Industrial Biotechnology.
Professor Debabrata Das.
Department of Biotechnology.
Indian Institute of Technology, Kharagpur.
Lecture-33.
Citric Acid Production.

Welcome back to my course Industrial Biotechnology now in the last class I shared with you the wine and vinegar fermentation process the I told you that wine there are different types of wine that has been marketed and broadly it can be classified into two types one is called still wine and another we called Sparkling wine. Still wine does not have any kind of carbon-di-oxide but still wine we have carbon-di-oxide it may be done by using carbonation or by natural fermentation process.

Then also that wine can be defined into other different category that has been explained but mostly the wine produced from the grape and grape should be sound and ripe, sound means I told you that texture of the grape should be should be hard and if it is there is possibility of microbial contamination in the fruit and then ripe should be there because the sugar content is more.

Now wine fermentation process is usually carried out in two different stages one is called primary fermentation another you call secondary fermentation process. Main problem associated with a wine is the storing because I told you that if you stored the wine at 2 for 2 years we get the dry wine dry wine means, it does not have sugar for taste perception. We cannot feel it the sweetness of the wine then we call it dry sugar concentration will be as minimum as possible and then also we have fine wine that require 5 years or more than because during the storing I mentioned that alcohol and acid the trace amount of acid that present in the wine they will react with each other and form the ester.

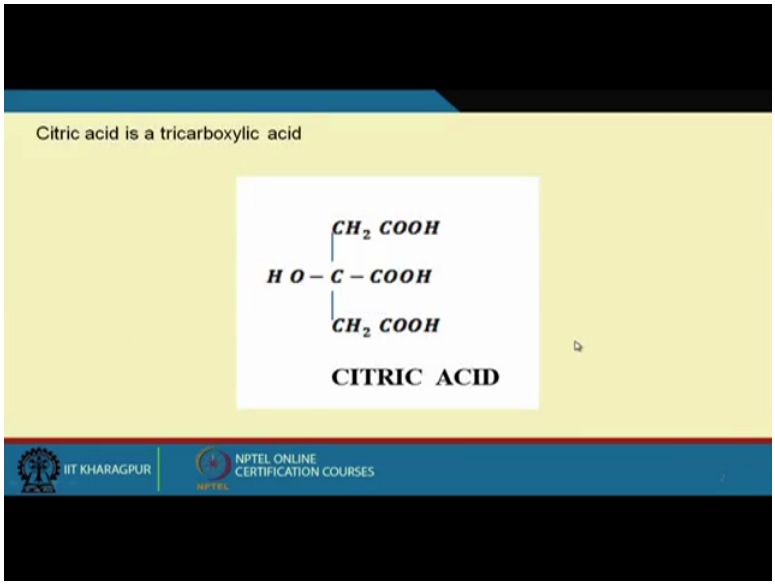
So harshness of the wine will go out and in place of that the some good flavour will be developed because we know the different esters are responsible for developing the flavour. Now again wine has two type of marketing one is short time and long time. In the short time market we just use some kind of preservative and do it in the market but in the long time it was the pasteurization of wine and so that it can be used for longer period of time and wine is used or you know alcohol is used for the production of vinegar.

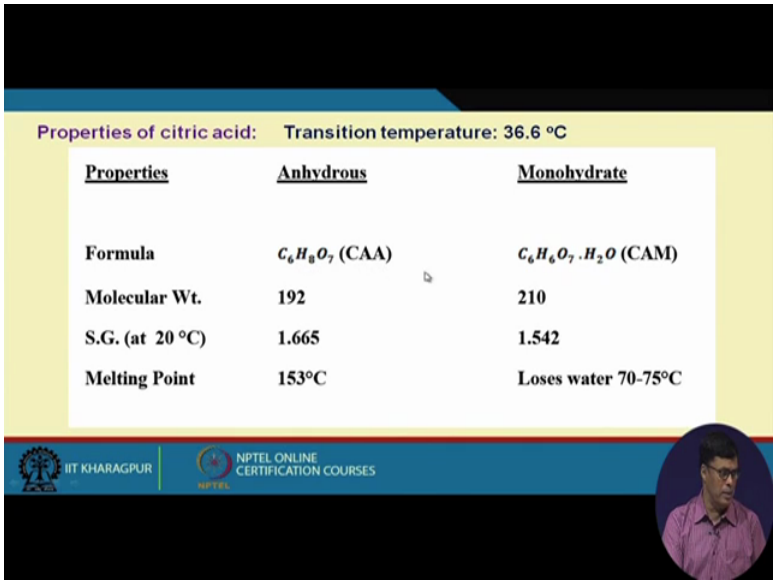
I told you vinegar is a Latin word and this is nothing this is something this is called soured, wine soured wine means the wine when in presence of some acetic acid bacteria, the example is the acetic acetic acid, acetobacter aceti that convert ethanol to acetic acid. So now concentration of acetic acid varies from 4 to 20 per cent the different type of vinegars are available cider vinegar is quite common in the market that is usually produced from apple juice.

Apple juice undergoes the alcohol fermentation process followed by the acetic acid fermentation process, now we have I tried to point out the difference between the natural vinegar and the synthetic vinegar. In case of synthetic vinegar we add we use the pure acetic acid for the preparation of the vinegar and this is available in two different forms one is colourless another is coloured.

In case of colour we add some kind of caramel caramel is nothing but when you heat the sugar, sugar undergoes caramalization reaction it gives the black colour we make your solution in water and that gives a colour that is the permissible colour as per food product organisation is concerned and then this is industry it is produced in two ways one is French process another is German process, I told you the French process is quite old process but we get good quality vinegar out of this but German process is easily little bit faster as compared to this French process.

(Refer Slide Time: 5:32)



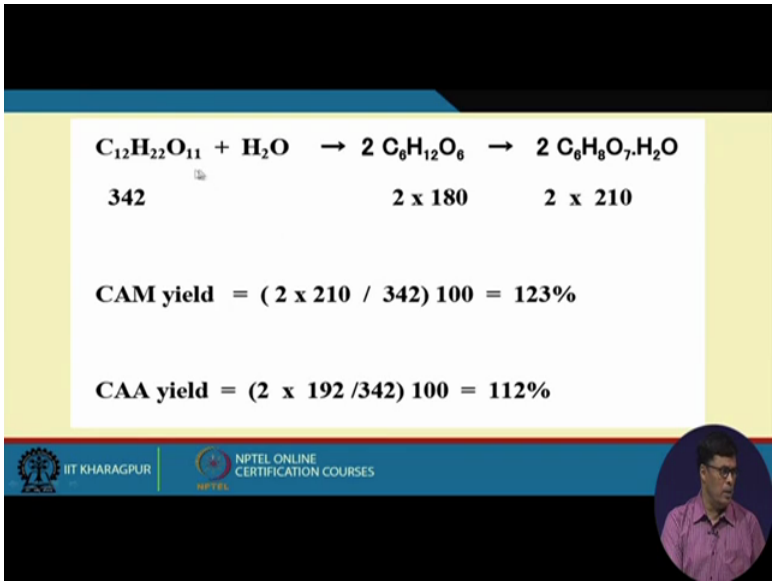


Now today I am very happy to share with you the another fermentation process that is citric acid fermentation process and where I have been worked for more than one and a half years. And citric acid such a wonderful that product that has been used in different purpose, for different purpose it can be used. Citric acid if you look at citric acid is considered as the tri-carboxylic acid though it has three carboxylic groups that we have and it has the property like this.

It is available in two different forms one is called anhydrous citric acid another is citric acid monomer. Citric acid monomer this formulae is little bit wrong you please correct it this should be H8 because 6 H8 O7 this also should be H8. So this is citric acid monomer this molecular weight is 100 the citric acid anhydrous molecular weight 192 and this monohy citric acid monohydrate that is what 210 and specific gravity mould is they are close to each other melting point 153 degree centigrade in citric acid.

Anhydrous and in case of CAM it is loses water at 70 to 75 degree centigrade. Most important thing I want to point out here the transition temperature is 36 point 6 degree centigrade because this anhydrous and monohydrate because when you do the crystallization as if we keep the temperature below 36 point 6 degree centigrade we get citric acid monohydrate and if we keep the temperature higher than that we produce the citric acid anhydrous.

(Refer Slide Time: 7:05)

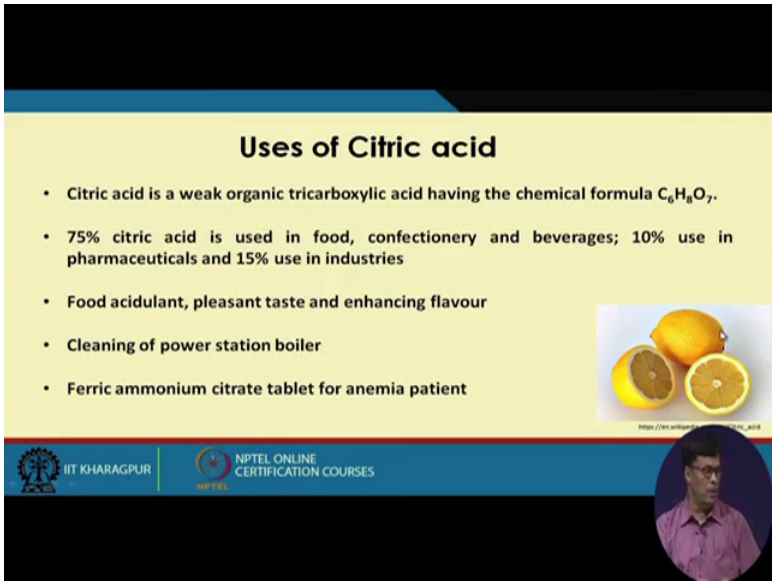


Now this is the stoichiometry of citric acid production this is this is how your sugar it produce the two moles of this you know sugar this comprises of glucose and fructose two two moles of C6 H12 O6 and then this produces two moles of the CAM what we call citric acid monomer. Now if you see the yield CAM yield it is calculated about 123 per cent and citric acid anhydrous if you calculate , if you here I want to draw your attention that the yield that you know it may be more than 100 per cent also.

Now here I want to just point out just in case of ethanol fermentation process we know that 180 gram glucose produces 92 gram of ethanol, so there the yield is about 51 per cent around 51 per cent but here in case of CAM it is here you see that 123 per cent so yield may be less than 100 per cent more than 100 per cent it depends on the type of product we are producing so that is thing.

I can give another example that (())(8:37) it can it has the glucose isomer as enzyme that can convert glucose to fructose, now glucose have the same molecular weight as that of the fructose so there the conversion is 100 per cent.

(Refer Slide Time: 8:53)



So now let me talk about 1 told you that citric acid has tremendous potentiality in the market, it has different use and I I worked with Citrozia biochemicals which is located in Surat and this is in collaboration in collaboration with John and Sterge limited UK.

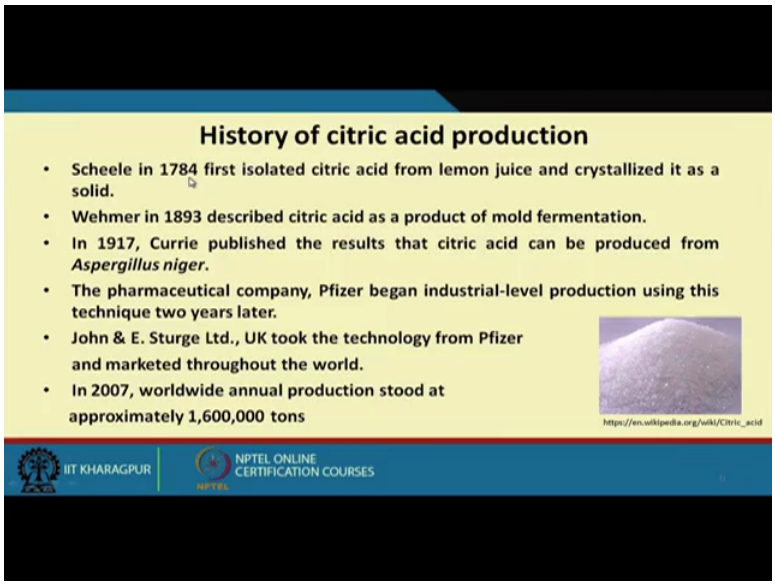
Now use of citric acid citric acid is the weak organic tricarboxylic acid having the empirical formulae this C6 H8 O7, 75 per cent citric acid is used in food confectionary and beverages. The most of the citric acid because not only it is used for acidifying the product but it gives a typical flavour and 10 per cent is used for pharmaceutical industries and 15 per cent used by the other industries, so 10 per cent the pharmaceutical industry mostly they use the citric acid analytes and 75 per cent citric acid and 15 per cent that industrial use mostly we have CAM.

Now it is used as a food acidulant because lot of jam, jelly , orange squash all places we use this citric acid, it has a pleasant taste and enhance the flavour. It is most important thing is the since it is the weak acid it can be used for cleaning the power station boiler because we know during the stem generation there is the deposition of the solid material on the boiler tubes that is usually cleaned by the by the citric acid. If we use the mineral acid like HCL, H2SO4 that affect may affect the material of construction so it is the recommended that we should use the weak acid for cleaning for cleaning the scale formation in the boiler tube.

Now there besides that the citric acid that other uses like ferric ammonium citrate tablet used for the anaemia patient also you know that in the biotechnology lab we use the citrate buffer then citric acid used also for the preservation of ascorbic acid which is called vitamin c also

for the stabilization of the fat. So it has tremendous potential in market and I can remember when I worked with Citrozia biochemicals I could not find any trace amount of product in our godown as soon as this produced it is marketed because citrozia biochemicals was the only company in India which was producing citric acid through the fermentation process.

(Refer Slide Time: 11:50)

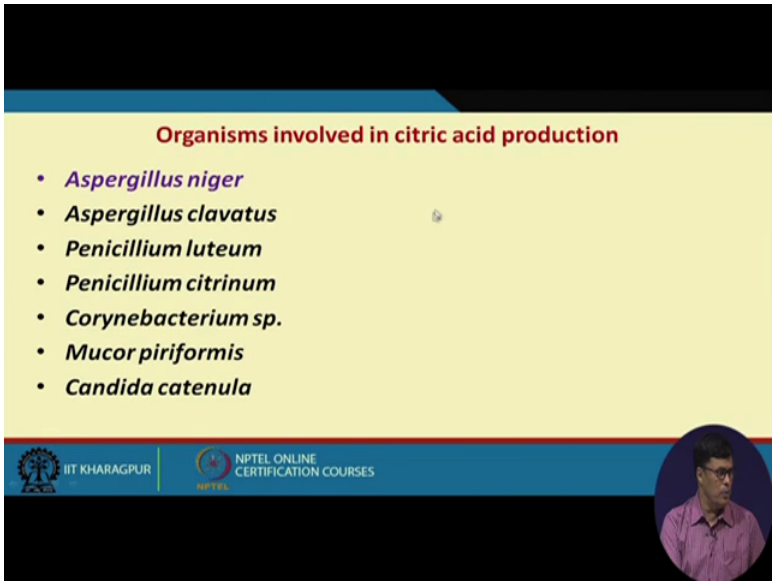


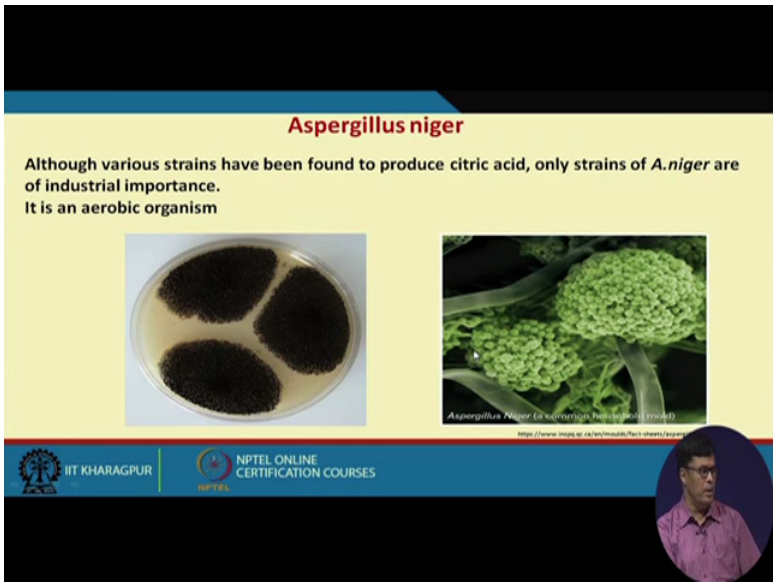
Other company was citrate India they usually imported the calcium citrate from China and you produce citric acid from calcium that is not a fermentation process that is a chemical process, now if you look at the history very is interesting the Scheale that in 1784 first isolated the citric acid from lemon juice we know the lemon juice contains what is called citrus food.

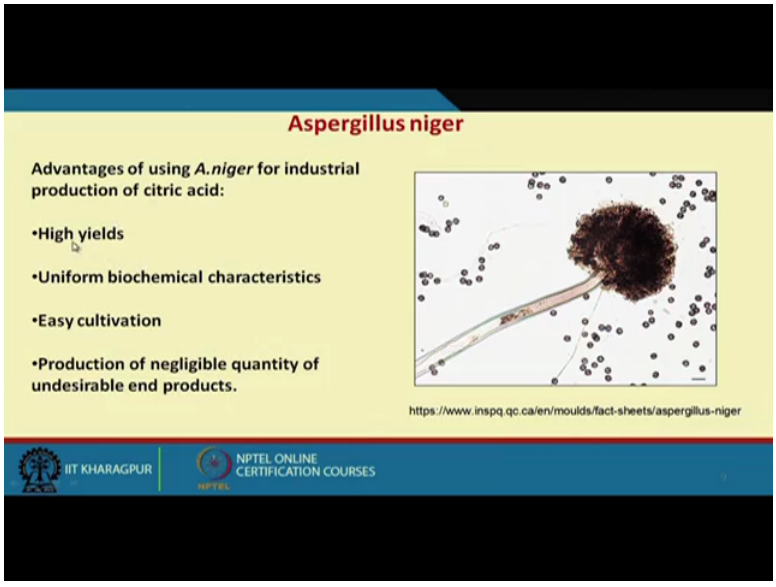
So citrus name is coming from the citric acid and crystallized it as a solid. I mentioned that two type of organic acids we have one is called volatile fatty acids another is non- volatile fatty acid. Volatile fatty acid means if you keep it to the atmosphere it will evaporate it out and non volatile fatty acid means if you keep it up to sometime that water will evaporated out and crystals of that acid will remain. So citric acid is considered as non-volatile fatty acid.

Wehmer this 1893 described the citric acid as the product of mold fermentation then 1917 the Currie published the results of citric acid can be produced from aspergillus niger. The pharmaceutical company Pfizer began the industrial level production using this technique but two years later then John E.Sturge limited UK took the technology you know purchased the technology from the Pfizer and marketed throughout the world, 2007 the worldwide annual production stood at approximately 1,600,000 that tonnes of products.

(Refer Slide Time: 13:19)



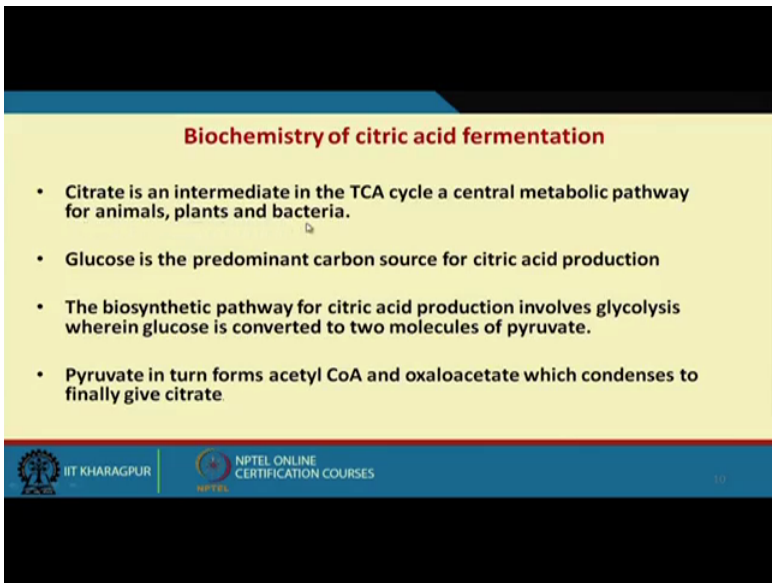




Now different organisms can be produce citric acid we have aspergillus different aspergillus species, aspergillus niger, aspergillus clavatus, penicillum luteum but among all these species the most the organism that is mostly used by the industry that is the aspergillus niger. Now aspergillus niger the various strains was found to produce citric acid that you know that is that aspergillus niger its fine has the industrial importance. Now this particular strain is available in most of the rotten citric citrus food. If we have in the market that any kind of rotten citrus food we will find the black spores formation is there this black spores is nothing but aspergillus niger.

Now if you pictorially it looks like this the black spores looks like this and under microscope it looks like, this you know beautiful that looking that that kind of flower type of thing that we can observe now the advantage of using aspergillus niger for industrial use is this. At the high yield uniform biochemical characteristics. The strain we use in the industry that is the industrial strain that is not the wild screen, naturally that productivity of the strain is very high. So it can produce high concentration of citric acid it can use the high concentration of sugar for the production of the citric acid.

(Refer Slide Time: 15:20)

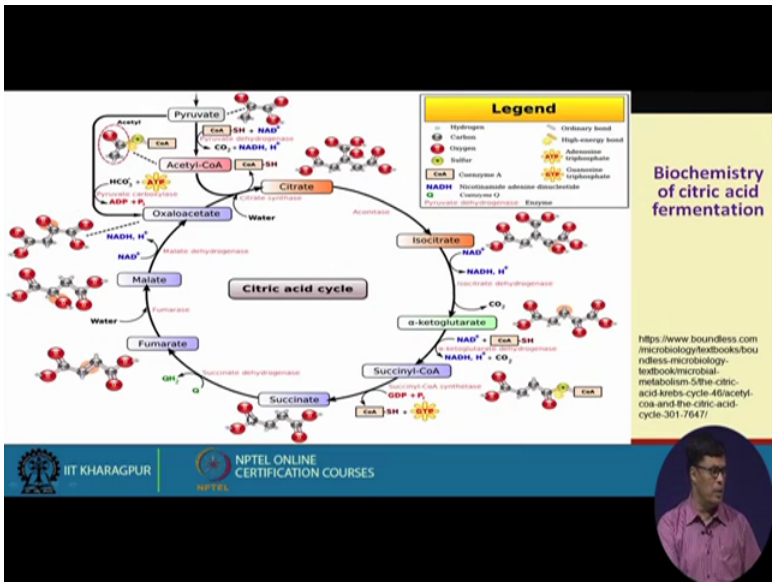


Now uniform biochemical characteristics, easy cultivation and production of negligible quantity of undesirable end product, and it looks like this another picture we have we have taken from the website it looks like that , that this is the spores you can see that. Now biochemistry of citric acid fermentation process citrate is the intermediate of the TCA cycle a central metabolic pathway of animal, plants and bacteria particularly aerobic organisms we

follow we have all the all the aerobes and anaerobes they have the indomyro pathway through which we can produce the glucose is converted to pyruvic acid.

But aerobes they have mostly the TCA cycle through the TCA cycle we can produce carbon-di-oxide and water. So it is like this the glucose is predominant carbon source for the citric acid I have already showed the stoichiometry biosynthetic pathway of citric acid production involve the glycolysis wherein the glucose is converted to two moles of pyruvic acid. The pyruvic in turn form the acetyl CoA and acetyl CoA in combination with oxaloacetate which condenses to finally to give the citrate.

(Refer Slide Time: 16:11)



So it is the metabolic pathway looks like this that you know we through the metabolic pathway that the indomyro pathway it produce pyruvic and this pyruvic produce the acetyl CoA, then oxaloacetate acid this is one of the metabolise of the TCA cycle when they combine together with the help of citrate synthesis they produce the citrate . And then again if you allow the citric acid product they allow the TCA cycle to continue then again it produce isocitrate alpha ketoglucotarate, succinyl CoA, succinate, fumarate, the malate and the oxaloacetate.

So you know that this is the cycle is going on through which we produce (())(16:58) I told you it produces carbon-di-oxide we know that that you know that when you take glucose in our system and the day to day life we take we get lot of energy and this energy comes from the energy molecule produced through this biochemical reactions. So this is during this the

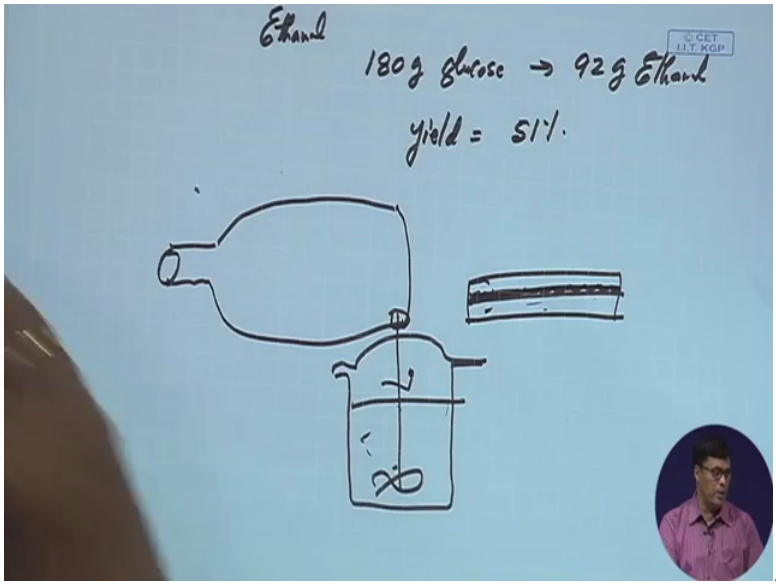
product is ultimate product is same as if you burn glucose you produce carbon-di-oxide in water if you take in our system glucose that also produce carbon-di-oxide the water.

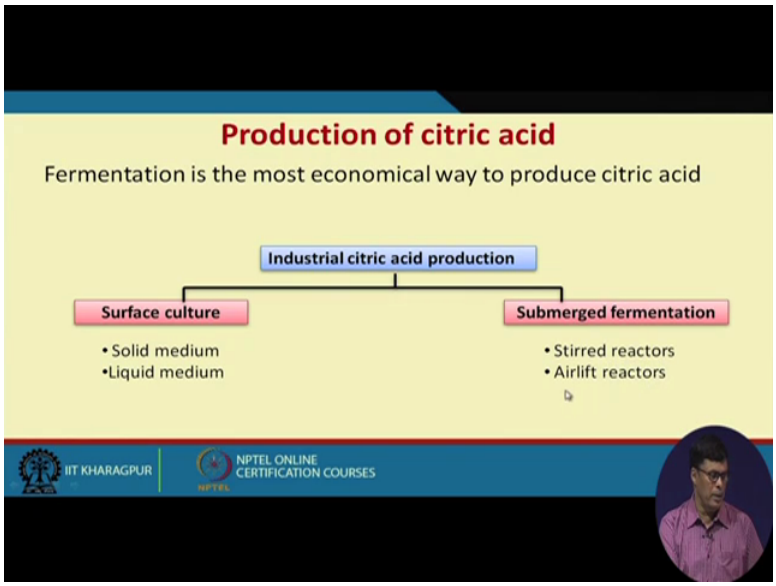
But only that difference is that when you burn glucose it produce heat but when you pass through this metabolic pathway we produce some kind of energy molecule that we use as per our requirement. But here we have the our interest is the citric acid production. So in the citric acid production the beauty is that if you give the inhibition to the enzyme responsible for the formation of high society alpha due to glutarate that is the enzymes is aconites and isocitrate dehydrogenase then the accumulation of citric acid will take place.

That is actually happen in case of citric acid fermentation process when we work with this process then we found that first 24 hours there is no accumulation of citric acid then the TCA cycle will be in operation but after say about 46 hours the 48 hours of fermentation there will be accumulation of citric acid and we find the inhibition of isocitrate dehydrogenase . And then if you if you continue after 96 hours of fermentation the total activity of acculates and isocitrate dehydrogenase will be inhibited.

So your accumulation wil of citric acid will be greatly taking place in the system. The normal time of citric acid fermentation is 124 to 144 hours that is approximately 6 days. Now production of citric acid can be produced in two different ways one is called surface culture another is submerged culture . Surface culture because this is this is the aerobic fermentation process , surface culture means that we do not we have we keep the more surface area so that natural that diffusion of oxygen that take place in the fermentation media and growth of the organism take place.

(Refer Slide Time: 19:39)





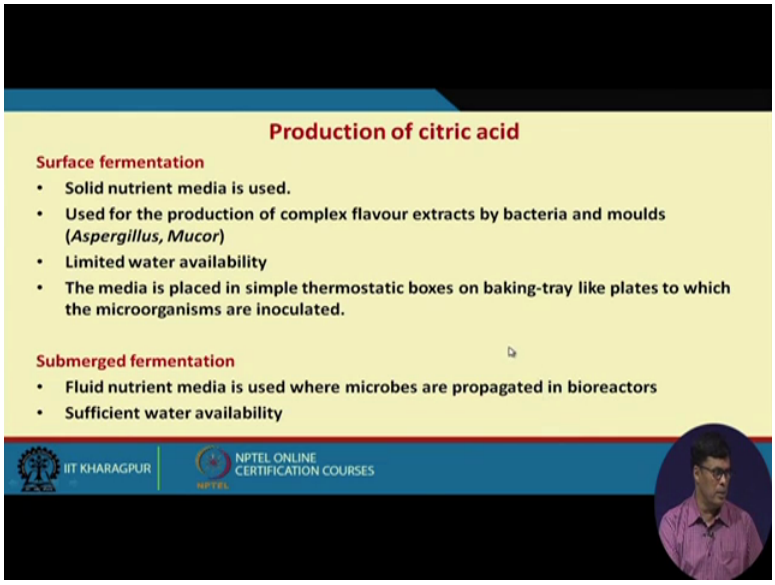
But this technique is very less used in the industry and mostly we use submerged the culture because suppose I can give the example suppose we have specialty white mouth aldemmier fox, we know that this is like this this is so this is white whte here we put the media and we and white the surface is there, so the diffusion of oxygen takes place at the surface of the media, the growth of the organism . If you take the side view it will like this , this is on the on the surface the the the microbial organism will grow on the surface of this and in this media you may get the citric acid production.

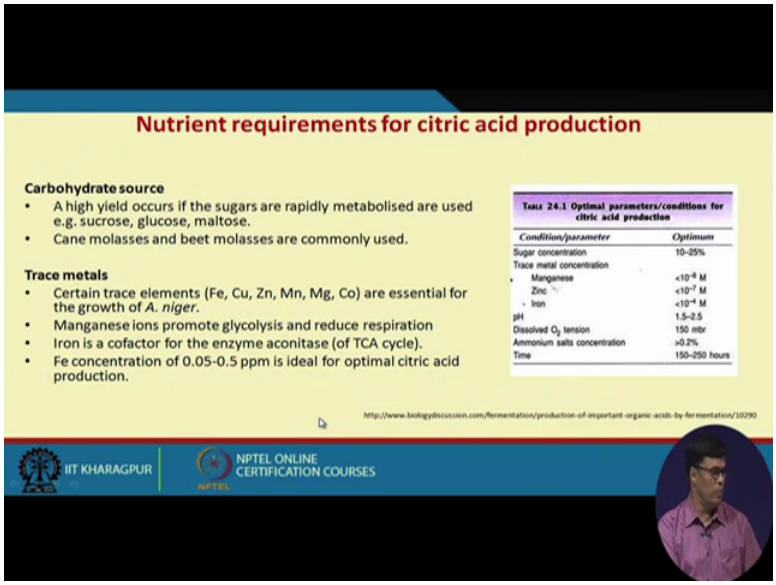
This is but this is very slow fermentation process as compared to submerged fermentation process in case of submerge fermentation process what is happening , that whole media is used for the growth of the organism, because here we have stirred and you put the organism

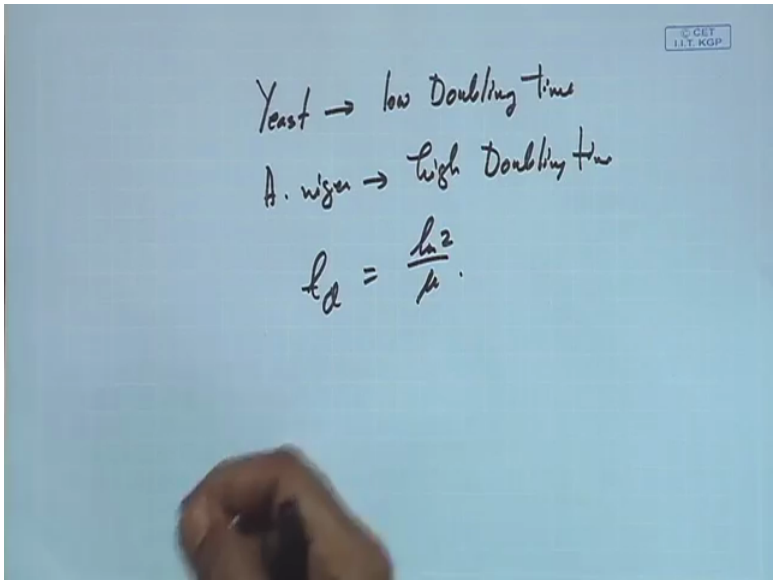
the whole here the organism growth only in a surface culture growth take place on the surface of the media.

But in case of submerged culture the growth of the medium will take place throughout the media that is the difference so here surface culture we have solid media we have liquid medium and and submerged culture we have stirred reactor and airlift reactor though we have mentioned two the mostly we use the stirred reactor for producing the citric acid.

(Refer Slide Time: 21:06)







Now now I shall discuss surface fermentation the solid nutrient media is used that in case then used for the production of complex flavour extracts by bacteria and molds that is the aspergillus and mucor. Limited water availability then water is placed in simple thermostatic box on baking tray like plates to which the microorgnisms are inoculated. So this is the surface culture that we have and surface culture as I pointed out fluid nutrient media is used are propagated in the bioreactor , sufficient water is availability is there. So in here growth is more and production of citric acid also will be more.

Now question comes that what are the nutrients required for this and this is very important as per citric acid fermentation is concerned. Because if you look at this carbohydrate sources a high yield occur if the sugar are rapidly metabolise are used as for example sucrose, glucose and maltose and cane molasses and beet molasses are commonly used. Now here we have little problem because when we use the cane molasses then cane molasses contents it is also a good media for the growth of yeast cell. Now yeast cell I I already point out yeast the saccharomyces cerevisiae they have very low doubling time and where is the fungi the aspergillus niger has high doubling time. So since we know the doubling time td equal to a ln2 by MU.

So MU is the specific growth rate ln2 by MU so it is inverse proportional to if a doubling time is less that means less, then MU will be high and if doubling time is high that means MU will be less. So since MU is that yeast that MU is more as compared to asperagillus niger then then that in the citric acid fermentation process the main problem we face with the yeast contamination problem because if any yeast enter into the system then it affect the citric acid production to a great extent.

Now trace element that place important role in the citric acid fermentation process. The certain trace elements like iron, copper, zinc, manganese, magnesium that cobalt are essential for the growth of aspergillus niger. Manganese ions promote the glycolysis and reduce the respiration, iron is a co-factor for the enzymes aconitase in the TCA cycle. Iron concentration 0.05 to 0.5 ppm is ideal for the optimal citric acid concentrate.

Now here I want to point out that citric acid concentrate that is mineral concentration plays very important role what you call pivotal role in the in the citric acid fermentation process. Particularly if we consider the iron and manganese this is, plays a very vital role as you have seen that iron is a cofactor for acconitase which is I have already shown you you can see here that this is acconitase, this is citric . If this enzyme is inhibited then and only then citric acid accumulation will take place in the system.

So that is why that that if iron we take out from this medium then and only then the activity of acconitase will be will be affected. So this is very important so so so this this iron and manganese that plays a very important role and manganese that phosphofroctukinase there is enzyme that is if we reduce this manganese concentration in the me media dium then citric acid inhibition for phosphofroctukinaseis that will be reduced. Because if the activity of phosphofroctukinase inhibited in the endomyro pathway then our citric acid yield in the fermentation process will be reduced to a great extent.

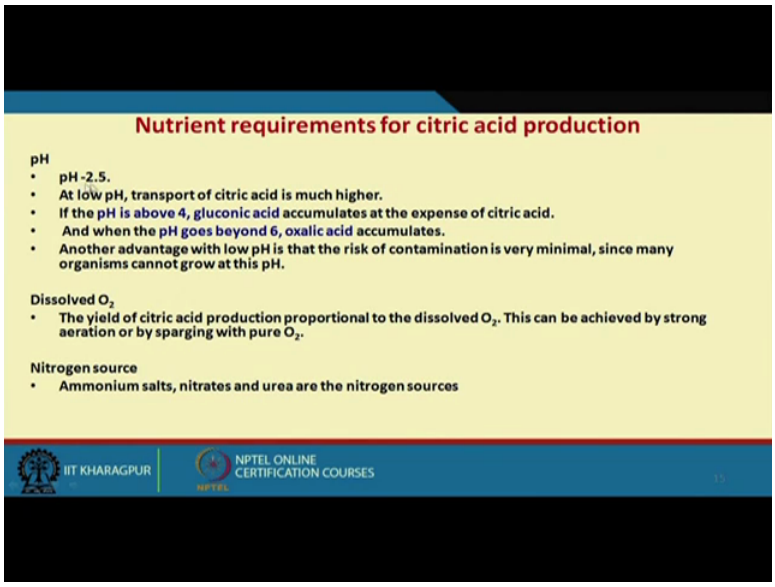
So this is the problem that we have so this is we we have observed that if manganese concentration it reduce by 1 ppm is increased by 1 ppm in other way our citric acid will reduce by 10 per cent, the reduction of citric acid will take place about 10 per cent. So this is that you can you can see that what is tremendous things that we have affect we have on this citric acid fermentation process.

So because as I just pointed out that cane molasses is found most suitable substrate for this citric acid fermentation process and it contains good amount of iron and manganese . So when the cane molasses is used for the inoculum vessel then I shall show you that how the inoculum cell production take place. Now in the inoculum vessel that when we use then we allow the citric acid to continue so we require the iron and manganese sufficient amount so that that is not a problem for the aspergillus niger .

But when you go for this production fermenter where you are looking for citric acid production this is to be removed. Now question comes how we remove citric acid from the

cane molasses . So we use some kind of chelating agent, potassium ferrous cyanide we we steam it , we boil it for half an hour so that whatever iron and manganese present in the cane molasses that will be precipitated out, this is how we can remove.

(Refer Slide Time: 27:29)



And this is the concentration of the media is given here then pH of the fermentation process is usually 2.5 is preferable at low pH transport citric acid is much higher as pH, above 4 the gluconic acid accumulation take place at a expense of citric acid.

Now here I want to point out that if you maintain the controlled pH that at 4 then we will be having the we will not get much of citric acid production but if you control the pH, it goes beyond 6 then we will get the oxalic acid production in the system but if we allow the pH to drop down, then we will find sufficient amount of citric acid production in the system. So another advantage at the low pH that risk of contamination is very minimum and since many organism can not grow at this pH.

Dissolved oxygen plays important role because dissolved oxygen because this is the aerobic fermentation process, so aeration is very important factor for this fermentation process. Now nitrogen source ammonium salt, nitrate and urea are the are used as a nitrogen source. Now here I want to point out another very interesting thing that since this is a filament cells that you know when we use any kind of stirred reactor and we inoculate the asperagillus niger, if we allow if we put on the agitator the growth of the organism will be affected drastically.

So initially there should be an ideal time for both the inoculum vessel and the production fermenter so that your organism can grow sufficiently then and only then we can put the (())

(29:05) on so that your organism can grow in the medium properly. Let me stop it here and next class I shall continue this lecture. Thank you.