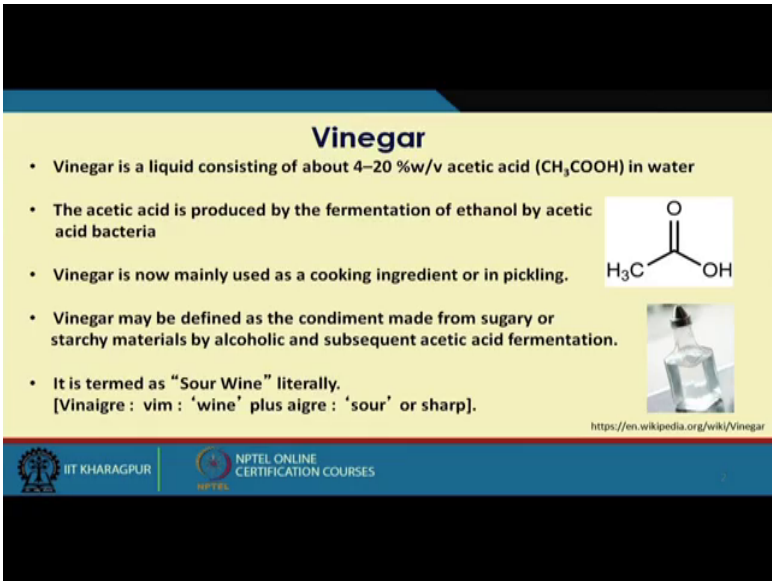
Course on Industrial Biotechnology
By Professor Debabrata Das
Department of Biotechnology
Indian Institute of Technology, Kharagpur
Lecture 32
Module 7
Vinegar Production

Let me start another very interesting topic that is the vinegar production which is largely used in the day to day life.

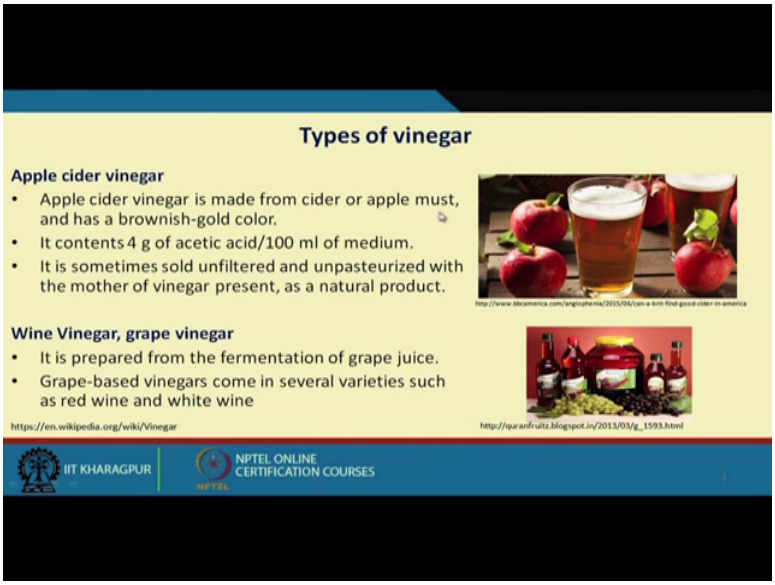
(Refer Slide Time: 0:26)



The vinegar is a liquid consisting of about 4 to 20 per cent of weight by volume of acetic acid, acetic acid is produced by fermentation of ethanol by acetic acid bacteria. Vinegar is now mainly used as a cooking ingredient or in pickling. The vinegar may be defined as the condiment made from sugary or starchy material by alcoholic and subsequent acetic acid fermentation.

Now what vinegar has come from the you know that it is like this the vim is called wine and aigre is called sour or as a sharp that means it is called sour wine. The vinegar is known as sour wine, sour wine means wine comprises of what alcohol. Now ethanol and this ethanol subsequently it again undergo the fermentation with the help of some acetic acid bacteria we call it acetic acid. So Acetobacter aceti, this acetobacter aceti convert the ethanol to acetic acid so that is why we call it sour wine.

(Refer Slide Time: 1:46)



Now typical types of vinegar we have apple cider vinegar apple cider vinegar is made from cider or apple must, and has a brownish colour brownish gold colour. You can see the how the apple cider vinegar looks and it contains 4 gram of acetic acid per 100 millilitre medium and it is sometimes sold unfiltered, unpasteurized with the mother of vinegar present as a natural product. So this is this is how it looks and wine vinegar it is it is usually from the grape juice we produce wine and then wine the alcohol that is present there that is converted to acetic acid.

It is prepared from the fermentation of grape juice and grape based vinegar comes from the several variety such red wine and white wine. Red and white wine which contains the alcohol this when we put some acetobacter aceti then they convert these two acetic acid then we call it wine vinegar.

(Refer Slide Time: 2:59)

Now then we have malt vinegar the raw materials is barley malt sometimes its called as alegar, alegar means it is produced from ale beer we have seen in the when we discuss in the brewing industry laggard beer and ale beer is mostly used.

Now if you use the ale beer for the production of this vinegar we call it alegar, it is typically light brown colour you can it is the light brown colour and in the United Kingdom and Canada malt vinegar is a traditional seasoning for fish and chips that is the use. The sugar vinegar raw materials is sugar syrup or molasses. It ranges dark yellow to golden brown colour.

(Refer Slide Time: 3:49)

Now types of vinegar that is the another type that is available that what you call spirit or distilled vinegar. This is usually produced from the distilled alcohol because I know brewing alcohol making process I told you that alcohol has the boiling point of 78 degree centigrade so alcohol is separated out by the distillation process. Now after separation this alcohol we take it out and we this alcohol is used for with the help of acetobacter aceti, this converted alcohol to acetic acid and then we call it is spirit vinegar, so it is like this spirit or distilled vinegar.

The product made from acetic acid fermentation of dilute, distilled alcohol. It contains 5 to 8 per cent acetic acid in water with a pH of 2.6 it is known as distilled spirit or virgin vinegar or white vinegar. It is used in cooking, baking, meat preservation, pickling as well as for medicinal laboratory and cleaning purposes. The different purposes the vinegar is used. Now here I want to point out very interesting thing what is the difference between the synthetic vinegar and the natural vinegar?

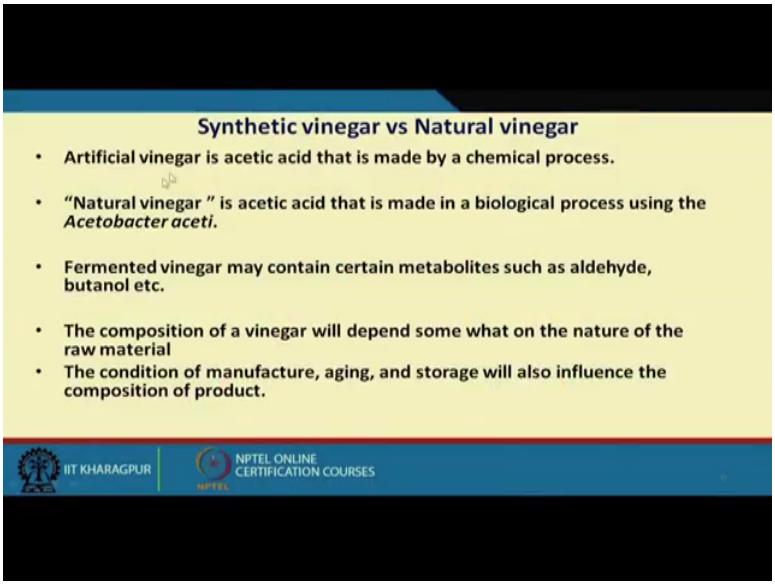
The synthetic vinegar means if we if we take the pure acetic acid because pure acetic acid can be produced chemically and take the and we make it 4 to 20 per cent and do the bottling then we get the what you call synthetic vinegar but natural vinegar is usually produced through the fermentation process. Now what is the difference you will get in the in the synthetic vinegar and the natural vinegar.

In the natural vinegar since it is produced through the fermentation process acetic acid concentration may be same but during fermentation process not only this your acetic acid is produced there is some other acid also produced and also since it is producing from alcohol ethanol some trace amount of ethanol also may be present in the vinegar. So in case of natural vinegar we have different type of component present in the vinegar but in the synthetic vinegar we have only the acetic acid.

Now vinegar synthetic vinegar also marketed into two different form one is one is white vinegar another is dark vinegar. Now you might be knowing the white vinegar the citric acid does not have any colour if you dilute into water it will perfectly white in colourless so you put it in the bottle this is one variety. Another is colour vinegar that you know the sugar when you heat I told I showed you that it undergo the dehydration process when dehydration process sugar will be caramelise caramelization of the sugar will take place and during caramelization sugar will be little darker in colour blackish in colour.

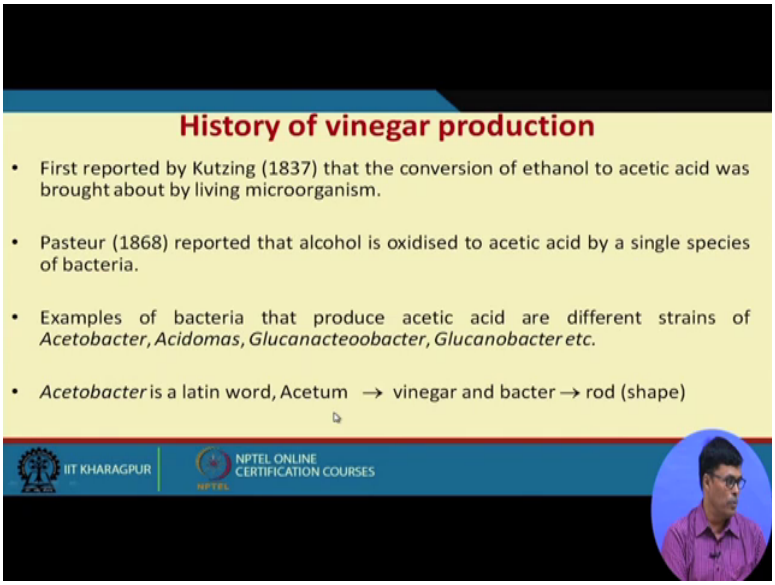
And this use as a colouring agent you make a solution in the water and mix with acetic acid solution then will be dark colour vinegar that is also marketed.

(Refer Slide Time: 7:23)



Now here the artificial vinegar is acetic acid that is made by a chemical process what I mentioned, natural vinegar is the acetic acid that is made from the biological process using acetobacter aceti. The fermented vinegar contains certain metabolise such as aldehyde, butanol and different other because may be alcohol some other components there the composition of vinegar will depend what on the nature of the raw material depends of the nature of the raw materials. The condition of the manufacture, aging, storage will also increase the composition of the product. So this is the very important point we should we should keep it in mind.

(Refer Slide Time: 8:03)

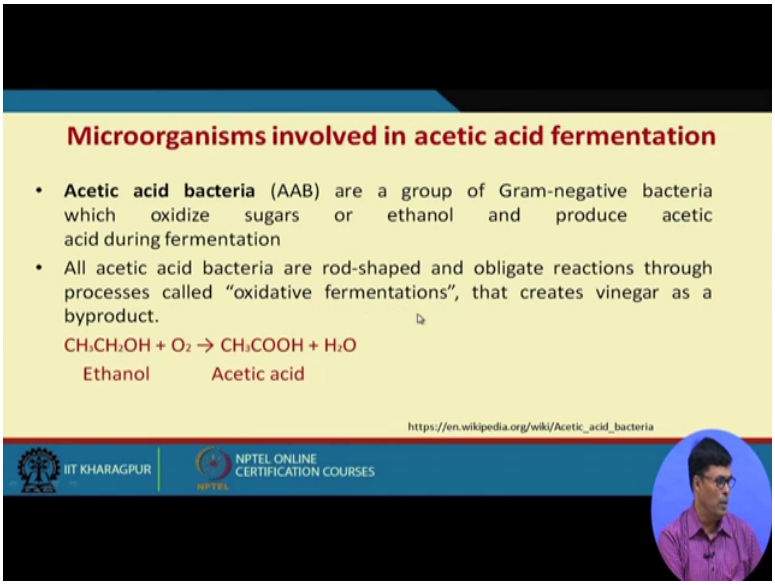


Now if you look at the what is the history behind this vinegar the first reported by Kutzing this is year 1937 convert the ethanol to acetic acid and then Pasteur 1868 reported the alcohol is oxidised to acetic acid by a single species of bacteria.

The different bacteria strains s available for this process one is the acetobacter then acidomas, then gluconacetobacter. The acetobacter is a Latin word acetum meaning is the vinegar and bacter means the rod shape that means aceto acetobacter aceti which is used mostly for vinegar fermentation process because it is it is rod shape and that is vinegar it produces vinegar that means it produce the acetic acid.

So name from the name it is it is how it is they put the name like this. The acetobacter is a Latin word the acetum stands for vinegar and bacter stands for the rod shape because if you see the microorganism under the microscope it will be rod shaped that is why it is rod shaped vinegar producing microorganism there is a acetobacter, okay.

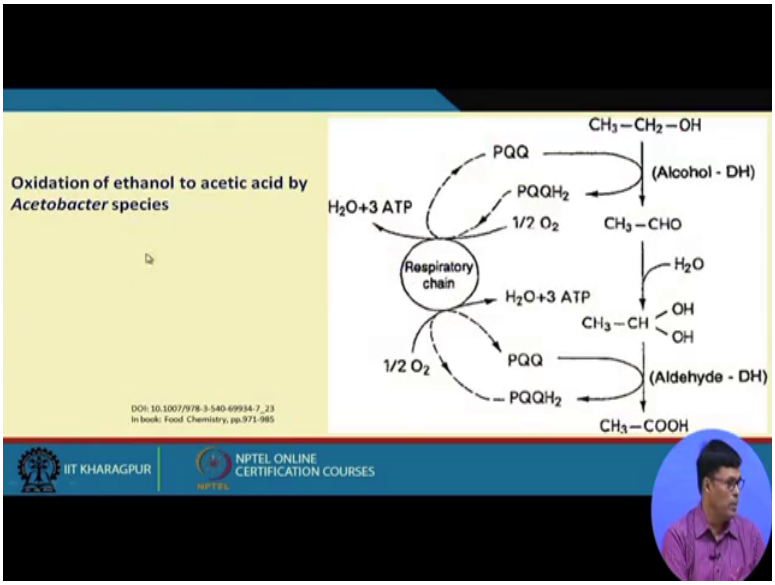
(Refer Slide Time: 9:27)



And then microorganism involve in the acetic acid fermentation process it is the call the gram negative bacteria which oxidize the sugar or ethanol or produce acetic acid, all acetic acid bacteria rod shape and obligation reaction processes the oxidation fermentation. This is this reaction take place in presence of oxygen, here I want to point out then the alcohol fermentation process the acetaldehyde to alcohol this is the reduction process.

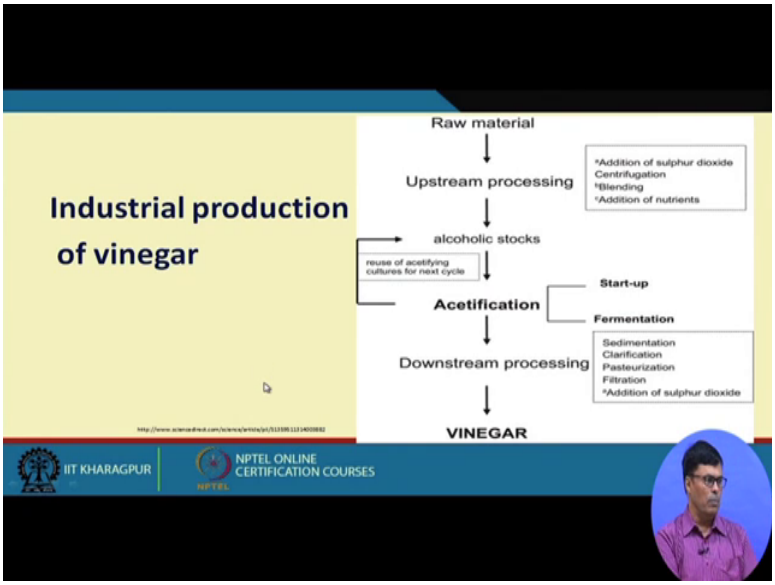
But here alcohol to acetic acid is a oxidation process. Your organism one is the organism is aerobic in nature because organism require absorb oxygen for the growth and metabolism also for the conversion of this ethanol to acetic acid we require oxygen, so this is this is the we have dual purpose.

(Refer Slide Time: 10:25)



Now this is the pathway that we have that is how ethanol that is converted to acetaldehyde then to acetic acid.

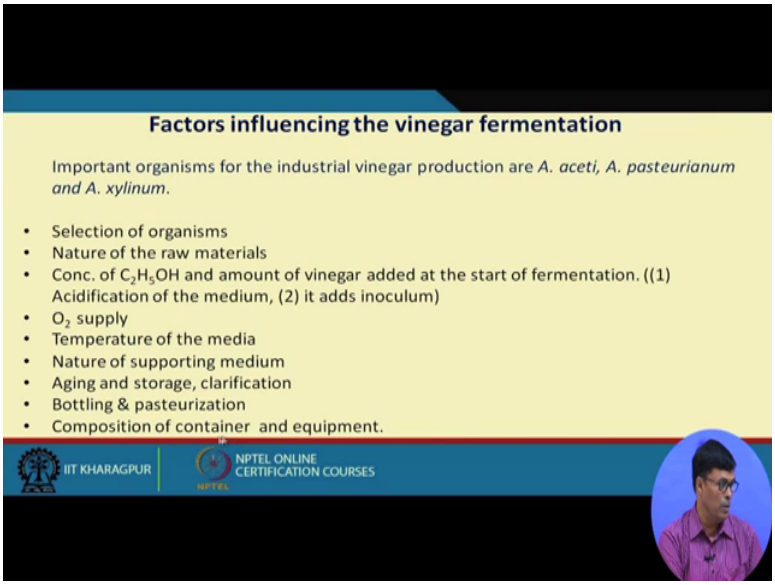
(Refer Slide Time: 10:38)



This is the oxidation of ethanol acetic by acetobacter species. Now question come what you how the industrially we produce vinegar? I shall discuss it in details, the raw materials we take it and then we have upstream processing addition of sulphur dioxide centrifuge blending and addition of nutrient whatever is required we will do this for preparation of the medium then we have alcoholic stock from that we can the alcoholic stock then we put the organism acetobacter aceti here we start up culture undergo fermentation process then after fermentation process we have downstream processing like sedimentation, clarification, pasteurization, filtration, addition of sulphur dioxide.

Then we this sulphur dioxide we add in case we do not do the pasteurization because this is used as a preservative material and we get the vinegar this is how we produce vinegar in the industry.

(Refer Slide Time: 11:34)



Now question comes what are the factors that include the vinegar fermentation process, the important organism that is used that is the acetobacter aecti acetobacter pasteurianum and acetobacter xylinum. So say the first factor is the selection of organism and nature of the raw materials they use.

Then then concentration of ethanol and amount of vinegar added at the start of fermentation because the vinegar itself they use lot of vinegar at the initial phase of fermentation one is it use of the inoculum another way it maintains the pH of the medium because it should be acidic around 3 to 3.6 that is maintain by adding the vinegar. Significant amount I shall discuss the process in details.

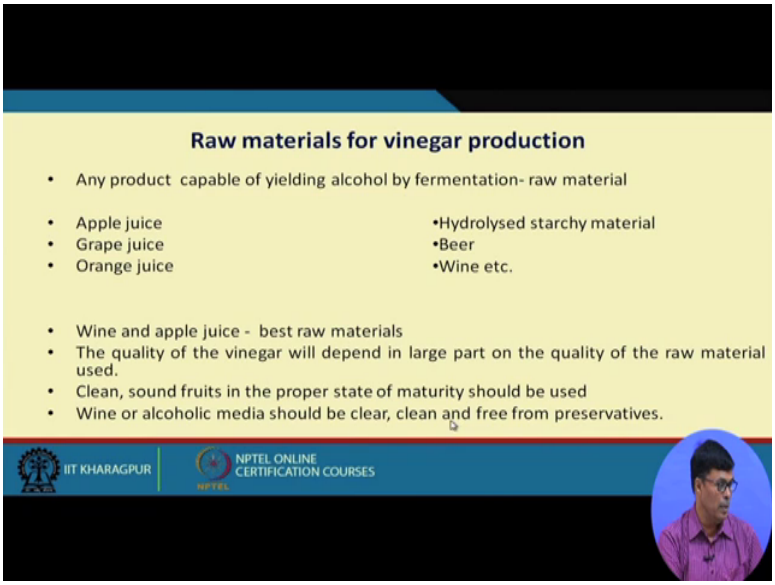
And then the concentration of ethanol and amount of vinegar added at the start of fermentation the acidification of the medium and its adds inoculum what I pointed out then oxygen is to be supplied this is aerobic fermentation process. Temperature of the medium is very important, nature of supporting material, nature of supporting material I shall discuss we know that two type of process that your organism can grow freely suspended in the liquid and produce acetic acid or we can but we can immobilize the cell on the solid metrics and pass the material through this immobilise solid metrics we need pass through this if we convert the ethanol is converted to acetic acid.

So that supporting material there talking that where the organism fixed on the surface of the solid matrix and then aging and storage where here also aging and storage is very important because during the aging process the alcohol some alcohol and acid they form the ester help

to develop some kind of flavours there so that is also clarification that is also takes place then we do the bottling and pasteurization.

Composition of the containers and equipment that also plays very important role the reason is that acetic acid is the acid and which has some corrosive property.

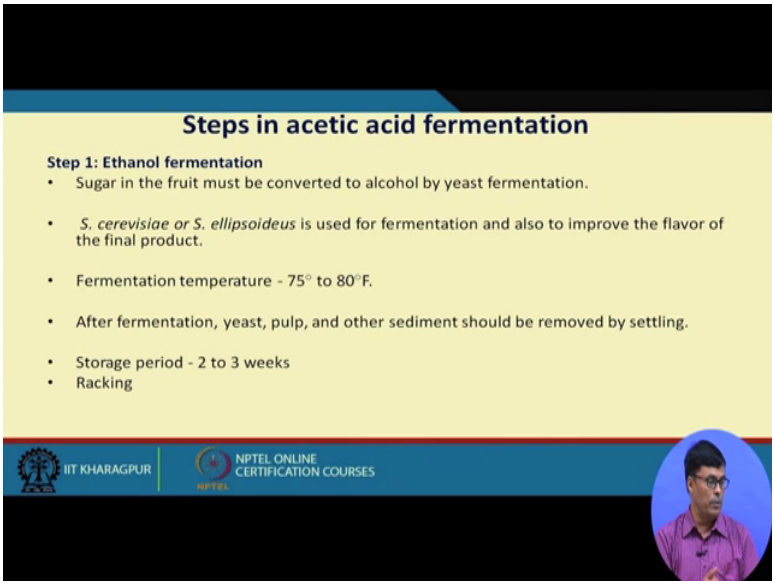
(Refer Slide Time: 13:59)

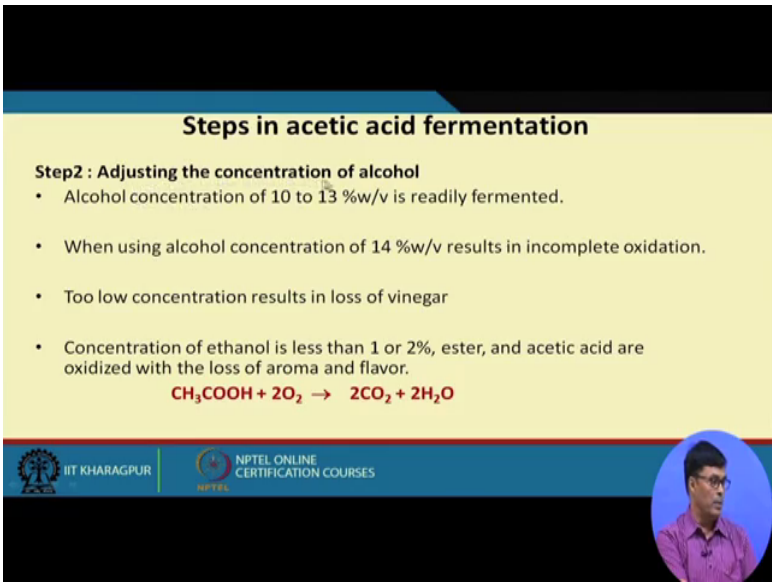


Now raw materials used for the vinegar fermentation process any product capable of yielding alcohol by fermentation that is raw materials. We use the apple juice, grape juice, orange juice.

We can use the hydrolyse starchy material, beer and wine the different types of raw material can be considered for this fermentation process and then wine apple juice is the best raw materials that find for the vinegar production the quality of the vinegar depends on the large part quality of the raw materials and clean and sound fruit in proper state of maturity should be used and wine alcohol media should be cleared and cleaned and free from preservatives so that your organism can grow properly.

(Refer Slide Time: 14:50)





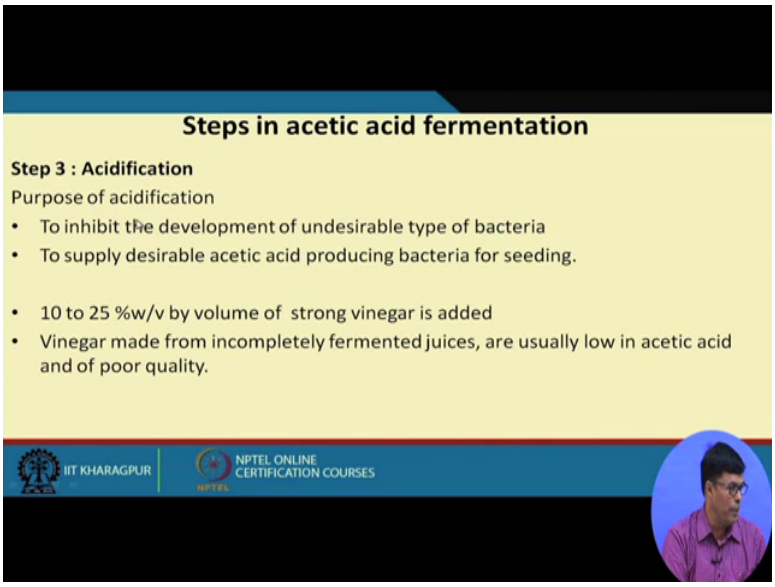
Now as the steps involved I told you their first step is the ethanol fermentation process where the sugar is converted to ethanol with the help of saccharomyces cerevisiae or saccharomyces ellipsoideus and then we maintain the temperature 75 degree, 75 to 80 degree Fahrenheit after the fermentation yeast pulp and others sediments should be removed by settling and the storage for 2 to 3 weeks then with the racking, racking also kind of a clarification process after that in the second state we adjusting the concentration of alcohol.

Now here I point out I want to point out the alcohol concentration plays very important role in acetic acid fermentation process, if the concentration is more that is undesirable more than 13 13 per cent it is undesirable if it is less than 13 per cent that is also undesirable. So we find that 13 per cent is very optimum for this for this acetic acid fermentation process. The alcohol

contains about 10 10 to 13 per cent now if it is 14 per cent it causes the incomplete oxidation if inward concentration results in loss of vinegar the concentration of an ethanol less than 1 to 2 per cent ester and acetic acid are oxidized with the loss of aroma and flavour.

So this is the reaction that we take place that that lower concentration this acetic acid in presence of oxygen that will oxidized to carbon-di-oxide and water.

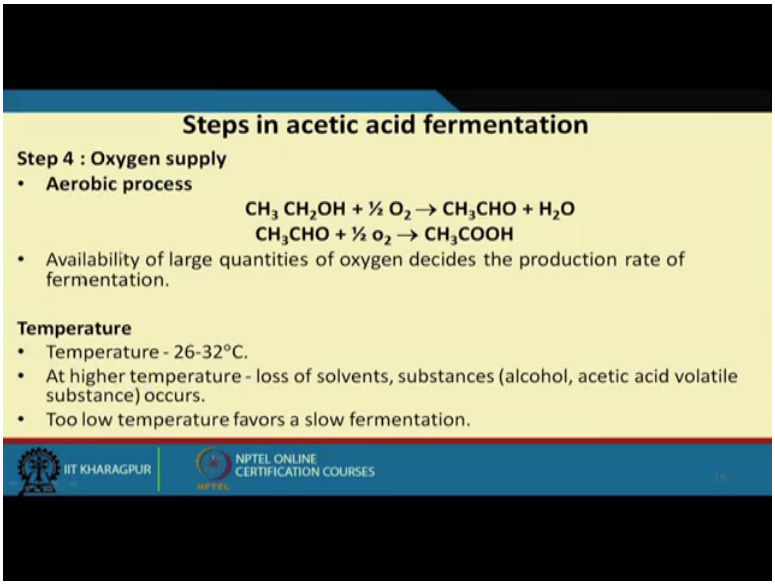
(Refer Slide Time: 16:34)



The acidification the purpose of acidification because I told you significant amount of vinegar is used as the initial stage of fermentation for dual purpose one is to maintain the pH, the purpose of acidification to inhibit the development of undesirable type of bacteria and to supply the desirable acetic acid producing bacteria for seeding purpose.

So this perhaps has dual purpose we increase the we decrease the pH so that other bacteria can grow and also we supply the acetobacter aceti so that alcohol can be converted to a acetic acid. 10 to 25 per cent weight by volume of strong vinegar is added and vinegar made from completely fermented juice are usually low in acetic acid and poor in quality. So this is very important that incomplete fermentation that give the lot of unwanted material and that resulted very poor quality of vinegar that we should keep it in mind.

(Refer Slide Time: 17:44)

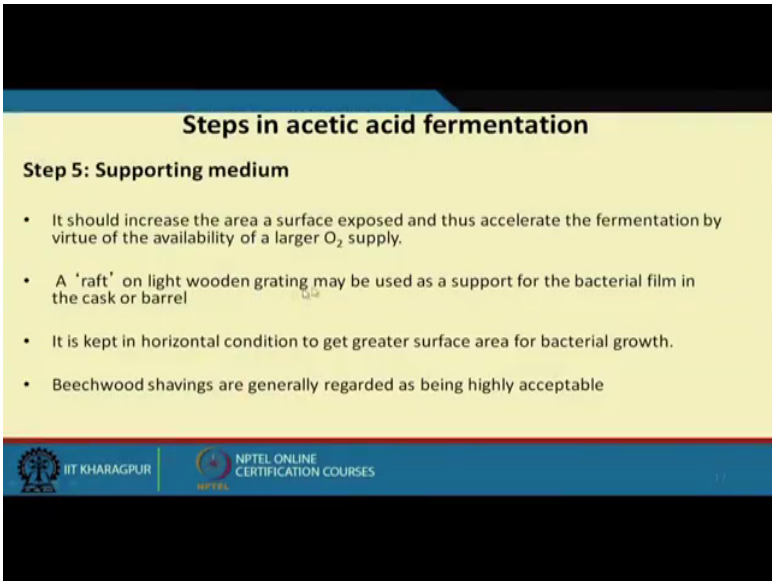


And then oxygen supply as I told you that oxygen is very much required because this is the oxidation process. The ethanol is oxidized to acetaldehyde and acetaldehyde is oxidized to acetic acid. The availability of large quantity of oxygen decides the production of fermentation and that is the production rate of the fermentation, temperature is 26 point to 32 degree centigrade at high temperature loss of solvents or substance like alcohol acetic acid volatile substances occurs.

So we shall have to maintain the temperature it will be 26 to 32 degree centigrade, low temperature favours the slow if you if you reduce the temperature the activity of the organism will be reduced then the rate of fermentation will be will be will be slow. The supporting material it should be increased the area of surface expose and thus the accelerate the fermentation by virtue of the availability of larger oxygen supply.

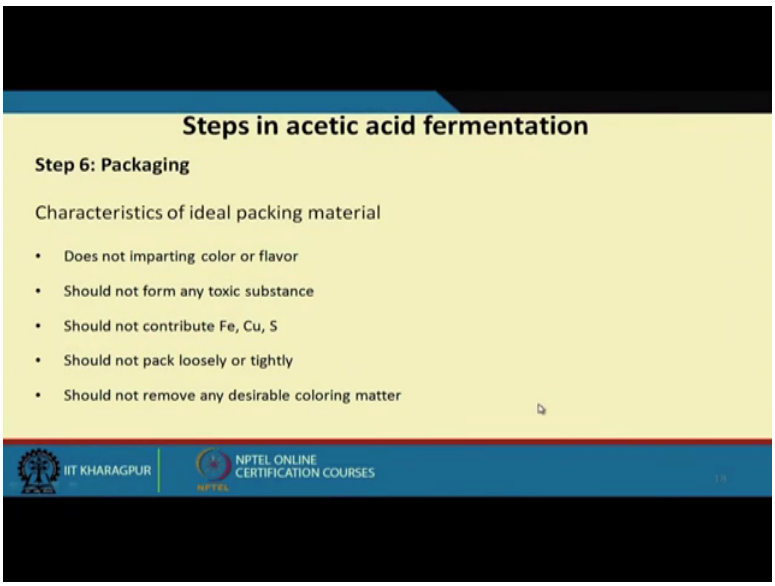
So because usually the beech wood shaving that is used as a packing material I shall explain the process and then it should be little lighter if it is lighter wood then lot of you know the population is there inside the wood lot of fruit we had inside the wood, so your oxygen can go through the fruit and organism also can immobilise on that and then that you know that your production ethanol to acetic acid production will be more.

(Refer Slide Time: 19:25)



The raft on light wooden grating may be used as a support for the bacterial film in the cask or barrels, it is kept in the horizontal condition to get the greater surface area for bacterial we know that more is the surface area more will be the that organism will be attach to the surface. So if the concentration of the organism is more then there is a rate of reaction rate of conversion of ethanol to acetic acid is expected to be more because this is what is the beech wood shaving is generally regarded as the as being highly acceptable material for this. This is for citric acid production.

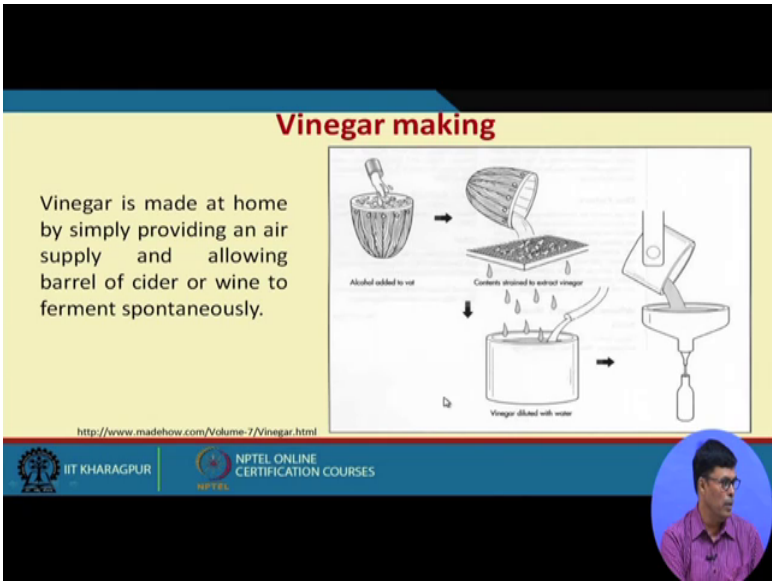
(Refer Slide Time: 20:08)



The characteristics of ideal packing material this is very important it does not imparting colour or flavour to the vinegar, it should not have any toxic substances. Should not

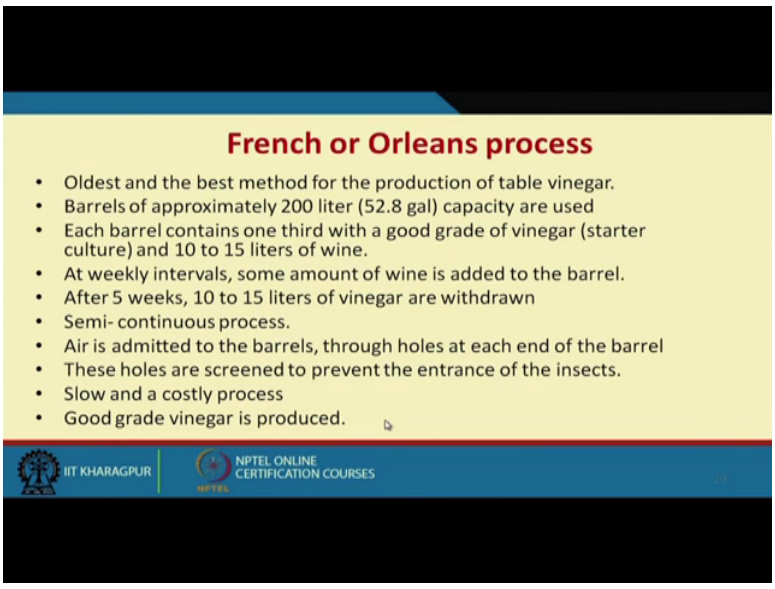
contribute to iron, copper and sulphur, sulphur should not pack loosely or tightly should not remove any desirable colour material present in the fermentation broth.

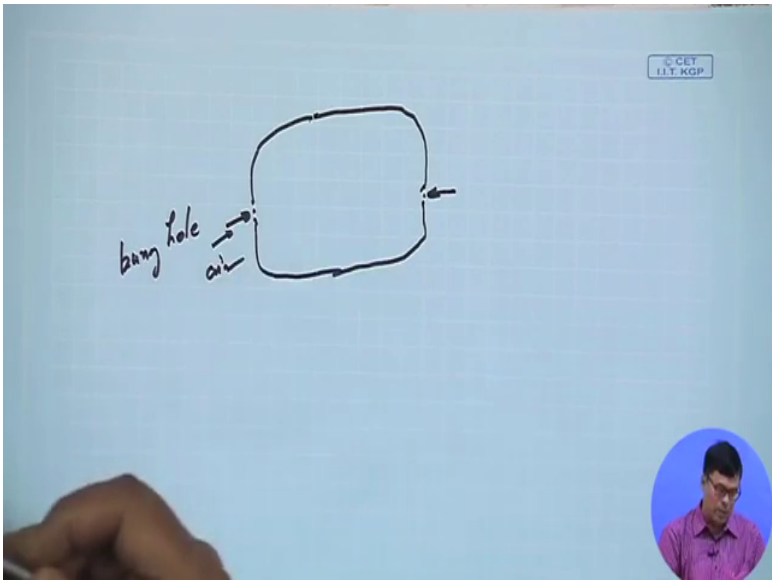
(Refer Slide Time: 20:32)



Now the vinegar this is homemade how it is homemade just one typical the follow that schematic diagram is given here the vinegar is made at home by simple providing the air supplying and allowing the barrel of cider or wine to ferment spontaneously. So here we have alcohol added in the barrel where we have vinegar then we put it here we take out this and vinegar diluted with water then we put it in the bottles like this, this is our homemade vinegar.

(Refer Slide Time: 21:08)





Now two process largely available for vinegar fermentation process one is called Orleans or of the French process another is we have German process, let me discuss about the French or Orleans process.

It is the oldest and the best method for the production of table vinegar. Barrels are approximately 200 litres capacity are used. Each barrel contains one third with a good grade of vinegar I told you that significant amount of vinegar is required at the start-up of the fermentation process, then one third means it is one third it means 200 litres is one third approximately about 66 litres.

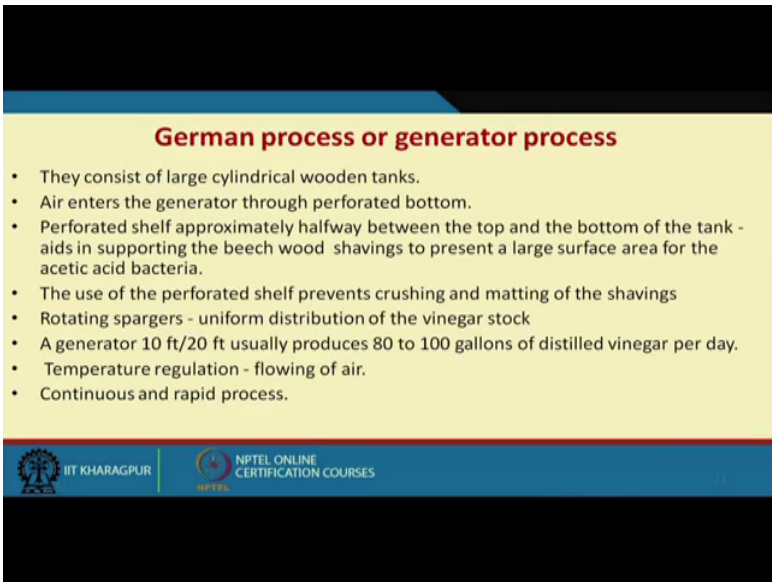
So 66 litres we add and from that we add 10 to 15 litres of wine and then at weekly intervals, some amount of wine is added in the barrel same amount of wine is added not some, same amount of wine is added because this same this is 10 to 15 litres same amount we keep on adding and then after 5 weeks that 10 to 15 litres vinegar we withdraw and same amount of wine we enter we take it in.

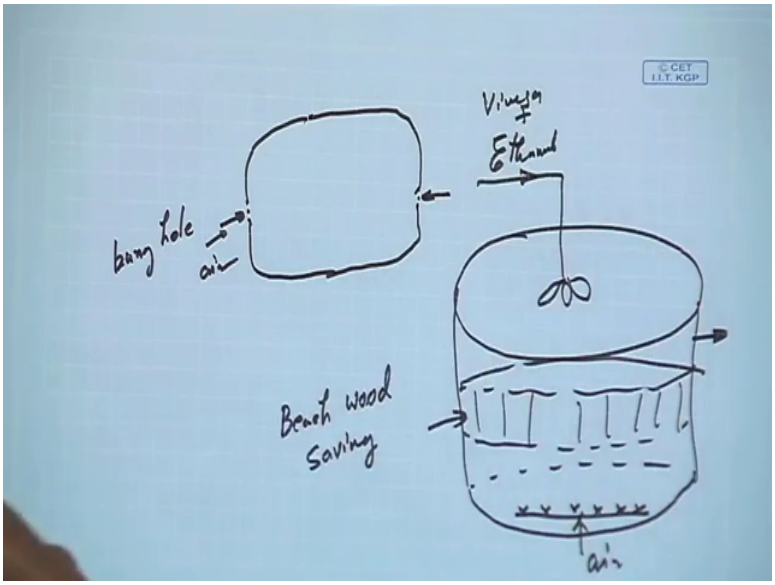
This is a semi continuous process air is admitted at the barrel through the holes at each sides of the barrel I can show you this it is looks like this, this is called bung hole this is the hole where the air goes but we put some kind of you know that membrane here so that no flies and other things can enter into the system at the same time here air can enter into the system through this.

So air is admitted in the barrel through the holes at the each end of the barrel and this holes are screened to prevent the entrance of the insects I told you that and slow and costly it is the slow and costly process and good grade vinegar can be produced through this process, so this

is a old process and this called the largely used from the old age they use largely but you know more advance process is considered as the German process or what you call generator process.

(Refer Slide Time: 23:54)





And this is consists of large cylinder, wooden tank so I can give the example that this is the wooden tank.

So here we can have beech wood shaving we can put the beech wood shaving this is this is beech wood shaving and so your organism will be immobilise on the surface then you pass your air through this and here there is a sprinkler so you can have a sprinkler like this, so we put ethanol here this sprinkle the organisms are remobilised on the surface when it goes then

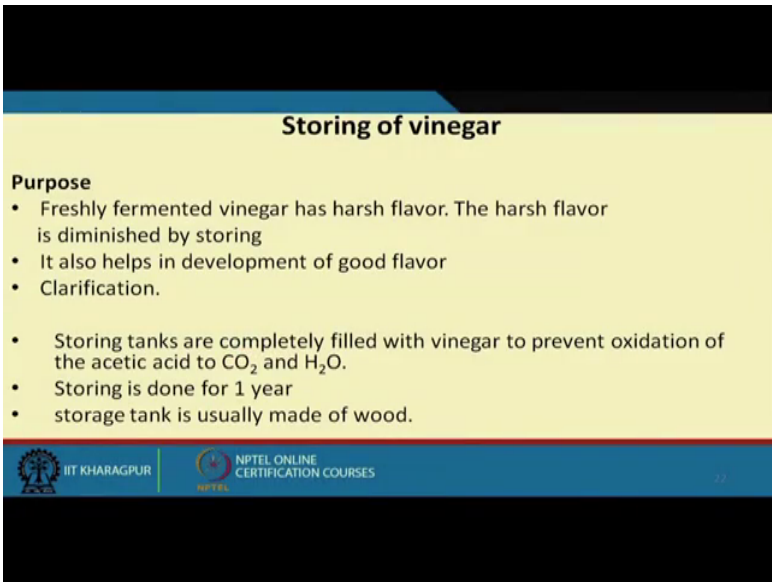
it comes in contact with this and they produce the acetic acid then you can take out the acetic acid form here you can take it out from.

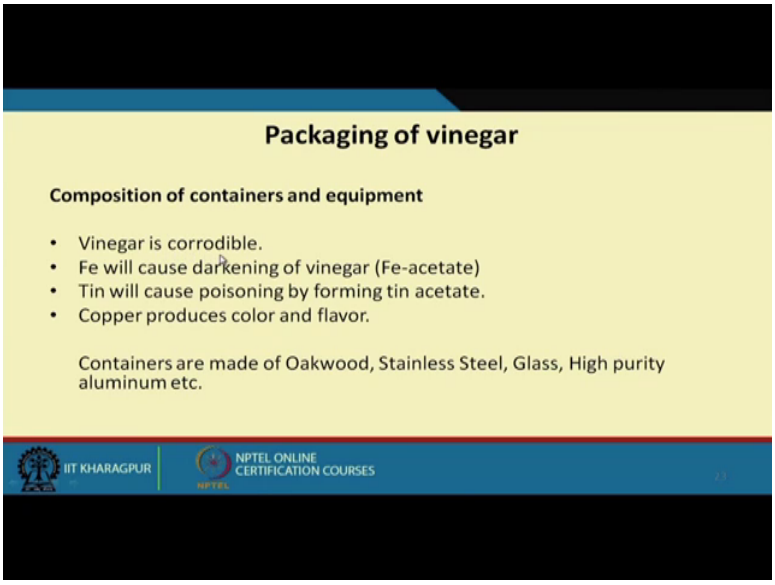
Large cylindrical tank wooden tank air is centred in the generator through the perforated bottom so we can have a distributor we can have the distributor like this. The perforated shelf approximately half way between the top and bottom of the tank and its supporting the beech wood shaving to present the large surface area for the acetic acid bacteria. The use of perforated shelf prevents crushing and matting of the shavings.

So perforation that is very much required for that and rotating spargers uniform distribution of the vinegar stock, this is the uniformly this we put some kind of vinegar stock we have vinegar plus ethanol both we put together vinegar stock we put together. A generator of this is usually that 10 to 23 the height and produces about 80 to 100 gallons of distilled vinegar per day and then temperature regulation is done by flowing the air and continuous this the continuous rapid process.

So this is how this process is used and we this rate of this that acetic acid production will be much higher as compared to the French or Orleans process.

(Refer Slide Time: 26:49)





Now next problem is the storing of vinegar the purpose is the freshly fermented vinegar has harsh flavour I told you during the fermentation process lot of fatty acid formation is there that gives the very harsh flavour so this harsh flavour would be diminished as soon as you stored because alcohol and acid they form the esters, it also help developing the good flavour and clarification because it removes some kind of suspended material.

Storing tanks are completely filled with vinegar to prevent the oxidation of acetic acid to carbon-di-oxide and water storage is done for one year and storage tank is usually made of wood that is that is usually the packing material the composition of the containers the vinegar is corrodible and iron will cause the darkening of the vinegar and tin will causes the poisoning by forming the tin acetate. Copper produce the colour and flavour.

So this is this is not required so we are the container should be of proper quality and the container should be made of either oak wood, stainless steel, glass or high purity aluminium that is used as a as a container. Thank you very much