Biomass Conversion and Biorefinery
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Module 06
Lecture-17
Details of Various Processes

Good morning students, today is lecture 2 under module 6. As you know that we are discussing various microbial conversion processes and in the last class we have discussed in brief the different processes and different types of equipments and the products under that lecture. So, in today's class we will be basically discussing about the processes in little detail - anaerobic digestion and fermentation. So, let us begin anaerobic digestion.

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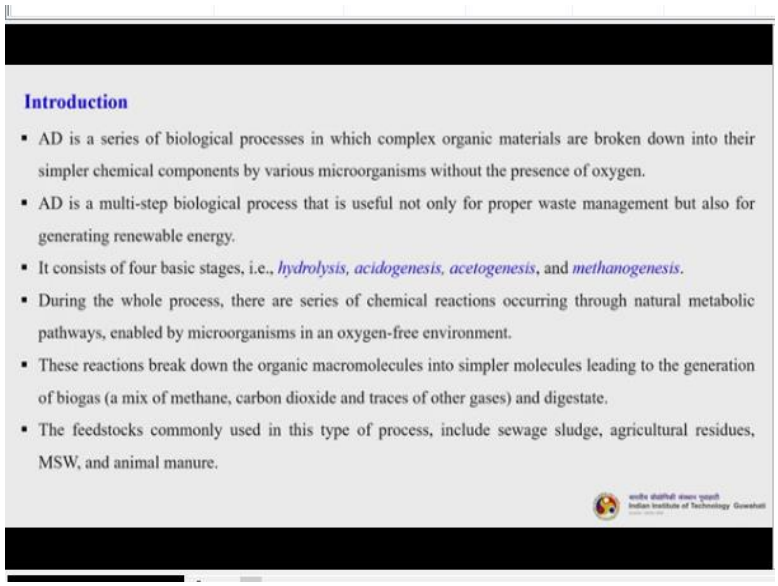

Anaerobic digestion is a series of biological processes in which complex organic materials are broken down into their simpler chemical components by various microorganisms without the presence of oxygen. It is a multi-step biological process that is useful not only for proper waste management but also for generating renewable energy like various types of biofuels. It consists of 4 basic stages hydrolysis, acidogenesis, acetogenesis and methanogenesis.

During the entire process there are series of chemical reactions occurring through natural metabolic pathways enabled by microorganisms in an oxygen free environment. Now these reactions break down the organic macromolecules into simpler molecules leading to the generation of biogas which is a mixture of methane, carbon dioxide and traces of other gases like hydrogen and carbon monoxide.

And apart from that the digestate or the solid part**.** So, the feedstocks that are commonly used in this type process include sewage sludge, agricultural residues, municipal solid residue, animal manure and there can be many other feedstocks also.

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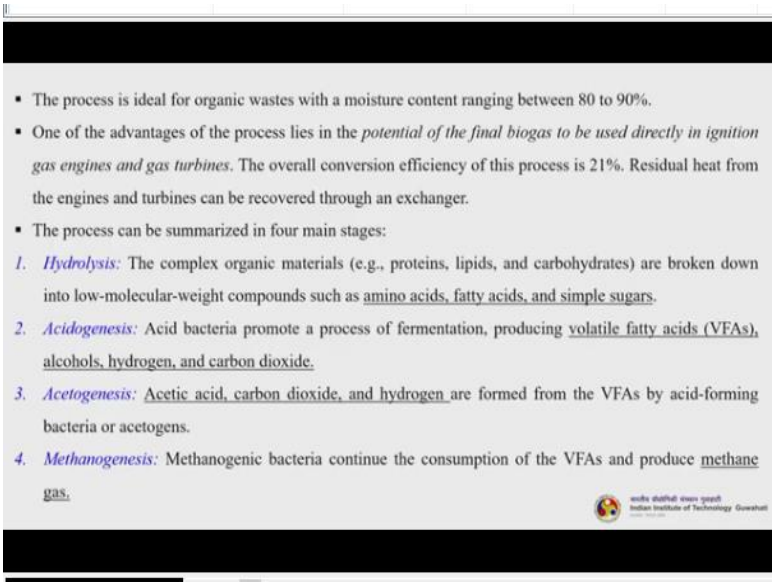

Now the process is ideal for organic waste with a moisture content ranging between 80 to 90%. One of the advantages of the process lies in the potential of the final biogas to be used directly in ignition gas engines and gas turbines. The overall conversion efficiency of this process is 21%, residual heat from the engines and turbines can be recovered through an exchanger. Now the process can be summarized in 4 main stages.

First is hydrolysis. So, in hydrolysis the complex organic materials for example proteins, lipids and carbohydrates - they are broken down into low molecular weight compounds such as amino acids, fatty acids and simple sugars. Under acidogenesis the acidic bacteria promote a process of fermentation producing the volatile fatty acids. Apart from volatile fatty acids there are alcohols, hydrogen and carbon dioxide also get produced. Then acetogenesis, here acetic acid, carbon dioxide and hydrogen are formed from the volatile fatty acids by acid forming bacteria, they are known as also acetogens. And in the last which is the most important step is the methanogenesis, here the methanogenic bacteria continue the consumption of the volatile fatty acids and produce the methane gas.

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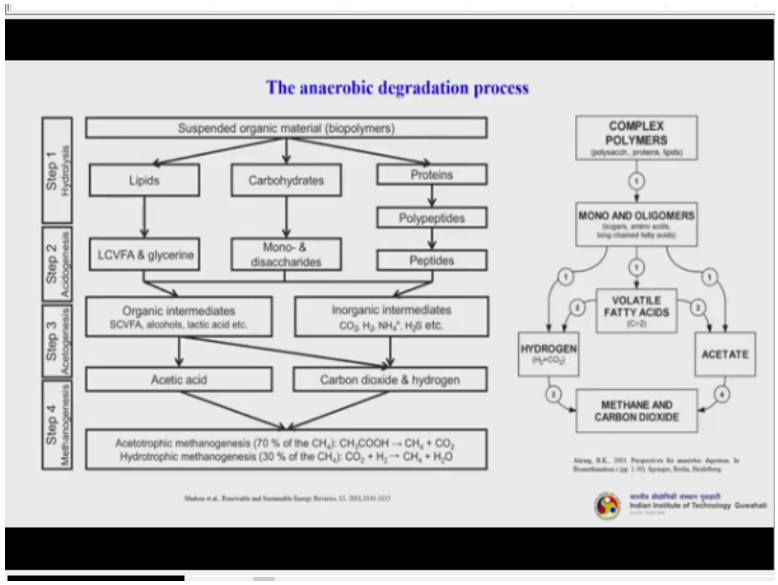

We will try to see in a nutshell - if you recall last class I have shown you one sketch, here this is little in an elaborate way it is being presented. So, let us quickly glance through it. So, the first step is hydrolysis here. The organic materials you can call them, group them and term them as biopolymers - they are getting converted under lipids, carbohydrates and proteins to various routes.

If you look at the first route the lipids are getting converted to LCVFA- the low carbon volatile fatty acids and glycerine**.** Now that can be converted to organic intermediates and alcohols, lactic acid - further to acetic acid by the step 2 and step 3. So, up to this. Now carbohydrates can be converted into mono and disaccharides and then they also can be converted either into organic intermediates or inorganic intermediates**.**

Similarly, the proteins get converted to polypeptides and again peptides and then again these peptides can be converted to either organic intermediates or inorganic intermediates. Now please understand that when I am telling that this conversion is happening it depends upon what type of microorganism is being present and what they are converting**.** So, that is the most important thing apart from other things.

Now before you come to the last one which is called the methanogenesis**.** So, you can see that methanogenesis can happen via 2 different routes, one is this acetate route - acetic acid route, another is the carbon dioxide and hydrogen route. So, either acetic acid can convert to methane and carbon dioxide via this reaction or the carbon dioxide plus hydrogen can be converted to methane plus water.

So, please understand that the final reaction again proceeds mostly by the methanogens via 2 different routes. Now if we use the acetotropic methanogens - so mostly this is for the 70% of the methane that is getting produced, this is the route, then we get the acetate route. And if we are using the hydrotropic methanogens then the next 30% of the entire methane that is being produced is coming from this particular route. So, the entire scheme is again presented there in a very a brief way.

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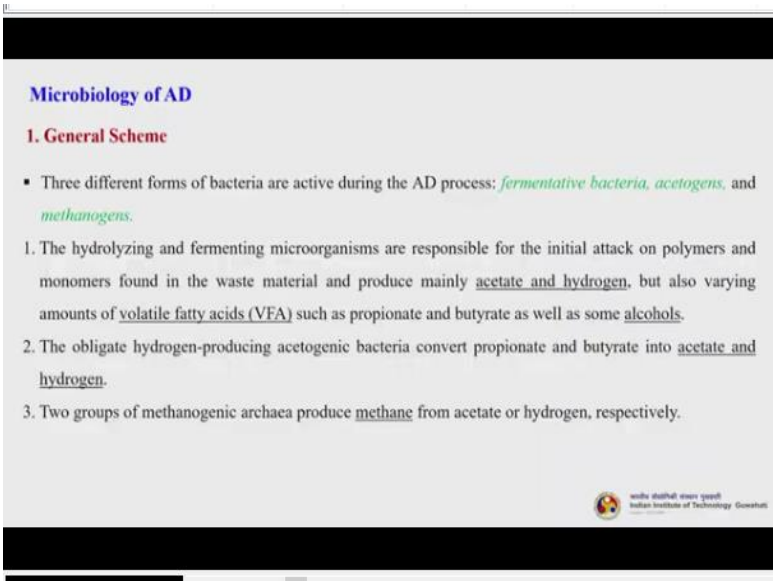

So, now we will try to understand the microbiology of the entire anaerobic digestion process. So, let us first talk about the general scheme. So, 3 different forms of bacteria are active during the AD process. So, they are fermentative bacteria, they are acetogens and methanogens. So, these are the main microflora which are responsible for the entire anaerobic digestion process for different reactions.

Now the hydrolyzing and fermenting microorganisms are responsible for the initial attack on polymers and monomers found in the waste material and produce mainly acetate and hydrogen, but also varying amounts of volatile fatty acids such as propionate and butyrate as well as some alcohols. Now the obligate hydrogen-producing acetogenic bacteria convert this propionate and butyrate into again acetate and hydrogen. So, 2 groups of methanogenic archaea produce methane from the acetate or hydrogen respectively.

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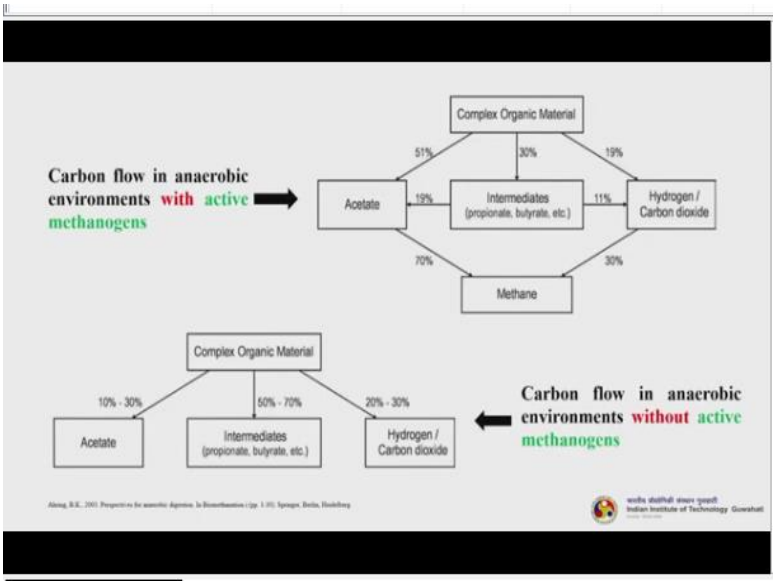

So, this is again we will try to understand. This is a schematic representation of how the carbon is flowing in the anaerobic environment with methanogen. So, this is for with methanogens and this is without methanogen. So, let us try to understand what is happening with the methanogens. So, when the complex organic materials are getting degraded in the presence of methanogens then 3 things will happen.

So, usually if you see this particular route from this side the left side, you can see 51% is getting converted through this route. So, the organic materials are degraded to acetate, acetate is degrading to methane. So, as I told you 2 slides before that the 70% of the methane that is produced from the anaerobic digestion comes via this route - acetate**.** Apart from that 51%, 30% is again converted to propionate and butyrate, which are further again converted to either acetate or hydrogen and carbon dioxide depending upon the process condition as well as depending upon the type of microorganisms present. And the next 19% is directly getting converted to hydrogen and carbon monoxide and this 30% of the entire methane that is getting produced coming via hydrogen plus carbon dioxide reaction. Now this entire scheme is when the methanogens is present.

Now when methanogens are not present then what is happening to the carbon cycle**?** So, here the complex material are getting converted to acetate, intermediates and hydrogen and carbon dioxide in various of course percentage and further processing is not happening because there are no methanogens available which will degrade these compounds into methane and carbon monoxide**.**

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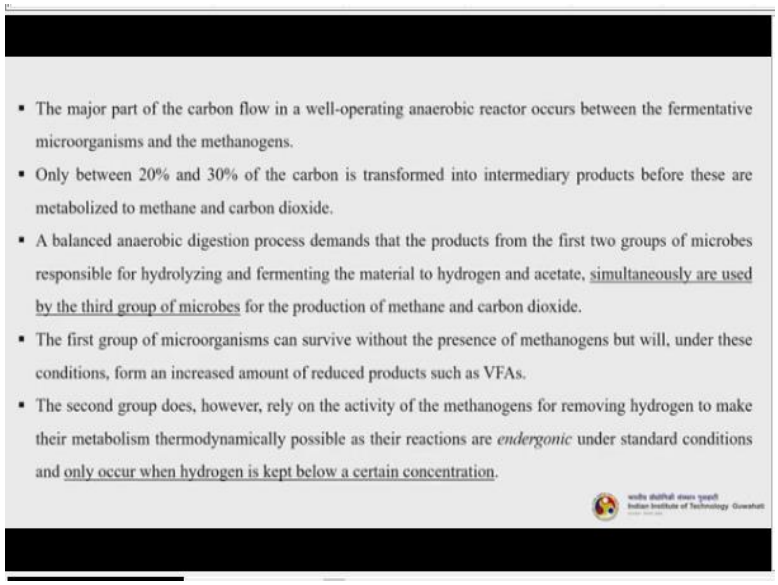

Now this scheme we have understood; now we will go ahead and try to understand in a gist that what the scheme is all about. So, the major part of the carbon flow in a well operating anaerobic reactor occurs between the fermentative microorganisms and the methanogens. Only between 20 to 30% of the carbon is transferred into intermediary products before these are metabolized into methane and carbon.

So, this is what I have shown you - the intermediate products are propionate, butyrate etc. Now again these will be converted either to acetate or hydrogen and carbon dioxide route**.** Before finally being converted to methane. Now a balanced anaerobic digestion process demands that, the products from the first 2 groups of microbes responsible for hydrolyzing and fermenting the material to hydrogen and acetate, simultaneously are used by the third group of microbes for the production of methane and carbon dioxide. So, this is very important. Now the first group of microorganisms can survive without the presence of methanogens but will under these conditions form an increased amount of the reduced products such as volatile fatty acids. The second group does however rely on the activity of methanogens for removing hydrogen to make their metabolism thermodynamically possible as their reactions are endergonic under standard conditions and only occurs when the hydrogen is kept below a certain concentration. Now endergonic reactions are such reactions in which the heat is actually absorbed. So, the net change of free energy is always positive.

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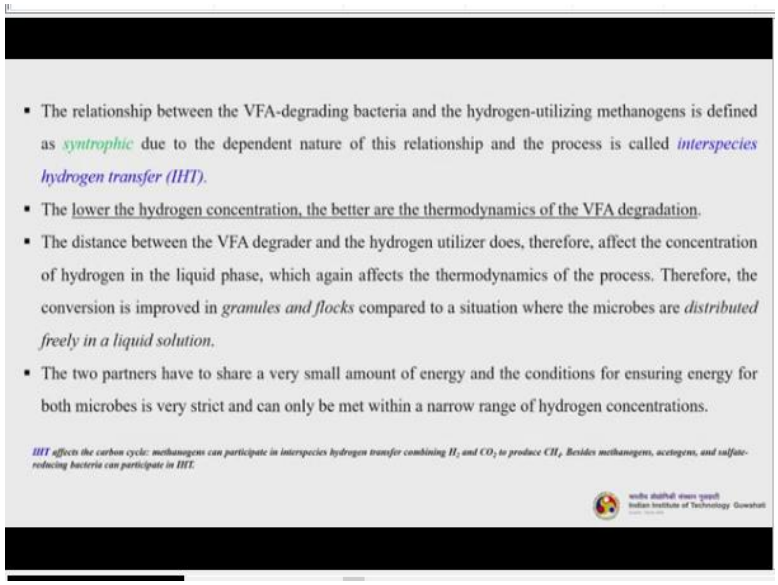

The relationship between the volatile fatty acid degrading bacteria and the hydrogen utilizing methanogens is defined as syntrophic due to the dependent nature of this relationship and the process is called interspecies hydrogen transport. Now syntrophic is a process or we can say that it is a technique by which even the microorganisms especially in such anaerobic digestion process coexist.

So, in this process let us say there are 2 different types of microorganisms are present in a syntrophic relationship; then basically they are syntrophic because they are co-feeding each other. So, the products are generated by one microorganism is being consumed by the other microorganisms. So, they are interdependent on each other, they are not actually parasite, they are interdependent and both are actually feeding on the products of each other**.** So, the interspecies hydrogen transfer actually affects the entire carbon cycle - I have mentioned here.

So, methanogens can participate in the interspecies hydrogen transfer combining hydrogen and carbon dioxide to produce methane. So, besides methanogens, acetogens and sulphate reducing bacteria can also participate in the IHT. So, the lower the hydrogen concentration, better are the thermodynamics of the volatile fatty acid degradation. So, the distance between the VFA degrader and the hydrogen utilizer that eventually affects the thermodynamics of the process.

Therefore, the conversion is improved in granules and flocks compared to a situation where the microbes are distributed freely in liquid solution. Essentially what is the meaning of that?

Microorganisms are grown in granules and flocks and when they are suspended freely in the liquid solution without forming flock**s**, the entire thermodynamics inside the process and the hydrogen utilization, actually the IHT is getting affected.

Two partners have to share a very small amount of energy and the conditions for ensuring energy for both microbes is very strict and can only be met within a narrow range of hydrogen concentrations.

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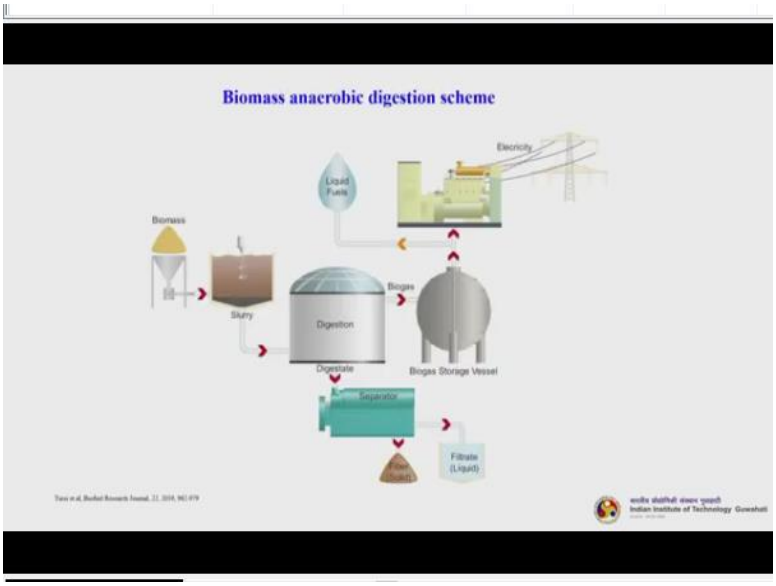

So, this is a schematic representation of the biomass anaerobic digestion scheme. It is a general representation. So, you can see just we will quickly glance. So the biomass, it has to be pre-processed - so you may have to sometimes chop it - mechanical pre-processing, then you can go for some slight thermal pre-processing where you remove moisture and all.

Bring them to a desired particle size and bring them to a desired moisture content before you feed them to the digester. Then they are made into slurry. Now you do not dump the entire solid biomass under the digester**.** So, you usually make them into a slurry. This slurry goes to the digester. Now here the anaerobic digestion is happening, so you have to give inoculum, if required, you have to supply certain other micro nutrients or nutrients and maintain the proper temperature inside the digester so that the anaerobic digestion happens. And it's strictly anaerobic process - dark fermentation. Now once the process starts happening, slowly you will see that day 3, day 4, day 5 and after that so biogas will start coming. Now this biogas whatever will come will be collected in a biogas storage vessel. From here you can

either convert it to liquid fuels by compressing it or you can send it to the gas turbine system where you can generate electricity directly.

And whatever left out here - the digestate or semi solid type of with having some moisture in that - it can go to a separator where you get the filtrate liquid, this also can be converted to some other value added products and then the fiber or solid again we can process it under thermochemical conversion process or you can use as cattle feed and some other value added products.

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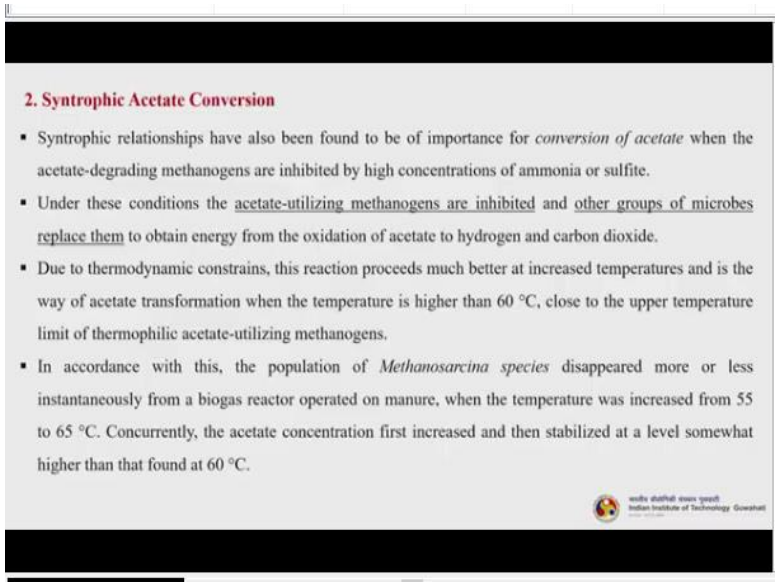

The second thing is that, this is what we talked about with the general scheme. Now we are discussing about the syntrophic acetate conversion process. Now the syntrophic relationships have also been found to be importance for the conversion of acetate when the acetate degrading methanogens are inhibited by concentrations of ammonia or sulfite. So, we discussed syntrophic for the IHT - interspecies hydrogen transfer.

Now we are discussing that, syntrophic relationship also having some importance when we talk about acetate conversion. Now under these conditions the acetate utilizing methanogens are inhibited and other groups of microbes replace them to obtain energy from the oxidation of acetate to hydrogen and carbon dioxide. Due to thermodynamic constants this reaction proceeds much better at increased temperatures and is the way of acetate transformation when the temperature is usually higher than the 60 degree centigrade.

So, that is the upper limit of the thermophilic acetate utilizing methanogens. So, in accordance to this, the population of *Methanosarcina species* which is one of the methanogen species disappeared more or less instantaneously from a biogas reactor operated on manure when the temperature was increased from 55 to 65 degree centigrade. Now concurrently the acetate concentration first increased and then stabilized at a level somewhat higher than that found in the 60 degree centigrade.

So, clearly telling us that beyond 60 degree centigrade some of these thermophilic activities are happening and the acetate utilizing methanogens are inhibited.

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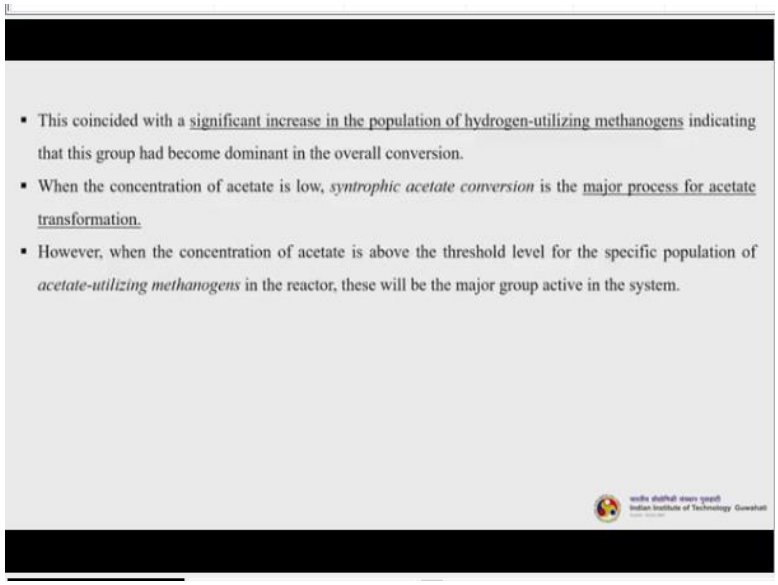

So, this coincided with a significant increase in the population of hydrogen utilizing methanogens indicating that this group had become dominant in the overall conversion. So, there will be more hydrogen production. When the concentration of acetate is low, syntrophic acetate conversion is the major process for acetate transformation. However, when the concentration of acetate is above the threshold level for the specific population of acetate utilizing methanogens in the reactor, these will be the major group active in the system.

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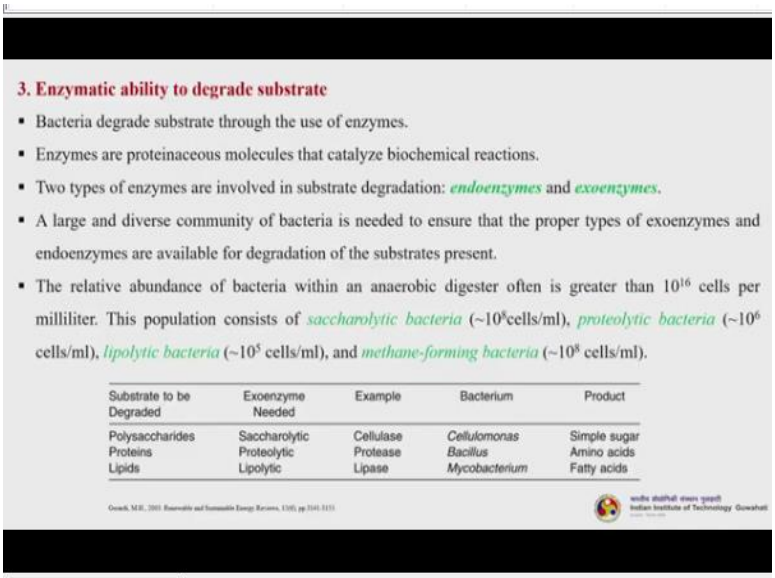

The next is enzymatic ability to degrade substrate. Now bacteria degrade substrate through the use of enzymes. Enzymes are proteinaceous molecules that catalyze biochemical reactions. Two types of enzymes are involved in the substrate degradation: endoenzymes and exoenzymes. Now a large and diverse community of bacteria is needed to ensure that proper types of exoenzymes and endoenzymes are available for the degradation of the substrates present.

The relative abundance of bacteria within an aerobic digester often is greater than 10^{16} cells per millilitre. This population consists of a saccharolytic bacteria, proteolytic bacteria, lipolytic bacteria and methane-forming bacteria. So, the table below gives an understanding about that substrates to be degraded, different types of exoenzyme that is required and examples.

Now we can see one case. Let us see the first one, the polysaccharides. So, this is the substrate that is getting degraded and the exoenzyme you need to degrade this substrate is saccharolytic exoenzyme. An example is cellulase. Cellulase is exactly it is the enzyme, that will do the degradation and the bacterium that is required is the *Bacillus* species, the *Cellulomonas* species and the product will be the simple sugar. So, similarly it is there for proteins and lipids which will be converted into amino acids and fatty acids by *Bacillus* and *Mycobacterium* species.

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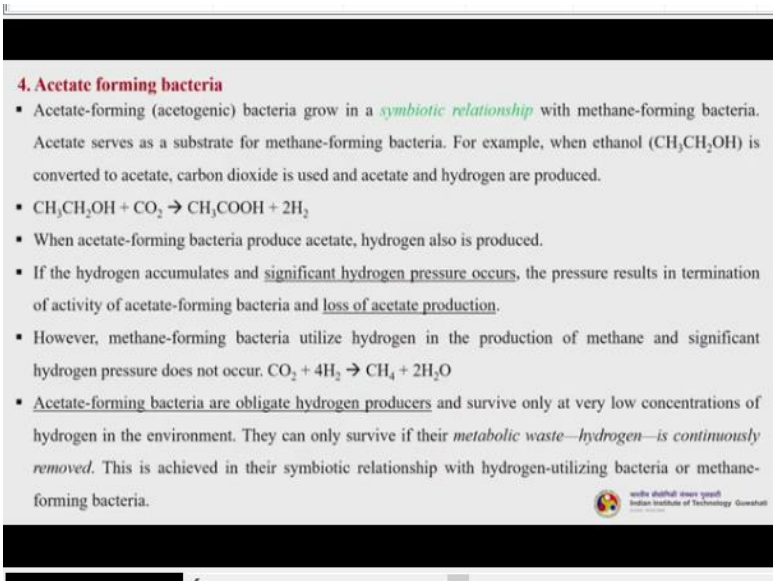

Next is acetate forming bacteria. Acetate forming bacteria or acetogenic bacteria grows in a symbiotic relationship with methane forming bacteria. Acetate serves as a substrate for methane forming bacteria. For example, when ethanol is converted to acetate, carbon dioxide is used and acetate and hydrogen are produced. So, this is the reaction:

$$CH_3CH_2OH + CO_2 \rightarrow CH_3COOH + 2H_2$$

When acetate forming bacteria produce acetate hydrogen is also produced. If the hydrogen accumulates and significant hydrogen pressure occurs, the pressure results in the termination of activity of acetate forming bacteria and loss of acetate production. So, this has to be controlled in the fermenters. However, methane forming bacteria utilize hydrogen in the production of methane and significant hydrogen pressure does not occur:

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

Acetate forming bacteria are obligate hydrogen producers and survive only at very low concentrations of hydrogen in the environment, they can only survive if their metabolic waste that is hydrogen is continuously removed or consumed by other microflora. Now this is achieved in their symbiotic relationship with hydrogen utilizing bacteria and/or methane forming bacteria.

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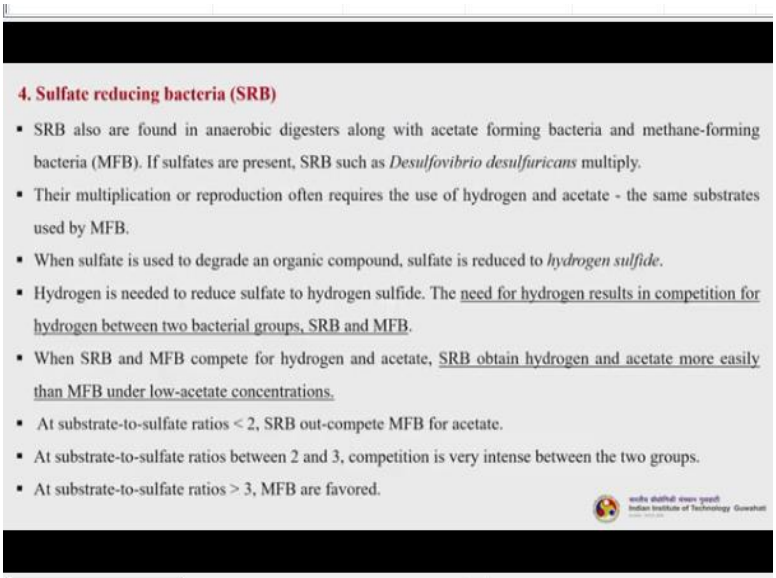

So, the next is sulphate reducing bacteria. So, SRB are also found in anaerobic digesters along with acetate forming bacteria and methane forming bacteria. If sulphates are present, then SRB such as *Desulfovibrio disulfuricans* multiply. So, this is one type of sulfate reducing bacteria. Their multiplication or reproduction often requires the use of hydrogen and acetate the same substrates used by the methane forming bacteria methanogens.

When sulfate is used to degrade an organic compound, sulphate is reduced to hydrogen sulfide. Hydrogen is needed to reduce sulfate to hydrogen sulphide. The need for hydrogen results in competition for hydrogen between 2 bacterial groups SRB and MFB. When SRB and MFB compete for hydrogen and acetate, SRB obtain hydrogen and acetate more easily than MFB under low acetate concentrations.

At substrate-to-sulfate ratios less than 2, SRB out compete MFB for acetate and at substrate-to-sulfate ratios between 2 and 3, competition is very intense between the 2 groups and when substrate-to-sulfate ratio is greater than 3, the methanogens are favoured.

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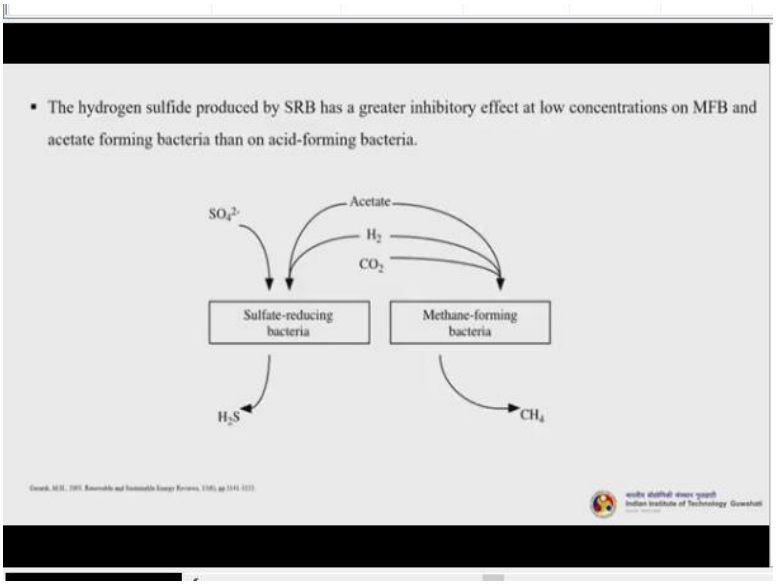

So, the hydrogen sulfide produced by SRB has a greater inhibitory effect at low concentrations on MFB and acetate forming bacteria than acid forming bacteria. This is one of the simple representation scheme that how the sulphate reducing bacteria and methane forming bacteria are surviving in a synergistic relationship between them - symbiotic. So, you can see that the sulphate is being reduced by the sulphate reducing bacteria to hydrogen sulfide.

And they are also consuming the hydrogen and acetate that is getting produced from the methane forming bacteria, as we have understood, then beyond certain limits of the hydrogen inside the fermenter or anaerobic digester the methane forming bacteria will cease to do their methanogenic activities. So, the hydrogen has to be continuously removed. Now in this symbiotic relationship the hydrogen is getting consumed by the sulphate reducing bacteria to hydrogen sulfide and the level of hydrogen is maintained in such a way that the methanogenesis reaction is getting favoured**.**

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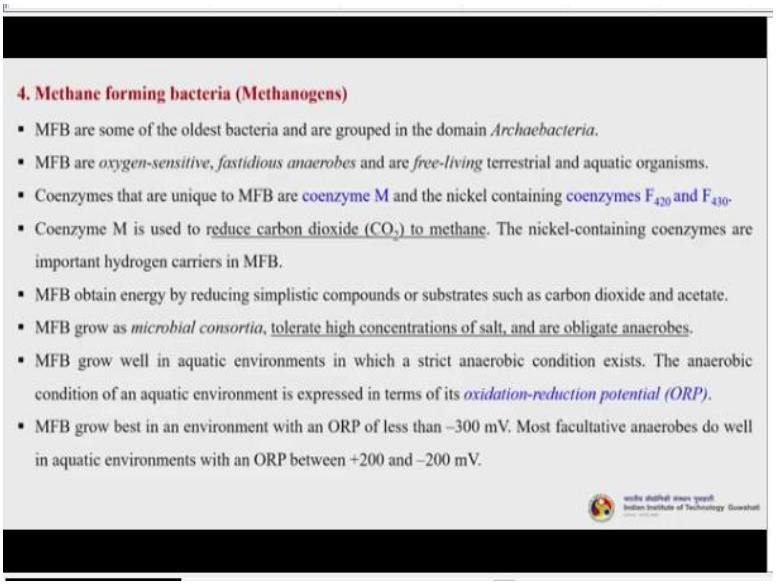

So, next is methane forming bacteria. MFB are some of the oldest bacteria and are grouped in the domain *Archaeabacteria*. MFB are oxygen sensitive, fastidious anaerobes and are free living terrestrial and aquatic organisms. Coenzymes that are unique to MFB are coenzyme M and the nickel containing coenzymes F 420 and F 430. Coenzyme M is used to reduce carbon dioxide to methane.

The nickel containing coenzymes are important hydrogen carriers in the methanogens. So, MFB obtain energy by reducing simplistic compounds or substrates such as carbon dioxide and acetate. MFB grow as microbial consortia, tolerate high concentrations of salt and are obligate anaerobes. MFB grow well in aquatic environments in which strict anaerobic condition exists.

The anaerobic condition of an aquatic environment is expressed in terms of it ORP or which is called the oxidation reduction potential. MFB grow best in an environment with an ORP of less than - 300 millivolt. Most facultative anaerobes do well in aquatic environments with ORP between + 200 and - 200 millivolt. So, facultative anaerobes are a group of microorganisms which do actually their metabolic activity in the presence of oxygen.

But when we deplete oxygen and they can also go for their metabolic activity without the presence of oxygen also. So, they are that is why called facultative anaerobes.

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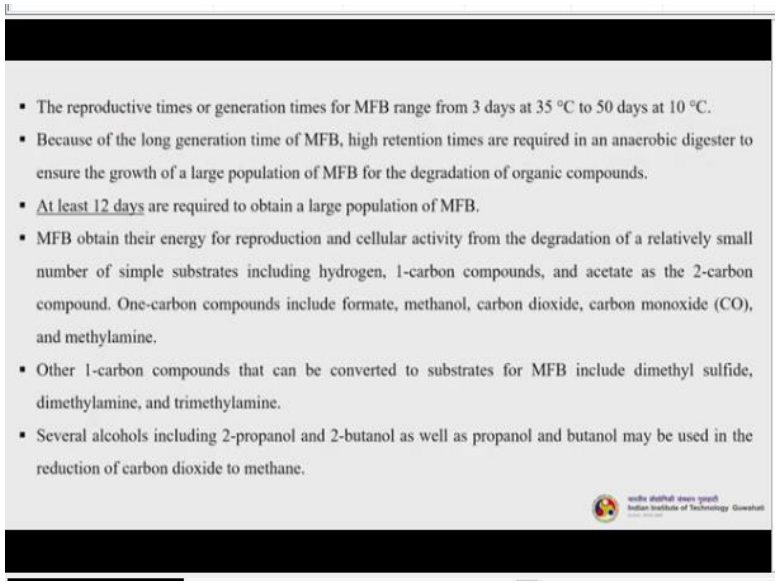

The reproductive times or generation times for MFB range from 3 days at 35 degrees centigrade to 50 days at 10 degree centigrade. Because of the long generation time of MFB high retention times are required in an anaerobic digester to ensure the growth of a large population of MFB for the degradation of organic compounds. At least 12 days are required to obtain a large population of MFB.

MFB obtain their energy for reproduction and cellular activity from the degradation of a relatively small number of simple substrates including hydrogen, 1 carbon compounds and acetate as the 2 carbon compound. 1 carbon compounds include formate, methanol, carbon dioxide, carbon monoxide and methylamine. Other one carbon compounds that can be converted to substrate for MFB include dimethyl sulfide, dimethylamine and trimethylamine.

Several alcohols including 2-propanol and 2-butanol as well as propanol and butanol may be used in the reduction of carbon dioxide to methane.

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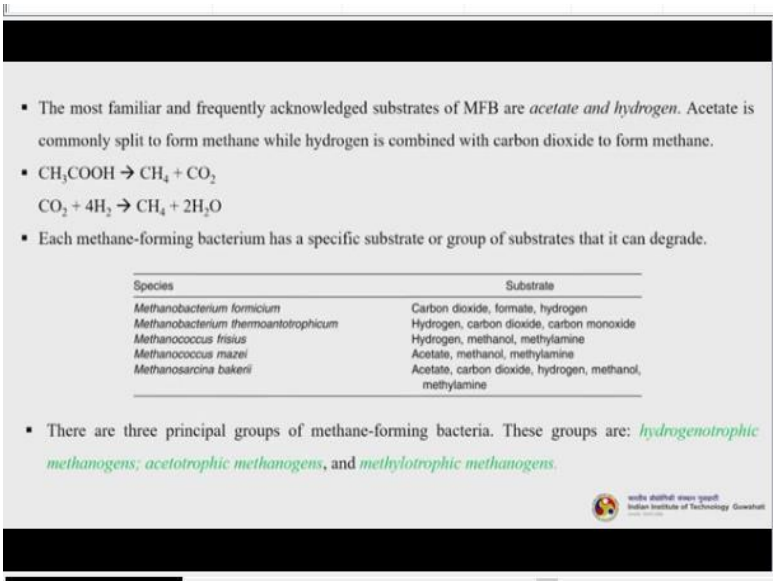

The most familiar and frequently acknowledged substrates of MFB are acetate and hydrogen. Acetate is commonly split to form methane while hydrogen is combined with carbon dioxide to form methane. So, these reactions we have seen many times, again it has been just reported here for the easy understanding and to maintain the flow.

$$CH_3COOH \rightarrow CH_4 + CO_2$$

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

So, each methane forming bacterium has a specific substrate or group of substrates that it can degrade. So, you can see here there are only 5 methanogens are being listed, there are many others. So, if you see the first one the *Methanobacterium formicicum*. So, what it does, its substrate is carbon dioxide, formate and hydrogen. If you talk about the last one *Methanosarcina bakerii*, so for it the substrate is acetate, carbon dioxide, hydrogen, methanol and methylamine.

Now there are 3 principal groups of methane-forming bacteria. So, these groups are hydrogenotrophic methanogens, acetotrophic methanogens and methylotrophic methanogens. Broadly grouped into 3 different types.

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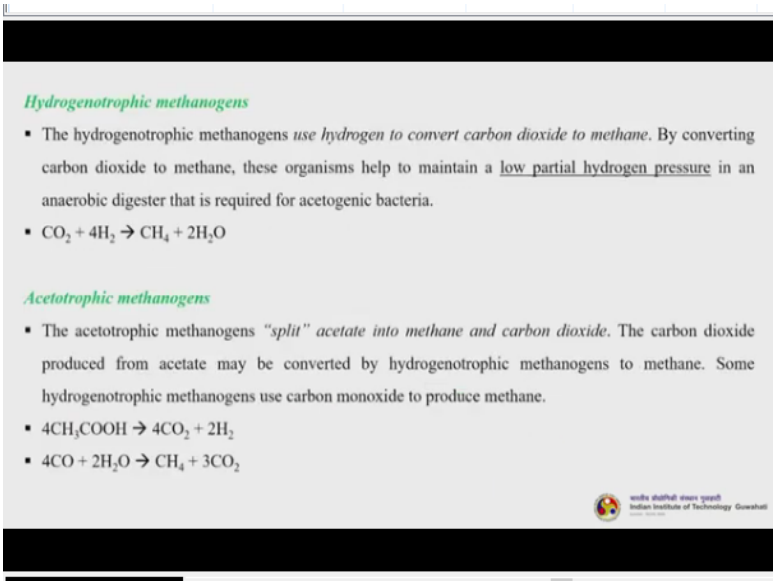

Let us see the hydrogenotrophic methanogens. The hydrogenotrophic methanogens use hydrogen to convert carbon dioxide to methane. By converting carbon dioxide to methane these organisms help to maintain a low partial hydrogen pressure in an anaerobic digester that is required for the acetogenic bacteria to do this reaction:

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

Now the acetotropic methanogens split acetate into methane and carbon dioxide. The carbon dioxide produced from acetate may be converted by hydrogenotrophic methanogens to methane. Some hydrogenotrophic methanogens use carbon monoxide also to produce methane. So, this is the reaction:

$$4CH_3COOH \rightarrow 4CO_2 + 2H_2$$

$$4CO + 2H_2O \rightarrow CH_4 + 3CO_2$$

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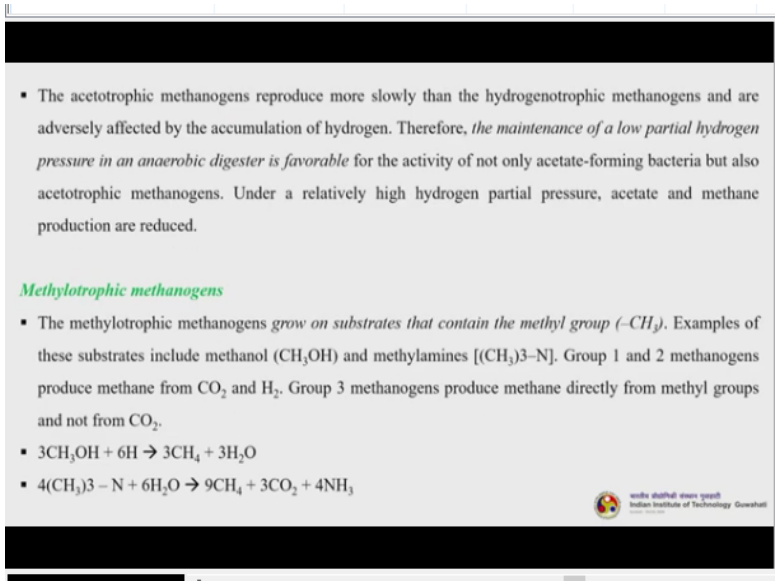

So, the acetotropic methanogens reproduce more slowly than the hydrogenotrophic methanogens and are adversely affected by the accumulation of hydrogen. Therefore, the maintenance of a low partial hydrogen pressure in an anaerobic digester is favourable for the activity of not only acetate-forming bacteria, but also acetotrophic methanogens. Under a relatively high hydrogen partial pressure acetate and methane production are reduced.

Now let us talk about the methylotrophic methanogens. The methylotrophic methanogens grow on substrates that contain the methyl group CH_3. Examples of these substrates include methanol and methylamines. Group 1 and group 2 methanogens produce methane from carbon dioxide and hydrogen, whereas group 3 methanogens produce methane directly from the methyl groups and not from the carbon dioxide.

$$3CH_3OH + 6H \rightarrow 3CH_4 + 3H_2O$$

$$4(CH_3)3 - N + 6H_2O \rightarrow 9CH_4 + 3CO_2 + 4NH_3$$

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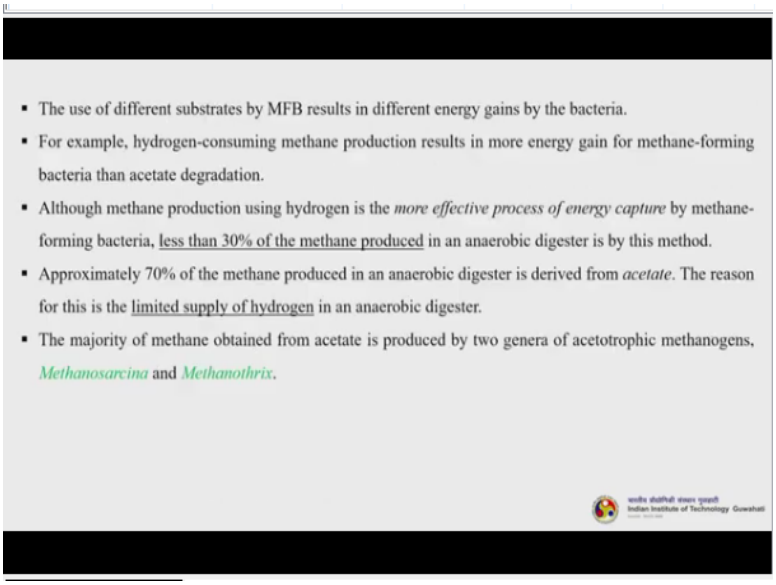

So, the use of different substrates by MFB results in different energy gains by the bacteria. For example hydrogen consuming methane production results in more energy gain for methane-forming bacteria than acetate degradation. Although methane production using hydrogen is the more effective process for energy captured by methane forming bacteria, less than 30% of the methane produced in anaerobic digester is by this method only.

Approximately 70% of the methane produced in an anaerobic digester is directly derived from the acetate pathway. The reason for this is the limited supply of hydrogen in an anaerobic digester. So, the majority of the methane obtained from acetate is produced by 2 genera of acetotrophic methanogens that is *Methanosarcina* and *Methanothrix*.

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Now we will discuss about the fermentation process in a bit more detail than what we discussed in our last lecture.

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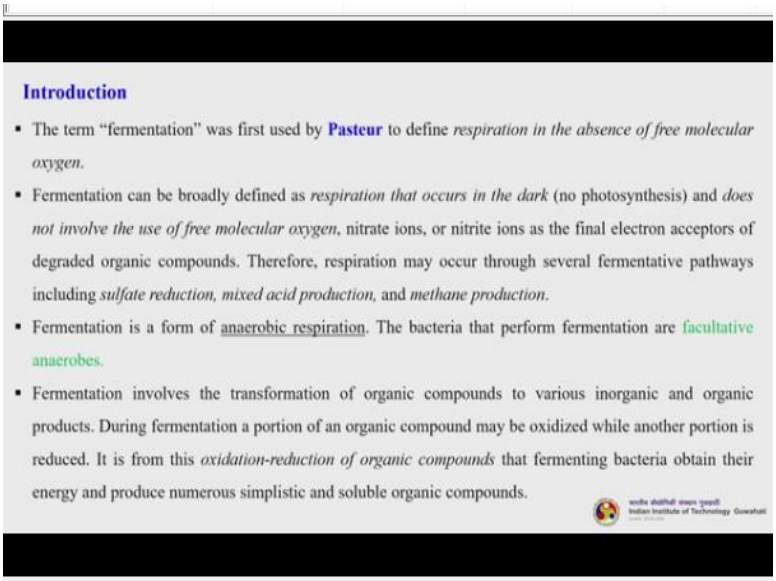

So, the term fermentation was first used by Louis Pasteur to define respiration in the absence of free molecular oxygen. Fermentation can be broadly defined as respiration that occurs in the dark and not involve the use of free molecular oxygen or nitrite ions as the final electron acceptors of the degraded organic compounds. Therefore, respiration may occur through several fermentative pathways including sulfate reduction, mixed acid production and methane production.

Fermentation is a form of anaerobic respiration. The bacteria that perform fermentation are facultative anaerobes. So, I have already explained what is facultative anaerobes. Fermentation involves the transformation of organic compounds to various inorganic and organic products. During fermentation a portion of an organic compound may be oxidized while another portion is reduced.

It is from this oxidation-reduction of organic compounds that fermenting bacteria obtain their energy and produce numerous simplistic and soluble organic compounds.

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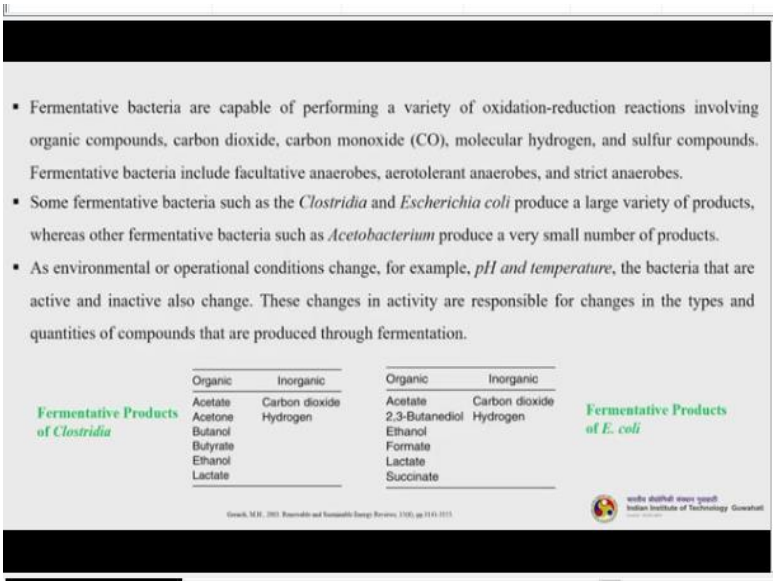

Fermentative bacteria are capable of performing a variety of oxidation-reduction reactions involving the organic carbon dioxide, carbon monoxide, molecular hydrogen and sulfur compounds. Fermentative bacteria include facultative anaerobes, aerotolerant anaerobes and strict anaerobes. Some fermentative bacteria such as *Clostridia* and *Escherichia coli* produce a large variety of products, whereas other fermentative bacteria such as *Acetobacterium* produce a very small number of products.

As environmental and operational conditions change for example the pH and temperature the bacteria that are active and inactive also change, because the environment has a huge effect on the different types of microorganisms. These changes in activity are responsible for changes in the types and quantities of compounds that are produced through fermentation. Let us see these 2 small tables are listed here.

The first one is the fermentative products of *Clostridium* species. You can see that organic products like acetate, acetone, butanol, inorganic carbon dioxide and hydrogen. And this one the second one is the fermentative products from *E. coli* or *Escherichia coli*, acetate, ethanol, formate everything under organic and under inorganic carbon dioxide and hydrogen.

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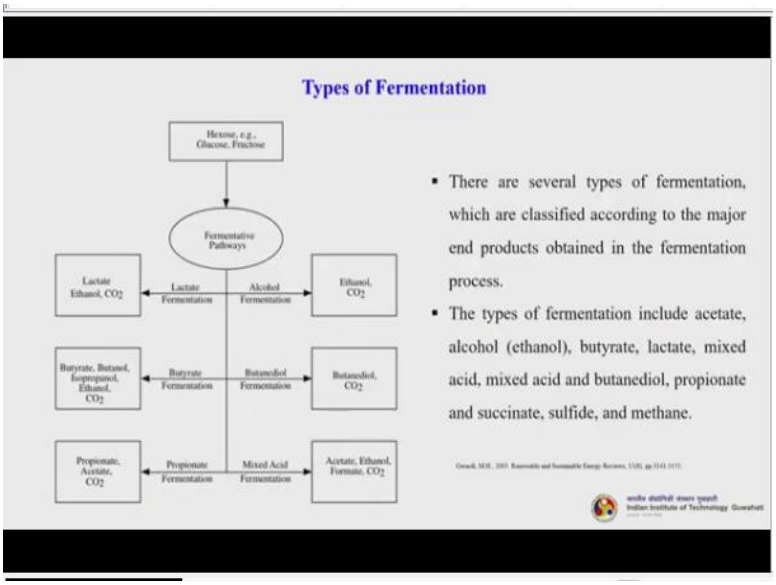

So, we can have a look at the different types of fermentation, this is presented in a nice scheme. So, different pathways are when you degrade hexoses, for example, glucose and fructose through different fermentative pathways. So, these are the different paths. So, when you go for the lactate fermentation you get lactate, ethanol and carbon dioxide. When you go for the alcohol fermentation it is ethanol and carbon dioxide, when you go for butyrate fermentation you get butyrate, butanol, isopropanol, ethanol, carbon dioxide and when you go for this butanediol fermentation you get butanediol and carbon dioxide.

Similarly, the propionate fermentation will give you propionate, acetate and carbon dioxide. And mixed acid fermentation will give you acetate, ethanol and carbon dioxide along with some formate, formic acid. Now there are several types of fermentation which are classified according to the major end products obtained in the fermentation process. Now these types of fermentation include acetate, alcohol or basically ethanol, butyrate, lactate, mixed acid, mixed acid and butanediol, propionate and succinate, sulfide and methane. So, these are different types of fermentation pathways we will see one by one.

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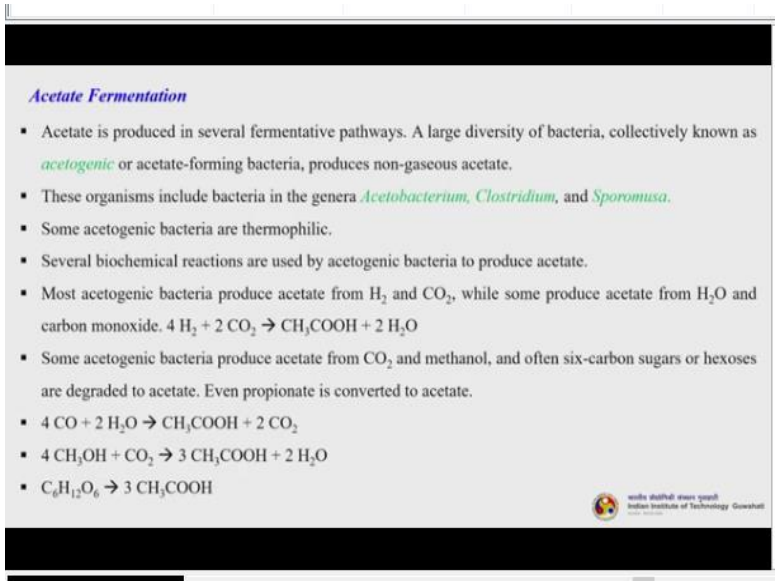

So, the first is acetate fermentation. Acetate is produced in several fermentative pathways. A large diversity of bacteria collectively known as acetogenic or acetate forming bacteria produces non gaseous acetate. These organisms include bacteria in the genera *Acetobacterium*, *Clostridium* and *Sporomusa*. Some acetogenic bacteria are of course thermophilic, but not all.

Several biochemical reactions are used by acetogenic bacteria to produce acetate. Most acetogenic bacteria produce acetate from hydrogen and carbon monoxide while some produce acetate from water and carbon monoxide by this particular reaction:

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

Some acidogenic bacteria produce acetate from carbon dioxide and methanol and often 6 carbon sugars or hexoses are degraded to acetone. Even propionate is converted to acetate. So, these are the reactions:

$$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2$$

$$4CH_3OH + CO_2 \rightarrow 3CH_3COOH + 2H_2O$$

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH$$

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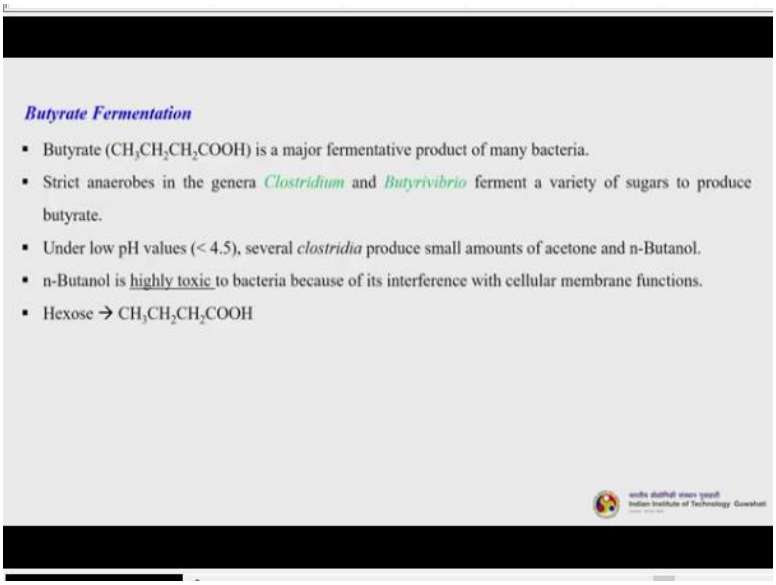

Then we will talk about the butyrate fermentation. Butyrate is a major fermentative product of many bacteria. Strict anaerobes in the genera of *Clostridium* and *Butyrivibrio* ferment a variety of sugars to produce butyrate. Under low pH values almost less than 4.5 several clostridia species produce small amounts of acetone and n-Butanol. Now n-Butanol is highly toxic to bacteria because of its interference with the cellular membrane functions. So, the hexose that is getting converted to butyrate:

$$Hexose \rightarrow CH_3CH_2CH_2COOH$$

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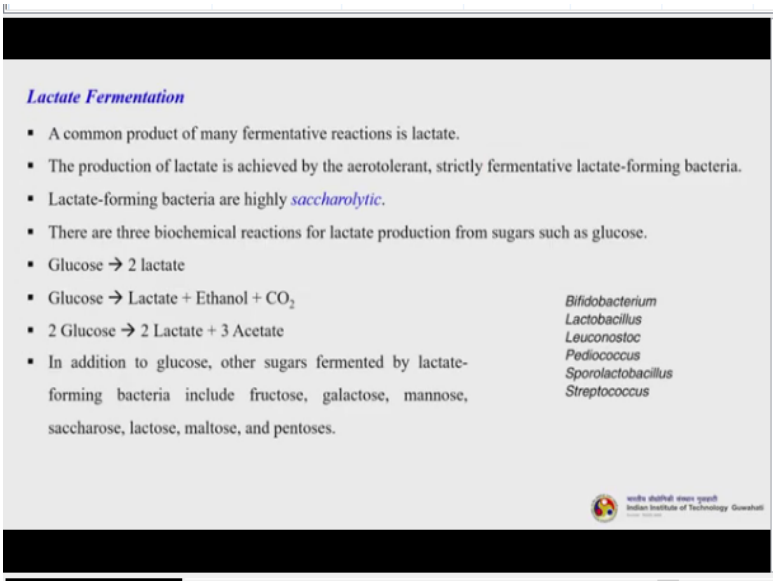

The next is lactate fermentation. A common product of many fermentative reaction is lactate. The production of lactate is achieved by the aerotolerant, strictly fermentative lactate forming bacteria and they are highly saccharolytic.

There are 3 biochemical reactions for lactate production from sugar such as glucose:

$$Glucose \rightarrow 2\ Lactate$$

$$Glucose \rightarrow Lactate + Ethanol + CO_2$$

$$2Glucose \rightarrow 2\ Lactate + 3\ Acetate$$

The above reactions depend on what type of bacterial species it is being used. So, these are some of the bacterial species are being shown in the other side of the slide. So, in addition to the glucose other sugars fermented by lactate forming bacteria include fructose, galactose, mannose, saccharose, lactose, maltose and some pentoses.

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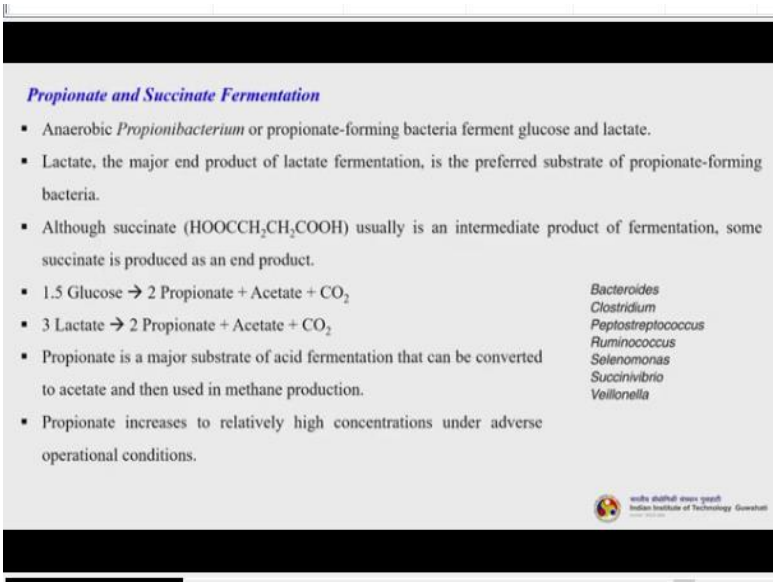

The next is propionate and succinate fermentation. Anaerobic *Propionibacterium* or propionate-forming bacteria ferment glucose and lactate. Lactate the major end product of the lactate fermentation is the preferred substrate for the propionate forming bacteria. Although succinate usually is an intermediate product of the fermentation some succinate is produced as an end product.

$$1.5\ Glucose \rightarrow 2\ Propionate + Acetate + CO_2$$

$$3\ Lactate \rightarrow 2\ Propionate + Acetate + CO_2$$

The above reactions depend upon which species is converting it or degrading it. These are some of the species responsible for doing these conversions of glucose and lactate to propionate is being listed there. So, propionate is a major substrate for acid fermentation that can be converted to acetate and then used in methane production. Propionate increases the relatively high concentrations under adverse operational conditions.

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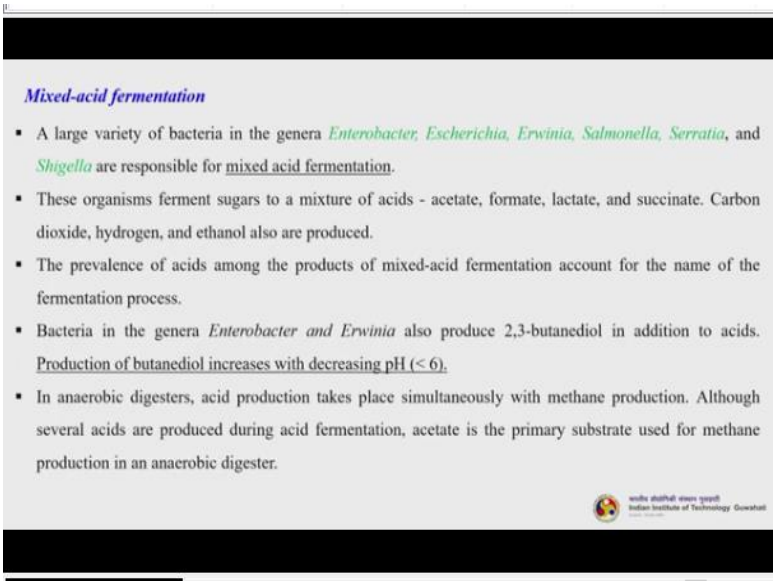

Then the next is mixed acid fermentation and it is sometimes combined with that of the butanediol production. Now a large variety of bacteria in the genera *Enterobacter*, *Escherichia*, *Erwinia*, *Salmonella*, *Serratia* and *Shigella* are responsible for the mixed acid fermentation. These organisms ferment sugars to a mixture of acids such as acetate, formate, lactate and succinate.

Carbon dioxide, hydrogen and ethanol are also being produced. The prevalence of acids among the products of mixed acid fermentation account for the name of the fermentation process. Bacteria in the genera *Enterobacter* and *Erwinia* also produce 2, 3 butanediol in addition to acids. The production of butanediol increases when the pH is decrease that means less than 6.

So, in anaerobic digester acid production takes place simultaneously with methane production. Although several acids are produced during acid fermentation, acetate is the primary substrate used for methane production in an anaerobic digester.

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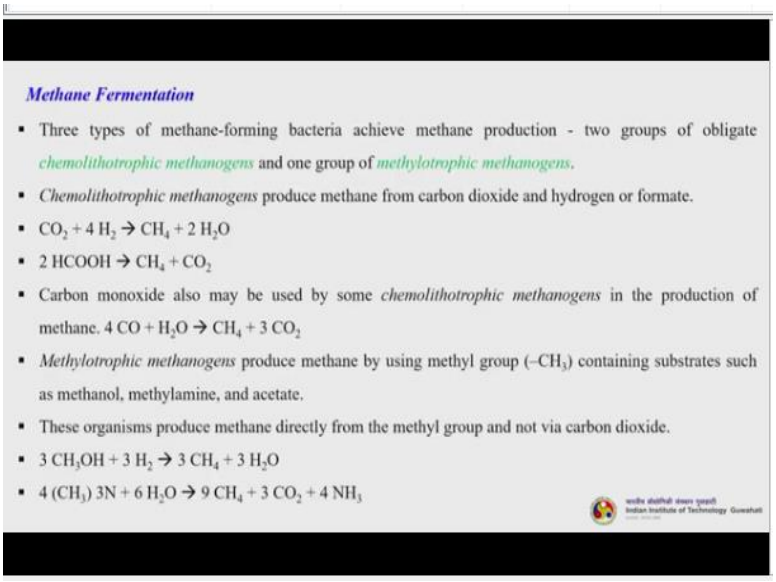

We will see the next one which is the methane fermentation. Three types of methane-forming bacteria achieve methane for production, 2 groups of obligate chemolithotrophic methanogens and one group of methylotrophic methanogens. Chemolithotrophic methanogens produce methane from carbon dioxide and hydrogen or formate by this reaction:

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

$$2HCOOH \rightarrow CH_4 + CO_2$$

Now carbon monoxide also may be used by some chemolithotropic methanogens in the production of methane, by this reaction:

$$4CO + H_2O \rightarrow CH_4 + 3CO_2$$

Now methylotrophic methanogens produce methane by using methyl group containing substrates such as methanol, methylamine and acetic and these organisms produce methane directly from the methyl group and not via carbon dioxide by these 2 following reactions:

$$3CH_3OH + 3H_2 \rightarrow 3CH_4 + 3H_2O$$

$$4(CH_3)3N + 6H_2O \rightarrow 9CH_4 + 3CO_2 + 4NH_3$$

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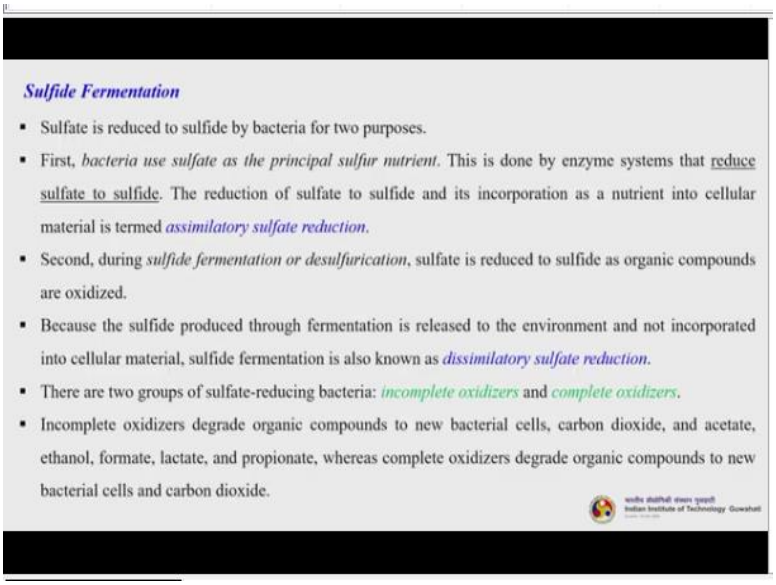

Then next is sulfide fermentation. Sulfate is reduced to sulfide by bacteria for 2 purposes. So, first is that bacteria use sulfate as the principal sulfur nutrient. Now this is done by enzyme systems that reduce sulfate to sulfide. The reduction of sulfate to sulfide and its incorporation as a nutrient into cellular material is termed as a assimilatory sulfate reduction. Second is that during sulfide fermentation or desulfurification, sulfate is reduced to sulfide as organic compounds are oxidized.

Because the sulfide produced through fermentation is released to the environment and not incorporated into the cellular material, sulfide fermentation is also known as dissimilatory sulfate reduction. There are 2 groups of sulfate reducing bacteria first group is called incomplete oxidizers and the second are complete oxidizers.

Incomplete oxidizers degrade organic compounds to new bacterial cells, carbon dioxide and acetate, ethanol, formate, lactate and propionate, whereas complete oxidizers degrade organic compounds to new bacterial cells and carbon dioxide. So, you can see that the incomplete oxidizers actually produce so many different types of products.

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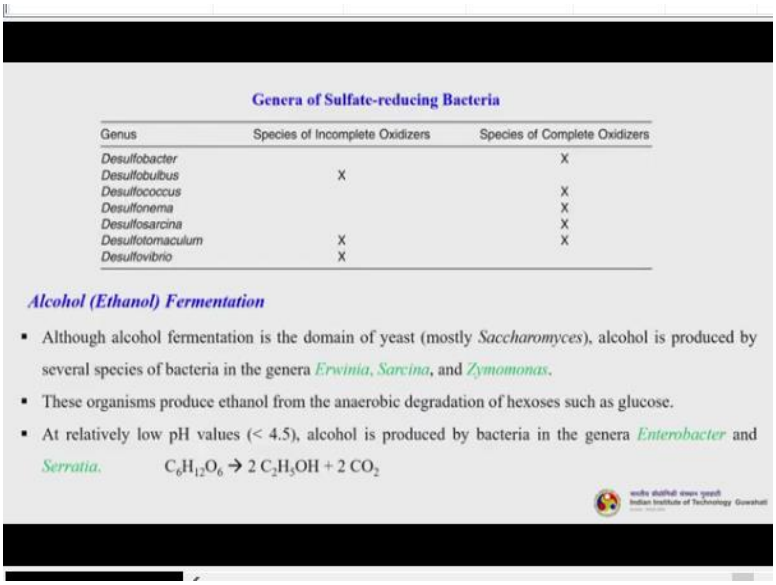

So, the table list actually genera of sulfate reducing bacteria. So, you can see different genus of sulfate reducing bacteria there and it is mentioned whether they are the species of incomplete oxidizers or they fall under the species of complete oxidizer. So, the *Desulfobacter* the first one. This is a complete oxidizer. The second one is *Desulfobulbus*, it is a incomplete oxidizer. Like similarly there are others also mentioned.

So, the next fermentation type is the alcohol or ethanol fermentation. Though alcohol fermentation is the domain of yeast, so mostly the *Saccharomyces*, alcohol is also produced by several species of bacteria in the genera of *Erwinia*, *Sarcina* and *Zymomonas*. Now these organisms produce ethanol from the anaerobic degradation of hexoses such as glucose.

At relatively low pH value less than 4.5, alcohol is produced by the bacteria in the genera *Enterobacter* and *Serratia*, by this reaction:

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

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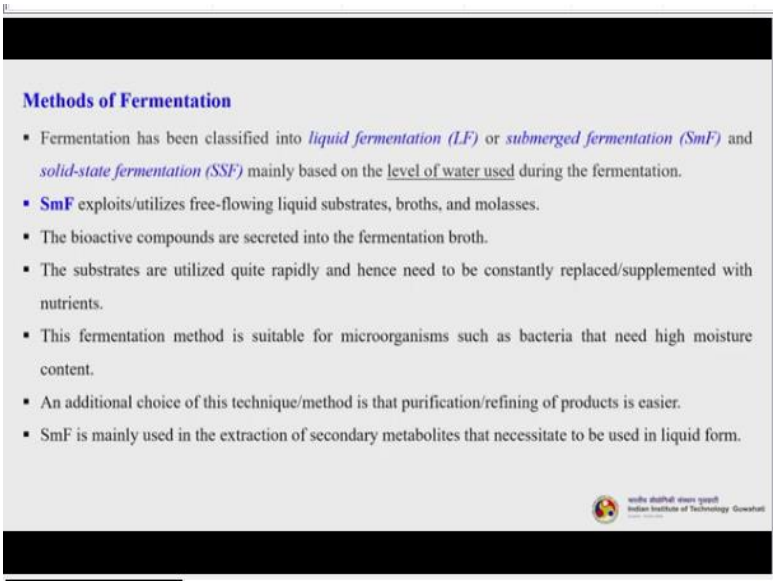

So, now we will quickly understand and the different methods of fermentation. Now fermentation has been classified into liquid fermentation, submerged fermentation or solid-state fermentation mainly based on the level of water used during the fermentation. So, SmF which is the submerged fermentation exploits or utilizes free flowing liquid substrate broths and molasses.

The bioactive compounds are secreted into the fermentation broth. The substrates are utilized quite rapidly and hence need to be constantly replaced or supplemented with nutrients. This fermentation method is suitable for microorganisms such as bacteria that need high moisture content. An additional choice of this technique/method is that purification and refining of products is easier. SmF is mainly used in the extraction of secondary metabolites that necessitate to be used in the liquid form.

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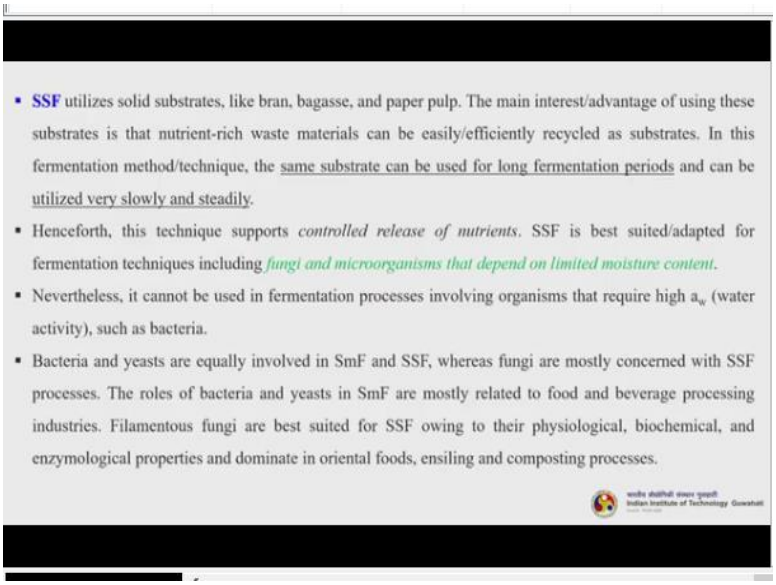

In contrast, SSF utilizes the solid substrate like bran, bagasse and paper pulp. The main interest and advantage of using this substrate is that nutrient-rich waste materials can be easily or efficiently recycled as substrate. In this fermentation method or technique, the same substrate can be used for a long fermentation period and can be utilized very slowly and steadily.

Henceforth, this technique supports controlled release of nutrients. SSF is best suited or adapted for fermentation techniques including fungi and microorganisms that depend on limited moisture content. Nevertheless, it cannot be used in fermentation process involving organisms they require a very high a_w value (a_w is the water activity value) such as most of the bacteria. So, bacteria and yeasts are equally involved in SmF and SSF, whereas fungi are mostly concerned with the SSF processes.

The roles of bacteria and yeast in SMF are mostly related to food and beverage processing industries. Filamentous fungi are best suited for SSF owing to their physiological, biochemical and enzymological properties and dominate in oriental foods, ensiling and composting processes.

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Factor	Liquid substrate fermentation	Solid substrate fermentation
Substrates	Soluble Substrates (sugars)	Polymer Insoluble Substrates: Starch, Cellulose, Pectines, Lignin
Aseptic conditions	Heat sterilization and aseptic control	Vapor treatment, non sterile conditions
Water	High volumes of water consumed	Limited Consumption of Water; low A_w ,
Metabolic heating	Easy control of temperature	Low heat transfer capacity
Aeration	Limitation of soluble oxygen. High level of air required	Easy aeration and high surface exchange air/substrate
pH control	Easy pH control	Buffered solid substrates
Mechanical agitation	Good homogenization	Static conditions preferred
Scale up	Industrial equipments available	Need for Engineering & new design Equipment
Inoculation	Easy inoculation, continuous process	Spore inoculation, batch
Contamination	Risks of contamination for single strain bacteria	Risk of contamination for low rate growth fungi
Energetic consideration	High energy consuming	Low energy consuming
Volume of equipment	High volumes and high cost technology	Low volumes and low costs of equipments
Effluent and pollution	High volumes of polluting effluents	No effluents, less pollution
Concentration of Substrate/Products	30–80 g/L	100/300 g/L

So, this is a table which gives us information about the different factors, the liquid substrate fermentation and the solid substrate fermentation. So, if you see the second one under aseptic condition; so the liquid substrate fermentation, there will be heat sterilization and aseptic control and the solid substrate fermentation, vapour treatment and non-sterile conditions.

So, when you talk about let us say the inoculation here - so easy inoculation and continuous process under the liquid substrate fermentation and under solid state fermentation spore inoculation and it is a batch process. Because mostly it is being done by the fungi. So, you can go through the table later on.

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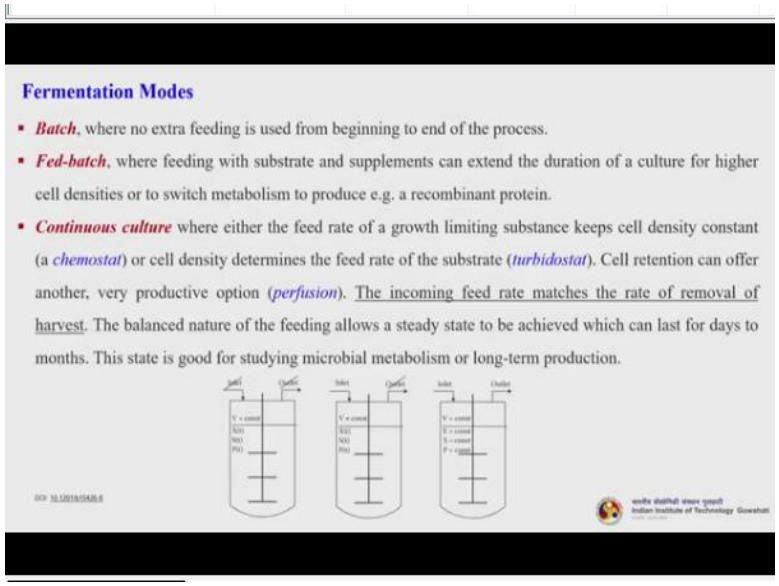

So, I am moving ahead. So, we will try to understand what are the different fermentation modes, how it can be done? Essentially there are 3, one is the batch one which is very much

is being practiced in most of the lab scales, then the fed-batch and then the continuous culture. Now what is batch? Now here you can see nicely I have depicted this particular figure - given this particular figure, from here you can directly understand what is a batch, what is the fed-batch and what is a continuous process from this. There is an inlet, there is outlet, you can see that under batch inlet and outlet both are strike down, what does it mean? So, this means that no extra feeding is used from the beginning to the end of the process. Once the substrate is fed to the batch reactor and the microorganisms and other necessary things are being supplied it is being closed and reaction will proceed. Once the reaction stops the products are formed you will open the reactor. So, this is what is batch.

Now what is fed-batch? So, you can see that outlet, there is no outlet**,** but there is intermittent inlet. So, once you supply the feed then you can intermittently also supply the feed. What does it mean? So, fed-batch is a process where feeding with substrate and supplements can extend the duration of a culture for higher cell densities or to switch metabolism to produce a recombinant protein for example. So, intermittently you are feeding.

The next is the continuous culture where inlet and outlet both are open throughout the process**.** That is why it is a continuous process. Continuous feeding and continuous taking out of the reaction products. So, it is mostly adapted in the industries. So, continuous culture where either the feed rate of a growth limiting substance keeps cell density constant (that reactor is called a chemostat) or cell density determines the fed rate of the substrate (That reactor is called a turbidostat). Now cell retention can offer another very productive option, that is called perfusion. The incoming feed rate matches the rate of the removal of the harvest. The balanced nature of the feeding allows a steady state to be achieved which can last for days to months. This state is good for studying microbial metabolism or long-term production.

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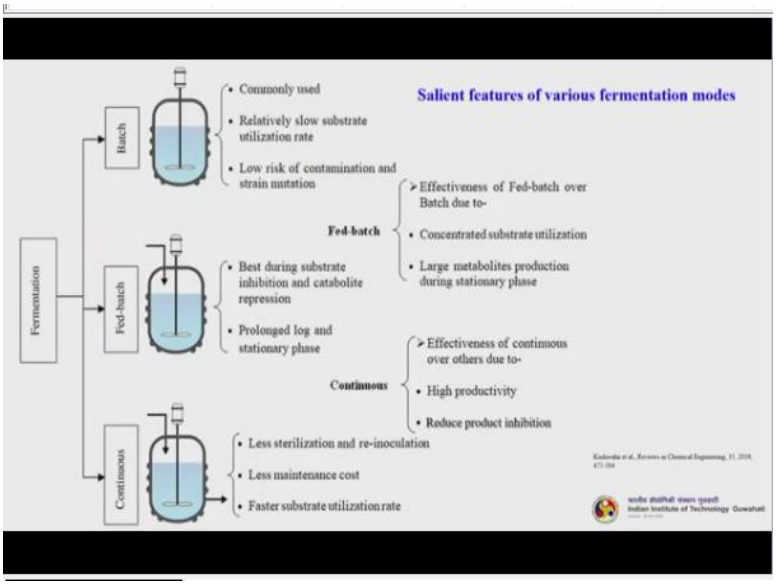

Now this is again described nicely under this particular schematic representation which tells us the salient features of various fermentative modes. We will quickly go through it. The batch, fed-batch and continuous. Let us see the batch**.** So, it is commonly used, relatively slow substrate utilization rate and low risk of contamination and strain mutation because it is a closed system**.** There is no feeding, there is no taking out of the products**.**

In the fed-batch it is best during substrate inhibition. When there is substrate inhibition you feed little more again the dilution factor actually increases inside the fermentation and it will dilute the inhibitory products. So, that inhibitory products under dilution will not more serve as inhibitory substances (*the inhibitory effect will be diminished*) and it has prolonged log and stationary phase of the microorganisms (growth phase we are talking about). So, when you compare fed-batch, we can say the effectiveness of fed-batch over batch due to concentrated substrate utilization and large metabolites production during stationary phase. Now this is the advantages of fed-batch with batch respectively.

Now let us talk about the continuous system. Now here less sterilization and re-inoculation is required because we are continuously feeding the substrate as well as continuously taking out the substrate. Less maintenance cost and fastest substrate utilization rate. Now if you compare continuous with fed-batch or batch we can say that it is more effective due to high productivity and reduce product inhibition. So, this is all about fermentation and how we can do the fermentation via various types of reactors or the various types of mode.

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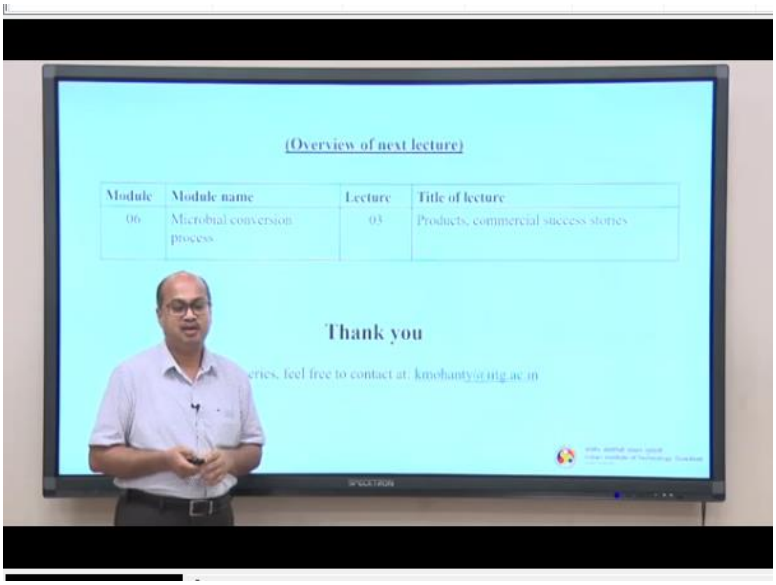

So, with this today I conclude my lecture and in our next lecture under this module we will be discussing about the various products of the microbial conversion processes and their utilities and some of the commercial success stories. So, thank you very much and if you have any query please register it under the Swayam portal or drop a mail to me at kmohanty.iitg.ac.in.