Course on Industrial Biotechnology
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Lecture 27
Module 6
Ethanol Fermentation

Welcome back to the Industrial Biotechnology course today, this first this is the first class in which we are going to discuss one industrial fermentation process where we will be and try to find out that how industry the industry how we work with this biochemical process and if you look at the ethanol fermentation process it appears to be the oldest fermentation process and in India we have more than 250 distilleries and and this produces ethanol from the mostly from cane molasses.

Some industry also use starchy raw materials like broken rice for the production of ethanol. Now today I am going to share my experience and the details of the ethanol fermentation process.

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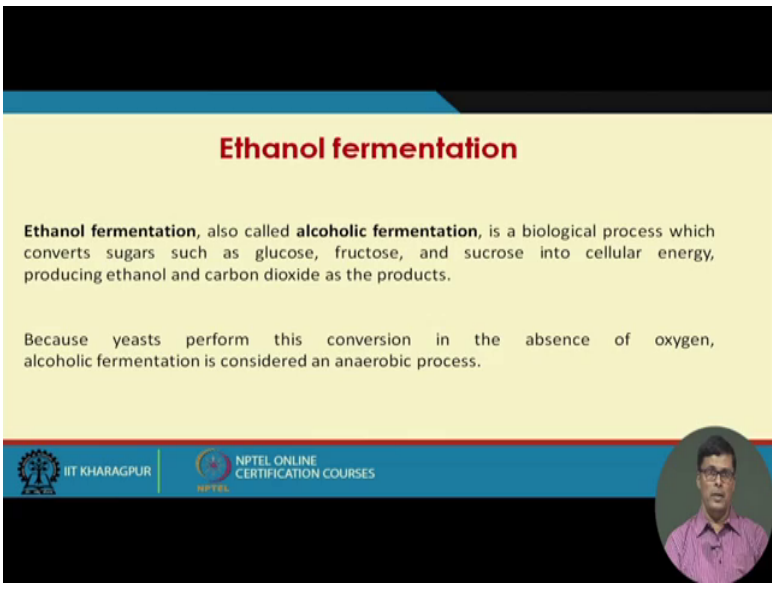

Now if you look at the ethanol fermentation process that it is basically it is called the alcoholic fermentation process and is a biological process which convert the sugar into glucose and fructose and sucrose into the cellular energy producing ethanol and carbon-di-oxide.

Let me explain that, because the sucrose because I told you the cane molasses is fine to be the very suitable raw materials for the ethanol fermentation process and cane molasses contains

about 50 per cent of sugar this sugar in presence of the enzymes invertase it is converted to glucose and fructose and this this glucose again when you pass through the Embden-Mayer Pathway it produces pyruvic acid and pyruvic acid when undergoes carboxylation reaction is produces the acetaldehyde and acetaldehyde when it is reduced it produces ethanol.

I shall explain the details of this biochemical pathways now the yeast is the is largely used for this ethanol fermentation process and yeast perform the conversion in the absence of oxygen ethanol fermentation is considered anaerobic process. So yeast can grow in two ways under aerobic condition it produce the cell mass what you call Baker's yeast where largely used in the bread making industry but under anaerobic condition sugar will be converted to ethanol.

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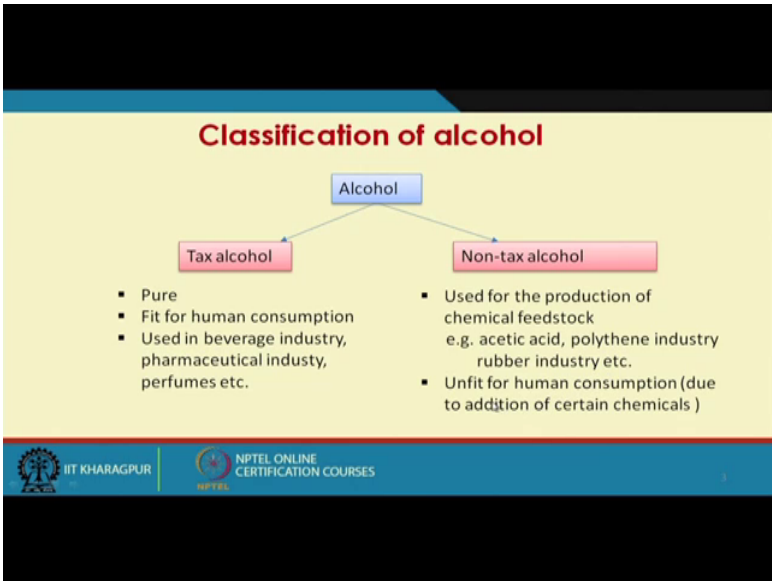

Now the classification of if you look the classification of ethanol we have two different classes one is called tax alcohol another we have non-tax alcohol. Tax alcohol is usually considered as a pure alcohol and non-tax alcohol is the alcohol that is used as a chemical feedstock in the industry. Now since tax alcohol is the pure alcohol this can be used for human that human consumption and used in the beverage industry, pharmaceutical industry and perfumes largely used.

But in non-tax alcohol used as a chemical feedstock like acetic acid, polythene industry and rubber industry etcetera but it is unfit for human consumption due to the addition of certain purposefully that we add certain chemicals to make the alcohol totally unfit for human consumption because the cost of non-tax alcohol is very less as compared to tax alcohol. So it

is in India since we are the tropical country the government of India they do not encourage to take alcohol for human consumption that is why they impose lot of tax on it.

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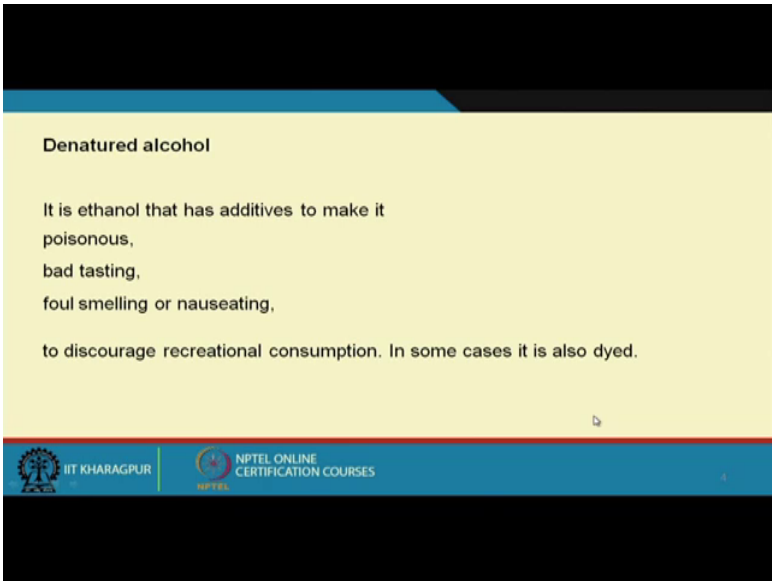

Now denatured alcohol this is non-tax alcohol basically it is the denatured alcohol this is as I told you that it is we add certain additives which makes it poisonous, bad in taste, foul smell or nauseating and to discourage the recreational consumption in some cases it is also dyed. So that everybody can understand that. So they have they use the different formulae for preparing the denature alcohol depending on the industry we are going to use because when they add different chemicals in the denature alcohol they take into account those chemicals should not affect that particular chemical production process.

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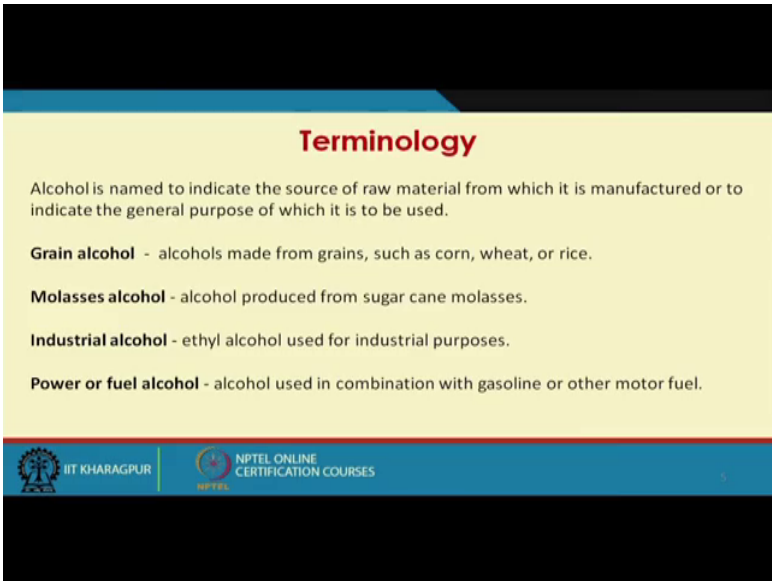

So on the basis of that they choose different formulas. Now the terminology different types of alcohol we have in the market we that we have different names we have like grain alcohol, molasses alcohol, industrial alcohol and power alcohol. Now power alcohol is very interesting now-a-days we have lot of you know power shortage problem though government they are insisting that gasoline as we know that India has a acute shortage of the petroleum because whatever reserves we have or petroleum is hardly for 30 years, so we mostly import the crude petroleum from outside and it which is very costly.

So government of India they always encourage that 10 to 20 per cent of gasoline that can be whether we can replace by ethanol. So when we use the ethanol as a substitute or gasoline we call it power alcohol.

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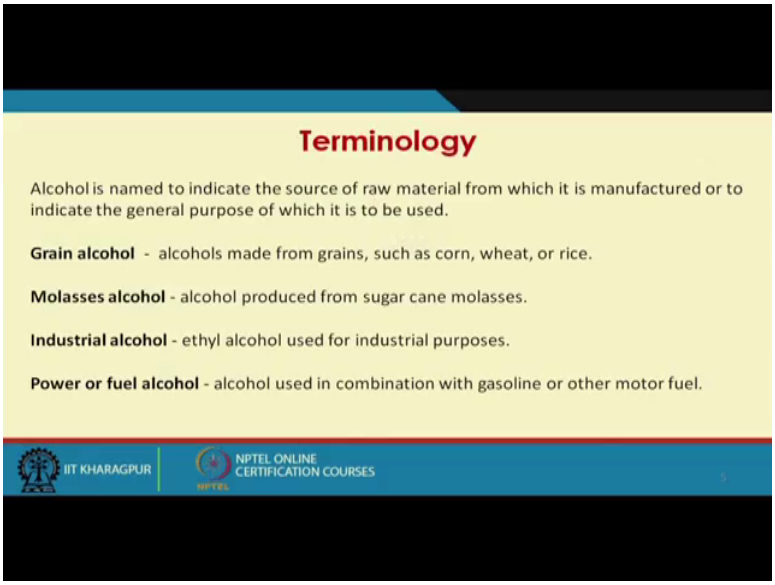

Now in case of grain alcohol means that this alcohol is produced from grains such as corn, wheat and rice. Molasses alcohol means it is produced from molasses maybe cane molasses maybe beet molasses. Industrial alcohol is used for the industrial purpose.

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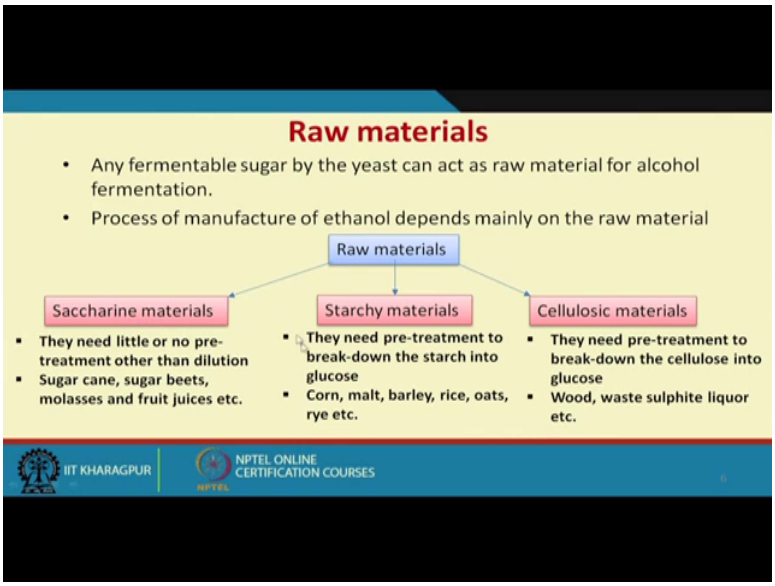

Now raw material that is used for the for these alcohol fermentation process has three different types one is we call that saccharine materials which mostly comprises of sugar type of material which easy to ferment and so you do not require any kind of pre-treatment but when we use the material like starchy materials and cellulosic material it require the pre-treatment like in case of starch material first starch has to degrade into glucose then glucose with the help of the Embden-Mayer pathway it produce the ethanol.

Now in case of cellulosic material we cellulose is break down to glucose with the help of the enzymes like cellulosic enzymes we have we have exoglucanase, endoglucanase, alpha, beta 1, 4 glycosides the different enzymes they participate in the reactions and then they converted into glucose. Now we use the wood and waste pulp liquor wood mostly we have lignin cellulosic material we remove the lignin then the cellulose we hydrolyse and produce glucose this glucose ultimately can be converted into ethanol.

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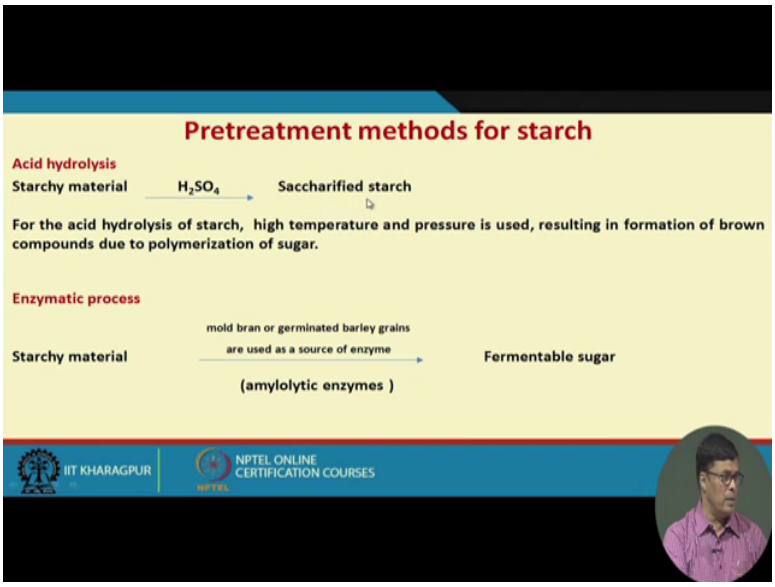

Now it is it can be explained in this way that starch can be chemically pre-treated with H2SO4 when hydrolyse in presence of acids we get the saccharified starch for the acid hydrolysis of starch this is take place at high temperature and pressure and which resulting the formation of brown compounds due to polymerization of sugar.

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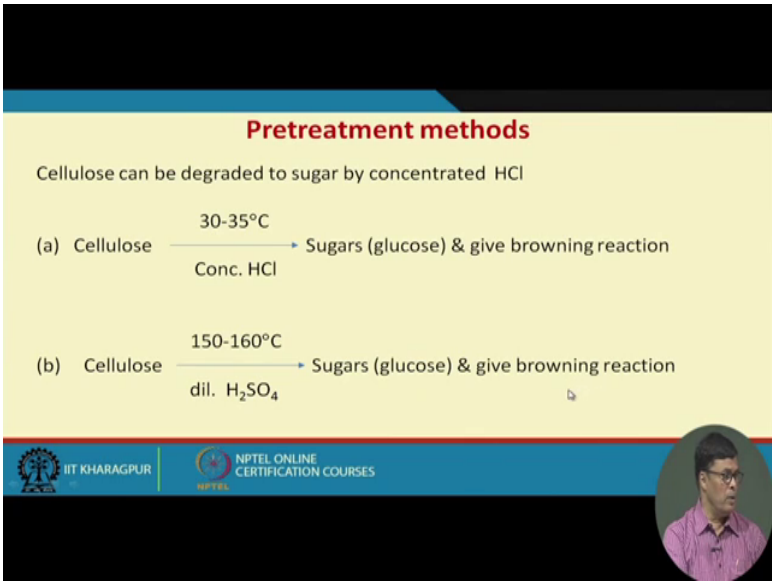

An enzymatic process we use the starchy material mostly we use the amylolytic enzymes for the conversion of the starch to sugar and amylolytic enzymes also different types we have alpha amylase we have beta amylase we have gluco amylase so you know different types of enzymes they participate in the reactor reaction and give the glucose. Now in case of cellulose mostly is hydrolyse by acid concentration HCl concentrated HCl or dilute H2SO4 to give the sugar but both the cases we get the have the brewing reactions but major problem and also I told you enzymatically also we can convert the cellulose into glucose in that case we shall have to use the cellulose enzymes.

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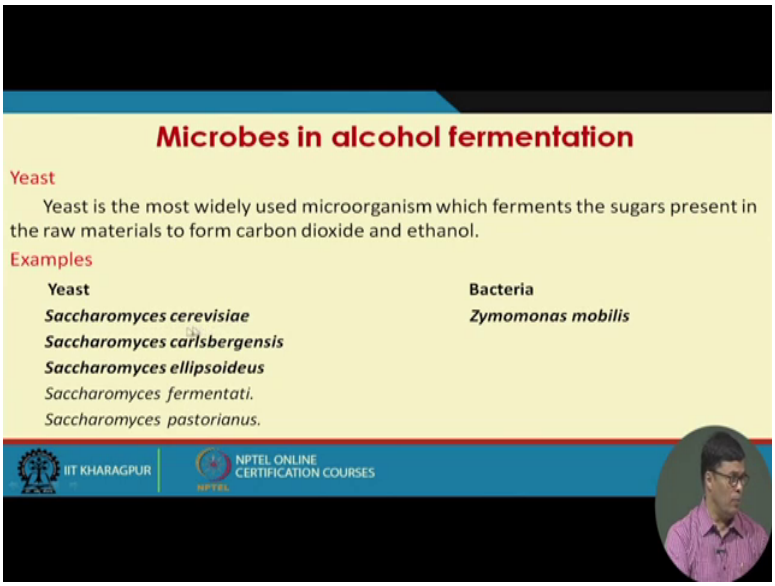

Now question comes what is the microorganism that used in the alcohol fermentation process. The two type of microorganism is usually preferred and one is called yeast largely used by the industries and bacteria also to some extent it is used the why the bacteria is chosen the reason is that bacteria we know the doubling time of bacteria is much less as compared to yeast that is why we prefer some cases we prefer the yeast bacteria but most of the cases we use saccharomyces cerevisiae which is easy to use it is very easy to use most of the cases for alcohol fermentation process we use saccharomyces cerevisisiae which is very hard cell wall very easy to grow.

Then saccharomyces carlsbergensis is also used for the for this fermentation process, saccharomyces cerevisae usually called top fermenting yeast saccharomyces carlbergensis is usually called bottom fermenting yeast. Saccharomycis ellipsoideus that is mostly used for wine making industries other saccharomyces species also used in some typical fermentation process and as for bacteria is concerned we use the zymomonas mabilis now has the potentiality to convert sugar into alcohol.

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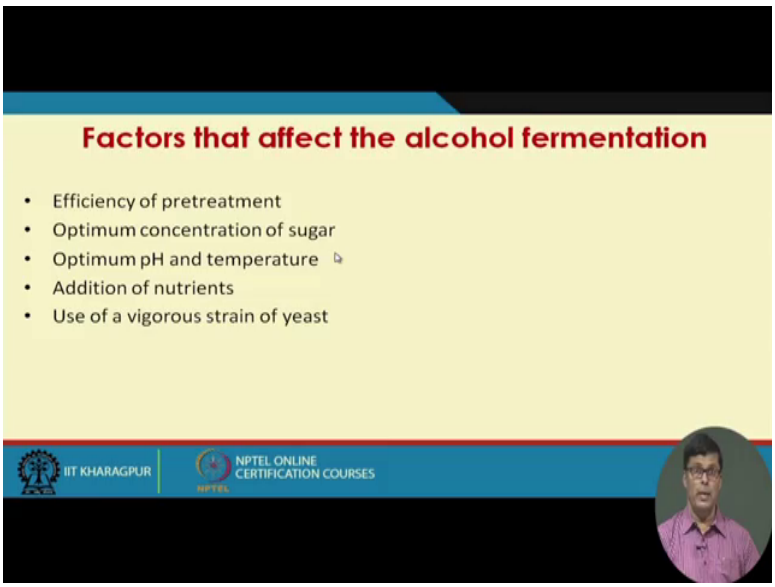

The factors that effects the ethanol fermentation process the efficiency of the pre-treatment this is very important then optimum concentration of sugar because we have observed that I told you in the in one of my lectures I mentioned that the cost recovery cost or purification cost depends on the that concentration of products in the fermentation broth. Now if the concentration of the product in the fermentation broth is high then recovery cost will be less. So usually we preferred the high concentration of the fermentation concentration of product in the fermentation broth.

Now in this case if we increase the ethanol concentration in the fermentation broth we shall have to use more sugar and if you use higher sugar concentration then it causes some kind of osmotic pressure and we shall have to develop some osmo tolerant yeast culture so that you know we can have it can withstand the high sugar concentration also it should withstand the high ethanol concentration. The osmo tolerant and alcohol tolerant culture is very much required.

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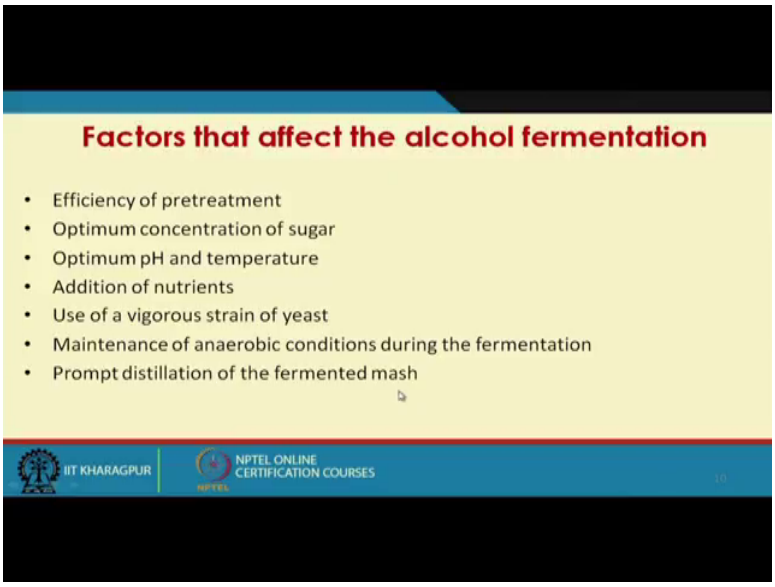

So optimum concentration of sugar is very important then optimum pH and temperature the yeast fermentation usually taken place as the acidic pH and at their own 4.5 to 5 and temperature is close to ambient temperature is equal to 30 degree centigrade, addition of nutrient as per because we required some nitrogen, magnesium or phosphorous that we sometimes we use as per the requirement and use of vigorous strain of yeast we use the high product strain that industrial strain we use which has high productivity.

Maintenance of anaerobic condition during fermentation process because that anaerobically that yeast can convert the sugar into alcohol, so anaerobic condition is very required. Now one thing I want to point out here the alcohol fermentation process is the fermentation process usually can take place in the open vat we do not require much of strain condition. The reason is that because as I mentioned that it produce in acidic pH acidic pH less amount of less number of organism can grow and also initially that I told you the yeast can grow under aerobic condition and it produce the cell mass under anaerobic condition it produce the alcohol. So initially whatever dissolved oxygen is there that will be used by the yeast cells for their growth and metabolism.

Then as soon as that is that is consumed the anaerobic condition prevail it convert the sugar into alcohol. So automatically the anaerobic condition and anaerobic condition a few number of organisms can grow and usually we I told I mentioned that cane molasses or molasses is fine suitable for the for the alcohol fermentation process I shall explain why molasses is suitable for the alcohol fermentation process in my next slides.

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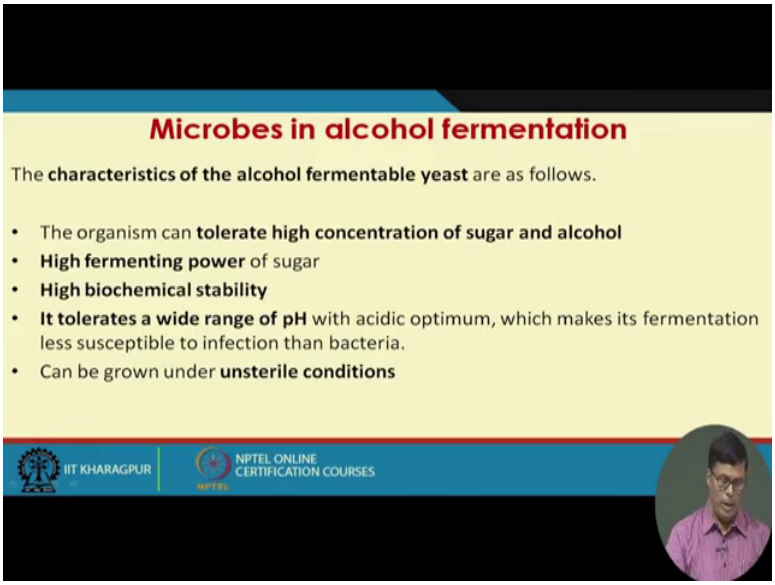

Now then then prompt distillation of the fermented mash that is also very much required, now the characteristics of alcohol fermentable yeast are as follows as I mentioned it should tolerate the high tolerate high concentration of sugar and alcohol, high fermenting power of sugar more rate of fermentation. I initially couple of slide I mentioned that characteristics of the organism we can determine with respect to case and Mu max value and I also mentioned that when I discussed the (())(15:01) model for product formation I told you that alcohol is the growth associated product that means rate of alcohol formation is proportional to the rate of cell mass formation.

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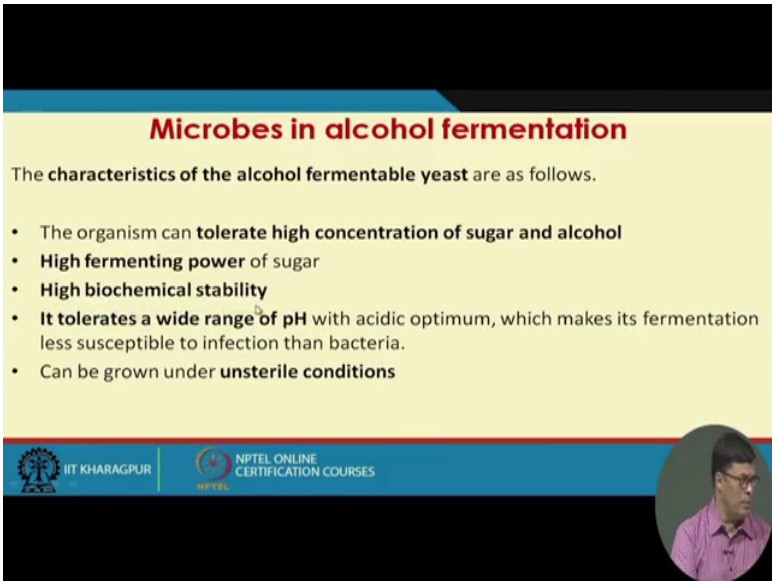

So say mode cell mass formation is there more alcohol formation will take place in the system. Now high fermenting power is very important high biochemical stability that is also very important more we have biochemical stability so that you know fermentation process we have uniform alcohol concentration which is very much desirable in the industry.

It tolerate the high wide range of pH with acidic optimum, which make the fermentation less susceptible to infection than bacteria can grow in unsterile condition. This is the major advantage of this alcohol fermentation process.

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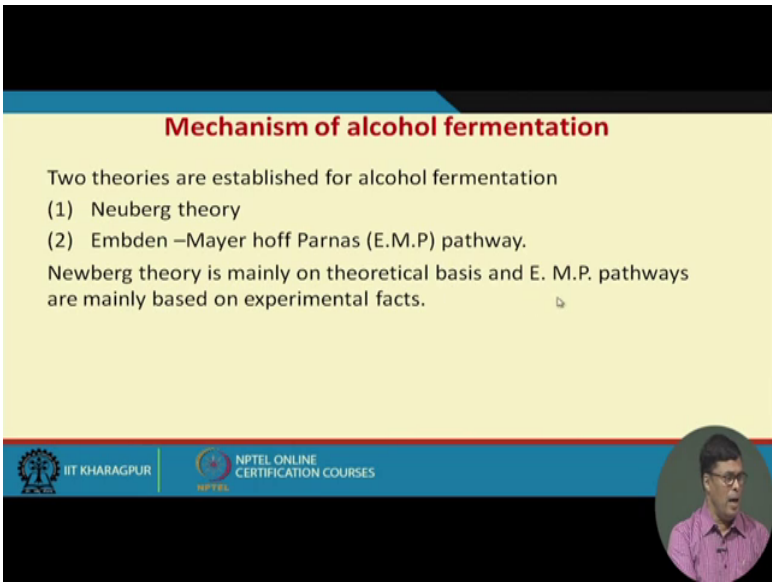

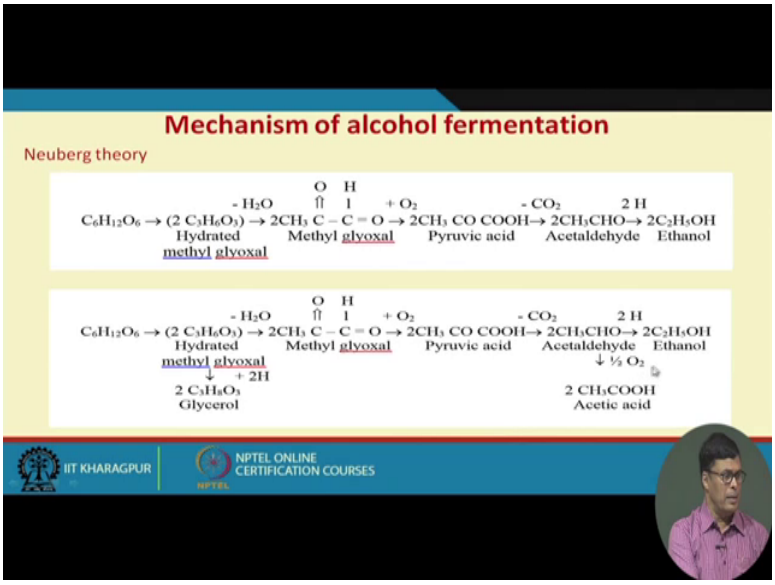

Now mechanism of this alcohol fermentation process can be explained with the help of this Newberg theory and the Embden-Mayer hoff Parnas pathway and Newberg theory is mainly the theoretical basis and EMP pathway is mainly based on the experimental facts and you find here the this is the glucose molecules then hydrate hydrated the methyl glyoxal this is converted to pyruvic acid then acid aldehyde then then ethanol.

This is the this is how it produce then other way one way it can produce glycerol another way it can produce the acetic acid also it can produce ethanol but Embden-Mayer pathway I do not like to mention because we know Emdben-Mayer pathway the glucose is converted to pyruvic acid one mole of glucose is converted two mole of pyruvic acid and then same procedure is used that it under (())(17:01) reaction we produce the acid aldehyde then when is reduced further reduction takes place it produce the ethanol.

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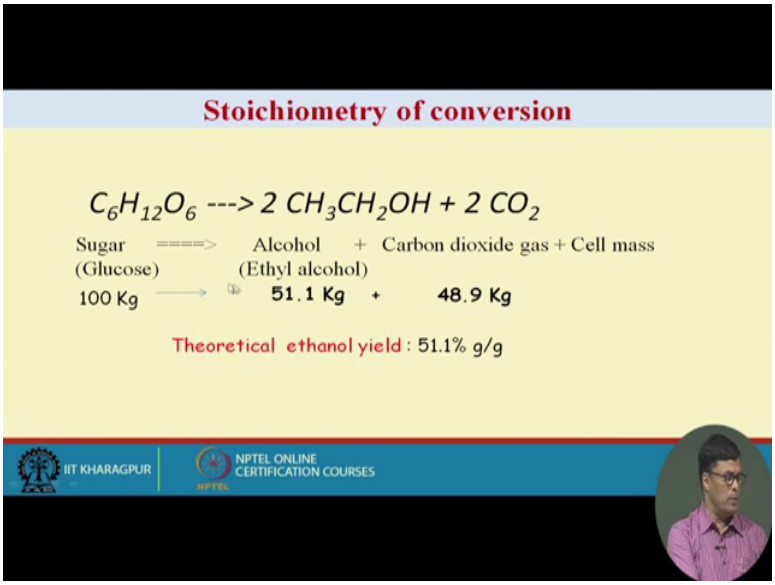

Now stoichiometry of this process is like this one mole of glucose can produce two mole of ethanol and two mole of carbon-di-oxide. So 100 grams if you see the 100 grams of glucose as first stoichiometry it produce 51.1 kg of ethanol and 48.9 kg of carbon-di-oxide and this carbon-di-oxide I want to mention it is pure carbon-di-oxide, so most of the some of industry they carry out the though this fermentation can be carried out in open vat but they usually carried out in the close vessel so that we can collect this carbon-di-oxide and high pressure it can convert into dry ice and they send it to the some cold cold drinks producing industries.

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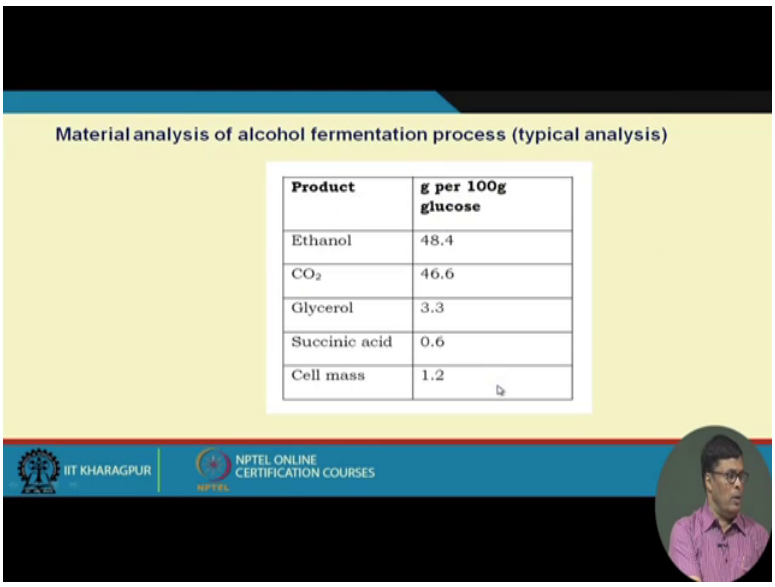

The theoretical yield is coming about 51.1 per cent that is. Now typical analysis of the fermentation product that I have given here that material analysis that 100 grams of glucose

produce 48 point 4 grams so you can see before the stoichiometric is a 51.1 kg but it is a actual practise will get less amount of ethanol then 46 point we can see here that 48.9 kg here we have 46.6 glycerol 3.6 succinic acid 0.6 and cell mass besides that it produces fusel oil.

Fusel oil is nothing but the higher alcohols that also it produce during this fermentation process.

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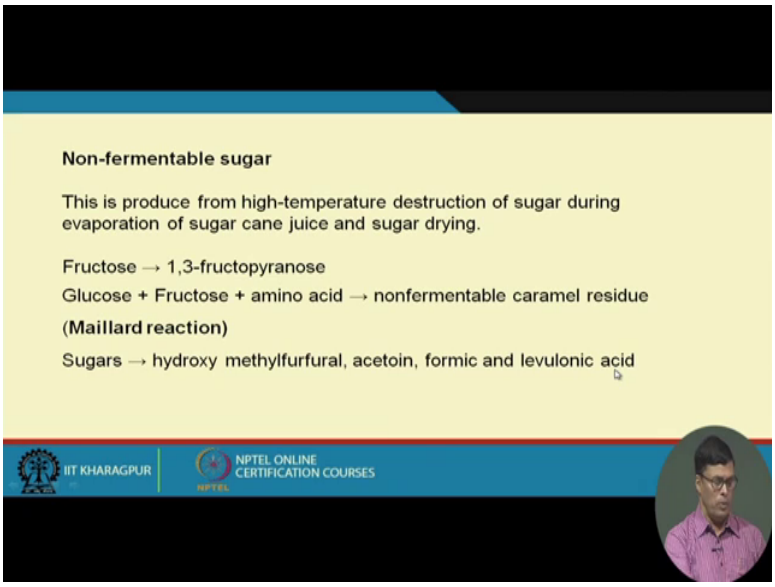

Now during the I told you that cane molasses is considered as a very effective raw materials for this ethanol fermentation process and cane molasses is the byproduct of the sugar industry it produce this is this is produce from the high temperature destruction of sugar during the evaporation of sugar and the of the cane juice and the sugar drying process.

So some kind of during heating process some destruction of the sugar take place as for example fructose will convert to 1, 3 fructpyranose this is simple dehydration we get this product glucose, fructose amino acid they form the non-fermentable caramel residue this is the mallard reactions. So this is also not fermentable then we know the pyrolysis that sugar when undergo pyrolysis it produce the hydrooxy methylfurfural acetoin, formic and levulonic acid

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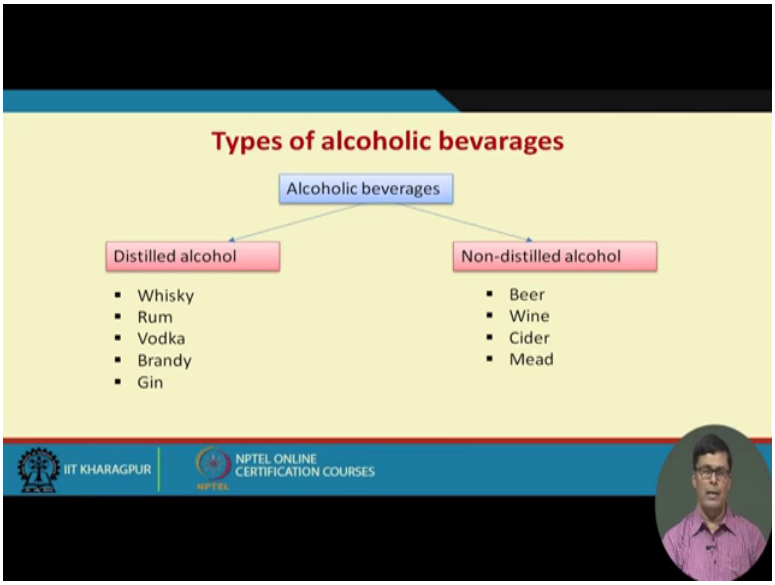

This are not produce this cannot be produced ethanol. So this non fermentable sugar also produce present in the in the media to some extent, so let me share the type of alcohol fermentation process that as for when alcohol is use as a beverage it has two it can be divided into two types one is called distilled alcohol another is non-distilled alcohol. Now what do you means by distilled alcohol? Distilled alcohol means that after the fermentation is over it passes through the distillation process and through the distillation process we get the pure alcohol pure means I am saying that normal distillation process maximum alcohol concentration we get about 95 per cent above 95 per cent it forms the azeotropic mixture with water in that case even through the distillation you cannot separate the alcohol from the water so you have to use the difference solvent like benzene to get the absolute alcohol.

Absolute alcohol means is contains the 100 per cent of 100 per cent of alcohol. Now concentration of alcohol in the industry usually expressed in degree proof.

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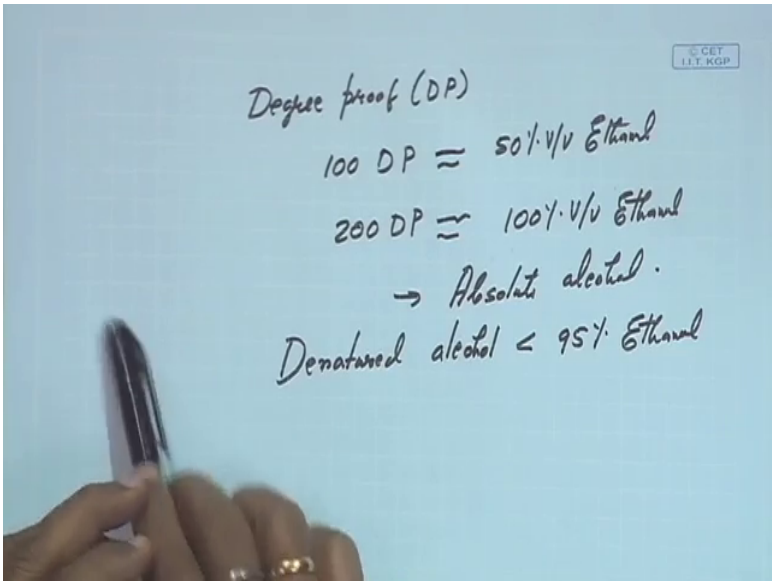

Now suppose we have 100 degree proof is approximately equal to this is equal to about 50 per cent volume by volume ethanol. So if you say 200 degree proof it contains about 100 per cent of volume by volume ethanol. So this is called absolute alcohol and we talk about the denatured alcohol in the denatured alcohol actually the denatured alcohol, usually contains less than 95 per cent alcohol ethanol. Now I told you denature alcohol is a purposefully makes unfit for human consumption by adding different ingredients.

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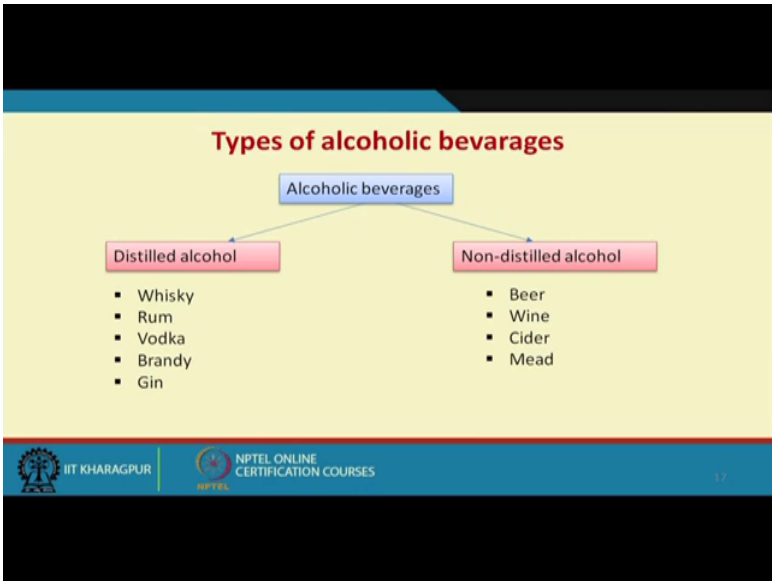

Now here the distilled alcohol means we through the distillation process we separate the alcohol and this alcohol we mix with different ingredient to give the different colour and flavour.

Now we have different distilled alcohol we have whisky, rum, vodka, brandy and gin but this actually whisky actually usually produced from wheat starch and rum usually produced from this this cane molasses, brandy usually produce from fruit juices so different distilled alcohol has different raw material different raw material that is used for this production process and different company they have different trade name that for the like whisky we have different company, they have different that you know that different company have different trade name.

And their taste is varies with respect to the spices because after the alcohol fermentation alcohol separation is taking place this alcohol mix with different spices to give different smell and different taste. So different taste and different that is depend on the formulae that what kind of spices they use to give and that is 100 per cent trade secret as per different industries. This never, scotch whisky I can give the example that is that is that is as typical flavour and typical colour. So this is rum we have we have typical flavour and typical colour, so vodka, brandy and gin.

Now here I want to point out the brandy usually produce from the fruit juices now when we go to the market and try to purchase the brandy that and if it is written in the in the bottle that artificial then it is not produces from the fruit juices because it artificial means may be it produce from cane molasses or some other processes and then they add some spices in such a way it give the taste of brandy and the taste it can it give the taste of brandy.

So that is that is how it is produced. Now non distilled liquor we have several we have beer we wine we have cider cider we have the mead. Now beer we largely use we have two type of beer is largely in market lager and ale beer and wine is also largely used. The cider is usually produced from apple juice and mead is usually produced from honey and we add some fruit juices in it. Now here I want to point out that alcohol concentration in the distilled liquor is much higher is more than 45 per cent 45 to 50 per cent whereas beer we have about 4 to 8 per cent and we have in case of wine it might be it might be 10 to 20 per cent. Cider we might be varies it may be 2 to 8 per cent and mead also it is 5 to maybe 20 per cent.

So we have different concentration as per different the so here in case of non-distilled liquor we can use directly the fermentation broth we just separate the cell mass and use that for and pass through the maturation process and then use for direct consumption.

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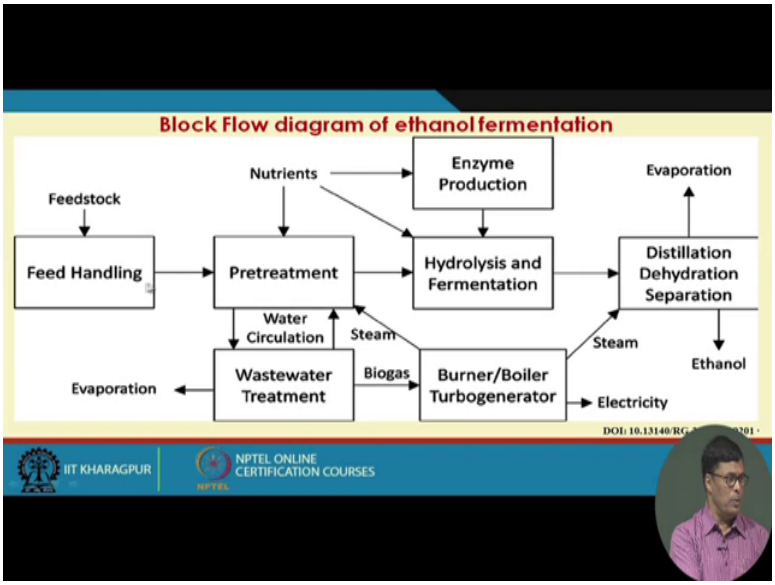

Now this is the block flow diagram of the ethanol fermentation process, how it is taking place this is feedstock we put it but we shall have to do the material handling then we do the pre-treatment process I mentioned in case of starch and in case of cellulose we require pre-treatment but in case of sugarcane molasses we do not require any much of pre-treatment then this is hydrolysis or fermentation, after the fermentation it passes through the distillation process and then through their process of evaporation we separate the ethanol and this this waste water that after the pre-treatment some waste is produced that we can use for the steam generation and the waste water treatment we produce some biogas and this biogas can be used for running the boilers and from the boilers we can use steam that for the distillation purpose.

So this is a very simple block flow diagram for the ethanol fermentation process I think in the next presentation I shall give the detail information of the ethanol fermentation process. Now in this presentation I give you the overview of the ethanol fermentation process how the ethanol fermentation take place, what is the classification of the different type of alcohol that is marketed I told you tax alcohol and non-tax alcohol. Tax alcohol is a pure alcohol, non-tax alcohol is the impure alcohol.

Non-tax alcohol usually use as a chemical feedstock by the chemical industry and but the cost of the non-tax alcohol is much cheaper as compared to tax alcohol so then then how this ethanol formation takes place we have I talk about the different metabolic pathways and we also discussed about the distilled alcohol and non-distilled alcohol. Thank you very much.