Industrial Biotechnology
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Module 2
Lecture No 9
Stoichiometry of Bioprocess

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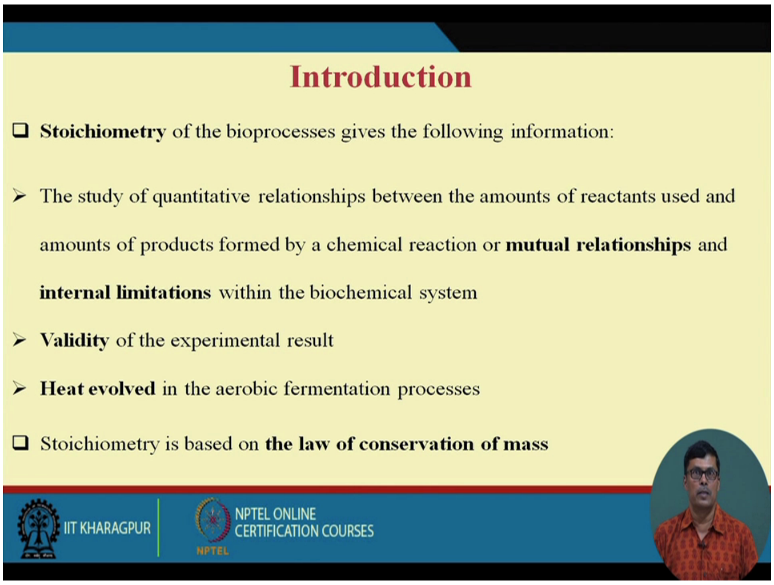

Today I am going to discuss the Stoichiometry of bioprocess, now if you look at the stoichiometry that of the bioprocess that give the three different information. First it give the information to study the quantitative relationship between the amount of the reactant used and the amount of product formed by a chemical reaction or mutual relationship and internal limitations within the biochemical system that means what we want to see how much of substrate react to give how much of products that information we can generate.

Then this also gives us the information about the validity of the experimental results this is this I shall show you how it can be done and another is the heat evolved in the aerobic fermentation process as you know most of the most of the biochemical reaction their (()) (01:23) in nature during the reaction some heat evolved takes place. So most of the biochemical reaction usually most of the microorganism they grow to push to the ambient temperature and atmospheric pressure.

So if the temperature shoots up particularly during summer we are in the topical country and summer about temperature as high as 40-45 degree centigrade and quite as the temperature of the fermenter is 30-35 degree centigrade naturally the temperature shoots up so we required some kind of kind of (())(02:00) arrangement. So if you want to calculate how much heat is evolved during this process that we can easily find it out and Stoichiometry is basically based on law of conservation of mass.

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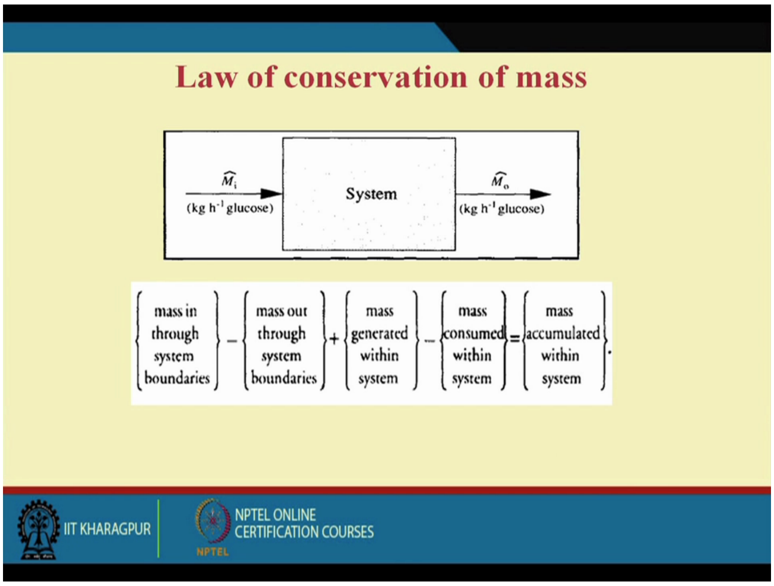

That if you if you look at this equation, the things will be very clear that mass in in through the system mass in through the system boundary what is coming in and mass out through the system boundary. So something is coming in and mass is coming in and mass is going out and and + mass generated within the system – the mass consumed within the system that is equal to mass accumulated within the system.

This is a kind of law of mass balance equation that we have in this particular system, now the elemental balance is we have a material balance on biological reactions can easily be written when the composition of the substrate product and cellular materials are known. So here I want to tell you that whenever we want to I want to do something of stoichiometry of bioprocess we should have some kind of information that what exactly going on in the biological process then and only then we can we can we can do the stoichiometry.

As for example suppose we want to use some kind of carbon source suppose we want to use glucose for the cell mass production, so glucose is converted to cell mass if not producing other than the cell mass but glucose when you produce ethanol we are targeting only ethanol not the cell mass. So literally that our Stoichiometry equation will be little bit different so this is very important.

Usually the electron-proton balance are the required in addition to elemental balances to determine the stoichiometric coefficient of the bioreactions. So this is I shall show you how it can be done, accurate determination of the composition of cellular material is a major problem. Typical cellular composition represented by CH 1.8 O 0.5 N 0.5, so biomass composition we approximately we approximated if we find it is variation is + – 5 percent so it is it is quite acceptable and one mole of biological material is defined as the amount containing one gram atom carbons such as CH alpha O beta and N delta. Now here the elemental pay balance I have shown in a particular equation.

The microscopic mass balance of the microbial system concerning the biomass production and another product can be written in its original form this is the substrate, I can write in the elemental form this is you call empirical formula, but this is on the basis of per carbon atom substrate, per carbon atom biomass per carbon atom product.

So this is as you know that for the formation of the cell mass we required nitrogen source and also most of the biochemical process they are aerobic in nature we required oxygen. So it gives the biomass then it gives the product then due to respiration and other reactions it produces carbon-dioxide and water. The composition of substrate biomass and the product in the equation are expressed by the elemental chemical analysis.

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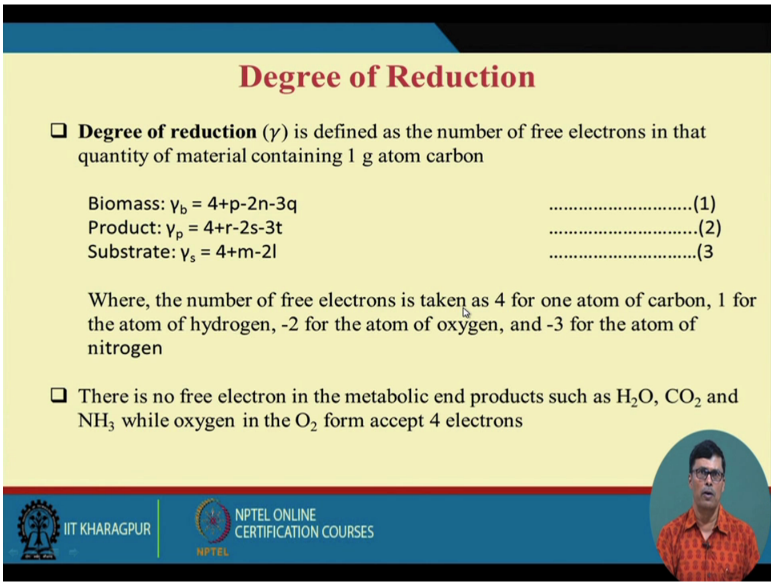

Now we come across one new term that is called degree of reduction. Degree of reduction means number of free electrons in that quantity material containing 1 gram atom carbon that I can I can tell you as per example biomass if you look at the biomass now in the biomass this is this is the formulae of biomass now this is the carbon, hydrogen, oxygen and nitrogen. Now what do you what do you what do you consider that in case of free electron if you look at in case of carbon we have 4 free free electrons.

In case of hydrogen we have 1 free electron and in case of oxygen it always take 2 electrons because it has 6 electrons in the outer most orbit, it can take 2 electrons and – the 3 electrons accept by the by the nitrogen. So this is how we can write the free electron balance now if we if we consider this is biomass (())(06:52) the (())(06:51) little bit hot 4 + P – 2 N – 3Q here what exactly you can you can find this was like this and similarly product also we can we can right click like this.

If you look at the product that is it is 4 + odd – 2S – 3t, in case of substrate what will be there 4 + M – 2L so this is this is how we can write that how much free electron present per gram atom of biomass per gram atom of product and per gram atom substrate, but there is no free electron of the metabolic end products such as the water, carbon-dioxide, ammonia.

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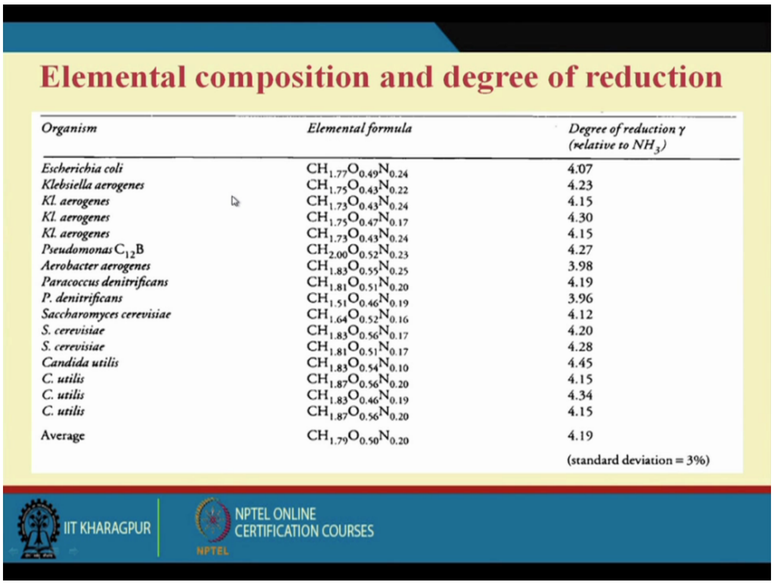

While oxygen in the form of O2 accepts 4 electrons so this is the basis on which we can do the Stoichiometry analysis so this is the this is the this is the point that we should have to keep it in mind when we do the Stoichiometry analysis of the bioprocess. Now I have given some typical examples in this table you can see the different biomass and I have given the empirical formulae of the biomass and you you see that if you if you do the do the free electron analysis of this biomass. You will you will get this figure so degree of reduction of the different biomass is different though they are very close to each other but they are different from each other, standard deviation is 3 per cent.

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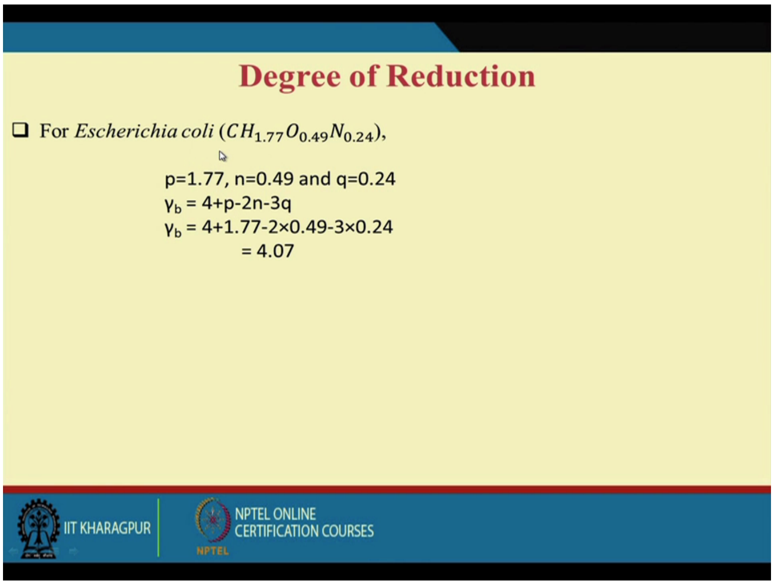

Now we have taken the example of one particular biomass what you call Escherichia coli, the recoil is largely used and its formulae estimated as CH 1.77 O 0.49 and N 0.24 now P will be what P one carbon 1.7N is 0.499 uses. So we can if you write the degree of reductions so it is what will be 4 + 1.77 – 2 into point 449 – 3 into 0.24 so it is coming 0.47, so we can easily do this calculation.

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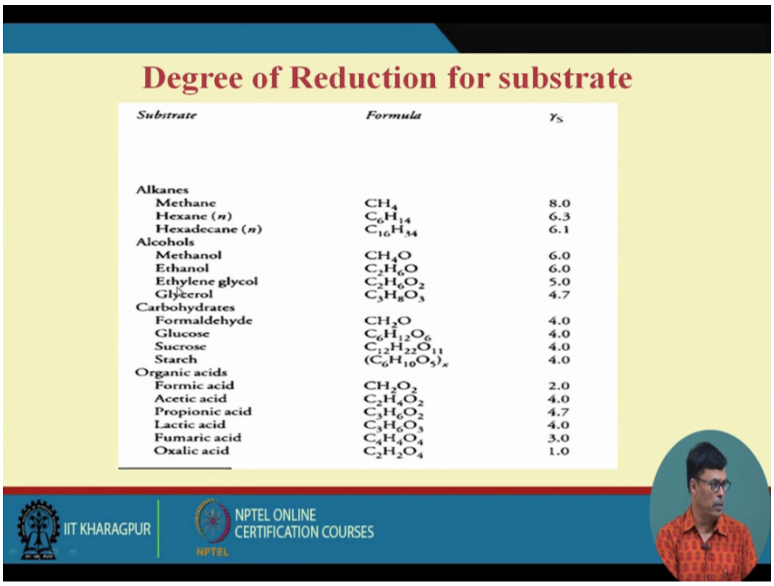

Now I have given other examples of different substrates as per example alkanes like methane, hexane, hexadecane, then alcohol methanol, ethanol that we have ethylene glycol, glycerin also different you can you can easily do by yourself and check whether you are coming have you are getting this figure or not.

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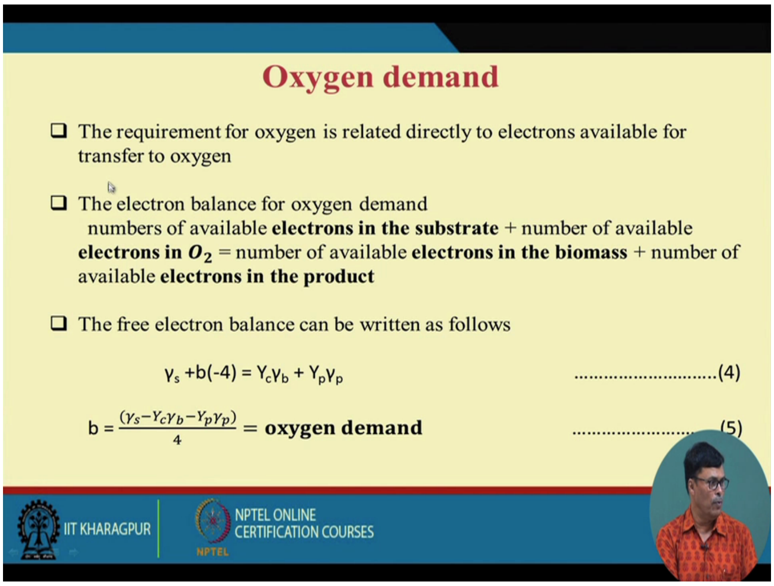

Now the oxygen requirement is directly related to the electron available for the transfer to oxygen because this is very important from these we can easily we can we can as I shall show you how you can find out the heat evolved in the fermentation process.

Now if you want to do the electron balance of this equation that we have we have the previous equation that we have here that we have substrate we have we have we have oxygen we have biomass, we have we have product because I already told you ammonia the free electron of ammonia will be 0, free electron of carbon dioxide is 0 and free electron of water is 0. So if you do this carbon free carbon balance then how you do that, number of available carbon in the substrate + number of available carbon in oxygen equal to number of available carbon in biomass and number of available carbon in product.

So if you do this you will get this equation the gamma is if the degree of reduction of this substrate and b (())(10:50) , of O2 one O2 give 4 electrons and then gamma the Yc, Yc is the if you if you look at Yc is the Yc one gram atom biomass. Yp one gram atom product like this that we have so if you that is why you what you have written here Yc into gamma p gamma p is the degree of reductions of biomass and Yp and gamma p this multiply by this.

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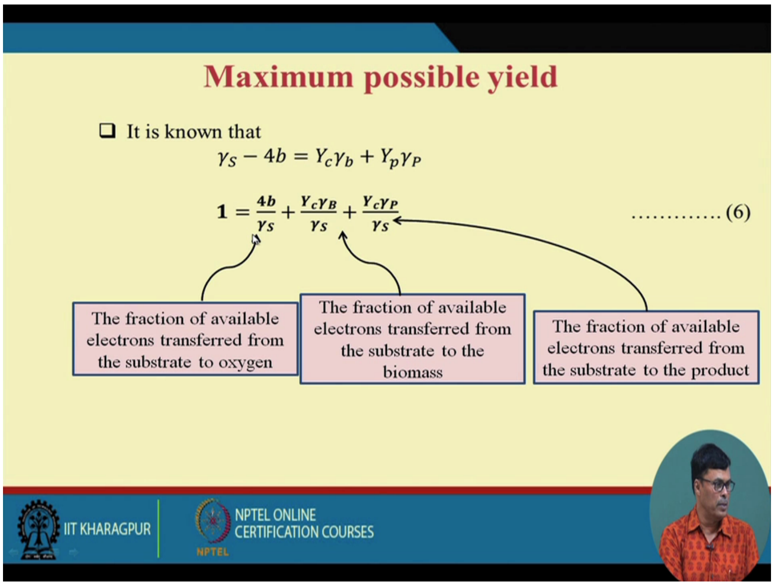

Now if you if you divide by 4 we will give the b and b is the oxygen than demand in the process. So we can easily calculate how much oxygen required in the system if you have this information, now if you if you in this equation if you we can we can defy by gamma S and you will come across this kind of equation and when you come across this kind of equation we have three different fractions.

This fractions indicate it is the fractions you of available electron transferred from the substrate to oxygen and fractions available electrons transferred from substrate to biomass this is biomass gamma b is the biomass and this is the gamma p so this is the fractions available electrons transferred from the surface, substrate to product so I hope it is clear that three different fractions indicate the different things different information, the three different informations.

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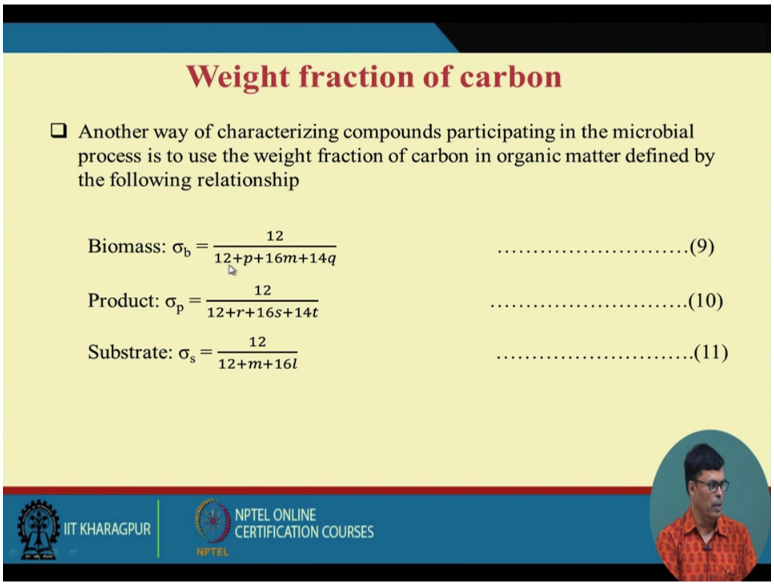

Now again the information that we have what you call eta equal to Yc gamma b by gamma s this is called energetic growth yield this is this is called energetic growth yield and if epsilon p it is called as energetic product yield. Two information is very much required that is why we require this because we know I shall show you the thermodynamic efficiency of any biological process depends on these two factors because this is nothing but eta + epsilon p now another way we can we can have the analysis that is the another way characterizing the compounds participating in the microbial process is to use the weight fraction of carbon in the organic matter defined by the following relationship as for example suppose we have biomass, we have the formula of biomass so we have one gram atom biomass that is equal to 12 gram because the atomic weight of biomass is 12.

Then if you look at the formula, formula is 12 what is the molecular weight of the biomass 1 gram atom of biomass that if you multiply with this oxygen will be 16, nitrogen will be 14 and hydrogen will be 1 if you if you put these value then you will get the sigma b sigma b then sigma p also you can do and sigma s also. So this fraction basically this with that the with respect to carbon, carbon per gram of biomass how much carbon is there that is this fraction builds it like this information we can get from this then in case of biomass production of the above formulae are not adequate for solution the unknown coefficient.

Another experimental quantity is required that is the biomass yield that is biomass yield we can easily calculate when you carry out any kind of biochemical processes if you if you look at suppose you carry out any fermentation process you grow the cell mass inside the reactor and after the (())(14:59) after some time if you take out the cell mass and determine how much how much cell mass is produced because that is nothing but equal to the final cell mass concentration – the initial cell mass concentration divide by how much substrate is consumed that is might s zero – s the initial substrate concentration and s is the substrate concentration of the sample that we have taken out from the reactor.

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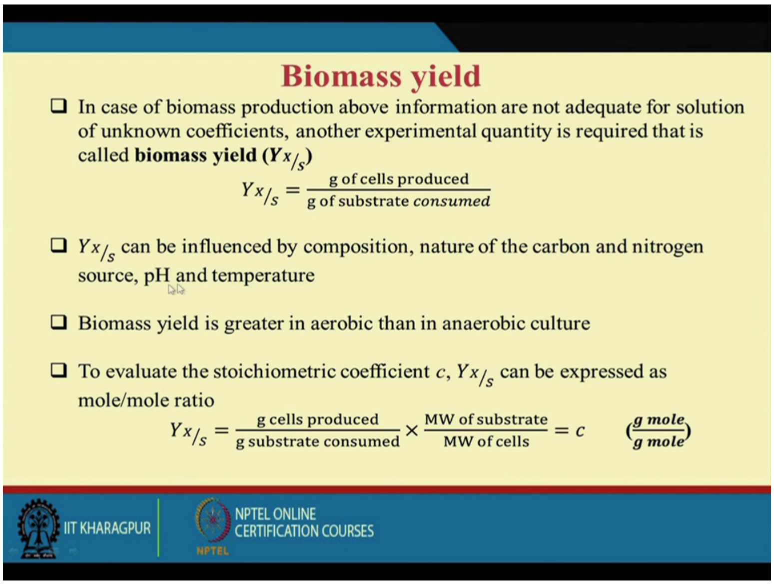

So you can easily calculate the yield coefficient this is gram of cell produced per gram of substrate consumed, so then we have Yx by s can be influenced by the composition, nature of carbon nitrogen source pH and temperature as you know biological system I told you now early + they very sensitive to the environment. As you change the environmental parameters your composition your cell mass yield everything it depends it is very because I have I have given the example that in our human system also suppose we if we increase the temperature of this room to 40 degree centigrade we will be reluctant to work because 40 degree centigrade is very high, 40, 45 degree.

Similar to the microorganism they are very sensitive to the environment so if you if you if you keep the condition as the good then and only then you will get the your yield will be more. The biomass is greater in the aerobic than the anaerobic culture this is very important because if you look at the aerobic fermentation process always we get more cell mass production as compared to anaerobic formation.

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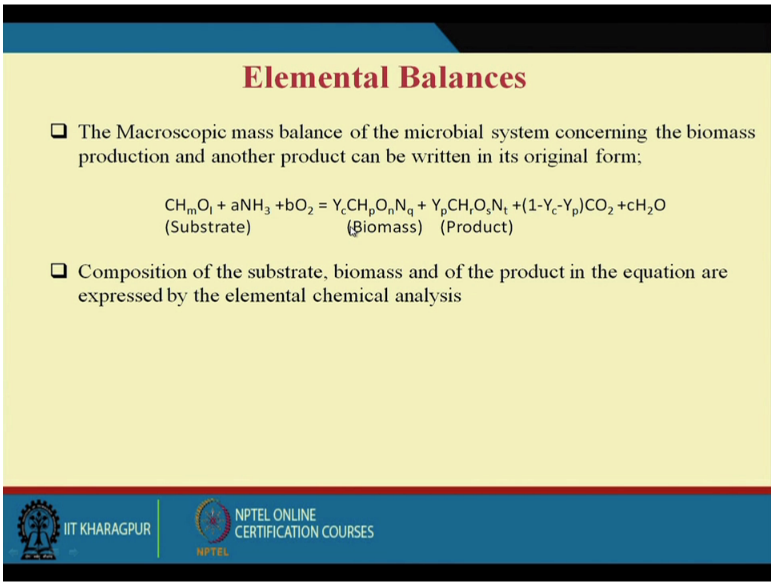

So in other way we can tell the nutritional requirement for the anaerobic fermentation process is much less as compared to the aerobic fermentation process and then then we can evaluate this Yx by s is the gram of cell produced per gram of substrate. Now if you multiply if you divide by the molecular weight of this substrate and molecular of the cell mass then you can easily convert it to c and this c is nothing but you can see that we have already if you look at our equation that here you have Yc what is the value of Yc you can what is this what is this is Yc equal to that gram atom of biomass produced per gram atom of substrate that is the ratio that you have that you can calculate here.

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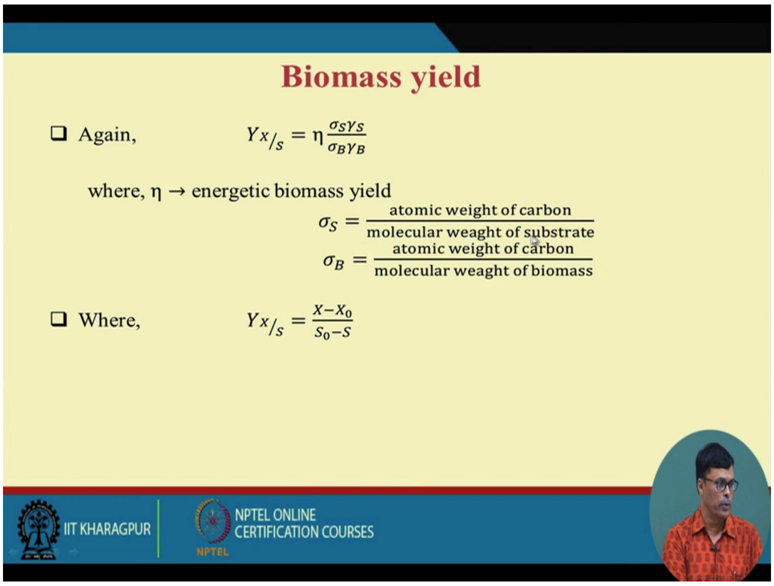

Here you can calculate if you divide by the molecular weight you can the gram moles per gram atom substrate you can easily find it out. Now this can be again expressed as Yx by s can be expressed as eta, eta is the energy integral coefficient sigma s gamma s sigma bs gamma b. So this this I have already explained the sigma s gamma s the atomic weight of carbon molecular weight of substrate and Yx by s it could nothing but x – x0, s0 – s so you can suppose if you know the why it is required because suppose you carry out some kind of I told you the Stoichiometry of the process gives you the information on the validity of (()) (18:35) experimental results.

Now suppose you experimentally you find out the cell mass yield that you can easily find it out because you know the final cell mass concentration – the initial cell mass concentration divide by initial substrate concentration – final substrate concentration. This ratio gives you the yield coefficient now this when you write the Stoichiometry equation from the empirical formula of the substrate and the biomass, you can easily find out the value of sigma s and gamma s sigma b and gamma b, you can easily estimate so if you know this and you can find out the eta value

Now I told you (())(19:18) yield coefficient that that is nothing but eta + epsilon p so from that we can find out that now in case of aerobic process I just show you this is that is from 0.5 to 0.6 and in case of anaerobic process it is 0.7. So if these values comes within these range that means your experimental result is right and if these results varies from that that means there is something wrong with the experimental results. I say we will solve some problems subsequently so I think the problem this things will be very clear to you.

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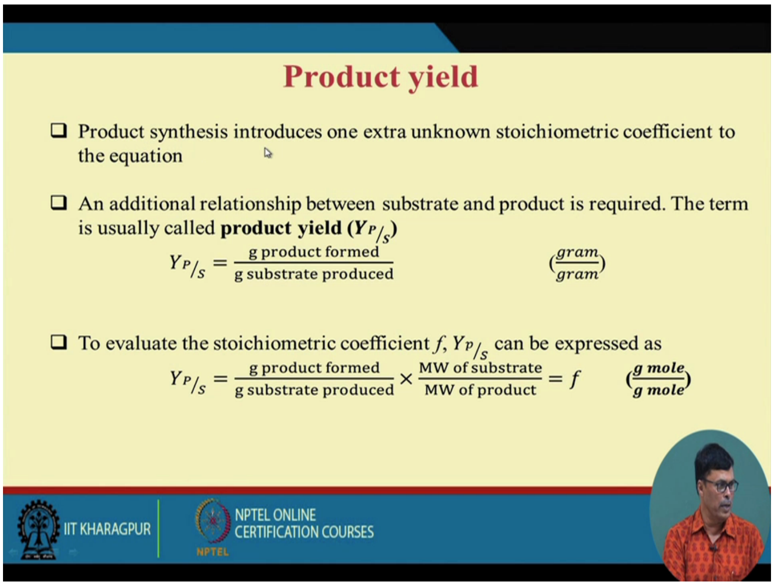

Now here the regarding the product synthesis introduces one extra unknown Stoichiometry coefficient to the equation and additional relationship between the substrate and product is required. The term is called product yield, what is this Yp by s is what gram of product from per gram of substrate produced, so this ratio is gram by gram there is the dimensionless. Now here also you can easily find out the value of in the terms of gram atom, gram mole of product from per gram of gram mole of substrate consumed. So in this equation that in your stoichiometric equation you have seen before that you know that here that the Yp value that you can easily calculate it form this equation that also you can easily find it out.

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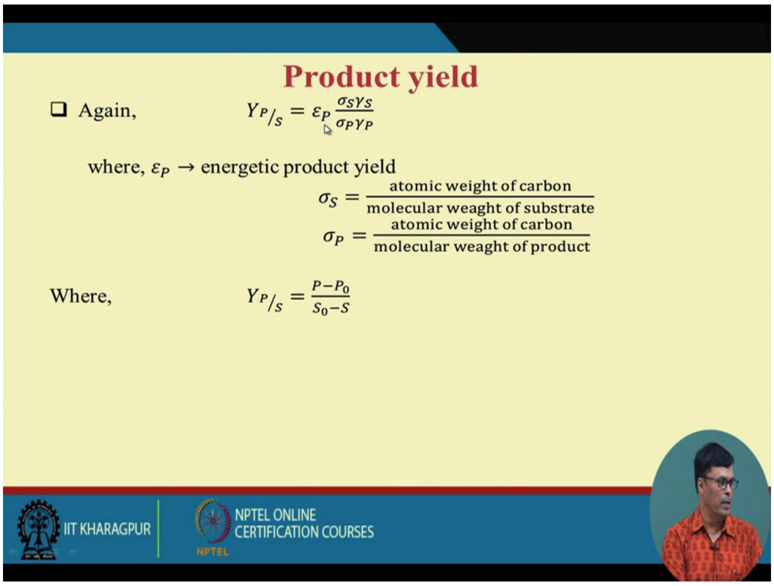

Now Yp by s again it can be written as epsilon p epsilon p energetic product yield and this is sigma s the sigma gamma s and sigma p by gamma p. So this ratio you can easily find out and Y p by s equal to p is the final product concentration – increased zero is the initial product concentration. S zero is the initial substrate concentration – s so you can find out that gram of product form per gram of substrate consumed. So similarly we can calculate Y x by x by O x by Yx by this is gram of cell produced per gram of oxygen consumed 3 eta to the sigma b gamma b 1 – eta – epsilon p. This also this equation we can use we can directly calculate how much how much cell mass is produced per gram of oxygen consumed.

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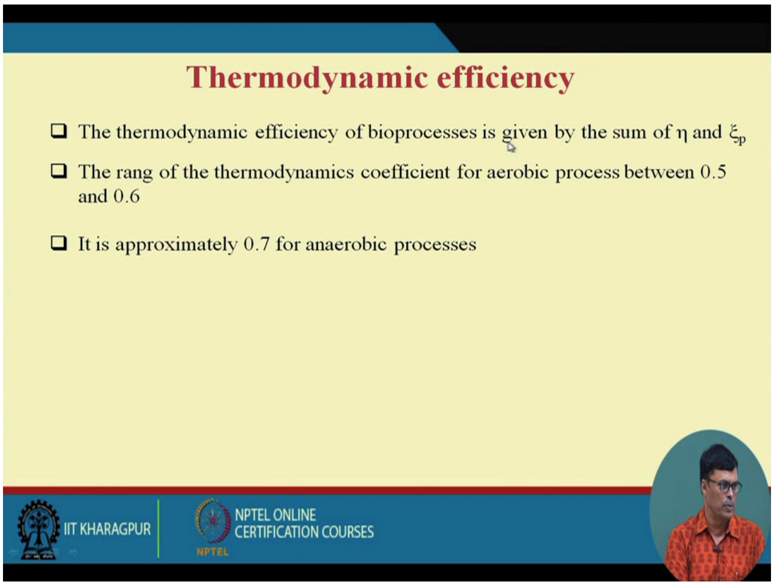

Now I was talking about the thermodynamic coefficient of the bioprocess is given by eta and epsilon and range of thermodynamic coefficient for the aerobic fermentation process is0.5 to 0.6 and approximately point 0.7 in case of anaerobic. This we have to remember that when you want to find out the Stoichiometry of the biology, suppose you do not have the information of eta and you know some I can I can I can give little bit of example.

Suppose anaerobic digestion process, anaerobic digestion process what we what we do basically we produce methane and carbon-dioxide from the organic waste now it is the anaerobic fermentation process through which we get the methane and carbon-di-oxide. Now here since it is anaerobic fermentation process, I told you the cell mass growth in aerobic process is usually 10 times as compared to that of anaerobic fermentation process.

So we can if we can ignore the amount of cell mass that produced in the anaerobic fermentation process so in that case the thermodynamics coefficient we can assume to be 0.7

because we know that eta that eta value in case of this anaerobic fermentation process the this thermodynamics coefficient it should be equal to 0.7. Now if there is a variation of the value from 0.7 then we shall have to check that (())(23:40) there will be possibility of the error so if you correct this error then you will find the value will be coming close to 0.7.

So it is the good method it is very nice way to detect the error of your experimental (()) (23:56). Now I told you another thing that heat evolved by the anaerobic fermentation process also can be determined with the help of Stoichiometry of the equation because particularly aerobic process we require oxygen and because oxygen is required for both for the aerobic and anaerobic process as we know that in the aerobic process the organism required the molecular oxygen aerobic process they required molecular oxygen but anaerobic process they take the oxygen which present in the compound like nitrate , sulphate, nitride all this compounds.

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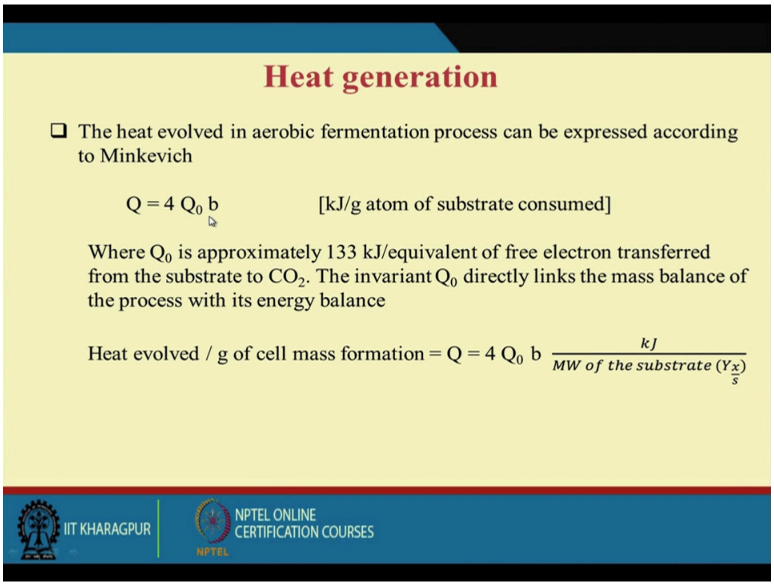

So they cannot they cannot use the molecular oxygen but they used oxygen present in the in the compound like nitrate sulphate and nitrite like this. So here that now here that the heat evolved in the aerobic fermentation process we can calculate with a help of the equation that is 4 into Q0 into b, Q0 is the approximately equal to 133, 133 a kilo Joules per equivalent free electron transferred from substrate to carbon-dioxide.

So if you multiply by b in that then we actually this will get the give you the information kilo Joule of per atom of substrate consumed. Now here I want to show you something that you know that Q actually Q is equal to 4 Q0 into b and what is the this is equal to kilo Joule per gram atom substrate, now question is that now in the biological process we try to find out per cell mass formation how much is the is the product formation.

So how you can find out so here if you multiply suppose you know that Yx by s is nothing but gram now so here what I can do I can multiply this with a molecular weight of molecular weight of substrate we can multiply and then Yx if we multiply molecular weight then you will get gram of substrate Kilo Joule heat produced per gram of substrate and Yx by s is what Yx by s is the gram of cells produced per gram of substrate.

So you know that so here I have written like this that if you defined by molecular weight of substrate by yield coefficient then you will get the information per gram cell mass formation how much heat is evolved that you can find out. Suppose you want to produce 10 kg of (()) (27:11) in the baker's fermentation process. For the production of 10 kg of bakers how much is heat is evolved you can easily calculate with the help of this equation. So this is all I wanted to tell about the Stoichiometry of bioprocess, next class I shall discuss about the different problem that we have with the Stoichiometry of bioprocess so then your conception will be reputed clear Thank you.