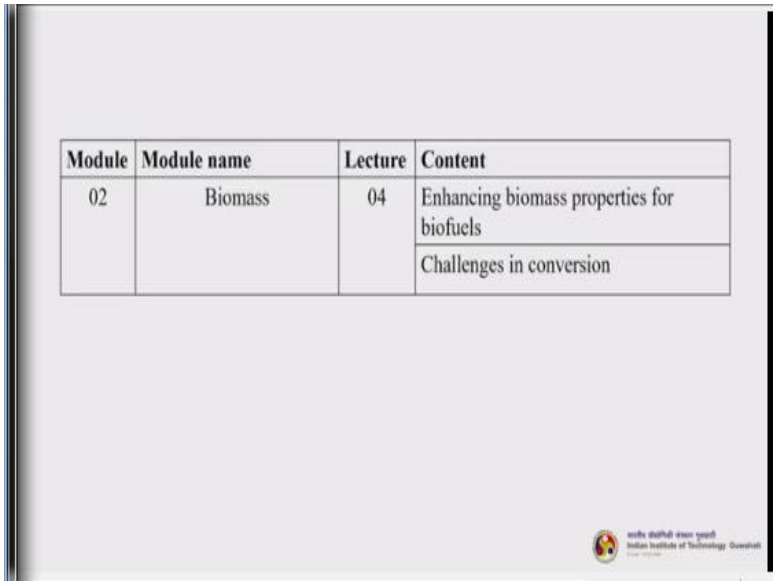
Biomass Conversion and Biorefinery
Prof. Kaustubha Mohanty
Department of Chemical Engineering
Indian Institute of Technology-Guwahati

Module 02
Lecture-06
Enhancing Biomass Properties

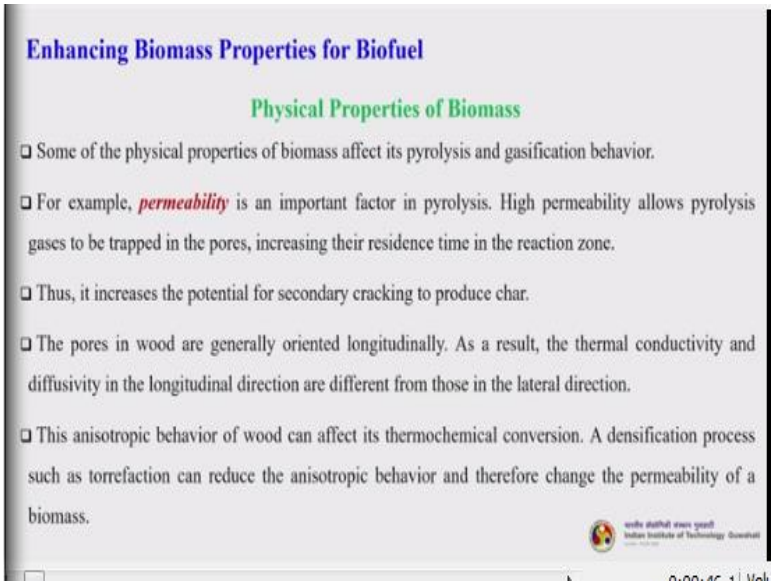
Good morning students, this is lecture 4 of module 2.

(Refer Slide Time: 00:30)



In today's lecture, we will be discussing about the biomass properties, how we can enhance some of these properties and what are the challenges in conversion of the biomass into biofuels?

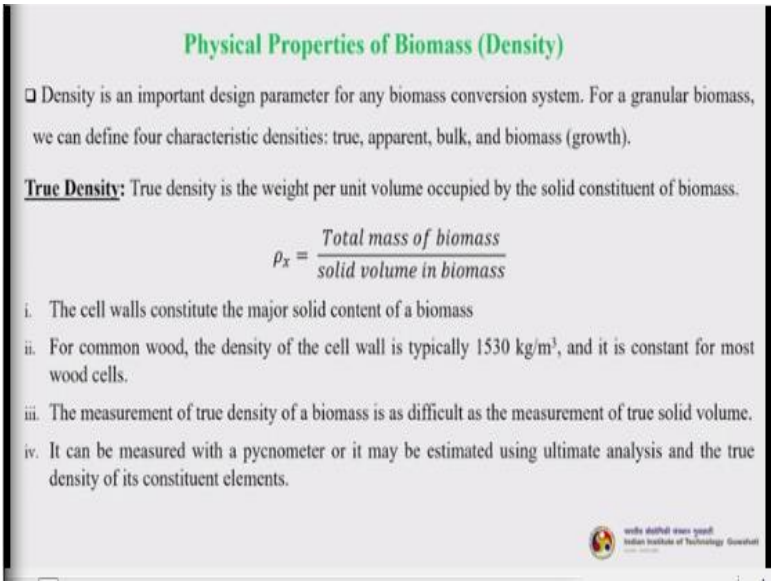
(Refer Slide Time: 00:43)



So, let us begin by discussing about physical properties of biomasses. So, some of the physical properties of biomass affect it is pyrolysis and gasification behavior (basically the thermochemical conversion). For example, permeability is an important factor in pyrolysis. High permeability will allow the pyrolysis gases to be trapped in the pores, increasing their residence time in the reaction zone.

Thus, it increases the potential for secondary cracking to produce char. The pores in wood are generally oriented longitudinally. As a result, the thermal conductivity and diffusivity in the longitudinal direction are different from those in the lateral direction. This anisotropic behavior of wood can affect its thermochemical conversion. A densification process such as torrefaction can reduce the anisotropic behavior and therefore change the permeability of biomass. Hence permeability is an important property with respect to the pyrolysis.

(Refer Slide Time: 01:38)

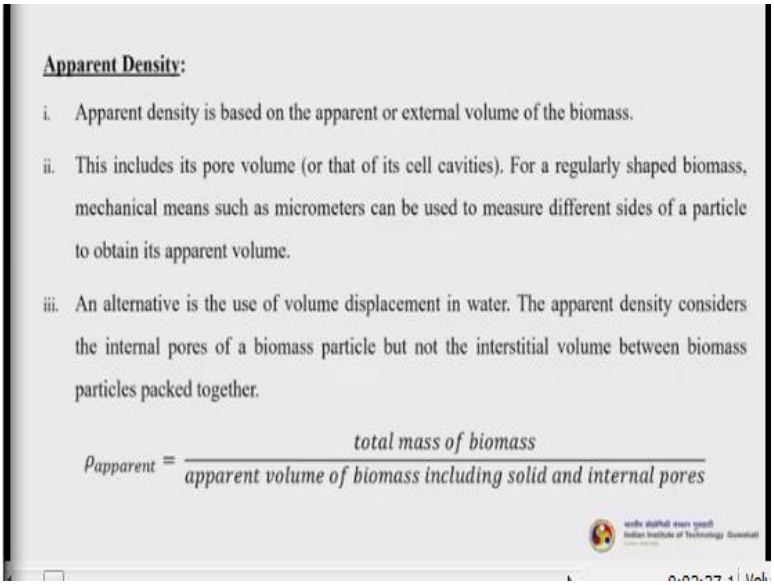


Density is an important design parameter for any biomass conversion system. For a granular biomass we can define four characteristic densities: true density, apparent density, bulk density and biomass (growth) density. Now true density is the weight per unit volume occupied by the solid constituent of biomass. So, it is given by total mass of biomass divided by solid volume in biomass.

$$\rho_x = \frac{Total\ mass\ of\ biomass}{Solid\ volume\ in\ biomass}$$

The cell walls constitute the major solid content of a biomass. For common wood the density of the cell wall is typically 1530 kg per meter cube and it is constant for most of the wood cells. The measurement of true density of a biomass is as difficult as the measurement of a true solid volume. So, it can either be measured with a pycnometer or maybe estimated using ultimate analysis and true density of the constituent elements.

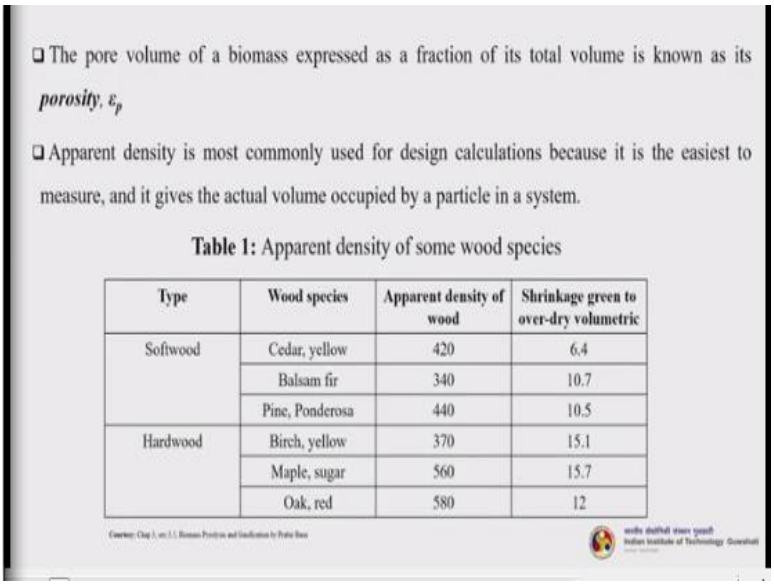
(Refer Slide Time: 02:26)



Next is apparent density; so it is based on the apparent or external volume of the biomass. This includes its pore volume or you can say the volume of all the cell cavities. For a regular shaped biomass, mechanical means such as micrometers can be used to measure different sides of a particle to obtain its apparent volume. An alternative is the use of volume displacement in water. The apparent density considered the internal pores of a biomass particle but not the interstitial volume between the biomass packed together. So this is the equation for the apparent density.

$$\rho_{apparent} = \frac{Total\ mass\ of\ biomass}{Apparent\ volume\ of\ biomass\ including\ solid\ and\ internal\ pores}$$

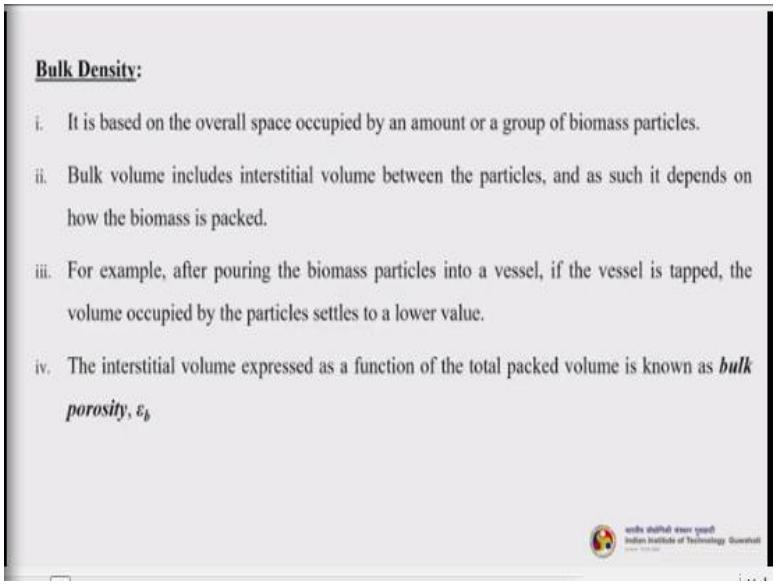
(Refer Slide Time: 02:59)



The pore volume of a biomass expressed as a fraction of it is total volume is known as it is porosity. Apparent density is most commonly used for design calculations because it is the easiest to measure and it

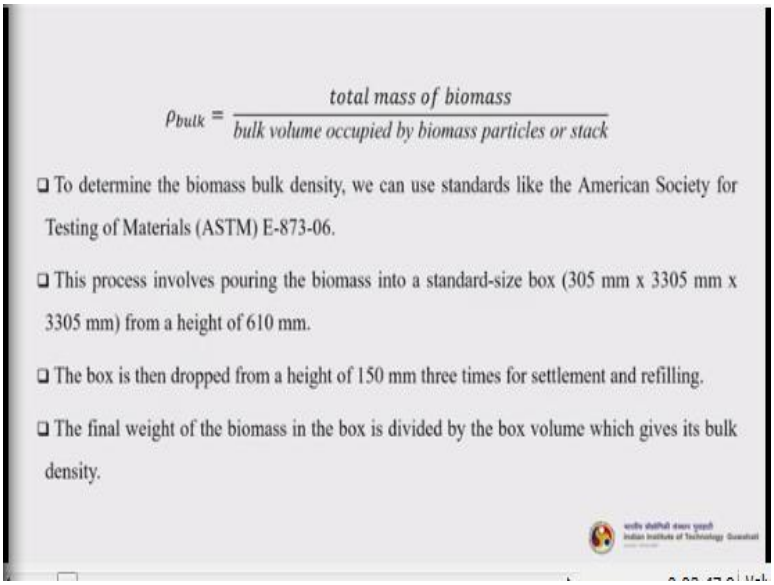
gives the actual volume occupied by a particle in a system. So, you can see the table 1 has given apparent density of some of the wood species.

(Refer Slide Time: 03:19)



Then the bulk density; So bulk density is based on the overall space occupied by an amount or a group of biomass particles. Bulk volume includes interstitial volume between the particles and as such it depends on how the biomass is packed. For example, after pouring the biomass particles into a vessel, if the vessel is tapped, the volume occupied by the particles settles to a lower value. The interstitial volume expressed as a function of the total packed volume is known as bulk porosity.

(Refer Slide Time: 03:48)

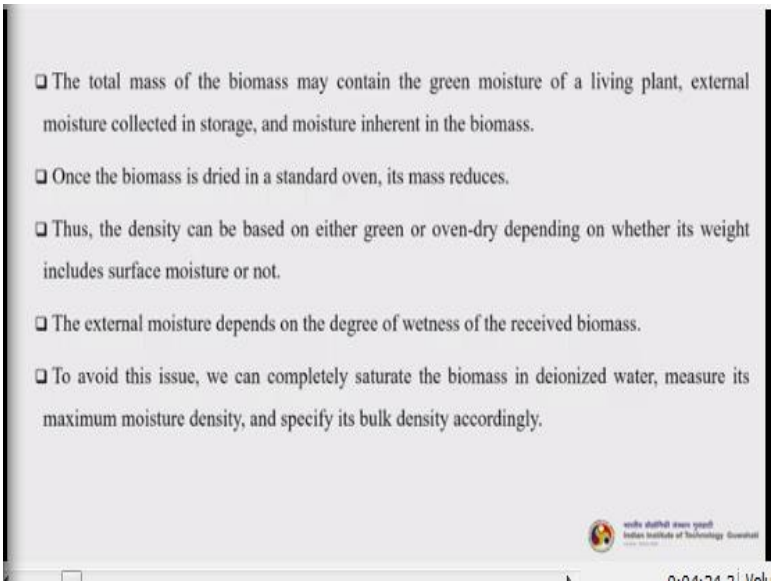


So, this is the bulk density equation.

$$\rho_{bulk} = \frac{Total\ mass\ of\ biomass}{Bulk\ volume\ occupied\ by\ biomass\ particles\ or\ stack}$$

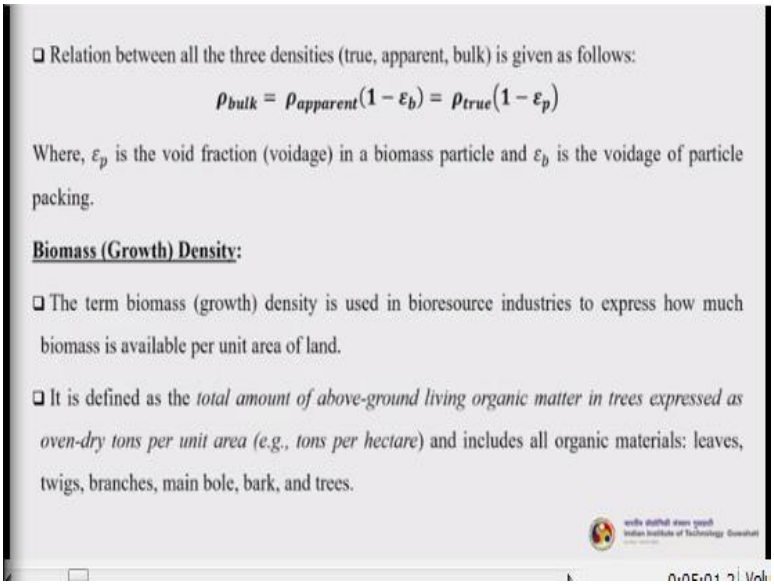
So to determine the biomass bulk density, we can use standard like the American Society for testing materials, E-873-06 standard. So, this process involves pouring the biomass into a standard sized box of a particular size (given here), from a height of 610 millimeters. The box is then dropped from a height of 150 millimeters three times for settlement and refilling. The final weight of the biomass in the box is divided by the box volume which gives its bulk density. This is how we can measure bulk density of the biomass.

(Refer Slide Time: 04:24)



The total mass of the biomass may contain the green moisture of a living plant, external moisture collected during storage and moisture inherent in the biomass. So, once the biomass is dried in a standard oven, its mass reduces. Thus, the density can be based on either green or oven-dry depending on whether its weight includes surface moisture or not. The external moisture depends on the degree of wetness of the received biomass. To avoid this issue, we can completely saturate the biomass in deionized water, measure its maximum moisture density, and specify it is bulk density accordingly.

(Refer Slide Time: 05:01)



So, there is a relation between these three densities as given here.

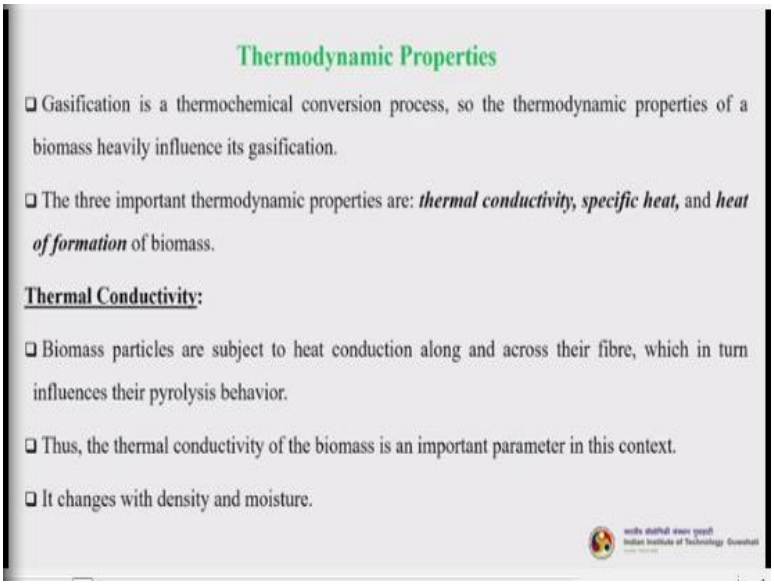
$$\rho_{bulk} = \rho_{apparent}(1 - \varepsilon_b) = \rho_{true}(1 - \varepsilon_p)$$

Where, epsilon p is the void fraction or voidage in a biomass particle and epsilon b is the voidage of particle packing.

So, then the next is the biomass growth density. It is specifically for biomass not for other materials. So, the term biomass growth density is used in bioresource industries to express how much biomass is available per unit area of land.

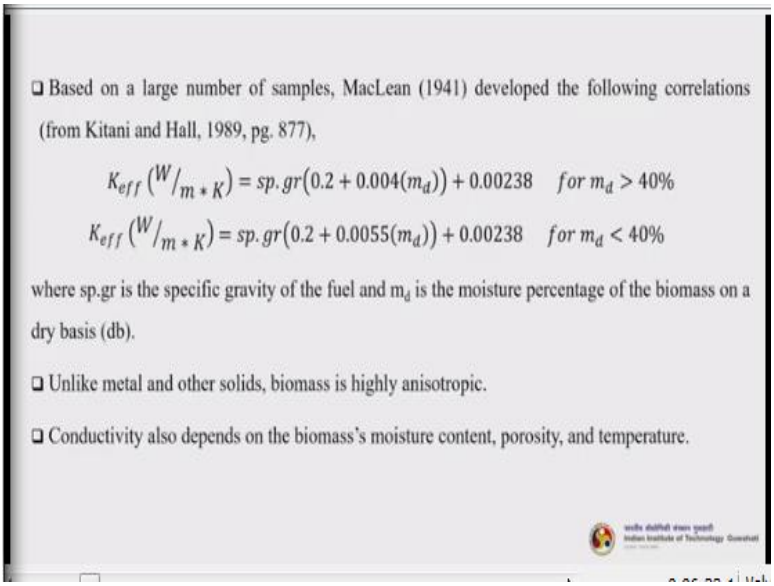
So, it is defined as the total amount of above-ground living organic matter in trees expressed as oven-dry tons per unit area (that is basically the tons per hectare) and includes all organic materials: whether it is leaves, twigs, branches, main bole, bark and the trees.

(Refer Slide Time: 05:44)



Then we will see some of the thermodynamic properties. When we talk about gasification, which is a thermo chemical conversion process, the thermodynamic properties of biomass heavily influence its gasification properties (so do for pyrolysis also). So, the three important thermodynamic properties are thermal conductivity, specific heat and heat of formation. Now what is thermal conductivity: biomass particles are subject to heat conduction along and across their fibre which in turn influences the pyrolysis behavior, and/or gasification behavior of course. Thus the thermal conductivity of the biomass is an important parameter in this context. It changes with density and moisture.

(Refer Slide Time: 06:22)



So, how it changes? We will see. So based on a large number of samples, MacLean in 1941, developed the following correlations (which is adopted from this Kitani and Hall 1989, a book is given in the page number 877). So, K effective watts per meter Kelvin, is specific gravity in bracket 0.2 + 0.004 into m d +

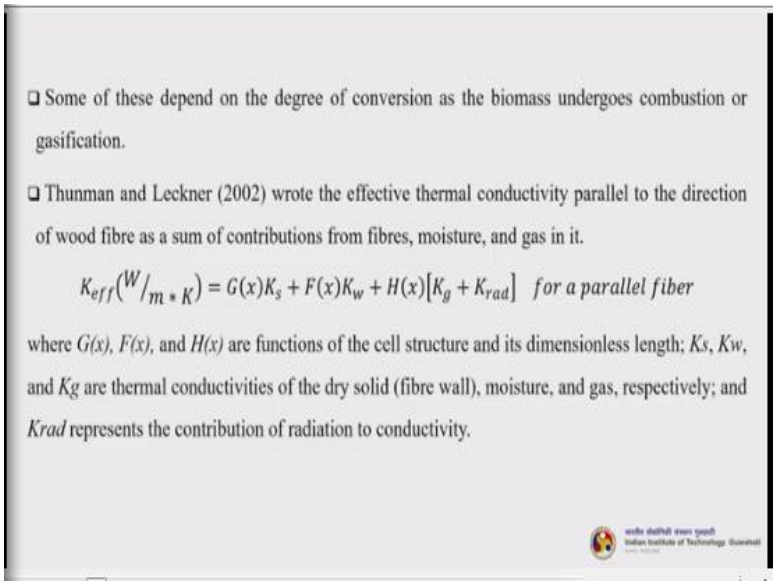
0.00238. So, this particular correlation, as you know correlations are valid for certain range, so for this particular correlation it is only valid when your m d is greater than 40%. Now another equation which is given by this, you can see that equation also (I am not reading it). So that is valid when m d is less than 40%.

$$K_{eff}\left({}^{W}/_{m*K}\right) = sp.gr\left(0.2 + 0.004(m_d)\right) + 0.00238 \quad for\ m_d > 40\%$$

$$K_{eff}\left({}^{W}/_{m*K}\right) = sp.gr\left(0.2 + 0.0055(m_d)\right) + 0.00238 \quad for\ m_d < 40\%$$

So, two equations or correlations were proposed, the first one is when the m d is greater than 40% and the second one is when the m d is less than 40%. So, m d is the moisture percentage of the biomass on a dry basis. So, unlike metal and other solids biomass is highly anisotropic. Conductivity also depends on the biomass's moisture content, porosity as well as temperature.

(Refer Slide Time: 07:33)

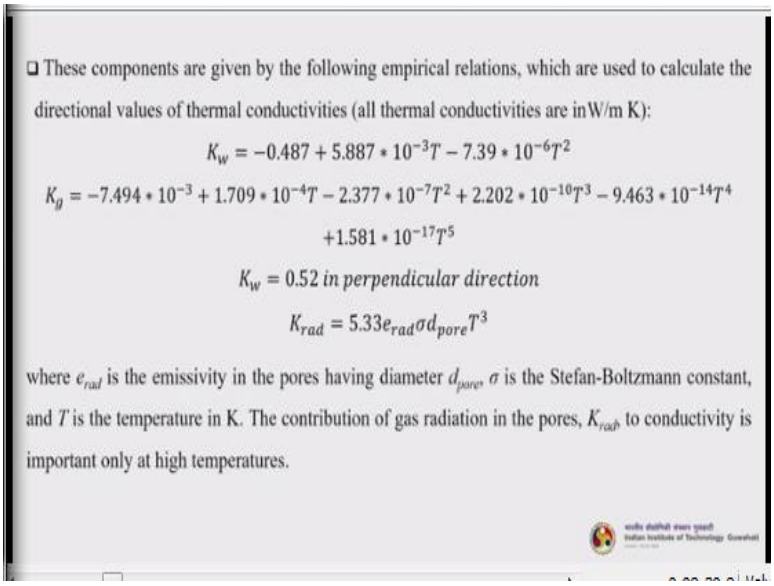


Some of these depend on the degree of conversion as the biomass undergoes combustion or gasification. Thunman and Leckner in 2002 wrote the effective thermal conductivity parallel to the direction of wood fibre as a sum of contributions from fibres, moisture and gas in it. It is a good equation which many of us working on the biomass sector they use it. So, K effective in watts per meter Kelvin, is G K s + F K + H into K g + K rad for a parallel fiber.

$$K_{eff}\left({}^{W}/_{m*K}\right) = G(x)K_s + F(x)K_w + H(x)\left[K_g + K_{rad}\right]\ for\ a\ parallel\ fiber$$

Where, G x, F x and H x are the functions of the cell structure and it is dimensionless length; K s, K w and K g are thermal conductivities of the dry solid (that is fibre wall), moisture and gas respectively; And K rad represents the contribution of radiation to conductivity; it is a very nice or excellent equation which is being adopted universally.

(Refer Slide Time: 08:29)



So, we will see few more equations. So these components are given by the following empirical relations, which are to be used to calculate the directional values of the thermal conductivities. Here all the thermal conductivities are measured in watts per meter Kelvin. So, Kw is given by this equation - 0.487 + 5.887 into 10 rise of - 3 into T - 7.39 into 10 power of - 6 T square. Now K z is given by this long equation, K w is 0.52 in perpendicular direction and K rad, so which is coming from the radiation is 5.33 e of radiation then sigma d pore and T cube.

$$K_w = -0.487 + 5.887 * 10^{-3}T - 7.39 * 10^{-6}T^2$$

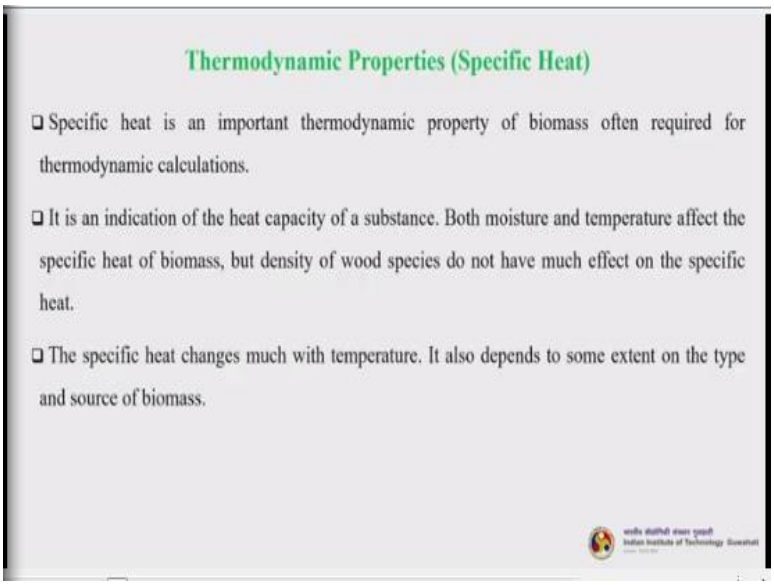
$$K_g = -7.494 * 10^{-3} + 1.709 * 10^{-4}T - 2.377 * 10^{-7}T^2 + 2.202 * 10^{-10}T^3 - 9.463 * 10^{-14}T^4 + 1.581 * 10^{-17}T^5$$

$$K_w = 0.52 \; in \; perpendicular \; direction$$

$$K_{rad} = 5.33 e_{rad} \sigma d_{pore} T^3$$

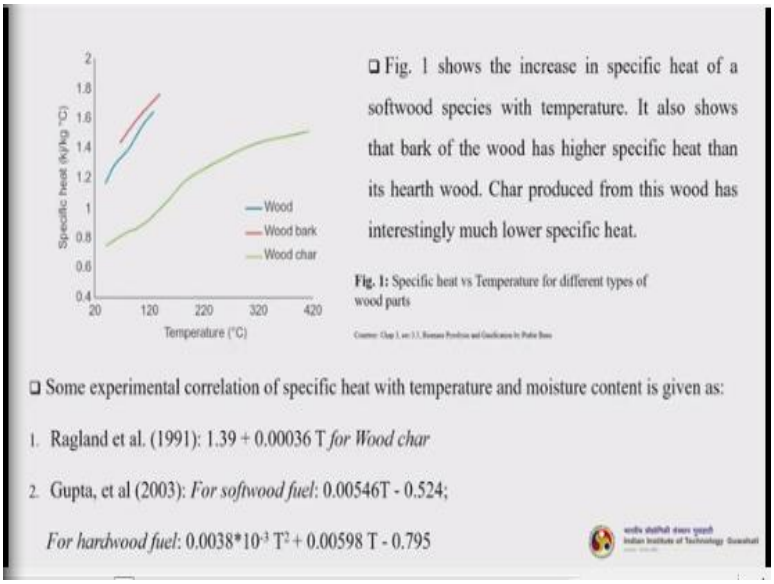
So, E rad is the emissivity of the pores having diameter d pore and sigma is the Stefan Boltzmann constant, and T is the temperature in Kelvin. The contribution of gas radiation in the pores K rad, to conductivity is important only at high temperatures.

(Refer Slide Time: 09:32)



Now we will talk about specific heat, another important thermodynamic property. So, specific heat of biomass is often required for thermodynamic calculations. So, it is an indication of the heat capacity of a substance. Both moisture and temperature affect the specific heat of biomass. But density of wood species do not have much effect on the specific heat. So, the specific heat changes much with temperature it also depends on to some extent on the type and source of the biomass.

(Refer Slide Time: 10:01)



So, please look at this particular figure. So, you can see there are three (specific heat of a softwood species parts) species temperature versus specific heat has been given here. So, the below one that is the wood char, the red one is the wood bark and the blue one is the wood. So, this figure shows the increase in specific heat of a softwood species with temperature. It also shows that bark of the wood has higher specific heat, when it is compared to the other two species.

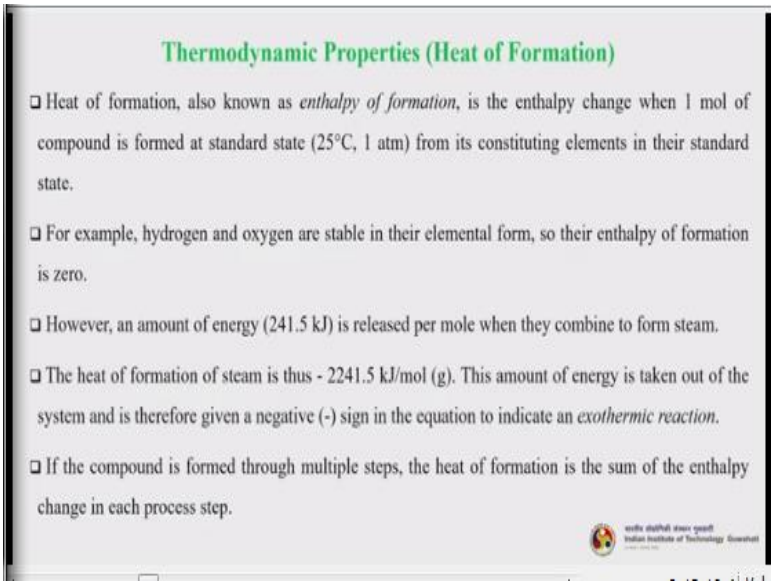
Char produced from this wood has interestingly much lower specific heat. Some experimental correlation of specific heat with temperature and moisture content is given as this Ragland et al equation 1.39 + 0.00036 T for the wood char, Gupta et al suggested for the softwood fuel 0.00546 into T - 0.524 and for the hardwood fuel 0.0038 into 10 power of - 3 T square + 0.00598 T - 0.795.

Ragland et al., (1991): 1.39 + 0.00036 T for *Wood char*
Gupta et al., (2003): For softwood fuel: 0.00546T – 0.524
For hardwood fuel: $0.0038 * 10^{-3}T^2 + 0.00598T – 0.795$

Now I want to say something about this so called relations; please note I do not know whether most of you are aware of the fact or not regarding these correlations. So, let us understand what is the meaning of correlation; why suddenly some particular number of 0.003, some x square some T square is coming into picture. Now please understand that any correlation is an equation which is developed by doing certain fixed number of experiments; it is all based on the experimental results.

That is why they have some specificity or limitation, like we are showing m d in the last equation. I told you that these particular two equations, one equation is valid when the moisture content is greater than 40%, another equation is valid when the moisture content is less than 40%. So, that the reason is that this is how the experiments are being done and this is how the equation has come from different experiments and mostly they are average values, there is a particular way to do it. So, you need to understand that any correlation are experimentally derived equations and has some limitations.

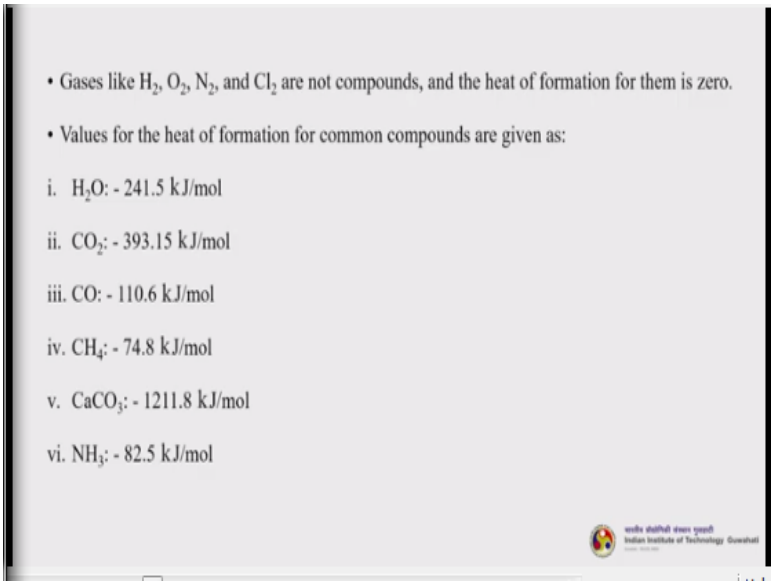
(Refer Slide Time: 12:16)



So, then let us understand the heat of formation; heat of formation also known as the enthalpy of formation is the enthalpy change when one mole of compound is formed at standard state, that is 25 degrees centigrade and 1 atmosphere from its constituting elements in their standard state. Now for example, hydrogen and oxygen are stable in their elemental form, so their enthalpy of formation is always zero, in elemental form.

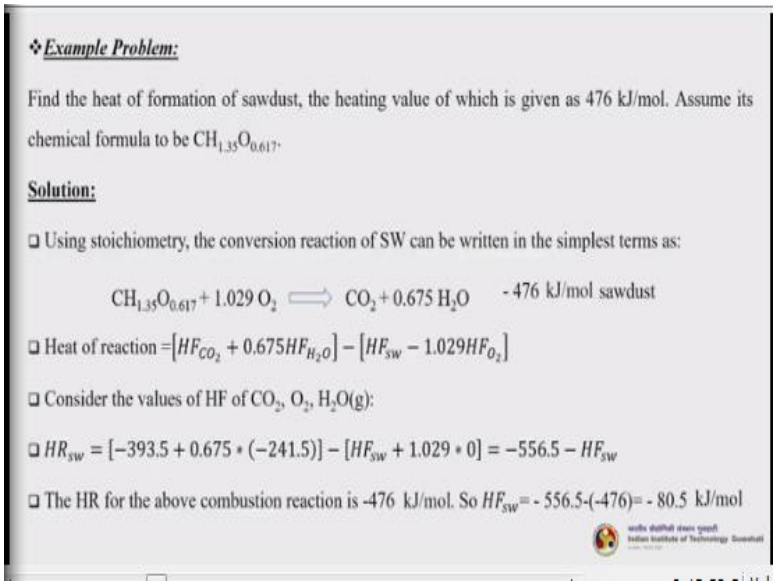
Now however an amount of energy 241.5 kilojoules is released per mole when they are combined to form steam, that means hydrogen and oxygen. So, the heat of formation of steam is thus - 2241.5 kilojoules per mole that is in the gaseous form. So, this amount of energy is taken out of the system and is therefore given a negative sign in the equation to indicate that it is an exothermic reaction. If the compound is formed through multiple steps, the heat of formation is the sum of the enthalpy change in each process step.

(Refer Slide Time: 13:17)



So, gases like hydrogen, oxygen, nitrogen and chlorine are not compounds and the heat of formation for them is zero. Values for heat of formationn for some of the compounds are given, you can see it later water is - 241, carbon dioxide is - 393, right there are a few were given just for your understanding.

(Refer Slide Time: 13:37)



Now this is a small example problem, you can just go through it. So, find that heat of formation of sawdust, the heating value of which is given as 476 kilojoules per mole, assume its chemical formula to be CH 1.35 O 0.617. Now stoichiometry has to be written. The conversion of SW can be written in the simplest term as CHO + 1.029 Oxygen will give **C** carbon dioxide + water 0.6575 water - 476 kilojoules per mole of sawdust is the isothermic reaction.

So, heat of reaction you can calculate like this, HF of carbon dioxide + 0.675 HF of water - HF of sawdust - 1.029 HF of oxygen. So, consider the values of HF of heat of formation of carbon dioxide, oxygen and water and substitute. So, you will get heat of reaction for the above combustion reaction - 476, it is given. So, you will calculate the heating value to be - 80.5 kilojoules per mole.

$$CH_{1.35}1.029\,O_2 \rightarrow CO_2 + 0.675\,H_2O \quad -476\,kJ/mol\ sawdust$$

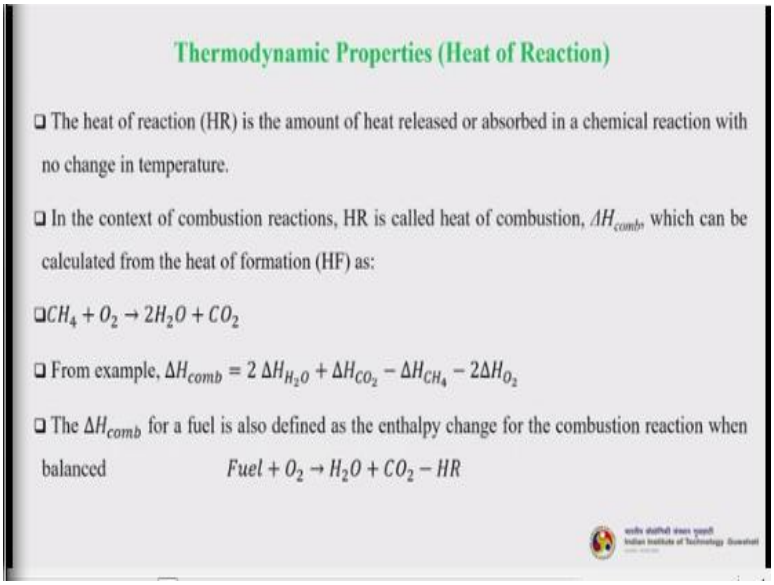
$$Heat\ of\ reaction = \left[HF_{CO_2} + 0.675HF_{H_2O}\right] - \left[HF_{sw} - 1.029HF_{O_2}\right]$$

Consider the values of HF of CO_2, O_2, H_2O (g):

$$HR_{sw} = [-393.5 + 0.675 * (-241.5)] - [HF_{sw} + 1.029 * 0] = -556.5 - HF_{sw}$$

The HR for the above combustion reaction is -476 kJ/mol. So, $HF_{sw} = -556.5(-476) = -80.5\,kJ/mol$

(Refer Slide Time: 14:46)



So, the heat of reaction is the amount of heat released or absorbed in a chemical reaction with no change in temperature. In the context of combustion reactions, heat of reaction is called the heat of combustion. deltaH comb submits a combination, which can be calculated from the heat of formation as: methane plus oxygen gives you 2 water plus carbon dioxide, so heat of combination will be 2 of deltaH water + deltaH carbon dioxide - deltaH methane - deltaH oxygen.

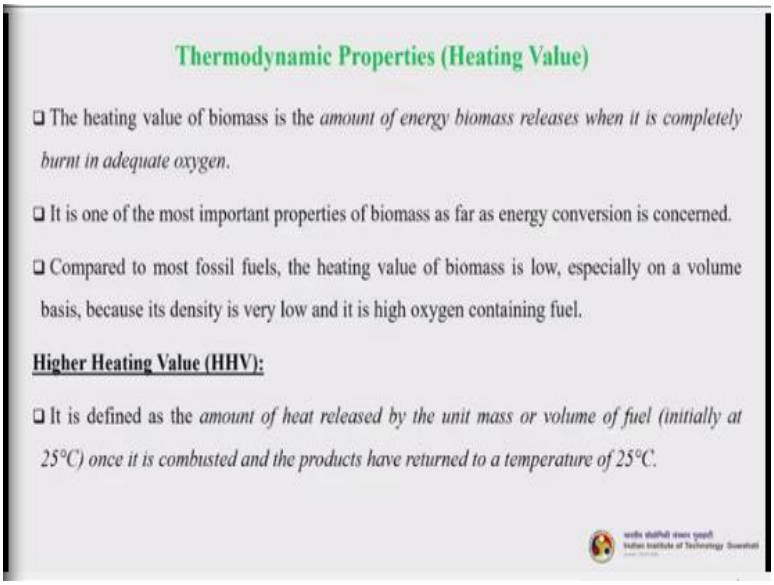
$$CH_4 + O_2 \rightarrow 2H_2O + CO_2$$

$$\Delta H_{comb} = 2\Delta H_{H_2O} + \Delta H_{CO_2} - \Delta H_{CH_4} - 2\Delta H_{O_2}$$

So, the deltaH comb, the heat of combustion of the combination for the entire fuel, can be defined as the enthalpy change for the combustion reaction when it is a balance. So, fuel plus oxygen will give you water plus carbon dioxide minus heat of reaction.

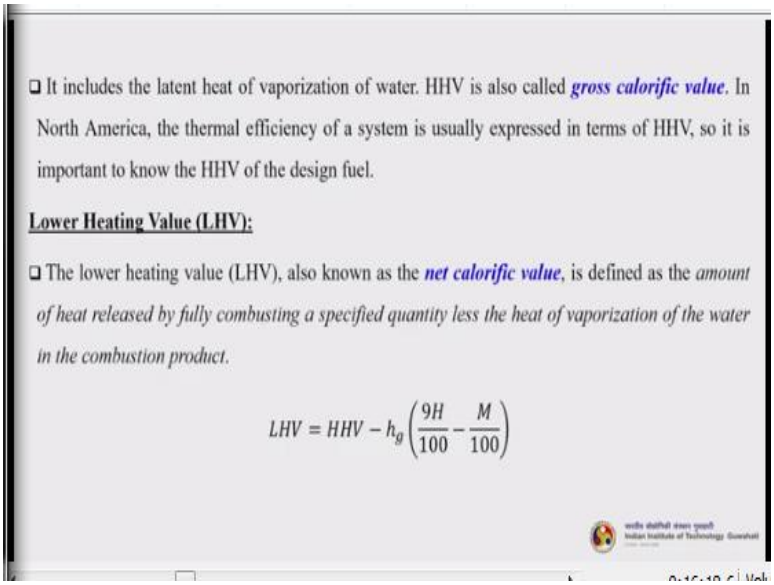
$$Fuel + O_2 \rightarrow H_2O + CO_2 - HR$$

(Refer Slide Time: 15:34)



So, the heating value of biomass is the amount of energy biomass releases when it is completely burnt in adequate oxygen. So, it is one of the most important properties of biomass as far as energy conversion is concerned. Compared to most fossil fuels, the heating value of biomass is low especially on a volume basis because its density is very low and it is high oxygen containing fuel. Higher heating value, what is higher heating value? This is also very important to understand. So, it is defined as the amount of heat released by the unit mass or volume of fuel, initially at 25 degrees centigrade, once it is combusted, and the products have returned to a temperature of 25 degrees centigrade.

(Refer Slide Time: 16:18)

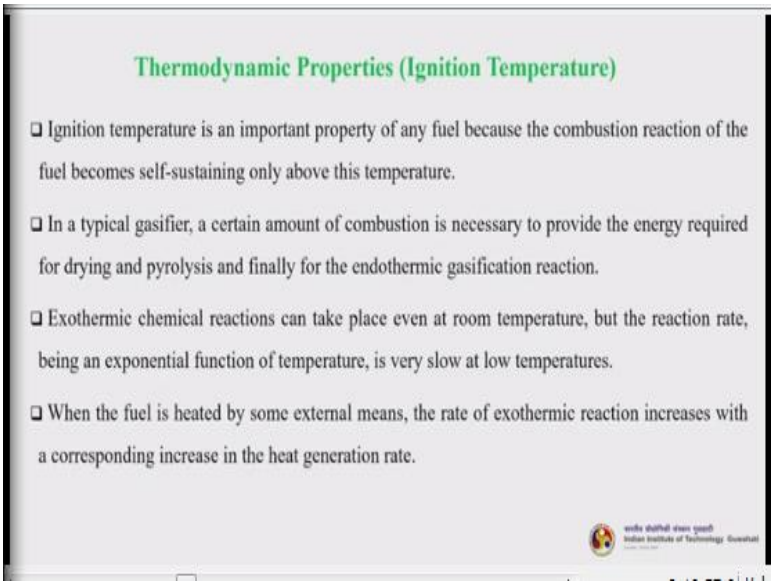


It includes the latent heat of vaporization of water. HHV is also called as the gross calorific value. In North America, the thermal efficiency of a system is usually expressed in terms of HHV, so it is important to know the HHV of the design fuel. Then there is something called LHV or lower heating value, so LHV is also known as net calorific value. HHV is gross calorific value and LHV is the net calorific value.

So, the lower heating value is defined is the amount of heat released by fully combusting a specified quantity less the heat of vaporization of the water in the combustion product, so this is the equation you can refer to.

$$LHV = HHV - h_g\left(\frac{9H}{100} - \frac{M}{100}\right)$$

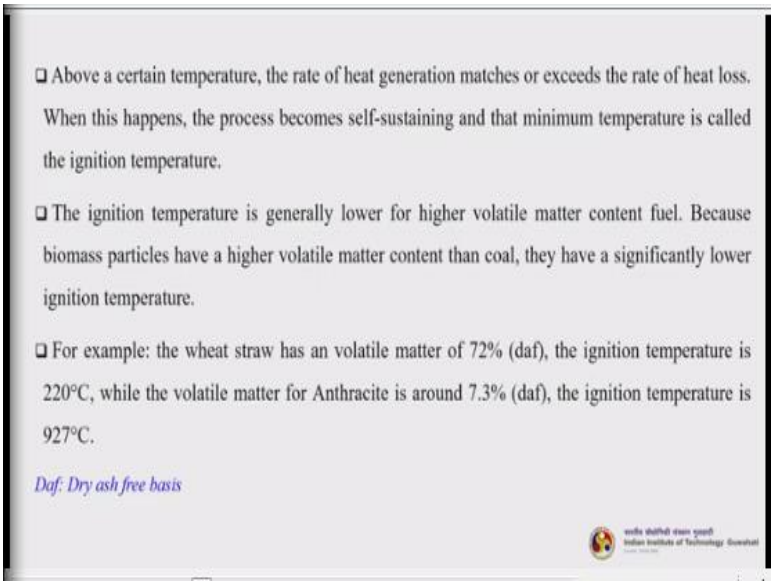
(Refer Slide Time: 16:55)



So, then the next thermodynamic property is ignition temperature. So, ignition temperature is an important property of any fuel because the combustion reaction of the fuel becomes self sustaining only above this temperature. So, above only this temperature it will ignite basically. So in a typical gasifier a certain amount of combustion is necessary to provide the energy required for drying and pyrolysis and finally for the endothermic gasification reaction.

Exothermic chemical reaction can take place even at room temperature but the reaction rate being an exponential function of temperature is very slow at low temperatures. So, when the fuel is heated by some external means, the rate of exothermic reaction increases with a corresponding increase in the heat generation rate.

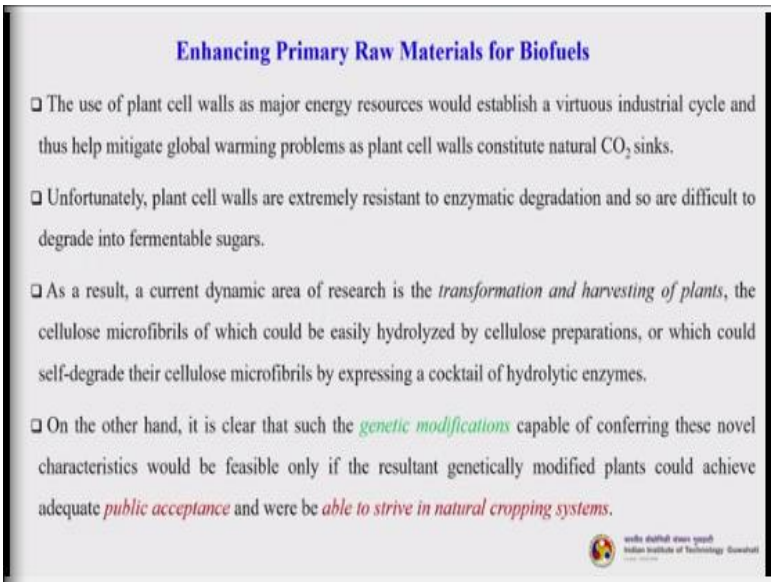
(Refer Slide Time: 17:39)



So, above a certain temperature the rate of heat generation matches or exceeds the rate of heat loss. When this happens the process becomes self sustaining and that minimum temperature is called the ignition temperature. So, the ignition temperature is generally lower for higher volatile matter content fuel because biomass particles have a higher volatile metal content than coal. So, usually they have significantly lower ignition temperature.

So, the inherent meaning is that, so biomass particles will ignite very fast compared to the coal. So, for example the wheat straw has a volatile matter of 72% (daf basis, so daf is the dry ash free basis). The ignition temperature is 220 degrees centigrade while the volatile matter of anthracite is only 7.3% but the ignition temperature is 927 degrees centigrade.

(Refer Slide Time: 18:34)



Now we will try to understand how we will enhance the primary raw materials for the biofuels and what are the different types of techniques that exist, including some of the genetic engineering aspects. Now the use of plant cell wall as major energy sources would establish a virtuous industrial cycle and thus help mitigate global warming problems as plant cell walls constitute the natural carbon dioxide sinks.

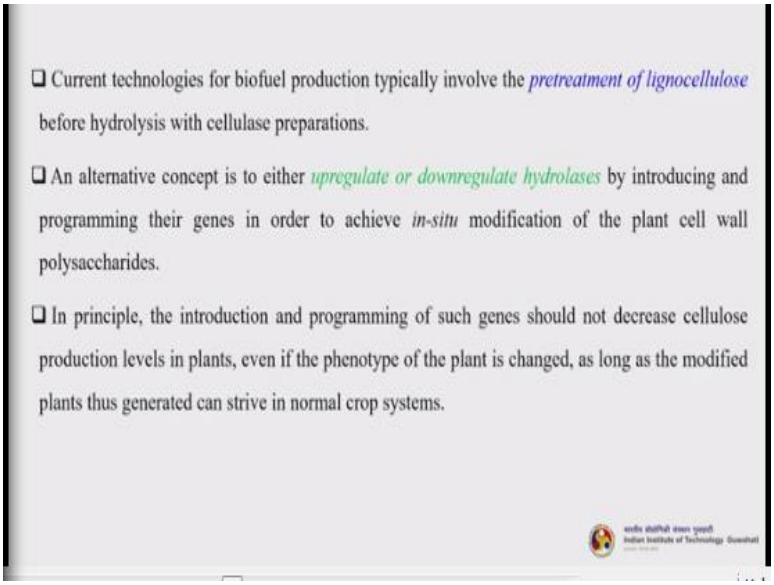
Unfortunately plant cell walls are extremely resistant to enzymatic degradation and so are difficult to degrade into fermentable sugars. Now that is the reason why as it is mentioned here; there is a need for the pretreatment of the biomass, (we will also discuss in our subsequent slides today itself). And due to this recalcitrant nature of the cell wall, huge amount of energy and effort is required to make it amorphous thus releasing the sugars which is responsible for producing alcohol.

As a result, current dynamic area of research is the transformation and harvesting of plants. So, the cellulose microfibrils which could be easily hydrolyzed by cellulose preparations or which could self degrade their cellulose microfibrils by expressing a cocktail of hydrolytic enzymes. On the other hand, now it is clear that such genetic modifications capable of conferring these novel characteristics would be feasible only if the resultant genetically modified plants could achieve adequate public acceptance and were able to strive in natural cropping system.

Now here I wish to tell you something very interesting. So, many of you will be knowing about this genetic modified crops. You remember few years back in India there is a lot of hue and cry regarding the genetic or transgenic brinjal. So, public perception about genetic modification till date is not so good. So, they feel that if a particular species is genetically modified and being consumed, by the humans or the animals it may have some bad effect, which I cannot give a right straightaway answer to that; we need to do more study on that actually, I cannot say whether it is good or bad in this platform. But we need to understand one thing, that the public perception is not so good and acceptability of such genetic crops actually needs more public awareness and you need to convince the public about what is the importance of this and whether there is any adverse effect if it is being consumed by the humans and/or animals. This is first thing.

Second thing; this so called genetically modified species, plants, crops, whatever it is, they must have the capacity of naturally cropping systems, that is one thing. There should also be able to withstand the usual natural environment as well as the climatic conditions. So, these are some of the challenges which still remain in the development of so called genetic engineering or genetic engineered species, crops or transgenic plants, you can call them.

(Refer Slide Time: 21:56)

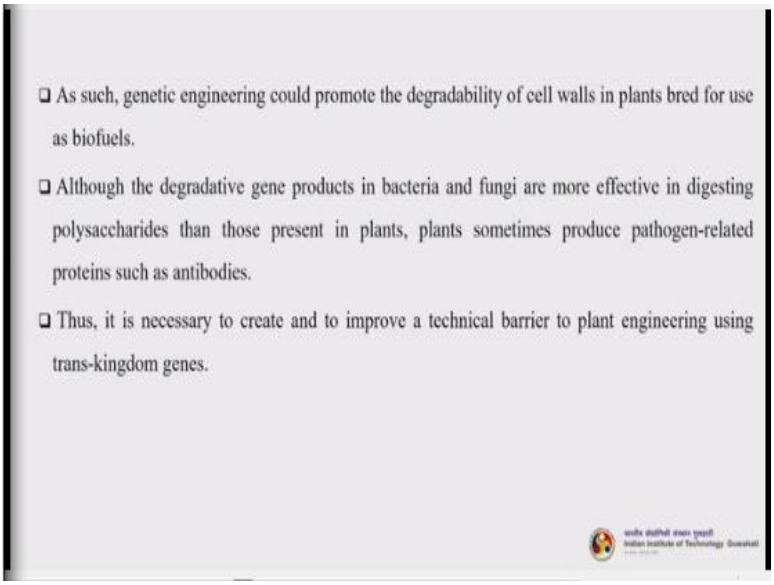


So, the current technologies for biofuel production typically involve the pretreatment of lignocellulose before hydrolysis with cellulase preparations. Cellulase is the enzyme which will degrade the cellulose to glucose. So, an alternative concept is to either upregulate or downregulate hydrolases by introducing and programming their genes in order to achieve in situ modification of the plant cell wall polysaccharides.

So, we can do in situ modification inside the plant cell itself by doing some genetic modifications by over expressing either certain genes or certain proteins which is responsible for a particular, let us say, either increasing the cellulose yield or carbohydrate yield or making it resistant to certain types of pathogens attack. There are many things. It is not that genetic engineering is being done only to have a higher yield of the biomass or have higher yield of cellulose, it is not so.

So, in principle the introduction and programming of such genes should not decrease cellulose production levels in plants otherwise it will have an adverse effect, so our main aim is not going to be achieved.

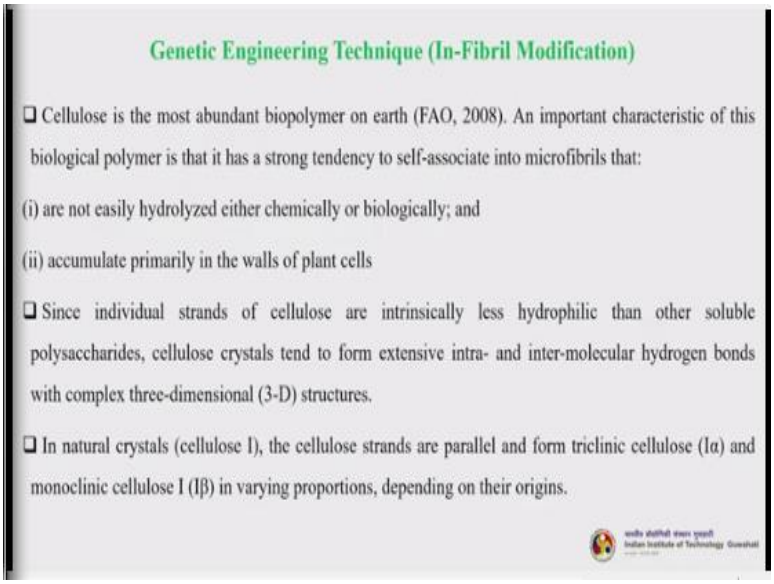
(Refer Slide Time: 23:15)



So, as such genetic engineering could promote the degradability of cell walls in plants bred for use as biofuels. Although the degradative gene products in bacteria and fungi are more effective in digesting polysaccharides than those present in plants, so plants sometimes produce pathogen related proteins such as antibodies. So, thus it is necessary to create and improve a technical barrier to plant engineering using trans-kingdom genes, I hope you all understand what is genes.

So, you can browse little more about these particular few slides and few of the particular words which you may not be aware of; please read it from literature.

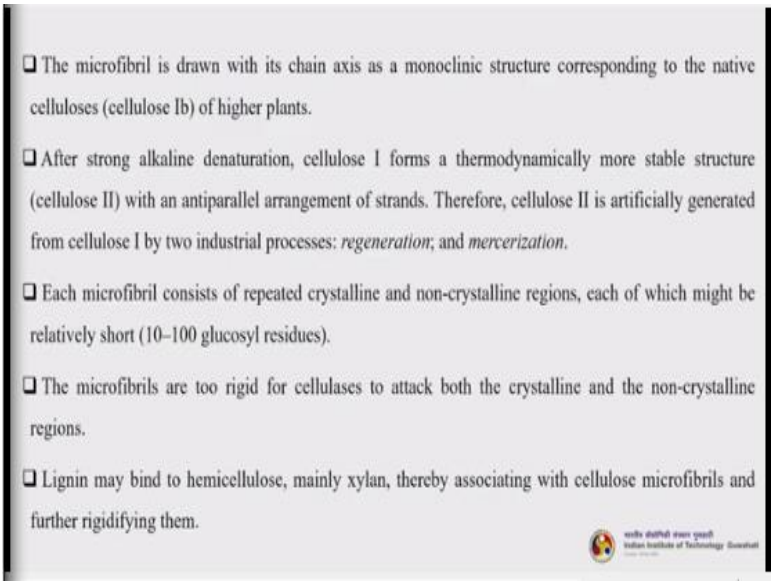
(Refer Slide Time: 23:57)



So, we will discuss how we can do this genetic engineering technique using the In-Fibril modification. Now cellulose is most abundant biopolymer on the earth. An important characteristic of this biological

polymer is that, it has a strong tendency to self-associate into microfibrils that: are not easily hydrolyzed either chemically or biologically and that accumulate primarily in the walls of the plant cells. Now since individual strands of cellulose are intrinsically less hydrophilic than other soluble polysaccharides, cellulose crystals tend to form extensive intra and intermolecular hydrogen bonds with complex 3 dimensional structures. In natural crystals, for example cellulose I, the cellulose strands are parallel and form triclinic cellulose, and monoclinic cellulose in varying proportions depending on their origins.

(Refer Slide Time: 24:53)



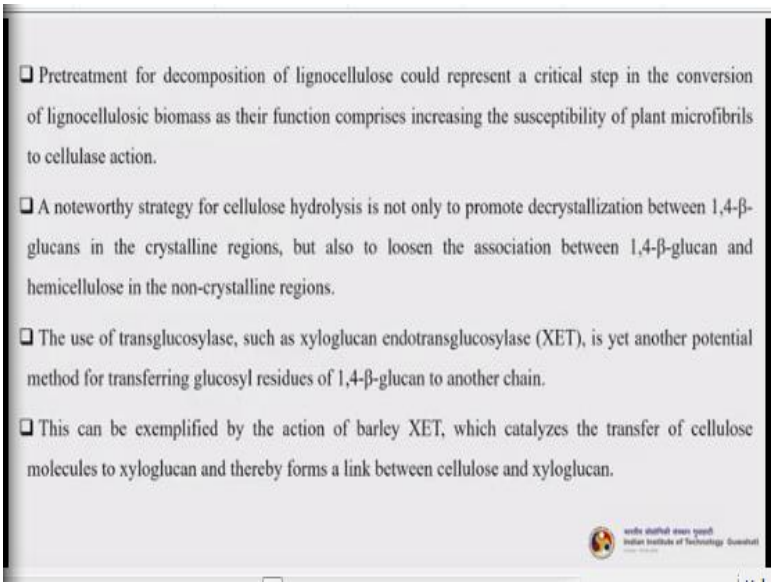
So, the microfibril is drawn with its chain axis as a monoclinic structure corresponding to the native cellulose of higher plants. After strong alkaline denaturation, cellulose I forms a thermodynamically more stable structure than that of the cellulose II with an anti parallel arrangement of strands. Therefore, cellulose II is artificially generated from cellulose I by two industrial processes, first is called regeneration and second is called the mercerization.

Each microfibril consist of repeated crystalline and non crystalline regions, each of which might be relatively short (almost around 10 to 100 glucosyl residues that it contains). The microfibrils are too rigid for cellulases to attack both the crystalline and the non crystalline regions. Lignin (another component, a very high class compound) may bind to hemicelluloses mainly xylan, thereby associating with cellulose microfibrils and further rigidifying them.

So, in a lignocellulosic biomass (next sometimes I will show you a structure), cellulose, hemicellulose and lignin are bound together in a very intricate manner. So, thereby making it more rigid to the cellulase attack, cellulase is the enzyme which we want to use for degrading the cellulose whatever is available. So,

that is the reason why we talk about this delignification process, the pretreatment is mostly about delignification. Not always it is delignification; but mostly, it is roughly understood as delignification. That means removing the lignin or separating lignin from cellulose and hemicellulose. Cellulose is C 6 sugar and hemicelluloses are C 5 sugars, pentose sugars basically.

(Refer Slide Time: 26:48)

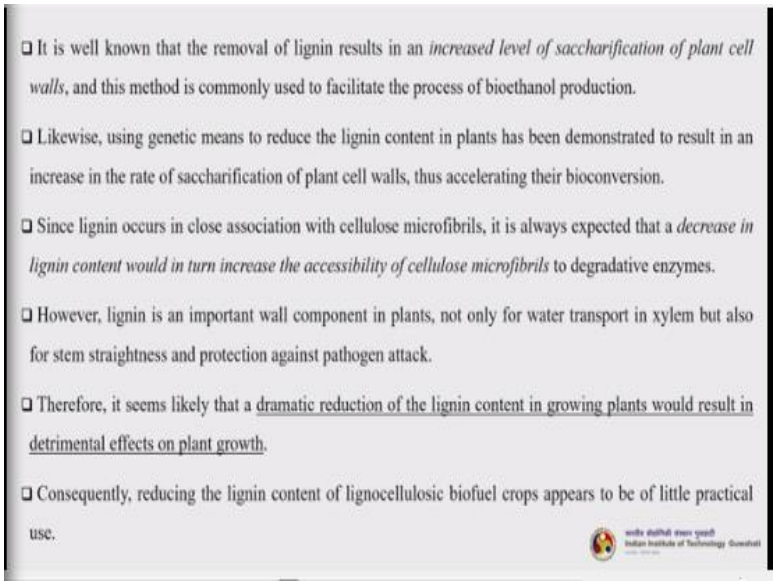


Pretreatment for decomposition of lignocellulose could represent a critical step in the conversion of lignocellulosic biomass, as their function comprises increasing the susceptibility of plant microfibrils to cellulase action. A noteworthy strategy for cellulose hydrolysis is not only to promote decrystallization between the so called 1, 4 beta glucans in the crystalline regions, but also to loosen the association between 1, 4 beta glucan and hemicellulose in the non-crystalline regions.

So, that is very important, this particular sentence is very important. So, the cellulose hydrolysis is not only doing the decrystallization between this 1, 4 beta glucans, but it is also losing the association between this 1, 4 beta glucan as well as others hemicelluloses in the non-crystalline regions. So, the use of transglucosylase, such as xyloglucan endotransglucosylase (which is known as XET), is yet another potential method for transferring glucosyl residues of 1, 4 beta glucan to another chain.

Now this can be exemplified by the action of barley XET, which catalyses the transfer of cellulose molecules to xyloglucan and thereby forms a link between cellulose and xyloglucan.

(Refer Slide Time: 28:08)



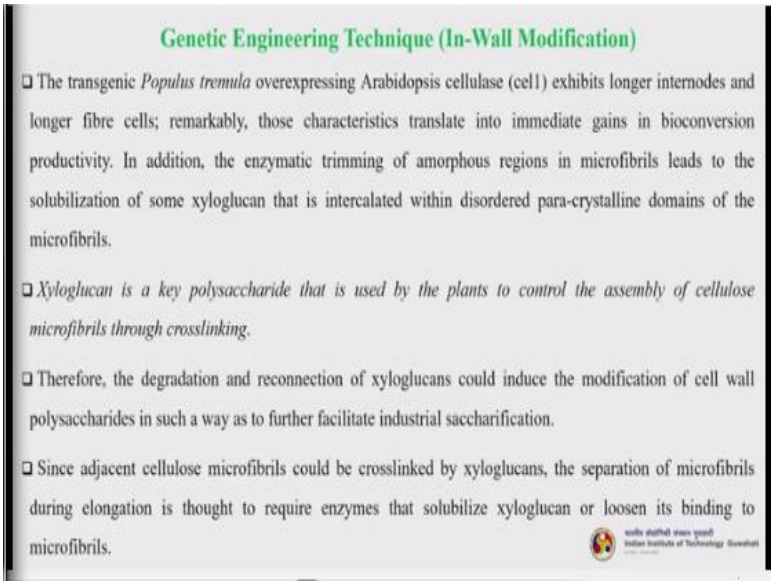
It is well known that the removal of lignin results in an increased level of saccharification of plant cell walls, and this method is commonly used to facilitate the process of bioethanol production. Lignin occurs in close association with cellulose microfibrils, it is always expected that a decrease in lignin content would in turn increase the accessibility of the cellulose microfibrils to degradative enzymes. Lignin is an important cell wall components, in the plants not only for water transport in xylem but also for stem straightness and protection against pathogen attack.

So, lignin provides some sort of mechanical support also, you can say that. Therefore, it seems likely that a dramatic reduction of the lignin content in the growing plants would result in a detrimental effects of the plant growth, so you need to balance it. So, consequently reducing the lignin content of lignocellulosic biofuel crops appears to be that of (little) practical use.

Let us understand that, if we want to reduce the lignin content of a dedicated energy crop in which the lignin content is already less (let us say miscanthus, switch grass, elephant grass - they are bush type of plants, they are grasses), then it is not going to have a much higher effect on the mechanical stability of the plants or these bushes (because they are grasses and bush basically).

But having said that, if we are drastically reducing the in-fibril lignin, for the hardwood or softwood trees, then we need to be careful about whether the plant can grow properly and erect and stand on the soil by itself, by having a good mechanical stability. So that is the question basically. That is how the genetic engineering or the engineers must ensure that there is no adverse effect on the growth of the plant.

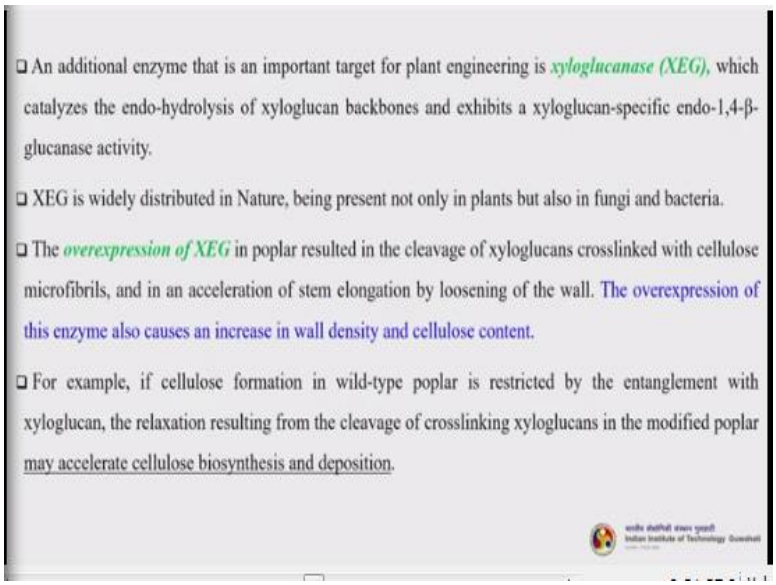
(Refer Slide Time: 30:16)



So, the transgenic *Populus tremula* overexpressing Arabidopsis cellulase (that is cell1) exhibits longer internodes and longer fibre cells; remarkably, those characteristics translate into immediate gains in bioconversion productivity. In addition, the enzymatic trimming of amorphous regions in the microfibrils leads to the solubilization of some xyloglucan that is intercalated with disordered para-crystalline domains of the microfibrils.

Xyloglucan is a key polysaccharide that is used by the plants to control the assembly of cellulose microfibrils through cross linking. So, therefore the degradation and reconnection of xyloglucans could induce the modification of cell wall polysaccharides in such a way, so as to further facilitate industrial saccharification. Since adjacent cellulose microfibrils could be crosslinked to xyloglucans, the separation of microfibrils during elongation is thought to require enzymes that solubilize xyloglucan or loosen its binding to microfibrils.

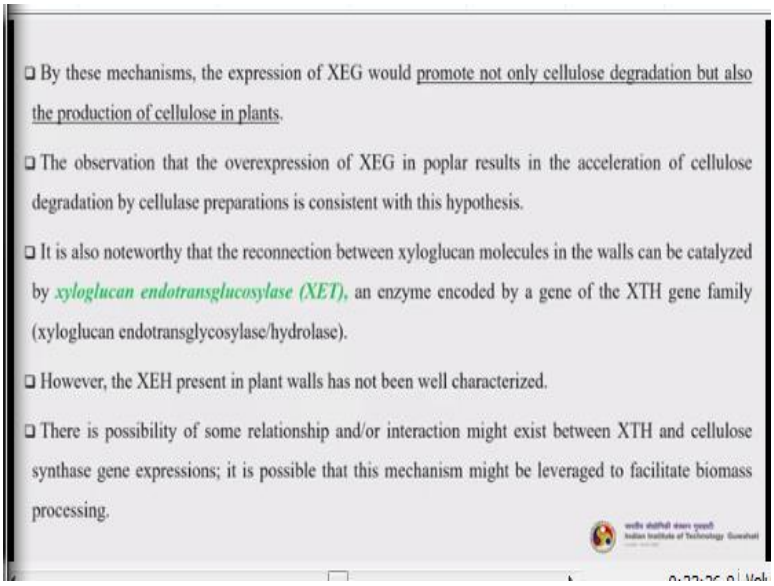
(Refer Slide Time: 31:24)



An additional enzyme that is an important target for plants genetic engineering is xyloglucanase, which is called XEG, which catalyses the endo-hydrolysis of the xyloglucan backbones and exhibits xyloglucan specific endo-1, 4-beta glucanase activity. XEG is widely distributed in nature, being present not only in plants but also in fungi and bacteria. So, the overexpression of XEG in poplar (poplar is a plant which we have discussed in our last class - a dedicated energy crop) resulted in the cleavage of xyloglucans crosslinked with cellulose microfibrils, and in an acceleration of stem elongation by loosening of the wall. The overexpression of this enzyme also causes an increase in wall density and cellulose content. So, I will tell you in a crude way, what is the meaning of overexpression. These are genetic engineering terms. So, as I told you please go back and read a little more about certain terms which you are not very clear about.

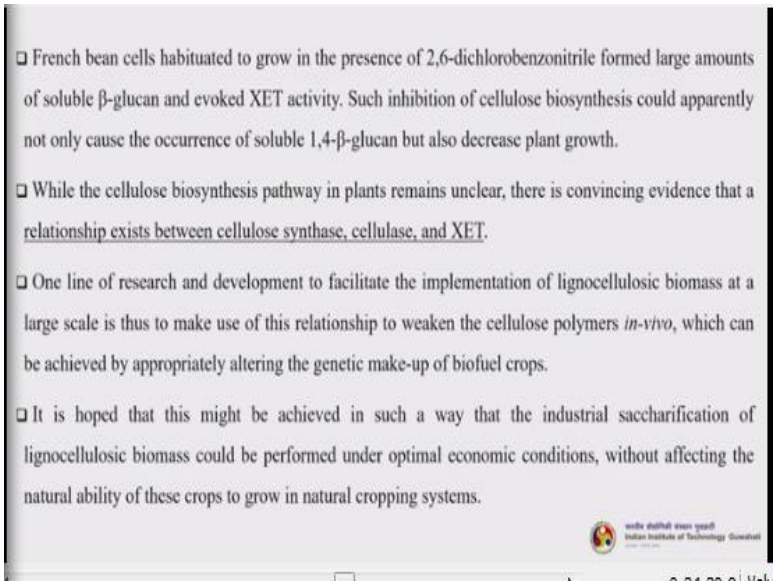
So, in this particular class, it is very difficult to make you understand each and every bit of the genetic engineering aspects, so that is not the scope of this course also. So, I will be telling in a nutshell; overexpression means making more copies of the parent protein, or gene. So, for example if cellulose formation in wild type poplar is restricted by the entanglement with xyloglucan, the relaxation resulting from the cleavage of crosslinking xyloglucans in the modified poplar may accelerate cellulose biosynthesis and deposition. So, the meaning of this particular sentence is that, when we overexpress XEG in the poplar, it has helped in cleavage of the xyloglucans which is cross-linked to the cellulose microfibrils.

(Refer Slide Time: 33:25)



So, by these mechanisms, the expression of XEG would promote not only cellulose degradation, but also the production of cellulose in plants. So, the observation that overexpression of XEG in poplar results in the acceleration of cellulose degradation by cellulase preparations is consistent with this hypothesis. Now it is also noteworthy that the reconnection between xyloglucan molecules in the walls can be catalysed by xyloglucan endotransglucosylase, which is called the XET, an enzyme encoded by the gene of XTH gene family. However, XEH present in plant cell walls has not been well characterized (another class of enzyme). So, there is possibility of some relationship and/or interaction might exist between XTH and cellulose synthase gene expressions; it is possible that this mechanism might be leveraged to facilitate biomass processing. More work is currently being done on whatever we have written in this last sentence here.

(Refer Slide Time: 34:28)

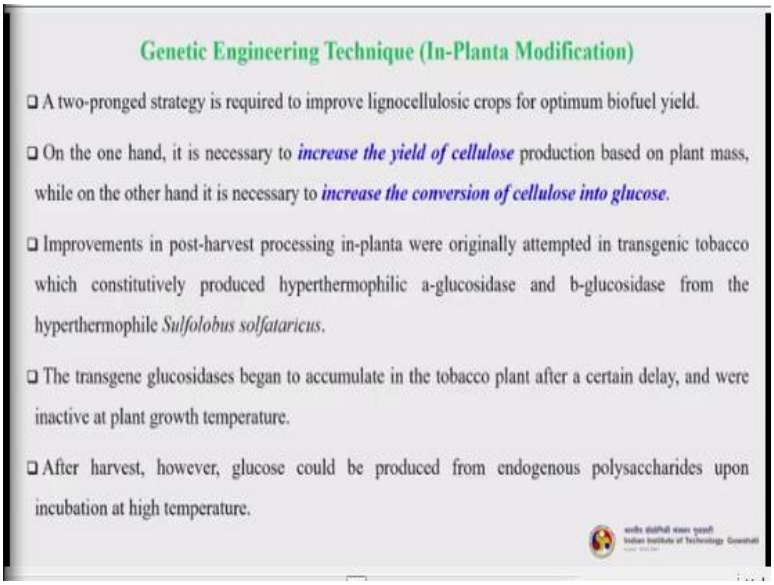


So, French bean cells habituated to grow in the presence of 2, 6 dichlorobenzonitrile formed large amounts of soluble beta glucan and evoked the XET activity. Such inhibition of cellulose biosynthesis would apparently not only cause the occurrence of soluble 1, 4 beta glucan but also decrease the plant growth. While the cellulose biosynthesis pathway in plants remain unclear, there is convincing evidence that a relationship exists between cellulose synthase, cellulase the enzyme, and the XET.

One line of research and development to facilitate the implementation of lignocellulosic biomass at a large scale is thus to make the use of this relationship to weaken the cellulose polymers in vivo, which can be achieved by appropriately altering the genetic makeup of biofuel crops. It is hoped that this might be achieved in such a way that the industrial saccharification of lignocellulosic biomass could be performed under optimal economic conditions without affecting the natural ability of these crops to grow in a natural cropping system.

Again this is what we have already discussed in one of the slides, as I told that everything is so good about the genetic engineering things. It has to be done in a proper way and proper understanding that if I am decreasing the lignin content, then it should not affect the growth of the plant. This is one of the foremost important thing.

(Refer Slide Time: 35:56)



Let us learn what is In-Planta modification. Like another genetic engineering technique. A two-pronged strategy is required to improve lignocellulosic crops for optimal biofuel yield. So, the first one, it is necessary to increase the yield of the cellulose production based on the plant mass, while on the other

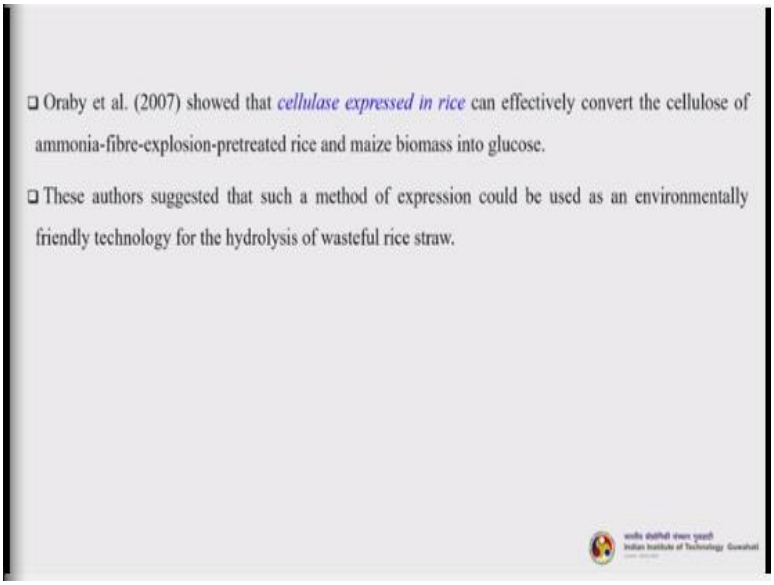
hand, it is also necessary to increase the conversion of cellulose into glucose. There are two things, first you increase the yield of cellulose, by means of the genetic engineering aspect.

Second thing is that, you increase cellulose; but cellulose is bound in such an intricate fashion with hemicellulose and lignin and their rigidity so high they are crystalline. So, that crystallinity has to be overcome so that the cellulose can be transformed into glucose. So, improvements in the post harvest processing in-planta were originally attempted in transgenic tobacco which constitutively produced hyperthermophilic a-glucosidase and b-glucosidase (two different types of enzymes) from the hyperthermophile *Sulfolobus solfataricus*.

So, this is one particular unicellular organism from which these two enzymes have been derived. Transgenic plant means genetically modified plant. So, the transgene glucosidases began to accumulate in the tobacco plant after a certain delay and were inactivate at plant growth temperature. After harvest, however glucose could be produced from endogenous polysaccharide upon incubation at high temperature.

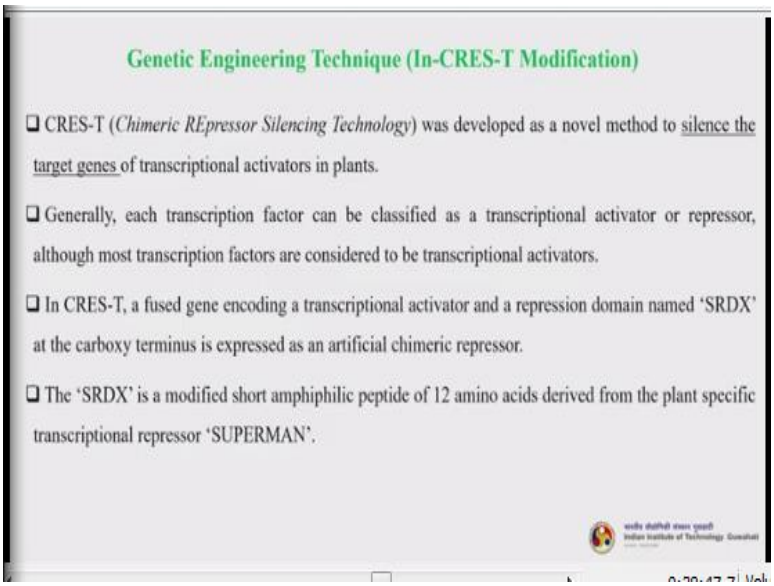
I would like to say that transgenic tobacco is being farmed in many of the Western countries, because not for the consumption of the tobacco leaves, but to purify one particular monoclonal antibody which is present in the transgenic tobacco. Usually it is present in a very small quantity, about 6 to 7% not more than that. Sometimes it is less than that depending upon the species, so it is a very high class antibody, needs to be purified, for that purpose this transgenic tobacco plants are been cultivated.

(Refer Slide Time: 38:08)



So Oraby et al in 2007, showed that cellulase expressed in rice can effectively convert the cellulose of ammonia-fibre-explosion-pretreated rice and maize biomass into glucose. Now ammonia fibre explosion pretreated rice, so ammonia fibre explosion is one of the pretreatment technique, we will read about more pretreatment techniques later in our subsequent lectures. We will discuss about ammonia fibre explosion als. So, these authors suggested that such a method of expression could be used as an environmentally friendly technology for the hydrolysis of wasteful rice straw.

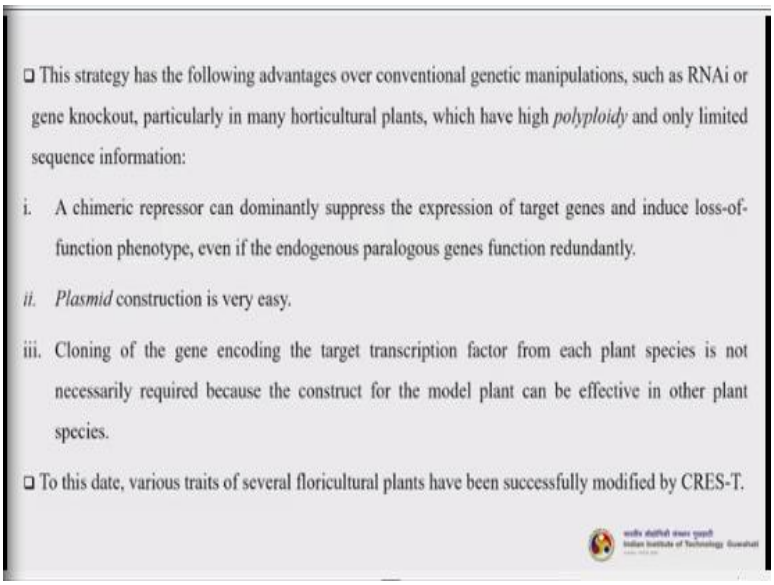
(Refer Slide Time: 38:46)



So, next genetic engineering technique is In-CRES-T modification. So, CRES-T means chimeric REpressor silencing technology; it was developed as a novel method to silence the target genes of transcriptional activators in plants. Transcription factor is a protein; and what is transcription? So, transcription means in a nutshell, transferring one particular information from the DNA to the messenger RNA or mRNA. So that process is called transcription.

You can read little more about these terminologies from the literature, so that things will be more clear. In CRES-T, a fused gene encoding a transcriptional activator and a repression domain named as SRDX at the carboxy terminus is expressed as an artificial chimeric repressor. The SRDX is a modified short amphiphilic peptide of 12 amino acids derived from the plant specific transcriptional repressor known as SUPERMAN.

(Refer Slide Time: 39:59)

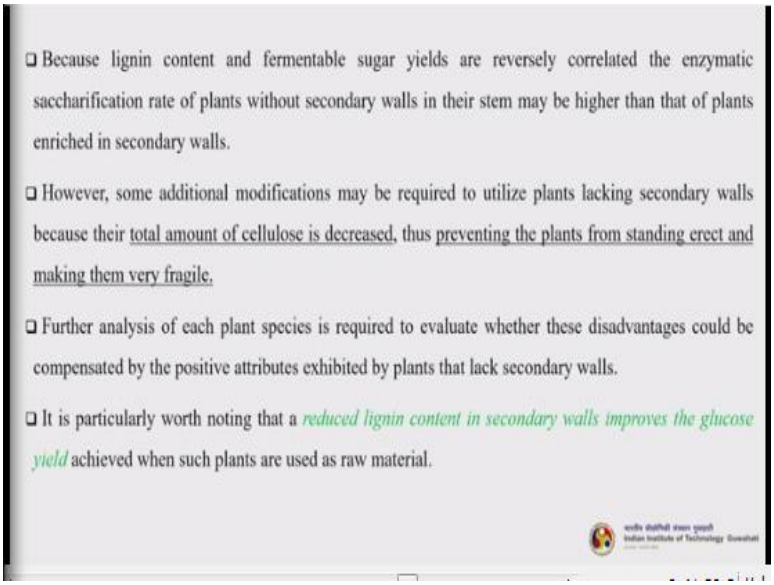


This strategy has the following advantages over the conventional genetic manipulations, such as RNAi or gene knockout particularly in many horticultural plants which have high polyploidy and only limited sequence information: (You know polyploidy; there is something called diploid, what is the meaning of that? So, when an offspring is actually born, it usually carries one set of chromosomes, in their genes from each of the parents. In polyploids, they will have two sets of genes, two from one parent and two form another parent. You can understand in a crude way.)

i) So, chimeric repressor can dominantly suppress the expression of target genes and induce loss-of-function phenotype, even if the endogenous paralogous genes function redundantly.
ii) Plasmid construction is very easy, what is plasmid? Plasmid is a small extra chromosomal DNA, i.e., not present in the chromosome itself.
iii) Cloning of the gene encoding the target transcription factor from each plant species is not necessarily required because the construct of the model plant can be effective in other plant species.

To this date various traits of several floricultural plants have been successfully modified by the CRES-T technique.

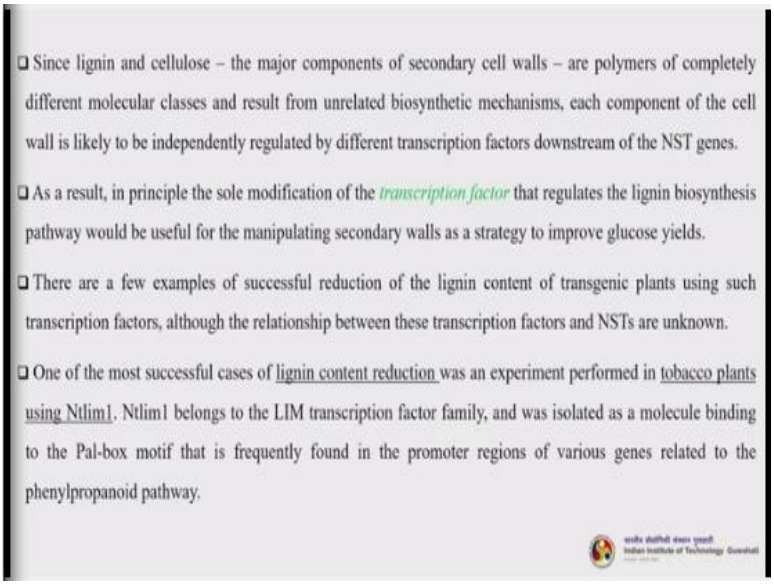
(Refer Slide Time: 41:27)



Because lignin content and fermentable sugar yields are reversibly correlated, the enzymatic saccharification rate of plants without secondary walls in their stem may be higher than that of the plants enriched in the secondary walls. Some additional modifications may be required to utilize plants lacking secondary walls because their total amount of cellulose is decreased, thus preventing the plants from standing erect and making them very fragile.

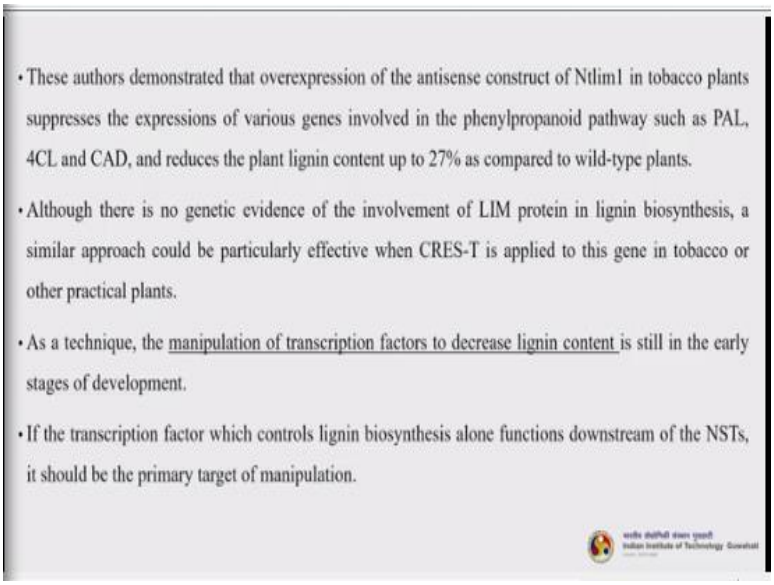
Further analysis of each plant species is required to evaluate whether these disadvantages could be compensated by the positive attributes exhibited by plants that lack secondary walls. It is particularly worth noting that a reduced lignin content in secondary walls improves the glucose yield. That is what we have already understood.

(Refer Slide Time: 42:12)



Since lignin and cellulose - the major components of secondary walls - are polymers of completely different molecular classes and result from unrelated biosynthetic mechanisms, each component of the cell wall is likely to be independently regulated by different transcription factors downstream of the NST genes. I am leaving it as it is, you please read this later on, if you have any query, please ask me. This little more detail about the genetic engineering aspect, though is not so much important for this course but I felt that I will basically write it, so you can later on read it. So, I am just moving ahead with the other material.

(Refer Slide Time: 42:52)



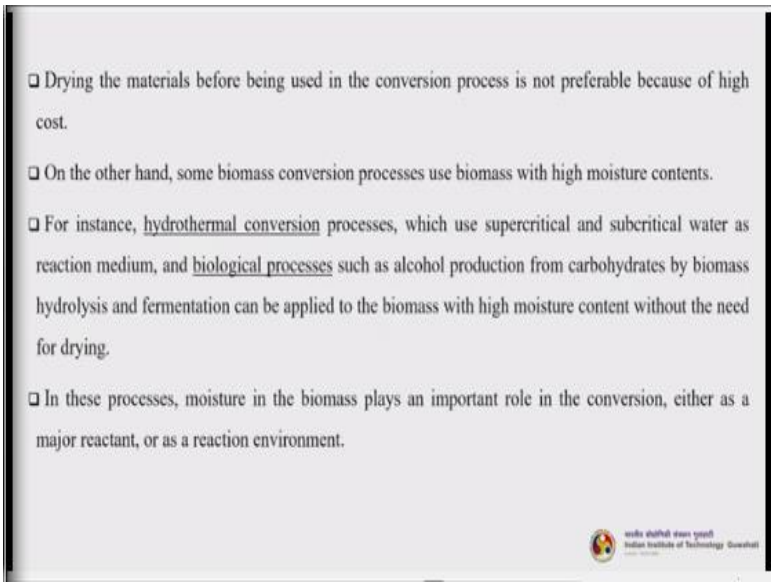
So, this also the same thing, I am just leaving it to you to read. In case you have any query, please feel free to write to me, I will be definitely happy to address those.

(Refer Slide Time: 43:05)

Now we will try to understand what are the challenges in conversion of biomass to biofuels. There are only few challenges. But that needs to be addressed suitably, so that we will have no problem in the conversion. The first one is the moisture content; we have discussed it in a nutshell earlier. So, biomass materials with high moisture content is not suitable feedstock for conventional thermochemical conversion technologies such as gasification, pyrolysis.

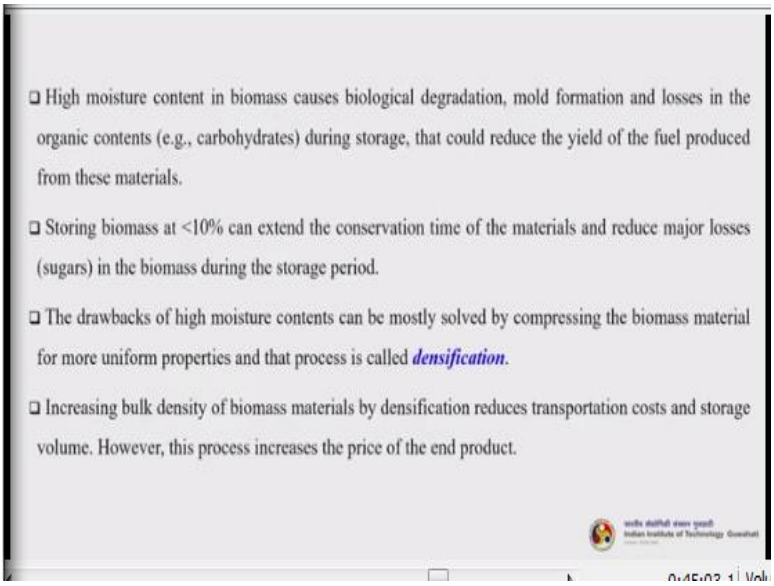
High moisture can reduce the effectiveness of conversion processes. Moisture in raw biomass materials is also undesired because fuel wood produced from these materials can contain more moisture. The fuels, which have high moisture contents cannot burn easily. Some part of the energy in the fuel are always consumed for the vaporization of water, which is present in the fuel. In order to maximize the heating value of the fuel produced from these materials the moisture content biomass should be always less than 20%.

(Refer Slide Time: 44:03)



Drying the materials before being used in the conversion process is not preferable because of high cost (because it is an energy intensive process). On the other hand, some biomass conversion processes use biomass with high moisture content. The first one is hydrothermal conversion process. This is a beautiful technology; it is currently being adopted in many industrial practices. So, in this particular technology, in a high pressure high temperature system, you are going to convert the high moisture content feedstock (it can be anything, any biomass or anything) to crude oil (basically biocrude). And in certain biological processes such as alcohol production from carbohydrates by biomass, high moisture content does not create any problem. So, in these processes, moisture in the biomass play an important role in the conversion either as a major reactant or as a reaction environment.

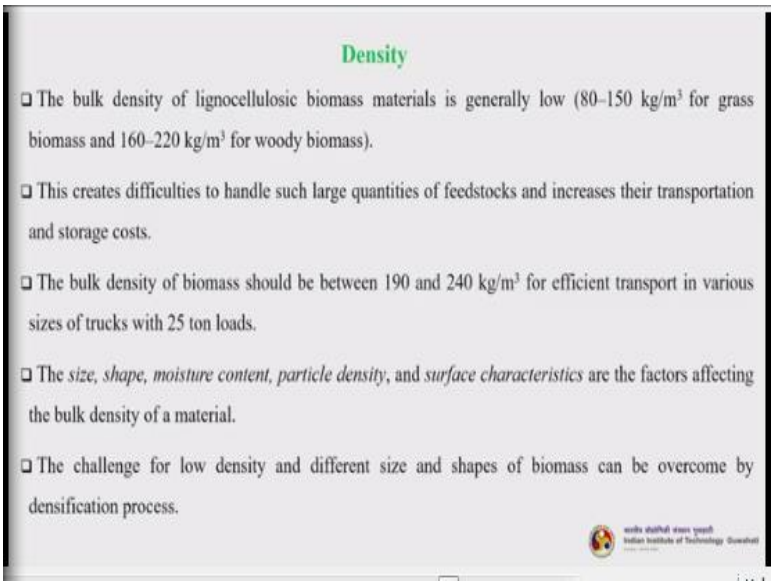
(Refer Slide Time: 45:03)



For example, high moisture content in biomass causes biological degradation, mold formation and losses in the organic content during storage, that could reduce the yield of the fuel wood from these materials. Storing biomass at less than 10% can extend the conservation time of the materials and reduce major losses (that means losses of the sugars) during the storage period. The drawbacks of high moisture content can be mostly solved by compressing the biomass material for more uniform properties and that process is called densification.

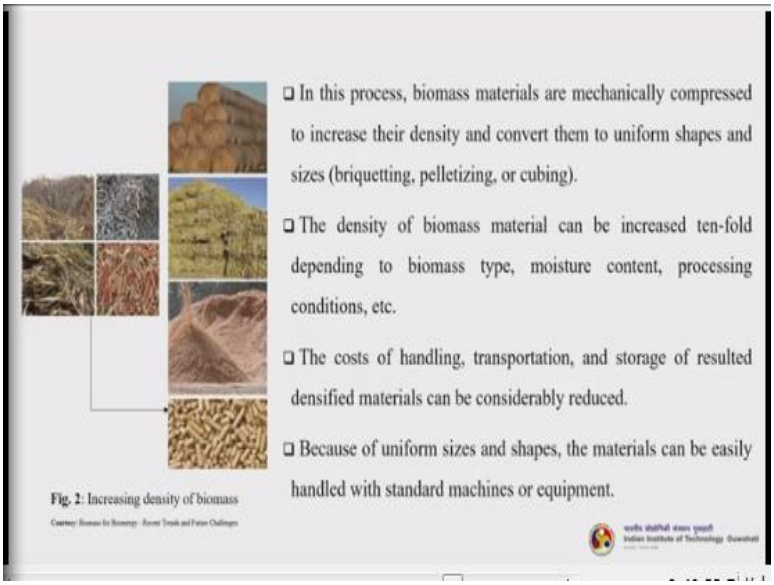
So, you must have heard about densification of biomass. So increasing bulk density of biomass materials by densification reduces transportation cost and storage volume. However, this process adds an extra cost, densification is an added process basically. So for any added process there is a cost to it and hence the overall cost increases.

(Refer Slide Time: 45:53)



Then density; bulk density of lignocellulosic biomass materials is generally low. This creates difficulties to handle such large quantities of feedstocks and increases the transportation and storage cost. The bulk density of biomass should be between 190 to 240 kg per meter cube for efficient transport in various sizes of trucks with approximately 25 ton loads. The size, shape, moisture content, particle density and surface characteristics are the factors affecting the bulk density of a material. The challenge for low density and different size and shapes of biomass can be overcome by densification process.

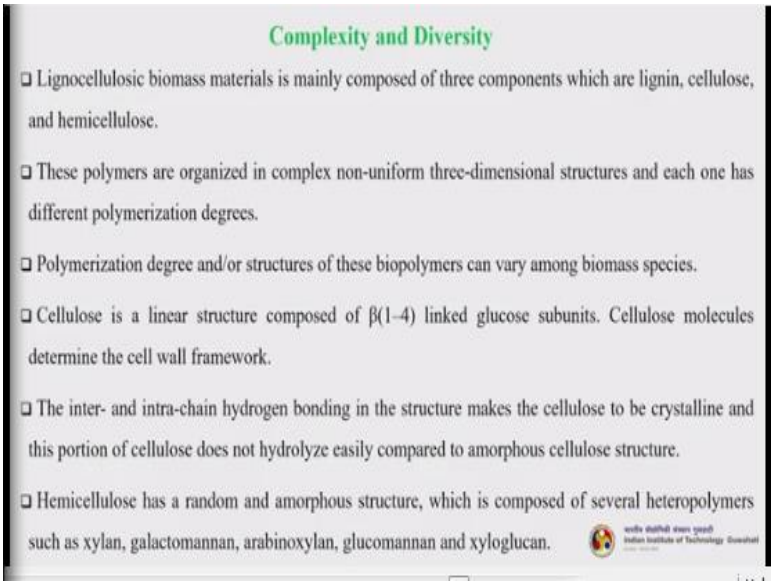
(Refer Slide Time: 46:33)



So, biomass in densification process; biomass materials are mechanically compressed to increase their density and convert them into uniform shapes and sizes. You can see, how these have been converted into particular shapes. These are powder, these are some sort of briquettes, these are some sort of rolls. You can briquette them, pelletizing them, cubing them. So, then density of biomass can be increased ten-fold

depending upon the biomass type, moisture content and processing condition. The costs of handling, transportation and storage of resulted densified materials can be considerably reduced. Now because of uniform size and shape the materials can be easily handled.

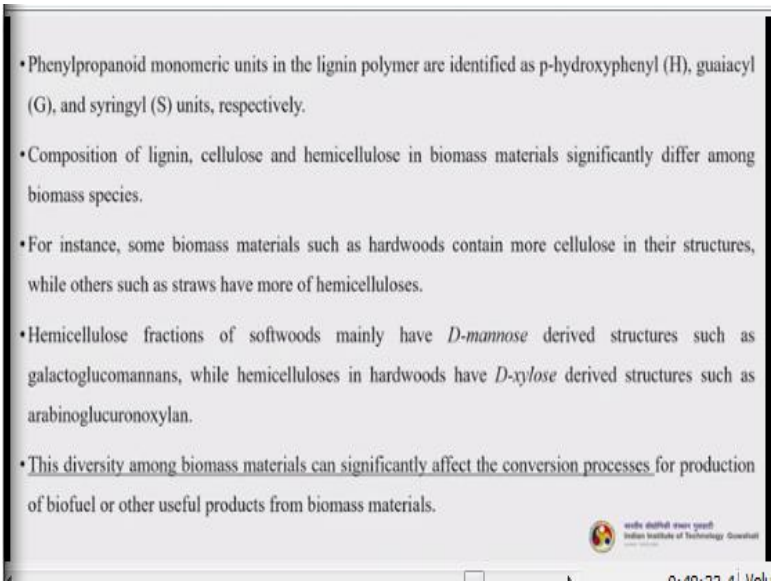
(Refer Slide Time: 47:14)



So, the next is complexity and diversity. Lignocellulosic biomass materials is mainly composed of three components lignin, cellulose and hemicellulose. These polymers are organized in the complex non uniform three dimensional structures and each one has different polymerization degrees. Polymerization degree and/or structures of these biopolymers can vary among the biomass species. Cellulose is a linear structure composite of beta 1-4 linked glucose subunits. Cellulose molecules determine the cell wall framework.

The inter and intra chain hydrogen bonding in the structure makes the cellulose to be crystalline and this portion of cellulose does not hydrolyze easily compared to the amorphous cellulose structure. And that is what we have understood during the genetic modification steps that we have discussed. How the cellulose can be available or more amenable to degradation, either by removing lignin**,** decrease the lignin content or overexpressing certain cellulases (enzymes basically). So, that whatever we want that will be fulfilled. Hemicellulose has a random and amorphous structure which is composed of several heteropolymers such as xylan, galactomannan, arabinoxylan, glucomannan and xyloglucan.

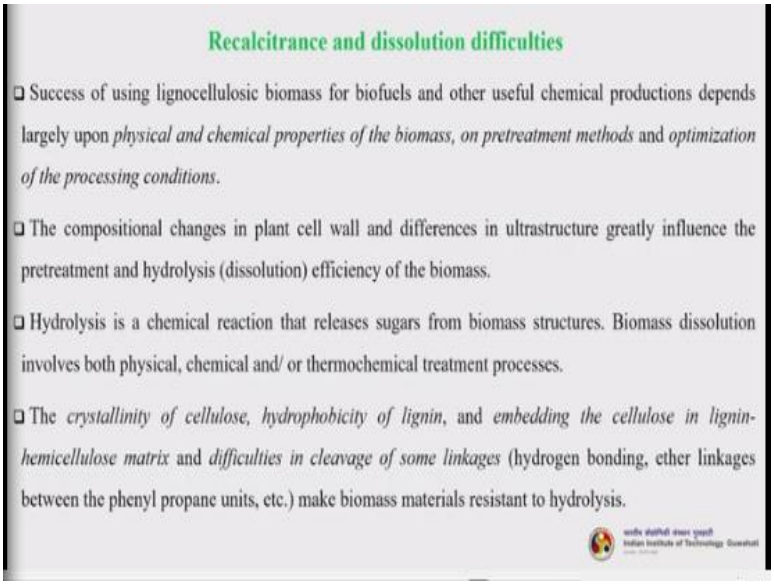
(Refer Slide Time: 48:32)



Phenylpropanoid monomeric units in the lignin polymer are identified as p-hydroxyphenyl, guaiacyl and syringyl units. Composition of lignin, cellulose and hemicellulose in biomass materials significantly differ among biomass species. For instance, some biomass materials such as hardwoods contain more cellulose in their structures while others such as straws have more hemicellulose. Hemicellulose fractions of softwoods mainly have D-mannose derived structures such as galactoglucomannans while hemicelluloses in hardwoods have D-xylose derived structures.

Now this diversity among biomass material can significantly affect the conversion process for production of biofuel and other useful products from the biomass materials.

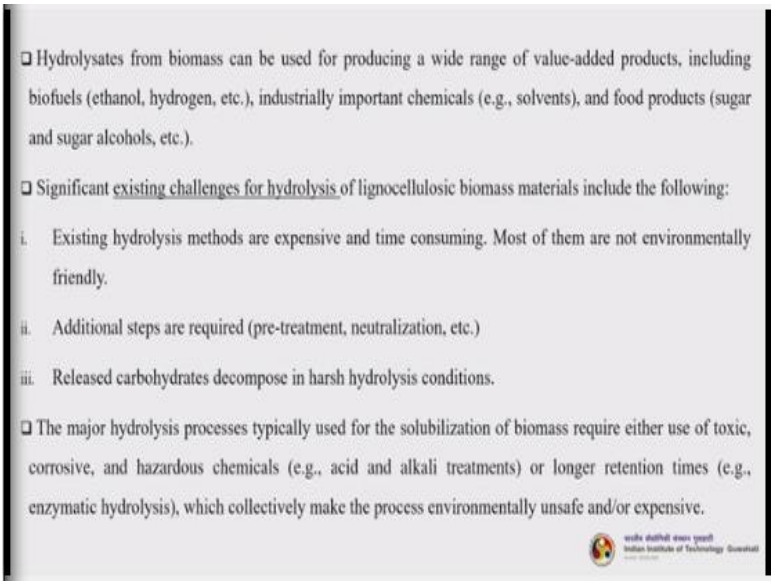
(Refer Slide Time: 49:18)



So, the next is recalcitrance and dissolution difficulties. Success of using lignocellulosic biomass for biofuels and other useful chemical productions depends largely upon the physical and chemical properties of the biomass, on pretreatment methods and optimization of the processing conditions. The compositional changes in plant cell wall and the differences in ultra structure greatly influence the pretreatment and hydrolysis efficiency of the biomass.

Hydrolysis is a chemical reaction that releases sugars from biomass structures. Biomass dissolution involves both physical, chemical and/or thermochemical treatment processes. We will read more about these techniques later on in our subsequent lectures. So, things will be clearer that time. So the crystallinity of cellulose, hydrophobicity of lignin, and embedding the cellulose in lignin-hemicellulose matrix and difficulties in cleavage of some linkages (for example hydrogen bonding, ether linkages between phenyl propane units) make biomass materials resistant to hydrolysis.

(Refer Slide Time: 50:20)



Hydrolysates from biomass can be used for producing a wide range of value added products, including biofuels (it can be ethanol, hydrogen, butanol any such things), industrially important chemicals (for example some of the solvents) and food products (sugar and sugar alcohols).

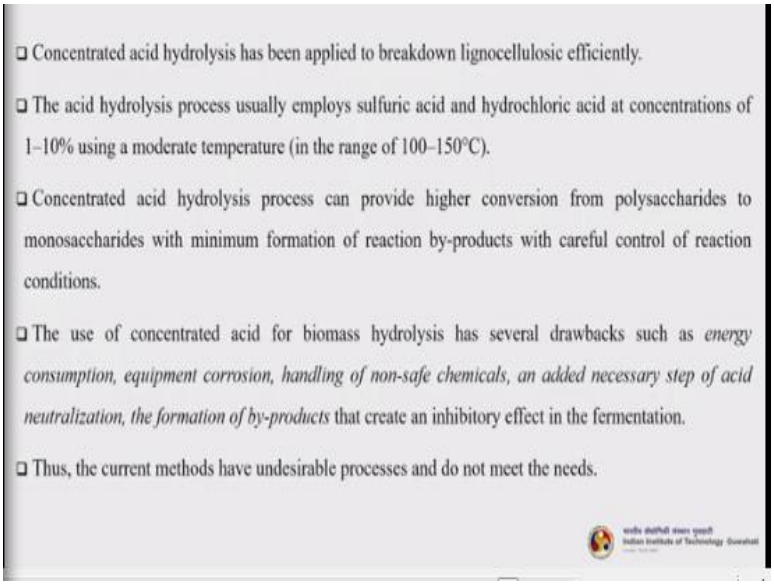
Significant existing challenges for hydrolysis of lignocellulosic biomaterials include the following. So, first is that existing hydrolysis methods are expensive and time consuming. Most of them are not environmental friendly. Second is that additional steps are required, just like here pretreatment, neutralization etc. Then third is, released carbohydrates decompose in harsh hydrolysis conditions which is prevalent during the hydrolysis process. So these are some of the challenges that needs to be tackled.

The major hydrolysis processes typically used for solubilization of biomass require either use of toxic, corrosive and hazardous chemicals (for example acids, alkali) or longer retention time (for example during enzymatic hydrolysis), which collectively make the process environmentally unsafe and/or expensive. That is why there is a huge work right now going on across the globe to develop different pre-treatment techniques. Basically different pre-treatment techniques; I'd rather say that efficient and sustainable pre-treatment techniques in which the yield will be more. The techniques should be environmentally benign. It should be a green approach.

So, huge work is going on. There are developments of hybrid techniques. We will discuss something; hybrid means basically combining more than one unit operations together. Because in one single unit operation, you may not achieve the yield which you are looking for; so you combine two processes. But having said all these, three things we should note with respect to the pre-treatment:

First is that, it should be a low-cost technique and it should be done at a very faster rate. So, time is directly related to money in industry. Second, it should result in a higher yield of the cellulose. Third is that, it should be a greener process.

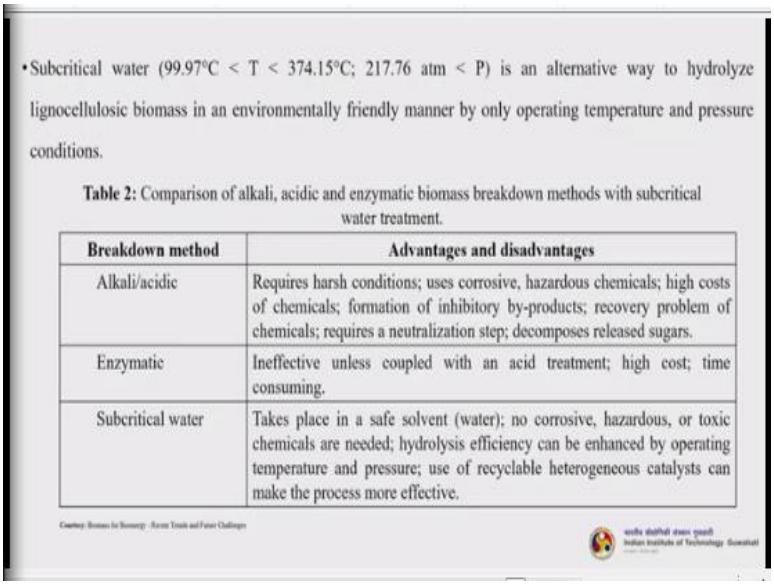
(Refer Slide Time: 52:32)



So, concentrated acid hydrolysis has been applied, but the problem with concentrated acid hydrolysis are several. So, though they provide higher conversion, but, there are environmental concerns, corrosion and so many other things. So, due to all these things some of these are listed here, please refer later on. Since almost two decades' researchers have focused their attention to dilute acid pre-treatment rather than concentrated acid pre-treatment.

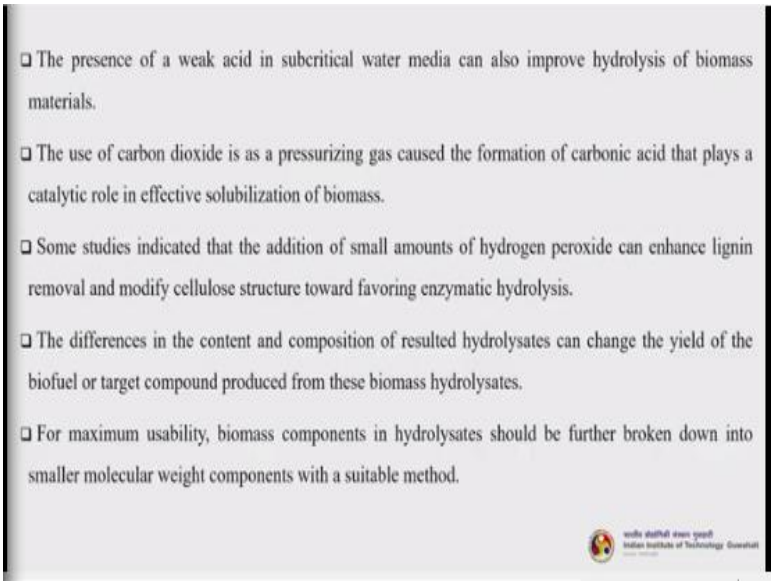
At dilute acid pre-treatment you will see hundreds and hundreds of literature reported by various researchers who have worked with so many different types of species and studied the pre-treatment using the dilute acid method. So, we will of course discuss more about that.

(Refer Slide Time: 53:22)



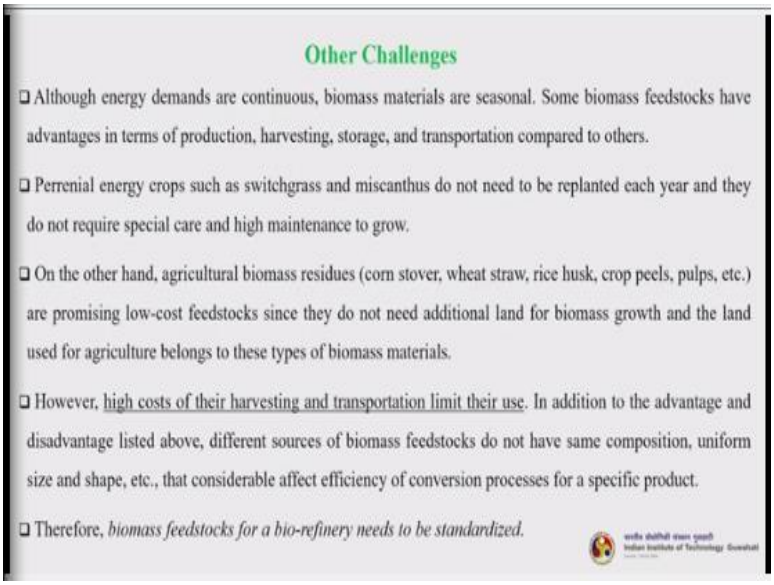
So, subcritical water is an alternative way to hydrolyze lignocellulosic biomass but please not that when you talk subcritical, supercritical the reactor in which we are going to achieve it, the initial investment is very high and you are going to again use higher energy to achieve that. Now this table will make you understand about certain breakdown methods, pre-treatment methods, alkali, acidic, enzymatic, subcritical water and their various advantages and disadvantages. So, please refer to it later on.

(Refer Slide Time: 53:56)



So, the presence of a weak acid in subcritical water media can also improve hydrolysis of biomass materials. The use of carbon dioxide as a pressurizing gas also caused formation of carbonic acid that plays catalytic role in effective solubilization of biomass. Some studies have indicated that the addition of small amounts of hydrogen peroxide can enhance lignin removal. The differences in the content and composition of resulted hydrolysates can change the yield of the biofuel, that is another concern again. So, for maximum usability, biomass components in hydrolysates should be further broken down into smaller molecular weight components with a suitable method.

(Refer Slide Time: 54:35)

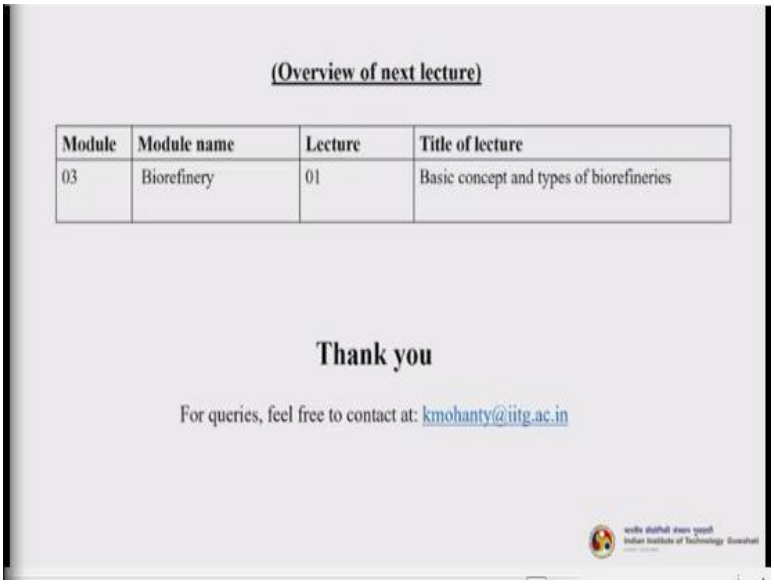


So, there are other challenges also, we will just quickly go through it. So, although energy demands are continuous, biomass materials are seasonal. So some biomass feedstocks have advantages in terms of production, harvesting, storage and transportation compared to others. So, perennial energy crops such as switch grass and miscanthus do not need to be replanted each year and they do not require special care and high maintenance to grow.

On the other hand, agricultural biomass residues, whether it is a corn stover, wheat straw, rice husk, crop peels, pulps etc. are promising low-cost feedstocks since they do not need additional land for biomass growth and the land used for agriculture belongs to these type of biomass materials. However, high cost of their harvesting and transportation limit their use. In addition to the advantages and disadvantages listed above, different sources of biomass feedstocks do not have the same composition, uniform size and shape etc. that considerably affect the efficiency of the conversion process for a specific product.

So, there are so many things that needs to be taken care of while you go and design for a particular conversion technology. Therefore, biomass feedstocks for a bio-refinery needs to be standardized, this is the ultimate thing and has to be done.

(Refer Slide Time: 55:50)



So, with this I windup. So thank you very much. In the next class that will be module 3, we will start discussing on bio-refinery. We will try to understand what is the concept of bio-refinery though in a nutshell I have covered it in the introduction class and what are the types of bio-refinery. So, thank you very much, if you have any query please drop a mail to me at kmohanty@iitg.ac.in or please drop your query in the swayam portal, thank you.