Biological Process Design for Wastewater Treatment
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Lecture: 09
Stoichiometry of Microbial Growth-I

Welcome everyone to this NPTEL Online Certification Course on Biological Process Design for Wastewater Treatment. So, in the previous lectures we have already studied the initial basics of the biological processes that occur, the microbiology and later on we studied the wastewater characterization. And in particular, we studied in detail the COD, BOD TOC and ThOD methods of characterization of wastewater which are directly useful during the biological process design.

Now, we are starting another section within this NPTEL online certification course. And from today onwards, we will be considering the modeling of biological processes via various reactions and their kinetics. So, today we will start with the various types of reactions or the which occur during the wastewater treatment, inside the wastewater, where the not only the substrate or the pollutants are utilized, also different types of biomass and their growth may occur. And during these reactions, various possibilities are occur and we will study the stoichiometry of these reactions which may occur with oxygen, without oxygen, under anoxic conditions etc. So, all these reactions today onwards, we will be studying in detail for few lectures. So, we will start with the some of the basics of the reactions kinetics in general.

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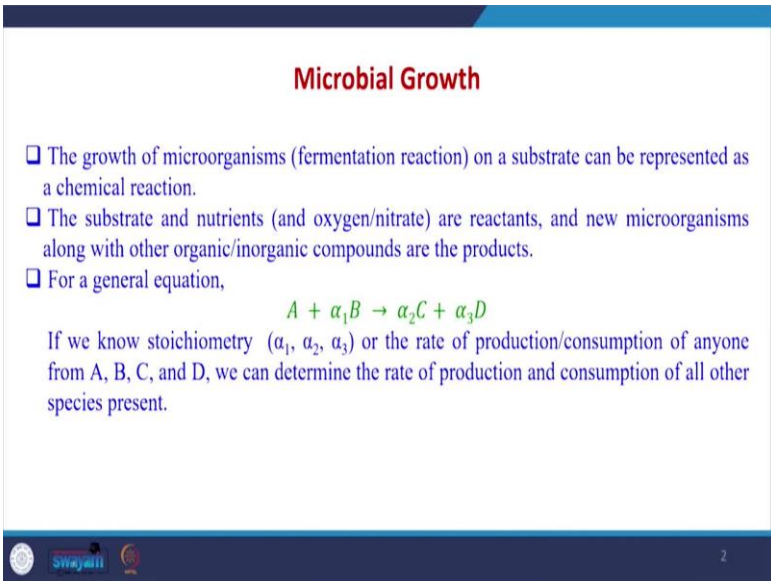

For a general equation,

$A + \alpha_1 B \rightarrow \alpha_2 C + \alpha_3 D$

So, for microbial growth, the microorganisms or the fermentation reaction or substrate can always be represented by some chemical reactions. And during these microbial growth or substrate utilization reactions, the substrate and the nutrients and oxygen oblique nitrate are the reactants, whereas, the various types of products may be the CO_2, ammonia, etc. may be the product and along with them, new type of microorganisms get formed, they are also the product.

In general, if we knew any other reaction, so, this is a general chemistry that if you know the reaction, where A and B are the reactants, and C and D are the products. So, under those conditions, what we have to do is that first initially we will have to balance the equation. So, what we do is that we try to find out the coefficients of the concentration of the respective reactants and products and these coefficients are called stoichiometry coefficient.

So, and these may be like further reactions given here, these are alpha 1, alpha 2, and alpha 3 and if we knew the stoichiometry, that means, these coefficients and the rate of production oblique consumption of any of the other raw material product, which are A, B, C, D.

So, if any of the rate of production or consumption of the A, B, C, D values are known, then we can find out the rate of production and consumption of all other species present in the that particular reaction. So, and this particular idea will be useful later on, when we study later section.

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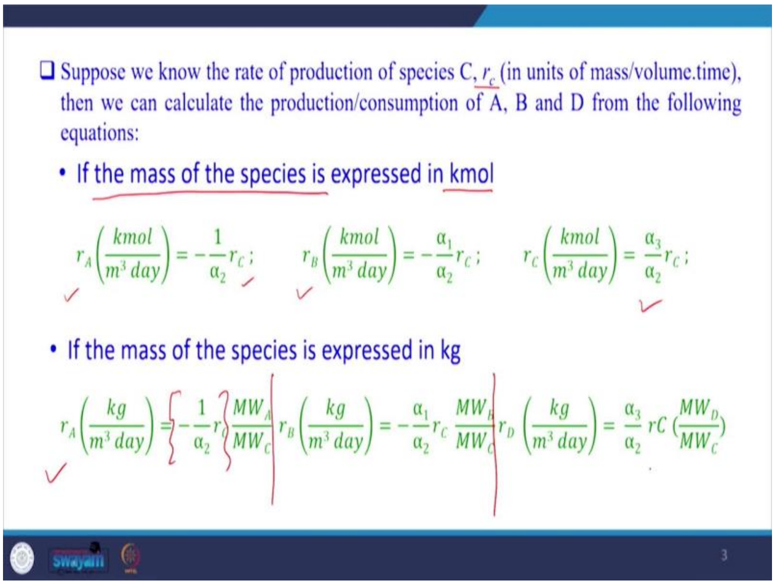

If the mass of the species is expressed in kmol

$$r_A\left(\frac{kmol}{m^3\ day}\right) = -\frac{1}{\alpha_2} r_C\ ;$$

$$r_B\left(\frac{kmol}{m^3\ day}\right) = -\frac{\alpha_1}{\alpha_2} r_C\ ;$$

$$r_C\left(\frac{kmol}{m^3\ day}\right) = \ \frac{\alpha_3}{\alpha_2} r_C\ ;$$

If the mass of the species is expressed in kg

$$r_A\left(\frac{kg}{m^3\ day}\right) = -\frac{1}{\alpha_2} r_C \frac{MW_A}{MW_C}\ r_B\left(\frac{kg}{m^3\ day}\right) = -\frac{\alpha_1}{\alpha_2} r_C\ \frac{MW_B}{MW_C} r_D\ \left(\frac{kg}{m^3\ day}\right) = \ \frac{\alpha_3}{\alpha_2}\ rC\ (\frac{MW_D}{MW_C})$$

Suppose, we know the rate of production of species C that means which is represented by r subscript c and its unit may be mass per unit volume per unit time, then we can calculate the production oblique consumption of A and B and D from the following equation. So, if the mass of the species is expressed in kilo mole, that means, we are using the molar concentration then the reaction with respect to r_A can be found out from r_C via this equation, where 1 by alpha 2.

Similarly, for r_B we can find out using a minus alpha 1 by alpha 2. So, we use the ratios of the both B as well as C for finding out these parameters. Similarly, for r_C also we can find out. If the mass is expressed in kg, so this is possible that we have the values known in kg per litre or something like this. So under those conditions, we will have to convert those kg values into the molar units.

And for doing this we will have to use the molecular weight. So, under those conditions, these, the above equations get modified as here. So, we can see the initial values are same, but we have to multiply together by the molecular weight of A divided by molecular weight of B. Similarly, for r_B we can find out and similarly for r_D also, we can find out, that means if the rate of production of a species C in any unit either mass per unit volume per unit time or moles per unit volume per unit time are known, we can easily find the other values.

And this idea will be used later on, because generally we perform the calculation experimentally with respect to rate of production or rate of consumption of one of the key elements, which can easily be measured and once it can be measured, and if we know the stoichiometry of the reaction, we can find out the rate of production oblique consumption of all other species via this.

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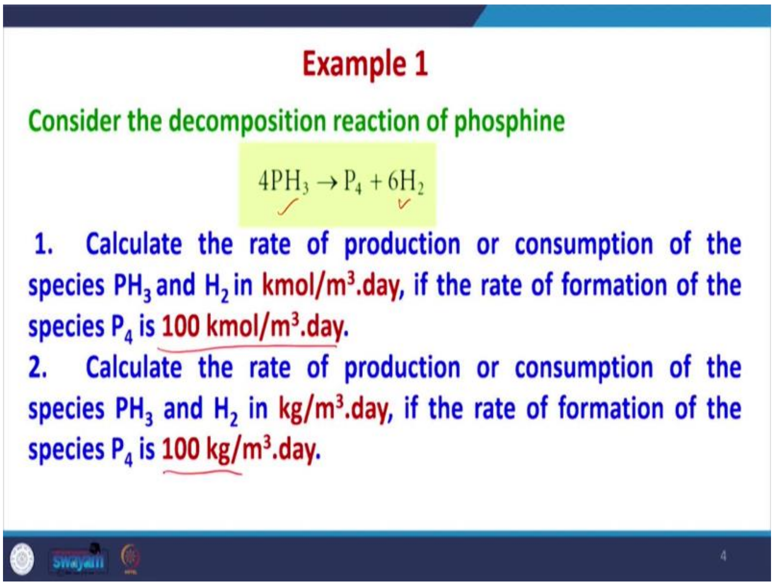

Consider the decomposition reaction of phosphine

$4PH_3 \rightarrow P_4 + 6H_2$

Now, here an example is given that the decomposition reaction of phosphine is given here and we calculate it is being asked that calculate the rate of production or consumption of the species PH_3 and H_2. So, we had to find out the rate of conjunction of PH_3 and rate of production of hydrogen in kilo moles per meter cube per day if the rate of formation of P_4 species is 100 kilo mole per metre cube per day.

So, that means, the rate of formation is in kilo mole and we have to find out the rate of production and consumption of all other species. Similarly, in the second part, it is being asked that if the rate of production on rate of formation of a species P_4 is 100 kg not kilo mole, then we have to find out the date of production and consumption of species PH_3 and H_2 in kg per meter cube per day.

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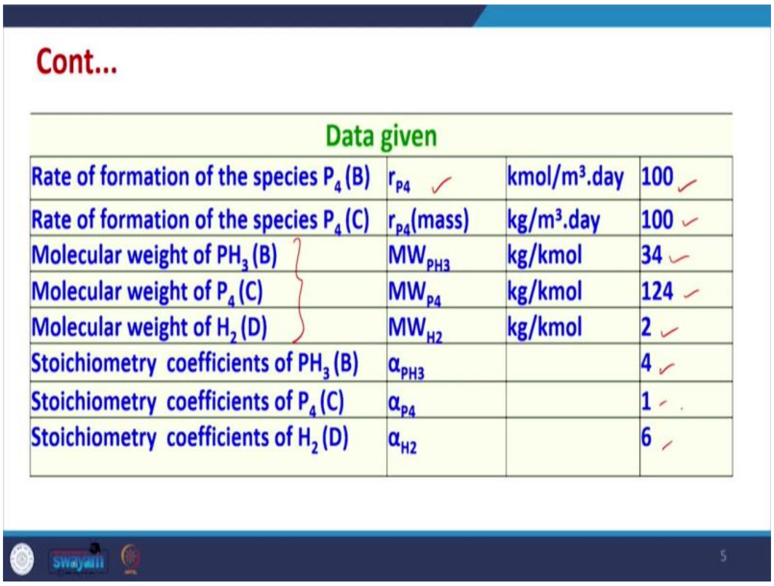

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Data given			
Rate of formation of the species P_4 (B)	r_{P4}	kmol/m³.day	100
Rate of formation of the species P_4 (C)	r_{P4}(mass)	kg/m³.day	100
Molecular weight of PH_3 (B)	MW_{PH3}	kg/kmol	34
Molecular weight of P_4 (C)	MW_{P4}	kg/kmol	124
Molecular weight of H_2 (D)	MW_{H2}	kg/kmol	2
Stoichiometry coefficients of PH_3 (B)	α_{PH3}		4
Stoichiometry coefficients of P_4 (C)	α_{P4}		1
Stoichiometry coefficients of H_2 (D)	α_{H2}		6

Now, that data given is already given with respect to P_4 which is in the first part this is 100 kilo mole per metre cube per day in the second part, it is kg 100 kg per metre cube per day. So, we will have to find out the molecular weight of all the species B, C, and D and that has been found here 34, 124, 2. Similarly, stoichiometry coefficients are known from the reaction itself 4, 1 and 6. So, these are written here 4, 1 and 6.

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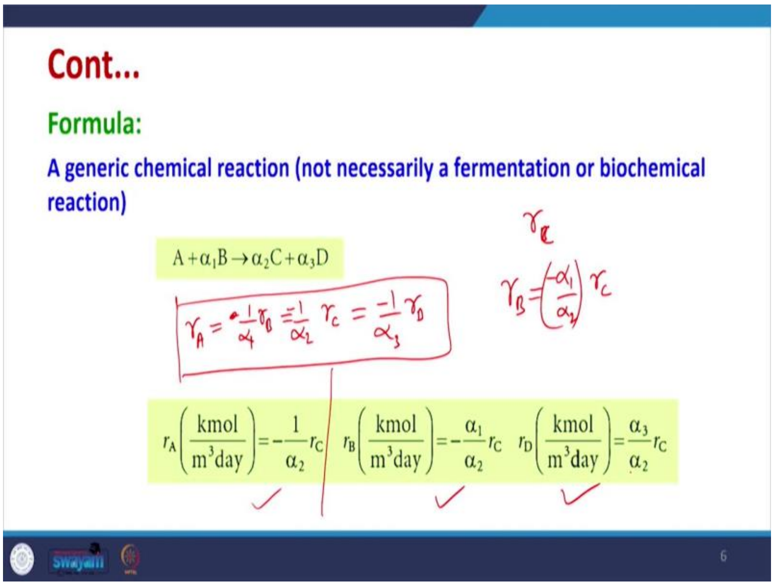

$$A + \alpha_1 B \rightarrow \alpha_2 C + \alpha_3 D$$

$$r_A\left(\frac{\text{kmol}}{\text{m}^3\text{day}}\right) = -\frac{1}{\alpha_2} r_C \quad r_B\left(\frac{\text{kmol}}{\text{m}^3\text{day}}\right) = -\frac{\alpha_1}{\alpha_2} r_C \quad r_D\left(\frac{\text{kmol}}{\text{m}^3\text{day}}\right) = \frac{\alpha_3}{\alpha_2} r_C$$

And if they are known, then we can use this particular general for any general chemical reaction not necessarily a fermentation or biochemical reaction, if this is known, we can use this particular type of equation where r_A with respect to r_C can always be found out. Similarly, r_A can be written with respect to r_B or r_C. So, any of these types of general reactions can be written.

So, this is will be minus 1 upon this will be alpha 1 with respect to r_B and this is same as r_C minus one upon alpha 2 is equal to minus alpha 3 r_D. So, this will be known, both these are negative whereas, first one is positive. So, using this reaction we can find out this equation the rate of production of any other species. So, far the present case the value of r_B is given okay. So, sorry the value of r_C has been given and we have to find out for r_B and r_D.

So, we can use this equation. So, from here we can easily see the r_B value is equal to r_C alpha 1 alpha 2 minus. So, this is this and thus we can find out this is the equation here and similarly for r_D we can find out using this equation. So, once this is known, we can easily solve.

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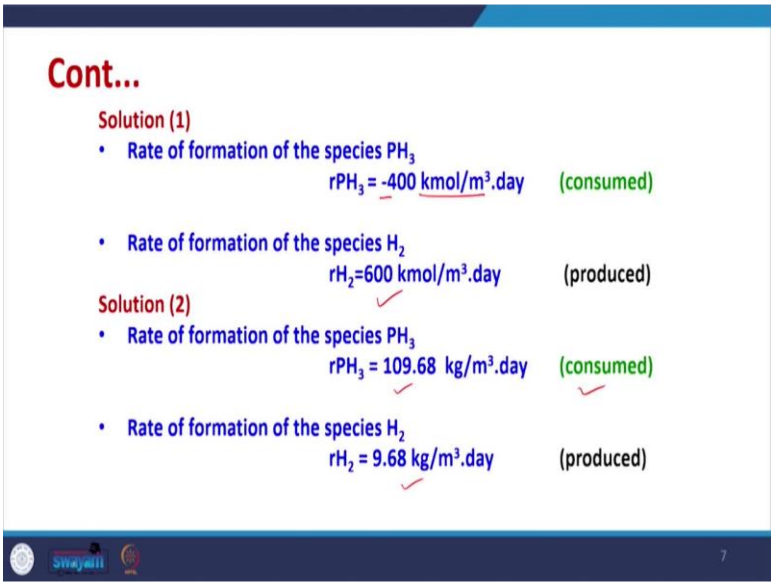

And the rate of formation in the initial case is in kilo moles. So, we can easily use this particular type of equation to solve it and it will come out as minus 600 kilo mole per metre cube per day. Remember we are keeping minus r PH_3 because rate of formation is given. So, this is there. Similarly, the rate of formation of species PH_3 which is getting consumed is minus this is 109.68 kg per day and data formation of a species H_2 is this.

So, we can use the rate of formation equations for finding out the rate of formation and conjunction of all other species.

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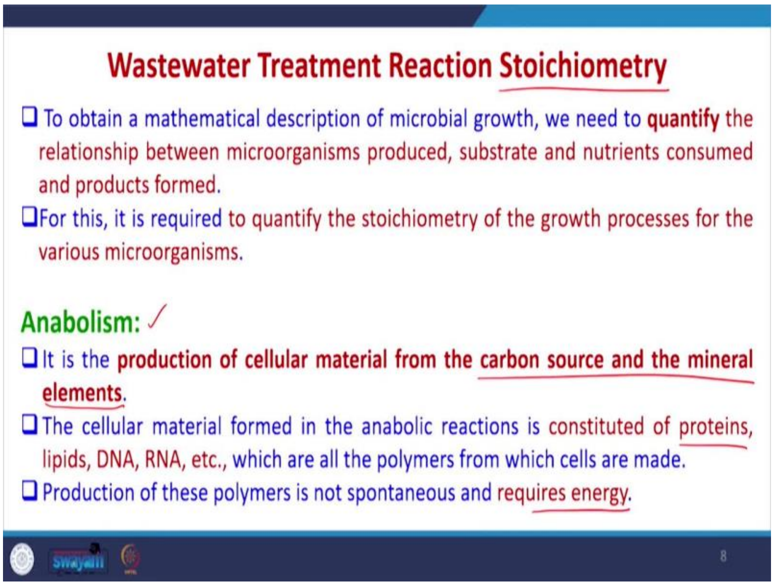

Now, we will go further and study in detail the wastewater treatment reaction stoichiometries. So, during wastewater treatment, a lot of reactions occur depending upon the environment depending upon the substrate microorganism, nutrient presence, etc. And there are various types of reactions which are possible that we will study in detail. So, we have to for better analysis design at sector we should find out the stoichiometry of all these reactions.

So, to obtain a mathematical description of microbial growth, we need to quantify the relationship between the amount of microorganisms and produce with respect to the substrate and then nutrients consumed and other projects found during the reaction so this is very, very important for understanding the microbial growth.

In this regard, it is required to quantify the stoichiometry of growth process for the various microorganisms under various conditions. So, during microbial growth, there are two types of basic reactions which occur one category is called anabolism and another is called catabolism. So, in the anabolism, it is the production of cellular material from the carbon source and the mineral elements.

So, what is anabolic reaction is the one in which the cellular materials are produced. So, then the microbial biomass the cellular materials get produced on the carbon source and the mineral elements and this reaction is called anabolism. The cellular material found in the anabolism reactions are constitute rate of proteins, lipids, DNA, RNA etc, which are all the polymers from which cells are made.

Now, production of these polymers is not a spontaneous and requires a sort of energy. So, this is not easy and this cannot be done without energy requirement. So, that means we require energy from some other reactions.

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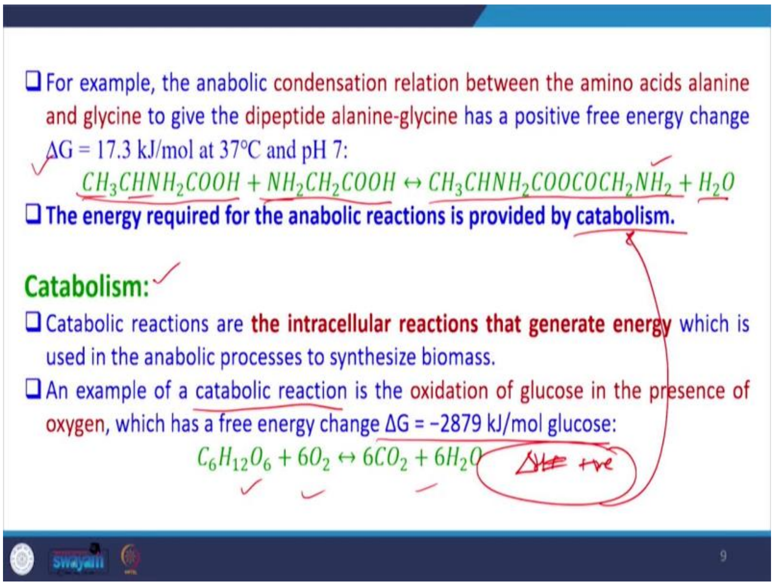

Anabolism:

$$CH_3CHNH_2COOH + NH_2CH_2COOH \leftrightarrow CH_3CHNH_2COOCOCH_2NH_2 + H_2O$$

Catabolism:

$$C_6H_{12}O_6 + 6O_2 \leftrightarrow 6CO_2 + 6H_2O$$

For example, the anabolic condensation relation between the amino acids alanine and glycine to give di peptide alanine-glycine has a positive free energy change delta G of this value at 37 degrees centigrade and pH 7 and this reaction can be represented here we can see the reactions happening between the glycine and amino acid alanine to give the di peptide alanine-glycine along with water, the energy required for this reaction will always be obtained from the catabolism reaction.

There are other types of reaction which are classified as catabolism in which the energy is obtained. Here what we are doing is that that we are utilizing the carbon source which contain lot of carbon along with some other elements maybe a nitrogen containing element to form the cell walls and other related materials. Now, during this reaction the energy is required.

Now, this energy will be obtained from catabolic reaction, which are the intracellular reactions that generate energy and during this energy process during this process, which are similar to like combustion reactions, the various types of carbon containing compounds in the presence of oxygen they react and give energy.

So, for example, a catabolic reaction is the oxidation of glucose in the presence of oxygen, which has a free energy change like this and for this reaction, the reaction can be represented here. And we can easily see that delta H will be highly positive for this reaction, and this energy will go from this particular reaction to this reaction where the energy will be utilized in the anabolism reactions.

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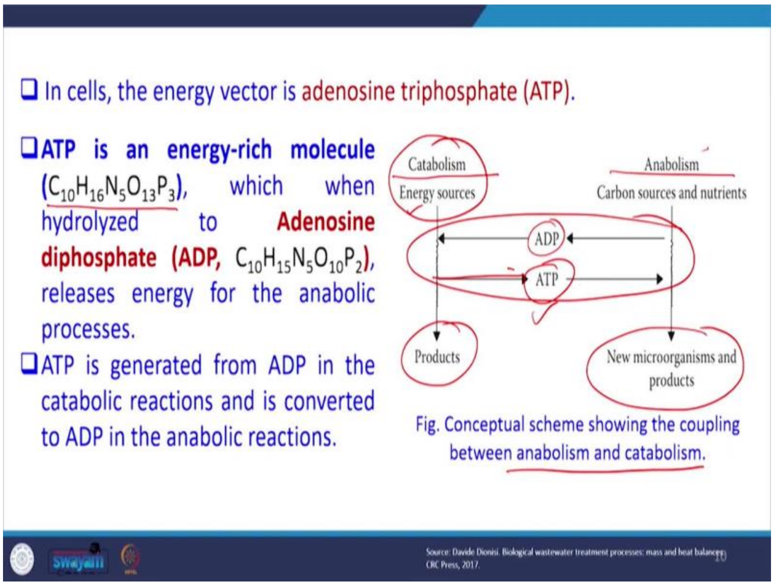

Now, in cells inside the cells, the energy vector is most important energy vector is ATP, ATP which is adenosine triphosphate. Now, this adenosine triphosphate gets found during the catabolism energy reaction so, we have a lot of energy sources the catabolism reaction happens we have some products which get formed.

But this ATP also get formed this ATP gets transferred into in the anabolic reaction where this ATP which is actually the energy source and this is utilized and we get new microorganisms and product and during this reaction ADP get found which is transferred back to the catabolic reaction.

Now, in cells thus the energy vector is this ATP and ATP is an energy rich molecule which can be represented by this particular formula $C_{10}H_{16}N_5O_{13}P_3$, which is when hydrolyze gives ADP, ADP is adenosine diphosphate in place of triphosphate we have diphosphate okay and during this reaction the energy is released to the anabolic process which occurs. ATP is generated from ADP back in the catabolic reactions and is converted to ADP again in the anabolic reactions.

This whole movement takes place from ATP to ADP and ADP to ATP vice versa thus exchanging energy between the catabolism reaction and the anabolic reactions. So, this is the conceptual scheme showing the coupling between anabolism and catabolism and ATP and ADP are the key molecules which actually help in the coupling process.

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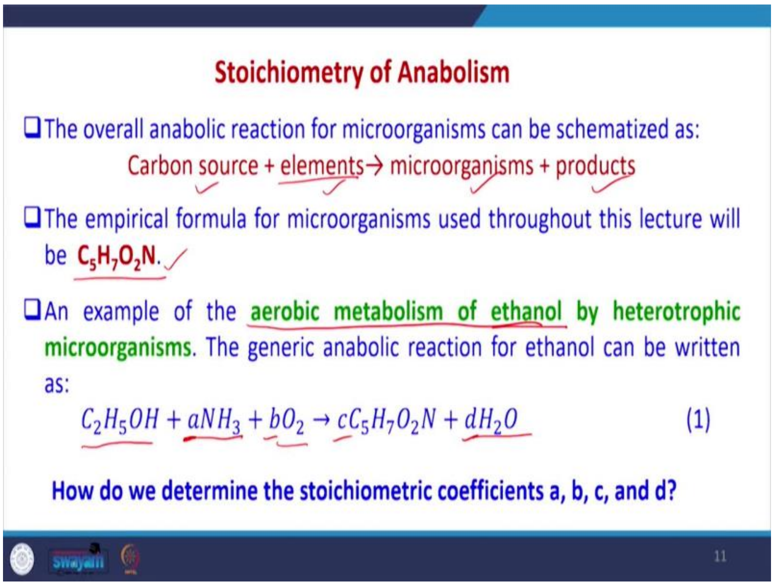

The generic anabolic reaction for ethanol can be written as:

$$C_2H_5OH + aNH_3 + bO_2 \rightarrow cC_5H_7O_2N + dH_2O$$

Now, what we will do is that we will study the anabolic reactions under various conditions in detail. Similarly, later on we will study the catabolic reactions under various conditions in detail. And later further, later on, we will combine both the reactions together to get a combined reaction mechanism under various conditions. So, we will study the anabolism first catabolism and then the combination reactions.

So, the overall anabolic reaction for microorganism can be schematized as some carbon source will be there, some other key elements will be there, we will be having microorganism which are getting formed and we have products which are getting released. Now, the empirical formula for microorganism that will be used all throughout will be $C_5H_7O_2N$ so, this is one of the generic formula which is used in the literature the same we are using here.

Now, what we will be doing is that we will be studying different types of metabolic reaction under aerobic condition, anaerobic condition using various types of substrate etc, or carbon source and under various conditions of fermentation, nitrification sector for and then we will study the anabolism stoichiometry.

Now an example with respect to aerobic metabolism of ethanol so, we are we are trying to understand starting with aerobic metabolism out by heterotrophic microorganism and we are taking an example of ethanol first. So, for this particular reaction that generic anabolic reaction can be written as that we have ethanol C_2H_5OH in element the key element which is generally required is nitrogen.

So, for nitrogen we are taking ammonia and oxygen is also required, so, that we can get formed this particular microorganism and we have water which gets released. So, we have this reaction which is written here. Now, in this reaction, if you see, the stoichiometric coefficients are written as A, B, C and D, and these values are unknown. So, how do we determine stoichiometric coefficients A, B, C and D.

So, for this I will suggest that you can go back a study that class 11th 12th or graduation level courses and where the it is described how to make balances with respect to is to stoichiometric coefficient equations. So, through balances we can find out the value of A, B, C, and D. So, we will be not going in very basic but somewhere wherever some complications are there we will be discussing otherwise we will be simply going with respect to the balance equation.

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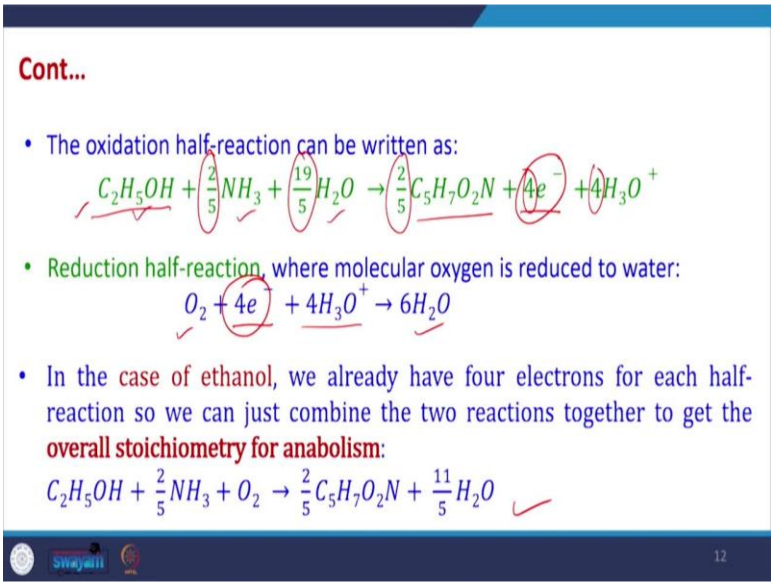

The oxidation half-reaction can be written as:

- $C_2H_5OH + \frac{2}{5}NH_3 + \frac{19}{5}H_2O \rightarrow \frac{2}{5}C_5H_7O_2N + 4e^- + 4H_3O^+$

Reduction half-reaction, where molecular oxygen is reduced to water:

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

The overall stoichiometry for anabolism:

- $C_2H_5OH + \frac{2}{5}NH_3 + O_2 \rightarrow \frac{2}{5}C_5H_7O_2N + \frac{11}{5}H_2O$

Now, the oxidation half reaction for this aerobic metabolism by heterotrophic microorganisms with respect to ethanol can be written here. So, what we do is that we are trying to find for half reaction, oxidation half reaction then reduction half reaction. So, in the oxidation half reaction the electrons are getting released, whereas, in the reduction of the reaction electrons are getting utilized.

So, first we write with respect to ethanol, ammonia and water that what are the what is how much amount of electron gets released and how much amount of microorganisms getting form. So, if you write this equation, we can balance this and the coefficients are written here 2 by 5, 19 by 5, 2 by 5 and 4 and this we do by keeping the value of stoichiometric coefficient of ethanol as 1.

So, this is one of the key compound we are understanding. So, we are keeping the stoichiometric coefficient 1 here. Now, similarly, the reduction half reaction, where molecule and oxygen is reduced to water is given here. So, oxygen is reduced to water via this particular reaction and the reaction is written here. Now, for the case of ethanol, we already have 4 electrons for each half reaction.

So, we need not multiply together if the values 4 electron and 4 electron would have been different then we would have multiplied by either of the half reaction, so, as to balance the electrons on both sides, but this is not required for ethanol. So, overall stoichiometry by just adding together both reactions can be written here. So, this is the stoichiometry of the ethanol with respect to aerobic metabolism, by heterotrophic microorganism.

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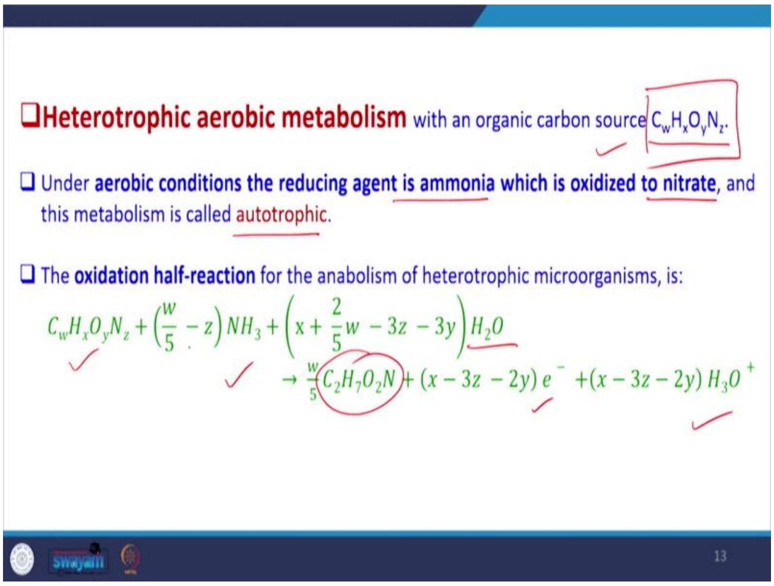

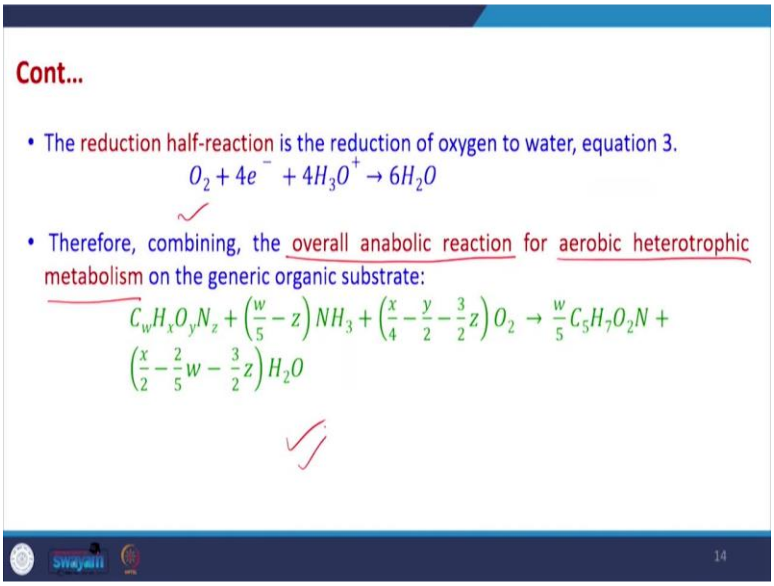

The oxidation half-reaction for the anabolism of heterotrophic microorganisms, is:

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

The reduction half-reaction is the reduction of oxygen to water:

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

The overall anabolic reaction for aerobic heterotrophic metabolism on the generic organic substrate:

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

Similarly, heterotrophic aerobic metabolic with an organic carbon source general carbon source which is $C_wH_xO_yN_z$ remember this formula we will be using a lot all throughout our this section. So, under aerobic condition the reducing agent is generally ammonia and which is oxidized to nitrate and this metabolism is called autotrophic.

So, this is there so, we have this particular carbon source, this is ammonia we have water and we are forming the microorganisms and we are releasing some amount of electron and some amount of H_3O plus. And using some balance we can always write because the $C_wH_xO_yN_z$ values are given we can easily balance this relationship or using some simple balance conditions which is taught earlier.

Similarly, the oxidation half reaction for the reduction of oxygen to water can be repeated in by this already we studied. Therefore, combining together overall anabolic reaction for aerobic heterotopic metabolism can be written like this, for ethanol case it was generally written earlier. For the general carbon source which is returned by this the reaction can be written here.

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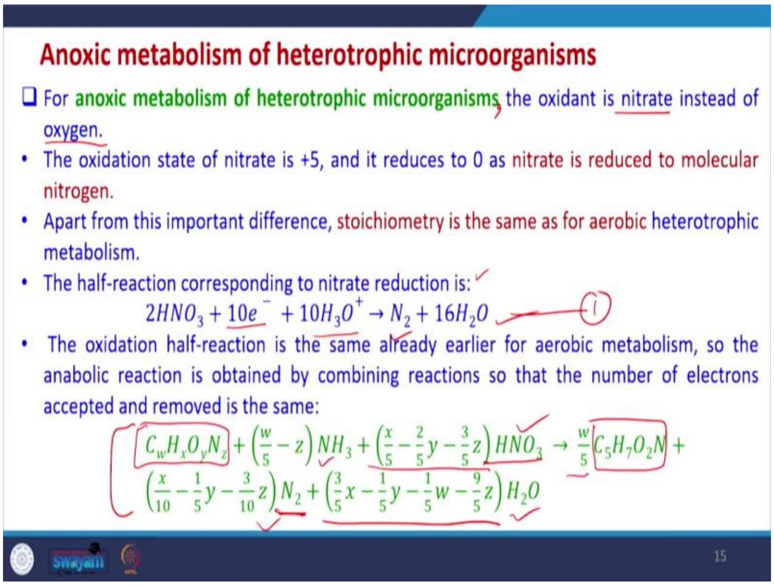

The half-reaction corresponding to nitrate reduction is:

- $2HNO_3 + 10e^- + 10H_3O^+ \rightarrow N_2 + 16H_2O$

The anabolic reaction:

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

Now, going further we can similarly find out the anoxic metabolism. Till now, we have studied the aerobic metabolism. Now, we will try to find out the anoxic metabolism of heterotrophic microorganisms, so, for anoxic metabolism of heterotrophic microorganisms, the oxygen which is used is nitrate instead of oxygen. So, remember we do not have oxygen, which is available, when the reaction in place of oxygen, we will be using HNO_3.

So, this is one difference which is there. Now, in the oxidation state of nitrate is plus 5 and it reduces to 0 as nitrate is being reduced to molecular nitrogen. So, we can see in this reaction overall reaction will be nitrogen which will be getting formed. So, in the anabolic reaction, which in place of oxygen we have a HNO_3 and HNO_3 is getting reduced to N_2.

Now, apart from this important difference stoichiometry is same for aerobic heterotrophic metabolism. The half reaction corresponding to nitrate reduction is same. For reduction reaction the reaction is different because we do not have oxygen so in place of oxygen which, we have a HNO_3. Now a HNO_3 combines with together with the electrons and H_3O plus to form nitrogen via this particular reaction.

Now, this particular reaction plus the oxidation reaction which was given earlier, they are combined together to form this particular reaction which is given here and we can see here the oxidation half reaction is same as earlier for aerobic metabolism. So, the anabolic reaction is obtained by combining reactions, so, that the number of electrons accepted and removed is the same.

And thus we have the substrate which is there which is the our general carbon source it reacts with ammonia with nitrate to form the microorganism which is given generally by this formula plus nitrogen and hydrogen. The key thing is how to find out this stoichiometric coefficient and that can be found out by general balance equations. So, we have got to know 2 types of aerobic metabolism, as well as anoxic metabolic for anabolic reactions.

Now, we will further study in detail other types of reactions in whole process, and we will continue further in the next class. Today we have we studied only two basic difference we have found out there are anabolic reactions, there are catabolic reactions, the anabolic reactions, the micro, the cell wall and other things are formed for which we require carbonaceous source and other elements.

But however, for this reaction to occur, we require energy and that energy is obtained from catabolic reactions. So, catabolic reactions actually generate ATP, ATP gets transferred to the anabolic reaction. And then anabolic reactions convert the ATP into ADP and use the energy for their reaction the ADP further gets transferred to the catabolic reactions where it again generates ATP and which again goes back to the anabolic reaction.

So, further on we studied few types of anabolic reactions, and their stoichiometry. There are many other different types of reactions that occur in the wastewater treatment inside the reactor. We will study the anabolic reactions of such anabolic stoichiometry of such reactions in the next class. And further we study the catabolic reactions also for all such type of reactions. So we will continue in the next class. Thank you very much.