Bioreactors
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Lecture - 04
Some important concepts

Welcome to lecture 4 in this NPTEL online certification course on Bioreactors, the 10-hour course. In the last lecture, lecture number 3, we had looked at how to solve problems in general and we solved a problem related to the sterilization kinetics. One of the things I would like you to note, keep in mind and check always, is after you arrive at the final answer, please check whether it makes some sense. For example, if you had seen or gotten an answer of about of 7,500 hours or something like that, then you need to go back and check your calculations. It is always good to check your calculations after you finish, but always keep the reality of the number that you get in mind. 7500 hours seems a little odd for any situation. Experience always helps you make better, to have a better idea of expected numbers, but even otherwise 7500 hours when you are looking at a bioreactor seems a little odd. At the same time if you had gotten an answer of about 10 power minus 3 seconds, it seems ridiculously short. So, under both these situations, I think it is good to check the approach, the math and the overall calculations.

This is something similar. Suppose you are working on, let us say pipe sizes, and you get an answer of about 50 meters, you know the pipe diameter is 50 meters. Just taking an odd example, 50-meter diameter is you know something unimaginable. The pipe size having a 50-meter diameter is unimaginable. So, you should always have this picture of your answer in mind and check it, please do it every time, I will again emphasise this when we do the next practice problem.

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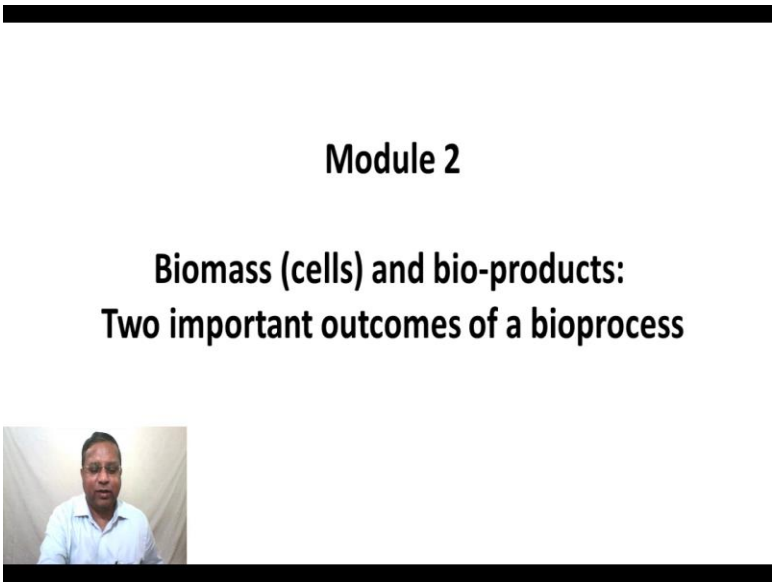

Now let us continue with module 2 is on Biomass (cells) and bio-products. Biomass or cells and bio-products, and these are the two important outcomes of any bioprocess. They could be cells themselves or they could be molecules made by these cells. Let us look at these one by one.

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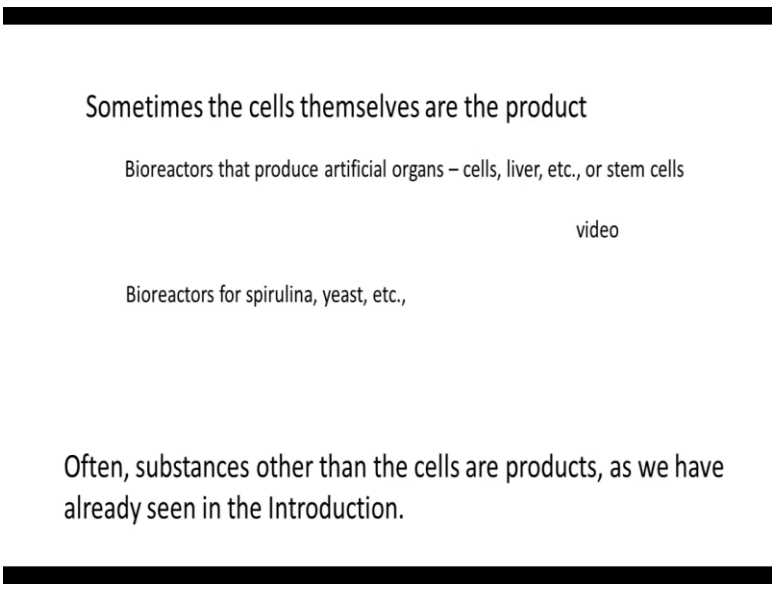

Sometimes the cells themselves are the product, might seem a little odd in the beginning but let us consider this. I am sure you would have heard of artificial organs. Artificial skin was, I think, the first popular organ that was made outside and then put on to burn

victims so that they could heal faster and so on.

Nowadays we have many different organs produced artificially in appropriate bio reactors. They might, the bioreactors themselves might look a little different. The cells grow on a scaffold that resemble the organ and therefore you get the shape and then the functionality is brought in and so on and so forth. So, that you have a functional organ which can be transplanted into, probably in the place of an organ that has gone bad. Definitely have liver, kidney and so on so forth that are made that way. We did talk of skin which is popular. You could also grow stem cells which are used for a variety of purposes using bioreactors. In all these situations, the cells themselves are the main product from the bioreactor and all effort goes to making sure that you have the required cell concentration, the required, they should grow in the required way and so on and so forth, and they should also proper functionally when grown in bioreactors.

I think I have a video here as a suggested video.

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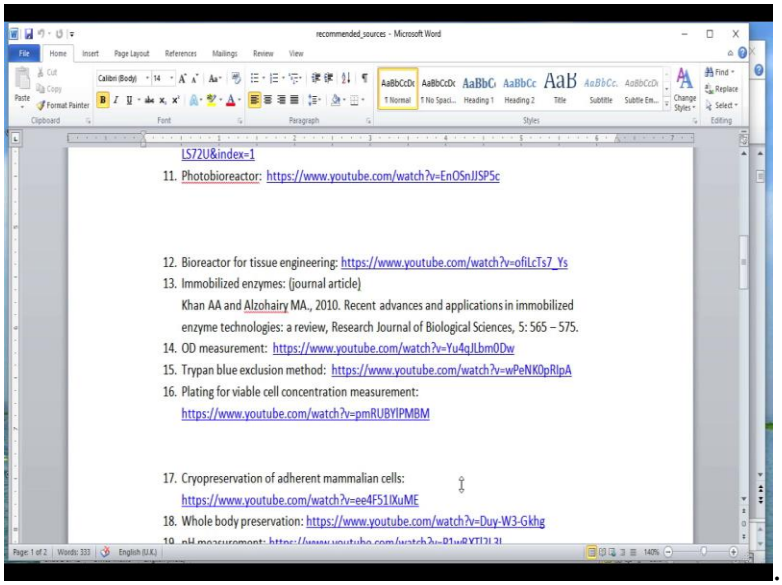

This is from the file that is available to you – websites, videos and papers recommended. I should also add this disclaimer that IIT Madras and I do not recommend or endorse any of the commercial brands that may be depicted in some of the sources below. The sources were chosen for their pedagogic value. This is number 12 that I just mentioned. Bioreactor for tissue engineering. This is the link, you can click on the link and go and see the video. It gives you some idea has to how bioreactors are used for making organs.

You may have heard or something called spirulina. Spirulina is an algae. It is grown and then processed and packaged and that is sold as nutraceuticals. Many people consume that. These are grown in large bioreactors. In fact they are grown in ponds which act as bioreactors for the appropriate production of spirulina and there the spirulina cells themselves become the product of interest.

Similar is the case with something called single cell protein. Single cell protein was very popular in the 1970's and 80's and so on, as fodder for cattle. They are nothing but yeast cells and they are also grown in bioreactors, processed and then provided as fodder for the cattle. In these 2 cases, spirulina and single cell protein, the cells themselves are the products. However, as we have already seen in the introduction, there are very many other products. I think we saw insulin, monoclonal antibodies, ethanol, bio-oil and so on and so forth, that the cells produce. These are the molecules that are produced by the cells and they are the products.

There are 2 major types of products, the cells themselves or the molecules that are made by the cells which are the main products from bioreactors.

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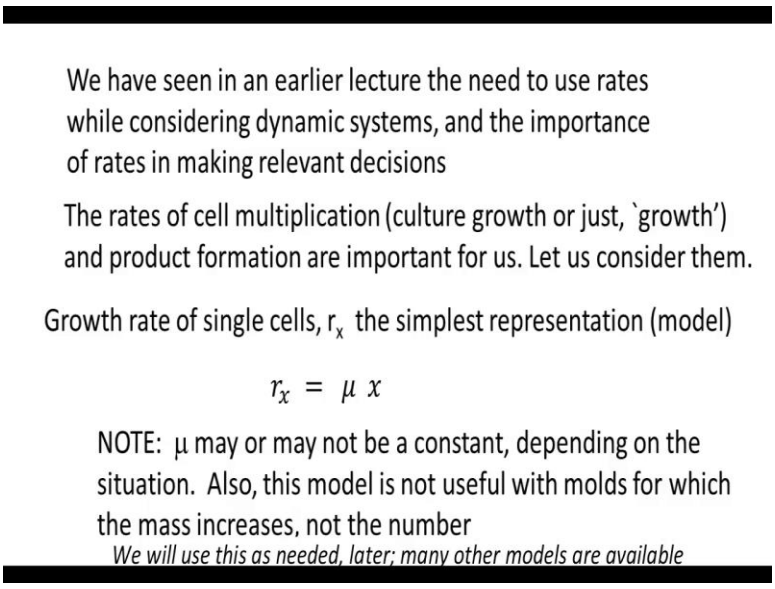

In the initial lectures, we saw the need to use rates while considering dynamic systems. We said rate is a central parameter on which we base our decisions, and so on. Therefore that is considered as the main parameter. We do not consider amounts and so on and so forth, which can get a little confusing for the kind of decisions that we need to make

while dealing with dynamic systems, engineering systems and so on. So, we will also consider rates, everything will be geared toward rates, the model will, models will be geared towards rates, mathematical models will be geared towards the rates, all the discussion would be geared towards rates and so on and so forth.

You need to internalize this. This takes a while for internalization. Especially, since some of these concepts were introduced to you at the school level from the point of view of a batch system, where some approximations work, but not in all cases and especially not in a continuous case. You need to internalize that, I will keep repeating this till it gets across. The rates of cell multiplication on one hand and the rate of product formation are the important rates for us, right, and what is cell multiplication? One cell becoming 2 cells in the bioreactor, becoming 4 cells and so on and so forth, ok.

In other words, the culture in the bioreactor is growing, right? Therefore, cell multiplication, culture growth, or just growth, all mean the same thing. The cells, you would have probably learnt in a basic biology course, they undergo something called a cell cycle and that is also sometimes seen as some sort of a growth, not always. But we are not talking about that, we are talking of the multiplication of cells. You need to keep this in mind. It is not the cell volume changing during the cell cycle and so on so forth, each cell's volume is changing during the cell cycle that is not what we consider growth here. This is culture growth or the cell multiplication.

If we consider single cell, the simplest mathematical representation or a mathematical model is as follows: the rate of cell growth as given by r_x is directly proportional to the cell concentration x, a first order model, or equals a certain mu into x.

$$r_x = \mu\, x$$

Note that mu may or may not be a constant, depending on the situation. You need to again internalize this. mu may or may not be a and also there are organisms called molds. For example, *Aspergillus niger*, is a common mold that grows on bread. They are these stringy organisms that grow by extension of their strands or the hyphae as they are called, you know they extend, their mass increases. But the number is still or limited to just that one - with the mass increasing. In such a case, this may not be valid if x is the number concentration of the cells. You need to keep this in mind.

We will use this model as needed. In this lecture I am going to present some concepts and we will use the concepts a little later. So let us just focus on the concepts in this particular lecture. This is not the only model that is available for cell growth. There are many such models and we will see some of them. This is an introductory course so will see only some of them.

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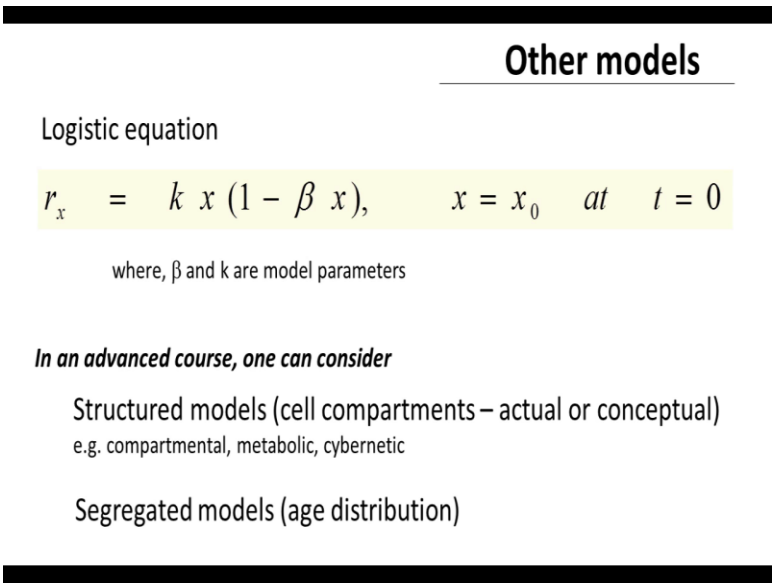

One other model, just I think I will just show you just one other model. It is called the logistic equation which is helpful in certain situations, where r_x is given as

$$r_x = kx(1 - \beta x)$$

where k and beta are the model parameters which change according to the system and this goes with the condition that x equals the initial cell concentration.

$$x = x_0\ at\ t = 0$$

This is called the Logistic equation which is valid under certain circumstances. And in an advanced course one can consider structured models, which essentially consist of or take into account compartmentalization in the cell. You know they could be actual compartments such as the nucleus, mitochondria, so on and so forth or they could be even conceptual.

Some examples of the compartmental or the structured models are compartmental

models, metabolic models, cybernetic models and so on so forth. This being an introductory course let us not get into the details, but you would need to probably be exposed to them so that you will able to recognize them when you see them in the future. Then there could be some models which take into account the distribution in ages of cells in the culture, the above models the r x equals mu x or r x equals k x into 1 minus beta x the logistic equation.

$r_x = kx(1 - \beta x)$

They considered all the cells to be at the same age in the bioreactor that need not be true, they could be up variation in the ages of cells and that distribution the variation is taken into account in the segregated models.

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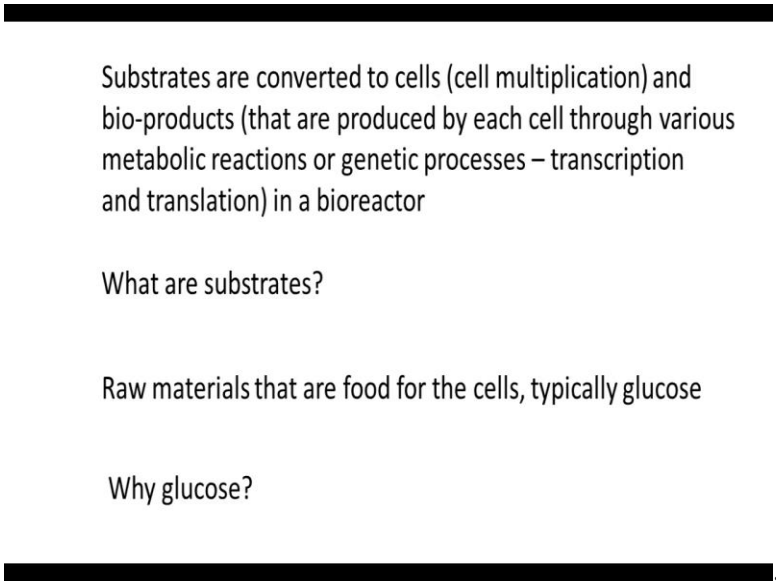

The substrates, you know something called substrates, we have already seen what a substrate is, we will see that in detail now, are converted to cells through cell multiplication and bio-products. The bio-products could be made by the metabolic reactions inside the cells or the genetic processes inside the cells. The genetic processes are transcription, translation, you know the gene which is a part of the DNA that gets transcribed, translated to probably a protein, the protein could be a product that is what we mean by the product resulting from a genetic process or it could be a metabolite that is made by the metabolic reactions in the bioreactor. So let us look closer at the substrates, what are substrates? In layman's terms, substrates are raw materials that are

food for the cells and a typical example is glucose C6H12O6. The carbohydrate glucose is a nice example of the substrate. Why are we looking at Glucose?

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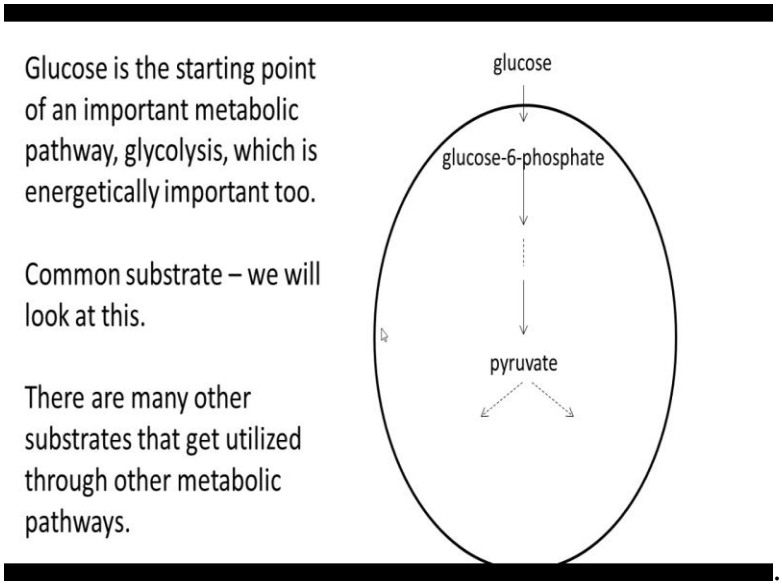

Because glucose is a starting point of an important metabolic pathway in the cell called glycolysis, and glycolysis has importance from the energy aspects to, they provide energy to the cell, it is necessary to provide energy to the cell and so on. Usually glucose which is provided in the extracellular space gets transported into the cell through glucose transporters in the membrane, then it gets converted to glucose 6 phosphate through an enzymatic reaction and to fructose 6 phosphate through another enzymatic reaction and so on so forth, until it reaches what is called a pyruvate which is an important branch point in the metabolic pathway. The glycolysis itself is supposed to be from glucose to pyruvate. Since this is an important set of reactions, important metabolic pathway that takes place in the cell, glucose is a typical substrate that is considered.

There are many other substrates that can get utilized through other such metabolic pathways in the cell. Let us not get into the details. Glucose is not the only substrate but it is a very common substrate. In fact, many times if there is a mixture of substrates, cells tend to prefer glucose sometimes.

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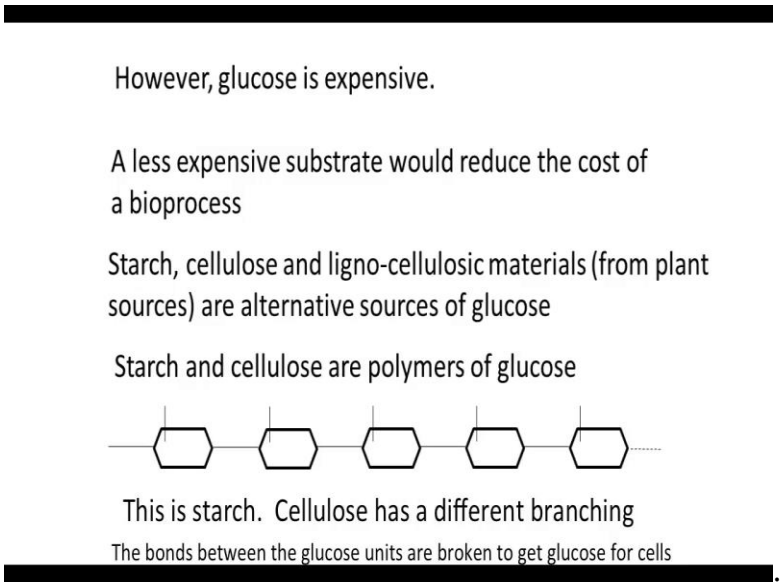

However, from an industry prospective, glucose is expensive. So, if you start with an expensive raw material, expensive substrate, the product is going to turn out to be more expensive. If you have an alternate of glucose, that is not as expensive, then that is much better for the industry, because it reduces the cost of the bioprocess. Very common alternatives for glucose are starch you know, the same starch that you know of. Cellulose, all the wood around you contains cellulose and ligno cellulosic materials. Ligno cellulosic materials are currently being researched heavily for, as a glucose source. Cellulose and ligno cellulosic materials are found abundantly in all the plants, wood, wood is all that and they are alternative sources of glucose. You know you may have, may or may not have considered this, the source for all the photosynthetic, the carbon source for all the photosynthetic organisms happens to be the CO2 in the air. So all the wood, the carbon in the wood that you find came from CO2 in the air. Although it is point naught 3 naught 4 percent now and is implicated in global warming and so on, that is the source of carbon for photosynthetic organisms.

Starch and cellulose happen to be polymers of glucose. For example, this is 1 glucose molecule here that you see, this is oxygen here, I have not indicated but this is oxygen, this is carbon 1, carbon 2, carbon 3, carbon 4, carbon 5, carbon 6, C6H12O6. So, this is the pyranose form as written here and then connected to other glucose molecules this way, this is one form of starch called amylose. So, if you break down these bonds from starch, then you can get glucose for the cells to consume, starch is found in abundance

and therefore it is less expensive then glucose and therefore, starch can be a source of glucose for the cells. The cellulose happens to have a slightly different branching you know amylose and amylopectin starch and then cellulose is slightly different bonding and branching and so on so forth. They are all polymers of starch, if you find a way by which you can break down the bonds in between them and release the monomers then they become available for the cells to consume and produce the product of interest.

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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.,)

In general, a medium, we had talked of media earlier, that is the complex substance, the substance in which cells grow. It provides all the nutrients for the cells to grow, that is for the culture to, cells to multiply, the culture to grow and so on.

The medium could either be complex or be defined, defined just means that you know everything that is there, complex means you may not know all the details of the medium. In general, a medium contains a carbon source, glucose is a very common carbon source, we did talk of carbon dioxide being the carbon source in the case of plants. And energy sources which leads to the formation of ATP and so on so forth, it could be the same as carbon source or it could be different. You know the glucose gets into this pathway glycolysis and then another pathway called the TCA cycle and then oxidative phosphorylation and thereby produces ATP, in this case the glucose itself is the energy source. Many times you could have other pathways that produce energy and the energy source could be different from the carbon source,

.

Glutamine is sometimes the energy source for mammalian cells and so on. And nitrogen source, because cells require nitrogen, carbon, nitrogen, oxygen, hydrogen, phosphorous and so on are the essential elements that are present in living systems in cells. The medium contains salts, the medium could contain trace nutrients such as vitamins, minerals and so on. So, this is what a medium in general contains.

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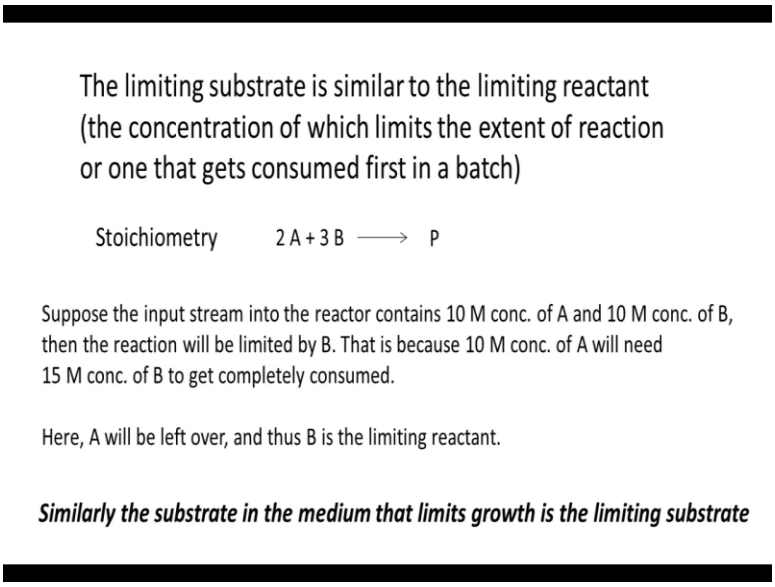

We looked at a substrate glucose, way to get glucose from other things and so on. Now we need to look at something called a limiting substrate, now we look at some 2 or 3 concepts or 3 or 4 concepts. We will not use them now, but we will try to understand what they are, use them later. The limiting substrate, is one such concept. The limiting substrate is similar to the concept of limiting reactant, you all know what a limiting reactant is in the case of chemical reactions. The limiting reactant is the one, the concentration of which limits the extent of reaction or the one that gets consumed first in a batch, in a batch is important there. We will say that the concentration of which limits the extent of the reaction, that is called a limiting reactant. Let us see an example to understand this a little better.

Let us consider the following stoichiometry,

$2A + 3B \rightarrow P$

In other words, 2 moles of A needs 3 moles of B to react completely to give you 1 mole of P. If it happens that the input stream to the reactor, let us say it is a continuous process, there is an input stream to the reactor, this contains 10 molar concentration of A and 10 molar concentration of B, this molar is nothing but moles per liter, this is concentration of A, concentration of B. Then the reaction is going to be limited by B, this is because the 10-molar concentration of A would require 3 by 2 into 10 moles molar concentration of B to react completely, there is 15 molar concentration of B to get completely consumed and since only 10 molar concentration of B is present, B will limit the reaction and A will be left over, and therefore B is the limiting reactant.

Similarly, the substrate in the medium that limits growth is called the limiting substrate. You could have many different substrates, we talked of carbon source, nitrogen source, energy source and all that. The one that is going to limit growth is going to be your limiting substrate.

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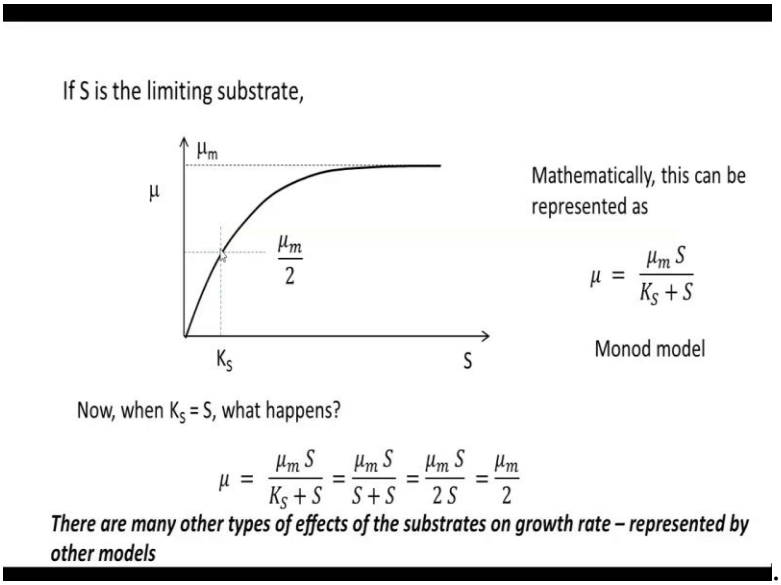

If we consider the limiting substrate and let us say if S is the limiting substrate, then the variation of the specific growth rate mu, remember that r x equals mu x we said,

$$r_x = \mu x$$

So let us say this is the same mu here, the variation of specific growth rate with substrate concentration is given in this graph. This is a rectangular hyperbola, it increases and then

it saturates somewhere here to a value of mu m, maximum specific growth rate. There is a way of mathematically representing this, this is a rectangular hyperbola, so rectangular hyperbola equation would describe this appropriately and this is the equation that we are taking about.

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

This is the well-known Monod model which gives the effect of substrate concentration on the growth rate of cells.

If it happens to be here, if you have enough substrate and you happen to be in this regime then mu becomes a constant equals mu m, that is a situation when you have mu as a constant. In general r x equals mu x need not be a constant, if you have enough substrate then mu equals mu m which happens to be a constant.

Let us look at what happens when K s equals S, if Ks equals S in the monod model we substitute S for Ks let us see what happens then. So, When $K_S = S$,

$$\mu = \frac{\mu_m S}{K_S + S} = \frac{\mu_m S}{S + S} = \frac{\mu_m S}{2 S} = \frac{\mu_m}{2}$$

Therefore, when S equals K s, you know when you have the substrate concentration equal to K s or when K s happens to be the substrate concentration, then your mu happens to be mu m by 2 or half maximal specific growth rate. So, K s is the substrate concentration at which you get half maximal growth rate, ok.

There are many other types of effects of substrate on growth rate many other ways of representing them, let us look at a few of them here.

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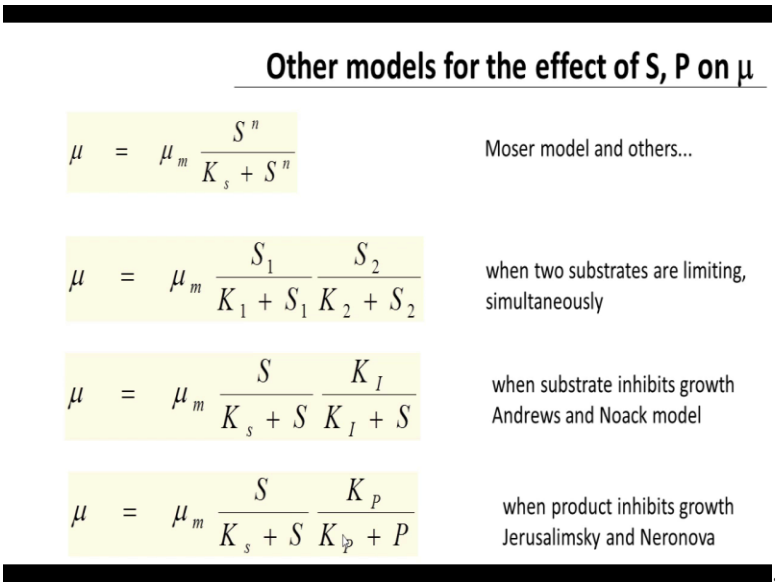

The other models for the effect of substrate and product also in this case you know, product could also affect the specific growth rate. For example, I think we did mention ethanol beyond a certain percentage, some 18 percent of so, it starts affecting the growth rate of cells, that can also be represented. I will give you some brief representations here. There are many models. Some of them are:

$$\mu = \mu_m \frac{S^n}{K_s + S^n}$$

This is called the Moser model, where you have mu m, K s and n as the parameters of the model.

This is one other model.

$$\mu = \mu_m \frac{S_1}{K_1 + S_1} \frac{S_2}{K_2 + S_2}$$

This model is very popular when 2 substrates are simultaneously limiting.

When the substrate happens to limit growth, some substrates do that, then, you could use this model, which was given by Andrews and Noack,

$$\mu = \mu_m \frac{S}{K_s + S} \frac{K_I}{K_I + S}$$

This is the Andrews and Noack model.

And I will probably mentioned one more model which is the Jerusalimsky and Neronova model, which gives actually the effect of product inhibition on the growth.

$$\mu = \mu_m \frac{S}{K_s + S} \frac{K_P}{K_P + P}$$

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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

Many other models are available.

So far we looked at models that describe growth, a specific growth rate and so on growth rate, specific growth rate also.

Now, let us look at the product if it happens to be another molecule apart from the cells or the product, the molecule made by the cells. A very simple model, a widely-used model is called the Luedeking-Piret model for the product formation rate, note that again these are all models for rates. The Luedeking-Piret model goes as something like this,

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

The product formation rate equals something called a growth dependent parameter times the rate of cell growth plus a growth independent parameter times the cell concentration. There are growth dependent products and growth independent products. Growth dependent products are sometimes called primary metabolites, growth independent

products are sometimes are called secondary metabolites. These are loose associations, but this is what we need to look at rp equals alpha times r x, the product formation rate depends on growth rate and the cell concentration. Sometimes beta could be 0, sometimes alpha could be 0 and so on. Many other models are available. We will probably not look at other models in this particular course, but just remember that many other models are available

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Yield coefficients

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

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

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

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

.

The next concept that we are going to look at, useful concept is called the yield coefficient. It is a very simple concept, but useful and you could define many yield coefficients. For example, Y of x slash s, yield of cells with respect to substrate, that is the way to read this, yield of cells with respect to substrate equals is, or is defined as the amount of cells produced divided by the amount of substrate consumed, it is a rate of the amounts of cells produced by the substrate consumed.

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

Similarly, you could define a yield of product with respect to substrate, which is amount of product formed divided by the amount of substrate consumed.

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

And one more, yield of cells with respect to product, amount of cells produced divided by the amount of product formed.

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

Both are formed in this case sometimes its formed, sometimes consumed you can define what ever yield coefficient that you can.

The advantage in doing this is that the yield coefficient can be assumed constant for a certain small region of interest, you know that is why it becomes very useful and the constancy is usually a good assumptions if the region happens to be small. We will see how this is done.

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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

.

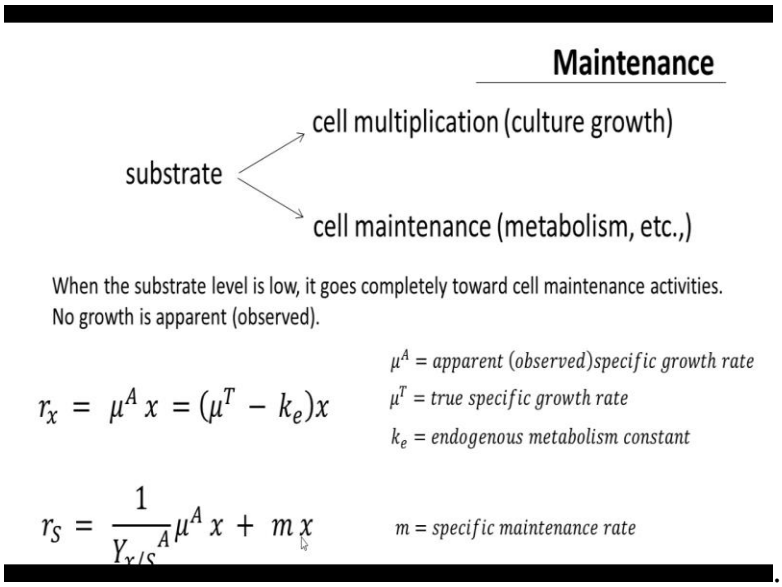
If the, the usefulness of this once it is defined, is that if you know 1 rate you could estimate the other rate from the relevant yield coefficient. For example, if the substrate consumption rate is needed and is not known, but the growth rate is known you could estimate the substrate consumption rate from the growth rate, if the yield coefficients is known and can be taken to be a constant. For example, the substrate consumption rate, r_S, is

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

You know this comes directly from the definition of the yield coefficient itself, amount of cells produced by the amount of substrate consume, those are amounts, is a yield coefficient therefore, if you are look looking at substrate it will be 1 by Y x s times r x, this is the rate of substrate consumption, rate of cell formation. So, going by our first model this can be written as r x equals mu x therefore, r s equals.

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

We will actually use these to compute some useful parameters in the next module, for now just know about this. (Refer Slide Time: 33:19) Probably tell you one more thing,



No yeah, one more concept and I think we will stop the, finish up this module here. This is the concept of Maintenance, you know the substrate glucose for example, goes towards cell multiplication or culture growth, cells multiplying culture growth but the cells are complex systems. They have a lot of reactions happening in them, they have a lot of processes happening in them, there is a lot of genes going to proteins, sort of transport happening in them ATP being made, ATP being used, all kinds of things are happening. And energy is required for all of them and the substrate needs to provide energy for such processes. So such processes that is metabolism, transport, genetic transformation, genetic processes and so on so forth are called cell maintenance. So, the

substrate goes for cell maintenance and once this is satisfied, then it goes for cell multiplication. We did not consider this explicitly so far, we need to consider this because when the substrate level is low, it goes completely towards cell maintenance activities and no growth is apparent, no growth is observed, apparent means observed, no growth is observed. So, it is all going to cell maintenance. There will be substrate consumption but it does not show up as growth, which means everything is going to maintain the cell, maintain the activities of the cell.

A way of representing this in terms of rates is, the rate of growth equals an apparent specific growth rate an observed specific growth rate times the cell concentration x, and this apparent specific growth rate can be defined as the difference between the true specific growth rate which is obtained when all the substrate goals towards cell multiplication alone, that is a concept, μ^T , true specific growth rate and the difference between μ^T and something called an endogenous metabolism constant, μ^A is apparent or the observed specific growth rate, μ^T is the true specific growth rate and ke is the endogenous metabolism constant. So, μ^Aequals μ^T minus ke.

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

And using the yield coefficient r s the substrate consumption rate is 1 by Y x/s apparent the yield, apparent yield times apparent specific growth rate into cell concentration plus something called m into cell concentration where m is the specific maintenance rate.

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

I think this is a nice place to close this particular lecture we did see quite a lot of concepts. Very briefly; we saw that the cells themselves could be products very quickly let us go through what all we saw and there could be other products that are made. We are looking at writing down expressions for rates of these, rate of growth or cell multiplication and rate of product formation. Initially we saw expressions for rate of growth r x equals mu x,

$$r_x = \mu x$$

μ may or may not be a constant depending on the situation, then we said that there are

other models, this is the Logistic equation and in an advanced course one can consider Structured models, Segregated models and many other things.

The substrates are converted into cells and products. We saw that glucose is a typical substrate because it participates in one of the important metabolic pathway in the cell called glycolysis and we saw some alternatives for glucose, because glucose is expensive from the context of the industry. Starch cellulose and ligno cellulosic materials are good alternatives to glucose, starch and cellulose are already heavily used, ligno cellulosic materials there is lot of research going on in use of ligno cellulosic materials to get glucose. Then we saw what a medium contained, a carbon source and energy source and nitrogen source salts and trace nutrients, any of them could be a limiting substrate. If it is a limiting substrate which limits growth, then the variation of specific growth rate with the variation in concentration is given by the Monod's model and there is an interpretation for this K s which is the substrate concentration at half maximal growth rate.

Then, we said that there are other models for the effect of substrate product concentration on mu we saw few of them, Moser model and the model when 2 substrates are simultaneously limiting and Andrews and Noack model which gives you the effect of substrate inhibition and the model by Jerusalimsky and Neronova which gives the effect of product inhibition on growth. Then we looked at the very popular model called the Luedeking-Piret model for the product formation rate, which goes as r_p equals alpha times r_x the growth dependent parameter plus beta times x the growth independent parameter.

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

And many other models are available. Then we saw what Yield coefficients were, many yield coefficients could be defined, it is essentially amount per amount and then they can be used to convert one rate into the other. That is one of the ways in which we will be using them, and then we looked at the concept of Maintenance which is the substrate going to maintain the activities in the cell first such as metabolism, transport and other things, genetic processes and then after satisfying this it goes to us multiplication.

Therefore, when the substrate level is very low, one may not see growth at all. That is

represented by the apparent growth rate and the true growth rate and an endogenous metabolism constant ke μ^T minus ke equals μ^A.

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

And also the rate of substrate consumption can be written in terms of the apparent specific growth rate and a specific maintenance rate.

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

I think that is good enough for lecture number 4, see you again in lecture number 5.