Biological Process Design for Wastewater Treatment
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Lecture: 10
Stoichiometry of Microbial Growth-II

Welcome again to this NPTEL Online Certification Course Lecture on Biological Process Designed for Wastewater Treatment. So, today we will continue with the new stoichiometric reaction that we started studying in the previous class, the new section that we had started. In the last lecture, we understood that there are 2 types of reactions which occur, which help in the overall growth of microbes.

So, those reactions were called as anabolic reactions and catabolic reaction. In the anabolic reaction, the carbon source is utilized along with the other elements to make microbes or microbial cell materials. And during this reaction, we require a lot of energy and that energy is obtained from catabolic reactions in which the ATP is generated which actually gets transferred into anabolic reactions where ATP is utilized and it is converted into di-phosphate which is ADP and ADP again transferred back into the catabolic reaction.

So, this continues. Later on in the previous reaction we studied the stoichiometry of two of the reaction that happened during the anabolism.

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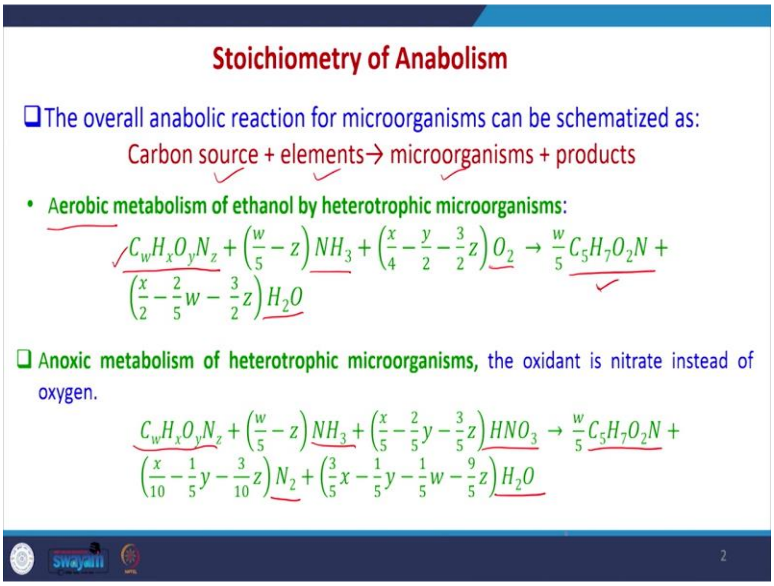

Aerobic metabolism of ethanol by heterotrophic microorganisms:

$$C_wH_xO_yN_z + \left(\frac{w}{5} - z\right) NH_3 + \left(\frac{x}{4} - \frac{y}{2} - \frac{3}{2}z\right) O_2 \rightarrow \frac{w}{5} C_5H_7O_2N + \left(\frac{x}{2} - \frac{2}{5}w - \frac{3}{2}z\right) H_2O$$

Anoxic metabolism of heterotrophic microorganisms, the oxidant is nitrate instead of oxygen.

$$C_wH_xO_yN_z + \left(\frac{w}{5} - z\right) NH_3 + \left(\frac{x}{5} - \frac{2}{5}y - \frac{3}{5}z\right) HNO_3 \rightarrow \frac{w}{5} C_5H_7O_2N + \left(\frac{x}{10} - \frac{1}{5}y - \frac{3}{10}z\right) N_2 + \left(\frac{3}{5}x - \frac{1}{5}y - \frac{1}{5}w - \frac{9}{5}z\right) H_2O$$

So, stoichiometry we started studying the stoichiometry of anabolic under which the overall anabolic reaction from micro exam was schematized as some carbon source, some elements they get converted into microorganism. For the microorganisms, we are using the general formula this, for the carbon source, we are using a generic formula of $C_wH_xO_yN_z$ and then we started understanding the metabolic reactions of heterotrophic microorganism under various conditions.

So, first condition that we studied was aerobic condition. So, we studied the aerobic metabolism and we found out that reaction can be represented by this particular equation where the carbon source along with ammonia and oxygen, they react to form the microorganisms and H_2O.

Now, in the anoxic condition, when the oxygen is not available to under that condition, we use nitrate along with ammonia and certainly the carbon source and these get converted again into microorganism, the nitrogen gas and water. So, in place of in the anoxic condition nitrate gets converted into N_2 whereas in the aerobic condition the oxygen is there, so oxygen is used.

Now we will further study other types of metabolic reactions under the anabolism category. And later on in the today's lecture we will study the same thing under catabolism. So, we will study that difference.

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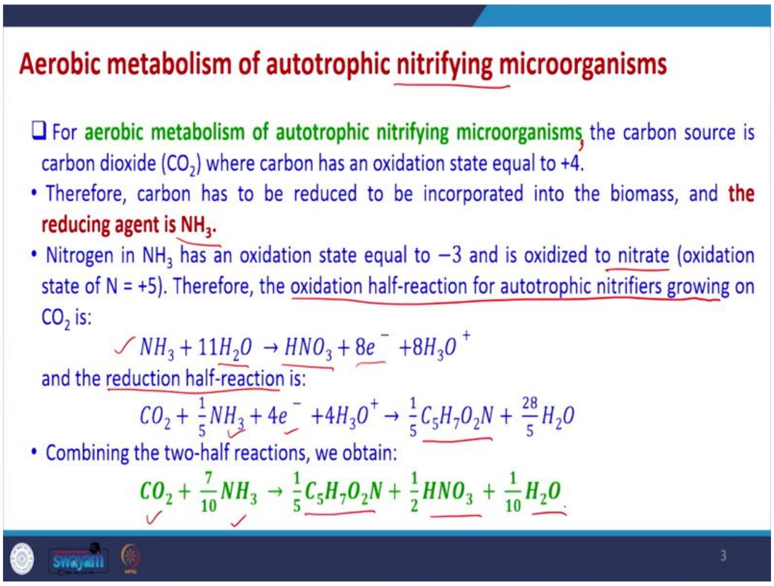

The oxidation half-reaction for autotrophic nitrifiers growing on CO_2 is:

$$NH_3 + 11H_2O \rightarrow HNO_3 + 8e^- + 8H_3O^+$$

The reduction half-reaction is:

$$CO_2 + \frac{1}{5}NH_3 + 4e^- + 4H_3O^+ \rightarrow \frac{1}{5}C_5H_7O_2N + \frac{28}{5}H_2O$$

Combining the two-half reactions, we obtain:

$$\boldsymbol{CO_2 + \frac{7}{10}NH_3 \rightarrow \frac{1}{5}C_5H_7O_2N + \frac{1}{2}HNO_3 + \frac{1}{10}H_2O}$$

Now for aerobic metabolism of autotropic nitrifying microorganism, now we are going for nitrification. For aerobic metabolism of autotropic nitrifying microorganism the carbon sources carbon dioxide, where carbon has an oxidation state of plus 4. Therefore, this carbon will have to be reduced to be incorporated into the biomass and for this the reducing agent is NH_3.

Now, nitrogen in NH_3 has an oxidation state equal to minus 3 and it gets oxidized to nitrate, which is having an oxidation state of plus 5. Therefore, the oxidation half reaction for this autotropic nitrifiers growing on CO_2 is this. So, this is the so, ammonia reacts with water to form HNO_3 with some electrons released. Similarly, in the reduction half reaction, the CO_2 is utilized along with the ammonia and these electrons to form the microorganism.

Now, this overall combining both reactions we have CO_2 which is getting utilize along with ammonia to form microorganism HNO_3 and H_2O. So, this is the overall aerobic metabolism for nitrifying microorganism.

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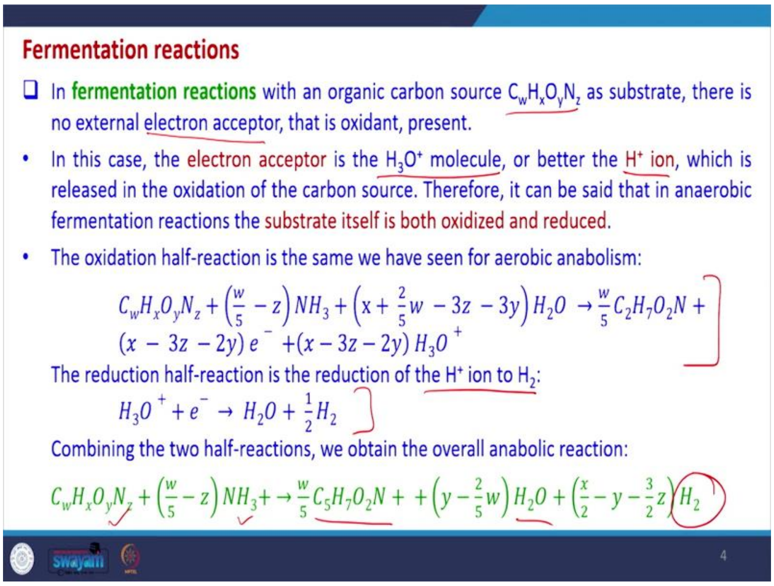

The oxidation half-reaction is the same we have seen for aerobic anabolism:

$$C_wH_xO_yN_z + \left(\frac{w}{5} - z\right) NH_3 + \left(x + \frac{2}{5}w - 3z - 3y\right) H_2O \rightarrow \frac{w}{5} C_2H_7O_2N + (x - 3z - 2y)\, e^- + (x - 3z - 2y)\, H_3O^+$$

The reduction half-reaction is the reduction of the H^+ ion to H_2:

$$H_3O^+ + e^- \rightarrow H_2O + \frac{1}{2} H_2$$

Combining the two half-reactions, we obtain the overall anabolic reaction:

$$C_wH_xO_yN_z + \left(\frac{w}{5} - z\right) NH_3 + \rightarrow \frac{w}{5} C_5H_7O_2N + + \left(y - \frac{2}{5}w\right) H_2O + \left(\frac{x}{2} - y - \frac{3}{2}z\right) H_2$$

Now, in the fermentation reactions what happened? So, there is another type of reactions which are possible in the wastewater treatment which are fermentation reactions. So, in fermentation reactions with an organic source, which was the source that we have been using in the previous lecture also, there is no external electron acceptor that is oxidant is not.

In this case the electron acceptor is the H_3O molecule or H plus ion which is released in the during the oxidation of carbon source. And therefore, it can be said that in anaerobic fermentation reaction, the substrate itself is both oxidized and reduced. So, the oxidation half reaction is same we have seen for aerobic anabolism. So, this is the reaction.

Now, this is the half reaction for aerobic anabolism. However, the reduced half the action is the reduction of H plus ions to H_2. So, this is the difference in the fermentation reaction. So, we have this reaction and combining together we have the carbon source combining together with ammonia to form the microorganism H_2O and H_2 gets released remember here H_2 is getting released in the fermentation reactions.

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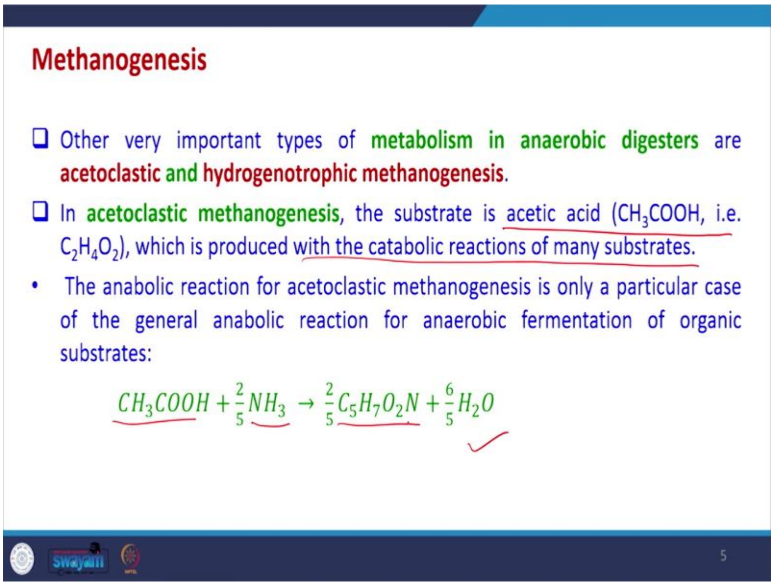

$$CH_3COOH + \frac{2}{5}NH_3 \rightarrow \frac{2}{5}C_5H_7O_2N + \frac{6}{5}H_2O$$

Now, there is another type of reactions which are possible in the biological treatment these are called methanogenesis reaction and this metabolism in the anaerobic digester where methane get released, there are 2 types of metabolism possible. One is called acetolastic and another is called hydrogenotrophic methanogenesis.

So, in the acetolastic methanogenesis the substrate is acetic acid for example, which is produced with the catabolic reactions of many substrate. The anaerobic reaction of the acetolastic methanogenesis is only a particular case for any other reaction, but it can be represented by this particular reaction where acetic acid is reacting with ammonia to form the microorganism along with water.

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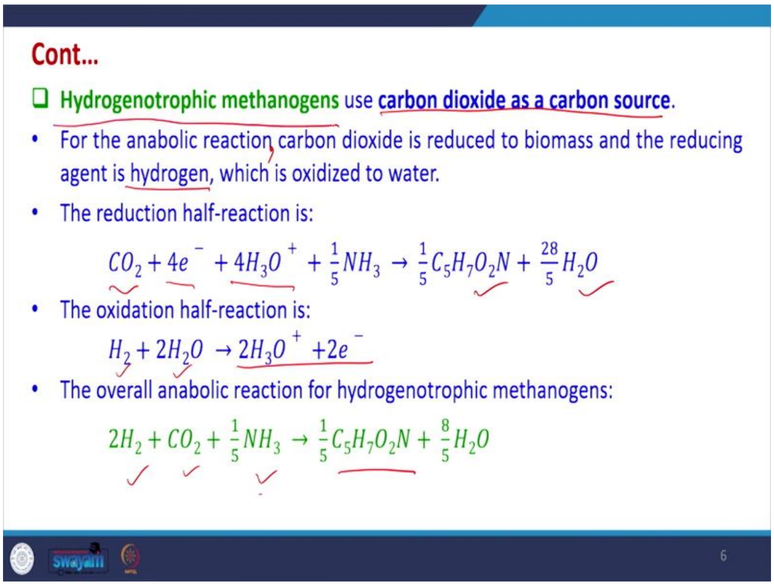

The reduction half-reaction is:

$$CO_2 + 4e^- + 4H_3O^+ + \frac{1}{5}NH_3 \rightarrow \frac{1}{5}C_5H_7O_2N + \frac{28}{5}H_2O$$

The oxidation half-reaction is:

$$H_2 + 2H_2O \rightarrow 2H_3O^+ + 2e^-$$

The overall anabolic reaction for hydrogenotrophic methanogens:

$$2H_2 + CO_2 + \frac{1}{5}NH_3 \rightarrow \frac{1}{5}C_5H_7O_2N + \frac{8}{5}H_2O$$

During the hydrogenotrophic methanogenesis the carbon dioxide is used as a carbon source and for the anabolic reaction carbon dioxide is reduced to biomass and the reducing agent is hydrogen. So, we have hydrogen which is used as the reducing agent so, CO_2 with 4 electron plus 4 H_3O plus and ammonia they produce the microorganism along with the water and H_2 along with the H_2O release the electrons and H_3O plus.

If we combine together overall reaction is this. Hydrogen plus CO_2 plus ammonia produced the microorganism during the hydrogenotrophic methanogens. So, this is the overall reaction that happens during these particular types of reactions. So, we have studied few types of anabolic reactions and overall their stoichiometric equations can be summarized here.

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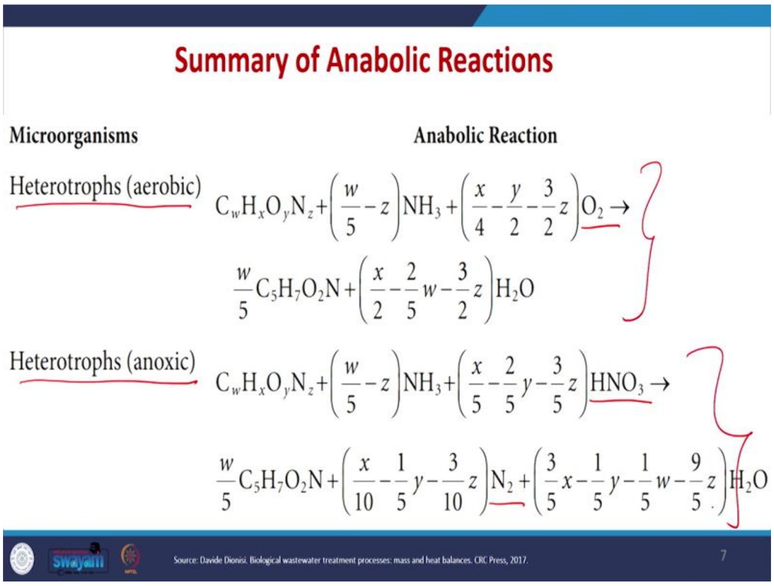

First we studied the heterotrophs under aerobic conditions. Then heterotrophs in the anoxic condition. In the aerobic condition oxygen was present whereas, in the anoxic condition oxygen was not present. So, we used HNO_3. HNO_3 was converted into N_2 and under anoxic condition where oxygen was utilized as such during aerobic condition and the reactions stoichiometry are given here respective by 2 reactions.

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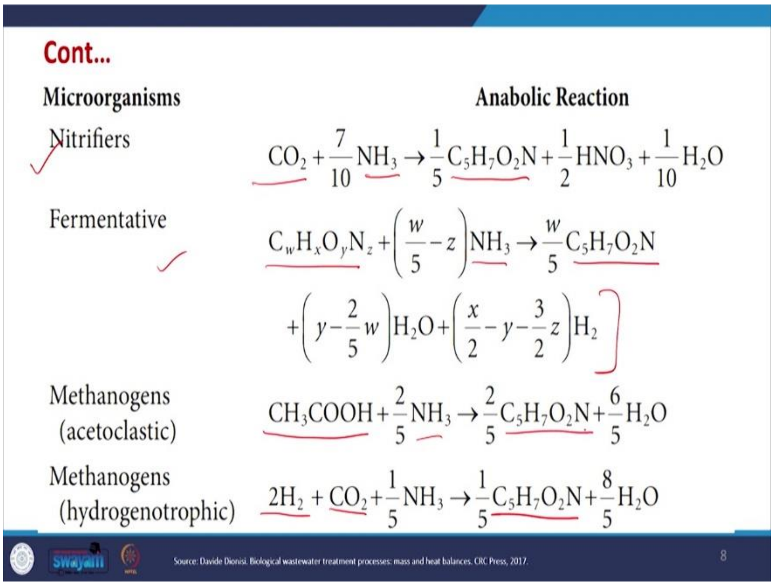

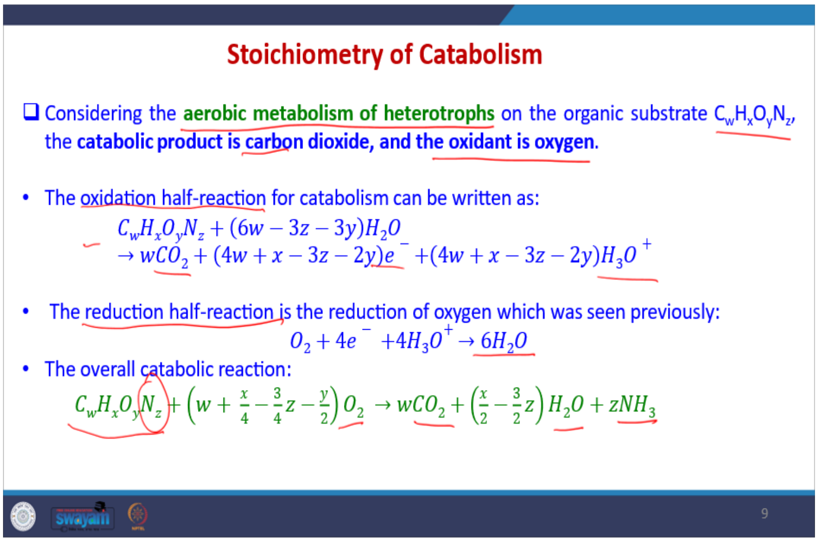

The oxidation half-reaction for catabolism can be written as:

$$C_wH_xO_yN_z + (6w - 3z - 3y)H_2O \rightarrow wCO_2 + (4w + x - 3z - 2y)e^- + (4w + x - 3z - 2y)H_3O^+$$

The reduction half-reaction is the reduction of oxygen which was seen previously:

$$O_2 + 4e^- + 4H_3O^+ \rightarrow 6H_2O$$

The overall catabolic reaction:

$$C_wH_xO_yN_z + \left(w + \frac{x}{4} - \frac{3}{4}z - \frac{y}{2}\right)O_2 \rightarrow wCO_2 + \left(\frac{x}{2} - \frac{3}{2}z\right)H_2O + zNH_3$$

Later on in today’s class we studied regained during that nitrifiers where we can see the carbon dioxide along with ammonia is used for the reaction. Similarly, during the fermentative process, the carbon substrate along with the ammonia get forms the microorganism along with water and hydrogen. In the hydrogenotrophic, again hydrogen is used along with the carbon dioxide to produce the biomass.

During the acetoclastic, the acid is used along with the ammonia to form the microorganism. So, these are the various anabolic reactions which have which occur during the wastewater where substrate is utilized for production of biomass. Now, there are catabolic reactions which actually provide the energy. So, now we will study the catabolic reactions in detail.

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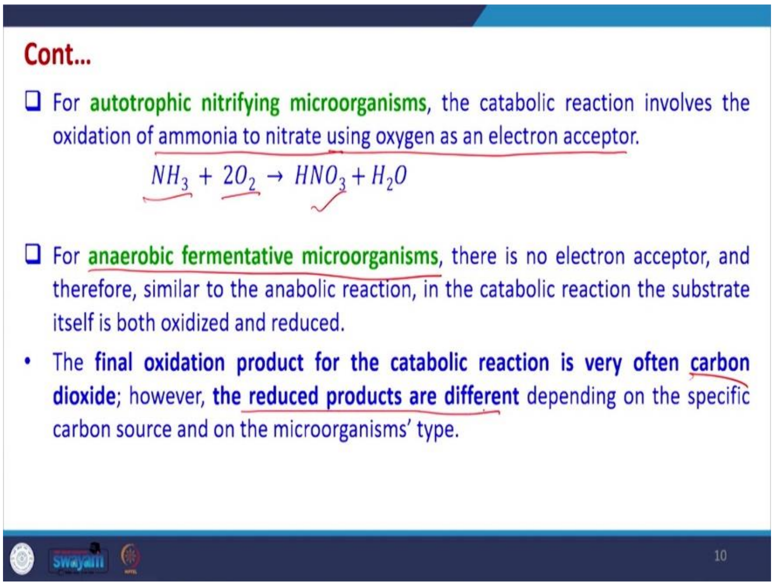

For autotrophic nitrifying microorganisms, the catabolic reaction:

$$NH_3 + 2O_2 \rightarrow HNO_3 + H_2O$$

So, stoichiometry of catabolism so we will start with this. So, considering the aerobic metabolism of heterotrophs. So, first we will start with the aerobic metabolism of heterotrophs on the organic substrate which was same as earlier. The catabolic product is carbon dioxide and the oxidant is oxygen.

So, for simple aerobic condition we have oxygen which acts as an oxidant and this oxygen works on the organic substrate to produce CO_2 electron and H_3O during the oxidation half reaction and during the reduction half reaction, this reduced oxygen which was seen previously it is utilized to produce water and if we combine together both the reactions A and B, so, we have overall catabolic reactions can be written as where some substrate is oxidized in presence of oxygen to CO_2 and H_2O and NH_3 if nitrogen is as a element.

So, already we have seen a such type of reaction during our theoretical calculation of ThOD. So same is being repeated here. Here they are called as catabolic reaction because they provide the energy during the aerobic metabolism of heterotrophs. Now, going further for autotrophs nitrifying microorganism the catabolic reaction involves the oxidation of ammonia to nitrate using oxygen as an electron acceptor.

So, this is used to produce the nitrate. So, here oxygen is there, ammonia is there. So, we have HNO_3 gets formed and plus H_2O. During the anaerobic fermentative microorganism reactions, there is no electron acceptor and therefore, it is similar to anabolic reaction which was shown earlier. So, in the catabolism reaction the substrate itself is both oxidized and reduced as it was the case earlier.

The final oxidation product for catabolic reaction is very often carbon dioxide and the reduced products are different, depending upon the carbon source and the microorganism type.

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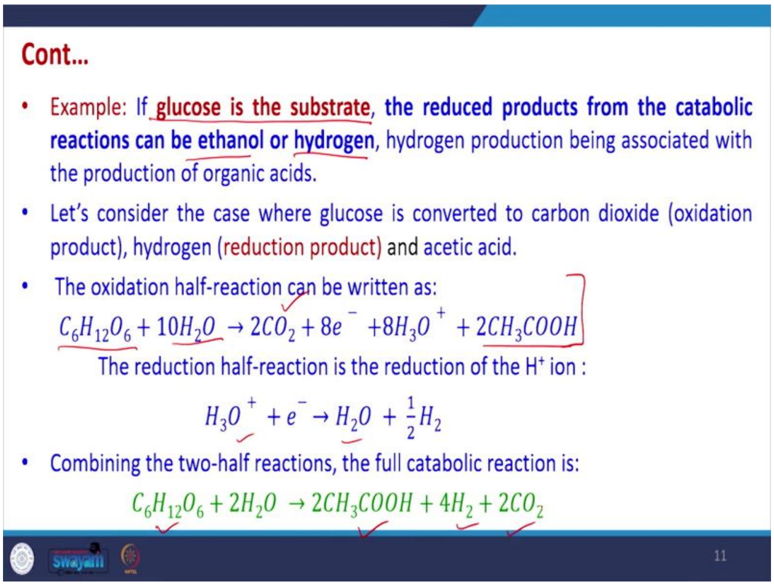

The oxidation half-reaction can be written as:

$$C_6H_{12}O_6 + 10H_2O \rightarrow 2CO_2 + 8e^- + 8H_3O^+ + 2CH_3COOH$$

The reduction half-reaction is the reduction of the H^+ ion:

$$H_3O^+ + e^- \rightarrow H_2O + \frac{1}{2}H_2$$

Combining the two-half reactions, the full catabolic reaction is:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 4H_2 + 2CO_2$$

So, if you take like glucose is the substrate, the reduced product from the catabolic reactions will be ethanol or hydrogen. The hydrogen production being associated with production of organic acid. So, let us consider the case where glucose is converted to carbon dioxide, hydrogen and acetic acid.

The oxidation half reaction for this case can be written like this, where we can see the glucose is being converted into the acetic acid in presence of water and CO_2 is getting released. The reduction half reaction can be written like this and combining together the glucose is getting converted into acid, hydrogen and CO_2. So, this is the catabolic reaction.

And in this reaction the energy gets released. During the methanogens there are 2 types of methanogens possible, the acetolastic and the hydrogenotrophic.

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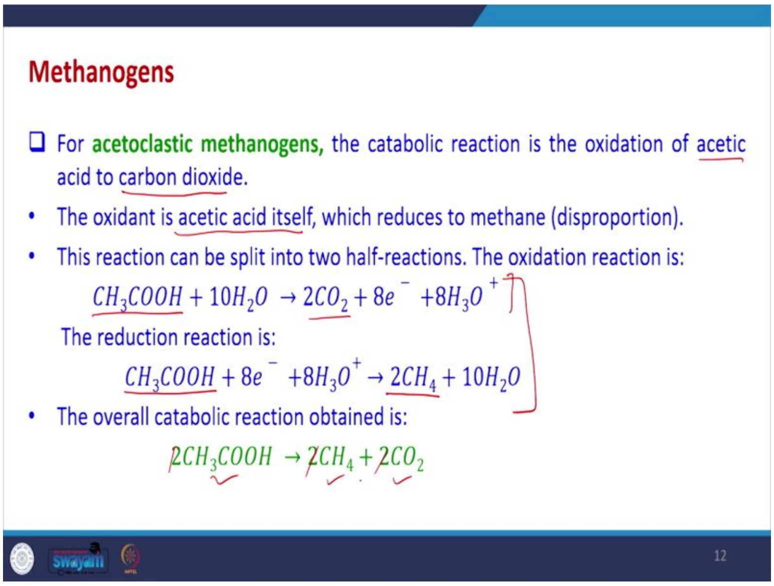

This reaction can be split into two half-reactions. The oxidation reaction is:

$$CH_3COOH + 10H_2O \rightarrow 2CO_2 + 8e^- + 8H_3O^+$$

The reduction reaction is:

$$CH_3COOH + 8e^- + 8H_3O^+ \rightarrow 2CH_4 + 10H_2O$$

The overall catabolic reaction obtained is:

$$2CH_3COOH \rightarrow 2CH_4 + 2CO_2$$

Now, in this acetolastic methanogens the catabolic reaction is the oxidation of acetic acid to carbon dioxide. So, here the conversion happens from acetic acid to carbon dioxide, the oxidant is acetic acid itself, which reduces to methane. So, we can write the half reaction for this reaction. These two half reactions are written and we can see the acid itself is getting reduced to methane, whereas, in the oxidation reaction the acidic acid is getting reduced getting oxidized to CO_2.

So, both acetic acid itself works as both oxidant as well as both reduction and oxidation of acetic acid is happening and we combined together both reactions to get 2 moles of acetic acid getting converted into 2 moles of methane and 2 moles of CO_2 or simply we can write without 2 also this is a straightforward reaction which can be written.

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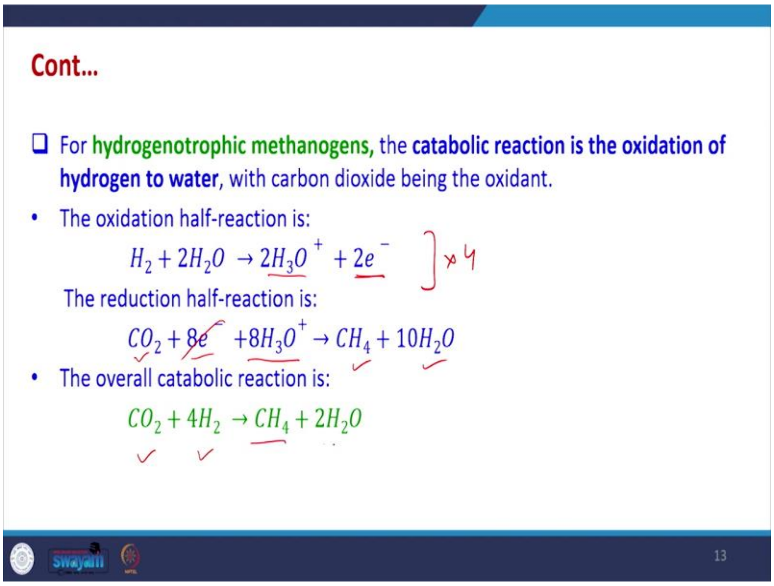

The oxidation half-reaction is:

$$H_2 + 2H_2O \rightarrow 2H_3O^+ + 2e^-$$

The reduction half-reaction is:

$$CO_2 + 8e^- + 8H_3O^+ \rightarrow CH_4 + 10H_2O$$

The overall catabolic reaction is:

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

Now containing four hydrogenotrophic methanogens the catabolic reaction is the oxidation of hydrogen to water. So, in this case with carbon dioxide being used as an oxidant. So, we can see here the carbon dioxide is being used as an oxidant in biological reaction, which is never possible in usual scenario. The oxidation half reactions are H_2 combining with water to form H_3O plus and 2 electrons. And in the reduction half reaction the CO_2 combining together with 8 electrons and H_3O plus to form methane and water.

Now we have to combine both reactions together. And for this we will have to multiply the first reaction by 4 so, that 8 electrons and 8 electrons go off. And overall the action can be written as CO_2 plus 4 H_2 produces methane plus water. So, this is the hydrogenotrophic methanogens reaction under catabolism conditions. Now, the summary of catabolic reactions can be written here.

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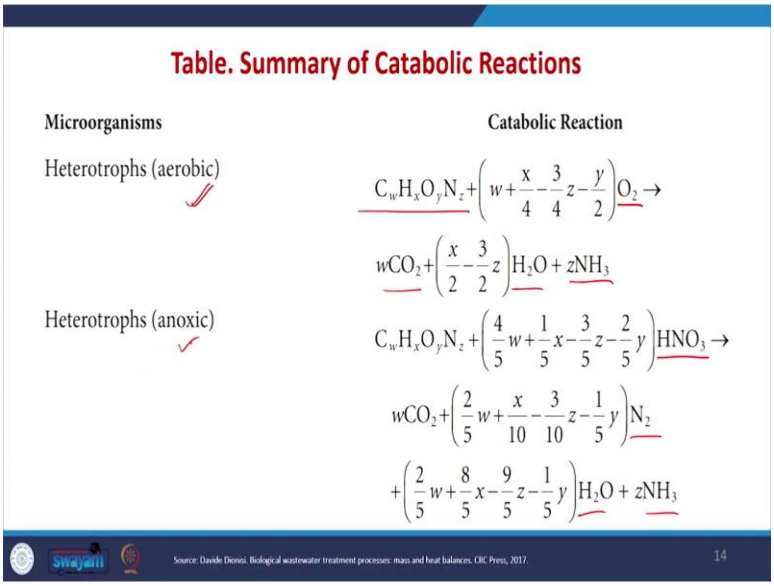

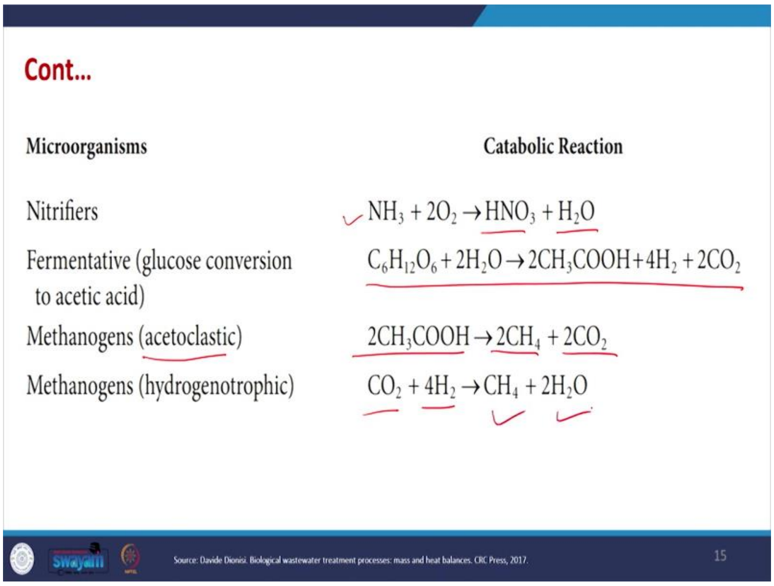

In the aerobic condition, we have oxygen, in the anaerobic condition, we have nitrate. Oxygen is getting converted the all the carbon sources getting converted into CO_2, H_2O and ammonia under aerobic condition, and in the anaerobic condition, this HNO_3 is getting converted into nitrate, H_2O and NH_3 and both these reactions are one is occurring under aerobic condition, another is occurring under anoxic condition.

Further on there are other types of reactions which are possible under catabolic reaction category and one is called nitrifiers we already studied and in the nitrifiers, ammonia combines with oxygen to form HNO_3 and water when the nitrifier microorganisms are present, when the fermentative microorganism are present and we are representing glucose conversion to acetic acid we can write like this equation. And we can see here that CO_2 is getting produced.

In another reaction when acetolastic conditions with respect to methanogens is there so, methane is getting produced along with CO_2 from acetic acid itself. In the second case, when the hydrogenotrophic reaction is happening, so, hydrogen is being used along with CO_2 to produce methane and water. So, till now, we have studied anabolic reactions, catabolic reactions separately and under various conditions when different types of microorganisms are present.

And depending upon the availability of whether oxygen is present or not present, all the reactions have been listed. Now, the challenge is that can we combine together these reactions and both anabolic and catabolic reactions together and come up with a combined reaction. So, this is the challenge and this is what we are going to study.

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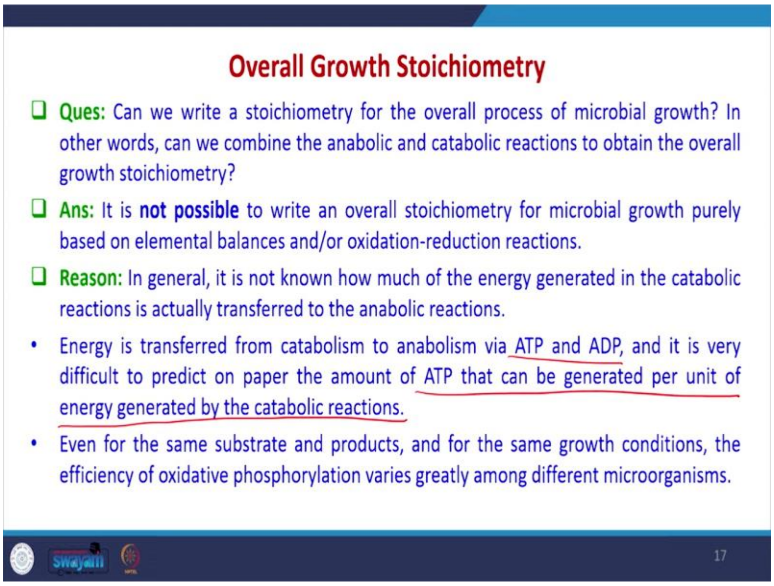

And so, we want to study the overall growth stoichiometry, will end with some this question. Can we write stoichiometry for overall process of microbial growth? In other words, can we combine the anabolic and catabolic reaction to obtain the overall stoichiometry? Answer is it is not possible to write an overall stoichiometry for microbial growth purely based on elemental balances oblique and or oxidation-reduction reactions.

Why this is so? In general, it is not known how much amount of energy is generated in the catabolic reactions and how much is actually being transferred to anabolic reactions. So, this is one of the basic regions where it is not easily possible to combine together both. Second is that the energy is transferred from catabolism to anabolism via ATP and ADP. And it is very difficult to predict on paper the amount of ATP that can be generated per unit of energy generated by catabolic reaction.

So, we can find out the energy generated by catabolic reactions, but it is not easy to find out how much ATP has been generated per unit of energy generated and even for the same substrate and the product and for the same growth condition, the efficiency of oxidative phosphorylation varies greatly among different microorganisms. So, because of this condition, the overall growth chemistry is stoichiometry is not easy to understand.

And we have to use certain other conditions to combine together. So, we will understand and we will start with a little bit studying the overall growth stoichiometry in the next class onwards, and we will combine together the anabolic and catabolic reactions. There is a difficulty in solving them together.

So, we will use a certain condition under which they can be overall combined together and then they can be used together for some inferences which can be derived out of the overall microbial growth stoichiometry. So, with this, we will end today’s lecture. Thank you very much.