

**Water and Wastewater Engineering**  
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**Anaerobic Treatment**  
**Lecture – 27**

Last few classes we were discussing about the wastewater treatment by aerobic processors. We have seen both suspended growth systems as well as attached growth systems. Today we will start the anaerobic wastewater treatment processes. It also includes both suspended growth systems as well as attached growth systems.

What is an anaerobic treatment system?

We have discussed about the different types of treatment or the different types of processors based upon the electron acceptors present. In anaerobic system what is happening is the molecular oxygen will be present in the system so the electrons whatever is released from the organic matter will be undergoing a series of oxidation reduction reactions and finally molecular oxygen will be accepting the electrons and water will be formed. But in anoxic system molecular oxygen will not be present but inorganic oxygen in form of inorganic components like nitrate, sulfate etc will be present so these components this nitrate, sulfate etc will be acting as the electron acceptor.

In anoxic system also electron transfer system is present. But in anaerobic process there is no electron transfer system involve or there is no oxidation reduction reactions taking place or the energy generated in the system is not by oxidation reaction but by substrate level **first for** relation. So that is the major difference between aerobic system and anaerobic system. Here molecular oxygen is not present at all and substrate itself is acting as the electron donor and electron acceptor and substrate itself will be getting reduced to methane and a portion is getting oxidized to carbon dioxide. So we will see **what are the** different suspended those systems and attached froth systems commonly used in anaerobic processors.

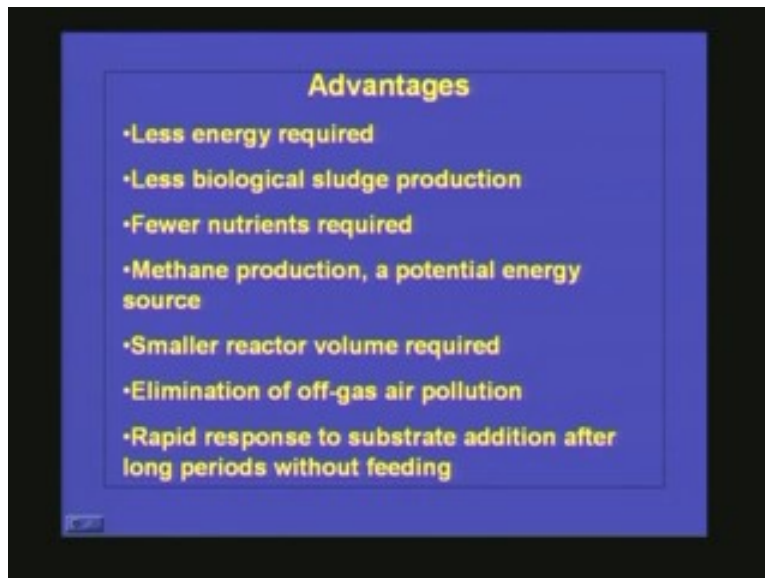
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Anaerobic suspended growth systems include up flow anaerobic sludge blanket and this is another attached growth system up flow and down flow anaerobic attached growth then fluidized bed attached growth system and anaerobic lagoon is an example is suspended growth system and membrane separation anaerobic process is an attached growth system.

Now we will see the advantages of this anaerobic system. Anaerobic system is having many advantages over aerobic system.

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The major one is it request less energy. The reason is in an aerobic system the oxidation of organic matter is taking place by the help of oxygen. So whatever is the organic lot in the wastewater corresponding to that one we have to supply air or oxygen so the oxygen supply to the system in involves lots of energy. **We have seen this in detail when we are doing the aerator design.** But in anaerobic system no oxygen is required. The substrate itself will be undergoing oxidation and reduction so there is no need of any external supply of oxygen so definitely in the system energy requirement is very very less compared to aerobic system.

Moreover, here since the aerobic process is much more efficient compared to anaerobic process what I mean is the energy generated by oxidative reduction process is much more compared to the substrate level **first for relation which we will discuss in detail when we talk about the anaerobic metabolism.** So because of this one the sludge generated in aerobic system is much more compared to anaerobic system. Moreover the sludge generated in anaerobic system is in the stabilized form which doesn't require any further treatment so sludge handling is not a problem in anaerobic system.

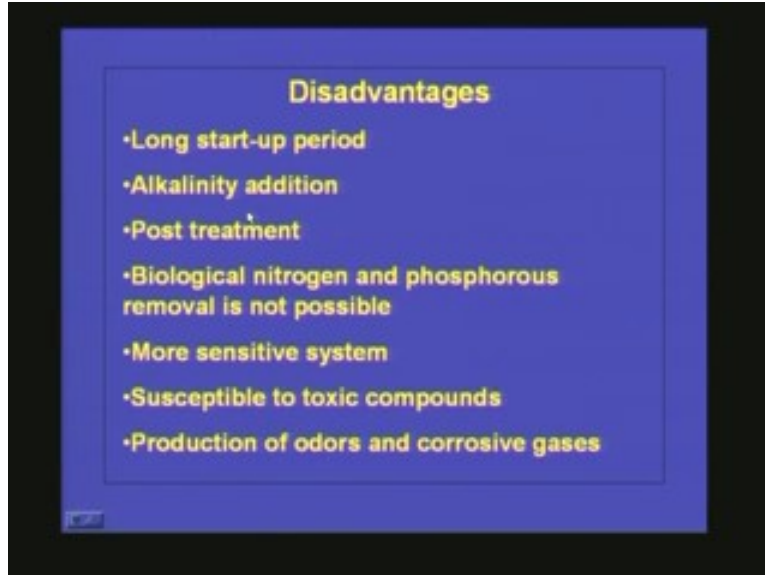
Another one is since the sludge generation is very very less the nutrient requirement is also less. As we have seen what is the molecular formula of a biomass empirical formula each unit requires a particular amount of nitrogen phosphorus and other micro nutrients. But if the total amount of sludge generated is less then definitely the micronutrients and macronutrients present in the system will be reduced considerably.

Second one is, the other advantages are elimination of off-gas air pollution. This means if we go for aerobic processes lot of carbon dioxide will be released into the atmosphere and we all know that carbon dioxide also contributes to the global warming. But when we talk about anaerobic system the byproduct is biogas which can be used as an energy source so the air pollution can be controlled. Moreover it gives rapid response to substrate addition after long periods without feeding. This anaerobic sludge can stay without any food up to six months and even after that if you add food or supply food to the sludge it will be activating at a faster rate. But in aerobic system the sludge cannot stay without food for longer period that is another advantage.

Hence, this process can be used for intermittent process. because some industries they are seasonal so in such cases if you go for aerobic system then as long as the wastewater supply is there the system will be working but when once the wastewater supply is cutoff the system will not be working anymore. But in the next season when we want to go for further treatment of the wastewater we have to start the system from the beginning again. But in anaerobic systems it is not a problem when the wastewater is not there the sludge will be remaining there inactive and as soon as you add more and more wastewater it will be activated by itself. These are the advantages.

Even if the process is having so many advantages why is it not becoming so popular in our country or worldwide? Definitely there will be many disadvantages for this process. We will see the disadvantages of this anaerobic process.

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The major one is it requires long start-up period and second one is because in the system addition of alkalinity is very very essential and this process is not able to remove nutrient like phosphate and nitrate so there is a requirement of post treatment. Just by an anaerobic treatment process the effluent will not be meeting the discharge standard. So after the anaerobic treatment we have to go for a post treatment mostly it is aerobic process or aerobic combined with other physical chemical processes.

Then biological nitrogen and phosphorous removal is not possible, that's what I told here. The post treatment is basically for this nutrient removal as well as the pathogens removal.

The system is more sensitive because the microbiology of the system is very very complicated that not only a single group of microorganisms are present. It's a group of microorganism which does the work in the system so the system is very very sensitive compare to aerobic system.

Susceptible to toxic compounds: It also produces odors and corrosive gases like hydrogen sulfite etc. these are the disadvantages of a system. So, if we can cut down the disadvantages definitely this anaerobic process will become the treatment options for the future.

What are the design considerations whenever we talk about the anaerobic process?  
It is almost same as that of aerobic process. We should know the characteristics of the wastewater because that defines what type of treatment or how much is the time require etc.

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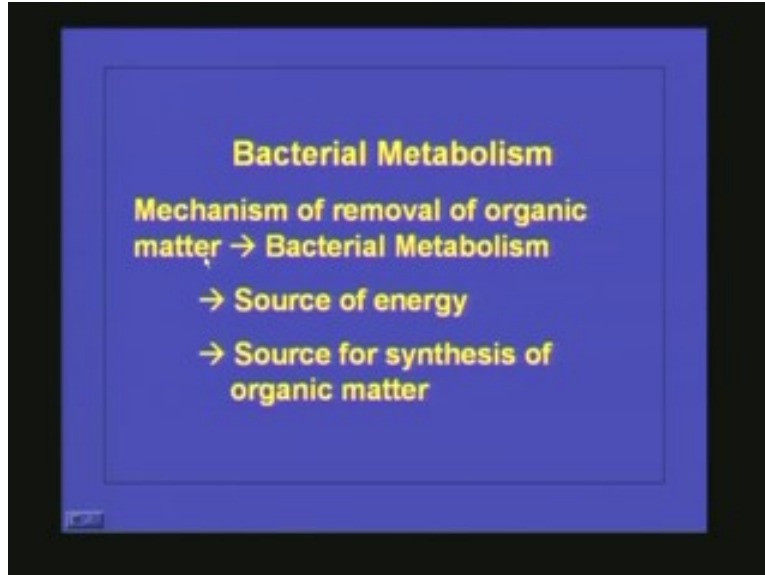


Then we have the flow and loading conditions, organic concentration and design temperature, fraction of colloidal and suspended organic material and wastewater alkalinity, nutrients, macronutrients, inorganic and organic toxic compounds and solid retention time.

These are the design considerations for any of the biological systems we have already discussed. So before going into the details of the anaerobic process first we will see what the mechanism is or how the metabolism is taking place in anaerobic process.

If you want to design a system we should know how the microorganisms are performing in anaerobic process then only we can optimize the system according to our requirement. So we know that because of the metabolism of the microorganism the organic matter removal is taking place in any biological treatment systems. **That is what I have given here.**

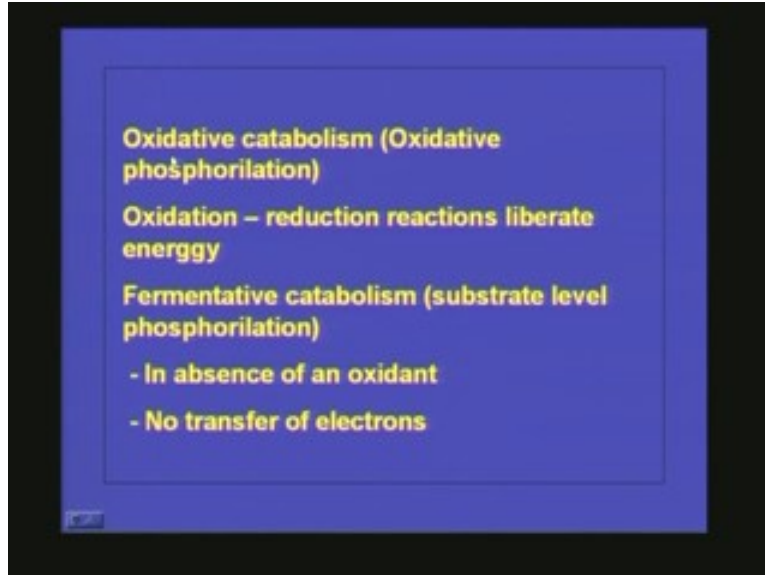
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The mechanism of removal of organic matter is because of bacterial metabolism and the metabolism is taking place or during the metabolism the organic matter is used as a source of energy and as a source of synthesis of organic matter. So in both the ways organic matter will be utilized.

We have already seen in the introduction of biological processors there are three different types of phosphorylation or energy releasing reaction. One is oxidative phosphorylation, another one is substrate level phosphorylation and the third one is photo phosphorylation. So, as I have already mentioned the oxidative phosphorylation is taking place in aerobic processes where electron transport system is present so it will be undergoing a series of oxidation reduction reactions with the help of enzymes and coenzymes and oxygen will be acting as the terminal electron acceptor. Or in anoxic process nitrate, sulfite etc will be acting as the terminal electron acceptor so lot of energy will be released in process.

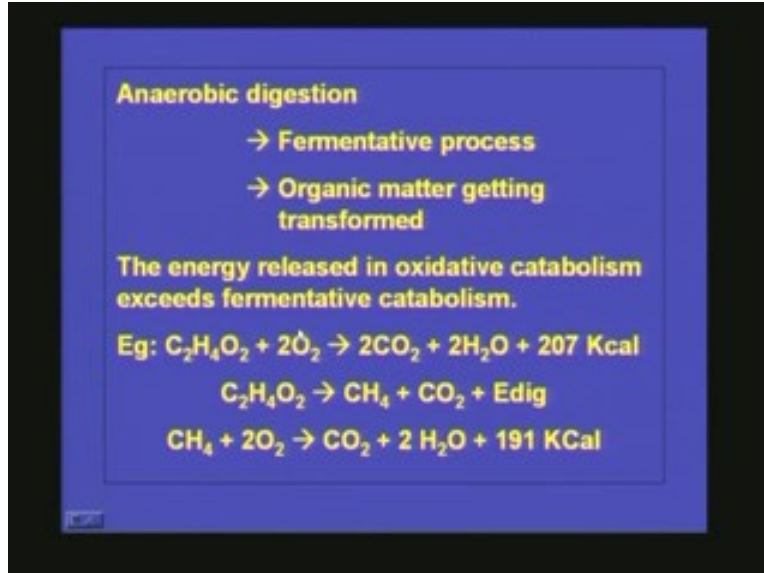
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The other phosphorylation is substrate level phosphorylation. In such systems electron transport system is not present at all. so here definitely the energy released will be less compared to the oxidative phosphorylation. In anaerobic system this substrate level phosphorylation is taking place and it is done by fermentative catabolism so the specialty of this fermentative catabolism is it is happening in the absence of an oxidant and no transfer of electrons are taking place but only the substrate is undergoing certain changes. That is the specialty of fermentative catabolism.

Or in other words in fermentation process the organic matter itself will be acting as the electron donor and electron acceptor so we don't have to give any external electron acceptors so as a result hydrogen gas will be generated in the fermentation processes. So if you talk about any anaerobic digestion it is a fermentative process and organic matter is getting transformed.

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**Anaerobic digestion**

- Fermentative process
- Organic matter getting transformed

The energy released in oxidative catabolism exceeds fermentative catabolism.

Eg:  $C_2H_4O_2 + 2O_2 \rightarrow 2CO_2 + 2H_2O + 207 \text{ Kcal}$

$C_2H_4O_2 \rightarrow CH_4 + CO_2 + E_{dig}$

$CH_4 + 2O_2 \rightarrow CO_2 + 2 H_2O + 191 \text{ KCal}$

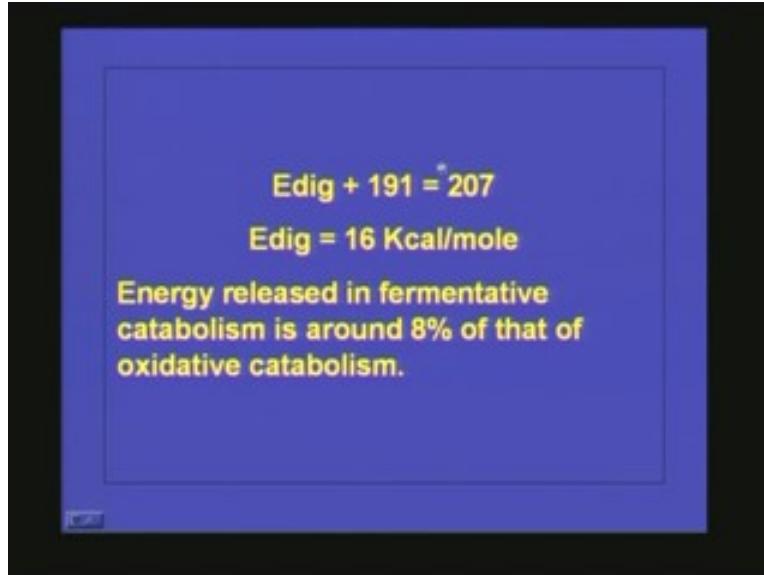
The energy released in oxidative catabolism exceeds fermentative catabolism. This we can show easily using these chemical or biological reactions. We can see an organic matter  $C_2H_4O_2$  when it gets oxidized. In aerobic system the complete oxidation of the organic matter will be taking place with the help of microorganism. So, if it is completely getting oxidized in presence of oxygen you will be getting carbon dioxide and water and apart from that one we will be getting 207 kilo calories of energy. That means one mole of  $C_2H_4O_2$  gives 207 kilo calories of energy if it undergoes oxidative phosphorylation. But if it undergoes anaerobic or fermentative phosphorylation then  $C_2H_4O_2$  will be getting converted to methane plus carbon dioxide plus some energy of digestion.

How can we find out this energy of digestion?

What we have to do is the total energy of the system will be a constant so we can find out what is the energy released from this methane if we go for complete oxidation. That is what is given here. We have a mole of methane and it is getting completely oxidized in presence of oxygen so we are getting carbon dioxide and water and by oxidizing methane we are getting 191 kilo calories of energy or methane will be having an energy content of 191 kilo calories. We know that the total energy of the system will be a constant so 191 kilo calories of energies stored by the methane so what will be the energy of this digestive process or anaerobic catabolism or fermentative catabolism.



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We can write like this;

Energy liberated in the digestive process plus 191 kilo calories will be equal to 207 kilo calories or the energy released during the digestive process is only 16 kilo calories per mole whereas in aerobic process or oxidative phosphorylation for the same one mole of organic matter we will be getting an energy of 207 kilo calories per mole. That is what I have written here; energy released in fermentative catabolism is around 8% of that of oxidative catabolism. This is very very important. This is an advantage as well as disadvantage of anaerobic process.

The advantage is since the energy produced in anaerobic process or anaerobic digestion is so low if the microorganism has to generate the energy required for their metabolism we have to use a large portion of the organic matter whatever is available for catabolism. That means if we take the same amount of organic matter the energy generated here is only 8% of that of aerobic process.

Therefore, if you have 1 Kg of organic matter the amount used for catabolism in aerobic process will be 8% of that of the one which is used in anaerobic process. So what will happen is most of the organic matter in anaerobic process will be used for the catabolism and only a little amount will be used for the anabolic purposes so definitely the cells synthesis will be very very less or the amount of sludge produced will be very very less. This is a positive point when we talk about the sludge handling because the sludge produced in anaerobic process is very very less so we don't have to go for any post treatment.

But in aerobic process the sludge generation will be so high because we have seen that the yield coefficient that means the biomass synthesis per unit mass of substrate utilization varies from 0.4 to 0.6. That means if 1 Kg of organic matter is converted by aerobic process we will be getting around 400 to 600g of biomass whereas in aerobic

process we will be getting only 8g of biomass so the sludge volume will be reduced drastically in anaerobic process.

But the major disadvantage of this is in aerobic process we can maintain a very high concentration of microorganism in the system because the microbial concentration is very very important in any biological process. The reason is the microorganisms are responsible for the treatment; they are the ones which utilize the pollutants present in the wastewater and convert it into environmental friendly byproduct like carbon dioxide, water, nitrogen, gas etc. So if the amount of microorganism present in the treatment unit is high then definitely the time require for the treatment will be very very less.

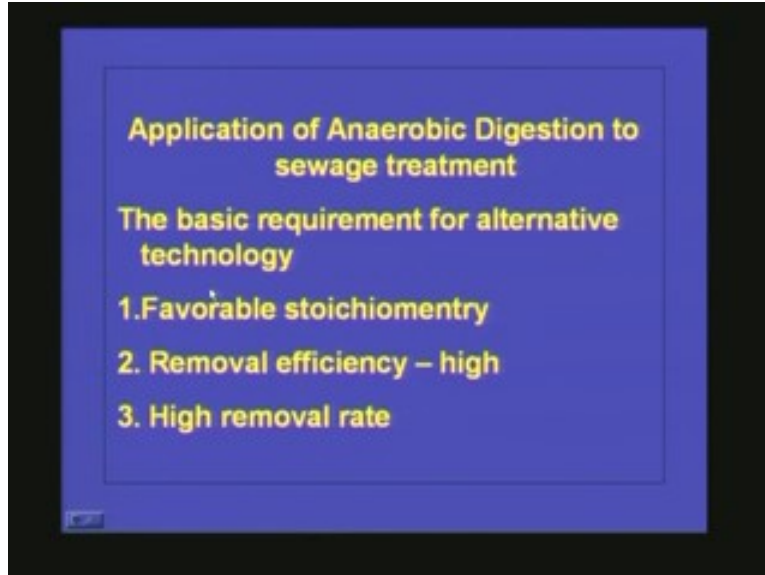
But in anaerobic process the anabolism is so less or the cells synthesis is very less compared to aerobic process so the amount of microorganism present in the system if you take the same time duration then it will be much less compared to the aerobic process. So the detention time required in anaerobic process will be considerably more compared to aerobic process that's why in convention activated sludge process we need to retain the wastewater only for 4 to 6 hours in the aeration time but when we talk about the conventional anaerobic process like septic tank or anaerobic sludge digesters the detention time is around 20 to 30 days.

This explains why we have to give such a long time in anaerobic process. Because of this one such a long duration is required for proper treatment, this process was not gaining any attention in the past the reason is such a long time is required so you can imagine what is the size of the reactor we have to provide if you want to treat the wastewater because the quantity of wastewater coming to the system is very very large and if you want to keep that one for 30 days or 20 days then the volume of the reactor will be increasing considerably. That's what I told, the catabolism or this metabolic process or whatever is happening in anaerobic process is having a positive effect as well as a negative effect but how can we overcome this one?

If you can maintain high microbial concentration in the system we can reduce the hydraulic retention time.

Now we will see how we can apply the anaerobic digestion to sewage treatment.

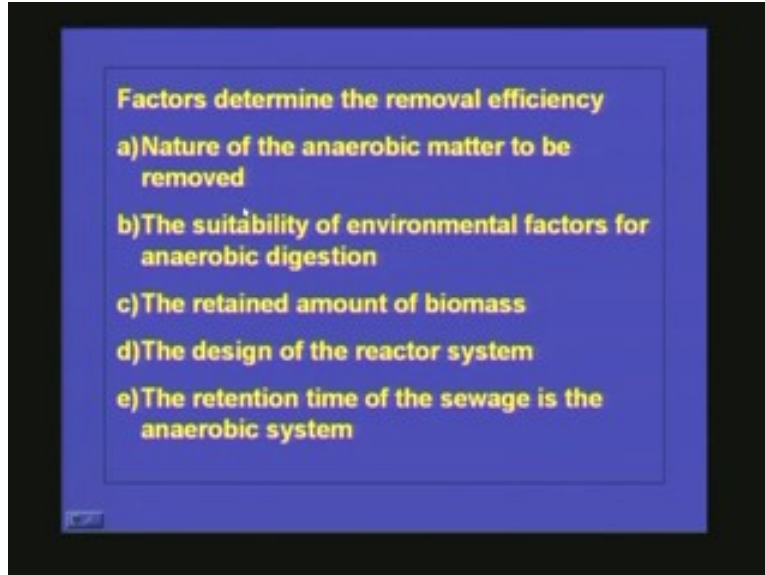
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The basic requirement for alternative technology: We have seen that anaerobic technologies are having many advantages over aerobic technology but those advantages alone will not make it an alternative technology unless it satisfies the following conditions. We should have a favorable stoichiometry. That means whatever reactions are happening it should be feasible and it should have very high removal efficiency and the removal rate should be very very high. If the removal rate is not high then we have to give a long time for the treatment so that is not preferable.

Now we will see the factors that determine the removal efficiency in an anaerobic system.

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Those are;

- Nature of the anaerobic matter to be removed
- The suitability of the environmental factors for anaerobic digestion
- The retained amount of biomass
- The design of the reactor system and
- The retention time of the sewage in the anaerobic system

This is the same for other biological systems also. If the organic matter whatever is coming to the system is easily degradable then definitely the treatment efficiency will be very very high. Another one is environmental conditions. We have already seen that anaerobic process is very very susceptible to change in environmental conditions. So if you can provide **conducive** environmental condition then we can definitely increase the removal efficiency in an anaerobic system.

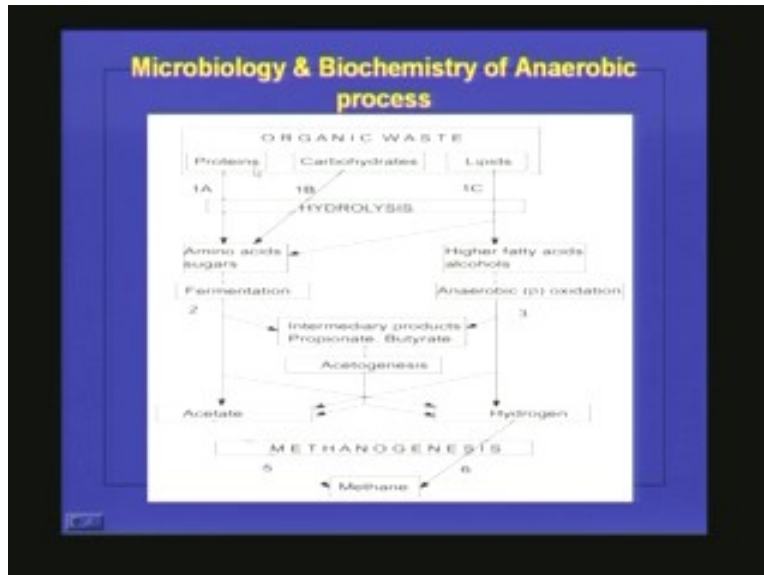
The third and most important one is the retained amount of biomass in the system. If we can retain a high concentration of biomass in the system definitely our treatment efficiency will be improved.

The fourth one is how we are designing the system. We have seen different reactors configurations; **CSTR** reactors, plug flow reactors and batch reactors so based upon how we are designing the reactor the efficiency of the system will be increasing.

The last one is the time we are allowing the wastewater to remain inside the reactor. If you retain the wastewater for long time in the system definitely the treatment efficiency will be increasing. So whenever we go for any system and if you want to increase the efficiency we have to consider each and every factor in detail and we have to make our

design accordingly. Now we will see what are the microbiology and biochemistry of anaerobic process.

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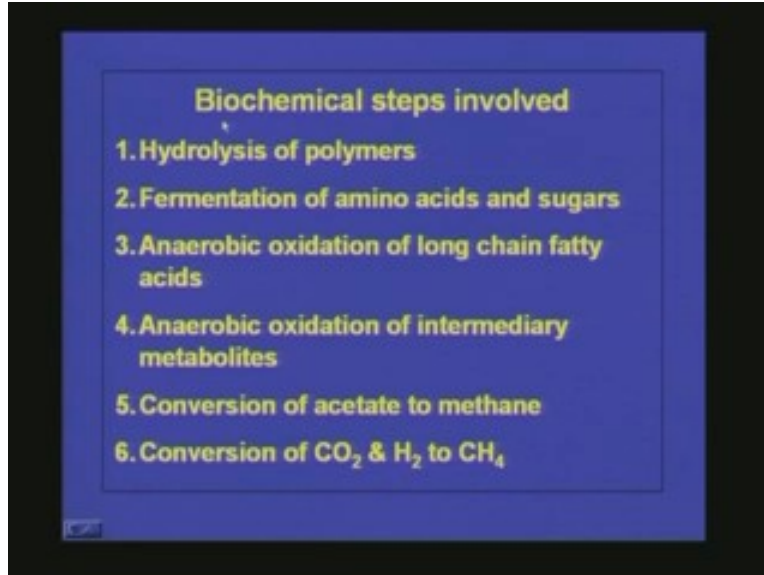


As I have already mentioned it is a very complicated process. That means many reactions are taking place and many groups of microorganisms are involved in this process. This picture shows the path-wise of anaerobic degradation. You have the organic ways and it consists of proteins, carbohydrates and lipids so any domestic wastewater we take it will be containing all these components. What will happen is as the first step hydrolysis will be taking place then after hydrolysis the proteins and lipids undergo fermentation.

The byproduct of hydrolysis are amino acids and sugars and this will be undergoing fermentation and we will be getting intermediary products like propionate, butyric etc and these lipids will be giving higher fatty acids and alcohols and this higher fatty acids and alcohols will be undergoing anaerobic oxidation and anaerobic beta oxidation and you will be getting again propionate and **butyric**. Then the next step is acetogenesis and here we will be getting acetate as well as hydrogen and this hydrogen will also produce some fermentation path as well as this anaerobic beta oxidation.

The last stage is methanogenesis. Methanogen or methane fermentation can take place from acetate and from the combination of hydrogen and carbon dioxide whatever is generated in the fermentation process. These are the six important steps of anaerobic treatment. First one is hydrolysis, second one is fermentation, third one is anaerobic oxidation, fourth one is acetogenesis, fifth one is methanogenesis. This methanogenesis is itself can be divided into two different categories; one is the methanogenesis from acetate and another one is the methanogenesis from hydrogen. So we will see this one in detail.

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The biochemical steps involved are;

- Hydrolysis
- Fermentation
- Anaerobic oxidation
- Anaerobic beta oxidation
- Conversion of acetate to methane and
- Conversion of carbon dioxide and hydrogen to methane

These six steps are carried out by three different groups of microorganisms.

First one is the hydrolytic and acidogenic bacteria, another one is obligate hydrogen producing acetogenic bacteria and the third one is methanogenic bacteria. **We will see the role of bacteria when we discuss the different steps in detail.** First we will see what is happening in the hydrolysis process. We know that the treatment of any wastewater is taking place because of the microbial metabolic activity.

How the metabolic activity is taking place?

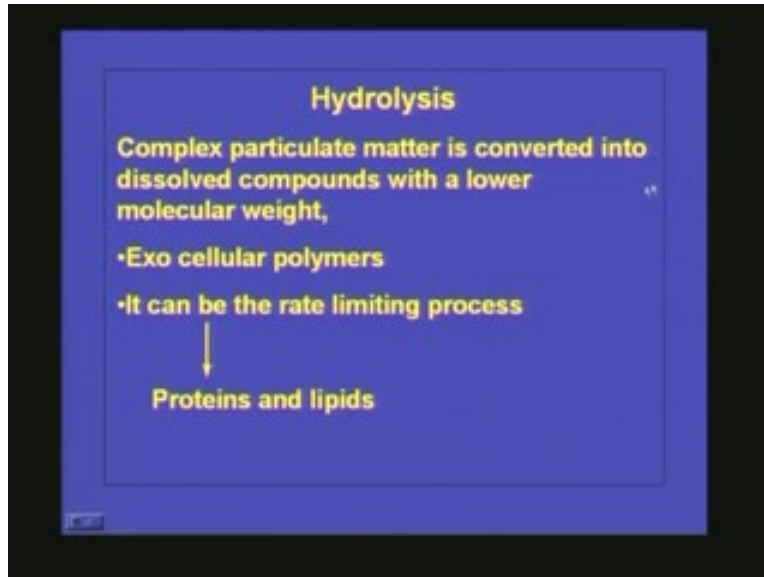
The substrate has to come and get attached to the cell and it has to penetrate through the cell and inside the cell the metabolic activity will be taking place. But we know that most of the organic molecules present in the wastewater is very large in size so it will not be able to penetrate through the cell.

The size of the molecule will be such that it will not be able to penetrate through the cell membrane so how can the cell take this organic matter?

First they send some extra cellular enzymes or produce some extra cellular enzymes so these extra cellular enzymes will hydrolyze the big size organic matter into small fractions. So in hydrolysis for the complex organic matter they will be adding water

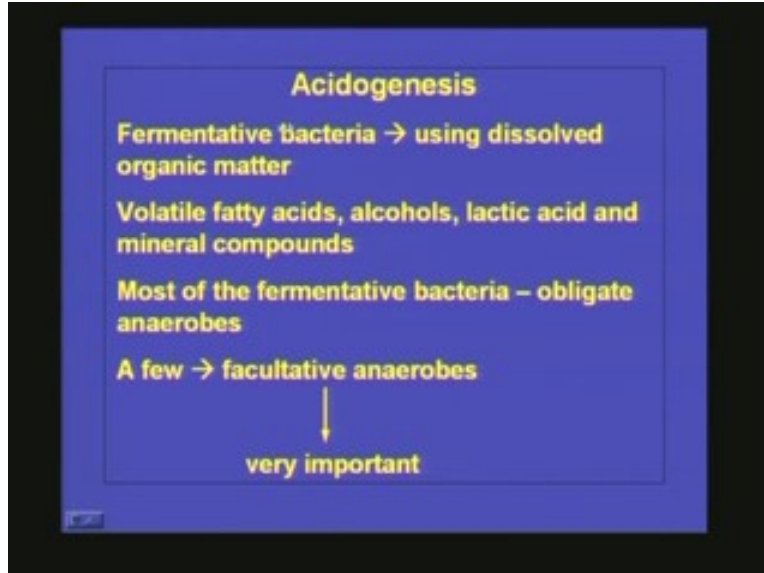
molecule. That is the hydrolysis process. In the process the large organic molecule will be split into small fractions. Once it is split into small small fractions it will be easily penetrating to the cell membrane and once it enters in the cell membrane it can undergo metabolism. That is what is happening in hydrolysis.

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Thus, complex particulate matter is converted into dissolved compounds with a lower molecular weight. This is happening with the help of exo cellular polymers and sometimes it can be the rate limiting process. If it is simple carbohydrates the hydrolysis will be very very easy. But whenever we talk about proteins and lipids if the wastewater contains more of proteins and lipids this hydrolysis can be the rate limiting step. Unless the hydrolysis takes place the other process will not be able to takes place because unless it is able to enter the cell membrane other process cannot takes place. Especially when the wastewater contains more of proteins and lipids hydrolysis can be the rate limiting step. Therefore, we have to be careful about the nature of the wastewater coming into the system. So whenever we design we have to design accordingly.

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Now the second stage is acidogenesis. In hydrolysis the complex organic compounds are split into small compounds like amino acids and sugars and the fermentative bacteria will be using this dissolved organic matter in the form of amino acids and sugars and they will be converting it into volatile fatty acids, alcohol, lactic acid and mineral compounds like hydrogen sulfite, ammonia, carbon dioxide etc so that is the acidogenesis step. The reason we are calling it as acidogenesis step is because of the fermentative process the amino acids and sugars will be splitting up into volatile fatty acids, lactic acids, alcohols and other organic acids so definitely the pH of the system will be coming down at this stage because lot of different types of organic acids will be generated due to the fermentative process.

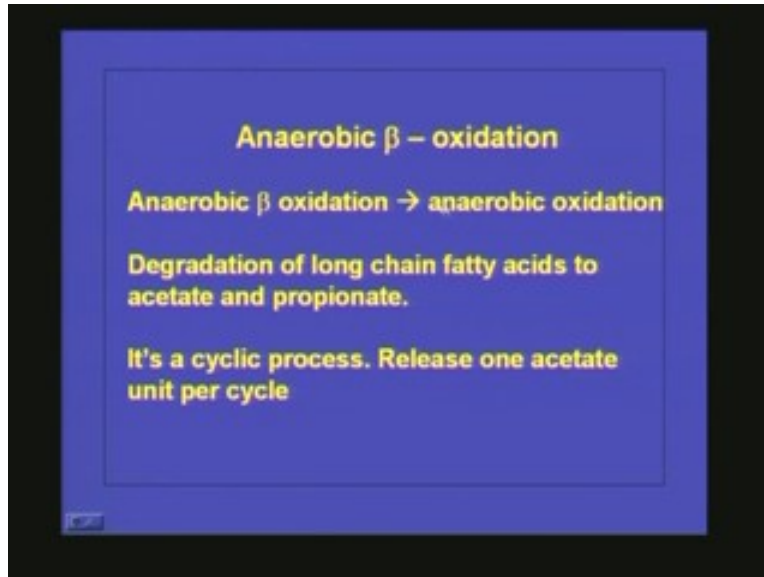
Most of the fermentative bacteria present in the system are obligate anaerobes. This hydrolysis and acidogenesis this bacteria most of them are obligate anaerobes but a few are facultative anaerobic bacteria which is doing this fermentative process and these facultative anaerobes that means the facultative bacteria are those which can survive in aerobic conditions as well as anaerobic conditions or they can undergo oxidative phosphorylation as well as this substrate level **phosphor relation**. So these facultative bacteria are very very important in anaerobic process.

The reason is whenever we talk about the domestic wastewater it will be open to the atmosphere so definitely there will be some dissolved oxygen present in the system. Hence, what will be able to take care of this oxygen as this oxygen is toxic to the obligate anaerobic bacteria they will not be able to perform in the presence of oxygen so what will be taking care of the dissolved oxygen present in the wastewater. The bacteria responsible for that one is the facultative bacteria, they will be consuming the oxygen and they will be undergoing that oxidative phosphorylation using to organic matter and all the oxygen that remains in the system will be getting removed so the system will be having an incomplete anaerobic condition which is conducive for other bacteria for example the



obligate, acetate, generating bacteria as well as methanogens they are obligate anaerobes though they cannot perform in the presence of oxygen they will not die in the presence of oxygen they will become inactive in the presence of oxygen and once the oxygen is removed from the system they will against restart their performance.

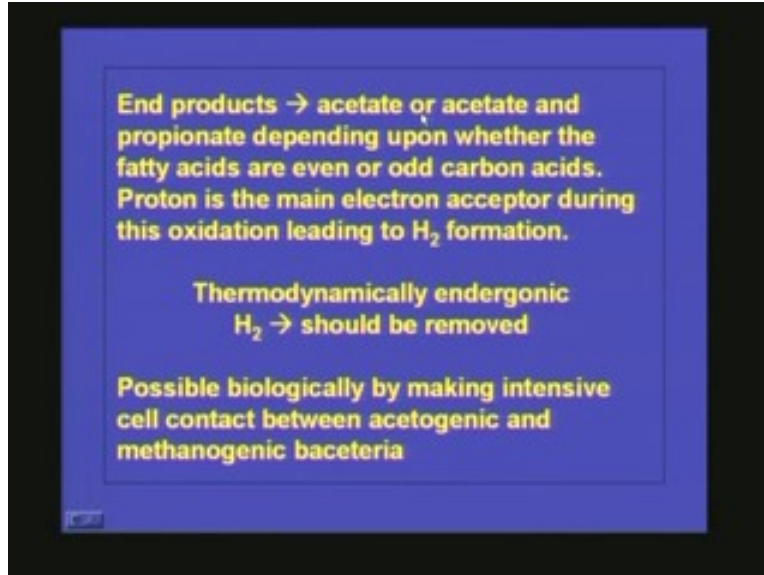
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Now we will see what this anaerobic beta oxidation is. We have seen that after the fermentation we will be getting long chain fatty acids. So these long chain fatty acids has to be converted to acetate for the methanogenesis or for the methane protection so how this long chain fatty acids are getting converted to acetate or acetate and propionate that is happening because of this anaerobic beta oxidation.

In anaerobic beta oxidation the degradation of long fatty acids to acetate and propionate is taking place. It is a cyclic process it release one acetate unit per cycle and it will also liberate hydrogen. But sometimes along with acetate it will be librating propionate also, it will be depending upon the number of carbon atoms present in the organic matter or number of carbon atoms present in the long chain fatty acids. **That is what I have written here.**

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The end products are acetate or acetate and propionate depending upon whether the fatty acids are even or odd carbon acids. If it is even number fatty acids then you will be getting only acetate and if it is an odd carbon acid then you will be getting acetate plus propionate. Because we know that acetate is having two carbon items and propionate is having three carbon items so depending upon whether it is having odd number of carbon atoms or even number of carbon atoms the end product will be changing. And along with this acetate and propionate we will be getting lot of hydrogen H<sub>2</sub> from the beta oxidation and this hydrogen production in a way it is good because we know that certain methanogenesis methanogenesis bacteria are methanogens can convert this hydrogen and carbon dioxide to methane.

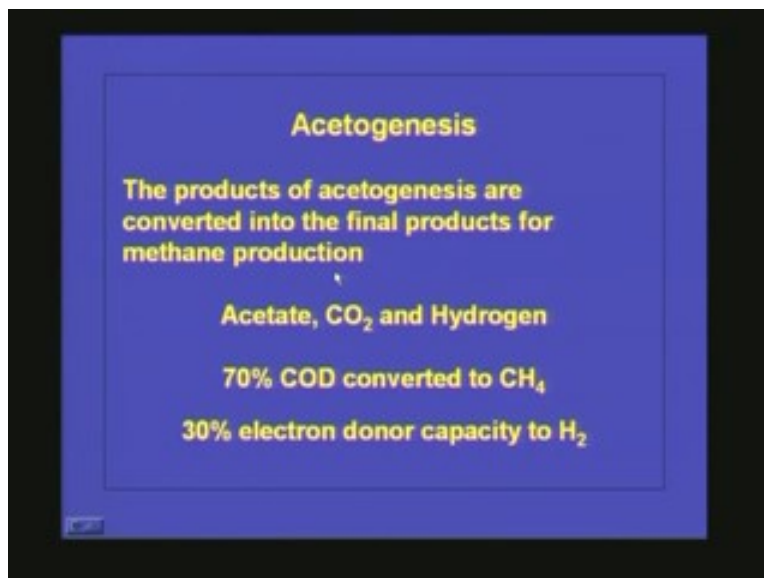
But if you talk about this anaerobic beta oxidation, if you see thermodynamics of the process it is an end organic process. That means the reaction is not feasible under standard condition. Therefore, as the reaction proceeds more and more acetate and propionate will be generated and along with that hydrogen molecule also will be liberated so the concentration of the product will be increasing. So in any reaction when the concentration of the products are more the reaction rate will be decreasing because we know the reaction rate so if you want to increase the rate of the reaction we have to remove the hydrogen atom or hydrogen molecule present in the system then only the reaction will be proceeding forward so that is where the help of hydrogen utilizing bacteria is coming into picture.

If the hydrogen whatever is generated during the anaerobic beta oxidation can be removed by certain other microorganism in the same rate then the hydrogen and concentration or the hydrogen molecule or hydrogen partial pressure in the system will be less and the reaction will be feasible, this is very very important. If the methanogens are not active in the system then this anaerobic beta oxidation will not be taking place and all the long chain fatty acids will be getting accumulated in the system. So if the long chain

fatty acids get accumulated in the system definitely the system pH will be coming down. Though it is organic acids any acid can reduce the system PH. But we all know that the methanogens can perform only under neutral condition. So once the methanogens are not able to remove the hydrogen partial pressure whatever is developed in the system for the production of methane then the beta oxidation will be stopped and the system pH will be coming down. Hence, as the system pH comes down the methanogens will become inactive because the environment is non consistent for the system thus the system will be failing or the efficiency of the system will be deteriorating day by day and we will not be able to retrieve the system at all. This is known as stock digesters.

This anaerobic beta oxidation and hydrogen partial pressure in the reactor is very very important for the proper functioning of the reactor. The reason is if the hydrogen partial pressure is higher then the anaerobic beta oxidation will not be taking place and if anaerobic beta oxidation is not taking place the fatty acids will be accumulating in the system so the pH will be going down and as the pH goes down the methanogenic activity will be further reducing.

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Now the first process is acetogenesis. We have seen that first hydrolysis is taking place and the products formed by the hydrolysis is undergoing fermentation and the fermentation byproduct the long chain fatty acids etc will be undergoing anaerobic beta oxidation and we are getting propionate, butyrate and many other products. So in acetogenesis what is happening is the product of acetogenesis is converted into final products for methane production. The final product for methane production is acetate, carbon dioxide and hydrogen because the raw materials that are whatever microorganisms used for methane production are acetate, carbon dioxide and hydrogen.

Hence, the acetogenesis step is the one which converts all the other byproducts of anaerobic beta oxidation and fermentation to the byproduct which is required for the

methane protection. Here we can see that acetate is there, carbon dioxide is there and hydrogen is there. There are many microorganisms which can use the carbon dioxide as well as the hydrogen to form methane. But there are only a limited group of microorganisms which can convert acetate to methane.

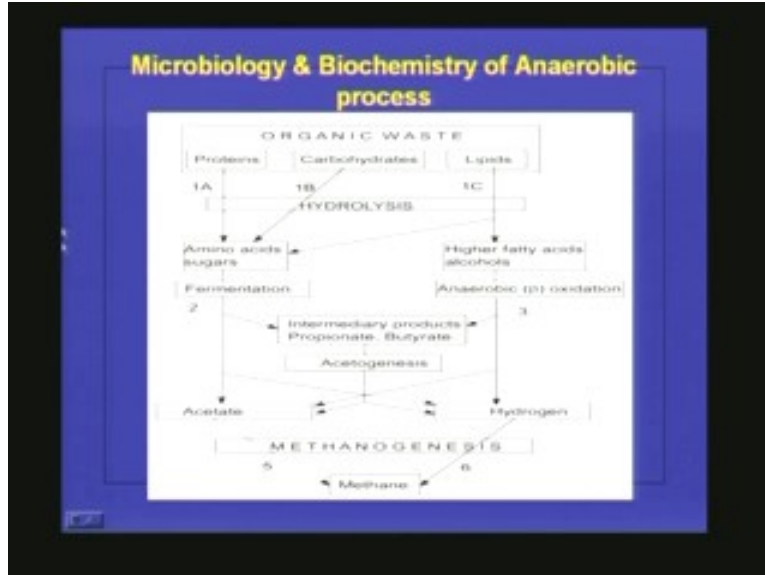
But if you see the methane formation around 70% of the methane is generated from acetate and only 30% is coming from carbon dioxide a hydrogen so the bacteria which is generating methane from the acetate or from hydrogen and carbon dioxide is known as methanogens and the bacteria which are responsible for the production of acetate and hydrogen are known as acetogens and the bacteria which are responsible for the hydrolysis and fermentation are known as hydrolytic and acetogenic bacteria and in this three different types of bacteria the acetogens and methanogens are obligate **entraps**.

Obligate **entraps** means they can survive only in anaerobic conditions or they will be performing only in complete anaerobic conditions. Or if you want to maintained anaerobic condition in terms of millivolts then it should be in the range of  $-350$  mV or something like that. That means the dissolved oxygen concentration will be very very low or it is almost negligible. But in the acetogens and fermentative bacteria some of them are facultative type of bacteria so if some oxygen is present in the system they will be able to remove that oxygen by oxidative phosphorelation and the system will be converted into complete anaerobic system.

This is the path way I showed earlier (Refer Slide Time: 38:22). So now this one will be much clearer. The organic waste is coming which contains proteins, carbohydrates and lipids so hydrolysis is taking place. In hydrolysis the splitting up of these molecules are taking place with the help of hydrolytic bacteria and in hydrolytic bacteria few are strict anaerobes and few are facultative type. These facultative type bacteria are very very important for anaerobic process because they will be taking care of the dissolved oxygen whatever is coming in the wastewater.

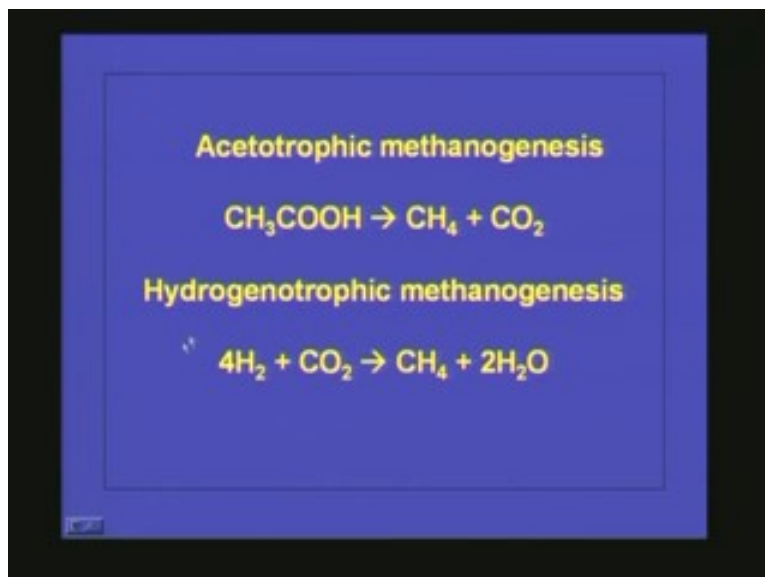
So once the hydrolysis is over you will be getting amino acids and sugars from proteins, carbohydrates and lipids and this amino acids and sugars are undergoing fermentation and you will be getting intermediary products like **propionate and butyrate** and lipids and after hydrolysis we will be getting certain higher fatty acids and alcohols, this will be undergoing anaerobic beta oxidation and you will be getting acetate as well as propionate and this (Refer Slide Time: 39:23) intermediary compounds whatever is present here with the help of acetogenesis or in the help of acetogens the acetate producing bacteria will be getting converted to acetate and hydrogen and fermentation will also produce hydrogen acetate and carbon dioxide. So this acetate, hydrogen and carbon dioxide in the presence of methanogenic bacteria will form methane.

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This is the complete picture of an anaerobic process. So we can see that so many steps are there and so many different classes of microorganisms are present in the system. So, if one step is not happening so entire reactor will be failing or if one process is taking lot of time the entire process will take lot of time. That is why the anaerobic process is complicated and earlier the biochemistry or microbiology of the anaerobic process was not known so people were not able to optimize the process. That was one of the reasons why this process was not becoming so popular.

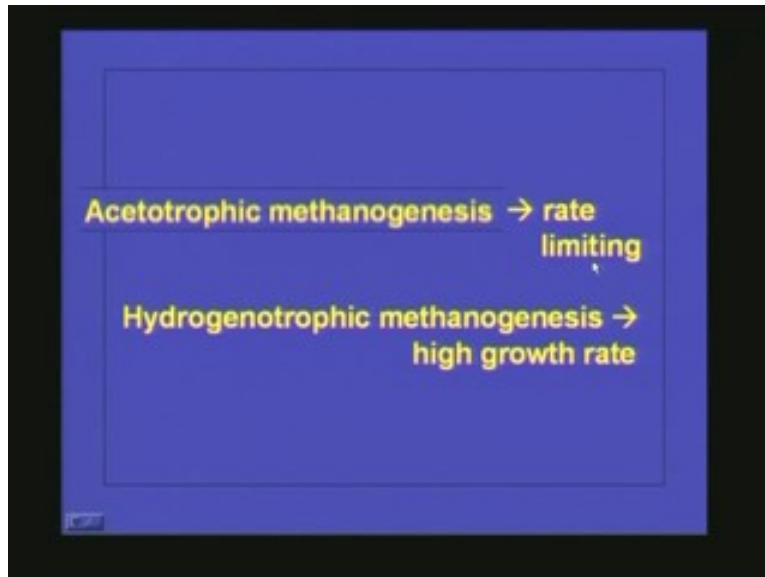
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This is the reaction that is taking place in methanogenesis. The methanogenesis can be done in two different ways. One is acetotrophic methanogenesis that means the methane production is taking place from the acetate so  $\text{CH}_3\text{COOH}$  is acetate so it will be forming methane and carbon dioxide. The other one is hydrogenotrophic methanogenesis. That means hydrogen plus carbon dioxide is reacting together to form methane and water.

So, if you see this acetotrophic methanogenesis and hydrogenotrophic methanogenesis this acetotrophic methanogenesis is rate limiting because acetogenesis or acetotrophic methanogens take long time for the regeneration whereas in the hydrogenotrophic methanogens the regeneration time is very less. This type of bacteria will be more in the system so that will not be the rate limiting step but the acetotrophic methanogens will be the rate limiting step in any anaerobic process. **That's what I have written here.**

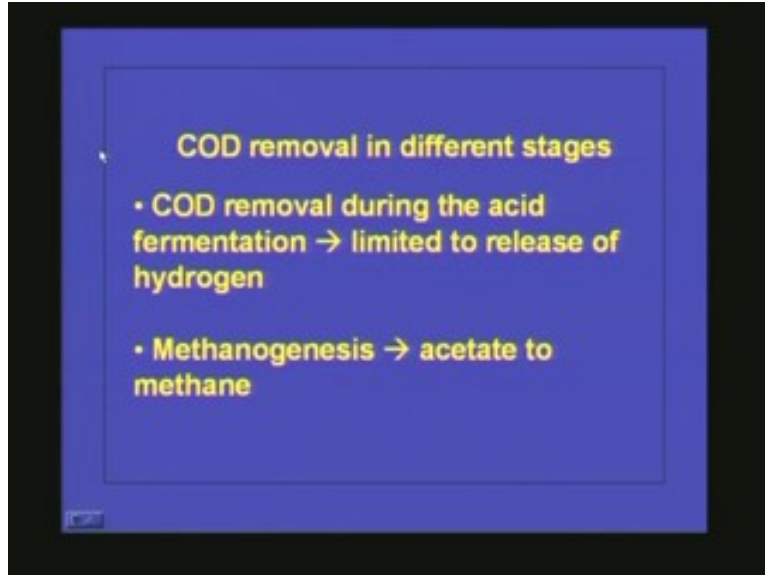
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Acetotrophic methanogenesis is rate limiting and in hydrogenotrophic methanogenesis because of the high growth rate you will be having large number of microorganisms coming under this category so this rate will be very very high whereas the microorganism which can form methane from acetate is only a very few so definitely this will be the rate limiting step. And if you want to see the COD removal in different stage status of an anaerobic process the major COD removal is taking place in methanogenesis.

Hence, what is happening is the acetate will be getting converted to methane and carbon dioxide and another COD reduction is taking place in fermentation so there the electron transferred whatever is there is released as hydrogen molecule. So whatever is the amount of hydrogen liberated that much of COD reduction is taking place in the fermentation process or the major contribution of the COD reduction is taking place only in the methanogenesis step.

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COD removal during the acid fermentation is limited to the release of hydrogen and in methanogenesis here lot of acetate is getting converted to methane so the maximum amount of COD reduction is taking place in this stage. So the most important problem whatever we face in anaerobic process is the souring of the reactor or the [s.....ck.....43:11] reactor.

We have already discussed the reason for that one. The reason is the methanogens are very very sensitive and in most of the anaerobic reactors this methanogenesis is rate limiting. If you want to have the COD removal or the treatment efficiency the COD should be removed from the system and COD removal mainly is taking place only in methanogenesis. So if the methanogenesis is rate limiting or the methanogenesis is not taking place in the system definitely there will not be any COD removal in the system so the efficiency of the system will be coming down.

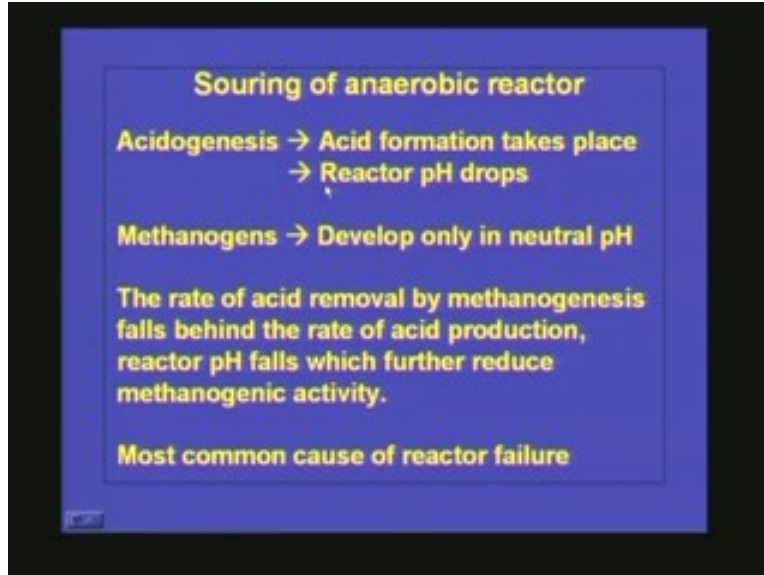
What will happen in an anaerobic system?

These methanogens will survive only in neutral PH. that means the optimum pH for methanogens is in the range of 6.8 to 7.6. So if the pH is less than that one these methanogenic bacteria will not be able to survive under that condition. So what will happen is during the fermentation or acidogenesis process and anaerobic beta oxidation we have seen that hydrogen molecules will be liberated and it will be increasing the partial pressure of hydrogen molecule in the system.

The acidogenesis process will be generating so many acids so definitely if these acids are getting accumulated in the system the pH of the system will be coming down. So if the methanogens are not working in the same rate what will happen this acids will be accumulating in the system then these methadations will not be able to survey so that is what is happening in a souring anaerobic reactor. **That is what I have written here.**



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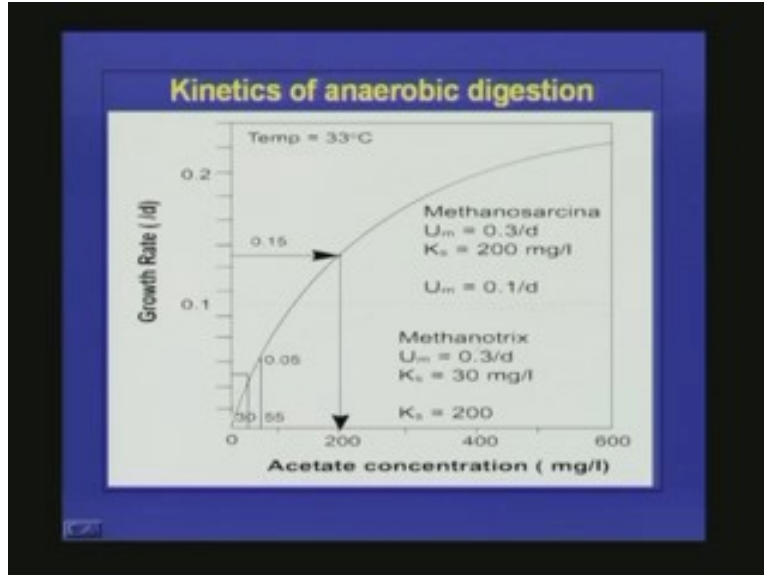


Acidogenesis: The acid formation takes place and because of this one the reactor pH drops and methanogens develop only in neutral PH. The rate of acid removal by methanogenesis falls behind the rate of acid production. The reactor pH falls and this further reduces the methanogenic activity. This is the most common cause of reactor failure.

If you want to control this one then we have to add lot of alkalinity to the reactor so that the pH variation in the system can be maintained within the specified limit, that is very very important. We have already seen the metabolism of anaerobic process. So if you want to see the kinetics of anaerobic digestion we have already seen that the microbial growth rate in anaerobic systems are much less compared to aerobic system and in anaerobic process the methanogens is the rate limiting step. The major methanogenic bacteria present in the systems are methanosarcina and methanotrix.



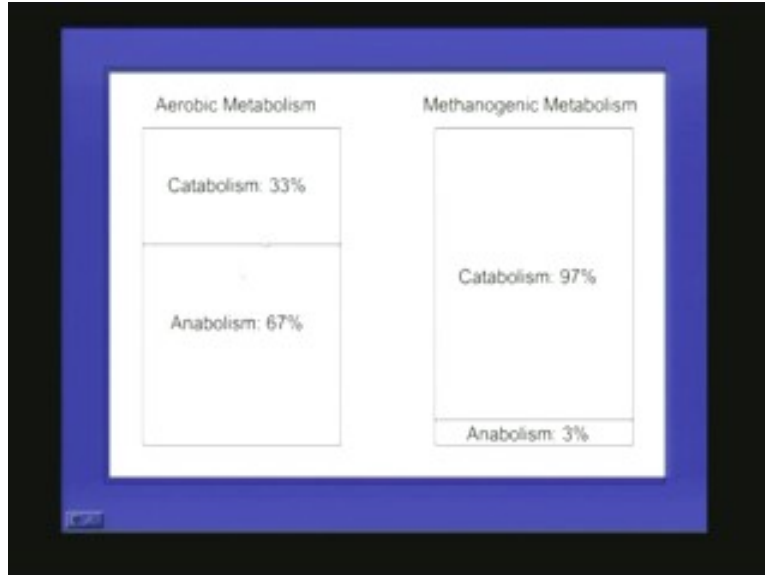
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This shows the growth curve of methanotrix and methanosarcina. Thus, we can see that this methanotrix is having a  $\mu_{max}$  value of 0.1 D that means maximum specific growth rate of 0.1 per day. That means if you want to design a system and if you want to maintain this microorganism in the system the biological sludge retention time should be 1 by this  $\mu_{max}$  value that means it should be 1 by 0.1 that means 10 days.

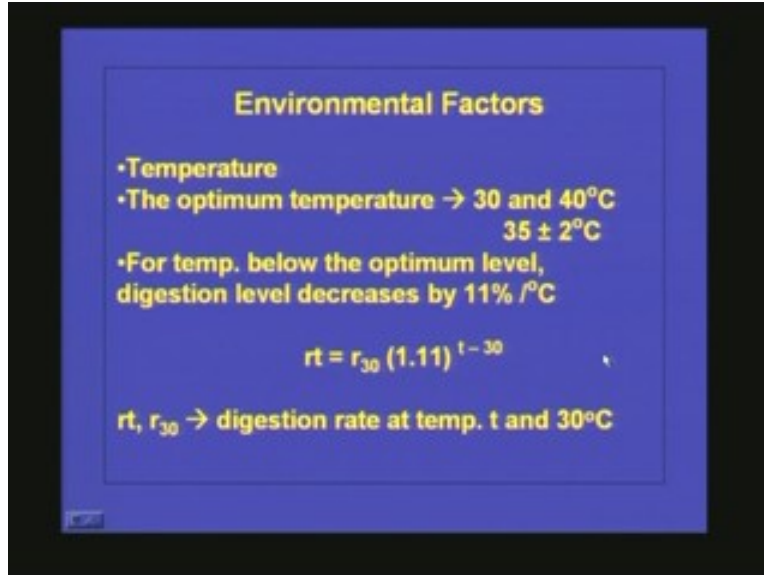
Therefore, if you design a system with **BSRT** less than 10 days then whatever the time we are providing that will not be enough for the regeneration of this methanotrix so whatever is present in the reactor will be washing away from the reactor if the BSRT is less than 10 days. So that is the reason we have to give a last hydraulic retention time as well as biological sludge retention time in anaerobic systems. If you can retain the microorganism in the system and only the liquid is going out of the reactor then we can reduce the hydraulic retention time. Otherwise we have to a high hydraulic retention time also in the system if we are not able to maintain high microbial concentration in the system.

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We have already seen this which shows the difference between aerobic metabolism and anaerobic metabolism. So in aerobic metabolism the catabolism is only 33% or whatever substrate we are giving around 33% is used for catabolism and for anabolism around 67% is used. It varies from microorganism to microorganism so we can take a range as 40 to 70. But when you come to the methanogenic metabolism the catabolism is around 97% and anabolism is only 3% so we can imagine the amount of biomass getting generated in the anaerobic system. This is the reason why the methanogenic process is becoming rate limiting. The biomass generation is so less so unless we give a large retention time or large biological sludge retention time then only we can get a significant amount of biomass in the system because most of the COD or most of the organic matter coming to the system is utilized for the catabolism or catabolic purposes.

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**Environmental Factors**

- Temperature
- The optimum temperature → 30 and 40°C  
35 ± 2°C
- For temp. below the optimum level,  
digestion level decreases by 11% /°C

$$r_t = r_{30} (1.11)^{t-30}$$

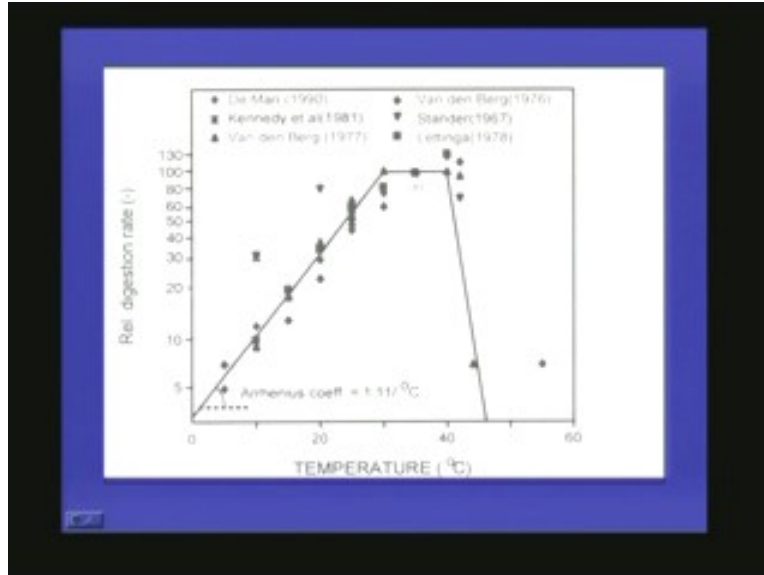
$r_t, r_{30}$  → digestion rate at temp. t and 30°C

Now we will see the favorable environmental factors for the anaerobic process.

One is temperature. Temperature is very very important for anaerobic process. The optimum temperature is 30 to 40 degree centigrade, the optimum being 35 plus or minus 2 degree centigrade and if the temperature is below the optimum level the performance will be coming down drastically and researches have shown that for every one degree decrease in the temperature the efficiency will be coming down by 11%. We can find out what is the digestion rate at any temperature if you know the digestion rate at 30 degree. We can use this formula to calculate the digestion rate;  $r_t$  is equal to  $r_{30}$  into 1.11 raised to  $t$  minus 30. This is the temperature dependent reaction or we can make out how the reaction rate is changing with respect to temperature.

From this formula itself we can see that for a temperature below the optimum level the digestion level decreases by 11% per degree centigrade. And here (Refer Slide Time: 49:55) this  $r_t$  and  $r_{30}$  represents the digestion rate at temp t and 30 degree centigrade.

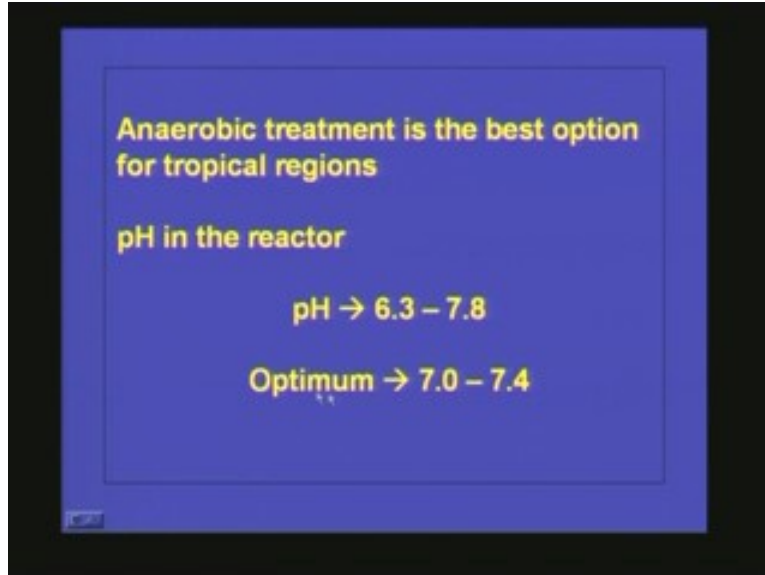
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This shows the effect of temperature in anaerobic process. This is the relative digestion rate, we can see that at low temperatures the digestion rate is so less and it keeps on increasing at a constant rate with respect to the temperature and it reaches a maximum value around 30 degree centigrade and 30 to 40 it will be almost a constant maximum being at 35 plus or minus 2 degree centigrade. This is the optimum rate and after this one the efficiency will be coming down drastically. We can see that if the temperature is more than 45 degree centigrade the anaerobic reactor is not going to work. So this is for the mesophilic microorganism.

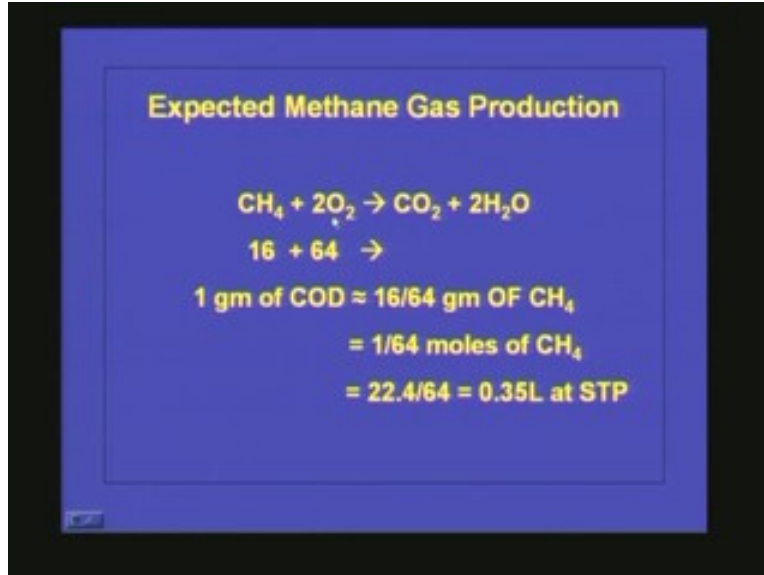
Nowadays people are developing thermophilic and the microorganism which can work at a lower temperature effectively. Whatever we have discussed so far we can make sure that anaerobic treatment is the best option for tropical region because in tropical region the temperature available will be in all the seasons around 30 or 35 degree centigrade so at this time the reaction rate will be at maximum and will be getting maximum efficiency. So anaerobic treatment is the best option for tropical regions and pH in the reactor we have to maintain in between 6.3 to 7.8 and optimum pH is 7 to 7.4.

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And we have seen that the anaerobic process as a byproduct we will be getting methane and it is having high calorific value and we can use it as a fuel. So if you want to find out what is the methane generation or what is the amount of methane generated in anaerobic process we can find out using this equation; we take methane and if you want to completely oxidize it in the presence of oxygen what will happen is methane plus oxygen will give carbon dioxide plus  $2\text{H}_2\text{O}$  that means 16 gm of methane combines to 64 gm of oxygen or 1 gram COD is equivalent to 16 by 64 grams of methane or 1 by 64 moles of methane or methane gas generated by 1 gm of COD is around 0.35 liters at STP.

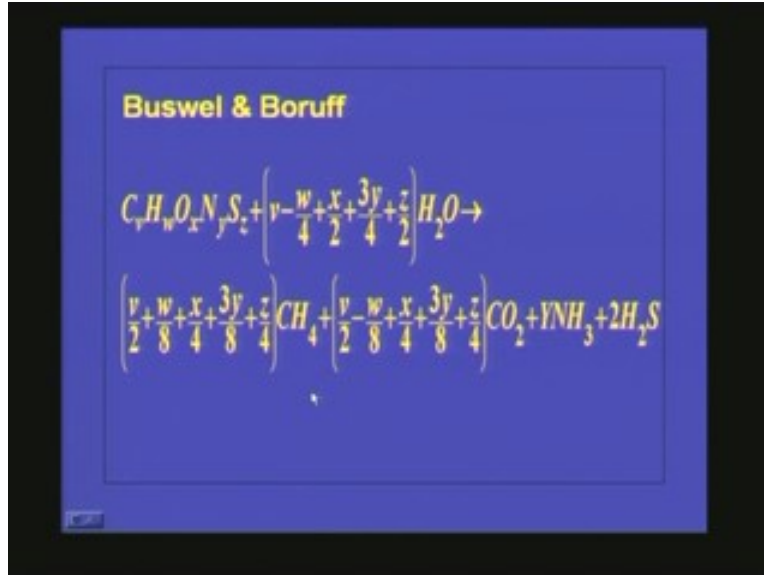
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So if we can convert 1 gm of COD to methane gas we will be getting 350 ml of methane gas at STP as per the **stochemistry**.

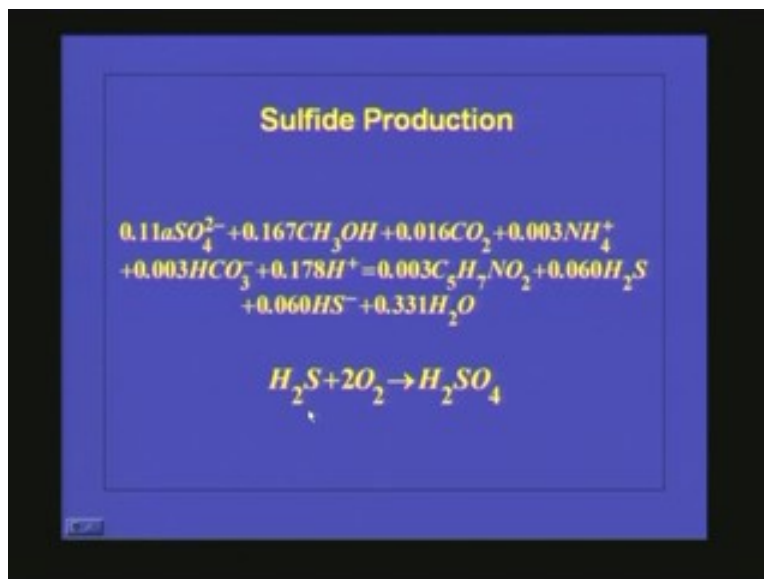
There are different other formula developed by other people depending upon the composition of the organic matter. So if you have v molecules of C and w molecules of H, x molecules of oxygen and y molecules of nitrogen and z molecules of sulfur in the system then this (Refer Slide Time: 53:13) will be undergoing a reaction with water and this is the byproduct. You will be getting methane carbon dioxide ammonia and hydrogen sulfite so we can find out what is the amount of methane produced and what is the amount of carbon dioxide produced and what is the amount of ammonia produced then hydrogen sulfite produced using this equation;  $v$  by 2 +  $w$  by 8 +  $x$  by 4 + 3  $y$  by 8 +  $z$  + 4 where v w x y z are the number of atoms of carbon, hydrogen, oxygen, nitrogen and sulfur present in the organic matter respectively.

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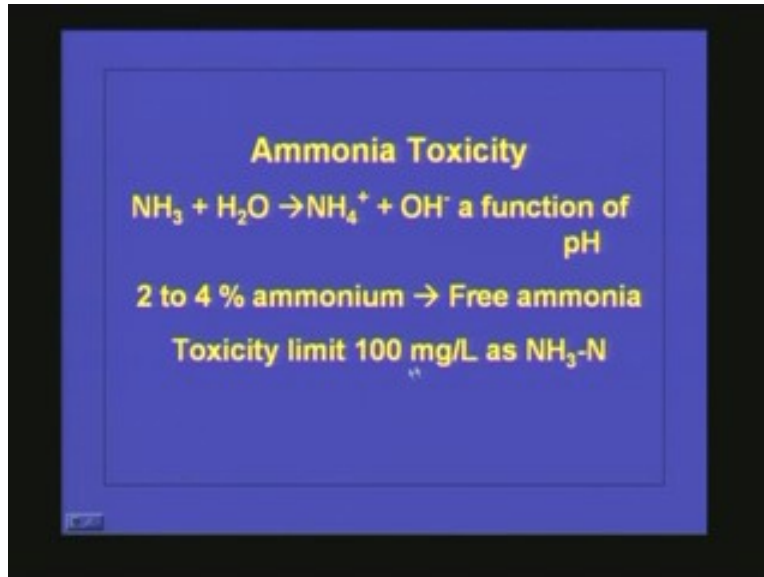
Similarly we can find out the amount of carbon dioxide produced and the amount of ammonia produced and hydrogen sulfite produced in the system. So if you want to find out the mole fractions same equation we can use and we can get the mole fractions of carbon dioxide, methane and hydrogen sulfite in the gas generated if you know the composition of the waste coming to the system. Similarly we can find out the sulfide production also from the system. If sulfide is there and organic matter is there and carbon dioxide and ammonia is present in the system then you will be getting hydrogen sulfite and HS and water.

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This hydrogen sulfide and oxygen will combine to form sulfuric acid. So this is the corrosive gas whatever is generated in the anaerobic system. So a portion of the hydrogen sulfide is essential for the anaerobic microbial growth because sulfide is an essential part of the molecular structure. But if it is in excess it will be a problem for the reactor system.

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Similarly another byproduct is ammonia. And if the ammonia concentration is a success it will also inhibit the process. If 2 to 4% of ammonium or is present in free ammonia form it will be inhibiting the reactor or the toxicity of ammonia in the system is 100mg per liter as ammonium nitrogen. If it is present in the ammonium form it will not be toxic but if it is present in the ammonia form it will be toxic and this ammonia conversion to ammonium is a pH dependent process.

We will see the things we discussed today. Today we were discussing what an anaerobic process is and how it is different from aerobic process and we have seen the advantages of anaerobic process. We have seen that it is less energy intensive and the sludge production is less, etc. But we have also seen the disadvantages of this anaerobic process and because of these disadvantages the anaerobic process was not popular till the recent past. But nowadays what is happening is the microbiology of the process is very clear and people are able to maintain large quantity of sludge in the anaerobic systems by the invention of hybrid reactors. The anaerobic technology is gaining much more attention nowadays.

We have seen the detail biochemical path way of anaerobic degradation and we have seen that mainly six steps are involved in the anaerobic process and three groups of microorganisms are present in the system. And depending upon the nature of the organic matter either hydrolysis or methanogenesis can be the rate limiting step. So by knowing the organic matter and the process we can design the system effectively.