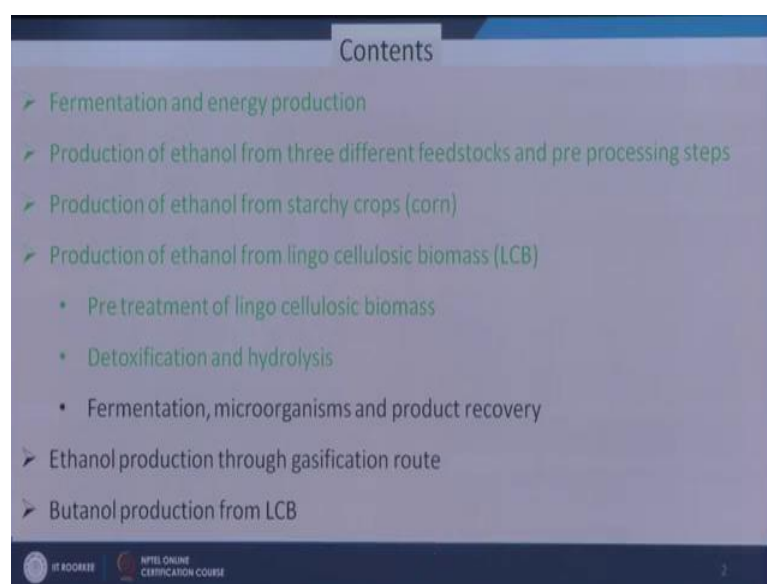


Waste to energy conversion
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Lecture – 31
Energy production from Organic wastes through Fermentation – 2

Hi friends. Now we will start discussion on the second part of the module Energy Production from Organic Wastes through Fermentation. In the first part of this module we have discussed on the fundamentals of fermentation and methanol fermentation reactions and different feed stocks means waste which can be processed through this biological routes for the production of ethanol.

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Contents	
➤	Fermentation and energy production
➤	Production of ethanol from three different feedstocks and pre processing steps
➤	Production of ethanol from starchy crops (corn)
➤	Production of ethanol from lingo cellulosic biomass (LCB)
•	Pre treatment of lingo cellulosic biomass
•	Detoxification and hydrolysis
•	Fermentation, microorganisms and product recovery
➤	Ethanol production through gasification route
➤	Butanol production from LCB

And we have started discussion on the production of ethanol from lingo cellulosic biomass. And we have covered the pretreatment of lingo cellulosic biomass and detoxifications and hydrolysis. And in this part of this module we will discuss on the fermentation microorganisms, and product recovery ethanol production through gasification route and butanol production from lingo cellulosic biomass.

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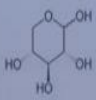
Fermentation

$$C_6H_{12}O_6 \text{ (Glucose)} \rightarrow 2C_2H_5OH + 2CO_2$$

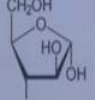
$$3C_5H_{10}O_5 \text{ (Xylose, arabinose)} \rightarrow 5C_2H_5OH + 5CO_2$$

$$C_{12}H_{22}O_{11} \text{ (cellulose)} + H_2O \rightarrow C_2H_5OH + CO_2$$

$$C_{10}H_{18}O_9 \text{ (Xylobiose)} + H_2O \rightarrow C_2H_5OH + CO_2$$



Xylose

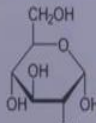


Arabinose



Ethanol yield

$$Y_{EtOH} = \frac{CV}{m}$$

Where, Y_{EtOH} is ethanol yield (g/kg),
 C is ethanol concentration (g/L),
 V is initial volume of liquid medium (L),
and m is the mass of the substrate (kg).



Glucose

So, we have seen that in fermentation process, the organic waste is converted to ethanol, and if we use the starch based feed stocks, then we get the glucose as sugar or hexose like say mannose galactose etcetera. So, only hexose sugar are present in this feed stocks, and so ethanol reaction takes place through this reaction, but in ligno cellulosic biomass due to the presence of cellulose lignin and hemicellulose, we get after lignin separation cellulose and xylose this hexose and pentose sugar. So, this pentose and hexose sugar in monosaccharide or maybe in terms of disaccharide can be fermented. So, we have 2, 4 feed stocks mean sugar that is glucose xylose cellulose and xylobiose. So, cellulose is the disaccharides of the glucose and xylobiose is the disaccharide of xylose or arabinose.

So, these are the reactions. So, from all those sugar we get ethanol and carbon dioxide. And these are that structure of xylose arabinose and glucose. So, 6 sugar 6 carbon sugar and 5 carbon sugar. Now how can we find out the ethanol yield? So, ethanol yield is defined as ethanol indicated to CV by m, where C is the ethanol concentration in gram per liter and V is the initial volume of liquid medium in liter and m is the mass of the substrate in kg then we will get ethanol yield that is Y_{EtOH} is that is in gram per kg.

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Ethanol productivity

Amount of ethanol produced per unit of substrate utilized per unit of time. It is typically determined when ethanol concentration is maximal.

$$Q_{\text{EtOH}} = \frac{CV}{mt} = \frac{1000Y_{\text{EtOH}}}{t}$$

Where, Q_{EtOH} is the ethanol productivity (mg/kg h), and t is the time at which the ethanol concentration produced on substrates is maximum (h).

The maximum theoretical ethanol yield :

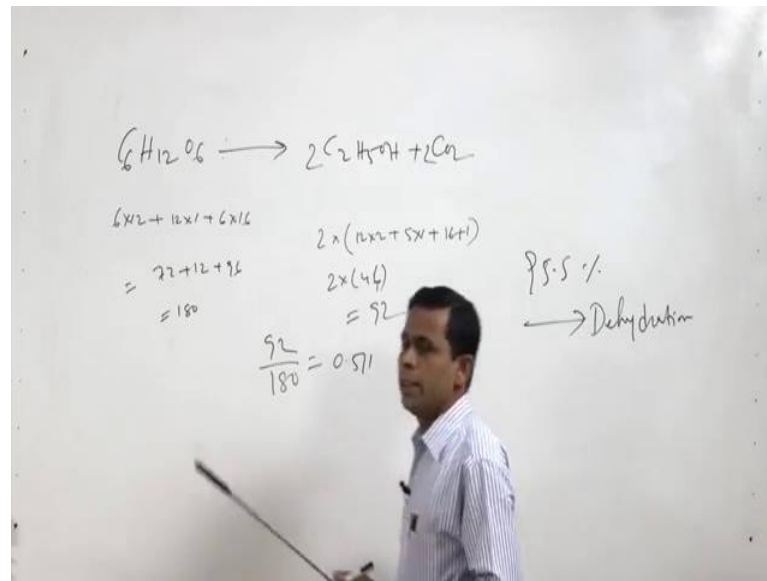
$$Y_{\text{max}}(\%) = \frac{\text{Ethanol produced in reactor (g)}}{\text{Initial sugar in reactor (g)} \times 0.511} \times 100$$

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Now, what is the ethanol productivity? If we can determine the ethanol yield, then if we divide it by time then we will get the ethanol productivity. The definition of ethanol productivity is the amount of ethanol produced per unit of substrate utilized per unit of time.

So, this can be written as Q_{EtOH} that is the productivity of ethanol that is equal to CV by m into t if it is gram per kg then it is fine otherwise if it can be expressed in mg per kg then we have to multiply it by one thousand so ethanol productivity is equal to one thousand into Y_{EtOH} by t in when the unit is mg per kg hour. Now what is the maximum yield? So, maximum ethanol yield is defined as ethanol production in reactor in gram divided by theoretically available ethanol that is sugar initially available in the reactor into 0.511 into 100. So, where from we are getting this expression?

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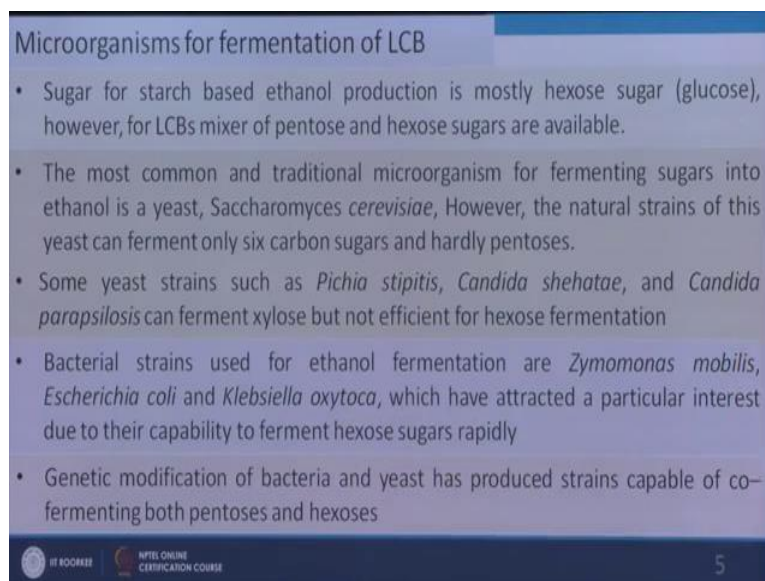


So, if we have this reaction say $\text{C}_6\text{H}_{12}\text{O}_6$ this is reacting and it is this sugar is converted to $\text{C}_2\text{H}_5\text{OH}$ plus CO_2 .

So, $\text{C}_2\text{H}_5\text{OH}$ plus CO_2 to $2\text{C}_2\text{H}_5\text{OH}$ plus 2CO_2 . So, this is the reaction here. So, glucose molecular weight is equal to 6 into 12 plus 12 into 1 plus 6 into 16. So, that is equal to 180 and here we are getting 2 into 12 into 2 plus 5 into 1 plus 16 plus 1; so 2 into 45. So, that is equal to 90. So, if we divide this 90 divided by 180 then it is becoming 0.511. So, this function which we have got that 0.511; that means, maximum with 100 percent conversion of this glucose takes place to ethanol then we can get 0.511 times of this.

So, that is why this expression is there the ethanol production is reactor divided by initial sugar in reactor in gram into 0.511 into 100 that is the maximum yield of ethanol production.

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Microorganisms for fermentation of LCB

- Sugar for starch based ethanol production is mostly hexose sugar (glucose), however, for LCBs mixer of pentose and hexose sugars are available.
- The most common and traditional microorganism for fermenting sugars into ethanol is a yeast, *Saccharomyces cerevisiae*. However, the natural strains of this yeast can ferment only six carbon sugars and hardly pentoses.
- Some yeast strains such as *Pichia stipitis*, *Candida shehatae*, and *Candida parapsilosis* can ferment xylose but not efficient for hexose fermentation
- Bacterial strains used for ethanol fermentation are *Zymomonas mobilis*, *Escherichia coli* and *Klebsiella oxytoca*, which have attracted a particular interest due to their capability to ferment hexose sugars rapidly
- Genetic modification of bacteria and yeast has produced strains capable of co-fermenting both pentoses and hexoses

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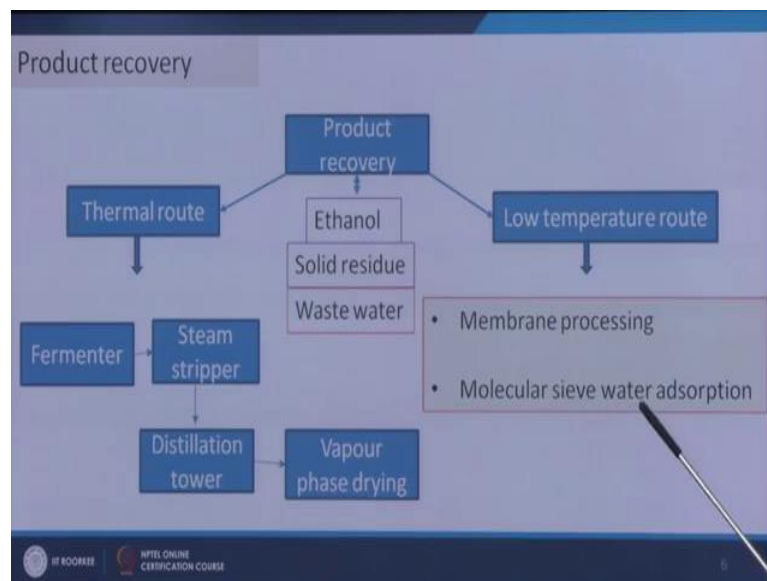
Now, we will see the microorganisms which are suitable for the production of ethanol from the ligno cellulosic biomass. As we have discussed that in ligno cellulosic biomass we have both pentose and hexose sugar. And yeast that is *saccharomyces cerevisiae* which is every conventional microorganisms for the production of ethanol from organic sugar is also applicable here because hexose sugar is present, but these natural strain cannot ferment on the pentose sugar, but some type of yeasts are there that is *pichia stipitis* and *candida* species basically.

So, *candida shehatae* and *candida parapsilosis*, so these 2 can be these 2 strains can work on the pentose sugar and converted into ethanol. So, these are the yeast not only yeast some bacterial species are also available which can work on this sugar and give the ethanol as a product. Some examples are given here that is your *zymomonas mobilis*. So, this is one bacteria *escherichia coli* is another one and this is one *klebsiella oxytoca*. So, these are some bacteria which can also work on the sugar for the production of ethanol.

Now efforts are on how to get more efficient strain those can be equally applicable or suitable to convert both pentose and hexose sugar. And genetically modified microorganisms are being developed and experiments are going on to develop new microorganisms genetic genetically modified which will be suitable for both type of sugar for the ethanol fermentation.

Now, once the ethanol is produced we have to separate it. As we have discussed in the first part of this module that when ethanol is produced in the fermenter the concentration is around say 8 to 10 percent and then we have to increase this concentration. So, we had used some distillation unit. So, do after dissolution it gives 95.5 percent ethanol; so 95.5 percent ethanol after distillation. And then we had dehydration unit. So, these are the steps we have discussed in the first part of this module.

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So, this is these are on the thermal route. So, for the recovery of the ethanol from the solid residue as well as the waste water in the fermenter we have 2 types of operations or 2 types of routes one is thermal route another is membrane based route.

So, low temperature route that is membrane and molecular sieve. These 2 processes are being used in recent years and conventionally this thermal route is available for the separations of ethanol from the fermenting media or the fermenter. So, from the fermenter we the steam stripper then distillation and distillation to vapor phase drying. So, a distillation if we think than this by distillation we can get 95.5 percent of ethanol.

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- The first process that was used in many of the earlier ethanol plants is the so-called azeotropic distillation or ternary distillation process (as opposed to a binary or two component distillation process). It consists of introducing a third component, benzene or cyclohexane, to the azeotropic solution which forms a heterogeneous azeotropic mixture. When this mixture is distilled, anhydrous ethanol is produced at the bottom of the distillation column.
 - ✓ (B.P. of ethanol 78.4 °C, water 100 °C, mixture 78.1 °C (95.5 % ethanol))
 - ✓ (B.P. of cyclohexane 80.7 °C, water 100 °C, mixture 69.8 °C (80 % cyclohexane))
- Another early method is called extractive distillation, which consists of adding a ternary component that increases the relative volatility of ethanol. In this case, anhydrous alcohol is produced and withdrawn at the top of the distillation column.
 - ✓ ethylene-glycol
- Some other methods: Salt distillation; Pressure swing distillation; Pervaporation

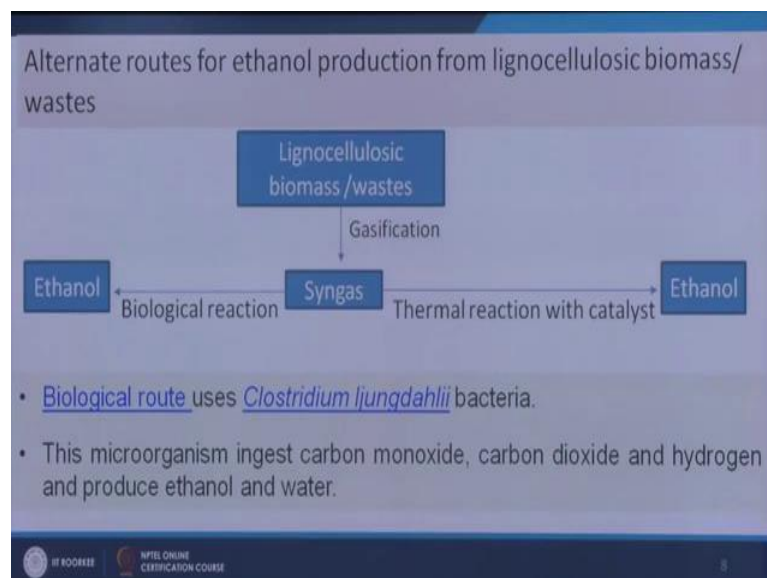
Then how can we get 99 percent pure 99.9 percent pure ethanol. Preliminary at the initial stage the attempt was like this azeotropic distillation was utilized as well as extractive distillation was utilized. So, azeotropic distillation one third components were added in this mixture that is azeotropic mixture of ethanol and water. So, we see here the ethanol boiling point is 70.4 degree centigrade water is 100 degree centigrade, but when it is a mixture azeotropic mixture the boiling point is 78.1 degree centigrade.

So, if we can add one external agent in it or external chemical in it, which can lower the boiling point further with respect to water and that particular compound as for example, we have cyclohexane and benzene anyone can be used. So, cyclohexane boiling point is 80.7 degree centigrade water is 100 degree centigrade, but when it is a mixture azeotropic mixture it is temperature is 69.8 degree centigrade, so when this cyclohexane is added and use in the distillation. So, cyclohexane and water will goes off whereas, ethanol will be remaining in the bottom of the distillation column.

So, that way we can separate the ethanol and that was the primitive approach then extractive distillation in this case another external component was added in this ethanol water azeotro which increases the relative volatility of the ethanol. So, as a result ethanol comes with the solvent or it is in the vapor phase and the water remains in the distillation column. So, ethylene glycol is an example of that extractive distillation. So, apart from

these some other methods like salt distillation pressure swing distillations and pervaporations are used for the separations of ethanol from the media.

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Now, we will discuss some other routes which are applicable for the production of ethanol. So, here two routes have been shown one is by the gasification the waste material can be converted to syngas. And that syngas can further we converted to ethanol through catalytic reaction we have discussed in previous modules that once syngas is produced that can be converted to ethanol or methanol or any other higher alcohol.

So, in case of methanol production kopperoxide best catalyst is used high pressure and temperatures reaction is applied and ethanol also other catalyst are used, but this is not commercially produced as on today. And another route that is biological route from syngas to ethanol that is also being tested in laboratory, but not it is applied in commercial scale some microorganisms have been developed which can convert syngas that is CO , CO_2 and hydrogen the microorganism consumed is and ethanol is produced. So, these are the other routes, but these routes are not commercial route.

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Butanol production from lignocellulosic biomass	Process
<ul style="list-style-type: none">• Butanol is a major co-product of ABE (acetone, butanol and ethanol) fermentation as the typical proportions of acetone, butanol and ethanol are 3:6:1.• <i>Clostridium</i> spp., particularly <i>C. acetobutylicum</i> or <i>C. beijerinckii</i> are capable of converting broad-ranging carbon sources (e.g. glucose, galactose, cellobiose, mannose, xylose and arabinose) to fuels and chemicals• The ABE fermentation is biphasic involving acidogenesis and solventogenesis.• Acidogenic phase involves the production of acids (e.g. acetic and butyric acid).• Solventogenic phase is related to the production of solvents (e.g. acetone, butanol and ethanol).• The ABE-producing bacteria can utilize both starchy and lignocellulosic substrates	

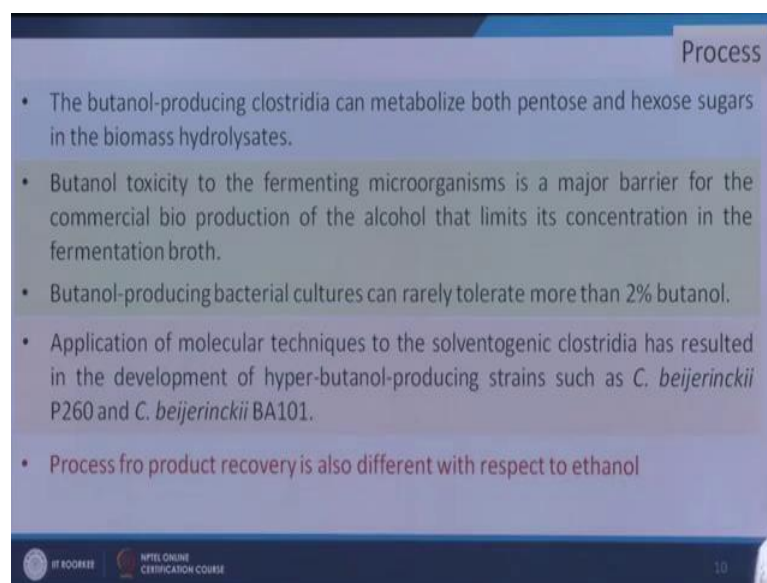
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Now, we are coming on butanol production. So, butanol can be produced through the fermentation, but here the process will be slight different. The separations of the butanol from the media will also be slightly different from that of ethanol production.

Let us see here the clostridium species, the bacteria that is clostridium acetobutylicum and clostridium beijerinckii are the 2 bacteria which have been found to suitable for the production of butanol from the sugar. Now when butanol is produced not only butanol is produced in the media other 2 components that is acetone and ethanol are also produced that is called ABE fermentation acetone butanol and ethanol fermentation. And in this fermentation the product is A is to B is to E that is acetone is to butanol is to ethanol is equal to 3 is to 6 is to 1. So, this is a typical ratio of these 3 components in this methanol for a butanol fermentation route or A B route now ABE fermise ABE fermentation is if by phasic fermentation that is acidogenic phase and solventogenesis phase.

So, acid acidogenesis and solventogenesis 2 phases are there, unlike ethanol fermentation. So, acidogenic phase involves the production of acids like say acetic acid butyric acid. And then solventogenesis converts these to solvents like acetone butanol ethanol etcetera. The ABE producing bacteria can utilize both starchy and lingo cellulosic biomass all type of biomass or waste can be used for the production of this A B E.

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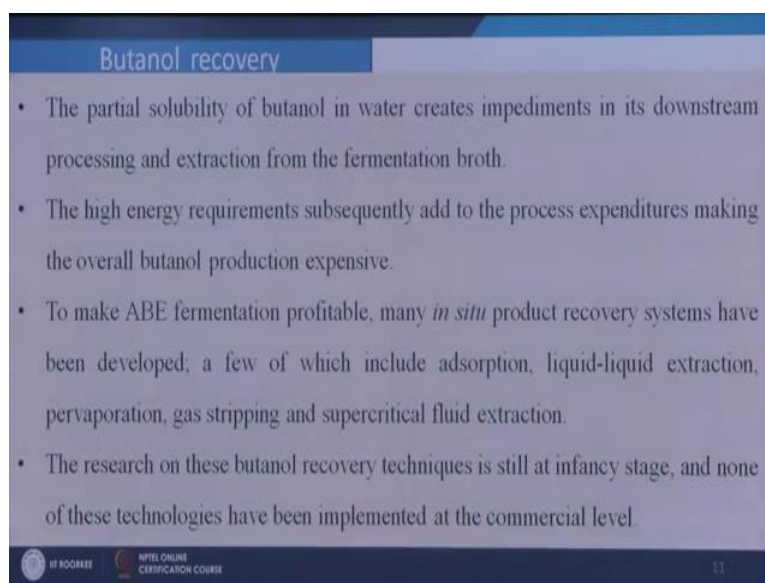
- The butanol-producing clostridia can metabolize both pentose and hexose sugars in the biomass hydrolysates.
- Butanol toxicity to the fermenting microorganisms is a major barrier for the commercial bio production of the alcohol that limits its concentration in the fermentation broth.
- Butanol-producing bacterial cultures can rarely tolerate more than 2% butanol.
- Application of molecular techniques to the solventogenic clostridia has resulted in the development of hyper-butanol-producing strains such as *C. beijerinckii* P260 and *C. beijerinckii* BA101.
- Process for product recovery is also different with respect to ethanol

Now, one important factor here is that butanol which is produced in the media is toxic to the microorganisms. And it has been found that if its concentration is more than say 2 percent. So, the microorganisms suffer their growth hampers. So, that is the main disadvantage of this process.

So, efforts are going on to develop new strains which are suitable to resist the concentration of butanol in the media, and application of molecular techniques to the solventogenic clostridia has resulted in the development of hyper butanol producing strains such as *Clostridium beijerinckii* p 260 and *C. beijerinckii* B A 1 0 1. So, these 2 strains have recently been reported that is modified genetically modified bacteria which are having more butanol production capacity and we have discussed this process is not a very similar to that of ethanol process because the mechanism the microorganisms are different, and the concentration of butanol present in the media is also very less with respect to ethanol only 2 percent.

So, we have to remove this butanol from the media. So, the methods will also be slightly different from that of ethanol separation process.

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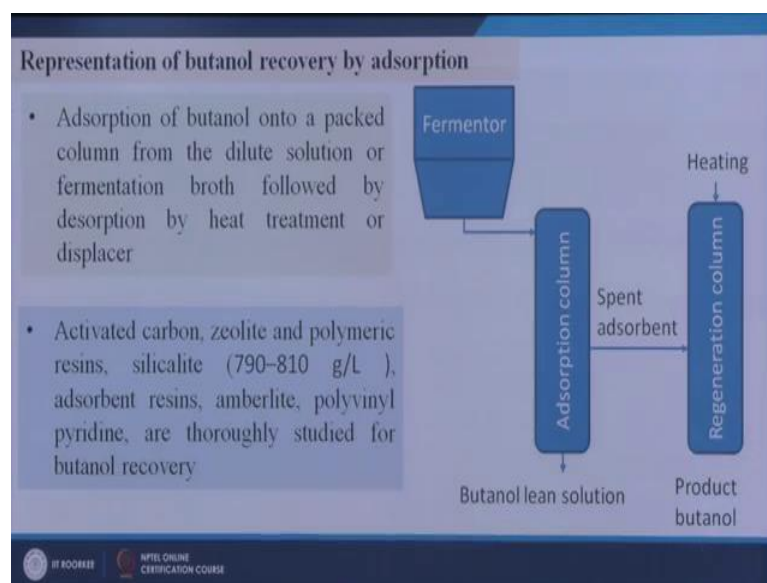
- The partial solubility of butanol in water creates impediments in its downstream processing and extraction from the fermentation broth.
- The high energy requirements subsequently add to the process expenditures making the overall butanol production expensive.
- To make ABE fermentation profitable, many *in situ* product recovery systems have been developed; a few of which include adsorption, liquid-liquid extraction, pervaporation, gas stripping and supercritical fluid extraction.
- The research on these butanol recovery techniques is still at infancy stage, and none of these technologies have been implemented at the commercial level.

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So, now we will see how the butanol can be recovered. The main problem for the recovery of the butanol from the media is that it is partial solubility, with the water and its boiling point is also high that is 117 degree centigrade boiling point. That is why for the separations it requires higher heat with respect to ethanol production through distillation. That is why to separate the butanol in the A B process some separation techniques are being developed.

So, out of those some important are adsorption liquid-liquid extractions pervaporation gas stripping and supercritical fluid extraction. However, one important thing is that all those techniques are in infancy stage. There are no any commercial plants and not here developed, but conceptually these are available and people are trying to develop these techniques.

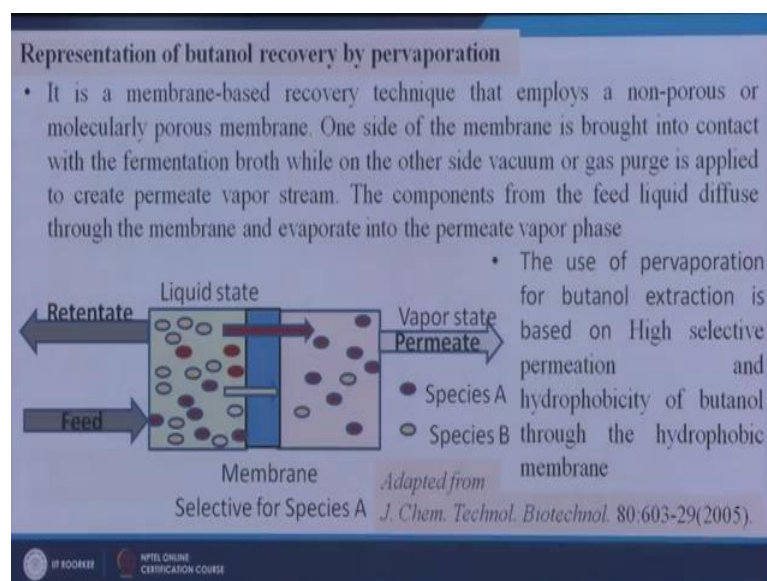
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So, now you will see the how the adsorption process or adsorption column can be used to separate butanol from the media, there is a fermenter. So, from the fermenter that is around 2 percent of butanol it is passed through the first column the absorption column. So, 2 percent butanol will be at adsorbed selectively on this adsorbent base bed and this adsorbent bed will be taken out another bed new bed will be put here then this old adsorbent bed will be regenerated by heating. So, butanol will be coming out or desorbed from the media or from the adsorbent and product butanol we can get. So, this is the mechanism of the separation of the butanol from the fermenter media, now the materials which has been used here. So, for that is activated carbon zeolite and polymeric resins silicalite adsorbent resins amberlite polyvinyl pyridine.

So, these are the adsorbents which have been tested by many researchers for the recovery of the butanol from the fermented broth.

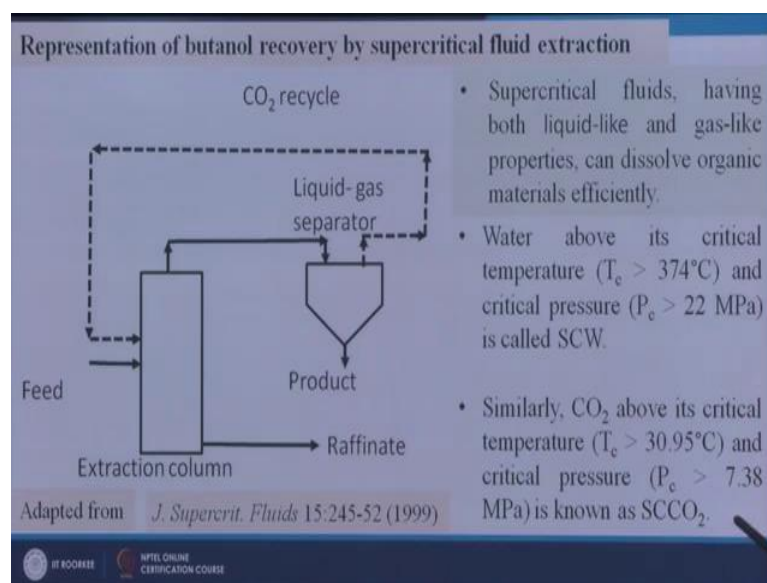
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Next we will discuss on pervaporation process. So, pervaporation process this is one membrane is required this membrane is not porous or molecularly porous; that means, size selective all molecules will not be able to pass through it some selective size of the molecules will be able to pass through it. So, one side we will send the broth. And through this membrane the butanol will pass through. Why it will pass through because this is selective to butanol and this is hydrophobic in nature butanol is hydrophobic in nature. So, butanol will come out through this membrane into this chamber. So, when it is coming in this chamber, this chamber is under vacuum or some gas part is there, so the produce the product which is coming in this side of this of the membrane that will be in vapor phase.

So, this is the mechanism of the separation of the butanol from the media using pervaporation process and from the vapor we will collect the butanol.

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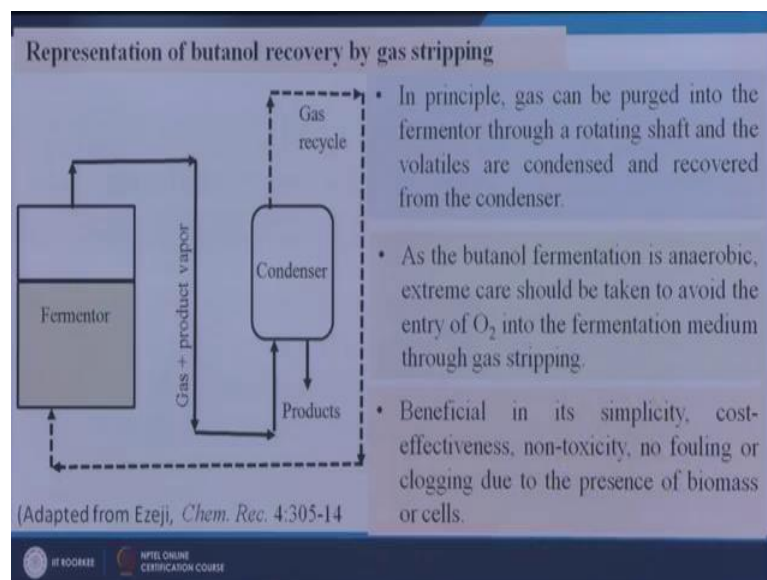
Next we will discuss on the supercritical fluid extraction to super critical fluid, if we have some fluid if we increase the pressure and temperature. So, it will reach it is super critical stage. So, beyond the critical point it will be supercritical fluid. And supercritical fluid is having some unique characteristics; it is a very good solvent. It can resolve organic molecules in it. So, we can have option for water supercritical water supercritical carbon dioxide etcetera. So, if we want to use the supercritical water, the condition is that the temperature and pressure is like this temperature is greater than 374 degree centigrade and pressure is also more than 220 atmospheric pressure.

So, there is for water. So, similarly if we use the carbon dioxide the temperature must be more than 30.95 degree centigrade. And pressure will be more than 73 or 74 atmospheric pressure. So, under these conditions the water and carbon dioxide will be behaving as a supercritical fluid, and if these fluids are used here when the feed is entering in this same extraction column. In this extraction column feed we are giving that is fermenter broth and here we have solvent we are giving supercritical solvent.

So, due to the very good solvent property of this supercritical fluid the organic molecules like butanol will be dissolved in it maybe other organic compounds like say acetone ethanol etcetera will also be coming and hear it will be coming and after condensation we will get the product. So, this by one stage we will not get the high purity of the butanol, but successive steps can give the improvement in the percentage of the butanol.

So, this is the mechanism of the separation of the butanol using supercritical solvent extraction method.

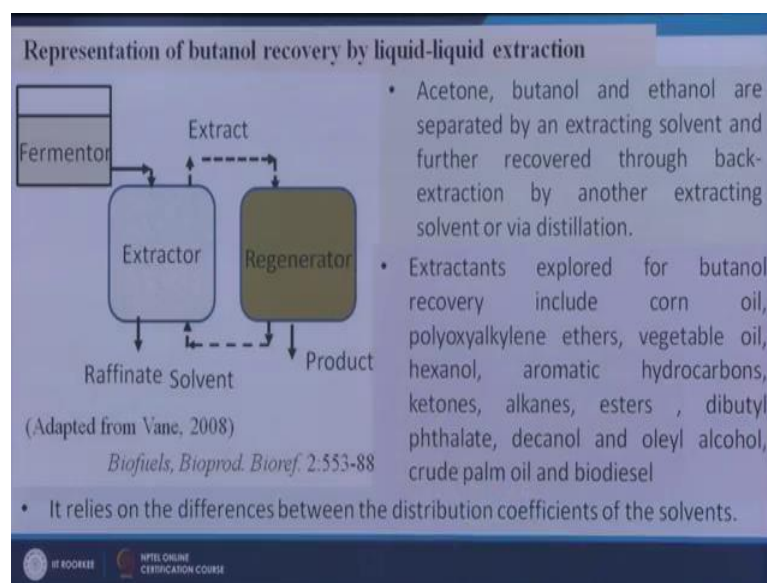
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Now, we will discuss on the gas stripping. So, gas stripping if we pass some gas hot gas through this media. So, if this is a fermenter. So, they are everything is there solid material is there butanol acetone everything all the other compounds also microorganisms. So, when we will pass the gas. The gas will take the volatiles compound butanol acetone etcetera or it will go up after condensation it will give us the product. So, hot gas is going the volatiles are going out with the hot gas and it is condensed and products have been separated. So, this is the mechanism for the separation of the butanol using gas stripping and when condensation takes place then the gas is again recycled.

So, this is the mechanism, but one important precaution is that the gas which we are sending that that must be free from oxygen, because the whole reaction takes place in anaerobic condition. So, oxygen will hamper the rate of reaction and there will be no fermentation. So, these has some beneficial. This method has some benefit that is it is simple and cost-effective and non toxicity and no fouling or clogging due to presence of biomass or cells.

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Next, liquid-liquid extractions; so fermenter broth can be contacted with some liquid which will be having some specific butanol capturing capacity. And this works on this the difference between the distribution coefficients.

So, if you use to one solvent the distribution coefficient of butanol will be more than the broth butanol will come here. So, when it will be going out the fluid that is extractant. So, it will be regenerated. So, we will get the product butanol. So, this regeneration can take place either by extraction using other solvent or it can also be done through distillation. So, after regeneration again solvent can be used. So, this is the mechanism for the liquid-liquid extractions and different type of solvents which has been used for these applications or for this purpose are presented here some examples are say corn oil polyoxyalkylene ethers vegetable oil hexanol aromatic hydrocarbons ketones alkanes esters dibutyl phthalate decanol and oleyl alcohol crude palm oil and biodiesel.

So, all those things have been used for the separation of the butanol from the neutron broth.

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Representation of butanol recovery by distillation

- Distillation is a traditional method for butanol recovery from the aqueous fermentation broth.
- Distillation requires high energy as butanol has a boiling point (117.7°C) greater than that of water.
- A heterogeneous distillation, especially involving an azeotrope is used to separate butanol from water mixture.

B.P. of n-butanol 117.8 °C , water 100 °C , mixture 92.4 °C (55 % butanol)

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Then distillation: distillation is also one process just like azeotropic distillations as discussed for ethanol separations. So, also be applicable here, but here we see the boiling point is 117.8 degree centigrade for butanol and water 100 degree. So, heterogeneous mixture is 92.4 degree centigrade when the butanol concentration is 55 percent. So, 55 percent butanol can be recovered, but it is high concentration is not possible we have to go for another we have that for other another molecules and extractive distillations and azeotropic distillations may be followed.

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Advantages and limitations of butanol recovery technologies			
Recovery technique	Advantages	Limitations	Energy requirement (MJ/kg)
Adsorption	(i) Easy operation. (ii) Less energy intensive.	(i) Low selectivity. (ii) High material cost. (iii) High adsorbent regeneration cost.	1.3-33
Distillation	Traditionally used. Less infrastructure requirement.	High operational cost due to - low concentration of butanol. Highly energy intensive.	

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Here we will see the advantage and limitations of these different techniques for the recovery of the butanol from the fermentation broth.

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Advantages and limitations of butanol recovery technologies			
Recovery technique	Advantages	Limitations	Energy requirement (MJ/kg)
Gas stripping	(i) High efficiency. (ii) Reduced butanol toxicity. (iii) Easy operation. (iv) No fouling.	Low selectivity. Low efficiency.	14-31
Liquid-liquid extraction	(i) Less energy intensive. (ii) High selectivity. (iii) High efficiency.	(i) High operational cost. (ii) Toxicity of extractant (iii) Emulsion formation	7.7-26

So, adsorption, distillation and gas stripping liquid-liquid extraction and pervaporations supercritical fluid extractions; so all those things we have presented in this table. So, here advantage and disadvantage are provided as well as energy requirement is also given. So, adsorption is easy operations and less energy intensive, but it has low selectivity and high material cost etcetera and distillations is a traditional onem and it is high operational cost that is high energy intensive process gas stripping it is having very high efficiency nontoxic it is a and easy operations no fouling, but low selectivity and low efficiency this is the power requirement energy requirement liquid-liquid extractions also less energy intensive high selectivity, but high operational cost also and toxicity of the extract and that is most important point for this the negative point that is toxicity of the extraction.

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Advantages and limitations of butanol recovery technologies			
Recovery technique	Advantages	Limitations	Energy requirement (MJ/kg)
Pervaporation	Selective removal of solvents. Less energy intensive. High flux	(i) High membrane cost. (ii) Vulnerable to temperature-sensitive compounds and cells.	2-145
Supercritical fluid extraction	(i) Cost-effective. (ii) Recyclable.	(i) Needs more research.	-

And pervaporations and supercritical fluid extraction is a good option, but it is not. So, matured it needs more research. So, these are the salient features and advantage and disadvantages of these different methods for the recovery of the butanol from the fermentation broth.

(Refer Slide Time: 26:57)

Lignocellulosic biomasses/ wastes and microbial strain used for butanol production	
Feedstock	Bacteria
Aspen wood, Corn stover, Pinewood	<i>Clostridium acetobutylicum</i> P262
Bagasse, Rice straw	<i>Clostridium saccharoperbutylacetonicum</i> ATCC 27022
Barley straw, Wheat straw, Switchgrass	<i>Clostridium beijerinckii</i> P260
Corn fiber	<i>Clostridium beijerinckii</i> BA101
Pinewood, Wheat straw, Timothy grass	<i>Clostridium beijerinckii</i> B-592
Wheat straw	<i>Clostridium acetobutylicum</i> IFP 921

Now, this slide gives us some example of bacteria and different types of feed stocks which have been used for the production of butanol through this fermentation route. So, here *clostridium acetobutylicum* p262 *clostridium beijerinckii* p260 *clostridium*

beijerinckii B A 101 clostridium beijerinckii b592 and some acetobutylicum this 920 one. So, some strains have been reported in recent years for the production of butanol from the biomass thank you very much; so up to this in this module.

Thank you very much for your patience.