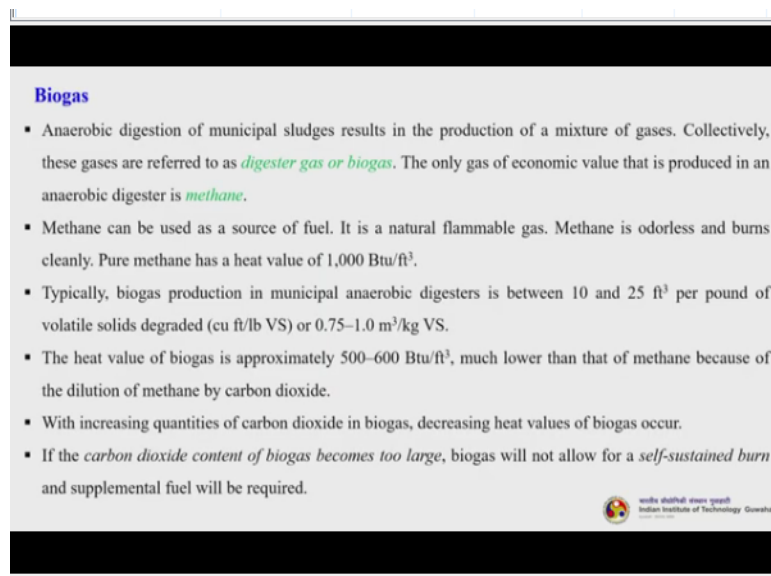


**Biomass Conversion and Biorefinery**  
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**Indian Institute of Technology-Guwahati**

**Module 06**  
**Lecture-18**  
**Products and Commercial Success Stories**


Good morning students. This is lecture 3 of module 6. As you know that in this module we are discussing about the microbial conversion processes. In today's lecture, we will discuss about the different types of microbial conversion products and few commercial success stories related to these products. So, let us begin.

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**Biogas**

- Anaerobic digestion of municipal sludges results in the production of a mixture of gases. Collectively, these gases are referred to as *digester gas or biogas*. The only gas of economic value that is produced in an anaerobic digester is *methane*.
- Methane can be used as a source of fuel. It is a natural flammable gas. Methane is odorless and burns cleanly. Pure methane has a heat value of 1,000 Btu/ft<sup>3</sup>.
- Typically, biogas production in municipal anaerobic digesters is between 10 and 25 ft<sup>3</sup> per pound of volatile solids degraded (cu ft/lb VS) or 0.75–1.0 m<sup>3</sup>/kg VS.
- The heat value of biogas is approximately 500–600 Btu/ft<sup>3</sup>, much lower than that of methane because of the dilution of methane by carbon dioxide.
- With increasing quantities of carbon dioxide in biogas, decreasing heat values of biogas occur.
- If the *carbon dioxide content of biogas becomes too large*, biogas will not allow for a *self-sustained burn* and supplemental fuel will be required.

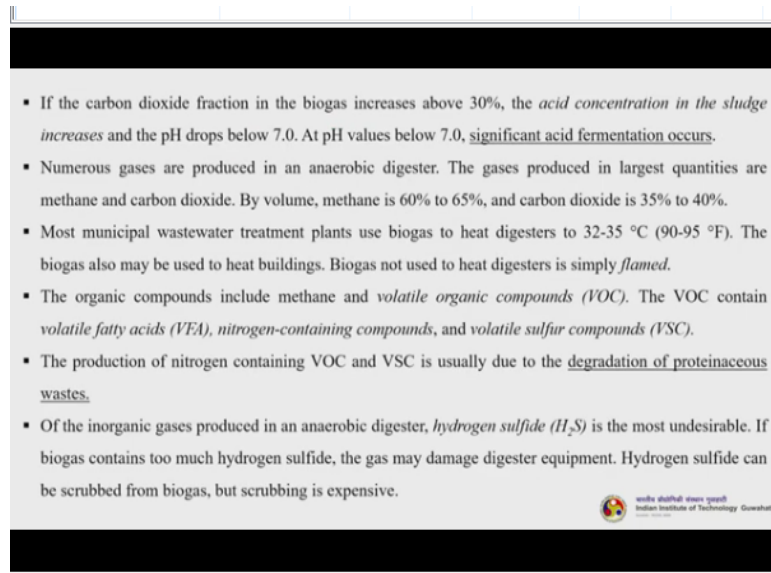
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So, the first and foremost important microbial conversion product is of course, biogas which is coming from the anaerobic digestion. So, AD of municipal sludges results in the production of a mixture of gases. Collectively these are referred as either digester gas or biogas. The only gas of economic value that is produced in an anaerobic digester is methane. Now, methane can be used as a source of fuel.


It is a natural flammable gas, methane is odourless and burns cleanly. Pure methane has a heat value of around 1000 British thermal unit per feet cube. Typically, biogas production in municipal anaerobic digesters is between 10 to 25 feet cube per pound of volatile solids degraded or 0.75 to 1 meter cube per kg of volatile solids. The heat value of biogas is approximately 500 to 600 Btu per feet cube, much lower than that of the methane because of the dilution of the methane by carbon dioxide.

Now, with an increase in the quantity of carbon dioxide in the biogas, its heat value will be decreasing. So, if the carbon dioxide content of the biogas becomes too large, biogas will not allow for a self sustained burn and supplemental fuel will be required. So, that means if carbon dioxide will be too much, then biogas will not ignite and you need to have some secondary fuel to do the ignition.

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- If the carbon dioxide fraction in the biogas increases above 30%, the *acid concentration in the sludge increases* and the pH drops below 7.0. At pH values below 7.0, significant acid fermentation occurs.
- Numerous gases are produced in an anaerobic digester. The gases produced in largest quantities are methane and carbon dioxide. By volume, methane is 60% to 65%, and carbon dioxide is 35% to 40%.
- Most municipal wastewater treatment plants use biogas to heat digesters to 32-35 °C (90-95 °F). The biogas also may be used to heat buildings. Biogas not used to heat digesters is simply *flamed*.
- The organic compounds include methane and *volatile organic compounds (VOC)*. The VOC contain *volatile fatty acids (VFA)*, *nitrogen-containing compounds*, and *volatile sulfur compounds (VSC)*.
- The production of nitrogen containing VOC and VSC is usually due to the degradation of proteinaceous wastes.
- Of the inorganic gases produced in an anaerobic digester, *hydrogen sulfide (H<sub>2</sub>S)* is the most undesirable. If biogas contains too much hydrogen sulfide, the gas may damage digester equipment. Hydrogen sulfide can be scrubbed from biogas, but scrubbing is expensive.

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So, if the carbon dioxide fraction in the biogas increases above 30%, the acid concentration in the sludge increases and the pH drops below 7. And at pH values below 7 significant acid fermentation occurs. Now that is not good for the anaerobic digestion, when we are talking about biogas or methane formation. Now numerous gases are produced in an anaerobic digester. The gases produced in largest quantities are methane and carbon dioxide by volume methane is about 60 to 65% and carbon dioxide is 35 to 40%.

Most of the municipal wastewater treatment plants use biogas to heat digesters to around 32 to 35 degrees centigrade. The biogas may also be used to heat buildings. Biogas not used to heat digesters is simply flamed up. So, the organic compounds include methane and volatile organic compounds. The VOC contains different types of other components such as volatile fatty acids, nitrogen containing compounds and volatile sulfur compound VSC.

The production of nitrogen containing VOC and VSC is usually due to the degradation of proteinaceous wastes that is present in different types of wastes like municipal solid waste. Now of the inorganic gases produced in an anaerobic digester, hydrogen sulfide is the most undesirable. The reason is that if the biogas contents too much of hydrogen sulfide, the gas may damage the digester equipment, this is basically due to the corrosion.

So, hydrogen sulfide can be scrubbed from biogas, but the scrubbing process is expensive, usually, you go for a chemical scrubbing. So, means you are adding more cost to the entire process.

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**Organic gases produced through microbial activity in AD**

Name	Formula	VFA	VSC
Acetate	CH <sub>3</sub> COOH	X	
Butyrate	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>3</sub> CH <sub>2</sub> COOH	X	
Caproic acid	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>5</sub> COOH	X	
Formate	HCOOH	X	
Propionate	CH <sub>3</sub> CH <sub>2</sub> COOH	X	
Succinate	CH <sub>3</sub> CHOHCOOH	X	
Valeric acid	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>4</sub> COOH	X	
Methane	CH <sub>4</sub>		
Cadaverine	H <sub>2</sub> N(CH <sub>2</sub> ) <sub>5</sub> NH <sub>2</sub>		
Dimethylamine	CH <sub>3</sub> NHCH <sub>3</sub>		
Ethylamine	C <sub>2</sub> H <sub>5</sub> NH <sub>2</sub>		
Indole	C <sub>8</sub> H <sub>7</sub> N		
Methylamine	CH <sub>3</sub> NH <sub>2</sub>		
Putrescine	H <sub>2</sub> N(CH <sub>2</sub> ) <sub>4</sub> NH <sub>2</sub>		
Propylamine	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> NH <sub>2</sub>		
Pyridine	C <sub>5</sub> H <sub>5</sub> N		
Skatole	C <sub>9</sub> H <sub>9</sub> N		
Trimethylamine	CH <sub>3</sub> NCH <sub>2</sub> CH <sub>3</sub>		
Allyl mercaptan	CH <sub>2</sub> =CHCH <sub>2</sub> SH		X
Benzyl mercaptan	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> SH		X
Dimethyl sulfide	(CH <sub>3</sub> ) <sub>2</sub> S		X
Ethyl mercaptan	C <sub>2</sub> H <sub>5</sub> SH		X
Methyl mercaptan	CH <sub>3</sub> SH		X
Thiocresol	CH <sub>3</sub> C <sub>6</sub> H <sub>4</sub> SH		X
Thioglycolic acid	HSCH <sub>2</sub> COOH		X

**Inorganic gases produced through microbial activity in AD**

Name	Formula
Ammonia	NH <sub>3</sub>
Carbon dioxide	CO <sub>2</sub>
Carbon disulfide	CS <sub>2</sub>
Carbon monoxide	CO
Hydrogen sulfide	H <sub>2</sub> S
Nitrogen	N <sub>2</sub>
Nitrous oxide	N <sub>2</sub> O

- The inorganic gases molecular nitrogen (N<sub>2</sub>) and nitrous oxide (N<sub>2</sub>O) are produced through *anoxic respiration* (denitrification) in the anaerobic digester.
- Anoxic respiration can occur with the transfer of nitrate ions (NO<sup>3-</sup>) to the digester with sludges or the addition of nitrate-containing compounds such as sodium nitrate (NaNO<sub>3</sub>) to increase digester alkalinity.

Genick, M.H., 2003. The microbiology of anaerobic digesters. John Wiley & Sons.

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So, let us look at this particular slide where I have given the organic gases that is produced through microbial activity. So, this particular table gives the different types of organic gases. And here we have different types of inorganic gases. So, you can see that inorganic gases are basically ammonia, carbon dioxide, carbon disulfide, carbon monoxide, hydrogen sulfide, nitrogen and nitrous oxide. And organic gases are so many - acetate, butyrate, caproic acid, formate, propionate, succinate and there is a big list basically.

So, the inorganic gases like molecular nitrogen and nitrous oxide are produced through anoxic respiration, the process is called denitrification in the anaerobic digester. Now, anoxic respiration can occur with the transfer of nitrate ions, to the digester with sludges or the addition of nitrate containing compounds, such as sodium nitrate to increase the digester alkalinity.

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**Lactic Acid (Lactate)**

- Lactic acid is an organic acid. It has a molecular formula  $\text{CH}_3\text{CH}(\text{OH})\text{COOH}$ . It is white in solid state and is miscible with water. While in liquid state (dissolved state), it is a colorless solution.
- Fermentation has virtually waived chemical synthesis of lactic acid (LA). The LA is produced anaerobically with a 95% (w/w) yield based on carbohydrate, a titer of over 100 g/L, and a productivity of over 2 g/L. This is comparable to processes employing *LA bacteria*.
- *Lactobacilli* produce mixed isomers, whereas *Rhizopus* forms L -(+)- LA exclusively.
- *Rhizopus oryzae* is favored for formation since it makes only the stereochemically pure L -(+)-LA.
- In *homolactic fermentation*, one molecule of glucose is ultimately converted to two molecules of lactic acid. *Heterolactic fermentation*, in contrast, yields *carbon dioxide and ethanol* in addition to lactic acid, in a process called the *phosphoketolase pathway*.
- *Lactate dehydrogenase* catalyzes the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD<sup>+</sup>.



So, the next product is lactic acid. So, lactic acid is an organic acid. It has molecular formula  $\text{CH}_3\text{CH}(\text{OH})\text{COOH}$ . It is white in solid state and is miscible with water. While in liquid state that means in the dissolved form, it is a colourless solution. Fermentation has virtually waived chemical synthesis of lactic acid. The lactic acid is produced anaerobically with a 95% w/w yield based on carbohydrate, a titer of over 100 grams per liter and a productivity of over 2 grams per liter. Now, this is comparable to process employing the lactic acid bacteria.

*Lactobacilli* produce mixed isomers, whereas *Rhizopus* forms L -(+)- LA exclusively, lactic acid exclusively. So, *Rhizopus oryzae* is favoured for formation since it makes only the stereochemically pure L plus lactic acid. In a homolactic fermentation, one mole of glucose is ultimately converted to 2 moles of lactic acid and under a heterolactic fermentation yields we get carbon dioxide and ethanol in addition to lactic acid in a process called the phosphoketolase pathway.

Now, lactate dehydrogenase that is the enzyme responsible for lactic acid formation, catalyzes the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD<sup>+</sup>. So, if you are interested, you can read a little more on the phosphoketolase pathway to understand the entire process in a better way.

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- Griffith and Compere (1977) described a fixed-film system for continuous LA production from WWs (contain readily recoverable sugar polymers) of the pulp, paper and fiberboard industries.
- Their fixed-film unit (2 inches x 6 inches) was seeded with *Lactobacilli* and lactose fermenting yeasts (*kefir culture*). The wood molasses substrate (wastewater concentrate) was pretreated with *cellulase, diastase and hemicellulases*. With a continuous feed rate of 60 g/g wood molasses over the seeded fixed-film unit, 31 to 32 g/g lactic acid yields were obtained.
- The production of calcium lactate from molasses by *Lactobacillus delbrueckii* has also been reported by Tewari and Vyas (1971).
- Two of the most common applications of lactic acid fermentation: *production of yogurt and sauerkraut*.
- Lactic acid is found primarily in sour milk products, such as koumiss, laban, yogurt, kefir, and some cottage cheeses. The casein in fermented milk is coagulated (curdled) by lactic acid. Lactic acid is also *responsible for the sour flavor* of sourdough bread.

Griffith and Compere, *Developments in Industrial Microbiology*, 18, 3071, 723.  
Tewari and Vyas, *Journal of Punjab Agricultural University*, 8, 1971, 402

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So, here we are trying to understand in brief what are the different types of products and of course, their end uses and other necessary things ascribed to these products. So, Griffith and Compere these 2 scientists in 1977 described a fixed film system for continuous lactic acid production from the wastewaters which contained readily recoverable sugar polymers from the pulp, paper and fibreboard industries.

Now their fixed film unit (it is 2 inches x 6 inches small one), was seeded with *Lactobacilli* and lactose fermenting yeast. So, usually the kefir culture we many times called it kephir. So, kephir. So, kefir culture is a symbiotic mesophilic mixed culture, where bacteria and yeast are both present in symbiosis. Now the wood molasses substrate wastewater concentrate was pre-treated with cellulase, diastase and hemicellulases. So, these are the enzymes.

Now with a continuous feed rate of 60 gram per gram wood molasses over the seed(ed) fixed film unit, 31 to 32 grams per gram per lactic acid yield was obtained, which is pretty good. So, the production of calcium lactate from molasses by *Lactobacillus delbrueckii* has also been reported by Tewari and Vyas in 1971. So, 2 of the most common applications of lactic acid fermentation is production of yogurt, and sauerkraut.

Now, yogurt all of us know and sauerkraut is a cabbage based product which is fermented - the cabbage is basically sliced into very thin strips and then fermented using the lactic acid bacteria. So, lactic acid is found primarily in sour milk products such as koumiss, laban, yogurt, kefir and some cottage cheeses. The casein in fermented milk is coagulated (curdled) by lactic acid. Lactic acid is also responsible for the sour flavour of the sourdough bread.

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### Acetic Acid (Acetate)

- Acetic acid can be produced by biomass fermentation in **five** different well-known methods.
- (1) *Anaerobic gasification of biopolymers to methane and CO<sub>2</sub>, followed by methanolic carbonylation to acetic acid.* This method essentially involves an anaerobic digestion to produce methane followed by introduction of the methane gas into a standard methanol carbonylation facility.
- (2) *Anaerobic yeast fermentation of hydrolyzed biopolymers to ethanol, followed by oxidation to acetaldehyde and then to produce acetic acid from acetaldehyde.* Oxygen-enriched air and acetaldehyde are fed into a reactor at 66 °C and 101.3 kPa, where they undergo a three-step chain reaction. The process is about 95% efficient with very few byproducts.
- (3) *Anaerobic yeast fermentation of hydrolyzed biopolymers to ethanol followed by aerobic bacterial fermentation to acetic acid.* The third method is the process currently used for *Vinegar production*.
- In this process, molasses, nutrients and 1% ethanol are used to start a submerged aerobic fermentation.




So, then acetic acid. Acetic acid can be produced by biomass fermentation in 5 different well known methods. So we will see one by one. The first one is anaerobic gasification of biopolymers to methane and carbon dioxide, followed by methanolic carbonylation to acetic acid. Now, this method essentially involves an anaerobic digestion to produce methane, followed by introduction of the methane gas into a standard methanol carbonylation facility.

So, the second one is anaerobic yeast fermentation of hydrolyzed biopolymers to ethanol followed by oxidation to acetaldehyde and then to produce acetic acid from acetaldehyde. Now, in this case, oxygen enriched air and acetaldehyde are fed into a reactor at around 66 degrees centigrade and 101.3 kilo Pascal, where they undergo a 3 step chain reaction. Now, this process is about 95% efficient with very few byproducts.

The next process is anaerobic yeast fermentation of hydrolyzed biopolymers to ethanol followed by aerobic bacterial fermentation to acetic acid. So, in this single process both aerobic and anaerobic processes are used. This is the third method, and the process is currently used for vinegar production, it is very widely adapted process for vinegar production. We will just discuss in a little more detail. So, in this process molasses, nutrients and 1% ethanol are used to start a submerged aerobic fermentation. That is the seed you can say, first, the primary feedstock.

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- The concentration of ethanol is kept at about 1% until the acetic acid concentration nears 10 to 11 %. This mixture of extractant and acetic acid is then put through a distillation chain to recover both. A major drawback to this process for acetic acid production is the *energy-intensive distillation step* which adds substantially to the cost of acetic acid production.
- (4) *Anaerobic bacterial homo-fermentation of biopolymers* is the fourth method that has generated much interest in recent years. In these fermentations, hydrogen is oxidized and CO<sub>2</sub> is reduced to acetic acid.
- (5) The fifth method consists of *anaerobic bacterial hetero-fermentation of biopolymers with simultaneous production of ethanol and other acids*. Hetero-fermentation of carbohydrates to acetic acids presents several purification problems. These problems are multiplied in hetero-fermentation by the presence of other organic products and a concomitant lower yield of acetic acid.



Now, the concentration of ethanol is kept about 1% until the acetic acid concentration nears to 10 to 11%. Now this mixture of extractant and acetic acid is then put through a distillation chain to recover both. A major drawback to this process for acetic acid production is the energy intensive distillation step, which adds substantially to the cost of acetic acid production.

Then the fourth process is anaerobic bacterial homo-fermentation of biopolymers. So, that has generated much interest in recent years. In this fermentation, hydrogen is oxidized and carbon dioxide is reduced to acetic acid.


In the next method that is an anaerobic bacterial hetero-fermentation of biopolymers with simultaneous production of ethanol and other acids. Now hetero-fermentation of carbohydrates to acetic acids present several purification problems. Now these problems are multiplied in hetero-fermentation by the presence of other organic products and the concomitant lower yield of acetic acid is usually reported.

So, we understand that there are 5 different processes through which we can produce acetic acid. So, we have discussed in a brief about this 5 different processes and their pros and cons.

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**Alcohol**

- Ethyl alcohol (C<sub>2</sub>H<sub>5</sub>OH) is a primary metabolite that can be formed from fermentation of a carbohydrate/sugar or a polysaccharide that can be depolymerized to a fermentable sugar.
- It has lower toxicity and is easily biodegradable, soluble in water, harmless to the environment and does not generate greenhouse gases.
- Yeasts are preferred for these fermentations, but the species used depends on the substrate employed.
- *Saccharomyces cerevisiae* is used for the fermentation of hexose, whereas *Candida* sp. or *Kluyveromyces fragilis* may be employed if pentose or lactose, respectively, is the substrate.
- Ethanol is produced in Brazil from cane sugar at 12.5 billion liters/year and is used as a 25% fuel blend or as a pure fuel. With regard to beverage ethanol, some 60 million tons of beer and 30 million tons of wine are produced each year.



So, the next important class of product from the microbial conversion is alcohol - so ethyl alcohol. So, ethyl alcohol is a primary metabolite that can be formed from fermentation of a carbohydrate or sugar or a polysaccharide that can be depolymerized to a fermentable sugar. Now it has lower toxicity and is easily biodegradable. It is soluble in water, harmless to the environment and does not generate greenhouse gases.

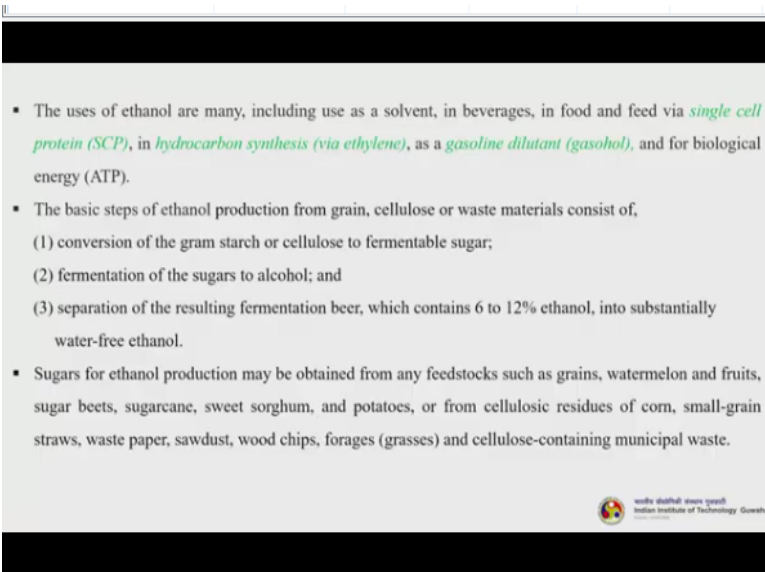
Mostly, yeasts are preferred for these fermentation. *Saccharomyces cerevisiae* is the well known yeast to do alcohol fermentation. But the species used depends on the substrate employed. Again, I am telling you, it is very important, which species you are going to use, *Saccharomyces* different strains are available. Now, that will depend on what is your substrate. For example, *Saccharomyces cerevisiae* is used for the fermentation of hexose, whereas *Candida* species or *Kluyveromyces fragilis* species may be employed if pentose or lactose is used as a substrate for the ethanol production.

Now, ethanol is produced in Brazil from cane sugar at 12.5 billion litres per year and is used as 25% fuel blend or as a pure fuel. With regard to beverage ethanol, some 60 million tons of beer and 30 million tons of wine are produced each year. Now, having said that, please understand that we are talking about Brazil here, sugar production from the sugar, sugar to ethanol in Brazil and various other countries.

Now, this we have already discussed when we discuss about bio-refinery fundamentals and concepts, we have discussed that we cannot do it in India and other developing countries because there is a food versus feed problem. So, in India, we cannot talk about or think about producing ethanol from this but certainly we can think about producing it from the waste, whether it is lignocellulosic waste from our agricultural product forest tellings and all or even molasses and other wastes.

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- The uses of ethanol are many, including use as a solvent, in beverages, in food and feed via *single cell protein (SCP)*, in *hydrocarbon synthesis (via ethylene)*, as a *gasoline dilutant (gasohol)*, and for biological energy (ATP).
- The basic steps of ethanol production from grain, cellulose or waste materials consist of,
  - (1) conversion of the gram starch or cellulose to fermentable sugar;
  - (2) fermentation of the sugars to alcohol; and
  - (3) separation of the resulting fermentation beer, which contains 6 to 12% ethanol, into substantially water-free ethanol.
- Sugars for ethanol production may be obtained from any feedstocks such as grains, watermelon and fruits, sugar beets, sugarcane, sweet sorghum, and potatoes, or from cellulosic residues of corn, small-grain straws, waste paper, sawdust, wood chips, forages (grasses) and cellulose-containing municipal waste.

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Now, the uses of ethanol are many, including the use as a solvent in beverages, in food and feed via single cell protein - which is very well known as a SCP, hydrocarbon synthesis via ethylene, as a gasoline dilutant as gasohol. So, gasohol is almost 10% ethanol plus 90% gasoline. And for biological energy generation, that is ATP and that happens during the metabolic pathway. So, the basic steps for ethanol production from grain, cellulose or waste materials consists of 3 steps.

The first one is the conversion of the gram starch or cellulose to fermentable sugar. So, then, this fermentable sugar is fermented to alcohol. And the third and one most important step is the separation of the resulting fermentation beer, which contains 6 to 12% ethanol into substantially water-free ethanol. So, sugars for ethanol production may be obtained from any feedstocks, such as grains, watermelon, and fruits, sugar beets, sugar cane, sweet sorghum, and potatoes and from cellulosic residues of corn, small grain straws, wastepaper, sawdust, wood chips, grasses or forages and cellulose containing municipal waste.

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**Glycerol**

- Glycerol is used in almost all chemical industries due to its particular combination of physical and chemical properties.
- The majority goes into the manufacturing of synthetic resins and ester gums, drugs, cosmetics, and toothpastes because glycerol is a good solvent of many compounds.
- One of the biochemical processes that produce glycerol is aerobic fermentation with *osmophilic yeast*.
- Glycerol is accumulated in yeast as a compatible solute during adaptation to high osmotic pressures or high sugar concentrations.
- *S. cerevisiae* uses glycerol as its sole compatible *osmolyte*. The process usually decreases the specific growth rate because of the limited oxygen transfer rates (OTRs) of industrial bioreactors.
- *Candida krusei* is another osmophilic yeast which can ferment glucose into glycerol.
- DuPont Corporate and Genencor have engineered biosynthetic pathways into an industrial strain of *E. coli* to directly convert glucose to 1,3-propanediol, a route not previously available in a single microorganism.

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So, the next product is or we can say that, many times say it is a byproduct, is glycerol. So, we have discussed about glycerol earlier that glycerol is one of the most important byproduct having lots of commercial application from the biodiesel industries. But glycerol does produce during the microbial conversion process also. So, glycerol is used in almost all chemical industries due to its particular combination of physical and chemical properties.

The majority goes into the manufacturing of synthetic resins and ester gums, drugs, cosmetics and toothpastes because glycerol is a good solvent for many compounds. So, one of the biochemical processes that produce glycerol is aerobic fermentation with osmophilic yeast. Glycerol is accumulated in the yeast as a compatible solute during the adaptation to high osmotic pressures or high sugar concentrations.

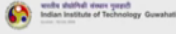
*Saccharomyces cerevisiae* uses glycerol as its sole compatible osmolyte. The process usually decreases the specific growth rate because of the limited oxygen transfer rates of industrial bioreactors. *Candida krusei* is another osmophilic yeast which can ferment glucose into glycerol. DuPont corporate and Genencor these are the 2 important industries which work on producing different types of enzymes and enzyme producing microorganisms.

So, they have different cocktails of enzymes also. So, these 2 companies have engineered biosynthetic pathways into an industrial strain of *E. Coli* to directly convert glucose to 1-3 propanediol, a route not previously available in a single microorganism. So, this is an important breakthrough in enzyme technology you can say.

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**Polymers and biodegradable plastics**

- Bio-based polymers include various synthetic polymers derived from renewable resources, *biopolymers* (nucleic acids, polyamides, polysaccharides, polyesters, and polyphenols), their derivatives, and their blends and composites.
- They are applied in the food, pharmaceutical, chemical, and petroleum industries, and are used as emulsifying agents, stabilizing agents, flocculating agents etc.
- Lactic acid produced from fermentation has been used to synthesize *biodegradable plastic* (polylactic acid).
- Biodegradable plastics have a high demand because they are thermoplastic, environmentally degradable, and help to reduce the disposal problem of non-degradable plastics.
- Several polyesters with properties comparable to conventional plastics, such as polybutylene succinate (PBS), polyester carbonate (PEC), poly-D-3-hydroxybutyrate (PHB), polypropiolactone (PPL), and poly-L-lactide (PLA), are used as biodegradable plastics.




So, the next class of products are polymers and biodegradable plastics. So, a lot of emphasis is being laid on biodegradable plastics due to various reasons of the plastic pollution and has been in market since almost a decade, the biodegradable plastics. So, bio based polymers include various synthetic polymers derived from renewable sources. So, biopolymers it can be nucleic acids, polyamides, polysaccharides, polyesters and polyphenols and their derivatives and their blends and composites.

So, they are applied in the food, pharmaceutical, chemical and petroleum industries and are used as a emulsifying agent, stabilizing agent and flocculating agents. Lactic acid produced from fermentation has been used to synthesize biodegradable plastics. So, that is polylactic acid - PLA. Biodegradable plastics have a high demand because they are thermoplastic and environmentally degradable and help to reduce the disposal problem of the non degradable plastics.

Several polyesters with properties comparable to conventional plastics such as polybutylene succinate, polyester carbonate, poly-D-3-hydroxybutyrate, polypropiolactone - PPL and poly-L-lactic acid (actually polylactic acid – PLA) are used as biodegradable plastics.

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- Many of these biopolymers, such as *PLA* and *polyglycolic acid*, have been accepted for use in the medical industry as medical devices or cell culture matrices.
- *Poly-glutamic acid (PGA)*, produced by the genus of *Bacillus*, can be used as the basis in drug delivery applications for cancer therapy. PGA-conjugation can provide more stable and water-soluble drugs, which control drugs exposure to tumor cells.
- *Polyhydroxyalkanoate (PHA)* is one of the largest groups of thermoplastic polyesters synthesized by numerous bacteria as an intracellular carbon and energy storage compound and accumulated as granules in the cytoplasm.
- PHA is regarded as a potentially useful alternative to petroleum-derived thermoplastics because it is biodegradable and biocompatible.
- PHA has been industrially produced by pure cultures of *Alcaligenes latus*, *Azotobacter vinelandii*, *Pseudomonas oleovorans*, *Ralstonia eutropha*, recombinant *Alcaligenes eutrophus*, and recombinant *E. coli*.




Many of these bio polymers such as polylactic acid and polyglycolic acid, have been accepted for use in the medical industry as medical devices or cell culture matrices. Poly-glutamic acid produced by genus *Bacillus* can be used as the basis in drug delivery applications for cancer therapy. Now PGA conjugation can provide more stable and water soluble drugs, which control drugs' exposure to tumour cells. Polyhydroxyalkanoate, is one of the largest group of thermoplastic polyesters synthesized by numerous bacteria as an intracellular carbon and energy storage compound and accumulated as granules in the cytoplasm.

PHA is regarded as a potentially useful alternative to petroleum derived thermoplastics because it is biodegradable and biocompatible. PHA has been industrially produced by pure cultures of *Alcaligenes latus*, *Azotobacter vinelandii*, *Pseudomonas oleovorans*, *Ralstonia eutropha*, recombinant *Alcaligenes eutrophus* and recombinant *E. Coli*.

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### Other Products

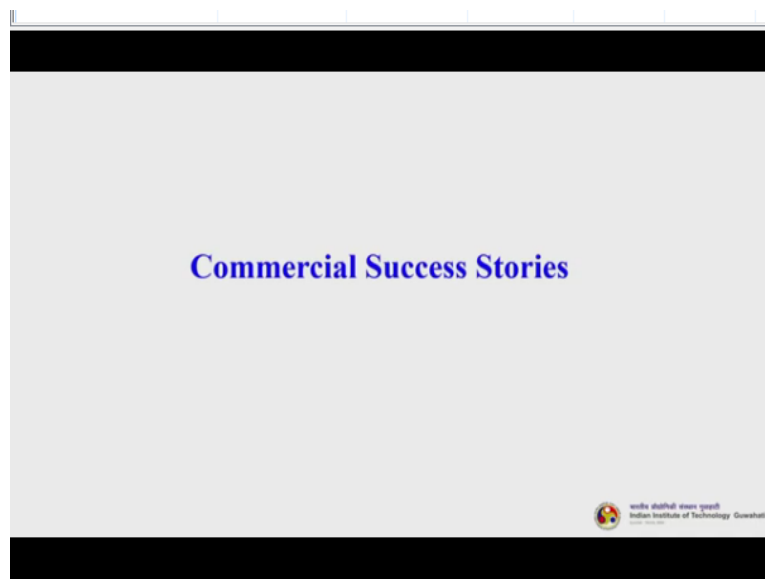
- *Microbial polysaccharides*: Xanthan gum, Dextrans, Mannans, Pullulan, Cellulose
- *Amino acids*: L-Alanine, L-Aspartic acid, L-Aspartic acid, L-Lysine, L-Phenylalanine, L-Threonine
- *Antibiotics*: Aminoglycosides, Aminoglycosides, Bacteriocin,  $\beta$ -Lactam, Nisin, Tetracyclines
- *Enzymes*: Alkaline proteases,  $\alpha$ -Amylases, Glucose isomerase,  $\beta$ -Lactamase, Lipases, Pullulanase
- *Vitamins*:  $\beta$ -carotene, Provitamin D2, Vitamin B12, Riboflavin



So, apart from all these products that we have discussed, which are actually the major products, there are other various products which are being produced in very small or minor quantities. So, some of these are having high industrial and commercial value, like microbial polysaccharides. So, xanthan gum, Dextrans, Mannans, Pullulan, and Cellulose. Then amino acids - L-Alanine, L-Aspartic acid, then L-Lysine, L-Phenylalanine, and L-Threonine.

Antibiotics such as Aminoglycosides, then Bacteriocin,  $\beta$ -Lactam, Nisin, Tetracyclines. Enzymes such as alkaline proteases,  $\alpha$ -Amylases, and there are many others and few vitamins like beta-carotene, Provitamin D 2, vitamin B 12 and Riboflavin.

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So, we will now see and discuss the different commercial success stories, how the microbial conversion process has been commercially adapted to produce different products. So we will see 2 or 3.

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**Solrod Biogas, Denmark**

- The Solrod Biogas Plant was taken into operation in 2015. The plant was established and is operated by Solrod Biogas A/S, founded May 28, 2014 with Solrod Municipality as shareholder.
- The idea to build a biogas plant in Solrod emerged from the need to find a sustainable solution to the community's *odour problem*, caused by seaweed fouling the beach.
- Simultaneously, the Solrod Municipality also wished to take concrete action concerning climate change challenges by producing green energy.
- The biogas plant has a treatment capacity of 200,000 tons feedstock/year. The biogas produced is used for *CHP generation* in a large gas engine.
- The power is sold to the grid and the heat is supplied to the local district heating system which is operated by Vestegnens Kraftvarmeselskab I/S and owned by 12 municipalities as stakeholders.



Source: IEA Bioenergy Task 17, Energy from Biogas, 2017

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So, the first one is that Solrod biogas plant in Denmark. So, the Solrod biogas plant was taken into operation in 2015 - very recently started. The plant was established and is operated by Solrod biogas company founded in May 28, 2014 with Solrod Municipality as the shareholder. The idea is to build a biogas plant in Solrod emerged from the need to find a sustainable solution to the community's odour problem, which is basically caused by the seaweed fouling the beach.

So, what happens in this particular province or the town of Solrod is that this huge amount of seaweeds is coming and getting deposited on the sea shore. Now, slowly, they are degrading under the attack of sunlight and of course water and they are getting degraded. So, when they are getting degraded, they started producing obnoxious gases, I am not telling it is toxic, but they are creating huge odour nuisance.

So, the people in the municipality of Solrod, they wanted to get rid of this odour in a permanent way. So, then this idea has started that how to convert these seaweeds into valuable products, so that they can get rid of this odour as well as they can produce some valuable products. So, then the story begins actually. So, simultaneously the Solrod Municipality also wish to take concrete action concerning climate change challenges by producing green energy.

So, the biogas plant has a treatment capacity of 200,000 tonnes feedstock per year, the biogas produced is used directly for the CHP generation in a large gas engine - combined heat generation system/cycle. So, the power is sold to the grid and the heat is supplied to the local district heating system, which is operated by a particular company like Vestegnens and owned by 12 municipalities as stakeholders.

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Input Output	
Treatment capacity biomass	200,000 tons/year
Methane production	6 million m <sup>3</sup> /year
Electricity production	23 GWh/year
Heat production (District Heating)	28 GWh/year

Biomass feedstock	(tonnes)	Share of biogas	Contribution to the value of the project
Seaweed*	7,400	0.5%	Nutrients supply and improved sea water quality
Manure	53,200	9.5%	Gas production and process stability
CP Kelco (pectin)	79,400	76.6%	Gas production booster
Chr. Hansen	60,000	13.5%	Nutrients supply and gas production booster
Total	200,000	100.0%	

\**Zostera maritima, Pilayella littoralis and Ectocarpus sp.*

Source: IIS Energy Tech 21, Energy from Biogas, 2017

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So, if you look at that input output system of the particular plant, so the treatment capacity is 200,000 tons per year, methane that is getting produced is 6 million meter cube per year, electricity production is 23 gigawatts hour per year, heat production is basically the district heating is again at 28 gigawatts hour per year. Now, if we talk about biomass feedstocks, the major feedstock is of course, the seaweed which was the initial target actually.

So, seaweed contains various types of *Zostera maritima*, *Pilayella littoralis* and *Ectocarpus sp.* So, usually 7400 tons, the share of biogas is about 0.5%. So, contribution to the value of the project is nutrient supply and improved sea water quality. So, this is very interesting the last column and then manure is being added of course, CP Kelco - that is a pectin, then Chr Hansen. So, altogether adds on to 200,000 tons, but if you see the shares so you can see that from the pectin the share of biogas is huge.

Seaweed, is not the major contributor however, in this way, they got rid of the entire odour creating problem and simultaneously produced fuels. So, if you talk about manure so it is contribution to the value of project is terms of gas production and process stability. Pectin, major contribution to the gas production booster and Hansen actually, again, nutrient supply and gas production booster.

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**Investment and Economy**

- Solrod Biogas A/S has a share capital of 16 million DKK, consisting of a cash contribution of 6.08 million DKK and 9.92 million DKK as assets, other than cash.

Investment	85 millions DKK (ex. CHP unit)
EU grant	0.5 millions EUR
Annual revenues	30 millions DKK

Source: IIA Bioregry Task 17, Energy from Biogas, 2017

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So, if you talk about investment and economy, the Solrod biogas has a share capital of 16 million Danish kroner consisting of a cash contribution of 6.08 million Danish kroner and 9.92 million Danish kroner as assets, other than the cash. So, investment is 85 million DKK which is excluding the CHP unit. Then the European Union grant is 0.5 million euro and the annual revenue that is coming right now is around 30 million Danish kroner. So, this is quite a success story, and it is been recently implemented.

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**Estimated benefits related to Solrod biogas plant**

- 60 GWh/year renewable energy production
- 104 local jobs, of which 14 permanent
- 40,100 tons CO<sub>2e</sub> saved/year (51% of the municipality target for 2025)
- Sustainable waste treatment and lower costs of waste transport
- Production of *digestate as biofertilizer* for farmers
- Reduced leaching of N to aquatic environment by 62 tonnes/year (70% of requirement for Køge Bay)
- Reduced leaching of P to the aquatic environment by 9 tonnes/year (100% of requirement for Køge Bay)
- *Reduced odour nuisance* from the beach/seaweed
- Improved sea water quality and higher recreational value of the maritime coastal area

Source: IIA Bioregry Task 17, Energy from Biogas, 2017

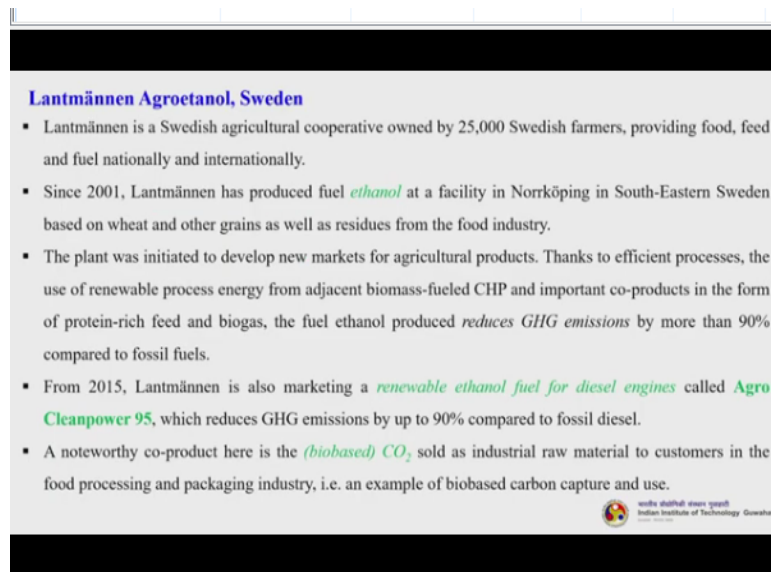
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So, if you talk about the estimated benefits related to the Solrod biogas plant, so it is 60 gigawatt hour per year in renewable energy production, which is a very good amount, 104 local jobs are being created out of which 14 are permanent jobs, 40,100 tons of carbon dioxide equivalent is almost saved per year, which is almost 51% of the municipality target for the 2025. The next is sustainable waste treatment and lower cost of the waste transport, production of digestate as bio-fertilizer for farmers.



So, this is another success story from this particular biogas plant, where the digestate or the solid part left out after the fermentation is being used as the biofertilizers and it is sold to different farmers. So, reduced leaching of nitrogen to aquatic environment by almost 62 tons per year - So, 70% requirement for the Koge Bay and reduced leaching of phosphate to the aquatic environment by 9 tons per year - 100% of requirement of the Koge Bay. So, reduced odour nuisance from the beach and seaweed which was their major target, then improved sea water quality and higher recreational value of the maritime coastal area.

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**Lantmännen Agroetanol, Sweden**

- Lantmännen is a Swedish agricultural cooperative owned by 25,000 Swedish farmers, providing food, feed and fuel nationally and internationally.
- Since 2001, Lantmännen has produced fuel *ethanol* at a facility in Norrköping in South-Eastern Sweden based on wheat and other grains as well as residues from the food industry.
- The plant was initiated to develop new markets for agricultural products. Thanks to efficient processes, the use of renewable process energy from adjacent biomass-fueled CHP and important co-products in the form of protein-rich feed and biogas, the fuel ethanol produced *reduces GHG emissions* by more than 90% compared to fossil fuels.
- From 2015, Lantmännen is also marketing a *renewable ethanol fuel for diesel engines* called **Agro Cleanpower 95**, which reduces GHG emissions by up to 90% compared to fossil diesel.
- A noteworthy co-product here is the *(biobased) CO<sub>2</sub>* sold as industrial raw material to customers in the food processing and packaging industry, i.e. an example of biobased carbon capture and use.

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So, the next success story that we are going to discuss is about the Lantmannen Agroetanol, Sweden. So, Lantmannen is a Swedish agricultural cooperative owned by 25,000 Swedish farmers, providing food, feed and fuel nationally and internationally. Now since 2001 Lantmannen has produced fuel ethanol at a facility in a Norrkoping in the South-Eastern Sweden, based on wheat and other grains as well as residues from the food industry.

The plant was initiated to develop new markets for agricultural products. Thanks to the efficient processes, the use of renewable process energy from adjacent biomass fuel CHP and important co-products in the form of protein rich feed and biogas, the fuel ethanol produced reduces the greenhouse gas emissions by more than 90% compared to the fossil fuels. Now this is the major outcome of this entire project.

So, from 2015 onwards Lantmannen is also marketing a renewable ethanol fuel for the diesel engines known as Agro Cleanpower 95, that is the trade mark which reduces the greenhouse gas emissions by up to 90% compared to the fossil diesel. A noteworthy co-product here is the biobased carbon dioxide that is sold as an industrial raw material to customers in the food processing and packaging industry, that is an example of the biobased carbon capture and use.

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- Yet, the ethanol production at the Lantmännen plant has had significant problems in terms of business performance. However, beginning in the second half of 2015, the plant has become profitable as a result of *increased ethanol volumes exported* instead of used within Sweden.
- This is a result of differences in policy structures: e.g., in Germany, policy measures rewards renewable fuels depending on GHG emission reduction potential whereas Swedish policy currently do not.
- This has made Lantmännen's ethanol highly competitive in other European markets and has resulted in substantial profits. In 2018, Sweden introduced similar policy measures as Germany.
- Agroetanol has an annual capacity to convert 600,000 tons of grains into 230,000 m<sup>3</sup> ethanol with 200,000 tons of protein feed as co-product, mainly for cattle, and 200,000 tons of CO<sub>2</sub> which is collected, liquefied and turned into *green carbonic acid* (mainly for beverage production).



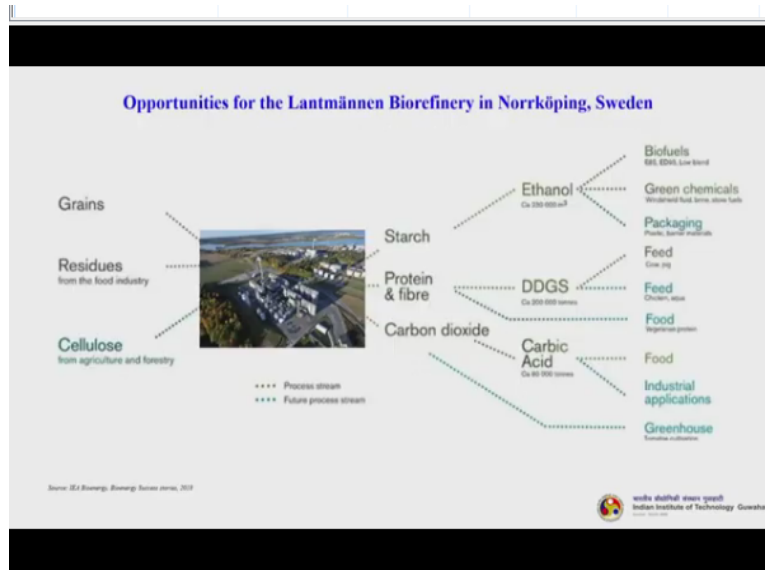
Yet the ethanol production at the Lantmannen plant has had significant problems in terms of business performance. However, beginning in the second half of 2015, the plant has become profitable as a result of increased ethanol volumes exported instead of used within the Sweden. So, actually what happened when they were using whatever the ethanol that is being produced inside the Sweden in the initial years, then they are not making profit.

Because of the policy problems that was there or existed that particular duration in the Sweden. Now, so they decided to sell it off. So, export it to various other countries. So, once they started doing that, their revenues have jumped like anything. Now, later on the Swedish government has also changed its policy so that this Lantmannen ethanol was being again now marketed inside Sweden.

So, this was actually as I told that there was policy problem. So, in Germany, the policy measures towards renewable fuels depending on the greenhouse gas emission reduction potential, whereas the Swedish policy currently do not. But this has made Lantmannen's ethanol highly competitive in other European markets and has resulted in substantial profits. However, in 2018 Sweden introduced similar policy measures as Germany.

So, Agroetanol has an annual capacity to convert 600,000 tons of grains to 230,000 meter cube of ethanol with 200,000 tons of protein feed as co-product mainly for cattle and 200,000 tons of carbon dioxide which is collected, liquefied and turned into green carbonic acid. So, that mainly goes for the beverage production.

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So, this is all about the Lantmannen biorefinery which is located at the Norrköping in Sweden. So, this is the plant top aerial view. So, the grains residues from the food industries, then cellulose from agricultural and forest tailings and forest residues are processed. So, they are into 3 distinct streams, the one is starch rich stream, another is a protein and fiber rich stream another is a carbon dioxide, the gas basically which is coming from the pre-processing.

Now, the starch goes to the ethanol platform, which gives us biofuels, green chemicals and packaging, again the solid whatever is left out. Then the protein and fiber goes to the DDGS platform where it is converted to feed and food. Then carbon dioxide, it goes to the carbic acid platform where it produced food, industrial applications and of course carbon dioxide also goes for this greenhouse gas emission. It is a bio based carbon dioxide capture and sequestration cycle basically you talk about.

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Year of implementation:	2005, updated 2015
Location:	Norrköping, Sweden
Technology:	<i>Ethanol biorefinery</i> Location of the ethanol plant close to biomass-based CHP ensures deliveries of renewable electricity and process heat.
Principle feedstocks:	Wheat and other grains, as well as starch-rich residues from the food industry
Products/markets:	Fuel ethanol and co-products in the form of protein-rich feed. A further co-product here is the (biobased) carbon dioxide that is captured and sold as industrial raw material to customers in the food processing and packaging industry.
Technology Readiness Level (TRL):	TRL 9 – actual system proven in operational environment

Source: ICA Bioenergy, Bioenergy Services series, 2012

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Then the year of implementation of this plant is 2005. But again, it is updated in or upgraded in 2015. Location is of course Norrköping in Sweden. The technology is ethanol biorefinery. So, the location of ethanol plant close to biomass based CHP ensures deliveries of renewable electricity and process heat. So, this is one of the most important take home message from this particular plant. Principle feedstocks are wheat and other grains as well as starch rich residues from the food industry.

If you talk about Products and markets, then fuel ethanol and co-products in the form of protein rich feed. A further co-product here is the biobased carbon dioxide that is captured and sold as industrial raw material to customers in the food processing and packaging industry. So, if you talk about the TRL the technology readiness level: it is TRL 9 - So, that is the actual system proven in operational environment and it is quite a success story.

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**Biowert grass Biorefinery – Biobased plastics, Germany**

- Biowert Industrie GmbH was founded by Michael Gass in 2000 as a Swiss-German company. The first Biowert grass refinery started operation in 2007 and is located in Brensbach, Germany, on an 18,000 m<sup>2</sup> site.
- The main products based on grass from permanent pastureland and arable land for crop production are *grass fibre insulation* (AgriCell<sup>BW</sup>), *natural fibre reinforced plastic* (AgriPlast<sup>BW</sup>) and *fertiliser* made from digestate (AgriFer<sup>BW</sup>).
- The facility has an annual throughput of about 2,000 t dry matter (equivalent to 8,000 t grass per year at 25% – 30% dry matter content).
- The integrated biogas plant produces 13,40,000 m<sup>3</sup> of biogas annually which is used in combined heat and power facilities, which in 2012 produced 5.2 GWh<sub>e</sub> of electricity.

Source: I&A Biowert, Part II, Energy from Biogas, 2012

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So, the next one is Biowert grass biorefinery, that is for the biobased plastics located in Germany. So, Biowert industries was founded by Michael Gass in 2000 as a Swiss-German company. The first Biowert grass refinery is started operation in 2007 and is located in Brensbach, Germany on an 18,000 m<sup>2</sup> site. The main products based on grass from permanent pastureland and arable land for crop production are grass fibre insulation (AgriCell<sup>BW</sup> is the trademark name), natural fibre reinforced plastic (AgriPlast<sup>BW</sup> is the trademark name) and fertilizer made from digestate (AgriFer<sup>BW</sup> that is the trademark name). So, the facility has an annual throughput of about 2000 tons dry matter equivalent to 8000 tons grass per year at 25 to 30% dry matter content. The integrated biogas plant produces 13,40,000 m<sup>3</sup> of biogas annually, which is used in combined heat and power facilities, which in 2012 produced 5.2 gigawatt hour (GWh<sub>e</sub>) electricity.

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- The first step after ensiling includes mechanical treatment of grass silage and isolation of grass fibres through pulping, drying and pressing processes.
- The grass fibres are further processed into AgriCell<sup>BW</sup> and AgriPlast<sup>BW</sup> synthetic granules.
- AgriPlast<sup>BW</sup> contains 30 – 50% grass fibres and 50 – 70% recycled polyolefine and is used for injection moulding for a range of uses.
- The grass juice remaining from mechanical pretreatment of grass silage is used as substrate in the biogas plant (together with local co-substrates such as food waste and slurry).
- The heat and electricity derived from the biogas facility is used to satisfy the energy in the biorefinery and excess electricity is exported to the electricity grid. Wastewater arising from the process is reused for pretreatment (slurrying) of grass silage.
- Digestate from the biogas plant is further processed to a concentrated and a liquid biofertilizer (AgriFer<sup>BW</sup>) used by local farmers. This closes the nutrient cycle in the circular economy.

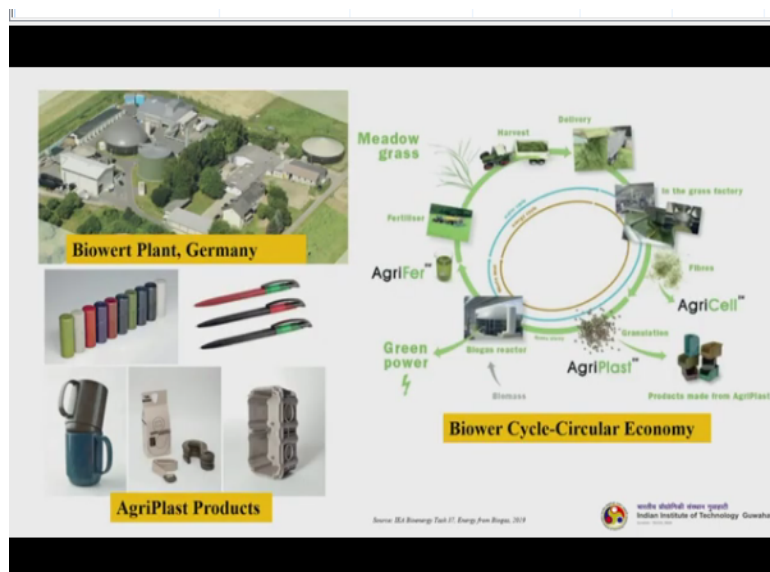
Source: IIT Bombay Tech II, Energy from Biogas, 2019



Now, the first step after ensiling includes mechanical treatment of the grass silage and isolation of grass fibres through pulping, drying and pressing processes. The grass fibres are further processed into AgriCell<sup>BW</sup> and AgriPlast<sup>BW</sup> synthetic granules. AgriPlast<sup>BW</sup> contains 30 to 50% grass fibres and 50 to 70% recycled polyolefine and is used for injection moulding for a range of uses.

The grass juice remaining from the mechanical pretreatment of grass silage is used as a substrate in the biogas plant together with local co-substrate such as food waste and slurry. The heat and electricity derived from the biogas facility is used to satisfy the energy in the biorefinery and excess electricity is exported to the electricity grid. Wastewater that is arising from the process is reused for pretreatment of the grass silage. Digestate from the biogas plant is further processed to a concentrated and a liquid biofertilizer used by the local farmers. Now, this closes the nutrient cycle in the circular economy.

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Source: IIT Bombay Tech II, Energy from Biogas, 2019



So, if you closely look at this particular plant and how it happens, this is the aerial view of the Biowert plant, these are the different types of agri-based products. Now, let us have a look here this is interesting actually. This is a cycle which is Biower cycle, which talks about the circular economy. So, there is a complete nutrient recycle here in this. If you start from the grass - Meadow grass, it is harvested, then it is delivered, processed in the grass factory.

So, the solid parts go to the fibre production in a name of AgriCell, then whatever the other products that comes here is again processed and granule into different products named as AgriPlast. Then the slurry - grass slurry, that goes to the biogas reactor - basically for the anaerobic digestion. Here the different other biomass also can be co-fed. So, the co-feedstock process here. So, whatever comes out is nothing but your green power.

Then whatever left out this can be used as AgriFer<sup>BW</sup> agri fertilizer basically the slurry it can be concentrated and made into your solid fertilizer also. Now, you can see that fertilizer is being again used as fertilizer in plantation to grow Meadow grass. So, thereby the nutrients which are present in the grass are getting recycled again. So, it is a complete nutrient recycle and circular economic concept.

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Input and output		
	Grass refinery	Biogas & CHP plant
<b>Input</b>		
Biomass	8,000 t / year	Grass juice: 1,942 t / year
Electricity demand	2.5 – 3 GWhel / year	Co-substrates in biogas facility: 15,260 t / year
<b>Output</b>		
Product	AgriCellBW: 1,410 t/year AgriPlastBW: 2,500 t/year	Biogas: Approximately 1,340,000 m <sup>3</sup> /year at methane concentration of 60 % AgriFerBW concentrated and AgriFerBW liquid digestate: 11,362 t/year
Electricity production		5.2 GWh in 2012

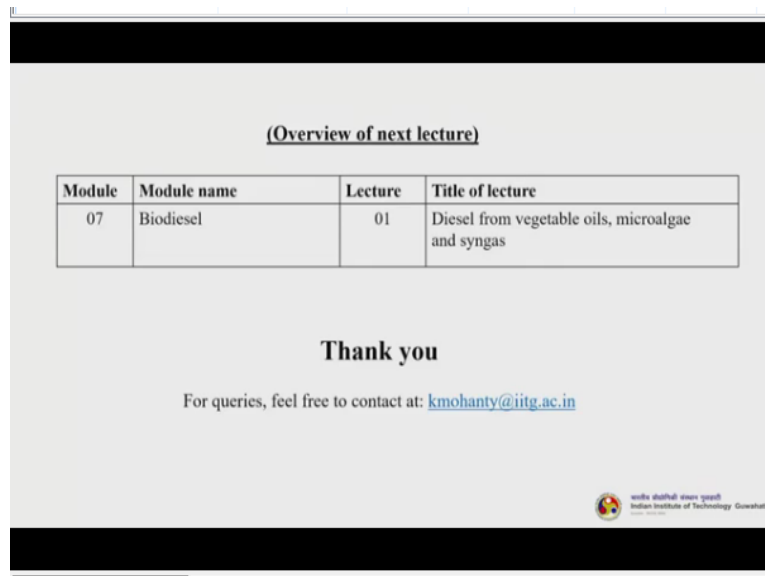
Source: IIT Bombay, Feb 17, Energy from Biogas, 2019

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So, if you look at the input and output for this particular plant, - the input is biomass and then electricity demand. So, biomass is about 8000 tons per year. And electricity demand is 2.5 to 3 gigawatt hour per year. And the biogas and CHP plant that produce actually grass juices - 1942 ton per year - that is required as a feedstock to be fed to the digester and then the co-substrates in biogas facility around 15,260 tons per year.

So what is the output? Outputs are this actually: AgriCell<sup>BW</sup> and AgriPlast<sup>BW</sup>, the solid by-products which are being converted into 2 different trade names and being marketed - and then biogas (approximately 13,40,000 m<sup>3</sup> per year of the methane concentration). And then of course, there is 5.2 gigawatt hour electricity generation. Now, please understand that - whatever it is getting produced here, the electricity, almost 50 to 60% of that is being utilized in the entire plant and the rest either is being sold or directly fed to the electricity grid.

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(Overview of next lecture)

Module	Module name	Lecture	Title of lecture
07	Biodiesel	01	Diesel from vegetable oils, microalgae and syngas

**Thank you**

For queries, feel free to contact at: [kmohanty@iitg.ac.in](mailto:kmohanty@iitg.ac.in)

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So, with this, I conclude today's lecture. So, in the next module, that is module 7, we will be discussing about bio-diesel. So, thank you very much in case you have any query please feel free to register your query in the Swayam portal or you can drop a mail to me at [kmohanty@iitg.ac.in](mailto:kmohanty@iitg.ac.in), Thank you.