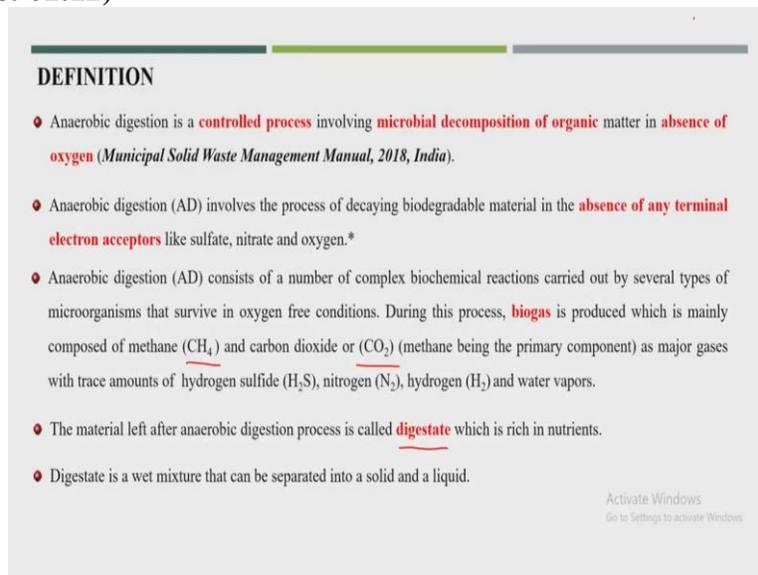


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**Lecture - 30**  
**Definition, stages and Factors Affecting Anaerobic Digestion**

So, hello students. Now we are on module 9, biological transformations- II and we will talk about the anaerobic digestion process. Here, we will have 3 lectures on the anaerobic digestion process and in this module, I shall discuss what exactly is the meaning of the anaerobic digestion process. The process depends on different types, different stages, different factors affecting and also the commercially available reactors. So, we are at the transformation. Today's lecture will be on definition, stages and factors affecting the anaerobic digestion process.

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**DEFINITION**

- ◆ Anaerobic digestion is a **controlled process** involving **microbial decomposition of organic** matter in **absence of oxygen** (*Municipal Solid Waste Management Manual, 2018, India*).
- ◆ Anaerobic digestion (AD) involves the process of decaying biodegradable material in the **absence of any terminal electron acceptors** like sulfate, nitrate and oxygen.\*
- ◆ Anaerobic digestion (AD) consists of a number of complex biochemical reactions carried out by several types of microorganisms that survive in oxygen free conditions. During this process, **biogas** is produced which is mainly composed of methane ( $\text{CH}_4$ ) and carbon dioxide or ( $\text{CO}_2$ ) (methane being the primary component) as major gases with trace amounts of hydrogen sulfide ( $\text{H}_2\text{S}$ ), nitrogen ( $\text{N}_2$ ), hydrogen ( $\text{H}_2$ ) and water vapors.
- ◆ The material left after anaerobic digestion process is called **digestate** which is rich in nutrients.
- ◆ Digestate is a wet mixture that can be separated into a solid and a liquid.

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So, first, we will go for definition. So, like the composting process, I explained that is an aerobic process. So, degradation of organic matter under the aerobic condition that also you can say is a composting process. Similar way the anaerobic digestion is a controlled process involving microbial decomposition of organic matter in the absence of oxygen. This was provided in our municipal service manual 2018.

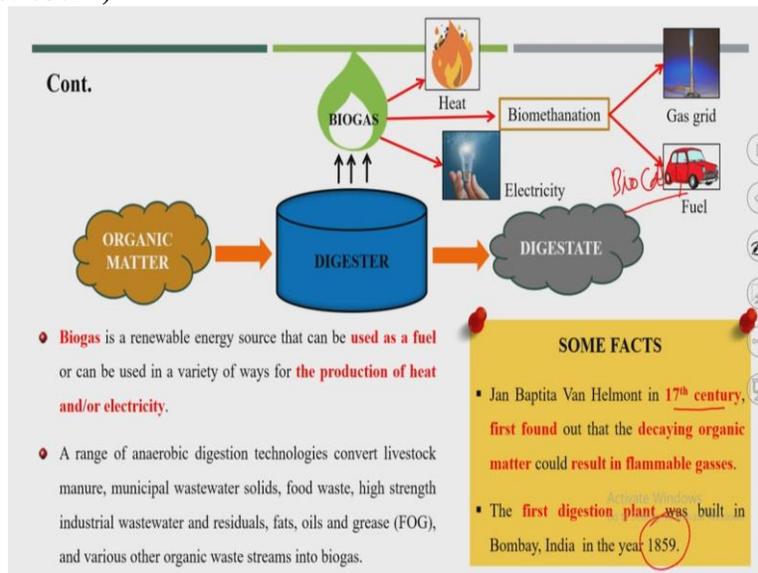
But, if you go through technically; anaerobic digestion involved the process of decaying organic matter in the absence of any terminal electron acceptor, like sulfate, nitrate, or oxygen. So, it is

the absence of any terminal electron acceptor and in this case, the major electron acceptors are oxygen. In the same way, we can say the absence of oxygen. Anaerobic digestion consists of a number of complex biochemical reactions carried out by several types of microorganisms that survive in oxygen-free conditions.

During this process, the biogas is produced which is mainly composed of methane and carbon dioxide as major gases with trace amounts of hydrogen sulfide, nitrogen, hydrogen and water vapors. But the major compositions are methane and carbon dioxide. The material left over the anaerobic digestion process is called digestate. This you remember, we also have a discussion on digestate which is rich in nutrient.

So, normally, the mini literature suggests that whatever the leftover or whatever is the outlet from the anaerobic digestion process can be used as in compost or as in fertilizer because it will be rich in nutrients. Digestate is a wet mixture that can be separated into solid and liquid.

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So, now here this is the digester, if you feed organic matter and finally you will get digested is an outcome of the reactor along with that the biogas will generate and this biogas majorly will be the methane is a highly combustible gas and along with the carbon dioxide will be also there, because of the degradation process. So, this biogas is a renewable energy source that can be used as fuel can be used as a variety of ways of production of heat and electricity.

This biogas directly can be used in the kitchen for cooking purposes directly we can use without any separation, only the problem is that this carbon dioxide is a highly corrosive gas. So, if that percentage is low, then easily we can use directly this biogas for cooking purposes. So whatever here directly we can use for heat production for the cooking process, through by biomethanation process.

Biomethanation process means the separation of carbon dioxide from the biogas directly gas we can put it into the gas grid or directly this methane if we are getting 90, 95% of methane means we can called as biocng. So, normally whatever the cng is available that contained 95 to 98% of methane, but that is chemically produced methane. Now, here through this biogas also if you are able to remove the carbon dioxide from the biogas and other gases like hydrogen sulfide, hydrogen, or ammonia.

And can come up with methane percentage 95 to 98% also can be used as in fuel and same this biogas also can be used for electricity or power production also is possible. A range of anaerobic digestion technologies convert livestock manure, municipal wastewater solids, food waste, high strength industrial wastewater and residual, fat, oil grease and various organic wastes stream into biogas. So, this anaerobic digestion process is very popular in the wastewater treatment process or not only for the sewage treatment.

But also for industrial wastewater treatment, this anaerobic digestion process is highly popular. Why it is very popular? Because sludge production is very low in anaerobic digestion processes like I talked about composting process where the glucose degradation to the compost or that final sludge that will go up to 30% but in the anaerobic condition whatever the sludge will produce that will have only 5%. So, sludge production is low that is the major benefit of the anaerobic digestion process.

And this anaerobic digestion also can take high purified wastewater so like in the aerobic system for wastewater treatment or sewage treatment in the aerobic system we can go up to 200, 300 milligram per liter purified but in the anaerobic system we can go up to 700- 800 milligram per liter purified value and there are a lot of successful processes and various scales of anaerobic

digestion process are available for the sewage treatment and some other industrial wastewater treatment process.

Similar way for anaerobic digestion process also is very popular for the organic waste that is solid in nature, but again that directly we cannot go for the solid one we have to make that particular material is in slurry kind of material and then easy to go for anaerobic digestion process. So, some facts if you see that, in the 17th century, the first found out the decaying of organic matter could result inflammable gases.

So first anaerobic digestion plant was built in Mumbai, India in 1859. So, you see here is a very old process we are knowing this anaerobic digestion process. Maybe I think you also heard of sometimes gobar gas plant. Even in the 90s, when I was very young, I was in the school and in the especially in the rural areas we used to see number of gobar gas plants and these gobar gas plants specially for the production of biogas for cooking purpose and highly successfully running these kinds of plants was available. But now, I think most of the places those plants are not working because of different issues.

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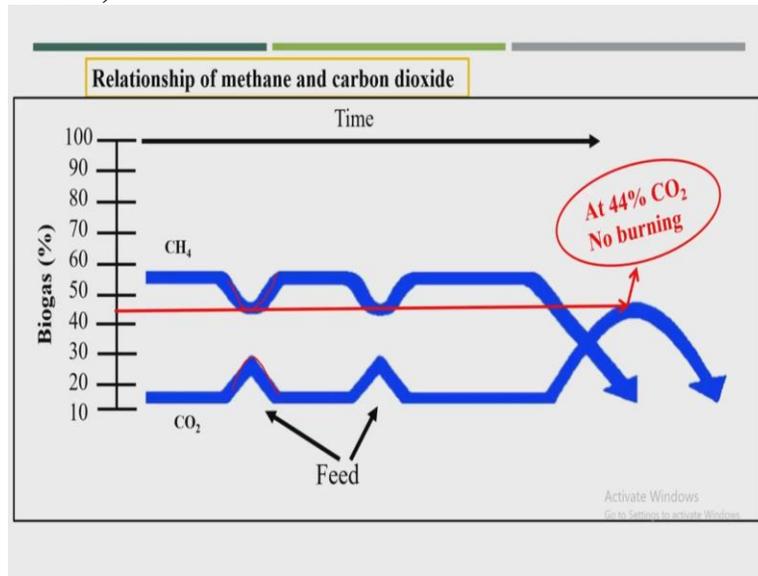
**COMPOSITION OF BIOGAS**

- Methane (CH<sub>4</sub>): 40-70 vol.% (65%)
- Carbon dioxide (CO<sub>2</sub>): 30-60 vol.% (35%)
- Other gases: 1-5 vol.% include
  - ✓ Hydrogen (H<sub>2</sub>) : 0-1 vol.%
  - ✓ Hydrogen sulfide (H<sub>2</sub>S): 0-3 vol.%

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So, when you talk about the composition of biogas, your methane percentage can go up to 70% also is possible. But if you are properly designed reactor can achieve 65 minimum 65% of methane, carbon dioxide could be 30 to 60% and other gases are in between 1 to 5%. But for proper reactors, these percentages of other gases never increase 1% or 2%.

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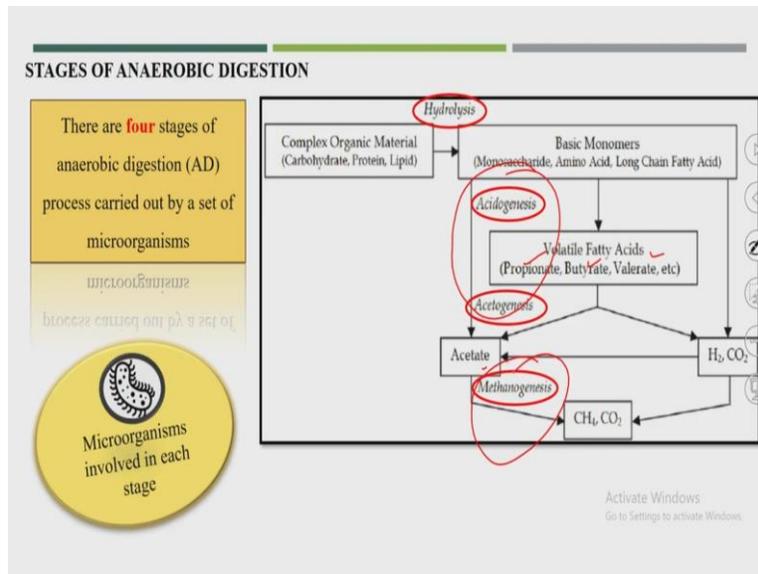


So, when you see the relationship of methane and carbon dioxide, so this is the time versus the biogas. So methane percentage is more and carbon dioxide percentage is low. And you see here, there is a change in or lower down in methane and increase in carbon dioxide in 2 different places is because of feed. Because time to time will feed the reactor, feed the unit with the fresh organic matter. So, these kinds of things are possible.

And a particular time it is possible that carbon dioxide percentage will be more if there is no proper anaerobic condition achieved. So, at 44% of carbon dioxide, if that is getting out from the reactor, there will not be any burning or there will not be any flame is possible means, the reactor is generating more than 45% of carbon dioxide cannot be useful for cooking purpose. And this is what the problem is with those gobar gas plants.

I think the feed has been changed because they were working onto the cow dung or mostly onto the cow dung, but later on, the other kind of feed also has been utilized in the biogas plants like kitchen waste other during the festival also a large amount of organic waste is getting generated and that also has been disposed into this reactor and this entire reactor become acidified because you know that kitchenware highly acidic in nature. So, because of that, the carbon dioxide percentage has been raised and those kinds of reactors have never come back again in the same conditions.

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If you see the stages of the anaerobic digestion process, it will have 4 different stages of the digestion process. First is the hydrolysis. So, hydrolysis is nothing but a simple breakdown of complex organic matter. Like the hydrolysis process, cooking at a household also is one kind of hydrolysis process, or we can say is a pretreatment process. So, why cooking is very important? Because that particular substrate we cannot digest or humans cannot digest very easily.

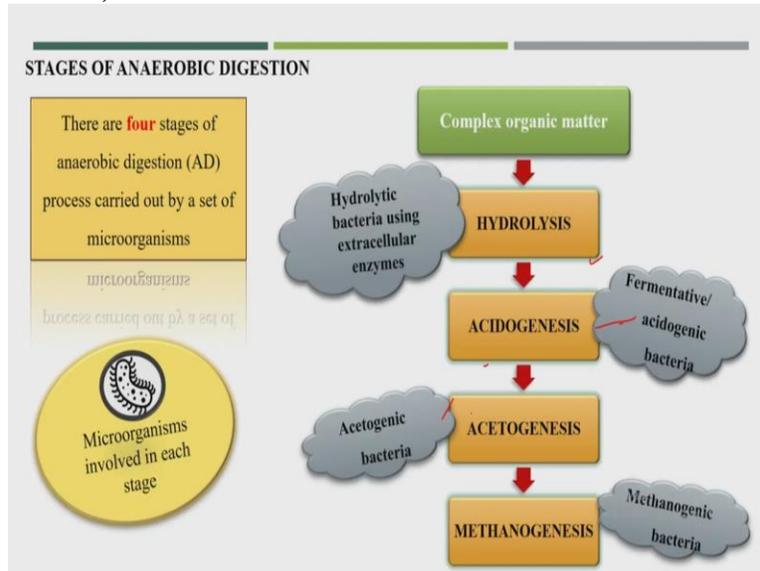
So, if you go for cooking it will get hydrolyzed and convert that food into a very simple material, which is very easy to get digested by humans. Next is the acidogenesis, so, here majorly all basic monomers will convert into volatile fatty acids, but these volatile fatty acids will be mostly propionic acid, butyric acid or valeric acids and these are somewhat long-chain carbon containing fatty acids.

That is in acidogenesis and acidogenesis followed the acetogenesis. So, only change in both the stages, is, in the acidogenesis, this propionic acid, butyric acid or valeric acid will be produced, but in acetogenesis, these acids will break down and convert to acetic acid and finally from acetic acid we will get methane and carbon dioxide as final products in biogas. And the important fact is that in all the stages, there are microorganisms involved in each stage.

So, if you simply can say that what kind of microorganism. So, for these, acidogenesis acetogenesis, normally those bacteria we call as acid-forming bacteria because here major

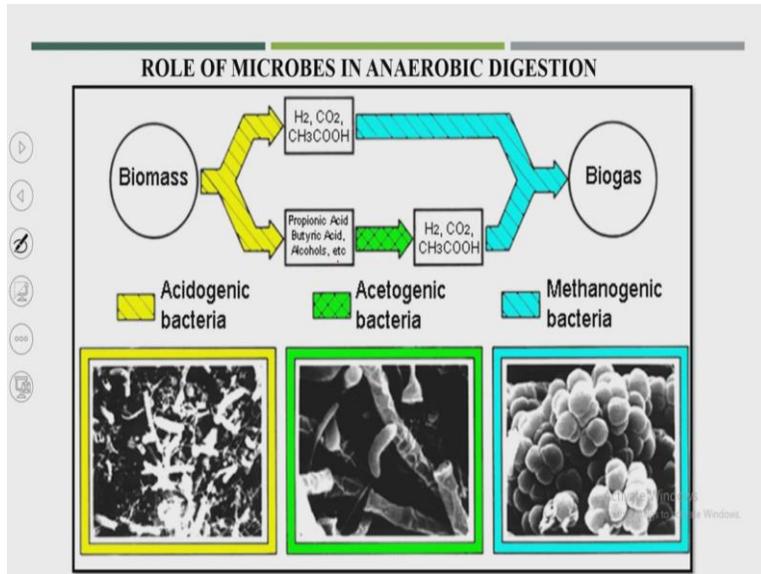
production is acid production and the methanogenesis stage normally that is a second stage or if you take the first stage is the hydrolysis, second is the acid-forming stage and the third stage will be the methane-forming bacteria. These are more abundant and should be there in the reactor.

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So, here you can see that complex organic matter through the first stage is hydrolysis, the second stage is acidogenesis, the third is acetogenesis and the fourth is methanogenesis stage. So, in hydrolysis, the hydrolytic bacteria using extracellular enzymes are involved in those microorganisms. In the acetogenesis, is fermentative or acidogenic bacteria will work and acetogenesis, this acetogenic bacteria but the group of these and these called as acid-forming bacteria. And finally, in methanogenesis, we will get methanogenic bacteria or methane-forming bacteria.

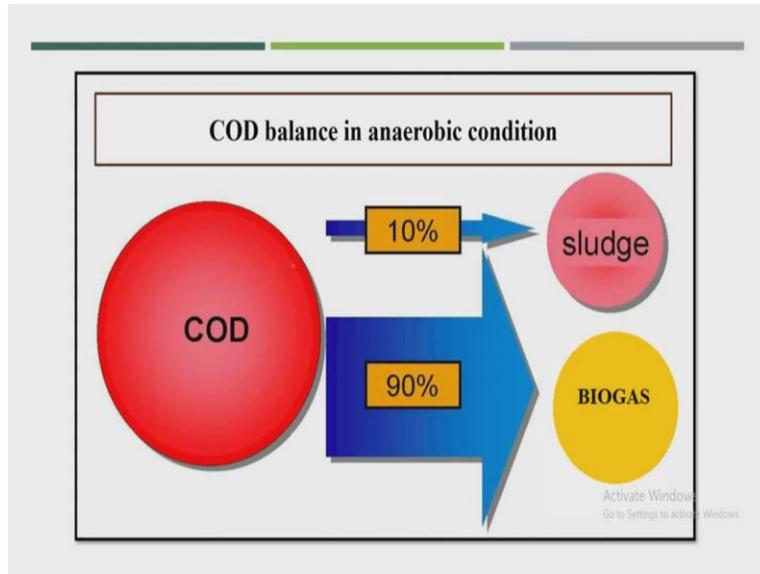
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So, what is the role of microbes in the anaerobic digestion process? So, now, here you see that, so, here I made it to 1 particular flow of degradation process. So, biomass will get after the hydrolysis process, it will produce fatty acids. So, this propionic acid, butyric acid or other acids will produce and parallelly this acetic acid also will produce and finally, this is both will be together and again this propionic acid or butyric acid will convert into acetic acid.

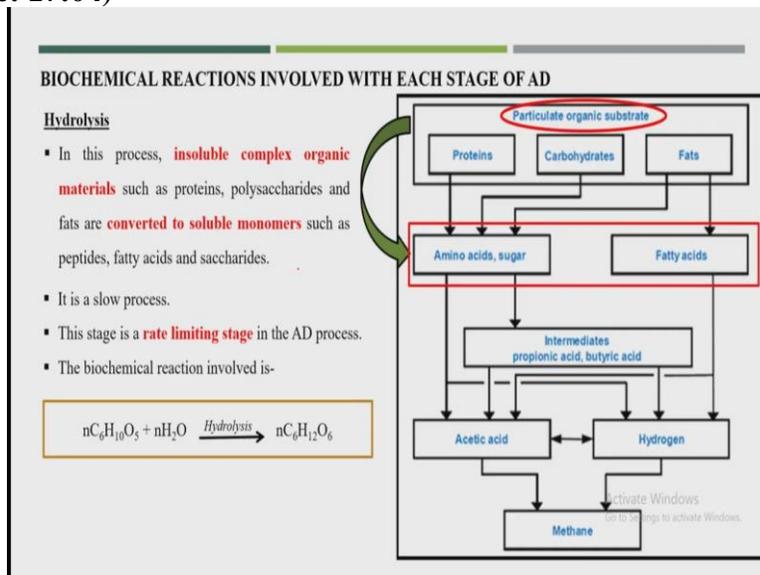
And finally, biogas will produce and you see here by color this yellow color by the production of fatty acids will by acidogenic bacteria and green color you will see that is acetogenic bacteria. These bacteria are required to convert from the large carbon-containing fatty acids to the simple carbon-containing as fatty acids and finally, methanogenic bacteria from the same acetic acid will convert into methane and carbon dioxide.

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So, if you see the COD balance or chemical oxygen demand nothing but as an organic matter in the anaerobic digestion process. So, your 90% will utilize for the biogas production and only 10% will go to the sludge production or digested production that is why the anaerobic digestion process is highly beneficial.

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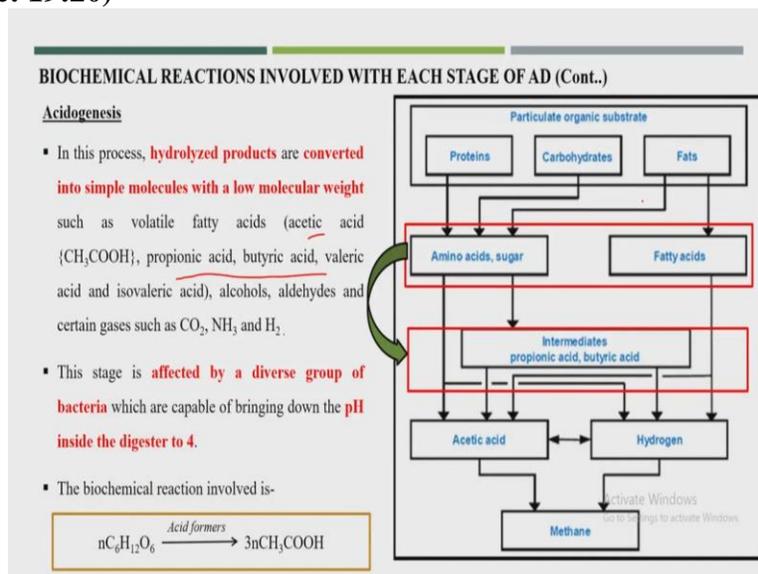
So, again the different stages of the anaerobic digestion process. So, the first is the hydrolysis process. In this process, the insoluble complex organic matters such as protein, polysaccharides, and fats are converted to soluble monomers such as a peptide, fatty acids, and saccharides. So, that is the first hydrolysis process. So, where this entire the simpler monomers mean amino acid,

sugar, and fatty acids. So, it is a slow process, this stage is a rate-limiting stage in the anaerobic digestion process.

This is very important to know that in the anaerobic digestion process, which stage is a rate-limiting stage is a and if you know the rate-limiting stage, we can work more on to rate-limiting stage or you can find different solutions for this rate-limiting stage. So, hydrolysis is the rate-limiting stage in the anaerobic digestion process. So, because it is a rate-limiting stage, so, many times if you feed waste into the digester or in the reactor, we already will go for mechanical shredding or grinding.

This mechanical grinding means we will convert that entire particle to 3 mm 4 mm size like in the composting I propose that to how the size of 1 centimeter or 2-centimeter size is good for the bacterial degradation process. So, here if you reduce the size of 3 mm, 4 mm size is highly beneficial for the anaerobic bacteria. So, mechanical grinding is a 1 kind of hydrolysis process or pretreatment process and direct the material will go to the acid-forming stage. So, what are the biochemical reaction involved here whatever organic matter will hydrolyze and will convert into simple monomers.

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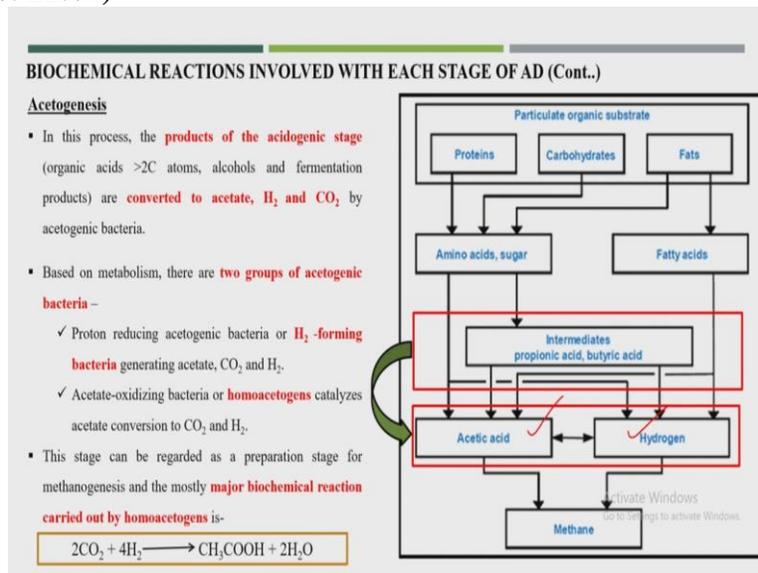
Now, the next stage is acidogenesis, or fermentation process. So, in this process, hydrolyzed products are converted into simple molecules with low molecular weight, such as volatile fatty acids like propionic acid, butyric acid, valeric acid and alcohols, aldehydes, and certain gases

such as carbon dioxide, ammonia, and hydrogen. So, this is the second stage and this stage is affected by a diverse group of bacteria which is capable of bringing down the pH inside the reactor to 4.

Now, this is also a very important fact in the anaerobic digestion process, because these bacteria are one of the strongest bacteria in the degradation process under anaerobic conditions. So, because of the production of this acetic acid, propionic acid although it is not very strong acids, these are the weak acids, but, because of this acid production, there is a chance of a drop in the pH to 4. And now, the problem once the pH is acidic, very difficult to produce the methane gas.

In such conditions, there will not be a possibility of growth of methanogenic bacteria or methane-forming bacteria at this low pH. So, biochemical reaction involves the acid formers and finally, it will convert into the fatty acids.

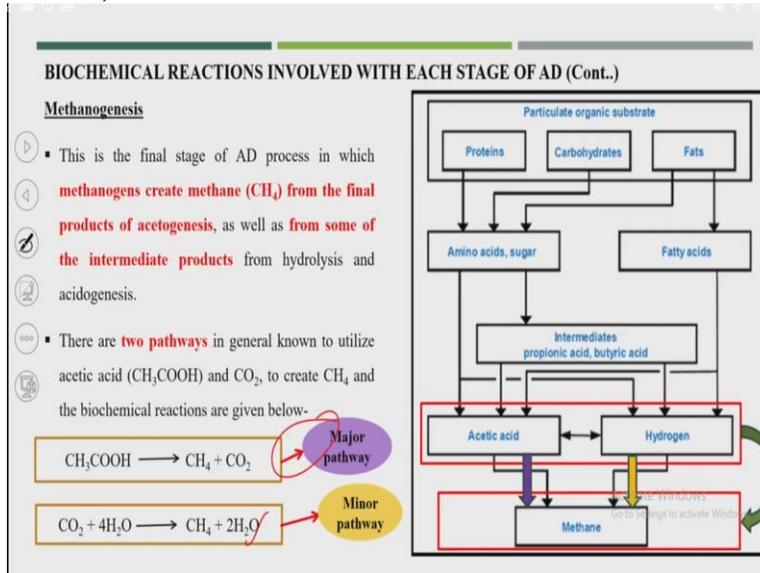
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Now, the next stage is acetogenesis. In this process, the products of acidogenesis stage are converted to acetate, H<sub>2</sub> and CO<sub>2</sub> by acetogenic bacteria. So, now here the production of acidic acid is a major along with the hydrogen. Based on metabolism, there are 2 groups of acetogenic bacteria, the first is proton reducing acetogenic bacteria or H<sub>2</sub> forming bacteria generating acetate, CO<sub>2</sub> and H<sub>2</sub>.

And the next is acetate-oxidizing bacteria or homoacetogens catalyze acetate conversion to CO<sub>2</sub> and H<sub>2</sub>. This stage can be regarded as a preparation stage for methanogenesis and mostly a major biochemical reaction carried out by homoacetogens. So, what will be the reactions could be CO<sub>2</sub> plus H<sub>2</sub> is CH<sub>3</sub> that acetic acid production.

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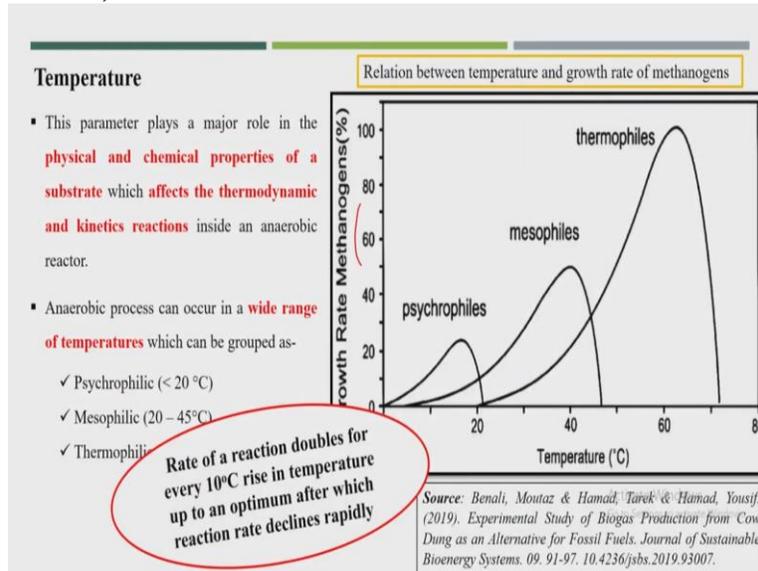
Now, the next stage is or the last stage is methanogenesis. So, this is the final stage in the other anaerobic digestion process in which methanogens create methane from the final product of acetogenesis as well as from some of the intermediate products from hydrolysis and acidogenesis. So; finally, methane production. So, there are 2 pathways to create methane, the first is this acetic acid means CH<sub>3</sub>COOH is converting into methane and carbon dioxide, but remember that this process could be only possible at neutral pH.

And again the problem is that the methane-forming bacteria is not that strong as compared to the acid-forming bacteria and that is why the buffer or buffering capacity always needs to be discussed in the anaerobic digestion process. So, this buffering could be possible that if some kind of alkalinity could generate in the reactor that is highly beneficial or some amount of ammonia production also could be highly favorable in the reactor.

So, that the neutral pH could be possible to maintain for the growth of methane forming bacteria or methanogenic bacteria and there is another way by that way methane will produce that is this carbon dioxide can react with water and will produce but I think we did not find much literature

that this pathway could be possible but the major pathway is this way where acetic acid is degrading and converting into methane and carbon dioxide. Now, once we know the different stages of the anaerobic digestion process, we will go for factors affecting the anaerobic digestion process.

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So, first is temperature. This parameter plays a major role in the physical, chemical property of substrate which affects the thermodynamics and kinetic reaction inside the reactor. So, the anaerobic process can occur in a wide range of temperatures which can be grouped as psychrophilic less than 20 degrees, mesophilic 20 to 45 degrees, and thermophilic 45 to 65 degrees centigrade. So, if you see here, the growth rate of methanogens, at which temperature the methanogens will be growing fast.

So, you see in the psychrophilic, you will not get much growth of methanogens, and in mesophilic, it is the production of methanogens, but in the thermophilic condition like temperature more than 45-degree centigrade, there is a very good chance of production of more methanogens, more methanogens production means more methane production in the process. So, the rate of reaction doubles for every 10-degree centigrade rise in the temperature up to an optimum after which the reaction rate decline rapidly.

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### Temperature (Cont.)

- **Thermophilic temperature range** generally has several effects over the lower ranges. Some **positive effects** are-
  - ✓ Increase in hydrolysed soluble product which make them more accessible to microorganisms.
  - ✓ Increased reaction kinetics in both chemical and biological process shortening the reaction time and hence the hydraulic retention time (HRT) of the reactor.
  - ✓ Improves the physico-chemical properties of the soluble substrate.
- Some **negative effects** of thermophilic temperature range are-
  - ✓ Increases the fraction of free ammonia (NH<sub>3</sub>) which is inhibitory to microorganisms.
  - ✓ Ammonia inhibition could disturb the whole AD process dynamics and may hamper the quality of the gases.
  - ✓ Reactor stability reduces.
  - ✓ High accumulation of VFAs occur which affects the growth rate of methanogens.
  - ✓ Effluent quality reduces and toxicity increases for certain types of substrates.

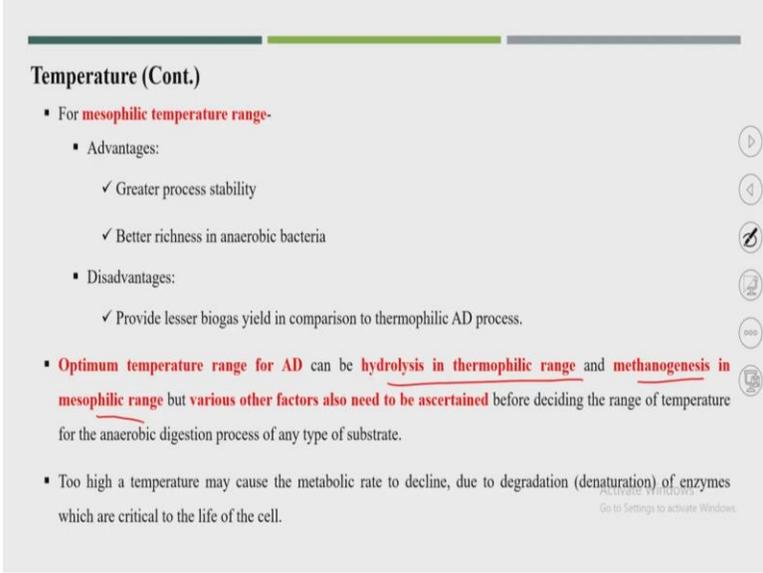
Now, the thermophilic temperature range generally has several effects over the lower range. Some positive effects are an increase in the hydrolysis soluble products which make them more accessible to the microorganism that is one very important benefit of thermophilic temperature range, increased reaction kinetics in both chemical and biological process shortening the reaction time. Hence the HRT of the reactor hydraulic retention time.

This is also an important facet of the anaerobic digestion process, if suppose, the HRT or hydraulic retention time is more normal in the commercial reactor, we find the HRT of 25 to 30 days means, you will be required very large size of the reactor for low quantity of organic field. So, as an engineer, I think we need to work more to reduce the HRT. So, this is also a good idea if you increase the temperature in the reactor.

Improves the physico-chemical properties of the soluble substrate, but, there are some negative effects of thermophilic temperature that the increase the fraction of free ammonia which is inhibitory it is not good for the microorganisms. Ammonia inhibition could disturb the whole AD anaerobic digestion process dynamics and may hamper the quality of the gases. The reactor stability reduces. High accumulation of VFAs occurs which affects the growth rate of methanogens.

So, there is a possibility that in the thermophilic temperature the production of volatile fatty acids will be more if this concentration goes to 2000, 10,000 milligrams per liter, even more than 4000 milligrams per liter volatile fatty acid production. So, that is also not good for the anaerobic digestion process, although we required volatile fatty acids for the production of methane, the high concentration also highly inhibitory for the anaerobic digestion process and effluent quality reduce and toxicity increase the certain type of substrate.

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**Temperature (Cont.)**

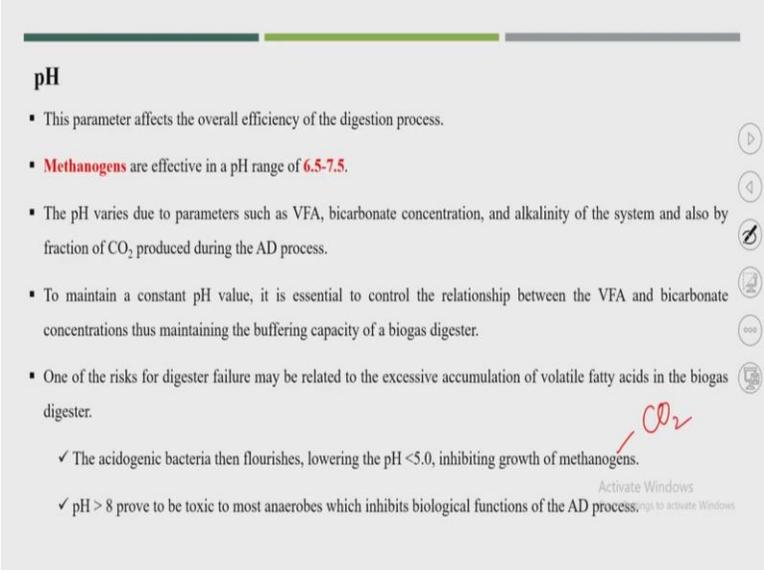
- For **mesophilic temperature range**-
  - Advantages:
    - ✓ Greater process stability
    - ✓ Better richness in anaerobic bacteria
  - Disadvantages:
    - ✓ Provide lesser biogas yield in comparison to thermophilic AD process.
- **Optimum temperature range for AD** can be **hydrolysis in thermophilic range** and **methanogenesis in mesophilic range** but **various other factors also need to be ascertained** before deciding the range of temperature for the anaerobic digestion process of any type of substrate.
- Too high a temperature may cause the metabolic rate to decline, due to degradation (denaturation) of enzymes which are critical to the life of the cell.

But for mesophilic temperature range, what are the advantages? Greater process stability, better richness in anaerobic bacteria and disadvantage is that the yield will not good as compared to the thermophilic one. But the optimum temperature range of anaerobic digestion can be hydrolysis in the thermophilic range and methanogens in the mesophilic range, but various other factors also need to be ascertained before deciding the range of temperature for the anaerobic digestion process.

So, here what it is explaining that why not the hydrolysis could do it thermophilic range and the methanogens is will be in the mesophilic range. So, that many times like I explained about the mechanical grinding that is a hydrolysis process or pretreatment process in the in this case what is suggested that in the hydrolysis increase the temperature for hydrolysis process and followed by the whatever the anaerobic digestion process you do it at a mesophilic temperature only.

So, there are many research papers available where the substrate gas kept in the oven for getting the thermophilic temperature had been used for the different way the temperature has been added into the mass so that the hydrolysis could be better followed by an anaerobic digestion process. That is under the mesophilic temperature. Too high a temperature may cause the metabolic rate to decline due to the degradation of enzymes which are critical to the life of the cell.

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**pH**

- This parameter affects the overall efficiency of the digestion process.
- **Methanogens** are effective in a pH range of **6.5-7.5**.
- The pH varies due to parameters such as VFA, bicarbonate concentration, and alkalinity of the system and also by fraction of CO<sub>2</sub> produced during the AD process.
- To maintain a constant pH value, it is essential to control the relationship between the VFA and bicarbonate concentrations thus maintaining the buffering capacity of a biogas digester.
- One of the risks for digester failure may be related to the excessive accumulation of volatile fatty acids in the biogas digester.
  - ✓ The acidogenic bacteria then flourishes, lowering the pH <5.0, inhibiting growth of methanogens.
  - ✓ pH > 8 prove to be toxic to most anaerobes which inhibits biological functions of the AD process.

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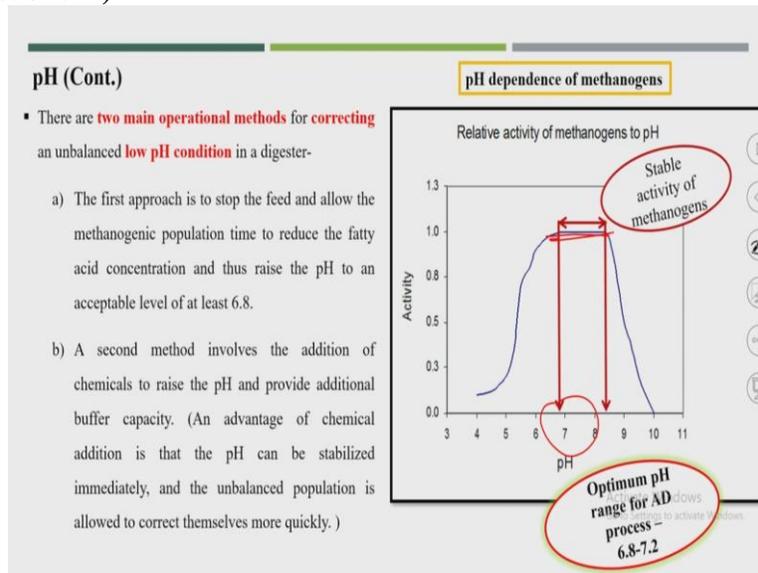
Now, the next parameter is pH, this parameter affects the overall efficiency of the digestion process. So, as I was telling that methanogens are very effective at a range of 6.5 to 7.5 means neutral pH. The pH varies due to parameters such as VFA, carbonate concentration, and alkalinity of the system also by a fraction of CO<sub>2</sub> produced during the anaerobic digestion process. So, from time to time we have to monitor the reactor, how the pH increase or reduction is possible in the reactor.

To maintain a constant pH, it is essential to control the relationship between the VFA and carbonate concentration thus maintaining the buffering capacity of the biogas reactor. So, one of the risks of the digester failure may be related to the excessive accumulation of volatile fatty acids in the biogas digester. So, this is what I was telling about, if there is an excessive amount of VFA production because the pH will drop drastically. So, that could be one of the risks of failure of biogas reactor.

The acidogenesis bacteria then flourish at this low pH, the acidogenic bacteria will flourish, lowering the pH 5, inhibiting the growth of methanogens. So, in this case, the major production of gas will be carbon dioxide, not methane. And you know that if carbon dioxide percentage more than 44% so, it will not be a combustible gas and pH more than it proves to be toxic to most anaerobic bacteria, which inhibit biological fraction up on the reduction process.

So, here important is that to maintain the pH and this is possible by the addition of a mix of different organic residues. So, suppose kitchen waste if you are planning so, it is good to mix with the other kind of material which will maintain the particular pH or your reactor has to be acclimatized into the neutral pH and slowly this acidic kitchen waste could be fed into the reactor not at a very high rate and by looking and checking the reactor pH.

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So, there are 2 main operational methods for correcting unbalanced low pH conditions in the digester. The first approach is to stop the feed and allow the methanogenic population time to reduce the fatty acid concentration thus raise the pH to an acceptable level of at least 6.8 or more than 6.5. So, the first approach is that do not feed the reactor, stop the feeding. So that the; microbes will have more time to reduce these volatile fatty acids.

Once you know that pH has been modified by reduction of volatile fatty acids, again we can start the feeding of the reactor. The second method involves the addition of chemicals to raise the pH which can provide the additional buffering capacity of that particular mass. And then what is the

advantage of this chemical addition is that pH can be stabilized immediately and the unbalanced population is allowed to correct themselves more quickly. So, because see in the first case, we have to wait for few days for feeding of the reactor.

But in the second stage, by addition of some kind of chemical these chemicals will be mostly alkali may be sodium hydroxide or potassium hydroxide addition with different normality we can add to reduce the pH immediately in the reactor. So we need not wait for a longer period to maintain the pH but it again depends upon the size of the reactor. If there is a big, very big size of the reactor, and maybe the size of the reactor is 500 tons per day or 200 tons to 100 pounds per day capacity, that much amount of chemical utilization would not be possible.

So, if you see here, the activity of the microbial activity at different pH, you see here the pH from this pH you will find the maximum activity of methanogenic bacteria. So, the optimum pH range to 6.8 to 7.2.

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**Buffering capacity (Alkalinity)**

- An anaerobic digestion process has its **own buffering capacity against pH drop due to the production of alkalinity** during AD process. [Example, degradation of protein present in any substrate releases ammonia (NH<sub>3</sub>), which reacts with carbon dioxide (CO<sub>2</sub>) producing ammonium carbonate (NH<sub>4</sub>HCO<sub>3</sub>) as alkalinity]  
$$\text{NH}_3 + \text{H}_2\text{O} + \text{CO}_2 \rightarrow \text{NH}_4\text{HCO}_3$$
- The **degradation of salt of fatty acids** may produce some alkalinity. The reaction below shows-  
$$\text{CH}_3\text{COONa} + \text{H}_2\text{O} \rightarrow \text{CH}_4 + \text{NaHCO}_3$$
- **Sulfate and sulfite reduction** also generate alkalinity, the reaction of which can be shown below-  
$$\text{CH}_3\text{COO}^- + \text{SO}_4^{2-} \rightarrow \text{HS}^- + \text{HCO}_3^- + 3\text{H}_2\text{O}$$
- When pH in an anaerobic system drops due to VFA accumulation, the alkalinity present within the system neutralizes the acid and prevents further pH drop. If the alkalinity present is insufficient to buffer the system pH, external alkalinity additions are necessary.

Now, if you see the buffering capacity or alkalinity, an anaerobic digestion process has its own buffering capacity against the pH drop due to the production of alkalinity during the anaerobic digestion process. As an example, the degradation of protein present in the substrate release ammonia which reacts with carbon dioxide and producing ammonium carbonate, NH<sub>4</sub>HCO<sub>3</sub> as alkalinity. So, because of that also we can maintain the pH in the reactor. So, this could be the reaction.

The degradation of salt of fatty acids may produce some alkalinity and this reaction could be possible like here. This also can be possible. So, this is the sodium alkalinity. Sulfate and sulfide reduction also generate alkalinity, the reaction could be like this. So, here also the carbonates or bicarbonates will get produce. When pH in the anaerobic system drop due to VFA accumulation, the alkalinity present within the system neutralizes the acid and prevents further pH drop. If the alkalinity presents insufficient to buffer the system pH, external alkalinity additions are necessary.

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**Carbon/Nitrogen**

- Nitrogen is the major nutrient required for the growth of microbes, and the synthesis of amino acids and proteins which in turn converts into ammonia, a buffer compound for the neutralization of the acidification process.
- If the **nitrogen content is low**, microbial populations remain less and it might take longer duration to digest the available carbon present in the substrate.
- If the **nitrogen content is high**, then excess nitrogen leads to production of too much ammonia (NH<sub>3</sub>) which inhibits the anaerobic process.
- **Carbon serves to be source of energy and nitrogen controls the microbial population** so the ratio of carbon and nitrogen (C/N) plays an important role for an effective AD process.

C/N ratio range of 20-35 has been found to be mostly suitable for various types of substrates

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Now, the other factor is carbon to nitrogen ratio. So, as we know that nitrogen is a major nutrient required for the growth of micro and synthesis of anaerobic acids and protein which convert into ammonia. If the nitrogen content is low, the microbial population remains less and it might take a longer duration to digest the available carbon present in the substrate means your HRT will be very high. If the nitrogen content is high, then excess nitrogen leads to the production of too much ammonia, which inhibits the anaerobic digestion process.

So, this is also another problem if you have more nitrogen. So, ammonia production could be possible although this ammonia is good for production to get more alkalinity but if more product that is also problematic. Carbon serves as a source of energy and nitrogen controls the microbial population. So, the ratio of carbon to nitrogen is very important in the anaerobic digestion

process. So, it is suggested that 20 to 30 is the best or optimum carbon to nitrogen ratio for the anaerobic digestion process.

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The slide features a title 'Organic loading rate (OLR)' followed by five bullet points. The text is presented in a clean, sans-serif font with key terms highlighted in red. A decorative horizontal bar with green and grey segments is at the top. A small 'Activate Windows' watermark is visible in the bottom right corner of the slide content area.

**Organic loading rate (OLR)**

- OLR refers to the **amount of volatile matter that is fed into an anaerobic reactor.**
- **High OLR** alters the digester environment inhibiting the microbial activity during the initial stages of acid fermentation.
- Increased OLR increases acid formers (hydrolytic/fermentative bacteria) activity as compared to that of the methane formers (methanogens), leading to high accumulation of fatty acids.
- **Low OLR** leads to underutilization of microorganisms (starvation) leading to very slow rate of AD process.
- OLR, therefore, should be **optimized based on the type of feedstock** and also **reactor temperature.**

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Now, another factor is OLR or organic loading rate. The organic loading rate refers to the amount of volatile matter that is fed into an anaerobic reactor. The high OLR alters the digested environment inhibiting the microbial activity during the initial stage of acid fermentation. So, if you add more amount of organic matter means more carbon that is also not beneficial for the degradation process.

Increased OLR increases acid former activity as compared to that methane former leading to a high accumulation of fatty acids. So, this is also a very important factor. And low OLR leads to neutralization of microorganisms leading to a very slow rate of the anaerobic digestion process. So, that OLR also has to be optimized in the reactor before starting or before the operation of the anaerobic digestion process.

So, OLR therefore should be optimized based on the type of feedstock and the reactor temperature. So, this study has to be conducted before starting the reactor, and also this OLR will be different for different substrate because the different amount of volatile matters will be available, has to be checked from time to time, and should find the optimum OLR.

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### Hydraulic Retention Time (HRT)

- HRT is the **average time that the organic substrate remains in the digester**.
- HRT affects the biological and chemical properties of organic substrates.
- It plays an important role in AD process especially for methanogenic bacteria in mesophilic temperature range.
- It is **desired to be long enough** to provide **sufficient methanogenic activity**.
- In general, HRT is shorter for AD at higher temperatures and vice-versa but mostly depends on the properties of feedstock.

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Now, the next parameter is a hydraulic retention time. So, HRT is the average time that the organic substance remains in the digester. So HRT affects the biological chemical property of the organic substrate. It plays an important role, especially for methanogenic bacteria in the mesophilic range. It is desired to be long enough to provide sufficient methanogenic activity. But this is another problem if you have long or large HRT, your reactor size requirement will be more.

In general, HRT is shorter for anaerobic digestion at higher temperatures and vice versa. Mostly depend on the property of feedstock. So, if you go for a thermophilic kind of reactor thermophilic range reactor, your HRT will be low, and Mesophilic your HRT will be somewhat high.

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**Volatile fatty acids (VFA)**

- VFAs are produced by acid-forming bacteria.
- Accumulation of VFA always affects the anaerobic digestion process by **lowering pH** and **suppressing methanogens**.
- During the **acidogenesis phase**, two VFAs namely **acetic acid** and **butyric acid** are known to **favor the methane formation**.
- **Acetic acid** contributes to almost **70% of methane** formation..
- Few other VFAs produced are-
  - ✓ Propionic
  - ✓ Valeric
  - ✓ Iso- butyric
  - ✓ Iso-valeric
- VFA concentration is a **good indicator** for **monitoring performance** of anaerobic digestion process (activity of acetogenic and methanogenic bacteria).

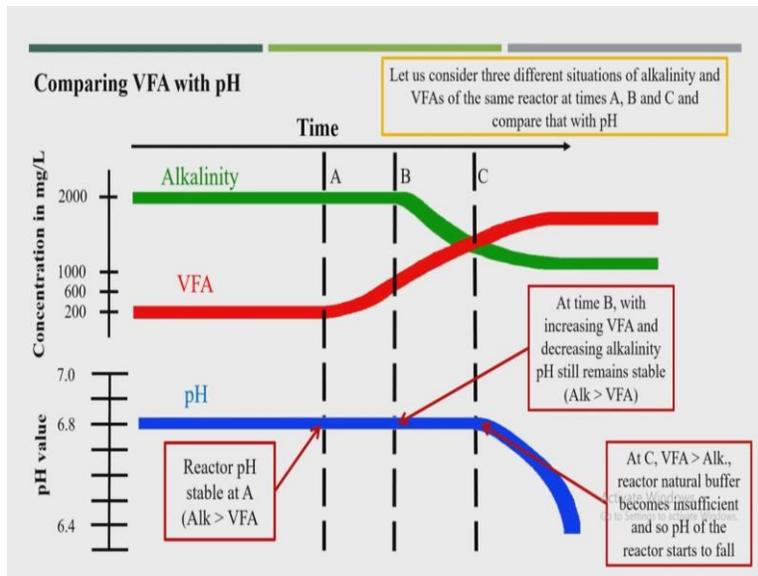
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Next is the VFA. So, this is what we were talking about. The VFAs are produced under the acid-forming bacteria. So the accumulation of VFA always affects the anaerobic digestion process by lowering the pH and suppressing methanogens. During the acidogenesis phase, 2 VFS namely acetic acid and butyric acid are known to be a favor methane formation. Acetic acid contributes almost 70% of methane formation.

So, the important VFA is acetic acid. Few other VFA produced like propionic acid, apart from butyric acid, valeric acid, iso-butyric, iso-valeric acids, and this contributes the remaining 30%. So, VFA concentration is a good indicator for monitoring the performance of the anaerobic digestion process. So, when you do the laboratory experiments of the anaerobic digestion process, I think apart from pH, VFA, and your COD change, that chemical oxygen change is the measurement of volatiles solid.

Either we can go volatiles solid measurement or COD measurement and also the VFA measurement also is very important, that is a good monitoring parameter for the anaerobic digestion process.

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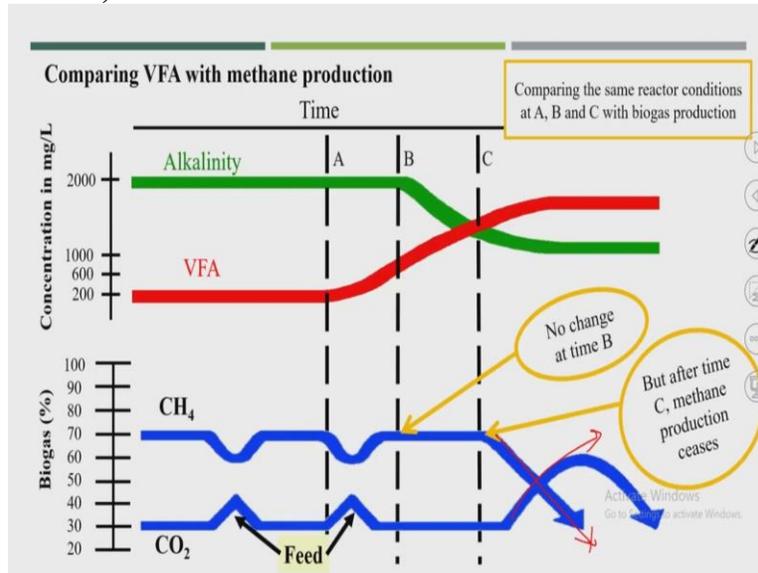
So, what will be the relation between volatile fatty acids the alkalinity? Let us consider an example of an anaerobic reactor showing variation in the concentration of alkalinity and VFA with digestion time. So, like you can see here the different concentrations. So, here you can see the green color is alkalinity and red color is a VFA. This graph shows that the digester has a good buffering capacity.

Now; because VFA production is less compared to alkalinity. So, low VFA accumulation at 200 milligrams per liter compared to the alkalinity of 2000 milligrams per liter. So, now, we can understand the reactor has a very good buffering capacity. And when you compare the VFA with pH, so, let us consider that 3 different situations of alkalinity and VFA at the same reactor at times A, B, and C, and then we will compare the pH of the reactor.

So, now, here we see that, now, when you started the reactor your VFA production was 200 milligram per liter, and alkalinity production is 2000 milligram per liter, but at one point, the VFA production has increased and alkalinity has been reduced. So, we take 3 different situations like A, the situation the A, B, and C. Now, when you see the pH in all 3 different situations, we started with the pH of 6.8 but at the situation C or stage 3, your pH is dropping and coming to the acidic condition.

So, in condition A, the pH is stable. So, in that case, alkalinity is more than VFA and at time B or stage B with increasing VFA, now, here you see that VFA was increasing, but alkalinity is slightly reducing and decreasing alkalinity, pH remains stable. So, until that also pH will not affect because in the same condition the alkalinity is more than VFA but at stage 3, the VFA now is more than alkalinity or almost equal, the reactor natural buffer becoming insufficient. So, the pH of the reactor starts to fall.

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Now, take the same example of gas production. So, as we saw in the previous one. At this condition, pH has been dropped, and when the same alkalinity and VFA conditions how the methane production could be possible. So, now, we also saw that methane production and carbon dioxide production. So, even there is no change in gas production or methane production until time B, where alkalinity is more than VFA but at time C, the methane production seized.

And after that, the methane production will be completely dropped down and the carbon dioxide increase will get started. So, see how important is alkalinity and VFA production in the reactor. So, when you monitor or when you operate the anaerobic digester from time to time you collect the sample and get it to analyze alkalinity also get it to analyze and VFA also is analyzed. Suppose you are not able to do the alkalinity, but at least VFA total VFA try to get analyze and see that this concentration should not increase up to 2000-3000 milligrams per liter VFA.

Because not only it will hamper the pH of the reactor also will affect the methane production in the reactor. So, thank you, and might you people have understood very well the different stages of the anaerobic digestion process and also there are different factors which are affecting the biogas production or methane production. So, thank you.