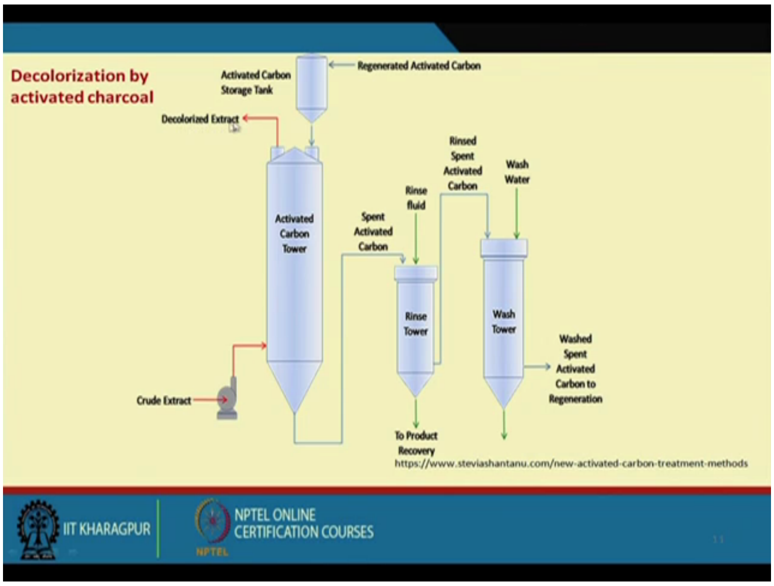

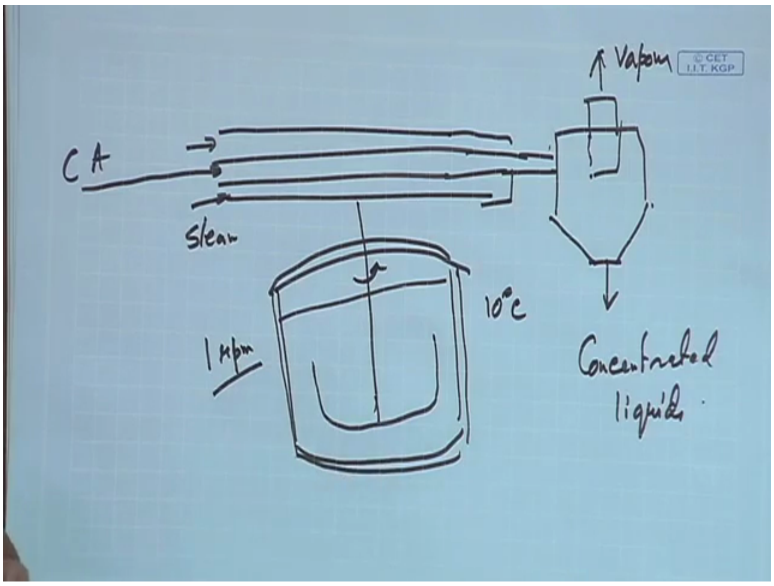

Like you know that we have cyclo separator type of things so when you throw it out here vapour will go out and here concentrated liquid will comes out concentrated liquid will comes out, so this is the process through which we can have concentrate the liquid and and then then this passes through then this is the process through which we can remove the colour we can we can we can pass the liquid extract here and this is activated charcoal then you can regenerate the activated charcoal here and this is this is again we take it here and fill it and we get the decolourized extract.

This is called now then we passes through the crystallizer I showed you the crystallizer that is the I told you that crystallizer is simple the kind of vessel that reacting vessel and this is usually that the insulated I told you this is insulated because we maintain the temperature as around 10 degree centigrade and I told you the citric acid has a transition temperature of 36.6 degree centigrade.

Lower than 36.6 degree centigrade citric acid monohydrate formation will be there now this is the agitator and anchor agitator might be used because which has less friction because and if this is usually move very low rpm 1 rpm, 1 rpm is very low because if we if we move this very high rpm this will affect the crystallization process to a great extent so little starting is required just which which help in the crystallization process.

The degree of purity of the citric acid depends on the on the purpose for which it is required for instance pure form of citric acid needed for the use of food preparation while the industrial purpose we require crude because even if there is a colour is there for industrial purpose, the crystals of citric acid looks like this perfectly white power. You can see how it looks.

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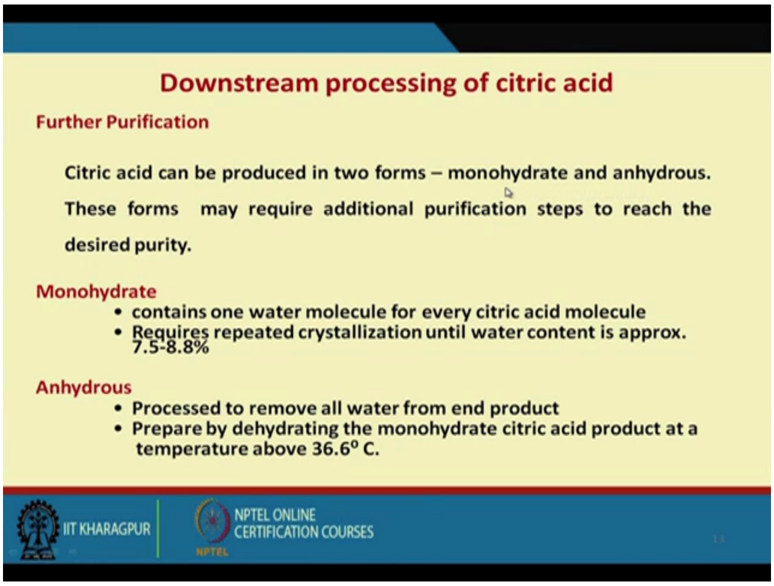

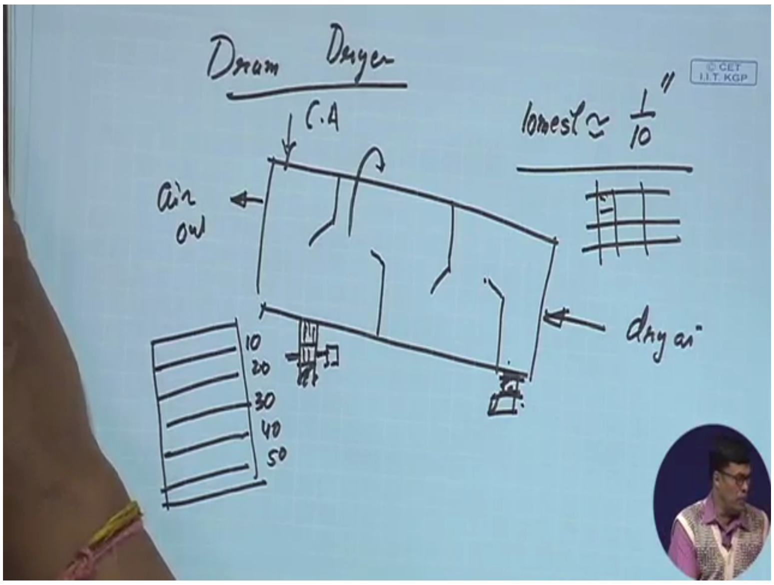

Now now citric acid can be produced in two form I already mentioned that monohydrate calcium of the citric acid monohydrate and citric acid anhydrous these forms are required additional purification step to reach the that desired purity. Now monohydrate contains 1 mole of water molecule every citric acid molecule and requires repeated crystallization under the water content is approximately 57.85 per cent.

Now after the crystallization process it passes through the centrifugation machine just in the centrifugal continuous centrifugal machine when you pass through the crystals will throw to the periphery of the of the crystallizer and liquid we will collect it from the middle that liquid contents about 30 per cent of citric acid that is that is called the mother liquor and solid crystals we take it out we wash it and then these crystals we passes through the dryer what you call drum dryer drum dryer also looks like this.

I can I can explain little bit how the drum, this is called drum dryer is used. This dryer is like this so we have some kind of arrestor like this suppose we put the crystals citric acid crystals here and here we pass the the dry air and this is rotated at the very low rpm this is rotated at the very low rpm that is. The gear that we have then this is gear this is connected with some this is might be motor with connected with motor, this is it is connected here and this is the supporting material.

This is kind of supporting material we have so it is rotated very low rpm suppose it is rotating like this and when you pass the crystals the air will go out, air is out air out so when it rotates like this and material roll in the in the drum like this and air comes like this and in this way the air that crystal will be totally dry. Then it put it to the sieving machine I showed you before also in the shaving machine we have difference sieving plates.

We have I told you maybe it a 10 mest size it may be 20 mest size 30 mest size 40 mest size 50 mest size so we can collect the different size of crystal 10 to 20, 20 to 30, 30 to 40, 40 to 50 and one mest is equal to approximately equal to 1 by that one mest is the 1 inch that is the 1 inch that means 10 mest equal to 1 by 10 inch. This is inch so this is the pore size because port size of the mest this is the pore size this pore size is 1 by approximate not exactly approximately equal to 1 by 10 inch.

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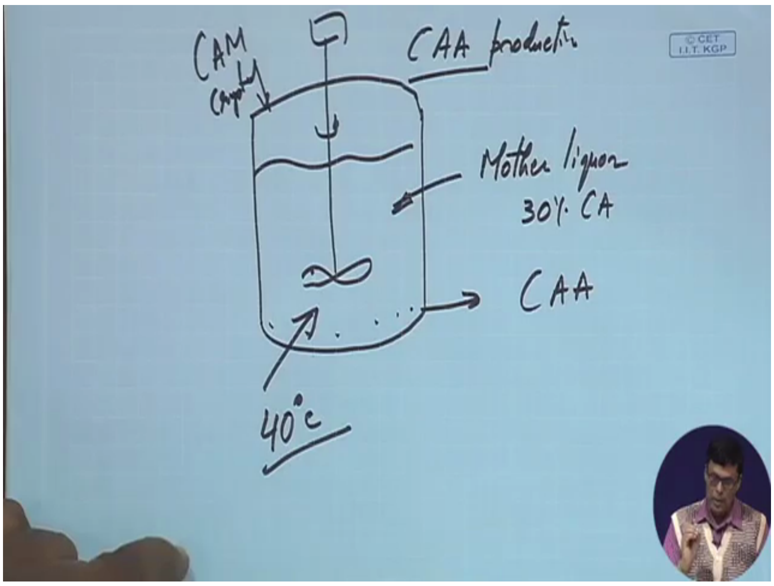

So now question comes how we produce the anhydrous citric acid, the anhydrous citric acid also very interesting that we take in a this is this is this is the vessel in the reactor vessel then we have a stirrer here and this is a motor and we put it this is the mother liquor and here we put a CAM and when put then water molecule present in the CAM that will go in the mother liquor. We will we will get here we get the citric acid anhydrous now here the temperature we maintain about 40 degree centigrade because transition temperature I told you that 36.6 degree centigrade for this is for the production of citric acid anhydrous.

So citric acid anhydrous production the let me repeat it again what is saying the just a kind of reactor vessel that here we take the mother liquor mother liquor contents 30 per cent of citric acid and the we take CAM CAM that is crystals, crystals we take we put it here we put the stirrer then water molecule present in the camp that will be soluble form and whatever solid that remain there that is citric acid anhydrous.

And it just looks like packet powder it is not a lake crystals will give you citric acid monohydrate will give you some gloss but this anhydrous it just like a powder. This is used in the pharmaceuticals industries. Now I want to solve you to two problems which is related with this citric acid industry I hope this will be useful for you.

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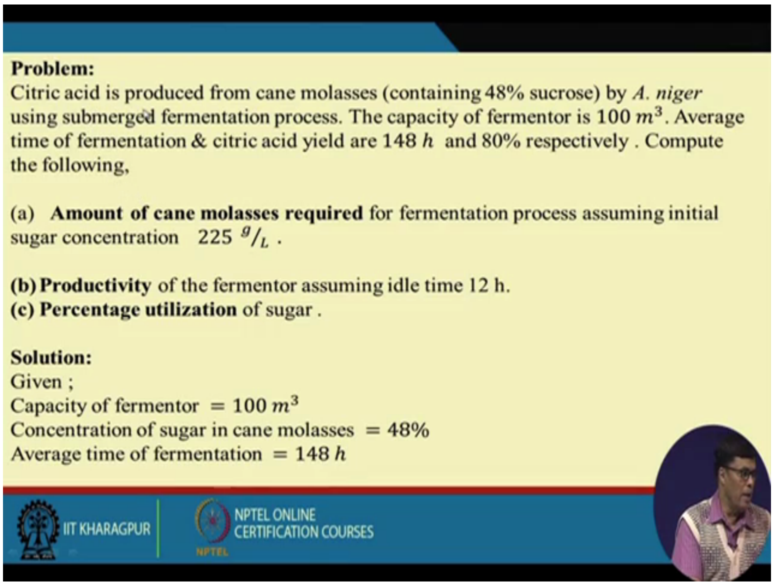

Citric acid is produced from cane molasses containing 48 per cent weight by value this maybe sucrose or you know volume whatever you consider because nothing is mentioned by aspergillus niger using submerge fermentation process.

The capacity of the fermenter is 100 cubic metre average time of fermenter, fermentation and citric acid is 148 hours and 80 per cent respectively. Compute the following. Amount of cane molasses required for the fermentation process assuming initial sugar concentration 225 gram per litre, productivity of the fermenter assuming the idle time of 12 hours and percentage utilization of sugar this is to be calculated.

Let us see it is very simple the capacity of the fermenter is 100 cubic metre then concentration of the concentration of sugar in the in the cane molasses is 48 per cent and then this is average time of fermentation is 148 hours this is given.

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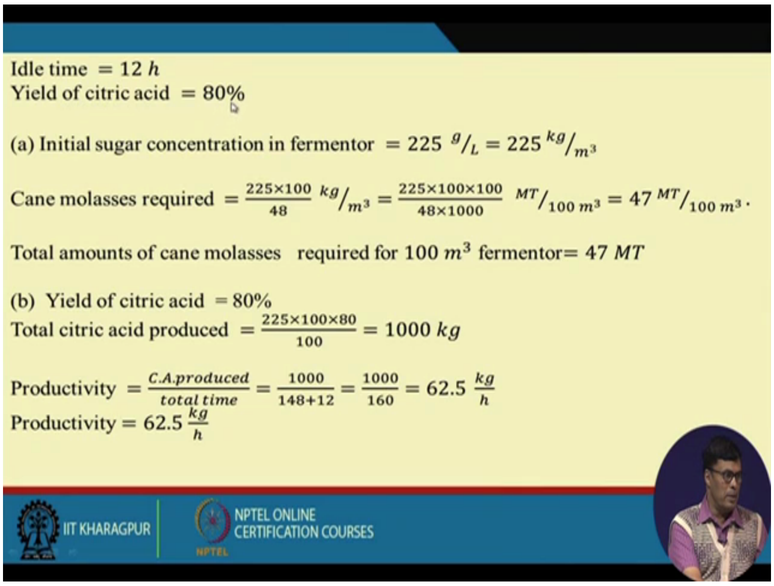

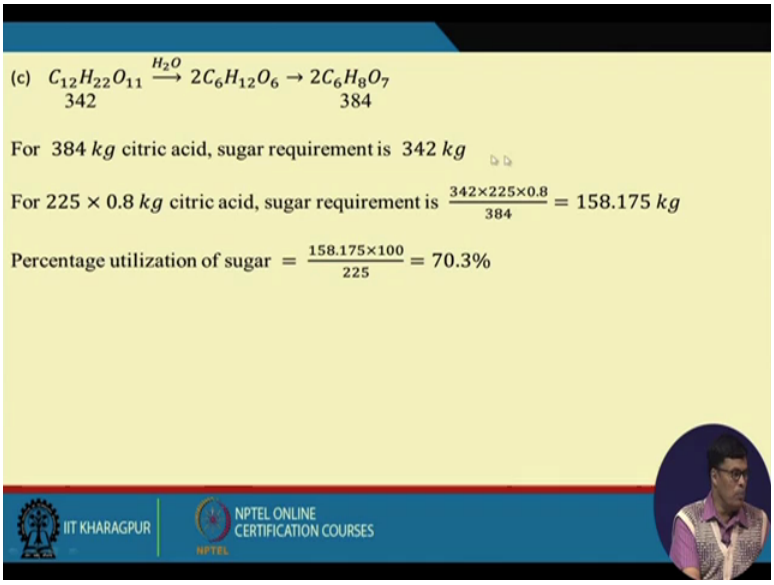

Now idle time is 12 hours and citric acid yield is 80 per cent so initial sugar concentration is 225 gram per litre. This is equal to 255 to 225 kg per cubic metre I showed you the calculation before and then you can find out how much metric tonnes of cane molasses because you see that how much this is the amount of sugar required you multiplied by the volume and this contains about 40 per cent of this sugar.

So it will so it will it will it will defied by 0.48 and then if will multiply by 100 then this this is coming around this 47 metric tonne per 100 cubic metre. Total amount of molasses required then about 47 metric tonne, yield is how we can calculate this is the total amount of sugar that is concentration initial concentration then this is the this is the volume of the fermenter if you multiplied this is this much kg of sugar, how much is the yield it is 80 per cent point 8 , so 80 divide by 100 so it is coming about 1000 kg.

Now productivity how we can calculate this is divided by total time of fermentation is coming about 62.5 kg per hour so this how we can calculate the productivity now most important thing is that how we can calculate the percentage utilization of sugar. This is that this is the stoichiometry we have this is the sugar with molecular weight is 342 and this produce two moles of h that glucose and then it produce two moles of this citric acid. And I will consider the anhydrous then if it is anhydrous then this gram and this much of citric acid is produced from the 342 kg of sugar so we have already calculated before that how much is that that sugar is produced about that total is 1000 kg.

So here here we calculated in different way that we we calculated that 0.8 per cent the 80 per cent that is recovery of this citric acid to 225 is the sugar you multiply by 0.5 then we get this this much of sugar is is converted to citric acid then from this stoichiometry we can find out how much actual sugar is required for getting this much of citric acid the initial concentration or initial amount of sugar is like this and amount of sugar that is required for producing this much of citric that citric acid is this much.

If you take the ratio it is 70.3 per cent so that is the percentage conversion because why I want to show you that this is not equivalent to the actually the yield that we have yield is 80 per cent but actual sugar utilization when we calculate we find it is 70.3 per cent.

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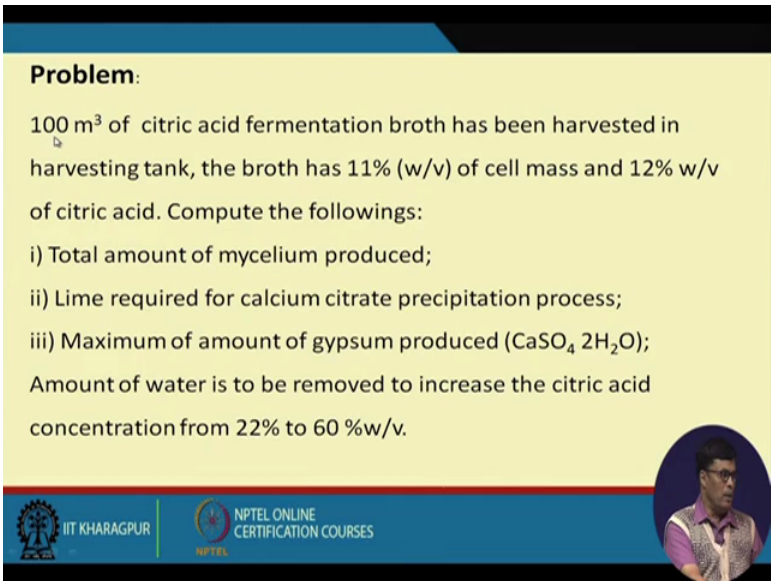

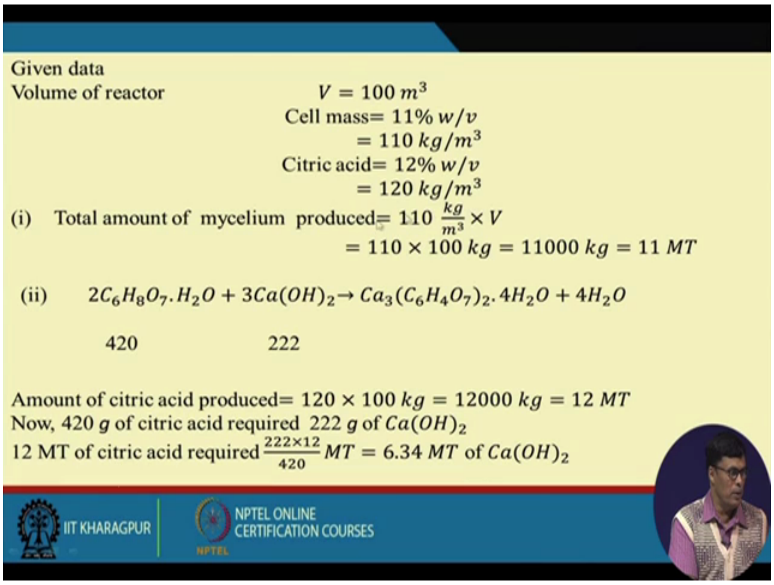

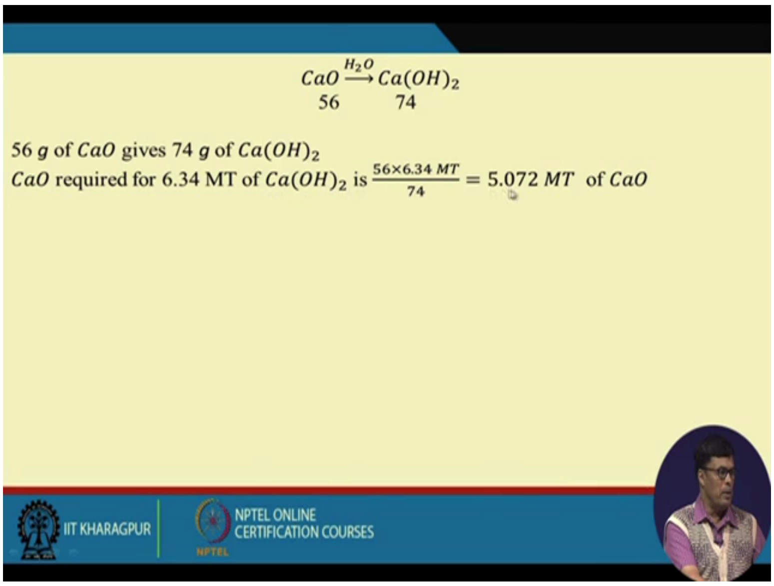

The another another problem also I have I have solve here that is 100 cubic metre citric acid fermentation broth has been harvested in the harvesting tank broth contains 11 per cent weight by volume cell mass, 12 per cent weight by volume of citric acid compute the followings.

Total amount of mycelium produced, lime required for calcium citric precipitation, maximum amount of gypsum produced, amount of water to be removed to increase the citric acid concentration 22 per cent to 60 per cent. It is very simple that volume is 100 cubic metre cell mass concentration element that means it is coming about 110 kg per cubic metre, citric acid 120 kg per cubic metre. So you can easily calculate how much how much cell mass is produced is produced around 11 metric tonnes is quite high and then we have the stoichiometry this much of citric acid monohydrate required this much of calcium hydroxide to produce how much of calcium citrate.

So amount of citric acid required produced this much of this much 12 metric tonnes of citric acid then from this stoichiometry we get 420 citric acid required 222 gram of calcium hydroxide so for this much 12 metric tonnes how much is required we can calcium hydroxide required we can easily calculate. So this is about 6.34 metric tonnes of calcium hydroxide required I told you calcium hydroxide is produced from calcium oxide.

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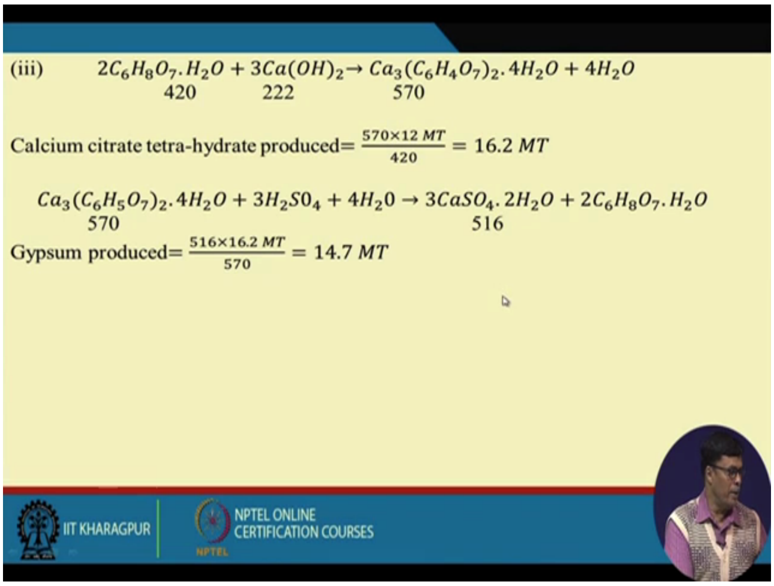

Now how much calcium oxide required that we can just this unitary method through which we can find out that how much calcium oxide is required, now finally we shall have to find out how much gypsum is produced now this is the stoichiometry we have. Now this is the this is the citric acid monohydrate with calcium hydroxide producing the calcium citrate.

Now question comes how much calcium citrate is produced it is 16.2 now this calcium citrate again convert it to 516 gram of gypsum from the from the stoichiometry we can find out how much gypsum is produced that is 14.7 metric tonnes then finally we want to find out how much water is to be removed to reduce to increase the increase the citric acid concentration from 22 per cent to 60 per cent. That this is simply material analysis, 2200 into 16 into V, we find out how much after when you make it 60 per cent how much volume will be remaining.

This much volume 36.66 millilitre volume will remaining so how much water is to be removed, 10 - 36.66 that means 63 point 36 millilitres so 60 that means if you fermentation if your fermenter has this much 100 cubic metre that fermenter harvesting tank than this much water is to be removed for increasing the citric acid concentration from 22 per cent to 60 per cent let me end this lecture. I hope you understand this citric acid fermentation process in details and I try to discuss this process in details and also at the end I tried to discuss the numericals associated with this problem. Thank you very much.