Bioreactors
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Lecture -18
Call view: stoichiometry : degree of reductance

Welcome to lecture 18, the NPTEL online certification course on bioreactors. We will begin module 5 today, which is the last module of this course.

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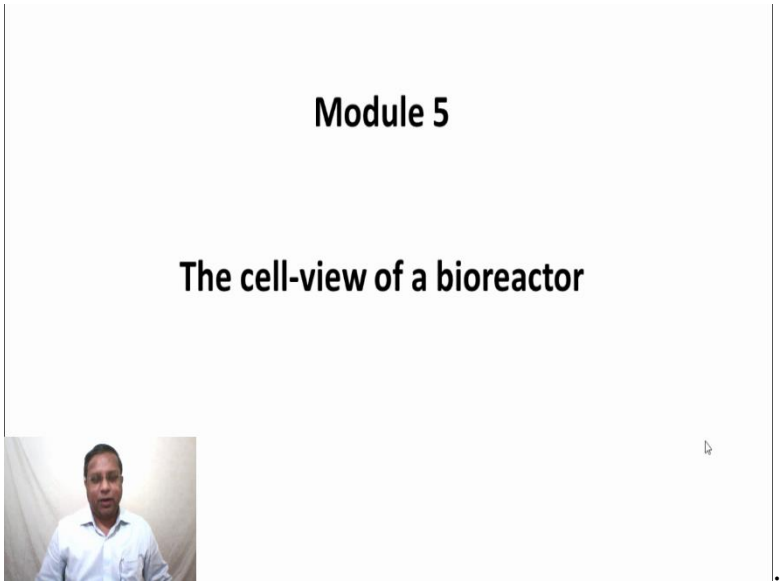

The title of this module is the cell-view of a bioreactor. We have always maintained that the cells are the actual factories that produce the product in the bioreactor. They could be products themselves. If you recall module 2, the title was two major outcomes of a bioreactor, the cells themselves, or what is known as the biomass, or the products that are made by cells, or through enzyme reactions. So, we are going to look closer into what is happening, or look into what is happening inside the cells in a bioreactor, and how we could use some of those to make bioreactors perform better.

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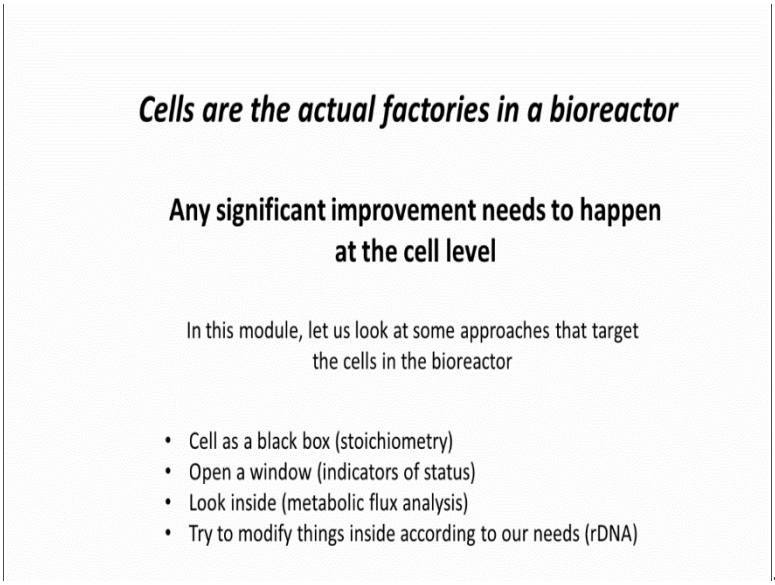

Just to repeat that, the cells are the actual factories in a bioreactor and any significant improvement needs to happen at the cell level, because, that is the more fundamental level. Understanding anything from a fundamental level is always much better for guaranteed improvement and that is what we are trying to do here.

We will look at some approaches that target the cells in the bioreactor. In fact, I have a theme for this. We will first look at the cell as a black box; you know, these stoichiometric aspects, black box, what is happening, what we could do. Then, we will open a window into that black box, through the indicators of "status" of the cell; the status is quote unquote. There are many statuses that are talked about. We will look at some aspects of that. Then, we look inside through the window, maybe from other directions also; maybe we will open up some doors also, and look inside. And, an example of that is metabolic flux analysis. And then, try to modify things according to our needs, such as DNA modification, use of recombinant DNA technologies and so on and so forth. We will just barely touch upon them, because, that is an entire field in itself, recombinant DNA technology. I will just talk about its relevance to improving the operation, or the outcomes from a bioreactor. So, that is going to be the theme of this particular module.

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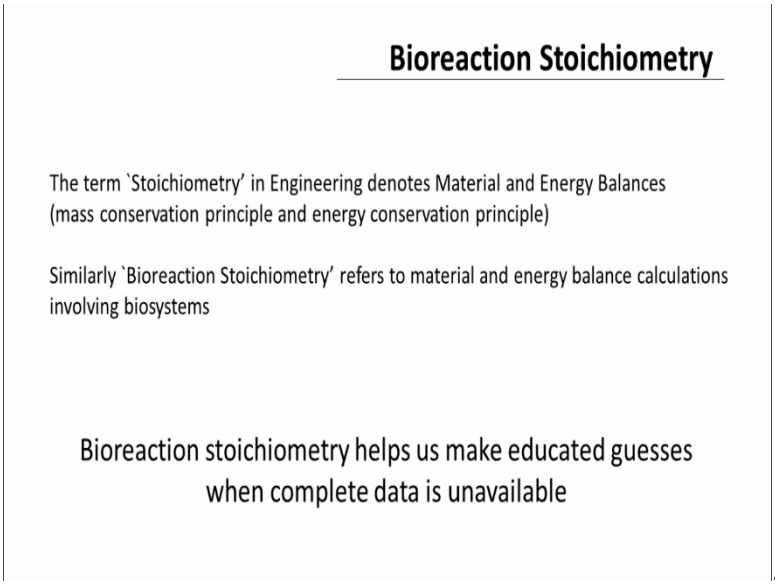

First, the bioreaction stoichiometry, which we will cover in this lecture. Then, let us look at a problem. Then, in the next lecture, we will solve the problem and then, go forward. Bioreaction stoichiometry. The term stoichiometry, it is used heavily in chemistry. It is also used in engineering. In engineering, it actually denotes the material and energy balances, or things that are based on the mass conservation principle and the energy conservation principle; that is what is called stoichiometry, in some fuzzy fashion in engineering. You have books on stoichiometry.

Similarly, bioreaction stoichiometry refers to material and energy balance calculations that involve biosystems. Bioreaction stoichiometry helps us make educated guesses when the complete data is unavailable; that is how we are going to look at it. We are, we have just started looking into the cell, at the cell level. We had led to this in our earlier discussions, but this is where we are actually getting to look in to the cell, and the first approach is that the cell is a black box and what we can gather from whatever we observed it as a black box, but with a thinking that there are things going on inside the cell.

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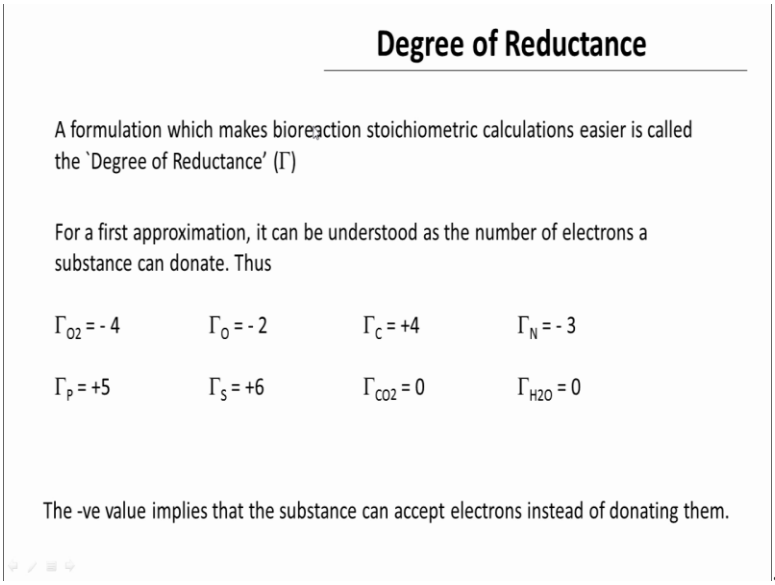

A very useful concept for the use of stoichiometry is what is called the Degree of Reductance. This is a formulation which makes bioreaction stoichiometry calculations easier, and it is denoted by the capital Greek symbol gamma as is given here, you see here; this is the degree of reductance. We have degree of reductance given for each substance. As a first approximation, it can be understood as the number of electrons that a substance can donate. So, it is reasonably fundamental, fundamental concept. It works at the electron level, electrons are conserved and so, you can work, you can do things there.

So, number of, it is understood as the number of electrons that a substance can donate. This is only the first approximation; this is not always valid. So, it is more of a number that is assigned to a certain molecule. We will see this. Thus, the number of electrons that oxygen can donate is - 4, and O_2 is - 4, O is minus 2, carbon is plus 4 and nitrogen is -3. It is the number of electrons that a substance can donate. The value for phosphorous is plus 5; sulphur is plus 6; CO_2 is 0 and H_2O is 0. We can just add the degree of reductances and arrive at the degree of reductance of a compound that is made up of these elements.

So, the basic things are O, C, N, P and S, and from that you can get oxygen, nitrogen, CO

2, H 2 O, sorry, Oxygen, CO 2, H 2 O and so on and so forth. So, the negative value actually implies that the substance can accept electrons instead of donating them. And, this is understood as the number of electrons that a substance can donate; that is the degree of reductance. Let us see what we can do with this concept.

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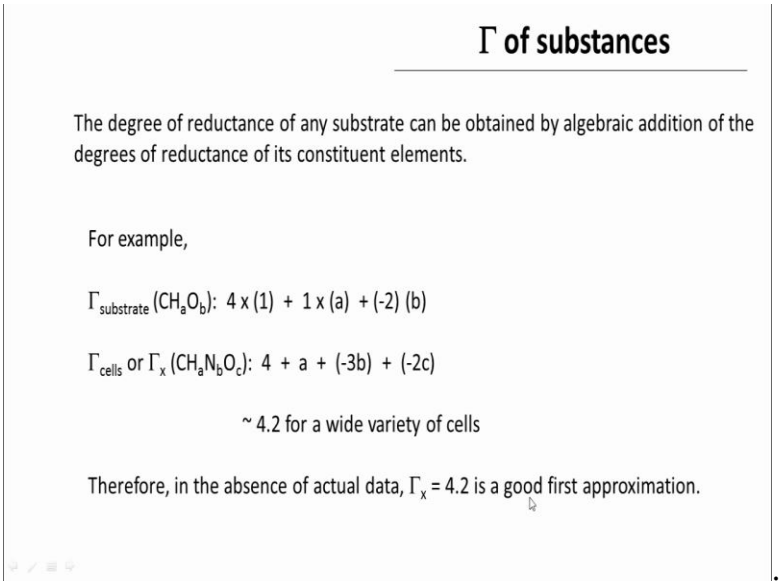

Before that, as was briefly mentioned earlier, the degree of reductance of any substance can be obtained by the algebraic addition of the degree of reductance, degrees of reductance of its constituent elements, of its constituent elements. For example, even for a complex molecule such as a substrate, you know, we had earlier looked at O_2, H_2O, CO_2, even if you have something like a carbohydrate here, CH_aO_b, the degree of reductance of the substrate is the degree of reductance of its constituent elements added together.

Thus the degree of reductance of :

Substrate (CH_aO_b): 4 x (1) + 1 x (a) + (-2) (b)

Cells ($CH_aN_bO_c$): 4 + a + (-3b) + (-2c)

This Γ_{cells} has been found to be about 4.2 for a wide variety of cells, and herein lies the strength of this method. You know, this has been estimated. And similarly, the parameters that we are going to look at, chiefly, the chief parameters have been estimated for a wide variety of substances. These happened to fall within a very narrow range, and therefore, if you take the average value of that range as an, as a representative value, we can do first level calculations very easily, and get insights into what is happening, which can in turn be used to improve the bioreactor performance. Therefore, in the absence of actual data, Γ_{cells} equals 4.2, is a good first approximation.

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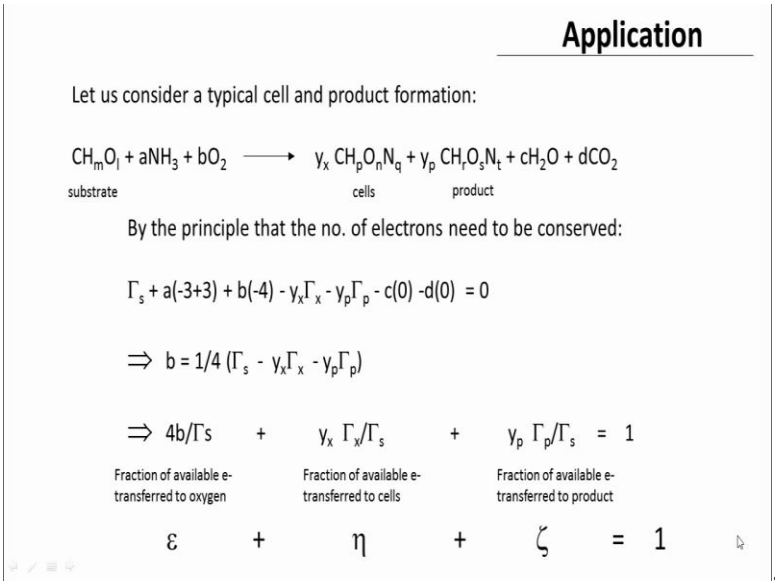

Let us look at the application of this, a couple of applications, and I will assign a problem. Let us consider a typical cell and product formation. This is a stoichiometric equation that represents the cell and product formation,

$$CH_mO_l + aNH_3 + bO_2 \rightarrow y_x\, CH_pO_nN_q + y_p\, CH_rO_sN_t + cH_2O + dCO_2$$

So, this equation, stoichiometric equation, represents the cell and the product formation in a reasonably general format. We will choose this as the equation for our analysis of a few things that we will do in this lecture.

By the principle that the no. of electrons need to be conserved:

$\Gamma_s + a(-3+3) + b(-4) - y_x \Gamma_x - y_p \Gamma_p - c(0) - d(0) = 0$

Where he subscripts s, x and p refer to substrate, cells and product respectively.

Rearranging the above equation,

$b = 1/4 (\Gamma_s - y_x \Gamma_x - y_p \Gamma_p)$

$4b/\Gamma s + y_x \Gamma_x/\Gamma_s + y_p \Gamma_p/\Gamma_s = 1$

$\varepsilon + \eta + \zeta = 1$

Electrons, we know, are conserved, right. So, by that principle, if you count the number of electrons on this side, that must equal the number of electrons on this side.

This is a powerful expression. This indicates the fraction of the available electrons from the substrate that has been transferred to oxygen, because b is the stoichiometric coefficient of oxygen; 4b is the number electrons that is associated with it and so on.

So, $4b/\Gamma s$ gives us the fraction of available electrons transferred to oxygen and is denoted by ε

$y_x \Gamma_x/\Gamma_s$ gives us the fraction of available electrons transferred to cells and is denoted by η

$y_p \Gamma_p/\Gamma_s$ gives us the fraction of available electrons transferred to the product and is denoted by ζ.

All these add up to 1 since these are all fractions.

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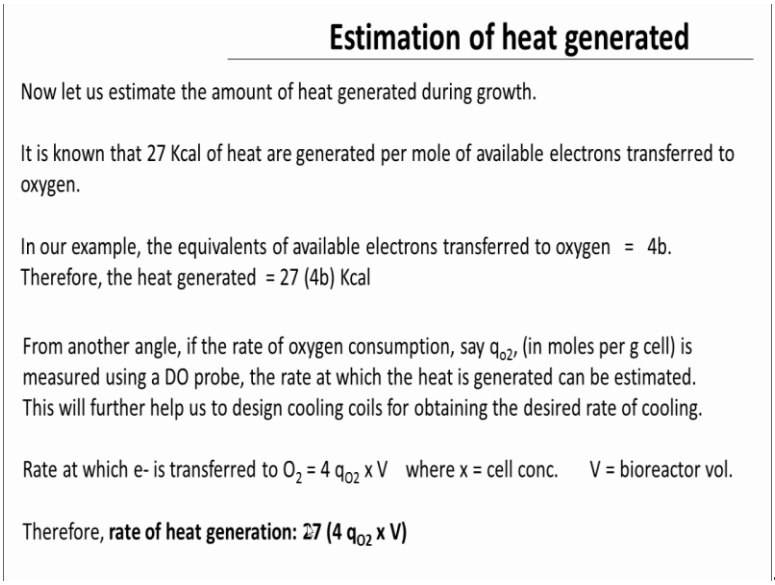

Now, using these let me show you how to estimate the heat that is generated from the cells in the bioreactor during growth. I said the power of this method comes because the rules of thumb values are known, right. So, it is known that 27 kilocalories of heat are generated per mole of available electrons transferred to oxygen. So you know ε. For every mole of electron transferred to oxygen, 27 kilocalories of heat are generated. This is known.

In our example, the equivalents of available electrons transferred to oxygen = 4b. Therefore, the heat generated = 27 (4b) Kcal

From another angle, if the rate of oxygen consumption, say q_{O_2} in moles per gram cell is measured using a DO probe, you know, if you process the DO data, you can get the oxygen consumption rate, the rate at which heat is generated can be estimated. Then, it becomes powerful. Once we know how much of heat is metabolically generated, we can a priori say what kind of a cooling design we need to look at. This will help us design cooling coils for obtaining the desired rate of cooling. If it is cooling, you can use a coil, because the temperature is not going to go down too much; it is not going to affect the cells; whereas, heating coils are not really used; we use the jacketed system.

Similarly, a jacketed system, it can also be used for cooling, but cooling coils are also used.

So Rate at which e^- is transferred to O_2 = 4 q_{O_2} x V, where x = cell concentration and V = bioreactor volume

q_{O_2} is the oxygen uptake rate in moles per gram cell. We need to multiply it by the cell concentration to get it in appropriate units, and then, multiply it by the bioreactor volume to get it in the units that we need. So, this is the rate at which electrons are transferred to oxygen, number of electrons per unit time, right.

Therefore, **rate of heat generation: 27 (4 q_{O2} x V)**

Which can be helpful for us to make many decisions.

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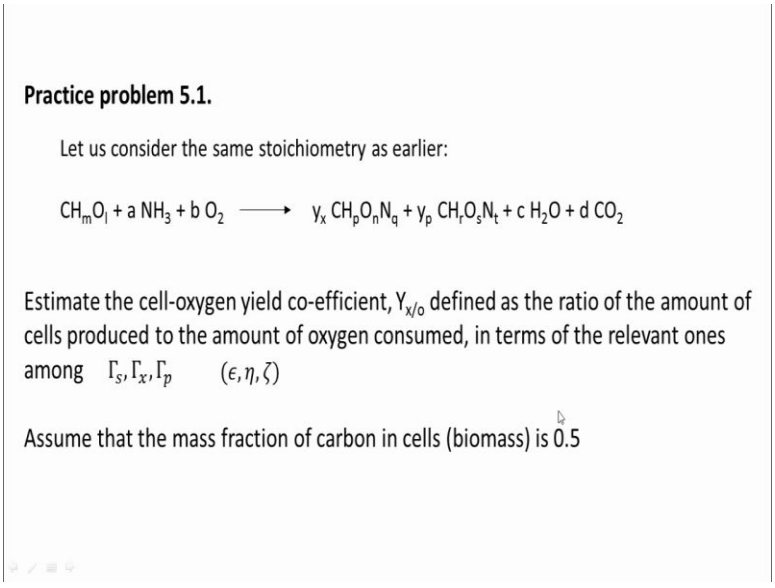

Now, let me assign this problem and we can finish up this lecture here. When we come back, we will solve the problem in the next lecture.

Practice problem 5.1.

For the following equation

$CH_mO_l + a\ NH_3 + b\ O_2 \rightarrow y_x\ CH_pO_nN_q + y_p\ CH_rO_sN_t + c\ H_2O + d\ CO_2$

Estimate the cell-oxygen yield co-efficient, $Y_{x/o}$ defined as the ratio of the amount of cells produced to the amount of oxygen consumed, in terms of the relevant ones among Γ_s, Γ_x, Γ_p (ϵ, η, ζ).

Assume that the mass fraction of carbon in cells (biomass) is 0.5.So, you can say that, the cells are growing aerobically. So, you get a fraction of 0.5; if it is, anaerobically it is around 0.1 or so.