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Lecture – 35 Aquaculture and Algal Biorefinery, Waste Biorefinery

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Good morning students. This is lecture 2 under module 12. And as you know that we are discussing integrated biorefinery concept and few examples. In today's lecture, we will discuss about aquaculture and algal biorefinery, that how aquaculture and algae biorefinery are clubbed together. Then we will also discuss a little about the waste biorefinery though few things we have already discussed in our module 2. Then we will talk about hybrid, chemical and biological conversion processes in the biorefinery concept. So, let us begin.

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So, as you can see from this particular slide, this is about the co-processing of algal and cellulosic feedstock. You can see this algal feedstock, so once you grow it, then you harvest it. You harvest, then harvested algal biomass. There are 2 things. First is that biomass you take it out. Second thing is that after you harvest biomass the culture water will remain. So, basically it is a wastewater. So, culture water containing cellulose and hemicellulose.

So that can go to some recovery unit and then finally it can also be used for the enzyme cocktail storage tank and residual culture water for the recycling and reuse purposes. So, the harvested algal biomass, once you separate the oil or the lipid content of that, so you get algal meal. So algal oil having so many different types of contents like AA, PUFA, then omega-3 and all these things, so that can be separated.

So you can get various products, not only fuels, but also some platform chemicals and others glycerin, biodiesel, 1, 3 propanediol and the list is endless, there are many. Similarly, if you talk about the cellulosic feedstock, once you harvest the crop, so compaction, transportation, pretreatment, detoxification, solid and liquid separation, then you go for the enzymatic hydrolysis of cellulose and hemicellulose after you remove the lignin part.

Then once that is done, so you can go for the fermentation. So, you get bioethanol, mostly if you talk about the fermentation pathway you get bioethanol. And other than biethanol, we have so many other products, some acids and some other products as the coproducts. They are also value-added products. Now, this is in a nutshell we try to understand how the algal biorefinery tried to clubbed with other systems.

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Aquaculture and Algal Biorefinery

□ Based on the unique advantages in productivity and the potential to avoid competition for arable land or biodiverse landscapes, algae have been considered as one of the most promising resources for hiofitels and hiomass

- □ Although they are not superior to higher plants with respect to photosynthetic efficiency, microalgae have extremely high growth rates and yield more oil than any higher plants.
- □ Algae can make use of waste streams as a nutrient source, which have great potential for wastewater bioremediation. More importantly, algae are the basis of the food chain supporting over 70% of the world's biomass.

 \Box With concerns of sustainability issues for the over utilization of marine resources, *aquaculture* is regarded as a future sustainable source of quality protein for the planet's growing population.

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So one classical example we will discuss is about aquaculture and algal biorefinery. So, based on the unique advantages in productivity and the potential to avoid competition for arable land or biodiverse landscapes, algae have been considered as one of the most promising resources for biofuels and biomass. So, this we have discussed during our discussion on algae long back. So although they are not superior to higher plants with respect to photosynthetic efficiency, the microalgae have extremely high growth rates and yield more oil than any higher plants. So, a plant takes time to grow and do its photosynthesis and other things. However, algae can grow very fast. So, algae can make use of waste stream as a nutrient source, which have great potential for wastewater. bioremediation. So, this is most interesting part actually particularly if you talk about algae vis-a-vis about other terrestrial plants.

So, more importantly algae are the basis of the food chain supporting over 70% of the world's biomass. With concerns of sustainability issues for the over utilization of marine resources, aquaculture is regarded as a future sustainable source of quality protein for the planet's growing population.

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□ Fish and shell fish mariculture thereby have become an important food sector globally and hold great promise for closing the nutritional gap of many people worldwide.

□ However, at present, the vast majority of aquaculture feed is sourced from wild fish populations. While reducing use of fish oils in aqua feed, an apparent challenge in aquaculture is to produce final products/sea foods with high levels of omega-3 fatty acids, health promoting for the consumers.

This growing concern is also a driving force for the marketing of non-fish omega-3 oils and alternative feed ingredients in aquaculture.

□ As algal omega-3 oil can be recovered from the extracted lipids intended for biofuel production, policy initiatives for the meaningful integration of algal biofuel production with aquaculture industries can provide many economic and sustainable outcomes to society.

Fish and shell fish mariculture thereby have become an important food sector globally and hold great promise for closing the nutritional gap of many people worldwide. However, at present the vast majority of aquaculture feed is sourced from wild fish populations. While reducing use of fish oils in aqua feed, an apparent challenge in aquaculture is to produce the final products or sea foods with high levels of omega-3 fatty acids, that is the health promoting agent for the consumers.

Now this growing concern is also a driving force for the marketing of non-fish omega-3 oils and alternative feed ingredients in aquaculture. Now, there are some aesthetic issues. Many consumers do not like to consume omega-3 that is derived from the fish oils. As algal omega-3 oil can be recovered from the extracted lipids intended for biofuel production, policy initiatives for the meaningful integration of algal biofuel production with the aquaculture industry has to be done and thereby it will provide many economic and sustainable outcomes to the society at a large.

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So, the discharged water from aquaculture could match the requirement for algal growth. So it has some nutrient content. So algae can grow fantastically over that discharge water. So similar has also been implemented in the aquaponic system that aims to simultaneously grow fish and vegetables in a single system; that has been very popular since few years, the aquaponic system.

So in fact disposal of aquaculture wastewater has become a major task to further improve production efficiency and decrease the environmental impacts of the aquaculture industry. However, this process is not so straightforward due to the cost associated with the nutrient removal, that process is very costly. So, the effluent from aquaculture production and facilities contains large amount of nitrates and phosphates which are also essential nutrients for the algal growth in production.

Therefore, the benefit of the ancillary industries between aquaculture and algal biofuel can offset energy and greenhouse gas emissions in the algal production and also reduce investment for the wastewater management in aquaculture. So, you save a lot of money in that.

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So, let us concentrate on this proposed scheme. So, this scheme talks about, this is the flow sheet for the microalgae biorefinery aiming at producing omega-3 fatty acids, animal feed and biodiesel as the main products simultaneously. So, algae grow with the presence of light, water and fertilizer or some nutrient source. While you are cultivating algae, so you can use wastewater and even carbon dioxide from different sources like power plants, the flue gas.

So, then you harvest algae biomass, so you get algal oil that can convert to omega-3 fatty acids. Remaining oil can be converted to biodiesel. You get glycerol. Glycerol can be converted to 1, 3 propaneiol through different routes. This we have already discussed how to do it, catalytically or biologically. Whatever meal that is left out, algal meal that is the residual biomass after the oil is extracted you can either convert into biogas or ethanol depending upon the process you choose thermochemical or biochemical.

You can have the animal feed also or other valuable products also can be found out from that. So an algae can produce proteins, carbohydrates, lipids and other valuable components for example pigments, antioxidants, fatty acids, vitamins, recombinant proteins. The concept of an algal biorefinery has to explore the possibility of large-scale parallel and complimentary production streams of these bioproducts from industrially grown algae.

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So, let us look at the potential of an algal biorefinery in aquaculture. The main applications of microalgae for aquaculture are associated with nutrition as a sole component or as a food additive to basic nutrients. Microalgae are required for larval nutrition during a brief period or even for adults, either for direct consumption in the case of mollusk or penaeid shrimp or directly or indirectly as food for the live prey fed to the small fish larva.

The use of microalgae in fish hatcheries is required for both production of live prey and maintaining the quality of the larva-rearing medium. A significant growth rate can be obtained with microalgae supplemented diets in many species such as prawn, oyster, abalone and scallop. The nutritional value of the dietary algae is not only dependent on the chemical composition, but also on factors such as the capability of the animal to ingest and digest the algae and to assimilate their nutrients.

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As aquafeed additives, algal biorefinery products have been shown to have positive effects on the immune system of the developing fish larvae and the regulation of immunity, gene expression as well as signalling. For example, feeding guppies with optimal concentration of a neutral lipid extract of the green microalgae *Parietochloris incisa* containing beta carotene and arachidonic acid as the major highly unsaturated fatty acids significantly reduced infection with the protozoan parasite, *Tetrahymena* species.

This is a very interesting work actually. So an additive effect of this *P. incisa* derived beta carotene and AA rich triacylglycerol TAG was evidenced by increased guppy survival under acute salinity stress. So as use of antibiotics in aquaculture is banned in many countries. algal aquafeed could be a more profitable venture rather than nutrient input alone. A lot of work has been going on on this particular aspect.

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The most frequently used aquaculture feed species are *Chlorella*, *Tetraselmis*, *Isochrysis*, *Pavlova*, then *Phaeodactylum*, then *Chaetoceros*, *Nannochloropsis*, *Skeletonema* and *Thalassiosira*. So, combination of different algal species provides better balanced nutrition and improves animal growth better than a diet composed of only one algal species.

In addition to providing protein and energy, they provide other key nutrients such as vitamins, essential polyunsaturated fatty acids, pigments and sterols which are transferred through the food chain. Protein and vitamin content is a major factor determining the nutritional value of microalgae. In addition, omega-3 PUFAs mainly consisting EPA, AA and DHA content is of the major importance as it has lot of commercial value.

So, please have a close look at this particular slide. Here the aquaculture and algal biorefinery has been shown to stress that how algal biorefinery can be coupled with the aquaculture system. So, you see that the first one this is the aquaculture is going on. So, the fish feed aquaculture is going on. So, algae cultivated with that wastewater. Then once you take the algal biomass it can go for so many different types; biofuels, bioplastics, then other valueadded products and many.

Let us not again go back to that, we have already covered it many times. Then whatever the water that is coming out after the algae is harvested you need to treat it a little further. So that it can be recycled back to either here or to here. Similarly here if you can see this is the schematic representation of a multiproduct algal biorefinery model. So, you grow algae in different systems.

So, this is how the algae has been cultivated either raceway pond, photobioreactors, indoor, outdoor anything. You get it, so it can go for feed and supplements that same thing can be used as in the aquaculture system. So, the residual biomass can be converted to biofertilizer, bio oil, biogas, so many other things, so even pharmaceuticals and high value natural products. So, this is how algal bioculture and aquaculture can be combined together for a win-win situation.

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It has been estimated that the production of microalgae for aquaculture could be more than 1000 tons, so 62% for mollusks, 21% for shrimps and 16% for fish. The worldwide annual production of algal biomass is estimated to be 5 million kg per year with a market value of about 1.25 billion US dollar per year. Its huge, every day it is growing. So, approximately one-fifth of this biomass is used to nourish the fish and shell fish that are cultivated in aquaculture hatcheries.

However, valuable extractable chemicals as above could be obtained from biofuel biorefineries. By producing various coproducts such as omega-3 fatty acids and biodiesel in a sequential biomass processing algal biorefineries have a strong potential to match the challenge in aquaculture to generate end products, seafood with high levels of healthpromoting nutrients for human consumption as well as it takes into account the aesthetic issues also without further depleting naturally occurring fish stocks.

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So, let us try to understand the trends in algal biorefinery with reference to the aquaculture systems. So with increased demand of second and third generation biofuels, private investment has sprung up now far exceeding public funding. Currently, no algal biorefinery is commercially operating. However, significant improvements to commercialization have been reported by companies like Sapphire Energy, CEHMM and Cellana.

Although algae have been recognized as a promising biofuel feedstock, it is worth noting that there are also some obstacles hindering the development of the microalgae biofuels. For instance, with current available photo-bioreactors the production cost of algal biomass is eight times higher than that of the fossil fuels. So, therefore maximizing the value derived from algal biomass feedstock seems to be essential for the algal biofuel development.

So as and when you produce more and more different value-added products from the algal biorefinery system, the price of the algal biofuel will come down significantly.

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□ At present, many other algal products are more profitable than biodiesel, and these products will be important to establish profitable algal biorefineries. □ However, it can be anticipated that demand for these products will be much more easily satisfied which will influence the price than the demand for biodiesel which aims to replace large proportions of fossil fuels. \Box At present, the productivity of microalgal biomass on average can reach 80 t/ha/year obtained from a microalgae plant. If "biofuel content" accounts for 30% of the biomass, the non-fuel production can easily exceed 50 t/ha/year. \Box *Vice versa*, the ongoing research of microalgae biofuels will likely continue to alleviate the production cost, which is also a desirable criterion for the algal biorefinery. **The Second Second Second Second** Indian Institute of Technology Guwahati

At present, many other algal products are more profitable than biodiesel and these products will be important to establish profitable algal biorefineries. However, it can be anticipated that demand for these products will be much more easily satisfied which will influence the price than the demand for biodiesel, which aims to replace large proportions of fossil fuels. At present, the productivity of microalgal biomass on average can reach 80 ton per hectare per year obtained from a microalgae plant.

If biofuel content accounts for 30% of the biomass, it is roughly into that range, the non-fuel production can easily exceed 50 tons per hectare per year. So this is a direct mass balance you can say taking into account 30% of the biomass goes for the biofuel production. So, vice versa the ongoing research of microalgae biofuels will likely continue to alleviate the production cost which is also a desirable criteria for the algal biorefinery.

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So, let us understand what is the future direction. Requirement of high cost and energy for the large-scale production of microalgae has been the major constraint for its commercial utilization. Reduction of excessive power expenditure through optimization of different methods and technologies for agitation, harvesting, drying of biomass is one of the key directions of future research as all these are energy intensive processes and costly.

Now moreover, efficient strain selection, genetic engineering and utilization of the wastewater and carbon dioxide for biomass production should be taken into consideration. So, microalgae species and strain selection is determined by many factors. Some of them are growth rate, optimal temperature range, lipid accumulation, harvesting properties and response to nutrient deprivation.

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Unsaturated fatty acids constitute the major proportions of lipids in microalgae, raising concerns for storage of biodiesel as the acids are prone to oxidation. One corrective measure is the partial catalytic hydrogenation of the oil. However, higher levels of polyunsaturated fats lower the cold filter plugging point, the temperature at which the fuel starts to form crystals or you can say solidifies and blocks the fuel filters of an engine.

It can be seen that the extent of unsaturation in oil lowers its melting point. Therefore, colder climates require a higher unsaturated lipid content to enable the fuel to perform at low temperatures. Linked to the cost of cultivation, the process byproducts as for example the exhausted growth medium and exploited biomass should be recycled or be considered as new substrates for other processes in an integrated biorefinery system with higher value and commercial suitability.

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For example, as fertilizer costs for nitrogen and phosphorus are likely to increase due to the high energy cost and depletion respectively, it can be envisaged that unwanted biomass can be used for biogas that is methane production whereby nutrients from the sludge can be recirculated. Similarly, fish waste can be used to create additional revenue streams through the growth of algae for biofuel and methane which is also called the Aqua-Sphere system.

To this end, the issue of bioaccumulation of heavy metals and bioactive chemicals need to be considered if animal feed is to be produced repeatedly. Furthermore, it is important to search for native microalgae species both for biofuel production and aquaculture due to the environmental and economic benefits they offer.

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In aquaculture, it has been already reported that native species exhibited superior survival than laboratory species in larval P. magellanicus.

If However, the process of isolating and testing new species of algae is very labor intensive, and hatcheries are unlikely to adopt new algal species without well-established culture methods and proven long-term success.

 \Box The enrichment of live algal feeds by altering culture conditions and by adding supplements to the culture water may be a very fruitful area of research. However, little is known about the effect of these modified feeds on larval performance.

□ Moreover, the direct addition of dissolved organic components such as sugars, amino-acids, and fatty acids to larval culture tanks may be a very direct and simple way to supply essential nutrients to larvae, although little research has been done in this area.

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| Indian Institute of Technology Guwahati In aquaculture, it has been already reported that native species exhibited superior survival

than laboratory species in in larval *P. magellanicus*. However, the process of isolating and testing new species of algae is extremely labor intensive and hatcheries are unlikely to adopt new algal species without well-established culture methods and proven long-term success. The enrichment of live algal feeds by altering culturing conditions and by adding supplements to the culture water may be a very fruitful area of research.

Many researchers are working on this particular aspect. However, little is known about the effect of this modified feeds on larval performance. More research is required on this aspect. Moreover, the direct addition of dissolved organic components such as sugars, amino acids and fatty acids to larval culture tanks may be a very direct and simple way to supply essential nutrients to larva, although little research has been done in this particular area.

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Now, we will talk about waste biorefineries. I think we discussed about waste biorefineries in one of our classes long back, but we are discussing this in an integrated biorefinery context. So, a waste biorefinery is a means to valorize waste as a renewable feedstock to recover biobased materials and energy through sustainable biotechnology. Now, this approach holistically integrates remediation and resource recovery.

You can see this is the petroleum based linear economy and this is the bio-based circular economy. Whatever you get the environmentally clean and renewable energy. It is a sustainable process, and this is a non-sustainable process. So it is ecologically and environmentally friendly, this is not so. Now if you come to here, we talk about an integrated waste biorefinery where different types of wastes can be recycled, reused as a feedstock for the waste biorefinery to have various value-added products.

Now we can have more than one different types of conversion process whether it is biological or thermochemical. And we can have different processes, unit operations inside a single integrated waste biorefinery basically as we have learned about integrated biorefinery. Integrated biorefinery is one in which we use different types of feedstocks, use different types of conversion technology and what we get is different types of products.

So, waste valorization has become vital necessity where biodegradable organic fraction of the wastes are treated employing biocatalyst under specifically maintained operating conditions. Now, this is a need of the hour. This is nothing more fancy nowadays, this is the need of the hour. So, we get biofuels, we get water that is treated can be recycled back to the process unit, we get different types of bio-based products including bioplastic and we get bioenergy. **(Refer Slide Time: 20:58)**

> Traditionally, waste remediation processes are intended to remove/reduce pollutants to safeguard the environment. However, the current pressing need to find alternative feedstocks has led to a transition from remediating waste towards refining waste to recover valuable resources.

> □ Any waste (solid, liquid, or gaseous) has an inherent net positive energy that can be recovered and reused to produce bio-based products and biofuels through a closed-loop bioprocesses cascade, enabling the shift towards a circular and low-carbon bio-economy.

> \Box The possibility is evident that bio-based products which include platform chemicals, biofuels, biocommodities, etc. can be derived from waste at a scale that is sufficiently large.

> □ Approximately 9 GT of CO₂, 650 km³ of wastewater, and 2.2 GT of solid waste are generated each year, making it possible to substantially satisfy both the current feedstock crisis and future sustainability requirements and creating independence from fossil-based precursors.

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So, traditionally waste remediation processes are intended to remove, reduce pollutants to safeguard the environment. Now, no more that is the case. The current pressing need to find alternative feedstocks has led to a transition from remediating waste towards refining waste to recover valuable resources. Any waste whether it is solid, liquid or gaseous has an inherent net positive energy that can be recovered and reused to produce bio-based products and biofuels through a closed loop bioprocesses cascading system (more than one process) enabling the shift towards a circular and low-carbon bioeconomy. The possibility is evident that bio-based products which include platform chemicals, biofuels, bio-commodities can be derived from waste at a scale that is sufficiently large.

Approximately 9 GT of carbon dioxide, 650 kilometer cube of wastewater and 2.2 GT of solid waste at generated each year making it possible to substantially satisfy both the current feedstock crisis and future sustainability requirements and creating independence from the fossil-based precursors.

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□ The integrated approach encompasses multifunctional bioprocesses in an optimized sequence to mine every constituent of waste, with the objectives of maximizing the productivity of mercantile intermediates and products and making the process economical.

Q Bioprocesses that utilize waste as a substrate for producing bio-based products require appropriate technologies including acidogenesis, bio-electrogenesis, photosynthesis, photo-fermentation, etc.

In this scenario, acidogenic fermentation is evolving as a focal process that enables a cascading progression of hydrolysis, acidogenesis, solventogenesis, and methanogenesis.

Q Current research is focused on navigating the acidogenic process to obtain *specific high-value products* instead of solely producing methane if the reaction progresses to completion.

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The integrated approach encompasses multifunctional bioprocesses in an optimized sequence to mine every constituent of waste (nothing is waste, everything can be converted into some valuable product by a particular technology), with the objectives of maximizing the productivity of mercantile intermediates and products and making the process more economical.

Bioprocesses that utilize waste as a substrate for producing bio-based products require appropriate technologies including acidogenesis, bio-electrogenesis, photosynthesis and proto-fermentation, etc. In this scenario, acidogenic fermentation is evolving as a focal process that enables a cascading progression of hydrolysis, acidogenesis, solventogenesis as well as methanogenesis.

Current research is focused on navigating the acidogenic process to obtain specific high-value products instead of solely producing methane if the reaction progresses to completion. So, nobody is talking about only methane production. When you produce methane, you are also producing other valuable chemicals, it is already being produced, nobody has ever thought of that, we have to trap them and further refine them to get a value-added product, now that is being done.

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Metabolically versatile acidogenic bacterias utilize various wastes along with carbon monoxide and carbon dioxide to produce a variety of high-value products including short and medium chain fatty acids, alcohols, biohydrogen biomethane, esters and other products depending on the process parameters and of course operational conditions. A systemized interlinking of bio processes is the key to constructing a waste biorefinery.

Effluents from acidogenic bioprocess could be used as substrates by photo biocapture clusters - which are very commonly known as PCCs - comprising photosynthetic organisms to advance treatment efficacy, assimilating gaseous waste basically as well as recover secondary products. Now, these photo biocapture clusters have shown notable progress in the production of biofuels and commercially viable bioproducts owing to their photosynthetic and chemoautotrophic machinery.

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Microalgae with malleable metabolisms can be cultivated in autotrophic and heterotrophic modes of nutrition utilizing carbon dioxide and the organic fraction of waste as their carbon sources. Because they can consume various feedstocks, microalgae can be employed to yield biodiesel, biomass or high-value chemicals such as pharmaceuticals, nutraceuticals, antioxidants, food supplements and biofertilizers.

Photo-bacteria have been widely applied to waste remediation through photo-fermentation to produce platform chemicals, bioelectricity, biohydrogen and biopolymers through photoanoxygenesis. Although there are certain challenges, the carbon dioxide bio-capture cluster has the innate potential to solve energy and climate change issues in a unified approach or in a more sustainable way also.

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The success of these technologies relies on the versatile metabolism of the biocatalysts and their ability to utilize waste. More work has been done on this particular development of the biocatalyst and many people are still working day and night to develop efficient biocatalyst. Tailoring syntrophic biocatalyst into synthetic consortia that means comprising several different organisms with this specific objective could escalate their potential to overcome their individual limitations and maximize their productivity.

In addition to producing various bio-based products, the reducing equivalents that is protons and electrons generated during the acidogenic process can be harvested as electrical energy in the presence of an electrode assembly in a microbial fuel cell through a process called bioelectrogenesis. We have already discussed about microbial fuel cell. Now, although the achievable power densities with MFCs are low at present, they can be improved with technological interventions combined with process optimization.

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 \square In addition to power, MFCs have many other applications, such as *bio-electrochemical treatment* (BET) for treating waste, microbial electrosynthesis (MES), and microbial electrolysis cells (MECs) for producing high-value products and biofuels.

Q Electro-fermentation (EF) is closely associated with MES for steering fermentation in a bioelectrogenic environment where in the reducing equivalents in the anodic chamber are channelized to synthesize high-value electrochemicals in the cathodic chamber through a defined circuitry control.

De Bio-cathodes are being introduced to bio-electrochemistry to balance the drawbacks of MFCs, providing economic feasibility and higher power outputs.

In addition to power, MFCs have many other applications such as bio-electrochemical treatment for treating waste, microbial electrosynthesis and microbial electrolysis cell for producing high-value products as well as biofuels. Electro-fermentation is closely associated with the MES for steering fermenters and in a bio-electrongenic environment wherein, the reducing equivalents in the anodic chamber are channelized to synthesize high-value electrochemicals in the cathodic chamber through a defined circuitry control. Bio-cathodes are being introduced to bio-electrochemistry to balance the drawbacks of MFCs providing economic feasibility and higher power outputs.

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□ Synthesis of various products, including short-, medium-, and long-chain fatty acids, alcohols, esters, and diols, are being researched using specifically enriched bacteria in microbial electrochemical systems.

Integrating PCCs with MES is hypothesized to enhance the photosynthetic efficiency, leading to increased biomass and greater synthesis of light-induced bio-products, including biodiesel, nutraceuticals, antioxidants, pigments, and colorants, through the transferred electron flux.

Photosynthetic bio-cathodes, with their ability to produce oxygen, develop a strongly electronegative environment for enhanced proton transport, thereby con-currently increasing the power output.

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Synthesis of various products including short, medium and long-chain fatty acids, alcohol, esters and diols are being researched using specifically enriched bacteria in microbial electrochemical systems. Integrating PCCs with MES hypothesized to enhance the photosynthetic efficiency leading to increased biomass and greater synthesis of light induced bio-products including biodiesel, nutraceuticals, antioxidants, pigments and colorants through that transport electron flux.

Photosynthetic bio-cathodes with their ability to produce oxygen develop a strongly electronegative environment for enhanced proton transport, thereby concurrently increasing the power output.

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Progressively retuning MFC configurations could make microbial electrochemical technologies a viable option for waste remediation, high-value product synthesis, and CO₂ sequestration in the context of a waste biorefinery, in turn ensuring environmental sustainability and establishing a viable bioelectro economy. Innovative and optimized process integrations to mine wastes to recover marketable products can begin to reform the current linear economy, leading to 'environmental biofactories'. Q Reconfiguring the current mindset to make the closed-loop approach a social norm will help to

transition from the petroleum refinery to waste biorefinery comprehensively addressing sustainable development goals (SDGs).

Progressively retuning MFC configurations could make microbial electrochemical technologies a viable option for the waste remediation, high-value product synthesis and carbon dioxide sequestration in the context of a waste biorefinery, in turn ensuring environmental sustainability and establishing a viable bio-electro economy.

Innovative and optimized process integrations to mine wastes to recover marketable products can begin to reform the current linear economy leading to environmental biofactories. Reconfiguring the current mindset to make the closed-loop approach a social norm will help to transition from the petroleum refinery to waste biorefinery comprehensively addressing sustainable development goals.

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 \Box Food waste (FW) is one of the mostly commonly generated bio-wastes in developing and developed countries

The disposal of FW pollutes the environment and results in a loss of valuable nutrients. In order to conserve natural resources and to meet the environmental discharge standards, the management of FW for the production of high value-added products has grown rapidly.

QFW biomass has been mostly used for the production of biofuels with the aim of partially or completely replacing highly polluting fossil-oriented fuels. The major disadvantages by these traditional treatment technologies are excessive time, poor feasibility, high cost, the emission of greenhouse gases, and sludge production.

The integration of a biorefinery is the most promising technology used to convert FW into value-added products.

Food waste is one of the most commonly generated bio-waste in developing and developed countries. The disposal of food waste pollutes the environment and results in a loss of valuable nutrients. In order to conserve natural resources and to meet the environmental discharge standards, the management of food waste for the production of high value-added products has grown rapidly.

Food waste biomass has been mostly used for the production of biofuels with the aim of partially or completely replacing highly polluting fossil-oriented fuels. The major disadvantages by these traditional treatment technologies are excessive time, poor feasibility, high cost, the emission of greenhouse gases and sludge production. The integration of a biorefinery is the most promising technology used to convert food waste into value-added products.

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So, this is a slide where integrated biorefinery concept diagram using food waste as the substrate or feedstock has been presented. So, you can see that so food waste, so we can have anaerobic fermentation. We get biohydrogen, biomethane, biohythane, VFA the volatile fatty acids and biofertilizers the leftover. From microbial electrolysis cell also you get all these things. We can have animal feed the left out things.

Yeast fermentation you get bioethanol, biodiesel. Bio-electriochemical process you get biochemicals, biopolymers. So, after this whatever the effluents is left out just look at this, the effluents basically the wastewater so you can further treat it to grow algae over that. What we have discussed a few minutes before, so algal growth. Biodiesel, biomass, nutraceuticals, animal feed. Again, the same thing whatever we are getting, of course a different product. Then you go for anoxygenesis, you get bioplastics.

You can use it in the microbial fuel cell you get bioelectricity. So then effluents, again whatever it is coming from this secondary effluents can be treated little more and can be directly used for agricultural and farming processes. The concept of biorefinery is the bioconversion of food waste into biofuels, power, heat and bio-products with the effective utilization of the food waste. The complexity of integrated biorefineries is based on the factors such as feedstock, byproducts and the applied technologies.

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Conventional biorefineries are designed around separate unit operations such as processing of feedstock, fermentation and post fermentation recovery of the biofuels. For instance, the processing of feedstocks such as corn or corn starch needs hydrolysis, followed by batch fermentation and then recovery of product. These individual processes necessitate improved principal and operational cost and production cost.

However, the progress made with these new technologies can permit these unit operations to be integrated as hard as possible, increasing the viability of the existing biorefineries. In the system, food waste and waste cooking oil are converted into biogas and biodiesel by anaerobic digestion and transesterification processes respectively. And this is the established protocol. Digested residue from the AD process can be further used as stand-alone fertilizer or as a coenzyme in the composting process.

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 \Box Integrated FW recycling by means of cultivation of *Rhodotorula glutinis* and AD for the fuel precursors. It was reported that dominant fatty acid methyl esters such as palmitic, stearic, and oleic acids were found to be perfect for biodiesel production. \Box The maximum yield of *methane* was achieved from AD of derived residual solids. \Box As mentioned in the previous section, AD is a complex sequential process and shows a balanced equilibrium that involves accumulation of VFAs within the digester system. The over accumulation of fatty acids inhibits the methanogenic process. Therefore, integrating hydrogen and methane production through two-stage fermentation is the possible route. wells shallfield steam yeard

Integrated food waste recycling by means of cultivation of the *Rhodotorula glutinis* and anaerobic digestion for the fuel precursors. It was reported that dominant fatty acid methyl esters such as palmitic, stearic and oleic acids were found to be perfect for the biodiesel production. The maximum yield of methane was achieved from the anaerobic digestion of the derived residual solids.

As mentioned in the previous section, AD is a complex sequential process and shows a balanced equilibrium that involves accumulation of the volatile fatty acids within the digester system. The over accumulation of fatty acids inhibits the methanogenic process. Therefore, integrating hydrogen and methane production through two-stage fermentation is the possible route.

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The blend of hydrogen and methane gas with a composition of 10-15% H₂, 50-55% CH₄, and 30-40% CO₂ is called *bio-hythane*, which is produced using two-stage anaerobic fermentation (AF) processes. The raw materials used for the production of hydrogen are sugars, starch, and carbohydrates. \Box Bio-hythane is considered as an energy carrier with a higher conversion efficiency, with the replacement of fossil fuels by the biological process. The addition of a lower amount of H₂ extends the flammability range of $CH₄$ due to its higher mass specific heating value than $CH₄$.

 \Box The burning speed of H₂ is about sevenfold higher than CH₄, thereby reducing the burning time of the engine. Furthermore, the presence of hydrogen reduces the emission of greenhouse gases into the atmosphere with decreases CO₂ gas production.

 \Box Bio-hythane is considered to be an *environment-friendly* gas.

The blend of hydrogen and methane gas with a composition of 10 to 15% hydrogen and 50 to 55% methane and 30 to 40% carbon dioxide is called bio-hythane which is produced using a two-stage anaerobic fermentation process. The raw materials used for the production of hydrogen are sugar, starch and carbohydrates. Bio-hythane is considered as an energy carrier with a higher energy conversion efficiency with the replacement of fossil fuels by the biological processes.

The addition of a lower amount of hydrogen extends the flammability range of methane due to its higher mass specific heating value than methane. The burning speed of hydrogen is about seven fold higher than methane thereby reducing the burning time of the engine. Furthermore, the presence of hydrogen reduces the emission of greenhouse gases into the atmosphere with decreased carbon dioxide gas production.

Bio-hythane is considered to be an environmental friendly gas. Basically, what is done is that, you know hydrogen and methane both are clean fuels. Hydrogen is a much more clean fuel, but it has its own limitations. Methane has its own limitation. So, if you combine them in different proportions and find some optimized proportion, then you can have a win-win situation that you have advantageous things from both the fuels.

And you are clubbing together so that the bio-hythane can be used without any limitations, which are already present with our hydrogen or otherwise methane if we talk about a standalone.

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Q Hydrogen is produced from FW rich in starch and cellulose. Dark fermentation produces hydrogen during the acidogenic stage of AD of organic matter, which is considered to be a promising technique relative to the conventional chemical process because the need for chemical energy is less and it is therefore more eco-friendly. □ Based on energy recovery, the output of energy is seven to nine times greater by the combined production of H_2 and CH₄ than conventional H_2 production alone, and 10-12% higher than the production of CH₄. Therefore, two-stage fermentation plays a significant role in maximum recovery of energy from FW substrate. D Effective biomethane production from non-gaseous fermentation products could make biological production of biohydrogen economically attractive.

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Numerous investigations have been carried out employing FW as substrate for bio-hythane production.

The generation of bio-hythane has been investigated both in lab-scale and semi pilot-scale extents. In the first stage, a higher hydrogen production of 66.7 L/kg VS was obtained, whereas in the second stage a higher biogas production of 0.72 m³/kg VS was obtained as distinctive bio-hythane composition.

 \Box One study executed a two-stage fermentation process for enhancement of bioenergy from FW. The results revealed the maximum yield of methane was observed by utilizing effluent from the first stage. In addition, COD removal of 70-90% was obtained under optimum conditions. The overall reaction rate and yield of biogas were improved by the production of combined hydrogen and methane in a twostage process related to the conventional two-phase process.

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□ Adapting older Biotech achievements, such as bioethanol from sugarcane or beets, into a biorefinery could make them more efficient. However, biorefineries using food crops suffer from the same sustainability issues as first-generation biofuels.

 \Box Of course, much of the hype surrounding biorefineries is the possibility (and technical challenge) of using biomass waste - or at least *biomass sources that are sustainable*. The field's close relationship with agriculture is one of the reasons there are so many incentives: there is the hope that biorefineries could regenerate rural areas.

One example of a company working towards this end is **Matrica**, which relies on plantations of thistle across Sardinia, in previously uncultivated plots of land. From the oil-rich thistle flower and plant biomass, Matrica produces not only energy, but also a range of products perhaps unfamiliar to the normal consumer but essential to much of our daily life - chemical intermediates, plasticizers, lubricants, components of cosmetic products and etc.

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Adapting older Biotech achievements such as bioethanol from sugarcane or beets into a biorefinery could make them more efficient. However, biorefineries using food crops suffer from the same sustainability issues as the first generation biofuels. Of course, much of the hype surrounding biorefinery is the possibility and the technical challenge of using the biomass waste or at least biomass resources that are sustainable.

The field's close relationship with agriculture is one of the reasons there are so many incentives. So, there is the hope that biorefineries could regenerate rural areas. So, one example of company working towards this end is Matrica, which relies on plantations of the thistle across Sardinia in previously uncultivated plots of the land, so unaerable lands basically.

From the oil rich thistle flower and plant biomass, Matrica produces not only energy, but also a wide range of products, perhaps unfamiliar to the normal consumer but essential to much of our daily life, chemical intermediates, plasticizers, lubricants, components of cosmetic products and there are many more..

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Meanwhile, Norway has Borregard which describes itself as the world's most advanced biorefinery. Listed on the Oslo Stock Market with a market cap of over 800 million euro Borregard specializes in using wood as a raw material. The resulting speciality chemicals are used in fields as diverse as construction and pharmaceuticals. But it is not only wood that is desirable raw material. In green biorefineries, grasslands are used instead.

For example, Biowert was founded in 2005 in Germany and now sells both bio-based plastics and the grass green juice from which amino acids and proteins can be extracted and used as flavoring agents or in cosmetics.

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This is another success story. This is Billund Biorefinery process that is in Denmark. Then let us talk about the waste, how waste can be converted. This is a classical success story. In this area Ynsect that is in France, a particularly curious case, so instead of enzymes or microbes, it uses insects for the bioconversion steps in the refinement process. Insects eat the agricultural waste such as fruit that did not meet the standards of supermarkets.

And the waste is then transformed into products from aquaculture feed to nutraceuticals. And then in Denmark the Billund Biorefinery goes after other waste energy that is sewage. Besides treating the water, this biorefinery utilizes the organic waste to produce biogas, fertilizer and bioplastics.

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Hybrid chemical and biological conversion processes

If Hybrid biomass conversion technologies have both biochemical and thermochemical steps. For example, heterogeneous catalytic processes for converting aqueous phase sugars into hydrocarbons have been developed.

The overall process still requires a clean sugar stream generated from the pretreatment and enzymatic hydrolysis of lignocellulosic biomass but uses supported metal oxide catalysts to make aromatic hydrocarbons instead of yeast to ferment sugars to ethanol.

□ Another hybrid under development uses gasification to deconstruct lignocellulosic biomass to produce syngas, then a biochemical conversion step to ferment the intermediate syngas to produce ethanol or other chemicals.

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So, now we will talk about the hybrid chemical and biological conversion process with respect to the integrated biorefinery concept. So, hybrid biomass conversion technologies have both biochemical and thermochemical steps. For example, heterogeneous catalytic processes for converting aqueous phase sugars into hydrocarbons have been developed. The overall process still requires a clean sugar stream generated from the pretreatment and enzymatic hydrolysis of lignocellulosic biomass, but uses supported metal oxide catalysts to make aromatic hydrocarbons instead of yeast to ferment sugars to ethanol. Now, if you recall many times I have told you during our other lectures that this catalyst, supported metal oxide catalyst has a huge role in the biorefinery concept. You talk about any other catalytic conversion process, you need an efficient catalyst.

Now, at present also the catalysts which are commercially available are extremely costly. So, there is a lot of research going on how to develop efficient catalysts from different types of waste, industrial waste, biowaste and other wastes. Another hybrid under development uses the gasification to deconstruct lignocellulosic biomass to produce syngas, then a biochemical conversion step to ferment the intermediate syngas to produce ethanol or other chemical. **(Refer Slide Time: 39:50)**

> \Box Syngas quality is still a challenge, as impurities can adversely affect the fermentation organisms, but the process is less sensitive to syngas composition (H₂:CO ratio, CO₂ and N₂ concentration). □ Syngas compression is also not needed for the ambient pressure process, but optimizing mass transfer is critical for efficiency.

> The main disadvantage of biochemical degradation and conversion processes is their inability to process the lignin fraction of the biomass feedstock. This inherently results in a loss of carbon and thus an inefficient feedstock usage.

> □ Purely thermochemical conversion processes, on the other hand, are characterized by their low product selectivity and thus relatively low yields.

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Syngas quality is still a challenge as impurities can adversely affect the fermentation microorganisms, but the process is less sensitive to syngas composition, that means the hydrogen to carbon monoxide ratio, carbon dioxide and nitrogen concentration. Syngas gas compression is also not needed for the ambient pressure process, but optimizing mass transfer is critical for efficiency.

The main disadvantage of biochemical degradation and conversion process is their inability to process the lignin fraction of the biomass feedstock. This inherently results in a loss of carbon and thus an inefficient feedstock use. Purely thermochemical conversion processes on the other hand are characterized by their own low product selectivity and thus relatively low yields.

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This is a syngas fermentation process flow chart where the feedstock is being gasified here. Then the syngas, there are many other processes as I told you many times this is just a simple schematic to make you understand, it goes to the fermenter. The syngas is getting fermented. Then it goes to a distillation column where you get ethanol is the distillate. Now, hybrid biorefineries employ both previously introduced bioconversion platform that is biological and thermochemical conversion platforms simultaneously.

By using a thermochemical degradation step like pyrolysis or gasification followed by a biochemical conversion step, the usage of the biomass feedstock and the production of the desired products is significantly increased. Now, these characteristics make the two platform biorefinery perhaps the most integrated design as of now.

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So, the fermentation of syngas from biomass gasification is another possible route for ethanol production from woody biomass. In this process, the ethanol synthesis is realized by a biological fermentation. So far only very limited information is available about complete process analysis for biomass derived syngas fermentation. Now, this process is realised in 5 major process steps discussed below or shown here.

Gasification, first we are getting flue gas and all these things. Then we have a gas cleaning. So, the gas goes to the gas cleaning process. From here it gets to the compressor where there is a fermentation happening here. From fermentation, one stream goes to the product separation part, we get ethanol, acetic acid and all these things. This is a distillation column, series of distillation columns. Another part goes to the compressor.

And basically to the CHP / combined heat and power generation unit. So we have gasification, we have gas cleaning unit, we have fermentation unit, we product separation as well as heat and power production unit. So, this is a classical flow sheet of the thermochemical conversion with gasification and syngas fermentation So, thermochemical conversion process is here, biochemical conversion process is here, both are integrated, This is what is the integrated biorefinery is all about.

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The gasification and gas cleaning is based on the same technology compared to the gasification and synthesis process. Like in the catalytic synthesis, methane present in the producer gas coming from the steam gasification process cannot be used within the syngas fermentation.

 \Box In the present analysis, CO₂ is not regarded as a critical component for syngas fermentation. Therefore, no CO, removal process is assumed in the gas cleaning section. This may not hold true for the layout of the fermentation reactor.

 \Box Large fractions of CO₂ present in the syngas will increase fermenter volume and power consumption for gas compression. Furthermore, significant amounts of CO, will be present in the water phase because of the significantly higher solubility compared to H₂ and CO.

□ Ethene, ethane, tars, particulates, sulphur and nitrogen compounds may have an inhibiting effect not fully investigated yet. Thus, further gas cleaning steps may be necessary.

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So, the gasification and gas cleaning is based on the same technology compared to the gasification and synthesis process. Like in the catalytic synthesis, methane present in the producer gas coming from the steam gasification process cannot be used within the syngas fermentation. In the present analysis, carbon dioxide is not regarded as a critical component of syngas fermentation.

Therefore, no carbon dioxide removal process is assumed in the gas cleaning section. This may not hold true for the layout of the fermentation reactor. Large fractions of carbon dioxide present in the syngas will increase fermenter volume and power consumption for gas compression. Furthermore, significant amounts of carbon dioxide will be present in the water phase because of the significantly higher solubility compared to hydrogen and carbon monoxide.

Ethene, ethane, tars, particulates, sulphur and nitrogen compounds may have an inhibiting effect - not fully investigated yet. Thus further gas cleaning steps may be necessary.

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For the fermentation of synthesis gas to ethanol, different bacteria are known so far. Beside ethanol, these microorganisms produce acetic acid as a byproduct. In the syngas fermentation process, carbon monoxide is consumed primarily by microorganisms, hydrogen is consumed to a lower extent and methane present in the producer gas cannot be converted.

With a conversion efficiency of approximately 85% of the carbon monoxide content of the syngas, the recycling of the unconverted gas does not appear promising because the partial pressures of the main substrates - carbon monoxide and hydrogen - would be reduced in the fresh feed leading to decreased mass transfer rates and increased fermenter volume. In syngas fermentation, the conversion is mainly limited by the mass transfer of hydrogen and carbon monoxide in the aqueous phase.

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So, Pardo-Planas et al in 2017 presented an Aspen Plus model of the production of ethanol and acetic acid with the use of syngas fermentation. The reference is given below. The considered biomass feedstock is switchgrass. The modelled process consists of a gasification unit, a fermentation unit and a downstream processing unit. The model for the gasification unit was based on an Auger gasifier.

Auger gasifiers have distinguished drying, pyrolysis and combustion zones. This allows for a tight control of how much feedstock carbon is converted into char. The fermentation module was modelled in Aspen Plus with the use of a stoichiometric reactor.

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The reactor operates at ambient pressure and 37 °C. Acetogenic bacteria, like Clostridium ljungdahlii, Clostridium carboxidivorans, Alkalibaculum bacchi, were present in the reactor and catalyzed the following reactions.

> $4CO + 2H₂O \rightarrow CH₃COOH + 2CO₂$ $CO₂ + 4H₂ \rightarrow CH₃COOH + 2H₂O$ $6CO + 3H₂O \rightarrow C₂H₅OH + 4CO₂$ $2CO_2 + 6H_2 \rightarrow C_2H_5OH + 3H_2O$

D Michailos et al. (2019) developed another Aspen Plus model of a syngas fermentation process, but modelled the gasifier as a circulating fluidized bed reactor and used a CSTR reactor for the fermentation process. The considered acetogenic bacterium was C. ljungdahlii, which converts, respectively, 70 and 50% of the CO and H₂ present in the syngas to ethanol.

So the reactor operates at ambient pressure and 37 degrees centigrade. Acetogenic bacteria like *Clostridium ljungdahlii, Clostridium carboxidivorans*, then *Alkalibaculum bacchi* were present in the reactor and catalyzed the following reactions. 4 carbon monoxide $+2$ water gives us CH 3 COOH + 2 carbon dioxide. Carbon dioxide + 4 hydrogen gives us CH 3 COOH + 2 water. 6 carbon monoxide + 3 water gives us C 2 H 5 OH + 4 carbon dioxide. Then 2 carbon dioxide + 6 hydrogen reacts to give us again C2 H 5 OH and 3 water.

> $4CO + 2H₂O \rightarrow CH₃COOH + 2CO₂$ $CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$ $6CO + 3H₂O \rightarrow C₂H₅OH + 4CO₂$ $2CO₂ + 6H₂ \rightarrow C₂H₅OH + 3H₂O$

And in another work Michailos et al in 2019 developed another Aspen Plus model of a syngas fermentation process, but modelled the gasifier as a circulating fluidized bed reactor and used a CSTR reactor for the fermentation process.

The considered acetogenic bacterium was *Clostridium ljungdahlii* which converts respectively 70 and 50% of the carbon monoxide and hydrogen present in the syngas to ethanol. References are given below. If you are interested more, so please go through this interesting work.

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So, with this I conclude my today's lecture and next lecture will be our last lecture for this course. In that lecture, we will be covering about one of the very important thing, everybody is talking about today, you develop some process what you will do with that? You have to study the techno-economical aspects as well as the lifecycle of that, especially if it is a biological system.

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So, we will be discussing about that and we will wind up our course with that particular lecture. So, thank you very much. If you have any query, please register in this Swayam portal or you are always free to drop a mail to me at [kmohanty@iitg.ac.in.](mailto:kmohanty@iitg.ac.in)