

Renewable Energy Engineering: Solar, Wind and Biomass Energy Systems
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Lecture – 25
Bioconversion of Substrates into Alcohol

Good morning everyone. Welcome to part 1 of lecture 1 under module 8. In this module, we will discuss about the bioconversion of substrates into alcohol and basic principles of the bioconversion.

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Module	Module name	Lecture	Content
08	Bioconversion of substrates into alcohol and Thermo-chemical conversion of biomass to solid, liquid and gaseous fuels	01 (Part I)	Bioconversion of substrates into alcohol Basic principles of bioconversion

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Bioconversion of substrates into alcohol (Ethanol production)

Ethanol production is based on two major techniques

- a. *Chemical synthesis:*
Acid-catalyzed hydration of ethylene forms ethanol which is referred to as a chemical process. Phosphoric acid is the most commonly used catalyst in the chemical process (Baeyens *et al.*, 2015).
- b. **Fermentation:**
Production of ethanol through fermentation process by using different microbial strains is called as biological process. Under anaerobic conditions, certain species of yeast (e.g., *Saccharomyces cerevisiae*) and bacteria (e.g. *Zymomonas mobilis*) metabolizes the sugars into ethanol and carbon dioxide (Zabed *et al.*, 2017)

Zabed, M. et al., (2017). Renewable and Sustainable Energy Reviews. Elsevier Ltd. pp. 475-501. doi: 10.1016/j.rser.2016.12.274.
Baeyens, J. et al., (2015). Progress in Energy and Combustion Science. Elsevier Ltd. 41, pp. 59-86. doi: 10.1016/j.pes.2014.10.002.

So, by conversion of substrate into alcohol, so, mainly here we will discuss about the ethanol production. So, ethanol production is based on the 2 major processes or we can say, the

techniques. The first one is the chemical process and the other one is a fermentation. So, the chemical process here, it is mainly carried out using acid as a catalyst. So, acid catalyst hydration of the ethylene it forms ethanol and which is referred to as a chemical process.

So, phosphoric acid is the most widely used catalyst for the chemical process and to produce ethanol by the hydration of the ethylene. Whereas the another process alternative to this chemical process is the fermentation technique. So, production of the ethanol through fermentation process by using different microbial strain is called as a biological process. So, under anaerobic condition, certain species of yeast that is *Saccharomyces cerevisiae* here is used for the ethanol production.

And the bacterial strain that is mainly *Zymomonas mobilis*, it metabolizes the sugar into ethanol and carbon dioxide. So, likewise, there are these 2 different processes are there, but in this particular course, our main focus is on the fermentation technique.

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Comparison of Biochemical and Thermochemical Routes for Biomass Conversion into Ethanol

Process	Biochemical Route (Sugar Fermentation)	Thermochemical Route
Feedstock	Sugarcane/Starch/Corn	Cellulosic Stock/Wood/MSW
Reactor Type	Batch	Continuous
Reaction Time	Days	Minutes
Water Usage	3.5 - 170 L/L ethanol	< 1 L/L ethanol
By-products	Distiller's dried grain	Syngas/Electricity
Yield*	~ 400 L/ton	~ 265 - 492 L/ton
Technology Maturity	> 100 plants in the United States	Pilot Plant

The fermentation of syngas from biomass gasification is another possible route for ethanol production from woody biomass.

* Courtesy: Biomass gasification, gasification and ethanol, by R. Kumar, 27 Feb 2016, Published 17

So, if you compare the biochemical and the thermo-chemical conversion of the biomass into ethanol, so, there are 2 different technologies are also there. One is called as a biochemical route, which mainly carried out using sugar fermentation and another is a thermo-chemical route. So, if you look at the technological availability and the mature technology which is available for the biochemical conversion of sucrose or starchy material to the ethanol, it plays an important role.

Because the particular platform for the conversion of this material into the ethanol is already available. And it can expand to again to the lignocellulosic biomass as well with slight modification in the process conversion technologies. Otherwise, the platform which is required for the conversion purpose from this particular sugar is already in place and it is widely being used for the commercial production as well.

But the expansion of this particular platform for the lignocellulose biomass it may need certain technological changes that too in the predetermined stage or that is in the sense of like pre processing of the biomass, so that it can be converted into the reducing sugar. And the produced reducing sugars can further be converted into the ethanol. But the technology which is required for the conversion of lignocellulosic biomass to the ethanol is most expensive.

As well as, there are certain issues are also involved in the conversion of this lignocellulosic biomass to ethanol. And it faces various challenges in terms of the high process efficiency. So, to alternative to this particular technology, thermo-chemical conversion of the woody biomass to produce a syngas using a gasification technology. And further these biomass derived syngas can be fermented to produce the ethanol.

So, as a result, there are 2 different technologies are already available and which are being used for the ethanol production. And in that fermentation is a major step by which the produced syngas is getting converted into the ethanol. Whereas, in the regular fermentation technology, the simple sugar is produced from these materials, either it is a lignocellulosic biomass or sucrose or the starchy material can be fermented to produce the ethanol.

But the fermentation of the syngas from biomass gasification is already which I mentioned is another process, but very limited information is available about this particular process. And the complete process analysis also is not available. As a result, this particular technology of biomass derived syngas to produce the ethanol is still is in the pilotscale. By looking at the comparative analysis of these processes, which can be evident that the feedstock which is used in the regular fermentation which is called a sugar fermentation technique.

It is in the form of sugarcane, starch and the corn whereas, in case of thermo-chemical route, the material handled by the process is cellulosic stock, wood and MSW. The reactor type in case of the sugar fermentation is a batch kind of reactor is used, whereas in case of thermo-

chemical route of conversion, it is a continuous reactor. That is the advantage here and the reaction time is basically in case of fermentation is some days. It required some days to convert the raw material to the ethanol, whereas here it is in minutes.

So, that is a big difference between these 2 processes. Apart from that the uses of water here if you see, it requires a significant amount of water during the process, whereas, when it is a thermo-chemical conversion route, the requirement of water is significantly less. And the byproduct which is produced from the regular fermentation, which is called alcoholic fermentation as well, it provides distiller's dried grain as a byproduct.

Whereas, in case of thermo-chemical route, syngas and electricity is the byproduct. Apart from that the yield which achieved in alcoholic fermentation, it is relatively high here, whereas, in case of the thermo-chemical route, it varies between this particular range, which is available in the literature. And technological maturity, if you see here, as I already mentioned, due to the technological maturity of the biochemical conversion process to produce the ethanol from this kind of substrate.

It plays an important role in terms of providing a platform for the expansion of this particular technology for the lignocellulosic biomass as well. But, as I mentioned, very limited information is available about the thermo-chemical conversion route. And as a result, this particular technology is tested only at a pilot scale. And there are, maybe some processes which must be locally producing this kind of material, but it is not widely known for at a commercial scale yet.

However, this particular technology, if you see, a number of plants are available in the United States as well as in the India for the production of ethanol using this kind of material. So, the focus point of this particular lecture is on the biochemical route of conversion of the feedstock into the ethanol. So, this is just for the comparison purpose and just to make you aware about the recent advancement in the conversion of this particular material to a ethanol.

So, there are alternative techniques are also available, which also utilises the fermentation pathway to produce the ethanol. But in this case, the raw material is nothing but the syngas produced during the gasification need to be cleaned first. And then after cleaning the syngas, it can be fermented using the suitable microorganism to produce the ethanol. So, likewise, the

advancement is also happening in this particular technology to convert the material more faster way and at a faster rate into the ethanol.

But as I mentioned, our main focus in this particular course is on the biochemical pathway to produce the ethanol.

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- *Saccharomyces cerevisiae*, is poisoned by C_2H_5OH concentrator greater than 10%, and so higher concentrations upto 95% are produced by distilling and fractionating.
- Production of bioethanol from sugars like Sucrose, Glucose, and Fructose is an industrially well-established process and it is found to be a safest and eco-friendly process as well (Vohra *et al.*, 2014).
- However, the first usage of ethanol blended gasoline as a fuel occurred in the 1920s and 1930s and was in high demand during World War II because of fuel shortages.
- Several renewable resources like rice, wheat, corn, sorghum grains, sugarcane, cassava, and sugar beet are generally used for the production of bioethanol.

Vohra, et al., 2010, Journal of Environmental Chemical Engineering, 2(1), pp. 273-284, doi: 10.1016/j.jece.2011.10.013

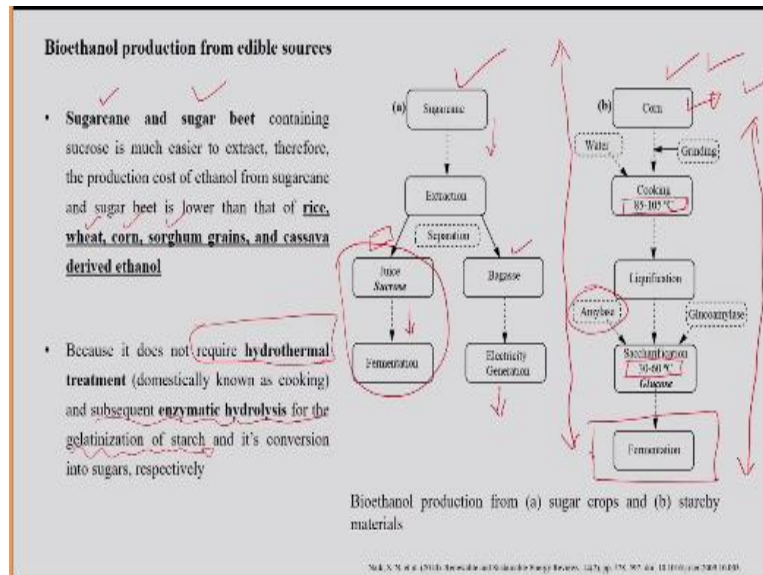
So, as I mentioned in the previous slide, *Saccharomyces cerevisiae*, which is the widely used microbial stain for the fermentation purpose. But, it is poisoned by ethanol concentrator which is greater than around 10%. And so, higher concentration of 95% are produced by distilling and fractionating technique. The concentration if it is going beyond 10% so, it will poison the *Saccharomyces cerevisiae*.

As a result, the ethanol which is produced using this fermentation technology, which is called as a accurate ethanol, this needs to be upgraded using the distillation technique to produce a 95% ethanol. The production of the ethanol from the sugars like sucrose, glucose and fructose is an industrially well established technology and is also considered as the safe technology and eco friendly process as well.

Apart from that, the first use of this ethanol blended gasoline as a fuel occurred in 1920s and 1930s. And it was in high demand during the World War 2 because of the fuel shortage. So, accordingly there are several renewable resources like rice, wheat, corn, sorghum, grains, sugar cane, cassava and sugar beet are generally used for the production of the ethanol. But now, if you look at this particular list of the resources which are mentioned here.

So, these particular resources are also divided into the 2 separate class. One is called as a class which contains a sucrose containing material and other is considered a starchy material.

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So, the sucrose containing material is mainly considered as a sugar cane and sugar beet because sugar cane and the sugar beet, it mainly contains the sucrose and it is much easier to extract the sucrose from the sugar cane as well as from the sugar beet. As a result, the production cost for this particular material is relatively less to produce the ethanol compared to the other material such as wheat, corn, sorghum, grain and cassava derived ethanol.

Why? Because in this particular feedstock, it does not require any hydrothermal treatment. That is the advantage of utilising this material that it does not require any hydrothermal treatment. Also, it does not require any enzymatic hydrolysis for the gelatinization of the starch and its conversion into the sugar. As a result, the extracted sucrose can directly be fermented to produce the ethanol.

So, just for the comparison purpose, we have shown here the conversion pathway of the sugar cane as well as the starchy material. So, if you just look at the conversion pathway for the sugar cane as a material or the sugar beet as a material. So, after the extraction, what happens is like, here the separation can be done that is nothing but juice and the sucrose fraction can be separated from the bagasse here.

And the produced sucrose or the juice can be fermented to produce the ethanol as well as the bagasse, which is produced during the process can be used to produce the heat or the electricity purpose. So, the number of steps which are involved in this particular process are relatively less. As a result the production cost for this particular process is lower than that of the starchy material.

Whereas, if you compare the conversion pathway for the starchy material, for example, is a corn. So, what happened in this case is like, first the material has to pass through a cooking process which is called a primary process. And it is carried out in the temperature range of say 85 to 105 degree C. So, once this particular cooking is carried out which is calling a primary operation in the starchy material.

So, what happened in this particular process? The gelatinization of the starch happens and then this particular material which is a gelatinization material of the starch, it undergoes hydrolysis process using certain enzyme which is called as amylase enzyme. And this particular hydrolysis is carried out in the temperature range of 30 to 60 degree C. And this hydrolysis process, it converts the starchy gelatinous material into a glucose that is called as a simple sugar.

And the produced simple sugars further can be converted into the ethanol. So, the number of steps if you see, in this particular process requires are relatively more than that of the sucrose material. As a result, the production cost of the ethanol from the sucrose containing material is lower than the production cost of the starchy material.

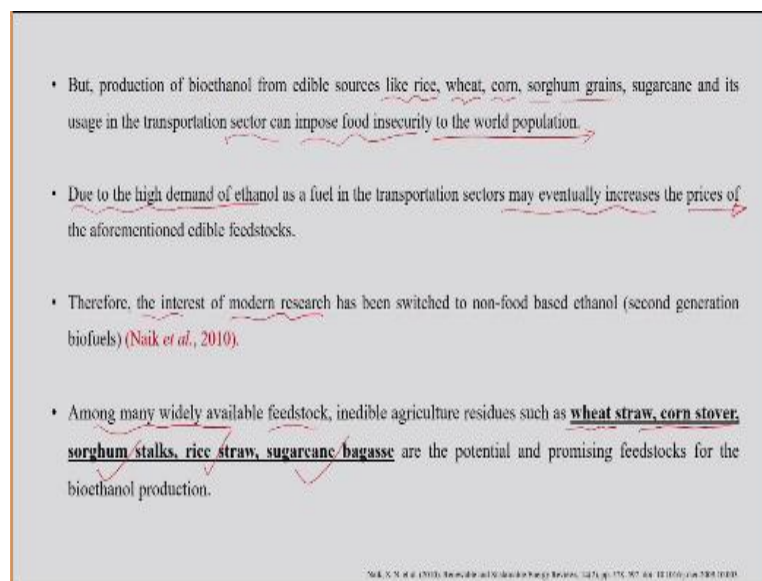
Apart from that, unlike sucrose fermentation technique, in case of the starchy material, the hydrolysis of the gelatinization of the material is carried out using a separate enzyme that means amylase. And the hydrolyzed solution which is obtained from the starch can be fermented using a *Saccharomyces cerevisiae* to obtain the ethanol. So, if you see the comparison wise as well, it requires 2 enzymes to convert the raw material to the product.

Whereas, in this particular case, single enzyme operation is sufficient to convert the raw material into the product. So, that is also a difference in the 2 processes which can be also visualised from this particular chart that it requires one enzyme in this particular step, which

is called the enzymatic hydrolysis process to produce the sugar simple sugar in the form of the glucose. And the produced sugar undergo fermentation here.

It also required another enzyme to produce the ethanol. Whereas, in this particular case, it is carried out using a single enzyme and it produces again the same product which is the ethanol. So, by this way, the conversion steps which is required for both this material is different. As a result, so, production costs for the starchy material is literally higher than that of the sucrose containing material. That also can be visualised from this schematic.

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So, now, the production of this bioethanol from the edible sources like rice, wheat, corn, sorghum grains, sugar cane and its usage in the transportation sector can impose food in security to the world population. That is one of the major concern about the utilisation of this material further by ethanol production. Because these all materials are the edible materials and utilisation of this material for the ethanol production.

And subsequent utilisation of this ethanol which is produced using this particular process in the transportation sector, it may impose food in security to the world population. Due to the high demand of the ethanol as a fuel in the transportation sector, it may eventually increase the price of the food grade material as well. So, that is also a negative impact of utilisation of this material for the ethanol production.

So, as a result, what happens? Therefore, the interest of modern research has been switched to non-food grade feedstock material. So, that is also termed as the second generation biofuel.

So, mostly the non-food grade feedstock material here is lignocellulosic material or the agriculture residues. So, among many widely available feedstock material, which is called the inedible feedstock material.

Or I would term it as an agriculture residues such as wheat straw, corn stover, sorghum stalks, rice straw and sugar cane bagasse can be considered as the potential and the promising feedstock for the production of the ethanol because these are the materials which are widely available. And if you look at the production of this particular material at the world level, so, significant amount of this particular material is available for the utilisation purpose.

And these abundantly available residues are basically used as an animal feed or as a domestic fuel for the heating purpose. Apart from that, there is no much use of this particular agriculture residues for any other purposes. Now, therefore, from this particular ability of this material, if you see, only lower amount of the material or very less amount of the material is being used as a animal feed, whereas, the rest of the material is disposed of or maybe it is disposed of by burning also.

The burning of this material in the open field, it emits greenhouse gases. And as a result, the amount of these greenhouse gases which is emitted during this burning, it could be in a very large amount, which could in principle be an inducer of the global warming as well. Thus, instead of burning this material in open field, it can also be effectively utilised for the production of the byproduct.

That too for the production of the bioethanol, which also gives certain benefit in terms of enhancement of the energy resources or you can say, strengthening the Sustainable Energy, increasing the rural economy as well as eco friendly system as well. Therefore, the utilisation of this feedstock can be effectively carried out to produce the bioethanol. Now, if you look at this particular material and the amount of this particular material available in this 4 countries itself.

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The amount of major agricultural crops residues burned in the year 2016 and their CO₂ emissions

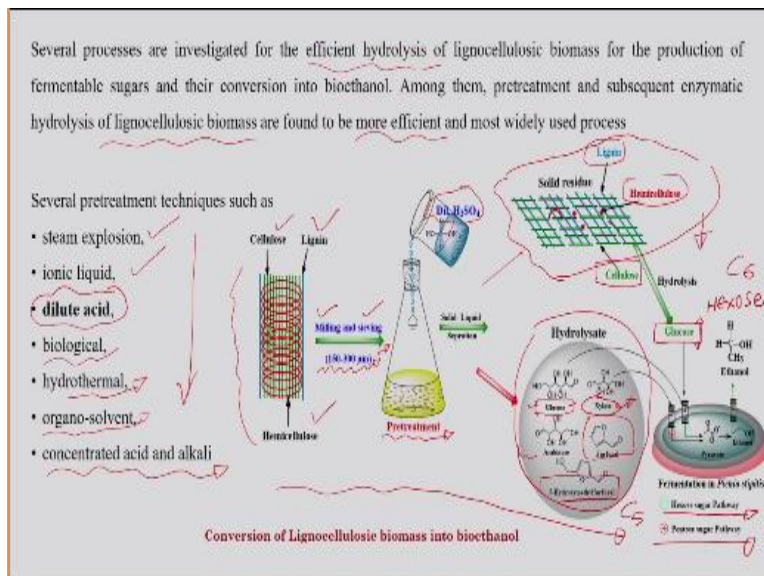
Country	Agriculture crop residues (Million dry ton/year)				Million ton/year
	Corn	Rice	Wheat	Sugarcane	CO ₂ emission
USA	35.11	0.07	7.10	0.24	3.73
India	10.20	23.63	12.09	3.22	4.25
China	38.98	16.75	9.74	1.09	5.75
Brazil	14.96	1.07	0.87	6.65	2.03

Due to the structural rigidity, bioethanol production from lignocellulosic biomass is difficult than that of sucrose (sugarcane) and starch-containing materials (corn, wheat, rice, sorghum grains, and cassava).

It gives a idea that the significant amount of these materials are available in the raw form and very small amount of these materials are only being used as a animal feed. And the remaining material is disposed off as a waste. Now, if you look at the amount of major agricultural crop residues that have been burned in the year 2016 and the CO₂ emission caused because of the burning of these resources, you can see in this particular table here.

So, this is a significant amount of the CO₂ contribution happened in the environment because of the burning of these resources. However, the utilisation of these resources for the ethanol production is also not that easy because, due to the structural rigidity, the bioethanol production from the lignocellulosic biomass is difficult than that of the sucrose and starchy material. And this is mainly because of the structural rigidity of the lignocellulosic biomass.

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And here is because of that, several processes have been investigated for the efficient hydrolysis of this lignocellulosic biomass for the conversion into the reducing sugar first and then subsequent conversion of the produce reducing sugar to the ethanol. But out of the several techniques, pretreatment and enzymatic hydrolysis of lignocellulosic biomass are found to be more efficient and most widely used process.

So, if you see this particular slide, number of pretreatment techniques have been enlisted here, which shows here the steam explosion, ionic liquid and dilute acid, biological process as well, hydrothermal technique, organo solvent and concentrated acid and the alkali technique. Among these techniques, the dilute acid technique is the most widely used and the most effective technique for the pretreatment of the lignocellulosic biomass.

So, the lignocellulosic biomass which is shown here in this particular diagram is contains cellulose, hemicellulose and the lignin fraction. As we already discussed this point before, the raw materials cannot be used in its raw form directly for the conversion purpose. As a result, it has to pass through certain pre processing stage. Similarly, in this particular stage, the raw feedstock first need to be built.

And then sieved to produce smaller particles of the raw material. So, produce smaller particles of raw materials is also have a large surface area for the reaction purpose as well. And then this particular produce material can undergo pretreatment step to hydrolyze the hemicellulose fraction of the material. So, this particular process is carried out using dilute acid technique.

So, the dilute sulfuric acid is used for the pretreatment of the this reduced material. So, as a result what happens is like in this particular stage, the hydrolysis of mainly hemicellulose fraction of the lignocellulosic biomass takes place and it makes the residual biomass containing cellulose more amenable for the enzymatic hydrolysis. So, this is termed as a residual biomass which can be obtained after the separation of the hydrolyzate from the residual biomass.

And in this case, if you see, it mainly contains the cellulose along with some fibre, lignin and some fraction of the hemicellulose which could not get converted in the pretreatment step here. So, as a result, this is called as a residual biomass, which is obtained after the

pretreatment of the processed raw material and the hydrolyzate produced in this particular case, it mainly contains the xylose and some fraction of the glucose.

Because during this pretreatment stage it may happen so, that some fraction of the cellulose it may undergo the hydrolysis. So, as a result you can find here that it also produced certain small fraction of the glucose, but mainly it contains the xylose as a pentose sugar along with the arabinose and furfural and the 5 HMF. So, these 2 compounds are considered as a inhibitor for the ethanol fermentation stage.

So, to avoid the formation of this particular compound, the process parameter need to be optimised and operated in such a way that the production of this particular inhibitors can be avoided in the pretreatment stage itself. And this particular residual biomass which mainly contains the cellulose fraction can be hydrolyzed enzymatically to produce mainly a hexose sugar in the form of glucose.

And then the produce C 6 fraction and the C 5 fraction can be fermented using the hexose sugar pathway and the pentose sugar pathway to produce the ethanol. And this is how the conversion of lignocellulosic biomass to ethanol takes place. So, if you see in this particular case, it all depends on the composition of the lignocellulosic biomass and accordingly it can provide the specific yield of the ethanol.

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Ethanol is produced by the action of microorganisms on carbohydrates (alcoholic fermentation). Carbohydrates (saccharides) can be divided into three major classes in order of increasing complexity.

- ✓ **Monosaccharides** (simple hydrocarbons, which cannot be hydrolyzed into simpler compounds),
glucose and fructose
 - More precisely, glucose and fructose can be represented by the formulae $\text{HCO}-(\text{CHO})_n-\text{CH}_2\text{OH}$ and $\text{CH}_2\text{OH}-\text{CO}-(\text{CHO})_n-\text{CH}_2\text{OH}$, respectively.
- ✓ **Oligosaccharides** (Yield few but definite numbers (2-10) of monosaccharide molecules on hydrolysis).
 - For example, a disaccharide (such as sucrose and maltose, both having the formula $(\text{C}_{12}\text{H}_{22}\text{O}_{11})$) produces two monosaccharide molecules on hydrolysis.
 - *Sucrose (common sugar)* occurs naturally in sugarcane and beetroot. *Maltose (malt sugar)* is derived from starch.

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The ethanol is produced by the action of microorganism on the carbohydrates and the carbohydrate here are majorly classified into 3 types in the order of increasing complexity.

That means if you talk about the monosaccharide, which is a simple hydrocarbon and which further cannot be hydrolyzed into a simpler compound, because, this is one of the most simple hydrocarbon which can be obtained from the complex carbohydrate structure of the lignocellulosic biomass and the example is glucose and the fructose.

More precisely, if you see the glucose and the fructose can be represented by the formula here and this is for the fructose respectively. So, another class of carbohydrate if you see, which is a oligosaccharide. So, this oligosaccharides it yield few but definite numbers of monosaccharide molecules on the hydrolysis. So, this is the difference between the monosaccharide and the oligosaccharide as well.

Because oligosaccharides it yield few, but definite number of monosaccharide molecule on the hydrolysis. And if you see the example here, a disaccharide molecule such as sucrose or maltose, both having the formula of like C 12 H 23 and O 11. This produces 2 monosaccharide molecules under hydrolysis. So, if you see the oligosaccharides, example is sucrose and the maltose.

So, hydrolysis of the sucrose and maltose it gives 2 monosaccharide molecules and the sucrose as we already discussed, which is a common sugar occurs naturally in the sugar cane and the beetroot whereas maltose which is also called as a malt sugar is derived from the starch material. So, this is the difference between the monosaccharide and the oligosaccharides.

So, monosaccharide is the simple sugar which further cannot be analysed to produce simpler compound whereas, oligosaccharide it yields definite number of the monosaccharide molecules on the hydrolysis.

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Polysaccharides (high-molecular mass carbohydrates, which yield a large number of monosaccharide molecules on hydrolysis).

- Examples are *starch and cellulose*; both having the general formula $(C_6H_{10}O_5)_n$. Large numbers (few hundreds to few thousands) of glucose units are joined together in a complex chain.
- *Starch* occurs naturally in all plants, particularly in seeds. The main sources are maize, barley, rice, wheat, potato, cassava and sorghum.
- *Cellulose* is the main constituent of cell walls of the plants. Wood contains **45-50%** while cotton contains **90-95%** cellulose.

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And polysaccharide is mainly a high molecular mass carbohydrate and which yield large number of monosaccharide molecules on hydrolysis. So, this is difference between the polysaccharides, oligosaccharides and the monosaccharides. Now, if you talk about the example here is mainly a starch and the cellulose and both having the formula is like C 6 H 10 O 5 and number of such glucose units are joined together in a complex chain manner.

And then it forms a cellulose compound. The starch it occurs naturally in all plants, particularly in the seeds. And the main sources are maize, barley, rice, potato, cassava and sorghum. Whereas, the cellulose is the main constituent of the plant cell wall. And this we already discussed in our previous lectures as well that cellulose is the main constituent of the plant cell wall.

And in wood, it varies in the range of 45 to 50%, while in cotton its percentage is around 90 to 95%. So, that is the reason the lignocellulosic biomass was just to be utilised for the ethanol production. So, it requires certain treatment stages so that it can be converted into the monosaccharide first, which is called as a simple sugar and then the produced monosaccharides can be utilised for the ethanol production.

Now, based on this carbohydrate, if you just try to see the actual sugar production from this particular carbohydrates, so, there are basically 3 materials.

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The hexose (glucose and/or fructose) required for ethanol fermentation is derived from (i) sucrose, (ii) starch; or (iii) cellulose as explained below.

i. Sucrose:

- Sucrose is the most common disaccharide and can be produced from sugarcane or beetroot.
- Usually, commercial sucrose is separated from cane juice, and the remaining molasses; which has low commercial value, is used for ethanol production.
- The molasses itself has about 55% sugar content and serves as very good raw material for ethanol production.
- On hydrolysis with dilute acids or enzymes, it gives equal amounts of glucose and fructose.

$$C_{12}H_{22}O_{11} \text{ (Sucrose)} \xrightarrow{\text{hydrolysis}} C_6H_{12}O_6 \text{ (Glucose)} + C_6H_{12}O_6 \text{ (Fructose)}$$

- Sucrose are readily available in a fermentable form, require least expensive preparation, but are generally most expensive to obtain.

One is the sucrose, starch and the cellulose. So, these materials can be effectively converted into the hexose sugar and the producing hexose sugars can be utilised for the ethanol production. So, let us discuss about these 3 materials one by one. Sucrose if we talk about here, it is the most common disaccharide as we already discussed in the previous slide and can be produced from the sugar cane and the beetroot.

So, the source material for the sucrose is sugar cane and the beetroot. And usually, commercial sucrose is separated from the cane juice and the molasses, which is produced during this process also has a low commercial value. So, as a result, the produced molasses can be effectively utilised for the ethanol production. So, that is the also the advantage of this particular material.

Instead of using the Sucrose material directly, the molasses part of this material can be converted into the ethanol. And the technology for the production of the ethanol from the molasses is well established technology and is being used commercially at a large scale for the production of the ethanol as well. So, the molasses itself has about 55% of the sugar content and it serves as a potential raw material for the ethanol production.

Now, on hydrolysis of this particular compound using dilute acid or the enzyme, it gives equal amount of glucose and fructose molecule. So, if you see here, the structure of the sucrose, this is a structure of the sucrose which undergoes hydrolysis to produce glucose and fructose molecules. The sucrose are readily available in a fermentable form it requires least expensive preparation, but are generally most expensive to obtain as well.

So, this is very important point about the sucrose conversion to the ethanol. So similarly, if you see the starch material.

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ii. Starch:

- On hydrolysis with dilute H_2SO_4 or enzymes, Starch breaks down to maltose and finally to glucose.

$$2(C_6H_{10}O_5)_n + nH_2O \rightarrow nC_{12}H_{22}O_{11} + nH_2O \rightarrow 2n C_6H_{12}O_6$$

(starch)
(Maltose)
(Glucose)
Hexose

Starch bearing materials are often cheaper, but require processing to solubilise and convert starch to sugars.

iii. Cellulose

- Cellulose is not hydrolyzed so easily as starch, but on heating with dilute sulphuric acid under pressure yields glucose.

$$(C_6H_{10}O_5)_n + nH_2O \rightarrow n C_6H_{12}O_6$$

(Cellulose)
(Glucose)

Cellulosic materials are readily available organic compound, but require most expensive and costly preparation. Finally, ethanol is obtained from fermentation of hexose sugars.

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In case of starch material, the hydrolysis of starch material with a dilute sulfuric acid or you can say the enzyme, the starch breakdown into maltose and finally, use glucose after subsequent hydrolysis of this maltose as well. So, this is a reaction scheme, which is shown here. Say for example, this is a starch molecule, which undergo hydrolysis to produce the maltose and the maltose which undergo subsequent hydrolysis to produce the simple sugar in the form of the glucose which is also called as a hexose sugar.

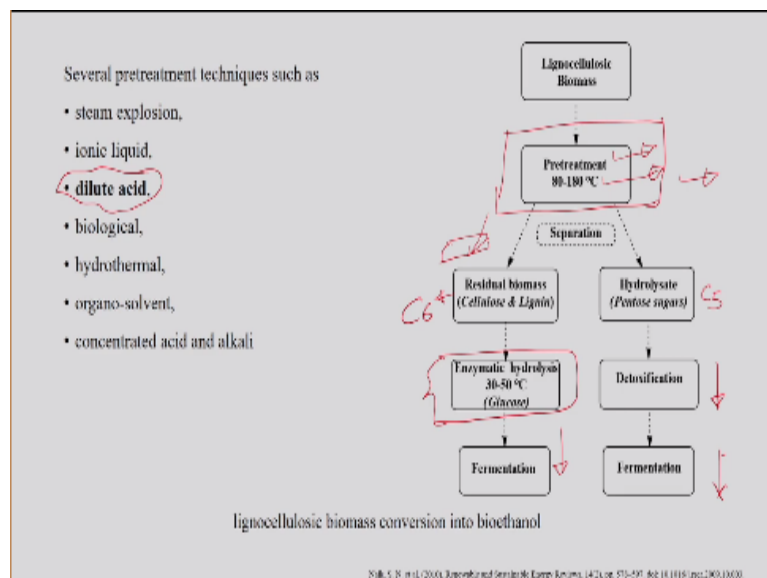
And the starch material are often cheaper, but required processing to solubilise and convert starch to sugar. Thus, the reason, as we already discussed this part in the previous slide, in terms of the comparison of the flowchart, the starch material it requires more steps to convert the material into the reducing sugar form that is in the form of the hexose sugar and then it can be subsequently converted into the ethanol compared to the sucrose containing material.

Whereas in case of the cellulose, if you see here, the cellulose is not hydrolyzed as easily as that of the starch. But on heating with dilute sulfuric acid under the high pressure, then cellulose converted into a reducing sugar as well. So, in this case, if you see, this is a cellulose molecules, which undergoes dilute acid hydrolysis to produce the glucose molecules. The cellulosic materials are readily available materials but require most expensive and costly preparation.

Finally, the reducing sugar which obtained from this cellulose material can be converted into the ethanol using the fermentation technique. But as I mentioned, the cellulosic fraction because of its complex and the rigid structure, require most expensive and harsh pretreatment technique sothat it can release the reducing sugar and then the produced reducing sugar can be further converted into the ethanol using the fermentation technique which is called as a alcoholic fermentation technique.

Now, in terms of the process point of view and the number of steps which are required for the conversion of the lignocellulosic biomass to the ethanol, if you look at the chart which is displayed in here on this particular slide. So, the number of steps which are required in this particular process are relatively more than that of the starch and the sucrose containing material. Whereas, the pretreatment step is the commerce step in the case of the lignocellulosic material.

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If we talk about any lignocellulosic material, it has to undergo certain pretreatment stage. And the temperature requirement in the pretreatment stage, it varies depending on the composition of the material as well. And if you see here the temperature ranges varies from 80 to 180 degree Celsius. So, most preferred technology for the pretreatment of the lignocellulosic biomass is a dilute acid pretreatment technique followed by the enzymatic hydrolysis of the residual biomass to produce the sugar.

So, in the case of enzymatic hydrolysis as well, as we have mentioned earlier, so, it also carried out using 2 different enzyme here because the lignocellulosic biomass mainly use C 5 and C 6 sugars. So, there is no efficient technology still available for effectively convert the C 5 and C 6 sugars together into the ethanol. As a result, C 5 and C 6 sugars need to be fermented using the hexose sugar pathway and the pentose sugar pathway to produce the ethanol.

Although the research is underway to have a common enzyme to produce the ethanol from the hexose and the pentose sugars together, but still there is no commercial scale operation in place which converts the pentose and the hexose sugars together in a single operation. So, in this particular process, after the dilute acid hydrolysis of the lignocellulosic biomass, so, it mainly hydrolyses the hemicellulose fraction of the biomass.

We use mainly a pentose sugar that is in the form of the C 5 sugar which is called as a xylose alongwith some fraction of glucose and arabinose. As this particular treatment need to be carried out very effectively as I mentioned, because it may produce certain inhibitory compound during the pretreatment stage. So, as a result, the process parameters need to be tuned accordingly, so, that it mainly produces only the xylose and cellulose alongwith some arabinose compound and sub-traces of the inhibitory compound.

So, that need to be restricted very effectively so that it will not inhibit the fermentation process later. And the residual biomass, which is mainly a cellulose fraction along with some lignin fibre and hemicellulose fraction, which could not get converted during this pretreatment stage, that residual biomass can be hydrolyzed enzymatically to produce the glucose fraction that is called as a C 6 sugars.

So, the C 6 sugars obtained after the enzymatic hydrolysis of the residual biomass, if you see here, this is called as a enzymatic hydrolysis stage to produce the glucose fraction, that is a C 6 sugar. And this C 6 sugars can be fermented to produce the ethanol. Similarly, the C 5 sugar fraction can be amended to produce the ethanol. And then the produced ethanol can be converted into a commercial grade ethanol by distillation technique.

So, the number of steps which are required for the conversion of lignocellulosic biomass to ethanol are comparatively more than that of the starch and the sucrose containing material.

As a result, this particular technique is more expensive and costly compared to the sucrose and the starchy material conversion into the ethanol. So, now, once we understand the process of production of the ethanol from the lignocellulosic fraction. So, let us talk about the fermentation system using the cellulosic material.

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Fermentation Systems (Cellulosic material)

The conversion of cellulose, starch and sugars to ethanol (C_2H_5OH):

$$(C_6H_{10}O_5)_n + n H_2O \rightarrow n C_6H_{12}O_6$$

(Cellulose) (Glucose)

$$C_{12}H_{22}O_{11} + H_2O \rightarrow 2C_6H_{12}O_6$$

glucose

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

ethanol

Thermal properties of ethanol

Parameters	Temperature ($^{\circ}C$)
Boiling point	78.22
Flash point	16.45
Auto-ignition temperature	424.85
Heat of combustion	26800 kJ/kg

* Source: Bio-chemical engineering, P. Ramani, 2nd Edition, 2004, Page 129

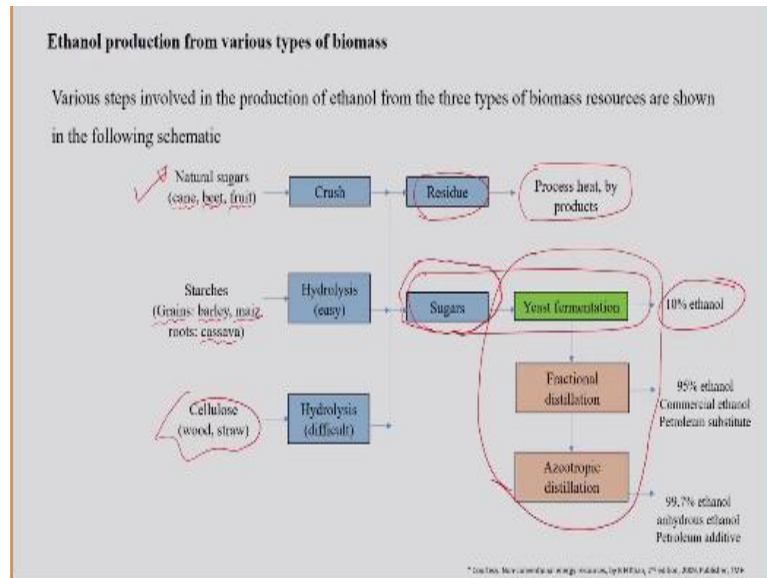
So, how it gets converted into the ethanol and the reaction involved in that particular process. So, here the cellulose material is first hydrolyzed to produce the glucose. So, maybe certain sucrose containing materials can also undergo hydrolysis step to produce the glucose molecule and the glucose, which is called as the simple monosaccharide molecule or the sugar compound produced during the hydrolysis steps can undergo a fermentation process to produce 2 moles of ethanol and 2 moles of carbon dioxide.

So, one mole of glucose molecule, it gives 2 moles of ethanol and 2 moles of carbon dioxide molecule during the fermentation of the glucose. So, thermal properties of this particular ethanol which is produced during this process, if you look at the thermal properties, so, the boiling point of ethanol is 78 degrees Celsius and the flashpoint is around 16.45 and the auto-ignition temperature is around 424.

But if you talk about the heat of combustion of ethanol, it is around 26.8 mega joule per kg, which is relatively good value for the combustion of the ethanol. So, now, up till this point we will discussed about the different materials, which can be effectively utilised for the production of the ethanol that is sucrose, starch material and the cellulose.

If you compare this material and just try to see how the number of steps involved in this particular conversion of raw material to ethanol are different, then it can be very easy to understand that why the production of the ethanol from cellulosic biomass is more expensive than that of the starch and the sucrose.

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So, for example, if the raw material is a natural sugar in the form of say, cane, beetroot and the fruit, so, simply this material can be crushed to produce the sugar. And the residual matter obtained during this process can be separated and can be used as a raw material to generate the heat as well as to produce the electricity. Whereas in case of starchy material, the examples barley, maize or if it is a root that is a cassava.

So, what happened in this case, that the material has to pass through certain primary pretreatment stage that is a cooking operation as we have discussed earlier and then after cooking, the gelatinization of the starch will occur and then the hydrolysis of the starch is carried out using the enzymatic hydrolysis type. And the produced sugar can be fermented to produce the ethanol.

So, if you see the number of steps required in this particular step is more than that of the sucrose containing material and hence, the production cost of this particular procedure is relatively higher than that of the sucrose containing material, even the remaining process are almost same. And that is why it is called as a well established technology when it is a fermentation process.

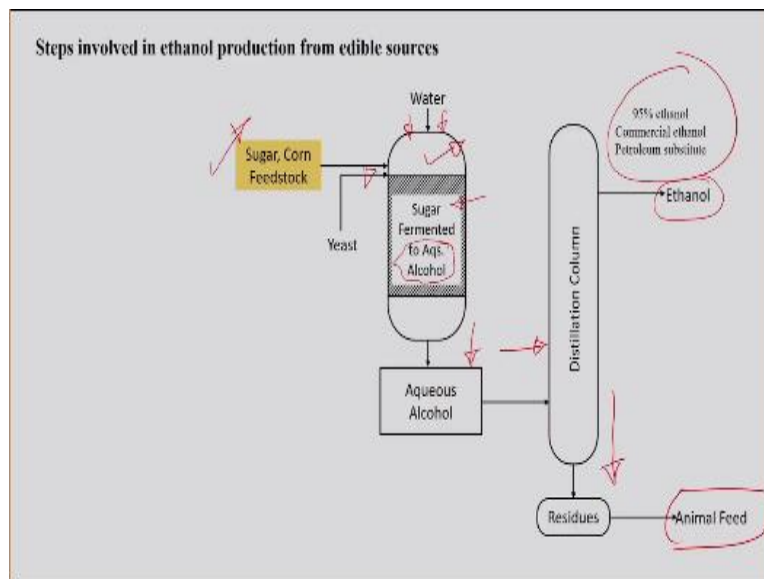
But, to produce the raw material for the fermentation and the pre-processing of the raw material to produce the reducing sugar is more expensive, if the feedstocks are getting changed. Now, if you see the example of the cellulose, so, in case of cellulose, the example is wood, straw as we have discussed or the agriculture residues. So, this kind of materials need to be pretreated first.

And after the pretreatment as we have discussed, the hexose fraction is get separated during the hydrolysis of the pretreatment message and the remaining residual matter undergoes the enzymatic hydrolysis to produce the simple sugar that is a glucose. And then the produced glucose can be fermented to produce the ethanol. So, likewise, the number of steps which are required in the conversion of lignocellulosic biomass to ethanol or again more than that of the starch and the sucrose containing material.

And as a result, this particular process is quite expensive and costly compared to that of the sucrose and the starch material. And the remaining fraction of the process are almost same. This is a aqueous alcohol, which is obtained during the fermentation and this aqueous alcohol can be upgraded using the suitable distillation technique.

Now, once we understand the production of this ethanol from different material, whether it is a edible source or non edible source.

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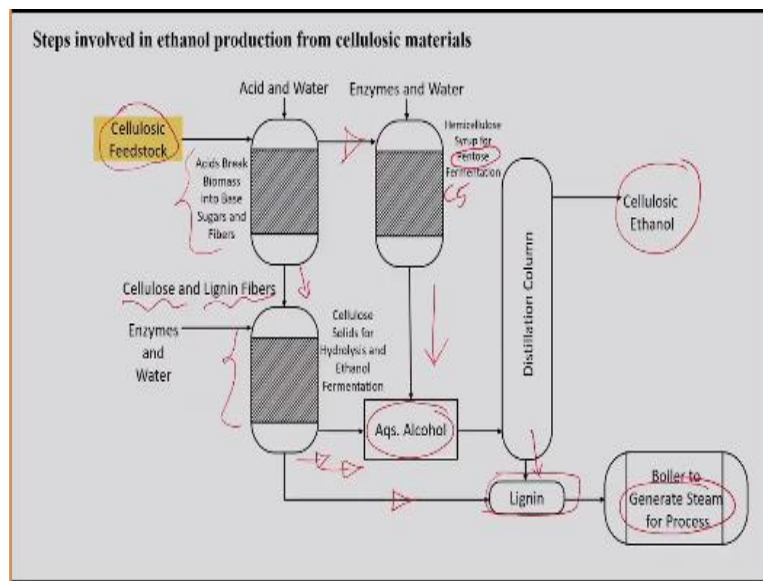
So, let us discuss about the production of ethanol from the edible source, example is sugar, corn as a feedstock. So, in this case, what happens is like the material need to be pre-

processed first and the pre-processed materials can directly undergo the fermentation using microorganism that is a yeast, in presence of water, so that the pH of the system can be maintained to the required level and then it can be fermented to produce the aqueous alcohol.

Because as we have discussed earlier, *Saccharomyces cerevisiae*, it is poisoned by the ethanol concentrator more than the 10%. Hence to maintain their concentration, it can be diluted with the same amount of water, so that we can maintain the pH in the proper range, so that it will not inhibit the microorganism during the fermentation process.

And to produce accurate ethanol can be distilled to obtain 95% commercial grade ethanol. Here, which is called as a by ethanol as well. And the result you produce during this particular process, it act as a animal feed. So, this is how the production of the ethanol takes place from the edible source as a raw material.

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However, if you discuss the ethanol production from the non-edible feedstock, now, the number of operations required in this process are relatively higher than that of the edible feedstock. Let us see the example of the cellulosic feedstock here. So the cellulosic material first need to be pretreated so that it can separate the hemicellulose fraction from the cellulose fraction here.

And the hemicellulose fraction obtained after the pretreatment can be separated in the form of the hydrolysate that contains mainly the C 5 sugar. And then the produced syrup of the hemicellulose fraction that is a pentose sugar, can be fermented to produce the ethanol. So,

this is the operation which carried out for the separation of the pentose sugar from the residual biomass after the pretreatment stage.

And once the residual biomass obtained after the pretreatment stage is mainly in the form of cellulose and the lignin fibres with small traces of hemicellulose fraction, which could not get treated in the acid pretreatment stage. So, this residual biomass can be enzymatically hydrolysed to produce the C 6 sugar, that is called as a glucose sugar. And this is called as a C 5 sugar, mainly a xylaine that is the Xylose.

So, the C 6 sugars obtained from the enzymatic hydrolysis of the residual biomass can undergo fermentation to produce again the aqueous alcohol. So, the aqueous alcohol obtained from the C 5 fraction and the aqueous alcohol which is obtained from the C 6 fraction can be distilled to produce the cellulosic ethanol. So, this is how is the difference between the production of the ethanol from the lignocellulosic biomass that is a non-edible feedstock and the production of the ethanol from the edible feedstock that is mainly of sugar and corn.

And the lignin fraction obtained from both this process is used to generate process steam. So, the residual matter which is produced during the entire process can be used to generate the process steam. So, this is how the entire process of the production of the ethanol from the lignocellulosic biomass is takes place.

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- Pyruvate serves as a key branching point in fermentations.
- In an ethanolic type of fermentation, pyruvate is metabolized to acetaldehyde and carbon dioxide, and acetaldehyde is then reduced to ethanol.
- Thus, in this process, it is theoretically possible to achieve 2 moles of ethanol for each mole of glucose converted, or on a weight basis, a 51 % conversion of glucose into ethanol.
- In practice only 90-95% of the theoretical amount of ethanol is produced as some of the pyruvate is consumed for cellular material during culture growth and is not available to serve as an electron acceptor.
- Also, low levels of higher alcohols are produced as metabolic waste products.

Type of fermentation	End products	Microorganisms
Ethanol	Ethanol, Carbon dioxide	Yeast
Mixed acid	Lactic acid, Formic acid, Acetic acid Carbon dioxide, Hydrogen, Ethanol	<i>Zymomonas</i> <i>Clostridium</i> and many enteric bacteria
Butanediol	As in mixed acid and 2,3-butanediol	<i>Bacillus</i> and other bacteria

So, now, if you consider the production of the ethanol from this individualistic biomass, in this case, the pyruvate is a key branching point in fermentation technique. Because, in the

ethanolic type of fermentation, the pyruvate is metabolised to produce acetaldehyde and carbon dioxide. And the produce acetaldehyde is further reduced to form the ethanol. So, this is how the pathway of ethanol production happens.

Because it mainly depends on the pyruvate, because it is considered as a key branching point in the fermentation process of which is mainly the ethanolic fermentation. And in this particular process, theoretically it is possible to achieve 2 moles of ethanol as I mentioned earlier. And if you remember our previous discussion in the previous slide, the one mole of glucose mainly produced 2 moles of ethanol by converting the glucose and 2 moles of CO₂.

And even on the weight basis if you see, it mainly produces around 51% conversion of glucose into the ethanol. So, that means, mainly it is a 51% of conversion of ethanol theoretically happens in the reaction to convert into the ethanol. If you just see the stoichiometry of the reaction scheme, it can be observed that it mainly produced around 51% of the ethanol stoichiometrically, that is called as the theoretical yield from the glucose molecule. That is called as a hexose sugar.

And in practice, only 90 to 95% of theoretical amount of ethanol can be achieved. Because, some of the pyruvate produced during the process is consumed for cellular material during the culture growth and it is not available to serve as an electron acceptor. So, because of that, the ethanol conversion which you can achieve is around like 90 to 95% compared to that of the theoretical amount of the ethanol.

Apart from that, some low level of the higher alcohols are also getting produced during this particular process. So, for the comparison purpose, we have shown here some type of fermentation technologies and the microorganism which is used for the fermentation purpose. So, if you see this particular table, the first point here is about the ethanolic fermentation. So, if you see the ethanolic fermentation here, which mainly carried out using the microorganism that is yeast and it produces ethanol as a product along with carbon dioxide.

However, if you consider the mix acid type fermentation process, in this particular case, the microorganism which is used for the mix acid fermentation is *Zymomonas*. And the product obtained during this particular process are mixed acid in the form of lactic acid, formic acid,

acetic acid and along with that it also produce the carbon dioxide, hydrogen and ethanol. But mainly it produces the mix acids along with the ethanol as well.

Whereas, if you see the third process that is a butanediol fermentation, so, in this case, it also produces mixed acid along with the butanol that is called as a 2, 3-butanediol and the microorganism which is used in this particular case is bacillus. So, this particular table is mainly used here just to compare the type of fermentation technology. Again, I am emphasising here our main focus in this particular course is about the alcoholic fermentation technique only.

Now, after understanding the process, which is used for the production of the ethanol, either it is a sucrose containing material, starch material or the lignocellulosic material, let us see how the theoretical ethanol yield can be calculated from the stoichiometry of the particular reaction. Because, if it is a lignocellulosic based material, then as I mentioned, it contains the C 5 and the C 6 sugars.

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Theoretical ethanol yield

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

$$3C_5H_{10}O_5 \rightarrow 5C_2H_5OH + 5CO_2$$

$$T_{EV}(\text{kg/ton}) = C_s(\text{kg/ton}) \times 0.51 \text{ kg ethanol}$$

$$V(\text{L/ton}) = \frac{\text{Mass (kg)}}{\rho (\text{kg/L})}$$

Where, T_{EV} represents the theoretical ethanol yield, C_s is the concentration of sugars (C_5 or C_6) present in the lignocellulosic biomass, 0.51 is theoretical ethanol yield (g/g), constant N , volume (L), and ρ is ethanol density (0.789 kg/L at 20 °C).

So, if you talk about the C 6 sugar, the C 6 sugars can be converted to yield 2 moles of ethanol along with 2 moles of carbon dioxide. Whereas, if it is a C 5 sugar, so, C 5 sugar in the form of C 5 H 10 O 5 which is called as a pentose sugar. It produces around 5 moles of ethanol and 5 moles of carbon dioxide. So, the theoretical ethanol yield for this particular sugar fractions can be calculated using the simple equation.

And C s in this particular equation, it represents the concentration of sugars either C 6 or C 5 which are present in the lignocellulosic biomass. Whereas, 0.51 is the theoretical ethanol yield constant, which is used for the calculation of the theoretical ethanol yield. And the theoretical ethanol yield obtained from this equation, it is in the form of kilogram per ton of biomass, which is used for the conversion purpose.

Whereas, if we need to convert this value into litre per ton, so, it can be divided using the density of the ethanol and it gives the value in the form of litres per ton of the material. So, this is how the theoretical ethanol yield for the lignocellulosic biomass or the sugars containing the lignocellulosic biomass can be calculated so, just to have the idea about the different lignocellulosic biomass and this theoretical ethanol yield.

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Theoretical bioethanol potential analysis

LCB	Structural Carbohydrate (kg/Ton)	TEY (kg/Ton)	TEY (L/Ton)
Rice straw	599	305.5	387
Wheat straw	616	314	398
Sugarcane bagasse	599	305.5	387
Corn Stover	596	304	385

This particular table is summarised here just to show the theoretical ethanol yield from the different lignocellulosic biomass. As we just discussed in the previous slide, the theoretical ethanol yield is calculated based on the sugar concentration in the lignocellulosic biomass. And as we already discussed this point multiple times, the composition of the cellulose and hemicellulose in the lignocellulosic biomass, it differs from hardwood material to the softwood material.

As a result even in the hardwood material, you can observe that there is a variation in the cellulose and the hemicellulose fraction present in the biomass. So, as a result, it is bound to have some changes into the theoretical ethanol yield of the lignocellulosic biomass. For

example, if you see the rice straw, the carbohydrate content in the rice straw is around 599 that means, this much kg per ton of carbohydrates are present in the rice straw.

So, now, based on this if you just try to calculate the theoretical ethanol yield for the rice straw, it comes to be around 30.5. This is on kilogram per ton basis. If you convert again this value in the form of litres per ton, so, it comes around 387. Similarly, if the material is a wheat straw, now, the carbohydrate content in this particular material is 616 kilogram per ton of the biomass.

So, basically the theoretical ethanol which can be achieved from this particular raw material is higher than the rice straw because the carbohydrate fraction itself is more in a wheat straw compared to the rice straw. And obviously, it will reflect the theoretical ethanol in the litres per ton as well. Whereas, when it is a sugar cane bagasse, again, a carbohydrate fraction is more or less similar to that of the rice straw.

And here is the theoretical ethanol yield in this particular case is close to or similar to the rice straw. While the corn stover, it has a carbohydrate fraction of 596. Accordingly, the theoretical ethanol yield is different than the remaining material. So likewise, the theoretical ethanol yield can be calculated for the specific feedstock which is being used for the ethanol production.

And based on that, also the process efficiency can be calculated for the specific feedstock. After understanding the theoretical ethanol yield from the lignocellulosic biomass, now, let us compare the liquid properties of the ethanol, which is produced from this particular raw material and compare this properties with the fuel grade material that is a conventional fuel as well.

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Liquid fuel characteristics of alcohols, diesel and gasoline

Characteristic	Gasoline	Diesel	Methanol	Ethanol	Butanol
Boiling point °C	32-210	204-343	65	78	118
Lower heating Value (MJ/kg)	44.5	43	19.6	26.9	33.1
MJ/lit.	31.9	38.9	15.4	21.1	26.7

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So, if you just try to compare these properties of the liquid fuel with alcohol, that is mainly the methanol and the ethanol, we can see that the boiling point in case of the ethanol is in one temperature, because ethanol is a single chemical. Whereas, in case of the fuel, it has a wide range of the chemical compound as a result, its boiling point is also having a certain wide range which varies from this particular range.

While in case of the lower calorific value if you see, the lower calorific value of the ethanol is 26.9 which is significantly lower than that of the fuels. This is mainly because the ethanol contains oxygen and the fuels do not. And that is what is the reason that the ethanol has significantly lower heating value than that of the conventional fuel.

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Uses of Ethanol

- Hydrous ethanol (95% by volume) or commercial ethanol is used as fuel in specially designed IC engines with 25% mileage penalty compared to conventional vehicles.
- Up to 22% blend of anhydrous ethanol (99.7% by volume) with petrol require no engine modification and incurring no mileage penalty and is being used by a large number of automobiles in the world.
- Anhydrous ethanol is required for the purpose of blending of petrol.
- The ethanol additive has antiknock properties and is preferred to the more commonly used tetraethyl lead, which produces serious air pollution.
- The excellent combustion properties of ethanol enables an engine to produce up to 20% more power as compared to that of petrol.

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Now, if we just talk about the uses of this ethanol, so the hydrous ethanol which is 55% by volume or they can say a commercial ethanol, it is used as a fuel in the specially designed IC engines with 25% mileage penalty compared to the conventional vehicles. It is blended up to 22% of this anhydrous ethanol, whereas, blending of this anhydrous ethanol which is 99.7% by volume with a petrol, it requires no engine modification and incur no mileage penalty and is being used by a large number of automobiles in the world.

But it should be an anhydrous ethanol, then it requires no engine modification as well and also it incurred no mileage penalty. Whereas, if it is a commercial grade ethanol which is 95% by volume, then it required some engine modification, moreover, it also incurred around 25% of the mileage penalty compared to the conventional vehicles. Apart from that, the anhydrous ethanol is required for the purpose of blending of petrol.

And the ethanol additive has antiknock properties and is preferred to more commonly used tetraethyl lead which produces serious air pollution. And that is the reason, ethanol is also blended in the petrol, so that the air pollution can be avoided because of this commonly used tetraethyl lead. And the excellent combustion properties of the ethanol enables an engine to produce around 20% more power than that of the conventional petrol as well.

So, this all talks about the uses and advantages of using ethanol as a petroleum blend in the conventional petrol. So, in this particular lecture, we discussed about the ethanolic fermentation, mainly the alcoholic fermentation technique and how it is important to convert the lignocellulosic biomass to ethanol rather than using the edible grade feedstock.

So, with this we end our lecture here and in the next lecture, we will discuss about the thermo-chemical conversion of biomass into solid, liquid and gaseous fuels. Regarding this lecture, if you have any doubt, feel free to contact me at vvgoud@iitg.ac.in. Thank you.