

**Aspects of Biochemical Engineering**  
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**Lecture – 08**  
**Stoichiometry of Biochemical Process III**

We will come back my course aspects of biochemical engineering. So, last two lectures, I tried to discuss about the Stoichiometry of bioprocess. Now this will be; I will be completing this Stoichiometry bioprocess by this lecture and here I shall discuss two other problems and I hope that you know this will cleared all the; that whatever problem that we will be having in the Stoichiometry, then I request all of you to into do this Stoichiometry of this equation by as you do not.

So, there you practice it and we have any problem please let me know now first problem that I am going to discuss in this lecture this is the anaerobic digestion process as you know the anaerobic digestion process is largely in operation in India and throughout the world particularly anaerobic digestion process because if we if we look the history of the wastewater treatment processes like this.

That he initially that that all the waste treatment processes, we considered this kind of wastage for the in the industry and why we have this wastewater treatment processes the reason is that no industry particularly chemical and biochemical industry they cannot discharge their waste water directly to the watercourses because as soon as they; this is the; these two; the watercourses that water will be polluted in a lot of bacteria will grow in the water and pollute our drinking water sources and it will give it is a serious concerned.

So, we shall have to treat the organic waste now it has been observed for the all the industry that ten percent of the total investment they keep for the wastewater treatment processes. Now initially, this I told you that 70 percent of this wastewater treatment processes are controlled by the biological mean and 30 percent maybe we are through chemicals are other means. So, now, this process when you go for the waste water treatment process, initially we thought that it is a kind of wastage of money. Now due to the due to the development of the anaerobic digestion process it is now possible that a part of the money that you spent for wastewater treatment process can be recovered.

I can give a typical example that in West Bengal, we have IB agro industry, they produce the ethanol from broken rice and they have the anaerobic the treatment process and they told me the 50 percent of their distillation cost installation energy recovery is taken care by the anaerobic digestion process that methane that is produced by this process now the problem that I am going to discuss. Now this is the anaerobic digestion process and this.

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**Problem 4:**  
 Elemental analysis of refuse that it content 76% (w/w) of organic matter of the composition  $CH_{2.1}O_{0.9}N_{0.15}$  the remaining 24% was ash. The minimum methane production by anaerobic digestion is to be calculated per Kg of dry refuse, also determine Stoichiometric equation of the process.

**Solution:**

*Ans +*  $CH_{2.1}O_{0.9}N_{0.15} \rightarrow bCH_4 + cCO_2 + dNH_3 + eH^+$   
 (Substrate) (Product)

$$\sigma_s = \frac{12}{12 + 2.1 + 16 \times 0.9 + 14 \times 0.15} = 0.392$$

$$\sigma_p = \frac{12}{12 + 4} = 0.75$$

$$\gamma_s = 4 + 2.1 - 2 \times 0.9 - 3 \times 0.15 = 3.85$$



Now, problem is like this that elemental analysis of refuse contains 76 percent weight by weight that is the organic matter the actually, we might be aware that organic matter only participate in the biochemical reaction mostly and inorganic matter also, you can participate that you use as a cofactor for different enzymatic reaction that is the only thing that we have now this empirical formula of the refuse is given here and remaining 24 percent gas as this kind of metal oxide and the minimum methane production through the anaerobic digestion is to be calculated per kg of diffuse also determine the stoichiometric of the of the process.

So, you know that the I hope the idea is very cleared that some organic waste is there we putting it into the anaerobic digestion process and we want to produce the methane. So, we shall have to find out that that what is the; what is, how much methane we can produce after that and not only that we shall have to also produce they find out what is the stoichiometric of this process.

Now, for doing; so, question comes what is the basic equation of this. So, a basic equation will be this. This is the substrate that we know that is already given here that is  $CH_2.1 O_{0.9}$  and  $N_{0.15}$ . This is this plus water molecule. This is not given here; maybe this should be given here. This is because I told you that waste material comprises of you know that lot of polymeric material like starch a glucose. It is kind of protein. So, you know this.

This undergo hydrolysis. Hydrolysis means the reaction which is take place in presence of water when a hydrolysis, if then it from those smaller molecules and these smaller molecules then participate if the reaction and produce methane carbon dioxide ammonia and  $H_2$  that is produced. So, we shall have to develop the stoichiometric equation on the basis of that. So, that is the whole thing that when you do the analysis of anaerobic digestion process the preliminary concept on the process is very much required.

Until unless we have the conception of the process we cannot write the stoichiometric of the equation a conception; I mean that what are the initial requirement of the process what is the final desired product. We are going to get if you have some idea then and only, then you can write that tentative equation and then you have whatever coefficient is there we can we can feed it up one after another I can show you.

Now this is the tentative; the reaction that we have in the anaerobic digestion process and as you know that anaerobic digestion process is usually carried out by 2 group of Michael for a 1 is the acid regions and methane regions acid region means that you know that convert the organic matter to the organic acid volatile fatty acid and parotid fatty acid will be convert it to the do methane and carbon dioxide now though this is the ultimate equation that we have here.

Now here we can find out the this that  $\sigma_s$  and  $\gamma$ ;  $\gamma$  is we can find out and  $\sigma_p$  and  $\gamma_p$  we can we can easily calculate on the basis of this if that the empirical relationship we can find out that.

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$\gamma_p = 4 + 4 = 8$

Calculation of  $Y_{p/s}$   
 For anaerobic process  $\epsilon_p = 0.7$

$$Y_{p/s} = \epsilon_p \frac{\sigma_s Y_s}{\sigma_p \gamma_p}$$

$$= 0.7 \frac{(0.392)(3.85)}{(0.75)(8)} \frac{\text{g of } CH_4}{\text{g of substrate}}$$

$$Y_{p/s} = 0.176 \text{ g of } CH_4 / \text{g of substrate} = 0.176 \times 0.76 \text{ g of } CH_4 / \text{g of refuse}$$

$$= 0.134 \text{ g of } CH_4 / \text{g of refuse}$$

Now, this anaerobic digestion process; what we have; we know this the thermodynamic coefficient is 0.47, I have already mentioned. So, if we and heard in this stoichiometric equation, we have seen that in this stoichiometric equation we have seen that in this stoichiometric equation, we have seen only product this product we have not we have not considered.

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**Problem 4:**  
 Elemental analysis of refuse that it content 76% (w/w) of organic matter of the composition  $CH_{2.1}O_{0.9}N_{0.15}$  the remaining 24% was ash. The minimum methane production by anaerobic digestion is to be calculated per Kg of dry refuse, also determine Stoichiometric equation of the process.

**Solution:**

$$CH_{2.1}O_{0.9}N_{0.15} \rightarrow bCH_4 + cCO_2 + dNH_3 + eH^+$$

(Substrate) (Product) *almost*

$$\sigma_s = \frac{12}{12 + 2.1 + 16 \times 0.9 + 14 \times 0.15} = 0.392$$

$$\sigma_p = \frac{12}{12 + 4} = 0.75$$

$$\gamma_s = 4 + 2.1 - 2 \times 0.9 - 3 \times 0.15 = 3.85$$

This is the product we have considered, but we did not consider any cell mass here cell mass here because the anaerobic. Anaerobic fermentation process cell mass production

we can appear to be negligible it is a one tenth and that anaerobic aerobic fermentation process. So, this we neglected now that. So,  $Y_{p \text{ by } s}$  is equal to  $\epsilon_p \sigma_s \gamma_s \sigma_p \gamma_p$ . So, in this the all coefficient is given here and so, if we solve this equation it is coming about 0.1, 0.78 a gram of methane produced from gamma of substrate.

So, this way we can we can we can find it out. Now here the gram of substrate when you consider this is the organic substrate, but this again comprises of 76 percent we have seen the diffused content 76 percent organic matter. So, by if you if you if you consider actual refused the actual biomass then what you have to do we shall have to multiplied by point seven 6 because seventy say 76 percent is the organic matter present in the biomass. So, if you multiply point 6 then we find out gram of refused that this much of gram of methane produced per gram of refuse I am previously it was my per gram of methane produce per gram of substrate now we can calculate it the per gram of refuse.

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Total methane produced =  $0.134 \text{ kg/kg of dry refuse}$   
 Calculation of stoichiometric co-efficient:

$$b + c = 1$$

$$c = 1 - b$$

$$c = 1 - 0.33 = 0.67$$

Nitrogen balance indicates

$$d = 0.15$$

Oxygen balance

$$a = 2c - 0.9$$

$$a = 2 \times 0.67 - 0.9 = 0.44$$

Hydrogen balance

$$2.1 + 2a = 4b + 3d + c$$

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So, this is coming at this has come at 0.134 gram methane per gram of refuse, then how we can calculate the stoichiometry of this process, we can do it very easily that if we know this, we can find out; we know the; what is the molecular weight of methane and what is the molecular weight of biomass.

So, from that we can we can find out if you look at our coefficient this we have what we have found out we have found out how much how much b is produced we can we can

easily calculate b value we can easily calculate if you know how much b value is produced then we can easily calculate the carbon dioxide because if you do the carbon balance one c one equal to b into a b plus c; am I right. So, if you know the; if we know this is 1 and this is b value we calculated. So, you can easily calculate the value of c.

So, this is how you can find out the; so, we can we can calculate the value of c here value of c you can calculate this is coming like this then we can we do the nitrogen balance oxygen balance hydrogen balance we go. As I mentioned in the previous lecture that we simple the kind of the balance equation that we if we if we if we if we do that we can easily solve this equation that this is like this. This is coming like this you can solve all this all this coefficient we can easily calculate.

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$$2.1 + 2 \times 0.44 = 4 \times 0.33 + 3 \times 0.15 + c$$

$$c = 2.98 - 1.77 = 1.21$$

**Stoichiometric equation**

$$\text{CH}_{2.1}\text{O}_{0.9}\text{N}_{0.15} + 0.4\text{H}_2\text{O} \rightarrow 0.33\text{CH}_4 + 0.67\text{CO}_2 + 0.15\text{NH}_3 + 1.21\text{H}^+$$

*0.33 g atom CH<sub>4</sub>  
g atom refuse  
g CH<sub>4</sub>  
g S.W*

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Now, when you have all the coefficient, then we can put this equation that we have already written previously this is the equation we will come. So, this will give you the total idea that what is the stoichiometry of this process now here I want to add something else suppose this is the molecular conversion, am I right, this is like this, if you like 0.33 gram atom methane per gram atom refused; am I right.

Now we know the what is the atomic weight of this and we know what is the molecular weight of this molecular weight and this molecular weight. So, we multiply by that we can we can we can multiply then we will get the gram of methane produced per gram of

gram of subsidy from that you can you can do that now. Now let me let me show you something else here.

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$2.1 + 2 \times 0.44 = 4 \times 0.33 + 3 \times 0.15 + c$   
 $c = 2.98 - 1.77 = 1.21$

**Stoichiometric equation**

$CH_{2.1}O_{0.9}N_{0.15} + 0.4H_2O \rightarrow 0.33CH_4 + 0.67CO_2 + 0.15NH_3 + 1.21H^+$

*Handwritten notes:*  
 $0.33 \text{ g atom C} \times 4 = 1.32 \text{ g atom C}$   
 $0.33 \text{ moles of } CH_4 \text{ NTP}$   
 $1 \text{ mole} = 22.4 \text{ L}$   
 $22.4 \times 0.33 \text{ L } CH_4$

Now, suppose you get this much of methane produce. So, you can you can you can find our point 0.33 moles of methane am I right. So, now we know at NTP into what is the NTP. NTP is normal temperature and pressure what is the normal temperature is pressure temperature is 0 degree centigrade and pressure is one atmospheric pressure.

That at NTP one mol of any gas this is the; what is the volume that we have 22.4 liter am I right. So, if you know this much of bamos. So, if you if you multiplied by. So, 22.4 in 2 0.33, then mean this liter of methane will be produced per gram atom of this refused. So, now if you divide by molecular weight of this reviews then you can find out the per gram of refused how much a methane is produced that you can easily calculate them.

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**Problem 5:**

The microorganism *Mycobacterium vaccae* is able to grow with ethane as the sole source of carbon and energy. It is grown (at 30 °C) in a CSTR reactor (chemostat) with continuous supply of gas (5 vol % ethane and 95 vol % air) and of a sterile aqueous medium which contains various minerals and N- source,  $(\text{NH}_4)_2\text{SO}_4$ . The limiting substrate is ethane.  $Y_{x/S} = 23.7 \text{ g cell mass (mol ethane)}^{-1}$ .

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Now, let me go to the next problem and next problem is very interesting if you look at here the microorganism micro bacterium hacking is able to grow with ethane as this whole sources of carbon and energy. Now here I want to point out one thing that you know that I discussed the single cell protein may be considered as a very very good source for the animal feed and it is not very good for the human food the reason is that it contains lot of nucleic acid and due to presence of nucleic acid it causes gout and kidney stores.

So, there is a train in the world that how we can produce the microbial proteins. So, different country they are producing microbial protein from the different sources now in our country we have very less petroleum reserve we have hardly we have whatever petroleum reserve we have mostly it is be consumed have and the only for 330 ,years not more; that is why most of the core petroleum we import from outside. Now in the petroleum producing country, they have lot of petroleum; they can use the hydrocarbons for the production of cell mass.

So, this is the problem related to that now they use the ethane as the source of carbon ethane is kind of hydrocarbons that this is used as a for the product production of mycobacterium vaccae, it grows at 30 degree centigrade in a CSTR chemostat with the continuous supply of gas five percent ethane and ninety five percent air and of a sterile aqueous media which contains various minerals nitrogen as the ammonium sulphate and



limited that substrate in is ethane what is the mean by limited substrate limited substrate which is the what constant what is the component that present in the limited amount.

As a since ethanol ethane ethane is the hydrocarbon is inspiringly soluble in in water and you know that mike organism can utilize the substrate which is soluble in water they cannot take the substrate which is which is insoluble. So, it is to first; it is to be solubilized the solubility is since the solubility of ethane is very less. So, that is why this the ethane solubility is a limiting factor in these particular reactions and yield coefficient now this is very important yield coefficient is 23.7 gram cell per mol of ethane that is the that is given there.

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**Problem 5:**

1. Except the small amount of S and P an analysis of dry cell mass is C: 47.40 wt%, N: 8.30 wt%, H: 7.43 wt% and ash: 4.00 wt%. The remainder is taken to be oxygen which cannot be detected in the analysis. Determine the stoichiometric formula for the (ash free) biomass,  $\text{CH}_a\text{O}_b\text{N}_c$  and the formula weight per C-atom. Determine  $Y_{X/S}$  in unit of C-mol biomass/C-mol ethane.
2. Calculate the oxygen consumption  $Y_{O/X}$  (mol  $\text{O}_2$ /C-mol biomass) when it is assumed that  $\text{CO}_2$  is the only product besides the biomass. Write the full stoichiometric equation. Determine the total heat evolved per kg dry cell mass.

Now, except for the small amount except for the small amount sulfur and phosphorus and analysis of dry mass carbon content is 47.4 percent weight percent weight person means weight by weight; that means, if you take the 100; come basis, this is 100 gram, then carbon content in the biomass is 47.4 gram, the nitrogen 8.3 gram, hydrogen is 7.43 gram as is 4 gram.

The remaining remainder is taken as the oxygen which cannot be detected in the analysis is very difficult to find out oxygen present in the substrate determination of the stoichiometric formula of the as free biomass is a H a O b and N c and the formula weight per gram; gram atom carbon that and determined Y X by S in the unit carbon mol biomass per carbon mol ethane. Now you can remember that previously what is given

this is this in this problem that is given this is like this the gram of cell mass 23.7 gram of cell mass produce per gram or ethane.

So, this you have to calculate the gram atom of the cell mass produced per gram atom of ethane that we shall have to we will have to find it out this is the this is the equation that we have gram mol biomass per gram this is then again, we shall have to calculate the oxygen consumption that the gram molecular oxygen per gram mol of biomass formation and when it is consume it is the assumed that carbon dioxide is the only product besides the biomass and write the full stoichiometry equation and determine the total heat evolved per kg of dry cell mass formation.



So, this is this is the very interesting in this is say the sense that that here you get the opportunity to find out; how to determine the empirical formula of the biomass and then you have to calculate that carbon mol biomass produce per carbon mol ethane consumed then you then you calculate the what is the oxygen consumed then followed by the stoichiometry of the whole process and finally, you have to find out the how much heat involved in this process.

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**Solution**

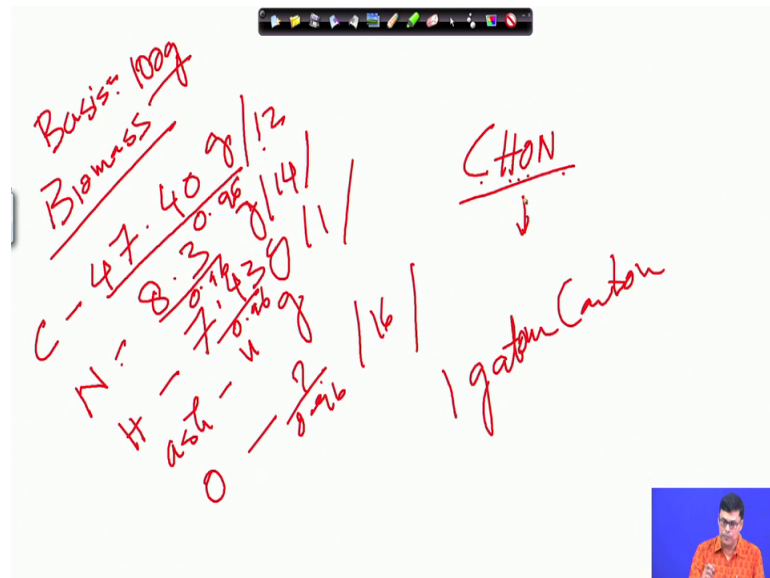
1. the stoichiometric formula of the 'ash free' biomass can be calculated in several different ways (on the basis of ash free or non-ash free biomass). The result should of course be the same if the calculation are consistent. Here we show the calculation on 'ash free basis' in table

Analysis	Wt % of total	'ash free basis'	Atoms per C- atom
C	47.40	49.375= 4.112 C-atom	1
H	7.43	7.739	1.881
N	8.30	8.645	0.150
'Ash'	4.00	—	—
O	32.87	34.24	0.52

So, how you are going to dissolve this? This is very interesting that. So, suppose that you know that in this and that you know if you look at the biomass.

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You look the biomass. So, we have carbon, am I right, carbon is 47 percent; 47.4.

This is basically; this is a basis is 100 gram, basis is 100 gram biomass, if a 100 gram biomass carbon present is 47, nitrogen is 8.3. This is all gram and then we have hydrogen this is 7.43 gram and ash is about 4 grams. Now remaining whatever after summation all these thing whatever we remaining that will be optimum remaining is the oxygen. So, what you want to point in an each contents about 4 percent ash.

So, when you when you do the stoichiometric analysis; we do the stoichiometric analysis with the respect to the organic substrate. So, naturally that this is to be converted to the 100 gram of not biomass; 100 gram of organic material, if you want to have convert to 100 gram of organic material, then with that carbon content, it will divide by we can 0.96, there 4 percent is there that you convert 100 percent of biomass.

So, this is also 0.96. This is also 0.96 and so we can do that this also 0.96. Now when you give this carbon; you know that what is that atomic carbon is 12 nitrogen is how much? 14 hydrogen is 1, oxygen is how much; 16. So, you know that. So, you if you can you can you can do the analysis carbon hydrogen oxygen you can permit then. So, you we shall have to find out the biomass empirical formula on the basis of one gram atom carbon; am I right; one gram atom carbon. So, how much carbon atom is there, this is divided by 12. Now this you have to divide with this; all this thing, you divide, then you can find our per gram atom of carbon how much nitrogen is there; how much hydrogen

is there how much oxygen is there. Now if you have this then you can put it in the equation like this; this is C H O N.

So, we can we can we can we can we can write the different figure that is one this is one, but this will be different this we can write it here. So, we can we can have the empirical formula like this and then we can find out the molecular weight of this particular formula. So, this is the exactly what he has it has done here you this is the what exactly we have done it here this is the this is the figure that we have and then we have find out like this; this has come like this now.

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**Solution**

Formula  $\frac{CH_{1.88}O_{0.52}N_{0.15}}{M_x = 24.30 \text{ g. (C mol)}^{-1}}$

$$Y_{X/S} = \frac{(0.96)(23.7)}{24.30} (\text{g biomass}). (\text{mol ethane})^{-1}$$

$$= 0.4681 (\text{C - mol biomass}). (\text{C - mol ethane})^{-1}$$

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Now, we got the formula like this. This is the formula we got and with the molecular weight this. Now we have only this figure 23.7. If you divide by 24 point this molecular weight, then we will get the gram mol biomass per gram mol of ethane. Now since it contains about of 4 percent of ash. So, you multiply by this, did you get the exact figure or should be the figure of gram mol of biomass per gram mol of ethane that you can easily find it out.

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**Solution**

2.  $\gamma_b = 4.39, \gamma_s = 7$  and  
 Suppose the stoichiometric equation

$$\text{C}_2\text{H}_6 + a \text{NH}_3 + b \text{O}_2 \rightarrow Y_c \text{CH}_{1.86}\text{O}_{0.52}\text{N}_{0.13} + (1 - Y_c) \text{CO}_2 + c \text{H}_2\text{O}$$

Where,

$$b = Y_{O/S}$$

$$Y_c = Y_{X/S} \quad \frac{\text{mol biomass}}{\text{C-mol ethane}}$$

we know

$$\text{oxygen demand} = b = \frac{(\gamma_s - \gamma_b \times Y_c)}{4} = \frac{(7 - 4.39 \times 0.4681)}{4} = 1.236 \frac{\text{mol O}_2}{\text{C-mol ethane}}$$

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Now, next the question is that how we can find out the oxygen requirement of the system. So, again in this system first we shall have to find out that; what is the tentative stoichiometric equation. So, this is the ethane this is the gram atom ethane this is the usually the formula is c ethane formula is C 2 H 6. The gram atom will be C H 3 and then ammonia is required here cell mass is produced and this is the oxygen is record.

This is the aerobic process this is the cell mass and this is carbon dioxide and this is water this is the; what we have the equation and from that we can find out different coefficient. This is substrate this is the cell mass that we can develop the gamma s value c gamma b value you can calculate and from that you can you can also determine this b value because the Y c value already you calculated the gram moles of the biomass produced per gram of ethane form.

So, if you know that you can put this equation here and this is coming about 1.236 that is moles of oxygen per gram atom of ethane.

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**Solution**

$$Y_{O/x} = \frac{Y_{O/s}}{Y_{X/s}} = \frac{1.236}{0.4681} = 2.64 \text{ mol } O_2 \cdot (\text{C - mol biomass})^{-1}$$

$CH_3 + a NH_3 + 1.236 O_2 \rightarrow 0.4681 CH_{1.88}O_{0.52}N_{0.15} + (1 - 0.4681) CO_2 + c H_2O$   
Balancing the equation

$$CH_3 + 0.0702 NH_3 + 1.236 O_2 \rightarrow 0.4681 CH_{1.88}O_{0.52}N_{0.15} + 0.5319 CO_2 + 1.165 H_2O$$

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Now, then we can also calculate the  $Y_{O/c}$  that is this I can show you  $Y_{O/x}$ ;  $Y_{O/x}$  means gram of oxygen required gram of cell mass, am I right.

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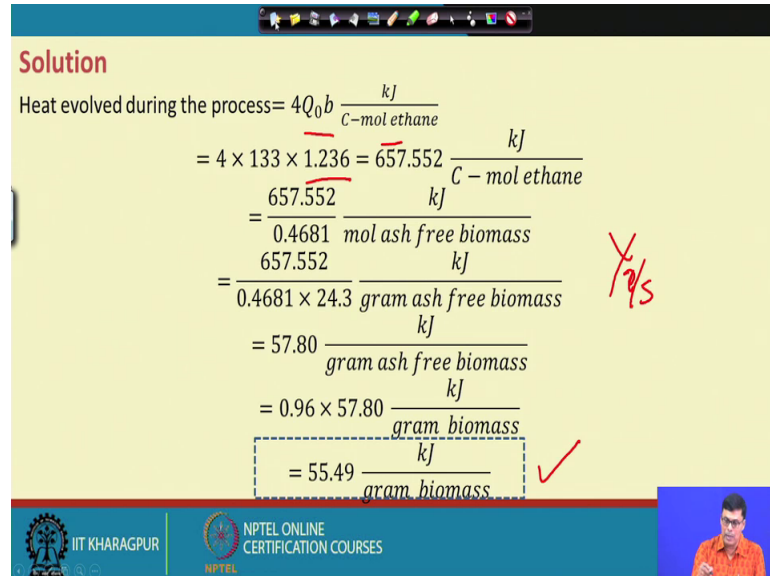
$$Y_{O/x} = \frac{\text{g } O_2}{\text{g Cell mass}} = \frac{Y_{O/s}}{Y_{X/s}}$$

So, I can I can write like this; this is a group  $Y_{O/s}$  and  $Y_{X/s}$ . So, we can we can easily do that because if you this s-s will cancel. So, this is exactly what is shown here; what is shown here. So, you can do that and then we if you do the balanced carbon balance and nitrogen balance oxygen balance oxygen; oxygen already we have

calculated we find this stoichiometric equation this stoichiometry equation you can calculate.

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**Solution**

$$\begin{aligned}
 \text{Heat evolved during the process} &= 4Q_0b \frac{\text{kJ}}{\text{C-mol ethane}} \\
 &= 4 \times 133 \times 1.236 = 657.552 \frac{\text{kJ}}{\text{C-mol ethane}} \\
 &= \frac{657.552 \text{ kJ}}{0.4681 \text{ mol ash free biomass}} \\
 &= \frac{657.552 \text{ kJ}}{0.4681 \times 24.3 \text{ gram ash free biomass}} \quad \text{Y/x/s} \\
 &= 57.80 \frac{\text{kJ}}{\text{gram ash free biomass}} \\
 &= 0.96 \times 57.80 \frac{\text{kJ}}{\text{gram biomass}} \\
 &= 55.49 \frac{\text{kJ}}{\text{gram biomass}} \quad \checkmark
 \end{aligned}$$


Then we can heat evolved we can find out like this 4, 4, 4, 3, 4, this is 4 Q 0 into b.

B already we calculated. So, we can find out this is kilo joule per mol of a substrate consumed and then I told you if you divide with the molecular weight you find out that how much it for a per gram of cell mass; how mol come of substitute, how can it produce, then you divide by the Y x by s, if you divide by Y x by s, the gram of cell produce per gram of substrate consumed then you find out or per gram of cell mass how much how much heat is default.

So, this is how you can calculate that total heat evolved in this process and so, in conclusion, I want to tell you that in the stoichiometry of the biochemical process, we; it can be done very easily first we shall we shall have to know the different empirical formulas of the substrate we should know the empirical formula of the biomass, we should know the empirical formula of the product and the empirical formula can be well, it is easily defined, we can the carbon hydrogen oxygen nitrogen we can assume that it will present there and then we can through the empirical analysis we can easily find out if you know the elemental composition of this material.

We can easily find out the empirical formula once you know this empirical formula, then we can write the different coefficient that is we required in this particular equation and when you write any kind of biochemical equation, we should have the some preliminary idea on the biochemical process or whether we are targeting for the cell mass formation, whether you are targeting for the product formation whether there is process is aerobic or anaerobic.

All this information if it is there then on the basis of that we can write the stoichiometry equation and also, I showed you that how you can find out the validity of the experimental results and also the amount of heat involved in the process. I hope this will be very much useful for do the mass and energy analysis of any kind of biochemical process.

Thank you very much.