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

Module 06
Lecture-16
Types, Fundamentals, Equipments, Applications

Good morning students. Today we are starting module 6, and under module 6 we will be discussing about the various microbial conversion processes. So, in today's lecture we will be discussing different types of microbial conversion processes, then the fundamentals basically and what are the equipments that are being used for the microbial conversion processes and few applications. So, let us begin.

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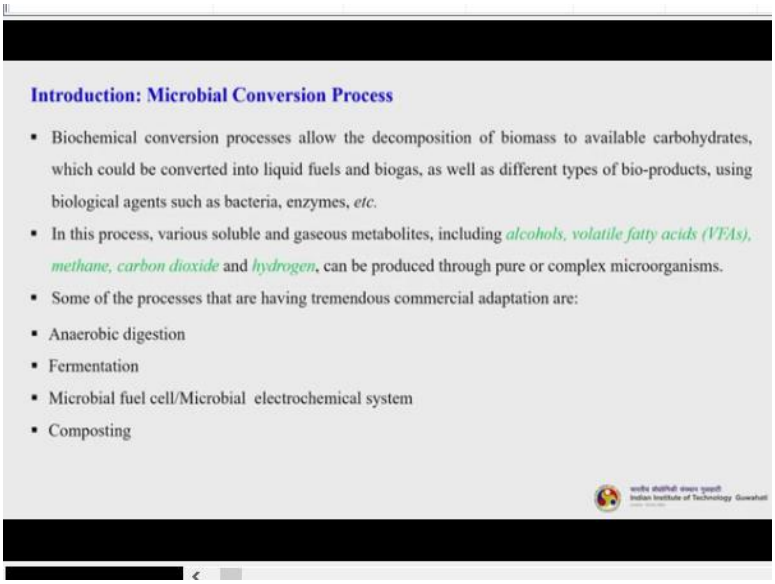

So, biochemical conversion processes allow the decomposition of biomass to available carbohydrates, which could be converted into liquid fuels and biogas as well as different types of bioproducts using biological agents such as bacteria and enzymes etc. Now, in this process, various soluble and gaseous metabolites including alcohols, volatile fatty acids, methane, carbon dioxide and hydrogen can be produced through pure and complex microorganisms.

Some of the processes that are having tremendous commercial application are anaerobic digestion, fermentation, microbial fuel cell or microbial electrochemical systems and composting.

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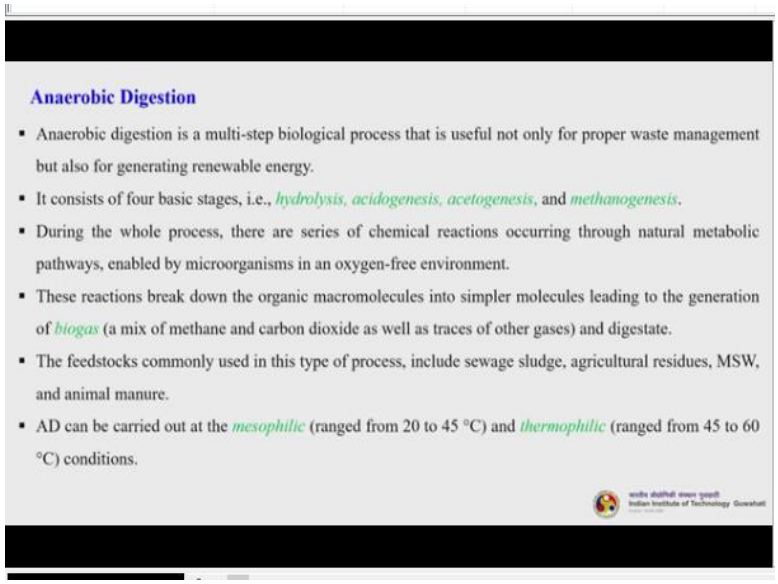

So, we will see all of these briefly. So, first one is anaerobic digestion. Now, anaerobic digestion is a multi-step biological process that is useful not only for proper waste management, but also for generating renewable energy. It consists of 4 basic stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Now, during the whole process, there are a series of chemical reactions occurring through natural metabolic pathways enabled by microorganisms in an oxygen free environment.

So, anaerobic digestion means basically, the entire process is happening without oxygen. Now, these reactions break down the organic macromolecules into simpler molecules, leading to the generation of biogas. So, biogas basically here, it is a mixture of methane and carbon dioxide, as well as traces of other gases and digestate.

Now, the feedstocks commonly used for this type of processes include sewage sludge, agricultural residues, the municipal solid residue and animal manure. Anaerobic digestion can be carried out at the mesophiclic which is basically from 20 to 45 degrees centigrade, or thermophilic range from 45 to 60 degrees centigrade conditions.

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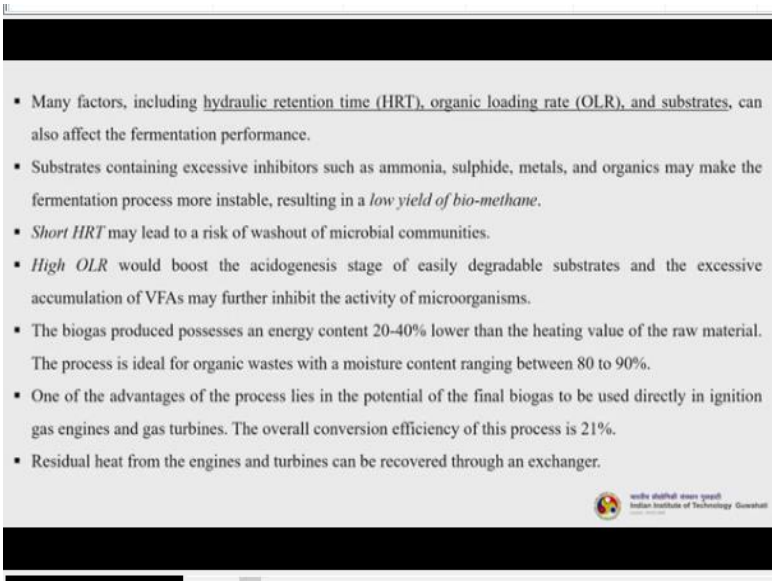

Many factors including hydraulic retention time, organic loading rate and substrates can also affect the fermentation performance. Now, substrates containing excessive inhibitors such as ammonia, sulphide, metals and organics may make the fermentation process more instable resulting in a low yield of bio-methane.

Actually, what happens during an anaerobic digestion process? So, when the process is happening, there are many byproducts that are produced. Now, some of these are actually not required for that particular environment. So, we can call them that, they are inhibitors or toxic compounds. Now, what way they are inhibiting basically? So beyond certain limit, if they are getting produced and again let us say produced and remained in the environment or in the process equipment itself, then they will hamper the growth of the microorganisms and their metabolic activities. Now, so, that is not good. So, in any such fermentation process, including anaerobic digestion, when such type of inhibitory compounds are getting formed, it is required that these compounds needs to be removed frequently. So, as to maintain their concentration inside the equipment, at a very small level, so that they are not going to inhibit the metabolic activity of the microorganisms.

Apart from that, if we go for a short hydraulic retention time, so that might lead to the risk of wash out of microbial communities. So, retention time basically means how much time the feed is going to be spent or going to be processed inside a particular reactor. So, a high OLR that means organic loading rate will boost the acidogenesis stage. So, that means the feedstock is very much enriched with the organic compound.

So, if you have a high OLR, so it will boost the acidogenesis stage of easily degradable substrates and the excessive accumulation of volatile fatty acids may further inhibit the activity of microorganisms. So, precisely this means that if you have a high organic loading so there will be faster degradation of the easily degradable substrates, basically during the acidogenesis stage, which usually results into the volatile fatty acids.

And even though volatile fatty acids are important, but beyond certain limit again, they will inhibit the activity of the microorganisms. The biogas produced possesses an energy content of 20 to 40% lower than the heating value of the raw material. Now, the process is ideal for organic waste with a moisture content ranging between 80 to 90%. One of the advantages of the process lies in the potential of the final biogas to be used directly in ignition gas engines and gas turbines.

The overall conversion efficiency of the process is 21%. Residual heat from the engines and turbines can be recovered through an exchange. So, I told you in the last class or even last to last class when we were discussing about thermochemical conversion process, I told you that when any such conversion processes whether it is thermochemical, biochemical or any other unit operations are going on, so, usually there is some heat generation. Now, that heat generation even if it is not so high also, if we can harness that heat generation by some waste heat recovery process and recycle back it to some other unit which requires the heat, maybe for steam generation, maybe for drying the biomass, then it will be a very good thing or we can say it will help us in a sustainable bio-refinery approach.

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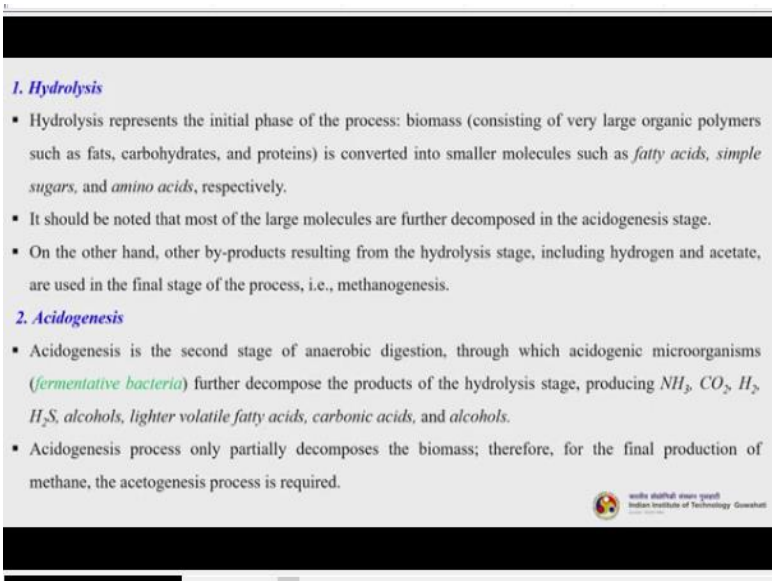

We will see the reaction steps. The first one is hydrolysis. So, hydrolysis represents the initial phase of the process. Biomass that consists of very large organic polymers such as fats, carbohydrates and proteins are converted into smaller molecules such as fatty acids, simple sugars and amino acids. It should be noted that most of the large molecules are further decomposed in the acidogenesis stage. On the other hand, other by-products resulting from the hydrolysis stage including hydrogen and acetate are used in the final stage of the process that is methanogenesis.

The second step is acidogenesis. Acidogenesis is the second stage of the anaerobic digestion through which acidogenic microorganisms basically fermentative bacteria, further decompose the products of the hydrolysis stage producing ammonia, carbon dioxide, hydrogen, hydrogen sulphide, alcohols, lighter volatile fatty acids, carbonic acid and certain alcohols. Acidogenesis process only partially decomposes the biomass, therefore for the final production of methane, the acetogenesis process is required.

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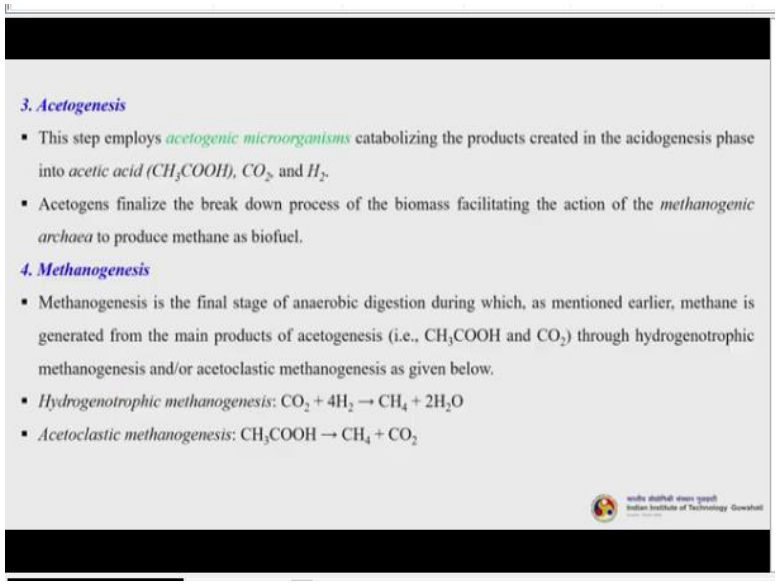

Now acetogenesis, this step employs acetogenic microorganisms catabolizing the products created in a acidogenesis phases into acetic acid CH_3COOH, carbon dioxide and hydrogen. Now acetogens finalize the breakdown process of the biomass facilitating the action of the methanogenic archaea to produce methane as biofuel.

Then the last step is the methanogenesis. And now this is the final stage of anaerobic digestion during which as mentioned earlier, methane is generated from the main products of acetogenesis that is acetic acid and carbon dioxide through hydrogenotrophic methanogenesis

and/or acetoclastic methanogenesis as given below. So, 2 different types of reactions take place:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

$$CH_3COOH \rightarrow CH_4 + CO_2$$

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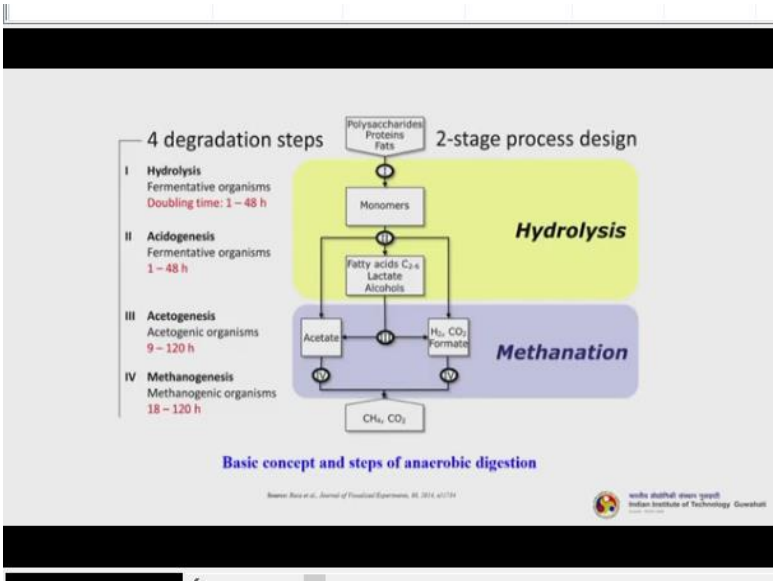

Have a look at this particular slide, this is the basic concept and steps for the anaerobic digestion process. So, whatever we have discussed it is given in a schematic representation here. So, the 4 degradation steps that what we just discussed - hydrolysis, acidogenesis, acetogenesis, methanogenesis. So, doubling time is 1 to 48 hour. This is again the second step is 1 to 48 hour. Acetogenesis takes more time 9 to 120 hours and methanogenesis is 18 to 120 hours.

So, the polysaccharides, proteins, fats. So, that gets converted to monomers, that again get converted to fatty acids, lactate, alcohols, acetate, hydrogen, carbon dioxide, formate. So, this is the methanation step, that is the hydrolysis steps. So, ultimately, we get methane and carbon dioxide by the final 2 reactions, which we just discussed in the previous slide.

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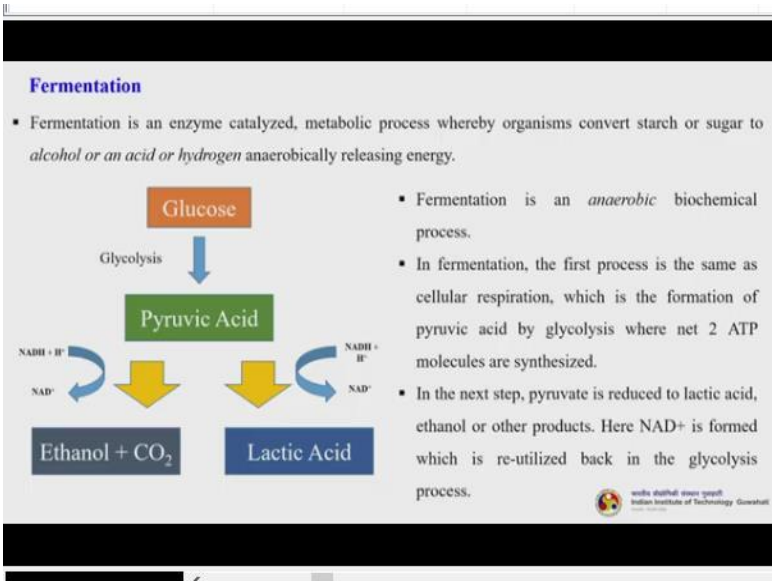

So, the next is fermentation. So, fermentation is an enzyme catalyzed metabolic process whereby organisms convert starch or sugar to alcohol or an acid or hydrogen, anaerobically, releasing energy. Now, fermentation is an anaerobic biochemical process. In fermentation the first process is the same as cellular respiration, which is the formation of pyruvic acid by glycolysis where 2 net ATP molecules are synthesized.

So, you can see this scheme, here nicely it is represented. So, that glucose goes through that glycolysis step and it provides the pyruvic acid, so this is the same as the respiratory cycle. And here 2 net ATP molecules are synthesized. Now then in the next step pyruvate is reduced to lactic acid. So, in this step**,** so pyruvate to lactic acid and ethanol plus carbon dioxide and other products.

So, here NAD+ is formed which is reutilized back in the glycolysis process. So, you can see the reaction here and NADH + H+ it gives us NAD+. Now, this NAD+ is again goes back here that means, whatever it is getting produced here will be consumed in that glycolysis step.

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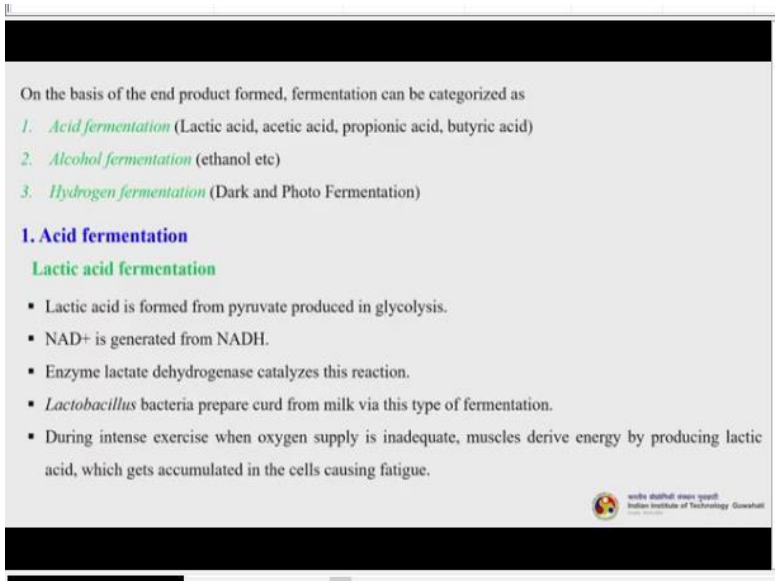

So, on the basis of the end product formed, fermentation can be categorized as acid fermentation, alcohol fermentation and hydrogen fermentation. So, we will see one by one what are those. Let us first discuss about the acid fermentation. So under acid fermentation lactic acid fermentation. So, lactic acid is formed from pyruvate produced in glycolysis. NAD+ is generated from NADH. Enzyme lactate dehydrogenase catalyze this reaction. So, lactate dehydrogenase is the enzyme that catalyzes this reaction. *Lactobacillus* bacteria prepare curd from milk via this type of lactic acid fermentation. Now, during intense exercise when oxygen supply is inadequate muscles derive energy by producing lactic acid, which gets accumulated in the cells causing fatigue and all of us have noticed this when we get stressed up.

So, the muscles basically pain and if you go for a this one some sort of we can say intense exercise, most of us have felt this lactic acid production and this lactic acid production inside the muscles actually causes the fatigue and sometimes pain also. So, when we go for a massage for the muscles, so, it basically it removes or disperses this lactic acid which is stored in a particular area of the muscles, thereby reducing the fatigue and pain.

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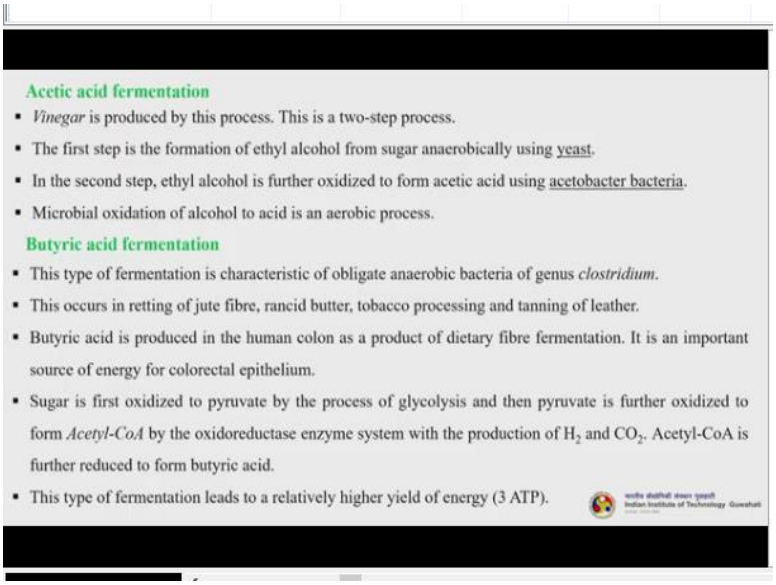

So, the next one is acetic acid fermentation. Vinegar, which is one of the most widely used product, in the food and beverage and this one restaurant industries is produced by this process. So, this is a 2 step process. The first step is the formation of ethyl alcohol from sugar anaerobically using yeast and in the second step ethyl alcohol is further oxidized to form acetic acid using acetobacter bacteria. Now microbial oxidation of alcohol to acid is an aerobic process.

So, the next one butyric acid fermentation. Now, this type of fermentation is characteristics of obligate anaerobic bacteria, genus *Clostridium*. This occurs in retting of jute fiber, rancid butter, tobacco processing and tanning of leather. Butyric acid is produced in human colon as a product of dietary fiber fermentation, it is an important source of energy for colorectal epithelium.

Sugar is first oxidized to pyruvate by the process of glycolysis. And then pyruvate is further oxidized to form acetyl coenzyme A by the oxidoreductase enzyme with the production of hydrogen and carbon dioxide. Now, this acetyl coenzyme A is further reduced to form butyric acid, this type of fermentation leads to a relatively higher yield of energy, a 3 ATP**.** We have seen that in glycolysis, it is 2 ATP, here in this case it is 3 ATP.

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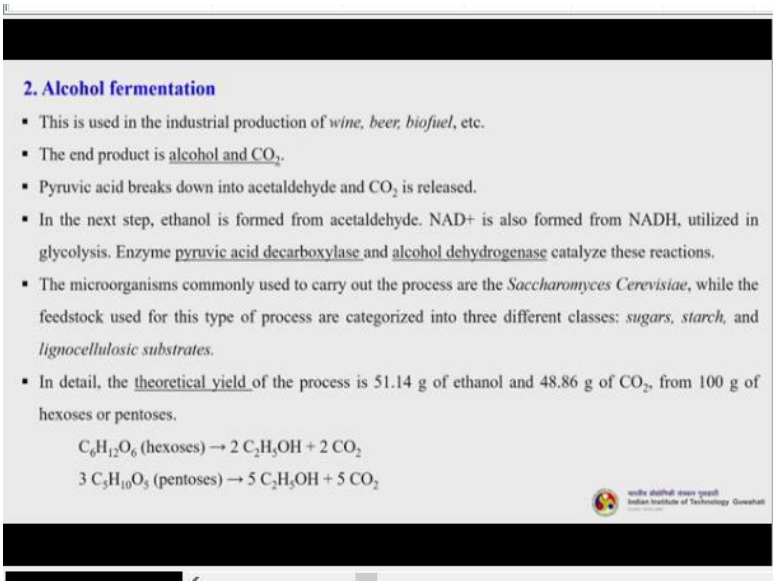

So, the next is alcohol fermentation. So, we have discussed about acid fermentation. So, we will now discuss about alcohol fermentation. So, this is used in the industrial production of wine, beer, biofuel etc. The end product is alcohol and carbon dioxide. Pyruvic acid breaks down into acetaldehyde and carbon dioxide is released. In the next step ethanol is formed from acetaldehyde.

NAD+ is also formed from NADH utilized in glycolysis. Enzyme pyruvic acid decarboxylase and alcohol dehydrogenase catalyzes these reactions. So, these are 2 enzymes which are responsible for doing these reactions. Now, microorganisms commonly used to carry out the process are *Saccharomyces cerevisiae*, while the feedstock used for this type of process are categorized into 3 different classes, sugars, starch and lignocellulosic structures.

In detail the theoretical yield of the processes is 51.14 gram of ethanol and 48.86 grams of carbon dioxide from 100 gram of hexoses or pentoses. So, this is the reaction:

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

$$3C_5H_{10}O_5\ (pentoses) \rightarrow 5C_2H_5OH + 2CO_2$$

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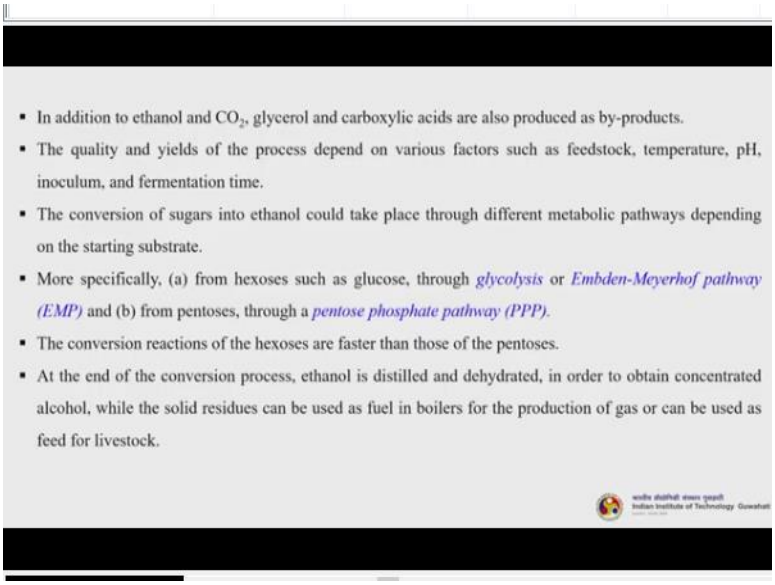

So, in addition to ethanol and carbon dioxide glycerol and carboxylic acids are also produced as by-products. The quality and yields of the process depends on various factors such as feedstock, temperature, pH, inoculum and fermentation time. The conversion of sugars into ethanol could take place through different metabolic pathways depending on the starting substrate.

More specifically from hexoses such as glucose through glycolysis or EMP pathway - the Embden-Meyerhof pathway and from pentoses through a pentose phosphate pathway, which is known as PPP pathway. So, the conversion reactions of the hexoses are faster than those of the pentoses. At the end of the conversion process ethanol is distilled and dehydrated in order to obtain concentrated alcohol while the solid residues can be used as fuel in boilers for the production of gas or can be used as feed for livestock.

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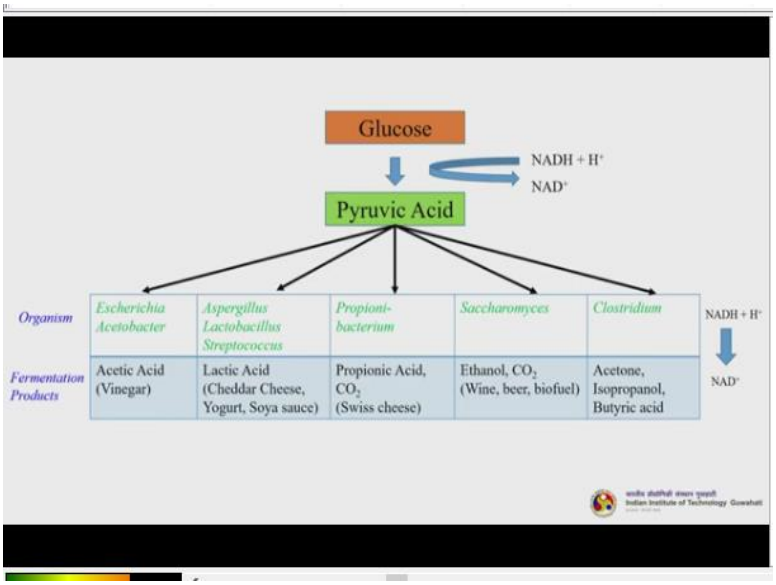

So, you can see this particular schematic representation how glucose is getting converted to pyruvic acid. Pyruvic acid is getting converted to different types of these are here the different types of organisms are written. And here the fermentation products are written, you can see the pyruvic acid depending upon the different types of organisms are going to give us different types of products.

If you are using the *Escherichia* or *Acetobacter* we will get acetic acid, that is vinegar. Pyruvic acid will be converted to lactic acid, Cheese, yogurt, soya sauce further processing, if we use as *Aspergillus*, *Lactobacillus*, *Streptococcus*, all these organisms. So, pyruvic acid can be converted to propionic acid, if we use *Propionibacterium*. So, further if you use *Saccharomyces* you will get ethanol plus carbon dioxide, if you use *Clostridium*, you will get acetone , isopropanol and butyric acid.

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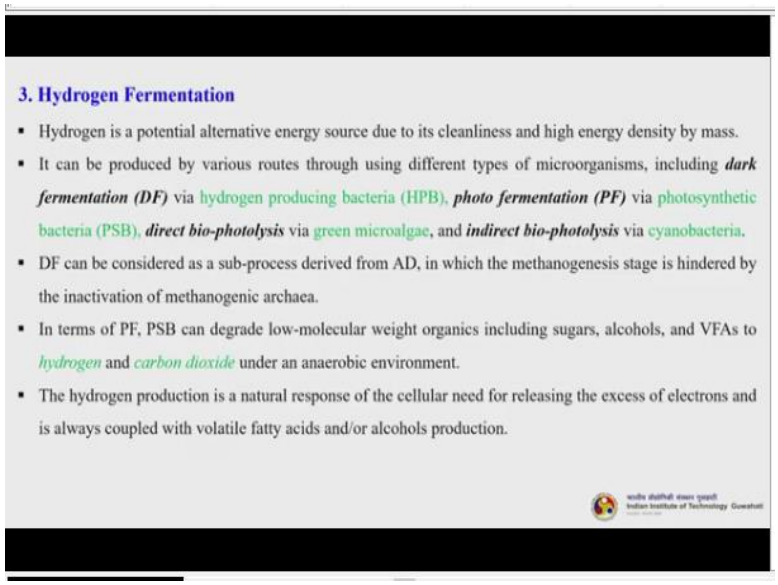

The next is hydrogen fermentation. Now, hydrogen is a potential alternative energy source due to its cleanliness and high energy density by mass. It can be produced by various routes through using different types of microorganisms, including dark fermentation via hydrogen producing bacteria, photo fermentation via photosynthetic bacteria and direct bio-photolysis using green microalgae or indirect bio-photolysis using cyanobacteria.

Now, dark fermentation can be considered as a sub process derived from anaerobic digestion, in which the methanogenesis stage is hindered by the inactivation of the methanogenic archaea. Now in terms of photo-fermentation and this photosynthetic bacteria, the photosynthetic bacteria can degrade low molecular weight organics, including sugars, alcohols and volatile fatty acids to hydrogen and carbon monoxide under an anaerobic environment.

So, this is more or less similar to the anaerobic digestion process, this particular step. So, the hydrogen production is a natural response of the cellular need for the releasing of the excess of electrons and is always coupled with volatile fatty acids and/or alcohol production.

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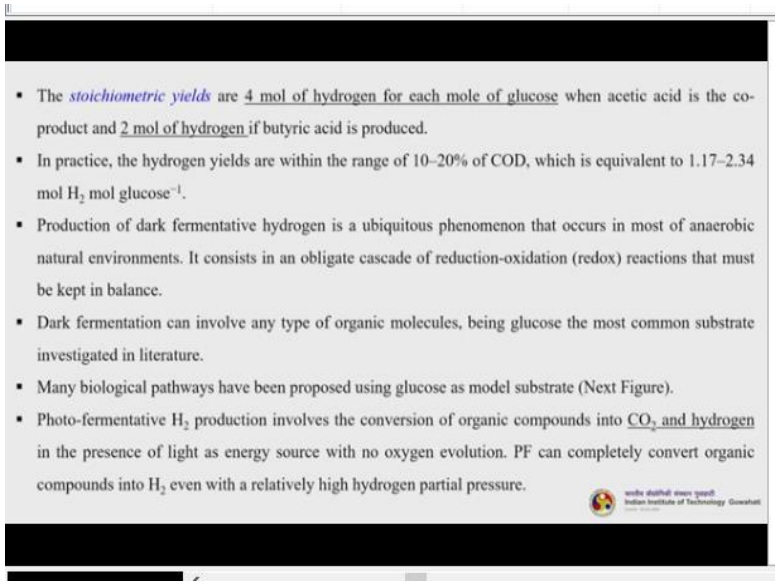

The stoichiometric yields are 4 moles of hydrogen for each mole of glucose, when acetic acid is the co-product and 2 moles of hydrogen if butyric acid is produced. So, many times what happens if you are looking for pure hydrogen production, then you have to suppress the path to produce butyric acid, we will always go for the acetic acid pathway where we will get more hydrogen yield per mole of glucose.

In practice the hydrogen yields are within the range of 10 to 20% of the COD the chemical oxygen demand, which is equivalent to 1.17 to 2.3 moles hydrogen per mole of glucose. Now, production of dark fermentative hydrogen is a ubiquitous phenomenon that occurs in most of the anaerobic natural environments. It consists in an obligate cascade of reduction oxidation or redox reactions that must be kept in balance.

Now dark fermentation can involve any type of organic molecules, glucose being the most common substructure investigated in literature. Many biological pathways have been proposed using glucose as model substrate. I will show you the next figure. And photo fermentative hydrogen production involves the conversion of organic compounds into carbon dioxide and hydrogen in the presence of light as an energy source with no oxygen evolution. Photo fermentation can completely convert organic compounds into hydrogen even with a relatively high hydrogen partial pressure.

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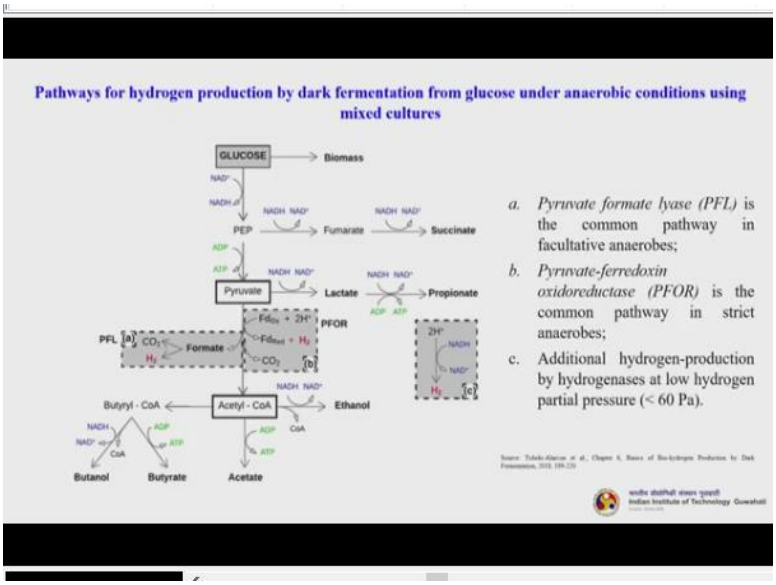

So, this is the pathways for the hydrogen production by dark fermentation from glucose under anaerobic conditions using mixed cultures. So, let us try to understand, so the glucose that is getting degraded to this PEP using NAD + and NADH that cycle. So, then it gives to fumarate and succinate. Now when it comes in the glycolysis pathway, this straight forward here, so, 2 ATPs are being produced.

So, it is getting converted to pyruvate. Now this pyruvate can be converted to lactate and again propionate, this is another pathway. Now when we come down here and we go for this acetyl coenzyme A production. Now this acetyl coenzyme A can further be converted to acetate to ethanol or butyryl coenzyme A via different, different pathways.

Now Pyruvate formate lyase, which is known as PFL, is the common pathway in the facultative anaerobes. So, pyruvate-ferredoxin oxidoreductase, which is known as PFOR, is the common pathway in strict anaerobes. Additional hydrogen production by hydrogenases at low hydrogen partial pressure less than 60 Pascal is also happens.

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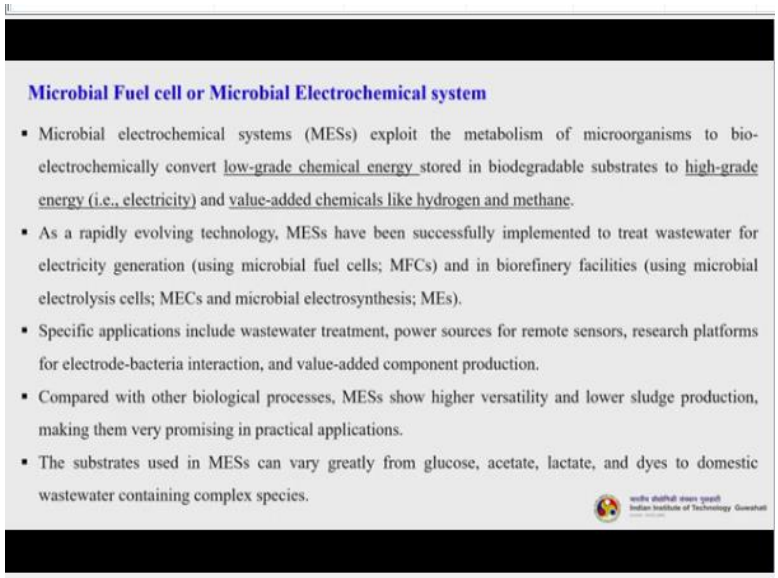

So, next is we will try to understand the basic concepts about the microbial fuel cell or microbial electrochemical systems. Now, microbial electrochemical systems exploit the metabolism of microorganisms to bio-electrochemically convert low grade chemical energy stored in biodegradable substrates to high grade energy, that is electricity and value added chemicals like hydrogen and methane.

As a rapidly evolving technology, this microbial electrolytic electrochemical system has been successfully implemented to treat wastewater for electricity generation using microbial fuel cells, and in bio-refinery facilities using microbial electrolysis cells and microbial electrosynthesis. Now, specific applications include wastewater treatment, power sources for remote sensors, research platforms for electrode-bacteria interaction and value added component production.

Compared with other biological processes, this MES show higher versatility and lower sludge production making them very promising in practical applications. In many other applications, if there is a high rate of sludge production, then sludge disposal is an another issue which needs to be tackled because that sludge has to be properly disposed, otherwise where you will keep the sludge.

So, that is of course, there are many applications of the sludge nowadays. So, many value added products are being produced, depending upon of course, the quality of the sludge. The substrates used in MES can vary greatly from glucose, acetate, lactate and dyes to domestic wastewater containing complex species.

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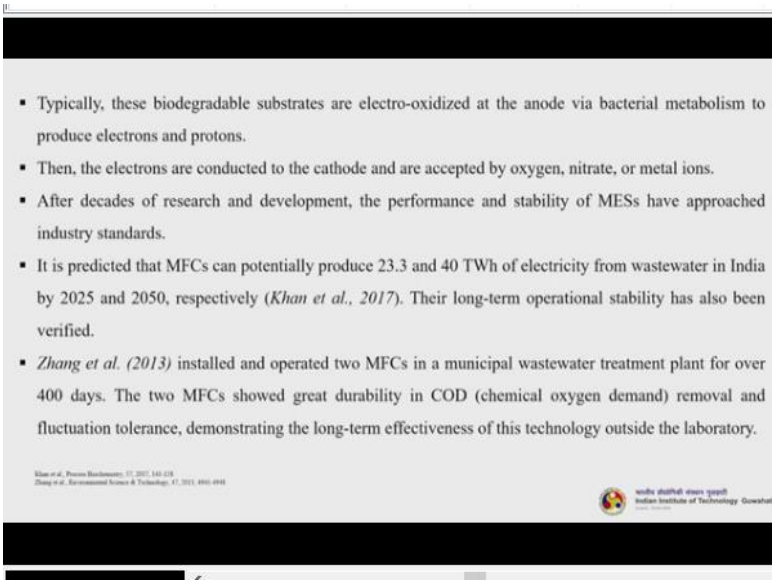

Typically, these biodegradable substances are electro-oxidized at the anode via bacterial metabolism to produce electrons and protons. Then the electrons are conducted to the cathode and are accepted by oxygen nitrate and metal ions. After decades of research and development, the performance and stability of MES have approached industry standards. It is predicted that MFCs can potentially produce 23.3 and 40 terawatt hour of electricity from wastewater in India by 2025 and 2050 respectively.

So, this is a projection or prediction you can say. The long term operational stability has also been verified. So Zhang et al installed and operated 2 microbial fuel cells in a municipal wastewater treatment plant for about 400 days. These 2 microbial fuel cells showed great durability in the COD removal and fluctuation tolerance, demonstrating the long term effectiveness of this technology outside the laboratory.

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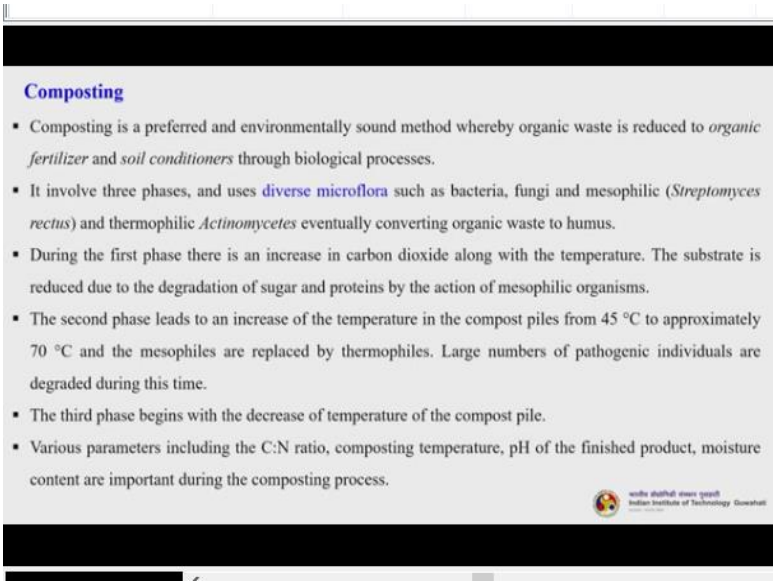

So, the next important microbial conversion process is composting. Composting is a preferred and environmentally sound method whereby organic waste is reduced to organic fertilizer and soil conditioners through biological processes. It involves 3 phases, and uses diverse microflora such as bacteria, fungi and mesophiclic and as well as thermophilic eventually converting organic waste to humus.

During the first phase there is an increase in carbon dioxide along with the temperature, the substrate is reduced due to the degradation of sugar and proteins by the action of mesophiclic organisms. The second phase leads to an increase of the temperature in the compost piles from 45 degrees centigrade to approximately 70 degrees centigrade and the mesophiles are replaced by thermophiles.

Large number of pathogenic individuals are degraded during this time; the third phage begins with the decrease of the temperature of the compost pile. Various parameters including the carbon nitrogen ratio, composting temperature, pH of the finished product, moisture content are important during the composting process.

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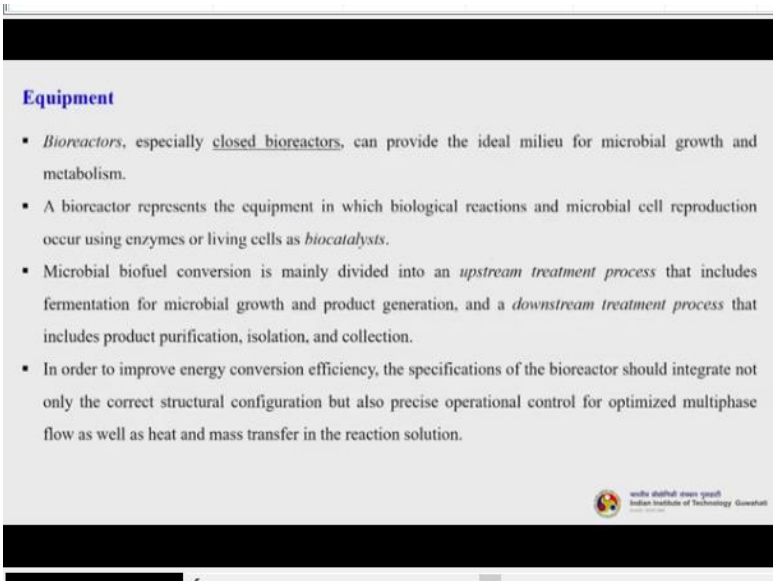

Now, let us understand the different equipments, those are essentially required to accomplish the microbial conversion processes. So, bioreactors, especially are closed bioreactors can provide the ideal milieu for the microbial growth and metabolism, because why we are talking about closed bioreactors, because we can easily control all the parameters in a closed system.

A bioreactor represents the equipment in which biological reactions and microbial cell reproduction occur using enzymes or living cell as bio-catalyst. Microbial biofuel conversion is mainly divided into an upstream treatment process that includes fermentation for microbial growth and product generation and a downstream treatment process that includes product purification, isolation and collection.

In order to improve energy conversion efficiency, the specifications of the bioreactor should integrate not only the correct structural configuration, but also precise operational control for optimized multiphase flow as well as heat and mass transfer in the reaction solution.

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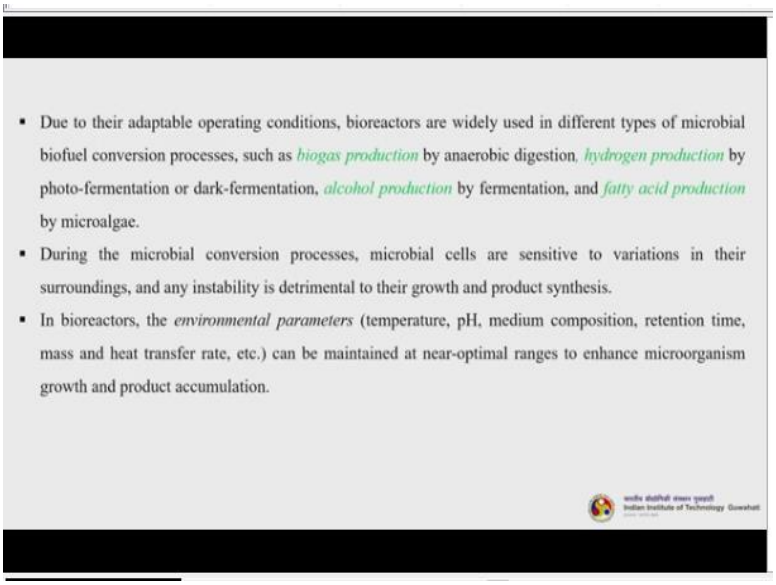

Due to their adaptable operating conditions, bioreactors are widely used in different types of microbial biofuel conversion processes, such as biogas production by anaerobic digestion, hydrogen production by photo-fermentation or dark fermentation, alcohol production by fermentation and fatty acid production by microalgae. During the microbial conversion processes, microbial cells are sensitive to variations in their surroundings and any instability is detrimental to their growth and product synthesis.

In bioreactors the environmental parameters there are many. So, some of these are noted here like temperature, pH, medium composition, retention time, mass and heat transfer rate. So, this can be maintained at near optimal ranges to enhance microorganism growth and product accumulation. So, whenever we are going to start a process using a bioreactor and a particular microorganism, a single strain or a mixed strain and the different types of substrates, you need to optimize the various process parameters which are written here in the last sentence and we have just discussed. So, this optimization is required because at that particular optimized conditions probably will get the highest yield of the product which is your desired product.

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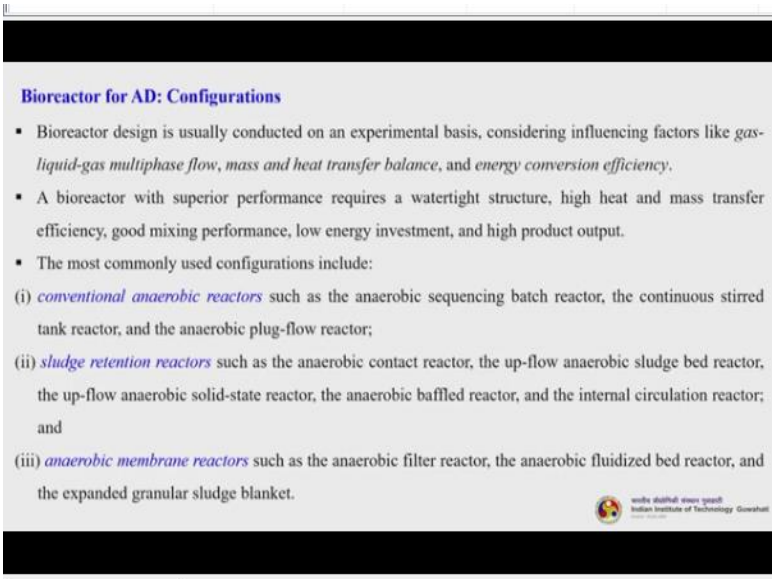

So, let us now discuss about the bioreactors for anaerobic digestion and their configurations. Bioreactor design is usually conducted on an experimental basis considering influencing factors like gas-liquid-gas multiphase flow, mass and heat transfer balance and energy conversion efficiency. A bioreactor with superior performance requires a watertight structure, high heat and mass transfer efficiency, good mixing performance, low energy investment and high product output.

The most commonly used configurations are: convectional anaerobic reactor, such as the anaerobic sequencing batch reactor, the continuous stirred tank reactor and the anaerobic plug flow reactor. Then, in the second category it is the sludge retention reactor, such as anaerobic contact reactor, the up-flow anaerobic sludge bed reactor, the up-flow anaerobic solid state reactor, the anaerobic baffled reactor and the internal circulation reactor.

And in the third category it's an anaerobic membrane reactor, such as the anaerobic filter reactor, the anaerobic fluidized bed reactor and the expanded granular sludge blanket.

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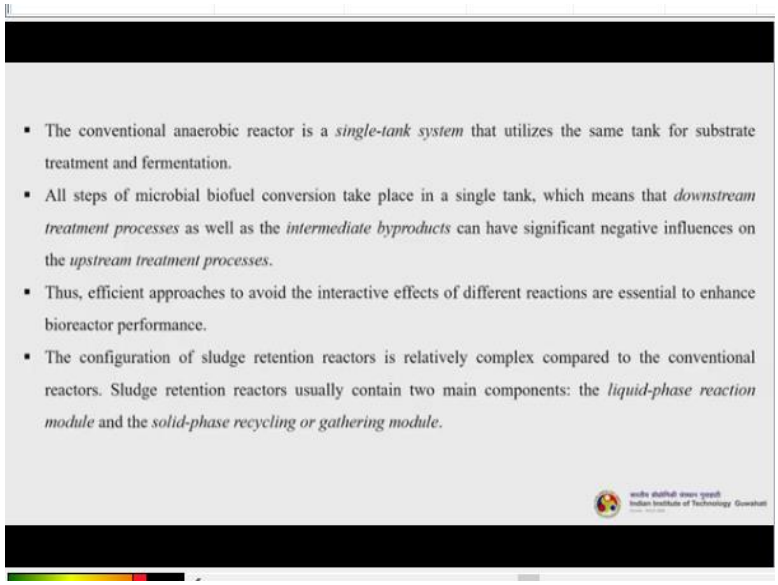

The conventional anaerobic reactor is a single-tank system that utilizes the same tank for substrate treatment and fermentation. It is the single equipment or the single reactor in which all sorts of reactions are happening. All steps of microbial biofuels conversion take place in a single tank, which means that downstream treatment process as well as the intermediate byproducts can have significant negative influences on the upstream treatment processes because in a single reactor it is happening.

So, when there is product formation that the amount of product as well as the inhibitory compounds that form due to certain secondary reactions, they are all retaining in the same reactor. So, it will further inhibit the growth of the microorganism and even stop the further reactions. Thus, efficient approaches to avoid the interactive effects of different reactions are essential to enhance bioreactor performance.

The configuration of sludge retention reactors is relatively complex compared to the conventional reactors, sludge retention reactors usually contain 2 main components, the liquid phase reaction module and the solid phase recycling or gathering module.

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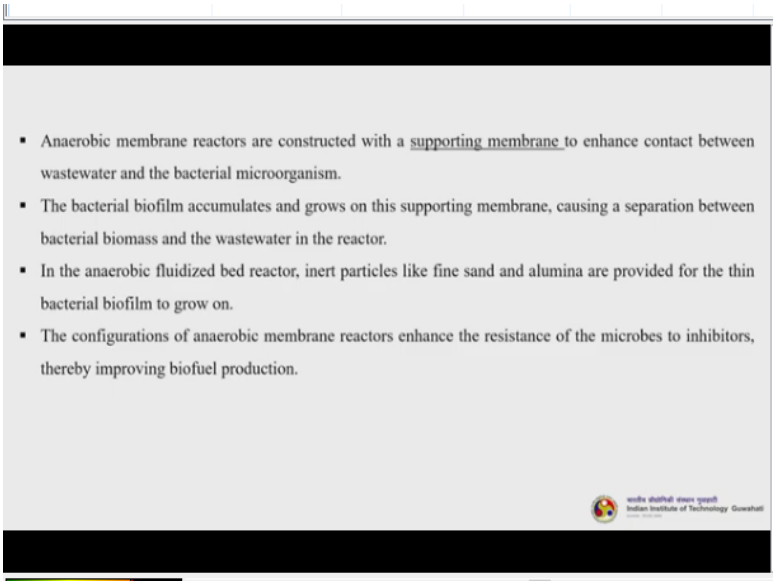

Anaerobic membrane reactors are constructed with a supporting membrane to enhance contact between wastewater and the bacterial microorganism. Now when there is the growth of this bacterial biofilm. So, it grows on the supporting membrane causing a separation between the bacterial biomass and the wastewater in the reactor. So, in the anaerobic fluidized bed reactor inert particles like fine sand and alumina are provided for the thin bacterial biofilm to grow on. The configurations of anaerobic membrane reactor enhance the resistance of the microbes to inhibitors, thereby improving biofuel production.

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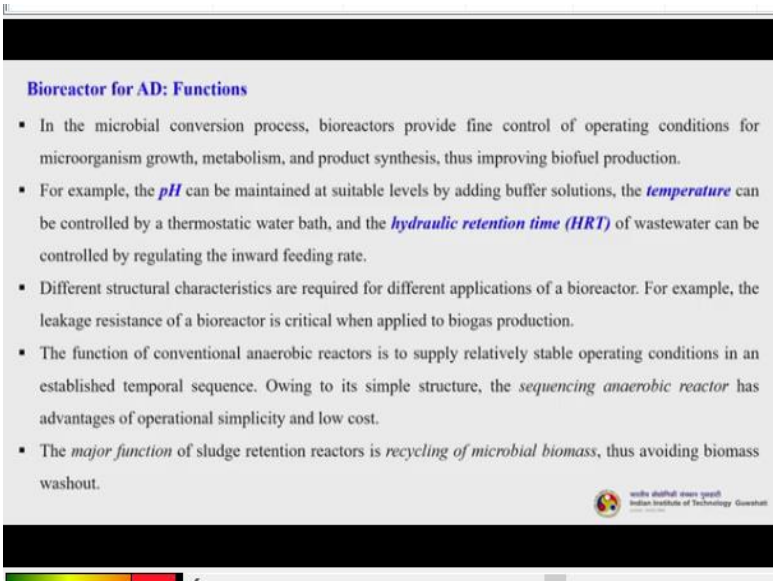

We will see the bioreactor functions. So, in that microbial conversion process bioreactors provide fine control of operating conditions for microorganisms' growth, metabolism and product synthesis thus improving the biofuel production. For example, the pH can be maintained at suitable levels by adding buffer solutions and the temperature can be controlled

by a thermostatic water bath or the hydraulic retention time of wastewater can be controlled by regulating the inward feeding rate.

Now, different structural characteristics are required for different applications of a bioreactor, for example the leakage resistance of a bioreactor is critical when applied to biogas production. The function of conventional anaerobic reactors is to supply relatively stable operating conditions in an established temporal sequence. Owing to its simple structure the sequencing anaerobic reactor has the advantages of operational simplicity and low cost. The major function of sludge retention reactor is the recycling of microbial biomass thus, avoiding biomass washout.

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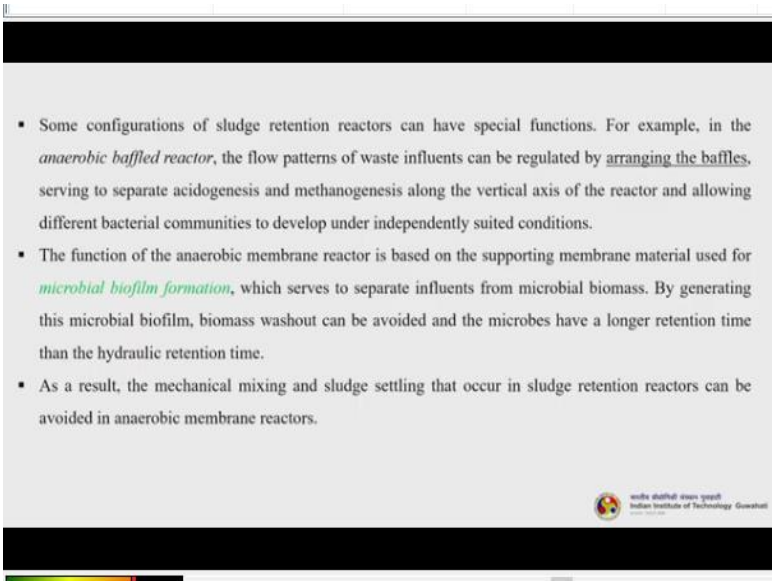

Some configurations of sludge retention reactors can have special functions. For example, in the anaerobic baffled reactor, the flow patterns of waste influents can be regulated by arranging the baffles, serving to separate acidogenesis and methanogenesis along the vertical axis of the reactor and allowing different bacterial communities to develop under independently suited conditions.

The function of the anaerobic membrane reactor is based on the supporting membrane material used for microbial biofilm formation, which serves to separate influents from the microbial biomass. By generating this microbial biofilm biomass washout can be avoided. So, this is one of the greatest advantage of using a solid membranes. And the microbes have a longer retention time than hydraulic retention time.

As a result the mechanical mixing and sludge settling that occur in sludge retention reactors can be avoided in anaerobic membrane reactors. So, these are very important class of membrane reactors.

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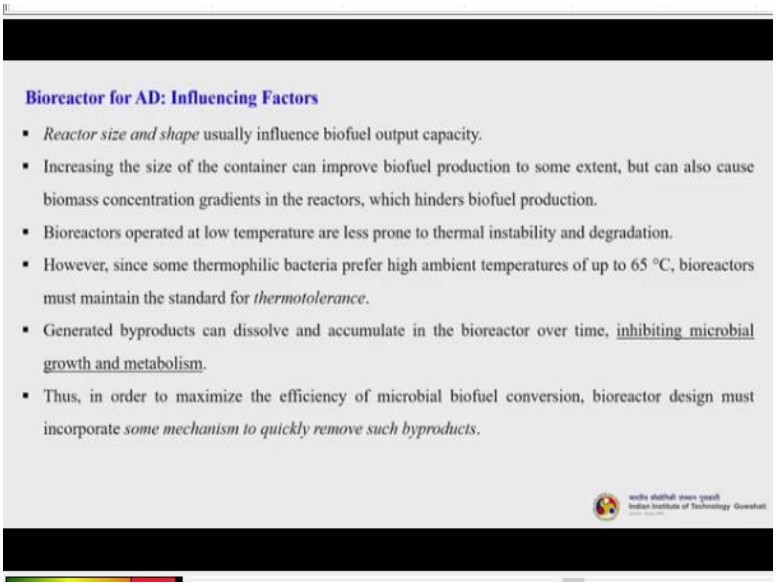

Now, let us understand the influencing factors for the bioreactors for anaerobic digestion. Reactor size and shape usually influence biofuel output capacity increasing the size of the container can improve biofuel production to some extent, but can also cause biomass concentration gradients in the reactors, which further hinders the biofuel production. Bioreactors operated at low temperature are less prone to thermal instability and degradation.

However, since some thermophilic bacteria prefer high ambient temperatures of up to 65 degrees centigrade, bioreactors must maintain the standard of thermotolerance. Generated byproducts can dissolve and accumulate in the bioreactor over time inhibiting microbial growth and metabolism. Thus, in order to maximize the efficiency of microbial biofuel conversion, bioreactor design must incorporate some mechanism to quickly remove such byproducts.

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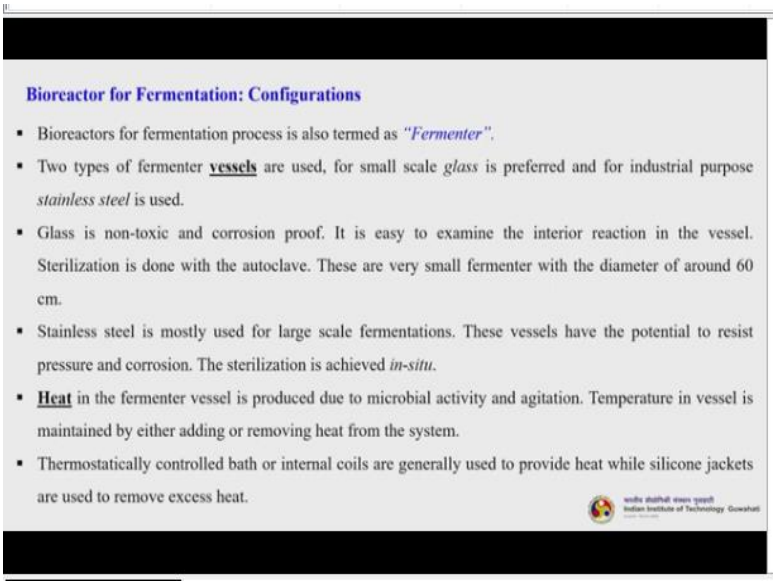

Let us understand the bioreactors for fermentation. So, their configuration. So, bioreactors for fermentation process are also termed as fermenters. Two types of fermenter vessels are used. So, the small scales are usually made up of glass and for the industrial purposes, we use stainless steel. So, glass is non toxic and corrosion proof, it is easy to examine the interior reaction what is happening inside the vessel.

Sterilization is easily done with the autoclaves. So, these are very small fermenters with a diameter of around 60 centimeter. Then stainless steel is mostly used for large scale fermentations, these vessels have the potential to resist pressure and corrosion, the sterilization is achieved in situ. So, heat in the fermenter vessel is produced due to microbial activity and agitation.

Temperature in the vessel is maintained by either adding or removing heat from the system. So, we can have jacketed system. I will show you one of the figure in which we can understand this. So, thermostatically controlled baths or internal coils of generally used to provide heat while silicon jackets are used to remove excess heat.

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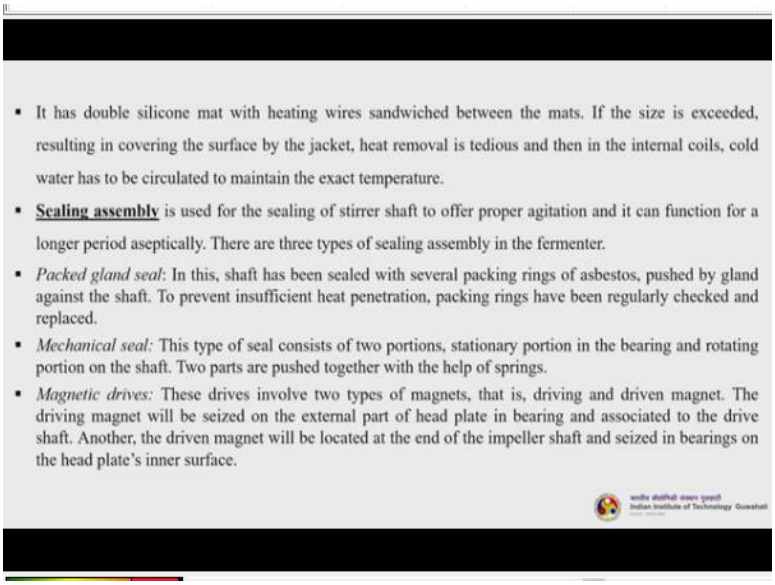

So, it has doubled silicon mat with heating wires sandwiched between the mats. Now, if the size is exceeded, resulting in covering the surface by the jacket heat removal is tedious and then in the internal coils cold water has to be circulated to maintain the exact temperature, it is always easy if you have an outside temperature control. Now, that is possible only when you have a smaller reactors or fermenters.

If we have large reactors or fermenters then some inside internal coiling facility has to be integrated, but that again creates problem for the proper mixing of the fermentation broth. Then next is sealing assembly. So, it is used for sealing of the stirrer shaft to offer proper agitation and it can function for a longer period aseptically. There are 3 types of sealing assembly in the fermenter.

Packed gland seal: so in this the shaft has been sealed with several packing rings of asbestos, pushed by gland against the shaft. To prevent insufficient heat penetration packing rings have been regularly checked and replaced. The second one is mechanical seal and the third one is magnetic drives. So, in the mechanical seal it consists of 2 portion, stationary portion in the bearing and rotating portion for the shaft. Two parts are pushed together with the help of springs. Under the magnetic drives, these are again of 2 types of magnets that is driving and driven magnet, the driving magnet will be seized on the external part of the head plate in bearing and associated to the drive shaft and another that driven magnet will be located at the end of the impeller shaft and seized in the bearings on the head plates' inner surface.

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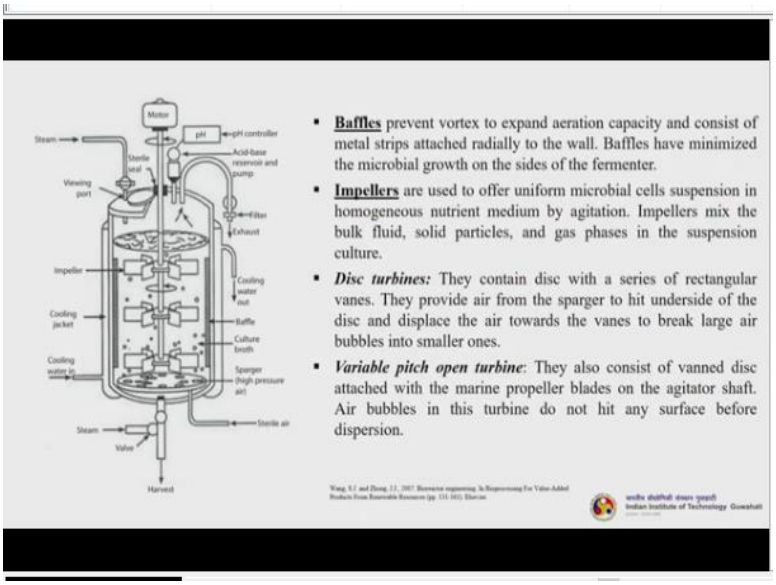

Now, let us see this particular schematic representation of a usual fermenter, it can be a lab scale fermenter, it can be industrial scale fermenter. Let us see. So, you can see that this is the reactor: there is a motor and this is the impeller. This is the impeller; you can see these are the small plates which are there. So, please understand that impellers there are so many different types of impeller designs are available, it is not that only this has to be used, this is a particular design.

Now, what impeller design you will choose that is the job of the engineer or the scientist who are basically designing the fermenters, that based on what type of substrate you are going to use inside the fermenter. So, this is about impeller. So, this whatever you were seeing here, this is an external jacket. So, that job is to remove the heat that is produced inside the fermenter.

So, how do you do that? So you can send in the cooling water - ice cold water and it will take away the excess heat what is being produced in the fermenter and not required and you will get the cooling water out here. So, it will have elevated temperature depending upon what temperature is there. Now, there is a sparger that is provided. Again I am telling you sparger there are so many different types of sparger designs are there.

You can use a single nozzle sparger, you can use 10 perforated whole plates, you can use 100 perforated whole plates or you can have different types of designs. Again you have to decide what is your requirement and whatever you are seeing this is a culture broth then we can have

baffles. Now baffles are not mandated everywhere, the necessity arises that if baffles are there the mixing will be good inside this.

So, impeller will be there, it is very slow, it will move very slowly. But if it moves too slow then the microorganisms will start depositing on the surface of the impeller plates. So, that is also not correct. So, there are so many other things are there you can see that steam can be put here. So, that the reason the steam is required to sterilize one particular batch is over, then you need to sterilize it in the big systems.

Or if it is a small fermenter that glass type, you can take it out remove the heads shaft, motor and everything and all accessories, take it and put it in autoclave where we can go for sterilization there. So, baffles are there, impellers, disc turbines, variable pitch open turbine. I have already told what is the job of baffles and impellers. So, let us move ahead.

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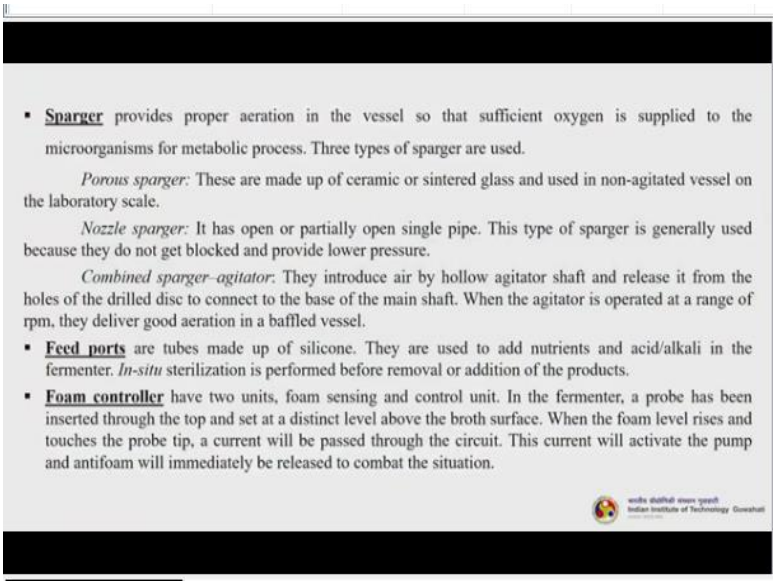

So, sparger provides proper aeration in the vessels so that sufficient oxygen is supplied to the microorganisms for metabolic process. Three types of spargers are used porous spargers, nozzle spargers and combined spargers and agitator. In the porous spargers these are made up of ceramic or sintered glass and used in non-agitated vessel on the laboratory scale. Nozzle sparger has opened or partially open single pipe.

Now, this type of sparger is generally used because they do not get blocked and provide lower pressure. So, in case of combined sparger and agitator they introduce air by hollow agitator shaft and release it from the holes of the drill disc to connect to the base of the main

shaft. When the agitator is operated at the range of RPM, the deliver good aeration in a baffled vessel.

Then there are feed ports, which are tubes made up of silicon. They are used to add nutrients and acid/alkali in the fermenter, in situ sterilization is performed before removal or addition of the product. Then, another very important thing for the fermenters are foam controller. Now they have 2 units foam sensing and control unit. In the fermenter a probe has been inserted through the top and set at a distinct level above the broth surface.

Now when the form level rises and touches this probe tip a current will be passed through the circuit. So, this current will activate the pump and antifoam will immediately be released to combat that situation, because foaming is not beneficial for the fermentation.

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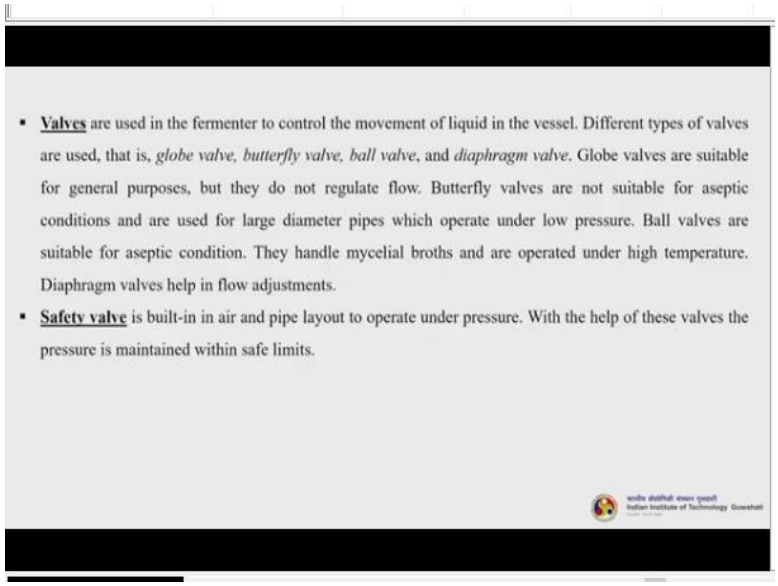

Then different types of valves are used in the fermenter to control the movement of liquid in the vessel, like globe valve, butterfly valve, ball valve and diaphragm value. Now globe valves are suitable for general purposes but they do not regulate flow. Butterfly valves are not suitable for aseptic conditions and are used for large diameter pipes which operate under low pressure. Ball valves are essentially suitable for aseptic conditions. They handle mycelial broths and are operated under high temperature. Diaphragm valves help in flow adjustment. Then apart from that we have safety valves. So, they are built-in in air and pipe layout to operate under pressure. With the help of these valves the pressure is maintained within the safe limits.

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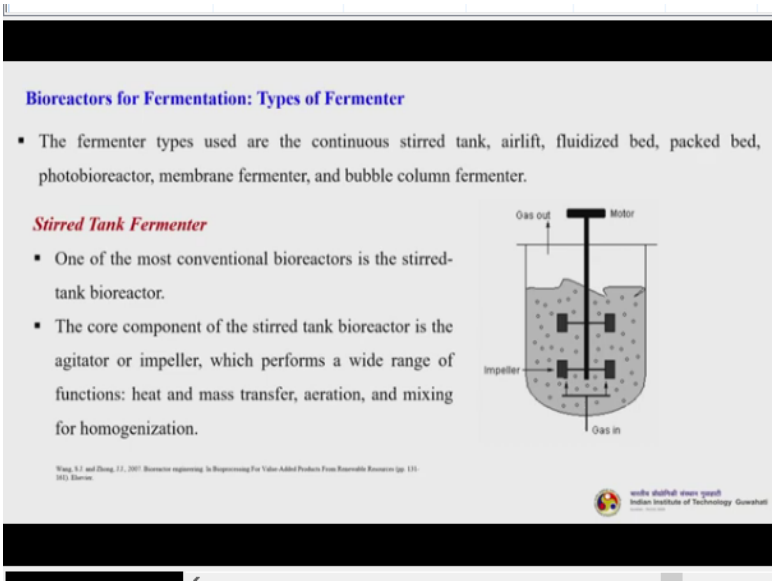

Now let us discuss about the types of fermenters. We will quickly discuss about the basics of the few fermenters which are essentially adapted in the lab scale as well as commercial scale. There are many types. So, the first one is the continuous stirred tank reactor, then we have airlift reactor, we have fluidized bed reactor, we have packed bed reactors, we have photobioreactors, membrane fermenters and bubble column fermemter.

So, we will see quickly all these reactors in a glance. So, the first one you can see the image. So, that is the stirred tank fermenter the simplest one. So, one of the most conventional bioreactors is the stirred tank bioreactor used in the lab scale as well as in the commercial scale also. The core component of the stirred tank bioreactor is the agitator or impeller which performs a wide range of functions.

So, it does the heat and mass transfer functions that means it helps in enhancing the heat and mass transfer rate, it does aeration also and it does mixing of the fermentation broth.

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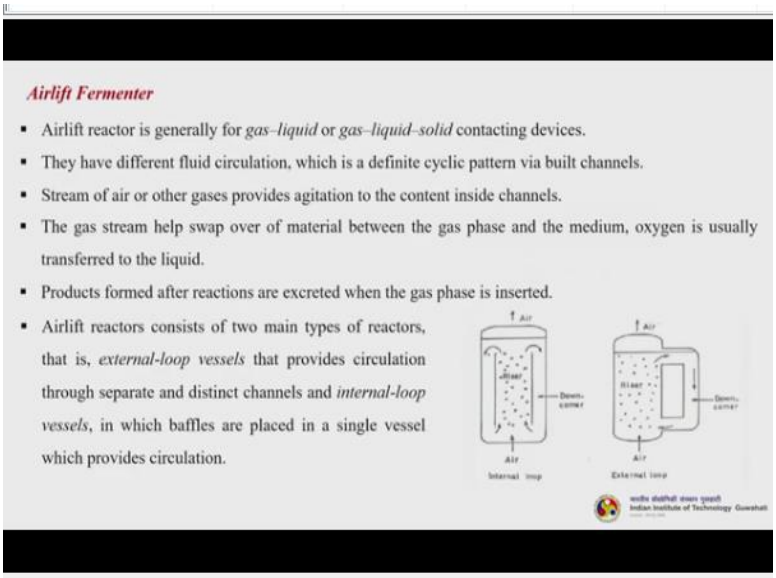

Second one is airlift fermenters. So, these are classic fermenters and are being used in industrial scale as well in the lab scale also. So, airlift reactor is generally for the gas liquid or gas liquid solid contacting devices. They have different fluid circulation, which is a definite cyclic pattern via built channels. Stream of air or other gases provides agitation to the content inside channels.

The gas stream helps swap over the material between the gas phase and the medium, oxygen is usually transferred to the liquid. Products formed after the reactions are excreted when the gas phase is inserted. Two types of airlift reactors are there: one is called the internal loop you can see the first image and then there is a second one which is called external loop. Now in the internal loop and in both the systems we have there are 2 things.

First one is called riser column, another is called downcomer. See this riser and downcomer differentiation you can easily understand in the external loop reactor where the downcomer is outside the main reactor. And here it is inside. So, you can you can see 2 pipes, big pipes. One big diameter pipe inside that a small diameter pipe if it is placed like that.

So, the inside one will be the riser through which the flow is basically happening like this and then it is coming. It is a circulating flow like this. And when it is in outside that is the external loop airlift reactor, most of the reactions are happening in the riser section and this is what helps in a proper heat and mass transfer rate as well as to maintain the microorganism growth. And some other activities like that you can talk about the HRT, OLR and all these things.

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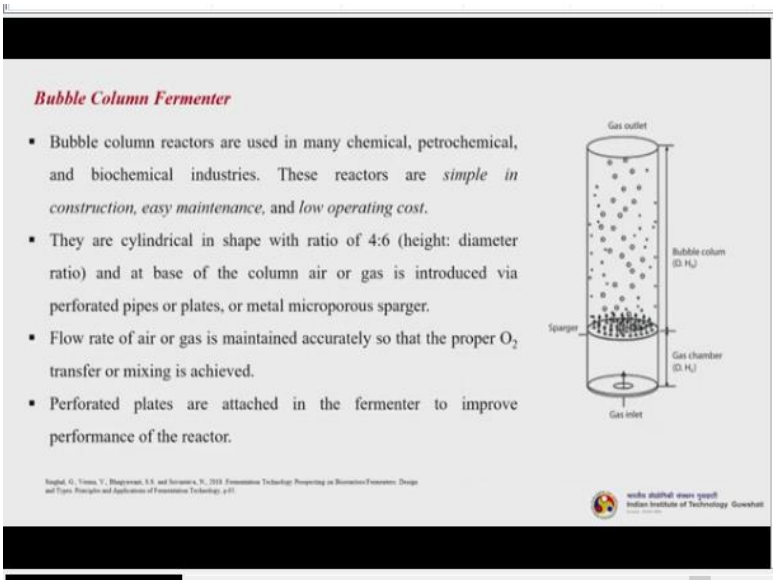

So, the next is bubble column fermenter. So, these are very classical reactors, which are used in many chemical, petrochemical and biochemical industries. Now, these reactors are simple in construction, they are easy to maintain and they have low operating costs. So, these are cylindrical in shape with a ratio of 4 is to 6 : height to diameter ratio basically and at the base of the column air or gas is introduced via perforated pipes or plates or metal microporous sparger.

So, you can see that, so, this is a column it is a big column, it can be made up of a glass, it can be made up of perspex, it can be any other material also. So, here there is a sparger that is provided – multiperforated/multiple hole sparger you can see, you can use different types of sparger also. So, gas is being passed through like this and through this particular sparger the gas will be pushed through and when it will come in contact with the fermentation broth, it will result in small, small bubbles.

Now, the concept in the entire the bubble column is that how the bubbles are getting created. The size that matters, and how they are getting coalesced with each other because bubbles has a tendency to coalesce with each other, so they will form a small to big bubbles. Now these big bubbles under agitation, mechanical agitation or any sorts of agitation inside the fermentation broth will again be ruptured into smaller bubbles.

So, what is the idea is that it is a continuous generation of bubbles, coalescence and again break down into smaller bubbles will create high mass transfer area. So, that is the basic

concept in the bubble column reactor. So, flow rate of air or gas is maintained accurately so that the proper oxygen transfer or mixing is achieved. Perforated plates are attached in the fermenter to improve the performance of the reactor.

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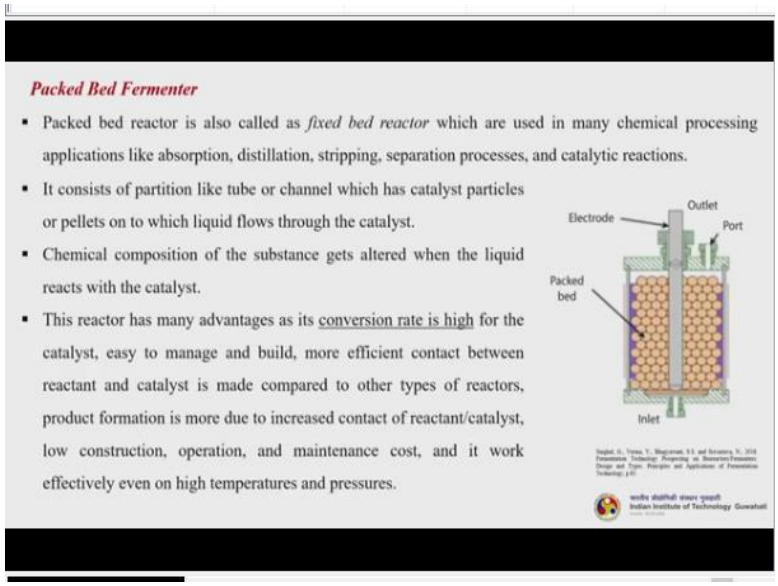

So, the next is packed bed fermenter. Now, packed bed fermenter reactors are also called as fixed bed reactors, which are used in many chemical processing applications like absorption, distillation, stripping, separation processes and catalytic reactions. It consists of partition like tube or channel which has catalytic particles of pellets on to which liquid flows through the catalyst.

Chemical composition of the substance gets altered when the liquid reacts with the catalyst. This reactor has many advantages as its conversion rate is high for the catalyst, easy to manage and build, more efficient contact between reactant and catalyst is made compared to other types of reactors, product formation is more due to the increased contact of reactant/catalyst. Please understand, if this is only one of the reactor in which there is intimate contact between the reactant and the catalyst.

So, the entire amount of the catalytic surface will be covered with the reactants, so, that there will be more product formation. So, these reactors work effectively even on high temperatures and pressures.

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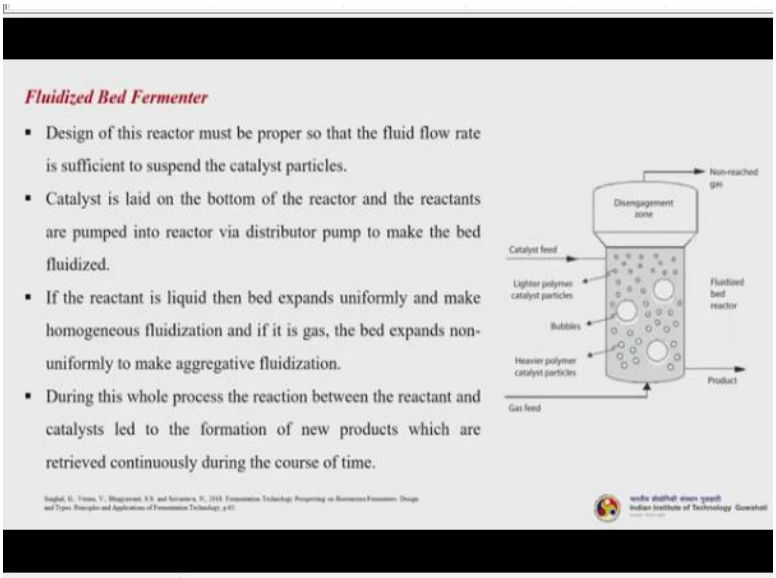

So, the next is another class of fermenters which are also industrially used and adapted. So, are called fluidized bed fermenters. Now, design of the reactor must be proper so that fluid flow rate is sufficient to suspend the catalyst particles. So, catalyst is laid on the bottom of the reactor and the reactants are pumped into reactor via distributor pump to make the bed fluidized.

If the reactant is liquid then bed expands uniformly and make homogeneous fluidization and if it is gas then bed expands non uniformally to make aggregative fluidization. During this whole process the reaction between the reactant and catalyst led to the formation of new products which are retrieved continuously during the course of time.

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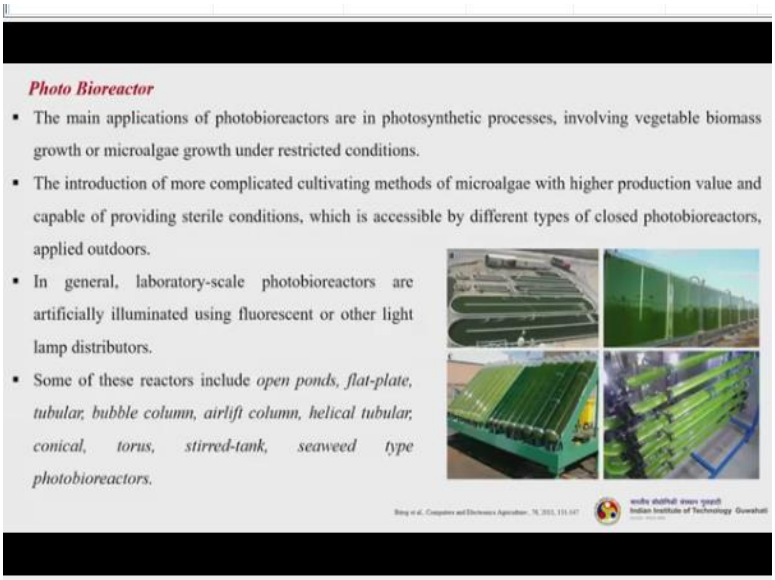

So, the next is photobioreactors. So, the main application of photobioreactors are in photosynthetic processes involving vegetable biomass growth or microalgae growth under restricted conditions. Now, the introduction of more complicated cultivating methods of microalgae with higher production value and capable of providing sterile conditions which is accessible by different types of close photobioreactors applied outdoors.

So, here whatever you are seeing now. So, in general, if you talk about the laboratory scale photobioreactors they are artificially illuminated because we need to provide light for the photosynthesis. So, here whatever you are seeing, so, this is an image of a (open/outdoor) raceway pond. These raceway ponds can also be constructed in-house where we can supply this one artificial lighting.

But this is an open to atmosphere and open to sunlight raceway pond. Now, these are plate type algal photobioreactor, these are tubular photobioreactors. Now, here this, this and this, these 3 are closed systems. So, as I told you that during this discussion this closed systems are always good because we can easily control the different process parameters inside a closed system and they are not susceptible to any other infectious bacteria, virus or this such environmental problems.

Whereas this type of raceway pond are always susceptible to environmental conditions - sometimes rains, let us say the rain happens it will immediately increase the amount of the broth inside the reactor. So, everything gets diluted, which is not happening here and here and here. Now, this is easy to maintain, the cost is low. However, these are initially very costly processes, but once you have this, so then you can maintain it easily and control it easily. So, that the yield will be better.

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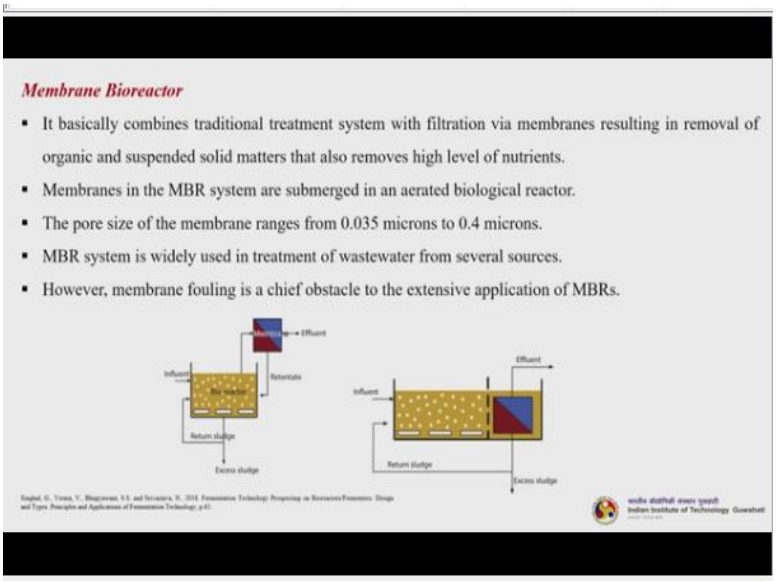

So, next is these are latest development in the environmental and energy sites actually the membrane bioreactors. So, it basically combines traditional treatment with filtration via membranes, resulting in the removal of organic and suspended solid matters that are also removes high level of nutrients. Now, membrane bioreactor systems are submerged in an aerated biological reactor. The pore size of the membrane ranges from 0.035 microns to 0.4 microns.

Membrane bioreactor system is widely used in treatment of wastewater from several sources. However, membrane fouling is a chief obstacle to the extensive application of membranes. So, you can see here 2 different images are there. So, here what is happening the membrane is outside. So, this is the bioreactor. So, membrane is used as separate. This is a 2 different unit operations; bioreactors, here everything is happening all the reactions, product formation, then you need to remove product and other value added products or inhibitory compounds use a membrane reactor. So, it will remove the effluent and the retentate can be recycled. This is one system.

In another system where we have this activated sludge ponds and such type of units the membrane bioreactors can be directly placed inside the aerobic ponds. So, it has its own advantages and disadvantages, this has its own advantages disadvantages. This is easy to control. Here the clogging and concentration polarization can be an issue, if we are putting it directly inside - because microorganisms will start growing on the surface of the membrane. So, there are issues into that but both are used.

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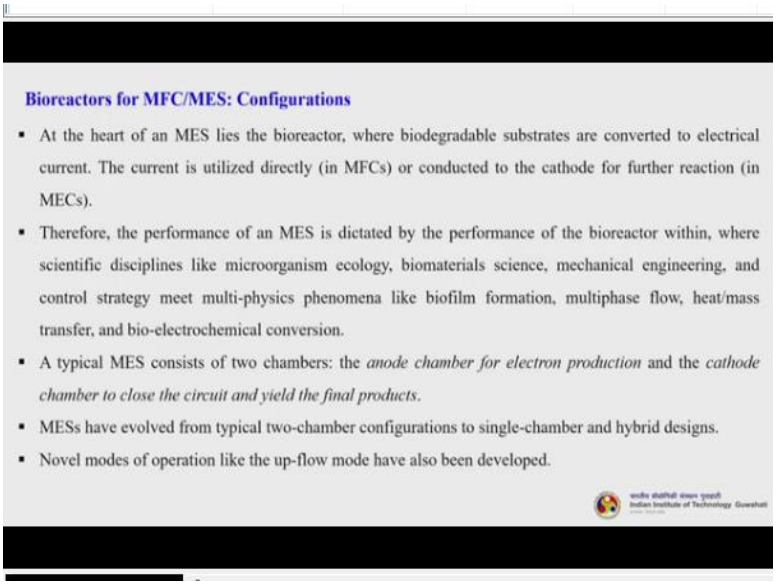

So, then we will talk about the bioreactors for microbial fuel cell and microbial electrolysis systems their configurations. At the heart of an MES lies the bioreactor where biodegradable substrates are converted to electrical current. The current is utilized directly in the MFCs or conducted to the cathode for further reaction in case of the MECs.

Now, therefore, the performance of an MES is dictated by the performance of the bioreactor within where scientific disciplines like microorganism ecology, biomaterial science, mechanical engineering and control strategy meet multiphysics phenomena like biofilm formation, multiphase flow, heat and mass transfer and bioelectrochemical conversion complicated systems.

So, many different types of parameters needs to be controlled and taken care of. A typical MES consists of 2 chambers the anode chamber for electron production and the cathode chamber to close the circuit and the yield of the final products. MES have evolved from typical 2 chamber configurations to single chamber and hybrid designs. Novel modes of operation like the up-flow mode have also been developed.

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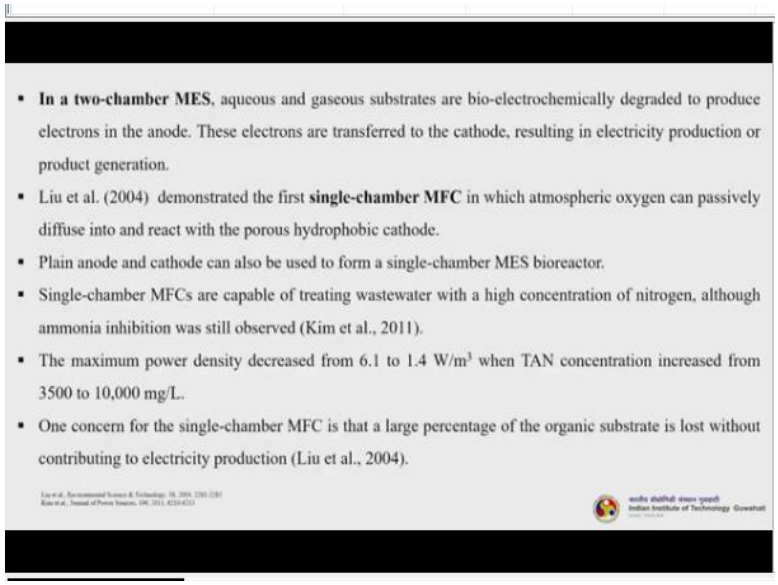

In a 2 chamber MES aqueous and gaseous substrates are bioelectrochemically degraded to produce electrons in the anode, these electrons are transferred to the cathode resulting in electricity production or product generation. Liu et al demonstrated the first single chamber MFC in which atmospheric oxygen can passively diffuse into and react with the porous hydrophobic cathode.

Plain anode and cathode can also be used to form a single chamber MES bioreactor. Single chamber MFCs are capable of treating wastewater with a high concentration of nitrogen, although ammonia inhibition was still observed. So, ammonia whatever is getting produced, if it is again produced a certain amount which is beyond a tolerable amount then it will suppress all the metabolic activities.

So, the maximum power density decreased from 6.1 to 1.4 watts per meter cube when TAN concentration increased from 3500 to 10,000 milligrams per liter**.** So, TAN is the total ammonia nitrogen. So, one concern for the single chamber MFC is that a large percentage of the organic substrate is lost without contributing to the electricity production.

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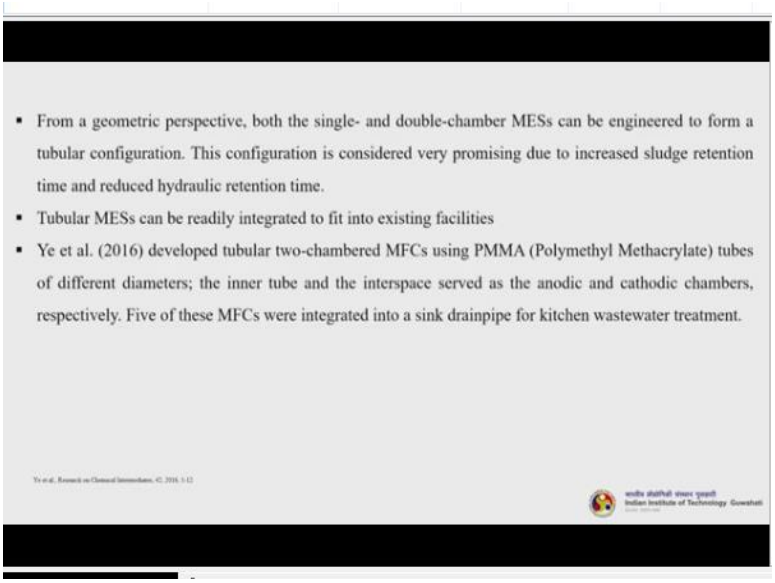

From a geometric perspective, both the single and double-chamber MESs can be engineered to form a tubular configuration. Now, this configuration is considered very promising due to increased sludge rentention time and reduced hydraulic retention time. Tubular MESs can be readily integrated to fit into existing facilities. Ye et al developed tubular 2 chamber MFCs using PMMA (polymethyl Methacrylate) tubes of different diameters; the inner tube and interspace served as the anode and cathodic chambers (respectively).

Five of these MFCs were integrated into a sink drainpipe for kitchen wastewater treatments. It is a very nice work. I have referred it down. Please read if you wish to read and learn more on this particular technology.

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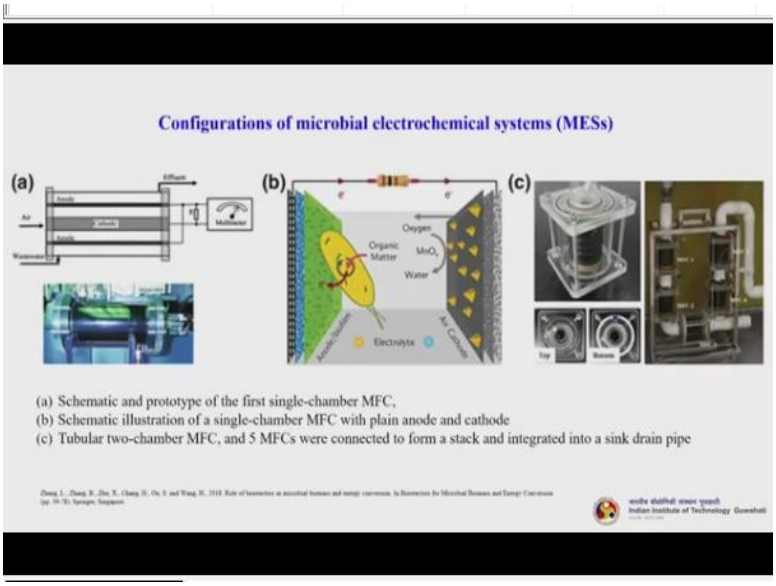

So, these are the configurations of the microbial electrolytic systems. So, the first one whatever you are seeing, this this schematic and prototype of the first single chamber MFC**.** So, it is a very simple system. And the second one, where it says a single chamber MFC with a plain anode and a cathode. So, this is the cathode here, Air cathode this is the anode here, you can see the organic matter and microorganisms start depositing on the surface of the anode.

In the third one so, it is a tublar 2 chamber MFC. Here in this case the 5 MFCs were connected to form a stack and integrated into a sink drain pipe. You can set the cascading basically or multistging.

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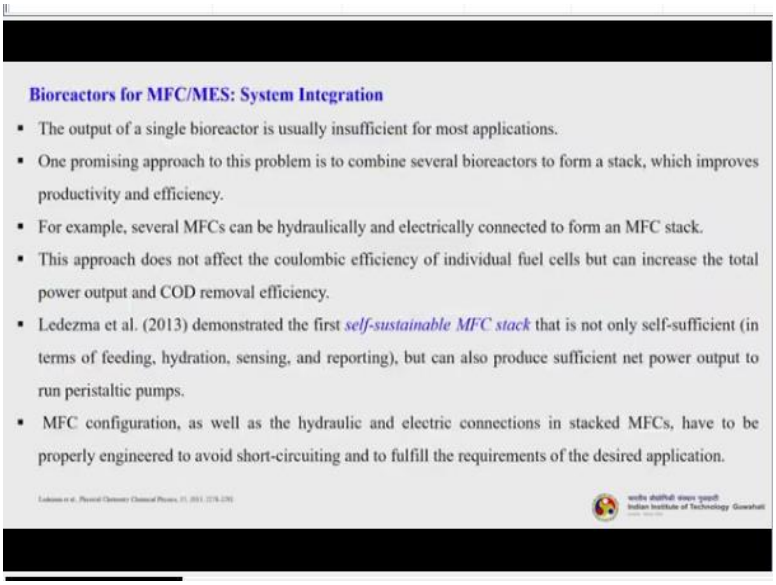

Now, let us understand the system integration of the bioreactors for MFCs and MES. So, the output of a single bioreactor is usually insufficient for most of the applications. One promising approach to this problem is to combine several bioreactors to form a stack, which improves productivity and efficiency. For example, several MFCs can be hydraulically and electrically connected to form an MFC stack.

This approach does not affect the columbic efficiency of individual fuel cells, but can increase the total power output and COD removal efficiency. Ledezma et al demonstrated the first self sustained MFC stack that is not only self sufficient in terms of feeding, hydration, sensing and reporting, but can also produce sufficient net power output to run peristaltic pumps.

So, peristaltic pumps are required to feed different types of materials, whether it can be your substrate, whether it can be different types of nutrients, even supplying buffers also. MFC configuration as well as the hydraulic and electric connections in stacked MFCs have to be properly engineered to avoid short circuiting and to fulfill the requirements of the desired application.

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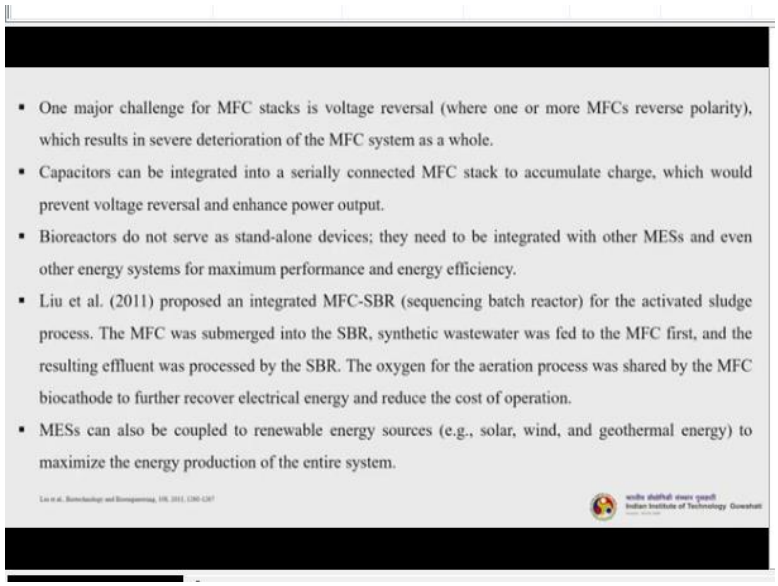

One major challenge for MFC stacks is voltage reversal. When one or more MFCs reverse polarity. So, this results in severe deterioration of the MFC system as a whole. Capacitors can be integrated into a serially connected MFC stack to accumulate charge, which should prevent voltage reversal and enhance power output. So, bioreactors do not serve as stand-alone devices, they need to be integrated with other MES and even other energy systems for maximum performance and energy efficiency.

In a classical study Liu et al proposed an integrated MFC-SBR (SBR is sequencing batch reactor) for the activated sludge process. The MFC was submerged inside this SBR, synthetic wastewater was fed to the MFC first and the resulting effluent was processed by the SBR. The oxygen for the aeration process was shared by the MFC biocathode to further recover electrical energy and reduce the cost of operation.

MESs can also be coupled to renewable energy sources like solar, wind and geothermal energy to maximize the energy production of the entire system.

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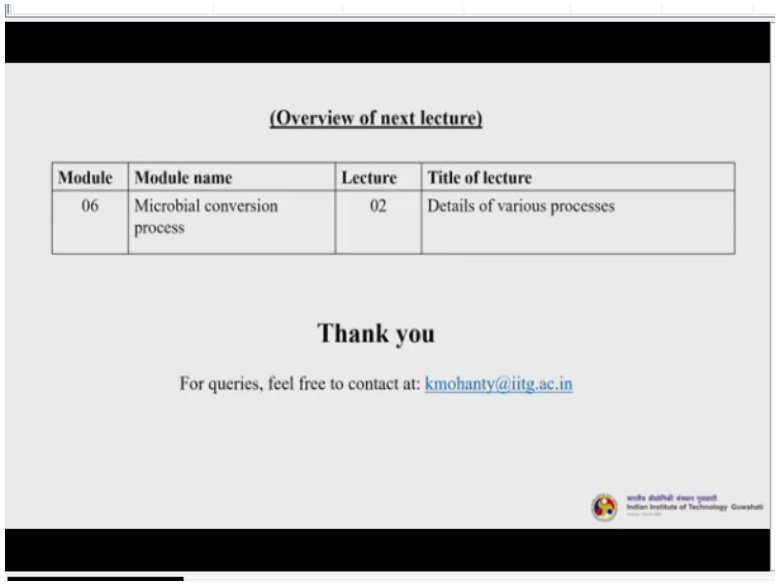

With this I conclude today's lecture. In our next lecture, we will be discussing about the details of the various microbial conversion processes. So, if you have any query regarding this lecture, please feel free to post your query in Swayam portal or drop a mail to me at kmohanty@iitg.ac.in. Thank you.