Bioreactors

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Lecture - 21
Course summary

Welcome to this summary lecture for the course on bioreactors, the NPTEL online certification course. This summary lecture would provide an overview of the things that we looked at in this course, and hopefully it will improve the understanding and appreciation of the material here. We first began with an introductory module; there were totally five modules, the first was introductory module, the second one looked at two major outcomes from a bioreactor; the cells themselves or what is known as biomass or the products made by the cells or through enzymatic reactions. The third module looked at analysis of a few common operating modes of the bioreactor, and inherent advantages in operating them, in those various modes over the other modes.

The fourth module looked at the bioreactor environment parameters. The variables that are usually measured and controlled, to make the bioreactor operate optimally, without understanding the actual factories that are inside the bioreactor to a great extent. Some surface level understanding is there, but to a great extent it is not understood, and the bioreactor environment parameter operation level, is limited to that. Then in the last module, module five we looked at some means by which we can understand, the cells themselves; what is going on inside the cells, hopefully to manipulate them towards better production of substances of interest to us; that is the overall a scheme for this course. Let us summarize some details from these various modules.

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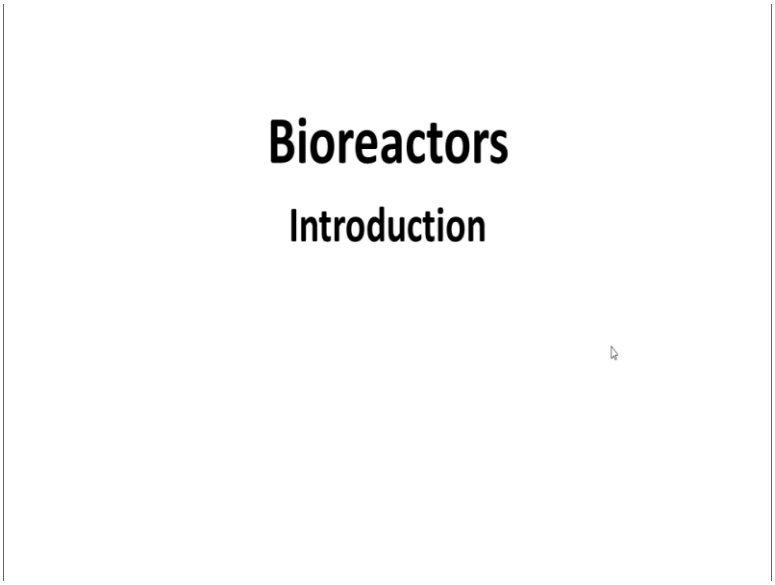

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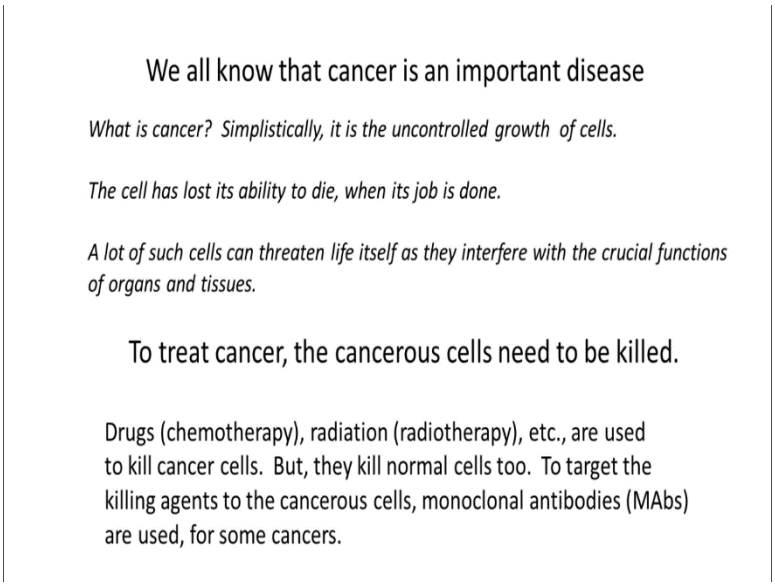

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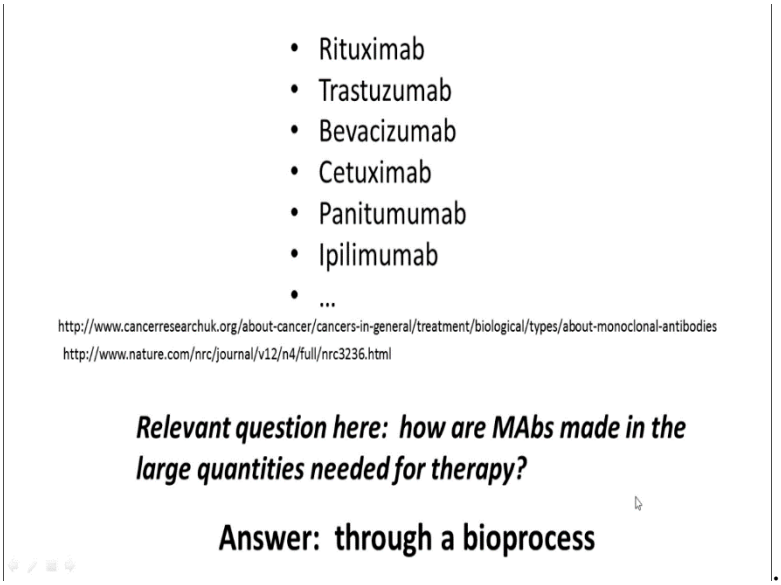

The introductory module we first saw what cancer was, and the drugs that are used to treat cancer, the monoclonal antibodies. And we saw that MAbs are made through bioreactors, through a bioprocess of which bioreactor is the important part.

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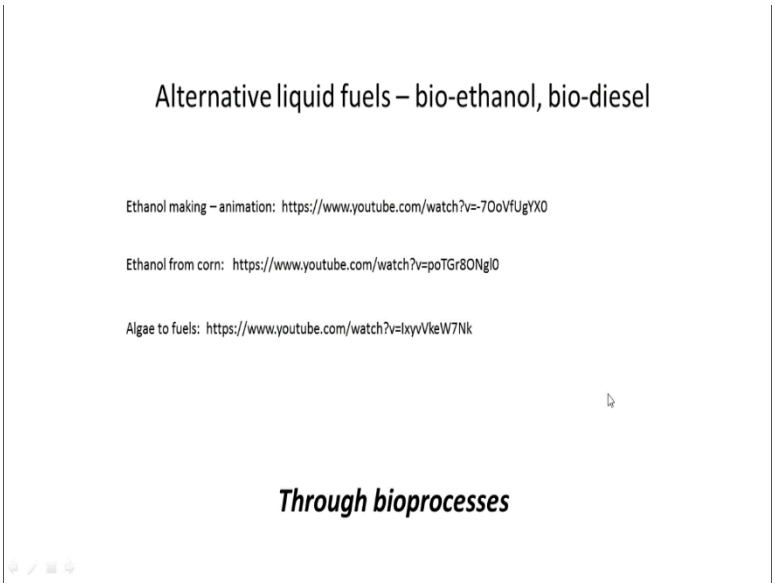

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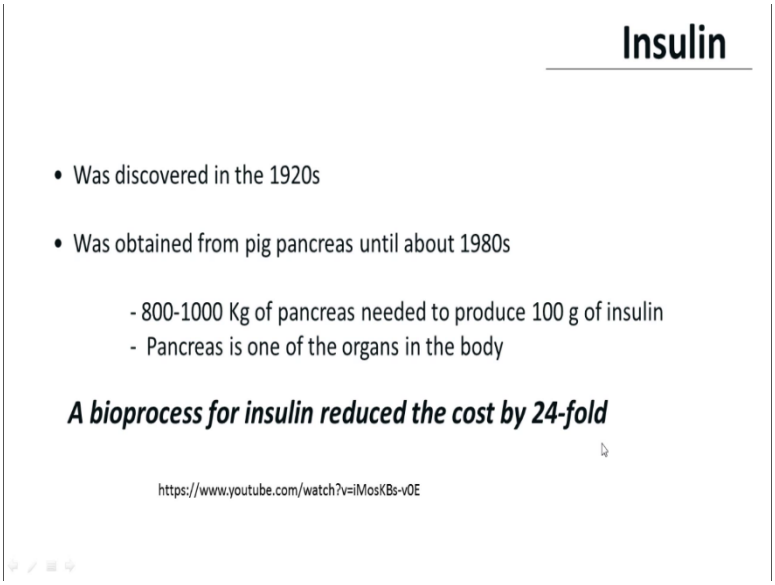

Similarly, this is another example, alternative liquid fuels such as bioethanol, biodiesel, also bioreactors play major role in them. Insulin which is a drug to treat diabetes is made through bioreactors, and even yogurt is made through bioreactors; a simple bioreactor at home.

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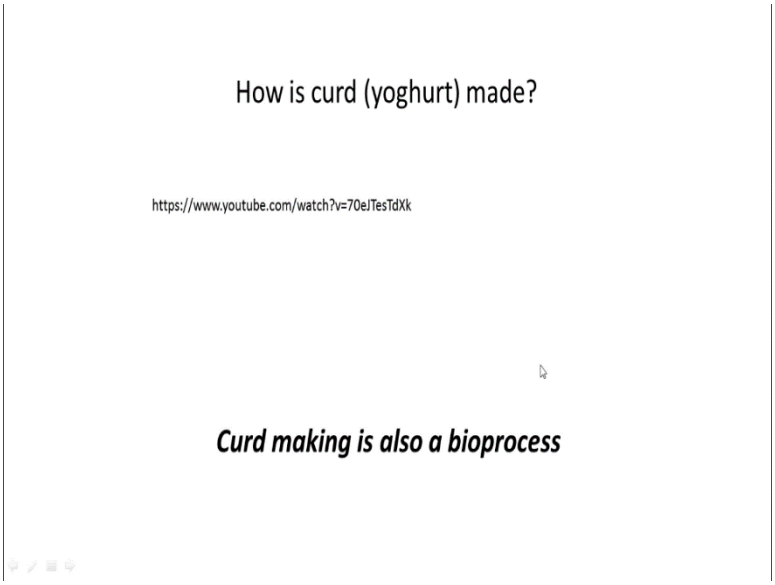

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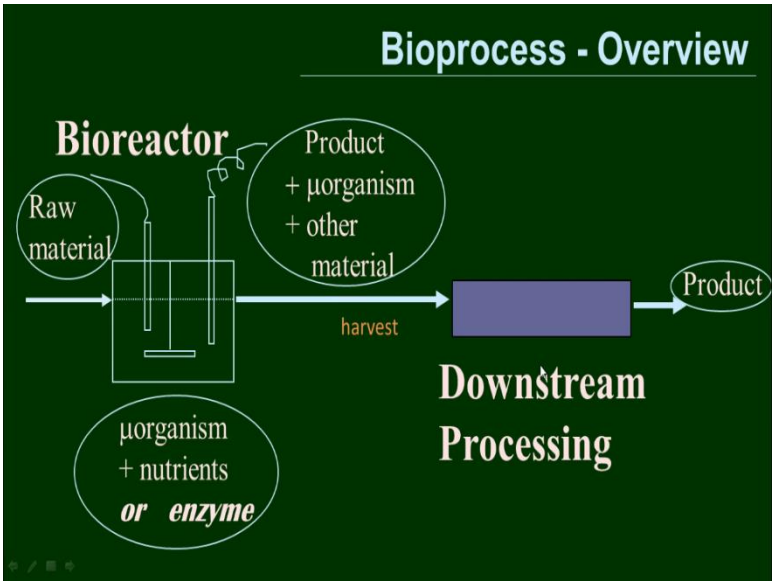

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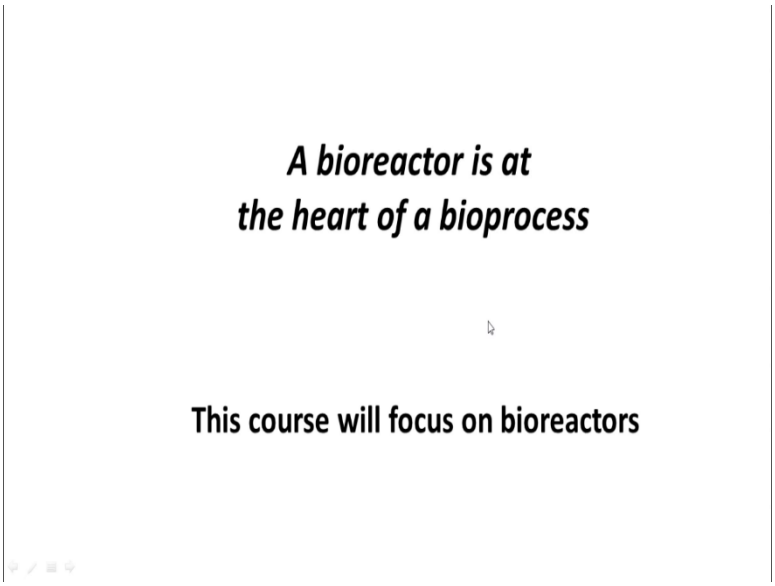

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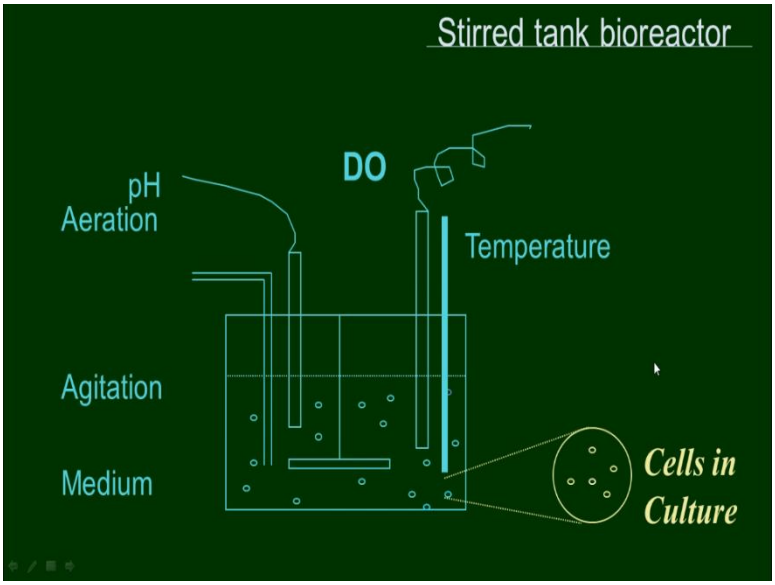

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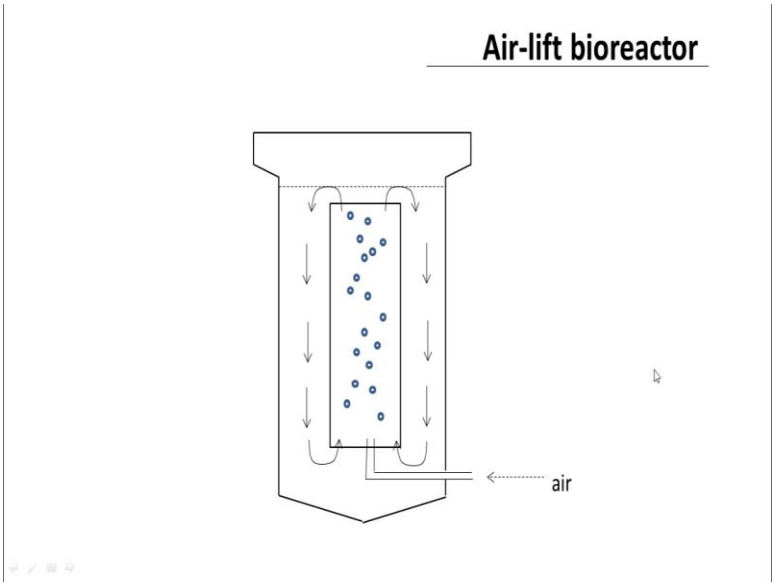

Then we looked at the bio process, the bioreactor. The common bioreactor types that are used; Such as stirred tank bioreactor, the air lift bioreactor, the packed bed bioreactor, solid state bioreactor, where there is no liquid usually, single use bioreactors such as disposable plastic vessels, and wave bioreactors which are good for certain operational reasons in the industry.

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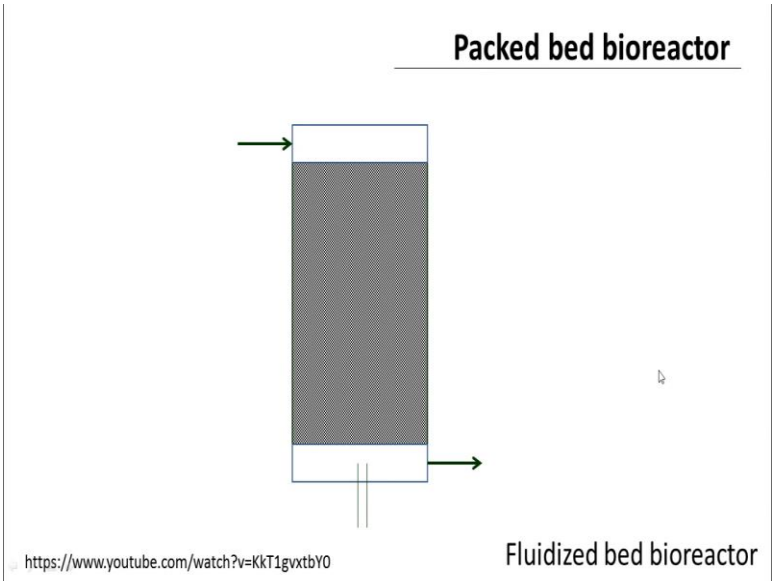

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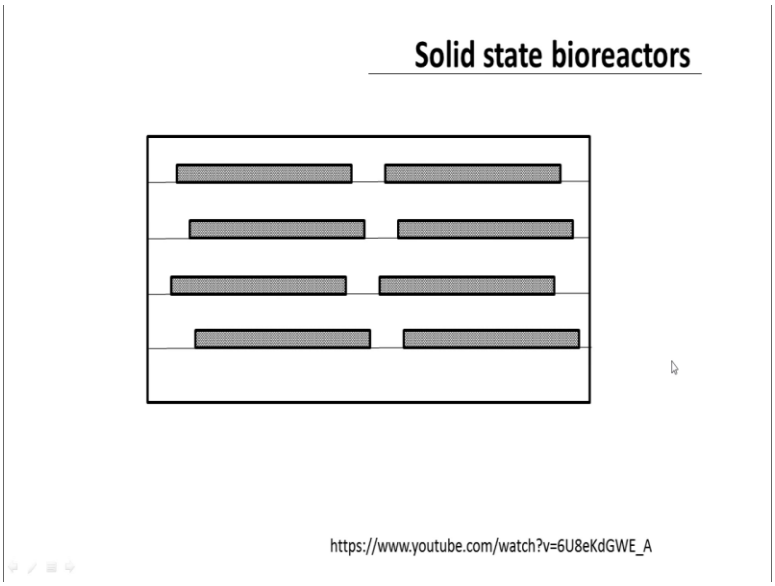

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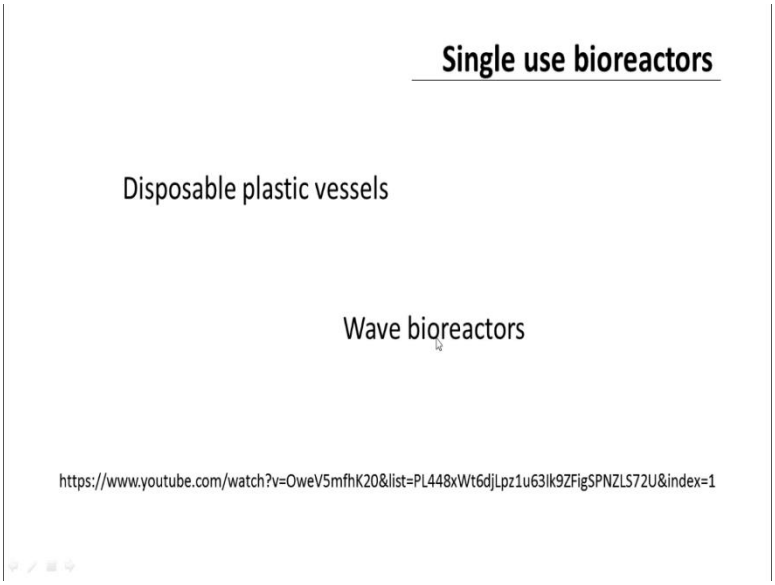

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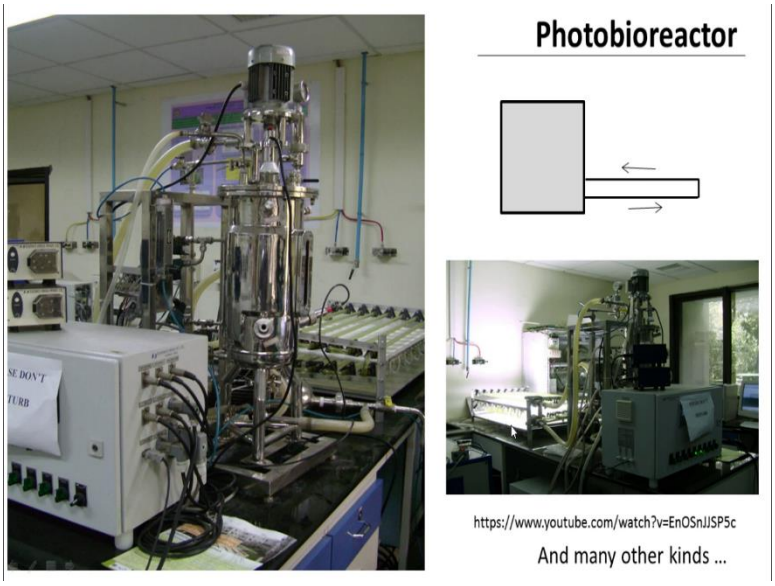

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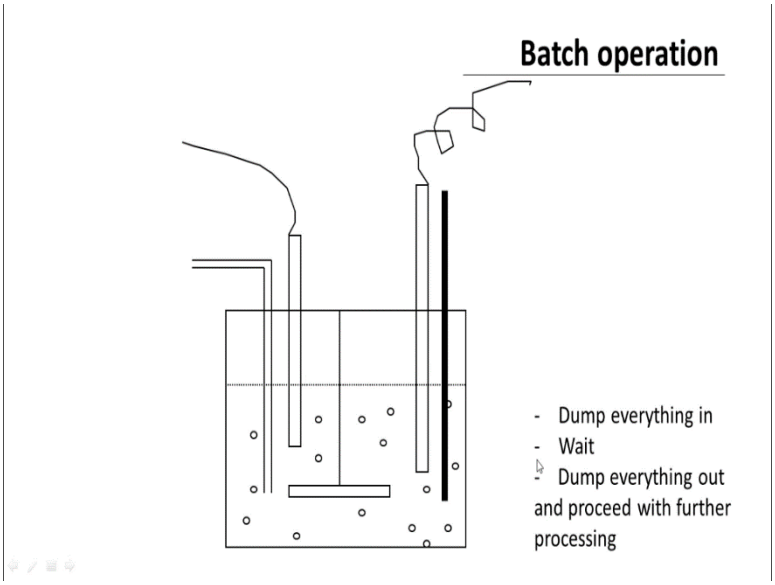

And photo bioreactor which is used for, cultivating photo synthetic organisms. These all we saw, and we said there are many other kinds of bioreactors also, these are some of the common once. Then we said that the bioreactor could be operated in a few operation modes. The batch, the where we dump everything in, let the process happen, and dump everything out and proceed with processing.

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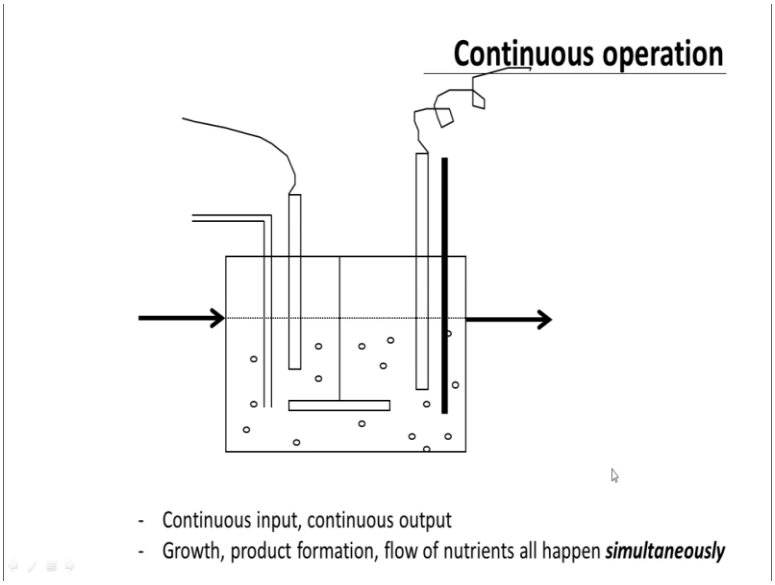

Continuous operation where there is a continuous input and a continuous output. We are usually interested in the steady state operation, which occurs for most of the time. The initial path could be at unsteady state, and the startup and the shutdown paths could be at unsteady state, which we are not normally interested in.

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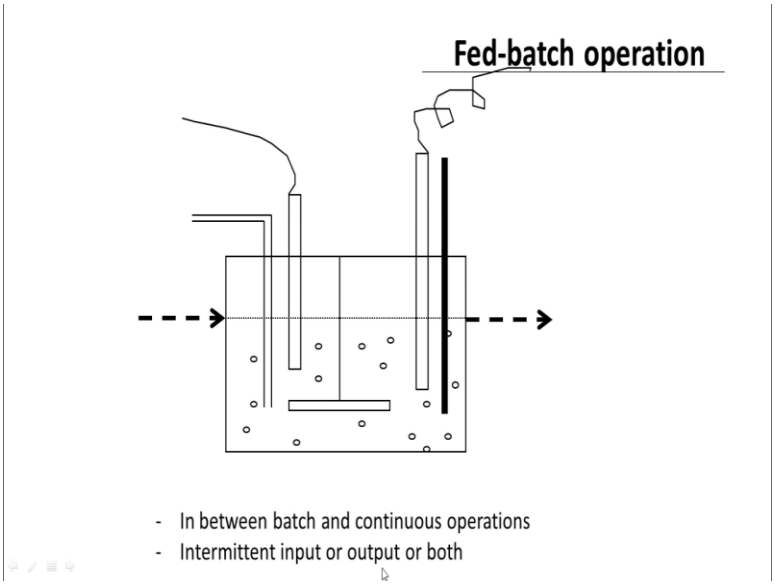

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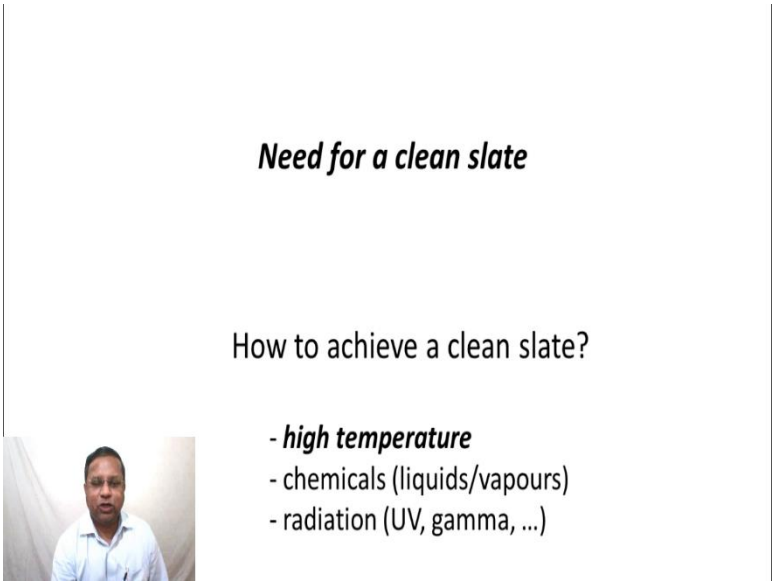

And then the fed batch operation which is somewhere in between a batch and a continuous operation may be, intermittent input or intermittent output or both. Then we looked at the need for a clean slate; first we kill all the microorganisms in the bioreactor. Then introduce a microorganisms of interest. So, that they can grow well and produce the product of interest to us. Some of the, means of achieving a clean slate is high temperature, chemicals, and radiation.

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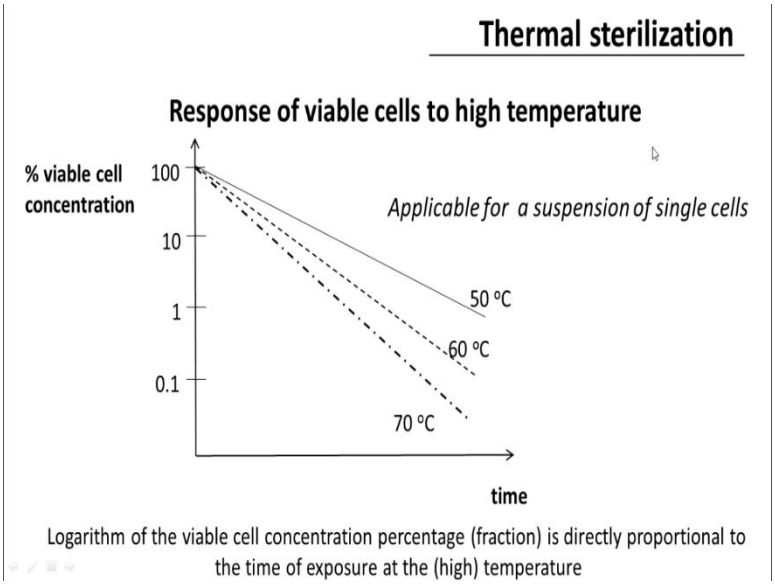

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Such a relationship results when the rate of decrease in viable cell concentration is directly proportional to the viable cell concentration present at any time. Let us see how that happens

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

$$r_d = k_d x_v \quad \text{Concentration basis}$$

Let us write a balance on cells taking the bioreactor broth as the system,

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

Let us review the basis for this equation

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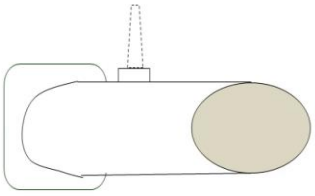
Let us say that we are filling a water tank of volume, V = 12,000 L



mass, m = ? 12,000 Kg

How long would it take, t, to fill a tank?

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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

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

We looked at some kinetics, corresponding to the high temperature sterilization or thermal sterilization. The concept of decimal reduction time the basis of that. I will not get into that details in the summery. We looked at the material balance basics and so, on. Yes, this was, this was the time that it takes for the cell concentration the time taken for the cell concentration, to go from a viable cell concentration initially x_{v0} to a final viable cell concentration x_v. It is given as

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

And then we saw the concept of a decimal reduction time, which is the time taken for a tenfold reduction in the viable cell concentration at a given temperature. These can be used for design purposes. And that is where we finished up module one, with a practice problem and a solution to that.

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The time taken for a 10-fold reduction in viable cell concentration at a given temperature is an important parameter for design of thermal sterilization. It is called the *decimal reduction time*, D

A 10-fold reduction in viable cell concentration means

$$\left(\frac{x_{v0}}{x_v}\right) = 10$$

Substituting this in the expression for time, we get

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

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Practice problem 1.1

A bioreactor needs to be sterilized before use. The solution in the bioreactor consists of single cells with similar thermal response characteristics. At 70 °C, it takes 5 min for the viable cell concentration to reduce to 20% of its original value.

a) Determine the decimal reduction time

b) How long would it take for the viable cell concentration to reduce to 0.1% of its original value under the same conditions?

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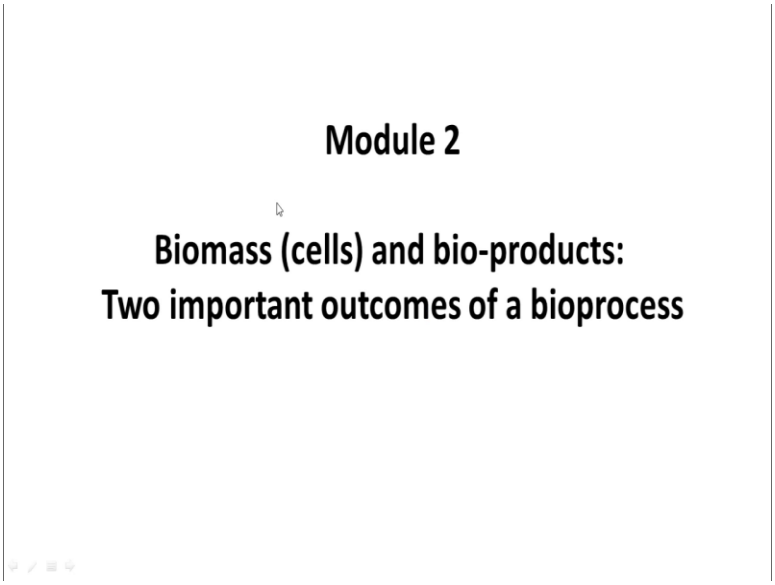

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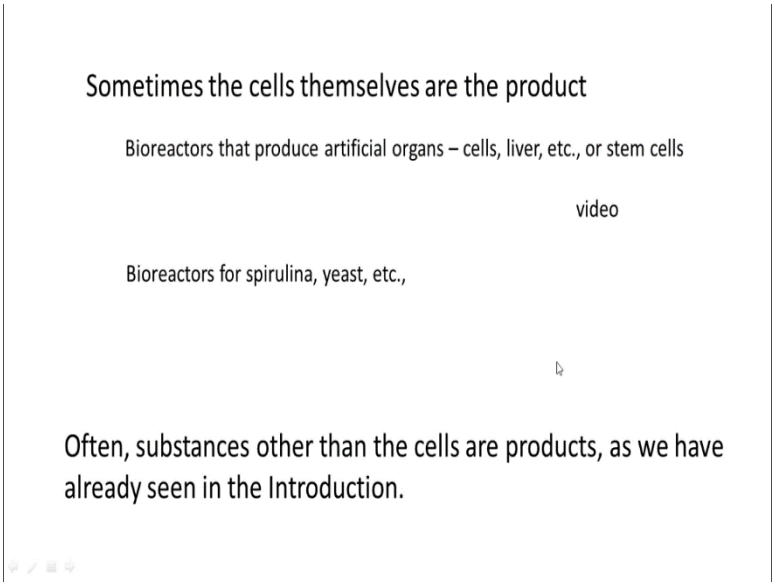

Then the module two looked at the two major outcomes of from a bioreactor or bioprocess, which are cells and the bioproducts and we saw examples where cells themselves could be important bioproducts.

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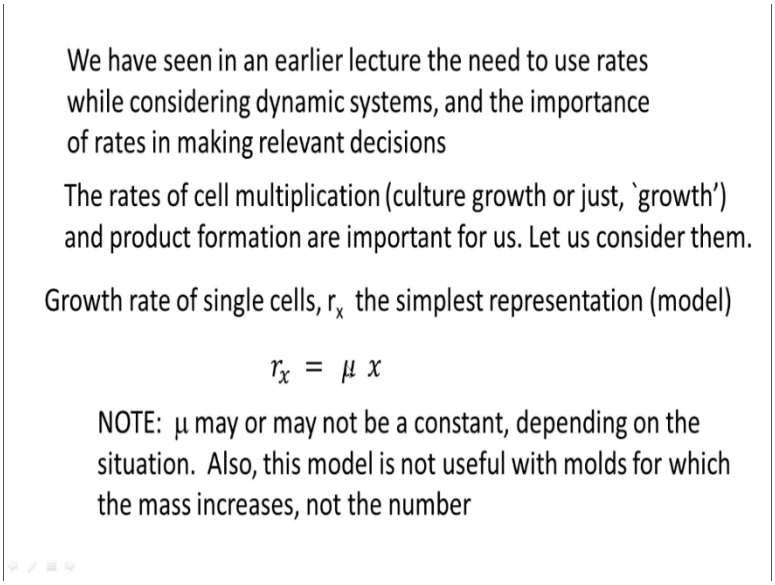

And otherwise there are many other molecules that come out of the cell. The models for growth when cells themselves are the bio-products, or you know each cell is going to produce a product. So, it makes sense to have more of cells, more concentration of cells; and therefore, in any case growth is important for us, or in either case growth is important for us. This is the simplest model

$$r_x = \mu x$$

the first order representation, μ may or may not be a constant. It is a constant in certain situations, it is not a constant in other situations.

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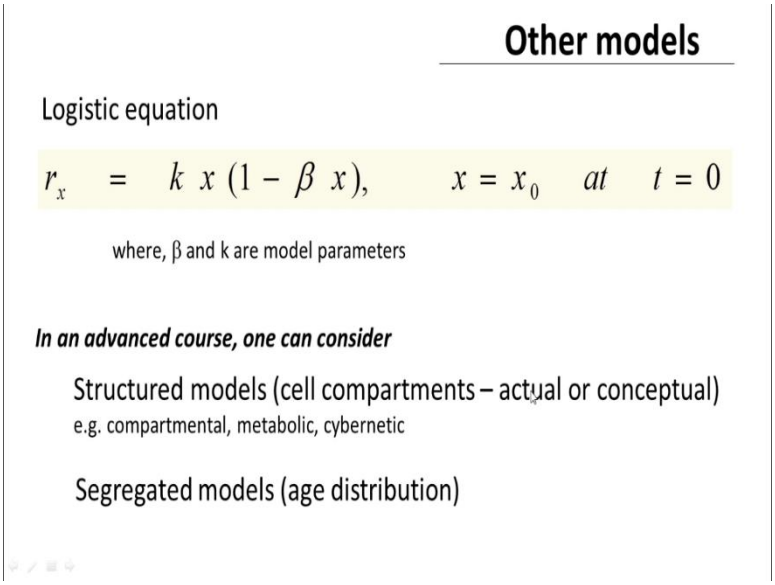

Then we looked at one other model the logistic equation, and then we said there are more complex models that are available, for people who are interested in advanced level learning of this aspect.

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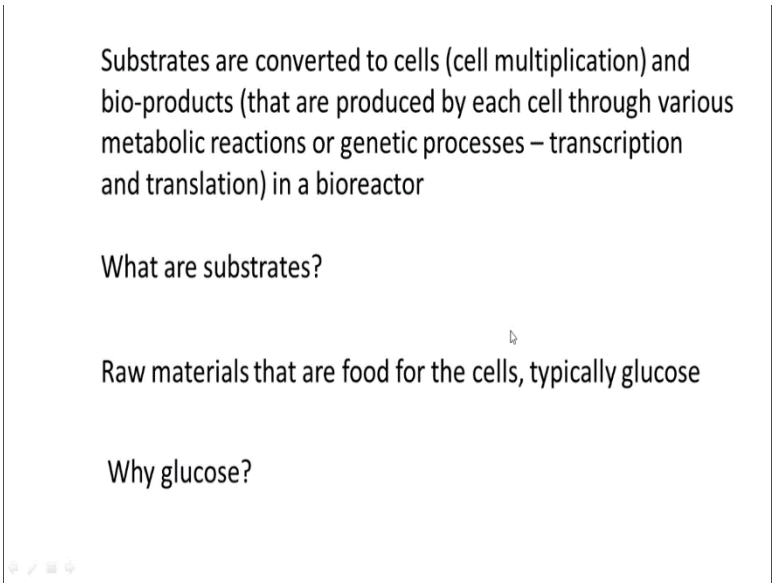

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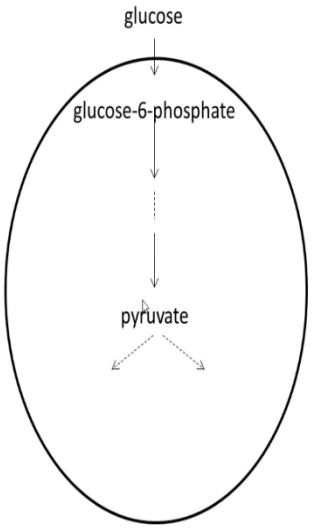
Glucose is the starting point of an important metabolic pathway, glycolysis, which is energetically important too.

Common substrate – we will look at this.

There are many other substrates that get utilized through other metabolic pathways.



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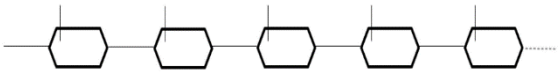
However, glucose is expensive.

A less expensive substrate would reduce the cost of a bioprocess

Starch, cellulose and ligno-cellulosic materials (from plant sources) are alternative sources of glucose

Starch and cellulose are polymers of glucose



This is starch. Cellulose has a different branching

The bonds between the glucose units are broken to get glucose for cells

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In general, a medium (complex or defined) contains

- a carbon source (e.g. glucose)
- an energy source (could be the same as C source or different)
- a nitrogen source
- salts
- trace nutrients (vitamins, minerals, etc.,)

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The limiting substrate is similar to the limiting reactant (the concentration of which limits the extent of reaction or one that gets consumed first in a batch)

Stoichiometry $2A + 3B \longrightarrow P$

Suppose the input stream into the reactor contains 10 M conc. of A and 10 M conc. of B, then the reaction will be limited by B. That is because 10 M conc. of A will need 15 M conc. of B to get completely consumed.

Here, A will be left over, and thus B is the limiting reactant.

Similarly the substrate in the medium that limits growth is the limiting substrate

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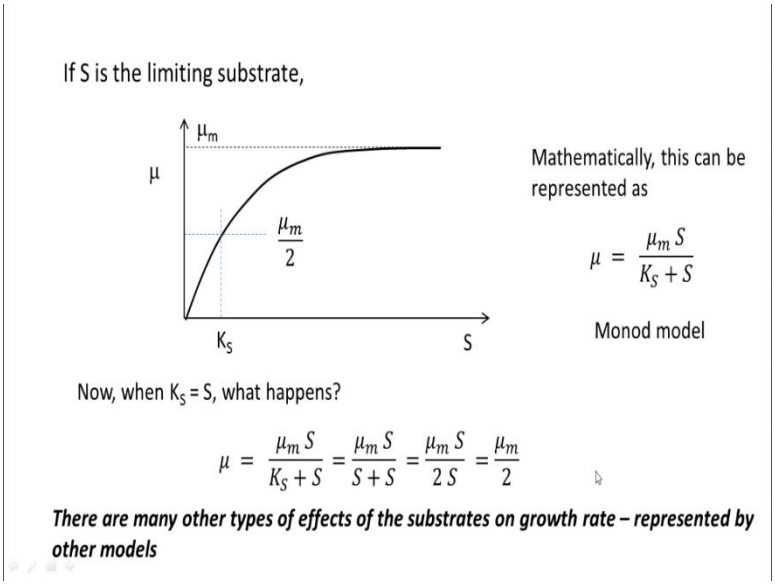

Then we said substrates are converted to products. And then we looked at some important concepts; such as a limiting substrate, a limiting and its relationship to specific growth rate, or the dependence of specific growth rate on the substrate concentration, which is given by the monod model, and then we said there are other models available also, such as the Moser model, the Andrews and Noack model.

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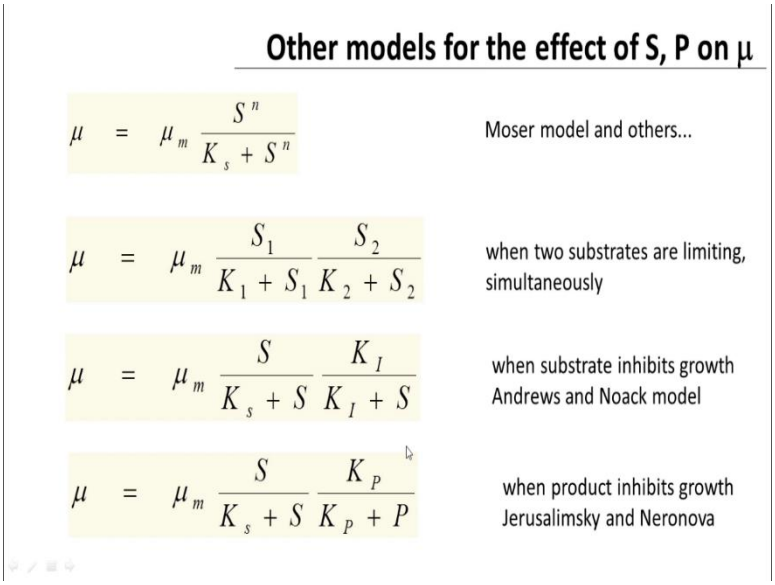

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A simple, but useful model for the product formation rate in a bioreactor is the Luedeking-Piret model

$$r_p = \alpha r_x + \beta x$$

r_p: rate of product formation
r_x: growth rate
x: cell concentration
α: growth dependent parameter
β: growth independent parameter

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Yield coefficients

Yield coefficients are useful parameters. There are many yield coefficients that can be defined as needed. For example,

$$Y_{x/S} = \frac{amount\ of\ cells\ produced}{amount\ of\ substrate\ consumed}$$

$$Y_{p/S} = \frac{amount\ of\ product\ formed}{amount\ of\ substrate\ consumed}$$

$$Y_{x/p} = \frac{amount\ of\ cells\ produced}{amount\ of\ product\ formed}$$

Assumed constant – usually a good assumption

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They can be used to estimate relevant rates from related rates. For example, the rate of substrate consumption can be found from the growth rate

$$r_S = \frac{1}{Y_{x/S}} r_x = \frac{1}{Y_{x/S}} \mu x$$

In the next module we will use the above parameters – rates of growth, product formation, and yield coefficients

Or the model that gives the limitation of two substrate simultaneously or Jerusalimsky and Neronova, which looks at the product inhibition. Then we looked at a model for product formation kinetics in the bioreactor; the luedeking piret model. Then we looked at what yield coefficients where, and how they can be used to inter convert; one rate to another rate, inter convert rates.

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Maintenance

substrate → cell multiplication (culture growth)

substrate → cell maintenance (metabolism, etc.,)

When the substrate level is low, it goes completely toward cell maintenance activities. No growth is apparent (observed).

$$r_x = \mu^A x = (\mu^T - k_e)x$$

μ^A = apparent (observed) specific growth rate

μ^T = true specific growth rate

k_e = endogenous metabolism constant

$$r_S = \frac{1}{Y_{x/S}^A} \mu^A x + m x$$

m = specific maintenance rate

Then we looked at the concept of maintenance, the substrate we said needs to go towards cell multiplication, as well as towards cell maintenance of metabolism, transport, genetic processes and so on. So, that is taken into account, especially when the substrate concentrations are low, and some ways of taking them into account have be given here.

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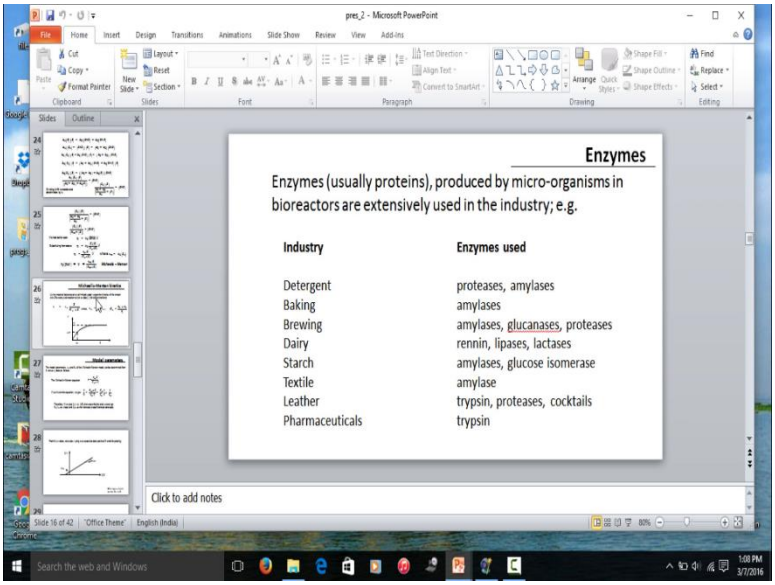

Then we looked at enzymes, we looked at enzyme kinetics, the Michaelis Menten enzyme kinetics. You can go through it in detail. The final derivation is

$$v = v_m \frac{S}{K_m + S}$$

Where

$$v_m = k_3 E_{total}$$

$$K_m = \frac{k_2 + k_3}{k_1}$$

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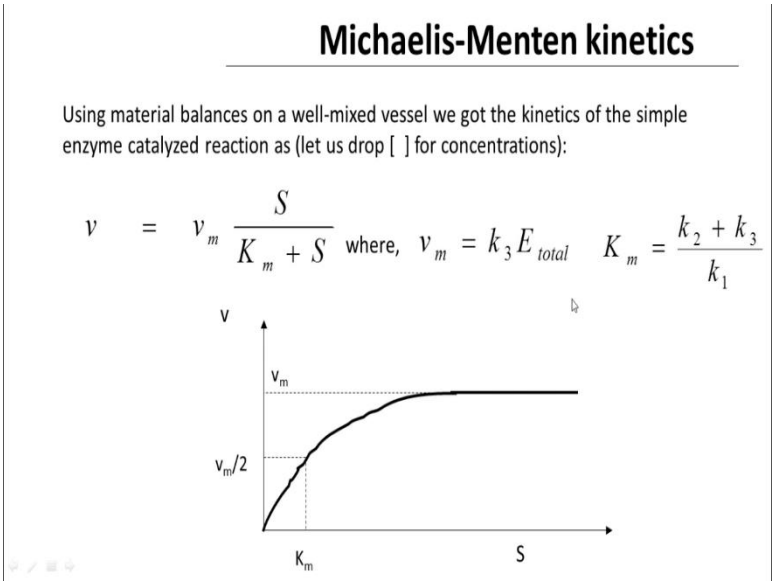

The same form as the Monod equation form, but of course this is entirely different. These correspond to the rate constants of individual steps in the mechanism, enzyme plus substrate giving you enzyme substrate complex, which further gives you enzyme and enzyme back and the product of interest. So, above is the representation in a graph.

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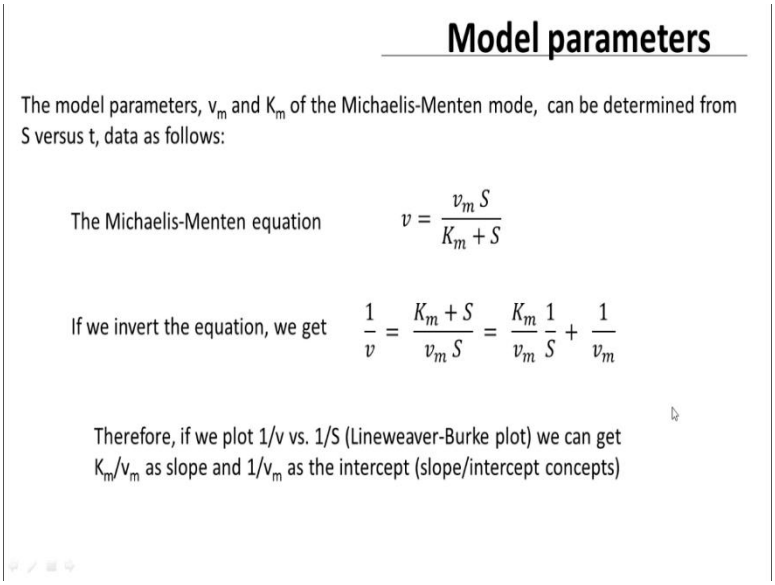

And then we found out a way to find out the model parameters v m and k m by the Lineweaver-Burk plot, and the basis for that.

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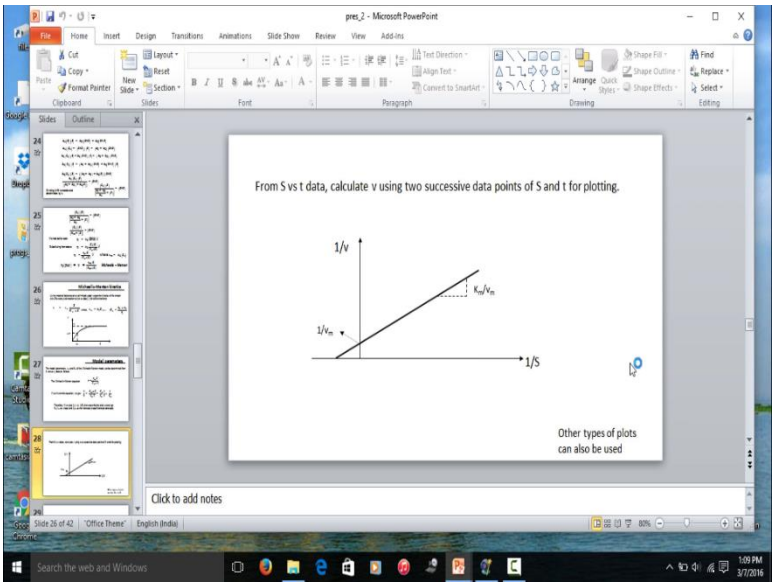

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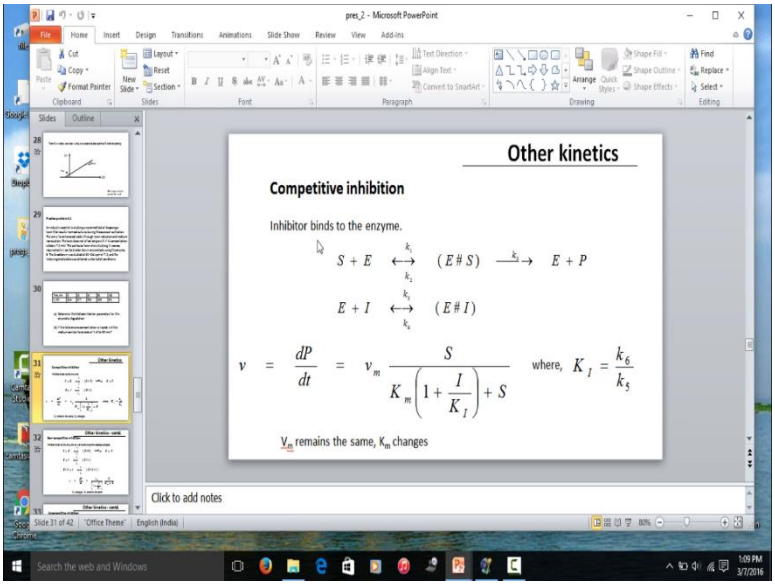

And we also did some practice problems 2.1, then we looked at different kinetics, other kinetics, especially the competition kinetics. We said that there could be three kinds of

common inhibitions; competitive inhibition, non-competitive inhibition, and un-competitive inhibition. In the competitive inhibition, the inhibitor binds the enzyme, and thereby inhibits the rate of the reaction.

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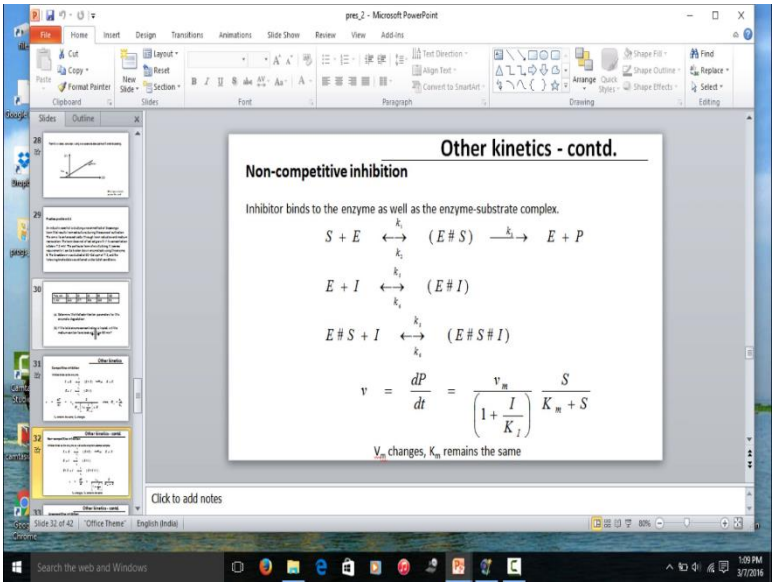

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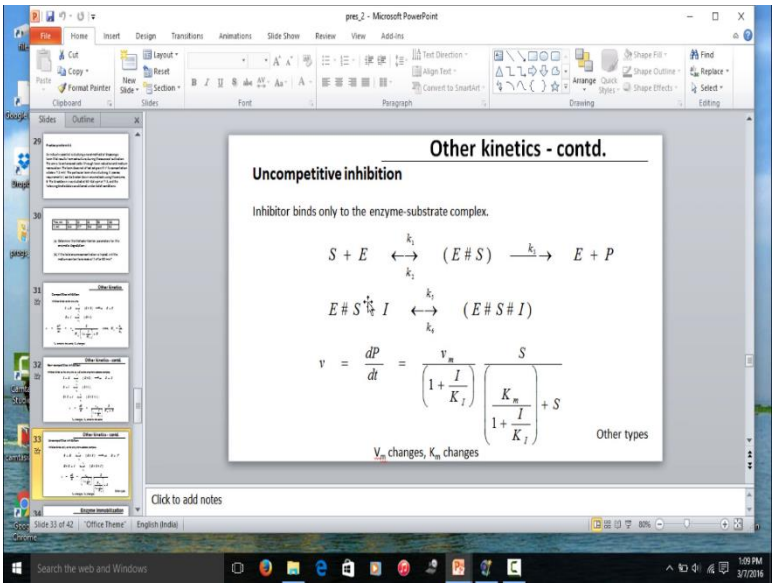

In the non-competitive inhibition, the inhibitor binds to the enzyme, as well as the enzyme substrate complex, and inhibits the reaction. In the uncompetitive inhibition it binds only to the enzyme substrate complex and inhibits. And in these three cases the v ms and k ms change. Only v m changes for competitive, only k m changes for non-competitive, and v m and k m change for uncompetitive.

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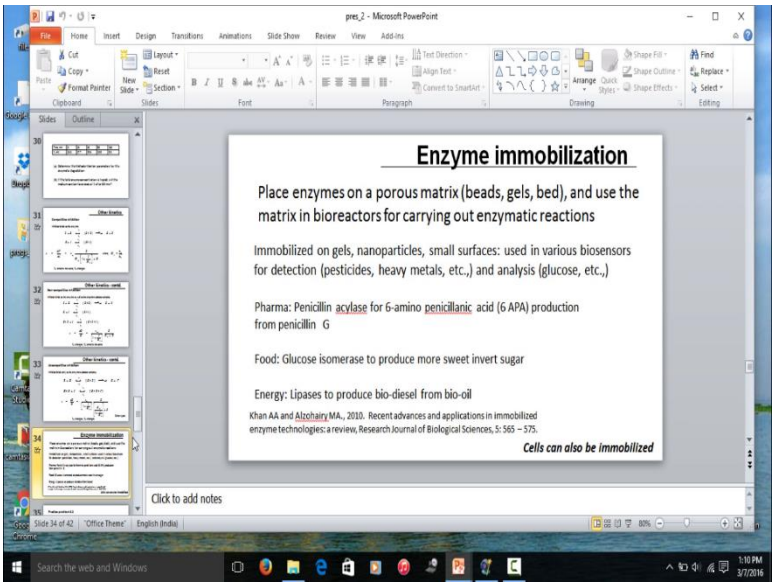

Then we looked at enzyme immobilization, whereby we can minimize the damage to the enzyme, but it comes at a cost. We also said that cells could be immobilized. These have been used in industrially in an immobilization mode. That is what we essentially saw in module two, and we also worked out a couple of problems. Hopefully the problem solutions would have been useful. The methodologies would have been useful. And then in module three.

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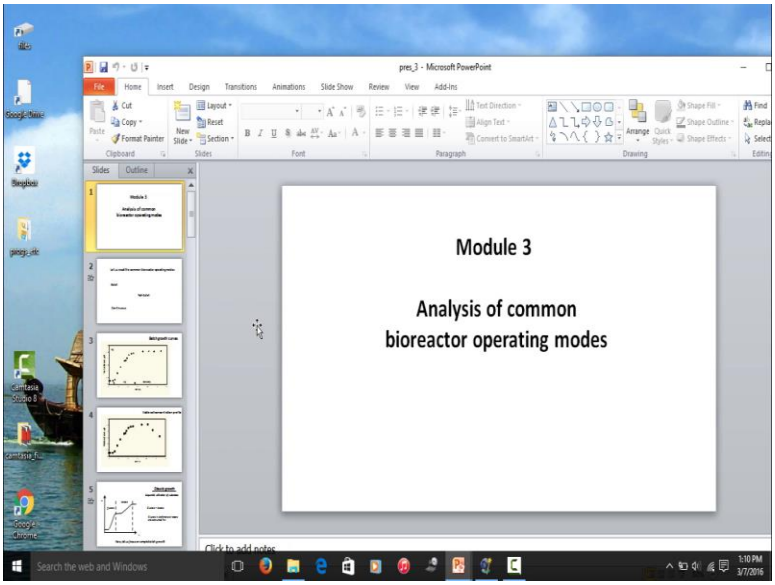

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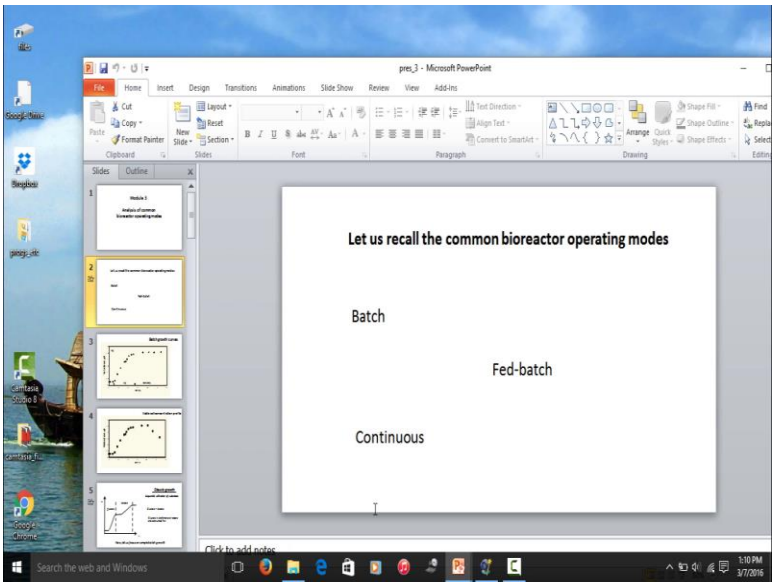

We looked at the analysis of common bioreactor operating modes; the batch mode, the continuous mode, and the fed batch mode. These two we looked at in great detail. Fed batch I just introduced you, because it is complex. I just introduced you a way of approaching that. So, in the batch mode, we did balances, and we got expressions, for the time of a batch, for the time to reach a certain desired cell concentration.

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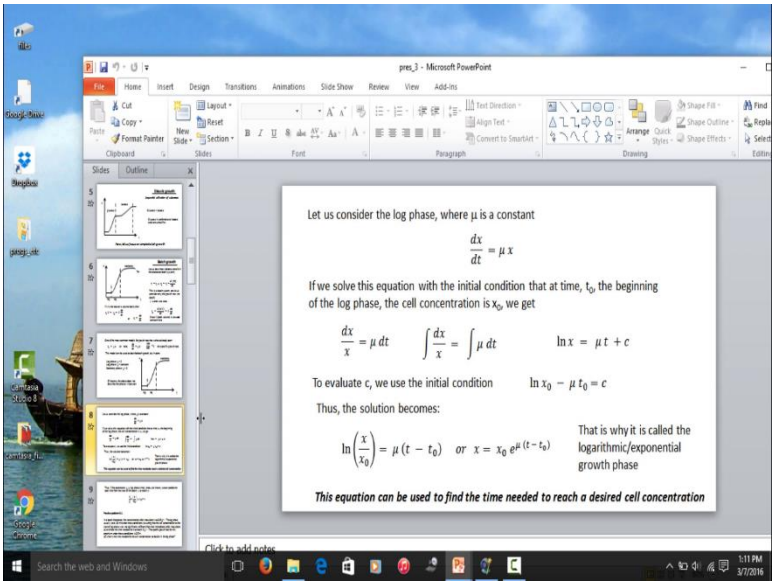

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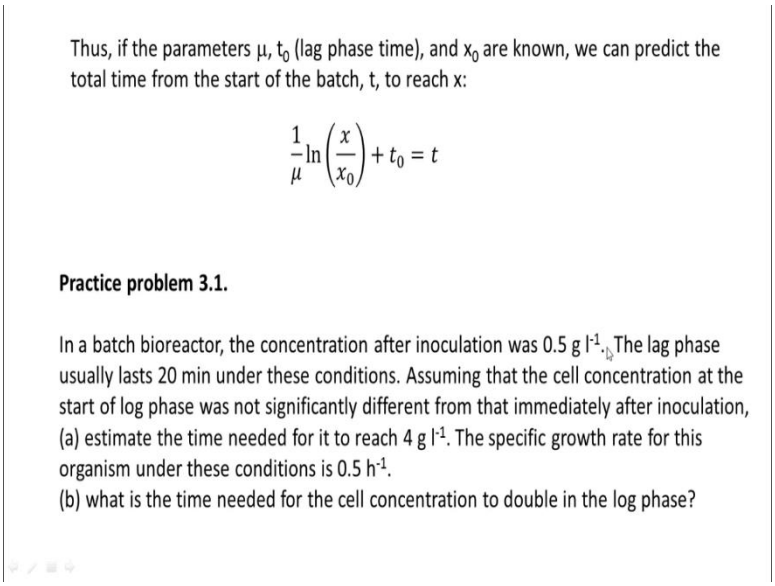

Starting from a certain initial concentration, and that is

$$\frac{1}{\mu}\ln\left(\frac{x}{x_0}\right) + t_0 = t$$

x_0 is the initial cell concentration, t_0 is a lag time, the sum of these is the a batch time. We worked on a problem to illustrate that.

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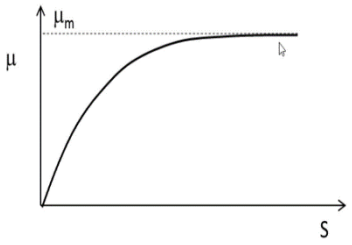
The above did not consider the effect of substrate on the specific growth rate, μ. We tacitly assumed that enough substrate was present, so that $\mu = \mu_m$. This need not always be the case. We have seen that



$$\mu = \frac{\mu_m S}{K_S + S}$$

Monod model

And then we brought in the effect of substrate concentration on the specific growth rate, and saw how things could change.

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If we incorporate the effect of the substrate, for the log phase,

$$\frac{dx}{dt} = \left(\frac{\mu_m S}{K_S + S}\right) x$$

Let us consider two cases: $S \gg K_S$ $S \approx K_S$

If $S \gg K_S$ $\frac{\mu_m S}{K_S + S} = \frac{\mu_m S}{S} = \mu_m$ which is the same case as earlier

If $S \approx K_S$ We cannot use the above approximation. This case is uncommon, and can happen when some crucial but unusual substrate becomes limiting. Otherwise, the culture would have reached stationary phase when this happens.

We have two quantities, x and S that vary with time in the same differential equation. It would be preferable to have only one dependent variable. We need to express S in terms of x. How do we do that?

When that has been brought in, it could be relevant in some situations, but it is more important for the continuous bioreactor situation. So, when we went and analyzed the continuous bioreactors, we got some very insightful, good insights into the operation, and we said at steady state.

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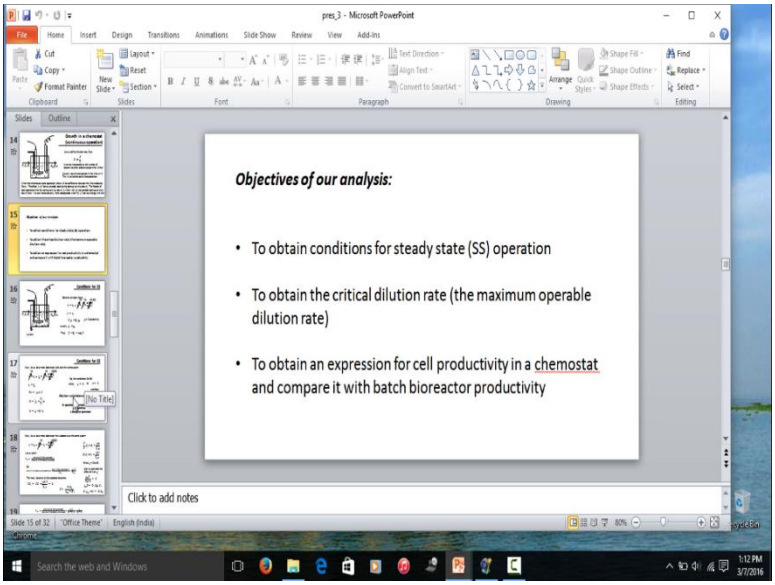

We obtain conditions for steady state operation. We obtained the critical dilution rate, which is the maximum operable dilution rate, before washout occurs, and we also obtained an expression for cell productivity in a chemostat and compared it with the batch bio reactor productivity. We said that this specific growth rate equals the dilution rate at steady state operation. Therefore, an operational parameter such a flow rate, and dilution rate is flow rate by the volume. So, the flow rate can be used to control a biological parameter which is the growth rate; that is the significance here. And then we obtained a critical dilution rate, beyond which there is actually no cell production by the cell. And then we said that a chemostat is inherently three to four times more productive, then a batch bioreactor. Although it is a lot more difficult to operate a chemostat. It is a lot more intensive to operative a chemostat. Then we looked at means of analysis. We have of course, worked out some problems.

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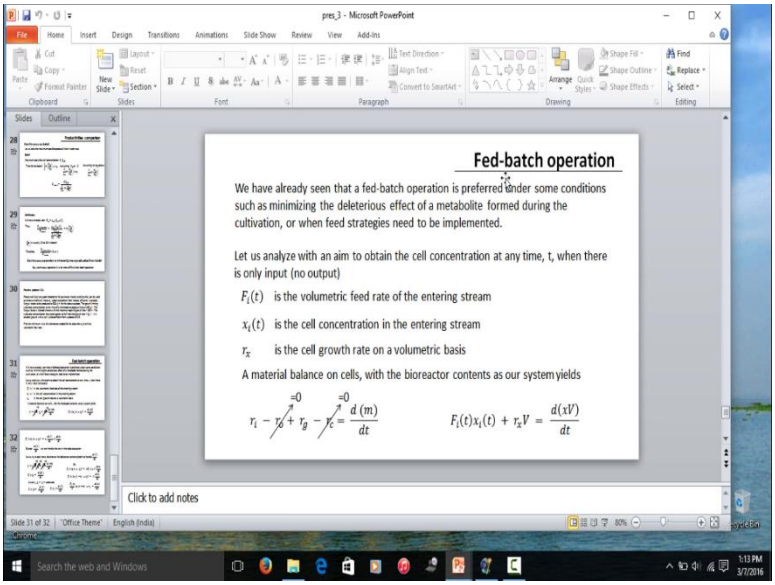

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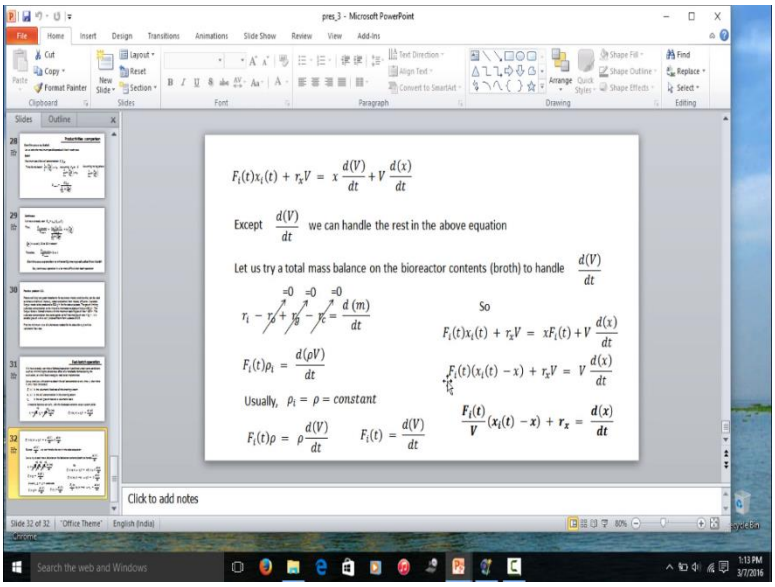

Then we looked at a brief method of analysis, or analysis in brief of a fed batch operation, where there is only input. Very briefly if we know the functions of the variation of the flow rate, input flow rate with time, and the variation of the input cell concentration with time, then we can use this equation to design appropriate aspects, is what we said. So, that was module three.

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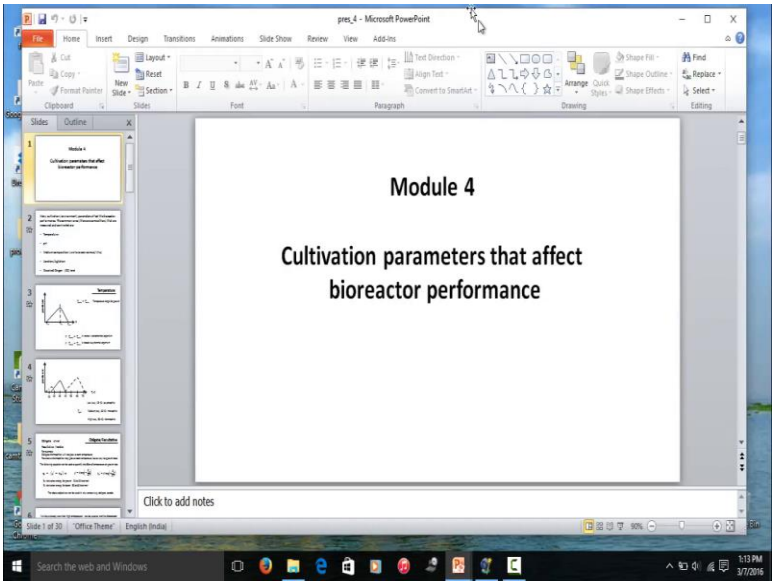

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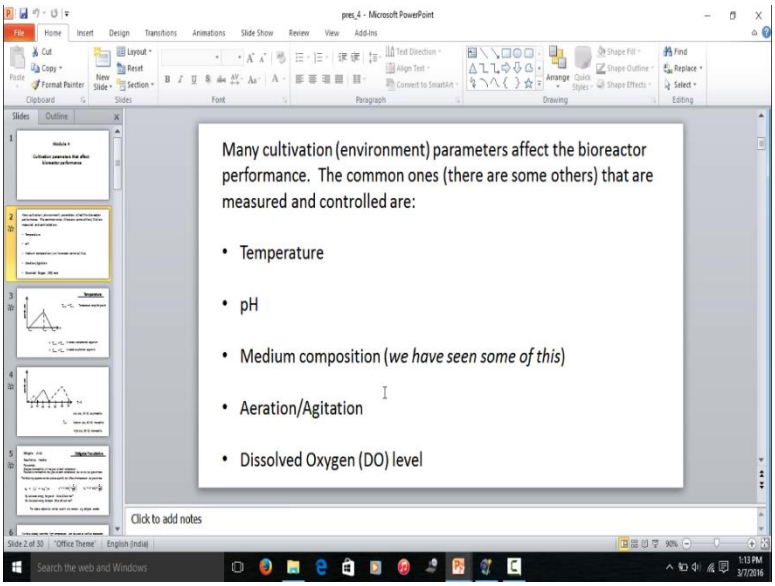

In module four we looked at cultivation parameters that affect bioreactor performance. The cultivation parameters, were temperature pH medium composition aeration and agitation, and dissolved oxygen level. Dissolved oxygen level we looked at in great detail, including k l a measurement methodology, the dynamic response method. We also

worked out a problem by which we got a better appreciation of the k l a determination. Then we looked at some aspects of scale up and scale down.

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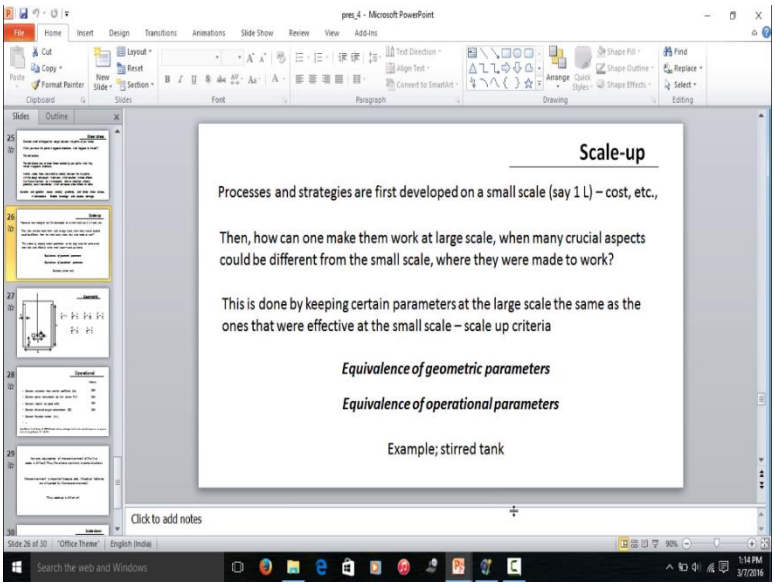

We said that we need to maintain the equivalence of geometric parameters, and the equivalence of operational parameters during scale up, only then can we expect, at least that whatever result we got at a lower scale, would be applicable at a higher scale.

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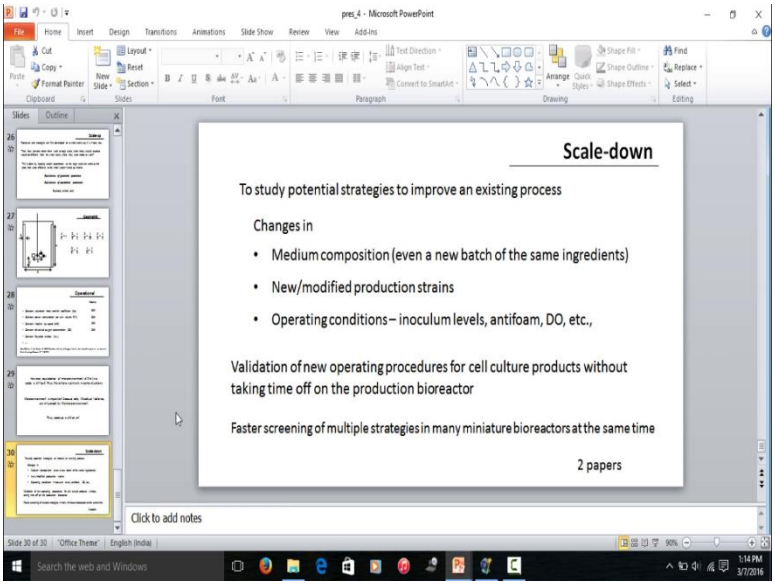

The scale down is important, because it gives us a means by which potential strategies to improve the existing process, such as changes in medium composition, new or modified production strains used, or operating conditions, different operating conditions can be tested and the validation of new operation operating procedures for cell culture products, can be tested without taking time off the production bioreactor, and without too much cost. So, that is the advantage of scale up scale down. I have given you a lot of references which include videos, websites, and papers, to provide a lot of background which would be interesting to people who want to know more about those aspects.

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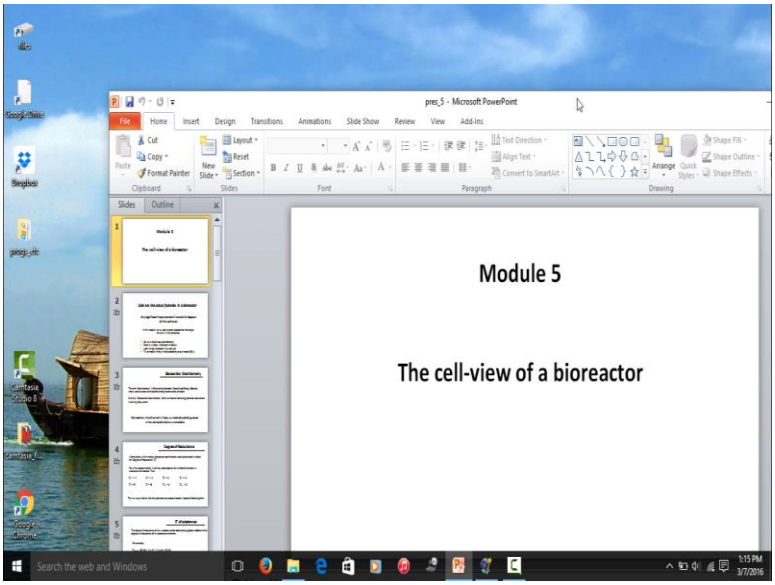

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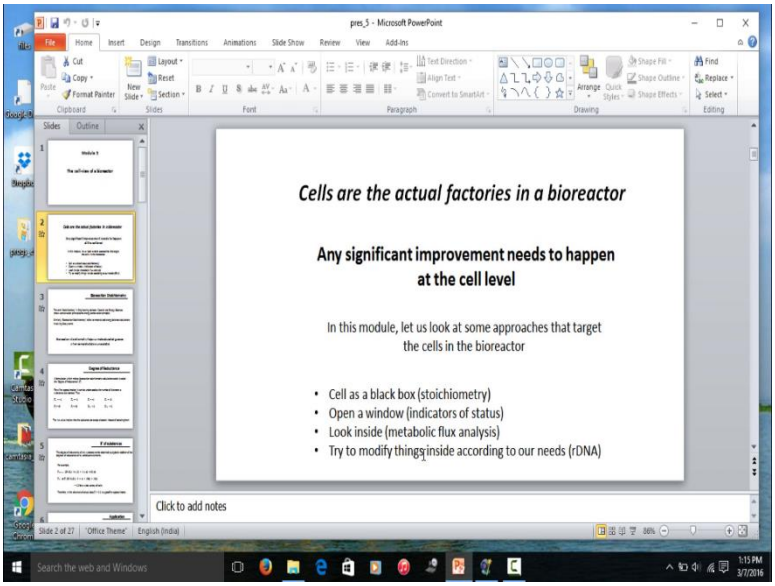

Then finally, we looked at the cell view of the bioreactor, because cells are the actual factories that are producing the product. We said that we would look at the cell as a black box initially, get some insights using bioreaction stoichiometry. Then open a window through cellular indicators; such as redox state energy state, and probably intracellular pH. and we can use those as control variable also; that is what we saw. And then we looked inside, we spent a lot of time looking inside through one technique called metabolic flex analysis. And we saw how it provided us directions to appropriately genetically modify the organism, and actually achieve threefold increase in yields; this

was the paper by Vallino and Stephanopoulos. And then I very briefly mentioned how the r DNA technology can be used to change what is inside itself, or probably we can look at techniques by which the entire cell is changed such as the hybridoma technology right. So, hopefully you had the course that would be helpful to you. Hopefully you had fun during the course and you enjoyed the course.

Wish you the very best and all success.