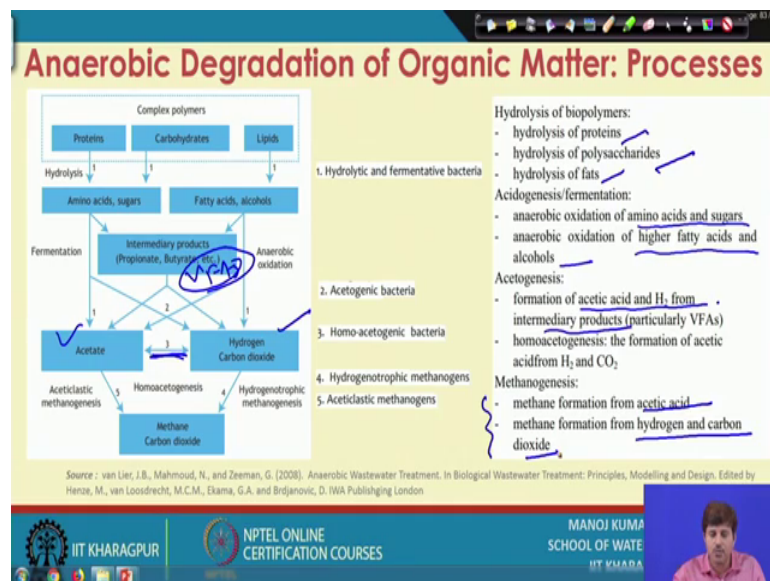


Wastewater Treatment and Recycling
Prof. Manoj Kumar Tiwari
School of Water Resources
Indian Institute of Technology Kharagpur

Lecture – 34
Anaerobic Degradation Processes

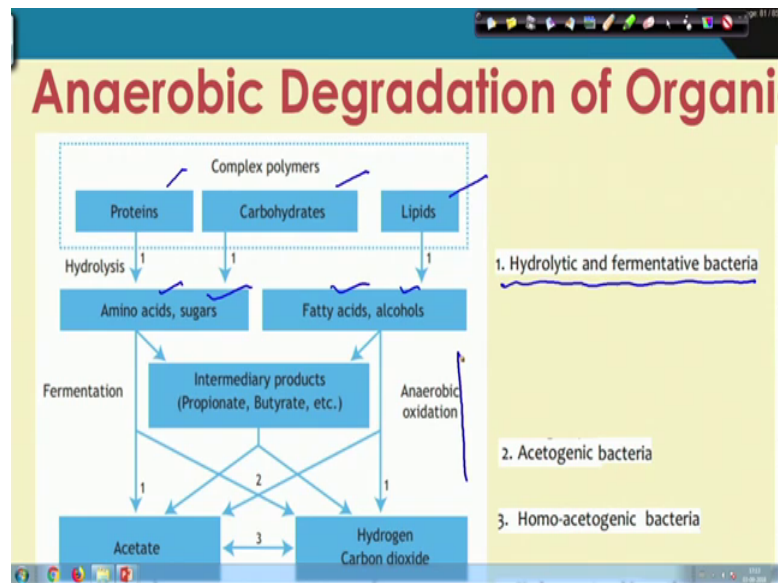
Welcome back everyone. So we will continue our discussions from the previous class when we are talking about the Anaerobic Degradation Processes. So, we did discuss why anaerobic treatment puts forward an attractive option as opposed to the aerobic treatment technology. And we were in the discussion about the different steps or different processes which are involved in the aerobic in the anaerobic degradation of the organic matter. So, will take forward that discussion further in this class and we will see what happens essentially in the detail.

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So, if you see the anaerobic degradation of organic matter. So, we as we are discussing in the previous class as well that there could be complex polymer in the form of protein carbohydrate lipids. So, there is the first step which is your hydrolysis is achieved by the hydrolytic and fermentative bacteria. So the one number here indicates about this type of bacteria this type of a species.

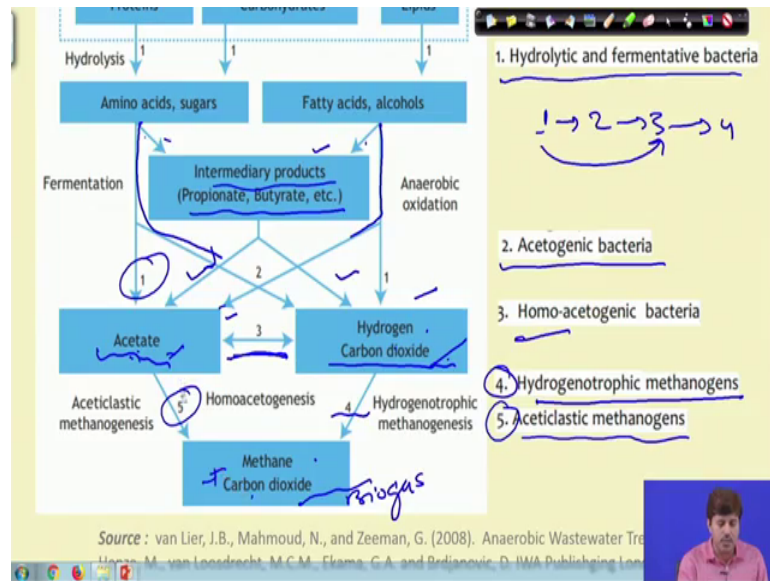
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Now, the this hydrolytic and fermentative bacteria that if you see here so what happens that proteins carbohydrates or lipids are converted through hydrolysis into amino acids sugars and these lipids can be converted to fatty acids and alcohol. And the kind of microorganisms involved here are the hydrolytic and fermentative bacteria. Now this again involves like the there would be hydrolysis of proteins, hydrolysis of carbohydrates, hydrolysis of lipids; so we can subclass that way.

Then what happens that these amino acid sugars or long chain fatty acids and alcohol could further be acted by this group of the same group of bacteria and could be converted to the simpler or smaller products where we can see that these through again by the action of the same hydrolytic group of bacteria, it can be converted to acetate or can be converted to the hydrogen carbonate.

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So, it is not as such a uni line step that 1 goes to 2 2 goes to 3 3 goes to 4; there is a possibility of this directly going here as well. So, this kind of like pathway is adopted. Now these fatty acids or alcohol which are there can be actually converted to intermediary byproducts like propionate butyrate and all that or can directly be converted to acetate or can directly be hydrolyzed to hydrogen and carbon dioxide.

So, if they are getting directly hydrolyzed it will be by the action of hydrolytic and fermentative bacteria. If they have been converted to intermediate byproduct like propionate butyrate can again be converted to acetate or can be converted to hydrogen and carbon dioxide through the group of acetogenic bacteria.

So, this second process is essentially the process of this becomes the process of acetogenesis and then this 2 step that we see here is the process of kind of acetogenesis. So, through the acetogenic bacteria, this can actually be converted to the acetate or this thing these directly could also be converted to acetate or these by the action of hydrolysis.

Similarly, your amino acids and sugars can also be converted to intermediate byproduct through form through the acetogenesis process. And then acetogenesis can convert these to either acetate or hydrogen and carbon dioxide or these directly could also be converted to either acetate or hydrogen either acetate or hydrogen and carbon dioxide through the action of acetogenesis bacteria.

This acetate and hydrogen are also interchangeable with the action of homoacetogenic bacteria. So, this homoacetogenic bacteria can actually convert acetate to hydrogen and carbon dioxide or alternatively can convert hydrogen and carbon dioxide to acetate. So, that way we have kind of products by the action of 1 2 and these 3 groups in the form of acetate and hydrogen and carbon dioxide and both of these can then further be converted to methane and carbon dioxide which we normally refer as biogas.

So, this biogas production can be either from the hydrogen and carbon dioxide with the help of hydrogenotrophic methanogens. So, this is hydrogenotrophic methanogenesis and hydrogenotrophic methanogenesis convert hydrogen to methane basically and this the group of bacteria that is involved is hydrogenotrophic methanogens.

Whereas, the acetoclastic methanogens which is the 5 number can convert acetate into methane and carbon dioxide; so that is the another group of bacteria acetoclastic methanogens which are involved and this is called that way if this is called acetoclastic methanogenesis and this is called hydrogenotrophic methanogenesis. So, that way we have 2 distinct through different type of methanogenesis involved over there.

So, if we look at the sub steps so hydrolysis of biopolymers can be a hydrolysis of protein hydrolysis of polysaccharides hydrolysis of fats, then acidogenesis, then acidogenesis and fermentation can lead to the way anaerobic oxidation of amino acids and sugar or anaerobic oxidation of higher fatty acids and alcohol and the acetogenesis there is formation of acetic acid and hydrogen from intermediate products. So, like the intermediate via phase whatever we have, so they convert to hydrogen or acetate those kind of thing as take acid or hydrogen. And then there is homoacetogenesis also has the formation of acetic acid from H_2 and CO_2 .

So, this homoacetogenesis step can also be will be a subclass of acetogenesis and then in the methanogenesis finally, again we have acetoclastic and hydrogenotrophic to subclasses for the methane formation. So, methane formation from acetic acid and methane formation from hydrogen and carbon dioxide; so that way we have all these different steps which leads to the formation of methane from there.

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Anaerobic Degradation Processes: Hydrolysis

- Bacteria unable to take up particulate organic matter as substrate.
- Enzymes excreted by fermentative bacteria converts complex undissolved form of organic matter into less-complex dissolved compounds which can cross the cell barrier.
- The process is very sensitive to temperature and temperature fluctuations
- Generally rate limiting step in the process of anaerobic digestion

Source : van Lier, J.B., Mahmoud, N., and Zeeman, G. (2008). Anaerobic Wastewater Treatment. In Biological Wastewater Treatment: Principles, Modelling and Design. Edited by Henze, M., van Loosdrecht, M.C.M., Ekama, G.A. and Brdjanovic, D. IWA Publishing London

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Now, if we see the hydrolysis step; so the bacteria typically why the hydrolysis takes place. So, it is basically because bacteria are unable to take up the particulate organic matter as a substrate, there are very few a species there are there are few a species which can take up that. But they are very few in numbers and general bacteria cannot take up particulate organic matter or the organic matter which is in the solid form which is not solubilize or not dissolved in the water as their source of substrate.

So, when there are microorganisms present and when there are organic matter present of that nature which bacteria cannot directly consume or cannot directly attack on or cannot directly take up the as a substrate. So, what they do? They excrete enzymes they excrete certain enzymes these fermentative bacteria excretes certain enzymes which converts these complex undissolved form of organic matter into less complex and dissolved compounds.

So, what happens when these undissolved and complex organic matter are converted into the less complex and dissolved organic matter. So these because of these organic matter which are products are relatively lesser complex and are dissolved in the water phase. So, they can actually cross the barrier of the cells. They can cross the membrane of the cell wall and enter into the system and provide the substrate required for the to be utilized as a source of carbon or source of energy.

So, that possibility actually enhances and that becomes the first and essential step for bacteria to be able to attack that, otherwise if you have let us say this kind of lipid. So, this lipid is a 2 complex structure for bacteria to directly attack on this.

So, what happens that it gets hydrolyzed with the lipase enzyme is released and then this is converted to the glycerol and a long chain fatty acid compound ok. So, because it has converted to now a long chain fatty acid compound and glycerol, so then these can actually be more easily utilized or mold more easily consumed as a substrate by the microorganisms as opposed to it is original structure.

So, that becomes the key of the microorganisms attacking the organic pollutant or organic matter present in the water and utilizing them as a substrate. So, for the purpose it has to be in this lesser complex form and solubilized form generally and it is the hydrolysis step that does this thing in the anaerobic system.

Hydrolysis is actually it is not only limited to anaerobic system. There are aerobic hydrolysis also, so it is the these complex molecules are hydrolyzed in the aerobic system as well. And in fact there are it can some of these compounds can just be hydrolyzed by the reaction of water. So, there is a possibility of abiotic hydrolyzed hydrolysis where there is no bacteria involved at all.

So, this process is very sensitive the process of typically hydrolysis and that too in the case of anaerobic degradation, so anaerobic hydrolysis is very sensitive to temperature and it is fluctuation. And that is why the like see it is essential step because without this the you are not going to produce compounds which can be taken up by the bacteria. So, that becomes an essential step but it is highly sensitive on temperature and it is fluctuation and some of the other parameters as well. And if we have a complex nature of substrate particularly ok, for easy decomposable substrate or the things that usually are there in the sieve age or those kind of thing which are easily degradable.

So, for them, this may not be the rate limiting step but for the complex substrates or the wastewater which is having high end organic chemicals which are pretty complex to degrade the hydrolysis becomes generally the rate limiting step in this entire anaerobic degradation process.

Now, rate limiting step means this is the slowest because as we saw there are there are 4 different stages. There is a 4 means there are 4 to 5 different reaction steps are there is hydrolysis. It could be converted to intermediate is fatty acids. Then the acetate then from acetate it could convert it to hydrogen or directly to the methane. So, there is a possible reaction chain and if we see the rate of these reactions the most slow rate at times or particularly for the complex organic matter is hydraulic.

So, that is why it becomes rate limiting step because of it is slowest nature. So, until on you may have lot of substrate present, but until unless it is hydrolyzed bacteria, I cannot act upon and as soon as it is hydrolyzed the subsequent process could become faster. So, once it is hydrolyzed the bacteria can quickly sort of break it down to the intermediate compounds and then the acetic acids and then probably the biogas.

So, the subsequent process may be faster it is the hydrolysis which slows which controls the entire rate. So, as the hydrolysis could become the rate limiting a step and that is why like controlling the temperature of the anaerobic system becomes important for such specific cases.

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Anaerobic Degradation: Acidogenesis & Acetogenesis

o Acidogenesis Reactions:

Reactions	ΔG° (kJ/mol)
$C_{12}H_{22}O_{11} + 9H_2O \rightarrow 4CH_3COO^- + 4HCO_3^- + 8H^+ + 8H_2$	-457.5
$C_{12}H_{22}O_{11} + 5H_2O \rightarrow 2CH_3CH_2CH_2COO^- + 4HCO_3^- + 6H^+ + 4H_2$	-554.1
$C_{12}H_{22}O_{11} + 3H_2O \rightarrow 2CH_3COO^- + 2CH_3CH_2COO^- + 2HCO_3^- + 6H^+ + 2H_2$	-610.5

o Acetogenesis Reactions:

Compound	Reaction	ΔG° (kJ/mol)
Lactate	$CH_3CHOHCOO^- + 2H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 2H_2$	-4.2
Ethanol	$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2H_2$	+9.6
Butyrate	$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2$	+48.1
Propionate	$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2$	+76.1
Methanol	$4CH_3OH + 2CO_2 \rightarrow 3CH_3COOH + 2H_2O$	-2.9
Hydrogen-CO ₂	$2HCO_3^- + 4H_2 + H^+ \rightarrow CH_3COO^- + 4H_2O$	-70.3
Palmitate	$CH_3(CH_2)_{14}COO^- + 14H_2O \rightarrow 8CH_3COO^- + 7H^+ + 14H_2$	+345.6

Change in free energy estimated assuming neutral pH at 25°C, and 1 atm. Pressure.

Source : van Lier, J.B., Mahmoud, N., and Zeeman, G. (2008). Anaerobic Wastewater Treatment. In Biological Wastewater Treatment: Principles, Modelling and Design. Edited by Henze, M., van Loosdrecht, M.C.M., Ekama, G.A. and Brdjanovic, D. IWA Publishing London

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Now, if we have a look at acidogenesis and acetogenesis reactions what happens in these processes. So, in acidogenesis we can have let us say long chain acids or long chain compounds, they convert to the acetate or propionate or butyrate those kind of thing they are converted into and could also be converted into like they will be leading some

bicarbonate. So, this alternative factor is also becomes important for particularly for this step or for many other step as well.

Then in acetogenesis, there is there is the compound like lactate ethanol butyrate propionate methanol then hydrogen and CO2 all these things may undergo different type of reactions like lactate can undergo like this.

So, it eventually will convert to the acetic acid, similarly your butyrate will again be eventually converting to acetic acid propionate again will be converting to acetic acid and hydrogen. So, these kind of reactions are formed where things get converted to the acetate acetic acid or acetate ions and then eventually it can be further decomposed or degraded.

Now, if you see the delta G of the reaction change in the free energy assuming at natural ph and 25 degree Celsius temperature and 1 atmospheric pressure. So, that gives an idea that spontaneity of the reactions and which process are likely to be the faster. So, the one which are with positive G values are not that likely to take place or not that easy, but the one with negative G values are will be quite a few like pretty spontaneous processes and that way can be like we can see them happening pretty rapidly as well in the system.

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Anaerobic Degradation: Methanogenesis

- $2\text{CH}_3\text{CH}_2\text{OH} + \text{CO}_2 \rightarrow 2\text{CH}_3\text{COOH} + \text{CH}_4$
- $\text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2$
- $\text{CH}_3\text{OH} \rightarrow \text{CH}_4 + \text{H}_2\text{O}$
- $\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$
- $\text{CH}_3\text{COO}^- + \text{SO}_4^{2-} + \text{H}^+ \rightarrow 2\text{HCO}_3^- + \text{H}_2\text{S}$
- $\text{CH}_3\text{COO}^- + \text{NO}_3^- + \text{H}_2\text{O} + \text{H}^+ \rightarrow 2\text{HCO}_3^- + \text{NH}_4^+$

Handwritten note: $\frac{dS}{dt} = \mu \frac{S}{K_S + S}$

Functional step	Reaction	ΔG° kJ/mol	μ_{max} 1/d	T_d d	K_s mgCOD/l
Acetotrophic methanogenesis	$\text{CH}_3\text{COO}^- + \text{H}_2\text{O} \rightarrow \text{CH}_4 + \text{HCO}_3^-$	0.12	0.12	5.8 ^a	30 ^a
		-31	0.71 ^b	1.0 ^b	300 ^b
Hydrogenotrophic methanogenesis	$\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$	-131	2.85	0.2	0.06

^a Two different methanogens belonging to ¹*Methanosarcino* spec. and ²*Methanosacta* spec.

Graph: A graph showing growth rate vs substrate concentration. It shows two curves for Methanosarcina and Methanosacta. Methanosarcina has a higher growth rate at low substrate concentrations (higher μ_{max} and lower K_s), while Methanosacta has a higher growth rate at high substrate concentrations (lower μ_{max} and higher K_s). A horizontal line is drawn at 25 mgCOD/l.

Source : van Lies, J.B., Mahmoud, N., and Zeeman, G. (2008). Anaerobic Wastewater Treatment. In Biological Wastewater Treatment: Principles, Modelling and Design. Edited by Henze, M., van Loosdrecht, M.C.M., Ekama, G.A. and Brdjanovic, D. IWA Publishing London

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Now, if we have a look at methanogenesis aspects, so again the there are methane formation could take place in various ways, from the direct like propionate and butyrate

can also be basically lead to the methane formation the acetate converts to the methane and carbon dioxide. The alcohol can also convert to carbon dioxide methane and H₂O then CO₂ and H₂O the hydrogen and carbon which is the hydrogenotropic methanogenesis, that also converts the methane and this thing. There is possibility of sulfate nitrate and those kind of thing they also might undergo some sort of reactions in the electronic substance this thing.

So, that way the methane production is observed or is seen in the reactor. Now if we see the different functional steps. So, for acetotropic methanogens or acetoclastic methanogens what we say the major reaction is acetate converting to methane. The delta G for this is minus 31 which is a pretty good and if we see the like the typical values so of these.

Now this a step acetogenic methanogenesis or acetotropic methanogenesis. There are 2 major a species which act upon. So, one is methanosarcina and another is methanosarcina if we see they have a different shape like earlier week we discussed about the biomass growth, we discussed about the monod and kinetics thing.

So, if we see their net growth rate on the monod kinetic depending on the substrate concentration. So, what we see that one will have the methanosarcina works far better when the substrate concentration is higher. So, the rate of growth will be this much and accordingly rate of decomposition because, we know that if we take them mono out this thing.

So, rate of substrate consumption is minus $\frac{1}{y}$ and then we have $\frac{dx}{dt}$ and from there on $\frac{dx}{dt}$. We can write as $\mu_{max} \frac{S}{K_s + S}$ right.

So, the higher the X value the greater is this thing. So, the rate of growth here will be much higher when it is beyond certain substrate concentration, but at lower substrate concentration it is the methanosarcina which works better. So, if we want to basically have very high COD value and want to bring it down ok. The substrate concentration is too high from very high level; if we want to bring it down to significant levels; we should go for methanosarcina is species because that gives the high rate of biomass growth and resulting high rate of substrate decomposition. But if we want to basically if we are working with relatively lower COD and want it to bring it down to further, so

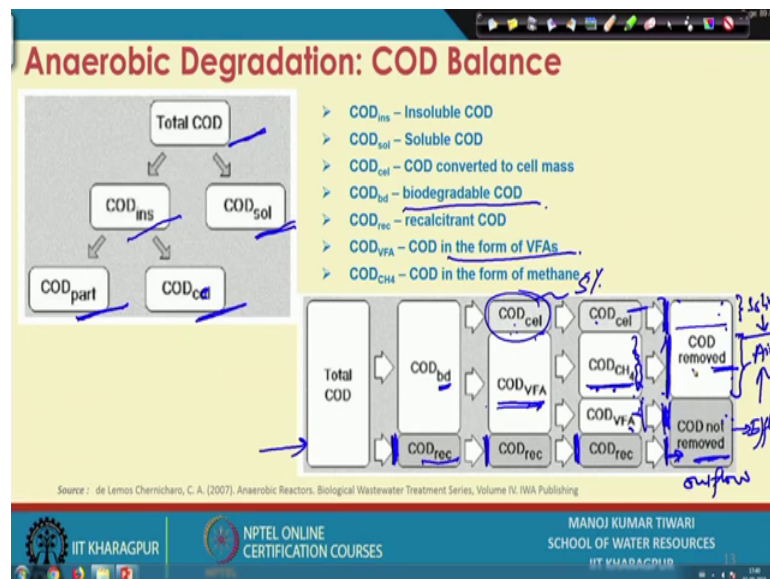
because if your reference COD is decreased if you are working in say this range; so then if and if you are working with methanol sarcina.

So, methanosarcina here will not be able to show the adequate growth methanosarcina here will have lesser growth rate as opposed to the methanoceta. So, depending on where or what kind of so let us say 25 milligrams ud per liter if we are working in a higher range, it is better to opt for methanosarcina. If we are working in a lower range, it is better to opt for methanosarceta. So, that way we can choose this a species depending on it is mu max and ks values.

There are hydrogenotropic methanogens which converts the carbon dioxide and hydrogen to methane and water. They also are response tenias reactions; they have pretty less values of delta G. So, the quite that way and the species which typically involved has K s value of 0.06. So, can actually be very effective the K s value low K s value means that it can actually work at even very low concentrations. It can give significant rates and mu max is 2.85 which is again a pretty high value.

So, that way it can means this will be a pretty fast process and which is revealed from it is delta G value as well thermodynamic parameter as well. So, as thermodynamics suggests this is going to be quite fast process, the hydrogenotropic methanogenesis is usually faster as opposed to the acetoclastic methanogenesis and acetoclastic methanogenesis will be depending on where which substrate range we are working with and whether we have the appropriate kind of species in present or not. So, that is about the methanogenesis how it is done.

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Now, if we have a look at the, what happens to the COD which is coming into the system into anaerobic reactor. So, if we see the mass balance of COD what happens that the total COD which is coming in one more point before we discuss.

So, while discussing activated sludge process or aerobic process in the previous week, we are more focused when we are discussing about the organic matter we are more frequently using the term BOD. So, BOD because as we measure BOD is the amount of oxygen required to aerobically degrade the biodegradable organic matter and since those processes was aerobic so we use the term BOD ok.

But if we go to monitor BOD in an anaerobic system or with the anaerobic microorganisms so because, anaerobic microorganisms cannot work upon the cannot work in presence of oxygen, oxygen might be toxic to them. So, if you end up measuring BOD with anaerobic microorganisms you are not going to get it. So, it is better it is advisable to work with COD in case of anaerobic degradation system. So, that we have a better idea of how much organic matter is present in the system.

So, that way that is why we are more focusing and more concerned about the chemical oxygen demand here instead of the biological oxygen demand for the anaerobic processes. So, the total COD that actually comes in the system may be of the 2 forms, there is there could be the soluble COD which is the amount of organic matter which are dissolved in the water which can easily be kind of like.

If you filter that it will pass through the filter so that becomes your soluble COD and then, there are insoluble COD or the part of organic matter the concentration of organic matter which are not dissolved in the water or which are actually in this suspended or insoluble stage in the water.

Now, this insoluble COD again is because of the particulate matters present in this. So, the particulate COD k COD present due to the particulate matters and COD presents due to the cell. So, whatever the cell mass is there the microorganisms is there they also have because that is also organic form of organic matter.

So, they will also have their own COD, so this insoluble COD because the particulate matter is in the suspended phase or particular particulate phase. So, that cannot get dissolved in the water that is one aspect, the cell mass or the cellular mass can also COD which is actually converted to the cell mass is also not dissolved in the water. So, there will be a constituent of the COD in the form of COD cell as well.

Now, what happens the total COD which enters the system, the total COD which enters the system would be of 2 type the COD which is biodegradable say bd ok, so COD bd is actually the biodegradable COD. So, the c COD which is biodegradable and COD which is recalcitrant means which cannot be degraded by microorganism. So, that recalcitrant COD or the non biodegradable COD we can say that is also there.

Now, whatsoever fraction of non biodegradable COD is coming into the system because, it is not being degraded it is not being reacted by the bacteria it will as it is come out of the system ok, so that will add to the COD which is not removed from the system. Now what happens to the COD which is biodegradable the COD which is biodegradable through like step of your hydrolysis and acetogenesis or acetogen acetogenesis and acetogenesis.

It will eventually convert to the in the volatile fatty acids because, the biodegradable portion of organic matter are going to convert to the volatile fatty acids and some are going to convert to this cell mass as we discussed this may be around just 5 percent of that. But still will be some COD which will be converted to the cell mass. So, COD cell is the COD which is converted to the cell mass and COD VFA is the COD in the form of volatile fatty acids because, they are also organic matter and they have their own oxygen demand. So, this is the COD in the form of VFA.

Now, the COD which is converted to the cell mass will again be basically coming as a cell mass only and if we have a good settling characteristic of this large if we design a reactor in such a way that our cell mass is retained in the reactor and does not go to the effluent. So, something which is retained in the reactor or which is phased out of the reactor will not be accounted into the outflow.

So, if you see this outflow so the cell mass is actually since it is getting settled, so it will be removed from the outflow from the water it will be removed. So, this COD due to the cell mass is form a constituent of the COD which is removed from the system because, the COD which is if the biodegradable COD which has converted to the cell mass and cell mass settle down. So, in the from water phase it has removed and that is why it will come as a COD removed.

Of the total VFAs there will be methane production, so significant amount of this goes to the methane or those kind of things; so, this biogas that is produced or the maintained that is produced. So, COD which is converted the total COD of the VFA will be converted to COD of the methane and there will still be some COD of the VFA which we still left in the system ok, which are still the volatile fatty acids which are dissolved in the system.

Now, the COD due to the methane COD which has been converted to the methane or COD in the form of methane will scape the system because methane will be taking a root of biogas and will not be present in the water. So, even this will be removed from the water and this COD from the methane will form another constituent of the COD remove. Whereas, the COD is still remaining in the form of volatile fatty acids because these VFAs are still in the water in the soluble state and these have this portion of the VFA has not been converted to biogas. So, this will again come in the form of effluent and will constitute in the COD in the effluent.

So, that COD is not removed because this portion is still coming in the outflow in affluent while this portion is removed from the effluent. So, removal is in the 2 phase this much has gone to the basically air in the form of a like gases and this much has been converted to the solid and settle down. So, this is basically moving up and this is settling down. So, that way we have the removal of COD and if we account for the mass balance of this mass balance of COD in anaerobic process this is what we are going to get.

So, this is how the different stages COD will change from one form to another form and eventually will either removed from the system or will get retained in the system in the form of either COD offered by the recalcitrant compound which are non biodegradable compound or COD in the form of volatile fatty acids still present in the system which has not been converted to your methane.

So, that is how the COD mass balance can get us an idea of what is the efficiency of this anaerobic degradation process would be in a specific cases. So, we will conclude this lecture here and subsequently we will discuss the other aspect of the anaerobic degradation and what are some of the reactor configurations that are available in the anaerobic for the purpose of anaerobic degradation of wastewater in the next class.

Thank you.