

Biological Process Design for Wastewater Treatment
Professor. Vimal Chandra Srivastava
Department of Chemical Engineering
Indian Institute of Technology, Roorkee
Lecture – 11
Stoichiometry of Microbial Growth - III


Welcome everyone in this NPTEL online certification course on biological process design for wastewater treatment. So, in the previous last two lectures, we studied regarding the overall stoichiometry of the two types of reactions that happen in the biological systems; and these reactions were anabolic and catabolic reactions that we have studied in detail in the previous two lectures. And we found that there are many reactions which happen under different conditions with different types of microorganisms, for both anabolic and catabolic reactions. So, is it possible to combine them together and write an overall growth stoichiometry for the overall balance of the reactions?

So, is it possible to combine them together? So, that is the what we are going to learn; and how we are going to combine them together. Generally, it is not possible to write an overall stoichiometry for microbial growth, purely based upon the elemental balances, and or oxidation/reduction reaction that have we have been writing for anabolic and catabolic reactions. The reason behind is that, that the much of the energy which is generated in the catabolic reaction that actually we studied earlier.

(Refer Slide Time: 01:56)

Overall Growth Stoichiometry

- ❑ **Ques:** Can we write a stoichiometry for the overall process of microbial growth? In other words, can we combine the anabolic and catabolic reactions to obtain the overall growth stoichiometry?
- ❑ **Ans:** It is **not possible** to write an overall stoichiometry for microbial growth purely based on elemental balances and/or oxidation-reduction reactions.
- ❑ **Reason:** In general, it is not known how much of the energy generated in the catabolic reactions is actually transferred to the anabolic reactions.
 - 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 the catabolic reactions.
 - Even for the same substrate and products, and for the same growth conditions, the efficiency of oxidative phosphorylation varies greatly among different microorganisms.

 2

It is not possible to determine that how much of that energy has been actually been transferred to the system. And energy transferred from the (ana) catabolism to anabolism via ATP and ADP is difficult to predict on the paper; because the amount of ATP that can be generated per unit energy generated by the catabolic reactions is difficult to predict. So, in general we cannot predict beforehand, or we cannot write that how much of energy which is generated by a catabolism reactions, have been actually been used by the anabolism reaction. So, it is very difficult to predict.

Also even for the same substrate and products, and for the same growth conditions, the efficiency of oxidative phosphorylation reactions varies greatly among different microorganism; and thus it is not possible in general to write an overall growth stoichiometry. But, how to go ahead further? There is some conditions that mathematical clarifications will be given in this slide.

(Refer Slide Time: 03:06)

Cont...

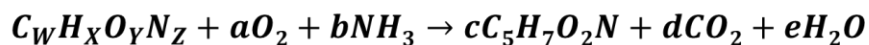
□ **Mathematical clarification**

- ❖ Consider overall growth stoichiometry for aerobic heterotrophic microorganisms growing on a substrate $C_xH_yO_zN_z$.
- ❖ The overall growth reaction will be a combination of the anabolic and catabolic reactions, therefore, the reactants will be the substrate, oxygen, and ammonia, while the products will be biomass, carbon dioxide, and water.
- ❖ The overall growth stoichiometry therefore will be something like:

$$C_wH_xO_yN_z + aO_2 + bNH_3 \rightarrow cC_5H_7O_2N + dCO_2 + eH_2O$$

The above equation cannot be solved without any additional information since there are four equations (the elemental balances for C, N, O and H) and five unknown coefficients.

The overall growth stoichiometry therefore will be something like:



We can see here that consider an overall growth stoichiometry of anaerobic heterotrophic microorganisms, growing on a substrate of the general formula $C_xH_yO_zN_z$. So, CWXY, actually this is $C_wH_xO_yN_z$. And the overall growth reaction will be a combination of both anabolic and

catabolic reactions. So, therefore the reactions will be substrate, will be based upon the substrate oxygen, ammonia; while the products will be biomass, carbon dioxide, and water. So, this will be the overall growth reaction under aerobic, heterotrophic microorganism, when they are working.

Now, the reaction can be written like this; and here we can see that some the stoichiometric coefficient a, b, c, d and e are written with respect to oxygen, ammonia, biomass, CO₂ and H₂O. The above equation cannot be solved without any additional information. Since, there are four equations we can write; because, the number of elements which are present in these reactions are carbon, nitrogen, oxygen and hydrogen. So, thus we can write four equations for four elements. Now, the unknowns are 5 a, b, c, d and e; so, there are 5 unknowns. So, mathematically also it is not possible to determine the value of a b c, d and e. So, what is the go ahead? What is the next step? How we can combine together and how we can find out the value of a b c, d and e?

(Refer Slide Time: 04:54)

Cont...

- ❖ The additional equation needed to be able to write the stoichiometry of microbial growth will be obtained from experimental data and is represented by growth yield.
- ❖ The growth yield is defined as **the amount of biomass formed per unit amount of substrate consumed**, that is:

$$Y_{X/S} = \left(\frac{\text{kg biomass}}{\text{kg substrate}} \right) = \frac{\text{biomass produced due to growth}}{\text{substrate remove due to growth}}$$
- ❖ The growth yield coefficient is usually obtained from experimental data.
- ❖ If the growth yield is known, this constitutes an additional equation relating the coefficients a, b, c, d, e of the growth, and the overall stoichiometry can be calculated.

The growth yield is defined as:

$$Y_{X/S} = \left(\frac{\text{kg biomass}}{\text{kg substrate}} \right) = \frac{\text{biomass produced due to growth}}{\text{substrate remove due to growth}}$$

So, the additional equations are required to be able to write the stoichiometry of microbial growth, and that is obtained from the experimental data, and is generally represented by growth yield. So, what we do is that we try to find out another parameter which is called as growth yield

and which is experimentally found out; and from this growth yield the parameters a b c, d and e, the stoichiometric coefficient are found out. The growth yield is defined as the amount of biomass formed per unit amount of substrate consumed. So, it is represented like this, where X represents the amount of biomass formed and S is the substrate.

So, kg of biomass formed per kg of substrate, we can write biomass produced due to the growth and substrate removed due to the growth. So, this is there. The growth yield coefficient is usually obtained from the experimental data. If the growth yield is known, so, if we can find out the growth yield, this constitutes an additional equation; and thus, we have now four elemental equation, one growth yield, so, we have 5 equations; and thus, we can find out the values of coefficient a b c, d in terms of the growth yield and the other parameters. So, this is how we can relate. So, for example, the example is given here.

(Refer Slide Time: 06:23)

Cont...

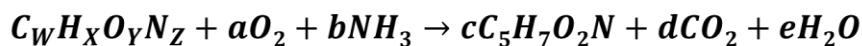
❖ Example: for **Aerobic metabolism** of the $C_wH_xO_yN_z$ substrate shown by equation below,

$$C_wH_xO_yN_z + aO_2 + bNH_3 \rightarrow cC_5H_7O_2N + dCO_2 + eH_2O$$

Knowledge of the growth yield $Y_{X/S}$ will give the following systems of five equations in five unknowns:

- $\frac{c.MW_{biomass}}{MW_{substrate}} = Y_{X/S}$ ✓
- $5c + d = w$ ✓ C balance ✓
- $2c + 2d + e = 2a + y$ ✓ O balance ✓
- $7c + 2e = 3b + x$ ✓ H balance ✓
- $5c + d = w$ ✓ N balance ✓

For Aerobic metabolism:



- $\frac{c.MW_{biomass}}{MW_{substrate}} = Y_{X/S}$
- $5c + d = w$ C balance
- $2c + 2d + e = 2a + y$ O balance
- $7c + 2e = 3b + x$ H balance
- $5c + d = w$ N balance

For aerobic metabolism of the reaction which was given earlier with respect to $C_wH_xO_yN_z$ as substrate is shown here; and we can see that the a, b, c, d values are there. Now, we have four equations we can write based upon the growth yield. So, growth yield is written here; and here the growth yield is defined as the amount of biomass formed, so amount of biomass formed is C into molecular weight of biomass, and divided by the molecular weight of substrate; because C is the amount of biomass which has been formed per unit amount of substrate. And here the since the coefficient is 1, so it is not written here. So, this is C into molecular weight of biomass, divided by molecular weight of substrate; so, this is the growth yield.

Now, there are four balances we can write here; so, we can write carbon, oxygen, hydrogen and nitrogen balance. For carbon balance, we can see here that we have 5c, which is here plus d; and the carbon is going here in CO_2 , and which is equal to w; which is because all the carbon is within the substrate, it is only the w amount is there. So, we have this carbon balance. Similarly, we can write for oxygen, hydrogen and nitrogen the way it is written here; and we can easily write this equation. Now, within this equation, there is the one thing that this all the values are known. Now, what we do? Going further; what we do is that, we solve by substitution.

(Refer Slide Time: 08:07)

Cont...

- Solving by substitution, we obtain the overall growth stoichiometry:

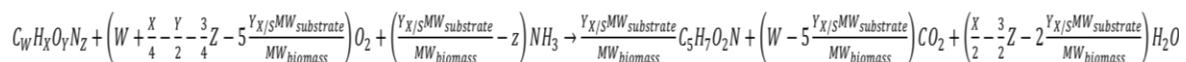
$$C_wH_xO_yN_z + \left(W + \frac{x}{4} - \frac{y}{2} - \frac{3}{4}Z - 5 \frac{Y_{X/S}MW_{substrate}}{MW_{biomass}} \right) O_2 + \left(\frac{Y_{X/S}MW_{substrate}}{MW_{biomass}} - z \right) NH_3 \rightarrow \frac{Y_{X/S}MW_{substrate}}{MW_{biomass}} C_5H_7O_2N + \left(W - 5 \frac{Y_{X/S}MW_{substrate}}{MW_{biomass}} \right) CO_2 + \left(\frac{x}{2} - \frac{3}{2}Z - 2 \frac{Y_{X/S}MW_{substrate}}{MW_{biomass}} \right) H_2O$$

- It is evident, therefore, that **once the growth yield $Y_{X/S}$ is known, the stoichiometry of microbial growth is fully defined** and can be calculated with the elemental balances described above.
- Example:** Substrate is glucose and growth yield is 0.3 kg biomass/kg glucose (reasonable value for aerobic growth on many organic substrates), above equation corresponds to the following overall growth stoichiometry:

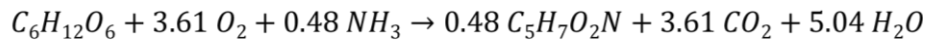
$$C_6H_{12}O_6 + 3.61 O_2 + 0.48 NH_3 \rightarrow 0.48 C_5H_7O_2N + 3.61 CO_2 + 5.04 H_2O$$

(Handwritten notes: $W=6, x=12, y=6, z=0, Y_{X/S}=0.3$)

The overall growth stoichiometry:



Example: Substrate is glucose, the following overall growth stoichiometry:



Cont...

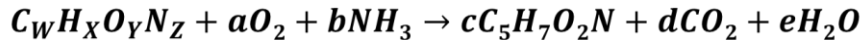
❖ Example: for **Aerobic metabolism** of the $C_wH_xO_yN_z$ substrate shown by equation below,

$$C_wH_xO_yN_z + aO_2 + bNH_3 \rightarrow cC_5H_7O_2N + dCO_2 + eH_2O$$

Knowledge of the growth yield $Y_{X/S}$ will give the following systems of five equations in five unknowns:

- $\frac{c \cdot MW_{biomass}}{MW_{substrate}} = Y_{X/S}$ $\Rightarrow c = \frac{Y_{X/S} \cdot MW_{sub}}{MW_{bi}}$
- $5c + d = w$ C balance ✓
- $2c + 2d + e = 2a + y$ O balance ✓
- $7c + 2e = 3b + x$ H balance ✓
- $5c + d = w$ N balance ✓

For Aerobic metabolism:



- $\frac{c \cdot MW_{biomass}}{MW_{substrate}} = Y_{X/S}$
- $5c + d = w$ C balance
- $2c + 2d + e = 2a + y$ O balance
- $7c + 2e = 3b + x$ H balance
- $5c + d = w$ N balance

And for substitution what we do is that, we write c in terms of $Y_{X/S}$. So, the c value is written in terms of $Y_{X/S}$ into molecular weight of substrate, divided by molecular weight of biomass. So, in this equation, we can easily write for c; and this c is further used. c is equal to $Y_{X/S}$ into molecular weight of substrate, divided by molecular weight of biomass. So, the c value is known to us and using all other parametric values, then can be found out using this. And the values of a, b, c can be found out in terms of W, X, Y, Z and the $Y_{X/S}$; so, this is what is written. We can find out the, all the a value is written in terms of W, X, Y, Z and the $Y_{X/S}$ which is the growth yield. So, we can substitute all bases and we can write this equation.

So, it is evident from this equation that once the growth yield $Y_{X/S}$ is known to us, the stoichiometry of microbial growth is fully defined and can be calculated with elemental balances I have described in the previous slide. So, for the another case if we take substrate is suppose glucose. So, in place of a generic substrate, if we write for glucose, which is $C_6H_{12}O_6$; so, if we write the balance equation for this. And if suppose the growth yield which is experimentally determined, it is point 3 kg biomass per kg glucose, and it is a reasonable value for aerobic growth on many organic substrates.

So, this value has been carried, the yield has been taken as point 3. And if we take the value of this point 3 and glucose, thus we know the value of W, X and Y. So, in this case the W value is 6, the X value is 12, the Y value is 6, and the Z value is 0; and the $Y_{X/S}$ value has been taken as point 3. So, if we take all these values together and substitute in this particular equation, we can easily write this particular equation.

So, thus, if the growth yield is known, the substrate formula is known, we can easily write the balance equation. So, overall we can combine together we can write the overall growth stoichiometric equations, if the growth yield is known beforehand. So, this is the keyword.

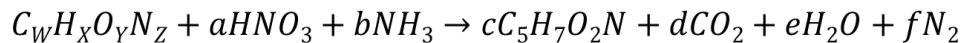
(Refer Slide Time: 10:56)

Cont...

- ❖ For **heterotrophs growing on the organic substrate using nitrate as the electron acceptor**, the overall growth reaction will have the form:

$$C_W H_X O_Y N_Z + a HNO_3 + b NH_3 \rightarrow c C_5 H_7 O_2 N + d CO_2 + e H_2 O + f N_2$$
- The overall growth stoichiometry, in this case, can still be obtained by introducing the growth yield $Y_{X/S}$, defined in the same way as for aerobic metabolism, and using the elemental balances for C, H, N, and O.
- However, this will give us five equations, but **there are six unknown coefficients here, due to the presence of molecular nitrogen.**
- ✓ A sixth equation can easily be obtained by noticing that **molecular nitrogen only comes from the reduction of nitrate, and that all the nitrate that reacts is converted to nitrogen.** Therefore, we have the following additional equation: $f = \frac{a}{2}$
- By solving the system of equations obtained stoichiometry for the overall growth of heterotrophic microorganisms on a carbon source using nitrate as electron acceptor is shown in Table (later).

The overall growth reaction will have the form:



Now, for heterotrophs growing on organic substrate using nitrate as an (elect) electron acceptor. So, in this case already we have studied regarding the catabolic reaction and anabolic reaction. So, we can write for this the reaction will be written here; it will be HNO_3 and ammonia. In place of oxygen, we are using HNO_3 and overall we can write. So, here we have in this case, we again introduced we have 6 coefficient a, b, c, d, e and f. So, now we have 6 unknown coefficients due to presence of molecular nitrogen which is coming into picture; because in the case of heterotrophs growing on organic substrates, which use nitrate as an electron acceptor, nitrogen gets formed.

Now, the sixth equation can be easily obtained by noticing some peculiarity in this equation that the molecular nitrogen comes from the reduction of nitrate and that all the nitrate that reacts is converted into nitrogen; so, this is the peculiarity. And from this what we can write is that, the f value is always equal to a by 2. So, this f is actually we can always write in place of f here a by 2.

So, in actual condition we have only five unknowns and four elemental balances we can write. So, thus using the growth coefficient, again the growth yield, we can write the overall balance. So, overall growth of the heterotrophic microorganisms on a carbon source using nitrate as an electron acceptor can be written as shown in the later table.

(Refer Slide Time: 12:51)

Nitrifying microorganisms

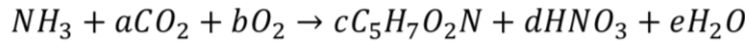
- ❖ For nitrifying microorganisms, combining the anabolic and catabolic reactions the general growth equation will have the form:

$$\underline{NH_3} + \underline{aCO_2} + \underline{bO_2} \rightarrow \underline{cC_5H_7O_2N} + \underline{dHNO_3} + \underline{eH_2O}$$
- For nitrifiers, the growth yield is usually expressed as biomass produced per unit mass of nitrate (as nitrogen) produced, that is:

$$Y_{XA/NO_3} \left(\frac{\text{kg biomass}}{\text{kg } NNO_3} \right) = \left(\frac{\text{Biomass produced due to growth}}{\text{Nitrate (as nitrogen) produced due to growth}} \right)$$
- Equation can be written as, introducing the molecular weights of biomass and nitrogen and with reference to reaction:

$$Y_{XA/NO_3} = \frac{c}{d} \cdot 8.07$$
- Combining Equation with the elemental balances of C, H, O and N, referred to reaction Equation, we obtain the overall growth stoichiometry for nitrifying microorganisms as shown in Table.

The general growth equation will have the form:



For nitrifiers the growth yield is usually expressed as:

$$Y_{\frac{XA}{NO_3}} \left(\frac{\text{kg biomass}}{\text{kg } NNO_3} \right) = \left(\frac{\text{Biomass produced due to growth}}{\text{Nitrate (as nitrogen) produced due to growth}} \right)$$

Now, there is another condition that we have nitrifying microorganism. For nitrifying microorganisms, combining the anabolic and catabolic reactions, the general growth equation will have the form of this. So, again we have five unknowns a, b, c, d and e. In this case, we can see that a CO₂ is being used along with the oxygen for nitrifying microorganism; and ammonia is the base substrate for nitrifies the growth yield is usually expressed as biomass produced per unit mass of nitrate as nitrogen produced.




So, there is some difference in the growth yield which is given here; so kg biomass produced per kg nitrate which has been produced, so, both in terms of production. And this equation can be written as introducing the molecular weights of biomass, and nitrogen, and with reference to the reaction. We can easily find that the value of this Y_{XA/NO₃}; it will be equal to c by d into 8.07. So, we can after solving, we can always get this particular equation. So, combining equation with the elemental balance of C, H, O and N and referred to reaction equation, this particular reaction equation; the overall growth can be written and it will be shown later on in the table.

(Refer Slide Time: 14:15)

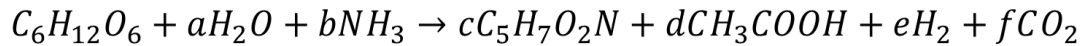
Growth under anaerobic conditions

- ❖ The same approach can be used to derive the stoichiometry of microbial growth under anaerobic conditions. For fermentative bacteria, the growth yield is always expressed exactly in the same way as for heterotrophic microorganisms.
- Due to the wide range of substrates and products that can be formed under fermentation conditions it is more practicable to refer, as an example, to a particular fermentation reaction.
- For example, with reference to the conversion of glucose to acetic acid and hydrogen, the general stoichiometry is:

$$C_6H_{12}O_6 + aH_2O + bNH_3 \rightarrow cC_5H_7O_2N + dCH_3COOH + eH_2 + fCO_2$$
- The six coefficients a, b, c, d, e, f in reaction can be calculated from the growth yield, four elemental balances and from the additional equation $d = f$, which comes from the fact that, for glucose fermentation to acetic acid, carbon dioxide is only produced in the catabolic reactions, which produce one mol of CO₂ per mol of acetic acid.




9

With reference to the conversion of glucose to acetic acid and hydrogen, the general stoichiometry is:



Similarly, for growth under anaerobic condition, so, here the same approach can be used to derive the stoichiometry of microbial growth under anaerobic condition. So, for fermenting bacteria, the growth yield is always expressed exactly in the same way as for heterotrophic microorganisms; so this is similar to that. Due to which the wide range of substrates and products that can be formed under fermentative condition, it is more practical to refer to particular fermentation reactions. With reference to like conversion of glucose to acetic acid and hydrogen.

So, if we case, for example, this particular equation. So, the equation can be written here, the glucose is there, and we can see that it is combining with water and ammonia to form the biomass, plus acetic acid. So, we are referring only where the conversion of glucose to acetic acid, biomass and hydrogen is taking place along with the carbon dioxide. So, we have a b c, d, e, and f.

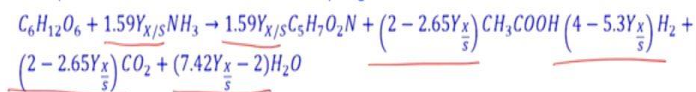
So, we have 6 unknowns and 6 coefficient that have to be found out. Again, we can write only four elemental balance. But, additional equation is that, that the value of d will always be equal to the value of f, which comes from the fact that for glucose fermentation to acetic acid, carbon dioxide is the only produced in the catabolic reactions, which produces one mole of CO₂ per mole of acetic acid.

So, and because of this known fact, the value of d and f are equal. So, we can write here in place of f, the value of d. So, thus we have only 5 unknowns and 4 elemental equations; and again we can use the growth yield definition to solve the problem.

(Refer Slide Time: 16:09)

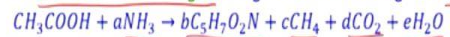
Cont...

- The equations can be solved in the usual way to give:

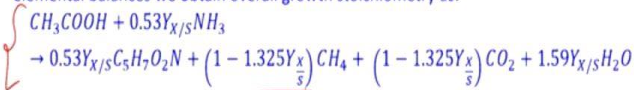


This represents overall growth stoichiometry for the fermentative microorganisms which convert glucose to acetic acid and hydrogen.

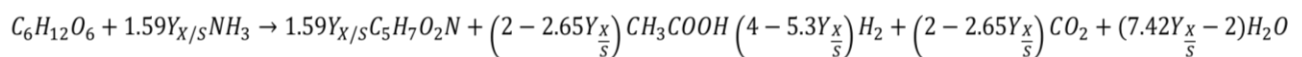
- For acetoclastic methanogens the general form of the growth stoichiometry is:



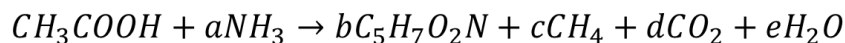
- The growth yield is defined in the usual way as kg biomass/kg acetic acid, and, by solving the elemental balances we obtain overall growth stoichiometry as:



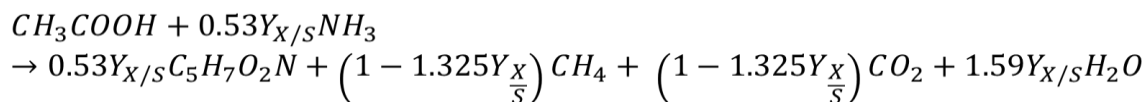
The equations can be solved in the usual way to give:



For acetoclastic methanogens the general form of the growth stoichiometry is:



We obtain overall growth stoichiometry as:



So, the equation can be solved usual to give this particular equation, and which is written here in terms of growth yield, this is written. We can solve, we already have found out; and all the value can be found out if the value of $Y_{X/S}$ is known to us. So, this represents the overall growth chemistry for the fermentative microorganism which convert glucose to acetic acid and hydrogen. So, we are referring to a particular equation only. Again, for acetolastic methanogens, the general form of the growth stoichiometry is here. So, here we are referring to a case where the acetic acid is getting converted.

So, acetic acid is combining with ammonia to form the microorganism, plus methane, CO_2 and H_2O . Now, the growth yield is again defined in the usual way in terms of kg biomass produced per kg of acetic acid. So, this is the usual definition, and combining this we can write the overall equation in the form of this; and we can always find out the exact value of the coefficient once

the growth yield is experimentally determined. So, this is the how we go ahead in solving different types of stoichiometric overall growth chemistry equation.

(Refer Slide Time: 17:33)

Cont...

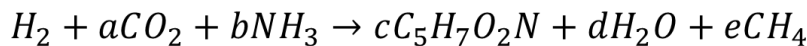
- ❖ For **hydrogenotrophic methanogens**, the overall growth stoichiometry has the form:

$$H_2 + aCO_2 + bNH_3 \rightarrow cC_5H_7O_2N + dH_2O + eCH_4$$
- The growth yield can be defined as kg biomass/kg hydrogen:

$$Y_{X/S} \left(\frac{\text{kg biomass}}{\text{kg hydrogen}} \right) = 56.5c$$
- From equation, and from the elemental balances the following overall growth stoichiometry can be obtained:

$$H_2 + \left(0.25 + 0.044Y_{X/S}\right)CO_2 + 0.0177Y_{X/S}NH_3 \rightarrow 0.0177Y_{X/S}C_5H_7O_2N + \left(0.5 + 0.053Y_{X/S}\right)H_2O + \left(0.25 - 0.044Y_{X/S}\right)CH_4$$

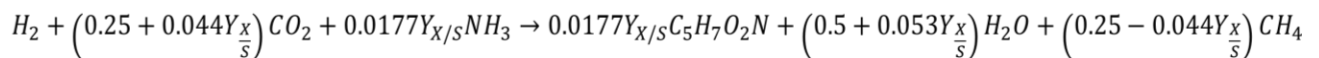
For hydrogenotrophic methanogens, the overall growth stoichiometry has the form:



The growth yield can be defined as kg biomass/kg hydrogen:

$$Y_{X/S} \left(\frac{\text{kg biomass}}{\text{kg hydrogen}} \right) = 56.5c$$

The following overall growth stoichiometry can be obtained:



Now, for hydrogenotrophic methanogens, in which the hydrogen combines with carbon dioxide and ammonia to form the biomass, plus H₂O and methane. So, for this, the growth yield is defined as kg of biomass produced per kg of hydrogen. And if we actually find out the value will be, if we just write the molecular weights of biomass and hydrogen; so, it will be 56.5c. So, this is the Y_{X/S} by definition.

So, we can see here the c value will be there, the molecular weight of biomass will be used, and molecular weight of hydrogen will be used. And using this particular equation and the elemental

balances, we can write the actual value of stoichiometric coefficient for each of the cases; and which can be solved once the value of yield coefficient is determined. So, this is how we can write for different equation.

(Refer Slide Time: 18:33)

Table. Summary of the Microbial Growth Reactions as a Function of the Growth Yield for Various Types of Microorganisms

Microorganisms	Growth Yield	Growth Stoichiometry
Heterotrophs (aerobic) ✓	$Y_{X/S}$ $\left(\frac{\text{kg biomass}}{\text{kg substrate}} \right)$	$C_w H_x O_z N_1 + \left(w + \frac{x}{4} - \frac{y}{2} - \frac{3}{4} z - 5 \frac{Y_{X/S} MW_{\text{substrate}}}{MW_{\text{biomass}}} \right) O_2 + \left(\frac{Y_{X/S} MW_{\text{substrate}}}{MW_{\text{biomass}}} - z \right) NH_3 \rightarrow$ $\frac{Y_{X/S} MW_{\text{substrate}}}{MW_{\text{biomass}}} C_1 H_1 O_1 N + \left(w - 5 \frac{Y_{X/S} MW_{\text{substrate}}}{MW_{\text{biomass}}} \right) CO_2 + \left(\frac{x}{2} - \frac{3}{2} z - 2 \frac{Y_{X/S} MW_{\text{substrate}}}{MW_{\text{biomass}}} \right) H_2 O$
Heterotrophs (anoxic) ✓	$Y_{X/S}$ $\left(\frac{\text{kg biomass}}{\text{kg substrate}} \right)$	$C_w H_x O_z N_1 + \left(\frac{4}{5} w + \frac{1}{5} x - \frac{3}{5} z - \frac{2}{5} y - 4 \frac{Y_{X/S} MW_{\text{subs}}}{MW_{\text{biomass}}} \right) HNO_3 + \left(\frac{Y_{X/S} MW_{\text{subs}}}{MW_{\text{biomass}}} - z \right) NH_3 \rightarrow$ $\frac{Y_{X/S} MW_{\text{subs}}}{MW_{\text{biomass}}} C_1 H_1 O_1 N + \left(w - 5 \frac{Y_{X/S} MW_{\text{subs}}}{MW_{\text{biomass}}} \right) CO_2 + \left(\frac{2}{5} w + \frac{3}{5} x - \frac{9}{5} z - \frac{1}{5} y - 4 \frac{Y_{X/S} MW_{\text{subs}}}{MW_{\text{biomass}}} \right) H_2 O$ $+ \left(\frac{2}{5} w + \frac{1}{10} x - \frac{3}{10} z - \frac{1}{5} y - 2 \frac{Y_{X/S} MW_{\text{subs}}}{MW_{\text{biomass}}} \right) N_2$

Source: David Deziel, Biological wastewater treatment processes: mass and heat balances, CRC Press, 2017. 12

Cont...

Microorganisms	Growth Yield	Growth Stoichiometry
Nitrifiers ✓	$Y_{X/(N-NO_3)}$ $\left(\frac{\text{kg biomass}}{\text{kg N-NO}_3} \right)$	$NH_3 + 5 \frac{Y_{X/(N-NO_3)}}{Y_{X/(N-NO_3)} + 8.07} CO_2 + \left(0.75 + \frac{10.1 - 5.75 Y_{X/(N-NO_3)}}{Y_{X/(N-NO_3)} + 8.07} \right) O_2 \rightarrow$ $\frac{Y_{X/(N-NO_3)}}{Y_{X/(N-NO_3)} + 8.07} C_1 H_1 O_1 N + \frac{8.07}{Y_{X/(N-NO_3)} + 8.07} HNO_3 + \left(1.5 - \frac{4.035 + 3.5 Y_{X/(N-NO_3)}}{Y_{X/(N-NO_3)} + 8.07} \right) H_2 O$
Fermentative (glucose conversion to acetic acid) ✓	$Y_{X/S}$ $\left(\frac{\text{kg biomass}}{\text{kg glucose}} \right)$	$C_6 H_{12} O_6 + 1.59 Y_{X/S} NH_3 \rightarrow 1.59 Y_{X/S} C_2 H_3 O_2 N + (2 - 2.65 Y_{X/S}) CH_3 COOH$ $+ (4 - 5.3 Y_{X/S}) H_2 + (2 - 2.65 Y_{X/S}) CO_2 + (7.42 Y_{X/S} - 2) H_2 O$
Methanogens (acetoclastic) ✓	$Y_{X/S}$ $\left(\frac{\text{kg biomass}}{\text{kg acetic acid}} \right)$	$CH_3 COOH + 0.53 Y_{X/S} NH_3 \rightarrow 0.53 Y_{X/S} C_1 H_1 O_1 N + (1 - 1.325 Y_{X/S}) CH_4 + (1 - 1.325 Y_{X/S}) CO_2$ $+ 1.59 Y_{X/S} H_2 O$
Methanogens (hydrogenotrophic) ✓	$Y_{X/S}$ $\left(\frac{\text{kg biomass}}{\text{kg hydrogen}} \right)$	$H_2 + (0.25 + 0.044 Y_{X/S}) CO_2 + 0.0177 Y_{X/S} NH_3 \rightarrow$ $0.0177 Y_{X/S} C_1 H_1 O_1 N + (0.5 + 0.053 Y_{X/S}) H_2 O + (0.25 - 0.044 Y_{X/S}) CH_4$

Source: David Deziel, Biological wastewater treatment processes: mass and heat balances, CRC Press, 2017. 13

And the summary of the microbial growth reaction as the function of growth yield for various types of microorganisms can be written here. So, we have already seen for heterotrophs aerobic reaction, heterotrophs anoxic reaction; the growth yield is defined exactly in the same manner, and this is how the equations are written. So, we have previously seen this equation. In these two reactions that difference is with respect to oxygen and HNO₃ used. And then we studied

regarding the nitrifiers, where the growth yield was defined like this, kg of biomass produced per kg of nitrate nitrogen.

And then again using the yield coefficient, we can write this particular balance equation. Similarly, for glucose conversion to acetic acid, the methanogens where acetolastic conditions were there; and similarly for methanogens, where hydrogenotrophic conditions are there. For this, the growth yields are little bit defined differently; kg biomass per kg glucose for fermentative, kg biomass per kg acetic acid for acetolastic, kg biomass per kg hydrogen for methanogens. And so, there is a difference in the growth yield definition; but, using these growth yield definitions we can write the overall balance, which is (going) here.

So, thus, using overall the growth yield definitions, we can write the overall growth stoichiometry under various microorganisms operation. So, this is, this can be done. Now, there is another thing parameter or another important information that we can gather from these equations is the upper limit of growth yield.

(Refer Slide Time: 20:26)

Upper limit of growth yield

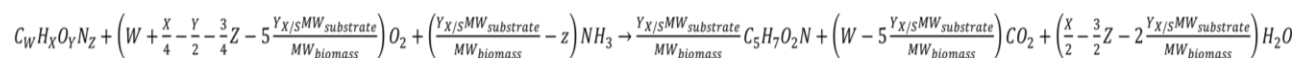
- From the overall growth stoichiometry, we can calculate some upper limits on the value of the growth yield $Y_{X/S}$.
- For metabolism to occur, both the anabolic and catabolic reactions need to take place, and the higher limit of $Y_{X/S}$ is the one for which no catabolism occurs.
- For example, the aerobic heterotrophic metabolism of a carbon source, which is represented by Equation.

$$C_W H_X O_Y N_Z + \left(W + \frac{X}{4} - \frac{Y}{2} - \frac{3}{4}Z - 5 \frac{Y_{X/S} MW_{substrate}}{MW_{biomass}} \right) O_2 + \left(\frac{Y_{X/S} MW_{substrate}}{MW_{biomass}} - z \right) N H_3$$

$$\rightarrow \frac{Y_{X/S} MW_{substrate}}{MW_{biomass}} C_5 H_7 O_2 N + \left(W - 5 \frac{Y_{X/S} MW_{substrate}}{MW_{biomass}} \right) CO_2 + \left(\frac{X}{2} - \frac{3}{2}Z - 2 \frac{Y_{X/S} MW_{substrate}}{MW_{biomass}} \right) H_2 O$$

14

The aerobic heterotrophic metabolism of a carbon source, which is represented by Equation:



So, can you find we have written so many equations? Now, can we find out the upper limit of growth yield using these equations? Yes, it is possible. So, from the overall growth

stoichiometry, we can calculate some upper limits on the value of growth yield. For like, for example, for metabolism to occur, and for both anabolic and catabolic reactions need to take place; under that condition the higher limit of $Y_{X/S}$ is the one in which no catabolic reactions have occurs. For example, for aerobic heterotrophic metabolism of a carbon source, the reaction can be written like this. So, already we have written earlier the equation.

(Refer Slide Time: 21:15)

- In order for **catabolism to occur**, the stoichiometric coefficient for oxygen needs to be higher than 0 (i.e. oxygen needs to be consumed), therefore it needs to be:

$$W + \frac{X}{4} - \frac{Y}{2} - \frac{3}{4}Z - 5 \frac{Y_{X/S} MW_{substrate}}{MW_{substrate}} > 0$$

- This means that:

$$Y_{X/S} < \left(W + \frac{X}{4} - \frac{Y}{2} - \frac{3}{4}Z \right) \frac{MW_{biomass}}{5 MW_{substrate}}$$

$W = 6$
 $X = 12$
 $Y = 6$
 $Z = 0$

- For example, if the substrate is **glucose (C₆H₁₂O₆)**, this condition means that it needs to be $Y_{X/S} < 0.75$ kg biomass/kg glucose.

In order for catabolism to occur,

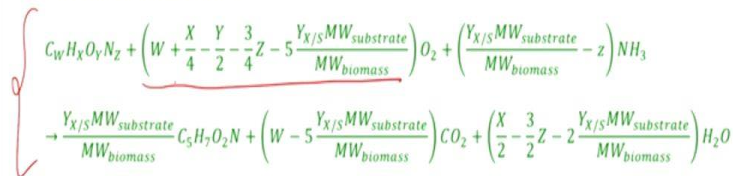
$$W + \frac{X}{4} - \frac{Y}{2} - \frac{3}{4}Z - 5 \frac{Y_{X/S} MW_{substrate}}{MW_{substrate}} > 0$$

This means that:

$$Y_{X/S} < \left(W + \frac{X}{4} - \frac{Y}{2} - \frac{3}{4}Z \right) \frac{MW_{biomass}}{5 MW_{substrate}}$$

Upper limit of growth yield

- From the overall growth stoichiometry, we can calculate some upper limits on the value of the growth yield $Y_{X/S}$.
- For metabolism to occur, both the anabolic and catabolic reactions need to take place, and the higher limit of $Y_{X/S}$ is the one for which no catabolism occurs.
- For example, the aerobic heterotrophic metabolism of a carbon source, which is represented by Equation.



In order for catabolism to occur,

$$W + \frac{X}{4} - \frac{Y}{2} - \frac{3}{4}Z - 5 \frac{Y_{X/S} MW_{substrate}}{MW_{substrate}} > 0$$

$$Y_{X/S} < \left(W + \frac{X}{4} - \frac{Y}{2} - \frac{3}{4}Z \right) \frac{MW_{biomass}}{5 MW_{substrate}}$$

Now, from this equation, in order for catabolism to occur, the stoichiometric coefficient for oxygen needs to be higher than 0. So, that means that using this equation for oxygen, this is the stoichiometric coefficient. Now, for this reaction to occur, at least this coefficient value should be greater than 0. So, this is the condition that we apply; so, overall this equation is written here.

Now, under if we solve this, actually the equation can further be written as the $Y_{X/S}$ values will always be less than this particular value. Now, for example, if the substrate is glucose, so, we have all the values are known to us. So, this condition means that overall we can solve the this particular value by substituting different values. So, we can substitute for W is equal to 6, this X is equal to 12, Y is equal to 6, and Z is equal to 0.

So, if we substitute in this particular equation and write the molecular weight of (biome); there is some mistake. This is substrate by biomass, so this, so this will be biomass by substrate. So, under this condition, we can find out the value of $Y_{X/S}$ less than some value; and that some value is point 75 kg biomass per kg glucose. So, that means, the upper limit of growth yield for this

particular reaction is point 75. Actually, it will be much lower and we have earlier taken the value to be point 3, which was very reasonable. So, for the growth yield of.

(Refer Slide Time: 23:03)

Cont...

- ❖ For the growth of **hydrogenotrophic methanogens**, the need for catabolism to occur means that some methane needs to be produced and this translates into the condition:

$$0.25 - 0.044Y_X > 0$$
 That is $Y_{X/S} < 5.68 \frac{\text{kg biomass}}{\text{kg hydrogen}}$
- ❖ It is important to observe, however, that **the upper limit for the growth yield calculated in this way refers only to the chemistry of the reaction and not necessarily to its energetics.**
- In practice, there needs to be some minimum energy generated from the catabolic reactions for the metabolism to occur, and this means that the **maximum limit of the $Y_{X/S}$ can be significantly lower than the upper limit calculated here.**
- However, it is always important to make a simple consistency check of the **experimental data to make sure that the upper limit for $Y_{X/S}$ is not exceeded.**

For the growth of hydrogenotrophic methanogens,

$$0.25 - 0.044Y_X > 0$$

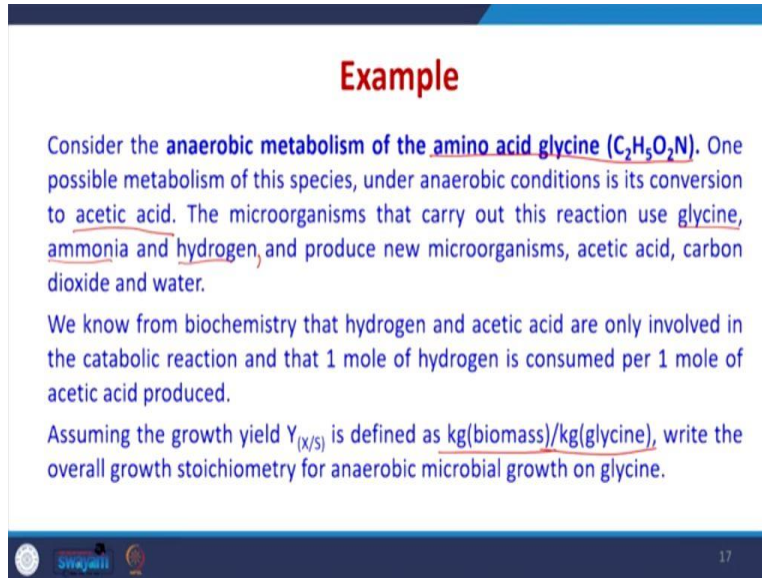
$$\text{That is } Y_{X/S} < 5.68 \frac{\text{kg biomass}}{\text{kg hydrogen}}$$

Similarly, we can find out for other types of reactions like for the growth of hydrogenotrophic methanogens, the need of catabolism to occur; means that some methane to be produced. So, that means the coefficient this coefficient should be greater than 0. Now, if you solve it, actually it will come out that; it should be less than 5.68. So, it is important to observe that the upper limit of growth yield calculated in this way refers only to the chemistry of the reaction, and not necessarily to the energetics. So, in practical, the actual value of the growth G will be much lower than the upper limit calculated here.

However, it is always important to make a simple consistency to check whether the experimental data is correct or not; and the value of $Y_{X/S}$ is not exceeded. So, sometimes say by mistake we can report such. So, as such we can find out for each of the reaction what is the upper limit of the

growth yield using the method which has been defined here. Now, we will take some examples before closing today's lecture.

(Refer Slide Time: 24:18)



Example

Consider the anaerobic metabolism of the amino acid glycine ($C_2H_5O_2N$). One possible metabolism of this species, under anaerobic conditions is its conversion to acetic acid. The microorganisms that carry out this reaction use glycine, ammonia and hydrogen, and produce new microorganisms, acetic acid, carbon dioxide and water.

We know from biochemistry that hydrogen and acetic acid are only involved in the catabolic reaction and that 1 mole of hydrogen is consumed per 1 mole of acetic acid produced.

Assuming the growth yield $Y_{(X/S)}$ is defined as kg(biomass)/kg(glycine), write the overall growth stoichiometry for anaerobic microbial growth on glycine.

17

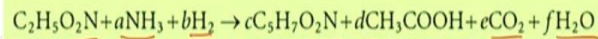
So, this is one example, in which it is being told that consider the anaerobic metabolism of amino acid glycine. One possible metabolism of this species, under anaerobic condition is its conversion to acetic acid. So, we are trying to convert amino acid glycine to acetic acid. The microcosm that carry out this reaction use glycine, ammonia and hydrogen. So, that means on the reactant side we have glycine, then ammonia, and then hydrogen; and they produce new microorganisms acetic acid, and carbon dioxide, and water. We know from biochemistry that hydrogen and acetic acid are only involved in the catabolic reactions.

And that one mole of hydrogen is consumed per mole of acetic acid produced; so, already we have written. Assuming that the growth yield is defined as kg of biomass produced per kg of glycine consumed, write the overall growth stoichiometry of anaerobic microbial growth on glycine. So, this is what we have to do. So, using the information which is given on in this example, we can easily write this particular equation.

(Refer Slide Time: 25:34)

Solution

Theory and formulae:



$$Y_{X/S} \left(\frac{\text{kg biomass}}{\text{kg glycine}} \right) = \frac{cMW_{\text{biomass}}}{MW_{\text{glycine}}} = 1.51c$$

$$\begin{cases} 5c + 2d + e = 2 & \text{C balance} \\ 2c + 2d + 2e + f = 2 & \text{O balance} \\ 7c + 4d + 2f = 3a + 2b + 5 & \text{H balance} \\ c = 1 + a & \text{N balance} \end{cases}$$



18

Example

Consider the anaerobic metabolism of the amino acid glycine ($C_2H_5O_2N$). One possible metabolism of this species, under anaerobic conditions is its conversion to acetic acid. The microorganisms that carry out this reaction use glycine, ammonia and hydrogen, and produce new microorganisms, acetic acid, carbon dioxide and water.

We know from biochemistry that hydrogen and acetic acid are only involved in the catabolic reaction and that 1 mole of hydrogen is consumed per 1 mole of acetic acid produced.

Assuming the growth yield $Y_{(X/S)}$ is defined as kg(biomass)/kg(glycine), write the overall growth stoichiometry for anaerobic microbial growth on glycine.



17

So, we have glycine which is reacting with ammonia and hydrogen to form the biomass acetic acid, CO₂ and H₂O. So, we have 6 coefficient and we have the this Y_{X/S} is defined. So, this will be c upon 1 into molecular weight of biomass, into divided by molecular weight of glycine. So, we can easily calculate the molecular weight of biomass and glycine because their formula is given. Using this we can find out that, the Y_{X/S} value is equal to 1.51c. Now, similarly, the value of this the for different C, O, H and N balance equations are given here; and already it is written that another condition is that that 1 mole of hydrogen is consumed per 1 mole of acetic acid produced. So, this is another condition which is given here.

(Refer Slide Time: 26:39)

Solution

Data Given		
1 mole of hydrogen is consumed per 1 mole of acetic acid produced or $b=d$		
Molecular mass of biomass	MW _{biomass}	113.11 ✓
Molecular mass of glycine	MW _{glycine}	75.07 ✓

$$C_2H_5O_2N + (0.66Y_{X/S} - 1)NH_3 + (1 - 2.2Y_{X/S})H_2 \rightarrow$$

$$0.66Y_{X/S}C_5H_7O_2N + (1 - 2.2Y_{X/S})CH_3COOH$$

$$+ 1.1Y_{X/S}CO_2 + 0.88Y_{X/S}H_2O$$

Solution

Theory and formulae:

$$C_2H_5O_2N + aNH_3 + bH_2 \rightarrow cC_5H_7O_2N + dCH_3COOH + eCO_2 + fH_2O$$

$$Y_{X/S} \left(\frac{\text{kg biomass}}{\text{kg glycine}} \right) = \frac{cMW_{\text{biomass}}}{MW_{\text{glycine}}} = 1.51c$$

$$\begin{cases} 5c + 2d + e = 2 & \text{Cbalance} \\ 2c + 2d + 2e + f = 2 & \text{Obalance} \\ 7c + 4d + 2f = 3a + 2b + 5 & \text{Hbalance} \\ c = 1 + a & \text{Nbalance} \end{cases}$$

$b = d$

So, using these conditions we can write, 1 mole of hydrogen is consumed per 1 mole of acetic acid produced. Or, that means, we have b is equal to d, which is this is b and this is d. So, this another condition that is coming here is b is equal to d. So, using this condition molecular weight of biomass and molecular weight of glycine, we can easily write the equation and overall stoichiometric can be written by this particular equation which is given here. So, this is one example.

(Refer Slide Time: 27:10)

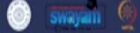
Example

Consider the anaerobic metabolism of palmitic acid ($C_{16}H_{32}O_2$).

Under anaerobic conditions palmitic acid is converted to acetic acid and hydrogen. The overall growth reaction includes the consumption of palmitic acid, ammonia, water and carbon dioxide and the production of microorganisms, acetic acid, hydrogen and carbon dioxide.

We know from biochemistry that acetic acid and hydrogen are only produced during catabolism of palmitic acid, and are not produced or consumed during anabolic reactions.

The growth yield is defined as kg (biomass)/kg (palmitic acid). Write the overall growth stoichiometry for the anaerobic metabolism of palmitic acid.

20

Another example we can take for the anaerobic metabolism of palmitic acid, which is given by the formula $C_{16}H_{32}O_2$; so, we have to find out for anaerobic metabolism. So, under this condition, the palmitic acid is assumed to be converted into acetic acid and hydrogen. The overall growth reaction involves the consumption of ammonia, water along with the palmitic acid, and production of microorganism, acetic acid, hydrogen and carbon dioxide.

And we know from biochemistry that acetic acid and hydrogen are only produced during catabolism of palmitic acid, and are not produced or consumed during anabolic reactions. And that yield coefficient definition is this. So write the overall stoichiometry of anaerobic metabolism of palmitic acid.

(Refer Slide Time: 28:05)

Cont...

Theory and formulae:

$$C_{16}H_{32}O_2 + aNH_3 + bH_2O + cCO_2 \rightarrow dC_5H_7O_2N + eCH_3COOH + fH_2$$

$$Y_{X/S} \left(\frac{\text{kg biomass}}{\text{kg palmitic acid}} \right) = \frac{dMW_{\text{biomass}}}{MW_{\text{palmitic}}} = 0.44d$$

$$C_{16}H_{32}O_2 + 14H_2O \rightarrow 8CH_3COOH + 14H_2$$

Carbon balance $(16+c=5d+2e)$
 Oxygen balance $(2+b+2c=2d+2e)$
 Hydrogen balance $(32+3a+2b=7d+e+2f)$
 Nitrogen balance $(a=d)$

So, we can write this equation. Here we can see that this palmitic acid with ammonia, H₂O and CO₂, they are reacting together to form the acetic acid, hydrogen along with the biomass. So, if we can easily write and this will be the equation with respect to growth yield. And once this is known, also we can easily see that the carbon balance will be given by this particular equation. And the nitrogen balance gives that a is equal to d. So, it is very easy, we can find out that a is equal to d. So, a will become equal to the Y_{X/S} by 0.44.

(Refer Slide Time: 28:48)

Solution

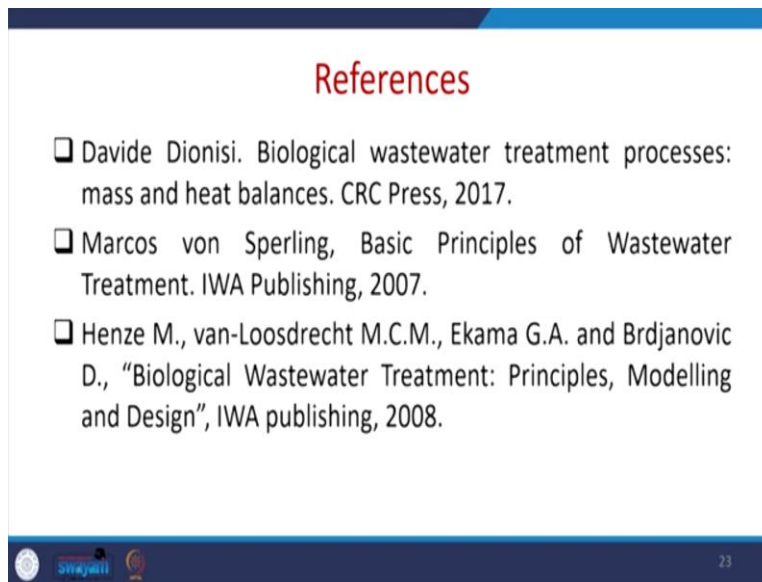
Data Given		
Molecular mass of biomass	MW _{biomass}	113.11
Molecular mass of palmitic acid (C ₁₆ H ₃₂ O ₂)	MW _{palmitic acid}	75.07

$$C_{16}H_{32}O_2 + 2.27Y_{X/S}NH_3 + (14 - 10.26Y_{X/S})H_2O + 3.45Y_{X/S}CO_2 \rightarrow$$

$$2.27Y_{X/S}C_5H_7O_2N + (8 - 3.95Y_{X/S})CH_3COOH + (14 - 6.91Y_{X/S})H_2$$

And using all these conditions and using the molecular weight of the biomass, and the molecular weight of palmitic acid, we can solve the overall. We can find out the overall coefficient in terms of $Y_{X/S}$, the growth yield; and the overall equation can be written like this. And once the experimentally we can determine the value of $Y_{X/S}$, we can exactly write the stoichiometric coefficients of this particular reaction. So, this way we can write the overall growth chemistry under various conditions, various conditions. And thus, we can find out the overall stoichiometry of the equations.

(Refer Slide Time: 29:29)



We have used the following references for preparing this slide; you can always refer to these books for further understanding the overall growth chemistry. In total, we have understood the catabolic reactions, the anabolic reactions, and the combination of both. So, all these reactions are highly helpful in determining the actual reactions, which are happening inside the bio-reactors or the wastewater treatment reactors, if the wastewater contain any of the conditions or the microorganisms, and the what type of substrates are in the wastewater itself.

So, we will be using this equation later on for various uses. And using this we can find out the mechanism, we can try to find out the overall growth yield et-cetera also. So, these equations are highly helpful in understanding the microbial degradation processes happening inside the reactor during the wastewater treatment. So, we will continue with further sections in the next lecture. Thank you very much.