Biomass Conversion and Biorefinery
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Lecture – 28
Biohydrogen Production, Metabolics, Microorganisms

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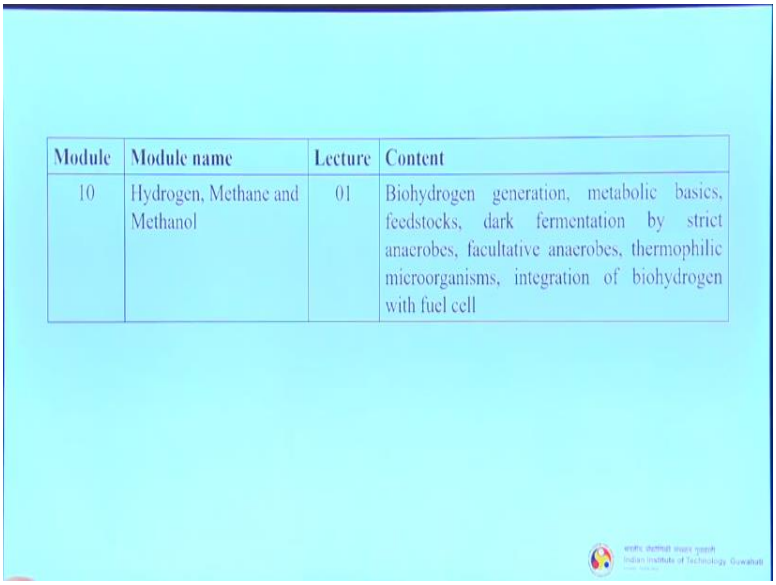

Good morning students. We are starting lecture 1 under module 10 today. So this module is dedicated to hydrogen, methane and methanol. So, three most important value added products from the fermentation and even other processes also. And today we will be discussing exclusively on biohydrogen generation, its metabolic pathways, basics and what are the different types of microorganisms that carry out this biohydrogen production. And we will also learn about facultative anaerobes, thermophilic microorganisms and microbial fuel cell. So, let us begin.

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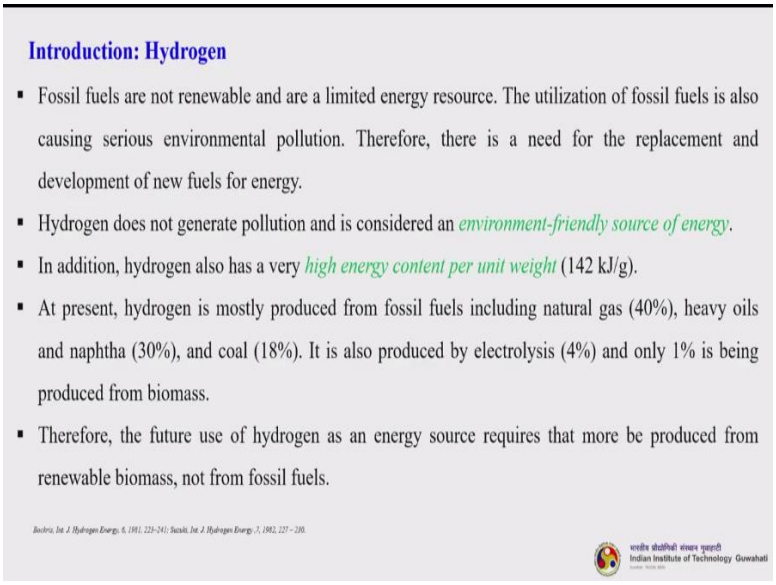

Fossil fuels are not renewable and are a limited energy source. The utilization of fossil fuels is also causing serious environmental pollution. Therefore, there is a need for the replacement and development of new fuels for energy. Hydrogen does not generate pollution and is considered as an environment friendly source of energy. In addition, hydrogen also has a very high energy content per unit weight.

So, almost 142 kilojoules per gram. At present, hydrogen is mostly produced from fossil fuels including natural gas, heavy oil sand naphtha as well as coal also. Now, it is also produced by electrolysis of water and only 1% is being currently produced from the biomass. Therefore, the future use of hydrogen as an energy source requires that more be produced from renewable biomass, not from the fossil fuels.

So most of the research is now concentrated on development of biomass based hydrogen production and its further utilization.

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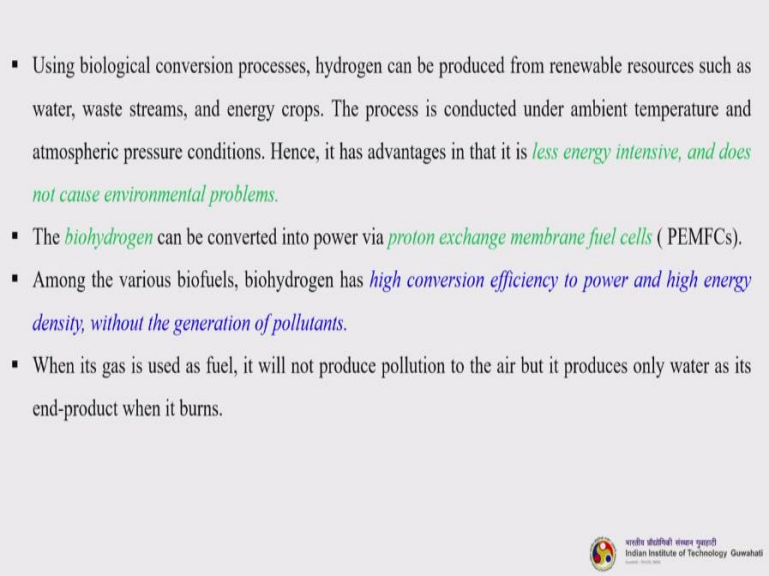

Using biological conversion processes, hydrogen can be produced from renewable sources such as water, waste streams and energy crops. The process is conducted under ambient temperature and atmospheric pressure conditions. Hence, it has the advantage that it is less energy intensive and it does not cause environmental problems. The biohydrogen can be converted into power via proton exchange membrane fuel cell.

Among the various biofuels biohydrogen has a high conversion efficiency to power and high energy density and without the generation of pollutants. So when its gas is used as fuel, it will not produce pollution to the air but it produces only water as its end product when it burns.

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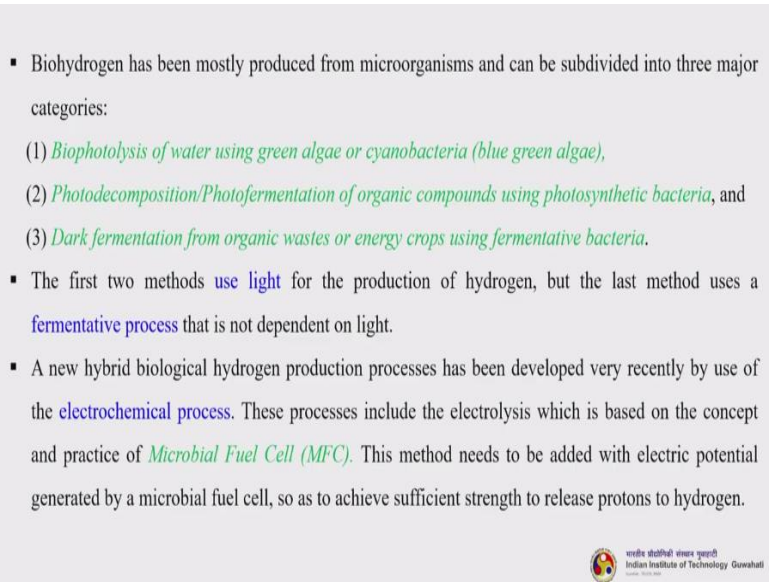

So biohydrogen has been mostly produced from microorganisms and can be subdivided into 3 major categories. So biophotolysis of water using green algae or cyanobacteria, which are

also known as blue green algae. Photodecomposition or photofermentation of organic compounds using photosynthetic bacteria and dark fermentation from organic waste or energy crops using fermentative bacteria.

Now, the first 2 methods use light for the production of hydrogen, but the last method uses the fermentative process that is not dependent on light. A new hybrid biological hydrogen production process has been developed very recently by use of the electrochemical process. Now these processes include the electrolysis, which is based on the concept and practice of microbial fuel cell. This method needs to be added with electrical potential generated by a microbial fuel cell so as to achieve sufficient strength to release protons to hydrogen.

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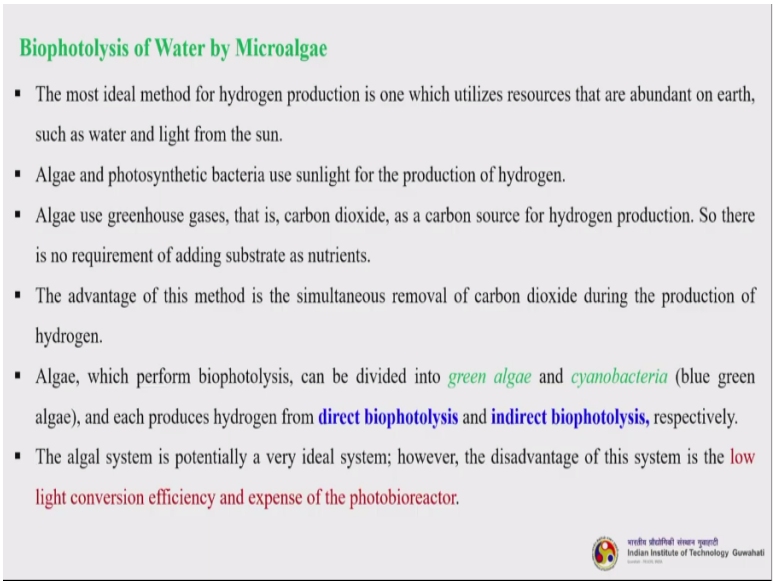

So, we will discuss one by one these 3 processes. The first is the biophotolysis of water by microalgae. The most ideal method for hydrogen production is one which utilizes resources that are abundant on earth such as water and light from the sun. Algae and photosynthetic bacteria use sunlight for the production of hydrogen. Algae use greenhouse gases that is carbon dioxide as the carbon source for hydrogen production.

So there is no requirement of adding substrate as nutrients. However, you may need to give some substrate or supply nutrients and micronutrients for its growth. Now, the advantage of this method is the simultaneous removal of carbon dioxide during the production of hydrogen. Algae which perform biophotolysis can be divided into green algae and cyanobacteria.

And each produces hydrogen from direct biophotolysis and indirect biophotolysis respectively. The algal system is potentially a very ideal system. However, the disadvantage of this system is the low light conversion efficiency and the expense of the photobioreactor.

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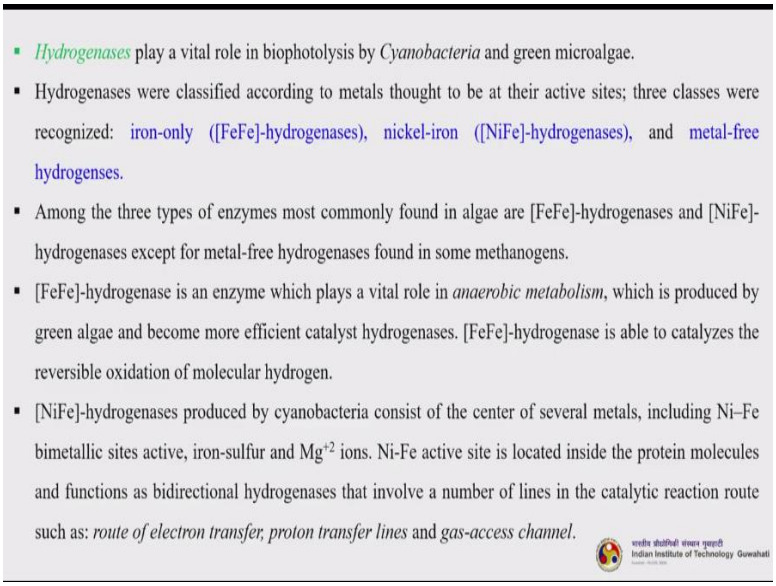

Hydrogenases plays a vital role in biophotolysis of cyanobacteria and green microalgae. Hydrogenases are the enzymes responsible to carry out hydrogen production. They were classified according to the metals thought to be at their active sites. So 3 classes of hydrogenases were recognized. The first is the iron only - iron-iron hydrogenases, then the nickel-iron hydrogenases and then metal-free hydrogenases.

Among the 3 types of enzymes most commonly found in algae are the iron hydrogenases and nickel-iron hydrogenases except for the metal-free hydrogenases found in some of the methanogens. Iron-iron hydrogenase is an enzyme which plays a vital role in anaerobic metabolism which is produced by green algae and become more efficient catalyst hydrogenases. Iron-iron hydrogenase is able to catalyze the reversible oxidation of the molecular hydrogen.

Now, coming to the nickel-iron hydrogenase; produced by the cyanobacteria consist of the center of several metals that includes nickel and iron bimetallic sites active, iron-sulfur and magnesium ions. Now nickel-iron active site is located inside the protein molecules and functions as the bidirectional hydrogenases that involve a number of lines in the catalytic reaction route such as route of the electron transfer, proton transfer lines as well as the gas-access channel.

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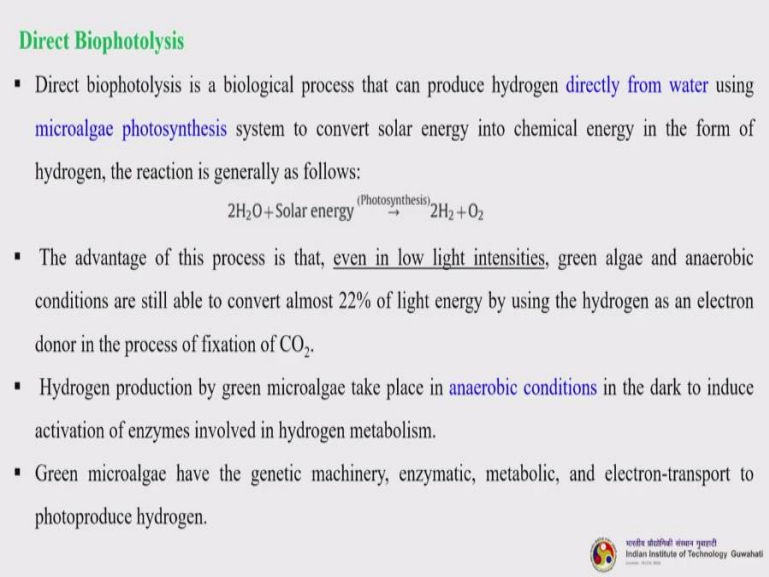

Now we will discuss about the direct biophotolysis. Direct biophotolysis is a biological process that can produce hydrogen directly from water using microalgae photosynthesis system to convert solar energy into chemical energy in the form of hydrogen. The reaction is given as two water plus solar energy under photosynthesis process will produce 2 molecules of hydrogen and oxygen.

$$2H_2O + \text{Solar energy} \xrightarrow{\text{(Photosynthesis)}} 2H_2 + O_2$$

The advantage of this process is that even in low light intensities, green algae and anaerobic conditions are still able to convert almost 22% of the light energy by using the hydrogen as an electron donor in the process of fixation of carbon dioxide. Hydrogen production by green microalgae takes place in anaerobic conditions in the dark to induce activation of enzymes involved in the hydrogen metabolism. Green microalgae have the genetic machinery, enzymatic, metabolic and electron transport to photoproduce hydrogen.

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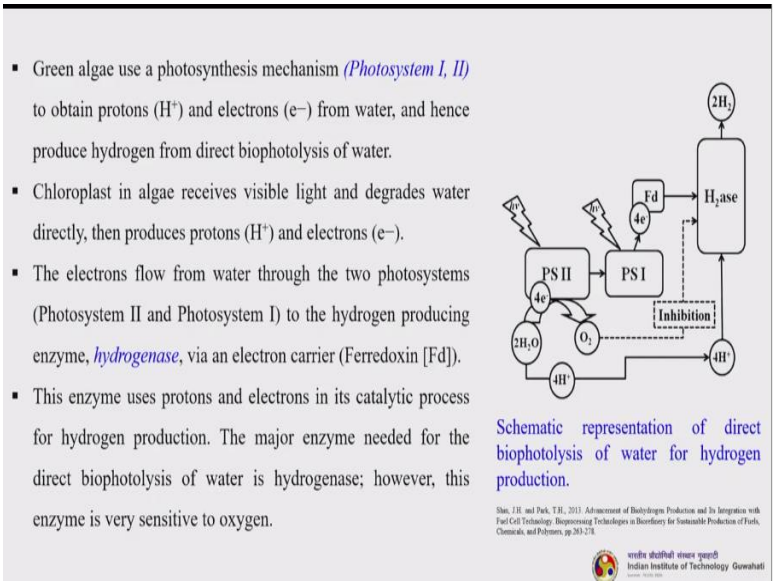

So, you can see this is the schematic representation of the direct biophotolysis of water for hydrogen production. I will explain this. So, green algae uses a photosynthetic mechanism. So, it can be photosystem 1 and photosystem 2 both basically to obtain protons and electrons from water and hence produce hydrogen from direct biophotolysis of water. You can read little more about photosystem 1 and photosystem 2 in a nutshell, I will just tell you that.

Photosystem 1 and photosystem 2, there are so many differences. The basic difference is that what type of light energy they are absorbing. So photosystem 1 basically absorbs higher wavelength or longer wavelength lights around 700 nanometer whereas photosystem 2 absorbs shorter wavelength around 630 nanometers a little less than that. And another differentiation is that photosystem 2 actually generates the electron e^- directly from the water.

Whereas photosystem 2 gets its electron from the process of the photosystem 2. So, chloroplast in algae receives visible light and degrades water directly, then produces protons and electrons. This is happening in the PS 2. Now, the electrons flow from the water through the two photosystem, PS 1 and PS 2 to the hydrogen producing enzyme, here in this case is hydrogenase, via an electron carrier (here it is ferredoxin) which is denoted by Fd, you can see that here. So, here from the water we get the electrons. Now, this electron is being used for the hydrogenase also and even the PS 1 also gets the e minus from the PS 2. And with the help of ferredoxin the hydrogenase is able to produce hydrogen. Now, this enzyme uses protons and electrons in its catalytic process for hydrogen production. The major enzyme needed for that direct biophotolysis of water is hydrogenase. However, this enzyme is very sensitive to oxygen.

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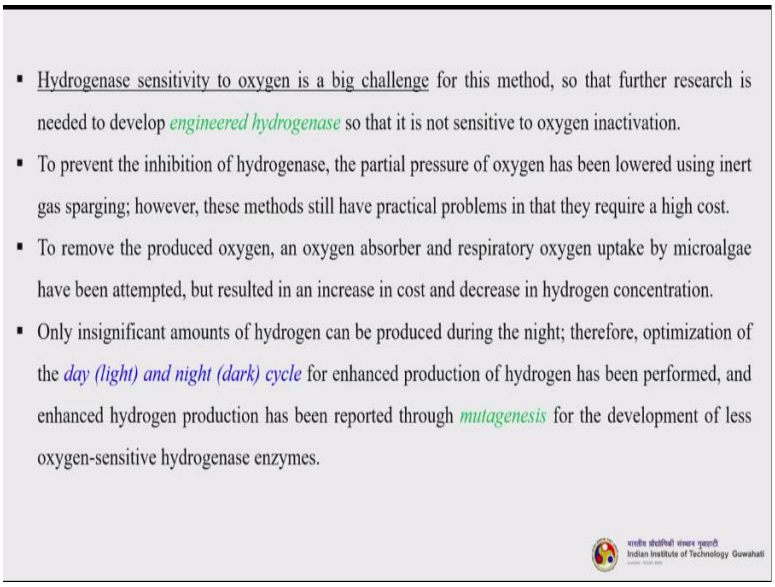

Now, hydrogenase sensitivity to oxygen is a big challenge for this method, so that further research is needed to develop engineered hydrogenase so that it is not sensitive to oxygen inactivation. Since oxygen is extremely sensitive for the hydrogenase mechanism, whatever oxygen is getting produced has to be scrapped out. Having said that it is not so easy to do that.

So to prevent the inhibition of hydrogenase the partial pressure of oxygen has been lowered using inert gas sparging; however, these methods still have practical problems in that they require a high cost. You are additionally making some mechanical enrichment of sparging gas, so of course you need pump or tank, sparger and all these things. So, you are adding cost to the entire process.

Now, to remove the produced oxygen, an oxygen absorber and respiratory oxygen uptake by microalgae have been attempted, but resulted again in an increase in the cost and decrease in the hydrogen concentration, not very effective. Now, only insignificant amounts of hydrogen can be produced during the night. Therefore optimization of the daylight and night dark cycle for enhanced production of hydrogen has been performed and enhanced hydrogen production has been reported through mutagenesis for the development of less oxygen sensitive hydrogenase enzymes.

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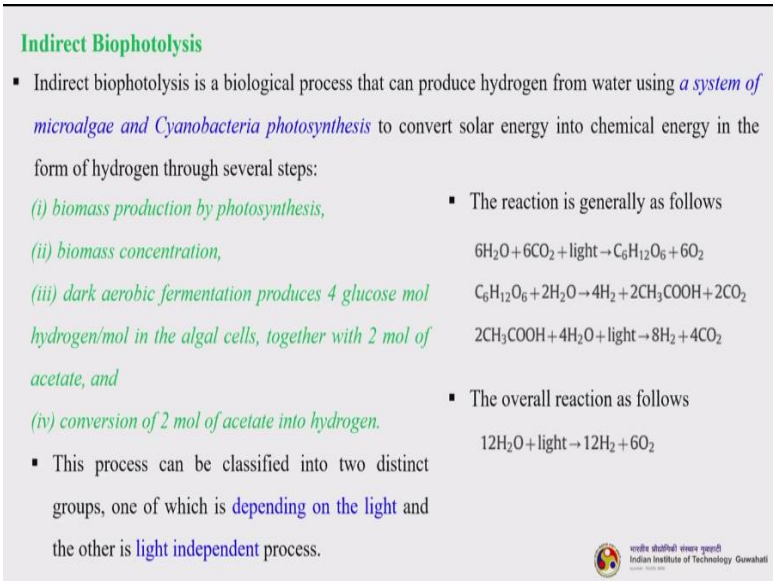

Now, we will discuss about indirect biophotolysis. Indirect biophotolysis is a biological process that can produce hydrogen from water using a system of microalgae and Cyanobacteria photosynthesis to convert solar energy into chemical energy in the form of hydrogen through several steps. Now, what are those steps? Essentially 4 steps. First is the biomass production by photosynthesis. Second is biomass concentration.

Third is dark aerobic fermentation which produces 4 glucose mol hydrogen per mol in the algal cells together with 2 moles of acetate, acetic acid and (fourth) conversion of 2 moles of acetic acid into hydrogen. Now, this process can be classified into two distinct groups one of which is dependent on the light and the other is not depending on the light. So the reactions you can see that 6 water + 6 carbon dioxide in the presence of light will give us glucose + 6 oxygen. Now this glucose + 2 water will give us 4 moles of hydrogen + 2 acetic acid and 2 carbon dioxide. Now these 2 acetic acid plus water plus light will again be converted to hydrogen and carbon dioxide.

$$6H_2O + 6CO_2 + light \rightarrow C_6H_{12}O_6 + 6O_2$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CH_3COOH + 2CO_2$$

$$2CH_3COOH + 4H_2O + light \rightarrow 8H_2 + 4CO_2$$

So overall reaction can be written as 12 water in the presence of light will give us 12 moles of hydrogen plus 6 moles of oxygen.

$$12H_2O + light \rightarrow 12H_2 + 6O_2$$

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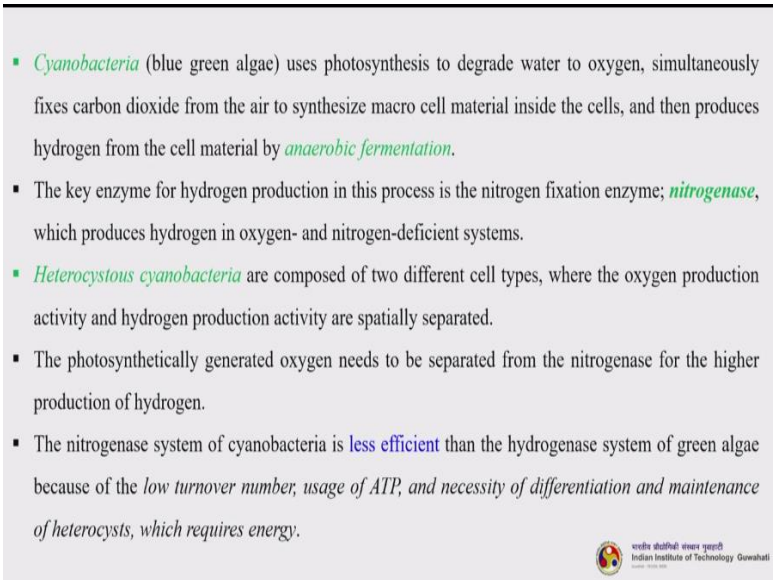

Cyanobacteria or the blue green algae uses photosynthesis to degrade water to oxygen, simultaneously fixes carbon dioxide from the air to synthesize macro cell material inside the cells and then produces hydrogen from the cell material by anaerobic fermentation. The key enzyme for hydrogen production in this process is the nitrogen fixation enzyme nitrogenase which produces hydrogen in the oxygen and nitrogen deficient systems.

Heterocystous cyanobacteria are composed of two different cell types where the oxygen production activity and the hydrogen production activity spatially separated. The photosynthetically generated oxygen needs to be separated from the nitrogenase for the higher production of hydrogen, otherwise it will inhibit basically.

The nitrogenase system of cyanobacteria is less efficient than the hydrogenase system of green algae because of the low turnover number, usage of the ATP that is continuously produced and getting used, the necessity for differentiation and maintenance of heterocysts which requires a lot of energy.

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- *Cyanobacteria* contain not only nitrogenase but also uptake hydrogenase, which consumes the hydrogen, thus hydrogen production is low. In order to resolve these problems, the uptake hydrogenase function should be removed from *cyanobacteria*.
- An uptake hydrogenase deleted mutant and replacement of gas phase with argon gas was previously shown to enhance the production of hydrogen.
- In indirect biophotolysis, separating spatially separated oxygen production and hydrogen production was attempted with the aim of *preventing inhibition of the hydrogen production activity*.
- Cell material is produced by CO_2 fixation and biophotolysis in stage 1, and then the cell material is converted to hydrogen by anaerobic fermentation in stage 2 (See Figure in next slide).
- *Chlamydomonas reinhardtii* has been mainly used for indirect biophotolysis. The hydrogenase system of *C. reinhardtii* was investigated to increase the hydrogen production rate and to remove the oxygen which inhibits the hydrogen production activity.

Laudblad et al., Int. J. Hydrogen Energy, 27, 2002, 1271 – 1281; Melis et al., Plant Physiol. 122, 2000, 127 – 135.

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Cyanobacteria contain not only nitrogenase but also uptake hydrogenase which consumes the hydrogen. Thus hydrogen production is getting low. Now, in order to resolve these problems, the uptake of hydrogenase function should be removed from cyanobacteria. An uptake hydrogenase deleted mutant and replacement of gas phase with argon gas was previously sought to enhance the production of hydrogen.

In indirect biophotolysis separated oxygen production and hydrogen production was attempted with the aim of preventing inhibition of the hydrogen production activity. Cell material is produced by carbon dioxide fixation and biphotolysis in stage 1, and then the cell material is converted to hydrogen by anaerobic fermentation in stage 2.

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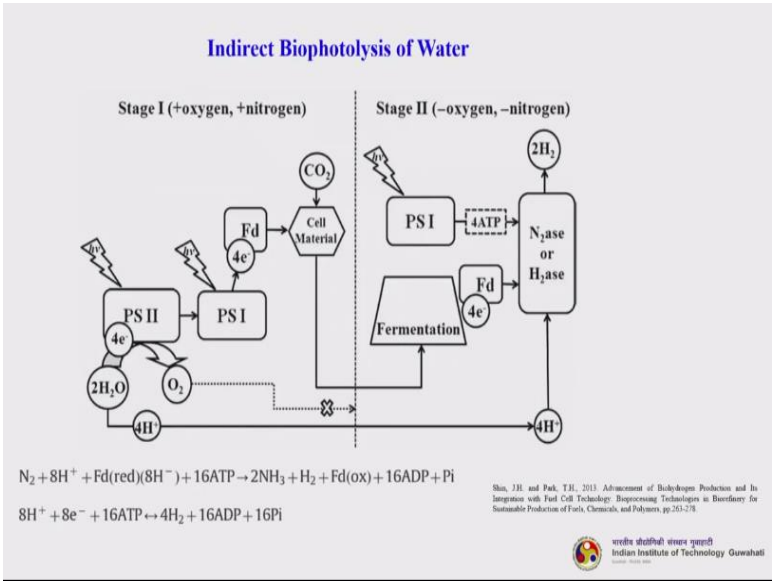

I will show you this. So, indirect biophotolysis, it consists of two distinct steps. This is actually more or less we can say that photosynthesis and this is your anaerobic fermentation. So, again the same which we have shown during the direct photolysis PS 2 and PS 1, photosystem 2 and photosystem 1 are doing their job. PS 2 is getting the electron directly from the water, then PS 1 it is getting it from the PS 2 system.

Then with the help of ferredoxin and hydrogease, the cell material is getting converted. Then it goes to the anaerobic process where fermentation is happening. And again this PS 1, whatever the ATP is getting generated that ATP is being used by the nitrogenase as well as the hydrogenase enzymes with the help of ferredoxin to convert biomass into hydrogen and of course other products little.

Chlamydomonas reinhardtii has been the main microorganism for the indirect biophotolysis. The hydrogenase system of *Chlamydomonas reinhardtii* was investigated to increase the hydrogen production rate and to remove the oxygen which inhibits the hydrogen production activity. This is the overall reaction. So 8 hydrogen + 8 electron + 16 ATP will gives 4 moles of hydrogen + 16 ADP + 16 Pi.

$$N_2 + 8H^+ + Fd(red)(8H^-) + 16ATP \rightarrow 2NH_3 + H_2 + Fd(ox) + 16ADP + Pi$$

$$8H^+ + 8e^- + 16ATP \leftrightarrow 4H_2 + 16ADP + 16Pi$$

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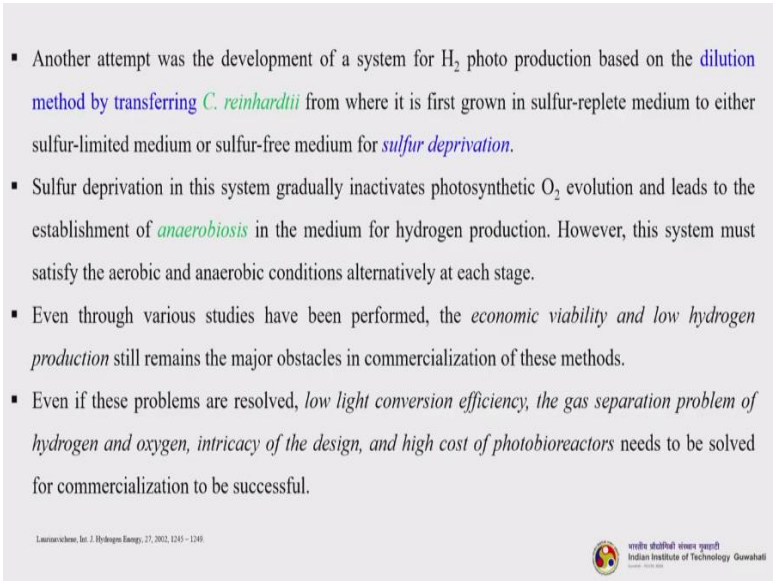

So, another attempt was the development of a system for hydrogen photoproduction based on the dilution method by transferring the *Chlamydomonas reinhardtii* from where it is first grown, usually in the sulfur-replete medium to either sulfur-limited medium or sulfur-free medium or sulfure deprivation. Now, sulfur deprivation in this system gradually inactivates the photosynthetic oxygen evolution and leads to the establishment of anaerobiosis in the medium for the hydrogen production which is required, anaerobic condition basically. However, this system must satisfy the anaerobic and aerobic conditions alternatively at each stage. Even though various studies have been performed, the economic viability and low hydrogen production still remains the major obstacles in the commercialization of these methods.

Even if these problems are resolved, low light conversion efficiency, the gas separation problem of hydrogen and oxygen, intricacy of the design and high cost of photobioreactors needs to be solved for the commercialization to be successful.

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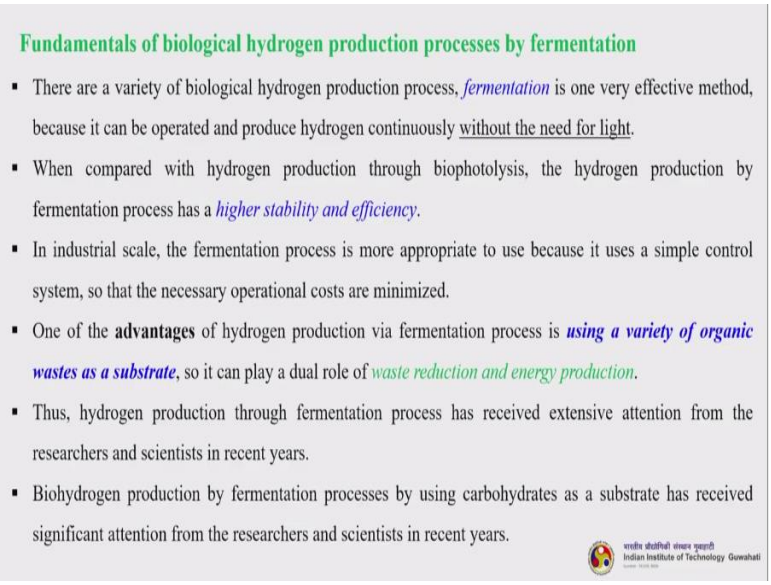

Now, we will discuss the fundamentals of biological hydrogen production processes by the fermentation pathway. Now, there are a variety of biological production processes. Fermentation is one of the most effective method because it can be operated and produce hydrogen continuously without the need for light. When compared with hydrogen production through biophotolysis, the hydrogen production by fermentation process has a higher stability and efficiency.

In industrial scale, the fermentation process is more appropriate to use because it uses a simple control system so that the necessary operational costs are minimized. And it can be integrated with other anaerobic process - that is one of the biggest advantages. One of the advantage of the hydrogen production by fermentation process is using a variety of organic waste as substrate - this is what I was telling.

When we are talking about the waste conversion, you can always couple waste conversion in the form of fermentation so that you can produce biohydrogen. So, it plays a dual role of waste reduction and energy production. So, basically waste to energy. Thus, hydrogen production through fermentation process has received extensive attention from the researchers and scientists in recent years.

Biohydrogen production by fermentation process by using carbohydrates as substrate has received significant attention from the researchers and scientists in the recent few years.

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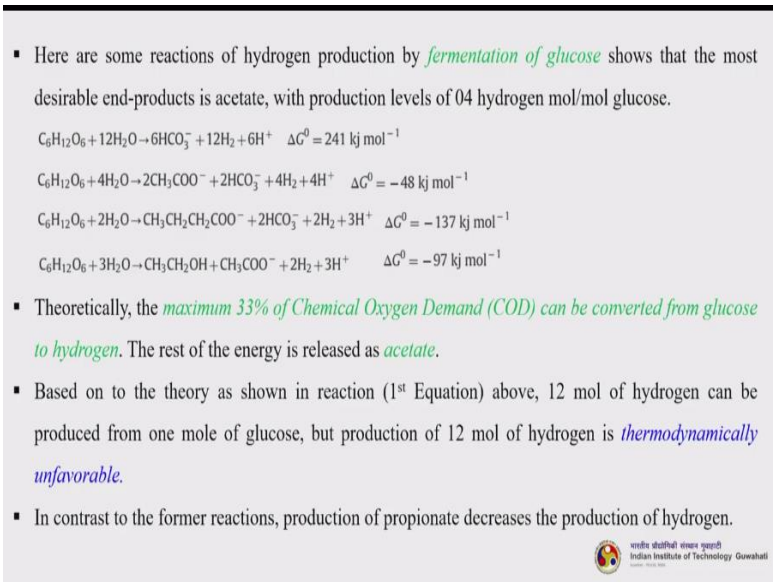

So, here are some reactions of hydrogen production by fermentation of glucose shows that the most desirable end product is acetate, acetic acid. With the production levels of 4 hydrogen mole per mole of glucose. So C 6 H 12 O 6 + 12 water will give us 6 HCO 3 minus bicarbonate + 12 moles of hydrogen and 6 H+. Similarly, I am not reading the reactions. You can go through later on. You can also see that delta G which given in the first one is 241 kilojoules per mole. For other all the 3 reactions it is negative –48, –137, –97.

$$C_6H_{12}O_6 + 12H_2O \rightarrow 6HCO_3^- + 12H_2 + 6H^+ \quad \Delta G^0 = 241 \text{ kj mol}^{-1}$$

$$C_6H_{12}O_6 + 4H_2O \rightarrow 2CH_3COO^- + 2HCO_3^- + 4H_2 + 4H^+ \quad \Delta G^0 = -48 \text{ kj mol}^{-1}$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2CH_2COO^- + 2HCO_3^- + 2H_2 + 3H^+ \quad \Delta G^0 = -137 \text{ kj mol}^{-1}$$

$$C_6H_{12}O_6 + 3H_2O \rightarrow CH_3CH_2OH + CH_3COO^- + 2H_2 + 3H^+ \quad \Delta G^0 = -97 \text{ kj mol}^{-1}$$

So, theoretically the maximum 33% of chemical oxygen demand can be converted from glucose to hydrogen, The rest of the energy is released as acetate. Based on the theory as shown in the reaction the first equation above, 12 moles of hydrogen can be produced from 1 mole of glucose, see this is theoretical.

But production of 12 mole of hydrogen is thermodynamically unfavorable. So it is never getting produced because you have to maintain the thermodynamic equilibrium. So in contrast to the former reactions, production of propionate decreases the production of hydrogen.

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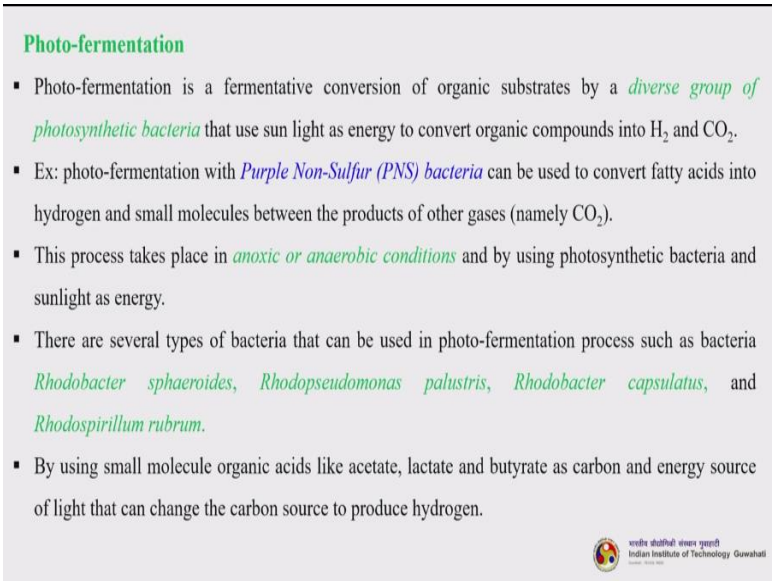

So, we will now discuss about photo-fermentation. Photo-fermentation is a fermentative conversion of organic substrates by a diverse group of photosynthetic bacteria that use sunlight as energy to convert organic compounds into hydrogen and carbon dioxide. For example photo-fermentation with a purple non-sulfur bacteria, which is well known as PNS bacteria, can be used to convert fatty acids into hydrogen and small molecules between the products of other gases, namely carbon dioxide.

Now, this process takes place in anoxic or anaerobic conditions and by using photosynthetic bacterium and sunlight as energy. There are several types of bacteria that can be used in photo-fermentation processes such as *Rhodobacter sphaeroides*, *Rhodopseudomonas palustris*, *Rhodobacter capsulatus*, and *Rhodospirillum rubrum*. Now by using small molecule organic acids such as acetate, lactate and butyrate as carbon and energy source of light, it can change the carbon source to produce hydrogen.

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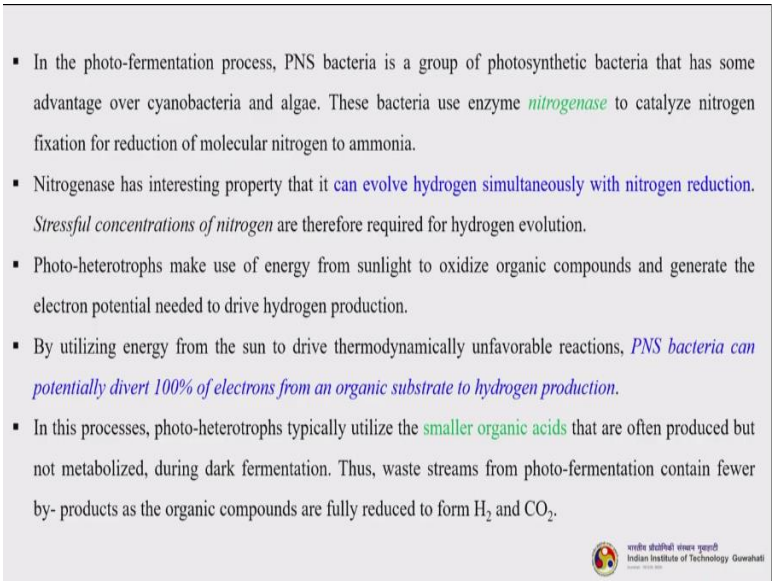

In the photo-fermentation process, PNS bacteria is a group of photosynthetic bacteria that has some advantage over cyanobacteria and algae. Now, these bacteria use enzyme nitrogease to catalyze nitrogen fixation for reduction of molecular nitrogen to ammonia. Nitrogenase has interesting property that it can evolve hydrogen simultaneously with nitrogen reduction. Stressful concentrations of nitrogen are therefore required for hydrogen evolution.

Photo-heterotrophs make use of energy from sunlight to oxidize organic compounds and generate the electron potential needed to drive hydrogen production. By utilizing energy from the sun to drive thermodynamically unfavorable reactions, PNS bacteria can potentially divert 100% of electrons from an organic substrate to hydrogen production. In these processes, photo-heterotrophs typically utilize the smaller organic acids that are often produced but not metabolized during the dark fermentation.

Thus, waste streams from photo-fermentation obtained contain fewer byproducts as the organic compounds are fully reduced to form hydrogen and carbon dioxide and that is one of the most important aspect of this entire process.

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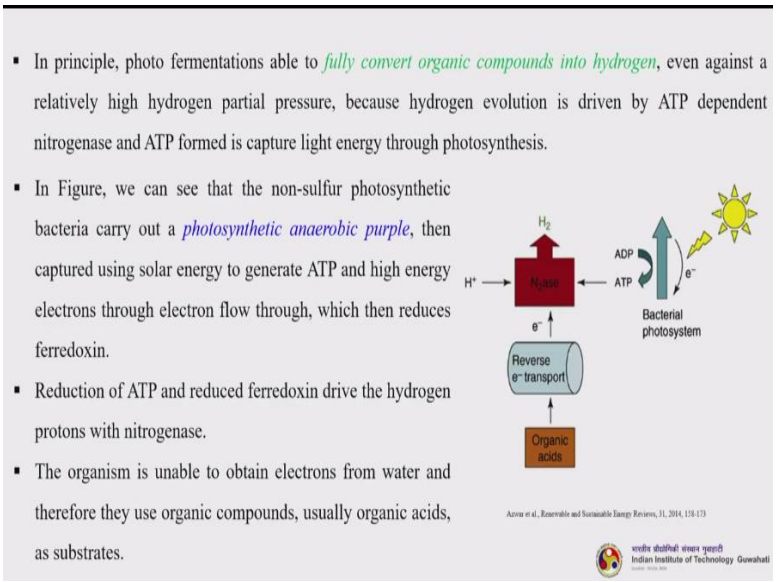

In principle, photo-fermentation able to convert fully organic compounds into hydrogen even against a relatively high hydrogen partial pressure because hydrogen evolution is driven by the ATP dependent nitrogenase and ATP formed is capture light energy through photosynthesis. Now, you can have a look at this particular image. Now, in the figure we can see that the non-sulfur photosynthetic bacteria can carry out photosynthetic anaerobic purple, then captured using solar energy to generate ATP and high energy electrons through electron flow through which then reduces ferredoxin. Now, reduction of ATP and reduced ferredoxin drive the hydrogen protons with nitrogenase. The organism is unable to obtain electrons from water and therefore they use organic compounds usually organic acids as substrates.

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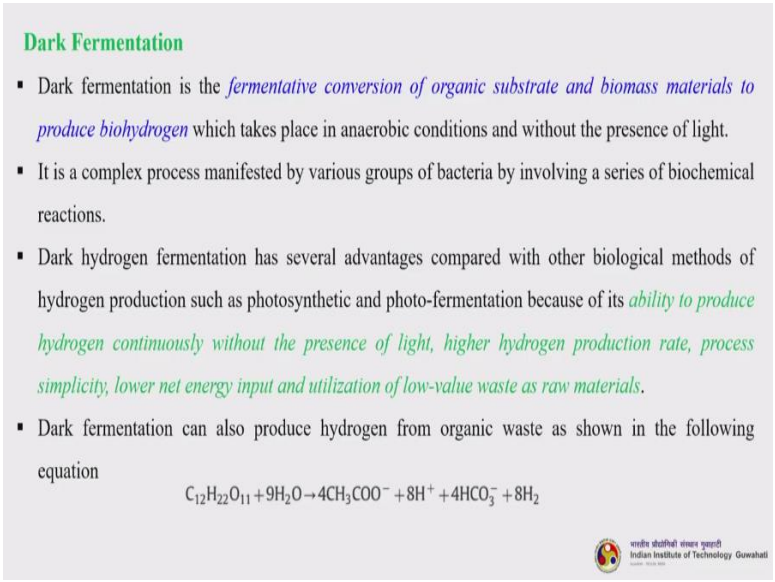

And now we will discuss about dark fermentation. Dark fermentation is the fermentative conversion of organic substrate and biomass materials to produce biohydrogen which takes place in anaerobic conditions and without the presence of light. It is a complex process manifested by various groups of bacteria by involving a series of biochemical reactions. Dark hydrogen fermentation has several advantages compared with other biological methods of hydrogen production, such as, photosynthetic and photo-fermentation because of its ability to produce hydrogen continuously without the presence of light, higher hydrogen production rate, process simplicity, lower net energy input and utilization of low value waste as raw materials. Dark fermentation can also produce hydrogen from organic waste as shown in the following equation. So, the organic waste plus water can give us CH 3 COO – H+ + 4 bicarbonate + 8 hydrogen.

$$C_{12}H_{22}O_{11} + 9H_2O \rightarrow 4CH_3COO^- + 8H^+ + 4HCO_3^- + 8H_2$$

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- In order to increase the yield of more hydrogen in the dark fermentation process, it is necessary to control several parameters namely *pH, organic food, nutrition feed rate, temperature, Solids Retention Time (SRT), and* P_{H2}.
- One of the most important parameters on hydrogen production is pH, because pH has direct influence on the activities of the enzyme *hydrogenase*. Several studies reported that the hydrogenase activity are directly correlated with dark fermentation of hydrogen, this indicates that the pH plays a very important role on hydrogen production.
- In general, pH value was maintained at a pH range of 5.5 to 8.0 either by adjusting the initial pH, buffer usage, or using an automatic pH controller. By applying these techniques, the maximum conversion efficiency has been increased by 60-70%.
- Several studies have been conducted for the hydrogen production on a *batch, anaerobic sequencing batch reactor (AnSBR), fed-batch, fluidized bed bioreactor (FBR), continuously stirred tank reactor (CSTR)* and continuous dark fermentation with different types of raw materials.

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So, in order to increase the yield of more hydrogen in the dark fermentation process, it is necessary to control several parameters namely pH, organic food, nutrition feed rate, temperature, solid retention time and the partial pressure of hydrogen. One of the most important parameters of hydrogen production is pH, because pH has a direct influence on the activities of the enzyme hydrogenase.

Several studies reported that the hydrogenase activity are directly correlated with dark fermentation of hydrogen. This indicates that pH plays a very important role on the hydrogen

production. Anyway, as I told you many times that pH always plays a very important role whether it is a biological system or whether it is a non-biological system, especially when you are dealing with some aqueous medium reaction.

Now, in general pH value was maintained at a pH range of 5.5 to 8 either by adjusting the initial pH, by using the buffer or by using an automatic pH controller. Now by applying these techniques, the maximum conversion efficiency has been increased by 60 to 70%. Several studies have been conducted for the hydrogen production using a batch reactor, anaerobic sequencing batch reactor, fed batch reactor, fluidized bed reactor and continuously stirred tank reactors and continuous dark fermentation with different types of raw materials. Extensive studies are being reported. So those who are interested to learn more under dark fermentative biohydrogen production please look for literature.

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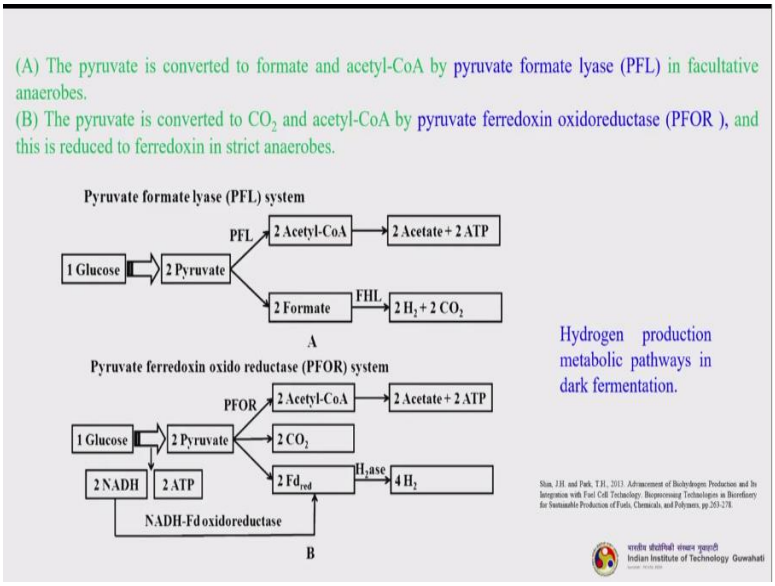

So, we will explain the hydrogen production metabolic pathway in the dark fermentation. Essentially you can see these, I will explain. Two different pathways. One is the first one this one - which is called the pyruvate. In this pathway, particularly the pyruvate is converted to formate and acetyl coenzyme A by pyruvate formate lyase, so PFL system. We call it as a PFL system in facultative anaerobes.

Whereas in case of the B it is a PFOR system; here the pyruvate is converted to carbon dioxide and acetyl coenzyme A by pyruvate ferredoxin oxidoreductase, PFOR and this is reduced to ferredoxin by strict anaerobes. So, you can see this, the first one the PFL system

here the glucose is getting converted to pyruvate, pyruvate is getting converted to formate as well as acetyl coenzyme A.

Now formate is getting converted to hydrogen and carbon dioxide. In case of PFOR system here the glucose is converted to pyruvate. Now this pyruvate is getting converted to carbon dioxide and acetyl coenzyme A and both these in the presence ferredoxin along with the hydrogenase is getting converted to 4 moles of hydrogen.

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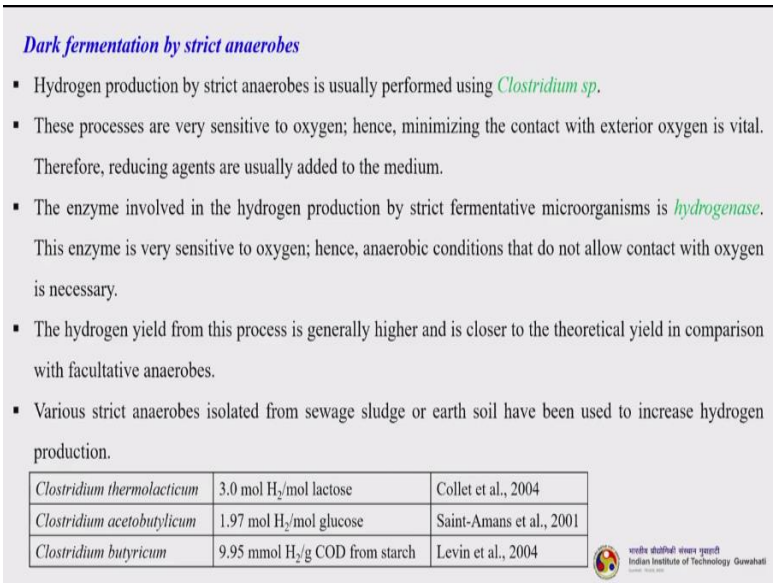

So dark fermentation by strict anaerobes: Hydrogen production by strict anaerobes is usually performed using *Clostridium* species. We have discussed about *Clostridium* when we discussed about ABE fermentation. Now, these processes are very sensitive to oxygen hence minimizing the contact with exterior oxygen is vital. Therefore, reducing agents are usually added to the medium.

The enzyme involved in the hydrogen production by strict fermentative microorganism is hydrogenase. This enzyme is very sensitive to oxygen. Hence, anaerobic conditions that do not allow contact with oxygen at utmost necessary. The hydrogen yield from this process is generally higher and is closer to the theoretical yield in comparison with the facultative anaerobes. Very strict anaerobes isolated from sewage sludge or earth soil have been used to increase hydrogen production.

Three are being listed here. *Clostridium thermolacticum* - so 3 moles of hydrogen per mole of lactose; *Clostridium acetobutylicum* - so 1.97 moles of hydrogen per mole of glucose; and

Clostridium butyricum - 9.95 millimoles of hydrogen per gram of COD from starch. You can see the first one is perhaps one of the best.

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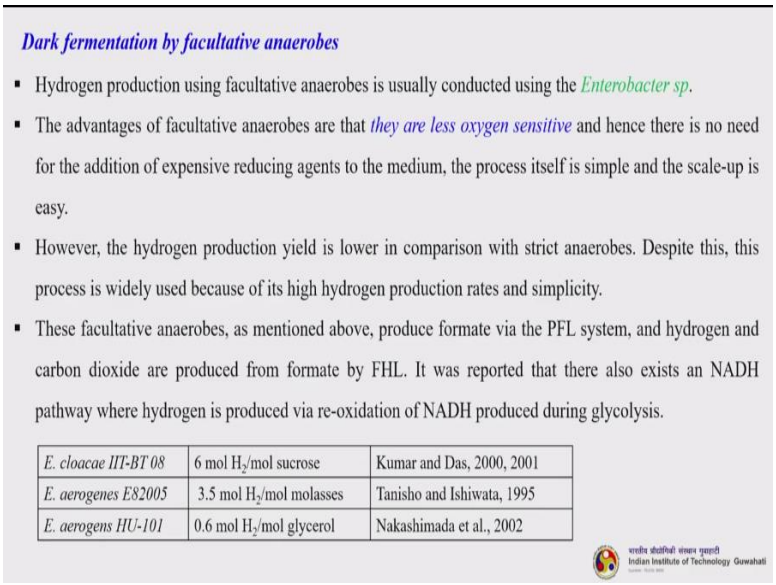

So, then dark fermentation by facultative anaerobes. Hydrogen production using facultative anaerobes is usually conducted using the *Enterobacter* species. The advantages of facultative anaerobes are that they are less oxygen sensitive and hence there is no need for the addition of expensive reducing agents to the medium. The process itself is simple and scale up is easy. However, the hydrogen production yield is lower in comparison with the strict anaerobes.

Despite this, this process is widely used because of its high hydrogen production rates and simplicity in operation. Now, these facultative anaerobes as mentioned above produce formate via the PFL system and hydrogen and carbon dioxide are produced from formate by FHL. It was reported that there also exists an NADH pathway where the hydrogen is getting produced via re-oxidation of NADH produced during the glycolysis pathway.

So, *Enterobacter cloacae* IIT-BT 08, this is developed by Professor Das group in IIT Kharagpur Biotechnology Department - 6 moles of hydrogen per mole of sucrose. *Enterobacter aerogenes* E82005 has given 3.5 moles of hydrogen per mole of molasses. *Enterobacter aerogenes*, HU-101 has given 6 moles of hydrogen per mole of glycerol. So excellent works are being reported and many works are being also commercially adapted in few biorefineries.

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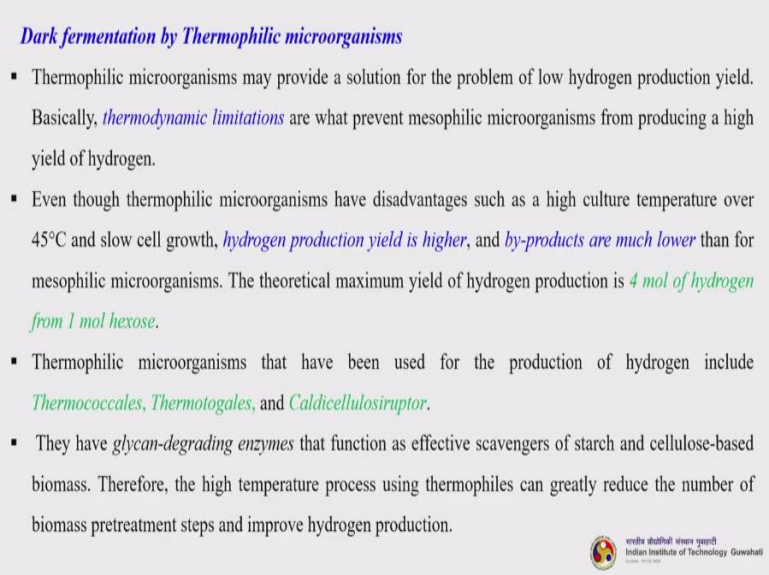

So then we will discuss about the dark fermentation by thermophilic microorganisms. Now, thermophilic microorganisms may provide a solution for the problem of low hydrogen production yield. Basically, thermodynamic limitations are what prevent mesophilic microorganisms from producing a high yield of biohydrogen. Even though thermophilic microorganisms have disadvantages such as a high culture temperature over 45 degrees centigrade and slow cell growth, hydrogen production yield is higher.

And byproducts are much lower than for the mesophilic microorganisms. The theoretical maximum yield of hydrogen production is 4 moles of hydrogen from 1 mole of hexose. Now thermophilic microorganisms that have been used for the production of hydrogen include *Thermococcales*, *Thermotogales* and *Caldicellulosiruptor*. Now they have glycan-degrading enzymes that function as effective scavengers of starch and cellulose-based biomass.

Therefore, the high temperature process using thermophiles can greatly reduce the number of biomass pretreatment steps and improve the hydrogen production.

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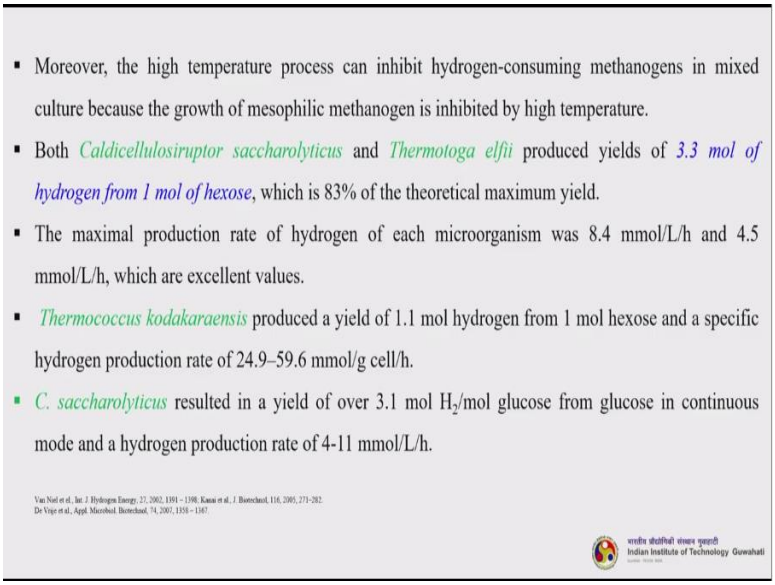

Moreover, the high temperature process can inhibit hydrogen-consuming methanogens in mixed culture because the growth of mesophilic methanogen is inhibited by high temperature. Both *Caldicellulosiruptor saccharolyticus* and *Thermotoga elfii* produced yields of 3.3 moles of hydrogen from 1 mole of hexose which is 83% of the theoretical maximum yield and is very good.

So, the maximal production rate of hydrogen of each microorganism was 8.4 millimole per liters per hour and 4.5 millimole per liter per hour which are excellent values. *Thermococcus kodakaraensis* produced a yield of 1.1 mole hydrogen from 1 mole of hexose and a specific hydrogen production rate of 24.9 to 59.6 millimol per gram cell per hour.

C. saccharolyticus resulted in a yield of over 3.1 mole hydrogen per mole of glucose from glucose in continuous mode and hydrogen production rate of 4 to 11 millimol per liter per hour, which is also a good one if you compare with other such microorganisms.

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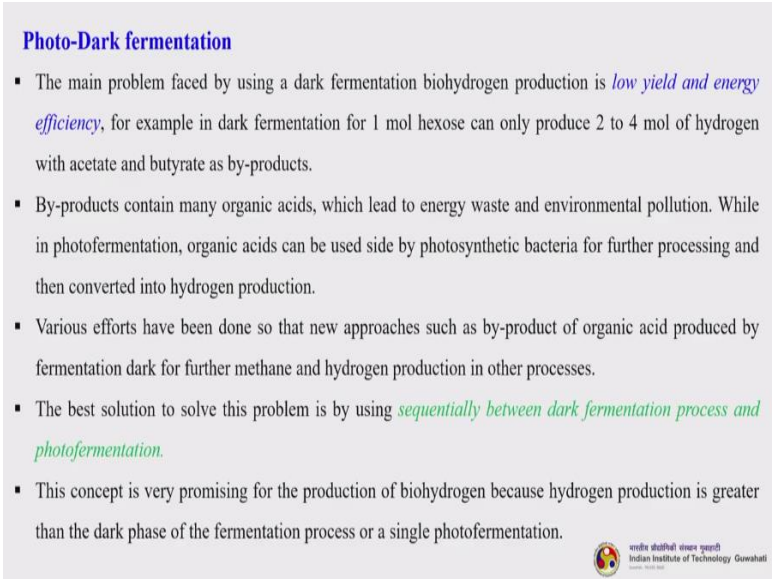

So, we will now discuss about photo-dark fermentation. The main problem faced by using a dark fermentation biohydrogen production is the low yield and energy efficiency. For example in dark fermentation for 1 mole hexose can only produce 2 to 4 moles of hydrogen with acetate and butyrate again as byproducts. Now, byproducts contain many organic acids, which lead to energy waste and environmental pollution.

While in photofermentation, organic acids can be used side by photosynthetic bacteria for further processing and then converted into hydrogen production. Now, various efforts have been done so that new approaches such as byproduct of organic acid produced by fermentation for further methane and hydrogen production in other processes. The best solution to solve this problem is by using sequentially between dark fermentation process and photofermentation.

So, basically you operate sequentially dark fermentation and photofermentation in separate units. Now, this concept is very promising for the production of biohydrogen because hydrogen production is greater than the dark phase of the fermentation process or a single photofermentation.

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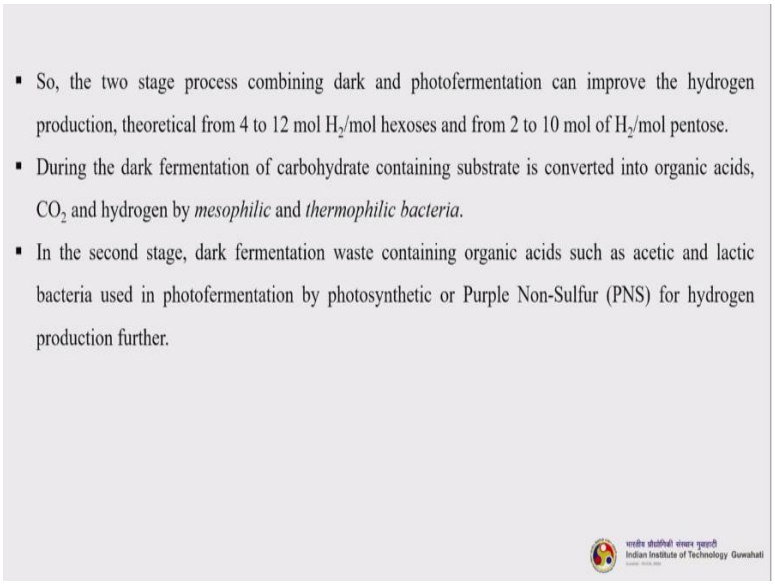

So, the two stage process combining dark and photo fermentation can improve the hydrogen production theoretically from to 4 to 12 moles of hydrogen per mole of hexose and from 2 to 10 moles of hydrogen per mole of pentose. During the dark fermentation of carbohydrate containing substrate is converted to organic acids, carbon dioxide and hydrogen by mesophilic and thermophilic bacteria.

In the second stage, dark fermentation waste containing organic acids such as acetic acid and lactic bacteria used in photofermentation by photosynthetic or PNS bacteria for hydrogen production further.

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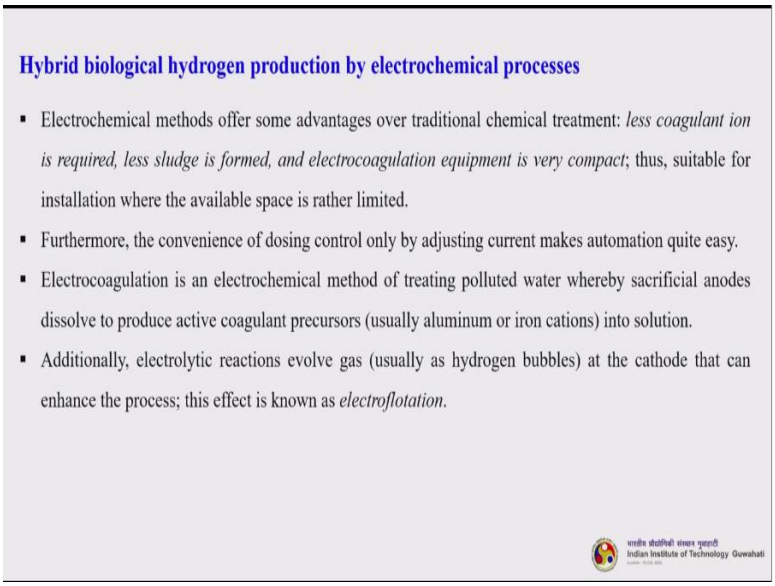

So, hybrid biological hydrogen production by electrochemical processes. Electrochemical methods offer some advantages over traditional chemical treatments. So, what are those?

Now less coagulant ion is required, less sludge is formed and electrocoagulation equipment is very compact. Thus, it is suitable for installation where the available space is rather limited. Furthermore, the convenience of dosing control only by adjusting current makes automation quite easy.

Electrocoagulation is an electrochemical method of treating polluted water whereby sacrificial anodes dissolve to produce active coagulant precursor usually aluminum or iron cations into the solution. Additionally, electrolytic reaction evolve gas usually the hydrogen in the form of bubbles at the cathode that can enhance the process. This effect is known as the electroflotation.

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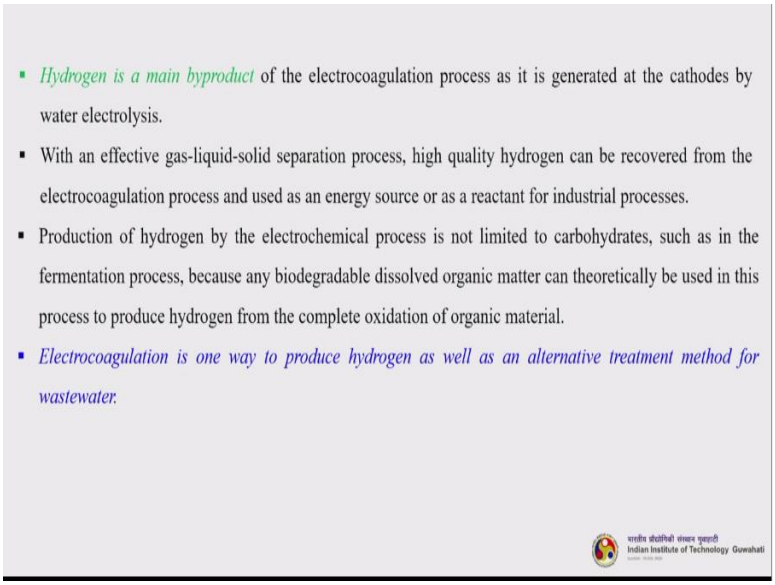

Hydrogen is a main byproduct of the electrocoagulation process as it is generated at the cathodes by water electrolysis. With an effective gas-liquid-solid separation process, high quality hydrogen can be recovered from the electrocoagulation process and used as an energy source or as a reactant for industrial processes. Production of hydrogen by the electrochemical process is not limited to carbohydrate as in the fermentation process actually, because any biodegradable dissolved organic matter can theoretically be used in this process to produce hydrogen from the complete oxidation of organic material. Electrocoagulation is one way to produce hydrogen as well as an alternative treatment method for the wastewater. So, basically, we can again say that water energy in excess or waste to energy.

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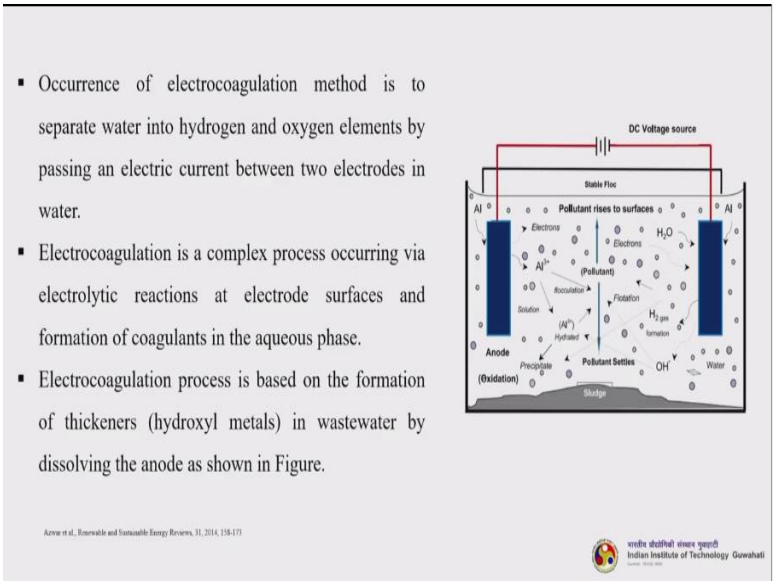

So, this is the representation that how actually it happens. So, this is the anode where oxidation is happening. This is the cathode. So, you can see that when the electric current is applied to both the anode and cathode, so the current flows through, the pollutants rises to the surfaces and are getting converted slowly by the available microorganisms to various products and byproducts.

Now, occurrence of electrocoagulation method is to separate water into hydrogen and oxygen elements by passing an electric current between the two electrodes in the water. Electrocoagulation is a complex process occurring via electrolytic reactions at electrode surfaces and formation of coagulants in the aqueous phase. Electrocoagulation process is based on the formation of thickeners, so basically hydroxyl metals in wastewater by dissolving the anode as shown in the figure.

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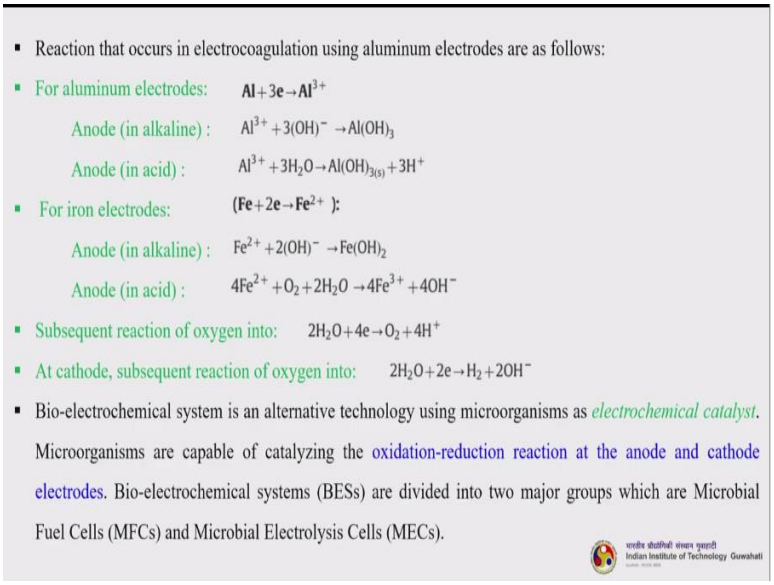

So, reaction that occurs in electrocoagulation using alumunium electrode are as follows. You can use other metal as a electrode also. So, for aluminum electrodes Al + 3 e gives Al 3+. So, in the anode in alkaline medium Al 3+ + 3 OH gives us Al OH 3. So, in case of acidic medium in anode we will get Al OH 3 that is in the form of solid plus 3 H+. Now, for iron electrodes, the ion plus 2 e will give us Fe 2+. Now, in alkaline medium we will get Fe OH 2 and in case of acidic medium we get 4 moles of Fe 3+ and 4 moles of OH minus. Now, subsequent reaction of oxygen that is getting generated by this reaction 2 water 4 e will give us oxygen + 4 H+. At cathode subsequent reaction of oxygen happens which is this 2 water + 2 e will give us hydrogen + 2 OH minus.

- **For aluminum electrodes:** $Al+3e\rightarrow Al^{3+}$
  - **Anode (in alkaline) :** $Al^{3+}+3(OH)^{-}\rightarrow Al(OH)_3$
  - **Anode (in acid) :** $Al^{3+}+3H_2O\rightarrow Al(OH)_{3(s)}+3H^{+}$
- **For iron electrodes:** $(Fe+2e\rightarrow Fe^{2+})$:
  - **Anode (in alkaline) :** $Fe^{2+}+2(OH)^{-}\rightarrow Fe(OH)_2$
  - **Anode (in acid) :** $4Fe^{2+}+O_2+2H_2O\rightarrow 4Fe^{3+}+4OH^{-}$
- **Subsequent reaction of oxygen into:** $2H_2O+4e\rightarrow O_2+4H^{+}$
- **At cathode, subsequent reaction of oxygen into:** $2H_2O+2e\rightarrow H_2+2OH^{-}$

Now, you can understand so many different types of reactions are happening simultaneously. So, controlling is of course an important issue. So, a bioelectrochemical system is an alternative technology using microorganisms as electrochemical catalysts. Microorganisms are capable of catalyzing the oxygen reduction reaction at the anode and cathode electrodes. Bioelectrical systems are divided into two major groups which are microbial fuel cells and microbial electrolysis cells, MFCs and MECs.

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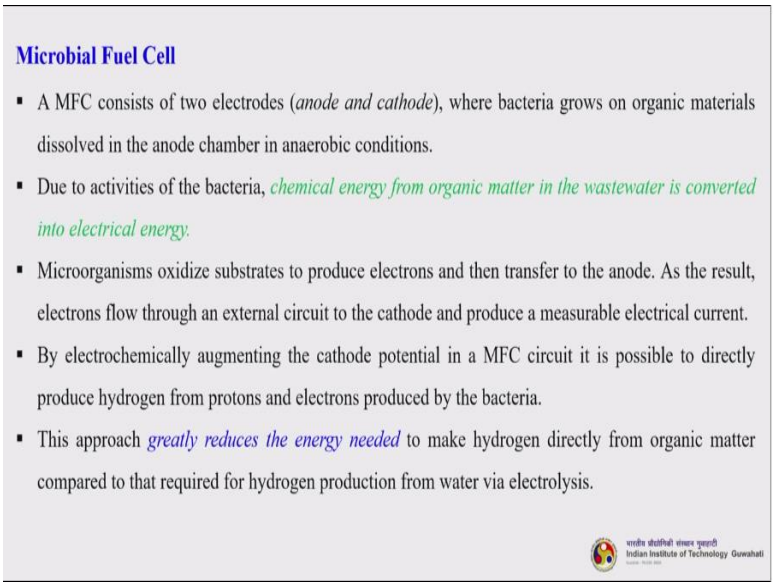

So, we will just in a glance understand about microbial fuel cell. MFC consists of two electrodes, anode and cathode, where bacteria grows on organic materials dissolved in the anode chamber in anaerobic conditions. Due to activities of the bacteria, chemical energy from organic matter in the wastewater is converted into electrical energy. Microorganisms oxidize substrates to produce electrons and then transport to the anode.

As a result, electrons flow through an external circuit to the cathode and produce a measurable electrical current. If you recall we have discussed MFC earlier also, but just to maintain the flow of this entire lecture we are discussing in a nutshell. So, by electrochemically augmenting the cathode potential in MFC circuit, it is possible to directly produce hydrogen from protons and electrons produced by the bacteria.

This approach greatly reduces the energy needed to make hydrogen directly from organic matter compared to that required for hydrogen production from water via electrolysis.

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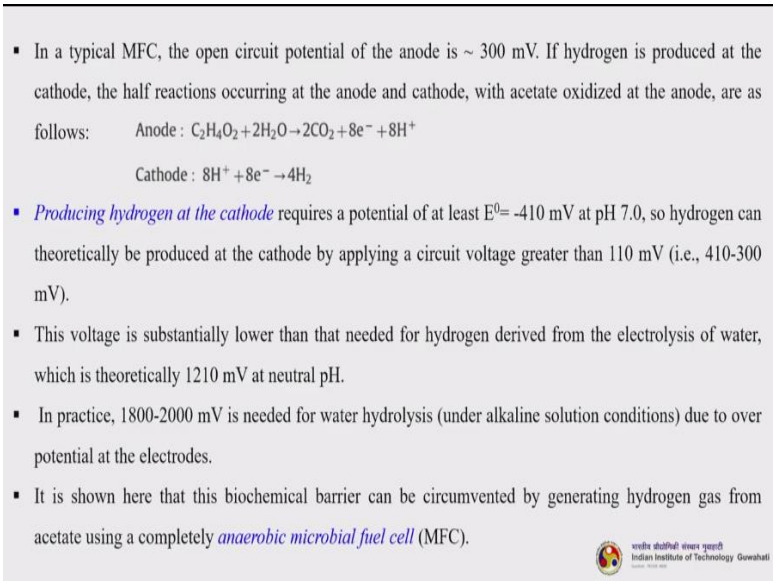

In a typical MFC, the open circuit potential of the anode is usually 300 millivolt. If hydrogen is produced at the cathode, the half reactions occurring at the anode and cathode with acetate being oxidized at the anode are as follows. So, at anode C 2 H 4 O 2 + 2 water will give us 2 moles of carbon dioxide, 8 electrons and 8 protons. At cathode 8 proton + 8 electrons again getting reacted to gives us 4 moles of hydrogen.

$$\text{Anode : } C_2H_4O_2+2H_2O\rightarrow 2CO_2+8e^-+8H^+$$

$$\text{Cathode : } 8H^++8e^-\rightarrow 4H_2$$

Now, producing hydrogen at the cathode requires a potential of at least –410 millivolt at pH 7. So, hydrogen can theoretically be produced at the cathode by applying a circuit voltage greater than 110 millivolts that is in the range of 410 to 300 millivolt. This voltage is substantially lower than that needed for the hydrogen derived from the electrolysis of water which is theoretically 1210 millivolt at neutral pH that is very high actually if you compare with this process.

In practice, 1800 to 2000 millivolt is needed for water hydrolysis under alkaline solution conditions due to over potential at the electrodes. It is shown here that this biochemical barrier can be circumvented by generating hydrogen gas from acetate using a completely anaerobic microbial fuel cell.

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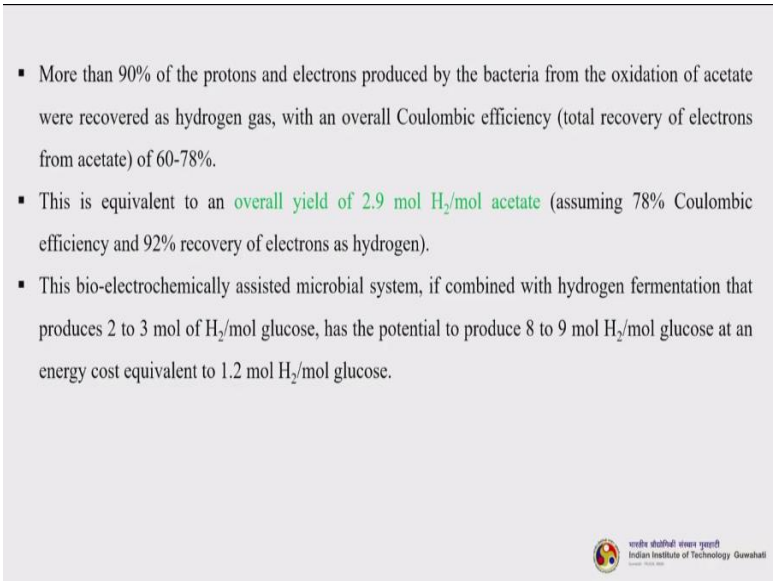

More than 90% of the protons and electrons produced by bacteria from the oxidation of acetate were recovered as hydrogen gas with an overall Coulombic efficiency - that is the total recovery of electrons from acetate - usually at around 60 to 78%. This is equivalent to an overall yield of 2.9 moles of hydrogen per mole of acetate if you assume 78% Coulombic efficiency and 92% recovery of electrons in the form of hydrogen.

Now, this bio-electrochemically assisted microbial system if combined with hydrogen fermentation that produces 2 to 3 moles of hydrogen per mole of glucose has the potential to produce 8 to 9 moles of hydrogen per mole of glucose at an energy cost equivalent to 1.2 mole of hydrogen per mole of glucose, which is excellent actually.

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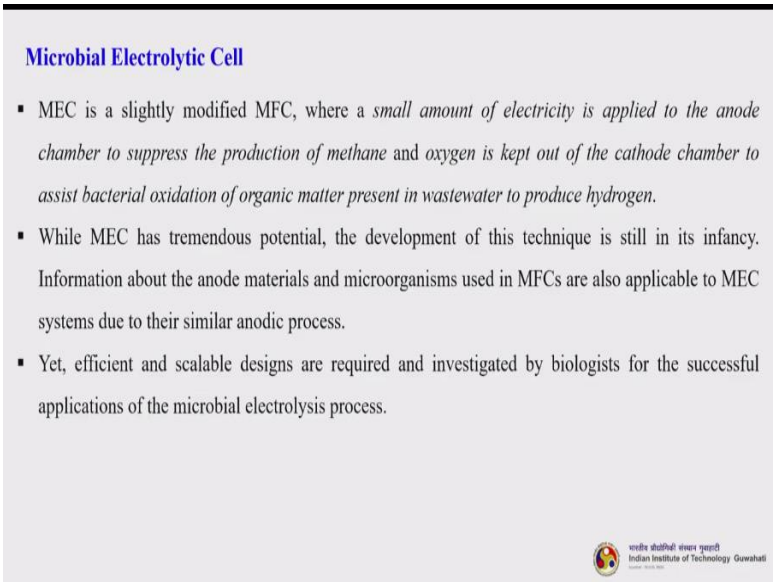

So, we will wind up our discussion talking about this MEC/microbial electrolytic cell: MEC is a slightly modified MFC, where a small amount of electricity is applied to the anode chamber to suppress the production of methane and oxygen is kept out of the cathode chamber to assist bacterial oxidation of organic matter present in wastewater to produce hydrogen.

While MEC has tremendous potential, the development of this technique is still in its infancy. Information about the anode materials and microorganisms used in MFCs are also applicable to MEC systems due to their similar anodic processes. Yet, efficient and scalable designs are required and investigated by biologists for the successful applications of the microbial electrolysis process.

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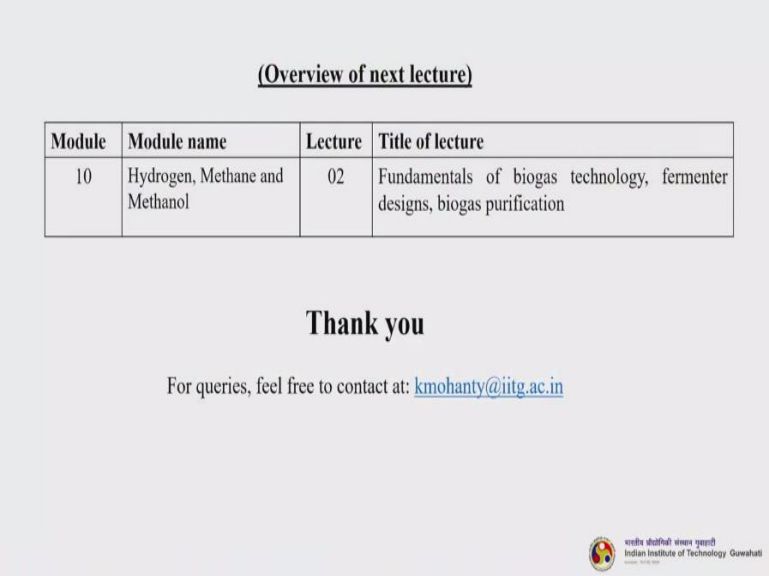

So with this, I wind up today's lecture. So in the next lecture, we will be discussing about the fundamentals of biogas technology, fermenter designs and biogas purification processes. So if you have any query, please feel free to register it in the Swayam portal or you can always drop a mail to me at kmohanty@iitg.ac.in. Thank you.