Bioreactors

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Lecture - 02
Sterilization

Welcome, to this second lecture on Bioreactors. This is a mooc on Bioreactors. In the last lecture, previous lecture, we looked at module one which is introduction. After mentioning the formalities of the course, we looked at some day-to-day examples.

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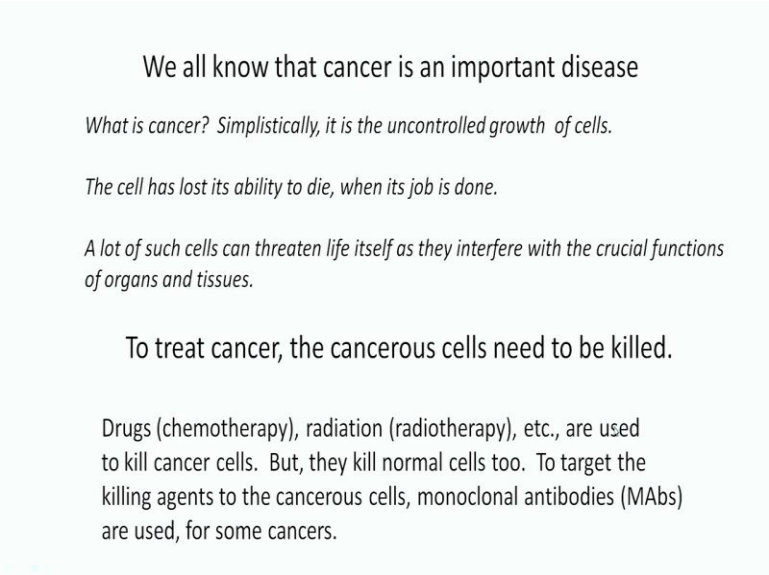

Such as cancer, concerns day to day concerns, cancer. We looked at what cancer was and how cancer is treated allopathically.

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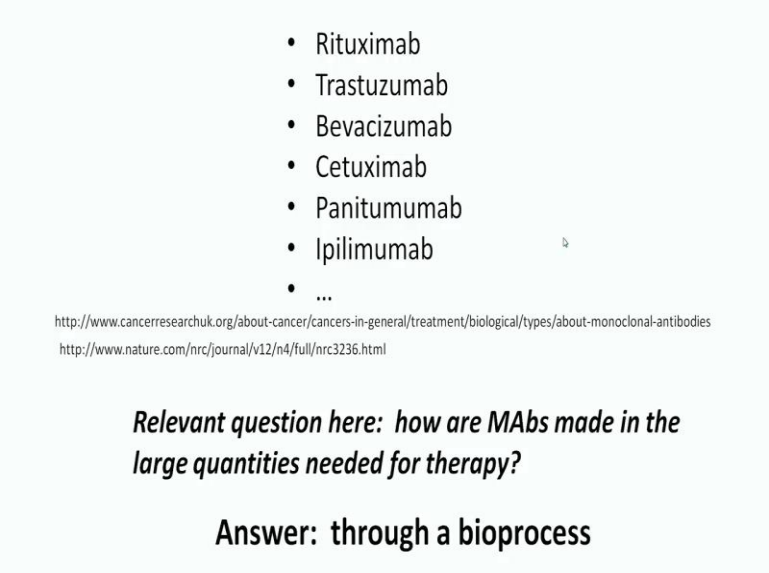

And then, we said that monoclonal antibodies are used to target the drugs that kill cancerous cells, more directly to the cancer cells. These monoclonal antibodies when required in large amounts are produced through bioreactors or bioprocesses and that is how we started. Then we looked at other examples.

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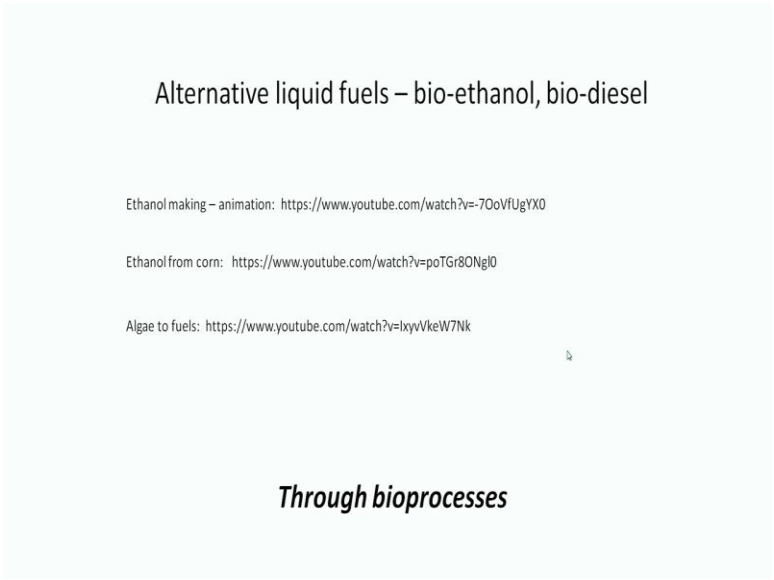

Such as Alternative liquid fuels: bio-ethanol and bio-diesel. And saw that they were also produced through bioprocesses. I had given you links to some information on ethanol

making, ethanol from corn, algae to fuels and so on. I hope you have seen them, they are interesting videos.

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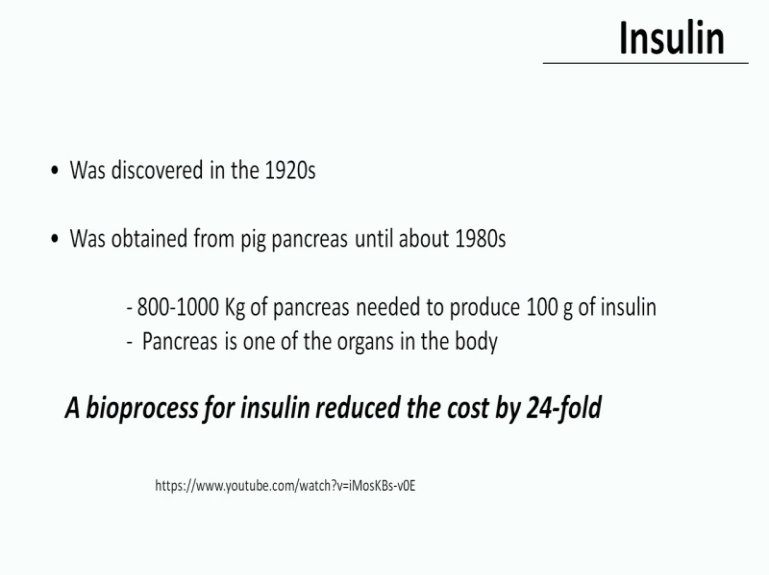

Then we looked at Insulin, which is a classic drug. In fact one of the first ones to be produced large scale, one of the first new ones to be produced large scale, using bio processes. And finally, we looked at curd making and said curd making is also a bioprocess.

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The vessel in which the curd is made is nothing but a bioreactor. Of course, it is a simpler form of a bioreactor. A bioreactor is nothing but, a vessel- highly instrumented and controlled vessel in which bio reactions take place and bio products are made, normally with the multiplication of sets.

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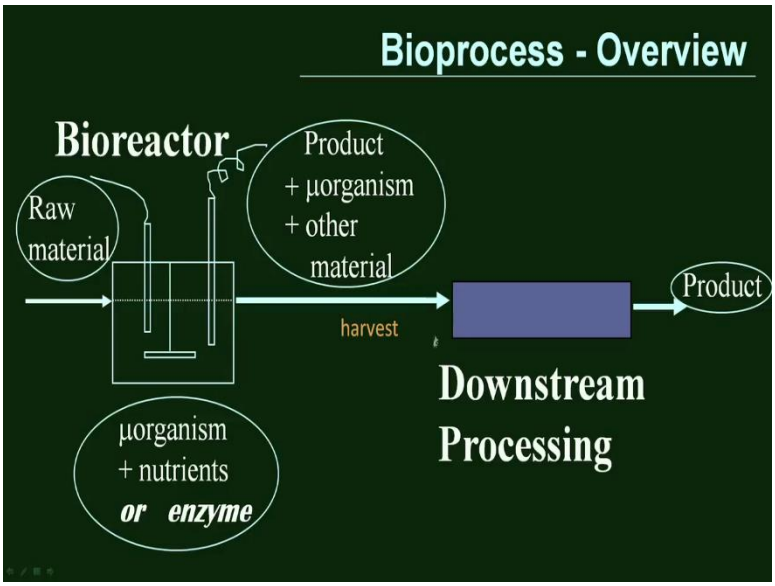

Then, we looked at the overview of the bioprocess. We said that it has two major aspects, the Bioreactor aspect or the Upstream aspect and the Downstream processing aspects. We said that the raw material goes into the bioreactor, which contains the microorganism and nutrients for it to grow and produce the product of interest. Or, it could be an enzyme which is doing an enzymatic conversion. And what is harvested from the bioreactor, this liquids stream that is harvested from the bioreactor contains the product of interest along with the microorganism and the other material that arose from these two. Since we are interested only in the product, we need to remove that from the other material. And that is what is done through downstream processing steps to achieve the final purified product.

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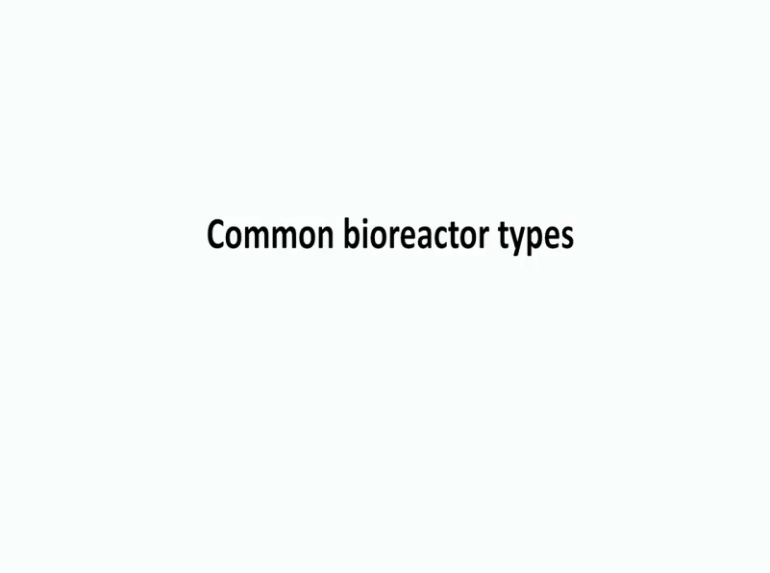

Then we looked at common bioreactor types, some common bioreactor types.

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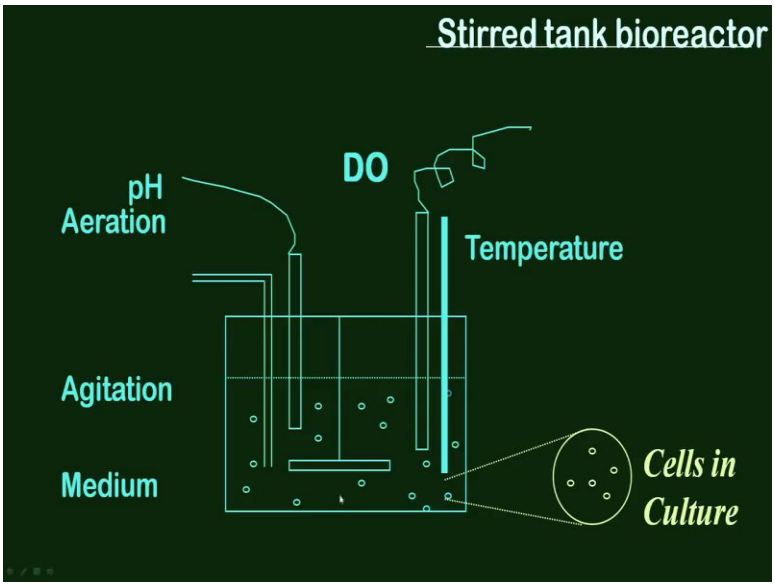

We said that the stirred tank bioreactor is one of the commonest types, it is nothing but a stirred tank.

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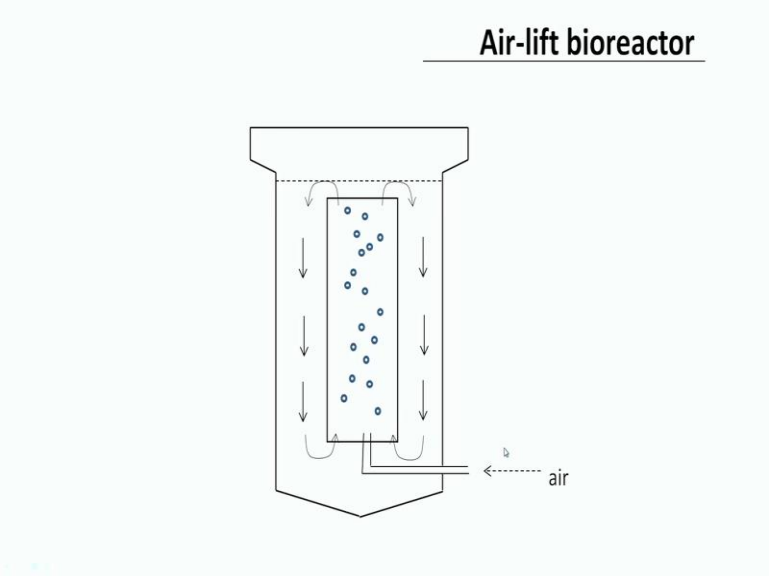

And then an air-lift bioreactor, a Packed bed bioreactor, a Fluidized bed bioreactor - which is nothing but a packed bed which jiggles around.

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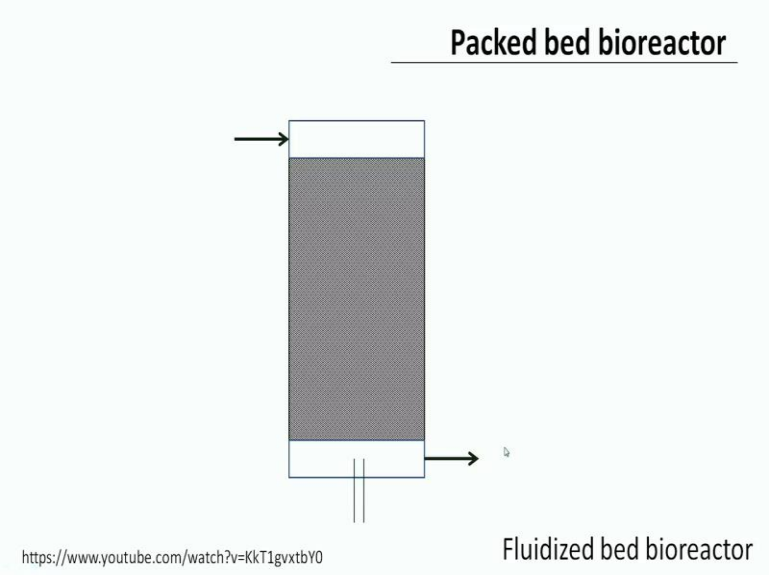

There are some advantages there. There was a video, which led you to some detail on the fluidized bed bioreactor.

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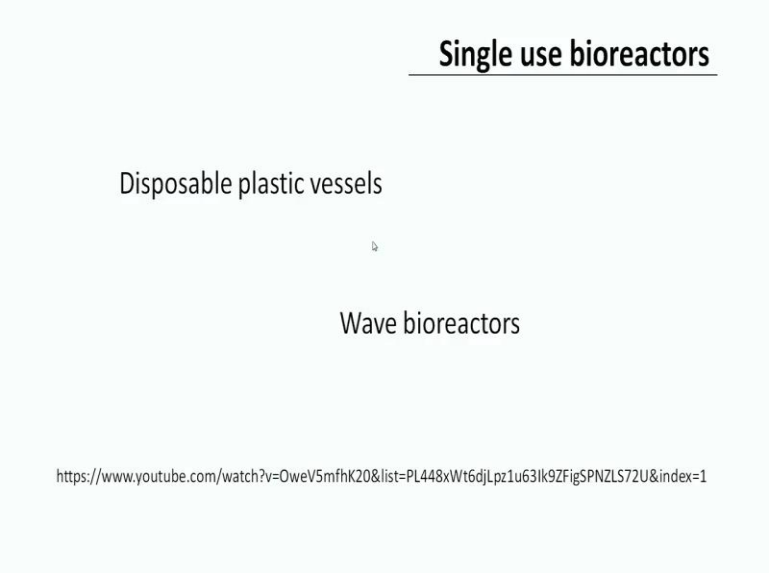

And then solid state bioreactors. Single use bioreactors that are helpful in meeting the stringent demands or stringent regulatory procedures for the production of any biological material.

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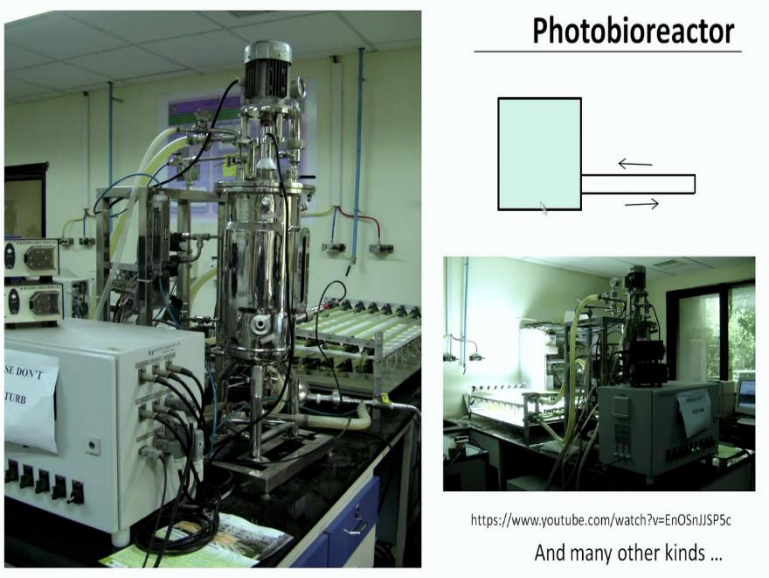

Then also, a Photobioreactor that is typically used with micro algae and other photosynthetic organisms. We said there are many other kinds of reactors. These are the typical ones, there could be other kinds too.

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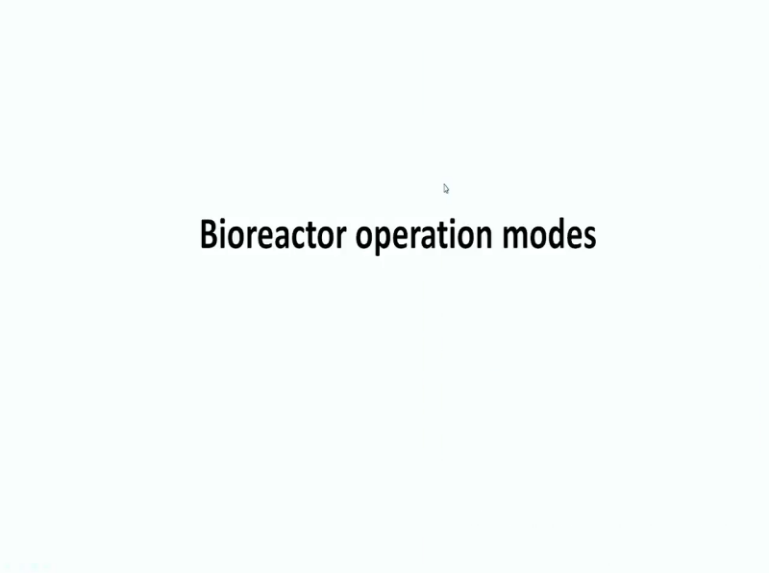

Then we said that any bioreactor or many bioreactors, where each bioreactor can be operated in 3 basic modes.

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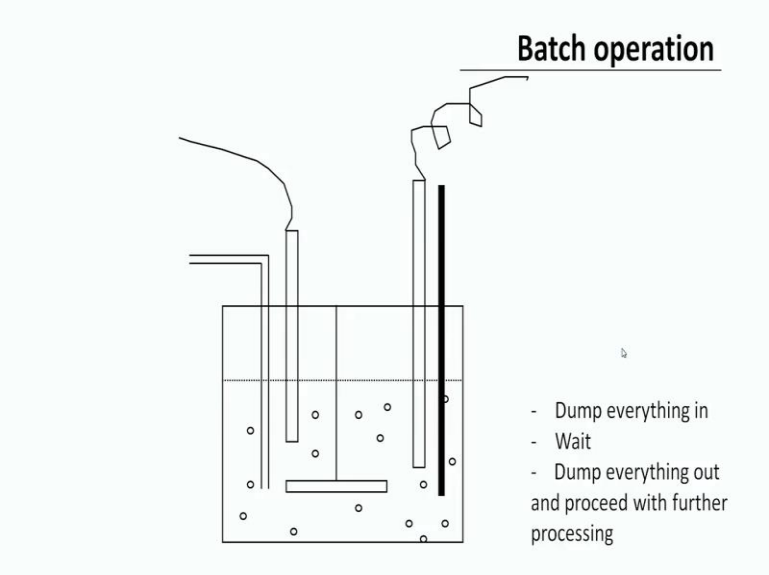

Either a batch operation, where we dump everything in, wait for the process to complete and dump everything out and proceed with further processing.

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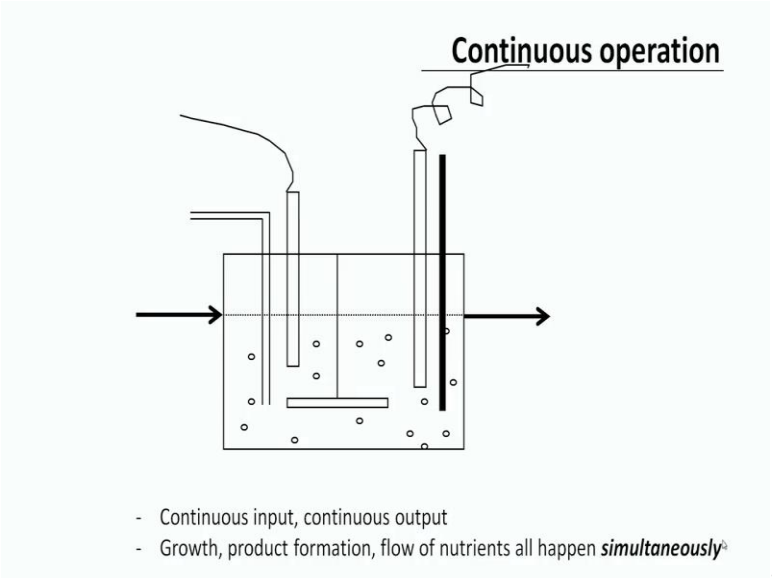

Or a continuous operation, where there is a continuous input, a continuous output. The growth, product formation, flow of nutrients - all happen simultaneously.

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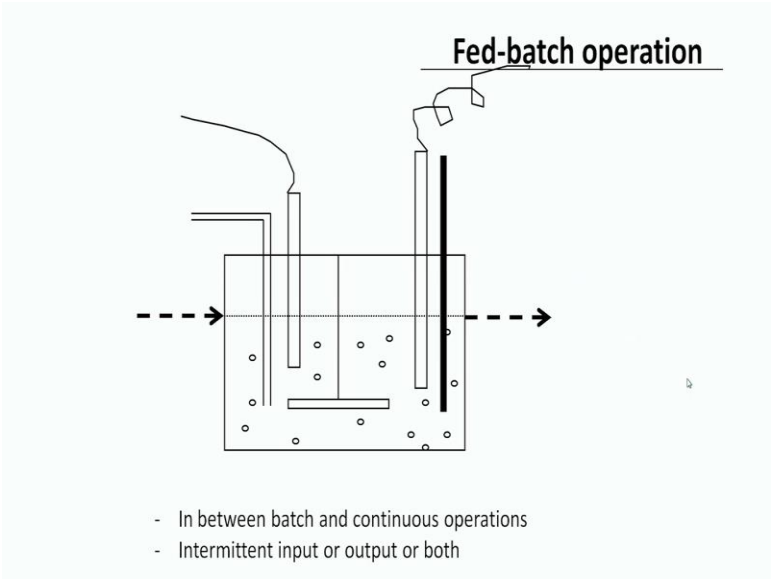

Then a fed-batch operation, which is in between a batch and a continuous operation where there could be intermittent input or output or both, both intermittent output and the intermittent input, at the same time. Some reactors we said, nicely fit into all 3 modes of the possibilities of operating them in all 3 modes of operation, some of them do not. This is think, where we finished up the last lecture.

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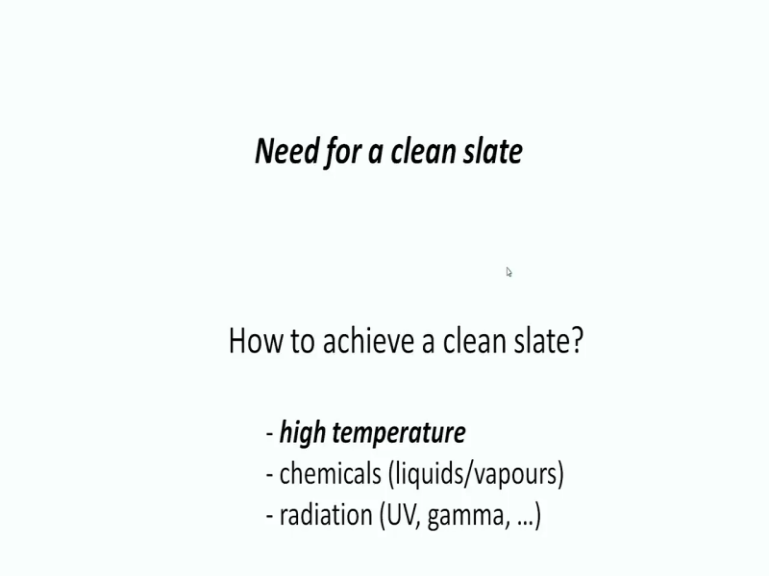

We said that, whenever a bioprocess is involved, we typically need a clean slate. We want only the organisms of our interest to grow in the bioreactor and therefore, the bioreactor should be a clean slate when we begin. The ways of achieving a clean slate is either through high temperature or a chemicals, vapors or through radiation such as UV and gamma, which kills all the organisms that are present in the bioreactor and then provides us with a clean slate, and then we introduce the organisms of our interest which grows, multiplies, produces the product of interest. So, let us move further in this lecture. From this point, we will look at high temperature in some details of achieving a clean slate or sterilization as it is called, using high temperature.

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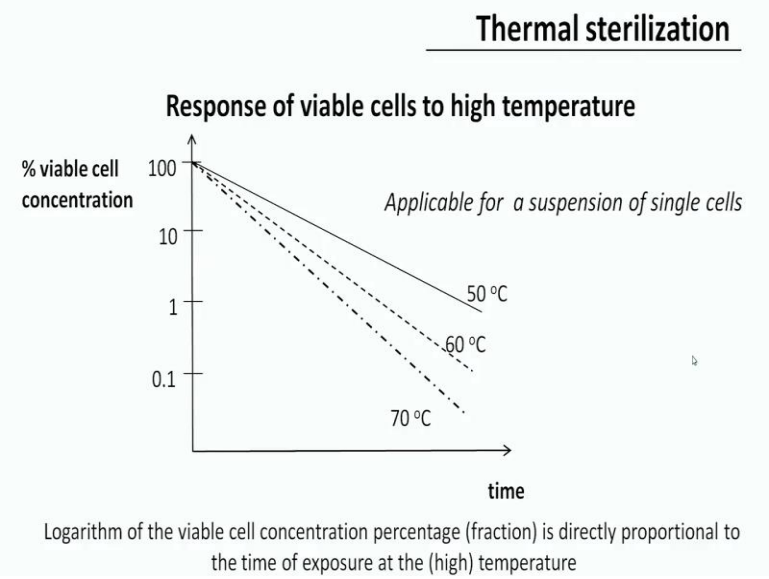

This is specifically thermal sterilization. The one that uses high temperature. If we plot the percent viable cell concentration versus time as is shown here. This percent viable cell concentration we are plotting on a log scale 100, 10, 1, 0.1. At a temperature of 50 which is typically a higher temperature, then, many organisms grow at 30-degree c, 37-degree c, we sum at 20, 27 oh sorry 25, 27 and so on. So, 50-degree c is somewhat high for most organisms. There are some thermophiles at grow well at high temperatures, but let us not consider them for the time being. If it is at 50-degree c, then the viable cell concentration decreases linearly with time, at 50-degree c. At 60 degree c again the response is linear, but it is steeper, it gets killed faster. And similarly at 70-degree c, the response is linear, but the slope of this curve is larger compared to the ones at 50-degree c or 60-degree c.

This is the typical response that is absorbed when viable cells are exposed to high temperature - temperature that is higher than their normal operating temperature. This kind of a behavior is applicable for a suspension of single cells, cells separately not clumped together and so on and so forth. And what this graph tells us is that the logarithm of the viable cell concentration, know this is on a log scale, so the logarithm of the viable cell concentration percentage or a fraction is, you know that fraction times under is percentage. So, fraction is directly proportion to the time of exposure at the high temperature. So, this is the kind of behavior that we see when we expose single cell suspensions to high temperature. So, how we are going to use this?

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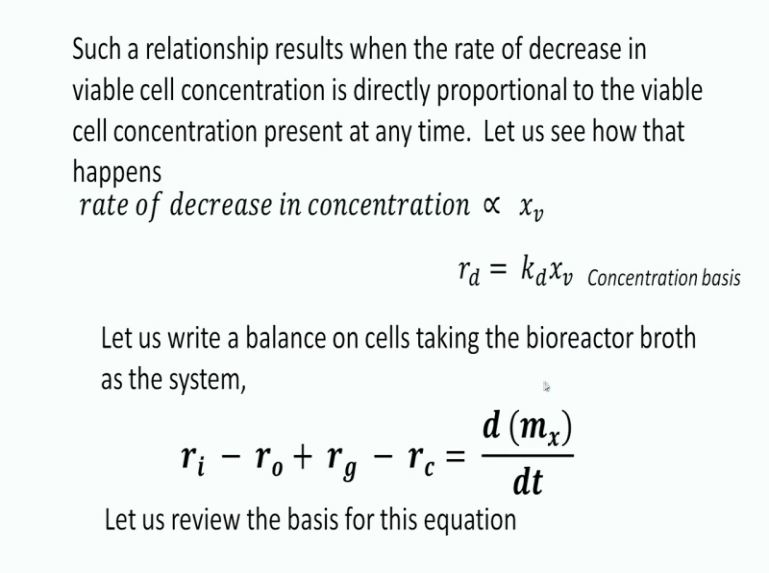

Before we look at how to use this, lets us understand this a little better. So, that it becomes easier to use this at any required situation, in any required situation. This kind of a relationship that we saw earlier results when the rate of decrease in viable cell concentration is directly proportion to the viable cell concentration present at any time. Now let me repeat this, the rate of decrease of viable cell concentration is directly proportional to the viable cell concentration present at any time. For example, this is what is, what we mentioned here, but written in mathematical terms. The rate of decrease in concentration of viable cells is proportional to the viable cell concentration x_v.

$$rate\ of\ decrease\ in\ concentration \propto x_v$$

If we call the rater_d, the rate of decrease in concentration as r_d. Since it is proportional, we can replace the proportionality by equal to sign and here we need to multiply x v with a constant. Let us call this constant k_d. You might be familiar with this kind of a first order expression in your earlier courses. So, we are using a first order kind of a representation here.

$$r_d = k_d x_v$$

Note, that this r_d is rate of decrease in concentration, which is the concentration decrease per unit time, mass or number of cells per unit volume, per unit time. That is the unit here. Therefore, the basis is the concentration basis. We need to remember this.

Now, let us write a balance on cells taking the bioreactor as the system. You have all gone through a course on material balances. So, we are going to write a material balance on cells and you all know that a balance can be written over a region of focus and that is called the system. The region of focus is called the system. In this case, the bioreactor broth, the liquid part in the bioreactor in which the cells grow, that is what we are going to take as the system and we are going to write a balance on cells on that system. You might recall this equation here.

$$r_i - r_o + r_g - r_c = \frac{d(m_x)}{dt}$$

The rate of input of the cells into the system, which is the bioreactor broth, minus the rate of output of cells from the bioreactor broth, plus the rate of generation of cells in the bioreactor broth, minus the rate of consumption of cells in the bioreactor broth, equals the rate of accumulation of cells in the bioreactor broth. This is, if you consider all that can happen to the species cells. This is all that can happen, it can either come into the system, go out of the system, get generated in the system or get consumed in the system and the net result of all these things must equal the accumulated rate or accumulated amount, expressed as a rate here. Because all these are rates, this is also a rate.

Also important to note that this is on a mass basis. This comes from the principle of mass conservation and that principle is what allows us to write this equal to sign. Mass is conserved, mass can be either be created nor destroyed. We are of course, not considering either nuclear reactions or traveling at the speed of light and in such situations, the mass of a species is conserved. The mass of the species before a process equals the mass of the species after the process. So, that is the basis for this. If it is still not clear, many students take a while to understand this. So, let us review the basis for this equation. You may have done some of this in your first course on material balances. So, you could take this as a review and try to understand this to the extent needed, to be able to manipulate or write balances according to our need.

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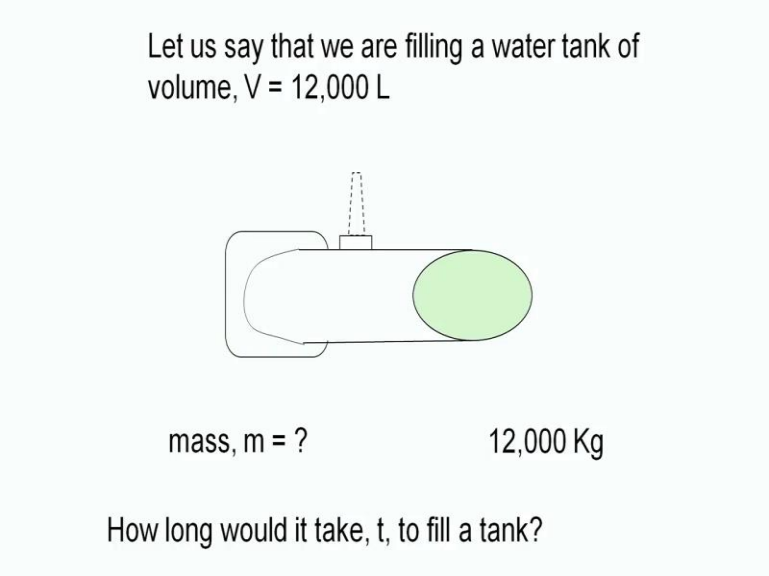

To do that, let us consider a situation that we are all, in Chennai atleast, very familiar with in summers. Now this is the use of water tankers. If water runs out, then water tankers are used to get water for daily needs. Let the typical size of a water tanker could be around 12,000 liters and this is my representation of a water tanker here. I would like you to use your imagination to see how this is a water tanker. This is the back of the lorry on which the water tanker rests and let us say that this is the filling of the water tanker with water at its source.

So, this typically is either 8,000 liters, 10,000 liters, 12,000 liters or 16,000 liters. Let us consider a tank of 12,000-liter capacity. This is the case, what is the mass of the water in the tank? All of you know that if we know the density, we can find out the mass of the water in the tank. And the mass happens to be 12,000 Kg because the density of water is 1 gram per cc or 1,000 kilogram per meter cubed, 1 kilogram per liter and therefore 12,000 liters comes out to be 12,000 kg. Now the question is, how long would it take to fill a tank? To fill this tank - 12,000-liter tank. That is the question. So, people who remember their material balance course or who are comfortable with this will immediately ask the question, what is the input rate of water that fills the tank? If you know the input rate of water, then you can figure out the time taken to fill the tank quite easily.

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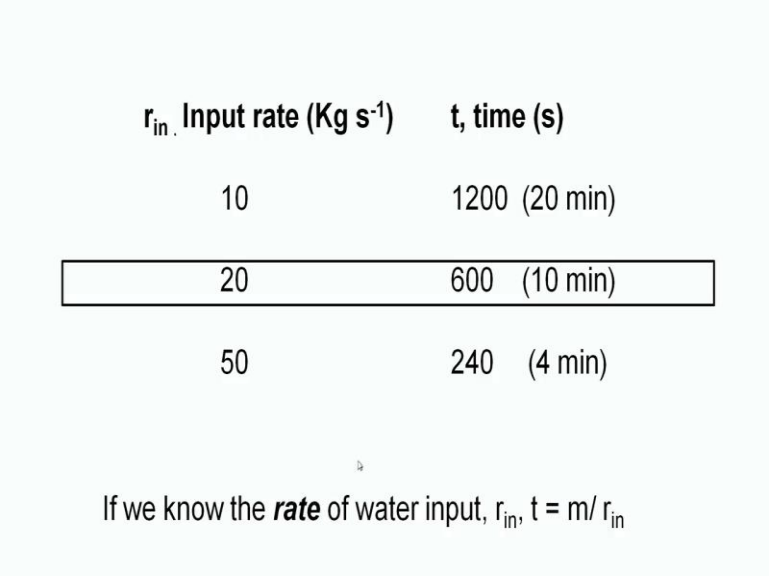

If the input rate happens to be 10 kilogram per second, the time would be 12,000, sorry 1,200 seconds or 20 minutes. If the input rate is 20, the time would be 600 seconds or 10 minutes. If the input rate is 50 kilogram per second, the time would be 240 seconds or 4 minutes. How did we get at this? If we know the rate of water input, the time is nothing but mass, is nothing but mass divided by the rate and that is what this turns out to be. For example; 12,000 kilogram by 10 kilogram per second gives you 1,200 seconds, which is 20 minutes and so on. A typical time in which the tank gets filled is about 10 minutes, and therefore let us choose this as the input rate for further discussion. Note that we are talking of rates. Now let us complicate this process a little bit.

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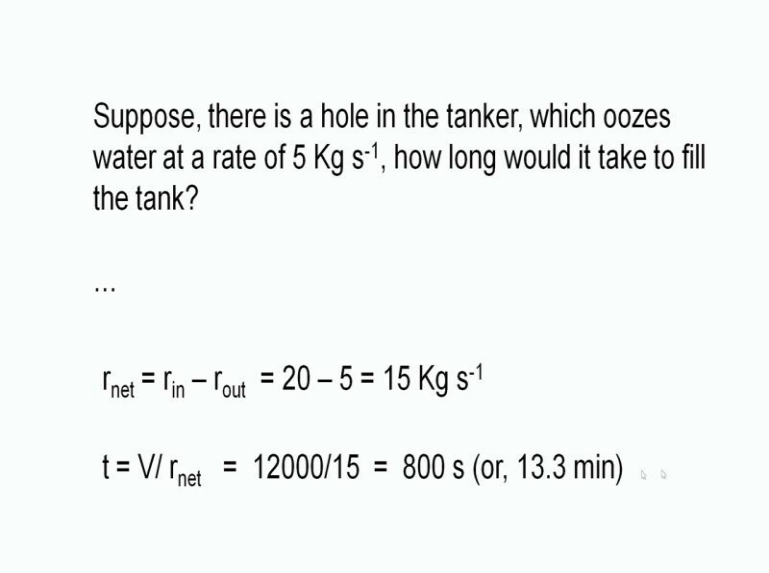

Suppose, there is a hole in the tanker, which oozes water at the rate of 5 kilogram per second. How long would it take to fill the tank? If you recall your course, you would say if I know the net rate, I can very easily find the time. So, that is the advantage here, if you work in terms of rate, all these very standard questions become easier to calculate. If you work in terms of volumes in it, becomes rather difficult to see what is happening and so on and so forth. Therefore, we work in terms of rate. And that is why rate is considered some sort of a standard parameter, while dealing with dyamic systems, engineering systems and so on. We will look at that in a little while.

The net rate in this case, there is a 10 kilograms coming in, 5 kilograms going out per second, 10 kilograms per second coming in. Sorry I think it is 20 kilograms per second coming in, 5 kilograms per second going out. So, the net rate happens to be 15 kilogram per second.

$r_{net} = r_{in} – r_{out} = 20 – 5 = 15 \text{ Kg } s^{-1}$

And once you know the net rate, you can find out the time, as the volume divided by the net rate. Or in this case the mass divided by the net rate.

$t = V/ r_{net} = 12000/15 = 800 \text{ s (or, 13.3 min)}$

This has to be m, 12,000 kg is divided by 15 kilogram per second or 800 seconds or about 13.3 minutes, is what we get.

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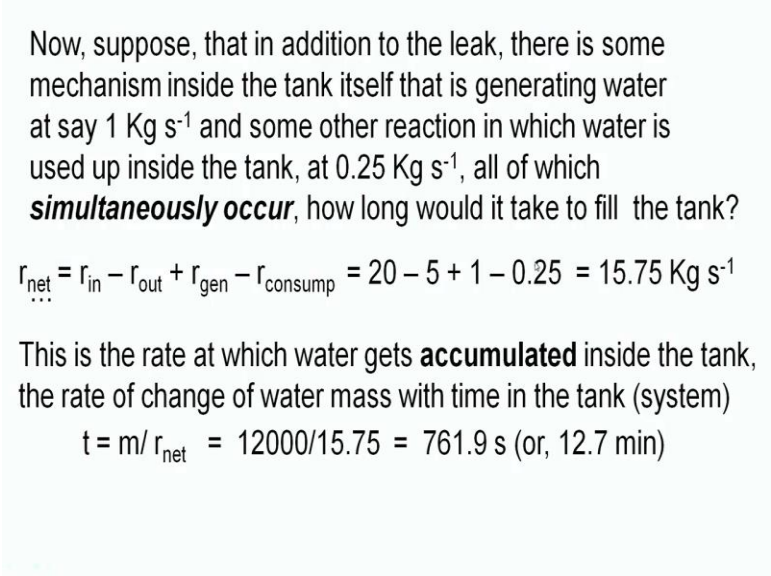

Now, let us complicate this in a creative fashion. Suppose, that in addition to the leak, there is some mechanism inside the tank itself that is generating water at 1 kilogram per second. And some other reaction in which the water is used up inside the tank, at 0.25 kilogram per second, all of which simultaneously occur. This is the crucial line here. They all simultaneously occur. If all these simultaneously occur, how long would it take to fill the tank? You are comfortable with rates by now so, you ask me, what is the net rate? If I know the net rate, I can find that out. How do you find the net rate? The net rate is rate of input minus rate of output plus rate of generation minus rate of consumption. Rate of input is 20 kilogram per second. Rate of output is 5 kilogram per second. That we have already seen. Now the rate of generation here, water is being generated at 1 kilogram per second and it is being consumed by a reaction at 0.25 kilogram per second. Therefore, the net rate turns out to be 15.75 kilogram per second.

$r_{net} = r_{in} - r_{out} + r_{gen} - r_{consump} = 20 - 5 + 1 - 0.25 = 15.75 \text{ Kg s}^{-1}$

So, this is the rate at which water gets accumulated inside the system or inside the tank, the rate of change of water mass with time in the tank. So, that will turn out to be 12,000 kg divided by the net rate which is 15.75 kilogram per second, which turns out to be 761.9 seconds or 12.7 minutes.

$t = m/ r_{net} = 12000/15.75 = 761.9 \text{ s (or, 12.7 min)}$

Now, I gave you this background to our equation. Rate of input minus rate of output plus rate of generation minus rate of consumption equals the rate of accumulation. And the rate of accumulation we write as the derivative so that the form is easy to directly use.

$$\boldsymbol{r_{net} = r_i - r_o + r_g - r_c = \frac{d\,(m)}{dt}}$$

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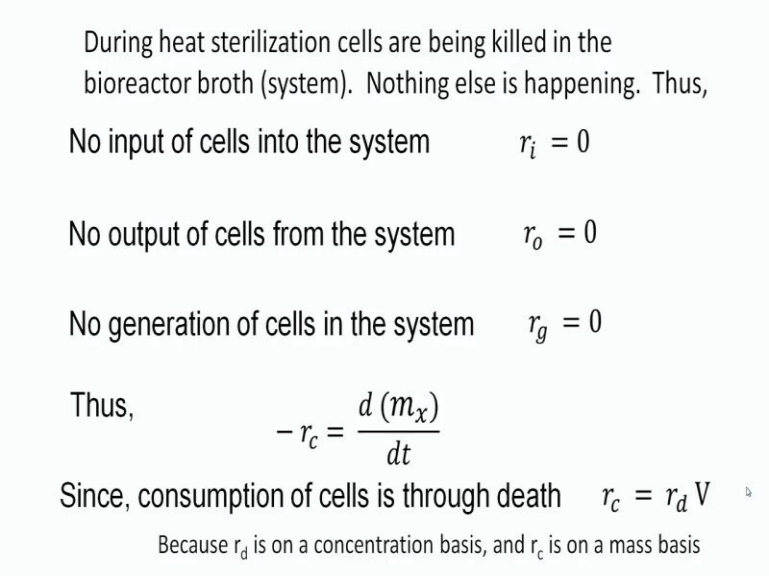

During heat sterilization of cells, you know you are sterilizing, let's say a stirred tank bioreactor containing medium which probably has some cells growing in it already. It is not sterilized and we are going to heat sterilize and kill all the cells that are present there. During that process, the cells are being killed in the bioreactor broth, which is our system. Nothing else is happening, there is no input, there is no output and so on so forth. Therefore, the rate of input of cells, mass of cells into the system is 0.

$$r_i = 0$$

There is no output of cells from the system. The rate of output of cells, again mass of cells or number of cells, is 0.

$$r_o = 0$$

There is no generation of cells in the system or let's assume that. Therefore, the rate of generation of cells is 0.

$$r_g = 0$$

And therefore, the only term that remains from r i minus r o plus r g minus r c is this. Minus r c and minus r c the rate of consumption of cells, r c is equal to d dt, the rate at which mass of cells gets accumulated in the system.

$$-r_c = \frac{d(m_x)}{dt}$$

This is what we get by considering it from first principles.

The consumption of cells is through the death of cells as they are getting exposed to the high temperature. Remember, this is the heat sterilization that we are talking about. Therefore, r c which is the rate of consumption of cells on a mass basis is r d which was on a volume basis, remember that was, a sorry, that was on a concentration basis, a concentration is nothing but mass divided by the volume, therefore to get mass from concentration, you need to multiply concentration by volume. Therefore, to get a rate that is mass based from a rate that is concentration based, you need to multiply the concentration based rate by volume.

$$r_c = r_d \, V$$

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Therefore,

$$-r_d V = \frac{d(m_x)}{dt}$$

From the definition of concentration

$$mass = concentration \times volume$$

$$m_x = x_v V$$

$$-r_d V = \frac{d(x_v V)}{dt} = V\frac{d(x_v)}{dt}$$

V is a constant here

Thus

$$-r_d = -k_d x_v = \frac{d(x_v)}{dt}$$

Therefore, this r d into V, which is the rate of consumption of cells or the rate of death of cells on a mass basis, this minus comes from our balance equation, equals the accumulation rate of the mass of cells inside the bioreactor, when it is being sterilized. Minus r d into V equals d dt of m x.

$$-r_d V = \frac{d(m_x)}{dt}$$

Now, we have everything on a mass basis, which is nice to have. Now, from the definition of concentration, mass is nothing but concentration times volume.

$$mass = concentration \times volume$$

Or in other words, concentration is mass by volume, that is the definition. And therefore, m x which we have here is x v, the concentration of viable cells, into the volume.

$$m_x = x_v V$$

If we use this to replace this m x, then we get this minus r d into V equals m x as x v into V, therefore, d dt of x v into V. And in this case, since volume is a constant, volume of the broth, volume of the system, that is a constant, we can take volume out of the derivative, V into d dt of x v.

$$-r_d V = \frac{d(x_v V)}{dt} = V\frac{d(x_v)}{dt}$$

Thus, if we, now we have a volume here, we have a volume here. We can cancel them out. They are the same volumes. Therefore, minus r d equals minus k d x v, which is what we had seen earlier. We had represented r d as a first order expression, k d into x v. The rate of death of cells equals a constant times the viable cell concentration. That is a first order expression. That equals d x v dt, since we have, we have cancelled the volumes here.

$$-r_d = -k_d x_v = \frac{d(x_v)}{dt}$$

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$$\frac{d\,(x_v)}{dt} = -\,k_d x_v$$

If we solve this first order differential equation, we get

$$\ln\left(\frac{x_{v0}}{x_v}\right) = k_d\,t$$

Or, the time needed for the viable cell concentration to go down to x_v starting from x_{v0} is

$$t = \frac{2.303}{k_d}\log_{10}\left(\frac{x_{v0}}{x_v}\right)$$

This is the final expression here, d d t of x v equals minus k d times x v.

$$\frac{d\,(x_v)}{dt} = -\,k_d x_v$$

This is the first order differential equation, which you are all familiar with. If we solve this first order differential equation, we get ln or natural log x v naught by x v equals k d times t time.

$$\ln\left(\frac{x_{v0}}{x_v}\right) = k_d\,t$$

Where x v naught is the concentration when we began the process of sterilization- heat sterilization and x v is the viable cell concentration at any time t. Or the way to look at this from our perspective, from a design perspective, is the time needed for the viable cell concentration to go down to x v starting from x v naught when the process started, is nothing but, I am converting the natural log to log base 10. You know that the conversation factor is 2.303. Therefore, natural log x v naught x v is 2.303 log to the base 10 x v naught x v and k d comes to the denominator here. Therefore, the time required for this amount of a decrease in viable cell concentration from x v naught by x v is this expression. 2.303 divided by k d, log to the base ten of x v naught by x v.

$$t = \frac{2.303}{k_d}\log_{10}\left(\frac{x_{v0}}{x_v}\right)$$

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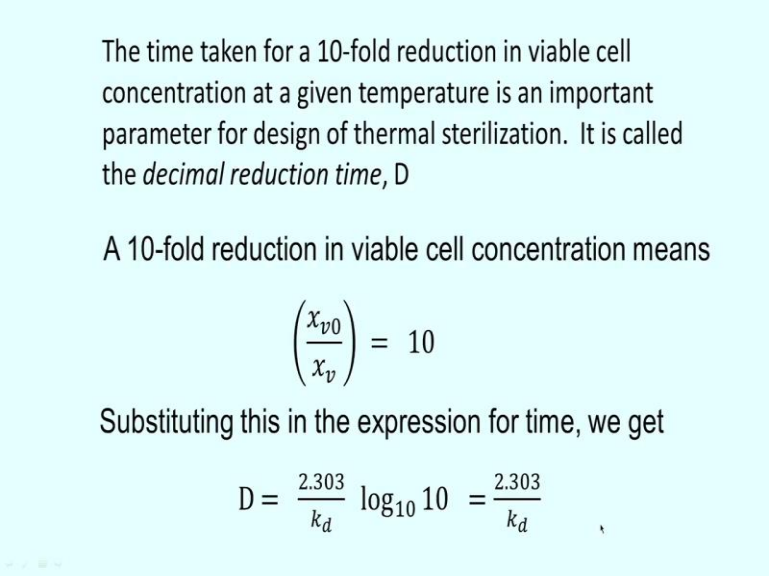

The time taken for a 10-fold reduction in the viable cell concentration. Now, when if it was initially a certain value, it has gone down to one-tenth of what it was initially. That is the 10-fold reduction in viable cell concentration, at any given temperature, is an important parameter for the design of thermal sterilization. We are figuring out means by which we collect the information so that it can be used elsewhere, without much information to design the sterilization process, to design the sterilization equipment and so on. This time taken for 10-fold reduction in viable cell concentration is called the decimal reduction time, typically expressed as capital D. In terms of whatever we have derived at 10-fold reduction in viable cell concentration essentially means, that the initial viable cell concentration x v naught divided by the viable cell concentration at that time t x v, must be 10, straight forward.

$$t = \frac{2.303}{k_d} \log_{10}\left(\frac{x_{v0}}{x_v}\right)$$

And if we substitute this in the expression for time that we had derived earlier, this expression the time taken for the concentration to go from x v naught to x v. Then, we would get an expression for the decimal reduction time, in terms of the variables that we would like. 2.303 divided by k d log 10 to the base 10. You all know log a to the base a as 1 and therefore, this reduces to 2.303 by k d.

$$D = \frac{2.303}{k_d} \log_{10} 10 = \frac{2.303}{k_d}$$

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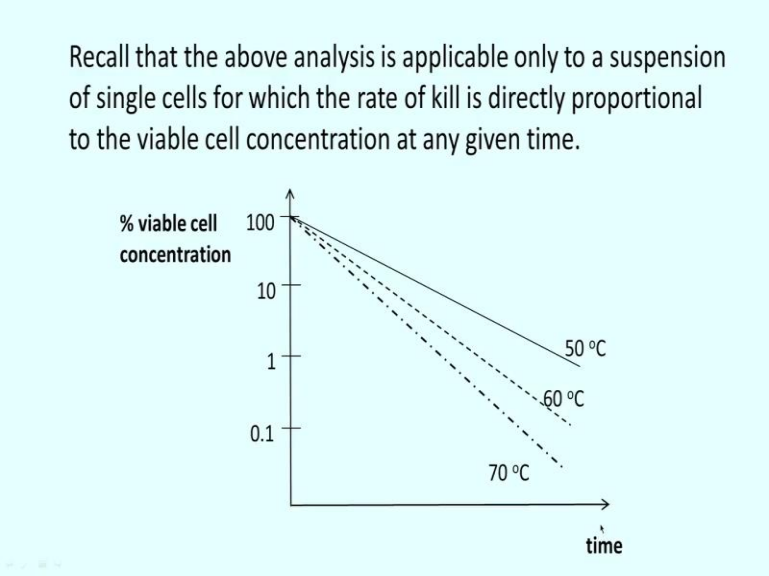

Recall that the above analysis is applicable only to a suspension of single cells for which the rate of kill is directly proportional to the viable cell concentration at any given time. Remember, this has been observed. And then this observation, we looked at representing mathematically and we said that the first order decrease would be a good way to represent this. That is, the rate of decrease of cell concentration at the high temperature is directly proportional to the viable cell concentration at any time. And that is what resulted in the earlier mathematical expression that we had. Mathematical expression always generalizes things. It makes it a lot more useful in situations that has not been seen earlier, relevant situations that have not been seen earlier and thereby leads to design of sterilization processes, in this case.

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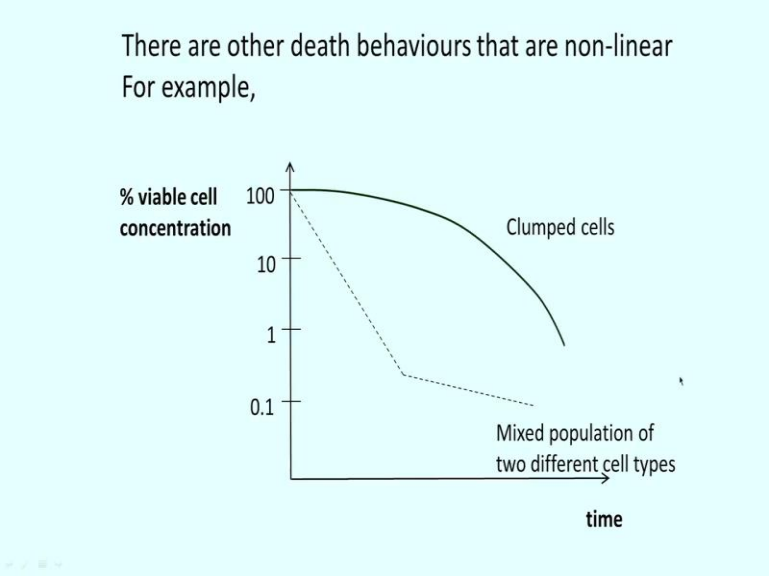

That was for a suspension of single cells. There are other death behaviors that are non-linear. Let us see whether you are able to guess this first. This is a plot of again, percent viable cell concentration 100, 10, 1, 0.1, therefore, on a log scale, versus time. And can you guess what would give a curve like this? Earlier it was linear, a linear decrease. In this case the decrease is something like this. It does not decrease much for quite a while and then it starts decreasing. Can you guess what kind of content or viable cells, viable cells at what form would give raise to this? The answer is, clumped cells. Now cells clump with each other. Then, it takes a while for the killing of the internal parts of the clump compared to the outer parts of the clump. So that results in this kind of a behavior for a plot of percent viable cell concentration versus time.

Let us look one other non-linear behavior, just for the variety. This is another non-linear behavior. As you can see it is represented as two lines, from 100 to let's say 0.2 or something like that, this is one line and then there is another line here with a different slope compared to this. When do you think such a behavior would be seen?

You know, we have already seen that this happens, in a suspension of single cells. Only thing is, that we have 2 lines with different slopes. So, this could arise when you have mixed population of 2 different cell types. The type differing, the difference in types arising from their response to a high temperature. This decreases, these type of cells decrease fast, these type of cells decreases slow, the viable cell concentration versus

time. And this is a kind of behavior that arises when you have a mixed population of two different cell types.

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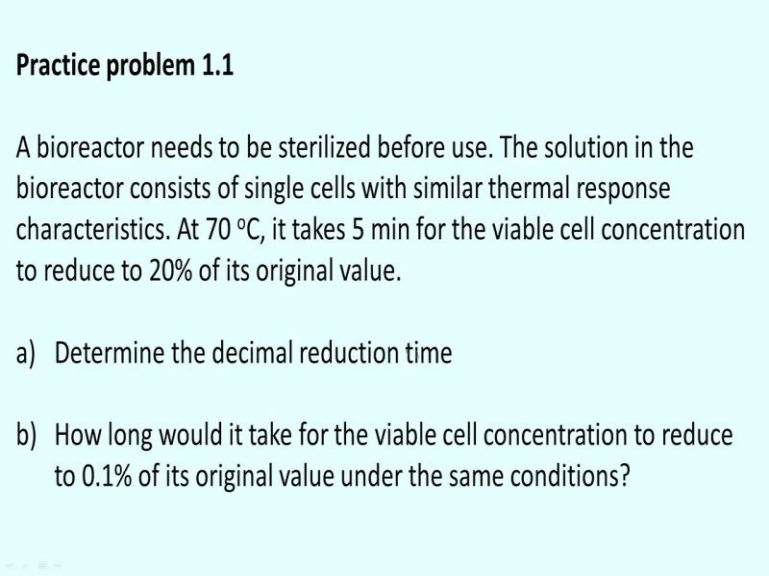

Now, I am going to present the first practice problem. This is, let me call it 1.1 because this is module 1. That is what the first number indicates and this is problem 1 of module 1, therefore, 1.1. I am going to assign this problem to you and you can solve it. You need not submit this problem. What we will, this will not count toward evaluation for this mooc. However, I believe that you need to pick up these skills of problem solving. Therefore, what I am going to do is, when we begin the next lecture, I am going to solve this first for you and then go forward.

The practice problem here reads; a bioreactor needs to be sterilized before use. The solution in the bioreactor consists of single cells with similar thermal response characteristics. So, it is going to be a single line that is dropping. At 70 degree c, it takes 5 minutes for the viable cell concentration to reduce to 20 percent of its original value. a) Determine the decimal reduction time. Remember, we said it was an important parameter. So determine that first, determine the decimal reduction time and b) How long would it take for the viable cell concentration to reduce to 0.1 percent of its original value under the same conditions? This is a problem for you, you need to know how to solve problems. We will probably touch up on that when we solve the problem or at least

I will show you some thinking behind the solution when we begin the next lecture. Therefore, this is what we did in the introduction; I guess we went through the earlier parts of the introduction when we began this lecture. Essentially some examples of bioprocesses and what exactly is a bioprocess and that the bioreactor is at the heart of the bioprocess.

Different types of bioreactors are commonly used. Some commonly used bioreactors we saw, such as the stirred tank bioreactor, the air-lift bioreactor, the solid state bioreactor, single use bioreactors, photobioreactors, packed bed bioreactors, fluidized bed bioreactors and so on. There are many different types. Bioreactor is nothing but, an instrumented controlled vessel in which bioreactions take place, typically mediated by cells or it could be an enzyme that mediates the reaction. That is exactly what a bioreactor is. Then we said that any bioreactor can be operated in different modes, some bioreactors lend themselves to operation in all the 3 basic modes and some do not.

For example, a stirred tank bioreactor can be operated in a batch mode, in a continuous mode as well as in a fed batch mode. Whereas, maybe a packed bed, it is a little difficult to operate only in a batch mode. You can but it takes a lot more effort. Rather it is normally not operated in a batch mode; it is operated in a continuous mode. Then, we looked at the need for a clean slate. That is, we want only our organisms to grow and produce the product of interest and therefore, we need to get rid of all other organisms that are normally present. We said that organisms are present in the air around us all the time and nothing prevents them from getting into the bioreactor broth that is open to the atmosphere. And therefore, we need to kill all the cells that are initially present there. The main modes of killing include thermal killing, a high temperature can be used to kill cells or you could use appropriate vapors or liquids. A 70 percent ethanol solution is used to kill cells on your hands when you handle these organisms, cell cultures and so on. Or formalin vapors, formaldehyde vapors from a formalin solution is used to kill the organisms in a space such as a lab.

And then you could also use gamma rays and so on. UV rays, gamma rays, gamma rays. Especially, if you are sterilizing, let us say syringes and things like that, that cannot be exposed to high temperature, they are made of plastics, they cannot be exposed to high temperature. So, those 3 modes of killing. Then we said we will look at the thermal

sterilization and some detail. We reaffirmed the need to be clear about the concept of rate as a basic parameter in any engineering system analysis, dynamic system analysis.

The rate by itself is important. The way you measure rate could be very different. For example, you have speed, which is equivalent of rate and you could measure an average speed by the distance traveled by time taken. But, speed is rather central to the kind of, to answer the kind of questions that we would be interested in when we deal with design operation of engineering systems, in this case biological engineering systems. And then, using rates, we said that the rate of killing of cells by high temperature could be directly proportional to the viable cell concentration at a particular time or first order kind of expression.

Then we saw that led to the definition of a decimal reduction rate, a standard parameter. And then also a more general expression before that, that gives the time, the time that it takes to go from, let us say an initial concentration of x v naught, initial viable cell concentrate of x v naught to a certain viable cell concentration of x v at a particular time t. Then we looked at this practice problem here. Before that, we did see that this linear killing is only one kind of a behavior. You could have other kinds of killing such as a curve when there are clumped cells and a combination of 2 straight lines with different slopes, if you have a mixed population of thermally different organisms. Then we looked at this practice problem. You might want to spend some time in solving this. It will be helpful. These are the kind of things that engineers are expected to do and it will be good to pick up these skills. When we meet next, we will solve this problem and start module 2.

See you in the next lecture.