Bioreactors
Prof G. K. Suraishkumar
Department of Biotechnology
Indian Institute of Technology, Madras

Lecture-17
Shear stress, scale up, scale-down

Welcome to lecture number 17, NPTEL online certification course on bioreactors. In the previous lecture, we solved a problem, the practice problem 4.1.

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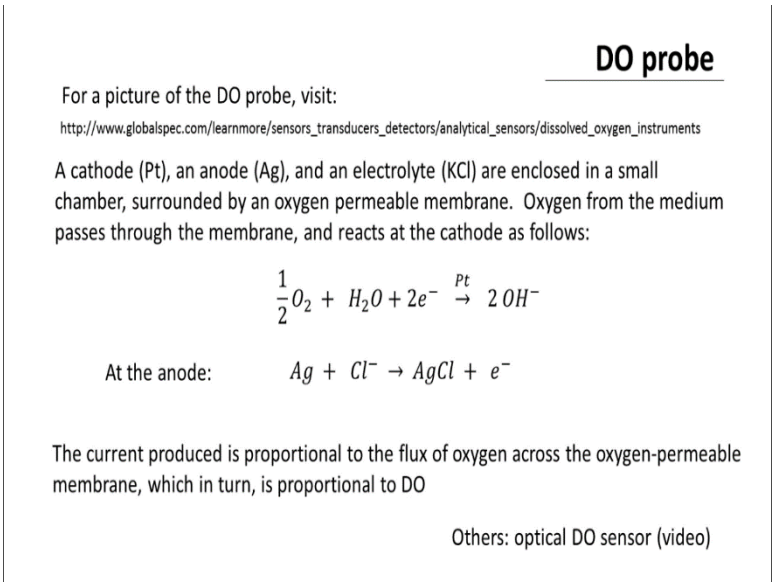

In the lecture before that, we had looked at certain bioreactor environment variables that affect bioreactor performance such as temperature, pH, medium composition, agitation aeration levels and dissolved oxygen levels. These are crucial, and so, they need to be properly measured and controlled. We looked at some aspects of that. In the previous lecture, we looked at, the lecture before the problem, we looked at the dissolved oxygen aspect. While talking about the DO probe, there was some slight confusion there; that is because of an error here; the cathode is platinum and anode is silver. I think I had mixed it around the last time; otherwise, it is all the same. So, the cathode is platinum; anode is silver.

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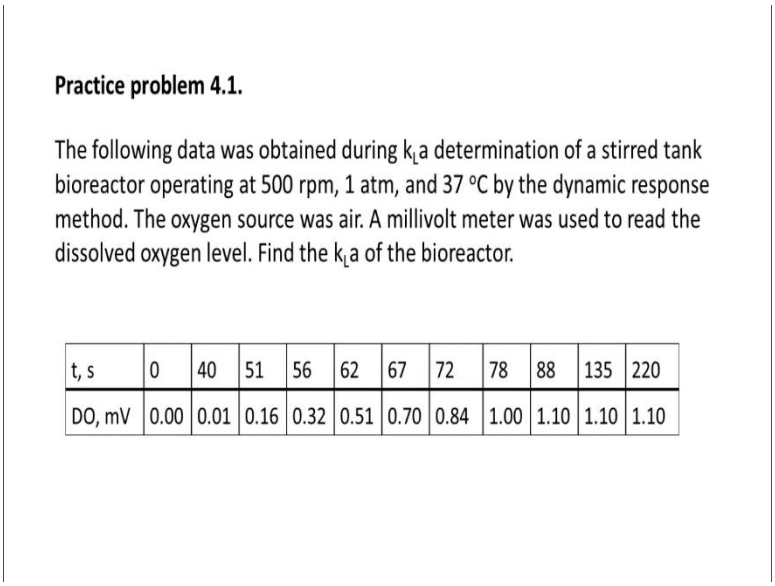

Then, that is more forward. I think, we had looked at this practice problem 4.1, its solution and so on. We will move forward from here. When we talked about agitation and aeration, we had said that both affect DO, as well as they cause shear stress in cells.

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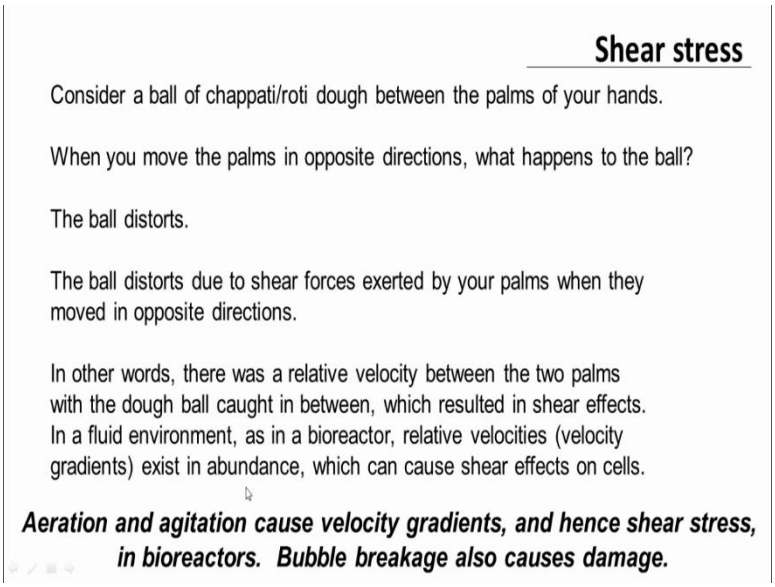

What exactly is shear stress? If you have done a course on Fluid Mechanics, you would already know; but that does not matter. Depending on your background, you may or may not have done it. Let us understand shear stress this way. Let us take a ball of chapati dough, or roti dough, and place that dough ball between the palms of your hands, right;

something like this. And then, when we slightly press and move the palms in opposite directions, what happens to the ball, right. The ball does get distorted, the chapati dough does get distorted. The ball distorts due to the shear forces exerted by the palms when they are moved in opposite directions. There are forces acting on the surface of the chapati dough ball, which distorts it, and such forces are called shear forces, they are surface forces. In other words, when there is a relative velocity between the two palms, this palm is moving in this direction, this palm is moving in this direction, or it could be both moving in the same direction, but at different velocities, as long as there is a relative velocity between the two palms, the dough ball that is caught in between is going to experience shear stress, which could deform the dough ball there.

In a fluid environment, such as in a bioreactor, relative velocities, or velocity gradients exist in abundance. Velocity gradient means, the difference in velocity with a certain distance, it is the derivative with respect to the distance of the velocity, dv/ d x , dv/ d y and so on. So, those are velocity gradients. As long as there are velocity gradients, there is going to be shear stress, and the cells are going to experience shear stress. So, shear stress is a given in the case of a bioreactor environment, fluid environment, where there are a lot of velocity gradients. The velocity gradients are brought about because the fluid is moving; the fluid is moving because of the agitator that is present there, or the agitation that is being caused, or the aeration that is being employed. The aeration also displaces the fluid. It causes velocity gradients. Therefore, it can cause shear stress.

So, aeration and agitation cause velocity gradients, and hence shear stress in bioreactors. Bubble breakage, the air bubble breakage could also cause damage, but the damage is through a different mechanism, that is through the energy released due to bubble breakage. In terms of shear stress, aeration and agitation cause shear stress, because they cause velocity gradients in the bioreactor. So, we need to worry about the shear stress. Some cells, such as the mammalian cells, animal cells, do not have a cell wall and therefore, they could be more susceptible to shear stress. Some cells, because they have a cell wall, they could be less susceptible. All these are may, may be not, because, even if you have a cell wall, you could have a lot of shear stress effects, because the cell wall is rigid. It does not allow easy motion, and if the contents inside need to move freely, and the cell wall does not allow it, that can also cause stress. So, shear stress, all cells experience shear stress in bioreactors. Some are able to withstand shear stress to a certain

extent; some are not able to, and we need to be aware of that, because that would determine, whether the bioreactor is going to turn out successful or not.

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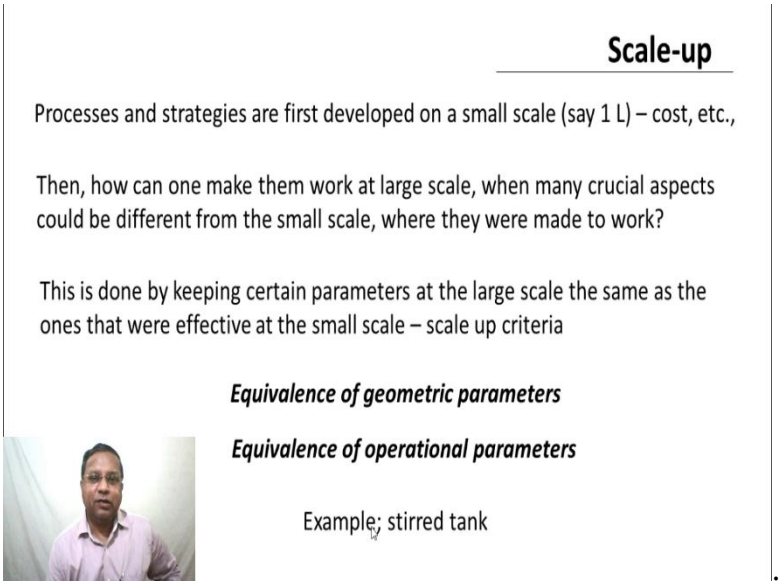

Having said that, let us look at scale-up of bioreactors. What does scale-up mean? When a process is developed, it is usually developed at a small scale, lab scale, first; maybe in a shake flask, and so on. And then, in a bioreactor, maybe a 1 liter, 2 liter bioreactor; and maybe at the, in the lab itself, if we have a 10, 20 liter bioreactor, there is a 10-fold scale-up that is happening there. What is, what was found at the small scale of 1 liter must be translatable to a large scale; that is what is meant by scale-up. Things need to be ensured, the scale-up needs to be ensured; otherwise, the process may not be viable at the industry level, where the scale-up could be in many orders of magnitude. The industrial reactors, as we saw, could be 1 lakh, 2 lakh reactors, 2 lakh liter reactors and so on; the lab scale is 1 liter. So, this scale-up is an important aspect of bioreactors.

We look at some aspects of those. They were, of course, developed at a small scale because of the cost; we do not even know whether the process is going to work. So, to minimize cost involved, even medium is expensive; the processes are developed first at small scale. So, as mentioned, how can one make them work at large scale, when many crucial aspects could be different from the small scale, where they were made to work, or where they were demonstrated to work. The scale-up is done by keeping certain parameters at the large scale the same as the ones that were effective at the small scale.

So, appropriate parameters at the large scale are kept as a same as the effective ones at the small scale. These are called scale-up criteria. During scale-up criteria, we cannot choose anything and have that as a scale-up criteria. We cannot say that, you know, the diameter of the impeller should be the same; then it will not work, right. Or, we should not say that, probably the shaft diameter should be the same; that does not make any sense. So, appropriate things need to be the same at 2 levels, at the small scale, as well as at the large scale. What is normally seen is, equivalence of geometric parameters as they are called, and equivalence of operational parameters. And, to show both these, or to look at the very surface level information on these, let us take the example of a stirred tank.

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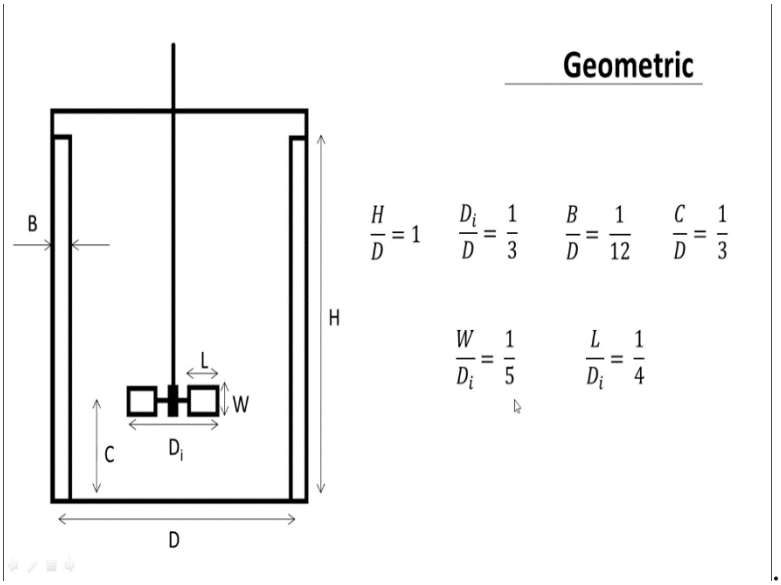

First, the geometric similarity. When there is a stirred tank, this is the stirred tank; the rectangle, actually, it is a cylinder; this is the tank, stirred tank. This is the impeller and this is impeller shaft, impeller blades. The various dimensions are given here. D i is the diameter from one, the tip of one impeller to the tip of a diametrically opposite impeller. W is the width of impeller; L is the length of the impeller blade; C is the distance from the bottom of the tank to the middle of the impeller. Only one set is used. We will just take this is as an example. D is the diameter of the vessel, H is the height of the liquid, in with the vessel is kept and B is what is called a baffle. Baffle, typically, there are about 4 or 6 baffles. Baffles are employed because, you know, what happens when you rotate a liquid, when you use an impeller to rotate a liquid, there is a vortex that gets formed.

Therefore, the liquid will be something like this on top; that is not very desirable. To stop the vortex formation, if you have strips, thin strips; there are about 4 strips at opposite diameters. Those strips, metal strips, can stop this vortex formation; that is the function of this baffle. So, for geometric similarity, these are the ones that are considered. The height to the diameter ratio must be 1. In the case of stirred tanks, these are very standard ones. If we take this down, the H by D should be 1. The D I, the impeller diameter, tip to tip, divided by the tank diameter should be one third; D i by D is 1 by 3. B, the baffle thickness divided by the diameter of the tank is 1 by 12; this should be maintained constant, at the small scale, as well as at the large scale for geometric similarity. C, which is the distance from the bottom of the impeller, bottom of the tank, to the impeller middle, to the diameter, the ratio should be 1 by 3.

Also, W, the width of the impeller blade divided by the impeller diameter should be one fifth and L the length of the impeller blade by the impeller diameter should be one fourth. So, there are four criteria based on, normalized with the tank diameter:

$\frac{H}{D} = 1$ $\frac{D_i}{D} = \frac{1}{3}$ $\frac{B}{D} = \frac{1}{12}$ $\frac{C}{D} = \frac{1}{3}$

And, there are 2 that are based on the impeller diameter;

$\frac{W}{D_i} = \frac{1}{5}$ $\frac{L}{D_i} = \frac{1}{4}$

So, as long as these two things are maintained the same, these are chosen, both at the small scale and at the large scale, we will have geometric similarity.

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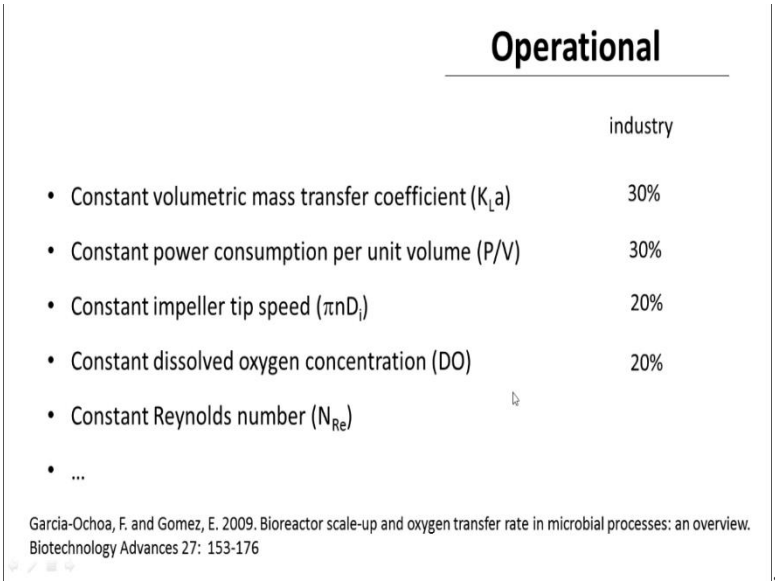

For the operational similarity, we look for maintaining a constant K L a. We have looked at K L a, and it is also an important scale-up parameter. We look for maintaining a constant volumetric mass transfer coefficient of K L a at the small and the large scales. We look for constant power consumption per unit volume (P/V). We look for constant impeller tip speed; impeller tip, you know, impeller diameter is Di; πDi would be the circle which it makes out, the distance that is traveled. This times n - the r p m, the speed of the impeller; gives you the variable that you need to keep constant, at the lower level and at the higher level or higher scale. And also, one looks for constant dissolved oxygen concentration and constant Reynolds number in the bioreactor. The industry and in fact, quite a few are looked at, these are the common ones.

This paper by Garcia-Ochoa and Gomez in 2009, this is published in Biotechnology Advances; the title is Bioreactor scale-up and oxygen transfer rate in microbial processes: an overview. This says that, in the industry, typically this scale-up is done on a K L a basis 30 percent of the time, on a constant power consumption per unit volume basis 30 percent of the time, constant impeller tip speed 20 percent of the time, constant DO value 20 percent of the time. That is, typically, the chosen parameters for scale-up in the industry.

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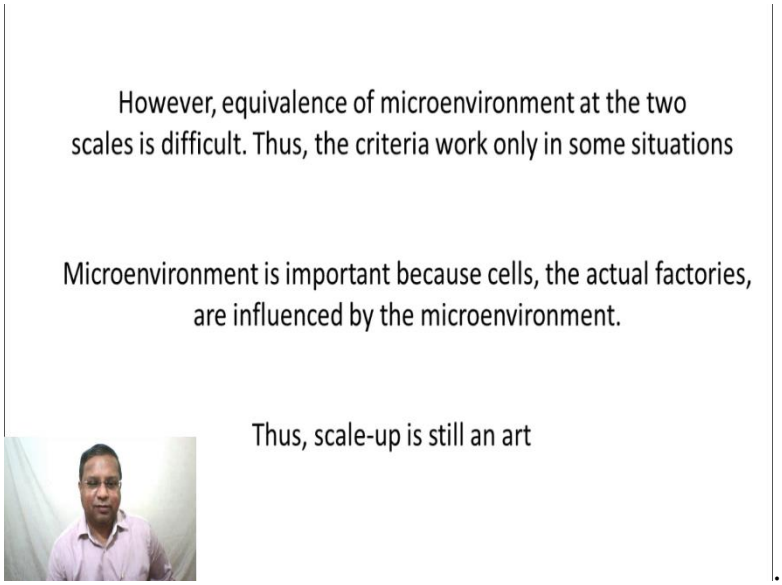

The equivalence of microenvironment of the two scales is rather difficult. You know, the, we know that, the cells are the actual factories that produce a product, and therefore, the microenvironment, or the environment of each cell needs to be kept the same for expecting the same level of the performance, same kind of performance, at these two levels. This is rather difficult. So, the criteria, usually work, but they do not work all the time. So, we need to be wary of that, and hope that, it works; if it does not work, we need to go and understand things at the cell level. In fact, that is what the next module is all about. This provides some reasoning for understanding things at the micro level, even from an industry point of view. Microenvironment is important, as we mentioned, because cells, the actual factories, are influenced by the microenvironment. The scale-up is still an art. It is not fully understood even today, and more work needs to be done to be able to bring it down to a firm science.

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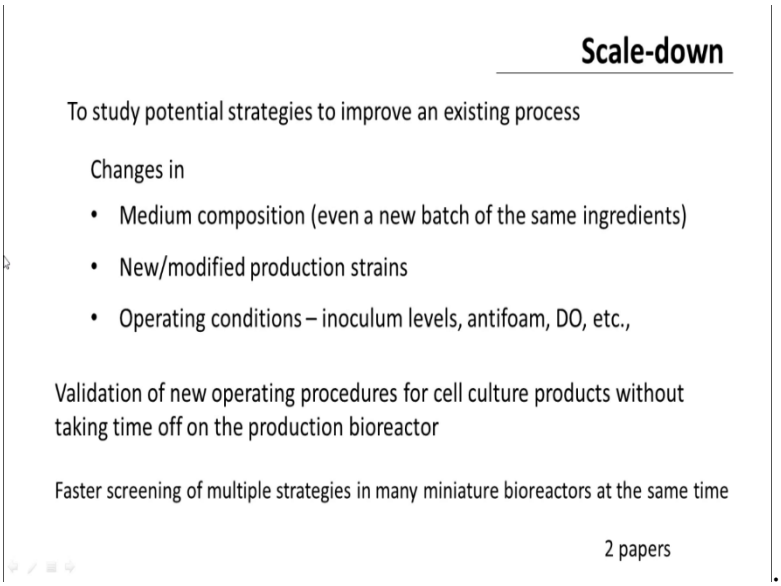

We talked of scale-up; scale-down might sound a little odd initially, because usually, you hear of scale-up, not scale-down, but scale-down is also important in bioreactors. It is something like this; we are already operating at the large scale; we need to do some changes to improve the process at the large scale, but they might be stuck for production, they might be used continuously for production. So, you cannot do experiments at the large scale. And typically, you do not want to spend so much money in doing these experiments also. What if the experiment does not work? Then, you would have lost the entire cost associated with trying out that experiment. So, what is done is, the things are built at the small scale to represent the large scale; and then, experiments could be done at the small scale, with the confidence that they would work at the large scale. And then, you could implement them at the large scale. So, scale-down is also an important aspect in bioreactors, to study potential strategies to improve an existing process, changes in medium composition, for example, even a new batch of the same ingredients is tested at the scale-down level. New or modified production strains are first tried on at the scale-down level. Operating conditions such as inoculum levels, antifoam use, dissolved oxygen level, etcetera, are tried out. And, validation of new operating procedures for cell culture products without taking time off from the production bioreactor. In all these situations, scale-down becomes important. So, and faster screening of multiple strategies in many miniature bioreactors at the same time also becomes possible if an effective scale-down has been made.

So, you screen various things at small, small reactors. They have all been exposed to representative set of conditions at the large scale and then, it can be… There is more confidence associated with the results from a scale-down process, especially at the industry level. There are 2 papers here, that I would like to point out. These are given in your reference document.

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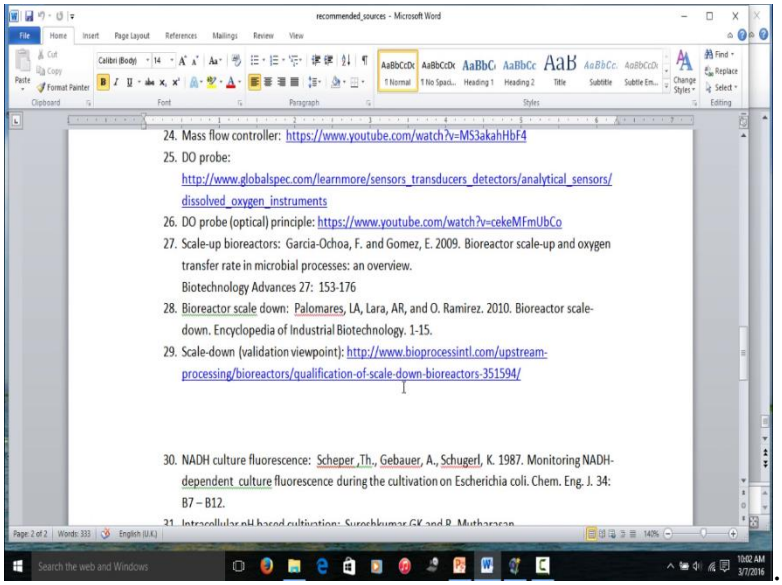

The number 27 is on scale-up; this we talked about, Garcia-Ochoa. 28 is on bioreactor scale-down, Palomares et al, Palomares, Lara, and Octavio Ramirez, 2010, Bioreactor scale-down, in the Encyclopedia of Industrial Biotechnology. And, the validation view point in scale-down has been given on, in this website; you can go on check out this website. That is it for module 4. In module 4, we looked at the effect of certain bioreactor environment parameters, on the bioreactor cultures, and thereby the bioreactor performance. The ones that we looked at were temperature, pH, medium composition, agitation and aeration levels and dissolved oxygen level. Dissolved oxygen level, we looked at in some depth. Then, we also looked at scale-up and scale-down of bioreactors in module 4. So, when we meet again in, when we meet next, we will take up module 5, which will be the last module for this course. See you then.