Biomass Conversion and Biorefinery
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Lecture 10
Barriers and Types

Good morning students. Today's class is lecture 1 under module 4 and in this module we will be discussing about the Biomass pretreatment. So, this is one of the most important aspect of the entire Biomass conversion or biorefinery concept. So, in today's class we will discuss about what are the barriers that exist for the lignocellulosic Biomass conversion and what are the different types of pretreatment technologies that are commercially adapted one by one.

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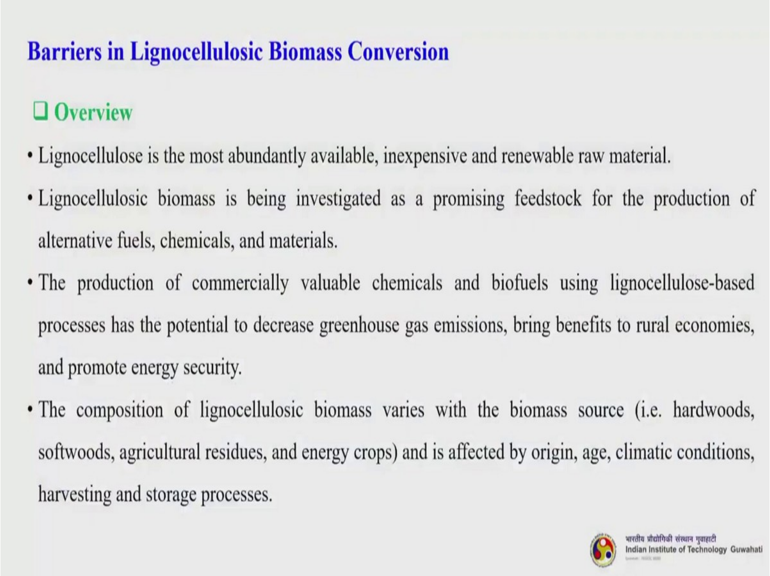

Barriers in Lignocellulosic Biomass Conversion

❑ Overview

- Lignocellulose is the most abundantly available, inexpensive and renewable raw material.
- Lignocellulosic biomass is being investigated as a promising feedstock for the production of alternative fuels, chemicals, and materials.
- The production of commercially valuable chemicals and biofuels using lignocellulose-based processes has the potential to decrease greenhouse gas emissions, bring benefits to rural economies, and promote energy security.
- The composition of lignocellulosic biomass varies with the biomass source (i.e. hardwoods, softwoods, agricultural residues, and energy crops) and is affected by origin, age, climatic conditions, harvesting and storage processes.

Lignocellulose is the most abundantly available, inexpensive and renewable raw material. Lignocellulosic Biomass is being investigated as a promising feedstock for the production of alternative fuels, Chemicals and materials. The production of commercially valuable Chemicals and biofuels using lignocellulose based processes has the potential to decrease Greenhouse gas emissions, bring benefits to rural economy, and promote energy security.

The composition of lignocellulosic biomass varies with biomass source. So hard wood, soft wood, agriculture residue, energy crops, municipal solid waste all these things and is affected by the origin, age, climatic conditions, harvesting and storage processes. Some of these things we already know; during our subsequent classes we have discussed this.

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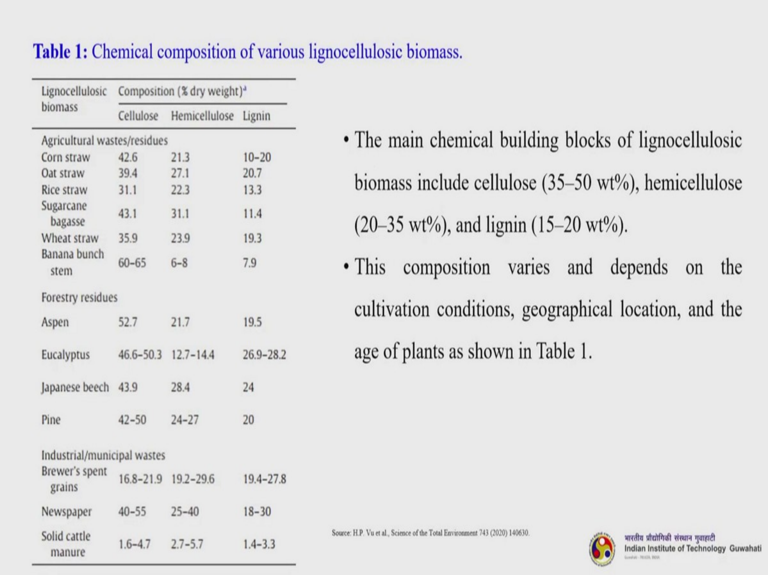

Table 1: Chemical composition of various lignocellulosic biomass.

Lignocellulosic biomass	Composition (% dry weight)[a]		
	Cellulose	Hemicellulose	Lignin
Agricultural wastes/residues			
Corn straw	42.6	21.3	10–20
Oat straw	39.4	27.1	20.7
Rice straw	31.1	22.3	13.3
Sugarcane bagasse	43.1	31.1	11.4
Wheat straw	35.9	23.9	19.3
Banana bunch stem	60–65	6–8	7.9
Forestry residues			
Aspen	52.7	21.7	19.5
Eucalyptus	46.6–50.3	12.7–14.4	26.9–28.2
Japanese beech	43.9	28.4	24
Pine	42–50	24–27	20
Industrial/municipal wastes			
Brewer's spent grains	16.8–21.9	19.2–29.6	19.4–27.8
Newspaper	40–55	25–40	18–30
Solid cattle manure	1.6–4.7	2.7–5.7	1.4–3.3

- The main chemical building blocks of lignocellulosic biomass include cellulose (35–50 wt%), hemicellulose (20–35 wt%), and lignin (15–20 wt%).
- This composition varies and depends on the cultivation conditions, geographical location, and the age of plants as shown in Table 1.

Source: H.P. Vu et al., Science of the Total Environment 743 (2020) 140630.

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Now, please have a look at this particular table, where the chemical composition of various lignocellulosic Biomass has been listed and the source has also been given. You can later on see. It is basically listed under three different categories: cellulose, hemicellulose and Lignin. Please understand that, apart from these there are some other materials also, there are proteins, there are volatile materials, ash all these things.

The main chemical building blocks of lignocellulosic biomass that includes actually cellulose - almost about 35 to 50%, hemicellulose - 20 to 35% and Lignin which vary from 15 to 20%. Now the composition varies and depends on cultivation conditions, geographical location and the age of plants. Now let us see, this corn straw or oat straw. You see this it is almost 39-42 in that range is the cellulose, and hemicellulose is from 27-21 in that range, and Lignin is 10 to 20 in that range.

Now, you come to bagasse, here the cellulose little more. Now you come to Aspen (this forestry residues) cellulose amount is increased basically, there are so many things. Now if you come to solid cattle manure, you can see the cellulose, hemicellulose and lignin all the three major components are very less. There are other components which are more predominantly in that particular material.

So likewise, the understanding is that, the cellulose, hemicellulose and Lignin varies apart from other materials. And the process or the technologies for converting this Biomass, including of course, the pre-treatment technologies, should be developed in such a way that any process can consider or can take the various multiple feedstocks rather than developing a process for a single or two feedstocks.

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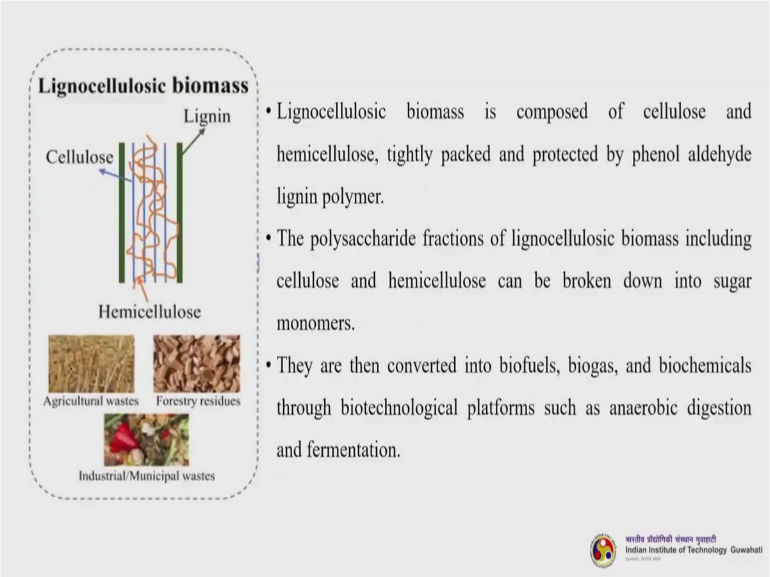

Lignocellulosic biomass

- Lignocellulosic biomass is composed of cellulose and hemicellulose, tightly packed and protected by phenol aldehyde lignin polymer.
- The polysaccharide fractions of lignocellulosic biomass including cellulose and hemicellulose can be broken down into sugar monomers.
- They are then converted into biofuels, biogas, and biochemicals through biotechnological platforms such as anaerobic digestion and fermentation.

Please have a look at this particular slide. You see that, one class long back I think we have discussed how the cellulose, hemicellulose and Lignin actually intricately bind together. This again it is telling, you please see that the cellulose is the blue part. Lignin is the green part, and the hemicellulose is the red part or the orange whatever it is will be visible from your side. So these are intricately bound together. So that is the reason why we are talking about in today's class pre-treatment.

Why the pre-treatment is required? Because I want or we want this cellulose and hemicellulose to be disintegrated. Because it is bound together - cellulose, hemicellulose and Lignin all are bound together and you need to disintegrate them. Then you need to take out the cellulose and hemicellulose part. We will go for hydrolysis, we will get C6 and C5 sugar, then that will be fermented to Bioethanol, alcohol platforms and other chemicals.

Now lignocellulosic Biomass is composed of cellulose and hemicellulose, tightly packed and protected by phenol aldehyde Lignin polymer. And the polysaccharide fractions of lignocellulosic biomass, including cellulose and hemicellulose can be broken down into sugar monomers. They are then converted into biofuels, biogas and biochemicals through biotechnological platforms such as anaerobic digestion and fermentation.

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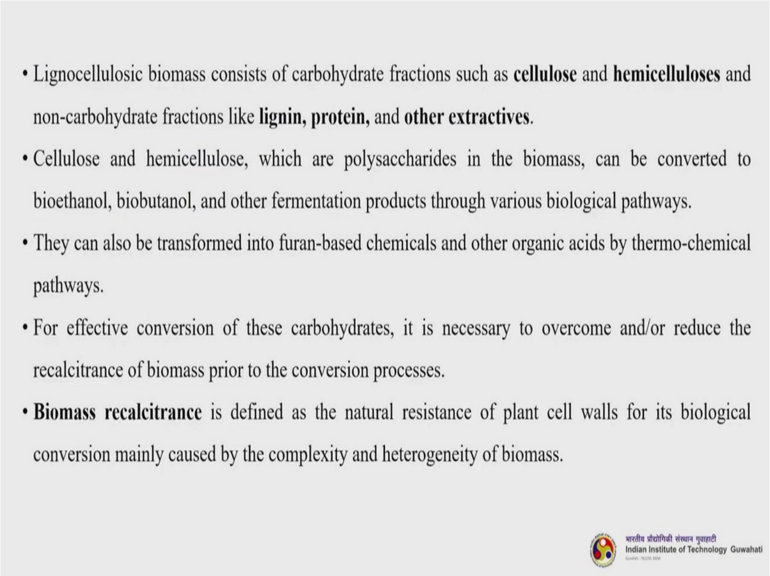

- Lignocellulosic biomass consists of carbohydrate fractions such as **cellulose** and **hemicelluloses** and non-carbohydrate fractions like **lignin, protein,** and **other extractives**.
- Cellulose and hemicellulose, which are polysaccharides in the biomass, can be converted to bioethanol, biobutanol, and other fermentation products through various biological pathways.
- They can also be transformed into furan-based chemicals and other organic acids by thermo-chemical pathways.
- For effective conversion of these carbohydrates, it is necessary to overcome and/or reduce the recalcitrance of biomass prior to the conversion processes.
- **Biomass recalcitrance** is defined as the natural resistance of plant cell walls for its biological conversion mainly caused by the complexity and heterogeneity of biomass.

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Lignocellulosic Biomass consists of carbohydrate fraction such as cellulose and hemicellulose and non-carbohydrate functions like lignin, protein and other extractives or volatile matters. So, cellulose and hemicellulose which are polysaccharides in the biomass can be converted to bioethanol, biobutanol and other fermentation products through various biological pathways. They can also be transformed into furan based chemicals and other organic acids by thermochemical pathways. Either it is pyrolysis and gasification or any such processes.

For effective conversion of these carbohydrates, it is necessary to overcome and/or reduce the recalcitrance of biomass prior to the conversion process. Bio recalcitrance is defined as the natural resistance of plant cell walls for its biological conversion mainly caused by the complexity and heterogeneity of biomass.

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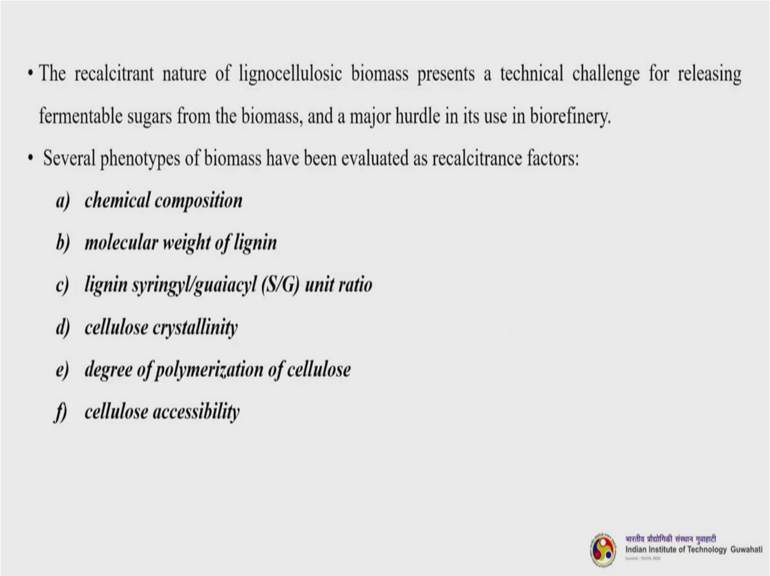

- The recalcitrant nature of lignocellulosic biomass presents a technical challenge for releasing fermentable sugars from the biomass, and a major hurdle in its use in biorefinery.
- Several phenotypes of biomass have been evaluated as recalcitrance factors:
  - *a) chemical composition*
  - *b) molecular weight of lignin*
  - *c) lignin syringyl/guaiacyl (S/G) unit ratio*
  - *d) cellulose crystallinity*
  - *e) degree of polymerization of cellulose*
  - *f) cellulose accessibility*

The recalcitrant nature of lignocellulosic biomass presents a technical challenge for releasing fermentable sugars from the Biomass and a major hurdle in its use in biorefinery. Several phenotypes of biomass have been evaluated as recalcitrance factors. So, some of these are listed here. First is the chemical composition and second is the molecular weight of lignin, lignin syringyl or guaiacyl unit (we will discuss about this later on little), Cellulose crystallinity, then degree of polymerization of cellulose and cellulose accessibility.

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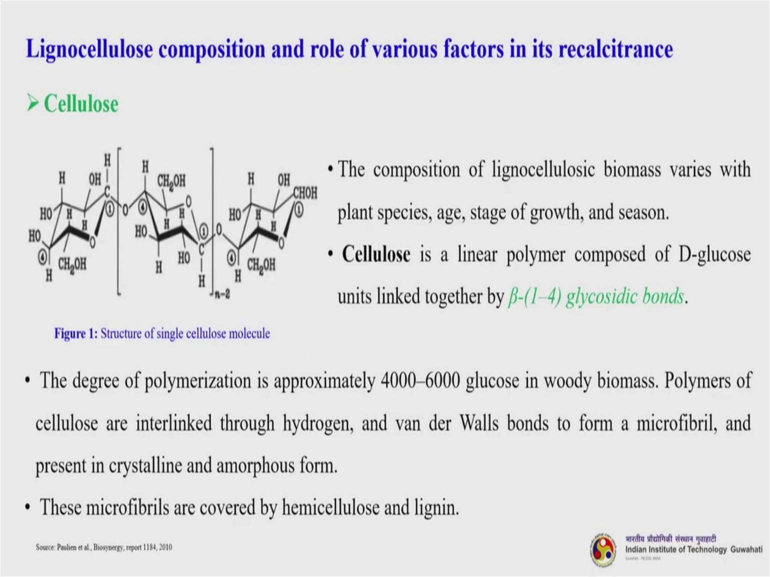

Lignocellulose composition and role of various factors in its recalcitrance

➤ Cellulose

Figure 1: Structure of single cellulose molecule

- The composition of lignocellulosic biomass varies with plant species, age, stage of growth, and season.
- **Cellulose** is a linear polymer composed of D-glucose units linked together by *β-(1–4) glycosidic bonds*.
- The degree of polymerization is approximately 4000–6000 glucose in woody biomass. Polymers of cellulose are interlinked through hydrogen, and van der Walls bonds to form a microfibril, and present in crystalline and amorphous form.
- These microfibrils are covered by hemicellulose and lignin.

Source: Paulien et al., Biosynergy, report 1184, 2010

Let us talk about cellulose. So we will try to understand what are the various components, and their composition, and their role in the recalcitrant nature of the lignocellulosic biomass. Now you know cellulose is one of the most abundantly available material on the earth (natural material). So, the composition of lignocellulosic biomass varies with plant species, age, stage of growth and season; this is well understood.

Cellulose is a linear polymer composed of D-glucose units linked together by the *β-(1-4) glycosidic bonds*. This is a classical structure of the single cellulose molecule. The degree of polymerization is approximately 4000 to 6000 glucose in Woody Biomass. Polymers of cellulose are interlinked through hydrogen and van der Waals bond to form a microfibril and present in crystalline and amorphous form. These microfibrils are covered by hemicellulose and Lignin and intricately actually pegged.

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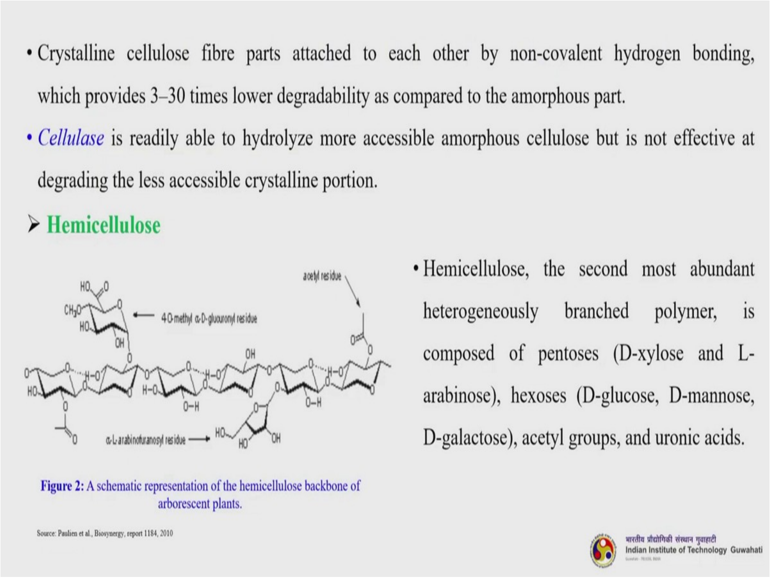

- Crystalline cellulose fibre parts attached to each other by non-covalent hydrogen bonding, which provides 3–30 times lower degradability as compared to the amorphous part.
- *Cellulase* is readily able to hydrolyze more accessible amorphous cellulose but is not effective at degrading the less accessible crystalline portion.

➢ **Hemicellulose**

Figure 2: A schematic representation of the hemicellulose backbone of arborescent plants.

Source: Paulien et al., Biosynergy, report 1184, 2010

- Hemicellulose, the second most abundant heterogeneously branched polymer, is composed of pentoses (D-xylose and L-arabinose), hexoses (D-glucose, D-mannose, D-galactose), acetyl groups, and uronic acids.

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So, crystalline cellulose fibre parts attached to each other by non-covalent hydrogen bonding, which provides 3 to 30 times lower degradability as compared to the amorphous part. Now cellulase, that is the enzyme which will degrade cellulose, is readily able to hydrolyse more accessible amorphous cellulose but it is not effective at degrading the less accessible crystalline portion. That is the reason why we are going for pre-treatment.

Now, let us understand hemicellulose. So, this is a classical structure of hemicellulose molecule. So, hemicellulose, the second most abundant heterogeneously branched polymer, is composed of pentoses (D-xylose and L-arabinose), hexoses (such as D-Glucose, D-mannose and D-galactose), apart from these sugar units, there are certain acetyl groups and uronic acids.

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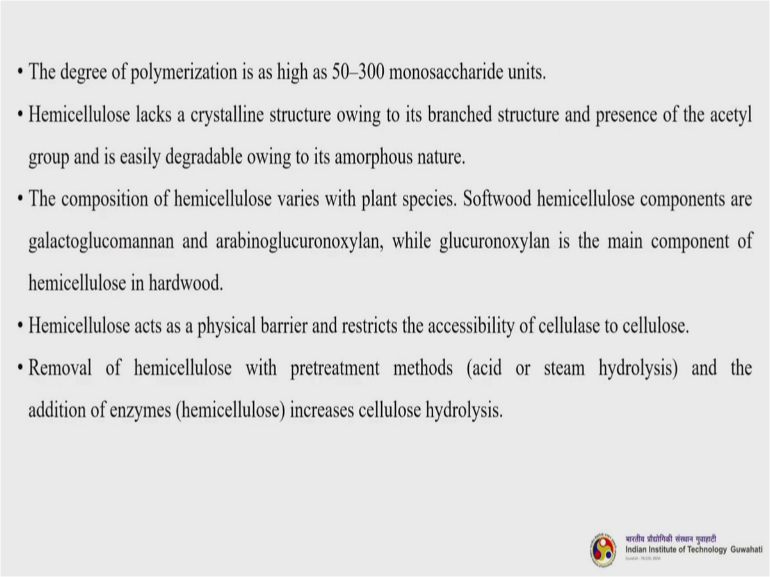

- The degree of polymerization is as high as 50–300 monosaccharide units.
- Hemicellulose lacks a crystalline structure owing to its branched structure and presence of the acetyl group and is easily degradable owing to its amorphous nature.
- The composition of hemicellulose varies with plant species. Softwood hemicellulose components are galactoglucomannan and arabinoglucuronoxylan, while glucuronoxylan is the main component of hemicellulose in hardwood.
- Hemicellulose acts as a physical barrier and restricts the accessibility of cellulase to cellulose.
- Removal of hemicellulose with pretreatment methods (acid or steam hydrolysis) and the addition of enzymes (hemicellulose) increases cellulose hydrolysis.

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So, the degree of polymerization is as high as 50 - 300 monosaccharide units. Hemicellulose lacks a crystalline structure owing to its branched structure and the presence of acetyl group and is easily degradable owing to its amorphous nature. This is the basic structural difference between cellulose and hemicellulose. Where cellulose is more recalcitrant in nature and hemicellulose is little less than cellulose in recalcitrance.

So, the composition of hemicellulose varies with plant species. Soft wood hemicellulose components are galactoglucomannan and arabinoglucuronoxylan, while glucuronoxylan is the main component of hemicellulose in hardwood. Hemicellulose acts as a physical barrier and restricts the accessibility of cellulase to cellulose. Removal of hemicellulose with pretreatment methods such as acid or steam hydrolysis and the addition of enzymes increases the cellulose hydrolysis.

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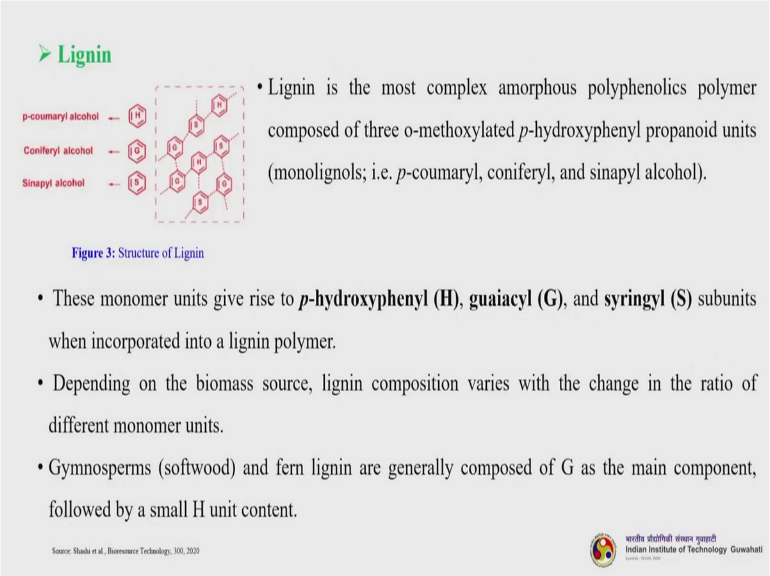

Lignin

- Lignin is the most complex amorphous polyphenolics polymer composed of three o-methoxylated *p*-hydroxyphenyl propanoid units (monolignols; i.e. *p*-coumaryl, coniferyl, and sinapyl alcohol).

Figure 3: Structure of Lignin

- These monomer units give rise to ***p*-hydroxyphenyl (H)**, **guaiacyl (G)**, and **syringyl (S)** subunits when incorporated into a lignin polymer.
- Depending on the biomass source, lignin composition varies with the change in the ratio of different monomer units.
- Gymnosperms (softwood) and fern lignin are generally composed of G as the main component, followed by a small H unit content.

Source: Shashi et al., Bioresource Technology, 300, 2020

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So then Lignin. Lignin is the most complex amorphous polyphenolic polymer composed of three o-methoxylated p-hydroxypenyl propanoid units (which are known as a monolignols as for example of p-coumaryl, coniferyl and sinapyl alcohol). So, you can see the structure of the p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol and that of the lignin. So, these monomer units give rise to p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) subunits when incorporated into a lignin polymer.

So, this H, G and S they are intricately bound together and linked together to form the complex lignin molecular structure. So, depending on the biomass source, lignin composition varies with the change in the ratio of different monomer units. So, gymnosperms or you can say that soft wood plants and Fern Lignin are generally composed of G as the main component followed by a small amount of H unit.

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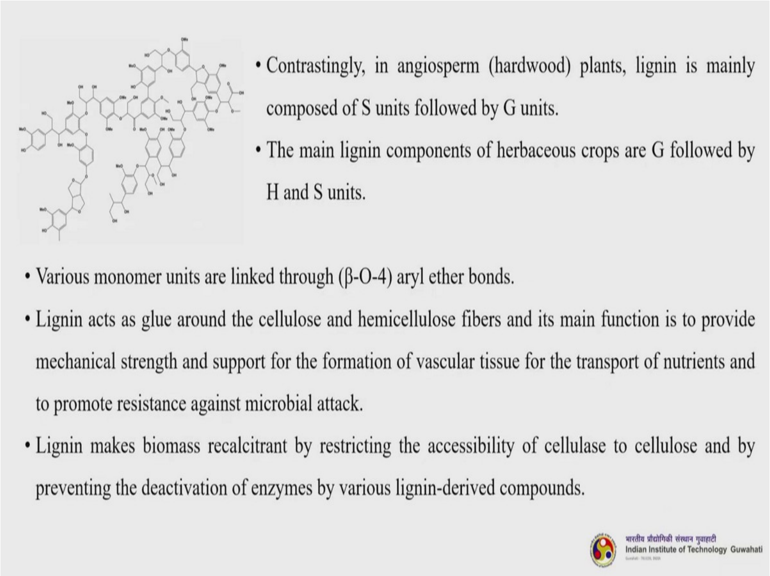

- Contrastingly, in angiosperm (hardwood) plants, lignin is mainly composed of S units followed by G units.
- The main lignin components of herbaceous crops are G followed by H and S units.
- Various monomer units are linked through (β-O-4) aryl ether bonds.
- Lignin acts as glue around the cellulose and hemicellulose fibers and its main function is to provide mechanical strength and support for the formation of vascular tissue for the transport of nutrients and to promote resistance against microbial attack.
- Lignin makes biomass recalcitrant by restricting the accessibility of cellulase to cellulose and by preventing the deactivation of enzymes by various lignin-derived compounds.

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Now contrastingly in angiosperm that is hard plants, Lignin is mainly composed of S units followed by G units. So more of S followed by less of G. So, the main lignin components of herbaceous crops are G followed by H and S. So, from this statement we will understand basically that this H, S and G, depending up on how much amount they are present, the softness or hardness is basically decided.

So various monomer units are linked through this β-O-4 aryl ether bonds and lignin acts as glue around the cellulose and hemicellulose fibres. And its main function is to provide mechanical strength and support for the formation of vascular tissue for the transport of nutrients and to promote resistance against microbial attack. This is the main function of Lignin in plants. And Lignin makes Biomass recalcitrant by restricting the accessibility of cellulase enzyme to cellulose and by preventing the deactivation of enzymes by various lignin derived compounds.

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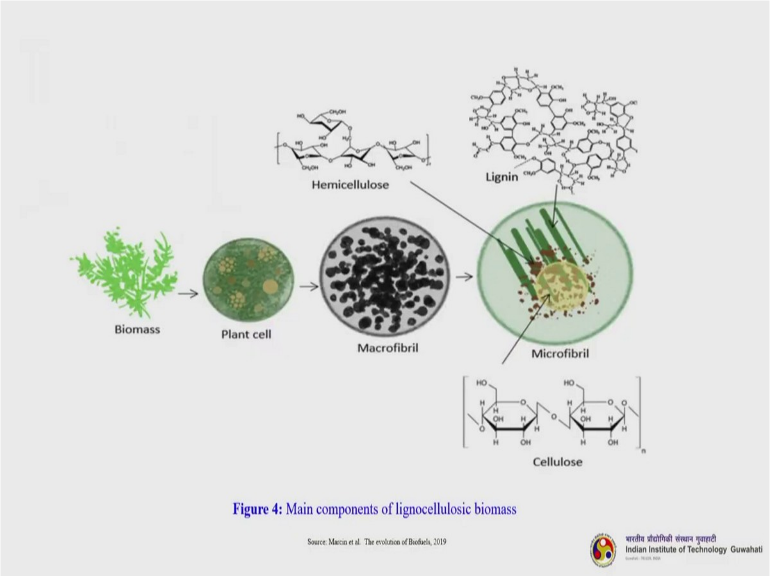

Figure 4: Main components of lignocellulosic biomass

Source: Marcin et al. The evolution of Biofuels, 2019

This is the figure which will tell about this main component of lignocellulosic biomass, how intricately they are actually bound together. So, the Biomass, this is a typical plant cell microscopic image. This is the Macro fibril and this is the microfibrils. In the microfibril you can see that how Lignin, which is this rod shaped green shown here, then cellulose and hemicellulose (this brown portion), so you can understand that, that looks like scattered but they are intricately bound together with each other.

So, we need to actually disintegrate this entire structure. So that we get cellulose and of course hemicellulose also.

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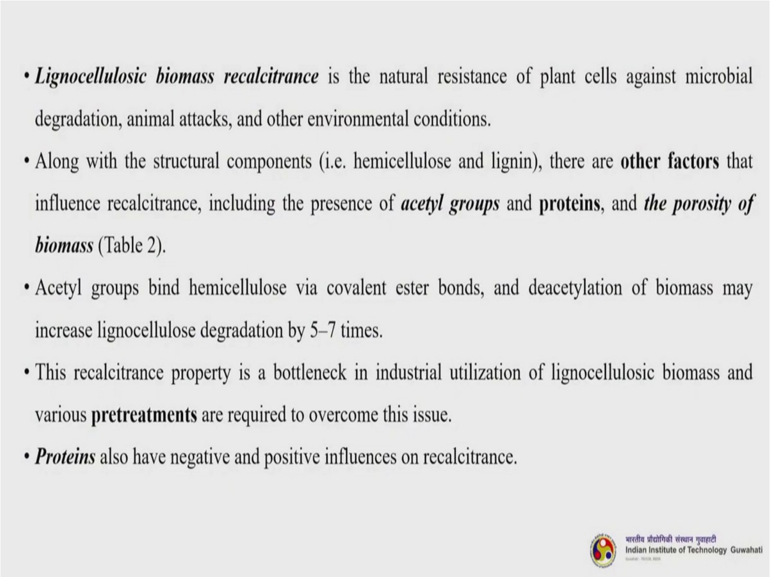

- ***Lignocellulosic biomass recalcitrance*** is the natural resistance of plant cells against microbial degradation, animal attacks, and other environmental conditions.
- Along with the structural components (i.e. hemicellulose and lignin), there are **other factors** that influence recalcitrance, including the presence of ***acetyl groups*** and **proteins**, and ***the porosity of biomass*** (Table 2).
- Acetyl groups bind hemicellulose via covalent ester bonds, and deacetylation of biomass may increase lignocellulose degradation by 5–7 times.
- This recalcitrance property is a bottleneck in industrial utilization of lignocellulosic biomass and various **pretreatments** are required to overcome this issue.
- ***Proteins*** also have negative and positive influences on recalcitrance.

Lignocellulosic biomass recalcitrance is the natural resistance of plant cells against microbial, degradation, animal attacks and other environmental conditions. Now along with the structural components, there are other factors that influence recalcitrance. So, some of them are acetyl groups and proteins and the porosity of the biomass. In table 2 we will learn more about that. So acetyl groups bind hemicellulose via covalent ester bonds and deacetylation of biomass may increase lignocellulose degradation by 5 to 7 times.

This recalcitrance property is a bottleneck in industrial utilisation of lignocellulosic biomass and various pre-treatments are required to overcome this issue. Proteins also have negative and positive influences on recalcitrance.

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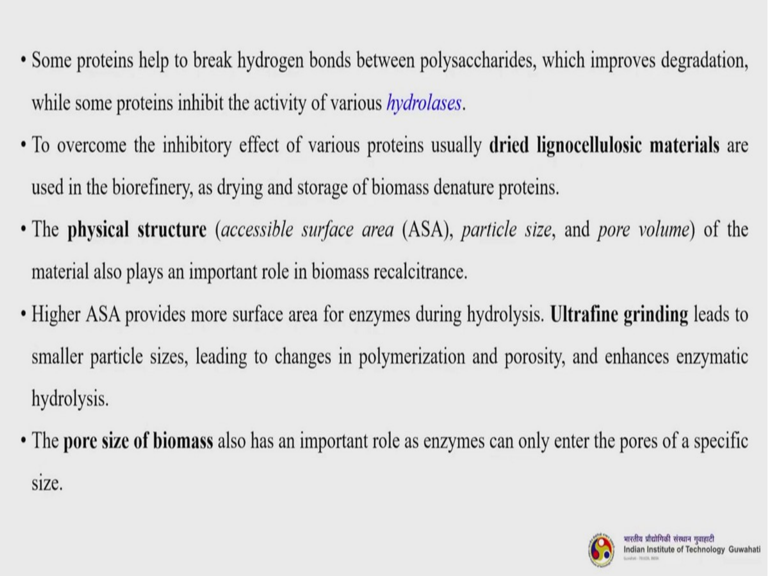

- Some proteins help to break hydrogen bonds between polysaccharides, which improves degradation, while some proteins inhibit the activity of various *hydrolases*.
- To overcome the inhibitory effect of various proteins usually **dried lignocellulosic materials** are used in the biorefinery, as drying and storage of biomass denature proteins.
- The **physical structure** (*accessible surface area* (ASA), *particle size*, and *pore volume*) of the material also plays an important role in biomass recalcitrance.
- Higher ASA provides more surface area for enzymes during hydrolysis. **Ultrafine grinding** leads to smaller particle sizes, leading to changes in polymerization and porosity, and enhances enzymatic hydrolysis.
- The **pore size of biomass** also has an important role as enzymes can only enter the pores of a specific size.

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Some proteins help to break hydrogen bonds between polysaccharides which improves degradation while some proteins inhibit the activity of various *hydrolases*. So, hydrolases are the enzymes that are responsible for doing the hydrolysis. So, to overcome the inhibitory effects of various proteins, usually dried lignocellulosic materials are used in the bio refinery, as drying and storage of biomass denature protein.

So, the physical structure which includes the accessible surface area (We call it ASA), particle size and pore volume of the material plays an important role in the biomass recalcitrance. Higher ASA provides more surface area for enzymes during hydrolysis. Ultrafine grinding leads to smaller particle sizes leading to changes in polymerization and porosity and enhances enzymatic hydrolysis. When you are grinding them to finer particle size, basically you are increasing the surface area and thereby resulting in more degradation. The pore size of the Biomass also has an important role, as enzymes which can only enter the pore of specific size, because enzyme has a certain size.

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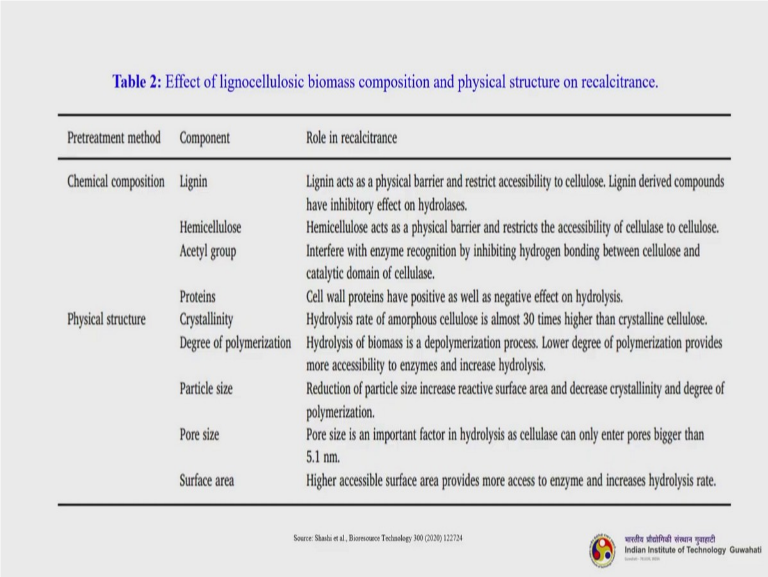

Table 2: Effect of lignocellulosic biomass composition and physical structure on recalcitrance.

Pretreatment method	Component	Role in recalcitrance
Chemical composition	Lignin	Lignin acts as a physical barrier and restrict accessibility to cellulose. Lignin derived compounds have inhibitory effect on hydrolases.
	Hemicellulose	Hemicellulose acts as a physical barrier and restricts the accessibility of cellulase to cellulose.
	Acetyl group	Interfere with enzyme recognition by inhibiting hydrogen bonding between cellulose and catalytic domain of cellulase.
	Proteins	Cell wall proteins have positive as well as negative effect on hydrolysis.
Physical structure	Crystallinity	Hydrolysis rate of amorphous cellulose is almost 30 times higher than crystalline cellulose.
	Degree of polymerization	Hydrolysis of biomass is a depolymerization process. Lower degree of polymerization provides more accessibility to enzymes and increase hydrolysis.
	Particle size	Reduction of particle size increase reactive surface area and decrease crystallinity and degree of polymerization.
	Pore size	Pore size is an important factor in hydrolysis as cellulase can only enter pores bigger than 5.1 nm.
	Surface area	Higher accessible surface area provides more access to enzyme and increases hydrolysis rate.

Source: Shashi et al., Bioresource Technology 300 (2020) 122724

So, this is the table 2. Let us understand what is being given here and this is the reference of this particular table. So, effect of lignocellulosic biomass composition and physical structure and recalcitrance. So, this is the pre-treatment method: chemical method and physical method. So the components are Lignin, hemicellulose, acetyl group, proteins. Then under physical we will be talking about crystallinity, degree of polymerization, particle size, pore size and surface area. This is what we have already discussed.

So, in a nutshell whatever we have discussed is represented in this particular table. So please refer to it later on; we will just quickly glance through it. So, lignin acts as a physical barrier and restricts accessibility to cellulose. Lignin derived compounds have inhibitory effects on hydrolysis. That is very important. So, when you do this hydrolysis, and there are certain by-products that will be created which are not required at all and which are many times are toxic to the entire process.

We need to get rid of them. So lignin plays a very important role in making such or generating such by-product. That is why lignin has to be removed completely. Hemicellulose acts the physical barrier and restrict the accessibility of cellulase enzyme to cellulose. Acetyl group interferes with enzymes recognition. Proteins will have both positive and negative effects on hydrolysis.

Crystallinity tells us that hydrolysis rate of amorphous cellulose is almost 30 times higher than the crystalline cellulose. That is why more crystalline the cellulose, we need to have a better pre-treatment technology and we need to spend the more energy, Chemicals and all these things to make the higher crystalline cellulose amenable to hydrolysis; and other things please go through it later on.

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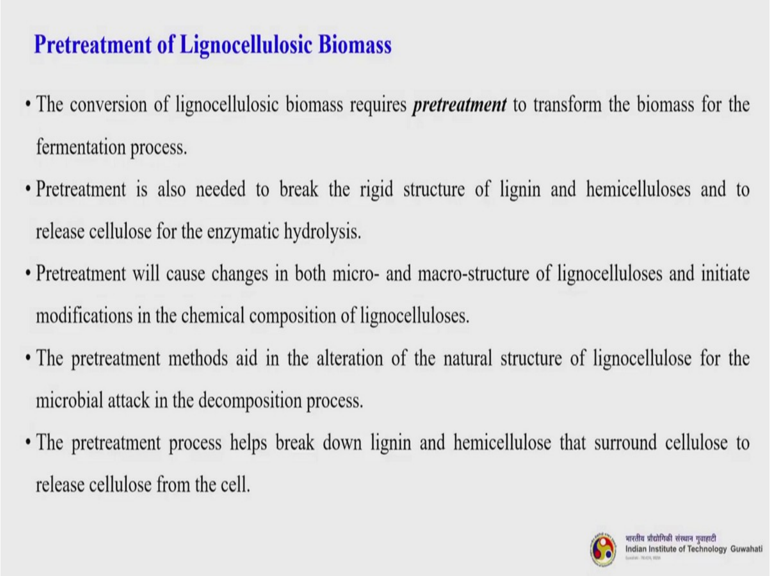

Pretreatment of Lignocellulosic Biomass

- The conversion of lignocellulosic biomass requires ***pretreatment*** to transform the biomass for the fermentation process.
- Pretreatment is also needed to break the rigid structure of lignin and hemicelluloses and to release cellulose for the enzymatic hydrolysis.
- Pretreatment will cause changes in both micro- and macro-structure of lignocelluloses and initiate modifications in the chemical composition of lignocelluloses.
- The pretreatment methods aid in the alteration of the natural structure of lignocellulose for the microbial attack in the decomposition process.
- The pretreatment process helps break down lignin and hemicellulose that surround cellulose to release cellulose from the cell.

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Now, we will discuss about the pre-treatment of lignocellulosic biomass, the different methods. The conversion of lignocellulosic biomass requires pre-treatment to transform the Biomass for the fermentation process. Pretreatment is also needed to break the rigid structure of Lignin and hemicellulose and to release cellulose for the enzymatic hydrolysis. Pretreatment will cause changes in both micro and macro structure of lignocelluloses and initiate modifications in the chemical composition of lignocelluloses.

The pre-treatment methods aid in the alteration of the natural structure of lignocellulose for the microbial attack in the decomposition process. Pretreatment process helps breakdown Lignin and hemicellulose that surround cellulose to release cellulose from the cell.

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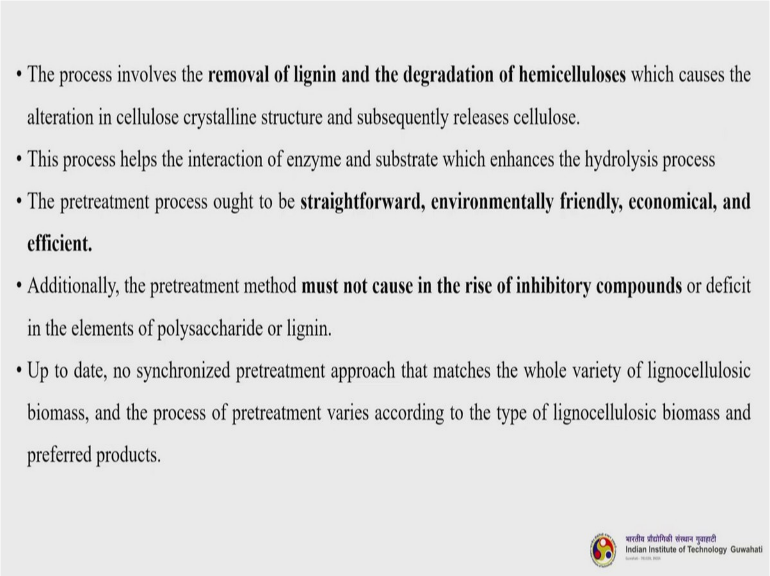

- The process involves the **removal of lignin and the degradation of hemicelluloses** which causes the alteration in cellulose crystalline structure and subsequently releases cellulose.
- This process helps the interaction of enzyme and substrate which enhances the hydrolysis process
- The pretreatment process ought to be **straightforward, environmentally friendly, economical, and efficient.**
- Additionally, the pretreatment method **must not cause in the rise of inhibitory compounds** or deficit in the elements of polysaccharide or lignin.
- Up to date, no synchronized pretreatment approach that matches the whole variety of lignocellulosic biomass, and the process of pretreatment varies according to the type of lignocellulosic biomass and preferred products.

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The process involves the removal of Lignin and the degradation of hemicelluloses which causes the alteration in cellulose crystalline structure and subsequently it releases cellulose. This process helps the interaction of enzyme and substrate which enhances the hydrolysis process. The pretreatment process ought to be straightforward, environmental friendly, economical and efficient. It has to be like that. Otherwise it will not be a sustainable process and cannot be a part of the biorefinery.

Additionally, the pretreatment method must not cause in the rise of inhibitory compounds. This is what I was just mentioning. So, these inhibitory compounds formation should be restricted by choosing a particular pre-treatment method which will not create a greater amount of such inhibitory compounds.

Up to date, no synchronised pre-treatment approach that matches the whole variety of lignocellulosic biomass, and the process of pre-treatment varies according to the type of lignocellulosic biomass and preferred products. This is what I was mentioned in the beginning of the class. You have to note; it is a very serious thing actually. When we are talking about these pre-treatment processes or when we are talking about the entire biorefinery concept, we need to understand that the pretreatment cost is almost 40%, if you talk about the entire cost of the product. Now, why it is 40% right now or almost in that range? The reason is that; first thing is that whatever pre-treatment techniques are available, these are suitable for certain particular types of biomass. So no pretreatment technology, not a single one or hybrid Technology has been developed which will take into account so many different types of multiple feedstocks; because different feedstocks will have different components. Some will have higher cellulose, and some will have very low amount of hemicellulose, some will have high amount of lignin; so it all depends. That is the reason why we need to work more to develop more efficient pre-treatment technologies, which will be economical, which will also take less time, which will not be energy-intensive and then which will be sustainable. And which will again, I am telling you that, must be able to take into account multiple feedstocks or it will be able to process multiple feedstocks, that is very important.

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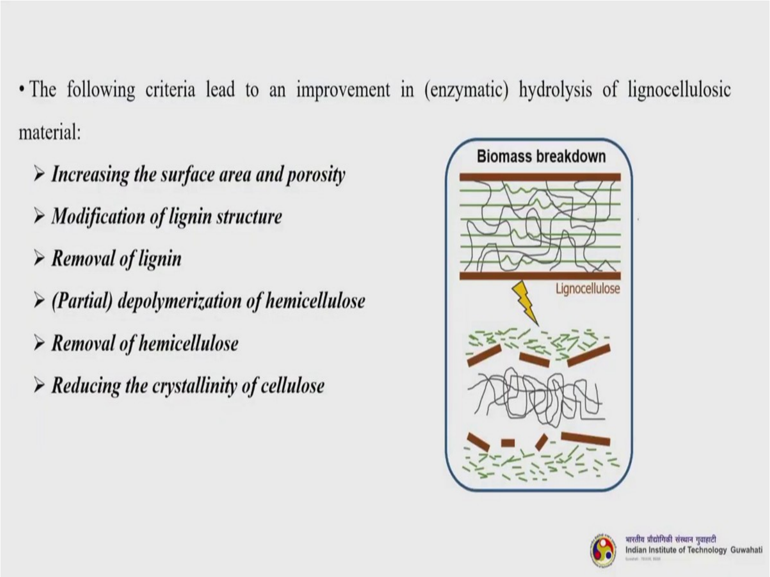

- The following criteria lead to an improvement in (enzymatic) hydrolysis of lignocellulosic material:
  - *Increasing the surface area and porosity*
  - *Modification of lignin structure*
  - *Removal of lignin*
  - *(Partial) depolymerization of hemicellulose*
  - *Removal of hemicellulose*
  - *Reducing the crystallinity of cellulose*

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So, the following criteria lead to an improvement in the enzymatic hydrolysis of lignocellulosic materials: The first is increasing the surface area and porosity which can be done by doing the mechanical processing; modification of the lignin structure (modification of the lignin structure can be done by genetic engineering - in one of our class we have discussed that); you have to remove Lignin, so this is a delignification process (you call this one pretreatment also); partial depolymerization of the hemicellulose (So, you have to release hemicellulose also or depolymerize so that hemicellulose which is bound to cellulose will be disintegrated); then remove hemicellulose, because hemicellulose also can be hydrolysed and converted to the sugar; and then reducing the crystallinity of the cellulose.

So, once you do the pre-treatment (you can see how it is intricately bound); so, this is the lignin, green one is cellulose and this thread type whatever it is being shown here is hemicellulose. You disintegrate, that means you treat it, delignify it and it will be disintegrated into something like this: hemicellulose pure, lignin in the purest form and cellulose in the purest form.

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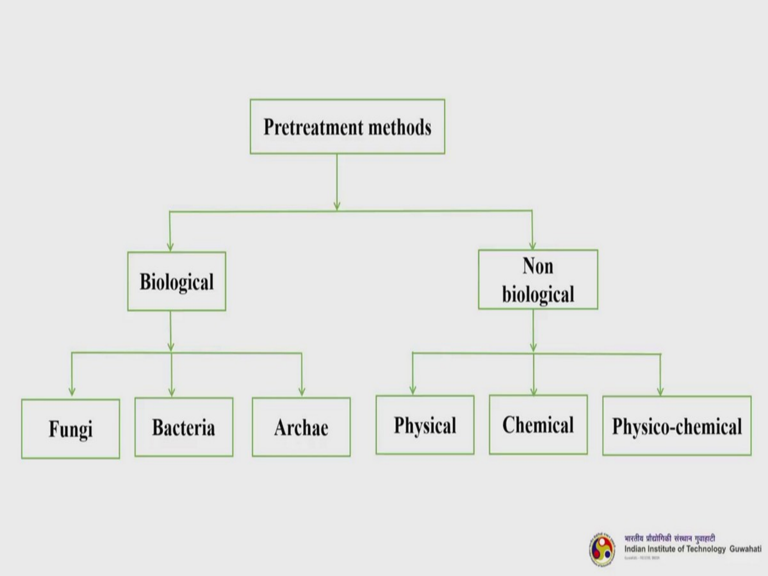

Pretreatment methods

Biological

Non biological

Fungi

Bacteria

Archae

Physical

Chemical

Physico-chemical

Let us understand the pretreatment methods. So we have two different broad categories of pretreatment methods: first one is biological and second is a non-biological. So, in the biological we have fungi based pretreatment methods, we can have bacteria based or Archae based. And under non biological we have varieties of pretreatment methods: some are physical, some are chemical and some are physico chemical. So in today's class we will discuss few of them and some we will discussion in our next class.

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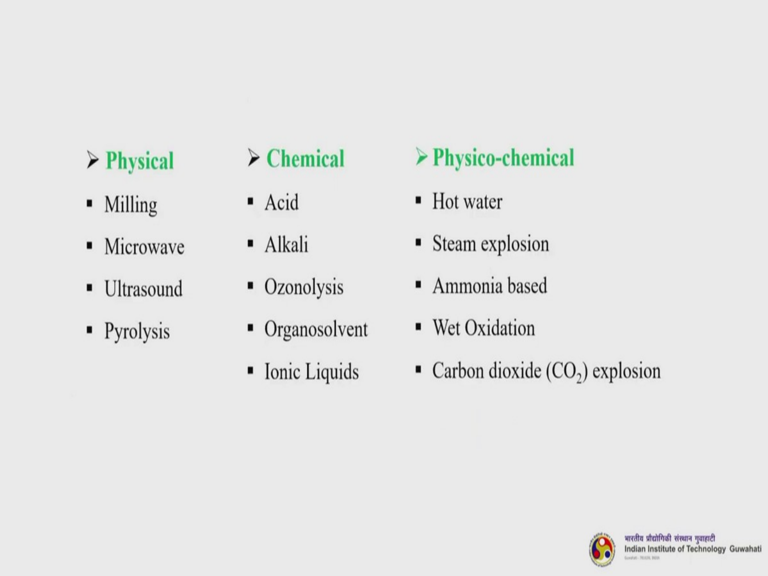

➢ **Physical**

- Milling
- Microwave
- Ultrasound
- Pyrolysis

➢ **Chemical**

- Acid
- Alkali
- Ozonolysis
- Organosolvent
- Ionic Liquids

➢ **Physico-chemical**

- Hot water
- Steam explosion
- Ammonia based
- Wet Oxidation
- Carbon dioxide (CO_2) explosion

These are some of the methods I have listed, there are many which are not listed here also. Physical: under physical it can be milling, microwave, ultrasound, pyrolysis. Under chemical it is acid treatment, alkali treatment, ozonolysis, organosolvent process and ionic liquids. And ionic liquids are excellent class of green solvents. And then physico-chemical: hot water treatment, steam explosion, Ammonia based treatment, wet oxidation and carbon dioxide explosion.

Some of these physico chemical treatment and chemical treatments are of course, they are costly.

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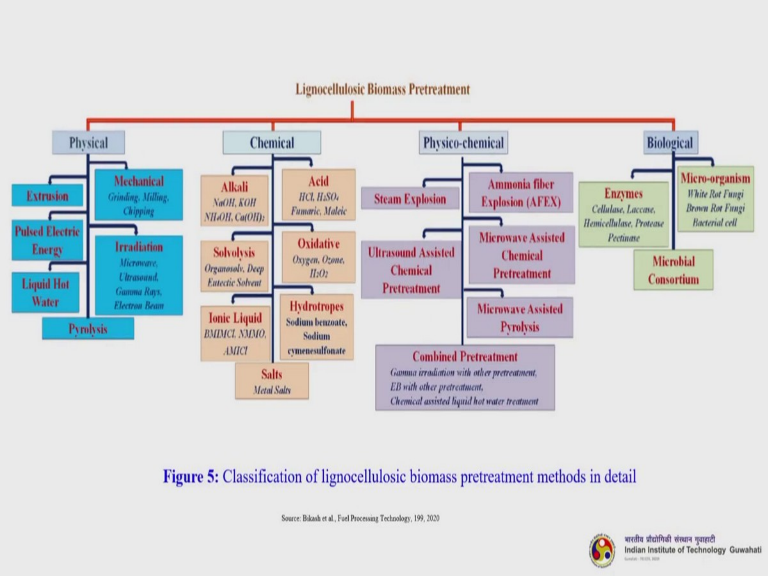

Figure 5: Classification of lignocellulosic biomass pretreatment methods in detail

Source: Bikash et al., Fuel Processing Technology, 199, 2020

Now this particular slide will show you whatever I have just told you in the earlier slide. It is presented in a better way here. So, classification of the lignocellulosic Biomass pre-treatment methods in detail. Physical: extrusion, pulsed electric energy based, liquid hot water, pyrolysis, irradiation (basically the microwave treatment), Mechanical milling, grinding, chipping.

When you come to chemical: It is again acid, alkali, solvolysis (that means using organic solvents), oxidative (using either oxygen, ozone, hydrogen peroxide), then ionic liquid - different types of ionic liquids are there, and hydrotropes like sodium benzoate and salts - certain metal salts.

Then we have physico-chemical. So, steam explosion, Ammonia fibre explosion (AFEX) - It is a very interesting and very efficient technique. But you have to do it in a proper way. Otherwise there are risks associated with that - anyway will discuss later on. Ultrasound-assisted chemical pre-treatment - this is one of the most efficient processes. But however, it has its own limitation. Now beyond certain limit, it cannot work and it's a low-cost technology though a certain amount of energy is required. Then microwave-assisted chemical treatment. Not only microwave, but microwave assisted chemical pre-treatment. Then microwave-assisted pyrolysis. Then combined pre-treatment. So, this is called hybrid - more work is being done here. So, it is a combination of one or two different pre-treatment methods. Then biological; based on enzymes, microorganisms and development of suitable microbial consortium to take care of the pretreatment.

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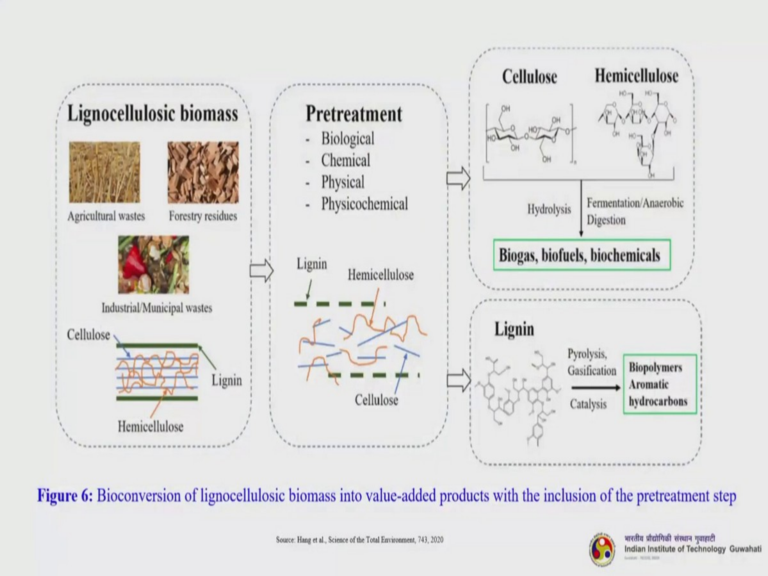

Figure 6: Bioconversion of lignocellulosic biomass into value-added products with the inclusion of the pretreatment step

Source: Hang et al., Science of the Total Environment, 743, 2020

So, this is the bioconversion of lignocellulosic biomass into value added products with the inclusion of the pretreatment steps. So this is your biomass and this is the structure. So you pre-treat here. Biological, chemical, physical, physico-chemical, anything we get and that will result in something, the disintegration of the structure of the Lignin, cellulose and hemicellulose.

Then you convert them: cellulose and hemicellulose will be basically purified. So, you hydrolyse them. So, then you can (subject to) any bio chemical or bio technological platform such as fermentation, anaerobic digestion, so you get alcohol platform. It can be bio-ethanol. It can be bio butanol. Then we can get biogas also. Next is lignin, so lignin either it can go for pyrolysis, gasification with or without the presence of catalysis.

So, we can get biopolymers, we will get aromatic hydrocarbons and other value-added chemicals.

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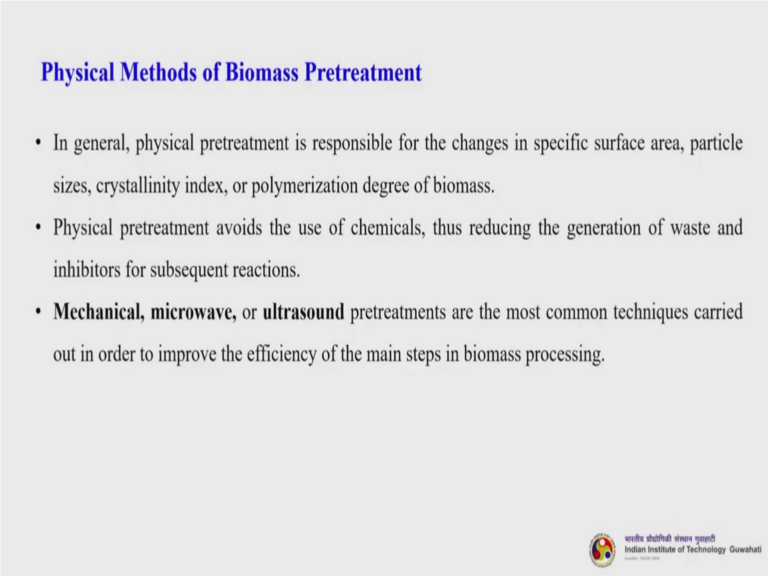

Physical Methods of Biomass Pretreatment

- In general, physical pretreatment is responsible for the changes in specific surface area, particle sizes, crystallinity index, or polymerization degree of biomass.
- Physical pretreatment avoids the use of chemicals, thus reducing the generation of waste and inhibitors for subsequent reactions.
- **Mechanical, microwave,** or **ultrasound** pretreatments are the most common techniques carried out in order to improve the efficiency of the main steps in biomass processing.

So, physical methods of biomass pre-treatment we will discuss. So, in general physical pre-treatment is responsible for the changes in specific surface area, particle sizes, crystallinity index and polymerization degree of biomass. Physical pre-treatment avoids the use of chemicals thus reducing the generation of waste and inhibitors for subsequent reaction. Now mechanical, microwave or ultrasound pretreatments are the most common techniques carried out in order to improve the efficiency of the main steps in biomass processing.

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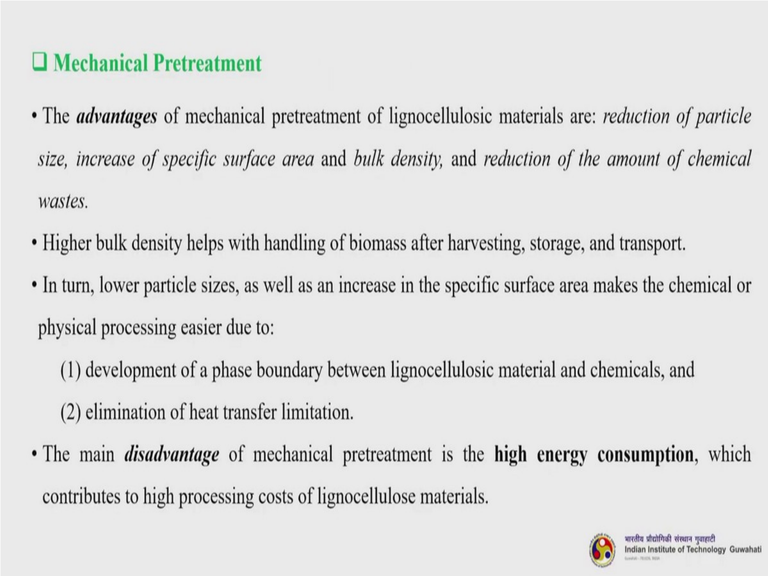

❑ Mechanical Pretreatment

- The ***advantages*** of mechanical pretreatment of lignocellulosic materials are: *reduction of particle size, increase of specific surface area* and *bulk density,* and *reduction of the amount of chemical wastes.*
- Higher bulk density helps with handling of biomass after harvesting, storage, and transport.
- In turn, lower particle sizes, as well as an increase in the specific surface area makes the chemical or physical processing easier due to:
  (1) development of a phase boundary between lignocellulosic material and chemicals, and
  (2) elimination of heat transfer limitation.
- The main ***disadvantage*** of mechanical pretreatment is the **high energy consumption**, which contributes to high processing costs of lignocellulose materials.

So, let us understand mechanical pretreatment. The advantages of mechanical pretreatment of lignocellulosic materials are: reduction of particle size, increase of specific surface area and bulk density and reduction of the amount of chemical waste. So higher bulk density helps with handling of biomass after harvesting, storage and transport. In turn lower particle sizes as well as an increase in the specific surface area makes the chemical or physical processing easier due to: (1) development of a phase boundary between lignocellulosic material and chemicals and; (2) elimination of heat transfer limitation. So, the main disadvantage of mechanical pre-treatment is the high energy consumption which contributes to high processing cost of lignocellulosic materials.

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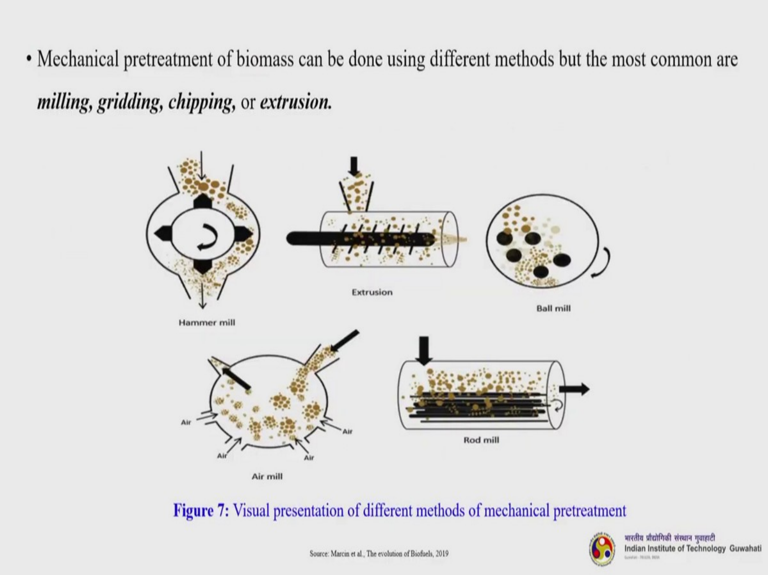

- Mechanical pretreatment of biomass can be done using different methods but the most common are ***milling, gridding, chipping,*** or ***extrusion.***

Figure 7: Visual presentation of different methods of mechanical pretreatment

Source: Marcin et al., The evolution of Biofuels, 2019

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These are some of the schematic representation of the mechanical pre-treatment processes. This is a hammer mill where milling is done. This is an extruder for the extrusion process. That is a ball mill where small balls are present to disintegrate or reduce the size of the Biomass. This is an Air mill and that is a roll mill. All are mechanical operation process basically.

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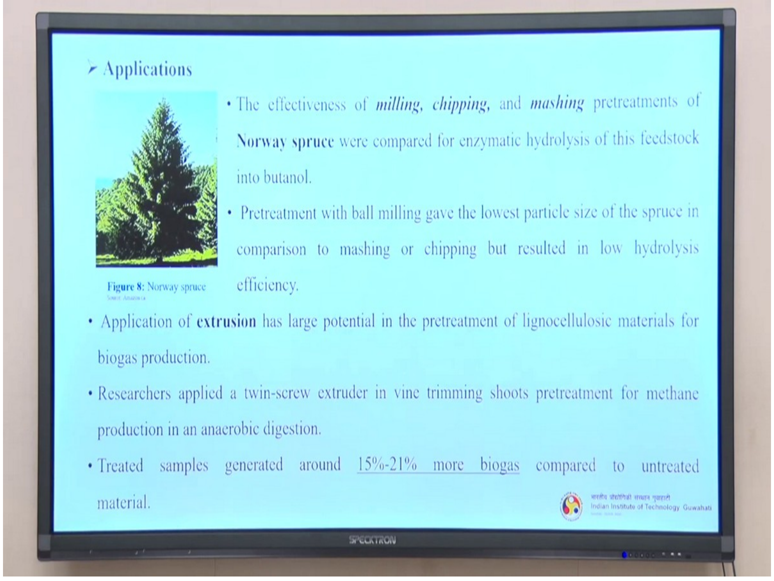

➤ Applications

- The effectiveness of ***milling, chipping,*** and ***mashing*** pretreatments of **Norway spruce** were compared for enzymatic hydrolysis of this feedstock into butanol.
- Pretreatment with ball milling gave the lowest particle size of the spruce in comparison to mashing or chipping but resulted in low hydrolysis efficiency.

Figure 8: Norway spruce

- Application of **extrusion** has large potential in the pretreatment of lignocellulosic materials for biogas production.
- Researchers applied a twin-screw extruder in vine trimming shoots pretreatment for methane production in an anaerobic digestion.
- Treated samples generated around 15%-21% more biogas compared to untreated material.

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So, where do we apply this actually. So the effectiveness of milling, chipping and mashing of pre-treatments of Norway spruce (it’s an example here) were compared for enzymatic hydrolysis of this feedstock into when it is being converted into biobutanol. So pre-treatment with ball milling gave the lowest particle size of the spruce in comparison to mashing or chipping but resulted in low hydrolysis efficiency.

Now application of extrusion has a large potential in the pretreatment of lignocellulosic materials for biogas production. Researchers have applied twin screw extruder in vine trimming shoots pre-treatment for Methane production in anaerobic digestion. Now treated samples generated around almost 15 to 21% of more biogas compared to untreated material. So, it is a very good finding with physical treatment.

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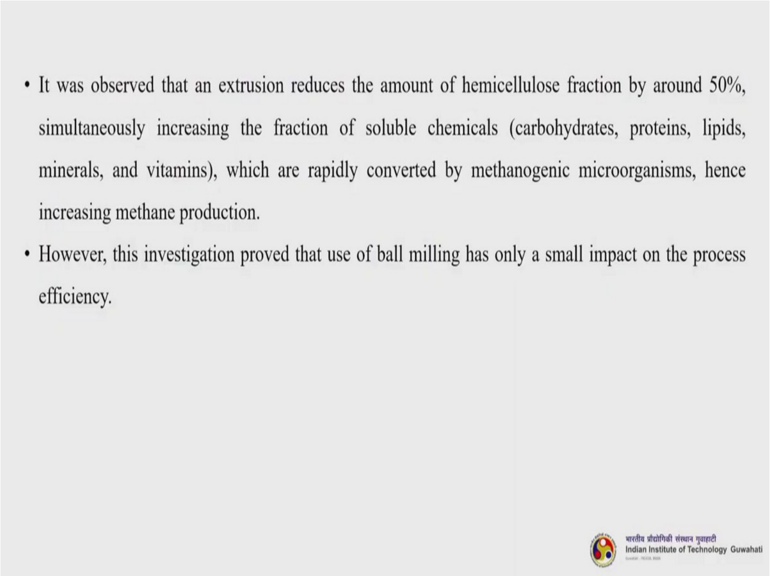

- It was observed that an extrusion reduces the amount of hemicellulose fraction by around 50%, simultaneously increasing the fraction of soluble chemicals (carbohydrates, proteins, lipids, minerals, and vitamins), which are rapidly converted by methanogenic microorganisms, hence increasing methane production.
- However, this investigation proved that use of ball milling has only a small impact on the process efficiency.

So, it was observed that an extrusion reduces the amount of hemicellulose fraction by around 50% simultaneously increasing the fraction of soluble Chemicals like carbohydrates proteins, lipids, minerals and vitamins which are rapidly converted by methanogenic microorganisms hence, increasing Methane production. However, this investigation proved that use of ball milling has only a small impact on the process efficiency.

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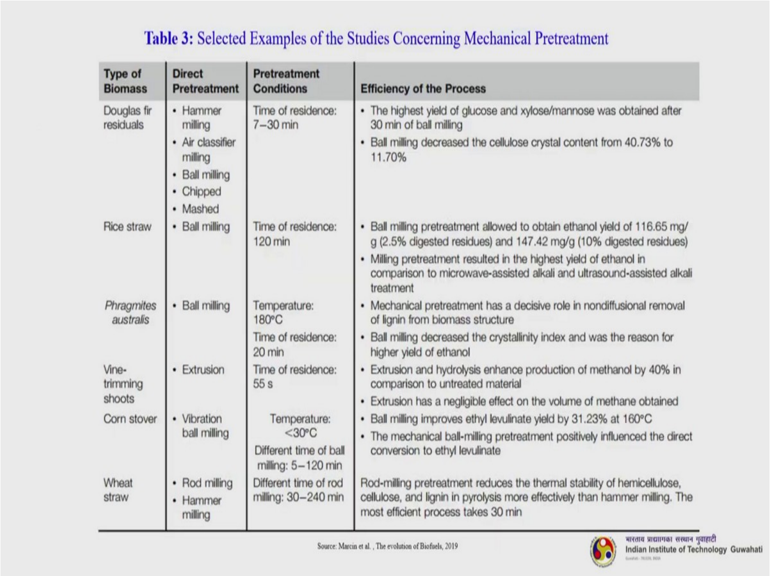

Table 3: Selected Examples of the Studies Concerning Mechanical Pretreatment

Type of Biomass	Direct Pretreatment	Pretreatment Conditions	Efficiency of the Process
Douglas fir residuals	• Hammer milling • Air classifier milling • Ball milling • Chipped • Mashed	Time of residence: 7–30 min	• The highest yield of glucose and xylose/mannose was obtained after 30 min of ball milling • Ball milling decreased the cellulose crystal content from 40.73% to 11.70%
Rice straw	• Ball milling	Time of residence: 120 min	• Ball milling pretreatment allowed to obtain ethanol yield of 116.65 mg/g (2.5% digested residues) and 147.42 mg/g (10% digested residues) • Milling pretreatment resulted in the highest yield of ethanol in comparison to microwave-assisted alkali and ultrasound-assisted alkali treatment
Phragmites australis	• Ball milling	Temperature: 180°C Time of residence: 20 min	• Mechanical pretreatment has a decisive role in nondiffusional removal of lignin from biomass structure • Ball milling decreased the crystallinity index and was the reason for higher yield of ethanol
Vine-trimming shoots	• Extrusion	Time of residence: 55 s	• Extrusion and hydrolysis enhance production of methanol by 40% in comparison to untreated material • Extrusion has a negligible effect on the volume of methane obtained
Corn stover	• Vibration ball milling	Temperature: <30°C Different time of ball milling: 5–120 min	• Ball milling improves ethyl levulinate yield by 31.23% at 160°C • The mechanical ball-milling pretreatment positively influenced the direct conversion to ethyl levulinate
Wheat straw	• Rod milling • Hammer milling	Different time of rod milling: 30–240 min	Rod-milling pretreatment reduces the thermal stability of hemicellulose, cellulose, and lignin in pyrolysis more effectively than hammer milling. The most efficient process takes 30 min

Source: Marcin et al. , The evolution of Biofuels, 2019

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This is the selected examples of the studies concerning mechanical pretreatment. So, I leave it to you. Please have a look later on. So, you can see that there are different types of biomasses are listed here, different types of pretreatment methods are listed here, a mechanical one. Then pretreatment conditions. How much time and all these things are given here. And what is the efficiency of the process?

So, we will just see the first one. The Douglas fir residues. So, the hammer milling, air classifier milling, ball milling, chipped, mashed all these things has been compared. So, the time of residence inside the mill is basically 7 to 30 minutes. The result is that, highest yield of glucose and xylose/mannose and was obtained after 30 minutes of ball milling. So ball milling decreases the cellulose crystal from 40.73% to 11.7% which is a significant decrease. Please have a look later on.

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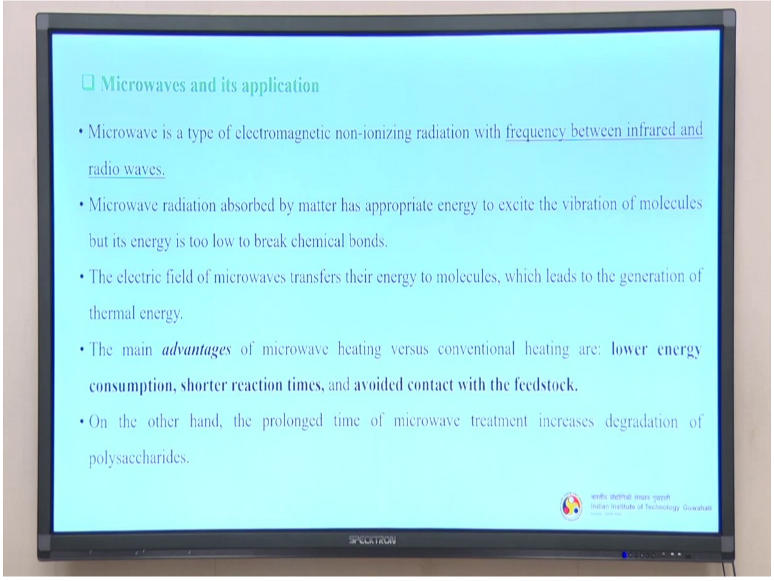
❑ Microwaves and its application

- Microwave is a type of electromagnetic non-ionizing radiation with frequency between infrared and radio waves.
- Microwave radiation absorbed by matter has appropriate energy to excite the vibration of molecules but its energy is too low to break chemical bonds.
- The electric field of microwaves transfers their energy to molecules, which leads to the generation of thermal energy.
- The main ***advantages*** of microwave heating versus conventional heating are: **lower energy consumption, shorter reaction times,** and **avoided contact with the feedstock.**
- On the other hand, the prolonged time of microwave treatment increases degradation of polysaccharides.

Now the next one Microwave and its application. So, Microwave is a type of electromagnetic non-ionizing radiation with frequency between the infrared and radio waves. Microwave radiation absorbed by matter has appropriate energy to excite the vibration of molecules, but its energy is too low to break chemical bonds. The electric field of microwave transfer their energy to molecules which leads to the generation of thermal energy.

The main advantages of microwave heating versus conventional heating are: lower energy consumption, shorter reaction time and avoided contact with the feedstock. On the other hand, the prolonged time of microwave treatment increases degradation of polysaccharides. So researchers have demonstrated advantages of the application of microwave in the increase of the yield of biogas in biomethanation of organic matter.

This is just one of the researches which I have listed here, there are many works. So, Microwave generated heat increases the solubility of Lignin, released soluble compounds and improved the rate of hydrolysis due to cellular disruption. However, the effectiveness of biogas production depended on the time of exposure and power of irradiation. A longer exposure time can lead to fractional degradation of reducing Sugars and the generation of inhibitors negatively affecting the biogas formation.

Nevertheless, appropriate time of microwave irradiation of biomass increased cell frangibility and improved enzymatic hydrolysis by disruption of biomass complex structure. It is a good process, it is easy to operate and not too energy-intensive also.

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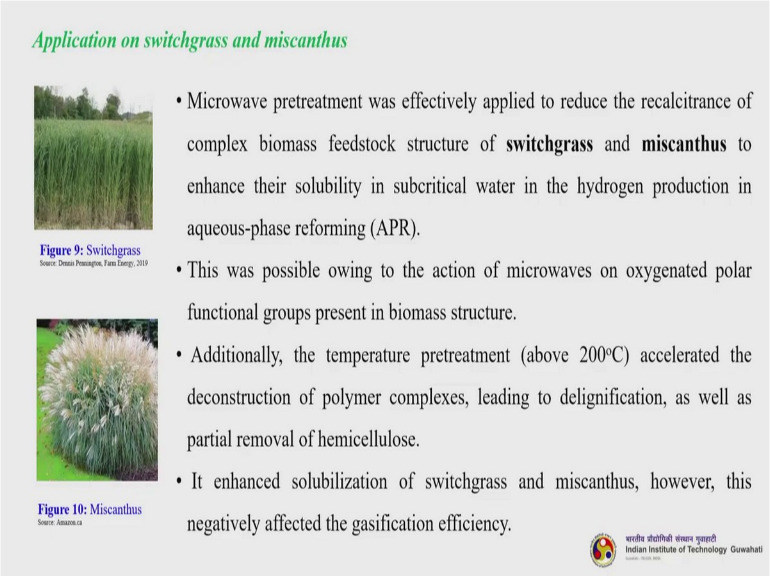

Application on switchgrass and miscanthus

- Microwave pretreatment was effectively applied to reduce the recalcitrance of complex biomass feedstock structure of **switchgrass** and **miscanthus** to enhance their solubility in subcritical water in the hydrogen production in aqueous-phase reforming (APR).
- This was possible owing to the action of microwaves on oxygenated polar functional groups present in biomass structure.
- Additionally, the temperature pretreatment (above 200°C) accelerated the deconstruction of polymer complexes, leading to delignification, as well as partial removal of hemicellulose.
- It enhanced solubilization of switchgrass and miscanthus, however, this negatively affected the gasification efficiency.

Figure 9: Switchgrass
Source: Dennis Pennington, Farm Energy, 2019

Figure 10: Miscanthus
Source: Amazon.ca

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So, let us talk about one of its application for converting the switchgrass and miscanthus into alcohol platform. So, microwave pretreatment was effectively applied to reduce the recalcitrance of complex biomass feedstock structure of switchgrass and miscanthus to enhance their solubility in subcritical water in the hydrogen production in aqueous phase reforming. It was the done for the hydrogen production process.

So, this was possible owing to the action of microwaves on oxygenated polar functional groups present in the biomass structure. Additionally, the temperature pretreatment above 200 degrees centigrade accelerated the deconstruction of polymer complexes leading to delignification as well as partial removal of hemicellulose. It enhances solubilisation switchgrass and miscanthus however this negatively affected the gasification efficiency.

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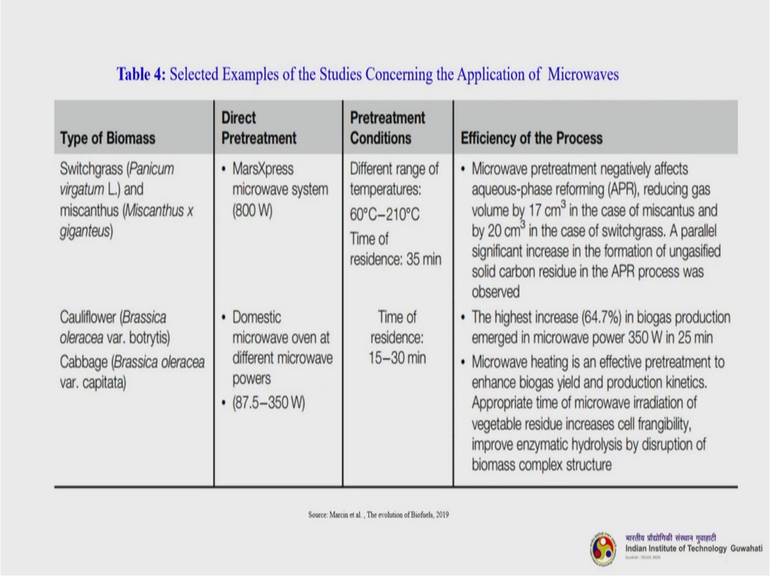

Table 4: Selected Examples of the Studies Concerning the Application of Microwaves

Type of Biomass	Direct Pretreatment	Pretreatment Conditions	Efficiency of the Process
Switchgrass (*Panicum virgatum* L.) and miscanthus (*Miscanthus x giganteus*)	• MarsXpress microwave system (800 W)	Different range of temperatures: 60°C–210°C Time of residence: 35 min	• Microwave pretreatment negatively affects aqueous-phase reforming (APR), reducing gas volume by 17 cm^3 in the case of miscantus and by 20 cm^3 in the case of switchgrass. A parallel significant increase in the formation of ungasified solid carbon residue in the APR process was observed
Cauliflower (*Brassica oleracea* var. botrytis) Cabbage (*Brassica oleracea* var. capitata)	• Domestic microwave oven at different microwave powers • (87.5–350 W)	Time of residence: 15–30 min	• The highest increase (64.7%) in biogas production emerged in microwave power 350 W in 25 min • Microwave heating is an effective pretreatment to enhance biogas yield and production kinetics. Appropriate time of microwave irradiation of vegetable residue increases cell frangibility, improve enzymatic hydrolysis by disruption of biomass complex structure

Source: Marcin et al., The evolution of Biofuels, 2019

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So, this table will again let us understand what are the different types of studies that concerns about the application of microwaves. You can see again switchgrass, is there and miscanthus, and then Cauliflower and cabbage is also mentioned here. So there are direct pre-treatment and then domestic microwave oven at different microwave power. This is a domestic microwave oven which is being used here. Time of residence is 15 to 30 min. You can see that highest increase in biogas production emerged in microwave power of 350 watts in 25 minutes.

And another in very interesting fact about microwave treatment is that, it is a very faster process. So, time is money in industries. So, we should choose such processes which are faster. So please have a look later on.

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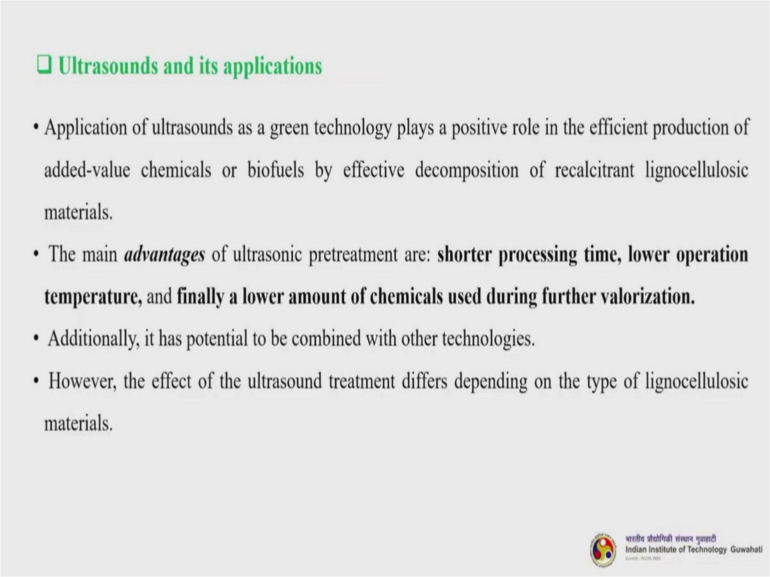

❑ Ultrasounds and its applications

- Application of ultrasounds as a green technology plays a positive role in the efficient production of added-value chemicals or biofuels by effective decomposition of recalcitrant lignocellulosic materials.
- The main ***advantages*** of ultrasonic pretreatment are: **shorter processing time, lower operation temperature,** and **finally a lower amount of chemicals used during further valorization.**
- Additionally, it has potential to be combined with other technologies.
- However, the effect of the ultrasound treatment differs depending on the type of lignocellulosic materials.

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The next one is ultrasound and its application. Application of ultrasound as a green Technology plays a positive role in the efficient production of added value Chemicals or biofuels by effective decomposition of recalcitrant lignocellulosic materials. The main advantages of ultrasound pre-treatment are: shorter processing time, lower operation temperature and finally a lower amount of chemicals used during further valorization. Additionally, it has potential to be combined with other technologies.

However, the effect of the ultrasound treatment differs depending on the type of lignocellulosic material.

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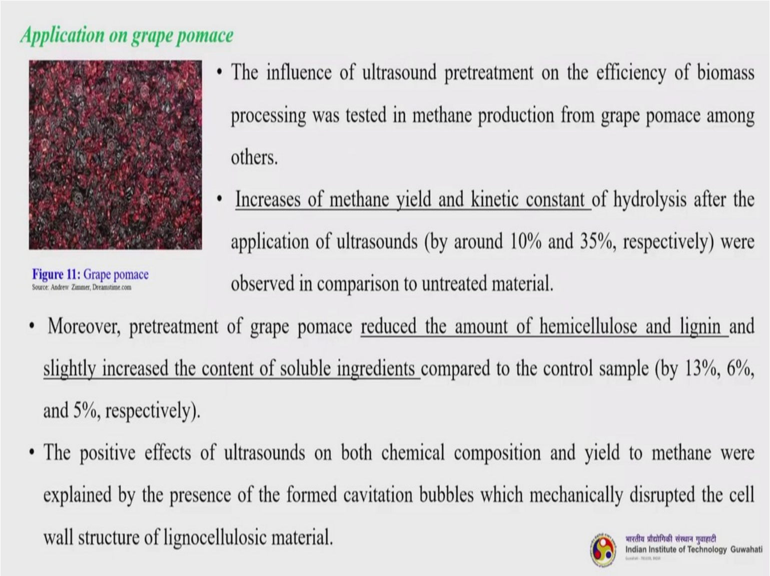

Application on grape pomace

- The influence of ultrasound pretreatment on the efficiency of biomass processing was tested in methane production from grape pomace among others.
- Increases of methane yield and kinetic constant of hydrolysis after the application of ultrasounds (by around 10% and 35%, respectively) were observed in comparison to untreated material.

Figure 11: Grape pomace
Source: Andrew Zimmer, Dreamstime.com

- Moreover, pretreatment of grape pomace reduced the amount of hemicellulose and lignin and slightly increased the content of soluble ingredients compared to the control sample (by 13%, 6%, and 5%, respectively).
- The positive effects of ultrasounds on both chemical composition and yield to methane were explained by the presence of the formed cavitation bubbles which mechanically disrupted the cell wall structure of lignocellulosic material.

One example of grape pomace has been listed here. So, the influence of ultrasound pre-treatment on the efficiency of biomass processing was tested for Methane production from grape pomace among others. So, increases of Methane yield and kinetic constant of the hydrolysis after the application of ultrasound by all most 10% and 35% respectively were observed in comparison to untreated material.

Moreover, pretreatment of grape pomace reduced the amount of hemicellulose and Lignin and slightly increased the content of soluble ingredients compared to control samples. It is almost by 13%, 6% and 5% respectively. So, the positive effects of ultrasounds on both chemical composition and yield to Methane were explained by the presence of the formed Cavitation bubbles which mechanically disrupted the cell wall structure of the lignocellulosic material.

So, it is an interesting technology actually technique. So simple one, where the Cavitation actually happens by the formation of bubbles and it will do the disruption of the cellular structure.

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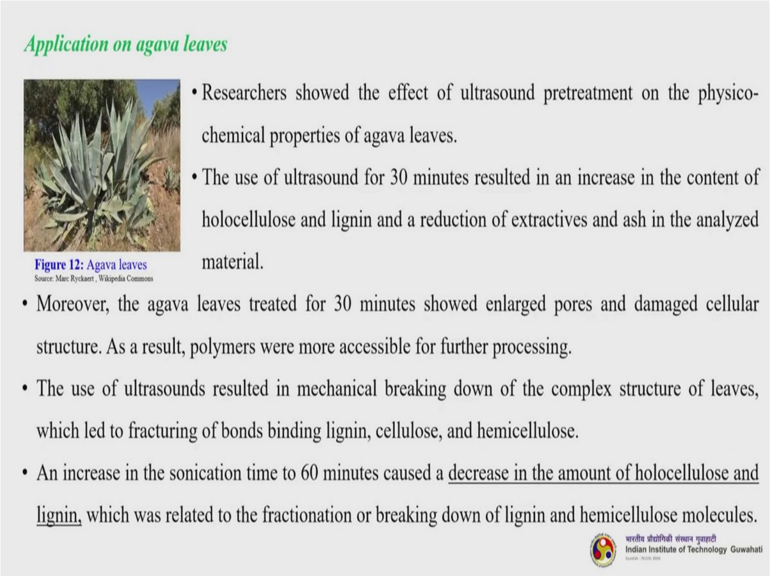

Application on agava leaves

- Researchers showed the effect of ultrasound pretreatment on the physico-chemical properties of agava leaves.
- The use of ultrasound for 30 minutes resulted in an increase in the content of holocellulose and lignin and a reduction of extractives and ash in the analyzed material.

Figure 12: Agava leaves

Source: Marc Ryckaert , Wikipedia Commons

- Moreover, the agava leaves treated for 30 minutes showed enlarged pores and damaged cellular structure. As a result, polymers were more accessible for further processing.
- The use of ultrasounds resulted in mechanical breaking down of the complex structure of leaves, which led to fracturing of bonds binding lignin, cellulose, and hemicellulose.
- An increase in the sonication time to 60 minutes caused a decrease in the amount of holocellulose and lignin, which was related to the fractionation or breaking down of lignin and hemicellulose molecules.

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So, we will see one of the applications on the Agava leaves. So, researchers, showed the effect of ultrasound pretreatment on the physico chemical properties of the Agava leaves. The use of ultrasound for 30 minutes resulted in an increase in the content of holocellulose and Lignin and a reduction of extractives and ash in the analysed material. Now holocellulose is the amount of water non-soluble carbohydrates basically.

So, moreover the agava leaves treated for 30 minutes showed enlarged pores and damaged cellular structure. As a result, Polymers were more accessible for further processing. The use of ultrasound resulted in mechanical breaking down of the complex structure of leaves which led to fracturing of bonds binding Lignin and cellulose and hemicellulose. An increase in the sonication time to 60 minutes, caused the decrease in the amount of holocellulose and Lignin which was related to the fractionation or breaking down of Lignin and hemicellulose molecules.

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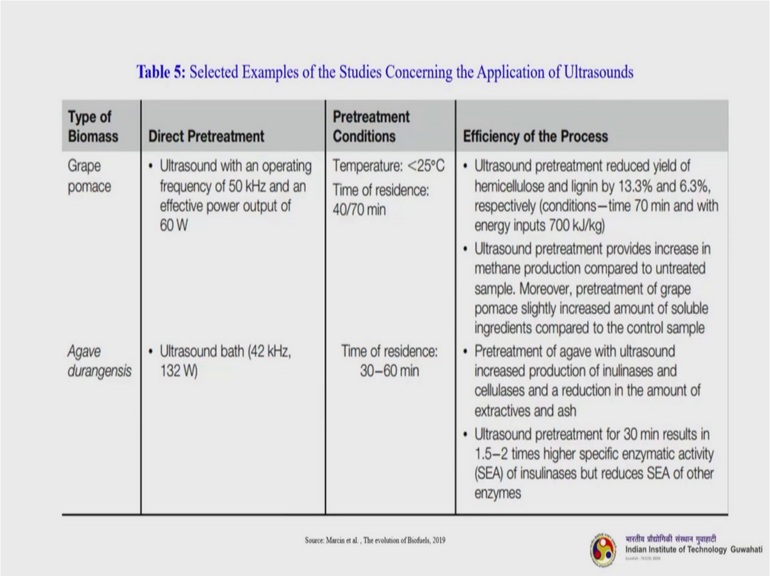

Table 5: Selected Examples of the Studies Concerning the Application of Ultrasounds

Type of Biomass	Direct Pretreatment	Pretreatment Conditions	Efficiency of the Process
Grape pomace	• Ultrasound with an operating frequency of 50 kHz and an effective power output of 60 W	Temperature: <25°C Time of residence: 40/70 min	• Ultrasound pretreatment reduced yield of hemicellulose and lignin by 13.3% and 6.3%, respectively (conditions—time 70 min and with energy inputs 700 kJ/kg) • Ultrasound pretreatment provides increase in methane production compared to untreated sample. Moreover, pretreatment of grape pomace slightly increased amount of soluble ingredients compared to the control sample
Agave durangensis	• Ultrasound bath (42 kHz, 132 W)	Time of residence: 30–60 min	• Pretreatment of agave with ultrasound increased production of inulinases and cellulases and a reduction in the amount of extractives and ash • Ultrasound pretreatment for 30 min results in 1.5–2 times higher specific enzymatic activity (SEA) of insulinases but reduces SEA of other enzymes

Source: Marcin et al. , The evolution of Biofuels, 2019

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So this particular table has given examples of studies concerning the application of ultrasound. You can see again that grape pomace and Agava leaves are being ultra sound treated and the different pretreatment conditions are given. You can see the efficiency of the process. So, the first one in case of grape pomace less than 25 degree centigrade almost 40 to 70 minutes is the time of Residence. So, ultrasound treatment reduced yield of hemicellulose and Lignin by 13.3 and 6.3% respectively. Ultrasound pre-treatment provided increase in Methane production compared to untreated sample. Moreover, pre-treatment of grape pomace slightly increased the amount of soluble ingredients compared to the control sample. Similarly, in case of the Agava leaves pre-treatment resulted in the production of inulinases and cellulases and a reduction in the amount of extractives and ash; these are the enzymes.

So, ultrasound pre-treatment for 30 minutes, results in 1.5 to 2 times higher specific enzymatic activity which is known as SEA of inulinases, but reduces SEA for other enzymes. So, these are some of the classical findings from the ultrasound-based treatment.

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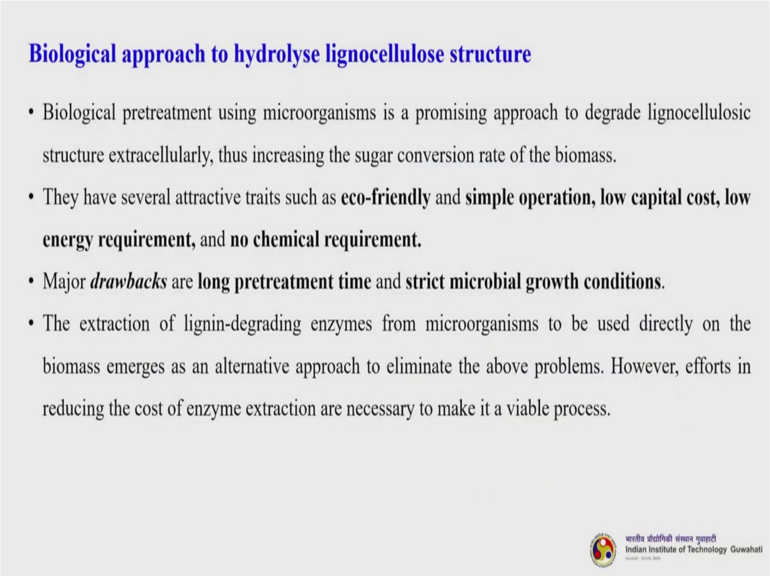

Biological approach to hydrolyse lignocellulose structure

- Biological pretreatment using microorganisms is a promising approach to degrade lignocellulosic structure extracellularly, thus increasing the sugar conversion rate of the biomass.
- They have several attractive traits such as **eco-friendly** and **simple operation, low capital cost, low energy requirement,** and **no chemical requirement.**
- Major ***drawbacks*** are **long pretreatment time** and **strict microbial growth conditions**.
- The extraction of lignin-degrading enzymes from microorganisms to be used directly on the biomass emerges as an alternative approach to eliminate the above problems. However, efforts in reducing the cost of enzyme extraction are necessary to make it a viable process.

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So now we will discuss the biological approaches to hydrolyse lignocellulosic structures. Biological pretreatment using microorganisms is a promising approach to degrade lignocellulose structure extracellularly thus increasing the sugar conversion rate of the biomass. Now they have several attractive traits such as eco-friendly and simple operation, low capital cost, low energy requirement and almost no chemical requirement.

However, the major drawback are the long pre-treatment time and strict microbial growth conditions. So, the extraction of Lignin degrading enzymes from microorganisms to be used directly on the Biomass emerges as an alternative approach to eliminate the above problems. However, efforts in reducing the cost of enzyme extraction are necessary to make it a viable process.

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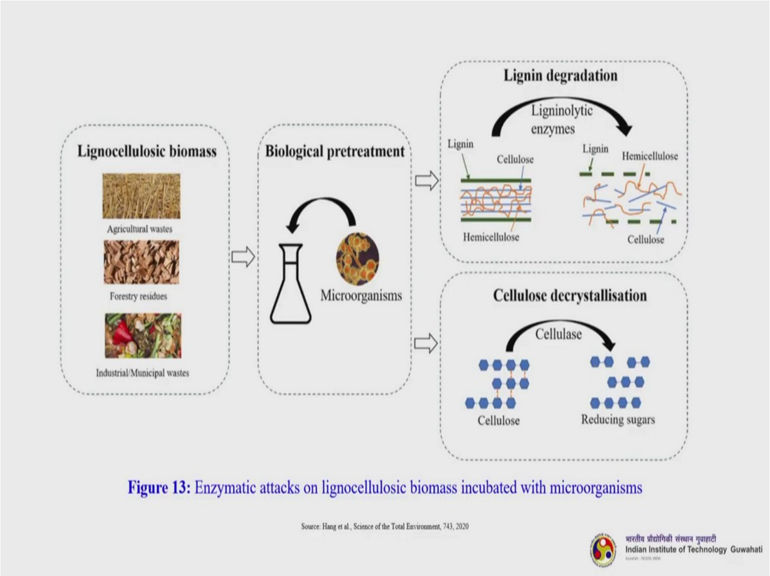

Figure 13: Enzymatic attacks on lignocellulosic biomass incubated with microorganisms

Source: Hang et al., Science of the Total Environment, 743, 2020

So, this is the schematic representation. So, here the lignocellulosic Biomass. Any such lignocellulosic Biomass, treat it with microorganisms. It can be a single strain microorganism. It can be a microbial consortium also; depends on what you are deciding actually. Then again actually it goes for the Lignin degradation, and the cellulose decrystallization.

So, from cellulose to reducing sugars using the cellulase enzyme. So then of course you can further ferment them to alcohol platforms.

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Cellulolytic and ligninolytic microorganisms

- The commonly used microorganisms are **bacteria** and **filamentous fungi** (e.g. ascomycetes and basidiomycetes), which are found ubiquitous in soil, living plants, and lignocellulosic waste material.
- The fungi can be classified into **brown rot, white rot,** and **soft rot fungi**. These microorganisms secret enzymes that are capable of selectively degrade lignin (*ligninolytic fungi*) or hydrolyse cellulose (*cellulolytic bacteria*).
- Biological pretreatment using microorganisms and enzymes extracted from them also offers a great opportunity to produce various high value-added chemicals from the waste-by-product lignin.
- Among the microorganisms, **white-rot fungi** have been extensively studied and proven to be the most effective lignin-degrading species.

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So, cellulolytic and ligninolytic microorganisms. So, the commonly used microorganisms are bacteria and filamentous fungi (for example *Ascomycetes*, *Basidiomycetes*), which are found ubiquitous in soil, living plants and lignocellulosic waste material. The fungi can be classified into brown rot, white rot and soft rot fungi. These microorganisms secrete enzymes that are capable of selectively degrading Lignin (they are known as the ligninolytic fungi) or hydrolyse cellulose (they are known as the cellulolytic bacteria). Now biological pretreatment using microorganisms and enzymes extracted from them also offer a great opportunity to produce various high value-added Chemicals from the waste-by-product lignin. Among the microorganisms, white rot fungi have been extensively studied and proven to be one of the most effective lignin degrading species. However again I am telling you that it is a time-consuming process.

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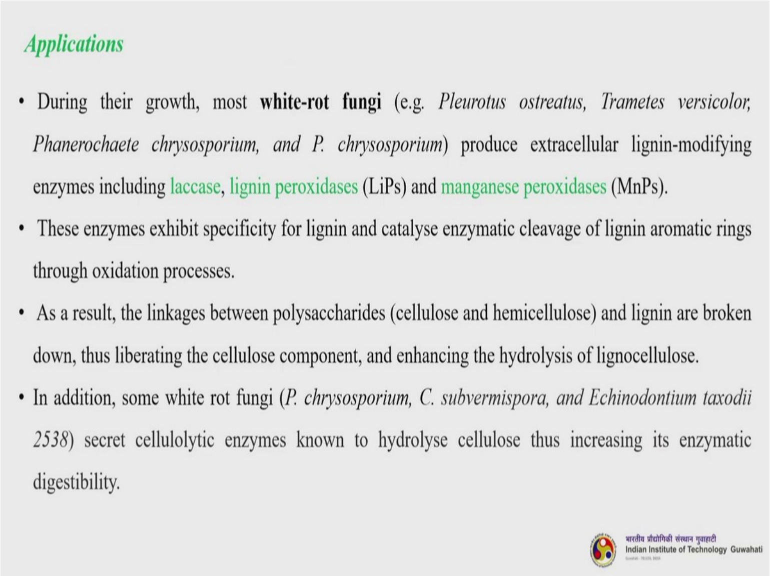

Applications

- During their growth, most **white-rot fungi** (e.g. *Pleurotus ostreatus, Trametes versicolor, Phanerochaete chrysosporium, and P. chrysosporium*) produce extracellular lignin-modifying enzymes including laccase, lignin peroxidases (LiPs) and manganese peroxidases (MnPs).
- These enzymes exhibit specificity for lignin and catalyse enzymatic cleavage of lignin aromatic rings through oxidation processes.
- As a result, the linkages between polysaccharides (cellulose and hemicellulose) and lignin are broken down, thus liberating the cellulose component, and enhancing the hydrolysis of lignocellulose.
- In addition, some white rot fungi (*P. chrysosporium, C. subvermispora, and Echinodontium taxodii 2538*) secret cellulolytic enzymes known to hydrolyse cellulose thus increasing its enzymatic digestibility.

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Let us talk about the applications. So during their growth most white rot fungi, whether it is *Pleurotus ostreatus*, whether it is *Trametes versicolor*, whether it is *Phanerochaete chrysosporium*, produce extracellular Lignin modifying enzymes including laccase, Lignin peroxidase (which are known as LiPs) and manganese peroxidases (which are known as MnPs). Now these are enzymes.

Now these enzymes exhibit specificity for Lignin and catalyse enzymatic cleavage of Lignin aromatic rings through oxidation processes. And these are all slow processes. So, as a result the linkages between polysaccharides and Lignin are broken down thus liberating the cellulose component and enhancing the hydrolysis of lignocellulose. In addition, some of the white rot fungi as mentioned here, for example the *Phanerochaete chrysosporium*, secrete cellulolytic enzymes known to hydrolyse cellulose thus increasing its enzymatic digestibility. So, it is happening simultaneously basically you can say in that spirit.

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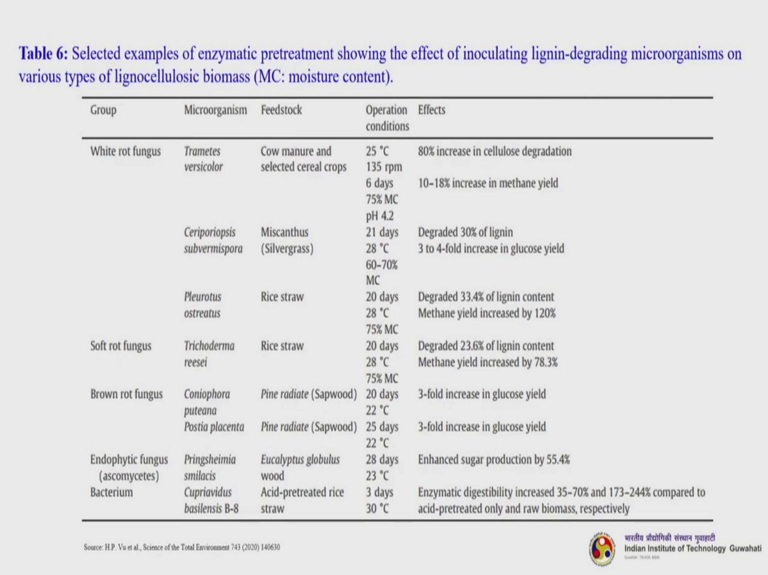

Table 6: Selected examples of enzymatic pretreatment showing the effect of inoculating lignin-degrading microorganisms on various types of lignocellulosic biomass (MC: moisture content).

Group	Microorganism	Feedstock	Operation conditions	Effects
White rot fungus	*Trametes versicolor*	Cow manure and selected cereal crops	25 °C 135 rpm 6 days 75% MC pH 4.2	80% increase in cellulose degradation 10–18% increase in methane yield
	Ceriporiopsis subvermispora	Miscanthus (Silvergrass)	21 days 28 °C 60–70% MC	Degraded 30% of lignin 3 to 4-fold increase in glucose yield
	Pleurotus ostreatus	Rice straw	20 days 28 °C 75% MC	Degraded 33.4% of lignin content Methane yield increased by 120%
Soft rot fungus	*Trichoderma reesei*	Rice straw	20 days 28 °C 75% MC	Degraded 23.6% of lignin content Methane yield increased by 78.3%
Brown rot fungus	*Coniophora puteana*	*Pine radiate* (Sapwood)	20 days 22 °C	3-fold increase in glucose yield
	Postia placenta	*Pine radiate* (Sapwood)	25 days 22 °C	3-fold increase in glucose yield
Endophytic fungus (ascomycetes)	*Pringsheimia smilacis*	*Eucalyptus globulus* wood	28 days 23 °C	Enhanced sugar production by 55.4%
Bacterium	*Cupriavidus basilensis* B-8	Acid-pretreated rice straw	3 days 30 °C	Enzymatic digestibility increased 35–70% and 173–244% compared to acid-pretreated only and raw biomass, respectively

Source: H.P. Vu et al., Science of the Total Environment 743 (2020) 140630

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So, some of the different types of fungus and bacteria and the feedstock and the operating conditions and what has happened after the pre-treatment process. So we will see one thing; the white rot fungus *T. versicolor*. Cow manure and selected cereal crops were being considered. Operation condition is 25 degrees centigrade, 135 RPM rotations per minute, 6 days, 75% MC. and pH is 4.2. So, the result is 80% increase in cellulose degradation. It is an excellent result.

But it has taken time, you can understand that it is 6 days' time. So, 10 to 18% increase in methane yield. So if you look at this particular table in a proper way you will understand that efficiency is not an issue. The only issue is that it is very time consuming and then since we are dealing with live organisms, we have to ensure each and every day that their growth conditions are maintained.

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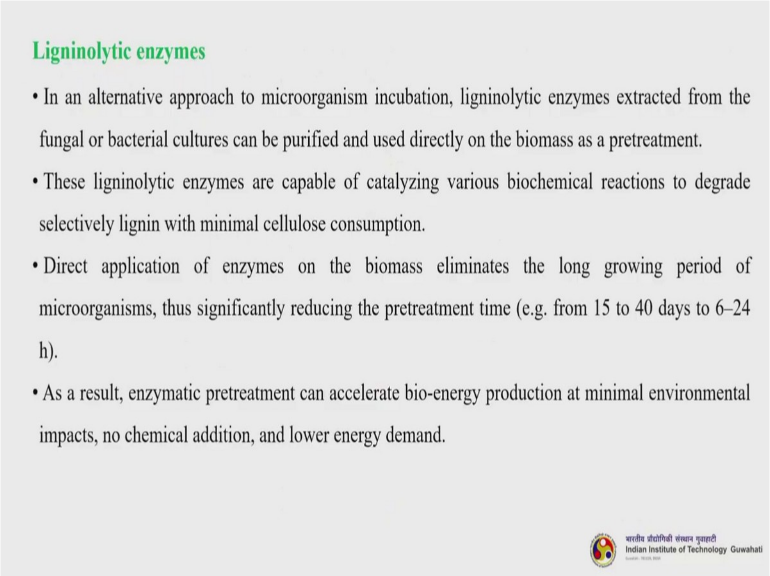

Ligninolytic enzymes

- In an alternative approach to microorganism incubation, ligninolytic enzymes extracted from the fungal or bacterial cultures can be purified and used directly on the biomass as a pretreatment.
- These ligninolytic enzymes are capable of catalyzing various biochemical reactions to degrade selectively lignin with minimal cellulose consumption.
- Direct application of enzymes on the biomass eliminates the long growing period of microorganisms, thus significantly reducing the pretreatment time (e.g. from 15 to 40 days to 6–24 h).
- As a result, enzymatic pretreatment can accelerate bio-energy production at minimal environmental impacts, no chemical addition, and lower energy demand.

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So ligninolytic enzymes. In an alternative approach to microorganism incubation, ligninolytic enzymes extracted from the fungal and bacterial cultures can be purified and used directly on the biomass as pre-treatment. Here what we are talking about that, instead of using the microorganisms directly we extract the enzymes which is responsible for this pre-treatment, delignification or disintegration, can be extracted and purified and then it will be used.

So now in this case, we are done away with the growth condition of the microorganisms. Only we have to maintain proper conditions for the enzymatic attack on to the plant cell or the biomass cells basically. But please understand that, it is a better technique. However, extracting and purifying enzymes from microorganisms is a very tedious job and is time-consuming as well as it is a high cost matter.

So, these ligninolytic enzymes are capable of catalysing various biochemical reactions to degrade selectively Lignin with minimal cellulose consumption. The direct application of enzymes on the Biomass eliminates the long growing period of microorganisms thus significantly reducing the pretreatment time. So, 15 to 40 days to almost 6 to 24 hours. That is fine, but again, the cost has a bigger role to play.

So as a result, enzymatic pre-treatment can accelerate bioenergy production at minimal environmental impacts, no chemical addition and lower energy.

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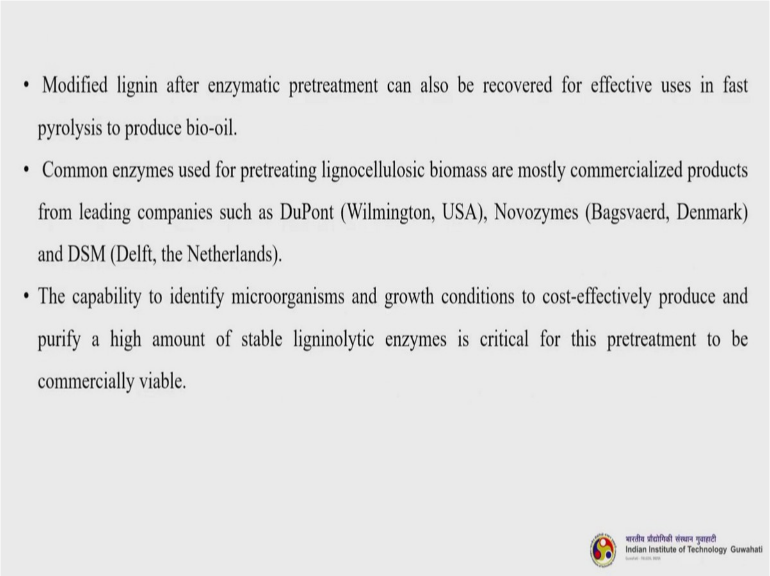

- Modified lignin after enzymatic pretreatment can also be recovered for effective uses in fast pyrolysis to produce bio-oil.
- Common enzymes used for pretreating lignocellulosic biomass are mostly commercialized products from leading companies such as DuPont (Wilmington, USA), Novozymes (Bagsvaerd, Denmark) and DSM (Delft, the Netherlands).
- The capability to identify microorganisms and growth conditions to cost-effectively produce and purify a high amount of stable ligninolytic enzymes is critical for this pretreatment to be commercially viable.

Modified Lignin after enzymatic pretreatment can also be recovered for effective uses in fast pyrolysis to produce biooil. This is one of the significant works. So, common enzymes used for prre-treating lignocellulosic Biomass are mostly commercialized products from leading companies such as DuPont, Novozymes and DSM. So these are some of the companies, the enzyme making companies basically and very well known.

The capability to identify microorganisms and growth conditions to cost-effectively produce and purify high amount of stable ligninolytic enzymes is critical for this pretreatment to be commercially viable.

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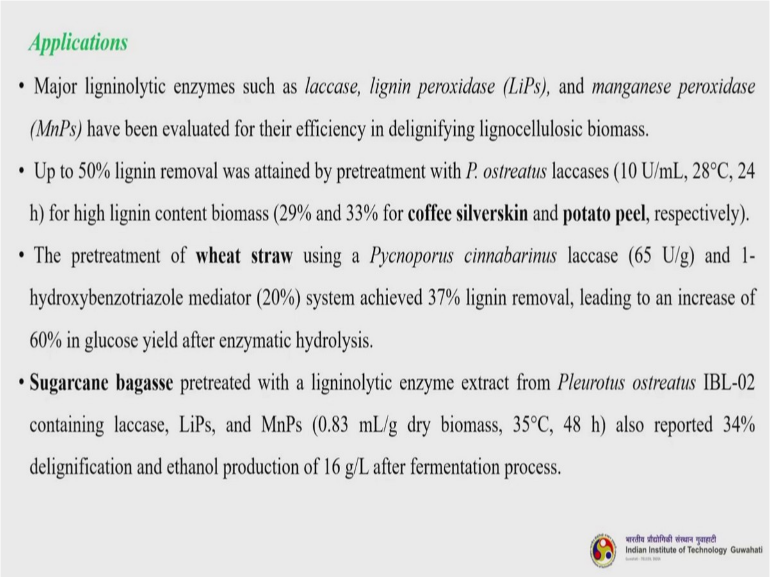

Applications

- Major ligninolytic enzymes such as *laccase, lignin peroxidase (LiPs),* and *manganese peroxidase (MnPs)* have been evaluated for their efficiency in delignifying lignocellulosic biomass.
- Up to 50% lignin removal was attained by pretreatment with *P. ostreatus* laccases (10 U/mL, 28°C, 24 h) for high lignin content biomass (29% and 33% for **coffee silverskin** and **potato peel**, respectively).
- The pretreatment of **wheat straw** using a *Pycnoporus cinnabarinus* laccase (65 U/g) and 1-hydroxybenzotriazole mediator (20%) system achieved 37% lignin removal, leading to an increase of 60% in glucose yield after enzymatic hydrolysis.
- **Sugarcane bagasse** pretreated with a ligninolytic enzyme extract from *Pleurotus ostreatus* IBL-02 containing laccase, LiPs, and MnPs (0.83 mL/g dry biomass, 35°C, 48 h) also reported 34% delignification and ethanol production of 16 g/L after fermentation process.

So major ligninolytic enzymes such laccase, LiPs which is called Lignin peroxidase and MnPs - Manganese peroxidase have been evaluated for their efficiency in delignifying lignocellulosic biomass. Up to 50% lignin removal was attained by pre-treatment with *P. ostreatus* laccases for high Lignin content biomass (29% and 33% for coffee silverskin and potato peel respectively).

The pre-treatment of wheat straw using a *P. cinnabarinus* laccase achieved 37% Lignin removal, leading to an increase of 60% in glucose yield after the enzymatic hydrolysis. Sugarcane bagasse pre-treated with ligninolytic enzyme extracted from *P. ostreatus* IBL-02 strain containing laccase, LiPs and MnPs also reported 34% delignification and ethanol production of 16 grams per litre after the fermentation process.

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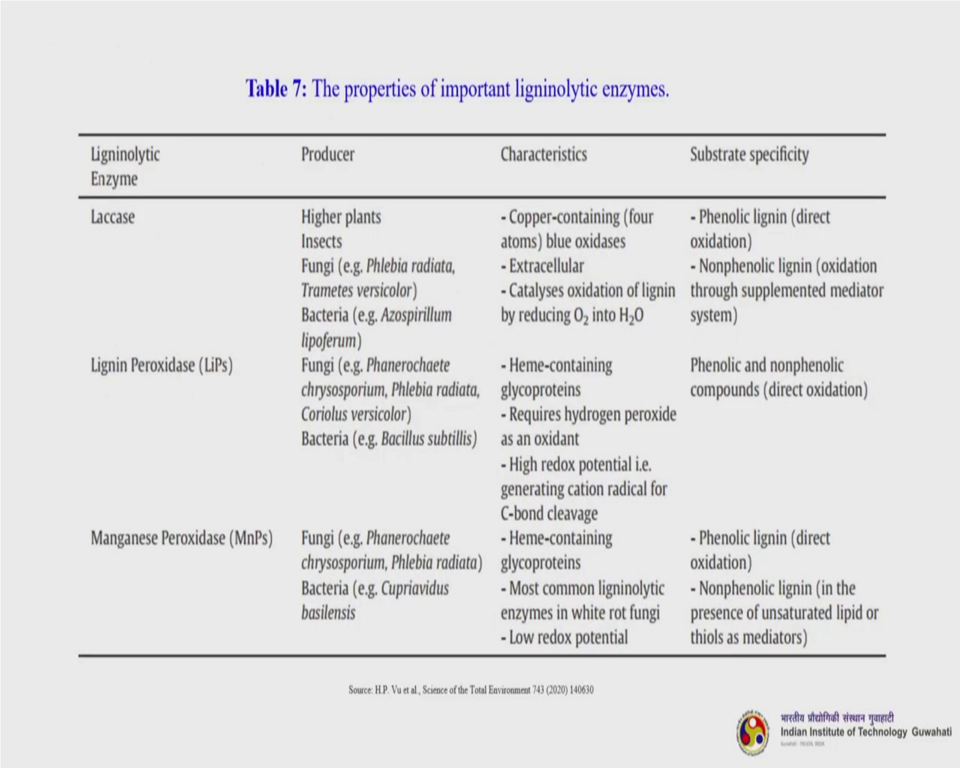

Table 7: The properties of important ligninolytic enzymes.

Ligninolytic Enzyme	Producer	Characteristics	Substrate specificity
Laccase	Higher plants Insects Fungi (e.g. *Phlebia radiata, Trametes versicolor*) Bacteria (e.g. *Azospirillum lipoferum*)	- Copper-containing (four atoms) blue oxidases - Extracellular - Catalyses oxidation of lignin by reducing O_2 into H_2O	- Phenolic lignin (direct oxidation) - Nonphenolic lignin (oxidation through supplemented mediator system)
Lignin Peroxidase (LiPs)	Fungi (e.g. *Phanerochaete chrysosporium, Phlebia radiata, Coriolus versicolor*) Bacteria (e.g. *Bacillus subtillis*)	- Heme-containing glycoproteins - Requires hydrogen peroxide as an oxidant - High redox potential i.e. generating cation radical for C-bond cleavage	Phenolic and nonphenolic compounds (direct oxidation)
Manganese Peroxidase (MnPs)	Fungi (e.g. *Phanerochaete chrysosporium, Phlebia radiata*) Bacteria (e.g. *Cupriavidus basilensis*	- Heme-containing glycoproteins - Most common ligninolytic enzymes in white rot fungi - Low redox potential	- Phenolic lignin (direct oxidation) - Nonphenolic lignin (in the presence of unsaturated lipid or thiols as mediators)

Source: H.P. Vu et al., Science of the Total Environment 743 (2020) 140630

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This table lists some of the properties of the important ligninolytic enzymes. So, you can see three enzymes are listed here: laccase, LiPs and MnPs. And different types of plant materials. What are the characteristic features, and what is the substrate specificity. So, you please go through it later on when you go through the lecture note. So, with this I conclude today's lecture.

And tomorrow we will be discussing dilute acid, alkali hydrolysis and Ozone treatment pretreatment methods. If you have any query, please feel free to write to me at kmohanty@iitg.ac.in or please register your query in the Swayam portal. So, thank you very much.