Aspects of Biochemical Engineering
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Lecture – 07
Stoichiometry of Biochemical Processes-II

Welcome back to my course Aspects of Biochemical Engineering. Now, in the last lecture I started this stoichiometry of biochemical process. And this stoichiometry biochemical process basically deal with the intermolecular relationship of different reactant molecule t grip the product and also I told you this stoichiometry of bioprocess to gives the two additional thing, one is it help us to find out the validity of experimental results and this is on the basis of thermodynamic coefficient of the process because I told you in the aerobic process the thermodynamic coefficient varies from 0.5 to 0.6 and in case of anaerobic process 0.7.

So, on the basis of that, now in this lecture I just show you how we can do the validity of the experiment results and how we can solve some numerical problems, how we can develop some kind of stoichiometry of kind of chemical biochemical processes.

Now, again let me point out that whenever we do any kind of stoichiometry analysis I told you first of all we shall have to find we shall have to have the idea of the process if you do not have the preliminary idea of the process it will be very difficult to analyze the system. So, I hope this would be very clear because coming to lectures I shall be concentrate on solving the different problems.

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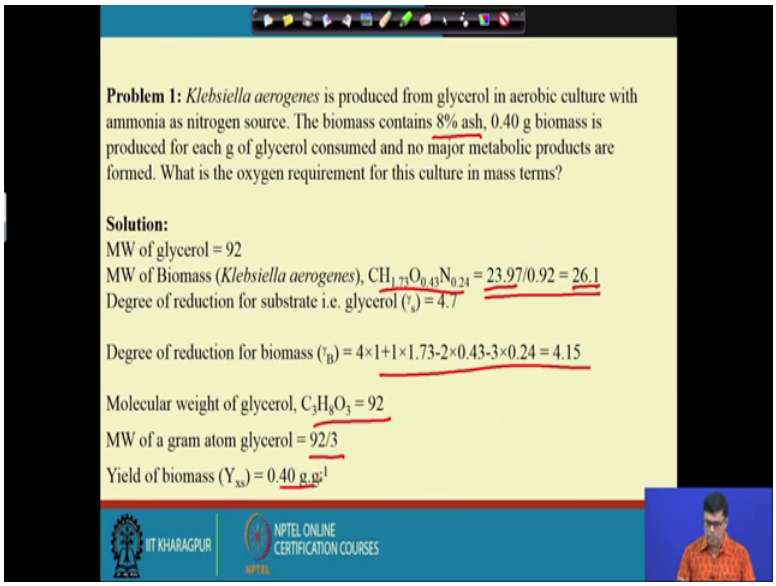

The first thing that I want to point out this particular problems the Klebsiella aerogenes is produced from glycerol in aerobic culture with ammonia as nitrogen source biomass content 8 percent of ash 0.8 gram of biomass is produced for each gram of glycerol consumed. So, no major metabolic products are formed. What is the oxygen requirement for this culture in mass terms?

So, basically this equation deals with that a glycerol is used as a carbon source for the production of biomass and here the biomass that is used that is the clips layer erosions that is the cell mass that is used and in this process that we get 0.4 grams of biomass is produced per each gram of glycerol forms.

Now, question comes how we can solve this problem that let us try this.

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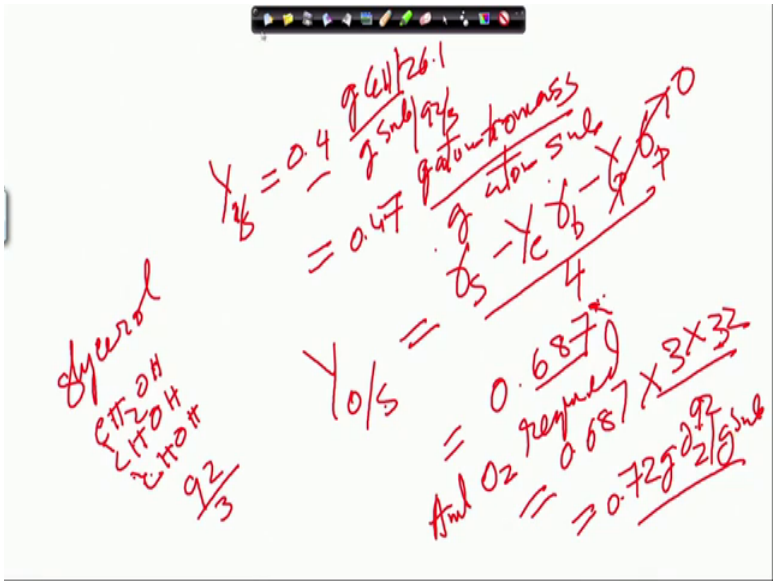

Now, as you know that you know that yx by s is equal to what 0.4 am I right, 0.4 is the Y x by s and what is the glycerol formula, glycerol is CH 2 OH, C H O H, C H O H am I right. So, this is the molecular weight of 92. Now, how many carbon atom is there? 12 3. So, a per atom per gram carbon atom how much you will be, what will be the molecular weight of 1 gram atom this glycerol? 93 by 2.

So, what I can do, this is 0.4 means gram of cells per gram of substrate am I right. Now, per gram if I can; what I can do I can divide that with respect to this molecular weight. So, this is a cell we know the molecular weight of the cell the formula and the last previous lecture I have already shown the average formula of the cell mass. So, if we take the molecular weight this is around 26.1 gram and this is 92 by 3. So, this will come around 0.47, 4 7 means that is this is gram atom biomass or cell biomass or cell whatever we considered by gram atom of substrate. This we can easily find out.

Now, Y o by s, Y o by s is what? That a gram of oxygen required per gram of substrate consumed it can be written like this is gamma s minus Y c into gamma b minus Y p and gamma p divided by 4. Now, if we here we do not have any product rather than biomass. So, we can assume this is p is 0 because p we have only biomass is the product. Now, if you put the value here you we will get this is 0.687. So, amount of, amount of o 0 required, o 2 required how we can calculate? This is 0.687 multiplied by 3.32 divided by

this is 92, this is 92 by 3 and this is 32. So, if you wonder this is coming around 0.72 gram oxygen per gram substrate. So, we can easily calculate.

So, let me repeat it again how we have solved. This in this problem I told that Klebsiella aerogenes is produced and this picture is given this is 0.4 gram of cell produced per gram of substrate. Now, if you want to convert it that how much a gram atom of biomass produced per gram atom substrate simple we do can divide by the molecular weight and then we can, we have this why you this that oxygen requirement per gram of substrate this is the formula that we have that we have already been the last lecture we have seen that. So, if we put the value of gamma s then gamma b here and Y c and by divided by 4 we will get this figure.

Now, this is the actually, this is the gram of oxygen required per gram of, this is that the moles of oxygen required per gram atom of atom of substrate. Now, if it is like this, what is the molecular weight of oxygen? This is 32. What is the gram atom molecular, gram atom one gram atom of substrate? 92 by 3. So, if you divide that we will get 0.7 2 gram of oxygen that is required per gram of substrate consumed.

Now, in this program if you go to the text I hope everything will be clear. Now, here what we have seen this is the, there is a formula of the biomass that we have if we assume that and this is how we have got the molecular weight because; how you got the molecular weight this is the actually the molecular weight, but it contains the 8 percent of ash. So, you divided by 0.92 we get the 20, so when 26, 100 gram. So, this will be the actually the molecular weight of the, this is the total mass of the biomass that we had.

Now, now here that degree of reduction that we can easily calculate like this and then molecular weight of glycerol I showed you this and this is the equation that we have then Y c, that is holiday calculi showed you this calculations and this is how we can do it here.

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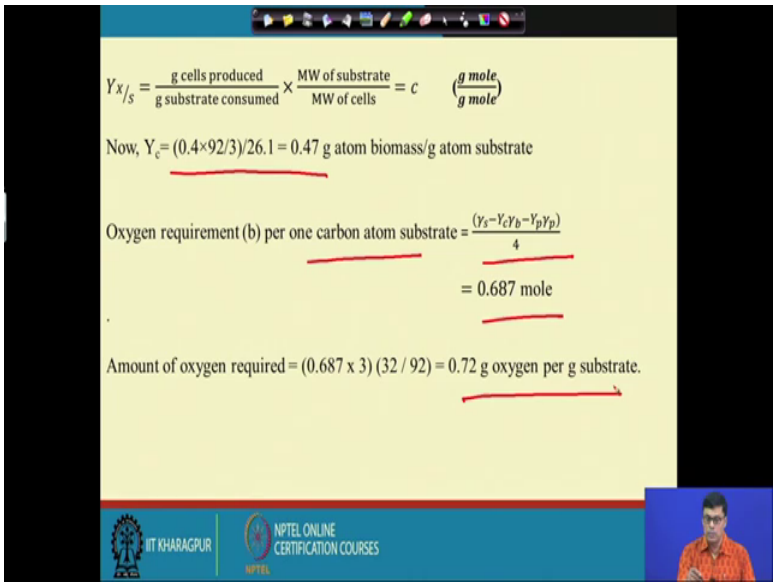

This whole calculation we have done it and this is per gram atom of carbon oxygen requirement this is the equation that we have, this is moles of oxygen they produce per gram atom of substrate consume. The mole so, the molecular weight of oxygen is 32 and then 72 by 3 to 3 is the gram molecular, molecular weight of 1 gram atom of substrate if you multiply you will get this substrate that how much oxygen required per gram of substrate. Now, this is how we can do that.

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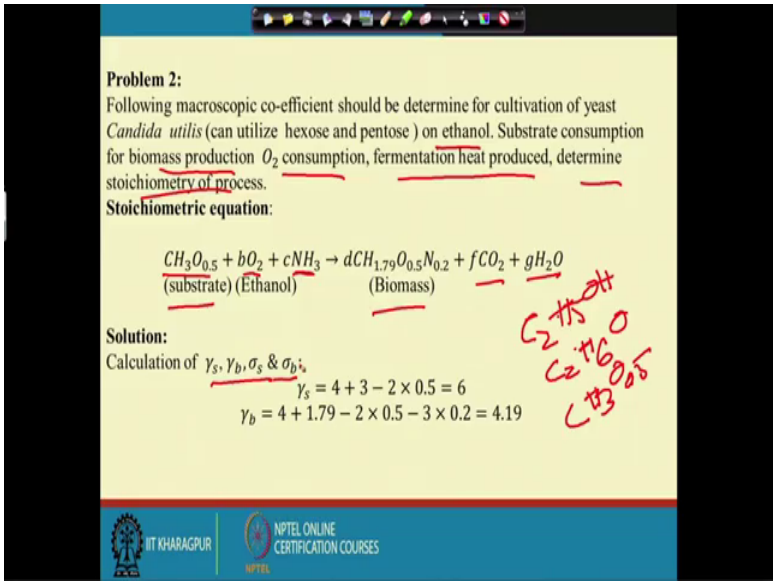

Now, next problem that we have this is little bit different that following the macroscopic coefficient should determine for cultivation of yeast Candida utilis on ethanol. The substrate consumption of biomass production oxygen consumption, fermentative heat produced, again determine the stoichiometry of the process.

So, here therefore, the question is little bit similar as compared to the previous equation. The reason is that in the previous problem we have considered glycerol as a carbon source. Now, an organism was Klebsiella and here in the we are using the ethanol as the carbon source and organism is Candida utilis, here we shall have to call the calculate not only the oxygen requirement, but also we shall have to calculate how much is the formula the heat evolved take place, how much substrate consumption take place, how much the what will be the stoichiometry of the. So, complete a complete analysis of the process that is to be taking place in this particular problem.

Now, as I told you as, I pointed out that when you analyze any kind of biochemical process first we should have that preliminary idea that how this process work. Now, here this is the substrate this is, what is the formula of ethanol? Ethanol formulas C 2 H 5 OH am I right. Now, this is this I can write C 2 H 6 O. Now, if you divide by 2 it is CH 3 O 0.5 this I can write. So, you know this is how it has come here. This is CH 3 and 0.5 then oxygen this is aerobic process and ammonia required for the cell mass formation and we get the biomass and also a carbon dioxide and water. Though this is idea should we should have what would be the reactant and what would be the product then and only then we can analyze the process also otherwise we cannot analyze the process.

Now, here we can degree of reduction for substrate biomass and we have that carbon atom the ratio, carbon mass ratio that of the substrate and biomass that we can easily calculate.

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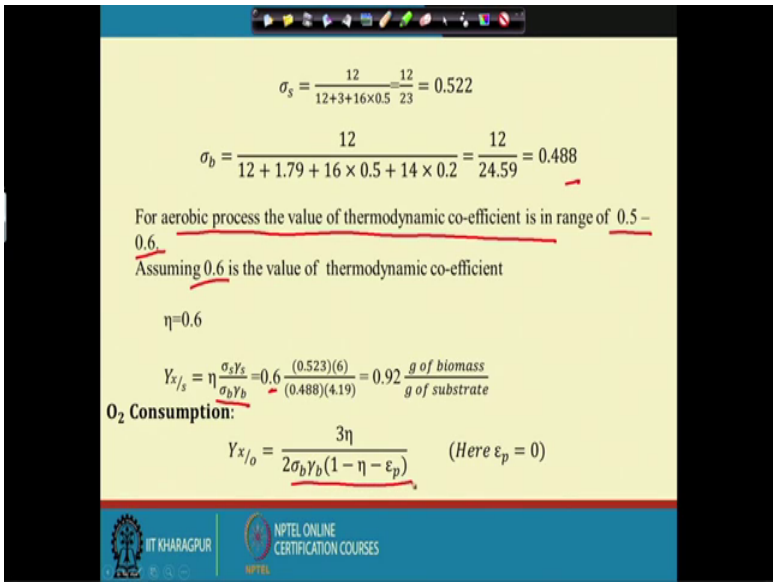

Now, this is sigma s and sigma b how we can calculate this has been shown here. Now, as you know I told you that aerobic process thermodynamic coefficient varies from 0.5 to 0.6. So, I can assume this is 0.6. Now, if it is 0.6 then I can this is Y x by s equal to this formula we know. So, we put the 0.6 way this we calculate at 0.92. Now, Y x by o it can be calculated 3 eta by this.

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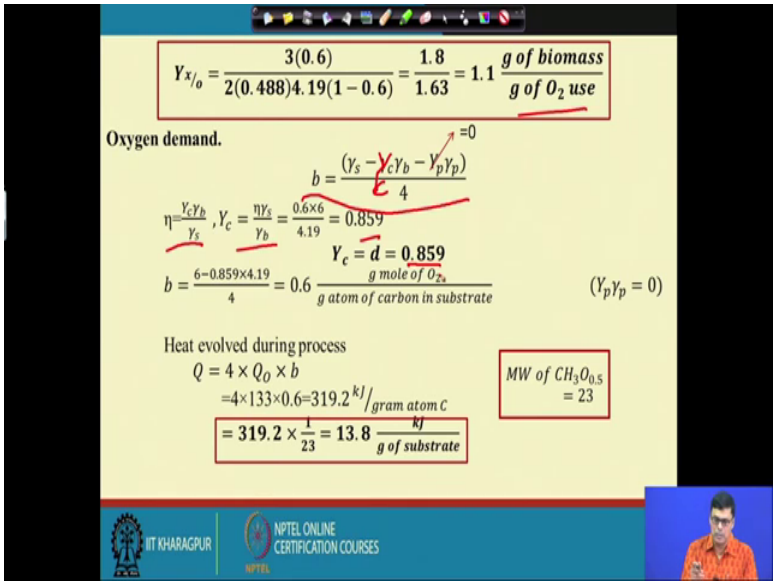

Now, then we can calculate as a gram of biomass produce per gram up gram up oxygen used that we can calculate from this. Here we can calculate, then we can calculate the

oxygen demand this we have done in the previous equation also, only the thing is that here it should be Y c naught gamma c, this Y c is this and eta is this, so we can calculate this. Y c is coming about this 0.856. Now, here if you look at this formula though we are talking about d Y c means this is here it will be d. So, this is this is the actually Y c, Y c is the gram atom of biomass produced per gram atom of substrate consumed.

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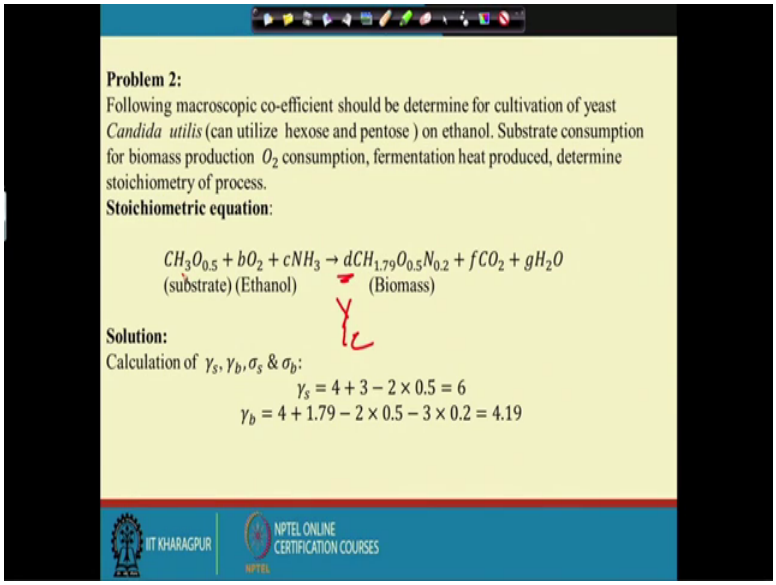

So, we can easily calculate and then from this is, that is why we have written this is equal to d and this is the 0.85. Then b we can calculate from this equation we will find out this and then we can find out that how much it evolved, if you this is 0.6 we get and then we 0.6 if we put it is gram atom of a carbon substrate and then I told you this we can convert to gram atom of biomass formation, you simple divide by the yield coefficient you can do it very easily.

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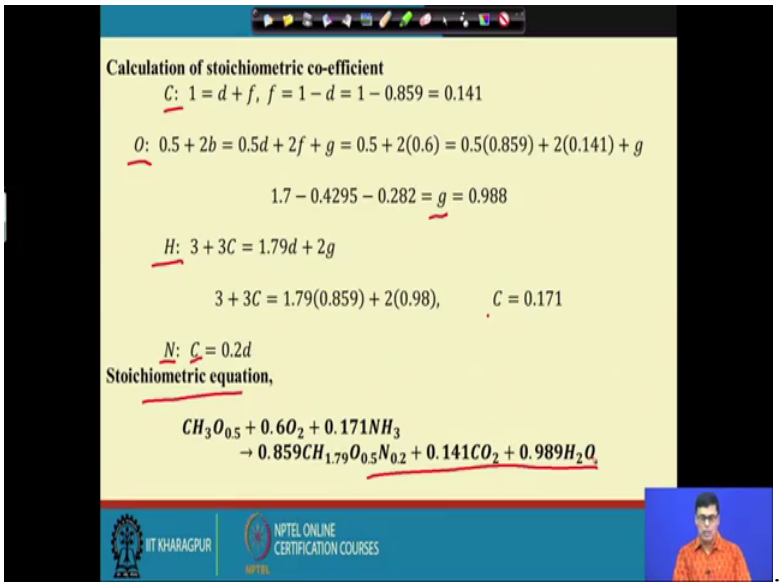

And then this is the simple elemental analysis. If you are in this equation if you do the elemental and this is the carbon balance, this is the oxygen balance, this is the hydrogen balance, this is the nitrogen balance. If you do, you will get all the coefficient value, C value we can, g value different coefficient value and if we put this stoichiometric equation we will find this equation is CH 3 0.6 O 2 and this you can find out.

So, this will give you some idea that what is the theoretical yield of the biomass from gram atom of substrate that we can easily calculate. So, you have to what I suggest you that you should practice it. Until unless you practice it will not be clear to you because it is simple this material analysis of the process if you do the material analysis of the process you can do it very nicely.

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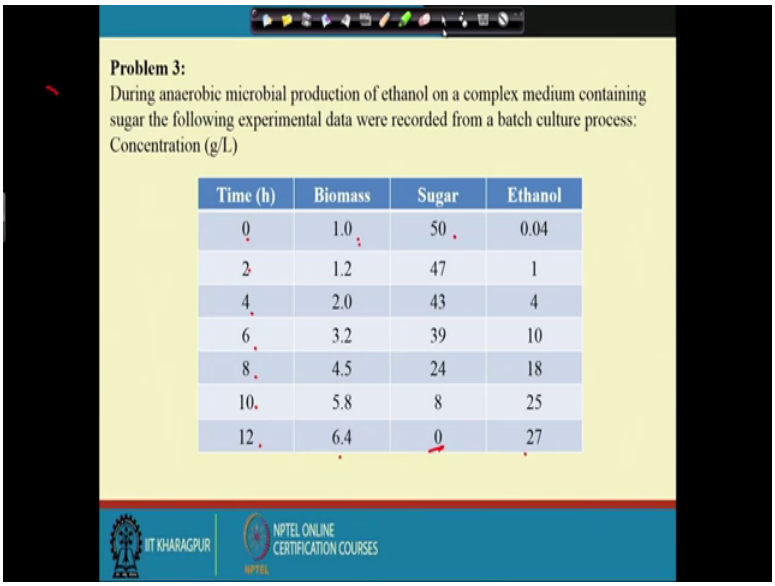

Time (h)	Biomass	Sugar	Ethanol
0	1.0	50	0.04
2	1.2	47	1
4	2.0	43	4
6	3.2	39	10
8	4.5	24	18
10	5.8	8	25
12	6.4	0	27

Now, as they as I pointed out that you know that stoichiometry of the bioprocess also give you the idea that about the validity of the experimental. Now, this particular problem deals with that. Now, what is this problem? Problem is that during the anaerobic microbial production of ethanol on complex formula media containing the sugar the following experimental data were recorded from a batch culture process whose concentration is this.

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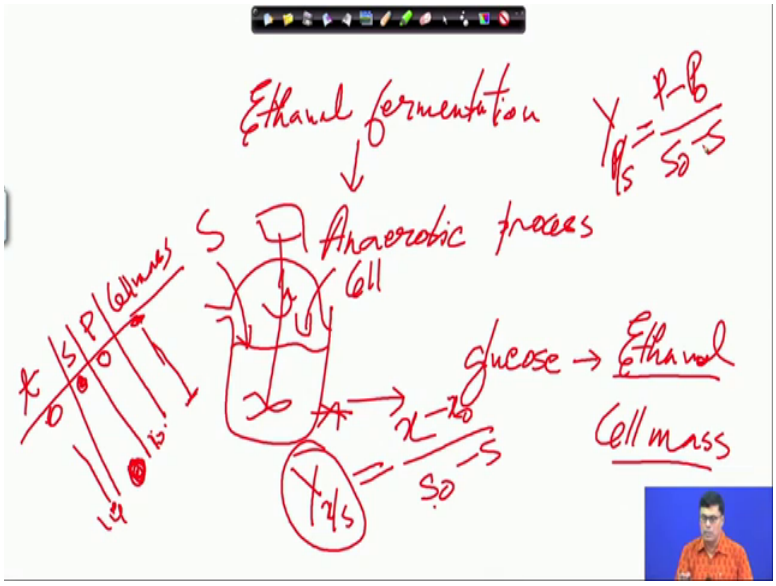

So, here I want to point out like this that ethanol fermentation, this is considered as anaerobic process. Now, I have already I explained that anaerobic process means that it organism does not required molecular oxygen for their growth and metabolism. Not only that in case of obligate analogue this molecular oxygen will be harmful for them. Now, these ethanol fermentation process that we carried out by the help of saccharomyces cerevisiae.

Now, present problem deals with that. So, it is, suppose this is a reactor this is a reactor in this reactor we put your substrate and put your cell mass and under anaerobic condition you run this process and what will be your product, your glucose will be converted to ethanol and also you it converted to a part of the cell mass. But one thing I want to point out here that since it is the anaerobic process the amount of cell mass production will be very less, because in case of aerobic process, repeat the aerobic then cell mass production will be much much higher ethanol will not be there. So, only under anaerobic condition ethanol will be produced.

So, in this present problem what we have done. We have we have carried out this experiment we draw the sample here and we prepared a table and this is with respect to time we find out the substrate concentration, we find out the product concentration, we find out the cell mass concentration, we find out that. So, we can we add different times we can find out the different this concentration.

Now, from that we can suppose this is the 0 then we have slowly slowly you come to the may be you have 14 hours I do not know what is the. So, initially you have some substrate concentration. So, then finally, if the substrate concentration usually should be 0 and product concentration easily 0 initially then finally, you have some substitute is converted to products cell mass you have small amount of cell mass concentration which is present at the inoculum and finally, this will little bit increase. These are the things we have.

Now, here we can easily calculate what is the x by s value. How we can calculate? If you know that what is the final x value and we know the initial x 0 value and we know s 0 minus s value, s 0 is the initial substrate concentration, s is the final substrate concentration here, so we can find out easily that what is the biomass in it.

Similarly I can calculate Y p by s that also you can calculate. Now, how we can calculate? This is p minus p 0 divided by s 0 minus s. So, I can easily calculate the, this is the product yield. So, this is how, this formula looks, this is how this you see that how this problem. This is the this is different time 0 to 2 hours, 4 hours, 6 hours, 8 hour, 10 hours and 12 hours as the biomass concentration keep on rising. This is initially it is 1 gram per liter and finally, it is 6.4 gram per liter. Sugar concentration was 50 gram per liter and finally, it is 0 and ethanol 0.04 when it is some amount and finally, it is 27 grams per liter.

Though this is the question that we have and we shall have to find out whether these experimental results the data that is reporting in this table whether it is right or wrong. This we shall have to find out. Let us see how we can find out how that the validity of the experimental results.

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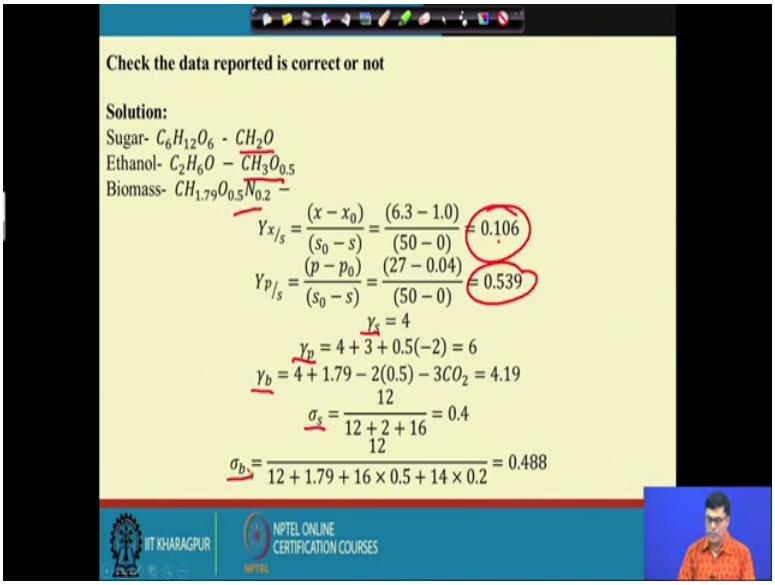

Now, what we can do first that this is the substrate, this is the glucose is converted the glucose if we consider per gram atom carbon, this is CH 2 O you know that. Now, ethanol also C 2 H 6 O this we can write CH 3 O 5 and biomass composition this average biomass composition is reported to this we can take into account. So, now, from the experimental results we can find out, from the experimental results we can find out what is the value of Y x by s what is the Y p by s this is the initial cell mass concentration you can sense the, you can find out this. You see that this is initial cell mass concentration,

this is the final cell mass concentration, initial sugar concentration, final sugar concentrate, initial the ethanol concentration, final that ethanol concentration.

So, here if you put this data we will get this two picture, one is that cell mass yield 0.106 gram of cell per gram of substrate consumed, Y p by s is 1.539 gram of ethanol per gram of substrate consumed. So, and then we can calculate from this empirical formula from this gram atom of carbon we can easily calculate what is the gamma s value, what is the gamma p value, gamma b value. We can calculate similarly sigma a sigma b and sigma p we can easily calculate that we can do the calculation.

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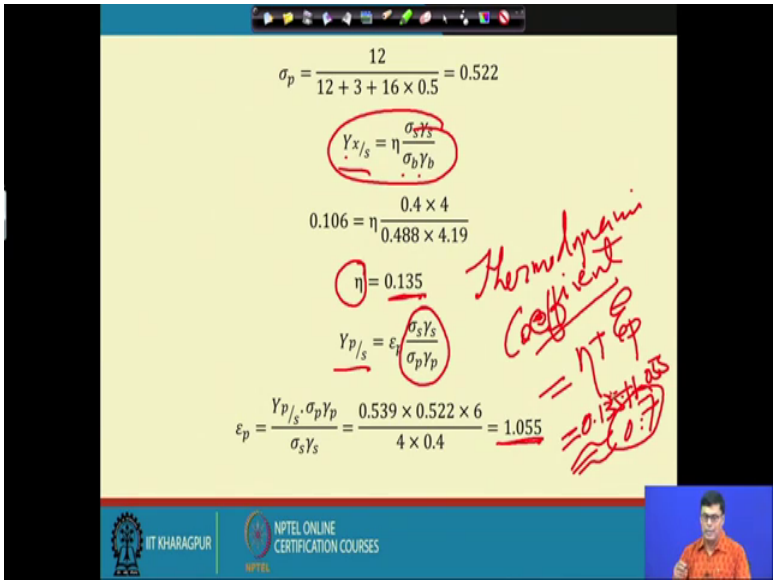

Though this is the value we obtained and then this is Y x by s equal to what this is the equation we have seen previously. Now, what we shall have to do? We shall have to put all the values this is the experimental results and this is the degree of reduction of substrate and carbon fraction of the say substrate and this is the carbon fraction of the biomass and degree of reduction of the biomass. Then if you put the all these values here you can find out the eta value.

Now, what is the eta value you get? It is 0.135 and then Y p by s I can write that is epsilon p equal to gamma s sigma s by gamma p by sigma p. So, again if this value we have already calculated and this value already we also calculated from the empirical formula and so, if we put all these values then we find this is 1.055. So, what you know that thermodynamic, what is the thermodynamic coefficient? What is the thermodynamic

coefficient? This is equal to eta plus epsilon p. So, what is the eta value? This is the eta value and this is the epsilon. So, it is coming what, this is 0.135 plus 1.055. So, we know that the anaerobic process the thermodynamic coefficient is how much? Thermodynamic is about this is about equal to 0.7. So, it is much higher than 0.7 am I right.

So, it is 1.131; 188 that is you know it is quite high value. So, it is clearly indicate the data that is reported here that is not preferred. There is some mistake in the data because this is not following the rule that you know that thermodynamic coefficient that we have or the anaerobic process.

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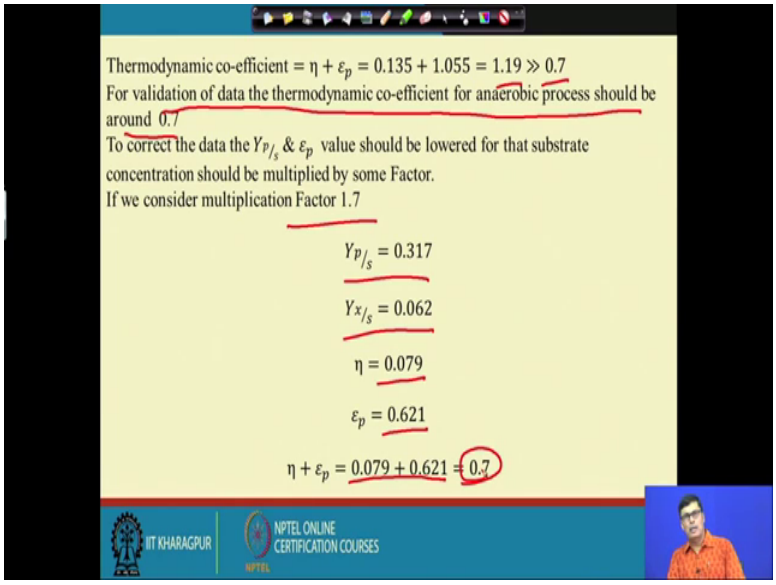

What we have what we have done, this is exactly here it is pointed out this is 1.19 which is more than 0.7. So, for validation of the data of thermodynamic coefficient anaerobic process should be done. Now, how we can validate? That you know what is this we shall have to find out where we might have committed the mistake.

Now, in the anaerobic digestion process what we have, we have what we estimated, we estimated the glucose concentration, we estimated the cell mass concentration, we estimated the ethanol concentration. Now, usually cell mass concentration estimated with the help of gravimetric analysis. It does not have much a mystic and say glucose estimation process we do colorimetrically and in case of the ethanol we usually do with the help of a gas chromatograph with very microscopic because a gas chromatography results are very reliable because it manually estimate the ethanol concentration.

So, what we can expect that by out of this data we can expect the colorimetric analysis of the substrate might be wrong. So, what we have done, we multiplied to some factor because with the substrate concentration and try to find out that what is the respective value of Y p by s and Y p by s and Y x by s and what we have observed if we multiplied by substrate concentration by 1.7 then Y p by base value is coming about 0.317 and Y x by s is 0.062.

So, then if we calculate the eta, eta value and epsilon p value is coming 0.079 and epsilon p 0.621, if you some do the two thing is coming about 0.7, but then and only then that results will be. So, form that we can conclude that if there is a something wrong in the substrate estimation and if we multiplied this substitute concentration by 1.7 then and only then your results will be ok.

This is how we can we can find out the validity of the experimental results. So, in this lecture I try to talk about how we can do the analysis of the biochemical process. By here I have given two example, one is the with respect to cell mass formation using the aerobic process and there is the anaerobic process, the anaerobic process we convert the carbon to cell mass and the anaerobic process we convert carbon to cell mass as well as the product. Finally, we try to find out how this, stoichiometric analysis help us to find out the validity of the experimental. I think from that you will get the idea that how this stoichiometric equation can be applied. Also we calculated the heat evolved for the production of per gram of biomass, per gram of substrate consumed that also can be easily estimated.

Thank you very much.