Industrial Biotechnology
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Module 2
Lecture No 10
Stoichiometry of Bioprocess (Contd)

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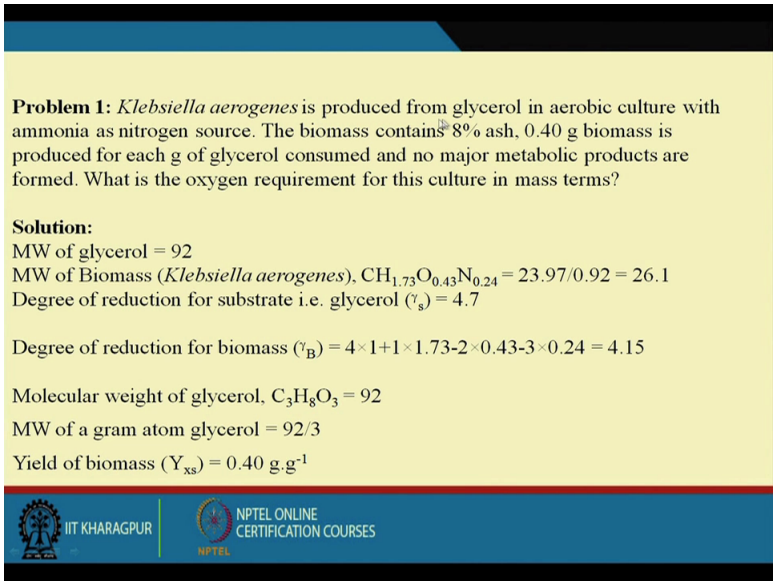

Now I am going to discuss some problems on stoichiometry of the bioprocess. If you look at this problem this is the Klebsiella aerogenes is produced from glycerol in aerobic culture with ammonia as nitrogen source, biomass content 8 per cent of ash, 0.4 gram biomass is produced each gram of glycerol consumed and no major metabolic products are formed. What is the oxygen requirement for this culture in mass terms?

Now this problem actually deal with you know that single cell protein, single cell protein is basically the microbial protein and this is produced by different ways we can use the carbohydrate for the production of cell mass, we can use the hydrocarbons also or produce in cell mass we can use the alcohol like methyl alcohol ethyl alcohol for the production of cell mass.

In this problem we use the Klebsiella that that is Klebsiella aerogenes that is used the glycerol, glycerol is the by product of the biodiesel producing in the industry because when biodiesel is produced from lipid the by product is the glycerol. Now this glycerol if they use for the production of this biomass, now we shall have to find out than this is the aerobic

fermentation process and we shall have to find out that how much oxygen is required for this production process and yield is 0.4 gram biomass per gram of glycerol consumed.

This is basically this is nothing but Yx by s Yx by s is 0.4 gram biomass per gram of glycerol that is given there. Now glycerol has the that empirical formulae C3 H8 O3 and this is equal to this molecular weight is about 92, now one if you if you consider the 1 gram carbon atom then 8 by 3 by O this is equal to 92 by 3, this is like this.

Now empirical formula of the Klebsiella ammonia that as show to this is CH 1.7 O 0.43 and at the end we assume it like this and actually the molecular weight is coming 23.97 but it contains 8 per cent ash, but that is why we divide by 0.92 to get the whole whole mass that you know that you know how much (())(03:41) how much is there that is we can 26.1 that we we get the the molecular weight of the biomass we get it like this.

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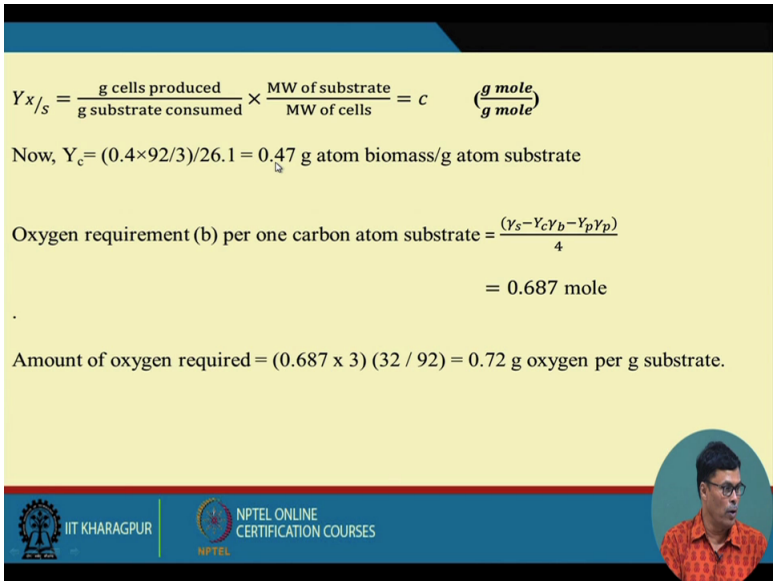

The degree of reduction of substrate we can easily calculate we have all the substrate is glycerol so we can easily find out the degree of reduction this is coming about 4.7 and degree of reduction for the biomass I have shown you before that also calculated it 4.15 and then we calculate the Yx by s this is this is gram of cells gram of cell produced per gram of substrate divide by their respective molecular weight we get gram of gram atoms of the cell produced per gram atom of substrate consumed.

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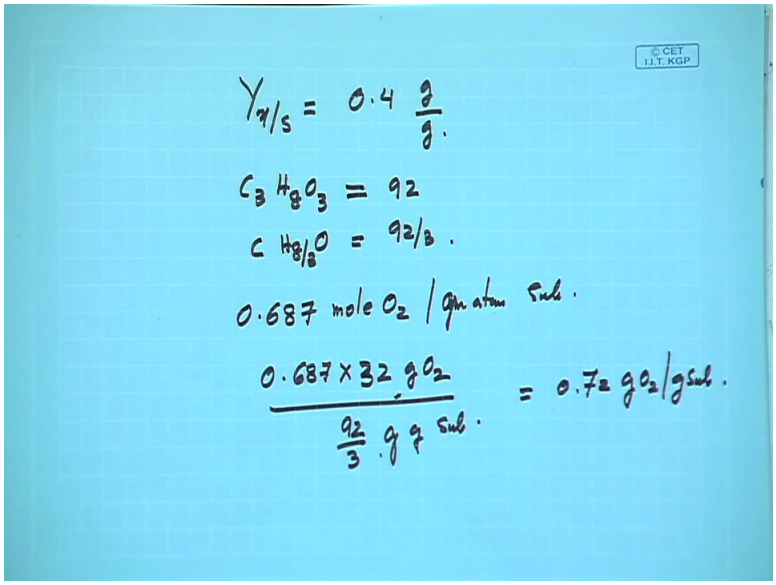

Now this is equal to Yc and this is this is this is coming about point 4 if they multiplied point 47 now if you then we we calculate this oxygen requirement per one carbon atom substrate is coming about 0.687 mole and if you then what is the total amount of that oxygen required per gram of substrate how we can calculate this is so actually we are getting 0.687 mole of O2 per gram atom atom substrate, am I right now what is the molecular weight of that oxygen that is 0.687 into 32 am I right, and what is the what is the gram atom of substrate this is gram of oxygen and gram atom of substrate is how much that is 1 gram atom of substrate is about 92 divide by 3. This is the gram of substrate, where the molecular weight one gram atom of substrate is 92 by 3. So if you divide by this it is coming about 0.72 gram of oxygen per gram of substrate, so this you can easily calculate there is not a problem for us.

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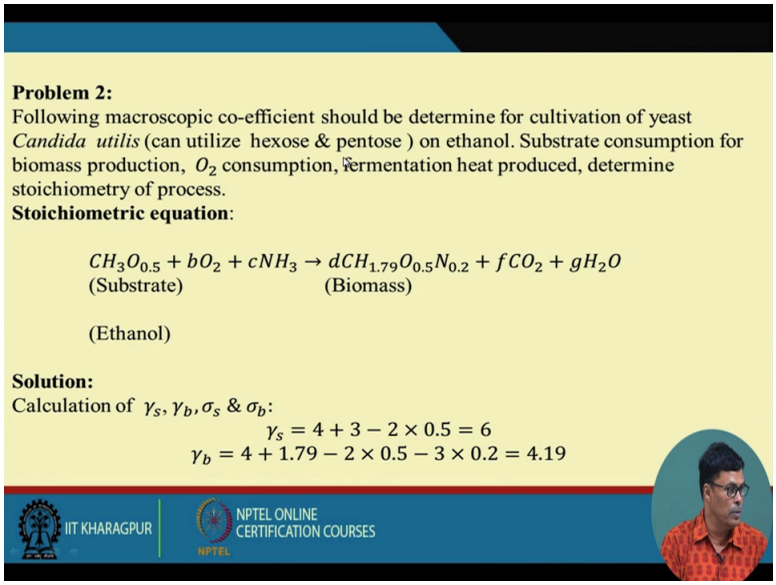

Now next problem that again this is with a Candida utilis. Following the microscopic coefficient should be determined for the cultivation of the yeast Candida utilis can utilize both hexose and pentose sugar on ethanol. Substrate consumption for biomass production oxygen consumption and fermentation heat produced and determination stoichiometry of the process. So these are the different parameters we have to determine and as I as I pointed out before that whenever we want to solve any kind of this any kind of stoichiometric problem. First we should have some idea about the process otherwise we cannot solve this problem. Now here Candida utilis is considered as the (())(07:14) this is used as a enable field now and the speciality of this yeast is that it cannot use both the hexose and pentose sugar.

Now here this Candida utilis is utilizing the ethanol as a substrate for the production of the biomass so we what we require basically that we require the per cell mass formation we required carbon as a substrate but + nitrogen source nitrogen also contribute for the cell mass formation and I told you the aerobic process is required aerobic, why aerobic process because the with the cell mass growth in the aerobic process is 10 times faster as compared to that of anaerobic fermentation process, so we required oxygen or so that is why in case of this stoichiometry equation when you write, we will find that this is ethanol that which is gram atom of substrate.

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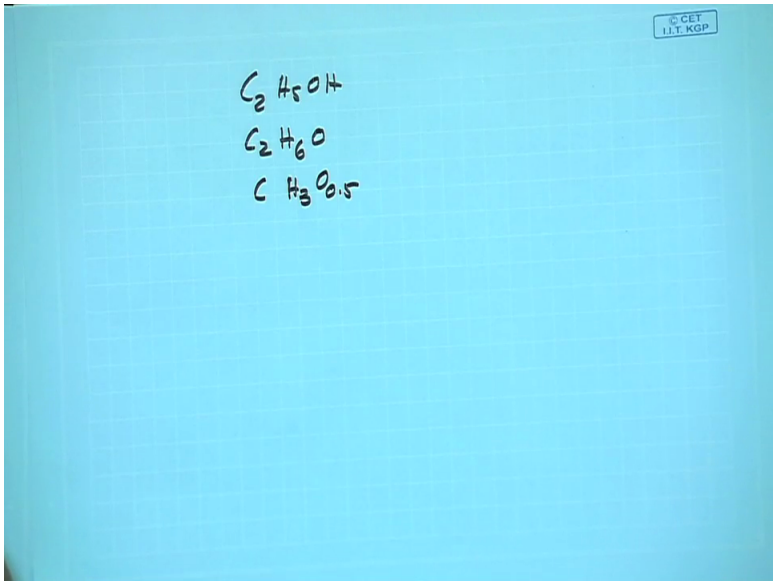

Ethanol when we write it is like this because ethanol I showed you before that C2 H5 OH now if you write in the empirical formulae like C2 H6 O and now you write per gram atom carbon it is like 3 O 0.5 so this is per gram atom carbon, this is exactly we have written, this is ethanol then b moles of their oxygen and c moles of ammonia when that in combination they form the d moles of per gram atom of biomass then if carbon-dioxide and g water. So here we this is the substrate and this is the substrate and this is the biomass.

So we can easily calculate the degree of reduction of substrate and degree b deduction of biomass and degree of deduction of substrate how we can calculate here you see the c stage as 4 and h is 3 and O is 2 into 0.5 this is coming about 6. Similarly we can calculate for biomass, it is c is 4, h is 1.79 then 2 into 0.5 – 3 into 0.2, it is coming about 0.19. Now similarly that sigma s and sigma b we can calculate by with respect to gram carbon this 12 divide by molecular weight of the substrate and 12 divide by the molecular weight of the biomass.

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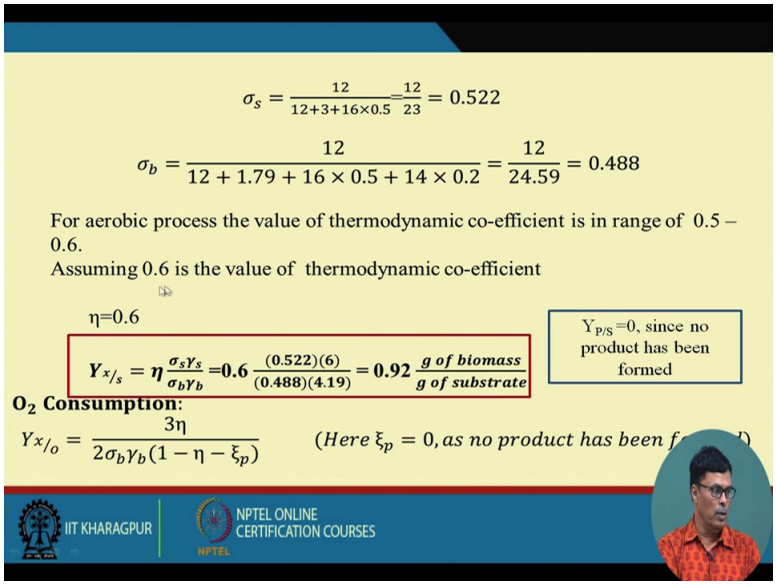

So all that coming is this is 0.522 and this 0.488, now when you this since it is aerobic forces the thermodynamic coefficient is assume to be 0. 5 to 0. 6 and we can assume 0.6 because and so and we assume there is no other product accepting biomass. So epsilon p can be assumed to be 0 so then eta will be equal to 0. 5 and eta Yx by s then we can calculate with the help of this degree of reduction and the you have sigma s, we can we can easily find out. And we find out that this is coming about 0.92 gram biomass per gram of substrate consumed and similarly we can calculate the oxygen consumption 3 eta divide by 2 sigma, there is sigma b gamma b 1 – eta – epsilon equal to 0 as no product has been formed other than biomass then we can calculate this is coming about 1.1 gram biomass gram of oxygen used.

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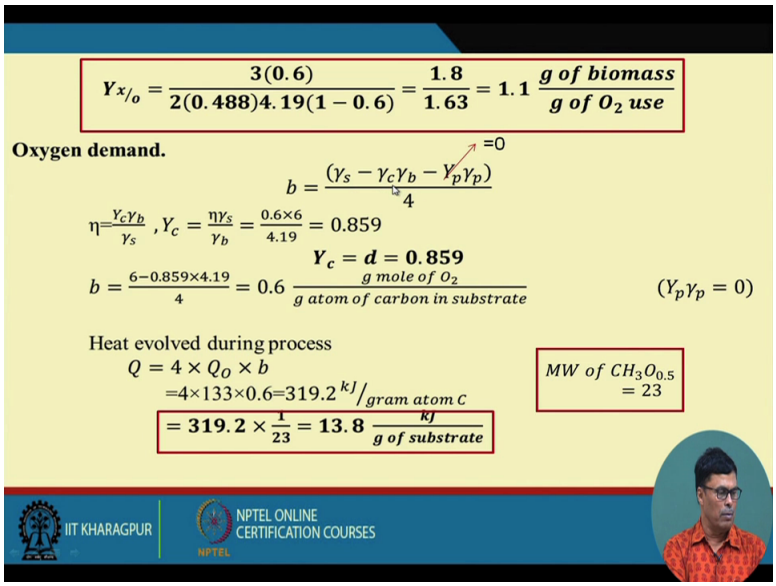

The oxygen demand b that can be calculated like this and b is coming around this is 0.6, this is gram mole of oxygen per carbon atom of substrate consumed so now what is the heat evolved in this reaction is equal to 4 into Q0 into b that is 4 into 133 into 0.6 that is around 319.2 kilo Joule per gram atom per substrate, now per gram atom substrate if you want to find out that per gram of substrate then we have to divide by the with respect to that the gamma atom molecular weight of the gram atom of substrate that is 23 and it is coming about 13.8 then kg per gram of substrate. So we can calculate the molecular weight of the substrate is about gram atom substrate is 23, so we can calculate like this.

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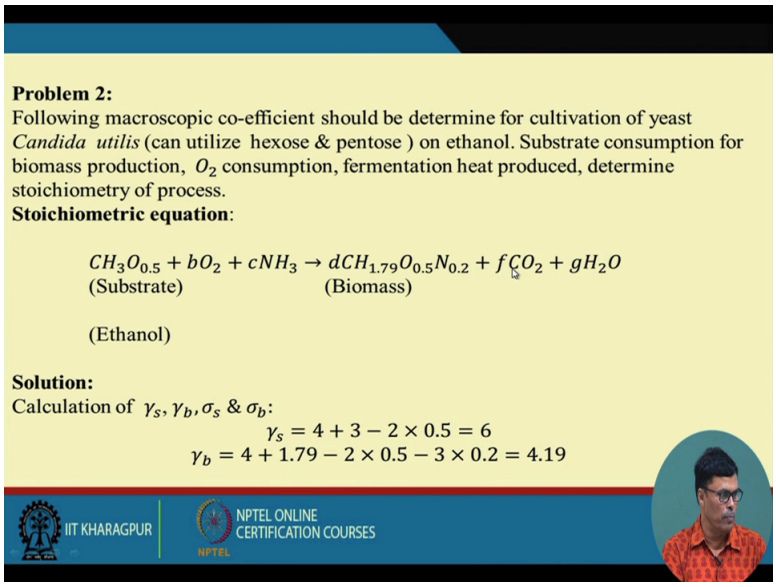

Now if you when you when you do the stoichiometric equation balance write the stoichiometry equation we can easily do the carbon balance. Now if you look at here this carbon is contributing at two different forms so if you do the carbon balance this is one carbon 1, 1 is equal to d + f am I right? d + f. So we have already can I calculate the d because yield coefficient already we calculated, so f equal to 1 – d we already calculated here.

D is coming here is point this is Yc we have Yc is the eta sigma s by sigma b, it is nothing but Yc is nothing but d this is equal to 0.859 and – this we will get the value we will get about 0.14, similarly we can do the oxygen balance, oxygen we have three different forms we have oxygen here oxygen here oxygen here oxygen here to all more less all excepting ammonia all places we have oxygen so we can make a oxygen balance.

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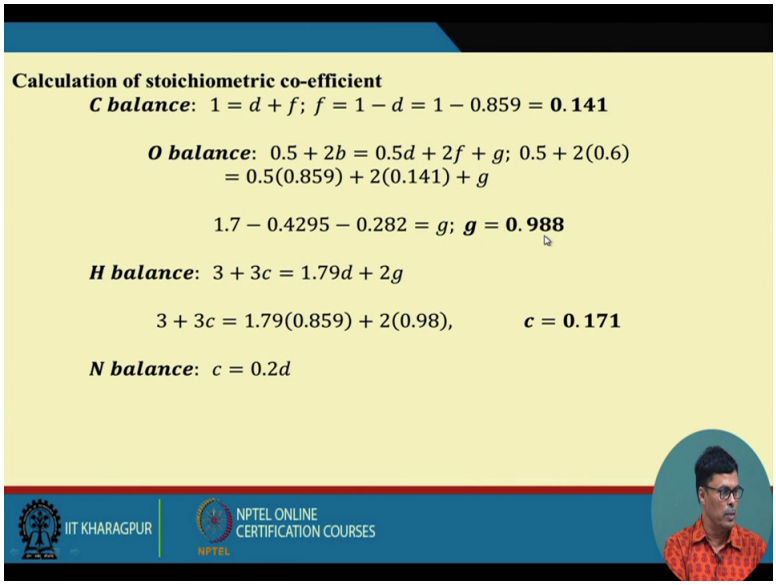

This is this is 0.5 into b into you see that how we can 2 into b because then 0.5 into d that 2 into f and g then if you this and this equation then we can find out g value point everything is known then we can find out g equal to this.

Similarly we can do the hydrogen balance also hydrogen we find here substrate ammonia and this biomass and water molecule then we can we can do this calculation and we can find out the c value is 0.15. And nitrogen balance is very simple, nitrogen only contribute for the for the biomass so this 0.2 into d equal to c so you know the value of d so you can find out the value of c. so you know once we know all this thing then we put this value that different coefficient we can put it here and we can put this equation we get the complete stoichiometric equation.

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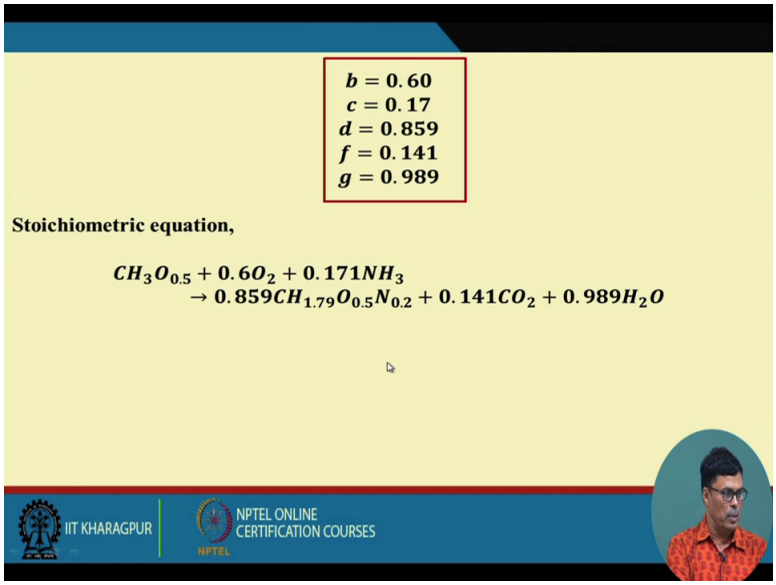

Now this why this equation is required because this gives you the idea of what is the how the different component contribute for the products and other by product formation process. This is very essential for the industry to find out the stoichiometry of this particular process.

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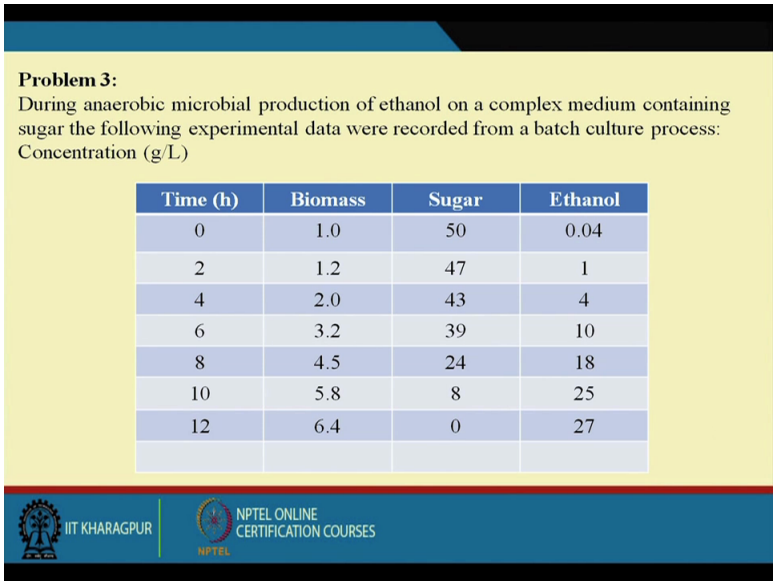

Time (h)	Biomass	Sugar	Ethanol
0	1.0	50	0.04
2	1.2	47	1
4	2.0	43	4
6	3.2	39	10
8	4.5	24	18
10	5.8	8	25
12	6.4	0	27

Now another thing I pointed out that stoichiometry not only give us the interrelationship of different molecules present in the reaction mixture but also gives the idea that on the validity of the experimental results, now this let us see how we can find out that. Now there is the problem this is called during the anaerobic microbial production of ethanol on complex media containing sugar the following experimental data were recorded from the batch culture process, the concentration is gram per litre this concentration is already in gram per litre.

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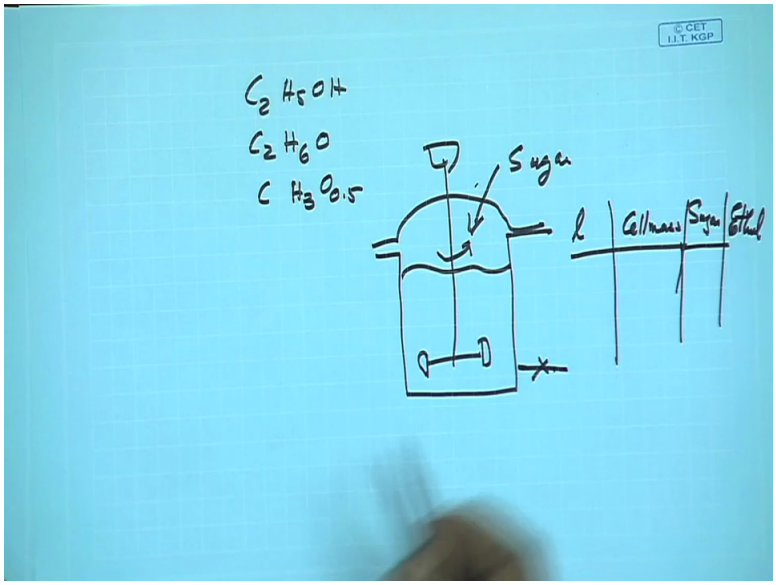

So this is the time of fermentation so this is a this is a batch process so batch process basically it looks like this, it is a batch process is like this so what do you do we put here ethanol we put here sugar in the medium and time to time withdraw the sample and we find out at different time what is the cell mass production what is the cell mass or biomass production then we have what is the sugar concentration and what is the ethanol concentration ethanol concentration that we have. So we can find out that you know different concentration we can find out in this reported here. Now the we shall have to find out the data that is reported here that is right or wrong and which data if you that require some kind of corrections, so take the data reported is correct or not so let us see how we can do the analysis.

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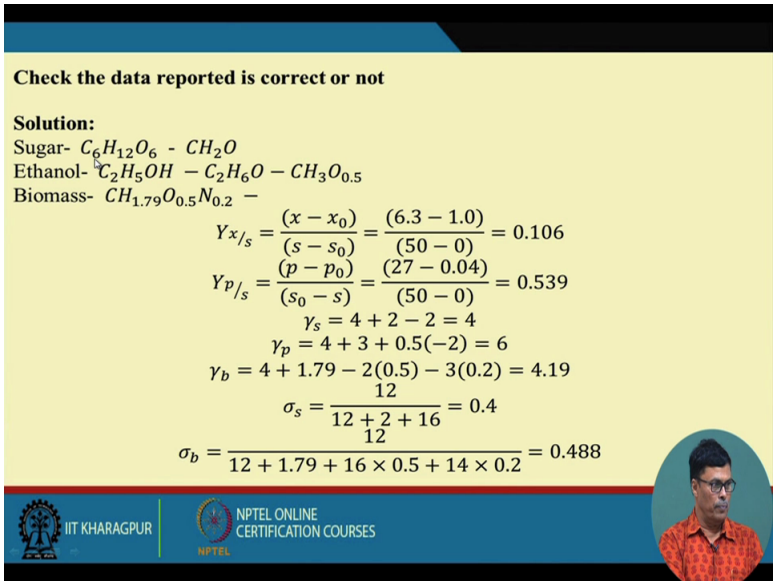

Now sugar has the formulae of that you know that sugar has the formula is what that is C20 C12 H22 O11 am I right. So this is the when it hydrolyse, it produce to this C6 H12 O6 this is in presence of water, it produce like this. So so this is this is consulate the carbohydrate this is C H2O so this is we write this empirical formula, this is in case of sugar, the CH2O, ethanol CH3 O 0.5 and biomass is a per gram atom carbon what is that that we found out. Now from the experimental results you see that we know the initial substrate concentration and final substrate concentration, initial product concentration and final product concentration initial biomass concentration and final biomass concentration that you know now since we know that we can easily calculate x – x0 there is biomass initial biomass concentration.

Now final biomass concentration and initial biomass concentration and it is the substrate concentration will be s0 – s that is initial substrate concentration where – final substrate concentration so this ratio is coming about 0.106. Now p by s is coming like this this is repeated mistake this should be s 0 – s and here it is p – p0 this is coming about 0.539, now from this empirical formula we can find out gamma s and gamma b and gamma p.

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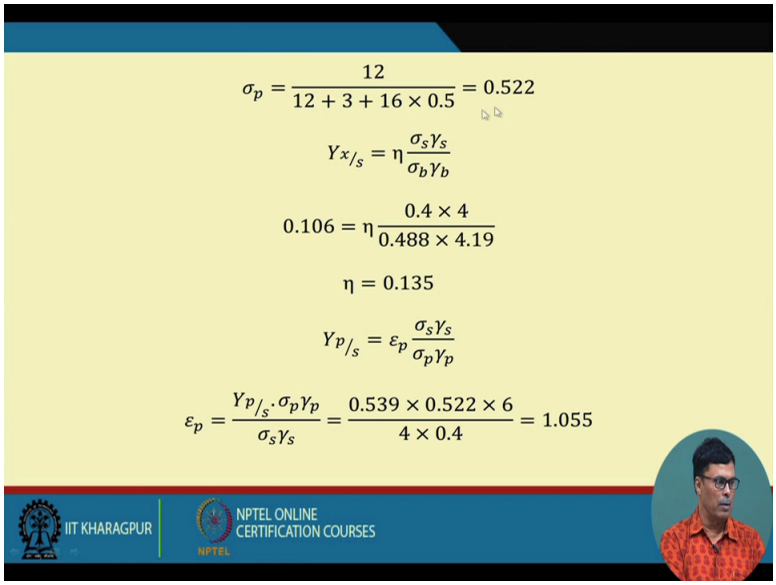

We can at the same time we can calculate the sigma, s sigma b and sigma p we can calculate and if you know that then you can find out Yx by s because eta sigma s, gamma s sigma b and gamma b so we can put this value and find out the what is the value of eta and surely Yp by s we know that then we can find out the what is the value of epsilon. Now we know that because this ethanol fermentation usually carry out under anaerobic conditions and under anaerobic condition we know the thermodynamics efficiency of the process is about 0.7.

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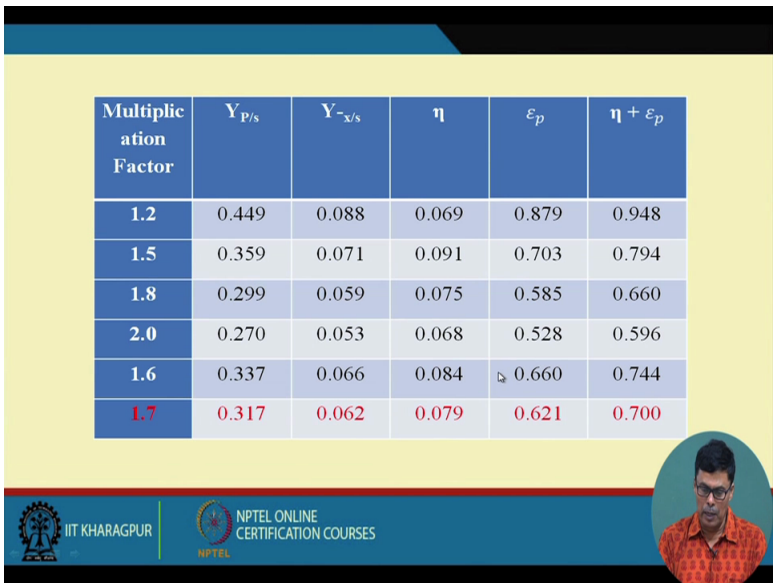

Multiplication Factor	$Y_{P/s}$	$Y_{-x/s}$	η	ε_p	$\eta + \varepsilon_p$
1.2	0.449	0.088	0.069	0.879	0.948
1.5	0.359	0.071	0.091	0.703	0.794
1.8	0.299	0.059	0.075	0.585	0.660
2.0	0.270	0.053	0.068	0.528	0.596
1.6	0.337	0.066	0.084	0.660	0.744
1.7	0.317	0.062	0.079	0.621	0.700

So now we shall have to check that whether this calculative value because what we reported here thermodynamic efficiency, this is equal to eta + epsilon ok now what we got this is about 0.135 + 0.1055, so 1055 so this is much greater than 0.7 because in the anaerobic forces this

is the anaerobic process, this thermodynamic efficiency is 0.7 so it is obvious that your results are some whatever data you have reported here that has some mistakes. So we shall have to find out that where is the mistakes now when we when we try to do that we find out that which component plays very important role may be might be having some error as for example that that you know ethanol we usually estimate by (())(21:54) we can find out the the ethanol concentration accurately.

So there will be there will not be much error we can expect that the estimation of ethanol does not have much of error and usually the sugar concentration in the lab we usually estimated by the DNAs method. And DNAs method is actually not for the sugar for actual sugar estimation this is used for finding out the reducing sugar concentration, so maybe may be due to some experimental error you know we get this some erroneous results here and then we try to what we have done we multiplied the substrate concentration with some other factor like 1.2, 1 0.5, 1.8 to 1.6, 1.7 different factors we multiplied.

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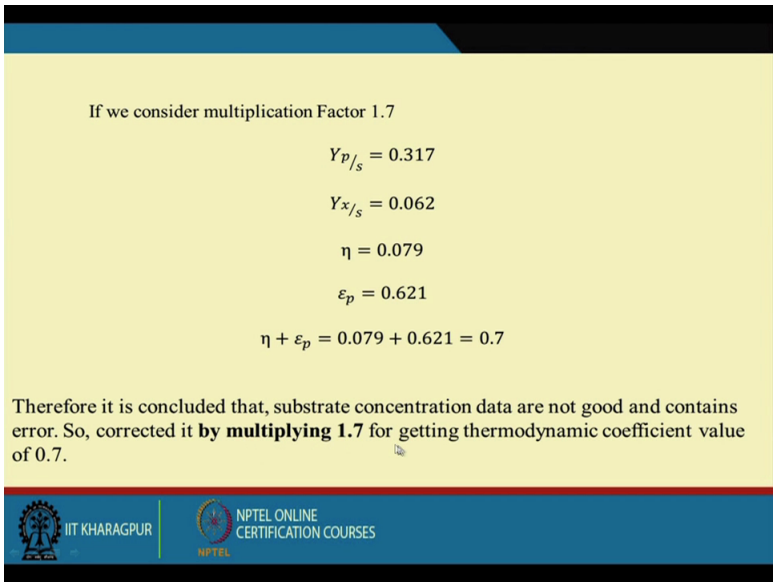

And tried to find out that what factor gives us the eta + epsilon value 0.7 and we find that when we multiplied by 1.7 substrate concentration then we get the eta + epsilon value epsilon p value that means that whatever data we reported for the cell mass concentration that is to be multiplied by 1.7 then we will get the correct results that is thermodynamics efficiency will be 0.7. This is how we can find out the validity of the experimental results.

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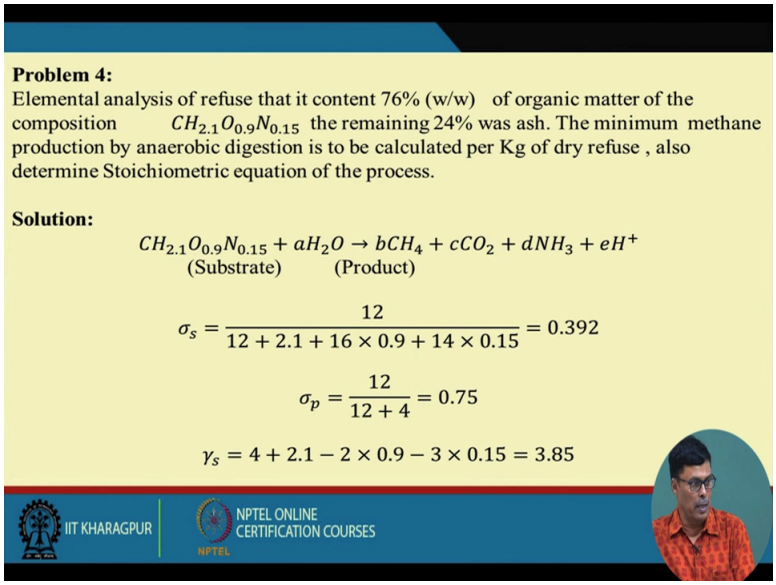

Now next problem that I want to discuss about the anaerobic digestion process which is largely in operation in the different places because we know that anaerobic digestion process we can we can produce methane and carbon-dioxide, and biogas we have largely used in different places not only in the industry nowadays of biogas is used for the operation of different bus and different vehicles, so this problem related to that and we have seen that this problem the elemental analysis of the refuse it contains about 76 per cent of weight by weight of organic matter and whose competition is this containing so 24 per cent is as.

And minimum methane produced by the anaerobic digestion is calculated by per kg of dry refuse also determine the stoichiometry of the process that you shall have to calculate. Now for the again I mention that whenever we shall have to do any kind of stoichiometric analysis first we shall have to know the process, in the anaerobic digestion process how we how we produced methane and carbon-dioxide because what is happening that organic residue comprises of lot of polymeric substances, this undergoes the hydrolysis as per example that glucose as per example cellulose, hemi cellulose starch and protein all these big big curricular even lipid there the bigger molecule.

They with the help of hydrolysing (())(25:29) like (())(25:31) then protease lipid they degraded to the smaller molecules then it will be becoming there will be it will be solubilised. After that this undergo the the with the help of (())(25:49) this undergo the metabolic pathways like embryonic myro pathway and then it produces different organic acid like (()) (25:57) organic acid like acetic acid, propane acid, butyric acid and then it is convert to

methane and carbon-dioxide. So basically that the reaction is that refuse reaction is first undergo hydrolysis then internally produce organic acid then produce methane and carbon-dioxide and also it produces ammonia and some kind of hydrogen.

So this is how we have written this equation, this is the substrate and then this is the water molecule then methane then it produce carbon-dioxide and ammonia and then hydrogen atom. Now here we have so we consider the methane as the product and we ignore we the cell mass produced because this since it is a anaerobic fermentation process we assume the biomass formation here is negligible.

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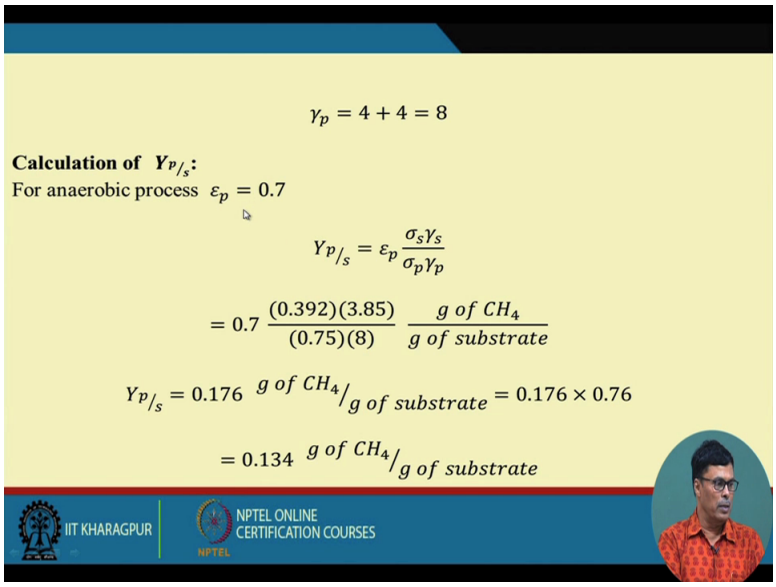

If you do that then sigma s we can calculate it with respect to this substrate and with respect to this product we can also calculate the gamma s and gamma p that we can calculate both the things we can calculate, then we can assume this anaerobic process epsilon p is 0.7 and if it is 0.7 we can we can easily calculate that how much gram of methane is produced per gram of substrate consumed because Yp by s is the yield coefficient, yield coefficient is the gram of product that is gram of methane produced per gram of substrate consumed.

So this is this is coming about 0.176 but this is this is we consider the per kg of organic biomass but organic biomass content about 75 percent the the total organic residual containing the 75 percent of organic biomass so we multiply by 0.76 then you will get 0.34 gram of methane per gram of substrate. So we can easily calculate how much minimum gram of methane can be produced. Then we can we can we can also find out this b value that different this component that we required for this we can one by one we can calculate we

know this equation, the b is a product that how much product that is Yc value we can easily calculate.

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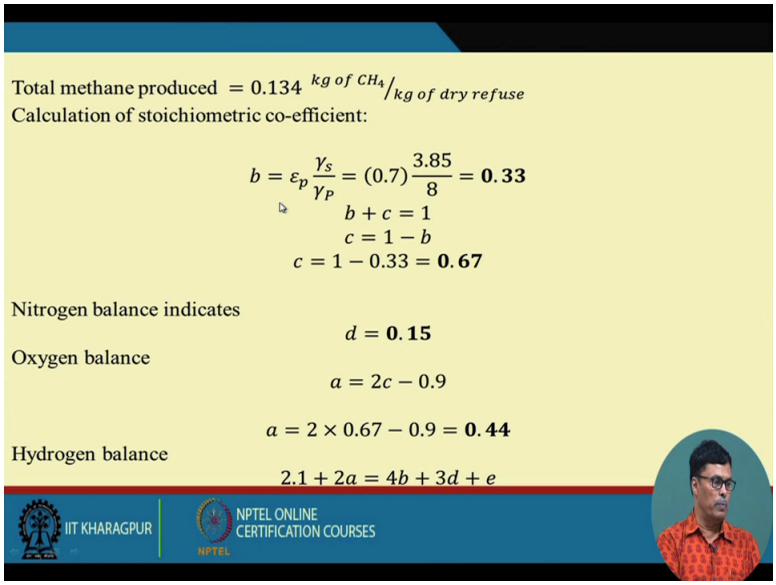

This is Yp value Yp value is we can calculate like this this ratio and once we know this b value we can find out the c value because b + you see that b + c, b + c carbon balance b + c equal to 1 and then we can easily calculate the c value like this we can find out the d and a value. And when you calculate all these values like before we put this coefficient here in this equation and we check the complete stoichiometric equation. So this is how we can we can we can write the complete stoichiometric equation, so this is very much required for all the fermentation process I hope this will be very useful for doing the material analysis of the process. Thank you.