Course on Industrial Biotechnology
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Lecture No 59
10m3 Pilot Plant Operations For Biohydrogen Production

Welcome back to our course Industrial Biotechnology. Today I think in the last lectures I tried to cover activated sludge process and the anaerobic digestion process for the treatment of industrial effluent and today I want to share some our research experience that we have on biohydrogen production. We consider the hydrogen should be fuel for the future the reason is that when hydrogen burns it produces only water and not only that hydrogen has highest energy density as compared to any fuel available with us.

So we want to share that kind of research activities that is going on at IIT Kharagpur and recently we carried out 10 cubic metre pilot plant study, the research the information on that also I am going to share with you and finally we will show you the video 1 operational video on the operation of pilot plant so that you can have some kind of experience how the industrially we operate the fermentation process.

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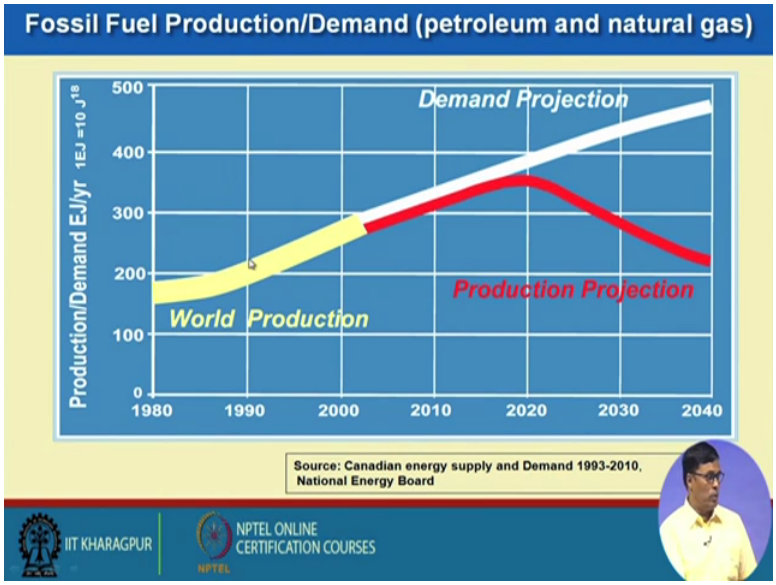

So let me start with that, this is the topic that I have chosen that is the 10 cubic metre pilot plant operation for Biohydrogen production at IIT Kharagpur and if you look at the fossil fuel that is production and demand it is demand keep on rising with respect to time due to the rapid industrialization and urbanization and you will find that after 2020 around that our demand keep on rising but the fuel production from the fossil fuel that will keep on declining.So there will be gap between the demand and the production of fuel from the frozen fuel, so naturally we shall have to find out some kind of alternative resources to fulfil the gap.

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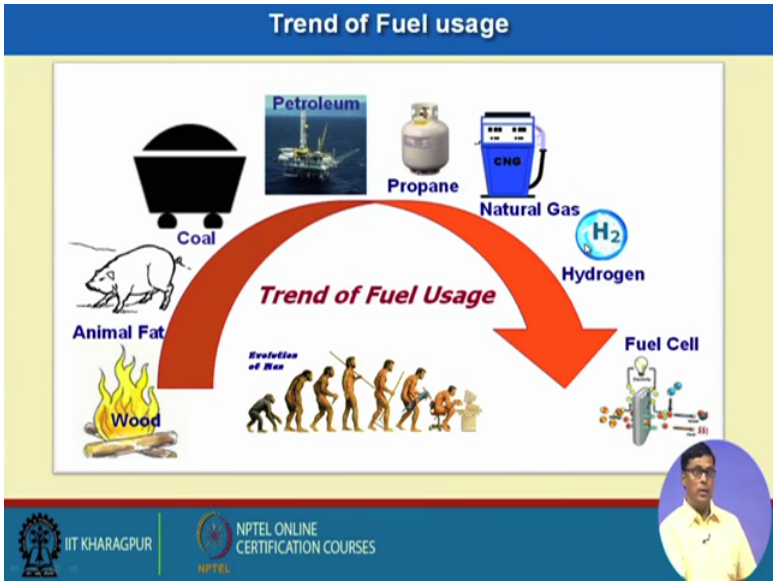

So this is the fuel usage it is very interesting, if you look at the human civilization we started with burning of wood as a source of energy and slowly we switch over to a particular area what you call hydrogen and fuel cell where we do not have any kind of pollution. In the fuel cell we can use the hydrogen as a fuel and it converted to electricity. So hydrogen will be converted to water here, no way it will be polluting the environment. So if we can do that we can save our environment and we can also solve our energy shortage problem.

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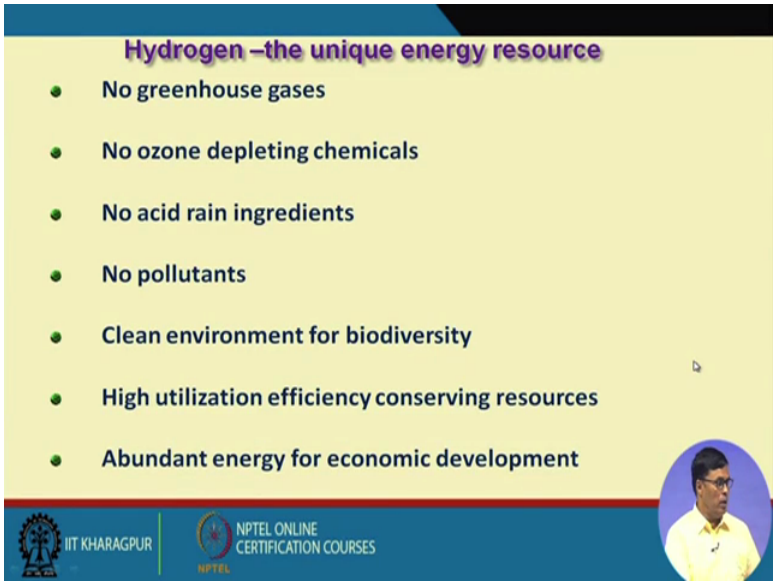

Now hydrogen the unique energy source the reason is that it has no greenhouse gasses no ozone depleting chemicals, no acid rain ingredients, no pollutants, clean environment for biodiversity, high utilization efficiency conserving resources and abundant energy for economic development.

Now if you look at the current technology for the production of hydrogen, there are mostly the chemical and the electrolysis process and these processes are as per example the Thermo chemical gasification coupled with water gas shift reaction and then fast pyrolysis followed by reforming of carbohydrate fraction of bio-oil, Direct solar gasification, miscellaneous novel gasification process, biomass derived syn-fuel conversion, supercritical conversion of biomass and microbial conversion of biomass. We are here microbial conversion of biomass because biomass is a renewal energy source if we can produce some kind of useful fuel from this biomass this will be sustainable.

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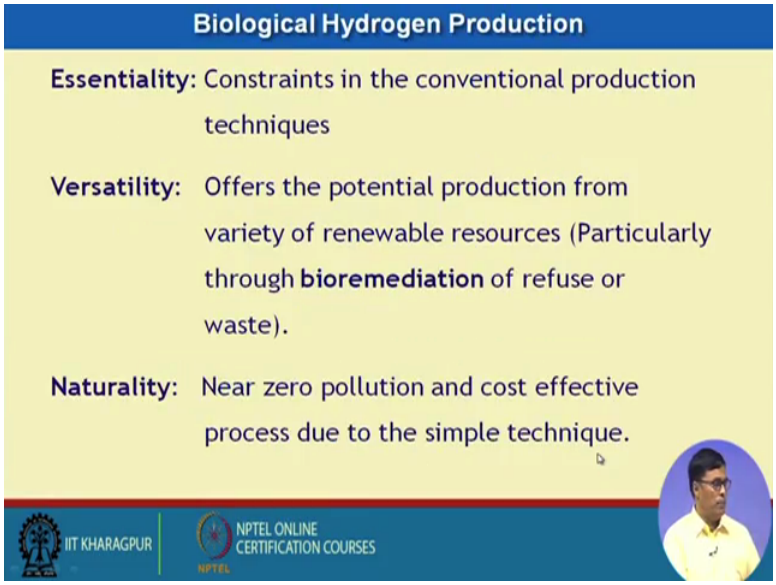

So now question comes why we are interested for biological hydrogen production processes? The reasons is three, that one is what you call essentiality, another is the versatility and another is the Naturality. Now if you look at the essentiality that means that constant that we have with a conventional technique, what is the constant we have? That is the high temperature high pressure and high energy intensity process but this is to be so replace by the, because biological process usually operated at ambient temperature and atmospheric pressure so energy requirement for this process is comparatively low.

And versatility is this energy can be derived from the renewable energy source particularly waste material which not only safeguard our environment but also the waste material can be used for the generation of energy. Naturality is the near zero pollution and cost effective process due to the simple technique.

Now if you look at the hydrogen production from microbial conversion of biomass, this is the industry that produces flue gas which polluted our environment this carbon-di-oxide can be fixed for sequestration process by using algae. We have algal biomass these algal biomass can be used for hydrogen production the raw material different agricultural residue, different organic waste can be used for the dark fermentation process and convert it here.

The spent media that can be used for biophotolysis can produce hydrogen and dark fermentation directly can produce hydrogen and when this material can be used in the microbial fuel cell that also produce hydrogen. So this hydrogen we can pass through the fuel cell for the generation of electricity here the product is water. This is how it is a sustainable

process because we do not pollute the environment at the same time we produce our energy for fruitful purpose.

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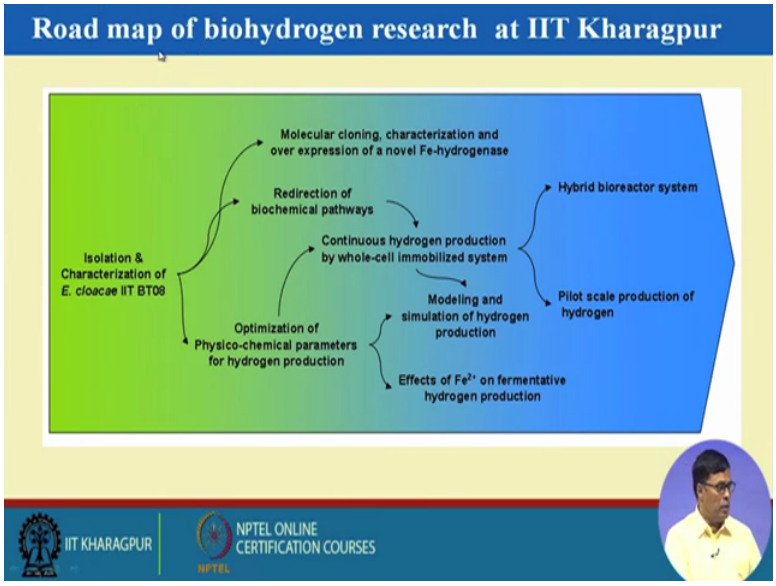

Now if you look at the roadmap of biohydrogen research at IIT Kharagpur, when we started this research work 1999, we isolate and characterize the E.cloacae IIT BT08 this organism appears to be the first hydrogen producing organism so far been reported and this is the organism we reported for the first time that this produce hydrogen and then we try to justify the reason behind that by doing some kind of molecular biology work, we did the redirection of metabolic pathway for increasing the hydrogen production then we use the continuous hydrogen production by whole sale immobilization system.

Optimization of physical chemical parameters for hydrogen production we did modelling and simulation for hydrogen production effect of iron for fermentative process then use the hybrid reactor and then now we have successfully operated the pilot plant. Further now we are looking for the industry for the commercialization of the process.

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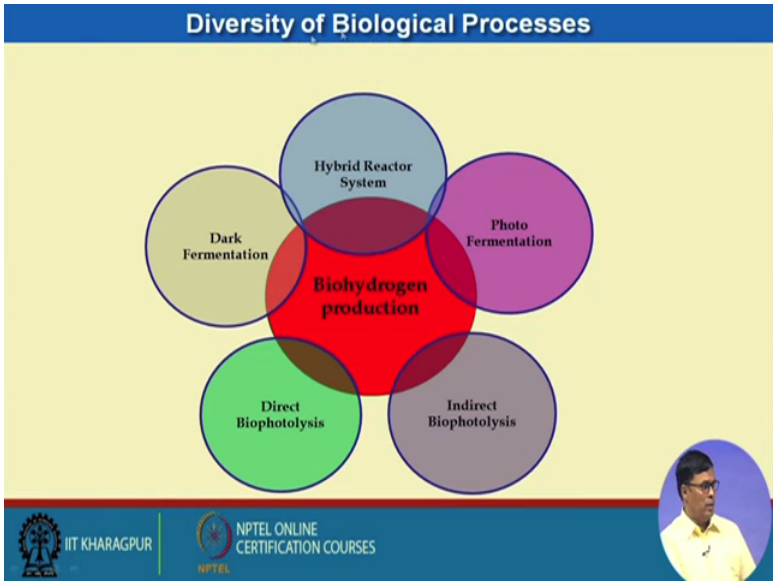

Now if you look at the biodiversity of the biological process is like this. This can be produced dark fermentation process appears to be the best process because we can produce hydrogen from the organic waste and we can produce hydrogen throughout the day because it does not require the presence of any kind of sunlight but whenever we work with the photo biological process it depends on the sunlight that is another additional requirement that we have.

So not only that this problem direct biophotolysis and indirect biophotolysis, one is with the help of cyanobacteria another will be help of green algae and this we found that when we use this organism and if you look at the history of Biohydrogen production it is very interesting that Mitsui that scientist from university of Miami that he found out that the algae can produce hydrogen from water and as we know the three fourth of the earth is water.

So if we can produce hydrogen from water we can solve our energy problem but the problem is that when we try to grow this algae we find that when water degraded it produce hydrogen and also molecular oxygen, this molecular oxygen give the inhibitory effect to the enzymes, hydrogenous which is responsible for hydrogen production. So initially the hydrogen production started and after sometime this is inhibited.

So this research is going on how to safeguard this problem but we find we do not have much of problem when you go for dark fermentation process one advantage is that we do not require any kind of sunlight, in addition to that hydrogen can be produced from the waste material and it can produce throughout the day and it produce hydrogen at very faster rate and another problem that we have with photo fermentation process is.

Photo fermentation process is produces some kind of pigment, when it grows it gives some kind of shading effect and so light penetration is a problem so that light energy conversion efficiency that will decrease with respect to time, as the time proceed the conversion efficiency decreases to a great extent this is the major problem that we face with the photo biological process.

Now here we can see the dark fermentation and we had the collaboration project with Oslo University and the technical University of Denmark and Uppsala university just to find out the potentiality of algae for the hydrogen production we have come to the conclusion that this is no good for hydrogen production and then with there is the another process called photo fermentation process.

In this photo fermentation process the photo fermented bacteria can utilize the metabolites which is produced in the dark fermentation process for the generation of hydrogen but the problem is that as I told you that as the organism grow that will be kind of shading effect and that causes the reduction of hydrogen production to a great extent. So we find that is why the dark fermentation is more suitable for the hydrogen production.

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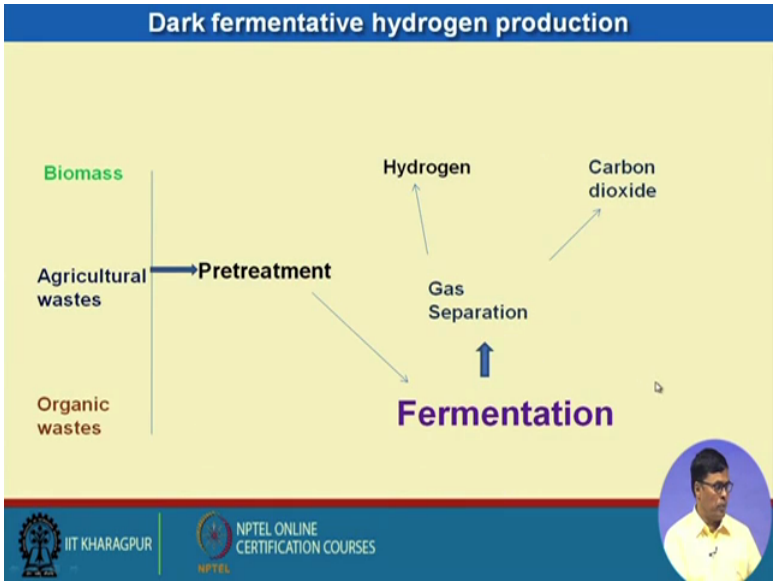

Now if you look at the dark fermentation process how it works, this is the raw materials biomass agricultural waste and we have organic waste this is biomass we mean the activated the sludge that we get from the activated sludge plant that is a biomass and we can get agricultural waste we have several other organic waste. First it should undergo pre-treatment, after the pre-treatment we take it in the fermenters where the fermented dark fermentation

takes place and then we get the gas, one way we get hydrogen another way we get carbon-di-oxide.

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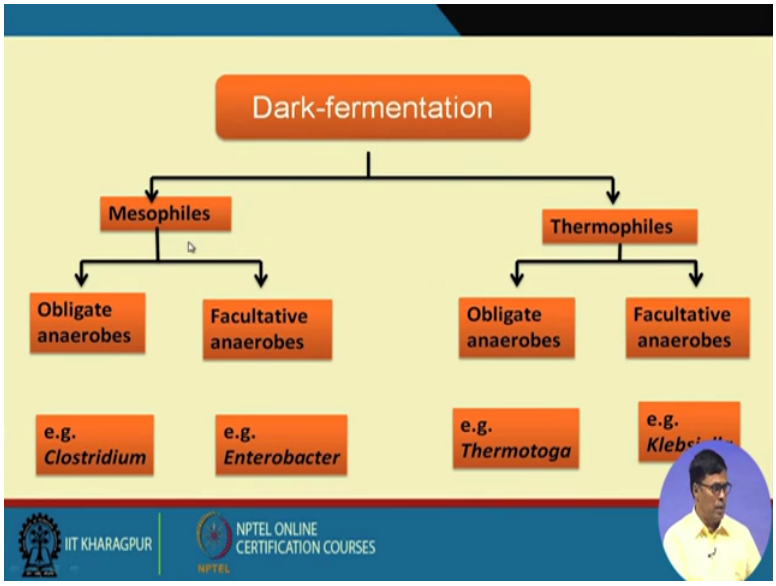

Now dark fermentation usually carried out by two groups of microfula one is called Mesophiles another is Thermophiles. Mesophiles we have two categories one is obligate anaerobe another is facultative anaerobe. Now facultative anaerobe is easy to handle, the reason is that this can tolerate little bit of dissolved oxygen concentration but obligate anaerobe does not tolerate they cannot tolerate that they little bit of oxygen concentration the organism will be killed in presence of oxygen.

So this is the problem of creating the anaerobic environment complete anaerobic environment but once we create that then the organism grow very nicely, the example of that is clostridium(())(12:48) that did not grow under obligate anaerobe. In the Thermophiles we get Thermotoga that can grow but in facultative anaerobes we have Enterobacter cloacae, the beauty of the Enterobacter cloacae is that it can grow even in absence of oxygen or in the presence of oxygen so it is easy to handle and facultative anaerobes of Thermophiles is the klebsiella.

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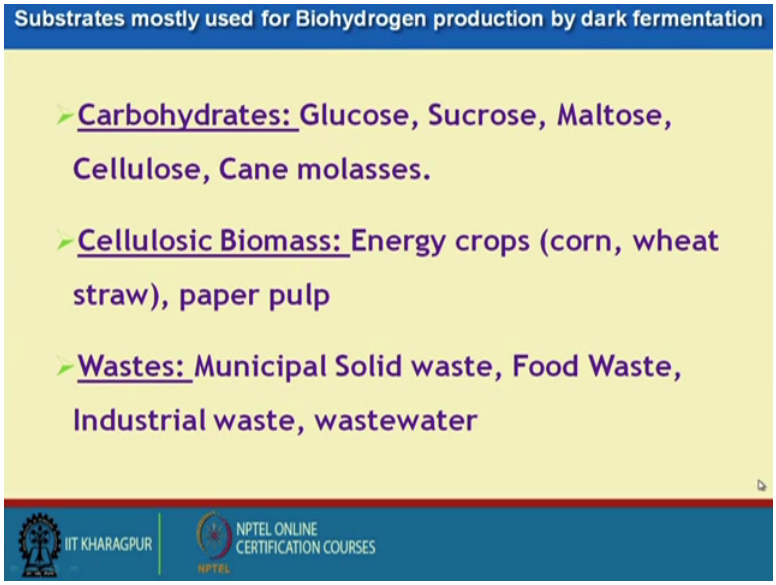

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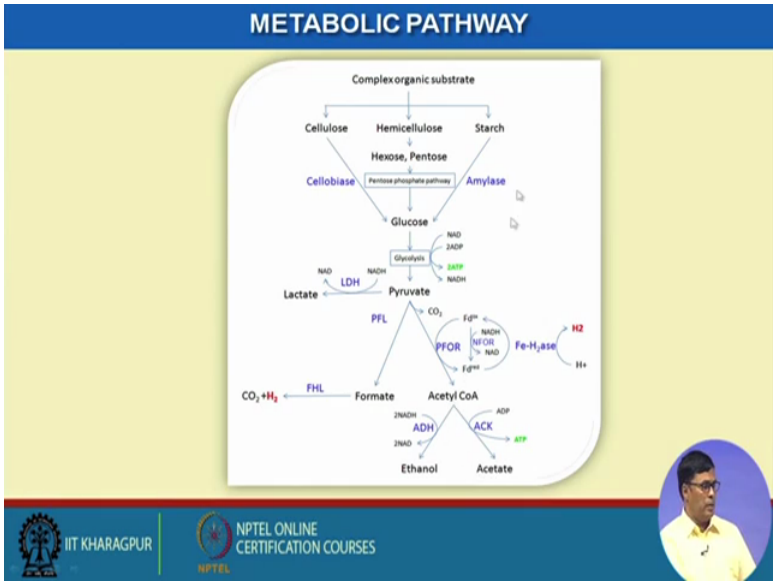

Now different substrates can be used for the Biohydrogen production process. The carbohydrates we have glucose, sucrose, maltose, cellulose and cane molasses. Cellulosic material we have energy crops like corn, wheat, wheat straw, paper pulp then waste we have municipal solid waste, food waste, industrial waste and the waste water and this is the metabolic pathway.

If you look at complex organic material when it hydrolyse they comprises of cellulose, semi cellulose and starch, when they hydrolyse they produce glucose and this glucose undergoes this glycolytic pathway what we call Embden Meyerhof pathway it produce the pyruvate acid

and this pyruvate acid when it forms a formate with the help of formate hydrogen lyase it produce carbon-di-oxide in hydrogen and here hydrogen it produce that NADH you can see that how it continues for the hydrogen production and also we have another product we have ethanol we have acetate we have lactate.

But only the problem is that if you look at the ethanol production is little bit undesirable because it consumed the NADH and also lactate also that NADH. So if we can block this pathway this NADH can be used for the hydrogen production that is how we have carried out some experiments, how we can rewrite the metabolic pathway to produce to increase the hydrogen production.

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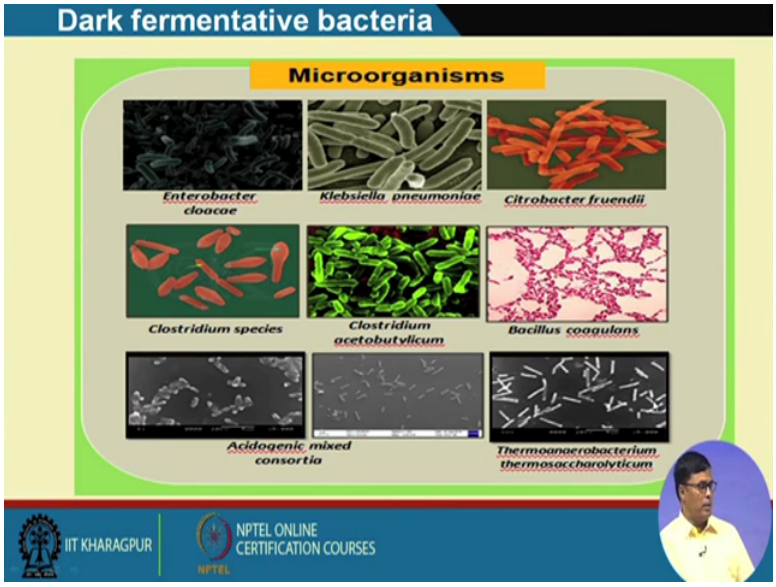

And this is the different bacteria isolated in our lab, one is initially this is Enterobacter cloacae this is identified by Chandigarh and then later on this bacteria identified at klebsiella pneumoniae because it has 99.8 per cent similarity, here they have 98 percent similarity as per (())(15:13) are in a consent then we have Citrobacter frundii we isolated from high oil containing soil we have clostridium species isolated from the anaerobic digester, clostridium acetobutylicum also isolated from the anaerobic digester. Bacillus coogulans isolated from sewage sludge and this is the thing that we develop in our lab we find that I explain the biomethanation process and I told you the biomethanation process comprises of two steps, one is Acidogenesis another is methanogenesis.

The acidogens they produce they convert the organic material to volatile fatty acid and if you look at that methanogens they convert the volatile fatty acid to methane and carbon-di-oxide,

now here little information is available that on how the potentiality on the this acidogens for the hydrogen production. We are one of the lab who reported that you know this acidogens has very good potential for the hydrogen production and we are quite successful in producing that and this is how this organism looks under the microscope and another as I told you we use the Thermophilic bacteria also to find out they are suitable for hydrogen production.

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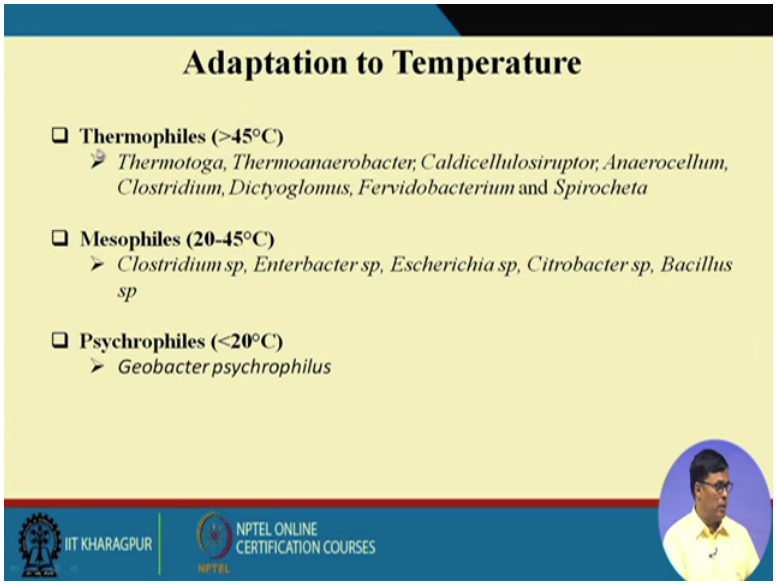

Now this is the three with respect to temperature we can classify the organism in three different groups one is Thermophiles another Mesophiles and Psychrophiles. Now Thermophiles we have Thermotoga, I told you that different organism is there clostridium species is there. Mesophiles we have Clostridium, Enterobacter Escherichia and Psychrophiles we have Geobacter Psychrophiles.

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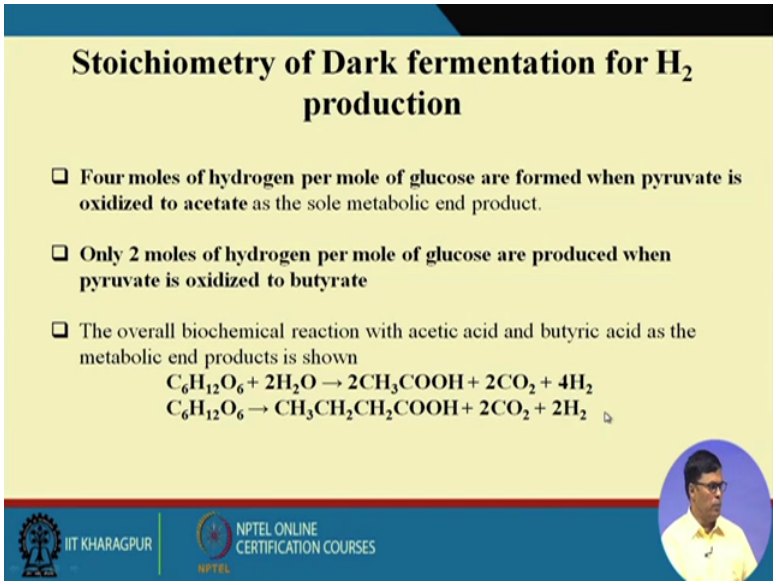

Now if you look at the stoichiometry of hydrogen production is very interesting, the one mole of glucose can convert two moles of acidic acid and four moles of hydrogen if it follows the acidic acid pathway but if it produce it follow the butyric acid pathway then it produce two moles of hydrogen. So our intention is that how this organism can be used by the acetate pathway? So that our hydrogen production can be increased to a great extent.

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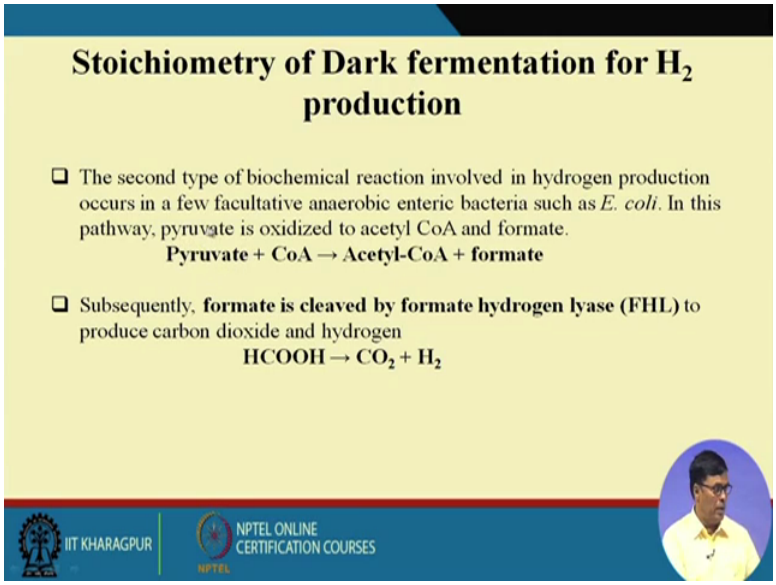

Now another interesting feature is that we have already shown that through the (())(17:52) pathway we get our organism produce pyruvate and this pyruvate we get the acetyl-CoA and formate and this formate in presence of formate hydrogen lyaes it produce carbon-di-oxide

and hydrogen, this is also present in our organism and this helps for the hydrogen production.

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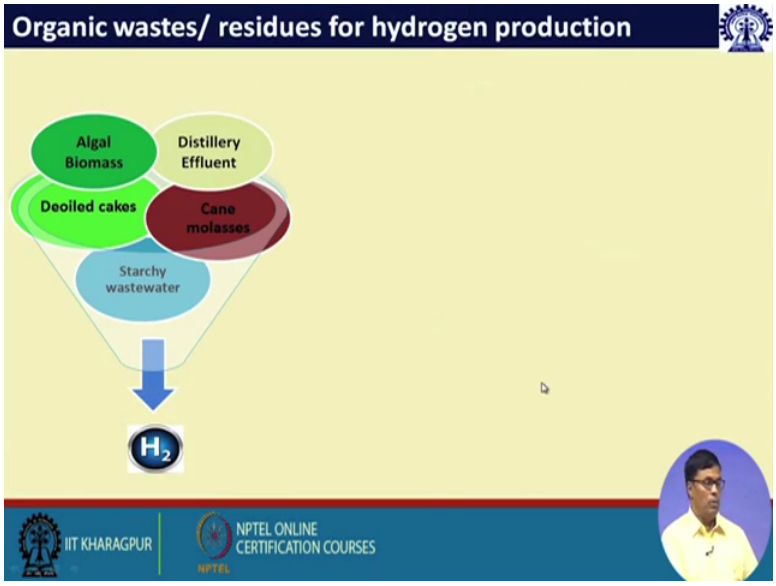

Now I am going to share some of our research finding, here I want to point out that ministry of new and renewable energy sources they made a hydrogen road map, a national hydrogen road map and there they identify three different areas, one is hydrogen production another is hydrogen storage, another is hydrogen utilization. So they have given because hydrogen can be produced through the chemical process, Thermochemical process it can be produced through the electrolysis process it can be produced through the biological process.

So this (())(18:57) they have given the responsibility to IIT Kharagpur to lead the research of Biohydrogen in India and we took 5 groups with us one is IRCT Hyderabad another is JNQ Hyderabad then Allahabad University, Banaras Hindu University and Tele New Delhi. So we walk together we try to find out the potentiality of different organic waste suitable for hydrogen production. Finally we came out with the two pilot plant 10 cubic metre pilot plants one is established at IIT Kharagpur another established at IRCT Hyderabad.

So I want to share our major finding, one of the major finding is that it is very interesting that when we use any kind of waste material for microbial degradation process as you know that waste material is a waste for a particular industry and it undergo several treatment processes, so what happens actually that during the treatment process the quality of the material is lost.

So I told you at the beginning for the growth of the bacteria we require carbon source, we require nitrogen source, we require minerals, we require vitamin. So if we do not give all this material properly then the organism cannot grow and multiply. So in this in this waste we add some kind of yeast extract or we add mold extract but this are the material which are very costly.

So when you talk about the energy generation process naturally we shall have to think for the substitution of this material and we came up with some kind of suggestion that we find out how this material can be replaced by some waste material, so that our process will be economically accepted.

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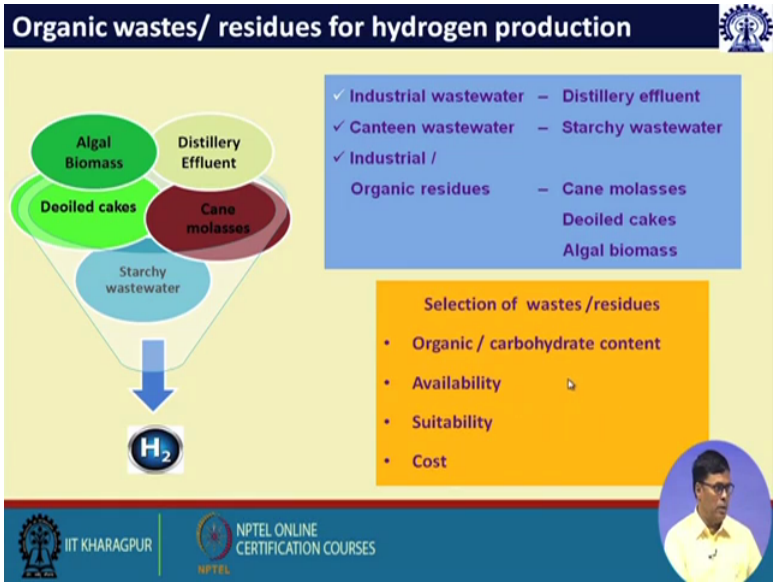

Now here show you that we use the different type of organic waste and ultimately we came out with the conclusion that algal biomass, distillery effluent, De oiled cake, cane molasses and starchy waste water is the most suitable raw materials for hydrogen production. Now industrial waste water we have distillery effluent, canteen waste water, we have starchy waste water, industrial and organic residue we have cane molasses, De oiled cake and algal biomass. Now this selection of waste and residue depends on organic and carbohydrate contents, also the availability suitability and the cost these are the different factors that affects on the selection of the raw material.

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Now here I want to show you the different types of De oiled cake we considered that the substrate one is ground nut De oiled cake it looks like this, coconut De oiled cake it looks this, mustard De oiled cake it looks like this, sesame De oiled cake it looks like this, rice bran it looks like that and soya bean De oiled cake it looks like this.

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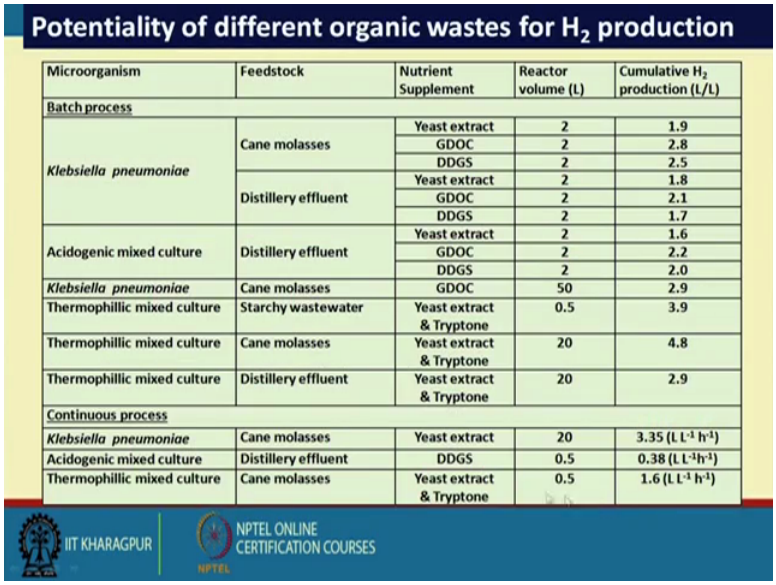

Microorganism	Feedstock	Nutrient Supplement	Reactor volume (L)	Cumulative H_2 production (L/L)
Batch process				
Klebsiella pneumoniae	Cane molasses	Yeast extract	2	1.9
		GDOC	2	2.8
		DDGS	2	2.5
	Distillery effluent	Yeast extract	2	1.8
		GDOC	2	2.1
		DDGS	2	1.7
Acidogenic mixed culture	Distillery effluent	Yeast extract	2	1.6
		GDOC	2	2.2
		DDGS	2	2.0
Klebsiella pneumoniae	Cane molasses	GDOC	50	2.9
Thermophillic mixed culture	Starchy wastewater	Yeast extract & Tryptone	0.5	3.9
Thermophillic mixed culture	Cane molasses	Yeast extract & Tryptone	20	4.8
Thermophillic mixed culture	Distillery effluent	Yeast extract & Tryptone	20	2.9
Continuous process				
Klebsiella pneumoniae	Cane molasses	Yeast extract	20	3.35 ($L L^{-1} h^{-1}$)
Acidogenic mixed culture	Distillery effluent	DDGS	0.5	0.38 ($L L^{-1} h^{-1}$)
Thermophillic mixed culture	Cane molasses	Yeast extract & Tryptone	0.5	1.6 ($L L^{-1} h^{-1}$)

Now if here our batch process and continuous process we make a comparative study that klebsiella pneumoniae when we use cane molasses as a raw materials we use the yeast extract then our hydrogen production is 1.9 litre per litre of the fermentation broth, when we use the that groundnut De oiled cake this is we find 2.8 that means even yeast as compared to yeast extract groundnut De oiled cake gives the better results.

Now this is distiller dry grains soluble solids this gives also better results this is also waste material of distillery industry then distillery effluent we also use this material we find that that groundnut De oiled cake is better as compared to yeast extract, so yeast extract is very costly material we can replace that then the process will be quite cheaper.

The acidogenic we tried it this the pure organism we try to replace with a mixed acidogenic mixed culture where we can use the unsterile conditions and using unsterile condition also we get better results in case of groundnut De oiled cake, then klebsiella pneumoniae with cane molasses and groundnut De oiled cake with 50 litre reactors we get 2.9 this is all 2 litre reactors and then Thermophilic mixed culture we use the starchy waste water, we get 3.9 litre per litre of the fermentation broth.

Then when we use cane molasses we get 4.8 distillery effluent is 2.9, so here we observed that distillery effluent get the comparatively less hydrogen production as compared to cane molasses by continuous system we operated klebsiella pneumoniae and acidogenic mixed culture and Thermophilic mixed culture and we find our production at the different level 20 litres with 3.3 litre per litre per hour distillery effluent is quite less is 0.3, 0.8 in case of

acidogenic mix culture, 3.38 litre per litre per hour and Thermophilic mixed culture is 1 point 6 litre per litre per hour.

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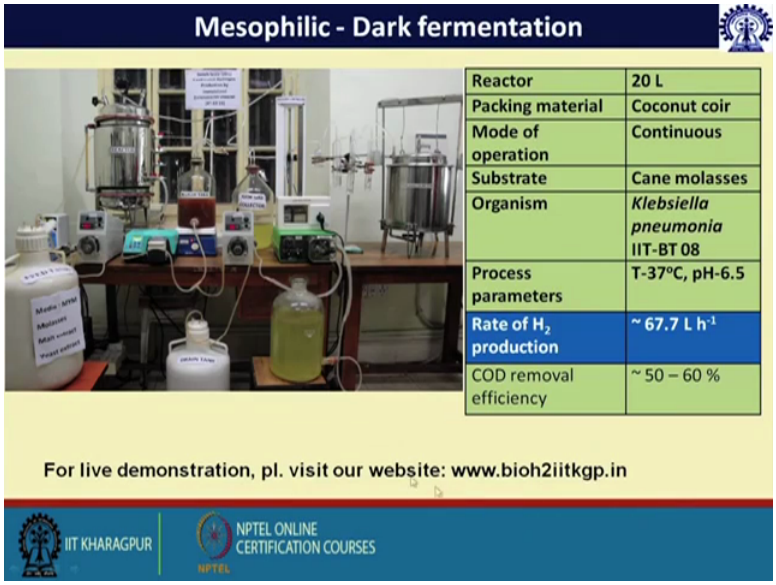

Now here this is the 20 litre reactor we operated this is the custom design custom made reactor we designed and fabricated there locally and we operated this fermenter and by operating the fermenter we get the hydrogen production about 1667.7 litre per hour, we use the immobilized column I discuss the immobilize column has the advantage as compared to this suspended cell culture because immobilized column you can fix the cell on the solid matrix and pass the substrate and it will be converted to hydrogen. So the cell mass leaking from the system would be very less.

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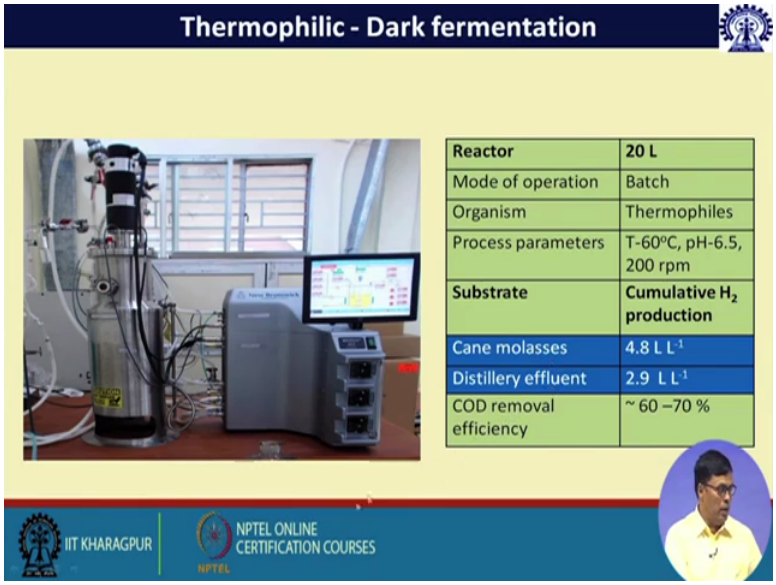

When we use the Thermophilic organism then this is the Thermophilic dark fermentation process we find cane molasses again this is the control fermenter but previous one is custom made fermenter that we designed by ourselves this is supplied by the (()) (25:49) and we get 4 .8 litre per litre of the fermentation broth in case of cane molasses and distillery effluent and 2.9 litre per litre fermentation broth and COD removal is 60 to 70 percent

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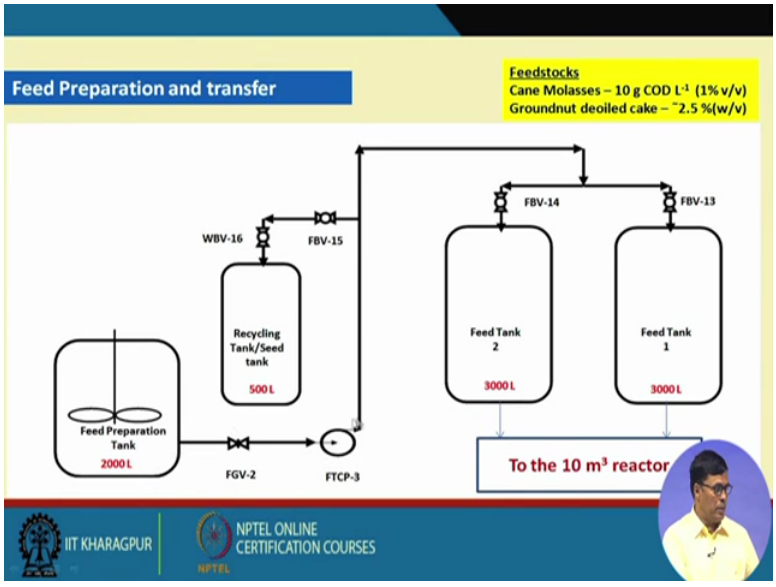

And this is the 10 cubic metre pilot plant that we operated this is the 10 cubic metre reactor, this is the 500 litre, this 50 litre, this is 3 cubic metre reactor you can see this is located here and we operated this successfully and we prepared the seed culture here and we feed

preparation with here and 2000 litre reactor we transferred in this reactor and then finally we transfer into the 10 cubic metre reactor.

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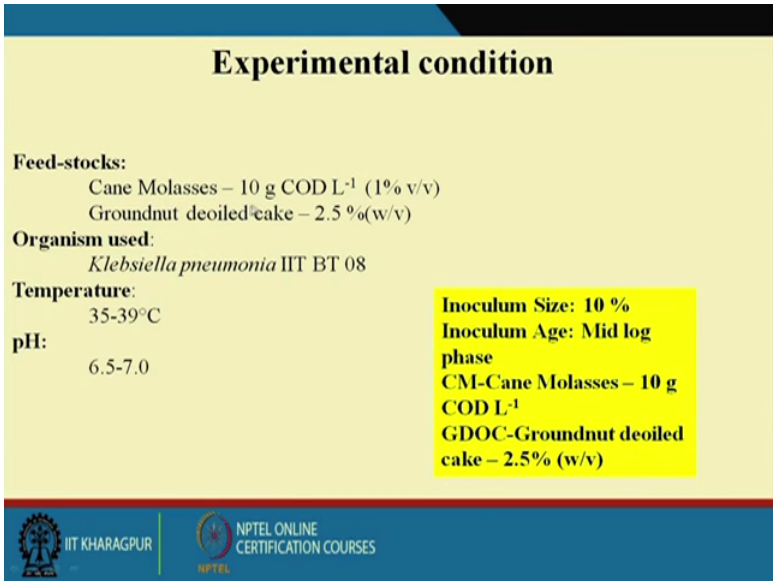

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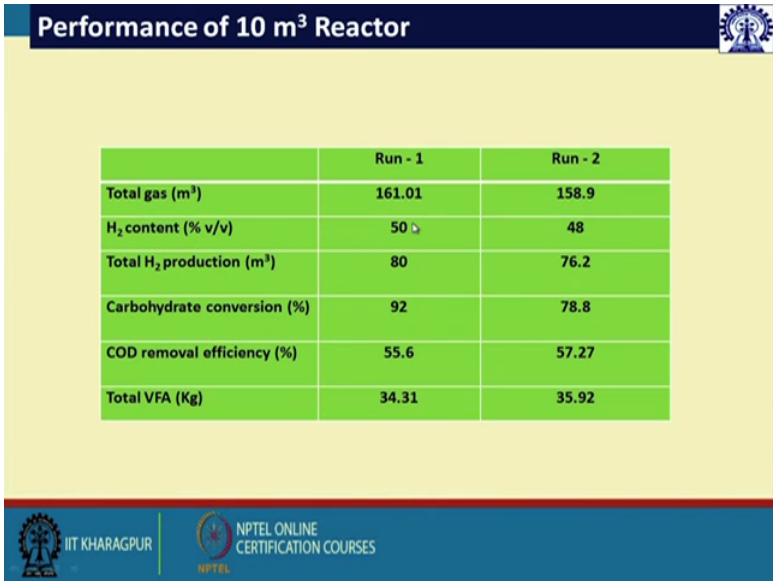

And this cane molasses and groundnut De oiled cake we use 2.5 per cent and finally we reduced to 1.5 per cent and we find that gas hydrogen content is about 50 cubic metre run1 and the second run we got it 48 cubic metre that is if volume by volume you can say 4.8 volume per volume of the fermentation broth.

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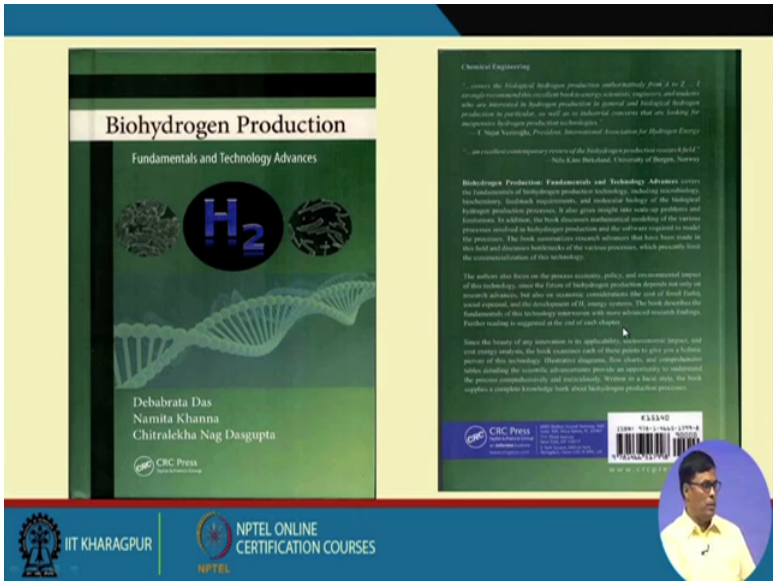

Now there are two books, one is on Biohydrogen production fundamentals and technology advances this is published by CRC press this is kind of textbook if anybody interested to develop the knowledge on this Biohydrogen production process I refer this book you should go through book I think you will learn that how this process can be operated.

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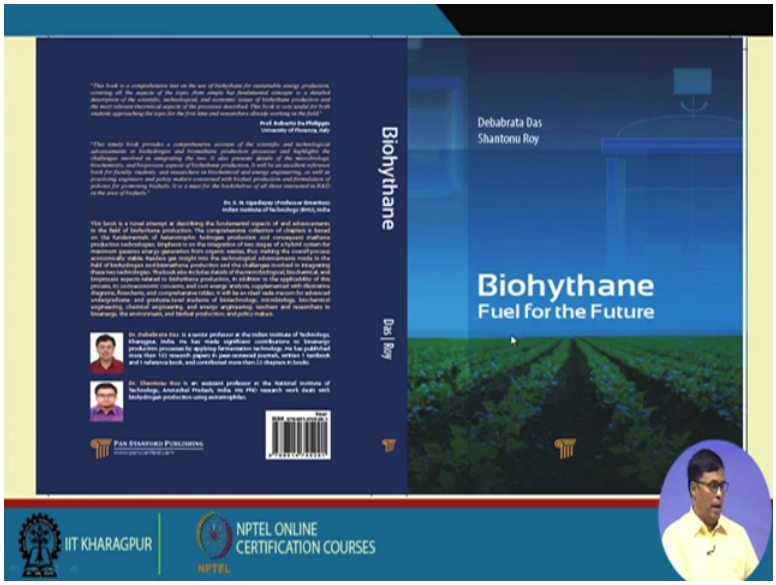

Another book we recently published, this is the first book on Biohythane in the world, Biohythane means hydrogen followed by methane production we find this process is sustainable and this will be with us for the future.

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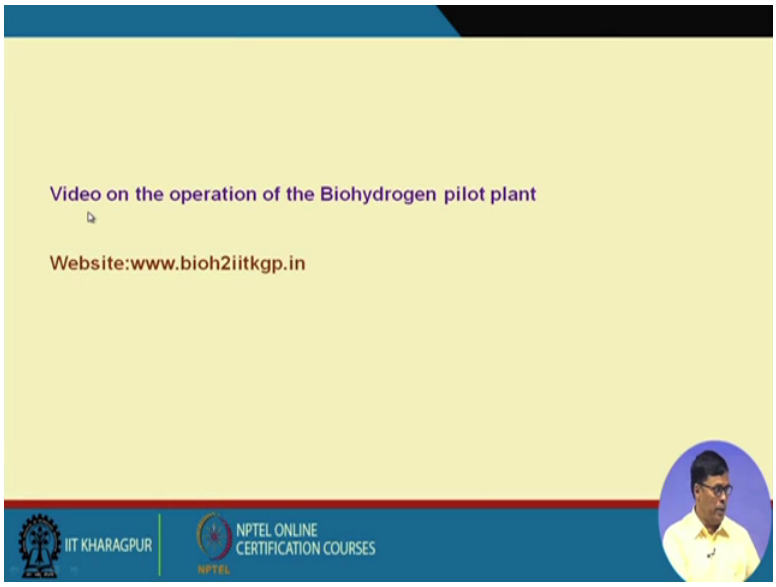

Now after this I shall we will operate a video on the operation of Biohydrogen pilot plant and anybody interested to get the information more on the process they can visit our website www. biohydrogenbioh2iitkgp.in.

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This video takes us on a virtual tour, demonstrating the operation and the functioning of Biohydrogen pilot plant at IIT Kharagpur. The bioprocess engineering lab has been working for the last 10 years on the development of various green technologies to produce hydrogen from organic wastes among them dark fermentation appeared to be the most promising technology.

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The plant is equipped with various feed tanks each of a capacity of 3 metre cube, it is also equipped with the seed tank of capacity 500 litre, a bioreactor with a working capacity of 10 metre cube, a gas collector with a collection capacity of 2 metre cube. Different accessories such as pumps, pH monitoring and control system, a flow metre, a steam generator, hot water bath, a compressor and a chiller.

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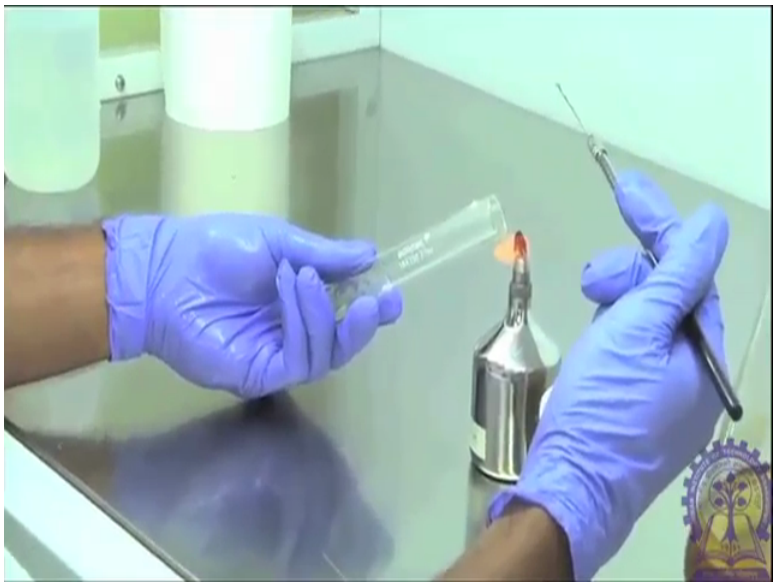

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To start the operation we use an indigenous lab Isolate klebsiella pneumoniae IIT BT08 which is a facultative gram negative anaerobe, our lab scale experiments demonstrated that this strain could generate up to 2.8 litres of hydrogen from 1 metre of organic waste. To initiate the plant operation pure cultures of this trade are carefully cultured under aseptic conditions. The inoculums preparation for the plant is carried out in several stages, in the first stage a loop full of the culture is transferred into 500 mL of nutrient broth media this nutrient broth media is a complex nutrient rich media that supports the growth of the organism.

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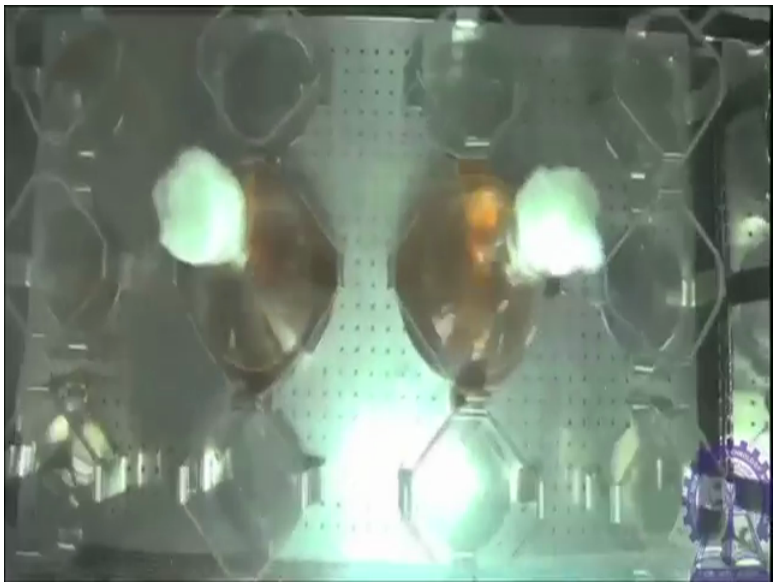

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This organism takes up to 10 hours to attain the mid lock phase which is ideal for the hydrogen production. In order to facilitate the growth of this organism this media is kept in a incubator shaker up to 10 hours, following the growth which can be visually seen in terms of turbidity of the media, this culture further acts as inoculum for the subsequent bioreactors.

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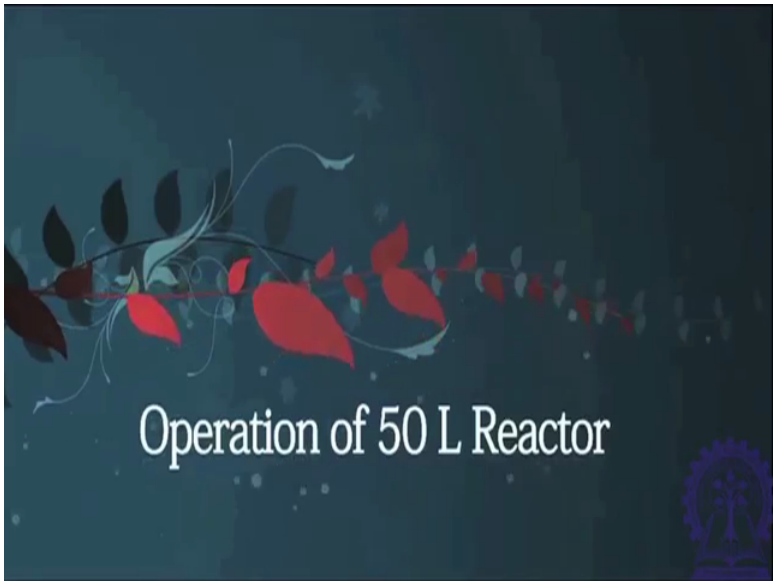

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In the stage two this organism is further added as inoculum to a 50 litre reactor the 50 litre reactor is equipped with a hot water bath to maintain the temperature and the gas collector through the inoculation port of the 50 litre reactor, media is carefully added into the reactor. Following this inoculation is done into the reactor where a concentration of 10 per cent volume by volume is maintained, this reactor is passed with nitrogen to maintain anaerobic condition and it is constantly monitored for its hydrogen production.

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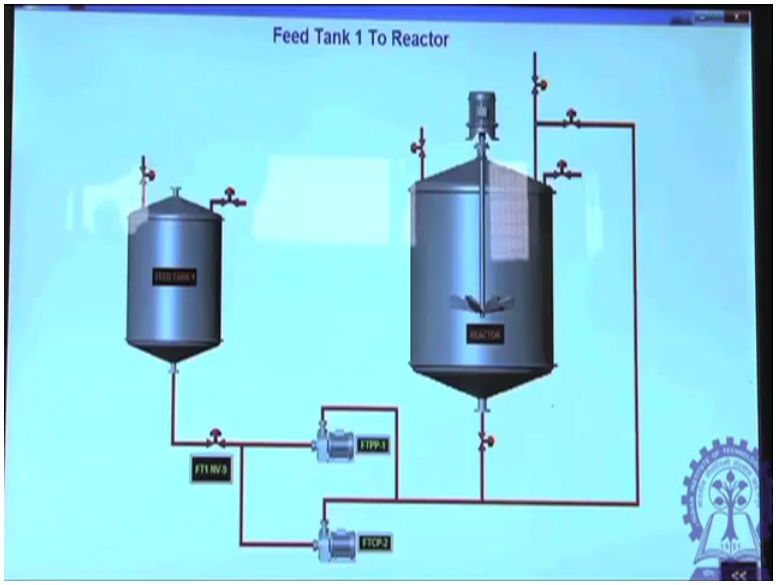

The plant is also equipped with a PLD controller which enables manual controls of individual processes of the plant, further an online monitoring system of various parameters such as temperature pH and the control of processes such as feed transfer to various reactors and the nitrogen supply etc is possible through the software.

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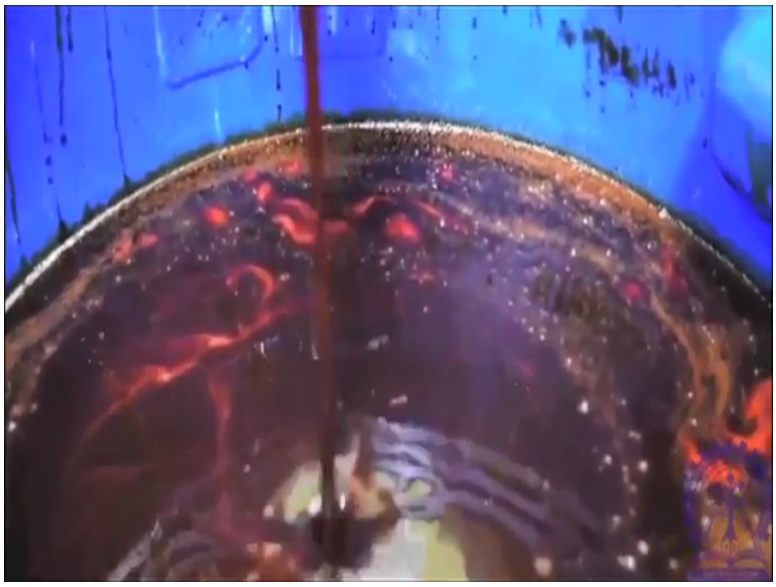

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For the media preparation cane molasses is used as the primary substrate, cane molasses is a byoproduct of the sugar processing industry. It is a viscous liquid consisting of approximately 40 to 50 per cent of sucrose, so to initiate the operation cane molasses at a concentration of 1 percent volume by volume is added to the feed preparation tank under constant mixing; in addition to the cane molasses groundnut De oiled cake is also added as a nutritional supplement to the media.

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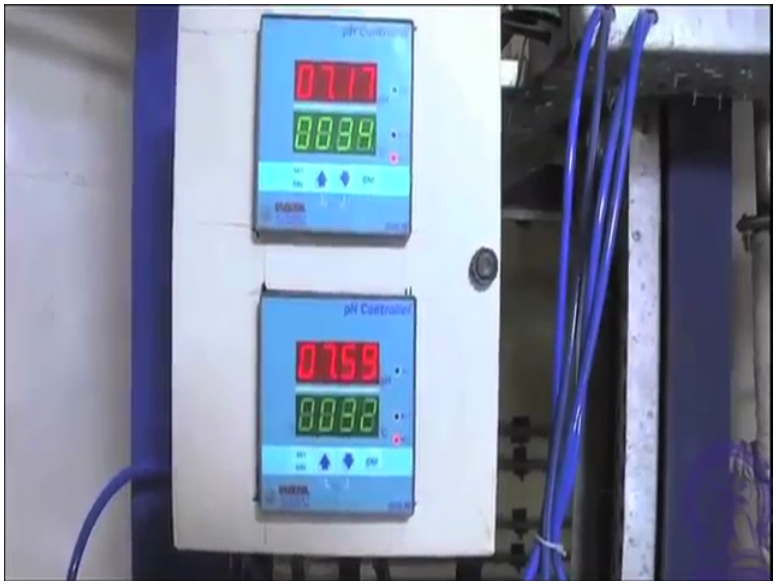

Groundnut De oiled cake is a byproduct of the agro processing industry; this product is rich in nitrogen, minerals and vitamins. Our lab scale experiments have shown that addition of groundnut De oiled cake to the media have significantly improved the hydrogen production. Upon thorough mixing it was essential to adjust the pH of the media, the plant is equipped with a pH dosing and a control system the final pH of the media was adjusted to 7.

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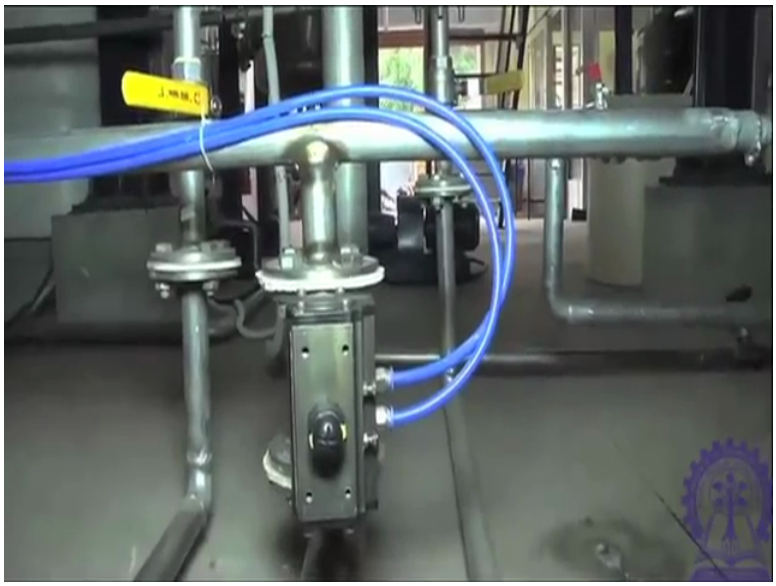

The feed in the feed preparation tank is transferred to various reactors with the help of pumps, in order to start the operation of the 10 metre cube reactor feed from the feed preparation tank is initially transferred to the feed tank 2 with the help of a centrifugal pump, nitrogen is passed in the feed tank 2 for sufficient time to maintain anaerobisity, this feed is further transferred from the feed tank2 to the 10 metre cube reactor.

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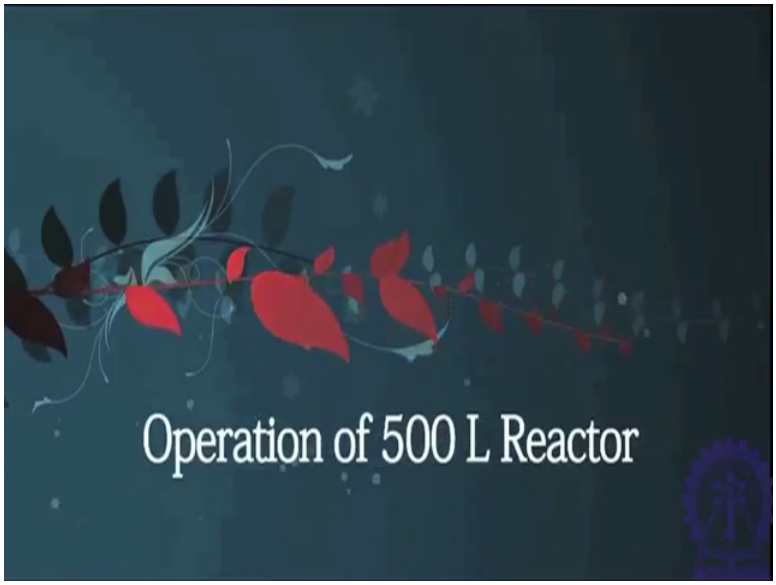

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The culture grown in the 50 litre reactor serves as the inoculum for the 500 litre reactor, once the maximum rate of hydrogen production is obtained in the 50 litre reactor, the culture in this reactor is carefully transferred into the 500 litre reactor with the help of a peristaltic pump. Following the transfer the 500 litre reactor is passed with nitrogen for sufficient time to maintain anaerobic condition.At regular intervals constant recirculation of media is done using a circulation pump to maintain homogenity in the 500 litre reactor.

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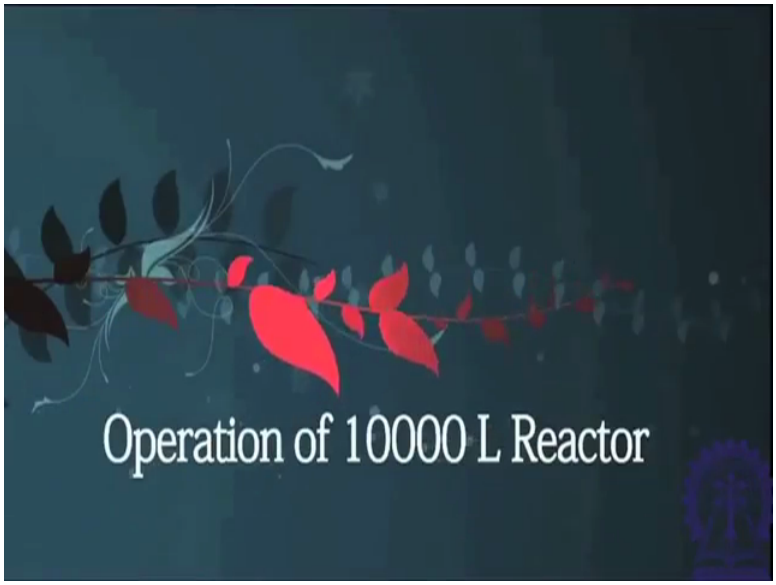

The culture that is grown in the 500 litre reactor further serves as the inoculum for the 10 meter cube reactor, after the media is transferred to the 10 meter cube reactor inoculation is done from the 500 litre reactor.

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Constant sampling of the 10 meter cube reactor is done to monitor parameters such as pH, COD and the volatile fatty acids, gas production can be observed in the reactor within 3 hours of inoculation, constant bubbling can be observed inside the reactor once the gas production starts. The gas produced in the reactor flows through the pipelines and finally reaches the gas collector; there is a provision to collect the gas in the gas collection tank for further use.

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A flow meter installed along the pipeline constantly measures the production rate of the gas inside the reactor. In case of excess gas production the gas can be exhausted through the flame arrested to the atmosphere, gas chromatography is an instrument which is used to analyse the purity of gas. An online gas analysing system is equipped with gc to check the composition of the gas produced in the reactor.

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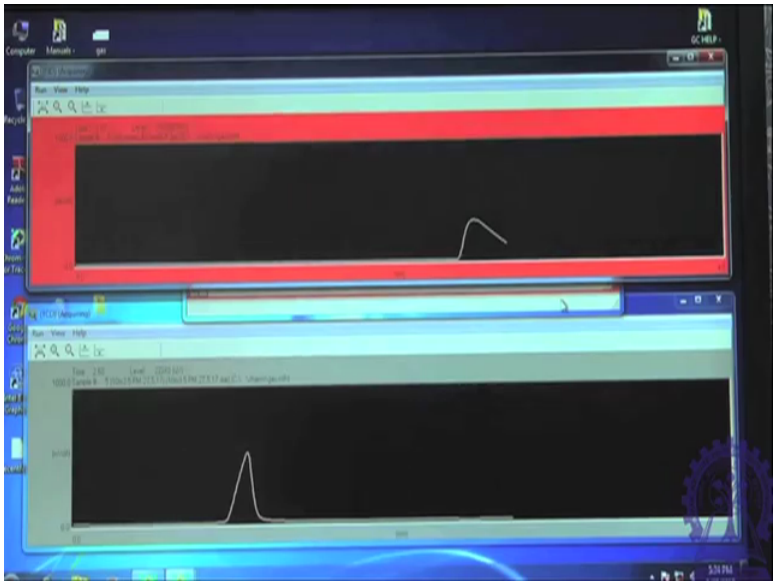

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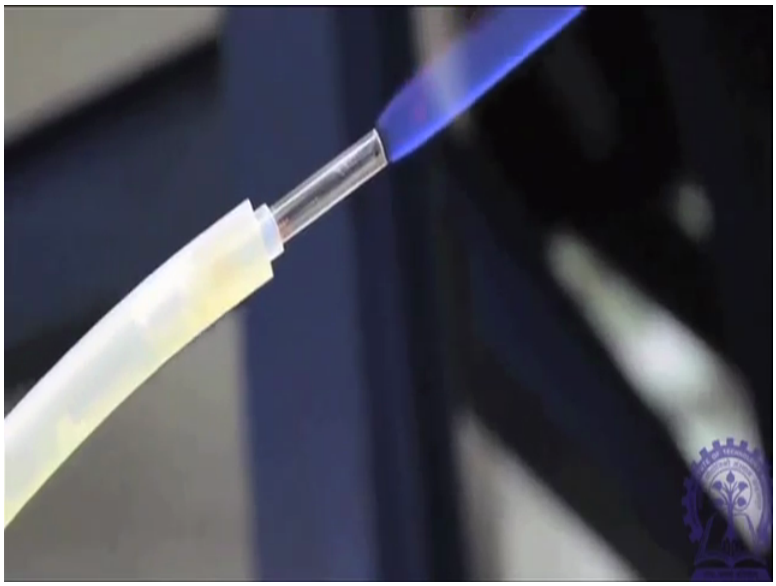

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The gas chromatograph is equipped with a thermal conductivity detector and a flame ionization detector the thermal conductivity detector in the gas chromatograph detects hydrogen as indicated in the chromatogram. Further some part of the gas was collected into a gas collector to manually check the flame properties upon ignition a blue flame was observed indicating the presence of hydrogen in the gas mixture. We hope that our endeavour can contribute for a cleaner energy generation and simultaneous bioremediation for the future generations, thank you.