Industrial Biotechnology.
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Lecture-53.
Butanol Production.

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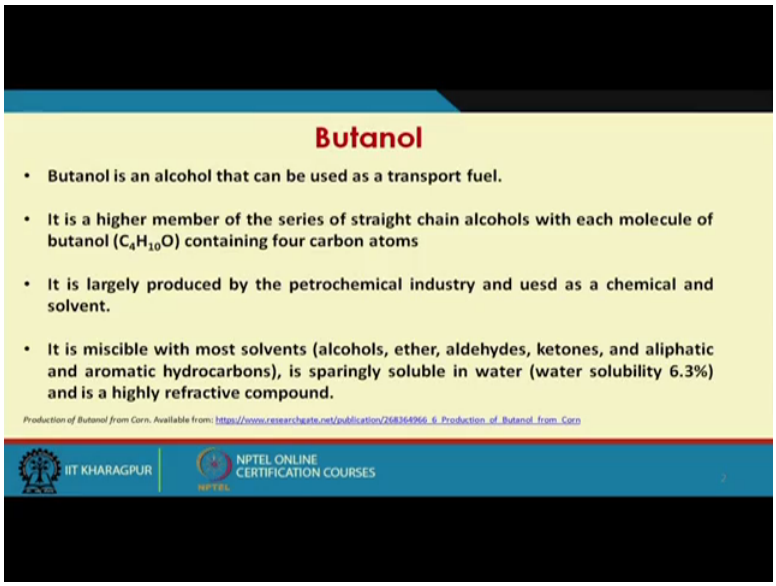

Welcome back to my course industrial biotechnology. In the last lecture I tried to cover the biodiesel which has appeared to be the one of the promising bioenergy source for the future and I told you that the biodiesel can be produced from the vegetable source, from the animal fat, also it can be produced from microalgae. And basically we extract this lipid or fat from these sources and then it undergoes that transesterification process in presence of methyl

alcohol and acid catalyst, it produces biodiesel and which is nothing but methyl esters of the higher fatty acids.

Now today I want to discuss another promising biofuel for the future, that is the bioethanol, butanol. Bio butanol is considered another very important source that we have and butanol can be produced both from the, both through the biological process as well as chemical process, we will be talking about how this butanol production takes place through the fermentation process. And here I want to add some history behind the, we will discuss the detailed history on the butanol production process and particularly importance of butanol production that has been given more stress during the World War II.

Because that time there was the acute shortage of crude petroleum, so the, so you know the USA was looking for some kind of substitute for this petroleum. So they called, they considered this butanol as because it has good calorific value and also it has higher cetane number which is very much required for running the vehicles. Now if you look at the butanol, it is a kind of higher alcohol and butanol is an alcohol that can be used for transport fuel, I told you it is the, it is there and I told you when I discussed the ethanol fermentation process, then also I discussed that with ethanol we produce some kind of that by product as higher alcohol, what you call fusion oil and this is also contains the butanol, amyl alcohols and other alcohols that we have.

And it is higher member series of straight-chain alcohol with each molecules of butanol, status C4 carbon, that has 4 carbon ring that we have, carbon chain which we have containing the 4 carbon atoms. It is largely produced by petrochemical industry, used as a chemical and solvent. It is miscible with the most of the solvents like alcohol, ether, aldehydes, ketones and aliphatic and aromatic hydrocarbons. It is sparingly soluble in water, water solubility is only 6.3 percent and is a highly attractive compound.

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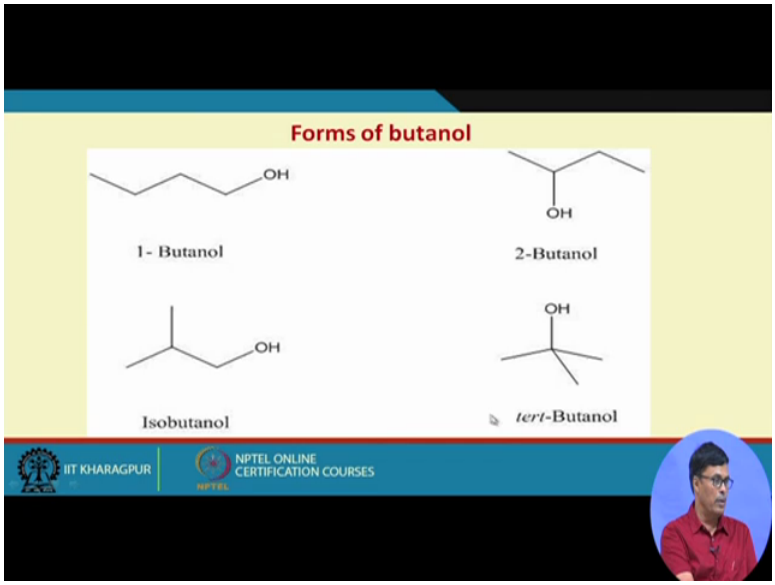

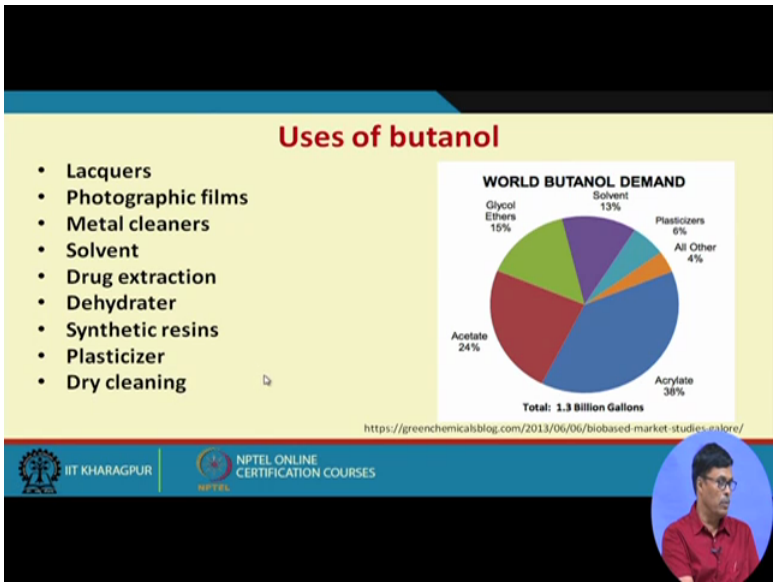

Now chemically it looks like this, this is one carbon, 2 carbon, 3 carbon and 4 carbon and this is the OH group at the end, we have 1 butanol, we have 2 butanol, we have iso butanol, we have tert butanol, that are the butanol that we have. If you look at the uses of butanol, it can be used as lacquers, then it is used as a for the production of this photographic film and metal cleaners and solvent, it is and drug extract, and dehydrator and synthetic resin and plasticiser and also dry cleaners. So these are the different things that we have.

Now here we have given some kind of information that world butanol demand that we have. This is total is 1.3 billion gallons and why it is used, that has been noted here. We can see most of the butanol used for acrylate production. And then we have acetate, then we have glycol ethers, then solvent and plasticiser.

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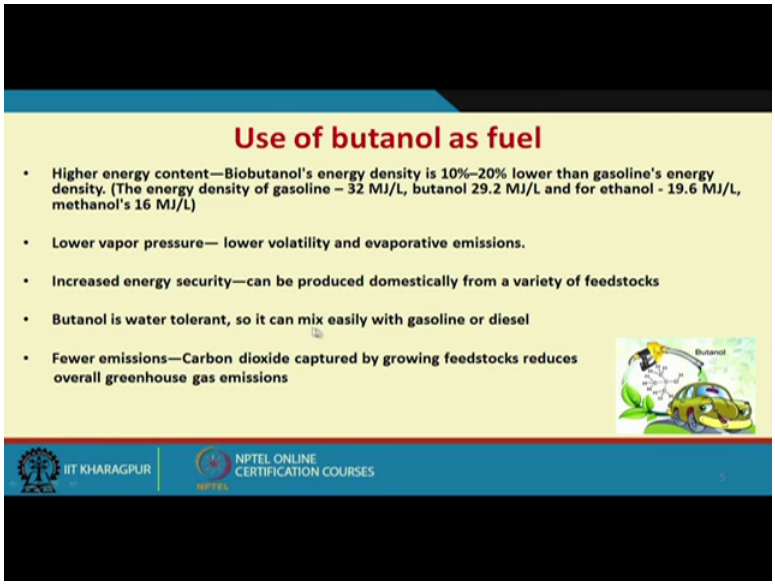

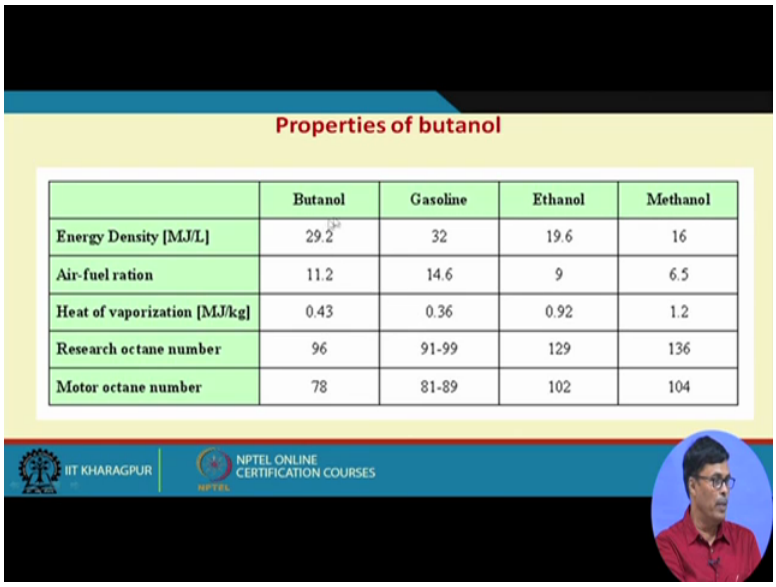

	Butanol	Gasoline	Ethanol	Methanol
Energy Density [MJ/L]	29.2	32	19.6	16
Air-fuel ration	11.2	14.6	9	6.5
Heat of vaporization [MJ/kg]	0.43	0.36	0.92	1.2
Research octane number	96	91-99	129	136
Motor octane number	78	81-89	102	104

Now use of butanol here, it is clear that why butanol is used as an energy source because butanol, the energy density is 10 to 20 percent lower than gasoline but it is higher than ethanol. You can see the gasoline has the calorific value is 32 megajoule per litre, whereas butanol is 29.2 megajoule per litre which is very close to gasoline but if you look at the ethanol as compared to ethanol, the calorific value is quite high and also as compared to methanol, it is also butanol has higher calorific value. Another advantage is that it has lower vapour pressure, lower volatility and evaporative emission which is very much required for the fuels.

And increased energy security – can be produced domestically from a variety of feedstocks that as I discussed, different organic matters can be used for the production of butanol.

Butanol is oxygen tolerant, so it can mix easily with gasoline or diesel. Fewer emissions – carbon dioxide captured by growing the feedstock produces overall greenhouse emissions due to the use of butanol. Now butanol we have made a comparative study how the characteristics of this differ from that of other fuel like gasoline, ethanol and methanol.

We have energy density here, I pointed out is very close to gasoline, then air fuel ratio that is also close to gasoline and heat of vaporisation, that is also close to gasoline a compare with ethanol and methanol, the heat of vaporisation is quite high and octane number, this is also very close to gasoline but in case of obviously ethanol we have octane number is quite high and motor octane number, that is also very close to butanol is very close to gasoline.

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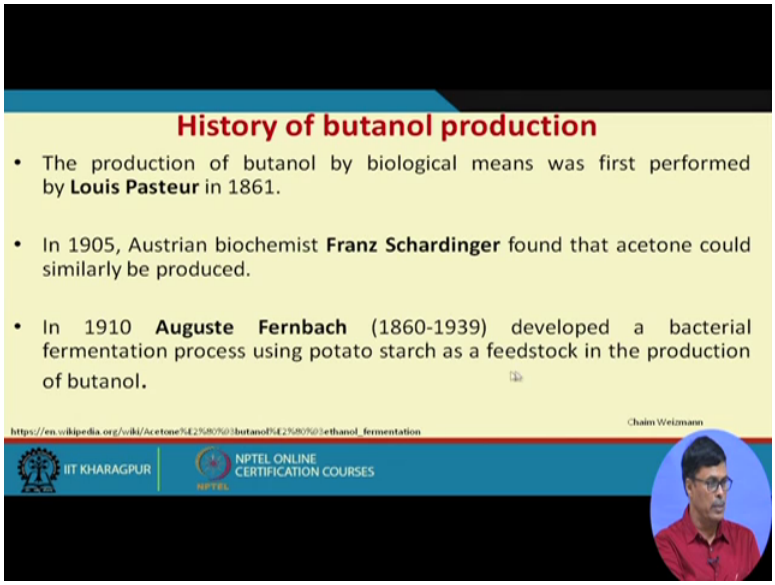

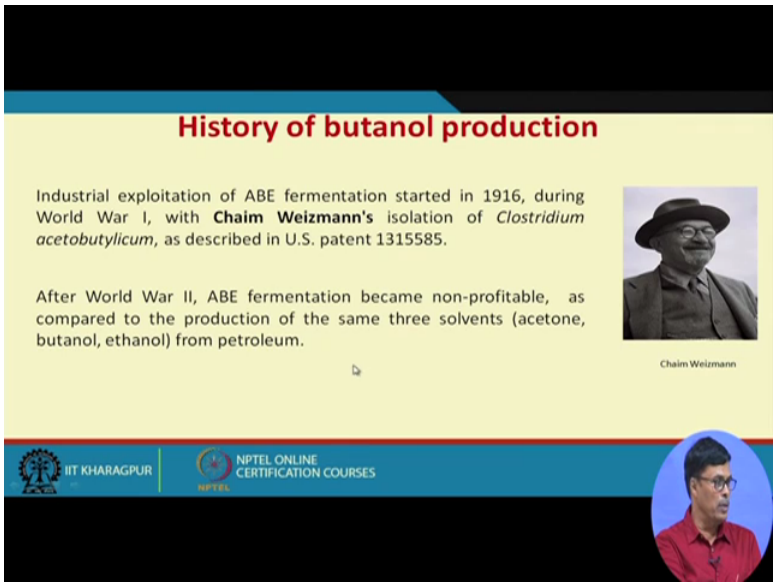

Now history of butanol production, that is the production of butanol by the biological means was 1st performed by Louis Pasteur in the year 1861. 1905, Austrian biochemist Franz Schardinger found that acetone could be similarly produced and 1910 then Fernbach developed then develop a bacterial fermentation process using the potato starch as the feedstock in the production of butanol. Now that butanol production through the fermentation process, one scientist played a very important role whose name is Weizmann Clostridium acetobutylicum, it is obligated anaerobe and he patented, he got the American patent which, because it has, produced high concentration of butanol.

After Second World War, acetone butanol fermentation became nonprofitable as compared to the production of the same, there were 3 solvents like acetone, butanol, ethanol as compared to that of petroleum, because acetone butanol fermentation process, butanol is produced through the acetone butanol fermentation process where it produces not only the butanol but also introduced acetone as well as ethanol. So that is the byproduct of that particular industry.

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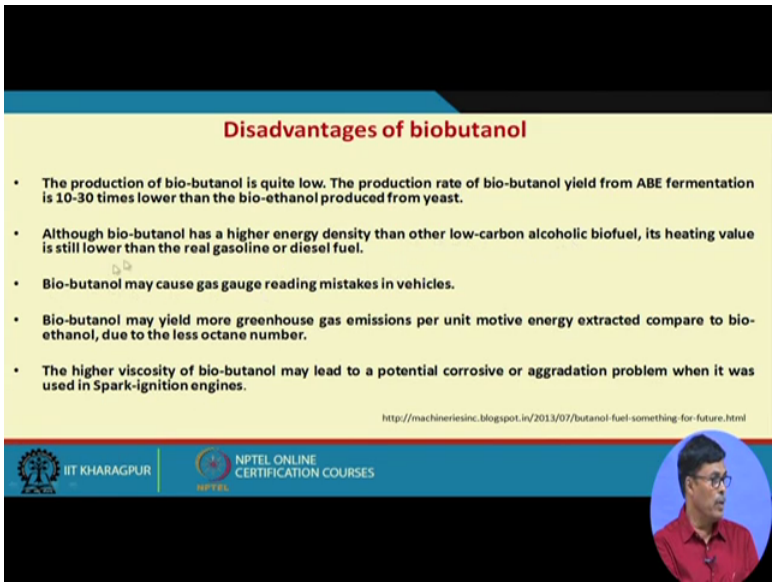

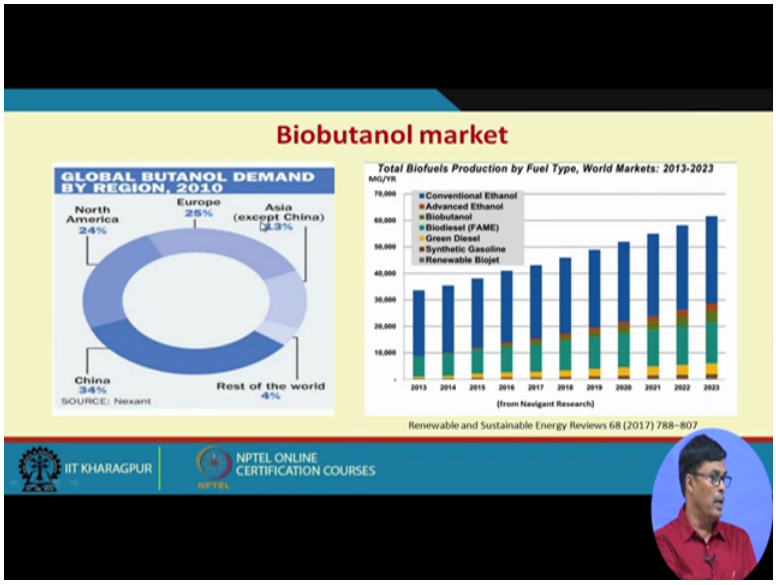

So that is the one of the major problems that we have. Now Disadvantage of bio butanol is several, the production of bio butanol is quite low, the concentration I already mentioned you that if the concentration is low, then the recovery cost will be very high, the cost of the product depends on the concentration of the particular product present in the fermentation broth. Now we find that through the fermentation broth, now it is reported in the literature, we got around 4 to 4.5 grams per litre which is quite less.

Now Bio butanol may cause the gas gauge reading mistakes in vehicles. Bio butanol may yield more greenhouse gas ignition per unit motive energy excited compared to bioethanol due to the less octane number. So this is the only high viscosity of bio butanol, may lead to the potential corrosive and aggregation problem when it was used in spark ignited engines. So this is the bio butanol market, we can, we can see the market that we have in Asia, except China it is 13 percent, Europe 25 percent, North America it is 24 percent, China itself has a very high market, that is 34 percent, rest of the world is 4 percent.

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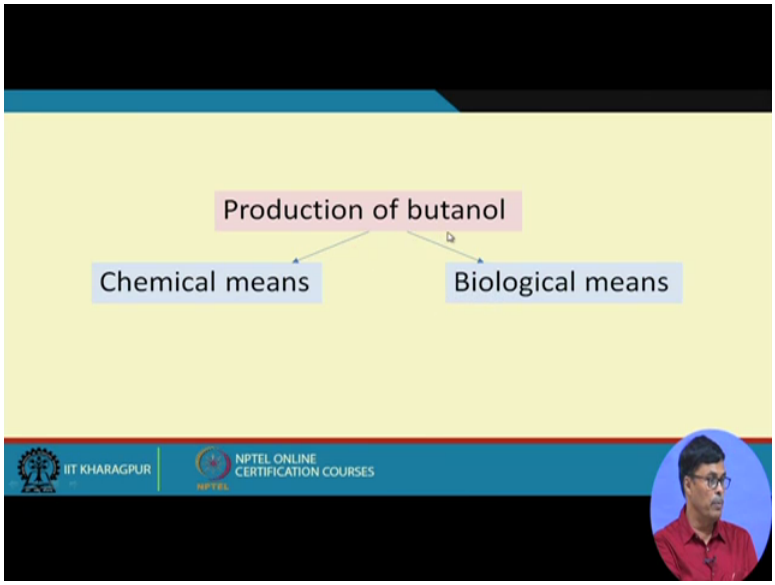

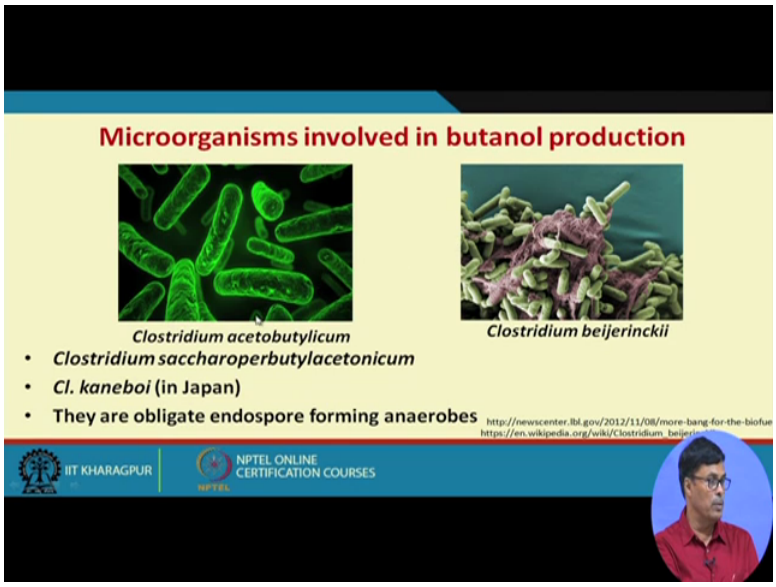

And this is the pattern of the use of bioethanol or production of bioethanol, butanol that is showing with respect to time, how it increases. Now as I pointed out before, the butanol can be produced by 2 ways, one is chemical means, another is biological means. Now biological means, it is produced, I told you it is acetone butanol fermentation where we get 3 solvents, one is acetone, another is butanol, another is ethanol. And in case of chemical process, it is used from the petrochemical industry and the microorganism that is largely used for bio butanol production is Clostridium acetobutylicum and Clostridium which are, these are the 2 organisms largely used.

And also we have Clostridium Kaneboi that is used in Japan. They are obligate, I told you obligate, and I want to point out that obligate anaerobe is little bit difficult to handle because

stressed amount of oxygen present in the fermentation broth will kill the microorganisms, we totally the oxygen, dissolved oxygen concentration of the fermentation broth we have to remove, then only then, your organism can grow in the fermentation medium. This is the only disadvantage we have, another problem with anaerobic fermentation process is that the rate of reaction is quite slow as compared to aerobic fermentation process.

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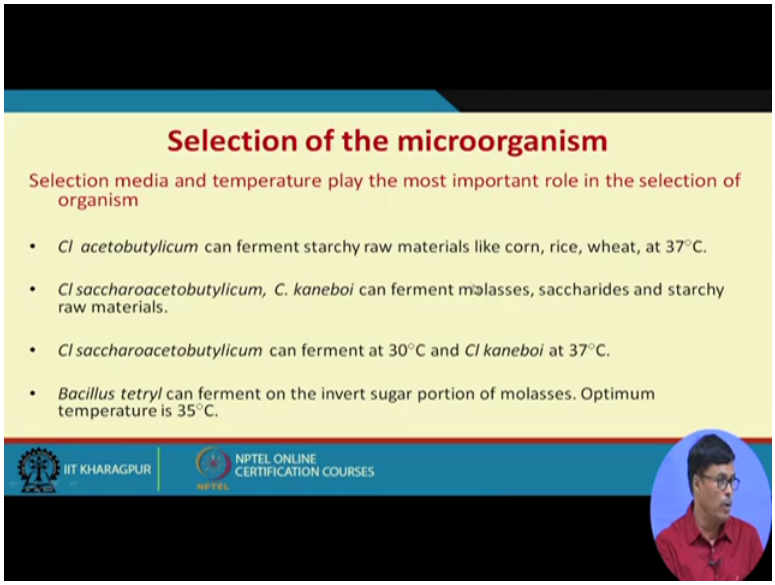

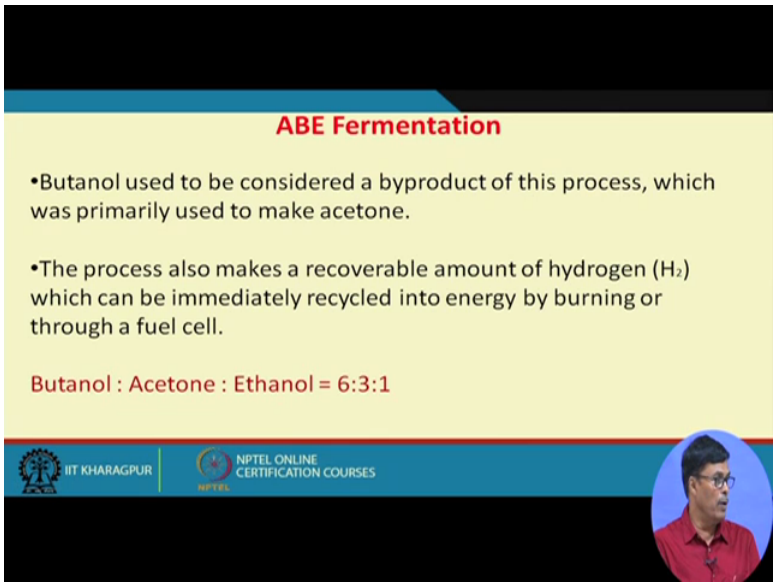

Now selection of microorganisms, that is very important, we have already shown before also that microorganism plays very important role. If you look at that Clostridium acetobutylicum can ferment the starchy raw material like corn, rice and wheat at 37 degrees centigrade. Clostridium Sachar acetobutylicum and Clostridium Kaneboi can ferment molasses, saccharides and starchy raw materials. Clostridium Sachar acetobutylicum can ferment at 30

degrees centigrade and close study Kaneboi at 37 degrees centigrade. Bacillus tetryl, this can ferment the invert sugar portion of molasses, optimum temperature is 35 degree centigrade.

Now acetone butanol fermentation process that, I told you 3 solvents will get in the fermentation broth, one is butanol, another acetone and ethanol. If you look at the ratio that how much is present there in the fermentation broth is 6 is to 3 is to 1. The process also makes the recoverable amount of hydrogen because this we observed, because this has 3 different phases, one called acid formation phase, another is solvent formation phase, what you called solvent Genesis and again it produces the asset.

It has 3 different phases, in initial phase, in the acid says, the acid formation takes place and we observe very good amount of hydrogen production that takes place in this particular phase. So we can we can collect the hydrogen and use as a source of energy. And simultaneously we will be getting the organic acid. Then in the 2nd phase this organic acid will be converted to butanol and acetone and ethanol.

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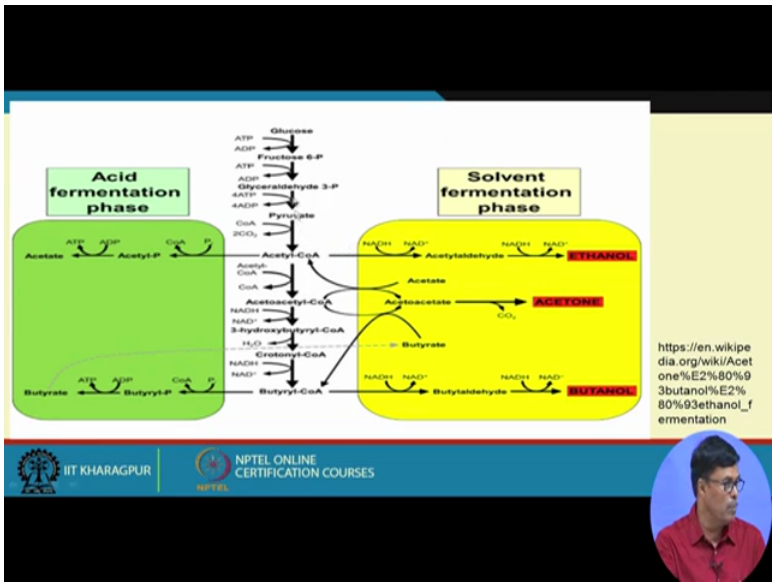

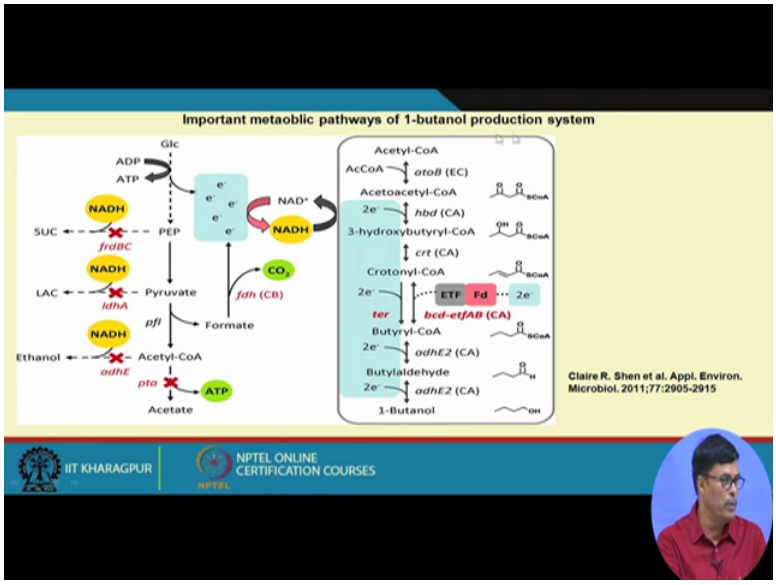

Now this is a metabolic part is how this formation takes place. We have acetate, butyrate and this forms and this also forms, when it is passed through the solvent Genesis process, it produces the ethanol acetone and butanol. So it has been shown here that this is the environmental path through which we get the acetal CoA and from this acetal CoA we get the butyric acid as well as we get butanol, we get acetate and also with this I will get acetone and ethanol.

This is the important metabolic pathway for one put in all production process also given here, how it is produced, butanol. So what is, what we want to point out that that after pyruvate acid, it produces acetate and also ethanol, now these 2 processes is to be is to be closed, you have to eliminate this process, then only then you can have reactive relation of butanol in your fermentation broth.

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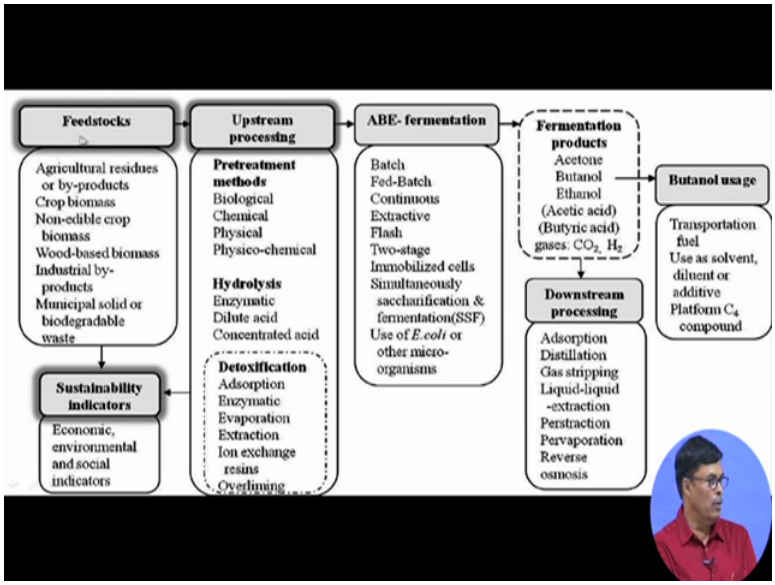

The stages involved in the butanol fermentation is several we have feedstock, then have upstream processing, then we have acetone butanol fermentation process, then we have the fermentation products and then we have, we can have we can see butanol usage. Now if you look at the feedstock, it is very interesting, it uses the different sources of raw materials like agriculture residue or byproducts of the crop biomass, nonedible crop of mass, wood-based biomass, industrial byproducts, municipal solid or biodegradable waste. Then we have sustainable, this is the sustainability indicator, economic, environmental and social indicators.

So that is, then the upstream processing we have pretreatment process, we have biological, chemical, physical and physicochemical. Now I can give a typical example that it will use any kind of cellulosic material as a raw material for this fermentation process. Then you have to go for either acid hydrolysis or alcoholic hydrolysis or you have to go to biological, that enzymatic hydrolysis process. So it is just to convert the cellulose to glucose.

And also you can have, in process you can have the physical and physicochemical system, physical treatment means you can you can you can cut the size of the solid particle into small size so that your bacteria or enzyme can act more with the raw materials. The hydrolysis and the Dilutive acid and the concentrated acid, that is used for this hydrolysis process. And detoxification, that is adsorption, enzymatic, evaporation, extraction, ion exchange, and resin and over liming, this is a process we can do for the detoxification of this particular residue that we get from this .

Then this affects the economy of the process obviously to a great extent. Acetone butanol fermentation process can be carried out by, both by batch process, fed batch process, then continuous, extractive, Flash, two-stage, immobilised cells, so different processes we have, we have simultaneous saccharification, fermentation that you know where both the saccharification your organism that is used for the saccharification of the starch cellulosic material and also it produces also the butanol. So usually we consider kind of mixed Micro product for doing all this thing, user of E. coli or other microorganisms that is used for this process.

The fermentation product we have major product, we have acetone, butanol and ethanol. And then this is separated out through the distillation process and then this can be used as a transport fuel, used as solvent, I mentioned before.

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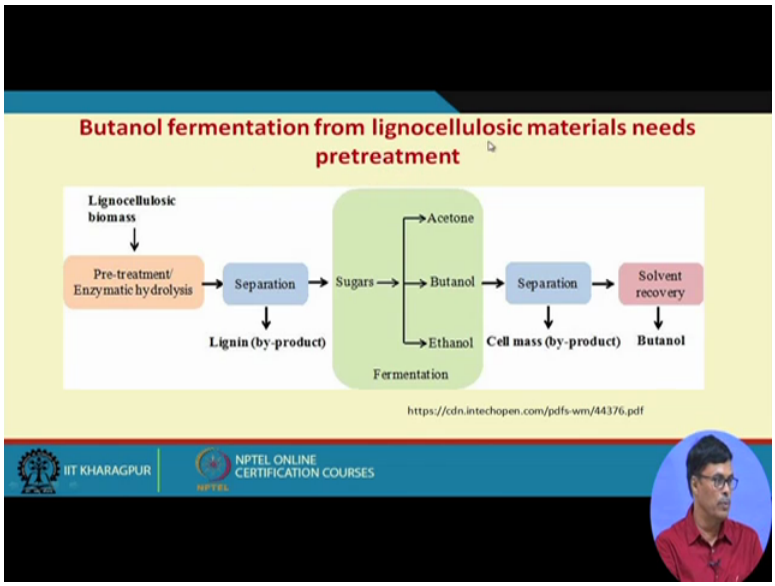

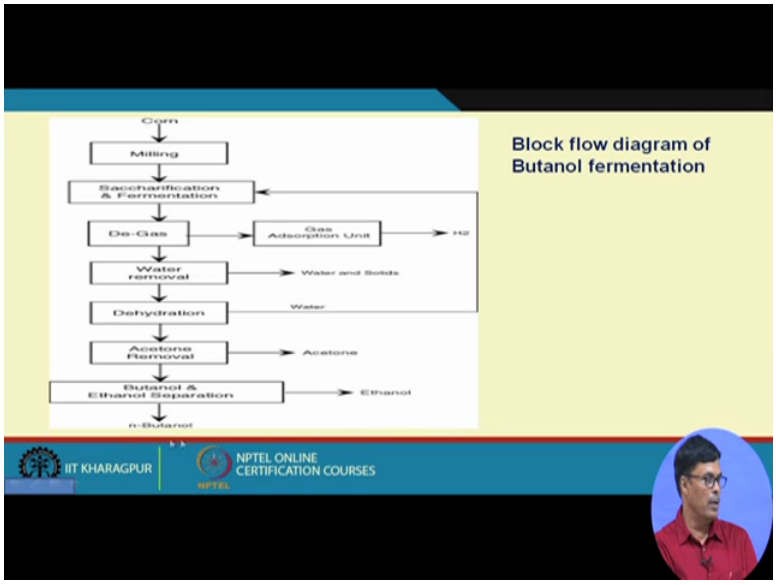

Now if you look at butanol fermentation process from Lignocellulosic material needs the pretreatment, I told you, this is enzymatic hydrolysis, we get the glucose, we separate the lignin, then sugar, it is converted to acetone, butanol and these are the suppression process, we separate the cell mass and the solvent recovered through that the distillation process, we get the butanol. Now this is the block flow diagram, block flow diagram about the production of butanol from cornstarch. Corn, we take out and we dry corn, we do the milling, milling means, it is a kind of, you are making it powdered form.

Then we make a dough and undergo the saccharification process, there is a soluble material containing the glucose that undergo the fermentation process, then we have degas, then water removable, dehydration, acetone removal and the butanol, ethanol removal, that that here, this glucose, whatever is produced, that is converted to acetone, butanol and ethanol. So we can purify it and finally we get the normal butanol.

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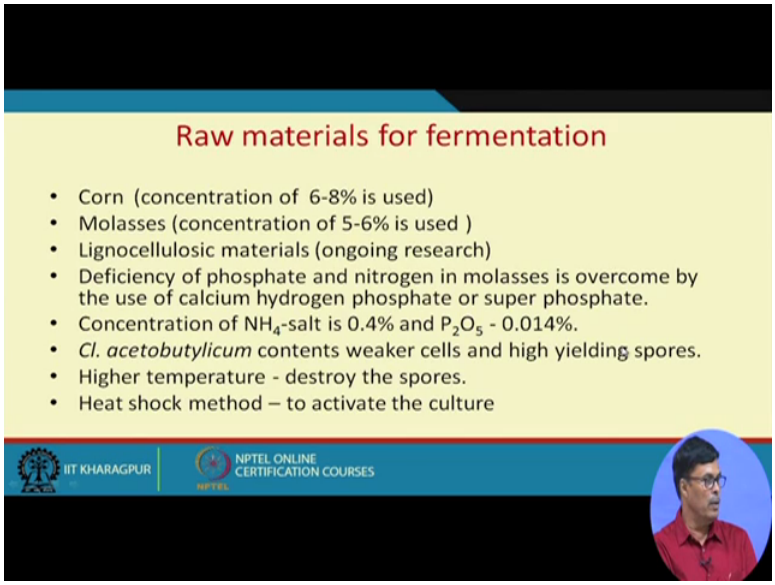

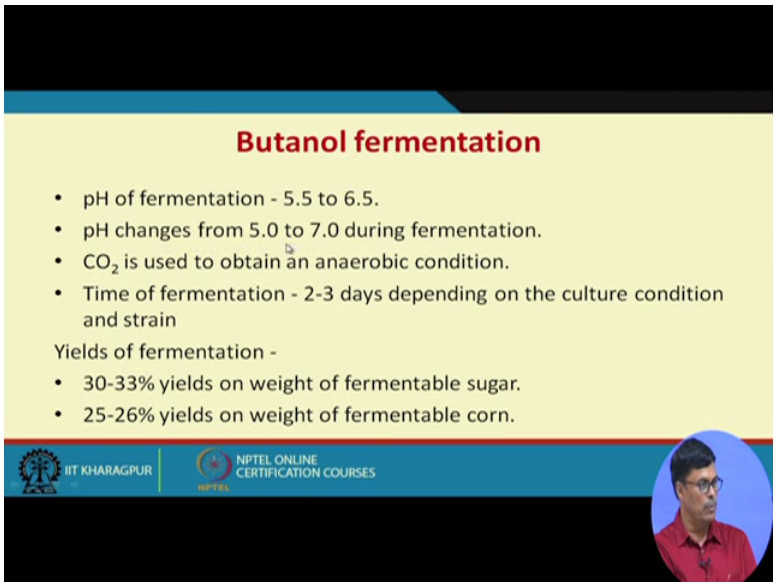

Now raw materials used as I mentioned before that we can use the corn, concentration is 6 to 8 percent, molasses concentration, 5 to 6 percent, Lignocellulosic materials, the research, this is on the research level. Deficiency of phosphate and nitrogen and molasses is overcome by adding the calcium hydrogen phosphate of super phosphate. The concentration of ammonium salt is about 0.4 percent and P2 O5, phosphorus pentoxide, is about phosphorus in the form of phosphorus pentoxide is 0.014 percent. Clostridium acetobutylicum contains the weaker cells and high yielding spores. The higher temperature – destroy the spores and heat shock Method – to activate the culture.

This is very important step we find when we try to use the Clostridium acetobutylicum, then we find the heat shock is very much required just to activate the culture. Now pH of the

fermentation process is 5.5 to 6.5, pH changes to 7 to read the fermentation process, the carbon dioxide is obtained in anaerobic condition. Time of fermentation is 2 to 3 days depending on the culture condition and strain. The yield is like this, 30 to 33 percent on the basis of fermentable sugar and 25 to 26 percent on the basis of fermentable corn.

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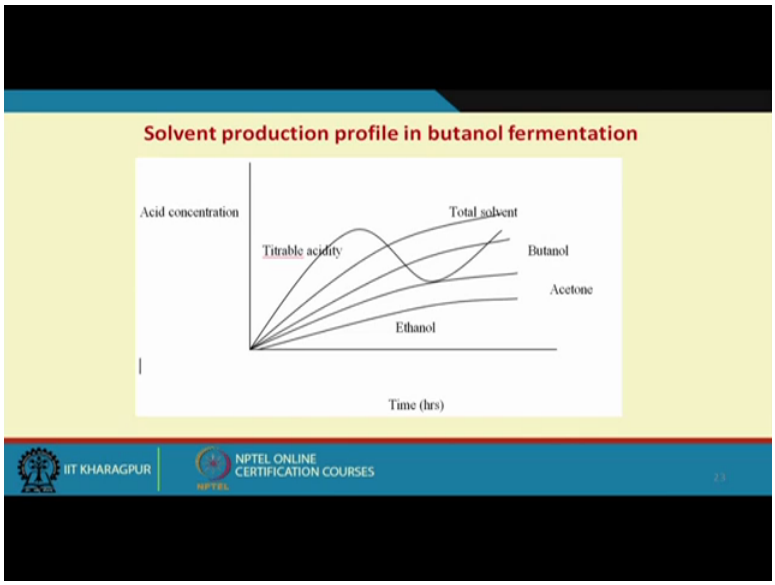

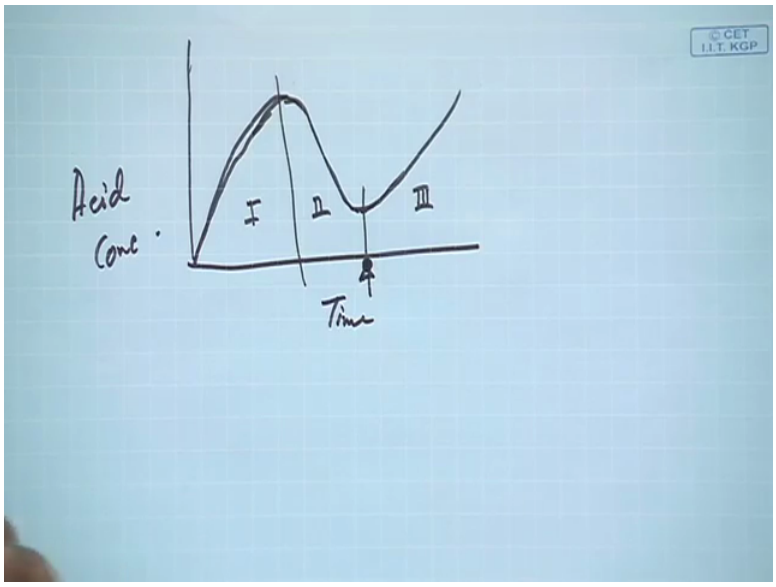

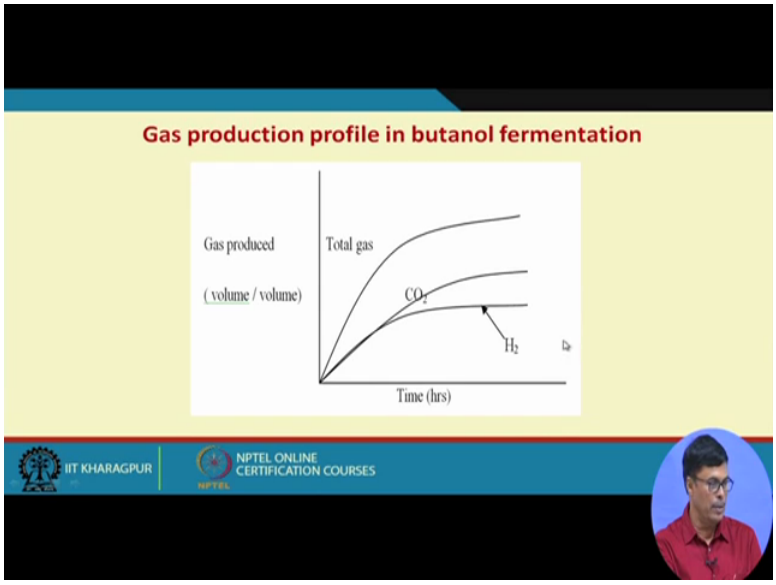

Now here we have very interesting thing that we have, that we have, I told you this acetone butanol fermentation takes place in the 3 different phases. This we consider phase 1, this consider phase 2 and this consider phase 3. This is time and this is your, you can acid concentration. So here we have acid formation increases and then, then it decreases. When it decreases, you have more active relation of solvent, again finally it increases. So usually we carry out the fermentation up to this level because this is the level carried out to have more solvent accumulation in the fermentation broth.

So this is, this is, you can see in this figure, this is the butanol concentration is quite high, then acetone, then ethanol. In this ratio I mentioned before 6 is to 3 is to1. And you know that I told you during the acid fermentation, acid production process the hydrogen gas is produced along with the carbon dioxide, this is the total, this hydrogen gas we can tap and use as an energy source.

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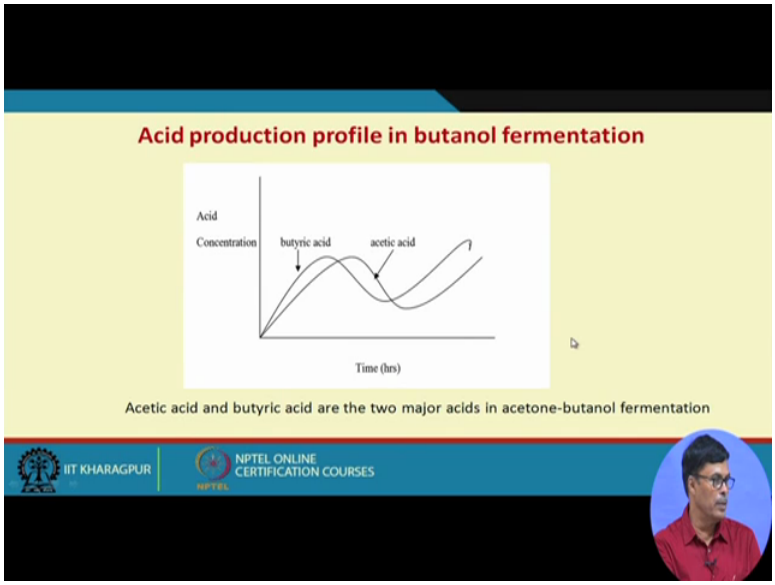

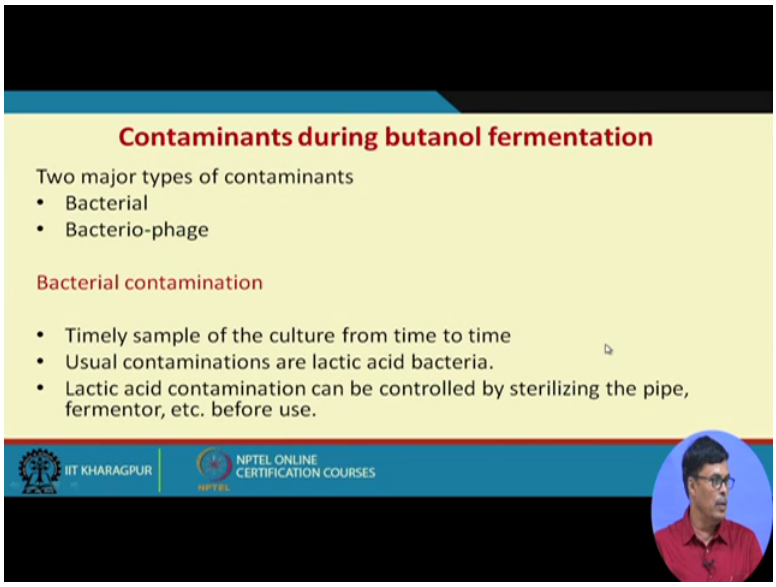

This is the profile of different acid, this is butyric acid profile, this is an acetic acid profile, how it changes with respect to time in the acetone butanol fermentation process. Now contamination during the butanol fermentation process is of 2 main types, one material and bacterio-phage. Now here I want to point out that in the bacterial fermentation process that only that you know some other bacteria, there is every possibility of some other bacteria also may interfere in the process but one advantage of this process is that, it is the obligatory anaerobe, so under obligatory anaerobe conditions, that, a few organisms can grow but anyhow that we face a lot of problem with the other bacteria because lot of other couple of bacteria can particularly I can give the example of methanogens, they also obligatory anaerobe, they also can grow here.

But only the difference is that, here the pH is quite low, so methanogens will not be problem, the condemnation problem never arises but in case of maybe the other acid producing bacteria area contaminate in this process. But bacteria phage is a common problem for bacterial culture because bacteria phage is present there, I told you when I discussed the cheesemaking industry that is a one important problem that we have with the cheesemaking industry that bacteria phage contamination problem. The cheese, the bacteria phage is kind of virus, they attack a particular bacteria and they kill the bacteria.

That is, that is a problem and that is why I told you you can remember that cheesemaking industry usually we prefer the mixed culture so that even one if one bacteria is killed, other bacteria will keep on functioning. So that is one of the way we can recover this kind of problem. But here, since we are using the pure culture, with the bacterial phage contamination, the whole process will be affected. Now bacterial contamination – timely sampling of the culture from time to time, usual contamination with the lactic acid bacteria, lactic acid contamination can be controlled by sterilising the pipe, fermentor, etc, before use.

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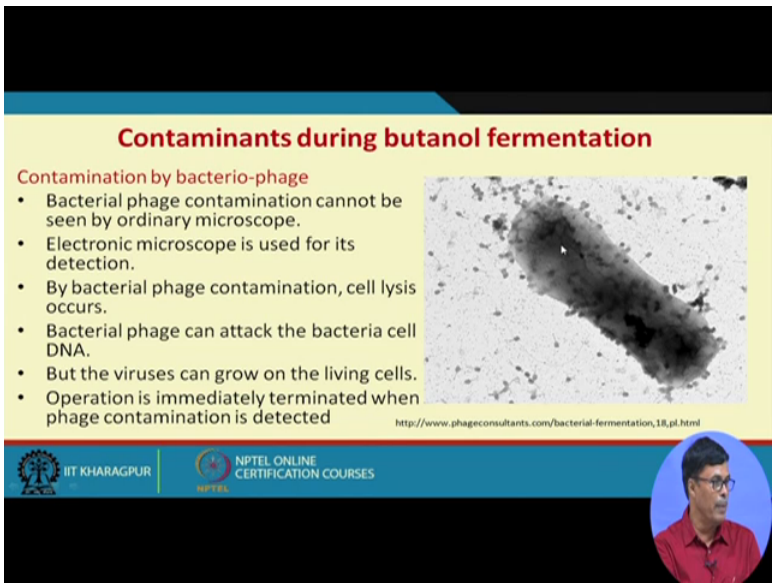

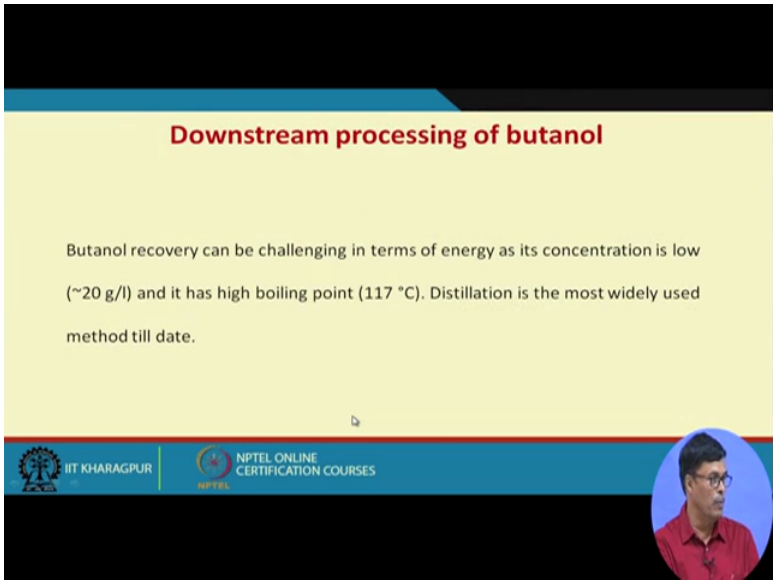

Now this is the bacteria phage, how it attacks the bacteria, how it attacks the bacteria, the contamination of bacteria phage, bacterial phage contamination cannot be seen by the ordinary, cannot be seen by ordinary microscope. This is because viruses are very small particle, so you cannot visualize under the microscope, it is much smaller than bacterial cell, we know the size of the bacteria is 0.5 to 2 microns, so it is much less than that, so it is very difficult to visualize in the ordinary microscope. So this is the problem that the bacterial phage can attack the bacterial cell DNA.

But the viruses can grow on the living cells and oppression is immediately terminated when the phage contamination is detected. That is the problem that we have. Now we have downstream processing of butanol, butanol recovery can be challenging in terms of energy as this concentration is very low, that is 20 gram per litre and it has a high boiling point of 117 degree centigrade. So distillation is most widely used method till that.

So in conclusion I want to tell you that butanol is considered as a promising fuel, bioenergy source for the future because if you look at the calorific value of butanol, it is very close to gasoline and not only that, we find the cetane number is also close to gasoline and vaporisation of this particular butanol is comparatively less as compared to ethanol, so it is more promising as compared to the ethanol, which I told you that ethanol can be used also as a powered alcohol and government of India or throughout the world, it has been found that if we replace the gasoline, 10 to 20 percent by ethanol, the same engine configuration can be used for running the vehicles.

So similarly but here the advantages that butanol, the calorific value is close to gasoline, so we do not have any energy problems, energy conversion problem but when we use the ethanol, the energy content, the combustible energy is quite less as compared to butanol. This is the problem that we have and butanol can be, bio butanol can be produced through the biological, different processes but this process is obligatory anaerobic fermentation process where this anaerobic fermentation, only the obligatory anaerobe that can grow, examples are Clostridium, acetobutylicum and other bacteria also used and I told you one important feature of this particular organism, it requires heat shock to activate the culture, we also carry out this experiment in our lab and we find that until and unless we do the heat shock, this organism will not grow properly.

So and usually that butanol is recovered through the distillation process, only the problem is that, the high boiling point, 117 degrees centigrade, so we can collect on the basis of fractional distillation. So in the fermentation broth, we get the 3 different solvents like butanol, acetone and ethanol in the proportion of 6 is to 3 is to 1. So I think it is all about the butanol fermentation process, in the next lecture I shall cover one very interesting topic, that is largely in operation in our country, that is bio fertiliser. And it has great prospects as for India and abroad is concerned. So thank you by much.