Biomass Conversion and Biorefinery
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Lecture – 25
Microorganisms, Current Industrial Ethanol Production Technology

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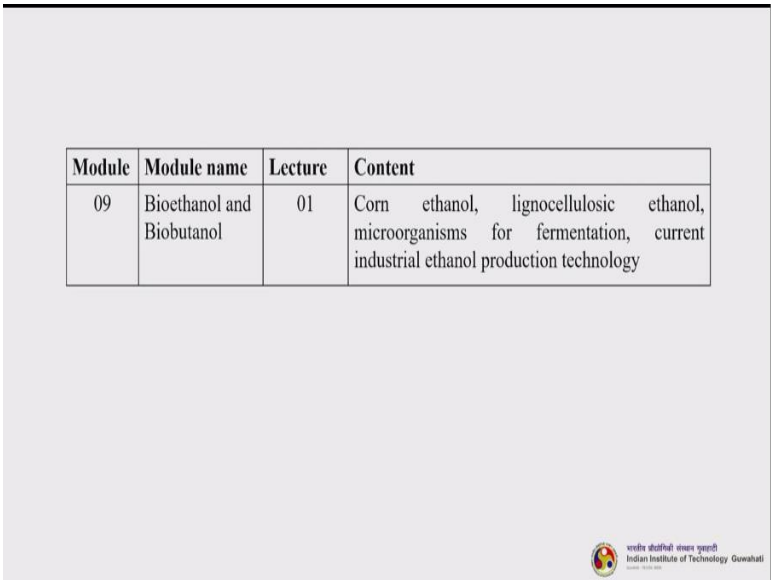

Module	Module name	Lecture	Content
09	Bioethanol and Biobutanol	01	Corn ethanol, lignocellulosic ethanol, microorganisms for fermentation, current industrial ethanol production technology

Good morning students. Today is lecture 1 under module 9. And as you know that in this module 9 we will be discussing basically about bioethanol and biobutanol, so two of the most important products from the fermentation pathway. So, let us begin our class today.

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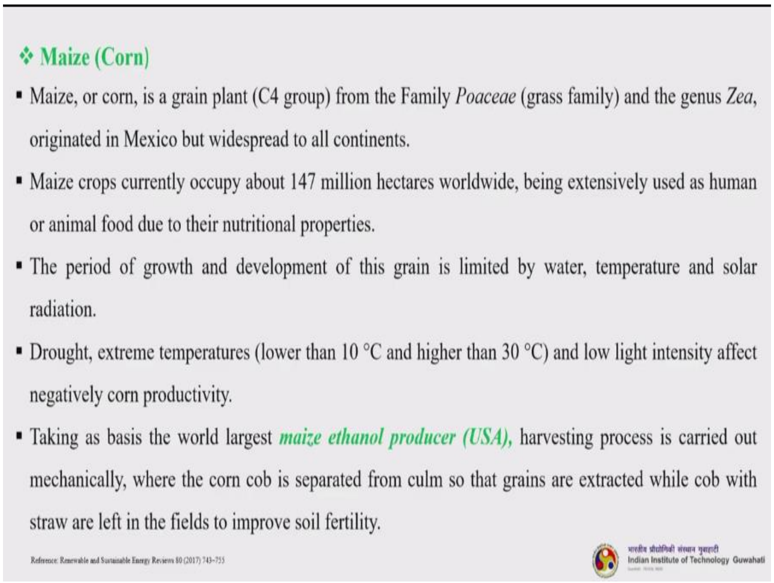

So, we will talk about corn ethanol first. So, as you know maize or corn is a grain plant from the family *Poaceae* and the genus *Zea*, originated in Mexico but widespread to all continents. Maize crops currently occupy about 147 million hectares worldwide being extensively used as human or animal food due to their nutritional properties. The period of growth and development of this grain is limited by water, temperature and solar radiation.

If you recall we have discussed about maize when we discussed about different types of biomass, lignocellulosic biomasses and other biomass which has been used for conversion. So, drought, extreme temperatures lower than 10 degrees centigrade and higher than 30 degrees centigrade and low light intensity affect negatively corn productivity. Taking as basis the world's largest maize ethanol producer that is United States, harvesting processes carried out mechanically where the corn cob is separated from the culm so that grains are extracted while the cob with the straw are left in the field to improve the soil fertility.

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Table 1: Global Corn Production and Yield From 2015–17 (in million metric tons)

	Production (million metric tons)			Yield (metric tons per hectare)		
Country/region	2015/16	2016/17	2017/18 projected	2015/16	2016/17	2017/18 projected
World	969.49	1070.51	1036.90	5.43	5.83	5.70
USA	345.51	384.78	362.09	10.57	10.96	10.72
China	224.63	219.55	215.00	5.89	5.97	6.14
Brazil	67.00	98.50	95.00	4.19	5.61	5.37
European Union	58.75	61.14	61.60	6.35	7.11	7.03
Argentina	29.00	41.00	40.00	8.29	8.37	8.16
Mexico	25.97	27.40	25.00	3.60	3.65	3.50
India	22.57	26.00	25.00	2.56	2.71	2.63
Rest of the world	196.06	212.14	213.21	—	—	—

Source: Data taken from Foreign Agricultural Services/USDA Office of Global Analysis

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So, this is a table which will make you understand about the global corn production and the yield from 2015 and 2017 in million metric tons. If you see the world is around actually projected if you look at projected to 1036.90 million metric tons that is the production in 2017 and 18 and it has been increased, but I could not find actually the updated statistics. So, I am presenting you the old one. This is taken from the Foreign Agricultural Services of the USDA office.

And you can see that apart from United States, China, Brazil, the entire European Union is clubbed into one group, Argentina, Mexico and even India is contributing towards the global production in a substantial way.

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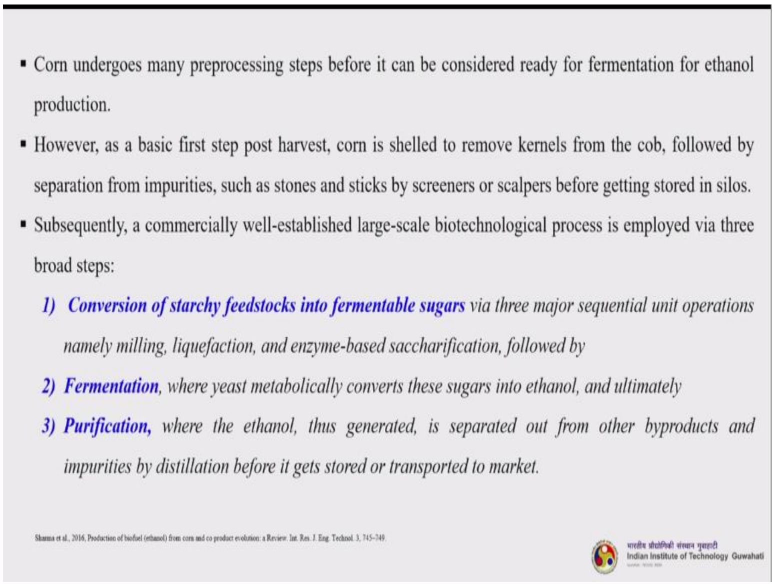

So corn undergoes many preprocessing steps before it can be considered ready for fermentation for ethanol production. However, as a basic first step post harvest corn is shelled to remove kernels from the cob, followed by separation from the impurities such as stones and sticks by screeners or scalpers before getting stored in the silos. Subsequently, a commercially well-established large-scale biotechnological process is employed via 3 broad steps.

So, the first step is the conversion of starchy feedstocks into fermentable sugars. So, this also happens via 3 major sequential unit operations namely milling, liquefaction and enzyme-based saccharification. Now, the second one is fermentation where the yeast metabolically converts these sugars into ethanol and then finally followed by the purification where the ethanol thus generated is separated out from other byproducts and impurities by distillation before it gets stored or transported to market.

Now, please understand that this is the basic scheme for any corn ethanol production or we can say ethanol production. So, this and this is well understood, anyway we are going to discuss and in purification step distillation is the one which is being commercially adapted in many corn ethanol refineries. But please understand that only distillation is not going to serve the purpose, there are many other recovery methods also there.

Depending upon what is your yield of the corn ethanol in the fermentative product basically, what is the concentration and what are the other byproducts present, so that will decide eventually whether you are going to have only distillation, a series of distillation or you will have to combine some other unit operations along with distillation so as to have a hybrid recovery process.

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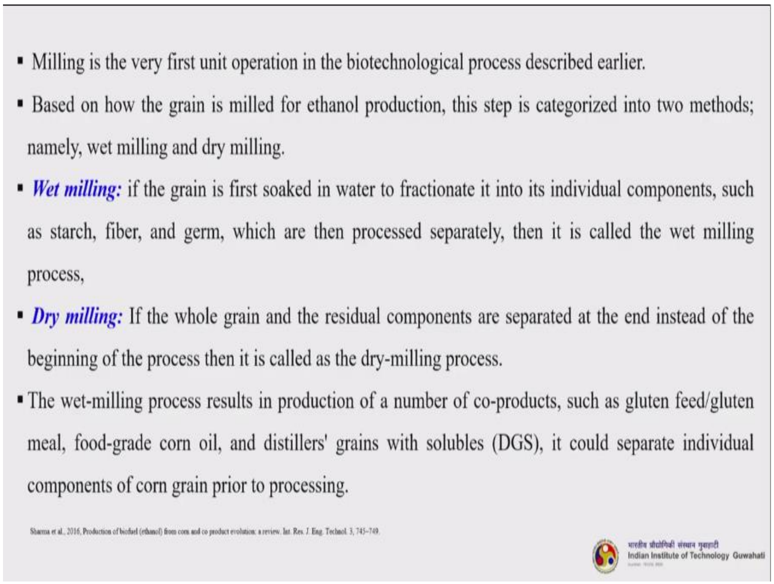

Milling is the very first unit operation in the biotechnological process described earlier. So, based on how the grain is milled for ethanol production, this step is categorized into 2 methods. First is wet milling and then dry milling. So, we will discuss in detail about these two. So wet milling is what? If the grain is first soaked into water to fractionate it into its individual components such as starch, fiber and germ which are then processed separately, then it is called wet milling process.

And what is dry milling? Now if the whole grain and the residual components are separated at the end instead of the beginning of the process then it is called the dry milling process. So, this is the basic difference between two milling processes. The wet milling process results in production of a number of co-products such as gluten feed, gluten meal, food-grade corn oil and distillers' grain with solubles which is called as DGS. It could separate individual components of corn grain prior to processing.

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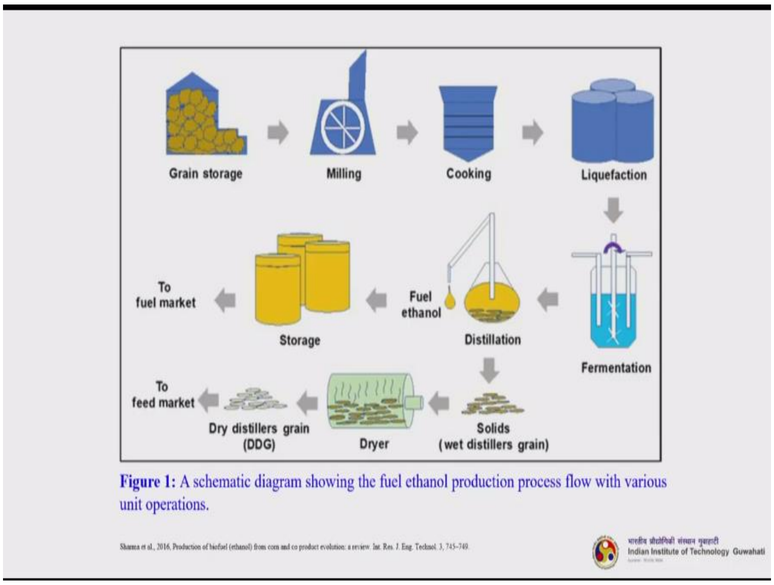

So, this is the schematic where you can understand that how the fuel ethanol is being produced along with the various unit operations. So, let us just glance through it in a quick way. So, this is the grain storage. Then it goes for milling. Now, this milling can be dry milling or wet milling. Then you go for cooking, liquefaction, then the fermentation. This is the main fermentation bioreactors.

Once it is done, so then you will go for distillation where your fuel ethanol will be recovered and you will get the solid residues and apart from that certain value-added components which are present as byproducts, but not essentially require in the fuel ethanol components. Then you go for storage and fuel to market. These solids products we can also make value added products out of these. So, you dry them, then it goes to DDG which is called a dry distillers grain and also can be sold out as a product.

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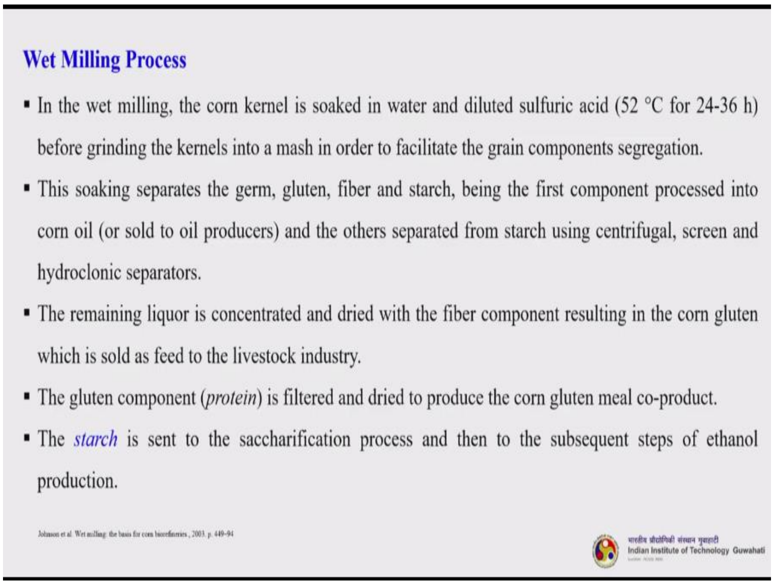

So, let us understand wet and dry milling in a better way again. So, in the wet milling the corn kernel is soaked in water and diluted sulfuric acid almost 24 to 36 hours at 52 degrees centigrade before grinding the kernels into mash in order to facilitate the grain components segregation. Now, this soaking separates the germ, gluten, fiber and starch being the first component processed into corn oil or sold to oil producers.

And the other separated from starch using centrifugal, screen and hydroclonic separators. The remaining liquor is concentrated and dried with the fiber component resulting in the corn gluten which is sold as feed to the livestock industry. The gluten component that is protein is filtered and dried to produce the corn gluten meal product. The starch is sent to the saccharification process and then to the subsequent steps of ethanol production. The usual steps followed by fermentation, distillation and all.

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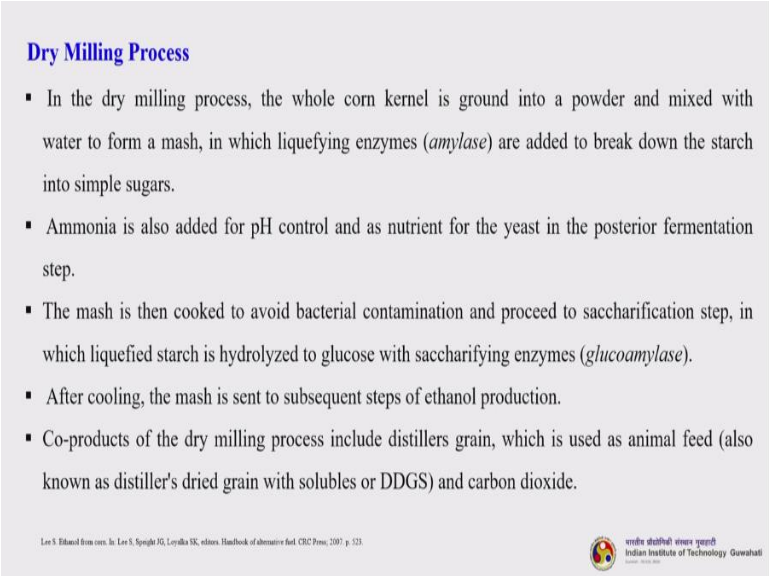

And in the dry milling process, the whole corn kernel is ground into a fine powder and mixed with water to form a mash in which the liquefying enzymes such as amylase are added to break down the starch into simple sugars. Now, ammonia is also added for pH control and as nutrient for the yeast in the posterior fermentation step. The mash is then cooked to avoid bacterial contamination and proceed to saccharification step in which liquefied starch is hydrolyzed to glucose with saccharifying enzymes such as glucoamylase. So, after cooling the mash is sent to subsequent steps for ethanol production. Co-products of the dry milling process include distillers grain which is used as an animal feed, also known as the distillers' dried grain with solubles or its short form is DDGs and carbon dioxide.

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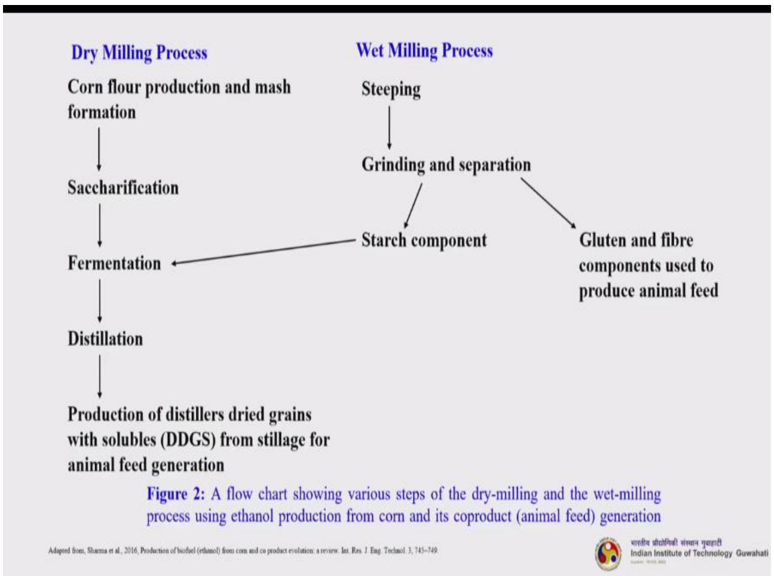

So, this is the flowchart showing various steps of the dry milling and wet milling process. So, corn flour production and mash formation in the dry milling process it starts, then it followed by saccharification, fermentation, distillation and then DDGS formation. Now, similarly in the wet milling also we start with steeping. Now steeping followed by grinding and separation where you remove starch and separate starch.

And then others are gluten and fiber components which can also be converted to animal feed. Now this starch again whatever it is there so it can go to fermentation, distillation and the usual process. So, from here down it is the usual common processes in both the wet milling and dry milling and the differences we have already discussed.

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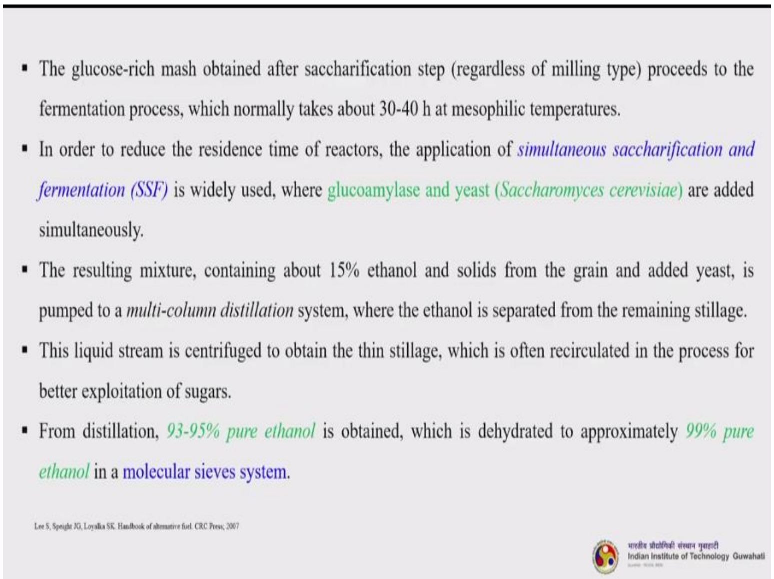

The glucose-rich mash obtained after saccharification step regardless of the milling type actually proceeds to the fermentation process which normally takes about 30 to 40 hours at mesophilic temperatures. Now in order to reduce the residence time of reactors, the application of simultaneous saccharification and fermentation which is known as SSF is widely where glucoamylase and yeast usually *Saccharomyces cerevisiae* are added simultaneously.

The resulting mixture containing about 15% ethanol and solids from the grain and added yeast is pumped to a multi-column distillation column where the ethanol is separated from the remaining stillage. This liquid stream is centrifuged to obtain the thin stillage which is often recirculated in the process for better exploitation of sugars. From distillation 93 to 95% pure ethanol is obtained, which is dehydrated to approximately 99% pure ethanol in a molecular sieve system.

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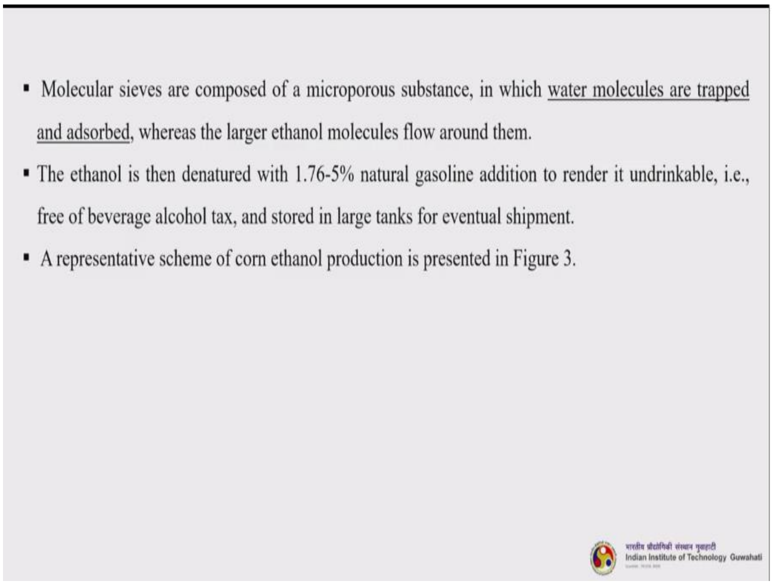

What this molecular sieve will do? Let us understand. So molecular sieves are composed of a microporous substance in which the water molecules are only trapped and adsorbed inside their molecular structure whereas the larger ethanol molecules will flow around them. So that means it will not adsorb them. So ethanol is then denatured with 1.76 to 5% natural gasoline that is being added actually to render it undrinkable.

So that is free of beverage alcohol tax and stored in large tanks for eventual shipment. A representative scheme for the corn ethanol is being shown in the figure, we will try to understand.

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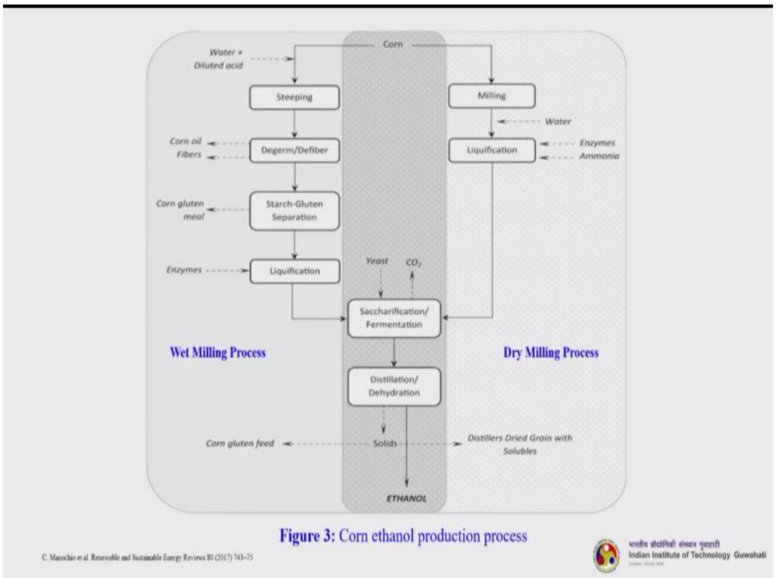

So, again two different processes. The wet milling is this side, the dry milling is this side and this particular porition is dedicated to the processes which are common to both. So, again you can see that steeping followed by degerm and defiber followed by the starch separation, then liquefaction, then it goes to saccharification, fermentation, distillation, dehydration and whatever solids you get that can be either corn gluten feed or can be DDGS.

Then you get here ethanol. Similarly, in the dry milling process it is milling - the mash formation actually - followed by liquefaction and then the usual process. So, this is an overall schematic representation of how corn ethanol can be produced using two different milling processes dry and wet.

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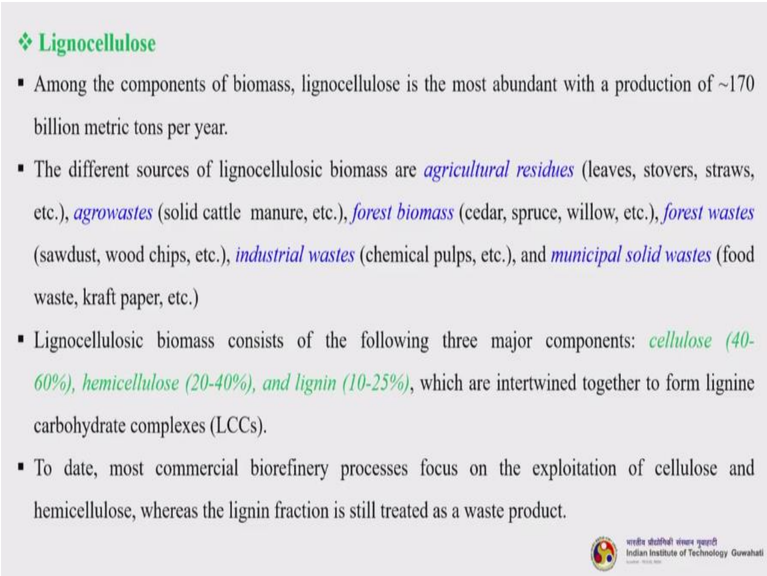

Now, we will discuss about lignocellulosic ethanol production. Among the components of biomass, lignocellulose is the most abundant with the production of almost 170 billion metric tons per year. The different sources of lignocellulosic biomass are agricultural residues, agrowastes, forest biomass, forest wastes, industrial wastes, municipal solid waste, etc. Now, if you recall we have already discussed about lignocellulosic biomass, different types of biomasses, their characteristic features everything earlier. So, we just quickly go through again in a nutshell. So, lignocellulosic biomass consists of the following three major components. Cellulose around 40 to 60%, hemicellulose around 20 to 40% and lignin is the rest 10 to 25% which are intertwined together to form a lignine carbohydrate complex which are also called as LCC.

To date, most commercial biorefinery processes focus on the exploitation of cellulose and hemicellulose whereas the lignin fraction is still treated as a waste product. But if you recall, we have already discussed about how and why lignin should also be treated at par with other components such as cellulose and hemicellulose because lignin is a very high value commercial chemical.

Now that lignin can be converted to various other components, it can be converted to fuel also and apart from that other chemicals via lignin biorefinery which we have already discussed. I hope you can recall that, right.

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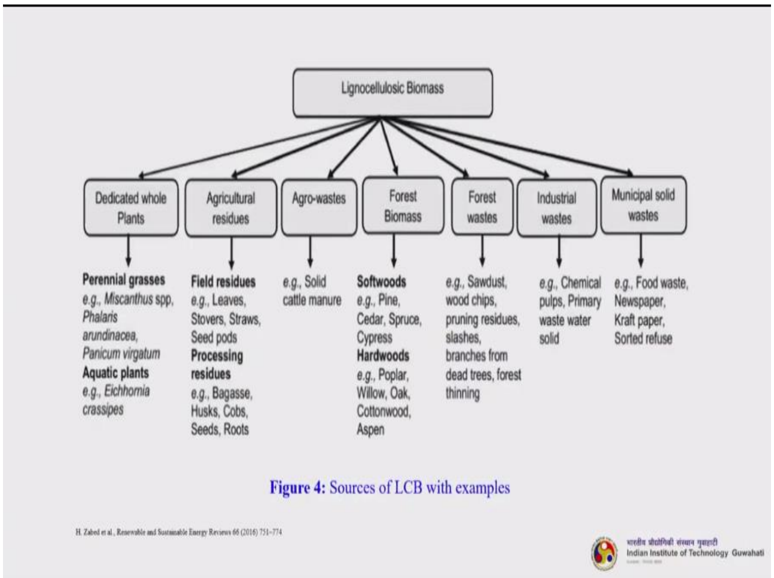

So, this is the different types of lignocellulosic biomasses, we have already discussed this in one of our subsequent classes, but I feel that it is better to show you once again in a nutshell so that the flow of this particular lecture will be retained. So, just quickly we are going through. We have different types of biomasses and what are listed under them. So dedicated whole plants, agricultural residues, agro-wastes, forest waste, industrial waste, municipal solid waste.

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Table 2: Brief description and bioethanol potential of different LCB sources

Sources	Brief description	Potential	Annual production	Potential ethanol yield (L/ton)
Perennial grasses	Twenty perennial grasses have been reported for potential ethanol feedstocks	• High biomass yield; 0.9–37 ton/ha • High cellulose content • Easy to grow and harvest • Can provide 50–70% of the total biomass for ethanol	0.9–37 ton dry matter /ha-year	160–460
Aquatic plants	Water hyacinth (*Eichhornia crassipes*) is the major and promising aquatic plant for ethanol	• Widely prevalent aquatic weeds • Abundant in certain parts of the world making it a suitable feedstock for distributed ethanol production • They grow in water bodies and do not compete with arable lands. • Exceptionally fast growing plant • High biomass productivity	39.5 ton/ha-year	-
Agricultural residues	Major residues include cereal straws, bagasse, oil palm biomass, natural non-wood plant fibers (Bast fiber, leaf fiber and see hair fiber)	• Easily available • Crop waste management to value added product • Minimizing the reliance on forest woody biomass and thus reduce deforestation • Short harvest rotation period • Global crop residues are estimated (million ton/year) 2802 for cereal crops, 3107 for 17 cereals and legumes, and 3758 for 27 food crops	2802–3758 million ton/year	235–450
Forest biomass and waste	Biomass includes softwoods (conifers and gymnosperm trees) and hardwoods (angiosperm trees); Wastes includes sawdust, wood chips, pruning residues, slashes, branches from dead trees, forest thinning	• One of the largest unexploited and underutilized LCBs, which is estimated, for example, nearly 60 million dry tons annually in the USA - • High density makes the transportation more economical. • Flexible harvesting times	-	220–275 for softwoods, and 280–285 for hardwoods
Municipal solid waste (MSW)	Mixed municipal solid waste consists of integrated cupboard, paper, food residues, garden waste, metal, glass, plastics and textile	• Prospects for both energy development and waste management	20.7 million ton/year for an urban area with 217 million population	154

H. Zabed et al., Renewable and Sustainable Energy Reviews 66 (2016) 751–774

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And this table 2 gives a brief description and bioethanol potential of different lignocellulosic biomass sources. So, if you look at quickly perennial grasses, so the potential can be 0.9 to 37 tons per hectare that is the biomass growth and you can see the potential ethanol yield is almost 160 to 460 litres per ton. Though not very good, but it is okay. So, because whenever you talk about lignocellulosic biomasses we never depend upon the single biomass.

So, there are various types of biomasses that can be combined together and processed so that entire biorefinery will become sustainable. If you look at the agricultural residue, so the ethanol production is almost 235 to 450 litres per ton. Similarly, municipal solid waste the amount is of course less 154, but again you need to understand that we are converting municipal solid waste which is already a waste into some value-added products.

So, it is always a win-win thing, only we need to take care of the process economics and sustainability.

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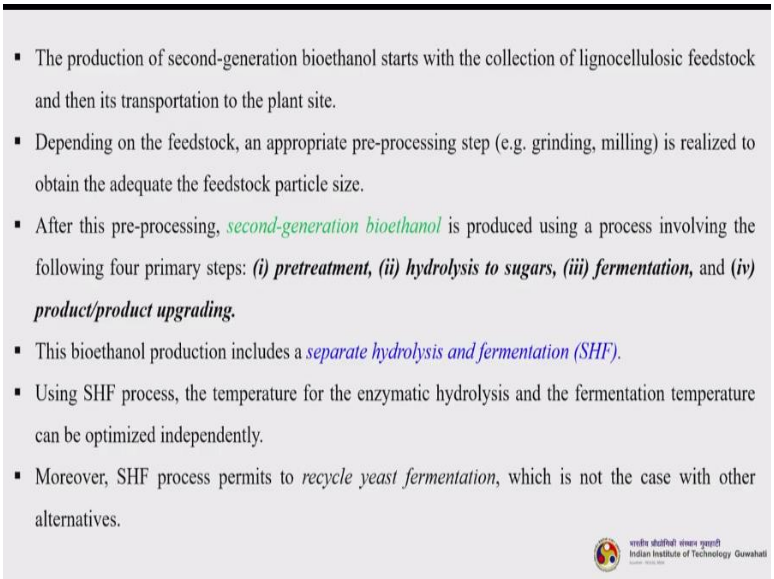

The production of second-generation bioethanol starts with the collection of lignocellulosic feedstock and then its transportation to the plant site. If you remember again recall, let me tell again - the transportation is one of the major costs that contributes to this lignocellulosic bioethanol production. Now, depending on the feedstock and appropriate pre-processing step, either grinding, milling, so all these mechanical pre-processing steps is realized to obtain the adequate feedstock particle size so that you can transport easily - something to do with your bulk density and all. So, after this pre-processing, second-generation bioethanol is produced using a process involving the following primary steps. So, the first is pretreatment followed by hydrolysis to sugars, then fermentation and product and product upgrading.

Now, there is a pre-treatment which is not present in the corn ethanol production. So you can tell sometimes that milling is a type of pre-treatment, but it is not separately grouped under a pre-treatment category. So, this biethanol production includes a separate hydrolysis and fermentation which is known as SHF. Now, using SHF process, the temperature for the enzymatic hydrolysis and fermentation temperature can be optimized independently.

So, this is one of the most important thing. Moreover SHF also permits to recycle the yeast fermentation, which is not the case with the other alternative like your SSFs because you are carrying out the entire thing in a single reactor system.

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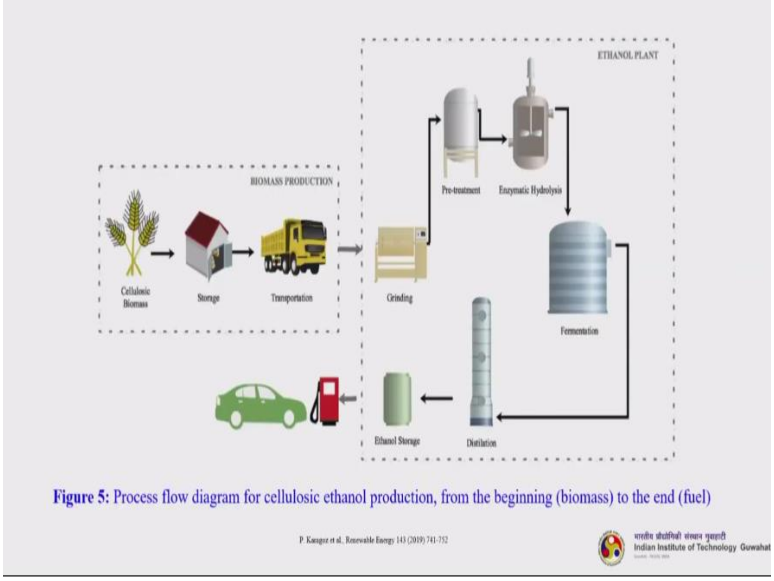

This is the process flow diagram for the cellulosic ethanol production from the beginning that is from the biomass procurement and storage to that of end fuel. So, lignocellulosic biomass that is procured, then stored, then transported. Transported to this biorefinery. Then you grind it, pre-treat it, enzymatic hydrolysis, then you go for fermentation, then go for distillation where you purify ethanol. Then ethanol storage and subsequent use.

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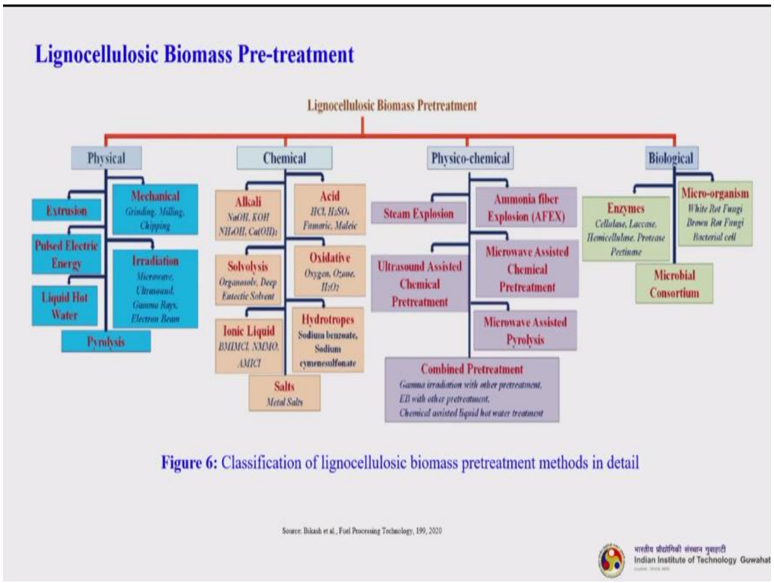

Now lignocellulosic biomass pre-treatment. If you try to recall we have discussed in extensively. I have told you what are the different types of pre-treatment methods, what are the pros and cons of each of the pre-treatment methods. So, we're are not going to discuss it once more. We will just quickly in a single sight try to understand what are the different pretreatment methods and try to recall it.

So, the physical: You can see under physical extrusion, pulsed electric energy, liquid hot water which is one of the most important one, mechanical, irradiation and pyrolysis. Then under chemical: so alkali, acid. So these are very important class of pre-treatment methods especially acid which is dilute acid. Solvolysis, oxidative processes, hydrotropes, ionic liquid, salts. Then we go for a combination of physical and chemical which is called physico-chemical.

Steam explosion, AFEX - the ammonia fiber explosion, ultrasound assisted chemical pretreatment, microwave assisted chemical pretreatment, microwave assisted pyrolysis which is also gaining a lot of research interest nowadays, combined pretreatment. Then we have biological. So biological pre-treatment processes they are benign process, they are good. The only thing is that they take long time.

So, when you are actually talking about a biorefinery concept, then biological somehow do not fit if you talk about the process time it takes. So enzyme based, microorganism based and of course you can have microbial Consortium.

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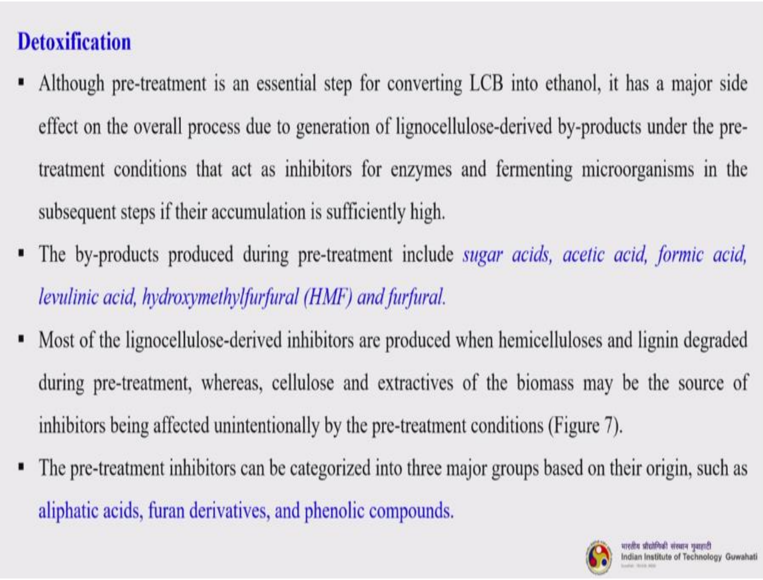

Let us talk about the detoxification. Although pretreatment is an essential step for converting the lignocellulosic biomass into ethanol, it has a major side effect on the overall process due to generation of lignocellulose derived byproducts under the pretreatment conditions that act as inhibitors for enzymes and fermenting microorganisms in the subsequent steps if their accumulation is sufficiently high.

The byproducts produced during pretreatment include sugar acids, acetic acid, formic acid, levulinic acid, HMF and furfural. You know HMF is one of the very high value chemical, it is a fuel additive. And other products are also having certain commercial value. Now, most of the lignocellulose derived inhibitors are produced when hemicellulose and lignin degraded during pretreatment.

Whereas cellulose and extractives of the biomass may be the source of inhibitors being affected unintentionally by the pretreatment conditions. I will show you it in figure 7, so the next I will show you. So, the pretreatment inhibitors can be categorized into three major groups based on their origins. So, first is the aliphatic acids, then furan derivatives and phenolic compounds.

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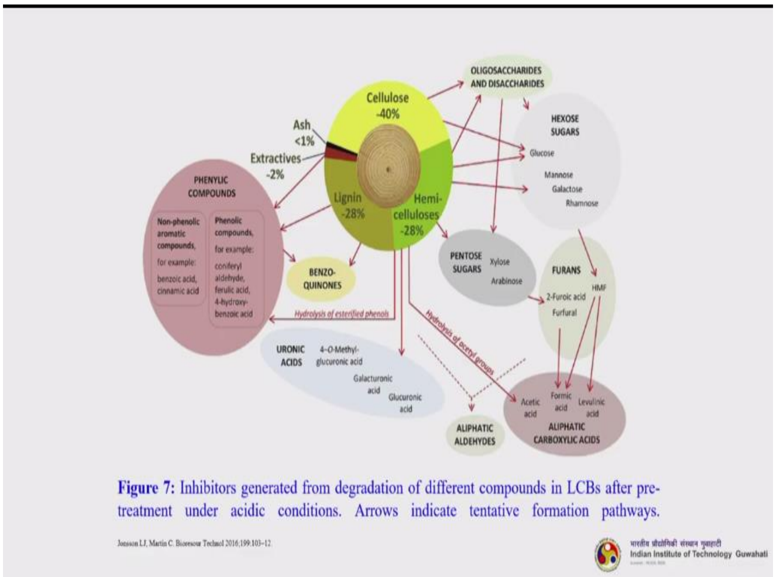

Figure 7: Inhibitors generated from degradation of different compounds in LCBs after pre-treatment under acidic conditions. Arrows indicate tentative formation pathways.

Jonsson LJ, Martin C. Bioresour Technol 2016;199:103–12

You can have a look here. So, this is the different types of inhibitors that is generated from degradation of different compounds in the lignocellulosic biomass after the pretreatment under acidic conditions. So, arrows indicate tentative formation pathways. So, you can see, let us look at this cellulose first. So, cellulose is giving us oligosaccharides and disaccharids, hexose sugars like glucose, mannose and all these things which we need actually.

Hemicellulose is giving us pentose and all, furans and then aliphatic carboxylic acids. Hemicellulose also degrades to these type of compounds which can be grouped as uronic acids. Now lignin, so lignin gives us benzoquinones, phenylic compounds, the major component of actually lignin, Lignin also can degrade and gives us certain very few amount of course uronic acids. Then apart from that we have extractives and ash.

So, this is a broad understanding that how different byproducts are getting generated and what is their pathway. So it is very complicated. It is not so easy and various researchers have tried to actually simulate then study the pathway through which actually these different byproducts are forming.

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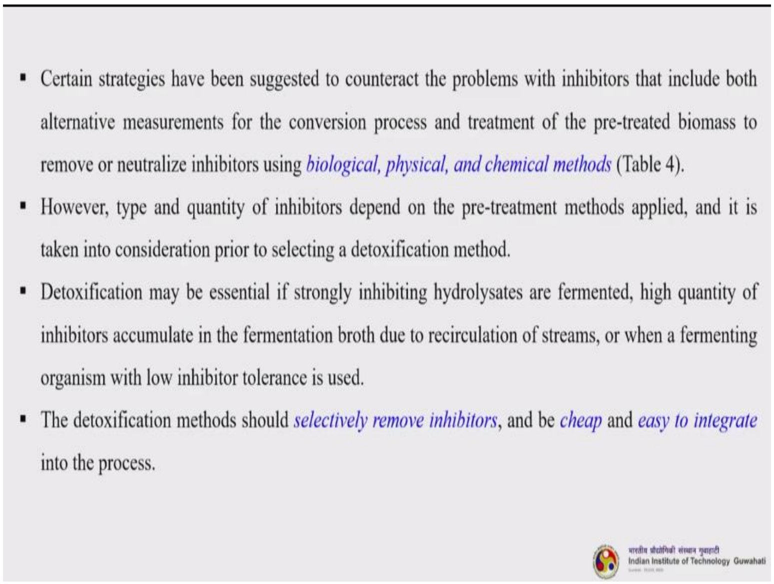

So certain strategies have been suggested to counteract the problems with inhibitors that include both alternative measurements for the conversion process and the treatment of the pretreated biomass to remove or neutralize inhibitors using biological, physical and chemical methods. However, type and quantity of inhibitors depend on the pretreatment methods applied and it is taken into consideration prior to selecting a detoxification method.

Detoxification may be essential if strongly inhibiting hydrolysates are fermented, high quantity of inhibitors accumulate in the fermentation broth due to recirculation of streams or when a fermenting organism with low inhibitor tolerance is used. The detoxification methods should selectively remove inhibitors and be cheap or low cost we can say and easy to integrate into the entire process scheme.

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Table 3: Strategies and techniques to overcome the inhibitors problems during ethanol production from LCB

Strategy	Approach	Remarks/Drawbacks
Alternative measurements		
Feedstock selection and engineering	• Selection of the biomass with less recalcitrance and those generate less inhibitors during pretreatment	• Limitation of using broad range of LCBs • Option for short-rotation crops dedicated to biorefining through sugar platform process
Culturing schemes	• Use of large inoculum size	• Effects on productivity and product yield • Additional costs for inoculum
Selection of microorganism	• Screening of microbial collections from natural or industrial environments	• Selection ought to be made primarily on the basis of specific productivity and product yields
Evolutionary engineering	• Adaptive evolution using specific inhibitors and lignocellulosic hydrolysates	• Cause of inhibition problems varies depending on feedstock, pretreatment conditions
Genetic/metabolic engineering	• Engineering of resistance to phenolics, furfural, and carboxylic acids	• GMO-based process
Treatment of the pretreated biomass		
Chemical additives	• Alkali [such as $Ca(OH)_2$, NaOH, NH_4OH] • Reducing agents [such as dithionite, dithiothreitol, sulfite]	• More chemicals needed • Some methods require additional process step
Enzymatic treatment	• Laccase • Peroxidase	• Requirement of costly enzymes and increase in process time
Heating and vaporization	• Evaporation • Heat treatment	• High energy consumption
Liquid-liquid extraction	• Ethyl acetate • Supercritical fluid extraction [such as supercritical CO_2] • Trialkylamine	• Requirement of additional chemicals and time
Liquid–solid extraction	• Activated carbon • Ion exchange • Lignin	• Requirement of additional equipment and time
Microbial treatment	• *Coniochaeta ligniaria* • *Trichoderma reesei* • *reibacillus thermosphaericus*	• Could be time-consuming and affect sugar content

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So, this table make you understand about the strategies and techniques to overcome the inhibitors problem during ethanol production from LCB. So, if you look at the strategy the first one is feedstock selection and engineering. So, what is the approach? Approach is that selection of biomass with less recalcitrance and those generate less inhibitors during pretreatment. So, this can be done when you screen biomass for fermentation.

So, remarks is that the drawbacks can be something like that the limitation of using broad range of LCB. There are many n number of LCBs, how many you are going to screen. So, this is an experimental screening you have to do to make you understand. Then the option for short rotation crops dedicated to biorefining through sugar platform process, this is one of the important outcome. Let us talk about one more, chemical additives.

So, treatment of the pretreated biomass. So, if you talk about alkalis, then more chemicals needed and reducing agents such as dithionite and all. So some methods require additional process step, to purify certain other chemicals. So, if you talk about then liquid-liquid extraction, so you use ethyl acetate, supercritical fluid extraction, though it is costly but it is a very good process, trialkylamine. So, requirement of additional chemicals and time. So, every strategies and techniques need to be fixed based upon what is your feedstock. Now, in lignocellulosic biorefinery the feedstocks keeps on varying. So, you have to optimize the process parameters again and again when you are actually mixing or changing a whole range of biomass from one particular biomass composition to another biomass composition that can also be done and it has been practiced also. Otherwise, you cannot have a sustainable biorefinery.

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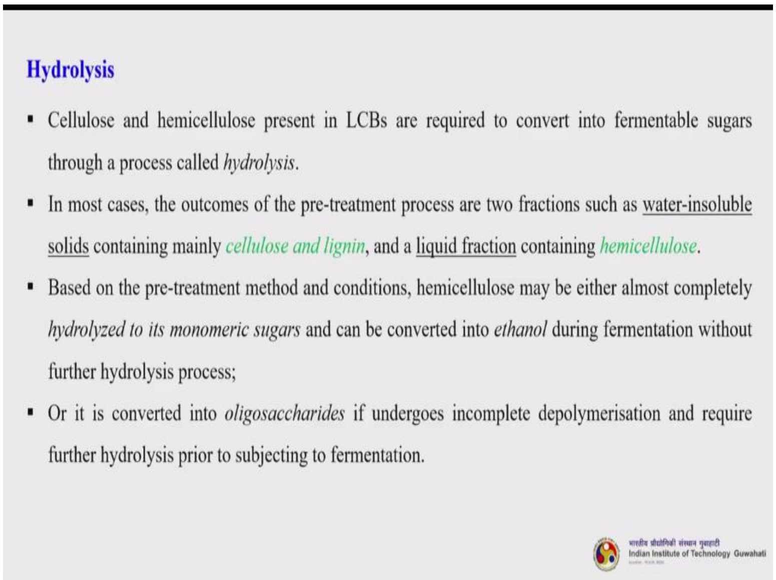

Then the next step is hydrolysis. So, cellulose and hemicellulose present in the lignocellulosic biomasses are required to convert into fermentable sugars through a process called hydrolysis. In most cases, the outcomes of the pretreatment process are two fractions such as first is the water-insoluble solids, so they contain mainly cellulose and lignin. And then a liquid fraction that contents the hemicellulose.

Now, based on the pretreatment method and conditions, hemicellulose may be either almost completely hydrolyzed into its monomeric sugars and then can be converted into ethanol during the fermentation without further hydrolysis process or it can be converted into oligosaccharides if it undergoes complete depolymerization and require further hydrolysis prior to subjecting to the fermentation process.

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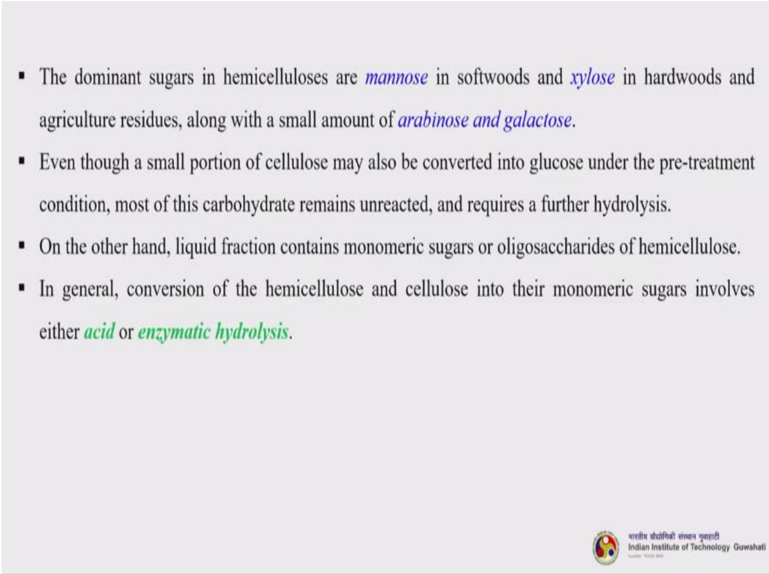

The dominant sugars in hemicellulose are mannose in softwoods and xylose in hardwoods and agricultural residues along with a small amount of arabinose and galactose. Even though a small portion of cellulose may also be converted into glucose under the pretreatment condition, most of this carbohydrate remains unreacted and requires further hydrolysis.

On the other hand, liquid fraction contains monomeric sugars or oligosaccharides of hemicellulose. In general, conversion of hemicellulose and cellulose into their monomeric sugars involves either acid or enzymatic hydrolysis.

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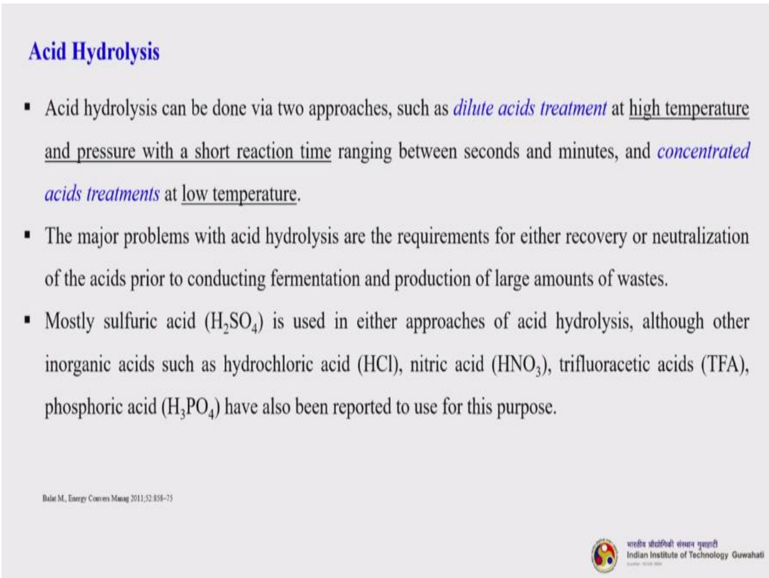

So, we will talk about acid hydrolysis. Acid hydrolysis can be done via two approaches such as dilute acid treatment at high temperature and pressure with a short reaction time ranging between seconds and minutes and then a concentrated acid treatments at low temperature and it takes a long time. The major problem with acid hydrolysis are the requirements of either recovery or neutralization of the acids prior to conducting fermentation and production of large amount of wastes.

Mostly sulfuric acid is used in either approaches of acid hydrolysis, although other inorganic acids such as hydrochloric acid, nitric acid, trifluoracetic acid, phosphoric acid have also been reported for use for this purpose.

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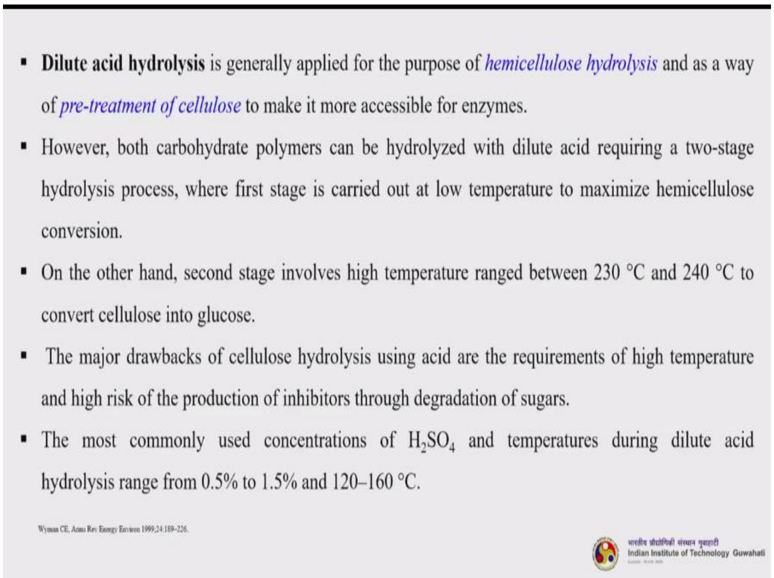

Dilute acid hydrolysis is generally applied for the purpose of hemicellulose hydrolysis and as a way of pretreatment of cellulose to make it more accessible to enzymes. However, both carbohydrate polymers can be hydrolyzed with dilute acid requiring a two-stage hydrolysis process where the first stage is carried out at low temperature to maximize the hemicellulose conversion. On the other hand, the second stage involves high temperature range between 230 degrees centigrade to 240 degrees centigrade to convert cellulose into glucose.

The major drawbacks of cellulose hydrolysis using acid are the requirements of high temperature and high risk of the production of inhibitors through degradation of sugars. The most commonly used concentrations of sulfuric acid and temperatures during dilute acid hydrolysis ranges from 0.5 to 1.5% and 120 to 160 degrees centigrade. So, this is the range. You need to optimize it for a particular biomass.

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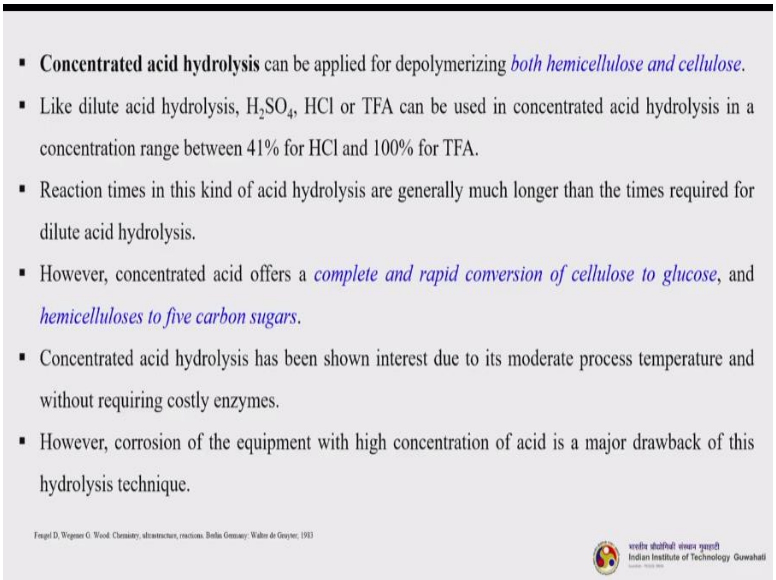

Then the next is the concentrated acid hydrolysis. Now, this can be applied for depolymerizing both hemicellulose and cellulose. So, this is the advantage of this process. Like dilute acid hydrolysis, sulfuric acid, hydrochloric acid or TFA can also be used in concentrated acid hydrolysis in a concentration range between 41% for HCl and almost 100% for the TFA.

Now, reaction times in this kind of acid hydrolysis are generally much longer than the times required for dilute acid hydrolysis. However, concentrated acid offers a complete and rapid conversion of cellulose to glucose and hemicelluloses to five carbon sugars. Again this is one of the best thing about this concentrated acid hydrolysis. So, this process has been shown interest due to its moderate process temperature and without requiring costly enzymes.

However, corrosion of the equipment with high concentration of acid is a major drawback of this hydrolysis technique. So, you need to invest a so much about the equipment.

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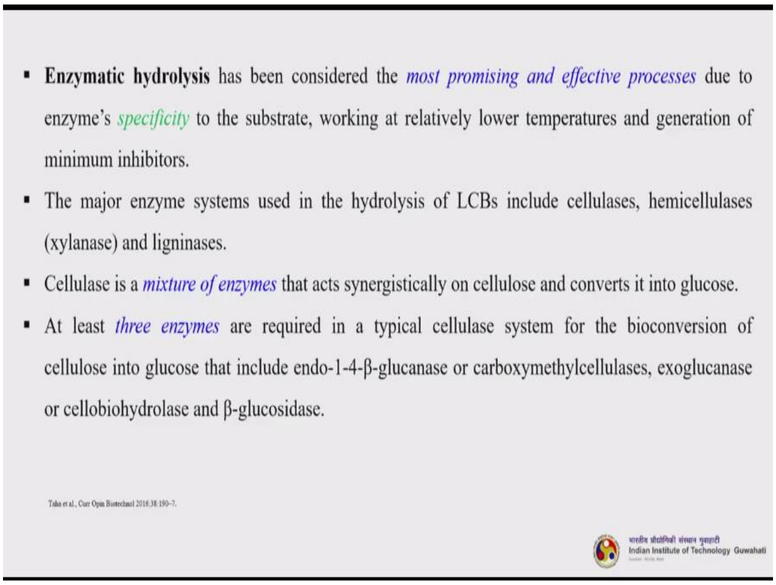

Then the enzymatic hydrolysis. Now, this has been considered as one of the most promising green and effective process due to enzyme specificity to the substrate working at relatively lower temperatures and generation of minimum inhibitors. So, the major enzyme systems used in the hydrolysis of lignocellulosic biomasses include cellulases, hemicellulases such as xylanase and ligninases.

So, cellulase is a mixture, it is a cocktail of enzymes, mixture of enzymes that acts synergistically on cellulose and converts it into glucose. So, you need at least 3 enzymes to make the cellulase cocktail - are required in a typical cellulase system for the bioconversion of cellulose into glucose. So, that include endo 1-4 beta glucanase or carboxymethylcellulases or then exoglucanase or cellobiohydrolase and beta glucosidase. So, these 3 are minimum required.

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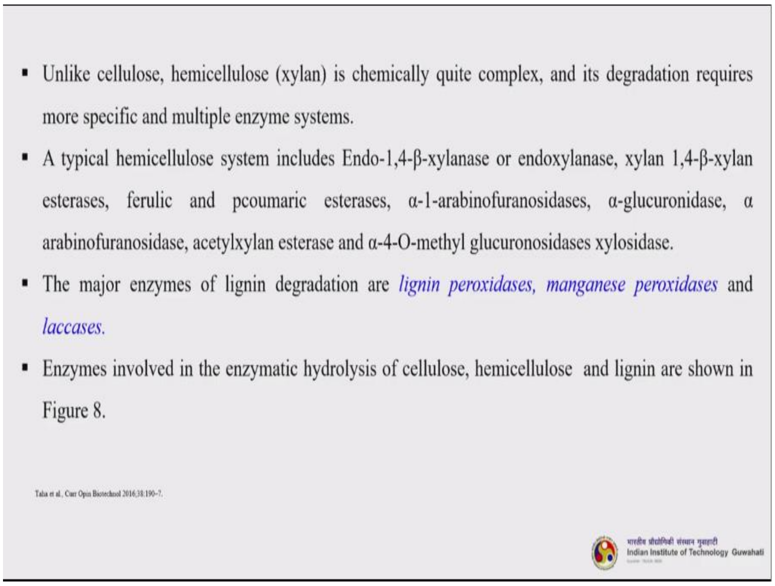

So, unlike cellulose, hemicellulose is chemically quite complex and its degredation requires more specific and multiple enzyme systems. A typical hemicellulose system includes endo 1, 4 beta xylanase or endoxylanase, xylan 1, 4 beta xylan esterases. Then there are so many other enzymes which are being listed here.

Now, the major enzymes of lignin degradation are lignin peroxidases, manganese peroxidases and laccases. So, enzymes involved in the enzymatic hydrolysis of cellulose, hemicellulose and lignin so I have shown, you a picture you can see this.

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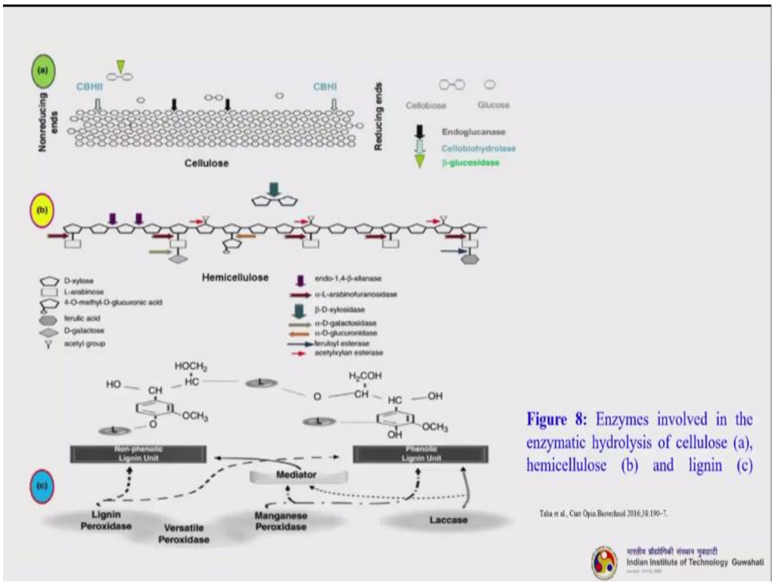

Figure 8: Enzymes involved in the enzymatic hydrolysis of cellulose (a), hemicellulose (b) and lignin (c)

So, these are the different types of enzymes that are involved in the enzymatic hydrolysis of cellulose, hemicellulose and lignin. So, the first one here this is cellulose, here this is hemicellulose structure and this is lignin. You can see different types of enzymes such as endoglucanases, cellobiohydrolase, beta glucosidase for the cellulose hydrolysis. Here for the hemicellulose hydrolysis there are so many different types of enzymes are there and how they are actually targeting.

So, these are components you can understand. So, this particular component corresponds to D xylose which is present in the hemicellulose. Similarly, this small one where the red enzyme is being shown - the red enzyme is basically acetylxylan esterase and this particular component is an acetyl group. And lignin is a very complex structure. So lignin is also being degraded using different enzymes such as lignin peroxidase, then versatile peroxidases, you can have a cocktail also and manganese peroxidase and of course laccases.

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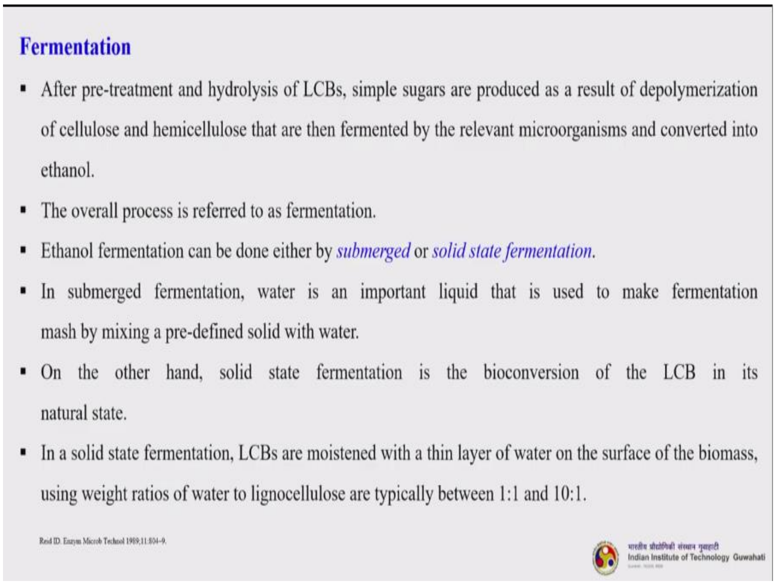

So, then the next is fermentation. So after pretreatment and hydrolysis of the lignocellulosic biomasses, simple sugars are produced as a result of depolymerization of cellulose and hemicellulose that are then fermented by relevant microorganisms and converted into ethanol. The overall process is referred to as the fermentation. Ethanol fermentation can be done either by submerged or solid state fermentation.

And what is the difference we will try to understand. So in submerged fermentation, water is an important liquid that is used to make fermentation mash by mixing a predefined solid with water. On the other hand, solid state fermentation is the bioconversion of the LCB in its natural state. So in a solid state fermentation, LCBs are moistened and with a thin layer of water on the surface of the biomass using weight ratios of water to lignocellulose -

approximately 1 : 1 ratio or 10 : 1 ratio in that, again varies for the different types of biomasses.

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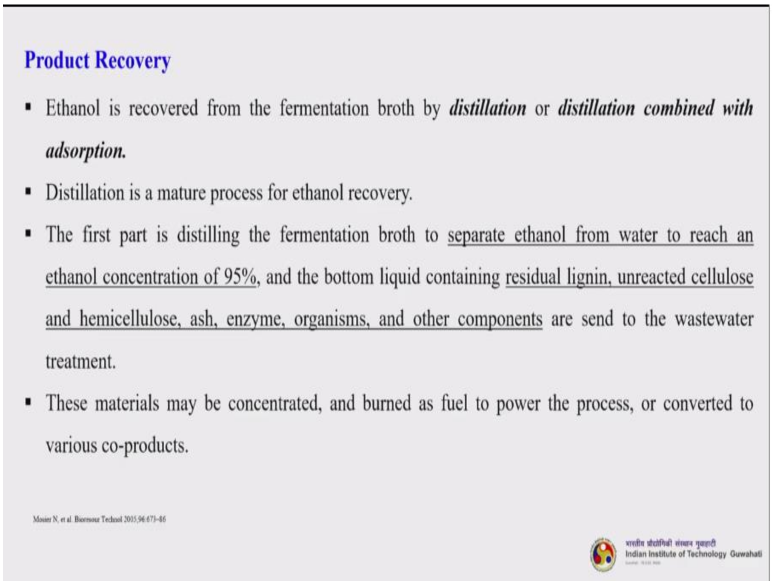

So the next is product recovery which is one of the most important steps. The reason is that this step consumes lot of energy, chemicals and almost 40% of the entire production cost belongs to this particular product recovery strategies. So, there is a lot of work is still going on how to reduce the cost of this particular process, the product recovery process. So, ethanol is recovered from the fermentation broth by distillation or distillation combined with adsorption.

It may be membrane also, now a lot of work has been reported with membrane. So, distillation is a mature process for ethanol recovery The first part is distilling the fermentation broth to separate ethanol from water to reach an ethanol concentration of around 95% and the bottom liquid contains residual lignin, unreacted cellulose and hemicellulose, ash, enzyme, organisms that means the microorganisms and other components and are send to the wastewater treatment.

So, there you can again recover some of the components. These materials may be concentrated and burned as fuel to power the process or converted to various co-products, the bottom part.

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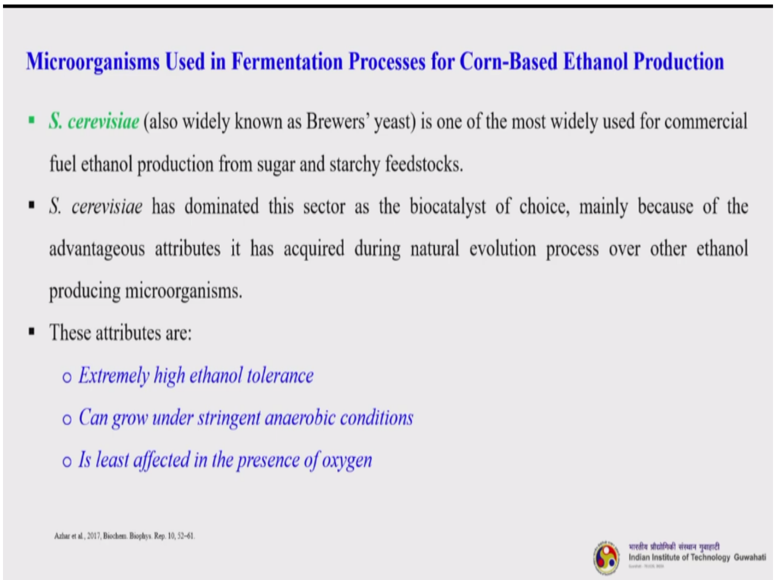

So, we will try to understand the different microorganisms which are required in the fermentation process for the corn based ethanol products. So, the most important is of course the Brewers' yeast which is *Saccharomyces cerevisiae* - the most widely used for commercial fuel ethanol production from sugar and starchy feedstocks. *Saccharomyces cerevisiae* has dominated this sector as the biocatalyst of choice mainly because of the advantageous attributes it has acquired during natural evolution process over the other ethanol producing microorganisms. So, what are these attributes? So, major are extremely high ethanol tolerance, this is the most important part of this *Sacchromyces cerevisiae* use. It can grow under stringent anaerobic conditions, is least affected in the presence of oxygen. So, these are very important attributes.

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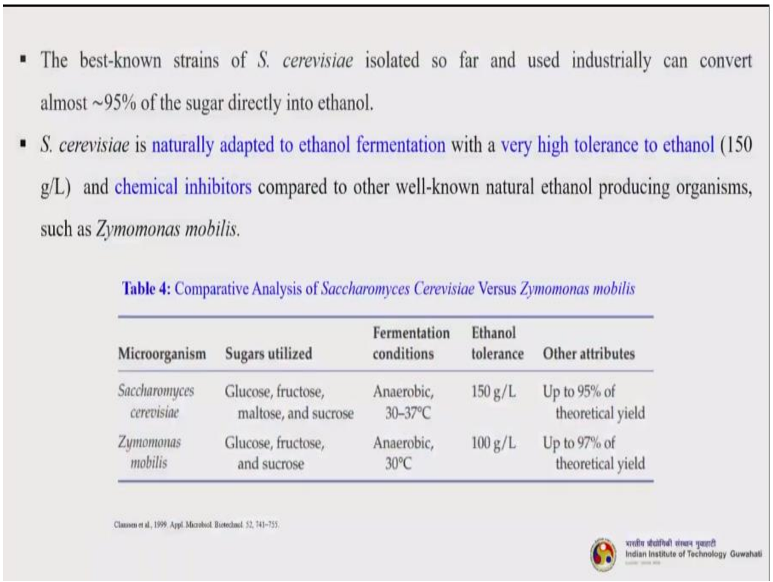

Microorganism	Sugars utilized	Fermentation conditions	Ethanol tolerance	Other attributes
Saccharomyces cerevisiae	Glucose, fructose, maltose, and sucrose	Anaerobic, 30–37°C	150 g/L	Up to 95% of theoretical yield
Zymomonas mobilis	Glucose, fructose, and sucrose	Anaerobic, 30°C	100 g/L	Up to 97% of theoretical yield

So, the best known strains of *Saccharomyces cerevisiae* isolated so far and used industrially can convert almost 95% of the sugar directly into ethanol. So, *Saccharomuces cerevisiae* is a naturally adapted ethanol fermenting microorganism with a very high tolerance to ethanol almost 150 grams per liter and chemical inhibitors compared to other well-known natural ethanol producing organisms such as *Zymomonas mobilis*.

So, you can have a look at this particular table which gives us a comparative analysis of the *Saccharomyces cerevisiae* versus *Zymomonas mobilis*. So, let us see the first one *Saccharomyces cerevisiae*, so the sugars that is utilized you can use any of these glucose, fructose, maltose, sucrose, etc. The fermentation condition is anaerobic almost 30 to 37 degrees centigrade. Ethanol tolerance 150 grams per liter. Other attributes is up to 95% of the theoretical yield.

If you use *Zymomonas mobilis* again the same sort of sugars that it can depolymerize. So, it is anaerobic around 30 degree centigrade, more or less same. Ethanol tolerance is a little less than that of the Saccharomyces cerevisiae around 100 grams per liter, but it can produce up to 97% of the theoretical yield. Now please understand this is theoretically, practically how much is getting produced that depends upon your process optimization and how nicely or how best you can do the process optimization.

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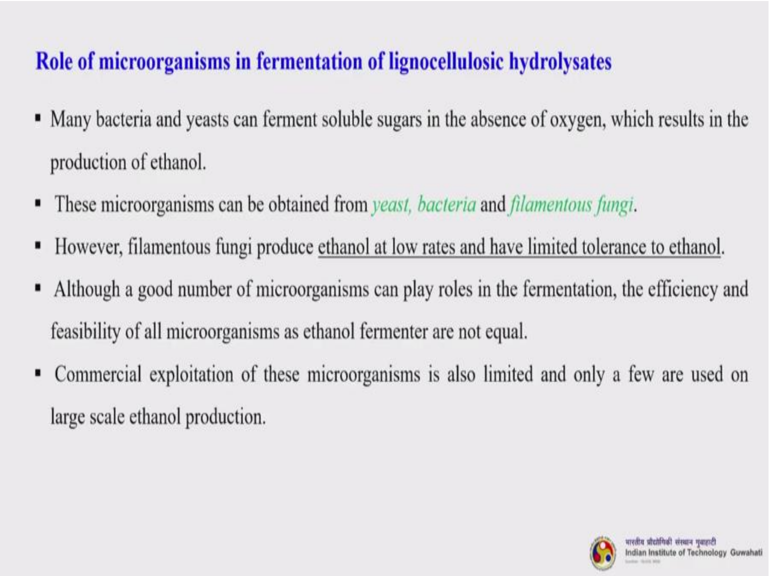

So, we will understand the role of microorganisms in fermentation of lignocellulosic hydrolysates. Many bacteria and yeasts can ferment soluble sugars in the absence of oxygen, which results in the production of ethanol. Now these microorganisms can be obtained from yeast, bacteria and filamentous fungi. However, filamentous fungi produce ethanol at low rates and have limited tolerance to ethanol.

Although a good number of microorganisms can play roles in the fermentation, the efficiency and feasibility of all microorganisms as ethanol fermenter are not equal. Commercial exploitation of these microorganisms is also limited and only a few are used on large scale ethanol production.

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The most common and widely used microorganism for ethanol fermentation is of course yeast which has been proved to be robust and well suited to fermentation of lignocellulosic hydrolysates. It can efficiently ferment 6 carbon sugars but hardly pentoses due to the lack of enzymes that convert xylose to xylulose. The common bacterial species used for ethanol fermentation is a gram negative bacteria which is this *Zymomonas mobilis*.

Some thermophilic anaerobic bacteria such as *Thermoanaerobacter ethanolicus*. *Clostridium*, then *Thermoanaerobacter mathranii*, *Thermoanaerobium brockii*, *Clostridium thermosaccharolyticum*, so these are all investigated for lignocellulosic ethanol production.

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The performance of any microorganism as ethanol fermenter can be evaluated based on the efficiency under the different process conditions. This I was talking about, process optimization condition and all these things. So these are the parameters that need to be optimized. Wide temperature range, pH range, ethanol tolerance, growth rate, ethanol productivity, osmotic tolerance, specificity, ethanol yield, genetic stability and inhibitor tolerance.

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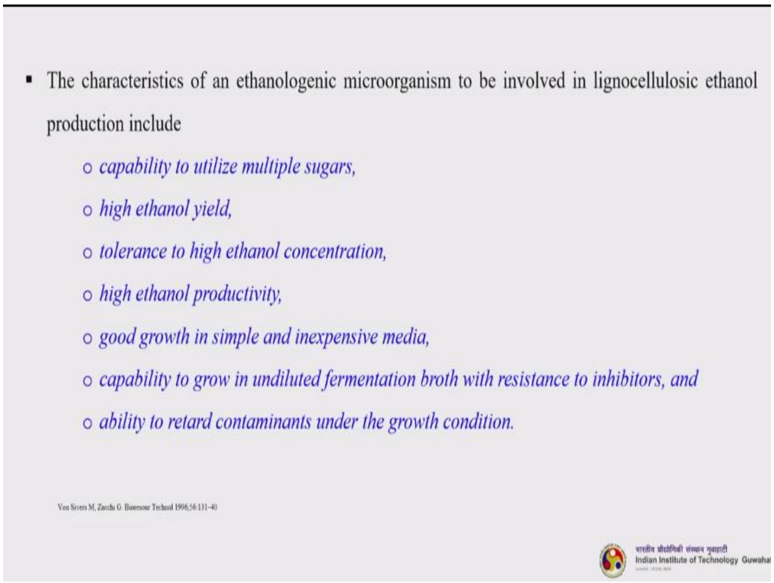

The characteristic of an ethanologenic microorganism to be involved in lignocellulosic ethanol production includes its capability to utilize multiple sugars - that is the very important thing because when you talk about LCB, we talk about cellulose, hemicellulose both. We want to convert both these sugars. So, then high ethanol yield. It must tolerate to high ethanol concentration.

It should have high ethanol productivity. Good growth in simple and inexpensive media. Capability to grow in undiluted fermentation broth with resistance to inhibitors and ability to retard contaminants under the growth condition.

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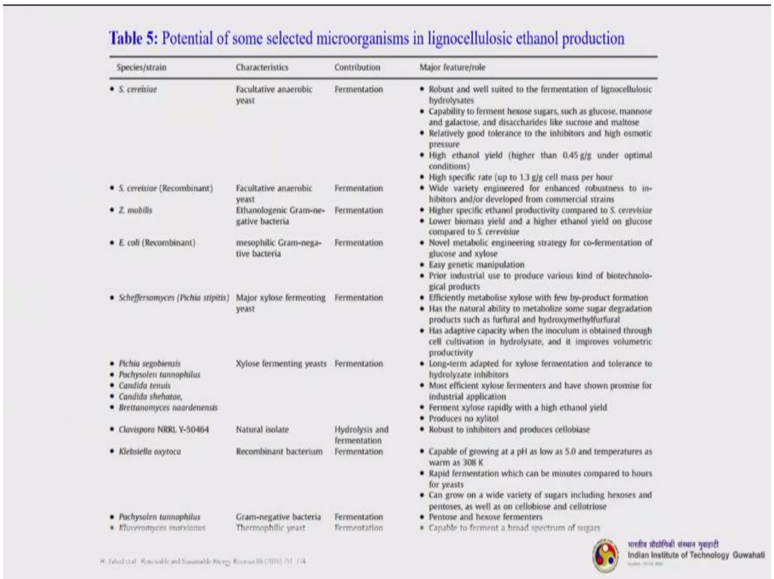

Table 5: Potential of some selected microorganisms in lignocellulosic ethanol production

Species/strain	Characteristics	Contribution	Major feature/role
• *S. cereisiae*	Facultative anaerobic yeast	Fermentation	• Robust and well suited to the fermentation of lignocellulosic hydrolysates • Capability to ferment hexose sugars, such as glucose, mannose and galactose, and disaccharides like sucrose and maltose • Relatively good tolerance to the inhibitors and high osmotic pressure • High ethanol yield (higher than 0.45 g/g under optimal conditions) • High specific rate (up to 1.3 g/g cell mass per hour
• *S. cereisiae* (Recombinant)	Facultative anaerobic yeast	Fermentation	• Wide variety engineered for enhanced robustness to inhibitors and/or developed from commercial strains
• *Z. mobilis*	Ethanologenic Gram-negative bacteria	Fermentation	• Higher specific ethanol productivity compared to *S. cerevisiae* • Lower biomass yield and a higher ethanol yield on glucose compared to *S. cerevisiae*
• *E. coli* (Recombinant)	mesophilic Gram-negative bacteria	Fermentation	• Novel metabolic engineering strategy for co-fermentation of glucose and xylose • Easy genetic manipulation • Prior industrial use to produce various kind of biotechnological products
• *Scheffersomyces (Pichia stipitis)*	Major xylose fermenting yeast	Fermentation	• Efficiently metabolise xylose with few by-product formation • Has the natural ability to metabolize some sugar degradation products such as furfural and hydroxymethylfurfural • Has adaptive capacity when the inoculum is obtained through cell cultivation in hydrolysate, and it improves volumetric productivity
• *Pichia segobiensis* • *Pachysolen tannophilus* • *Candida tenuis* • *Candida shehatae*, • *Brettanomyces naardenensis*	Xylose fermenting yeasts	Fermentation	• Long-term adapted for xylose fermentation and tolerance to hydrolyzate inhibitors • Most efficient xylose fermenters and have shown promise for industrial application • Ferment xylose rapidly with a high ethanol yield • Produces no xylitol
• *Clavispora* NRRL Y-50464	Natural isolate	Hydrolysis and fermentation	• Robust to inhibitors and produces cellobiase
• *Klebsiella oxytoca*	Recombinant bacterium	Fermentation	• Capable of growing at a pH as low as 5.0 and temperatures as warm as 308 K • Rapid fermentation which can be minutes compared to hours for yeasts • Can grow on a wide variety of sugars including hexoses and pentoses, as well as on cellobiose and cellotriose
• *Pachysolen tannophilus*	Gram-negative bacteria	Fermentation	• Pentose and hexose fermenters
• *Kluveromyces marxianus*	Thermophilic yeast	Fermentation	• Capable to ferment a broad spectrum of sugars

So, this particular table will tell you about the potential of some of the selected microorganisms in lignocellulosic biethanol production. So, you can have a look at it later. The font sizes are very small. So, if you take a screenshot and then you can read later on. So, all species are listed here, mostly which had been used commercially also. *Saccharomyces cerevisiae*, then recombinant *Saccharomyces cerevisiae* that means genetically modified.

Zymomonas mobilis, *E. coli*, recombinant *E. coli* and there are many others also and their characteristics features, their contribution, where it is being used, only fermentation or fermenters plus hydrolysis both. And then what is the major feature and role.

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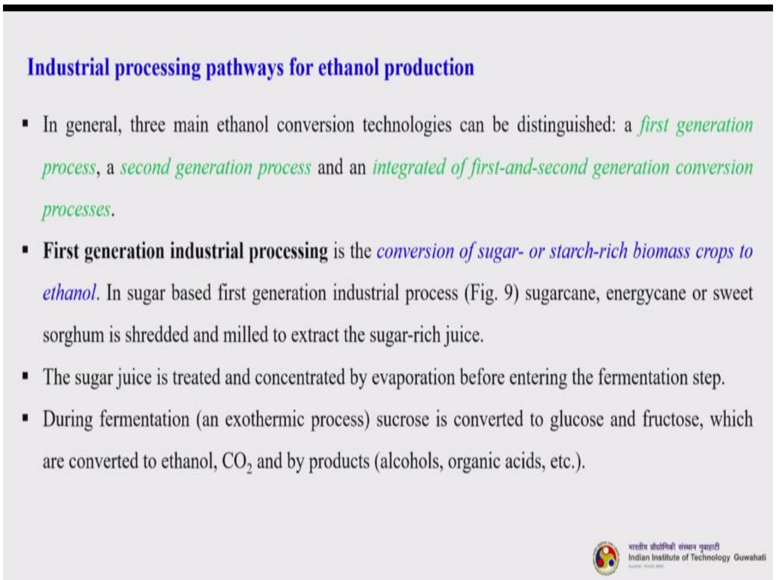

So, then let us discuss about the industrial processing pathways for the ethanol production. So, in general three main ethanol conversion technologies can be distinguished. So, the first one is the first generation process, then second generation process and the integrated of first and second generation conversion process. If you recall, the biofuels have also been known as like that. First generation biofuels, second generation biofuel, third generation biofuels, all these things.

So the first generation industrial processing is the conversion of sugar and starch rich biomass crops to ethanol. In sugar based first generation industrial processes, I will show you the figures later in the next slide, sugarcane, energycane or sweets sorghum is shredded and milled to extract the sugar rich juice. The sugar juice is treated and concentrated by evaporation before entering the fermentation step.

During fermentation which is an exothermic process, sucrose is converted to glucose and fructose which are converted to ethanol, carbon dioxide and byproducts like alcohols, organic acids, etc.

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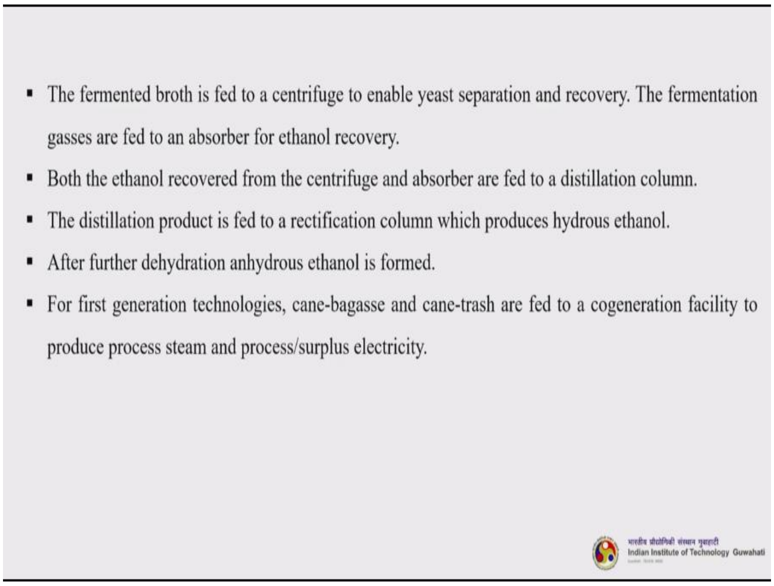

So, the fermented broth is fed to a centrifuge to enable yeast separation and recovery. The fermentation gases are fed to an absorber for ethanol recovery. Both the ethanol recovered from the centrifuge and absorber are fed to a distillation column. So, actually the similar process of saccharification followed by fermentation, distillation and the usual procedure. The distillation product is fed to a rectification column which produces hydrous ethanol.

After further dehydration anhydrous ethanol is formed. So, for first generation technologies, cane bagasse and cane trash are fed to a cogeneration facility to produce process steam and process surplus electricity. So in a closed loop birefinery concept.

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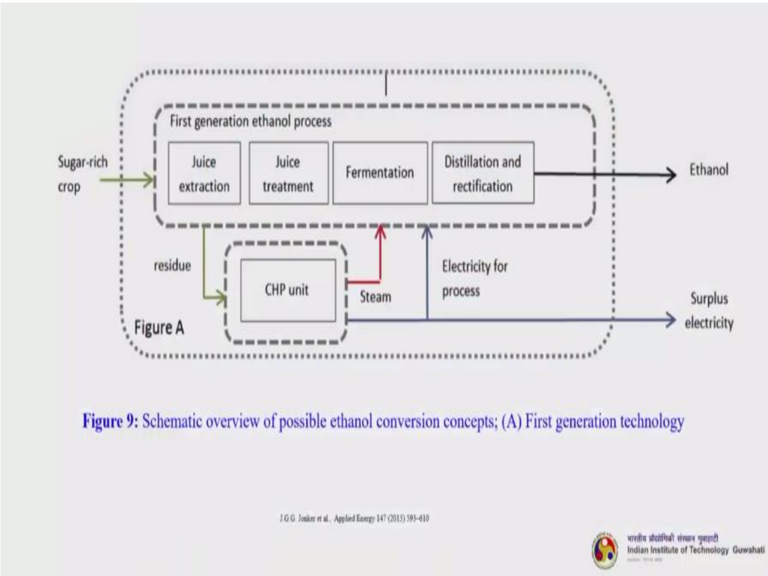

So, this is the schematic of the possible ethanol conversion concepts for the first generation technology. Quickly we will go through this. So, the sugary rich crops like sugarcane and any other starchy crops. So, you extract the juice and the juice is getting treated. Then fermented, distillation and rectification, So, you get ethanol. So, whatever the residue is coming here it goes to the combined heat generation process, heat and power generation unit.

So, you get a steam. The steam is being used for other processes like distillation and all. And then you can also have electricity. And whatever surplus electricity after you use it in the entire scheme can also be send to your grid or can be sold out.

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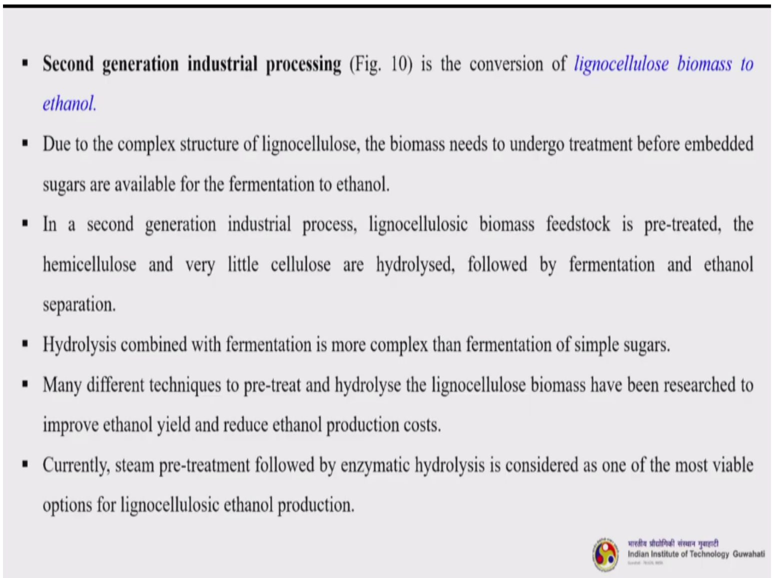

So, the next is the second generation industrial processing. So, here the conversion of lignocellulosic biomass to ethanol happens. So, due to the complex structure of lignocellulose, the biomass needs to undergo treatment for embedded sugars that are available for the fermentation to ethanol. In a second generation industrial process, lignocellulosic biomass feedstock is pretreated and the hemicellulose and very little cellulose are hydrolyzed followed by fermentation and ethanol separation.

Hydrolysis combined with fermentation is more complex than fermentation of simple sugars. Many different techniques to pretreat and hydrolyse the lignocellulose biomass have been researched to improve ethanol yield and to reduce ethanol production cost. Currently, steam pretreatment followed by enzymatic hydrolysis is considered as one of the most viable options for lignocellulosic ethanol production.

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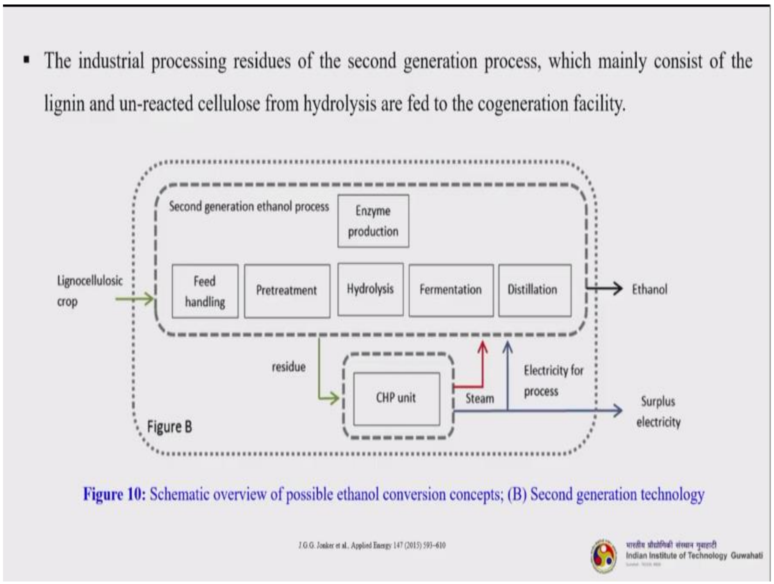

So, this is the scheme actually. So the feed handling, so basically you go for pretreatment and all these things. In mechanical preprocessing, then you go for the pretreatment. Any one the pretreatment process that we have already discussed. Hydrolysis, fermentation, distillation, the common steps. Then whatever the residue, solid residue it goes to a CHP unit. Again steam is being used here, electricity being used here, whatever produced surplus electricity can be taken back and sold out.

One of the important feature here is of course the enzyme that is required - you can have to produce it in a mass or large scale. So, the industrial processing residues of the second generation process which mainly consists of lignin and the unreacted cellulose from hydrolysis are fed to the cogeneration facility. So, the CHP unit basically.

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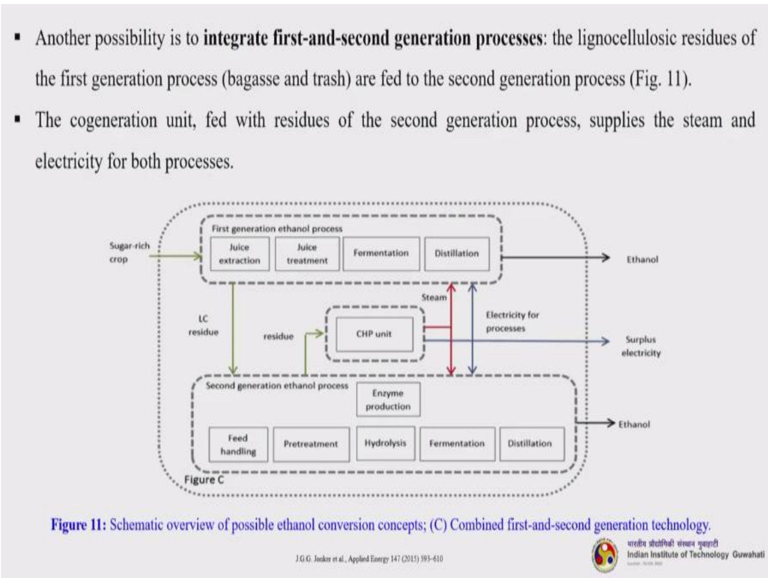

So, another possibility is to integrate first-and-second generation process that is the lignocellulosic residues of the first generation process as for example your bagasse and trash are fed to the second generation process. How it happens? So, whatever it is the lignocellulosic residues coming from the first generation - this is the first generation, this is the second generation - are being fed to the second generation process because they are lignocellulosic rich component. And the entire rest process schemes are same.

So, the cogeneration unit fed with residues of the second generation process supplies the steam and electricity for both the processes. So, you have a basically a combined CHP unit, single CHP unit. This is the advantage of combining or integrating both the first generation and second generation that you can have a single CHP unit which will produce enough steam and electricity which will be sufficient for managing both first generation and second generation processes together.

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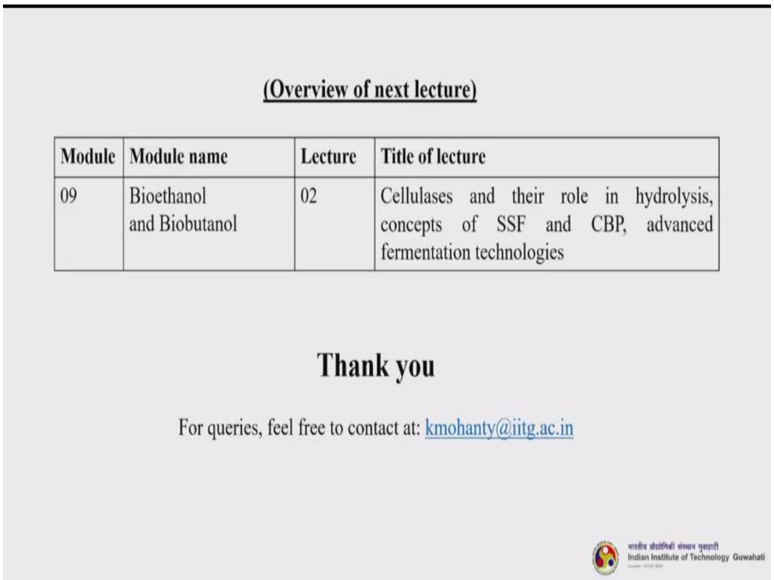

Module	Module name	Lecture	Title of lecture
09	Bioethanol and Biobutanol	02	Cellulases and their role in hydrolysis, concepts of SSF and CBP, advanced fermentation technologies

So, I think with this I conclude today's lecture. In case if you have any query, please register it in the Swayam portal and I will answer it. You can also drop a mail to me directly at kmohanty@iitg.ac.in. So, thank you very much. And in the next class, we will be discussing about cellulases - that is the enzymes and their role in hydrolysis, concepts of SSF - simultaneous saccharification and fermentation and CBP - the consulated bioprocessing and then advanced fermentation technologies. Thank you.